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An Updated mtDNA Phylogeny for *Sauromalus* and Implications for the Evolution of Gigantism

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Populations of the large, herbivorous chuckwalla lizard (*Sauromalus*) differ substantially with regard to body size throughout their range. Among populations of *S. obesus*, body size varies from approximately 180 g to 380 g, or 175 mm to 218 mm UDL (upper decile snout-vent length). Size differences are partly determined by vegetation density, which is closely linked to winter rainfall (Case 1976). On islands in the Sea of Cortés, two endemic species attain substantially larger body sizes: *S. hispidus* on Ángel de La Guarda island (App fig. 8.1; locations 1 and 2) reaches about 1400g, 219 mm UDL, while *S. varius* on San Esteban reaches 1900 g, 301 mm UDL (Case 1982).

A central question is whether the gigantic species diverged early from the rest of the genus, as proposed by Shaw (1945). This would imply that the gigantic chuckwallas may have simply retained large body size from ancestors shared with other large-bodied iguanine genera (*Iguana, Conolophus, Amblyrhynchus, Cyclura, Ctenosaura*). Alternatively, large body size may have evolved recently in response to the island environment (Case 1982).

Earlier, we tested Shaw's (1945) hypothesis by reconstructing a phylogeny of *Sauromalus* using mtDNA (cyt. *b*) sequence variation (Petren and Case 1997). Results showed that the island gigantics diverged from other *Sauromalus* on the nearby Baja Peninsula *after* most of the other populations across the entire range were established. Furthermore, the two gigantic species were firmly supported to be sister taxa, an unexpected result given the extensive phenotypic divergence between them. The resulting phylogenetic topology supported the conclusion that body size had undergone two major changes: the ancestor of all chuckwallas became substantially smaller than the closest iguanine relative (*Iguana*), and the ancestor of the gigantics evolved large size again after becoming isolated on a deepwater island.

Our sampling of populations has been extended further to reveal the patterns of genetic differentiation in *Sauromalus*. Humans have historically moved gigantic forms to other islands to provide a source of food (see Nabhen, Chap 15; Murphy, Chap 8). For instance, *S. hispidus* has been introduced to islands near the Baja peninsula (App fig. 8.1, locations 3-4). Sampling was extended beyond historically human-occupied regions to rule out recent gene flow. Populations on the southern islands of San Francisco and Espíritu Santo (formerly referred to as subspecies *S. ater ater*) extend our sampling to the southern edge of the genus' range.

To reconstruct a molecular phylogenetic hypothesis, a total of 902 bp of the cytochrome *b* gene was sequenced (Petren and Case 1997). *Iguana iguana* sequence was used as the outgroup, as it is most closely related to *Sauromalus*. The mean Jukes-Cantor distance to all *Sauromalus* cytochrome *b* haplotypes is 13.3% for *Iguana*, 15.0% for *Ctenosaura* and 15.5% for *Conolophus*; Petren and Case 1997). Using different outgroups (e.g *Ctenosaura* or *Conolophus*) does not change our results. Unweighted maximum parsimony, neighbor-joining and quartet puzzling maximum likelihood were used to reconstruct trees (Swofford 1999, Strimmer and von Haeseler

1996). All methods agree with respect to the divisions of the four major clades, as well as the branching order among them (App fig. 8.2). The resulting topology is very similar to the previous one (Petren and Case 1997) and does not alter our original conclusions regarding body size evolution in *Sauromalus*. The northern peninsular populations cluster with other nearby populations, while the southern island populations cluster as expected with the southern peninsular and island populations. Four main clades are well supported by bootstrap analysis: The northern/eastern, the southern, the north Baja Peninsular and the island gigantics. Roughly, these same geographic divisions for mainland forms were also found by Hollingsworth (1998, his fig. 46) based on morphological characters, although his phylogeny is very different (also see Murphy, Chap 8 and app 8.2).

Within the northern clade, the more isolated populations from the eastern side of the Sea of Cortés form a well-supported clade corresponding to their classification as *S. o. townsendi*. In contrast, the more northern subspecies *S. o. tumidus* is not clearly distinct from *S. o. obesus*. The widely distributed *S. o. obesus* shows large genetic distances among populations, reaching an extreme with the genetically distinct northern and southern Baja California clades. The result is that based on cytochrome *b*, *S. obesus* is a paraphyletic taxon with regard to other *Sauromalus* species.

The distinct Northern Baja California clade suggests that there may have been two distinct vicariant boundaries in the peninsular region. However among *Sauromalus,* genetic distance correlates strongly with geographic distance (Mantel test; $r = 0.77$; permuted $P < 0.005$, approximated $P < 0.0001$). Thus, caution must be used in interpreting molecular genetic discontinuities in regions that have not been extensively sampled. Nevertheless, the possible existence of a genetic discontinuity in the Northern peninsular region agrees with patterns found in other taxa (see Murphy, Chap 8).

The pattern of isolation by distance is evident even at reduced spatial scales, as nearby populations contain substantially different haplotypes (Lamb et al. 1992, Petren and Case 1997). Previous taxonomic classifications of species and subspecies based on phenotype are reflected in the molecular data primarily where taxa are isolated geographically, as in the eastern and southern clades. Mitochondrial DNA is maternally inherited, thus female migration and gene flow appear to be limited. We hypothesize that this may be a consequence of the strong preference of mainland chuckwallas for rocky outcrops, and their reduced tendency to cross sandy arroyos, where they may be vulnerable to predators. This island-like population structure reduces gene flow and enhances divergence rates, both from drift and selection. Similar color pattern "classes" on the mainland have discontinuous ranges (Hollingsworth 1998).

Based on the phylogenetic topology, we previously concluded that gigantism evolved in response to the island environment. The timing of events supports this interpretation. The island of Ángel de la Guarda is between 1 to 3 my old, and was apparently formed by block-fault movement of a large landmass away from the Baja peninsula (Gastil *et al*. 1983, Carreño, Chap 2). Mitochondrial DNA sequence of the endemic *S. hispidus* from this island is approximately 2% divergent from chuckwallas found in the region from where the island originated. This is in line (or if anything, less divergent) with the widely used estimated rate of mtDNA mutation of 2% per million years. The much greater sequence divergence among mainland forms (up to 7%) strongly suggests that these lineages were established before the island gigantic species arose.

An alternative interpretation is that all mainland and island populations were once large-bodied, and all of the mainland forms evolved smaller size (Shaw 1945, Hollingsworth 1998). This is unlikely because of the large number of independent evolutionary events that must be invoked: each mainland lineage must have responded dramatically and in parallel to some selective force. The selective factors responsible would have had to change dramatically and in concert less than one million years ago across the entire range of the genus on the mainland.

The selective factors involved in the evolution of large size in *S. hispidus* and *S. varius*, are reviewed in Chap 9. We have proposed a model (Petren and Case 1997) whereby predation pressure from a diverse suite of mainland predators has acted to constrain the body size of mainland chuckwallas soon after their divergence from *Iguana* relatives. Large size may also be favored because of variation in the food supply (Case 1979). Both islands experience long periods of drought that can last many years. Larger lizards can store more energy during periods of greater resource availability, and therefore are able to withstand longer periods without adequate resources. However, Case (Chap 9) finds that the largest individuals on Ángel suffer disproportionately higher mortality rates during drought years than smaller and presumably younger individuals.

Iguanines show substantial variation not only in body size, but also in sexual dimorphism and behavioral traits such as territoriality. For this reason they have been characterized as a model system for the study of the evolution of these traits (Burghard and Rand 1982, Carothers 1994). The gigantic chuckwallas have relaxed territorial tendencies and show reduced sexual dimorphism in body size when compared to mainland forms. The selective factors shaping these traits may also be linked to predation, and food availability. Manipulative field experiments would be of great value for understanding the interplay of the factors affecting body size in *Sauromalus*.

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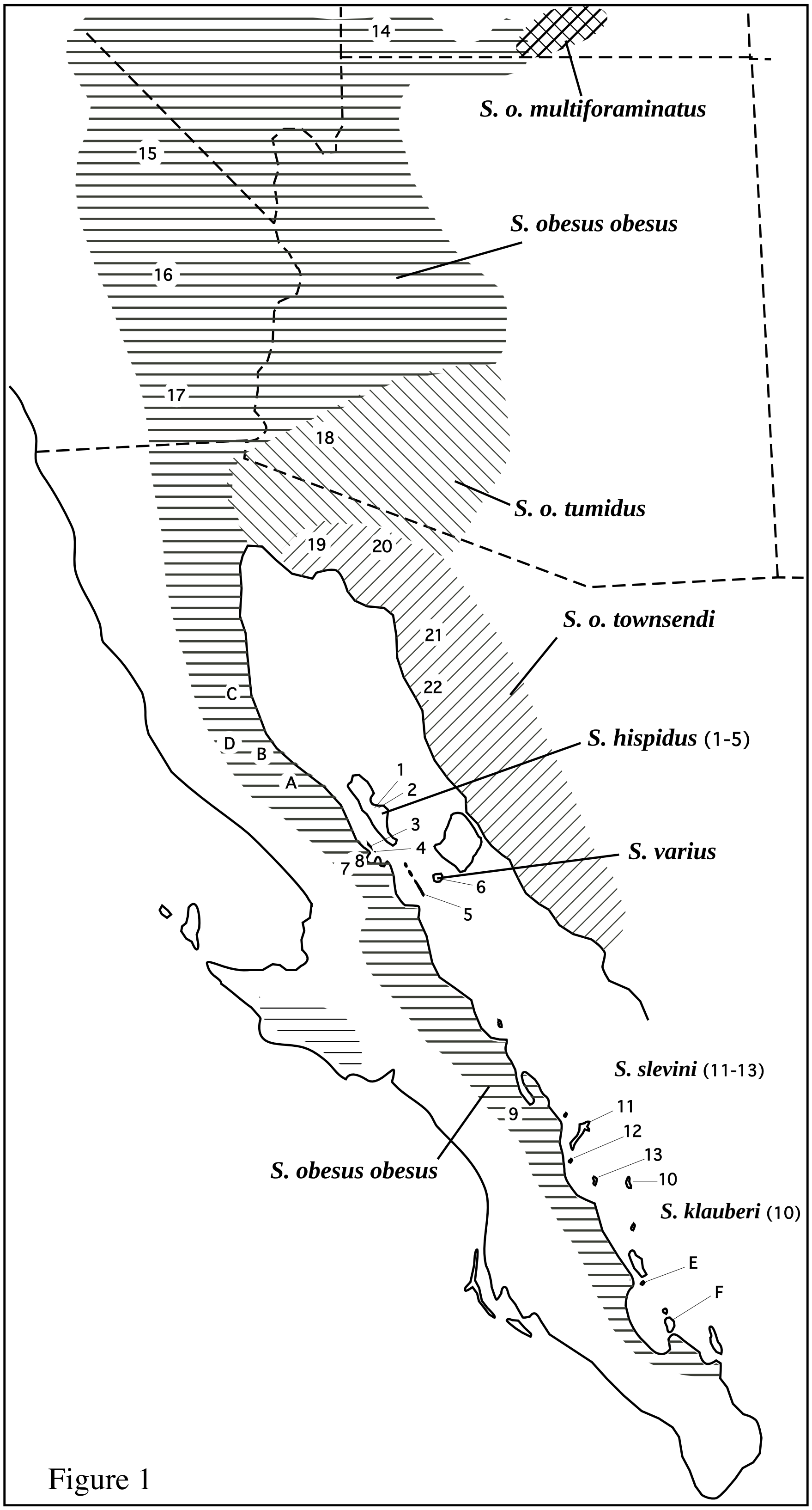
Figure 1. Distribution of *Sauromalus* species and geographic locations of sampled populations (Shaw 1945, Petren and Case 1997). Numbered locations correspond to Petren and Case (1997). Six individuals from new populations were added to previous analyses. Individuals *A-D* were sampled from the Northern Baja California Peninsula, while *E* and *F* were taken from individuals on the islands of San Francisco and Espiritu Santo, respectively. Note the well-supported *S. slevini* clade on the islands of Carmen (11), Danzante (12), and Monserrate (13). Hollingsworth (1998), based on morphological characters, does not include Danzante in this group, but rather within *S. obesus* (*ater*).

Figure 2. A phylogeny of *Sauromalus* based on Petren and Case (1997) and newly sampled populations (*A-F*). A total of 902 bp of the cytochrome *b* gene was sequenced. *Iguana iguana* was used as the outgroup. One of sixteen most parsimonious trees is shown, with branch lengths proportional to inferred numbers of base changes. The percentage representations in 1000 bootstrap pseudoreplicates is given for maximum parsimony (above node) and neighbor-joining (below node). Discrepancies among the 16 maximum parsimony trees, the neighbor joining tree and the quartet puzzling tree are confined to rearrangements within the southern and northern/eastern clades. The composition of the four main clades and their relative arrangement was consistent among methods. These relationships remain unchanged from the original analysis, except for the addition of new taxa. Sequences are deposited in Genbank under accession numbers AF020223-AF020259.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S. o. multiforaminatus
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S. obesus obesus
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S. o. tumidus
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S. o. townsendi
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C
S. hispidus (1-5)
D
B
A
1
2
3
4
8
7
S. varius
6
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S. slevini (11-13)
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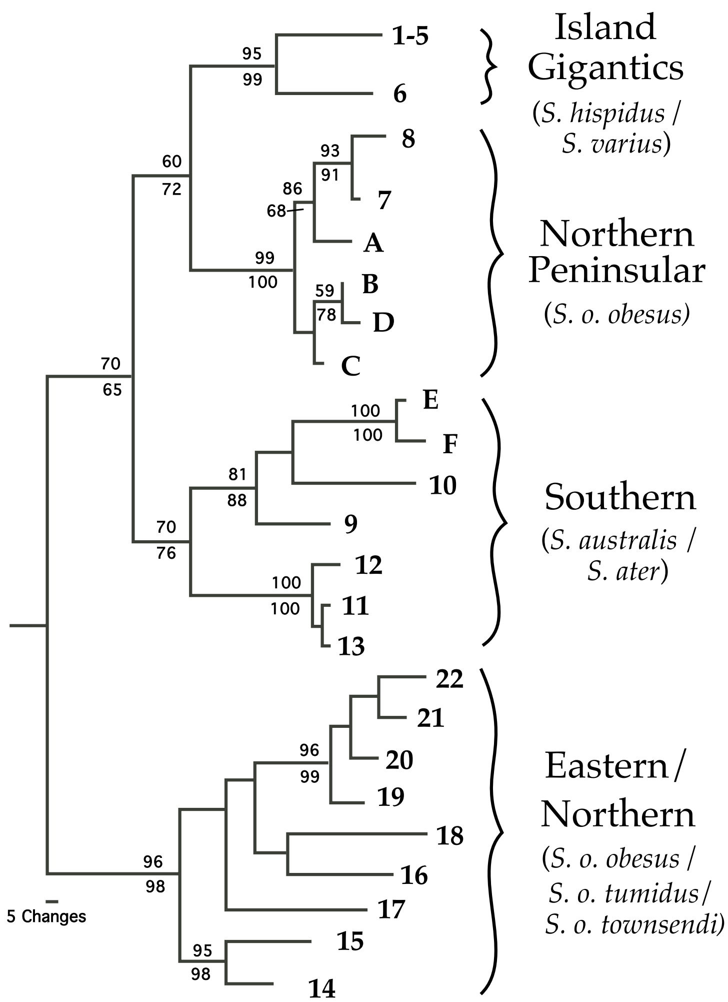
S. obesus obesus
S. klauberi (10)
E
F
Figure 1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5 Changes
Island
Gigantics
(S. hispidus /
S. varius)
Northern
Peninsular
(S. o. obesus)
Southern
(S. australis /
S. ater)
Eastern/
Northern
(S. o. obesus /
S. o. tumidus/
S. o. townsendi)

Figure 2