Thirteen new species of butterflies (Lepidoptera: Hesperiidae) from Texas

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Abstract

Analyses of whole genomic shotgun datasets, COI barcodes, morphology, and historical literature suggest that the following 13 butterfly species from the family Hesperiidae (Lepidoptera: Papilionoidea) in Texas, USA are distinct from their closest named relatives and therefore are described as new (type localities are given in parenthesis): *Spicauda atelis* Grishin, **new species** (Hidalgo Co., Mission), *Urbanus* (*Urbanus*) *rickardi* Grishin, **new species** (Hidalgo Co., nr. Madero), *Urbanus* (*Urbanus*) *oplerorum* Grishin, **new species** (Hidalgo Co., Mission/Madero), *Telegonus tsongae* Grishin, **new species** (Starr Co., Roma), *Autochton caballo* Grishin, **new species** (Hidalgo Co., 6 mi W of Hidalgo), *Epargyreus fractigutta* Grishin, **new species** (Hidalgo Co., McAllen), *Aguna mcguirei* Grishin, **new species** (Cameron Co., Brownsville), *Polygonus pardus* Grishin, **new species** (Hidalgo Co., McAllen), *Arteurotia artistella* Grishin, **new species** (Hidalgo Co., Mission), *Heliopetes elonmuski* Grishin, **new species** (Cameron Co., Boca Chica), *Hesperia balcones* Grishin, **new species** (Travis Co., Volente), *Troyus fabulosus* Grishin, **new species** (Hidalgo Co., Peñitas), and *Lerema ochrius* Grishin, **new species** (Hidalgo Co., nr. Relampago). Most of these species are known in the US almost exclusively from the Lower Rio Grande Valley in Texas. Nine of the holotypes were collected in 1971–1975, a banner period for butterfly species newly recorded from the Rio Grande Valley of Texas; five of them collected by William W. McGuire, and one by Nadine M. McGuire. At the time, these new species have been recorded under the names of their close relatives. A **Neotype** is designated for *Papilio fulminator* Sepp, [1841] (Suriname). **Lectotypes** are designated for *Goniurus teleus* Hübner, 1821 (unknown,

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likely in South America), *Goniloba azul* Reakirt, [1867] (Mexico: Veracruz) and *Eudamus misitra* Plötz, 1881 (Mexico). Several taxonomic changes are proposed. The following taxa are species (not subspecies): *Spicauda zalanthus* (Plötz, 1880), **reinstated status** (not *Spicauda teleus* (Hübner, 1821)), *Telegonus fulminator* (Sepp, [1841]), **reinstated status** (not *Telegonus fulgerator* (Walch, 1775), *Telegonus misitra* (Plötz, 1881), **reinstated status** (not *Telegonus azul* (Reakirt, [1867])), *Autochton reducta* (Mabille and Boullet, 1919), **new status** (not *Autochton potrillo* (Lucas, 1857)), *Epargyreus gaumeri* Godman and Salvin, 1893, **reinstated status** (not *Epargyreus clavicornis* (Herrich-Schäffer, 1869)), and *Polygonus punctus* E. Bell and W. Comstock, 1948, **new status** (not *Polygonus savigny* (Latreille, [1824])). *Urbanus ehakernae* Burns, 2014 and *Epargyreus socus chota* Evans, 1952 are junior subjective synonyms of *Urbanus alva* Evans, 1952 and *Epargyreus clavicornis* (Herrich-Schäffer, 1869), respectively, and *Epargyreus gaumeri tenda* Evans, 1955, **new combination** is not a subspecies of *E. clavicornis*.

Keywords

Cryptic species; biodiversity; skipper butterflies; genomics; speciation; nomenclature; taxonomy

Introduction

From prehistoric times, people noticed that animals and plants mostly exist as discrete varieties or groups (i.e., kinds, sets, series) of individuals. Such individuals look similar within a group, but differ between groups, and nearly no intermediates exist. This discreteness is a fact that needs conceptualization. These groups reflecting discreteness observed in nature have been called "species." Practically, species have been defined by their identifiability. If individuals can be identified as belonging to their group, such groups were regarded as species.

New approaches to old problems reveal surprises. Initially, species of butterflies were described on the basis of wing patterns and shapes (Linnaeus 1758), because that was how they were easily identified. Since Godman and Salvin (1893–1899), species delineation in Hesperiidae (Lepidoptera) relies heavily on differences in genitalia. This approach, cemented by W. H. Evans (Evans 1937, 1949, 1951, 1952, 1953, 1955), has been used since. Consistent differences in genitalia of either sex are viewed as the strongest evidence of speciation, and populations differing in wing patterns but similar in genitalia are treated as subspecies. Some species can only be distinguished by genitalia and not by facies (Burns 2000), an observation hardly causing a surprise today. Identifiability by genitalia is currently a cornerstone of Hesperiidae species delineation. Presently, species confidently identifiable only by DNA sequences cause questions.

Theoretically, concepts of species have been reasonably well defined (Mallet 1995; Mayr 1996; de Queiroz 2005; Mallet 2020). Most biologists tend to conceptualize speciation in terms of a (possibly porous) reproductive barrier. While this barrier may not be as absolute as in the original biological species concept (Mayr 1942), the reproductive isolation is the most straightforward way to connect discreteness of life forms we observe to biological reality. Indeed, the reproductive barrier that is strong enough to prevent equilibration of the

gene pool between species would keep species distinct from each other as discrete units of biological diversity that can be identified.

Practically, at least in Hesperiidae, species were largely delineated by differences in genitalia. If quantifiable differences in either male or female (or both) genitalia separate all inspected specimens of the two species, such differences are taken as the decisive evidence of species distinction. While not necessarily directly linked to the reproductive barrier and incompatibilities between species, the differences in hard morphological structures tend to be more consistently discrete than rather plastic and variable wing patterns, and therefore may simply reflect genetic differentiation.

Genetic differentiation is typically linked to speciation. A general logic is that when populations spend sufficient time in isolation (e.g., allopatry), random genetic differences that accumulate through mutations and spread throughout each population would by chance contain some that are phenotypically expressed as incompatibilities, and these populations become reproductively isolated. The underlying logic behind COI barcode utility (Hebert et al. 2003, 2004) is exactly that: this tiny 658 base pair piece of DNA is used as a "ruler" to measure genetic differentiation. While in many cases barcodes give a reasonable approximation, they frequently fail for a number of reasons (Rubinoff et al. 2006), most important of which are their short length (i.e., not enough statistical power) and frequent introgression (i.e., barcodes exchange between species).

Extrapolating from the barcode approximation to the total, whole genomic analysis is expected to give the ultimate answer to species delineation and identifiability. Overall genetic differentiation can be measured from genomic sequences and correlated with traditional criteria, such as genitalic differences in Hesperiidae. We argue that genomic analysis is a more direct measure of natural reproductive isolation between populations than genitalic distinction, and possibly than any other conceivable approach. In the absence of reproductive isolation, genetic material would exchange rather freely between populations, and this exchange will prevent populations from diverging genetically. Conversely, populations that genetically diverged at the level typical of well-studies species would likely belong to distinct species. We can also directly detect genomic segments that exchanged recently between populations, i.e., find genomic regions in a specimen of one population that are more similar to another population than to its own.

Measuring genetic differentiation using F_{st} (fixation index reflecting divergence within populations relatively to that between populations) (Hudson et al. 1992) and gene exchange using G_{min} (Geneva et al. 2015) for a number of pairs of butterfly taxa across the central Texas suture zone, we find that $F_{st} > 0.20$ and $G_{min} < 0.05$ (i.e., 5%) computed on protein-coding regions from the Z chromosome usually correspond to distinct species (Cong et al. 2019a). For comparison, different human populations are typically characterized by $F_{st} < 0.2$ (the same species) and humans have less than 4% of Neanderthal DNA (different species) (Harris and Nielsen 2016).

Inspections of genome-level phylogenetic trees reveals discreteness. Species typically correspond to strongly supported clades (due to limited gene exchange between clades)

that do not usually have strongly supported subclades (due to free gene flow within a clade). Such clades corresponding to species look more like "combs" (i.e., a star topology) than fully bifurcated trees, because gene flow between individuals within a species equalizes them and hinders deviation from the star tree topology, a deviation that would naturally arise in the absence of gene equilibration (Zhang et al. 2022d).

Finally, we also compare COI barcodes and calculate distances between them. Combined with the analysis of nuclear and mitochondrial genome trees, COI barcodes offer a standard and better explored measure of genetic differentiation. Coupled with either phenotypic differences or congruent nuclear genome trees, 2% difference typically corresponds to distinct species (Hebert et al. 2003; Lukhtanov et al. 2016), however, many exceptions exist and should be expected. For example, all *Celastrina* Tutt, 1906 specimens from the USA and Mexico we sequenced possess 100% identical COI barcodes.

We applied these criteria largely based on genome-scale analyses to delineate species of Texas butterflies. Theoretically, our species concept is more similar to the genomic integrity species concept of Sperling (2003), i.e., a naturally occurring and porous reproductive barrier is nevertheless strong enough to maintain genomic distinctness of a group of populations defined as a species and prevents this genomic cluster (or lineage) from disappearing and merging with other such lineages. Practically, we assume that a genomic sequence represents its organism, because it encodes the entire phenotype of this organism, not only wing patterns and genitalia, but also all life stages, such as caterpillars and pupae, their foodplants, and ecological preferences. We arrive at decisions about whether the two groups of populations are species based on their F_{st}, G_{min}, phylogenetic tree structure, and COI barcode differences, as discussed above. As to the species identifiability, because they were delineated using DNA sequences, they are confidently identified by DNA sequences. While in all cases we report phenotypic characters to identify these species, it is best to consider such characters preliminary and in need of further investigations.

Although this study is aimed at specifics of several Texas butterflies, we also observe some general principles. First, under our criteria, there are only a few species with ranges spanning both North and South America. One of such species is *Spicauda simplicius* (Stoll, 1790), which is genetically homogeneous across both Americas (Zhang et al. 2022c). For most others, a North American counterpart (i.e., the sister group of populations) is a species distinct from its South American counterpart. The major suture zone is either in Costa Rica/Panama, or the Colombian Andes (or both, in which case there are more than two species). These counterpart species are sometimes cryptic and can presently be identified with confidence only by their DNA sequences. Second, in agreement with previously reported estimates (Funk and Omland 2003; McKay and Zink 2010; Ross 2014), we find the incongruence between nuclear and mitochondrial genome trees to be common and expected it in at least 15% of cases. Due to this incongruence caused by introgression, some distinct species do not differ in COI barcodes, while others may have several distinct barcode haplotypes accumulated through exchange with other species. Therefore, one should exercise caution when deriving conclusions exclusively from COI barcodes or even the entire mitochondrial DNA. Third, phylogenetic trees constructed from protein-coding

regions on the Z chromosome reveal discreteness better than autosome trees; and strongly supported clades with nearly star topology within them typically correspond to species.

Materials and Methods

Traditionally, new species are identified through visual comparisons of facies and/or genitalia, sometimes complemented with field observations about their life histories and ecology. Here, we use a genomic screen approach to species discovery. First, we obtain whole genome shotgun sequence datasets of various butterfly species across their ranges using our previously established experimental protocols (Li et al. 2019; Zhang et al. 2019). Typically, a leg of a dry pinned specimen is used for DNA extraction. Specimens of any age are amenable to this protocol (Cong et al. 2021). Second, these genomic datasets composed of 150 bp (or less) DNA segments are subjected to computational analysis to identify and assemble (i.e., stitch together) protein-coding regions using DIAMOND (Buchfink et al. 2015) and a reference set of all proteins encoded in a previously assembled genome of a relative. In this work, we used our published reference genome of *Cecropterus lyciades* (Geyer, 1832) (Shen et al. 2017). This procedure results in a master-slave alignment of all these regions (i.e., coding regions in each specimen are aligned to the reference) and these alignments, which are too large (about 18 million positions) for time-efficient phylogenetic analysis, are randomly subsampled for 300,000 positions (by codon) to be used in construction of phylogenetic trees as described previously (Zhang et al. 2022b). Third, we construct phylogenetic trees using IQ-tree v1.6.12 under the GTR+GAMMA model (Nguyen et al. 2015) from these randomly sampled positions in nuclear (autosomes and Z chromosome separately) and mitochondrial genomes, and estimate statistical significance by standard codon resampling from the original complete alignment for nuclear genomes, but use ultrafast bootstrap (Hoang et al. 2018) for mitogenomes. These three trees are visualized using FigTree (Rambaut 2018) and visually compared to each other.

Inspecting the genomic-level trees, we look for confident clades close to the leaves that visually appear like combs (i.e., star subtrees). Such clades are candidates for representing distinct species. Preference is given to the Z chromosome tree because most of the genes important in speciation (pheromone production, wing pattern control, differences between sexes) are encoded by this chromosome, which, in addition, is more resistant to introgression (Pazhenkova and Lukhtanov 2021). In some instances, only a single specimen of a species is available, and we compare its genetic distance from others with distances between specimens of the same species. Then, we assign available names to these clades. In many instances, the assignment is supported by sequenced primary type specimens included in the trees: the species represented by the clade receives the name of the type of the oldest valid name in this clade. If there are no valid names, available names in the clade serve as the basis for naming (and resurrection from synonymy). In the absence of sequenced primary types, identifications are made by a traditional phenotype-based method: comparing facies and genitalia with those of extant primary type specimens or, if types could not be found, with original descriptions while taking type localities into account. The clades that cannot be assigned available names represent potential new species and become the focus of this study.

For the clades without names, we compute F_{st} and G_{min} statistics and COI barcode difference comparing them with their named sister. If these statistics are comparable to those typical of distinct species, we decide that the clade without a name is a new species and look for phenotypic differences from its sister species. In addition to phenotypic diagnosis, we provide diagnostic DNA characters, both in nuclear genome and COI barcode. DNA characters are found in nuclear protein-coding regions using our previously developed procedure (see SI Appendix to Li et al. 2019). The logic behind the character selection was detailed in Cong et. al (2019b). The character states are provided in species diagnoses as abbreviations. E.g., aly728.44.1:G672C means position 672 in exon 1 of gene 44 from scaffold 728 of the *Cecropterus lyciades* (Geyer, 1832) (formerly in *Achalarus* Scudder, 1872, thus aly) reference genome (Shen et al. 2017) is C, changed from G in the ancestor. When characters are given for the sister clade of the diagnosed taxon, the following notation is used: aly5294.20.2:A548A (not C), which means that position 548 in exon 2 of gene 20 on scaffold 5294 is occupied by the ancestral base pair A, which was changed to C in the sister clade (so it is not C in the diagnosed taxon). The same notation is used for COI barcode characters, but without a prefix ending with ':'. The sequences of exons from the reference genome with the positions used as character states highlighted in green are given in the supplemental file deposited at <https://osf.io//vkj6d/>. Linking to these DNA sequences from this publication ensures that the numbers given in the diagnoses can be readily associated with actual sequences. Whole genome shotgun datasets we obtained and used in this work are available from the NCBI database <https://www.ncbi.nlm.nih.gov/> as BioProject PRJNA895865, and BioSample entries of the project contain the locality and other collection data of the sequenced specimens shown in the trees. Additionally, specimen data are summarized in Table S1 of the supplemental file <https://osf.io//vkj6d/>. COI barcode sequences have been deposited in GenBank with accessions OP762098–OP762116 and OP984702–OP984705. All new names have been registered with ZooBank.

Wherever possible, we illustrate descriptions with live photographs of the new species and their closest relatives, mostly taken from iNaturalist (2022). Links to observations by observation number reported in figure legends are <https://www.inaturalist.org/observations/xxx>, where xxx is the number. None of the individuals shown on these photographs were sequenced or dissected. Therefore, identification was done purely by facies to the best of our ability. While there is little doubt that the poses shown reflect how these species would look like in nature, there is some uncertainty about these identifications, not only due to misidentifying known species, but also in light of other cryptic species that possibly remain to be discovered. For example, many neotropical *Epargyreus* Hübner, [1819] are exceedingly similar in appearance and additional cryptic species are expected. For others, like *Hesperia* Fabricius, 1793 from central Texas, identifications by facies should be reliable.

The specimens were examined and sampled for sequencing in the following collections (abbreviations, which are not necessarily acronyms of the current names of these institutions, are given in parentheses and used in Table S1 of the supplemental file <https://osf.io//vkj6d/>): American Museum of Natural History, New York, NY, USA (AMNH), Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA (ANSP), Natural History Museum, London, UK (BMNH), Burke Museum of Natural History and Culture, Seattle, WA, USA (BMUW), California Academy of Sciences, San Francisco, CA

(CAS), Carnegie Museum of Natural History, Pittsburgh, PA, USA (CMNH), Colorado State University Collection, Fort Collins, CO, USA (CSUC), Field Museum of Natural History, Chicago, FL, USA (FMNH), Los Angeles County Museum of Natural History, Los Angeles, CA, USA (LACM), Museum für Naturkunde, Berlin, Germany (MFNB), McGuire Center for Lepidoptera and Biodiversity, Gainesville, FL, USA (MGCL), Muséum National d'Histoire Naturelle, Paris, France (MNHP), Museum für Tierkunde, Dresden, Germany (MTD), Texas A&M University Insect Collection, College Station, TX, USA (TAMU), Biodiversity Center, University of Texas at Austin, Austin, TX, USA (TMMC), Bohart Museum of Entomology, University of California, Davis, CA, USA (UCDC), National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), University of Texas Southwestern, freezers of the Grishin lab, Dallas, TX, USA (UTSW), Zoologische Staatssammlung München, Germany (ZSMC), and research collections of Ernst Brockmann, Germany (EBrockmann), Matthew J. W. Cock, UK (MJWCock), Bill Dempwolf, USA (WRDempwolf), Bernard Hermier, French Guiana (BHermier), Nick V. Grishin, USA (NVG), John Morrall (JMorrall), and John A. Shuey, USA (JAShuey). Type status abbreviations used are: HT holotype, LT lectotype, NT neotype, ST syntype, PT paratype, and PLT paralectotype.

Results and Discussion

Genomic analysis of Hesperiidae species across their ranges reveals 13 distinct unnamed phylogenetic lineages that are described below as species with type localities in the United States. These species are genotypically unique lineages separated from other similar lineages. They are characterized by genetic homogeneity across their range and genetic distinction from other similar lineages. Most of them are likely to be allopatric with their closest relatives, but the transition from one species to another in genotype is abrupt, without detected intermediates. Specific rationale with relevant statistics for each species distinction is given below in their "Definition and diagnosis." Most of the new species (with some notable exceptions) enter south Texas as the northernmost point of their distribution. The 13 new species are not immediately recognizable new butterflies. On the contrary, these butterflies have been observed before and have been included in the US faunistic lists, but under existing names. Using the evidence presented below, they are split from the species they have been placed with previously. Most commonly, their sister species were described from specimens collected in South America (e.g., Suriname), and the south Texas populations represent species distinct from those found in South America.

The 13 newly described cryptic species look exceedingly similar to their closest named relatives, and, due to phenotypic variation, some can be confidently identified only by DNA sequences (nuclear, and in most cases mitochondrial). Therefore, these species escaped recognition in the era dominated by visual inspection of wing patterns and genitalia shapes. Only with the advent of DNA sequencing (nuclear genomes in particular) we find the decisive evidence of their phylogenetic distinction, unique evolutionary history, and quantifiable differences from the named species. Therefore, they deserve to be recognized as unique genotypes, differentiated from previously known species at the level known for traditionally defined and less cryptic species. Although the center of distribution for most of these species may be somewhere in Mexico or Central America, we select the Lower

Rio Grande Valley in Texas, USA as their type locality. Being at the northern boundary of their range and thus farthest away from populations of their more southern sister species, specimens from these localities may represent the most genetically divergent or "pure" populations receiving less introgression from their southern counterparts.

Species descriptions below are accompanied by other nomenclatural acts (designation of a neotype and lectotypes) and taxonomic adjustments required to support the new species. Finally, we note that a species' description is the first (and not the ultimate) step in its studies, and we consider our work an invitation to take next steps in learning about their biology.

Lectotype designation for *Goniurus teleus* Hübner, 1821

The name *Goniurus teleus* Hübner, 1821 (type locality not given) was proposed (Hübner 1821) for the four illustrations of at least two specimens stated to be a male and female on plate [154] (the number penciled in later) misidentified as "*Urbanus fortis dorantes*" (Hübner [1808]). The whereabouts of these specimens remain unknown and are being investigated by us. While illustration number 1 (top of the page, dorsal image) agrees with what Evans (1952: 93) called "*Urbanus teleus*"—a species currently placed in the genus *Spicauda* Grishin, 2019 (Li et al. 2019), illustration number 2 (just below No. 1, also dorsal image) does not, and in our opinion could be a species known today as *Spicauda procne* (Plötz, 1881) (type locality in Brazil). This is because the forewing discal band in No. 2 is narrower, broken, missing a spot at the base of the cell M_3-CuA_1 (at least on the left forewing), and the spot in the cell CuA_2-1A+2A is offset distad and entirely disconnected from the band. Instead, illustration No. 1 shows a broader and continuous band from the costa to before the middle of the cell CuA_2-1A+2A, cut by brown veins: the spot at the base of the cell M_3-CuA_1 bulges outwards from the band, the spot in the cell CuA_2-1A+2A is situated at the distal end of the band and is about half the width of the spot in cell CuA_1-CuA_2; four apical spots. This phenotype is consistent with specimens from the Guianas and Brazil currently identified as *S. teleus*. Therefore, because of a polytypic type series, to stabilize nomenclature and the current application of the name, N.V.G. hereby designates the syntype with dorsal side illustrated as No. 1 (top image) on plate [154] from Hübner ([1808]), diagnostic characters listed above, as the **lectotype** of *Goniurus teleus* Hübner, 1821. It remains unknown if the lectotype is still extant.

Spicauda zalanthus (Plötz, 1880), reinstated status, is a species distinct from *Spicauda teleus* (Hübner, 1821)

Genomic sequencing and analysis of a syntype of *Goniurus zalanthus* Plötz, 1880 (type locality in Brazil: Rio Grande do Sul) NVG-15029E02 in MFNB places it in the clade with other specimens from Southeastern Brazil (Fig. 1 green, 3d, e) that is distinct from specimens we identify as *Spicauda teleus* (Hübner, 1821) (type locality not specified) (Fig. 1 blue). F_{st}/G_{min} statistics are 0.43/0.007 and their COI barcodes differ by 3.2% (21 bp). Therefore, we **reinstate** it as a species-level taxon *Spicauda zalanthus* (Plötz, 1880).

Spicauda atelis Grishin, new species

https://zoobank.org/7F4E9AFE-1998-487A-BD6F-1841F6CB11EB

(Fig. 1 part, 2, 3a, b, 4)

Definition and diagnosis.—In addition to species-level status of *S. zalanthus*, phylogenetic analysis of nuclear genome datasets of specimens identified as *Spicauda teleus* (e.g., Z chromosome genes, Fig. 1a) reveals their partitioning into 2 clades. One clade (Fig. 1 blue) consists of specimens from South America and Panama, including specimens from the Guianas and Brazil, a region with the likely type locality of *S. teleus*. The other clade (Fig. 1 red) is North American and does not have an available name. F_{st}/G_{min} statistics for comparison of these two clades (red and blue) are 0.32/0.003 suggesting that they correspond to species-level taxa. Curiously, neither of the two species is monophyletic in mitogenomes (Fig. 1b) and their COI barcodes differ by only 0.3–0.6% (2–4 bp). There are no positions in the barcode that consistently differentiate the two species in all specimens we sequenced, suggesting mitochondrial introgression. The new species, represented by the red clade, is sister to *S. teleus* and keys to it (C.13.13) in Evans (1952). It differs from *S. teleus* by a longer terminal spike in male genitalia and by a more robust and humped ampulla-costa, which is higher relative to the harpe and its spike than in *S. teleus*. Due to the cryptic nature of this species, most reliable identification is achieved by nuclear DNA comparison (not the COI barcodes!) and a combination of the following base pairs in the nuclear genome is diagnostic: aly5021.7.12:C2856T, aly1779.5.1:G723A, aly2085.2.4:T831A, aly536.8.1:T1305C, and aly536.8.1:C869G.

Barcode sequence of the holotype.—Sample NVG-14112D07, GenBank OP984702, 658 base pairs:

AACTTTATATTTTATTTTCGGAATTTGAGCAGGATTAGTTGGAACTTCATTAAGATT
ACTTATTCGAACTGAATTAGGAATACCAGGATCTTTAATTGGAGATGATCAAATTTA
TAATACTATTGTAACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTATTAT
AATTGGAGGATTTGGAAATTGATTAATTCCTTTAATATTAGGAGCTCCTGATATAGC
TTTCCCTCGTATAAATAATATAAGATTTTGATTATTACCCCCATCTTTAACCCTTTTA
ATTTCAAGAAGCATTGTTGAAAATGGAGCAGGAACTGGATGAACTGTATACCCCC
CTTTATCTTCTAATATTGCCCATCAAGGAGCATCAGTAGATTTAGCAATTTTTTCTTT
ACATCTTGCAGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACTATTATT
AATATACGAATTAATAATTTATCATTTGATCAAATACCTTTATTTATTTGAGCTGTTG
GAATTACAGCTTTATTATTATTACTTTCATTACCTGTTTTAGCAGGTGCTATTACTATA
TTATTAACTGATCGAAATTTAAATACCTCATTTTTTGATCCTGCTGGGGGAGGAGAT
CCTATTTTATACCAACACCTATTT

Type material.—Holotype: ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 2a, bears the following five rectangular labels, four white: [TEXAS: | Hɪᴅᴀʟɢᴏ Cᴏᴜɴᴛʏ | city of Mission | 10th Street at | irrigation ditch], [coll. | 11 Sep 1972 | Roy O. Kendall | & C. A. Kendall], [HESPERIIDAE, | Pyrginae: | Urbanus teleus | ♂ (Hubner, 1821) | det. R.O. Kendall | M. & B. No. 28], [DNA sample ID: | NVG-14112D07 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Spicauda atelis | Grishin]. **Paratypes:** 6♂♂ and 2♀♀: <u>USA</u> 1♀ NVG-14112D08 Texas, Hidalgo Co., McAllen, 18-Oct-1973, W. W. McGuire [TAMU], GenBank accession OP762098 (Fig. 2b); <u>Mexico</u>: 1♂ NVG-3280 San Luis Potosi, El Salto

Falls, 24-Dec-1972, Roy O. Kendall and C. A. Kendall leg., genitalia NVG150111-96, [TAMU] (Fig. 3b, 4); others in USNM, 1♂ NVG-19121B11 Hidalgo, 40 mi N of Jacala, 18-Aug-1967, Gary F. Hevel leg; 1♂ NVG-19121A06 Oaxaca, Candelaria Loxicha, 6-Jul-1974; Guatemala 1♂ NVG-19121A07 Peten, Finca Ixobel S of Poptun, 5-10-Jun-2003, R. Leuschner leg.; Honduras 1♀ NVG-19121A08 Las Minas, 30-Jul-1972, R. D. Lohman; El Salvador 1♂ NVG-19121A09 2 km N San Isidro, 22-Oct-1967, E. L. Todd; Costa Rica 1♂ NVG-17106B07, 07-SRNP-55853 Area de Conservación Guanacaste, Guanacaste Prov., Sector Mundo Nuevo, Mariano Pereira leg. ex larva, eclosed on 23-May-2007.

Type locality.—USA: Texas, Hidalgo Co., Mission, 10th Street at irrigation ditch.

Etymology.—The name of its sister species in Greek is τέλειος (téleios): perfect, complete, and this new one is incomplete: ατελής (atelís), because we do not completely know yet how to unambiguously identify this species by its phenotype. The name is a noun in apposition.

English name.—Atelis longtail.

Distribution.—From the Lower Rio Grande Valley in South Texas to Costa Rica.

Comment.—The 5th forewing apical spot is sometimes present in this species, e.g., in the paratype NVG-3280 (Fig. 3b), which is not *U. tanna* Evans, 1952 by genomics (Fig. 1) and genitalia (Fig. 4).

Urbanus ehakernae Burns, 2014 is a junior subjective synonym of *Urbanus* (*Urbanus*) *alva* Evans, 1952

Genomic analysis reveals that the type specimens of *Urbanus ehakernae* Burns, 2014 (type locality in Costa Rica) are intermixed in the same clade with specimens from Mexico (including a specimen from Veracruz) and Belize that we identified as *Urbanus alva* Evans, 1952 (type locality Mexico: Veracruz, Atoyac) (Fig. 5a purple). Their COI barcodes are 100% identical and all these specimens are phenotypically similar, including the holotype of *U. alva*. In particular, the iridescent overscaling is green (rather than blue), even partly yellowish, extensive on hindwing, reaching up to the distant third (females) or quarter (males), rather sharply transitioning to the dark brown submarginal area. This overscaling is less prominent on forewings, yellower in color, particularly distad, green scales confined to the base, but extensive olive-yellow scales approach the discal band composed of large (compared to other species) spots. The ventral hindwing discal band is only slightly forked towards the costal margin in males. Therefore, we propose to treat *Urbanus ehakernae* Burns, 2014, **new synonym**, as a junior subjective synonym of *Urbanus alva* Evans, 1952.

Urbanus (*Urbanus*) *rickardi* Grishin, new species

https://zoobank.org/68326A84-5C57-42DA-B975-8B33D2A99386

(Fig. 5 part, 6, 7)

Definition and diagnosis.—Sister to *Urbanus pronus* Evans, 1952 (type locality Ecuador: Ambato). In both nuclear and mitochondrial genome trees (Fig. 5 orange), separated from closely clustered South American *U. pronus* (Fig. 5 blue) specimens by a gap, and its COI barcodes differ from them by 2.1% (14 bp). Therefore, proposed as a species-level taxon. Phenotypically, similar to *U. pronus* and keys to it in Steinhauser (1981), and to C.13.5 in Evans (1952) (wrong genitalic sketch, see Steinhauser 1981: 22), but the overscaling at the wing bases and body above is greener, less extensive and yellower towards hindwing tail, disappearing more gradually towards it (Fig. 6); in male genitalia (Fig. 7), the caudal end of the valva is less round and longer, the uncus arms are farther apart especially near the base, the aedeagus is terminally narrower and more extended. Because the species is confidently known from one specimen, the extent of variation in these phenotypic characters has not been studied. Therefore, confidently identified by genotype: in DNA, a combination of the following base pairs is diagnostic in nuclear genome: aly320.19.2:G97T, aly5719.2.5:A132C, aly1188.10.5:C120G, aly100186.1.3:C54C(not T), aly5719.2.5:G144G(not C), and aly967.3.5:A84A (not G), and COI barcode: T106C, T193C, A208G, A253G, T263T(not C), A316G, T397T(not C), and A477G.

Barcode sequence of the holotype.—Sample NVG-14111G01, GenBank OP762099, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACTTCTTTAAGATTA
CTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGATGACCAAATTTA
TAATACTATTGTTACAGCTCACGCATTTATTATAATTTTTTTTATAGTTATACCTATTAT
AATTGGAGGATTTGGTAACTGACTAGTACCTTTGATAATAGGAGCCCCTGATATAGC
TTTCCCCCGTATAAATAACATGAGATTTTGATTATTACCCCCTTCTTTAACTTTATTA
ATTTCAAGAAGAATTATTGAAAATGGGGCTGGAACAGGATGAACAGTTTATCCCC
CTCTTTCATCTAATATTGCTCATCAAGGAGCCTCTGTTGATTTAGCAATTTTTTCCC
TTCATCTTGCTGGAATTTCATCAATTCTTGGAGCTATTAATTTTATTACAACAATTAT
TAATATACGAATTAATAGATTATCTTTTGATCAAATACCATTATTTGTATGAGCTGTA
GGAATTACAGCATTATTATTATTACTTTCTTTACCTGTTTTAGCTGGAGCTATTACTAT
ATTATTAACTGATCGAAATTTAAATACTTCATTTTTTGACCCTGCTGGAGGAGGGG
ATCCAATTTTATATCAACATTTATTT

Type material.—**Holotype:** ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 6, 7, bears the following six rectangular labels, five white: [TEXAS: | HIDALGO COUNTY | Tex Hwy 1016 S of | Mission nr Madero | TEXAS:], [coll. | 19 Oct 1971 | Michael A. Rickard], [Urbanus | pronus ♂ det | James Scott], [HESPERIIDAE, | Pyrginae: | Urbanus pronus | Evans, 1952 | ♂ det. R.O. Kendall | M. & B. No. 25], [DNA sample ID: | NVG-14111G01 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Urbanus | rickardi Grishin].

Type locality.—USA: Texas, Hidalgo Co., Urban Road No. 1019 S of Mission, near Madero.

Etymology.—The name honors Michael A. Rickard, the collector of the holotype, and contributor of many remarkable butterfly records from the Lower Rio Grande Valley in

Texas (McGuire and Rickard 1976; Rickard 2010; Rickard and Grishin 2010) among his many other contributions to our knowledge of Texas butterflies. The name is a noun in the genitive case.

English name.—Rickard's longtail.

Distribution.—Currently known only from the holotype from the Lower Rio Grande Valley in South Texas as a possible stray from Mexico.

Urbanus (*Urbanus*) *oplerorum* Grishin, new species

https://zoobank.org/D236B083-93CB-4975-ACA5-F42BF02C1C62

(Fig. 5 part, 8)

Definition and diagnosis.—Previously identified and reported as *Urbanus viterboana* (Ehrmann, 1907) (type locality in Colombia) (Bordelon 2011) and keys to it in Steinhauser (1981), and to C.13.2(b) in Evans (1952). However, genomic sequencing of the holotype of *U. viterboana* and phylogenetic analysis reveals that it (Fig. 5 red) is not conspecific with *U. viterboana* (Fig. 5 green) and instead is sister to *Urbanus bernikerni* Burns, 2014 (type locality in Costa Rica) (Fig. 5 cyan). Differs from *U. bernikerni* by 3.3% (22 bp) in the COI barcode, and from *Urbanus viterboana* (Ehrmann, 1907) by 5% (33 bp), forms a separate distinct clade in genomic trees and therefore is a species-level taxon. In facies (Fig. 8), differs from *U. viterboana* in having larger, rectangular, and hourglass-shaped (rather than rounder and some being nearly triangular) hyaline forewing spots; from *U. bernikerni* by more brilliant-blue (rather than greenish) dorsal overscaling and yellower hyaline spots; and from *Urbanus segnestami* Burns, 2014 (type locality in Costa Rica) by less extensive, and bluer, overscaling that does not extend past the basal half of hindwing. Due to individual variation and similarity between these semi-cryptic species, confident identification is achieved by genotype. In DNA, a combination of the following base pairs is diagnostic in nuclear genome: aly216.58.7:G39A, aly669.9.1:A231G, aly770.19.8:G63A, aly1405.5.10:G81A, and aly216.58.7:G42T, and COI barcode: T64C, A202T(not C), T277T(not A), T283C, T287C, T409T(not C), and T514A(not C).

Barcode sequence of the holotype.—Sample NVG-14112E06, GenBank OP762100, 658 base pairs:

AACCTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACTTCTTTAAGATTA
CTTATCCGAACAGAATTAGGAACCCCAGGATCTTTAATTGGAGATGACCAAATTTA
TAATACTATTGTAACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTAT
AATTGGAGGATTTGGTAATTGATTAGTTCCTTTAATAATAGGTGCTCCTGATATAGC
TTTCCCCCGTATAAATAATATAAGATTTTGATTATTACCCCCCTCTTTAACCTTACTA
ATTTCAAGAAGAATTGTTGAAAATGGTGCTGGTACTGGATGAACAGTTTACCCCCC
CTCTTTCATCTAATATTGCCCATCAAGGAGCCTCAGTTGATCTAGCAATTTTTTCCC
TTCATCTTGCTGGAATTTCATCTATTCTTGGAGCTATTAATTTTATTACAACAATTAT
TAATATACGAATTAATAAATTAACTTTTGATCAAATACCTTTATTTGTATGAGCAGTA
GGAATTACAGCATTATTATTATTACTTTCTTTACCTGTTTTAGCTGGAGCTATTACCA

TATTATTAACTGATCGAAATTTAAATACATCATTTTTTGATCCTGCTGGAGGAGGAG
ATCCAATTTTATATCAACATTTATTT

Type material.—Holotype: ♀ deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), illustrated in Fig. 8a, bears the following three rectangular labels, two white: [TX: Hidalgo Co., | Mission/Madero | 4-VI-10 | Mike Rickard], [DNA sample ID: | NVG-14112E06 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Urbanus | oplerorum Grishin]. **Paratype:** 1♀ Mexico: Tamaulipas, Gomez Farias, leg. Paul A. Opler and Evi M. Buckner-Opler 13-14-Oct-2003, NVG-17067E10, CSU_ENT1038946, in CSUC.

Type locality.—USA: Texas, Hidalgo Co., Mission/Madero.

Etymology.—The name honors Paul A. Opler and Evi M. Buckner-Opler who collected the paratype on one of their many expeditions that contributed most significantly to our knowledge of Lepidoptera. Paul's lifelong contributions to Lepidopterology cannot be overstated, being immensely broad and far-reaching: in science, education, conservation, and dissemination of knowledge through best-selling books. During the last several years, Paul and Evi have been collecting and preserving butterflies for genomic analysis, and their excellent material forms the foundation for the genomic studies of western USA species. The name is a noun in the genitive case.

English name.—The Oplers' longtail.

Distribution.—Presently known from South Texas and Mexico: Tamaulipas.

Neotype designation for *Papilio fulminator* Sepp, [1841]

Having designated the neotype of *Papilio fulgerator* Walch, 1775 (type locality in Suriname) recently (Zhang et al. 2022a), shown here for comparison (Fig. 9a, b), we proceed here with analyses of related taxa. *Papilio fulminator* Sepp, [1841] was described from an unstated number of specimens (Sepp [1841]). From the original description and illustrations, we regard that *P. fulminator* is differentiated from other taxa by the following characters: extensive shiny overscaling on the dorsal side of thorax, abdomen, and wing bases is greenish rather than blue, extending nearly up to the discal hyaline forewing band; this greenish overscaling is also extensive on ventral forewing near costa, nearing the discal band; forewing with four (not three or five) subapical spots; the spot in the forewing cell M_3-CuA_1 is offset distad from the discal hyaline band but still just adjoins it; hindwing with two darker bands on a paler ground color and a darker outer margin, fringes somewhat checkered.

We searched for *P. fulminator* syntypes in the collections that contain many historical specimens and inspected their Hesperiidae holdings looking for old specimens that match the information we gathered about this species: Natural History Museum, London, UK (BMNH), Museum für Naturkunde, Berlin, Germany (MFNB), Muséum National d'Histoire Naturelle, Paris, France (MNHP), Naturalis Biodiversity Center, Leiden, Netherlands (RMNH), and Zoologische Staatssammlung München, Germany (ZSMC). None were found,

and we therefore believe they were lost, together with most other type material of Sepp names. Hence, we proceeded with the neotype designation, because there is an exceptional need to define *P. fulminator* objectively by a single specimen due to cryptic species in the *T. fulgerator* complex (Hebert et al. 2004; Brower 2010; Zhang et al. 2022a). While the identity of *Telegonus fulgerator* (Walch, 1775) is defined by its neotype (Zhang et al. 2022a), the identity of *P. fulminator* remains unclear and DNA information from its neotype specimen is critical for future studies of the complex, because its cryptic diversity has been revealed by DNA analysis. We looked for candidate neotype specimens in several collections across the world to find one that fits best what we know about *P. fulminator*. After these investigations, N.V.G. designates a specimen shown in Fig. 9c as the **neotype** of *Papilio fulminator* Sepp, [1841].

Our neotype of *P. fulminator* satisfies all requirements set forth by the ICZN Article 75.3, namely: 75.3.1. It is designated to clarify the taxonomic status of *Papilio fulminator* Sepp, [1841], which has been in question due to many cryptic species closely related to *T. fulgerator*; 75.3.2. The characters for the taxon are listed above and include, in particular, broadly greenish wing bases on the dorsal side and two dark bands on ventral hindwing; 75.3.3. The neotype specimen is a female bearing the following two labels, one green [Surinam | V. – IX | Fruhstorfer] and the other white [DNA sample ID: | NVG-18057D08 | c/o Nick V. Grishin]; 75.3.4. Our search for syntypes, described above, was unsuccessful, and this is the reason we believe that they were lost; 75.3.5. The neotype is consistent with the original drawings as evidenced by the presence of described characters in the specimen shown in Fig. 9c, except that these characters are somewhat exaggerated in the original illustration (Sepp [1841]), and in the neotype, the greenish overscaling is less extensive, contrast between the darker ventral hindwing bands and paler ground color is less pronounced, in particular, darker submarginal area transitions gradually to paler postdiscal area, and fringes are less distinctly checkered; 75.3.6. The neotype is from Suriname, the type locality specified in the original description; 75.3.7. The neotype is in the Zoological State Collection, Munich, Germany (Zoologische Staatssammlung München, ZSMC). The COI barcode sequence of the neotype, sample NVG-18057D08, GenBank OP762102, 658 base pairs is:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACTTCACTAAGATT
ACTTATTCGAACTGAATTGGGAACTCCAGGATCTTTAATTGGAGATGACCAAATTT
ATAATACAATTGTTACAGCTCACGCATTTATTATAATTTTTTTTATAGTTATACCTATT
ATAATTGGAGGATTCGGAAATTGACTAGTCCCATTAATAATAGGTGCCCCAGATATA
GCTTTCCCCCGTATAAATAATATAAGATTTTGATTATTGCCCCCATCTTTAACTTTAT
TAATTTCAAGAAGAATTGTTGAAAATGGGGCTGGTACAGGATGAACAGTTTATCCC
CCTCTTTCATCCAACATTGCCCATCAAGGAGCTTCTGTTGATTTAGCAATTTTTTCT
CTTCATCTTGCCGGTATTTCATCAATTCTTGGAGCTATTAATTTTATTACAACAATTA
TTAATATACGAATTAATAATTTATCTTTTGATCAAATACCATTATTTGTTTGAGCTGT
AGGAATTACAGCATTATTATTATTACTTTCATTACCTGTCTTAGCAGGTGCTATCACT
ATATTACTAACAGACCGAAATTTAAATACTTCTTTTTTTGATCCTGCAGGTGGAGGA
GATCCAATTTTATATCAACATTTATTT

Searching the BOLD database (Ratnasingham and Hebert 2007) with this barcode, we found a single closest barcode (differing by just 1 bp) that belongs to a specimen from French Guiana (sample ID IngaHerbiv_1224, specimen record https://boldsystems.org/index.php/Public_RecordView?processid=JANIH1224-13), which is a caterpillar that looks rather similar to the original illustration of *P. fulminator* caterpillar (Sepp [1841]) in having narrow yellow bands across darker segments. However, judging by its head pattern, the original illustration shows the 4th or 5th instar, and the BOLD caterpillar is an earlier instar that might change color patterns with further development.

Telegonus fulminator (Sepp, [1841]) is a species distinct from *Telegonus fulgerator* (Walch, 1775)

Papilio fulminator Sepp, [1841] was placed in synonymy with *Telegonus fulgerator* (Walch, 1775) by Godman and Salvin (1893) and kept at this status since. Genomic sequencing of the neotypes of both taxa, both from Suriname, and their analysis together with other specimens of *Telegonus* reveal that they are not monophyletic in the nuclear genome tree (Fig. 10a purple and orange) and distinct at the level typical of other related species. They differ phenotypically in that *P. fulminator* hindwing is with a pair of more clearly defined darker bands and smaller white basal area near costa beneath, more extensive greenish overscaling above, and more conspicuously checkered fringes (Fig. 9). Therefore, we **reinstate** *Telegonus fulminator* (Sepp, [1841]) as a species-level taxon. However, COI barcodes of the neotypes of these two sympatric species differ by only 0.5% (3 bp) indicating that barcodes do not always diverge strongly between some *Telegonus* species, which is consistent with previous findings (these species were previously placed in *Astraptes* Hübner, [1819]) (Hebert et al. 2004).

Lectotype designation for *Goniloba azul* Reakirt, [1867]

Goniloba azul Reakirt, [1867] was described from an unstated number of specimens (Reakirt [1867]). From the original description, we regard that *G. azul* is differentiated from other taxa by the following characters: shiny overscaling over the dorsal side of thorax, abdomen and wing bases is brilliant-blue rather than greenish, reaches basal third of both wings on the dorsal side; forewing with five (not three or four) subapical spots; discal forewing hyaline band consists of six spots; ventral hindwing from its base to the middle with a broad white area by the costal margin.

The original description credits type specimen(s) that came from "Mexico, near Vera Cruz" to William H. Edwards. We searched for these types in the collections known to have W. H. Edwards specimens: the Field Museum of Natural History, Chicago, IL, USA (FMNH), the Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA (ANSP), and the Carnegie Museum of Natural History, Pittsburgh, PA, USA (CMNH). The search was not limited to specimens labeled as types or to curated drawers. Every specimen in the Hesperiidae cabinets was checked. The original description mentioned three rather uncommon characters: "shining blue" overscaling, we assume of a very high brilliance, five spots at the forewing apex, and large size: wingspan of 2.5 inches. Looking for an old specimen that has all these characters, we came up with only one option, a specimen in CMNH, female, shown in Fig. 9d. The specimen had a wingspan of 2.48 inches, was

on an old pin characteristic of Edwards specimens and had a single label [Exch. A.N.S.P, | C.M.Acc.20359]. We sampled a leg for sequencing, adding a label [DNA sample ID: | NVG-15099B05 | c/o Nick V. Grishin].

The genomic sequencing results unambiguously placed the specimen with similar-looking specimens characterized by particularly brilliant-blue overscaling, from Mexico: Veracruz, the type locality of *G. azul* (Fig. 10). Also, sequencing reads were short (30–70 bp and not 150 bp) due to DNA degradation and generally poor quality, implying that the specimen is more than a century old. The specimen matched the original description almost perfectly. The only inconsistency with the original description is the basal area of wings: on the forewing, "a yellow spot at their base" is barely noticeable, and yellow scales are largely removed on the right forewing, and hindwing is missing "a small brown spot at the shoulder" of hindwing. It is entirely rubbed off on the right, and only a few brown scales remain among the white and yellowish scales on the left. This last character casts doubt on the syntype status of this specimen, because of its present condition it is difficult to imagine that anyone would describe these few scales as a "brown spot." Therefore, either this specimen is not the one described by Reakirt, or the scales were lost from the bases of wings on the ventral side, especially on the right wings. Such loss might have occurred during spreading (the species could have been described from an unspread specimen), or later, for instance, due to damage by psocids.

It seems highly unlikely to find a historical specimen (judging from its pin and short sequencing reads due to DNA damage) that nearly perfectly matches the original description, is deduced (per genomic comparison) to come from the purported type locality, and is in the collection that houses the largest number of W. H. Edwards specimens, unless this specimen is a syntype of *G. azul*. Therefore, we regard that we discovered a syntype of *G. azul*. To ensure unambiguous identification of this species, N.V.G. hereby designates the syntype in CMNH shown in Fig. 9d as the **lectotype** of *Goniloba azul* Reakirt, [1867]. The lectotype is missing both antennae and has damaged wings, in particular, several segments are chipped of its left hindwing. The COI barcode sequence of the lectotype, sample NVG-15099B05, GenBank OP762103, 658 base pairs is:

AACTTTATATTTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACTTCATTAAGATTA
CTTATTCGAACTGAATTAGGAACTCCAGGATCTTTAATTGGAGATGATCAAATTTAT
AATACAATTGTTACAGCTCACGCATTTATTATAATTTTTTTTATAGTTATACCTATTAT
AATTGGAGGATTCGGAAATTGATTAGTCCCATTAATAATAGGTGCCCCAGATATAGC
TTTCCCCCGTATAAATAATATAAGATTTTGATTATTACCTCCATCTTTAACTTTATTAA
TTTCAAGAAGAATCGTTGAAAATGGGGCTGGTACAGGATGAACAGTTTATCCCCC
TCTTTCATCCAACATTGCCCATCAAGGAGCTTCTGTTGATTTAGCAATTTTTTCTCT
TCATCTTGCCGGTATTTCATCAATTCTTGGAGCTATTAATTTTATTACAACAATTATT
AATATACGAATTAATAATTTATCTTTTGATCAAATACCATTATTTGTTTGAGCTGTAG
GAATTACAGCATTATTATTATTACTTTCATTACCTGTCTTAGCAGGTGCTATTACTATA
TTATTAACAGACCGAAATTTAAATACTTCTTTTTTTGATCCTGCAGGAGGAGGAGA
TCCAATTTTATATCAACATTTATTT

Genomic sequencing of the lectotype and placing it in the phylogenetic context of its relatives (Fig. 10) suggests that it may be conspecific with the species dubbed INGCUP in

Hebert et al. (2004), but additional research is needed to investigate this relationship further. *Telegonus azul* has been regarded as a species-level taxon by Li et al. (Li et al. 2019), and not a subspecies of *T. fulgerator*, a status that we confirm here (Fig. 10 green vs. purple).

Lectotype designation for *Eudamus misitra* Plötz, 1881

Two syntypes of *Eudamus misitra* Plötz, 1881 (type locality in Mexico), numbers 4.983, 4.984 in MFNB as mentioned in the original description (Plötz 1881), belong to different species according to genomic sequencing results (Fig. 10). The male syntype is already labeled as the lectotype, although the designation was not published. To ensure unambiguous identification of *E. misitra*, N.V.G. hereby designates its male syntype in MFNB bearing the following seven rectangular labels, the first two red, the fifth green, and others white: [Lectotypus], [Type], [4983], [misitra | Pl. typ.!], [Mexico Deppe], [{QR code} http:// coll.mfn-berlin.de/u/ | e1f9cb], and [DNA sample ID: | NVG-15031B02 | c/o Nick V. Grishin] as the **lectotype** of *Eudamus misitra* Plötz, 1881. The second type specimen, a female, not conspecific with the lectotype, is nevertheless a paralectotype. The COI barcode sequence of the lectotype, sample NVG-15031B02, GenBank OP762104, 658 base pairs is: AACCCTATATTTTATTTTTGGAATTTGAGCAGGACTAATTGGAACTTCCTTAAGATT ACTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGATGATCAAATTT ATAATACAATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTA TAATTGGAGGATTTGGAAATTGACTAGTTCCATTAATAATAGGTGCCCCAGACATA GCTTTTCCCCGTATAAATAACATAAGATTTTGATTATTACCTCCATCTTTAACTTTAT TAATTTCAAGAAGAATTGTTGAAAACGGAGCTGGCACAGGATGAACAGTTTATCC CCCTCTTTCATCAAATATTGCCCATCAAGGAGCATCTGTTGATTTAGCAATTTTTTC CCTTCATCTTGCTGGTATTTCATCAATTCTTGGAGCTATTAATTTTATCACAACAATT ATTAATATACGAATTAATAACTTATCTTTTGATCAAATACCATTATTTGTTTGAGCTG TGGGAATTACAGCATTATTATTATTACTTTCATTACCCGTTTTAGCAGGTGCTATTAC TATATTATTAACAGATCGAAATTTAAATACTTCTTTTTTTGATCCTGCAGGAGGAGG AGATCCAATCTTATACCAACACTTATTT

Because Ferdinand Deppe collected in Mexico in 1824–1829 (Stresemann 1954), the lectotype was likely collected during this time period.

Telegonus misitra (Plötz, 1881) is a species distinct from *Telegonus azul* (Reakirt, [1867])

Genomic sequencing of the lectotype of *Eudamus misitra* Plötz, 1881 (type locality in Mexico) and placing it in the phylogenetic context of its relatives reveal that its clade (Fig. 10 blue) is not monophyletic with *Telegonus azul* (Reakirt, [1867]) (type locality in Mexico: Veracruz) (Fig. 10 green) and instead is far removed from it. The COI barcodes of the two lectotypes differ by 2.1% (14 bp). Therefore, we **reinstate** *Telegonus misitra* (Plötz, 1881) as a species distinct from *T. azul*. Furthermore, phylogenetic trees (Fig. 10) suggest that the species dubbed SENNOV in Hebert et al. (2004) is *T. misitra*.

Telegonus tsongae Grishin, new species

https://zoobank.org/926DF925-40ED-45AF-A379-451262C255D6

(Fig. 10 part, 11–20)

Definition and diagnosis.—Genomic comparison of *Telegonus fulgerator* group species reveals that specimens from the US, together with Mexican specimens, form a distinct clade that cannot be associated with any available name and therefore represents a new taxon (Fig. 10 red). It is closest to *T. misitra* (Plötz, 1881) (type locality in Mexico) (Fig. 10 blue) and the two taxa are sisters in the mitochondrial genome tree, but their COI barcodes differ by 2.6% (17 bp) and F_{st}/G_{min} statistics for them are 0.53/0.003 suggesting strong genetic differentiation. The caterpillar patterns (Fig. 16–20) and foodplants (Fig. 14), both used as characters to differentiate a complex of sympatric species (Hebert et al. 2004), differ as well. Therefore, the red clade is a new species. Distinguished from its relatives by a combination of blue (not greenish) wing bases and body above, usually 4 nearly equal in size forewing hyaline spots in a row, straight discal band of hyaline spots, narrower than in many other species, spot at the base of cell M_3-CuA_1 typically small, within the band, spot in cell CuA_1-1A+2A also small or missing, aligned with the band or a little offset distad; the body beneath is pale-yellow (specimens reared in the lab) to orange-yellow (most adults collected in the field), the white segment at the base of hindwing near costa barely reaches the middle of costal margin from the base. Caterpillar with pattern distinct from other *Telegonus* species (Hebert et al. 2004): each segment is dorsally black with a yellow crossband in distal half, the band narrowing laterally and in the middle, where it may be separated into two spots; there are two white streaks behind each segment of the band, lower half of the body and the head reddish. The yellow bands are deeper in color, different from pale-yellow bands in *T. misitra*, and are broader than narrow and typically uniform in width bands of *T. misitra*. The caterpillar feeds on the leaves of *Karwinskia* Zucc. (Rhamnaceae), also recorded as a rarely used (in addition to mostly various Fabaceae Lindl.) foodplant of *T. misitra* (i.e., SENNOV) (Hebert et al. 2004). Due to known and potentially undiscovered variation in the phenotypic characters, including caterpillar patterns, the new species can be reliably diagnosed by DNA, and a combination of the following base pairs is diagnostic in nuclear genome: aly173.14.1:C1732T, aly1080.27.6:C1648G, aly173.14.1:T1902C, aly383.21.1:C4023T, and aly1080.27.6:A1301T, and COI barcode: A229G, T283C, T292C, T578C, and T596C.

Barcode sequence of the holotype.—Sample NVG-3614, GenBank OP762105, 658 base pairs:

AACCTTATATTTTATTTTTGGAATTTGAGCAGGACTAATTGGAACTTCCTTAAGATT
ACTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGATGATCAAATTT
ATAATACAATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTA
TAATTGGAGGATTTGGAAATTGACTAGTTCCATTAATAATAGGTGCCCCAGATATGG
CTTTCCCCCGTATAAATAACATAAGATTTTGATTATTGCCCCCATCTTTAACCTTATT
AATCTCAAGAAGAATTGTTGAAAATGGAGCTGGTACAGGATGAACAGTTTATCCC
CCTCTTTCATCAAATATTGCCCATCAAGGAGCATCTGTTGATTTAGCAATTTTTTCC
CTTCATCTTGCTGGTATTTCATCAATTCTTGGAGCTATTAATTTTATTACAACAATTA
TTAATATACGAATTAATAATTTATCTTTTGATCAAATACCTTTATTTGTTTGAGCTGT
AGGAATTACAGCATTATTATTATTACTTTCATTACCTGTTTTAGCAGGTGCTATTACT
ATACTATTAACAGATCGAAATCTAAATACTTCTTTTTTTGATCCTGCAGGAGGAGGA
GATCCAATCTTATACCAACACTTATTT

Type material.—**Holotype:** ♂ deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), illustrated in Fig. 11, bears the following three rectangular labels, two white: [USA: TEXAS: Starr Co., | Roma, near International | Bridge, 26.40472, −99.01859 | 14-Jun-2015, leg. Qian Cong], [DNA sample ID: | NVG-3614 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Telegonus | tsongae Grishin]. **Paratypes:** 30♂♂ and 21♀ U̲S̲A̲: Texas: 1♂ 11-BOA-15610C09 Val Verde Co., Devil's River State Natural Area, 3rd side canyon off Dolan Creek, GPS 29.9120, −100.9841, 17-Apr-2004, Tony Gallucci leg. [TMMC]; 1♂ 11-BOA-15610C10 Travis Co., Austin, Upper Stillhouse Hollow (Wild Park), 2-May-1985, C. J. Durden leg. [TMMC]; 2♂♂ NVG-3422 (Fig. 13), NVG-3427, 1♀ NVG-3426 (latter 2 in copula) Cameron Co., 2.5 mi SW of Sebastian, 30-May-2015, N. V. Grishin leg.; Starr Co., Roma, by the international bridge, GPS around 26.4048, −99.0185, 31-May-2015: Denise Tan leg. 1♂ NVG-3456; N. V. Grishin leg.: 6♂♂ NVG-3453,–3455, –3457, –3458, –3459, –3460 (Fig. 12e–m); 1♂ NVG-3613 14-Jun-2015; ex larvae, eclosed in 2015: 4♂♂ NVG-4191 18-Jul, NVG-4374 2-Aug, NVG-4372 3-Aug, NVG-4568 24-Aug and 6♀♀ NVG-4206 18-Jul, NVG-4373 2-Aug, NVG-4391 6-Aug, NVG-4392 7-Aug, NVG-4471 13-Aug, NVG-4518 16-Aug; Roma, GPS 26.4148, −99.0224, 28-Jun-2015, N. V. Grishin leg.: 2♂♂ NVG-3831, –3834 and 1♀ NVG-3833; Roma Creek, GPS 26.4222, −99.0307, ex larvae, N. V. Grishin leg., eclosed: 1♂ NVG-511 3-Jan-2008, 1♂ 6-Jan-2008; 1♀ 20-Jan-2008; Falcon Heights, ex ♀ M. Reid leg., ex ova, eclosed: 8♂♂: 8-, NVG-514 11-, 15-, 16-, 17-, 17-, 21-, 23-Jan-2008 and 11♀♀: NVG-512 9-, NVG-513 11-, 13-, 15-, 16-, 18-, 20-, NVG-517 21-, 24-, 26-, 27-Jan-2008; M̲e̲x̲i̲c̲o̲: Nuevo Leon: 1♂ 11-BOA-15610D01 Santiago, Cola de Caballo, GPS 25.36854, −100.1571, 19-Aug-1977, C. J. Durden leg. [TMMC]; 1♂ NVG-17113A11 60 mi SE Monterrey, Cumbres de Monterrey National Park, 13-Oct-1980, J. Robb leg. [TMMC]; 1♀ NVG-15610C12 San Luis Potosi, Maiz, El Salto, 28-Jul-1981, C. J. Durden leg. [TMMC].

Type locality.—USA: Texas, Starr Co., Roma, by the international bridge, GPS 26.4047, −99.0186.

Etymology.—The name honors the collector of the holotype, Qian Cong (pronounced as Tsien Tsong, hence the spelling of the species name), who pioneered genomic sequencing in our lab and developed unique protocols to obtain whole genome shotgun datasets of historical specimens in collections regardless of their age. Without her contributions, this and all other genomics studies by our group would have been simply impossible. The name is a noun in the genitive case.

English name.—Qian's flasher, pronounced as Tsien's flasher.

Distribution.—Southern Texas and Mexico, currently confirmed in the states of Nuevo Leon and San Luis Potosí.

Caterpillar foodplant and habitat.—In Texas, we are aware of a single caterpillar foodplant, which is Coyotillo: *Karwinskia humboldtiana* (Schult.) Zucc. (Rhamnaceae) (Fig. 14a, b, d). Plants growing in the open are not widely utilized (Fig. 14a) and plants in the shaded areas, frequently on the slopes and near water (Fig. 14c), are selected for oviposition.

Life history and rearing.—Eggs are glued singly or in small numbers to the underside of fully opened leaves of *K. humboldtiana* growing in the shade (Fig. 16b, d), both on small plants near the ground, or on older plants above 2 meters. Caterpillars go through five instars and live in shelters they build from leaves (Fig. 16, 19j), in which they pupate. We have found all caterpillar instars and pupal exuviae in nature, but to document the life history, we reared this species from oviposition by females in captivity.

Coyotillo branches with cut ends wrapped into moist paper towel and covered with foil to prevent evaporation were secured with scotch tape near the bottom of a plastic transparent container in a manner to create enough space for the female to move around but block it from freely reaching the bottom. When this container is turned over (Fig. 15a), the female typically ends up sitting on the leaves after each brief flight. Placed under a lamp to emulate bright day conditions and provide heat, the female failed to oviposit after several days. However, many eggs were obtained (Fig. 15b) when the container with the female was moved for an hour (checked every 15 min to prevent overheating) into a car parked outside, partly in the shade provided by trees. The temperature inside the car nevertheless reached 110°F and possibly beyond. This procedure was repeated daily for a week, and the female fed every other day with a diluted sugar (sucrose) solution absorbed by a piece of paper towel (Fig. 15c). The proboscis needed to be uncoiled by an insect pin (female held by wings) and dipped into the moist towel to initiate feeding. After this forced initiation, a grip on the female wings was released and she continued to feed for several minutes.

Eggs are white, dome-shaped, nearly spherical (Fig. 17a–d), develop 5–7 days, and turn orange-brown (caterpillar head and body showing through the transparent shell) before hatching (Fig. 17e). The hatched caterpillar is yellow with brown-orange head and black collar (Fig. 17g, h); it crawls to a leaf edge and starts feeding. Caterpillar becomes greenish-yellow upon feeding (Fig. 17i, j) and starts building a shelter (Fig. 17k). Two narrow cuts are chewed out from the leaf with a leaf segment between them several times wider than a caterpillar. Then, a silk pad is made at the base of this segment between the two cuts (Fig. 16h). As silk threads contract with time, they folds the leaf segment (with the caterpillar on it) over and place it on top of the rest of the leaf (Fig. 16e, f, i). When the tip of the segment comes close to the leaf surface, the caterpillar secures it to the leaf with silk (Fig. 16j, arrow 2). All shelters we observed were folded over the upper surface of the leaf, with lower surface of the leaf segment exposed.

The second-instar caterpillar is darker, olive-green, with yellow-green dots in rows (Fig. 17q–z). By the next molt, a central yellow crossband on each segment develops in addition to the dots, and this band is clearly defined in the third instar (Fig. 18a–d). Besides the yellow band, the growing third instar gains a narrow white crossband on each segment and loses yellow-green dots. The ventral side and the distal part of the last abdominal segment become red. The fourth instar is black above, red beneath, and each segment with yellow and white bands, mostly narrower or broken in the middle (Fig. 18e–h). Head brown-red, covered with setae. The fifth and final instar is similar, but setae on the head more extensive and longer, and the head is larger comparatively to the body, which is covered in sparse white very fine setae (Fig. 119a–i). Head darker in the middle and with 3 paler longitudinal stripes on each side (Fig. 20a).

Out of several dozen caterpillars, both wild-collected and reared from eggs, only this color morph was observed, without variation, although the width of yellow bands in the last two instars varied somewhat (Fig. 18e–h, 19a–i). Male caterpillars can be distinguished from females by testes seen through the skin of the 8th segment (Fig. 20b). The testes appear as a doublet of orange-red oval bodies in the middle of the segment. In females, the middle area of the 8th segment around the yellow band is black (Fig. 20c).

The prepupal caterpillar builds a roomy shelter for pupation, with extra space needed for the wing cases to expand significantly in the pupa for it to form properly. A Y-shaped girdle supports the prepupa resting on it and hooked by the last pair of legs to the silk pad (Fig. 19j). It appears that the lack of contact with the leaf in a prepupa suspended this way is important for pupation and proper expansion of wing cases, because damage to the girdle sometimes resulted in asymmetrical pupae with reduced wing cases.

The freshly formed pupa turns brown with orange dorsal stripes (Fig. 19k) that partly fade within a day and the pupa develops white bloom covering its entire surface except around the spiracles and some joints (Fig. 19l). This bloom is a very fine powder that can be rubbed off the pupa upon the slightest touch. Removal of the bloom, typical of many Eudaminae pupae, did not affect pupal development in captivity. Pupae eclosed in 15–17 days.

Autochton reducta (Mabille and Boullet, 1919), new status, is a species distinct from *Autochton potrillo* (Lucas, 1857)

The genome-level phylogeny of *Autochton potrillo* (Lucas, 1857) (type locality in Cuba) reveals that sequenced specimens of *Cabares potrillo* var. *reducta* Mabille and Boullet, 1919 (type locality in Venezuela), including the holotype (NVG-18086C03), form a distinct and prominent clade (Fig. 21 green). F_{st}/G_{min} statistics for these two clades are 0.40/0.006, however, their COI barcodes do not differ much, 0.6% (4 bp). Due to genetic differentiation in nuclear genome comparable to that of species-level taxa we propose to treat it as a species *Autochton reducta* (Mabille and Boullet, 1919), **new status**. Phenotypically, as the name suggests, this species is characterized by reduced hyaline spots (Fig. 23e), and the forewing discal cell spot that is more saddle-shaped or horseshoe-like in the namesake *A. potrillo* is frequently straight or reduced to one or two small spots.

Autochton caballo Grishin, new species

https://zoobank.org/A49A7A6F-99C4-4405-8A50-E689FEAE9538

(Fig. 21 part, 22, 23a, b)

Definition and diagnosis.—The Z chromosome tree reveals that in addition to *Autochton reducta* (Mabille and Boullet, 1919) (type locality in Venezuela) that we regard as a species (Fig. 21 green), a possible sister to *Autochton potrillo* (Lucas, 1857) (type locality in Cuba) (Fig. 21 blue), continental populations identified as *A. potrillo* form a clade of their own (Fig. 21 red) thus constitute a distinct taxon. F_{st}/G_{min} statistics for comparing the red and the blue clades are 0.31/0.009, but their COI barcodes do not show distinction: 0.3% (2 bp), even less so than those of *A. reducta*. Due to nuclear genome differentiation, the red clade represents a species distinct from *A. potrillo*, and differs from

it by having larger hyaline spots on forewing (Fig. 22, 23a, b), the discal cell spot is more saddle- (or horseshoe-) shaped, not broken into two spots as in many insular *A. potrillo* specimens (Fig. 23c, d) or much reduced as in *A. reducta* (Fig. 23e), both upper and lower shoulders of the spot are better developed; yellow overscaling is less extensive than in both *A. potrillo* and *A. reducta*, and the wings have less pebbly appearance as a result. Due to phenotypic variation, reliable identification is achieved by DNA characters: a combination of the following base pairs is diagnostic in the nuclear genome: aly16576.3.5:C27T, aly291.18.6:C69T, aly259.28.6:T123C, aly2007.4.2:A90G, and aly345.8.16:T81C, and COI barcode: T100T(not C), T250T(not C), and T364T(not C).

Barcode sequence of the holotype.—Sample NVG-15101G06, GenBank OP762106, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAGTAGGTACTTCTTTAAGCTTA
TTAATTCGAACTGAATTAGGAACTCCTGGATCATTGATTGGTGATGATCAAATTTAT
AATACTATTGTAACAGCTCATGCTTTTATTATAATTTTCTTTATAGTAATACCTATTAT
AATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATTAGGAGCACCTGACATAGC
TTTCCCCCGTATAAACAATATAAGATTTTGATTATTACCCCCATCCCTAACTCTTTTA
ATTTCTAGTAGTATTGTAGAAAATGGTGCTGGAACAGGATGAACTGTTTATCCTCC
TCTTTCTTCTAATATTGCTCACCAAGGAGCTTCAGTTGATTTAGCAATTTTTTCCCT
TCATCTTGCAGGTATTTCTTCTATTCTTGGAGCCATTAATTTTATTACAACAATTATT
AATATACGTGTTAATAATTTATCATTTGACCAAATACCTCTTTTCGTTTGAGCTGTAG
GAATTACAGCTCTTCTTCTTTTACTTTCTTTACCTGTTTTAGCTGGAGCTATTACTAT
ATTATTAACAGATCGAAATTTAAATACCTCATTTTTTGATCCTGCAGGAGGAGGAG
ACCCAATTTTATATCAACATTTATTT

Type material.—**Holotype:** ♂ deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), illustrated in Fig. 22, bears the following three rectangular labels, two white: [Hidalgo Co., TEX. | 6 mi W of Hidalgo | 19/X/74 | -W^m McG.], [DNA sample ID: | NVG-15101G06 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Autochton | caballo Grishin]. **Paratypes:** 3♂♂ and 2♀♀, in TAMU, collected by Roy O. Kendall and C. A. Kendall: <u>USA</u> 1♂ NVG-19013H09 Texas, Bexar Co., north of San Antonio near NE Preserve, 4-Apr-1959; <u>Mexico</u>: 1♀ NVG-19013H11 Nuevo Leon, Cola de Caballo, 23-Oct-1979; 1♂ NVG-19013H12 San Luis Potosi, EI Naranjo, 1-Mar-1976; 1♂ NVG-19013H10 Coahuila, Hwy 57 ca. 24 km ESE Saltillo at truck rest area, 15-Sep-1977; and in USNM 1♀ NVG-5715, 09-SRNP-57000 <u>Costa Rica</u>: Area de Conservación Guanacaste, Guanacaste Prov., Sector Mundo Nuevo, ex larva, eclosed on 29-Aug-2009, Mariano Pereira leg., genitalia NVG160214-76.

Type locality.—USA: Texas, Hidalgo Co., 6 mi W of Hidalgo.

Etymology.—The name (horse in Spanish) is for the saddle- or horseshoe-shaped spot in the forewing discal cell in this sister of *A. potrillo* (foal), typically with a less developed spot. The name is a masculine noun in apposition.

English name.—Caballo skipper.

Distribution.—Continental species: from South Texas, USA to Costa Rica.

Epargyreus socus chota Evans, 1952 is a junior subjective synonym of *Epargyreus clavicornis* (Herrich-Schäffer, 1869)

Genomic sequencing of two syntypes of *Eudamus clavicornis* Herrich-Schäffer, 1869 (type locality from USA to Tropical America), in MFNB, reveals that they are closely clustered with *Epargyreus socus chota* Evans, 1952 (type locality in Trinidad) (Fig. 24 orange). Their COI barcodes are 100% identical and the specimens are phenotypically similar. Therefore, we propose that *Epargyreus socus chota* Evans, 1952, **new synonym,** is a junior subjective synonym of *Epargyreus clavicornis* (Herrich-Schäffer, 1869). The type locality of *E. clavicornis* may be in Venezuela because *E. socus chota* has been recorded from Trinidad and Venezuela, and we are not aware of many mid-18th century specimens collected in Trinidad.

Epargyreus gaumeri Godman and Salvin, 1893, reinstated status, is a species distinct from *Epargyreus clavicornis* (Herrich-Schäffer, 1869), and *Epargyreus gaumeri tenda* Evans, 1955, new combination, as its subspecies

The two taxa: *Epargyreus gaumeri* Godman and Salvin, 1893 (type locality Honduras: Roatán Island) and *Epargyreus clavicornis tenda* Evans, 1955 (type locality in Colombia) that Evans (1952) regarded as subspecies of *Epargyreus clavicornis* (Herrich-Schäffer, 1869) (type locality from USA to Tropical America, probably in Venezuela) are not conspecific with the syntypes of *E. clavicornis*, (Fig. 24 cyan and orange). In the absence of other available names, we **reinstate** *Epargyreus gaumeri* Godman and Salvin, 1893 as a species and, following Evans (1955), keep *E. clavicornis tenda* conspecific with it, but as its subspecies *Epargyreus gaumeri tenda* Evans, 1955, **new combination**.

Epargyreus fractigutta Grishin, new species

https://zoobank.org/BCB1E347-AD55-48AE-85D6-1B2E4DEF3F81

(Fig. 24 part, 25, 26a–f)

Definition and diagnosis.—Genomic analysis of *Epargyreus* Hübner, [1819] reveals that specimens identified as *Epargyreus cruza* Evans, 1952 (type locality Mexico: Veracruz, Cordova) partition into two clades (Fig. 24 red and blue). F_{st}/G_{min} statistics for these two clades are 0.48/0.004, their COI barcodes differ by 1.5% (10 bp), and specimens from these clades are likely sympatric in Costa Rica and Panama suggesting that they correspond to species-level taxa. Currently, there is no available name associated with the red clade (Fig. 24) and it represents a new species. In both nuclear and mitochondrial genome trees, this new species is sister to *E. cruza* and keys to it (C.2.9(a)) in Evans (1952). To facilitate phenotypic comparison, the entire type series of the new species is illustrated in Fig. 26b–f, together with *E. cruza* (Fig. 26g–k). In facies, the following characters separate the new species from *E. cruza*: on the forewing, the hyaline spot in cell CuA_2-1A+2A is offset farther distad from spot in cell CuA_1-CuA_2, (the two spots are closer together in *E. cruza* and are frequently larger) and the discal cell spot is rounder, not as strongly crescent-shaped as in typical *E. cruza*; on the ventral hindwing, the silver spot near the end of the discal cell

more offset basad from the larger silver spot and is slightly more distant from that larger spot than in *E. cruza*, the area between the silver spots (both, in discal cell and between veins 1A+2A and M_3) and a postdiscal band of faint white spots is mostly of dark ground color and largely devoid of pale overscaling, in particular between the discal cell spot and the postdiscal spot between the veins M_1 and M_3 (this overscaling is frequently extensive in *E. cruza*, including the holotype, Fig. 26g), the white spots in the postdiscal band are narrower and less diffuse that in *E. cruza*, pale lavender submarginal overscaling reaches the white postdiscal band, in particular in the middle and mostly in the cell CuA_1-CuA_2 (separated from the band with ground color brown scales in *E. cruza*, noticeably in the cell CuA_1-CuA_2, especially if white overscaling is poorly expressed between the discal silver spots and the postdiscal white band). In other words, lavender/white overscaling in *E. cruza* is expressed first between the discal silver spots and the postdiscal white band, but in the new species, it is expressed first between the postdiscal while band and marginal pale-lavender area (which are frequently merged with each other as a result). The holotypes of both taxa reflect this tendency well (Fig. 26b, g). In male genitalia, harpe narrower distad than in *E. cruza* and less constricted before distal end. However, because this new cryptic species was discovered using genomic analysis and the extent of phenotypic variation across its range remains to be studied, DNA offers definitive identification that should be relied upon. In DNA, a combination of the following base pairs is diagnostic in nuclear genome: aly2487.16.2:A89T, aly281.7.1:C1490T, aly84.5.6:C1196A, aly84.5.6:A1197C, and aly281.7.1:G1473T, and COI barcode: T82C, T106T(not C), T530C, T547C, A559G, and 637C(not T).

Barcode sequence of the holotype.—Sample NVG-14111F08, GenBank OP762107, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGATTAATTGGAACTTCATTAAGATTA
CTTATTCGAACTGAATTAGGAACCCCAGGATCTTTAATTGGAGATGATCAAATCTAT
AATACTATTGTAACAGCTCATGCTTTCATTATAATTTTTTTCATAGTTATACCAATTAT
AATTGGAGGATTTGGAAATTGATTAATTCCCCTTATATTAGGAGCTCCAGATATAGC
TTTCCCCCGTATAAATAATATAAGATTTTGATTATTACCCCCATCACTAACTCTTTTA
ATTTCAAGAAGTATTGTTGAAAATGGAGCTGGAACAGGATGAACTGTTTACCCCC
CTCTTTCTTCCAATATTGCCCATCAAGGATCTTCTGTAGATTTAGCAATTTTTTCTTT
ACACTTAGCTGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACAATTATT
AATATACGAATTAATAATTTATCTTTTGATCAAATACCTTTATTTGTTTGAGCAGTTG
GAATTACAGCTCTATTATTATTACTTTCCTTACCTGTATTGGCAGGTGCTATTACTATA
TTATTAACTGACCGAAATTTAAATACCTCTTTTTTTGATCCTGCAGGAGGAGGAGA
CCCCATTTTATACCAACATTTATTT

Type material.—Holotype: ♀ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 25, 26b, bears the following six rectangular labels, five white: [first TEXAS | record], [McAllen, Texas | Hidalgo Co. | 18/X/73 | W. W. McGuire, Collr.], [Epargyreus | exadeus cruza Evans | Det. '75 | W. W. McGuire], [HESPERIIDAE, | Pyrginae: | Epargyreus exadeus | cruza Evans, 1952 | det. R.O. Kendall | ♀ M. & B. No. 8a], [DNA sample ID: | NVG-14111F08 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Epargyreus | fractigutta Grishin].

Reported by McGuire and Rickard (1976) as "Epargyreus exadeus cruza". **Paratypes:** 4♂♂, one from each locality: <u>Mexico</u>: NVG-17097A08 San Luis Potosi, Axtla Junction, Laurel Canyon, 21-Mar-1980, W. H. Howe leg. [USNM]; NVG-19124E11 Veracruz, 3.9 km N of Omealca, 6-Aug-1981, C. J. Durden leg. [TMMC]; NVG-17097A09 Chiapas, San Jeronimo, 19-Jul-1979 [USNM]; and <u>Panama</u>: NVG-17098A07 El Valle, 31-Jan-1965, S. S. Nicolay leg., genitalia vial H670 [USNM].

Type locality.—USA: Texas, Hidalgo Co., McAllen.

Etymology.—The name is a fusion of Latin words 'fracti' for broken and 'gutta' for drop, tear, or spot, reflecting a discontinuous (broken) silver spot on ventral hindwing, a modified English name for the US sightings of this species that reflects larger separation between the two ventral hindwing sliver spots, i.e., the silver spot is "more broken" in this new species than in *E. cruza*. The name is a noun in apposition.

English name.—Broken silverdrop.

Distribution.—From the Lower Rio Grande Valley in South Texas to Panama.

Comments.—Using a combination of phenotypic characters detailed above, a live individual photographed recently in the Mission area of Hidalgo Co. in Texas (details "obscured", Fig 26a) is more likely to be this new species (or others), but not *E. cruza*. We are not aware of reliable *E. cruza* records from the US. We suggest changing the English name of *E. cruza* (if it needs one) to "bruised silverdrop" to have the "ruz" sound from the name *cruza* and reflect the "bruising" of the silver spot, an impression created by surrounding pale scales (Fig. 26g–k) that are mostly missing in the new species (Fig. 26a–f), so that the US species is still called "broken silverdrop."

Aguna mcguirei Grishin, new species

https://zoobank.org/F99C0D22-EDB9-4FBB-9195-6A5CC88C9A85

(Fig. 27 part, 28, 29a, b, 30)

Definition and diagnosis.—Genomic sequencing of *Aguna metophis* (Latreille, [1824]) (type locality in Brazil) reveals that North American specimens, including one from the US, cluster together and away from a specimen from Brazil: Rio de Janeiro in both nuclear and mitochondrial genes (Fig. 27). Their COI barcodes are 2.4% (16 bp) different, including specimens from Brazil: Rondônia not shown in genomic trees but having barcodes only 1 bp different from the Rio specimen. This genetic differentiation combined with genetic uniformness within each group across their ranges suggests that North American specimens are a species distinct from Brazilian *A. metophis*, and because there is no available name to assign to them, represent a new species. This new species keys to *A. metophis* (C.5.11) in Evans (1952) and differs from it by typically narrower pale discal band on ventral hindwing (Fig. 28, 29a, b), in particular, the streak in cell CuA_2-1A+2A is mostly narrower and smaller than in *A. metophis* (Fig. 29c, d); forewing hyaline spots are usually larger (although some *A. metophis* possess large spots as well) and less angular, and the spot in the

cell CuA_1-CuA_2 is not as strongly hourglass-like as in *A. metophis*, who also frequently has corners of this spot extending distad (most strongly along the vein CuA_2) into a sharp triangle, mostly lacking in the new species; uncus arms are longer (comparatively to the tegumen length) than in *A. metophis*, the gnathos is shorter, the ventral margin of valva is more concave in the middle, the dorsal tooth of the harpe (near ampulla) is large and more prominent, the harpe is more extended and pointed distally. Phenotypic differences from *A. metophis* are rather subtle and, therefore, confident identification is made by DNA characters: a combination of the following base pairs is diagnostic in nuclear genome: aly283.2.7:A147C, aly283.2.7:T141A, aly686.41.7:A132C, aly164.1.4:G95A, and aly16576.4.6:C654T, and COI barcode: T85C, T212C, T340C, T346C, T562C, and T574A.

Barcode sequence of the holotype.—Sample NVG-5526, GenBank OP762108, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCTGGATTAATTGGAACTTCTTTAAGATTA
CTTATTCGTACTGAATTAGGTACCCCCGGATCTTTAATTGGTGATGATCAAATTTAT
AATACTATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATA
ATTGGAGGATTTGGAAATTGATTAATTCCTTTAATACTAGGAGCCCCAGATATAGCA
TTCCCTCGAATAAATAATATAAGATTTTGACTTTTACCCCCTTCTTTAACTCTTTTAA
TTTCTAGAAGTATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCC
CCTTTCTTCTAATATTGCTCATCAAGGAGCTTCTGTAGATTTAGCAATTTTCTCTTTA
CATTTAGCAGGAATTTCTTCTATTCTTGGAGCTATTAATTTTATTACAACAATTATTA
ATATACGAATTAATAATTTATCATTTGATCAAATATCTTTATTTATTTGAGCAGTTGG
AATTACAGCTTTATTATTATTACTTTCTTTACCAGTTTTAGCCGGAGCTATTACAATAT
TATTAACTGATCGAAATTTAAATACATCATTTTTTGATCCAGCAGGAGGAGGAGATC
CTATTCTATATCAACATTTATTT

Type material.—Holotype: ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 28, 30 bears the following six rectangular labels, five white: [TEXAS: | Cameron County | Brownsville], [coll. | 20 Oct 1973 | W. W. McGuire], [HESPERIIDAE, | Pyrginae: | Aguna metophis | (Latreille, [1824]) | det. R.O. Kendall | ♂ M. & B. No. 15], [DNA sample ID: | NVG-5526 | c/o Nick V. Grishin], [genitalia | NVG160110-62 | Nick V. Grishin] and one red [HOLOTYPE ♂ | Aguna mcguirei | Grishin]. **Paratypes:** 6♂♂ and 6♀♀: <u>USA</u>: Texas: 1♀ NVG-19013E12 Galveston Co., Galveston, 7-Aug-1973, W. W. McGuire leg. [TAMU]; Hidalgo Co.: 1♀ NVG-15091H07 Madero, ex egg ex ♀ 6-Nov-2005, reared on *Bauhinia mexicana*, eclosed 17-Feb-2006, R. W. Boscoe leg. [MGCL]; 1♀ NVG-15091H08 McAllen, 1-Oct-1972, F. D. Fee leg. [MGCL]; 1♂ NVG-15105A07 Santa Ana National Wildlife Refuge, 5-Nov-1972, J. W. Tilden leg. [CAS]; Mexico: 1♂ NVG-5506 Tamaulipas, nr. Los Kikos, Gonzales, Ranch, ex larva coll. 1-Jan-1975 on *Bauhinia mexicana*, R. O. Kendall and C. A. Kendall leg., genitalia NVG160110-43 [TAMU]; 1♀ NVG-19013F01 San Luis Potosi, El Salto Falls, 24-Dec-1972, R. O. Kendall and C. A. Kendall leg. [TAMU]; Sinaloa: Mazatlan, Aug [old], Kusche leg. [USNM]: 1♂ NVG-5042, genitalia NVG151101-93; 1♀ NVG-5043, genitalia NVG151101-94; 1♂ NVG-15105A08 Yucatan, Piste, 18-Sep-1959, E. C. Welling leg. [CAS]; <u>Costa Rica</u> 1♂ NVG-17098B11, 05-SRNP-41056, Area de Conservación Guanacaste, Alajuela Prov., Sector Rincon Rain Forest, ex larva, eclosed on 21-May-2005,

Minor Carmona leg., genitalia X-6837 J. M. Burns 2010 [USNM]; <u>Panama</u>: Canal Area, Paraiso, G. B. Small leg. [USNM]: 1♂ NVG-5039 16-Jul-1978, genitalia NVG151101-90; 1♀ NVG-5044 27-Jun-1977, genitalia NVG151101-95.

Type locality.—USA: Texas, Cameron Co., Brownsville.

Etymology.—Named in honor of William W. McGuire, the collector of the holotype (and also the northernmost paratype, from Galveston in Texas), whose contributions to lepidopterology cannot be overstated, starting from many new butterfly records (McGuire and Rickard 1976), collecting the holotypes of five species described in this work (one more is collected by Nadine M. McGuire), and his illustrious taxonomic studies, particularly on *Hesperia*, to the establishment and most generous support of the premier institute for the studies of Lepidoptera, the McGuire Center. The name is a noun in the genitive case.

English name.—McGuire's Aguna.

Distribution.—From the Lower Rio Grande Valley in South Texas to Panama.

Comment.—Although proper Latinization may call for replacing "mc" with "mac" in the species epithet (Vendetti and Garland 2019), to avoid confusion of people who may not be familiar with this tradition and keeping the spelling consistent, the original "mc" was not altered.

Polygonus punctus E. Bell and W. Comstock, 1948, new status, is a species distinct from *Polygonus savigny* (Latreille, [1824])

Genomic analysis of the lectotype of *Hesperia savigny* Latreille, [1824] (type locality not stated), currently in the genus *Polygonus* Hübner, [1825], and the holotype of *Polygonus manueli* E. Bell and W. Comstock, 1948 (type locality Brazil: Santa Catarina, New Bremen) together with a more recently collected specimen from Southeast Brazil confirms that they are conspecific (Fig. 31 green), but reveals that the holotype of *Polygonus manueli punctus* E. Bell and W. Comstock, 1948 (type locality St. Vincent), currently regarded as a subspecies of *P. savigny*, together with other specimens from Lesser Antilles and Isla Margarita, Venezuela are in a distinct clade (Fig. 31 blue), prominently separated from the green clade in both nuclear and mitochondrial genome trees. COI barcodes of *P. savigny savigny* and *P. savigny punctus* differ by 3% (20 bp). In the presence of phenotypic differences, this genetic differentiation suggests that *Polygonus punctus* E. Bell and W. Comstock, 1948, **new status,** is a species-level taxon. Due to genetic similarities, we hypothesize that the type locality of *H. savigny* Latreille, [1824] may be in Southeast Brazil.

Polygonus pardus Grishin, new species

https://zoobank.org/5D5511FC-2D68-46F5-AD2E-062F10DD20D4

(Fig. 31 part, 32, 33a, 34)

Definition and diagnosis.—Phylogenetic analysis of genomic sequences reveals that *Polygonus savigny* Latreille, [1824] (type locality not given, possibly in Southeast Brazil)

is not monophyletic, and Mexican populations currently assigned to *P. savigny* (Fig. 31 red) form a clade sister to *Polygonus punctus* E. Bell and W. Comstock, 1948 (type locality St. Vincent) (Fig. 31 blue) rather than Brazilian *P. savigny* (Fig. 31 green). Therefore, the red clade is not *P. savigny* but, because there are no names available for these populations, represents a new taxon closely related to *P. punctus*. F_{st}/G_{min} statistics for the comparison of the two taxa are 0.22/0.02, but their COI barcodes differ by 0.5% (3 bp), although consistently in all specimens (Fig. 31b). Due to genetic differentiation, the new taxon is a species. It differs from its closest relative *P. punctus* (Fig. 33b) by larger hyaline spots on the forewing (Fig. 32, 33a), in particular, the spot in the cell CuA_1-CuA_2 is nearly square, not narrower and rectangular as in *P. punctus*. Compared to a more distant relative *P. savigny* (Fig. 33c), dorsal hindwing spots are usually defined weaker, not as contrasting against ground color. In male genitalia, the valva is narrower (in lateral view), not broadening towards vinculum and the ampulla is more concave. These phenotypic differences are subtle, and the new species is best diagnosed by DNA. A combination of the following base pairs is diagnostic in nuclear genome: aly3507.2.1:C65T, aly3507.2.1:G70A, aly925.11.10:C284G, aly5411.1.24:G60C, and aly490.12.1:A4311C, and COI barcode: T169C, T283C, and T412T(not C).

Barcode sequence of the holotype.—Sample NVG-14111F12, GenBank OP762109, 658 base pairs:

AACTTTATATTTTATTTTTGGTATTTGAGCAGGTATAGTAGGTACATCTCTTAGATTA
CTAATTCGAACAGAATTAGGAACCCCTGGATCTTTAATTGGAGATGATCAAATTTA
TAATACTATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTAATACCCATTAT
AATTGGAGGATTTGGTAATTGATTAGTTCCTCTTATACTTGGAGCTCCTGATATAGC
TTTCCCTCGAATAAATAATATAAGATTTTGACTATTACCCCCTTCTTTAACCCTATTA
ATTTCAAGAAGAATTGTTGAAAATGGAGCAGGTACAGGTTGAACAGTTTACCCCC
CTCTTTCAGCTAATATTGCTCATCAAGGTTCTTCAGTAGATTTAGCAATTTTTTCCT
TACATTTAGCTGGAATTTCCTCTATTTTAGGAGCTATTAATTTTATTACTACAATTATT
AATATACGAATTAGAAATTTATCTTTTGATCAAATACCTTTATTCGTTTGAGCTGTTG
GAATTACAGCTTTATTATTACTTCTTTCTTTACCTGTATTAGCAGGAGCTATTACTATA
CTTTTAACAGATCGAAATTTAAATACTTCCTTTTTTGATCCTGCAGGAGGAGGAGA
TCCTATTTTATATCAACATTTATTT

Type material.—**Holotype:** ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 32, 34 bears the following six rectangular labels, five white: [TEXAS: | HIDALGO COUNTY | McAllen], [coll. | 1-IX-1972 | W. W. McGuire], [Polygonus | manueli ♂ | Det. 7 | W. W. McGuire], [HESPERIIDAE, | Pyrginae: | Polygonus manueli | manueli Bell and | W.P. Comstock, 1948 | det. R.O. Kendall | ♂ M. & B. No. 10], [DNA sample ID: | NVG-14111F12 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Polygonus | pardus Grishin]. **Paratypes:** 3♂♂ and 1♀: Mexico: 1♀ NVG-15116H12 Sinaloa, MX Hwy 40, near Jct. of Mex 15, 400', 30-Aug-1967, R. W. Holland leg. [CSUC]; 1♂ NVG-5028 Oaxaca, Isthmus, road to San Miguel Chimalapa, ca. 100 ft, 13-Aug-1992, J. Kemner leg., genitalia NVG151101-79 [USNM]; 1♂ NVG-5029 Yucatan, Piste, 15-Aug-1962, E. Welling leg., genitalia NVG151101-80 [USNM]; 1♂ NVG-15099F01 Chiapas, 10 mi NW Bonampak, 1-Aug-1988, J. Kemner leg. [CMNH].

Type locality.—USA: Texas, Hidalgo Co., McAllen.

Etymology.—There is *Polygonus leo*, and now there will be *Polygonus pardus*, two related species and two parts of the word *leopardus*. More, its brown-spotted tawny hindwing lives up to the name, which is a masculine noun in apposition.

English name.—Spotted polygon.

Distribution.—From the Lower Rio Grande Valley in South Texas, USA to Costa Rica.

Arteurotia artistella Grishin, new species

https://zoobank.org/477809F5-8458-48A4-B38E-FEEABD6B998B

(Fig. 35 part, 36, 37a–c, 38)

Definition and diagnosis.—Inspection of phylogenetic trees constructed from protein-coding regions in the nuclear genome reveals that specimens identified as *Arteurotia tractipennis tractipennis* A. Butler and H. Druce, 1872 (type locality in Costa Rica) from the northern parts of its range form a separate and strongly supported clade (e.g., Z chromosome tree, Fig. 35a red) sister to a strongly supported clade of all others (Fig. 35a blue and green). F_{st}/G_{min} statistics for the comparison of the two taxa are 0.40/0.003 suggest that the red clade is a distinct species. Curiously, in mitochondrial genome (Fig. 35b), this new species is sister to the nominotypical *A. tractipennis* from Central America, and the South American subspecies *Arteurotia tractipennis contractipennis* Mabille and Boullet, 1916 (type locality in Venezuela) is sister to them both. This incongruence between nuclear and mitochondrial genomes is likely caused by mitochondrial introgression at some point in the past, because F_{st} between *A. t. tractipennis* and *A. t. contractipennis* computed on the Z chromosome is low (0.13) suggesting that they are conspecific. Therefore, the COI barcodes of the new species are more similar to the barcodes of the nominotypical *A. tractipennis* (0.8%, 5 bp) than to the barcodes of *A. t. contractipennis* (1.2%, 8 bp). The new species is similar to *A. tractipennis* (Fig. 37d, e) and differs from it by the apical hyaline forewing spots being closer together, in particular, the spot in the cell R_3-R_4 is typically closer to the spot in the cell R_4-R_5 in the new species (Fig. 36, 37a–c) than in *A. tractipennis*, the apical black patch is usually smaller, and the hindwing androconial patch comes closer to the wing outer margin; long prong (from the ampulla) of the left valva (asymmetric genitalia) is longer and more gracile (Fig. 38). Due to individual variation, this new species is best diagnosed by DNA. A combination of the following base pairs is diagnostic in nuclear genome: aly1313.21.3:A339G, aly1603.80.1:T144A, aly1603.29.3:C411T, aly770.4.1:A1470G, and aly925.19.1:C24T, and COI barcode: A34G, A302G, C421C(not T), C505C(not T), and A628A(not G).

Barcode sequence of the holotype.—Sample NVG-5485, GenBank OP762110, 658 base pairs:

TACTTTATATTTTATTTTTGGTATTTGATCAGGGATAGTAGGAACATCTTTAAGTATA CTTATTCGATCAGAATTAGGAATACCTGGATCTTTAATTGGAGATGATCAAATTTAT AATACTATCGTAACTGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATCAT

AATTGGAGGATTCGGAAATTGATTAGTACCCCTTATATTAGGAGCACCTGATATAGC
TTTTCCCCGAATAAATAACATAAGATTTTGACTTTTACCTCCTTCTTTAATATTATTA
ATTTCAAGAAGTGTTGTAGAAAATGGTGCTGGAACAGGTTGAACTGTTTATCCCC
CCCTTTCAGCTAATATTGCTCATCAAGGATCTTCTGTAGATTTAACTATTTTTTCCCT
TCATTTAGCTGGAATTTCCTCTATTTTAGGGGCAATTAATTTTATTACAACTATTATT
AATATACGAATTAATAATTTATCTTTTGATCAAATACCTCTATTCGTATGAGCTGTAG
GTATTACTGCTTTACTTTTATTATTATCTTTACCCGTTTTAGCTGGAGCTATTACCATA
TTATTAACTGATCGTAATTTAAACACATCTTTTTTTGATCCAGCTGGAGGAGGAGAT
CCAATTTTATATCAACATTTATTT

Type material.—Holotype: ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 36a, 38, bears the following eight rectangular labels, seven white: [FIRST | UNITED STATES | RECORD], [TEXAS: | HIDALGO COUNTY | city of Mission | 10th Street at | irrigation ditch], [coll. | 2-IX-1972 | N. M. McGuire], [Allyn Museum photo | No. 051079-7-8], [HESPERIIDAE, | Pyrginae: | Arteurotia tractipennis | tractipennis | Butler & H. Druce, 1872 | ♂ det. R.O. Kendall | M. & B. No. 62.5], [DNA sample ID: | NVG-5485 | c/o Nick V. Grishin], [genitalia | NVG160110-26 | Nick V. Grishin], and one red [HOLOTYPE ♂ | Arteurotia | artistella Grishin]. **Paratype:** 1♂ <u>Mexico</u>: Tamaulipas, Ciudad Victoria, Rio San Marcos, ca 1000 ft, GPS 23.9167, −98.8833, J. Kemner leg., 4-Jun-1992, NVG-18059D02, in USNM.

Type locality.—USA: Texas, Hidalgo Co., Mission.

Etymology.—The name is for the dense cluster of spots at the forewing apex, from Latin 'artus' for narrow, close, fitted, confined, dense and 'stella' star. The name is a feminine noun in apposition.

English name.—Artistarred skipper.

Distribution.—Presently known from South Texas and Mexico: Tamaulipas. Notably, El Salvador and Costa Rica are inhabited by a different species, *A. tractipennis*. It is unknown if the two species are sympatric. However, this geographical boundary somewhere in southern Mexico or Guatemala, is not the typical suture zone in Panama or Colombia that separates the faunas of North and South Americas that we see in other cases discussed in this work.

Heliopetes elonmuski Grishin, new species

https://zoobank.org/93636723-E1FE-4D3E-9A3D-5F2CC96141E5

(Fig. 39 part, 40, 41a–c, 42)

Definition and diagnosis.—Genomic analysis reveals that *Heliopetes arsalte* (Linnaeus, 1758) (type locality "Indiis", Honey and Scoble (2001) suggested "South America, probably the Guianas") is not monophyletic in both nuclear and mitochondrial genome trees (Fig 39 blue and red), and instead northern populations identified as *H. arsalte* (Fig 39 red) are sister to *Heliopetes marginata* Hayward, 1940 (type locality Ecuador: Balzapamba) (Fig

39 green). The three clades are well-differentiated genetically: F_{st}/G_{min} statistics for their comparison are 0.39/0.007, and therefore represent three distinct species. Searching for possible names that may apply to the northern populations (red clade), we see that *Pyrgus figara* Butler, 1870 was not definitively assigned to a particular locality. Butler (1870) wrote about the species he described in the publication that proposed the name *figara*: "The greater part of these are in the Kaden collection, now in the possession of Mr. Herbert Druce; and many of the species are from Venezuela." Indeed a syntype of *Pyrgus figara* Butler, 1870, female in BMNH, is labeled from "Druce Coll. ex Kaden Coll.", and although it lacks the locality label, it is consistent with phenotype of *H. arsalte* from Venezuela (or other South American countries) in that it is a dark specimen with heavy overscaling along hindwing veins, especially by the outer margin, and broader dark marginal border on forewing (covering distal third of the wing) than in any North American specimens we have seen. Forewing costal cell is completely brown, and in North American specimens it is either mostly white and if brown then with a white streak at the base and along costa (Fig. 40, 41b, c). Therefore, this evidence suggests that *P. figara* is of South American, possibly Venezuelan, origin, and this name does not apply to the red clade (Fig. 39), indeed being a synonym of *H. arsalte*. In the absence of other available name candidates (*Papilio niveus* Cramer, 1775 is from Suriname and is quite similar to South American *H. arsalte*, both having very narrow dark borders on wings, typical for males from the Guianas), the red clade represents a new species. Curiously, the COI barcodes are 1.7% (11 bp) different between the new species and *H. arsalte*, despite not being monophyletic with it, but 2.1% (14 bp) different from its sister *H. marginata*. This is because the evolutionary rate in the mitogenome is slower in *H. arsalte* (Fig. 39b blue) compared to the other two species (Fig. 39b red and green). The new species keys to C.2.7(a) in Evans (1953) and differs from *H. arsalte* by male genitalia (Fig. 42): the tegumen is longer, bulkier; the valva is narrower, the harpe is straighter, separated from the ampulla by a wide cleft, the phallobase is shorter; and by the dark form females (Fig. 41c, d) with narrower forewing dark border, especially towards tornus, and more extensive pale scales (sometimes a ray) in the forewing costal cell towards the wing base. Due to phenotypic differences being slight, best diagnosed by DNA. A combination of the following base pairs is diagnostic in nuclear genome: aly3766.1.5:G62C, aly2379.11.15:G51A, aly16576.4.4:C143T, and aly274.30.2:T84C, and COI barcode: A160G, A181T, C367C(not T), T562A, and C610T.

Barcode sequence of the holotype.—Sample NVG-7557, GenBank OP762111, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCTTTAAGATTA
TTAATTCGAACTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATTTAT
AATACTATTGTTACAGCTCATGCTTTTATTATAATTTTCTTTATGGTAATACCAATTAT
AATTGGTGGATTTGGAAATTGATTAGTACCTTTAATATTAGGAGCCCCAGATATAGC
ATTTCCTCGTATAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAACATTATTAA
TTTCAAGAAGTGTAGTAGAAAACGGAGCAGGAACTGGTTGAACAGTTTACCCCCC
TCTCTCAGCTAATATTGCACATCAAGGTTCTTCTGTTGATTTAGCTATTTTTTCTTTA
CATTTAGCAGGAATTTCATCTATCTTAGGAGCTATTAATTTTATTACAACTATTATTA
ATATACGTATTAGAAATATATCATTTGATCAAATACCTTTATTTGTATGAGCAGTAGG
TATTACAGCTTTATTATTATTATTATCATTACCTGTTTTAGCAGGTGCTATTACTATATT

ATTAACAGATCGAAATTTAAATACATCATTTTTTGATCCTGCAGGAGGAGGAGATC
CTATTTTATATCAACATTTATTT

Type material.—Holotype: ♀ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 40, bears the following seven rectangular labels, six white: [FIRST | UNITED STATES | RECORD 2 of 2], [TEXAS: | Cameron Co. | Boca Chica], [coll. | 20-X-73 | W. W. McGuire], [HESPERIIDAE, | Pyrginae: | Heliopetes arsalte | (Linnaeus, 1758) | ♂ det. R.O. Kendall | M. & B. No. 112], [DNA sample ID: | NVG-7557 | c/o Nick V. Grishin], [genitalia | NVG170107-13 | Nick V. Grishin], and one red [HOLOTYPE ♀ | Heliopetes | elonmuski Grishin]. **Paratypes:** 6♂♂ and 2♀♀: 1♀ the same data as the holotype; Mexico: Tamaulipas: 1♀ NVG-7559 Ciudad Mante, Los Arcos Ct., 2-Jul-1977, R. O. Kendall and C. A. Kendall leg. [TAMU], genitalia NVG170107-15; 1♂ NVG-20062H05 0.5 km SW of Gomez Farias, 400 m, 24-Dec-1972, C. J. Durden leg. [TMMC]; San Luis Potosi: 1♂ NVG-7558 ca. 10 mi E Cd. Valles, grounds Hotel Taninul, 5-Feb-1980, R. O. Kendall and C. A. Kendall leg. [TAMU], genitalia NVG170107-14; 1♂ NVG-20062H08 Maiz, El Salto, 28-Jul-1981, C. J. Durden [TMMC]; Veracruz 1♂ NVG-19091C08, Boca del Rio, 9-Jun-1994, R. Segura leg. [USNM]; Guatemala 1♂ NVG-19091C09 Peten District, Finca Ixobel, S Poptun, 1700 ft, GPS 16.3039, −89.4222, 5-10-Jun-2003, Ron Leuschner leg. [USNM]; Honduras 1♂ NVG-19091C10 San Pedro Sula, Aug-1975, N. L. H. Krauss leg. [USNM].

Type locality.—USA: Texas, Cameron Co., Boca Chica.

Etymology.—The name is inspired by the type locality. Boca Chica is now a site for SpaceX, a company founded by Elon Musk that opens unprecedented opportunities for the exploration of the unknown. It is our hope that nature of the Boca Chica area will be preserved, and genetically unique species like this one will thrive there and around the area. The name is a noun in the genitive case.

English name.—SpaceX white-skipper.

Distribution.—From South Texas to Costa Rica.

Hesperia balcones Grishin, new species

https://zoobank.org/D8965D03-EFD6-4958-802C-C08F42FC86E4

(Fig. 43 part, 44, 45a–d, 46a–d, 47)

Definition and diagnosis.—Genomic analysis of *Hesperia woodgatei* (Williams, 1914) (type locality in USA: New Mexico, Los Alamos Co., Jemez Mts.) across its range reveals a prominent split into two clades. One clade (Fig. 43 blue) included a syntype of *H. woodgatei* (Fig. 43 magenta) and in addition to the specimens from the USA (New Mexico and Arizona) there were also specimens from Mexico (Coahuila). The other clade (Fig. 43 red) consists of the central Texas populations. F_{st}/G_{min} statistics for their comparison are 0.39/0.006, suggesting that the two clades correspond to distinct species. COI barcodes differ between them by 1.2% (8 bp). In contrast, F_{st}/G_{min} between populations of *H.*

woodgatei (Fig. 43 blue) from the US and from Mexico are 0.13/0.06, suggesting that they are conspecific. The red clade does not have an available name associated with it and therefore represents a new species. The new species is similar to *H. woodgatei* and keys to it (M.10.2) in Evans (1955), but differs in having browner and grayer (instead of more greenish) ventral side of wing with typically smaller spots, especially on the forewing (Fig. 44, 45a–d); in male genitalia (Fig. 46a, b, e, f), the tegumen with uncus is smaller and shorter, broader in dorsal view and narrower towards the base in lateral view; the saccus is shorter and broader (the shape and number of valval teeth appears to be an individually variable character); in female genitalia (Fig. 46c, d, g, h), the lamella postvaginalis is straighter and mostly convex along its distal margin and not with protruding central more sclerotized area concave in the middle, as in *H. woodgatei*. In DNA, a combination of the following base pairs is diagnostic in nuclear genome: aly1603.68.1:G108A, aly5294.15.2:C156T, aly6841.61.2:G318A, aly315.1.25:C75T, and aly151.14.4:C48A, and COI barcode: T118T(not C), T235T(not C), T274T(not C), and T287T(not C).

Barcode sequence of the holotype.—Sample NVG-22031H10, GenBank OP984704, 658 base pairs:

AACTTTATATTTTATTTTTGGTATTTGAGCAGGTATATTAGGAACTTCTTTAAGTTTAT
TAATTCGAACAGAATTAGGTAATCCTGGATCTTTAATTGGAGATGACCAAATTTATA
ATACTATTGTCACAGCACATGCTTTTATTATAATTTTTTTTATAGTTATACCAATTATA
ATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATTAGGAGCTCCTGATATAGCT
TTTCCACGTATAAATAATATAAGATTTTGAATATTACCCCCTTCATTAACATTATTAAT
TTCAAGAAGAATTGTAGAAAATGGTACTGGAACAGGTTGAACTGTTTATCCCCCTT
TATCTTCTAATATTGCTCATCAAGGATCTTCTGTTGATTTAACAATTTTTTCTCTTCA
TTTAGCTGGAATTTCATCTATTCTAGGAGCTATTAATTTTATTACAACAATTATTAAC
ATACGAATTAAAAATTTATCTTTTGATCAAATACCTTTATTTGTTTGATCTGTAGGA
ATTACAGCATTATTATTACTTTTATCTTTACCTGTATTAGCAGGAGCTATTACTATATT
ACTTACTGATCGAAATTTAAATACTTCTTTTTTCGATCCAGCAGGAGGAGGAGATC
CAATTTTATATCAACATTTATTT

Type material.—**Holotype:** ♂ deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), illustrated in Fig. 44, bears the following 3 rectangular labels, 2 white: [USA: Texas, Travis Co. | Volente, along Lime Creek Rd. | between 30.4611, −97.9064 | and 30.4605, −97.9011 | 9-Oct-2008 N. V. Grishin leg.], [DNA sample ID: | NVG-22031H10 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Hesperia | balcones Grishin]. **Paratypes:** 17♂♂ and 27♀♀: <u>USA</u>: Travis Co.: the type locality, N. V. Grishin leg.: 2♂♂ 9-Oct-2005; 2♂♂ and 2♀♀ 6-Oct-2007; ex ♀ ex ovum, 1♂ eclosed 9-Feb-2008; 1♂ NVG-22031H11, 4♂♂, and 12♀♀ 9-Oct-2008; ex ♀ ex ovum, eclosed: 1♀ 27-Feb-2009, 1♂ 6-Mar-2009, 1♀ 21-Mar-2009; Volente, W. R. Dempwolf leg.: 1♀ NVG-17113E04 30-Sep-2016, GenBank accession OP762112; 1♂ NVG-17113E09 6-Oct-2017; 1♂ NVG-17113E08 7-Oct-2016; 1♀ NVG-17113E05 7-Oct-2016; Austin, FM2769 nr. Bullick Hollow Rd., N. V. Grishin leg.: 30.4410, −97.8696: 3♀♀ 9-Oct-2005; ex ♀ ex ovum, eclosed: 1♂ 18-Apr-2006, 1♀ 27-Apr-2006; 30.44033, −97.87318: 1♀ 9-Oct-2005; 1♂ and 2♀♀ 9-Oct-2008; Bee Cave Rd., ca. 9 mi WNW of

Austin, 10-Oct-1976, R. O. Kendall and C. A. Kendall, leg. [TAMU]: 2♂♂ NVG-19012H10 and NVG-19012H11, 1♀ NVG-19012H1; and 1♀ NVG-19071E02 Kerr Co., [probably Lacey's ranch,] about 1900, Barnes Collection [USNM]. Reared specimens developed continuously without diapause and aestivation, and eclosed during spring the following year, which is unnatural. In nature, there is only one flight in the fall, not spring.

Type locality.—USA: Texas, Travis Co., Volente, along Lime Creek Rd. between GPS 30.4611, −97.9064 and 30.4605, −97.9011.

Etymology.—The name is for the Hill Country that contains the entire distribution of this species: an area in Central Texas bounded on the east by the Balcones Fault. In 1756, Bernardo De Miranda, a Spanish explorer, named this formation "Los Balcones", meaning "Balconies" (Spearing 1991). Limestone cliffs and hills of the Balcones Escarpment is the habitat of this species. The name is a masculine noun in apposition.

English name.—Hill country skipper.

Distribution.—Currently known only from central Texas, USA.

Life history.—While we have not observed immature stages in nature, we reared this species from eggs laid by captive females. White eggs were placed singly on living or dry leaves and stems of grasses (Fig. 47a, b). Feeding on *Cynodon dactylon* (L.) Pers. (for convenience of access to this grass), caterpillars are greenish with jet-black head and collar in the first two instars (Fig. 47c–f, cream-colored before starting to feed), turning brown towards maroon-purple with white neck, and from the 3rd instar developing a pair of yellowish vertical stripes on the forehead (Fig. 47g–l). In the laboratory conditions, caterpillars did not undergo diapause or aestivation and pupated after six instars. Pupa pale, cream-colored with brown mottled pattern and pinkish abdomen (Fig. 47m–o).

Troyus fabulosus Grishin, new species

https://zoobank.org/15B54334-E76A-44D0-853C-B15E1B423C4D

(Fig. 48 part, 49, 50a, b, 51)

Definition and diagnosis.—Inspection of genomic trees of *Troyus* A. Warren and Turland, 2012 (type species *Troyus turneri* A. Warren and Turland, 2012) reveals that specimens of *Troyus fantasos* (Cramer, 1780) (type locality in Suriname) are not monophyletic in the mitochondrial genome tree (Fig. 48b blue and red) and they are correspondingly partitioned into two clades in nuclear DNA. F_{st}/G_{min} statistics for the comparison of the two clades are 0.38/0.006, suggesting that they represent distinct species. The blue clade contains more southern populations including a specimen from French Guiana, and these specimens agree better with the original illustration of *T. fantasos*, thus being this species. The red clade consists of northern populations (southernmost of those sequenced was from Honduras) and does not have a name, therefore being a new species. COI barcodes of the new species differ from those of *T. fantasos* by 4.3% (28 bp). However, it shares the barcodes (and the rest of mitochondrial DNA, Fig. 48b) with sympatric

Troyus onaca (Evans, 1955) and *Troyus diversa maeon* (Mabille, 1891). Moreover, we see mitochondrial DNA introgression between *T. aurelius* (Plötz, 1882) (Fig. 48b olive) and *T. phyllides* (Röber, 1925) (Fig. 48b cyan), suggesting evolutionary peculiarities in this genus and underscoring the importance of nuclear DNA analysis (Fig. 48a). The new species keys to "*Vettius fantasos fantasos*" (J.45.12(b)) in Evans (1955), and differs from it by generally paler and less contrasting ventral hindwing: area between veins M_1 and M_3 that is mostly pale distad of the postdiscal rust-colored cross-bar (Fig. 49, 50a, b), but is dark in typical *T. fantasos* (Fig. 50c, d); the cross-bar is usually more distal in *T. fantasos* than in the new species, making the postdiscal pale spot appear smaller in the new species than in *T. fantasos*; the dorsal hindwing pale spots are whiter than more typically brownish-orange spots of *T. fantasos*; the valva is broader (in lateral view), the harpe is less robust and its posterior spike-like projection is thinner (Fig. 51a, b) compared to *T. fantasos* (Fig. 51c, d). In DNA, a combination of the following base pairs is diagnostic in nuclear genome: aly528.39.5:A75G, aly1409.3.1:A174T, aly669.14.1:A672T, aly536.145.4:G198A, and aly2085.2.4:T943C, and COI barcode differs from the phenotypically similar *T. fantasos*: C49C(not T), C82C(not T), C121C(not T), C145C(not T), and C343C(not A), but is shared with the phenotypically distinct *T. onaca* and *T. diversa maeon*.

Barcode sequence of the holotype.—Sample NVG-10654, GenBank OP762113, 658 base pairs:

AACTTTATACTTTATTTTTGGAATTTGAGCAGGAATACTAGGAACATCCTTAAGTTT
ATTAATTCGAACAGAATTAGGTAACCCTGGATCTTTAATTGGAGATGATCAAATTTA
TAATACCATTGTAACAGCTCATGCTTTTATCATAATTTTTTTTATAGTTATACCAATTA
TAATTGGAGGATTTGGAAATTGATTAGTCCCATTAATATTAGGAGCTCCCGATATAG
CATTCCCACGAATAAATAATATAAGATTTTGAATATTACCTCCTTCTTTAATACTTTT
AATTTCAAGAAGAATTGTAGAAAATGGTGCAGGAACAGGATGAACAGTTTACCCC
CCTCTTTCATCTAATATTGCCCACCAAGGAGCTTCTGTTGATTTAGCAATTTTTTCT
CTTCATTTAGCTGGGATTTCATCAATTCTAGGAGCAATTAATTTTATTACTACAATCA
TTAATATACGAATTAGAAATTTATCATTTGATCAAATACCTTTATTTGTTTGATCAGT
AGGTATTACAGCATTATTATTACTTTTATCACTACCGGTATTAGCAGGAGCTATTACT
ATACTTTTAACTGATCGAAATTTAAACACATCTTTTTTTGATCCTGCTGGAGGAGG
AGATCCAATCTTATATCAACATTTATTT

Type material.—Holotype: ♂ deposited in the Texas A&M University Insect Collection, College Station, Texas, USA (TAMU), illustrated in Fig. 49, bears the following seven rectangular labels, six white: [TEXAS: | Hɪᴅᴀʟɢᴏ Cᴏᴜɴᴛʏ | Penitas], [coll. | 24-Oct-1975 | Edward C. Knudson], [Allyn Museum photo | No. 810610 7–8], [HESPERIIDAE, | Hesperiinae: | Vettius fantasos | (Stoll, [1780]) | ♂ det. R.O. Kendall | [M. & B. No. 137.5]], [DNA sample ID: | NVG-10654 | c/o Nick V. Grishin], [genitalia | NVG180106-71 | Nick V. Grishin], and one red [HOLOTYPE ♂ | Troyus fabulosus | Grishin]. **Paratypes:** 7♂♂ in TMMC unless specified otherwise: <u>Mexico</u>: Tamaulipas: 1♂ NVG-20062D06 1–4 km N of Gomez Farias, 350 m, 10-Aug-1981, C. J. Durden; 2♂ NVG-20062F07 and NVG-20062F08, 13 km NNE of Chamal, 1600 ft, 13-Jan-1969; San Luis Potosi: 1♂ NVG-20062D07 Maiz, El Sabinito, 14-Jun-1979, C. J. Durden; 1♂ NVG-17111A03 El Platanito,24-Jun-1983, W. H. Howe leg. [LACM]; 1♂ NVG-20062D05 Veracruz, 4.5 km

SW of Omealca, 7-Aug-1981, C. J. Durden; <u>Honduras</u> 1♂ NVG-19022F03 San Pedro Sula, 27-Dec-1978, Robert D. Lehman leg. [USNM].

Type locality.—USA: Texas, Hidalgo Co., Peñitas.

Etymology.—The name implies that this northern counterpart of South American *T. fantasos* is no less fantastic than it. The name is a masculine adjective.

English name.—Fabulous skipper.

Distribution.—Currently known from South Texas, USA, Mexico, and Honduras.

Comment.—Incongruence between nuclear and mitochondrial DNA trees (Fig. 48a vs. b) is remarkable in *Troyus*. It highlights the dangers of relying exclusively on mitochondrial DNA in phylogenetic studies.

Lectotype designation for *Goniloba parumpunctata* Herrich-Schäffer, 1869

Although the locality for *Goniloba parumpunctata* Herrich-Schäffer, 1869 was not given in the original description, its syntypes were assumed to be South American by several sources. For instance, inspecting the originals of unpublished drawings that included a number of species proposed by Herrich-Schäffer, Godman (1907) selected a specimen from Venezuela as the closest match to the drawing of *G. parumpunctata*. This specimen, according to its labels, originally from the Kaden collection, then in the Druce collection, then in the Godman-Salvin collection, presently in BMNH, bears a label "Compared with Plotz's drawing of parumpunctata ♂ H-S," typical for the specimens chosen by Godman that he found most similar to the drawings. Draudt (1921–1924) gave "Venezuela, Brazil" as localities for his *Lerema parumpunctata*.

We sequenced a male and female syntypes of *G. parumpunctata* (♂ NVG-15035F12 and ♀ NVG-15035G03, in MFNB), and they were placed among eastern US specimens of *Lerema accius* (J. E. Smith, 1797) (type locality in USA: Georgia) in genomic trees (Fig. 52 magenta in blue clade). Due to this genetic similarity (Fig. 52), first, we suggest that the type locality of *G. parumpunctata* is in eastern USA and, second, we confirm that *G. parumpunctata* is a junior subjective synonym of *L. accius*. To stabilize this treatment, in case other syntypes are not conspecific, N.V.G. hereby designates the sequenced male syntype bearing the following eight rectangular labels [Origin.], [parumpunctatus | abgebildet m.], [78:2.], [Coll. H.— Sch.], [Coll. | Staudinger], [parumpuncta- | ta | H. Sch.], [{QR code} http://coll.mfn-berlin.de/u/ | 3226f7], and [DNA sample ID: | NVG-15035F12 | c/o Nick V. Grishin] as the **lectotype** of *Goniloba parumpunctata* Herrich-Schäffer, 1869. The lectotype is missing the abdomen (genitalia prepared by O. Mielke but do not correspond to this species according to his label), the right antenna (except a stub) and has a deep tear in the left hindwing. The COI barcode sequence of the lectotype, GenBank OP762114, 658 base pairs is:

AACTTTATATTTTATCTTTGGAATTTGAGCAGGAATATTAGGAACTTCTTTAAGTTTA
TTAATTCGAACAGAATTAGGAAATCCAGGATCTTTAATTGGAGATGATCAAATTTAT
AATACTATTGTCACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATA
ATTGGAGGATTTGGTAATTGATTAGTCCCATTAATACTAGGAGCTCCTGATATAGCA

TTTCCACGAATAAATAACATAAGATTTTGAATACTACCTCCCTCATTAATACTATTAA
TTTCAAGTAGAATTGTAGAAAATGGTGCAGGAACAGGATGAACAGTTTATCCACC
TTTATCCTCTAATATTGCCCATCAAGGAGCATCAGTTGATTTAGCAATTTTTTCTCTT
CATTTAGCAGGTATTTCTTCAATTTTAGGAGCTATTAATTTTATTACTACAATTATTAA
TATACGAGTTAAAAATTTATCTTTTGATCAAATACCTTTATTTGTTTGATCTGTCGGA
ATTACAGCATTATTATTATTACTTTCACTACCTGTATTAGCTGGAGCTATTACTATACT
TTTAACTGATCGAAATCTTAATACTTCTTTTTTTGATCCTGCAGGAGGTGGTGATCC
TATTTTATACCAACATTTATTT

The second label of the lectotype agrees with Herrich-Schäffer's handwriting ("abgebildet" was probably added later) and 'm.' is for 'mihi' (Latin for 'of me'), placed after a species name as an attribution of the new species to the writer. This notation was common more than a century ago instead of the author's name written directly. This 'm.' confirms that the label was written by Herrich-Schäffer and offers additional evidence that this specimen was a syntype. The third label gives the number of this species (genus 78, species 2) in the Mabille catalogue (1904).

Lerema (*Lerema*) *ochrius* Grishin, new species

https://zoobank.org/3700C6D0-BE94-48C9-B78F-905885E911C0

(Fig. 52 part, 53a, b, 54b, 55a, b, 56, 57a–f, 58)

Definition and diagnosis.—First, we noticed a COI barcode split in *Lerema accius* (J. E. Smith, 1797) (type locality in USA: Georgia): 1.8%–2.1% (12–14 bp) difference between the two groups and a clear partitioning into two clades in the mitochondrial genome tree (Fig. 52b red and blue). The blue clade corresponds to eastern USA *L. accius* that includes other available names currently associated with it. The red clade encompasses southern and southwestern populations that do not have a name; therefore this clade represents a new taxon. F_{st}/G_{min} statistics for the comparison of the two clades are 0.205/0.008 suggesting that the new taxon is a species. Curiously, while *L. accius* (Fig. 52a blue) forms a strongly supported clade separated by a prominent branch from other specimens, the new species represented by the red clade in the mitogenome (Fig. 52b) is not monophyletic in the Z chromosome tree (Fig. 52a) and appears as a set of weakly supported bifurcations. This topology is a likely result of introgression from other taxa. Introgression from *L. accius* will bring specimens closer to the blue branch, and introgression from Mexican and Central American species, such as *Lerema pattenii* Scudder, 1872 (type locality in Guatemala), *Lerema liris* Evans, 1955, or *Lerema lucius* Grishin, 2022 (type locality in Panama) will "pull" the tree branch with the specimen closer to the root. Therefore, the specimens are spread out in the nuclear tree instead of forming a clade. The new species is phenotypically similar to *L. accius* and keys to it (J.39.2(a)) in Evans (1955), and differs from it (Fig. 53c, d, 54a, 55c, d) by being more ocherous on the ventral side (Fig. 53a, b, 54b, 55a, b) instead of rusty-brown in *L. accius*. This difference in hue (yellower vs. redder) may be most obvious by the forewing apex distad of subapical hyaline spots. Among caterpillars that we inspected, we note the following head capsule differences (numbered cyan arrows point to characters in Fig. 57a, ordered by their possible reliability). Generally, the dark-brown

pattern is reduced compared to *L. accius*, but the vertical band is wider towards the mouth (no. 1 in Fig. 57a) and only in very dark *L. accius* this band extends towards eyes (Fig. 57l, m); dark framing of the head capsule central groove is generally wider towards the middle (no. 2) but is the widest at the head apex in *L. accius*; the vertical band is comparatively narrower near its middle (no. 3), not as wide as in *L. accius*, where it may be the widest in the middle; the central area right above the mouth is typically yellow-orange in *L. accius*, but is paler, less saturated in color in the new species (no. 4). Due to seasonal forms and extreme phenotypic variation in both species (including caterpillar head patterns and colors), reliable identification is achieved by DNA sequences: a combination of the following base pairs is diagnostic in nuclear genome: aly1357.16.2:G181A, aly173.37.11:G247A, aly84.20.1:C210A, aly6654.1.1:A1734T, and aly768.1.1:A351G, and COI barcode: T55C, T127T(not C), T247C, T340C, and T436C.

Barcode sequence of the holotype.—Sample NVG-22031H12, GenBank OP984705, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATATTAGGAACTTCTTTAAGCTTA
TTAATTCGAACAGAATTAGGAAATCCAGGATCTTTAATTGGAGATGATCAAATTTAT
AATACTATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATA
ATTGGAGGATTTGGTAATTGATTAATCCCATTAATACTAGGAGCTCCTGATATAGCA
TTTCCACGAATAAACAATATAAGATTTTGAATATTACCTCCTTCATTAATACTATTAA
TTTCAAGTAGAATTGTAGAAAATGGTGCAGGAACAGGATGAACAGTTTACCCACC
TTTATCTTCTAATATTGCCCATCAAGGAGCATCAGTTGATTTAGCAATTTTTTCTCTT
CATTTAGCAGGTATTTCTTCAATTTTAGGAGCCATTAATTTTATTACTACAATTATTA
ATATACGAATTAGAAATTTATCTTTTGATCAAATACCTTTATTTGTTTGATCTGTCGG
AATTACAGCATTATTATTATTACTTTCACTACCTGTATTAGCTGGAGCTATTACTATAC
TTTTAACTGATCGAAATCTTAATACTTCTTTTTTTGATCCTGCAGGAGGTGGTGATC
CTATTTTATACCAACATTTATTT

Type material.—**Holotype:** ♀ deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), illustrated in Fig. 53b, bears the following three rectangular labels, two white: [26.0682, –97.8912 | USA: Texas, Hidalgo Co. | 1.5 air mi SE of Relampago | Old Rio Rico Rd., ex ♀ | ex ovum, ecl. 14-Jun-2015 |Nick V. Grishin leg.], [DNA sample ID: | NVG-22031H12 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Lerema | ochrius Grishin]. **Paratypes:** 22♂♂ and 16♀♀: USA: Texas: Cameron Co.: E of Brownsville: 1♂, 1♀ 10-Nov-1996; 1♀ 11-Nov-1996; ex ♀ ex ovum, eclosed: 1♂ 12-Mar-2003, 1♂ 13-Mar-2003, 1♂ and 1♀ 14-Mar-2003, 1♂ and 1♀ 15-Mar-2003, 1♂ 16-Mar-2003; River Dr., 1.4 mi S of Santa Maria: 1♂ NVG-3350 23-May-2015 [UTSW]; 1♀ 14-Jun-2015; 1♀ NVG-3650 2.5 mi SW of Sebastian, 13-Jun-2015 [UTSW]; 1♂ NVG-3198 Brownsville, 22-Oct-1972, R. O. Kendall and C. A. Kendall leg., genitalia NVG15011-14 [TAMU]; Hidalgo Co.: 1♂ NVG-22054A05 Edinburg, ex larva, eclosed on 17-Jun-2015; 1.5 air mi SE of Relampago, Old Rio Rico Rd., 26.0682, –97.8912: 1♀ NVG-3380 [UTSW], 1♀ 24-May-2015; 2♂♂ ex ♀ ex ovum, eclosed 7-Jul-2015 and 24-Sep-2015; 1♂ NVG-3993 10-Jul-2015 [UTSW], GenBank accession OP762116; 1♀ ex larva, eclosed 23-Sep-2015; 1♂ Mission, Military Rd. W of Urban Road No. 1016, 25-Oct-2004; 1♂ NVG-3258 Bentsen-Rio Grande Valley State Park, World Birding Center, 27-Oct-2004,

J. and F. Preston leg., genitalia NVG15011-74 [TAMU]; 1♀ NVG-5177 Chihuahua, 15-Nov-2015 [UTSW]; Peñitas, around GPS 26.2260, −98.4347: 1♀ 4-Nov-2005; ex larva, eclosed 1♀ 26-Mar-2006; ex ♀ ex ovum, eclosed: 1♂ and 1♀ 22-Jan-2005, 1♂ 23-Jan-2005, 2♂♂ 25-Jan-2005; Starr Co.: 1♂ Rio Grande City, Fort Ringgold, 14-Nov-2015; Roma, nr. international bridge [UTSW]: 1♀ NVG-3808 28-Jun-2015; 1♂ NVG-3997 11-Jul-2015; 1♂ NVG-22054A04, Maverick Co., Eagle Pass 21-Mar-2009; Brewster Co., Big Bend National Park: 1♀ NVG-3197, Chisos Basin, 5280', 6-Oct-1966, R. O. Kendall and C. A. Kendall leg., genitalia NVG15011-13 [TAMU]; 1♂ NVG-3256 15-Aug-1968, J. E. Hafernik leg., genitalia NVG15011-72 [TAMU]; Rio Grande Village: 1♀ NVG-22054A03 ex larva, eclosed 1-Jun-2005 (Fig. 54b). All N. V. Grishin leg., unless indicated otherwise.

Type locality.—USA: Texas, Hidalgo Co., 1.5 air mi SE of Relampago, Old Rio Rico Rd., GPS 26.0682, −97.8912.

Etymology.—The name is for the ocherous (brownish-yellow) color typical of this species ventral side. The name is a noun in apposition, similar to those of its close and similar-looking relatives *L. accius* and *L. lucius*.

English name.—Ocherous skipper.

Distribution.—Currently known from South and West Texas and Mexico. Curiously, the new species was found in sympatry with *L. accius* in the Big Bend National Park (USA: Texas, Brewster Co.). Two specimens (NVG-22054A02 and NVG-22054A03), both females, were reared in the lab from caterpillars collected in the Rio Grande Village campground area. Genetically (Fig. 52) and phenotypically (Fig. 54) they are identified as two different species. *Lerema accius* specimen (Fig. 54a, NVG-22054A02) is not a result of accidental introduction with the locally growing foodplant into the lab in Dallas, because it is genetically different from Dallas specimens. However, the Rio Grande Village campground, where the caterpillar was found, is an area that receives many travelers, campers, and nature enthusiasts. *Lerema accius* is one of the most common Hesperiidae species throughout Texas and eastern US with caterpillars feeding on roadside grasses. Therefore, accidental introduction of *L. accius* to the campground area cannot be excluded, and additional studies of the two *Lerema* species in west Texas are of interest.

Life history.—Eggs white, glued to leaves either singly or in small groups (Fig. 58a–c), developing caterpillar heads can be seen through the transparent eggshell (Fig. 58c). Caterpillars hatch white (Fig. 58d, e), become green upon feeding (Fig. 58f–i), head and collar in the 1st and 2nd instars jet-black, in the 3rd to 5th instars head whitish with a characteristic brown pattern, frequently orangish on top and in front to varying degree (Fig. 56a–f, 58j–n), body yellowish-green with darker, greenish dots, spots, and several longitudinal bands, anal plate concolorous with body, collar dark-brown. Feed on a variety of grasses. Pupa green with a narrow conical projection on the head.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Evans WH. 1952. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part II. Pyrginae. Section I. The Trustees of the British Museum (Natural History); London. v + 178 p., pls. 10–25.

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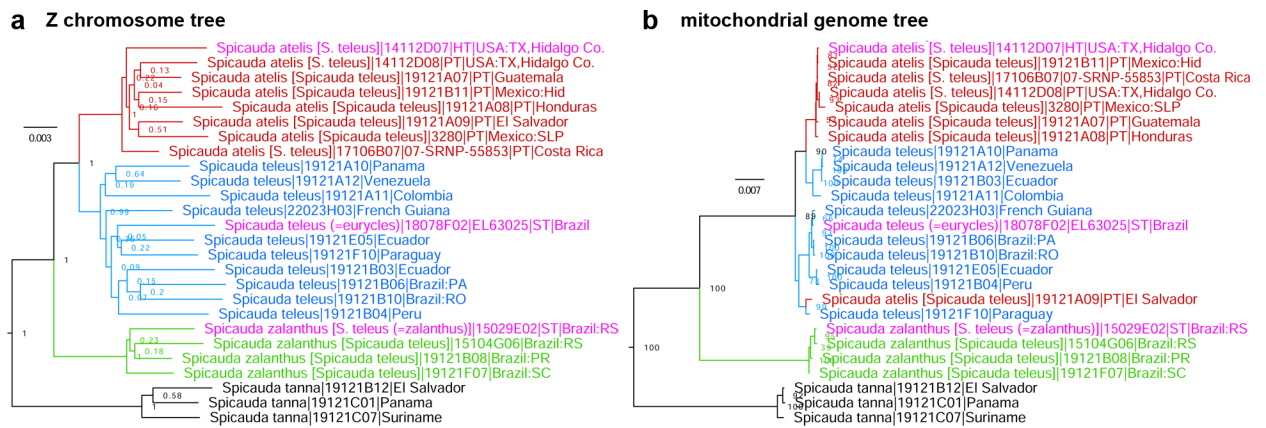
Figure 1.

Trees of *Spicauda teleus* group constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *S. atelis* **sp. n.** (red), *S. teleus* (blue), and *S. zalanthus* (green) rooted with *S. tanna* (black). Primary type specimens are labeled in magenta. Statistical bootstrap support values are shown at nodes (regular in nuclear genome trees and ultrafast in mitogenome trees). For each specimen, the name adopted in this work is given first, and a previously used name is listed in square brackets (if different), supplemented with the DNA sample number, type status (see Materials and Methods for abbreviations) and general locality. See Table S1 in the Supplemental file <https://osf.io//vkj6d/> or NCBI database entries for additional data about these specimens. Synonyms are given in parentheses preceded by "=". The type status refers to this synonym, if the synonym name is provided. The same notations are used throughout this work in other figures showing phylogenetic trees.
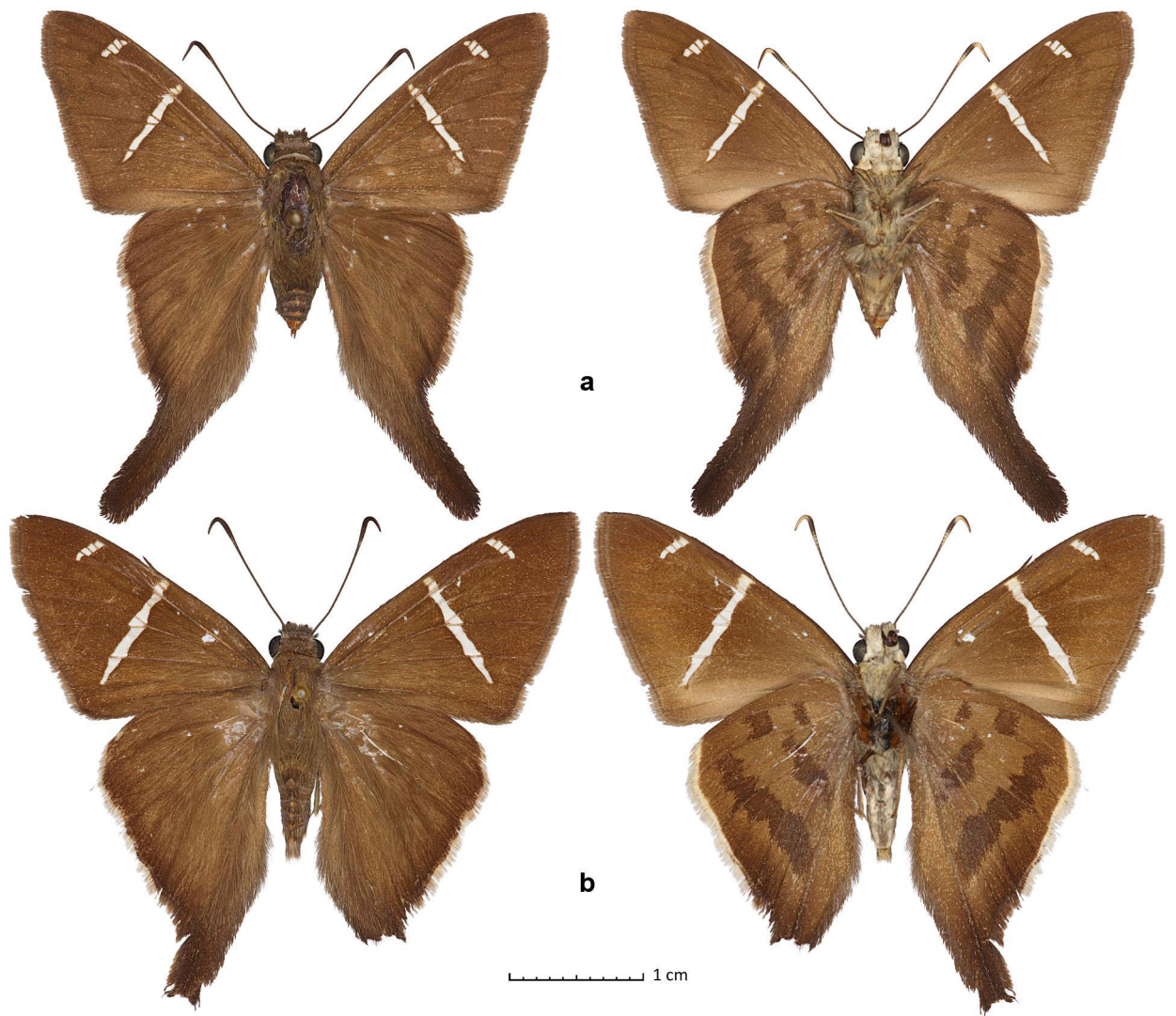
Figure 2.
Spicauda atelis **sp. n**. **a)** holotype ♂ NVG-14112D07, **b)** paratype ♀ NVG-14112D08 dorsal (left) and ventral (right) views, data in text.
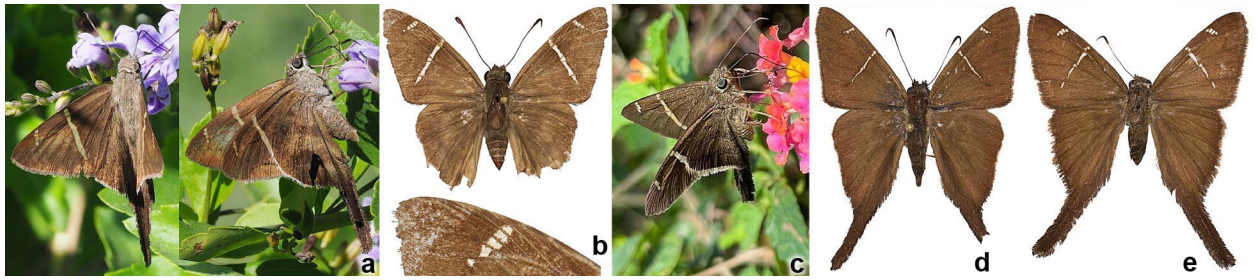
Figure 3.

Three species of *Spicauda*. **a–b)** *S. atelis* **sp. n**. **a)** USA: TX, Hidalgo Co., Mission, 3-Nov-2020, iNaturalist observation 64178872 © Mike Rickard. **b)** paratype NVG-3280, data in text, genitalia in Fig. 4, note the 5th subapical forewing spot—section of the wing magnified below. **c)** *S. teleus*, Trinidad: Couva-Tabaquite-Talparo, 4-May-2022, iNaturalist observation 115731394 © ralytt. **d–e)** *S. zalanthus*. **d)** NVG-19121B08 ♂ Brazil: Paraná, Curitiba, 900 m, 26-Oct-1969, O. Mielke leg. [USNM]. **e)** NVG-19121F07 ♀ Brazil: Santa Catarina, Apr-1945 (no other data) [USNM]. Some images were color-corrected and/or rotated and iNaturalist photographs are available under CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/
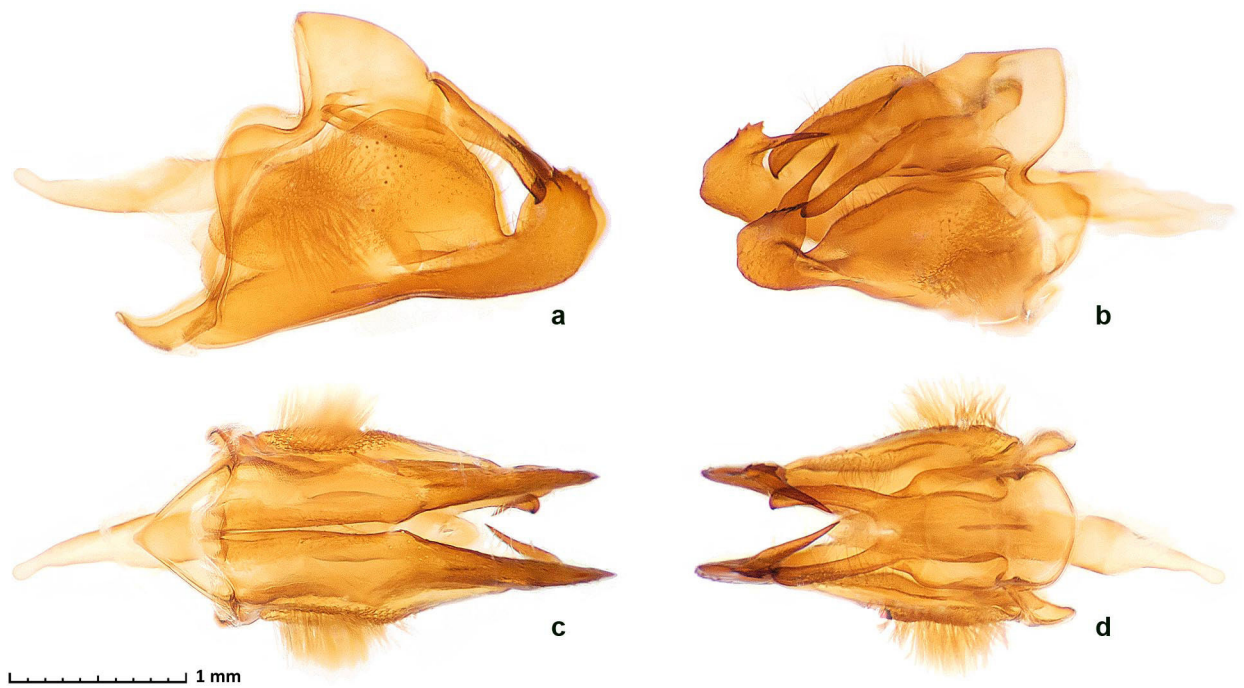
Figure 4.
Male genitalia of *Spicauda atelis* **sp. n.** paratype NVG-3280 (data in text) in different views.
a) left lateral, **b)** right dorsolateral, **c)** ventral, **d)** dorsal.
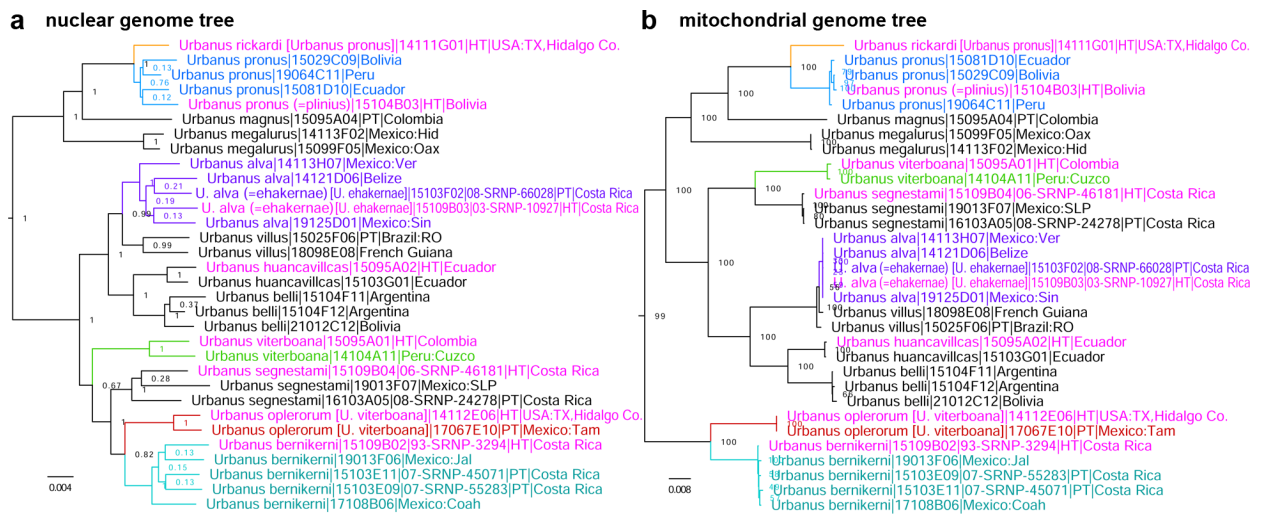
Figure 5.

Trees of selected *Urbanus* (*Urbanus*) species constructed from protein-coding regions in **a)** nuclear and **b)** mitochondrial genomes: *U. rickardi* **sp. n.** (orange), *U. pronus* (blue), *U. alva* (purple), *U. viterboana* (green), *U. oplerorum* **sp. n.** (red), and *U. bernikerni* (cyan) among their relatives (black). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
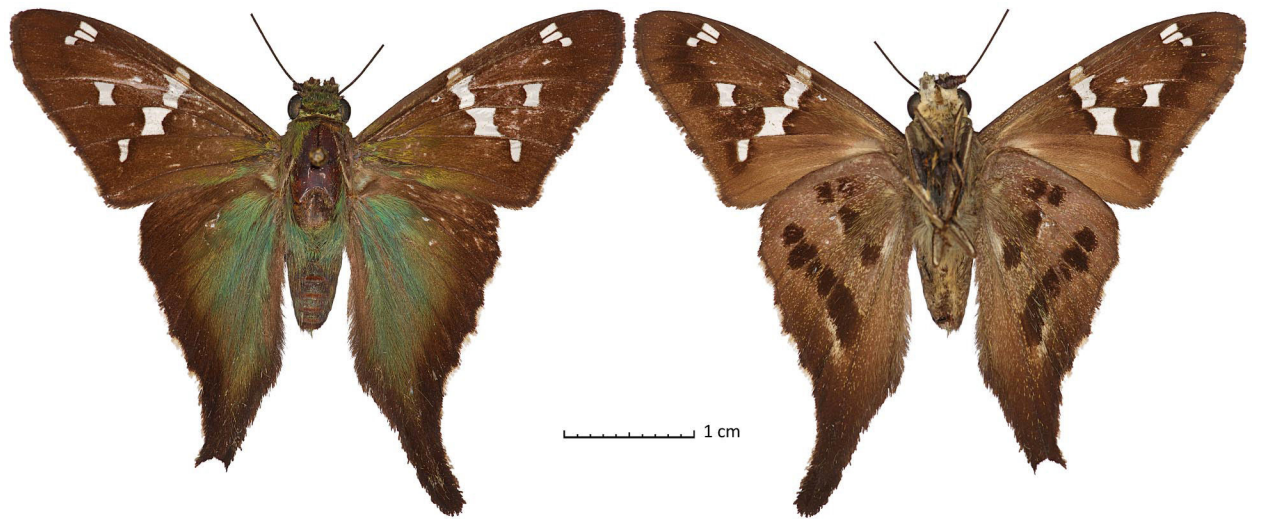
Figure 6.

Holotype of *Urbanus* (*Urbanus*) *rickardi* **sp. n.** dorsal (left) and ventral (right) views, data in text.
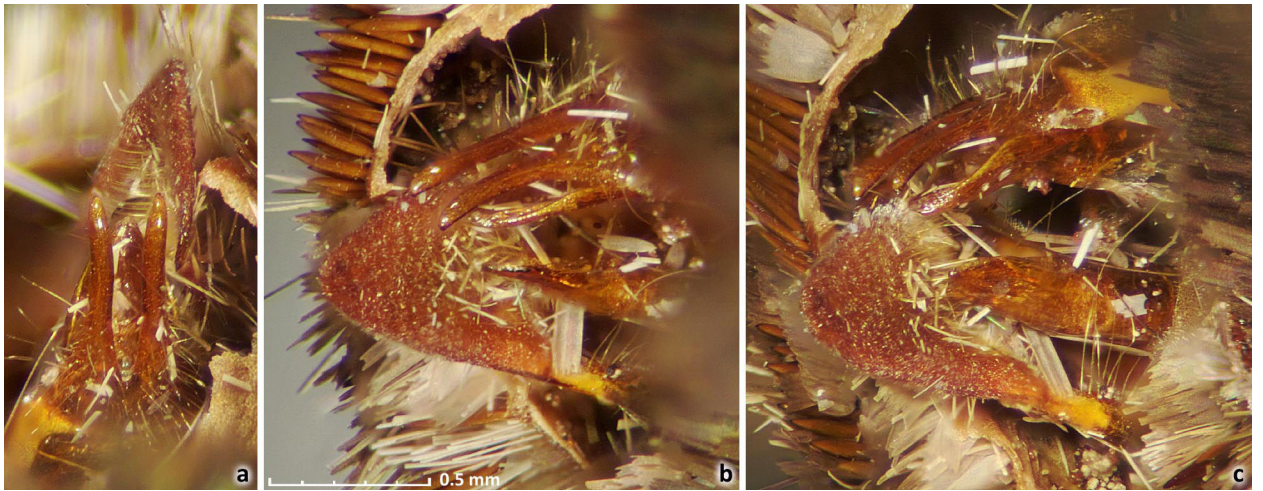
Figure 7.

Male genitalia of *Urbanus* (*Urbanus*) *rickardi* **sp. n.** holotype in situ (data in text) in different views. **a)** dorsal, caudal end up, **b)** right dorsolateral, **c)** right lateral. Genitalia were dry-dissected and right valva removed (likely by James Scott).
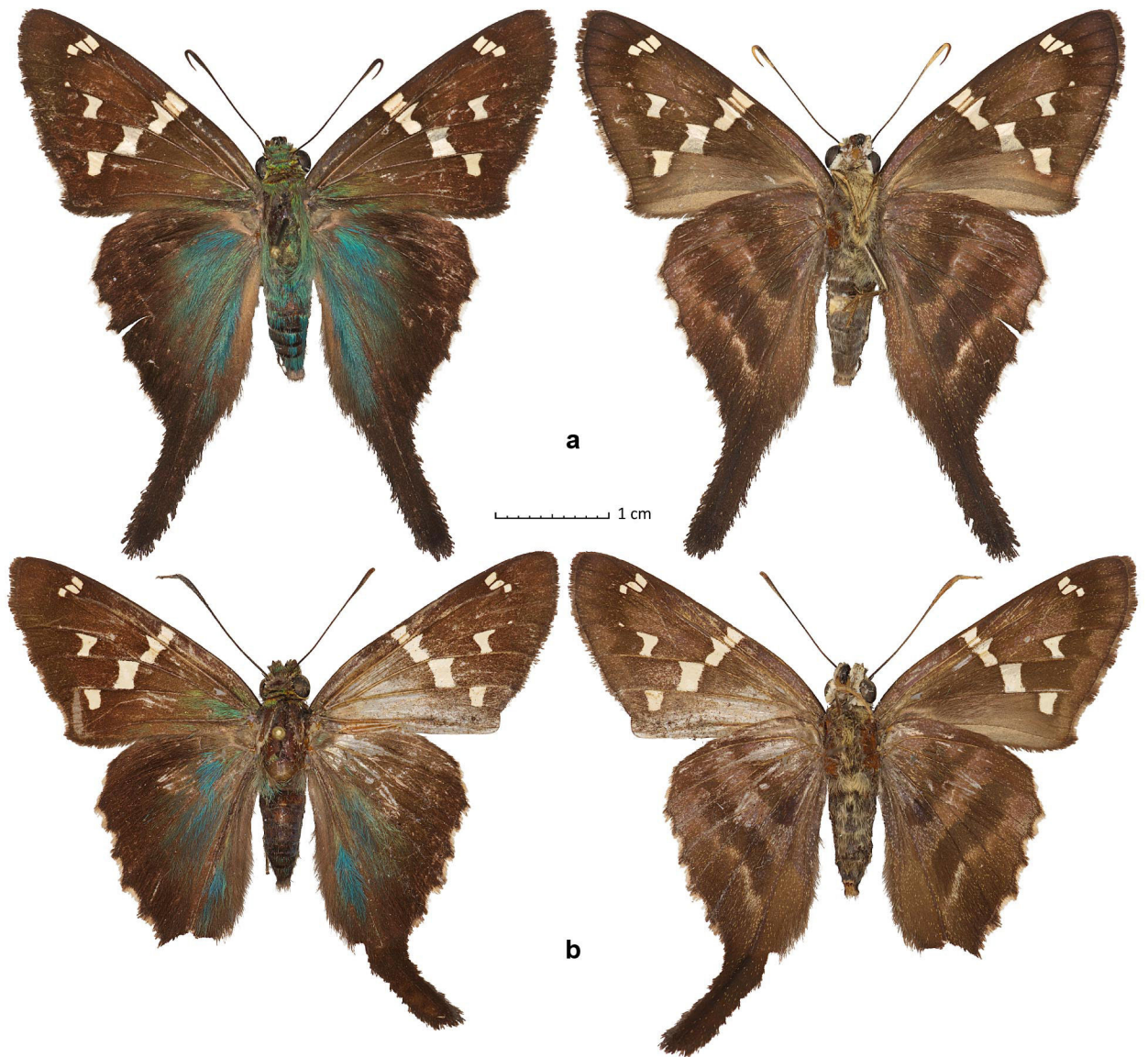
Figure 8.
The type series of *Urbanus* (*Urbanus*) *oplerorum* **sp. n**. **a)** holotype ♀ NVG-14112E06, **b)** paratype ♀ NVG-17067E10, dorsal (left) and ventral (right) views, data in text.
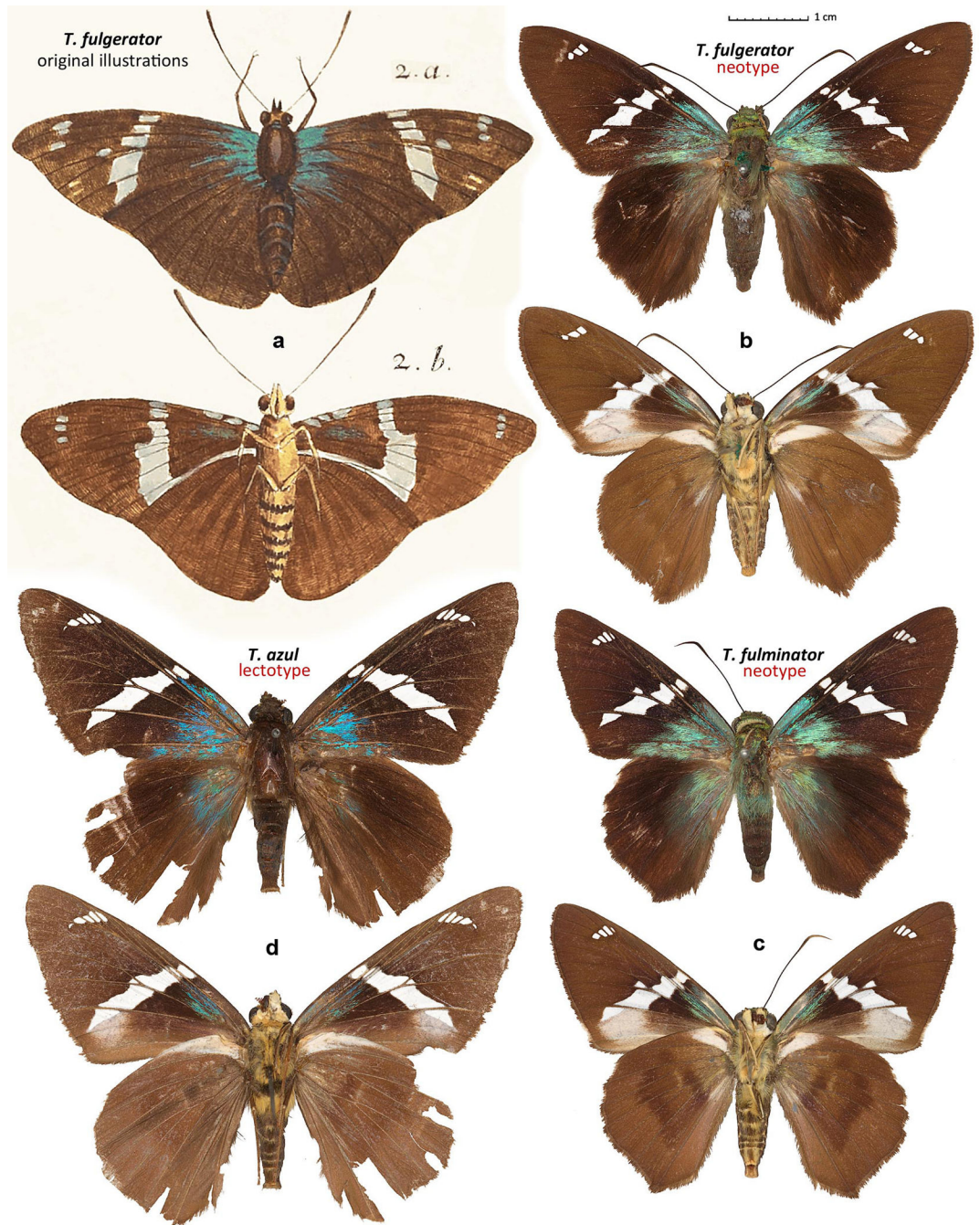
Figure 9.

Telegonus primary types. **a)** *Telegonus fulgerator* original illustrations of the holotype (now lost), rotated from the original, labels (2.a. and 2.b.) repositioned on the images. **b)** *Telegonus fulgerator* neotype ♀ NVG-18057D11 from Suriname in ZSMC. **c)** *Telegonus fulminator* neotype ♀ NVG-18057D08 from Suriname in ZSMC, designated herein. **d)** *Telegonus azul* lectotype ♀ NVG-15099B05 from Mexico: Veracruz in CMNH, designated herein. Dorsal (above each panel label) and ventral (below each panel label) views are shown.
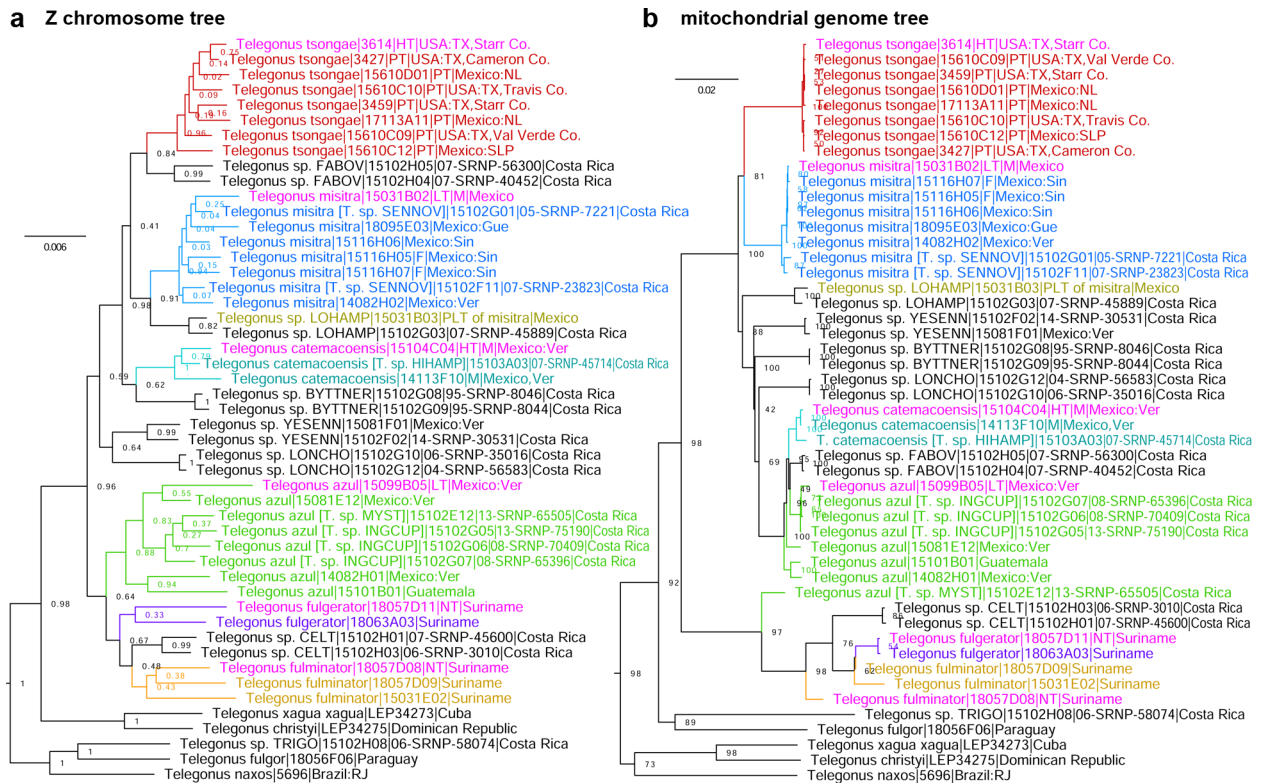
Figure 10.

Trees of *Telegonus fulgerator* complex constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *T. tsongae* **sp. n.** (red), *T. misitra* (blue), *T. catemacoensis* (cyan), *T. azul* (green), *T. fulgerator* (purple), and *T. fulminator* (orange) among their relatives (black). Primary type specimens are labeled in magenta and paralectotype of *T. misitra* in olive. See Fig. 1 legend for other notations.

Figure 11.
Holotype of *Telegonus tsongae* **sp. n.** dorsal (left) and ventral (right) views, data in text.
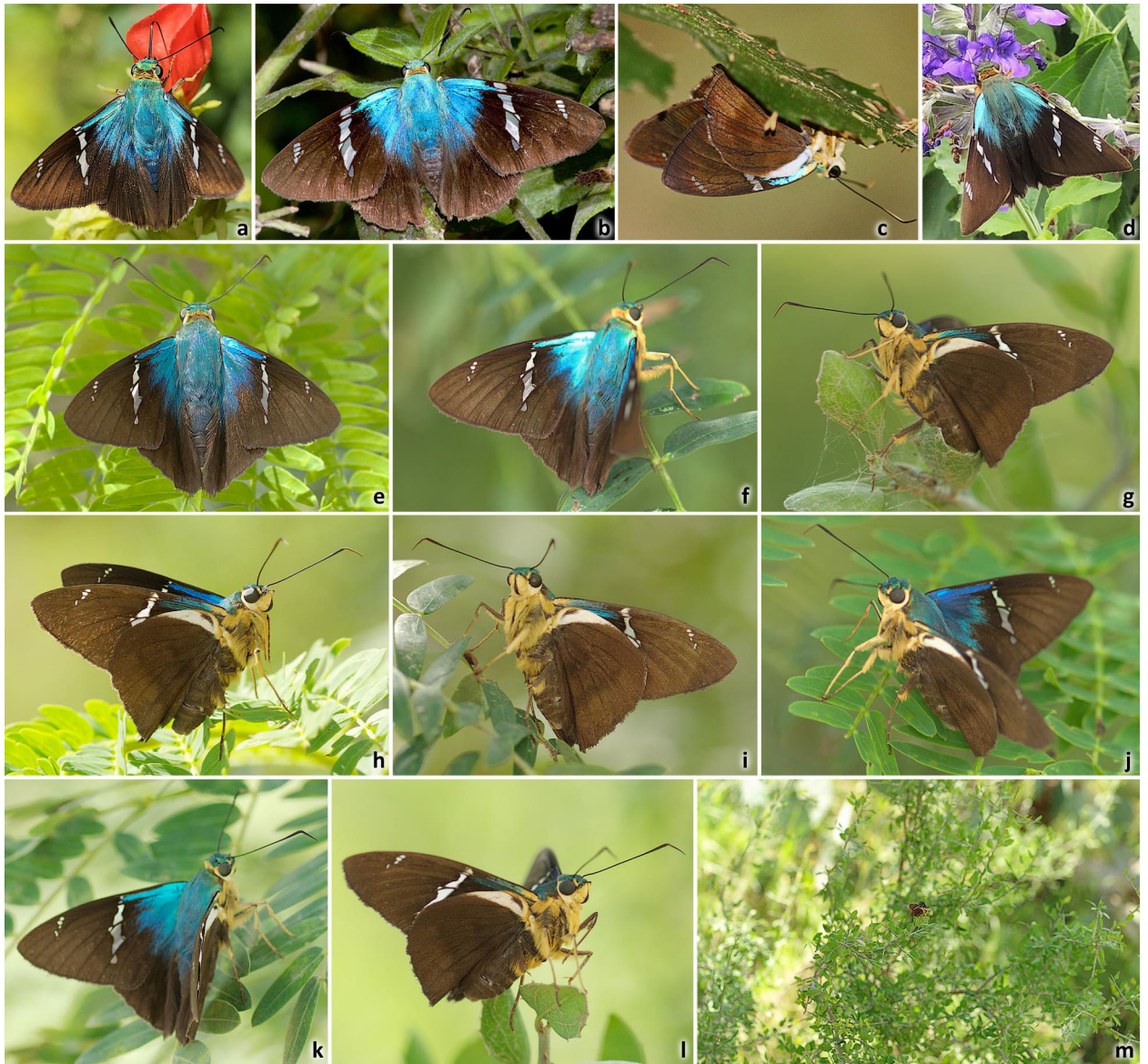
Figure 12.

Telegonus tsongae **sp. n.** in nature, USA: Texas. **a–d)** iNaturalist observations. **a–b)** Hidalgo Co., Bentsen-Rio Grande Valley State Park: 108107791 ♂ 27-Oct-2008 © John Rosford and 110234060 ♀ 25-Dec-2014 © Mark + Holly Salvato, respectively; **c)** 2090814 ♀ Uvalde Co., Cooks Slough Nature Park, 12-Sep-2015 © Diana-Terry Hibbitts; **d)** 787547 ♀ Williamson Co., Liberty Hill, 16-Jul-2014 © Goolsbygirl. Some images were color-corrected and are available under CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/; **e–m)** paratype ♂ NVG-3460 at the type locality, perching in various poses under varying (natural) lighting, 31-May-2015 © Nick V. Grishin, **m)** overall view of the perch with the paratype in the middle of the image, pose magnified in **l)**.
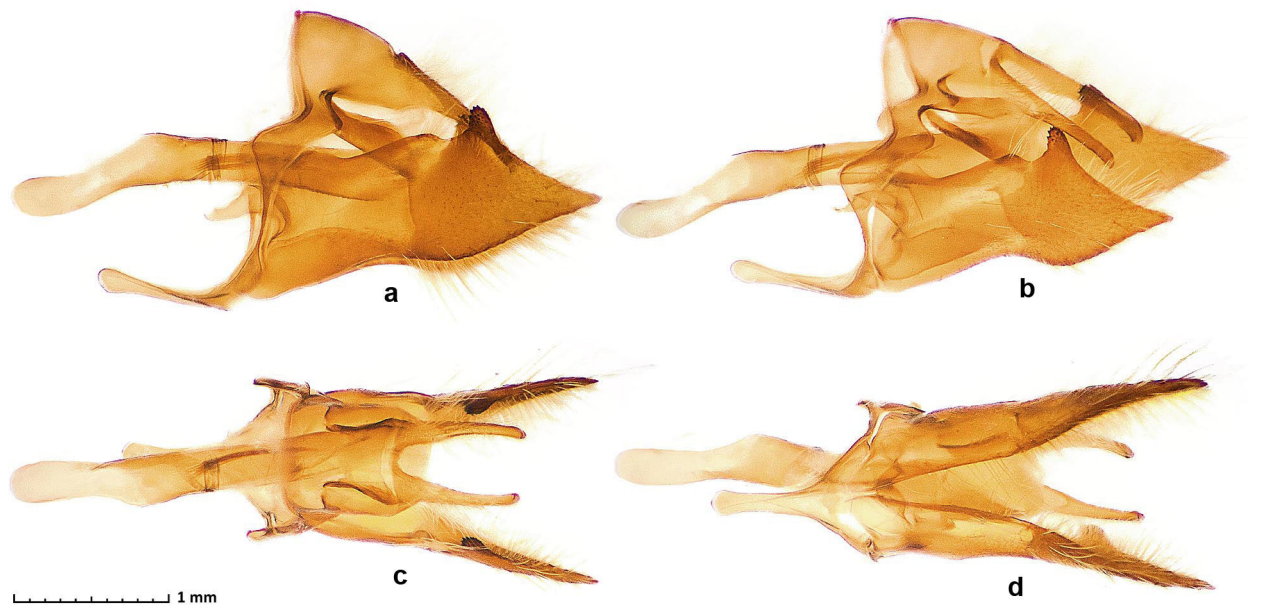
Figure 13.
Genitalia of *Telegonus tsongae* **sp. n.** paratype NVG-3422 (data in text) in different views.
a) left lateral, **b)** left posterolateral, **c)** dorsal, **d)** ventral.

Figure 14.

Caterpillar foodplant and habitat of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., Roma Creek, 3-Nov-2007. **a**, **b**, **d)** *Karwinskia humboldtiana*, **a)** growing in the open, not readily used for oviposition, **b)** flowers, **c)** shaded section of an arroyo (destroyed by now), a typical habitat of immature stages, **d)** enlarged leaves.

Figure 15.
Inducing oviposition in a captive female of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co.
a) a plastic container with a female on the caterpillar foodplant *Karwinskia humboldtiana*,
27-Oct-2007, **b)** many eggs obtained and **c)** the female feeding on sugar solution, 4-
Nov-2007.
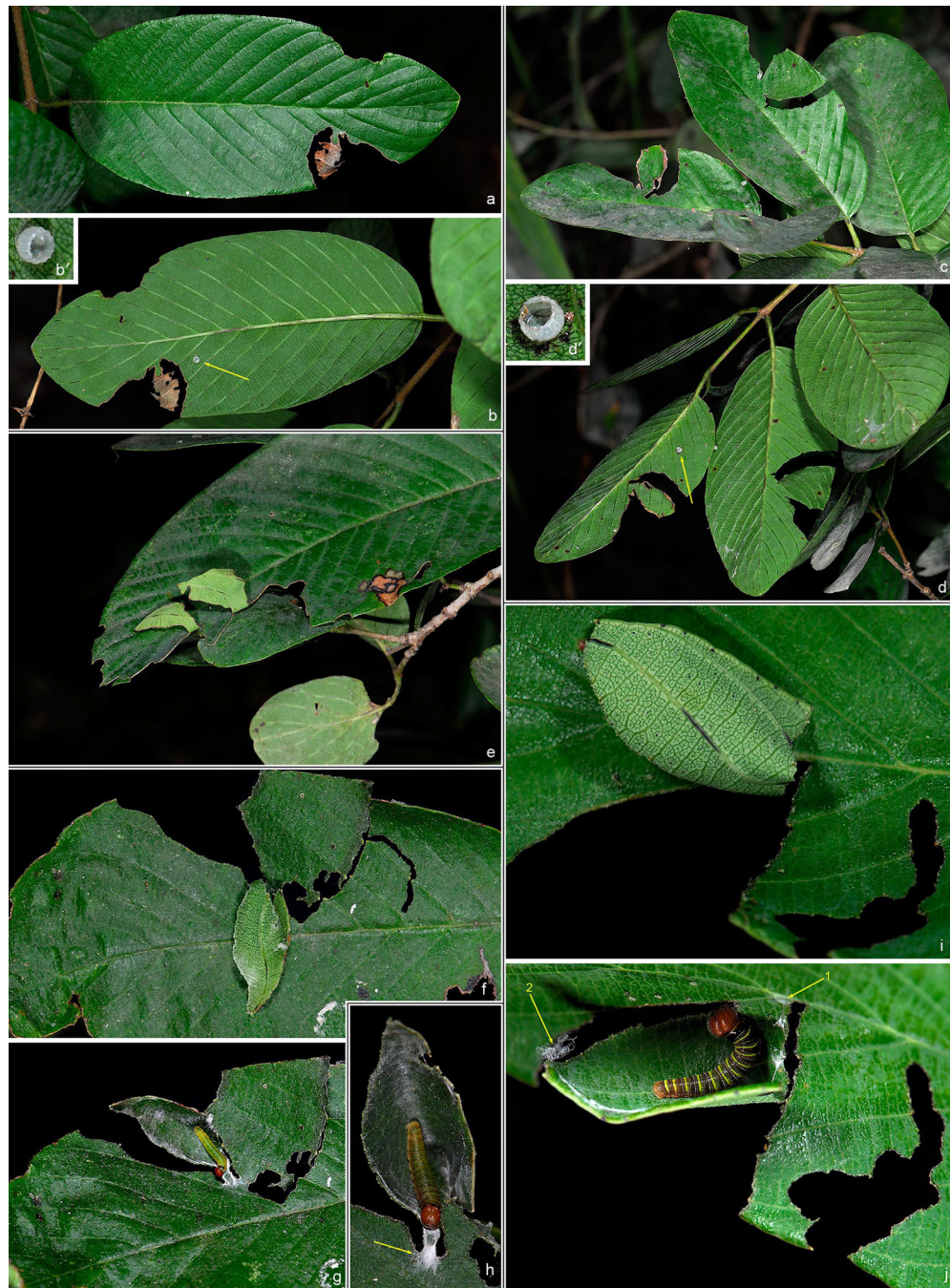
Figure 16.

Shelters of early instar caterpillars of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., Roma Creek, 3-Nov-2007 (except **i–j**. on 7-Nov). **a–e)** leaves with feeding damage and abandoned shelters, **b**, **d)** an eggshell beneath the leaves, indicated by a yellow arrow and enlarged in **b'**) and **d'**); **e)** two shelters on one leaf; **f–j)** shelters with caterpillars, closed (natural appearance, **f**, **i**) and teared-open (for photography, **g**, **h**, **j**); arrows indicate silkpads to fold the shelter (at the base, 1) and to secure it to the leaf (at the tip, 2).

Figure 17.

Eggs and earlier instar caterpillars of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., 2007. **a–e)** eggs, **f)** eggshell, **g–p)** 1st instar, **q–z)** 2rd instar. **d**, **f**, **k**, **m)** 3-Nov, **a**, **b**, **c**, **l**, **n**, **o)** 4-Nov, **p)** 5-Nov, **r–s)** 6-Nov, **e**, **q**, **t**, **v)** 7-Nov, **g**, **h**, **i**, **j**, **w**, **x)** 8-Nov, **u**, **y**, **z)** 20-Nov.

Figure 18.
Late instar caterpillars of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., 2007. **a–d)** 3rd instar, **e–h)** 4th instar. **a)** 16-Nov, **b**, **c**, **d)** 18-Nov, **e)** 19-Nov, **h)** 20-Nov, **f)** 23-Nov, **g)** 28-Nov, **i)** 3-Dec.

Figure 19.

Ultimate instar caterpillars and pupa of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., 2007. **a–i)** 5th instar caterpillar, **j)** prepupa, **k)** pupa within an hour of molting of the caterpillar shown in **j)**, **l)** the same pupa with developed white bloom several hours after molting; **a–c)** 28-Nov, the same individual, **d)** 3-Dec, **e**, **i.** 9-Dec, the same individual, **f)** 16-Dec, **j)** 17-Dec, **k)** 18-Dec, **g**, **l)** 19 Dec; **h)** 30-Dec.

Figure 20.
Ultimate (5th) instar caterpillar of *Telegonus tsongae* **sp. n.**, USA: Texas, Starr Co., 2007.
a) head in anterodorsal view, **b)** ♂ **c)** ♀ 8th segment in dorsal view, a pair of orange testes seen through in ♂, **d)** last segments in dorsal view; **a**, **d)** 25-Dec, the same individual; **b–c)** 30-Dec.

a Z chromosome tree

b mitochondrial genome tree
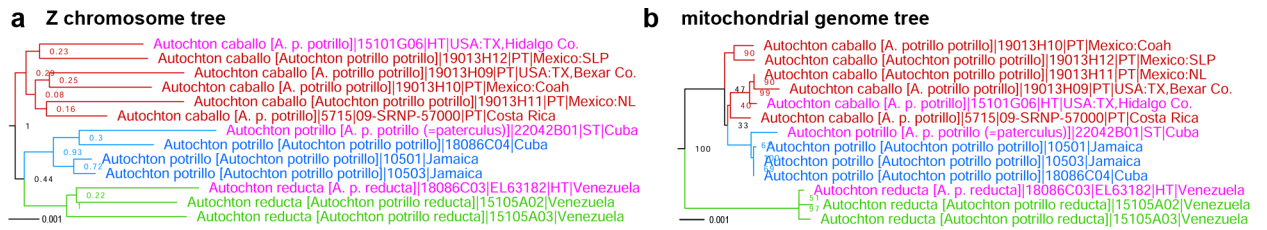
Figure 21.

Trees of *Autochton potrillo* group constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *A. caballo* **sp. n.** (red), *A. potrillo* (blue), and *A. reducta* (green). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
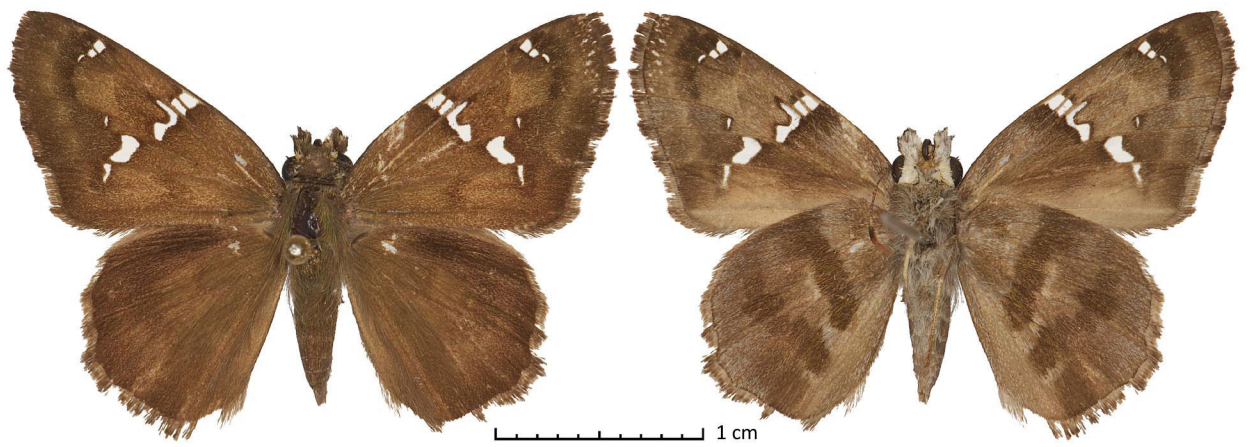
Figure 22.
Holotype of *Autochton caballo* **sp. n.** dorsal (left) and ventral (right) views, data in text.
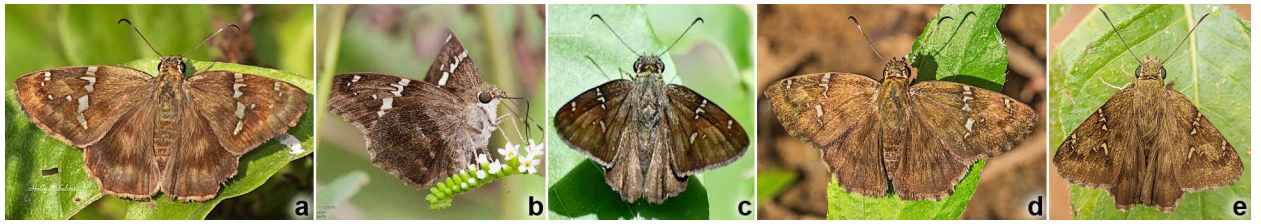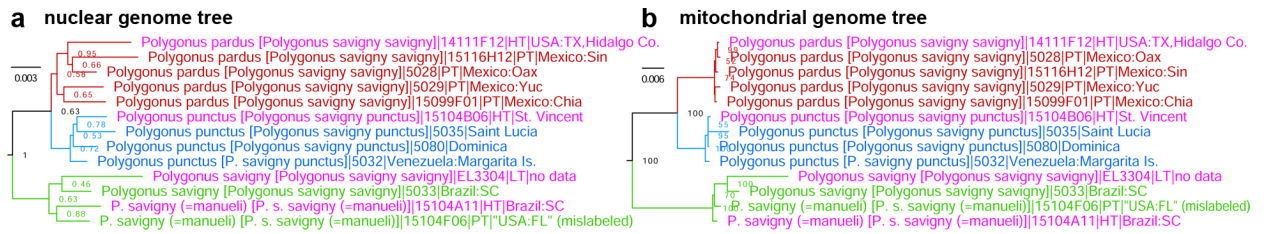
Figure 23.

Three species of *Autochton*, iNaturalist observations. **a–b)** *A. caballo* **sp. n**. USA: TX, Hidalgo Co., Mission. **a)** 104493477 11-Nov-2017 © Mark + Holly Salvato. **b)** 116272640 22-Nov-2010 © John Rosford. **c–d)** *A. potrillo*. **c)** 57177194 Cuba: Guamá, 14-Aug-2020 © Alexis Felipe. **d)** 40695160 Dominican Republic: Hato Mayor del Rey, 7-Jan-2020 © djhiker. **e)** *A. reducta* 11287957 Colombia: Atlántico, Tubará, 3-Jan-2018 © djhiker. Some images are color-corrected and/or rotated. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.
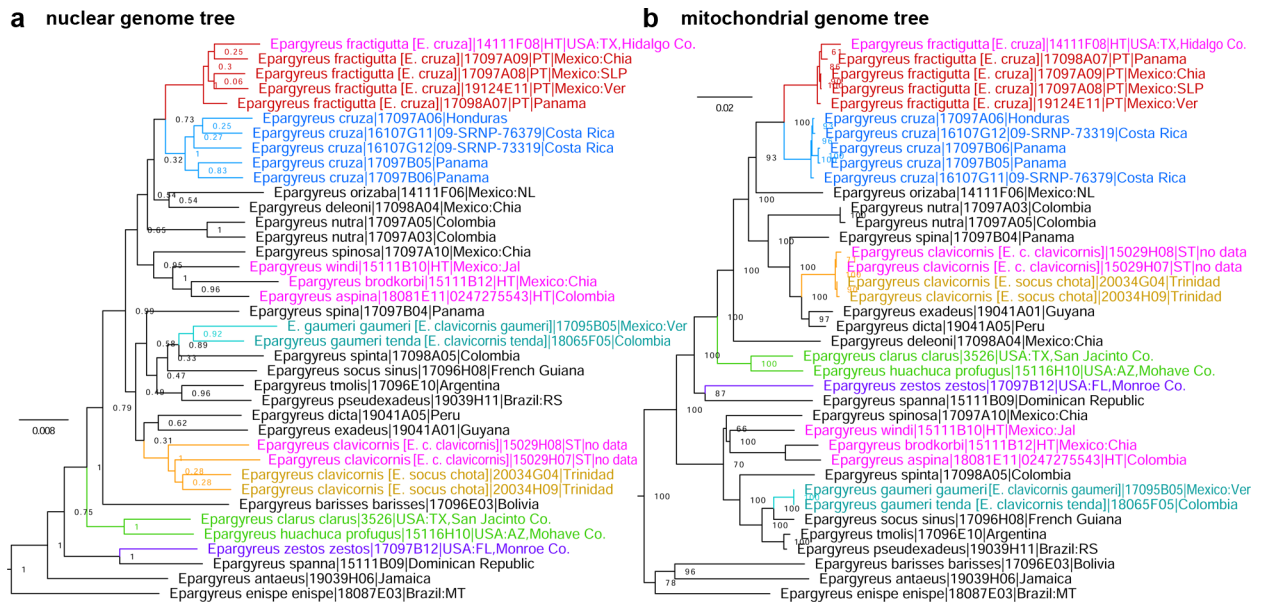
Figure 24.

Trees of *Epargyreus* constructed from protein-coding regions in **a)** nuclear and **b)** mitochondrial genomes: *E. fractigutta* **sp. n.** (red), *E. cruza* (blue), *E. gaumeri* (cyan), *E. clavicornis* (orange), *E. clarus* with *E. huachuca* (green), and *E. zestos* (purple) among their relatives (black). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.

Figure 25.
Holotype of *Epargyreus fractigutta* **sp. n.** dorsal (left) and ventral (right) views, data in text.
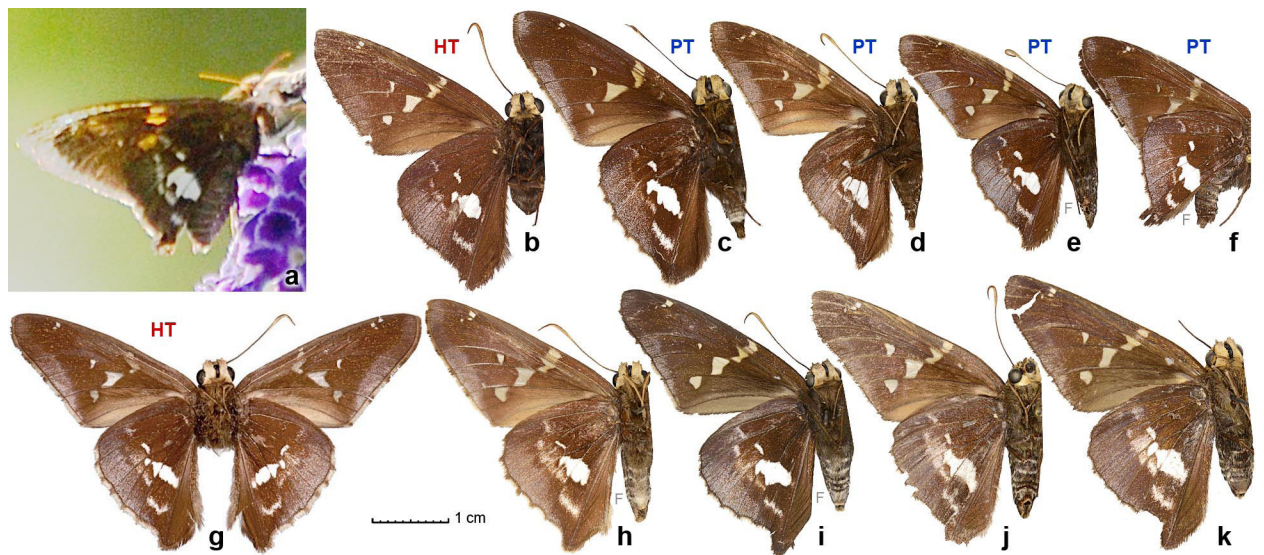
Figure 26.

Two species of *Epargyreus*. **a)** Possible *E. fractigutta* **sp. n.**, iNaturalist observation 61025624 USA: Texas, Hidalgo County, Mission area, details "obscured", Sep-2020 © Beverly Pardue, the image is brightened, flipped, and rotated. **b–f)** The type series of *E. fractigutta* **sp. n. b)** holotype, NVG-14111F08 USA: TX, others are paratypes, detailed data in text. **c)** NVG-17097A09 MX: Chiapas, **d)** NVG-17097A08 MX: SLP, **e)** NVG-17098A07 Panama, **f)** NVG-19124E11 MX: Veracruz. **g–k)** *Epargyreus cruza*. **g)** holotype [BMNH] © of the Trustees of the Natural History Museum London, others are in USNM. **h)** NVG-17097A06 Honduras. **i)** NVG-16107G11, 09-SRNP-76379 Costa Rica. **j)** NVG-17097B06 Panama, **k)** NVG-17097B05 Panama. Gray F indicates that the image is flipped (left-right inverted). Photographs **a)** and **g)** are made available under CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.

**a** Z chromosome tree

b mitochondrial genome tree
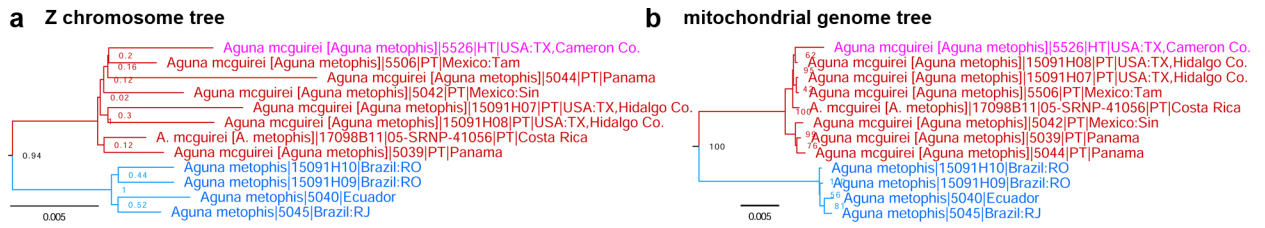
Figure 27.

Trees of *Aguna metophis* group constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *A. mcguirei* **sp. n.** (red), and *E. metophis* (blue). The holotype is labeled in magenta. See Fig. 1 legend for other notations.

Figure 28.
Holotype of *Aguna mcguirei* **sp. n.** dorsal (left) and ventral (right) views, data in text.
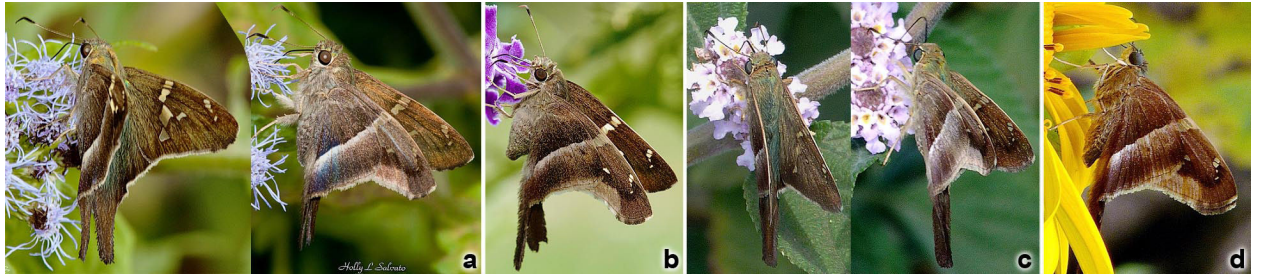
Figure 29.

Two species of *Aguna*, iNaturalist observations. **a–b)** *A. mcguirei* **sp. n.** USA: TX, Hidalgo Co: **a)** 110235614 Estero Llano Grande State Park, 5-Nov-2014 © Mark + Holly Salvato. **b)** 123646178 Mission, 1-Nov-2012 © John Rosford. **c–d)** *A. metophis*. **c)** 130511769 Brazil: Minas Gerais, 12-Aug-2022 © Carlos Otávio Gussoni. **d)** 122340621 Brazil: São Paulo, Rio Claro, 7-Jan-2020 © Carlos Otávio Gussoni. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.
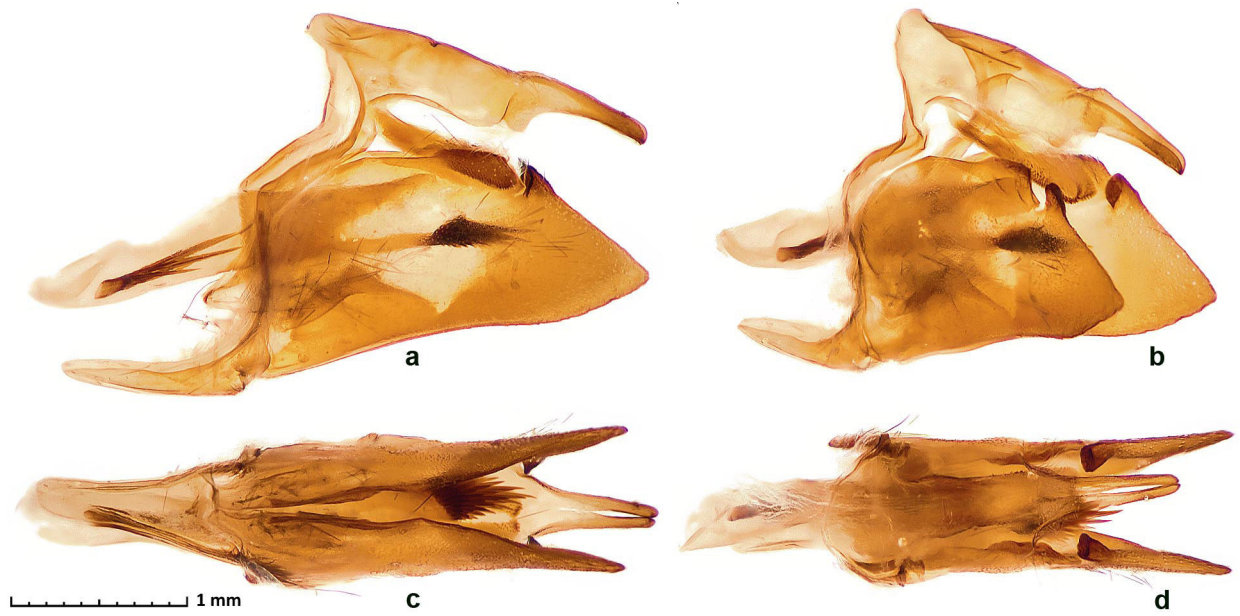
Figure 30.
Genitalia of *Aguna mcguirei* **sp. n.** holotype (data in text) in different views. **a)** left lateral,
b) left posterolateral, **c)** ventral, **d)** dorsal.

a nuclear genome tree

b mitochondrial genome tree

Figure 31.

Trees of *Polygonus* constructed from protein-coding regions in **a)** nuclear and **b)** mitochondrial genomes: *P. pardus* **sp. n.** (red), *P. punctus* (blue), and *P. savigny* (green). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
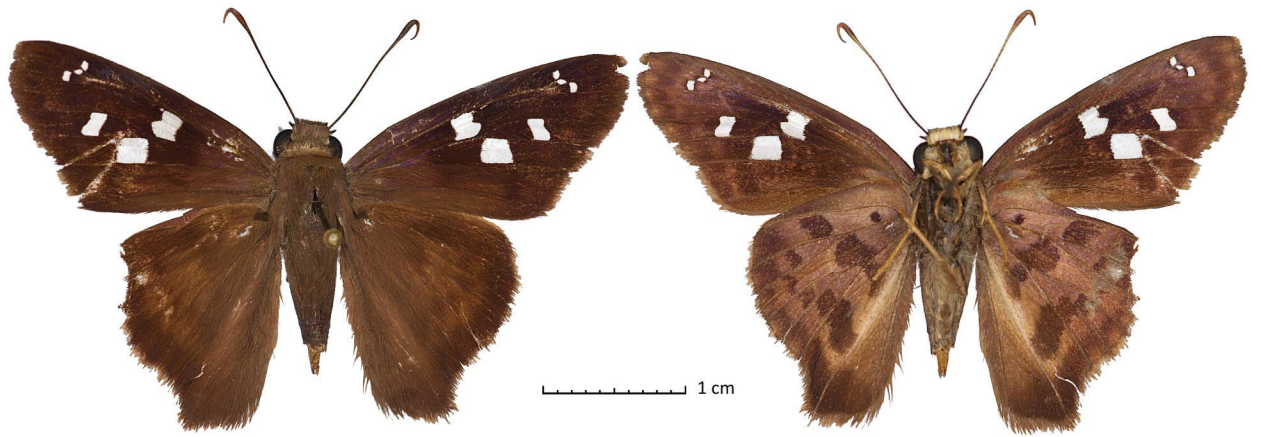
Figure 32.
Holotype of *Polygonus pardus* **sp. n.** dorsal (left) and ventral (right) views, data in text.

Figure 33.

Three species of *Polygonus*, iNaturalist observations. **a)** *P. pardus* **sp. n.** 21129976 USA: TX, Hidalgo Co., Estero Llano Grande State Park, 16-Nov-2018 © Susan Blayney. **b)** *P. punctus* 90418403 Guadeloupe: Terrede-Haut, 15-Jun-2021 © ombeline_sculfort. **c)** *P. savigny* 40923980 Brazil: Paraná, Curitiba, 8-Mar-2020 © sergiomessias. Some images are color-corrected and/or rotated. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.
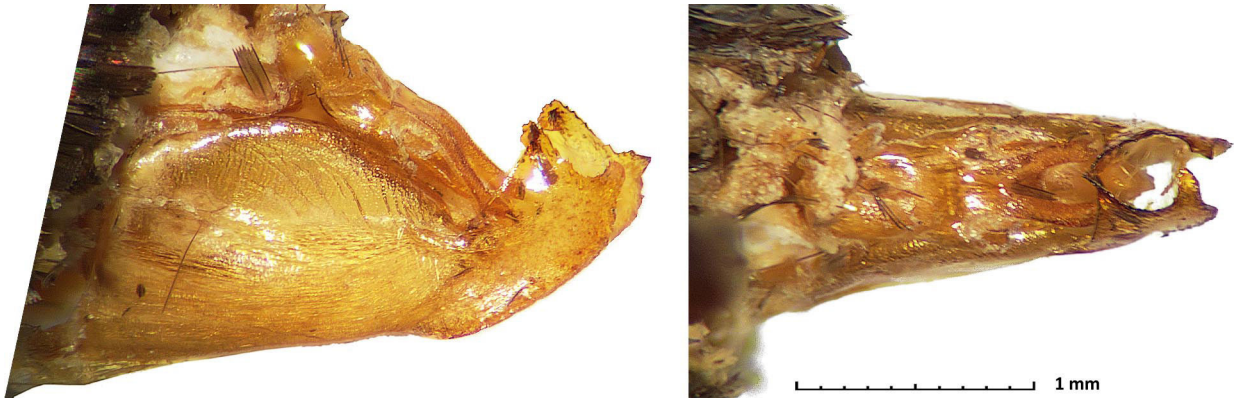
Figure 34.
Genitalia of *Polygonus pardus* **sp. n.** holotype (data in text) in situ, left lateral (left) and dorsal (right) views.

a Z chromosome tree

b mitochondrial genome tree
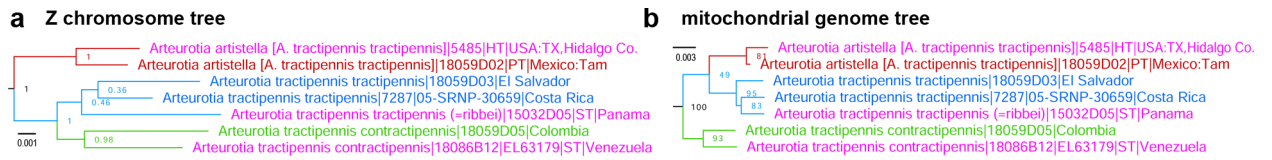
Figure 35.

Trees of *Arteurotia* constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *A. artistella* **sp. n.** (red), *A. tractipennis tractipennis* (blue), and *A. tractipennis contractipennis* (green). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
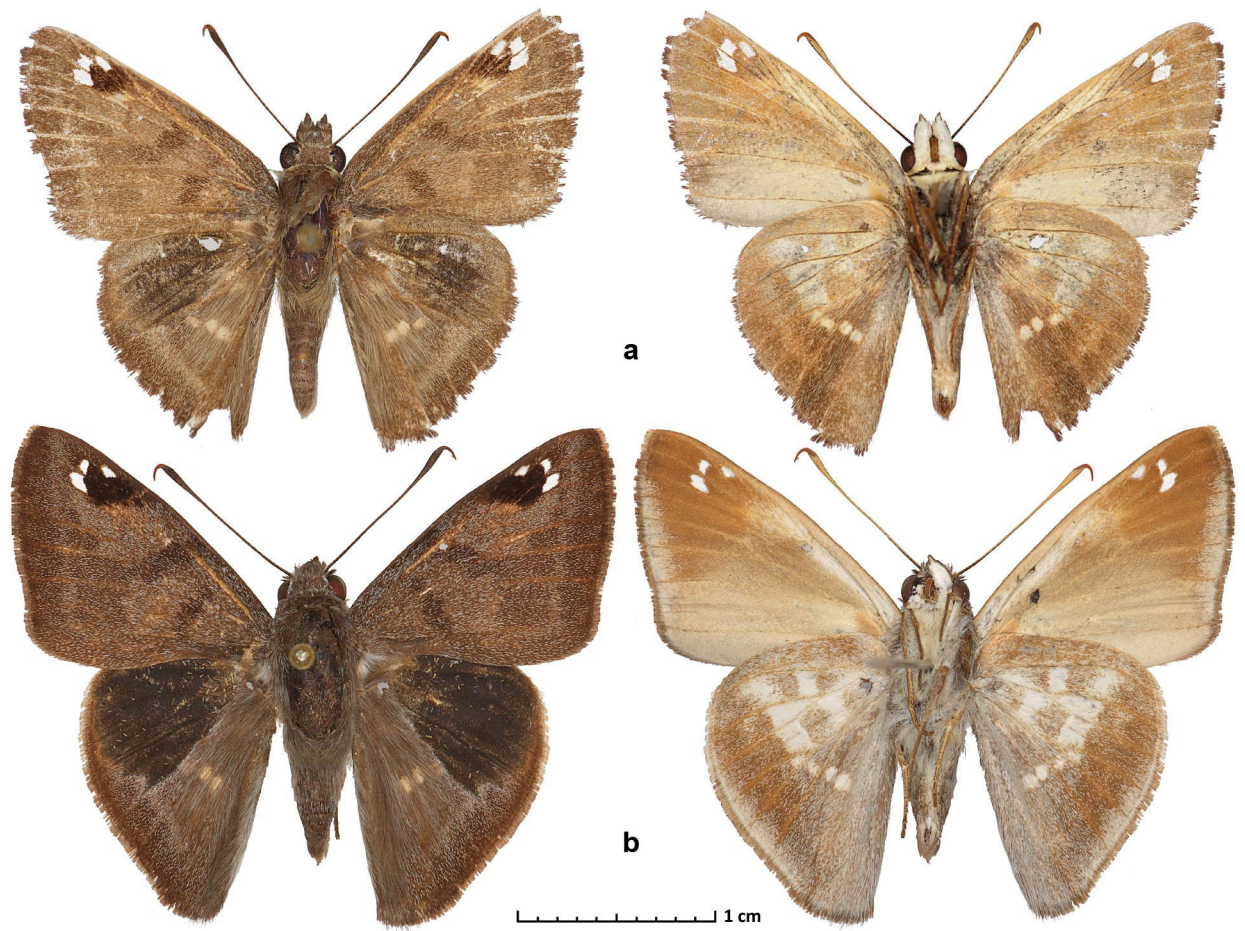
Figure 36.
The type series of *Arteurotia artistella* **sp. n**. **a)** holotype ♂ NVG-5485, **b)** paratype ♂
NVG-18059D02, dorsal (left) and ventral (right) views, data in text.
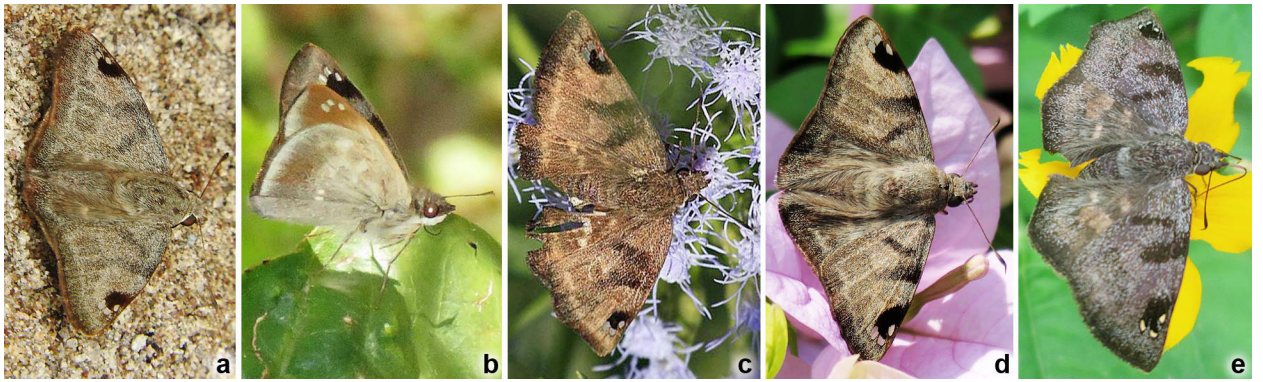
Figure 37.
Two species of *Arteurotia*, iNaturalist observations. **a–c)** *A. artistella* **sp. n**. **a)** 29447636 Mexico: San Luis Potosí, Aquismón, 16-Jul-2019 © Carlos G Velazco-Macias. **b)** 65660684 Mexico: Guanajuato, Victoria, Rancho Viejo, 8-Nov-2020 © Ma. Eugenia Mendiola González. **c)** 9615777 USA: Texas, Hidalgo Co, Mission, 3-Nov-2010 © upupamartin. **d–e)** *A. tractipennis*. **d)** 43115536 Honduras: Francisco Morazán, Santa Ana, 24-Apr-2020 © John van Dort. **e)** 69372846 Colombia: Sucre, San Benito Abad, 6-Sep-2017 © Jeir Ortega Galvan. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.
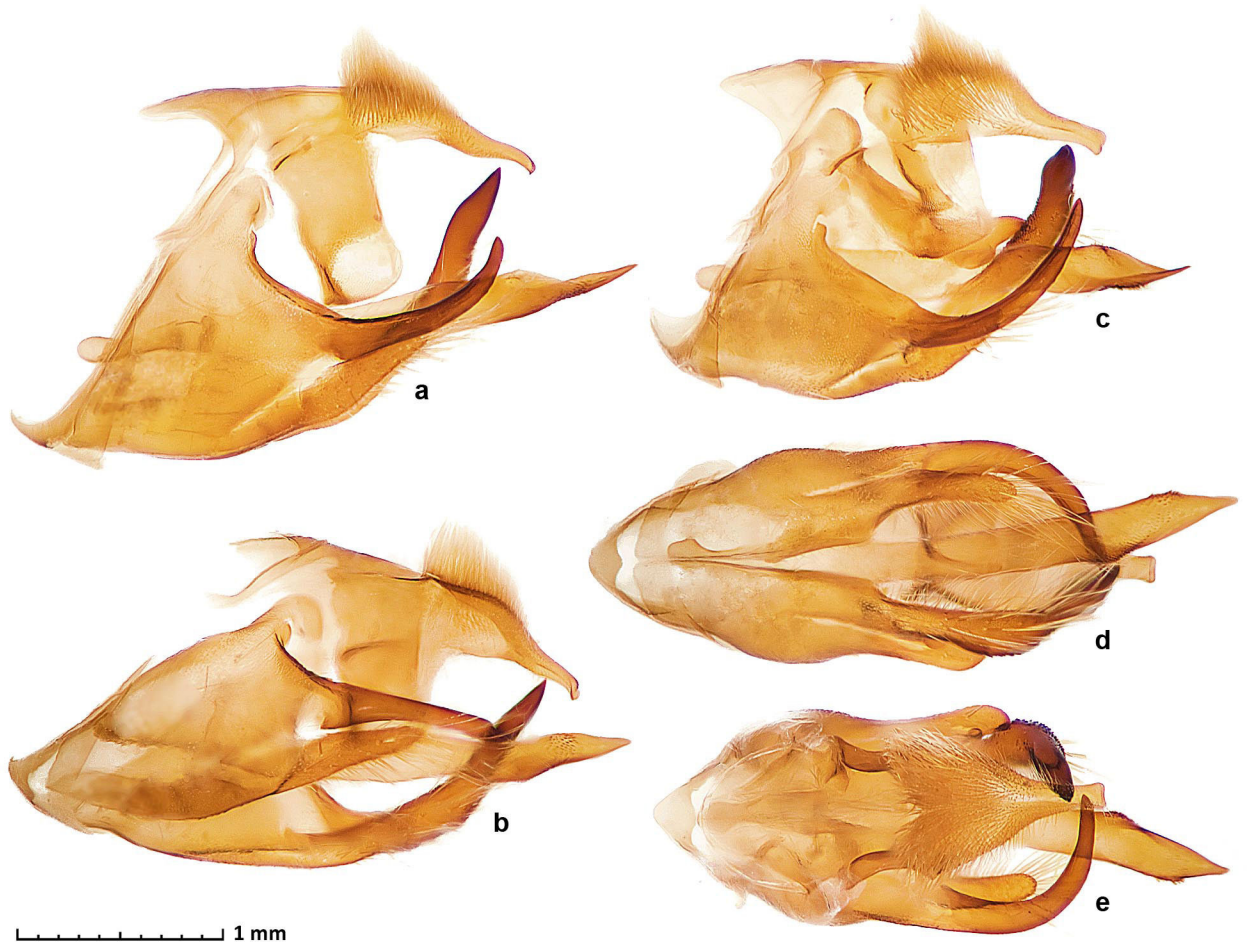
Figure 38.
Genitalia of *Arteurotia artistella* **sp. n.** holotype (data in text) in different views. **a)** left lateral, **b)** left ventrolateral, **c)** left posterolateral, **d)** ventral, and **e)** dorsal.

Author Manuscript
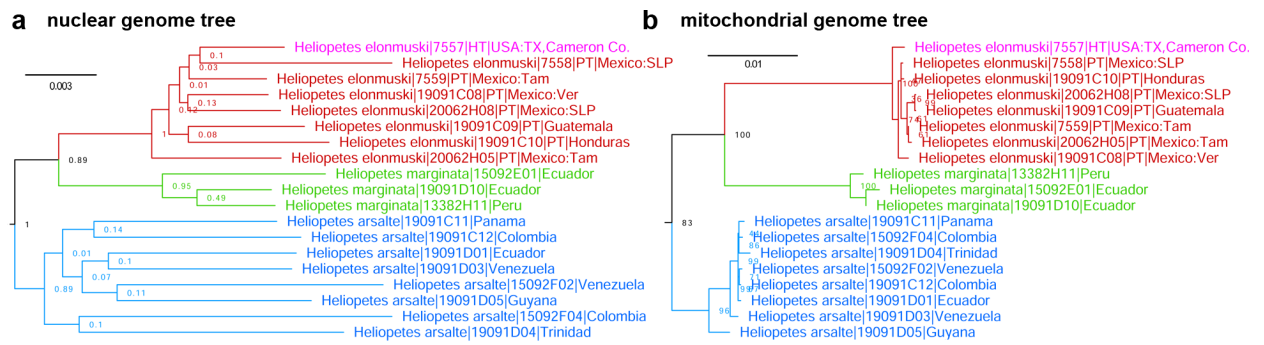
Figure 39.

Trees of *Heliopetes arsalte* group constructed from protein-coding regions in **a)** nuclear and **b)** mitochondrial genomes: *H. elonmuski* **sp. n.** (red), previously regarded as conspecific with *H. arsalte* (blue), and *H. marginata* (green). The holotype is labeled in magenta. See Fig. 1 legend for other notations.
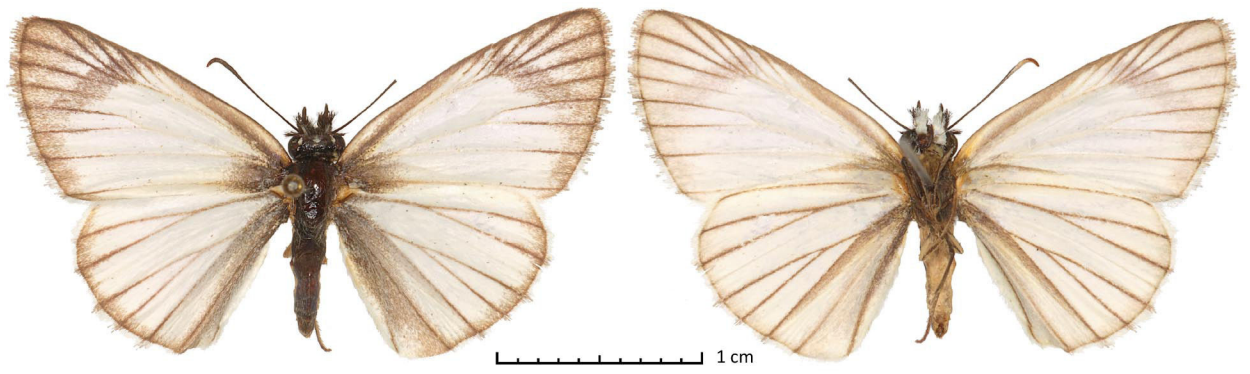
Figure 40.
Holotype of *Heliopetes elonmuski* **sp. n.** dorsal (left) and ventral (right) views, data in text.
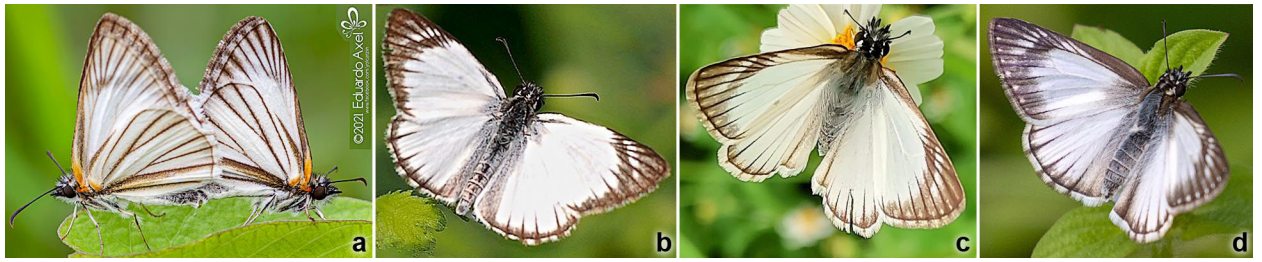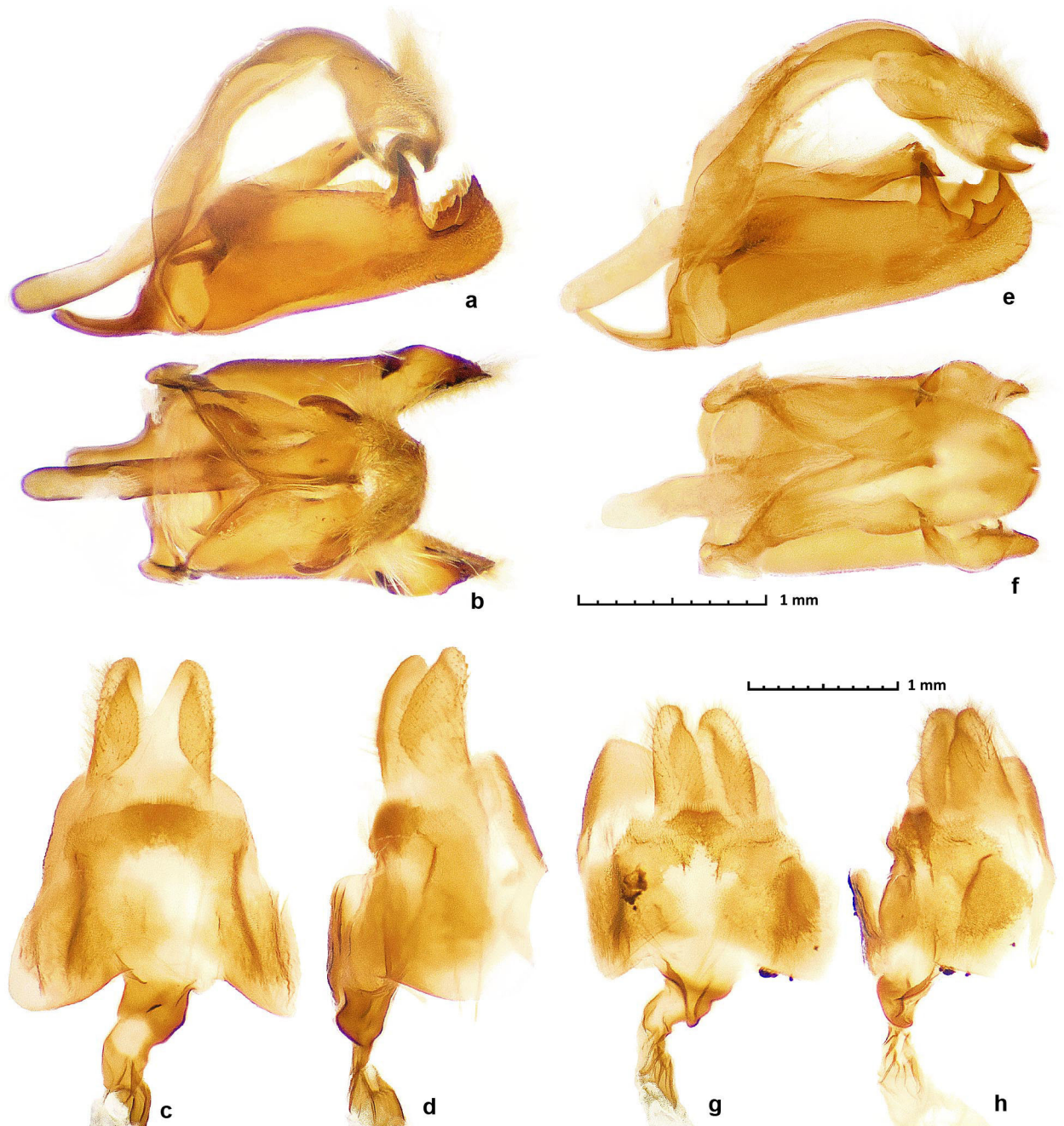
Figure 41.
Two species of *Heliopetes*, iNaturalist observations. **a–c)** *Heliopetes elonmuski* **sp. n**. **a)** in copula ♀ (left), ♂ (right) 101984200 Mexico: Veracruz, vic. Huejutla de Reyes, 13-Nov-2021 © Eduardo Axel Recillas Bautista. **b)** ♂ 31651109 Belize: Crooked Tree, 29-Jul-2015 © shirdipam. **c)** ♀ 131414102 Mexico: Chiapas, Ocosingo, 18-Aug-2022 © Silvano LG. **d)** ♀ *H. arsalte* 117179611 Brazil: Paraiba, Pitimbu, 14-May-2022 © Thomaz de Carvalho Callado. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.
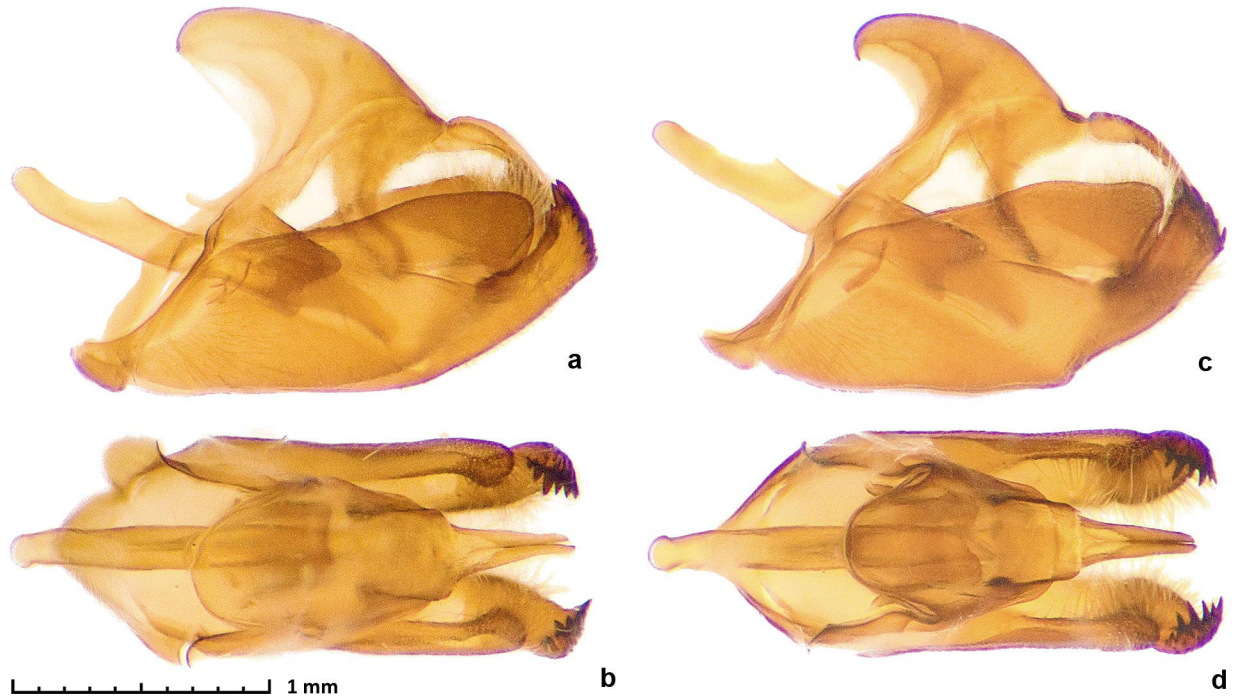
Figure 42.
Male genitalia of *Heliopetes*. **a–b)** Paratype of *H. elonmuski* **sp. n.** NVG-20062H08 (data in text) in different views: **a)** left lateral, **b)** dorsal. **c–d)** *H. arsalte* NVG-20062H07 from Brazil: Rondônia, 6–8 km NE of Cacaulandia, 23-Apr-1992, C. J. Durden leg. [TMMC]: **c)** left lateral, **d)** dorsal.
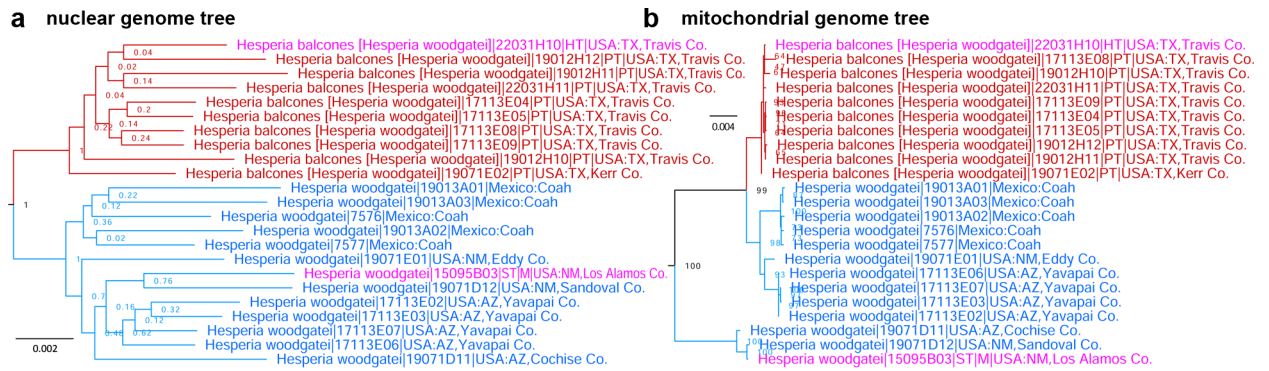
Figure 43.

Trees of *Hesperia woodgatei* group constructed from protein-coding regions in **a)** nuclear and **b)** mitochondrial genomes: *H. balcones* **sp. n.** (red) and *H. woodgatei* (blue). A syntype is labeled in magenta. See Fig. 1 legend for other notations.

Figure 44.
Holotype of *Hesperia balcones* **sp. n.** dorsal (left) and ventral (right) views, data in text.
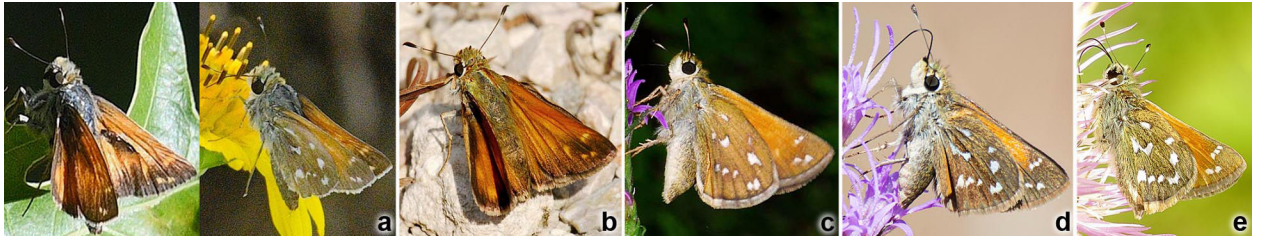
Figure 45.

Two species of *Hesperia* from the USA. Numbers are given for iNaturalist observations. **a–d)** *H. balcones* **sp. n.**, Texas. **a)** 99404027 Burnet Co., Balcones Canyonlands National Wildlife Refuge, 17-Oct-2021 © Jack Cochran. **b–c)** Travis Co. Volente, 6-Oct-2007 © Nick V. Grishin. **d)** 2146572 Burnet Co., Balcones Canyonlands National Wildlife Refuge, 16-Oct-2015 © Roger Shaw. **e)** *H. woodgatei* 16707845 NM: Crant Co., Lake Roberts, 16-Sep-2018 © Bill Carrell. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.

Figure 46.
Genitalia of *Hesperia*. **a–d)** *H. balcones* **sp. n.** paratypes, data in text: **a–b)** NVG-22031H11 ♂ and **c–d)** NVG-19071E02 ♀. **e–h)** *H. woodgatei* from USA: New Mexico [USNM]: **e–f)** NVG-19071D12 ♂ topotype, Jemez Springs, 7000 ft, Aug-1930, J. Woodgate leg. and **g–h)** NVG-19071E01 ♀ Eddy Co., Guadalupe Mountains National Park, N McKittrick Canyon, 1-Oct-1983, S. J. Cary leg., in different views: **a, e)** right lateral, **b, f)** dorsal, **c, g)** ventral, **d, h)** right lateral. Female genitalia (corpus bursae is not shown) are slightly reduced (smaller scalebar) compared to male genitalia (larger scalebar).

Figure 47.

Immature stages of *Hesperia balcones* **sp. n.** from the type locality. **a–b)** An egg in different views, 22-Oct-2007. **c–l)** Caterpillars of different instars: 1st [**c)** 22-Oct-2007, **d–e)** 21-Oct-2007], 2nd [**f)** 8-Nov-2007], 3rd [**g)** 18-Nov-2007], 4th [**h)** 26-Nov-2007, **i)** 3-Dec-2007], 5th [**j)** 3-Dec-2007, **k)** 22-Dec-2007], 6th [**l)** 10-Jan-2008]. **m–o)** A pupa in different views, 26-Jan-2008: **m)** dorsal, **n)** ventral, **o)** right lateral. All images are to scale.
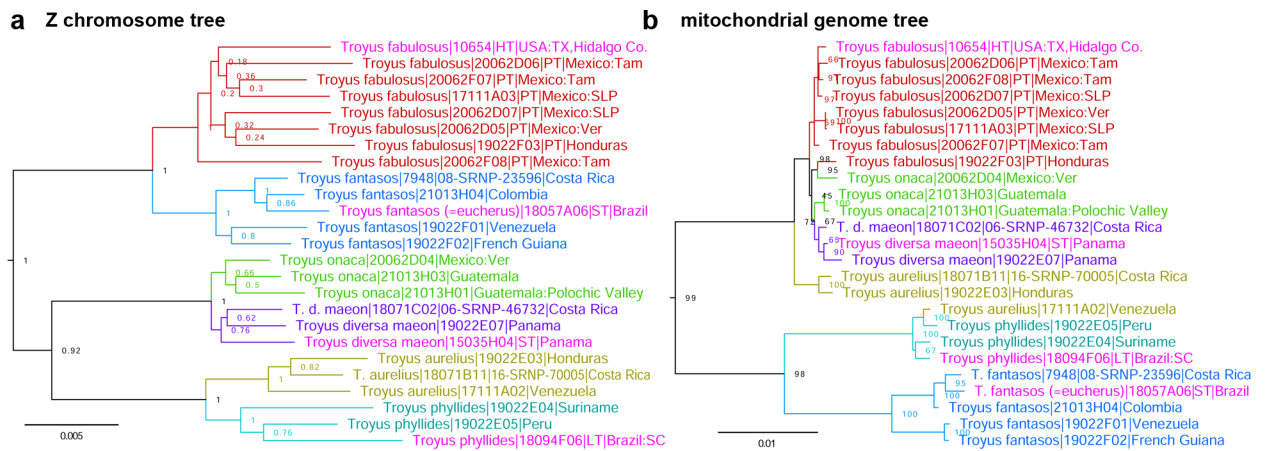
Figure 48.

Trees of *Troyus* constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *T. fabulosus* **sp. n.** (red), previously regarded as conspecific with *T. fantasos* (blue), *T. onaca* (green), *T. diversa maeon* (purple), *T. aurelius* (olive), and *T. phyllides* (cyan). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
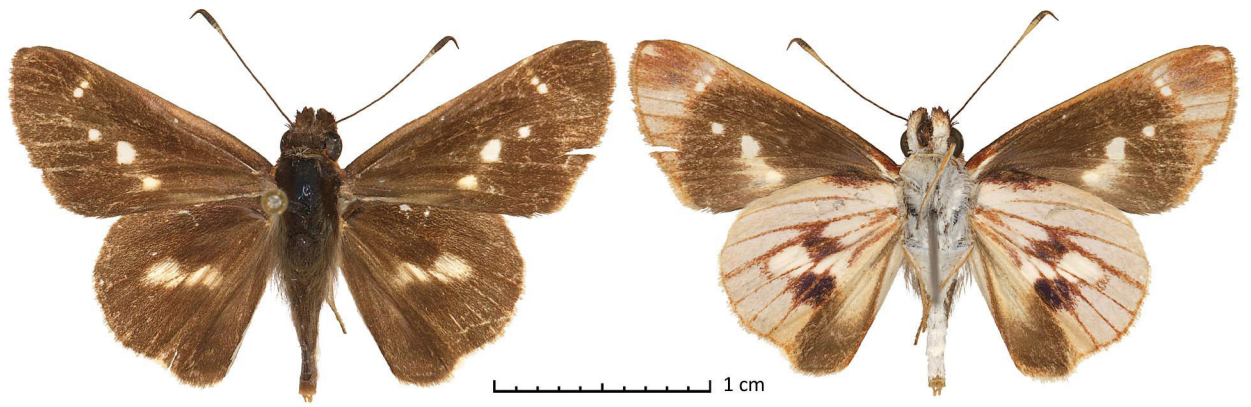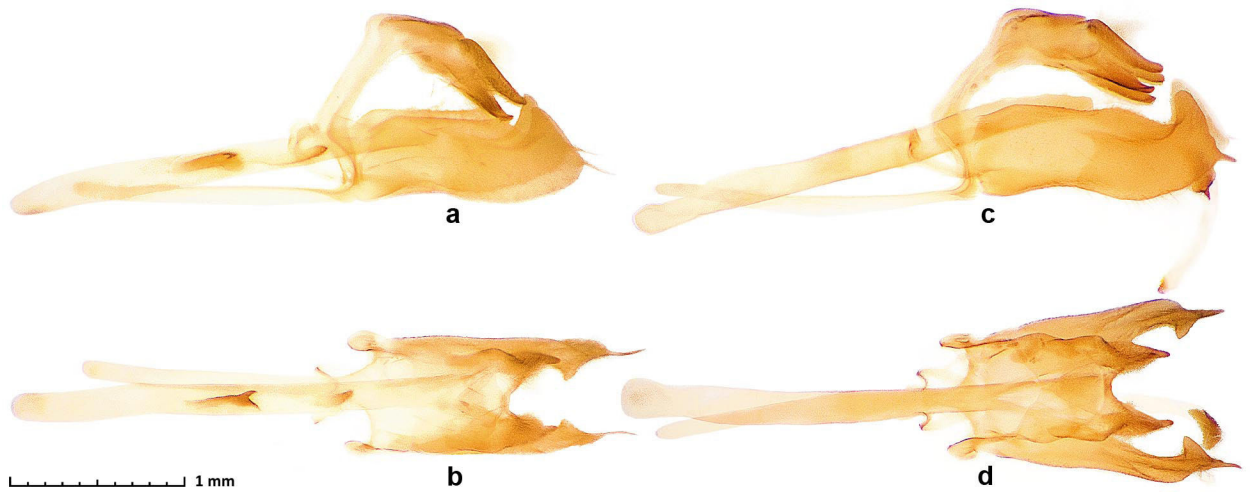
Figure 49.

Holotype of *Troyus fabulosus* **sp. n.** dorsal (left) and ventral (right) views, data in text.
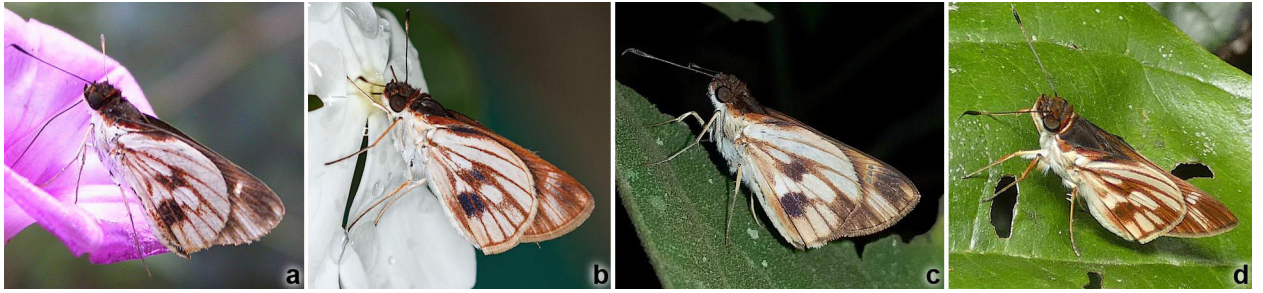
Figure 50.
Two species of *Troyus*, iNaturalist observations. **a–b)** *T. fabulosus* **sp. n.**, Mexico. **a)** 2698712 Nuevo León, Santiago, 21-Oct-2003 © Nick Block. **b)** 55341546 Querétaro, Jalpan de Serra, 27-Jul-2020 © Felix Fleck. **c–d)** *T. fantasos.* **c)** 101683213 Colombia: Cundinamarca, Nocaima, 21-Nov-2021 © Oscar Enciso. **d)** 98977311 Brazil: Pernambuco, Paudalho, 10-Oct-2021 © Helio Lourencini. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.

Figure 51.

Male genitalia of *Troyus*. **a–b)** Paratype of *T. fabulosus* **sp. n.** NVG-20062F08 (data in text) in different views: **a)** left lateral, **b)** dorsal. **c–d)** *T. fantasos* NVG-21013H04 (vesica everted) from Colombia: Dept. Magdalena, Bonda, 250 ft, Sep-[about 1920], Holland collection [CMNH]: **c)** left lateral, **d)** dorsal.
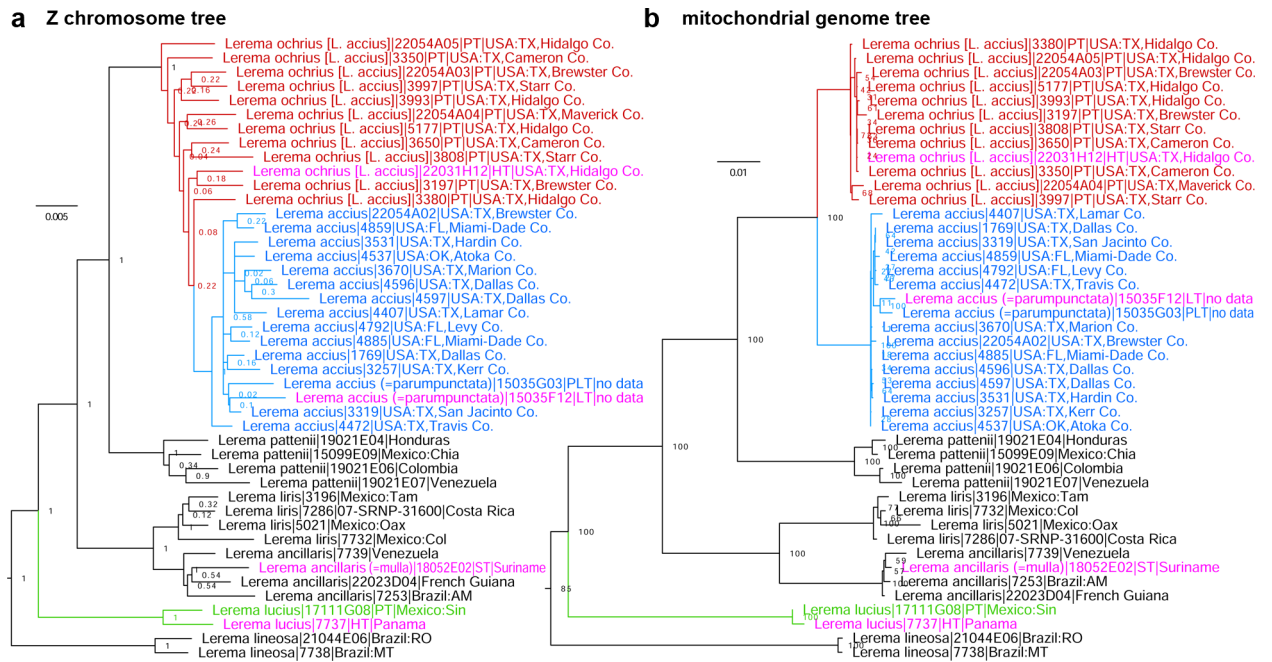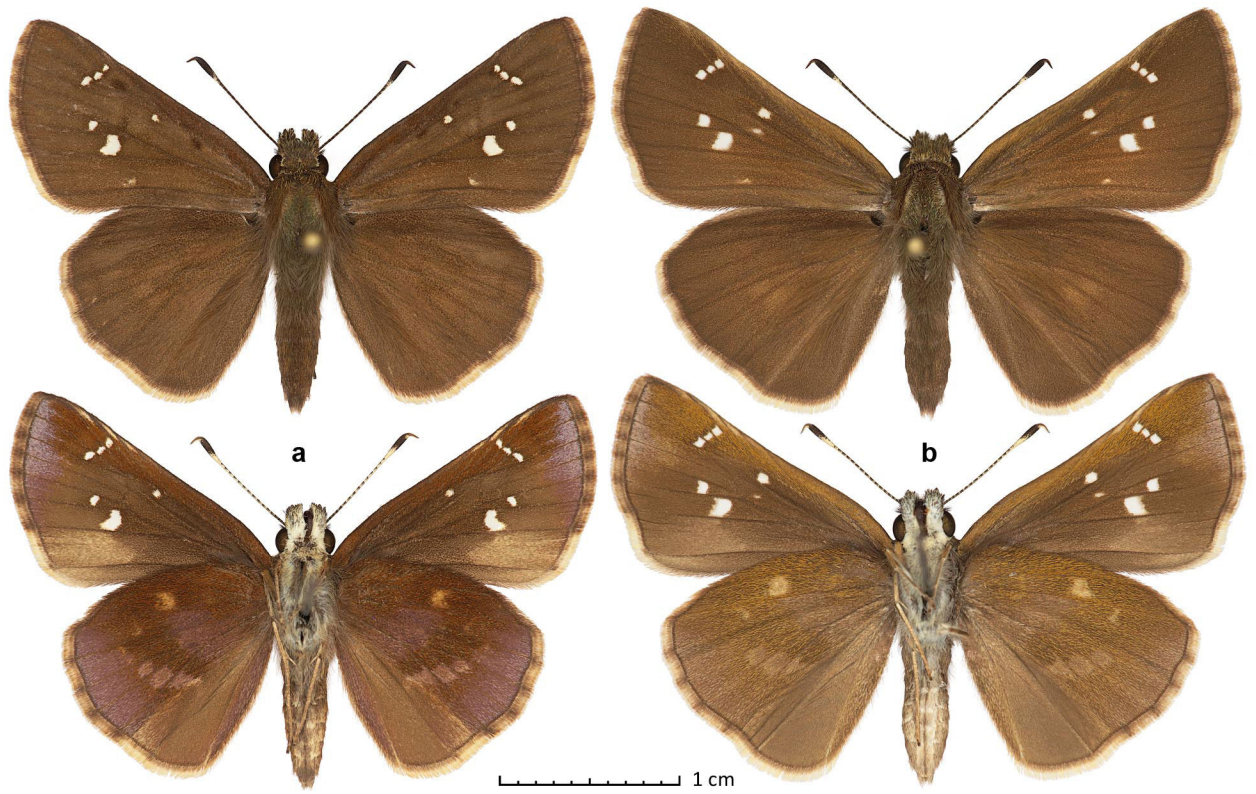
Figure 52.

Trees of *Lerema* constructed from protein-coding regions in **a)** Z chromosome and **b)** mitochondrial genome: *L. ochrius* **sp. n.** (red), *L. accius* (blue), and *L. lucius* (green) among their relatives (black). Primary type specimens are labeled in magenta. See Fig. 1 legend for other notations.
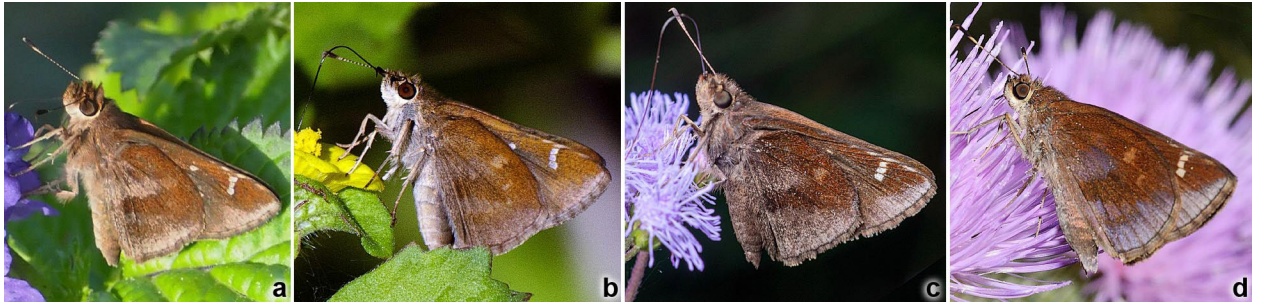
Figure 53.

Reared specimens of *Lerema* from USA: Texas, N. V. Grishin leg., in dorsal (left of the letter) and ventral (right of the letter) views. **a–b)** *L. ochrius* **sp. n.** from Hidalgo Co., 1.5 air mi SE of Relampago, Old Rio Rico Rd.: **a)** paratype ♂, eclosed 7-Jul-2015; **b)** holotype ♀ NVG-22031H12, eclosed 14-Jun-2015. **c–d)** *L. accius* from Denton Co., Flower Mound, nr. Grapevine Lake: **c)** ♂ eclosed 31-Jul-1997; **d)** ♀ eclosed 29-Sep-1997.
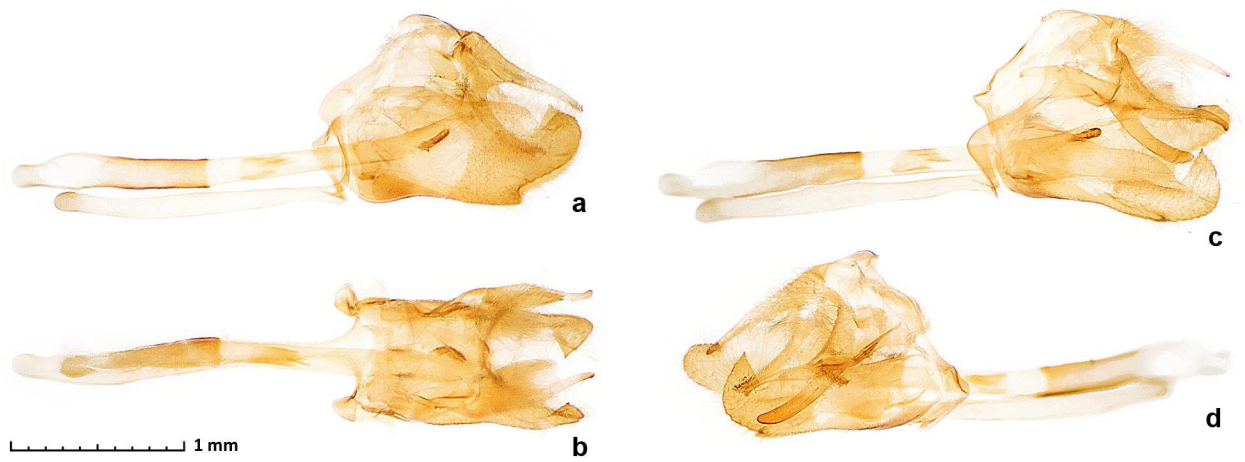
Figure 54.
Reared specimens of *Lerema* from USA: Texas, Brewster Co., Big Bend National Park, Rio Grande Village campground, N. V. Grishin leg., females, ex larva, in dorsal (above) and ventral (below) views. **a)** *L. accius* NVG-22054A02 eclosed 23-Jun-2005. **b)** paratype of *L. ochrius* **sp. n.** NVG-22054A03 eclosed 1-Jun-2005.
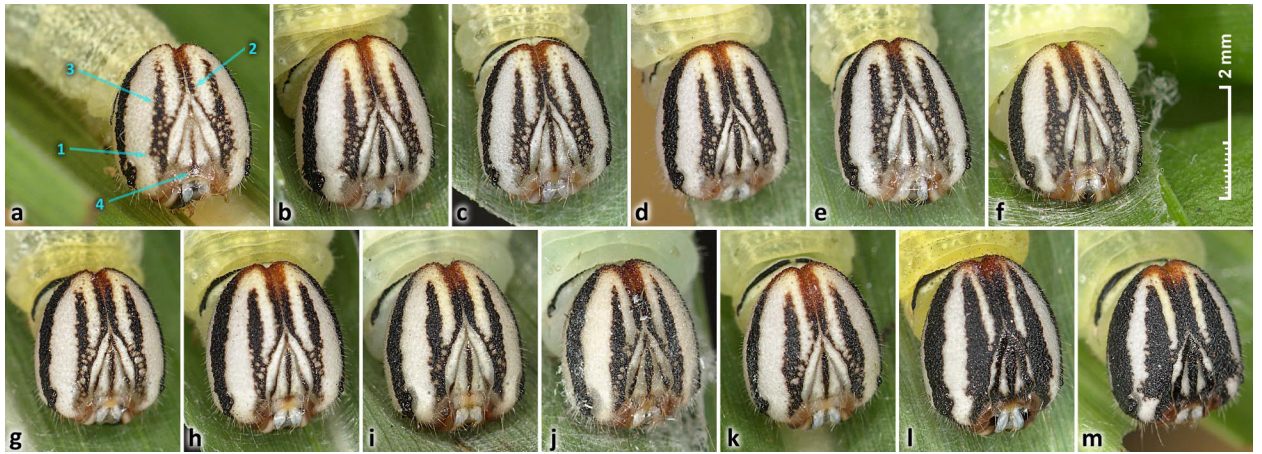
Figure 55.

Two species of *Lerema* from the USA, iNaturalist observations. **a–b)** *L. orchius* **sp. n.**, Texas, Hidalgo Co. **a)** 104144271 Mission, 1-Jan-2022 © Cin-Ty Lee. **b)** 130093189 Edinburg, 8-Oct-2021 © Scott. **c–d)** *L. accius.* **c)** 138909684 Georgia, Clarke Co., Athens, 15-Oct-2022 © Diego Huet. **d)** 136277302 Illinois, Johnson Co., Cypress, 22-Sep-2022 © Harlan Ratcliff. Some images are color-corrected, rotated, and/or flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.

Figure 56.
Male genitalia of *Lerema ochrius* **sp. n.** paratype NVG-3258 (data in text) in different views:
a) left lateral, **b)** dorsal (uncus twisted to the right to expose harpe), **c)** left dorsolateral, **d)** right dorsolateral.

Figure 57.

Heads of *Lerema* 5th instar caterpillars from USA: Texas, 2015. **a–f)** *Lerema ochrius* **sp. n**. **a)** Starr Co., Roma 7-Aug. **b–e)** The type locality, 7-Sep. **f)** Cameron Co., River Dr., 1.4 mi S of Santa Maria, 25-Jun. **g–m)** *Lerema accius*. **g–h)** Dallas Co., Dallas: Norbuck Park, 7-Aug. Moss Park: **i)** 7-Aug, **j)** 11-Aug, **l)** 19-Jul. **k, m)** nr. White Rock Lake, 22-Jul. Numbered cyan arrows in 57a refer to characters discussed in text.

Figure 58.
Eggs and caterpillars of *Lerema ochrius* **sp. n.** from USA: Texas, 2015. Photographs taken on the same date show the same individuals, except 58k, which is a different individual from the caterpillar in 58l–n. **a–c)** Eggs. **d–n)** Caterpillars of different instars: 1st (**d–i**), 3rd just molted with exuviae behind and the head capsule in front (**j**), 4th feeding (**k**), 5th (**l–n**). Cameron Co., River Dr., 1.4 mi S. of Santa Maria: **a–b)** 19-Jun, **d–e)** 23-Jun; **f–g)** 2.5 mi SW of Sebastian, 25-Jun; Starr Co., Roma: **c)** 5-Jul, **h–i)** 9-Jul, **j)** 27-Jul, **k–n)** 4-Aug.