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Phylogeny, phylogeography, and geographic variation of *Sylvisorex howelli* (Soricidae), an endemic shrew of the Eastern Arc Mountains, Tanzania

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Abstract

The Eastern Arc Mountains of eastern Africa are notable for the high levels of endemism exhibited by various forest-dwelling organisms of this ancient montane archipelago. There has been virtually no assessment of the variation among populations of small mammal species living on these unique mountains, but recent faunal surveys have produced sufficient material to initiate such studies. Cranial morphometric and DNA sequence data were examined from six populations of *Sylvisorex howelli* Jenkins, 1984, an endemic shrew found in several different massifs of the Eastern Arc Mountains, to assess variation across the archipelago in the context of various hypotheses of historical biogeography. Twenty-two cranial measurements were analysed using principal components analysis. Age classes (based on tooth wear) and sex had little effect on the variation exhibited by the variables studied. Overall, specimens of *S. howelli* from the East Usambara Mountains are smaller than specimens from other known populations. The mitochondrial ND2 and 12S rRNA genes from representatives of each montane population of *S. howelli* in addition to several crocidurine taxa from eastern Africa and three soricine outgroup species were sequenced to assess phylogenetic relationships among these taxa. Neither maximum likelihood, maximum parsimony, nor Bayesian analyses support monophyly of the genus *Sylvisorex*, but *S. howelli* populations were consistently recovered as a well-supported clade. Over 40 individuals of *S. howelli* from six disjunct montane ranges, comprising the entire known distribution of the species, were sequenced for 504 base pairs of ND2 to investigate phylogeographic patterns. Phylogenetic analysis recovered six reciprocally monophyletic haplotype clades grouped by locality. Branch lengths are consistent with relatively long periods of isolation among populations from the Uluguru, Ukaguru, Nguru, Nguu, East Usambara and West Usambara Mountains, with low levels of diversity observed within each population. These results are interpreted within the historical context of the Eastern Arc Mountains.

Key words: *Sylvisorex howelli*, biogeography, Eastern Arc Mountains, shrews, Tanzania

INTRODUCTION

The Eastern Arc Mountains of Tanzania and south-eastern Kenya are renowned for high levels of endemism exhibited by both the flora and fauna of this ancient montane archipelago (Rodgers & Homewood, 1982*a*,*b*; Lovett, 1988; Scharff, 1992; Howell, 1993; Stanley & Hutterer, 2000). This unique biota, however, is severely threatened by rapid habitat degradation, and our understanding of the natural history of this endangered montane habitat is extremely poor. Knowledge of the fauna of these mountains will help elucidate the evolutionary history of these groups and may provide critically needed data for effective conservation strategies.

Sylvisorex howelli Jenkins, 1984 is an endemic crocidurine shrew, known only from montane forests of the Eastern Arc Mountains in Tanzania (Jenkins, 1984; Hutterer, 1986; Stanley, Kihaule *et al*., 1998). The species was first described based on one specimen from the Uluguru Mountains (Jenkins, 1984). Hutterer (1986) subsequently described a subspecies (*S. howelli usambarensis*) based on a second specimen from the West Usambara Mountains. Recent faunal surveys of the small mammals of the Eastern Arc Mountains have produced large series of this endemic shrew that now allow us to describe the distribution of the species as well as variation among isolated populations and to investigate hypotheses of faunal historical biogeography of the Eastern Arc Mountains. While recent studies have investigated the biogeography of Eastern Arc Mountain birds (Stuart, 1981; Roy, Arctander & Fjeldså, 1998; Roy, Sponer & Fjeldså, 2000; Bowie, 2003; Beresford, Fjeldså & Kiure, 2004; Bowie *et al*., 2004), this is the first study combining morphological and molecular data for shrews of these mountains. Because of their expected lower vagility, these non-volant small mammals may provide unique

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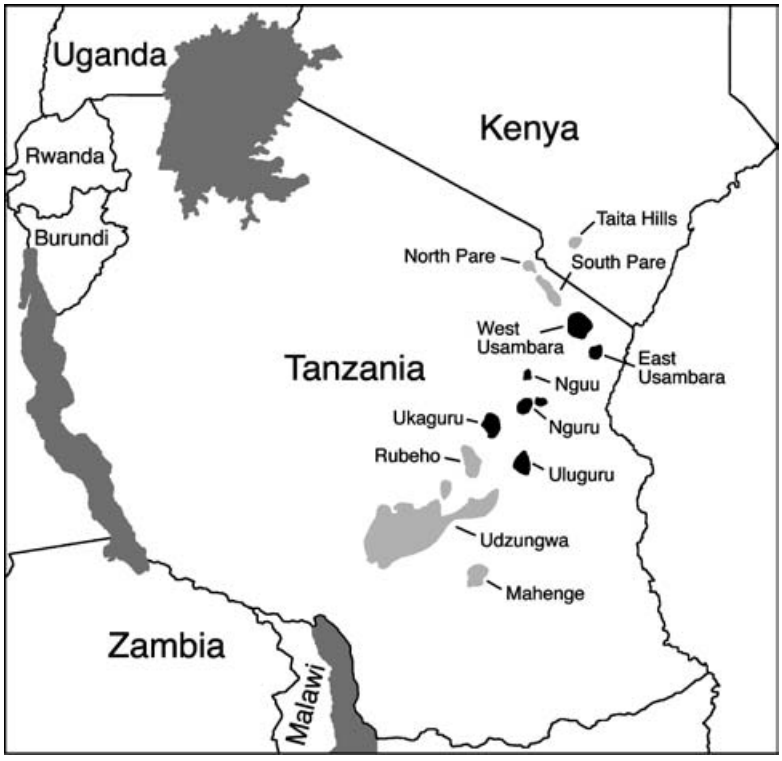

Fig. 1. The Eastern Arc Mountains of Tanzania and south-eastern Kenya. Mountains in black are localities where *Sylvisorex howelli* has been documented and where samples reported in this paper were collected.

insight into the biogeographic history of this montane archipelago.

METHODS AND MATERIALS

Fieldwork

Specimens were collected during small mammal surveys of the Eastern Arc Mountains that involved the first author and biologists from the University of Dar es Salaam. *Sylvisorex howelli* were collected on 6 of the ranges visited including (from north to south) the East and West Usambara, Nguu, Nguru, Ukaguru and Uluguru Mountains (Fig. 1). Shrews were collected using pitfall lines consisting of 11 buckets (15 l) buried in the ground such that the top of the bucket was flush with the ground. The buckets were 26 cm deep, set 5 m apart, and had a 50 cm high plastic fence running over the top and through the centre of each bucket (Stanley, Goodman & Hutterer, 1996). Animals that fell into the buckets were retrieved and handled in accordance with American Society of Mammalogists guidelines (Animal Care and Use Committee, 1998). Specimens were prepared as skins, skulls and skeletons or in fluid and deposited in the Field Museum of Natural History (FMNH). Heart, kidney and liver tissues were stored in liquid nitrogen. External measurements were taken by WTS from each specimen at the time of collection and include total length (TL, tip of nose to last discernable caudal vertebra), head and body (HB, tip of nose to where tail inserts on body), tail length (Tail, from where tail inserts on body to last discernable caudal vertebra), hind foot (HF, from heel to tip of claw), ear (Ear, proximal notch to distal tip of ear) and weight (WT). All measurements are in mm except weight (g). This study also included a sample of 10 specimens (skulls only) that were collected by Frontier-Tanzania (Society for Environmental Exploration and University of Dar es Salaam) on Mount Nilo in the East Usambara Mountains.

Morphometrics

Only skulls from adults (based on complete fusion between the basioccipital and basisphenoid bones) were measured. Specimens were assigned to 1 of 4 toothwear categories following the definitions of Dippenaar (1997), although our focus was constrained to the wear exhibited by the first upper molar. There were no specimens that met the definitions of category I. FMNH 151144, 150017, and 161242 exemplified categories II, III, and IV, respectively (*sensu* Dippenaar, 1977), and these specimens were subsequently used as a reference series.

Craniodental measurements were recorded to the nearest 0.01 mm using digital callipers (Fig. 2). These measurements (following Dippenaar, 1977; van Zyll de Jong & Kirkland, 1989; Carraway, 1990) and their abbreviations include condylo-incisive length (CI), basal length (BL), post-palatal length (PPL), length of entire upper toothrow (UTRL), length of complex teeth in upper toothrow (CUTRL), least interorbital width (LIW), bimaxillary width (BW), nasal width (NW), greatest

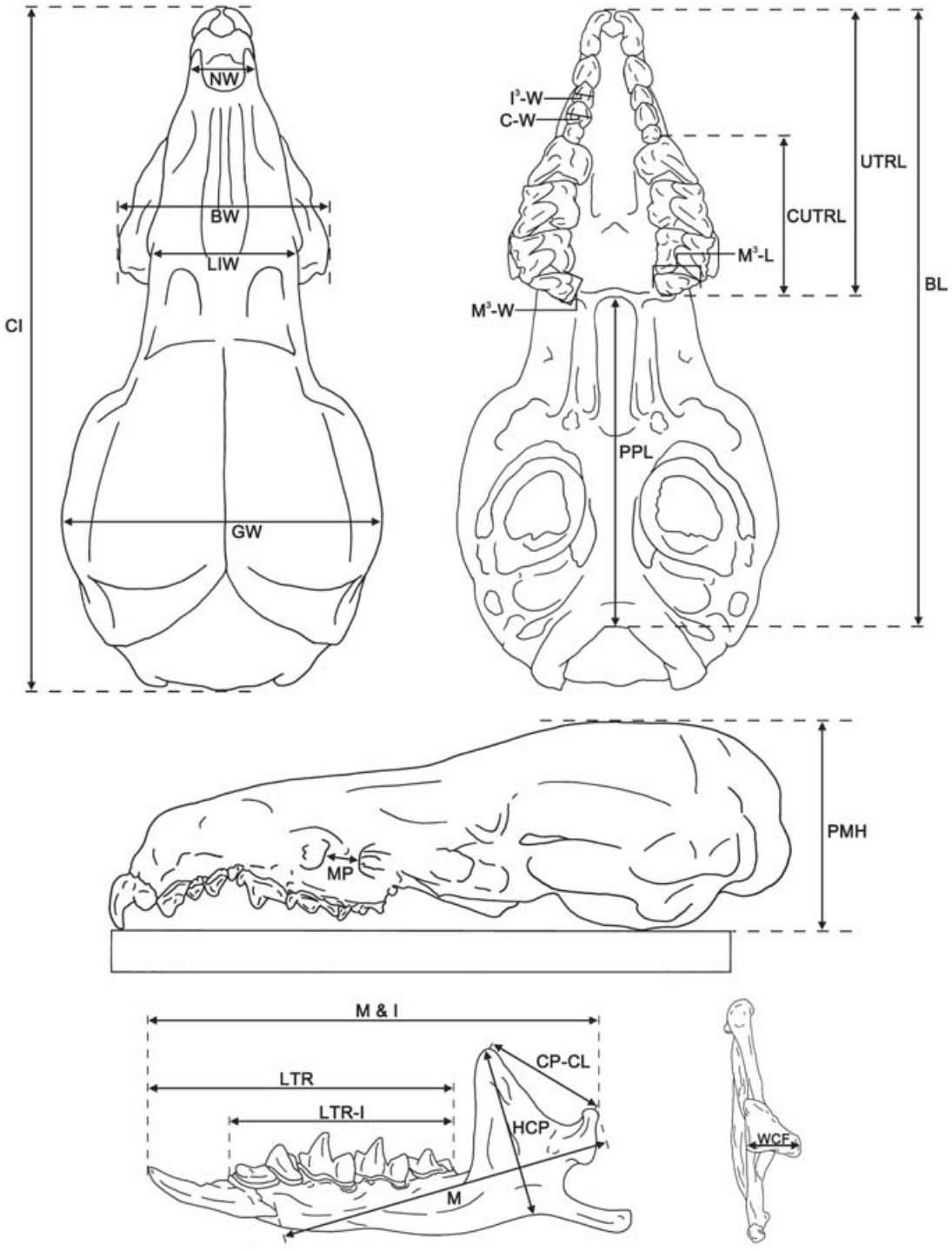

Fig. 2. Diagram of the cranium and dentary showing the various measurements and associated landmarks used in this study. See Methods and Materials for definitions of the abbreviations.

width of the braincase (GW), height of the braincase (PMH; measured by placing skull on microscope slide, measuring from the ventral surface of the slide to the highest point of the cranium and then subtracting the thickness of the slide from that measurement; J. Patton, pers. comm.), width of third upper incisor (I^3-W), width of upper canine (C-W), length of third upper molar (M^3-L), width of third upper molar (M^3-W), least distance across the maxillary plate parallel to the alveolar line (MP), length of mandible including the first incisor (M&I), length of mandible not including first incisor (M), length of mandibular toothrow including first incisor (LTR), length of mandibular toothrow not including first incisor (LTR-I), height of coronoid process (HCP), coronoid process-condyle length (CP-CL), and width of lower condylar facet (WCF).

Standard descriptive statistics (mean, range, and standard deviation) were calculated for each population.

One-way analyses of variances (ANOVA) were used to determine whether gender or age had a significant effect on non-geographic population variation in both external and cranial variables in all populations. To test for and assess significant geographical variation, a 1-way ANOVA (effect = mountain) and the post-hoc Scheffé test were used to identify characters that differed significantly among populations. Principal components and canonical variates were extracted from a variance–covariance matrix using the cranial variables converted to natural logarithms. All univariate and multivariate statistical analyses were conducted using Systat (version 10, 2002).

DNA sequencing and analysis

Genomic DNA was extracted from fresh frozen or buffered tissues from 62 specimens representing 14 species of shrew (Appendix 1). These include 11 species of crocidurines and 3 soricines. Whenever possible, a minimum of 10 individuals was sampled from each population of *S. howelli*, with a total resulting sample size of 49. The entire mitochondrial NADH dehydrogenase subunit 2 and 12S rRNA genes (ND2 and 12S, respectively, hereafter) were amplified separately using the primers given in Appendix 2 and either sequenced (both strands) directly from purified initial PCR products or from purified re-amplified PCR products. For phylogenetic analysis within *S. howelli* (see below), only the first 504 bases of ND2 were sequenced. Amplification and sequencing strategies for each specimen are provided in Appendix 3. Additional information on DNA extraction, amplification, purification, and sequencing can be found in Olson, Goodman & Yoder (2004). All sequences generated in this study have been deposited in GenBank under accession numbers AY691809–AY691886. The resulting ND2 sequences were aligned by eye with reference to the translated amino acid sequence using MacClade 4.0 (Maddison & Maddison, 2000). The 12S sequences were aligned by eye to the secondary structure model of Springer & Douzery (1996) for mammalian 12S rRNA. Ambiguously aligned regions were excluded from all analyses. Published sequences for both genes from 2 additional soricine taxa (*Sorex unguiculatus*, GenBank accession AB061527 and *Soriculus fumidus* AF348081) and 12S sequences from *Blarina brevicauda* (M95110) and *Sorex palustris* (U97343) were also included in our analyses. A complete, annotated alignment has been submitted to TreeBASE (www.treebase.org).

Phylogenetic analyses were conducted in 2 stages. An initial higher-level analysis was performed to determine whether *S. howelli*, as currently recognized, represents a monophyletic taxon. For the higher-level analysis, complete sequences of both genes were included for all outgroup species and a single representative of *S. howelli* from each of the 6 populations. Subsequent analysis within *S. howelli* was based on the first 504 bases of ND2 sequenced for 49 specimens. Ten individuals were sequenced from each locality (Fig. 1), except for the Nguu Mountains, where the total available sample was 8, and the East Usambara Mountains, where only a single specimen was available. The 5 soricine species were used to root all trees in the higher-level analysis, whose results were used to identify the appropriate outgroup in the second, lower-level analysis. Both the maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were used to estimate phylogeny using PAUP* 4.0 (Swofford, 2002). For MP analyses, all characters were treated as unordered and equally weighted. Heuristic tree searches were conducted using stepwise addition (100 random addition sequences) and the tree bisection–reconnection (TBR) branch-swapping algorithm. Nodal support was estimated by bootstrap re-sampling of 1000 pseudoreplicates (10 random-addition replicates per pseudoreplicate) under otherwise identical conditions, with starting trees obtained by stepwise addition. For ML tree searches, the best-fit model of nucleotide substitution was estimated using the program Modeltest 3.5 (Posada & Crandall, 1998). Model estimation was performed separately on the 12S + ND2 data matrix and the ND2 matrix for the higher-level and intraspecific analyses, respectively. Parameter values based on the preferred model according to the likelihood ratio test were then fixed in a ML search using 50 heuristic replicates with TBR branch-swapping. Bootstrap support was estimated from 250 pseudoreplicates using the NNI branch-swapping algorithm on starting trees obtained by stepwise addition.

In addition to bootstrap proportions, nodal support based on Bayesian posterior probabilities was estimated using the program MRBAYES (v. 3.0; Ronquist & Huelsenbeck, 2003). Three data partitions were recognized in the higher-level analysis and model parameters for each partition were estimated separately (i.e. 'unlinked'). Partitions included: (1) paired stem positions in the 12S rRNA gene according to the secondary structure model (see above), with each pairing interaction specified; (2) non-pairing (primarily loop) positions in the 12S gene; (3) protein-coding (ND2). For the pairing partition, the doublet model of nucleotide substitution, which is appropriate for modelling stem regions of ribosomal genes (Ronquist & Huelsenbeck, 2003), was specified. For the remaining 2 partitions, a model with 6 categories of base substitution was specified, with a gamma-distributed rate parameter and a proportion of invariant sites. Four MCMC chains (3 heated, 1 unheated) were allowed to proceed for 5 million generations, sampling trees every 100 generations. Trees sampled before the attainment of stable likelihood values were discarded as burn-in and all subsequently sampled trees in each MCMC run were included in 50% majority-rule consensus trees to obtain posterior probabilities. Posterior probabilities were similarly estimated for the analysis among *S. howelli* populations, but without any partitions specified in the model.

RESULTS

Morphometrics

External measurements revealed the East Usambara Mountains population to be the smallest in four of the

Table 1. External measurements of individuals of *Sylvisorex howelli* from six different mountains in the Eastern Arc Mountains (sexes combined). Mean ± SD and range. See text for definitions

	East Usambara ($n = 2$)	West Usambara ($n = 38$)	Uluguru ($n = 35$)	Nguu ($n = 21$)	Ukaguru ($n = 91$)	Nguru ($n = 26$)
TL	100.0 ± 2.8	104.7 ± 4.6	106.4 ± 4.7	106.6 ± 3.5	107.1 ± 4.0	110.8 ± 4.4
	98–102	95–112	95 –119	101–113	96–117	100–119
HB	54.5 ± 3.5	63.4 ± 3.8	61.1 ± 3.4	63.1 ± 2.6	60.7 ± 2.7	62.4 ± 3.2
	52–57	55–72	54–68	57–66	54–68	57–70
Tail	42.5 ± 2.1	41.9 ± 2.3	44.5 ± 2.6	43.3 ± 2.5	46.0 ± 2.9	47.2 ± 2.7
	41–44	38–46	40–51	39–47	38–58	42–52
HF	10.5 ± 0.7	11.1 ± 0.6	11.2 ± 0.5	11.7 ± 0.6	11.1 ± 0.3	11.6 ± 0.5
	10–11	9–13	10–12	11–13	11–12	11–12
Ear	8.0 ± 0.0	8.4 ± 0.8	8.6 ± 0.5	9.0 ± 0.3	8.9 ± 0.4	8.7 ± 0.5
	8–8	7.5–12	8–9	8–10	7–9	8–10
WT	3.8 ± 0.4	3.9 ± 0.4	4.2 ± 0.4	4.5 ± 0.4	4.4 ± 0.5	5.1 ± 0.5
	3.5–4	2.5–5	3.3–5.3	3.9–5.4	3.3–5.8	4.2–6.2

five criteria, and lightest in weight, although the sample size was small ($n = 2$, Table 1). While not as striking, the Nguru population was the largest and heaviest. The majority of the specimens we studied fell into age classes II and III, and we saw no examples of age class I. A one-way analysis of variance to test the effect of age on the variation of cranial variables revealed only six dimensions that varied significantly among age groups within certain populations. Each of these dimensions exhibited significant variation among age groups in only one of the six populations, with the exception of the length of the third upper molar (M^3-L), which differed significantly among age cohorts in two populations. In most cases age class IV was the cause of this significance. These differences were sporadic and not consistent, and may be the result of a Type 1 sampling error, and therefore it was felt that grouping age classes together for subsequent analyses was justified.

Sexual dimorphism was detected in 10 cranial dimensions. While the East Usambara population was included in this analysis, the sample size was relatively small ($n = 9$; three males, six females). As with age groups, these differences were not consistent across populations and were minor relative to the inter-locality differentiation (see below). Based on these observations, the sexes were combined in all subsequent analyses.

F-values produced by the one-way ANOVA to test the null hypothesis of no significant geographic variation were all highly significant (Table 3), with the greatest amount of morphologic heterogeneity exhibited by the width of third upper incisor (I^3-W) and upper canine (C-W), greatest width of the skull (GW), and the least interorbital width (LIW). In general, cranial dimensions were smallest in the East Usambara sample (19 of the 22 characters), and the a posteriori Scheffé test showed this population was significantly smaller than all other populations in 45% (10 of 22) of the characters (Fig. 3). Though less striking, the three gross measurements (greatest length, breadth and height of the skull) showed the Nguru population as having the largest skull (Table 2). Hutterer's (1986) earlier observations of the *S. howelli* from the Uluguru Mountains being larger than those from the West Usambara Mountains is supported in 77% (17 of 22) of the characters.

Principal components analysis resulted in skull length measurements generally having positive and moderately high loadings with the first component. The variable with the highest loading on PC 1 was the breadth of the maxillary plate (MP). PC 2 was most heavily influenced by the width of the third upper incisor (I^3-W). The first two components explained 32.5% and 24.2% of the variation, respectively (Table 3). The East Usambara sample is the most discrete aggregation on the first two components (Fig. 4) with narrow mastoid plates and third upper incisors.

Discriminant function analysis correctly classified specimens by their respective collecting localities with the following levels of success: East Usambara, 100%; West Usambara, 97.4%; Nguu, 95.2%; Nguru, 88.5%; Ukaguru, 89%; and Uluguru, 94.3%. As in the principal components analysis, the East Usambara sample is the most distinctive and only slightly overlaps with the West Usambara sample in a plot of the scores on the first two factors (explaining 38.5% and 26.7% of the variation, respectively). The Ukaguru and Uluguru specimens are separated from one another in multidimensional space, but overlap with the West Usambara, Nguru and Nguu samples (Fig. 5).

Sequence characteristics and molecular phylogenetics

The length of the 12S rRNA gene in shrews sequenced for this study ranged from 962 to 972 bases. The exclusion of alignment-ambiguous positions resulted in 821 characters, of which 178 were variable and 123 were parsimony informative. No length variation was observed in ND2, and visual inspection of the alignment in MacClade did not suggest the occurrence of hidden insertion/deletion events. Of the 1044 bases in the ND2 gene in shrews, 618 were variable and 523 of those were parsimony informative. Uncorrected distances within *S. howelli* ranged from 1.1% to 8.9% in the entire ND2 gene but only 0.1–1.7% in 12S, suggesting that ND2 is a much more appropriate marker for investigating phylogeographic patterns within *S. howelli*. Within the larger *S. howelli* sample (used for phylogeographic analysis), 83 of the 504

Table 2. Cranial measurements of individuals of *Sylvisorex howelli* from six different mountains in the Eastern Arc Mountains (sexes combined). Mean ± SD and range. See text and Fig. 2 for character definitions

	East Usambara ($n = 10$[a])	West Usambara ($n = 38$)	Uluguru ($n = 35$)	Nguu ($n = 21$)	Ukaguru ($n = 91$)	Nguru ($n = 26$)
CI	16.01 ± 0.26	16.46 ± 0.25	16.62 ± 0.29	16.54 ± 0.24	16.38 ± 0.34	16.76 ± 0.23
	15.68–16.36	15.91–16.86	16.21–17.27	16.22–17.03	15.50–17.19	16.42–17.28
BL	14.37 ± 0.23	14.67 ± 0.26	14.92 ± 0.26	14.81 ± 0.24	14.71 ± 0.32	14.99 ± 0.24
	14.07–14.76	14.15–15.14	14.49–15.55	14.44–15.23	13.91–15.38	14.64–15.58
PPL	7.58 ± 0.18	7.66 ± 0.16	7.88 ± 0.20	7.66 ± 0.14	7.75 ± 0.19	7.84 ± 0.14
	7.19–7.80	7.16–7.96	7.58–8.44	7.41–7.90	7.34–8.12	7.52–8.13
UTRL	6.58 ± 0.20	6.85 ± 0.13	7.01 ± 0.13	7.01 ± 0.10	6.89 ± 0.16	7.00 ± 0.10
	6.28–6.82	6.47–7.14	6.74–7.35	6.82–7.21	6.57–7.18	6.80–7.16
CUTRL	3.76 ± 0.08	3.85 ± 0.09	3.92 ± 0.09	3.91 ± 0.06	3.88 ± 0.09	3.94 ± 0.07
	3.60–3.85	3.65–4.00	3.75–4.16	3.77–4.03	3.67–4.05	3.80–4.06
LIW	3.48 ± 0.09	3.52 ± 0.09	3.65 ± 0.09	3.74 ± 0.07	3.69 ± 0.10	3.73 ± 0.11
	3.35–3.63	3.36–3.70	3.47–3.83	3.64–3.85	3.48–3.95	3.56–4.06
BW	4.80 ± 0.20	4.94 ± 0.11	4.96 ± 0.12	5.11 ± 0.08	4.95 ± 0.11	5.03 ± 0.08
	4.52–5.09	4.72–5.18	4.70–5.16	4.95–5.25	4.71–5.17	4.83–5.17
NW	1.57 ± 0.06	1.56 ± 0.06	1.49 ± 0.06	1.59 ± 0.06	1.62 ± 0.07	1.64 ± 0.06
	1.46–1.65	1.36–1.67	1.34–1.59	1.52–1.75	1.43–1.75	1.52–1.75
GW	7.10 ± 0.26	7.56 ± 0.14	7.53 ± 0.13	7.62 ± 0.11	7.61 ± 0.15	7.83 ± 0.17
	6.51–7.46	7.28–7.90	7.20–7.84	7.41–7.82	7.27–7.99	7.39–8.10
PMH	4.86 ± 0.29	4.84 ± 0.12	4.85 ± 0.10	4.79 ± 0.22	4.90 ± 0.19	4.97 ± 0.10
	4.53–5.36	4.54–4.99	4.70–5.12	4.11–5.19	4.43–5.40	4.77–5.16
I^3-W	0.44 ± 0.04	0.51 ± 0.03	0.52 ± 0.03	0.55 ± 0.02	0.56 ± 0.03	0.55 ± 0.04
	0.38–0.49	0.40–0.57	0.40–0.58	0.51–0.59	0.48–0.64	0.46–0.61
C-W	0.48 ± 0.05	0.58 ± 0.03	0.58 ± 0.03	0.61 ± 0.02	0.58 ± 0.03	0.58 ± 0.03
	0.42–0.55	0.52–0.62	0.50–0.63	0.57–0.64	0.53–0.64	0.53–0.63
M^3-L	1.05 ± 0.04	1.11 ± 0.04	1.14 ± 0.06	1.12 ± 0.04	1.14 ± 0.05	1.12 ± 0.04
	0.95–1.12	1.03–1.20	1.04–1.28	1.06–1.19	0.98–1.23	1.05–1.19
M^3-W	0.50 ± 0.02	0.53 ± 0.03	0.57 ± 0.04	0.55 ± 0.02	0.55 ± 0.03	0.55 ± 0.03
	0.46–0.53	0.48–0.60	0.47–0.65	0.51–0.60	0.47–0.68	0.49–0.63
MP	0.72 ± 0.07	0.78 ± 0.07	0.91 ± 0.08	0.80 ± 0.07	0.77 ± 0.07	0.81 ± 0.07
	0.62–0.85	0.64–0.89	0.75–1.06	0.67–0.98	0.58–0.94	0.68–0.95
M&I	9.45 ± 0.18	9.81 ± 0.19	9.94 ± 0.20	10.02 ± 0.18	9.82 ± 0.25	10.01 ± 0.19
	9.14–9.71	9.43–10.13	9.64–10.50	9.73–10.29	9.14–10.31	9.70–10.50
M	7.23 ± 0.16	7.42 ± 0.16	7.44 ± 0.21	7.57 ± 0.11	7.39 ± 0.21	7.51 ± 0.19
	7.01–7.49	7.06–7.69	6.99–7.84	7.36–7.75	6.80–7.81	7.12–8.01
LTR	6.00 ± 0.21	6.36 ± 0.13	6.52 ± 0.12	6.45 ± 0.13	6.35 ± 0.16	6.49 ± 0.10
	5.69–6.35	5.95–6.60	6.27–6.77	6.15–6.64	5.98–6.70	6.24–6.67
LTR-I	4.62 ± 0.10	4.76 ± 0.09	4.84 ± 0.11	4.84 ± 0.10	4.76 ± 0.12	4.84 ± 0.07
	4.42–4.74	4.54–4.92	4.60–5.06	4.56–5.06	4.42–5.02	4.69–4.99
HCP	3.59 ± 0.07	3.77 ± 0.10	3.66 ± 0.11	3.72 ± 0.09	3.68 ± 0.10	3.69 ± 0.07
	3.50–3.72	3.62–3.94	3.46–3.86	3.55–3.89	3.45–3.90	3.57–3.79
CP-CL	3.15 ± 0.09	3.15 ± 0.10	3.08 ± 0.10	3.15 ± 0.09	3.20 ± 0.12	3.14 ± 0.08
	3.03–3.28	2.95–3.34	2.91–3.33	3.00–3.34	2.92–3.44	3.00–3.32
WCF	1.61 ± 0.07	1.67 ± 0.06	1.65 ± 0.05	1.72 ± 0.06	1.67 ± 0.07	1.66 ± 0.06
	1.51–1.69	1.45–1.76	1.54–1.75	1.60–1.88	1.49–1.82	1.56–1.78

[a] Sample size = 9 for I^3-W, C-W, M&I, LTR and LTR-I.

nucleotides sequenced for ND2 were variable, and 68 of those were parsimony informative. The majority of the variable positions (66; 56 parsimony informative) were at third positions, followed by first (12; 10) and second (5; 2) positions. Nineteen unique haplotypes were recovered in *S. howelli*, with the largest number (7; haplotypes A–G hereafter) found in the Uluguru population, followed by 6 (H–M) in the Ukaguru sample, 2 each in the West Usambara (N–O) and Nguru (P–Q) samples, and one each in the Nguu and East Usambara populations.

For the first (higher-level) analysis of the combined ND2 (complete sequence) and 12S dataset, the heuristic tree search under the maximum parsimony criterion resulted in a single most-parsimonious tree 2777 steps long (not shown; see below). For maximum likelihood analysis, the preferred model of nucleotide substitution according to a hierarchical likelihood ratio test was equivalent to the TrN model with site-specific rate variation approximated by the gamma (Γ) distribution with a proportion of invariant sites (I) (parameter values are provided in the data matrix available on TreeBASE (www.treebase.org). The single ML tree ($-\ln L = 13642.10947$) with associated ML and corresponding MP bootstrap values is shown in Fig. 6. For the most part, ML and MP analyses resulted in congruent topologies, and no node receiving $\geq 70\%$ bootstrap support in either analysis was conflicted in the other. Likelihood scores converged on a stable value within the first 1000 sampled generations in both Bayesian analyses;

Table 3. Results of principal component analyses and one-way ANOVA (effect = locality) for *Sylvisorex howelli* from the six sites sampled

Cranial variables	PC 1	PC 2	PC 3	*F* (locality)
CI	0.59	0.19	0.52	13.6
BL	0.63	0.18	0.49	11.1
PPL	0.51	0.10	0.27	10.6
UTRL	0.66	0.28	0.36	19.7
CUTRL	0.62	0.30	0.30	10.4
LIW	0.42	0.43	− 0.02	33.0
BW	0.39	0.46	0.35	14.2
NW	− 0.16	0.42	0.04	23.9
GW	0.35	0.45	0.18	35.7
PMH	0.17	0.26	0.25	3.6
I^3-W	0.32	0.78	− 0.44	43.4
C-W	0.45	0.60	− 0.14	34.1
M^3-L	0.45	0.44	0.13	8.1
M^3-W	0.56	0.25	0.09	10.9
MP	0.84	− 0.51	− 0.13	24.6
M&I	0.65	0.24	0.51	11.8
M	0.47	0.18	0.50	6.0
LTR	0.69	0.21	0.30	20.5
LTR-I	0.57	0.24	0.32	10.0
HCP	0.32	0.25	0.57	7.7
CP-CL	0.18	0.35	0.52	6.8
WCF	0.38	0.29	0.65	5.3
Eigenvalue	0.015	0.011	0.005	
%variance	32.5	24.2	10.6	
Cumulative% variance	32.5	56.7	67.3	

* All F values significant at $P \leq 0.01$.

the first 100 000 generations were therefore conservatively excluded as burn-in. A basal position for *Myosorex* with respect to the remaining sampled crocidurines was recovered with strong bootstrap and Bayesian support. *Sylvisorex granti* and *Sylvisorex lunaris* formed a well-supported sister group to *Sylvisorex johnstoni* in both analyses. Although the node uniting these three species received relatively low MP bootstrap support, likelihood and Bayesian analyses strongly support it. The two species of *Suncus* did not form a monophyletic clade; instead, *Suncus murinus* was recovered as the sister taxon to a clade comprised of *Suncus madagascariensis* and *Crocidura hildegardeae*. As with the previously discussed node, neither of these groupings was strongly supported by MP bootstrap values, in contrast to the high likelihood and posterior probability values. Finally, the six specimens of *S. howelli*, representing the entire known geographic range of the species (Fig. 1), formed a very well-supported clade. The phylogenetic position of *S. howelli* to the remaining crocidurine taxa sampled, however, was not resolved in our analyses. Furthermore, neither MP, ML, nor Bayesian analyses supported a monophyletic *Sylvisorex*, although the nested position of the *Suncus* + *Crocidura* clade within *Sylvisorex* was also not strongly supported.

Based on the single optimal tree recovered under ML (Fig. 6), *Suncus madagascariensis*, *Suncus murinus*, and *Crocidura hildegardeae* were used to root all trees in the second round of analyses. Sixty equally parsimonious trees (not shown) were obtained (length = 60 steps) in the MP analysis of the first 504 nucleotides sequenced for ND2 in *S. howelli*. The preferred model of nucleotide substitution for ML analysis was equivalent to the GTR (general time-reversible) + Γ + I. The single tree obtained in the heuristic ML analysis (− lnL = 2020.81078) with associated ML, MP, and Bayesian support values is shown in Fig. 7. Phylogenetic analysis under both optimality criteria recovered six reciprocally monophyletic haplotype clades grouped by locality, with relationships among populations congruent with those recovered in the higher-level analysis with single representatives of each population. Although relationships among the East Usambara, West Usambara, and Nguu + Nguru haplotype clades were not resolved with confidence in the phylogeographic analysis (Fig. 7), the higher-level phylogenetic analysis, with nearly four times as much sequence data, provided strong support for every node uniting these populations (Fig. 6). This, combined with the reciprocal monophyly of each population in the phylogeographic analysis, leads us to accept the population phylogeny for *S. howelli* from the higher-level analysis (Fig. 6).

DISCUSSION

Phylogenetic analysis of > 1800 nucleotides of mtDNA in 11 species of African crocidurine shrews did not support monophyly of the genus *Sylvisorex* (Fig. 6). This is not surprising in light of the similar findings of Quérouil *et al*. (2001), who conducted phylogenetic analyses of 549 bases of the mitochondrial 16S rRNA gene sequenced for 24 crocidurine species. The results of their study are not exhaustively compared with ours here owing to the disparate and largely non-overlapping taxon and character sampling (e.g. their sample did not include *S. howelli*, *S. lunaris* or *S. granti*, while ours lacked several of the non-*Sylvisorex* species used in their study). One interesting discrepancy, however, is noted. In some (but not all) of the analyses of Quérouil *et al*. (2001), *Sylvisorex megalura* was recovered as more closely related to *Suncus etruscus* than either species was to *Suncus murinus* or other *Sylvisorex* species (including *Sylvisorex ollula* and *S. johnstoni*). In contrast, our results (Fig. 6) strongly support a clade consisting of both species of *Suncus* and *Crocidura hildegardeae* to the exclusion of *Sylvisorex megalura*, and although poorly supported by parsimony bootstrap values, the sister relationship between *Sylvisorex megalura* and *Sylvisorex ollula* received moderate ML boostrap support and a posterior probability of 0.97. Quérouil *et al*. (2001) suggested that their results were in agreement with the karyotypic results of Schlitter *et al*. (1999), who showed that *Sylvisorex megalura* possesses a higher diploid and fundamental number than any other member of the group, including *Sylvisorex ollula* and *Sylvisorex johnstoni*. If our results are correct, then the karyotypic distinction of *Sylvisorex megalura* may represent a relatively recent apomorphy.

In contrast to the lack of resolution among major lineages of *Sylvisorex* included in our sample, strong support was found for monophyly of *S. howelli*. Although its

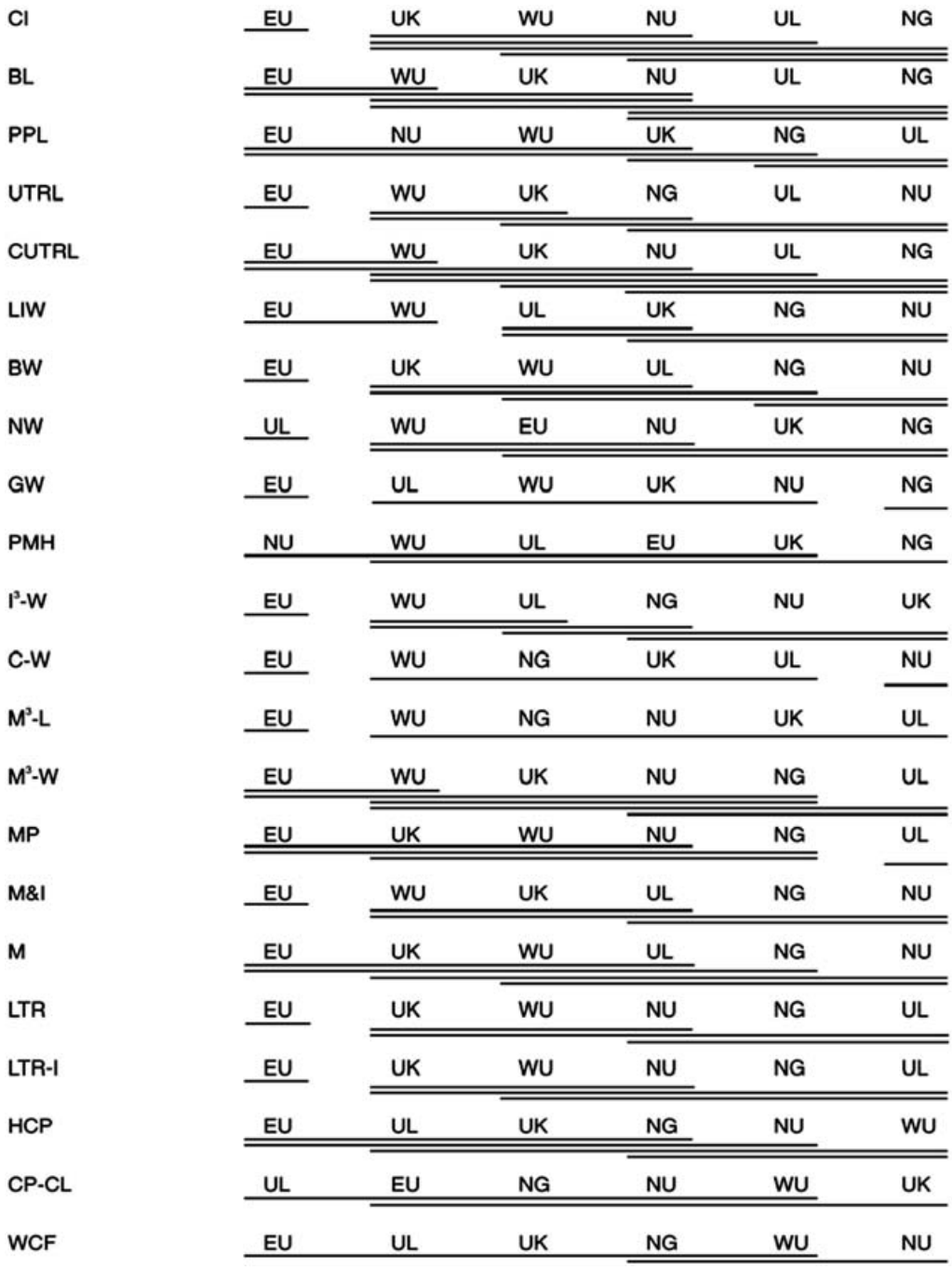

Fig. 3. Results of the a posteriori Scheffé test following the one-way ANOVA (effect = locality) in *Sylvisorex howelli*. Populations are arranged smallest to largest from left to right for each character. Statistically homogenous subsets are designated by lines below localities. Comparisons where all populations did not differ significantly are not represented by a line. WU, West Usambara; EU, East Usambara; NG, Nguru; NU, Nguu; UK, Ukaguru; UL, Uluguru.

closest relative remains equivocal, phylogenetic relationships among populations of *S. howelli* are strikingly concordant with geography. No haplotypes were shared among different populations, and branch lengths (Fig. 6) suggest relatively long periods of population isolation, especially for those in the Ulugurus and Ukagurus. These results are consistent with a vicariance model of range fragmentation. That is, during different climatic regimes of the past, habitat currently restricted to higher elevations was more continuously distributed between mountains. Climatic perturbations caused this habitat to retreat to higher elevations (deMenocal, 1995), presumably isolating it and any dependent inhabitants. While several workers have proposed various scenarios for the climatic history of eastern Africa (e.g. Griffiths, 1993; Livingstone, 1993; deMenocal, 1995), the specific chronology of fragmentation among the various Eastern Arc Mountains is still relatively unresolved, making explicit predictions

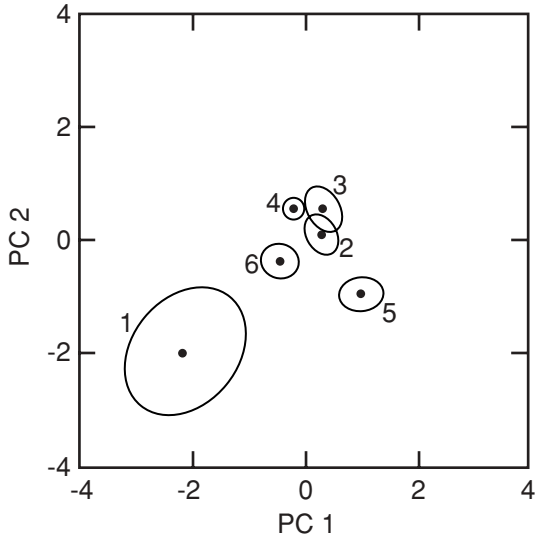

Fig. 4. Projection of group centroids on first two principal components for the six populations of *Sylvisorex howelli* studied. Ellipses, 95% confidence limits of a group's centroid. Mountains: 1, East Usambara; 2, Nguru; 3, Nguu; 4, Ukaguru; 5, Uluguru; 6, West Usambara.

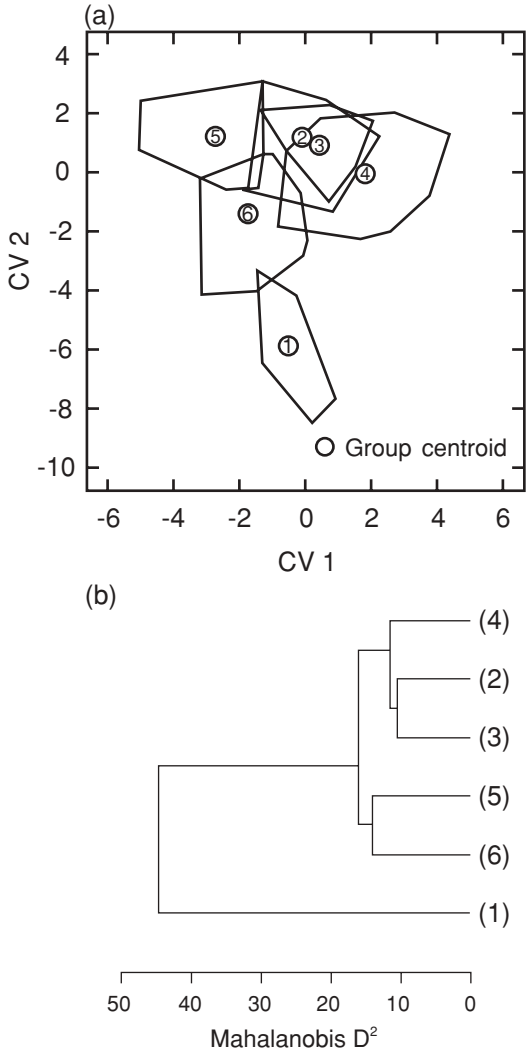

Fig. 5. Results of discriminant function analysis of cranial characters in *Sylvisorex howelli*. (a) Projection of scores onto the first two canonical variates; polygons include the maximal specimen score dispersion around the group centroid. (b) Phenogram resulting from clustering of Mahalanobis' distances among group centroids. Mountains: 1, East Usambara; 2, Nguru; 3, Nguu; 4, Ukaguru; 5, Uluguru; 6, West Usambara.

of biogeographic and phylogeographic patterns difficult. None the less, the results shown in Figs 6 & 7 are believed to be inconsistent with a dispersal hypothesis given the numerous dispersal events required to have resulted in the current distribution of *S. howelli*. All evidence to date suggests that *S. howelli* is not capable of dispersing across unsuitable habitats. It has not been found in any other Eastern Arc mountain range, even after intensive small mammal surveys of the forests of other mountains (Stanley, Goodman *et al*., 1996; Stanley, Kihaule *et al*., 1998). For example, the South Pare Mountains are in close proximity to the West Usambara Mountains (Fig. 1), yet *S. howelli* has not been recorded in the South Pare, despite intensive sampling efforts with the same methodology used in other Eastern Arc Mountains (Stanley, Goodman *et al*., 1996). Although the habitat of the South Pare is generally more arid than mountains where *S. howelli* is known, the Udzungwa Mountains are not, and *S. howelli* is not known from these mountains either, despite similarly rigorous surveys in multiple forests of this range (Stanley, Kihaule *et al*., 1998).

Not only is *S. howelli* apparently unable to disperse between isolated montane forests, our results suggest that there is limited dispersal *within* forest fragments as well. For example, in the Uluguru Mountains, samples were obtained at 1345 and 1535 m on one east-facing slope of the range and we found only one haplotype shared between the two sites, but two and four haplotypes unique to the lower and higher elevations was found, respectively (Appendix 1, Fig. 7). The distance between these two sampling sites is < 3 km. In the Ukaguru Mountains, two sites approximately 0.75 km apart but at similar elevations (1840 and 1900 m) were sampled and three unique haplotypes were found at each site, with no haplotypes shared among sites (Appendix 1). These results suggest that *S. howelli* is decidedly non-vagile, even within the same forest block.

There is evidence that within forest fragments where it is known to occur, *S. howelli* is more abundant at higher elevations. Based on the surveys that have been conducted to date, it seems that *S. howelli* rarely occurs, or is less abundant, at elevations below 1000 m. For example, in the Nguru Mountains, the trap success for *S. howelli* was much lower at 1000 m than it was at 1500 m (W. T. Stanley, pers. obs.). In the East Usambara Mountains, the trap success was very poor at *c*. 1000 m near Amani, but greater at Mount Nilo at 1500 m (W. T. Stanley & K. M. Howell, pers. obs.). These results lead us to conclude that *S. howelli* prefers moist montane environments. Similar patterns have been reported for other shrews endemic to the Eastern Arc Mountains (e.g.

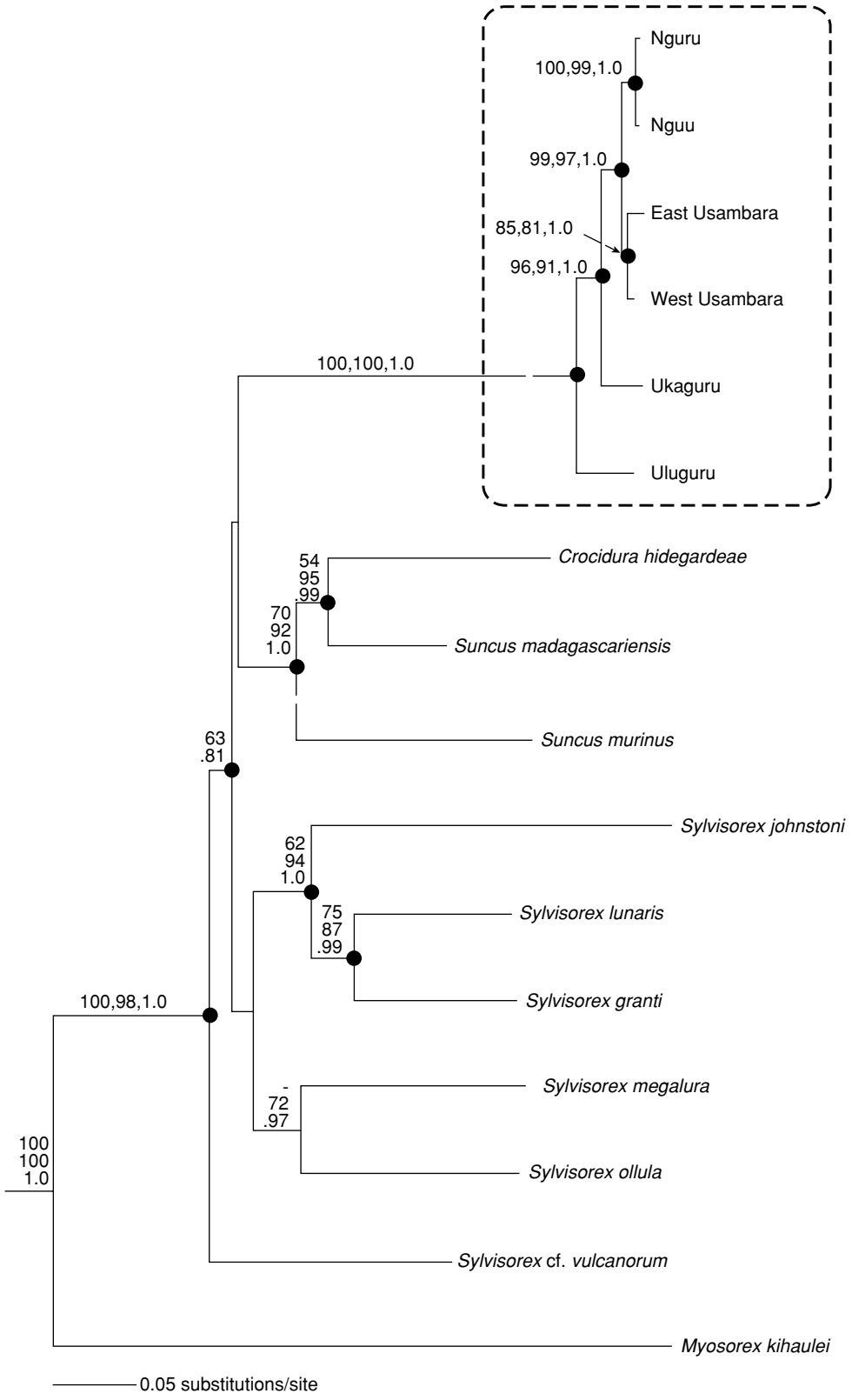

Fig. 6. Results of the higher-level phylogenetic analysis. Tree shown is the single tree recovered in ML heuristic tree search (the five soricine outgroups are not shown). Black circles, nodes recovered in the optimal tree in MP analysis. MP bootstrap, ML bootstrap, and posterior probabilities, respectively, are shown next to nodes. -, Bootstrap values < 50%. Representatives of *Sylvisorex howelli* are identified by locality and enclosed inside the dashed line (see Fig. 1, Appendix 1).

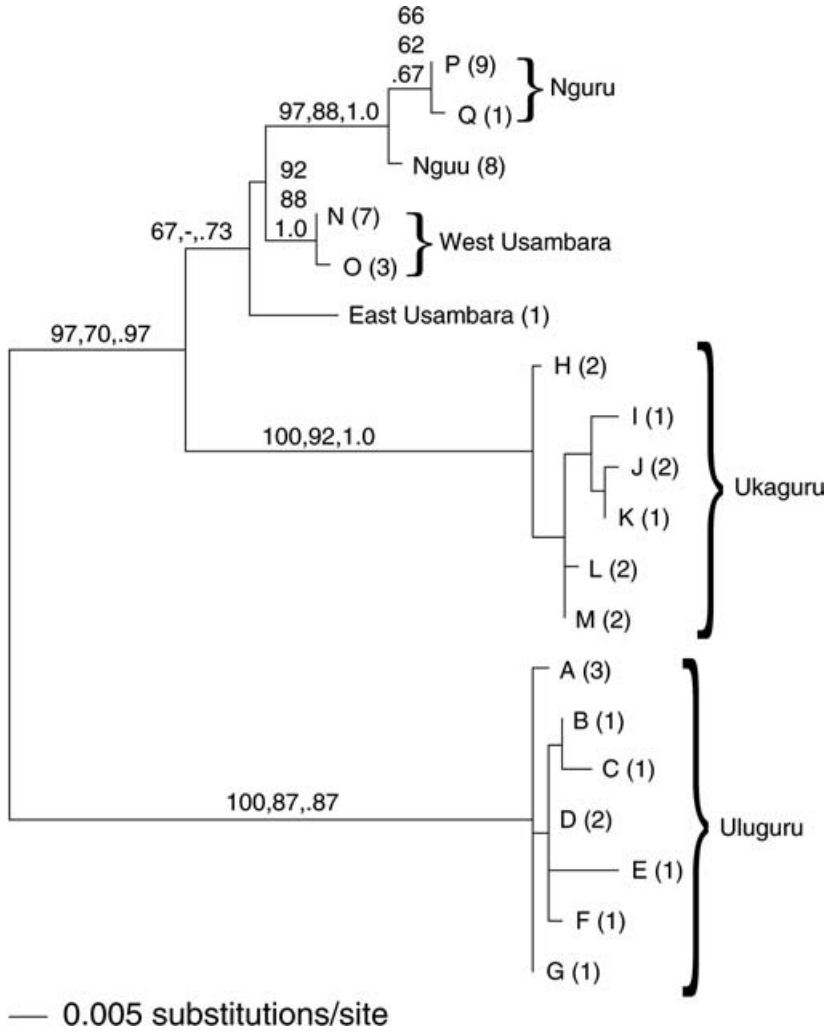

Fig. 7. Results of the phylogenetic analysis of populations of *Sylvisorex howelli*. Tree shown is single tree recovered in ML heuristic search (outgroups not shown). Support values as in Fig. 6 (not shown for nodes within each population). Different haplotypes within locality clades are identified by letters, and the number of each haplotype is given in parentheses.

Myosorex kihaulei; Stanley & Hutterer, 2000; Stanley, Kihaule *et al*., 1998).

We suggest that aridification of habitats at lower elevations and the concomitant upslope retreat of moist forest habitats during historic climatic perturbations best explain the isolation of the various populations of *S. howelli* within the Eastern Arc. We further propose an area cladogram for forest fragmentation that is consistent with Fig. 7 is further proposed. Under this scenario, the Ulugurus became isolated first, followed by the separation of the Ukagurus from the remaining block of forest habitat. The subsequent fragmentation of the latter into the remaining four refugia may have occurred much more rapidly, based on the relatively shorter branch lengths in this part of the phylogeny of *S. howelli* (Figs 6 & 7). We intend this to serve as a framework against which detailed studies of historical climatic changes in eastern Africa can be compared, as well as phylogeographic patterns of other Eastern Arc endemics.

There is considerable morphological and molecular variation among the six isolated populations of *S. howelli*. In general, the pattern of molecular differentiation follows the geographical arrangement of the various Eastern Arc Mountains. The pattern shown by the molecular data, however, is in contrast to that observed in the morphological analyses. The East Usambara sample is the most distinctive with regard to cranial morphometry, particularly in its smaller overall size, the narrow width of the third upper incisor and canine, and narrow breadth of the mastoid plate. However, the East Usambara population does not represent a particularly basal divergence within *S. howelli* and, as such, its morphological distinctiveness is unexpected. We hypothesize that the cause for the differences in these mensural cranial characters between the East Usambara population and those of the other massifs may be character displacement. This is because the East Usambara Mountains is the only range where another similarly sized shrew (*Crocidura hildegardeae*) is known to occur in syntopy with *S. howelli* (W. T. Stanley, pers. obs.). None of the other mountains where we have documented the occurrence of *S. howelli* has a sympatrically occurring shrew species in the same size range

(2.5–8 g). We propose that the sympatry of *S. howelli* and *C. hildegardeae* may have driven the divergent evolution of the skull in one or both species, a hypothesis that will be explored in future investigations. The possible influences of elevational and environmental gradients on the body size of *S. howelli* should also be investigated.

This study marks the first attempt to investigate and characterize the morphological variation, phylogenetic position, and intraspecific phylogeography of the recently described and previously poorly studied species *S. howelli*. Collectively, these results contribute to our growing knowledge of the patterns and processes responsible for the unique biota of the Eastern Arc Mountains as well as the evolutionary history of eastern Africa's diverse shrew fauna. Because *S. howelli* is an Eastern Arc endemic, exhibits substantial genetic variation both across its known range and within individual isolated forest fragments, and is clearly dependent upon the wet forest habitats of this archipelago, it may be representative of other unique organisms that rely on these habitats. Because of this, all efforts should be made to protect the individual forests where this shrew occurs.

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APPENDIX 1. VOUCHER INFORMATION

All specimens used in this study are housed at the Field Museum of Natural History; numbers below are FMNH catalogue numbers. Specimens: plain text, measured only; underlined, sequenced only; bold, measured and sequenced. Letters in parentheses correspond to haplotypes as shown in Fig. 7. Asterisks, *Sylvisorex howelli* specimens sequenced for the complete ND2 and 12S genes in the higher-level analysis.

Sylvisorex howelli:

Tanzania, Morogoro Region, Kilosa District, Ukaguru Mts, Mamiwa-Kisara Forest Reserve, 1 km E, 0.75 km S Mount Munyera, 1900 m:

166780(J), **166781**(K), 166782–166787, **166790**(M), **166796**(M), **166797**(J), 166799, 166800, 166802–166809, 166811–166822, 166824, 166829–166833, 166835, 166838–166843, 166845–166850, 166853, 166855, 166856, 166859, 166861, 166863–166865.

Tanzania, Morogoro Region, Kilosa District, Ukaguru Mts, Mamiwa-Kisara Forest Reserve, 1 km E, 1.5 km S Mount Munyera, 1840 m:

166630(H), **166633**(H), **166868***(I), **166869–166870**(L), 166872, 166873, 166877–166880, 166882, 166884–166902

Tanzania, Morogoro Region, Morogoro District, Nguru Mts, Manyangu Forest Reserve, 8 km N, 3 km W Mhonda, 1000 m:

161223(P)

Tanzania, Morogoro Region, Morogoro District, Nguru Mts, Nguru South Forest Reserve, 6 km N, 6 km W Mhonda, 1500 m:

161224–161228, 161230–161241, 161243*(Q), 161244–161247(P), 161251(P), **161252–161254**(P), 161255, 161256, 161258, 161261, 161262

Tanzania, Morogoro Region, Morogoro District, Uluguru Mts, Uluguru North Forest Reserve, 3 km W, 1.3 km N Tegetero, 1345 m:

158303(A), 158304*(A), 158305(A), 158306(B), **158423**(D)

Tanzania, Morogoro Region, Morogoro District, Uluguru Mts, Uluguru North Forest Reserve, 5.1 km W, 2.3 km N Tegetero, 1535 m:

158308(D), 158309(G), 158310(E), 158311(C), 158312(F), 158313–158317, 158319–158327, 158329–158332, 158424, 158430, 158432, 158433, 158444, 158445, 158448, 158450–158453, 158456–158458, 158461, 158462

Tanzania, Tanga Region, Handeni District, Nguu Mts, Nguru North Forest Reserve, 3.6 km E, 4.7 km S Gombero, 1430 m:

168165, 168166*, 168167, 168298–168301, **168303**, 168304–168320

Tanzania, Tanga Region, Korogwe District, West Usambara Mts, 12.5 km NW Korogwe, Ambangulu Tea Estate, 1300 m:

147184–147187, 150001, 150007, 150009, 150010, **150011**(N), 150012, **150013***(N), 150014(N), **150019**(N), **150020**(N), 150022, 150024–150036, 151139, 151146, **151147**(O), **151148**(O), 151149(O), **151150**(N), 151151(N), 151152–151155, 151385, 151386

Tanzania, Tanga Region, Muheza District, East Usambara Mts, 4.5 km ESE Amani, Monga Tea Estate, 870 m:

150023*

Tanzania, Tanga Region, Muheza District, East Usambara Mts, Nilo Forest Reserve, 800–1025 m:

171761–171763, 171767, 171769, 171772–171774, 171778, 171781

Outgroups:

Sylvisorex johnstoni (162197), *Sylvisorex lunaris* (144229), *Sylvisorex megalura* (150379), *Sylvisorex granti* (174131), *Sylvisorex* cf. *vulcanorum* (144240), *Sylvisorex ollula* (162189), *Crocidura hildegardeae* (155538), *Myosorex kihaulei* (155622), *Suncus*

madagascariensis (159734), *Suncus murinus* (156339), *Blarina brevicauda* (Field Museum Prep Lab 5145), *Sorex cinereus* (159789), *Sorex palustris* (163073).

APPENDIX 2. PRIMERS

Several published primers were used in this study, including 12S-1 and 12S-2 (Kocher *et al*., 1989), 12S-C (Springer, Hollar & Burk, 1995), and all of the ND2 primers except those given below (Olson *et al.*, 2004). Only new (unpublished) primers are listed here. 12S-G is slightly modified from the synonymous primer reported in Springer *et al*. (1995). Direction of primers: F, forward; R, reverse.

Gene	Primer	Direction	Sequence (5′-3′)
ND2	SF1	F	TAACTTGACAAAAAATTGCCCC
ND2	SR1	R	CGTAATTGTGTTTGGTTAAGGCC
12S	16R1	R	TACAGAACAGGCTCCTCTAG
12S	16R2	R	AACCAGCTATCACCAGGCTCG
12S	AF4	F	CTTAAAGGACTTGGCGGT
12S	AF6	F	AACGTTAGGTCAAGGTGTA
12S	AR3	R	GCTGAAGATGGCGGTATA
12S	AR6	R	TGAAATCTTCTGGGTGTA
12S	G	R	TTTCATCTTTTCCTTYCGGTAC
12S	LOF1	F	AAGGAGGATTTAGYAGTAA

APPENDIX 3. AMPLIFICATION AND SEQUENCING STRATEGY

Amplification and sequencing strategy for each specimen sequenced in this study. Primer pairs used to generate overlapping sequences are listed as Forward/Reverse for each section sequenced. First and last primers listed for each specimen were used to generate initial PCR products. Internal primers were used in sequencing reactions (and, in some cases, re-amplification followed by sequencing; see Olson *et al*., 2004). See Appendix 2 for primer sequences.

ND2

Sylvisorex johnstoni 162197: Met-1/SR1, 3TX/Trp-2T
Sylvisorex megalura 150379: Met-1/SR1, 3TX/Trp-2T
Sylvisorex vulcanorum 144219: Met-1/SR1, SF1/Trp-2
Sylvisorex granti 162249: Met-1/SR1, 3TX/Trp-2T
Sylvisorex vulcanorum 144240: Met-1/SR1, SF1/Trp-2
Sylvisorex ollula 162189: Met-1/SR1, SF1/Trp-2T
Crocidura hildegardeae 155538: Met-1/LOR2, 3TX/Trp-2T
Myosorex geata 155622: Met-1/LOR2, 3TX/Trp-2T
Suncus madagascariensis 159734: Met-1/LOR2, 3TX/Trp-2T
Suncus murinus 156339: Met-1/LOR2, SF1/Trp-2T
Blarina brevicauda PL 5145: Met-1/LOR2, 3TX/Trp-2
Sorex cinereus PL 4230: Met-1/LOR2, 3TX/Trp-2T
Sorex palustris 163073: Met-1/SR1, SF1/Trp-2
Sylvisorex howelli 166868: Met-1/SR1, 3TX/Trp-2. 150013 and 168166: Met-1/SR1, 3TX/Trp-2T. 150023, 158304, and 161243: Met-1/SR1, SF1/Trp-2. All other specimens: Met-1/SR1.

12S

Sylvisorex johnstoni 162197: C/AR3, AF4/G
Sylvisorex lunaris 144229: C/AR3, AF4/AR6
Sylvisorex megalura 150379: C/AR3, AF4/AR6
Sylvisorex granti 162249: C/AR3, AF4/AR6
Sylvisorex vulcanorum 144240: C/16R1, 1/2, LOF1/G
Sylvisorex ollula 162189: C/AR3, AF4/AR6
Crocidura hildegardeae 155538: C/LOR2, 1/2, LOF1/G
Myosorex geata 155622: C/16R1, 1/2, LOF1/G
Suncus madagascariensis 159734: C/AR3, AF4/AR6
Suncus murinus 156339: C/LOR2, 1/2, LOF1/G
Sorex cinereus PL 4230: C/16R1, 1/2, AF6/G
Sylvisorex howelli 150013, 158304, 161243, 166868: C/16R1, 1/2, LOF1/G.
150023: C/AR3, AF4/AR6. 168166: C/AR3, AF4/16R2