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Genomic analysis reveals new species and subspecies 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 ¹Biophysics, ²Biochemistry, and ³Eugene 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. Large-scale genomic sequencing of butterfly taxa reveals new findings that are presented here. While we focus on detecting species by comparative genomics and define subspecies as groups of populations genetically differentiated from each other but not as strongly as species (i.e., subspecies as "species in the making"), we report other adjustments to butterfly classification. As a result, 4 subgenera, 11 species, and 6 subspecies are proposed as new. New subgenera are: Rapis Grishin, subgen. n. (type species Papilio rapae Linnaeus, 1758, genus Pieris Schrank, 1801) in Pieridae Swainson, 1820 and Callitera Grishin, subgen. n. (type species Eurygona? pulcherrima Herrich-Schäffer, [1853], genus Isapis E. Doubleday, 1847), Matizada Grishin, subgen. n. (type species Themone poecila H. Bates, 1868, genus Isapis E. Doubleday, 1847), and Parapanara Grishin, subgen. n. (type species Lyropteryx diadocis Stichel, 1910, genus Paraphthonia Stichel, 1910) in Riodinidae Grote, 1895 (1827). New species are (type localities in parenthesis): Chlosvne pardelina Grishin, sp. n. (USA: Texas, Duval Co.) in Nymphalidae Rafinesque, 1815; Erythia paracheles Grishin, sp. n. (Panama: Canal Zone), Erythia borrosa Grishin, sp. n. (Panama: Panama), and Cremna telarania Grishin, sp. n. (Bolivia: La Paz) in Riodinidae; and Euriphellus colombiensis Grishin, sp. n. (Colombia: Río Dagua), Euriphellus ecuadoricus Grishin, sp. n. (Ecuador: Canelos), Urbanus (Urbanus) cubanus Grishin, sp. n. (Cuba: Havana), Gorgythion guyanus Grishin, sp. n. (Guyana: Essequibo), Lon co Grishin, sp. n. (Mexico: Guerrero), Lon ma Grishin, sp. n. (Panama: Chiriquí), and Lon chia Grishin, sp. n. (Mexico: Chiapas) in Hesperiidae Latreille, 1809. New subspecies are (type localities in parenthesis): Chlosyne definita dolosa Grishin, ssp. n. (Mexico: Chihuahua) in Nymphalidae and Limochores mystic nino Grishin, ssp. n. (USA: Arizona, Coconino Co.), Hesperia pahaska hannawackeri Grishin, ssp. n. (USA: Utah, San Juan Co.), Pseudocopaeodes eunus ash Grishin, ssp. n. (USA: Nevada, Nve Co.), Ochlodes napa kaibab Grishin, ssp. n. (USA: Arizona, Coconino Co.), and Lon melane sur Grishin, ssp. n. (Mexico: Baja California Sur) in Hesperiidae. Furthermore, we confirm 1 genus, resurrect 2 subgenera, change the rank of 3 genera to subgenera, synonymize 3 genera, 1 species, and 1 subspecies, and present evidence to support 17 taxa (1 confirmed) as species instead of subspecies or synonyms. Namely, we confirm Perpheres Hirowatari, 1992 as a valid genus, not a junior subjective synonym of Danis [Fabricius], 1807 (Lycaenidae); treat Sinopieris H. Huang, 1995 and Artogeia Verity, 1947 as subgenera (not genera or synonyms) of Pieris Schrank, 1801 (Pieridae); regard these genera as subgenera: Serradinga G. Henning & S Henning, 1996 of Dingana van Son, 1955 (Nymphalidae), Necyria Westwood, 1851 of Lyropteryx Westwood, 1851 (Riodinidae), and Nothodanis Hirowatari, 1992 of Danis [Fabricius], 1807 (Lycaenidae); and propose that the following are junior subjective synonyms, not genera, species, or subspecies: Eugrumia Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 of Sinerebia Nakatani, 2017 in Nymphalidae: Satyrini: Ypthimina Reuter, 1896, new placement (not Erebiina Tutt, 1896), Pistoria Hemming, 1964 of Caleta Fruhstorfer, 1922 and Upolampes Bethune-Baker, 1908 of Thaumaina Bethune-Baker, 1908 in Lycaenidae, and Synargis orestessa Hübner, [1819] of Synargis soranus (Stoll, 1781) and Mesosemia eumene furia Stichel, 1910 of *Ectosemia erinnya* (Stichel, 1910) in Riodinidae. The following taxa are species, not subspecies or synonyms: Pontia edusa (Fabricius, 1777) (not Pontia daplidice (Linnaeus, 1758)) and Pontia johnstonii (Crowley, 1887) (not Pontia helice (Linnaeus, 1764)) in Pieridae; Chlosyne anastasia (Hemming, 1934) (not Chlosyne definita (E. Aaron, [1885])) and Chlosyne bollii (W. H. Edwards, 1878) (not Chlosyne theona (Ménétriés, 1855)) in Nymphalidae; Ectosemia attavus (J. Zikán, 1952) (not Ectosemia eumene (Cramer, 1776)), Cremna dentata (Stichel, 1910) (not Cremna radiata (Godman & Salvin, 1886)), Cremna pupillata Stichel, 1915 (not Cremna alector (Gever, 1837)), Lasaia peninsularis Clench, 1972 (not Lasaia sula Staudinger, 1888), and Synargis arche (Hewitson, 1865) (not Synargis orestessa Hübner, [1819]) in Riodinidae; and Quadrus (Zera) difficilis (Weeks, 1901) (confirmed as not Quadrus (Zera) zera (A. Butler, 1870)), Gorgythion marginata Schaus, 1902 (not Gorgythion begga (Prittwitz, 1868)), Pardaleodes murcia (Plötz, 1883) (not Pardaleodes incerta (Snellen, 1872)), Pardaleodes pusiella Mabille, 1877 (not Pardaleodes sator (Westwood, 1852)), Ochlodes napa (W. H. Edwards, 1865) and Ochlodes santacruza J. Scott, 1981 (not Ochlodes sylvanoides (Boisduval, 1852)), and Lon vitellina (Herrich-Schäffer, 1869) and Lon poa (Evans, 1955) (not Lon melane (W. H. Edwards, 1869)) in Hesperiidae. In addition, we propose new genus-

species combinations: Ectosemia nesti (Hewitson, 1858) (not Semomesia Westwood, 1851), Ectosemia acuta (Hewitson, 1873) and Ectosemia eurythmia (Stichel, 1915) (not Mesosemia Hübner, [1819]), Eugelasia satyroides (Lathy, 1926), Eugelasia modesta (H. Bates, 1868) and Pelolasia leucophryna (Schaus, 1913) (not Euselasia Hübner, 1819), Lyropteryx melaniae (Stichel, 1930) (not Melanis Hübner, [1819]), Isapis pulcherrima (Herrich-Schäffer, [1853]) and Isapis poecila (H. Bates, 1868) (not Themone Westwood, 1851), Paraphthonia diadocis (Stichel, 1910) (not Lyropteryx Westwood, 1851), Tarucus clathratus W. Holland, 1891 (not Castalius Hübner, [1819]), and Vidius tanna (de Jong, 1983) (not Cobalopsis Godman, 1900); new species-subspecies combination Ochlodes santacruza catalina J. Emmel & T. Emmel, 1998 (not Ochlodes sylvanoides (Boisduval, 1852)); and transfer junior subjective synonyms between taxa: Parnassius smintheus var. niger W. G. Wright, 1905 of Parnassius smintheus smintheus E. Doubleday, 1847 (not of Parnassius smintheus behrii W. H. Edwards, 1870), Goniurus proteoides Plötz, 1881 of Urbanus proteus domingo (Scudder, 1872) (not of Urbanus proteus proteus (Linnaeus, 1758)), Bolla subgisela Strand, 1921 of Staphylus melangon epicaste Mabille, 1903 (not of Bolla eusebius (Plötz, 1884)), Gorgythion beggoides Schaus, 1902 of Gorgythion begga begga (Prittwitz, 1868) (not of Gorgythion plautia (Möschler, 1877)), and Pamphila milo W. H. Edwards, 1883 of Ochlodes agricola verus (W. H. Edwards, 1881) (not of Ochlodes agricola nemorum (Boisduval, 1852)). Furthermore, by finding an additional specimen, we confirm Semalea malawi Grishin, 2023 as a species-level taxon using genomic analysis; we conclude that populations of Hesperia pahaska Leussler, 1938 in most of New Mexico and the White Mountains, Arizona are the nominal subspecies, not H. pahaska williamsi Lindsey, 1940; and report a natural hybrid between Chlosyne bollii (W. H. Edwards, [1878]) and Chlosyne chinatiensis (Tinkham, 1944). Lectotypes are designated for 6 names: Erebia atramentaria Bang-Haas, 1927 (type locality in China: Gansu) in Nymphalidae; Mesosemia eumene erinnya Stichel, 1910 (type locality in Peru: Pozuzo) and Mesosemia eumene furia Stichel, 1910 (type locality Bolivia: La Paz, Farinas) in Riodinidae; and Goniurus proteoides Plötz, 1881 (type locality in the Antilles), Hesperia erratica Plötz, 1883 (type locality likely in the USA, not Guatemala, as deduced by genomic comparison), confirming this name as a junior subjective synonym of Lon zabulon (Boisduval & Le Conte, [1837]), and Lerodea? rupilius Schaus, 1913 (type locality Mexico: Jalisco, Guadalajara, not Costa Rica: Guápiles), not a nomen dubium, but a subspecies of Atrytonopsis edwardsi W. Barnes & McDunnough, 1916, in Hesperiidae. A neotype is designated for Cobalus vitellina Herrich-Schäffer, 1869 (type locality becomes Mexico: Oaxaca). Hesperia amanda Plötz, 1883 is regarded as a nomen dubium, not a junior subjective synonym of Ochlodes sylvanoides napa (W. H. Edwards, 1865).

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

ZooBank registration: http://zoobank.org/4594F1CA-9EE8-4A80-A0CA-792676139D20

INTRODUCTION AND METHODS

This work extends our studies that stem from genomic sequencing of butterflies and employs similar principles and methods (Cong et al. 2019a, b, 2020, 2021; Li et al. 2019; Zhang et al. 2019a–d, 2020, 2021, 2022b, c, 2023b–d; Robbins et al. 2022). The objective is to enhance the classification of butterflies by analyzing genomic data. The chosen method involves screening various specimens of butterfly taxa across the world. The focus is on species found in the United States, with specimens collected from their entire geographical range. These specimens are primarily sourced from museum and private collections (see the acknowledgments section for details), with ages ranging from approximately 250 years to recently collected. Whenever feasible, we sequence the DNA of primary type specimens to establish an unbiased reference for their names (Zhang et al. 2022a). DNA extraction typically utilizes the legs of specimen's DNA is already short due to age and is then sequenced using the Illumina next-generation sequencing platform with 150 bp reads. Our approach does not rely on the amplification of specific genes or regions; instead, we sequence every extracted DNA segment. Consequently, the protocol is effective even with very old specimens, whose DNA may be fragmented into 30–50 bp segments.

Sequence data, specifically segments that are 150 base pairs or shorter, from each specimen are employed to construct exons of protein-coding genes. This construction is guided by a reference genome from the species most closely related according to phylogeny. These protein-coding genes serve as the basis for inferring the phylogenic trees. Three separate trees are generated using IQtree v1.6.12, utilizing the GTR+GAMMA model (Nguyen et al. 2015): one tree is derived from autosomes in the nuclear genome, another from the gene predicted to be located in the Z chromosome, and the third from the mitochondrial genome. To reduce the computational workload, a random selection of 100,000 codons is made, representing approximately 2% of the total dataset, for use in constructing the nuclear trees

(300,000 base pairs). Statistical support for the tree branches is determined based on 100 replicates, each composed of 10,000 codons randomly sampled from the complete set of codons. 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. For further methodological details, refer to our earlier publications (Li et al. 2019; Zhang et al. 2022b).

The phylogenetic trees were visualized, rotated, and colored using FigTree (Rambaut 2018). We superimposed the current taxonomic classification onto these trees to identify taxa that are not monophyletic and to pinpoint clades corresponding to taxa that lack names. Genomic trees often unveil "levels," representing specific points in time when diversification occurred independently in multiple lineages (Zhang et al. 2021). These instances of "synchronized" diversification arise from geological events that affect major lineages simultaneously. They present an opportunity to align taxonomic ranks (such as tribe, subtribe, genus, or subgenus) with the levels observed in genomic trees. This approach results in a more objective and internally consistent classification that takes into account both genetic differentiation and paleontological history. In making classification decisions, we heavily rely on genomic trees, with morphological considerations as supplementary evidence to justify the outcomes. This preference stems from the fact that genomes provide a comprehensive view of an organism, encompassing more information than the morphology of adult specimens traditionally used in butterfly classification. Genomes carry encoded data about life histories, habitat preferences, mating behavior, and dietary sources. While we may not yet have the means to extract and predict phenotypic traits from this genetic information, we can employ its genetic equivalent, derived from an aggregate of random codons from all protein-coding genes. This allows us to deduce a taxonomic classification that is rooted in phylogeny and sound from an evolutionary perspective.

The taxa we define are monophyletic and correspond to prominent clades. By "prominent," we refer to branches within the tree with strong statistical support, typically with 100% agreement among replicates, and are usually longer than neighboring branches. The length of a branch is directly proportional to the number of base-pair substitutions that have occurred along that branch. Not only do longer branches receive high statistical support, but their larger number of base-pair substitutions is likely to result in more noticeable phenotypic changes. These changes might be reflected in various morphological characteristics, which may not necessarily manifest in adults but could be apparent in immature stages or other aspects of the phenotype. However, it is important to note that the relationship between the number of genetic changes and visually significant phenotypic differences is highly nonlinear (Zhang et al. 2019a). This means there can be short tree branches that correspond to visually distinguishable taxa. Each case needs to be evaluated individually. Nonetheless, it remains unclear whether a significant phenotypic change in the appearance of adults, brought about by a small number of genetic changes, such as a single genomic segment inversion, justifies the erection of a distinct taxon for that lineage. This is especially true if all other characteristics, like those of caterpillars, remain quite similar to the relatives of this lineage. Importantly, our taxonomic proposals consider the current classification, and 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 address a number of higher classification issues in this work, such as altering some genera, proposing new subgenera, and transferring species between genera to restore monophyly, the focus is on the species and subspecies levels. Species are delineated by a combination of criteria that include genetic differentiation in the Z chromosome measured by F_{st} (>0.20 usually corresponds to distinct species) and gene exchange G_{min} (<0.05 for distinct species) (Cong et al. 2019a), COI barcode difference (typically >2% for distinct species) (Hebert et al. 2003) and its correlation with phenotypic differences (Lukhtanov et al. 2016), and the prominence of species-level clades (Zhang et al. 2022c). However, COI barcodes (together with mitochondria) frequently introgress between species (Bachtrog et al. 2006; Cong et al. 2017a), and some distinct species may possess highly similar or identical barcodes (Burns et al. 2008; Zhang et al. 2023a). See the "Species, subspecies, and genomics" section in Zhang et al. (2022a) for further discussion.

Traditionally, subspecies are defined as groups of populations from different geographical areas that possess recognizable phenotypic differences (e.g., 70% of individuals can be identified by phenotype without knowing their locality) but can successfully interbreed (Mayr 1982; Monroe 1982). In practice, the "successfully interbreed" criterion is difficult to assess, and typically, wing pattern difference in butterflies from different localities is the sole criterion for subspecies definition. It nearly always remains unknown whether these wing pattern differences are genetically encoded or are a consequence of environmental factors. Working with genomic sequences allows us to compare populations in their genotypes. New subspecies names are proposed in this work for genetically differentiated populations that form distinct clades in at least one of the genomic trees, but their genetic differentiation is lower than that we use to delineate species. Thus, our subspecies are "species in the making:" differentiated populations, but to a lesser extent than species. After we delineate subspecies in the genomic trees, we inspect the wing patterns of these specimens and figure out wing pattern characters that may statistically diagnose these subspecies. As for most subspecies, these phenotypic diagnoses are statistical, i.e., they may apply to \sim 70% of specimens, and exceptions should be expected. However, because our subspecies are delineated as clades in the genomic trees, DNA-based characters that support these clades are expected to be much stronger than wing pattern characters and to hold for nearly all specimens. Therefore, we also provide DNA-based diagnoses for all newly described subspecies.

Sections of this work are arranged in taxonomic order deduced from genome-scale phylogeny complemented by phenotypic considerations. For the new taxa, in addition to brief phenotypic diagnoses sometimes accompanied by references that discuss and illustrate morphological characters in greater detail, we provide diagnostic DNA characters in the nuclear genome and (when meaningful) in the COI barcode. DNA characters are found in nuclear protein-coding regions using our previously developed procedure (see SI Appendix to Li et al. 2019). The logic behind the character selection was described in Cong et al. (2019b) and is aimed at finding more robust characters likely to stand when additional specimens and species are sequenced.

The character states are given in species diagnoses as abbreviations for one of the four reference genomes: *Pieris rapae* (Linnaeus, 1758) (pra) (Shen et al. 2016), *Heliconius melpomene* (Linnaeus, 1758) (hm) (Davey et al. 2016), *Calephelis nemesis* (W. H. Edwards, 1871) (cne) (Cong et al. 2017b), 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 < <u>https://osf.io/akhmg/</u>>. 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 obtained and used in this work are available from the NCBI database < https://www.ncbi.nlm.nih.gov/ > as BioProject PRJNA1051313 and BioSample entries of the project contain the locality and other collection data of the sequenced specimens shown in the trees. 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; 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 <u>OR837724–OR837745</u> and <u>OR939283–OR939284</u>. Abbreviations or acronyms for collections are listed in the acknowledgments section.

Family Papilionidae Latreille, [1802]

Parnassius smintheus var. niger W. G. Wright, 1905 is a junior subjective synonym of Parnassius smintheus smintheus E. Doubleday, 1847 and not of Parnassius smintheus behrii W. H. Edwards, 1870

Genomic analysis of the holotype of *Parnassius smintheus* var. niger W. G. Wright, 1905 (type locality USA: California, Sierra Co. Donner Summit, but no locality label on the holotype, sequenced as NVG-22098G08) (Fig. 1 red, highlighted yellow) currently treated as a junior subjective synonym of Parnassius smintheus behrii W. H. Edwards, 1870 (type locality USA: California, Tioga Pass) is not monophyletic with it (Fig. 1 magenta) and is instead in the clade with more eastern subspecies of Parnassius smintheus E. Doubleday, 1847 (type locality in "Rocky Mountains," possibly Canada: Alberta, vicinity of Rock Lake) (Fig. 1 blue, olive and cyan). While the affinity of *P. smintheus* var. niger to these eastern subspecies and not to P. s. behrii or northern and western subspecies (P. s. sternitzkvi McDunnough, 1937, P. s. olympianna Burdick, 1941 and P. s. yukonensis Eisner, 1969) is confident, it is more challenging to assign it to one of the eastern taxa using the specimens we sequenced. Tentatively, in accord with the nuclear genome tree (Fig. 1), we place P. smintheus var. niger as a junior subjective synonym of Parnassius smintheus smintheus E. Doubleday, 1847, and hypothesize that its type locality may have been incorrect. Sequencing of additional specimens across the range, including those from around the Donner Pass area in Sierra Co., California, is needed to determine its locality and synonymy more precisely. Our current genomic analysis reveals that *Parnassius smintheus maximus* Bryk & Eisner, 1937 (type locality in USA: Montana, Fergus Co.) is closely related to the nominotypical P. smintheus (Fig. 1 blue), and it is possible that *P. smintheus* var. *niger* is synonymous with the former taxon, or all



Fig. 1. The phylogenetic tree of the *Parnassius smintheus* subspecies inferred from protein-coding regions of the nuclear genome (autosomes): 3,763,728 bp positions and ultra-fast bootstrap (Minh et al. 2013) were used for this tree. The holotype of *P. smintheus* var. *niger* is shown in red and highlighted in yellow, and *P. smintheus behrii* is colored in magenta.

three are synonyms. Before sequencing the lectotype of *P. smintheus smintheus* that is expected to shed light on the situation, we avoid replacing the name *P. smintheus maximus* with *P. smintheus niger*.

Family Pieridae Swainson, 1820

Pontia edusa (Fabricius, 1777) is confirmed as a species distinct from *Pontia daplidice* (Linnaeus, 1758)

Although it cannot be readily identified by external appearance, *Pontia edusa* (Fabricius, 1777) (type locality in Germany) is most strongly differentiated genetically from *Pontia daplidice* (Linnaeus, 1758) (type locality in South Europe and Africa), with $F_{st}/G_{min}/COI$ barcode difference of 0.85/0.000/8.2% (54 bp) (Fig. 2). This difference is significantly more than usual for close relatives and is more in line with the difference between species in different subgenera. Therefore, we confirm that *Pontia edusa* (Fabricius, 1777) is a species distinct from *Pontia daplidice* (Linnaeus, 1758). Finally, we note a curious incongruence between the nuclear (Fig. 2a) and mitochondrial (Fig. 2b) genome trees. As expected from their phenotypes, *Pontia glauconome* (Klug, 1829) (type locality in Egypt) is sister to both *P. daplidice* and *P. edusa* in the nuclear genome tree. However, *P. edusa* gets within the *P. glauconome* clade in the mitochondrial genome tree, likely due to mitochondrial introgression.



Fig. 2. Phylogenetic trees of selected *Pontia* species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes. Different species are shown in different colors: *P. daplidice* (blue), *P. edusa* (red), *P. johnstonii* (magenta), *P. helice* (green), and *P. glauconome* (black).

Pontia johnstonii (Crowley, 1887) is a species distinct from *Pontia helice* (Linnaeus, 1764)

Synchloe johnstonii Crowley, 1887 (type locality Tanzania: Kilimanjaro) currently treated as a subspecies of *Papilio helice* Linnaeus, 1764 (type locality South Africa: Tulbagh) in the genus *Pontia* [Fabricius], 1807 (type species *Papilio daplidice* Linnaeus, 1758) is genetically differentiated from it at the level characteristic of distinct species (Fig. 2), e.g., COI barcodes differ by 3.8% (25 bp), and is phenotypically distinguished by nearly black framing around olive overscaling of veins on hindwing. Therefore, we propose that *Pontia johnstonii* (Crowley, 1887), **stat. rest.** is a species distinct from *Pontia helice* (Linnaeus, 1764).

Sinopieris H. Huang, 1995 and Artogeia Verity, 1947 are subgenera of Pieris Schrank, 1801

At times treated as a distinct genus or placed in *Pontia* [Fabricius], 1807 (type species *Papilio daplidice* Linnaeus, 1758), *Sinopieris* H. Huang, 1995 (type species *Sinopieris gongaensis* H. Huang, 1995) originates within *Pieris* Schrank, 1801 (type species *Papilio brassicae* Linnaeus, 1758) according to our genomic trees (Fig. 3). Therefore, *Sinopieris* belongs to *Pieris*. However, due to the visual distinction of some of these species resulting in their confusion with *Pontia*, instead of synonymizing *Sinopieris* with *Pieris*, we propose to treat the former as a subgenus of the latter. We note that *Pieris extensa* Poujade, 1888 (type locality in China) belongs to the subgenus *Sinopieris* (Fig. 3). Then, to restore the monophyly of the subgenus *Pieris*, we propose to treat *Artogeia* Verity, 1947 **stat. rev.** (type species *Papilio napi* Linnaeus, 1758), which is sister to *Sinopieris* (Fig. 3), as another subgenus of *Pieris*.

Rapis Grishin, new subgenus

http://zoobank.org/CDE5789D-03D6-4AEF-8974-8F1C57995DD5

Type species. Papilio rapae Linnaeus, 1758.

Definition. Genomic phylogeny of the genus Pieris Schrank, 1801 (type species Papilio brassicae Linnaeus, 1758) reveals several prominent clades that could be regarded as subgenera, including the nominotypical (Fig. 3 violet) and Artogeia Verity, 1947 (type species Papilio napi Linnaeus, 1758) (Fig. 3 blue). Although Sinopieris H. Huang, 1995 (type species Sinopieris gongaensis H. Huang, 1995) (Fig. 3 red) is not supported by a very prominent branch, this group of species is confidently monophyletic, originates around the same time as other subgenera and is phenotypically distinct. The remaining fourth clade (Fig. 3 green) is prominent and cannot be confidently included in other subgenera: it is sister to the subgenus *Pieris* in the genomic tree, but only with 88% support (not above 95%). Therefore, this green clade represents the fourth subgenus, and it does not have a name. This new subgenus constitutes the P. rapae group of Robbins and Henson (1986), who described and illustrated diagnostic characters for it. In brief, species in the new subgenus are distinguished from the nominotypical subgenus by shorter (less than half the size) and onion-shaped, rather than elongated, and roconia and from Artogeia and Sinopieris by the lack of posterior process on signum (Robbins and Henson 1986) and, additionally, by the lack of overscaling along ventral hindwing veins. In DNA, a combination of the following characters is diagnostic in the nuclear genome: pra6360.8.e1:T87A, pra590.15.e1:A181C, pra82.57.e2:G168T, pra82.57. e2:T177C, pra283.114.e1:A531T and in COI barcode: T163A, A205T, T421T, G512G, C533C, T535T, T589C, C641C.

Etymology. The name is a fusion of the type species name with its genus name: Rap[ae] + [Pier]is. The name is a feminine noun in the nominative singular.

Species included. The type species (i.e., *Papilio rapae* Linnaeus, 1758), *Papilio canidia* Linnaeus, 1768, *Pieris krueperi* Staudinger, 1860, *Pontia mannii* J. Mayer, 1851, and *Pieris tadjika* Grum-Grshimaïlo, 1888 (Robbins and Henson 1986), including their closest relatives sometimes regarded as distinct species.

Parent taxon. Genus Pieris Schrank, 1801.



Fig. 3. The phylogenetic tree of selected Pierini species inferred from protein-coding regions of the nuclear genomes (autosomes). Different subgenera of *Pieris* are shown in different colors: *Rapis* subgen. n. (blue), *Pieris* (violet), *Artogeia* (blue), and *Sinopieris* (red). Note the several-fold difference in substitution rates reflected in branch lengths, e.g., low in some *Pontia* and high in *Ascia* Scopoli, 1777.

Family Nymphalidae Rafinesque, 1815

Eugrumia Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 is a junior subjective synonym of *Sinerebia* Nakatani, 2017, which is a sister genus of *Paralasa* Moore, 1893 (Satyrini: Ypthimina)

Entirely dark-brown butterfly Erebia atramentaria Bang-Haas, 1927 (type locality China: Gansu Province, Oinling Mountains, Datong River, syntype sequenced as NVG-22126F02) visually similar to a better-known Erebia magdalena Strecker, 1880 (type locality in USA: Colorado, Clear Creek Co.) has been kept in its original genus Erebia Dalman, 1816 (type species Papilio ligea Linnaeus, 1758), the type genus of the subtribe Erebiina Tutt, 1896, since its description until it was designated the type species of Sinerebia Nakatani, 2017, a genus sometimes synonymized with Erebia. Genomic phylogeny places a syntype of Sinerebia atramentaria as a close sister to Eugrumia herse (Grum-Grshimaïlo, 1891) (type locality in China), which is the type species of the genus Eugrumia Della Bruna, Gallo, Lucarelli & Sbordoni, 2000 in the subtribe Ypthimina Reuter, 1896, not Erebiina (Fig. 4 red), with the COI barcode difference of only 0.8–1.2% (5–8 bp), despite the remarkable difference in their wing patterns. Therefore, we confidently propose that Eugrumia syn. nov. is a junior subjective synonym of Sinerebia Nakatani, 2017. Sinerebia is sister to Paralasa Moore, 1893 (type species Erebia kalinda Moore, 1865) (Fig. 4), and some of Sinerebia species were previously included in Paralasa, which is supported by the genetic similarity between the two genera, e.g., COI barcodes of their type species differ by 6.1% (40 bp). This small difference is more characteristic of subgenera than genera. However, pending further studies, we keep Sinerebia and Paralasa as distinct genera due to much closer relationships among species within each genus than between these genera (Fig. 4).

We suspect that the absence of wing patterns in *Sinerebia atramentaria* hindered its taxonomic classification until it was revealed by genomic sequencing. Finally, to define the taxonomic identity of this species objectively, N.V.G. hereby designates a syntype in the MTD collection, a male with the following five printed labels, the 4th yellow, and others white: [Kansu sept.occ. | Hsining | Nanshan mont. | Tatung | 3500m. Juli], [Staudinger | Ankauf 1948], [711], [atramentaria O. Bang-Haas, 1927 |



Fig. 4. Phylogenetic trees of selected Satyrini Boisduval, [1833] (1820) inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes. Different subtribes are colored differently: Erebina (purple with *Erebia* labeled in purple), Ypthimina (blue with *Sinerebia* colored and labeled in red and *Paralasa* labeled in blue), and Callerebina Grishin, 2021 (olive). The sequence of SAMN18673646 is taken from the alignment provided in Kawahara et al. (2023).

SYNTYPUS | Y. Nekrutenko det. 11.09.2000], and [DNA sample ID: | NVG-22126F02 | c/o Nick V. Grishin] as the **lectotype** of *Erebia atramentaria* Bang-Haas, 1927. The lectotype has noticeable areas on wings with scales partially rubbed off and a small nick by the apex of the left forewing.

Serradinga G. Henning & S Henning, 1996 is a subgenus of Dingana van Son, 1955

Genomic sequencing of type species of genera *Serradinga* G. Henning & S Henning, 1996 (*Leptoneura bowkeri* Trimen, 1870) and *Dingana* van Son, 1955 (*Leptoneura dingana* Trimen, 1873) reveals that they are closely related to each other (Fig. 5 red and blue), e.g., their COI barcodes differ by 5.8% (38 bp), which is typical for closely related congeners. Therefore, we propose to treat *Serradinga* G. Henning & S Henning, 1996 as a subgenus of *Dingana* van Son, 1955.



Fig. 5. Phylogenetic trees of the type species of available genus-group names in the tribe Dirini Verity, 1953 inferred from protein-coding regions of **a**) the Z chromosome and **b**) the mitochondrial genome. The genus *Dingana* is colored blue, with its subgenus *Serradinga* in red.

Chlosyne pardelina Grishin, new species

http://zoobank.org/4BB18B88-5D31-42AF-B99C-5B7D74DDCD1F

(Figs. 6 part, 7)

Definition and diagnosis. Genomic sequencing of specimens identified as Chlosyne endeis (Godman & Salvin, 1894) (type locality in Mexico: Nayarit) reveals that they are either not monophyletic (in nuclear trees) or prominently separated into two clades (in the mitochondrial genome) (Fig. 6). F_{st}/COI barcode difference between the specimens in two clades are 0.38/1.5% (10 bp), typical for closely related but distinct species of *Chlosyne* Butler, 1870. Therefore, the specimens we sequenced belong to one of the two distinct species. We identify specimens from Nayarit, Mexico, the state with the type locality of C. endeis as that species. Specimens from south Texas (USA) and eastern Mexico are not C. endeis and belong to a different species. This species was at times regarded as a subspecies of C. endeis under the name "pardelina," which was attributed either to Higgins (Lamas 2004) or to Scott (Pelham 2008). However, neither Higgins (1960) nor Scott (1986) made the name available. Higgins proposed "form pardelina forma nov." for "male specimens of endeis ..., in which the ground-colour is yellow" (Higgins 1960). However, according to Articles 45.6.1 and 45.6.4.1 of the ICZN Code (ICZN [International Commission on Zoological Nomenclature] 1999), this name is infrasubspecific because it was applied to an infrasubspecific entity and not "adopted" before 1985, and therefore is unavailable. The glossary of the ICZN Code defines "infrasubspecific entity" as "... Specimen(s) within a species differing from other specimens in consequence of intrapopulation variability (e.g., opposite sexes, ...," and Higgins applied the name to male specimens. Scott did not establish this name either because he merely applied it (not even referencing Higgins) to the subspecies of C. endeis "in the U.S." without description, definition, or bibliographic reference to such (fails Art. 13.1). Therefore, this species lacks an available name, and is new. This new species is generally similar to C. endeis in having brown wings with yellow or white spots, some in discal bands separated by veins, and two patches of submarginal red spots (sometimes vestigial) distad of the yellow discal band on the dorsal hindwing, by the apex and tornus. The new species differs from C. endeis in having a yellow to orange rather than a white discal band of dorsal hindwing (and frequently on forewing) and typically larger patches of red hindwing spots. Due to phenotypic variability, definitive identification is provided by DNA, and a combination of the following characters is diagnostic



Fig. 6. Phylogenetic trees of selected *Chlosyne* species inferred from protein-coding regions of a) the nuclear genome (autosomes), b) the Z chromosome, and c) the mitochondrial genome. Different taxa are shown in different colors: *C. definita* (blue, with *C. definita dolosa* ssp. n. in red), *C. anastasia* stat. rest. (green), *C. pardelina* sp. n. (magenta), and *C. endeis* (violet). One tree branch was truncated, as indicated by dots.

in the nuclear genome: hm2012952-RA.1:A156G, hm2012952-RA.1:A543T, hm2010701-RA.4:C66T, hm2018077-RA.9:G69C, hm2006719-RA.4:C78T and in COI barcode: A286C, C451T, 562T, 574C, A625G. Barcode sequence of the holotype. Sample NVG-17117C06, GenBank <u>OR837724</u>, 658 base pairs:

Type material. Holotype: of deposited in the Texas A&M University Insect Collection, College Station, TX, USA [TAMU], illustrated in Fig. 7, bears six labels: five white [TEXAS: | DUVAL COUNTY | Texas Hwy 16 ca | 15 mi (24 km) S | of Freer at Parrilla Creek], [ex larva | (had larval diapause) | 11 Sep 1980 | Roy O. Kendall | and C. A. Kendall], [Larval foodplant: | ACANTHACEAE | Carlowrightia | parviflora (Buckl. | Wasshausen (foliage)], [NYMPHALIDAE: | Chlosyne endeis | pardelina | of Higgins, 1960 | det. Roy O. Kendall | [M. & B. No. 601.b]], [DNA sample ID: | NVG-17117C06 | c/o Nick V. Grishin], and one red [HOLOTYPE of | Chlosyne pardelina | Grishin]. Its pupal case and the last instar caterpillar exuvium are in a gelatine capsule pinned under the specimen. The date given on the label refers to eclosion. **Paratypes:** 299: 19 the same data as the holotype, but eclosed on 13-Sep-1980 (NVG-17117C05) and 19 Mexico: San Luis Potosí, Rte 80, 2–7 mi NW Ciudad del Maíz, 16-Jul-1988, D. Mullins leg. (NVG-19086A10, USNMENT 01314130) [USNM].

Type locality. USA: Texas, Duval Co., SH16 ca. 15 mi south of Freer at Parrilla Creek, GPS 27.6478, -98.6572.



Fig. 7. Holotype of Chlosyne pardelina sp. n. in dorsal (left) and ventral (right) views, data in text.

Etymology. In the interest of stability, the name used by Higgins and Scott is kept. In Spanish, the word *pardelina* refers to any small, spotted, or mottled bird, and it fits the general appearance of this species. The name is a feminine noun in apposition.

Distribution. South Texas and northeastern Mexico.

Chlosyne anastasia (Hemming, 1934) is a species distinct from Chlosyne definita (E. Aaron, [1885])

Melitaea anastasia Hemming, 1934, a replacement name for *Melitaea beckeri* Godman, [1901] (type locality Mexico: Durango, Durango City), which is a junior primary homonym of *Melitaea artemis* var. *beckeri* Herrich-Schäffer, 1851 (type locality in Spain), currently treated as a subspecies of *Chlosyne definita* (E. Aaron, 1885) (type locality in USA: Texas, Nueces Co.) is genetically distant from it with F_{st} /COI barcode difference of 0.27/3% (20 bp). The COI barcode difference is large because the mitochondrial DNA of *M. anastasia* is closest (0.3%, 2 bp between the COI barcodes, likely due to introgression) to *Chlosyne endeis* (Godman & Salvin, 1894) (type locality in Mexico: Nayarit) (Fig. 6c), a species more distant from *M. anastasia* according to the nuclear genome tree (Fig. 6a). Because of this genetic differentiation, we propose that *Chlosyne anastasia* (Hemming, 1934), **stat. rest.** is a species distinct from *Chlosyne definita* (E. Aaron, [1885]). Both *C. definita* and *C. anastasia* **stat. rest.** have been recorded from the state of Durango, where the former is known from the north, and the latter is documented from the south (Fig. 6). *Chlosyne anastasia* **stat. rest.** does not occur in the United States.

Chlosyne definita dolosa Grishin, new subspecies

http://zoobank.org/0CE8273D-D38A-47D7-A73E-A15A3354B0CC

(Figs. 6 part, 8)

Definition and diagnosis. Before this work, western populations of *Chlosyne definita* (E. Aaron, 1885) (type locality in USA: Texas, Nueces Co.), including those in northwestern Mexican states of Sonora and Chihuahua have been placed within the subspecies *Chlosyne definita anastasia* (Hemming, 1934) (type locality Mexico: Durango, Durango City) due to their phenotypic similarity in having less extensive dark markings and narrower white band and spots on wings venter. Genomic trees reveal that C. d. anastasia is not monophyletic, and the populations near its type locality are genetically differentiated at the species level (i.e., C. anastasia stat. rest., see above) (Fig. 6), but more northern populations differ from them by 3.3% (22 bp) in COI barcode and are closer related to the nominotypical C. definita (COI barcode difference of 0.6% 4 bp). Therefore, we regard these northwestern Mexico populations as conspecific with C. definita, but due to their phenotypic and genetic differences propose that they constitute a distinct subspecies. This new subspecies is phenotypically more similar to C. anastasia stat. rest. and differs from it in the following characters: the central white band on the ventral hindwing is less broad but broader than in the nominotypical subspecies; black markings are more extensive but less expressed than in the nominotypical subspecies, e.g., the basal white band on the ventral hindwing is typically cut through (or even cut short) by black overscaling around vein 1A+2A. Due to phenotypic variability, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: hm2010867-RA.6:C66T, hm2010867-RA.6:G87A, hm2003966-RA.6:T1521C, hm200 3966-RA.6:C4239T, hm2014195-RA.2:A709G and in COI barcode: A40G, 169T, A205T, T283C, T475T. Barcode sequence of the holotype. Sample NVG-22088C12, GenBank OR837725, 658 base pairs:

Type material. Holotype: σ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 8, bears four printed (text



Fig. 8. Holotype of Chlosyne definita dolosa ssp. n. in dorsal (left) and ventral (right) views, data in text.

in italics handwritten) labels: three white [Chihuahua, Mex. | 19.4 miles E. of | Tomochic *Oak-Pine* | July 29, 1984. | *ca 7000' Leg D. Mullins*], [J. D. Turner ex | Malcolm Douglas | colln. | MGCL Accession | # 2009-26], [DNA sample ID: | NVG-22088C12 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Chlosyne definita | dolosa Grishin]. **Paratypes:** 299 Mexico, Sonora, 5 mi NW of Yecora, plateau edge, 25-Jul-1987, M. Smith leg. (NVG-22088D10) and 28/29-Jul-1987 (NVG-22088D01) [MGCL].

Type locality. Mexico: Chihuahua, 19.4 mi E of Tomochic, elevation ca. 7000'.

Etymology. In Latin, *dolosus* means cunning, deceitful, crafty, or sly. The name is given for the deceitful nature of this subspecies, which was hidden behind the name *Chlosyne anastasia* before it was revealed by genomic sequence comparison. The name is a feminine adjective.

Distribution. Northwestern Mexico, recorded from the states of Sonora and Chihuahua.

Chlosyne bollii (W. H. Edwards, 1878) is a species distinct from *Chlosyne theona* (Ménétriés, 1855)

Our previous genomic analysis demonstrated that *Chlosyne chinatiensis* (Tinkham, 1944) (type locality in USA: Texas, Presidio Co.) is a species distinct from *Chlosyne theona* (Ménétriés, 1855) (type locality in Nicaragua) (Zhang et al. 2020) in agreement with Cassie et al.(2001). Sequencing of additional specimens across the range from Arizona and Texas to Costa Rica reveals that *Melitaea bollii* W. H. Edwards, 1877 (type locality USA: Texas, Bexar Co., San Antonio), currently regarded as a subspecies of *C. theona* (Fig.



Fig. 9. Phylogenetic trees of selected *Chlosyne* species inferred from protein-coding regions of a) the nuclear genome (autosomes), b) the Z chromosome, and c) the mitochondrial genome. Different taxa are shown in different colors: *C. chinatiensis* (green), *C. bollii* stat. rest. (red), *C. theona* (blue, with nominotypical subspecies in violet color), and a hybrid specimen *C. bollii* (father) \times *C. chinatiensis* (mother) (olive, highlighted in yellow). One tree branch was truncated, as indicated by dots.

9 red) forms a prominent clade that is sister to *C. chinatiensis* in the tree from autosomes (Fig. 9a) and in the mitochondrial genome tree (with some possible introgression, Fig. 9c) (i.e., not monophyletic with *Chlosyne theona*), but is sister to *C. theona* in the Z chromosome tree (Fig. 9b). All other subspecies of *C. theona* we sequenced (including a nominotypical specimen from Nicaragua, NVG-22097C09) clustered together and were not strongly separated from each other despite large geographic distances between these populations, e.g., *Chlosyne theona thekla* (W. H. Edwards, 1870) (type locality in USA: AZ, Pima co.) and *Chlosyne theona costaricensis* (Austin & M. Smith, 1998) (type locality in Costa Rica), and their phenotypic distinction. The genetic differentiation of *C. theona bollii* from *C. chinatiensis* and all other sequenced *C. theona* subspecies is at the level characteristic of distinct species, i.e., $F_{st}/G_{min}/COI$ barcode difference of 0.62/0.001/0.6% (4 bp, they essentially share mitochondrial DNA) (*C. chinatiensis*) and 0.46/0.003/1.2% (8 bp) (*C. theona*). Therefore, we propose that Chlosyne *bollii* (W. H. Edwards, 1878), **stat. rest.** is a species distinct from *Chlosyne theona* (Ménétriés, 1855).

A natural hybrid between *Chlosyne bollii* (W. H. Edwards, [1878]) and *Chlosyne chinatiensis* (Tinkham, 1944)

Sequencing of a suspected hybrid between Chlosvne bollii (W. H. Edwards, [1878]), stat. rest. (type locality USA: Texas, Bexar Co., San Antonio) and Chlosyne chinatiensis (Tinkham, 1944) (type locality in USA: Texas, Presidio Co.), a female NVG-19072B01 collected in USA: Texas, Brewster Co., along FM2627 25 mi SE of USH385 on 23-Mar-1994 by Steve M. Spomer (Fig. 10) places it in different positions in the three trees (Fig. 9 olive) thus confirming its hybrid origin. In the nuclear genome tree constructed from autosomes (Fig. 9a), this specimen is sister to C. chinatiensis, suggesting that it has a significant fraction of C. chinatiensis genes. In the Z chromosome tree (Fig. 9b), this specimen is within C. bollii, suggesting that its Z chromosome, which in females is inherited from the father (in butterflies, ZZ are males and ZW are females), came from C. bollii. In the mitochondrial genome tree (Fig. 9c), the specimen is placed within C. chinatiensis, suggesting that its mitochondrial DNA, which is inherited from the mother, came from C. chinatiensis. Therefore, we confirm this female as a natural interspecies hybrid and conclude that its father was C. bollii, and its mother was C. chinatiensis.



Fig. 10. *Chlosyne bollii* (father) \times *C. chinatiensis* (mother) hybrid in dorsal (top) and ventral (bottom) views. \bigcirc Steve Spomer

Family Riodinidae Grote, 1895 (1827)

Euselasia satyroides Lathy, 1926 and *Eurygona modesta* H. Bates, 1868 belong to the genus *Eugelasia* Grishin, 2021

Genomic trees reveal that *Euselasia satyroides* Lathy, 1926 (type locality in Argentina) is sister to *Eugelasia brevicauda* (Lathy, 1926) (type locality in Bolivia) and, therefore, originates within the genus *Eugelasia* Grishin, 2021 (type species *Eurygona eugeon* Hewitson, 1856) (Fig. 11). Although we have not sequenced *Eurygona modesta* H. Bates, 1868, currently in the genus *Euselasia* Hübner, 1819 (type species *Euselasia gelaena* Hübner, 1819, which is a junior subjective synonym of *Papilio gelon* Stoll, 1787), it is phenotypically similar to *E. satyroides* and its relatives (Santos et al. 2014). Therefore, we propose the following **new combinations**: *Eugelasia satyroides* (Lathy, 1926) and *Eugelasia modesta* (H. Bates, 1868).



Fig. 11. Phylogenetic trees of selected Euselasiini species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes. Different genera are colored differently, and species transferred between genera are labeled in different colors: *Eurygona leucophryna* **comb. nov.** (magenta) and *Eugelasia satyroides* **comb. nov.** (orange).

Eurygona leucophryna Schaus, 1913 belongs to the genus Pelolasia Grishin, 2021

Genomic sequencing of a syntype of *Eurygona leucophryna* Schaus, 1913 (type locality in Costa Rica: Cachí) reveals that it is sister to *Pelolasia cataleuca* (R. Felder, 1869) (Fig. 11) in the genus *Pelolasia* Grishin, 2021 (type species *Eurygona pelor* Hewitson, 1853). This close relationship with *P. cataleuca* was mentioned in the original description of *E. leucophryna* (Schaus 1913). Thus, we propose *Pelolasia leucophryna* (Schaus, 1913), **comb. nov**.

Erythia paracheles Grishin, new species

http://zoobank.org/E7E4D9E8-760B-4D0F-BFC7-DD0060AC7FBB

(Figs. 12, 13 part)

Definition and diagnosis. Genomic analysis reveals that an orange female from central Panama (Figs. 12, 13 orange) initially identified as an aberration of *Erythia aurantiaca* (Salvin & Godman, 1868) (type locality in Guatemala) is instead sister to but genetically differentiated from *Erythia cheles* (Godman & Salvin, 1889) (type locality in Panama: Chiriquí, holotype sequenced as NVG-21123B03) (Fig. 13), e.g., COI barcode difference of 4.4% (29 bp). We sequenced two specimens of *E. cheles* (the holotype and another female, NVG-19036F06), and they are genetically close to each other (Fig. 13 blue). However, due to strong genetic differentiation, the orange female represents a distinct species that, according to our investigation, does not have a name. The female of this new species differs from its relatives in the nearly uniform orange coloration of the dorsal side of wings, only with a hint of the brown outer margin, more developed by the apex of the forewing, and is similarly orange, only slightly yellower, on the ventral side, with a thin postdiscal darker orange band on both wings and no other markings. In females of other species, the apex of the dorsal forewing and usually the outer margin are largely brown, and there are at



Fig. 12. Holotype of Erythia paracheles sp. n. in dorsal (left) and ventral (right) views, data in text.



Fig. 13. Phylogenetic trees of selected *Erythia* species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes. Different species are colored in different colors: *E. cheles* (blue), *E. paracheles* **sp. n.** (orange), *E. borrosa* **sp. n.** (magenta), and *E. aurantiaca* (violet).

least traces of black submarginal spots on the ventral hindwing. While it remains unclear whether this specimen is an aberration, we are confident that it is a species distinct from both *E. cheles* and *E. aurantiaca* due to its prominent genetic differentiation, and, therefore, it is described as a new species. To confidently identify this new species despite the unknown phenotypic variation, we provide a diagnostic combination of DNA characters in the nuclear genome: cne11073.6.7:T54C, cne3970.3.2:T111C, cne20880. 1.4:A84G, cne10214.9.8:A66G, cne4577.3.8:C18T, cne1935.4.1:C1113C (not T), cne1935.4.1:C1558C (not A), cne84.2.2:C1860C (not T), cne15258.2.1:A612A (not T), cne14561.1.14:C79C (not T) and in the COI barcode: T16C, 88C, T142C, T169C, T250C, T361C, T391A, T400C, A577G, T619C.

Barcode sequence of the holotype. Sample NVG-19036F07, GenBank OR837726, 658 base pairs:

Type material. Holotype: $\$ deposited in the National Museum of Natural History, Washington, DC, USA [USNM], illustrated in Fig. 12, bears four printed labels: three white [Riodinidae? 3/28/76 | Las Cruces Trail, CZ], [DNA sample ID: | NVG-19036F07 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 00939912], and one red [HOLOTYPE $\$ | Erythia paracheles | Grishin].

Type locality. Panama: Canal Zone, Las Cruces Trail.

Etymology. The prefix "para" means alongside, near, beyond, or similar to. This new species is sister to *E. cheles* and is similar to it. The name is treated as a masculine noun in apposition.

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

Erythia borrosa Grishin, new species

http://zoobank.org/86B396F7-EF80-4EC8-AE29-A4D674DCDDDF

(Figs. 13 part, 14)

Definition and diagnosis. Genomic phylogeny inferred from all sequenced specimens of Euselasiini Kirby, 1871 (1867) reveals that a specimen from central Panama (Figs. 13 magenta, 14) is sister to the clade of Erythia cheles (Godman & Salvin, 1889) (type locality in Panama: Chiriquí) with Erythia *paracheles* sp. n. (type locality in Panama: Canal Zone) and therefore represents a species distinct from them (Fig. 13), also being strongly differentiated genetically, e.g., COI barcode difference of 4.9% (32 bp) from E. cheles and 5.9% (39 bp) from E. paracheles. These three species form a clade sister to Erythia aurantiaca (Salvin & Godman, 1868) (type locality in Guatemala). Even in wing patterns, the specimen from Panama appears different from the named taxa, and these differences, supported by genetic differentiation, suggest that this specimen belongs to a new species. Males of this new species differ from their relatives in a diffuse boundary between brown framing along wing margins and orange interior on the dorsal side: brown overscaling partially extends into orange areas. The brown/orange boundary is sharper and could even be rather crips in closely related species, or orange areas are more restricted on forewing to the area between the inner margin and discal cell. The costal area of the dorsal hindwing is brown from its base, and the discal cell is largely orange but brown towards the costa; the dorsal hindwing has a brown batch at the apex and a brown margin of decreasing width and disappearing towards the tornus; the submarginal area is browner than the brighter orange discal area from costa to mid-wing. The



Fig. 14. Holotype of Erythia borrosa sp. n. in dorsal (left) and ventral (right) views, data in text.

ventral side of the wings is pearly-pinkish with a posdiscal pale-brown line on all wings (close to a submarginal row of spots on the hindwing) and orange-brown narrow marginal framing. Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: cne5785.3.5:A96T, cne7688.1.2:T150G, cne3970. 3.2:G96A, cne254625.2.3:G270A, cne10780.4.1:T1347A, cne4782.4.3:T282T (not C), cne3195.11.14:A54A (not G), cne563.4.3:G216G (not A), cne563.4.3:G219G (not A), cne3970.3.2:T111T (not C) and in COI barcode: T4C, T56T, T197T, T202C, T206T, T274C, T550C.

Barcode sequence of the holotype. Sample NVG-19036H09, GenBank OR837727, 658 base pairs:

Type material. Holotype: σ deposited in the National Museum of Natural History, Washington, DC, USA [USNM], illustrated in Fig. 14, bears four printed (2nd and 3rd lines on the 1st label handwritten) labels: three white [Panama:Panama | Cerro Campana | 800m. 17.III.1973 | G. B. Small], [DNA sample ID: | NVG-19036H09 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01544858], and one red [HOLOTYPE σ | Erythia borrosa | Grishin].

Type locality. Panama: Panama Province, Cerro Campana, elevation 800 m.

Etymology. In Spanish, *borrosa* means blurry or fuzzy. The name refers to edges between brown and orange in this species that lack the sharpness of its relatives, and brown gradually dissolves into orange, or orange is overscaled with brown towards the margins. The name is a Latinized feminine adjective.

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

Mesosemia nesti Hewitson, 1858, *Mesosemia acuta* Hewitson, 1873, and *Mesosemia eurythmia* Stichel, 1915 belong to the genus *Ectosemia* Grishin, 2021

Genomic sequencing of *Mesosemia nesti* Hewitson, 1858 (type locality in French Guiana)—the species transferred to *Semomesia* Westwood, 1851 (type species *Papilio croesus* Fabricius, 1777) in Callaghan and Lamas (2004)—and *Mesosemia acuta* Hewitson, 1873 (type locality in Brazil, possibly Rio de Janeiro) reveals that they are not monophyletic with *Mesosemia* Hübner, 1819 (type species *Mesosemia philoclessa* Hübner, 1819) that includes *Semomesia* as its junior subjective synonym, but instead originate within the genus *Ectosemia* Grishin, 2021 (type species *Papilio eumene* Cramer, 1776) (Fig. 15). Therefore, we propose the following **new combinations**: *Ectosemia nesti* (Hewitson, 1858) and

Ectosemia acuta (Hewitson, 1873). Although we have not sequenced *Mesosemia eurythmia* Stichel, 1915 (type locality in Brazil: Amazonas), we tentatively place it in *Ectosemia* due to wing pattern similarities: *Ectosemia eurythmia* (Stichel, 1915), **comb. nov**.



Fig. 15. Phylogenetic trees of selected Mesosemiini species inferred from protein-coding regions of a) the nuclear (autosomes) and b) the mitochondrial genomes. Different genera of Mesosemiina are colored in different colors: *Ectosemia* (blue, with *Ectosemia acuta* comb. nov. and *Ectosemia nesti* comb. nov. labeled in red; *Ectosemia attavus* stat. nov. in magenta; and *Ectosemia erinnya* in cyan), *Mesosemia* (violet), and *Endosemia* (olive). TS - type species.

Mesosemia eumene furia Stichel, 1910 is a junior subjective synonym of Ectosemia erinnya (Stichel, 1910)

Genomic analysis of syntypes of *Mesosemia eumene erinnya* Stichel, 1910 (type locality in Ecuador and Peru, sequenced as NVG-21126B12 and NVG-21126C01), currently a valid species of *Ectosemia* Grishin, 2021 (type species *Papilio eumene* Cramer, 1776) and *Mesosemia eumene furia* Stichel, 1910 (type locality in Bolivia and Peru, sequenced as NVG-21126B11) kept in the same status since its description and, as a consequence of being a subspecies of the type species (Zhang et al. 2021) of *Ectosemia*, transferred in this genus, reveals that they are genetically close (Fig. 15), do not segregate into separate clades, and most likely are conspecific. Therefore, we propose that *Mesosemia eumene furia* Stichel, 1910, **syn. nov.** is a junior subjective synonym of *Ectosemia erinnya* (Stichel, 1910). To define these taxa objectively and to clarify their type localities, their lectotypes are designated below.

First, N.V.G. hereby designates a syntype in the MFNB collection, a female with the following six printed (but 4th handwritten) labels, the 1st red, 4th greenish-gray, and others white: [Typus], [Süd Peru | Pozuzo | e.c.H.Stichel], [2266], [erinnya | Stich.], [ex coll. | H. STICHEL], and [DNA sample ID: | NVG-21126B12 | c/o Nick V. Grishin] as the **lectotype** of *Mesosemia eumene erinnya* Stichel, 1910. The lectotype is a specimen in good condition with half of its right antenna broken off, and some scales rubbed off near the middle of the forewing outer margin. The type locality of *Ectosemia erinnya* becomes Peru: Pozuzo. According to our genomic analysis, the lectotype is conspecific with the paralectotype (NVG-21126C01) from Ecuador: Archidona (Fig. 15).

Second, N.V.G. hereby designates a syntype in the MFNB collection, a male with the following six printed (but 4th handwritten) labels, the 1st red, 4th greenish-gray, and others white: [Typus], [Bolivia La Paz | Farinas | e.c.H.Stichel], [2265], [furia | Stich.], [ex coll. | H. STICHEL], and [DNA sample ID: | NVG-21126B11 | c/o Nick V. Grishin] as the **lectotype** of *Mesosemia eumene furia* Stichel, 1910. The lectotype lacks the abdomen, and outer-marginal segments of the right forewing are chipped off from the middle to the tornus. The type locality of *M. e. furia* becomes Bolivia: La Paz, Farinas.

Mesosemia eumene race attavus J. Zikán, 1952 is a species distinct from Ectosemia eumene (Cramer, 1776)

The genomic comparison reveals that *Mesosemia eumene* race *attavus* J. Zikán, 1952 (type locality in Brazil: Amazonas, São Gabriel da Cachoeira municipality, Rio Negro), currently a subspecies of *Ectosemia eumene* (Cramer, 1776) (type locality in Suriname), is strongly differentiated genetically from the latter (Fig. 15), e.g., COI barcode difference of 4.4% (29 bp). These genetic and also pronounced phenotypic differences in wing patterns (narrower dark brown dorsal hindwing bands) and shapes (more

convex outer hindwing margin) suggest that *Ectosemia attavus* (J. Zikán, 1952), stat. nov. is a species distinct from *Ectosemia eumene* (Cramer, 1776).

Cremna telarania Grishin, new species

http://zoobank.org/8EF9AA2F-9A47-4201-9E26-C1507FDC9FD0

(Figs. 16, 17 part)

Definition and diagnosis. Genomic sequencing of *Cremna* E. Doubleday, 1847 (type species *Papilio actoris* Cramer, 1776) reveals a clade consisting of a pair from Bolivia (Fig. 16) distinct from other species in the *C. actoris* group (Fig. 17): COI barcode difference of 1.8% (12 bp) with a syntype of *Cremna meleagris* Hopffer, 1874 (type locality in Peru: Chanchamayo), a junior subjective synonym of *Cremna heteroea* H. Bates, 1867 (type locality in Brazil: Amazonas), sister to the clade representing the new species. This new species differs from its relatives in smaller size, paler wings (especially beneath), more prominent cream-colored marginal spots above, and boomerang-shaped, narrower on the dorsal side postdiscal (in addition to submarginal) spots on both wings (weak on dorsal forewing in the female). These spots are broader and rounder in other species and are crescent-shaped only in *Cremna calitra* Hewitson, 1869 (type locality in Ecuador), a species with mostly larger spots on the dorsal side, but smaller spots along the outer wing margins (especially on the hindwing) and on the ventral side.

Barcode sequence of the holotype. Sample NVG-22112E04, GenBank OR939283, 658 base pairs:



Fig. 16. Cremna telarania sp. n. in dorsal (above) and ventral (below) views, data in text:a) holotype of NVG-22112E04 and b) paratype Q NVG-22112E11.



Fig. 17. Phylogenetic trees of selected *Cremna* species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genome: *C. telarania* **sp. n.** (red), *C. heteroea* (blue), *C. actoris* (green), and *C. calitra* (violet).

Type material. Holotype: σ deposited in the Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 16a, bears five rectangular labels, the first two handwritten and others printed: four white [Bolivia | Torochita | 90. Garl.], [meleagris | Hopff.], [Coll. | Satudinger], [DNA sample ID: | NVG-22112E04 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Cremna telarania | Grishin]. **Paratype:** 19 with the same data as the holotype (NVG-22112E11, GenBank barcode <u>OR939284</u>, Fig. 16b).

Type locality. Bolivia: La Paz Department, Mapiri.

Etymology. In Spanish, la telaraña means spider web. The name is given for the cobweb wing pattern of this species. The name is a feminine noun in apposition.

Distribution. Currently known only from the La Paz Department in Bolivia.

Cremna dentata (Stichel, 1910) is a species distinct from *Cremna radiata* (Godman & Salvin, 1886)

Genomic analysis reveals that *Voltinia radiata dentata* Stichel, 1910 (type locality in Colombia), currently treated as a junior subjective synonym of *Cremna radiata* (Godman & Salvin, 1886) (type locality Costa Rica: Irazú), is genetically differentiated from it at the level characteristic of distinct species (Fig. 18), e.g., COI barcode difference of 2.6% (17 bp) in the presence of phenotypic distinction (Lukhtanov et al. 2016): *C. dentata* typically has longer white rays between veins on wings. Therefore, we propose that *Cremna dentata* (Stichel, 1910), **stat. nov.** is a species distinct from *Cremna radiata* (Godman & Salvin, 1886).



Fig. 18. Phylogenetic trees of selected *Cremna* and *Napaea* species inferred from protein-coding regions of a) the nuclear (autosomes) and b) the mitochondrial genomes: *C. radiata* (blue), *C. dentata* stat. rest. (red), and their sister *C. theata* (green).

Cremna pupillata Stichel, 1915 is a species distinct from Cremna alector (Geyer, 1837)

Genomic analysis reveals that specimens identified as *Cremna alector* (Geyer, 1837) (type locality in Brazil) partition into two genetically differentiated clades suggestive of species level (Fig. 19): e.g., their COI barcodes differ by 3.6% (24 bp). Upon phenotypic inspection, we find that specimens in one clade have white spots by forewing costa in the postdiscal blue band, and specimens in the other clade lack them, among other differences discussed by Hall (2005). While syntypes of *C. alector* are likely lost, original illustrations of this species show the lack of spots (Geyer 1837). Inspecting these illustrations, we

conclude that they are detailed enough to depict the spots if they were present. The lectotype of *Cremna alector pupillata* Stichel, 1910 (type locality in Brazil: Espírito Santo, sequenced as NVG-21125A10) has the spots and thus, according to our genomic results, is a species different from *C. alector*, represented by two specimens without spots from Linhares, Espírito Santo in Brazil (Fig. 19). Therefore, we propose that *Cremna pupillata* Stichel, 1915, **stat. nov.** is a species distinct from *Cremna alector* (Geyer, 1837).



Fig. 19. Phylogenetic trees of selected *Cremna* species inferred from protein-coding regions of **a**) the Z chromosome and **b**) the mitochondrial genome: *C. alector* (blue) and *C. pupillata* stat. nov. (red).

A species list of Napaeina J. Hall, 2003 assigned to genera

The list below is mostly based on previously published results (Callaghan and Lamas 2004; Hall 2005; Seraphim et al. 2018; Zhang et al. 2021) guided by the genome-level phylogeny (Fig. 20) and is given to correct some ambiguities and mistakes. For example, Zhang et al. (2021) gave an erroneous combination *Napaea sanarita* (Schaus, 1902) (type locality in Brazil: Rio de Janeiro) while correctly resurrecting the genus *Eucorna* Strand, 1932 of which *Eucora sanarita* Schaus, 1902 is the type and the only species



Fig. 20. Phylogenetic trees of Napaeina species inferred from protein-coding regions of **a**) the nuclear genome (autosomes) and **b**) the Z chromosome. Different genera are colored in different colors: *Hyphilaria* (cyan), *Eucorna* (magenta), *Cremna* (green), *Napaea* (blue, with "*Napaea*" *thasus* in orange), and *Ithomiola* (purple).

(Rosa et al. 2023). We note that *Cremna calitra* Hewitson, 1869 (type locality in Ecuador) belongs to *Cremna* E. Doubleday, 1847 (type species *Papilio actoris* Cramer, 1776), not *Napaea* Hübner, 1819 (type species *Cremna eucharila* Bates, 1867) (Fig. 20).

Assignment of species to genera follows our study (Zhang et al. 2021), and we attempt arranging species in the list to maximize the phenotypic similarity of the neighbors but without disrupting a phylogenetic order given by genomic trees (Fig. 20): i.e., a strongly supported clade in the trees is a continuous segment in the list. We start with *Hyphilaria*, but the order of the entire list can be reversed. We put *Cremna* and *Napaea* next to each other because species in these genera are similar (Hall 2005). To maintain phylogenetic order, the considerations above necessitate placing *Ithomiola* last. Finally, we situate *Eucorna* next to *Cremna*. Similar arguments were applied within each genus. Further suggestions about the order to optimize similarity in appearance between neighbors are encouraged.

Only valid names of genera and species are given below; for subspecies, see the Butterflies of America website (Warren et al. 2023); for synonyms and taxonomic discussions, see other publications (Callaghan and Lamas 2004; Hall 2005). Type genus (for family-group names) or type species (for genus-group names) names are given in parenthesis, and names of type species are underlined.

Tribe Mesosemiini Grote, 1898 (Mesosemia Hübner, [1819]) Subtribe Napaeina J. Hall, 2003 (Napaea Hübner, [1819]) Genus Hyphilaria Hübner, [1819] (Hyphilaria nicia Hübner, [1819]) Hyphilaria anthias (Hewitson, 1874) Hyphilaria nicia Hübner, [1819] Hyphilaria parthenis (Westwood, 1851) Genus *Eucorna* Strand, 1932 (Eucora sanarita Schaus, 1902) Eucorna sanarita (Schaus, 1902) Genus Cremna E. Doubleday, 1847 (Papilio actoris Cramer, 1776) Cremna alector (Geyer, 1837) Cremna pupillata Stichel, 1915, stat. nov. Cremna radiata (Godman & Salvin, 1886) Cremna dentata (Stichel, 1910), stat. nov. Cremna theata (Stichel, 1910) Cremna actoris (Cramer, 1776) Cremna heteroea H. Bates, 1867 Cremna telarania Grishin, sp. n. Cremna calitra Hewitson, 1869 Genus Napaea Hübner, [1819] (Cremna eucharila Bates, 1867) Napaea sylva (Möschler, 1877) Napaea beltiana (H. Bates, 1867) Napaea dramba (J. Hall, Robbins & Harvey, 2004) [fossil] Napaea danforthi A. Warren & Opler, 1999 Napaea umbra (Boisduval, 1870) Napaea loxicha (RG. Maza & J. Maza, 2016) Napaea maya (J. Maza & Lamas, 2016) Napaea necaxa (RG. Maza & J. Maza, 2018) Napaea totonaca (RG. Maza & J. Maza, 2016) Napaea rufolimba J. Hall, 2005 Napaea tumbesia J. Hall & Lamas, 2001 Napaea fratelloi J. Hall & Harvey, 2005 Napaea eucharila (H. Bates, 1867) Napaea frustatoria Brévignon, 2019 Napaea phryxe (C. Felder & R. Felder, 1865) Napaea agroeca Stichel, 1910 Napaea cebrenia (Hewitson, [1873]) Napaea zikani Stichel, 1923 Napaea elisae (J. Zikán, 1952) Napaea joinvilea J. Hall & Harvey, 2005 Napaea melampia (H. Bates, 1867) Napaea mellosa J. Hall & Harvey, 2005 Napaea gynaecomorpha J. Hall, Harvey & Gallard, 2005 Napaea merula (Thieme, 1907)

Genus "new genus 1" Seraphim et al. 2018 "Napaea" thasus (Stoll, 1780) comb. nov. [placed in its possible sister genus for now just to have a genus name]

Genus Ithomiola C. Felder & R. Felder, 1865 (Ithomiola floralis C. Felder & R. Felder, 1865) Ithomiola eburna (J. Hall & Harvey, 2005) Ithomiola candidata (Hewitson, 1874) Ithomiola oweni (Schaus, 1913) Ithomiola calculosa J. Hall & Harvey, 2005 Ithomiola theages (Godman & Salvin, 1878) Ithomiola cribralis (Stichel, 1915) Ithomiola neildi (J. Hall & Willmott, 1998) Ithomiola bajotanos J. Hall, 2005 Ithomiola tanos (Stichel, 1910) Ithomiola nepos (Fabricius, 1793) Ithomiola orpheus (Westwood, 1851) Ithomiola callixena (Hewitson, 1870) Ithomiola buckleyi J. Hall & Willmott, 1998 Ithomiola floralis C. Felder & R. Felder, 1865

Lasaia peninsularis Clench, 1972 is a species distinct from Lasaia sula Staudinger, 1888

Genomic analysis reveals that *Lasaia sula peninsularis* Clench, 1972 (type locality Mexico: Yucatán, Pisté) is genetically differentiated from *Lasaia sula* Staudinger, 1888 (type locality in Honduras) at the level characteristic of distinct species (Fig. 21) with $F_{st}/G_{min}/COI$ barcode difference of 0.52/0.005/1.5% (10 bp), with the barcode difference computed between lectotypes of both names. Therefore, we propose that *Lasaia peninsularis* Clench, 1972, **stat. nov.** is a species distinct from *Lasaia sula* Staudinger, 1888.



Fig. 21. The phylogenetic tree of selected Riodinini species inferred from protein-coding regions of the nuclear genome (autosomes). Taxa discussed in the text are shown in different colors, and those transferred between genera (green arrows indicate the direction of transfer) are labeled in a color different from the rest of the genus. Genus-group names (genera in bold italics and subgenera in italics) are shown by corresponding branches. Levels in the tree that approximately correspond to genus and subgenus are labeled on top. New subgenera are highlighted in yellow.

Necyria Westwood, 1851 is a subgenus of Lyropteryx Westwood, 1851

Although traditionally treated as distinct (and monophyletic) genera for more than 170 years, *Lyropteryx* Westwood, 1851 (type species *Lyropteryx apollonia* Westwood, 1851) and *Necyria* Westwood, 1851 (type species *Necyria bellona* Westwood, 1851) are genetically (Fig. 21) and phenotypically close. COI barcodes of their type species differ by 2.7% (18 bp), which is typical for closely related sister species, not different genera, and both genera are characterized by lyre- or harp-like wing patterns resulting from metallic overscaling between the veins complemented with red spots or stripes. A novice cannot easily assign a species to a genus by wing patterns. For all these reasons, we propose to treat these monophyletic groups as subgenera. *Necyria* and *Lyropteryx* were proposed in the same work issued on the same day (Westwood 1851), and being the first revisers, we give precedence to *Lyropteryx* because this name is more descriptive of a butterfly appearance: its wing ($\pi t \le \rho t \le r$), resembles the musical instrument lyre ($\lambda \omega \rho a - lyra$). Therefore, we propose that *Necyria* Westwood, 1851, **stat. nov.** is a subgenus of *Lyropteryx* Westwood, 1851.

Lymnas melaniae Stichel, 1930 belongs to the genus *Lyropteryx* Westwood, 1851 and not *Melanis* Hübner, [1819]

Genomic phylogeny reveals that Lymnas melaniae Stichel, 1930 (type locality in Brazil: Santa Catarina), currently in the genus Melanis Hübner, [1819] (type species Papilio melander Stoll, 1780), is not monophyletic with it and instead is a close sister to Lyropteryx Westwood, 1851 (type species Lyropteryx apollonia Westwood, 1851) (Fig. 21): COI barcode difference of 2.3% (15 bp), which is typical for closely related congeners. To restore the monophyly of Melanis, we transfer L. melaniae to the genus Lyropteryx, forming a new combination Lyropteryx melaniae (Stichel, 1930), comb. nov.

Eurygona? pulcherrima Herrich-Schäffer, [1853] and *Themone poecila* H. Bates, 1868 belong to the genus *Isapis* E. Doubleday, 1847 and not *Themone* Westwood, 1851

Eurygona? pulcherrima Herrich-Schäffer, [1853] (type species in Suriname) and *Themone poecila* H. Bates, 1868 (type locality Brazil: Amazonas, Ega [= Tefé]) currently placed in the genus *Themone* Westwood, 1851 (type species *Helicopis pais* Hübner, [1820]) are not monophyletic with it and instead form a clade together with *Isapis* E. Doubleday, 1847 (type species *Papilio agyrtus* Cramer, 1777) and are close to it genetically (Fig. 21). To restore monophyly of *Themone*, we transfer the two species to *Isapis* forming new combinations *Isapis pulcherrima* (Herrich-Schäffer, [1853]), **comb. nov.** and *Isapis poecila* (H. Bates, 1868), **comb. nov.** Furthermore, we note that the clade with these species is sister to *Melanis* Hübner, [1819] (type species *Papilio melander* Stoll, 1780), and they are closely related to it, i.e., COI barcodes of the type species of *Melanis* and *Isapis* differ by 7.3% (48 bp). Therefore, *Isapis* can be included in *Melanis*, a step we are not taking here but proposing for consideration.

Callitera Grishin, new subgenus

http://zoobank.org/261B10A3-E22E-49EA-97B3-466C4393884E

Type species. Eurygona? pulcherrima Herrich-Schäffer, [1853].

Definition. As shown above, *Eurygona? pulcherrima* Herrich-Schäffer, [1853] (type locality in Suriname) belongs to the genus *Isapis* E. Doubleday, 1847 (type species *Papilio agyrtus* Cramer, 1777) and not to *Themone* Westwood, 1851 (type species *Helicopis pais* Hübner, [1820]) (Fig. 21). However, it is genetically differentiated from the type species of *Isapis* at the subgenus level, e.g., their COI barcodes differ by 6.5% (43 bp). Therefore, we propose that the lineage with *Isapis pulcherrima* represents a new subgenus. This subgenus differs from its relatives by a combination of the following characters: each wing is blackish-brown with a yellow stripe by its base beneath (as in the type species of *Isapis*) and

discal white streaks sometimes framed with metallic-green scales on the dorsal side and could be vestigial on the hindwing. In DNA, a combination of the following characters is diagnostic in the nuclear genome: cne3301.6.2:T166A, cne3301.6.2:C186T, cne178.3.20:T1233A, cne178.3.20:T1632A, cne37196.1.3:A87T and in COI barcode: T67A, T82C, A211G, 223A, A268T, T625G.

Etymology. The name of the type species, *pulcherrima*, is a Latin word meaning very beautiful, most beautiful, or prettiest. The name of the new subgenus is formed from the Greek word K $\alpha\lambda\lambda$ itep η (kalliteri), which means most beautiful. The name is a feminine noun in the nominative singular.

Species included. Only the type species.

Parent taxon. Genus Isapis E. Doubleday, 1847.

Matizada Grishin, new subgenus

http://zoobank.org/203DA466-AC54-4A5E-9BC5-44CE0AFD1F2F

Type species. Themone poecila H. Bates, 1868.

Definition. As shown above, *Themone poecila* H. Bates, 1868 (type locality Brazil: Amazonas, Ega [= Tefé]) belongs to the genus *Isapis* E. Doubleday, 1847 (type species *Papilio agyrtus* Cramer, 1777) and not to *Themone* Westwood, 1851 (type species *Helicopis pais* Hübner, [1820]) (Fig. 21). However, it is genetically differentiated from the type species of *Isapis* at the subgenus level, e.g., their COI barcodes differ by 8.1% (53 bp) and is sister to the clade of two subgenera: *Isapis* and *Callitera* **subgen. n**. Therefore, we propose that the lineage with *Isapis poecila* represents a new subgenus. This subgenus differs from its relatives by a combination of the following characters: each wing is blackish-brown with a yellow-orange area towards the base and a central yellow spot, which may be vestigial on the dorsal side. In DNA, a combination of the following characters is diagnostic in nuclear genome: cne792.14.1:C927T, cne792.14.1:A132T, cne2411.1.1:A348T, cne5004.10.6:A822T, cne5004.10.6:G633A, cne5335.1.1:C114C (not T), cne5335.1.1:T129T (not C), cne5331.3.1:A53A (not G), cne5331.3.1:T237T (not C), cne573.8.1: C63C (not G) and in COI barcode: A4C, C81T, T88A, A278A, 421C, A586A.

Etymology. The name of the type species, *poecila*, typically refers to colorful or variegated markings or patterns. It is derived from the Greek word $\pi \sigma \kappa i \lambda \sigma \zeta$ (poikilos), which means varied, diverse, or multicolored. The name of the new subgenus is formed from the Spanish word matizado, which means variegated or mottled. The name is treated as a feminine noun in the nominative singular.

Species included. Only the type species.

Parent taxon. Genus Isapis E. Doubleday, 1847.

Lyropteryx diadocis Stichel, 1910 belongs to the genus *Paraphthonia* Stichel, 1910 and not *Lyropteryx* Westwood, 1851

Genomic phylogeny reveals that Lyropteryx diadocis Stichel, 1910 (type locality in Brazil: Amazonas) kept in its original genus is not monophyletic with Lyropteryx Westwood, 1851 (type species Lyropteryx apollonia Westwood, 1851) and instead is sister to Paraphthonia Stichel, 1910 (type species Monethe molione Godman, 1903) (Fig. 21). Because Paraphthonia itself is already quite closely related to Brachyglenis C. Felder & R. Felder, 1862 (type species Brachyglenis esthema C. Felder & R. Felder, 1862) and Themone Westwood, 1851 (type species Helicopis pais Hübner, [1820]) (Fig. 21): COI barcode difference of 6.2% (41 bp) and 6.8% (45 bp), respectively, we restore monophyly of Lyropteryx by including L. diadocis in the genus Paraphthonia to form a new combination Paraphthonia diadocis (Stichel, 1910), comb. nov. Due to the genetic closeness of these three genera (Paraphthonia, Brachyglenis, and Themone), it is conceivable to combine them in a single genus Themone, a step we are not taking here but proposing for consideration.

Parapanara Grishin, new subgenus http://zoobank.org/F41BC3E1-EAB0-44E4-A4F3-E9C9F8D04775

Type species. Lyropteryx diadocis Stichel, 1910.

Definition. As shown above, *Lyropteryx diadocis* Stichel, 1910 (type locality in Brazil: Amazonas) belongs to the genus *Paraphthonia* Stichel, 1910 (type species *Monethe molione* Godman, 1903) and not to *Lyropteryx* Westwood, 1851 (type species *Lyropteryx apollonia* Westwood, 1851) (Fig. 21). However, it is genetically differentiated from the type species of *Paraphthonia* at the subgenus level, e.g., their COI barcodes differ by 6.1% (40 bp). Therefore, we propose that the lineage with *Paraphthonia diadocis* represents a new subgenus. This subgenus differs from its relatives by a combination of the following characters: forewing vein R₂ originates at the anterior distal corner of the discal cell, the forewing with the orange-yellow band from mid-costa to near tornus, and the hindwing with metallic-green overscaling around veins in distal half. In DNA, a combination of the following characters is diagnostic in the nuclear genome: cne3461.1.26:A146G, cne3461.1.26:A1989G, cne6404.2.4:C87T, cne945.5.1:A342T, cne945.5.1: T352C, cne3615.3.2:A132A (not G), cne4618.3.1:C31C (not G), cne4618.3.1:C37C (not G), cne6843.7.6: T352A, A604G.

Etymology. In its appearance, the type species of this subgenus resembles some species from the genus *Panara* E. Doubleday, 1847 (type species *Papilio jarbas* Drury, 1782), and the prefix "para" means alongside, near, beyond, or similar to. The name is a feminine noun in the nominative singular.

Species included. Only the type species.

Parent taxon. Genus Paraphthonia Stichel, 1910.

Synargis orestessa Hübner, [1819] is a junior subjective synonym of *Synargis soranus* (Stoll, 1781), and *Synargis arche* (Hewitson, 1865) is a valid species

Synargis orestessa Hübner, [1819] was proposed as a replacement name for *Papilio orestes* Cramer, 1780 (type locality in Suriname) preoccupied by *Papilio orestes* Meerburgh, 1777 (in Papilionidae). Original illustrations (dorsal and ventral) (Cramer 1775–1780) of *P. orestes* and a possible syntype specimen in RMNH that agrees with the illustrations and would simultaneously be a syntype of the replacement name *S. orestessa* look more similar to the original illustrations of *Synargis soranus* (Stoll, 1781) (type locality in Suriname) and specimens referred to by the name "*soranus*," than to specimens of the species currently referred to by the name "*orestessa*." Genomic analysis shows the presence of two species (Fig. 22) that differ by 3.2% (21 bp) in their COI barcodes: females of one possess broader yellow-orange bands on the forewing, and of the other have narrower and whiter bands usually separated into spots, among other differences. For these reasons, we propose that *Synargis orestessa* Hübner, [1819], **stat. nov.** is a junior subjective synonym of *Synargis soranus* (Stoll, 1781). The oldest name for the species that was referred to by the name "*orestessa*" is *Nymphidium arche* Hewitson, 1865 (type locality in Brazil: Amazonas), and, hence, *Synargis arche* (Hewitson, 1865) is a valid species.



Fig. 22. Phylogenetic trees of several *Synargis* species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes: *S. soranus* (red), *S. arche* stat. rest. (blue), and their sister *S. abaris* (green).

Family Lycaenidae [Leach], [1815]

Nothodanis Hirowatari, 1992 is a subgenus of Danis [Fabricius], 1807

Genomic phylogeny of Polyommatini Swainson, 1827 reveals that *Nothodanis* Hirowatari, 1992 (type species *Lycaena schaeffera* Eschscholtz, 1821) (Fig. 23 cyan) is closely related to its sister genus *Danis* [Fabricius], 1807 (type species *Papilio danis* Cramer, 1775) (Fig. 23 violet), e.g., their COI barcodes differ by 10.2% (67 bp) and the two diverged from each more recently than most other genera (Fig. 23, on the right of the green line). Therefore, we propose to treat *Nothodanis* Hirowatari, 1992, **stat. nov.** as a subgenus of *Danis* [Fabricius], 1807.



Fig. 23. Phylogenetic trees of selected Polyommatini species inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genomes. Taxa discussed in the text are shown in color. The green line delineates genera. The sequence of SAMN18674175 is taken from the alignment provided in Kawahara et al. (2023).

Perpheres Hirowatari, 1992 is confirmed as a valid genus

Perpheres Hirowatari, 1992 (type and the only species *Thysonotis perpheres* (H. H. Druce & Bethune-Baker, 1893) (Fig. 23 olive) was at times lumped with *Danis* [Fabricius], 1807 (type species *Papilio danis* Cramer, 1775), but is in a clade different from *Danis* (subtribe Danina Koçak & Seven, 1997) and the same clade with *Castalius* Hübner, [1819] (type species *Papilio rosimon* Fabricius, 1775, subtribe Castaliina Distant, 1884) (Fig. 23). Therefore, the placement of *T. perpheres* in *Danis* is incorrect and, because *Perpheres* is genetically distant from genera that are closest to it (Fig. 23), we confirm *Perpheres* as a valid genus in the subtribe Castaliina.

Pistoria Hemming, 1964 is a junior subjective synonym of Caleta Fruhstorfer, 1922

Adding DNA segments of *Mambara nigropunctata* Bethune-Baker, 1908 (type locality in Papua New Guinea, biosample SAMN18674175), the type species of currently valid genus *Pistoria* Hemming, 1964, taken from the alignment provided in Kawahara et al. (2023) (Fig. 23 red) to our genomic datasets of its relatives, we find that *Pistoria nigropunctata* originates within *Caleta* Fruhstorfer, 1922 (type species *Lycaena caleta* Hewitson, 1876), rendering it paraphyletic. To restore the monophyly, due to the genetic closeness of *Pistoria nigropunctata* and *Caleta caleta* (COI barcode difference of 5.9%, 39 bp), we propose that *Pistoria* Hemming, 1964, **syn. nov.** is a junior subjective synonym of *Caleta* Fruhstorfer, 1922.

Upolampes Bethune-Baker, 1908 is a junior subjective synonym of *Thaumaina* Bethune-Baker, 1908

Genomic trees reveal that *Upolampes* Bethune-Baker, 1908 (type species *Upolampes striata* Bethune-Baker, 1908, which is a junior subjective synonym of *Lycaena evena* Hewitson, 1876) (Fig. 24b, Fig. 23

orange) and *Thaumaina* Bethune-Baker, 1908 (type species *Thaumaina uranothauma* Bethune-Baker, 1908) (Fig. 24a, Fig. 23 green) are closely related to each other despite the remarkable difference in their wing patterns (Fig. 24): e.g., their COI barcode differ by 6.5% (43 bp) and therefore should be synonymous. The two names, *Upolampes* and *Thaumaina*, were published in the same work issued on the same date (Bethune-Baker 1908). As the first revisers, we give precedence to *Thaumaina* (two valid species, fewer name changes) over *Upolampes* (one valid species) and propose that *Upolampes* Bethune-Baker, 1908, **syn. nov.** is a junior subjective synonym of *Thaumaina* Bethune-Baker, 1908.



Fig. 24. Males of *Thaumaina* from Papua New Guinea in dorsal (left) and ventral (right) views: a) *T. uranothauma deliciosa* Wind & Clench, 1945, Wau, 5000 ft, 18-22-Apr-1972, R. H. Carcasson leg. (NVG-22038B01) [USNM]; b) *T. evena* comb. nov., Madang, genitalia of this specimen are illustrated on pl. 5, f. 14 in Fruhstorfer (1918) (NVG-22027A02) [ZSMC].

Tarucus clathratus W. Holland, 1891, comb. rest.

Genomic sequencing of the holotype by monotypy: "the type, a male," per original description (Holland 1891), of *Tarucus clathratus* W. Holland, 1891 (type locality in Sulawesi, sequenced as NVG-20124E10) (Fig. 25, Fig. 23 magenta), currently placed in the genus *Castalius* Hübner, [1819] (type species *Papilio rosimon* Fabricius, 1775), reveals that it is not monophyletic with its type species and is in the same clade with *Tarucus* F. Moore, 1881 (type species *Hesperia theophrastus* Fabricius, 1793) (Fig. 23 blue), where it was originally placed. Therefore, we return it to the genus *Tarucus*, as originally proposed: *Tarucus clathratus* W. Holland, 1891, **comb. rest**. We note that the photograph of *T. clathratus* holotype in the original description (Holland 1891) (Fig. 25b) on a casual look does not appear particularly similar to



Fig. 25. Holotype of *Tarucus clathratus*, data in text: **a)** photographs taken on 28-Jun-2021 by N.V.G., dorsal (left) and ventral (right) views of the specimen with its labels (below); labels reduced by one third compared to the specimen; larger scale bar refers to the specimen, smaller scale bar refers to labels and genitalia vial; **b)** photograph of the holotype in ventral view reproduced from pl. 5, f. 8 in Holland (1891).

the holotype specimen (Fig. 25a). The photograph shows a specimen with narrower dark bands (likely overexposed or possibly re-touched) and a different position of antenna (was re-attached later). However, despite these differences, the wing shape and outline of the spread with the right hindwing closer to the forewing than the left pair, the position of tails, and the tear along the Rs vein in the middle of the right hindwing match between the likely holotype and the photograph. The specimen is in CMNH, bears the label "Holland Collection", agrees with the original description better than the photograph (broad dark bands per description), and is labeled in Holland's handwriting as "Type" in a manner similar to all other type specimens he described and labeled. Therefore, there is little reason to doubt that the specimen we sequenced (NVG-20124E10) is the holotype.

Family Hesperiidae Latreille, 1809

Euriphellus colombiensis Grishin, new species

http://zoobank.org/466927D6-598F-4C73-8835-F3D4305F40BB

(Figs. 26 part, 27a, c, 28a-b)

Definition and diagnosis. The Z chromosome analysis of *Euriphellus* Austin, 2008 (type species *Papilio* euribates Stoll, 1782) reveals that four specimens from Colombia and Ecuador form a clade sister to several *Euriphellus* species that is genetically differentiated from them (Fig. 26a). Therefore, these specimens belong to species distinct from the rest. The two pairs of specimens are genetically differentiated from each other, e.g., COI barcode differences of 4.9% (32 bp) between them (possible introgression with Euriphellus lama (Evans, 1952), Fig. 26b), and belong to two different new species. The one from western Colombia is described here, and the one from eastern Ecuador is described below. The new species from Colombia keys to D.4.2(b) in Evans (1952), and differs from its relatives by the following combination of characters in male: dorsal wing color yellower in hue, forewing without submarginal hyaline spots in cells M₁-M₂, M₂-M₃, or R₃-R₄, only two yellow hyaline subapical spots in cells R₄-R₅ and R₅-M₁, hindwing with six well-developed and nearly collected into a band postdiscal brown spots on dorsal side, one in each cell between veins RS and 1A+2A, ventrally with prominent yellow spots, including near the base of cell Sc+R₁-RS (Fig. 27a), tegumen narrower in dorsal view, harpe longer than in relatives, humped along ventral margin, expanded into a keel with several small teeth on dorsal side and narrows to a point, ampulla with a nearly square process, flattened along its somewhat irregular dorsoposterior margin (Fig. 28a, b); and female with larger discal forewing hyaline spots, the spot in cell M_3 -CuA₁ overlaps the spot in cell CuA₁-CuA₂ by most of its width (Fig. 27c). Due to unknown phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly2582.35.2:G1861A, aly2582.35.2:C1862G, aly767.18.5:A88T, aly767.18.5:T117A, aly54.32.1:C215G and in COI barcode: A181G, T259C, C343T, T364C, T376A, T484T, T553A.



Fig. 26. Phylogenetic trees of selected *Euriphellus* species inferred from protein-coding regions of a) the Z chromosome and b) the mitochondrial genome: *E. marian* (violet), *E. lama* (cyan), *E. phraxanor* (blue), *E. mena* (green), *E. colombiensis* sp. n. (red), *E. ecuadoricus* sp. n. (magenta), *E. euribates* (olive), and *E. polygius* (black).



Fig. 27. Type specimens of *Euriphellus*: **a)** *E. colombiensis* **sp. n.** σ holotype, **b)** *E. ecuadoricus* **sp. n.** σ holotype, **c)** *E. colombiensis* **sp. n.** φ paratype, **d)** *E. ecuadoricus* **sp. n.** φ paratype in dorsal (left) and ventral (right) views, data in text.



Fig. 28. Genitalia of holotypes: a-b) Euriphellus colombiensis sp. n. and c-d) Euriphellus ecuadoricus sp. n. in dorsal (a, c) and left lateral (b, d) views.

Barcode sequence of the holotype. Sample NVG-18052E08, GenBank OR837728, 658 base pairs:

Type material. Holotype: σ deposited in the Museum für Naturkunde, Berlin, Germany [MFNB], illustrated in Fig. 27a, bears four printed labels: 1st green, two white [W.Columb. | Rio Dagua | 600-1000m | W.Hopp S. | 2 - 5] (the last line is rotated 90° to the left and printed on the right margin of the label), [DNA sample ID: | NVG-18052E08 | c/o Nick V. Grishin], [DNA sample ID: | NVG-22111G11 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Euriphellus | colombiensis Grishin]. The first NVG number corresponds to a sampled leg, and the second is for the abdomen DNA extraction followed by genitalia dissection. **Paratype:** 19 with the same data as the holotype (NVG-18052E11, GenBank barcode <u>OR837729</u>, Fig. 27c).

Type locality. Colombia: Río Dagua, 600–1000 m.

Etymology. The name is given for the country of the type locality. The name is a masculine adjective. **Distribution.** Currently known only from Colombia.

Euriphellus ecuadoricus Grishin, new species

http://zoobank.org/462B5465-AC20-4876-93A3-4803BB75CD2C

(Figs. 26 part, 27b, d, 28c-d)

Definition and diagnosis. Genomic analysis of *Euriphellus* Austin, 2008 (type species *Papilio euribates* Stoll, 1782) reveals that four specimens from Colombia and Ecuador form a clade sister to several *Euriphellus* species that is genetically differentiated from them (Fig. 26). Therefore, these specimens belong to species distinct from the rest. The two pairs of specimens are genetically differentiated from each other, e.g., COI barcode differences of 4.9% (32 bp) between them, and belong to two different new species. The one from eastern Ecuador is described here, and the one from western Colombia is described above. The new species from Ecuador keys to D.4.2(b) in Evans (1952), and differs from its relatives by the following combination of characters in males: wings not as rounded as in *Euriphellus mena* (Evans,

1952) (type locality in Ecuador), dorsal wing color redder in hue, forewing may be with small submarginal hyaline spots in cells M₁-M₂, M₂-M₃, and R₃-R₄, and larger hyaline subapical spots in cells R₄-R₅ and R₅-M₁, hindwing with five weaker-developed and separated postdiscal brown spots on dorsal side, one in each cell between veins RS and CuA₂, ventrally with prominent yellow spots, but not near the base of cell Sc+R₁-RS (Fig. 27b), tegumen broader in dorsal view, harpe shorter than in *Euriphellus colombiensis* **sp. n.**, only weakly humped along ventral margin, no dorsal keel, and narrows to a point, ampulla with a rounded thumb-like process with somewhat irregular margins (Fig. 28c, d); and female with smaller discal forewing hyaline spots, the spot in cell M₃-CuA₁ offset distad from the spot in cell CuA₁-CuA₂ not overlapping with it (Fig. 27d). Due to unknown phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly331.26.8:C109A, aly331.26.8:G267A, aly331.26.8:T291C, aly536.154.1:A618G, aly536. 154.1:T631C and in COI barcode: A28G, T91A, A229G, C343A, G474A, A538G, T544A, T607C, T634C. Barcode sequence of the holotype. Sample NVG-18057G01, GenBank OR837730, 658 base pairs:

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Type material. Holotype: σ deposited in the Zoologische Staatssammlung München, Germany [ZSMC], illustrated in Fig. 27b, bears five printed labels: four white [Canelos | Ecuador or.], [Collection | v.Rosen], [DNA sample ID: | NVG-18057G01 | c/o Nick V. Grishin], [DNA sample ID: | NVG-23012A09 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Euriphellus | ecuadoricus Grishin]. The first NVG number corresponds to a sampled leg, and the second is for the abdomen DNA extraction followed by genitalia dissection. **Paratype:** 19 with the same data as the holotype (NVG-18057G02, GenBank barcode <u>OR837731</u>, Fig. 27d).

Type locality. Ecuador: Canelos.

Etymology. The name is given for the country of the type locality. The name is a masculine adjective. **Distribution.** Currently known only from Ecuador.

Goniurus proteoides Plötz, 1881 is a junior subjective synonym of *Urbanus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus* (Linnaeus, 1758)

Genomic analysis of a syntype of *Goniurus proteoides* Plötz, 1881 (type locality in North America, NVG-15029D03) currently considered a junior subjective synonym of *Urbanus proteus proteus* (Linnaeus, 1758) (type locality in America) (Mielke 2005) reveals that it is not monophyletic with the latter and instead placed within specimens of *Urbanus proteus domingo* (Scudder, 1872) (type locality in Haiti), in agreement with Evans (1952) (Fig. 29). Therefore, we regard *Goniurus proteoides* Plötz, 1881 as a junior subjective synonym of *Urbanus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus proteus proteus domingo* (Scudder, 1872) and not of *Urbanus domingo* (Scudder) and *domingo* (Scudder) and



Fig. 29. Phylogenetic trees of selected *Urbanus* species inferred from protein-coding regions of **a**) the nuclear genome (autosomes) and **b**) the mitochondrial genome: *U. proteus* (blue branches) with *U. proteus proteus* (blue labels) and *U. proteus domingo* (specimens from Cuba are labeled in magenta, the lectotype of *Goniurus proteoides* in orange, and others in violet), *U. cubanus* **sp. n.** (red), and their sister *U. velinus* (green).

(Linnaeus, 1758). In agreement with this conclusion, Godman (1907), who inspected the unpublished drawing t[afel].33 by Plötz of *G. proteoides*, wrote that he had its "Specimens from the Lesser Antilles in the G. & S. coll." To stabilize nomenclature, N.V.G. hereby designates the sole syntype curated in the MFNB collection, a male with the following five labels, the 2nd handwritten and others printed, the 1st red and others white: [Type], [proteoides | Pl. 104], [Coll. H.—Sch], [{QR Code} http://coll.mfn-berlin.de/u/ | e1f97d], and [DNA sample ID: | NVG-15029D03 | c/o Nick V. Grishin] as the **lectotype** of *Goniurus proteoides* Plötz, 1881. The type locality of *G. proteoides* is in the Antilles (unclear if the Greater or the Lesser). Sequencing additional specimens of *U. p. domingo* across its range may pinpoint the type locality more precisely.

Urbanus (Urbanus) cubanus Grishin, new species

http://zoobank.org/E1311827-75D9-4699-8E88-D7A317D9792A

(Figs. 29 part, 30, 31a-e)

Definition and diagnosis. The nuclear genome tree reveals a prominent clade of two specimens from Cuba (Fig. 29 red) initially identified as *Urbanus proteus domingo* (Scudder, 1872) (type locality in



Fig. 30. Urbanus (Urbanus) cubanus sp. n. in dorsal (left) and ventral (right) views, data in text:a) holotype \$ NVG-18057F12 and b) paratype \$ NVG-21126H02.



Fig. 31. Female genitalia of *Urbanus (Urbanus) cubanus* **sp. n.: a–c)** holotype NVG-18057F12 and **d–e)** paratype NVG-21126H02, data in text, and **f–g)** *Urbanus (Urbanus) proteus domingo* NVG-23012A04 from Cuba: Havana, 3-Mar-1927 [ZMSC] in ventral (a, b, d, f) and right ventrolateral (c, e, g) views. Complete genitalia with ductus and corpus bursae are shown in a) and reduced two times (as indicated by smaller scale) compared to other images. Blue arrows point at the antrum.

Haiti) that is sister to all other *Urbanus proteus* (Linnaeus, 1758) (type locality in America) we sequenced (Fig. 29 blue branches), and is placed approximately halfway between *U. proteus* and its sister species *Urbanus velinus* (Plötz, 1881) (type locality in Brazil: Bahia) (Fig. 29 green). The two specimens are strongly differentiated genetically from *U. p. domingo* (Fig. 29 violet, orange, and magenta labels), including two other specimens from Cuba (southeastern region) (Fig. 29 magenta labels): $F_{st}/G_{min}/COI$ barcode difference of 0.51/0.00/0.8% (5 bp, barcodes are similar between the two species). Therefore, these two specimens represent a species distinct from *U. proteus*. This new species keys to C.13.1(b) in Evans (1952) and differs from its closest relative *U. proteus* in broader and straighter ventral hindwing dark brown bands and a darker area by mid-costa, hyaline spot in forewing cell CuA₁-CuA₂ closer aligned with the spot in discal cell rather than shifted distad, absent or small submarginal hyaline spots in forewing cells M₁-M₂ and M₂-M₃, and narrower antrum (Fig. 31 blue arrows). Due to unexplored phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly103.33.9:A90G, aly103.33.9:T160C, aly103.33.9:G162C, aly207.9.6: A180G, aly103.50.3:T60C and in COI barcode: C220C, T322C, T385C, T610C, C616T.

Barcode sequence of the holotype. Sample NVG-18057F12, GenBank OR837732, 658 base pairs:

Type material. Holotype: 9 deposited in the Zoologische Staatssammlung München, Germany [ZSMC], illustrated in Fig. 30a, bears six labels: four white, the 3rd greenish [CUBA, La Habana, | Boyeros, Finca La | Chata (23.036 N, - | 82.376 W), July 9 2014 | R. Núñez leg.], [RNA-1-171], [BC ZSM Lep 92903], [DNA sample ID: | NVG-18057F12 | c/o Nick V. Grishin], [DNA sample ID: | NVG-23012A03 | c/o Nick V. Grishin], and one red [HOLOTYPE 9 | Urbanus (Urbanus) | cubanus Grishin]. The first NVG number corresponds to a sampled leg, and the second is for the abdomen DNA extraction followed by genitalia dissection. **Paratype:** 19 Cuba, Gundlach leg., Coll. Thieme, genitalia vial NVG-22111G12 (NVG-21126H02, GenBank barcode <u>OR837733</u>, Fig. 30b) [MFNB].

Type locality. Cuba: Havana, Boyeros, Finca La Chata, GPS 23.036, -82.376.

Etymology. The name is given for the country of the type locality. The name is a masculine adjective. **Distribution.** Cuba; currently confirmed from the northwestern region (Havana).

Comment. The ground color difference between the holotype and paratype (darker brown vs. paler reddish-brown, Fig. 30) is due to fading with age: the paratype was collected more than a century ago.

Quadrus (Zera) difficilis (Weeks, 1901) is confirmed as a species distinct from Quadrus (Zera) zera (A. Butler, 1870)

Although *Quadrus (Zera) difficilis* (Weeks, 1901) (type locality in Bolivia) is treated as a distinct species and not a subspecies of *Quadrus (Zera) zera* (A. Butler, 1870) (type locality in Venezuela), on the Butterflies of America website (Warren et al. 2023), this taxonomic opinion has not been substantiated numerically. Genomic sequencing of several specimens of both taxa reveals prominent genetic differentiation between them (Fig. 32), e.g., F_{st} /COI barcode difference of 0.30/2.7% (18 bp), thus confirming *Quadrus (Zera) difficilis* (Weeks, 1901) as a species-level taxon.



Fig. 32. Phylogenetic trees of selected *Quadrus* (*Zera*) species inferred from protein-coding regions of **a**) the nuclear genome (autosomes) and **b**) the mitochondrial genome: *Q. zera* (blue) and *Q. difficilis* (red).

Bolla subgisela Strand, 1921 is a junior subjective synonym of *Staphylus melangon epicaste* Mabille, 1903 and not of *Bolla eusebius* (Plötz, 1884)

Genomic analysis of the holotype of *Bolla subgisela* Strand, 1921 (type locality in Colombia), currently regarded as a junior subjective synonym of *Bolla eusebius* (Plötz, 1884) (type locality in Central America) reveals that it is not monophyletic with *Bolla* Mabille, 1903 (type species *Bolla pullata* Mabille, 1903 treated as a junior subjective synonym of *Staphylus imbras* Godman and Salvin, 1896), but instead is placed within specimens of *Staphylus melangon* (Mabille, 1883) (type locality in South America) and away from *Staphylus tucumanus* (Plötz, 1884) (type locality in Argentina), a sister species of *S. melangon* (Fig. 33). Therefore, *B. subgisela* is conspecific with *S. melangon*. The phylogenetic analysis we used does not differentiate between subspecies of *S. melangon* (Fig. 33), and we assign *B. subgisela* to subspecies by a combination of wing pattern characters and locality. Only *Staphylus melangon epicaste* Mabille, 1903 (type locality in Brazil) possesses brown ventral hindwing without dominant white overscaling toward tornus and inner margin, similar to *B. subgisela*, and it is the only subspecies documented from Colombia (Evans 1953). Therefore, we propose that *Bolla subgisela* Strand, 1921 is a junior subjective synonym of *Staphylus melangon epicaste* Mabille, 1903.



Fig. 33. Phylogenetic trees of selected *Bolla* (violet) and *Staphylus* inferred from protein-coding regions of **a**) the nuclear genome (autosomes) and **b**) the mitochondrial genome: *S. melangon* (blue, with *Bolla subgisela* Strand, 1921, which is a junior subjective synonym of *S. m. epicaste*, in magenta) and its sister species *S. tucumanus* (green).

Gorgythion marginata Schaus, 1902 is a species distinct from Gorgythion begga (Prittwitz, 1868)

Gorgythion marginata Schaus, 1902 (type locality in Peru) (Fig. 34 red) currently regarded as a junior subjective synonym of Gorgythion begga pyralina (Möschler, 1877) (type locality in Suriname) (Fig. 34 blue, part) is genetically differentiated from it and generally from Gorgythion begga (Prittwitz, 1868) (type locality in Brazil: Rio de Janeiro) at the species level (Fig. 34), e.g., F_{st} /COI barcode difference of 0.26/2.9% (19 bp). Therefore, we propose that Gorgythion marginata Schaus, 1902, stat. rest. is a species-level taxon distinct from Gorgythion begga (Prittwitz, 1868).



Fig. 34. Phylogenetic trees of *Gorgythion* species inferred from protein-coding regions of **a**) the Z chromosome and **b**) the mitochondrial genome. Different species are shown in different colors: *G. begga* (blue, with *G. beggoides* syntype labeled in orange), *G. guyanus* **sp. n.** (magenta), *G. marginata* **stat. rest.** (red), *G. beggina* (violet), *G. plautia* (cyan), *G. canda* (olive), *G. vox* (green), and *G. alcandra* (black).

Gorgythion beggoides Schaus, 1902 is a junior subjective synonym of *Gorgythion begga begga* (Prittwitz, 1868), not of *Gorgythion plautia* (Möschler, 1877)

Gorgythion beggoides Schaus, 1902 (type locality in Trinidad) (Fig. 34 orange) currently treated as a junior subjective synonym of *Gorgythion plautia* (Möschler, 1877) (type locality in Suriname) (Fig. 34 cyan) is not monophyletic with it and is instead placed within specimens of *Gorgythion begga* (Prittwitz, 1868) (type locality in Brazil: Rio de Janeiro) (Fig. 34 blue). Hence, we propose that *G. beggoides* and *G. beggoides* are conspecific. Due to extensive expression of white scaling around the tornus on the ventral hindwing, *G. beggoides* belongs to the nominotypical subspecies and not to *Gorgythion begga pyralina* (Möschler, 1877) (type locality in Suriname). Therefore, we propose that *Gorgythion beggoides* Schaus, 1902 is a junior subjective synonym of *Gorgythion begga begga* (Prittwitz, 1868), not of *Gorgythion plautia* (Möschler, 1877).

Gorgythion guyanus Grishin, new species

http://zoobank.org/75920045-0024-46CF-83F0-F53371D32E59

(Figs. 34 part, 35, 36)

Definition and diagnosis. Genomic analysis of *Gorgythion* Godman & Salvin, 1896 (type species *Helias pyralina* Möschler, 1877) reveals that a specimen from Guyana (NVG-15043F10) (Figs. 34 magenta, 35) while being sister to *Gorgythion begga* (Prittwitz, 1868) (type locality in Brazil: Rio de Janeiro) (Fig. 34 blue), is not grouping closely with any of the described species (Fig. 34) and therefore is new. It exhibits COI barcode differences of 2.4% (16 bp) from *Gorgythion begga pyralina* (Möschler, 1877) (type locality in Suriname). This new species keys to E.36.1(a) in Evans (1953) and differs from its relatives in nearly unmarked dark-brown dorsal hindwing with convex outer margin, rounder than in *Gorgythion plautia* (Möschler, 1877) (type locality in Suriname) (Fig. 34 cyan), ventral hindwing without white area towards tornus, forewing not prominently truncate or produced at the apex, with developed markings and broad



Fig. 35. Holotype of Gorgythion guyanus sp. n. in dorsal (left) and ventral (right) views, data in text.



Fig. 36. Genitalia of Gorgythion guyanus sp. n. holotype in a) dorsal, b) posterolateral, c) left, and d) right lateral views.

pale-brown areas (Fig. 35); left valva broader at the base, and expansion of its ampulla curved inward, appearing truncate in lateral view (Fig. 36). Due to unknown phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly1313.36.6:C75T, aly1497.9.9:A87G, aly361.8.3:T111C, aly361.8.3:C126T, aly13198.6.3: G318C, aly1204.4.2:G54G (not A), aly1166.4.2:A30A (not C), aly1166.4.2:T42T (not C), aly770.15.7:A12A (not G), aly770.15.7:G30G (not A) and in COI barcode: T59C, T172T, A181G, T280T, T463C, T574C.

Barcode sequence of the holotype. Sample NVG-15043F10, GenBank OR837734, 658 base pairs:

Type material. Holotype: σ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 35, bears five labels: four white [GUYANA: ESSEQUIBO | Mt. Wokomung, 3500 ft. | XI ,1993; S. Fratello], [Genit. Vial No. | SRS-4628], [Allyn Museum | Acc. 1994-5], [DNA sample ID: | NVG-15043F10 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Gorgythion | guyanus Grishin].

Type locality. Guyana: Essequibo, Mt. Wokomung, elevation 3500 ft.

Etymology. The name is given for the country of the type locality. The name is a masculine adjective. **Distribution.** Currently known only for the holotype collected in Guyana.

Pardaleodes murcia (Plötz, 1883) is a species distinct from Pardaleodes incerta (Snellen, 1872)

Genomic analysis reveals that *Hesperia murcia* (Plötz, 1883) (type locality not specified, syntype sequenced as NVG-21116G06), currently treated as a subspecies of *Pardaleodes incerta* (Snellen, 1872) (type locality in Angola), is not monophyletic with it and is sister to the clade of *P. incerta* (Stoll, 1781) together with *Pardaleodes edipus* (Stoll, 1781) (type locality in South Africa) (Fig. 37), genetically differentiated from them with $F_{st}/G_{min}/COI$ barcode difference of 0.61/0.00/4.6% (30 bp) (*P. incerta*) and 0.64/0.00/5.0% (33 bp) (*P. edipus*). Therefore, we propose that *Pardaleodes murcia* (Plötz, 1883), **stat. rest.** is a species distinct from *Pardaleodes incerta* (Snellen, 1872). Sequencing a series of specimens



from additional localities in western Africa and comparing them with genomic sequences of *P. murcia* syntypes may help in determining the type locality of this species more precisely.

Pardaleodes pusiella Mabille, 1877 is a species distinct from Pardaleodes sator (Westwood, 1852)

Genomic analysis reveals that *Pardaleodes pusiella* Mabille, 1877 (type locality in Angola), currently regarded as a subspecies of *Pardaleodes sator* (Westwood, 1852) (type locality in Guinea), is genetically differentiated from it at the species level (Fig. 37): e.g., $F_{st}/G_{min}/COI$ barcode difference of 0.62/0.00/ 3.2% (21 bp). Therefore, we propose that *Pardaleodes pusiella* Mabille, 1877, **stat. rest.** is a species distinct from *Pardaleodes sator* (Westwood, 1852).

Semalea malawi Grishin, 2023 is confirmed as a species-level taxon

Genomic sequencing of Hesperiidae from the CAS collection revealed a second confirmed specimen of *Semalea malawi* Grishin, 2023 (type locality in Malawi, holotype sequenced as NVG-19043B12), which is also a male as the holotype but was collected in northeastern Tanzania (Tanga District, Usambara Mts., Amani Malaria Station, elevation 300 ft, 6-Jan-1970, M. E. Irwin & E. S. Ross leg., NVG-22108B02,



Fig. 38. Semalea malawi Grishin, 2023: a) the holotype and b) a specimen from Tanzania, data in text.



Fig. 39. Phylogenetic trees of selected *Semalea* 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: *S. rega* (violet), *S. vibius* (blue), and *S. malawi* (red). The sequence of SAMN18587799 is taken from the alignment provided in Kawahara et al. (2023).

CASENT 8568645) (Figs. 38b, 39). Compared to the holotype (Fig. 38a), it is yellower in the hue of paler scales (forewing patch, ventral overscaling, palpi, and cheeks), with a slightly shorter forewing orange patch, and has a small orange subapical spot on the forewing, more expressed on the ventral side (Fig. 39b). The COI barcode sequence of this specimen (GenBank <u>OR837735</u>) matches all 7 diagnostic characters given in the original description (Zhang et al. 2023b) but differs by 5 bp from the holotype. With this additional specimen, we carried out F_{st}/G_{min} test to obtain the values 0.34/0.000 (with *S. vibius* (Hewitson, 1878)) and 0.55/0.000 (with *S. rega* (Mabille, 1889)) confirming *S. malawi* as a species.

Limochores mystic nino Grishin, new subspecies

http://zoobank.org/E1AE8324-1FFE-4A14-916D-8BCC85B96105

(Figs. 40 part, 41)

Definition and diagnosis. Genomic sequencing of specimens from the southwesternmost population of *Limochores mystic* (W. H. Edwards, 1863) (type locality in USA: NY, Greene Co., Huner) reveals that they are sister to all other subspecies of *L. mystic* in the tree inferred from the nuclear genome (autosomes only) (Fig. 40a), although they fall among other *L. mystic* populations in the Z chromosome (not shown) and mitogenome trees (Fig. 40b), and their COI barcodes differ only due to variation. Therefore, this population is likely conspecific with *L. mystic*; however, being most divergent from all others, it represents a separate and new subspecies. In its duller look with more diffuse boundaries between brown ground color and yellow-orange spots, this subspecies is most similar to *Limochores mystic dacotah* (W.



Fig. 40. Phylogenetic trees of *Limochores mystic* (blue, with *L. mystic dacotah* in cyan and *L. mystic nino* ssp. n. in magenta) and *Limochores sonora* (black, with *L. sonora utahensis* in violet color) inferred from protein-coding regions of a) the nuclear (autosomes) and b) the mitochondrial genomes.



Fig. 41. Limochores mystic nino ssp. n. in dorsal (left) and ventral (right) views, data in text: a) holotype of and b) paratype Q.

H. Edwards, 1871) (type locality in USA: Colorado, Clear Creek Co.) and differs from it by the following characters. Males have reduced orange scaling, spots and bands are narrower, e.g., the orange on the dorsal hindwing is reduced to a band approximately the same width as the brown margin, and the band is separated from the orange discal cell by a brownish belt. Both sexes have darker ventral sides of wings. Due to extensive phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly499.49.4:G66C, aly848.2.19:T51C, aly838.7.2:C48T, aly838.7.2:T63C, aly1838.42.3:C34T.

Barcode sequence of the holotype. Sample NVG-22102C07, GenBank OR837736, 658 base pairs:

Type material. Holotype: σ deposited the California Academy of Sciences, San Francisco, CA, USA [CAS], illustrated in Fig. 41a, bears seven printed (text in italics handwritten) labels: six white [Circle Bar Draw at | Tillman Ranch, 7100' | Coconino Co. AZ], [9 June 1988 | collected by Kilian Roever], [COLLECTION OF | C. D. MacNeill], [Polites mystic | ssp. nov. | Det.C.D.MacNeill '98], [DNA sample ID: | NVG-22102C07 | c/o Nick V. Grishin], [{QR Code} CASENT | 8566979], and one red [HOLOTYPE σ | Limochores mystic | nino Grishin]. **Paratype:** 19 same data as the holotype (NVG-22102C08, CASENT 8566980, Fig. 41b) [CAS].

Type locality. USA: Arizona, Coconino Co., Circle Bar Draw at Tillman Ranch, 7100'.

Etymology. The name is formed from the name of the county of the type locality [Coco]*nino* and is a noun in apposition.

Distribution. Only known from central Arizona, USA. Populations in southwestern Colorado should be studied to determine their taxonomic identity.

Hesperia pahaska Leussler, 1938 populations in most of New Mexico and the White Mountains, Arizona are the nominal subspecies, not H. p. williamsi Lindsey, 1940

Genomic trees of *Hesperia pahaska* Leussler, 1938 (type locality in USA: Nebraska, Sioux Co.) reveal that a specimen from southeastern New Mexico (Lincoln Co., NVG-22057C03) and specimens from the White Mountains in Arizona are not in the same clade with *Hesperia pahaska williamsi* Lindsey, 1940 (type locality in USA: Arizona, Pima Co. Baboquivari Mts., holotype sequenced as NVG-15096B10), a subspecies they were usually attributed to, but are within specimens of the nominal *H. pahaska* (Fig. 42). Therefore, we deduce that populations in most of New Mexico and eastern Arizona are *H. pahaska pahaska*, while the specimens from southwestern New Mexico remain to be studied.

Hesperia pahaska hannawackeri Grishin, new subspecies

http://zoobank.org/FC3A2704-0C47-4FEC-8476-2947275E52FD

(Figs. 42 part, 43)

Definition and diagnosis. Populations of *Hesperia pahaska* Leussler, 1938 (type locality in USA: Nebraska, Sioux Co.) from southeastern Utah and southwestern Colorado are usually included in *Hesperia pahaska martini* MacNeill, 1964 (type locality USA: California, San Bernardino Co., 4.5 mi SE of Ivanpah). However, they form a distinct clade in the Z chromosome tree and possess a distinct mitochondrial genome haplotype (Fig. 42 red), representing distinct subspecies. This new subspecies differs from *H. p. martini* in better outlined and contrasting with fulvous ground color brown outer margins, smaller forewing subapical spots, and usually smaller ventral hindwing white spots; from the nominal *Hesperia pahaska* by less contrasting with the fulvous colors subapical and submarginal dorsal forewing spots, which are typically paler in *H. p. pahaska*, and usually more extensive fulvous areas penetrating fuscous margins in females on wings above (more similar to *H. p. williamsi* in this aspect) and



Fig. 42. Phylogenetic trees of selected *Hesperia* species, including *H. viridis* (green) and *H. pahaska* with its subspecies *H. p. pahaska* (violet), *H. p. hannawackeri* **ssp. n.** (red), *H. p. williamsi* (blue), and *H. p. martini* (cyan) inferred from protein-coding regions of **a**) the Z chromosome and **b**) the mitochondrial genome.



Fig. 43. Hesperia pahaska hannawackeri ssp. n. in dorsal (above) and ventral (below) views, data in text:a) holotype of NVG-20045F06 and b) paratype Q NVG-20045F05.

from *Hesperia pahaska williamsi* Lindsey, 1940 (type locality in USA: Arizona, Pima Co., Baboquivari Mts) by generally larger white ventral hindwing spots. Due to extensive phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly5021.3.8:C60T, aly7690.1.10:C45A, aly7690.1.10:C204T, aly4196.3.1:C333G, aly4196. 3.1:A415G and in COI barcode: T10C, T19C, G101A, 328C, T646C.

Barcode sequence of the holotype. Sample NVG-20045F06, GenBank OR837737, 658 base pairs:

Type material. Holotype: σ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 43a, bears four printed labels: two white [Pack Creek Day Use Area | La Sal Mountains | San Juan Co, UT | 2 June 2016 | Robb Hannawacker], [Pahaska Skipper | male | Hesperia pahaska], [DNA sample ID: | NVG-20045F06 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Hesperia pahaska | hannawackeri Grishin]. **Paratypes:** 5 $\sigma\sigma$ 29: USA: 19 Utah, San Juan Co., Poison Canyon, el. 8500', 5-Jun-2020, R. Hannawacker leg. (NVG-20045F05) (Fig. 43b); Colorado: Mesa Co.: $2\sigma\sigma$ Black Ridge Breaks, West Reef, 7050 ft, 24-25-May-2007, M. S. Fisher leg. (NVG-22055G08 & NVG-22055G09); and 1 σ BLM lands W of Gateway, Unaweep Seep Natural Area, 10-Sep-2017, Paul A. Opler and Evi M. Buckner-Opler leg. (PAO566); 1 σ Delta Co., 4.6-7.3 mi. SE of Austin, 6000-6600 ft, 12-Jun-1983, M. S. Fisher leg. (NVG-22055G10); 1 σ 19 Montrose Co., Gunnison Gorge NWA, Wave-Eagle Trail Loop, 6000-6300 ft, 1- and 3-Jun-2016, M. S. Fisher leg. (NVG-22055G12).

Type locality. USA: Utah, San Juan Co., La Sal Mountains, Pack Creek Picnic Area.

Etymology. The name honors Robb Hannawacker, the collector of the holotype and a female paratype from Utah. Robb is a dedicated Lepidopterist and the author of the book on the butterflies of southeastern Utah. He helped our lab tremendously with genomic studies of butterflies from his region (southeastern Utah) by collecting specimens and connecting us with others who can help further. The name is a masculine noun in the genitive case.

Distribution. Southeastern Utah and southwestern Colorado in the USA.

Pseudocopaeodes eunus ash Grishin, new subspecies

http://zoobank.org/9EB3AEE9-E29D-4684-9A7D-1EB65A9C6FB5

(Figs. 44 part, 45a)

Definition and diagnosis. Genomic sequencing of *Pseudocopaeodes eunus* (W. H. Edwards, 1881) (type locality USA: Kern Co., the bottoms of Kern River, near Bakersfield) populations reveals that specimens from the Ash Meadows area in southern Nevada are not monophyletic with *Pseudocopaeodes eunus alinea J.* Scott, 1981 (type locality in USA: California, San Bernardino Co., Afton Canyon) despite the similarity in being less marked than other populations, and form a distinct clade with genetic differentiation larger than for some other *P. eunus* subspecies (Fig. 44), e.g., their COI barcodes differ by 1.1% (7 bp). Therefore, the Ash Meadows population represents a new subspecies. This subspecies is most similar to *P. e. alinea* in appearance and differs from it in having less conspicuous and thinner



Fig. 44. Phylogenetic trees of *Pseudocopaeodes eunus* inferred from protein-coding regions of **a**) the nuclear genome (autosomes) and **b**) the mitochondrial genome. Different subspecies are shown in different colors: *P. e. obscurus* (violet), *P. e. flavus* (green), *P. e. eunus* (blue), *P. e. alinea* (cyan), and *P. e. ash* ssp. n. (red). One tree branch was truncated at dots.

stigma and by being paler and whiter on the ventral side of wings (Fig. 45a) instead of yellower in color. In particular, palpi beneath, cheeks, area by ventral forewing costa at the wing base, forewing apex, and hindwing overall are whiter (and wing venter redder) than in *P. e. alinea*, which is yellower (Fig. 45b). Dorsal hindwing by the apex is usually less dark, and dark scales by the costa are confined mostly to the base. Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly1022.2.1:G558A, aly1781.2.2:C384T, aly1781.2.2:G444A, aly4645.18. 2:C183T, aly4645.18.2:T213A and in COI barcode can be distinguished from other subspecies, except *Pseudocopaeodes eunus obscurus* Austin & J. Emmel, 1998 (type locality in USA: Nevada, Carson City): A214A, T220C, A484A, T514T, 604C.

Barcode sequence of the holotype. Sample NVG-21049E06, GenBank OR837738, 658 base pairs:

Type material. Holotype: σ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 45a, bears four labels, 1st handprinted others printed: three white [ASH MEADOWS | NYE CO. NEV. | 2 SEPT. 1989 | LEG:P.SAVAGE], [P Savage colln. | MGCL Acc. | 2006-15], [DNA sample ID: | NVG-21049E06 | c/o Nick V. Grishin], and one red [HOLOTYPE σ | Pseudocopaeodes | eunus ash Grishin]. **Paratypes:** 2 $\sigma\sigma$ 29 φ same locality as the holotype: 2 $\sigma\sigma$ 6-Sep-1987, P. Savage colln. (NVG-22076F04 and NVG-22076F05) [MGCL], 1 φ 7-Sep-1988, P. Savage leg. (NVG-21049E07) [MGCL], and 1 φ 12-Aug-1984 G. T. Austin leg. (NVG-20063G12, CSU_ENT1028906) [CSUC].



Fig. 45. Holotypes of *Pseudocopaeodes*: a) *P. eunus ash* ssp. n. (data in text) and b) *P. eunus alinea* NVG-19061F04 USA: CA, San Bernardino Co., Afton Canyon, 9-Sep-1965, Oakley Shields leg. [LACM] in dorsal (left) and ventral (right) views.

Type locality. USA: Nevada, Nye Co., Ash Meadows.

Etymology. The name is given for the type locality and the ashier appearance: paler, whiter, ventrally dusted with white compared to other subspecies. The name is treated as a masculine noun in apposition.

Distribution. Southern Nevada; known only from the Ash Meadows area.

Comments. First, we note that where *P. eunus* is double-brooded, the two broods differ in appearance. The first one produces darker specimens with broader dark-framed veins and more conspicuous two pale rays of the ventral hindwing, thus having a classical *P. eunus* appearance. The second brood produces paler specimens. Therefore, wing patterns within the broods should be compared between populations to reach meaningful conclusions. Second, we see that genetic differentiation between the two subspecies *Pseudocopaeodes eunus obscurus* Austin & J. Emmel, 1998 (type locality in USA: Nevada, Carson City) and *Pseudocopaeodes eunus flavus* Austin & J. Emmel, 1998 (type locality in USA: Nevada, Churchill Co.) is limited compared to others (Fig. 44), suggesting that they are not particularly distinct from each other, despite their phenotypic difference in wing patterns. Thus, wing patterns can differ with very few genetic changes. Third, we observe the genetic similarity between the lectotype of *Copaeodes wrightii* W. H. Edwards, 1882 (type locality USA: California, San Bernardino Co., nr. Victorville) currently treated as a junior subjective synonym of the nominal *P. eunus* and the type series of *Pseudocopaeodes eunus alinea* J. Scott, 1981 (type locality USA: California, San Bernardino Co., Afton Canyon) (Fig. 44). While additional analyses are required, it may be that *C. wrightii* is not a synonym of *P. e. eunus*, but instead is a valid subspecies and the same taxon as *P. e. alinea*, with the latter being its junior subjective synonym.

Pamphila milo W. H. Edwards, 1883 is a junior subjective synonym of Ochlodes agricola verus (W. H. Edwards, 1881)

The holotype of *Pamphila milo* W. H. Edwards, 1883 was stated to be from "Mt. Hood, Oregon", and later from Thurston Co., Washington, where this species is not known to occur (Pelham 2008; Pelham 2023). Genomic analysis of the holotype (NVG-15036C12) places it within specimens of *Ochlodes agricola verus* (W. H. Edwards, 1881) (type locality USA: California, Kern Co., Havilah) from Kern Co.,

California that include the lectotype of the latter (NVG-15096F09) (Fig. 46). Therefore, we propose that *Pamphila milo* W. H. Edwards, 1883 is a junior subjective synonym of *Ochlodes agricola verus* (W. H. Edwards, 1881), and not of *Ochlodes agricola nemorum* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) as currently treated. While sequencing of additional specimens of *Ochlodes agricola (Boisduval, 1852)* (type locality in USA: California, Marin Co.) for population analysis is needed for confident conclusions, our phylogenetic analysis tentatively suggests that the type locality of *P. milo* might have been in Kern Co., California, possibly even "Havilah", together with *O. a. verus*.



Hesperia amanda Plötz, 1883 is a nomen dubium

Suggested as a probable variation of *Hesperia ottoe* W. H. Edwards, 1866 (type locality in USA: Kansas) by Godman (1907) and placed as a junior subjective synonym of *Ochlodes sylvanoides napa* (W. H. Edwards, 1865) (type locality in USA: Colorado, Clear Creek Co.) by Evans (1955), *Hesperia amanda* Plötz, 1883 (type locality not stated and unknown) was described in an identification key from at least one male with a forewing length of 13 mm (Plötz 1883). Relevant parts of the key are translated and combined here as: "Upperside reddish-yellow. Forewing with a diagonal, wide, dark brown stigma that

starts broad in cell 1 and narrows in cell 3, and a brown longitudinal spot in cell 5. Forewing margin widely brown. Hindwing margin less wide, especially on the inner margin, narrow and pale brown. Hindwing underside reddish-yellow with a faded yellow band". Published on plate 180 (row i, images 4 and 5 from the left) illustration of *H. amanda* in Draudt (1921–1924) (Fig. 47) is, according to our reading of Evans (1955), paler than inspected by Evans copy of Plötz's original drawing t[afel]. 617. Indeed, Draudt's illustration agrees well with the original description and likely reflects the appearance of this species. In our opinion, *H. amanda* is conspecific neither with *H. ottoe* nor with *O. s. napa* largely because the stigma in *H. amanda*



extends into cell 3 (i.e., M_3 -CuA₁), forewing brown streak distad of the discal cell is confined to the cell 5 (i.e., M_1 - M_2), and the inner margin of dorsal hindwing is only narrowly brown, with a paler-brown outer margin by the tornus. These characters do not match the former two species. The stigma reaches into cell 3 in many Indo-Australian Hesperiidae, but we are not able to associate *H. amanda* with any species known to us. Therefore, we propose to treat this name as *nomen dubium*, pending further research.

Ochlodes napa (W. H. Edwards, 1865) and Ochlodes santacruza J. Scott, 1981 are species distinct from Ochlodes sylvanoides (Boisduval, 1852)

Genomic analysis of specimens identified as *Ochlodes sylvanoides* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) reveals their partitioning into three prominent clades genetically differentiated at the level typical for distinct species (Fig. 48 green, red with magenta, and blue). The first clade (Fig. 48 green) corresponds to populations from the Islands of the California coast: Santa Cruz Island and Santa Catalina Island. The senior name for these populations is *Ochlodes sylvanoides santacruza* J. Scott, 1981 (type locality USA: California, Santa Barbara Co., Santa Cruz Island). The second clade (Fig. 48 red and magenta, two clades in mitochondrial genome tree Fig. 48b, likely due to introgression) includes populations from the eastern part of the range (Colorado, southeastern Utah, and Arizona) and is currently represented by one name, *Ochlodes sylvanoides napa* (W. H. Edwards, 1865)



Fig. 48. Phylogenetic trees of selected *Ochlodes* species, including *O. santacruza* (green) and *O. napa* with its subspecies *O. n. napa* (red) and *O. n. kaibab* **ssp. n.** (magenta), and *O. sylvanoides* (blue) inferred from protein-coding regions of **a**) the nuclear (autosomes) and **b**) the mitochondrial genome.

(type locality in USA: Colorado, Clear Creek Co.). The third clade (Fig. 48 blue) comprises all other populations (Oregon, mainland California, Nevada) and includes the nominotypical subspecies *O. sylvanoides sylvanoides*. $F_{st}/G_{min}/COI$ barcode difference among these clades are: 0.43/0.003/2.1% (14 bp) (*O. s. sylvanoides* vs. *O. s. napa*), 0.33/0.01/1.7% (11 bp) (*O. s. sylvanoides* vs. *O. s. santacruza*), and 0.54/0.008/2.6% (17 bp) (*O. s. napa* vs. *O. s. santacruza*). Therefore, we propose that *Ochlodes napa* (W. H. Edwards, 1865), **stat. rest.** and *Ochlodes santacruza* J. Scott, 1981, **stat. nov.** are species distinct from *Ochlodes sylvanoides* (Boisduval, 1852) and form a new species-subspecies combination: *Ochlodes santacruza catalina* J. Emmel & T. Emmel, 1998, **comb. nov**.

Ochlodes napa kaibab Grishin, new subspecies

http://zoobank.org/0CF5A2B5-E6B9-460A-999F-500EFEFE3921

(Figs. 48 part, 49)

Definition and diagnosis. Genomic sequencing reveals that specimens of *Ochlodes napa* (W. H. Edwards, 1865), **stat. rest.** (type locality in USA: Colorado, Clear Creek Co.) from the southwestern part of the range are genetically differentiated from the rest (Fig. 48) with the COI barcode difference of 2.0% (13 bp). This difference is large because they possess mitochondrial genomes (and therefore COI barcodes) more similar to *Ochlodes sylvanoides* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) than to *O. napa* (Fig. 48b). Due to this genetic differentiation, these populations from Coconino Co. in Arizona represent a distinct taxon that currently does not have a name and therefore is new. We consider it to be a subspecies of *O. napa* because genetic differentiation in the nuclear genome is not prominent (Fig. 48a), and we are not aware of this new taxon being sympatric with *O. napa*. This new subspecies is characterized by a darker appearance, sharper edges of brown areas likely caused by reduced fulvous overscaling over the brown areas, especially near their edges (e.g., the brown spot distad of the discal cell on forewing), and submarginal spots in cells M_1-M_2 and M_2-M_3 are better separated from fulvous areas of the forewing. Its females tend to have more developed fulvous areas in the forewing



Fig. 49. *Ochlodes napa kaibab* **ssp. n.** in dorsal (above) and ventral (below) views, data in text: **a)** holotype of NVG-21113C07 and **b)** paratype **P** NVG-21113C06.

discal cell and wing bases above. Due to extensive phenotypic variation, definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly1500.7.2:A162T, aly1500.7.2:T170A, aly3598.15.2:G447A, aly3598.15.2:C459T, aly378.20.4:A390G and in COI barcode: A217A, A256C, T439C, T505C, T583T, T616C.

Barcode sequence of the holotype. Sample NVG-21113C07, GenBank OR837739, 658 base pairs:

Type material. Holotype: σ deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA [MGCL], illustrated in Fig. 49a, bears four printed labels: three white [South Canyon Spring | Kaibab Plateau, AZ | 30 July 2021 | Robb Hannawacker], [Woodland Skipper | Ochlodes sylvanoides | male], [DNA sample ID: | NVG-21113C07 | c/o Nick V. Grishin], and one red [HOLOTYPE σ Ochlodes napa | kaibab Grishin]. **Paratype:** 19 same data as the holotype (NVG-21113C06) (Fig. 49b).

Type locality. USA: Arizona, Coconino Co., Kaibab Plateau, South Canyon Spring.

Etymology. The name is a noun in apposition taken from the type locality of this species.

Distribution. Northern Arizona, USA. Populations in southeastern Utah are the nominal subspecies, and those in southwestern Utah should be studied to determine their identity.

Lectotype designation for *Hesperia erratica* Plötz, 1883

Hesperia erratica Plötz, 1883 (type locality in Guatemala), currently a junior subjective synonym of *Lon zabulon* (Boisduval & Le Conte, [1837]) (type locality in North America, possibly USA: Georgia), was described from an unstated number of specimens from Guatemala (Plötz 1883). One specimen, shown in Fig. 50a, is curated in the MFNB collection as a syntype of *H. erratica*. We determine that this specimen is indeed a syntype. First, it agrees with the original description, which we translate as: "Yellow on both sides, all wings dark at the base and outer margin. Hindwing underside straw-yellow, at the base palebrown with yellow spot, cell 1b is overscaled with pale-brown. Three such spots are in an oblique line in cell 1c, 2, and 3, one spot in the discal cell near the brown base, and a patch in the corner of cell 6. In cell 7 at the apex, there is a small brown spot, like the previous ones, and in cell 6, the narrow uneven border begins, ending at vein 1b. Upperside dark-yellow, forewing with brown-powdered [refers to the following list], the apical spots ending at the costal margin, narrow in cells 4 and 5, and a dark brown cross vein. Hindwing with a brown costal margin, narrow in cells 4 and 5, then rapidly widening outer border, and a broad inner margin. Fringes of the forewing light brown, and of the hindwing yellow." The description does not mention a diffuse discal spot in hindwing cell 5 (i.e., M₁-M₂) beneath, present in the syntype, but all its other characters are in very good agreement with the description.

Second, according to its labels, this candidate syntype specimen from the Weymer collection was seen by Plötz, who identified it at the time as "zabulon Bd." ("best[immt]. v[on]. Plötz"). Subsequently, Plötz likely changed his mind because, in his publication with the key describing *H. erratica*, he placed it after his "zabulon", which was actually *Lon hobomok* (T. Harris, 1862) (type locality in USA: Massachusetts) (Plötz 1883). This is also corroborated by the opinion of Godman (1907), who inspected the original Plötz drawings of "zabulon" (t[afel]. 655) "from Buffalo" and concluded that they "represent *A. hobomok*." Thus, Plötz's "zabulon" was *L. hobomok*, and Plötz probably proposed the name *erratica* for the true *L. zabulon*, represented by this specimen, after he realized that two species were involved (see specimen labels below). Third, the specimen bears a label with "Erratica Plötz i 1." in Weymer's handwriting, meaning that this name was given to Weymer by Plötz before publication of the name (therefore "i. 1.", for "in litteris"). Fourth, Godman (1907), who inspected Plötz original drawing of *H. erratica* (t[afel]. 656), identified it as male "Atrytone zabulon" in accord with the identity of the syntype.

We were not able to find other syntypes, and to stabilize nomenclature, N.V.G. hereby designates the sole syntype in the MFNB collection, a male with the following seven printed (but 2nd, 3rd, and 4th

handwritten) labels, the 1st red and others white: [Typus], [zabulon Bd. | $n^{\underline{0}}$ 92 best. v. Plötz | ist mogl and. Art], [Erratica Plötz i l. | taf. 656. Guatemala], [Erratica Pltz | i.l. | Guatemala], [Coll. Weymer], [{QR Code} http://coll.mfn-berlin.de/u/ | 44a0bc], and [DNA sample ID: | NVG-18052B03 | c/o Nick V. Grishin] as the **lectotype** of *Hesperia erratica* Plötz, 1883. The last two lines on the 2nd label are abbreviated and should read "n^{$\underline{0}$} 92 bestimmt von Plötz | ist möglicherweise andere Art", which we translate as "n^{$\underline{0}$} 92 identified by Plötz | is possibly a different species", an indication that Plötz would change his opinion about the determination of this specimen as "zabulon." The COI barcode sequence of *H. erratica* lectotype, sample NVG-18052B03, GenBank <u>OR837740</u>, 658 base pairs, is:



Fig. 50. Primary type specimens of *Lon* in dorsal (left) and ventral (right) views, data in text: a) lectotype of *Hesperia erratica*, which is a specimen of *Lon zabulon*; b) holotype of *Lon co* sp. n.; and c) holotype of *Lon ma* sp. n. Larger scale bar refers to specimens and smaller scale bar refers to labels, which are reduced in half compared to specimens.

Hesperia erratica Plötz, 1883 (type locality in the USA, not Guatemala) is confirmed as a junior subjective synonym of *Lon zabulon* (Boisduval & Le Conte, [1837])

Genomic tree of specimens identified as *Lon zabulon* (Boisduval & Le Conte, [1837]) (type locality in North America, possibly USA: Georgia) reveals that the lectotype of *Hesperia erratica* Plötz, 1883 (type locality in Guatemala, sequenced as NVG-18052B03, Fig. 50a) is in the clade with specimens from the USA (Fig. 51 violet) and not with specimens from Mexico and Central America, including El Salvador and Costa Rica (Fig. 51 blue, a species different from *L. zabulon*, see below). Therefore, we confirm *H. erratica* as a junior subjective synonym of *L. zabulon* but propose that its type locality given as 'Guatemala' in the original description and on the lectotype labels was erroneous and should be corrected to the USA. Sequencing of *L. zabulon* specimens across the range will allow us to determine the type locality more precisely.

Even from the wing patterns of the lectotype, as also hinted in the original description of H. *erratica*, it is more likely to be from the United States than Guatemala. First, orange-yellow on the dorsal forewing is more extensive than in Central American specimens, i.e., the triplet of subapical spots is connected to the doublet of submarginal spots (Fig. 50a), while in Central American specimens, they are typically well-separated from each other (Fig. 50b). This character is also described for H. *erratica* by Plötz (1883) as a "long spot in cells 4 and 5", meaning that there is a yellow background (i.e., "upperside dark-yellow") that is formed by subapical and submarginal yellow spots together with the rest of the wing (except the marginal brown border) and there is a separate brown spot on this background. Instead, specimens from Central America would be described as having 3 yellow subapical and 2 submarginal spots on a brown background by the apex. Second, the ventral hindwing brown border by the outer margin is described by Plötz as "narrow uneven" (Fig. 50a), which is more typical for specimens from the US. This border is usually broader and more even in Central American specimens (Fig. 50b).

Looking more into the discrepancy about the type locality, we find that only two species of Hesperiidae proposed by Plötz have the type locality listed as "Guatemala." In addition to H. erratica, the second one is Achlvodes gorgona Plötz, 1884, a junior subjective synonym of Gesta invisus (Butler & H. Druce, 1872). A possible syntype of A. gorgona is from the Möschler collection (now in MFNB). It was collected in Guatemala in 1884 according to its dedicated locality/collector/date green label, which is likely correct. The type(s) of *H. erratica* would have been from an earlier collection event because the name was published in 1883. Moreover, unlike A. gorgona, it lacks a dedicated locality label. Therefore, it is unclear whether the type locality in Guatemala is accurate for *H. erratica*. Localities for the specimens collected in the US were known to be incorrect or missing. At least two mistakes have been documented. First, Goniloba parumpunctata Herrich-Schäffer, 1869 (type locality not stated in the original description, but later assumed to be in South America, possibly Venezuela), which is a junior subjective synonym of *Lerema accius* (J. E. Smith, 1797) (type locality in USA: Georgia) had the locality of the lectotype (male) and at least one female paralectotype deduced to be in eastern US by genomic sequence comparison (Zhang et al. 2023a). Second, Pyrgus argina Plötz, 1884 (type locality given as "Brisbane" [Australia]), which is a junior subjective synonym of Amblyscirtes hegon (Scudder, 1863) (type locality in USA: New Hampshire, White Mountains), is only known from the USA (Evans 1949).



Fig. 51. Phylogenetic trees of selected *Lon* species inferred from protein-coding regions of **a**) the nuclear genome (autosomes), **b**) the Z chromosome, and **c**) the mitochondrial genome: *L. zabulon* (violet), *L. co* (blue), and *L. ma* (red). The sequence of SAMN18587728 is taken from the alignment provided in Kawahara et al. (2023).

Lon co Grishin, new species

http://zoobank.org/AA859D18-CFC6-47F8-9032-AB5B6D79EB69

(Figs. 50b, 51 part, 52)

Definition and diagnosis. Genomic trees of specimens identified as *Lon zabulon* (Boisduval & Le Conte, [1837]) (type locality in North America, possibly USA: Georgia) reveal their partitioning into three clades: from the USA, which is *L. zabulon* in accord with its phenotype and the type locality, and two others that do not have names (Fig. 51). One of these clades consists of specimens from Mexico and Central America (Fig. 51 blue) and differs from *L. zabulon* by $F_{st}/G_{min}/COI$ barcode of 0.49/0.01/3% (20 bp) thus representing a new species. This species differs from *L. zabulon* in reduced orange-yellow areas on wings, e.g., broader brown borders and a smaller, disconnected triplet of subapical spots and a doublet of submarginal spots on forewing; ventral hindwing with broader and more even outer border and larger spots (Fig. 50b); the process of aedeagus is more robust (Fig. 52a, e–j). Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly596.8.5:A189G, aly596.8.5:A192T, aly806.32.1:T876C, aly806.32.1:A1101G, aly1097.21.1:G46A and in COI barcode: T82C, G101A, T292C, C376T, T457C, T478C.

Barcode sequence of the holotype. Sample NVG-18115B03, GenBank OR837741, 658 base pairs:

Type material. Holotype: of deposited the National Museum of Natural History, Washington, DC, USA



Fig. 52. Genitalia of *Lon co* **sp. n.** paratypes, data in text: **a–b**) NVG-22056F05 complete genital capsule (right valva tilted ventrad) and **c–p**) NVG-22056E08 partly disassembled: **c**) left valva, **c**) right valva, **e–j**) aedeagus, **k**) tegumen and uncus, **l**) genital ring (uncus, tegumen, vinculum, and saccus rotated to align with the pane of the ring), **m–p**) juxta. Views: dorsal (a, e, m), left lateral (b, f, n), ventral (g, k, o), right ventrolateral (h), right lateral (c, d, i), and posterior (j, l, p). Directions: posterior on the right (a, b, e–i, m–o), posterior on the left (c, d), posterior on top (k), dorsal on top (b, c, d, f, j, l, n, p), ventral on top (i).

[USNM], illustrated in Fig. 50b, bears six printed labels: five white [MEX:Guerrero, | 5–7 km NW] Taxco, IX-14-82 | 1850–1900 m], [J. A. Powell | J. A. Chemsak | collectors], [Poanes zabulon (Boisduval & Le Conte) | o | det. J. M. Burns 1992], [DNA sample ID: | NVG-18115B03 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01531551], and one red [HOLOTYPE or | Lon co | Grishin]. Paratypes: 800: Mexico: 10 Tamaulipas, 11 km NW Gomez Farias, 6 km W Rancho Cielo, el. 5200-5500 ft, 9-Jul-1965, genitalia vial NVG231115-03 (NVG-22056F05) [TMMC]; 13 Veracruz, 10 km W Coscomatepec, el. 1800 m, 12-Aug-1987, Brown & Powell leg, genitalia vial J. M. Burns X-2953 (NVG-19068D12, USNMENT 01559668) [USNM]; 1^{or} Puebla, 6 mi N Chapulco, el. 7000', 4-Oct-1975, J. Powell, T. Eichlin & T. Friedlander leg. (NVG-18115B02, USNMENT 01531550) [USNM]; Oaxaca, 5-10 mi N of Oaxaca, el. 6000-7000 ft, J. Kemner leg.: 13 22-Aug-1992 (NVG-18115B04, USNMENT 01531552) [USNM] and 1 of 30-Aug-1989, genitalia vial NVG231115-02 (NVG-22056E08) [TMMC]; 1 of Chiapas, 12 km S of Las Casas, 26-28-Mar-1959, T. C. Emmel leg. (NVG-18115B05, USNMENT 01531553) [USNM]; 1or El Salvador, 2 mi down from Cerro Verde summit, 20-Aug-1972, C. F. & S. Hevel leg. (NVG-18115B06, USNMENT 01531554) [USNM]; 1d Costa Rica, Puntarenas Prov., Monteverde, el. 1300 m, 18-May-1985, J. A. Chemsak leg. (NVG-18115B07, USNMENT 01531555) [USNM]. **Type locality.** Mexico: Guerrero, 5–7 km NW of Taxco.

Etymology. The name is the last syllable of the country name of the type locality: [Mexi]*co*. The name is a noun in apposition.

Distribution. Mexico to Costa Rica.

Lon ma Grishin, new species

http://zoobank.org/74F97D16-BF2C-414A-B090-BEDAEED53343

(Figs. 50c, 51 part)

Definition and diagnosis. Genomic trees of specimens identified as *Lon zabulon* (Boisduval & Le Conte, [1837]) (type locality in North America, possibly USA: Georgia) reveal their partitioning into three clades: from the USA, which is *L. zabulon* in accord with its phenotype and the type locality, and two others that do not have names (Fig. 51). One of these clades (Fig. 51 blue) is described as a new species above. The second clade consists of specimens from Panama (Fig. 51 red) and differs from *L. zabulon* by $F_{st}/G_{min}/COI$ barcode of 0.50/0.009/2.3% (15 bp) and from *L. co* **sp. n.** by 0.47/0.008/3.2% (21 bp), thus representing a new species. This species differs from *L. zabulon* in being brighter colored and more orange; the orange-yellow patch on dorsal hindwing smaller, more like a patch than the entire hindwing being orange with brown borders, brown border wider; beneath brown spots larger; and from *L. co* **sp. n.** in more extensive orange-yellow areas on the forewing, e.g., submarginal and subapical forewing spots larger, on ventral side subapical triplet of spots more orange, like submarginal doublet, not yellower than it; and the absence of pale ray along dorsal hindwing 1b vein that is usually expressed in *L. co* **sp. n.** and *L. zabulon*. Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly525.115.3:G328A, aly2336.10.2:C108T, aly2336.10.2:G116A, aly923.19.4:C393T and in COI barcode: T79C, T169C, T206C, A349G, A577G.

Barcode sequence of the holotype. Sample NVG-18115B09, GenBank OR837742, 658 base pairs:

Type material. Holotype: σ deposited in the National Museum of Natural History, Washington, DC, USA [USNM], illustrated in Fig. 50c, bears four printed (date handwritten) labels: three white [PANAMA: Chiriqui | Volcan Baru 1800 m | 5 Dec.'76 | leg. S. S. Nicolay], [DNA sample ID: | NVG-18115B09 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01531557], and one red [HOLOTYPE σ | Lon ma | Grishin]. **Paratype:** 1 σ Panama, Chiriqui, Volcan Baru, el. 1759 m, GPS 8.683, -82.500, 14-Feb-1981, G. B. Small leg. (NVG-18115B08, USNMENT 01531556) [USNM].

Type locality. Panama: Chiriquí, Volcán Barú, elevation ca. 1800 m.

Etymology. The name is the last syllable of the country name of the type locality: [Pana]*ma*. The name is a noun in apposition.

Distribution. Currently known only from Chiriquí, Panama.

Neotype designation for *Cobalus vitellina* Herrich-Schäffer, 1869

Cobalus vitellina Herrich-Schäffer, 1869 was described within the identification key from an unstated number of specimens without locality data (Herrich-Schäffer 1869). The description was rather general and can apply to several species. Our translation from German of relevant segments assembled from the Herrich-Schäffer key is: "forewing cell 3 with a pale spot before its middle, discal cell unmarked; hindwing above with three yellow spots in cells 3–5, on the underside with a continuous yellow band." Primary types for several names proposed in the same work are curated in MFNB. These types came from the Herrich-Schäffer collection (label "Coll. H.—Sch.") through the Staudinger collection (label "Coll. Staudinger") and frequently bear identification labels in Herrich-Schäffer handwriting. To confidently infer the taxonomic identity of *C. vitellina*, we searched for such specimens in MFNB that agreed with the original description. We were not able to find syntypes of *C. vitellina* and turned to additional resources, such as publications and specimens collected around the time of *C. vitellina* description identified as this species or agreeing with the original description.

According to Godman (1907), Plötz illustrated "nearly all" species described by Herrich-Schäffer. While these drawings are not located to this day, Godman found specimens in his collection that, in his opinion, matched each drawing closely and obtained drawing copies of species he could not identify (1907). While there is no certainty that Plötz illustrated Herrich-Schäffer syntypes and not some other specimens determined by him or by Herrich-Schäffer to be these species, we use Plötz's (1883) description of C. vitellina, which he placed in the genus Hesperia Fabricius, 1793 (type species Papilio comma Linnaeus, 1758), to learn more about this taxon. Plötz's description of his drawings (rather than actual specimens) is more detailed than Herrich-Schäffer's and gives "Mexico" as the locality. We translate the description from German as: "Ventral side red-brown, forewing with basal half black, hindwing infused with rust-red, past the middle with a distorted rust-yellow band, which in cell 1c projects a ray towards the fringe. Dorsal side brown, forewing with deep red-yellow spots in cells 1–3 and 6-8; a small dot in cell 4. Hindwing with 3 yellow spots in cells 3-5 and yellow fringes. Antenna half as long as the forewing." Both this description and Godman's (1907) assessment, which noted that Plötz illustrated both a male and female from Mexico, agree with H. vitellina being either conspecific with or closely related to Lon melane (W. H. Edwards, 1869) (type locality in USA: California). Moreover, Draudt (1921–1924) frequently used (inferior) copies of Plötz's drawings as illustrations (Nakahara et al. 2022), and his figures of *melane* (plate 182e) might be copied from Plötz's *H. vitellina*, which Draudt synonymized with L. melane. The dorsal side shows a female, which is either an atypical specimen or not this species (instead reminding of *Buzyges rolla* (Mabille, 1883)) because the spots in cells M₃-CuA₁ and CuA₁-CuA₂ are nearly aligned with each other (in Lon species, these two spots do not overlap in most specimens), unless this is an imperfection of the reproduction from the original. The ventral side is of a male and is identifiable as L. melane or its close relative.

Furthermore, we found two specimens of interest in MFNB. The first specimen (NVG-21116G04), from the Möschler collection collected in Mexico in 1876 and identified by Möschler as "*vitellina*", agrees with all characters of this taxon presented above, except that the three spots on the dorsal hindwing are barely visible. This specimen cannot be a syntype because it was collected after the description of *C. vitellina*. The second specimen (NVG-22091C05) is from Herrich-Schäffer's collection, also from Mexico, and bears the identification label "marmorosa HS" in Herrich-Schäffer's handwriting. This specimen is one of those Godman (1900) mentioned within his treatment of "*Atrytone melane*" and identified as such. It is probably not a syntype of *C. vitellina* either because it possesses four (or even five), and not three, as per the original description, yellow spots on the dorsal hindwing. This is a boldly patterned specimen with a very wide ventral hindwing orange-yellow band occupying nearly a third of the wing area, and it is possible that Herrich-Schäffer viewed it as a new species that he planned to call



Fig. 53. Neotype of Cobalus vitellina Herrich-Schäffer, 1869 in dorsal (left) and ventral (right) views, data in text.

"marmorosa", a name that was never published. Nevertheless, both specimens (NVG-21116G04 and NVG-22091C05) fall within the current concept of "*Paratrytone melane vitellina*" as outlined by Evans (1955) and have not been questioned since (Mielke 2005).

Not finding syntypes, we proceeded with the neotype designation because there was an exceptional need to clarify both the taxonomic identity and the type locality of *C. vitellina*. Although the name has been consistently applied to the Mexican subspecies of *L. melane*, the potential for destabilization of nomenclature arises due to the existence of additional species in this group in Mexico and Central America (see below) unless the name *C. vitellina* is objectively defined by the neotype that also provides details about the type locality. A number of Hesperiidae species from Mexico described in the second half of the 19th century were likely based on specimens from Oaxaca, possibly collected by Deppe in 1824–1829. Therefore, we selected a neotype from Oaxaca. Hereby, N.V.G. designates a specimen in USNM illustrated in Fig. 53 (DNA sample NVG-18115F05) as the **neotype** of *Cobalus vitellina* Herrich-Schäffer, 1869. This neotype corroborates the current application of the name for a relative of *L. melane* from Mexico, as stated by Plötz (1883) and Godman (1907), supported by Evans (1955), and followed since in all literature (Mielke 2005).

This neotype satisfies all requirements set forth by the ICZN Article 75.3, namely: 75.3.1. It is designated to clarify the taxonomic identity of Cobalus vitellina Herrich-Schäffer, 1869, which is necessary because additional species are present among its close relatives, and to define the type locality that was not stated in the original description; **75.3.2.** The characters to differentiate this taxon from others were given in the original description (Herrich-Schäffer 1869), further elaborated by Plötz (1883). We regard them as follows: forewing brown with orange yellow spots in cells R₃-R₄, R₄-R₅, R₅-M₁, M₃-CuA₁, CuA_1 - CuA_2 , and CuA_2 -1A+2A, and a dot in cell M₂-M₃, discal cell unmarked; forewing beneath with nearly black basal half; hindwing above brown with three yellow spots in cells M₁-M₂, M₂-M₃, and M₃-CuA₁; hindwing beneath rust-colored with a continuous orange-yellow band; antenna about half of the forewing in length; 75.3.3. The neotype specimen is a male bearing three labels: [MEXICO: OAXACA | c. 3 mi. E La | Trinidad, 8500 ft | 3-VIII-1992 | J. Kemner], [DNA sample ID: | NVG-18115F05 | c/o Nick V. Grishin], [USNMENT | {QR Code} | 01531599] and illustrated in Fig. 53; the neotype has a tear along SC vein from costa on the right forewing; 75.3.4. We carefully searched for syntypes of C. vitellina in the MFNB collection (see above) because most of the Herrich-Schäffer Hesperiidae types are in this collection, and a study by Häuser et al. (2003) did not locate the syntypes in Stuttgart. We also checked the ANSP collection, where several Herrich-Schäffer types of Caribbean taxa are curated. We failed to find syntypes of C. vitellina among Hesperiidae holdings in these collections and, therefore, believe that they were lost; **75.3.5.** The neotype closely agrees with the original description of *C. vitellina* in all characters, as evidenced by comparing the neotype illustrated in Fig. 53 with the characters for this taxon given in the original description (Herrich-Schäffer 1869) and listed above (75.3.2.); 75.3.6. The neotype is from Mexico: Oaxaca, ca. 3 mi E of La Trinidad, 8500 ft, and the type locality was not specified in the original description but was stated as "Mexico" by Plötz (1883); 75.3.7. The neotype is in

the National Museum of Natural History, Washington, DC, USA (USNM). The COI barcode sequence of *C. vitellina* neotype, sample NVG-18115F05, GenBank <u>OR837743</u>, 658 base pairs, is:



Fig. 54. Phylogenetic trees of selected *Lon* species inferred from protein-coding regions of **a**) the nuclear genome (autosomes), **b**) the Z chromosome, and **c**) the mitochondrial genome. Different taxa are shown in different colors: *L. melane* (blue, with *L. melane sur* ssp. n. in cyan), *L. vitellina* (violet), *L. chia* sp. n. (red), and *L. poa* (green). The names of new taxa by corresponding tree branches are highlighted in yellow.

Lon vitellina (Herrich-Schäffer, 1869) and *Lon poa* (Evans, 1955) are species distinct from *Lon melane* (W. H. Edwards, 1869)

Genomic trees reveal that *Cobalus vitellina* Herrich-Schäffer, 1869 (type locality in Mexico: Oaxaca) and *Paratrytone melane poa* Evans, 1955 (type locality Costa Rica: Mount Poás), currently treated as subspecies of *Lon melane* (W. H. Edwards, 1869) (type locality in USA: California, likely San Francisco Bay area), are genetically differentiated from it at the species level (Fig. 54), e.g., $F_{st}/G_{min}/COI$ barcode difference from *L. melane* of 0.57/0.002/3.2% (21 bp) for *C. vitellina* and 0.66/0.001/2.3% (15 bp) for *P. melane poa*. Therefore, we propose that *Lon vitellina* (Herrich-Schäffer, 1869), **stat. rest.** and *Lon poa* (Evans, 1955), **stat. nov.** are species distinct from *Lon melane* (W. H. Edwards, 1869).

Lon melane sur Grishin, new subspecies

http://zoobank.org/A41D2EC4-85A8-4728-AD8E-233BF09A90DB

(Figs. 54 part, 55)

Definition and diagnosis. Genomic sequencing of the two specimens from Baja California Sur, Mexico, identified as a possible subspecies or a distinct geographical segregate of "*Paratrytone melane*" in previous works (Powell 1958; MacNeill 1962; Brown et al. 1992) reveals that they are indeed closely related to *Lon melane* (W. H. Edwards, 1869) (type locality in USA: California, likely San Francisco Bay area) (Fig. 54): e.g., their COI barcodes differ by 0.3–0.6% (2–4 bp), and, therefore, we consider them to be conspecific with it. However, the BCS specimens differ from the nominotypical *L. melane* in reduced fulvous overscaling at wing bases above, smaller orange spots on the forewing, more diffuse and brownish instead of orange dorsal hindwing spots, and weakly spotted more uniformly colored ventral hindwing. Therefore, they represent a distinct subspecies, which is new. A more detailed description of this subspecies was given by MacNeill (1962: 110–111), who called it "*Paratrytone melane* subsp." without proposing a formal name. Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly770.31.1:A393C, aly3721.1.4:G45A, aly93.14. 4:C408T, aly322.23.3:A87G, aly65.5.1:T199C and in COI barcode: T56C, T379A, T418C, T530C, C646C.



Fig. 55. Holotype of Lon melane sur ssp. n. in dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype. Sample NVG-22101H10, GenBank OR837744, 658 base pairs:

Type material. Holotype: σ deposited in the California Academy of Sciences, San Francisco, CA, USA [CAS], illustrated in Fig. 55, bears eight labels: seven white [La Laguna, | Sierra Laguna, | L.Cal.X-14-41], [melane Edw. | Det. by | F H Rindge], [Ross & Bohart | Collectors], [σ], [melane subspecies], [DNA sample ID: | NVG-22101H10 | c/o Nick V. Grishin], [{QR Code} CASENT | 8566940], and one red [HOLOTYPE σ | Lon melane | sur Grishin]. **Paratype:** 1 σ same data as the holotype (NVG-22101H09, CASENT 8566939).

Type locality. Mexico: Baja California Sur, Sierra de La Laguna.

Etymology. The name, a masculine noun in apposition, is the last word in the type locality state name, also meaning that this is the southernmost subspecies of *L. melane*.

Distribution. Mountains of the Cape region in Baja California Sur, Mexico.

Lon chia Grishin, new species

http://zoobank.org/E4498D7B-4A5E-4411-9CB0-E336AA04311F

(Figs. 54 part, 56)

Definition and diagnosis. The genomic tree reveals that specimens identified as *Lon poa* (Evans, 1955) (type locality Costa Rica: Mount Poás), **stat. nov.** partition into two clades (Fig. 54). One clade includes specimens from Costa Rica and Panama, being the true *L. poa* by locality and phenotype. The other clade is genetically differentiated from the first one with $F_{st}/G_{min}/COI$ barcode difference of 0.41/0.004/0.9% (6 bp) and represents a new species. This species keys to "*Paratrytone melane poa*" M.23.1(c) in Evans (1955) and is distinguished from the true *L. poa* by less extensive yellow overscaling on the ventral side of wings, in particular, on the hindwing; this overscaling is whiter, and the ground color in redder and browner than yellower. As a result, there is less contrast between the darker inner half and subapical half of the ventral forewing, which is paler in the apical half and contrasting dark brown towards the inner margin in *L. poa*. Definitive identification is provided by DNA, and a combination of the following characters is diagnostic in the nuclear genome: aly3177.11.6:A36C, aly3177.11.6:A39G, aly128.24.1: C189T, aly128.24.1:A235C, aly318.14.6:G672A and in COI barcode: T4C, T346C, T505C, A550A, 586T.

Barcode sequence of the holotype. Sample NVG-22105H05, GenBank OR837745, 658 base pairs:



Fig. 56. Holotype of Lon chia sp. n. in dorsal (left) and ventral (right) views, data in text.

Type material. Holotype: σ deposited in the California Academy of Sciences, San Francisco, CA, USA [CAS], illustrated in Fig. 56, bears five labels, the first two handwritten, others printed: four white [Rancho Belen, Chis. | Mex. IV-17-69 | Robert Wind], [P. m. poa], [DNA sample ID: | NVG-22105H05 | c/o Nick V. Grishin], [{QR Code} CASENT | 8568431], and one red [HOLOTYPE σ | Lon chia | Grishin]. **Paratypes:** 5 $\sigma\sigma$ 1 \circ : Mexico, Chiapas: 1 σ Comitan, Laguna Chamula, el. 7100 ft, 13-May-1987, C. J. Durden leg. (NVG-22056D01) [TMMC]; San Cristobal, La Almolonga, ca. 7500 ft: 1 σ 3-May-1988, J. Kemner leg. (NVG-18115F06, USNMEND 01531600) [USNM]; 1 σ 9-Jul-1988, C. J. Durden leg. (NVG-20062F09) [TMMC]; 1 σ 5-Jul-1992, J. Kemner & A. Vasquez leg. (NVG-18115F08) [USNM]; 1 σ <u>Guatemala</u>, Quiche department, above Chichicastenango, 11-Jan-1990, C. J. Durden leg. (NVG-22056C06) [TMMC]; and 1 \circ <u>El Salvador</u>, 2 mi down from Cerro Verde summit, 20-Aug-1972, G. F. & S. Hevel leg. (NVG-18115F09, USNMENT 01531603) [USNM].

Type locality. Mexico: Chiapas, ca. 20 km S of San Cristóbal, Rancho Belén.

Etymology. Like *poa* formed from "Mt. Poas", the name *chia* is formed from Chiapas, for the type locality of this species. The name is a noun in apposition.

Distribution. Confirmed from Mexico: Chiapas, Guatemala, and El Salvador.

Lerodea? rupilius Schaus, 1913 is a subspecies of *Atrytonopsis edwardsi* W. Barnes & McDunnough, 1916

Lerodea? *rupilius* Schaus, 1913 (type locality given as "Guapiles" [Costa Rica] in the original description) was regarded as *nomen dubium* by Burns (1983), who concluded that its syntype in USNM was "phony": it differed in some aspects from the original illustration in Schaus (1913) and was labeled from "Guadljara



Fig. 57. Phylogenetic trees of *Atrytonopsis edwardsi* (blue, with *A. e. rupilius* in red) and *Atrytonopsis ovinia* (violet) inferred from protein-coding regions of **a**) the Z chromosome and **b**) the mitochondrial genome.

Mex" and not from "Guapiles." However, our inspection of the syntype reveals that it agrees closely with the original description and bears labels in a style typical of all syntypes by Schaus. One of them is the identification label in his handwriting with this species' name and the word "type." It is difficult for us to imagine that this specimen is not a true syntype, provided that all other species Schaus proposed based on USNM material have extant syntypes in the collection. However, illustrations of specimens were not known to be particularly accurate, and we hypothesize that there was a mistake in stating the locality of a syntype in the original description: Guadljara was erroneously replaced with Guapiles in the publication (Schaus 1913), maybe because both words start with "Gua". Therefore, we regard this female "type," possibly the only specimen Schaus based his description of *L. rupilius* on, as a true syntype. To stabilize nomenclature, N.V.G. hereby designates this specimen in the USNM collection bearing the following four labels, 3rd red and others white: [Guadljara | Mex], [Lerodea ? | rupilius | type Schs], [Type | No. 16817 | U.S.N.M.], [GENITALIA NO. | X-1060 | J. M. Burns 1981] as the **lectotype** of *Lerodea* ? *rupilius* Schaus, 1913.

Morphologically, Burns (1983) identified the lectotype of *L. rupilius* as *Atrytonopsis edwardsi* W. Barnes & McDunnough, 1916 (type locality in USA: Arizona, Pima Co.), therefore, *L. rupilius* is not a junior subjective synonym of *Atrytonopsis ovinia zaovinia* Dyar, 1913 (type locality in Mexico: Puebla)—currently a junior subjective synonym of *Atrytonopsis ovinia* (Hewitson, 1866), (type locality in Nicaragua)—as treated by Evans (1955). Genomic analysis confirms this assessment and places the lectotype as sister to another specimen from Mexico: Jalisco (Fig. 57), thus also confirming the type locality as Mexico: Jalisco, Guadalajara. The two specimens from Jalisco (Fig. 57 red) are genetically differentiated from *A. edwardsi* specimens collected in the USA: Arizona and Texas and Mexico: Sonora (Fig. 57 blue), forming a separate clade. Due to this genetic differentiation, we propose that *L. rupilius* is a subspecies of *Atrytonopsis edwardsi* W. Barnes & McDunnough, 1916: *Atrytonopsis edwardsi rupilius* (Schaus, 1913), **comb. nov., stat. nov**. Despite a large gap in their distributions, we note that neither COI barcodes nor the whole mitochondrial genomes differentiate these subspecies, and we also see mitochondrial introgression from *A. ovinia* to *A. edwardsi* (Fig. 57b, red and violet within the blue clade).

Vidius tanna (de Jong, 1983) comb. nov.

Genomic sequencing of the holotype of *Cobalopsis tanna* de Jong, 1983 (type locality in Suriname), currently kept in its original genus, reveals that it is not monophyletic with *Cobalopsis* Godman, 1900 (type species *Pamphila edda* Mabille, 1891, which is a junior subjective synonym of *Hesperia autumna* Plötz, 1882) and instead originates within *Vidius* Evans, 1955 (type species *Narga vidius* Mabille, 1891) (Fig. 58). Therefore, we transfer this species from *Cobalopsis* to *Vidius* forming a new combination *Vidius tanna* (de Jong, 1983), **comb. nov**.



Fig. 58. Phylogenetic trees of *Vidius* (blue, with *V. tanna* comb. nov. in red) and *Cobalopsis* (violet) inferred from proteincoding regions of a) the nuclear (autosomes) and b) the mitochondrial genomes.

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