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Taxonomic advances driven by the genomic analysis of butterflies

Jing Zhang[1,2,3], Qian Cong[1,3], Jinhui Shen[1,2], Leina Song[1,2], and Nick V. Grishin[1,2*]

Departments of [1]Biophysics, [2]Biochemistry, and [3]Eugene McDermott Center For Human Growth & Development, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9050, USA;
[*]Corresponding author: *grishin@chop.swmed.edu*

ABSTRACT. This study presents new findings based on a large-scale analysis of butterfly genomic sequences. Focusing on species identification through comparative genomics, we define subspecies as populations differentiated to a lesser extent than distinct species ("species in the making"). Additionally, we propose further adjustments to the current butterfly classification. As a result, 3 subgenera, 12 species, and 4 subspecies are described as new. New subgenera are (type species in parenthesis): *Hyalaus* Grishin, **subgen. n.** (*Papilio epidaus* E. Doubleday, 1846) of *Eurytides* Hübner, [1821] (Papilionidae Latreille, [1802]) and *Astria* Grishin, **subgen. n.** (*Lycaena astraea* Freyer, 1851) of *Glaucopsyche* Scudder, 1872 and *Afroidium* Grishin, **subgen. n.** (*Lycaena metophis* Wallengren, 1860) of *Brephidium* Scudder, 1876 (Lycaenidae [Leach], [1815]). New species are (type localities in parenthesis): *Ithomiola* (*Ithomiola*) *tesseroides* Grishin, **sp. n.** (Panama: Chiriquí), *Ithomiola* (*Ithomiola*) *coladoris* Grishin, **sp. n.** (Panama: Cerro Jefe), *Ithomiola* (*Ithomiola*) *perutanos* Grishin, **sp. n.** (Peru: Cuzco), *Lasaia pallida* Grishin, **sp. n.** (Venezuela: Maracay), *Synargis regina* Grishin, **sp. n.** (Peru: Chanchamayo), *Synargis reginella* Grishin, **sp. n.** (Brazil: Pará), *Synargis flavicauda* Grishin, **sp. n.** (Peru: Rio Pachitea, Monte Alegre), *Synargis tenebritorna* Grishin, **sp. n.** (Brazil: Bahia), and *Synargis latidifa* Grishin, **sp. n.** (French Guiana) in Riodinidae Grote, 1895 (1827) and *Heliopetes* (*Heliopetes*) *acuta* Grishin, **sp. n.** (Mexico: Oaxaca), *Metrocles nun* Grishin, **sp. n.** (Brazil: Goiás), and *Hedone miracla* Grishin, **sp. n.** (Peru: Lima, Chancay River valley) in Hesperiidae Latreille, 1809. New subspecies are: *Microtia elva bogotana* Grishin, **ssp. n.** (Colombia: Bogota), and *Boloria astarte alaskia* Grishin, **ssp. n.** (USA: Alaska, Seward Peninsula) in Nymphalidae Rafinesque, 1815; *Lasaia sessilis oaxacensis* Grishin, **ssp. n.** (Mexico: Oaxaca) in Riodinidae; and *Cupido* (*Everes*) *comyntas orientalis* Grishin, **ssp. n.** (USA: North Carolina, Mecklenburg Co.) in Lycaenidae [Leach], [1815]. A number of taxonomic changes are proposed. The following taxa are species (not subspecies): *Eurema ella* (Röber, 1909) and *Eurema flavescens* (Chavannes, 1850) (not *Eurema elathea* (Cramer, 1777) and *Eurema millerorum* Llorente & Luis, 1987 (not *Eurema agave* (Cramer, 1775)) (Pieridae Swainson, 1820); *Ithomiola tessera* J. Hall, 2005 (not *Ithomiola theages* Godman & Salvin, 1878), *Lasaia callaina* Clench, 1972 (not *Lasaia agesilas* (Latreille, [1809])), and *Synargis attilius* (Stichel, 1925) (not *Synargis regulus* (Fabricius, 1793)) (Riodinidae); and *Gerosis cnidus* (Plötz, 1884) (not *Gerosis phisara* (Moore, 1884)), *Vettius prona* Evans, 1955 (not *Vettius phyllus* (Cramer, 1777)), *Vettius pica* (Herrich-Schäffer, 1869) (not *Vettius lafrenaye* (Latreille, [1824])), and *Phlebodes yalta* (Evans, 1955) (not *Phlebodes fuldai* (E. Bell, 1930)) (Hesperiidae). The following taxa are junior subjective synonyms: *Lycaena sissona* W. G. Wright, 1905 of *Cupido comyntas* (Godart, [1824]) (Lycaenidae) and *Punta* Evans, 1955 of *Paracarystus* Godman, 1900 (Hesperiidae). *Coladenia hamiltoni* Nicéville, 1889 and *Caprona*? *kuki* Tytler, 1915 are junior subjective synonyms of *Gerosis cnidus*, not *Gerosis phisara*. *Albulina* Tutt, 1909 is a genus distinct from *Agriades* Hübner, [1819]. *Eugrumia* Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 is a valid genus, and *Sinerebia* Nakatani, 2017 is its junior subjective synonym. The following are subgenera, not genera or synonyms: *Oraidium* Bethune-Baker, 1914 of *Brephidium* Scudder, 1876, *Vacciniina* Tutt, 1909 of *Agriades* Hübner, [1819], and *Arianna* Bálint, 2022 of *Plebejus* Kluk, 1780 (Lycaenidae) and *Uniphylus* Lemes, Siewert, O. Mielke, Casagrande & A. Warren, 2023 of *Bolla* Mabille, 1903 (Hesperiidae). We propose new genus-subgenus combinations *Erythia fayneli* (Gallard, 2006) (not in *Euselasia* Hübner, [1819]) and *Gallio coda* Evans, 1955 (not in *Tigasis* Godman, 1900) and new species-subspecies combination *Eurema flavescens obsoleta* (Jörgensen, 1932) (not *Eurema elathea* (Cramer, 1777)). We present the genomic phylogeny of all known species of *Euriphellus* Austin, 2008 (Hesperiidae). **Lectotypes** are designated for *Polyommatus comyntas* Godart, [1824] (type locality in USA: California, as deduced by genomic comparison) (Lycaenidae) and *Achlyodes cnidus* Plötz, 1884 (type locality unknown, possibly in eastern India or Bangladesh) (Hesperiidae).

Additional keywords: taxonomy, subspecies, classification, genomics, phylogeny, biodiversity.

ZooBank registration: http://zoobank.org/2B44E674-0784-4977-ADE5-A8AD69E30582

INTRODUCTION AND METHODS

Building upon the previous research in butterfly genomics, this study focuses on different taxa utilizing our established techniques (Cong et al. 2019a, b, 2020, 2021; Li et al. 2019; Zhang et al. 2019a–d, 2020, 2021, 2022b, c; Zhang et al. 2023c, f, g; Robbins et al. 2022). The goal is to improve the taxonomic classification of butterflies through genomic analysis. The selected methodology involves the examination of diverse butterfly specimens from around the world. These specimens are predominantly obtained from museum and private collections (refer to the acknowledgments section for specifics), spanning ages from approximately 250 years to recently gathered samples. Whenever possible, we conduct DNA sequencing on primary type specimens to establish an objective reference for their nomenclature (Zhang et al. 2022a). DNA extraction mostly employs the legs of specimens, and our non-destructive procedure preserves them. The extracted DNA undergoes fragmentation unless the specimen's DNA is inherently short due to aging, after which it is sequenced using the Illumina next-generation sequencing platform with 150 bp reads. Our approach avoids relying on the amplification of specific genes or segments; instead, we sequence each extracted DNA fragment. As a result, the protocol proves effective even with historical specimens, whose DNA may be fragmented into 30–50 bp segments.

Utilizing sequence data, particularly segments of 150 base pairs or less from each specimen, we construct exons of protein-coding genes. This construction is based on an available reference genome of a species most closely related based on phylogeny. The protein-coding genes are used for inferring phylogenetic trees. Three trees are constructed using IQtree v1.6.12 under the GTR+GAMMA model (Nguyen et al. 2015): one tree from autosomes in the nuclear genome, another from the gene predicted to be located in the Z chromosome, and a third from the mitochondrial genome. To streamline the computational workload, we randomly select 100,000 codons, representing approximately 2% of the total dataset, to construct the nuclear trees (equivalent to 300,000 base pairs). Statistical support for tree branches is determined based on 100 replicates, each composed of 10,000 codons randomly sampled from the complete set. Trees are constructed for each replicate, and the statistical support value, ranging from 0 to 100, corresponds to the number of replicates with a bipartition identical to that in the 100,000-codon tree. Additional details are given in our previous publications (Li et al. 2019; Zhang et al. 2022b).

The phylogenetic trees undergo visualization, rotation, and colorization using FigTree (Rambaut 2018). We overlay the current taxonomic classification onto these trees to identify non-monophyletic taxa and pinpoint clades corresponding to taxa lacking names. Genomic trees often reveal "levels," representing specific points in time when diversification occurred independently in multiple lineages (Zhang et al. 2021). These instances of "synchronized" diversification result from geological events impacting major lineages simultaneously, offering an opportunity to align taxonomic ranks with observed levels in genomic trees. This approach results in an objective and internally consistent classification considering both genetic differentiation and paleontological history. Classification decisions heavily rely on genomic trees, with morphological considerations as supplementary evidence. Genomes provide a comprehensive view of an organism, encompassing more information than morphology traditionally used in butterfly classification. Genomes encode data about life histories, habitat preferences, mating behaviors, and dietary sources. While predicting phenotypic traits from genetic information may be limited, we use its genetic equivalent from random codons to deduce a taxonomic classification rooted in statistically supported phylogeny.

The taxa we establish are monophyletic and align with prominent clades. When we say "prominent," we refer to branches in the tree with robust statistical support, typically showing 100% agreement among replicates and generally longer than neighboring branches. The length of a branch directly correlates with the number of base-pair substitutions along it. Longer branches not only receive strong statistical support but also harbor a higher count of base-pair substitutions, likely leading to more noticeable phenotypic changes. These changes may be evident in various morphological characteristics, not necessarily confined to adults but potentially observable in immature stages or other aspects of the phenotype. However, the relationship between the number of genetic changes and visually significant phenotypic differences is highly non-linear (Zhang et al. 2019a). Consequently, short tree branches can

correspond to visually distinguishable taxa and each case requires individual evaluation. Nevertheless, the question arises as to whether a significant phenotypic change in adult appearance, induced by a small number of genetic changes (such as a single genomic segment inversion), justifies the establishment of a distinct taxon for that lineage. This consideration is particularly pertinent if all other characteristics, such as those of caterpillars, remain similar to the relatives of this lineage. Our taxonomic proposals derive from the existing classification. We use currently recognized names and their respective taxonomic ranks as reference points for defining levels within the trees and establishing new taxa.

While we deal with several higher classification questions in this study, such as proposing new subgenera, changing ranks of genera to subgenera, and synonymizing others, our primary emphasis is on the species and subspecies levels. The delineation of species involves a combination of criteria, including genetic differentiation in the Z chromosome measured by F_{st} (typically >0.20, indicating distinct species) and gene exchange G_{min} (<0.05 for distinct species) (Cong et al. 2019a). Additionally, we consider COI barcode differences (typically >2% for distinct species) and their correlation with phenotypic characters (Lukhtanov et al. 2016), along with the prominence of species-level clades (Zhang et al. 2022c). We note that COI barcodes, along with mitochondria, often exhibit introgression between species (Bachtrog et al. 2006; Cong et al. 2017a), and some distinct species may share highly similar or identical barcodes (Burns et al. 2008; Zhang et al. 2023b). For further discussion, refer to the "Species, subspecies, and genomics" section in Zhang et al. (2022a).

Traditionally, subspecies have been defined as groups of populations from distinct geographical areas exhibiting recognizable phenotypic differences (e.g., where approximately 70% of individuals can be identified by phenotype without knowledge of their locality) and are capable of successful interbreeding (Mayr 1982; Monroe 1982). However, assessing the "successfully interbreed" criterion in practice is challenging, and in butterflies, wing pattern differences in individuals from different locations are often the primary criterion for defining subspecies. It is frequently unclear whether these wing pattern differences result from genetic encoding or environmental factors. Working with genomic sequences provides an opportunity to compare populations at the genotype level. In this study, new subspecies names are proposed for genetically differentiated populations forming distinct clades in at least one of the genomic trees. However, the genetic differentiation for subspecies is lower than the threshold used to delineate species. Therefore, our subspecies can be seen as "species in the making," i.e., groups of populations with some level of differentiation, though less than that required for full species status. Once subspecies are delineated in the genomic trees, we examine the wing patterns of these specimens to identify statistically diagnostic wing pattern characters for these subspecies. Phenotypic diagnoses for most subspecies are statistical and generally apply to around 70% of specimens. However, because subspecies are delineated as clades in the genomic trees, DNA-based characters supporting these clades are expected to be much stronger than wing pattern characters and hold for nearly all specimens. Therefore, we also provide DNA-based diagnoses for all newly described subspecies.

Families and species discussed below follow the taxonomic order deduced from genome-scale phylogeny, complemented by phenotypic considerations. For the newly proposed taxa, in addition to concise phenotypic diagnoses, occasionally accompanied by references that elaborate and illustrate morphological characters in greater detail, we present diagnostic DNA characters in both the nuclear genome and, where possible, the COI barcode. The DNA characters in nuclear protein-coding regions are identified using our previously developed protocol (refer to the SI Appendix in Li et al. 2019 for details). The selection of characters follows a conceptual framework outlined in Cong et al. (2019b) and aims to identify robust characters likely to withstand scrutiny as additional specimens and species are sequenced.

The character states are given in species diagnoses as abbreviations for one of the five reference genomes: *Pterourus glaucus* (Linnaeus, 1758) (pgl) (Cong et al. 2015), *Heliconius melpomene* (Linnaeus, 1758) (hm) (Davey et al. 2016), *Calephelis nemesis* (W. H. Edwards, 1871) (cne) (Cong et al. 2017b), *Calycopis cecrops* (Fabricius, 1793) (cce) (Cong et al. 2016), or *Cecropterus lyciades* (Geyer, 1832) (aly, because this species was formerly in the genus *Achalarus* Scudder, 1872) (Shen et al. 2017). E.g., aly728.44.1:G672C means position 672 in exon 1 of gene 44 from scaffold 728 of the *Cecropterus*

lyciades (Geyer, 1832) 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 in the supplemental file deposited at < https://osf.io/g4zft/ >. This link to the DNA sequences accessible from this publication ensures that DNA characters given in the diagnoses can be readily associated with actual sequences.

Whole genome shotgun datasets we sequenced and used in this study are deposited in the NCBI database < https://www.ncbi.nlm.nih.gov/ > as BioProject PRJNA1072724 and BioSample entries of the project provide the locality and other collection data of the specimens used in the trees. Moreover, for each specimen in tree figures, the following information is provided (separated by |): taxon name with comments in square brackets, DNA sample code, type status, general locality, and year of collection ('old' if not dated and likely collected 100–150 years ago). Type status abbreviations are: HT holotype, LT lectotype, ST syntype, T type (could be ST, LT, paralectotype, or HT, status not investigated), PT paratype, PLT paralectotype; and if a synonym name is given (in parenthesis, preceded by =, and in addition by ‡ for unavailable names), type status refers to the synonym. COI barcode sequences reported here have been deposited in GenBank with accessions PP254243–PP254260. Abbreviations and acronyms for collections are listed in the acknowledgments section. Three photographs shown in this work are taken from iNaturalist (2023). Links to observations by observation number reported in figure legends are < https://www.inaturalist.org/observations/xxx >, where xxx is the number.

Family Papilionidae Latreille, [1802]

Hyalaus Grishin, new subgenus

http://zoobank.org/3F5F76FC-9BDA-48A2-A6C2-53C0C2E49C2D

Type species. *Papilio epidaus* E. Doubleday, 1846.

Definition. Genomic phylogeny that includes type species of all available genus-group names in *Eurytides* Hübner, [1821] (type species *Eurytides iphitas* Hübner, [1821]) reveals a lineage consisting of *Eurytides epidaus* (E. Doubleday, 1846) (type locality in Mexico (Yucatan) and Honduras) (Fig. 1 magenta) that is sister to the subgenus *Mimoides* K. Brown, 1991 (type species *Papilio ariarathes* Esper, 1788). The subgenus *Boreographium* Grishin, 2021 (type species *Papilio marcellus* Cramer, 1777) is sister to both *Mimoides* and the lineage with *E. epidaus*. To keep the classification monophyletic, this

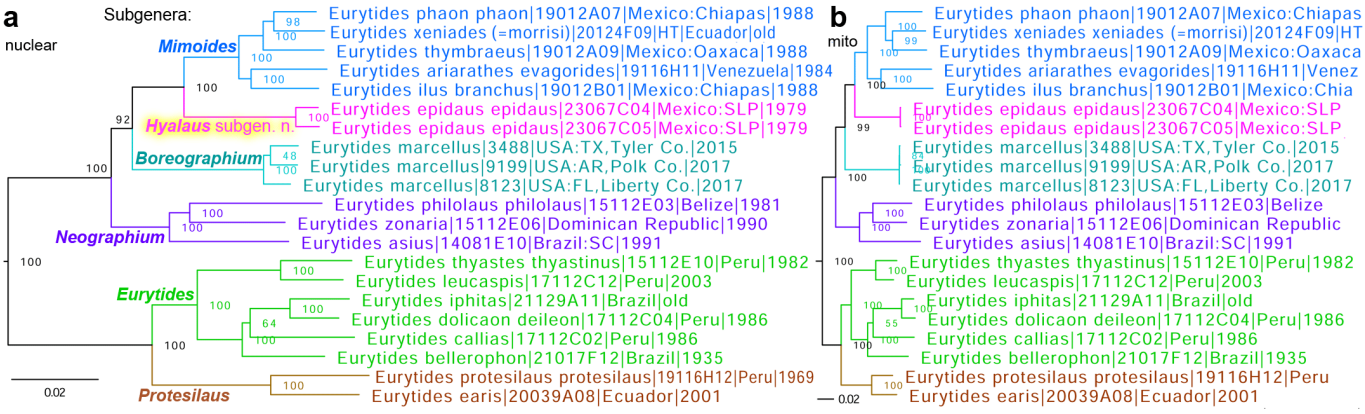

Fig. 1. Phylogenetic trees of the genus *Eurytides* inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different subgenera are labeled in the nuclear tree and colored differently: *Mimoides* (blue), *Hyalaus* **subgen. n.** (magenta, name highlighted in yellow), *Boreographium* (cyan), *Neographium* (purple), *Eurytides* (green), and *Protesilaus* (brown).

lineage can either be included in the subgenus *Mimoides*, or a new subgenus should be erected for it. *Mimoides,* as originally circumscribed, includes species unified by certain recognizable appearance, and *E. epidaus* does not resemble this phenotype, having a different look (Fig. 2) more similar to *Protesilaus* W. Swainson, 1832 (type species *Papilio protesilaus* Linnaeus, 1758). Moreover, *Mimoides* is a genetically compact group (Fig. 1), and *E. epidaus* is separated from *Mimoides* by a prominent tree branch. The COI barcode difference between *E. epidaus* and *E. ariarathes* (the type species of *Mimoides*) is 6.8% (45 bp), which is not atypical for species in different subgenera. For all these reasons, we propose to treat the lineage with *E. epidaus* as a new subgenus. This new subgenus differs from others by the presence of a hyaline area on the forewing distad of the postdiscal discal dark band; more produced, rounder forewing apex similar in shape to that of *Mimoides*; harpe with hook-shaped process in the middle directed anteroventrad, also present in *Boreographium* but absent in others, e.g., in *Mimoides*, and differs from *Boreographium* by a broader dorsal keel and excavate distal margin. In DNA, a combination of the following characters is diagnostic in the nuclear genome: pgl8036.1.5:T51A, pgl231.44.1:G809A, pgl231.44.1:T816C, pgl2266.11.4:T147C, pgl3034.5.5:T39A and in COI barcode: T133A, A352C, T364A, T454A, A541T.

Fig. 2. *Eurytides epidaus* from Nicaragua, 27-Mar-2019, showing hyaline areas on forewings. iNaturalist observation 34925339.

Etymology. In Greek, hyalos (ὕαλος) means glass; named for the glassy (i.e., hyaline) areas on forewings of the type species. The name is a masculine noun in the nominative singular.

Species included. Only the type species (i.e., *Papilio epidaus* E. Doubleday, 1846).

Parent taxon. Genus *Eurytides* Hübner, [1821].

Comment. As a result, we partitioned the genus *Eurytides* into six subgenera: *Mimoides*, *Hyalaus* **subgen. n.**, *Boreographium*, *Neographium* Möhn, 2002 (type species *Papilio philolaus* Boisduval, 1836), *Eurytides*, and *Protesilaus* W. Swainson, 1832 (Fig. 1).

Family Pieridae Swainson, 1820

Eurema ella (Röber, 1909) is a species distinct from *Eurema elathea* (Cramer, 1777)

Genomic phylogeny of all known species of *Eurema* Hübner, [1819] (type species *Papilio delia* Cramer, 1780, a primary junior homonym, valid name for this species is *Pieris daira* Godart, 1819) *sensu stricto* reveals that *Eurema elathea ella* (Röber, 1909) (type locality in Ecuador) (Fig. 3 orange) is not monophyletic with *Eurema elathea* (Cramer, 1777) (type locality in "Virginia", possibly Haiti) (Fig. 3 green) and is closer related to several species that include *Eurema daira* (Godart, 1819) (type locality in USA: Virginia) (Fig. 3 purple), but genetically differentiated from them. Therefore, we propose that *Eurema ella* (Röber, 1909), **stat. nov.** is a species distinct from *Eurema elathea* (Cramer, 1777).

Eurema flavescens (Chavannes, 1850) is a species distinct from *Eurema elathea* (Cramer, 1777)

Genomic analysis reveals that *Terias flavescens* Chavannes, 1850 (type locality in Brazil: São Paulo) (Fig. 3 magenta), currently regarded as a subspecies of *Eurema elathea* (Cramer, 1777) (type locality in

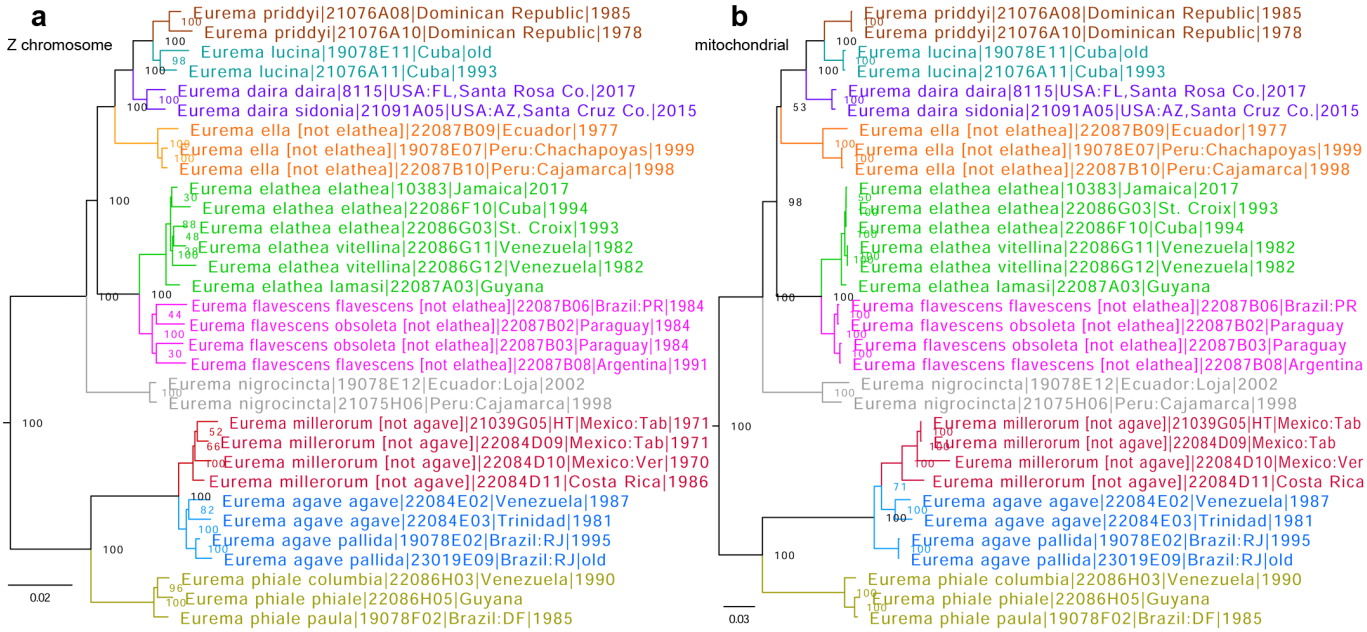

Fig. 3. Phylogenetic trees of all known species of *Eurema* inferred from protein-coding regions of **a)** the Z chromosome (best for species delineation) and **b)** the mitochondrial genome. Different species are colored differently: *E. priddyi* (Lathy, 1898) (brown), *E. lucina* (Poey, [1852]) (cyan), *E. daira* (purple), *E. ella* **stat. nov.** (orange), *E. elathea* (green), *E. flavescens* (Chavannes, 1850), **stat. rest.** (magenta), *E. nigrocincta* Dognin, 1889 (gray), *E. millerorum* **stat. nov.** (red), *E. agave* (blue), and *E. phiale* (Cramer, 1775) (olive).

"Virginia", possibly Haiti) (Fig. 3 green) is genetically differentiated from it at the species level, e.g., their COI barcodes differ by 2.9% (19 bp). Therefore, we propose that *Eurema flavescens* (Chavannes, 1850), **stat. rest.** is a species distinct from *Eurema elathea* (Cramer, 1777). Out of all other subspecies of *E. elathea*, *Terias elathea obsoleta* Jörgensen, 1932 (type locality in Paraguay) is closely related to *E. flavescens*, while others remain with *E. elathea* (Fig. 3). Thus, we additionally propose a new species-subspecies combination *Eurema flavescens obsoleta* (Jörgensen, 1932).

Eurema millerorum Llorente & Luis, 1987 is a species distinct from *Eurema agave* (Cramer, 1775)

Genomic sequencing reveals that *Eurema agave millerorum* Llorente & Luis, 1987 (type locality in Mexico: Tabasco) (Fig. 3 red) is genetically differentiated from *Eurema agave* (Cramer, [1775]) (type locality in Suriname) (Fig. 3 blue) at the species level, e.g., their COI barcodes differ by 3.2% (21 bp). Therefore, we propose that *Eurema millerorum* Llorente & Luis, 1987, **stat. nov.** is a species distinct from *Eurema agave* (Cramer, 1775). Due to comparatively smaller genetic differentiation in the nuclear genome (Fig. 3a), we leave *Terias pallida* Chavannes, 1850 (type locality in Brazil: São Paulo) as a subspecies of *E. agave* but note its distinct mitochondrial genome (Fig. 3b) needing further investigation.

Family Nymphalidae Rafinesque, 1815

Eugrumia Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 is a valid genus, and *Sinerebia* Nakatani, 2017 is its junior subjective synonym

Correcting a nomenclatural mistake in Zhang et al. (2023d), we state that *Sinerebia* Nakatani, 2017, **syn. nov.** (type species *Erebia atramentaria* Bang-Haas, 1927) is a junior subjective synonym of *Eugrumia* Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 (type species *Erebia herse* Grum-Grshimaïlo, 1891), which is a **valid genus**. These two names were erroneously swapped in Zhang et al. (2023d), and this mistake is corrected here to follow the priority of the two names (2000 vs. 2017). The arguments for the

synonymy of *Sinerebia* and *Eugrumia* and the validity of the genus that encompasses the species placed in *Sinerebia* and *Eugrumia* are the same as those discussed previously (Zhang et al. 2023d). As a result, we have *Eugrumia atramentaria* (Bang-Haas, 1927), **comb. nov**. We are grateful to Gian C. Bozano for kindly informing us about this error.

Microtia elva bogotana Grishin, new subspecies

http://zoobank.org/89EA04FE-2674-42EA-9B2E-AE4752B17097

(Figs. 4 part, 5)

Definition and diagnosis. Genomic analysis of *Microtia elva* H. Bates, 1864 (type locality in Guatemala and Nicaragua) reveals that while more boldly patterned southern populations included in the subspecies *Microtia elva horni* Rebel, 1906 (type locality in Mexico: Oaxaca) (Fig. 4 labeled in cyan) are not phylogenetically separated from the nominotypical subspecies (Fig. 4 labeled in blue), the two specimens from Bogota in Colombia, which corresponds to the southernmost known record of the species, are genetically differentiated from the rest (Fig. 4 magenta) and we consider them to represent a new subspecies of *M. elva*. This new subspecies differs from its relatives by dark legs, brighter orange color of bands and spots, wider forewing band, and larger and rounder inner forewing margin spot than in the nominate subspecies, but narrower discal band on the hindwing, especially beneath. A combination of the following DNA characters is diagnostic in the nuclear genome: hm2012473-RA.1:G201A, hm2012473-RA.1:C210T, hm2011393-RA.1:T1506A, hm2011393-RA.1:A1725G, hm2015159-RA.5:C1024A and in COI barcode: T13T, G38G, A43A, T589C, T513T.

Barcode sequence of the holotype. Sample NVG-22117B06, GenBank PP254243, 658 base pairs:

TACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGAACATCTTTAAGACTTTTAATTCGAACTGAATTAGGAAACCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCCCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTTCCATTAATATTAGGAGCTCCTGATATAGCTTTCCCTCGAA
TAAATAATATAAGATTTTGATTACTACCTCCATCACTTATATTATTAATTTCTAGTAGTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCACTTTCCTCCAATATTGC
TCATAGCGGATCATCTGTTGATTTAGCAATTTTTTCTTTACATTTAGCAGGAATCTCTTCAATTTTAGGAGCTATTAATTTTATTACTACAATTATTAATATACGTATTAATAATATATCA
TTTGATCAAATACCTTTATTTGTTTGAGCAGTAGGTATCACAGCTCTTTTATTATTATTATCATTACCAGTATTAGCAGGAGCTATTACCATACTTTTAACTGACCGAAATATTAATACAT
CATTTTTTGACCCAGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 5, bears four labels, 1st handwritten, others printed: three white [13 | VII], [Bogota | Nolcken], [DNA sample ID: | NVG-22117B06 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Microtia elva | bogotana Grishin]. **Paratype:** 1♀ the same locality, collector, and repository as the holotype (NVG-22117B07, bears an additional erroneous handwritten label “Brasilien”).

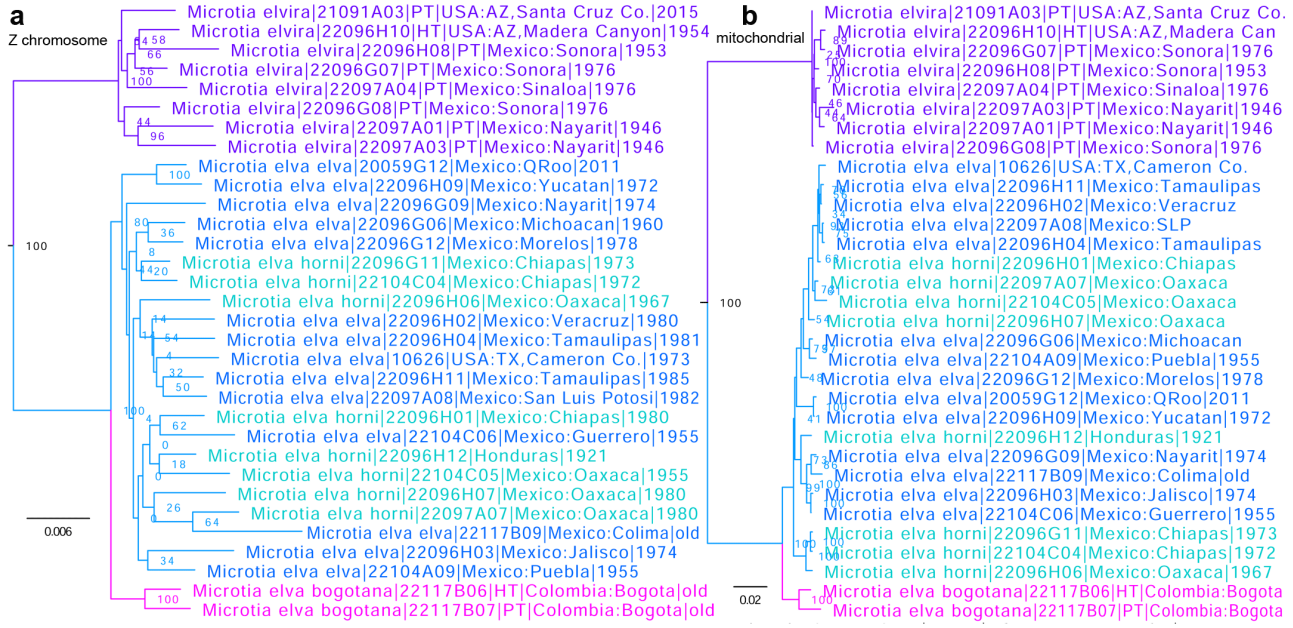

Fig. 4. Phylogenetic trees of selected *Microtia* species inferred from protein-coding regions of **a)** the Z chromosome (best for species delimitation) and **b)** the mitochondrial genome: *M. elvira* (purple) and *M. elva* (blue with *M. elva bogotana* **ssp. n.** colored in magenta and *M. elva horni* labeled in cyan).

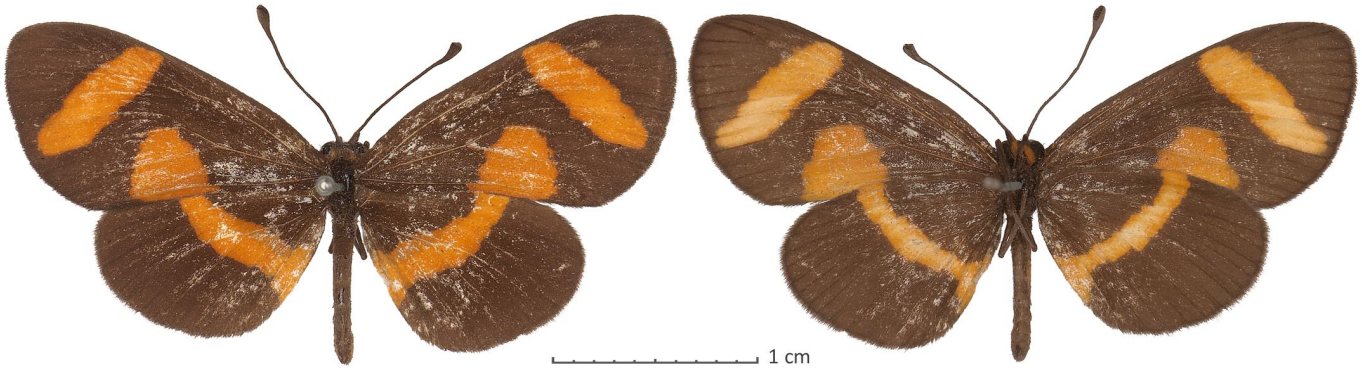

Fig. 5. Holotype of *Microtia elva bogotana* **ssp. n.** in dorsal (left) and ventral (right) views, data in text.

Type locality. Colombia: Bogota.

Etymology. The name refers to the type locality.

Distribution. Colombia.

Comments. The lack of separation of subspecies into clades in the genomic trees (e.g., *M. elva horni* vs. *M. elva horni*) does not mean that the subspecies are not valid, it only means that this phylogenetic method does not separate them. Such a situation can arise due to limited genetic differentiation and/or extensive gene flow. Population analysis is needed to define groups of populations that are best treated as subspecies if phylogenetic tree methods fail. Finally, further research and additional specimens are needed to investigate whether *M. elva bogotana* **ssp. n.** might be a species-level taxon.

Boloria astarte alaskia **Grishin, new subspecies**

http://zoobank.org/2B44E674-0784-4977-ADE5-A8AD69E30582

(Figs. 6 part, 7a)

Definition and diagnosis. Genomic analysis that includes the holotype of *Boloria astarte tschukotkensis* (Wyatt, 1961) (type locality in Russia: Chukotka Mts., sequenced as NVG-20095A04) reveals that *Boloria astarte* (E. Doubleday, [1847]) (type locality in Canada: Alberta) partitions into four, not three, distinct clades. Three clades correspond to subspecies of *B. astarte*: the nominate (Fig 6 green), *Boloria astarte distincta* (A. Gibson, 1920) (type locality in Canada: Yukon, Harrington Creek) (Fig 6 blue), and *B. astarte tschukotkensis* (Fig 6 purple). The fourth clade (Fig 6 red) comprises populations from Alaska, USA, that are currently called *B. astarte tschukotkensis* but, as our analysis shows, are genetically distinct from it. While forming a prominent clade in the nuclear genome tree (Fig. 6a), these populations from Alaska exhibit only 0.3% (2 bp) difference in the COI barcode from the nominate *B. astarte*. Therefore, this Alaskan clade represents a new subspecies. This new subspecies differs from others by its generally smaller size, less extensive white overscaling beneath, e.g., near the outer margin of the ventral hindwing, the submarginal row of spots is not covered with white, and white scaling is mostly restricted to the belt between the marginal and submarginal row of spots. The submarginal spots are rounder on the ventral side, not triangular as in *B. astarte distincta* (Fig. 6a vs. b). A notable difference is that the submarginal spots on the ventral forewing point outside (towards the margin) with their sharper ends in the new subspecies but inside (towards the wing base) in *B. astarte distincta*. The new subspecies differs from *B. astarte tschukotkensis* by better defined submarginal spots and weaker expressed and narrower dark framing in the discal area of ventral hindwing. A combination of the following DNA characters in the nuclear genome is diagnostic: hm2009446-RA.6:T96A, hm2010326-RA.1:G46A, hm2002793-RA.5:C72T, hm2010724-RA.2:T198A, hm2010724-RA.2:C255A but COI barcodes do not identify it consistently.

Barcode sequence of the holotype. Sample NVG-21068B11, GenBank PP254244, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACATCTCTTAGTTTACTAATTCGAACTGAATTAGGTAATCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGTAATTGATTAGTACCTTTAATATTAGGAGCCCCAGATATAGCATTTCCCCGTA
TAAATAATATAAGATTTTGACTTTTACCCCCATCTTTAATTTTACTTATTTCAAGTAGAATTGTCGAAAATGGAGCAGGAACAGGATGAACAGTGTACCCCCCACTTTCATCTAATATTGC
TCATAGAGGAGCTTCAGTAGACCTAGCAATTTTTTCTCTACATTTAGCTGGTATTTCCTCCATCCTAGGAGCTATTAATTTTATTACCACAATTATTAATATACGGATTAATAATATATCT
TTTGATCAAATACCATTATTTGTATGAGCTGTAGGTATTACAGCTTTATTACTTTTATTATCTTTACCAGTTTTAGCAGGAGCTATTACAATACTTTTAACTGATCGTAATTTAAATACTT
CATTTTTTGATCCTGCAGGAGGAGGAGATCCCATTTTATATCAACATTTATTT

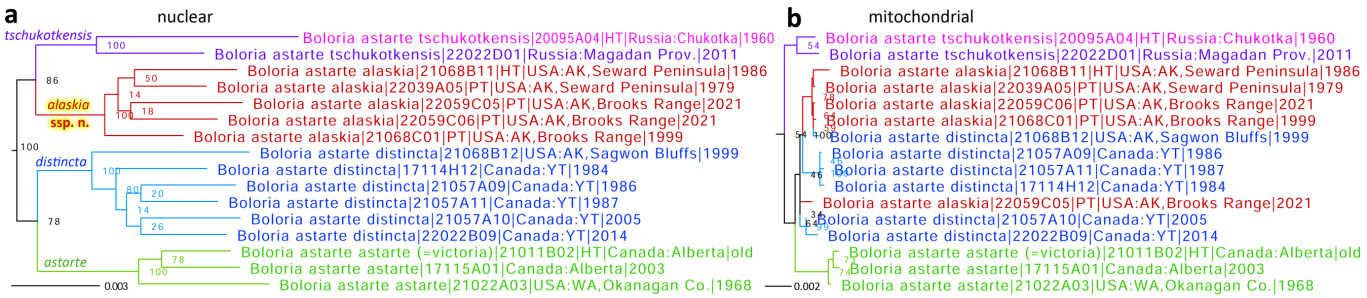

Fig. 6. Phylogenetic trees of *Boloria astarte* subspecies inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different subspecies are colored differently: *B. astarte tschukotkensis* (purple, the holotype labeled in magenta), *B. astarte alaskia* **ssp. n.** (red), *B. astarte distincta* (blue), and *B. astarte astarte* (green).

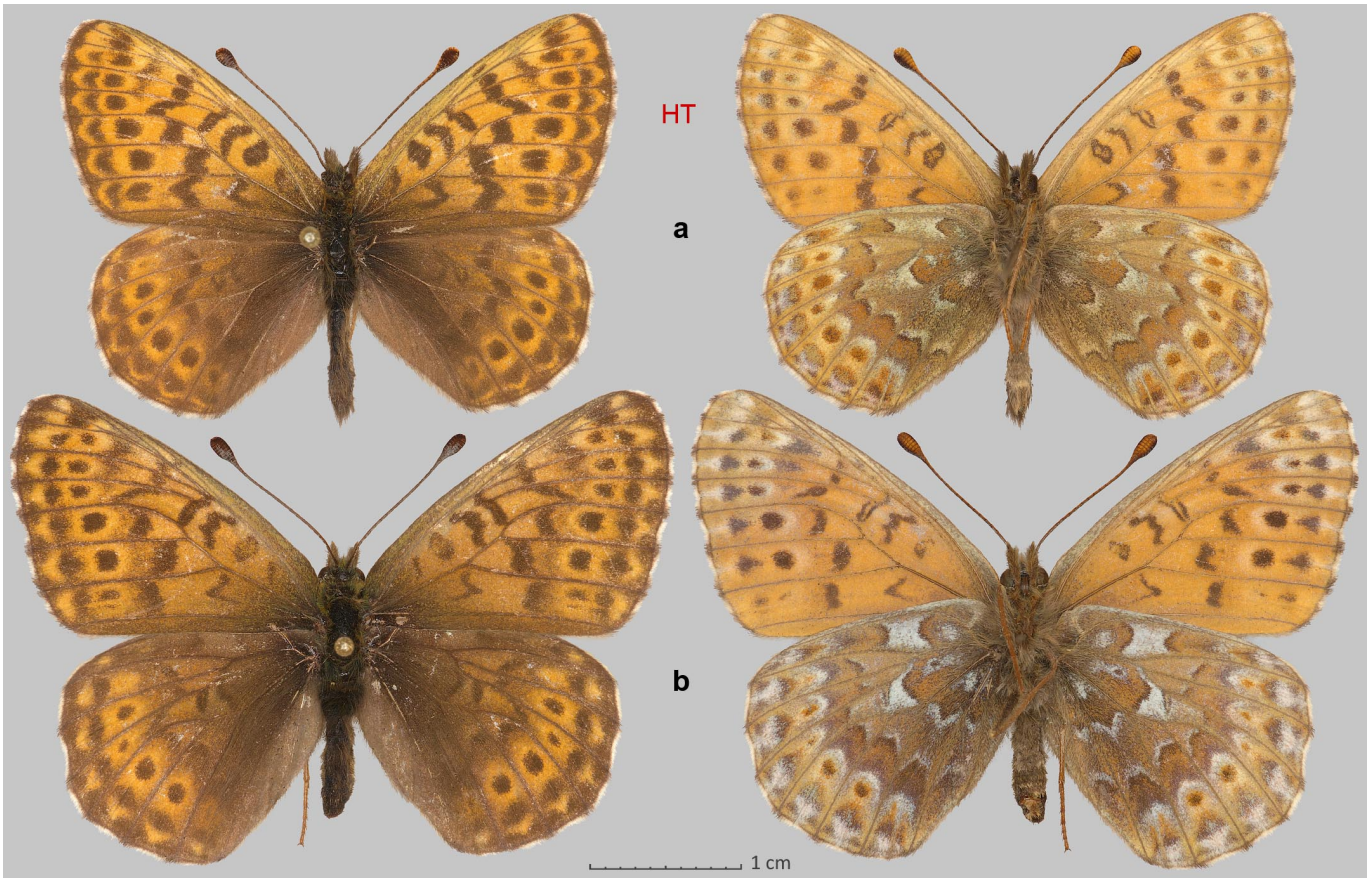

Fig. 7. *Boloria astarte* specimens from USA: Alaska [MGCL] in dorsal (left) and ventral (right) views: **a)** the holotype of *Boloria astarte alaskia* **ssp. n.** ♂ NVG-21068B11 Darby Mtns., Omilak, 24-Jun-1986, J. & L. Troubridge leg.; **b)** *Boloria astarte distincta* ♀ NVG-21068B12 Sagwon Bluffs, Dalton Hwy, mile 353, 11-Jul-1999, Jack L. Harry leg.

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 7, bears four labels, 1st handwritten, others printed: three white [Omilak, Darby Mtns | Alaska | June 24, 1986 | Collectors | J + L Troubridge], [J. & F. Preston Coll. | Allyn Museum | Acc. 1989-21], [DNA sample ID: | NVG-21068B11 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Boloria astarte | alaskia Grishin]. **Paratypes:** 2♂♂ and 2♀♀: from USA: Alaska: 1♂ Seward Peninsula, Kougarok Road, mile 48, Big Creek, elevation 1100 ft, 16-Jul-1979, Joseph D. Zeligs leg. (NVG-22039A05, fig. James Scott book, from James A. Scott collection); 1♀ Brooks Range, Chandalar Shelf Ridge, W of Dalton Hwy mile 237.5, 24-25-Jun-1999, Malcolm G. Douglas leg. (NVG-21068C01) [MGCL]; North Slope Borough, Dalton Highway, W. R. Dempwolf leg.: 1♂ N of Atigun Pass, elevation 2784', GPS 68.1558, −149.4361, 12-Jul-2021 (NVG-22059C05, WRD 20230) and 1♀ near Atigun Pass, elevation 3700', GPS 68.1397, −149.4439, 11-Jul-2021 (NVG-22059C06, WRD 20229).

Type locality. USA: Alaska, Seward Peninsula, Darby Mts., Omilak.

Etymology. The name refers to the state of the type locality.

Distribution. From the Seward Peninsula to the Brooks Range in Alaska, USA.

Family Riodinidae Grote, 1895 (1827)

Euselasia fayneli Gallard, 2006 belongs to the genus *Erythia* Hübner, [1819]

Genomic analysis of the holotype of *Euselasia fayneli* Gallard, 2006 (type locality in French Guiana, sequenced as NVG-23024F11) (Fig. 8 magenta) and representative Euselasiini Kirby, 1871 (1867) including type species of all available genus-group names (Fig. 8) reveals that it does not belong to *Euselasia* Hübner, [1819] (type species *Euselasia gelaena* Hübner, 1819, which is a junior subjective synonym of *Papilio gelon* Stoll, 1787), but instead originates within the genus *Erythia* Hübner, 1819 (type species *Papilio labdacus* Stoll, 1780). Therefore, we place it in this genus as *Erythia fayneli* Gallard, 2006, **comb. nov**.

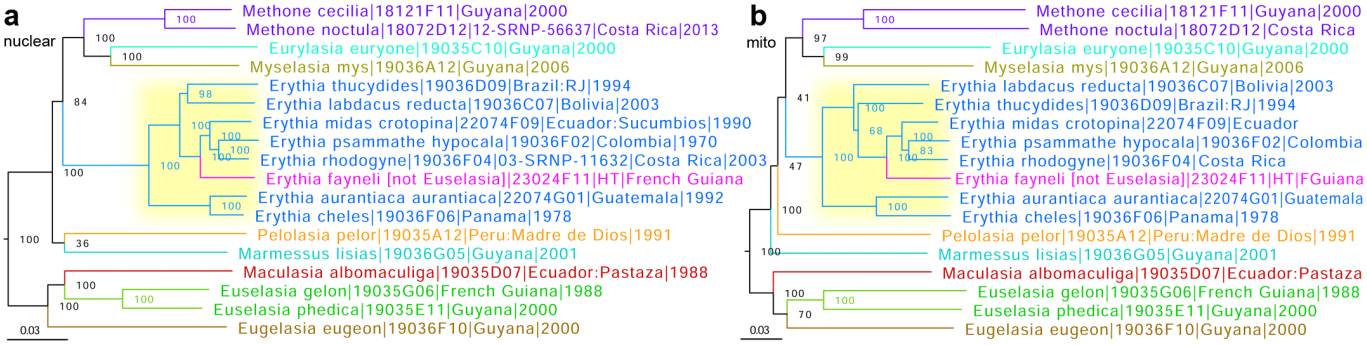

Fig. 8. Phylogenetic trees of *Euselasia* and relatives inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different genera are colored differently: *Methone* E. Doubleday, 1847 (purple), *Eurylasia* Grishin, 2021 (aquamarine), *Myselasia* Grishin, 2021 (olive), *Erythia* (blue with *Erythia fayneli* **comb. nov.** in magenta), *Pelolasia* Grishin, 2021 (orange), *Marmessus* Hübner, [1819] (cyan), *Maculasia* Grishin, 2021 (red), *Euselasia* (green), and *Eugelasia* Grishin, 2021 (brown). The *Erythia* clade is highlighted in yellow.

Ithomiola tessera J. Hall, 2005 is a species distinct from *Ithomiola theages* (Godman & Salvin, 1878)

Originally described and currently treated as a subspecies of *Ithomiola theages* (Godman & Salvin, 1878) (type locality in Costa Rica) (Fig. 9 purple), *Ithomiola theages tessera* J. Hall, 2005 (type locality in

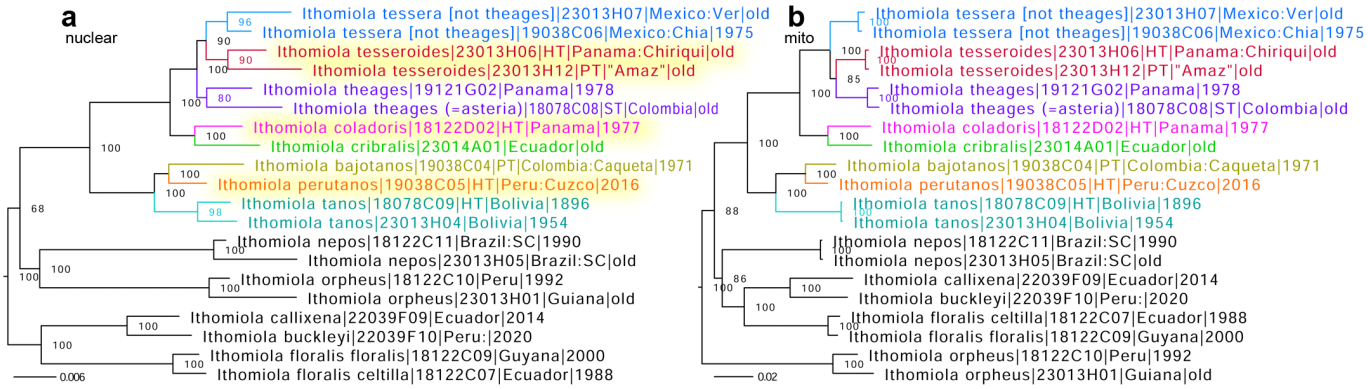

Fig. 9. Phylogenetic trees of selected *Ithomiola* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Species discussed in the text are colored differently, and new species names are highlighted in yellow in a): *I. tessera* **stat. nov.** (blue), *I. tesseroides* **sp. n.** (red), *I. theages* (purple), *I. coladoris* **sp. n.** (magenta), *I. cribralis* (green), *I. bajotanos* (olive), *I. perutanos* **sp. n.** (orange), and *I. tanos* (cyan).

Mexico: Veracruz) (Fig. 9 blue) is genetically differentiated from it at the species level (Fig. 9), e.g., their COI barcodes differ by 3.5% (23 bp). Moreover, the two taxa differ by the size and alignment of white discal spots on the forewing (Hall 2005). Therefore, we propose that *Ithomiola tessera* J. Hall, 2005, **stat. nov.** is a species distinct from *Ithomiola theages* (Godman & Salvin, 1878). Furthermore, inspection of genomic trees reveals three new species that differ from the previously described ones due to their genetic differentiation even as single specimens (Fig. 9, yellow highlight in 9a). These three new species are described below.

Ithomiola (*Ithomiola*) *tesseroides* Grishin, new species

http://zoobank.org/3400B799-BD7D-4126-95CE-0CF0766956D6

(Figs. 9 part, 10a)

Definition and diagnosis. Genome-based phylogeny of *Ithomiola* (*Ithomiola*) C. Felder & R. Felder, 1865 (type species *Ithomiola floralis* C. Felder & R. Felder, 1865) reveals that specimens from Panama (Fig. 9 red) identified as *Ithomiola tessera* J. Hall, 2005, **stat. nov.** (type locality in Mexico: Veracruz) (Fig. 9 blue) due to their large and blotchy white discal spots on the forewing are genetically differentiated from it at the species level (Fig. 9), e.g., their COI barcodes differ by 3.0% (20 bp), and therefore represent a new species. This species differs from its relatives by large and aligned discal white macules on the forewing that touch each other, as in *I. tessera*, from which its females can be differentiated by typically larger discal macules on both wings (but usually smaller postdiscal spots) and hindwing discal macule stronger separated from costa by brown: small white spot in cell Sc+R_1-Rs instead of a larger spot in typical *I. tessera*; and putative males (none sequenced) differ by more extensive blue scaling in the tornal area of dorsal hindwing. Because phenotypic variation of this species has not been explored and due to the absence of confirmed males, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne2399.14.5:A156G, cne6395.2.1:T631C, cne1958.7.3:T204C, cne1958.7.3:C477T, cne1661.11.5:T813C and in COI barcode: A277C, C343T, T382A, T595C, T634C.

Barcode sequence of the holotype: Sample NVG-23013H06, GenBank PP254245, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGTACATCTTTAAGTTTATTAATTCGTATAGAATTAGGTATACCTGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGTTTTGGAAATTGACTTGTTCCTTTAATATTAGGAGCTCCAGATATAGCTTTTCCTCGTA
TAAATAATATAAGATTTTGACTTCTTCCTCCTTCCTTATTTCTTCTTATTTCAAGAAGAATTGTTGAAAATGGTGCAGGTACTGGATGAACTGTTTACCCTCCTTTATCTTCTAACATTGC
TCATGGAGGATCTTCTGTAGATTTAGCAATTTTCTCATTACATTTAGCAGGTATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTTTATTTGTATGATCTGTAGGAATTACAGCATTATTATTACTTTTATCTTTACCTGTATTAGCAGGTGCAATTACTATATTATTAACAGACCGAAACTTAAATACTT
CATTTTTTGACCCTGCTGGAGGAGGTGACCCTATTCTTTATCAACATTTATTT

Type material. Holotype: ♀ deposited in the Zoologische Staatssammlung München, Germany [ZSMC], illustrated in Fig. 10a, bears three printed labels: two white [Chiriqui], [DNA sample ID: | NVG-23013H06 | c/o Nick V. Grishin] and one red [HOLOTYPE ♀ | Ithomiola (Ithomiola) | tesseroides Grishin]. **Paratype:** 1♀ with two original labels [920], [Theages | Godm. & Salv. | Amaz.], likely mislabeled from Panama and might also be from Chiriqui (NVG-23013H12, GenBank barcode PP254246) [ZSMC].

Type locality. Panama: Chiriqui.

Etymology. Formed from the name of *I. tessera*, which is a more northern species with similarly large and squarish adjoining discal forewing spots. In Latin, *tessera* means a square piece of stone, and it was given to that species for the forewing spots. The name is an adjective.

Distribution. Panama (at least).

Ithomiola (*Ithomiola*) *coladoris* Grishin, new species

http://zoobank.org/6C7200DE-F7A0-4DC0-A72D-317ACE584322

(Figs. 9 part, 10b)

Definition and diagnosis. Genome-based phylogeny of *Ithomiola* (*Ithomiola*) C. Felder & R. Felder, 1865 (type species *Ithomiola floralis* C. Felder & R. Felder, 1865) reveals that a specimen from Panama (Fig. 9 magenta) identified as *Ithomiola cribralis* (Stichel, 1915) (type locality in Ecuador) (Fig. 9 green)

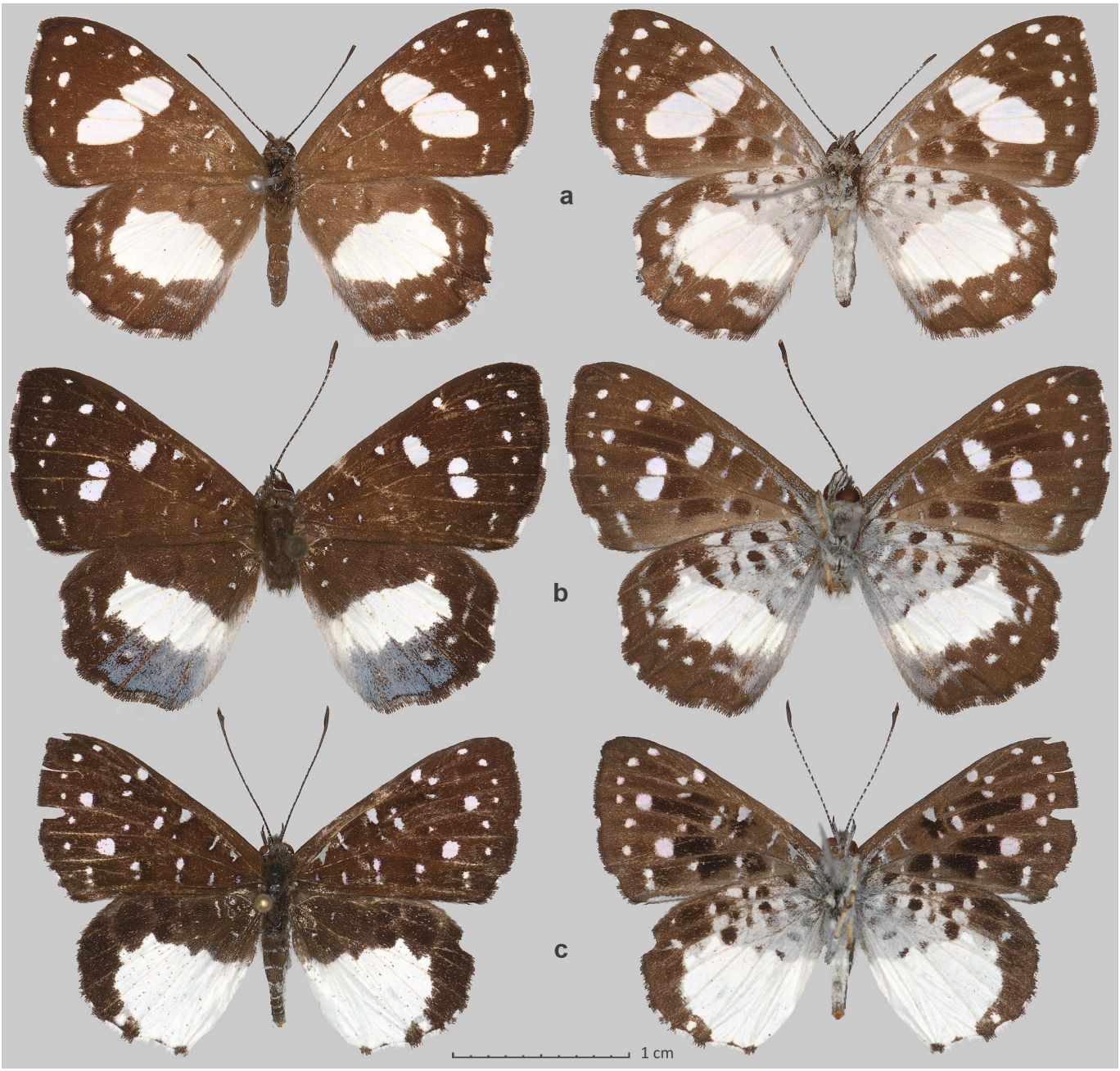

Fig. 10. Holotypes of new *Ithomiola* species in dorsal (left) and ventral (right) views, data in text: **a)** *I. tesseroides* **sp. n.**, **b)** *I. coladoris* **sp. n.**, and **c)** *I. perutanos* **sp. n**.

due to phenotypic similarities, is genetically differentiated from it at the species level (Fig. 9), e.g., their COI barcodes differ by 2.3% (15 bp), and therefore represents a new species. This species is most similar to its closest relative, *I. cribralis*, and differs from it by larger forewing white spots in both sexes (compare within each sex), larger hindwing discal white patch, and blue scaling extending farther from the tornus along the hindwing outer margin. For additional illustrations, see Figs. 62A (holotype), 62B (paratype), and 146 (male genitalia of the holotype) in Hall (2005), who has not illustrated true *I. cribralis* showing this species instead. Because the phenotypic variation of this species has not been extensively explored, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne10210.1.1:A505C, cne10210.1.1:A507G, cne804.5.4:A99G, cne41.6.2:T747C, cne41.6.2:A777G, cne9763.1.7:C72C (not T), cne5785.2.3:C93C (not T), cne191.3.2:T87T (not C), cne10809.1.1:T36T (not C), cne6006.2.2:T132T (not A) and in COI barcode: T40A, T184C, T376C, A403G, T500C, T653C.

Barcode sequence of the holotype: Sample NVG-18122D02, GenBank PP254247, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACATCTTTAAGATTATTAATTCGTATAGAATTAGGTATACCTGGATCTTTAATTGGTGATGATCAAATTTATAACACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGCTTTGGAAATTGACTTGTTCCTTTAATGTTAGGAGCTCCAGATATAGCTTTTCCTCGTA
TAAATAACATAAGATTTTGACTTTTACCTCCTTCATTAATTCTGCTTATTTCAAGAAGAATTGTTGAAAATGGTGCAGGTACTGGATGAACTGTTTACCCCCCTTTATCTTCTAATATTGC
ACATGGAGGATCCTCTGTTGACTTAGCAATTTTTTCATTGCATTTAGCAGGTATTTCATCTATTTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTCTATTTGTTTGATCTGTAGGAATTACAGCATTACTATTACTTTTATCTTTACCTGTATTAGCAGGTGCTATTACTATATTATTAACAGATCGAAATTTAAATACTT
CATTTTTTGATCCTGCTGGAGGAGGTGATCCTATTCTTTATCAACATCTATTT

Type material. Holotype: ♂ deposited in the National Museum of Natural History, Washington, DC, USA [USNM], illustrated in Fig. 10b, bears five printed (text in italics handwritten) labels: four white [PANAMA: PANAMA | *Cerro Jefe 900 m.* | *IV-22* .1977 | G. B. Small], [Genitalia Dissection | #1999 - 62 | Donald J. Harvey], [DNA sample ID: | NVG-18122D02 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01532041], and one red [HOLOTYPE ♂ | Ithomiola (Ithomiola) | coladoris Grishin]. **Paratype:** 1♀ the same data as the holotype, illustrated by Hall (2005) as Fig. 62B (not sequenced).

Type locality. Panama: Panama, Cerro Jefe, elevation 900 m.

Etymology. In Latin, *cribrum* means sieve or strainer and is a possible root of the name *cribralis*, given by Stichel to the sister of the new species, likely for the pattern of small white spots resembling small holes of a sieve. The new species has bigger spots, more similar to holes in colander, or colador in Spanish, hence the name, which is an adjective.

Distribution. Known only from Panama.

Ithomiola (*Ithomiola*) *perutanos* Grishin, new species

http://zoobank.org/69184170-F545-4A05-9FEC-6A440A3747B0

(Figs. 9 part, 10c)

Definition and diagnosis. Genome-based phylogeny of *Ithomiola* (*Ithomiola*) C. Felder & R. Felder, 1865 (type species *Ithomiola floralis* C. Felder & R. Felder, 1865) reveals that a specimen from Cuzco, Peru (Fig. 9 orange) identified as *Ithomiola bajotanos* J. Hall, 2005 (type locality in Ecuador: Tungurahua) (Fig. 9 green) due to phenotypic similarities is genetically differentiated from it at the species level (Fig. 9), e.g., its COI barcode differs by 2.1% (14 bp) from *I. bajotanos* paratype from Colombia, and therefore represents a new species. This species is most similar to its closest relative, *I. bajotanos*, in size and less developed blue spots in the basal area of the dorsal forewing, and differs from it by smaller white spots in the ventral hindwing discal area in cells Sc+R_1-Rs and Rs-M_1. These spots are larger than in a typical *Ithomiola tanos* (Stichel, 1910) (type locality in Bolivia, holotype sequenced as NVG-18078C09). Furthermore, forewing spots are, on average, smaller, and darker brown areas basad of spots on the ventral side are longer. Because phenotypic variation of this species has not been explored, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne7381.3.1:A60G, cne5239.2.2:T39C, cne5239.2.2:A129C, cne10306.1.1:C858T, cne7597.1.2:T96C, cne3123.5.1:T249T (not C), cne8031.2.1:C384C (not A), cne8031.2.1:T390T (not A), cne224.6.2:T48T (not A), cne123.8.6:C521C (not T) and in COI barcode: A46T, T124C, T205C, T265C, A474G, T499C, 508G.

Barcode sequence of the holotype: Sample NVG-19038C05, GenBank PP254248, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAATCGGAACTTCTTTAAGTTTATTAATTCGAATAGAATTAGGTACTCCAGGATCTTTAATTGGTGATGATCAAATTTATAACACT
ATCGTTACAGCTCATGCTTTTATTATAATTTTCTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGACTTGTTCCCTTAATATTAGGAGCTCCAGATATAGCTTTCCCTCGTA
TAAATAATATAAGATTTTGACTCCTTCCCCCTTCATTATTTCTTCTTATTTCAAGAAGAATTGTTGAAAATGGAGCAGGTACTGGATGAACTGTATATCCTCCTTTATCTTCTAATATTGC
CCATGGAGGATCTTCTGTTGATTTAGCAATTTTCTCTTTACATTTAGCAGGTATTTCATCAATTTTAGGAGCTATTAATTTTATTACTACTATTATTAATATACGTATTAGTAATTTATCA
TTTGATCAAATACCCTTATTTGTGTGATCAGTTGGTATTACAGCATTATTATTACTTTTATCTCTTCCTGTATTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACTT
CATTTTTTGATCCTGCTGGAGGAGGAGATCCTATTCTTTATCAACACTTATTT

Type material. Holotype: ♂ currently deposited in the National Museum of Natural History, Washington, DC, USA [USNM], illustrated in Fig. 10c, bears four printed labels: three white [PERU:Cuzco 1194 m. | Quebrada Santa Isabel | Cosnipata Valley 4738 | 27-III-2016 Kinyon], [DNA sample ID: | NVG-19038C05 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01544965], and one red [HOLOTYPE ♂ | Ithomiola (Ithomiola) | perutanos Grishin].

Type locality. Peru: Cuzco, Cosñipata Valley, Quebrada Santa Isabel, elevation 1194 m, approximate GPS −13.017, −71.500.

Etymology. Similarly to how the name *bajotanos* was formed (bajo for "lower" + *tanos*, the sister species name), *perutanos* means *tanos* from Peru. The name is a noun in apposition.

Distribution. Known only from the holotype collected in Cuzco, Peru.

Lasaia callaina Clench, 1972 is a species distinct from *Lasaia agesilas* (Latreille, [1809])

Genomic analysis of *Lasaia agesilas callaina* Clench, 1972 (type locality in Mexico: San Luis Potosí) including its holotype (NVG-15099E07) reveals that it is genetically differentiated from the nominate *Lasaia agesilas* (Latreille, [1809]) (type locality in Peru) at the species level (Fig. 11) with F_{st}/G_{min} of 0.27/0.008, although their COI barcodes differ only by 0.3% (2 bp). Moreover, it is sister to the clade of both *L. agesilas agesilas* and *Lasaia aerugo* Clench, 1972 (type locality in Peru). Therefore, we propose that *Lasaia callaina* Clench, 1972, **stat. nov.** is a species distinct from *Lasaia agesilas* (Latreille, [1809]).

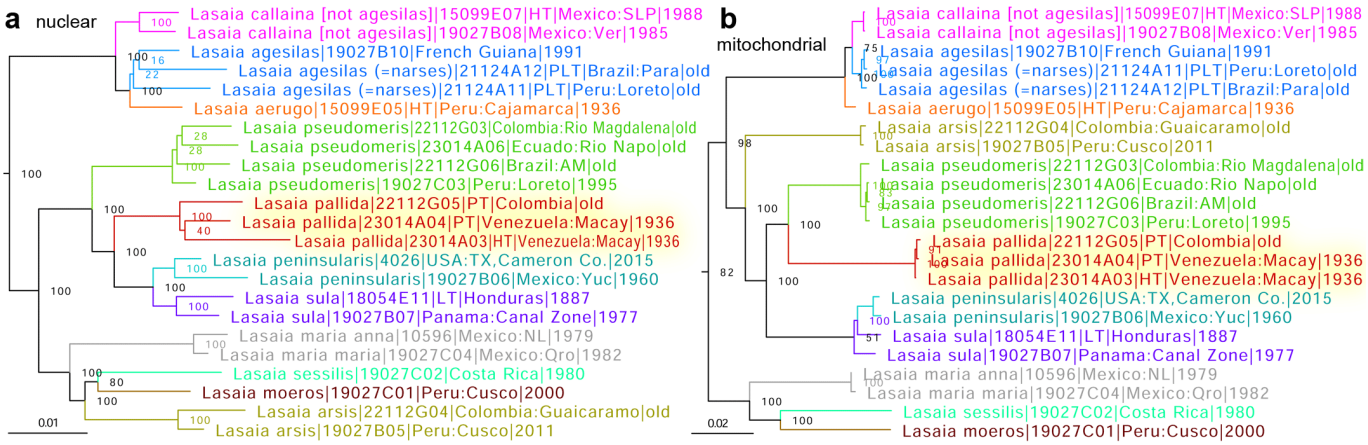

Fig. 11. Phylogenetic trees of selected *Lasaia* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different species are colored differently: *L. callaina* **stat. nov.** (magenta), *L. agesilas* (blue), *L. aerugo* (orange), *L. pseudomeris* (green), *L. pallida* **sp. n.** (red, highlighted in yellow), *L. peninsularis* (cyan), *L. sula* (purple), *L. maria* Clench, 1972 (gray), *L. sessilis* Schaus, 1890 (aquamarine), *L. moeros* Staudinger, 1888 (brown), and *L. arsis* Staudinger, [1887] (olive). Note the incongruence in the position of *L. arsis* in the two trees.

Lasaia pallida Grishin, new species

http://zoobank.org/846287C6-261D-4A8E-AF3D-2AC49CA0895C

(Figs. 11 part, 12)

Definition and diagnosis. Genomic analysis of *Lasaia* H. Bates, 1868 (type species *Papilio meris* Stoll, 1781) reveals a clade of three specimens from Colombia and Venezuela (Fig. 11 red) sister to both *L. peninsularis* Clench, 1972 (type locality in Mexico: Yucatan) and *L. sula* Staudinger, 1888 (type locality in Honduras) in the nuclear genome tree (Fig. 11 cyan and purple). This clade consists of paler in appearance specimens not associated with any available name. In the COI barcodes, specimens from this clade differ by 4.7% (31 bp) from *L. pseudomeris* Clench, 1972 (type locality in Bolivia) and by 5.6% (37 bp) from *L. sula*. Therefore, this clade represents a new species. This new species differs from its relatives by a combination of the following characters: generally paler and greener, with less developed black spotting above mostly constrained to the forewing apex and anterior portion, paler areas towards costa on the dorsal side of both wings, two prominent subapical pale spots, hindwing with brown spots weakly developed, restricted to the area near the costa and in some specimens the base; ventrally darker than many other species, hindwing with paler basal third, most prominent nearly white area anterior of discal cell, but towards the outer margin it is only somewhat paler than the discal area, without contrasting pale patches in the submarginal area. The entire type series is illustrated (Fig. 12) because the three specimens differ in their appearance, giving a range of phenotypic variation in this species. Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome:

cne23779.2.3:G132A, cne12599.1.1:G84A, cne16401.2.2:G51A, cne16401.2.2:G96A, cne7546.2.3:A96T and in COI barcode: T103C, A295T, T478C, T586G, A643G.

Fig. 12. The type series of *Lasaia pallida* **sp. n.** in dorsal (left or above the panel letter) and ventral (right or below the letter) views, males, data in text: **a)** the holotype NVG-23014A03 and paratypes **b)** NVG-22112G05 and **c)** NVG-23014A04.

Barcode sequence of the holotype. Sample NVG-23014A03, GenBank PP254249, 658 base pairs:

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACATCTTTAAGTTTATTAATTCGTATAGAATTAGGTATGCCAGGATCATTAATTGGTGACGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTCATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTACCTTTAATATTAGGAGCTCCTGATATAGCATTTCCACGAA
TAAATAATATAAGATTTTGACTTTTACCTCCATCTTTATTTCTACTAATTTCTAGAAGTATTGTAGAAAACGGAGCAGGAACTGGATGAACAGTTTACCCCCCACTGTCTTCTAATATTGC
TCATGGAGGATCTTCTGTAGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCTTCAATTTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGAATTAATAACTTATCC
TTTGATCAAATACCACTTTTTGTCTGATCAGTTGGTATTACTGCTTTATTATTATTATCATTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACGGATCGTAATTTAAATACAT
CTTTTTTTGATCCTGCAGGAGGAGGTGATCCAATTCTGTATCAACATTTATTC

Type material. Holotype: ♂ deposited in the Zoologische Staatssammlung München, Germany [ZSMC], illustrated in Fig. 12a, bears four labels, 2nd handwritten, others printed: three white [Venezuela | Maracay | ges.P.Vogl], [Lasaia | meris | Stoll], [DNA sample ID: | NVG-23014A03 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Lasaia pallida | Grishin]. **Paratypes:** 2♂♂: the same data as the holotype, 1936 (NVG-23014A04) and Colombia, Karsten leg. (NVG-22112G05) [MFNB].

Type locality. Venezuela: Maracay.

Etymology. In Latin, *pallidus* means pale or pallid. The name refers to the paler appearance of this species, particularly towards the costa on both wings above and the basal third of the hindwing beneath, and is a feminine adjective.

Distribution. Colombia and Venezuela.

Comments. Although we have not yet sequenced *Lasaia meris* (Stoll, 1781) (type locality in Suriname) and *Lasaia maritima* J. Hall & Lamas, 2001 (type locality in Peru: Piura), *Lasaia pallida* **sp. n.** is a species distinct from them. From the original illustration, *L. meris* is a strongly patterned species with well-developed pale submarginal areas on the hindwing (Stoll 1780–1782), and thus differs from least-patterned *Lasaia pallida* **sp. n**. Querying BOLD database (Ratnasingham and Hebert 2007) with COI barcodes of our sequenced specimens reveals that *L. maritima* is a species closely related to *Lasaia agesilas* (Latreille, [1809]) (type locality in Peru), similarly to *Lasaia aerugo* Clench, 1972 (type locality in Peru: Cajamarca), and thus different from *Lasaia pallida* **sp. n**.

Lasaia sessilis oaxacensis Grishin, new subspecies

http://zoobank.org/B74DED29-8359-4AF6-AB20-E564AC968987

(Figs. 13 part, 14)

Definition and diagnosis. Genomic analysis of *Lasaia* H. Bates, 1868 (type species *Papilio meris* Stoll, 1781) reveals that a specimen from Oaxaca, Mexico (Fig. 13 magenta, 14) is genetically differentiated from *Lasaia sessilis* Schaus, 1890 (type locality in Mexico: Veracruz, Coatepec, syntype sequenced as NVG-18048A05) (Fig. 13 blue), but not very prominently, e.g., their COI barcodes differ by 1.4% (9 bp). Therefore, this specimen represents a new taxon that we regard as a subspecies. This new subspecies is most similar to *L. sessilis* but differs from it by forewing dark dashes that are narrower, more connected with each other (rather than forming separate spots), and weaker developed than in *L. sessilis*, but hindwing dashes are better expressed on the dorsal side, especially towards the inner margin where they typically fade in *L. sessilis*, and costal area of dorsal hindwing paler than in a typical *L. sessilis*. Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne3288.5.1:C402G, cne205.20.3:T84C, cne640.6.18:A55G, cne640.6.18:T75C, cne7.2.1:T15A, cne7425.7.4:A24A (not G), cne1806.4.1:T119T (not C), cne790.10.6: C57C (not G), cne10214.8.11:G63G (not A), cne49345.1.3:T60T (not C) and in COI barcode: T49A, A241A, A295A, T403C, T616C.

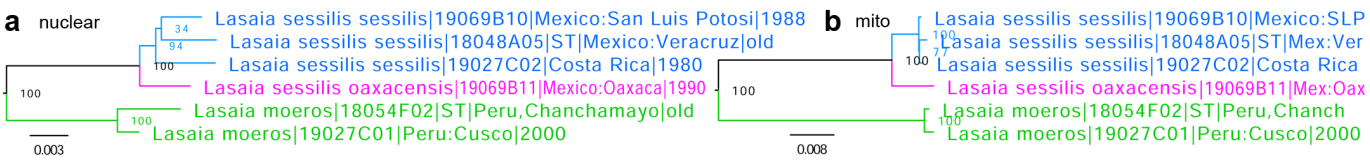

Fig. 13. Phylogenetic trees of *Lasaia sessilis* and relatives inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different taxa are colored differently: *L. sessilis sessilis* (blue), *L. sessilis oaxacensis* **ssp. n.** (magenta), and *L. moeros* (green).

Fig. 14. Holotype of *Lasaia sessilis oaxacensis* **ssp. n.** in dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype. Sample NVG-19069B11, GenBank PP254250, 658 base pairs:

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACATCATTAAGTTTATTAATTCGTATAGAATTAGGTATACCAGGATCATTAATTGGTGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATCATAATTGGAGGTTTTGGAAATTGATTAGTACCATTAATATTAGGAGCACCTGATATAGCATTTCCACGAA
TAAATAATATAAGATTCTGACTTCTTCCCCCATCTTTATTTCTTTTAATTTCAAGAAGTATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCACTGTCTTCTAATATTGC
TCATAGAGGATCCTCAGTAGATTTAGCTATTTTTTCTCTCCATTTAGCTGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGTATTAATAATTTATCT
TTTGATCAAATACCATTATTTATCTGATCAGTTGGTATTACTGCTTTATTATTATTATCTTTACCAGTTTTAGCAGGAGCTATTACAATATTATTAACAGACCGTAATTTAAATACAT
CTTTTTTTGACCCAGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the University of Texas Biodiversity Center insect collection, Austin, TX, USA [TMMC], illustrated in Fig. 14, bears five labels: 3rd handwritten, others printed; 2nd and 5th red, others white: [Mexico: Oaxaca, | La Soledad – Buena Vista | ~5000' 5-6.v.1990 | J. Kemner leg. #200], [Texas Memorial | Museum –UTexas | JKemner Spec. | 1197], [200], [DNA sample ID: | NVG-19069B11 | c/o Nick V. Grishin], and [HOLOTYPE ♂ | Lasaia sessilis | oaxacensis Grishin]. The numbers 1197 and 200 refer to the specimen and the locality, respectively, in the Kemner files in TMMC collection. The first label was made for the holotype using data in these files and added to the specimen together with the last (holotype) label. Only the 2nd and 3rd labels were original labels on this specimen.

Type locality. Mexico: Oaxaca, Sierra Madre del Sur, La Soledad – Buena Vista, elevation ca. 5000'.

Etymology. The name refers to the type locality and is a feminine adjective.

Distribution. Currently known only from the holotype collected in Oaxaca, Mexico.

Comment. Further research and additional specimens are needed to investigate whether *L. sessilis oaxacensis* **ssp. n.** could be a species-level taxon.

Synargis attilius (Stichel, 1925) is a species distinct from *Synargis regulus* (Fabricius, 1793)

Genomic analysis of two syntypes of *Nymula regulus attilius* Stichel, 1925 (type locality in Brazil: Rio de Janeiro; sequenced as NVG-21119F06 and NVG-21119F07) reveals that, together with other specimens from Southeast and South Brazil (Fig. 15 blue), they are sister to and genetically differentiated from *Synargis regulus* (Fabricius, 1793) (Fig. 15 olive) at the species level, e.g., their COI barcodes differ by 6.7% (44 bp). Therefore, we propose that *Synargis attilius* (Stichel, 1925) **stat. nov.** is a species distinct from *Synargis regulus* (Fabricius, 1793).

Zimsen (1964) did not specify repositories of *S. regulus* syntypes, which were from the Drury collection. We are not aware of their whereabouts and are researching this question. Presently, short of a neotype designation if no syntypes can be located, our identification of this species is based on the Jones' illustration (Oxford University Museum of Natural History 2021), which shows a comparatively large

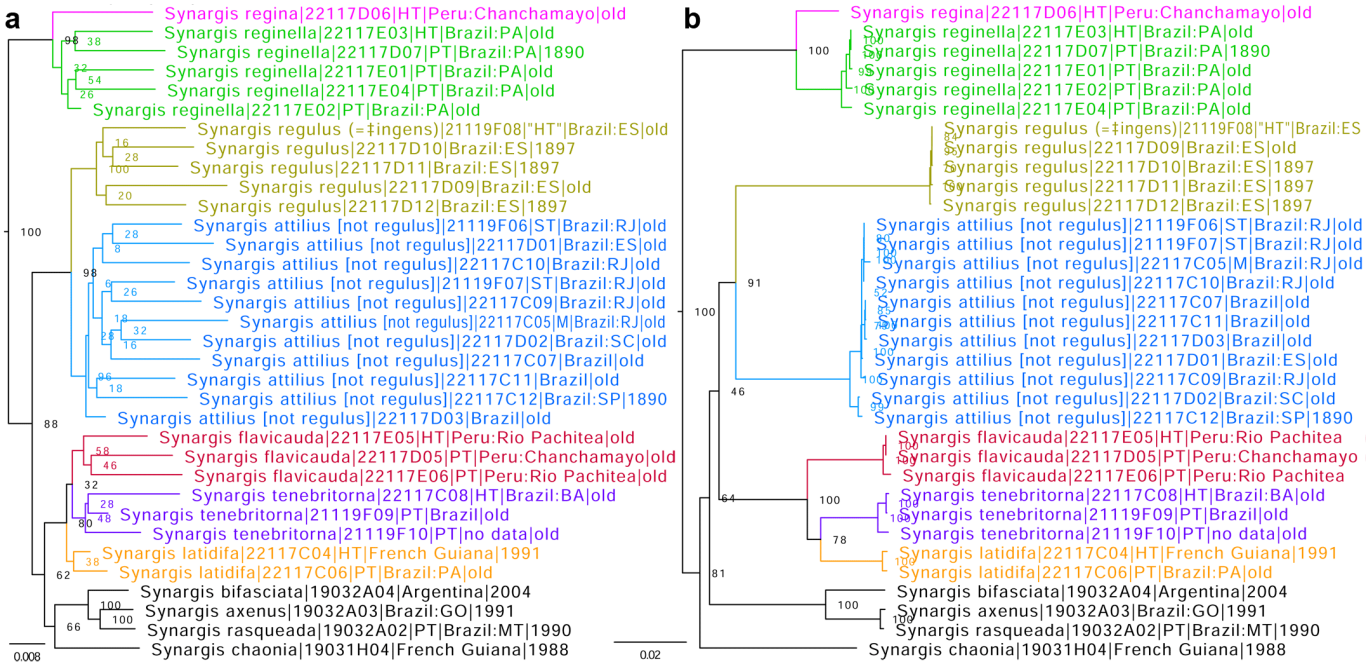

Fig. 15. Phylogenetic trees of selected *Synargis* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Species discussed in the text are shown in different colors: *S. regina* **sp. n.** (magenta), *S. reginella* **sp. n.** (green), *S. regulus* (olive), *S attilius* **stat. nov.** (blue), *S. flavicauda* **sp. n.** (red), *S. tenebritorna* **sp. n.** (purple), and *S. latidifa* **sp. n.** (orange).

female with broad pale-yellow markings and a ventral hindwing brown border without a pair of yellow marginal spots inside it. According to our analysis, only one species of the *S. regulus* complex lacks these yellow spots. It is a Southeast Brazilian species, where it may be sympatric with *S. attilius*.

We identify the "holotype" of an infrasubspecific name *Nymula regulus regulus* forma *ingens* Stichel, 1925 (from Brazil: Espirito Santo, sequenced as NVG-21119F08) referring to a "giant form" of the species (Stichel 1925) as *S. regulus*, in agreement with Stichel. However, not all *S. regulus* specimens are that large, and NVG-21119F08 is smaller than an average *S. attilius*. Besides the absence of yellow marginal spots inside the brown ventral hindwing marginal area, males of *S. regulus* can be distinguished from *S. attilius* by a more concave outer edge and more convex inner edge of the postbasal yellow band near the inner margin of the dorsal hindwing (i.e., more crescent-shaped postbasal dorsal hindwing band). In addition to *S. regulus* and *S. attilius* being distinct species, the genomic analysis revealed five more species in the *S. regulus* complex (Fig. 15). All five are new and they are described below.

Synargis regina Grishin, new species

http://zoobank.org/0AD77735-F840-4304-BD2A-5562AC4181AC

(Figs. 15 part, 16a)

Definition and diagnosis. A sole specimen from the *S. regulus* group that we sequenced from Chanchamayo, Peru (Fig. 15 magenta) is genetically differentiated from its sister clade composed of specimens from Brazil: Pará (which belong to another new species described below) (Fig. 15 green) at the species level: e.g., their COI barcodes differ by 2.3% (15 bp). Therefore, this female with a unique phenotype (Fig. 16a) represents a new species. This new species differs from its relatives by extensive and broad yellow areas on wings (even broader than in *S. regulus*), including broader submarginal macules on the forewing that are nearly touching each other, narrower broad bands, smaller marginal yellow spots on ventral side near each wing's tornus (absent in *S. regulus*) and lacking yellow marginal spot in cell M_3-CuA_1 (absent in *S. regulus* but present in species with broad yellow bands). Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne30383.1.1:C324T, cne30383.1.1:A327C, cne2564.25.13:G51A,

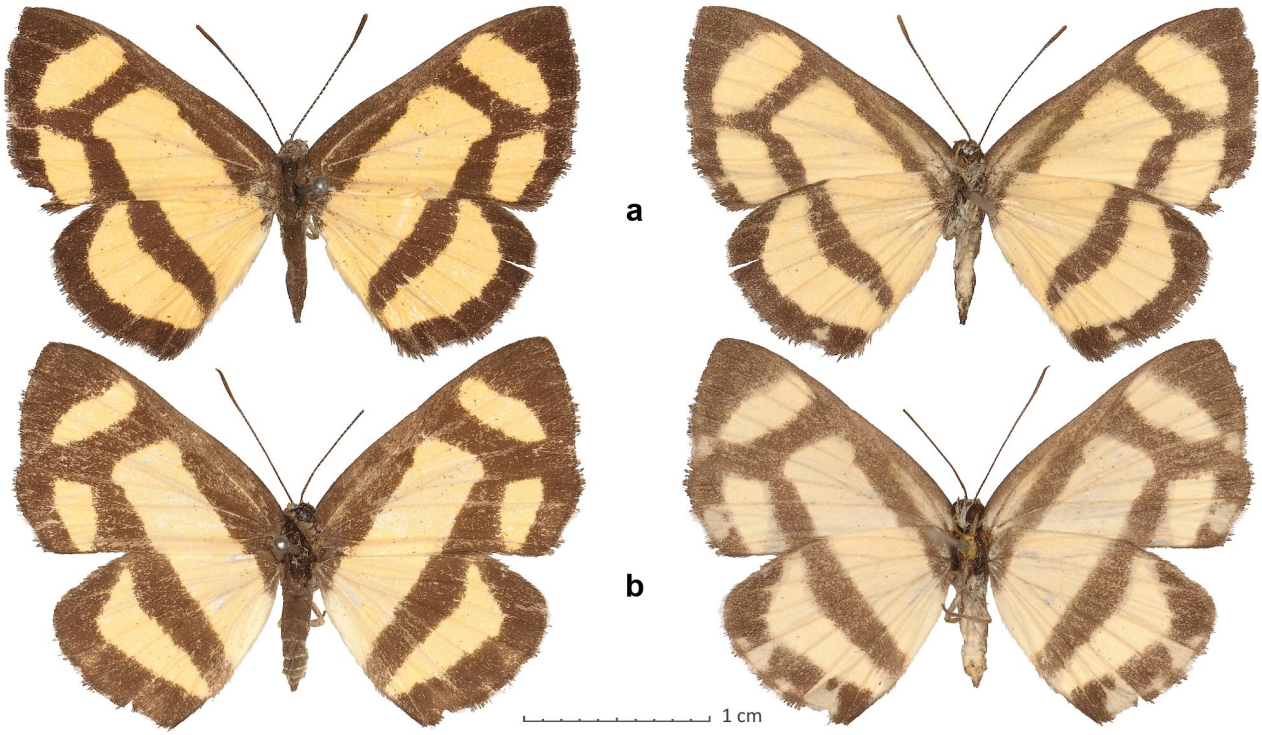

Fig. 16. Holotypes of new *Synargis* species in dorsal (left) and ventral (right) views (same scale as in Fig. 17), data in text: **a)** *S. regina* **sp. n.** and **b)** *S. reginella* **sp. n**.

cne3039.2.4:C36T, cne3039.2.4:A141T, cne2462.3.2:C153C (not T), cne8137.3.1:G420G (not T), cne5683.4.1:T929T (not A), cne17882.2.1:A79A (not G), cne1029.3.3:C114C (not T) and in COI barcode: T85C, G337G, T400C, A562G, A619C.

Barcode sequence of the holotype. Sample NVG-22117D06, GenBank PP254251, 658 base pairs:

AACTTTATATTTTATTTTTGGAATCTGAGCAGGTATAATAGGAACATCTCTTAGTTTATTAATTCGAATAGAATTAGGAATTCCCGGTTCTTTAATTGGAAATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGGAGCTCCAGATATAGCTTTCCCTCGTA
TAAATAATATAAGATTTTGATTATTACCCCCATCTTTATTTTTATTAATTTCTAGAAGAATTATTGAAAATGGAGCAGGAACTGGATGAACTGTGTACCCCCCACTTTCATCTAATATTGC
TCATAGAGGAGCTTCTGTTGATTTAGCTATTTTTTCCCTTCATTTAGCTGGAATTTCATCAATTTTAGGTGCAATTAATTTTATTACAACTATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTTTATTTATTTGATCTGTAGGAATTACTGCTCTTCTTCTTTTATTATCTTTACCTGTTTTAGCGGGAGCTATTACTATACTACTTACAGATCGAAATTTAAATACAT
CTTTTTTTGATCCCGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Type material. Holotype: ♀ currently deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 16a, bears five labels: 2nd handwritten and others printed; 1st green, 5th red, and others white [Chanchamayo | G.Tessmann], [spec. | (cf. zonata) | ♀ | 583] (the number is rotated 90° counterclockwise relative to the rest of the text and written along the right side of the label), [ex coll. | H. STICHEL], [DNA sample ID: | NVG-22117D06 | c/o Nick V. Grishin], and [HOLOTYPE ♀ | Synargis | regina Grishin].

Type locality. Peru: Chanchamayo.

Etymology. In Latin, *regulus* means little king or prince. It is a diminutive form of *rex*, which means king. In Latin, *regina* means queen, and this name is given to this brightest species of the group. The name is a noun in apposition.

Distribution. Currently known only from the holotype collected in central Peru.

Synargis reginella Grishin, new species

http://zoobank.org/8F95EA60-6CD3-4B47-93CA-78E8E3A130B0

(Figs. 15 part, 16b)

Definition and diagnosis. Several specimens from the *S. regulus* group collected in Brazil: Pará (Fig. 15 green) are genetically distinct from all others at the species level, e.g., COI barcode difference from their sister *S. regina* **sp. n.** (Fig. 15 magenta) is 2.3% (15 bp). Therefore, they represent a new species. This new species differs from its relatives by broader yellow bands and submarginal yellow macules, which are

smaller than in *S. regina* **sp. n.** and are separated from each other, prominent marginal yellow spots on the ventral side (including a comparatively large yellow spot in cell M_3-CuA_1) fully penetrating the brown border and connected with the postdiscal yellow area, and dorsally brown abdomen in males. Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne294.1.1:G44T, cne294.1.1:C59G, cne2806.2.1:T90C, cne935.2.3:G351A, cne935.2.3:C354T and in COI barcode: A94G, C235T, T283C, T454C, T646C.

Barcode sequence of the holotype. Sample NVG-22117E03, GenBank PP254252, 658 base pairs:

AACTTTATATTTTATTTTTGGAATCTGAGCAGGTATAATAGGAACATCTCTTAGTTTATTAATTCGAATAGAATTAGGAATTCCTGGTTCTTTGATTGGAAATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAATTCCATTAATATTAGGAGCTCCAGATATAGCTTTTCCTCGTA
TAAATAATATAAGATTTTGATTATTACCTCCATCTTTATTCTTATTAATTTCTAGAAGAATTATTGAAAATGGAGCAGGAACTGGATGAACTGTATATCCCCCACTTTCATCTAATATTGC
TCATGGAGGAGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCATCAATTTTAGGTGCAATTAATTTTATTACAACCATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTTTATTTATTTGATCTGTAGGAATTACTGCTCTTCTTCTTTTATTATCTTTACCTATTTTAGCAGGAGCTATTACTATACTACTTACAGATCGAAATTTAAATACAT
CTTTTTTTGACCCCGCAGGAGGTGGAGATCCAATTTTATACCAACATTTATTT

Type material. Holotype: ♀ currently deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 16b, bears four printed labels: three white [Itait. | Mich.], [ex coll. | H. STICHEL], [DNA sample ID: | NVG-22117E03 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Synargis | reginella Grishin]. According to the 1st label, the holotype was collected in Itaituba (Pará, Brazil) by Michael, probably in 1890. This year is on a similarly styled label of the topotypical paratype. A female is chosen as the holotype for best comparison with female primary types of *S. regina* **sp. n.** and Jones' drawing *S. regulus*. **Paratypes:** 2♂♂ and 2♀♀ from Brazil, Pará [MFNB]: 1♂ the same data as the holotype, 1890 (NVG-22117D07) and from Santarem: 1♂ (NVG-22117E01, Stichel collection number 1620), 1♀ (NVG-22117E02, Stichel Nº 2088), and 1♀ (NVG-22117E04, Stichel Nº 315).

Type locality. Brazil: Pará, Itaituba.

Etymology. In Latin, *regulus* means little king or prince. It is a diminutive form of *rex*, which means king. In Latin, *reginella* is a diminutive of *regina*, which means queen, and the name is given to this species that resembles *regina* but is not that bright. The name is a noun in apposition.

Distribution. Lower Amazonian region.

Synargis flavicauda Grishin, new species

http://zoobank.org/BA27EC2B-4A69-4C68-8273-F82B8FCE2E74

(Figs. 15 part, 17a)

Definition and diagnosis. Genomic analysis of the *S. regulus* group reveals a clade that, being distinct from all other species, itself consists of three species-level undescribed taxa (Fig. 15 red, purple, and orange). The first species (Fig. 15 red) with specimens sequenced from Peru differs in COI barcode from each of the other two species by 2.6% (17 bp). This new species is differentiated from its relatives by narrower than in several others yellow bands and submarginal macules, strongly developed marginal yellow spots inside brown border on the ventral side of wings, including the spot in cell M_3-CuA_1; this spot is connected or nearly connected with the subapical elongated macule. Many males have a dorsally yellow caudal half of the abdomen (yellow ventrally as in other species). Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne664.10.2:C508A, cne664.10.2:C519T, cne2800.7.1:C655G, cne2800.7.1:C660A, cne9234.1.8:T88C and in COI barcode: T103C, C340T, T407C, A451C, T578C.

Barcode sequence of the holotype. Sample NVG-22117E05, GenBank PP254253, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGTATAGTAGGAACATCTCTTAGTTTACTAATTCGAATAGAATTAGGAACTCCTGGATCTTTAATTGGAGACGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAACTGATTAGTTCCATTAATATTAGGAGCTCCTGATATAGCTTTCCCCCGTA
TAAATAATATAAGATTTTGATTATTACCTCCTTCTTTATTTTTATTAATCTCCAGAAGAATTGTTGAAAATGGAGCAGGAACTGGATGAACAGTATATCCCCCACTTTCATCTAATATTGC
TCATAGAGGAACTTCTGTTGATTTAGCTATTTTTTCTCTTCATCTAGCTGGAATTTCATCAATCTTAGGTGCAATTAATTTTATTACCACTATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTTTATTTGTTTGATCAGTAGGAATTACTGCTCTTCTTCTTTTATTATCATTACCTGTTTTAGCGGGAGCTATTACTATACTACTTACTGATCGAAATTTAAACACAT
CTTTTTTTGATCCTGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Type material. Holotype: ♂ currently deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 17a, bears four labels: 2nd handwritten and others printed; 1st green, 4th red, and others white [Mt. Alegre, Rio | Pachitea O. Peru | G.Tessmann], [regulus F. | f. sylvarum Bat.], [DNA sample ID: | NVG-22117E05 | c/o Nick V. Grishin], and [HOLOTYPE ♂ | Synargis |

flavicauda Grishin]. **Paratypes:** 3♂♂ from Peru [MFNB]: 2♂♂ the same data as the holotype (NVG-22117D04 and NVG-22117E06) and 1♂ from Peru: Chanchamayo, G. Tessmann leg. (NVG-22117D05).

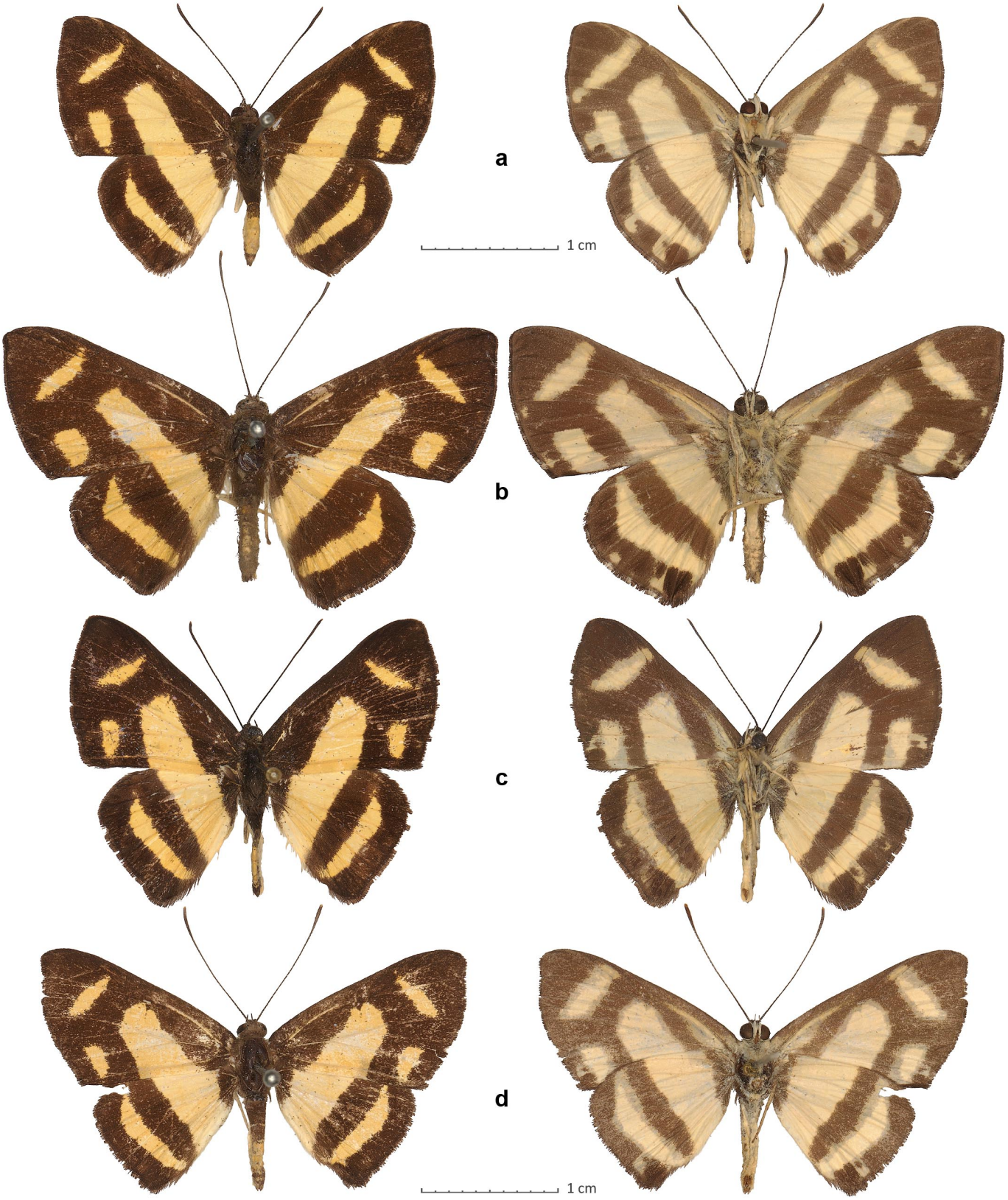

Fig. 17. Holotypes (unless indicated) of new *Synargis* species in dorsal (left) and ventral (right) views (same scale as in Fig. 16), data in text: **a)** *S. flavicauda* **sp. n.**, **b)** *S. tenebritorna* **sp. n.**, and **c–d)** *S. latidifa* **sp. n.**, paratype shown in d).

Type locality. Peru: Rio Pachitea, Monte Alegre. This is also the type locality of *Pseudophaloe tessmanni* Hering, 1925 (Erebidae: Arctiinae) and *Hylesia natex* Draudt, 1929 (Saturniidae).

Etymology. In Latin, *flavus* means yellow or golden, and *cauda* means tail. The compound word *flavicauda* refers to the yellow distal half of the abdomen dorsal side in males of this species. This coloration may also be present in other species of the group but is typically less pronounced. The name is an adjective.

Distribution. Central and East-central Peru.

Synargis tenebritorna Grishin, new species

http://zoobank.org/A7E8E1AF-38EC-4843-8120-1F42E07E6601

(Figs. 15 part, 17b)

Definition and diagnosis. Genomic analysis of the *S. regulus* group reveals a clade that, being distinct from all other species, itself consists of three species-level undescribed taxa (Fig. 15 red, purple, and orange). The second species (Fig. 15 purple) with specimens sequenced from Brazil: Bahia and with incomplete or missing data (likely from Southeast Brazil) differs in COI barcode by 2.6% (17 bp) from *S. flavicauda* **sp. n.** and by 2.0% (13 bp) from the new species described next. This new species is differentiated from its relatives by narrower than in nearly all other species yellow bands and submarginal macules, discal bands not much wider than submarginal bands and macules, weaker developed (but present) marginal yellow spots inside brown border on the ventral side of wings, including a small spot in the cell M_3-CuA_1 that does not reach subapical elongated macule, more strongly defined than in other species dark brown area by tornus on the ventral hindwing, mostly dorsally brown abdomen, and larger size, similar to that of many specimens of *S. regulus*. Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne4191.9.4:T63C, cne1381.2.3:C702T, cne3677.1.5:C150T, cne3677.1.5:C151T, cne1498.4.1:C82A and in COI barcode: G200A, T367C, A451C, T526A, T542C.

Barcode sequence of the holotype. Sample NVG-22117C08, GenBank PP254254, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGTATAATAGGAACATCTCTTAGTTTATTAATTCGAATAGAATTAGGAACTCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAATTATACCTATTATAATTGGAGGATTTGGAAACTGATTAATTCCATTAATATTAGGAGCTCCAGATATAGCTTTCCCCCGTA
TAAATAATATAAGATTTTGATTATTACCTCCTTCTTTATTTTTATTAATCTCCAGAAGAATTGTTGAAAATGGAGCAGGAACTGGATGAACAGTGTACCCCCCACTTTCATCTAATATTGC
TCACAGAGGAACTTCTGTTGATTTAGCCATTTTTTCTCTTCATTTAGCTGGAATTTCATCAATCTTAGGTGCAATTAATTTTATTACCACTATTATTAATATACGTATTAATAATTTATCA
TTTGATCAAATACCTTTATTTGTTTGATCTGTAGGAATTACAGCTCTTCTTCTTTTACTATCATTACCTGTTTTAGCAGGAGCTATTACTATATTACTTACTGATCGAAATTTAAATACAT
CTTTTTTTGATCCTGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Type material. Holotype: ♂ currently deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 17b, bears four printed (text in italics handwritten): three white [*Bahia, Para* | *Sello* | *Sieber* / Hist. Coll. | Nr. *3827*] (text after / is on the other side of the label), [ex coll. | H. STICHEL], [DNA sample ID: | NVG-22117C08 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Synargis | tenebritorna Grishin]. The 1st label of the holotype was added during the subsequent curation of historical collections by Andree Salk. Originally, the holotype was an unlabeled specimen in a series with the handwritten header label [Regulus | Fan God Don. | Baeotis Regulus | Wstw | Bah. Sello, Pará Sieb] on the specimen bearing a label with the number 3827 (a paratype of the species described next). Some specimens in this series are from Bahia, and others from Pará. The series consists of two species. The second species (described next) is Amazonian, and we deduce that it was collected in Pará by Friedrich Wilhelm Sieber (1775–1831). Therefore, we hypothesize that this species was collected in Bahia, Brazil, by Friedrich Sello[w] (1789–1831). **Paratypes:** 1♂ from Brazil, Maassen collection (NVG-21119F09) and 1♀ no data, Weymer collection (NVG-21119F10), both in MFNB.

Type locality. Brazil: Bahia.

Etymology. In Latin, *tenebrosus* means dark and describes something full of shadows or gloom, emphasizing the atmosphere of darkness. The name is a compound word of *tenebrosus* and *tornus* to indicate the dark ventral hindwing tornus. The name is an adjective.

Distribution. Brazil, specifically recorded from Bahia.

Synargis latidifa Grishin, new species

http://zoobank.org/986F2867-69C7-416F-8B88-FA3092BBFAC6

(Figs. 15 part, 17c, d)

Definition and diagnosis. Genomic analysis of the *S. regulus* group reveals a clade that, being distinct from all other species, itself consists of three species-level undescribed taxa (Fig. 15 red, purple, and orange). The first species (Fig. 15 orange) with specimens sequenced from French Guiana and Brazil: Pará differs in COI barcode by 2.0% (13 bp) from *S. tenebritorna* **sp. n.** from and by 2.6% (17 bp) from *S. flavicauda* **sp. n**. This new species is differentiated from its relatives by narrower than in some others yellow bands and submarginal macules, discal band at least twice the width of the submarginal band and macules (the hindwing discal band is even broader comparatively to the submarginal band in the paratype, Fig. 17d), weakly developed marginal yellow spots inside brown border on the ventral side of wings, and the spot in cell M_3-CuA_1 that is either missing or vestigial at least on the forewing, darker tornal area on ventral hindwing is visible but weaker than in *S. tenebritorna* **sp. n**. Males could be with dorsally yellow caudal half of abdomen (yellow ventrally as in other species). Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne1775.24.4:C93A, cne5268.4.1:T390C, cne656.6.1:C168T, cne5798.2.3:T168C, cne2799.9.1:A501G, cne8392.1.3:G87A, cne5383.1.4:A156G, cne5265.4.1:G5775A, cne11854.1.1:A493G, cne6377.3.2:T159T (not C) and in COI barcode: G87A, A409G, T487C, T526T, T542C.

Barcode sequence of the holotype. Sample NVG-22117C04, GenBank PP254255, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGTATAGTAGGAACATCTCTTAGTTTATTAATTCGAATAGAATTAGGAACTCCTGAATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAACTGATTAGTTCCATTAATATTAGGAGCTCCAGATATAGCTTTTCCCCGTA
TAAATAATATAAGATTTTGATTATTACCTCCTTCTTTATTTTTATTAATCTCCAGAAGAATTGTTGAAAATGGAGCAGGAACTGGATGAACAGTGTACCCCCCACTTTCATCCAATATTGC
TCATAGAGGAACTTCTGTTGATTTAGCCATTTTTTCTCTTCATTTGGCTGGAATTTCATCAATCTTAGGTGCAATTAATTTTATTACCACTATTATTAATATACGTATTAATAATTTATCA
TTCGATCAAATACCTTTATTTATTTGATCAGTAGGAATTACTGCTCTTCTTCTTTTACTATCATTACCTGTTTTAGCTGGAGCTATTACTATATTACTTACTGATCGAAATTTAAATACAT
CTTTTTTTGATCCTGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the collection of Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 17c, bears four labels, 2nd handwritten on glassine paper likely cut out of the envelope that contained this specimen, others printed: three white [French Guyana | Roura, Galion | 28.04.1991 | leg. C. Brévignon], [28.IV.1991 | Galion] (has other marks), [DNA sample ID: | NVG-22117C04 | c/o Nick V. Grishin], and one red [HOLOTYPE ♂ | Synargis | latidifa Grishin]. **Paratype:** 1♂ from Brazil: Pará, F. Sieber leg. (NVG-22117C06, GenBank barcode PP254256; the header specimen with the historical collection number label 3827; see the type material section of the previous species for the deduction of the locality of this specimen, Fig. 17d) [MFNB].

Type locality. French Guiana: Roura, Galion.

Etymology. In Latin, *latitudinum differentia* means "difference in width," referring to the widths of the discal (broader) and submarginal (narrow) bands: *lati*[tudinum]+*dif*[ferenti]*a*. The name is an adjective.

Distribution. Lower Amazonian region; recorded from Brazil: Pará and French Guiana.

Family Lycaenidae [Leach], [1815]

Astria Grishin, new subgenus

http://zoobank.org/132F96BD-9572-444E-A4F8-D2E0D21ED23B

Type species. *Lycaena astraea* Freyer, 1851.

Definition. Nuclear genome phylogeny reveals that *Glaucopsyche astraea* (Freyer, 1851) (type locality in Turkey, syntype sequenced as NVG-22119A10) is sister to all other species of *Glaucopsyche* Scudder, 1872 (type species *Polyommatus lygdamus* E. Doubleday, 1841), including type species of all its subgenera and their available synonyms: *Polyommatus lygdamus* E. Doubleday, 1841 of *Glaucopsyche* Scudder, 1872; *Lycaena catalina* Reakirt, 1866 (a junior subjective synonym of *Lycaena piasus* Boisduval, 1852) of *Phaedrotes* Scudder, 1876; *Polyommatus melanops* Boisduval, 1828 of *Apelles* Hemming, 1931; *Glaucopsyche* (*Sinia*) *leechi* Forster, 1940 of *Sinia* Forster, 1940; *Lycaena barine* Leech, 1893 (a subspecies of *Lycaena divina* Fixsen, 1887) of *Shijimiaeoides* Beuret, 1958; and *Lycaena argali*

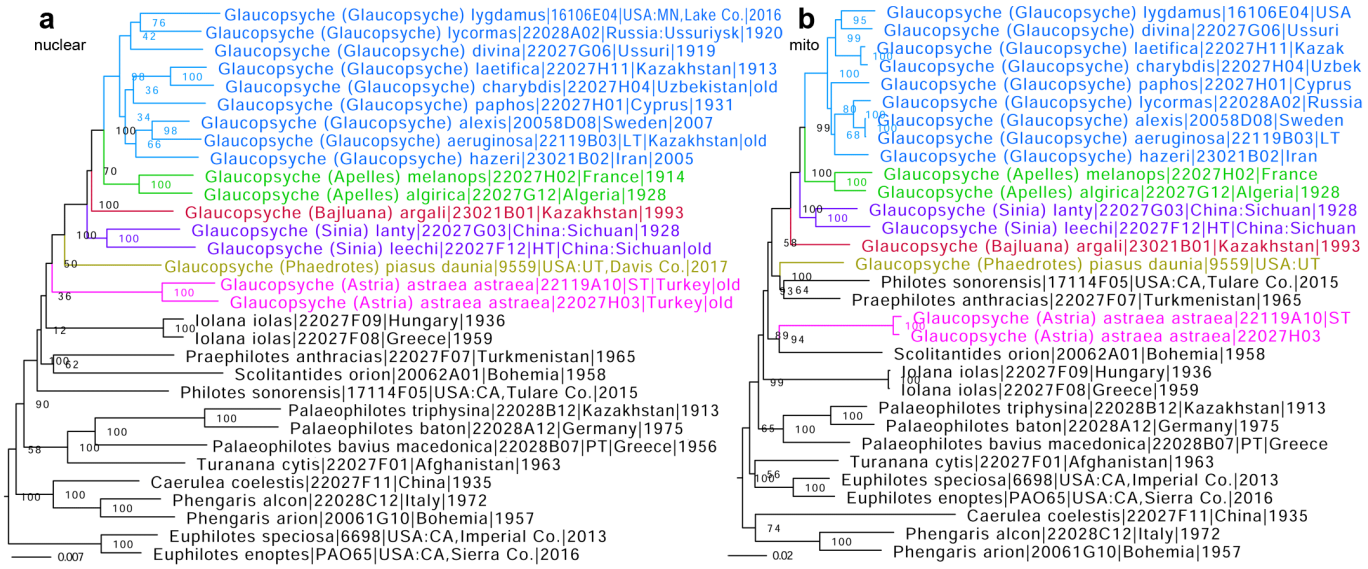

Fig. 18. Phylogenetic trees of selected *Glaucopsyche* species and relatives inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different subgenera of *Glaucopsyche* are colored differently: *Glaucopsyche* (blue), *Apelles* (green), *Bajluana* (red), *Sinia* (purple), *Phaedrotes* (olive), and *Astria* **subgen. n.** (magenta).

Elwes, 1899 of *Bajluana* Korshunov, 1990 (Fig. 18a). We note that *Phaedrotes* (a subgenus of *G. piasus*) that causes problems by rendering *Glaucopsyche* paraphyletic in trees inferred from a small number of gene markers, especially when using a larger fraction of positions from mitochondrial genes (Nazari et al. 2024) or complete mitogenomes (Fig. 18b), is closer related (with 100% statistical support) to the subgenus *Glaucopsyche* than *G. astraea*. In agreement with morphological considerations, *Glaucopsyche* is monophyletic in the nuclear genome tree, with *G. astraea* being its most divergent member. Therefore, *G. astraea* is not monophyletic with any described subgenera of *Glaucopsyche* and does not belong to any of them. Hence, its lineage represents a new subgenus. This new subgenus differs from its relatives by ventrally not darkened marginal area and submarginal spots in forewing cells M_3-CuA_1 and CuA_1-CuA_2 being closer to the margin than in other species and forming a line nearly parallel to the outer margin, while the other three spots of the band are in a straight line with each other and the 4th spot (in cell M_3-CuA_1), i.e., the spot nearest to costa is not offset from the rest. In DNA, a combination of the following characters is diagnostic in the nuclear genome: cce62262.1.1:A120T, cce62262.1.1:C123T, cce748.19.2: C39T, cce748.19.2:T78C, cce2404.9.1:C165T and in COI barcode: A34T, A94T, T448C, T484C, T598C.

Etymology. The name of the type species likely refers to Astraea (Ἀστραῖα), a Greek goddess of justice, innocence, purity, and precision. A different spelling of this name (Astria) is taken as the genus name, which is a feminine noun in the nominative singular.

Species included. Only the type species (i.e., *Lycaena astraea* Freyer, 1851).

Parent taxon. Genus *Glaucopsyche* Scudder, 1872.

Comment. *Glaucopsyche* is an example of confident incongruence between nuclear and mitochondrial genomes (Fig. 18a vs. b) with subgenera *Phaedrotes* and *Astria* **subgen. n.** not being in the same clade as the rest of the genus in the mitogenomic tree, probably due to mitochondrial introgression. Further studies of this incongruence will shed light on the role of hybridization in speciation and adaptation.

Oraidium Bethune-Baker, 1914 is a subgenus of *Brephidium* Scudder, 1876

Genomic analysis of all valid species of *Oraidium* Bethune-Baker, 1914 (type species *Lycaena barberae* Trimen, 1868) and *Brephidium* Scudder, 1876 (type species *Lycaena exilis* Boisduval, 1852), including all but one subspecies, reveals three groups that are approximately equidistant from each other genetically (Fig. 19 blue, purple, and red). Moreover, as the nuclear tree from autosomal codons suggests, *Brephidium* may be paraphyletic with respect to *Oraidium*, and the two African species of the group form

a clade (Fig. 19a) despite genitalic differences between them (Bethune-Baker 1914; Stempffer 1967). Similar, although less confident, grouping is seen in the mitochondrial genome tree (Fig. 19c). The COI barcode difference between the type species of *Oraidium* and *Brephidium* is 6.1% (40 bp), compared to somewhat larger difference between *Brephidium metophis* (Wallengren, 1860) and *Brephidium exilis* (Boisduval, 1852) of 8.1% (53 bp), also indicating approximately equal distance between these three species (Fig. 19). These results may reflect the actual relationship, implying fast evolution in genitalia of *Oraidium*, or caused by more frequent introgression between African species that resulted in convergent overall genetic similarity. Regardless of the evolutionary scenario, we do not see strong genomic support for maintaining *Oraidium* as a distinct genus. Moreover, the two African species, *B. metophis* and *O. barberae*, are quite similar to each other in wing patterns and are frequently misidentified (iNaturalist 2023). Nevertheless, *Oraidium* possesses distinct genitalia (Bethune-Baker 1914; Stempffer 1967), and instead of synonymizing it with *Brephidium*, we propose to treat *Oraidium* Bethune-Baker, 1914, **stat. nov.** as a subgenus of *Brephidium* Scudder, 1876.

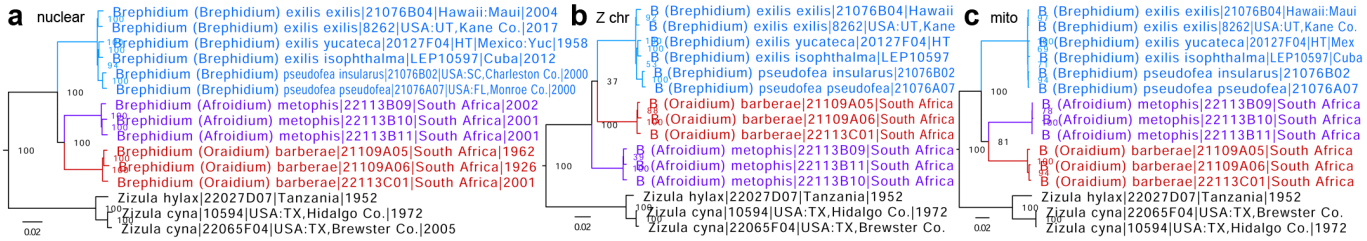

Fig. 19. Phylogenetic trees of *Brephidium* species inferred from protein-coding regions of **a)** the nuclear genome (autosomes), **b)** the Z chromosome, and **c)** the mitochondrial genome. Different subgenera are colored differently: *Brephidium* (blue), *Afroidium* **subgen. n.** (purple), and *Oraidium* **stat. nov.** (red).

Afroidium Grishin, new subgenus

http://zoobank.org/8FC2EE60-CF08-4749-83C9-A772D99E42D0

Type species. *Lycaena metophis* Wallengren, 1860.

Definition. As genomic analysis demonstrates, all species of *Oraidium* Bethune-Baker, 1914 (type species *Lycaena barberae* Trimen, 1868) and *Brephidium* Scudder, 1876 (type species *Lycaena exilis* Boisduval, 1852) (Fig. 19) partition into three groups approximately equidistant from each other (Fig. 19). Above, we proposed to treat *Oraidium* as a subgenus of *Brephidium*, which may render *Brephidium* paraphyletic (Fig. 19). To avoid possible non-monophyletic taxa, the third groups should also be given a rank of subgenus. This new subgenus differs from *Oraidium* by its aedeagus, which is not saddle-shaped in its internal part but is bulbous, and the external part is similar to a small beak (shorter than the internal part) and not divided into two long (about twice the length of the internal part in *O. barberae*) and slender processes; and from both *Oraidium* and *Brephidium* by the tegumen lobe, which is with the terminal process not as long and rod-like as in *Oraidium*, and is broader and bulkier than in *Brephidium*, terminally with five shorter bristles (not two longer ones), and the base dorsally with a hump (not a lobe as in *Oraidium* and not a hook-like process with sharp small teeth as in *Brephidium*). For further details about the morphology of these species and illustrations of their genitalia see Bethune-Baker (1914) and Stempffer (1967). In DNA, a combination of the following characters is diagnostic in the nuclear genome: cce22066.7.13:A106C, cce462.35.4:G159A, cce10386.1.3:C46T, cce1353.3.2:A63T, cce2452.1.6:C78T and in COI barcode: A88T, T127C, T163A, A202G, T616C.

Etymology. The name reflects the Afrotropical distribution of this subgenus and is formed similarly to *Brephidium* and *Oraidium*: *Afro*[tropical Breph]*idium*. The name is a neuter noun in the nominative singular.

Species included. Only the type species (i.e., *Lycaena metophis* Wallengren, 1860).

Parent taxon. Genus *Brephidium* Scudder, 1876.

Lycaena sissona W. G. Wright, 1905 is a junior subjective synonym of *Cupido comyntas* (Godart, [1824])

Genomic sequencing of two syntypes of *Polyommatus comyntas* Godart, [1824] (type locality in North America; NVG-23027C03 and NVG-23027C04) in the Muséum National d'Histoire Naturelle, Paris, France (MNHP) reveals that contrary to the current interpretation they are not monophyletic with specimens from the eastern USA (Fig. 20 green), but instead are in the same clade with specimens from California (Fig. 20 purple), together with the lectotype of *Lycaena sissona* W. G. Wright, 1905 currently treated as a valid subspecies, *Cupido comyntas sissona*, as proposed by Austin (2002). The same relationship is observed in both nuclear and mitochondrial genome trees (Fig. 20a and b), not revealing introgression scenarios that are commonly encountered in closely related groups of populations and even species. Moreover, specimens of *Cupido comyntas texana* (F. Chermock, 1945) form a clade of their own (Fig. 20 blue), thus confirming the validity of this subspecies distributed from southern Texas to Panama. However, specimens collected in the San Antonio area more recently than the type series of *C. comyntas texana* (1992 vs. 1920) (Fig. 20 labeled in cyan) partition into the two clades and may be intergrades between the eastern and southern subspecies.

As a result of the genomic analysis, we suggest that the type locality of *P. comyntas* Godart is likely in California and not in the eastern USA, and propose that *Lycaena sissona* W. G. Wright, 1905, **syn. nov.** is a junior subjective synonym of *Cupido comyntas* (Godart, [1824]). Visual assessment of the syntypes' wing pattern agrees with this conclusion: the ventral forewing nearly lacks marginal and submarginal spots towards the apex, and white framing of dark ventral spots is weakly defined. These characters were mentioned by Austin (2002) to distinguish *C. comyntas sissona* from other subspecies. To stabilize nomenclature, N.V.G. hereby designates one of the two syntypes in MNHP, a male, bearing the following six rectangular labels, 1st red, 4th green, and others white: [TYPE], [comyntas, god. | ♂], [EVERES | COMYNTAS GOD.], [MUSÉUM PARIS], [DNA sample ID: | NVG-23027C04 | c/o Nick V. Grishin], and [MNHN, Paris | EL83734 {QR code}] as the **lectotype** of *Polyommatus comyntas* Godart, [1824]. The style and handwriting on the second label are characteristic of Godart's type specimens, confirming this specimen as a syntype. The lectotype has a small nick at the apex of the right forewing and its head is rotated to the right.

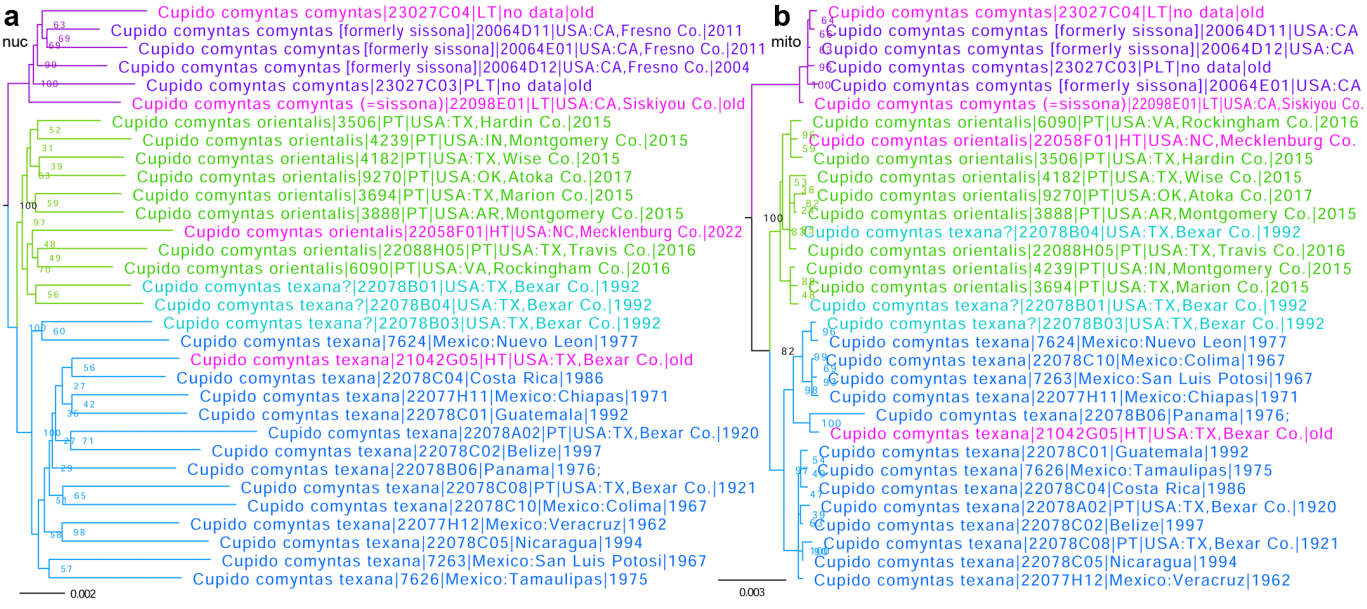

Fig. 20. Phylogenetic trees of *Cupido comyntas* subspecies inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different subspecies are shown in different colors (primary types are labeled in magenta): *C. comyntas comyntas* (purple, including *Lycaena sissona* W. G. Wright, 1905 as its synonym), *C. comyntas orientalis* **ssp. n.** (green), and *C. comyntas texana* (blue). Specimens more recently collected near the type locality of *C. comyntas texana* are labeled in cyan.

Cupido (*Everes*) *comyntas orientalis* Grishin, new subspecies

http://zoobank.org/7169288F-1A58-4B26-8776-F2566A73DB1B

(Figs. 20 part, 21)

Definition and diagnosis. As detailed above, the nominate *Cupido comyntas* (Godart, [1824]) is the western subspecies with a likely type locality in California. As a result, no available name applies to the eastern USA subspecies formerly known as "*Cupido comyntas comyntas*". Only two names, both infrasubspecific, have been proposed for the eastern US populations. *Everes comyntas* ab. *watermani* Nakahara, 1926 (from New York, Tompkins Co., Ithaca) is infrasubspecific according to the ICZN Art. 45.6.2 because it refers to an aberration ("ab.") (ICZN 1999). *Everes comyntas* f. *meinersi* W. D. Field, 1938 (from Kansas. Douglas Co. Lawrence) is infrasubspecific because, in accord with the ICZN Art. 45.6.4, the "author expressly used … "f."" and "also expressly gave it infrasubspecific rank" by stating that "This is the spring brood" (Field 1938). According to the ICZN Glossary, "infrasubspecific entity" refers to "Specimen(s) within a species differing from other specimens in consequence of intrapopulation variability … (e.g. … seasonal forms, … or … differing generations)" (ICZN 1999). The spring brood is a different generation within a species and a seasonal form. Furthermore, the name *meinersi* has not been used as a valid name for a species or subspecies (Art. 45.6.4.1). Therefore, the eastern US subspecies of *C. comyntas* is new and is described here.

The eastern subspecies (Fig. 20 green) is genetically differentiated from the nominate *C. comyntas*, which forms a clade sister to other populations (Fig. 20 purple), and the COI barcode difference between the western and eastern subspecies is 0.6% (4 bp). The eastern subspecies is closer genetically to the southern *Cupido comyntas texana* (F. Chermock, 1945) (type locality USA: Texas, Bexar Co., near San Antonio) (Fig. 20 blue), but forms a clade distinct from it (Fig. 20 green), although their COI barcodes generally do not differ but by one or two base pairs. This new subspecies differs from other *C. comyntas* subspecies by the following characters: darker and browner (vs. grayer) ventral side of wings with more distinct whitish framing of dark spots; typically a complete row of marginal and submarginal spots beneath, particularly on the forewing (these spots are usually poorly expressed or lacking towards the apex in other subspecies); and brighter orange (vs. paler and yellower) ventral hindwing tornal crescents (Fig. 21). See further details in Austin (2002), who referred to *C. comyntas comyntas* by the name of its junior subjective synonym, *sissona*, and to the new subspecies as "*comyntas comyntas*." A combination of the following DNA characters is diagnostic in the nuclear genome: cce2265.4.2:G66A, cce2896.5.6:A57G, cce2896.5.6:T84G, cce13103.2.4:C74T, cce13103.2.4:C59A and in COI barcode differs from the nominate subspecies by: 412A, 508T, 556A, 641T (COI barcodes do not generally differ from *C. comyntas texana*).

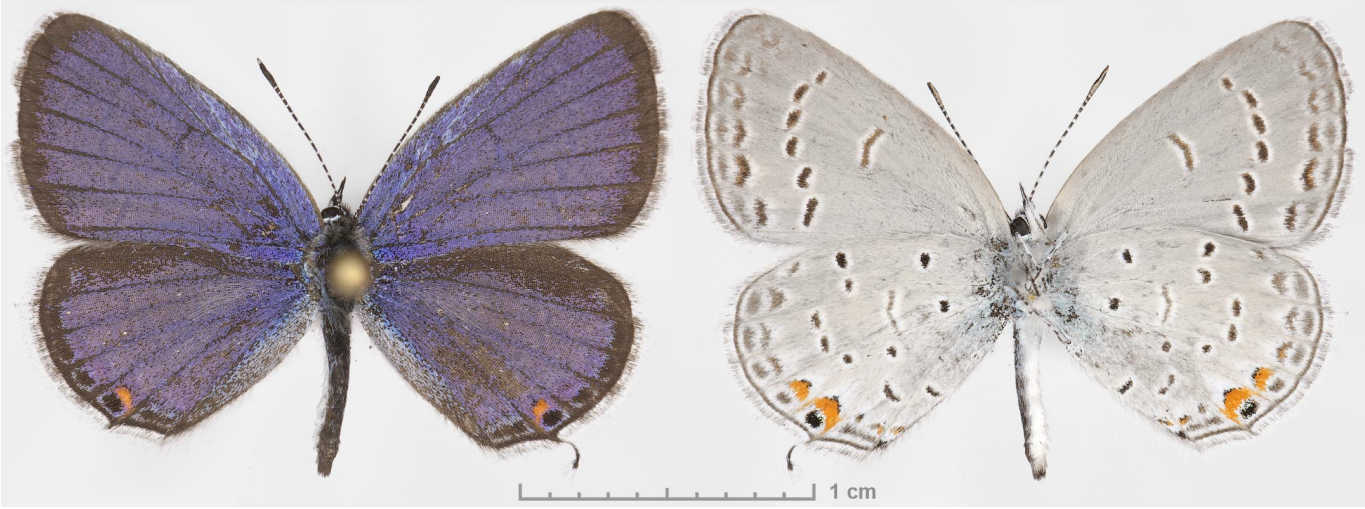

Fig. 21. Holotype of *Cupido comyntas orientalis* **ssp. n.** in dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype. Sample NVG-22058F01, GenBank PP254257, 658 base pairs:

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATATTAGGAACATCTTTAAGAATCTTAATTCGAATAGAATTAGGAACTCCAGGCTCATTAATTGGAGATGATCAAATTTATAATACT
ATTGTCACAGCTCATGCTTTTATTATAATTTTTTTCATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCATTAATATTAGGTGCTCCAGATATAGCATTTCCTCGAA
TAAATAATATAAGATTTTGATTATTACCTCCATCATTAATATTATTAATTTCAAGAAGAATCGTAGAAAATGGAGCAGGAACAGGATGAACAGTGTACCCCCCACTTTCATCAAATATTGC
CCATGGAGGATCATCTGTAGATTTAGCAATTTTTTCTTTACATTTAGCAGGAATCTCTTCAATTTTAGGAGCAATTAATTTTATTACAACTATTATTAATATACGAGTTAATAATTTATCA
TTTGATCAAATATCTCTATTTATTTGAGCTGTAGGAATTACAGCATTATTATTATTATTATCATTACCTGTATTAGCTGGGGCTATTACAATATTATTAACTGATCGAAATTTAAATACCT
CATTTTTTGATCCTGCTGGAGGAGGAGACCCAATCTTATATCAACATTTATTT

Type material. Holotype: ♂ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 21, bears five printed labels: four white [NC: Mecklenburg Co. | Charlotte, W. Arrowood Rd & | Green Ridge Dr, near Sugar Creek May 20, 2022 | Leg: W. Dempwolf], [Cupido comyntas | ♂ | Coll of: W R Dempwolf], [DNA sample ID: | NVG-22058F01 | c/o Nick V. Grishin], [WRD 20,914], and one red [HOLOTYPE ♂ | Cupido comyntas | orientalis Grishin]. **Paratypes:** 6♂♂ and 4♀♀ from USA: 1♂ Virginia, Rockingham Co., Briery Branch Rd., 7.3 mi WNW Briery Branch, GPS 38.4734, −79.2042, 07-May-2016, N. V. Grishin & Q. Cong leg. (NVG-6090); 1♀ Indiana, Montgomery Co., Shades State Park, 39.9312, −87.0666, N. V. Grishin, 1-Aug-2015 (NVG-4239); 1♂ Arkansas, Montgomery Co., Ouachita National Forest, Big Brushy Creek, along NF6, GPS 34.6589, −93.8345, 4-Jul-2015, N. V. Grishin leg. (NVG-3888); 1♂ Oklahoma, Atoka Co., McGee Creek, GPS 34.3737, −95.8886, 11-Jul-2017, N. V. Grishin leg. (NVG-9270); and Texas: 1♀ Marion Co., Caddo Lake region, along SH43, GPS 32.7957, −94.1755, 20-Jun-2015, N. V. Grishin leg. (NVG-3694); 1♂ Wise Co., LBJ National Grassland, Cottonwood Lake, GPS 33.3828, −97.5719, 19-Jul-2015, N. V. Grishin leg. (NVG-4182); 1♀ Hardin Co, 4.4 mi SW Kountze, along FM770, GPS 30.3389, −94.3678, 7-Jun-2015, N. V. Grishin leg. (NVG-3506); Travis Co., Barton Creek Greenbelt, Camp Craft Road entrance, 28-Mar-2016, W. R. Dempwolf, leg.: 1♂ (NVG-22088H04, WRD 9243) and 1♀ (NVG-22088H05, WRD 9244); and 1♂ Blanco Co., USH281 ca. 1 mi N of USH290, 3-Oct-2015, W. R. Dempwolf, leg. (NVG-22088H03, WRD 3649).

Type locality. USA: North Carolina, Mecklenburg Co., Charlotte, W. Arrowood Rd. and Green Ridge Dr. near Sugar Creek.

Etymology. In Latin, *orientalis* means eastern. This way, the eastern Eastern Tailed Blue gets its eastern name. The name is an adjective.

Distribution. In the eastern half of the USA, southwards to central Texas and Florida.

Albulina Tutt, 1909 is a genus distinct from *Agriades* Hübner, [1819]

Genomic phylogeny of *Agriades* Hübner, 1819 (type species *Papilio glandon* Prunner, 1798) and relatives reveals nuclear-mitochondrial incongruence (Fig. 22). *Papilio orbitulus* de Prunner, 1798, a valid name for the type species of *Albulina* Tutt, 1909 (type species *Papilio pheretes* Hoffmansegg, 1804, a replacement name for *Papilio atys* Hübner, [1804], regarded as a junior subjective synonym of *P. orbitulus*), currently in the genus *Agriades*, is indeed placed within *Agriades* in the mitochondrial genome tree (Fig. 22c magenta and green). However, nuclear genome trees (both autosomes Fig. 22a and the Z

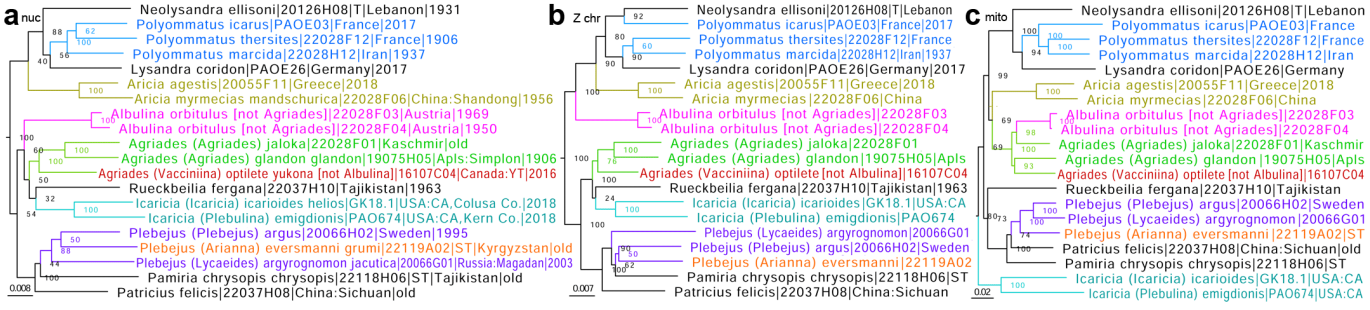

Fig. 22. Phylogenetic trees of selected Polyommatina species inferred from protein-coding regions of **a)** the nuclear genome (autosomes), **b)** the Z chromosome, and **c)** the mitochondrial genome. Different subgenera are colored differently: *Polyommatus* Latreille, 1804 (blue), *Aricia* Reichenbach, 1817 (olive), *Albulina* **stat. rev.** (magenta), *Agriades* (green with subgenus *Vacciniina* **stat. rev.** labeled in red), *Icaricia* Nabokov, 1945 (cyan), and *Plebejus* (purple with subgenus *Arianna* **stat. nov.** labeled in orange).

chromosome Fig. 22b) reveal that *A. orbitulus* is not monophyletic with *Agriades*, and the Z chromosome tree shows strong statistical support for the lack of monophyly (Fig. 22b). We consider that the nuclear genome represents the organism, and the mitochondrial genome is more prone to evolutionary irregularities such as introgression and possibly complete replacement by a mitogenome of a relative. Therefore, *Agriades* is not monophyletic if it includes *A. orbitulus*. To restore the monophyly of *Agriades*, we propose to treat *Albulina* Tutt, 1909, **stat. rev.** as a genus distinct from *Agriades* Hübner, [1819]. The example of nuclear-mitochondrial incongruence in *Agriades*-*Albulina* is remarkable in its taxonomic significance and should be investigated further in more detail.

Vacciniina Tutt, 1909 is a valid subgenus of *Agriades* Hübner, [1819]

Vacciniina Tutt, 1909 (type species *Papilio optilete* Knoch, 1781) is currently treated as a junior subjective synonym of *Albulina* Tutt, 1909 (type species *Papilio pheretes* Hoffmansegg, 1804, a replacement name for *Papilio atys* Hübner, [1804], regarded as a junior subjective synonym of *Papilio orbitulus* de Prunner, 1798), is not monophyletic with it, and instead remains in the genus *Agriades* Hübner, 1819 (type species *Papilio glandon* Prunner, 1798) (Fig. 22). Currently, *A. optilete* is assigned to a subgenus different from the nominotypical. To keep this hierarchy, we propose that *Vacciniina* Tutt, 1909, **stat. rest.** is a valid subgenus. This view is consistent with higher genetic differentiation of *A. optilete* from other *Agriades*.

Arianna Bálint, 2022 is a subgenus of *Plebejus* Kluk, 1780

Originally proposed as a genus, *Arianna* Bálint, 2022 (type species *Lycaena eversmanni* H. C. Lang, 1884) originates within *Plebejus* Kluk, 1780 (type species *Papilio argus* Linnaeus, 1758) rendering it polyphyletic (Fig. 22). Willing to neither split *Plebejus* into several genera nor synonymize *Arianna*, we propose to treat *Arianna* Bálint, 2022, **stat. nov.** as a subgenus of *Plebejus* Kluk, 1780. Some other related genera are likely to become subgenera of *Plebejus* after their genomic data are analyzed.

Family Hesperiidae Latreille, 1809

Genomic phylogeny of *Euriphellus* Austin, 2008

Four new species of *Euriphellus* Austin, 2008 (type species *Papilio euribates* Stoll, 1782) have been recently proposed: *E. panamicus* Grishin, 2023 (type locality in Panama: Panama), *E. panador* Grishin, 2023 (type locality in Ecuador: Esmeraldas), *E. colombiensis* Grishin, 2023 (type locality in Colombia: Río Dagua), and *E. ecuadoricus* Grishin, 2023 (type locality in Ecuador: Canelos) (Zhang et al. 2023a; Zhang et al. 2023d), but not all of them have been included together in the same phylogenetic tree. Here we show genome-based phylogeny of all known *Euriphellus* species (Fig. 23). The genus partitions into two distinct clades: the *E. euribates* group that consists of three species: *E. cebrenus* (Cramer, 1777) (type locality in Suriname), *E. euribates* (Stoll, 1782) (type locality in Suriname), and *E. polygius* (Latreille, [1824]) (type locality in South Brazil, as deduced by genomic sequencing) and the *E. phraxanor* group that includes all other species. *Euriphellus cebrenus* and *E. euribates* could be conspecific (Zhang et al. 2022b) pending further research and possible neotype designations. We find that the three trees are incongruent in the *E. phraxanor* group. However, the topology of the Z chromosome tree is not strongly supported (Fig. 23b). Most notable irregularities are in the mitochondrial genome tree (Fig. 23c): *E. panador* and *E. colombiensis* essentially share the mitochondrial DNA, despite not being sisters in the nuclear genome, and *E. lama* (Evans, 1952) (type locality in Guatemala) is similar to them while being a more distant species according to the nuclear genome. Comparing the topologies of the three trees, we hypothesize that *E. colombiensis* and *E. lama* experienced mitochondrial DNA introgression from *E. panador* but at different time points.

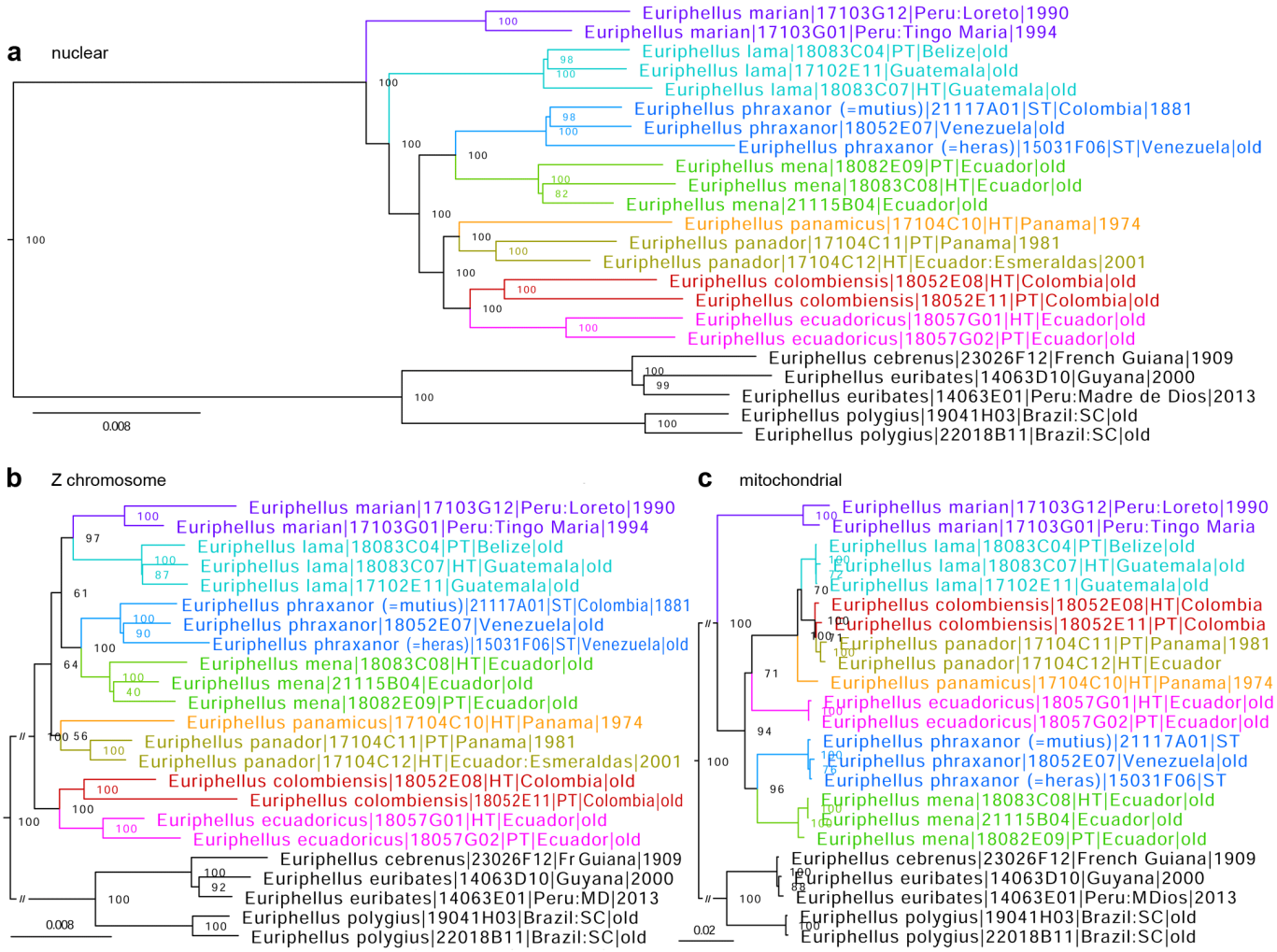

Fig. 23. Phylogenetic trees of all described *Euriphellus* species inferred from protein-coding regions of **a)** the nuclear genome (autosomes, about 3 million positions and ultrafast bootstrap (Minh et al. 2013) were used), **b)** the Z chromosome, and **c)** the mitochondrial genome. Different species of the *E. phraxanor* group are colored differently: *E. marian* (violet), *E. lama* (cyan), *E. phraxanor* (blue), *E. mena* (green), *E. panamicus* (orange), *E. panador* (olive), *E. colombiensis* **sp. n.** (red), and *E. ecuadoricus* **sp. n.** (magenta). Sections of the Z chromosome and mitogenome trees corresponding to long branches were removed (as indicated by //) to fit in the allotted space.

Uniphylus Lemes, Siewert, O. Mielke, Casagrande & A. Warren, 2023 is a subgenus of *Bolla* Mabille, 1903

Proposed as a genus for a single species *Staphylus evemerus* Godman & Salvin, 1896 (type locality in Costa Rica) based on a detailed study that integrated evidence from morphology, biology, and COI barcodes (Lemes et al. 2023), *Uniphylus* Lemes, Siewert, O. Mielke, Casagrande & A. Warren, 2023 is a confidently supported sister to a group of four species formerly limped under *Bolla zorilla* (Plötz, 1886) (type locality in Panama), (Zhang et al. 2023a, e). Genetic similarity between *S. evemerus* and the *B. zorilla* group is also reflected in the shape of uncus and tegumen (no side processes), short saccus, straight and short aedeagus, and a similar base of valva. Moreover, valvae of these species possess a process from the ampulla and differ from most other relatives by a straighter and expanded harpe. Although harpe is expanded into a different shape in *S. evemerus* and the *B. zorilla* group, to avoid monotypic genera in the presence of confidently identified relatives, we place the *B. zorilla* group species in *Uniphylus*.

This newly expanded *Uniphylus* is sister to the clade of species that were previously included in *Staphylus* Godman and Salvin, 1896 (type species *Helias ascalaphus* Staudinger, 1876) and recently transferred to *Bolla* Mabille, 1903 (type species *Bolla pullata* Mabille, 1903, treated as a junior subjective synonym of *Staphylus imbras* Godman and Salvin, 1896) (Zhang et al. 2023e). This group of species

contains *Pholisora balsa* E. Bell, 1937 (type locality in Peru), which is the type species of *Stolla* Grishin, 2023. While it is conceivable to treat *Uniphylus* and *Stolla* as synonyms, they stand as prominent phylogenetic groups in the nuclear genomic tree (Zhang et al. 2023e), and genetic differentiation between them is non-trivial, e.g., COI barcodes of the type species differ by 10.5% (69 bp, note rapid evolution of mitogenomes in some Pholisorina Grishin, 2023). Their genitalia also differ: e.g., *Stolla* species bear side processes (vestigial in some) on tegumen and their valva is shaped differently, both at the base and the harpe (Evans 1953; Zhang et al. 2023e). Therefore, *Uniphylus* and *Stolla* can be treated as valid taxa of equivalent rank. All these species were placed in *Bolla* by Zhang et al. (2023e) due to their monophyly, overall similarity in appearance, and to keep the traditional genera *Bolla* and *Staphylus* after minimal rearrangements required to restore monophyly.

For all these reasons, we propose to treat *Uniphylus* Lemes, Siewert, O. Mielke, Casagrande & A. Warren, 2023, **stat. nov.** as a subgenus of *Bolla* Mabille, 1903, among its other subgenera: sister subgenus *Stolla* Grishin, 2023 (type species *Pholisora balsa* E. Bell, 1937), and more distant relatives *Sebia* Grishin, 2023 (type species *Nisoniades eusebius* Plötz, 1884), *Bovaria* Grishin, 2023 (type species *Achlyodes cyclops* Mabille, 1876), and *Bolla*.

Heliopetes (*Heliopetes*) *acuta* Grishin, new species

http://zoobank.org/E7DBBCD3-4C99-4421-B3AB-D755D72CD7F3

(Figs. 24 part, 25)

Definition and diagnosis. Genome-based phylogeny places one specimen from Oaxaca, Mexico, tentatively identified by us as *Heliopetes* (*Heliopetes*) *lana* Grishin, 2023 (type locality in Guatemala) as sister to the clade of three species: *H. lana*, *Heliopetes alana* (Reakirt, 1868) (type locality in Colombia), and *Heliopetes chimbo* Evans, 1953 (type locality in Ecuador) (Fig. 24) and, therefore, represents a species distinct from them. The COI barcode of the new species differs by 2.1% (14 bp) from *H. lana*, 1.7% (11 bp) from *H. alana*, and 1.8% (12 bp) from *H. chimbo*. Curiously, the geographically closest and possibly sympatric *H. lana* has the COI barcode most different from the new species. This new species keys to *H. alana* (G.2.12) in Evans (1953) and differs from its relatives by better defined and larger pale triangles with sharper points at the outer margin of the ventral forewing, particularly at the apex, and brownish gray anal fold on the dorsal hindwing (typically white in *H. lana* and *H. alana*) (Fig. 25). Because the phenotypic variation of this species has not been explored, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly2532.4.3:A174G, aly2532.4.3:T345A, aly2532.4.3:C360T, aly2532.4.3:A363G, aly2633.1.13:T141C, aly259.26.1:C599C (not G), aly259.26.1:T619T (not C), aly235.14.1:C2784C (not T), aly235.14.1:A2829A (not G), aly1603.14.1:A294A (not G) and in COI barcode: C3T, C133T, T178T, T235T, T376A, C610T, T613T.

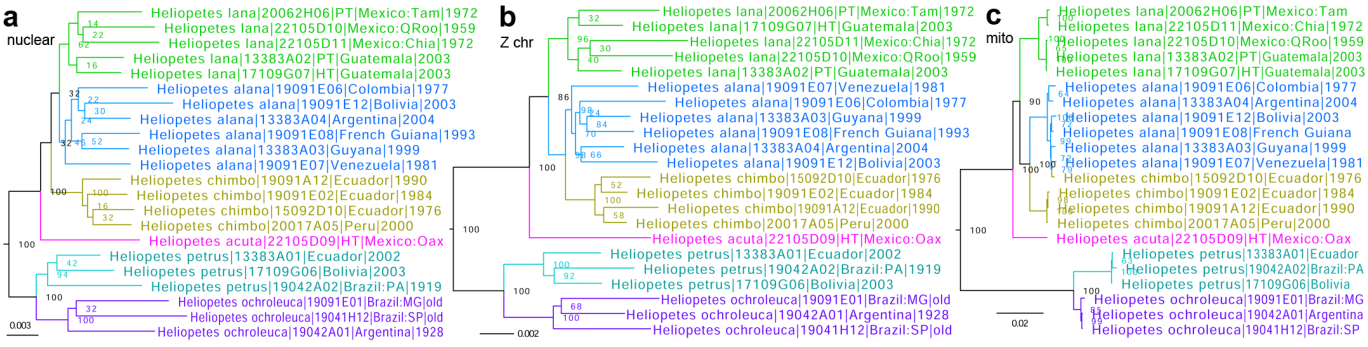

Fig. 24. Phylogenetic trees of selected *Heliopetes* (*Heliopetes*) species inferred from protein-coding regions of **a)** the nuclear genome (autosomes), **b)** the Z chromosome, and **c)** the mitochondrial genome. Different species are shown in different colors: *H. lana* (green), *H. alana* (blue), *H. chimbo* (olive), *H. acuta* **sp. n.** (magenta), *H. petrus* (Hübner, [1819]) (cyan), and *H. ochroleuca* J. Zikán, 1938 (purple). Note the introgression of *H. alana* mitochondrial DNA into one *H. chimbo* specimen (NVG-15092D10).

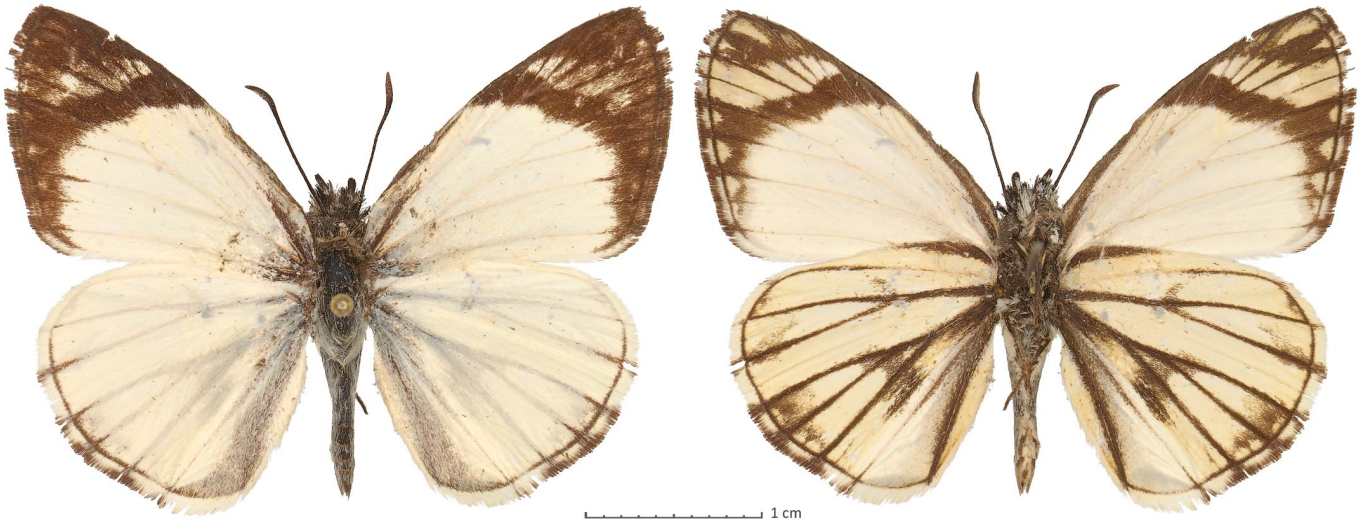

Fig. 25. Holotype of *Heliopetes* (*Heliopetes*) *acuta* **sp. n.** in dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype: Sample NVG-22105D09, GenBank PP254258, 658 base pairs:

AATTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCTTTAAGTTTATTAATTCGAACTGAATTAGGAAATCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCTTTTATTATAATTTTTTTCATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATTAGGAGCCCCAGATATAGCATTTCCTCGTA
TAAATAATATAAGATTTTGACTTTTACCCCCATCCCTAACATTATTAATTTCAAGAAGTGTAGTAGAAAATGGAGCAGGAACTGGTTGAACAGTTTACCCCCCTCTCTCGGCTAATATCGC
CCATCAAGGATCATCTGTTGATTTAGCTATTTTTTCTTTACATTTAGCTGGAATTTCATCTATCTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGTATTAGAAATATATCA
TTTGACCAAATACCTTTATTTGTATGAGCAGTAGGAATTACTGCTTTATTACTACTATTATCATTACCTGTTTTAGCAGGTGCTATTACAATATTATTAACAGATCGAAATTTAAATACAT
CATTTTTTGATCCTGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTC

Type material. Holotype: ♂ deposited in the California Academy of Sciences, San Francisco, CA, USA [CAS], illustrated in Fig. 25, bears seven printed (dates and the species name on the 4th label handwritten): six white [MEX.: Oaxaca, | Candelaria Loxicha | 500 m; IX-15-73], [rec'd from | P. Hubbell], [Collection of | C. D. MacNeill], [Heliopetes | alana (Reak.) | Det. C.D. MacNeill '75], [DNA sample ID: | NVG-22105D09 | c/o Nick V. Grishin], [{QR Code} CASENT | 8568387], and one red [HOLOTYPE ♂ | Heliopetes (Heliopetes) | acuta Grishin].

Type locality. Mexico: Oaxaca, Candelaria Loxicha.

Etymology. In Latin, *acutus* means sharp, pointed, or keen, from which we form the noun *acuta* and use it in apposition. The name refers to sharp and pointed marginal white triangles on the ventral forewing of this species.

Distribution. Known only from Oaxaca in Mexico.

Gerosis cnidus (Plötz, 1884) is a species distinct from *Gerosis phisara* (Moore, 1884), with revised synonymy

Inspecting Hesperiidae holdings in MFNB, we found a syntype of *Achlyodes cnidus* Plötz, 1884 (type locality not specified), currently regarded as a junior subjective synonym of *Gerosis phisara* (Moore, 1884) (type locality in India: Assam, Khasi Hills). According to its labels (Fig. 26b), this specimen shown in Fig. 26a was identified as *cnidus* by Plötz ("best[immt]. v[on]. Plötz"). This identification label was added to the specimen before the publication because it cited an unpublished name ("i[n]. l[itteris]"). The specimen is from the Weymer collection that contains primary types of many of Plötz's names. The specimen agrees with the original description and Godman's copy of the unpublished illustration by Plötz (Fig. 26c). Because this is a rarely encountered phenotype of *Gerosis*, it is possible that it was the only specimen known to Plötz, and the specimen illustrated. Godman's copies appear rather sketchy and not particularly detailed (it remains unclear whether the originals were more detailed), and the agreement between the illustration (Fig. 26c) and the specimen (Fig. 26a) is reasonable.

To stabilize nomenclature, N.V.G. hereby designates the specimen in MFNB shown in Fig. 26a, a male, bearing the following five rectangular white labels, 3rd and 5th printed, others handwritten: [cnidus

Plötz | Nº 161 best. v. Plötz], [Cnidus Plötz | i.l], [Coll. Weymer], [75:5.], and [DNA sample ID: | NVG-21115E07 | c/o Nick V. Grishin] as the **lectotype** of *Achlyodes cnidus* Plötz, 1884. The lectotype has a fingerprint mark at the left forewing apex and is missing a part of the right hindwing fringe towards the tornus. The number 161 likely refers to the Weymer collection, and we were not able to associate it with any published information. The label 75:5 gives a genus number (75 - *Gindanes*) and a species number (5 - *cnidus*) in the Mabille catalog (Mabille 1903) that was used as a guide to arranging the Hesperiidae collection in Berlin. The type locality that was not specified in the original description and not given on the lectotype labels remains unknown and will eventually be deduced by genomic sequencing and comparison with *G. cnidus* specimens from known localities.

Genomic sequencing of the *A. cnidus* lectotype does not place it close to *G. phisara* but instead revealed that it is distant from other *Gerosis* Mabille, 1903 (type species *Coladenia hamiltoni* Nicéville, 1889, currently treated as a junior subjective synonym of *Satarupa phisara* (Moore, 1884) (Fig. 27). Therefore, we propose that *Gerosis cnidus* (Plötz, 1884), **stat. rest.** is a valid species distinct from *Gerosis phisara* (Moore, 1884) in particular, and from other *Gerosis* in general. Because we sequenced only one specimen of *Gerosis cnidus*, it remains unclear whether its unique for *Gerosis* wing pattern is an aberration as suggested by Evans (1949) or a color morph (in which case the typical striped and spotted form has not been found yet), or represents the typical (and the only?) wing pattern form of this species.

Although we have not sequenced type specimens of *Coladenia hamiltoni* Nicéville, 1889 (type locality in Bangladesh: Sylhet) and *Caprona*? *kuki* Tytler, 1915 (type locality in India: Lushai Hills), due to their wing pattern similarity with *G. cnidus*, we propose to treat them as its junior subjective synonyms instead of keeping them as synonyms of *Gerosis phisara*, pending further research. As a result of this analysis, the valid name for the type species of *Gerosis* becomes *Gerosis cnidus* (Plötz, 1884), **stat. rest**.,

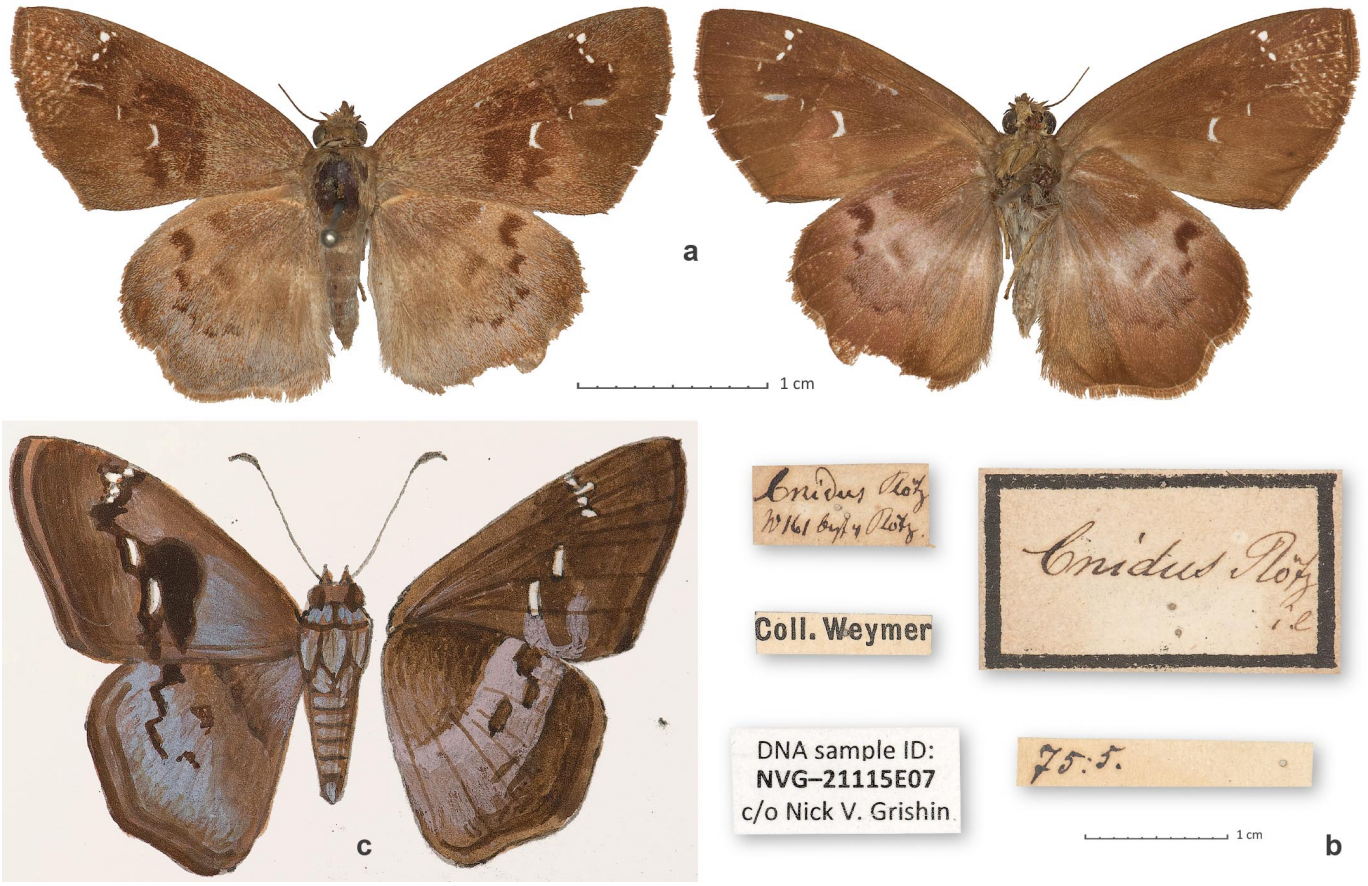

Fig. 26. *Gerosis cnidus* **stat. rest. a)** lectotype, **b)** its labels (reduced by a quarter compared to the specimen, scale below), and **c)** Godman's copy of the unpublished Plötz's drawing of *Achlyodes cnidus*, © of the Trustees of the Natural History Museum London and is made available under Creative Commons License 4.0 (https://creativecommons.org/licenses/by/4.0/). Dorsal and ventral views are shown on the left and right of the figure panel letter.

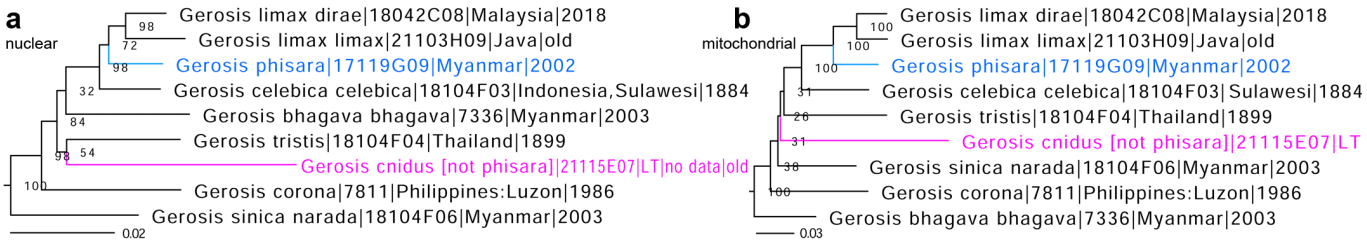

Fig. 27. Phylogenetic trees of selected *Gerosis* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome: *Gerosis phisara* (blue) and *Gerosis cnidus* **stat. rest.** (magenta).

and we hypothesize that its type locality is in eastern India or Bangladesh. If the wing pattern of *G. cnidus* represents an unusual color morph, this morph may be present in other species of *Gerosis*, in which case *C. hamiltoni* and/or *C. kuki* might be some species other than *G. cnidus*.

***Metrocles nun* Grishin, new species**

http://zoobank.org/40F78E1E-A010-4A7C-83CD-A1B5C9DFBBE1

(Figs. 28 part, 29, 30a)

Definition and diagnosis. Genomic analysis of an unusually patterned specimen from Goiás Brazil (Fig. 29) somewhat resembling *Metrocles schrottkyi* (Giacomelli, 1911) (type locality in Argentina) (Fig. 30b) in its wing pattern and a tri-partite brand that is nearly shaped into a stigma, places it in *Metrocles* Godman, 1900 (type species *Metrocles leucogaster* Godman, 1900) sister to *Metrocles argentea* (Weeks, 1901) (type locality in Bolivia) (Fig. 28). This specimen represents a new species that differs from all similar species by a combination of a nearly straight white discal band on reddish-brown ventral hindwing with its white inner margin that continues along the sides of the thorax, behind the eyes and onto the collar, thus forming a continuous hairpin-shaped white framing from tornus of one hindwing to the other, and the lack of white spots in the forewing discal cell. *Metrocles schrottkyi* lacks this white hairpin framing. In DNA, a combination of the following characters is diagnostic in the nuclear genome: aly2850.3.4:C63T, aly103.44.1:C60T, aly1591.7.3:T331C, aly127.37.1:G699A, aly127.37.1:C721A, aly2850.3.4:C75C (not T), aly6398.4.4:G66G (not A), aly6398.4.4:C72C (not G), aly499.16.2:C159C (not T), aly3268.8.1:C138C (not T) and in COI barcode: T49C, T197C, T235C, T529A, T595C.

Barcode sequence of the holotype. Sample NVG-18117A01, GenBank PP254259, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACTTCCCTAAGATTATTAATTCGAACTGAATTAGGAGCTCCTGGATCATTAATTGGAGATGATCAAATTTATAATACT
ATTGTTACAGCTCATGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGACTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCATTCCCTCGAA
TAAATAATATAAGATTTTGAATATTACCCCCATCATTAACTTTATTAATTTCTAGAAGAATTGTAGAAAATGGTGCAGGTACTGGTTGAACAGTTTATCCTCCTTTATCTTCTAATATTGC
CCATCAAGGATCTTCTGTTGATTTAGCAATTTTTTCACTTCATTTAGCTGGTATCTCATCAATCTTAGGAGCTATTAACTTTATCACAACAATTATTAATATACGAATTAGAAATATATCA
TTTGATCAAATACCTTTATTTGTATGATCTGTAGGAATTACAGCATTATTATTACTTTTATCTTTACCTGTTCTAGCTGGAGCTATTACTATATTACTTACTGATCGAAACTTAAATACTT
CATTTTTTGATCCTGCTGGAGGAGGTGATCCTATTTTATATCAACATTTATTT

Type material. Holotype: ♂ currently deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA [USNM], illustrated in Fig. 29, bears seven printed labels (text in italics handwritten): six white [24 kil. E. Formoso, | Go., Brazil | May 16, 1956 | F. S. Truxal], [MACHRIS BRAZILIAN | EXPEDITION – 1956 | LOS ANGELES | COUNTY MUSEUM], [genitalia | slide/vial # | *H 78* | Prep. S.S. Nicolay], [*Chalcone* | *zisa* ♂ | Det. *Plotz* | S.S. Nicolay], [DNA sample ID: | NVG-18117A01 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01531662], and one red [HOLOTYPE ♂ | Metrocles | nun Grishin].

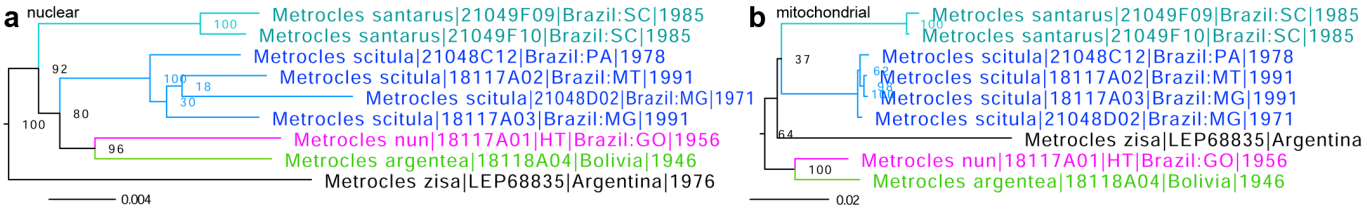

Fig. 28. Phylogenetic trees of selected *Metrocles* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different species are colored differently: *M. santarus* (E. Bell, 1940) (cyan), *M. scitula* (Hayward, 1951) (blue), *M. nun* **sp. n.** (magenta), *M. argentea* (green), and *M. zisa* (Plötz, 1882) (black).

Fig. 29. Holotype of *Metrocles nun* **sp. n.** in dorsal (left) and ventral (right) views, data in text.

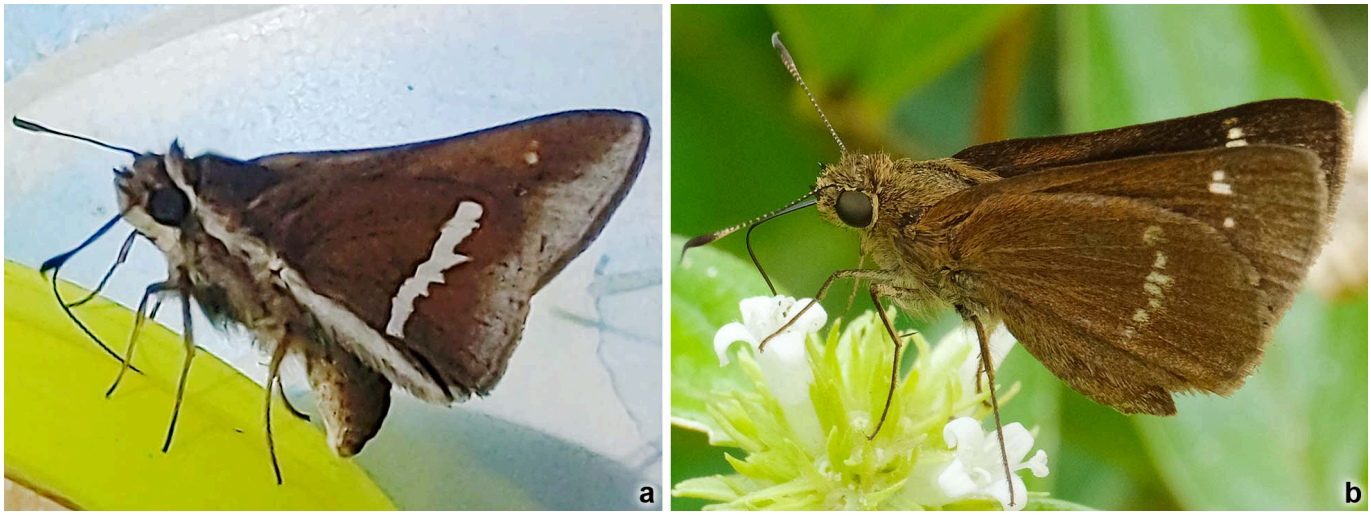

Fig. 30. Two species of *Metrocles*, iNaturalist observations from Brazil: **a)** *Metrocles nun* **sp. n.** (possible, or a close relative) 111942398 Brasília, Distrito Federal, 18-Apr-2022 © Antônio (ajcaguiar); **b)** *Metrocles schrottkyi* 155279605 São Paulo, Santo André, Paranapiacaba, 28-Jan-2023 © rick_costa. Images are color-corrected, rotated, cropped, and b) flipped. CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/.

Type locality. Brazil: Goiás, 24 km east of Formoso.

Etymology. A resting individual of this species, with its dark color and white framing from collar to tornus, resembles a nun (Fig. 30a), hence the name, which is a noun in apposition.

Distribution. Currently known from Central Brazil.

Comments. Although we have not yet sequenced *M. schrottkyi* (Fig. 30b), querying the BOLD database (Ratnasingham and Hebert 2007) with COI barcodes of our sequenced specimens reveals that it is a species different from either *Metrocles scitula* (Hayward, 1951) (type locality in Brazil: Mato Grosso) and this new species, although closely related to them (~2.5% difference).

Hedone miracla Grishin, new species

http://zoobank.org/81924866-9D81-4CAF-AA05-4BCC49DAF282

(Figs. 31 part, 32)

Definition and diagnosis. Genomic analysis of *Hedone* Scudder, 1872 (type species *Hesperia brettus* Boisduval & Le Conte, [1837], a junior subjective synonym of *Thymelicus vibex* Geyer, 1832) reveals

that a female collected north of Lima in Peru is sister to *Hedone mira* Grishin & Lamas, 2022 (type locality in Peru: Apurímac) but is genetically differentiated from it at the species level (Fig. 31), e.g., their COI barcodes differ by 2.4% (16 bp). Therefore, this female represents a new species. This new species differs from other *Hedone* species (except *H. mira*) by rusty-colored ventral hindwing with a yellowish broken discal band and only slightly scalloped dark outer border of forewing, and differs from *H. mira* by redder and broader (but not as broad and continuous as in *Hedone bittiae* (Lindsey, 1925), type locality in Peru) discal band on ventral hindwing, more diffuse marginal brown on dorsal hindwing blending with orange ground color, smaller forewing subapical spots, and submarginal spots more offset towards the forewing margin. Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly103.11.2:C760T, aly103.11.2:A1569G, aly159.18.1:T114A, aly159.18.1:C198T, aly499.1.3:G42A, aly1487.2.21:G57G (not A), aly1487.2.21:C60C (not A), aly577.49.5:A174A (not G), aly331.3.6:C165C (not T), aly569.1.2:C109C (not T) and in COI barcode: T124C, T284C, T343A, T532A, T596C.

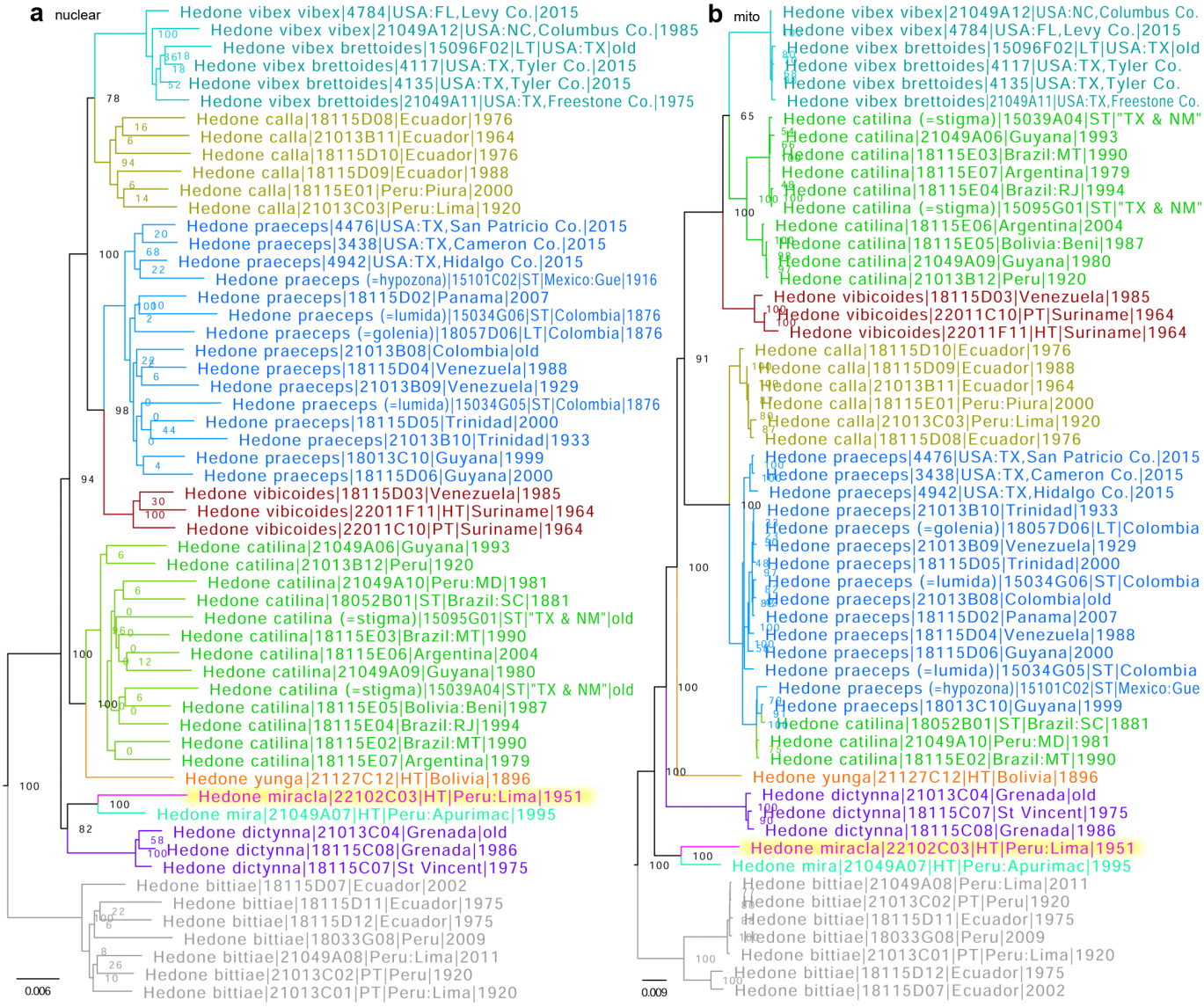

Fig. 31. Phylogenetic trees of selected *Hedone* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different species are colored differently: *H. vibex* (Geyer, 1832) (cyan), *H. calla* (Evans, 1955) (olive), *H. praeceps* Scudder, 1872 (blue), *H. vibicoides* (de Jong, 1983) (maroon), *H. catilina* (Plötz, 1886) (green), *H. yunga* Grishin, 2023 (orange), *H. miracla* **sp. n.** (magenta, label highlighted in yellow), *H. mira* Grishin & Lamas, 2022 (aquamarine), *H. dictynna* (Godman & Salvin, 1896) (purple), and *H. bittiae* (Lindsey, 1925) (gray).

Fig. 32. Holotype of *Hedone miracla* **sp. n.** in dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype. Sample NVG-22102C03, GenBank PP254260, 658 base pairs:

AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATATTAGGAACTTCCTTAAGTTTATTAATTCGAACAGAATTAGGTAATCCTGGTTCTTTAATTGGAGATGATCAAATTTATAATACT
ATCGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATATTAGGAGCTCCTGATATAGCTTTTCCTCGAA
TAAATAACATAAGATTTTGAATATTACCTCCTTCACTAACACTATTAATTTCAAGAAGAATTGTAGAAAATGGTGTAGGAACAGGTTGAACAGTTTATCCACCTTTATCTTCTAATATTGC
TCATCAAGGATCTTCTGTTGATTTAGCAATTTTTTCTCTTCATTTAGCTGGAATTTCTTCTATTTTAGGAGCTATTAATTTTATTACAACAATTATCAATATACGAATTAAAAATTTATCT
TTTGATCAAATACCTTTATTTGTATGATCTGTTGGAATTACAGCTCTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATTACTATATTACTTACAGATCGAAATCTAAATACTT
CTTTTTTTGATCCAGCTGGAGGAGGAGATCCAATCTTATATCAACATTTATTT

Type material. Holotype: ♀ currently deposited in the California Academy of Sciences, San Francisco, CA, USA [CAS], illustrated in Fig. 32, bears seven labels, 3rd and 4th handwritten (below, text in italics handwritten) and others printed: six white [Chancay, | PERU.III-15-51| River valley], [Ross and | Michelbacher | Collectors], [*♀6113* | *P. vibex ?* | *C. D. MacNeill'93*], [*Polites vibex* | *bittiae ? Lindsey* | Det. C.D. MacNeill *'93*], [DNA sample ID: | NVG-22102C03 | c/o Nick V. Grishin], [{QR Code} CASENT | 8566975], and one red [HOLOTYPE ♀ | Hedone miracla | Grishin].

Type locality. Peru: Lima Department, ~80 km north of Lima, Chancay River valley.

Etymology. The name is formed from the sister species, *H. mira*, and is a noun in apposition.

Distribution. Currently known only from the holotype collected in coastal Peru north of Lima.

Punta Evans, 1955 is a junior subjective synonym of *Paracarystus* Godman, 1900

Genomic phylogeny places *Punta punta* Evans, 1955 (type locality in Brazil: Pará), the type and the only species of *Punta* Evans, 1955, as a close sister to or even within *Paracarystus* Godman, 1900 (type species *Cobalus hypargyra* Herrich-Schäffer, 1869) (Fig. 33). Genetic differentiation between the two genera is low, e.g., COI barcodes of their type species differ by 6.1% (41 bp) and *Punta* does not stand out as a prominent separate lineage (Fig. 33). Therefore, we propose that *Punta* Evans, 1955, **syn. nov.** is a junior subjective synonym of *Paracarystus* Godman, 1900.

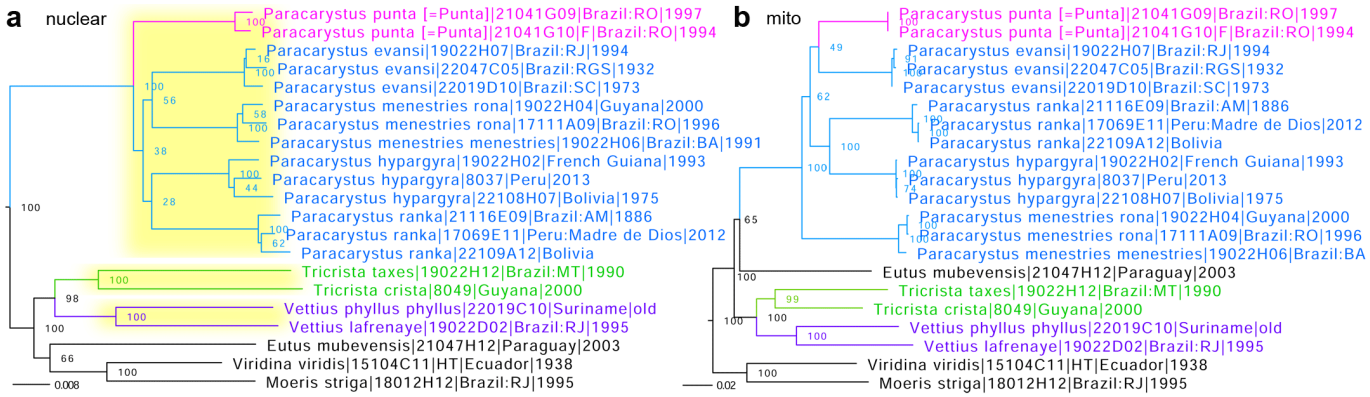

Fig. 33. Phylogenetic trees of *Paracarystus* species and relatives inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Genera with several species shown are colored: *Paracarystus* (blue, with *Paracarystus punta* **comb. nov.** in magenta), *Tricrista* Grishin, 2019 (green), and *Vettius* Godman, 1901 (purple). These genera are highlighted in yellow to illustrate comparable genetic differentiation within them (horizontal dimension of the highlight).

Vettius prona Evans, 1955 is a species distinct from *Vettius phyllus* (Cramer, 1777)

Genomic comparison of subspecies of *Vettius phyllus* (Cramer, 1777) (type locality in Suriname) reveals that *Vettius phyllus prona* Evans, 1955 (type locality in Brazil: São Paulo) is genetically differentiated from others at the species level (Fig. 34), e.g., its COI barcode differs from the nominate *V. phyllus* by 2% (13 bp). Therefore, we propose that *Vettius prona* Evans, 1955, **stat. nov.** is a species distinct from *Vettius phyllus* (Cramer, 1777).

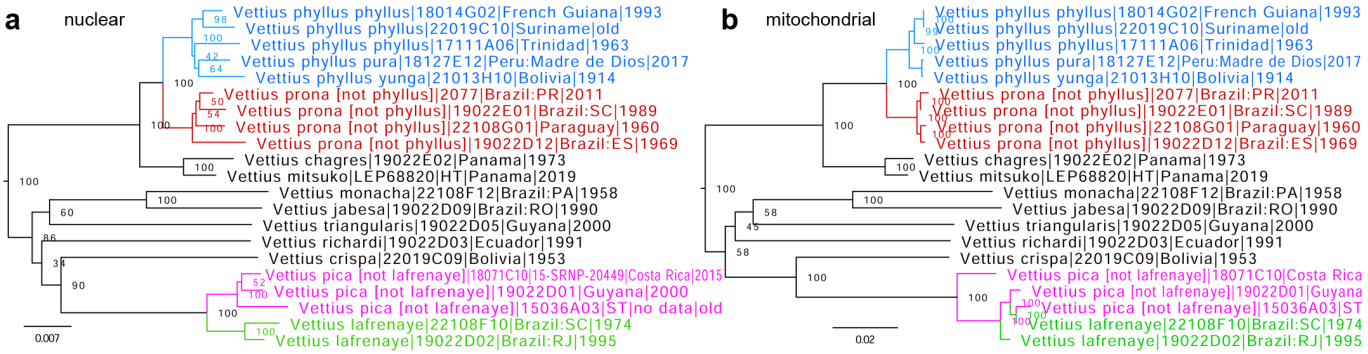

Fig. 34. Phylogenetic trees of selected *Vettius* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. The species discussed in the text are colored: *V. phyllus* (blue), *V. prona* **stat. nov.** (red), *V. pica* **stat. nov.** (magenta), and *V. lafrenaye* (green).

Vettius pica (Herrich-Schäffer, 1869) is a species distinct from *Vettius lafrenaye* (Latreille, [1824])

Genomic sequencing reveals that *Vettius lafrenaye pica* (Herrich-Schäffer, 1869) (type locality not specified) is genetically differentiated from *Vettius lafrenaye lafrenaye* (Latreille, [1824]) (type locality in Brazil) at the species level (Fig. 34), e.g., their F_{st}/G_{min} statistics are 0.62/0.002, but the COI barcodes introgress between them, with the specimen from Costa Rica showing a difference of 1.5% (10 bp) from others. Therefore, we propose that *Vettius pica* (Herrich-Schäffer, 1869), **stat. rest.** is a species distinct from *Vettius lafrenaye* (Latreille, [1824]).

Tigasis coda Evans, 1955 belongs to the genus *Gallio* Evans, 1955

Genomic phylogeny of Moncina A. Warren, 2008 reveals that *Tigasis coda* Evans, 1955 (type locality in Ecuador) is not monophyletic with *Tigasis* Godman, 1900 (type species *Tigasis zalates* Godman, 1900) and instead originates within *Gallio* Evans, 1955 (type species *Stomyles gallio* Mabille, 1904, which is a junior subjective synonym of *Vehilius carasta* Schaus, 1902) and is sister to the clade consisting from *Gallio garima* (Schaus, 1902) (type locality in Trinidad) and *Gallio massarus* (E. Bell, 1940) (type locality in Brazil: Santa Catarina) (Fig. 35). Therefore, we place *T. coda* in the genus *Gallio* to form *Gallio coda* (Evans, 1955), **comb. nov**.

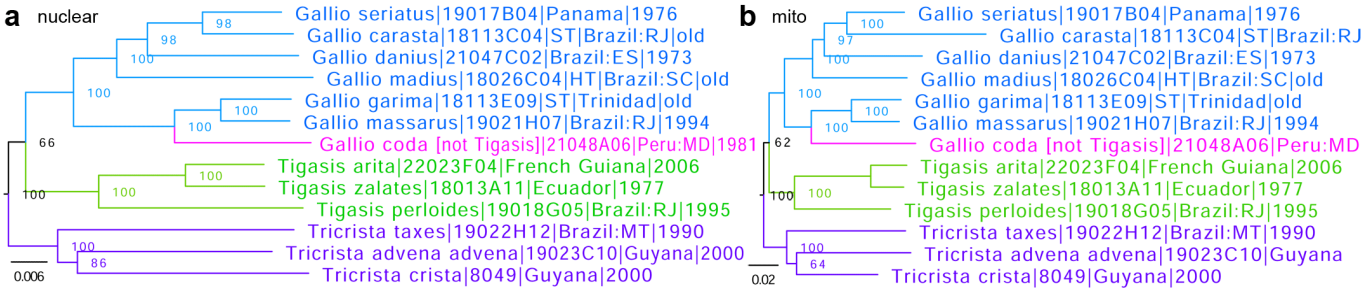

Fig. 35. Phylogenetic trees of selected *Gallio* species and relatives inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome. Different genera are colored differently: *Gallio* (blue, with *Gallio coda* **comb. nov.** in magenta), *Tigasis* (green), and *Tricrista* (purple).

Phlebodes yalta (Evans, 1955) is a species distinct from *Phlebodes fuldai* (E. Bell, 1930)

Genomic sequencing and analysis reveal that *Vettius yalta* Evans, 1955 (type locality in Brazil: Espírito Santo) is genetically differentiated from *Euroto fuldai* Bell, 1930 (type locality in Colombia: Simiti), currently in the genus *Phlebodes* Hübner, [1819] (type species *Papilio pertinax* Stoll, 1781), at the species level (Fig. 36), e.g., their COI barcodes differ by 4.3% (28 bp). Therefore, we propose that *Phlebodes yalta* (Evans, 1955), **stat. rest.** is a species distinct from *Phlebodes fuldai* (E. Bell, 1930).

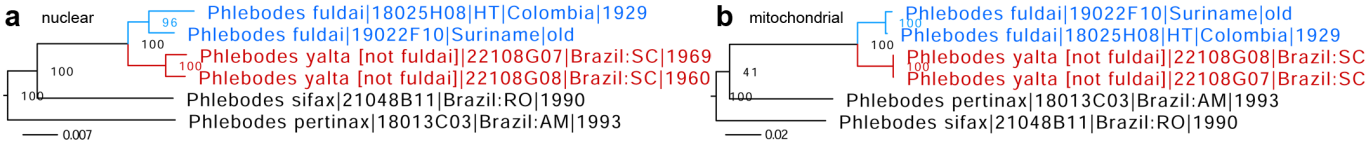

Fig. 36. Phylogenetic trees of selected *Phlebodes* species inferred from protein-coding regions of **a)** the nuclear (autosomes) and **b)** the mitochondrial genome: *Phlebodes fuldai* (blue) and *Phlebodes yalta* **stat. rest.** (red).

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