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Author(s): Jessica L. Hoisington-Lopez, Lisette P. Waits and Jack Sullivan
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Species limits and integrated taxonomy of the Idaho ground squirrel (*Urocitellus brunneus*): genetic and ecological differentiation

Jessica L. Hoisington-Lopez, Lisette P. Waits, and Jack Sullivan*

Department of Fish and Wildlife, CNR Room 105, P.O. Box 441136, University of Idaho, Moscow, ID 83844-1136, USA (JLH, LPW)
Department of Biological Sciences, LSS Room 252, P.O. Box 443051, University of Idaho, Moscow, ID 83844-3051, USA (JS)

* *Correspondent: jacks@uidaho.edu*

Allocation of evolutionary divergence into species level versus subspecies ranks is critical for conservation and management. *Urocitellus brunneus* (Idaho ground squirrel; formerly *Spermophilus brunneus*) is currently apportioned into 2 subspecies, *U. b. brunneus* and *U. b. endemicus*, but recent studies have suggested elevation to distinct species based on differences in pelage, bacular morphology, genetic data, timing of life-history cycle, and behavior. Following recent movements toward integrated taxonomy, we use the cohesion species concept to test whether both genetic and ecological data support species-level classification of *U. b. brunneus* and *U. b. endemicus*. Eight microsatellite loci and mitochondrial DNA (mtDNA) sequence data were generated for 339 individuals from 14 localities for *U. b. brunneus* and 312 individuals from 11 localities for *U. b. endemicus*. The null hypothesis of genetic interchangeability was tested with 2 independent data sets. First, we estimated an mtDNA gene tree from control region and cytochrome-*b* sequences via parsimony, maximum-likelihood, and Bayesian analyses. We then tested for evidence of recent migration using Bayesian clustering and coalescence analyses of microsatellite data. The 2nd null hypothesis, that of ecological exchangeability, was tested using an ecological niche-model analysis and a review of the literature based on morphology, habitat characteristics, and behavior. Although divergence of mtDNA sequences between the subspecies was modest (<1%), there are no haplotypes shared between the 2 taxa. There is strong support for monophyly of mtDNA haplotyes of *U. b. endemicus* (posterior probability = 0.94), with those from *U. b. brunneus* forming a basal grade. No evidence of recent gene flow was detected; the Bayesian clustering algorithms of mutlilocus genotype data indicated separate ancestry for both *U. b. brunneus* and *U. b. endemicus*. The ecological-niche model showed a nonoverlapping niche for each taxon, allowing for the possibility of differential adaptation. We reject both null hypotheses based on the data, which supports elevation of *U. b. brunneus* and *U. b. endemicus* as distinct species.

Key words: cohesion species concept, control region, cytochrome *b*, microsatellites, phylogeny, species delimitation, *Urocitellus brunneus*

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Species limits within the genus *Urocitellus* and relatives (Helgen et al. 2009) have long been uncertain, and this is particularly true of the taxonomy of *U. brunneus* (Idaho ground squirrel). *U. brunneus* was 1st collected in 1913 and described by Howell (1928) as a subspecies of *Spermophilus townsendii*. Subsequently, Howell (1938) raised it to the rank of species (and reallocated taxa from *S. townsendii* to *S. washingtoni*), a taxonomic conclusion that has been unchallenged for more than half a century. Recent molecular studies (Harrison et al. 2003; Herron et al. 2004) have demonstrated the paraphyly of the long-standing circumscription of *Spermophilus* (Howell 1938), and the recent revision by Helgen et al. (2009) allocated a clade of 12 species to the genus *Urocitellus*. Yensen (1991) conducted a thorough study of the morphology and distribution of *U.* (*S.*) *brunneus* and suggested the recognition of 2 subspecies: *U.* (*S.*) *b. brunneus* (northern Idaho ground squirrel) and *U.* (*S.*) *b. endemicus* (southern Idaho ground squirrel). Several studies have

suggested that *U.* (*S.*) *b. brunneus* and *U.* (*S.*) *b. endemicus* are more appropriately recognized as distinct species based on differences in pelage, bacular and cranial morphology, allele frequencies, and timing of life-history cycle and behavior (Garner et al. 2005; Gavin et al. 1999; Gill and Yensen 1992; Yensen 1991; Yensen and Sherman 1997).

Both subspecies of *U. brunneus* are narrow endemics that occur in a region where changing land-use patterns are degrading the habitat. *U. b. brunneus* is found in Adams and Valley counties of west-central Idaho (Fig. 1) and was listed as threatened under the United States Endangered Species Act in 2000 (United States Fish and Wildlife Service 2000). The primary threat is invasion of meadows by conifers due to fire exclusion (Yensen and Sherman 1997). *U. b. endemicus* is found in Washington, Gem, and Payette counties (Fig. 1) and is a candidate for listing under the Endangered Species Act. Primary threats to *U. b. endemicus* include habitat deterioration from rangeland activities and the introduction of invasive plants such as *Bromus tectorum* (cheatgrass) and *Taeniatherum asperum* (medusa head—Yensen 1999; Yensen et al. 1992).

Given the vulnerability of the species and the uncertainty in taxonomic status, additional research is necessary to evaluate the hypothesis that *U. b. endemicus* and *U. b. brunneus* should be recognized as distinct species. The topic of species delimitation has been greatly debated in the literature (e.g., Bacon and Bailey 2006; Harrison et al. 2003; Jouventin et al. 2006; Stockman and Bond 2007; Yu et al. 2004), and this debate has become increasingly contentious with the ascendance of DNA barcoding (e.g., Papadopoulou et al. 2009; Rubinoff 2006). This current debate also overlaps the classical discussion of numerous species concepts such as biological (Mayr 1969), ecological (Wiley 1978), genetic (Nei 1972; Palmer and Zamir 1982), phylogenetic (Cracraft 1983), evolutionary (Simpson 1951), cladistic (Wiley 1978), and cohesion (Templeton 1989). Furthermore, the definition and use of the subspecies concept has been controversial (e.g., Burbrink et al. 2000; Haig et al. 2006; Zink 2004). Multiple definitions have been proposed (Avise and Ball 1990; Haig et al. 2006; Mayr 1969; Templeton 2001), whereas some have argued that subspecies have no real objective meaning (Burbrink et al. 2000; Wilson and Brown 1953; Zink et al. 2000). Our view is that formal recognition of groups of populations that are differentiated from others at multiple independent loci or character systems, or both (e.g., Giannasi et al. 2001; Page and Charleston 1997), is both pragmatically useful and biologically relevant.

Because most conservation legislation assigns priority based on some index of species diversity, species delimitation is critical to conservation, and there is consensus that species delimitation should integrate several sources of information (e.g., Rissler and Apodaca 2007). One species concept that explicitly calls for integration, the cohesion species concept (Templeton 1989), has received considerable attention as being both general (e.g., Hull 1997) and applicable (Avila et al. 2006; Pons et al. 2006; Templeton 2001; Weisrock and

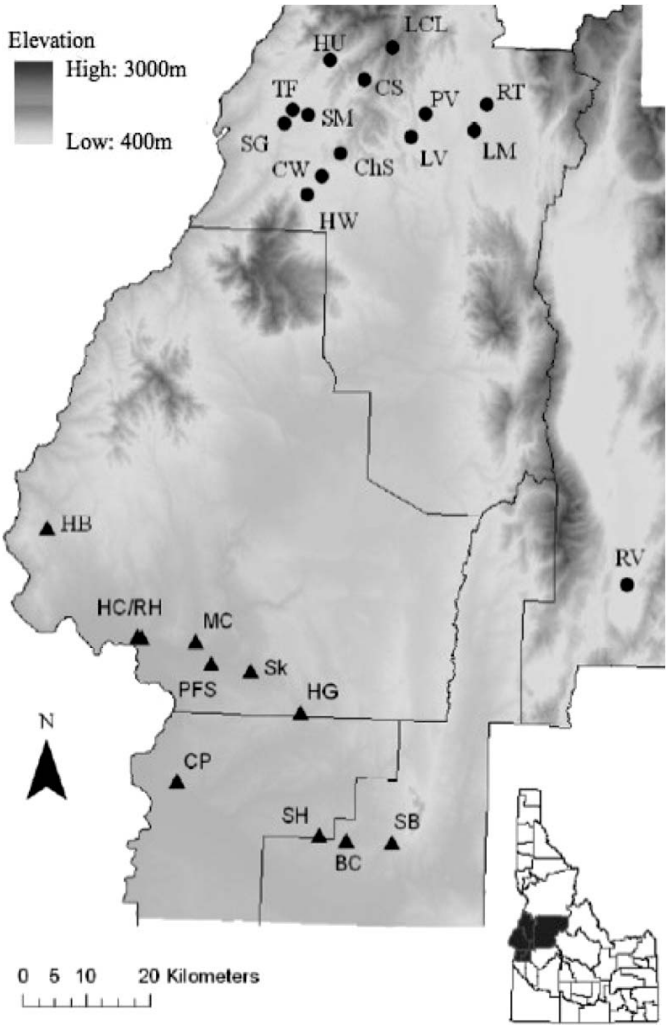

Fig. 1.—Spatial locations of the sampling areas for both subspecies. The dark counties in the Idaho map are shown in detail. Circles represent sampling areas for *Urocitellus brunneus brunneus* and triangles are areas for *U. b. endemicus.* Abbreviations follow Table 1. RH and HC are represented by 1 data point as RH/HC because of their close proximity. Background is from the National Elevation Dataset (http://ned.usgs.gov), with darker colors representing higher elevations.

Larson 2006). The cohesion species concept incorporates ideas from several definitions by requiring both genetic and ecological data, and addresses some of the weaknesses of focusing solely on 1 type of data (Sites and Crandall 1997). Additionally, the cohesion species concept is both predictive, and therefore testable, and applicable to situations where putatively distinct species are allopatric, as is the case between the 2 subspecies of *U. brunneus*.

Here, we apply the cohesion species concept (Templeton 1989) to the evaluation of the taxonomic status of *U. brunneus*. The current taxonomic hypothesis (that there are 2 subspecies) leads to the prediction that detectable gene flow has occurred between *U. b. brunneus* and *U. b. endemicus*. The hypothesis that each taxon is a distinct species (Yensen 1991) is based on strongly differentiated bacular morphology; under the cohesion species concept the morphologically based taxonomic hypothesis predicts ecological differentiation

between the taxa as well as genetic differentiation. Thus, genetic differentiation is a necessary, but not sufficient, condition for recognition of each taxon as distinct species. Here, we collect mitochondrial DNA (mtDNA) sequence data and microsatellite data at 14 sampling sites for *U. b. brunneus* and 11 sites for *U. b. endemicus* and utilize phylogenetic and population genetic analyses, as well as ecological niche modeling, to evaluate the taxonomic status of *U. brunneus* under the cohesion species concept (Templeton 1989).

Materials and Methods

Species concept.—Application of the cohesion species concept requires the assessment of 2 null hypotheses. The 1st of these is that the organisms sampled are derived from a single evolutionary lineage (Templeton 2001), which must be evaluated from a phylogenetic context. If the 1st null hypothesis is rejected, the 2nd hypothesis of genetic or ecological interchangeability, or both (Templeton 2001), can be tested, for example, using ecological niche modeling (Rissler and Apodaca 2007). Rejection of only 1 of the 2 hypotheses is evidence for subspecies classification and rejection of both is evidence for species-level classification (Templeton 2001). Presence of recent migration would be evidence for retention of current classification. We view this hypothesis-testing framework as a major strength of the concept and as justification for its use.

Sample collection.—Hair samples were collected in 2002 (Garner et al. 2005) and 2006 (this study) from 14 sampling areas for *U. b. brunneus* and 11 areas for *U. b. endemicus* (Fig. 1; Table 1) using methods described by Garner et al. (2005). Briefly, individuals were livetrapped and marked with a small patch of dye. We then plucked approximately 50 hairs from the tails of each individual. In the 2006 field season, we added 7 sampling areas for *U. b. brunneus* (HU, HW, CW, LCL, LM, RT, and RV; Fig. 1; Table 1) and 2 sites for *U. b. endemicus* (MC and PFS) that were not sampled in 2002. We also resampled 5 populations of *U. b. brunneus* (ChS, CS, LV, SG, and TF) and 4 populations of *U. b. endemicus* (BC, RH, HC, and SB). All procedures were approved by the University of Idaho Animal Care and Use Committee (2006-35), Idaho Fish and Game state permit (060308), and federal permit for *U. b. brunneus* (subpermit FWSSRBO-5) and are consistent with guidelines for use of wild mammals in research (Sikes et al. 2011). Hair samples were stored in paper envelopes in a plastic bag with silica and kept out of direct sunlight and freezing temperatures. The desiccated samples were then transferred to a −80°C freezer upon return to the laboratory until DNA extraction. DNA was extracted from 10–15 hairs using a modified protocol from the Qiagen tissue kit (Qiagen, Valencia, California—Garner et al. 2005) and stored in 200 µl of AE buffer in a −20°C freezer until polymerase chain reaction amplification, which was conducted in a room dedicated to low-quantity DNA. A negative extraction control was included with every 16 samples to monitor for contamination.

Table 1.—Sampling area and abbreviation (Abb.) used for A) *Urocitellus brunneus brunneus* and B) *U. b. endemicus*. Sample size (No.), allelic richness (AR; based on sample size of 6), and expected heterozygosity (H_E) are shown for the microsatellite data set. Sample size (No.) and haplotype diversity (*h*) measures are shown for the concatenated mitochondrial DNA (mtDNA) data set.

		Microsatellite			mtDNA	
Sampling area	Abb.	No.	AR_6	H_E	No.	*h*
A)						
Chipmunk Springs	ChS	18	3.24	0.576	9	0.2222
Cold Springs	CS	35	3.36	0.577	6	0.0000
Cottonwood	CW	6	3.00	0.584	7	0.5238
Huckleberry	HU	21	2.56	0.465	7	0.2857
Halfway	HW	17	2.92	0.595	7	0.4762
Lick Creek Lookout	LCL	18	3.08	0.564	7	0.0000
Little Mud	LM	13	2.94	0.536	7	0.2857
Lost Valley	LV	29	3.51	0.617	7	0.8095
Price Valley	PV	28	3.38	0.603	8	0.2500
Ridgetop	RT	13	3.59	0.658	6	0.3333
Round Valley	RV	23	2.12	0.393	6	0.0000
Summit Gulch	SG	35	3.27	0.589	7	0.2857
Squirrel Manor	SM	18	3.41	0.618	7	0.2857
Tree Farm	TF	65	3.27	0.547	7	0.2857
Average		24.21	3.12	0.566	7.00	0.2888
B)						
Bissel Creek	BC	65	3.32	0.514	6	0.9330
Clay Peak	CP	24	2.93	0.624	7	0.5238
Henley Basin	HB	12	1.77	0.308	7	0.9524
Holland Gulch	HG	10	3.16	0.664	8	0.7857
Mann Creek	MC	20	2.66	0.473	7	0.2857
Phillips Farm South	PFS	22	2.70	0.549	7	0.8095
Rolling Hills	RH	26	2.55	0.548	4	0.5000
Hillcrest Cemetery	HC	40	2.35	0.435	3	0.0000
Squaw Butte	SB	53	2.69	0.479	7	0.5238
Sand Hollow	SH	21	2.96	0.481	8	0.9286
Skow	Sk	19	3.05	0.567	7	0.9524
Average		23.92	2.74	0.513	6.45	0.6541

Mitochondrial DNA.—A 472 base-pair (bp) sequence of the control region (CR) was obtained using the modified primer pair L15774 and H16498 (Paetkau and Strobeck 1996; Shields and Kocher 1991). Conditions for polymerase chain reaction were described in Garner et al. (2005). Twenty individuals from each sampling area were analyzed at the CR. A subsample of ~7 individuals per study area was then sequenced for the full 1,097-bp cytochrome-b (*Cytb*) gene. The *Cytb* gene was sequenced in 2 shorter sections with a 200-bp overlap because of the degraded nature of DNA extracted using hair samples (Taberlet et al. 1999). We used a primer pair from Harrison et al. (2003) and developed internal primers using Primer3 (Rozen and Skaletsky 2000). The 1st primer pair was L15033 (CCAACGGCGCATCTATATTT) and H15915 and the 2nd pair was L14725 and H15326 (GGAATGCGAAAAATCGT). Polymerase chain reaction for both *Cytb* primer pairs included negative controls and consisted of 35 cycles (94°C for 30 s, 53°C for 60 s, and 72°C for 2 min), with an initial denaturation step of 94°C for 5 min and final extension of 72°C for 5 min. Polymerase chain reaction products for both loci were cleaned with ExoSAPit (United States Biochemical Corp., Cleveland,

Ohio), sequenced, and analyzed on an ABI 3130 automatic sequencer (Applied BioSystems, Bath, United Kingdom). Two outgroups were sequenced for inclusion in the phylogenetic analyses; sequences from *U. washingtoni* and *U. columbianus* were obtained using the same polymerase chain reaction and sequencing described above.

The raw sequences were aligned and verified in Sequencher (version 4.5; Gene Codes Corporation, Inc., Ann Arbor, Michigan) and MacClade (version 4.06; Sinauer Associates, Sunderland, Massachusetts). Unique haplotypes were identified using TCS (version 1.2.1—Clement et al. 2000). All haplotypes that differed by 1 or 2 mutations were verified using the original chromatogram files at all (if >3 individuals) or 3 randomly selected individuals. Haplotypes found in only 1 individual were reamplified and sequenced a 2nd time to double-check the accuracy of the sequence.

Microsatellites.—Samples collected in the 2006 field season were analyzed at the same 8 loci as samples collected in 2002 (Garner et al. 2005). Most loci were dinucleotides except for D117, a tetranucleotide, and IGS110b, a trinucleotide repeat (Garner et al. 2005). Polymerase chain reaction amplifications were run in 7-μl reactions using the Qiagen multiplex kit (Qiagen) with 3.5 μl of 2× multiplex polymerase chain reaction master mix, 0.7 μl of 6 mM multiplex PCR Buffer (Q-solution; Qiagen, Valencia, California), and 0.2 μM of each primer (except 0.1 μM of IGS110b, 0.25 μM of GS26, and 0.3 μM of GS3). The loci were divided into 2 multiplex reactions as follows: A) GS12, GS17, GS26, and IGS110b; and B) B109, B126, D117, and GS3. The polymerase chain reaction amplification was the following: 1 cycle of 95°C for 15 min; a step-down procedure (0.3°C for A and 0.4°C for B) per cycle of 94°C for 30 s, 53°C (54°C)–50°C for 90 s, 72°C for 60 s for 10 cycles; followed by 30 cycles of 94°C for 30 s, 50°C for 90 s, 72°C for 60 s; and a final extension of 60°C for 30 min. All polymerase chain reactions contained a negative control. Because forward primers were fluorescently labeled (6-FAM, HEX, VIC, or NED) and size ranges of primers using the same dyes were distinct, 1 μl of A and 1 μl of B of the same individual were mixed with 10 μl of formamide and 0.33 μl of LIZ size standard and denaturated at 95°C for 2 min. The denatured products were run on an ABI 3130xl (Applied Biosystems, Carlsbad, California) and then analyzed in Genemapper (version 3.7; Applied Biosystems). The data collected for the 2006 season were then combined with the data from Garner et al. (2005). In order to compare allele sizes, four 2002 samples were rerun and used to obtain bin ranges using the same allele sizes as Garner et al. (2005).

Phylogenetic analyses.—We created a minimum spanning network (TCS—Clement et al. 2000), breaking the loops using the criteria outlined in Crandall and Templeton (1993) and Panchal and Beaumont (2007) to evaluate the relationship between haplotypes. The mtDNA gene tree was estimated via parsimony, maximum-likelihood, and Bayesian analyses using PAUP* (Swofford 1998) and MrBayes (Huelsenbeck and Ronquist 2001). Model selection was performed using DT-ModSel (Minin et al. 2003); this method has an advantage over the Akaike information criterion (AIC) and hierarchical likelihood-ratio tests (e.g., Frati et al. 1997; Sullivan and Swofford 1997) usually implemented in ModelTest (Posada and Crandall 1998) in that it generally selects simpler models that perform as well or better than the models selected by likelihood-ratio tests and AIC (Abdo et al. 2005; Minin et al. 2003). Model selection was completed individually for the CR and *Cytb* loci and for the combined concatenated data set. For *Cytb*, the sequence was divided into multiple partitions using 1st, 2nd, and 3rd codon positions because rates usually differ among the positions (e.g., Irwin et al. 1991). In addition, we incorporated higher order structure of the *Cytb* locus by partitioning the sequences into transmembrane, inner-membrane, and outer-membrane sections using the sequences and structure identified in Esposti et al. (1993). For each scheme (see Table 2), we ran MrBayes for 5 million generations. Convergence of Markoc chain Monte Carlo to the target distribution was assessed by examining the standard deviation of split frequencies. The harmonic mean was then calculated for each partitioning scheme and the Bayesian information criterion (BIC—Schwartz 1978) and ΔBIC scores were calculated.

Phylogenetic trees also were estimated using a parsimony and maximum-likelihood framework in PAUP* (version .4.0). Searches were conducted under a heuristic framework using the model selected for the concatenated data set. These searches used tree-bisection-reconnection branch swapping, on a starting tree generated by stepwise addition with 100 random-addition replicates. To estimate nodal support, nonparametric bootstrap analyses were run with 200 replicates and only a single tree retained (i.e., max-trees = 1). In order to determine statistical support for the hypothesis of reciprocal monophyly between the 2 taxa, we conducted constrained searches to find the best tree consistent with reciprocal monophyly of the 2 taxa.

Analyses of genetic structure and gene flow using nuclear DNA (nDNA) and mtDNA.—F_{ST} was calculated for mtDNA in Arlequin (Excoffier et al. 2005) and for nDNA in GENEPOP (Raymond and Rousset 1995). An evaluation of the hierarchical partitioning of mtDNA and nDNA genetic variation within populations, among populations within subspecies, and among subspecies was conducted with an analysis of molecular variance (AMOVA—Excoffier et al. 1992) using Arlequin (Excoffier et al. 2005). Additionally, we used the microsatellite data set to evaluate the genetic distinctiveness of subspecies by implementing Bayesian clustering algorithms and estimating migration rates between the subspecies.

We estimated the number of migrants per generation in GeneClass (version 2.0—Piry et al. 2004) using a Bayesian approach with $\alpha = 0.05$ (Rannala and Mountain 1997). Probabilities were estimated with 10,000 simulated individuals using the same simulation algorithm as Paetkau et al. (2004). These calculations estimated the probability of an individual being a resident from the sampling area (i.e., the probability that an individual sampled is from the appropriate taxon). In addition, we conducted assignment tests using the

Table 2.—Different partitioning schemes used in DT-ModSel showing the partitioning scheme and model selected. MrBayes was run for 5 million generations for each scheme under the conditions listed in the text and the posterior probability of *Urocitellus brunneus brunneus* (Ubb) and *U. b. endemicus* (Ube) monophyly and the probability of exclusivity was estimated. BIC = Bayesian information criterion; see text for definition of partitions.

Scheme	Partitions	Model	No. free parameters	Average −lnL	BIC	ΔBIC	*P* (Ubb)	*P* (Ube)	*P* (exclusivity)
CR3codon	CR	HKY+I	16	−3,207.9	6,466.9	0.0	0.94	0.37	0.99
	CytB1st	K2P							
	CytB2nd	HKY							
	CytB3rd	GTR							
Twogene	CR	HKY+I	10	−3,258.7	6,549.4	82.5	0.58	0.50	0.99
	CytB	HKY+Γ							
CRinoutrans	CR	HKY+I	18	−3,260.0	6,577.5	110.7	0.59	0.52	0.98
	inner	HKY							
	outer	HKY							
	transmembrane	HKY+Γ							
CRsurftrans	CR	HKY+I	14	−3,268.6	6,582.0	115.1	0.62	0.49	0.98
	surface	HKY							
	transmembrane	HKY+Γ							
CRsurfQ	CR	HKY+I	22	−3,269.4	6,609.1	142.2	0.66	0.46	0.98
	surface	HKY							
	transmembrane	HKY+Γ							
	Q1	HKY							
	Q2	HKY							
CRinouQ	CR	HKY+I	26	−3,268.3	6,619.6	152.7	0.63	0.48	0.98
	inner	HKY							
	outer	HKY							
	transmembrane	HKY+Γ							
	Q1	HKY							
	Q2	HKY							
All	CR+CytB	HKY+I+Γ	6	−3,391.5	6,802.2	335.3	0.72	0.35	0.99

program BayesAss (version 1.3—Wilson and Rannala 2003), which was run for 3 million iterations with a sampling frequency of 2,000 and a burn-in of 1 million generations. Two independent runs with different initial seed values were conducted to ensure convergence.

To determine the number (k) of genetic clusters (populations) in the data set without predefined population or subspecies units, we used a Bayesian clustering approach. Structure (version 2.1—Pritchard et al. 2000) was used to conduct several analyses under different clustering models (k = 2–20 clusters), each run had a burn-in period of 50,000 Markov chain Monte Carlo generations followed by sampling 100,000 generations. The optimum value of k (i.e., the number of clusters suggested by the data) was determined using criteria outlined in Pritchard et al. (2000) and by calculating Δk (Evanno et al. 2005).

In addition, we analyzed the mtDNA data set under the divergence with migration model (e.g., Hey and Nielsen 2004) implemented in IMa2 (http://genfaculty.rutgers.edu/hey/software#IMa2) to estimate migration rates between the 2 taxa and to provide an estimate of time since divergence. We used uniform priors, with upper bounds on θ set to 200, upper bounds for migration parameters of 5, and time since divergence set to an upper bound of 10; we assumed a conventional mutation rate of 10^{-6} mutations per site per generation and a single generation per year (Yensen and Sherman 1997). We conducted multiple runs of 10 million generations, sampling every 100 and with a 10,000-generation burn in. All independent runs converged to similar parameters estimates.

Ecological interchangeability.—Even with a small number of data points (as few as 5), one can predict potential species distributions using ecological niche-modeling approaches (Pearson et al. 2007). Thus, to test the hypothesis of ecological interchangeability, we conducted ecological niche-model analyses (Maxent version 3.0.6-beta—Phillips et al. 2006) with 30 arc-second (approximately 1 km^2) data layers of minimum temperature, maximum temperature, precipitation, altitude, and 19 bioclimate variables (worldclim version 1.4—Hijmans et al. 2005; Table 3). We used the 25 localities where genetic data were sampled (14 localities for *U. b. brunneus* and 11 for *U. b. endemicus*). Analyses were run with response curves, predictions, and a jackknife procedure to measure variable importance with 10,000 simulations. Model performance was evaluated using the receiver operating characteristic analysis. This model evaluation is a sensitivity analysis of the presence and absence points predicted correctly by the model (Phillips et al. 2006). The area under the receiver operating characteristic curve (AUC) provides an indication of the fit of the data to the true distribution, where 0.5 = random distribution and 1 = perfect fit (e.g., Carstens and Richards 2007).

Results

Mitochondrial DNA diversity and structure.—Seventeen unique haplotypes were detected for the *Cytb* gene region; 7 in *U. b. brunneus* and 10 in *U. b. endemicus*. Twenty unique

Table 3.—Parameters estimated in the ecological niche model that contributed to either the *Urocitellus brunneus brunneus* (Ubb) or *U. b. endemicus* (Ube) model. The highest 3 variable contributors for each taxon are highlighted in bold.

Variable	% Ubb contribution	% Ube contribution
Vegetation	58.7	24.9
Mean diurnal range (°C)	13.1	0.7
Annual precipitation (mm)	12.4	0.0
Elevation (m)	7.7	0.0
Precipitation seasonality (coefficient of variation)	4.3	6.1
Annual mean temperature (°C)	1.6	26.0
Min temperature of coldest month (°C)	1.2	1.2
Precipitation of coldest quarter (mm)	0.8	0.0
Isothermality (P2/P7; %)	0.2	0.0
Precipitation of warmest quarter (mm)	0.2	0.0
Average monthly precipitation (mm)	0.0	10.9
Average monthly maximum temperature (°C)	0.0	3.3
Maximum temperature of warmest month (°C)	0.0	6.6
Temperature annual range (°C)	0.0	0.2
Mean temperature of wettest quarter (°C)	0.0	0.6
Mean temperature of driest quarter (°C)	0.0	8.6
Precipitation of wettest month (mm)	0.0	1.9
Precipitation of driest month (mm)	0.0	0.3
Precipitation of driest quarter (mm)	0.0	8.9

haplotypes were detected for the CR, 6 in *U. b. brunneus* (Appendix I) and 14 in *U. b. endemicus* (Appendix II). The concatenated data set contained 43 haplotypes, 13 in *U. b. brunneus* and 30 in *U. b. endemicus*. There were no haplotypes shared among subspecies. All sequences obtained were deposited in GenBank (accession numbers JQ679123–JQ679293). Haplotype diversity (*h*) within sampling areas ranged from 0 to 0.95 (Table 1). Haplotype diversity was higher in *U. b. endemicus* (0.65) than in *U. b. brunneus* (0.29). Pairwise F_{ST} for mtDNA among populations within subspecies ranged from −0.17 to 1 (0.55 average) for *U. b. brunneus* and from −0.09 to 0.79 (0.28 average) for *U. b. endemicus*, whereas pairwise F_{ST} between subspecies ranged from 0.12 to 1.0 (0.52 average). The AMOVAs detected significant substructure at all hierarchical levels; 10.1% of the variation was explained by subspecies groupings, 42.7% by variation among populations within subspecies, and 47.2% within populations (Table 4).

Phylogenetic analyses.—For the CR, there were 73 variable sites, of which 40 were parsimony informative; removal of the 2 outgroups resulted in a decrease to 29 and 20, respectively. For *Cytb*, there were 138 variable sites and 55 informative sites; without outgroups there were 21 and 16, respectively. The best partitioning scheme tested employed 4 partitions; the, CR and *Cytb* into 1st, 2nd, and 3rd codon positions. This resulted in a drastic improvement over a single partition (ΔBIC = 335.3; Table 2). Assigning the sequences into 2 partitions, CR and *Cytb*, also drastically improved the likelihood score over a single partition (ΔBIC = 82.5; Table 2), whereas partitioning *Cytb* using the structure of the gene improved the likelihood score from a single partition (ΔBIC = 110.7–152.7); however, this was not the best partitioning scheme based on ΔBIC.

Table 4.—Hierarchical analysis of genetic variation using AMOVA. mtDNA = mitochondrial DNA; nDNA = nuclear DNA.

Marker type	Source of variation	*F*stat	% variation	*P*
mtDNA	Among subspecies	0.10	10.1	0.0010
	Among populations within subspecies	0.47	42.7	>0.001
	Within populations	0.53	47.2	>0.001
nDNA	Among subspecies	0.18	17.7	>0.001
	Among populations within subspecies	0.18	14.6	>0.001
	Within populations	0.32	67.7	>0.001

Regardless of partitioning scheme for the Bayesian analyses, the mtDNA gene tree did not exhibit reciprocal monophyly between the 2 *U. brunneus* taxa (average *P*-value [exclusivity] = 0.98; Table 2) for the concatenated data or the CR and *Cytb* analyzed alone. The probability of monophyly in either the *U. b. brunneus* or *U. b. endemicus* varied among schemes, although a trend of a lower *P*-value (higher probability) for *U. b. endemicus* (0.37 versus 0.94 [*brunneus*] for best partitioning scheme) was observed (Table 2).

The phylogenetic tree obtained from the concatenated sequences indicated that the haplotypes of *U. b. brunneus* were paraphyletic with respect to the haplotypes of *U. b. endemicus*, with the 2 sequences from ChS (Ubb26 and Ubb38) the earliest diverging (Fig. 2). Bootstrap values from the parsimony and mzximum-likelihood analysis were low (<70%), with the Bayesian posterior probabilities higher (<0.99). There is evidence from the concatenated data set (posterior probability = 0.94) for monophyly of *U. b. endemicus*, but the Bayesian analysis for the 2 genes separately indicated lower probability for monophyly of *U. b. endemicus* (posterior probability = 0.50 [CR] and 0.36 [*Cytb*]). The analysis on the CR sequence alone showed the haplotype from ChS (UbbR) was the most basal on the tree (not shown). For analysis of the *Cytb* sequences only, the ChS haplotype (UbbI) was basal to the haplotypes of *U. b. endemicus*. The basal haplotypes for the whole tree (UbbVII, UbbXII, and UbbXI) were found throughout the range of the *U. b. brunneus* (not shown).

The minimum-spanning network (Fig. 3) indicated that the 2 taxa form distinct clades that are separated by 8 mutations (uncorrected p-distance = 0.0073) in the concatenated data set. Although each taxon forms a distinct clade, the distance between the 2 taxa is less than the distance found within each taxon. The haplotype from RV (Ubb40) is intermediate between subspecies (Fig. 3).

Results from IMa2 indicate virtually no migration between the 2 subspecies (Fig. 4); this is not surprising given the observed exclusivity of haplotypes. Furthermore, divergence time was estimated to be 5.045, which corresponds to an absolute time estimate of 32,548 years ago. Although the credibility interval is large (18,300–63,580 years ago) it excludes a post-Pleistocene divergence (Fig. 4).

Microsatellite diversity.—For *U. b. brunneus*, an average of 24 individuals per sampling area were analyzed at 8

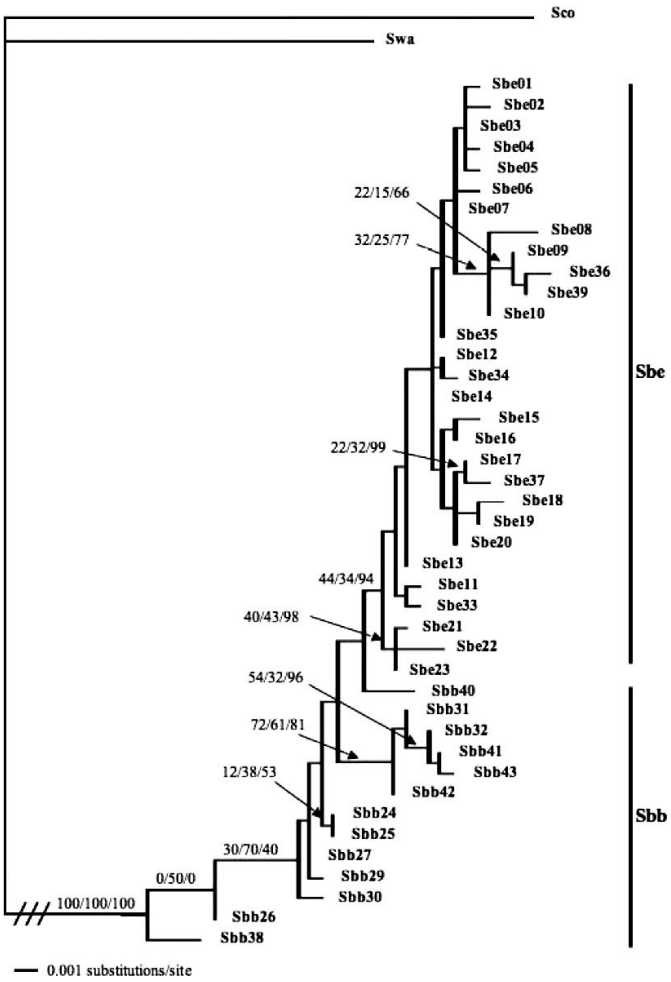

Fig. 2.—Phylogenetic tree estimated under heuristic search with the HKY+I+Γ model using the concatenated haplotypes. Values above lines are bootstrap values greater than 50% for parsimony, likelihood, and Bayesian analyses, respectively. Abbreviations used: *Urocitellus brunneus brunneus* (Ubb), *U. b. endemicus* (Ube), *U. washingtoni* (Uwa), and *U. columbianus* (Uco) haplotypes.

microsatellite loci (Table 1A). Average allelic richness was 3.1, with a range of 2.1 (RV) to 3.51 (LV). Expected heterozygosity (H_E) ranged from 0.39 (RV) to 0.62 (SM). For *U. b. endemicus*, an average of $n = 28.4$ individuals per sampling area was analyzed at 8 loci (Table 1B). Average allelic richness was 2.7 alleles per locus per sampling area with a range from 1.7 (HB) to 3.3 (BC). H_E ranged from 0.53 (HB) to 0.66 (HG). The average allelic richness (AR) was higher for *U. b. brunneus* (AR = 3.1) than for *U. b. endemicus* (AR = 2.74; $P = 0.023$). H_E was higher for *U. b. brunneus* ($H_E = 0.57$) than for *U. b. endemicus* ($H_E = 0.51$), but this difference was not statistically significant ($P = 0.132$).

Nuclear DNA structure and gene flow.—Nuclear DNA pairwise F_{ST} among populations within subspecies ranged from 0.03 to 0.46 (0.17 average) for *U. b. brunneus* and from 0.04 to 0.41 (0.19 average) for *U. b. endemicus*, whereas pairwise F_{ST} between subspecies ranged from 0.18 to 0.55 (0.34 average; Appendix III). The AMOVAs indicated that 17.7% of the variation was explained by subspecies groupings, 14.6% by variation among populations within subspecies, and 67.7% within populations (Table 4). The analysis conducted with GeneClass identified 0 recent migrants between the 2 taxa. Rates derived using BayesAss were 0.00040 migrants ($\sigma^2 = 0.00049$) from *U. b. brunneus* to *U. b. endemicus* and 0.00037 migrants ($\sigma^2 = 0.00045$) from *U. b. endemicus* to *U. b. brunneus*.

The plot of log-likelihood values from Structure (Fig. 5) indicated an increase in likelihood values until an asymptote at $k = 15$, with plausible values ranging from $k = 2$ to $k = 9$. A Δk statistic (Evanno et al. 2005) indicated the largest rate of change in log-likelihood scores between $k = 1$ and 2; this is strong evidence of 2 clusters at the highest hierarchical structure in our data set (Fig. 5). The plot of the ancestry values for each individual when $k = 2$ indicates that the split between genetic clusters corresponds precisely to the taxonomic division (Fig. 6); the strongest signal in the microsatellite data corresponds to differentiation between the taxa.

Ecological exchangeability.—We were able to explain a great deal of the habitat variation in our niche modeling: for *U. b. brunneus*, AUC = 0.965 and for *U. b. endemicus*, AUC = 0.971. The ecological niche models indicated nonoverlapping niches for each taxon (Fig. 7). An analysis of the relative contribution of explanatory variables demonstrated the distinct differences between the niches of the 2 taxa (Table 3). For *U. b. brunneus*, vegetation (59%), mean diurnal range (13%), and elevation (12%) defined the niche, whereas for *U. b. endemicus*, vegetation (25%), annual mean temperature (26%), and average monthly precipitation (11%) defined the habitat. For both taxa, vegetation explained most of the habitat variation, and this information was not contained in any of the other model variables.

Discussion

One of the long-standing criticisms of the biological species concept is its inherent reliance on the criterion of reproductive isolation (e.g., Templeton 1989). More recent discussions have pointed out that reproductive isolation is best viewed as an eventual result of differentiation (e.g., Wu 2001) and differentiation characteristic of species often occurs prior to the establishment of full reproductive isolation. This, coupled with increasing evidence for the preponderance of speciation via divergence with gene flow (e.g., Nadachowska and Babik 2009; Reid et al. 2012), undermines the centrality of demonstration of reproductive isolation in delimiting species. In cases of alloparty, then, the judgment relies not on an assessment of potential to interbreed, but instead on an assessment of the evolutionary distinctness of putative species. To us, these issues argue heavily in favor of a more integrated criterion for species delimitation, the genetic and ecological exchangeability criteria of the cohesion concept (Templeton 1989) for such integration.

Examination of our data sets suggests that, under the cohesion species concept, *U. b. brunneus* and *U. b. endemicus* could be treated as separate species. They are on separate

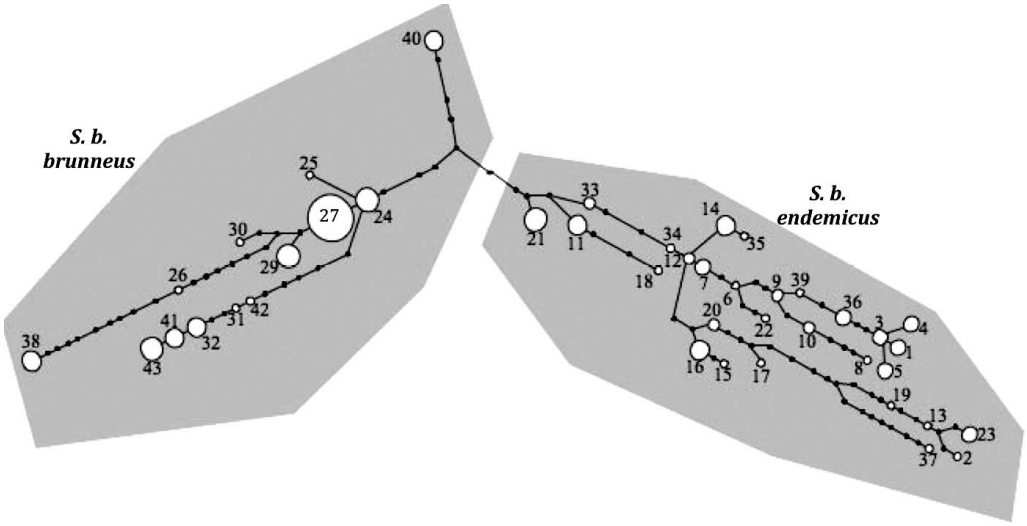

FIG. 3.—Network showing relationship between the 13 haplotypes of *Urocitellus brunneus brunneus* and 30 haplotypes of *U. b. endemicus*. The figure is based on 1,569 base pairs on the combined control region (CR) and cytochrome-*b* (*Cytb*) concatenated mitochondrial DNA haplotypes. Each single line represents 1 mutational step and a small black circle represents an inferred haplotype that was not sampled or is extinct. The size of the white circles (haplotypes) represents the frequency of the haplotype in the data set.

evolutionary trajectories, because there is little to no migration between populations of these taxa, and apparently has not been gene flow since their late-Pleistocene divergence (see Fig. 4). Furthermore, they occupy different, nonoverlapping niches. Therefore, the 2 taxa exhibit neither demographic nor genetic exchangeability and conform well to the cohesion species concept. In addition, the 2 taxa differ greatly in morphology and behavior (Yensen 1991; Yensen and Sherman 1997), which further supports their status as distinct species.

We applied a variety of different partitioning schemes to the data set to determine the best option for our data set. Because the CR is noncoding and has a higher average mutation rate (Greenberg et al. 1983), we treated it as a single partition. On the other hand the *Cytb* sequence is a functional gene (Esposti et al. 1993), and separating it into 1st, 2nd, and 3rd codon positions improved model fit and the average log-likelihood of the maximum-likelihood tree considerably (ΔBIC = 70.4; data not shown). However, incorporation of the structural and functional information for the *Cytb* gene did not result in substantially better model fit. This is most likely attributable to overparameterization because some of the partitions had only a few variable sites to estimate numerous parameters in each model (Rannala 2002).

The validity of a using a concatenated data set has been an issue of considerable debate (e.g., Bull et al. 1993; Chen and Li 2001; Kubatko and Degnan 2007; Miyamoto and Fitch 1995; Rokas and Carroll 2005). We used 2 mtDNA regions that are located close together and inherited as a single unit with the mitochondrial genome. Thus, the *Cytb* and CR should be reflecting the same gene tree (e.g., Sullivan 1996; Tchaicka et al. 2007). The phylogeny estimated using the concatenated data set showed higher support values than the trees obtained using the CR or *Cytb* sequences separately, likely a result of additivity of signal and hidden support (Gatesy et al. 1999) as been observed in other studies (e.g., Sullivan 1996).

We found strong support for monophyly of haplotypes of *U. b. endemicus* (posterior probability = 0.94) but low support for monophyly of *U. b. brunneus* (0.31) using the concatenated data set. However, paraphyly of haplotypes of *U. b. brunneus* also received little support. Reciprocal monophyly is a requirement for several species concepts (Mishler and Theriot 2000), but much recent research has shown that species-level monophyly is less common than previously thought. For example, Funk and Omland (2003) demonstrated that 17% of the 469 mammalian species surveyed exhibited paraphyletic or polyphyletic haplotype relationships, and one of the main sources of polyphyletic phylogenetic trees is incomplete lineage sorting (Funk and Omland 2003). The divergence of *U. brunneus* from other *Urocitellus* was estimated by Harrison et al. (2003) to be 0.4 million years ago (mya; range: 0–0.8 mya), which is one of the more recent divergences in this genus, but comparable to estimated divergence times of other recognized species with in the genus (*U. mollis* and *U. townsendii*—0.6 mya; *U. elegans* and *U. richardsonii*—0.5 mya). These estimates were conducted using phylogenetic techniques, whereas our estimate of 32.5 thousand years ago was derived using coalescent approaches. Given the recent divergence estimated for the species, it is not surprising that we observed short internal branches (Rosenberg 2003) and a lack of reciprocal monophyly between *U. b. brunneus* and *U. b. endemicus*.

The sequence divergence among the lineages in the concatenated data set was 0.7%, which is lower than the sequence divergence within each taxon (<1.5%). Furthermore, Baker and Bradley (2006) found that in the majority of cases, a *Cytb* distance of greater than 5% was observed between sister species and suggests that this criterion be used to provisionally identify mammalian species. The 0.7% divergence between subspecies also is lower than the pairwise sequence divergence (uncorrected p-distance) among

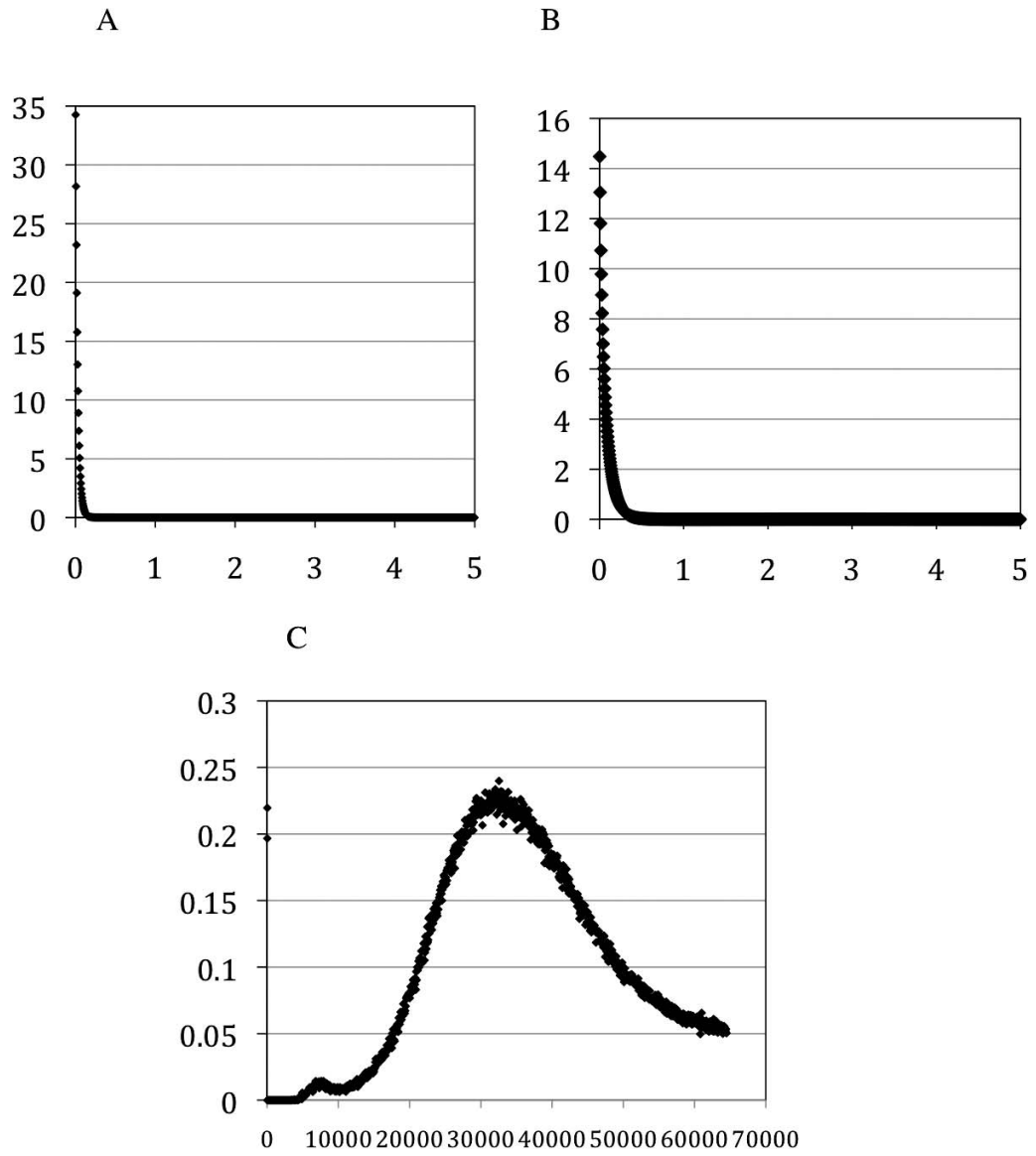

Fig. 4.—Posterior probabilities of parameters of the isolation with migration model calculated from mitochondrial DNA data using IMa2. There is no evidence of migration from A) *Urocitellus brunneus brunneus* into *U. b. endemicus* or B) *U. b. endemicus* into *U. b. brunneus*. C) The 2 taxa diverged 32,548 years ago, with a 95% credibility interval of 18,300–63,580 years.

species within marmotines, which range from 1% to 18.3% (Harrison et al. 2003). Thus, if we only had mtDNA data, we would recommend that *U. b. brunneus* and *U. b. endemicus* remain as subspecies and others may justifiably take this position.

However, we found no evidence of recent migration between the 2 taxa in the microsatellite data set, the mtDNA suggests no historical migration, and a greater amount of genetic variation was explained at the subspecies level than among populations within species (Table 4). Results from the Bayesian clustering analysis (Structure) indicate 2 well-differentiated genetic clusters in our data set ($\Delta k = 2$), corresponding to the 2 taxa; we detected no coancestry between them. This is significant because there are no a priori assignments of individuals to taxon in the Structure runs, and multiple runs were conducted with different populations structure (i.e., varying k); the strongest signal of genetic substructure in the data set divided individuals into 2 groups (Fig. 5) that coincide precisely with taxonomy (Fig. 6). Similarly, analyses with GENECLASS and BayesAss resulted in very low estimates of migration rate between taxa. Additionally, we found no mtDNA haplotypes shared between *U. b. brunneus* and *U. b. endemicus* and mtDNA migration rates indistinguishable from zero. Thus, these 2 taxa are not currently exchanging genes and have not exchanged genes since their divergence in the late Pleistocene. This is particularly relevant considering that the 2 taxa were parapatric in historical times (E. Yensen and P. Sherman, in litt.).

Assessment of species status under the cohesion species concept requires an examination of ecological as well as genetic differentiation. The ecological niche modeling (Fig. 7)

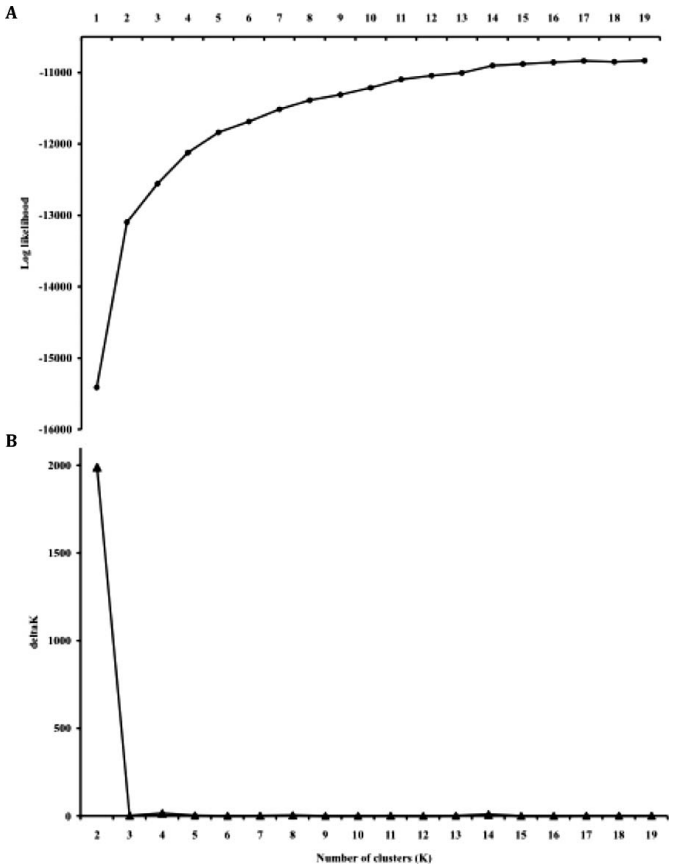

Fig. 5.—A) Log-likelihood plot from Structure and B) Evanno et al.'s (2005) Δk plot for $k = 1$–19. Values for each k-value were obtained from an average of 10 Structure runs.

demonstrates that the 2 taxa inhabit strongly different niches and have different habitat requirements. This ecological differentiation allows for (without necessarily demonstrating) the possibility of differential adaptation between the 2 taxa. *U. b. brunneus* has the most restricted geographical range of any *Urocitellus* species and one of the smallest ranges of any North American mammal (Gill and Yensen 1992). It occurs in an area of 30 × 100 km (Gavin et al. 1999) in xeric montane meadows with shallow, dry, rocky meadows associated with deeper, well-drained soils and surrounded by coniferous forests of *Pinus ponderosa* (ponderosa pine) and *Pseudotsuga menziesii* (douglas-fir—Yensen 1991) at elevations of about 1,150–1,550 m in Adams and Valley counties of Idaho (Yensen and Sherman 1997). In fact, all but one of the known populations is limited to an area of 30 × 10 km (Yensen and Sherman 1997). Conversely, *U. b. endemicus* is found in an area of 81 × 61 km of shrub–steppe habitat of the lower Weiser and Payette river basins at elevations between 670 and 975 m in Gem, Payette, and Washington counties in Idaho (E. Yensen and P. Sherman, in litt). It inhabits an area that was once dominated by *Artemisia tridentata* (big sagebrush), *Purshia tridentata* (bitterbrush), and other native forbs and bunchgrasses (Yensen 1991).

Furthermore, these 2 taxa differ morphologically. The pelage of *U. b. brunneus* appears to be dark reddish gray as the result of a mixture of black unbanded and yellowish red banded guard hairs (Yensen 1985, 1991). The pelage of *U. b. endemicus* is grayish brown as a result of less-intense pigment in the banded guard hairs (Yensen 1991). The difference in pelage is hypothesized to be attributable to differences in soil type; *U. b. brunneus* inhabits shallow reddish parent soils of basaltic origin, whereas *U. b. endemicus* lives in paler colored soils formed by granitic sands and clays (Yensen 1985, 1991). The mean length of *U. b. brunneus* is 233.7 mm (± 2.5 mm) for males and 225.9 mm (± 1.9 mm) for females (Yensen 1991; Yensen and Sherman 1997). *U. b. endemicus* has a mean length of 240.7 mm (± 1.7 mm) and 233.4 mm (± 2.0 mm) for males and females, respectively. *U. b. endemicus* is significantly larger than *U. b. brunneus* for 10 of 16 female and 14 of 16 male external and cranial characters (Yensen 1991). Additionally, the baculum of *U. b. endemicus* is significantly larger and has a larger number of spines than that of *U. b. brunneus* (Yensen 1991); this degree of bacular differentiation is comparable to that observed between other pairs of ground squirrel species (Yensen 1991). The difference in bacular morphology is particularly relevant because this is typically used as a key taxonomic character in sciurids (Patterson and Thaeler 1982; Yensen 1991). In fact, the differences in bacular morphology between *U. b. brunneus* and *U. b. endemicus* appear equal to, or greater than, the differences between 2 recognized species (*U.* (*S.*) *townsendii idahoensis* and *U.* (*S.*) *washingtoni*—Yensen 1991).

There are also behavioral differences identified between the 2 taxa, although they both spend much of the year hibernating underground. *U. b. brunneus* emerges in late March or early April (Yensen 1991), whereas *U. b. endemicus* emerges in late January or early February (Yensen and Sherman 1997). The

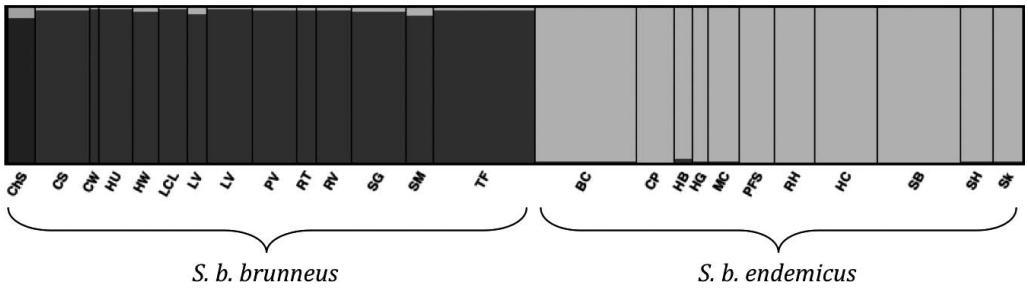

Fig. 6.—Ancestry values for each individual, separated by sampling area for $k = 2$ estimated in Structure. Cluster 1 ancestry is represented by dark gray and cluster 2 ancestry is represented by light gray. y-axis indicates the percentage of ancestry in each cluster.

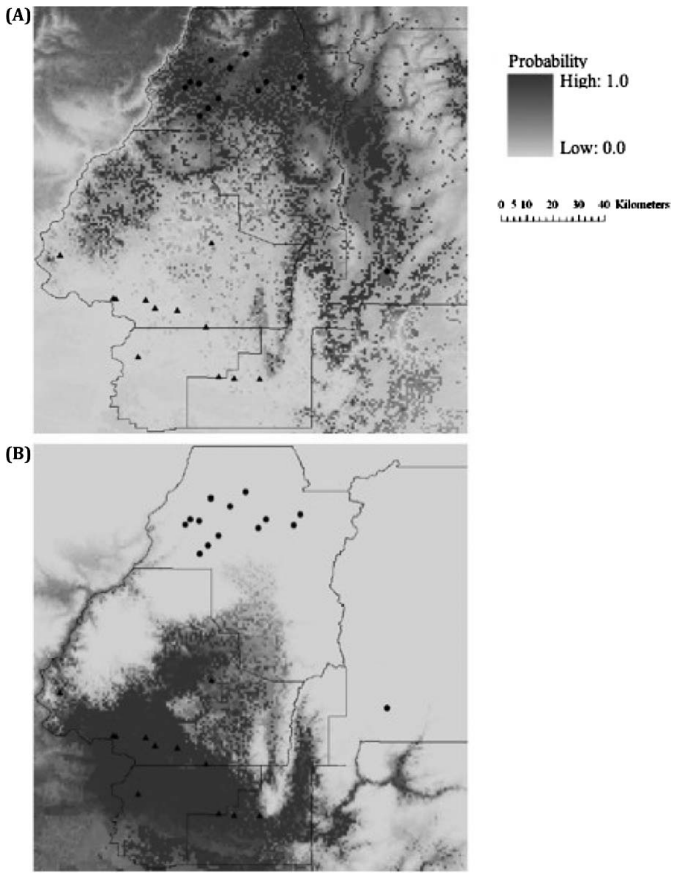

Fig. 7.—Ecological niche modeling for A) *Urocitellus brunneus brunneus* and B) *U. b. endemicus*. Sampling areas are indicated with circles (*brunneus*) and triangles (*endemicus*) and follow Fig. 1. Dark gray shading indicates suitable habitat.

difference in emergence times is attributed to the difference in elevation because emergence is timed with snowmelt (Yensen 1991; Yensen and Sherman 1997). Similarly, litters for *U. b. brunneus* emerge in late March–early April and for *U. b. endemicus* in late May–early June. The latter taxon also has significantly larger litter sizes (Yensen 1991; Yensen and Sherman 1997). However, these behavioral differences may be attributed to the different elevations the 2 taxa occupy.

Thus, we argue that the null hypothesis of ecological exchangeability can be rejected because the 2 taxa are significantly different in ecological niche (this study), bacular morphology, cranial morphometrics, pelage color and texture, annual cycles (Yensen 1991), allozyme frequencies (Gill and Yensen 1992), microsatellite frequencies, and mtDNA structure (this study; Garner et al. 2005). Although it would be valuable to undertake ecological experiments to test ecological exchangeability further (Rader et al. 2005), this type of experiment would be difficult to implement on a United States federally listed species.

Delimiting appropriate taxonomic status is challenging and sometimes controversial (e.g., King et al. 2006; Ramey et al. 2005, 2006, 2007; Vignieri et al. 2006), and this is particularly true in cases where there is neither parapatry nor sympatry. Here, we apply integrated taxonomy to address the delimitation of 2 taxa currently recognized as subspecies of *S. brunneus* using the criteria outlined for the cohesion species concept (Templeton 2001). We have demonstrated with nDNA and mtDNA that differentiation between the 2 subspecies is high (nDNA, F_{ST} = 0.34; mtDNA, F_{ST} = 0.52) and there has been no migration between *U. b. brunneus* and *U. b. endemicus* since their divergence. Furthermore, we demonstrated that the 2 taxa inhabit different niches, which provides the opportunity for divergence to occur via differential adaptation. These data, coupled with morphological differentiation documented by Yensen and Sherman (1997), provide compelling evidence that *U. b. brunneus* and *U. b. endemicus* should be elevated to species status, as advocated by Yensen and Sherman (1997).

Others may focus on the low level of mtDNA differentiation, following Baker and Bradley (2006), and opt to retain subspecific status in this difficult case. Similarly, others may wish to apply different species concepts and their accompanying different criteria for species delimitation. The appropriate taxonomic status to confer upon *brunneus* and *endemicus* may be resolved by generation of multilocus sequence data; however, it is cases such as this, where it is difficult to categorize the continuity of the speciation process, where we can learn most about speciation. The IMa2 runs indicate that the 2 taxa diverged very late in the Pleistocene, and this implies that the substantial ecological, morphological, and behavioral differentiation has evolved very rapidly.

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APPENDIX I

Haplotypes found for *Urocitellus brunneus brunneus* with the concatenated (concat), control region (CR), and cytochrome-*b* (*Cytb*) haplotype labels and frequencies for the concatenated data set found in each sampling area. Abbreviations follow Table 1.

			Sampling area													
Concat	CR	*Cytb*	ChS	CS	CW	HU	HW	LCL	LM	LV	PV	RT	RV	SG	SM	TF
Ubb24	J	VII	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.9
Ubb25	J	XII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Ubb26	K	I	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ubb27	K	VII	0.0	1.0	0.1	0.1	0.3	1.0.0	0.0	0.4	0.9	0.0	0.0	0.9	0.9	0.1
Ubb29	K	XI	0.0	0.0	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ubb30	K	XVIII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Ubb31	L	VII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Ubb32	L	X	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Ubb38	R	I	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ubb40	T	XVII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
Ubb41	U	X	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Ubb42	U	XI	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ubb43	U	XVIII	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.8	0.0	0.0	0.0	0.0

Appendix II

Haplotypes found for *Urocitellus brunneus endemicus* with the concatenated (concat), control region (CR), and cytochrome-*b* (*Cytb*) haplotype labels and frequencies for the concatenated data set found in each sampling area. Abbreviations follow Table 1.

			Sampling areas										
Concat	CR	*Cytb*	BC	CP	HB	HG	MC	PFS	RH	HC	SB	SH	Sk
Ube01	A	II	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube02	A	XIII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Ube03	A	XV	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1
Ube04	A	XVI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
Ube05	B	XV	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube06	C	VIII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Ube07	C	XV	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Ube08	D	VI	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube09	D	VIII	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Ube10	D	XVI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Ube11	E	VI	0.0	0.7	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Ube12	E	XV	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube13	F	XIII	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Ube14	F	XV	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Ube15	G	VIII	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Ube16	G	XV	0.0	0.0	0.0	0.0	0.0	0.0	0.8	1.0	0.0	0.0	0.0
Ube17	H	V	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube18	H	VI	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube19	H	XIII	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube20	H	XV	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube21	I	III	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.7	0.1	0.0
Ube22	I	VIII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Ube23	I	XIII	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
Ube33	N	III	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Ube34	N	XV	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Ube35	O	XV	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube36	P	XIV	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Ube37	Q	IV	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ube39	S	VIII	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX III

F_{ST} (θ) for microsatellite loci (below diagonal) and Φ_{ST} for mitochondrial DNA sequence data (above diagonal) for all populations. Abbreviations follow Table 1.

	ChS	CS	CW	HU	HW	LCL	LM	LV	PV	RT	RV	SG	SM	TF	BC	CP	HB	HC	HG	MC	PFS	RH	SB	SH	Sk
ChS		0.866	0.643	0.750	0.664	0.875	0.750	0.510	0.765	0.733	0.866	0.750	0.750	0.750	0.472	0.643	0.443	0.829	0.508	0.750	0.510	0.689	0.643	0.438	0.443
CS	0.115		0.673	0.820	0.644	0.000	0.846	0.261	0.040	0.833	1.000	0.024	0.024	0.820	0.533	0.719	0.496	1.000	0.563	0.846	0.570	0.805	0.719	0.487	0.496
CW	0.203	0.104		0.587	0.114	0.694	0.595	0.290	0.566	0.565	0.719	0.539	0.539	0.587	0.280	0.476	0.262	0.634	0.340	0.595	0.333	0.485	0.476	0.267	0.262
HU	0.267	0.165	0.188		0.603	0.833	0.714	0.417	0.652	0.692	0.846	0.674	0.622	0.167	0.407	0.595	0.381	0.794	0.453	0.714	0.452	0.634	0.595	0.380	0.381
HW	0.177	0.131	0.074	0.254		0.667	0.619	0.267	0.523	0.591	0.744	0.496	0.496	0.603	0.306	0.500	0.286	0.666	0.363	0.619	0.357	0.515	0.500	0.289	0.286
LCL	0.200	0.108	0.139	0.197	0.152		0.857	0.292	0.018	0.847	1.000	0.000	0.000	0.833	0.563	0.738	0.524	1.000	0.586	0.857	0.595	0.824	0.738	0.513	0.524
LM	0.225	0.125	0.219	0.249	0.203	0.116		0.441	0.733	0.082	0.846	0.714	0.714	0.714	0.407	0.595	0.381	0.794	0.453	0.714	0.452	0.634	0.595	0.380	0.381
LV	0.128	0.116	0.153	0.250	0.131	0.126	0.116		0.165	0.416	0.570	0.134	0.134	0.417	0.131	0.333	0.119	0.456	0.203	0.452	0.190	0.316	0.333	0.129	0.119
PV	0.137	0.067	0.129	0.215	0.107	0.088	0.088	0.056		0.714	0.856	0.070	0.153	0.652	0.442	0.621	0.414	0.813	0.482	0.733	0.483	0.664	0.621	0.411	0.414
RT	0.114	0.062	0.144	0.194	0.095	0.072	0.080	0.066	0.026		0.833	0.692	0.692	0.692	0.367	0.565	0.342	0.771	0.419	0.692	0.416	0.599	0.565	0.343	0.342
RV	0.309	0.287	0.316	0.462	0.294	0.401	0.442	0.221	0.284	0.357		0.846	0.846	0.846	0.533	0.719	0.496	1.000	0.563	0.846	0.570	0.805	0.719	0.487	0.496
SG	0.126	0.079	0.108	0.157	0.151	0.166	0.213	0.137	0.116	0.096	0.285		0.077	0.674	0.407	0.595	0.381	0.794	0.453	0.714	0.452	0.634	0.595	0.380	0.381
SM	0.142	0.056	0.088	0.165	0.096	0.138	0.167	0.112	0.086	0.095	0.279	0.105		0.622	0.407	0.595	0.381	0.794	0.453	0.714	0.452	0.634	0.595	0.380	0.381
TF	0.134	0.076	0.172	0.238	0.167	0.171	0.227	0.177	0.140	0.135	0.319	0.125	0.093		0.407	0.595	0.381	0.794	0.453	0.714	0.452	0.634	0.595	0.380	0.381
BC	0.203	0.252	0.343	0.397	0.253	0.347	0.354	0.279	0.232	0.216	0.386	0.256	0.248	0.277		0.280	0.034	0.400	0.145	0.407	0.039	0.254	0.052	0.039	0.057
CP	0.188	0.258	0.383	0.435	0.277	0.361	0.372	0.274	0.230	0.216	0.428	0.264	0.260	0.270	0.040		0.262	0.634	0.340	0.595	0.258	0.485	0.476	0.267	0.262
HB	0.364	0.367	0.526	0.497	0.407	0.499	0.492	0.365	0.361	0.365	0.550	0.327	0.342	0.333	0.304	0.332		0.371	0.133	0.381	0.119	0.235	0.262	0.060	0.048
HC	0.186	0.252	0.361	0.421	0.253	0.332	0.354	0.260	0.221	0.194	0.428	0.250	0.255	0.272	0.036	0.071	0.407		0.458	0.794	0.456	0.091	0.634	0.372	0.371
HG	0.281	0.316	0.421	0.436	0.349	0.427	0.423	0.331	0.315	0.294	0.475	0.289	0.295	0.303	0.168	0.207	0.137	0.208		0.453	0.203	0.325	0.328	0.143	0.084
MC	0.228	0.294	0.421	0.480	0.298	0.383	0.405	0.305	0.261	0.253	0.465	0.301	0.291	0.281	0.061	0.049	0.357	0.053	0.201		0.452	0.634	0.595	0.380	0.381
PFS	0.314	0.320	0.436	0.459	0.378	0.432	0.426	0.323	0.320	0.301	0.462	0.283	0.316	0.299	0.236	0.267	0.234	0.255	0.129	0.252		0.316	0.162	0.097	0.101
RH	0.352	0.351	0.483	0.498	0.422	0.442	0.452	0.351	0.346	0.326	0.498	0.330	0.356	0.325	0.274	0.288	0.346	0.285	0.231	0.263	0.064		0.485	0.243	0.235
SB	0.287	0.298	0.410	0.467	0.298	0.420	0.409	0.326	0.291	0.282	0.452	0.313	0.289	0.329	0.064	0.121	0.349	0.146	0.242	0.163	0.302	0.344		0.196	0.231
SH	0.215	0.276	0.382	0.419	0.284	0.366	0.383	0.294	0.244	0.238	0.417	0.271	0.276	0.295	0.040	0.045	0.374	0.095	0.222	0.113	0.293	0.327	0.143		0.060
Sk	0.194	0.231	0.372	0.417	0.272	0.333	0.340	0.244	0.210	0.184	0.429	0.246	0.234	0.257	0.053	0.051	0.337	0.059	0.204	0.081	0.221	0.218	0.091	0.101	