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# A NEW SPECIES OF HORNED LIZARD (GENUS *PHRYNOSOMA*) FROM GUERRERO, MÉXICO, WITH AN UPDATED MULTILOCUS PHYLOGENY

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ABSTRACT: We describe a new species of *Phrynosoma* from central northeastern Guerrero, México; perform a phylogenetic analysis of mitochondrial and nuclear sequence data to estimate its phylogenetic relationships; and investigate the monophyly of *Phrynosoma asio*, *P. braconnieri*, and *P. taurus*. The new species can be distinguished from all of its congeners by the possession of a unique combination of morphological characteristics. The molecular genetic data include three fragments of the mitochondrial genome and six nuclear genes (2419 and 3909 base pairs in total, respectively) for 31 samples belonging to the 16 previously recognized species of *Phrynosoma* and the new species. The new species is strongly supported in maximum likelihood analyses of both the concatenated mitochondrial and nuclear data as a monophyletic, distinct evolutionary lineage sister to, and moderately divergent from, *P. taurus*, and highly divergent from all of the other species of *Phrynosoma*. A Bayesian species tree analysis also strongly supports the monophyly of the Brevicauda clade, and a sister relationship between *P. taurus* and the new species.

RESUMEN: Se describe una especie nueva de *Phrynosoma* del centro-noreste de Guerrero, México; se realiza un análisis filogenético de secuencias de ADN mitocondrial y nuclear para estimar sus relaciones filogenéticas, y se investiga la monofilia de *P. asio*, *P. braconnieri*, y *P. taurus*. La nueva especie puede distinguirse de todos sus congéneres por la posesión de una combinación única de caracteres morfológicos. Los datos genéticos moleculares incluyen secuencias de tres fragmentos del genoma mitocondrial y seis genes nucleares (2419 y 3909 pares de bases en total, respectivamente) para 31 muestras de las 16 especies de *Phrynosoma* previamente reconocidas y la nueva especie. La nueva especie es fuertemente apoyada en análisis de máxima verosimilitud de los datos concatenados (tanto mitocondriales como nucleares) como un linaje evolutivo distinto y monofilético, hermano y moderadamente divergente de *P. taurus* y altamente divergente de todas las otras especies de *Phrynosoma*. Un análisis Bayesiano de árbol de las especies también apoya fuertemente la monofilia del clado Brevicauda y la relación de especies hermanas entre *P. taurus* y la nueva especie.

*Key words:* *Phrynosoma brevicauda* clade; Phrynosomatidae; Species tree; Systematics; Taxonomy

HORNED lizards (genus *Phrynosoma*) are among the most distinctive of North and Middle American reptiles because of their unusual morphology and life history (Leaché and McGuire, 2006). The genus is composed of 16 currently recognized species distributed from Canada to Guatemala: *Phrynosoma asio*, *P. blainvillii*, *P. braconnieri*, *P. cerroense*, *P. cornutum*, *P. coronatum*, *P. ditmarsi*, *P. douglasii*, *P. goodei*, *P. hernandesi*, *P. mcallii*, *P. modestum*, *P. orbiculare*, *P. platyrhinos*, *P. solare*, and *P. taurus* (Leaché and McGuire, 2006; Leaché et al., 2009). With the exception of *P. ditmarsi* described in 1906, all of these species were described in the 19th century or, in the case of *P. orbiculare* (Linnaeus, 1758), even earlier. However, only 12 of them were recognized before 1997. Since then, the number of species within *Phrynosoma* has increased to 16, mainly because of reevaluations of species limits within polytypic groups, and the elevation of subspecies (Zamudio et al., 1997; Montanucci, 2004; Leaché and McGuire, 2006; Mulcahy et al., 2006; but see Bryson et al., 2012).

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Of the 16 species of *Phrynosoma*, only four occur south of the Mexican Transvolcanic Belt: *Phrynosoma asio*, *P. braconnieri*, *P. orbiculare*, and *P. taurus* (Leaché and McGuire, 2006). *Phrynosoma braconnieri* and *P. taurus* have consistently been recovered as sister species in a number of phylogenetic studies (e.g., Montanucci, 1987; Zamudio et al., 1997; Reeder and Montanucci, 2001; Hodges and Zamudio, 2004; Leaché and McGuire, 2006). These species are viviparous (Montanucci, 1979; Zamudio and Parra-Olea, 2000), have extremely short tails (Smith and Taylor, 1950; Reeve, 1952; Leaché and McGuire, 2006), and have been reported to lack blood-squirting capabilities (Sherbrooke et al., 2004; Leaché and McGuire, 2006). However, it was recently reported that *P. taurus* does squirt blood (García-Vázquez and Canseco-Márquez, 2006). In their phylogenetic study, Leaché and McGuire (2006) provided a phylogenetic taxonomy for the genus and proposed the name Brevicauda for the clade composed of *P. braconnieri* and *P. taurus*.

*Phrynosoma braconnieri* and *P. taurus* have relatively small distributions in southern México (Leaché and McGuire, 2006). *Phrynosoma braconnieri* is known from Puebla and Oaxaca (Montanucci, 1979; Zamudio and Parra-Olea, 2000), whereas *P. taurus* is known from Guerrero, Morelos, Puebla, northern Oaxaca, and possibly northwestern Veracruz (Montanucci, 1979; Zamudio and Parra-Olea, 2000; García-Vázquez and Canseco-Márquez, 2006; Leaché and McGuire, 2006; Canseco-Márquez and Gutiérrez-Mayén, 2010). However, Montanucci (1979) reported considerable ecological diversity in both of these species. *Phrynosoma braconnieri* has been collected in pine–oak forests at approximately 2438 m in elevation and also in xeric, thorn–scrub vegetation from 1950 to 2255 m in elevation (total range, 1600–2500 m in elevation; Zamudio and Parra-Olea, 2000), whereas *P. taurus* has been collected in oak–chaparral in Guerrero and also in desert areas in Puebla (locality elevation ranges from 1300 to 1900 m; Zamudio and Parra-Olea, 2000). Furthermore, Montanucci (1979) reported color and morphological differences between specimens from pine–oak forests and xeric, thorn–scrub vegetation in *P. braconnieri* and suggested that recognition of two geographic races of this species might be warranted should these differences be consistent when more material is available. Montanucci (1979) also reported some color difference between specimens from oak–chaparral and desert areas in *P. taurus*, although too few specimens were available to determine whether this difference was constant. Although several phylogenies of *Phrynosoma* have been published (see above), the monophyly of *P. braconnieri* and *P. taurus*, as well as that of *P. asio*, has not been tested.

In May 2011, herpetologists from the Universidad Autónoma de Guerrero and the Universidad Nacional Autónoma de México collected one specimen of *Phrynosoma* in the vicinity of Tenexatlajco, in the Sierra Madre del Sur of Guerrero, that appeared to represent an undescribed species of this genus. Thirteen additional specimens collected subsequently in this, and other, localities in the Sierra Madre del Sur of Guerrero confirmed this notion. The undescribed species is small, has an extremely short tail, and most closely resembles *P. braconnieri*. Herein, we describe the new species, perform a phylogenetic analysis of *Phrynosoma* based on mitochondrial DNA (mtDNA) and nuclear gene sequence data to investigate phylogenetic relationships and provide some information about the ecology and reproductive biology of the new species. Also, we investigate for the first time the monophyly of *P. asio*, *P. braconnieri*, and *P. taurus*.

## Materials and Methods

### *Morphological Data*

We examined 14 specimens of the undescribed species of *Phrynosoma* from several different localities in the state of Guerrero: 11 from the type locality in the municipality of Chilapa de Álvarez and three from two localities in the municipality of Olinalá. Tissue samples were taken from eight specimens collected on 18 June 2012 at the type locality (field numbers ADL 4173–4176, 4178–4181), and the tissues were used for the molecular phylogenetic analyses (see below). Three of the specimens from the type locality (ADL

TABLE 1.—Samples of *Phrynosoma* included in the phylogenetic analyses. UWBM = University of Washington Burke Museum. ANMO = Adrián Nieto-Montes de Oca, CDR = Christopher D. Rivera, JAC = Jonathan A. Campbell, RRM = Richard R. Montanucci.

| Species | Samples | Vouchers |
|---|---|---|
| *P. asio* | 3 | MVZ 161505, CDR 279, UWBM:HERP:7280 |
| *P. blainvillii* | 1 | MVZ 150066 |
| *P. braconnieri* | 4 | UWBM:HERP:7282, UWBM:HERP:7283, ANMO 2167, ANMO 2161 |
| *P. cerroense* | 1 | MVZ 161188 |
| *P. cornutum* | 1 | MVZ 238582 |
| *P. coronatum* | 1 | MVZ 137778 |
| *P. ditmarsi* | 1 | RRM 2459 |
| *P. douglasii* | 1 | UWBM:HERP:7227 |
| *P. goodei* | 1 | CAS 229922 |
| *P. hernandesi* | 1 | MVZ 245875 |
| *P. mcallii* | 1 | CAS 229923 |
| *P. modestum* | 1 | MVZ 238583 |
| *P. orbiculare* | 1 | UWBM:HERP:7284 |
| *P. platyrhinos* | 1 | MVZ 161495 |
| *P. solare* | 1 | MVZ 241510 |
| *P. taurus* | 3 | UWBM:HERP:7295, UWBM:HERP:7296, JAC 22525 |
| *P.* sp. nov. | 8 | UWBM:HERP:7286, (MZFC 28101), UWBM:HERP:7287, (MZFC 27898), UWBM:HERP:7288, UWBM:HERP:7289, (MZFC 27899), UWBM:HERP:7291, (MZFC 27900), UWBM:HERP:7292, (MZFC 27896), UWBM:HERP:7293, UWBM:HERP:7294, (MZFC 27897) |

4175, 4177, 4180) were released upon their examination. The remaining eight specimens from this locality and the three specimens from the municipality of Olinalá were fixed in 10% buffered formalin, stored in 70% ethanol, and deposited at the Museo de Zoología *Alfonso L. Herrera* of the Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC).

The diagnosis is based on the examined specimens and the relevant literature (Gentry, 1885; Smith and Taylor, 1950; Reeve, 1952; Montanucci, 1987). Because the new species exhibited a combination of characters that readily distinguishes it from all other species of *Phrynosoma* except those in the Brevicauda clade (i.e., *P. braconnieri* and *P. taurus*), we compared it directly only to the latter species. A list of the specimens examined is provided in the Appendix. Institutional abbreviations for museums and collections follow Sabaj-Pérez (2010), except for the Burke Museum of the University of Washington (UWBM) and the herpetological collection at the Universidad de la Sierra Juárez (ICAH).

Nomenclature of scales follows Smith (1946), Reeve (1952), and Montanucci (1987, 2004). Scale counts were performed with the aid of a dissecting microscope. Measurements followed Montanucci (2004) and were taken with calipers (±0.1 mm). Color descriptions and codes (in parentheses) follow Smithe (1975). In the case of characters examined on both the left and right sides of each specimen, the corresponding conditions are reported in this order, separated by a slash. The superciliary, occipital, and temporal spines were measured on the right side of the specimens.

### *DNA Sequencing*

We collected new DNA sequence data for all species of *Phrynosoma*, and a total of 31 samples are included in the phylogenetic analyses. The majority of these specimens were used in previous studies of *Phrynosoma* (Reeder and Montanucci, 2001; Hodges and Zamudio, 2004; Leaché and McGuire, 2006; Leaché et al., 2009), and we augmented these with new specimens for *P. asio*, *P. braconnieri*, *P. douglasii*, *P. orbiculare*, and *P. taurus* (Table 1). Multiple individuals of *P. asio* ($n = 3$), *P. braconnieri* ($n = 4$), *P. taurus* ($n = 3$), and the new species ($n = 8$) were included to test the monophyly of these taxa.

Six nuclear exons were polymerase chain reaction (PCR) amplified and sequenced, including brain-derived neurotrophic factor (*BDNF*, 529 base pairs [bp]), exophilin 5 (*EXPH5*, 609 bp), NK-tumor recognition protein (*NKTR*, 639 bp), RNA fingerprint protein 35 (*R35*, 704 bp), recombination activating gene-1 (*RAG1*, 1054 bp), and suppressor of cytokine signaling 5 (*SOCS5*, 374 bp). We also sequenced three fragments of the mitochondrial genome (Table 2), including the 12S rRNA gene (*12S*, 769 bp), NADH1 (*ND1*, 969 bp) and NADH4 (*ND4*, 681 bp) protein coding genes, and several tRNA genes (histidine, serine, and leucine). Standard methods of DNA extraction and

TABLE 2.—Variation in the molecular sequences collected for *Phrynosoma* and source of primer sequences. Gene tree monophyly values are bootstrap percentages obtained from 100 replicates using maximum likelihood.

| Gene | Source | Length (bp) | Variable sites | *P. taurus* monophyly | *P. braconnieri* monophyly | *P.* sp. nov. monophyly |
|---|---|---|---|---|---|---|
| *12s* | Wiens et al., 1999 | 769 | 218 | 0 | 99 | 100 |
| *ND4* | Arévalo et al., 1994 | 681 | 284 | 93 | 100 | 94 |
| *ND1* | Leaché and Reeder, 2002 | 969 | 352 | 99 | 100 | 100 |
| mtDNA combined | | 2419 | 854 | 89 | 100 | 100 |
| *BDNF* | Leaché and McGuire, 2006 | 529 | 29 | 0 | 96 | 0 |
| *EXPH5* | Portik et al., 2012 | 609 | 49 | 0 | 0 | 100 |
| *NKTR* | Townsend et al., 2011 | 639 | 48 | 0 | 99 | 0 |
| *R35* | Leaché, 2009 | 704 | 69 | 97 | 99 | 92 |
| *RAG1* | Leaché and McGuire, 2006 | 1054 | 111 | 69 | 77 | 99 |
| *SOCS5* | Alföldi et al., 2011 | 374 | 14 | 0 | 91 | 0 |
| Nuclear combined | | 3909 | 320 | 0 | 100 | 99 |

PCR amplification were used (see Leaché and McGuire, 2006; Portik et al., 2012), and purified PCR products were sequenced using an ABI 3730 automated sequencer. All new sequences are deposited in GenBank (accessions KJ123951–KJ124157) and alignments are provided in the Dryad Digital Repository, http://datadryad.org (DOI:10.5061/dryad.n7h53).

Sequences were edited using Sequencher version 4.9, and multiple sequence alignments were generated using Muscle version 3.8.31 (Edgar, 2004). Open reading frames for the protein-coding genes were identified using MacClade version 4.08 (Maddison and Maddison, 2005). The *12S* alignment was guided using previous alignments generated for *12S* in *Phrynosoma* guided by secondary structure models (Reeder and Montanucci, 2001). For the nuclear genes, heterozygous sites were coded using ambiguity codes.

### *Phylogenetic Analyses*

Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian species tree estimation. ML gene trees were estimated for each mtDNA and nuclear gene separately, the combined mtDNA data, and the combined nuclear data. We did not conduct analyses of combined mtDNA + nuclear data because previous phylogenetic studies of *Phrynosoma* have identified strong conflict between mtDNA and nuclear genes resulting from hybridization and mtDNA introgression (Leaché and McGuire, 2006). ML analyses were conducted with RAxML version 7.0.4 (Stamatakis, 2006) with the GTR+GAMMA model of nucleotide substitution. All ML analyses executed 100 rapid bootstrap replicates followed by a thorough ML search under the specified model.

We used *BEAST version 1.7.4 (Heled and Drummond, 2010) to obtain a species tree for *Phrynosoma* that accommodates incomplete lineage sorting of the nuclear genes. The species tree analysis contained 31 samples belonging to 17 species. Species tree methods produce rooted phylogenetic trees; however, we conducted species tree analyses both with, and without, an outgroup to test the sensitivity of root inference at the base of the *Phrynosoma* phylogeny. We used the closely related sand lizard species *Holbrookia maculata* as an outgroup for this purpose (Wiens et al., 2013). The site models, clock models, and partition trees were unlinked, and we applied the HKY model of nucleotide substitution to each gene. We assumed a Yule tree prior and uncorrelated lognormal model for branch variation. Two replicate runs were conducted with random starting seeds and chain lengths of 100 million generations, with parameters sampled every 10,000 steps. Long chains were necessary for achieving high effective sample sizes (ESSs) for parameters, and ESS values $\geq$ 200 were used as a proxy for convergence of parameters. Species trees were summarized after discarding the first 25% of trees as burn-in. The post burn-in samples were analyzed in TreeAnnotator to produce a maximum clade credibility tree.

## Results

The combined nuclear data matrix contains 3909 base pairs and 320 variable sites, whereas the mtDNA data include 2419 base pairs and 854 variable sites (Table 2). The gene trees provide strong support (bootstrap $\geq$ 70%) for the monophyly of *P. braconnieri* more often than they do for *P. taurus* or the new species (Table 2). mtDNA genes that support each of these three species as monophyletic include *ND1* and *ND4*; the *12S* gene does not support the monophyly of *P. taurus*. The nuclear genes contain less variation and, as a result, they support the monophyly of these species less frequently than mtDNA. For example, only two of the nuclear genes, *R35* and *RAG-1*, support the monophyly of *P. taurus*, and these are the only nuclear genes that support the monophyly of all three species.

The ML analysis of the concatenated mtDNA data (Fig. 1A) is highly congruent with previous mtDNA-based estimates of *Phrynosoma* relationships. The new *Phrynosoma* samples from Guerrero form a clade that is sister to *P. taurus* (bootstrap = 100%) within the Brevicauda clade. The concatenated nuclear data also support the monophyly of the new samples from Guerrero and place them sister to *P. taurus* as well (Fig. 1B). In contrast, other aspects of the nuclear and mtDNA trees indicate opposing relationships that receive strong support in the separate analyses. For example, the mtDNA tree places *P. modestum* as sister to the remaining short-horned species (*P. ditmarsi*, *P. douglasii*, *P. hernandesi*, and *P. orbiculare*), whereas the nuclear data places *P. modestum* as sister to a clade containing *P. goodei* and *P. platyrhinos* (Fig. 1). In addition, the mtDNA and nuclear data disagree on the placement of the clade containing the short-tailed species (*P. braconnieri*, *P. taurus*, and *P.* sp. nov.); this clade diverges early at the base of the mtDNA gene tree but has a more recent divergence in the nuclear tree (Fig. 1).

The species tree analysis of the six nuclear loci (Fig. 2) provides strong support (posterior probability $\geq$0.95) for relationships at shallow levels in the tree, but the initial divergences in the group have low posterior probability support ($\leq$0.5). *Phrynosoma cornutum* is

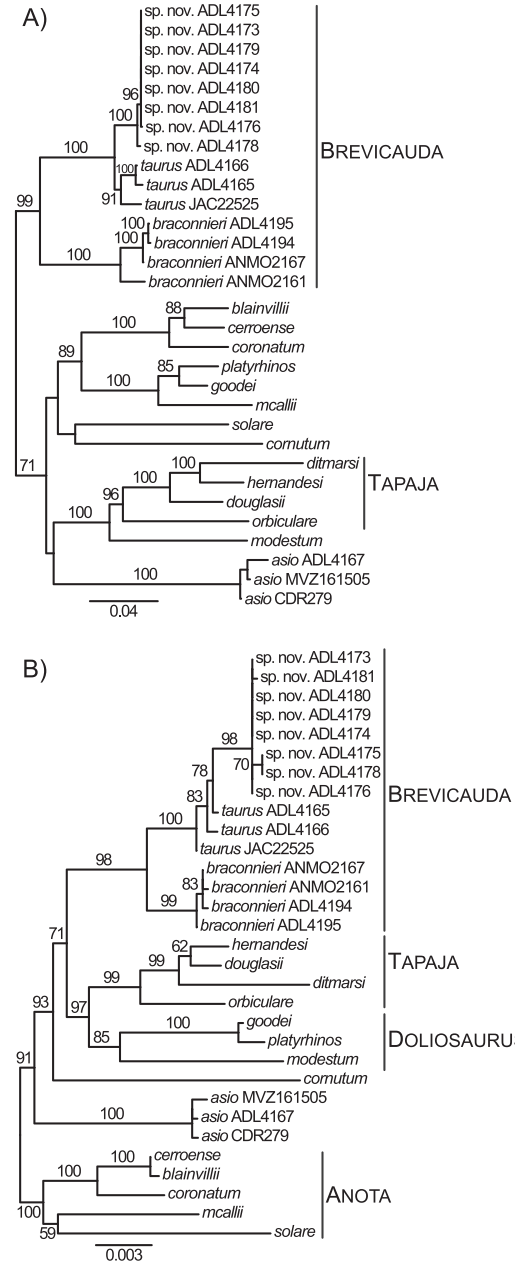


Fig. 1.—Phylogenetic relationships of *Phrynosoma* based on a maximum likelihood analysis of (A) concatenated mtDNA data and (B) concatenated nuclear data. Bootstrap values $\geq$50% are indicated on nodes. The trees are rooted with *Holbrookia maculata*. Phylogenetic taxonomy is noted where applicable.

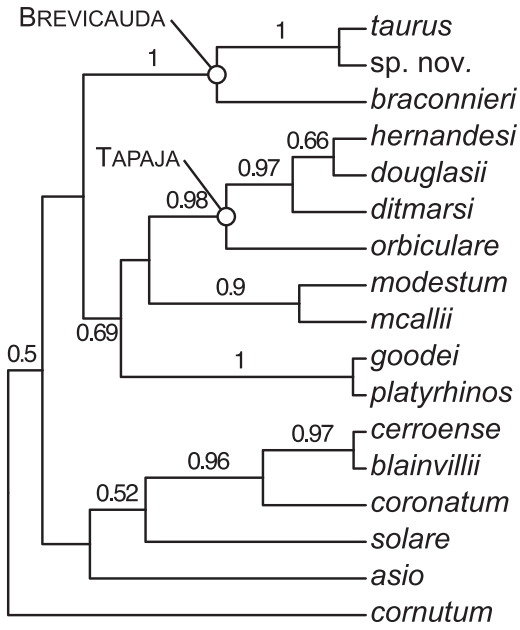


FIG. 2.—Species tree analysis of *Phrynosoma* showing the phylogenetic position of *Phrynosoma* sp. nov. in the Brevicauda clade of viviparous and short-tailed species endemic to México. Nodes supported by posterior probability values ≥ 0.50 are indicated. The outgroup, *Holbrookia maculata*, is not shown. Phylogenetic taxonomy is noted where applicable.

sister to the remaining species; however, the support for this relationship is low (posterior probability = 0.5). Including *H. maculata* as an outgroup does not change this placement of *P. cornutum* at the base of the tree. The remaining species are arranged as follows: *P. asio* is placed as sister to *P. solare* and the *P. coronatum* complex; *P. goodei* and *P. platyrhinos* form a clade sister to a *P. mcallii* and *P. modestum* clade that is sister to viviparous and short-horned species in the Tapaja clade.

The relative divergence times between the new species and *P. taurus* are comparable to those between the pairings of *P. goodei* and *P. platyrhinos*, *P. hernandesi* and *P. douglasii*, and *P. cerroense* and *P. blainvillii* (Fig. 2).

## Phrynosoma **sherbrookei** *sp. nov.*
*(Figs. 3–5)*

*Holotype*.—MZFC 28101 (ADL 4173), a male from Tenexatlajco, municipality of Chilapa de Álvarez, Guerrero, México, 17.55437°N, 99.26973°W (datum = WGS84 for all localities), 1997 m in elevation, collected on 18 June 2012 by D. Arenas-Moreno, M.A. Días-Ojendis, M. García-Pareja, P.P. González-Alvarado, J. Grummer, A. Hernández-Ríos, R. Lara-Reséndiz, A.D. Leaché, A. Nieto-Montes de Oca, and W.C. Sherbrooke.

*Paratypes*.—Ten specimens, all from Guerrero, México: three males (MZFC 27894, 27896, 27897) and four females (MZFC 27893, 27898–27900) from the same locality as the holotype (MZFC 27893–27894 collected on 2011 or 2012 by unrecorded collectors; MZFC 27896–27900 collected on 18 June 2012 by the same collectors as the holotype), and three specimens from the municipality of Olinalá: one female (MZFC 27895) from near La Encinera (Los Terrenos), 17.85202778°N, 98.75125°W, 1794 m elevation, collected on 19 July 2012 by W. Gramajo, and two specimens, one female and one juvenile of undetermined sex (MZFC 28303 and 28304, respectively) from 1 km NW Xixila, 17.94564°N, 98.85996°W, 1677 m elevation, collected on 1 October 2011 by E. Rosendo, V.H. Jiménez-Arcos, and S. Santa Cruz-Padilla.

*Diagnosis*.—*Phrynosoma sherbrookei* may be distinguished from *P. blainvillii*, *P. cerroense*, *P. coronatum*, *P. douglasii*, *P. goodei*, *P. hernandesi*, *P. mcallii*, *P. modestum*, *P. orbiculare*, *P. platyrhinos*, and *P. solare* by having keeled ventral scales (vs. ventral scales smooth in the other species).

Among the species with keeled ventral scales, *Phrynosoma sherbrookei* differs from *P. asio* and *P. cornutum* by having a smaller adult body size (snout–vent length [SVL] < 63.0 mm vs. SVL > 140.0 mm in the other species; Smith and Taylor, 1950), a shorter tail (tail length < 23% of SVL vs. tail length ≥ 50% of SVL in the other species; Smith and Taylor, 1950), and one lateral abdominal row of fringe scales (vs. two lateral abdominal rows of fringe scales in the other species; Smith and Taylor, 1950; Montanucci, 1987). *Phrynosoma sherbrookei* can be distinguished from *P. ditmarsi* by having well-developed occipital and temporal spines (vs. occipital and temporal spines absent, replaced by low, rounded protuberances in *P. ditmarsi*; Smith and

Taylor, 1950; Reeve, 1952) and one or two short rows of sublabials (vs. five or six rows of sublabials in *P. ditmarsi*; Reeve, 1952).

*Phrynosoma sherbrookei* is most closely related to the two species in the Brevicauda clade (*P. braconnieri* and *P. taurus*). The new species can be distinguished from *P. braconnieri* by having the outer temporal part of the skull prolonged posteriodorsally into two temporal spines (vs. the outer temporal part of the skull prolonged posteriodorsally into three temporal spines in *P. braconnieri*), posterior chinshields larger than the postlabials (vs. posterior chinshields smaller than at least the posterior-most postlabial in *P. braconnieri*), and more numerous sublabials (10–18, $\bar{X} = 14.7$, $n = 9$) usually arranged in two rows (vs. 1–7, $\bar{X} = 4.8$, $n = 9$, sublabials in one row in *P. braconnieri*).

*Phrynosoma sherbrookei* differs from *P. taurus* by having a smaller adult body size (<54 and <63 mm SVL in males and females, respectively, vs. 55.5–77.0 and 65.0–90.0 mm SVL in *P. taurus* males and females, respectively; Zamudio and Parra-Olea, 2000); by having outer temporal spines only slightly longer than the occipital spines (length ratio 1.1–1.6, $\bar{X} = 1.4$, $n = 12$) and not or barely exceeding the occipital spines in posterior extension (vs. outer temporal spines much longer than the occipital spines [length ratio 2.1–3.0, $\bar{X} = 2.5$, $n = 6$] and far exceeding the occipital spines in posterior extension in *P. taurus*), and in lacking enlarged postcloacal scales (vs. the presence of enlarged postcloacal scales in *P. taurus*).

*Description of holotype*.—Adult male, SVL 53.3 mm; head length 13.1 mm (24.6% of SVL), head width 13.5 mm; shank length 13.7 mm (25.6% of SVL); tail length 8.9 mm (67.9% of head length, 16.7% of SVL; Figs. 4 and 5).

Dorsal surface of head with polygonal, juxtaposed scales arranged in no discernible pattern; their surface texture granular. Postrostral scales small, flat, usually keeled. Frontal and supraocular scales small to mid-sized except for two somewhat enlarged azygous scales; usually with one central, slightly elevated granule and occasionally one or more keels converging on granule. Parietal scales mid-sized, conical, with numerous, conspicuous granules and keels. Abruptly differentiated transverse series of preoccipital tubercles absent. Rostral small, slightly more than twice as wide as high (width 1.3 mm, height 0.5 mm). External nares

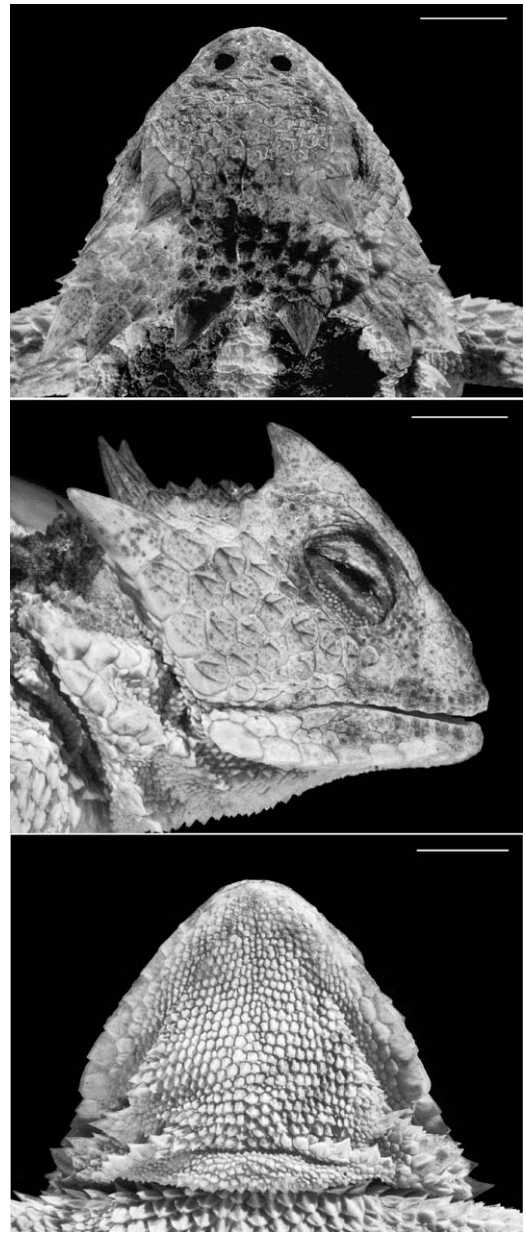

FIG. 3.—*Phrynosoma sherbrookei* sp. nov. Head scales of holotype (MZFC 28101) in dorsal (top), lateral (middle), and ventral view (bottom). Horizontal bars represent 5 mm.

FIG. 4.—Holotype of *Phrynosoma sherbrookei* sp. nov. (MZFC 28101). Photograph was taken at the type locality in Guerrero, México.

situated within canthal lines, dorsally pierced. Eleven scales along midline between rostral and interparietal. Interparietal to rostral distance 9.1 mm (69.2% of head length); interparietal length 2.0 mm; parietal shelf length 4.3 mm (32.7% of head length). Two comparatively small occipital spines (maximum width at base 2.12 mm, length 3.1 mm, or 23.6% of head length), projecting posterodorsally at angle of about 60° to longitudinal axis, widely separated from each other (distance between bases of spines 2.8 mm, or 21.6% of head length) and from temporal spines (distance between bases of occipital and inner temporal spines 2.0 mm/1.8 mm). Interoccipital and parietotemporal spines absent.

Lateral surface of head with polygonal, juxtaposed scales arranged in no discernible pattern except on temporal region; their surface texture granular. Canthals and superciliaries intermediate in size, keeled or striate; superciliary ridge terminating in a well-developed superciliary spine (maximum width at base 2.16 mm, length 2.2 mm, or 16.8% of head length) projecting posterodorsally at angle of about 60° to longitudinal axis. Loreals small, usually keeled. Three scale rows between orbit and supralabials; rows immediately below orbit and above supralabials composed of small, keeled scales; middle row composed of increasingly larger, keeled scales extending posteriorly from loreal region, merging with temporal scales. Temporal scales large, each with one slightly elevated granule near its posterior border and one or more keels converging on granule; scales arranged in oblique, parallel rows, gradually becoming larger posteriorly; scales in posterior-most two rows gradually becoming larger dorsally; those at their dorsal end abruptly differentiated into two adjacent, moderately large temporal spines; outer temporal row composed of three large scales followed dorsally by outer and inner temporal spines. Inner temporal spine smaller than outer one (maximum width at base and height = 1.84 and 2.36 mm, and 3.0 and 4.1 mm, respec-

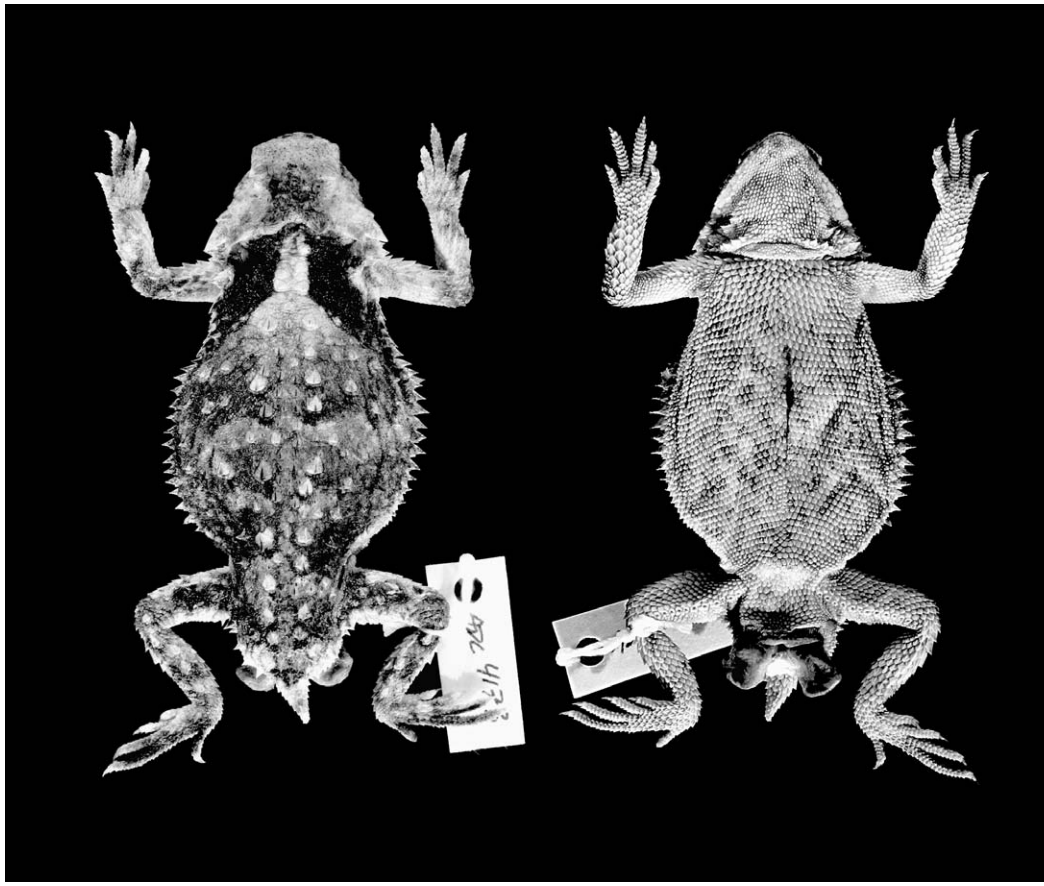

FIG. 5.—Holotype of *Phrynosoma sherbrookei* sp. nov. (MZFC 28101) in dorsal (left) and ventral (right) view.

tively; length of outer spine 31.2% of head length) or about as large as, and distinctly larger than, occipital spines, respectively; temporal spines projecting both posterolaterally and posterodorsally at angle of about 45° to longitudinal axis; projecting slightly farther posteriorly (about 1.2 mm) than occipital spines. Supralabials small, keeled, vertically oriented, not flared; some slightly scalloped along buccal margin of row. Tympanum exposed; skin fold above tympanum absent.

Mental scale small, shallow. Scales in infralabial-postlabial row moderately large, keeled; gradually becoming convex, triangular posteriorly in row from about its middle, with laterally projecting keeled edge; infralabial-postlabial row separated from chinshield row by 10/14 small sublabials arranged in two rows. Chinshields 8+ (row partially mis-shaped)/10, left and right rows abutting each other below mental-infralabials row; scales in each row gradually becoming larger, convex, triangular, keeled posteriorly in series, with keeled edge directed slightly lateroventrally, forming a serrate series barely visible from dorsal view. Posterior chinshields markedly larger than postlabials. Approximately 41 longitudinal gular scale rows between last chinshields. Pregular fold weakly developed, composed of a single transverse row of approximately five moderately enlarged, conical, keeled, mucronate scales on each side of ventral surface of neck. Gular fold well developed. One row of slightly enlarged gulars on each side of throat extending posteriorly to level of posterior end of chinshield row. Two rows of enlarged, keeled, mucronate scales on each side of neck: one row extending poste-

riorly from ear to gular fold, then curving dorsally bordering dorsal end of fold, and one row of increasingly larger scales extending posterodorsally from posterolateral corner of throat, at level of posterior end of chinshield row, to first scale row on neck at its junction with margin of gular fold.

Dorsal body scales heterogeneous, composed of enlarged scales interspersed among irregular, juxtaposed, smaller scales arranged in no discernible pattern, often keeled on middorsum; enlarged scales slightly oval, their posterior margin slightly rounded, arranged in: (1) two short paravertebral rows, each composed of three juxtaposed scales increasingly larger, keeled, and mucronate posteriorly between levels of anterior and posterior insertions of forelimbs; (2) one enlarged scale on each side of paravertebral rows at level of anterior insertion of forelimb, with several strong converging keels and strong mucrone projecting dorsally from center; (3) two poorly defined paravertebral rows of slightly enlarged, keeled, not or slightly mucronate scales extending between levels of axilla and cloaca; scales of each row separated from nearest scales in row by distance usually equal to, or shorter than, their own diameter; and, (4) six poorly defined longitudinal scale rows extending between levels of axilla and cloaca (four medial-most rows) or between levels of axilla and groin (two lateral-most rows); scales in each row strongly keeled; one median keel and two short lateral keels converging near posterior margin; strongly mucronate; mucrone originating at posterior end of median keel, near posterior edge of scale, projecting dorsally; medial-most rows straight, composed of largest scales on dorsum; remaining rows increasingly curved, paralleling body contour, and composed of gradually smaller scales laterally; scales of each row separated from nearest scales in row by distance usually equal to, or greater than, their own diameter.

Dorsal scales of arm and forearm imbricate, keeled, mucronate; those of thigh and shank heterogeneous, composed of enlarged scales interspersed among much smaller, irregular, juxtaposed, usually keeled scales arranged in no discernible pattern; enlarged scales rounded, their posterior margin slightly rounded, strongly keeled; one median keel and two short lateral keels converging near posterior margin; strongly mucronate; mucrone originating at posterior end of median keel, near posterior edge of scale, projecting dorsally.

Ventral body scales keeled, juxtaposed to slightly imbricate, not mucronate or with exceedingly small mucrone directed posteriorly. One lateral abdominal fringe scale row extending from level between anterior and posterior insertions of arm to nearly groin on each side; first two scales in row separated from rest of row by short gap at level of axilla. Approximately 40 transverse scale rows along midline between levels of axilla and groin; about 71 longitudinal scale rows between lateral abdominal fringe scale rows at level of midbody. Approximately 27/26 scales in lateral abdominal fringe scale row. Lateral abdominal fringe scale length at level of midbody ≈1.6 mm. Enlarged postcloacal scales absent. One single row of 12/12 femoral pores; pores surrounded anteriorly by single scale; bordered posteriorly by two to three small scales. Approximately 16 precloacal scales between femoral pore rows. Ventral scales of arms and legs imbricate, keeled, slightly mucronate. Subdigital lamellae under fourth toe 14/14. Tail short, subcylindrical, slightly wider than deep.

*Coloration of holotype in life*.—Dorsal surface of head, except for temporal areas and temporal spines, smoke gray (45); dorsal and lateral surfaces of temporal areas, temporal spines, and remaining lateral surfaces of head pale horn (92); frontal, supraocular, and especially parietal scales and superciliary and occipital spines darkened by black granules and keels; parietal and temporal scales and temporal spines moderately stippled with Peach Red (94). Ventral surface of head white, with irregular, scattered, indistinct gray spots (Fig. 4).

Dorsal background color of body glaucus (80). Sulfur yellow (157) middorsal stripe extending from posterior end of head to level of axilla (i.e., on paravertebral rows of enlarged, juxtaposed scales between levels of anterior and posterior insertions of arm), bordered on each side by large, bright jet black (89) blotch extending laterally to lateral abdominal scale row, between posterior end of head and level of axilla along its medial

margin, and between posterior end of head and level of about one-fifth distance from axilla to groin along its lateral margin. Six indistinct, middle neutral gray (84) crossbands on rest of body and tail: three on body (one between anterior jet black blotches and level of midbody and two between levels of midbody and groin), one above level of cloaca, and two on tail. Crossbands on body wide, with ill-defined, concave anterior and posterior margins, broadly connected to each other along midline; that above level of cloaca wide, broadly U-shaped; those on tail narrow. Irregular, poorly defined, medium gray spots and bars on arms, legs, and digits. Ventral surface white; chest and venter with irregular, scattered, indistinct gray spots and bars. A photograph of the preserved specimen is provided in Fig. 5.

*Variation*.—Except as noted, description of variation in scalation and morphometric ratios is based on all paratypes, except the juvenile specimen (MZFC 28304) and the three released specimens ($n = 12$; nine females, SVL 41.5–62.4 mm, and three males, SVL 46.7–53.1 mm). Morphometric ratios are given in Table 3. Variation in color pattern (Fig. 6) is based on the eight specimens collected at the type locality, in addition to the holotype.

Scales between the rostral and interparietal scales 11–13 ($\bar{X} = 12.4$). Superciliary and occipital spines approximately equal in size in all of the specimens; both spines from slightly smaller to slightly larger than the inner temporal spine in all of the specimens except about twice as large as latter spine in one specimen (MZFC 27893); from about two-thirds to nearly as large as the outer temporal spine. Superciliary spines projecting posterodorsally at angle of about 60° to longitudinal axis in six of the specimens; at angle of about 45° in five. Occipital and temporal spines projecting posterodorsally at angle of about 30°–40°, and about 40° to longitudinal axis, respectively, in all of the paratypes included ($n = 9$). Outer temporal spines slightly exceeding the occipital spines in posterior extension in 10 specimens; occipital spines slightly exceeding the outer temporal spines in posterior extension in two. Chinshields (right side) 10–11 ($\bar{X} = 10.7$). Sublabials (right side) 10–18 ($\bar{X} = 14.7$, $n = 9$). Longitudinal rows of gular scales between last chinshields 37–43 ($\bar{X} = 41.2$, $n = 9$). Transverse rows of ventral scales along midline between levels of axilla and groin 35–44 ($\bar{X} = 39.1$); ventral scales between lateral fringe scale rows at level of midbody 66–78 ($\bar{X} = 71.3$). Scales in lateral abdominal fringe scale row (right side) 25–32 ($\bar{X} = 28.5$). Femoral pores (right side) 11–15 ($\bar{X} = 12.0$). Precloacal scales between femoral pore rows 11–18 ($\bar{X} = 15.3$). Subdigital lamellae under fourth toe 13–16 ($\bar{X} = 14.8$).

In general, all specimens showed a dorsal color pattern similar to that of the holotype. However, the dorsal background color was a different tone of pale horn (92), vinaceous (3), and glaucus (80) in each of four, two, and two specimens, respectively (Fig. 6). All specimens had the parietal area and superciliary and occipital spines darkened to some degree by black granules and keels, and the temporal areas and temporal spines sparsely to densely speckled with red peach (94). All of the specimens also exhibited a pale middorsal stripe (in one of several tones of pale horn [92]) on the paravertebral rows of enlarged, juxtaposed scales; a black blotch on each side of the latter stripe, and several, sometimes indistinct, dark crossbands (in one of several tones of glaucus [80]) on the body and tail. In all of the specimens, the dark crossbands had poorly defined, concave anterior and posterior margins, and were broadly connected with each other along the midline. However, the dark crossbands on the body varied in distinctness (from moderately to clearly distinct in seven specimens; barely distinguishable in one), number (three, in six specimens; two indistinct ones, in two), and width (wide in five specimens; narrow in three). In five of the specimens, the flanks were heavily speckled with red peach (94). In all specimens there was a wide, often indistinct, dark crossband on the level of the cloaca, and two narrow crossbands on the short tail. The specimens also had irregular, dark marks and bars on the limbs and digits, and a white throat, chest, and belly with irregular, scattered, sometimes indistinct gray spots occasionally interconnected forming short bars.

TABLE 3.—Variation in selected morphometric ratios in the paratypes of *Phrynosoma sherbrookei* sp. nov., excluding a juvenile (MZFC 28304), and including three released females. Snout–vent length (SVL) is in mm.

| Field (ADL) no. | MZFC no. | SVL | Head length/SVL | Parietal shelf length/head length | Interparietal-rostral distance/head length | Superciliary spine length/head length | Occipital spine length/head length | Outer temporal spine length/head length | Outer temporal spine length/occipital spine length | Shank length/SVL | Tail length/head length | Tail length/SVL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Females | | | | | | | |
| | 27893 | 50.4 | 25.0 | 29.4 | 70.9 | 16.7 | 17.6 | 26.5 | 1.5 | 23.1 | 89.9 | 22.5 |
| | 27895 | 44.2 | 27.0 | 34.3 | 73.3 | 12.8 | 15.6 | 20.2 | 1.3 | 24.7 | 74.0 | 20.0 |
| 4174 | 27898 | 62.4 | 22.5 | 33.2 | 72.6 | 18.1 | 18.7 | 25.3 | 1.4 | 24.3 | 81.6 | 18.4 |
| 4176 | 27899 | 61.2 | 23.2 | 32.3 | 72.2 | 17.3 | 16.3 | 26.2 | 1.6 | 24.1 | 64.1 | 14.9 |
| 4178 | 27900 | 61.9 | 22.1 | 28.5 | 75.2 | 20.8 | 20.0 | 29.1 | 1.5 | 23.0 | 62.5 | 13.8 |
| | 28303 | 51.2 | 24.9 | 31.5 | 73.0 | 18.5 | 15.4 | 20.9 | 1.4 | 25.2 | 65.0 | 16.2 |
| 4177 | Released | 41.5 | 27.7 | 32.3 | 71.1 | 16.2 | 15.2 | 17.1 | 1.1 | 22.5 | 64.6 | 17.9 |
| 4175 | Released | 46.8 | 27.5 | 33.2 | 69.2 | 16.8 | 16.8 | 18.7 | 1.1 | 25.2 | 70.9 | 19.5 |
| 4180 | Released | 52.3 | 25.1 | 31.7 | 71.2 | 19.0 | 17.8 | 24.3 | 1.4 | 26.0 | 81.4 | 20.5 |
| | Mean | 52.4 | 25.0 | 31.8 | 72.1 | 17.4 | 17.1 | 23.2 | 1.4 | 24.2 | 72.7 | 18.2 |
| | Range | 41.5–62.4 | 22.1–27.7 | 28.5–34.3 | 69.2–75.2 | 12.8–20.8 | 15.2–20.0 | 17.1–29.1 | 1.1–1.6 | 22.5–26.0 | 62.5–89.9 | 13.8–22.5 |
| | | | | | Males | | | | | | | |
| | 27894 | 52.0 | 24.3 | 26.1 | 72.1 | — | 17.1 | 27.6 | 1.6 | 24.3 | 87.3 | 21.3 |
| 4179 | 27896 | 53.1 | 23.7 | 31.2 | 72.5 | 17.7 | 17.2 | 26.5 | 1.5 | 23.1 | 77.2 | 18.3 |
| 4181 | 27897 | 46.7 | 25.6 | 33.4 | 70.3 | 11.8 | 15.5 | 17.9 | 1.2 | 23.6 | 69.2 | 17.7 |
| | Mean | 50.6 | 24.5 | 30.2 | 71.6 | 14.8 | 16.6 | 24.0 | 1.4 | 23.7 | 77.9 | 19.1 |
| | Range | 46.7–53.1 | 23.7–25.6 | 26.1–33.4 | 70.3–72.5 | 11.8–17.7 | 15.5–17.2 | 17.9–27.6 | 1.2–1.6 | 23.1–24.3 | 69.2–87.3 | 17.7–21.3 |

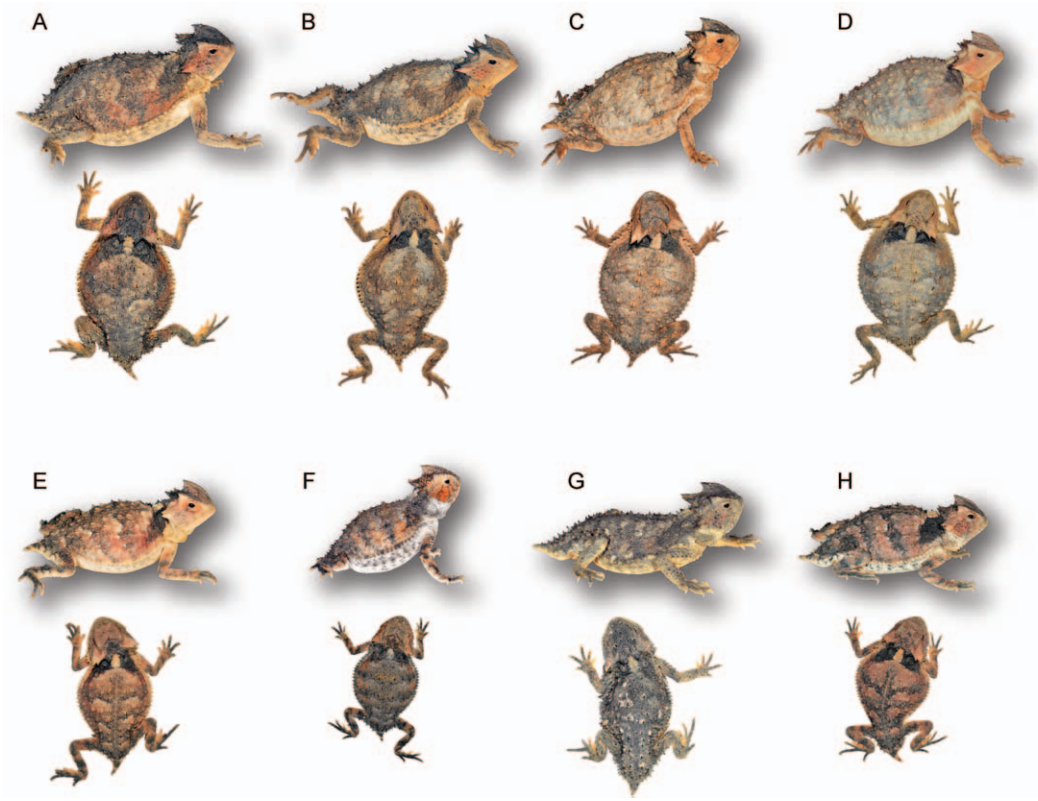


FIG. 6.—Color pattern variation in the paratype series of *Phrynosoma sherbrookei* sp. nov. from the type locality, both lateral and dorsal views. Females (A–F) and males (G–H) are arranged in descending snout-to-vent length as follows: (A) 62.4 mm (ADL 4174), (B) 61.9 mm (ADL 4178), (C) 61.2 mm (ADL 4176), (D) 52.3 mm (ADL 4180), (E) 46.8 mm (ADL 4175), (F) 41.5 mm (ADL 4177), (G) 53.1 mm (ADL 4179), and (H) 46.7 mm (ADL 4181).

*Etymology*.—The specific epithet is a patronym for Wade C. Sherbrooke in recognition of his many and significant contributions to the knowledge of horned lizards.

*Distribution and ecology*.—*Phrynosoma sherbrookei* is currently known from three localities at intermediate elevations in central northeastern Guerrero: the type locality, at 1800–2040 m in elevation in the vicinity of Tenexatlajco, municipality of Chilapa de Álvarez, near central Guerrero, and two localities, near La Encinera and near Xixila, between 60 and 65 km to the northeast, at 1677–1794 m in elevation in the municipality of Olinalá in northeastern Guerrero (Fig. 7). It seems likely that the species has a wider distribution in eastern Guerrero and adjacent Puebla.

The climate at the type-locality is temperate and subhumid with precipitation in the summer, and the substratum is composed of clays and sedimentary rocks (INEGI, 2009). The vegetation is oak forest with patches of grassland and scrub with *Agave* spp. and other herbaceous plants (Rzedowski, 1978). The area is heavily impacted by farming and soil erosion (INEGI, 2009). The type series was collected during the day in a small patch of grassland hillside with heavy erosion (Fig. 8).

*Life history*.—Field observations of one individual and fecal analysis of five specimens indicate that *P. sherbrookei* feeds on ants, as is true for most *Phrynosoma* species (Sherbrooke, 2003). The mean body temperature of 15 specimens was 30.25 ± 1.61°C. The species is viviparous. Mating occurs in spring and parturition in fall.

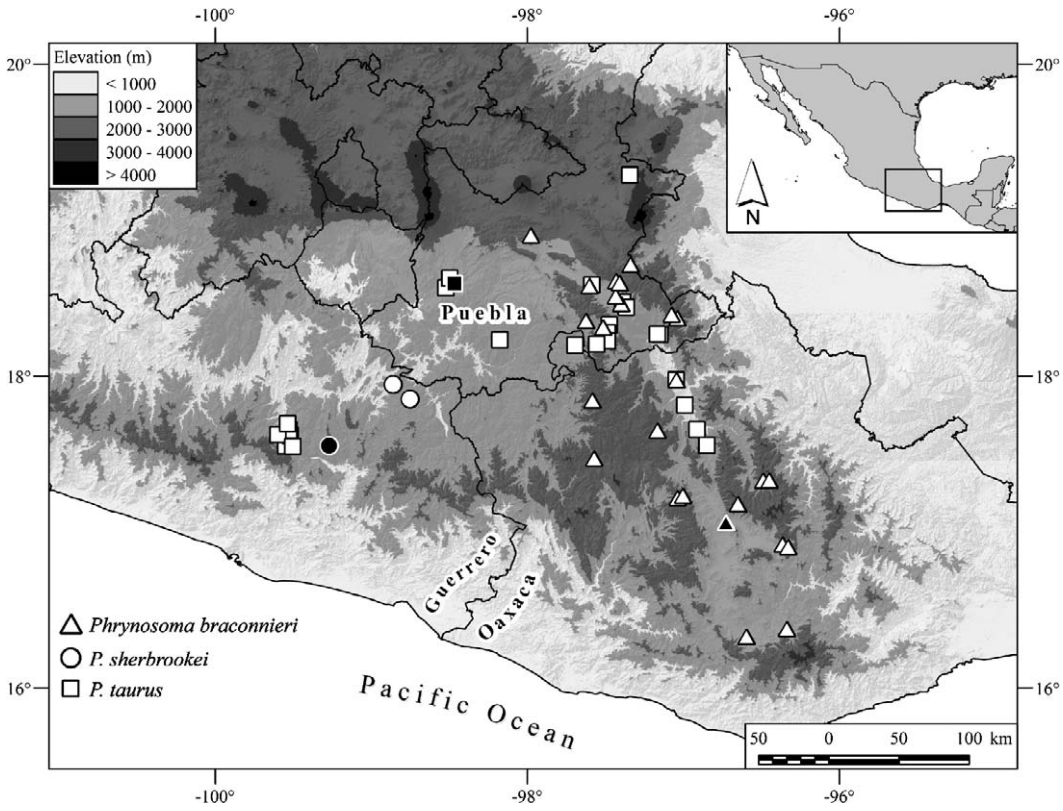


FIG. 7.—Geographic distribution of *Phrynosoma sherbrookei* sp. nov. within México (inset). Distributions of *P. braconnieri* and *P. taurus* are based on representative localities from Zamudio and Parra-Olea (2000) and Canseco-Márquez and Gutiérrez-Mayén (2010) in addition of those from this study. Closed symbols represent type localities.

Antipredator blood squirting has not been observed in *P. sherbrookei* in the field or induced in the lab.

## DISCUSSION

The distinctness of *P. sherbrookei* is supported by both morphological and molecular data. The possession of keeled ventral scales separates *P. sherbrookei* from all but five species in the genus, and the combination of several other characters (e.g., small body size, short tail, distinctive horns) clearly distinguishes it from those remaining five species. Also, *P. sherbrookei* was strongly supported in both the mitochondrial and nuclear trees as a distinct, monophyletic evolutionary lineage, moderately divergent from its sister species (*P. taurus*) and highly divergent from all of the other species of *Phrynosoma* (Figs. 1 and 2).

The phylogenetic placement of *P. sherbrookei* within the Brevicauda clade is not surprising based on external morphology and geographic distribution. The species in this clade (*P. braconnieri*, *P. taurus*, and *P. sherbrookei*) have a short tail and two paravertebral rows of enlarged, keeled, juxtaposed scales on the dorsal area of the neck (Montanucci, 1987). Also, the three species are viviparous and occur in southern México south of the Mexican Transvolcanic Belt. The known geographic distribution of *P. sherbrookei* (central northeastern Guerrero) is geographically intermediate between the records of *P. taurus* in central Guerrero (≈26 km to the west) and those in southern Puebla (Fig. 7). *Phrynosoma sherbrookei* was strongly supported as sister taxon to *P. taurus* rather than to *P. braconnieri* despite it being more similar to the latter species in body size,

FIG. 8.—Habitat of *Phrynosoma sherbrookei* sp. nov. at the type locality, Guerrero, México.

morphology of the occipital and temporal spines, and overall scalation.

The phylogenetic taxonomy established for *Phrynosoma* by Leaché and McGuire (2006) is supported by ML analysis of the concatenated nuclear genes, but it receives mixed support using mtDNA (Fig. 1). All analyses provide strong support for the monophyly of Brevicauda (containing *P. braconnieri*, *P. sherbrookei*, and *P. taurus*) and Tapaja (containing *P. douglasii*, *P. ditmarsi*, *P. hernandesi*, and *P. orbiculare*), and this includes the species tree analysis of the nuclear data. The Anota clade, which contains *P. solare*, *P. mcallii*, and the *P. coronatum* complex, is not supported by the mtDNA; instead, *P. mcallii* is placed sister to *P. goodei* and *P. platyrhinos* (Fig. 1). This conflicting result is expected because it is hypothesized that introgressive hybridization has replaced authentic *P. platyrhinos* and *P. goodei* mtDNA haplotypes with *P. mcallii* copies (Leaché and McGuire, 2006). This aberrant placement of *P. mcallii* also disrupts the monophyly of the Doliosaurus clade, which contains *P. goodei*, *P. modestum*, and *P. platyrhinos* (Fig. 1). The multilocus species tree analysis conducted using *BEAST also fails to support the monophyly of Anota and Doliosaurus, both of which are disrupted by the placement of *P. mcallii* sister to *P. modestum* (Fig. 2). Whereas the relationships that conflict with the phylogenetic taxonomy are strongly supported by the mtDNA data (i.e., 100% bootstrap support), the species tree analysis only provides weak support (i.e., $\leq$0.9 posterior probability) for such conflicts. Phylogenetic support is typically lower for coalescent-based analyses versus concatenation (Leaché, 2010), but the factor(s) causing *P. mcallii* to change phylogenetic positions under the coalescent model requires further investigation. Such studies will benefit from phylogenetic analyses that combine large numbers of loci with multiple samples for each species.

The monophyly of *P. braconnieri* and *P. asio* was strongly supported in the analyses of both the concatenated mitochondrial and nuclear data, whereas the monophyly of *P. taurus* was supported by the concatenated mitochondrial data (bootstrap = 91%; Fig. 1) and not

supported in the concatenated nuclear tree (Fig. 1), which supports *P. taurus* as paraphyletic with respect to *P. sherbrookei*. Nuclear genes contain less variation; thus, they support the monophyly of species less frequently than mitochondrial genes; they also take longer to sort to monophyly (Edwards and Beerli, 2000). Further research is needed to investigate the potential existence of multiple lineages within *P. asio*, *P. braconnieri*, and *P. taurus*.

Newly described species are usually not considered in Red Lists of threatened species or similar lists. *Phrynosoma braconnieri* and *P. taurus* are listed by the Mexican government as "threatened" and "under special protection," respectively (SEMARNAT, 2010). Given that the biology of *P. sherbrookei* seems similar to that of the former species, and its geographic distribution is much smaller, we suggest that *P. sherbrookei* also deserves conservation attention and therefore recommend that it be added to the above-mentioned list.

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## Appendix

### *Specimens Examined*

ANMO is an abbreviation for a field number of an uncataloged specimen at the MZFC.

*Phrynosoma braconnieri*.—PUEBLA: Tecali de Herrera (ANMO 2161); Tehuacán (MZFC 174); OAXACA: Ixtlán (ICAH 60, 111); Miahuatlán (ANMO 3278–3280); Santiago Yolomécatl (ANMO 847, 2167).

*Phrynosoma taurus*.—GUERRERO: Zumpango del Rio (ANMO 3059); Municipality of Eduardo Neri, Zumpango de Neri, Cerro del Tepetlayo (MZFC uncatalogued, 4 specimens); PUEBLA: Zapotitlán de las Salinas (ANMO 3066).

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