

Defining the Desert Tortoise(s): Our First Priority for a Coherent Conservation Strategy

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ABSTRACT. – Many populations of tortoises within the *Gopherus agassizii* – *G. berlandieri* complex could be designated as species, subspecies, Distinct Population Segments (DPSs), Evolutionarily Significant Units (ESUs), or Management Units (MUs). However, the appropriate designations for populations remain incompletely resolved. Ambiguities regarding the phylogenetic relationships and taxonomic status of desert tortoises impede precise and efficient legal protection, and compromise extrapolations from the studies of one population to another. Herein, we (1) identify the impediments to constructing a phylogenetic taxonomy and both genetic and ecological determinations of conservation units, (2) examine the consequences of delaying such resolution, (3) summarize the current data base available for systematic studies, (4) compare taxonomic solutions in other terrestrial chelonians, and (5) suggest remedies. A standardized program of sampling that includes all major populations across the entire range of both *G. agassizii* and *G. berlandieri* is proposed to complete the identification of populations and their assignments to mtDNA-based ESU and MU categories. Populations in potential contact zones should be sampled more extensively to determine the absence or extent of gene flow among different ESUs to resolve the identity of species. Finally, morphological, ecological, behavioral, and physiological distinctions among populations would establish a complete and broadly based array of DPSs.

KEY WORDS. – Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; *Gopherus berlandieri*; phylogenetic systematics; morphology; genetics; haplotypes; distinct population segments; evolutionarily significant units; management units; conservation; Nevada; California; Utah; Arizona; USA; Mexico

This issue of *Chelonian Conservation and Biology* is co-sponsored by the Desert Tortoise Council to celebrate more than 25 years of annual Desert Tortoise Council symposia. Two years ago, scientists and students conducting research on the extant gopherine tortoises were invited to submit papers for this special focused issue on the species of *Gopherus*. We are very pleased that this volume of 28 contributions contains 22 papers on desert tortoises (*G. agassizii*), three papers on gopher tortoises (*G. polyphemus*), one paper on Texas tortoises (*G. berlandieri*), one paper on *Mycoplasma* tests applicable to both desert and gopher tortoises, plus this introductory overview. Most papers were given at Desert Tortoise Council symposia, and several are the products of graduate student research.

Since the desert tortoise was Federally listed in the northern one-third of its geographic range (U.S. Fish and Wildlife Service, 1990), more than 100 research papers have been published on the species in peer-reviewed journals, and in proceedings of symposia (e.g., *Herpetological Monographs*, No. 8; Bury and Germano, 1994; Van Abbema,

1997; Van Devender, 2002a). Most recent studies are site- and population-specific.

Gopherus agassizii occupies portions of three major deserts linearly spanning more than 1000 km (Fig. 1). In the northern third of its range, it occurs from the edge of Great Basin Desert scrub in central Nevada and southwestern Utah south into saltbush scrub, creosote bush scrub, and tree yucca woodlands typical of the Mojave Desert. In California, populations extend southward into valleys and desert pavements with creosote bush and ocotillo broken by microphyll woodland washes typical of the western Sonoran Desert (Fig. 2; U.S. Fish and Wildlife Service, 1994).

Habitats in the central part of the geographic range of *G. agassizii* include rocky outcrops and palo verde–saguaro cactus communities of the Sonoran Desert uplands as well as ecotonal desert grasslands (Martin, 1995; Averill-Murray et al., 2002a; Van Devender, 2002b). In Mexico, habitats include Sonoran Desert thornscrub and the dark, wet floors of tropical deciduous forest (Bury et al., 2002). These landscapes and their tortoise populations are fragmented by

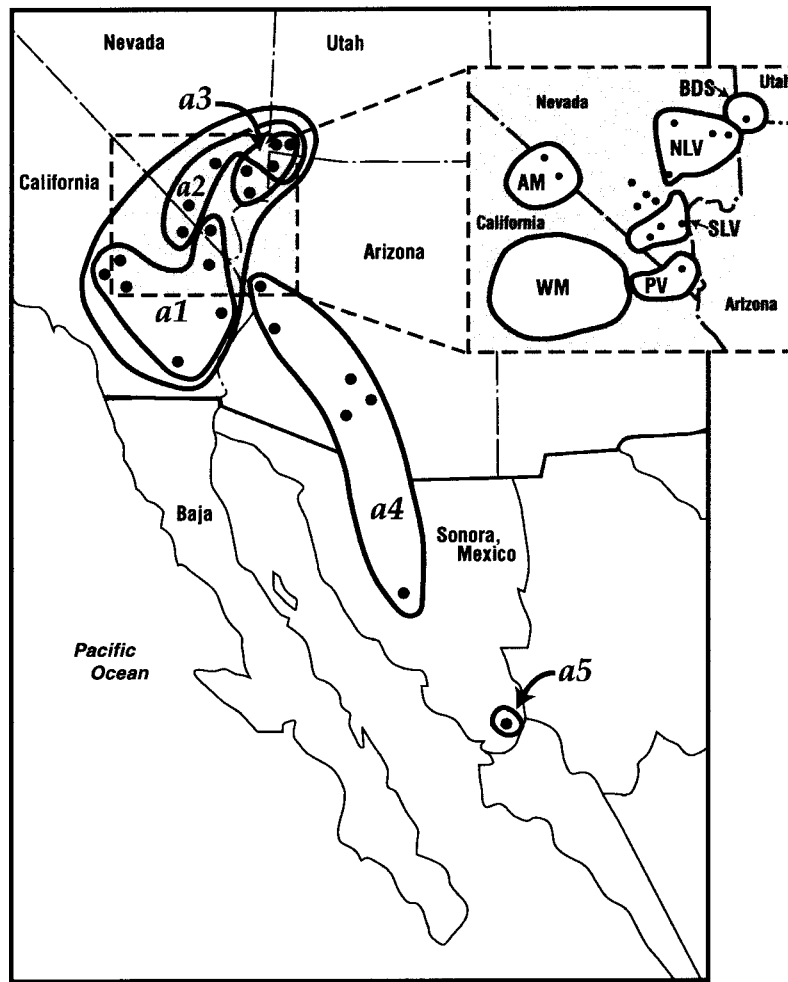


Figure 1. Map of the distribution of the major Evolutionarily Significant Units (ESUs) or haplotypes (a1–a5) of *G. agassizii* (Lamb et al., 1989; Lamb and McLuckie, 2002), and the Management Units (MUs) (inset) from Britten et al. (1997) for populations in the northeastern Mojave Desert. The Management Units generally fall within the borders of pre-existing Evolutionarily Significant Units. Haplotypes (ESUs) are as follows: a1 = Western Mojave Desert; a2 = Nevada; a3 = Utah; a4 = Sonoran Desert; a5 = Sinaloan. Management Units are as follows: AM = Amargosan; BDS = Beaver Dam Slope; NLV = North Las Vegas; PV = Piute; SLV = South Las Vegas; WM = West Mojave. Black circles = sampled populations (omitted only from WM because of its wide geographical distribution).

natural geographic barriers and in some cases have been separated for millions of years (Lamb et al., 1989; McLuckie et al., 1999; Lamb and McLuckie, 2002). When *G. berlandieri* is considered as a member of the *G. agassizii* group, the distribution extends across Texas to the Gulf of Mexico.

Desert tortoises exhibit substantial genetic (Lamb et al., 1989; Lamb and Lydeard, 1994), morphological (Weinstein and Berry, 1987; Germano, 1993), physiological (Turner et al., 1986; Wallis et al., 1999; Averill-Murray, et al., 2002a; Averill-Murray, 2002c), and behavioral (e.g., Woodbury and Hardy, 1948; Burge, 1977; Averill-Murray et al., 2002b) variation among populations. Differences recognized prior to 1994 were formalized for Mojave and western Sonoran populations in the *Desert Tortoise (Mojave Population) Recovery Plan* (Recovery Plan) (U.S. Fish and Wildlife Service, 1994). To ensure continuance of the recognized or putative diversity, the Recovery Plan recommended protection of distinct population segments (DPSs) or evolutionarily significant units (ESUs) in six “Recovery Units” (Ryder, 1986; Waples, 1991). Recently, evidence has supported the

hypothesis that Sonoran *G. agassizii* are different organisms from conspecific “Mojave” tortoises (VanDevender, 2002a). Indeed, the less studied “Sinaloan” haplotypic form is genetically and ecologically distinctive as well (Lamb and McLuckie, 2002; Bury et al., 2002).

Little attention has been given to a critical subject: “What is the desert tortoise?” Is *G. agassizii* a polymorphic or polytypic species, a complex of sibling species, subspecies, or ecomorphs? In terms of conservation, does it have diagnosable DPSs, ESUs, or management units (MUs) (Moritz, 1994a; Waples, 1998; Moritz, 2002)? A stable phylogeny is a prerequisite to extrapolating recent discoveries on one population to new environmental circumstances or different tortoise populations. But none exists. Effective conservation requires extrapolations and rigorous conservation genetics (Funk et al., 2002; Moritz, 2002). Herein, we provide an “assessment of need” for extant populations of *G. agassizii*. We (1) identify impediments to constructing a phylogenetic taxonomy and both the genetic and ecological determination of conservation units, (2) examine the conse-

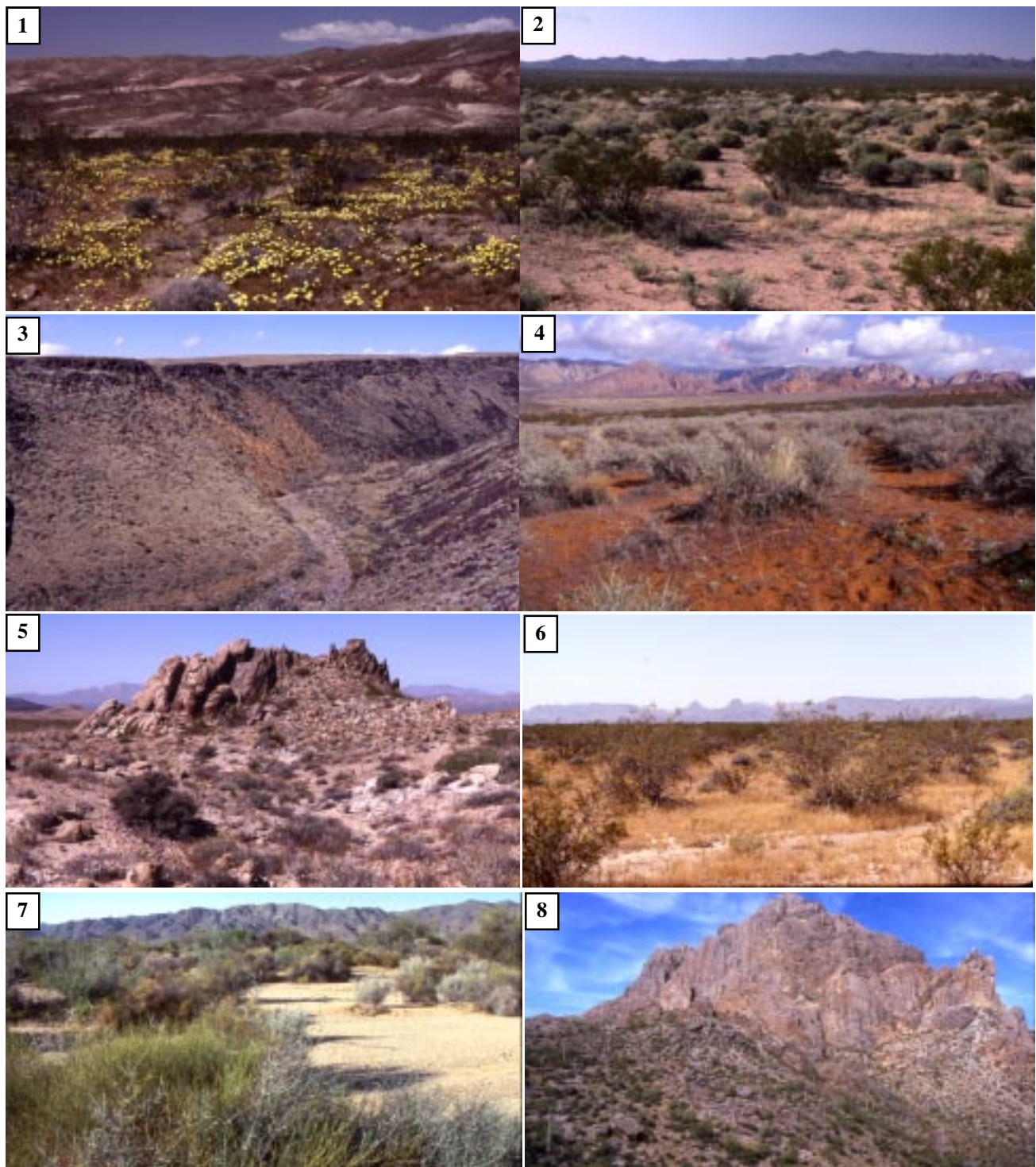


Figure 2. Diversity of habitats and phenotypes across the distributional range of the desert tortoise, *Gopherus agassizii*, and the Texas tortoise, *G. berlandieri*. **1.** Typical habitat of *G. agassizii* in creosote bush scrub, Stoddard Valley, southern Mojave Desert, California, where high densities of desert tortoises were reported in the 1970s and 1980s. Photo by Gary Bolton. **2.** Typical habitat of *G. agassizii* in creosote bush scrub with galleta grass, Fenner Valley, eastern Mojave Desert, Mojave National Preserve, California, where high densities of desert tortoises occurred through the early 1990s. Photo by Betty Burge. **3 and 4.** Specialized habitats of *G. agassizii* in the northeastern Mojave Desert at the Red Cliffs Desert Preserve, Utah, where tortoises live in dune sand and on very steep slopes. Photos by Kristin Berry. **5.** Typical habitat of *G. agassizii* in creosote bush scrub with ocotillo and cactus, Ward Valley, northern Colorado (a.k.a. western Sonoran) Desert, California, where moderate to high densities of desert tortoises occurred through the early 1990s. Photo by Tim Shields. **6.** Typical habitat of *G. agassizii* in creosote bush scrub, Chemehuevi Valley, northern Colorado (a.k.a. western Sonoran) Desert, California, where moderate to high densities of desert tortoises occurred through the early 1990s. Photo by Mike Walker. **7.** Typical habitat of *G. agassizii* in the eastern Colorado (a.k.a. western Sonoran) Desert, Chuckwalla Bench Area of Critical Environmental Concern, California, where high densities of desert tortoises occurred through the mid-1980s in microphyll woodland washes that cut through desert pavement. Photo by Peter Woodman. **8.** Typical habitat of *G. agassizii* in the Sonoran Desert: ironwood trees, palo verde, and saguaro vegetation at Ragged Top study area at Ironwood Forest National Monument, Pima County, Arizona. Photo by Roy C. Averill-Murray. (Fig. 2 continues on next page)

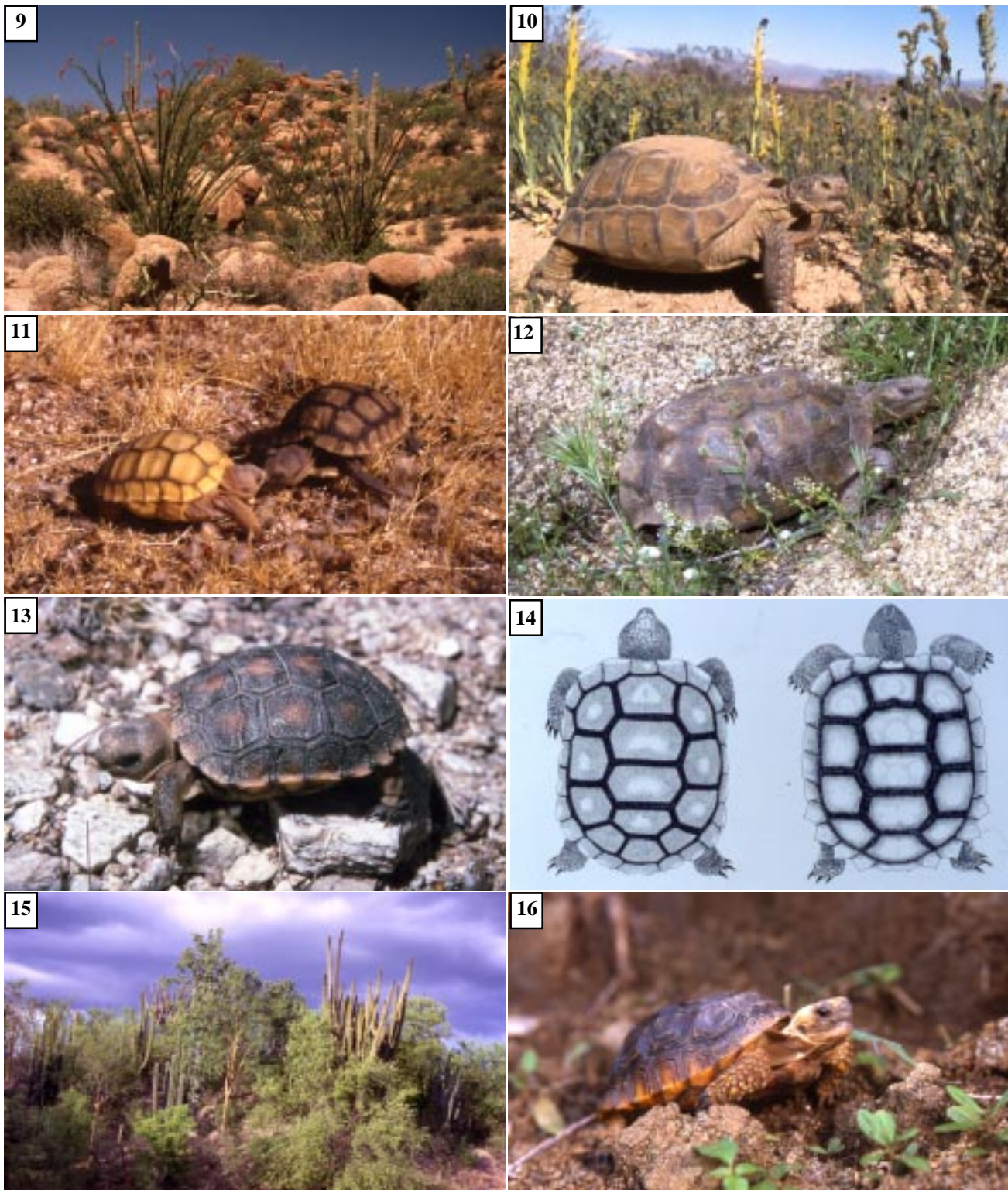


Figure 2. (continued) **9.** Typical habitat of *G. agassizii* in the Sonoran Desert: palo verde-saguaro vegetation at the Sugarloaf study site, Maricopa County, Arizona. Photo by Roy C. Averill-Murray. **10.** A round, high-domed *G. agassizii* from the western Mojave Desert, California. Photo by Bev Steveson. **11.** Light (xanthic) and dark colored neonate *G. agassizii*, possibly siblings, from the southern Mojave Desert, Marine Corps Air Ground Combat Center at 29 Palms, San Bernardino County, California. Photo by Curtis Bjurlin. **12.** Pear-shaped, flattened adult *G. agassizii* from the Sugarloaf study site, Maricopa County, Arizona. Photo by Roy C. Averill-Murray. **13.** Juvenile *G. agassizii* (estimated 1–2 yrs old) from the Sonoran Desert, Pima County, Arizona. Note the dark shell with orange-brown centers on some scutes. Photo by Roy C. Averill-Murray. **14.** Dorsal view comparison of Sonoran (left) and Mojave (right) juvenile carapace and head squamation of *G. agassizii*. Note more “dentate” morphology in the posterior marginals of the carapace of the Mojave juvenile (From Joyner-Griffith, 1991). **15.** Typical tropical deciduous forest habitat for the Sinaloan genotype of *G. agassizii* from Alamos, Sonora, Mexico. Photo by permission of T. Wiewandt. **16.** Juvenile *G. agassizii* of the Sinaloan genotype from Alamos, Sonora, Mexico. Photo by permission of T. Wiewandt. Note similar dark and orange hues in the juvenile of the Sonoran genotype in Fig. 2.13. (Fig. 2 continues on next page)

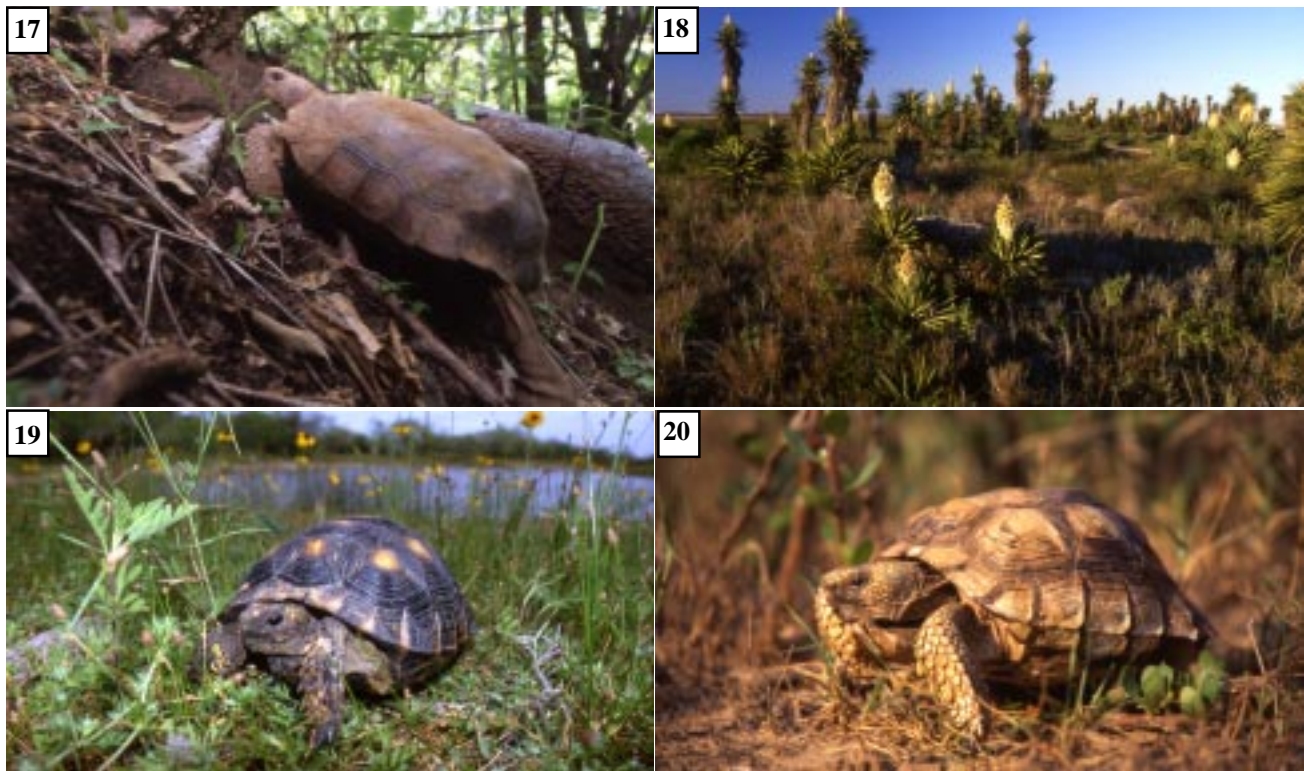


Figure 2. (continued) **17.** Adult *G. agassizii* of the Sinaloan genotype from Alamos, Sonora, Mexico. Photo by permission of T. Wiewandt. **18.** Typical Yucca-Mesquite grassland habitat of *G. berlandieri* in Lower Rio Grande Valley, Texas. Photo by permission of L. Ditto. **19.** Subadult *G. berlandieri* from Texas (with black and yellow contrasting pattern). Photo by permission of L. Ditto. **20.** Old, adult *G. berlandieri* from Texas, illustrating the more uniform horn-colored carapace that occasionally replaces the contrasting pattern in large or older tortoises. Photo by permission of L. Ditto.

quences of delaying such resolution, (3) summarize the database currently available for systematic studies, (4) compare taxonomic solutions applied to other terrestrial chelonians, and (5) suggest remedies.

The Problem and Why It Continues

Although populations of *G. agassizii* differ genetically and ecologically, we do not know the extent to which these populations differ from one another. Do discrete boundaries exist between populations and if so, where are they located—perhaps along major riverine systems (Weinstein and Berry, 1987; Lamb et al., 1989)? Several underlying problems compound this issue. Funding has focused on subjects necessary for managing declining populations: geographic distribution, demographic attributes of populations (densities, size-age class structure, recruitment, sex ratios), status and trends in populations, causes of mortality and mortality rates, habitat preferences and requirements, and fundamental information on physiology and ecology. Until recently, data did not reveal significant differences among populations. Second, relatively few studies have addressed problems in systematics (Bramble, 1982; Crumly, 1994; Crumly and Grismer, 1994; Lamb and Lydeard, 1994; Britten et al., 1997; McLuckie et al., 1999; Lamb and McLuckie, 2002). Most samples for genetic or morphometric analyses have been taken from previously existing study plots, specifically study plots established for monitoring

population status and trends, conducting research on health and disease, or established for a limited research project. Consequently, few analyses were designed to determine critical boundaries (Britten et al., 1997; McLuckie et al., 1999), to gather adequate samples, and to span the entire range of the desert tortoise (Jennings, 1985; Glenn et al., 1990; Lamb and McLuckie, 2002).

Other factors have contributed to the lack of focus. All North American species of tortoises are allopatric, and allopatry holds for most major population segments of the desert tortoise, although not for all proposed MUs, especially in northeastern Nevada and adjacent Arizona and Utah (Ernst et al., 1994; Britten et al., 1997; Lamb and McLuckie, 2002). There has been no need for diagnostic keys, taxonomic definitions, other identification tools, or a phylogenetic framework to extrapolate results from one study to another. In addition, researchers must obtain Federal and State permits from several jurisdictions in the U.S. and Federal permits in Mexico. Additional permits are necessary to work in parks and military reservations. In areas where populations have been depleted by disease or anthropogenic habitat degradations, sampling is likely to be especially time-consuming, e.g., in the western Mojave Desert, and central Sonora, Mexico.

Genetic studies add another layer of complexity. Previously, most yielded data either insensitive to very small changes, such as allozymes, or were entirely restricted to maternal lineages, such as mtDNA. These data sets might

not have registered subtle aspects of gene flow or its absence. And even when all this information is used to define species, weak and inconsistently applied terminology still impede our efforts (Crandall et al., 2000). Still, another issue involves environmental vs. genetic effects. Many morphological features reflect epigenesis in which both gene expression and environmental influences act on the ontogeny of the phenotype. Environmental influences on tortoise development are considerable, ranging from sex determination (Spotila et al., 1994) to body shape and absolute size (Jackson et al., 1976, 1978). Even such features as growth rings on scutes are subject to environmental influences (Berry, 2002).

Populations of *G. agassizii* differ regionally, but we do not know by how much, the nature of boundaries between populations, or whether they are discontinuous and isolated in both ecological and genetic terms. Without resolving these dual gaps in genetic data from nuclear genes and from key geographic areas, indisputable taxonomic conclusions may be elusive. Resolution of the current taxonomic ambiguity is our first priority for a coherent tortoise conservation program (e.g., Lovich and Gibbons, 1997). Why?

Consequences of Leaving Desert Tortoise Systematics and Categorization Unresolved

Our ultimate objective is to conserve the variation in populations of *G. agassizii* in accord with accurate taxonomic and legal designations. Although the “Mojave Population” is listed as threatened, limited data distinguish its features or delimit the precise boundaries of its range (U.S. Fish and Wildlife Service, 1994). Six “recovery units” were identified as the focal points in the Recovery Plan. For each, genetic, phenotypic, and environmental differences were tabularized and described.

In the U.S., legal protection of organisms is generally confined to species and subspecies. In Mexico, Procuraduría Federal de Protección al Ambiente (PROFEPA) is the responsible agency. Unlike state agencies within the U.S., Mexico’s federal *Ley General de Vida Silvestre* (General Law for Wildlife) designates the ecological population as the focal point for conservation, and, in principle, has the ability to consider populations individually for differing levels of legal protection. At the international level, the IUCN Red List recognizes taxa as the primary conservation units, regardless of hierarchical rank (e.g., species, subspecies, genus, family), although regional populations are occasionally listed (e.g., the Mediterranean population of *Trionyx triunguis*). With no recognized subspecies of *G. agassizii*, and less formal units being poorly defined, protection of genetically, ecologically, or biogeographically important subunits ranges from difficult to impossible.

The recovery units recognized in the Recovery Plan were constructed with genetic information (in particular, Lamb et al., 1989). A recent study of the genetics of the Coachella fringe-toed lizard, *Uma inornata*, revealed that the recovery plan for this endangered species forfeited the

protection of the only genetically diverse populations (Trépanier, 2002). Due to subsequent extirpation, that error is now irrevocable. Misdirected priorities, based upon short-term ecological considerations only, could render similar harm to the desert tortoise.

We echo a cover caption on a 1990 issue of *Nature*: “Bad Taxonomy Kills!” Is bad taxonomy allowing critical populations of the desert tortoise to slip away? The legislatively mandated monotypic treatment of the genus *Sphenodon* ignored the existence of three species which led to the extinction of one species and compromised the protection of another (Daugherty et al., 1990). Although these consequences and their remedies were revisited in the recent *Systematic Biology* issue dedicated to Biodiversity Systematics, and Conservation (Funk et al., 2002; Moritz, 2002), perhaps the most succinct summary was made by May (1990:130):

“Without taxonomy to give shape to the bricks, and systematics to tell us how to put them together, the house of biological science is a meaningless jumble.”

Land managers and agencies may need to extrapolate data in critical decision-making for the desert tortoise. Such extrapolations could prove disastrous if data from the “Mojave” desert tortoise are used, for example, to guide conservation of the Sonoran desert tortoise (Van Devender, 2002a). However, genetic extrapolations may occur in response to increasing societal pressure to translocate tortoises to reduce “take” or to augment depleted or extirpated populations. Such actions may introduce poorly adapted animals into alien habitats, and induce long-term reductions in genotypic fitness due to outcrossing depression. *Testudo kleinmanni*, which was recently split into two species, *T. wernerii* and *T. kleinmanni* (Perälä, 2001), provides an example. Confiscated *T. kleinmanni* were translocated into the gene pool of *T. wernerii*, initiating gene swamping (Rhymer and Simberloff, 1996; Perälä, 2001). Recent progress in desert tortoise biology will not be utilized to its full potential and conservation priorities will not be able to target populations most in need of protection until the taxonomy of these tortoises and their interrelationships are more thoroughly established phylogenetically.

Generic Assignments and Species Definitions: What’s in a Name?

The fundamental premise of phylogenetic taxonomy is that taxa are grouped and named on the basis of their phylogenetic relationships (e.g., Farris et al., 2001; Brooks and McLennan, 2002), and the relationships are based on shared derived character states (Hennig, 1966). For tortoises, both extant and extinct, current hypotheses are based on osteology and draw upon a robust array of evidence from fossils (Bramble, 1982; Crumly, 1994; but see McCord, 2002 for major gaps in the late Tertiary record). Fossils provide the anatomical perspective, especially the relationships among species groups within the gopherines. However, ambiguities remain regarding whether fossil species outside the verified clade of *G. polyphemus*–*G. flavomarginatus* (pro-

posed by Bramble [1982] as *Scaptochelys*, a junior synonym of *Xerobates* [Bour and Dubois, 1984]) are a monophyletic group or several divergent lineages grouped by shared primitive characters. No synapomorphies unite the fossil species of *Xerobates*. Since, within a phylogenetic context, taxonomic groupings must be based on synapomorphies, Bramble's genus and taxonomy is inappropriate and indeed, the genus *Xerobates* is now rarely used.

Extant *G. berlandieri* and the various populations of *G. agassizii* share a number of synapomorphies (Germano, 1993; Morafka et al., 1994; Lamb and Lydeard, 1994). Synapomorphies merely establish monophyly and close relationships for a small group including both species, and are not a justification for elevating the whole array of living and fossil "*Xerobates*" from an less specialized "grade" to a phylogenetic clade serving as a sister group to the clade of *G. polyphemus*–*G. flavomarginatus*.

The fundamental unit of evolution is the species. Species are the means of measuring biodiversity and the primary unit of conservation. Species are formed by the irreversible splitting of ancestral lineages regardless of the mechanism—vicariance, dispersal, ecology, or behavior (Dobzhansky, 1970). Although fundamental and essential, a universal definition remains contentious. Mayden (1997) noted that the problem derives from combining two distinct systems: concept and operation. Species concepts based on phylogenetic relationships, i.e., "historical concepts of species" (Brooks and McLennan, 2002), concern us.

Among a variety of historical concepts, most are derivations of the evolutionary species concept of Simpson (1951) and Wiley (1978). The concept contains the essential elements of persistence and divergence through time, as historical entities and cohesive wholes (Brooks and McLennan, 2002). But how does one recognize species? The evolutionary species concept does not have an operational basis or set of criteria. Rather it recognizes as species those units that may be diagnosed, which are internally cohesive (have actual or potential gene flow) and are the end points of a phylogenetic tree. This is much more conceptual than operational.

The operational basis of species starts with a phylogenetic analysis. The problem is simply how to divide a tree into individual species. Three operational tree-based concepts have been proposed (Mayden, 1997; Brooks and McLennan, 1999, 2002), two based on the phylogenetic species concept and one on the composite species concept (Kornet, 1993; Kornet and McAllister, 1993). All concepts recognize species on the basis of branching patterns and evidence of character state evolution. The composite species concept requires fixed changes, but the others typically do not. Whereas one version of the phylogenetic species concept allows for persistent ancestors (and peripheral isolates), the other two do not. Regardless, how do we evaluate species of tortoises?

Among populations of sexually reproducing organisms, such as desert tortoises, we can look for evidence of reproductive isolation, historical lineages, and diagnosable entities. Here the focus is on the functional differences and relationships between demographic units as determined

through population genetics. If independent historical lineages are not interbreeding, and each lineage expresses character state evolution, then each lineage can be recognized as a species, irrespective of the desirability of naming every isolated population. To determine how many recognizable species might currently be referred to as *G. agassizii*, we need to evaluate how the lineages were formed and how they maintain their identities. Populations isolated by geographical barriers that will not disappear during the next glaciation could certainly be considered as permanently isolated (Frost and Hillis, 1990). This is the relationship between *G. berlandieri* and *G. agassizii*. Evidence of interbreeding of nearby populations during the last glacial episode (i.e., an ephemeral isolation) can be directly assessed from nuclear gene markers, such as microsatellites, or potentially inferred from mtDNA based on multiple shared haplotypes.

The problem with *G. agassizii* is that we do not have the required phylogeny. To some extent, the maternal history of the "species" has been investigated and at least some maternal lineages have been identified (Lamb and Lydeard, 1994; Britten et al., 1997). However, these relationships do not form a phylogeny because they are not indicative of gene flow and genetic recombination; they are tokogenetic (Hennig, 1966), female lineages. Unabated gene flow would be so reticulate with homoplasy (= character conflict owing to convergence, parallelism, reversal, or particularly introgressive gene flow) that no dichotomous relationships would be resolved. If phylogenetic relationships can be resolved within *G. agassizii*, then multiple evolutionary species can be recognized. Unfortunately we do not have sufficient data to address this most basic question: what is *G. agassizii*?

Much of the anatomical differentiation is geographically constrained. Whether this variation reflects epigenetic or genetic effects is not known. More importantly, we cannot associate the anatomical variation with historical lineages. This leads to another problem, that of potentially recognizing subspecies of *G. agassizii*. Subspecies may be described and recognized, but they do not represent exclusive historical units. Thus, they are unacceptable within a phylogenetic context. Regardless of whether the naming of subspecies affords greater legal protection, only ephemerally isolated, diagnosable insular populations may be so recognized in contemporary taxonomy (Frost and Hillis, 1990; Murphy and Aguirre-León, 2002b). If history can be associated with isolated lineages of tortoises, it might be possible to recognize subspecies, but then it may be preferable to make them species so long as the naming corresponds to the conceptual and operational criteria.

Academics and law differ in their definitions of "species." Terminology has evolved independently in both legal and scientific communities and, unfortunately, the two communities often pursue different paths. Whereas one group will favor a term or definition, the other will subordinate or discard it. For example, the disuse of subspecies in academics (Frost and Hillis, 1990) is a problem for taxon-based legal

protection. The history of legal and academic interactions has been variously reviewed (Pennock and Dimmick, 1997; Waples, 1998; and indirectly, Moritz, 2002).

Conservation and Law

Legal terminology is important in conservation. The 1978 amendment to the 1973 U.S. Endangered Species Act (ESA) introduced DPS. Ryder (1986) contributed ESU. Moritz (1994a, 1994b, 2002) refined the definition of ESU and added MU. These terms bring a variety of concepts by which to categorize geographical variation in *G. agassizii*.

Distinctive Population Segments (DPS). — The DPS is the primary unit for protection under the current ESA (U.S. Department of the Interior and U.S. Department of Commerce, 1996) and is used by both the U.S. Fish and Wildlife Service and National Marine Fisheries Service (NMFS). DPS units are designated on the basis of three elements: (1) discreteness of the population segment in relation to the remainder of the species to which it belongs; (2) the significance of the population segment to the species to which it belongs; and (3) the conservation status of the population in relation to the ESA standard for listing.

Although the General Accounting Office requested that ESA protection of populations be revoked because it was too broad in its potential applications, the U.S. Senate retained coverage with the suggestion that DPS protection be used “sparingly” and “be well justified biologically” (U.S. Senate, 1979). Subsequently, the U.S. Department of the Interior and U.S. Department of Commerce (1996) redefined discreteness to be satisfied by a marked separation from other conspecific populations as a consequence of physiological, ecological or behavioral factors. Physical separation was also acceptable, both as a result of natural geographical isolation and along international boundaries where “discreteness” was created by differential protection/exploitation of populations on each side of a border.

Several criteria gauge “significance of the DPS:” persistence in an unusual or unique ecological setting for that species; geographical distribution that would create a gap in the range of the species given local extinction/extirpation; the only surviving population within its historic range (even if introduced populations are more common elsewhere); marked differences from conspecific populations in genetic characteristics. Conservation status is determined separately for each DPS within a species using ESA criteria. Individual vertebrate DPSs may be unlisted, threatened, endangered, and delisted. In effect, they are treated as separate species in terms of legal status. The ESA has differentially protected populations of five U.S. chelonians, including *G. agassizii*, *G. polyphemus*, the flattened musk turtle, *Sternotherus depressus*, and two sea turtles, *Chelonia mydas* and *Lepidochelys olivacea* (Pennock and Dimmick, 1997).

Evolutionarily Significant Units (ESU) and Management Units (MU). — Like the DPS, the definition ESU has evolved to become more explicit. Originally, it allowed zoo biologists to prioritize the propagation of groups within a

species that “actually represent significant adaptive variation” and “evolutionarily significant units within species” (Ryder, 1986). It evolved to identify reproductively isolated populations that are “separate from other populations and have unique or different adaptations” (Waples, 1991). This definition became the official policy of NMFS, essentially their formal definition for Pacific salmon (U.S. Department of Commerce, 1991). Subsequently, ESU was redefined in terms of phylogenetic species (Volger and DeSalle, 1994), or evolutionary species (Mayden and Wood, 1995). The definitions of Moritz (1994a, 1994b, 2002) rest upon units/populations that are mutually monophyletic for mtDNA and are significantly divergent from one another in their frequencies for nuclear loci as well. Moritz (2002) added a subordinate unit to the ESU, the MU, to designate genetically distinctive subdivisions. MUs could reflect distinctive allelic frequencies (restriction fragment length polymorphisms in mtDNA, allozymes, or sequenced DNA fragments), or lesser differences within mtDNA sequences. The MU recognizes and protects demographically distinctive populations to sustain the evolutionary viability of the larger ESU (Moritz, 2002). Britten et al. (1997), in part using allozyme differences among populations of *G. agassizii* in the northeastern Mojave Desert, suggested that these MUs were equivalent to individual Recovery Units of the Recovery Plan. However, the Recovery Plan equated Recovery Units with ESUs. The MU has never enjoyed the formal legal recognition afforded to either the DPS or ESU. The use of MUs by Britten et al. (1997) most closely approaches their use in a legal context.

The ESU and MU gave conservation a genetic basis and extended the principles of phylogenetic systematics to progressively smaller demographic units. However, these particular units exclude many of the criteria listed in the formal Federal definition of DPS, especially when only biogeographical, ecological, physiological, or ethological criteria are invoked. More recently, and paraphrasing Frankel (1974), Moritz (2002: 239) stated that the goal of biological conservation was “To maintain evolutionary processes and the viability of species and the functional landscapes necessary to achieve this.” Three elements were identified for conservation planning: (1) the combinations of areas and populations which maximize representation of species; (2) areas that represent specific evolutionary processes or admixtures of historically isolated populations (corridors, edaphic communities, ecotones, etc.); and (3) within areas identified in 1 and 2, the protection of contiguous habitats of sufficient area across major environmental gradients to ensure persistence. Moritz (2002: 251) concluded that “Conservation strategies for species may be improved, and made more flexible, by considering separately the genetic divergence that arises from adaptive rather than vicariant processes.” Only the product of vicariance would be designated as the ESU, based on mtDNA data most often involving presumptively neutral allelic alternatives. Perhaps the ESU is better characterized as being the result of deep historical processes including, but by no means confined to, vicariance. The adaptive process

involves both the genetic response and the environmentally induced selection.

There are opportunities for and difficulties in reconciling DPSs, ESUs, and MUs with each other and with the Recovery Units and Desert Wildlife Management Areas (DWMAs) described in the Recovery Plan (U.S. Fish and Wildlife Service, 1994). Collectively, the ESU and MU integrate into the broader, legally binding concept of DPS. The recognition of dynamic interactions between gene pools and selective environments can define populations but need not be strictly genetic definitions. Although the ESU and MU provide rigorous definitions for protection, DPS provides latitude in more than a genetic sense for protecting the aforementioned adaptive processes that have or potentially could produce these units. Likewise, recognition of the importance of unique habitats, habitat gradients, corridors, ecotones (Smith et al., 1997) and edaphic zones justifies the continued utility of the DPS, especially in the context of the Department of the Interior and Department of Commerce (1996) definition. These issues are important to defining the desert tortoise(s).

Considering Mojave and Sonoran populations, Lamb and McLuckie (2002: 81) stated that Recovery Units “demonstrate concordant geographical variation for certain nuclear genes (Jennings, 1985; Glenn et al., 1990; Britten et al., 1997), morphology (Weinstein and Berry, 1987) and behavior (Barrett, 1990).” Congruence between ESUs represented by Lamb and McLuckie’s (2002) major (mtDNA) genotypes, *a1*, *a2*, and *a3*, in their map and the subordinate six MUs described by Britten et al. (1997) are shown in Fig. 1. Three MUs — West Mojave, Piute Valley and South Las Vegas — are subsumed within genotype *a1*. The Amargosan MU falls within the boundaries of genotype *a2*, and the MUs of North Las Vegas and Beaver Dam Slope are both encompassed by Lamb and McLuckie’s (2002) genotype/ESU *a3*.

Legal applications of these units create challenges. Definitions of both ESUs and DPSs continue to evolve academically and in the application of the ESA. On one hand, DPS and Recovery Units are the legal units of protection, and on the other hand ESUs and MUs are advancing theory. The Recovery Plan (U.S. Fish and Wildlife Service, 1994) adheres closely to ESUs as defined by Waples (1991), and equates them with DPSs. Recently the definition of ESU has expanded, contracted, and been revised. In addition, molecular systematics has advanced, and now includes the widespread use of nuclear microsatellite DNA data. Consequently, delineations of Recovery Units of *G. agassizii* and some of their subordinate DWMAs need to be reconsidered, as was done by Britten et al. (1997) for a localized area centered in southern Nevada. Given the rigor with which the original Recovery Units were designated, none would be invalidated, although they might be reshaped or subdivided to better protect recently discovered MUs. Recovery Units may need to be re-prioritized for protection. Revision might also include designating more DPS, even if not defined in strictly genetic terms. In building upon the Recovery Plan, we are reminded of Pennock and Dimmick (1997: 611): “A

strict redefinition of distinct population segments as evolutionarily significant units will compromise management efforts because the role of demographic and behavioral data will be reduced. Furthermore, strictly cultural, economic, or geographic justifications for listing populations as threatened or endangered will be greatly curtailed.”

Precedence

Conservation requires law, and the operational basis of law is precedence. Two models from other chelonians are available as precedence. The *Terrapene carolina* complex and the genus *Testudo* are both composed of small, terrestrial, moderately diverse (5–10 taxa) and predominantly temperate species. Their phylogeny, character states by which each taxonomically recognized population is defined, and evidence for gene flow are used to resolve the taxonomic status of individual units.

The *T. carolina* clade (Minx, 1996) presents parallels to the *G. agassizii* – *G. berlandieri* complex. Both consist of two nominal species, one of which has much greater distribution and regional diversity than the other. *Terrapene coahuila* is allopatric and confined to a single isolated wetland in the state of Coahuila, Mexico. *Terrapene carolina* has six subspecies, four of which are parapatric and intergrade (e.g., Shannon and Smith, 1949; Smith and Sanders, 1952; Blaney 1968; Ward, 1968). Two subspecies are allopatric in southeastern Mexico (Ernst and McBreen, 1991). Morphometrics, skeletal anatomy, and the semi-aquatic behavior of *T. coahuila* have made its recognition unambiguous. Some diagnostic character states are primitive with respect to *T. carolina* (Minx, 1996; Bramble, 1974). Many defining character states of subspecies (Buskirk, 1993; Ernst et al., 1994; Dodd, 2001) are comparable to those used to define species among other taxa. However, a broad belt of intergradation occurs between the four contiguous subspecies across northern Florida, western Georgia, and Alabama (Dodd, 2001). These traditional subspecies might well be assigned DPS status today, and if supported genetically, categorized as ESUs. The two allopatric Mexican subspecies of *T. carolina* remain problematic. Unlike *T. coahuila*, they lack the profound morphological (especially osteological) differences that define the former as species. Perhaps molecular analyses will further resolve their taxonomic status.

The small, Mediterranean and west-to-central Asian tortoises of the genus *Testudo* express similar diversity, but more of them have been ranked as species: *T. graeca*, *T. hermanni*, *T. horsfieldi*, *T. kleinmanni*, *T. marginata*, and *T. wernerii* (Ernst and Barbour, 1989; Perälä, 2001). The genus consists of six species (Ernst and Barbour, 1989), divided into three subgenera (Loveridge and Williams, 1957; Khozatsky and Mlynarski, 1966): *Testudo*, *Pseudotestudo*, and *Agrionemys*. Both *T. graeca* and *T. hermanni* have subspecies. Anatomical differences among the species are equivalent to those of the subspecies of *Terrapene carolina*. Several species, *Testudo graeca* and *T. hermanni* in particular, have complementary ranges. Some Asian populations of

Testudo graeca are sympatric with *T. horsfieldi* (Stubbs, 1989a, 1989b). In central and southern Greece *T. hermanni* is frequently sympatric with the endemic *T. marginata* (Stubbs, 1989a, 1989b, 1989c). Despite the proximity and/or overlap of these forms, hybrids are absent. Their moderate morphological differentiation from one another belies the fact that they are species. Like both *Terrapene carolina* and some *G. agassizii*, allopatric populations of *Testudo graeca* pose taxonomic problems. A similar issue arises where the Nile River separates *Testudo kleinmanni* and *T. wernerii* (Perälä, 2001), much like the Colorado River approximately separates two major haplotypes (ESUs) of *G. agassizii* (Lamb and McLuckie, 2001). In both cases these allopatric (or nearly parapatric) products of vicariance fragmentation are difficult to assign taxonomically.

Likewise, these similar species of *Testudo* have expressed differential antibody production and susceptibility to viral diseases (rhinitis, pharyngitis, and stomatitis) when exposed to both Sendai virus (Jackson and Needham, 1983) and herpesvirus (Frost and Schmidt, 1997). Differential responses to diseases provoke greater interest in establishing an accurate systematics for “populations” of *G. agassizii*, where potentially different susceptibilities to infection are also a major concern.

These examples provide precedence for the *G. agassizii* – *G. berlandieri* complex. First, the extent of morphological differentiation among putative DPSs may not, by itself, determine whether they are operational species. Several subspecies of *T. carolina* are more distinctive than the species of *Testudo*. Second, the inability to recover a phylogeny and difficulty in evaluating degrees of differentiation are puzzles when the entire body of evidence is phenotypic, as it is in both these cases. Third, areas of real or potential contact are very valuable for resolving the degree and nature of gene flow between populations. Genetic comparisons may identify allopatric, or even sympatric, populations with relatively “shallow” histories of separation.

An Action Plan:

Resolving the Phylogeny and Taxonomy Among the *Gopherus agassizii* – *G. berlandieri* Complex

An action plan for the *G. agassizii* – *G. berlandieri* complex requires an evaluation of available resources and deficits in databases. Current, comparative data encompass genetics, morphology, developmental biology, ecology, physiology (including nutrition), behavior, and life history. Our first priority is an assessment of the taxonomic status of the isolated populations and distinctive morphological forms of *G. agassizii*. Since the maternal genealogy may strongly reflect deep history, especially when cladogenic patterns are repeated in several other taxa (e.g., Riddle et al., 2000; Murphy and Aguirre-León, 2002a), we recommend that gaps be filled in the existing mtDNA data sets. Subsequent analysis would identify and locate the boundaries of historical maternal lineages. Areas to be sampled thoroughly include, but are not limited to: populations at (1) edges and

centers of existing Recovery Units, both west and east of the Colorado River (McLuckie et al., 1999), (2) Red Cliff Desert Preserve in Utah, (3) west of Tucson, Arizona, and (4) throughout the Mexican state of Sonora, but especially between Guaymas and Alamos (Bury et al., 2002; Lamb and McLuckie, 2002), and (5) along the Rio Yaqui. The latter task may prove daunting, because the route of the major Mexican federal Highway 15 traverses unfavorable habitat and cultivated plains in the Sonoran lowlands (Bury et al., 2002), at some distance from the montane rocky slopes, which the tortoises appear to occupy in this part of Mexico (Fritts and Jennings, 1994).

MtDNA data are not indicative of gene flow. Thus, investigations of nuclear genes across these maternal boundaries must be pursued simultaneously. Microsatellite DNA data would be critical in unambiguously demonstrating the genetic integrity of maternal clades, i.e., the historical absence of gene flow among isolated or parapatric populations (e.g., Edwards et al., 2002, for selected Sonoran Desert populations). Such gene comparisons require large sample sizes (≥ 30 individuals) collected uniformly across the entire range of *G. agassizii* and *G. berlandieri*. As a part of the genetic studies, karyotypes (Dowler and Bickham, 1982) should be revisited in light of contemporary banding and immunofluorescence techniques.

Morphological differentiation is the next priority. Among existing morphological studies, Weinstein and Berry (1987) focused primarily upon differentiation of shell shape within the U.S., Germano (1992) addressed morphological ontogeny, and Germano (1993) evaluated shell morphology. The genetic geographical gaps also apply to morphological studies. In particular, studies need to be extended across Arizona and Mexico. Work needs to be directed toward a more detailed evaluation of shell shape, including proportions and angularity of individual scute and bony elements (e.g., see commentary by Weinstein and Berry, 1987) by sex, size-class, and age. Deep osteological variation needs to be pursued, as does superficial shell coloration and pigmentation. Figure 2 illustrates the graphic differences between adult *G. agassizii* and *G. berlandieri* and three major haplotypic forms within *G. agassizii*. The variation is comparable to that of species of *Testudo* and subspecies of *Terrapene carolina*. Figure 2 also highlights the ontogenetic changes in carapacial shape, pigment, and pattern. Figure 2.14 illustrates the dramatic differences in carapacial and dorsal head squamation in Sonoran vs. Mojave juvenile tortoises. Of particular interest, the pointed posterior marginals are well developed only in Mojavean juveniles. Similar differences, contingent on ontogenetic stage, have been used to separate *Geochelone carbonaria* from *G. denticulata* (Ernst and Barbour, 1989) and *T. kleinmanni* from *T. wernerii* (Perälä, 2001). The pronounced differences in individuals and populations in Fig. 2 illustrate the potential importance of many morphological and pigmentational characters used to define taxa in *Terrapene* and *Testudo*.

Aspects of physiology are important, but have a lower priority. The more striking findings between tortoise popu-

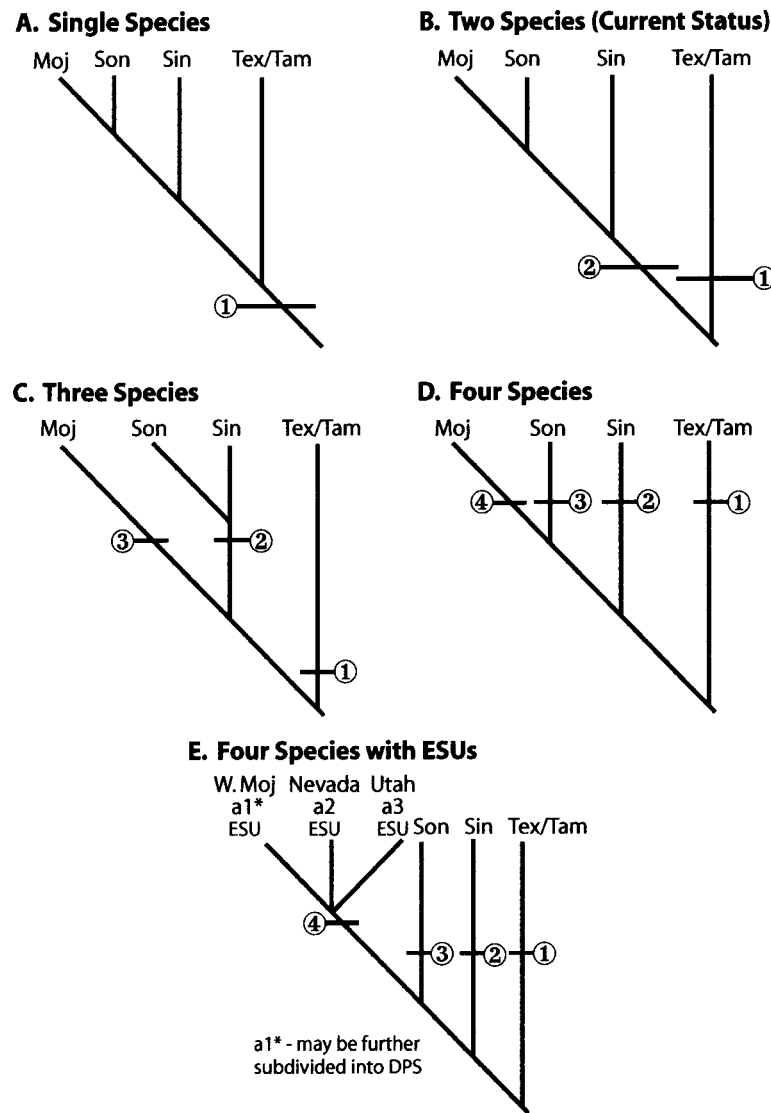


Figure 3. Alternative taxonomic arrangements of the *Gopherus agassizii* – *G. berlandieri* complex based on current estimates of phylogenetic relationships. This array of alternatives neither includes all possible permutations nor selects among alternatives: (A) all potential taxonomic units are subspecies of *G. polyphemus* (taxonomy of Mertens and Wermuth, 1955); (B) the taxonomic *status quo*, with two recognized species; (C) the Sonoran and Sinaloan genotypes are combined (Lamb et al., 1989) as a new species separate from both *G. agassizii* and *G. berlandieri*; (D) both Sonoran and Sinaloan genotypes are new species; (E) similar to D, but the three major Mojave Desert genotypes (Lamb et al., 1989) are Evolutionarily Significant Units (ESUs), with the option of further subdividing these into subordinate Management Units (MUs), or non-genetically based Distinct Population Segments (DPS).

lations occurring north and west of the Colorado River (“Mojave population”) and Sonoran population in Arizona include differences in reproductive physiology. Size at sexual maturity varies substantially between Mojave and Sonoran tortoises (Turner et al., 1986; Averill-Murray et al., 2002a; Averill-Murray, 2002). Mojave tortoises may initiate egg laying at ca. 180 mm carapace length (CL), while Sonoran tortoises have not been observed to lay eggs until 220 mm CL. The number of clutches produced per season, timing of oviposition, and numbers of eggs in clutches are also markedly different. This work needs to be extended to include the Colorado (western Sonoran) Desert in California, the Red Cliffs Preserve, and Mexico.

Comparative research on the physiology of field metabolic rates and water balance has been conducted on west-

ern, eastern, and northern Mojave populations (e.g., Peterson, 1996; Henen et al., 1998). Similar work needs to be undertaken in the Colorado Desert of California, as well as in Sonora and Sinaloa.

Ecological differentiation is also important. It is summarized on the broadest scales, and with a geographically complementary emphasis in the Recovery Plan, especially its Section F, Distinct Population Segments of the Desert Tortoise (U.S. Fish and Wildlife Service, 1994). Comparative work is also available in Van Devender (2002a).

If historical relationships can be resolved, and cohesiveness demonstrated via genetic isolation, then multiple species can and should be recognized. An array of possible outcomes is illustrated by Fig. 3, with the probable outcomes ranging from one species, a version of which was proposed

by Mertens and Wermuth (1955) who treated all *Gopherus* as subspecies of *G. polyphemus*, the current two, to four (or more) species and ESUs. However, if nuclear gene flow is demonstrated among historical maternal units, then perhaps *G. agassizii* should not be considered polytypic because recognition of multiple species or subspecies would not meet the operational criteria of evolutionary species, i.e., phylogenetic species.

Apart from taxonomic uses, molecular analyses will be critical for tortoise management. Genetically discernable units could be designated as ESUs or MUs (within the legal terminology of DPS). Predictive extrapolation of data from one unit to another can be made on cladograms. These clades might also correspond with demographic boundaries of other types of DPSs identified from morphological, ecological, physiological, and behavioral characteristics. Regardless of congruence, all of these data are required to observe patterns and set priorities to protect the full diversity in desert tortoises as keystone or flagship species in diverse ecosystems and evolutionary processes across a wide geographic range. This is the primary goal of desert tortoise conservation, yet it is a goal that will be delayed until this task is accomplished.

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