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Short Communication

Meroles revisited: complementary systematic inference from additional mitochondrial genes and complete taxon sampling of southern Africa's desert lizards

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1. Introduction

The desert lizards (*Meroles*: Lacertidae) form a small clade of ground-dwelling taxa inhabiting arid southwest Africa (Arnold, 1991). All seven species have some portion of their range in the Namib Desert, and four of the seven are Namib endemics. Their close geographic ties to the Namib are hypothesized to reflect an interesting pattern of speciation, one in which the lineage infiltrated progressively extreme desert habitats through successive rounds of adaptation (Arnold, 1981, 1990, 1991). Ecological competition plays a key role in Arnold's (1981) speciation model, establishing a sequence of displacement, subsequent adaptation, and species formation. In this iterative process, the lineage's inchoate species came to occupy increasingly xeric environments, culminating in successful entry to aeolian dunes of the Namib's vast "sand seas."

A phylogenetic tree reflecting this iterative mode of speciation should yield a pectinate topology, and, indeed, Arnold (1991) generated just such a tree for *Meroles*, one fully resolved with little character conflict, in a parsimony analysis of morphological data. To test Arnold's phylogenetic estimate and address independently its progression of morphological adaptation, Harris et al. (1998) conducted a molecular phylogenetic analysis of the desert lizards, using sequence data from the mitochondrial 12S and 16S ribosomal genes. Their molecular phylogeny corroborated the monophyly of *Meroles*, with its inclusion of the formerly monotypic *Aporosaura anchietae* [transferred to *Meroles* by Arnold (1991) and supported independently by albumin evolution (Mayer and Benyr, 1994)]. However, relationships within *Meroles* were less well supported by the mitochondrial data: maximum parsimony produced an unresolved consensus tree, and the maximum likelihood tree had very short internal branches. Moreover, one of the seven species, the Namib endemic *Meroles micropholidotus*, was not available for analysis.

Here we revisit the molecular systematics of desert lizards, offering complete taxon sampling and additional sequence data from two protein-coding mitochondrial genes. We combine these data with extended 12S and 16S rDNA gene sequences to generate a fully resolved phylogeny, complementing Harris et al.'s (1998) mitochondrial estimate of relationships within *Meroles*.

2. Materials and methods

Tissue samples were processed at field collection sites and preserved in saturated salt–DMSO buffer or 95% ethanol. With the exception of *M. micropholidotus*, we sequenced least two specimens of each species. Two species from the closely related lacertid genus *Pedioplanis* (*Pedioplanis gaerdesi* and *Pedioplanis inornata*) served as outgroups. Sampled taxa, together with collection localities and museum voucher numbers, are compiled in Appendix A.

Genomic DNA was extracted from liver, muscle, or blood using Qiagen's QIAamp DNA Mini kit. Regions from two protein-coding mitochondrial genes, cytochrome *b* (cyt*b*; ≅400 bp) and subunit 2 of NADH dehydrogenase (ND2; ≅500 bp), were amplified using the respective primer sets LGL 765 (Bickham et al.,

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1995) + H15149 (Meyer et al., 1990), and L 4437 + H4995 (Macey et al., 1997). We also sequenced both mitochondrial ribosomal genes, 12S (≅350 bp) and 16S (≅450 bp), using the primers of Bickham et al. (1996), which generated fragments slightly longer than those reported in Harris et al.'s (1998) survey. Gene fragments were amplified for 32 cycles involving 45 s denaturation at 92 °C, 35 s annealing at 54 °C (cyt*b*, 12S, 16S) or 60 °C (ND2), and 1 min extension at 72 °C. Amplification products, purified over Centri-sep columns, served as templates in cycle-sequencing reactions employing dye-labeled terminators (Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems), followed by electrophoresis in an ABI 377 automated DNA sequencer. Forward and reverse sequences were generated for each sample and their complementarity confirmed using ABI Sequence Navigator software.

Sequences were initially aligned using the CLUSTAL X program, applying default settings (Thompson et al., 1997). Given the indel variation commonly observed in rDNA sequences, we examined 12S and 16S alignments in greater detail, exploring gap placements for a series of gap opening (= 5, 10, 15, and 20) and extension costs (= 0.10 and 5.0) with the Multiple Alignment Parameters option in CLUSTAL X. Regions of these ribosomal RNA sequences whose nucleotide position homologies varied across different gap parameters were considered alignment-ambiguous. The regions so identified, largely coincident with the questionable regions reported by Harris et al. (1998), were excluded from phylogenetic analysis.

Phylogenetic inference was based on maximum parsimony (MP) and maximum likelihood (ML) analyses implemented in PAUP* 4.0 (Swofford, 1999). To assess combinability of the four gene data sets, we performed the incongruence length difference (ILD) test of Farris et al. (1994). ILD analysis involved 1000 replicates using the partition homogeneity test in PAUP*, excluding parsimony-uninformative characters from data sets prior to analysis. Results of the ILD test showed no significant conflict among data sets ($P = 0.490$), which were combined for analysis (but see Darlu and Lecointre, 2002).

We conducted an exhaustive search for the MP analysis, in which all characters were weighted equally and remaining gaps were treated as missing data. Prior to ML analysis, we used the MODELTEST program (ver. 3.06; Posada and Crandall, 1998) to identify the substitution model most appropriate for the combined data. We used the program's default settings to generate a neighbor-joining tree as a test tree and, subsequently, to compare substitution models ranging from simple (Jukes-Cantor) to increasingly parameter rich (general time-reversible). ML analysis entailed heuristic searches with 10 replicates of random stepwise addition with tree bisection reconnection branch rearrangement. Bootstrap analyses involving 1000 (MP) and 500 (ML) pseudoreplicates were conducted to estimate confidence limits for topological patterns revealed by these two procedures.

3. Results and discussion

Upon exclusion of ambiguously aligned regions from 12S and 16S sequences (50 nucleotides), the combined mtDNA data set comprised 1588 nucleotides, of which 528 were variable and 386 were parsimony informative. Cyt*b* and ND2 sequences contributed 73% of the informative sites, greatly bolstering the phylogenetic information originally provided by the two ribosomal genes. Patterns of variation for the four genes are summarized in Table 1, listed together with GenBank accession numbers. Levels of sequence divergence within species of *Meroles* were quite low (e.g., 0–2%, cyt*b*) with the exception of *Meroles anchietae*. Divergence levels of 7.0% (ND2) and 7.9% (cyt*b*) place the genetic structure detected between northern versus southern sand sea populations of *M. anchietae* in accord with observed allozymic variation (Nei distance >0.25; Gordon and Griffin (1989)). We also detected a 3-bp deletion in the ND2 gene in *M. anchietae*. Additional sequencing confirmed this deletion for all seven specimens in our possession, representing localities from both sand seas.

The exhaustive search recovered a single most-parsimonious tree (Fig. 1) consistent with the MP consensus tree reported by Harris et al. (1998). However, improved resolution over their consensus tree reveals patterns of relationship within *Meroles* unrecoverable by parsimony analysis of the 12S/16S data alone. For example, *M. anchietae* is not only placed firmly in *Meroles* but clearly falls within a strongly supported clade composed of *Meroles ctenodactylus*, *Meroles cuneirostris*, and *Meroles micropholidotus*. This clade is subtended by *Meroles reticulatus*, which, in turn, is subtended by *Meroles suborbitalis*. *Meroles knoxii* assumes a basal position in the tree. All nodes received high bootstrap support (Fig. 1), exceeding values of 90%, with one exception (79%).

The MODELTEST search identified the general time reversible substitution model (GTR, Rodríguez et al., 1990) with some sites assumed to be invariable (I) and variable sites assumed to follow a gamma distribution (Γ) as the best fit for the combined data. The ML tree derived from the MODELTEST parameter estimates is nearly identical to that of the MP tree, displaying comparable bootstrap support for the patterns of relationship observed for respective parsimony clades (Fig. 2).

Our mtDNA sequence data produced a phylogenetic estimate for *Meroles* that is essentially the same as (for ML, perfectly congruent with) the phylogeny based on morphology (Arnold, 1991). MP and ML trees display a

Table 1
Sequence variation, sequence divergence (% pairwise distances), and GenBank accession numbers for the mitochondrial genes used in the systematic survey of *Meroles*

Data partition	cyt*b*	ND2	12S	16S
No. of nucleotides	370	461	349[a]	404[a]
No. of variable sites	134	216	83	91
No. of informative sites	106	154	64	59
No. of informative sites First codon position	19	41	–	–
No. of informative sites Second codon position	4	15	–	–
No informative sites Third codon position	83	98	–	–
% Pairwise distances[b] (HKY85)	13.2–20.9	15.9–28.8	6.0–11.1	4.1–8.5
GenBank Accession Nos.	AY184400–AY184409	AY184390–AY184399	AY192426–AY192435	AY192416–AY192425

[a] No. of nucleotides after excluding ambiguous indel regions from the alignment.
[b] Interspecific distances within *Meroles*.

pectinate topology consistent with the successive rounds of ecological displacement, adaptation and species formation outlined in Arnold's (1981) model of speciation. In Arnold's (1991) view, the *Meroles* clade "shows steady progression from relatively firm substrates into very stringent habitats based on loose aeolian sand" and that this habitat shift "appears to have elicited many novel morphological features that are necessary for the survival in the extreme environments that the group has entered."

Meroles knoxii, the demonstrated sister taxon to other *Meroles* in both morphological and molecular analyses, is an ecological generalist (Branch, 1998; Mayer and Richer, 1990). Conversely, 17 derived be-

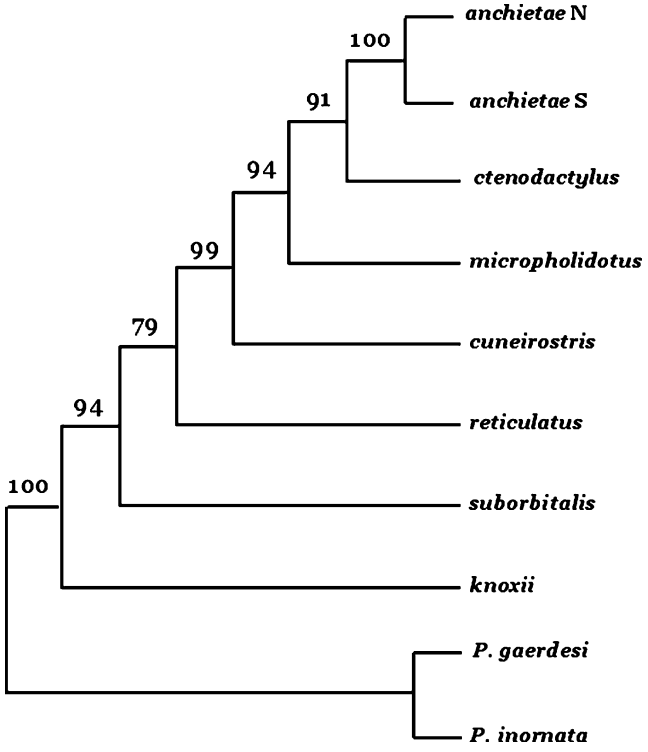

Fig. 1. Single most-parsimonious tree (TL = 1078, CI = 0.66, RI = 0.56) recovered from the combined mtDNA dataset; numbers at nodes are bootstrap proportions. *anchietae* N represents the Namib's northern sand sea and *anchietae* S the southern sand sea.

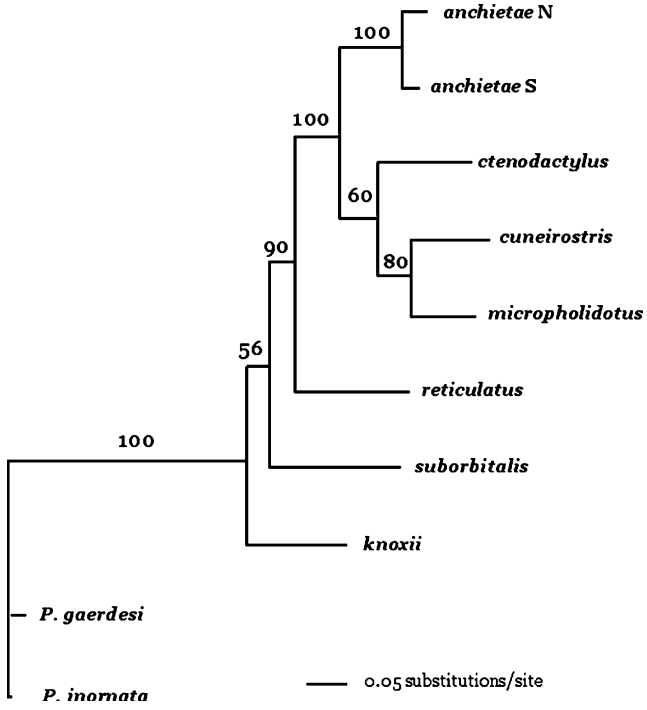

Fig. 2. Phylogram recovered by ML analysis for the combined mtDNA dataset using a GTR + I + Γ model of nucleotide substitution; $-\ln L = 6705.2$. Numbers at nodes are bootstrap proportions (>50%). *anchietae* N represents the Namib's northern sand sea and *anchietae* S the southern sand sea.

havioral and morphological characters associated with functioning in loose sand define the clade comprising *M. ctenodactylus*, *M. cuneirostris*, and *M. micropholidotus*, collectively recognized as the subgenus *Saurites* (Boulenger, 1921). Further, Boulenger (1921) considered the ultraspammophile *M. anchietae* (formerly *Aporosaura*) to be closely related to *Saurites*. Parallel evolution of these derived traits, though less tenable, merits consideration given the convergence (e.g., modified digits, countersunk lower jaw, and tympanic shields) often observed among dune-dwelling lizards (Arnold, 1995). To this end, phylogenetic inference from molecular data can be used to disclose functional convergence (or parallelism). Our fully resolved mtDNA phylogeny provides such an independent source. The congruence between mitochondrial and morphological data offers a robust phylogenetic hypothesis for *Meroles* and lends strong support to Arnold's (1990, 1991) speciation model for the desert lizards of the Namib.

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Appendix A

Tissue sample voucher specimens

Collection acronyms: AMB, Aaron M. Bauer field series; CAS, California Academy of Sciences; LSUMZ, Louisiana State University; PEM, Port Elizabeth Museum; and SMW, National Museum of Namibia. Acronyms in brackets indicate ultimate repository for specimens not yet accessioned into public museum collections.

Meroles anchietae. AMB 5912, CAS 206946 (south bank of Kuiseb River near Rooibank Rd., Walvis Bay District, Erongo Region, Namibia); CAS 214584–214585 (Rooibank, Walvis Bay District, Erongo Region, Namibia—23°11′21″S, 14°38′30″E); three tissue samples, no voucher specimens (21 km North Terrace Bay on Rd. D2302, Khorixas District, Kunene Region, Namibia).

Meroles ctenodactylus. LSUMZ 57357 (Lekkersing Rd., 37.1 km South Alexander Bay-Sendelingsdrif Rd., Northern Cape Province, South Africa—28°42′55″S, 16°59′38″E); CAS 206816 (McDougall's Bay, Northern Cape Province, South Africa—28°17′45″S, 16°52′50″E).

Meroles cuneirostris. AMB 4318 [PEM] (Sendelingsdrif Rd., 14 km from Alexander Bay, Northern Cape, South Africa—28°32′49″S, 16°35′18″E); AMB 5906 [SMW] (North bank of Kuiseb River at Rooibank Rd., Walvis Bay District, Erongo Region, Namibia—23°10′53″S, 14°38′47″E).

Meroles knoxii. CAS 200006 (Port Nolloth dump, Northern Cape Province, South Africa—29°15′27″S, 16°54′38″E); CAS 207029 (South of Kleinsee, deBeers Mining Farmlands near Farm Brazil, Melkbospunt, Northern Cape Province, South Africa—29°49′16″S, 17°04′48″E).

Meroles micropholidotus. ABN 01, Chemosystematic Laboratory Naturhistoriches Museum Wien (Lüderitz, Diamantsperrgebiet II, Lüderitz District, Karas Region, Namibia—26°31′S, 15°09′E).

Meroles reticulatus. AMB 5921, AMB 5925 [SMW] (Hentiesbaai Rd., 11.3 km South Cape Cross, Swakopmund District, Erongo Region, Namibia—21°49′54″S, 14°04′13″E).

Meroles suborbitalis. CAS 201868 (Farm Wittputs, Karasburg District, Karas Region, Namibia—28°34′51″S, 17°59′27″E); CAS 214504 (Farm Ballater, Northern Cape, South Africa—26°52′20″S, 21°11′10″E).

Pedioplanis gaerdesi. CAS 214746 (Purros Rd., 29 km West of Sesfontein, Opuwo District, Kunene Region, Namibia—19°03′01″S, 13°29′26″E).

Pedioplanis inornata. CAS 214787 (South of Karibib, junction of Rd. D 1914 and Rd. D 1952, Karibib District, Erongo Region, Namibia—22°16′13″S, 15°34′29″E).

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