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Phylogenomics, male internal genitalia, a new species, and other notes on New World *Stenopelmatus* Jerusalem crickets (Orthoptera: Stenopelmatoidea: Stenopelmatini)

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Abstract

Based on past and expanded DNA sampling, the orthopteran families Stenopelmatidae and Anostostomatidae, as currently structured, are shown to be non-monophyletic. The splay-footed cricket genus *Comicus* is confirmed to be genetically distinct from all Stenopelmatidae. We add two specimens to our previously published phylogenetic tree for New World *Stenopelmatus* Jerusalem cricket species and report the first multilocus DNA recovery for *S. ater* from Costa Rica. Male internal genitalia may be of systematic value in Jerusalem crickets, but we believe they should be analyzed when in their unfolded, "physiologically functional" configuration, where morphological characters can be seen in more detail when compared to their preserved, folded state. We describe *Stenopelmatus nuevoguatemalae* n. sp. from Guatemala.

Key words: Target enrichment, phylogenetics, Anostostomatidae, Schizodactylidae, *Comicus, Oryctopterus*, phallic lobes, *Stenopelmatus nuevoguatemalae,* rattlesnake predation, *Crotalus campbelli,* Deep Look video

Introduction

The orthopteran superfamily Stenopelmatoidea, which currently consists of three families (Anostostomatidae, Gryllacrididae, and Stenopelmatidae), is a monophyletic lineage containing more than 1,200 extant species within the suborder Ensifera. Despite generating the most complete DNA phylogeny for the Stenopelmatoidea, Vandergast *et al.* (2017), utilizing two nuclear and one mitochondrial gene, were unable to resolve some relationships among the families examined; although the monophyly of both the superfamily and worldwide Gryllacrididae were robustly recovered. Specifically, they failed to recover families Anostostomatidae and Stenopelmatidae as monophyletic. Song *et al*. (2020) conducted a phylogenomic analysis of Orthoptera using transcriptome and mitochondrial genome data and found each of the three families within Stenopelmatoidea to be monophyletic, but did not include key taxa to thoroughly test the monophyly of those families. To resolve these conflicting issues, we have continued with these efforts, adding several key species and now employing phylogenomic analysis using Orthoptera-specific target enrichment (OR-TE) probes that can capture more than 1,000 loci that can broadly resolve across phylogeny of Orthoptera (Shin *et al.* 2024).

While molecular phylogenetic studies have consistently recovered the monophyly of Stenopelmatoidea, with the three aforementioned families as its members (Song *et al*. 2015; Vandergast *et al*. 2017; Song *et al*. 2020), Gorochov (2021) did not agree with these results based on his own concepts. He proposed that Stenopelmatoidea only includes two families, Stenopelmatidae and Anostostomatidae, and treated Gryllacrididae as a subfamily of Stenopelmatidae. Furthermore, he considered the aberrant ensiferan lineage splay-footed crickets (genera *Comicus*

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and *Schizodactylus*) as a subfamily within Stenopelmatidae. In contrast, all previous molecular phylogenetic studies have so far recovered the splay-footed crickets as distinct (Schizodactylidae) and well separated from Stenopelmatidae.

In this study, we aim to answer the following two questions based on our phylogenomic analysis of expanded taxon sampling: 1) Are both Stenopelmatidae and Anostostomatidae, as currently considered by the Orthoptera Species File (OSF) (Cigliano *et al.* 2024), monophyletic? Or, are both families, as shown by Vandergast *et al.* (2017), non-monophyletic? 2) To what family do the extant splay-footed crickets (genera *Comicus* and *Schizodactylus*) belong? Schizodactylidae, per OSF and all previous molecular phylogenetic studies (Song *et al*. 2015; Vandergast *et al*. 2017; Song *et al*. 2020); or Stenopelmatidae, per Gorochov (2021)?

Weissman *et al.* (2021) revised the New World *Stenopelmatus* Jerusalem crickets (JC), describing 14 new taxa from Mexico, Honduras, and Ecuador. They estimated that as little as 30% of the Mexico and Central American JC fauna could be known, this low percentage subsequently confirmed by Gutiérrez-Rodríguez *et al.* (2022) and Ruiz-Mendoza *et al.* (2023). Because data from the latter two references indicated more cryptic species diversity in this genus than even we anticipated, we present sequence data from two individuals that, initially, were not thought important to examine.

While internal male genitalia can have considerable phylogenetic and taxonomic value in the Orthoptera, they have been analyzed only sparingly in JCs (e.g., Gorochov 2021), possibly because these structures are largely unsclerotized. Recently, Suastes-Jiménez *et al.* (2023) used in situ, folded, internal male genitalia as a major character in their description of four new species of *Stenopelmatus* from Mexico. We comment why we have reservations about their conclusions, and propose several improvements.

In 1888 Brunner von Wattenwyl described *Stenopelmatus guatemalae* (probably an adult female) and *S. vicinus* (a late instar male) from "Guatemala". Both of these taxa were designated nomen dubium by Weissman *et al.* (2021), since neither name could be accurately applied to specimens from Guatemala because the types lacked good collection data. Both names also lacked an adult male, calling drums, DNA, and karyotype information. Through the generosity of Oscar Cadena-Castañeda, we recovered DNA from an adult female JC from Guatemala, which we presented (specimen F2332) in Fig. 2 in Vandergast *et al.* (2017) and Figs 9 & 10 in Weissman *et al.* (2021); we now formally describe this taxon.

We also take this opportunity to correct a few minor typos in our 2021 paper.

Materials and Methods

Taxon sampling. We sampled a total of 38 specimens belonging to key lineages within Anostostomatidae, Stenopelmatidae, and Schizodactylidae. As ingroup taxa, we included 23 specimens of New World *Stenopelmatus* representing 17 species; and 5 specimens of Old World members of Stenopelmatidae representing *Oryctopterus*, *Sia,* and *Maxentius*. For outgroup sampling, we increased to eight species, compared to the three included in Weissman *et al*. (2021): We included two specimens of *Comicus calcaris* (Schizodactylidae) and eight specimens of Anostostomatidae (*Anabropsis* [2 species], *Cooloola*, *Penalva*, *Lezina*, *Cnemotettix*, and *Glaphyrosoma*). See Table 1 for specimen collection data.

Phylogenomic data generation and analyses. We used the Orthoptera-specific target enrichment (OR-TE) probes (Shin *et al.* 2024) to capture 1,828 loci from genomic DNA. See Weissman *et al.* (2021) and Shin *et al.* (2024) for DNA extraction, library preparation, hybrid capture, and data processing. We inferred the phylogeny using a coalescent-based species tree analysis in the program ASTRAL 5.7.3 (Zhang *et al*. 2018). Specifically, we created 1,236 individual datasets (one for each gene). The gene trees were generated from each gene-specific alignment in RAxML 8 (Stamatakis 2014) using 100 rapid bootstrap pseudoreplicates and the GTR GAMMA I model. From these 1,236 maximum likelihood trees, we collapsed any node with a bootstrap support value less than 10. Using these trees, we generated a summary species tree using the default options in ASTRAL. The alignments, individual trees used for the ASTRAL analysis, as well as the final tree file were deposited in DRYAD (https://doi.org/10.5061/dryad.hdr7sqvr7).

Imaging. Photographs in Figs 4–6 were taken with a Keyence VHX-7000 digital microscope.

Results

With this expanded survey, we still recovered all orthopteroid taxa as previously published (Vandergast *et al.* 2017; Weissman *et al.* 2021), except for *Stenopelmatus piceiventris* Walker. The latter was recovered at the base of the New World JC tree in our two earlier publications, a reasonable position given that this taxon has the highest chromosome count (2n♂=27) of any known JC species. (See White 1973, p. 452, for discussion about evolutionary trends in chromosome numbers within various animal groups). On the other hand, its different placement in our latest tree (Fig. 1) appears to indicate that its recovered position can vary depending on the markers used and their analysis.

Non-*Stenopelmatus* additions to our 2017 data set

OSF (Cigliano *et al.* 2024) considers the "Jerusalem cricket" family Stenopelmatidae to contain one subfamily (Stenopelmatinae). In contrast Gorochov (2021) considers the Stenopelmatidae to contain three subfamilies (Stenopelmatinae, Schizodactylinae, and Gryllacridinae). Both references agree with each other that the Stenopelmatinae contain the same four or five tribes. Two of these tribes were discussed in Vandergast *et al.* (2017), where we sequenced three species of the South African genus *Maxentius* (Tribe Maxentiini, and all labeled as *Sia* species in our 2017 paper), and recovered them as closest relatives of the New World JC genera *Stenopelmatus* and *Ammopelmatus* (Tribe Stenopelmatini). In our current study, with the addition (Fig. 1) of *Sia ferox* Giebel (Tribe Siini), from Java; and *Oryctopterus varuna* Hiremath & Prathapan (Tribe Oryctopterini), from India, these latter two genera are now recovered in a clade with South African *Maxentius*, and closest to a clade containing the Anostostomatidae genera *Lezina, Glaphyrosoma*, and *Cnemotettix.* But, with the addition of this new material, we found that the three Old World Stenopelmatinae tribes form a distinct clade which does not share the most recent common ancestor with the New World JCs. This renders Stenopelmatidae paraphyletic.

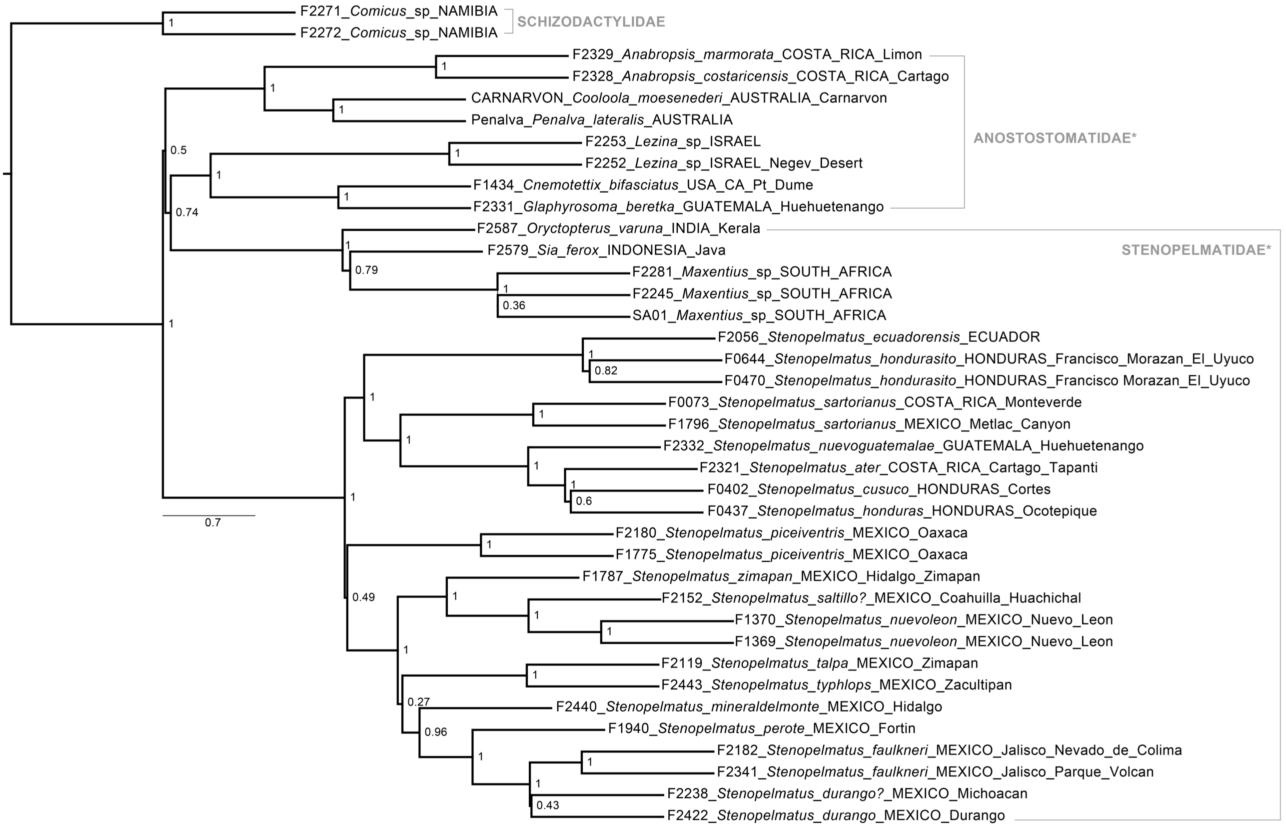

FIGURE 1. Orthoptera-specific target enrichment (OR-TE) analysis. We find both families Anostostomatidae and Stenopelmatidae (as indicated by an *) to be paraphyletic. Family Schizodactylidae is shown to be distantly related to both Anostostomatidae and Stenopelmatidae.

The anostostomatid relationship found in Fig. 1, between New World *Glaphyrosoma* and *Cnemotettix*, and Old World *Lezina*, was previously reported in Vandergast *et al.* (2017) when only 3 genes were analyzed. With our current analysis of 1,236 genes, these latter three genera again form a monophyletic group, which does not share the most recent common ancestor with other anostostomatids included in the analysis. Based on this finding, we consider Anostostomatidae paraphyletic.

Lastly, we note that our two splay-footed cricket samples (genus *Comicus*) are recovered in Fig. 1, distinct from all other Stenopelmatidae; a situation also found by Vandergast *et al.* (2017). This finding is not surprising (see discussion in Vandergast *et al.*, 2017, p. 27 & 28), given that the only karyotyped, splay-footed cricket, *Schizodactylus monstrosus* (Drury) (McClung & Asana 1933), is known to have all rod-shaped chromosomes, including both sex chromosomes. This finding contrasts with the entire superfamily Stenopelmatoidea (Vandergast *et al.* 2017), where all examined species have some metacentric autosomes, and always a metacentric X chromosome. Despite this information, Gorochov (2021) continues to place the splay-footed crickets as a subfamily (Schizodactylinae) within the family Stenopelmatidae, while dismissing overwhelming evidence from molecular and cytological data.

***Stenopelmatus* additions to our 2021 data set**

(1) S. ater: One of only two named Costa Rican *Stenopelmatus* species (the other being the fully winged *S. sartorianus* Saussure), we sequenced material (Fig. 1, specimen F2321), provided by Oscar Cadena-Castañeda, of a male *S. ater* from Parque Nacional Tapantí, Cartago Province, Costa Rica. The male's multilocus DNA was recovered with *S. nuevoguatemalae* n. sp., *S. honduras* Weissman, and *S. cusuco* Weissman. Jorge Gutiérrez-Rodríguez has also informed us (personal communication to DBW, 18-ii-2023) that he has a third, but undescribed, Costa Rican species (Fig. 2) from Ascensión Chirripó, Cartago Province, 9.463525° -83.571862°, that, according to Alejandro Zaldívar-Riverón (personal communication to DBW, 18-ii-2023), is recovered, despite being apterous, in the broad Central American species group of Gutiérrez-Rodríguez *et. al.* (2022). Interestingly, when DBW examined the Costa Rican INBio collection in the early 2000s, he only saw specimens of the two described taxa.

(2) S. hondurasito Weissman: We sequenced leg F0470 of a second individual, from the type locality, of this non-jumping species that confirmed no error when the leg of the first individual (F0644) was previously recovered (Weissman *et al.* 2021) in a larger clade with several jumping species.

FIGURE 2. Third, but unnamed, species of *Stenopelmatus* from Costa Rica, this specimen from Ascensión Chirripó, Cartago Province, 9.463525° -83.571862°. Photo Jorge Gutiérrez-Rodríguez.

(3) S. nuevoleon Weissman: Sample F1369, from a paratype female, was sequenced in Weissman *et al.* (2021) from a locality (DBW Stop 03-105) some 80 km due north of the type locality. We herein present the results of the allotype female (F1370), from the type locality, and report (Fig. 1) that both samples are recovered closest to each other and apparently represent the same taxon.

In summary, our latest results add further information (see Fig. 1) to our previous multilocus tree (see Fig. 10 in Weissman *et al.* 2021); continue to demonstrate the monophyletic origins of both New World JC genera *Stenopelmatus* and *Ammopelmatus*; and confirm the phylogenetic relationship of the pure black, hopping, wingless Costa Rican endemic *S. ater* Saussure & Pictet, which was recovered in our 3-gene Bayesian analysis (in Fig. 9, Weissman *et al.* 2021) but not in our multigene ASTRAL analysis (in Fig. 10, Weissman *et al.* 2021).

New JC species from Mexico and male internal genitalia

Suastes-Jiménez *et al.* (2023) revised the *Stenopelmatus talpa* species group, naming four new taxa–for which they had at least one adult male–based on combined evidence from the cytochrome *c* oxidase subunit 1 mitochondrial barcoding gene (COI) and male internal genitalia. They also recognized, but did not name, three other taxa for which they lacked an adult male. While we previously acknowledged (Weissman *et al.* 2021) the probable underestimated diversity of *Stenopelmatus* in Mexico, we believe their assessment of seven new species warrants further review for the following reasons: 1) Barcoding using the COI gene is notoriously unreliable in Orthoptera because so many groups appear to have recent, extensive speciation (Weissman & Gray 2019, p. 16; Ortego *et al.* 2023), amongst other reasons (e.g., Hawlitschek *et al.* 2017; Kock *et al.* 2024), including rampant nuclear integration of mtDNA (numts) (Moulton *et al.* 2010; Pereira *et al.* 2021). Other mtDNA genes, CYTB and COX3, were less frequently found as numts than COI, at least in some Orthoptera (Pereira *et al.* 2021). The nuclear ITS2 gene was also found to be a more reliable marker for delineating species in other Orthoptera Groups (Gray *et al.* 2020; Uluar & Çýplak 2020). These genes could make better "barcoding" genes for Orthoptera moving forward, although we are not sure why Suastes-Jiménez *et al.* (2023) did not use the extensive genetic data available from their prior studies on this group (see Gutiérrez-Rodríguez *et al.* 2022; Ruiz-Mendoza *et al.* 2023). 2) While we commend Suastes-Jiménez *et al.* (2023) for examining male internal genitalia, we are concerned, and discuss further in the next paragraph, that they compared genitalia while in their "folded" position instead of when "unfolded" and in their "physiologically functional" conformation. 3) We are concerned by a lack of adult male specimens to assess intraspecific variation in internal genitalia. Specifically, the sample size in four of their six named species, in their Figs 6 & 7, was only one adult male; while the fifth species, *S. tlaxcalli* Suastes-Jiménez, Barrientos-Lozano & Zaldívar-Riverón, was known from two adult males. The authors had 21 adult males available for the sixth species, *S. chilango* Suastes-Jiménez, Barrientos-Lozano & Zaldívar-Riverón, and apparently examined 9, but they made no comment on variation seen. To wit, how consistent, between species, are the minor differences seen in the ventral folds of the internal genitalia? 4) The whole-body color photographs presented in their Fig. 3 do not detail whether the pictured individuals were adults or nymphs. This staging is important because JCs are not only color-variable as adults, but can also change color patterns as they mature (Weissman *et al.* in prep). 5) And, lastly, given the large sample size (112 specimens) that Suastes-Jiménez *et al.* (2023) collected, we are disappointed that they did not note which specimens hopped, what are the drum calling songs of adult males (field collected adult females are usually mated and rarely drum in the laboratory), or make any apparent effort to raise male nymphs to obtain karyotypes from late instars and to increase their sample size of adults. And many of their specimens were collected after our paper (Weissman *et al.* 2021) pointed out the potential significance of such information in this very difficult group.

The function of male internal genitalia, during mating, in both New World *Stenopelmatus* and *Ammopelmatus* JCs, is illustrated elsewhere (Weissman 2001; Weissman *et. al.* 2008; Weissman *et. al.* 2021; and called "phallic lobes" therein). Basically, after the adult male anchors his hooks under the posterior edge of the female's 6th (or 7th?) abdominal sternite, he simultaneously inflates and unfolds his phallic lobes (Fig. 3), which quickly envelop the female's ovipositor and terminalia, before rapidly passing a bilobed spermatophore. Watching the KQED San Francisco Deep Look 4K video (https://www.youtube.com/watch?v=mHbwC-AIyTE&t=147s), and utilizing the slow speed playback option of 0.25, beginning around 2:48 in the video, one can watch the inflated phallic lobes apparently empty as the spermatophore enlarges, as seen in the photographed virgin mating pair of *Ammopelmatus* sp. *f*rom Santa Barbara County, California. Utilizing a live adult male, of a different *Ammopelmatus* sp. from Santa Clara County, California, we could artificially expand these lobes (Fig. 4) by using a forceps to lower the subgenital plate while simultaneously squeezing the male's abdomen. In this situation, the lobes appear diaphanous and empty, and without crease "lines" indicating a lack of physical demarcation between lateral and ventral fields. The lobes

FIGURE 3. Male (top) *Ammopelmatus* sp. with yellowish colored phallic lobes (arrow) fully unfolded and almost making contact with female below him. Photo DBW.

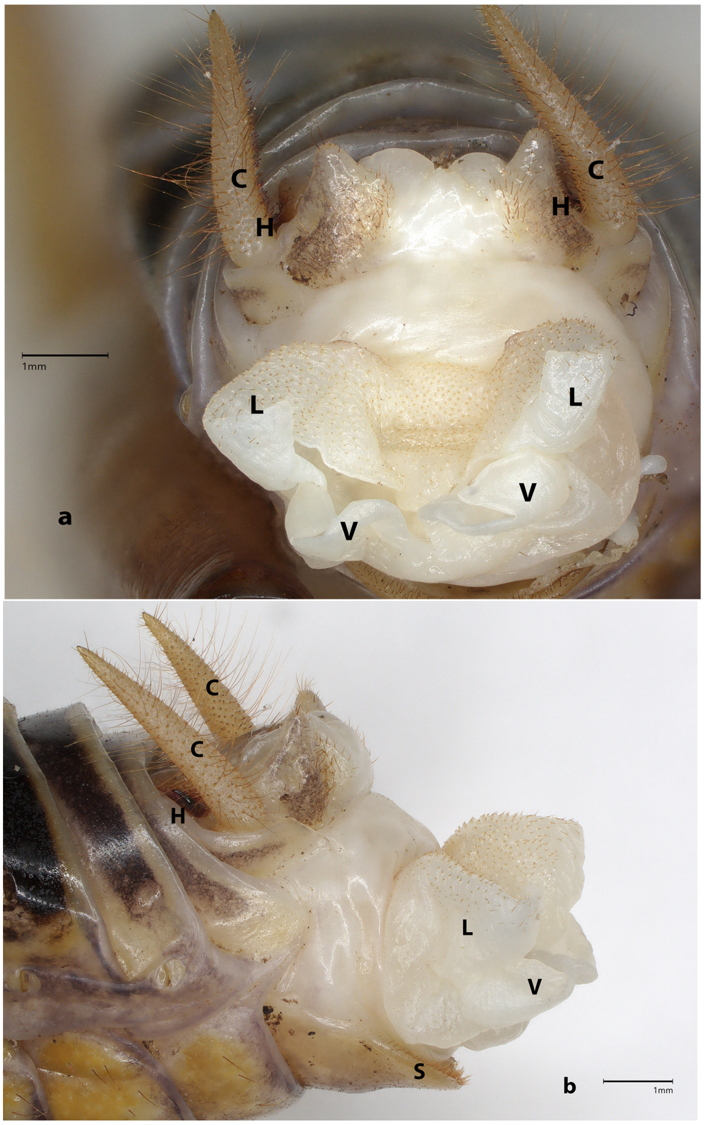

FIGURE 4. Inflated and unfolded phallic lobes, as prepared in live *Ammopelmatus* sp., from Santa Clara County, California: a. Posterior view. b. Lateral view. (C) cerci; (H) black hooks, confirming adult status; (L) lateral lobes; (S) subgenital plate; (V) ventral lobes. Photos Chris Grinter.

could be kept (mostly) expanded by injecting the male's abdomen, using a 27g or 30g hypodermic needle, with 75% ethanol, a technique described by Hubbell (1977) for camel crickets (family Rhaphidophoridae). These extruded lobes turned semi-opaque soon after the male was killed. We thus feel that while folded phallic lobes may have some taxonomic utility, such lobes are best assessed in their physiological, dynamic, unfolded state, as seen in Fig. 4, because important details could otherwise be hidden in the folds. Any morphological differences between individuals will be more visible and more amenable to comparison, even if live males are required for such preparations. In any case, because spermatophore transfer takes only seconds, and without any evidence of male penetration, we wonder if any of these minor morphological differences in lobes, as discussed by Suastes-Jiménez *et al.* (2023), are good species' delimiting characters, especially in the absence of any discussion about intraspecific variation. We also suspect that such small differences in folds would not be any kind of mating barrier. In contrast, we do feel that differences in drumming patterns, karyotype numbers, and whether or not individuals in populations hop or jump, are more convincing of species' distinctiveness.

We also note that both folded ventral and lateral lobes, as seen in the *Stenopelmatus* species in Suastes-Jiménez *et al*. (2023), are narrower and more separated than those seen in three species of adult male *Stenopelmatus* (Fig. 5) that we reported upon earlier (Weissman *et al*. 2021). These differences do not originate from our different preservation technique of first using Williams' fixative followed by 75% ethanol (see Weissman *et al.* 2021, p. 11), since examination of only 75% ethanol-preserved genitalia (not illustrated) also appear the same as our Fig. 5 ones. We particularly note that this difference persists in our Fig. 5C, an adult male of *S. typhlops* Rehn, the same taxon illustrated by Suastes-Jiménez *et al*. (2023) in their Figs 6C & 6D. Interestingly, in dorsal perspective (Fig. 5D), the surface of the genitalia of *S. typhlops* appears to have a number of surface structures that might be of taxonomic usefulness in distinguishing taxa.

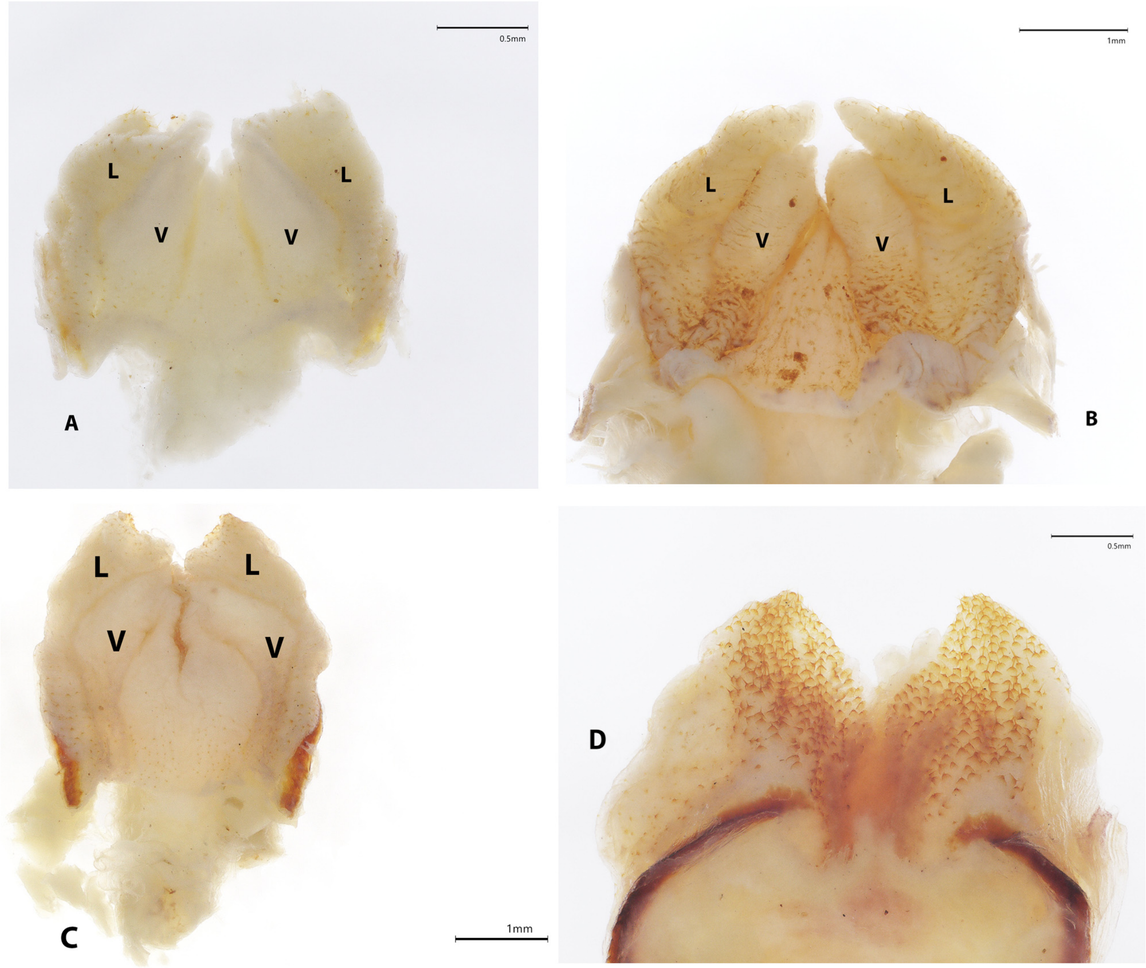

FIGURE 5. Ventral view of folded phallic lobes, in adult males, all from type localities. (A) *S. zimapan* (DBW Stop 11-66); (B) *S. piceiventris* (DBW Stop 06-36); (C) *S. typhlops* (DBW Stop 08-44); (D) Dorsal view, same specimen as in Fig. 5C. (L) lateral lobe; (V) ventral lobe. Photos Chris Grinter.

These narrower lobes of Suastes-Jiménez *et al*. (2023) also appear to be unrelated to generic differences between *Stenopelmatus* and *Ammopelmatus*. Fig. 6 shows the alcohol-only preserved folded lobes from the same species of *Ammopelmatus*, as seen in unfolded Fig. 4. We note no difference in lobe thickness when compared to those *Stenopelmatus* in Fig 5. Additionally, with careful visualization, we can confirm that, in situ, folded phallic lobes in live males in this same taxon are shaped similar to those seen in Fig. 6.

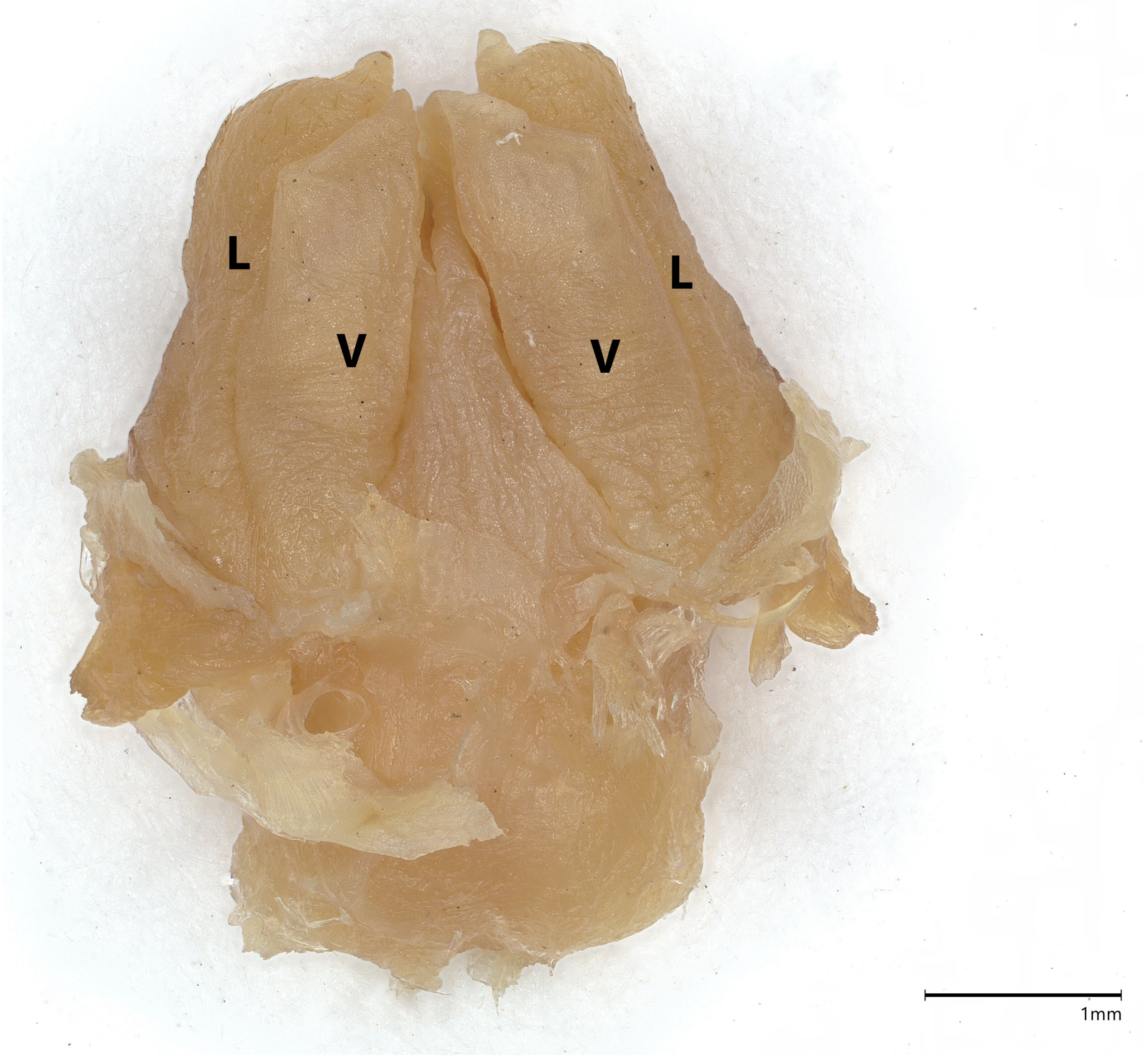

FIGURE 6. Ventral view of folded phallic lobes, in adult male *Ammopelmatus* sp., from Santa Clara County, California. Same species as illustrated in Fig. 4. (L) lateral lobe; (V) ventral lobe. Photo Chris Grinter.

Lastly, our immersion of genitalia in 10% KOH–meant to dissolve support tissue–also does not appear to affect folded lobe shape, which might be predicted since the lobes are not attached to any support tissue. In personal communication with Miguel Suastes-Jiménez and Alejandro Zaldívar-Riverón (December, 2023), they expanded upon their 2023 published methods, in that *both* pinned and 70% alcohol preserved adult males were initially treated for 45 minutes in hot water, before the internal genitalia were removed; and then for 20-50 minutes in heated KOH. Perhaps their treatment somehow dehydrates, and shrinks, both the ventral and lateral lobes, although we could not duplicate their results. But the source of their narrower lobes is important because only such narrow lobes permit visualization of structures that would normally be hidden within the folded lobes (as in our Figs 5 and 6).

We wonder if mating status might somehow be important in folded lobe thickness since Weissman (2001, p. 365) documented how spermatophore ampulla and spermatophylax size were reduced with each subsequent mating?

Stenopelmatus nuevoguatemalae **Weissman, n. sp.**

Guatemala Jerusalem Cricket
Figs 1, 7, 8, 9, 10

Distribution. Known only from the type locality.

Recognition characters. Unique specimen (F2332) that was first sequenced in Vandergast *et al.* (2017), and also reported in Weissman *et al.* (2021), and here (Fig. 1). Medium-large sized JC (Fig. 7) with apparent dark brown/black body, head, and pronotum but all legs and antennae reddish. Combination of color, size, and rear leg tibial spines separate this taxon from all current species of *Stenopelmatus* (see Weissman *et al.* 2021, Table 1). *S. nuevoguatemalae* differs from *S. guatemalae* and *S. vicinus*, the only *Stenopelmatus* species previously known from Guatemala (and both described from there), by having 4 inner and 4 outer rear leg tibial spines while *S. guatemalae* has 4 inner and 3 outer and *S. vicinus* has 3 inner and 3 outer rear leg tibial spines. The latter two taxa were described by Brunner von Wattenwyl, in 1888, and both were subsequently designated nomen dubium by Weissman *et al.* (2021). Genetically similar, but distinct, from *S. cusuco* Weissman, from Honduras (see Weissman *et al.* 2021), which it probably closely resembles in color when alive. *S. cusuco* has 3 or 4 inner rear leg tibial spines and 4 or 5 outer rear leg tibial spines. The type (and only known) localities of *S. nuevoguatemalae* and *S. cusuco* are separated by some 320 km. Documentation of different calling drums would be important in confirming that they are not conspecific.

Holotype. Adult female (Fig. 7), in alcohol. **Guatemala,** Department Huehuetenango, Barillas Unión Las Palmas, 15.931100° -91.299310°, 1444m, 15-v-2012, collected as adult. F2332. Collectors J. Monzón & F. Camposeco. Deposited California Academy of Sciences (CAS), CASENT type #20223. Measurements in mm: Body length 36.19, hind femur length 14.08, hind femur width 4.65. Fore leg tibia with 3 ventral spurs; middle leg tibia with 7 calcars, 2 ventral spurs; rear leg tibia (Fig. 8) with 4 outer (most proximal represented by a bump on both legs) and 4 inner spines, 2 ventral spurs. Ovipositor Fig. 9. Outline of furrow on face (Fig. 10).

FIGURE 7. Holotype adult female *S. nuevoguatemalae*. Photo Dan Weissman.

FIGURE 8. Left hind leg tibia, holotype female *S. nuevoguatemalae*. Photo Dan Weissman.

FIGURE 9. Ovipositor, holotype adult *S. nuevoguatemalae.* Photo Dan Weissman.

Male. Unknown.

Drum. Unknown.

Derivation of name. Since the name *S. guatemalae* is preoccupied, we choose this variation to indicate its country of origin.

Habitat. Mountain forests, according to the collectors.

Behavior. We predict that this species will hop.

Life cycle and seasonal occurrence. Unique adult holotype collected 15-v-2012.

DNA. F2332 recovered (Fig. 1) in a subclade with *S. ater*, *S. cusuco*, and *S. honduras.*

Karyotype. Unknown.

Discussion. Despite absence of a drum and karyotype, we nevertheless name this taxon because its DNA, precise locality, and distinctive set of morphological characters will permit future identification.

We thank Oscar Cadena-Castañeda for giving us permission to describe this new taxon, and for donating the holotype to the CAS.

Minor corrections to Weissman *et al.* (2021)

1. Abstract, p. 4, *Stenopelmatus erythromelas* Walker misspelled.
2. *S. chiapas* Weissman, p. 27, now CASENT type #20201.
3. *S. cusuco* Weissman, p. 29, now CASENT type #20200.
4. *S. sanfelipe* Weissman, p. 85, entries for Drum, Karyotype, and DNA duplicated from p. 84; entry for Habitat expanded on p. 85 and more information in Discussion on p. 86.
5. *S. sartorianus* Saussure, p. 91, under Systematics, we discussed how *S. sallei* Saussure has line priority over *S. sartorianus* and *S. sumichrasti* Saussure. Subsequently, Thomas Pape (personal communication to DBW, 20-v-2021) corrected our misunderstanding in that page priority, when assigning published names, does not currently apply. Given that information, we could have made both *S. sallei* and *S. sumichrasti* junior synonyms of *S. sartorianus*, as opposed to designating the former two taxa nomem dubium. On the other hand, Gutiérrez-Rodríguez *et al.* (2022, Fig. 4) show that *S. sartorianus* may be a sister species complex of long winged taxa.

FIGURE 10. Face, holotype adult female *S. nuevoguatemalae*. Photo Dan Weissman.

TABLE 1. Collection data for specimens, in the same sequence as they appear in Figure 1.

Specimen code	Species name	Collection information
F2271	*Comicus calcaris*	Nimibia, Namib Desert, Gunsbewys Farm. 26-v-2010. R. Lakes-Harlan
F2272	*Comicus calcaris*	Nimibia, Namib Desert, Gunsbewys Farm. 26-v-2010. R. Lakes-Harlan
F2329	*Anabropsis marmorata*	Costa Rica, Limon, Turrialba Volcan, 655m, 23-iv-2012, O. Cadena-Castañeda
F2328	*Anabropsis costaricensis*	Costa Rica, Cartago, Reserva Indigena Bajo Chiripo, 1280m, 24-iv-2012, O. Cadena-Castañeda
CARNARVON	*Cooloola moesenederi*	Australia, Queensland, Mt. Moffatt, Carnarvon National Park, S25.018° E147.895°, 714m, 13-xi-2010, N. Starick
Penalva	*Penalva lateralis*	Australia, Queensland, Kuranda, 19 Butler Dr., 16.48°S 145.38°E, 335m, 16-31-iii-2010, DCF Rentz
F2253	*Lezina concolor*	Israel, Negev Desert, Meshash Sands between Beer Sheva and Dimona, 12-iv-2012. D. Simon
F2252	*Lezina concolor*	Israel, Negev Desert, Meshash Sands between Beer Sheva and Dimona, 12-iv-2012. D. Simon
F1434	*Cnemotettix bifasciatus*	USA, California, Los Angeles Co., Pt Dume, 18-viii-2004, DBW
F2331	*Glaphyrosoma beretka*	Guatemala, Huehuetenango, Barillas, San Ramon, near Rio Bravo. 550m. 18-v-2012, O. Cadena-Castañeda
F2587	*Oryctopterus varuna*	India, College of Agriculture, Vellayani, Kerala, S. Hiremath
F2579	*Sia ferox*	Indonesia, Java, xi-2018, purchased, in 2019, on eBay by DC Lightfoot
F2281	*Maxentius sp.*	South Africa, Western Cape, CederbergNature Reserve, Wolfberg Cracks, 4-i-2011. P Naskrecki, CS Bazelet
F2245	*Maxentius sp.*	South Africa, Western Cape, Jonkersshoek Nature Reserve 1.3 km from entrance kiosk, 15-viii-2011, 207m. DB Weissman, CS Bazelet
SA01	*Maxentius sp.*	South Africa, Western Cape, Groot Winterhoek Nature Reserve. 28-xi-2008. S32.99885° E19.06147°. CS Bazelet
F2056	*Stenopelmatus ecuadorensis*	Ecuador, Napo Province, Estacion Cientifica Yasuni, 215m, 14-17-viii-1997, F. Andrews, A. Gillogly
F0644	*Stenopelmatus hondurasito*	Honduras, Department Francisco Morazán, Reserva Biologica Uyuca, 14.017335° -87.082974°, 1509m, 11-xii-1996, DBW
F0470	*Stenopelmatus hondurasito*	Honduras, Department Francisco Morazán, Reserva Biologica Uyuca, 14.017335° -87.082974°, 1509m, 11-xii-1996, DBW
F0073	*Stenopelmatus sartorianus*	Costa Rica, Puntarenes, Monteverde, 1524m, 16-vi-1995, DBW
F1796	*Stenopelmatus sartorianus*	Mexico, Veracruz, Metlac Canyon, 634m, 20-vi-2006, DBW, DC Lightfoot
F2332	*Stenopelmatus nuevoguatemalae*	Guatemala, Huehuetenango, Barillas, Union Las Palmas, 1444m, 15-v-2012, J. Monzón, F. Camposeco
F2321	*Stenopelmatus ater*	Costa Rica, Cartago Province, NNP Tapantí, 1655m, 21-22-iv-2012, O. Cadena-Castañeda
F0402	*Stenopelmatus cusuco*	Honduras, Cortes, Cusuco National Park, 1493m, 5-xii-1996, DBW
F0437	*Stenopelmatus honduras*	Honduras, Ocotepeque, RB Guisayote 20 km E Nueva Ocotepeque, 2048, 7-xii-1996, DBW
F2180	*Stenopelmatus piceiventris*	Mexico, Oaxaca, Cerro Iquana, 2286m, 4-viii-2007, MH Van Dam
F1775	*Stenopelmatus piceiventris*	Mexico, Oaxaca, Hwy 175 at km post 129.2, 2749m, 19-vi-2006, DBW, DC Lightfoot

......*Continued on the next page*

APPENDIX 1. (Continued)

Specimen code	Species name	Collection information
F1787	*Stenopelmatus zimapan*	Mexico, Hidalgo, Zimapan, 1707m, 23-vi-2006, DBW, DC Lightfoot
F2152	*Stenopelmatus saltillo?*	Mexico, Coahuila, Huachichal, 2163m, 19-ix-2008, MH Van Dam
F1370	*Stenopelmatus nuevoleon*	Mexico, Nuevo Leon, Hwy 61, Km marker 182, 24.699513° -100.032477°, 1776m, 20-ix-2003, DBW, DC Lightfoot
F1369	*Stenopelmatus nuevoleon*	Mexico, Nuevo Leon, Hwy 20, 20.8 road km SW Santiago, 1371m, 20-ix-2003, DBW, DC Lightfoot
F2119	*Stenopelmatus talpa*	Mexico, Hidalgo, Hwy 85 17 km S Jacala, 1959m, 3-vi-2008, DBW, DC Lightfoot
F2443	*Stenopelmatus typhlops*	Mexico, Hidalgo, Hwy 105, 6 km N Zacualtipan, 2139m, 18-vi-2015, DBW, DW Weissman
F2440	*Stenopelmatus mineraldelmonte*	Mexico, Hidalgo, Mineral del Monte, 2804m, 17-vi-2015, DBW, DW Weissman
F1940	*Stenopelmatus perote*	Mexico, Veracruz, 13.3 km S Hwy 140 on road to Cofre de Perote, 3108m, 22-vi-2006, DBW, DC Lightfoot
F2182	*Stenopelmatus faulkneri*	Mexico, Jalisco, Nevado de Colima, 28-vii-2007, MH Van Dam
F2342	*Stenopelmatus faulkneri*	Mexico, Jalisco, Parque Nacional Volcan Nevado de Colima, 3048m, 10-vii-2012, DBW, DW Weissman
F2238	*Stenopelmatus durango?*	Mexico, Michoacan, 4.1 km W Nuevo San Juan, 2090m, 30-vi-2011, DBW, DW Weissman
F2422	*Stenopelmatus durango*	Mexico, Durango, Hwy 40 libre 2 km NE Llano Grande, 2468m, 20-vii-2014, DBW, DW Weissman

Discussion

With the exception of *S. piceiventris*, discussed above, the addition of three new *Stenopelmatus* specimens in Fig. 1 has resulted in no substantive changes in the relationships of the recovered taxa when compared to the Fig. 10 tree in Weissman *et al.* (2021). Even more recently, much needed progress has been made in describing the diversity of *Stenopelmatus* in Mexico and Central America (Gutiérrez-Rodríguez *et al.* 2022; Ruiz-Mendoza *et al.* 2023). Comparing our Fig. 1 tree with that of Fig. 4 in Gutiérrez-Rodríguez *et al.* (2022), we note at least three potentially important differences: 1) *Stenopelmatus* appears polyphyletic in the Gutiérrez-Rodríguez *et al.* (2022) COI tree based on the placement of the Piceiventris species group, although with weak branch support values (<70). While this group may be difficult to place based on molecular characters alone, Weissman *et al.* (2021, p. 20) discussed why *Ammopelmatus* and *Stenopelmatus*, as delineated therein, are likely each valid, monophyletic genera. 2) The Gutiérrez-Rodríguez *et al.* (2022) proposed Faulkneri species group, composed of *S. faulkneri* Weissman, *S. zimapan* Weissman, and *S. mineraldelmonte* Weissman, is polyphyletic in our Fig. 1, and so may reflect multiple independent lineages upon further analysis. 3) Lack of additional material prevented us from performing multilocus analysis on the unique type of *S. chiapas* Weissman. In Vandergast *et al.* (2017), we presented results (see Fig. 2, F2172, then labeled as "*S. sallei*?") for this individual and we recovered it adjacent to their Central America species group, and not in the Piceiventris species group, as Gutiérrez-Rodríguez *et al.* (2022) predicted. Although the unique *S. chiapas* male has wing pads like that seen in both *S. piceiventris* and *S. sanfelipe* Weissman, in addition to short hairs, of unknown function, underneath his wing pads, and similar to those seen in the latter two taxa, we withhold our opinion pending analysis of fresh DNA material. The holotype of *S. chiapas* was collected and pinned in 1973, but we did not remove a middle leg and place it into 95% ethanol until December, 2009. Elsewhere (Weissman & Gray 2019, p. 16), we documented our concerns when dealing with old, pinned *Gryllus* field crickets, because of the annomalous DNA results that we had obtained in several species. On the other hand, Gutiérrez-Rodríguez *et al.* (2022) have sequenced two individuals, from Chiapas, that they called "*S. chiapas,* or near", and they were recovered in their Piceiventris species group, providing compelling evidence of a close relationship between these species. Overall, topological differences in the two molecular phylogenies appear to reflect a combination of different sets of molecular markers and taxa included in various analyses, and underscore the importance of preserving and having available ample tissue for molecular analysis, querying a diverse array of genetic loci, along with standardizing morphological measurements, and reporting on other behaviors that have been found to be important in species delineation in this group, such as drumming and hopping (Weissman *et al*. 2021).

Finally, we note that Carbajal-Márquez *et al.* (2022) document the predation of "*Stenopelmatus faulkneri*", by Campbell's Dusky Rattlesnake (*Crotalus campbelli*). Their report adds several other references of similar predation to those listed in Weissman *et al.* (2021), but we caution that their prey identification could need further verification given the extensive cryptic JC diversity in Mexico documented by Gutiérrez-Rodríguez *et al.* (2022) and Ruiz-Mendoza *et al.* (2023).

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