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Genomic evidence suggests further changes of butterfly names

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ABSTRACT. Further genomic sequencing of butterflies by our research group expanding the coverage of species and specimens from different localities, coupled with genome-scale phylogenetic analysis and complemented by phenotypic considerations, suggests a number of changes to the names of butterflies, mostly those recorded from the United States and Canada. Here, we present evidence to support these changes. The changes are intended to make butterfly classification more internally consistent at the genus, subgenus and species levels. I.e., considering all available evidence, we attempt to assign similar taxonomic ranks to the clades of comparable genetic differentiation, which on average is correlated with the age of phylogenetic groups estimated from trees. For species, we use criteria devised by genomic analysis of the genetic differentiation across suture zones and comparison of sympatric populations of closely related species. As a result, we resurrect 4 genera and 1 subgenus from subgeneric status or synonymy, change the rank of 8 currently used genera to subgenus, synonymize 7 genus-group names, summarize evidence to support 19 taxa as species instead of subspecies and 1 taxon as subspecies instead of species, along with a number of additional changes. One new genus and one new subspecies are described. Namely, the following taxa are treated as genera *Tharsalea* Scudder, 1876, *Helleia* Verity, 1943, *Apangea* Zhdanko, 1995, and *Boldenaria* Zhdanko, 1995. *Tetracharis* Grote, 1898 is a valid subgenus (not a synonym of *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833]) that consists of *Anthocharis cethura* C. Felder & R. Felder, 1865 (Müller, 1764), *Anthocharis midea* (Hübner, [1809]), and *Anthocharis limonea* (A. Butler, 1871). The following are subgenera: *Speyeria* Scudder, 1872 of *Argynnis* Fabricius, 1807; *Aglais* Dalman, 1816 and *Polygonia* Hübner, [1819] of *Nymphalis* Kluk, 1780; *Palaeonympha* Butler, 1871 of *Megisto* Hübner, [1819]; *Hyponephele* Muschamp, 1915 of *Cercyonis* Scudder, 1875; *Pyronia* Hübner, [1819] and *Aphantopus* Wallengren, 1853 of *Maniola* Schrank, 1801 and *Pseudonymphidia* Callaghan, 1985 of *Pachythone*. *Lafron* Grishin, **gen. n.** (type species *Papilio orus* Stoll, [1780], parent subfamily *Lycaeninae* [Leach], [1815]) is described. *Dipsas japonica* Murray, 1875 is fixed as the type species of *Neozephyrus* Sibatani & Ito, 1942. The following taxa are junior subjective synonyms: *Falcapica* Klots, 1930 of *Tetracharis* Grote, 1898; *Habrodais* Scudder, 1876, *Favonius* Sibatani & Ito, 1942, *Neozephyrus* Sibatani & Ito, 1942, *Quercusia* Verity, 1943, *Chrysozephyrus* Shirôzu & Yamamoto, 1956, and *Sibataniozephyrus* Inomata, 1986 of *Hypaurotis* Scudder, 1876; *Plesioarida* Trujano & García, 2018 of *Roeberella* Strand, 1932; *Papilio temenes* Godart, 1819 (lectotype designated herein) of *Heraclides aristodemus* (Esper, 1794), *Speyeria hydaspe conquista* dos Passos & Grey, 1945 of *Argynnis hesperis tetonia* (dos Passos & Grey, 1945), and *Erycides imbreus* Plötz, 1879 of *Phocides polybius polybius* (Fabricius, 1793). The following are revised genus-species combinations: *Pachythone lencates* (Hewitson, 1875) *Pachythone flocculus* (Brévignon & Gallard, 1993), *Pachythone floccus* (Brévignon, 2013), *Pachythone heberti* (P. Jauffret & J. Jauffret, 2007), *Pachythone marajoara* (P. Jauffret & J. Jauffret, 2007) and *Cissia cleophes* (Godman & Salvin, 1889). The following species are transferred between subgenera: *Anthocharis lanceolata* Lucas, 1852 belongs to *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833] instead of *Paramidea* Kuznetsov, 1929 and *Danaus eresimus* (Cramer, 1777) belongs to *Danaus* Kluk, 1780, and not to *Anosia* Hübner, 1816. The following taxa are distinct species rather than subspecies (of species shown in parenthesis): *Heraclides ponceana* (Schaus, 1911) (not *Heraclides aristodemus* (Esper, 1794)), *Colias elis* Strecker, 1885 (not *Colias meadii* W. H. Edwards, 1871), *Argynnis irene* Boisduval, 1869 and *Argynnis nausicaa* W. H. Edwards, 1874 (not *Argynnis hesperis* W. H. Edwards, 1864), *Coenonympha california* Westwood, [1851] (not *Coenonympha tullia* (Müller, 1764)), *Dione incarnata* N. Riley, 1926 (not *Dione vanillae* (Linnaeus, 1758)), *Chlosyne coronado* (M. Smith & Brock, 1988) (not *Chlosyne fulvia* (W. H. Edwards, 1879)), *Chlosyne chinatiensis* (Tinkham, 1944) (not *Chlosyne theona* (Ménétriés, 1855)), *Phocides lilea* (Reakirt, [1867]) (not *Phocides polybius* (Fabricius, 1793)),

Cecropterus nevada (Scudder, 1872) and *Cecropterus dobra* (Evans, 1952) (not *Cecropterus mexicana* (Herrich-Schäffer, 1869)), *Telegonus anausis* Godman & Salvin, 1896, (not *Telegonus anaphus* (Cramer, 1777)), *Epargyreus huachuca* Dixon, 1955 (not *Epargyreus clarus* (Cramer, 1775)), *Nisoniades bromias* (Godman & Salvin, 1894) (not *Nisoniades rubescens* (Möschler, 1877)), *Pholisora crestar* J. Scott & Davenport, 2017 (not *Pholisora catullus* (Fabricius, 1793)), *Carterocephalus mandan* (W. H. Edwards, 1863) and *Carterocephalus skada* (W. H. Edwards, 1870) (not *Carterocephalus palaemon* (Pallas, 1771)), *Amblyscirtes arizonae* H. Freeman, 1993 (not *Amblyscirtes elissa* Godman, 1900), and *Megathymus violae* D. Stallings & Turner, 1956 (not *Megathymus ursus* Poling, 1902). Resulting from these changes, the following are revised species-subspecies combinations: *Heraclides ponceana bjorndalae* (Clench, 1979), *Heraclides ponceana majasi* L. Miller, 1987, *Argynnis irene dodgei* Gunder, 1931, *Argynnis irene cottlei* J. A. Comstock, 1925, *Argynnis irene hanseni* (J. Emmel, T. Emmel & Mattoon, 1998), *Argynnis nausicaa elko* (Austin, 1984), *Argynnis nausicaa greyi* (Moeck, 1950), *Argynnis nausicaa viola* (dos Passos & Grey, 1945), *Argynnis nausicaa tetonia* (dos Passos & Grey, 1945), *Argynnis nausicaa chitone* W. H. Edwards, 1879, *Argynnis nausicaa schellbachi* (Garth, 1949), *Argynnis nausicaa electa* W. H. Edwards, 1878, *Argynnis nausicaa dorothea* (Moeck, 1947), and *Argynnis nausicaa capitanensis* (R. Holland, 1988), *Argynnis zerene atossa* W. H. Edwards, 1890, *Dione incarnata nigrior* (Michener, 1942), *Chlosyne coronado pariaensis* (M. Smith & Brock, 1988), *Cecropterus nevada aemilea* (Skinner, 1893), *Cecropterus nevada blanca* (J. Scott, 1981), *Telegonus anausis annetta* (Evans, 1952), *Telegonus anausis anoma* (Evans, 1952), *Telegonus anausis aniza* (Evans, 1952), *Epargyreus huachuca profugus* Austin, 1998, *Carterocephalus mandan mesapano* (Scudder, 1868) and *Carterocephalus skada magnus* Mattoon & Tilden, 1998. American *Coenonympha* subspecies placed under *C. tullia* other than *Coenonympha tullia kodiak* W. H. Edwards, 1869, *Coenonympha tullia mixturata* Alpheraky, 1897 and *Coenonympha tullia yukonensis* W. Holland, 1900 belong to *C. california*. *Heraclides ponceana latefasciatus* Grishin, **ssp. n.** is described from Cuba. *Argynnis coronis carolae* dos Passos & Grey, 1942 is considered a subspecies-level taxon. Unless stated otherwise, all subgenera, species, subspecies and synonyms of mentioned genera and species are transferred together with their parent taxa, and others remain as previously classified.

Key words: taxonomy, classification, genomics, phylogeny, biodiversity.

ZooBank registration: http://zoobank.org/9A8DCBC8-A9D5-4083-B640-BA5101827478

INTRODUCTION

DNA-based phylogenies (Zuckerkandl and Pauling 1965) revolutionized the way we view animal classification and taxonomy, including butterflies (Mutanen et al. 2010). Previously relying on several carefully selected gene markers, DNA methods have evolved towards sequencing and comparison of whole genomes. Genome-scale approaches aim at utilizing all DNA of an organism and thus are most comprehensive and accurate, frequently revealing inconsistencies between phylogeny and current classification (Kawahara and Breinholt 2014; Espeland et al. 2018; Allio et al. 2019; Li et al. 2019; Zhang et al. 2019a; Zhang et al. 2019b). Our research group genome-sequenced all butterfly species recorded from the United States and Canada (USC) (Zhang et al. 2019d) and proposed refinements to butterfly taxonomy (Zhang et al. 2019c). Currently we are working on extending our genomic datasets to cover subspecies and populations, in addition to species from other parts of the world. Review of these datasets suggests additional changes based on the analysis of phylogenetic trees and genetic variation between and within species. Here, we present these results after explaining the general logic and methods behind them.

Total DNA is extracted from a leg (or other parts of a butterfly), fragmented into short pieces (unless the specimen is old and DNA is already degraded) and sequenced in 150 base pair (bp) segments. Thus, every fragment of DNA present in the sample is getting sequenced, resulting in the coverage of the entire genome, both nuclear and mitochondrial, coding and non-coding (Zhang et al. 2019a). Because this approach can sequence very short DNA (e.g. 25-50 bp), even very old specimens with degraded DNA yield usable data (Cong et al. 2019a). In this work, we mostly use DNA sequences of protein-coding genes, which are computationally selected from the total pool of DNA sequences to match a known protein set of a butterfly. Butterflies typically have nearly 15 thousand genes, and the total number of base pairs that we get for phylogenetic analysis is about 10 million (Cong et al. 2015; Zhang et al. 2019d). For comparison, a good dataset of gene markers typically covers less than 10 thousand base pairs. Due to the vast size of genomic datasets, phylogenies resulting from them are usually reliable. Reliability of each node in the tree is indicated by a number next to that node, the closer it is to 1, the more reliable is the node. Furthermore, the genotype encodes phenotype, thus, all the features of wing patterns and

morphology of butterflies and their life stages (including caterpillar foodplant preferences) are encoded in these genomic sequences. For this reason, the genomic dataset we sequence may be the best representation of a butterfly (richer than a pinned adult specimen that we visually inspect), given a meaningful approach to analyze these genomes.

Armed with genome-scale phylogenetic trees, we have ventured to propose taxonomic changes. The majority of the changes discussed below deal with a change of rank in the classification. For genus-group names, we have changed the rank of taxa from genus to subgenus, or move between valid names and synonyms. For species-group names, mainly we have changed the rank of subspecies to species. The reason for these changes is to bring these categories in better agreement with their definitions, although such definitions are never precise and are continuously being refined with additional knowledge gained.

A genus is defined as a monophyletic group (a clade in an accurate phylogenetic tree) of related species that is below the level of a tribe or subtribe. Currently, there are no objective criteria for grouping species into genera. However, the genus level is probably more important than any other classification level above species, because a species name contains a genus name as the first word. Therefore, for its optimal practical use, a genus should be defined neither too stringently to encompass only a small group of very close relatives, nor should it be too broadly defined to include too distant relatives. Subjectively, taxonomists follow their own intuition about where to draw a boundary of a genus. Our thoughts on the criteria for a genus are discussed in the Taxonomic Appendix to Li et al. (2019) and the Introduction in Grishin (2019). First, a genus should be a major and prominent phylogenetic group (i.e., it is best if the phylogenetic tree branch leading to the last common ancestor of the genus is longer than the nearby branches). Secondly, genetic diversity is a function of the time since the origin of a genus. Theoretically, a genus may be defined as all ancestors of a species that lived at some given point in the past. Practically this definition can be applied through a narrow slice across a time-calibrated phylogenetic tree at a specific time point (Talavera et al. 2012; Li et al. 2019). While it is not possible to establish a distance cutoff using COI barcodes (or any other gene markers) when defining a genus due to the reasons explained by Trujano-Ortega et al. (2020), a comparison of COI distances may be instructive and should be used as evidence combined with other considerations. Provided these general criteria, it is also best if most genera are in agreement with how they are currently defined (mostly using phenotypic considerations, such as similarity in appearance), to avoid additional name changes. Further examples of how we apply these principles to specific cases are given in the Discussion section below.

Meaningful groups of species within a genus can be given a rank of subgenus. Subgenera are useful to define clades that are not as prominent as a genus, but represent groups of very close relatives, mostly recognizable immediately by their appearance as such. Although frequently frowned upon and synonymized or treated as a genus instead, a subgenus is one of just eight levels of the ICZN Code hierarchy of names (ICZN 1999). The number of levels in a phylogenetic tree is larger than these eight categories. Therefore, it seems wasteful to ignore the level that is aimed at refining the classification and indicating phylogenetic substructure within a large and diverse genus. We use subgenus category freely and suggest that many groups currently viewed as genera may be subgenera instead: it seems more valuable to stress their relatedness by uniting them in a broader genus, yet indicating their distinction by keeping them as subgenera. We believe that the consistency criterion is of importance here as well. I.e., groups defined as subgenera in one lineage should correspond to subgenera of comparable genetic differentiation and time of origin in another lineage.

Species definitions (=concepts) have been extensively addressed in the literature (Mallet 1995). Comparing many species concepts that have been proposed (Aldhebiani 2018), our view is closest to what Claridge (2017) described as a "broadly based biological species concept", which seems similar to the "genomic integrity species definition" of Sperling (2003). I.e., species are defined by some reproductive barrier, which is not absolute but porous (Mallet et al. 2016); hybrids are characterised by lower fitness and usually are eliminated from the population, thus allowing each species to maintain its genetic uniqueness (=integrity) that persists in time despite on-going hybridization with other species. This definition does not include phenotypic distinction between species, and allows for a possibility of "cryptic" species that look superficially indistinguishable, but are to some extent reproductively isolated

from each other. Equally, this definition does not exclude hybrid species, i.e. those that originate by a significant gene influx of one species into a population of another that occurred over a relatively short period of time. If this population that experienced the influx persists in time and is reproductively isolated from both of its parent species to the extent comparable to isolation in other related species, then it can be called a hybrid species, in particular, if it expands its range and further diversifies.

Traditionally, species are defined by morphological distinctiveness. Morphological distinctiveness accumulates with time as a result of reproductive isolation, but it does not necessarily contribute to it. Therefore, using a morphological consideration does not provide direct evidence of reproductive isolation and thus speciation. On the contrary, using genomic DNA sequences, we can study reproductive isolation directly and look for segments of DNA from different species in a species' genome under study. Presence of such segments would indicate hybridization and the fraction of such segments would indicate the extent of hybridization. Thus, analysis of genomic sequences may be the best practical approach to species definition available today.

We carried out a study to devise genomic criteria for distinguishing different species from populations of the same species (Cong et al. 2019b). We studied sister butterfly populations across a central Texas suture zone. Suture zones are geographic boundaries common for many pairs of closely related species (counterparts) (Remington 1968). This central Texas suture zone is old (Newton 2003), therefore pairs that attained species status across it are likely to be more strongly isolated reproductively than more recently speciated pairs. Therefore, our criteria obtained using this old zone are likely to be conservative. We analyzed transcriptomes (mostly protein-coding RNA) of 25 pairs of western and eastern counterparts around the central Texas suture zone. Some of these counterparts were different species like *Heraclides cresphontes* (Cramer, 1777) and *H. rumiko* Shiraiwa & Grishin, 2014, others were populations of the same species from the north and south Texas, e.g. *Hylephila phyleus* (Drury, 1773).

We found that two DNA-based measures best separate distinct species from conspecific populations. These measures were fixation index (Fst) and the extent of gene flow (measured by Gmin), both computed on the sex Z chromosome-encoded genes. Male butterflies have two Z chromosomes, while females have one Z and one W, which is a special female chromosome. Fst is frequently used in population genomics. Fst compares DNA divergence of specimens within each population (or species) to DNA divergence between populations (species). Fst ranges between 0 and 1. If Fst between two populations is low, near 0, then these populations are similar to each other. If Fst is high, above 0.5, then these two populations differ and are likely to be different species. Gmin is the fraction of segments of a different population (species) in a genome of a given population (species), thus it gives an estimate of gene exchange between populations or species (Geneva et al. 2015). Values of Gmin near 0 indicate the lack of gene exchange between populations suggesting that these populations are different species. Higher Gmin corresponds to more gene exchange and the lack of reproductive barrier meaning conspecificity.

These two measures (Fst and Gmin) partition the 25 pairs of counterparts into two distinct and well-separated groups: the one corresponding to little genetic isolation and thus being the same species, and the other characterized by higher Fst (more than 0.2) and smaller Gmin (less than 0.06, i.e. about 6%), which corresponds to distinct species. Studies on human populations show that maximal Fst between human populations is about 0.2 (Nelis et al. 2009) and our species (*Homo sapiens*) has about 1.5% - 2.1% genes introgressed from Neanderthals (*Homo neanderthalensis*) (Wall and Yoshihara Caldeira Brandt 2016). Therefore, our criteria applied to humans confirm that modern humans are the same species, but Neanderthal was a distinct species.

In this work, we compute Fst/Gmin on population pairs that we test for a possibility that they represent distinct species. When the values are, for instance 0.45/0.01, then it is most likely that we are dealing with distinct species (Fst is above 0.2 and Gmin is below 0.06). However, if the values we get are 0.16/0.2, then these populations are conspecific (Fst is below 0.2 and Gmin is above 0.06). These criteria are useful, but they should not be taken absolutely and separately from other evidence. While large Fst values above 0.5 and small Gmin values below 0.01 are more definitive indicators of speciation, when they are closer to the "gray zone" (e.g. Fst is between 0.18 and 0.25 and Gmin between 0.03 and 0.07) further evidence is necessary. Additional problem may arise with siblings that are low in genetic diversity,

because their genomes are various recombinations of their parents' genomes, or with inbred populations, for a similar reason. Inclusion of these closely related individuals elevates Fst due to low diversity within siblings or inbred populations. To avoid this undesirable effect, we used specimens from different localities when possible.

We also analyzed COI barcode divergence, which for distinct species is typically above 2% in the presence of phenotypic distinctiveness (Hebert et al. 2003) and inspect genomic trees. It should be taken into account that less than 2% barcode divergence has been reported for distinct species (Burns et al. 2007). Conversely, due to introgression, conspecific individuals may differ in their barcodes by more than 2% (Zakharov et al. 2009; Cong et al. 2017).

Furthermore, distinct species usually form distinct and well-supported clades (support values near 1 by these clades) in phylogenetic trees. Branches supporting distinct species are typically longer than internal branches within a subtree of conspecific specimens. Each terminal branch in the tree is usually long, because of individual variation and uniqueness of each specimen, and also because sequencing errors and contaminations (not being common for any pair of specimens) tend to be reflected in the terminal branch. For conspecific populations, specimens may be intermixed in the trees, or support values for separation of populations are smaller (closer to 0 than to 1), due to significant gene exchange between these populations, when some genes cluster individuals differently from other genes.

Yet another advantage of genomic analysis is its robustness to small number of specimens analyzed. Phenotypic analysis must rely on a large number of specimens to gauge the range of variation, which is shaped by the interactions of genotype with the environment. DNA variation is not affected by the environment, and each specimen contains two genomes: from mother and from father, which in turn contain information from "grandparents". Therefore, even a couple of specimens from distant localities (i.e. no close kinship) is sufficient to estimate intraspecific variation with reasonable accuracy.

For brevity, we do not review phenotypic differences between most of the taxa we deal with, and interested readers should consult other publications. All these taxa have been previously described and their phenotypic characters given elsewhere. These characters do not contribute to our decision to change ranks of these taxa, although they may be looked at as complementary supporting evidence that increases confidence in the results. The evidence presented here is based on genomic analysis. The data we offer are new and they allow us to interpret known phenotypic differences between these taxa from a different and complementary perspective. The purpose of this work is to propose taxonomic changes that are gleaned from genomic data, so that the new name combinations can be used in other publications. A more detailed evolutionary analysis of genomic data will be published elsewhere.

The taxonomic rearrangements we propose are supported by genome-scale phylogenetic trees, Fst and Gmin statistics, and sometimes complemented by phenotypic considerations. The sections are presented in standardized format. The taxonomic act is the title of each section. Relevant genera, subgenera and their type species, are specified. When the species are listed with their original genus name, author names are given without parenthesis. For each species and subspecies with revised rank, type locality is given. A section is usually illustrated with a small segment of a nuclear genomic tree (or other trees as stated in the text) including taxa necessary to support the conclusions. Previous, not newly proposed, genus-species and species-subspecies combinations are used in the figures (per Pelham 2020 <http://www.butterfliesofamerica.com/US-Can-Cat.htm>, version revised 7 August 2020, except those name changes adopted before this publication based on our genomic results). New name combinations are given in the text. Taxa of major focus are shown in red, other taxa of interest are shown in blue, magenta or green. The section ends with a conclusion and in many cases with a list of species with revised genus-species or species-subspecies name combinations. The sections are ordered by family and typically in their taxonomic order from Zhang et al. (2019d) (except in Nymphalidae, where the arrangement was altered to better fit larger images on pages while keeping them next to relevant text). Finally, 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 PRJNA672791, and BioSample entries of the project contain the locality and collection data of the specimens sequenced.

Heraclides ponceana (Schaus, 1911) is a species distinct from *Heraclides aristodemus* (Esper, 1794)

Initially proposed as a species and currently treated as a subspecies of *Heraclides aristodemus* (Esper, 1794) (type locality Haiti), *Papilio ponceana* Schaus, 1911 (type locality USA: Florida, Miami) shows profound genetic differentiation from *H. aristodemus* by a magnitude characteristic of species-level taxa. Phylogenetic trees reveal partitioning of the taxa previously placed in *H. aristodemus* into two prominent clades, including the tree constructed from proteins encoded by the Z chromosome (Fig. 1). The Fst between *H. a. ponceana* and *H. aristodemus* populations is 0.43, which indicates their strong differentiation, and Gmin is 0.02, which suggests low gene exchange between them. COI barcodes of the two also differ by 3.2% (~21 different base pairs). Moreover, it is possible (see below) that *H. a. ponceana* and *H. aristodemus* are (or were) sympatric in Cuba. Therefore, we **reinstate** *Heraclides ponceana* (Schaus, 1911) as a species-level taxon. Due to their genetic and morphological similarities, we consider *Heraclides ponceana bjorndalae* (Clench, 1979) (type locality Bahamas: Great Inagua Island) and *Heraclides ponceana majasi* L. Miller, 1987 (type locality Bahamas: Crooked Island) to be subspecies of *H. ponceana*, **new combinations**.

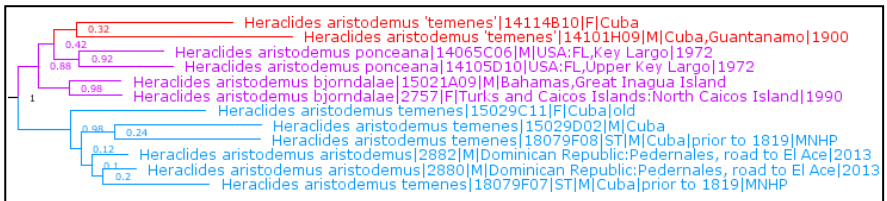

Fig. 1. *Heraclides ponceana* (red: new ssp., magenta) and *H. aristodemus* (blue).

Papilio temenes Godart, 1819 is a junior subjective synonym of *Heraclides aristodemus* (Esper, 1794)

The short version of the original description of *Papilio temenes* Godart, 1819 (page 21) is: "Dessus des ailes d'un brun-noirâtre, avec deux bandes jaunes, maculaires, disposées en sautoir sur les supérieures: les inférieures en queue: le dessous de celles-ci jaunâtre, avec une bande bleue, flexueuse, sur le milieu" (Godart 1819). It can be translated word-for-word as: "Above the wings of a blackish-brown, with two bands yellow, macular, arranged in sautoir [=crosswise] on the forewings: the hindwings [end] in tail: the underside of these [hindwings, not tails] yellowish, with a band blue, flexuous, in the middle", and interpreted as: "Wings above blackish-brown, forewings with two yellow macular bands that cross each other, hindwings with tails, underside of hindwings yellowish with a blue flexuous band in the middle." Perhaps the most significant word here is "**sautoir**" defined in Dictionary.com as "A ribbon, chain, scarf, or the like, tied around the neck in such a manner that the ends cross over each other" (Dictionary.com LLC 2020), and by Merriam-Webster dictionary as "a chain, ribbon, or scarf worn about the neck with the ends forming a St. Andrew's cross in front" (Merriam-Webster 2020). It is important because this character (crosswise vs. more parallel arrangement of yellow forewing bands) is diagnostic of *H. aristodemus* (crosswise) vs. *H. ponceana* (more parallel, images in Warren et al. (2016)). Today, the name *temenes* is applied to a broad-banded form of *H. ponceana* that flies in Cuba (see below). Here we argue that this is a misidentification, and the Godart's *temenes* is *H. aristodemus* instead.

We see that, although the Godart's description of *P. temenes* is brief, two of its details agree better with *H. aristodemus* than with the broad-banded subspecies of *H. ponceana* from Cuba: crisscrossing bands and blue flexuous band. In the broad-banded subspecies, the outer band is more parallel to the inner band and does not give an impression of crossing it. And the blue spots on the hindwing underside look more like a row of lunules than an irregular blue band. In *H. aristodemus*, forewing bands indeed give an impression of crossing bands, and the blue spots on the hindwing below look like an irregular blue band. Furthermore, an extended description of *P. temenes* on page 63 states that the forewing bands are narrow and macular ("étroites, maculaires") (Godart 1819), instead of being broad and continuous. Besides this

additional detail, the extended description reiterates other points of the short description. We note that some *H. aristodemus* females may have broader and more continuous yellow bands that are more similar to those in *H. ponceana* (Fig. 2e), but the *P. temenes* description does not mention this possibility, simplifying the application of the name.

Next, we inspected all potential syntypes of *P. temenes*, two specimens in Paris, France (Fig. 2ab) and one (only photograph inspected) in Edinburg, UK (Bland 2019). These 3 specimens closely agree with the original description of *P. temenes*, carry historical labels and labels indicating their type status ("type", "co-type", or "?co-type") and therefore are likely to be true syntypes of this taxon. These specimens phenotypically are *H. aristodemus* and not *H. ponceana*, differing from the broad-banded subspecies from Cuba. Furthermore, we sequenced two syntypes in Paris (one labeled "TYPE", the other labeled "CO-TYPE") and they are *H. aristodemus* by genomic DNA (Fig. 1 blue), in agreement with their wing patterns. In conclusion, our analysis of the original description and the likely syntypes leaves little doubt about the identity of *P. temenes* as *H. aristodemus*, which is a species different from the broad-banded *H. ponceana* "*temenes*" found on Cuba today (Fig. 1 red).

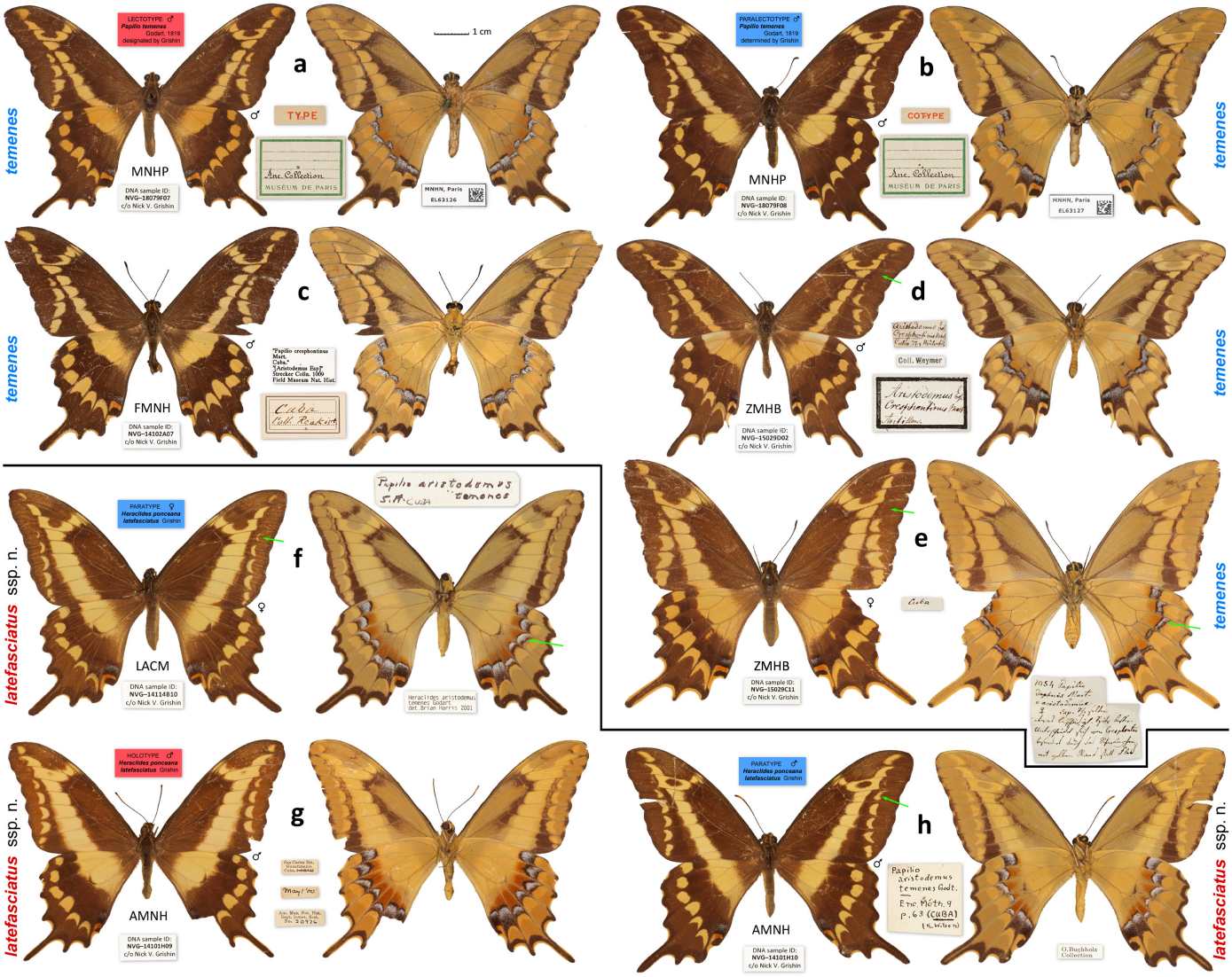

Fig. 2. *Heraclides aristodemus* (**a**–**e**) and *H. ponceana* (**f**–**h**). Green arrows point at equivalent positions in different specimens.

Due to misapplication of the name *temenes* to a taxon different from that in the original description and for the future stability of the name, we designate the syntype in the Muséum National d'Histoire Naturelle, Paris, France (MNHP) shown in Fig. 2a and possessing all the characters stated in the original description, with the following 4 rectangular white (some faded to brownish) labels: printed red || TYPE ||;

framed, lined and printed with green, first line handwritten in black || Anc. Collection | MUSÉUM DE PARIS ||; printed, with a square barcode on the right side || MNHN, Paris | EL63126 ||; printed || DNA sample ID: | NVG-18079F07 | c/o Nick V. Grishin ||, the **lectotype** of *Papilio temenes* Godart, 1819. The red, rectangular, printed label || LECTOTYPE ♂ | *Papilio temenes* | Godart, 1819 | designated by Grishin || will be added to this specimen. This specimen was chosen as the lectotype because it is labeled "type" rather than "co-type", and it yielded genomic dataset of a good quality for a specimen that old.

It appears that Oberthür (1897) was the first to incorrectly apply the name *P. temenes* to the broad-banded subspecies of *H. ponceana* from Cuba, inconsistently with the original description and now with the identity of the lectotype of *P. temenes*. Oberthür illustrated one such specimen. While we have not investigated the reasons behind Oberthür's mistake, we note that it has been followed in subsequent literature. Interestingly, we found 3 century-old *H. aristodemus* (i.e. true *P. temenes*) specimens labeled "Cuba", in ZMHB and FMNH (Fig. 2c–e). Further studies may answer the question whether both species (*H. aristodemus* and *H. ponceana*) co-occurred in Cuba, or the old records from Cuba were mislabeled.

In summary, we conclude that *Papilio temenes* Godart, 1819, **syn. n.**, is a junior subjective synonym of *Heraclides aristodemus* (Esper, 1794). The type locality of *temenes* should remain as stated on page 63 (Godart 1819): "Antilles & dans l'Amérique septentrionale", i.e., "West Indies and North America", which is not necessarily Cuba. It remains to be investigated whether *H. aristodemus* has been found or still occurs in Cuba. In any case, as detailed above, the broad-banded subspecies of *H. ponceana* from Cuba does not have a name, and it is described here as new.

***Heraclides ponceana latefasciatus* Grishin, new subspecies**

http://zoobank.org/C835D721-548C-4822-9280-E241A1C94866

(Figs. 1, 2f–h)

Definition. This taxon differs from *H. aristodemus* in that the outer band of yellow spots on the forewing is more parallel to the inner band (and the outer margin) rather than approaching the inner band at an angle and giving an appearance of crossing the inner band; on the hindwing below there are more prominent red spots and more crescent-shaped blue spots that are better separated from each other, rather than forming a continuous band. This subspecies differs from all other *H. ponceana* subspecies by broader yellow central bands on both wings, less extensive brown coloration on the forewings below, a paler basal area and the lack of red spotting in the postdiscal area on hindwing above.

Type locality. Cuba: Guantánamo province, Rio Seco, San Carlos Estate.

Distribution. Known only from Cuba.

Etymology. The broad yellow bands are the most distinctive feature of this subspecies. The name is formed from the Latin words *latus* (wide, broad) and *fascia* (band, stripe). The name is an adjective.

Type material. Holotype male (Fig. 2g), deposited in the American Museum of Natural History, New York, NY, USA (AMNH), with the following 4 rectangular white (some faded to brownish) labels: printed, the date crossed out || San Carlos Est. | Guantanamo | Cuba. ~~4-8 X '13~~ ||; handwritten || May 1 '00 ||; printed with the numbers handwritten || Am. Mus. Nat. Hist. | Dept. Invert. Zool. | No. 20976 ||; printed || DNA sample ID: | NVG-14101H09 | c/o Nick V. Grishin ||. The red, rectangular, printed label || HOLOTYPE ♂ | *Heraclides ponceana* | *latefasciatus* Grishin || will be added to this specimen. Ten paratypes (when known, localities are given in parenthesis after specimen numbers): 5 males (NVG-14101H10 in AMNH and NVG-14106A11 (Matanzas), NVG-14106A12 (Santiago), NVG-14106B01 (Guantanamo) & NVG-14106B02 in USNM) and 5 females (NVG-14114B09 & NVG-14114B10 in LACM and NVG-14106B03 & NVG-15104A01 (both from Santiago) & NVG-15104A02 in USNM).

Barcode sequence of the holotype. AACATTATATTTTATTTTTGGTGTTTGAGCAAGAATATTAGGAACTTCTCTTAGTTTATTA
ATTCGAACTGAATTAGGAACTCCAGGTTCTTTAATTGGAGATGATCAAATTTATAATACCATTGTTACAGCTCATGCTTTTATTATAATTTTTT
TTATGGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTTCCATTAATATTAGGAGCCCCTGATATAGCTTTCCCTCGAATAAATAA
TATAAGATTTTGACTTTTACCTCCTTCTTTAACTCTTTTAATTTCAAGTATAATTGTCGAAAATGGAGCTGGAACTGGATGAACTGTTTATCCT

CCCCTTTCTTCTAATATTGCTCATGGAAGAAGTTCAGTAGATTTAGTTATTTTTTCTCTTCATTTAGCGGGTATTTCTTCAATTTTAGGAGCAA
TTAATTTTATTACTACTATTATTAACATGCGAATTAATAGAATATCCTTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACAGCTTT
ATTATTACTCTTATCCTTACCCGTTTTAGCTGGAGCTATTACTATATTATTAACTGATCGAAATTTAAATACTTCATTCTTTGATCCTGCAGGA
GGAGGAGATCCTATTCTATACCAACACTTATTT

Instead of proposing a new name for the Cuban broad-banded subspecies of *H. ponceana*, we entertained a possibility to request ICZN to designate one such specimen as the neotype of *P. temenes*, consistent with the current usage of this name, but contrary to the original description and the identity of three extant syntypes. However, we decided against this route for the following reasons. First, the name *H. a. temenes* is not in very wide use being applied to an uncommon endemic of a single island to warrant a special consideration by ICZN. Second, it seems most fair to respect original research that lead to creation of this name, and the original identity of this species that is quite clear even from its description alone (see above). Third, it is conceivable that the true *P. temenes* occurred (or even still occurs) in Cuba, and further research may show that it is not a synonym, but a valid subspecies of *H. aristodemus*, in which case it will be without a name if the neotype is designated to preserve the current usage of "*temenes*" as a subspecies of *H. ponceana*. It would create a nuisance situation when the original *P. temenes* would need a new name. Finally, a valid name that is suggestive of a diagnostic character (*latefasciatus*) has an advantage of being easier to attribute to the taxon (compared to *temenes*) and thus may be easier to remember.

Family Pieridae Swainson, 1820

Colias elis Strecker, 1885 is a species distinct from *Colias meadii* W. H. Edwards, 1871

Previously considered a subspecies of *Colias meadii* W. H. Edwards, 1871 (type locality USA: Colorado), *Colias elis* Strecker, 1885 (type locality Canada: Alberta) is not monophyletic with *C. meadii* in the genomic trees (Fig. 3, although with low support), including the tree constructed from Z chromosome-encoded genes. The identity of the *C. elis* clade is supported by the sequence of its syntype from the Field Museum National History collection (Fig. 3). Furthermore, *C. elis* and *C. meadii* show genetic differences that are larger than a number of other *Colias* species pairs. e.g. *Colias eriphyle* W. H. Edwards, 1876 (type locality Canada: British Columbia) and *Colias eurytheme* Boisduval, 1852 (type locality USA: California). Fst/Gmin statistics for the comparison of *C. elis* and *C. meadii* are 0.40/0.03, but those for *C. meadii meadii* and *C. meadii lemhiensis* are 0.18/0.14 (can be used as a control of conspecific taxa). Therefore, we **reinstate** *Colias elis* Strecker, 1885 as a species-level taxon.

Fig. 3. *Colias elis* (red), *C meadii* (blue) and others w/ patch (magenta).

Tetracharis Grote, 1898 is a valid subgenus that includes *Anthocharis cethura* C. Felder & R. Felder, 1865 (Müller, 1764), *Anthocharis midea* (Hübner, [1809]), and *Anthocharis limonea* (A. Butler, 1871)

The genomic tree of representative species of *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833] (type species *Papilio cardamines* Linnaeus, 1758) including all type species of available genus-

group names considered subgenera or junior subjective synonyms of *Anthocharis* revealed an unexpected but highly confident clade consisting of *Anthocharis cethura* C. & R. Felder, 1865 (type locality USA: California, Los Angeles Co.), *Anthocharis midea* (Hübner, [1809]) (type locality USA: Georgia, Wilmington Island) and *Anthocharis limonea* (A. Butler, 1871) (type locality Mexico) (Fig. 4 red). The clade is unexpected, because currently *A. cethura* is placed in the subgenus *Anthocharis*, while the other two species are placed in the subgenus *Paramidea* Kuznetsov, 1929 (type species: *Anthocharis scolymus* Butler, 1866). Curiously, neither of these species in the red clade are monophyletic with the type species of subgenera they are currently assigned to: *A. (Anthocharis) cardamines* is in the blue clade (Fig. 4) and *A. (Paramidea) scolymus* is in the green clade. Therefore, the current classification is incorrect. Out of genus-group names that are available for the red clade, *Tetracharis* Grote, 1898 (type species *Anthocharis cethura* C. & R. Felder, 1865) is older than *Falcapica* Klots, 1930 (type species *Papilio genutia* Fabricius, 1793, which is a junior homonym of *Papilio genutia* Cramer, 1779, the oldest available name for this species is *Mancipium midea* Hübner, [1809]). As a result, we treat *Tetracharis* as a valid subgenus, **new status**, that includes three species: *A. cethura*, *A. midea*, and *A. limonea*, making *Falcapica* its junior subjective synonym.

Fig. 4. Subgenera *Anthocharis* (blue), *Tetracharis* (red) and *Paramidea* (green) with *Euchloe* as outgroups.

Anthocharis lanceolata Lucas, 1852 belongs to subgenus *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833] instead of *Paramidea* Kuznetsov, 1929

Our genome-level phylogeny strongly supports the placement of *Anthocharis lanceolata* Lucas, 1852 (type locality "Californie") as sister to the *Anthocharis sara* Lucas, 1852 (type locality "Californie") species group, which belongs to the subgenus *Anthocharis* Boisduval, Rambur, [Duménil] & Graslin, [1833] (type species *Papilio cardamines* Linnaeus, 1758) (Fig. 4, blue). The *A. sara* group taken together with *A. lanceolata* is sister to the subgenus *Tetracharis* (type species *Anthocharis cethura* C. & R. Felder, 1865) and thus is not monophyletic with *Anthocharis scolymus* Butler, 1866, the type species of *Paramidea* Kuznetsov, 1929. In other words, *Paramidea* Kuznetsov, 1929 is sister to a clade formed by subgenera *Anthocharis* and *Tetracharis*. Therefore, *A. lanceolata* does not belong to *Paramidea*, but instead is in the subgenus *Anthocharis*. Apparently, the falcate shape of the forewing vs. rounded forewing apex is not a character that indicates phylogenetic groupings within the genus *Anthocharis* and has originated more than once.

Family Lycaenidae [Leach], [1815]

Tharsalea Scudder, 1876, *Helleia* Verity, 1943, *Apangea* Zhdanko, 1995 and *Boldenaria* Zhdanko, 1995 are genera distinct from *Lycaena* [Fabricius], 1807

Discovering non-monophyly of *Lycaena* [Fabricius], 1807 (type species *Papilio phlaeas* Linnaeus, 1760) in a pioneering DNA-based phylogenetic analysis (van Dorp 2004) but stopping short of imminent taxonomic lumps or splits, de Jong and van Dorp (2006) concluded: "we propose that first the interrelationships as suggested by the present study are confirmed by further genetic markers." We accepted this challenge and used not some further markers, but all protein-coding genes in 34 Lycaeninae species, including the type species of 22 out of 28 available genus-group names (two of which share valid name of the type species with another genus-group name). The results confirm that *Lycaena* is not monophyletic (Fig. 5). Notably, *Iophanus* Draudt, 1920 (type and the only species *Chrysophanus* (?) *pyrrhias* Godman & Salvin, 1887) originates within *Lycaena* and is sister to most other American species

with strong support. This placement is unexpected due to the prominent phenotypic similarities between *Iophanus* and *Melanolycaena* Sibatani, 1974 (type species *Melanolycaena altimontana* Sibatani, 1974). Even using genitalic morphology, *Iophanus pyrrhias* (type locality Guatemala) was associated with the (largely) Palearctic clade (Fig. 5 blue) by Klots (1936) who wrote: "*pyrrhias* ... relation-ship is undoubtedly with the Palaearctic rather than with the Nearctic series, and is possibly rather ancient." As the genomic tree suggests, Klots was incorrect on both counts: *I. pyrrhias* is a relatively recent offshoot of the (largely) Nearctic clade (Fig. 5 red). To the contrary, *Melanolycaena* is sister to *Lycaena boldenarum* White, 1862 (type locality New Zealand) and they form a clade that is sister to all other Lycaeninae we have sequenced, including *Heliophorus* Geyer, [1832] (type species *Heliophorus belenus* Geyer, [1832], considered to be a junior subjective synonym of *Polyommatus epicles* Godart, [1824]). Thus, we were impressed by the intuition of Sibatani (1974) who stated in the last sentence of his work: "the possibility is not completely ruled out that the New Guinean *Melanolycaena* and the coppers of New Zealand are monphyletic [sic!]." Indeed, *Melanolycaena* is sister to *Boldenaria* Zhdanko, 1995 (type species *Lycaena boldenarum* White, 1862) from New Zealand (Fig. 5), despite phenotypic dissimilarities. For these reasons, genomic phylogeny implies that the division of Lycaeninae into two sections (*Lycaena* and *Heliophorus*) as suggested by Eliot (1973) was indeed tentative and needs to be revised because these sections are not monophyletic.

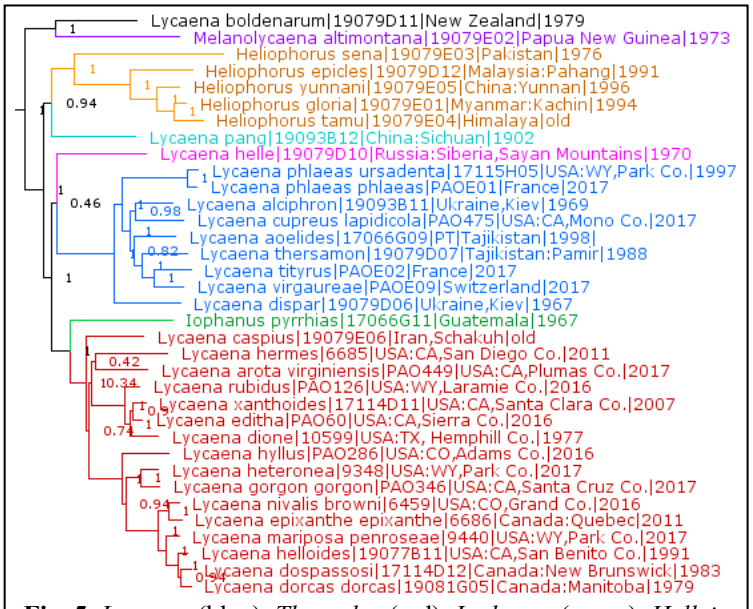

Fig. 5. *Lycaena* (blue), *Tharsalea* (red), *Iophanus* (green), *Helleia* (magenta), *Apangea*, (cyan), *Melanolycaena* (purple), *Heliophorus* (orange-brown) and *Boldenaria* (black).

Here, armed with genomic data for ~80% of the available genus-group names, we attempt such a revision. One solution to restore monophyly is to treat the whole subfamily as a single genus *Lycaena* that subsumes *Melanolycaena* and *Heliophorus* among others. This super-lumping solution may be in agreement with relatively low genetic diversification among all these species. Indeed, Lycaeninae experienced some of the slowest evolutionary rates among Lycaenidae as revealed by relatively shorter branches within the Lycaeninae clade compared to others (Zhang et al. 2019d). The difference in COI barcodes between distantly related Lycaeninae species ranges from about only 5% (35 bp, *L. boldenarum* and *L. phlaeas*, a difference common for closely related congeners) to about 8% (53 bp, *Heliophorus sena* Kollar, 1844 and *Lycaena pang* Oberthür, 1886, a difference typical for distantly related congeners). This high similarity in COI underscores the idea that it is meaningless to impose a strict cutoff on divergence values due to the differences in evolutionary rates in different lineages. However, even American species of *Lycaena* are estimated to have diverged over 20 million years ago (Zhang et al. 2019d), which is larger than for a typical diverse genus; i.e., it is larger than the divergence between *Anthocharis* and *Euchloe* Hübner, [1819] (Pieridae) and about the same as between *Vanessa* [Fabricius], 1807 and *Nymphalis* Kluk, 1780 (Nymphalidae). Furthermore, *Heliophorus* has been traditionally maintained as a genus-level taxon. Therefore, we reject the super-lumping solution of a monotypic subfamily Lycaeninae.

The opposite extreme would be to find a meaningful level closest to the leaves of the tree that defines genera. Ideally, there would be situations in the tree where many lineages diverge at about the same level (i.e. at the same distance from the root, meaning at about the same time in the past) and then stay as single lineages for some time (i.e. form longer branches). This rapid diversification immediately followed by a relative lack of further diversification creates a level in the classification, i.e. the tree looks more like a bush or a comb than a bifurcating tree at that point. Taking the (largely) Nearctic group (Fig. 5 red), we see exactly this situation: at its base, this group diversifies into five prominent clades, and then two of

these clades diversify further, also at approximately the same time point in the past. These five clades form a level in the tree and can be used as genera, offering the splitting solution. Notably, every one of these clades already has a genus-group name (Pelham 2008), including Palearctic *Hyrcanana* Bethune-Baker, 1914 (type species *Polyommatus caspius* Lederer, 1870). Apparently, these clades were also obvious from phenotypes: that is how they were defined and named to begin with (Scudder 1876; Klots 1936; Miller and Brown 1979). This level of classification can be propagated to other parts of the tree, although they are currently poorly covered by species. It is a meaningful level that can be chosen to define genera, but a significant number of such genera will be monotypic (e.g. two out of five in the Nearctic clade), and excepting knowledgeable aficionados of this group, such genera carry little information about their interrelationships. Hence, we looked for a compromise between the splitting and lumping solutions.

Inspection of the tree reveals a rapid diversification point between its root and the diversification of the red clade (Fig. 5): i.e. orange-brown, cyan, magenta, blue and red + green clades diverged at approximately the same time in the past. This divergence is followed by the lack of immediate further divergence, creating long and prominent branches in the tree and resulting in a meaningful level for classification. We have chosen to take this intermediate level to suggest division of Lycaeninae into genera. Most of these genera are unambiguously apparent from the tree: black, purple, orange-brown, cyan, magenta, blue and red clades stand for seven genera, all of which have previously proposed names. Two instances require further elaboration. First, *Heliophorus sena* (Kollar, [1844]) stands out from the rest in the genus (Fig. 5 orange). The type species of subgenus *Nesa* Zhdanko, 1995, it may be a genus-level taxon. However, it is monophyletic with *Heliophorus* and we leave it there as a subgenus to emphasize this relationship, awaiting further studies. Second, *Iophanus* (Fig. 5 green) is at about the same divergence from the rest of Nearctic species (Fig. 5 red) as *H. sena* from other *Heliophorus*. For now, we decided to keep this monotypic genus, because it is currently treated as such, and because its earlier divergence time sets it apart from the rapid diversification of the red clade. The name for the red clade is *Tharsalea* Scudder, 1876 (type species *Polyommatus arota* Boisduval, 1852), as chosen by Klots (1936), probably because this name was proposed before others in the paper (Scudder 1876).

In summary, we refrain from partitioning Lycaeninae into tribes and **revise the status** of the following names treating them as genera: *Tharsalea* Scudder, 1876, *Helleia* Verity, 1943 (type species *Papilio helle* Denis & Schiffermüller, 1775), *Apangea* Zhdanko, 1995 (type species *Chrysophanus pang* Oberthür, 1886) and *Boldenaria* Zhdanko, 1995. Furthermore, in agreement with previous studies (Sibatani 1974; van Dorp 2004; de Jong and van Dorp 2006), we conclude that the endemic South African species currently placed in *Lycaena* represent the 9th genus of Lycaeninae that is named next. We are looking forward to testing this hypothesis with genomic data.

Lafron **Grishin, new genus**

http://zoobank.org/0DB9D8C5-E666-46A3-822B-50E96448C82A

Type species. *Papilio orus* Stoll, [1780].

Definition. In male genitalia (Fig. 2 in de Jong and van Dorp 2006), differs from others in the subfamily Lycaeninae, except *Melanolycaena*, by a saccus-like pouch on juxta (Sibatani 1974); separated from *Melanolycaena* (Fig. 4 in Sibatani 1974) by juxta connected to valva at its more ventral part, as in other *Lycaena*. In wing patterns and shape, resembles a sympatric hairstreak *Chrysoritis lycegenes* (Trimen, 1874) (possible mimicry), i.e. wings are more rounded than most *Lycaena* and forewing black spots are closer to the margin, hindwing without tails and patterned more similar to Polyommatinae than to most *Lycaena*: pale-brown with darker marginal lunules and with paler spots usually darker in the middle.

Etymology. The name is a masculine noun in the nominative singular, formed as *L*[ycaen]*a* + [A]*fr*[ica] + *on* to indicate African origin of the genus and reach gender agreement with the type species name.

Species included. The type species and *Lycaena clarki* Dickson, 1971, both from South Africa.

Parent taxon. Subfamily *Lycaeninae* [Leach], [1815].

Lycaeninae genera, subgenera and their available synonyms

Here, we update the Appendix of Sibatani (1974) and suggest the following treatment of Lycaeninae grouped into nine genera. Placements of *Lafron* Grishin, **gen. n.** and *Phoenicurusia* Verity, 1943 are provisional due to the lack of both genomic data and unambiguous phenotypic evidence, and follow published works based on morphology and limited DNA analysis (Klots 1936; Sibatani 1974; van Dorp 2004; de Jong and van Dorp 2006). The list is preliminary and further changes are expected in groups poorly covered by our genome-based phylogeny. Junior subjective synonyms are preceded by "=". Unavailable names are not listed. Type species are given in parenthesis with their original genus name.

Genus ***Lafron*** Grishin, **gen. n.** (*Papilio orus* Stoll, [1780])
Genus ***Lycaena*** [Fabricius], 1807 (*Papilio phlaeas* Linnaeus, 1760)
Subgenus *Lycaena* [Fabricius], 1807 (*Papilio phlaeas* Linnaeus, 1760)
Subgenus *Thersamolycaena* Verity, 1957 (*Papilio dispar* Haworth, 1802)
Subgenus *Heodes* Dalman, 1816 (*Papilio virgaureae* Linnaeus, 1758)
=*Loweia* Tutt, 1906 (*Papilio dorilis* Hufnagel, 1766)
=*Thersamonia* Verity, 1919 (*Papilio thersamon* Esper, 1784)
=*Palaeochrysophanus* Verity, 1943 (*Papilio hippothoe* Linnaeus, 1760)
=*Alciphronia* Koçak, 1992 (*Papilio alciphron* Rottemburg, 1775)
=*Mirzakhania* Koçak, 1996 (*Chrysophanus kasyapa* F. Moore, 1865)
Genus ***Helleia*** Verity, 1943 (*Papilio helle* Denis & Schiffermüller, 1775)
Genus ***Tharsalea*** Scudder, 1876 (*Polyommatus arota* Boisduval, 1852)
Subgenus *Epidemia* Scudder, 1876 (*Polyommatus epixanthe* Boisduval & Le Conte, [1835])
=*Hyllolycaena* L. Miller & F. Brown, 1979 (*Papilio hyllus* Cramer, 1775)
=*Hellolycaena* Koçak, 1983 (=*Polyommatus thoe* Guérin-Méneville, [1832], which is *Papilio hyllus* Cramer, 1775)
Subgenus *Chalceria* Scudder, 1876 (*Chrysophanus rubidus* Behr, 1866)
=*Gaeides* Scudder, 1876 (*Chrysophanus dione* Scudder, 1868)
Subgenus *Tharsalea* Scudder, 1876 (*Polyommatus arota* Boisduval, 1852)
Subgenus *Hermelycaena* L. Miller & F. Brown, 1979 (*Chrysophanus hermes* W. H. Edwards, 1870)
Subgenus *Hyrcanana* Bethune-Baker, 1914 (*Polyommatus caspius* Lederer, 1870)
=*Sarthusia* Verity, 1943 (*Polyommatus sarthus* Staudinger, 1866)
Subgenus *Phoenicurusia* Verity, 1943 (*Polyommatus phoenicurus* var. *margelanica* Staudinger, 1881)
=*Athamanthia* Zhdanko, 1983; (*Polyommatus athamantis* Eversmann, 1854)
Genus ***Iophanus*** Draudt, 1920 (*Chrysophanus* (?) *pyrrhias* Godman & Salvin, 1887)
Genus ***Heliophorus*** Geyer, [1832] (=*H. belenus* Geyer, [1832], which is *Polyommatus epicles* Godart, [1824])
Subgenus *Heliophorus* Geyer, [1832] (=*H. belenus* Geyer, [1832], which is *Polyommatus epicles* Godart, [1824])
=*Ilerda* E. Doubleday, 1847 (*Polyommatus epicles* Godart, [1824])
=*Kulua* Zhdanko, 1995 (*Polyommatus tamu* Kollar, 1844)
Subgenus *Nesa* Zhdanko, 1995 (*Polyommatus sena* Kollar, 1844) [not a homonym! *Nesa* Leach, 1818 is a misspelling]
Genus ***Apangea*** Zhdanko, 1995 (*Chrysophanus pang* Oberthür, 1886)
Genus ***Melanolycaena*** Sibatani, 1974 (*Melanolycaena altimontana* Sibatani, 1974)
Genus ***Boldenaria*** Zhdanko, 1995 (*Lycaena boldenarum* White, 1862)

Habrodais Scudder, 1876, *Favonius* Sibatani & Ito, 1942, *Neozephyrus* Sibatani & Ito, 1942, *Quercusia* Verity, 1943, *Chrysozephyrus* Shirôzu & Yamamoto, 1956, and *Sibataniozephyrus* Inomata, 1986 are junior subjective synonyms of *Hypaurotis* Scudder, 1876

Inspecting genomic phylogenetic trees of US butterfly species (Zhang et al. 2019d), we noticed a close relationship between the only two New World genera from the tribe Theclini Swainson, 1830: *Hypaurotis* Scudder, 1876 (type species *Thecla crysalus* W. H. Edwards, 1873) and *Habrodais* Scudder, 1876 (type species *Thecla grunus* Boisduval, 1852). Despite dissimilar wing patterns and colors, divergence between the type species of these genera is indeed comparable to that of congeners (Fig. 6 top) and is even lower than the divergence in some compact genera, such as *Chlorostrymon* Clench, 1961, *Ministrymon* Clench, 1961 or *Electrostrymon* Clench, 1961 (Fig. 6 top), and particularly in more diverse genera such as *Strymon* Hübner, 1818, *Callophrys* Billberg, 1820, or *Satyrium* Scudder, 1876 (Zhang et al. 2019d). COI barcodes of *Hy. crysalus* and *Ha. grunus* are only 4.3% (28 bp) different: divergence similar to that at

times reported for different individuals of the same species (Zakharov et al. 2009; Kodandaramaiah et al. 2013), strongly suggesting that these two species are congeneric. The combination *Hypaurotis grunus* has been used previously in publications (Garth 1934), however, we failed to find the combination *Habrodais crysalus* published. Also, *H. crysalus* has a slightly broader distribution and is a more familiar butterfly. Therefore, among the two names published in the same work (Scudder 1876), we use *Hypaurotis* as valid, and *Habrodais* as its junior subjective synonym, resulting in *Hypaurotis grunus* (Boisduval, 1852) and *Hypaurotis poodiae* J. Brown & Faulkner, 1982, **revised** and **new combinations**.

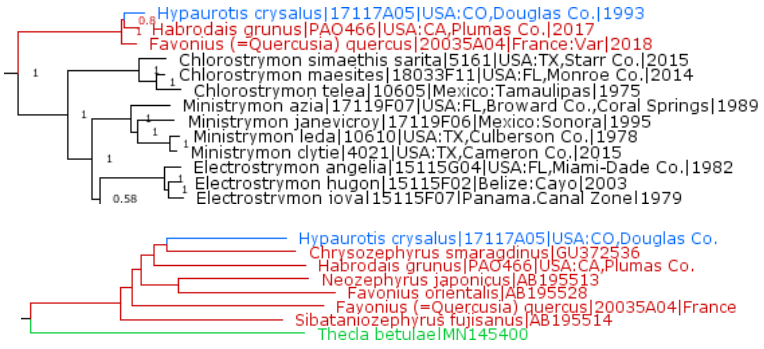

Fig. 6. *Hypaurotis*, *Habrodais* and others. Nuclear genome tree above, COI barcode dendrogram below. Specimens without locality given are from GenBank and their accession numbers are indicated.

Next, when a genomic dataset of an Old World species *Favonius quercus* (Linnaeus, 1758) was included in the Theclinae tree, it clustered closely with *Hypaurotis* (Fig. 6 top). The COI barcodes of *H. crysalus* and *F. quercus* differ by 4.7% (31 bp). Because we lack genomic data for other genus-group names from the *Thecla* section of Eliot (Eliot 1973), we downloaded available COI barcode data from GenBank (Sayers et al. 2020). We find that while for most species pairs, e. g., *H. crysalus* and *Favonius orientalis* (Murray, 1875), the barcodes are more similar (6%), for others, e. g., *H. crysalus* and *Thecla betulae* (Linnaeus, 1758) (the type species of *Thecla* Fabricius, 1807) the difference is larger (8.2%). The COI barcode distance dendrogram computed using BioNJ (Gascuel 1997) as implemented by the phylogeny.fr server (Dereeper et al. 2008) reveals close clustering of species we propose to place in *Hypaurotis* (Fig. 6 bottom, red) and separation of *T. betulae* (green) from this cluster. In the absence of genomic data, this COI barcode analysis of their type species suggest that in addition to *Habrodais* Scudder, 1876 and *Quercusia* Verity, 1943 (type species *Papilio quercus* Linnaeus, 1758), the following four genus-group names *Sibataniozephyrus* Inomata, 1986 (type species *Zephyrus fujisanus* Matsumura, 1910), *Neozephyrus* Sibatani & Ito, 1942 (type species given as *Thecla taxila* Bremer, 1861, which was, however, a misidentified *Dipsas japonica* Murray, 1875; according to the Art 70.3.2. of the ICZN Code the actual taxonomic identity of this species is chosen and *japonica* is **fixed** as **type species**), *Chrysozephyrus* Shirôzu & Yamamoto, 1956 (type species *Thecla smaragdina* Bremer, 1861) and *Favonius* Sibatani & Ito, 1942 (type species *Dipsas orientalis* Murray, 1875) are **junior subjective synonyms** of *Hypaurotis* Scudder, 1876, which is a genus distinct from *Thecla* Fabricius, 1807. In accord with genetic similarities, all these species are similar in appearance (Eliot 1973). We expect that future studies will reveal additional synonyms and possibly a subgeneric structure of *Hypaurotis*.

Finally, even from a practical standpoint of American butterfly knowledge, it seems more instructive to treat *H. crysalus* and *H. grunus* as congeneric emphasizing on their close kinship (despite apparent phenotypic dissimilarity), instead of placing them in two monotypic (or nearly monotypic) genera that accentuate their tenuous (but superficial) uniqueness. Finding their close relatives in the Old World places *Hypaurotis* among other Holarctic Theclinae genera such as *Callophrys* and *Satyrium* and emphasizes somewhat unusual but recurrent pattern revealing the connection between the Old and the New Worlds.

Family Riodinidae Grote, 1895

Plesioarida Trujano & García, 2018 is a junior subjective synonym of *Roeberella* Strand, 1932

Plesioarida Trujano & García, 2018 (type species *Apodemia walkeri* Godman & Salvin, 1886) was described as a genus (Trujano-Ortega et al. 2018) and was treated as a subgenus of *Apodemia* C. Felder & R. Felder,

1865 (type species *Lemonias mormo* C. Felder & R. Felder, 1859) by Zhang et al. (2019e) to present a more internally consistent classification of the tribe Emesidini Seraphim, Freitas & Kaminski, 2018. Continuing with the genomic sequencing of Riodinidae, we were surprised to find that a syntype of the type species of the genus *Roeberella* Strand, 1932, i.e., *Lemonias calvus* Staudinger, 1887 (type locality Peru: Chanchamayo), was in the same clade with *A. walkeri* (type locality Mexico: Guerrero), rendering *Plesioarida* paraphyletic in all three trees that we routinely construct (Fig. 7). Statistical support for the placement of *Roeberella calvus* within the subgenus *Plesioarida* as sister to both *A. walkeri* and *Apodemia hepburni* (Godman & Salvin, 1886) (type locality

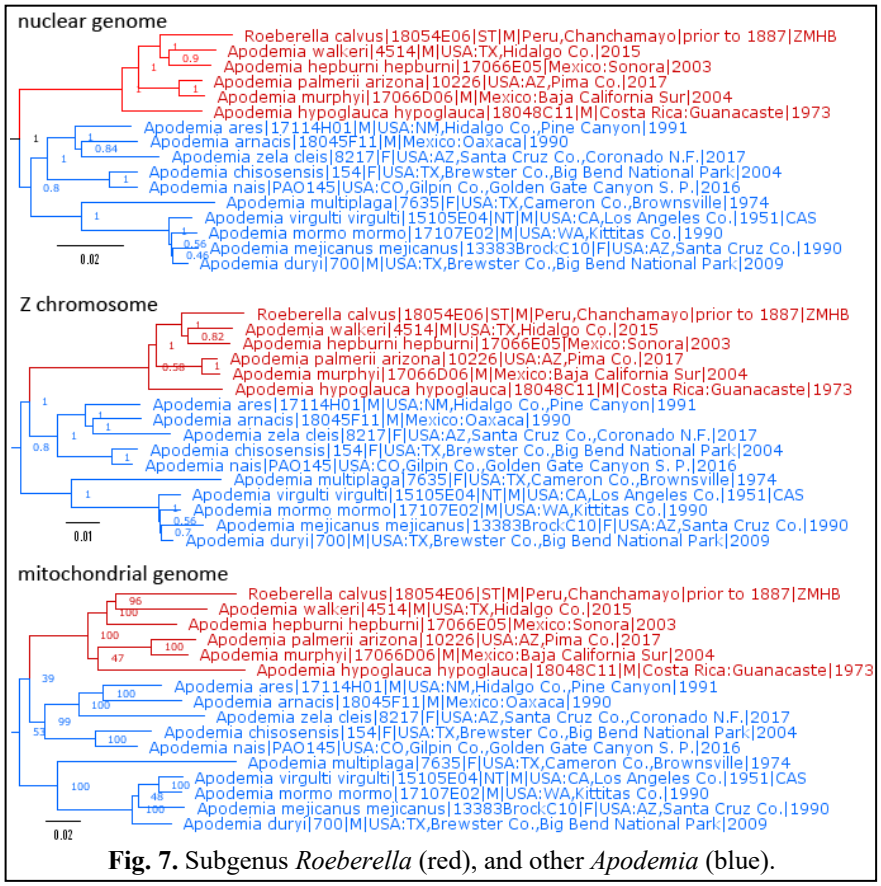

Fig. 7. Subgenus *Roeberella* (red), and other *Apodemia* (blue).

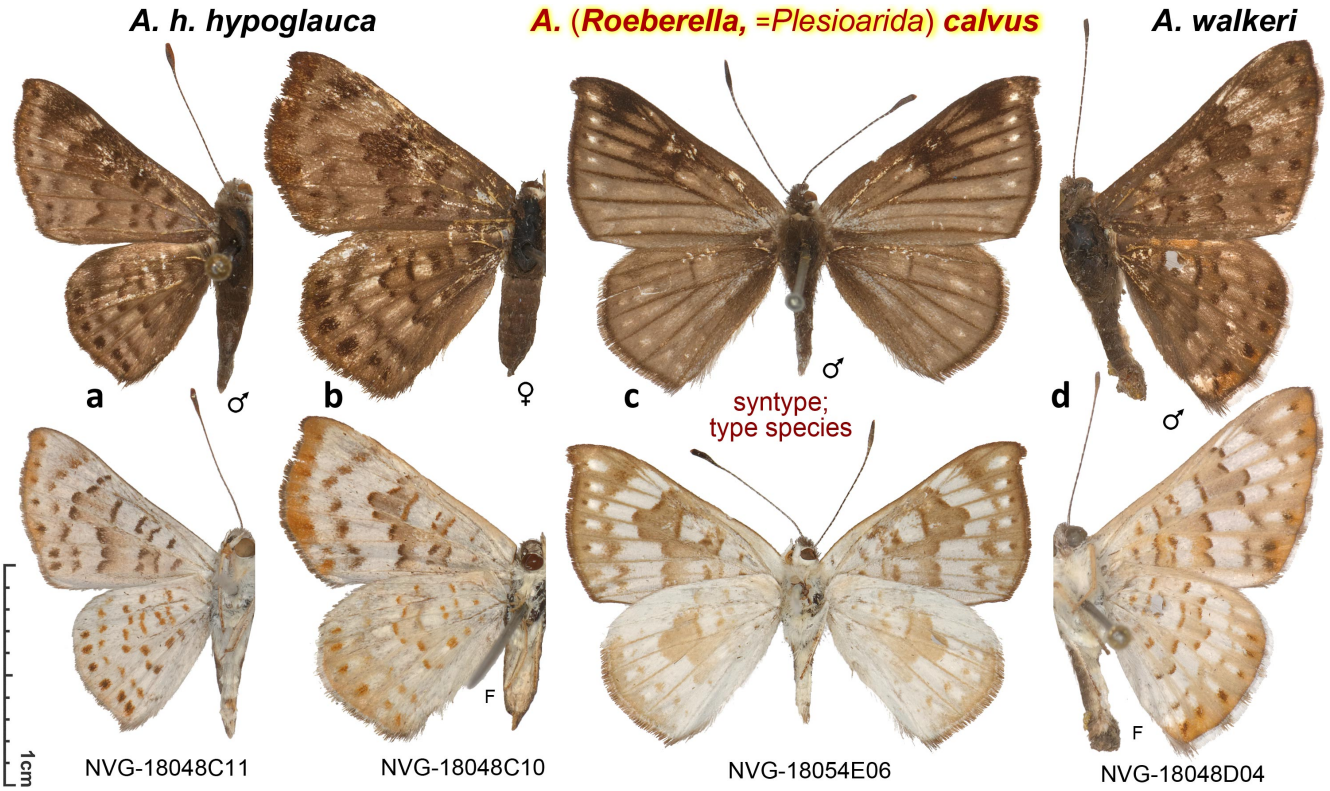

Fig. 8. Previously unnoticed but apparent phenotypic similarity between *Roeberella calvus* (**c**, syntype and the type species of *Roeberella*, Peru: Chanchamayo, NVG-18054E06 in ZMHB, here placed in *Apodemia*), and representative species of the subgenus *Plesioarida* (here synonymized with *Roeberella*): *Apodemia walkeri* (**d**, the type species of *Plesioarida*, Mexico: Morelos, NVG-18048D04 in USNM) and *Apodemia hypoglauca* (**a**, Costa Rica, NVG-18048C11; **b**, Mexico: Morelos, NVG-18048C10 both in USNM). Dorsal above, ventral below. This similarity was uncovered by genomic analysis.

Mexico: Chihuahua) is strong in the protein coding regions of the entire nuclear genome, Z-chromosome and mitochondrial genome: 100% of all segment trees contained the clade of these three species. Such a result was unexpected, because the type species of *Roeberella* (South American) was not previously compared with *Apodemia* (North American), and we suspected a possibility of error or contamination. However, COI barcodes of *R. calvus* and *A. walkeri* differ by only 5.2% (34 base pairs), a difference smaller than that between *A. walkeri* and *A. mormo* (8.5%, 56 bp). Moreover, *R. calvus* is phenotypically similar to *Plesioarida* species (Fig. 8) and shares a falcate forewing with *Apodemia hypoglauca* (Godman & Salvin, 1878) (type locality "Mexico"). The similarities are most prominent in the ventral wing pattern (Fig. 8bc bottom). Interestingly, the wing shape of *R. calvus* appears more like that of female *Plesioarida* (Fig. 8b). Dorsally, *R. calvus* reminds us of an aberrant *Plesioarida*. Therefore, due to genetic and phenotypic similarities, we conclude that *Plesioarida* **syn. n.** is a junior subjective synonym of *Roeberella*, and consequently we place *Roeberella* as a subgenus (**new status**) of *Apodemia*.

We think this find is particularly interesting for several reasons. First, it extends the range of *Apodemia*, previously not recorded from South America, to Peru, with all evolutionary and biogeographical implications of this fact. Second, it underscores the importance of a more comprehensive phylogenetic analysis before proposing new genus-group names to avoid creation of unnecessary synonyms (Trujano-Ortega et al. 2018; Trujano-Ortega et al. 2020). Third, it reiterates the power of genomic approaches and the value of the type concept, both in species and genus-group names. Sequencing of the syntype of the type species of the genus-group name *Roeberella* solidifies our conclusions, eliminating a possibility of misidentification or incorrect inference from a non-type species.

Lemonias lencates Hewitson, 1875 currently placed in *Roeberella* Strand, 1932 belongs to *Pachythone* H. Bates, 1868

Genomic sequencing of a specimen of *Roeberella lencates* (Hewitson, 1875) (type locality not given, species recorded from Costa Rica to Brazil) and comparisons among the type species of available genus-group names reveal that *R. lencates* (Fig. 9, red) is not monophyletic with the type species of *Roeberella* Strand, 1932 (*Lemonias calvus* Staudinger, 1887, type locality Peru: Chanchamayo, a syntype sequenced, Fig. 9, magenta), but instead belongs to *Pachythone* H. Bates, 1868 (type species *Pachythone erebia* Bates, 1868) (Fig. 9, blue). The tree includes the type species of all available genus-group names in Emesidini placed among other Riodinidae (Fig. 9, taxa currently placed in *Apodemia* are in green font). Therefore, we transfer *R. lencates* and a number of its close allies as suggested by their phenotypic similarities from *Roeberella* to *Pachythone*, forming the following **new combinations**: *Pachythone lencates* (Hewitson, 1875), *Pachythone flocculus* (Brévignon & Gallard, 1993), *Pachythone floccus* (Brévignon, 2013), *Pachythone heberti* (P. Jauffret & J. Jauffret, 2007) and *Pachythone marajoara* (P. Jauffret & J. Jauffret, 2007). Furthermore, due to close clustering in the genomic tree (Fig. 9) comparable to that within other genera (e.g., *Periplacis* Geyer, 1837 in addition to *Emesis* Fabricius, 1807 and *Apodemia*) and phenotypic similarities in wing patterns (e.g., compare with *Pachythone strati* (Kaye, 1925) and *Pachythone rubigo* (H. Bates, 1868)), we suggest that *Pseudonymphidia* Callaghan, 1985 (type species *Emesis clearista* Butler, 1871. Fig. 9, cyan) is a subgenus (**new status**) of *Pachythone* (Fig. 9, the clade labeled with the name in red font highlighted in yellow).

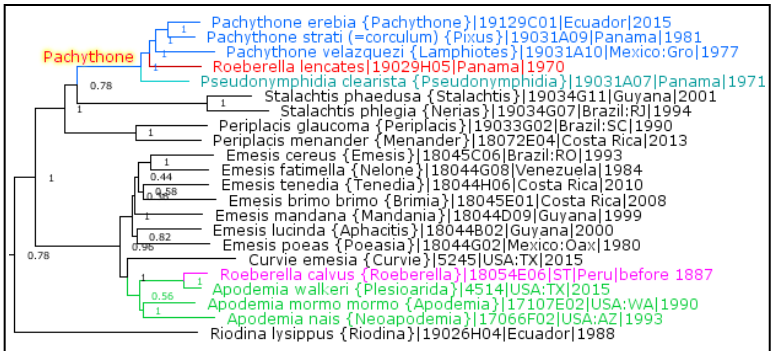

Fig. 9. Emesidini and *Pachythone* among others. Genus-group names each species is the type of are given in brackets.

Speyeria Scudder, 1872 is a subgenus of *Argynnis* Fabricius, 1807

A close relationship between the New World genus *Speyeria* Scudder, 1872 (type species *Papilio idalia* Drury, 1773) and the Old World genus *Argynnis* Fabricius, 1807 (type species *Papilio paphia* Linnaeus 1758) has been suggested (Simonsen 2006; Simonsen et al. 2006). In these studies, Simonsen proposed to treat *Speyeria* as a subgenus of *Argynnis*. Although this suggestion has been followed in a number of works (Wells et al. 2011; Scott and Fisher 2014), it has not been universally accepted (Pelham 2008; De Moya et al. 2017; Pelham 2020), likely due not to scientific but historical reasons. Several generations of American naturalists were raised being accustomed to the name *Speyeria* and are less familiar with the name *Argynnis*, thus being resistant to abandoning *Speyeria* as a genus name. Our genomic studies also support the view that *Speyeria* should be considered a subgenus within *Argynnis*. The two groups are very close to each other genetically (Fig. 10). The COI barcode difference between the type species of *Speyeria* and *Argynnis* is 8.2%, and the estimated time of divergence is 9.1 Mya according to De Moya et al. (2017) (*Brenthis* Hübner, [1819], which is sister to the clade that includes *Argynnis* and *Speyeria* diverged from them 11.1 Mya), but likely about 7.5 Mya according to Chazot et al. (2019), who show that *Brenthis* diverged from *Argynnis* at 9.2 Mya (9.1*9.2/11.1≈7.5). This divergence is nearly the same as (or less than) between the two species of *Euptoieta* E. Doubleday, 1848 (Fig. 10): COI barcode difference 8.8%, estimated time of divergence about 8 Mya according to Zhang et al. (2019d). Moreover, *Argynnis* is also quite close to *Boloria* Moore, 1900 (type species *Papilio pales* [Denis & Schiffermüller], 1775) (estimated divergence 14.6 Mya (Chazot et al. 2019)), and they together form a more prominent group in the phylogenetic tree than either of them does separately (Fig. 10). Thus, it is even conceivable to take the next step and treat *Boloria* sensu lato as a subgenus of *Argynnis*. Currently we refrain from their unification, because the genetic distance between *Argynnis* and *Boloria* is still within the limits possible for distinct genera, and the pronounced phenotypic distinction between these two genera exists making their visual recognition straightforward. However, butterfly classification would be more inconsistent if *Speyeria* stays a genus distinct from *Argynnis*. Therefore, we agree with Simonsen et al. (2006) and place *Speyeria* as a subgenus within *Argynnis*.

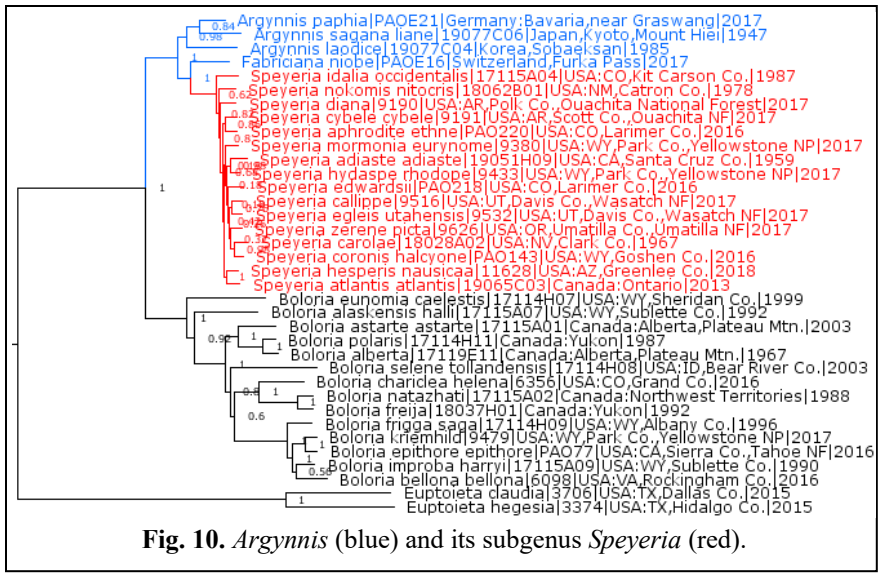

Fig. 10. *Argynnis* (blue) and its subgenus *Speyeria* (red).

Argynnis irene Boisduval, 1869 and *Argynnis nausicaa* W. H. Edwards, 1874 are species distinct from *Argynnis hesperis* W. H. Edwards, 1864

Argynnis atlantis W. H. Edwards, 1862 (type locality USA: New York, Green Co., mostly eastern in distribution) and *Argynnis hesperis* W. H. Edwards, 1864 (type locality USA: Colorado, Jefferson Co., mostly western in distribution) form a species complex that requires further investigation (Dunford 2009). We obtained whole genome shotgun sequences for nearly all its taxa considered valid by Pelham (2020). A number of these taxa were represented by their primary type specimens to ensure correct application of their names (Fig. 11, indicated as HT for holotype and LT for lectotype). Because protein-coding regions

of species in the subgenus *Speyeria* are quite conserved in their sequences, in order to provide better discrimination between taxa, we used all genomic sequences mapped to *Argynnis (Speyeria) diana*

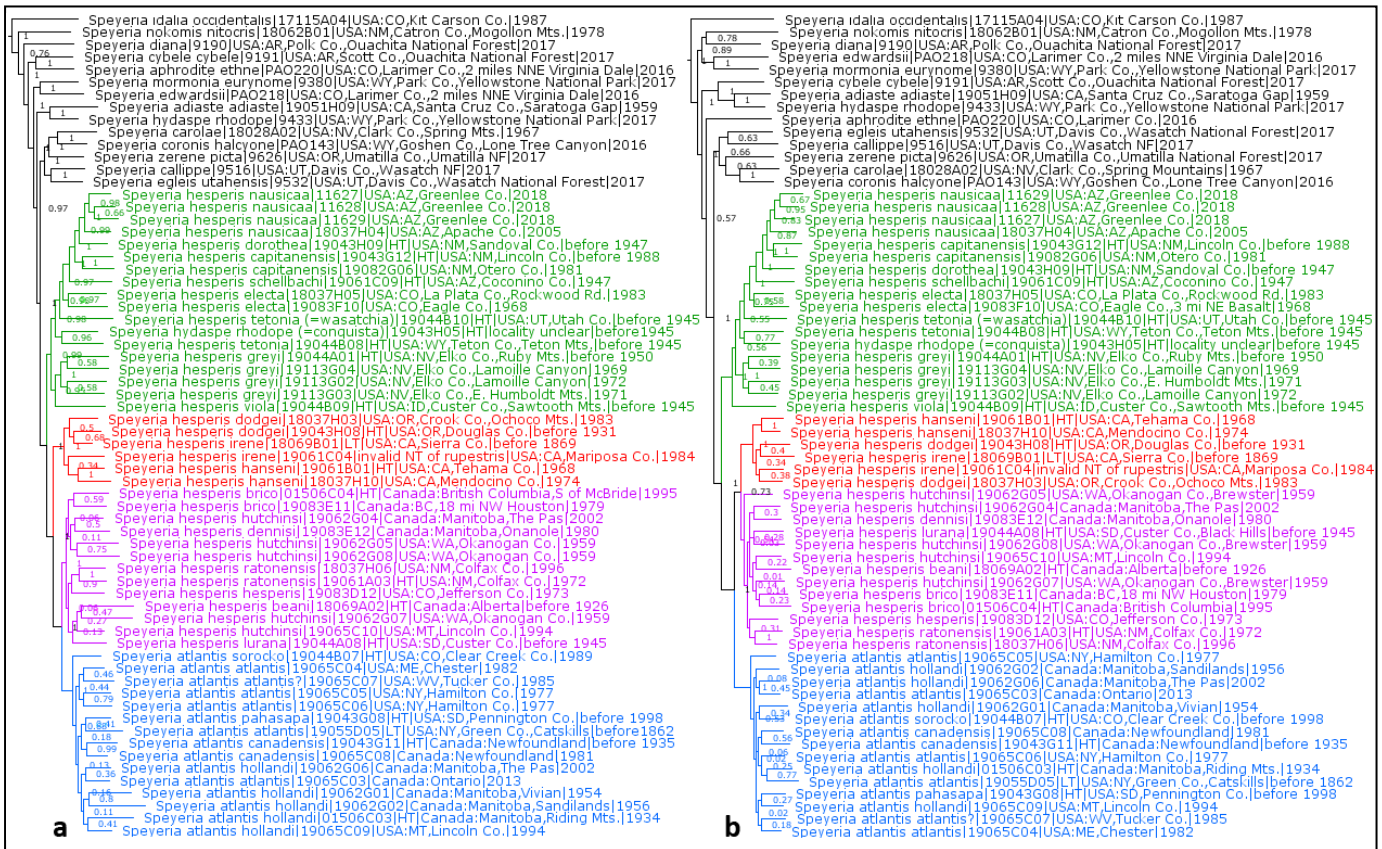

Fig. 11. *Argynnis irene* (red), *hesperis* (magenta), *atlantis* (blue) and *nausicaa* (green).
Trees constructed from nuclear genomic regions from (**a**) autosomes and (**b**) Z chromosome.

reference genome (Zhang et al. 2019d). Furthermore, we considered the autosomes and Z chromosome separately due to their distinct roles in evolution and differences in resistance to introgression (Cong et al. 2019b). The trees constructed from concatenated coding and non-coding regions of autosomes (Fig. 11a) and Z chromosome (Fig. 11b) revealed evolutionary complexities of the taxa included. First, as expected, the entire group consisting of *A. hesperis* (Fig. 11 green, red and magenta) and *A. atlantis* (Fig. 11 blue) is monophyletic in both trees. Second, in both trees, *A. hesperis* is paraphyletic with respect to *A. atlantis*, and a clade composed of south-central subspecies of *A. hesperis* (Fig. 11, green), including *A. hesperis nausicaa* W. H. Edwards, 1874 (type locality USA: Arizona, Graham Co.) is sister to all other taxa combined. Third, the position of the clade consisting of northern and eastern subspecies of *A. hesperis* that include the nominotypical subspecies (Fig. 11 magenta) is different in autosome and Z chromosome trees. In the autosome tree (Fig. 11a), this clade is sister to *A. atlantis*, and in the Z chromosome tree (Fig. 11b), it is sister to the clade of western subspecies of *A. hesperis* that include *A. hesperis irene* (Boisduval, 1869) (type locality USA: California, Sierra Co.).

Thus, the trees reveal four groups of taxa in this complex, and applying the oldest name in each group, we call them *atlantis*, *hesperis* (sensu stricto), *irene* and *nausicaa*. To probe whether these groups are species, we used the Fst/Gmin Z chromosome tests (Cong et al. 2019b) obtaining the following statistics. First, traditionally treated as distinct species, *atlantis* and *hesperis* groups show differences consistent with their species-level distinction: 0.23/0.07, albeit marginally (Fst for distinct species is typically above 0.2, with 0.5 and above indicating strong differentiation, and Gmin is less than 0.1, with 0.02 and below indicating strong isolation). Second, the differences between the *atlantis* and *nausicaa* groups are even more pronounced than those for *atlantis* and *hesperis*: 0.36/0.026, which is in agreement with the *nausicaa* group being sister to the clade consisting of the three other groups in both autosome and Z chromosome trees (Fig. 11). Third, the differences between *hesperis* and *nausicaa* are of about the same magnitude as for others: 0.26/0.05, indicating that *A. nausicaa* is a distinct species rather than a

group of subspecies within paraphyletic *A. hesperis*. Fourth, the *hesperis* and *irene* groups did not reveal species-level differences in the Z chromosome: 0.13/0.13, suggesting that they may be conspecific, which is in agreement with their close clustering together in the Z chromosome tree (Fig. 11b).

Although the *irene* group is not strongly different from the *hesperis* group in Z chromosome, it is placed differently in the autosome tree: as sister to both *hesperis* and *atlantis* groups, rendering the species *A. hesperis* that includes *irene* paraphyletic. While a species paraphyletic in a tree built from concatenated genomic alignments is not inconceivable due to the possibility of extensive introgression from some other species in a part of the species range, such a situation calls for further investigation. Analysis of the trees built from various segments of the nuclear genome revealed that some segments in the *hesperis* group are similar to the *atlantis* group, while other segments are similar to the *irene* group. Hence, we hypothesize that *A. hesperis* is a hybrid species of *A. irene* and *A. atlantis*, because it shares 20% and 67% of its autosome-linked genome, and 71% and 20% of its Z-linked genome with the latter two species respectively, while it possesses only 0.17% of unique polymorphisms, compared to 0.5% and 0.28% unique polymorphisms in *A. irene* and *A. atlantis*, respectively. We see that a significant fraction of the *A. hesperis* genome is shared with either *A. irene* or *A. atlantis*, and the number of unique mutations in the *A. hesperis* lineage is smaller than that compared to either of its putative parental species, suggesting a hybrid origin of *A. hesperis*. Consequently, we consider *A. irene* (consisting of four westernmost subspecies presently associated with *A. hesperis*, Fig. 11) to be a species-level taxon, and the Z chromosome similarity with *A. hesperis* is therefore explained by the hybrid origin of *A. hesperis*, which inherited larger segments of this chromosome from *A. irene*. This scenario of species originating by hybridization is not covered by the Fst/Gmin Z chromosome test for species distinction (Cong et al. 2019b). The COI barcodes of *A. irene* are closer to *A. atlantis* (2.5%, 17 bp difference) than to *A. hesperis* (5%, 33 bp difference), probably because *A. hesperis* possesses mitogenomes introgressed from *A. nausicaa* and does not reveal differences in the barcodes with the latter species. As a side note, the earlier-named species *A. hesperis* is likely to a be a hybrid species with one of the parental species being a named later (*A. irene*), illustrating that biological reality has little to do with the order species were named in.

Finally, we find (Fig. 11) that the holotype of *Speyeria hydaspe conquista* dos Passos & Grey, 1945 (type locality USA: New Mexico, Santa Fe Co., presumed to be in error), presently placed as a synonym of *Argynnis hydaspe rhodope* W. H. Edwards, 1874, clusters closely with the holotype of *Argynnis hesperis tetonia* (dos Passos & Grey, 1945) (type locality USA: Wyoming, Teton Co.) and is therefore placed as a synonym of *tetonia*, **new placement**.

In summary, genomic data suggest that the *atlantis-hesperis* complex consists of four species: *A. atlantis*, *A. hesperis*, and two others with **reinstated status**: *A. irene* and *A. nausicaa*. The following subspecies are assigned to *A. irene* to form **new combinations**: *Argynnis irene dodgei* Gunder, 1931, *Argynnis irene cottlei* J. A. Comstock, 1925, and *Argynnis irene hanseni* (J. Emmel, T. Emmel & Mattoon, 1998). The following subspecies are assigned to *A. nausicaa* to form **new combinations**: *Argynnis nausicaa elko* (Austin, 1984), *Argynnis nausicaa greyi* (Moeck, 1950), *Argynnis nausicaa viola* (dos Passos & Grey, 1945), *Argynnis nausicaa tetonia* (dos Passos & Grey, 1945), *Argynnis nausicaa chitone* W. H. Edwards, 1879, *Argynnis nausicaa schellbachi* (Garth, 1949), *Argynnis nausicaa electa* W. H. Edwards, 1878, *Argynnis nausicaa dorothea* (Moeck, 1947), and *Argynnis nausicaa capitanensis* (R. Holland, 1988). The names for other taxa in this complex remain unchanged.

Argynnis zerene atossa W. H. Edwards, 1890, new combination, is not a subspecies of *Argynnis adiaste* W. H. Edwards, 1864

Described as a species *Argynnis atossa* W. H. Edwards, 1890 (type locality USA: California, Kern Co.), this likely extinct butterfly was placed as a subspecies of *Argynnis adiaste* W. H. Edwards, 1864 (type locality USA: California, Santa Cruz Co.) due to its prominent wing pattern similarities, in particular, the washed out ventral hindwing devoid of silvery spots and reduced black markings above, especially on its hindwing. To our surprise, none of the trees (concatenated protein-coding regions of the entire nuclear

genome, of Z chromosome and mitochondrial genome) placed *atossa* with *adiaste* (Fig. 12). Instead, the *atossa* clade originated within *Argynnis zerene* Boisduval, 1852 (type locality USA: California, possibly Plumas Co.), which is phenotypically strongly spotted, both in back above and silvery below. *Argynnis adiaste* was sister to *Argynnis hydaspe* Boisduval, 1869 (type locality USA: California, possibly Sierra Co.) in a clade remote from *A. zerene*. Using Fst and Gmin statistics, *atossa* and *A. adiaste adiaste* are characterized by 0.48 and 0.012, respectively, indicating strong genetic isolation. However, *atossa* and *A. zerene* show the values Fst=0.13 and Gmin=0.15, which are within the range characteristic of conspecificity. For comparison, *A. adiaste adiaste* and *A. adiaste clemencei* J. A. Comstock, 1925 (type locality USA: California, San Luis Obispo Co.) exhibit Fst/Gmin of 0.06/0.21, showing less genetic differentiation from each other than *atossa* from *A. zerene*. COI barcodes of *atossa* and *adiaste adiaste* lectotypes differ by 2.7% (18 bp), but the *A. zerene myrtleae* (dos Passos & Grey, 1945) holotype and *atossa* lectotype differ by 1.2% (8 base pairs). We think that the evidence presented here to support that *atossa* is not *A. adiaste* is strong. However, while currently we do not have data to justify it, a possibility that *atossa* is a species distinct from *A. zerene* exists, if *A. zerene* is later found to be a complex of several distinct species. For the lack of a better option, we place *atossa* as a subspecies of *A. zerene*, where it fits genetically not worse than a number of other *A. zerene* subspecies, although it differs strongly from all other *A. zerene* subspecies in wing patterns.

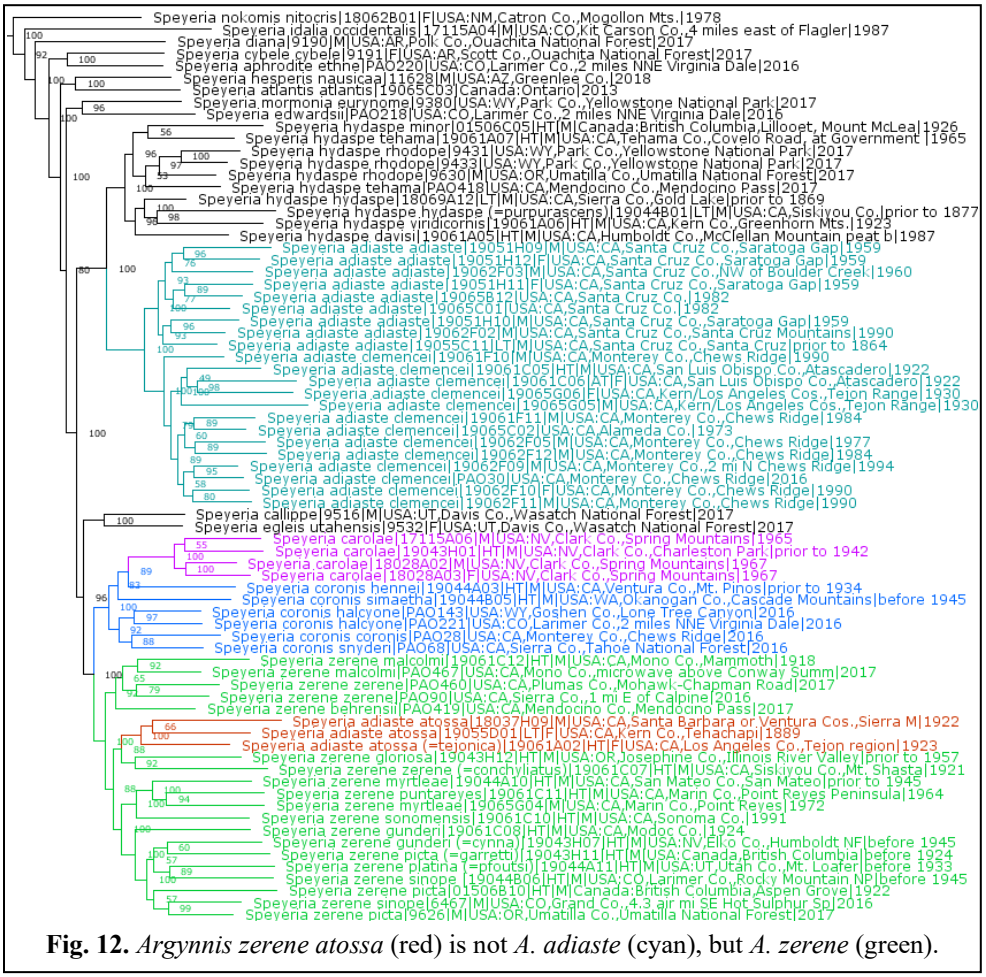

Fig. 12. *Argynnis zerene atossa* (red) is not *A. adiaste* (cyan), but *A. zerene* (green).

The association of *atossa* with *zerene* came as a surprise due to their phenotypic dissimilarity. The reasons behind the wing pattern similarities between *atossa* and *adiaste*, and the lack of such similarity between *atossa* and *zerene* remain unclear. We sequenced the primary types of both *atossa* (in the Carnegie Museum of Natural History, collected prior to its description in 1890) and *tejonica* J. A. Comstock, 1925 (in the Los Angeles County Museum, considered a subjective junior synonym of *atossa*, has spots weakly silvered, collected in 1923 in Los Angeles County, California) in addition to a specimen from Colorado State University collection (collected in 1922 in Sierra Madre Mountains, California). These specimens were collected in different localities and different years, and handled differently throughout the years, but they cluster together in all trees, forming a distinct clade within *zerene*. It does not seem likely that legs of yet unsequenced population of *zerene* were glued to all these 3 specimens, or similar contaminant affected these samples processed in our lab on different days (and for some of them, years) and sequenced in different lanes and batches. Therefore, we conclude that *Argynnis zerene atossa* W. H. Edwards, 1890, **new placement**, represents an unusual example of phenotypic convergence that hindered its taxonomic placement, now revealed through genomic sequencing.

Argynnis coronis carolae dos Passos & Grey, 1942 is a subspecies-level taxon

Described as a subspecies of *Argynnis (Speyeria) coronis* Behr, 1864 (type locality USA: California, possibly Santa Clara Co.), *Argynnis coronis carolae* dos Passos & Grey, 1942 (type locality USA: Nevada, Clark Co.) was elevated to species by Emmel and Austin (Emmel and Austin 1998) due to a number of its unique features, including the isolated locality. Our genomic trees revealed that *carolae* forms a compact clade consistent with its isolation, however, not separately, but deep within *A. coronis*, sister to *Argynnis coronis hennei* Gunder, 1934 (type locality USA: California, Ventura Co.) and near the nominal *A. coronis* (Fig. 12 magenta inside blue clade). This placement in the tree is geographically meaningful, but makes it difficult to accept the species status of *carolae* because it renders *coronis* non-monophyletic. Furthermore, Fst/Gmin for *carolae* vs. *coronis* are 0.23/0.11. Fst is somewhat elevated due to genetic closeness of individuals within apparently strongly inbred *carolae* population, but the gene flow between *carolae* and *coronis* is more than two times higher than the 0.05 threshold characteristic of different species. The COI barcodes of the *A. carolae* holotype and *A. coronis* differ by ~0.5% (3 bp). Therefore, we **reinstate** this taxon as a subspecies: *Argynnis coronis carolae* dos Passos & Grey, 1942.

Aglais Dalman, 1816 and *Polygonia* Hübner, [1819] are subgenera of *Nymphalis* Kluk, 1780

In agreement with others (Opler and Malikul 1992; Layberry et al. 1998; Savela 2020), we propose that *Aglais* Dalman, 1816 (type species *Papilio urticae* Linnaeus, 1758) and *Polygonia* Hübner, [1819] (type species *Papilio c-aureum* Linnaeus, 1758) are better treated as subgenera of *Nymphalis* Kluk, 1780 (type species *Papilio polychloros* Linnaeus, 1758) rather than as distinct genera. These three distinct phylogenetic groups are close to each other genetically with genetic distances between them of the same magnitude as those for taxa considered congeneric in closely related lineages, such as *Vanessa* [Fabricius], 1807 (type species *Papilio atalanta* Linnaeus, 1758) (Fig. 13). The times of divergence between *Nymphalis* and *Polygonia* and between *Nymphalis* and *Aglais* have been estimated as ~7 and ~11 Mya, respectively (Chazot et al. 2019). COI barcode difference between *N. polychloros* and *P. c-aureum* is 6.7% (44 bp), and between *N. polychloros* and *A. urticae* is 7.9% (52 bp). This divergence is comparable to that between *Vanessa annabella* (W. D. Field, 1971) and *Vanessa atalanta* (Linnaeus, 1758) at 6.2% (41 bp), but smaller than the divergence between *V. atalanta* and *N. polychloros* of 9.7% (64 bp). While it is not possible establish a meaningful COI cutoff for the genus-level divergence, these numbers comparatively indicate genetic similarity of these butterflies, and they also loosely correlate with their divergence times. Inspection of the genomic tree (Fig. 13) reveals that the most prominent internal branches (the longest) are indeed those that support *Vanessa* and its sister clade consisting of *Nymphalis*, *Polygonia* and *Aglais*. The clade of the latter three taxa is compact (Fig. 13), prominent, and genetic divergence within it agrees with the expected divergence within a genus. Therefore, unification of the three genera under a single genus (*Nymphalis*) would be more consistent with how other genera (e.g. *Vanessa*) are classified. These showy butterflies are quite diverse in their wing patterns and attracted significant attention, which is likely responsible for their oversplit classification. Also, we confirm the expected sister relationship between *Nymphalis antiopa*

Fig. 13. *Aglais* (red) and *Polygonia* (magenta) are *Nymphalis* (blue).

(Linnaeus, 1758) and *N. cyanomelas* (E. Doubleday, [1848]) (Fig. 13). In summary, in a move towards a more consistent classification, we suggest treating *Aglais* and *Polygonia* as subgenera of *Nymphalis*.

Coenonympha california Westwood, [1851] is species distinct from *Coenonympha tullia* (Müller, 1764)

In agreement with Kodandaramaiah and Wahlberg (2009), we find substantial genetic differentiation between European *Coenonympha tullia* (Müller, 1764) (type locality Denmark: Zealand Island) and North American *Coenonympha california* Westwood, [1851] (type locality USA: California, near San Francisco) (Warren et al. 2016). The latter has frequently been treated as a subspecies of the former (Pelham 2008; Pelham 2020). Fst/Gmin statistics for *C. tullia* vs. *california* are 0.22/0.02, indicating very limited gene exchange between these taxa, typical of closely related species rather than subspecies. This differentiation strongly suggests that *C. california* is indeed a species distinct from *C. tullia*. Moreover, we find that the northernmost American taxa from this complex form a clade that is sister to *C. tullia* (Müller, 1764) and not to *C. california* Westwood, [1851] (Fig. 14). These populations are currently attributed to subspecies *C. tullia kodiak* W. H. Edwards, 1869 (type locality USA: Alaska, Kodiak), *C. tullia mixturata* Alpheraky, 1897 (type locality Russia: Kamchatka; it remains to be investigated if this name applies to the Nearctic taxon) and *C. tullia yukonensis* W. Holland, 1900 (type locality Canada: Yukon and USA: Alaska). Compared to European *C. tullia*, they show Fst/Gmin of 0.14/0.05, which are in the range for conspecific populations, however with a more limited gene exchange than typical (Gmin is less than 0.1). Therefore, until further research shows otherwise, we leave these three subspecies with *C. tullia*. Future genomic studies of *Coenonympha tullia viluiensis* Ménétries, 1859 (type locality Russia: Vilyuy River) are also needed, because if it falls in the same clade with the three subspecies of *C. tullia* from North America, it would be the oldest name in this clade. And if this clade is found to be a species distinct from *C. tullia*, it will be the name of this species. In summary, according to our genomic analysis, both *C. tullia* and *C. california* are present in North America, and all American taxa of the *tullia* complex other than the northernmost subspecies *C. t. kodiak*, *C. t. mixturata* and *C. t. yukonensis* belong to *C. california*.

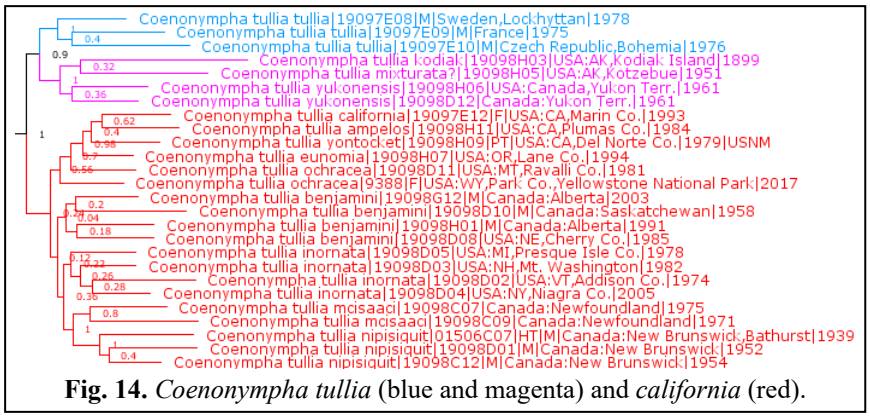

Fig. 14. *Coenonympha tullia* (blue and magenta) and *california* (red).

Palaeonympha Butler, 1871 is a subgenus of *Megisto* Hübner, [1819]

The New World genus *Megisto* Hübner, [1819] (type species *Papilio eurytus* Fabricius, 1775, a junior homonym, considered a synonym of *Papilio cymela* Cramer, 1777) became monotypic after the transfer of *Euptychia rubricata* W. H. Edwards, 1871 (type locality USA: Texas, McLennan Co.) to *Cissia* Doubleday, 1848 (type species *Papilio clarissa* Cramer, [1780], a junior subjective synonym of *Papilio penelope* Fabricius, 1775) (Zacca et al. 2018). A genus *Palaeonympha* Butler, 1871 was proposed for a newly described species *P. opalina* Butler, 1871 from China and remained monotypic since. In wing patterns, it

Fig. 15. *Palaeonympha* (red) is *Megisto* (blue); *Cissia* (magenta), *Vanima* (brown). IDs with "SA" are from NCBI database.

bears an uncanny resemblance to *M. cymela* (Fig. 16), and was indeed sister to *Megisto* both in gene marker-based (Zacca et al. 2018) and genome-scale phylogenies (Espeland et al. 2019). Genomic sequencing confirms a close relationship between monotypic *Megisto* from the New World and monotypic *Palaeonympha* from the Old World (Fig. 15 blue and red), closer than many species of *Cissia* are to each other (Zacca et al. 2018; Espeland et al. 2019) and about the same divergence as two species of *Vanima* Zacca, Casagrande & O. Mielke (Fig. 15 brown). The COI barcodes of *cymela* and *opalina* differ by 8.8% (58 bp), within the range of many congeners. To emphasize this close kinship between these two disjunct species (*cymela* and *opalina*) apparent from their genotypes and phenotypes, we propose that, instead of each being placed in its own monotypic genus, they are congeneric. We assign a **new status** of subgenus to *Palaeonympha*, resulting in *Megisto opalina* Butler, 1871, **new combination**. Thus, *Megisto* becomes a Holarctic genus, yet again indicating elaborate connections between the Old and the New Worlds.

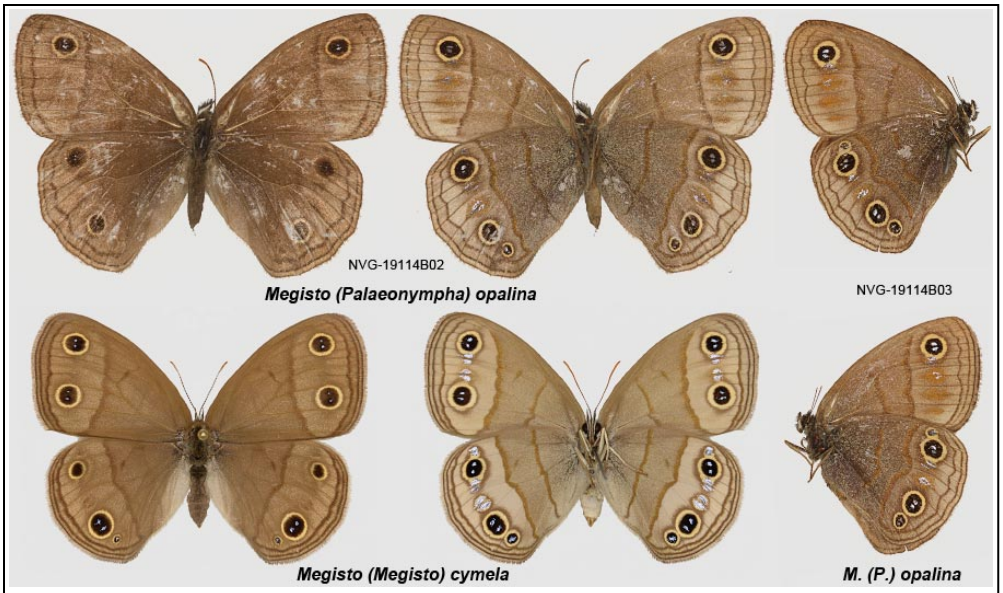

Fig. 16. *Megisto opalina* (Taiwan, sequenced as NVG-19114B02 & NVG-19114B03) and *M. cymela* (USA: OK, Carter Co., Murray Lake, 9-Apr-1999).

Cissia cleophes (Godman & Salvin, 1889) does not belong to *Megisto* Hübner, [1819]

Placed in *Megisto* recently on the basis of morphological similarities (Zacca et al. 2020), *Euptychia cleophes* Godman & Salvin, 1889 (type locality Mexico, Guerrero, Fig. 17) is not monophyletic with the type species of *Megisto* (Fig. 15 magenta vs. blue) and therefore does not belong to *Megisto*, unless this genus is expanded to include species placed in other genera, such as *Yphthimoides* Forster, 1964, *Vanima* and *Cissia*. In our tree (Fig. 15), *cleophes* falls within *Cissia*: it is in the clade with *C. penelope*, which is a valid name for the type species of *Cissia*. Therefore, because the tree strongly supports that *cleophes* does not belong to *Megisto*, we suggest to leave this species in *Cissia* (where it may belong and was placed previously, but statistical support for this clade is weaker) pending genomic analysis of other species from this and nearby clades.

Fig. 17. *Cissia cleophes* sequenced as NVG-19118B01.

Hyponephele Muschamp, 1915 is a subgenus of *Cercyonis* Scudder, 1875

In the genomic tree (Fig. 18), *Hyponephele* Muschamp, 1915 (type species *Papilio lycaon* Rottemburg, 1775) is sister to *Cercyonis* Scudder, 1875 (type species *Papilio alope* Fabricius, 1793, placed as a

subspecies of *Papilio pegala* Fabricius, 1775), in agreement with previous findings (Peña et al. 2006). The tree reveals that the genetic divergence between *Hyponephele* and *Cercyonis* is smaller than that within *Erebia* Dalman, 1816 (type species *Papilio ligea* Linnaeus, 1758). The COI barcodes of *H. lycaon* and *C. pegala* differ by 7.9% (52 bp). We give *Hyponephele* a **new status** of a subgenus within *Cercyonis*. This change may not be welcomed by the Old World Lepidopterists who are used to the name *Hyponephele* applied to its many species, similar to how *Argynnis* is not welcomed in America to include *Speyeria* as its subgenus. However, this name change highlights the close relationship between the two subgenera (*Hyponephele* and *Cercyonis*) making *Cercyonis* a Holarctic genus, similar to *Erebia* Dalman, 1816 (type species *Papilio ligea* Linnaeus, 1758) in divergence and distribution. This is yet another step towards more internally consistent genus-level classification in butterflies.

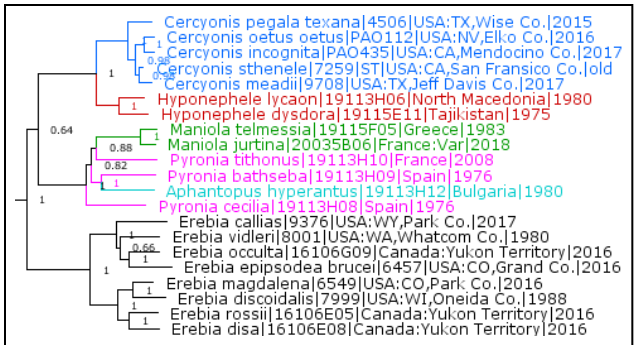

Fig. 18. *Cercyonis* (blue), *Hyponephele* (red), *Maniola* (green), *Pyronia* (magenta), and *Aphantopus* (cyan).

Pyronia Hübner, [1819] and *Aphantopus* Wallengren, 1853 are subgenera of *Maniola* Schrank, 1801

The genomic tree reveals that *Pyronia* Hübner, [1819] (type species *Papilio tithonus* Linnaeus, 1771) is not monophyletic (Fig. 18), and *Pyronia bathseba* (Fabricius, 1793) is sister to *Aphantopus* Wallengren, 1853 (type species *Papilio hyperantus* Linnaeus, 1758) with strong support (Fig. 18). Two other *Pyronia* species we sequenced are not monophyletic either: the type species of the genus is sister to *Maniola* Schrank, 1801 (type species *Maniola lemur* Schrank, 1801, which is a junior subjective synonym of *Papilio jurtina* Linnaeus, 1758). To restore monophyly, it is possible to break *Pyronia* into smaller genera, and these already have names available: *Pasiphana* de Lesse, 1952 (type species *Papilio bathseba* Fabricius, 1793) and *Idata* de Lesse, 1952 (type species *Epinephele ida* var. *cecilia* Vallantin, 1894). Alternatively, they can be grouped in some ways to form more inclusive monophyletic genera. The tree (Fig. 18) reveals three clusters of species of equivalent rank. One of these clusters is the genus *Erebia*. The other one is the genus *Cercyonis* as we presently define it (including *Hyponephele* as a subgenus). Therefore, it is meaningful to treat the third group as a single genus as well. Hence, we propose that *Aphantopus*, *Pyronia*, *Pasiphana* and *Idata* are subgenera of *Maniola*, **new status**. It is unfortunate that genomic data suggest abandoning the familiar *Aphantopus* and *Pyronia* as genera, however breaking this more inclusive but genetically prominent genus *Maniola* into four or five very small genera is even less appealing to us.

Dione incarnata N. Riley, 1926 is a species distinct from *Dione vanillae* (Linnaeus, 1758)

Inspection of a nuclear genomic tree reveals pronounced divergence between *Dione vanillae* (Linnaeus, 1758) (type locality South America, probably Surinam) specimens from Jamaica and from the USA. (Fig. 19). This divergence is of about the same magnitude as that between the distinct species *Dione moneta* Hübner, [1825] and *Dione juno* (Cramer, 1779), and somewhat smaller than that between more distant relatives

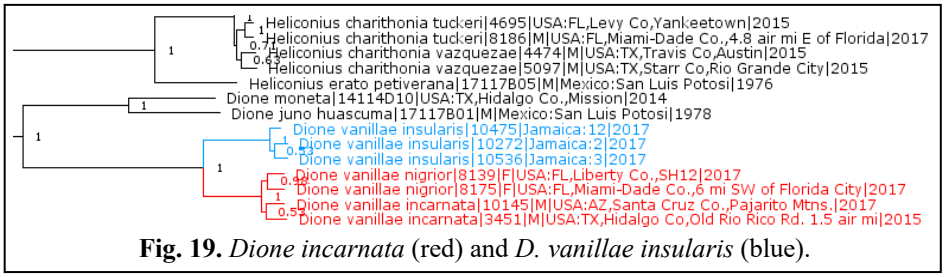

Fig. 19. *Dione incarnata* (red) and *D. vanillae insularis* (blue).

Heliconius charitonia (Linnaeus, 1767) and *H. erato* (Linnaeus, 1758) (Fig. 19). In contrast to their separation from *Dione vanillae insularis* (Maynard, 1889) (type locality Bahamas, Fig. 19 blue), the name currently applied to Jamaican populations, the two USA subspecies: eastern *Dione vanillae nigrior* (Michener, 1942) (type locality USA: Florida, Monroe Co.) and western *Dione vanillae incarnata* N. Riley, 1926 (type locality Mexico: Durango), cluster closely with each other (Fig. 19 red). The Fst/Gmin statistics for the two groups (red vs. blue) are 0.81/0.0002, indicating nearly absent gene exchange between these groups, the smallest of all sister species we studied in this work. Search of the BOLD database (Ratnasingham and Hebert 2007) reveals that Jamaican specimens group closely by their COI barcodes with specimens from other Caribbean Islands (Cuba, Dominican Republic, Puerto Rico), and in particular with specimens from the Bahamas (the type locality of *D. v. insularis*. Therefore, Jamaican populations may indeed be referred to as *D. v. insularis*. The COI barcode differences between Jamaican *insularis* and USA *incarnata* is 2.9% (19 bp). For these reasons, *D. vanillae insularis* is apparently a species distinct from the USA species consisting of subspecies *nigrior* and *incarnata*.

These two species (*insularis* and the USA species) are distinct phenotypically with wing shapes and patterns, as described by Maynard (1889). In fact, Maynard proposed *Agraulis insularis* as a species, and he considered the USA species to be *Agraulis vanillae*, characterized by longer wings, smaller black spots, white dots in forewing black spots compared to *insularis*. However, both the Merian illustration (1705), which was mentioned in the original description of *D. vanillae* (Linnaeus, 1758), and the lectotype specimen (Honey and Scoble 2001) reveal the *insularis* phenotype (e.g. extended black band on the forewing, broader wings) that differs from the USA specimens. In his revision, Michener gave a key to the *vanillae* complex taxa (Michener 1942). The first doublet separates *incarnata* with *nigrior* from all other taxa, including the nominotypical *vanillae*. Therefore, the USA specimens are not *D. vanillae*, but a species that may be referred to by the oldest name applicable to North American populations: *D. incarnata*, and we currently leave *insularis* as a subspecies of *D. vanillae*. Michener also raised the possibility that *incarnata* may be a distinct species, based on *incarnata* specimen from Colombia, in which case it would be sympatric with *D. vanillae*. However, these Colombian specimen might have been mislabeled. Due to the evidence presented here, *Dione incarnata* N. Riley, 1926, **new status**, appears to be a distinct species, with *D. incarnata nigrior* (Michener, 1942), **new combination**, being its subspecies.

Danaus eresimus (Cramer, 1777) belongs to subgenus *Danaus* Kluk, 1780, together with *Danaus plexippus* (Linnaeus, 1758), and not to subgenus *Anosia* Hübner, 1816 together with *Danaus gilippus* (Cramer, 1775)

Danaus eresimus (Cramer, 1777) (type locality Suriname) looks superficially similar to *Danaus gilippus* (Cramer, 1775) (type locality Brazil: Rio de Janeiro) and at times it is a challenge to distinguish these two species. On the contrary, *Danaus plexippus* (Linnaeus, 1758) (type locality USA: New York, Orleans Co.) is superficially more different from either *D. eresimus* or *D. gilippus* (Warren et al. 2016). Due to these superficial similarities and differences, traditionally, only the former, as the type species, belonged to *Danaus* Kluk, 1780, and the latter two species were placed in *Anosia* Hübner, 1816 (type species *Papilio gilippus* Cramer, 1775) (Ackery and Vane-Wright 1984; Pelham 2008; Pelham 2020). However, among these three species, genomic data (both nuclear and mitochondrial genomes) place *D. eresimus* as a sister to *D. plexippus* with high confidence (Fig. 20), and *D. gilippus* is a sister to that clade of the two species, in agreement with previous DNA-based analyses (Zhan et al. 2014; Aardema and Andolfatto 2016). Therefore, we transfer *Danaus eresimus* (Cramer, 1777) from the subgenus *Anosia* where it does not belong, to the subgenus *Danaus* in accord with the phylogeny of these three species. This change has already been implemented

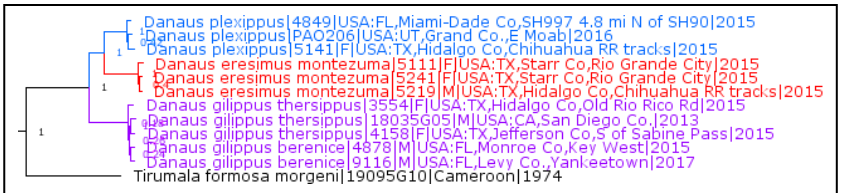

Fig. 20. *Danaus plexippus* (blue), *eresimus* (red) and *gilippus* (magenta).

by Pelham in the most recent version of the catalogue (2020) after the discussion of our genomic data and previous works with NVG. Here, we simply formalize this change in a publication.

Chlosyne coronado (M. Smith & Brock, 1988) is a species distinct from *Chlosyne fulvia* (W. H. Edwards, 1879)

In the *leanira* group, *Chlosyne fulvia coronado* (M. Smith & Brock, 1988) (type locality USA: Arizona, Pima Co.) has been a subspecies-level taxon since its description (Smith and Brock 1988). The genomic tree of the group revealed that *Chlosyne fulvia* (W. H. Edwards, 1879) (type locality USA: "Western Texas") is paraphyletic with respect to *Chlosyne cyneas* (Godman & Salvin, 1878) (type locality Mexico: Oaxaca), and *C. fulvia coronado* with *Chlosyne fulvia pariaensis* (M. Smith & Brock, 1988) (type locality USA: Utah, Kane Co.) form a clade more prominent than *Chlosyne leanira* (C. Felder & R. Felder, 1860) (type locality USA: California, Plumas Co.) (Fig. 21). The Fst/Gmin statistics for *fulvia* and *coronado* are 0.49/0.015, comparable to those of *fulvia* vs. *leanira*: 0.40/0.012. COI barcodes are 2.7% (18 bp) different between *fulvia* and *coronado*, but are 0% between *coronado* and *pariaensis*. Furthermore, according to the map of sequenced specimens (Fig. 21), *fulvia* and *coronado* may be sympatric in north-central Arizona. For all of these reasons, we propose species status for *Chlosyne coronado* (M. Smith & Brock, 1988) **new status**, and *Chlosyne coronado pariaensis* (M. Smith & Brock, 1988) **new combination**, as its subspecies.

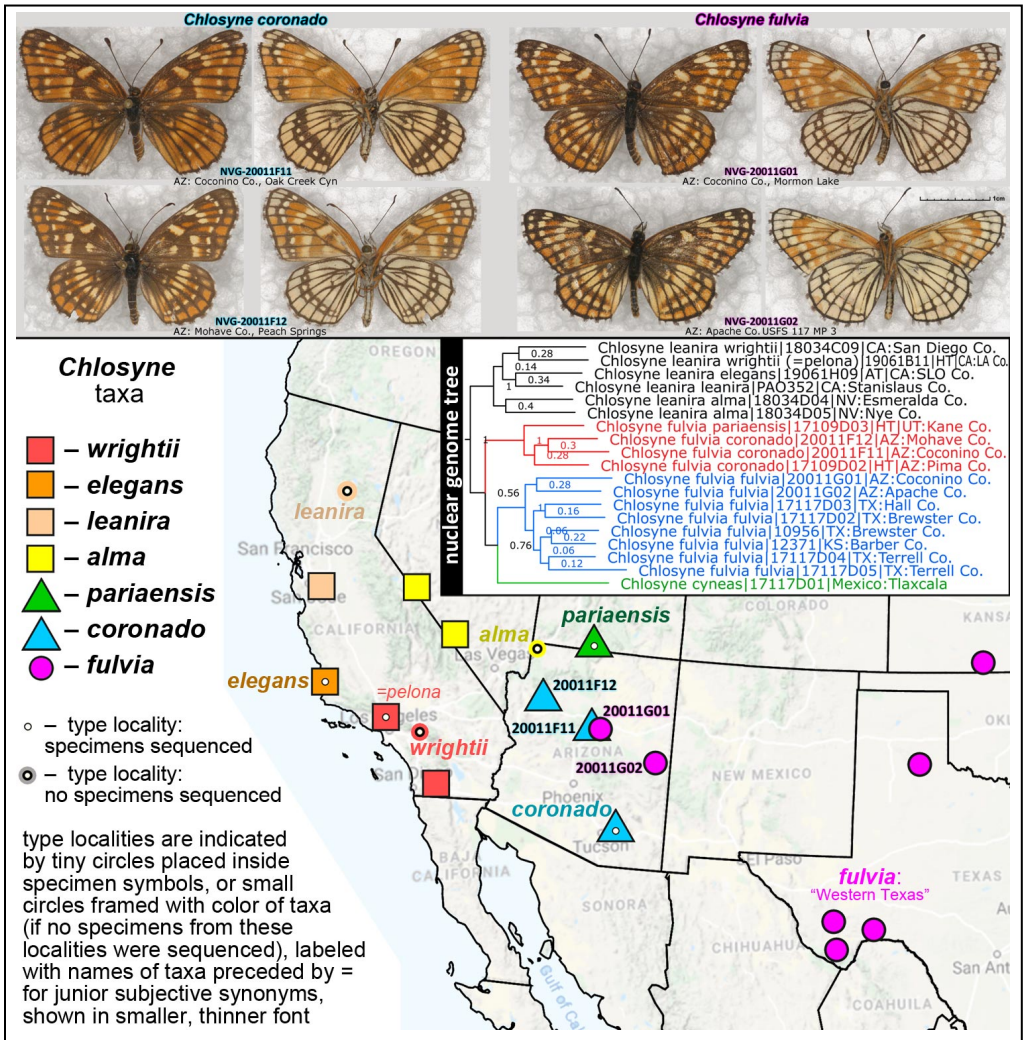

Fig. 21. *Chlosyne fulvia* and *coronado* with relatives. Four specimens from Arizona are shown (dorsal: right and ventral: left) and are labeled with their DNA sample numbers on the map that shows the localities of all sequenced specimens from the USA included in the tree.

Chlosyne chinatiensis (Tinkham, 1944) is a species distinct from *Chlosyne theona* (Ménétriés, 1855)

In their revision of the *theona* group, Austin and Smith (Austin and Smith 1998) placed *Melitaea chinatiensis* Tinkham, 1944 (type locality USA: Texas, Presidio Co.) as a subspecies of *Chlosyne theona*

(Ménétriés, 1855) (type locality Nicaragua). While accepting subspecies-level treatment of *chinatiensis*, Pelham (2008) writes: "There is considerable reason to consider this a distinct species from *theona*. More investigation is required." We carried out our genomic investigation by sequencing of three *chinatiensis* specimens from the US and Mexico (Fig. 22) and found that their comparison with *theona* specimens from across the range (from Arizona, Texas and Costa Rica) results in the following Fst/Gmin statistics: 0.35/0.019, indicating genetic differentiation and low gene exchange consistent with *chinatiensis* being a species-level taxon. The COI barcodes of *C. chinatiensis* and *C. theona thekla* differ by 1.4% (9 bp), but those of *C. chinatiensis* and *C. theona bolli* differ by 0.6% (4 bp). Moreover, *C. chinatiensis* is sympatric with *C. theona bolli* in west Texas, e.g. in the Big Bend National Park. Given this evidence, we **reinstate** *Chlosyne chinatiensis* (Tinkham, 1944) as a species.

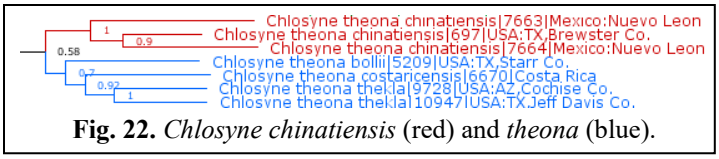

Fig. 22. *Chlosyne chinatiensis* (red) and *theona* (blue).

Family Hesperiidae Latreille, 1809

Phocides lilea (Reakirt, [1867]) is a species distinct from *Phocides polybius* (Fabricius, 1793)

A tree constructed from protein-coding regions of their nuclear genomes reveals that specimens of *Phocides polybius* (Fabricius, 1793) (type locality "Indiis", likely Suriname) partition into two distinct clades (Fig. 23). One of the clades consists of *Phocides polybius lilea* (Reakirt, [1867]) (type locality Mexico: Veracruz, Fig. 23 red). The other clade includes all other taxa of this species (Fig. 23 blue). Fst/Gmin statistics for the Z chromosome comparison of these two clades are 0.39/0.021, suggesting that they represent two distinct species. Moreover, COI barcodes of *lilea* from Mexico and *polybius* from Guyana show 3% (20 bp) difference. Therefore, we conclude that *Phocides lilea* (Reakirt, [1867]) is a species-level taxon, **reinstated status**.

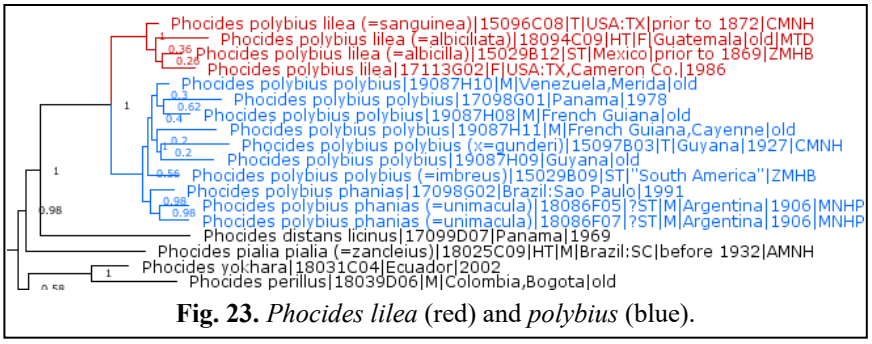

Fig. 23. *Phocides lilea* (red) and *polybius* (blue).

Furthermore, we sequenced the only known syntype of an enigmatic taxon *Erycides imbreus* Plötz, 1879 from the ZMHB collection, illustrated in Warren et al. (2016). This specimen is a syntype because it is a uniquely patterned specimen that carries appropriate labels, agrees with the original description and looks similar to the unpublished Godman copy of the Plötz illustration (in BMNH, inspected by NVG) (Godman 1907). It is an unusual specimen lacking an orange bar in the forewing discal cell (usually extending to costa) typical for *P. polybius*. Evans (1952) treated this name as a distinct species *Phocides imbreus* Plötz, 1879 based on a rather poor illustration of this specimen in Draudt (1921)—it is unlikely that Evans saw the actual specimen. Mielke & Casagrande (2002) inspected the syntype and synonymized the name with *P. polybius lilea* due to general phenotypic similarity and the lack of orange coloring on the fringe around the hindwing tornus. According to our genomic results (Fig. 23), *imbreus* is confidently placed with *Phocides polybius polybius*, **revised placement of a synonym**, and is probably an aberrant specimen of *polybius*, not *lilea*, lacking any orange coloration on its wings, not just on the fringe, but also a forewing orange bar. However, the head of the syntype retains the usual orange patterns including orange palpi and cheeks. Our revised synonymy is further supported by the label data on the specimen stating "Am. m.", which probably stands for America meridionalis (South America), where *P. lilea* is not known to occur.

Cecropterus nevada (Scudder, 1872) and *Cecropterus dobra* (Evans, 1952) are species distinct from *Cecropterus mexicana* (Herrich-Schäffer, 1869)

Genomic analysis of *Cecropterus mexicana* (Herrich-Schäffer, 1869) (type locality Mexico) reveals a pronounced divergence between its subspecies (Fig. 24) that was analyzed further. The genomic tree shows separation between some of them comparable to that from *Cecropterus diversus* (E. Bell, 1927) (type locality USA: California, Plumas Co.). While *Cecropterus mexicana aemilea* (Skinner, 1893) (type locality USA: Oregon, Klamath Co., male syntype sequenced), *C. mexicana blanca* (J. Scott, 1981) (type locality USA: California, Mono Co.) and *C. mexicana nevada* (Scudder, 1872) (type locality USA: California, Sierra Nevada) group closely together (all three unified under the name *nevada* below), *C. mexicana dobra* (Evans, 1952) (type locality USA: Arizona, Graham Co.) forms a clade distinct from them and *C. mexicana*. The Fst/Gmin statistics for these clades are: *mexicana* vs. *dobra*: 0.34/0.021, *mexicana* vs. *nevada*: 0.37/0.010, *nevada* vs. *dobra*: 0.30/0.055. We see that *nevada* and *dobra* exchange genes more frequently with each other than do each of them with *mexicana*. Differences between COI barcodes in pairs of these species are: *mexicana* and *dobra*: 1.8% (12 bp), *mexicana* and *nevada*: 1.1% (7 bp), *nevada* and *dobra*: 1.7% (11 bp). For comparison, the COI barcodes of *aemilea*, *blanca* and *nevada* are 100% identical. Curiously, in contrast to nuclear genomes (Fig. 24), mitochondrial genomes (as reflected by barcodes) place *mexicana* closer to *nevada*, and *dobra* farther away from them, which is yet another example of the peculiarity of mitochondrial evolution. Deriving further support from genitalic and wing pattern differences mentioned by Evans (1952), we suggest that *Cecropterus nevada* (Scudder, 1872), **reinstated status**, and *Cecropterus dobra* (Evans, 1952), **new status**, are species-level taxa, not subspecies of *Cecropterus mexicana* (Herrich-Schäffer, 1869). Then, we treat *Cecropterus nevada aemilea* (Skinner, 1893) and *Cecropterus nevada blanca* (J. Scott, 1981), **new combinations**, as subspecies of *C. nevada*.

Fig. 24. *Cecropterus nevada* (red), *dobra* (magenta) and *mexicana* (blue).

Telegonus anausis Godman & Salvin, 1896, is a species distinct from *Telegonus anaphus* (Cramer, 1777), and *Telegonus anausis annetta* (Evans, 1952) is its subspecies

A polytypic species *Telegonus anaphus* (Cramer, 1777) (type locality Suriname), in addition to nominal, includes four subspecies (Evans 1952), all of which we sequenced and analyzed. Among them, there is a single representative of the USA fauna: *Astraptes anaphus annetta* Evans, 1952 (type locality Costa Rica). The genomic tree of these taxa reveals a split between the nominotypical *T. anaphus* and all others (Fig. 25 blue vs. red clade). The oldest name in the second (red) clade is *Telegonus anausis* Godman & Salvin, 1896 (type locality St. Vincent, Grenada, Dominica, Hispaniola). The Fst/Gmin statistics computed on some of these subspecies are: *anaphus* vs. *annetta* 0.40/0.01 and *anaphus* vs. *anausis* 0.45/0.009, indicating species-level differentiation. The COI barcodes

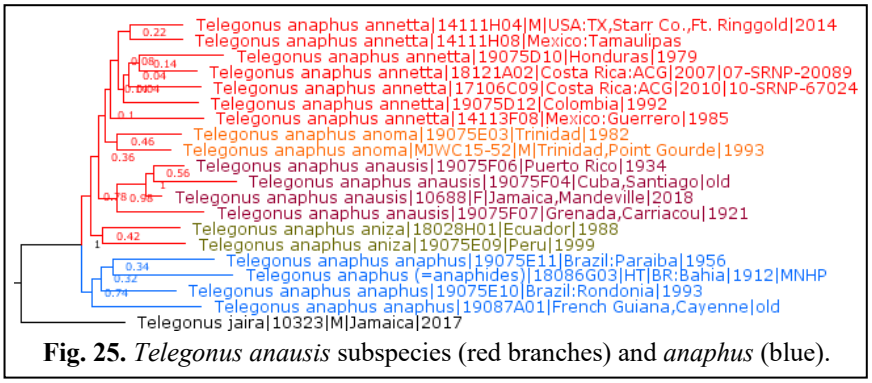

Fig. 25. *Telegonus anausis* subspecies (red branches) and *anaphus* (blue).

are 4.3% (28 bp) different between *anaphus* and *annetta*, which is larger than a typical difference between closely related species. Comparing subspecies within the second cluster (Fig. 25 red clade), we were not able to gather evidence for their change of status to species. For instance, Fst/Gmin of *anausis* vs. *annetta* is 0.16/0.11, which by itself does not justify Central American *annetta* as a species distinct from the Caribbean *anausis*. Therefore, we **reinstate** *Telegonus anausis* Godman & Salvin, 1896 as a species and transfer all (except the nominotypical) subspecies currently placed in *T. anaphus* to *T. anausis* to form the following **new combinations**: *Telegonus anausis annetta* (Evans, 1952), *Telegonus anausis anoma* (Evans, 1952), and *Telegonus anausis aniza* (Evans, 1952). A curious accident here is that as a result of genomic work, the name originally proposed (Godman and Salvin 1896) as *Telegonus anausis* is now returned to its original combination and status nearly 125 years later.

Epargyreus huachuca Dixon, 1955 is a species distinct from *Epargyreus clarus* (Cramer, 1775)

We obtained whole genome shotgun sequences of specimens from all known distinct groups of US populations currently assigned to *Epargyreus clarus* (Cramer, 1775) (type locality "Suriname", later corrected to USA: Virginia, Rockingham Co.). A tree made from protein-coding regions of the Z chromosome (Fig. 26) reveals a prominent split into two clades (blue and red) with divergence comparable to that between *Epargyreus orizaba* Scudder, 1872 and *Epargyreus cruza* Evans, 1952, shown as outgroups. The holotypes of both *E. clarus huachuca* Dixon, 1955 (type locality USA: Arizona, Cochise Co.) and *E. clarus profugus* Austin, 1998 (type locality USA: Nevada, Clark Co.) have been sequenced, along with a possible type specimen of *E. clarus californicus* MacNeill, 1975 (type locality USA: California, El Dorado Co.). The Fst and Gmin between *E. clarus clarus* and *E. clarus huachuca* are, 0.35 and 0.03 respectively, compared to those of 0.04 and 0.16 between *E. clarus huachuca* and *E. clarus profugus*. The difference in COI barcodes of *E. clarus clarus* and *E. clarus huachuca* is about 2% (~13 bp difference). For these reasons, we suggest that *Epargyreus huachuca* Dixon, 1955 is a distinct species, **new status**, and it includes *Epargyreus huachuca profugus* Austin, 1998, **new combination**, as a subspecies. We found that genetic differentiation between populations of *E. clarus californicus* is lower than between others (Fig. 26, a tight cluster of specimens with shorter terminal branches) suggesting either a recent dispersal or a bottleneck. Whether this genetic purge resulted in any degree of reproductive isolation of *E. clarus californicus* from other groups of *E. clarus* populations remains to be investigated.

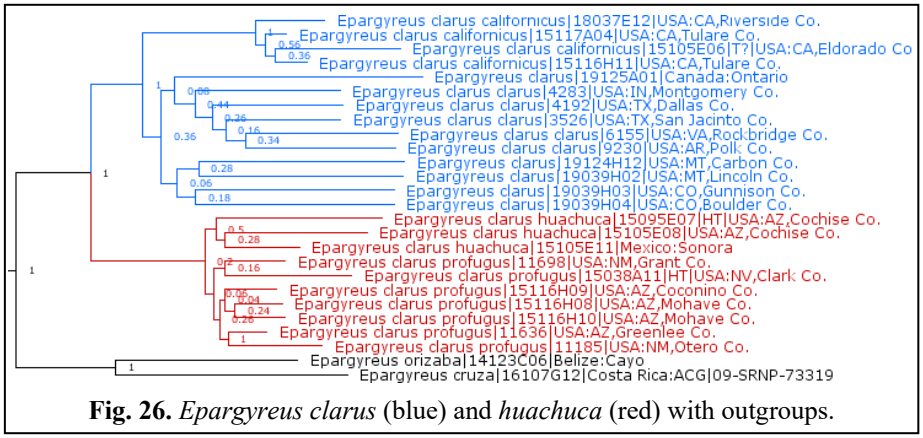

Fig. 26. *Epargyreus clarus* (blue) and *huachuca* (red) with outgroups.

Nisoniades bromias (Godman & Salvin, 1894) is a species distinct from *Nisoniades rubescens* (Möschler, 1877)

Considered a junior subjective synonym of *Nisoniades rubescens* (Möschler, 1877) (type locality Suriname), *Pellicia bromias* Godman & Salvin, 1894 (type locality Mexico, Guatemala, Costa Rica, Panama) reveals 1.2% (8 bp)

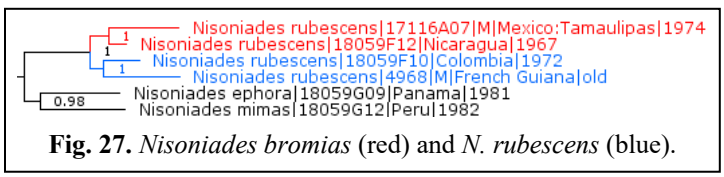

Fig. 27. *Nisoniades bromias* (red) and *N. rubescens* (blue).

difference in COI barcode, which by itself is not large enough to draw definitive conclusions, but it prompted further investigation. The Fst/Gmin statistics computed on two pairs of specimens from distant localities (Fig. 27) were 0.27/0.05, suggesting that *Nisoniades bromias* (Godman & Salvin, 1894), **reinstated status**, is a distinct species.

Pholisora crestar J. Scott & Davenport, 2017 is a species distinct from *Pholisora catullus* (Fabricius, 1793)

Recently described as a subspecies on the basis of wing pattern differences (Scott et al. 2017), *Pholisora catullus crestar* J. Scott & Davenport, 2017 (type locality USA: California, Tulare Co.) was synonymized with *Pholisora catullus* (Fabricius, 1793) (type locality "Indiis", likely eastern US) by Pelham (2020). As a part of on-going genomic sequencing inventory of the primary type specimens of Hesperiidae, we obtained and analyzed whole genome shotgun reads of the holotype and two paratypes of *crestar*. Surprisingly, their comparison with *P. catullus* populations from several distant localities revealed prominent genetic differentiation (Fig. 28). Moreover, one of the *crestar* paratypes (from CA: Mono Co., NVG-17066H12, Fig. 28) apparently is *P. catullus*, not *crestar*. Fst/Gmin statistics for the *crestar*/*catullus* comparison are 0.34/0.014, suggesting distinctness of *crestar* as a species. Gene exchange between *catullus* and *crestar* (0.014) is very low (for conspecific populations it is typically above 0.1), strongly supporting reproductive isolation between these taxa. Peculiarities of COI barcode evolution in *Pholisora* have been reported previously by Pfeiler (2018) and COI barcodes of the *crestar* holotype and the *catullus* specimen from Texas (NVG-3990) differ by 1.7% (11 bp). Due to strong genetic differentiation, we suggest that *Pholisora crestar* J. Scott & Davenport, 2017, **new status**, is a species-level taxon.

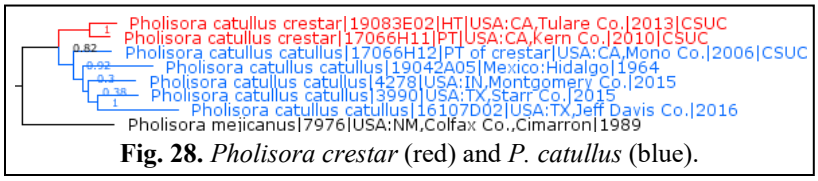

Fig. 28. *Pholisora crestar* (red) and *P. catullus* (blue).

Carterocephalus mandan (W. H. Edwards, 1863) and *Carterocephalus skada* (W. H. Edwards, 1870) are species-level taxa and not subspecies of *Carterocephalus palaemon* (Pallas, 1771)

Proposed as a species, *Hesperia mandan* W. H. Edwards, 1863 (type locality Canada: Manitoba) has mostly been considered a subspecies of *Carterocephalus palaemon* (Pallas, 1771) (type locality Russia: Samara Oblast) (Pelham 2008). Although it has been recently reinstated as a species (Pohl et al. 2010), this suggestion was not universally followed (Pelham 2020). Genomic comparison of the Old and New World *palaemon*-like populations reveals three clusters in the tree (Fig. 29): two corresponding to the abovementioned taxa (red and blue), and the third one (green) for *Carterocephalus palaemon skada* (W. H. Edwards, 1870) (type locality USA: AK, Kodiak) together with *Carterocephalus palaemon magnus* Mattoon & Tilden, 1998 (type locality USA: California, Sonoma Co.). The largest separation is observed between the Old World *C. palaemon* and the New World taxa. The Fst/Gmin statistics for the comparison of pairs of these 3 clusters (we call the green cluster by its oldest name: *skada*) are: *palaemon* vs. *mandan* 0.50/0.016, *palaemon* vs. *skada* 0.56/0.005, and *mandan* vs. *skada* 0.36/0.025. All of these numbers indicate strong genetic differentiation and very low gene exchange between clusters. Analysis of COI barcodes reveals an unusual situation.

Fig. 29. *Carterocephalus mandan* (red), *skada* (green) and *palaemon* (blue).

First, barcode difference of *palaemon* vs. *mandan* is the same as *palaemon* vs. *skada*: 1.5% (10 bp). Second, *mandan* barcodes are not much different from *skada* (0.3%, 2 bp), which can be explained by introgression, but the *mandan* neotype and *magnus* holotype exhibit larger difference of 0.76% (5 bp) between them. Third, for comparison, two widely sympatric Old World species *C. palaemon* and *Carterocephalus silvicola* (Meigen, 1829) exhibit Fst/Gmin of 0.72/0.0006 (indicating very strong isolation), but barcode difference between them is only 0.6% (4 bp). A number of similar instances of distinct butterfly species not strongly different in their barcodes have been documented (Burns et al. 2008; Cong et al. 2017), thus barcode differences and similarities cannot be considered separately from all other evidence. In summary, we suggest to **reinstate** *Carterocephalus mandan* (W. H. Edwards, 1863) and *Carterocephalus skada* (W. H. Edwards, 1870) as species, and additionally propose the following **revised combinations**: *Carterocephalus mandan mesapano* (Scudder, 1868) and *Carterocephalus skada magnus* Mattoon & Tilden, 1998.

Amblyscirtes arizonae H. Freeman, 1993 is a species distinct from *Amblyscirtes elissa* Godman, 1900

Described as a subspecies of *Amblyscirtes elissa* Godman, 1900 (type locality Mexico: Guerrero), *A. e. arizonae* H. Freeman, 1993 (type locality USA: Arizona, Santa Cruz Co.) has not caused much attention being invariably kept as a subspecies (Pelham 2008). We sequenced primary type specimens of both *A. e. arizonae* and *A. elissa* and compared them with specimens from other localities. The genomic tree revealed two prominent clusters with separation similar to that between *A. belli* H. Freeman, 1941 and *A. celia* Skinner, 1895, and between *A. carolina* (Skinner, 1892) and *A. reversa* F. Jones, 1926 (Fig. 30). The Fst/Gmin statistics for them were 0.60/0.002, implying strong genetic differentiation and virtually no gene exchange between these taxa. The COI barcodes of *A. elissa* and *A. e. arizonae* primary type specimens differ by 2.6% (17 bp). For these reasons, we suggest that *Amblyscirtes arizonae* H. Freeman, 1993 is a distinct species, **new status**.

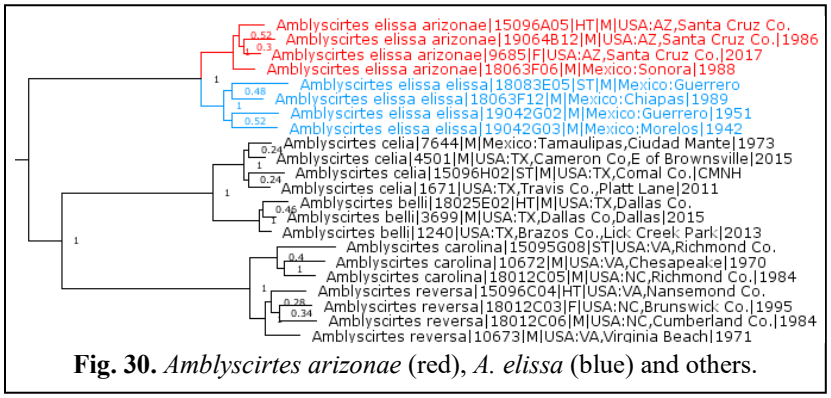

Fig. 30. *Amblyscirtes arizonae* (red), *A. elissa* (blue) and others.

Megathymus violae D. Stallings & Turner, 1956 is a species distinct from *Megathymus ursus* Poling, 1902

Initially proposed as a species, *Megathymus violae* D. Stallings & Turner, 1956 (type locality USA: New Mexico, Eddy Co.) was placed as a subspecies of *Megathymus ursus* Poling, 1902 (type locality USA: Arizona, Pinal Co.) by dos Passos (1960). Genomic comparison of specimens of both taxa across their ranges, including the holotypes, revealed their prominent separation in the tree with the distance close to that between *Megathymus yuccae* (Boisduval & Le Conte, [1837]) and

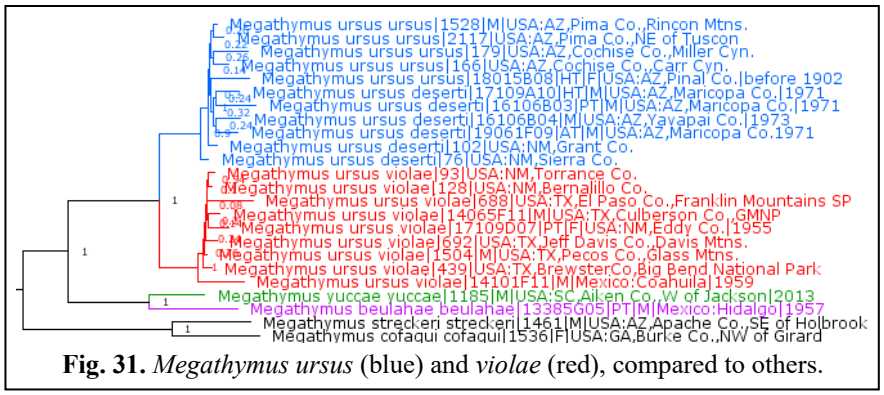

Fig. 31. *Megathymus ursus* (blue) and *violae* (red), compared to others.

Megathymus beulahae D. Stallings & J. Turner, 1958 (Fig. 31). The Fst/Gmin statistics for comparison of *ursus* and *violae* groups are 0.56/0.001 (note close to 0 gene exchange between these taxa). The COI barcodes of the *M. ursus* and *M. violae* holotypes differ by 1.8% (12 bp). For these reasons, we **reinstate** *Megathymus violae* D. Stallings & Turner, 1956 as a species-level taxon.

Discussion: genomic trees, branch lengths and genera

Near the end, coming back to the Introduction, we elaborate on and illustrate the reasons behind the classification decisions that we have chosen to make about genera. Traditionally, species were grouped into genera by phenotypic characters. For butterflies, these were mostly wing patterns and shapes, and genitalic morphology. When differences in these phenotypic aspects were deemed to be significant enough according to a subjective opinion of an individual researcher, they formed a basis for defining a genus. This system served its purpose until a consensus opinion was formed among taxonomists that each genus should be monophyletic. It is exceedingly difficult to predict monophyletic taxa from their phenotypes, and DNA-based phylogenetic trees provide the most reliable inference of monophyletic groups. Therefore, genera should be defined using phylogenetic trees constructed from DNA sequences.

Each individual feature of an organism can experience rapid evolution and fool researchers into making incorrect classification decisions. Genitalia that are commonly used in Lepidoptera classification are prone to such rapid changes as well. For instance, Steinhauser (1989) proposed a genus *Thessia* on the basis of unique shape of genitalic valvae. However, even a very short, 654 base pair region of DNA, such as the COI barcode, reveals the paraphyly of *Achalarus* Scudder, 1872 (as it was circumscribed at that time) with respect to *Thessia* (Pfeiler et al. 2016), suggesting that the unique valva is a result of accelerated evolution within *Achalarus* rather than a character originated after *Thessia* and *Achalarus* have (supposedly) diverged from each other. Therefore, a decision to erect the genus *Thessia* was a mistake, because *Thessia* is a subclade within (as it was then defined) *Achalarus*. Nevertheless, the barcode DNA region itself is a single feature, and as any other such feature, can experience evolutionary irregularities. To reduce such mistakes, it is better to use information from as many features as feasible. Complete genomes offer the ultimate DNA dataset for classification decisions. Genomic analysis suggests that *Achalarus* itself is a junior subjective synonym of the subgenus *Thorybes* Scudder, 1872, and *Thessia* is actually a junior subjective synonym of the subgenus *Murgaria* E. Watson, 1893 (Li et al. 2019).

Genomic trees summarize integral information about the entire organism, not just some of its features. For this reason, we use them to make decisions about classification of genera. Here, we explain how we arrive to these decisions using examples from this work and our previous publication (Zhang et al. 2019c). A maximum likelihood tree constructed using IQ-TREE program (model GTR+I+G) (Minh et al. 2020) from concatenated protein-coding regions of nuclear genomes is shown in Fig. 32. To best follow our logic, a reader may close the tree on the right (Fig. 32b, the final result) and look only at the tree on the left (Fig. 32a), which is the same as the tree on the right, but without the final results being marked in order not to bias the reader. This tree was constructed without assuming a molecular clock and reveals differences in evolutionary rates between species: i.e., species names are placed at difference distance from the left side of the page (=from the root of the tree). We see that *Emesis* evolved the fastest (the farthest from the left), and *Ephyriades* Hübner, [1819] evolved the slowest (closest to the left). In a tree, only horizontal (left-to-right) distances matter. Vertical (top to bottom) distances are arbitrary and are set to place species names evenly along vertical dimension, so that the names do not overlap and are not too far away from each other to save space.

Tree branches have different lengths. Again, only horizontal branches have evolutionary meaning, and vertical lines in the tree are set to avoid overlap of names and to connect branches to nodes. The length of a horizontal branch is proportional to the number of estimated changes in DNA (=fixed mutations) that happened along the branch. The tree has a scale bar near the bottom (Fig. 32). The length of that bar, as indicated, corresponds to 6 changes per 100 base pairs (=0.06, or 6%). Using this bar, we can measure evolutionary distances between taxa in DNA changes. Long branches correspond to many

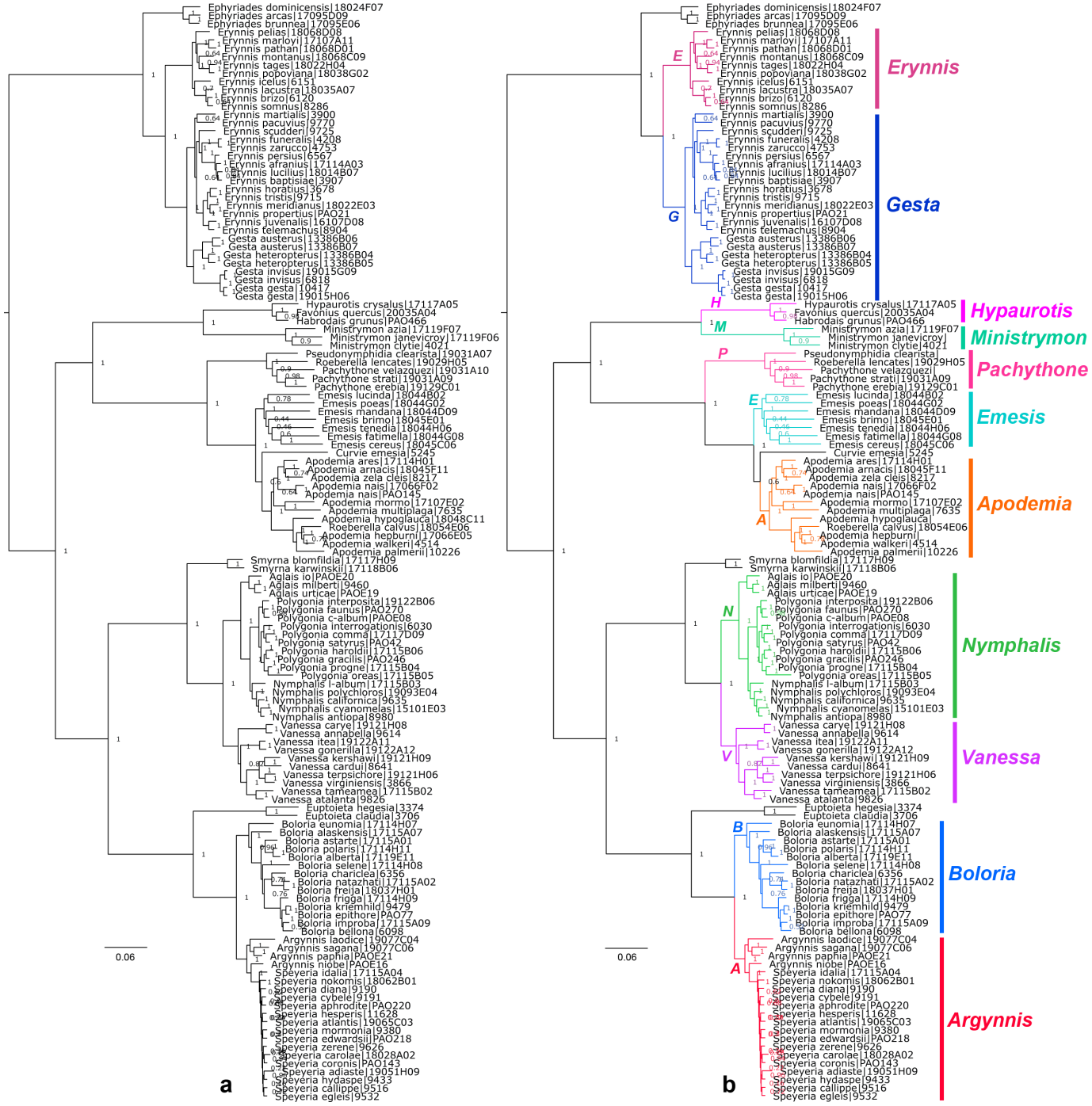

Fig. 32. Delineating genera using a tree constructed from nuclear genomes: unmarked (**a**) and colored by suggested genera (**b**).

changes in genomic DNA. Short branches correspond to few changes in genomic DNA. Because larger number of DNA changes are expected to result in larger number of phenotypic changes, longer branches correspond to more phenotypic changes on average. These are integral changes and some of them may be in genitalia, others may be in caterpillar morphology. Regardless of where these changes are, longer branches are more important than shorter branches. In addition to larger number of changes, longer branches are also more reliable and support clades that are more likely to be correct. The statistical reliability of every clade is indicated by a number next to each node. This number is a fraction of trees (out of 100 trees constructed from various subsets of genomic segments) that contain this node, e.g. a genome was divided into 100 segments and each segment was used to generate a tree. If a particular node is present in all 100 trees, the number by that node is 1. Therefore, this number measures consistency between trees constructed from different partitions of the data. If every DNA segment supports a clade, it

has a number 1 next to it. If 94 out of 100 segments support the clade, the number is 0.94.

A genus should be a prominent, major clade in the tree that is above species level and below tribe and subtribe levels. Phenotypic features are difficult to quantify, and due to the possibly uneven speed of evolution, it is a challenge to determine which phenotypic changes correspond to major clades. Total genomic changes can be used as a yardstick to quantify each clade. The number of total genomic changes is proportional to branch lengths in genomic trees (Fig. 32a). Therefore, the task of identifying genera may be viewed as a task of identifying prominent (i.e. supported by longer branches compared to surrounding branches) clades in genomic trees that on average correspond to how genera are defined currently (to avoid unnecessary taxonomic changes). Additionally, we believe that each genus should not be very different from another genus in terms of genetic differentiation of species placed in a genus, i.e. genera could be defined consistently, so that genera correspond to clades of approximately the same differentiation within. Defined consistently, the genus becomes a level (as meant by this word) of a classification instead of several varying levels, i.e., we can expect a genus to be a group of species bearing about the same relatedness among them as that in other genera. It would seem unnatural if one phylogenetic group is oversplit into genera, i.e. genera in that group correspond to very closely related species, but another group is undersplit, and genera in it correspond to species that are only distantly related. The measure of closeness as we use it, is overall genomic divergence.

Looking at the clade of Hesperiidae at the top of the tree (Fig. 32a) we see three major clades, not two and not four. The first clade is *Ephyriades* and is sister to all other taxa. Then all others split into two clades of similar genetic differentiation within each clade. We see that each of these clades resembles a tight bush or a comb, rather than an evenly bifurcating tree, i.e. the internal branches in either clade are much shorter than a branch that supports the entire clade. The clade with *Gesta* bifurcates into two subclades, one consists of *Gesta* sensu stricto (s. s.). Species from the other subclade were called "*Erynnis*" previously (and are called *Erynnis* in the tree to facilitate communication): it is a subgenus *Erynnides* Burns, 1964 (type species *Nisoniades propertius* Scudder & Burgess, 1870). If we consider these two subclades to be major clades, then the Hesperiidae tree would consist of four major clades (*Ephyriades*, *Erynnis* s. s., *Erynnides* and *Gesta*). However, the branches supporting the two subclades (*Erynnides* and *Gesta*) are nearly three times shorter than the branches supporting the clades *Erynnis* s. s. and a clade combining *Erynnides* with *Gesta*. Therefore, the Hesperiidae subtree should not be partitioned into four major clades, because two of these clades (*Erynnides*, *Gesta*) would be minor compared to the other two, and more importantly, compared to the clade combining *Erynnides* with *Gesta*.

The remaining alternative to a three-clade partitioning would be a two major clade partition, where *Erynnis* s. s., *Erynnides* and *Gesta* are all joined together into *Erynnis* sensu lato (s. l.) The branch supporting this clade is only slightly shorter than the branch supporting *Erynnis* s. s., and therefore this clade is rather prominent in the tree. We reject this solution for the two reasons. First, *Erynnis* s. l. is not a homogenous group of species, which we think a genus should be, i.e. the *Erynnis* s. l. clade does not look like a bush or a comb. Instead, it splits into two major clades: *Erynnis* s. s. and *Erynnides* + *Gesta*, (we call this clade *Gesta* s. l. from now on) each of which individually looks more like a comb than when they are combined. In other words, *Erynnis* s. l. itself is composed of two major clades, and does not represent a single group of species, but two major groups of species.

The second reason stems from consistency between different genera, i.e. an idea that different genera should represent the same level in the classification (Fig. 33). Being a level, genera should be groups of species with comparable divergence within each genus. In this tree (Fig. 32a), where all branches are to scale, we can compare divergence between *Erynnis* s. s. and *Gesta* s. l. to the divergence in Nymphalidae previously placed in genera *Aglais*, *Polygonia*, *Nymphalis*, and *Vanessa*. These two subtrees (*Erynnis* and *Vanessa*) are illustrated in Fig. 33. Genetic differentiation of a clade is proportional to the average distance (average sum of branch lengths) from the last common ancestor of the clade (=node that supports the entire clade) to the leaves (=species) in the clade. In other words, it is a linear distance (in horizontal dimension) from the base of the clade to the tips of the tree. On the one hand, we see that *Polygonia* divergence is rather small, perhaps comparable to the divergence of the *Erynnides* subclade with *horatius* and *juvenalis*, and definitely smaller than the divergence within either *Erynnis* s. s., or *Gesta* s. l. On the

other hand, the divergence of *Erynnis* s. l. is larger than the divergence of *Aglais*, *Polygonia*, *Nymphalis* and *Vanessa* combined. Therefore, having *Erynnis* s. l. as a genus is inconsistent with having *Polygonia* as a genus: these two groups represent different levels in the classification. Coming back to Nymphalidae, we see that branches supporting *Aglais*, *Polygonia* and *Nymphalis* individually are much shorter than the branches supporting *Erynnis* s. s. or *Gesta* s. l. Only the branch supporting *Vanessa* is somewhat comparable, although shorter. However, the branch supporting the first three clades together (*Nymphalis* s. l.) is more prominent and is about the same as the branch supporting *Vanessa*.

In summary, *Erynnis* s. l. is comparable to *Vanessa* s. l. (*Nymphalis* s. l. + *Vanessa* s. s.). A system of two genera (*Erynnis* s. s. and *Gesta* s. l.) is comparable to two genera *Nymphalis* s. l. and *Vanessa* s. s. We attempt to choose an internally consistent solution that agrees the most with how these species are assigned to genera in the current classification. Therefore, we choose the 2-genus solution for both of these cases, as shown in Figs. 32b (colored clades E: *Erynnis*, G: *Gesta*, N: *Nymphalis* and V: *Vanessa*) and 33 (shaded clades). These four genera represent a similar level in the classification and correlate with the current classification of these butterflies. The choice of *Erynnis* s. l. would correspond to a consistent choice of joining all four Nymphalidae genera in *Vanessa*, which may represent too much of a lump and more name changes (Fig. 33).

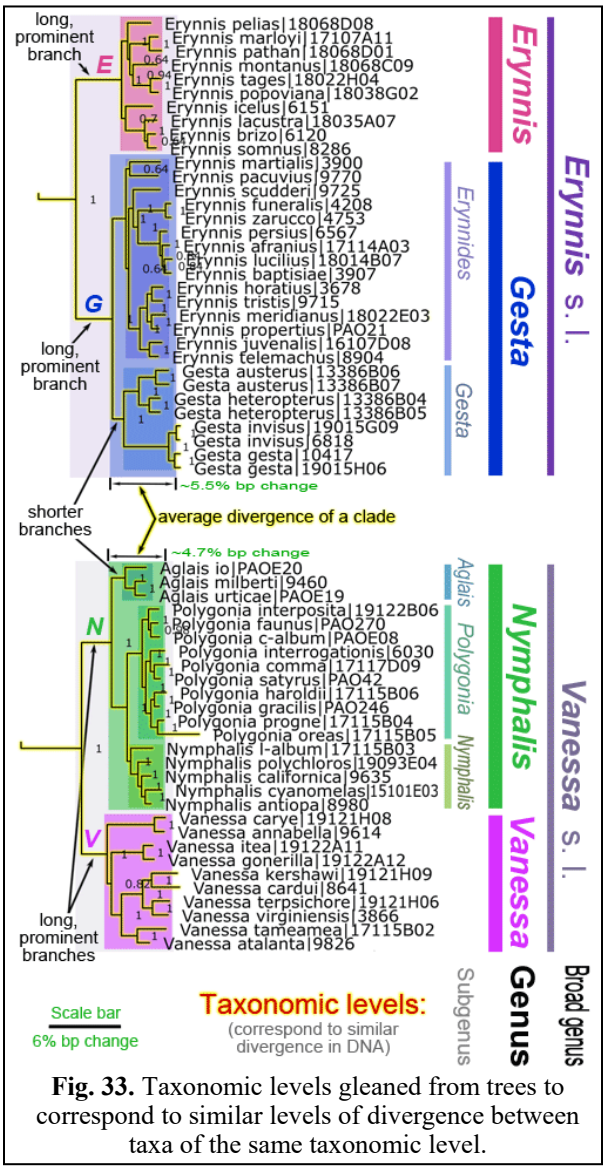

Fig. 33. Taxonomic levels gleaned from trees to correspond to similar levels of divergence between taxa of the same taxonomic level.

Another point is that genetic differentiation can be used to estimate divergence times of these clades through the tree rescaling and calibration with fossils (primary calibration) (Chazot et al. 2019) or other time-calibrated trees (secondary calibration) (Zhang et al. 2019a). As we have seen in Hesperiidae (Li et al. 2019), the genus level typically corresponds to divergence between 10 and 15 million years ago (Mya). Divergence of *Erynnis* s. l. was estimated to be about 27 Mya, which is larger than the divergence between *Vanessa* s. s. and *Nymphalis* s. l., at about 22 Mya (Zhang et al. 2019d). However, divergences within *Gesta* s. l. (~16 Mya), *Vanessa* s. s. (~16 Mya) and *Nymphalis* s. l. (~14 Mya) (Zhang et al. 2019d) are very much comparable to each other, and these genera represent groups of about the same level. It should be noted that the divergence times are only approximate, should be considered with caution, and may have errors of possibly up to 50%, especially in groups with large differences in evolutionary rates. However, the relative comparison of divergence times estimated within the same tree using the same method is expected to be more accurate.

Finally, a question arises about how these considerations of trees, branch lengths, divergence and geological times correlate with genera definition based on phenotypic characters. Because phenotypic characters are encoded by the genotype, longer branches in the tree that correspond to more changes in a genotype (these are integral genomic trees, not based on several gene markers) should translate to more changes in the phenotype. We advocate a method to delineate genera from genomic trees first, and then come back to phenotypic analysis to find the phenotypic characters that correspond to these genera. In the case of *Erynnis* and *Gesta*, the retrospective inspection of morphological characters yields substantial differences in male genitalia that have been noted and illustrated previously (Evans 1953; Burns 1964). The uncus is asymmetric, terminally broad in *Gesta*, but is symmetric, extending into a "beak" in *Erynnis*.

The valvae are strongly asymmetric with at least one extended harpe in *Gesta*, but are more symmetric with shorter harpes in *Erynnis*. Other differences are stated in the diagnosis of *Erynnides* by Burns (1964).

Comparing the clades of other groups in Fig. 32a with *Erynnis*/*Gesta* and *Nymphalis*/*Vanessa* we see that divergence within *Speyeria* and *Roeberella* (a clade containing *R. clavus* and with *Apodemia hypoglauca* at its base), and divergence between *Hypaurotis*, *Favonius* and *Habrodais* is much smaller than that in the groups we define as genera. We also see that the colored clades (with letters denoting corresponding genera by each clade) in Fig. 32b are more or less equivalent to each other in terms of genetic differentiation (distance from the base of the clade to its tips) and prominence (length of the branch supporting the clade). For these reasons, we suggest that these clades can be treated as genera: they are prominent, consistent, and reasonably well correspond to how genera have been defined previously. The changes we suggest combine some more compact in terms of genetic (and phenotypic) differentiation genera into more internally diverse genera that become more consistent with the differentiation within many classic genera such as *Emesis*, *Ministrymon*, *Vanessa*, and *Boloria*.

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LITERATURE CITED

Aardema, M. L., and P. Andolfatto. **2016**. Phylogenetic incongruence and the evolutionary origins of cardenolide-resistant forms of Na(+) ,K(+) -ATPase in Danaus butterflies. Evolution 70(8): 1913–1921.

Ackery, P. R., and R. I. Vane-Wright. **1984**. Milkweed butterflies: their cladistics and biology. British Museum (Natural History); London. x+425

Aldhebiani, A. Y. 2018. Species concept and speciation. Saudi J Biol Sci 25(3): 437–440.

Allio, R., C. Scornavacca, N. Benoit, A. L. Clamens, F. A. H. Sperling, and F. L. Condamine. **2019**. Whole genome shotgun phylogenomics resolves the pattern and timing of swallowtail butterfly evolution. Systematic Biology 69(1): 38–60.

Austin, G. T., and M. J. Smith. **1998**. Revision of the *Thessalia theona* complex (Lepidoptera: Nymphalidae: Melitaeinae). *In*: Emmel, T. C. (Ed.). Systematics of Western North American Butterflies. Mariposa Press; Gainesville, pp. 359–396.

Bland, K. P. 2019. Name-bearing types of butterflies (Lepidoptera, Papilionoidea), in the National Museums of Scotland, Edinburgh. Zootaxa 4559(1): 57–89.

Burns, J. M. 1964. Evolution in skipper butterflies of the genus *Erynnis*. University of California Press; Berkeley and Los Angeles. iv + 214

Burns, J. M., D. H. Janzen, M. Hajibabaei, W. Hallwachs, and P. D. Hebert. **2008**. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservacion Guanacaste, Costa Rica. Proc Natl Acad Sci U S A 105(17): 6350–6355.

Burns, J. M., D. H. Janzen, M. Hajibabaei, W. Hallwachs, and P. D. N. Hebert. **2007**. DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (Hesperiidae) can differ by only one to three nucleotides. J. Lep. Soc. 61(3): 138–153.

Chazot, N., N. Wahlberg, A. V. L. Freitas, C. Mitter, C. Labandeira, J. C. Sohn, R. K. Sahoo, N. Seraphim, R. de Jong, and M. Heikkila. **2019**. Priors and Posteriors in Bayesian Timing of Divergence Analyses: The Age of Butterflies Revisited. Syst Biol 68(5): 797–813.

Claridge, M. F. 2017. Insect Species – Concepts and Practice. *In*: Foottit, R. G., and P. H. Adler (Eds.). Insect Biodiversity: Science and Society. John Wiley & Sons; Chichester, UK, pp. 527–546.

Cong, Q., D. Borek, Z. Otwinowski, and N. V. Grishin. **2015**. Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. Cell Reports 10(6): 910–919.

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. Shen, J. Zhang, W. Li, L. N. Kinch, J. V. Calhoun, A. D. Warren, and N. V. Grishin. **2019a**. Genomics reveals the origins of ancient specimens. bioRxiv BIORXIV/2019/752121.

Cong, Q., J. Zhang, and N. V. Grishin. **2019b**. Genomic determinants of speciation. bioRxiv BIORXIV/2019/837666.

de Jong, R., and K. van Dorp. **2006**. The African copper connection. Entomologische Berichten. Amsterdam 66(4): 124–129.

De Moya, R. S., W. K. Savage, C. Tenney, X. Bao, N. Wahlberg, and R. I. Hill. **2017**. Interrelationships and diversification of *Argynnis* Fabricius and *Speyeria* Scudder butterflies. Systematic Entomology 42(4): 635–649.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J. F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J. M. Claverie, and O. Gascuel. **2008**. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36(Web Server issue): W465–W469.

Dictionary.com LLC. **2020**. <https://www.dictionary.com/browse/sautoir>. Accessed 1 Nov. 2020.

dos Passos, C. F. 1960. Taxonomic notes on Nearctic Rhopalocera. 1. Hesperioidea. Journal of the Lepidopterists' Society 14(1): 24–36.

Draudt, M. W. K. 1921. B. Grypocera, breitköpfige Tagfalter. *In*: Seitz, A. (Ed.). Die Gross-Schmetterlinge der Erde. Alfred Kernen; Stuttgart, p. 850, pl. 165f.

Dunford, J. C. 2009. Taxonomic overview of the greater fritillary genus *Speyeria* Scudder and the *atlantis* - *hesperis* species complexes, with species accounts, type images, and relevant literature (Lepidoptera: Nymphalidae). Insecta Mundi 0090: 1–74.

Eliot, J. N. 1973. The higher classification of the Lycaenidae (Lepidoptera): a tentative Arrangement. Bulletin of the British Museum of Natural History (Entomology) 28(6): 373–505.

Emmel, T. C., and G. T. Austin. **1998**. What is *Argynnis carolae*? The cytotaxonomy and systematic position of a *Speyeria* relic from Nevada (Lepidoptera: Nymphalidae). *In*: Emmel, T. C. (Ed.). Systematics of western North American butterflies. Mariposa Press; Gainesville, pp. 443–450.

Espeland, M., J. Breinholt, K. R. Willmott, A. D. Warren, R. Vila, E. F. A. Toussaint, S. C. Maunsell, K. Aduse-Poku, G. Talavera, R. Eastwood, M. A. Jarzyna, R. Guralnick, D. J. Lohman, N. E. Pierce, and A. Y. Kawahara. **2018**. A comprehensive and dated phylogenomic analysis of butterflies. Current Biology 28(5): 770–778.

Espeland, M., J. W. Breinholt, E. P. Barbosa, M. M. Casagrande, B. Huertas, G. Lamas, M. A. Marin, O. H. H. Mielke, J. Y. Miller, S. Nakahara, D. Tan, A. D. Warren, T. Zacca, A. Y. Kawahara, A. V. L. Freitas, and K. R. Willmott. **2019**. Four hundred shades of brown: Higher level phylogeny of the problematic Euptychiina (Lepidoptera, Nymphalidae, Satyrinae) based on hybrid enrichment data. Mol Phylogenet Evol 131: 116–124.

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. British Museum (Natural History). London. v + 178 pp., pls. 10–25.

Evans, W. H. 1953. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part III. Pyrginae. Section 2. British Museum (Natural History); London. v + 246 pp., pls. 26–53.

Garth, J. S. 1934. Butterflies of Yosemite National Park. Bulletin of the Southern California Academy of Sciences 34: 37–75.

Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 14(7): 685–695.

Geneva, A. J., C. A. Muirhead, S. B. Kingan, and D. Garrigan. **2015**. A new method to scan genomes for introgression in a secondary contact model. PLoS One 10(4): e0118621.

Godart, J. B. 1819. Pp. 13-328, in: Latreille, P. A., and J. B. Godart (Eds.), Encyclopédie Méthodique. Histoire naturelle. Entomologie, ou histoire naturelle des crustacés, des arachnides et des insectes. veuve Agasse; Paris, pp. 328.

Godman, F. D. 1907. Notes on the American species of Hesperiidae described by Plötz. Annals and Magazine of natural History (7)20(16): 132–155.

Godman, F. D., and O. Salvin. **1896**. On the butterflies of St. Vincent, Grenada, and the adjoining islands of the West Indies. Proceedings of the zoological Society of London 1896(2): 513–520.

Grishin, N. V. 2019. Expanded phenotypic diagnoses for 24 recently named new taxa of Hesperiidae (Lepidoptera). The Taxonomic Report of the International Lepidoptera Survey 8(1): 1–15.

Hebert, P. D., A. Cywinska, S. L. Ball, and J. R. deWaard. **2003**. Biological identifications through DNA barcodes. Proc Biol Sci 270(1512): 313–321.

Honey, M. R., and M. J. Scoble. **2001**. Linnaeus' butterflies (Lepidoptera: Papilionoidea and Hesperioidea). Zoological Journal of the Linnean Society 132(3): 277–399.

ICZN. **1999**. International Code of Zoological Nomenclature. Fourth edition. The International Trust for Zoological Nomenclature; London, UK.

Kawahara, A. Y., and J. W. Breinholt. **2014**. Phylogenomics provides strong evidence for relationships of butterflies and moths. Proceedings of the Royal Society B: Biological Sciences 281(1788): 20140970.

Klots, A. B. 1936. The interrelationships of the species of the genus *Lycaena* Fabricius (Lepidoptera, Lycaenidae). Bulletin of the Brooklyn Entomological Society 31: 154–170.

Kodandaramaiah, U., T. J. Simonsen, S. Bromilow, N. Wahlberg, and F. Sperling. **2013**. Deceptive single-locus taxonomy and phylogeography: *Wolbachia*-associated divergence in mitochondrial DNA is not reflected in morphology and nuclear markers in a butterfly species. Ecol Evol 3(16): 5167–5176.

Kodandaramaiah, U., and N. Wahlberg. **2009**. Phylogeny and biogeography of *Coenonympha* butterflies (Nymphalidae: Satyrinae) - patterns of colonization in the Holarctic. Systematic Entomology 34: 315–323.

Layberry, R. A., P. W. Hall, and J. D. Lafontaine. **1998**. The butterflies of Canada. University of Toronto Press; Toronto, Buffalo & London. 280 pp.

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.

Mallet, J. 1995. A species definition for the modern synthesis. Trends Ecol Evol 10(7): 294–299.

Mallet, J., N. Besansky, and M. W. Hahn. **2016**. How reticulated are species? Bioessays 38(2): 140–149.

Maynard, C. J. 1889. Description of a new species of butterfly from the West Indies. Contributions to Science (Newtonville) 1(2): 88–92.

Merian, M. S. 1705. Metamorphosis insectorum surinamensium. In qua erucae ac vermes surinamenses, cum omnibus suis transformationibus, ad vivum delineantur et describuntur, singulis eorum in plantas, flores et fructus

collocatis, in quibus reperta sunt; tunc etiam generatio ranarum, bufonum rariorum, lacertarum, serpentum, araneorum et formicarum exhibetur; omnia in America ad vivum naturali magnitudine picta atque descripta. Gerard Valk; Amsterdam. [iv] + 60 pp., 60 pls.

Merriam-Webster. **2020**. <https://www.merriam-webster.com/dictionary/sautoir>. Accessed 1 Nov. 2020.

Michener, C. D. 1942. A review of the subspecies of *Agraulis vanillae* (Linnaeus) (Lepidoptera: Nymphalidae). American Museum Novitates 1197: 1–8.

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.

Miller, L. D., and F. M. Brown. **1979**. Studies in the Lycaeninae (Lycaenidae). 4. The Higher Classification of the American Coppers. Bulletin of the Allyn Museum 51: 1–30.

Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. von Haeseler, and R. Lanfear. **2020**. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol 37(5): 1530–1534.

Mutanen, M., N. Wahlberg, and L. Kaila. **2010**. Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. Proc Biol Sci 277(1695): 2839–2848.

Nelis, M., T. Esko, R. Magi, F. Zimprich, A. Zimprich, D. Toncheva, S. Karachanak, T. Piskackova, I. Balascak, L. Peltonen, E. Jakkula, K. Rehnstrom, M. Lathrop, S. Heath, P. Galan, S. Schreiber, T. Meitinger, A. Pfeufer, H. E. Wichmann, B. Melegh, N. Polgar, D. Toniolo, P. Gasparini, P. D'Adamo, J. Klovins, L. Nikitina-Zake, V. Kucinskas, J. Kasnauskiene, J. Lubinski, T. Debniak, S. Limborska, A. Khrunin, X. Estivill, R. Rabionet, S. Marsal, A. Julia, S. E. Antonarakis, S. Deutsch, C. Borel, H. Attar, M. Gagnebin, M. Macek, M. Krawczak, M. Remm, and A. Metspalu. **2009**. Genetic structure of Europeans: a view from the North-East. PLoS One 4(5): e5472.

Newton, I. 2003. Speciation and biogeography of birds Academic Press; Amsterdam, pp. xii + 668.

Oberthür, C. 1897. Description de lépidoptères nouveaux. Bulletin de la Société entomologique de France 1897(11): 173–180.

Opler, P. A., and V. Malikul. **1992**. A field guide to eastern butterflies. Houghton Mifflin Co.; Boston, New York & London.

Pelham, J. P. 2008. Catalogue of the Butterflies of the United States and Canada. Journal of Research on the Lepidoptera 40: 1–658.

Pelham, J. P. 2020. Catalogue of the Butterflies of the United States and Canada. Revised 7 Aug. 2020. Accessed 1 Nov. 2020. http://www.butterfliesofamerica.com/US-Can-Cat.htm.

Peña, C., N. Wahlberg, E. Weingartner, U. Kodandaramaiah, S. Nylin, A. V. Freitas, and A. V. Brower. **2006**. Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. Mol Phylogenet Evol 40(1): 29–49.

Pfeiler, E. 2018. DNA barcoding and taxonomic challenges in describing new putative species: examples from sootywing and cloudywing butterflies (Lepidoptera: Hesperiidae). Diversity 10(4): 111.

Pfeiler, E., M. Ramírez, and T. A. Markow. **2016**. Polyphyly in *Urbanus* and *Astraptes* (Hesperiidae: Eudaminae) assessed using mitochondrial DNA barcodes, with a reinstated status proposed for *Achalarus*. J. Lep. Soc. 70(2): 85–95.

Pohl, G. R., G. G. Anweiler, B. C. Schmidt, and N. G. Kondla. **2010**. An annotated list of the Lepidoptera of Alberta, Canada Zookeys 38: 1–549.

Ratnasingham, S., and P. D. Hebert. **2007**. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7(3): 355–364.

Remington, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. *In*: Dobzhansky, T., M. K. Hecht, and W. C. Steere (Eds.). Evolutionary Biology. Springer; Boston, pp. 321–428.

Savela, M. 2020. Lepidoptera and some other life forms.

Sayers, E. W., M. Cavanaugh, K. Clark, J. Ostell, K. D. Pruitt, and I. Karsch-Mizrachi. **2020**. GenBank. Nucleic Acids Res 48(D1): D84–D86.

Scott, J. A., K. E. Davenport, N. G. Kondla, P. A. Opler, and M. S. Fisher. **2017**. New taxa and geographic variation of western North American butterflies: based on specimens in the C. P. Gillette Museum of Arthropod Diversity, Colorado State University. Papilio (New Series) 26: 1–54.

Scott, J. A., and M. S. Fisher. **2014**. *Argynnis* (*Speyeria*) *nokomis nokomis* : geographic variation, metapopulations, and the origin of spurious specimens (Nymphalidae). Papilio (New Series) 21: 1–31.

Scudder, S. H. 1876. Synonymic list of the butterflies of North America, north of Mexico. Part 2, Rurales. Bulletin of the Buffalo Society of Natural Sciences 3: 98–129.

Sibatani, A. 1974. A new genus for two new species of Lycaenidae (s. str.) (Lepidoptera: Lycaenidae) from Papua New Guinea. Journal of Australian Entomological Society 13: 95–110.

Simonsen, T. J. 2006. Fritillary phylogeny, classification and larval hostplants: reconstructed mainly on the basis of male and female genitalic morphology (Lepidoptera: Nymphalidae: Argynnini). Biological Journal of the Linnean Society 89: 627–673.

Simonsen, T. J., N. Wahlberg, A. V. Z. Brower, and R. de Jong. **2006**. Morphology, molecules and Fritillaries: approaching a stable phylogeny for Argynnini (Lepidoptera: Nymphalidae). Insect Systematics & Evolution 37: 405–418.

Smith, M. J., and J. P. Brock. **1988**. A review of the *Thessalia leanira* complex in the southwestern United States with a description of two new subspecies of *Thessalia fulvia*. Bulletin of the Allyn Museum 118: 1–21.

Sperling, F. A. H. 2003. Butterfly molecular systematics: from species definitions to higher level phylogenies. *In*: Boggs, C., P. Erlich, and W. Watt (Eds.). Ecology and Evolution Taking Flight: Butterflies as model study systems. University of Chicago Press; Chicago, USA, pp. 431–458.

Steinhauser, S. R. 1989. Taxonomic notes and descriptions of new taxa in the Neotropical Hesperiidae. Part I. Pyrginae. Bulletin of the Allyn Museum 127: 1–70.

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.

Trujano-Ortega, M., U. O. Garcia-Vazquez, C. J. Callaghan, O. Avalos-Hernandez, M. A. Luis-Martinez, and J. E. Llorente-Bousquets. **2018**. Two new genera of metalmark butterflies of North and Central America (Lepidoptera, Riodinidae). Zookeys 729: 61–85.

Trujano-Ortega, M., U. O. Garcia-Vazquez, C. J. Callaghan, O. Avalos-Hernandez, M. A. Luis-Martinez, and J. E. Llorente-Bousquets. **2020**. Challenges for organismic taxonomical revisions in the age of phylogenomics: A response to Zhang et al. (2019). Zookeys 4838(3): 436–440.

van Dorp, K. 2004. Molecular systematics of *Lycaena* F., 1807 (Lepidoptera: Lycaenidae) – some preliminary results. Proceedings of the Netherlands Entomological Society 15: 65–70.

Wall, J. D., and D. Yoshihara Caldeira Brandt. **2016**. Archaic admixture in human history. Curr Opin Genet Dev 41: 93–97.

Warren, A. D., K. J. Davis, E. M. Stangeland, J. P. Pelham, K. R. Willmott, and N. V. Grishin. **2016**. Illustrated Lists of American Butterflies. [21-XI-2017].

Wells, C. N., L. Edwards, R. Hawkins, L. Smith, and D. Tonkyn. **2011**. A rearing method for *Argynnis* (*Speyeria*) *diana* (Lepidoptera: Nymphalidae) that avoids larval diapause. Psyche: A Journal of Entomology 2011: 940280: 1–6.

Zacca, T., M. M. Casagrande, O. H. H. Mielke, B. Huertas, E. P. Barbosa, A. V. L. Freitas, L. M. Magaldi, M. Espeland, S. Nakahara, and K. R. Willmott. **2018**. Systematics of the butterfly genus *Cissia* Doubleday, 1848 (Lepidoptera: Nymphalidae: Satyrinae) using an integrative approach. Arthropod Systematics & Phylogeny 76(2): 349–376.

Zacca, T., M. M. Casagrande, O. H. H. Mielke, B. Huertas, M. Espeland, A. V. l. Freitas, K. R. Willmott, S. Nakahara, and G. Lamas. **2020**. Revalidation of *Vareuptychia* Forster, 1964, description of *Vanima* gen. nov., and notes on *Euptychia cleophes* Godman & Salvin, 1889 (Lepidoptera: Nymphalidae: Satyrinae). Zootaxa 4858(1): 1–34.

Zakharov, E. V., N. F. Lobo, C. Nowak, and J. J. Hellmann. **2009**. Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity (Edinb) 102(6): 590–599.

Zhan, S., W. Zhang, K. Niitepold, J. Hsu, J. F. Haeger, M. P. Zalucki, S. Altizer, J. C. de Roode, S. M. Reppert, and M. R. Kronforst. **2014**. The genetics of monarch butterfly migration and warning colouration. Nature 514(7522): 317–321.

Zhang, J., Q. Cong, J. Shen, E. Brockmann, and N. V. Grishin. **2019a**. Genomes reveal drastic and recurrent phenotypic divergence in firetip skipper butterflies (Hesperiidae: Pyrrhopyginae). Proceedings of the Royal Society B: Biological Sciences 286(1903): 20190609.

Zhang, J., Q. Cong, J. Shen, E. Brockmann, and N. V. Grishin. **2019b**. Three new subfamilies of skipper butterflies (Lepidoptera, Hesperiidae). Zookeys 861: 91–105.

Zhang, J., Q. Cong, J. Shen, P. A. Opler, and N. V. Grishin. **2019c**. 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. **2019d**. Genomics of a complete butterfly continent. bioRxiv BIORXIV/2019/829887.

Zhang, J., J. Shen, Q. Cong, and N. V. Grishin. **2019e**. Genomic analysis of the tribe Emesidini (Lepidoptera: Riodinidae). Zootaxa 4668(4): 475–488.

Zuckerkandl, E., and L. Pauling. **1965**. Molecules as documents of evolutionary history. J Theor Biol 8(2): 357–366.

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