Dr. Daniel D. Beck (right) with Martin Villa at the Centro Ecologia de Sonora, in Hermosillo, Mexico. Dr. Beck is holding a near-record length Río Fuerte beaded lizard (*Heloderma horridum exasperatum*). *Photo by Thomas Wiewandt.*

Taxonomic reassessment and conservation status of the beaded lizard, *Heloderma horridum* (Squamata: Helodermatidae)

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Abstract.—The beaded lizard (*Heloderma horridum*) and Gila monster (*H. suspectum*) are large, highly venomous, anguimorph lizards threatened by human persecution, habitat loss and degradation, and climate change. A recent DNA-based phylogenetic analysis of helodermatids (Douglas et al. 2010. *Molecular Phylogenetics and Evolution* 55: 153–167) suggests that the current infraspecific taxonomy (subspecies) of beaded lizards underestimates their biodiversity, and that species status for the various subspecies is warranted. Those authors discussed "conservation phylogenetics," which incorporates historical genetics in conservation decisions. Here, we reassess the taxonomy of beaded lizards utilizing the abovementioned molecular analysis, and incorporate morphology by performing a character mapping analysis. Furthermore, utilizing fossil-calibrated sequence divergence results, we explore beaded lizard diversification against a backdrop of the origin, diversification, and expansion of seasonally dry tropical forests (SDTFs) in Mexico and Guatemala. These forests are the primary biomes occupied by beaded lizards, and in Mesoamerica most are considered threatened, endangered, or extirpated. Pair-wise net sequence divergence (%) values were greatest between *H. h. charlesbogerti* and *H. h. exasperatum* (9.8%), and least between *H. h. alvarezi* and *H. h. charlesbogerti* (1%). The former clade represents populations that are widely separated in distribution (eastern Guatemala vs. southern Sonora, Mexico), whereas in the latter clade the populations are much closer (eastern Guatemala vs. Chiapas, Mexico). The nominate subspecies (*Heloderma h. horridum*) differed from the other subspecies of *H. horridum* at 5.4% to 7.1%. After diverging from a most-recent common ancestor ~35 mya in the Late Eocene, subsequent diversification (cladogenesis) of beaded lizards occurred during the late Miocene (9.71 mya), followed by a lengthy stasis of up to 5 my, and further cladogenesis extended into the Pliocene and Pleistocene. In both beaded lizards and SDTFs, the tempo of evolution and diversification was uneven, and their current distributions are fragmented. Based on multiple lines of evidence, including a review of the use of trinomials in taxonomy, we elevate the four subspecies of beaded lizards to full species: *Heloderma alvarezi* (Chiapan beaded lizard), *H. charlesbogerti* (Guatemalan beaded lizard), *H. exasperatum* Río Fuerte beaded lizard), and *H. horridum* (Mexican beaded lizard), with no changes in their vernacular names. Finally, we propose a series of research programs and conservation recommendations.

Key words. mtDNA, ATPase, nuclear genes, character mapping, genomics, seasonally dry tropical forests, reptiles

Resumen.—El escorpión (*Heloderma horridum*) y el monstruo de Gila (*H. suspectum*) son lagartijas grandes, anguimorfas, y muy venenosas que están sufriendo diversas amenazas como resultado de la persecución humana, degradación y pérdida del hábitat y el cambio climático global. Un análisis filogenético reciente basado en ADN de este grupo (Douglas et al. 2010. *Molecular Phylogenetics and Evolution* 55: 153–167) sugiere que la actual taxonomía intraespecífica (subspecies) del escorpión está subestimando la diversidad biológica, y el reconocimiento de especies es justificable. Estos autores discuten la utilidad del enfoque denominado "conservación filogenética", que hace hincapié en la incorporación de la genética histórica en las decisiones de conservación. En este estudio, reevaluamos la taxonomía del escorpión utilizando el análisis molecular antes mencionado e incorporamos la morfología en un análisis de mapeo de caracteres. Así mismo, con los resultados de la secuencia de divergencia calibrada con fósiles, se explora la diversificación del escorpión en forma yuxtapuesta al origen, la diversificación y la expansión de los bosques tropicales estacionalmente secos (SDTFs) en México y Guatemala. Estos bosques son los principales biomas ocupados por los escorpiones, y en Mesoamérica la mayoria son considerados amenazados, en peligro o

extirpados. Los valores de la secuencia de divergencia neta por pares (%) fueron mayores entre *H. h. charlesbogerti* y *H. H. exasperatum* (9,8%) y menores entre *H. h. alvarezi* y *H. h. charlesbogerti* (1%). El primer grupo representa a poblaciones que están muy distantes una de la otra en su distribución (este de Guatemala vs. sur de Sonora, México), mientras que las poblaciones en el segundo grupo están mucho más relacionadas (este de Guatemala vs. Chiapas, México). La subespecie denominada (*Heloderma h. horridum*) difirió de las otras subespecies de *H. horridum* entre un 5,4% a 7,1%. Después de la separación de un ancestro común más reciente, ~35 mda a finales del Eoceno, ocurrió una diversificación (cladogénesis) posterior de *Heloderma* a finales del Mioceno tardío (9,71 mda), seguida de un estancamiento prolongado de hasta 5 mda, con una cladogénesis posterior que se extendió hasta el Plioceno y Pleistoceno. En ambos grupos, escorpiones y bosques tropicales estacionalmente secos, los procesos de evolución y diversificación fueron desiguales, y su distribución fue fragmentada. Hoy en día, el escorpión está distribuido de manera irregular a lo largo de su amplio rango geográfico. Basándonos en varias líneas de evidencia, incluyendo una revisión del uso de trinomios taxonómicos, elevamos las cuatro subespecies del escorpión al nivel de especie: *Heloderma alvarezi* (escorpión de Chiapas), *H. charlesbogerti* (escorpión Guatemalteco), *H. exasperatum* (escorpión del Río Fuerte), y *H. horridum* (escorpión Mexicano), sin cambios en los nombres vernáculos. Por último, proponemos una serie de programas de investigación y recomendaciones para su conservación.

Palabras claves. ADNmt, ATPasas, genes nucleares, mapeo de caracteres, genómica, bosque tropical estacionalmente seco, reptiles

The century-long debate over the meaning and utility of the subspecies concept has produced spirited print but only superficial consensus. I suggest that genuine consensus about subspecies is an impossible goal ... the subspecies concept itself is simply too heterogeneous to be classified as strict science.

Fitzpatrick 2010: 54.

Introduction

The beaded lizard (*Heloderma horridum*) is a large, highly venomous, anguimorph (Helodermatidae) squamate with a fragmented distribution in Mesoamerica that extends from northwestern Mexico (Sonora, Chihuahua) to eastern Guatemala (Bogert and Martín del Campo 1956; Campbell and Vannini 1988; Campbell and Lamar 2004; Beck 2005; Beaman et al. 2006; Anzueto and Campbell 2010; Wilson et al. 2010, 2013; Domínguez-Vega et al. 2012). Among the reptilian fauna of this region, the beaded lizard (in Spanish, known as the "escorpión") is well known to local inhabitants, yet its natural history is surrounded by mystery, notoriety and misconception. Consequently, it is frequently slaughtered when encountered (Beck 2005).

Adding to this anthropogenic pressure, beaded lizard populations, with rare exceptions (Lemos-Espinal et al. 2003; Monroy-Vilchis et al. 2005), occur primarily in seasonally dry tropical forests, SDTFs (Campbell and Lamar 2004; Beck 2005; Campbell and Vannini 1988; Domínguez-Vega et al. 2012), the most endangered biome in Mesoamerica owing to persistent deforestation for agriculture, cattle ranching, and a burgeoning human population (Janzen 1988; Myers et al. 2000; Trejo and Dirzo 2000; Hoekstra et al. 2005; Miles et al. 2006; Stoner and Sánchez-Azofeifa, 2009; Williams-Linera and Lorea 2009; Beck 2005; Pennington et al. 2006; Wilson et al. 2010, 2013; Dirzo et al. 2011; De-Nova et al. 2012; Domínguez-Vega et al. 2012; Golicher et al. 2012). Furthermore, drought and fires escalate the above threats (Beck 2005; Miles et al. 2006), and recent predictive models of climate change show that the persistence of SDTFs in this region is highly dubious (Trejo and Dirzo 2000; Miles et al. 2006; Golicher et al. 2012).

Despite its large size and charismatic nature, our knowledge of the ecology, geographical distribution, and status of populations of *H. horridum* remains limited (Beck and Lowe 1991; Beck 2005; Ariano-Sánchez 2006; Douglas et al. 2010; Domiguez-Vega et al. 2012). Furthermore, based on multiple lines of evidence, a taxonomic reevaluation of this group of lizards is long overdue (Beck 2005; Douglas et al. 2010).

Here, we continue the dialogue concerning the infraspecifc (subspecific) taxonomy and conservation status of beaded lizards. We reviewed recent publications by Beck (2005) and Domínguez-Vega et al. (2012), and augment their conclusions based on personal (DDB) field research in Mexico. We reassess the taxonomic status of the populations of *H. horridum* using morphology, biogeography, and a recent molecular-based (mtDNA, nDNA) analysis conducted by Douglas et al. (2010). Although Douglas et al. (2010) commented on the mo-

lecular diversity of *Heloderma*, especially in *H. horridum*, they did not provide explicit taxonomic changes. In this paper, therefore, we reevaluate and expand upon their conclusions. To gain insights into phenotypic (morphological) evolution of extant *Heloderma*, with emphasis on *H. horridum*, we conduct a character mapping analysis (Brooks and McLennan 1991; Harvey and Pagel 1991; Martins 1996; Maddison and Maddison 2011), utilizing the phylogenetic information (trees) recovered by Douglas et al. (2010).

Overview of Morphology and Molecules in the genus *Heloderma*

1. Morphological assessment

Published over half a century ago, Bogert and Martín del Campo's (1956) detailed and expansive monograph of extant and fossil helodermatid lizards remains the definitive morphological reference (reviewed in Campbell and Lamar, 2004; Beck, 2005), and it contains the diagnoses and descriptions of two new subspecies (*Heloderma horridum alvarezi* and *H. h. exasperatum*). Thirty-two years later, Campbell and Vannini (1988) described a new subspecies (*H. h. charlesbogerti*), from the Río Motagua Valley in eastern Guatemala, in honor of Charles Bogert's pioneering work on these lizards. With few exceptions, such as Conrad et al. (2010) and Gauthier et al. (2012), who examined higher-level relationships of the Helodermatidae and other anguimorphs, a modern phylogeographic analysis of morphological diversity for extant helodermatids is lacking. However, as we illustrate in our character mapping analysis, the morphological characters used by Bogert and Martín del Campo (1956) in diagnosing and describing the subspecies of beaded lizards, though somewhat incomplete, remains useful in analyzing phenotypic variation.

2. Diagnosis, description, and distribution of *Heloderma horridum*

Diagnosis and description.—Bogert and Martín del Campo (1956) and Campbell and Vannini (1988) provided diagnoses and descriptions of the subspecies of *Heloderma horridum*. Recent information on the biology, systematics, and taxonomy of *H. horridum* and *H. suspectum* is summarized and critiqued by Campbell and Lamar (2004) and Beck (2005), and Beaman et al. (2006) provided a literature reference summary of the Helodermatidae. Presently, four subspecies of *H. horridum* are recognized (Figs. 1–5).

Mexican beaded lizard: *H. h. horridum* (Wiegmann 1829)
Río Fuerte beaded lizard: *H. h. exasperatum* Bogert and Martín del Campo 1956

Chiapan beaded lizard: *H. h. alvarezi* Bogert and Martín del Campo 1956
Guatemalan beaded lizard: *H. h. charlesbogerti* Campbell and Vannini 1988

The four subspecies of *H. horridum* were diagnosed and described on the basis of scutellation, color pattern, and geographical distribution, and we refer the reader to the aforementioned works for detailed descriptions and taxonomic keys. The characters used by Bogert and Martín del Campo (1956) and Campbell and Vannini (1988) to diagnose the subspecies have been reevaluated as to their stability, albeit informally (Campbell and Lamar 2004; Beck 2005). Poe and Wiens (2000) and Douglas et al. (2007) discussed the problem of character stability in phylogenetic analyses. Kraus (1988), for example, commented that reasonable evidence for character stability, and thus its usefulness as a shared-derived character (apomorphy), was the occurrence of a discrete trait in adults at a frequency of 80% or greater. In our character mapping analysis using published morphological characters (discussed below), character stability was a major assumption. Consequently, further research is warranted for substantiation.

Geographic distribution.—The geographic distribution of *Heloderma horridum* extends from southern Sonora and adjacent western Chihuahua, in Mexico, southward to eastern and southern Guatemala (Campbell and Lamar 2004; Beck 2005; Anzueto and Campbell 2010; Domiguez-Vega et al. 2012).

The Río Fuerte Beaded Lizard (*H. h. exasperatum*) inhabits the foothills of the Sierra Madre Occidental, within the drainage basins of the Río Mayo and Río Fuerte of the Sonoran-Sinaloan transition subtropical dry forest in southern Sonora, extreme western Chihuahua, and northern Sinaloa (Campbell and Lamar 2004; Beck 2005). Its distribution closely matches the fingers of SDTFs within this region, but it has also been encountered in pine-oak forest at 1,400 m near Alamos, Sonora (Schwalbe and Lowe 2000). Bogert and Martín del Campo (1956) commented that as far as their records indicated, a considerable hiatus existed between the distribution of *H. h. exasperatum* (to the north) and *H. h. horridum* (to the south), but owing to the narrow contact between the supranasal and postnasal in *H. h. horridum* from Sinaloa, intergradation might be found in populations north of Mazatlán. Based on this information, Beck (2005: 24) stated, "…in tropical dry forest habitats north of Mazatlan, Sinaloa, *H. h. exasperatum* likely intergrades with *H. h. horridum*." Definitive data on intergradation remains unreported, however, and published distribution maps have incorporated that assumption (e.g., Campbell and Lamar 2004; Beck 2005). Campbell and Lamar (2004, p. 104) show a single example of *H. suspectum* from El Dorado in west-central Sinaloa, Mexico (deposited in the American Museum of Natural History [90786]), a locality 280 km south from northern records in Río del Fuerte, Sinaloa.

Fig. 1. A. Adult Río Fuerte beaded lizard (*Heloderma horridum exasperatum*) in a defensive display (Alamos, Sonora). **B.** Adult Río Fuerte beaded lizard raiding a bird nest (Alamos, Sonora). *Photos by Thomas Wiewandt.*

Fig. 2. Adult Mexican beaded lizard (*H. h. horridum*) observed on 11 July 2011 at Emiliano Zapata, municipality of La Huerta, coastal Jalisco, Mexico. *Photo by Javier Alvarado.*

Fig. 3. Adult Chiapan beaded lizard (*Heloderma horridum alvarezi*) from Sumidero Canyon in the Río Grijalva Valley, east of Tuxtla Gutiérrez, Chiapas, Mexico. *Photo by Thomas Wiewandt.*

Fig. 4. Adult Guatemalan beaded lizard (*Heloderma horridum charlesbogerti*) from the Motagua Valley, Guatemala. *Photo by Daniel Ariano-Sánchez.*

Fig. 5. A. Juvenile *Heloderma horridum exasperatum* (in situ, Álamos, Sonora, Mexico). *Photo by Stephanie Meyer.*
B. Neonate *Heloderma h. horridum* (wild-collected July 2011, Chamela, Jalisco). *Photo by Kerry Holcomb.*
C. Neonate *Heloderma horridum alvarezi* (Río Lagartero Depression, extreme western Guatemala).
Photo by Quetzal Dwyer.
D. Neonate *Heloderma horridum charlesbogerti* (hatched at Zoo Atlanta in late 2012). *Photo by David Brothers, courtesy of Zoo Atlanta.*

Owing to this unusual location, we suggest a re-examination of this museum specimen to verify its identity. Neonates and juveniles of *H. h. exasperatum* resemble adults in color pattern (Fig. 5a), but they show greater contrast (i.e., a pale yellow to nearly white pattern on a ground color of brownish-black). Also, their color pattern can be distinguished from that of adults (e.g., no yellow speckling between the tail bands), and an ontogenetic increase in yellow pigment occurs (Bogert and Martín del Campo 1956; Beck 2005).

The Mexican beaded lizard (*H. h. horridum*), the subspecies with the most extensive distribution, occurs primarily in dry forest habitats from southern Sinaloa southward to Oaxaca, including the states of Jalisco, Nayarit, Colima, Michoacán, and Guerrero, and inland into the states of México and Morelos (Campbell and Lamar 2004; Beck 2005). Monroy-Vilchis et al. (2005)

recorded an observation of this taxon at mid elevations (e.g., 1861 m) in pine-oak woodlands in the state of México. Campbell and Vannini (1988), citing Álvarez del Toro (1983), indicated the probability of areas of intergradation between *H. h. horridum* and *H. h. alvarezi*, in the area between the Isthmus of Tehuantepec and Cintalapa, Chiapas. Nonetheless, Álvarez del Toro (1983) stated that individuals of beaded lizards with yellow markings (a coloration character present in *H. h. horridum*) are found in the region from Cintalapa to the Isthmus of Tehuantepec, as well as in dry areas along the coast from Arriaga (near the Isthmus of Tehuantepec) to Huixtla (near the Guatemalan border). Literature information on intergradation between these two subspecies is inconclusive and, therefore, will require further investigation. Neonates and juveniles of *H. h. horridum*, like those of *H. h. exasperatum*, resemble adults in color pattern (Fig. 5b), but their color contrast is greater (Bogert and Martín del Campo 1956; Beck 2005).

The Chiapan beaded lizard (*H. h. alvarezi*) inhabits dry forests in the Central Depression (Río Grijalva

Depression) of central Chiapas and the Río Lagartero Depression in extreme western Guatemala (Campbell and Lamar 2004; Beck 2005; Johnson et al. 2010; Wilson et al. 2010: p. 435). This taxon is unique among the subspecies in that it undergoes an ontogenetic increase in melanism, whereby it tends to lose the juvenile color pattern (Bogert and Martín del Campo 1956; Beck 2005). Neonates and juveniles often are distinctly marked with yellow spots and bands, including on the tail (Fig. 5c), whereas the color pattern of adults gradually transforms to an almost uniform dark brown or gray. Black individuals, however, are uncommon. Yellow banding on the tail, a characteristic typical of the other subspecies of beaded lizards, (Fig. 2), is essentially absent in adults (Bogert and Martín del Campo 1956; Beck 2005).

The Guatemalan beaded lizard (*H. h. charlesbogerti*) inhabits the Río Motagua Valley, in the Atlantic versant of eastern Guatemala (Campbell and Vannini 1988). Recently, however, Anzueto and Campbell (2010) reported three specimens from two disjunct populations on the Pacific versant of Guatemala, to the southwest of the Motagua Valley. Neonates resemble adults in color pattern, though they tend to be paler (Fig. 5d).

In summary, the distribution of *H. horridum* is fragmented throughout its extensive range and corresponds closely with the patchy distribution of SDTFs in Mexico and Guatemala (Beck 2005; Miles et al. 2006; Domínguez-Vega et al. 2012). The distribution of the Guatemalan beaded lizard (*H. h. charlesbogerti*) is distinctly allopatric (Campbell and Vannini 1988; Beck 2005; Ariano-Sánchez 2006; Anzueto and Campbell 2010).

3. Molecular assessment

Douglas et al. (2010) provided the first detailed molecular-based (mtDNA, nDNA) analysis of the phylogeographic diversity of helodermatid lizards, which is available at www.cnah.org/cnah_pdf.asp. Two authors (GWS, DDB) of this paper were co-authors. Specifically, Douglas et al. (2010) used a "conservation phylogenetics" approach (Avise 2005, 2008; Avise et al. 2008), which combines and emphasizes the principles and approaches of genetics and phylogeography and how they can be applied to describe and interpret biodiversity.

Methods.—Douglas et al. (2010) sampled 135 locality-specific individuals of *Heloderma* (48 *H. horridum*, 87 *H. suspectum*) from throughout their range (their ingroup). The outgroup taxa included multiple lineages of lizards and snakes, with an emphasis on anguimorphs. Based on both morphological and DNA-based analyses, all authorities have recognized the extant helodermatid lizards as monotypic (a single genus, *Heloderma*), and as members of a larger monophyletic assemblage of lizards termed the Anguimorpha (Pregill et al. 1986; Estes et al. 1988; Townsend et al. 2004; Wiens et al. 2010, 2012; Gauthier et al. 2012). This lineage includes the well-known varanids (*Varanus*), alligator lizards and their relatives

(Anguidae), as well as such relatively obscure taxa as the Old World Lanthanotidae (*Lanthanotus*) and Shinisauridae (*Shinisaurus*), and the New World Xenosauridae (*Xenosaurus*). The mtDNA analyses in Douglas et al. (2010) were rooted with the tuatara (*Sphenodon punctatus*), and Bayesian and maximum parsimony (MP) analyses were conducted using Mr. Bayes (Hulsenbeck and Rohnquist 2001).

Douglas et al. (2010) used sequence data from both mitochondrial (mt) DNA and nuclear (n) DNA as molecular markers in their phylogenetic analyses. Specifically, they discussed reasons for selecting mtDNA regions ATPase 8 and 6, and the nDNA introns alpha-enolase (ENOL) and ornithine decarboxylase (OD). The utility of combining mt- and nDNAs (supertree) in recovering phylogenetic signals has been discussed (Douglas et al. 2007, 2010), yet each of these markers and the procedure of combining sequence data have both benefits and pitfalls (Wiens 2008; Castoe et al. 2009). Long-branch attraction and convergence, for example, can result in misleading relationships (Bergsten 2005; Wiens 2008; Castoe et al. 2009). The tools for detecting and potentially correcting these problems have been discussed (e.g., Castoe et al. 2009; Assis and Rieppel 2011).

Results and discussion.—Douglas et al. (2010) recovered the genus *Heloderma* as monophyletic (Helodermatidae), with *H. horridum* and *H. suspectum* as sister taxa. In a partitioned Bayesian analysis of mtDNA, Helodermatidae was recovered as sister to the anguimorph clade (*Shinisaurus* (*Abronia* + *Elgaria*)), which in turn was sister to the clade *Lanthanotus* + *Varanus*. Recent molecular studies of squamates by Wiens et al. (2012, see references therein) recovered a similar topology to that of Douglas et al. (2010). However, an extensive morphological analysis by Gauthier et al. (2012) supported a traditional topology of *Heloderma* as sister to varanids and *Lanthanotus borneensis* (see Estes et al. 1986; Pregill et al. 1988). In Douglas et al. (2010), a partitioned Bayesian analysis of the nuclear marker alpha-enolase (intron 8 and exon 8 and 9), however, recovered *Heloderma* as sister to a monophyletic *Varanus*. Using a combined analysis of morphology (extant and fossil data), mitochondrial, and nuclear markers, Lee (2009) recovered Varanidae as sister to the clade Helodermatidae + Anguidae. In a combined approach, Wiens et al. (2010) recovered results that were similar to those of Lee (2009). A recent DNA-based analysis of Squamata by Pyron et al. (2013) examined 4151 species (lizards and snakes), and they recovered Helodermatidae as sister to the clade Anniellidae + Anguidae. Moreover, they recovered the clade Varanidae + Lanthanotidae as sister to Shinisauridae.

How do systematists deal with this type of incongruity (discordance) in studies that use different types (e.g., morphology vs. molecular) of phylogenetic markers? Recently, Assis and Rieppel (2011) and Losos et al. (2012) discussed the common occurrence of discordance between molecular and morphological phylogenetic

analyses. Specifically, with respect to discordance, Assis and Rieppel (2011) stated that, "...the issue is not to simply let the molecular signal override the morphological one. The issue instead is to make empirical evidence scientific by trying to find out why such contrastive signals are obtained in the first place." We concur with their opinions, and thus further research is warranted to resolve such conflicts in the phylogeny of anguimorph squamates.

Relationships among the four subspecies of *H. horridum* recovered in the analysis by Douglas et al. (2010, p. 158–159, fig. 3a, b) are depicted in Fig. 6. This topology was derived from a partitioned Bayesian analysis of the mtDNA regions ATPase 8 and 6. The Gila monster (*H. suspectum*) was the immediate outgroup. Two sets of sister pairs of beaded lizards were recovered: *H. h. exasperatum* (HHE) + *H. h. horridum* (HHH), and *H. h. alvarezi* (HHA) + *H. h. charlesbogerti* (HHC). The current subspecific designations for *H. horridum* were robustly supported (concordant) by these genetic analyses. Unlike results obtained for Gila monsters (*H. suspectum*), haplotype and genotype data for *H. horridum* were both diverse and highly concordant with the designated subspecies and their respective geographic distributions.

Douglas et al. (2010) generated pair-wise net sequence divergence (%) values based on their recovered relationships (Table 1, Fig. 6). The greatest divergence was between HHE and HHC (9.8%), and the least between HHA

and HHC (1%). The former pair represents populations widely separated in distribution (southern Sonora, Mexico vs. eastern Guatemala), whereas the latter are much more closely distributed (Chiapas, Mexico vs. eastern Guatemala). The nominate subspecies (*Heloderma h. horridum*) differed from the other three subspecies of beaded lizards, from 5.4% to 7.1%.

Table 1. Pair-wise net sequence divergence (%) values between the four subspecies of the beaded lizard (*Heloderma horridum*) derived from a partitioned Bayesian analysis of the mtDNA regions ATPase 8 and 6 (modified from Douglas et al. 2010, pp. 157–159, 163; fig. 3a, b, tables 1 and 3). Values in parentheses denote evolutionary divergence times, which represent mean age. Mean age is the time in millions of years (mya) since the most-recent common ancestor (tree node) and is provided for the sister clades HHE-HHH and HHA-HHC (Fig. 6). Beaded lizards and Gila monsters (*H. suspectum*) are hypothesized to have diverged from a most-recent common ancestor in the late Eocene ~35 mya (Douglas et al. 2010, p. 163). Percent sequence divergence was greatest for HHC-HHE, and was lowest for HHA-HHC. See text for further details.

	HHA	HHC	HHE	HHH
HHA	—			
HHC	1% (3.02)	—		
HHE	9.3%	9.8%	—	
HHH	5.4%	6.2%	7.1% (4.42)	—

HHA = *H. h. alvarezi*; HHC = *H. h. charlesbogerti*; HHE = *H. h. exasperatum*; HHH = *H. h. horridum*.
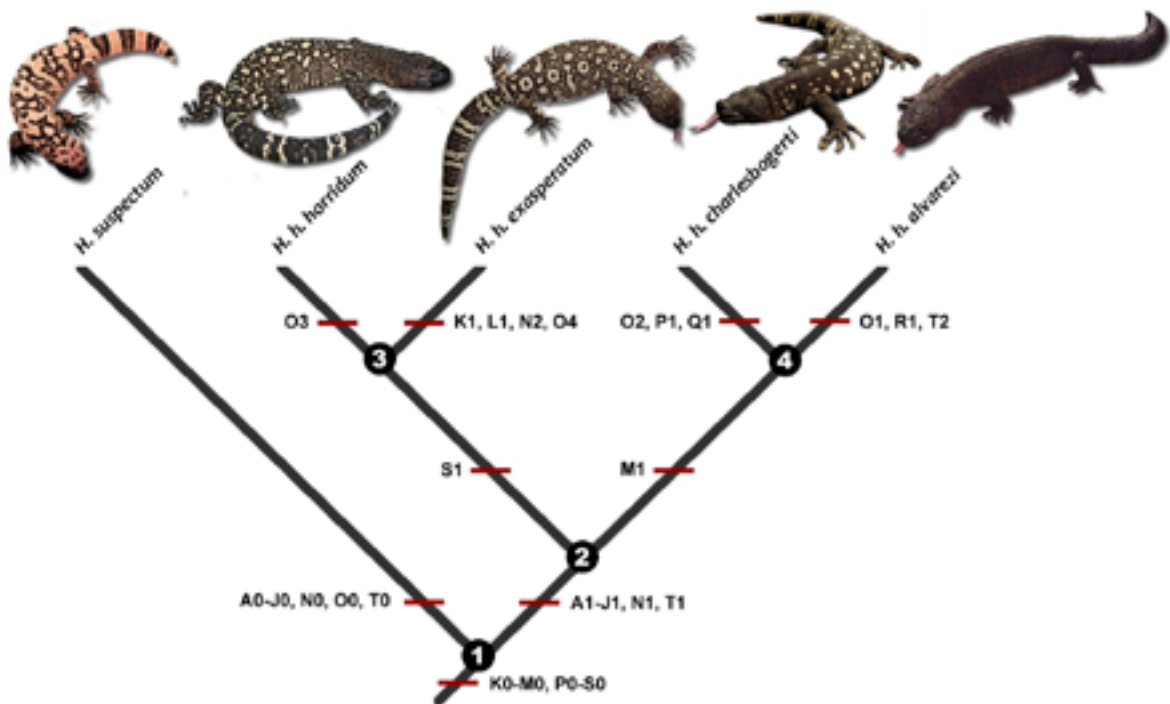
Fig. 6. Character mapping analysis. Tree topology and node dates based on Douglas et al. (2010). Morphological characters (Table 2) were mapped via parsimony and outgroup methods using the software program Mesquite (Maddison and Maddison 2011). Node 1 = Late Eocene (~35 million years ago, mya); Node 2 = 9.71 mya; Node 3 = 4.42 mya; and Node 4 = 3.02 mya (see Table 1). See text for details of the analysis.

Table 2. Morphological characters used for the character mapping analysis (see Table 1, Fig. 6). See text for details.

Character	State	Designation
Tail length	41–55% of snout-to-vent length	A0
	≥ 65% of snout-to-vent length	A1
Number of caudal vertebrae	25–28	B0
	40	B1
Number of transverse rows of ventromedial caudal scales (vent to tail tip) greater than 62	absent	C0
	present	C1
Usually one pair of enlarged preanal scales	present	D0
	absent	D1
First pair of infralabials usually in contact with chin shields	present	E0
	absent	E1
Number of maxillary teeth	8–9	F0
	6–7	F1
Upper posterior process of splenial bone	overlaps inner surface of coronoid	G0
	does not overlap coronoid	G1
Number of black tail bands (including black terminus on tail of juveniles)	4–5	H0
	6–7	H1
Adult total length	< 570 mm	I0
	> 600 mm	I1
Tongue color	black or nearly so	J0
	pink	J1
Supranasal-postnasal association	in contact	K0
	separated by first canthal	K1
Association of second supralabial and prenasal/nasal plates	in contact	L0
	separated by lorilabial	L1
Shape of mental scute	shield-shaped (elongate and triangular)	M0
	wedge-shaped (twice as long as wide)	M1
Dominant adult dorsal coloration	orange, pink	N0
	black or dark brown	N1
	yellow	N2
Adult dorsal yellow spotting	absent	O0
	extremely low	O1
	low	O2
	med	O3
	high	O4
Mental scute	scalloped edges absent	P0
	moderately scalloped edges	P1
Enlarged preanal scutes in some females	absent	Q0
	present	Q1
Ontogenetic melanism	absent	R0
	present	R1
Spots on tail in adults	absent	S0
	present	S1
Bands on tail	black	T0
	yellow	T1
	absent	T2

4. Character mapping analysis

A character mapping analysis (CMA) is one of several robust tools used in comparative biology to comprehend the distribution of traits (e.g., morphology), often by explicitly utilizing molecular phylogenetic information (Brooks and McLennan 1991; Harvey and Pagel 1991; Martins 1996; Freeman and Herron 2004; Maddison and Maddison 2011; for a critique, see Assis and Rieppel 2011). Specifically, the CMA aims to provide insights to the origin, frequency, and distribution of selected traits formally expressed onto a tree (e.g., Schuett et al. 2001, 2009; Fenwick et al. 2011). These procedures also are potentially useful in disentangling homology from homoplasy (Freeman and Herron 2004). Furthermore, the CMA provides a framework for testing hypotheses of adaptive evolution and the identification of species (Harvey and Pagel 1991; Futuyma 1998; Freeman and Herron 2004; Schuett et al. 2001, 2009; Maddison and Maddison 2011). However, CMA does not replace a strict phylogenetic analysis of morphological traits (Assis and Rieppel 2011).

Here, we used character mapping to investigate the morphological traits of the four subspecies of *H. horridum*, to gain insights on the distribution, divergence, and homology (e.g., shared-derived traits, such as possible autapomorphies) of these traits.

Methods.—We used published morphological data on *Heloderma* (Bogert and Martín del Campo 1956; Campbell and Vannini 1988; Campbell and Lamar 2004; Beck 2005) and selected 20 morphological characters for the CMA (Table 2). All characters were coded as binary (i.e., 0, 1) or multi-state (e.g., 0, 1, 2). Non-discrete multi-state characters (e.g., color pattern) were ordered from lowest to highest values. Character polarity was established by using *H. suspectum* as the outgroup. The CMA traced each character independently by using the outgroup analysis and parsimony procedures in Mesquite (Maddison and Maddison 2011), and we combined the individual results onto a global tree.

Results and discussion.—The CMA results (Fig. 6) show that multiple morphological traits are putative apomorphies or autapomorphies (traits unique to a single taxon) for the various *H. horridum* clades (subspecies) delimited in the molecular tree recovered by Douglas et al. (2010). Although we had a priori knowledge of specific and unique traits (presumptive autapomorphies) used to diagnose each of the subspecies, the CMA presents them in a phylogenetic and temporal framework. Our results show trends in scutellation (e.g., presence-absence, relative positions), relative tail length, and body color pattern, including ontogenetic melanism. Are the characters we used in the CMA stable in the subspecies? That question remains for future investigation; however, we have no evidence to the contrary. Indeed, we anticipate that these characters, and others likely to be revealed through detailed studies, will exhibit stability.

Importantly, each of these traits is amenable to further investigation and formal tests. For examples, what is the evolutionary and ecological significance of tongue color differences in beaded lizards (always pink) and Gila monsters (always black), the extreme differences in adult dorsal color pattern in *H. h. exasperatum* (yellow is predominant) vs. *H. h. alvarezi* (dark brown and patternless predominate), and ontogenetic melanism in *H. h. alvarezi*? As we discussed, beaded lizards occupy similar seasonally dry tropical forests, yet each of the subspecies exhibits pronounced molecular and morphological differentiation.

Similar types of questions concerning adaptation have used a CMA to explore social systems and sexual dimorphisms in lizards (Carothers 1984), male fighting and prey subjugation in snakes (Schuett et al. 2001), types of bipedalism in varanoids (Schuett et al. 2009), and direction of mode of parity (oviparous vs. viviparous) in viperids (Fenwick et al. 2011).

Subspecies and the Taxonomy of Beaded Lizards

Introduced in the late 19th century by ornithologists to describe geographic variation in avian species, the concept of subspecies and trinomial taxonomy exploded onto the scene in the early 20th century (Bogert et al. 1943), but not without controversy. The use of subspecies has been both exalted and condemned by biologists (see perspectives by Mallet 1995; Douglas et al. 2002; Zink 2004; Fitzpatrick 2010). Thousands of papers have been published in an attempt to either bolster the utility and promulgation of subspecies, or to denounce the concept as meaningless and misleading in evolutionary theory (Wilson and Brown 1953; Zink 2004). What is the problem? One common critical response is that the subspecies concept lacks coherence in meaning, and hence is difficult to comprehend (Futuyma 1998; Zink 2004). Moreover, the use of subspecies often masks real diversity (cryptic species, convergence) or depicts diversity that is non-existent or only trivial (e.g., lack of support in DNA-based analyses; Zink 2004). Indeed, as John Fitzpatrick attests (2010, p. 54), "The trinomial system cannot accurately represent the kind of information now available about genetic and character variation across space. Instead, even more accurate tools are being perfected for quantitative, standardized descriptions of variation. These analyses—not subspecies classifications—will keep providing new scientific insights into geographic variation."

Even with the identification of a variety of problems, many authors recommend that complete abandonment of the trinomial category in taxonomy is not necessary nor advised (e.g., Mallett 1995, Hawlitschek et al. 2012). Unfortunately, a consensus among biologists concerning the use of subspecies is not likely to emerge (Fitzpatrick 2010). In step with Fitzpatrick's (2010) comments, we

contend that the plethora of variation detected in organisms must be approached in a modern sense that does not rely upon a cumbersome and outdated taxonomic system. Indeed, we anticipate that the description of geographic variation in organisms, once emancipated from infraspecific taxonomy, will actually accelerate our understanding of variation and its complexities. In our view, the confusion in recognizing subspecies can also mislead conservation planning, and it has on more than one occasion (e.g., the dusky seaside sparrow, see Avise and Nelson 1989). We thus agree with Wilson and Brown (1953), Douglas et al. (2002), Zink (2004), Fitzpatrick (2010) and others in their insightful criticisms leveled at the subspecies concept and the use of trinomials in taxonomy. Other authors have echoed similar views (Burbrink et al., 2000; Burbrink 2001; Douglas et al. 2007; Tobias et al. 2010; Braby et al. 2011; Hoisington-Lopez 2012; Porras et al. 2013).

Given our reassessment of molecular (mt- and nDNAs), phylogeographic, morphological, and biogeographic evidence, we elevate the subspecies of *Heloderma horridum* to the rank of full species (Wiley, 1978; Zink 2004; Tobias et al. 2010; Braby et al. 2011; Porras et al. 2013). Indeed, Douglas et al. (2010, p. 164) stated that, "… unlike *H. suspectum*, our analyses support the subspecific designations within *H. horridum*. However, these particular lineages almost certainly circumscribe more than a single species … Thus, one benefit of a conservation phylogenetic perspective is that it can properly identify biodiversity to its correct (and thus manageable) taxonomic level." Accordingly, based on multiples lines of concordant evidence, we recognize four species of beaded lizards. They are:

> Mexican beaded lizard: *Heloderma horridum* (Wiegmann 1829)
>
> Río Fuerte beaded lizard: *Heloderma exasperatum* (Bogert and Martín del Campo 1956)
>
> Chiapan beaded lizard: *Heloderma alvarezi* (Bogert and Martín del Campo 1956)
>
> Guatemalan beaded lizard: *Heloderma charlesbogerti* (Campbell and Vannini, 1988)

In the above arrangement, we do not recognize subspecies and vernacular names remain unchanged. The geographic distribution of the four species of beaded lizards is presented in Fig. 7. Locality data for the map were derived from Bogert and Martín del Campo (1956), Campbell and Vannini (1988), Schwalbe and Lowe (2000), Lemos-Espinal et al. (2003), Campbell and Lamar (2004), Beck (2005), Monroy-Vilchis et al. (2005), Ariano-Sánchez and Salazar (2007), Anzueto and Campbell (2010), Domiguez-Vega et al. (2012), and Sánchez-De La Vega et al. (2012). The "?" on the map (coastal Oaxaca, municipality: San Pedro Tututepec) denotes a jet-black adult specimen photographed by Vicente Mata-Silva (pers. comm.) in December 2010. The validity of this record is questionable owing to its striking coloration resemblance to *H. alvarezi* from the Central Depression (Río Grijalva Depression) of Chiapas and extreme western Guatemala, rather than to *H. horridum*. Although the individual might represent an isolated population of *H. alvarezi*, further study in this area of Oaxaca is required to rule out human activity as an agent (e.g., displacement).

Beaded Lizards and Seasonally Dry Tropical Forests

The key to understanding the evolution and biogeography of beaded lizards and the prospects for implementing meaningful conservation measures is through a recognition of the biomes they occupy, which we emphasize are the widely but patchily distributed low elevation seasonally dry tropical forests (SDTFs; see Trejo and Dirzo 2000; Campbell and Lamar 2004; Beck 2005; Ariano-Sánchez 2006; Miles et al. 2006; Pennington et al. 2006; Dirzo et al. 2011; Domiguez-Vega et al. 2012).

The evolution of SDTFs in Mesoamerica is a complex evolutionary scenario (Stuart 1954, 1966), and our understanding of their origin and temporal diversification is in its infancy (Janzen, 1988; Becerra 2005; Pennington et al. 2006; Dirzo et al. 2011; De-Nova et al. 2012). One approach to grapple with complex issues such as the origin and historical construction of SDTFs in Mesoamerica has been to examine a single but highly diverse plant taxon within a phylogenetic (phylogenomic) backdrop. This approach, accomplished by Becerra (2005) and more recently by De-Nova et al. (2012), uses the woody plant (tree) *Bursera* (Burseracae, Sapindales), a highly diverse genus (> 100 species) with a distribution in the New World and emblematic of most dry forest landscapes (De-Nova et al. 2012). Owing to this diversity, coupled with extensive endemism, this taxon has yielded valuable information that serves as a reasonable proxy for diversification and expansion of the SDTF biomes (Dick and Pennington 2012). Hence, plant (angiosperm) species richness and expansion of SDTF biomes in Mesoamerica is hypothesized to parallel the diversification of *Bursera* (Dick and Wright 2005).

Based on both plastid and nuclear genomic markers that were analyzed using fossil-calibrated techniques and ancestral habitat reconstruction, the origin of *Bursera* in Mesoamerica is hypothesized to be in northwestern Mexico in the earliest Eocene (~50 mya), with subsequent extensive diversification and southern expansion along the Mexican Transvolcanic Belt in the Miocene, especially ~7–10 mya (De-Nova et al. 2012). Accelerated clade diversification of *Bursera* and its sister genus *Commiphora* occurred during the Miocene, a period of increased aridity likely derived from seasonal cooling and rain shadow effects (Dick and Wright 2005). Although causal connections are complex, they include global tectonic pro-
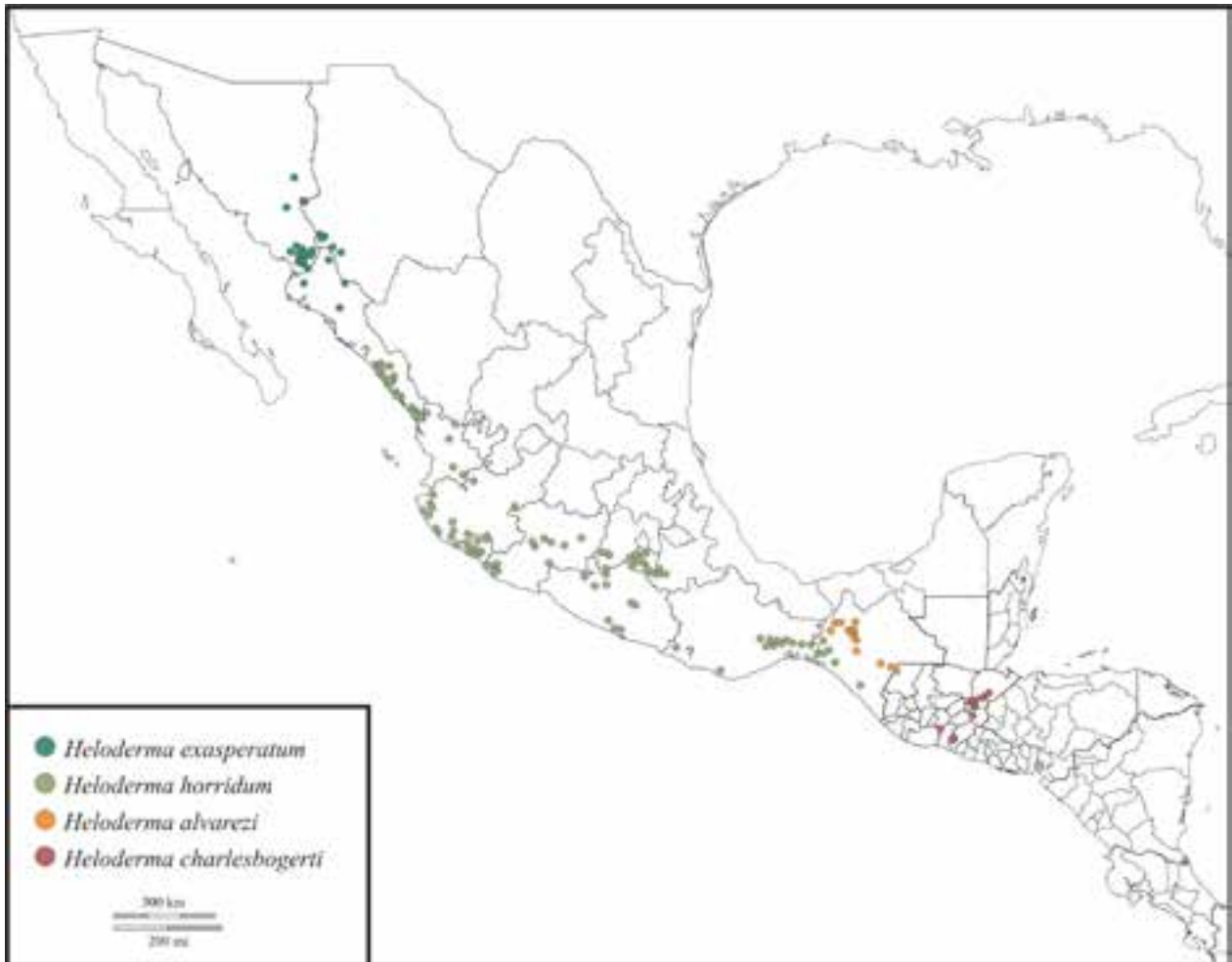
Fig. 7. The distribution of beaded lizards in Mexico and Guatemala. Colored dots represent verified sightings (populations) and museum records. Note the fragmented populations of all four species, which closely approximates the patchy distribution of seasonal dry tropical forests (see map in Brown and Lowe [1980]). See text for explanation of question marks ("?") and other details.

cesses, orogenic activities (uplifting of the Sierra Madre Occidental and Sierra Made Oriental) and local volcanism (Dick and Wright 2005; De-Nova et al. 2012). De-Nova et al. (2012) concluded by emphasizing that their phylogenomic analysis of *Bursera* points to high species diversity of SDTFs in Mesoamerica that derives from within-habitat speciation rates that occurred in the envelope of increasing aridity from the early Miocene to the present. Furthermore, they stated (p. 285), "This scenario agrees with previous suggestions that [angiosperm] lineages mostly restricted to dry environments in Mexico resulted from long periods of isolated evolution rather than rapid species generation...."

Beaded Lizard Evolution and Diversification

The phylogenetic analyses of *Heloderma horridum* (sensu lato) by Douglas et al (2010) provided fossil-calibrated estimates of divergence times, which allow us to draw connections to the origin and diversification of SDTFs in Mesoamerica (Table 1, Fig. 6). Based on those analyses, *H. horridum* (sensu lato) and *H. suspectum* are

hypothesized to have diverged from a most-recent common ancestor in the late Eocene (~35 mya), which corresponds to the establishment of *Bursera* in northwestern Mexico. Subsequent diversification (cladogenesis) of the beaded lizards occurred during the late Miocene (9.71 mya), followed by a lengthy period of stasis of up to 5 my, with subsequent cladogenesis extending into the Pliocene and Pleistocene. Of particular interest is that this scenario approximately parallels the diversification and southern expansion of SDTFs (Dick and Wright 2005; De-Nova et al. 2012). Accordingly, based on the above discussion of SDTFs and phylogenetic analyses, we suggest that beaded lizard lineage diversification resulted from long periods of isolated (allopatric) evolution in SDTFs. Douglas et al. (2010) referred to the fragmented tropical dry forests of western Mexico as "engines" for diversification. The extralimital distribution of *H. exasperatum* and *H. horridum* into adjacent pine-oak woodland and thorn scrub biomes appears to be relatively uncommon (Schwalbe and Lowe 2000; Beck 2005; Monroy-Vilchis et al. 2005).

Conservation of Beaded Lizards

A primary aim of this paper is to provide a useful and accurate synthesis of information on the taxonomy of beaded lizards that will lead to informed decisions regarding their conservation (see Douglas et al., 2010). Until recently, *H. horridum* (sensu lato) was designated as Vulnerable on the World Conservation Union (IUCN) Red List. In 2007, that designation was changed to Least Concern based on more stringent criteria (Canseco-Marquez and Muñoz 2007; categories and criteria version 3.1). The 2007 IUCN Red List also determined that, "Additional research is needed into the taxonomic status, distribution and threats to this species" (Canseco-Marquez and Muñoz 2007). The critically endangered status of *H. h. charlesbogerti* (sensu lato) in Guatemala (Ariano-Sánchez 2006; Ariano-Sánchez and Salazar 2007) has not altered the current IUCN Red List designation of this taxon, because population trends of other beaded lizards in Mexico remain "unknown" (www.iucnredlist.org/search; see International Reptile Conservation Foundation, IRCF; www.ircf.org). As more information on the population status of the newly elevated beaded lizards becomes available, in view of their fragmented distributions and threats to their habitats, the IUCN likely will designate these taxa as Vulnerable or a higher threat category (see our EVS analysis below). For example, *H. exasperatum*, *H. alvarezi*, and *H. charlesbogerti* all occupy limited areas of SDTF (Beck 2005).

In Mexico, helodermatid lizards are listed as "threatened" (amenazadas) under the Mexican law (NOM-059-SEMARNAT-2010), legislation comparable to that in the United States Endangered Species Act. The threatened category from Mexican law coincides, in part, with the "Vulnerable" category of the IUCN Red List. This document defines "threatened" as species or populations that could become at risk of extinction in a short to medium period if negative factors continue to operate that reduce population sizes or alter habitats. *Heloderma h. charlesbogerti* (sensu lato) is listed on the Guatemalan Lista Roja (Red List) as "endangered," with approximately 200–250 adult individuals remaining in under 26,000 ha of its natural habitat of SDTF and thorn scrub, (Ariano-Sánchez 2006).

Furthermore, *H. h. charlesbogerti* (sensu lato) is listed on CITES Appendix I, a designation that includes species threatened with extinction (see CITES document appended to Ariano-Sánchez and Salazar 2007). Trade in CITES Appendix I species is prohibited except under exceptional circumstances, such as for scientific research (CITES 2007). The remaining taxa of *Heloderma horridum* (sensu lato) (*H. h. alvarezi*, *H. h. exasperatum*, and *H. h. horridum*) are listed on Appendix II of CITES (CITES 2007). International trade in Appendix II species might be authorized under an export permit, issued by the originating country only if conditions are met that show trade will not be detrimental to the survival of the species in the wild. The United States Fish & Wildlife Service issues permits only if documentation is provided proving legal origin, including a complete paper trail back to legal founder animals. This procedure allows the importation of beaded lizards into the United States to be tightly regulated (in theory), and also subjects such imports to provisions of the Lacey Act that control commerce in illegally obtained fish and wildlife (Beck 2005).

Beaded Lizards: Denizens of Endangered SDTFs

Although occasional sightings of beaded lizards have been reported from mid elevation pine-oak woodlands, all four species primarily inhabit lowland SDTFs and rarely in associated thorn scrub, in both Mexico and Guatemala (Schwalbe and Lowe 2000; Lemos-Espinal et al. 2003; Campbell and Lamar 2004; Beck 2005; Monroy-Vilchis et al. 2005; Ariano and Salazar 2007; Domiguez-Vega et al. 2012). Thus, the optimal measure to reduce threats to beaded lizards is to maintain the integrity of their tropical dry forest habitats. Current threats to beaded lizards throughout their range include habitat loss, road mortality, poaching, and illegal trade (Beck 2005; Miles et al. 2006; Golicher et al. 2012). Habitat loss takes many forms, from the conversion of SDTFs to areas of agriculture and cattle ranching, to forest fragmentation owing to roads and other forms of development (Pennington et al. 2006). Degradation from human-introduced invasive (exotic) organisms and fire also are contributing factors (Beck 2005).

When the Spaniards arrived in the Western Hemisphere, Mesoamerican SDTFs covered a region stretching from Sonora (Mexico) to Panama, an area roughly the size of France (~550,000 km^2). Today, only 0.1% of that region (under 500 km^2) has official conservation status, and less than 2% remains sufficiently intact to attract the attention of conservationists (Janzen 1988; Hoekstra et al. 2005). Of all 13 terrestrial biomes analyzed by Hoekstra et al. (2005), the SDTF biome has the third highest conservation risk index (ratio of % land area converted per % land area protected), far above tropical wet forest and temperate forest biomes (Miles et al. 2006).

Mexico ranks among the most species rich countries in the world (García 2006; Urbina-Cardona and Flores-Villela 2010; Wilson and Johnson 2010; Wilson et al. 2010, 2013). Nearly one-third of all the Mexican herpetofaunal species are found in SDTFs (García 2006; De-Nova et al. 2012). Neotropical dry forests span over 16 degrees of latitude in Mexico, giving way to variation in climatic and topography that results in a diversity of tropical dry forest types, and a concurrent high proportion of endemism of flora and fauna (García 2006; De-Nova et al. 2012; Wilson et al. 2010; 2013). Mexican seasonally tropical dry forest, classified into seven ecoregions that encompass about 250,000 km^2, has enormous conservation value and has been identified as a hotspot

for conservation priorities (Myers et al. 2000; Sánchez-Azofeifa et al. 2005; García 2006; Urbina-Cardona and Flores-Villela, 2010; Wilson et al. 2010, Mittermeier et al. 2011). The vast majority (98%) of this region, however, lies outside of federally protected areas (De-Nova et al. 2012). With few exceptions, most of the protected areas in Mexico occur in the states of Chiapas and Jalisco, leaving much of the region (e.g., Nayarit and Sinaloa) without government (federal) protection (García 2006).

In Guatemala, less than 10% of an estimated 200,000 ha of original suitable habitat have been established as protected critical habitat in the Motagua Valley for the endangered *H. charlesbogerti* (Nájera Acevedo 2006). A strong effort led by local citizens, conservation workers, biologists, government officials, NGOs, and conservation organizations (e.g., The Nature Conservancy, International Reptile Conservation Association, Zoo Atlanta, and Zootropic) negotiated to have *H. h. charlesbogerti* (sensu lato) placed on CITES Appendix I, to purchase habitat, conduct research, employ local villagers in monitoring the lizards, and promote environmental education (Lock 2009). Similar efforts for beaded lizards have been underway for many years in Chiapas (Mexico), spearheaded at ZooMAT (Ramírez-Velázquez 2009), and in Chamela, Jalisco (www.ibiologia.unam.mx/ebchamela/www/reserva.html). Such efforts will need to expand in the years ahead and will doubtless play a crucial role if we hope to retain the integrity of existing SDTFs inhabited by beaded lizards throughout their range.

Discussion

In this paper, we reassessed the taxonomy of *Heloderma horridum* (sensu lato) using both published information and new analyses (e.g., CMA). We concluded that diversity in beaded lizards is greater than explained by infraspecific differences and that the recognition of subspecies is not warranted, as it obscures diversity. Our decision to elevate the four subspecies of *H. horridum* to full species status is not entirely novel (Beck 2005; Douglas et al. 2010). Furthermore, our taxonomic changes are based on integrative information (i.e., morphology, mt- and nDNA sequence information, biogeography) and changing perspectives on the utility of formally recognizing infraspecific diversity using a trinomial taxonomy (Wilson and Brown 1953; Douglas et al. 2002; Zink 2004; Porras et al. 2013). This decision not only adds to a better understanding of the evolution of helodermatids, but also provides an important evolutionary framework from which to judge conservation decisions with prudence (Douglas et al. 2002).

Below, we delineate and discuss prospective research and conservation recommendations for beaded lizards based on our present review. Borrowing some of the guidelines and recommendations for future research and conservation for cantils, also inhabitants of SDTFs, by Porras et al. (2013), we outline similar ones for the four

species of beaded lizards (*H. alvarezi*, *H. charlesbogerti*, *H. exasperatum*, and *H. horridum*).

Future Research and Conservation Recommendations

1. Throughout this paper we emphasized the importance of SDTFs in the distribution of beaded lizards, yet most SDTFs within their distribution are not Protected Natural Areas (PNAs; Beck 2005; Urbina-Cardona and Flores-Villela 2009; Domiguez-Vega et al. 2012). Accordingly, emphasis should be placed on those areas of SDTFs for prospective research, new conservation projects, and for establishing new PNAs. The protection of beaded lizards must be placed into a larger context of conservation planning. Proper stewardship of SDTFs and other biomes must include meaningful (scientific) protective measures for all of the flora and fauna, rather than piecemeal (e.g., taxon-by-taxon) approaches that lack a cohesive conservation plan (Douglas et al. 2010).

We applaud the efforts of Domíguez-Vega et al. (2012) in identifying conservation areas for beaded lizards; however, we do not agree with all of their conclusions. In particular, based on field experiences by one of us (DDB), we contend that the potential (predicted) range of *H. exasperatum* in Sonora (Mexico) based on the results of their habitat suitability modeling, appears exaggerated and thus may be misleading. In our opinion, their distribution maps (figs. 2 and 3) overestimate the extent of true SDTFs in Sonora, showing their occurrence in a type of biome that is more accurately classified as Sinaloan Thorn Scrub (see the excellent maps in Brown and Lowe 1980; Robichaux and Yetman 2000). In Sonora, beaded lizards (*H. exasperatum*) are rarely found in association with pure thorn scrub, while Gila monsters, in contrast, are frequently encountered in that type of habitat (Schwalbe and Lowe 2000; Beck 2005).

2. With few exceptions, the population viability of beaded lizards is largely unknown (Beck 2005; Ariano-Sánchez 2006; Ariano-Sánchez et al. 2007; Domíguez-Vega et al. 2012). We highly recommend that modern assessments of the four species occur at or near localities where they have been recorded (e.g., Jiménez-Valverde and Lobo 2007). Whereas *H. charlesbogerti*, and to a lesser degree *H. alvarezi* (Ramírez-Velázquez 2009), are receiving international conservation attention, we feel that similar consideration is necessary for *H. exasperatum* owing to its relatively limited geographic range (Sonora, Chihuahua, Sinaloa), the large extent of habitat destruction and fragmentation (Fig. 8), and limited areas receiving protection (Trejo and Dirzo 2000; Domíguez-Vega et al. 2012; see http://www.conanp.gob.mx/regionales/). In 1996, about 92, 000 hectares in the Sierra de Álamos and the upper drainage of the Río Cuchujaqui were declared a biosphere reserve by the Secretary of the Environment and Natural Resources (SEMARNAT 2010), called the

Área de Protección de Fauna y Flora Sierra de Álamos y Río Cuchujaqui (Martin and Yetman 2000; S. Meyer, pers. comm.). Efforts continue in Sonora to set aside additional habitat for conservation, but, other than Alamos, no other areas with true SDTFs presently exist (Robichaux and Yetman 2000; S. Meyer, pers. comm.).

3. Conservation management plans for each of the species of beaded lizards should be developed from an integrative perspective based on modern population assessments, genetic information, and ecological (e.g., soil, precipitation, temperature) and behavioral data (e.g., social structure, mating systems, home range size).

Fig. 8. Destruction of seasonally dry tropical forest near Alamos, Sonora, Mexico. *Photo by Daniel D. Beck.*

Fig. 9. A dead-on-the-road (DOR) *H. exasperatum* (sensu stricto) near Álamos, Sonora, Mexico. Vehicles on paved roads are an increasing threat to beaded lizards, Gila monsters, and other wildlife. *Photo by Thomas Wiewandt.*

Such a conservation plan is in place for the Guatemalan beaded lizard (*H. charlesbogerti*) by CONAP-Zootropic (www.ircf.org/downloads/PCHELODERMA-2Web.pdf). Also, aspects of burgeoning human population growth must be considered, since outside of PNAs these large slow-moving lizards generally are slaughtered on sight, killed on roads by vehicles (Fig. 9), and threatened by persistent habitat destruction primarily for agriculture and cattle ranching (Fig. 10). For discussions on conservation measures in helodermatid lizards, see Sullivan et al. (2004), Beck (2005), Kwiatkowski et al. (2008), Douglas et al. (2010), Domíguez-Vega et al. (2012), and Ariano-Sánchez and Salazar (2013).

In Mexico, the IUCN lists *Heloderma horridum* (sensu lato) under the category of Least Concern. Recently, Wilson et al. (2013) reported the Environmental Vulnerability Score (EVS) for *H. horridum* (sensu lato) as 11. Briefly, an EVS analysis assesses the potential threat status of a given species based on multiple criteria and provides a single score or index value (Wilson and McCranie 2004; Porras et al. 2013; Wilson et al. 2013). High EVS scores (e.g., 17), for example, signify vulnerability. With the taxonomic changes we proposed for beaded lizards, an EVS assessment is thus required for each species. Using the new criteria developed by Wilson et al. (2013; see Porras et al. 2013), we recalculated the EVS for the species of beaded lizards, which are presented below:

H. horridum: 5 + 4 + 5 = 14
H. exasperatum: 5 + 7 + 5 = 17
H. alvarezi: 4 + 6 + 5 = 15
H. charlesbogerti: 4 + 8 + 5 = 17

These recalculated values fall into the high vulnerability category (Wilson et al. 2013; Porras et al. 2013), underscoring the urgency for the development of conservation management plans and long-term population monitoring of all species of beaded lizards. These values thus need to be reported to the appropriate IUCN committees, so immediate changes in status can be made and conservation actions implemented.

Fig. 10. Agave cultivation in Mexico results in the destruction of seasonally dry tropical forests. *Photo by Thomas Wiewandt.*

Fig. 11. Antonio Ramirez Ramírez-Velázquez, a herpetologist, discusses the beauty and importance of beaded lizards (*H. alvarezi,* sensu stricto) to a group of enthusiastic children and their teacher at Zoo Miguel Álvarez del Toro (ZooMAT) in Tuxtla Gutiérrez, Chiapas, Mexico. The zoo was named in honor of its founding director, Señor Miguel Alvarez del Toro, who had a keen academic and conservation interest in beaded lizards. He collected the type specimen of *H. alvarezi* (described in Bogert and Martín del Campo, 1956), which was named in his honor. ZooMAT offers hands-on environmental education programs to schoolchildren and other citizens of southern Mexico. *Photo by Thomas Wiewandt.*

4. We recommend the establishment of zoo conservation (AZA) educational outreach programs, both ex situ and in situ, such as those currently in progress for *H. charlesbogerti* (www.IRCF.org;www.zooatlanta.org) and for *H. alvarezi* in Chiapas (Ramírez-Velázquez, 2009, see Fig. 11). Because of its limited range, destruction of its

natural habitat, small population size (200–250 adults) and endangered status, *H. charlesbogerti* is currently listed as CITES Appendix I (Ariano-Sánchez and Salazar 2007). Given the taxonomic elevation of these taxa, conservation agencies can use these charismatic lizards as flagship species in efforts to publicize conservation efforts in their respective countries at all levels of interest and concern, including education and ecotourism (Beck 2005). Eli Lilly Co., Disney Worldwide Conservation Fund and The Nature Conservancy support the conservation of *H. charlesbogerti* (Ariano-Sánchez and Salazar 2012). Such corporate involvement provides funds and positive public exposure (e.g., social network advertising) that otherwise would not be possible.

5. One of the major conclusions of this paper is that our knowledge of the taxonomy and phylogeography of beaded lizards remains at an elementary level. As discussed, a robust phylogeographic analysis using morphological characters is not available. Our character mapping exercise, for various reasons, is not a substitute procedure for detailed phylogenetic analyses using morphology (Assis 2009; Assis and Rieppel 2011). Other authors have made similar pleas concerning the importance of morphology, including fossils, in phylogenetic reconstruction (Poe and Wiens 2000; Wiens 2004, 2008; Gauthier et al. 2012). Moreover, further studies on the historical biogeography of helodermatids (e.g., ancestral area reconstruction) are needed (e.g., Ronquist 1997, 2001; Ree and Smith 2008). Detailed morpho-logical analyses can be conducted with new tools such as computed tomography (CT) scans of osteological characters of both extant and fossil specimens (Gauthier et al. 2012), and geometric morphometric approaches to external characters (Davis 2012). Furthermore, in the expanding field of "venomics" new venom characters in beaded

lizards will likely be discovered, which might prove useful in phylogenetic analyses (Fry et al. 2009, 2010).

As we progress into the "Age of Genomics" with ever-growing computational advancements (e.g., bioinformatics; Horner et al. 2009), new and exciting methods to explore organismal diversity are opening, including such next-generation approaches as pyrosequencing (microsatellite isolation), establishing transcriptome databases, and whole-genome sequencing (Wiens 2008; Castoe et al. 2011; Culver et al. 2011). Currently, plans are underway to apply pyrosequencing methods to helodermatids to generate a nearly inexhaustible supply of microsatellite markers for a variety of proposed analyses (W. Booth and T. Castoe, pers. comm.). Standing on the shoulders of The Human Genome Project (Culver et al. 2011), and reaping the success of genome projects in other reptilian taxa (Castoe et al. 2011), it is now possible to establish a "Helodermatid Genome Project." Beaded lizards and the Gila monster are especially good candidates for such an investment, especially given the importance of their venom components in medical research and recent pharmaceutical applications (Beck 2005; Douglas et al. 2010; Fry et al. 2009, 2010).

6. An important take-home message from Douglas et al. (2010) is that future conservation efforts will require a robust understanding of phylogenetic diversity (e.g., conservation phylogenetics) to make sensible (logical) and comprehensive conservation plans. For example, the range of *H. horridum* (sensu stricto) is the most expansive of the species of beaded lizards and has not been fully explored with respect to genetic diversity. Accordingly, sampling throughout its range may yield cryptic genetic diversity, perhaps even new species. We emphasize that viable conservation planning must incorporate all intellectual tools available, including those that incorporate old methods (e.g., paleoecological data) but viewed through a new lens (Douglas et al. 2007, 2009; Willis et al. 2010). Wisely, Greene (2005) reminds us that we are still grappling with understanding basic and essential issues concerning the natural history of most organisms. To that end, we must continue in our efforts to educate students and the public of the need for and importance of this branch of science.

7. The new taxonomic arrangement of beaded lizards we proposed will affect other fields of science, such as conservation biology and human medicine (Beck, 2005; Douglas et al., 2010). In Fry et al. (2010, p. 396, table 1), toxins are matched to the subspecies of beaded lizards and Gila monsters. Yet as noted by Beck (2005) and Douglas et al. (2010), the banded Gila monster (*H. s. cinctum*) is not a valid subspecies, which is based on several levels of analysis (i.e., morphology, geographic distribution, and haplotype data). Individuals assigned to

H. s. cinctum based on color and pattern, for example, have been found in southwestern Arizona near the Mexican border and in west-central New Mexico (Beck 2005). Furthermore, most venom researchers, including those who study helodermatids, often obtain samples from captive subjects in private collections and zoological institutions. Many of these animals have been bred in captivity and result from crossing individuals of unknown origin or from different populations (D. Boyer, pers. comm). Among other negative outcomes, such "mutts" will confound results of the true variation of venoms. Geographic and ontogenetic variation in venom constituents is well established in other squamates (Minton and Weinstein 1986; Alape-Girón et al. 2008; Gibbs et al. 2009), which is apparently the case in helodermatids (Fry et al. 2010). Thus, we strongly encourage researchers investigating helodermatid venoms for molecular analysis and pharmaceutical development to use subjects with detailed locality information, as well as age, gender, and size, and to provide those data in their publications.

8. Owing to problems that many scientists, their students, and other interested parties from Mesoamerica have in gaining access to primary scientific literature, we highly recommend that authors seek Open Access peer-reviewed journals as venues for their publications on beaded lizards, an important factor in our choice for selecting the present journal (www.redlist-ARC.org) as a venue for our data and conservation message.

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