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GENIC DIFFERENTIATION AND PHYLOGENETIC RELATIONSHIPS AMONG TROPICAL HARVEST MICE (*REITHRODONTOMYS*: SUBGENUS *APORODON*)

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To assess genic differentiation and phylogenetic relationships among selected species in the subgenus *Aporodon*, we screened 31 presumptive genetic loci in 6 species in the subgenus *Aporodon* (*R. creper, R. gracilis, R. mexicanus, R. microdon, R. spectabilis*, and *R. tenuirostris*) and in 4 species in the subgenus *Reithrodontomys* (*R. chrysopsis, R. fulvescens, R. megalotis*, and *R. sumichrasti*). The resulting phylogenetic trees were compared with the results of previous molecular and morphological studies. Results demonstrate that the subgenus *Aporodon* is monophyletic. Relationships determined on the basis of allozymes are largely congruent with an earlier analysis based on morphological and molecular characters. However, samples of *R. mexicanus* do not form a monophyletic lineage. Accordingly, populations from north-central Oaxaca and Costa Rica each likely represent an undescribed species.

Key words: allozymes, *Aporodon*, harvest mice, monophyly, paraphyly, phylogeny, *Reithrodontomys*

Harvest mice of the genus *Reithrodontomys* are distinguished from other genera of peromyscine rodents (sensu Carleton 1989) by possession of sulcate (grooved) upper incisors (Le Conte 1853). The only comprehensive analysis of relationships among species of *Reithrodontomys* subsequent to those by Allen (1895) and Howell (1914) was performed by Hooper (1952). His review emphasized Mexican and Central American forms and evaluated systematic relationships based on a series of cranial and external characters, pelage color, and distribution data. According to Hooper (1952), the 2 subgenera originally recognized by Howell (1914) are distinguished by a series of morphological traits. Specializations possessed by species in the subgenus *Aporodon* include an increase in complexity and size of the cheek teeth (crown length of M3 and m3 about three-fourths the length of M2 and m2, respectively, rather than about one-half the length of M2 or m2 as in species belonging to the subgenus *Reithrodontomys*) and in size of mesopterygoid fossa (larger in *Aporodon*—Hooper 1952; Rinker and Hooper 1950).

Later, Carleton (1980) characterized selected species in the subgenus *Aporodon* (represented in his analysis by *R. creper* and *R. mexicanus*) as having more than 36 caudal vertebrae, a discoglandular gastric epithelium, and a plantar surface on the heel of the hind feet that is either naked or

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slightly furred. In contrast, members of the subgenus *Reithrodontomys* (as represented by *R. fulvescens, R. humulis, R. megalotis, R. montanus,* and *R. sumichrasti*) have less than 36 caudal vertebrae, an intermediate gastric epithelium, and a plantar surface of the hind foot that is densely furred to the thenar pad. In addition, Carleton (1980) identified 3 character states involving the relative positions (either alternate, intermediate, or opposite) of the protoconid–metaconid and hypoconid–entoconid cusps on M1. Species of *Aporodon* examined had the ''intermediate'' condition, whereas species surveyed in the subgenus *Reithrodontomys* possessed the ''alternate'' state for this character.

Hooper (1952) divided species in the subgenus *Reithrodontomys* into 2 species groups. The *R. fulvescens* species group consisted of *R. fulvescens* and *R. hirsutus,* whereas the *R. megalotis* species group included 7 species (*R. burti, R. chrysopsis, R. humulis, R. megalotis, R. montanus, R. raviventris,* and *R. sumichrasti*). More recently, Hood et al. (1984) recognized *R. megalotis zacatecae* as a full species. Within the subgenus *Aporodon,* Hooper (1952) placed *R. creper, R. microdon, R. rodriguezi,* and *R. tenuirostris* in the *R. tenuirostris* species group and set them apart from *R. brevirostris, R. darienensis, R. gracilis,* and *R. mexicanus*—with the latter 4 species forming the *R. mexicanus* group. Jones and Lawlor (1965) and Jones and Genoways (1970) described *R. spectabilis* and *R. paradoxus,* respectively, and assigned both to the *R. mexicanus* species group. Currently, the genus *Reithrodontomys* consists of 20 species divided evenly between the 2 subgenera.

Few studies have assessed systematic relationships within the genus *Reithrodontomys* since Hooper's (1952) review, and none is comprehensive in scope. Studies of standard and differentially stained karyotypes (Carleton and Myers 1979; Engstrom et al. 1981; Hood et al. 1984; Robbins and Baker 1980) indicate that chromosomal characters are not useful in partitioning species of harvest mice along subgeneric or even species group (sensu Hooper 1952) boundaries. Moreover, species of *Reithrodontomys* considered by Hooper (1952) to be the most derived morphologically (*R. tenuirostris* species group) possess diploid and fundamental numbers hypothesized to be similar to the proposed ancestral condition for *Reithrodontomys* (Carleton and Myers 1979; Hood et al. 1984; Robbins and Baker 1980) and other cricetine rodents (Koop et al. 1984).

Several papers have addressed phylogenetic relationships among selected species of harvest mice based on molecular data. Arnold et al. (1983) and Nelson et al. (1984) both used allozyme data and focused on species belonging to the subgenus *Reithrodontomys*. The study by Arnold et al. (1983) was unable to resolve relationships among 8 species of harvest mice examined, whereas the follow-up paper by Nelson et al. (1984) uncovered synapomorphic characters that served to define 3 clades among 7 species belonging to the subgenus *Reithrodontomys*. Later, Bell et al. (2001) examined mitochondrial deoxyribonucleic acid cytochrome-*b* sequence data for the same set of taxa examined by Nelson et al. (1984) and recovered 2 of the 3 clades recognized by Nelson and coworkers.

The objectives of this study were 4-fold: to examine genic differentiation as assayed by protein electrophoresis for a more complete set of taxa; to develop a molecular-based phylogenetic hypothesis of relationships emphasizing species in the subgenus *Aporodon*; to test for monophyly of the subgenera *Aporodon* and *Reithrodontomys*; and where appropriate, to suggest changes in the current taxonomy.

Materials and Methods

Liver, kidney, and heart tissue samples were obtained from 71 specimens representing 10 species of *Reithrodontomys* and *Peromyscus maniculatus* as listed in Appendix I. A total of 31 genetic loci were examined from liver or

combined kidney and heart homogenate (Murphy et al. 1996); genetic loci, abbreviations, Enzyme Commission numbers (EC), and buffer systems used are summarized in Arellano (1994). Enzyme mobility was determined from horizontal electrophoretic gels, and alleles for each locus were labeled in alphabetical order using the most-anodal migration as ''a.''

Data were summarized as single genotypes for each individual and locus and were analyzed using the BIOSYS-1 computer program (Swofford and Selander 1989), which calculated average individual heterozygosity, percentage of polymorphism, and genetic distances. Rogers' (1972) coefficient was calculated for comparison with other studies. Although sample sizes in this study were small, Gorman and Renzi (1979) documented that samples as small as 2 individuals/population are sufficient to generate heterozygosity estimates within 2.5% of those calculated with much larger sample sizes. Archie et al. (1989) demonstrated that small sample size reduces variance of values for genetic distances and can cause instability in phenetic trees. However, use of small samples can be justified when values for heterozygosity and percentage of polymorphism are low and allele frequencies are equal or very close to 0 or 1, indicating that alleles move toward fixation. In this study, heterozygosity and polymorphism values were not high, and the majority of samples were distinguished by fixed allelic differences. Therefore, phylogenetic hypotheses developed from these data likely reflect those derived from larger sample sizes (Hafner et al. 1994).

Data were subjected to parsimony analyses using PAUP* software of Swofford (1999). Uninformative characters (monomorphic loci or autapomorphies) were not used in the original data matrix. We used the step matrix option in which each locus was considered as a single character, and alleles and each possible combination of them were considered as character states (Mabee and Humphries 1993). Although fixed characters provide the most-phylogenetic signal (Wiens 1995), we also included polymorphic characters because they also are phylogenetically informative (Wiens 1995; Wiens and Servedio 1997). Characters, as defined in Table 1, were treated as reticulate (unordered), assuming that all character-state transformations were possible instead of imposing a specific pathway. The combinations of alleles we used were those inferred to be present in ancestral nodes to reduce dimensions of the step matrix (Mabee and Humphries 1993; Mardulyn and Pasteels 1994). We used PAUP* version 4.07b (Swofford 1999) to reconstruct the array of plesiomorphic character states consistent with the most parsimonious tree(s), based on the character matrix (Table 1) and on the distances stored in the step matrix (Harris and Rogers 1999; Mardulyn and Pasteels 1994; Table 2).

We used the out-group method to root our trees (Watrous and Wheeler 1981) and selected *P. maniculatus*, a member of the genus thought to be the sister group to *Reithrodontomys* (Carleton 1980; Hooper and Musser 1964). To resolve relationships among species of *Aporodon*, we used single and multiple out-group combinations representing the subgenus *Reithrodontomys*.

Tree reconstruction was based on the heuristic search algorithm in PAUP* version 4.07b (Swofford 1999), including stepwise-addition sequence, 100 replications, and tree bisection and reconstruction swapping. Consensus trees (50% majority rule) were generated when more than 1 parsimonious tree resulted from the analysis. The most parsimonious tree obtained was compared with Hooper's (1952) phylogenetic hypothesis using the constraint option of PAUP*. Templeton's (1983) Wilcoxon signed-rank test was used to test for significant differences between tree topologies.

Crania of selected voucher specimens were measured (in mm) using hand-held digital calipers accurate to 0.05 mm. Although 13 craniodental variables were recorded, only a subset is reported in this study.

RESULTS

Two genetic loci (*MDH-2* and *IDDH*) were fixed for the same allele across all taxa examined, including the out-group, whereas all species of *Reithrodontomys* shared the same allele for *LDH-2* and *AAT-2* (Appendix II). Average polymorphism, based on samples of *Reithrodontomys* with $n > 4$, was 12.1%. Mean heterozygosity (H; direct count method) was 2.9%.

Reithrodontomys fulvescens, *R. gracilis*, and *R. microdon* were each represented by 2 populations in our analysis. *R. fulvescens* was sampled from Texas and southern Veracruz, Mexico. These 2 populations dif-

TABLE 1.—Data matrix with 29 polymorphic characters coded for 18 samples of *Reithrodontomys* and 1 of *Peromyscus maniculatus* based on allelic designations as presented in Appendix II. Characters correspond to genetic loci as follows: 1 = *LDH-1*; 2 = *LDH-2*; 3 = *AAT-1*; 4 = *AAT-2*; 5 = *MDH-1*; 6 = *SOD-1*; 7 = *SOD-2*; 8 = *IDH-1*; 9 = *IDH-2*; 10 = *G3PDH*; 11 = *PGM*; 12 = *PNP*; 13 = *GPI*; 14 = *PPA*; 15 = *PPB*; 16 = *PPD*; 17 = *PPF*; 18 = *ALB*; 19 = *PGDH*; 20 = *MPI*; 21 = *ADA*; 22 = *ADH*; 23 = *MDHP*; 24 = *α-GLUS*; 25 = *β-GLUS*; 26 = *β-GLUR*; 27 = *α-MAN*; 28 = *AK*; and 29 = *CK*. Character codes proceed from numerals 1 to 9 and then by letters of the alphabet (A to Z) as described in Table 2. Blanks indicate missing data.

	Characters																										
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
mexicanus-1	1	1	3	2	2	2	1	2	2	1	2	2	1	1	3	2	2	1	2	3	P	2	4	2	5	2	2	1	1
mexicanus-2	1	1	3	2	2	2	1	2	9	1	2	9	1	I	3	2	2	1	2	3	T	2	4	2	4	2	2	1	2
mexicanus-3	1	1	3	2	2	2	1	2	2	1	2	2	1	2	3	2	2	1	2	3	4	2	4	2	X	2	2	1	2
R. sp. A	2	1	3	2	2	2	1	2		1	2	2	2	1	I	2	3	1	4	2	3	4	3	2	5	2	2	1	2
R. sp. B	1	1	8	2	2	2	1	2	3	1	2	2	1	1	3	2	2	1	2	3	V	T	L	O	5	2	2	1	2
microdon-1	1	1	5	2	9	2	1	2	2	1	2	4	1	1	E	2	2	1	2	3	3	4	4	2	4	2	2	1	2
microdon-2	1	1	5	2	2	2	1	2	2	9	I	4	1	2	O	I	2	1	2	3	3	4	4	2	X	2	2	1	1
creper	1	1	5	2	2	2	1	2	2	1	2	L	1	1	3	I	2	2	E	3	Q	6	4	3	5	2	2	1	2
tenuirostris	1	1	N	2	2	2	1	2	2	1	2	4	1	2	3	2	3	1	2	3	1	4	4	3	4	2	3	1	9
spectabilis	1	1	5	2	2	1	1	2	2	1	2	9	1	1	3	2	2	1	2	3	T	4	4	2	5	2	3	1	2
gracilis-1	1	1	5	2	2	1	1	2	2	9	2	I	1	1	3	9	2	1	I	I	T	2	4	2	5	2	3	1	2
gracilis-2	1	1	5	2	2	1	1	2	2	1	I	9	1	1	I	I	2	1	2	3	4	2	4	2	5	2	3	1	2
sumichrasti	9	1	H	2	2	1	3	9	2	1	2	6	1	1	3	2	3	1	6	2	3		4	2	3	2	2	1	2
chrysopsis	2	1	5	2	2	2	3	9	2	1	2	4	1	1	3	2	1	1	6	2	4		3	2	3	1	2	1	2
fulvescens-1	2	1	7	2	2	2	3	2	2	1	9	T	4	2	I	1	2	1	2	I	1	4	1	2	3	2	2	2	1
fulvescens-2	2	1	7	2	2	2	3	2	2	1	2	5	3	2	3	1	2	1	2	2	1	3	1	2	3	2	1	2	1
megalotis	2	1	7	2	2	2	2	2	2	1	I	2	9	9	3	9	2	1	6	9	I	4	4	2	3	2	2	1	9
maniculatus	2	2	W	1	1	2	1	2	3	2	9	U	4	1	O	1	2	1	R	3	B	9	5	2	9	2	2	1	3

fered from each other by 3 fixed alternate alleles (*GPI, ADH,* and *β-MAN*) and by frequency differences at another 4 loci. The resulting Rogers' (1972) distance-value was 0.13. For the 2 populations of *R. gracilis* from Campeche and Yucatan, Mexico, frequency differences at 8 loci (Appendix II) contributed to yield a Rogers' (1972) distance-value of 0.05. *R. microdon* was examined from Chiapas, Mexico, and north-central Guatemala. These 2 populations differed by fixed alternate alleles at 2 loci (*PEP-A* and *CK*) and by frequency differences at another 2 loci. In addition, nonfixed autapomorphic alleles were present in both populations of *R. microdon* for *MDH-1, G3PDH, PGM, PEP-B,* and *PEP-D,* resulting in a Rogers' (1972) distance-value of 0.14.

Five populations of *R. mexicanus* (1 each from Costa Rica, El Salvador, and Mexico, and 2 from Guatemala) were examined. The sample from Oaxaca, Mexico (referred to hereafter as *Reithrodontomys* sp. A), differed by alternate fixed alleles at 6 loci (*LDH-1, GPI, PEP-F, PGDH, MPI,* and *MDHP*) as compared with samples of *R. mexicanus* (Appendix II). The majority of genetic variation within the 4 remaining samples of *R. mexicanus* was partitioned between the Costa Rican population and the 3 samples from El Salvador and Guatemala. These ''northern'' and ''southern'' *R. mexicanus* differed by fixed alternate alleles at 3 genetic loci (*AAT-1, IDH-2,* and *α-GLUS*) and by frequency differences at another 3 loci (*ADA, ADH,* and *MDHP*). The mean Rogers' (1972) distance-value among these 4 populations of *R. mexicanus* was 0.14 (range 0.07–0.22). However, the mean Rogers' (1972) distance-value among the 3 ''northern'' samples was 0.09, whereas the mean distance-value between the Costa Rican population (referred to hereafter as *Reithrodotomys* sp. B) and any of the other 3 was 0.19.

Cranial measurements recorded from the 4 voucher specimens of *Reithrodontomys* sp. B used in this study are more similar to examples of *R. m. cherrii* than to the other species of harvest mouse, *R. brevirostris,* which also is known from moderate elevations in central Costa Rica (Hall 1981). For example, mean values (with range in parentheses) of greatest length of skull, zygomatic breadth, and length of rostrum for *Reithrodontomys* sp. B are 24.25 (24.05–24.40), 12.75 (12.60–12.90), and 8.85 (8.50–9.05), respectively. Means for the same 3 cranial measurements (Hooper 1952) of *R. m. cherrii* ($n = 23$) are 23.7 (22.6–24.5), 12.1 (11.5–12.7), and 8.1 (7.6–8.5), and of *R. brevirostris* ($n = 4$) are 23.7 (22.6–24.5), 22.3 (22.1–22.5), and 7.9 (7.7–8.3), respectively (Hooper 1952).

A data matrix with 29 informative characters (Table 1) was subjected to phylogenetic analysis to resolve relationships among all species of *Reithrodontomys* included in this study using *P. maniculatus* as the out-group. A step matrix was used to establish the number of steps required in a transition between any 2 character states (Table 2). Cladistic reconstruction produced 12 most parsimonious trees with lengths of 191 steps. In the resulting consensus tree (Fig. 1), all samples of *Reithrodontomys* formed a single clade relative to the out-group, with *R. fulvescens* recovered as the basal taxon. In fact, all species of *Reithrodontomys* were recovered as a monophyletic group regardless of whether or not *P. maniculatus* was designated as the out-group taxon. Within this tree, samples representing the subgenus *Aporodon* (with the exception of *Reithrodontomys* sp. A) also formed a monophyletic group. However, species belonging to the subgenus *Reithrodontomys* did not. Within the clade formed by species in the subgenus *Aporodon,* 3 species represented by 2 or more samples (*R. gracilis, R. mexicanus,* and *R. microdon*) did not form monophyletic groups.

To further resolve relationships within the subgenus *Aporodon,* we performed a series of phylogenetic analyses using single and multiple species in the subgenus *Reith-*

TABLE 2.—Step matrix used to code character states used in the phylogenetic analysis and listed in TABLE 1. A total of 35 characters (1–9 and A–Z) were identified. Numbers in the matrix represent steps required for every character transformation. For example, the number of steps required to change from character 9 (presence of alleles a and b) to character 1 (presence of allele a) is 1 (loss of allele b). Likewise, it requires 2 steps to move from character 1 (presence of allele a) to character 2 (presence of allele b—gain of allele b and loss of allele a).

	1	2	3	4	5	6	7	8	9	A	B	C	D	E	F	G
[1-a]	—	2	2	2	2	2	2	2	1	2	3	4	2	1	2	6
[2-b]	2	—	2	2	2	2	2	2	1	2	3	4	2	3	4	8
[3-c]	2	2	—	2	2	2	2	2	3	2	3	4	4	1	2	6
[4-d]	2	2	2	—	2	2	2	2	3	4	3	4	2	3	2	8
[5-e]	2	2	2	2	—	2	2	2	3	4	5	4	4	3	4	6
[6-f]	2	2	2	2	2	—	2	2	3	4	5	6	4	3	4	6
[7-g]	2	2	2	2	2	2	—	2	3	4	5	6	4	3	4	6
[8-h]	2	2	2	2	2	2	2	—	3	4	5	6	4	3	4	6
[9-ab]	1	1	3	3	3	3	3	3	—	1	2	3	1	2	3	7
[A-abc]	2	2	2	4	4	4	4	4	1	—	1	2	2	1	2	6
[B-abc]	3	3	3	3	5	5	5	5	2	1	—	1	1	2	1	7
[C-abcde]	4	4	4	4	4	6	6	6	3	2	1	—	2	3	2	6
[D-abd]	2	2	4	2	4	4	4	4	1	2	1	2	—	3	2	8
[E-ac]	1	3	1	3	3	3	3	3	2	1	2	3	3	—	1	5
[F-acd]	2	4	2	2	4	4	4	4	3	2	1	2	2	1	—	6
[G-acefghi]	6	8	6	8	6	6	6	6	7	6	7	6	8	5	6	—
[H-af]	1	3	3	3	3	1	3	3	2	3	4	5	3	2	3	5
[I-bc]	3	1	1	3	3	3	3	3	2	1	2	3	3	2	3	7
[J-bcd]	4	2	2	2	4	4	4	4	3	2	1	2	2	3	2	8
[K-bcefghi]	8	6	6	8	6	6	6	6	7	6	7	6	8	7	8	2
[L-bd]	3	1	3	1	3	3	3	3	2	3	2	3	1	4	3	9
[M-bde]	4	2	4	2	2	4	4	4	3	4	3	2	2	5	4	8
[N-be]	3	1	3	3	1	3	3	3	2	3	4	3	3	4	5	7
[O-cd]	3	3	1	1	3	3	3	3	4	3	2	3	3	2	1	7
[P-cde]	4	4	2	2	2	4	4	4	5	4	3	2	4	3	2	6
[Q-ce]	3	3	1	3	1	3	3	3	4	3	4	3	5	2	3	5
[R-ceghi]	7	7	5	7	5	5	5	5	8	7	8	7	9	6	7	1
[S-cefghi]	6	6	4	6	4	6	4	4	7	6	7	6	8	5	6	2
[T-de]	3	3	3	1	1	3	3	3	4	5	4	3	3	4	3	7
[U-defg]	5	5	5	3	3	3	3	5	6	7	6	5	5	6	5	5
[V-df]	3	3	3	1	3	1	3	3	4	5	4	5	3	4	3	7
[W-dg]	3	3	3	1	3	3	1	3	4	5	4	5	3	4	3	7
[X-ef]	3	3	3	3	1	1	3	3	4	5	6	5	5	4	5	5
[Y-efghi]	6	6	6	6	4	4	4	4	7	8	9	8	8	7	8	2
[Z-fh]	3	3	3	3	3	1	3	1	4	5	6	7	5	4	5	5

rodontomys as out-groups. *Reithrodontomys* sp. A was constrained as part of the in-group in both analyses because its external, cranial, and dental morphologies were typical of species of *Aporodon*. Regardless of the out-group used, these analyses resulted in a fully resolved, single most parsimonious tree (Fig. 2) with a length of 155 steps. This tree differed from that shown in Fig. 1, in that *R. creper* groups with *R. microdon* and *R. tenuirostris*. We constrained our allozyme data to Hooper's (1952) tree topology (for comparable taxa) and conducted an additional phylogenetic analysis using *P. maniculatus* to polarize characters. The resulting tree was significantly longer ($P \leq 0.01$—11 steps) than the tree depicted in Fig. 1.

Overall, bootstrap values were low; values greater than 50% were obtained for

TABLE 2.—Extended.

H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	3	4	8	3	4	3	3	4	3	7	6	3	5	3	3	3	6	3
3	1	2	6	1	2	1	3	4	3	7	6	3	5	3	3	3	6	3
3	1	2	6	3	4	3	1	2	1	5	4	3	5	3	3	3	6	3
3	3	2	8	1	2	3	1	2	3	7	6	1	3	1	1	3	6	3
3	3	4	6	3	2	1	3	2	1	5	4	1	3	3	3	1	4	3
1	3	4	6	3	4	3	3	4	3	5	6	3	3	1	3	1	4	1
3	3	4	6	3	4	3	3	4	3	5	4	3	3	3	1	3	4	3
3	3	4	6	3	4	3	3	4	3	5	4	3	5	3	3	3	4	1
2	2	3	7	2	3	2	4	5	4	8	7	4	6	4	4	4	7	4
3	1	2	6	3	4	3	3	4	3	7	6	5	7	5	5	5	8	5
4	2	1	7	2	3	4	2	3	4	8	7	4	6	4	4	6	9	6
5	3	2	6	3	2	3	3	2	3	7	6	3	5	5	5	5	8	7
3	3	2	8	1	2	3	3	4	5	9	8	3	5	3	3	5	8	5
2	2	3	7	4	5	4	2	3	2	6	5	4	6	4	4	4	7	4
3	3	2	8	3	4	5	1	2	3	7	6	3	5	3	3	5	8	5
5	7	8	2	9	8	7	7	6	5	1	2	7	5	7	7	5	2	5
—	4	5	7	4	5	4	4	5	4	6	7	4	4	2	4	2	5	2
4	—	1	5	2	3	2	2	3	2	6	5	4	6	4	4	4	7	4
5	1	—	6	1	2	3	1	2	3	7	6	3	5	3	3	5	9	5
7	5	6	—	7	6	5	7	6	5	1	2	7	5	7	7	5	2	5
4	2	1	7	—	1	2	2	3	4	8	7	2	4	2	2	4	7	4
5	3	2	6	1	—	1	3	2	3	7	6	1	3	3	3	3	6	5
4	2	3	5	2	1	—	4	3	2	6	5	2	4	4	4	2	5	4
4	2	1	7	2	3	4	—	1	2	6	5	2	4	2	2	4	7	4
5	3	2	6	3	2	3	1	—	1	5	4	1	3	3	3	3	6	5
4	2	3	5	4	3	2	2	1	—	4	3	2	4	4	4	2	5	4
6	6	7	1	8	7	6	6	5	4	—	1	6	4	6	6	4	1	4
7	5	6	2	7	6	5	5	4	3	1	—	5	5	7	5	5	2	5
4	4	3	7	2	1	2	2	1	2	6	5	—	2	2	2	2	5	4
4	6	5	5	4	3	4	4	3	4	4	5	2	—	2	2	2	3	2
2	4	3	7	2	3	4	2	3	4	6	7	2	2	—	2	2	5	2
4	4	3	7	2	3	4	2	3	4	6	5	2	2	2	—	4	5	4
2	4	5	5	4	3	2	4	3	2	4	5	2	2	2	4	—	3	2
5	7	9	2	7	6	5	7	6	5	1	2	5	3	5	5	3	—	3
2	4	5	5	4	5	4	4	5	4	4	5	4	2	2	4	2	3	—

only 2 and 5 nodes, respectively, shown in Figs. 1 and 2. However, we found that if we increased the number of characters by duplicating our original data matrix (holding all else equal) and then recalculating bootstrap percentages (1,000 replicates), the resulting bootstrap values always increased. For example, when we doubled our data matrix (58 instead of 29 characters), the number of nodes with bootstrap percentages ≤50 in Fig. 1 increased from 2 to 10. Increasing the original data matrix 4-fold (now 116 characters) added 2 additional nodes (a total of 10) with bootstrap values ≤50% (ranges 50–87%). Enlarging the data matrix also increased the bootstrap values as given in Fig. 1. For example, doubling the data matrix increased the bootstrap value for the node defining *R. gracilis–R. spectabilis* from 68% to 84%. Likewise, the

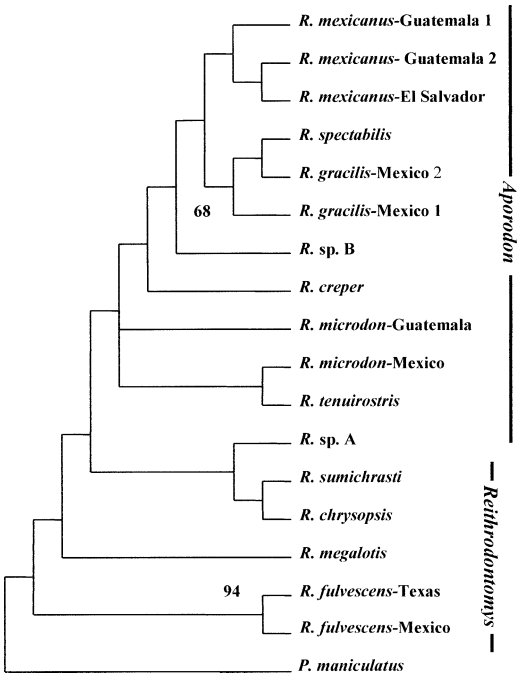

FIG. 1.—Maximum-parsimony, consensus cladogram (50% majority rule) derived from 12 equally parsimonious trees (length = 191 steps) showing phylogenetic relationships among members within the subgenus *Aporodon* using *Peromyscus maniculatus* as the out-group. Numbers on branches are bootstrap percentages based on 1,000 iterations.

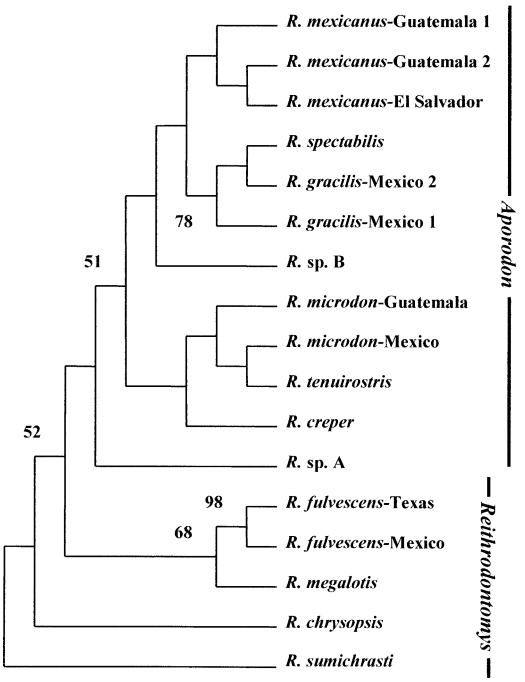

FIG. 2.—Single most parsimonious cladogram (length = 155 steps) derived using members of the subgenus *Reithrodontomys* as a composite out-group. Numbers on branches are bootstrap percentages based on 1,000 iterations.

node uniting the 2 samples of *R. fulvescens* increased from 94% to 96%. A 4-fold increase in the data set increased the bootstrap percentages to 98% and 97%, respectively.

DISCUSSION

Intraspecific differentiation.—Compared with other rodents, levels of polymorphism and heterozygosity detected among samples of *Reithrodontomys* are low (Avise and Aquadro 1982) but are characteristic of other species of peromyscine rodents with ''insular'' distributions (Kilpatrick 1981; Werbitsky and Kilpatrick 1987). This is consistent with our finding that the majority of intraspecific genetic variation in harvest mice is partitioned among populations. Whether this genetic structure is due to stochastic processes such as population bottlenecks or founder events, a reflection of cladogenic events, or other genetic factors intrinsic to *Reithrodontomys* cannot be determined from these data.

Before the present study, information on genetic differentiation within species of *Reithrodontomys* was limited to species in the subgenus *Reithrodontomys*. Arnold et al. (1983) examined 2 or more populations each of *R. fulvescens*, *R. humulis*, *R. megalotis*, and *R. montanus* and detected intraspecific variation in *R. fulvescens* and *R. humulis*. Three groups were delimited within the 8 populations of *R. fulvescens* evaluated by Arnold et al. (1983). One group showed no electrophoretic variation across 6 samples encompassing a geographic area from Oklahoma and Texas south to Tamaulipas, Puebla, and Morelos, Mexico. However, samples of *R. fulvescens* from Duran-

go and Chiapas, Mexico, differed from the other 6 populations and from each other by 2 (different) fixed alleles. We examined 2 samples of *R. fulvescens* and detected 3 fixed allelic differences between a population from south Texas and another from central Veracruz, Mexico. Direct comparison of allelic mobilities across studies is not possible. However, if we assume that our sample from Texas was similar to those evaluated by Arnold et al. (1983), then *R. fulvescens*, as presently constituted, likely includes at least 2 phyletic entities. One is distributed from Texas and Oklahoma south through Tamaulipas, Mexico, and into the Central Plateau of Mexico south of Mexico City. In addition, 1 or more allozymically distinct forms occurs in the Mexican states of Durango, Chiapas, and Veracruz. Additional sampling among Mexican populations of *R. fulvescens* should be conducted to further delimit the distributions of these groups.

Bell et al. (2001) presented mitochondrial cytochrome-*b* sequence data for 2 populations (1 individual from each) for 3 species in the subgenus *Reithrodontomys* (*R. megalotis, R. raviventris*, and *R. zacatacae*). Their analysis recovered the population pairs within each of these 3 species as ''sister taxa'' (Bell et al. 2001:84), but no values for genetic distance were provided.

Reithrodontomys microdon occurs only in a few scattered localities at elevations greater than about 2,300 m in central and southern Mexico and northern Middle America. The 2 samples compared in this study are referable to *R. m. microdon*, the southernmost of 3 recognized subspecies (Hall 1981). As in *R. mexicanus* (see below) and *R. fulvescens*, the among-population differentiation within *R. microdon* is large and exceeds that which usually characterizes a single species of peromyscine rodent (Calhoun et al. 1989; Rennert and Kilpatrick 1987; Rogers and Engstrom 1992; Werbitsky and Kilpatrick 1987).

Relatively high values of genetic distance were documented among samples originally referable to *R. mexicanus*. Based on our cladistic analyses of relationships among *Aporodon* taxa (Figs. 1 and 2), the populations from Oaxaca, Mexico (*Reithrodontomys* sp. A), and Costa Rica (*Reithrodontomys* sp. B) are phylogenetically distinct. Excluding these 2 putative species, the remaining 3 populations of *R. mexicanus* from Guatemala and El Salvador exhibit levels of genic divergence more typical of that documented within a single species of peromyscine rodent (Sullivan et al. 1991). *Reithrodontomys* sp. A currently is known from a single specimen collected in the southern portion of the Mexican Sierra Madre Oriental, and *Reithrodontomys* sp. B is known only from a single locality in the central highlands of Costa Rica.

Phylogenetic relationships.—Because our results indicate that there is a relationship between increasing bootstrap values and size of the data matrix, we suspect that bootstrap values for relatively small data sets are unreliable. More importantly, we believe that the low bootstrap values we obtained should not be necessarily interpreted as to the degree of confidence one should place on the stability of internal nodes depicted in Figs. 1 and 2.

Species in the subgenus *Aporodon* formed a monophyletic group in all analyses (Fig. 1) with the exception of *Reithrodontomys* sp. A. However, our results are not congruent with the monophyletic origin of the subgenus *Reithrodontomys* (Fig. 1) as suggested by Nelson et al. (1984) and assumed by Bell et al. (2001). Rather, our data suggest that the *Aporodon* clade is derived from ancestral forms most similar to *R. sumichrasti* and *R. chrysopsis*. According to Hooper (1952), these latter taxa originated in the humid highlands of southern Mexico and Central America, occupy equivalent habitats, and have ecological requirements similar to the more specialized species of *Aporodon* such as *R. creper* and *R. tenuirostris*.

Within the subgenus *Aporodon*, species are divided into 2 clades (Fig. 2) that mirror

the composition of the *R. mexicanus* (*R. gracilis, R. mexicanus,* and *R. spectabilis*) and the *R. tenuirostris* (*R. creper, R. microdon,* and *R. tenuirostris*) species groups delimited by Hooper (1952). With the exception of the placement of *R. creper*, relationships among these taxa are not altered (compare Figs. 1 and 2), regardless of the out-group taxon (or taxa) used to root the trees. Specifically, the sister group relationship between *R. microdon* and *R. tenuirostris* is supported.

Reithrodontomys microdon is paraphyletic relative to *R. tenuirostris*. We sampled *R. microdon* from 2 localities; central Guatemala and central Chiapas, Mexico. The kinship between the Chiapan sample of *R. microdon* and *R. tenuirostris* may indicate that *R. tenuirostris* evolved from a *R. microdon*–like ancestor in central Chiapas, Mexico, because both the Chiapan *R. microdon* and *R. tenuirostris* were collected from the same locality (Cerro Tzontehuitz).

The close relationship between *R. gracilis* and *R. spectabilis* as suggested by Jones and Lawlor (1965) is supported. In our results, *R. gracilis* also is paraphyletic with respect to *R. spectabilis*. *R. spectabilis*, a form endemic to Cozumel Island, is cladistically associated with the population of *R. gracilis* found on the Yucatan Peninsula close to Isla Cozumel rather than with the other sample of *R. gracilis* included in our analysis. The 2nd sample of *R. gracilis* was collected approximately 400 km to the southwest on the opposite side of the Yucatan Peninsula. It seems likely, therefore, that *R. spectabilis* evolved from *R. gracilis* stock located on the Yucatan Peninsula adjacent to Isla Cozumel.

The phylogenetic affinity of *Reithrodontomys* sp. A to other species of *Reithrodontomys* is problematic. It morphologically resembles other species of *Aporodon*, such as *R. mexicanus*, in traits such as dental pattern (M. Carleton, pers. comm.), but its ambiguous phylogenetic position and relatively large number of autapomorphic characters indicate that this form is an undescribed species of uncertain phylogenetic affinities.

Sullivan et al. (2000) demonstrated that the major physiogeographic feature separating populations of the codistributed taxa *Peromyscus aztecus–hylocetes* and *R. sumichrasti* in southern Mexico and Central America was the Isthmus of Tehuantepec. Inasmuch as *R. mexicanus* (sensu Hall 1981) has a distribution that is similar to both *P. aztecus* and *R. sumichrasti*, it is possible that *Reithrodontomys* sp. A occurs north of the Isthmus in the Sierra Madre Oriental and *R. mexicanus* (sensu Hall 1981) occurs only south of the Isthmus in Chiapas, Mexico, and other portions of Middle America. However, populations of *R. mexicanus* have not been sampled from the Sierra Madre Occidental in Mexico or immediately south of the Isthmus of Tehuantepec, neither were any samples of *R. mexicanus* included from the Sierra Madre Oriental. Additional sampling of Mexican as well as Middle and South American *R. mexicanus* is critical to delimit the southern distribution of *R. mexicanus* (sensu Hall 1981) as well to establish the distributional limits of *Reithrodontomys* sp. B (see below) in Central America and northern South America.

We believe that the cladistic relationships among samples previously regarded as *R. mexicanus* indicate that the population from Costa Rica (*Reithrodontomys* sp. B) is a distinct species allied with the *R. mexicanus* species group. This proposal is supported by several apomorphic characters that delimit the Costa Rican populations from samples of *R. mexicanus* and by the fact that the clade that comprised *R. gracilis* and *R. spectabilis* is imbedded within the samples of *R. mexicanus* (Figs. 1 and 2). The sample representing *Reithrodontomys* sp. B is referable to *R. mexicanus cherrii*, a morphologically distinct subspecies found in central and eastern Costa Rica and in western Panama. This subspecies is characterized by a relatively large size and brighter pelage (Hooper

1952). Additional sampling of other central American harvest mice (*R. brevirostris, R. paradoxus, R. rodriguezi*), as well as additional forms now considered subspecies of *R. mexicanus* (*garichensis* and *potrerograndei*—Hall 1981:651) should be undertaken so that phylogenetic relationships and distributional limits of these taxa can be determined.

Relationships among the 4 species of the subgenus *Reithrodontomys* (*R. chrysopsis, R. sumichrasti, R. megalotis,* and *R. fulvescens*; Fig. 1) are congruent with Hooper's (1952) morphological summary that arranges *R. chrysopsis* and *R. sumichrasti* as sister taxa. Placement of *R. fulvescens* as basal to other representatives of the subgenus *Reithrodontomys* also is consistent with Hooper's (1952) arrangement as well as that of Bell et al. (2001) based on mitochondrial cytochrome-*b* sequence data.

Hooper (1952) hypothesized that the archetypal (least specialized) harvest mouse probably was small, short-tailed, with a short and broad skull and relatively simple cusp patterns of the molar teeth. This ancestral form likely inhabited ''semiarid grasslands'' (Hooper 1952:195). However, Carleton and Myer (1979) provided an alternate hypothesis in which they proposed that the ''common ancestor of *Reithrodontomys* may have resembled a medium-sized, scansorial species'' that was similar either to *R. fulvescens* or to *R. gracilis* (Carleton and Myers 1979:311). Our data and those of Bell et al. (2001) support the latter hypothesis. A clearer picture likely will emerge with increased taxon sampling, particularly among species now allocated to the subgenus *Reithrodontomys* such as *R. burti, R. humulis,* and *R. montanus*. On the other hand, our genic data support Hooper's contention that species of *Aporodon* are more specialized. Taxa referable to the subgenus *Reithrodontomys* are basal to the more derived species belonging to the subgenus *Aporodon* in all phylogenetic analyses using *P. maniculatus* as the out-group (summarized in Fig. 1). We also support Hooper's (1952:196) proposal that the ''generalized'' species concentrate in the northern part of the range of the genus and specialized species in the southern part of the range. Therefore, we consider it likely that ecological (and corresponding morphological) specializations associated with the evolution of an arboreal lifestyle are derived conditions in the genus *Reithrodontomys*.

Resumen

Para estimar la diferenciación genética y las relaciones filogenéticas entre especies selectas del subgénero *Aporodon,* evaluamos 31 loci genéticos para 6 especies del subgénero *Aporodon* (*R. creper, R. gracilis, R. mexicanus, R. microdon, R. spectabilis,* and *R. tenuirostris*) y para 4 especies del subgénero *Reithrodontomys* (*R. chrysopsis, R. fulvescens, R. megalotis,* and *R. sumichrasti*). Los árboles filogenéticoas resultantes fueron comparados con resultados de previos estudios moleculares y morfológicos. Los resultados demuestran que el subgénero *Aporodon* es monofilético. Las relaciones obtenidas con base en aloenzimas son, en su mayoría, congruentes con un análisis previo basado en caracteres morfológicos. Sin embargo, las muestras de *R. mexicanus* no forman un linaje monofilético. Por consiguiente, las poblaciones del norte-centro de Oaxaca y de Costa Rica probablemente representen 2 especies separados no descritas.

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APPENDIX I

Specimens examined.—The 71 specimens examined are listed below by taxa, collecting locality, and museum acronym (Hafner et al. 1997). Abbreviations for voucher numbers are as follows: ASNHC = Angelo State Natural History Collections; BYU = Monte L. Bean Life Science Museum, Brigham Young University; CNMA = Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México, Mexico; LSUMZ = Louisiana State University Museum of Zoology; and ROM = Royal Ontario Museum. Numbers following species names indicate geographic samples within species, and these numbers correspond with designations in Table 2 and Appendix II.

Reithrodontomys chrysopsis.—MEXICO. Veracruz, 3.1 km S Puerto del Aire, 2,300 m (CNMA 34239).

Reithrodontomys creper.—COSTA RICA. Cartago [Province]: Rio Birris, 12 km N Potrero Cerrado, 2,800 m (ROM 97318, 97319, 97920–97922).

Reithrodontomys fulvescens.—(1) MEXICO. Veracruz: 18 km NW Teocelo, 1,300 m (CNMA 35313). (2) Texas: Nueces County, Port Aransas, Mustang Island (ASNHC 11632, 11633, 11438, ASK 3539 and 3546).

Reithrodontomys gracilis.—(1) MEXICO. Campeche: 52 km SW Champoton (ROM 95890–95894). (2) MEXICO. Yucatan, Laguna Becanchen (ASNHC 6369–6372).

Reithrodontomys megalotis.—Utah: Utah County, 8 mi W, 3.7 mi S Lehi, 1,500 m (BYU 14721–14725).

Reithrodontomys mexicanus.—(1) GUATEMALA. Baja Verapaz: 5 km E Puruhla, 1,550 m (ROM 98467–98470 and 98515). (2) GUATEMALA. Zacapa: 2 km N San Lorenzo, Sierra de la Minas, 2,150 m (ROM 99875, 99876, 99879, 99880, and 99892). (3) EL SALVADOR. Santa Ana: Parque Nacional Montecristo, 1,850 m (ROM 101508, 101534–101536).

Reithrodontomys microdon.—(1) GUATEMALA. Huehuetenango: 12 km NW Santa Eulalia, 2,730 m (ROM 98300, 98320, 98343, and 98382). (2) MEXICO. Chiapas: Municipio Chamula, Cerro Tzontehuitz, 13 km NE San Cristobal de las Casas, 2,880 m (BYU 14474–14479).

Reithrodontomys spectabilis.—MEXICO. Quintana Roo: Isla Cozumel, 30 km SE San Miguel (ASNHC 2139–2142 and CNMA 33045).

Reithrodontomys sumichrasti.—MEXICO. Veracruz: Zongolica (CNMA 34241).

Reithrodontomys tenuirostris.—MEXICO. Chiapas: Municipio Chamula, Cerro Tzontehuitz, 13 km NE San Cristobal de las Casas, 2,880 m (BYU 14479, 14480).

Reithrodontomys sp. A.—MEXICO. Oaxaca: Municipio Puerto de la Soledad, Puerto de la Soledad, 2,600 m (CNMA 33895).

Reithrodontomys sp. B.—COSTA RICA. San Jose Province: 1 km SW Poas, 1,500 m (LSUMZ 25164, 25165, 25375, and 25376).

Peromyscus maniculatus.—Utah: Utah County, 8 mi W, 3.7 mi S Lehi, 1,500 m (BYU 13278–13285).

Appendix II

Genetic variability at 29 polymorphic loci across samples of mice in genera *Reithrodontomys* and *Peromyscus*. Numbers below species abbreviations are sample sizes, numbers following species abbreviations are population samples as indicated in Appendix I, *n* = number of alleles per locus, letters refer to alleles, numbers in parenthesis are allele frequencies at a single locus. Taxon abbreviations are as follows: *R. mex* = *R. mexicanus*; *R. mic* = *R. microdon*; *R. crep* = *R. creper*; *R. tenu* = *R. tenuirostris*; *R. spec* = *R. spectabilis*; *R. gra* = *R. gracilis*; *R. sumi* = *R. sumichrasti*; *R. chry* = *R. chrysopsis*; *R. ful* = *R. fulvescens*; *R. mega* = *R. megalotis*; and *P. mani* = *P. maniculatus*.

Locus	*n*	*R. mex-1* 5	*R. mex-2* 5	*R. mex-3* 4	*R.* sp. *A* 1	*R.* sp. *B* 4	*R. mic-1* 4	*R. mic-2* 6	*R. crep* 5	*R. tenu* 2
LDH-1	2	a	a	a	b	a	a	a	a	a
LDH-2	2	a	a	a	a	a	a	a	a	a
AAT-1	8	c	c	c	c	h	e	e	b (0.20) e (0.80)	e
AAT-2	2	b	b	b	b	b	b	b	b	b
MDH-1	2	b	b	b	b	b	a (0.75) b (0.25)	b	b	b
SOD-1	2	b	b	b	b	b	b	b	b	b
SOD-2	3	a	a	a	a	a	a	a	a	a
IDH-1	2	b	b	b	b	b	b	b	b	b
IDH-2	3	b	a (0.20) b (0.80)	b	—	c	b	b	b	b
G3PDH	2	a	a	a	a	a	a	a (0.83) b (0.17)	a	a
PGM	3	b	b	b	b	b	b	b (0.83) c (0.17)	b	b
PNP	7	b	a (0.20) b (0.80)	b	b	b	d	d	b (0.50) d (0.50)	d
GPI	4	a	a	a	b	a	a	a	a	a
PEP-A	3	a	a (0.10) b (0.90)	b	a	b	a	b	a	b
PEP-B	4	c	c	c	b (0.50) c (0.50)	c	a (0.12) c (0.88)	c (0.92) d (0.08)	c	c
PEP-D	3	b	b	b	b	b	b	b (0.83) c (0.17)	b (0.90) c (0.10)	b
PEP-F	3	b	b	b	c	b	b	b	b	c
ALB	2	a	a	a	a	a	a	a	b	a
PGDH	9	b	b	b	d	b	b	b	a (0.70) c (0.30)	b
MPI	3	c	c	c	b	c	c	c	c	c
ADA	6	c (0.20) d (0.50) e (0.30)	d (0.20) e (0.80)	d	c	d (0.67) f (0.33)	c	c	c (0.40) e (0.60)	a
ADH	6	b	b	b	d	d (0.33)	d e (0.67)	d	f	d
MDHP	5	d	d	d	c	b (0.75) d (0.25)	d	d	d	d
α-GLUS	4	b	b	b	b	c (0.67)	b d (0.33)	b	c	c
β-GLUS	6	e	d	e (0.88) f (0.12)	e	e	d	e (0.80) f (0.20)	e	d
β-GLUR	2	b	b	b	b	b	b	b	b	b
α-MAN	3	b	b	b	b	b	b	b	b	c
AK	2	a	a	a	a	a	a	a	a	a
CK	3	a	b	b	b	b	b	a	b	a (0.50) b (0.50)

APPENDIX II.—Extended.

n	*R. spec* 5	*R. gra-1* 5	*R. gra-2* 4	*R. sumi* 1	*R. chry* 1	*R. ful-1* 1	*R. ful-2* 2	*R. mega* 5	*P. mani* 8
2	a	a	a	a (0.50) b (0.50)	b	b	b	b	b
2	a	a	a	a	a	a	a	a	b
8	e	e	e	a (0.50) f (0.50)	e	g	g	g	d (0.21) g (0.79)
2	b	b	b	b	b	b	b	b	a
2	b	b	b	b	b	b	b	b	a
2	a	a	a	a	b	b	b	b	b
3	a	a	a	c	c	c	c	b	a
2	b	b	b	a (0.50) b (0.50)	a (0.50) b (0.50)	b	b	b	b
3	b	b	b	b	b	b	b	b	c
2	a	a (0.80)	a b (0.20)	a	a	a	a	a	b
3	b	b	b (0.87) c (0.13)	b	b	b	a (0.10) b (0.90)	b (0.90) c (0.10)	a (0.07) b (0.93)
7	a (0.50) b (0.50)	b (0.80) c (0.20)	a (0.13) b (0.87)	f	d	e	d (0.20) e (0.80)	b	d (0.50) e (0.36) f (0.07) g (0.07)
4	a	a	a	a	a	c	d	a (0.80) b (0.20)	d
3	a	a	a	a	a	b	b	a (0.20) b (0.80)	a
4	c	c	b (0.13) c (0.87)	c	c	c	b (0.40) c (0.60)	c	c (0.86) d (0.14)
3	b	a (0.40) b (0.60)	b (0.87) c (0.13)	b	b	a	a	a (0.40) b (0.60)	a
3	b	b	b	c	a	b	b	b	b
2	a	a	a	a	a	a	a	a	a
9	b	b (0.90) c (0.10)	b	f	f	b	b	f	c (0.07) e (0.22) f (0.07) g (0.43) h (0.14) i (0.07)
3	c	b (0.30) c (0.70)	c	b	b	b	b (0.75) c (0.25)	a (0.10) b (0.90)	c
6	d (0.90) e (0.10)	d (0.80) e (0.20)	d	c	d	a	a	b (0.40) c (0.60)	b (0.71) c (0.21) d (0.08)
6	d	b	b	—	—	c	d	d	a (0.86) b (0.14)
5	d	d	d	d	c	a	a	d	e
4	b	b	b	b	b	b	b	b	b
6	e	e	e	c	c	c	c	c	a (0.07) b (0.93)
2	b	b	b	b	a	b	b	b	b
3	c	c	c	b	b	a	b	b	b
2	a	a	a	a	a	b	b	a	a
3	b	b	b	b	b	a	a	a (0.20) b (0.80)	c