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Two new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from Southern Mexico, with comments on the taxonomy of related species and their advertisement calls

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Abstract.—An analysis of morphological and molecular data is presented here, as well as notes on advertisement calls from populations of the *Eleutherodactylus nitidus* species group (subgenus *Syrrophus*) from southern Mexico, and two new species are described based on the results. *Eleutherodactylus maculabialis* sp. nov. is unique among its congeners by a combination of characters including widely expanded finger tips, indistinct but visible inguinal glands, a dark venter, and conspicuous orange or pale cream spots present on the upper lip. *Eleutherodactylus sentinelus* sp. nov. is unique among its congeners by the combination of its advertisement call, smooth dorsal and ventral skin, and a unique combination of a brown dorsum, pale interorbital bar and mid-dorsal stripe, and bright yellow inguinal flash coloration. The molecular phylogenetic analysis indicates that both species are part of the *Eleutherodactylus nitidus* species group, and closely related to one another. Known collecting localities for both new species suggest they are restricted to small ranges in moist pine-oak woodland, cloud forest, and oak-tropical deciduous forest on the windward slopes of the Sierra Madre del Sur in Guerrero. We discuss the relationships of the new species to all the species in the *Eleutherodactylus nitidus* species group and the validity of some related taxa based on the results of our phylogenetic analysis. The male advertisement calls of the new species are presented graphically, along with those of the closely related species occurring in the Sierra Madre del Sur. Range maps are presented for all species of the *Eleutherodactylus nitidus* species group, as the group is currently understood.

Keywords. Amphibia, common names, conservation, Guerrero, phylogenetics, Sierra Madre del Sur

Resumen.—Presentamos un análisis de datos morfológicos, moleculares y de cantos de poblaciones del grupo *Eleutherodactylus nitidus* (subgénero *Syrrophus*) del sur de México y describimos dos nuevas especies basado en nuestros resultados. *Eleutherodactylus maculabialis* sp. nov. es única entre sus congéneres por una combinación de caracteres, incluyendo la punta de los dedos ampliamente expandida, la presencia de glándulas inguinales poco distinguibles y presentar coloración con un fondo oscuro y manchas naranjas o pálidas en el labio superior. *Eleutherodactylus sentinelus* sp. nov. es única entre sus congéneres por la siguiente combinación de caracteres que incluyen su canto de advertencia, piel lisa en el dorso y zona ventral y una combinación única de coloración dorsal café, una barra interorbital pálida, una línea a lo largo del dorso y una coloración inguinal amarillo brillante. El análisis filogenético molecular indica que ambas especies pertenecen al grupo *Eleutherodactylus nitidus*, y están cercanamente relacionadas. Las localidades de colecta conocidas para ambas especies sugieren que las mismas tienen una distribución restringida a pequeños rangos en bosques húmedos de pino-encino, bosque mesófilo de montaña y bosque tropical cauducifolio con encinos en las pendientes de la Sierra Madre del Sur de Guerrero. Discutimos la relación entre todas las especies asociadas al grupo *Eleutherodactylus nitidus* así como la validez de algunos taxones relacionados basado en nuestro análisis filogenético. Analizamos los cantos de advertencia de las nuevas especies y las especies relacionadas que ocurren en la Sierra Madre del Sur. Finalmente presentamos mapas de distribución de todas las especies del grupo *Eleutherodactylus nitidus*, ya que actualmente no se entiende bien dicho grupo.

Palabras clave. Anfibios, conservación, filogenética, Guerrero, Sierra Madre del Sur

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Two new *Eleutherodactylus* species from Mexico

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Introduction

The frogs of the genus *Eleutherodactylus* Dumeril and Bibron, 1841 are among the most diverse and taxonomically challenging groups of amphibians in the New World (Hedges et al. 2008). The genus consists of five subgenera (Hedges et al. 2008), four of which are native to the West Indies (*Eleutherodactylus*; *Euhyas* Fitzinger 1843; *Pelorius* Hedges 1989; and *Schwartzius* Hedges et al. 2008), and a fifth (*Syrrhophus* Cope 1878), which is native to continental North America and Cuba. The native range of *Syrrhophus* extends from west-central Texas and central Sonora south through Mexico and into Guatemala and Belize, with two species in western Cuba. One species, *E. campi*, has been introduced to some urbanized parts of Texas (Dixon 2000), Louisiana (Hardy 2004), Alabama (McConnell et al. 2015), and Arizona (D. Ortiz, in press), presumably via the horticultural trade.

Whereas the systematics of the West Indian subgenera has been relatively well studied (Hedges et al. 2008), less attention has been given to the systematics of the subgenus *Syrrhophus* until recently. A recent effort by the authors sampled all of the currently recognized species of *Syrrhophus* in the United States, Mexico, and Guatemala. This resulted in Reyes et al. (2015) describing two distinctive new species of *Eleutherodactylus* from western Mexico based on mitochondrial DNA (mtDNA) sequence data, morphology, and advertisement calls.

In a continuation of that study, Grünwald et al. (2018) analyzed the morphological and molecular data of all known species within the subgenus, as well as the taxonomic history of *Syrrhophus*. That review led to the assignment of the continental North American species to two species series: the *Eleutherodactylus longipes* species series, which contains only the *Eleutherodactylus longipes* species group; and the *Eleutherodactylus nitidus* species series, which contains the *Eleutherodactylus nitidus* species group and the *Eleutherodactylus modestus* species group.

Those authors reviewed the *Eleutherodactylus modestus* species group in detail, describing six new species, synonymizing *E. nivicolimae* (Dixon and Webb, 1966) with *E. rufescens* (Duellman and Dixon, 1959), and providing data for the distinctiveness of *E. modestus* (Taylor, 1942), *E. pallidus* (Duellman, 1958), *E. teretistes* (Duellman, 1958), and *E. wixarika* Reyes-Velasco et al., 2015. Furthermore, those authors presented molecular evidence that *E. orarius* (Dixon, 1957) is distinct from *E. nitidus* (Peters, 1870). Recently, Palacios-Aguilar and Santos-Bibiano (2020) described a new species of saxicolous *Eleutherodactylus* from the lowlands of Guerrero, near the town of Tierra Colorada. With that

addition, there are currently 34 recognized species in the subgenus *Syrrhophus* in continental North America, plus two more in Cuba.

Here, we review the *Eleutherodactylus nitidus* species group in detail, and describe two more new species in that species group (*sensu* Grünwald et al. 2018) from the state of Guerrero. The morphological and molecular data for all named continental species of the subgenus *Syrrhophus* are analyzed to define the two new species. In addition, two subspecies are elevated to the species level and two other species are synonymized based on the results. These rearrangements result in 37 named species recognized as valid in the subgenus *Syrrhophus*, with (potentially) more awaiting description pending the completion of ongoing investigations.

Materials and Methods

Taxonomic sampling. From 2003–2019, the authors collected specimens of all known species of the subgenus *Syrrhophus* in Mexico, the United States, and Central America (Reyes-Velasco et al. 2015; Grünwald et al. 2018). Specimens of all currently recognized species of the subgenus (Frost 2020) were examined, and specimens of most species were measured, with the exceptions of *E. verruculatus* (Peters 1870) and the recently described *E. erythrochomus* (Palacios-Aguilar and Santos-Bibiano 2020). Comparisons of the new species described herein with the recently described species used the photos and data provided by the authors in the description (Palacios-Aguilar and Santos-Bibiano 2020). The validity of *E. verruculatus* has been questioned by various authors (Firschein 1954; Lynch 1970; Grünwald et al. 2018), and it is currently considered to be an enigmatic name with no known locality, genetic material, or recently collected specimens available.

All frogs were photographed alive—including dorsal, lateral, and ventral profiles, as well as photographs of each one showing the colors of flanks and flash colors on the groin and thigh. The frogs were euthanized with 10% ethanol or with topical benzocaine and tissue samples were obtained from thigh muscle or liver upon death and preserved in 96% ethanol. Specimens were preserved in 10% formalin and transferred to 70% ethanol for storage.

Measurements were taken from additional specimens of the subgenus *Syrrhophus* in the Museo de Zoología, Facultad de Ciencias (MZFC) of Universidad Nacional Autónoma de México (UNAM) and in the Amphibian and Reptile Diversity Research Center (ARDRC) of the

University of Texas at Arlington (UTA). Measurements were not taken directly for the type specimens of some previously described taxa so this study used the measurements of the type specimens provided in their original descriptions and published literature. Measurements of *Eleutherodactylus dilatus* (Davis and Dixon 1955), *E. fuscus* (Davis and Dixon 1955) (= *E. maurus*), and *E. albolabris* (Taylor 1943) are given in their original descriptions and in Dixon (1957a,b). Measurements for *E. nitidus* (Peters 1870) and *E. petersi* (Duellman 1954) were taken from Dixon (1957a,b), while measurements for *E. orarius* (Duellman 1958) and *E. syristes* (Hoyt 1965) were taken from their original descriptions. For *Eleutherodactylus pipilans* (Taylor 1940), *E. nebulosus* (Taylor 1943), and “*E. rubrimaculatus*” (Taylor and Smith 1945) this study used the measurements provided in their original descriptions and in Lynch (1970). Measurements of each of the above species were also taken from specimens collected during this study from the type localities or nearby.

The material collected is deposited at the Museo de Zoología, Facultad de Ciencias (MZFC) of the Universidad Nacional Autónoma de México (UNAM) in Mexico City. Specimen numbers for all material examined are provided in Appendix 1. While the specimens collected were formally catalogued at the MZFC, several of the examined specimens from both the MZFC and UTA collections have not been catalogued, and in such cases the original field numbers and their respective museums are listed.

Morphological measurements. The characters and terminology used herein follow those of Lynch and Duellman (1997), Savage (2002), and Grünwald et al. (2018). The following measurements were taken for each specimen (abbreviations listed in parentheses): snout-vent length (SVL); head length (HL); head width (HW); eyelid width (EW); interorbital distance (IOD); internarial distance (IND); eye-naris distance (END); diameter of eye (ED); width of tympanum (TW); height of tympanum (TH); eye-tympanum distance (ETD); upper arm length (UpL); forearm length (FoL); hand length (HaL); length of 1st finger (F1L); width of pad on 1st finger (F1PW); width of 1st finger (F1W); length of 2nd finger (F2L); width of pad on 2nd finger (F2PW); width of 2nd finger (F2W); length of 3rd finger (F3L); width of pad on 3rd finger (F3PW); width of 3rd finger (F3W); length of 4th finger (F4L); width of pad on 4th finger (F4PW); width of 4th finger (F4W); inner palmar tubercle length (IPTL); middle palmar tubercle length (MPTL); outer palmar tubercle length (OPTL); femur length (FeL); tibia length (TL); tarsal length (TaL), foot length (FL), total foot length (TotFL); length of 2nd toe (T2L); width of pad on 2nd toe (T2PW); width of 2nd toe (T2W); length of 3rd toe (T3L); width of pad on 3rd toe (T3PW); width of 3rd toe (T3W); length of 4th toe (T4L); width of pad on 4th toe (T4PW); width of 4th toe (T4W); length of 5th

toe (T5L); width of pad on 5th toe (T5PW); width of 5th toe (T5W); inner metatarsal tubercle length (IMTL); and outer metatarsal tubercle length (OMTL). Hand length (HA) was measured from the tip of the longest finger to the base of the palm, and foot length (FL) was measured from the tip of the longest toe to the base of the tarsus. The outer palmar tubercle refers to a small tubercle on the outer surface of the palm, but not one of the larger supernumerary tubercles. While these tubercles are usually present in *Syrrhophus*, they are generally absent in some species and their presence is variable in others.

Molecular analysis: DNA extraction and PCR amplification. A detailed description of the DNA extraction and PCR amplification protocols can be found in Grünwald et al. (2018). DNA was extracted from tissue samples using a standard potassium acetate protocol and a fraction of the 16s rRNA mitochondrial gene was sequenced using either the primers LX12SN1a (forward) and LX16S1Ra (reverse) of Zhang et al. (2013), or the modified primers 16Sar and 16Sbr of Bossuyt and Milinkovitch (2000). Unpurified PCR products were then shipped for sequencing to BGI Tech Solutions (Hong Kong).

Molecular analysis: sequence alignment and phylogenetic analysis. Regions with poor quality base calls were removed by manually trimming the 5' and 3' ends of all sequences using the program Geneious v6.1.6 (Biomatters Ltd., Auckland, New Zealand). All sequences were then aligned in Muscle (Edgar 2004), with a final alignment of 560 base pairs. Additionally, multiple sequences already available in GenBank were included. Two Cuban species of the subgenus *Syrrhophus* (*E. symingtoni* and *E. zeus*) were included as outgroups in the phylogenetic analysis. The final alignment included 104 individuals, of which 65 are new. All the new sequences have been deposited in GenBank and their accession numbers are shown in Appendix 2.

Bayesian inference of phylogeny (BI) was performed in MrBayes v3.2.2 (Ronquist et al. 2012), implemented on the CIPRES Science gateway server (Miller et al. 2010). First, the best-fit models of nucleotide substitution for the 16s rRNA mitochondrial gene were selected using the Bayesian Information Criterion (BIC) implemented in PartitionFinder v1.1.1 (Lanfear et al. 2012). The Bayesian analysis consisted of four runs of 10 million generations each, with four chains (one cold and three heated), and sampling every 1,000 generations. Tracer v1.6 (Drummond and Rambaut 2012) was used to confirm the convergence of the independent runs, based on overlap in likelihood and parameter estimates among runs, as well as effective sample size (ESS) and Potential Scale Reduction Factor value estimates (PSRF). PSRF indicated that individual runs had converged by 100,000 generations, so the first 25% of the runs were discarded as burn-in. Finally, posterior probability values were annotated on the

resulting topology using the program TreeAnnotator v1.8.3 (Rambaut et al. 2014). Additionally, genetic distances were obtained for the members of the group through the use of Mega X (Kumar et al. 2018).

Advertisement call graphing. Vocalizations were recorded for two individuals of each new species described here, as well as all other members of the *Eleutherodactylus* (*Syrrhophus*) *nitidus* species group (*sensu* Grünwald et al. 2018). The frogs were recorded while they were actively calling in the field, using the WavePad free recording software (NCH Software 2015) on various Apple iPhones. The calls were recorded at distances ranging from 50–150 cm, although distances within 100 cm of the frog were used whenever possible. Ambient temperatures were not obtained at the time of recording, but the time of day when each recording was made was noted.

The individual calls were isolated from other calls and background noise using Adobe Audition CC, and the calls were then analyzed using the software Raven Pro 1.5 (The Cornell Lab of Ornithology 2014). The Raven Pro settings were as follows: window size = 256 samples; window type = Hanning; overlap = 50%; DFT size = 256 samples; and grid spacing = 188 Hz. Temporal and spectral properties of isolated calls were analyzed using the Seewave version 1.6.4 package (Sueur et al. 2008) of the R statistical environment, version 3.3.2 (R Core Team 2016). Dominant frequency was estimated using a fast Fourier transform and fundamental frequency was estimated via short-term cepstral transform (Hanning window length = 256 samples with 80% overlap between successive windows for both spectral properties). 2D spectrograms were visualized using a sliding window analysis of short-term Fourier transform calculations.

Species descriptions. In order to simplify the identification of the two new species, their descriptions include comparisons of all the related species. To avoid confusion as to the point at which each species is formally described, the new species names are followed by “**sp. nov.**” until the point in the paper where a holotype is designated. Furthermore, for the sake of clarity the epithet “**sp. nov.**” is also used in the tables, figures, maps, appendices, and the molecular tree. The epithet “**sp.**” is used for the new species which are not described herein.

Results

Eleutherodactylus maculabialis sp. nov.

Spot-lipped Trilling Frog, *Rana trinadora* de labios manchados.

Figs. 1–2, 7A.

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Holotype. MZFC 33312 (CIG 00921). Adult male (Fig. 1), 11.4 km S of Puerto de Gallo, Municipality of Atoyac

de Álvarez (17.4672, -100.1916, datum = WGS84; 1,980 m asl), Guerrero, Mexico (Fig. 8A), collected on 15 July 2016 by Christoph I. Grünwald and Héctor Franz-Chávez.

Paratypes (n = 13; Fig. 2). MZFC 33307–311, 33313–314 (CIG 00916–920; 00922–923), seven adult males, collected at same locality and on same date as holotype; MZFC 33315–316 (CIG 00940–941), two adult males, 1.0 km S of bridge between Nueva Delhi and El Paraíso, Municipality of Atoyac de Álvarez (17.4150, -100.1924 datum = WGS84; 1,320 m asl), Guerrero, Mexico, collected on 15 July 2016 by Christoph Grünwald and Héctor Franz-Chávez; MZFC 33317–319 (CIG 00945–00947), two adult males, Yerba Santa, Municipality of General Heliodoro Castillo (17.5202, -99.9639, 2,003 m asl; datum = WGS84), Guerrero, Mexico collected on 16 July 2016 by Christoph Grünwald and Héctor Franz-Chávez; MZFC 33321 (CIG 00949), one adult male, 8.5 km N of Yerba Santa on road to Carrizal de Bravo, Municipality of General Heliodoro Castillo (17.5269, -99.9371, 1,880 m asl; datum = WGS84), Guerrero, Mexico, collected on 16 July 2016 by Christoph Grünwald and Héctor Franz-Chávez.

Diagnosis. Based on the phylogenetic analysis, this is a member of the genus *Eleutherodactylus*, subgenus *Syrrhophus*, as defined by Hedges et al. (2008). It is in the *Eleutherodactylus* (*Syrrhophus*) *nitidus* species series and the *Eleutherodactylus* (*Syrrhophus*) *nitidus* species group as defined by Grünwald et al. (2018) based on the condition of the tympanic annuli, ventral epidermis, and visceral peritoneum. A small frog, adult males measure 17.9–24.7 mm SVL; vocal slits are present in males, readily visible under partially translucent ventral epidermis; digital tips are expanded, 1.5–2.1 times the width of the narrowest part the finger on the third and fourth fingers; fingers moderately long, finger lengths are I-II-IV-III with third finger length ranging from 17–19% of SVL; compact lumbar gland above the inguinal region present, indistinct, barely visible in live specimen; ventral epidermis is partially translucent and visceral peritoneum is clear, not white, thus abdominal vein is not clearly visible against a white background on the venter of live specimens, and viscera are partially visible through translucent dark gray ventral epidermis; limbs moderate, TL/SVL ratio is 0.44–0.50, FeL/SVL ratio is 0.41–0.47 and TotFL/ SVL ratio is 0.66–0.73; snout relatively short, END/ SVL ratio is 0.10–0.12; tympanum small, indistinct and round, tympanic annuli not visible in live specimen; TW/ED ratio is 0.25–0.29. The dorsal, lateral, and ventral skin is smooth or slightly shagreen. Dorsal coloration variable, orange, tan, or brown, with darker brown marbling; loreal region dark brown, with conspicuous large white or orange spots present on upper labial region, and 1–3 orange spots on lower loreal region and upper portion of upper labia; pale interorbital

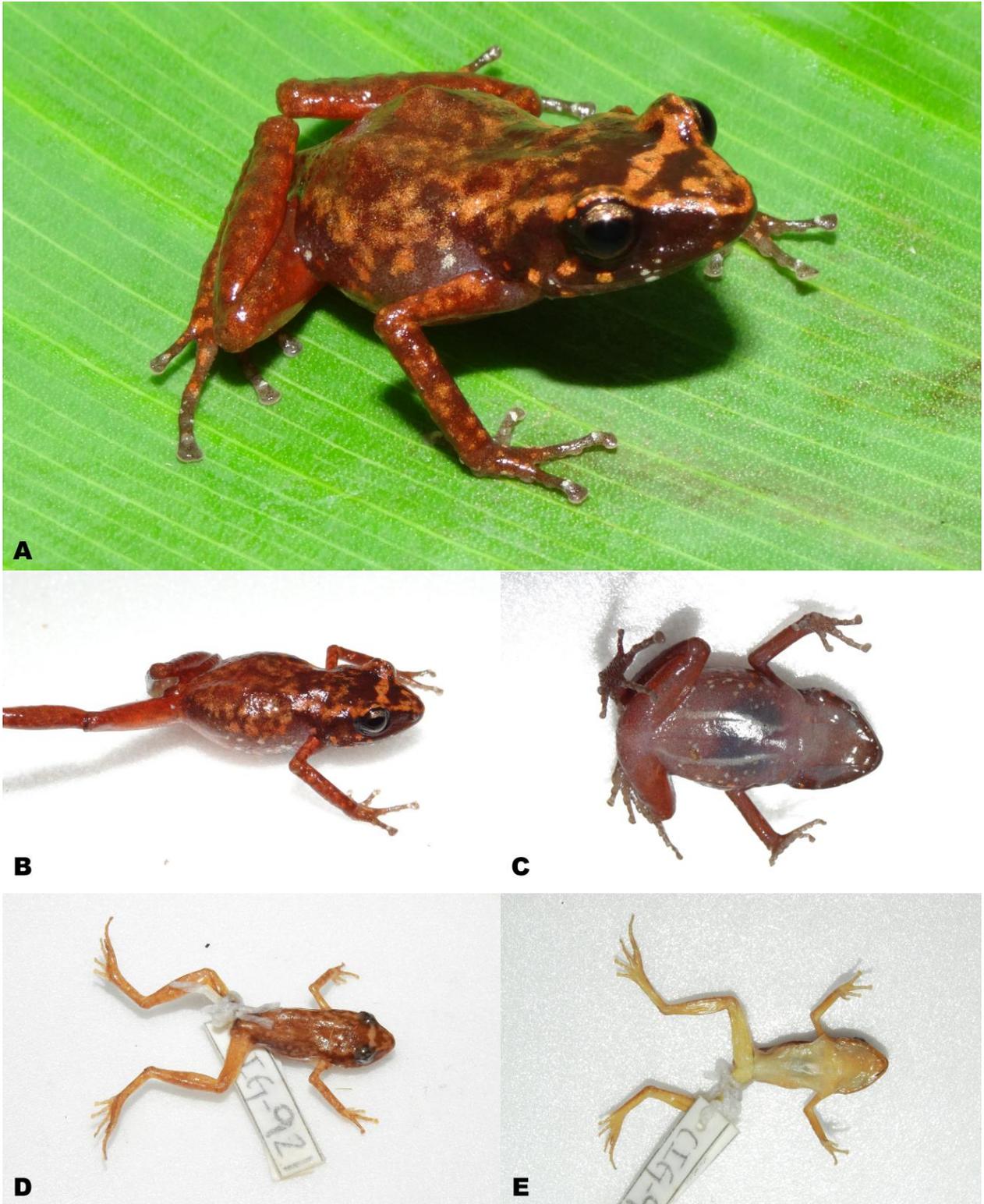


Fig. 1. Holotype of *Eleutherodactylus maculabialis* sp. nov., MZFC 33312 (CIG 00921) from 11.4 km S of Puerto de Gallo, Municipio de Atoyac de Álvarez, Guerrero, Mexico. (A) Dorsolateral perspective in life. (B) Lateral perspective in life. (C) Ventral perspective in life. (D) Dorsal perspective in preservative. (E) Ventral perspective in preservative.



Fig. 2. Some of the paratypes of *Eleutherodactylus maculabialis* sp. nov. in life. (A–C) MZFC 33310 (CIG 00919); (D–F) MZFC 33311 (CIG 00920); (G–I) MZFC 33314 (CIG 00923) all from type locality; (J–L) MZFC 33321 (CIG 00949); (M–O) MZFC 33318 (CIG 00946); (P–R) MZFC 33317 (CIG 00945) all from the vicinity of Yerba Santa on road to Carrizal de Bravo, Municipality of General Heliodoro Castillo, Guerrero, Mexico.

bar present, orange or tan, same color as palest dorsal coloration; indistinct transverse bands present on legs; upper arms same coloration and pattern as forearms; no pale mid-dorsal stripe; upper flanks same color as dorsum, lower flanks lavender or gray with white spots and marbling; venter gray to dark gray with sparse white spots or marbling present. Red, reddish, or orange inguinal flash colors sometimes present on thighs and groin; however, when present these colors are not more vivid than the coloration of the light interorbital bar and the characteristic colored spots on the upper lip. The mating call of adult males is a short trill (see below; Fig. 3).

Comparisons. *Eleutherodactylus maculabialis* can be distinguished from all species in the *Eleutherodactylus* (*Syrrhophus*) *longipes* species series by: possessing a small, indistinct tympanum with no tympanic annulus visible and with a diameter less than 30% of diameter of the eye; by possessing a ventral epidermis which is not clear, and combined with a visceral peritoneum which is not white, an abdominal vein on the venter which is not clearly evident against a white background in life; and by possessing a distinct, raised lumbar gland above the inguinal region.

Eleutherodactylus maculabialis can be distinguished from species of the *Eleutherodactylus* (*Syrrhophus*) *modestus* species group by the combination of possessing a compact, protruding lumbar gland above

the inguinal region, digital tips which are expanded more than 1.5 times the width of the narrowest part of the finger on the third and fourth fingers, and the presence of an interorbital bar.

Within its own species group, *E. maculabialis* can be distinguished from most species by possessing a compact inguinal gland that is indistinct but visible in live specimens. This character may or may not be visible in preserved specimens depending on how they were preserved. This species differs from *E. pipilans*, *E. erythrochomus*, and *E. nebulosus*, which lack a visible compact lumbar gland altogether. All other known species in the *E. (Syrrhophus) nitidus* species group have readily visible compact lumbar glands above the inguinal region. *Eleutherodactylus maculabialis* can be further distinguished from *E. pipilans* and *E. nebulosus* by possessing digital tips which are expanded more than 1.5 times the width of the narrowest part of the finger, and from *E. erythrochomus* by possessing digital tips which are more than 1.5 times but less than 3.0 times the width of the narrowest part of the finger. It may be distinguished from *E. nitidus*, *E. petersi*, and *E. orarius* by the combination of smoother skin, longer limbs, and tips of digits which are expanded more than 1.5 times the narrowest part of the finger on the third and fourth fingers. It is distinguished from *E. albolabris* by the following characters (characters of *E. albolabris* in parentheses): smaller size, 17.9–24.7 mm (23.0–26.8 mm), venter which is partially translucent and partially

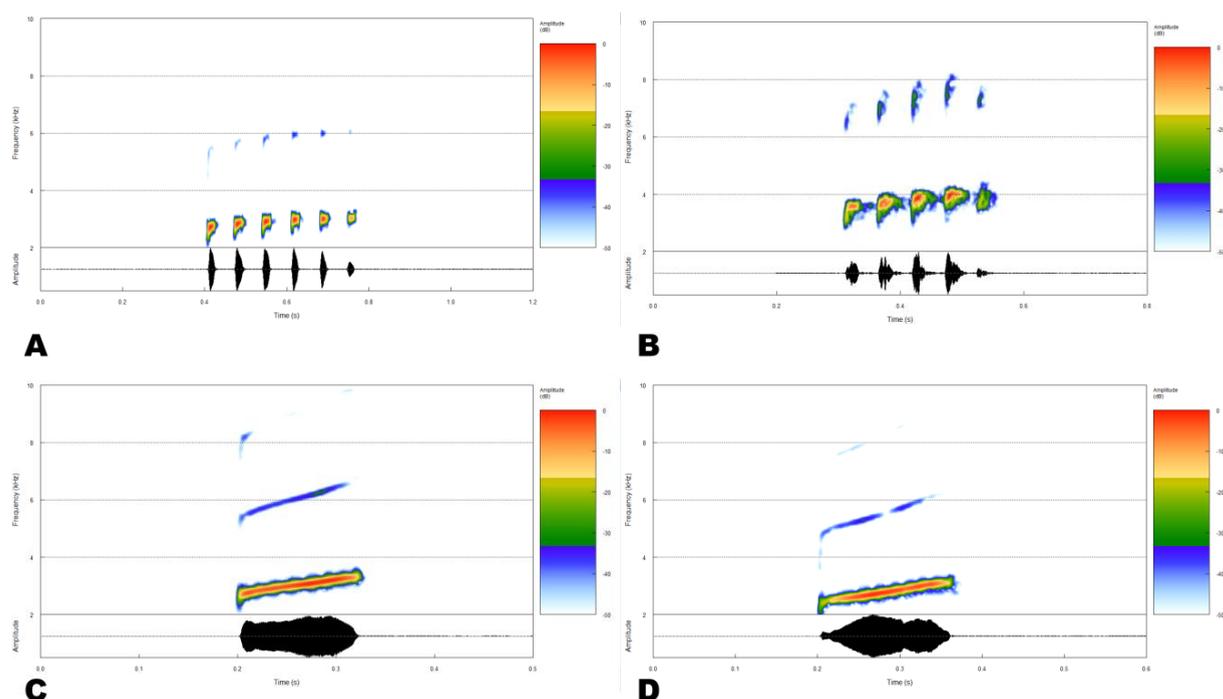


Fig. 3. Oscillograms and spectrograms of the advertisement calls of *Eleutherodactylus maculabialis* sp. nov. and *Eleutherodactylus sentinelus* sp. nov. (A) Adult male *E. maculabialis* sp. nov. from type locality. (B) Adult male *E. maculabialis* sp. nov. from 1.0 km S of bridge between Nueva Delhi and El Paraíso, Municipality of Atoyac de Álvarez, Guerrero, Mexico. (C) Adult male *Eleutherodactylus sentinelus* sp. nov. from Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico. (D) Adult male *Eleutherodactylus sentinelus* sp. nov. from type locality.

white with black markings (entire venter white with black markings), pale longitudinal blotch absent in mid-dorsal area (pale mid-dorsal blotch usually present in mid-dorsal area), smaller tympanum with TW/ED 0.25–0.28 (larger tympanum with TW/ED 0.27–0.32). *Eleutherodactylus maculabialis* may be distinguished from *E. dilatatus* by the following characters (characters of *E. dilatatus* in parentheses): smooth dorsal and ventral skin (rugose), dark gray ventral coloration with white spots (white ventral coloration with dark gray marbling), conspicuous white or orange spots on labial region (dark labial region, sometimes with white or pale yellow speckling), flash color absent, or when present not brighter than the pale coloration on dorsum and head (bright yellow flash coloration in inguinal region). *Eleutherodactylus maculabialis* may be distinguished from *E. maurus* by the following characters (characters of *E. maurus* in parentheses): presence of pale interorbital bar same color as snout (no pale interorbital bar), dark gray ventral coloration with white spots (white ventral coloration with dark gray marbling), conspicuous white or orange spots on labial region (dark labial region, sometimes with white or pale yellow speckling), smooth dorsal and ventral skin (rugose or slightly rugose dorsal and ventral skin), no pale mid-dorsal stripe present and upper arms same color as rest of limbs (thick, pale brown mid-dorsal strip usually present and upper arms pale ground coloration, unmarked and lighter than other limbs). *Eleutherodactylus maculabialis* is most similar to *E. syristes*. Both species share smooth dorsal and ventral skin, distinct compact lumbar glands above the inguinal region which are variably marked, a variable dorsal coloration of orange, brown, or tan with darker marbling, and similar red, reddish, or orange flash colorations. *Eleutherodactylus maculabialis* may be distinguished from *E. syristes* by the following characters (characters of *E. syristes* in parentheses): digital tips expanded more than 1.5 times the width of the narrowest part the finger on the third and fourth fingers (digital tips which are expanded 1.1–1.5 times the width of the narrowest part the finger on the third and fourth fingers), ventral coloration dark, with pale orange or white spots (ventral coloration white, with dark gray or black marbling and reticulations), conspicuous white or orange spots on labial region (labial region dark, occasionally with light gray or white speckling). Furthermore, *E. maculabialis* differs from all species in its species group by its advertisement call, which is a short trill. The only other species in the species group which has an advertisement call that is a trill is *E. syristes*, but the call of that species is much more drawn-out (see below, Fig. 11A–B).

Description of the holotype. Male, relatively small (23.5 mm SVL); head longer (7.8 mm) than wide (6.9 mm), head slightly wider than body; snout subovoid from a dorsal view and rounded from a lateral profile;

tympanum indistinct, rounded with no supra-tympanic fold present; tympanum small, circular, greatest width of tympanum 0.7 mm; greatest diameter of eye 2.7 mm; tympanum width to eye diameter 0.27; eyelid width 1.5 mm, approximately 32% of the IOD, first finger shorter than second finger; finger lengths from shortest to longest I-II-IV-III with two and three equal; digital pads on fingers two, three, and four expanded, 1.9 times the narrowest point of the digit on fingers three and four; expanded finger pads slightly rounded, three palmar tubercles; inner palmar tubercle about 70% as large as middle palmar tubercle, outer palmar tubercle about 45% the size of middle palmar tubercle (Fig. 6A); toe lengths from shortest to longest I-V-II-III-IV, TL2 and TL5 very similar; outer metatarsal conical with a round base, moderate, approximately 66% of inner metatarsal tubercle; inner metatarsal tubercle spherical shape with oval base, large, approximately 0.9 mm in length. Dorsal skin smooth, lateral skin slightly shagreened, ventral skin smooth to slightly areolate. Skin was smooth in life. Vocal slits present.

In life the holotype had a reddish-tan dorsal coloration on the back (Fig. 1A–C), with darker brown mottling that was increasingly dense towards the mid-dorsal area. The pale orange was present on flanks, interorbital bar and spots on labial region. The flanks were reddish-tan with indistinct darker brown mottling and small white spots. The hind legs and arms were reddish-tan with irregular brown transverse bars. Bright reddish-orange flash colors on groin or thighs. Ventral coloration was gray with white and orange spots on throat and upper chest.

Coloration in preservative is light brown dorsum, with darker brown markings and a pale tan interorbital bar. The dorsal surfaces of the legs are light brown and the groin and posterior surfaces of the thighs are brown. The venter is white, with light brown on throat (Fig. 1D–E).

Measurements of the holotype (in mm). IND 2.5, IOD 4.8, END 2.5, ETD 0.9, UpL 6.3, FoL 7.5, HaL 5.5, F1L 1.8, F1PW 0.5, F1W 0.4, F2L 2.4, F2PW 0.7, F2W 0.4, F3L 3.9, F3PW 0.9, F3W 0.5, F4L 2.4, F4PW 0.8, F4W 0.4, IPTL 0.7, MPTL 1.0, OPTL 0.4, FeL 10.5, TL 11.5, TaL 5.9, FL 10.2, T2L 3.5, T2PW 0.8, T2W 0.5, T3L 4.6, T3PW 0.8, T3W 0.5, T4L 7.2, T4PW 0.9, T4W 0.5, T5L 3.1, T5PW 0.6, T5W 0.4, IMTL 0.9, OMTL 0.6, FeL/SVL 45%, TL/SVL 49%, Ha/SVL 23%, FL/SVL 43%, HL/SVL 33%, HW/SVL 29%.

Variation. SVL from 17.9–24.7 mm (21.65 ± 1.77). Expanded finger pads vary from 1.5–2.1 times the narrowest part of the digit on the third and fourth fingers, with average of 1.7 ± 0.21 on the third finger and average of 1.73 ± 0.21 on the fourth finger. Dorsal ground coloration ranged from different shades of tan, reddish, or brown, always with darker brown coloration

on the dorsum. Different degrees of dark mottling are present on the dorsal surfaces. Venter always gray, but with varying amounts of white spots. Characteristic bright orange spots on upper part of upper labial and lower part of loreal region present in most individuals (MZFC 33307–314); however, these spots may be faded or white in individuals from lower elevations (MZFC 33315–19, 33321), and one specimen (MZFC 33316) has only one pale spot on the right side, and none on the left. In some individuals, orange coloration may be lacking on the lip (MZFC 33315–19, 33321), in these individuals it is lacking everywhere, including the inguinal region and on the interorbital bar. Morphological variation is presented in Table 1.

Distribution and ecology. This species is known from two municipalities in the Sierra Madre del Sur of central Guerrero (Fig. 7A). It has been collected in tropical evergreen forest, cloud forest, oak woodland, pine-oak forest, and riparian vegetation between 900–2,100 m asl. All specimens were collected from low-growing vegetation or rocks along road cuts while calling in the month of July. This species has been collected in sympatry with *E. albolabris* and *E. nitidus*.

Etymology. From the combination of the Latin words *maculatus* and *labialis*, meaning spotted-lip, named for the conspicuous bright colored spots on the upper lip and above the rictus, present in varying degrees, which along with the advertisement call help to readily distinguish this species in the field.

Referred specimens. CIG 01484–01485, 01501, three adult males collected at the type locality on 29 June 2019 by Christoph I. Grünwald, Karen I. Morales-Flores, Janelle Morales-Flores; JAC 22216, one adult male, collected between Nueva Delhi and Puerto del Gallo, Municipality of Atoyac de Álvarez (17.4668, 100.198, 2,020 m asl, datum WGS84), Guerrero, Mexico on 15 June 2002 by Jonathan A. Campbell; JAC 25643–25646, four adult males, collected at Nueva Delhi, Municipality of Atoyac de Álvarez (17.4113, -100.1954, 1,239 m asl, datum WGS84), Guerrero, Mexico on 6 July 2004 by Jonathan A. Campbell.

***Eleutherodactylus sentinelus* sp. nov.**

El Balsamo Peeping Frog, *Rana piadora* del Puerto El Balsamo.

Figs. 4–5, 7B.

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Holotype. MZFC 33306 (CIG 00913). Adult male (Fig. 4), 8.9 km SW of Puerto El Balsamo, Municipality of José Azueta (17.9549, -101.2253, 1,354 m asl; datum = WGS84), Guerrero, Mexico (Fig. 8B), collected on 14 July 2016 by Christoph I. Grünwald and Héctor Franz-Chávez.

Paratypes (*n* = 7; Fig. 5). MZFC 33302–33305 (CIG 00907–910), four adult males, collected at Puerto El Balsamo, Municipality of José Azueta (17.9813, -101.2291, 1,900 m asl; datum = WGS84), Guerrero, Mexico on 14 July 2016 by Christoph I. Grünwald and Héctor Franz-Chávez; MZFC 33031–33033 (CIG 00333–335), three adult males, collected at Puerto El Balsamo, Municipality of José Azueta (17.9812, -101.2292, 1,900 m asl; datum = WGS84), Guerrero, Mexico on 5 June 2015 by Christoph I. Grünwald, Nadia Pérez-Rivera, and Héctor Franz-Chávez.

Diagnosis. Based on the phylogenetic analysis, this species is a member of the genus *Eleutherodactylus*, subgenus *Syrrhophus*, as defined by Hedges et al. (2008); and in the *Eleutherodactylus* (*Syrrhophus*) *nitidus* species series and the *Eleutherodactylus* (*Syrrhophus*) *nitidus* species group as defined by Grünwald et al. (2018) based on the condition of the tympanic annuli, ventral epidermis, and visceral peritoneum. A small frog, adult males measure 23.3–25.3 mm SVL; vocal slits are present in males; digital tips are expanded, 1.4–2.3 times the width of the narrowest part the finger on the third and fourth fingers; finger lengths are I-II-IV-III, fingers moderately long, with third finger length ranging from 13–21% of SVL; compact lumbar gland in the inguinal region present but indistinct, visible in live specimen; ventral epidermis semi-translucent and the visceral peritoneum is clear, not white, abdominal vein barely visible on the venter of live specimens against the background of the viscera; limbs moderate, TL/SVL ratio is 0.41–0.56, FeL/SVL ratio is 0.38–0.46 and TotFL/ SVL ratio is 0.61–0.74; snout short, END/ SVL ratio is 0.10–0.11; tympanum small, indistinct and round, tympanic annuli not visible in live specimen; TW/EW ratio is 0.26–0.28. The dorsal skin is smooth to slightly pustulate. Dorsal coloration is reddish-tan or brown. A pale brown or reddish interorbital bar always present, and a pale mid-dorsal blotch of the same color as the interorbital bar is present. Upper arms pale and unmarked, dark transverse bands present on forearms and legs, and inguinal flash coloration orange or yellow present on groin and sometimes on posterior portion of thighs. Ventral coloration whitish or gray with some darker gray spots or indistinct marbling. The mating call of adult males is a quick chirp (“peep,” see below; Fig. 3).

Comparisons. *Eleutherodactylus sentinelus* can be distinguished from all species in the *Eleutherodactylus* (*Syrrhophus*) *longipes* species series by: possessing a small, indistinct tympanum with no tympanic annulus visible and with a diameter less than 30% of diameter of the eye; by possessing a visceral peritoneum which is not white, so that the abdominal vein on the venter is not clearly evident against a white background in life; and by possessing a distinct, raised lumbar gland above

Table 1. Morphological measurements of *Eleutherodactylus maculibialis* sp. nov. specimens. See text for definitions of measurement acronyms.

| | CIG 916 | CIG 917 | CIG 918 | CIG 919 | CIG 920 | CIG 921 | CIG 922 | CIG 923 | CIG 940 | CIG 941 | CIG 945 | CIG 946 | CIG 947 | CIG 949 |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| SVL | 19.26 | 22.27 | 21.05 | 22.05 | 22.95 | 23.47 | 24.65 | 21.3 | 20.46 | 17.9 | 23.88 | 22.01 | 20.13 | 21.67 |
| HL | 6.86 | 7.45 | 7.3 | 7.43 | 7.57 | 7.78 | 8.07 | 7.18 | 7 | 6.51 | 7.77 | 7.2 | 6.8 | 7.27 |
| TW | 0.68 | 0.71 | 0.68 | 0.72 | 0.73 | 0.73 | 0.74 | 0.72 | 0.67 | 0.63 | 0.67 | 0.67 | 0.66 | 0.67 |
| ED | 2.38 | 2.78 | 2.57 | 2.56 | 2.6 | 2.68 | 2.75 | 2.53 | 2.44 | 2.23 | 2.73 | 2.5 | 2.41 | 2.48 |
| EIW | 1.28 | 1.37 | 1.3 | 1.4 | 1.39 | 1.51 | 1.51 | 1.25 | 1.33 | 1.2 | 1.51 | 1.43 | 1.47 | 1.46 |
| IOD | 4.12 | 4.35 | 4.38 | 4.41 | 4.66 | 4.76 | 4.73 | 4.23 | 3.82 | 3.41 | 4.18 | 4.08 | 4.12 | 4.11 |
| IND | 2.15 | 2.32 | 2.36 | 2.34 | 2.41 | 2.48 | 2.53 | 2.22 | 2.15 | 2.04 | 2.45 | 2.22 | 2.2 | 2.22 |
| END | 2.24 | 2.66 | 2.4 | 2.36 | 2.43 | 2.51 | 2.5 | 2.31 | 2.2 | 2.08 | 2.51 | 2.3 | 2.26 | 2.31 |
| ETD | 0.74 | 0.81 | 0.85 | 0.84 | 0.86 | 0.88 | 0.89 | 0.85 | 0.84 | 0.84 | 0.9 | 0.88 | 0.84 | 0.87 |
| UpL | 5.03 | 5.33 | 5.06 | 5.08 | 6 | 6.31 | 5.9 | 5.51 | 5.1 | 4.22 | 5.6 | 5.28 | 4.84 | 4.94 |
| Fol | 6.12 | 6.6 | 6.07 | 6.51 | 7.17 | 7.47 | 7.18 | 6.52 | 6.15 | 5.24 | 6.9 | 6.15 | 5.86 | 5.9 |
| HaL | 4.72 | 5.55 | 5.07 | 5.22 | 5.3 | 5.5 | 5.46 | 5 | 4.53 | 4.05 | 5.54 | 4.95 | 5.06 | 5.12 |
| F3PW/F3W | 1.8 | 1.6 | 2 | 2.1 | 1.7 | 1.9 | 1.8 | 1.7 | 1.6 | 1.4 | 1.6 | 1.4 | 1.8 | 1.5 |
| F4PW/F4W | 1.9 | 1.7 | 2 | 2.1 | 1.6 | 1.9 | 1.9 | 1.6 | 1.8 | 1.5 | 1.6 | 1.5 | 1.8 | 1.4 |
| FeL | 9.05 | 10.06 | 9.65 | 9.66 | 10.03 | 10.49 | 10.44 | 8.75 | 8.6 | 8.22 | 9.92 | 9.78 | 9.04 | 9.09 |
| TL | 9.75 | 10.46 | 10.23 | 10.18 | 11 | 11.5 | 10.97 | 9.9 | 9.4 | 8.6 | 10.89 | 10.17 | 9.95 | 9.75 |
| TotFL | 14.08 | 15.75 | 15.13 | 15.43 | 15.81 | 16.12 | 16.4 | 14.53 | 13.4 | 12.42 | 15.83 | 14.6 | 14.57 | 13.93 |
| ITP | 0.56 | 0.6 | 0.57 | 0.55 | 0.68 | 0.67 | 0.69 | 0.58 | 0.49 | 0.39 | 0.51 | 0.58 | 0.47 | 0.4 |
| MPT | 0.78 | 0.9 | 0.79 | 0.84 | 0.92 | 0.96 | 0.95 | 0.77 | 0.75 | 0.69 | 0.73 | 0.73 | 0.7 | 0.7 |
| OPT | 0.28 | 0.45 | 0.4 | 0.37 | 0.46 | 0.43 | 0.44 | 0.37 | 0.3 | 0.25 | 0.34 | 0.35 | 0.35 | 0.32 |
| IMTL | 0.79 | 0.83 | 0.76 | 0.73 | 0.79 | 0.91 | 0.95 | 0.68 | 0.67 | 0.64 | 0.8 | 0.78 | 0.74 | 0.72 |
| OMTL | 0.44 | 0.55 | 0.45 | 0.45 | 0.56 | 0.6 | 0.63 | 0.37 | 0.32 | 0.4 | 0.45 | 0.43 | 0.41 | 0.45 |
| TW/ED | 0.29 | 0.26 | 0.26 | 0.28 | 0.28 | 0.27 | 0.27 | 0.28 | 0.27 | 0.28 | 0.25 | 0.27 | 0.27 | 0.27 |

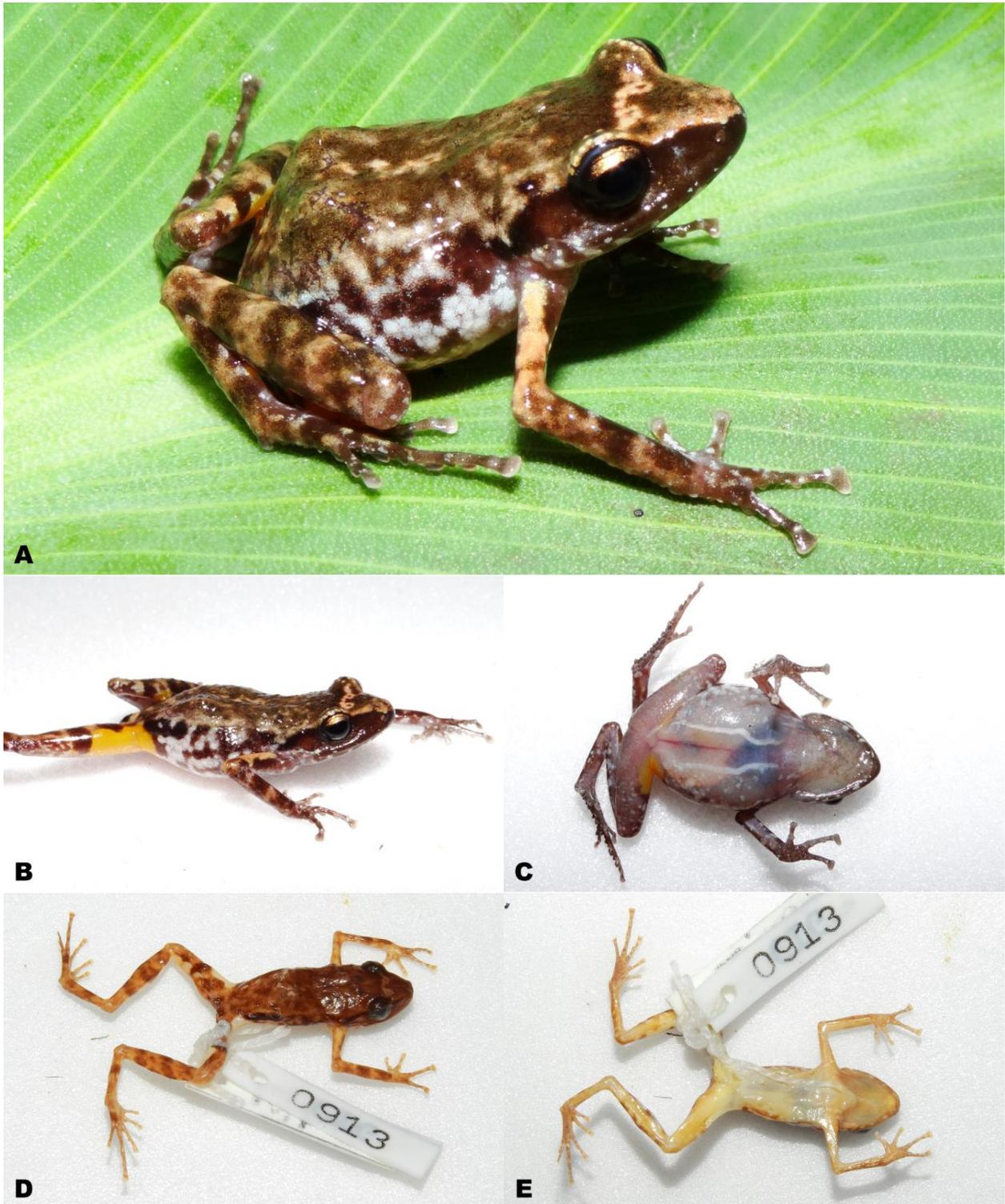


Fig. 4. Holotype of *Eleutherodactylus sentinelus* sp. nov., MZFC 33306 (CIG 00913) from 8.9 km SW of Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico. **(A)** Dorsolateral perspective in life. **(B)** Lateral perspective in life. **(C)** Ventral perspective in life. **(D)** Dorsal perspective in preservative. **(E)** Ventral perspective in preservative.



Fig. 5. Some of the paratypes of *Eleutherodactylus sentinelus* sp. nov. in life. (A–C) MZFC 33305 (CIG 00910); (D–F) MZFC 33304 (CIG 00909); (G–I) MZFC 33032 (CIG 00334); (J–L) MZFC 33031 (CIG 00333); (M–O) MZFC 33033 (CIG 00335) from Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico.

the inguinal region.

Eleutherodactylus sentinelus can be distinguished from species of the *Eleutherodactylus* (*Syrrhophus*) *modestus* species group by the combination of possessing a compact, protruding lumbar gland above the inguinal region, digital tips which are expanded more than 1.4 times the width of the narrowest part the finger on the third and fourth fingers, and the presence of an interorbital bar.

Within its own species group, *E. sentinelus* can be distinguished from most species by possessing a compact inguinal gland which is indistinct but visible in live specimens. This differs from *E. pipilans*, *E. erythrochomus*, and *E. nebulosus*, which lack a distinct visible inguinal gland altogether. All other known species in the *E. (Syrrhophus) nitidus* species group have readily visible compact lumbar glands above the inguinal region. *Eleutherodactylus sentinelus* can be further distinguished from *E. pipilans*, *E. erythrochomus*, and *E. nebulosus* by the presence of distinct pale interorbital bar and pale-yellow inguinal flash coloration. It may be distinguished from *E. nitidus*, *E. petersi*, and *E. orarius* by the combination of smoother skin, longer limbs, and tips of digits which are expanded more than 1.4 times the narrowest part of the finger on the third and fourth fingers. It further differs from these three species by call, which is a short chirp rather than a whistle. It is distinguished from *E. albolabris* by the following characters (characters of *E. albolabris* in parentheses): lip dark, never white, with some flecking (lip white, immaculate), inguinal flash coloration always yellow (always fiery orange), ventral coloration translucent and white, with some black markings (ventral coloration completely white with bold black markings), tympanum small with TW/ED 0.25–0.28 (tympanum slightly larger with TW/ED 0.27–0.32). *Eleutherodactylus sentinelus* may be distinguished from *E. maurus* by the following (characters of *E. maurus* in parentheses): by the presence of pale interorbital bar same color as snout (*E. maurus* presents no pale interorbital bar), smooth dorsal and ventral skin (rugose or slightly rugose dorsal and ventral skin), bright yellow or orange flash colors on thighs (flash colors absent, or barely discernible), pale interorbital stripe distinct between darker head coloration (pale interorbital stripe absent or same color as dorsal surface of snout). *Eleutherodactylus sentinelus* may be distinguished from *E. syristes* and *E. maculabialis* by its mating call, which is a rapid chirp (“peep”) as opposed to a trill. *Eleutherodactylus sentinelus* can be further distinguished from the closely related *E. syristes* by having more expanded finger tips, 1.4–2.3 times the narrowest part of the digit on fingers three and four, and usually more than 1.6 (vs. 1.1–1.5 times the narrowest part of the digit on fingers three and four), and by having yellow or yellowish-orange flash colors (vs. orange or reddish flash colors). *Eleutherodactylus sentinelus* is most similar to *E.*

dilatatus, from which it may be distinguished by the by much smoother skin on both the dorsum and venter (*E. dilatatus* has rugose dorsal skin and pustulate venter), smaller, less distinct inguinal glands (*E. dilatatus* has large, distinct inguinal glands), and a paler dorsal ground coloration (*E. dilatatus* is dark brown).

Description of the holotype. Small frog (24.1 mm SVL); male; head slightly longer (7.1 mm) than wide (6.4 mm), head slightly wider than body; snout subovoid from a dorsal view and rounded from a lateral profile; tympanum indistinct, rounded with no supra-tympanic fold present; tympanum small, circular, greatest width of tympanum 0.8 mm; greatest diameter of eye 2.9 mm; tympanum width to eye diameter ratio 0.28; eyelid width 1.6 mm, a third of the IOD; first finger shorter than second finger; finger lengths from shortest to longest I-II-IV-III; digital pads on fingers two, three, and four slightly expanded, 1.6 times the narrowest point of the digit on second finger and 2.3 times the narrowest point of the digit on fingers three and four; expanded finger pads truncate; three palmar tubercles; inner palmar tubercle about 75% as large as middle palmar tubercle, outer palmar tubercle about half the size of middle palmar tubercle (Fig. 6B); toe lengths from shortest to longest I-II-V-III-IV; outer metatarsal conical with a round base, small, approximately 50% of inner metatarsal tubercle; inner metatarsal tubercle spherical shape with oval base, large, approximately 0.9 mm in length; dorsal skin smooth, lateral skin slightly shagreened, ventral skin smooth. Vocal slits present.

In life, the holotype had a dark reddish-brown dorsal ground coloration, with pale reddish interorbital bar, mid-dorsal blotch, and upper arm coloration, with the upper arm the palest. Lateral portions of the head were dark brown coloration, with small white flecks on the labial and loreal regions. Lateral coloration was brown and white. Legs and arms were ochre with dark brown transverse bars. Yellow flash colors present on the groin. Ventral coloration flesh colored with white spots and black melanophores. See Fig. 4A–C for photographs of the holotype in life.

Coloration in preservative is brown dorsum, with some paler brown areas on the lower parts of the back. Indistinct cream-colored interorbital bar, upper arms and groin pale tan. Inguinal gland black. White marbling on lateral surfaces. Legs and arms pale tan with darker brown transverse bands. Ventral surfaces white, unmarked, throat yellowish (Fig. 4D–E).

Measurements of the holotype (in mm). IND 2.4, IOD 4.8, END 2.5, ETD 1.1, UpL 6.2, FoL 7.9, HaL 6.3, F1L 2.3, F1PW 0.5, F1W 0.3, F2L 2.7, F2PW 0.6, F2W 0.4, F3L 4.0, F3PW 1.0, F3W 0.4, F4L 3.1, F4PW 1.0, F4W 0.4, IPTL 0.6, MPTL 0.9, OPTL 0.4, FeL 11.3, TL 12.3, TaL 7.2; FL 10.9, T2L 3.1, T2PW 0.7, T2W 0.4, T3L 4.9, T3PW 0.7, T3W 0.4, T4L 7.9, T4PW 0.8, T4W 0.4,

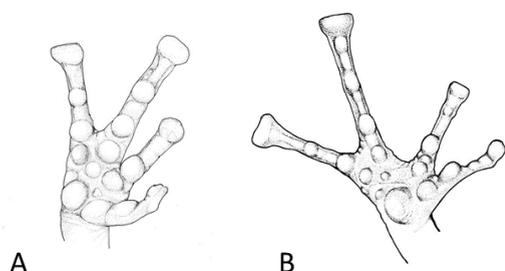


Fig. 6. (A) Ventral aspect of hand of holotype of *Eleutherodactylus maculabialis* sp. nov., MZFC 33312 (CIG 00921) from 11.4 km S of Puerto de Gallo, Municipio de Atoyac de Álvarez, Guerrero, Mexico. (B) Ventral aspect of hand of holotype of *Eleutherodactylus sentinelus* sp. nov., MZFC 33306 (CIG 00913) from 8.9 km SW of Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico.

T5L 3.5, T5PW 0.5, T5W 0.4, IMTL 1.0, OMTL 0.6, FeL/SVL 46%, TL/SVL 50%, Ha/SVL 25%, FL/SVL 44%, HL/SVL 33%, HW/SVL 29%.

Variation. SVL from 23.3–25.3 mm (24.2 ± 0.81). Expanded finger pads on third and fourth fingers vary from 1.4–2.3 times the narrowest part of the digit, with average 1.85 ± 0.26 on the third finger and average 1.78 ± 0.36 on the fourth finger. Dorsal ground coloration ranges across different shades of reddish brown (MZFC 33304), tan (MZFC 33031, 33306), and brown (MZFC 33032–3, 33304–5). Venter typically gray, with white and black markings, although these markings range from sparse to almost complete reticulation. Morphological variation is presented in Table 2.

Distribution and ecology. This species is known only from the vicinity of the type locality in the western-most extension of the Sierra Madre del Sur of Guerrero (Fig. 7A). It has been collected at elevations ranging from 1,300–1,900 m asl, on steep mountain sides in humid pine-oak forest, oak woodland and pine-oak woodland, and tropical deciduous forest ecotone. At the type locality, this species is sympatric with *E. petersi*. All individuals of *E. sentinelus* have been observed after the onset of the rainy season in the months of June and July. Individuals were found calling on small bushes or rocks. All were found active at night.

Etymology. This species is named after latin *sentinel*, meaning guard or outpost, in reference to its type locality, a mountain which stands out from the north and west as the first outpost of the Sierra Madre del Sur of Guerrero.

Summary Data for *Eleutherodactylus nitidus* Species Group Members

To help differentiate between the two new species

described above, and the related species in the *Eleutherodactylus nitidus* species group, important morphological, mensural, and call differences of all of the species in this group are collated into a single table (Table 3), and photographs of related species in the *E. nitidus* species group are provided (Figs. 9–10). The distributions of the species in the *E. nitidus* group are mapped in Figs. 7 and 13.

Differentiating the Advertisement Calls of Closely Related *Eleutherodactylus nitidus* Species Group Members

Recordings were made from 120 calling males of all 24 species of *Eleutherodactylus* from western Mexico. The calls of the species analyzed were found to fall into five different categories of a rapid burst whistle (trill), a drawn-out whistle (whistle), a strong high-pitched chirp (peep), a soft high-pitched chirp (chirp), and a drawn-out chirp (pipe). Of the species analyzed herein, four (*E. dilatatus*, *E. nebulosus*, *E. pipilans*, *E. sentinelus*) produce a strongly high-pitched chirp (peep), four (*E. albolabris*, *E. nitidus*, *E. orarius*,

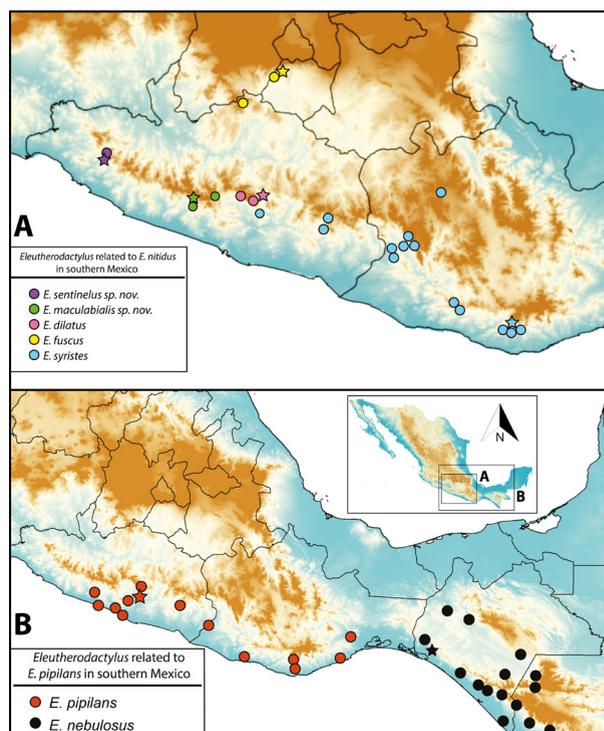


Fig. 7. (A) Map showing the type localities and distribution of *Eleutherodactylus* species related to *E. nitidus* in southern Mexico. The stars represent type localities and circles represent additional localities, with each color coded for the species: *E. sentinelus* sp. nov. (purple), *E. maculabialis* sp. nov. (green), *E. dilatatus* (pink), *E. maurus* (yellow), and *E. syristes* (blue). (B) Map showing the type localities and distribution of *Eleutherodactylus* species related to *E. pipilans* in southern Mexico. The red star represents the type locality of *E. pipilans* and red circles represent additional localities. The black star represents the type locality of *E. nebulosus* and black circles represent additional localities.



Fig. 8. (A) Type locality of *Eleutherodactylus maculabialis* sp. nov. at 11.4 km S of Puerto de Gallo, Municipio de Atoyac de Álvarez, Guerrero, Mexico. (B) Type locality of *Eleutherodactylus sentinelus* sp. nov. at 8.9 km SW of Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico.

E. petersi) produce a drawn-out whistle (whistle), two (*E. maculabialis* and *E. syristes*) produce multi-note or pulsed calls (trill) of various rates and notes, and one (*E. maurus*) produces a slow and drawn-out chirp (pipe). While a detailed account is given of the calls of the species we describe here, as well as some of their relatives, we refrain from giving detailed descriptions of the calls of *E. nitidus*, *E. albolabris*, *E. orarius*, *E. petersi*, *E. pipilans*, *E. nebulosus*, and “*E. rubrimactulatus*” pending a more detailed taxonomic study of this species complex.

***Eleutherodactylus dilatatus*.** The advertisement call of *E. dilatatus* is a single high-pitched “chirp” best described as a “peep” (Fig. 11C). Calls recorded at the type locality consist of a rapid chirp, with an average duration of 110 ms, and a dominant frequency ranging between starting at 2,550 kHz and 2,780 kHz.

***Eleutherodactylus maculabialis*.** The advertisement call of *E. maculabialis* is a relatively slow multi-note “trill” (Fig. 3). Calls recorded at the type locality consist of slow six-note trills with a duration of 180–210 ms, and a dominant frequency starting at 2,600–2,900 kHz and rising to 3,050–3,200 kHz. The call interval is about 40–55 ms (Fig. 3A). At a nearby

locality at 1,320 m asl (Fig. 3B), calls were recorded of two males which varied from the type locality in consisting of five-note trills with an average duration of 230 ms and a dominant frequency starting at 3,630 kHz and reaching 4,000 kHz (Table 4).

***Eleutherodactylus maurus*.** The advertisement call of *E. maurus* is a single, drawn-out “chirp” best described as a “pipe.” Calls recorded near Ocuilan, in Estado de México, consisted of a single chirp with a duration of 205 ms, and a dominant frequency starting at 2,760 kHz and rising to 3,150 kHz. The amplitude was strongest at the middle of a call (Fig. 11D). At a locality near Taxco, Guerrero, a call of *E. maurus* was recorded which consisted of a single, drawn-out chirp. However, the call was longer, averaging 275 ms and the amplitude was distributed in three sub-pulses. This indicates a further need to investigate this population.

***Eleutherodactylus sentinelus*.** The advertisement call of *E. sentinelus* is a single high-pitched “chirp” best described as a “peep” (Fig. 3). Calls recorded at the type locality consist of a rapid chirp, with an average duration of 160 ms, and a dominant frequency starting at 2,560 kHz and rising to 3,100 kHz (Fig. 3D). Calls recorded above the type locality at 1,900 m asl (Fig. 3C) are slightly shorter and of higher frequency, with an average duration of 120 ms, and a dominant frequency starting at 2,700 kHz and finishing at 3,300 kHz (Table 4).

***Eleutherodactylus syristes*.** The advertisement call of *E. syristes* is a rapid but long multiple-pulse “trill” (Fig. 11A–B). Calls recorded at two different localities in Guerrero consisted of 49–60 pulses, about 10 ms apart, with a total duration of the call averaging 500–560 ms. The dominant frequency at Agua de Obispo, Guerrero, was between 2,700–3,170 kHz, starting lower, peaking, and then lowering again (Fig. 11A). At a second locality, slightly lower than Agua de Obispo, east of Highway 95, the calls tended to have a dominant frequency ranging between 3,000–3,390 kHz (Fig. 11B).

***Eleutherodactylus albolabris*.** The advertisement call of *E. albolabris* is a single, multi-pulsed whistle, with the call continuous amongst the pulses and with a duration of approximately 150–250 ms (Fig. 11F). Calls recorded at the type locality at Agua de Obispo had a duration of 160 ms, and started at a dominant frequency of 2,840 kHz rising to 3,015 kHz. Calls varied between 7–10 continuous pulses. At a second locality, near Vallecitos in the Municipality of José Azueta, Guerrero, the calls had a duration of 240 ms, and started at a dominant frequency of 2,740 kHz which rose to 2,915 kHz. Calls started with a multi-pulsed trill and then ended with several distinct pulses. This population’s call is best described as a combination of a “trill” with a “whistle” (Fig. 11E).

Table 2. Morphological measurements of *Eleutherodactylus sentinelus* sp. nov. specimens. See text for definitions of measurement acronyms.

| | CIG-333 | CIG-334 | CIG-335 | CIG-907 | CIG-908 | CIG-909 | CIG-910 | CIG-913 |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|
| SVL | 24.37 | 24.48 | 25.30 | 25.1 | 23.31 | 23.41 | 23.25 | 24.54 |
| HL | 8.58 | 8.70 | 8.67 | 7.88 | 7.1 | 7.15 | 7.1 | 8.03 |
| TW | 0.74 | 0.76 | 0.80 | 0.76 | 0.74 | 0.74 | 0.74 | 0.81 |
| ED | 2.63 | 2.68 | 2.77 | 2.67 | 2.77 | 2.8 | 2.73 | 2.85 |
| EIW | 1.55 | 1.54 | 1.6 | 1.58 | 1.53 | 1.54 | 1.54 | 1.6 |
| IOD | 4.80 | 4.83 | 4.87 | 4.73 | 4.54 | 4.5 | 4.5 | 4.8 |
| IND | 2.58 | 2.37 | 2.55 | 2.34 | 2.31 | 2.33 | 2.28 | 2.44 |
| END | 2.80 | 2.74 | 2.78 | 2.59 | 2.4 | 2.41 | 2.38 | 2.52 |
| ETD | 1.03 | 1.00 | 1.01 | 1.03 | 0.97 | 0.95 | 0.96 | 1.07 |
| UpL | 5.76 | 6.26 | 6.40 | 4.52 | 5.32 | 5.4 | 5.37 | 6.2 |
| FoL | 6.74 | 7.43 | 7.56 | 7.15 | 6.8 | 6.93 | 6.87 | 7.92 |
| HaL | 5.5 | 5.4 | 6.2 | 5.25 | 5.11 | 5 | 5.05 | 6.25 |
| F3PW/F3W | 1.70 | 1.80 | 1.80 | 2.2 | 1.7 | 1.7 | 1.6 | 2.3 |
| F4PW/F4W | 1.60 | 1.90 | 1.70 | 2.3 | 1.4 | 1.5 | 1.5 | 2.3 |
| FeL | 10.26 | 10.58 | 11.00 | 9.62 | 9.35 | 9.26 | 9.3 | 11.28 |
| TL | 11.03 | 11.65 | 11.33 | 10.31 | 9.95 | 10.12 | 9.9 | 12.33 |
| TotFL | 16.4 | 16.3 | 17.5 | 15.4 | 15.51 | 15.35 | 15.4 | 18.05 |
| IPT | 0.58 | 0.70 | 0.74 | 0.5 | 0.58 | 0.6 | 0.59 | 0.65 |
| MPT | 0.84 | 1.00 | 1.07 | 0.77 | 0.88 | 0.86 | 0.87 | 0.86 |
| OPT | 0.4 | 0.45 | 0.45 | 0.32 | 0.39 | 0.42 | 0.42 | 0.4 |
| IMTL | 0.50 | 0.58 | 0.46 | 0.52 | 0.47 | 0.46 | 0.47 | 0.64 |
| OMTL | 0.90 | 0.92 | 1.04 | 0.74 | 0.84 | 0.85 | 0.83 | 0.99 |
| TW/ED | 0.28 | 0.28 | 0.29 | 0.28 | 0.27 | 0.26 | 0.27 | 0.28 |

Molecular Analysis of the *Eleutherodactylus nitidus* Species Group

The molecular phylogeny based on the mitochondrial rRNA 16S recovered a well-supported *Eleutherodactylus nitidus* species group (posterior probability (pp) 1; Figs. 12 and S1), which is sister to the *E. modestus* group as defined by Grünwald et al. (2018). As with previous phylogenies of the group based on 16S, some of the intermediate nodes in the phylogeny are not well supported (pp < 0.5), and we have collapsed all nodes below this value.

In the *Eleutherodactylus nitidus* species group, we recovered two main clades. The first one is composed of *E. pipilans*, *E. erythrochomus*, and *E. nebulosus*, while the second group includes all the other species. In the second group, many of the intermediate nodes have little to no support. However, individual species do show strong support (pp = 1) in most cases, including *E. dilatatus*, *E. sentinelus*, *E. maurus*, and *E. syristes*, while *E. maculabialis* has a posterior support of 0.86.

All the remaining species in the group form a second, well-supported clade (pp = 0.99). In this clade, we recovered an early split between

Eleutherodactylus albolabris and the other species, which include *E. orarius*, *E. petersi*, *E. nitidus*, and a hitherto undescribed form from Jalisco and Nayarit (Fig. 13). We refrain from describing the western-most form at this time, as material from the type locality of *E. petersi* is unavailable, so the relationship of the western form with *E. petersi* remains unclear.

We believe that a more detailed study of the taxa related to *E. nitidus* is needed, when more thorough sampling is available and employing more population-level specific tools, such as genome-wide SNP data, in order to better understand the patterns of gene-flow and introgression.

Discussion

With the descriptions of the two species here, the number of taxa in the subgenus *Syrrhophus* increases to 37, with 35 in continental North America and two in Cuba. Guerrero is one of the most speciose states, with nine recognized species and several undescribed taxa awaiting description (Grünwald, pers. obs.). All members of the subgenus *Syrrhophus* recorded from Guerrero thus far belong to the *Eleutherodactylus nitidus* species group as defined by Grünwald et

Table 3. Key comparative characters of members of the *Eleutherodactylus nitidus* species group.

| | <i>E.</i> | | | | | | | | | | | |
|-------------------------------|------------------------|---------------------------|-------------------------|-----------------------------------|---------------------------|----------------------|--------------------------|--------------------------|--------------------------|--------------------|----------------------------------|---|
| | <i>E. albolabris</i> | <i>E. dilatatus</i> | <i>E. erythrochomus</i> | <i>E. maculabilis</i> sp. nov. | <i>E. maurus</i> | <i>E. nebulosus</i> | <i>E. nitidus</i> | <i>E. orarius</i> | <i>E. petersi</i> | <i>E. pipilans</i> | <i>E. sentinelus</i> sp. nov. | <i>E. syristes</i> |
| Size | Medium | Medium | Large | Small | Small | Medium | Medium | Medium | Medium | Large | Medium | Small |
| SVL adult males (range) | 23.0–26.8 | 23.8–25.7 | 24.9–30.0 | 17.9–24.7 | 20.7–24.3 | 22.9–28.3 | 24.3–26.3 | 24.6–28.0 | 23.9–26.3 | 25.5–29.6 | 23.3–25.3 | 21.7–24.6 |
| Condition of interorbital bar | Indistinct, pale | Present, pale | Absent | Present, pale | Present, pale | Absent | Indistinct, pale | Indistinct, pale | Indistinct, pale | Absent | Present, pale | Present, pale |
| Pale mid-dorsal blotch | Sometimes present | Absent | Absent | Absent | Absent | Absent | Present | Sometimes present | Present | Absent | Present | Absent |
| Coloration of lip | White | Dark with pale flecking | As head | Dark with pale spots | Dark with pale flecking | Dark with pale spots | Mottled | White, mottled with dark | Mottled | As head | Dark with pale flecking | Dark, variously spotted |
| Inguinal flash coloration | Orange | Yellow | Absent | Varied, yellow / orange | Yellow | Absent | Faint, yellow-orange | Faint, yellow-orange | Faint, yellow-orange | Absent | Yellow | Varied, yellow / orange |
| Ventral coloration | White with black spots | Gray with white and black | Transparent | Gray with white and black | Gray with white and black | Transparent | White with dark mottling | White with dark mottling | White with dark mottling | Transparent | Transparent with white and black | Transparent and white, with black spots |
| Dorsal skin texture | Smooth | Not smooth | Smooth | Smooth | Not smooth | Smooth | Not smooth | Not smooth | Not smooth | Smooth | Smooth | Smooth |
| Ventral skin texture | Slightly rugose | Rugose | Smooth | Smooth | Rugose | Smooth | Slightly rugose | Slightly rugose | Slightly rugose | Smooth | Smooth | Smooth |
| Condition of inguinal gland | Very distinct | Very distinct | Indistinct | Distinct | Very distinct | Indistinct | Very distinct | Very distinct | Very distinct | Indistinct | Very distinct | Distinct |
| 3FPW/3FW | 1.3–1.9 | 1.5–1.7 | 2.3–3.8 | 1.4–2.1 | 1.3–1.6 | 1.1–1.5 | 1.1–1.5 | 1.2–1.4 | 1.1–1.7 | 1.5–1.9 | 1.7–2.3 | 1.1–1.9 |
| 4FPW/4FW | 1.3–1.9 | 1.5–1.8 | — | 1.4–2.1 | 1.3–1.7 | 1.1–1.5 | 1.1–1.5 | 1.2–1.4 | 1.1–1.7 | 1.5–2.0 | 1.5–2.3 | 1.2–1.8 |
| TW/ED | 0.27–0.32 | 0.25–0.35 | 0.33–0.51 | 0.25–0.28 | 0.28–0.31 | 0.35–0.38 | 0.25–0.29 | 0.25–0.29 | 0.25–0.29 | 0.30–0.36 | 0.26–0.28 | 0.25–0.29 |
| Call | Whistle | Peep | Peep | Trill | Pipe | Peep | Whistle | Whistle | Whistle | Peep | Peep | Trill |

Table 4. Advertisement call data of *Eleutherodactylus maculabialis* sp. nov. and *Eleutherodactylus sentinelus* sp. nov.

| | <i>E. maculabialis</i> | <i>E. sentinelus</i> |
|-----------------------------------|------------------------|----------------------|
| Call type | Trill | Peep |
| Dominant frequency (kHz) | 3.28 ± 532.5 | 2.91 ± 85.0 |
| Call length (ms) | 212.5 ± 20.55 | 140 ± 20.0 |
| Call rate (m⁻¹) | 5.49 ± 1.1 | 2.84 ± 0.19 |
| Call rise time (ms) | 18.5 ± 2.25 | 13 ± 4.0 |
| Pulse rate | 5.5 ± 0.25 | 1 ± 0 |
| Call interval | 19.87 ± 4.28 | 36.94 ± 10.52 |

al. (2018). Based on our phylogenetic analysis, *E. dilatatus*, *E. maurus*, and *E. syristes* are closely related and restricted to high-elevation moist mountainous areas. The new taxa described herein are closely related to these species, with *E. maculabialis* being a sister taxon to *E. syristes* and *E. sentinelus* being a sister taxon to *E. dilatatus*. They collectively have an allopatric distribution which covers most of Guerrero's high mountains.

Based on collection locality information, *E. maculabialis* has a known distribution of approximately 150 km² in the Sierra Madre del Sur. Widespread on the windward slopes of the great Cerro Teotepec, it has been collected at several localities along the road that ascends it, and also at some localities in the central Sierra Madre del Sur north of the Cerro Teotepec (e.g., La Guitarra, Yerba Santa). It appears to be isolated from the nearest locality of its closest relative, *E. syristes* (Agua de Obispo), by the combination of the dry and tropical Rio Papagayo Valley and the cooler pine forests of Omiltemi area which is inhabited by *E. dilatatus*. *Eleutherodactylus maculabialis* seems to be separated from *E. dilatatus* primarily by habitat, with the former inhabiting humid pine-oak forest, cloud forest, and tropical wet forest-oak woodland ecotone, whereas the latter is restricted to slightly drier pine forest and pine-oak woodland. A specimen (MZFC 12930) which appears to be *E. maculabialis*, was collected at the western extreme of the Omiltemi State Park. Unfortunately, it is not well preserved, and the absence of molecular data makes it impossible to definitively assign this specimen to this species. However, if this population is indeed *E. maculabialis*, it would suggest a much larger range for this species and closer proximity to the range of *E. dilatatus*.

The limited number of known collecting localities for *E. sentinelus* suggest that it has the smallest range of the *E. nitidus* species group, seconded by its close relative, *E. dilatatus*. As currently understood, the distribution of *E. sentinelus* is approximately 22 km², while *E. dilatatus* has a known distribution of approximately 76 km². These two species appear to be separated by around 160 km of Sierra Madre del Sur where neither has been collected, and only the lower elevation species of *E. nitidus*, *E. petersi*, *E. albolabris*, and *E. maculabialis* have been collected. More sampling is needed to determine the relationship of the distributions of *E. sentinelus* and

E. maculabialis in the western portions of the Sierra Madre del Sur.

Eleutherodactylus maculabialis is a species that seems to be abundant where it occurs. While exact man-hours per specimen collected were not logged while collecting this species, numerous specimens were collected with each attempt to locate the species, and many more were heard calling. *Eleutherodactylus sentinelus* seems to be less abundant than its congeners. During four attempts to collect specimens during the breeding season (June and July), no specimens were heard or collected during one of them, only three specimens were heard during another, and the remaining two attempts resulted in more than a dozen specimens heard and collection of the type series. This contrasts with our experiences with closely related *E. dilatatus*, *E. maculabialis*, and *E. syristes*, of which dozens of specimens were heard calling at all localities during all attempts to collect these species.

From a conservation perspective, *E. maculabialis* does not appear to have any major threats at this time. Small-scale agriculture is present at some locations throughout its range, but does not seem to largely impact this species, and the species continues to be present in disturbed plots. However, both illegal logging activity and illegal poppy farming were previously rampant on the lower slopes of Cerro Teotepec. In addition to destroying the forest being logged directly, the illegal logging also causes heavy siltation and landslides which destroy large swaths of the surrounding vegetation. The illegal poppy plantations also caused large swaths of suitable habitat to be completely cleared. We propose this species to be provisionally classified as Endangered B1ab(iii), based on IUCN Red List criteria that the range is more than 100 km² but smaller than 5,000 km², it occurs in only a couple of threat-defined locations, and there is ongoing decline in the extent and quality of its habitat due to small-scale agriculture, cattle ranching, illegal logging, opium poppy farming, road construction, and the resulting siltation due to these activities.

Habitat transformation due to small-scale agriculture is widely present within the distribution range of *E. sentinelus*, which seems to be limited to approximately 22 km². While these frogs seem to persist in disturbed areas, they are much less abundant than congeners in similar areas (Grünwald, pers. obs., see above). Due to the relative rarity of these frogs combined with the small known distribution, we



Fig. 9. Comparison photos of *Eleutherodactylus* species related to *E. nitidus* in life. (A–I) *E. syristes* from the vicinity of Agua de Obispo, Municipality of Chilpancingo, Guerrero, Mexico. (J–O) *E. albolabris* from Agua de Obispo, Municipality of Chilpancingo, Guerrero, Mexico. (P–R) *E. nitidus* from Yerba Santa, Municipality of General Heliodoro Castillo, Guerrero, Mexico.



Fig. 10. Comparison photos of *Eleutherodactylus* species related to *E. nitidus* in life. (A–I) *E. dilatatus* from Omiltemi, Municipality of Chilpancingo, Guerrero, Mexico. (J–O) *E. petersi* from Puerto El Balsamo, Municipality of José Azueta, Guerrero, Mexico. (P–R) *E. pipilans* from Acahuizotla, Municipality of Chilpancingo, Guerrero, Mexico.

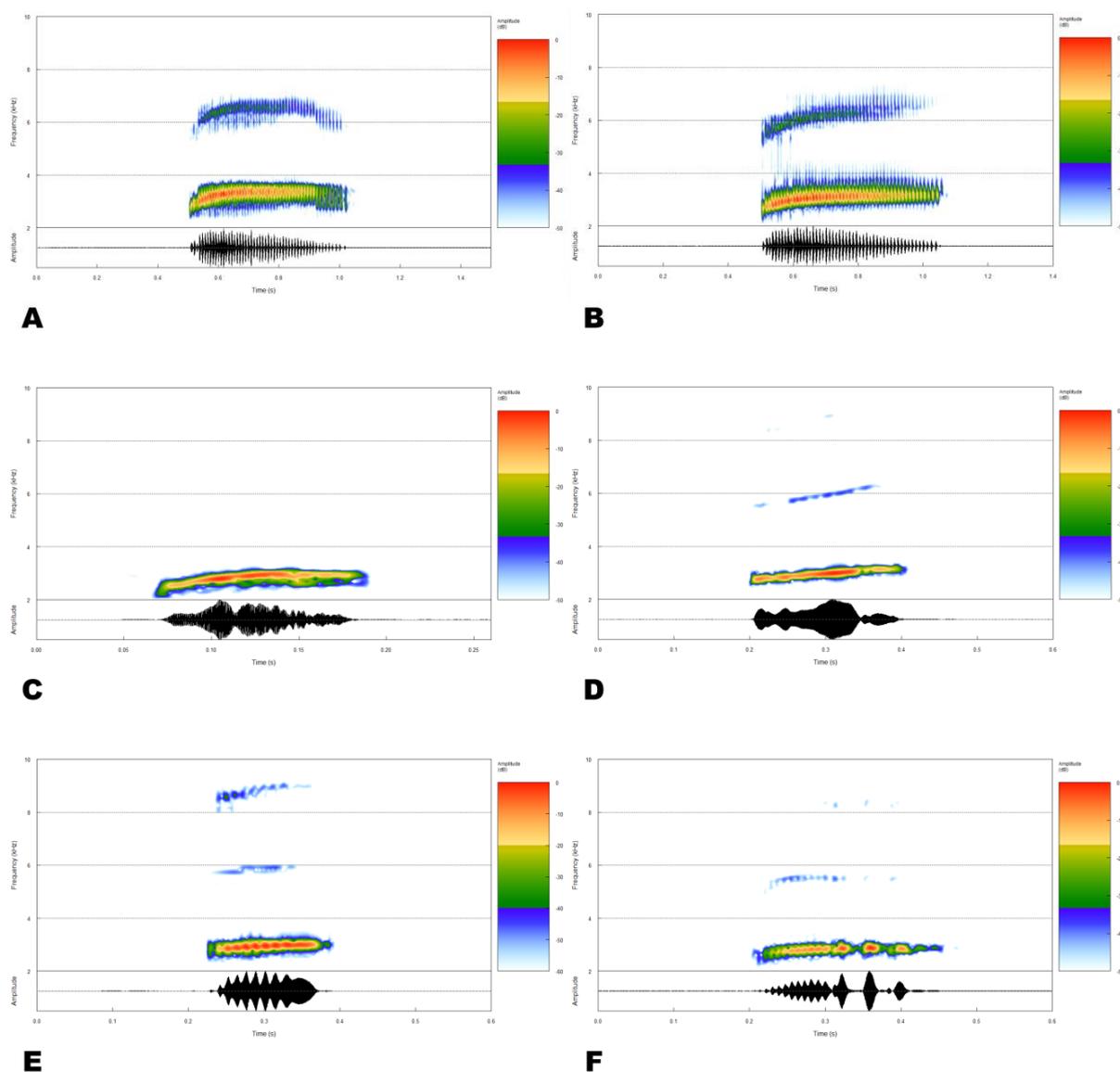


Fig. 11. Oscillograms and spectrograms of the advertisement calls of adult males of *Eleutherodactylus* species related to *E. maculabialis* sp. nov. and *E. sentinelus* sp. nov. (A) *E. syristes* from Agua del Obispo, Guerrero, Mexico. (B) *E. syristes* from east of Hwy. 95, near Acahuizotla, Guerrero, Mexico. (C) *E. dilatatus* from Municipality of Chilpancingo, Guerrero, Mexico. (D) *E. maurus* from Municipality of Ocuilán, Estado de México, Mexico. (E) *E. albolabris* from Municipality of Agua de Obispo, Guerrero, Mexico. (F) *E. albolabris* from Vallecitos, Guerrero, Mexico.

propose this species to be provisionally classified as Critically Endangered Blab(iii), based on the IUCN Red List criteria that its occurrence is less than 100 km², it occurs in only one threat-defined location, and there is ongoing decline in the extent and quality of its habitat due to small-scale cattle ranching and maize farming.

The species richness of the subgenus *Syrrophus* in Guerrero (9 species) represents 27% of the species richness in Mexico (35 species). The number of species known in this state will likely increase as more isolated mountain regions of Guerrero are explored. Guerrero currently has 83 species of amphibians, which represents 20.3% of the approximately 407 species currently recognized for Mexico (AmphibiaWeb 2020). More research in Guerrero is necessary to

continue documenting the incredible biodiversity of this state, as rampant habitat destruction and illegal logging continue to destroy the unique amphibian habitats in the forests of the Sierra Madre del Sur (Lips et al. 2004).

On the Validity of *Eleutherodactylus nebulosus* Taylor, 1943 and *E. rubrimaculatus* Smith and Taylor, 1945 as Species

Based on our phylogenetic results and genetic distances, the populations referred to as “*E. rubrimaculatus*” appear to be conspecific with *E. nebulosus*, as these two species are paraphyletic with respect to each other. Additionally, the genetic distances between the two taxa are very low (less than

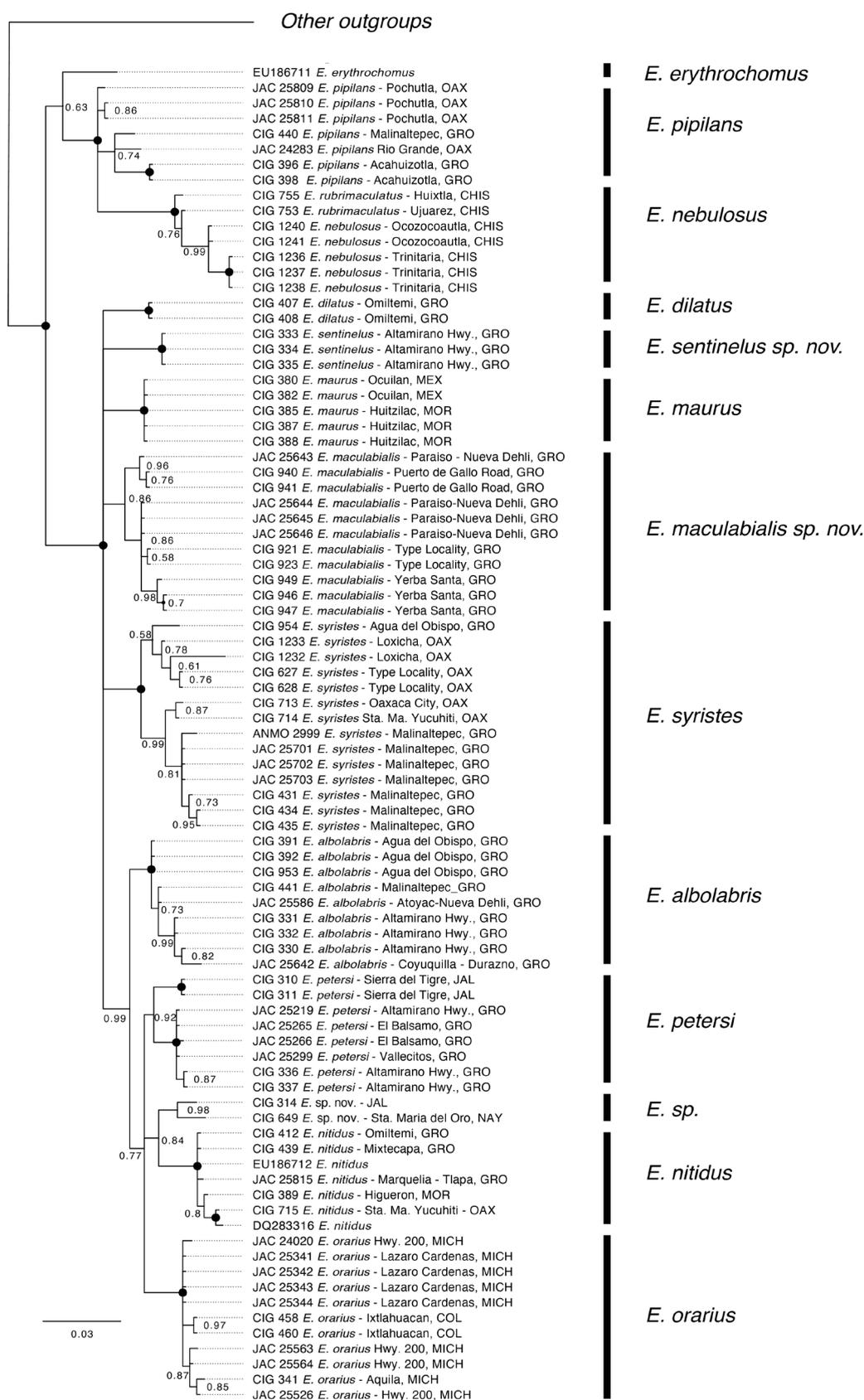


Fig. 12. Bayesian phylogenetic inference of members of the *Eleutherodactylus* subgenus *Syrrophopus*, with a focus on the *E. nitidus* species group, based on the mitochondrial loci 16S rRNA. Black circles represent nodes with a posterior support of 1. All nodes with support of less than 0.5 are collapsed.

Table 5. Uncorrected genetic distances for the 16S rRNA among members of the *Eleutherodactylus nitidus* species group.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1. <i>E. rufescens</i> | - | 0.053 | 0.071 | 0.080 | 0.075 | 0.070 | 0.069 | 0.059 | 0.074 | 0.065 | 0.072 | 0.065 | 0.067 | 0.075 |
| 2. <i>E. pipilans</i> | 0.053 | - | 0.037 | 0.040 | 0.052 | 0.051 | 0.056 | 0.048 | 0.058 | 0.055 | 0.055 | 0.050 | 0.052 | 0.061 |
| 3. <i>E. rubrimaculatus</i> | 0.071 | 0.037 | - | 0.013 | 0.076 | 0.066 | 0.072 | 0.062 | 0.069 | 0.079 | 0.073 | 0.074 | 0.069 | 0.082 |
| 4. <i>E. nebulosus</i> | 0.080 | 0.040 | 0.013 | - | 0.072 | 0.064 | 0.071 | 0.062 | 0.065 | 0.073 | 0.066 | 0.067 | 0.063 | 0.070 |
| 5. <i>E. dilatatus</i> | 0.075 | 0.052 | 0.076 | 0.072 | - | 0.044 | 0.033 | 0.026 | 0.038 | 0.035 | 0.030 | 0.037 | 0.027 | 0.042 |
| 6. <i>E. seninelus</i> sp. nov. | 0.070 | 0.051 | 0.066 | 0.064 | 0.044 | - | 0.033 | 0.031 | 0.040 | 0.042 | 0.043 | 0.037 | 0.043 | 0.055 |
| 7. <i>E. maurus</i> | 0.069 | 0.056 | 0.072 | 0.071 | 0.033 | 0.033 | - | 0.027 | 0.035 | 0.033 | 0.035 | 0.030 | 0.030 | 0.041 |
| 8. <i>E. maculabialis</i> sp. nov. | 0.059 | 0.048 | 0.062 | 0.062 | 0.026 | 0.031 | 0.027 | - | 0.030 | 0.026 | 0.029 | 0.032 | 0.032 | 0.039 |
| 9. <i>E. syristes</i> | 0.074 | 0.058 | 0.069 | 0.065 | 0.038 | 0.040 | 0.035 | 0.030 | - | 0.041 | 0.037 | 0.035 | 0.036 | 0.043 |
| 10. <i>E. albolabris</i> | 0.065 | 0.055 | 0.079 | 0.073 | 0.035 | 0.042 | 0.033 | 0.026 | 0.041 | - | 0.022 | 0.028 | 0.026 | 0.030 |
| 11. <i>E. petersi</i> | 0.072 | 0.055 | 0.073 | 0.066 | 0.030 | 0.043 | 0.035 | 0.029 | 0.037 | 0.022 | - | 0.025 | 0.025 | 0.027 |
| 12. <i>E. sp.</i> | 0.065 | 0.050 | 0.074 | 0.067 | 0.037 | 0.037 | 0.030 | 0.032 | 0.035 | 0.028 | 0.025 | - | 0.025 | 0.027 |
| 13. <i>E. nitidus</i> | 0.067 | 0.052 | 0.069 | 0.063 | 0.027 | 0.043 | 0.030 | 0.032 | 0.036 | 0.026 | 0.025 | 0.025 | - | 0.028 |
| 14. <i>E. orarius</i> | 0.075 | 0.061 | 0.082 | 0.070 | 0.042 | 0.055 | 0.041 | 0.039 | 0.043 | 0.030 | 0.027 | 0.027 | 0.028 | - |

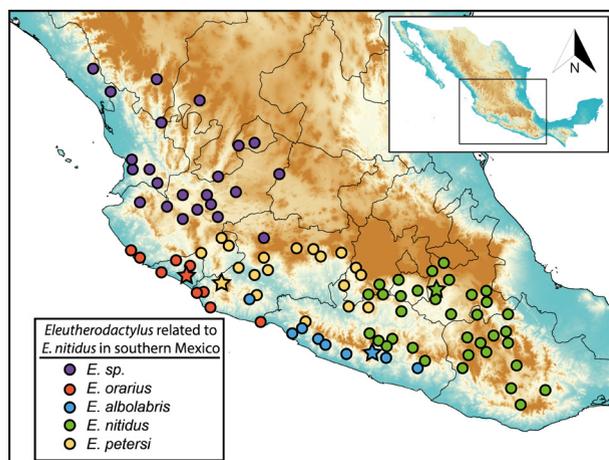


Fig. 13. Map of type localities and distributions of members of the *Eleutherodactylus nitidus* species group. Purple circles represent localities of an undescribed species related to *E. petersi*. For the other four species, stars represent the type localities and circles represent additional localities, which are color coded for species: *E. orarius* (red), *E. albolabris* (blue), *E. nitidus* (green), and *E. petersi* (orange).

1.5%; Table 5).

Taylor (1940) first described *E. nebulosus* from Arriaga, Chiapas, in 1943; and Smith and Taylor (1945) then described “*E. rubrimaculatus*” from Finca La Esperanza, near Escuintla, Chiapas. We did not collect this species at the type locality, however (CIG 755) is near-topotypic, from less than 19 km away on the same slope of the same mountain. The authors differentiated between these species predominately based on size and color pattern. However, those characters appear to be clinal when comparing photos of individual frogs from six localities along Pacific Chiapas (Fig. 14)—from west (Arriaga) to east (Union Juárez) this is evident in the color pattern shifting from the *E. nebulosus* pattern (Arriaga) to the “*E. rubrimaculatus*” pattern (Union Juárez). While molecular material was not available from all the individuals associated with these photos, our analysis presented here did include molecular material from two localities of “*E. rubrimaculatus*” and several localities of *E. nebulosus* around Chiapas. While closely related species of *Syrhophus* occurring in sympatry or near sympatry normally have very different advertisement calls, populations of *E. nebulosus* at Arriaga, Chiapas, and populations of “*E. rubrimaculatus*” at Huixtla, Chiapas, produce the same single note “peep” (Grünwald, pers. obs.). This suggests they are not reproductively isolated. Furthermore, the genetic distances are very low. (1.1–1.3%; Table 5). We thus conclude that “*E. rubrimaculatus*” is most likely a junior synonym of *E. nebulosus*. Our molecular analysis of frogs related to *E. pipilans* lend support for the recognition of two clades within *E. pipilans*, which are consistent with *E. pipilans* from Guerrero and Oaxaca and *E. nebulosus* + “*E. rubrimaculatus*” from southeastern Oaxaca, Chiapas, and Guatemala (Fig. 7B). Herein we recognize two species, *E. pipilans* and *E. nebulosus*. However, we stress that the lack of genetic sampling

between south-central Oaxaca and northwestern Chiapas may be exacerbating the actual genetic distances between these two populations. We suspect that further sampling around Cerro Quiengola, Tehuantepec, and the Sierra Sacamecate may prove these taxa to be two subspecies of a wide-ranging species.

Common Names for Members of the *Eleutherodactylus nitidus* Species Group

Although species of *Syrhophus* are often difficult to distinguish, their advertisement calls are useful for distinguishing species in the field. As these frogs are often located during breeding season by following their advertisement calls, it is helpful to know what type of call each species emits. Grünwald et al. (2018) defined vernacular names for the *E. modestus* species group based on the nature of their distinct advertisement calls. Similarly, we propose common names for the species described herein, as well as other species of the *E. nitidus* species group in Appendix 3.

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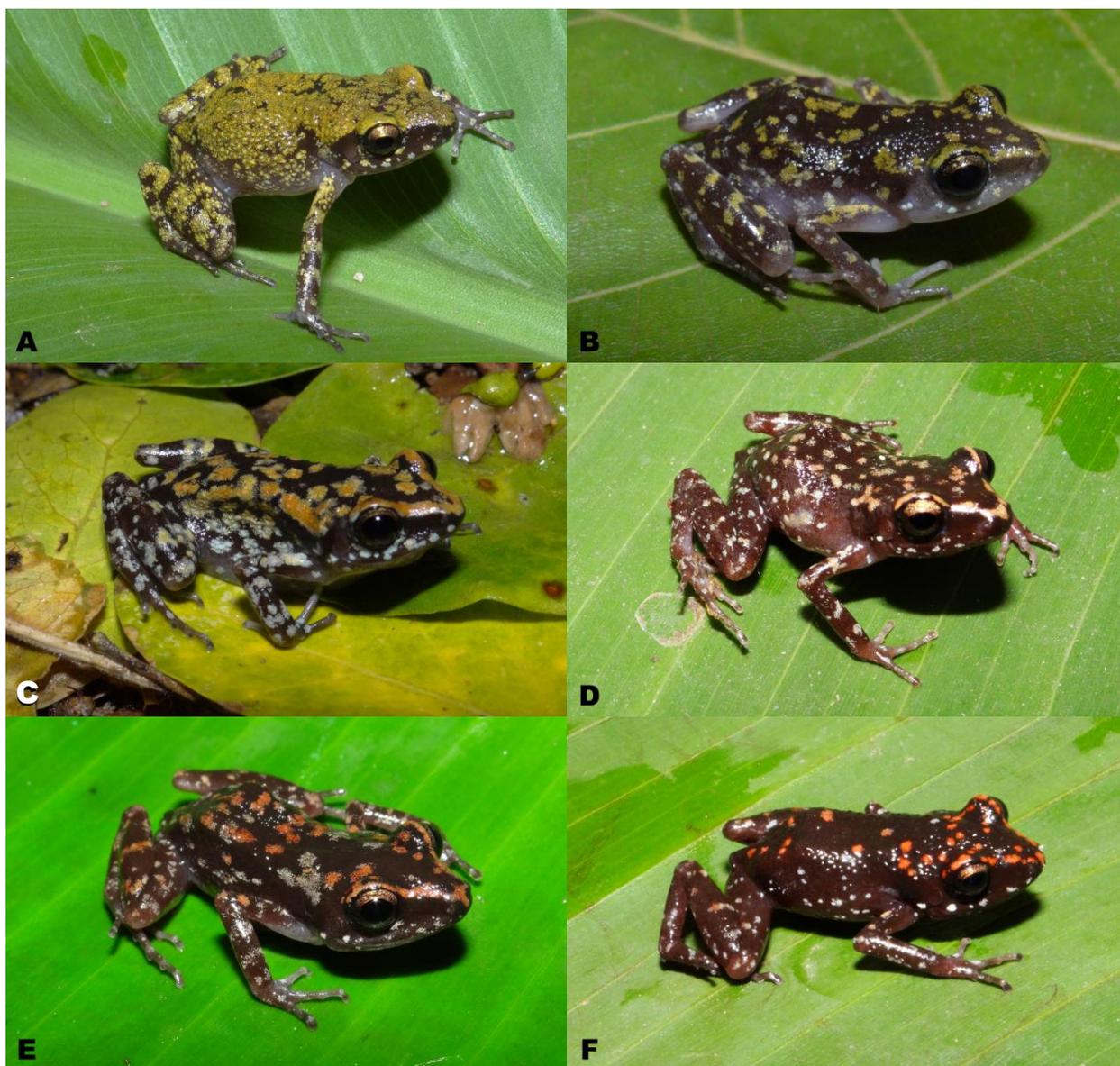


Fig. 14. *Eleutherodactylus nebulosus* in life, including specimens formerly assigned to “*Eleutherodactylus rubrimaculatus*.” (A) *E. nebulosus* from the Municipality of Cintalapa, Chiapas. (B) *E. nebulosus* from the Municipality of Pijijiapan, Chiapas. (C) *E. nebulosus* from the Municipality of Mapastepec, Chiapas. (D) “*E. rubrimaculatus*” (= *E. nebulosus*) from the Municipality of Huixtla, Chiapas. (E) “*E. rubrimaculatus*” (= *E. nebulosus*) from Belisario Dominguez, Chiapas. (F) “*E. rubrimaculatus*” (= *E. nebulosus*) from the Municipality of Union Juárez, Chiapas.

Literature Cited

AmphibiaWeb. 2020. *AmphibiaWeb*. University of California, Berkeley, California, USA. Available: <https://amphibiaweb.org> [Accessed: 13 August 2020].

Bossuyt F, Milinkovitch MC. 2000. Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences of the United States of America* 97: 6,585–6,590.

Center for Conservation Bioacoustics. 2014. *Raven Pro: Interactive Sound Analysis Software, Version 1.5*. The Cornell Lab of Ornithology, Ithaca, New York, USA. Available: <http://ravensoundsoftware.com/>

Cope ED. 1878. A new genus of Cystignathidae from

Texas. *American Naturalist* 12: 252–253.

Davis WB, Dixon JR. 1955. Notes on Mexican toads of the genus *Tomodactylus* with the descriptions of two new species. *Herpetologica* 11: 154–160.

Dixon JR. 1957a. Geographic variation and distribution of the genus *Tomodactylus* in Mexico. *Texas Journal of Science* 9: 379–409.

Dixon JR. 1957b. Geographic variation and distribution of the genus *Tomodactylus* in Mexico. M.S. Thesis, Texas A&M University, College Station, Texas, USA. 122 p.

Dixon JR, Webb RW. 1966. A new *Syrhophus* from Mexico (Amphibia: Leptodactylidae). *Contributions in Science of the Natural History Museum of Los Angeles County* 102: 1–5.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the

- BEAST 1.7. *Molecular Biology and Evolution* 29: 1,969–1,973.
- Duellman WE. 1954. The amphibians and reptiles of the Jorullo Volcano, Mexico. *Occasional Papers of the Museum of Zoology, University of Michigan* 560: 1–24.
- Duellman WE. 1958. A review of the frogs of the genus *Syrrhophus* in western Mexico. *Occasional Papers of the Museum of Zoology, University of Michigan* 594: 1–15.
- Duellman WE, Dixon JR. 1959. A new frog of the genus *Tomodactylus* from Michoacán, Mexico. *Texas Journal of Science* 11: 78–82.
- Duméril AMC, Bibron G. 1841. *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles. Volume 8*. Librairie Encyclopedique de Roret, Paris, France. 659 p.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1,792–1,797.
- Firschein IL. 1954. Definition of some little-understood members of the leptodactylid genus *Syrrhophus*, with a description of a new species. *Copeia* 1954: 48–58.
- Frost DR. 2020. *Amphibian Species of the World: An Online Reference*. Version 6.1. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 8 August 2020].
- Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, De Sa RO, Channing A, Wilkinson M, Donnellan SC, et al. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297: 1–371.
- Grünwald CI, Reyes-Velasco J, Franz-Chávez H, Morales-Flores KI, Ahumada-Carrillo IT, Jones JM, Boissinot S. 2018. Six new species of *Eleutherodactylus* (Anura: Eleutherodactylidae: subgenus *Syrrhophus*) from Mexico, with a discussion of their systematic relationships and the validity of related species. *Mesoamerican Herpetology* 5(1): 6–83.
- Günther ACLG. 1900. Reptilia and Batrachia. Part 155. Pp. 213–220 In: *Biologia Centrali Americana. Volume 7*. Editors, Salvin O, Godman FD. R.H. Porter and Dulau and Company, London, United Kingdom. 326 p.
- Hedges SB. 1989. Evolution and biogeography of West Indian frogs of the genus *Eleutherodactylus*: slow-evolving loci and the major groups. Pp. 305–370 In: *Biogeography of the West Indies: Past, Present, and Future*. Editor, Woods CA. Sandhill Crane Press, Gainesville, Florida, USA. 878 p.
- Hedges SB, Duellman WE, Heinicke MP. 2008. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1–182.
- Hoyt DL. 1965. A new frog of the genus *Tomodactylus* from Oaxaca, México. *Journal of the Ohio Herpetological Society* 5: 19–22.
- Joglar RL. 1989. Phylogenetic relationships of the West Indian frogs of the genus *Eleutherodactylus*. Ph.D. Dissertation. University of Kansas, Lawrence, Kansas, USA. 142 p.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1,547–1,549.
- Lanfear RB, Calcott S, Ho Y, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1,695–1,701.
- Lips KR, Mendelson III JR, Muñoz-Alonso A, Canseco-Márquez L, Mulcahy DG. 2004. Amphibian population declines in montane southern Mexico: resurveys of historical localities. *Biological Conservation* 119: 555–564.
- Lynch JD. 1970. A taxonomic revision of the leptodactylid frog genus *Syrrhophus* Cope. *University of Kansas Publications, Museum of Natural History* 20: 1–45.
- Lynch JD. 1971. Evolutionary relationships, osteology, and zoogeography of leptodactylid frogs. *Miscellaneous Publications of the University of Kansas Museum of Natural History* 53: 1–238.
- Lynch JD, Duellman WE. 1997. *Frogs of the Genus Eleutherodactylus (Leptodactylidae) in Western Ecuador: Systematics, Ecology, and Biogeography*. Museum of Natural History, University of Kansas, Lawrence, Kansas, USA. 236 p.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *Gateway Computing Environments Workshop (GCE), 2010*. IEEE: Piscataway, New Jersey, USA.
- Palacios-Aguilar R, Santos-Bibiano R. 2020. A new species of direct-developing frog of the genus *Eleutherodactylus* (Anura: Eleutherodactylidae) from the Pacific lowlands of Guerrero, Mexico. *Zootaxa* 4750: 250–260.
- Peters WCH. 1870. Über mexicanische Amphibien, welche Hr. Berkenbusch in Puebla auf Veranlassung des Hr. Legationsrathes von Schlözer dem zoologischen Museum zugesandt hat. *Monatsberichte der Königlichen Preussische Akademie der Wissenschaften zu Berlin* 1869: 874–881.
- Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. *Tracer v1.6*. Beast Developers, Edinburgh, United Kingdom. Available: <http://beast.bio.ed.ac.uk/Tracer>
- Reyes-Velasco J, Ahumada Carrillo IT, Burkhardt T, Devitt, TJ. 2015. Two new species of *Eleutherodactylus* (subgenus *Syrrhophus*) from western Mexico. *Zootaxa* 3914: 301–317.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica*. University of Chicago Press, Chicago, Illinois, USA. 934 p.
- Schwartz A. 1957. A new species of *Eleutherodactylus* (Amphibia: Leptodactylidae) from Cuba.

- Proceedings of the Biological Society of Washington* 70: 209–212.
- Schwartz A. 1958. Another new large *Eleutherodactylus* (Amphibia: Leptodactylidae) from western Cuba. *Proceedings of the Biological Society of Washington* 71: 37–42.
- Smith HM, Taylor EH. 1948. An annotated checklist and key to the Amphibia of Mexico. *Bulletin of the United States National Museum* 194: 1–118.
- Taylor EH. 1940. A new *Syrrhophus* from Guerrero, Mexico. *Proceedings of the Biological Society of Washington* 53: 95–98.
- Taylor EH. 1942. New Caudata and Salientia from Mexico. *University of Kansas Science Bulletin* 28: 295–323.
- Taylor EH. 1943. Herpetological novelties from Mexico. *University of Kansas Science Bulletin* 29: 343–361.
- Taylor EH, Smith HM. 1945. Summary of the collections of amphibians made in Mexico under the Walter Rathbone Bacon traveling scholarship. *Proceedings of the United States National Museum* 95: 521–613.
- Zhang P, Liang D, Mao RL, Hillis D, Wake D, Cannatella D. 2013. Efficient sequencing of anuran mtDNAs and a mitogenomic exploration of the phylogeny and evolution of frogs. *Molecular Biology and Evolution* 30: 1,899–1,915.

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Jacobo Reyes-Velasco is originally from Colima, Mexico, although he has worked around the world. Jacobo received his Bachelor’s degree in Biology from the Universidad de Guadalajara (CUCBA), and later received his Ph.D. from the University of Texas at Arlington. He is currently leading field expeditions in Mexico, teaching molecular biology, and writing a book on the herpetofauna of Colima. He has published numerous papers on the herpetofauna of western Mexico and is the co-founder of Entorno Biótico A.C. and HERP.MX A.C., two NGOs that focus on conservation initiatives and sustainable development in western Mexico.



Hector Franz-Chávez is a herpetologist and marine mammal specialist originally from Guadalajara, México. Hector Franz is a student of biology at the University of Guadalajara (CUCBA) and has been an avid herpetologist since childhood. His main interests are biogeography, natural history, and ecology of the herpetofauna of Mexico. He is also an avid nature photographer and marine tour guide in the Sea of Cortez, and has collaborated on ten species descriptions as well as numerous herpetological inventories of different parts of Mexico. Currently stationed out of La Paz, Baja California Sur, Hector is working on photographing and documenting the herpetofauna and marine life of the Baja California Peninsula.



Two new *Eleutherodactylus* species from Mexico



Karen I. Morales-Flores is a herpetologist originally from Guadalajara, Mexico, but she grew up in the United States where she developed an appreciation for nature, and specifically the reptiles in the deserts of Nevada. A student of biology at the University of Guadalajara (CUCBA), Karen (or “Kim”) has participated in numerous field expeditions with the HERP.MX Field Team. Her skills in the laboratory have encouraged her to take on ambitious projects like measuring every external body part of 500 direct-developing frogs. Her research has resulted in the description of nine new species so far, with many more to come. Kim’s ambitions as a herpetologist are only matched by her ambitions as an explorer, and she is currently travelling the world looking for exciting research opportunities.



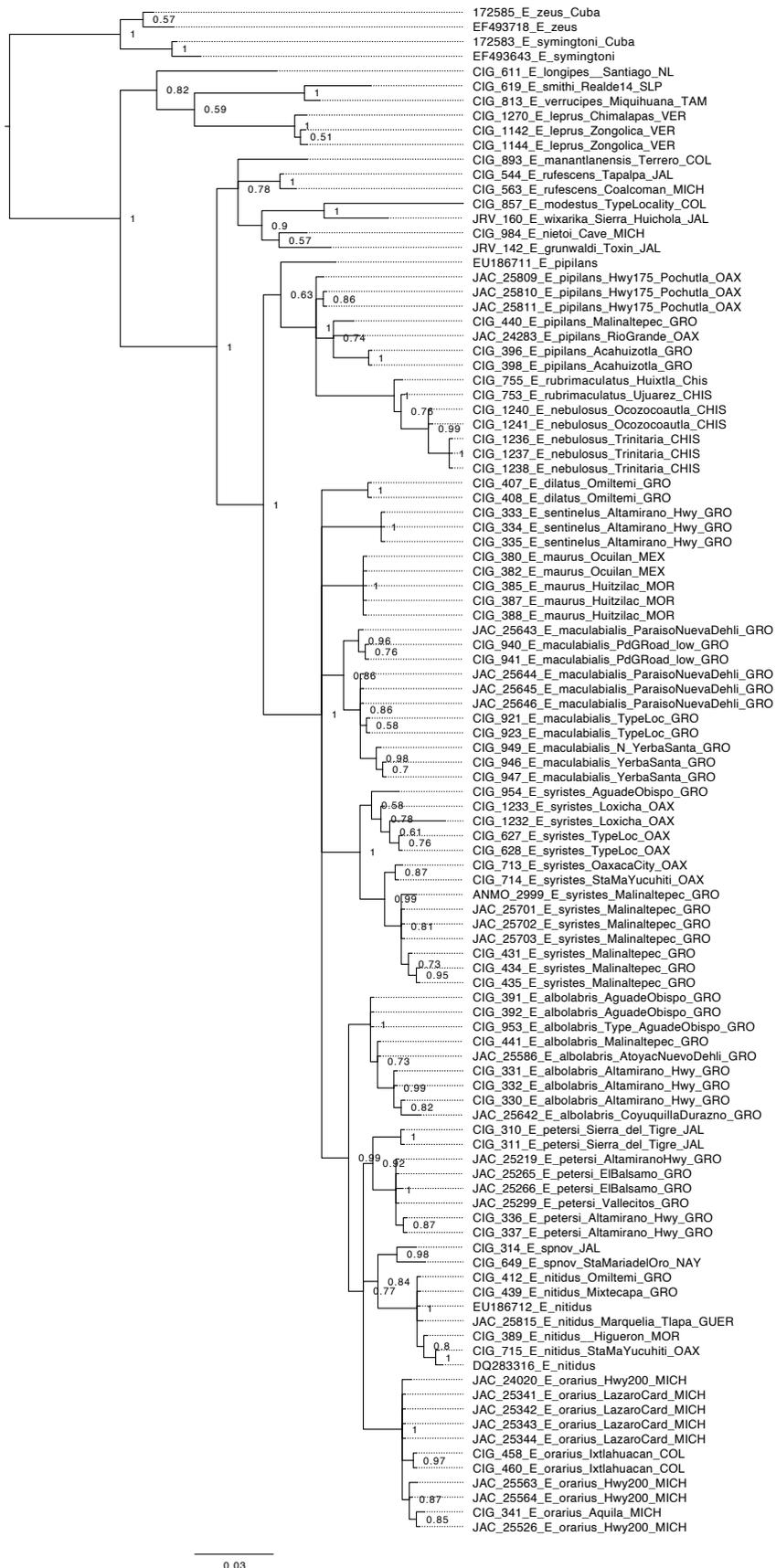
Ivan T. Ahumada-Carrillo is a Mexican herpetologist from Guadalajara, who received his degree from the University of Guadalajara (CUCBA). Ivan is currently an independent investigator who focuses on the biogeography of reptiles and amphibians in western Mexico. He has documented dozens of range extensions and state records, and has authored and co-authored various papers on biogeography, as well as the book *Anfibios y Reptiles del Bosque La Primavera*. Another interest of his is wildlife photography, and his work has been published throughout Mexico in educational materials, web sites, scientific magazines, and books. Ivan has now co-authored 11 new species descriptions, as well as numerous range extensions and state records from Mexico.



Christopher M. Rodriguez is a herpetologist from Los Angeles, California, who studied biology at California State University. Chris is an avid breeder of endangered reptiles and amphibians. Working at the Los Angeles Zoo, Chris has led captive breeding projects for endangered herpetofauna from around the world, as well as extremely delicate local species such as the Mountain Yellow-legged Frog (*Rana muscosa*). When not saving species, Chris has led field expeditions around the world, including Madagascar, Indonesia, Thailand, and above all Mexico. As a member of the HERP.MX Field Team, Chris has helped with the initial field work for several of the new species the Team has discovered.



Jason M. Jones was born and raised in southern California, where he studied biology and computer science at the University of California, Irvine. Currently residing in Colima, he has spent the past 17 years studying and photographing the herpetofauna of Mexico. A specialist in Mexican pitvipers, Jason co-led the rediscovery of *Crotalus lannomi*, and collaborated in the recent descriptions of *Crotalus campbelli*, *Crotalus tlaloci*, and *Ophryacus smaragdinus*. Jason co-founded HERP.MX which, in collaboration with Biodiversa, A.C., is currently developing strategies for the conservation of at-risk reptile species.



Supplementary Fig. 1. Bayesian phylogenetic inference of members of the *Eleutherodactylus* subgenus *Syrrhophus*, based on the mitochondrial loci 16S rRNA. All nodes with support of less than 0.5 are collapsed.

Two new *Eleutherodactylus* species from Mexico

Appendix 1. List of subgenus *Syrrophus* specimens examined in this study. Museum codes: MZFC = Museo de Zoología de la Facultad de Ciencias, Universidad Autónoma de México (UNAM); UTA = Amphibian and Reptile Diversity Research Center, University of Texas at Arlington. Field number codes: ANMO = Adrian Nieto Montes de Oca, uncatalogued at MZFC; CIG = Chris Grünwald, uncatalogued at MZFC; JAC = Jonathan A. Campbell, uncatalogued at UTA; JHM = John Malone, uncatalogued at UTA; JRV = Jacobo Reyes-Velasco, uncatalogued at MZFC.

Specimens Examined ($n = 495$)

Eleutherodactylus albolabris ($n = 20$): MEXICO: Guerrero: MZFC 33025–33030 (CIG 00327–00332), 33082–33085 (CIG 00390–00393), MZFC 33108–33109 (CIG 00441–00442), MZFC 33230 (CIG 00668), MZFC 33300–33301 (CIG 00903–00904), MZFC 33323 (CIG 00953), MZFC 33325–33326 (CIG 00955–00956), JAC 25586, 25642.

Eleutherodactylus angustidigitorum ($n = 20$): MEXICO: Jalisco: MZFC 33127–33130 (CIG 00476–00479), MZFC 33224–33225 (CIG 00662–00663), MZFC 33386–33388 (CIG 00991–00993), JAC 24912; Michoacán: MZFC 33015–33017 (CIG 00316–00318), MZFC 33065–33070 (CIG 00373–00378), JAC 26977.

Eleutherodactylus campi ($n = 13$): MEXICO: Nuevo León: MZFC 33195–33198 (CIG 00606–00609); UNITED STATES: Texas: JHM 1390–1394.

Eleutherodactylus colimotl ($n = 20$): MEXICO: Colima: MZFC 29282 (CIG 00468), MZFC 33115–33120 (CIG 00462–00467), MZFC 33237–33239 (CIG 00682–00684), MZFC 33299 (CIG 00901), MZFC 33329–33330 (CIG 00960–00961), JAC 30498–30499, 30631; Michoacán: MZFC 33036 (CIG 00340), JAC 23999–24001.

Eleutherodactylus cystignathoides ($n = 6$): MEXICO: Veracruz: MZFC 33351–33353 (CIG 01163–01165), MZFC 33354 (CIG 01170), JAC 30000–30001.

Eleutherodactylus dennisi ($n = 13$): MEXICO: Tamaulipas: MZFC 33255–33261 (CIG 00822–00828), UTA 59516–59521.

Eleutherodactylus dilatatus ($n = 19$): MEXICO: Guerrero: MZFC 33089–33094 (CIG 00405–00410), MZFC 33097 (CIG 00428), MZFC 33231 (CIG 00669), UTA 4017–4020, 4023–4024, 5269, 5276–5279.

Eleutherodactylus erendirae ($n = 25$): MEXICO: Jalisco: MZFC 33000–33008 (CIG 00300–00309), MZFC 33226–33229 (CIG 00664–00667), MZFC 33232 (CIG 00673), MZFC 33234–33235 (CIG 00679–00681); Michoacán: MZFC 29274, 33019–33024 (CIG 00319–00325).

Eleutherodactylus floresvilleani ($n = 12$): MEXICO: Michoacán: MZFC 33053–33064 (CIG 00361–00372).

Eleutherodactylus grandis ($n = 1$): MEXICO: Ciudad de México: UTA 56845.

Eleutherodactylus grunwaldi ($n = 12$): MEXICO: Colima: MZFC 27467–27475, MZFC 27484, MZFC 33298 (CIG 00898); JRV 00230.

Eleutherodactylus guttillatus ($n = 10$): MEXICO: Guanajuato: MZFC 33367–33369 (CIG 01248–01250); San Luis Potosí: MZFC 33200–33206 (CIG 00619–00625).

Eleutherodactylus interorbitalis ($n = 7$): MEXICO: Sinaloa: MZFC 33186–33187 (CIG 00584–00585), MZFC 33190–33194 (CIG 00600–00604).

Eleutherodactylus jaliscoensis ($n = 15$): MEXICO: Jalisco: MZFC 33131–33141 (CIG 00480–00490), MZFC 33274–33276 (CIG 00861–00863), MZFC 33280 (CIG 00876).

Eleutherodactylus leprus ($n = 7$): MEXICO: Veracruz: MZFC 33345–33350 (CIG 01139–01144), CIG 01270.

Eleutherodactylus longipes ($n = 3$): MEXICO: Nuevo León: MZFC 33199 (CIG 00611); Querétaro: UTA 59421–59422.

Eleutherodactylus maculabialis sp. nov. ($n = 27$): MEXICO: Guerrero: MZFC 33307–33319 (CIG 00916–00923, 00940–00941, 00945–00947), MZFC 33321 (CIG 00949), MZFC 33323 (CIG 00953), CIG 01484–01485, 01501, JAC 25643–25646.

Eleutherodactylus marnocki ($n = 3$): USA: Texas: JHM 1427–1429.

Eleutherodactylus manantlanensis ($n = 14$): MEXICO: Colima: MZFC 33372–33377 (CIG 00530–00535), MZFC 33379–33381 (CIG 00646–00648), MZFC 33292–33296 (CIG 00892–00896).

Eleutherodactylus maurus ($n = 11$): MEXICO: Estado de México: MZFC 33071–33076 (CIG 00379–00384), MZFC 33355 (CIG 01174); Morelos: MZFC 33077–33080 (CIG 00385–00388).

Eleutherodactylus modestus ($n = 34$): MEXICO: Colima: MZFC 26888–26889, MZFC 33263–33270 (CIG 00850–00857), MZFC 33291 (CIG 00891), MZFC 33297 (CIG 00897); Jalisco: MZFC 33144–33149 (CIG 00493–00498), MZFC 33150–33154 (CIG 00505–00509), MZFC 33161 (CIG 00522), MZFC 33183–33185 (CIG 00570–00572), MZFC 33217–33223 (00655–00661).

Eleutherodactylus nebulosus ($n = 9$): MEXICO: Chiapas: MZFC 33249–33251 (CIG 00753, 00755–00756), MZFC 33361–33366 (CIG 01236–01241).

Eleutherodactylus nietoi ($n = 13$): MEXICO: Michoacán: MZFC 33121 (CIG 00299), MZFC 33042–33045 (CIG 00346–00349), MZFC 33050–33052 (CIG 00355–00357), MZFC 33336–33337 (CIG 00974–00975), MZFC 33342–33343 (CIG 00983–00984), MZFC 33344 (CIG 00994).

Eleutherodactylus nitidus ($n = 31$): MEXICO: Estado de México: JAC 27237; Guerrero: MZFC 33096–33097 (CIG 00411–00412), MZFC 33104–33105 (CIG 00437–00438), JAC 25815; Morelos: MZFC 33081 (CIG 00389); Oaxaca: MZFC 33357–33358 (CIG 01211–01212); Puebla: MZFC 33356 (CIG 01181), JAC 27256–27276.

Eleutherodactylus orarius ($n = 13$): MEXICO: Colima: MZFC 26890, MZFC 33262 (CIG 00849); Michoacán: MZFC 33037 (CIG 00341), MZFC 33335 (CIG 00973), JAC 24020, 25526, 25563–25564, 29107, 30500–30501, 30517, 30625.

Eleutherodactylus pallidus ($n = 13$): MEXICO: Jalisco: MZFC 33271–33272 (CIG 00858–00859); Nayarit: MZFC 33189 (CIG 00588), MZFC 33212–33216 (CIG 00650–00654), MZFC 33243–33245 (CIG 00688–00690), MZFC 33018 (CIG 00995); Sinaloa: MZFC 33188 (CIG 00586).

Eleutherodactylus petersi ($n = 25$): MEXICO: Guerrero: MZFC 33034–33035 (CIG 00336–00337); JAC 25219, 25265–25266, 25299; Jalisco: MZFC 33010–33014 (CIG 00310–00314), MZFC 33034–33035 (CIG 00336–00337), MZFC 33110 (CIG

00457), MZFC 33273 (CIG 00860), JAC 28612; Michoacán: MZFC 33382–33385 (CIG 00675–00677), JAC 26947; Nayarit: MZFC 33211 (CIG 00649), MZFC 33240–33242 (CIG 00685–00687).

Eleutherodactylus pipilans ($n = 15$): MEXICO: Guerrero: MZFC 33086–33088 (CIG 00396–00398), MZFC 33106–33107 (CIG 00439–00440), MZFC 33322 (CIG 00952), CIG 1465; Oaxaca: MZFC 33210 (CIG 00645), JAC 24283, 25809–25811.

Eleutherodactylus rufescens ($n = 40$): MEXICO: Jalisco: MZFC 33122–33126 (CIG 00471–00475), MZFC 33162–33164 (CIG 00527–00529), MZFC 33165–33174 (CIG 00544–00553), MZFC 33385 (CIG 00678); Michoacán: MZFC 33038–33041 (CIG 00342–00345), MZFC 33046–33049 (CIG 00350–00353), MZFC 33175–33182 (CIG 00559–00566), MZFC 33233 (CIG 00674), MZFC 33338 (CIG 00976), MZFC 33339–33341 (CIG 00980–00982).

Eleutherodactylus saxatilis ($n = 4$): MEXICO: Sinaloa: MZFC 26893, 26896, 26898–26899.

Eleutherodactylus sentinelus sp. nov. ($n = 8$): MEXICO: Guerrero: MZFC 33031–33033 (CIG 00333–00335), MZFC 33302–33306 (CIG 00907–00913).

Eleutherodactylus syristes ($n = 21$): MEXICO: Guerrero: ANMO 2999; MZFC 33098–33103 (CIG 00431–00436), MZFC 33324 (CIG 00954), MZFC 33327–33328 (CIG 00957–00958) JAC 25701–25703; Oaxaca: MZFC 33207–33208 (CIG 00627–00628), MZFC 33209 (CIG 00644), 33378 (CIG00643), MZFC 33246–33247 (CIG 00713–00714), MZFC 33359–33360 (CIG 01232–01233).

Eleutherodactylus teretistes ($n = 5$): MEXICO: Jalisco: MZFC 33142–33143 (CIG 00491–00492), MZFC 33277–33279 (CIG 00864–00866).

Eleutherodactylus verrucipes ($n = 3$): MEXICO: MZFC 33253–33254 (CIG 00813–00814), CIG 01273.

Eleutherodactylus wixarika ($n = 3$): MEXICO: Jalisco: MZFC 27477–27479.

Two new *Eleutherodactylus* species from Mexico

Appendix 2. Specimen information and GenBank accession numbers.

| Field number | Species | Museum number | Locality | GenBank number |
|--------------|---|---------------|---------------------|----------------|
| — | <i>Eleutherodactylus symingtoni</i> | — | Cuba: Pinar del Río | EF493643 |
| — | <i>Eleutherodactylus nitidus</i> | AMCC118239 | Mexico: Puebla | EU186712 |
| — | <i>Eleutherodactylus erythrochomus</i> | MZFC 16254 | Mexico: Guerrero | EU186711 |
| — | <i>Eleutherodactylus zeus</i> | USNM335740 | Cuba: Pinar del Río | EF493718 |
| ANMO2999 | <i>Eleutherodactylus syristes</i> | — | Mexico: Guerrero | MT872423 |
| CIG1142 | <i>Eleutherodactylus leprus</i> | MZFC33348 | Mexico: Veracruz | MT872424 |
| CIG1144 | <i>Eleutherodactylus leprus</i> | MZFC33350 | Mexico: Veracruz | MT872425 |
| CIG1232 | <i>Eleutherodactylus syristes</i> | MZFC33359 | Mexico: Oaxaca | MT872426 |
| CIG1233 | <i>Eleutherodactylus syristes</i> | MZFC33360 | Mexico: Oaxaca | MT872427 |
| CIG1236 | <i>Eleutherodactylus nebulosus</i> | MZFC33361 | Mexico: Chiapas | MT872428 |
| CIG1237 | <i>Eleutherodactylus nebulosus</i> | MZFC33362 | Mexico: Chiapas | MT872429 |
| CIG1238 | <i>Eleutherodactylus nebulosus</i> | MZFC33363 | Mexico: Chiapas | MT872430 |
| CIG1240 | <i>Eleutherodactylus nebulosus</i> | MZFC33365 | Mexico: Chiapas | MT872431 |
| CIG1241 | <i>Eleutherodactylus nebulosus</i> | MZFC33366 | Mexico: Chiapas | MT872432 |
| CIG1270 | <i>Eleutherodactylus leprus</i> | — | Mexico: Veracruz | MT872433 |
| CIG310 | <i>Eleutherodactylus petersi</i> | MZFC33010 | Mexico: Jalisco | MT872487 |
| CIG311 | <i>Eleutherodactylus nitidus</i> | MZFC33011 | Mexico: Jalisco | MG857033 |
| CIG314 | <i>Eleutherodactylus nitidus</i> | MZFC33014 | Mexico: Jalisco | MG857034 |
| CIG330 | <i>Eleutherodactylus albolabris</i> | MZFC33028 | Mexico: Guerrero | MT872481 |
| CIG331 | <i>Eleutherodactylus albolabris</i> | MZFC33029 | Mexico: Guerrero | MT872482 |
| CIG332 | <i>Eleutherodactylus albolabris</i> | MZFC33030 | Mexico: Guerrero | MT872483 |
| CIG333 | <i>Eleutherodactylus sentinelus</i> sp. nov. | MZFC33031 | Mexico: Guerrero | MT872484 |
| CIG334 | <i>Eleutherodactylus sentinelus</i> sp. nov. | MZFC33032 | Mexico: Guerrero | MT872485 |
| CIG335 | <i>Eleutherodactylus sentinelus</i> sp. nov. | MZFC33033 | Mexico: Guerrero | MT872486 |
| CIG336 | <i>Eleutherodactylus nitidus</i> | MZFC33034 | Mexico: Guerrero | MG857032 |
| CIG337 | <i>Eleutherodactylus petersi</i> | MZFC33035 | Mexico: Guerrero | MT872473 |
| CIG341 | <i>Eleutherodactylus orarius</i> | MZFC33037 | Mexico: Michoacán | MG857041 |
| CIG380 | <i>Eleutherodactylus maurus</i> | MZFC33072 | Mexico: Mexico | MG857011 |
| CIG382 | <i>Eleutherodactylus maurus</i> | MZFC33074 | Mexico: Mexico | MT872478 |
| CIG385 | <i>Eleutherodactylus maurus</i> | MZFC33077 | Mexico: Morelos | MT872479 |
| CIG387 | <i>Eleutherodactylus maurus</i> | MZFC33079 | Mexico: Morelos | MT872480 |
| CIG388 | <i>Eleutherodactylus maurus</i> | MZFC33080 | Mexico: Morelos | MG857010 |
| CIG389 | <i>Eleutherodactylus nitidus</i> | MZFC33081 | Mexico: Morelos | MG857029 |
| CIG391 | <i>Eleutherodactylus albolabris</i> | MZFC33083 | Mexico: Guerrero | MG856955 |
| CIG392 | <i>Eleutherodactylus albolabris</i> | MZFC33084 | Mexico: Guerrero | MT872468 |
| CIG396 | <i>Eleutherodactylus pipilans</i> | MZFC33086 | Mexico: Guerrero | MG857054 |
| CIG398 | <i>Eleutherodactylus pipilans</i> | MZFC33088 | Mexico: Guerrero | MG857055 |
| CIG407 | <i>Eleutherodactylus dilatus</i> | MZFC33091 | Mexico: Guerrero | MG856973 |
| CIG408 | <i>Eleutherodactylus dilatus</i> | MZFC33092 | Mexico: Guerrero | MG856974 |
| CIG412 | <i>Eleutherodactylus nitidus</i> | MZFC33096 | Mexico: Guerrero | MG857031 |
| CIG431 | <i>Eleutherodactylus syristes</i> | MZFC33098 | Mexico: Guerrero | MT872471 |
| CIG434 | <i>Eleutherodactylus syristes</i> | MZFC33101 | Mexico: Guerrero | MT872472 |
| CIG435 | <i>Eleutherodactylus syristes</i> | MZFC33102 | Mexico: Guerrero | MG857071 |
| CIG439 | <i>Eleutherodactylus nitidus</i> | MZFC33106 | Mexico: Guerrero | MT872474 |
| CIG440 | <i>Eleutherodactylus pipilans</i> | MZFC33107 | Mexico: Guerrero | MT872475 |

Appendix 2 continued. Specimen information and GenBank accession numbers.

| Field number | Species | Museum number | Locality | GenBank number |
|--------------|--|---------------|-------------------------|----------------|
| CIG441 | <i>Eleutherodactylus albolabris</i> | MZFC33108 | Mexico: Guerrero | MT872476 |
| CIG458 | <i>Eleutherodactylus orarius</i> | MZFC33111 | Mexico: Colima | MT872477 |
| CIG460 | <i>Eleutherodactylus orarius</i> | MZFC33113 | Mexico: Colima | MG857042 |
| CIG544 | <i>Eleutherodactylus rufescens</i> | MZFC33165 | Mexico: Jalisco | MG857039 |
| CIG563 | <i>Eleutherodactylus rufescens</i> | MZFC33179 | Mexico: Michoacán | MG857060 |
| CIG611 | <i>Eleutherodactylus longipes</i> | MZFC33199 | Mexico: Nuevo Leon | MG857006 |
| CIG619 | <i>Eleutherodactylus guttilatus</i> | MZFC33200 | Mexico: San Luis Potosí | MG856994 |
| CIG627 | <i>Eleutherodactylus syristes</i> | MZFC33207 | Mexico: Oaxaca | MT872467 |
| CIG628 | <i>Eleutherodactylus syristes</i> | MZFC33208 | Mexico: Oaxaca | MG857073 |
| CIG649 | <i>Eleutherodactylus sp. nov.</i> | MZFC33211 | Mexico: Nayarit | MT872469 |
| CIG713 | <i>Eleutherodactylus syristes</i> | MZFC33246 | Mexico: Oaxaca | MT872470 |
| CIG714 | <i>Eleutherodactylus syristes</i> | MZFC33247 | Mexico: Oaxaca | MG857072 |
| CIG715 | <i>Eleutherodactylus nitidus</i> | MZFC33248 | Mexico: Oaxaca | MG857030 |
| CIG753 | <i>Eleutherodactylus nebulosus</i> | MZFC33249 | Mexico: Chiapas | MG857056 |
| CIG755 | <i>Eleutherodactylus nebulosus</i> | MZFC33250 | Mexico: Chiapas | MG857057 |
| CIG813 | <i>Eleutherodactylus verrucipes</i> | MZFC33253 | Mexico: Tamaulipas | MG857079 |
| CIG857 | <i>Eleutherodactylus modestus</i> | MZFC33270 | Mexico: Colima | MG857021 |
| CIG893 | <i>Eleutherodactylus manantlanensis</i> | MZFC33293 | Mexico: Colima | MG857007 |
| CIG921 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33312 | Mexico: Guerrero | MT872460 |
| CIG923 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33314 | Mexico: Guerrero | MT872461 |
| CIG940 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33315 | Mexico: Guerrero | MT872462 |
| CIG941 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33316 | Mexico: Guerrero | MT872463 |
| CIG946 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33318 | Mexico: Guerrero | MT872464 |
| CIG947 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33319 | Mexico: Guerrero | MT872465 |
| CIG949 | <i>Eleutherodactylus maculabialis</i> sp. nov. | MZFC33321 | Mexico: Guerrero | MT872466 |
| CIG953 | <i>Eleutherodactylus albolabris</i> | MZFC33323 | Mexico: Guerrero | MG856956 |
| CIG954 | <i>Eleutherodactylus syristes</i> | MZFC33324 | Mexico: Guerrero | MG857070 |
| CIG984 | <i>Eleutherodactylus nietoi</i> | MZFC33343 | Mexico: Michoacán | MG857028 |
| JAC24020 | <i>Eleutherodactylus orarius</i> | UTAA59508 | Mexico: Michoacán | MT872434 |
| JAC24283 | <i>Eleutherodactylus pipilans</i> | UTAA64104 | Mexico: Oaxaca | MT872435 |
| JAC25219 | <i>Eleutherodactylus petersi</i> | UTAA61561 | Mexico: Guerrero | MT872436 |
| JAC25265 | <i>Eleutherodactylus petersi</i> | UTAA61562 | Mexico: Guerrero | MT872437 |
| JAC25266 | <i>Eleutherodactylus petersi</i> | UTAA61563 | Mexico: Guerrero | MT872438 |
| JAC25299 | <i>Eleutherodactylus petersi</i> | UTAA61568 | Mexico: Guerrero | MT872439 |
| JAC25341 | <i>Eleutherodactylus orarius</i> | UTAA62400 | Mexico: Michoacán | MT872440 |
| JAC25342 | <i>Eleutherodactylus orarius</i> | UTAA62401 | Mexico: Michoacán | MT872441 |
| JAC25343 | <i>Eleutherodactylus orarius</i> | UTAA62402 | Mexico: Michoacán | MT872442 |
| JAC25344 | <i>Eleutherodactylus orarius</i> | UTAA62403 | Mexico: Michoacán | MT872443 |
| JAC25526 | <i>Eleutherodactylus orarius</i> | UTAA62090 | Mexico: Michoacán | MT872444 |
| JAC25563 | <i>Eleutherodactylus orarius</i> | UTAA61575 | Mexico: Michoacán | MT872445 |
| JAC25564 | <i>Eleutherodactylus orarius</i> | UTAA61576 | Mexico: Michoacán | MT872446 |

Two new *Eleutherodactylus* species from Mexico

Appendix 2 continued. Specimen information and GenBank accession numbers.

| Field number | Species | Museum number | Locality | GenBank number |
|--------------|--|---------------|------------------|----------------|
| JAC25586 | <i>Eleutherodactylus albolabris</i> | UTAA61577 | Mexico: Guerrero | MT872447 |
| JAC25642 | <i>Eleutherodactylus albolabris</i> | UTAA61578 | Mexico: Guerrero | MT872448 |
| JAC25643 | <i>Eleutherodactylus maculabialis</i> sp. nov. | UTAA62091 | Mexico: Guerrero | MT872449 |
| JAC25644 | <i>Eleutherodactylus maculabialis</i> sp. nov. | UTAA64131 | Mexico: Guerrero | MT872450 |
| JAC25645 | <i>Eleutherodactylus maculabialis</i> sp. nov. | UTAA64130 | Mexico: Guerrero | MT872451 |
| JAC25646 | <i>Eleutherodactylus maculabialis</i> sp. nov. | — | Mexico: Guerrero | MT872452 |
| JAC25701 | <i>Eleutherodactylus syristes</i> | UTAA61580 | Mexico: Guerrero | MT872453 |
| JAC25702 | <i>Eleutherodactylus syristes</i> | UTAA61581 | Mexico: Guerrero | MT872454 |
| JAC25703 | <i>Eleutherodactylus syristes</i> | UTAA61582 | Mexico: Guerrero | MT872455 |
| JAC25809 | <i>Eleutherodactylus pipilans</i> | UTAA62404 | Mexico: Oaxaca | MT872456 |
| JAC25810 | <i>Eleutherodactylus pipilans</i> | UTAA62405 | Mexico: Oaxaca | MT872457 |
| JAC25811 | <i>Eleutherodactylus pipilans</i> | UTAA62406 | Mexico: Oaxaca | MT872458 |
| JAC25815 | <i>Eleutherodactylus nitidus</i> | UTAA61584 | Mexico: Guerrero | MT872459 |
| JRV142 | <i>Eleutherodactylus grunwaldi</i> | — | Mexico: Jalisco | MG856993 |
| JRV160 | <i>Eleutherodactylus wixarika</i> | MZFFZ27477 | Mexico: Jalisco | MG857081 |

Appendix 3. Proposed standardized common names for the *Eleutherodactylus nitidus* species group members.

| | |
|---|------------------------------|
| <i>Eleutherodactylus albolabris</i> | White-lipped Whistling Frog |
| <i>Eleutherodactylus dilatus</i> | Omitemi Peeping Frog |
| <i>Eleutherodactylus erythrochomus</i> | Tierra Colorada Peeping Frog |
| <i>Eleutherodactylus maurus</i> | Dusky Piping Frog |
| <i>Eleutherodactylus nitidus</i> | Shiny Whistling Frog |
| <i>Eleutherodactylus orarius</i> | Coastal Whistling Frog |
| <i>Eleutherodactylus petersi</i> | Peters' Whistling Frog |
| <i>Eleutherodactylus pipilans</i> | Guerrero Peeping Frog |
| <i>Eleutherodactylus nebulosus</i> | Clouded Peeping Frog |
| <i>Eleutherodactylus maculabialis</i> sp. nov. | Spot-lipped Trilling Frog |
| <i>Eleutherodactylus sentinelus</i> sp. nov. | El Balsamo Peeping Frog |
| <i>Eleutherodactylus syristes</i> | Oaxaca Trilling Frog |
