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Additional taxonomic refinements suggested by genomic analysis of butterflies

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ABSTRACT. Comparative analyses of genomic data reveal further insights into the phylogeny and taxonomic classification of butterflies presented here. As a result, 2 new subgenera and 2 new species of HesperIIDae are described: *Borna* Grishin, **subgen. n.** (type species *Godmania borincona* Watson, 1937) and *Lilla* Grishin, **subgen. n.** (type species *Choranthus lilliae* Bell, 1931) of *Choranthus* Scudder, 1872, *Cecropterus* (*Murgaria*) *markwalkeri* Grishin, **sp. n.** (type locality in Mexico: Sonora), and *Hedone yunga* Grishin, **sp. n.** (type locality in Bolivia: Yungas, La Paz). The **lectotype** is designated for *Aethilla toxeus* Plötz, 1882. The type locality of *Dion uza* (Hewitson, 1877) is likely in southern Brazil. A number of taxonomic changes are proposed. The following taxa are subgenera, not genera: *Plebulina* Nabokov, 1945 of *Icaricia* Nabokov, 1945; *Sinia* Forster, 1940 of *Glaucopsyche* Scudder, 1872; *Pseudophilotes* Beuret, 1958 of *Palaeophilotes* Forster, 1938; and *Agraulis* Boisduval & Le Conte, [1835] of *Dione* Hübner, [1819]. *Asbolis* Mabille, 1904 is a subgenus of *Choranthus* Scudder, 1872 rather than its synonym. The following are species, not subspecies or synonyms: *Glaucopsyche algerica* (Heyne, 1895) (not *Glaucopsyche melanops* (Boisduval, 1829)), *Chlosyne flavula* (W. Barnes & McDunnough, 1918) (not *Chlosyne palla* (Boisduval, 1852)), *Cercyonis hypoleuca* Hawks & J. Emmel, 1998 (not *Cercyonis sthenele* (Boisduval, 1852)), *Cecropterus coyote* (Skinner, 1892) and *Cecropterus nigrociliata* (Mabille & Boulet, 1912) (not *Aethilla toxeus* Plötz, 1882), *Aguna malia* Evans, 1952 (not *Aguna megaeles* (Mabille, 1888)), *Polygonus arizonensis* (Skinner, 1911), *Polygonus histrio* Röber, 1925, *Polygonus pallida* Röber, 1925, and *Polygonus hagar* Evans, 1952 (not *Polygonus leo* (Gmelin, [1790])), *Viola kuma* (Bell, 1942), **comb. nov.** (not *Pachyneuria helena* (Hayward, 1939)), *Tamela maura* (Snellen, 1886) (not *Tamela othonias* (Hewitson, 1878)), *Tamela diocles* (Moore, [1866]) (not *Tamela nigrita* (Latreille, [1824])), *Vinius phellus* (Mabille, 1883) (not *Vinius exilis* (Plötz, 1883)), *Vinius sophistes* (Dyar, 1918) (not *Vinius tryhana* (Kaye, 1914)), and *Rhinthon andricus* (Mabille, 1895) and *Rhinthon aqua* (Evans, 1955) (not *Rhinthon braesia* (Hewitson, 1867)). The following are new and revised species-subspecies combinations: *Cercyonis sthenele damei* W. Barnes & Benjamin, 1926 (not *Cercyonis meadii* (W. H. Edwards, 1872)) and *Chlosyne flavula blackmorei* Pelham, 2008 and *Chlosyne flavula calydon* (W. Holland, 1931) (not *Chlosyne palla*). The following are valid subspecies resurrected from synonymy in new and reinstated species-subspecies combinations: *Chlosyne palla pola* (Boisduval, 1869) (not *Chlosyne gabbii gabbii* (Behr, 1863)) and *Cercyonis meadii mexicana* R. Chermock, 1949 (not *Cercyonis sthenele damei* W. Barnes & Benjamin, 1926, **comb. rev.**). The following are new junior subjective synonyms: *Aethilla toxeus* Plötz, 1882 of *Cecropterus albociliatus* (Mabille, 1877) and *Viola dagamba* Steinhauser, 1989 of *Viola kuma* (Bell, 1942), **comb. nov., stat. rest.** *Leucochitonea janice* Ehrmann, 1907 is a junior subjective synonym of *Helioptetes alana* (Reakirt, 1868) and not of *Helioptetes petrus* (Hübner, [1819]). The holotype of *Hermeuptychia sinuosa* Grishin, 2021 is illustrated after being spread.

Keywords: taxonomy, classification, genomics, phylogeny, biodiversity.

ZooBank registration: <http://zoobank.org/0782F3E3-1EC3-4CB5-B548-1F9DCF6C7917>

This report is a direct continuation of our previous publications (Zhang et al. 2019, 2020, 2021, 2022a, 2022b, 2022c), and the general philosophy employed is best summarized in the introduction to Zhang et al. (2023). Details of experimental and computational protocols are provided in the Appendix to Li et al. (2019). We sequence specimens of any age, with collection year specified in illustrated trees, “old” means

that the specimen comes from old collections, and no date is given on its labels, probably collected more than 100 years ago, around the turn of the 20th century (most specimens) or before. Phylogenetic tree construction was carried out as described in the Materials and Methods section by Zhang et al. (2022a).

For each set of specimens, we illustrate at least two trees: constructed from nuclear genomic regions (either Z chromosome or autosomes) and from the mitogenome DNA. While the two trees frequently agree, we see instances of confident (i.e., with strong statistical support and therefore unlikely caused by insufficient or internally inconsistent data) incongruence between nuclear and mitochondrial genomic trees. Comparing the two trees highlights the pitfalls of relying exclusively on mitochondrial DNA (and COI barcodes alone) in classification decisions. Even more, including a larger fraction of mitochondrial genes in a gene marker set used in the PCR amplification approach may bias the tree toward a mitogenomic signal and thus deviate from the nuclear DNA evolution scenario. In some cases, we show all three trees: autosomes, Z chromosome, and mitogenome. Their comparison may be most instructive for the analysis of evolutionary peculiarities.

It is important to note that we do not use specific gene markers. We sequence the whole genomic shotgun and assemble all protein-coding genes present in the sample by mapping them to a complete set of genes from a reference genome. This representation gives an unbiased view of the genomic content of an organism. For computing efficiency, the trees are constructed from a random sample of 100,000 codons (3 bp each) from the entire gene set, which is approximately 6 million codons. Statistical support is computed on 100 random samples of 10,000 codons each from the original pool of genes. All protein-coding genes are used in mitochondrial DNA trees, and their statistical support is computed using ultrafast bootstrap (Hoang et al. 2018).

In addition to phenotypic diagnoses of new taxa, we provide diagnostic DNA characters, both in the nuclear genome and COI barcode. DNA characters are found in nuclear protein-coding regions using our previously developed procedure (see SI Appendix to Li et al. 2019). The logic behind the character selection was detailed in Cong et al. (2019b). The character states are provided in species diagnoses as abbreviations. E.g., aly728.44.1:G672C means position 672 in exon 1 of gene 44 from scaffold 728 of the *Cecropterus lyciades* (Geyer, 1832) (formerly in *Achalarus* Scudder, 1872, thus aly) reference genome (Shen et al. 2017) is C, changed from G in the ancestor. When characters are given for the sister clade of the diagnosed taxon, the following notation is used: aly5294.20.2:A548A (not C), which means that position 548 in exon 2 of gene 20 on scaffold 5294 is occupied by the ancestral base pair A, which was changed to C in the sister clade (so it is not C in the diagnosed taxon). The same notation is used for COI barcode characters, but without a prefix ending with ‘.’. The sequences of exons from the reference genome with the positions used as character states highlighted in green are in the supplemental file deposited at < <https://osf.io/nxd5y/> >. Linking to these DNA sequences from this publication ensures that the numbers given in the diagnoses can be readily associated with actual sequences. Whole genome shotgun datasets we obtained and used in this work are available from the NCBI database < <https://www.ncbi.nlm.nih.gov/> > as BioProject PRJNA927657, and BioSample entries of the project contain the locality and other collection data of the sequenced specimens shown in the trees. COI barcode sequences have been deposited in GenBank with accessions [OQ311404–OQ311413](#). Several photographs shown in this work are taken from iNaturalist (2022). Links to observations by observation number reported in figure legends are < <https://www.inaturalist.org/observations/xxx> >, where xxx is the number.

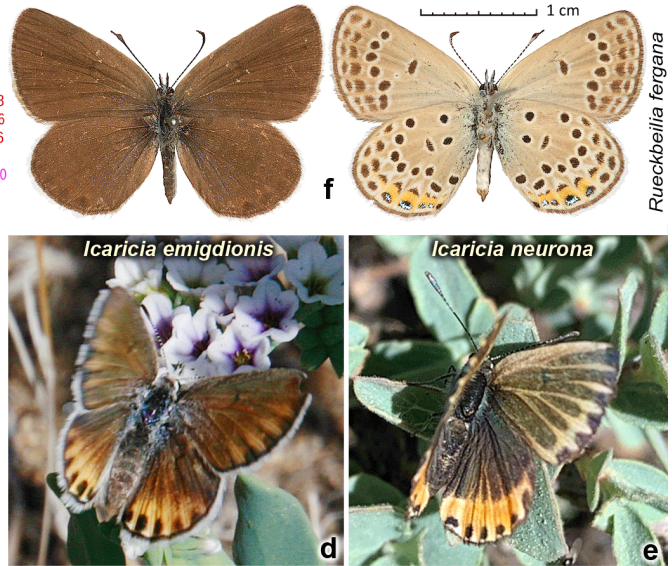
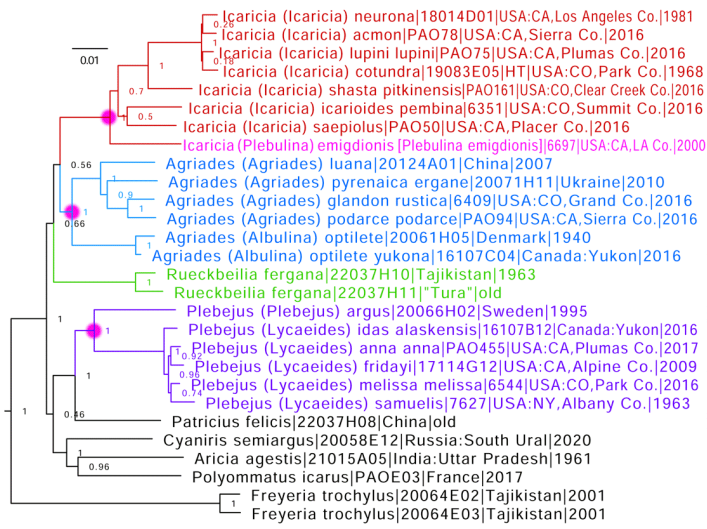
Family Lycaenidae [Leach], [1815]

***Plebulina* Nabokov, 1945 is a subgenus of *Icaricia* Nabokov, 1945**

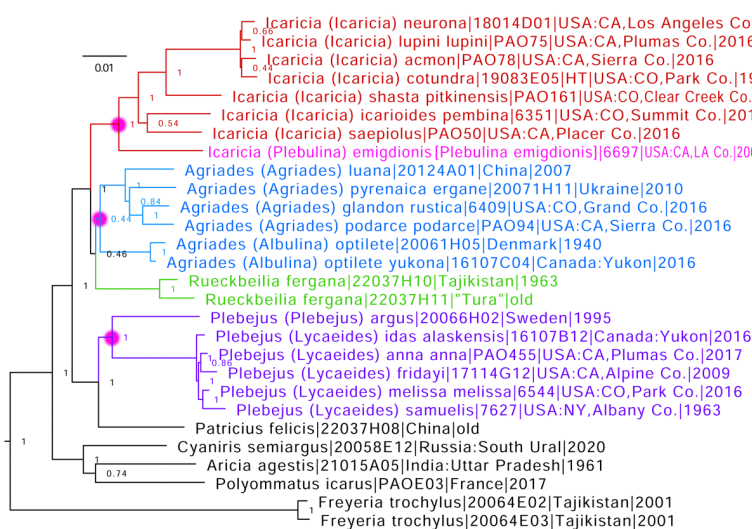
Genomic sequencing and comparison of the crown group of Polyommata Swainson, 1827 with a focus on species found in North America, confirm that the monotypic genus *Plebulina* Nabokov, 1945 (type species *Lycaena emigdionis* F. Grinnell, 1905) is closely related to *Icaricia* Nabokov, 1945 (type species *Lycaena icarioides* Boisduval, 1852) (Fig. 1a–c). In their pioneering work, Talavera et al. (2012)

proposed to treat *Plebulina* as a distinct genus because in their time-calibrated tree constructed from several gene markers, *Plebulina* was at a larger distance from *Icaricia* than their (somewhat flexible) cutoff for congeneric relationship.

a nuclear (autosome) tree



b Z chromosome tree



c mitochondrial genome tree

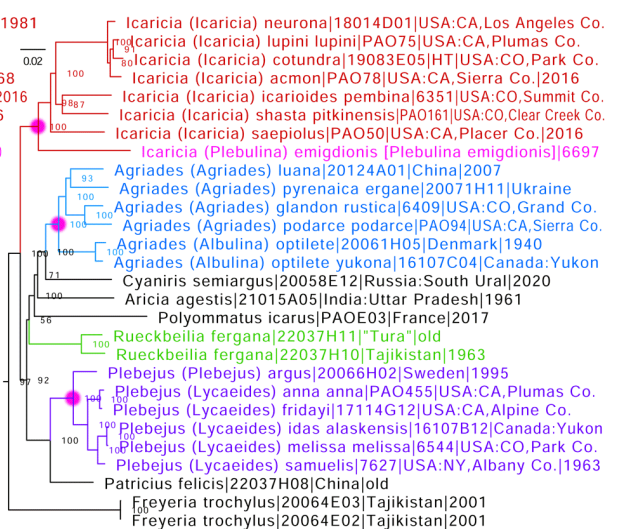


Fig. 1. *Plebulina* as a subgenus of *Icaricia*. **a–c.** Phylogenetic trees constructed from protein-coding regions in autosomes (**a**), Z chromosome (**b**), and mitochondrial genome (**c**): *Icaricia* (red, with *Icaricia (Plebulina) emigdionis* **comb. nov.** name shown in magenta), *Agriades* (blue), *Ruckbeilia* (green), and *Plebejus* (purple). Magenta dots mark diversification nodes of genera *Icaricia*, *Agriades*, and *Plebejus*. For each specimen, the name adopted in this work is given first, and a previously used name is listed in square brackets (if different), supplemented with the DNA sample number, type status (HT holotype, LT lectotype, NT neotype, ST syntype, PT paratype, and PLT paralectotype), general locality, and year. NCBI database entries in BioProject PRJNA927657 give additional data about these specimens. Synonyms are given in parentheses preceded by “=”. The type status refers to this synonym if the synonym name is provided. The same notations are used throughout this work in figures showing phylogenetic trees. **d–e.** Live females of *Icaricia* from USA: California, iNaturalist observations (data “observed”): **d.** *I. (Plebulina) emigdionis* 26900315 Kern Co., May-2007 © Nature Ali; **e.** *I. (Icaricia) neurona* 53685349 Ventura Co., Jul-2020 © Chris. Images are rotated and cropped. CC BY-NC 4.0 <https://creativecommons.org/licenses/by-nc/4.0/>. **f.** *Ruckbeilia fergana* ♀ NVG-22037H11 “Tura”, B. Neumögen collection [USNM], dorsal (left) and ventral (right) views.

In our genome-level phylogeny that includes all known species of *Icaricia*, the clade consisting of *Icaricia* and *Plebulina* taken together is well separated from all others by a prominent branch (Fig. 1) in both nuclear genome trees (autosomes Fig. 1a and Z chromosome Fig. 1b). However, *Icaricia* is not separated from *Plebulina* by a prominent branch, and the branch from the last common ancestor of *Icaricia* + *Plebulina* to the last common ancestor of *Icaricia* is shorter compared to the previous branch.

Furthermore, in the nuclear genome trees (Fig. 1a, b), the distance from the root to the last common ancestor of *Icaricia* + *Plebulina* is larger than that to the last common ancestors of genera *Agriades* Hübner, [1819] (type species *Papilio glandon* de Prunner, 1798) (Fig. 1 blue) and *Plebejus* Kluk, 1780 (type species *Papilio argus* Linnaeus, 1758) (Fig. 1 purple) (nodes marked with magenta dots in Fig 1) suggesting that the divergence between *Icaricia* and *Plebulina* might have occurred more recently than that within *Agriades* and *Plebejus*. Finally, the average distances from the leaves to the last common ancestors of *Icaricia* + *Plebulina*, *Agriades*, and *Plebejus* are approximately the same, implying that genetic differentiation in the nuclear genome is similar in the genera *Agriades*, *Plebejus*, and a group consisting of both *Icaricia* and *Plebulina*. Therefore, due to these genetic similarities and the phylogenetic tree structure, we propose to treat *Plebulina* Nabokov, 1945, **stat. nov.** as a subgenus of *Icaricia* Nabokov, 1945. *Plebulina* and *Icaricia* were proposed as genera in the same work issued on the same date. Being the first reviser, here we give precedence to *Icaricia* because more species are currently included in *Icaricia* than monotypic *Plebulina*, resulting in fewer name changes.

Despite a number of morphological differences, including a unique caterpillar foodplant of *Plebulina* (Nabokov 1945; Talavera et al. 2012; Ballmer 2022), phenotypic similarities between *Plebulina* and *Icaricia* are also notable. For instance, females of both species may have orange streaks along veins on the dorsal forewing wing surface (Fig. 1d, e). Furthermore, we hold an opinion that monotypic genera (such as *Plebulina*) should be reserved for species without apparent close relatives. In the presence of such relatives, it seems more instructive to indicate this relationship with the generic name. In contrast to *Plebulina*, genomic analysis supports the distinctness of (nearly monotypic) *Rueckbeilia* Lukhtanov, Talavera, Pierce & Vila, 2013 (type species *Lycaena loewii* var.? *fergana* Staudinger, 1881, Fig. 1f) (Fig. 1 green), because, while the statistical support for its placement in the same clade with *Agriades* and *Icaricia* + *Plebulina* is strong in the Z chromosome tree (1, i.e., 100%, Fig. 1b), it is not strongly associated with either of the two genera (less than 0.5). Therefore, if *Icaricia* and *Agriades* are treated as distinct genera, *Rueckbeilia* would also be distinct.

Finally, we offer a hypothesis about why *Plebulina* was more distinct from *Icaricia* in the phylogeny obtained by Talavera et al. (2012) than in our nuclear genome trees. In the mitochondrial genome tree (Fig. 1c), we observe this more distant relationship: the magenta dot for the red clade (*Icaricia* + *Plebulina*) is closer to the root (left) and farther from the leaves (which are at about the same level) than the magenta dots for the blue (*Agriades*) and purple (*Plebejus*) clades. Because a significant number of base pairs in the gene markers used by Talavera et al. (2012) came from mitochondrial genes, we suspect that these genes might have biased the branch lengths around *Plebulina* in their tree. The evolution of mitochondrial genomes experiences irregularities such as introgression and may not represent the organism as well as its nuclear genome. Therefore, we default to nuclear genome results (with an emphasis on the Z chromosome) for taxonomic classification.

***Sinia* Forster, 1940 is a subgenus of *Glaucopsyche* Scudder, 1872**

Sinia Forster, 1940 (type species *Glaucopsyche* (*Sinia*) *leechi* Forster, 1940) was originally proposed as a subgenus of *Glaucopsyche* Scudder, 1872 (type species *Polyommatus lygdamus* E. Doubleday, 1841) that also included *Lycaena lanty* Oberthür, 1886 (type locality China: Sichuan Prov., Kangding) and *Lycaena divina* Fixsen, 1887 (type locality in North Korea). Recently, *Sinia* has been treated as a valid genus (Lukhtanov and Gagarina 2022). However, *Lycaena divina* has been segregated into a separate genus, *Shijimiaeoides* Beuret, 1958 (type species *Lycaena barine* Leech, [1893], which is a synonym or subspecies of *L. divina*). Based on the genomic comparison, we placed *Shijimiaeoides* as a junior subjective synonym of *Glaucopsyche* (Zhang et al. 2022b). Here, we analyze genomic data on *Sinia* (Fig. 2 red) and find that it originates within *Glaucopsyche* (Fig. 2 blue), being sister to all others except the subgenus *Phaedrotes* Scudder, 1876 (type species *Lycaena catalina* Reakirt, 1866, which is a junior subjective synonym of *Lycaena piasus* Boisduval, 1852) in all three trees (Fig. 2a–c). Therefore, *Sinia* is a subgenus unless *Phaedrotes* is treated as a genus. Hence, we propose to regard *Sinia* Forster, 1940 a

subgenus of *Glaucopsyche* Scudder, 1872 as originally described. We also note wing pattern similarities between the type species of *Sinia*, *G. leechi* **comb. rest.** from China (Fig. 2d) and North American *G. piasus* (Fig. 2e): e.g., in the placement of white “arrowheads” on the ventral hindwing.

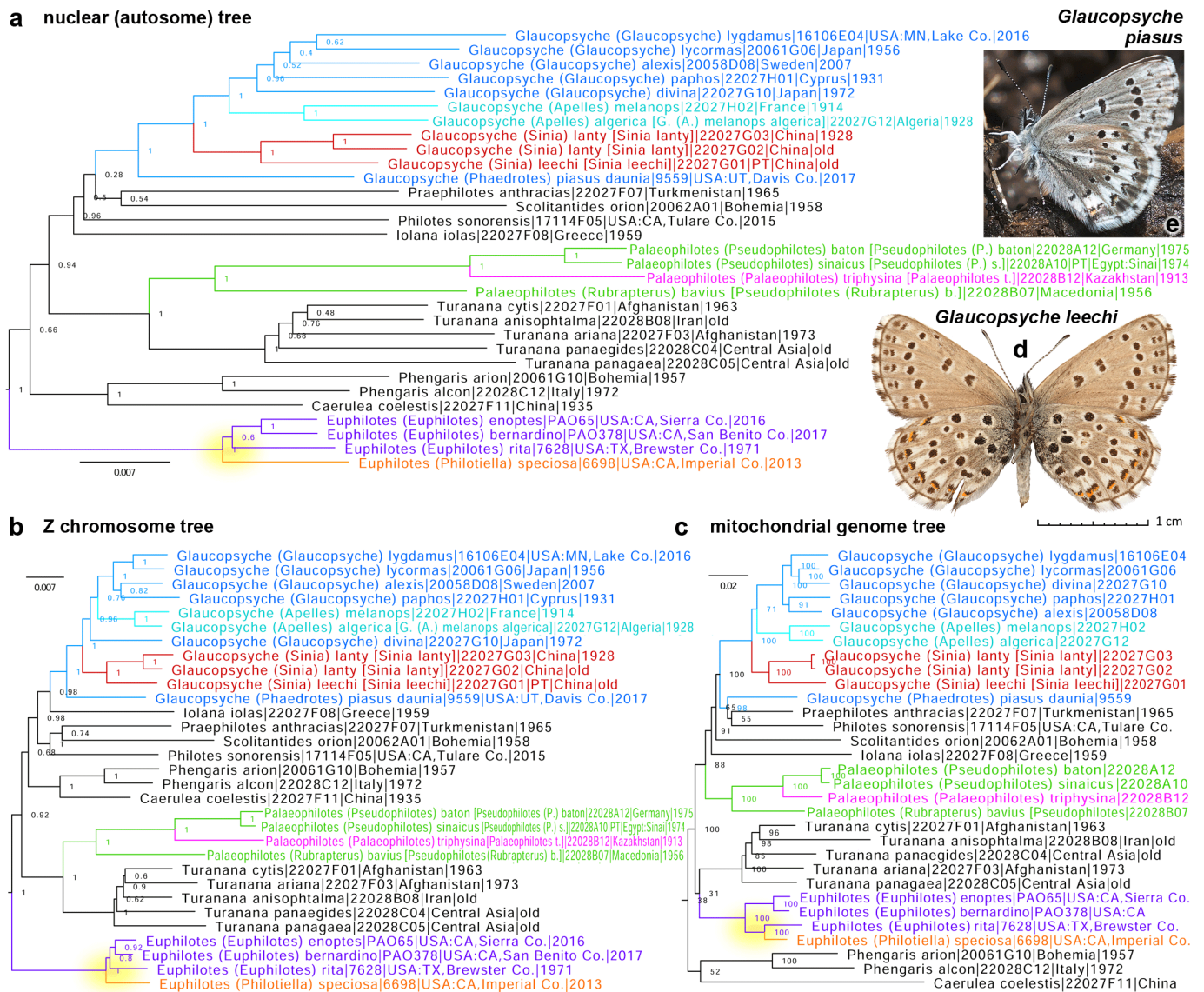


Fig. 2. Trees of *Scolitantidina* species constructed from protein-coding regions in **a.** autosomes, **b.** Z chromosome, and **c.** mitochondrial genome: *Glaucopsyche* (blue) with subgenera *Apelles* (cyan) and *Sinia* (red), *Palaephilotes* (green, nominotypical subgenus in magenta), and *Euphilotes* (purple) with its subgenus *Philotiella* (orange). Yellow highlights a case of strongly supported incongruence between nuclear and mitochondrial genome trees. **d.** *Glaucopsyche* (*Sinia*) *leechi* **comb. rest.** ♀ paratype ventral view, NVG-22027G01 China: Sichuan Prov., Songpan Co. [ZSMC]. **e.** *Glaucopsyche* (*Phaedrotes*) *piasus* iNaturalist observation 125272854 USA: California, Modoc Co., Modoc, 25-Jun-2022 © Paul G. Johnson. The image is rotated and cropped. CC BY-NC 4.0 <https://creativecommons.org/licenses/by-nc/4.0/>.

Finally, we note incongruence between the three trees (Fig. 2a–c). While *Glaucopsyche* is most confidently monophyletic in both nuclear genome trees (support 1, i.e., 100%, Fig. 2a, b), subgenus *Phaedrotes* is not in the same clade with the rest of *Glaucopsyche* in the mitogenome tree (Fig. 2c), cautioning about the reliance on mitochondrial DNA for organism-level phylogenies or a set of gene markers that may be dominated by mitochondrial genes. More weakly supported incongruence is in the relative position of *Apelles* Hemming, 1931 (type species *Polyommatus melanops* Boisduval, [1828]), treated as a valid subgenus by Lukhtanov and Gagarina (2022) and *G. divina*. Regardless of these incongruences that would be interesting to understand further, the position of *Sinia* is the same in all three trees. Therefore, our conclusion about its placement within *Glaucopsyche* is strongly supported.

***Glaucopsyche algerica* (Heyne, 1895), stat. nov. is a species distinct from *Glaucopsyche melanops* (Boisduval, 1829)**

Genomic sequencing of *Glaucopsyche melanops* (Boisduval, 1829) (type locality in France) and *Lycaena melanops* var. *algerica* Heyne, 1895 (type locality in Algeria), currently a valid subspecies of *G. melanops*, reveals genetic differentiation between them more in line with that known for distinct species among its relatives (Fig. 2 cyan): e.g., their COI barcodes differ by 1.8% (12 bp), and the genetic distance between them in the mitogenome is similar to that between *Glaucopsyche paphos* Chapman, 1920 (type locality in Cyprus) and *Glaucopsyche alexis* (Poda, 1761) (type locality in Austria), or between *Glaucopsyche lygdamus* (Doubleday, 1841) (type locality in USA) and *Glaucopsyche lycormas* (Butler, 1866) (type locality in Japan) (Fig. 2c). Therefore, we propose that *Glaucopsyche algerica* (Heyne, 1895), **stat. nov.** is a species-level taxon.

***Pseudophilotes* Beuret, 1958 is a subgenus of *Palaeophilotes* Forster, 1938**

Genomic sequencing of *Palaeophilotes* Forster, 1938 (type species *Lycaena triphysina* Staudinger, 1892, Fig. 3a) (Fig. 2 magenta) treated as a distinct genus by Lukhtanov and Gagarina (2022) due to the lack of its DNA sequences and pronounced phenotypic differences from other genera reveals that it is sister to the nominotypical subgenus of *Pseudophilotes* Beuret, 1958 (type species *Papilio baton* Bergsträsser, 1779, Fig. 3b) and is much closer to it genetically than the two subgenera of *Pseudophilotes*—nominotypical and *Rubrapterus* Korshunov, 1987 (type species *Lycaena bavus* Eversmann, 1832)—are to each other (Fig. 2). This was a surprise given the differences in the appearance of these species (Fig. 3a, b).



Fig. 3. Ultrafast phenotypic evolution in *Palaeophilotes*. Dorsal view of males, all in ZSMC. **a.** *P. (Palaeophilotes) triphysina* NVG-22028C01 Central Asia, old, coll. Erhardt. **b.** *P. (Pseudophilotes) baton* NVG-22028A12 Germany: Bavaria, vic. Jura, 17-May-1975, W. Schätz leg. **c.** *P. (Pseudophilotes) sinicus* paratype NVG-22028A10 Egypt: Sinai, Wadi Jibal, 1900 m, 26-May-1974, I. Nakamura leg. COI barcodes differ between (a) and (b) by 2.1% and between (b) and (c) by 0% (probably not a result of introgression, but the actual lack of genetic differentiation).

We observe that the tree branches in nuclear genome trees (Fig. 2a, b) leading to *Palaeophilotes* and the subgenus *Pseudophilotes* are longer (=reach farther to the right) than for all other Scolitantidina Tutt, 1907 we sequenced, indicating accelerated evolution. This elevated evolutionary rate in the group is likely the cause of the observed phenotypic disparity. Notably, the mitochondrial genome tree does not show elevated rates in this group: branches reach about the same level on the right (Fig. 2c). In accord with this mitogenome conservation, COI barcodes of *Pseudophilotes (Pseudophilotes) baton* (Fig. 3b) and *Pseudophilotes (Pseudophilotes) sinicus* Nakamura, 1976 (Fig. 3c) are 100% identical (despite visually apparent differences in facies) and those of *Palaeophilotes triphysina* (Fig. 3a) and *Pseudophilotes (Pseudophilotes) baton* (Fig. 3b) differ by only 2.1% (14 bp), which is in the range typical for the closest congeners. To confirm these unexpected results, we sequenced four specimens of *P. triphysina* (NVG-22027F04, NVG-22027F05, NVG-22028B12, and NVG-22028C01), and their COI barcodes were 100% identical (Genbank [OQ311404–OQ311407](https://doi.org/10.26434/chemrxiv-2024-00311)):

AACTTTATATTTTATTTTCGGAATTTGAGCAGGAATATTAGGAACATCTTTAAGAATTTTAAATTCGTATAGAATTAGGAACACCTGGATCTTTAATGGAGATGATCAAATTTATAACACT
ATTGTAACAGCTCATGCCTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTTGGAGGATTTGGAAATTTGACTAGTACCCTTAATATTAGGAGCACCTGATATAGCATTTCACAGAA
TAAATAATATAAGATTTTGATTATTACCTCCATCATTAAATATTAAATTTCAAGTAGAATCGTAGAAAATGGAGCAGGAACAGGATGAACAGTGTACCCCCACTTTTCATTAATATTGC
TCATAGAGGTTTCATCTGTGATTAGCAATTTTTCACCTTCATTAGCAGGAATTTTCATCAATTTTAGGAGCAATTAATTTTATTACTACAATTAATATATACGAGTAAATATATATCA
TTTGATCAAAATATCATTATTTTGGAGCAGTAGGTATTACAGCATTACTATTATTATTATCTTTACCTGTTTTAGCAGGTGCAATTACTATATTATTAACAGATCGAAATCTTAATACCT
CTTTTTTGACCTGCTGGAGGAGGATCCAATTTTATATCAACATTTATTT

Therefore, we propose to treat species of *Palaeophilotes* and *Pseudophilotes* as congeneric. However, due to the elevated rate of evolution in the nuclear genome that resulted in significant phenotypic differences, we conservatively place *Pseudophilotes* Beuret, 1958 as a subgenus of *Palaeophilotes* Forster, 1938 instead of synonymizing it. Due to the priority of names based on their dates, this action results in many name changes: we place all species of *Pseudophilotes* in the genus *Palaeophilotes*. However, if *Pseudophilotes* is kept as a genus, the classification of the group becomes genetically inconsistent and would require the elevation of *Rubrapterus* to the genus level (Fig. 2), which seems unwarranted provided its genetic and phenotypic similarities with *Pseudophilotes*.

In summary, the variability of evolutionary rates between taxa and rapid phenotypic evolution in some lineages pose challenges for taxonomic classification because recently diverged species can look very different from each other (Fig. 3). As a solution, we propose to keep the classification of genera largely consistent with the estimated divergence times and genetic differentiation, but to use subgenus as an indicator of phenotypic differences. This approach generally results in a smaller number of genera, frequently due to the elimination of monotypic genera, which in our opinion, should be used only to indicate the genetic uniqueness of taxa in the absence of close relatives.

Subgenus *Euphilotes* Mattoni, [1978] is paraphyletic in mitochondrial DNA

Zhang et al. (2019) placed *Philotiella* Mattoni, [1978] (type species *Lycaena speciosa* Hy. Edwards, 1877) as a subgenus of *Euphilotes* Mattoni, [1978] (type species *Lycaena enoptes* Boisduval, 1852) due to their genetic similarity, as recently confirmed by Lukhtanov and Gagarina (2022). Adding to these results, here we show that *Philotiella* and *Euphilotes* are not only very close to each other genetically (Fig. 2a, b yellow highlight) but also that *Philotiella* renders *Euphilotes* paraphyletic in the mitochondrial genome tree with confident statistical support (Fig. 2c yellow highlight), suggesting introgression of mitochondrial DNA between the subgenera and a possibility of future synonymization of these two names. Even phenotypically, some populations of *Euphilotes pallescens* (Tilden & Downey, 1955) may be superficially more similar to *Philotiella* than to *Euphilotes* species due to reduced spotting and the lack of orange spots on ventral hindwing. Finally, our current and more comprehensive trees (Fig. 2) strongly support the distinction of the genus *Euphilotes* (that includes *Philotiella* as a subgenus) from other genera in the subtribe Scolitantidina Tutt, 1907: nuclear genome trees place *Euphilotes* as sister to all other sequenced members of the subtribe, with higher confidence (0.92) in the Z chromosome tree (Fig. 2b). Although members of the New World genus *Euphilotes* have been placed within some of the Old World genera in the past, we show that unless the entire subtribe Scolitantidina is unified under a single genus (*Scolitantides* Hübner, 1819), *Euphilotes* cannot be combined with any other genus to keep it monophyletic.

Family Nymphalidae Rafinesque, 1815

***Agraulis* Boisduval & Le Conte, [1835] as a subgenus of *Dione* Hübner, [1819]**

From the position of consistency and uniformity of taxonomic classification, based on the genetic closeness of *Agraulis* Boisduval & Le Conte, [1835] (type species *Papilio vanillae* Linnaeus, 1758) and *Dione* Hübner, [1819] (type species *Papilio juno* Cramer, 1779) we proposed to regard the former as a subgenus of the latter, rather than keeping the two as separate genera (Zhang et al. 2019). Treating *Agraulis* and *Dione* as congeneric was not a novel concept (Scott 1986). Recent publications are divided between the two options: some argue for keeping *Agraulis* as a genus (Núñez et al. 2022; Penz 2022), while others use *Agraulis* as a subgenus of *Dione* (Farfán et al. 2022a; Farfán et al. 2022b; Pelham 2022).

In our opinion, this argument reflects lumping vs. splitting viewpoints. For every monophyletic lineage, one may find a sufficient number of characters, be it morphological or molecular, to “support” the distinction of the lineage at the taxonomic rank deemed appropriate. It seems impossible to formulate criteria for which morphological character differentiates between genera and which refers to subgenera. Therefore, it is not likely that genus/subgenus disagreement will be resolved by additional studies of *Agraulis* morphology. It seems equally unlikely that additional genomic sequencing of *Agraulis* and *Dione* will bring us closer to resolution because the data we have at hand (Zhang et al. 2020; Núñez et al. 2022) confidently resolve the phylogeny of the group and provide reliable estimates of evolutionary distances within the group and among their relatives.

While experts in their phylogenetic groups usually find it more aesthetically appealing to split them into many smaller genera (i.e., the more you study something, the more significant seem the differences), we think that a more inclusive treatment with a smaller number of distinctive and confidently monophyletic genera is more practical, both for general biologists (who do not have to learn additional names) and newcomers. While genetic similarity may be harder to relate to, similarity in facies between North American species of *Agraulis* and *Dione* is illustrated here in Fig. 4 and needs no explanation. Provided these species are closely related (as judged by genomic analysis), it seems meaningful to treat them as congeneric. From a broader perspective, we do not see an imperative reason to place in different genera two closely related species that can sometimes be misidentified for each other. We think that genera are for broader use, and subgenera could be for specialists.



Fig. 4. Two species of *Dione*, iNaturalist observations: **a.** *Dione (Agraulis) incarnata* 140735334 USA: Texas, Cameron Co. 31-Oct-2022 © Jeff Chapman; **b.** *Dione (Dione) moneta* 131933192 Mexico: Oaxaca, Tepelmeme Villa de Morelos, 6-Sep-2017 © Tepelmeme Villa de Morelos. Images are color-corrected, rotated, cropped, and/or flipped. CC BY-NC 4.0 <https://creativecommons.org/licenses/by-nc/4.0/>.

Genomics-based arguments for *Agraulis* as a subgenus of *Dione* from the position of internal consistency and uniformity of taxonomic classification were stated by Zhang et al. (2019). COI barcodes of *Agraulis* and *Dione* differ by less than 8%. From an even broader perspective of consistent application of ranks to clades depending on their evolutionary distance, humans (*Homo sapiens* Linnaeus, 1758) and chimps (*Pan troglodytes* (Blumenbach, 1775)), currently attributed to different genera, are 9.6% (63 bp) different in their barcodes (GenBank accessions MF479728 and HM068586), not 2% as specified by Penz (2022). Finally, subgenera *Agraulis* and *Dione* consist of a small number of species. Unifying them in one genus does not cause much inconvenience by creating a large genus that is difficult to navigate. On the contrary, the unification results in a more conveniently sized genus and emphasizes closer evolutionary connections within it. For all these reasons, we still support the treatment of *Agraulis* as a subgenus of *Dione*; however, placing them in separate genera is not obviously incorrect either.

***Chlosyne flavula* (W. Barnes & McDunnough, 1918), stat. rest.
is a species distinct from *Chlosyne palla* (Boisduval, 1852)**

Our recent finding that *Melitaea sterope* W. H. Edwards, 1870 (type locality in USA: Oregon, Wasco Co.) is not conspecific with *Chlosyne acastus* (W. H. Edwards, 1874) (type locality in USA: Utah, probably Utah Co.) and instead is a subspecies of *Chlosyne palla* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) (Zhang et al. 2022b) prompted further discussions and investigations. Genomic sequencing of all *C. palla* subspecies partitions them into two clades in the tree constructed from protein-coding regions in autosomes (Fig. 5a blue and red). While not strongly different in mitogenomes (Fig. 5b), the two clades are genetically differentiated in the Z chromosome with F_{st}/G_{min} of 0.2/0.016. This differentiation is approximately the same as in the following pairs of species (Fig. 5a): *Chlosyne whitneyi* (Behr, 1863) and *Chlosyne damoetas* (Skinner, 1902), *Chlosyne gabbii* (Behr, 1863) and *C. acastus*, and *Chlosyne hoffmanni* (Behr, 1863) and *Chlosyne harrisii* (Scudder, 1863). Therefore, the two clades represent two distinct species possibly coming in contact at least in Washington state as *C. palla sterope* (formerly *C. acastus*) and *Chlosyne palla blackmorei* Pelham, 2008 (type locality Canada: British Columbia, Lytton). The blue clade (Fig. 5) includes the lectotypes of *C. palla palla* and *C. palla sterope*. The oldest name in the red clade (Fig. 5) is *Melitaea flavula* W. Barnes & McDunnough, 1918 (type locality USA: Colorado, Garfield Co., Glenwood Springs, a syntype sequenced as NVG-22036E05). Therefore, we propose that *Chlosyne flavula* (W. Barnes & McDunnough, 1918), **stat. rest.** is a species distinct from *Chlosyne palla* (Boisduval, 1852). We place the following valid taxa (and their synonyms) as subspecies of *Chlosyne flavula*: *Chlosyne palla blackmorei* Pelham, 2008 and *Melitaea calydon* W. Holland, 1931 (type locality in USA: Colorado, Jefferson Co.). Other subspecies listed by Pelham (2022) remain with *C. palla*.

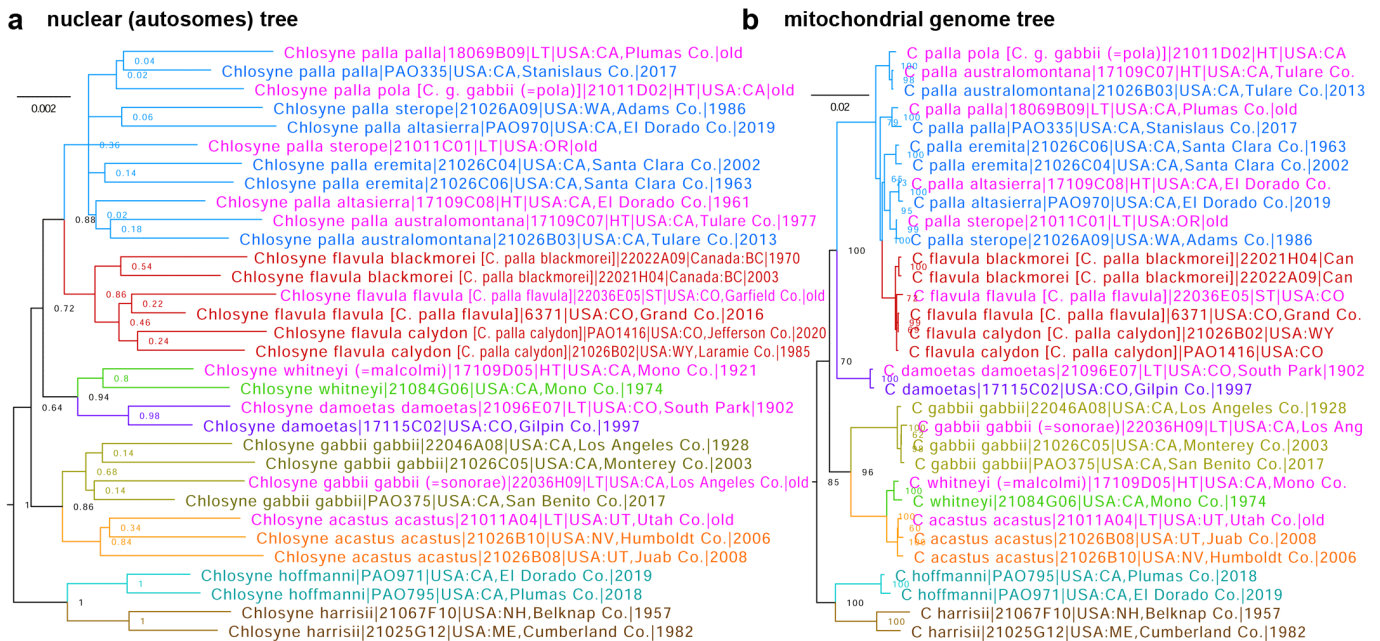


Fig. 5. Trees of *Chlosyne* species constructed from protein-coding regions in **a.** autosomes and **b.** mitochondrial genome: *C. palla* (blue), *C. flavula* **stat. rest.** (red), *C. whitneyi* (green), *C. damoetas* (purple), *C. gabbii* (olive), *C. acastus* (orange), *C. hoffmanni* (cyan), and *C. harrisii* (brown). Primary type specimens are labeled in magenta.

***Chlosyne palla pola* (Boisduval, 1869), comb. nov., stat. rev. is not a junior subjective synonym of *Chlosyne gabbii gabbii* (Behr, 1863)**

Genomic sequencing of the holotype of *Melitaea pola* Boisduval, 1869 (type locality “Sonora”, hypothesized to be USA: California, Los Angeles Co., La Tuna Canyon) currently regarded as a junior subjective synonym of *Chlosyne gabbii gabbii* (Behr, 1863) (type locality USA: California, Los Angeles

Co., La Tuna Canyon) is not in the same clade with it, but instead is a specimen of *Chlosyne palla* (Boisduval, 1852) (type locality in USA: California, Plumas Co.) (Fig. 5). Therefore, the hypothesized type locality of *M. pola*, which is the same as that of *C. gabbii*, is most likely incorrect. Could it be that the original type locality stated as “Sonora” was accurate, but instead of Sonora, Mexico, it represents a homonymous town currently in Tuolumne Co., California? While genomic sequencing of additional specimens is necessary to solve this problem confidently, we see that the holotype of *M. pola* shares mitochondrial DNA with *Chlosyne palla australomontana* J. Emmel, T. Emmel & Mattoon, 1998 (type locality in USA: California, Tulare Co., holotype sequenced as NVG-17109C07) (Fig. 5b), and specimens of similar appearance are known from Tuolumne Co., suggesting possible synonymy. Until a confidently supported synonymization solution is found, we conservatively propose to treat *M. pola* as a valid subspecies *Chlosyne palla pola* (Boisduval, 1869), **comb. nov.**, **stat. rev.**

***Cercyonis hypoleuca* Hawks & J. Emmel, 1998, stat. nov. is a species distinct from *Cercyonis sthenele* (Boisduval, 1852)**

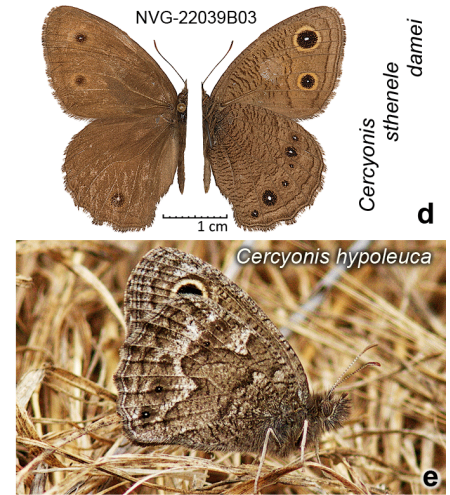
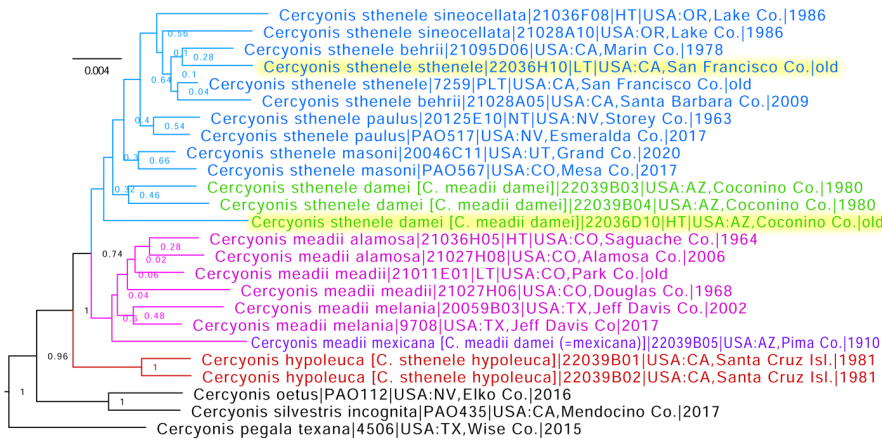
Genomic sequencing of *Cercyonis* Scudder, 1875 (type species *Papilio alope* Fabricius, 1793, which is a subspecies of *Papilio pegala* Fabricius, 1775) specimens reveals that *Cercyonis sthenele hypoleuca* Hawks & J. Emmel, 1998 (type locality USA: California, Santa Barbara Co., Santa Cruz Island) is sister to both *Cercyonis sthenele* (Boisduval, 1852) (type locality USA: California, San Francisco, lectotype sequenced as NVG-22036H10) and *Cercyonis meadii* (W. H. Edwards, 1872) (type locality USA: Colorado, Park Co. Bailey, lectotype sequenced as NVG-21011E01) in all three trees (Fig. 6). Thus, *C. meadii* renders *C. sthenele* that includes *C. sthenele hypoleuca* paraphyletic. Moreover, *C. sthenele hypoleuca* is genetically differentiated from *C. sthenele* at the level characteristic of distinct species: e.g., COI barcodes of *C. sthenele* lectotype and *C. hypoleuca* differ by 3.3% (22 bp). Therefore, we propose that *Cercyonis hypoleuca* Hawks & J. Emmel, 1998, **stat. nov.** is a species-level taxon. Interestingly, the ventral hindwing with a contrasting whitish pattern of *C. hypoleuca* (Fig. 6e) is superficially like that of nominotypical *C. sthenele*, which is genetically different from it. Instead, *C. sthenele sthenele* is genetically most similar to its geographical neighbor *Cercyonis sthenele behrii* (F. Grinnell, 1905) (type locality in USA: California, Marin Co.) that generally lacks the white pattern.

***Cercyonis sthenele damei* W. Barnes & Benjamin, 1926, comb. rev. is not a subspecies of *Cercyonis meadii* (W. H. Edwards, 1872)**

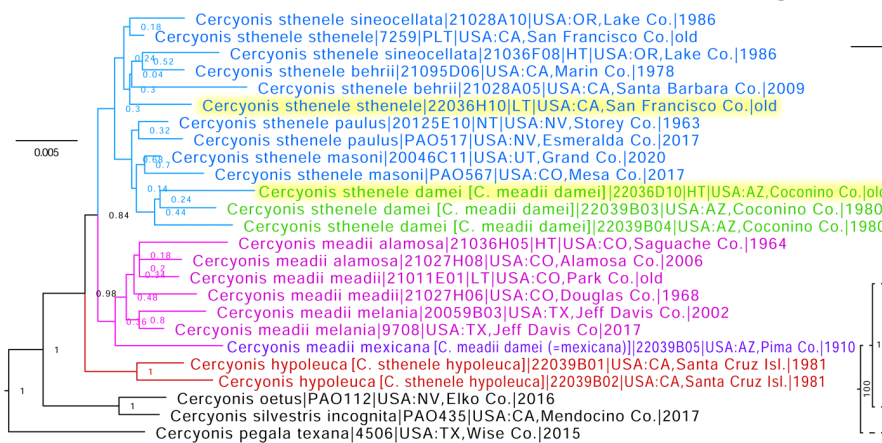
Genomic sequencing of the holotype of *Cercyonis damei* W. Barnes & Benjamin, 1926 (type locality USA: Arizona: Coconino Co., Grand Canyon) a taxon treated as a valid subspecies of *Cercyonis meadii* (W. H. Edwards, 1872) (type locality USA: Colorado, Park Co. Bailey, lectotype sequenced as NVG-21011E01) is not conspecific with it and instead is placed among specimens of *Cercyonis sthenele* (Boisduval, 1852) (type locality USA: California, San Francisco, lectotype sequenced as NVG-22036H10) in nuclear genome trees (Fig. 6a, b). Two more recently collected specimens from the general vicinity of the type locality of *C. damei* (e.g., Fig. 6d) are in the same clade with the holotype in the Z chromosome tree (Fig. 6b), which usually agrees well with speciation scenarios (Cong et al. 2019a). These specimens, including the *C. damei* holotype, are dark and lack reddish patches of *C. meadii*, being more similar phenotypically to *C. sthenele*. Therefore, from both its nuclear genomic sequences and superficial appearance, *Cercyonis damei* is not a subspecies of *C. meadii* but a subspecies of *C. sthenele*: *Cercyonis sthenele damei* W. Barnes & Benjamin, 1926, **comb. rev.** However, in the tree constructed from the protein-coding regions of autosomes (which usually harbor a larger number of introgressed genes), the three sequences specimens of *C. sthenele damei* do not form a clade. They are placed at the base of the *C. sthenele* clade (Fig. 6a), suggesting some introgression from *C. meadii*. These introgressed genomic regions “pull” these specimens closer to *C. meadii* in the tree and, thus, closer to the base of the clade. Nevertheless, the amount of introgression is insufficient to “move” any of these specimens into the *C. meadii* clade. Furthermore, two *C. sthenele damei* specimens, including the holotype, possess

mitochondrial DNA of *C. meadii* and not *C. sthenele* (Fig. 6c green-labeled in the magenta clade), directly indicating introgression from *C. meadii*. The third specimen (Fig. 6c green-labeled in the blue clade) has mitochondrial DNA of *C. sthenele*. This polyphyly of *C. sthenele damei* in the mitochondrial DNA tree indicates a varying extent of limited hybridization and introgression with *C. meadii* in its various genomic regions rather than supporting this taxon’s hybrid origin.

a nuclear (autosome) tree



b Z chromosome tree



c mitochondrial genome tree

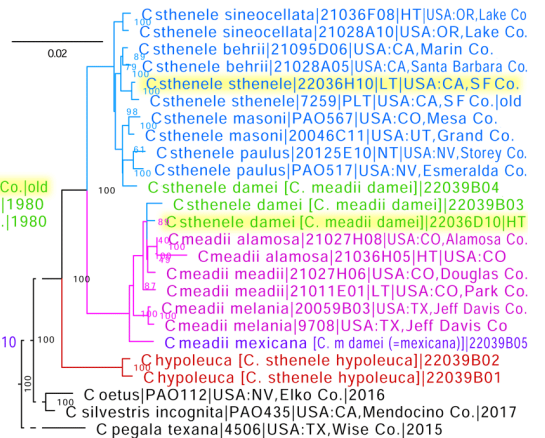


Fig. 6. Trees of *Cercyonis* species constructed from protein-coding regions in **a.** autosomes, **b.** Z chromosome, and **c.** mitochondrial genome: *C. sthenele* (blue) with *C. sthenele damei* **comb. rev.** labeled in green, *C. meadii* (magenta) with *C. meadii mexicana* **stat. rest.** labeled in purple, and *C. hypoleuca* **stat. nov.** (red). The lectotype of *C. sthenele sthenele* and the holotype of *C. sthenele damei* are highlighted in yellow. Gaps in branches in (c) indicate where vertical slices of the tree were removed to reduce its horizontal dimension (to allow an increase of the font size), i.e., branches with gaps are longer than shown. **d.** *C. sthenele damei* ♂ dorsal (left) and ventral (right) views, NVG-22039B03 USA: Arizona, Coconino Co., 17-Aug-1980, J. A. Scott leg. **e.** *C. hypoleuca* **stat. nov.**, iNaturalist observation 4540707 USA: California, Santa Barbara Co., Santa Cruz Island, Channel Islands National Park, 3-Jul-2013 © Nature Ali. The image is color-corrected and cropped. CC BY-NC 4.0 <https://creativecommons.org/licenses/by-nc/4.0/>.

***Cercyonis meadii mexicana* R. Chermock, 1949, stat. rest.
is a valid subspecies and not a junior subjective synonym
of *Cercyonis sthenele damei* W. Barnes & Benjamin, 1926, comb. rev.**

Genomic sequencing of a specimen from southeastern Arizona identified as *Cercyonis meadii mexicana* R. Chermock, 1949 (type locality in Mexico: Chihuahua) (Fig. 6 labeled in purple), a taxon treated as a junior subjective synonym of “*Cercyonis meadii damei*” W. Barnes & Benjamin, 1926 (type locality USA: Arizona: Coconino Co., Grand Canyon) by Pelham (2022) reveals that it is in a clade different from the holotype of *Cercyonis damei* (Fig. 6), which, as we have shown above, is a subspecies of *Cercyonis*

sthenele (Boisduval, 1852) (type locality USA: California, San Francisco, lectotype sequenced as NVG-22036H10) and instead belongs to *Cercyonis meadii* (W. H. Edwards, 1872) (type locality USA: Colorado, Park Co. Bailey, lectotype sequenced as NVG-21011E01), being sister to all its other subspecies in all three trees (Fig. 6 magenta) and thus is distinct from them. Therefore, we reinstate it as a valid subspecies *Cercyonis meadii mexicana* R. Chermock, 1949, **stat. rest.**

The holotype of *Hermeuptychia sinuosa* Grishin, 2021

The original description illustrated the holotype of *Hermeuptychia sinuosa* Grishin, 2021 (type locality Guatemala: El Progreso, Morazán) that was pinned through its side, unspread (Cong et al. 2021). Here, we use this opportunity and publish photographs of the holotype after it has been spread (Fig. 7). Its genitalia vial is pinned on the same pin as the specimen. The holotype is in the University of Texas Insect Collection, Austin, TX, USA.



Fig. 7. Holotype of *Hermeuptychia sinuosa* Grishin, 2021 dorsal (left) and ventral (right) views, data in text.

Family HesperIIDae Latreille, 1809

***Aethilla toxeus* Plötz, 1882, syn. nov. is a junior subjective synonym of *Cecropterus albociliatus* (Mabille, 1877)**

Genomic sequencing of the syntype of *Aethilla toxeus* Plötz, 1882 (NVG-15032A10, type locality in Mexico) in MFNB reveals that it is clustered with *Cecropterus albociliatus* (Mabille, 1877) (type locality in Colombia, Panama, and Guatemala) (Fig. 8 blue) and not within a species currently called *Cecropterus toxeus* (Fig. 8 green and purple). The sequenced specimen is a syntype (possibly the only one ever in existence) because it matches the original description and carries a label with the number 5054, as stated in the description. In the interest of stability of nomenclature, N.V.G. hereby designates this specimen in MFNB, a female, bearing the following six rectangular labels, the first is red, the third is green, and others are white: [Typus], [5054], [Mexico Deppe], [toxeus | Pl. | type 5054.], [{QR code} http://coll.mfn-berlin.de/u/ | 940b65], and [DNA sample ID: | NVG-15032A10 | c/o Nick V. Grishin] as the **lectotype** of *Aethilla toxeus* Plötz, 1882. Because Ferdinand Deppe collected in Mexico in 1824–1829 (Stresemann 1954), the lectotype was likely collected during that time period. The lectotype is missing nearly all its fringes (the white portion is represented literally by a couple of remaining scales), and all its wings except the right forewing are chipped at the margins. However, paler postdiscal spots in forewing cells M₃-CuA₁ and CuA₁-CuA₂ overlap; therefore, this specimen keys out to *Achalarus albociliatus* in Evans (1952), and not to Evans' "*Achalarus toxeus*." Therefore, *Aethilla toxeus* Plötz, 1882, **syn. nov.** is a junior subjective synonym of *Cecropterus albociliatus* (Mabille, 1877).

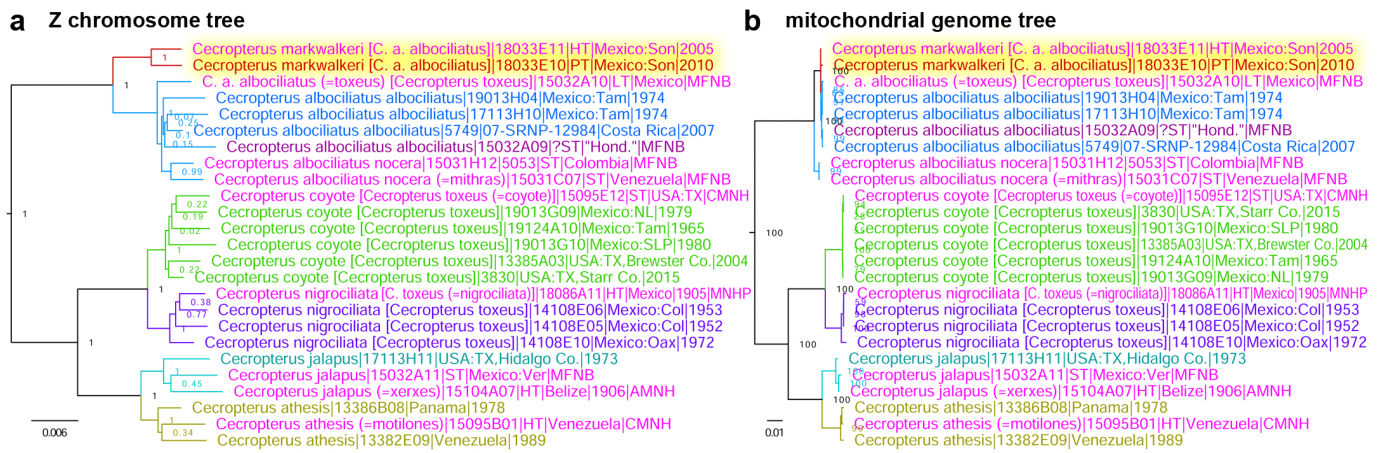


Fig. 8. Trees of selected *Cecropteris* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *C. markwalkeri* sp. n. (red, highlighted in yellow), *C. albociliatus* (blue), *C. coyote* stat. rest. (green), *C. nigrociliata* stat. rest. (purple), *C. jalapus* (cyan), and *C. atthesis* (olive). Primary type specimens are labeled in magenta, and a specimen curated as a possible syntype is labeled in plum color.

Cecropteris coyote (Skinner, 1892), stat. rest. and *Cecropteris nigrociliata* (Mabille & Boulet, 1912), stat. nov. are species distinct from *Aethilla toxeus* Plötz, 1882

As shown above, a species currently known as *Cecropteris toxeus* (Plötz, 1882) (type locality in Mexico) loses its name to *Cecropteris albociliatus* (Mabille, 1877) (type locality in Colombia, Panama, and Guatemala) because the lectotype of the former is conspecific with the latter. The next oldest name for the species currently misidentified as *C. toxeus* is *Cecropteris coyote* (Skinner, 1892) (type locality in USA: Southern Texas). Genomic sequencing of a syntype of *Eudamus coyote* places it in the clade with specimens from the US and eastern Mexico that constitute a species distinct from others (Fig. 8 green). Moreover, we find that the holotype of *Murgaria albociliata* var. *nigrociliata* Mabille & Boulet, 1912 (type locality in Mexico), a taxon currently regarded as a junior subjective synonym of *Cecropteris toxeus* (Plötz, 1882) and, therefore, given our findings, possibly conspecific with *C. coyote*, is in the clade sister to *C. coyote*, together with several specimens from southwestern Mexico (Fig. 8 purple). The two clades differ genetically at the level characteristic of distinct species alike genetic differentiation between sisters *Cecropteris jalapus* (Plötz, 1881) (Fig. 8 cyan) and *Cecropteris atthesis* (Hewitson, 1867) (Fig. 8 olive). COI barcodes of the primary types of *E. coyote* and *M. albociliata* var. *nigrociliata* differ by 1.8% (12 bp). Hence, we propose that *Cecropteris coyote* (Skinner, 1892), stat. rest. and *Cecropteris nigrociliata* (Mabille & Boulet, 1912), stat. nov. are species-level taxa.

Cecropteris (Murgaria) markwalkeri Grishin, new species

<http://zoobank.org/F1805F4A-DB94-47C9-A148-82B20B46BA6E>

(Figs. 8 part, 9, 10a–b, 11)

Definition and diagnosis. Genomic sequencing of the subgenus *Murgaria* E. Watson, 1893 (type species *Telegonus albociliatus* Mabille, 1877) reveals that two specimens from Sonora, Mexico are sister to a compact clade of *Cecropteris albociliatus* (Mabille, 1877) (type locality in Colombia, Panama, Guatemala) (Fig. 8). The latter clade included a specimen identified by Mabille as “*Teleg. albociliatus*” and curated as a type, in addition to the lectotype of *Aethilla toxeus* Plötz, 1882 (type locality in Mexico) and syntypes of *Aethilla nocera* Plötz, 1882 (type locality in Colombia) and *Telegonus mithras* Mabille, 1888 (type locality in Venezuela), the latter being a junior subjective synonym of the former, which is regarded as a subspecies of *C. albociliatus*. The two specimens from Sonora (Fig. 8 red) show prominent genetic differentiation from *C. albociliatus* (Fig. 8 blue) in the Z chromosome (F_{st}/G_{min} 0.61/0.001), which is larger than that between *Cecropteris jalapus* (Plötz, 1881) (Fig. 8 cyan) and *Cecropteris atthesis* (Hewitson, 1867) (Fig. 8 olive). Therefore, the Sonoran specimens represent a species distinct from *C.*

albociliatus, and because no available name applies to this species, it is new. Curiously this new species shares COI barcodes with its sister *C. albociliatus* (100% identical) but differs from it in male genitalia morphology (Fig. 10). The new species keys to “*Achalarus albociliatus albociliatus*” C.17.3(a) in Evans (1952) sharing with it the lack of costal fold, white (with a gray tint and some scales are more translucent) hindwing and brownish forewing fringes, but differing in the shape of valva in male genitalia: the valva is broader, ampulla is less developed, and is more in line with costa, separated from it by a slight concavity (Fig. 10a), instead of ampulla strongly bulging posterodorsad, separated from costa by a large concavity in *C. albociliatus* (Fig. 10c–e); harpe enlarged posteriad and broader, but relatively shorter compared to a narrower and longer harpe of *C. albociliatus*. A diagnostic combination of nuclear genome characters is: aly1651.25.1:T210C, aly1539.8.1:C888T, aly1089.5.3:G91A, aly1089.5.3:G121A, aly1222.33.2:T630C.



Fig. 9. Holotype of *Cecropterus markwalkeri* sp. n. dorsal (left) and ventral (right) views, data in text.

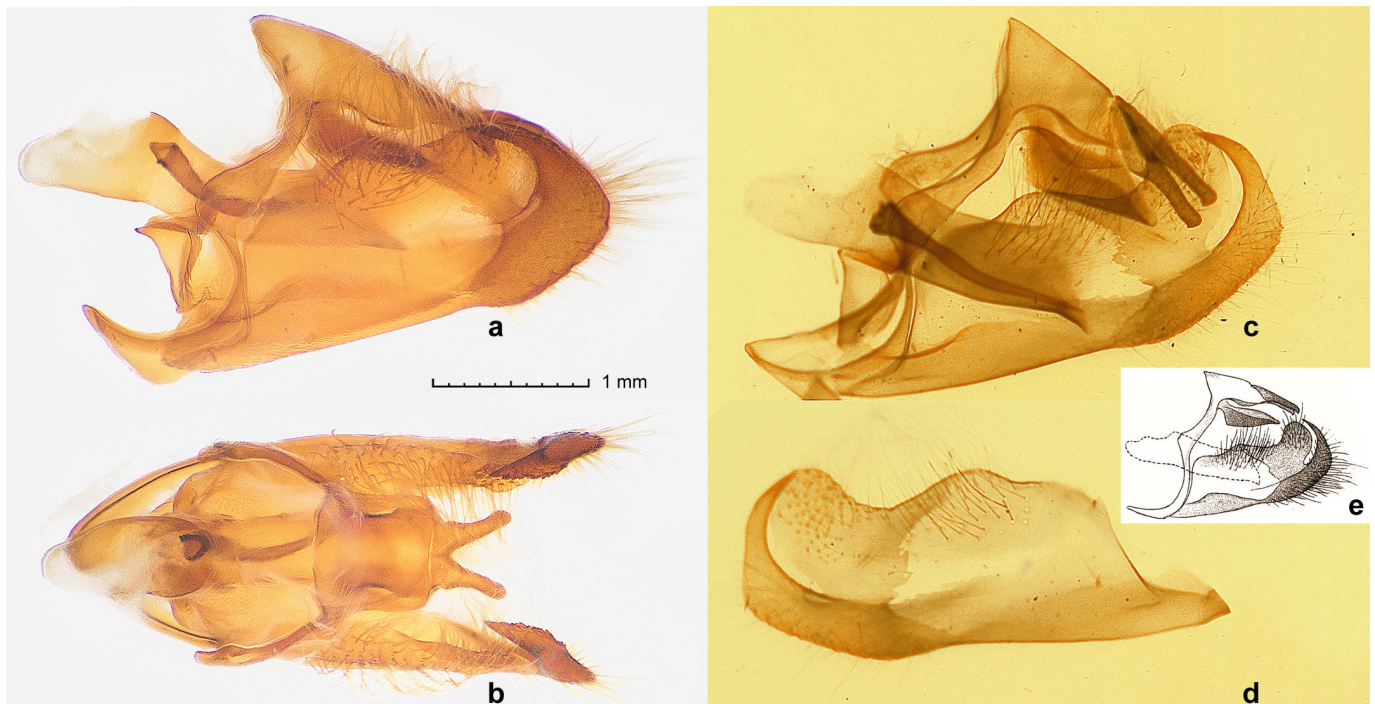


Fig. 10. Genitalia of *Cecropterus* (*Murgaria*). **a, b.** *C. markwalkeri* sp. n. holotype in left lateral (**a**) and dorsal (**b**) views. **c–e.** *C. albociliatus albociliatus* from Mexico: Veracruz, BMNH(E) 1717074, Godman’s mini-slide preparation [BMNH]: genital capsule in left lateral view with left valva removed (**c**), left valva in right lateral view (**d**), and genitalia illustration from Godman & Salvin (1894: pl. 80, fig. 14), not to scale (**e**). Photographs **c** and **d** are © The Trustees of the Natural History Museum London and are made available under Creative Commons License 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

Aguna malia Evans, 1952, stat. nov. is a species distinct from *Aguna megaeles* (Mabille, 1888)

Genomic sequencing of *Aguna megaeles* (Mabille, 1888) (type locality in Brazil: Santa Catarina) specimens, including its lectotype (NVG-15029D11) reveals that *Aguna megaeles malia* Evans, 1952 (type locality in Venezuela) (Fig. 12 red) is genetically differentiated from the nominotypical subspecies (Fig. 12 blue) at the level characteristic of species: e.g., their COI barcodes differ by 5.3% (35 bp). Therefore, we propose that it is a species-level taxon *Aguna malia* Evans, 1952, **stat. nov.**

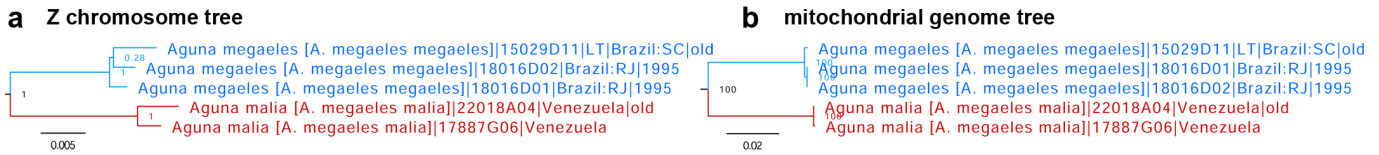


Fig. 12. Trees of *Aguna* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *A. megaeles* (blue) and *A. malia* **stat. nov.** (red).

Polygonus arizonensis (Skinner, 1911), **stat. nov.**, *Polygonus histrio* Röber, 1925, **stat. rest.**, *Polygonus pallida* Röber, 1925, **stat. nov.**, and *Polygonus hagar* Evans, 1952, **stat. nov.** are species distinct from *Polygonus leo* (Gmelin, [1790])

Genomic sequencing and analysis of *Polygonus leo* (Gmelin, [1790]) (type locality America, likely in Hispaniola) specimens from across the range reveal that all five subspecies of *P. leo* are genetically differentiated at the species level in both nuclear (Fig. 13a) and mitochondrial (Fig. 13b) DNA. E.g., COI barcodes of the closest taxa, i.e., the nominotypical *P. leo* (Fig. 13 purple) and *Polygonus (Acolastus) histrio* Röber, 1925 (type locality “vermutlich aus Panama”, but likely Cuba as suggested by DNA comparison) (Fig. 13 green) differ by 2.1% (14 bp). Therefore, we propose that *Polygonus arizonensis* (Skinner, 1911), **stat. nov.**, *Polygonus histrio* Röber, 1925, **stat. rest.**, *Polygonus pallida* Röber, 1925, **stat. nov.**, and *Polygonus hagar* Evans, 1952, **stat. nov.** are species, not subspecies.

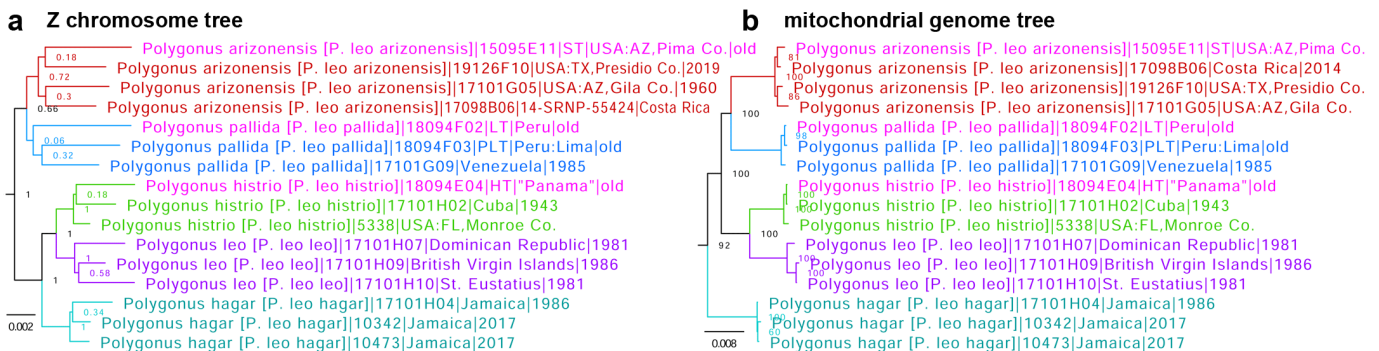


Fig. 13. Trees of *Polygonus leo* species complex constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *P. arizonensis* **stat. nov.** (red), *P. pallida* **stat. nov.** (blue), *P. histrio* **stat. rest.** (green), *P. leo* (purple), and *P. hagar* **stat. nov.** (cyan). Primary type specimens are labeled in magenta.

Viola dagamba Steinhauser, 1989 is a new junior subjective synonym of *Viola kuma* (Bell, 1942), **comb. nov.**, **stat. rest.**

Sequencing of the holotypes of *Viola dagamba* Steinhauser, 1989 (type locality in Guyana), currently a valid species, and *Pellicia kuma* Bell, 1942 (type locality in Venezuela), currently a junior subjective synonym of *Pachyneuria helena* (Hayward, 1939) (type locality in Ecuador: Rio Topo) reveals that they are conspecific (e.g., their COI barcodes are 100% identical) and belong to the genus *Viola* Evans, 1953 (type species *Staphylus alicus* Schaus, 1902), not *Pachyneuria* Mabille, 1888 (type species *Pachyneuria obscura* Mabille, 1888) (Fig. 14). Therefore, we reinstate *Viola kuma* (Bell, 1942), **comb. nov.**, **stat. rest.** as a species and treat *Viola dagamba* Steinhauser, 1989, **syn. nov.** as its junior subjective synonym.

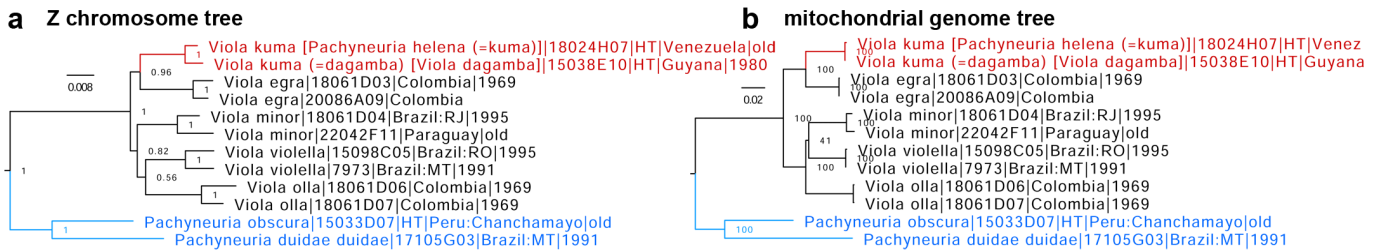


Fig. 14. Trees of *Viola* and *Pachyneuria* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *V. kuma* **comb. nov., stat. rest.** (red) and *Pachyneuria* (blue).

***Leucochitonea janice* Ehrmann, 1907 is a junior subjective synonym of *Heliopetes alana* (Reakirt, 1868) and not of *Heliopetes petrus* (Hübner, [1819])**

Genomic sequencing of the holotype of *Leucochitonea janice* Ehrmann, 1907 (NVG-15095C05, type locality Venezuela: Suapure) (Fig. 15 magenta) in CMNH, a taxon treated by Mielke (2005) as a junior subjective synonym of *Heliopetes petrus* (Hübner, [1819]) (type locality not given) (Fig. 15 red), reveals that it is not conspecific with it and instead is placed among specimens of *Heliopetes alana* (Reakirt, 1868) (type locality in Colombia) (Fig. 15 blue) in both nuclear (Fig. 15a) and mitochondrial (Fig. 15b) DNA trees. Therefore, we conclude that *Leucochitonea janice* Ehrmann, 1907 is a junior subjective synonym of *Heliopetes alana* (Reakirt, 1868) and not of *Heliopetes petrus* (Hübner, [1819]).

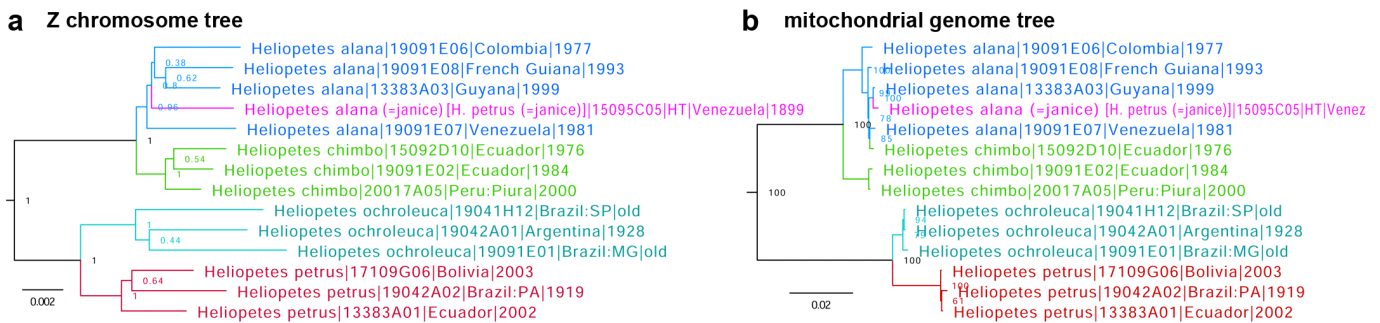


Fig. 15. Trees of *Heliopetes* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *H. alana* (blue) with the holotype of *Leucochitonea janice* Ehrmann, 1907 (magenta), *H. chimbo* Evans, 1953 (green), *H. ochroleuca* J. Zikán, 1938 (cyan), and *H. petrus* (red).

***Tamela maura* (Snellen, 1886), **stat. rest.**, and *Tamela diocles* (Moore, [1866]), **stat. rest.**, are species distinct from *Tamela othonias* (Hewitson, 1878) and *Tamela nigrita* (Latreille, [1824]), respectively**

Based on COI barcodes and morphological evidence, Xue et al. (2022) suggested recently that *Tamela othonias* (Hewitson, 1878) (type locality in Borneo) (Fig. 16 purple) and *Tamela fumatus* (Mabille, 1876) (type locality in the Philippines) (Fig. 16 cyan) are species distinct from *Tamela nigrita* (Latreille, [1824])

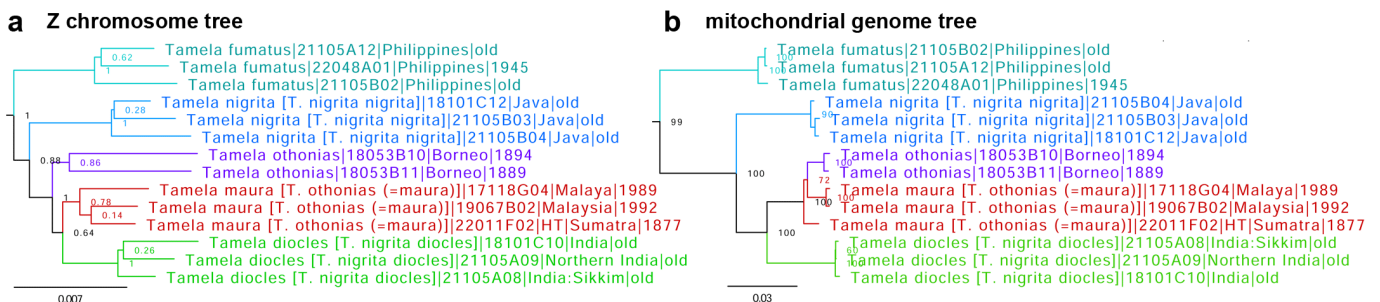


Fig. 16. Trees of *Tamela* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *T. fumatus* (cyan), *T. nigrita* (blue), *T. othonias* (purple), *T. maura* **stat. rest.** (red), and *T. diocles* **stat. rest.** (green).

(type locality in Java) (Fig. 16 blue). Our genomic results confirm that but also reveal that *Tamela nigrita diocles* (Moore, [1866]) (type locality in Bengal) (Fig. 16 green) is not monophyletic with *T. nigrita*, but instead is sister to *Tagiades maura* Snellen, 1886 (type locality in Sumatra) (Fig. 16 red), which Xue et al. (2022) regarded as a junior subjective synonym of *T. othonias*. While the association of *T. n. diocles* with *T. nigrita* is clearly wrong (Fig. 16), it is conceivable to place both *T. maura* and *T. n. diocles* in *T. othonias* as subspecies or synonyms. However, both taxa show genetic distinction from *T. othonias*, so we propose treating them as distinct species: *Tamela maura* (Snellen, 1886), **stat. rest.** and *Tamela diocles* (Moore, [1866]), **stat. rest.**, separate from either *T. othonias* or *T. nigrita*.

Hedone yunga Grishin, new species

<http://zoobank.org/8575DF83-2451-49B1-B6C4-AA4F9C595FF1>

(Figs. 17 part, 18)

Definition and diagnosis. Genomic sequencing of *Hedone* Scudder, 1872 (type species *Hesperia brettus* Boisduval & Le Conte, [1837]) reveals that one specimen from Bolivia was placed in the trees separately from all others, being sister to *Hedone catilina* (Plötz, 1886) (type locality Brazil: Santa Catarina, Blumenau; syntype NVG-18052B01 sequenced), but genetically differentiated from it at the level characteristic of species (Fig. 17). E.g., its COI barcode differs from that of the *H. catilina* syntype by 3.3% (22 bp). Therefore, this specimen represents a new species. This new species keys to “*Polites vibex catilina*” M.13.1(d) in Evans (1955) that is also known from Bolivia (Fig. 17) at a lower elevation but differs from it in females (male unknown) by the pattern of the ventral hindwing, which is whiter (instead

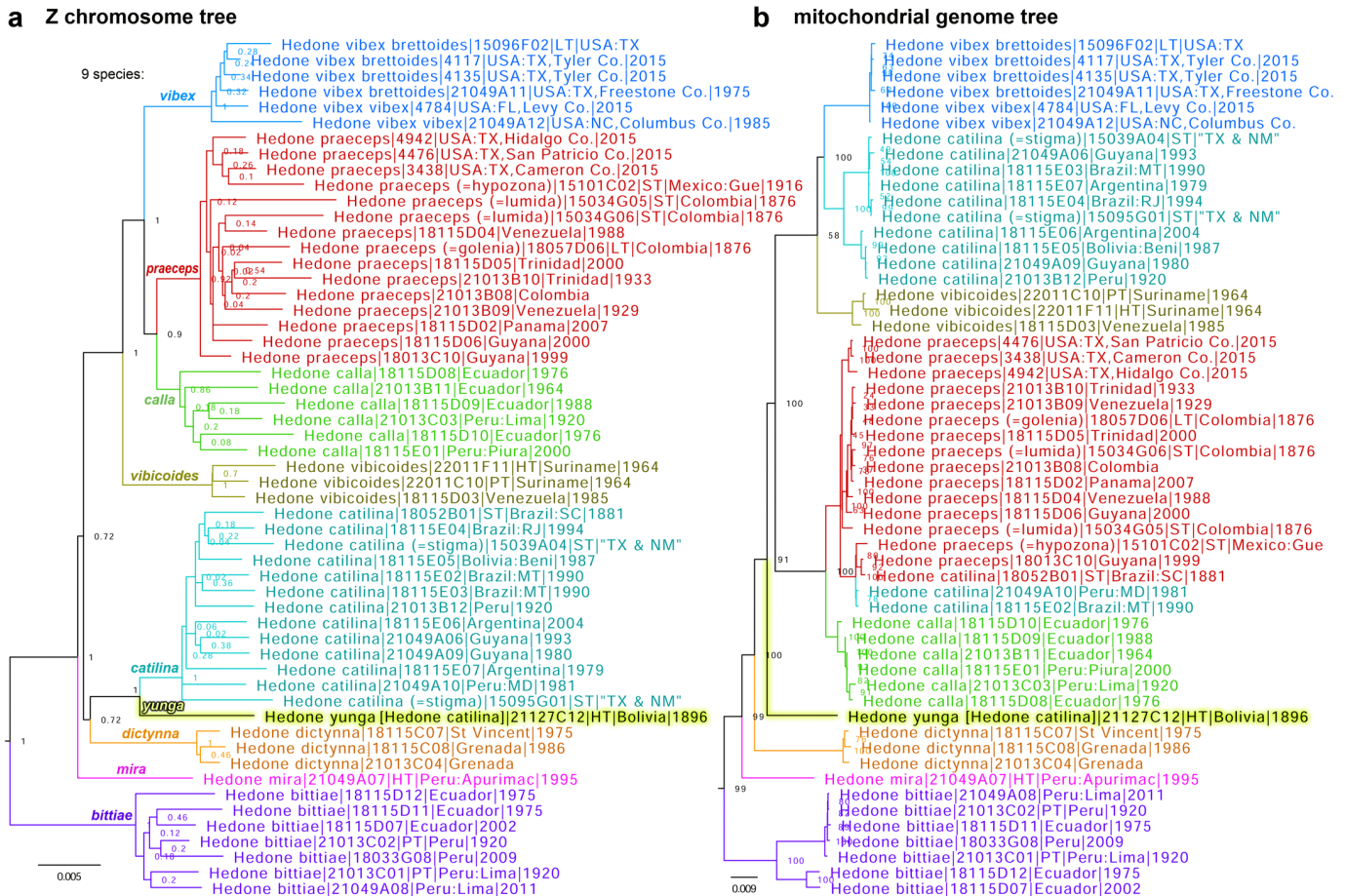


Fig. 17. Trees of nine *Hedone* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *H. vibex* (Geyer, 1832) (blue), *H. praeceps* Scudder, 1872 (red), *H. calla* (Evans, 1955) (green), *H. vibicoides* (de Jong, 1983) (olive), *H. catilina* (Plötz, 1886) (cyan), *H. yunga* sp. n. (highlighted in lime color), *H. dictynna* (Godman & Salvin, 1896) (orange), *H. mira* Grishin & Lamas, 2022 (magenta), and *H. bittiae* (Lindsey, 1925) (purple).

of yellow) overscaling, with more contrasty and better defined (rather than more diffuse) brown spots and a darker area by the end of the discal cell. In the absence of known males and without probing female variation, the most reliable identification is achieved by DNA, and a combination of the following base pairs is diagnostic in the nuclear genome: aly86.14.2:A4695C, aly23605.1.46:T819C, aly23605.1.46:G3606A, aly23605.1.46:T900A, aly23605.1.46:T2070G, aly127.52.1:T621T (not C), aly671.3.4:A189A (not G), aly1139.42.1:C165C (not T), aly1060.6.1:C1050C (not T), aly1042.7.1:A2148A (not G), and COI barcode: T4C, T412C, T406C, T547T, T646C.

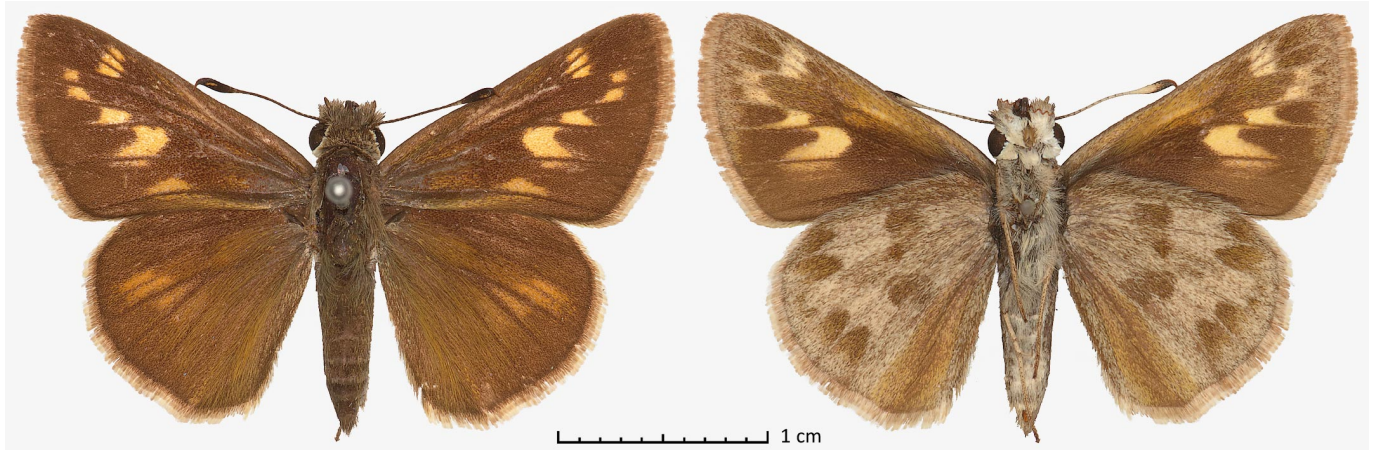


Fig. 18. Holotype of *Hedone yunga* sp. n. dorsal (left) and ventral (right) views, data in text.

Barcode sequence of the holotype: Sample NVG-21127C12, GenBank [OQ311411](https://www.ncbi.nlm.nih.gov/nuclseq/OL6811411.1), 658 base pairs:

```
AACCTTATATTTTATTTTGGTATTTGAGCAGGAATATTAGGAACCTCTTTAAGTTTATTAATTCGAACAGAATTAGGTAATCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACT
ATTGTAACAGCTCATGCTTTTATATAATTTTATAGTTATACCTATTATAATTTGGAGGATTTGGAAATGATTAGTCCATTAAATATTAGGAGCTCCTGATATAGCTTTCCCTCGAA
TAAATAATATAAGATTTTGAATATTACCCCTCATTAACATTATTAATTTCAAGAAGAATTGTAGAAAATGGTGCAGGAACAGGTTGAACAGTTTATCCTCCTTTATCTTCAAATATTGC
TCACCAAGGATCTTCTGTTGATTTAGCAATTTTCTCTTCACTTAGCCGGAATTTCTTCTATTTTAGGAGCTATTAATTTTATTACAACAATTTAATATAACGAATTTAAATTTATCT
TTTGATCAAATACCTTATTTGTATGATCTGTTGGAATTACAGCTCTTTTATTATTATCTTTACCTGTTTAGCTGGAGCTATTACTATATTACTTACAGATCGAAATTTAAATACTT
CATTTTTGATCCAGCAGGTTGGAGGAGATCCAATTTTATACCAACATTTATTT
```

Type material. Holotype: ♀ deposited in the Museum für Naturkunde, Berlin, Germany (MFNB), illustrated in Fig. 18, bears the following five rectangular labels, four white: [San Antonio (1800) | Bolivia (Yungas) | 1895—6. Garlepp], [Coll. | Staudinger], [] (no text on this label), [DNA sample ID: | NVG-21127C12 | c/o Nick V. Grishin], and one red [HOLOTYPE ♀ | Hedone | yunga Grishin].

Type locality. Bolivia: Yungas Region, La Paz Department, San Antonio.

Etymology. The name is given for the type locality. The name is a feminine noun in apposition.

Distribution. Currently known only from the holotype collected in the Yungas Region of Bolivia.

Comments. Without genomic sequencing that confidently supports the distinctness of this species based on a single female specimen, it would have been a challenge to describe this species without finding other specimens, including its male. Although wing patterns of the female holotype are recognizably different from other *Hedone* species, it is conceivable to hypothesize that it could have been an individual variation or aberration. On another note, a comparison of nuclear (Fig. 17a) and mitochondrial (Fig. 17b) DNA trees reveals incongruence typical for many species complexes in Lepidoptera and non-trivial evolutionary scenarios of mitogenome evolution that is likely riddled with introgression: *Hedone catilina* (Plötz, 1886) is polyphyletic (Fig. 17b cyan), and *H. catilina* with *Hedone bittiae* (Lindsey, 1925) (Fig. 17b purple) show several distinct mitogenome clusters that are not evident in nuclear DNA (Fig. 17a). Similar scenarios have been documented in other species groups (Zakharov et al. 2009; Cong et al. 2017).

***Vinius phellus* (Mabille, 1883), stat. rest. is a species distinct from *Vinius exilis* (Plötz, 1883)**

Genomic sequencing reveals prominent genetic differentiation between *Hesperia exilis* Plötz, 1883 (type locality “Californien”, in error, a possible (as hypothesized by G. Lamas) syntype in MFNB from Brazil:

Santa Catarina sequenced as NVG-21116A10) (Fig. 19 blue), currently a valid species in the genus *Vinius* Godman, 1900 (type species *Vinius arignote* Godman, 1900, which is a junior subjective synonym of *H. exilis*) and *Pamphila phellus* Mabilles, 1883 (type locality “Malaisie”, in error, probably French Guiana: Cayenne) (Fig. 19 red), currently a subspecies of the former species: e.g., their COI barcodes differ by 4% (26 bp). Therefore, we propose that *Vinius phellus* (Mabilles, 1883), **stat. rest.** is a species-level taxon.

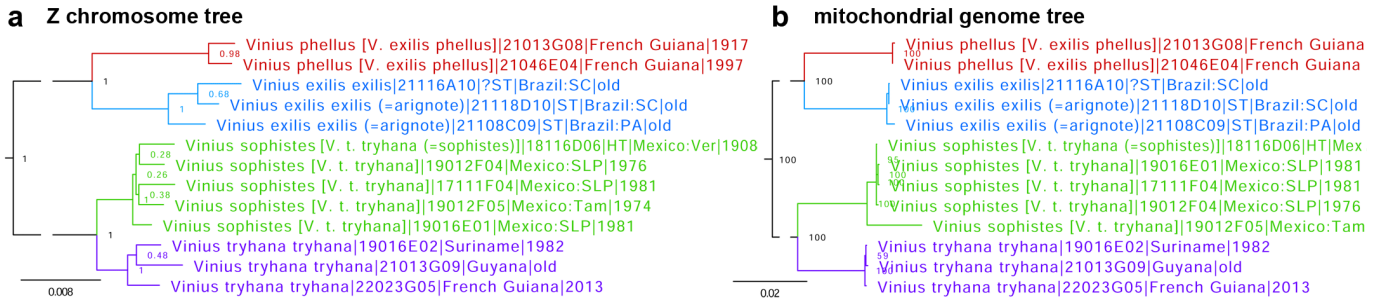


Fig. 19. Trees of *Vinius* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *V. phellus* (red), *V. exilis* (blue), *V. sophistes* (green), and *V. tryhana* (purple). Gaps in branches indicate where vertical slices of the tree were removed to reduce its horizontal dimension (to allow an increase of the font size), i.e., branches with gaps are longer than shown.

Vinius sophistes (Dyar, 1918), **stat. rest.** is a species distinct from *Vinius tryhana* (Kaye, 1914)

Genomic sequencing reveals prominent genetic differentiation between *Padraona tryhana* Kaye, 1914 (type locality in Trinidad) (Fig. 19 green), currently a valid species in the genus *Vinius* Godman, 1900 (type species *Vinius arignote* Godman, 1900, which is a junior subjective synonym of *Hesperia exilis* Plötz, 1883) and *Padraona sophistes* Dyar, 1918 (type locality in Mexico: Veracruz) (Fig. 19 purple), currently a junior subjective synonym of the former species: e.g., their COI barcodes differ by 3.6% (24 bp). Therefore, we propose that *Vinius sophistes* (Dyar, 1918), **stat. rest.** is a species-level taxon.

Rhinthon andricus (Mabilles, 1895), **stat. rest.** and *Rhinthon aqua* (Evans, 1955), **stat. nov.** are species distinct from *Rhinthon braesia* (Hewitson, 1867)

Genomic analysis of *Rhinthon* Godman, 1900 (type species *Proteides chiriquensis* Mabilles, 1889, a junior subjective synonym of *Hesperia osca* Plötz, 1882) specimens reveals that *Proteides andricus* Mabilles, 1895 (type locality in Brazil: Santa Catarina) (Fig. 20 red) currently treated as a subspecies of *Rhinthon braesia* (Hewitson, 1867) (type locality in Brazil: Pará) (Fig. 20 blue) is not monophyletic with it and is distinct from other species. Therefore, we reinstate it as a species-level taxon *Rhinthon andricus* (Mabilles, 1895), **stat. rest.** Furthermore, *Neoxeniades braesia aqua* Evans, 1955 (type locality Colombia: Rio Dagua) is genetically differentiated from the nominotypical *R. braesia* at the level characteristic of distinct species (Fig. 20a, F_{st}/G_{min} 0.48/0.003): e.g., compare with the pair *Rhinthon molion* (Godman, 1901) and *Rhinthon bajula* (Schaus, 1902). Therefore, we propose to treat *Rhinthon aqua* (Evans, 1955),

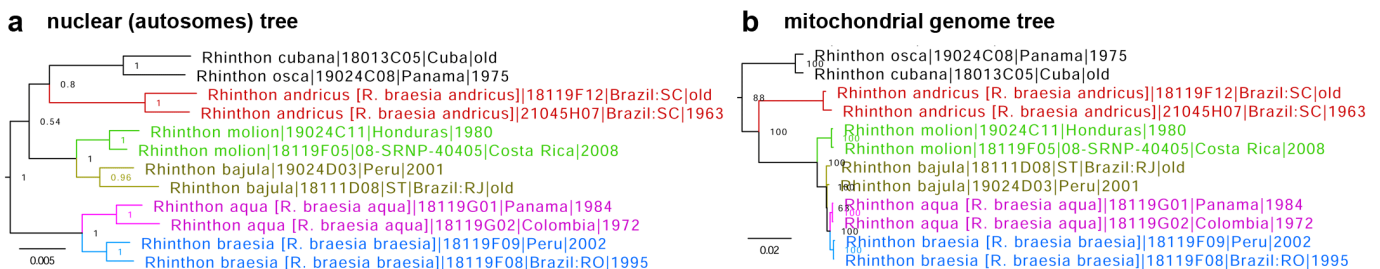


Fig. 20. Trees of *Rhinthon* species constructed from protein-coding regions in **a.** autosomes and **b.** mitochondrial genome: *R. andricus* **stat. rest.** (red), *R. molion* (green), *R. bajula* (olive), *R. aqua* **stat. nov.** (magenta), and *R. braesia* (blue).

stat. nov. as a species-level taxon. As a result of this analysis, *R. braesia* becomes monotypic. The taxon originally proposed as *Neoxeniades bajula peri* (Evans, 1955) (type locality in Brazil: Para) has been regarded as a valid species of *Niconiades* Hübner, [1821] (type species *Niconiades xanthaphes* Hübner, [1821]) by Zhang et al. (2022a). Finally, we note that mitochondrial DNA is largely shared among four species of *Rhinthon* (Fig. 20b), likely due to introgression.

The type locality of *Dion uza* (Hewitson, 1877) is likely in southern Brazil, and *Dion agassus* (Mabille, 1891) is confirmed as a valid species

The lectotype of *Hesperia uza* Hewitson, 1877 (type locality not stated) in MFNB designated by Mielke and Casagrande (2002) sequenced as NVG-18052D10 was subsequently designated as the neotype of *Hesperia pruinosa* Plötz, 1882 (type locality South America) in Zhang et al. (2022a), and this species was placed in the genus *Dion* Godman, 1901 (type species *Carystus gemmatus* Butler, 1872). The provenance of this specimen is unknown: no locality data were given on its labels or in the original description.

Here, we report genomic sequencing of several *Dion* specimens we found in MFNB that were collected at about the same time period as the *D. uza* lectotype. Although none of these specimens is as uniformly blue on the ventral hindwing as the lectotype, three of them cluster closely with the lectotype in both nuclear (Fig. 21a) and mitochondrial (Fig. 21b) DNA trees and therefore are conspecific with it. According to their labels, all three specimens are from southern Brazil: Espírito Santo (Southeast region of Brazil) and Santa Catarina (South region of Brazil). While it is not possible to pinpoint the type locality of *D. uza* with better precision using these specimens due to their genetic similarities, it is most likely that the lectotype was collected in southern Brazil, possibly in the states of Santa Catarina, Rio de Janeiro, or Espírito Santo.

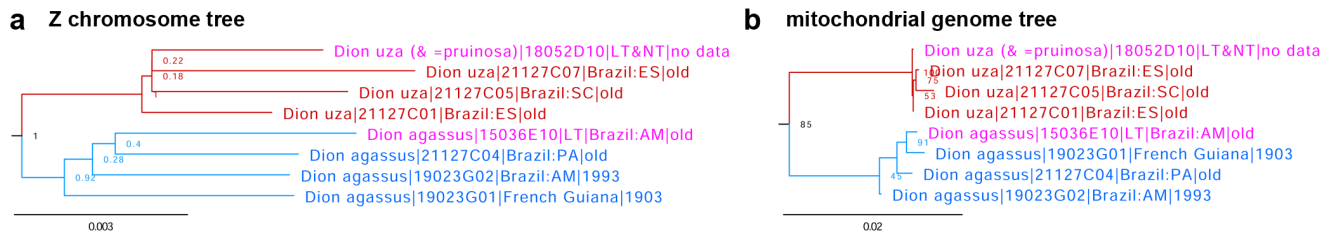


Fig. 21. Trees of *Dion* species constructed from protein-coding regions in **a.** Z chromosome and **b.** mitochondrial genome: *D. uza* (red) and *D. agassus* (blue). Primary type specimens are labeled in magenta.

Furthermore, Zhang et al. (2022a) treated *Dion agassus* (Mabille, 1891) (type locality Brazil: Amazonas, Massauary) as a species distinct from *D. uza* based on COI barcodes differences and phenotypic comparison of their lectotypes. Here, we confirm this treatment based on genomic sequencing of four specimens of each species (including their lectotypes) that support genetic distinction between the two species (Fig. 21) and report that COI barcodes of lectotypes of *Dion uza* (Hewitson, 1877) and *Dion agassus* (Mabille, 1891) differ by 2.9% (19 bp) (not 2.3% as stated in Zhang et al. (2022a) by mistake). The COI barcode sequence of the lectotype/neotype of *D. uza/H. pruinosa*, sample NVG-18052D10, GenBank accession [OQ311412](https://www.ncbi.nlm.nih.gov/nuccore/OQ311412), 658 base pairs is:

```
AACTTTATATTTTATTTTGGTATTTGAGCAGGAATATTAGGAACCTCTTAAGTTTACTAATTTCGAACAGAATTAGGTAATCCTGGCTCTTTAATTGGAGATGATCAAATTTATAACT
ATTGTAACAGCTCATGCTTTTATATAATTTTTCATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTCCCTTAATACTAGGAGCACCTGATATAGCTTTCCCGGAA
TAAATAATATAAGATTTGAATACTGCCACCCCTCCCTTATACTATTAACTTTAGTAGAATTGTAGAAAAGTGGAGCAGGACTGGATGAACAGTTTATCCCCCTCTTCTTCTAATATTGC
TCATCAAGGTTCTTCAGTTGATTTAGCAATTTTTCATTACATTTAGCAGGAATTTCTTCTATTAGTGTATTAATTTTATTACAACAATTTAATACATACGAATTAATAACTTATCA
TTTGATCAAATACCTTTATTTGTGTGATCTGTAGGTATTACAGCCTTATTACTATTATCTTTACCAGTATTAGCAGGAGCTATTACAATACTTCTTACTGATCGAAATTTAAATACTT
CTTTTTTGTATCCAGCAGGAGGAGGATCCAATTTTATATCAACATTTATTT
```

The COI barcode sequence of the lectotype of *D. agassus*, sample NVG-15036E10, GenBank accession [OQ311413](https://www.ncbi.nlm.nih.gov/nuccore/OQ311413), 658 base pairs is:

```
AACTTTATATTTTATTTTGGTATTTGAGCAGGAATATTAGGAACCTCTTTAAGTTTACTAATTTCGAACAGAATTAGGTAATCCTGGCTCTTTAATTGGAGACGATCAAATTTATAACT
ATTGTAACAGCTCATGCTTTTATATAATTTTTCATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTCCCTTTAATACTAGGAGCACCTGATATAGCTTTCCCGGAA
TAAATAATATAAGATTTGAATACTGCCACCCCTCCCTTATACTATTAACTTTAGTAGAATTGTAGAAAAGTGGAGCAGGACTGGATGAACAGTTTATCCCCCTCTTCTTCTAATATTGC
TCATCAAGGTTCTTCAGTTGATTTAGCAATTTTTCATTACATTTAGCAGGAATTTCTTCTATTAGTGTATTAATTTTATTACAACAATTTAATACATACGAATTAATAACTTATCA
TTTGATCAAATACCTTTATTTGTGTGATCTGTAGGTATTACAGCCTTACTGTTACTACTATCTTACCAGTATTAGCAGGAGCTATTACAATACTTCTTACTGATCGAAATTTAAATACTT
CTTTTTTGTATCCAGCAGGAGGAGGATCCAATTTTATACCAACATTTATTT
```


***Borna* Grishin, new subgenus**

<http://zoobank.org/C86F2094-5A1C-417C-AAC4-A9985ED28EDA>

Type species. *Godmania borincona* Watson, 1937.

Definition. This subgenus is represented in all trees by a clade sister to all other known *Choranthus* Scudder, 1872 (type species *Hesperia radians* Lucas, 1857) species (Fig. 22). Keys to M.24.6 in Evans (1955). Distinguished from its relatives by the following combination of characters: mid-tibiae smooth, ventral hindwing with dark, brownish (not bright orange) scales, in females uniformly colored, not much paler by the anal margin, dorsal hindwing in females brown with orange overscaling; in males, subapical orange spots on forewing form a nearly continuous orange band with other postdiscal spots; valva terminally deeply indented, shaped like a crab-claw. In DNA, a combination of the following base pairs is diagnostic in the nuclear genome: aly235.7.6:T37C, aly525.48.6:A129G, aly54.29.3:C201T, aly1022.3.14:A90G, aly904.12.5:A78T, and COI barcode: G101A, T112C, T115C, A376C, C483T, T568C.

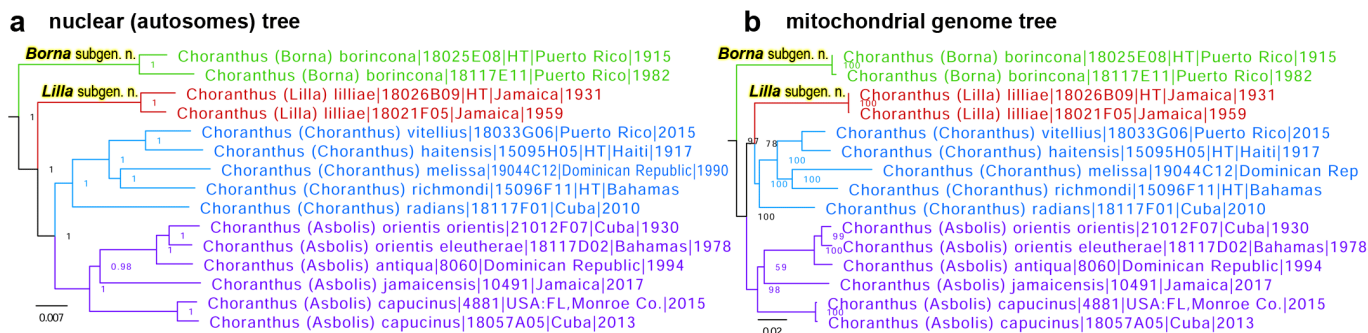


Fig. 22. Trees of *Choranthus* species constructed from protein-coding regions in **a.** autosomes and **b.** mitochondrial genome colored by subgenus: *Borna* **subgen. n.** (green), *Lilla* **subgen. n.** (red), *Choranthus* (blue), and *Asbolis* (purple).

Etymology. The name is a feminine noun in the nominative singular formed from the type species name *Bor[inco]na*.

Species included. Only the type species.

Parent taxon. Genus *Choranthus* Scudder, 1872.

***Lilla* Grishin, new subgenus**

<http://zoobank.org/7AAEDCAD-E796-4E4E-B3F8-37EFED5BCF15>

Type species. *Choranthus lilliae* Bell, 1931.

Definition. This subgenus is represented in the autosome genes tree by a clade sister to all other known *Choranthus* Scudder, 1872 (type species *Hesperia radians* Lucas, 1857) species except those in the subgenus *Borna* **subgen. n.** (Fig. 22). Keys to M.24.5 in Evans (1955). Distinguished from its relatives by the following combination of characters: mid-tibiae smooth, ventral hindwing with dark, brownish (not bright orange) scales, in females uniformly colored, not much paler by the anal margin, dorsal hindwing in females brown with a weak orange postdiscal band; in males, subapical orange spots on forewing clearly separated from other orange spots; uncus undivided, rounded, valva nearly elliptical in shape, terminally rounded, harpe not separated from the ampulla. In DNA, a combination of the following base pairs is diagnostic in the nuclear genome: aly1449.3.1:G59C, aly1603.21.2:C96T, aly145.9.4:G1015A, aly2487.37.1:A115T, aly274.10.13:C109T, and COI barcode: A43T, T121C, A274T, T367C, A430G, T533C.

Etymology. The name is a feminine noun in the nominative singular formed from the type species name *Lill[i]a[e]*.

Species included. Only the type species.

Parent taxon. Genus *Choranthus* Scudder, 1872.

***Asbolis* Mabille, 1904 is a subgenus of *Choranthus* Scudder, 1872**

Both *Asbolis* Mabille, 1904-[IV] (type and the only species *Goniloba sandarac* Herrich-Schäffer, 1865, a junior subjective synonym of *Eudamus capucinus* Lucas, 1857) and *Pyrrhocalles* Mabille, 1904-[V] (type species *Pamphila antiqua* Herrich-Schäffer, 1863) were regarded by Zhang et al. (2022a) as junior subjective synonyms of *Choranthus* Scudder, 1872 (type species *Hesperia radians* Lucas, 1857) due to their genetic similarities. Nevertheless, while being very closely related to each other (COI barcodes differ by 5.6%, 37 bp), contrasting with their difference in appearance, *Asbolis* and *Pyrrhocalles* show larger genetic differentiation from *Choranthus* in the nuclear genome (Fig. 22). Treating *Asbolis* combined with *Pyrrhocalles* as a subgenus would mean that the clades of *C. borincona* and *C. lilliae* should be considered subgenera as well, because *Asbolis* is closer related to *Choranthus* than *Choranthus* to *C. borincona* and *C. lilliae*. Presently, because these two clades have been defined as subgenera **Borna subgen. n.** and **Lilla subgen. n.**, it is meaningful to propose that *Asbolis* Mabille, 1904 is a subgenus of *Choranthus* Scudder, 1872 rather than its synonym (Fig. 22). Then, due to genetic similarities, we treat *Pyrrhocalles* as a junior (by about a month) subjective synonym of *Asbolis*. As a result, the subgenus *Asbolis* consists of all taxa listed by Mielke (2005) under *Pyrrhocalles* and *Asbolis*.

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LITERATURE CITED

- Ballmer, G. R. 2022.** Life History and Ecology of the San Emigdio Blue Butterfly (Lepidoptera: Lycaenidae). The Taxonomic Report of the International Lepidoptera Survey 10(9): 1–16.
- Cong, Q., E. P. Barbosa, M. A. Marín, A. V. L. Freitas, G. Lamas, and N. V. Grishin. 2021.** Two new species of *Hermeuptychia* from North America and three neotype designations (Nymphalidae: Satyrinae). The Taxonomic Report of the International Lepidoptera Survey 9(7): 1–20.
- Cong, Q., J. Shen, D. Borek, R. K. Robbins, P. A. Opler, Z. Otwinowski, and N. V. Grishin. 2017.** When COI barcodes deceive: complete genomes reveal introgression in hairstreaks. Proceedings of the Royal Society B: Biological Sciences 284(1848): 20161735.
- Cong, Q., J. Zhang, and N. V. Grishin. 2019a.** Genomic determinants of speciation. bioRxiv BIORXIV/2019/837666.
- Cong, Q., J. Zhang, J. Shen, and N. V. Grishin. 2019b.** Fifty new genera of HesperIIDae (Lepidoptera). Insecta Mundi 0731: 1–56.
- Evans, W. H. 1952.** A catalogue of the American HesperIIDae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part II. Pyrginae. Section I. The Trustees of the British Museum (Natural History); London. v + 178 pp., pls. 10–25.
- Evans, W. H. 1955.** A catalogue of the American HesperIIDae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part IV. HesperIIDae and Megathyminae. The Trustees of the British Museum (Natural History); London. v + 499 pp., pls. 54–88.
- Farfán, J., J. Cerdeña, W. Huanca-Mamani, H. A. Vargas, G. L. Gonçalves, and G. R. P. Moreira. 2022a.** Host plant variation and lack of genetic differentiation in populations of *Dione (Agraulis) dodona* Lamas & Farfan (Lepidoptera: Nymphalidae). Insects 13(819): 1–14.
- Farfán, J., J. Cerdeña, H. A. Vargas, G. L. Gonçalves, G. Lamas, and G. R. P. Moreira. 2022b.** A peculiar new species of *Dione (Agraulis)* Boisduval & Le Conte (Lepidoptera: Nymphalidae: Heliconiinae) associated with *Malesherbia* Ruiz & Pavón (Passifloraceae) in xeric western slopes of the Andes. Zookeys 1113: 199–227.
- Godman, F. D., and O. Salvin. 1894.** Pl. 80 in *Biologia Centrali-Americana*. Insecta. Lepidoptera-Rhopalocera. Dulau & Co., Bernard Quaritch; London. v. 3 (plates).
- Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018.** UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35(2): 518–522.
- iNaturalist. 2022.** A Community for Naturalists – iNaturalist. Available from <https://www.inaturalist.org>. Accessed 30 January 2023.
- Li, W., Q. Cong, J. Shen, J. Zhang, W. Hallwachs, D. H. Janzen, and N. V. Grishin. 2019.** Genomes of skipper butterflies reveal extensive convergence of wing patterns. Proceedings of the National Academy of Sciences of the United States of America 116(13): 6232–6237.

- Lukhtanov, V. A., and A. V. Gagarina. 2022.** Molecular Phylogeny and Taxonomy of the Butterfly Subtribe Scolitantidina with Special Focus on the Genera *Pseudophilotes*, *Glauropsyche* and *Iolana* (Lepidoptera, Lycaenidae). *Insects* 13(12): 1110.
- Mielke, O. H. H. 2005.** Catalogue of the American Hesperioidea: HesperIIDae (Lepidoptera). Sociedade Brasileira de Zoologia; Curitiba, Paraná, Brazil. xiii + 1536 pp.
- Mielke, O. H. H., and M. M. Casagrande. 2002.** Notas taxonômicas em HesperIIDae neotropicais, com descrições de novos taxa (Lepidoptera). *Revista brasileira de Zoologia* 19(Suplemento 1): 27–76.
- Nabokov, V. 1945.** Notes on the Morphology of the Genus *Lycaeides* (Lycænidæ, Lepidoptera). *Psyche: A Journal of Entomology* 51(3/4): 104–138.
- Núñez, R., K. R. Willmott, Y. Álvarez, J. A. Genaro, A. R. Pérez-Asso, M. Quejereta, T. Turner, J. Y. Miller, C. Brévignon, G. Lamas, and A. Hausmann. 2022.** Integrative taxonomy clarifies species limits in the hitherto monotypic passion-vine butterfly genera *Agraulis* and *Dryas* (Lepidoptera, Nymphalidae, Heliconiinae). *Systematic Entomology* 47(1): 152–178.
- Pelham, J. P. 2022.** Catalogue of the Butterflies of the United States and Canada. Revised 2 February 2022. <<http://www.butterfliesofamerica.com/US-Can-Cat.htm>> Accessed 30 January 2023.
- Penz, C. M. 2022.** Reinstated status of the butterfly genus *Agraulis* (Lepidoptera, Nymphalidae, Heliconiinae). *Zootaxa* 5209(3): 394–398.
- Scott, J. A. 1986.** The Butterflies of North America: A Natural History and Field Guide. Stanford University Press; Stanford, CA. xiii + 583 pp
- Shen, J., Q. Cong, D. Borek, Z. Otwinowski, and N. V. Grishin. 2017.** Complete genome of *Achalarus lyciades*, the first representative of the Eudaminae subfamily of skippers. *Current Genomics* 18(4): 366–374.
- Stresemann, E. 1954.** Ferdinand Deppe's travels in Mexico, 1824-1829. *Condor* 56(2): 86–92.
- Talavera, G., V. A. Lukhtanov, N. E. Pierce, and R. Vila. 2012.** Establishing criteria for higher-level classification using molecular data: the systematics of *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae). *Cladistics* 29: 166–192.
- Xue, G., P. Qu, M. Li, H. Chiba, and W. Li. 2022.** Molecular and morphological evidences confirm the statuses of *Ancistroides othonias* (Hewitson, 1878) and the subspecies of *A. nigrita* (Latreille, [1824]), *sensu* Evans 1949 (Lepidoptera, HesperIIDae). *Zootaxa* 5205(5): 481–490.
- Zakharov, E. V., N. F. Lobo, C. Nowak, and J. J. Hellmann. 2009.** Introgression as a likely cause of mtDNA parphyly in two allopatric skippers (Lepidoptera: HesperIIDae). *Heredity (Edinb)* 102(6): 590–599.
- Zhang, J., Q. Cong, and N. V. Grishin. 2023.** Thirteen new species of butterflies (Lepidoptera: HesperIIDae) from Texas. *Insecta Mundi* 0969: 1–58.
- Zhang, J., Q. Cong, J. Shen, and N. V. Grishin. 2022a.** Taxonomic changes suggested by the genomic analysis of HesperIIDae (Lepidoptera). *Insecta Mundi* 0921: 1–135.
- Zhang, J., Q. Cong, J. Shen, P. A. Opler, and N. V. Grishin. 2019.** Changes to North American butterfly names. *The Taxonomic Report of the International Lepidoptera Survey* 8(2): 1–11.
- Zhang, J., Q. Cong, J. Shen, P. A. Opler, and N. V. Grishin. 2020.** Genomic evidence suggests further changes of butterfly names. *The Taxonomic Report of the International Lepidoptera Survey* 8(7): 1–40.
- Zhang, J., Q. Cong, J. Shen, P. A. Opler, and N. V. Grishin. 2021.** Genomics-guided refinement of butterfly taxonomy. *The Taxonomic Report of the International Lepidoptera Survey* 9(3): 1–54.
- Zhang, J., Q. Cong, J. Shen, L. Song, R. Gott, J., P. Boyer, C. S. Guppy, S. Kohler, G. Lamas, P. A. Opler, and N. V. Grishin. 2022b.** Taxonomic discoveries enabled by genomic analysis of butterflies. *The Taxonomic Report of the International Lepidoptera Survey* 10(7): 1–59.
- Zhang, J., Q. Cong, J. Shen, L. Song, and N. V. Grishin. 2022c.** Genomic DNA sequencing reveals two new North American species of *Staphylus* (HesperIIDae: Pyrginae: Carcharodini). *The Taxonomic Report of the International Lepidoptera Survey* 10(4): 1–13.

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