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A NEW SPECIES OF ELEPHANT-SHREW (AFROTHERIA: MACROSCELIDEA: *ELEPHANTULUS*) FROM SOUTH AFRICA

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Elephant-shrews (also called sengis, order Macroscelidea) are small-bodied insectivorous mammals with a strictly African distribution. Fifteen species currently are recognized, of which 9 occur in the southern African subregion. On the basis of molecular, cytogenetic, and morphological evidence, *Elephantulus edwardii*, the only strictly South African endemic species, is shown to comprise 2 closely related taxa. The new *Elephantulus* taxon described herein is from the central Nama-Karoo region of Western Cape and Northern Cape Provinces. Important genetic distinctions underpin its delimitation. Sequence data from the mitochondrial cytochrome-*b* gene and the hypervariable control region as well as 7th intron of the nuclear fibrinogen gene show these 2 taxa to be reciprocally monophyletic. They are separated by 13.8% sequence divergence (uncorrected) based on the 2 mitochondrial segments, and 4.2% based on the nuclear intron sequences. In addition, fixed cytogenetic differences include a centromeric shift, heterochromatic differences on autosomal pairs 1–6, and the number of nucleolar organizer regions. The new species has several subtle morphological and phenotypic characters that distinguish it from its sibling species *E. edwardii*, the most striking of which is the presence of a tail-tuft, as well as the color of the flanks and the ventral pelage. The abundance, detailed distribution of the new form, and its life-history characteristics are not known, and further studies clearly are needed to determine its conservation status.

Key words: Afrotheria, cytogenetics, DNA sequencing, morphology, nomenclature, sengi, taxonomy

The order Macroscelidea (elephant-shrews) is nested within Afrotheria, an endemic African clade of mammals that comprises 6 orders whose recognition is based almost exclusively on DNA sequences and other genomic data (Proboscidea [elephants], Sirenia [dugong and manatees], Hyracoidea [hyraxes], Afrosoricida [tenrecs and golden moles], Tubulidentata [aardvark], and Macroscelidea [elephant-shrews]—e.g., Amrine-Madsen et al. 2003; Kriegs et al. 2006; Nikaido et al. 2003; Nishihara et al. 2005, 2006; Robinson et al. 2004; Ruiz-Herrera and Robinson 2007; Springer et al. 1997, 1999; Stanhope et al. 1998a, 1998b; Waters et al. 2007). Fifteen species are recognized within the Macroscelidea, which, with the exception of *Elephantulus rozeti*, have a strict sub-Saharan distribution (Corbet and Hanks 1968). Elephant-shrews (also called sengis) are small-bodied, capable of rapid movement (jumping and running), and insectivorous. They display social monogamy (Rathbun 1979). Two extant subfamilies, the Macroscelidinae and Rhynchocyoninae, are recognized within the order. The Macroscelidinae includes 3 of the 4 currently recognized genera: the monotypic *Macroscelides*, which is a southwestern African gravel-plain specialist, the monotypic *Petrodromus* (with a southern, eastern, and central African forest distribution), and *Elephantulus*, which includes 10 species found throughout a diverse array of habitats (Corbet and Hanks 1968). The 2nd subfamily, Rhynchocyoninae, is represented by 3 extant east and central African forest species within *Rhynchocyon*. This genus includes a new species from the Udzungwa Mountains in Tanzania (Rovero and Rathbun 2006; Rovero et al. 2008).

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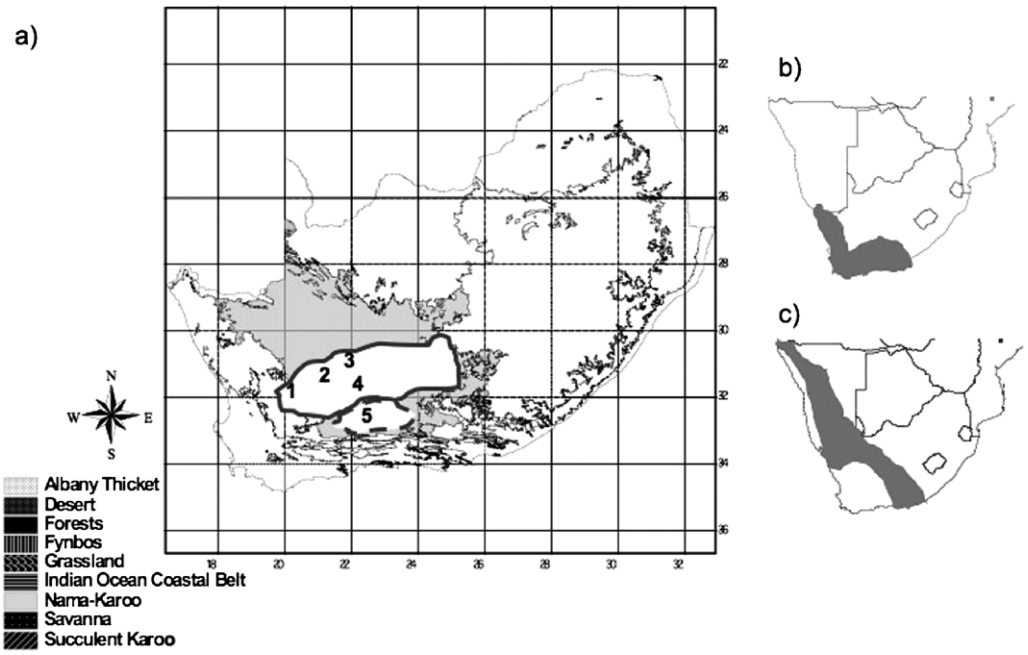

Fig. 1.—a) Map of South Africa showing the various vegetation biomes following Mucina and Rutherford (2006). The collection localities representing the Karoo lineage are indicated: 1) Calvinia, 2) Williston, 3) Carnarvon, 4) Loxton, and 5) Beaufort West. The approximate borders of the Upper Karoo bioregion (solid gray lines) and the Lower Karoo bioregion (dashed gray lines) in the Nama Karoo are provided. Distributions of the 2 species of rock elephant-shrew that overlap in range with the new form of *Elephantulus*, namely b) *E. edwardii* and c) *E. rupestris*, are given on a similar gray-scaled southern African map (ranges taken and redrawn from the Global Mammal Assessment sengi maps—G. Rathbun, pers. comm.).

Information on the number of existing species per biome, region, or continent is important in making informed conservation decisions (Medellín and Soberón 1999). However, this information is incomplete even for supposedly well-known groups of animals such as mammals, where reliable estimates of the number of species remain elusive (Morell 1996). For example, new mammalian species continue to be recognized that include the giant elephant-shrew from East Africa (Rovero et al. 2008); the Laotian rock rat (*Laonastes aenigmamus*), a rodent species from the Khammouan region of Laos (Jenkins et al. 2004); and the African forest elephant (*Loxodonta cyclotis*), previously thought to be a subspecies of the African elephant (*Loxodonta africana*—Roca et al. 2001). The description of a newly reported species should preferably be based on a number of character types including molecular, morphological, and anatomical data. However, these are often cryptic, or have only a few subtle characters that distinguish them from sibling species. In these instances genetics has become a powerful tool in providing the 1st clues in the recognition of new species (e.g., *Laniarius* [shrike]—Smith et al. 1990; *Pneumocystis wakefieldiae* [rat]—Cushion et al. 2004; *Microcebus* [mouse lemur]—Olivieri et al. 2007; *Microgale jobihely* [shrew tenrec]—Goodman et al. 2006; *Spermophilus taurensis* [Taurus ground squirrel]—Gündüz et al. 2007 and *Mormopterus acetabulosus* [bat]—Goodman et al. 2008). This is exemplified by elephant-shrews, where the genetic distinctiveness of a lineage from the central South African Nama-Karoo (Karoo clade) was identified by analysis of mitochondrial sequences (Smit et al. 2007). This novel lineage clustered as the sister to *E. edwardii* within a larger clade that also included *E. myurus* (Smit et al. 2007).

The Karoo clade (herein proposed to represent a previously unrecognized species of elephant-shrew) overlaps in distribution with the Cape rock elephant-shrew (*E. edwardii*; Fig. 1b), the western rock elephant-shrew (*E. rupestris*; Fig. 1c), and the round-eared elephant-shrew (*Macroscelides proboscideus*) in the South African Karoo. All of the southern African species of rock elephant-shrew (including *E. myurus*, which does not occur in this region) are morphologically very similar, but are phenotypically distinct from *M. proboscideus* (Corbet and Hanks 1968).

This study extends the investigation of Smit et al. (2007) through the addition of 10 specimens and provides evidence for the formal recognition of a new elephant-shrew species from South Africa. A multidisciplinary approach is followed that includes sequencing of mitochondrial and nuclear gene segments and comparative cytogenetics. This study assesses several phenotypic characters (principally those of Corbet and Hanks [1968]) for their usefulness in species identification and in so doing, expands the existing macroscelid key (Corbet 1974) to include the morphological identification of the new species described herein, and its delimitation from the phenotypically similar and largely sympatric *E. rupestris* and *E. edwardii*.

Table 1.—Specimens of the new species, *Elephantulus edwardii*, and *E. rupestris* included in the present study. Specimens for which mitochondrial (m), nuclear (n), and cytogenetic (c) data were available are indicated (CAS = California Academy of Sciences; MMK = McGregor Museum; TM = Transvaal Museum; HS = Stellenbosch University).

Species	Locality	Province	Country	Latitude, longitude	No. specimens			Source material
					m	n	c	
Elephantulus new species	Beaufort West	Western Cape	South Africa	32°12′S, 22°19′E	5	3		Museum specimen TM 29496–29498, TM 29528, TM 29529
	Williston	Northern Cape	South Africa	31°06′S, 21°21′E	2			Museum specimen TM 27303, TM 27304
	Carnarvon	Northern Cape	South Africa	30°30′S, 22°06′E	5	4		Museum specimen MMK/M/2167–2171
	Calvinia	Northern Cape	South Africa	31°48′S, 19°49′E	3	3	3	Soft tissue MMK/M/7305–7307
	Loxton	Northern Cape	South Africa	31°36′S, 22°36′E	2			Museum specimen CAS27648, CAS27649
E. edwardii	Melkboom, Namaqua National Park	Northern Cape	South Africa	29°25′S, 17°33′E		1		Soft tissue HS5
	Kamieskroon	Northern Cape	South Africa	30°07′S, 17°34′E	1			Soft tissue HS116
	Clanwilliam	Western Cape	South Africa	32°06′S, 18°29′E	1			Soft tissue HS22
	Cederberg	Western Cape	South Africa	32°14′S, 19°03′E	1		2	Soft tissue HS12, HS14
	Wellington	Western Cape	South Africa	32°48′S, 18°30′E	1	3		Soft tissue HS20, HS81, HS82
	Grabouw	Western Cape	South Africa	33°29′S, 18°24′E	1		1	Soft tissue HS84
	Napier	Western Cape	South Africa	33°29′S, 18°17′E	1	3	3	Soft tissue HS33, HS36, HS38
E. rupestris	Melkboom, Namaqua National Park	Northern Cape	South Africa	29°15′S, 17°33′E	1	2		Soft tissue HS1, HS2
	Paulshoek, Kamieskroon	Northern Cape	South Africa	30°23′S, 18°17′E	1	3		Soft tissue HS114, HS117, HS120
	Windhoek		Namibia	23°48′S, 17°06′E	1			Soft tissue HS63
	Goegap Nature Reserve, Springbok	Northern Cape	South Africa	29°13′S, 17°30′E	2	2		Soft tissue HS137, HS156
	Oudtshoorn	Western Cape	South Africa	33°18′S, 22°12′E	1	1		Soft tissue HS161

Materials and Methods

Sample Collection

Seventeen specimens of the new species were sequenced; these included 3 specimens that were livetrapped in the field, 7 specimens from the Transvaal Museum (TM, South Africa), 5 specimens from the McGregor Museum (MMK, South Africa), and 2 specimens from the California Academy of Sciences (CAS, United States; Table 1). Sequence data from the complete mitochondrial protein-coding cytochrome-*b* (*Cytb*) gene and the 5′ side of the hypervariable control region of the 17 specimens representative of the new species were compared to the 2 species of rock elephant-shrew with which they co-occur, *E. edwardii* and *E. rupestris*. In addition, 360 base pairs (bp) of the 7th intron of the nuclear fibrinogen gene were sequenced for the new species ($n = 10$) as well as representatives of *E. edwardii* ($n = 7$) and *E. rupestris* ($n = 8$). Specimens were collected under permits issued by Northern Cape Nature (permit 0938/05), Cape Nature (permit 373/2003), Department of Economic Affairs, Environment and Tourism, Eastern Cape (permits WRO 43/03WR and WRO 13/03WR), and South African National Parks. Collection protocols were approved by the ethics committee of Stellenbosch University (clearance number 2006B01008). In addition, guidelines on animal use, as approved by the American Society of Mammalogists, were followed (Gannon et al. 2007).

DNA Extraction and Sequencing

DNA was extracted from museum specimens using a commercial kit (DNeasy Tissue Kit; Qiagen, Doncaster, Australia). Total genomic DNA was obtained from fresh tissue collected in the field using a standard Proteinase K digestion followed by a phenol–chloroform extraction (Maniatis et al. 1982). Species-specific primers were designed and used in conjunction with the universal primers of Pääbo and Wilson (1988), Kocher et al. (1989), Irwin et al. (1991), Rosel et al. (1994), and Seddon et al. (2001). To minimize destructive sampling of museum specimens, dried tissue was preferentially taken from within the skull cavity using sterile forceps. Polymerase chain reaction blanks were invariably clean, and reextraction and amplification always produced the same sequences. GenBank blast searches confirmed their status as elephant-shrews.

Polymerase chain reaction amplification followed standard procedures. Amplification was carried out in a GeneAmp PCR 2700 system (Applied Biosystems, Foster City, California) with a thermal profile involving an initial denaturation step of 3 min at 95°C followed by 35 cycles at 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s. Successful amplification of museum tissue required the addition of bovine serum albumin (4 μl of 0.001 g/ml to a 30-μl reaction). Amplicons were electrophoresed in 1% agarose gels. Sequencing cocktails were cleaned using Centrisep spin columns (Princeton Separations,

Table 2.—External and cranial measurements of the new species, *Elephantulus edwardii*, and *E. rupestris*. External measurements for *E. edwardii* and *E. rupestris* are from specimen labels, whereas all cranial measurements were taken by HAS.

	New species						*E. edwardii*					
	Males			Females			Males			Females		
Measurement[a]	$\bar{X} \pm$ SD	*n*	Range	$\bar{X} \pm$ SD	*n*	Range	$\bar{X} \pm$ SD	*n*	Range	$\bar{X} \pm$ SD	*n*	Range
TL (mm)	246 ± 14.2	6	226–266	243 ± 6.7	6	232–252	241 ± 7.8	2	235–246	255 ± 17.2	12	220–280
T (mm)	129 ± 14.6	6	112–151	128 ± 6.1	6	121–135	127 ± 2.8	2	125–129	135 ± 10.7	12	112–147
E (mm)	29 ± 2.1	6	25–31	30 ± 1.2	6	29–32	27 ± 2.1	2	25–28	28 ± 1.9	12	25–30
HF c.u. (mm)	34 ± 1.4	6	32–36	34 ± 0.5	6	34–35	34.5 ± 0.7	2	34–35	36 ± 1.2	12	34–38
Mass (g)	45 ± 4.3	6	40–52	49 ± 6.9	6	38–59	41 ± 7.1	2	36–46	41 ± 8.8	6	31–52
GLS (mm)	34.0 ± 0.7	6	33.2–35.1	34.7± 0.4	6	34.2–35.1	34.0 ± 1.5	4	31.8–34.7	35.2 ± 1.9	18	30.3–37.7
RL (mm)	15.3 ± 0.4	6	14.8–15.7	15.9 ± 1.0	6	14.8–17.3	16.0 ± 0.6	4	15.1–16.5	16.5 ± 1.6	18	12.9–19.0
ZB (mm)	19.0 ± 0.7	6	18.3–20.0	19.3 ± 0.3	6	19.0–19.8	19.5 ± 0.5	4	19.0–20.0	19.5 ± 0.6	18	18.2–20.2
LIB (mm)	7.10 ± 0.4	6	6.5–7.5	7.47 ± 0.3	6	7.0–7.8	7.50 ± 0.5	4	7.1–8.2	7.30 ± 0.4	18	6.8–8.0

[a] TL = total length; T = tail length; E = ear length; HF c.u. = hind-foot length; GLS = greatest length of skull; RL = rostrum length; ZB = zygomatic breadth; LIB = least interorbital breadth.

[b] Mass was excluded from statistical analyses because of limited sample size.

Inc., Adelphia, New Jersey) and the products were analyzed on a 3100 ABI automated sequencer using BigDye chemistry (version 3; Applied Biosystems). Electropherograms of the raw data were checked manually and edited with Sequence Editor software version 1.0.3a (Applied Biosystems). Sequences have been submitted to GenBank under accession numbers DQ901212–DQ901218, DQ901250–DQ901256, and EU076240–EU076283.

Data Analysis

Analyses of the sequence data followed Smit et al. (2007). In short, maximum-likelihood and parsimony analyses were performed in PAUP* (Swofford 2001—1,000 nonparametric bootstrap replicates estimated clade support) together with a Bayesian inference approach (20×10^6 generations) as implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist 2001). The optimal evolutionary models for the various data partitions were determined in Modeltest (Posada and Crandall 2001—GTR+I+G model for the combined mitochondrial DNA and Trn+I model for the nuclear data set).

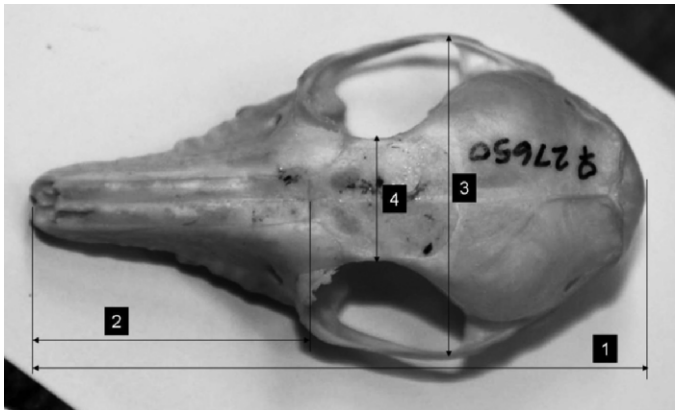

Fig. 2.—Cranial measurements superimposed on the dorsal view of a representative *Elephantulus* skull (*E. edwardii*; CAS 27650). 1) Greatest length of skull (GLS), 2) rostrum length (RL), 3) zygomatic breadth (ZB), and 4) least interorbital breadth (LIB).

Chromosome and Standard Karyotype Preparation

Metaphase chromosome spreads were obtained from fibroblast cultures (*E. edwardii*, $n = 6$; new species, $n = 3$). These were established from tail biopsies and cultivated in tissue culture medium supplemented with 15% fetal calf serum and maintained at 37°C and 5% CO_2. Metaphase chromosomes were harvested following conventional procedures and subjected to G-banding (Seabright 1971), C-banding (Sumner 1990), and silver staining (Goodpasture and Bloom 1975). The chromosomes ($2n = 26$) were numbered in decreasing size and arranged following the format in Robinson et al. (2004). The specimens that were analyzed cytogenetically are listed in Table 1.

Phenotypic Comparison

The morphological distinction between the new species and the elephant-shrew species with which it co-occurs, and that are morphologically very similar to it (*E. edwardii* and *E. rupestris*), was based on an analysis of 17 specimens of the new form and 25 specimens of adults from each of *E. edwardii* and *E. rupestris*; all specimens of *E. edwardii* and *E. rupestris* examined are housed in the mammal collections of the TM with the exception of CAS27650 and CAS27986. The characters examined follow Corbet and Hanks (1968) and include the color of the pelage, dental morphology, a number of standard external body measurements, as well as selected cranial measurements (Table 2; Fig. 2). Cranial measurements of the new species, *E. edwardii*, and *E. rupestris* were taken by HAS; those of CAS27648/9 (new species), CAS27650 (*E. edwardii*), and CAS27986 (*E. rupestris*) were taken by G. Rathbun. External measurements were recorded directly from museum labels with the exception of 3 specimens of the new species (MMK/M/7305/6/7) that were taken by HAS. Adults were defined by the presence of a fully erupted permanent dentition (Skinner and Chimimba 2005). Five of the 17 specimens of the new species were classified as subadults–juveniles and excluded from the metric analyses and qualitative dental comparisons. Measurements were taken with digital calipers.

TABLE 2.—Extended.

	E. rupestris								
	Males			Females			Kruskal–Wallis ANOVA		
Measurement[a]	$\bar{X} \pm$ SD	*n*	Range	$\bar{X} \pm$ SD	*n*	Range	*H*	*n*	*P*
TL (mm)	268 ± 15.8	9	248–297	273 ± 15.9	8	247–288	16.394	43	0.003
T (mm)	143 ± 9.7	9	131–160	140 ± 18.6	8	107–160	8.384	43	0.015
E (mm)	25 ± 2.4	9	21–29	26 ± 2.3	8	24–31	16.060	43	0.003
HF c.u. (mm)	37 ± 2.4	9	33–40	36 ± 1.7	8	35–39	12.382	43	0.002
Mass (g)	64 ± 6.5	6	58–76	59 ± 13.3	8	54–76	—[b]		
GLS (mm)	37.4 ± 1.1	13	36.0–39.5	37.2 ± 1.3	9	35.4–39.7	29.042	56	<0.001
RL (mm)	17.9 ± 1.0	13	16.0–19.9	17.5 ± 1.0	9	16.6–20.1	22.812	56	<0.001
ZB (mm)	20.1 ± 0.6	13	19.2–21.4	20.2 ± 0.5	9	19.2–20.8	15.177	56	<0.001
LIB (mm)	7.70 ± 0.5	13	7.1–8.4	7.60 ± 0.5	9	7.0–8.5	5.476	56	0.064

External measurements included total length (TL), tail length (T), ear length measured from the notch of the ear (E), and hind-foot length from the heel to the end of the longest claw (HF c.u.). Means and ranges are reported separately for sexes.

Four cranial measurements were recorded (see Fig. 2)—these include greatest length of skull (GLS: from the anteriormost point of the premaxilla [rostrum] to the posteriormost point of the skull, that is, posterior point of the occipital bone, along the

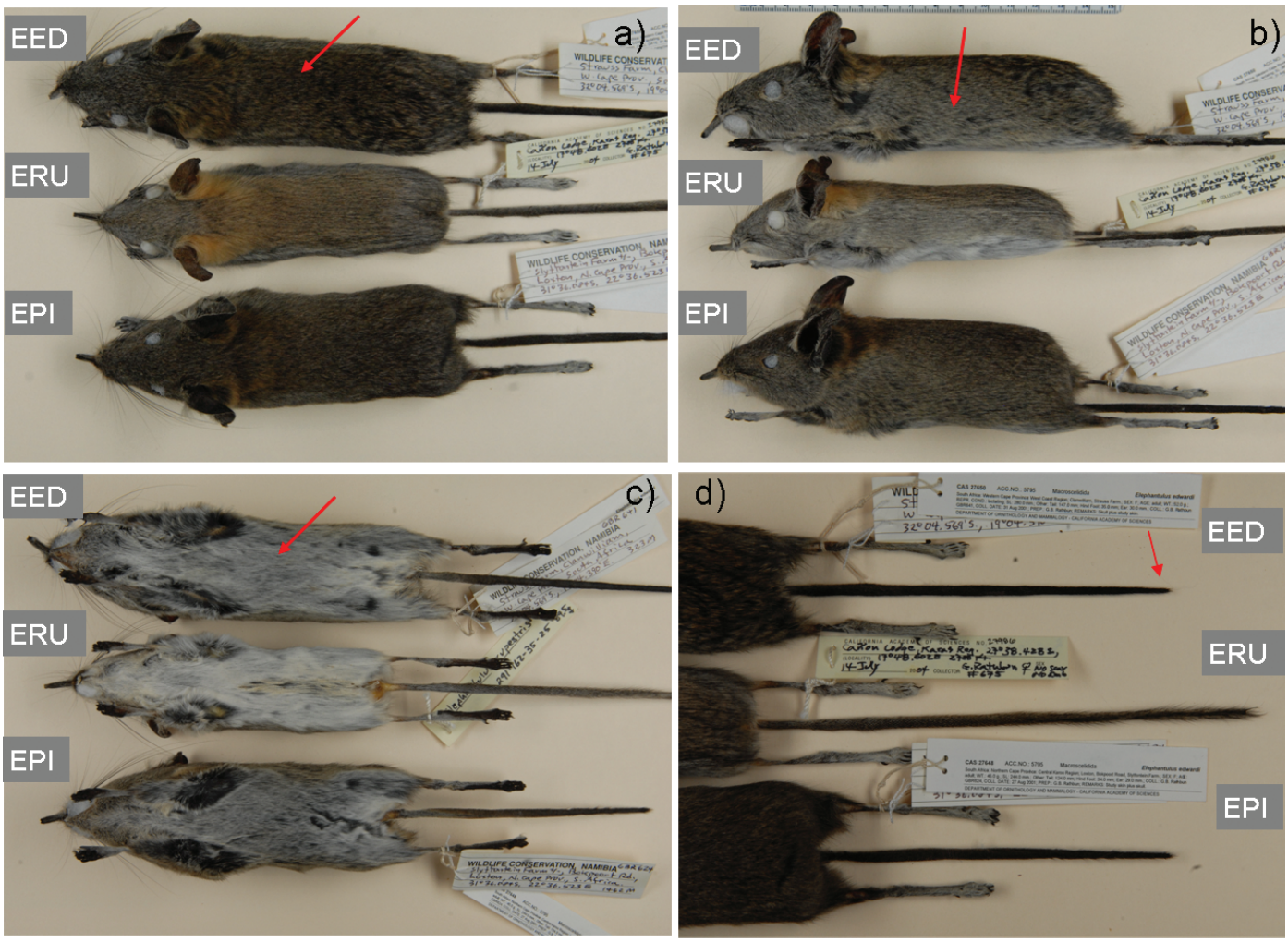

FIG. 3.—Differences in a) dorsal, b) flank, and c) ventral pelage between *Elephantulus edwardii* (EED), *E. rupestris* (ERU), and *E. pilicaudus* (EPI). d) The tail of *E. pilicaudus* is considerably more tufted toward the tip than that of *E. edwardii*, but less so than that of *E. rupestris*. Specimens correspond to CAS27650 (*E. edwardii*), CAS27986 (*E. rupestris*), and CAS27648 (*E. pilicaudus*) and are housed in the California Academy of Sciences (CAS).

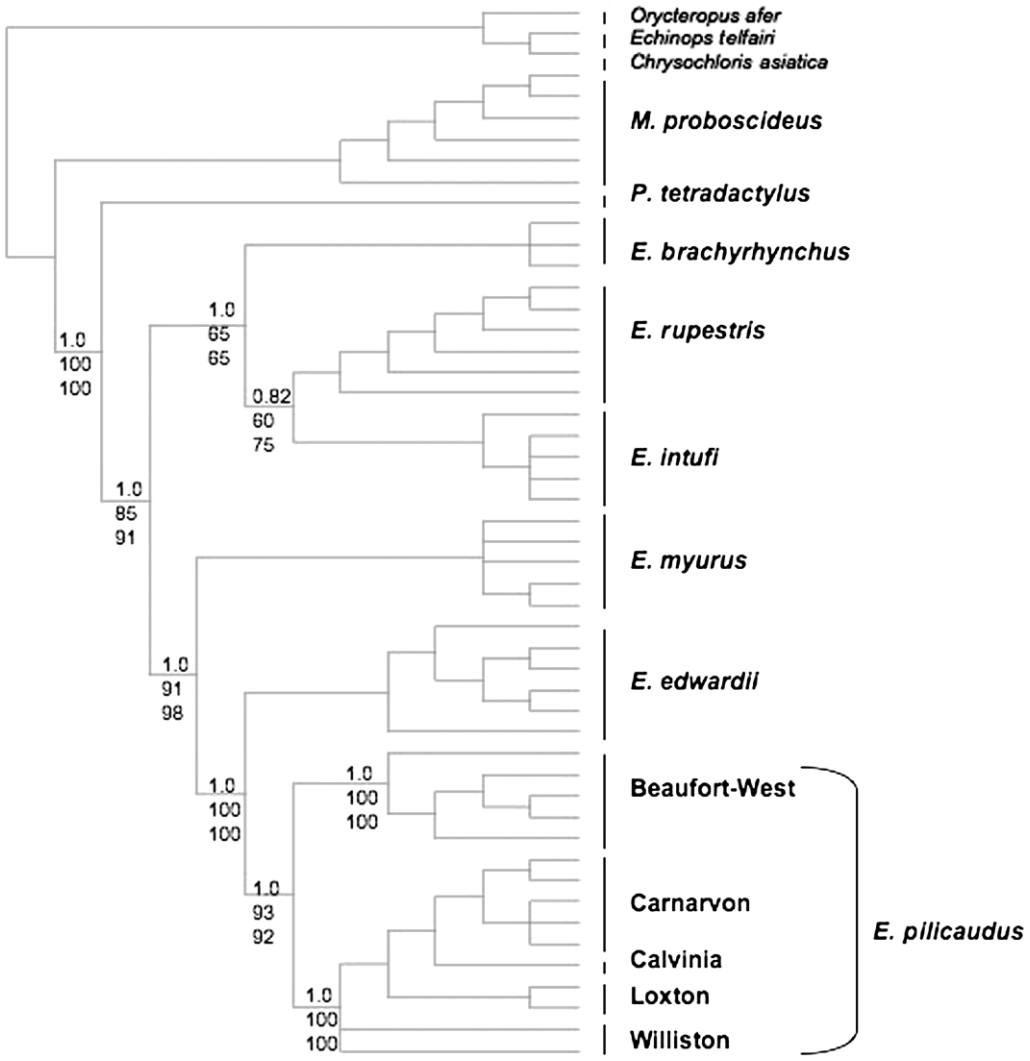

FIG. 4.—Bayesian tree based on the combined sequences from the cytochrome-*b* gene and control region showing the phylogenetic relatedness of *Elephantulus pilicaudus*, *E. edwardii*, and *E. rupestris* (see Table 1 for geographic localities). The tree is rooted on *Macroscelides proboscideus* and other Afroinsectiphillia. Values above the nodes indicate posterior probabilities from a 20 million generation run, and values below the nodes represent nonparametric bootstrap support for maximum parsimony (top) and maximum likelihood (bottom) for 1,000 replicates. The monophyly of each species was supported by a posterior probability of 1.0 and 100% bootstrap support.

TABLE 3.—Uncorrected sequence divergences separating the new species (*Elephantulus pilicaudus*) from *E. edwardii* and *E. rupestris*, the rock elephant-shrew species with which it co-occurs in parts of its range. Values are based on 1,381 bp of mitochondrial and 360 bp of nuclear sequence data. Values in boldface type represent intraspecific genetic variation.

			New species	
	E. rupestris	*E. edwardii*	Beaufort West	Calvinia/Carnarvon/Williston/Loxton
Mitochondrial data				
E. rupestris	**1.10**	22.88	22.17	
E. edwardii		**1.57**	13.80	
E. pilicaudus—Beaufort West			**0.45**	9.84
E. pilicaudus—Calvinia/Carnarvon/Williston/Loxton				**2.58**
Nuclear data				
E. rupestris	**0.2**	17.14	15.45	
E. edwardii		**0.15**	4.19	
E. pilicaudus			**0.01**	

Table 4.—Diploid chromosome numbers (2n) reported for Macroscelidinae species.

Species	2n	Reference
Elephantulus pilicaudus (Karoo rock elephant-shrew)	26	Present study
Elephantulus edwardii (Cape rock elephant-shrew)	26	Tolliver et al. 1989
Elephantulus rupestris (western rock elephant-shrew)	26	Wenhold and Robinson 1987; Tolliver et al. 1989
Elephantulus myurus (eastern rock elephant-shrew)	30	Ford and Hamerton 1956; Tolliver et al. 1989
Elephantulus brachyrhynchus (short-snouted elephant-shrew)	26	Stimson and Goodman 1966; Tolliver et al. 1989
Elephantulus intufi (bushveld elephant-shrew)	26	Tolliver et al. 1989
Elephantulus rozeti (North African elephant-shrew)	28	Matthey 1954
Macroscelides proboscideus (round-eared elephant-shrew)	26	Wenhold and Robinson 1987; Tolliver et al. 1989; Svartman et al. 2004
Petrodromus tetradactylus (four-toed elephant-shrew)	28	Wenhold and Robinson 1987; Tolliver et al. 1989

longitudinal axis of skull), rostrum length (RL: from the anteriormost point of the premaxilla to the anteriormost point of the suture at the border between the nasal and frontal bones), zygomatic breadth (ZB: greatest distance between the outer margins of the zygomatic arches), and least interorbital breadth (LIB: least distance dorsally between the orbits). There was no significant sexual dimorphism within either the new species, *E. edwardii*, or *E. rupestris* as determined by a Mann–Whitney *U*-test, and the sexes were combined for analysis of the external and cranial data (Kruskal–Wallis analysis of variance [ANOVA] and post hoc multiple comparisons of mean ranks for all groups). All statistical analyses were done in Statistica version 8.0 (StatSoft, Tulsa, Oklahoma). The qualitative dental characters were evaluated for their usefulness in distinguishing *E. edwardii* from *E. rupestris*. These include the presence of lingual and labial cusps on P1 and P2 as well as the shape of P2.

Results

Elephantulus pilicaudus Smit, new species

Holotype.—Adult female captured at Vondelingsfontein Farm on 19 September 2006 by HAS. Voucher specimen placed in the McGregor Museum, Kimberley (MMK), South Africa (MMK/M/7305). Fresh DNA sample (heart and liver) stored at Stellenbosch University (HS451).

Paratypes.—TM 27303 (adult male), TM 27304 (subadult female) collected at Goraas Farm (31°06′S, 21°21′E), Williston, Northern Cape Province, South Africa, on 10 February 1977 by I. Rautenbach et al. TM 29496 (adult female), TM 29497 (subadult male), TM 29498 (adult male), TM 29528 (adult male), TM 29529 (subadult male) collected at the Karoo National Park (32°12′S, 22°19′E), Beaufort West, Northern Cape Province, South Africa, on 21–22 January 1979 by I. Rautenbach et al. MMK/M/2167 (male), MMK/M/2168 (male), MMK/M/2169 (male), MMK/M/2170 (female), MMK/M/2171 (female) collected at Carnarvon Commonage (30°30′S, 22°06′E), Carnarvon, Northern Cape Province, South Africa, in 1983 by H. Erasmus. MMK/M/7306 (juvenile male), MMK/M/7307 (juvenile male) collected at Vondelingsfontein Farm, Calvinia (31°48′S, 19°49′E), Northern Cape Province, South Africa, on 19 September 2006 by HAS. CAS27648 (adult female), CAS27649 (adult female) collected at Slytfontein Farm, Loxton (31°36′S, 22°36′E), Northern Cape Province, South Africa, on 27–28 August 2001 by G. Rathbun.

Type Locality.—Vondelingsfontein Farm, Calvinia, Northern Cape Province, South Africa (31°48′S, 19°49′E; 1,449 m above sea level).

Distribution.—*Elephantulus pilicaudus* is confined to rocky habitat with an elevation of ≥1,300 m above sea level. This species is restricted (endemic) to the Upper and Lower Karoo Bioregions of the Nama-Karoo, South Africa (Fig. 1a).

Etymology.—The specific epithet refers to a morphological character (1 of a suite of traits that are collectively diagnostic)—

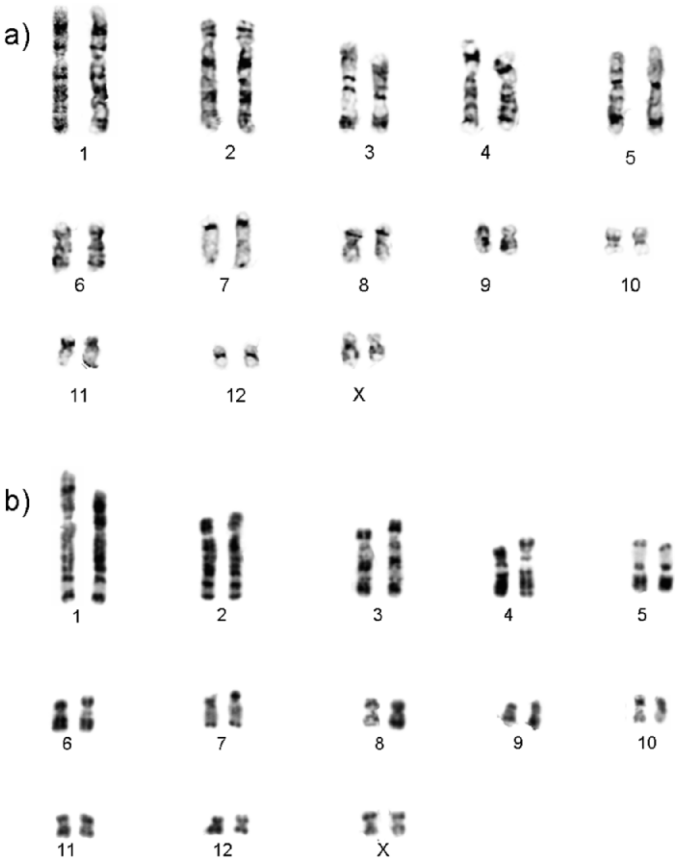

Fig. 5.—G-banded karyotypes of a) *Elephantulus edwardii* and b) *E. pilicaudus*. Chromosomes are ordered according to size and centromere position.

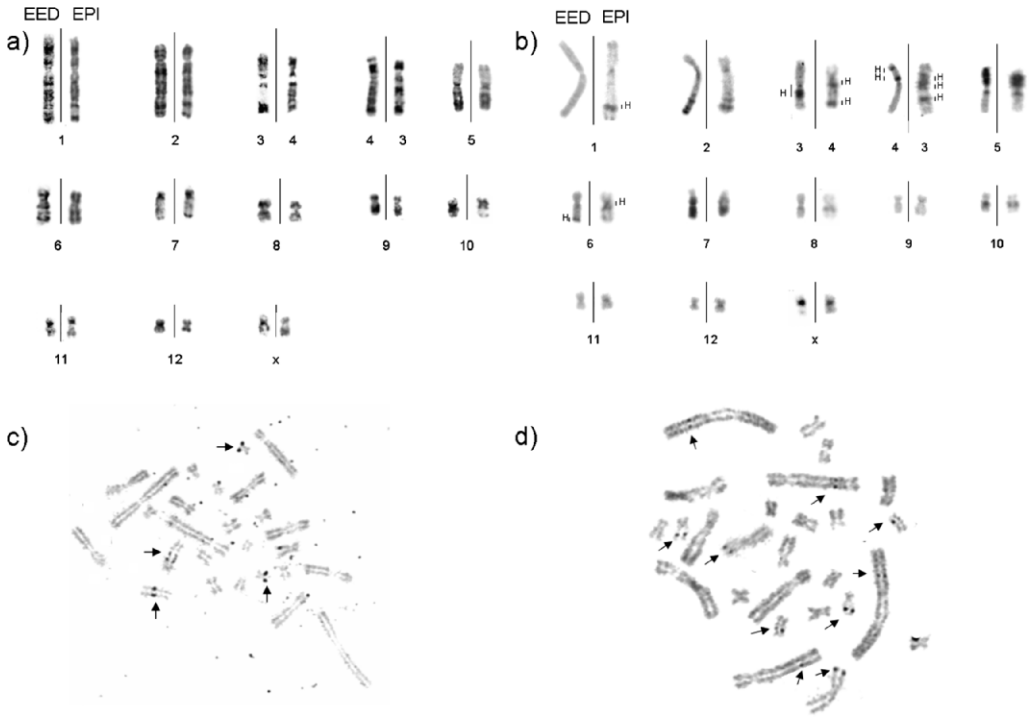

Fig. 6.—a) Half-karyotype G-band comparisons of *Elephantulus edwardii* EED (left) and *E. pilicaudus* EPI (right). b) Half-karyotype C-band comparisons of *E. edwardii* EED (left) and *E. pilicaudus* EPI (right); chromosome identification was done by sequential banding. Both centromeric and interstitial C-bands are evident. Nucleolar organizer regions (NORs) are shown in representative cells of c) *E. edwardii* ($n = 4$) and d) *E. pilicaudus* ($n = 10$).

the tail-tip is considerably more tufted in this species than in *E. edwardii*, its sister species, but less so compared to *E. rupestris*. ''Pili'' = hair and ''caudus'' = tail; gender masculine (see Fig. 3). It is recommended that the English name should be ''Karoo rock elephant-shrew,'' representative of its geographic occurrence in the South African Nama-Karoo.

Description.—The upper parts of the body and forehead are gray-brown tinged yellow and grizzled with blackish brown. This extends to the flanks and contrasts sharply with the gradual change in color evident between the dorsal and flanking regions of *E. edwardii* and *E. rupestris*, the 2 southern African species of rock elephant-shrew with which it shares overlapping ranges (see Figs. 3a and 3b). There is a dorsal diffuse black-brown pencil line along the midline of the proboscis that becomes lighter toward the forehead. The vibrissae are black. Ears are proportionately large, broad at the base with rounded tips. Postauricular region is tawny rufous tinged with pale yellow-brown rather than orange and extends behind the neck; it is less conspicuous than in *E. rupestris* but slightly more so than in *E. edwardii*. The under parts are mottled or blotchy gray. The eye-ring is yellow-cream and more prominent at the bottom, almost broken above to the right with the inner hair of the ear margins being similar in color. The tail is entirely black distally but proximally black above and paler below. The dark-colored hair that covers the tail is more dense toward the tip (<4 mm), where it ends in a definite tuft that is more pronounced than in *E. edwardii* (<4 mm), but less so than in *E. rupestris* (>6 mm) (see Fig. 3d). Total, tail, hind-feet, and ear length as well as body mass of adults are reported in Table 2. Tail length exceeds head-and-body length and is similar to that of *E. edwardii* and *E. rupestris*. The dental formula is i 3/3, c 1/1, p 4/4, m 2/2, total 40.

Comparisons.—A number of distinct characters distinguish *E. pilicaudus* from other elephant-shrew species. These include mitochondrial and nuclear sequence differences, fixed cytogenetic characters, and several subtle morphological features.

Mitochondrial and nuclear evidence.—The monophyly of *E. pilicaudus* is supported by all methods of analysis (Fig. 4) and is consistent with the phylogeny based on mitochondrial and nuclear markers reported by Smit et al. (2007). The sequence divergences separating *E. pilicaudus* from *E. edwardii* and *E. rupestris* are given in Table 3; they are comparable to those distinguishing other well-recognized species within this clade (see Smit et al. 2007). An uncorrected p-distance of 13.8% (calculated from the combined mitochondrial protein-coding *Cytb* gene and the control region sequences) separates *E. pilicaudus* from its sister species, *E. edwardii*. In the case of the 7th intron of the fibrinogen gene, an uncorrected p-distance of 4.2% separates *E. pilicaudus* from *E. edwardii*. There are 2 monophyletic groups within *E. pilicaudus* that correspond to the geographical localities Beaufort West and Carnarvon/Calvinia/Williston/Loxton (see Fig. 1a). These 2 groups are well supported by bootstrap values and posterior probabilities. In addition, a 75-bp insertion is present in the control region (data not included) of all Calvinia/Carnarvon/Williston/Loxton

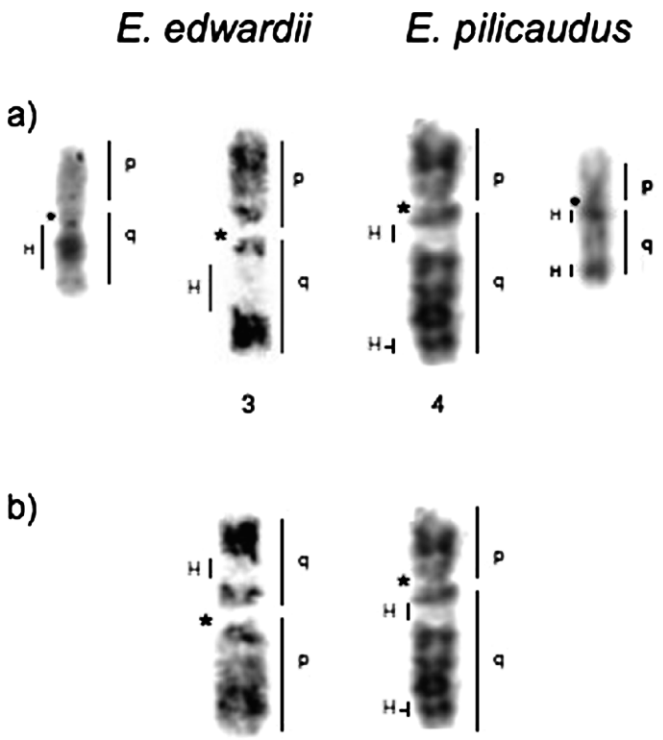

FIG. 7.—a) Side-by-side comparison of the G- and C-banded *Elephantulus pilicaudus* chromosome EPI 4 and its ortholog in *E. edwardii* EED 3. (C-banded chromosomes are presented in a contracted state to the left and right of the G-banded chromosomes of each species.) b) A reconstruction showing that the chromosomes differ through a centromeric shift and heterochromatic amplification in the long arm of EED 3, as well as by the presence of a heterochromatic band near the distal end of EPI 4. In this reconstruction, EED 3 is inverted and the heterochromatic block in the q arm is trimmed to match the size of the corresponding region in EPI 4.

specimens, and this distinguishes the clade from the Beaufort-West lineage.

Cytogenetic evidence.—Elephantulus pilicaudus has a diploid number of 26, identical to that of *E. edwardii* and most other Macroscelidinae (see Table 4). These include *E. rupestris*, *E. brachyrhynchus*, *E. intufi*, and *M. proboscideus* (Robinson et al. 2004; Svartman et al. 2004; Tolliver et al. 1989; Wenhold and Robinson 1987). However, several fixed cytogenetic differences separate *E. pilicaudus* and *E. edwardii*.

The *E. edwardii* (EED) and *E. pilicaudus* (EPI) G-banded karyotypes are shown in Figs. 5a and 5b. The *E. edwardii* karyotype presented herein is identical to that of Robinson et al. (2004—reported as *E. rupestris* [ERU] by these authors but subsequently identified in the present study as *E. edwardii* based on sequence data). A comparison of the G- and C-banded chromosomes of *E. edwardii* and *E. pilicaudus* is shown in Figs. 6a and 6b. The karyotypes of *E. edwardii* and *E. pilicaudus* are largely identical at the level of G-band resolution obtained in these analyses. Differences in the amount of heterochromatin and a centromere shift account for the positional changes in the respective karyotypes (discussed below; see Fig. 7). Silver staining of nucleolar organizer regions (NORs) in *E. edwardii* and *E. pilicaudus* and examination of published data on *E. rupestris* (Wenhold and Robinson 1987) show the presence of 2 pairs of NOR-bearing chromosomes (i.e., 4 NORs in total) in both *E. edwardii* (Fig. 6c) and *E. rupestris*; this contrasts sharply with the 10 NORs (corresponding to 5 autosomal pairs) detected in *E. pilicaudus* (Fig. 6d). Taken collectively these data argue for an absence of gene flow between *E. pilicaudus* and *E. edwardii*. This further underpins their uniqueness based on the sequence data and strengthens the case for their recognition as distinct species.

A comparison of the chromosome EPI 4 of *E. pilicaudus* and its ortholog in *E. edwardii* EED 3 is presented in Fig. 7a. The reconstruction shows that EED 3 and EPI 4 differ by a centromeric shift and heterochromatic amplification in the long arm of EED 3, as well as by the presence of a heterochromatic band near the distal end of EPI 4q (Fig. 7b). It is noteworthy that although EPI 4 appears to be similar in morphology and G-banding pattern to ERU 3 (Wenhold and Robinson 1987), the latter does not show the same C-bands as either *E. edwardii* or *E. pilicaudus*.

Phenotypic characteristics.—Although *E. pilicaudus* is phenotypically very similar to *E. edwardii* and *E. rupestris*, a suite of subtle features (no single diagnostic trait) supports its recognition as a distinct species (see Fig. 3 and Table 5). The most reliable of these are presented below. The descriptions of *E. edwardii* and *E. rupestris* follow Cobet and Hanks (1968).

1) The dorsal pelage is similar in *E. pilicaudus* and *E. edwardii*, being darker grayish brown tinged yellow and grizzled with blackish brown (rather than reddish brown), but is paler grayish brown in *E. rupestris* (Fig. 3a). The inconspicuous tawny rufous (tinged with yellow-brown) patches behind the ears in both *E. pilicaudus* and *E. edwardii* contrast sharply with the prominent orange-buff patches of *E. rupestris* (Fig. 3a). The dorsal coloring extends to the flanks in *E. pilicaudus* as opposed to the presence of a gradual change from dorsal pelage (gray-brown) to the flanks (entirely gray) in both *E. edwardii* and *E. rupestris* (Fig. 3b). The ventral pelage is distinctly different in all 3 species appearing mottled or blotched yellow-gray in *E. pilicaudus*, gray in *E. edwardii*, and white (less gray) in *E. rupestris* (Fig. 3c).
2) The tail-tuft, a characteristic that separates *E. pilicaudus* from both *E. edwardii* and *E. rupestris*, is noticeably more dense (<4 mm) in *E. pilicaudus* than in *E. edwardii* (<4 mm), but less so than in *E. rupestris* (>6 mm; Fig. 3d); there is no consistent difference in tail color between *E. pilicaudus*, *E. edwardii*, or *E. rupestris*; the tail is black above and tends to be paler on the ventral surface toward the base, but is completely black distally in all 3 species. Tail length exceeds head-and-body length in *E. pilicaudus*, *E. edwardii*, and *E. rupestris*, but more so in *E. rupestris*.
3) The light buffy color above the mouth at the base of the nose and posterior to the angle of the mouth and dorsal on the cheek in *E. pilicaudus* appears absent in both *E. edwardii* and *E. rupestris*.
4) The eye-ring (broken to the right above) in *E. pilicaudus* is

TABLE 5.—Morphological differences distinguishing the new species (*Elephantulus pilicaudus*) from *E. edwardii* and *E. rupestris* (taken from Corbet and Hanks [1968]; also for illustrations of cranial and dental features).

	E. pilicaudus	*E. edwardii*	*E. rupestris*
Tail	Black above; pale below at base but distal half black all around; tufted toward tip; considerably more tufted toward tip than *E. edwardii* but less than *E. rupestris* (<4 mm)	Black above; pale below at base but distal half black all around; tufted toward tip (<4 mm)	Black above; slightly lighter on the under surface toward the base; elongated brush at tip (>6 mm)
Dorsal pelage	Gray-brown, tinged yellowish and grizzled with blackish brown; extending to flanks	Gray-brown, tinged yellowish and grizzled with blackish brown; sharply separated from gray flanks	Gray-brown, although paler (grayer) than in new species and *E. edwardii*, becoming almost pure gray on flanks
Flank color	Similar to dorsal pelage	Gray	Gray
Ventral pelage	Appears mottled or blotched yellow-gray	Appears gray	Appears white (less gray)
Buffy patches behind ears	Tawny rufous/yellow-brown hair patch; less conspicuous than in *E. rupestris* (but slightly more so than in *E. edwardii*)	Tawny rufous/yellow-brown hair patch; less conspicuous than in *E. rupestris*	Rufous/yellow-orange hair patch extending to neck—prominent
Cheek color	Light buff	Absent (appears gray)	Absent (appears gray)
Ears	Proportionally large; broad at base with rounded tips; supratragus and tragus slightly developed	Proportionally large; broad at base with rounded tips; supratragus and tragus slightly developed	Proportionally large; more pointed tips than *E. edwardii* and new species; supratragus and tragus not developed
Eye-ring	Broken to the right above (not consistent); prominent at bottom; yellow-cream	Solid; white-gray	Distinct; broken (above and below); white
Suture between premaxilla and maxilla	Straight	Straight	Sinuous
Skull	Swollen ectotympanic; less-inflated entotympanic bullae	Swollen ectotympanic; less-inflated entotympanic bullae	Ectotympanic not inflated; inflated entotympanic bullae
P1	Lacking lingual cusp; reduction of all but 1 principal cusp	Lacking lingual cusp; reduction of all but 1 principal cusp	With lingual cusp
P1 and P2	Well-developed anterior but poorly developed posterior labial cusps	Well-developed anterior but poorly developed posterior labial cusps	Anterior and posterior well developed
P2	Sectorial	Sectorial	Molariform
P2 lingual cusp	Single lingual cusp present or absent	Single lingual cusp present or absent	Two lingual cusps present

yellow-cream compared to the solid whitish gray eye-ring of *E. edwardii* and the white eye-ring of *E. rupestris*; however, it should be noted that the shape of the eye-ring was not always consistent between specimens within the 3 species.

5) The ears of all 3 species are proportionately large; the ears are rounded at the tip in *E. pilicaudus* and *E. edwardii* but more pointed in *E. rupestris*; although the supratragus and tragus are slightly developed in both *E. pilicaudus* and *E. edwardii*, these characters are absent in *E. rupestris* (see Corbet and Hanks [1968] for an illustration of the tragus and supratragus in *E. edwardii*).

No phenotypic distinctions could be made between specimens of the 2 monophyletic lineages detected within *E. pilicaudus* (the Beaufort West and the Calvinia/Carnarvon/Williston groups; see above). External body and cranial measurements are reported in Table 2. There are no statistically supported differences in external measurements between *E. pilicaudus* and *E. edwardii*. However, *E. rupestris* is larger than both *E. pilicaudus* and *E. edwardii* in overall size as measured by total length (TL; $P < 0.001$; $P = 0.042$, respectively), greatest length of skull (GLS; $P < 0.001$; $P < 0.001$), rostrum length (RL; $P < 0.001$; $P = 0.010$), and zygomatic breadth (ZB; $P < 0.001$; $P = 0.018$; see Table 2). *E. rupestris* is similarly significantly different from *E. pilicaudus* in tail length (T; $P = 0.012$), ear length (E; $P < 0.001$), and hind-foot length (HF c.u.; $P = 0.002$). Least interorbital breadth (LIB) was not significantly different among the 3 species based on a Kruskal–Wallis ANOVA ($P = 0.065$; see Table 2). Mass was excluded from the statistical analysis because of limited sample size.

The adult dental formula i 3/3, c 1/1, p 4/4, m 2/2, total 40 is identical in *E. pilicaudus*, *E. edwardii*, and *E. rupestris* (and most other Macroscelidinae). A set of qualitative dental characteristics clearly separates *E. edwardii* and *E. pilicaudus* from *E. rupestris*. These characters include the absence of lingual cusps on P1 in *E. edwardii* and *E. pilicaudus*, and their presence in *E. rupestris*; anterior labial cusps well developed but posterior cusps poorly so in P1 and P2 of *E. edwardii* and *E. pilicaudus*, whereas both anterior and posterior labial cusps are well developed in *E. rupestris*; and P2 is sectorial in *E. edwardii* and *E. pilicaudus* but with variable lingual cusps that contrast with a molariform upper P2 and the 2 lingual cusps present in *E. rupestris* (see Table 5; for *E. edwardii* and *E. rupestris*, see Corbet and Hanks [1968] and Skinner and Chimimba [2005]; see Corbet and Hanks [1968] for dental illustrations). Root

characteristics of the lower p1 were not examined in the present study because verification would have resulted in damage to the skulls preserved in museum collections.

Key to the Species of Elephantulus

The key is taken from Corbet (1974) and expanded to include data presented above.

1. Pectoral gland present, a naked or short-haired patch in center of thorax 2
   Pectoral gland absent 4
2. Prominent brown mark behind eye; 2 lower molars, that is, 10 lower teeth 3
   No brown mark behind eye; 3 lower molars . . . . . *E. fuscipes*
3. Hair of tail becoming long toward the tip, forming a brush; tail about 120% of head and body; I2 equal in size to I1 and I3 . . . . . *E. revoili*
   Hair of tail not forming a brush; tail about equal to head and body; I2 smaller than I1 . . . . . *E. rufescens*
4. Tail shorter than head and body; 3 lower molars, that is, 11 lower teeth . . . . . *E. brachyrhynchus*
   Tail not shorter than head and body; 2 lower molars 5
5. P1 with a lingual cusp; P2 molariform, with 2 well-developed lingual cups; ventral pelage superficially white 6
   P1 lacking a lingual cusp; P2 sectorial with or without small lingual cusps; ventral pelage showing gray, except in the northern African 7
6. Size larger; upper toothrow > 18.7 mm; tail about 115% of head and body, distinctly tufted toward the tip, predominantly black above; white eye-ring narrow, broken above and below the eye; P2 and P3 with 3 cusps, arranged in a triangle, behind the principal cusp . . . . . *E. rupestris*
   Size smaller; upper toothrow < 18.7 mm; tail about 106% of head and body, not distinctly tufted, speckled above; white eye-ring conspicuous and unbroken; P2 and P3 with only 2 cusps, arranged transversely, behind the principal cusp . . . . . *E. intufi*
7. Ectotympanic parts of bullae inflated to same level as entotympanic parts; I2 equal to I1 and I3 (southern Africa) 8
   Ectotympanic parts of bullae much less inflated that entotympanic parts; I2 larger than I1 and I3 (northern Africa) . . . . . *E. rozeti*
8. P2 with 1, occasionally 2, lingual cusps; supratragus small and fairly thick; premaxillary suture slightly sinuous; tail bicolored throughout its length, yellow-brown above, entirely short-haired . . . . . *E. myurus*
   P2 without a lingual cusp; supratragus large and thin; premaxillary suture straight; tail black above, distal half black all round and slightly tufted 9
9. Tail less tufted at tip (hairs < 4 mm); dorsal pelage (gray-brown tinged with yellow) separated from gray flanks; ventral pelage pure gray . . . . . *E. edwardii*
   Tail considerably more tufted toward tip (hairs < 4 mm); dorsal pelage (gray-brown tinged with yellow) extends to flanks; ventral pelage mottled or blotched yellow-gray . . . . . *E. pilicaudus*

Notes on Conservation Status

Information on the conservation status of the species is lacking. Importantly, despite numerous field excursions in the region only 17 specimens of the new species (3 livetrapped by HAS, 2 trapped by Dr. Galen Rathbun, and 12 museum specimens) have been collected from the Nama-Karoo. This is taken to indicate that *E. pilicaudus* is regionally limited, and rarely encountered. Concerted efforts should be made to assess its relative abundance and to determine potential threats to its habitat.

Discussion

The assignment of the monophyletic Karoo clade to either of the available species names within *E. edwardii* (*E. capensis* or *E. karoensis*—both names had previously been synonomized within *E.* edwardii—Corbet and Hanks 1968; Meester et al. 1986) was conclusively ruled out by Smit et al. (2007). DNA sequencing of the type specimen of *E. capensis* (TM 2312, GenBank DQ901249—Roberts 1924:62) placed this specimen firmly within *E. edwardii*, whereas sequence from the type specimen of *E. karoensis* (TM 688, GenBank DQ901238—Roberts 1938:234) was found to cluster within *E. rupestris* (Smit et al. 2007).

In this paper, compelling evidence is provided for the recognition of a new *Elephantulus* species, *E. pilicaudus*. The description is based on analysis of mitochondrial and nuclear DNA sequences, and comparative cytogenetic data. An identification scheme is provided that distinguishes *E. pilicaudus* from other species of rock elephant-shrews with which it co-occurs. The recognition of *E. pilicaudus* increases the number of species within *Elephantulus* (subfamily Macroscelidinae) to 11. The southern African rock elephant-shrews are consequently considered to include *E. pilicaudus*, *E. edwardii*, *E. rupestris*, and *E. myurus*. Of these, *E. pilicaudus* and *E. edwardii* are endemic to South Africa, further underscoring the region′s rich elephant-shrew biodiversity. Seven of the 15 extant species (and 3 of the 4 genera) occur within its borders. The new species is regionally limited to the Nama Karoo, which borders on 2 biodiversity hotspots, the Succulent Karoo to the west, and the Cape Floristic Kingdom to the south (Low and Rebelo 1996). This vegetation biome is subdivided into Bushmanland and the Upper and Lower Karoo Bioregion vegetational units (Mucina and Rutherford 2006; see Fig. 1a). It is noteworthy that specimens that group within the Calvinia/Carnarvon/Williston/Loxton clade are referable to the Upper Karoo Bioregion, whereas specimens with the Beaufort West genetic profile all occur in the Lower Karoo Bioregion.

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Literature Cited

Amrine-Madsen, H., K. P. Koepfli, R. K. Wayne, and M. S. Springer. 2003. A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. Molecular Phylogenetics and Evolution 28:225–240.

Corbet, G. B. 1974. Family Macroscelididae. An identification manual, part 1.5. Pp. 1–6 in The mammals of Africa (J. Meester and H. W. Setzer, eds.). Smithsonian Institution Press, Washington, D.C.

Corbet, G. B., and J. Hanks. 1968. A revision of the elephant-shrews, family Macroscelididae. Bulletin of the British Museum (Natural History), Zoology Series 16:47–111.

Cushion, M. T., S. P. Keeley, and J. R. Stringer. 2004. Molecular and phenotypic description of *Pneumocystis wakefieldiae* sp. nov., a new species in rats. Mycologia 96:429–438.

Ford, C. E., and J. L. Hamerton. 1956. Chromosomes of five rodent species. Nature 177:140–141.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Goodman, S. M., B. Jansen Van Vuuren, F. Ratrimomanarivo, J.-M. Probst, and R. C. K. Bowie 2008. Specific status of populations in the Mascarene Islands referred to *Mormopterus acetabulosus* (Chiroptera: Molossidae), with description of a new species. Journal of Mammalogy 89:1316–1327.

Goodman, S. M., C. J. Raxworthy, C. P. Maminirnua, and L. E. Olson. 2006. A new species of shrew tenrec (*Microgale jobihely*) from northern Madagascar. Journal of Zoology (London) 270:384–398.

Goodpasture, C., and S. E. Bloom. 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma 53:37–50.

Gündüz, I., M. Jaarola, C. Tez, C. Yeniyurt, P. D. Polly, and J. B. Searle. 2007. Multigenic and morphometric differentiation of ground squirrels (*Spermophilus*, Scuirdiae, Rodentia) in Turkey, with a description of a new species. Molecular Phylogenetics and Evolution 43:916–935.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

Jenkins, P. D., C. W. Kilpatrick, M. F. Robinson, and R. J. Timmins. 2004. Morphological and molecular investigations of a new family, genus and species of rodent (Mammalia: Rodentia: Hystricognatha) from Lao PDR. Systematics and Biodiversity 2:419–454.

Kocher, T. D., et al. 1989. Dynamics of mitochondrial DNA evolution in animals. Amplification with conserved primers. Proceedings of the National Academy of Sciences of the USA 86:6196–6200.

Kriegs, J. O., G. Churakov, M. Kiefmann, U. Jordan, J. Brosius, and J. Schmitz. 2006. Retroposed elements as archives for the evolutionary history of placental mammals. PLoS Biology 4:e91.

Low, A. B., and A. G. Rebelo. 1996. Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs and Tourism, Pretoria, South Africa.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning. Cold Spring Harbor Press, Cold Spring Harbor, New York.

Matthey, R. 1954. Les chromosomes de *Macroscelides rozeti* (Duverroy) (mammalia—Insectivora). Existe-t-il une série polyploide chez les Macroscelididae. Revue Suisse de Zoologie 61:669–677.

Medellín, R. A., and J. Soberón. 1999. Predictions of mammal diversity on four land masses. Conservation Biology 13:143–149.

Meester, J. A. J., I. L. Rautenbach, N. J. Dippenaar, and C. M. Baker. 1986. Classification of southern African mammals. Transvaal Museum Monograph 5:314.

Morell, V. 1996. New mammals discovered by biology's new explorers. Science 273:1491.

Mucina, L., and M. C. Rutherford. 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19. South African National Biodiversity Institute, Pretoria, South Africa.

Nikaido, M., H. Nishihara, Y. Hukumoto, and N. Okada. 2003. Ancient SINEs from African endemic mammals. Molecular Biology and Evolution 20:522–527.

Nishihara, H., M. Hasegawa, and N. Okada. 2006. Pegasoferae, an unexpected mammalian clade revealed by tracking ancient retroposon insertions. Proceedings of the National Academy of Sciences 103:9929–9934.

Nishihara, H., Y. Satta, M. Nikaido, J. G. M. Thewissen, M. J. Stanhope, and N. Okada. 2005. A retroposon analysis of afrotherian phylogeny. Molecular Biology and Evolution 22:1823–1833.

Olivieri, G., et al. 2007. The ever-increasing diversity in mouse lemurs: three new species in north and northwestern Madagascar. Molecular Phylogenetics and Evolution 43:309–327.

Pääbo, S., and A. C. Wilson. 1988. Polymerase chain reaction reveals cloning artefacts. Nature 334:387–388.

Posada, D., and K. A. Crandall. 2001. Intraspecific phylogenetics: trees grafting into networks. Trends in Ecology and Evolution 16:37–45.

Rathbun, G. B. 1979. The social structure and ecology of elephant-shrews. Zeitschrift für Tierpsychologie 20:3–76.

Roberts, A. 1924. *Elephantulus capensis*. Annals of the Transvaal Museum 10:62.

Roberts, A. 1938. *Elephantulus karoensis*. Annals of the Transvaal Museum 19:234.

Robinson, T. J., B. Fu, M. A. Ferguson-Smith, and F. Yang. 2004. Cross-species chromosome painting in the golden mole and elephant shrew: support for the mammalian clades Afrotheria and Afroinsectiphillia but not Afroinsectivora. Proceedings of the Royal Society of London, B. Biological Sciences 271:1477–1484.

Roca, A. L., N. Georgiadis, J. Pecon-Slattery, and S. J. O'Brien. 2001. Genetic evidence for two species of elephant in Africa. Science 293:1473–1477.

Rosel, P. E., A. E. Dizon, and J. E. Heyning. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). Marine Biology 119:159–167.

Rovero, F., and G. B. Rathbun. 2006. A potentially new giant sengi (elephant-shrew) from the Udzungwa Mountains, Tanzania. Journal of East African Natural History 95:111–115.

Rovero, F., et al. 2008. A new species of giant sengi or elephant-shrew (genus *Rhynchocyon*) highlights the exceptional biodiversity of the Udzungwa Mountains of Tanzania. Journal of Zoology (London) 247:126–133.

Ruiz-Herrera, A., and T. J. Robinson. 2007. Chromosomal instability in Afrotheria: fragile sites, evolutionary breakpoints and phylogenetic inference from genome sequence assemblies. BMC Evolutionary Biology 7:199.

Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet 2:971–972.

Seddon, J. M., F. Santucci, N. J. Reeve, and G. M. Hewitt. 2001. DNA footprints of European hedgehogs, *Erinaceus europaeus* and

E. concolor: Pleistocene refugia, postglacial expansion and colonization routes. Molecular Ecology 10:2187–2198.

SKINNER, J. D., AND C. T. CHIMIMBA. 2005. The mammals of the southern African subregion. 3rd ed. Cambridge University Press, Cambridge, United Kingdom.

SMIT, H. A., T. J. ROBINSON, AND B. JANSEN VAN VUUREN. 2007. Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidea). Molecular Ecology 16:2680–2692.

SMITH, E. F. G., P. ARCTANDER, J. FJELDSA, AND O. G. AMIR. 1990. A new species of shrike (Laniidae: *Laniarius*) from Somalia, verified by DNA sequence data from the only known individual. Ibis 133:227–235.

SPRINGER, M. S., H. M. AMRINE, A. BURK, AND M. J. STANHOPE. 1999. Additional support for Afrotheria and Paenungulata, the performance of mitochondrial versus nuclear genes, and the impact of data partitions with heterogeneous base composition. Systematic Biology 48:65–75.

SPRINGER, M. S., ET AL. 1997. Endemic African mammals shake the phylogenetic tree. Nature 388:61–64.

STANHOPE, M. J., O. MADSEN, V. G. WADDELL, G. C. CLEVEN, W. W. DE JONG, AND M. S. SPRINGER. 1998a. Highly congruent molecular support for a diverse superordinal clade of endemic African mammals. Molecular Phylogenetics and Evolution 9:501–508.

STANHOPE, M. J., ET AL. 1998b. Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. Proceedings of the National Academy of Sciences of the USA 95:9967–9972.

STIMSON, C., AND M. GOODMAN. 1966. Chromosomes of the elephant shrew *Nasilio brachyrhynchus brachyrhynchus*. Mammalian Chromosome Newsletter 22:188.

SUMNER, A. T. 1990. Chromosome banding. Unwin Hyman, London, United Kingdom.

SVARTMAN, M., G. STONE, J. E. PAGE, AND R. STANYON. 2004. A chromosome painting test of the basal eutherian karyotype. Chromosome Research 12:45–53.

SWOFFORD, D. L. 2001. PAUP*: phylogenetic analysis using parsimony (*and other methods), beta version 4.0b2. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

TOLLIVER, D. K., L. W. ROBBINS, I. L. RAUTENBACH, D. A. SCHLITTER, AND C. G. COETZEE. 1989. Biochemical systematics of elephant shrews from southern Africa. Biochemical Systematics and Ecology 17:345–355.

WATERS, P. D., G. DOBIGNY, P. J. WADDELL, AND T. J. ROBINSON. 2007. Evolutionary history of line-1 in the major clades of placental mammals. PLoS ONE 2:e158.

WENHOLD, B. A., AND T. J. ROBINSON. 1987. Comparative karyology of three species of elephant-shrew (Insectivora: Macroscelididae). Zeitschrift für Säugetierkunde 52:1–9.

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