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Original Article

A new skipper butterfly from Panama and Colombia with its genome (Lepidoptera: Hesperiidae: Eudaminae)

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ABSTRACT

We name and describe a new butterfly species in the family Hesperiidae (Lepidoptera), *Fulvatis regalodelcielo* sp. nov. from Panama and Colombia. In addition to generating mitochondrial and draft nuclear genome assemblies for the type series, we utilized available whole-genome sequencing data and reconciled a species tree using a full-likelihood multispecies coalescent method, as well as producing two concatenated maximum-likelihood trees from nuclear and mitochondrial genome datasets. All three phylogenetic hypotheses recovered *F. regalodelcielo* sp. nov. as a member of the tribe Phocidini (Eudaminae) with a high support. *Fulvatis regalodelcielo* sp. nov. is most closely related to *Fulvatis fulvius* (Plötz, 1882). Coalescent-based species tree analysis suggests that these two taxa diverged less than 1 million years ago. We provide diagnostic morphological characters for congeneric species and designate a lectotype for *Telegonus fulvius* Plötz, 1882 to remove any ambiguity regarding its identity. We draw attention to both advantage and disadvantage of generating classification schemes based on genomic data, and highlight the importance of biological classification as a tool to effectively communicate about the diversity of life.

Keywords: archives; multispecies coalescent, Phocidini, species tree, taxonomy

INTRODUCTION

Historically, the classification of skipper butterflies (Lepidoptera: Papilionoidea: Hesperiidae) was dominated by morphological studies such as the monumental revisionary works of William Harry Evans (Evans 1937, 1949, 1951, 1952, 1953, 1955). Despite the taxonomic breadth of these works, the diversity of the group has made it difficult for systematists to determine synapomorphies and other defining morphological characteristics that allow for an informative classification scheme for the group. Since the establishment of the genus *Hesperia* by Fabricius (1793), the skippers have been considered by many authors as having their own superfamily (Hesperioidea) distinct from the rest of the butterflies (Papilionoidea) until their recent placement in Papilionoidea (Heikkilä *et al.* 2012). Although their placement in butterflies is now considered solid (Espeland *et al.* 2018, Kawahara *et al.* 2023), there remain many taxa within the Hesperiidae whose higher-level classification has been in flux until recently, or still remains in flux. One such example is *Euschemon rafflesia* (MacLeay, 1826), whose frenulo-retinacular wing coupling of the male individuals is a character commonly shared with moths, and *E. rafflesia* is the only butterfly species to possess a frenulum and a retinaculum except for members of the Hedylidae. The presence of a frenulum and a retinaculum, coupled with its elongated antennae caused much disagreement among researchers about the species' systematic placement, which ranged mutually exclusive from 'Heterocera' (e.g. Watson 1893), to its own family 'Euschemonidae' (e.g. Lindsey *et al.* 1931), to

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'Pyrginae' (e.g. Evans 1949). Owing to the recent rise of molecular techniques, a consensus regarding its placement in the Hesperiidae is starting to emerge (e.g. Sahoo *et al.* 2016).

Indeed, the rapid development of high-throughput DNA sequencing techniques over the past decade or so, coupled with the increasing availability of computing technology, is enabling us to settle some of the hardest morphological conundrums across the tree of life, including within the Hesperiidae (Cong *et al.* 2019, Li *et al.* 2019, Zhang *et al.* 2019a, b, 2022, 2023a, b, d, Brockmann *et al.* 2022). These works resulted in over 70 new genus-group names and more than 160 new species-group names in the family. These molecular genomic studies, including sequencing type specimens, combined with morphological analyses have done much to refine Hesperiidae classification, but refinement continues, and a seemingly countless number of new species await description.

During the course of field work aimed at surveying Panamanian butterfly fauna, coauthors John R. MacDonald (J.R.M.) and Albert Thurman (A.T.) and collaborators discovered an undescribed skipper from eastern Panama. The primary purpose of this study is to describe and name this new species of skipper butterfly and place it within the hierarchy of Hesperiidae taxonomy. We present draft genome assemblies for this new species, add it to compiled and assembled published genomic data for Hesperiidae, and infer a species tree to determine the taxonomic placement of this taxon. In addition, we illustrate and discuss morphological characters to support generic classification. Information from archival records is incorporated to strengthen our findings.

MATERIALS AND METHODS

Field work

As part of an ongoing faunistic study of Panamanian butterflies, which initially began in the 1970s by J.R.M., A.T., and collaborators, field work has been conducted throughout the country totalling over 1500 field days since 2001. Butterflies were sampled with various collecting techniques such as hand-netting and trapping, including light traps at night using mercury vapor and/or UV light. Information regarding collecting permits relevant to the present study is provided in the Acknowledgements section.

Morphological study

We studied adult external morphology using a Leica MZ12 stereomicroscope with a camera lucida to produce wing venation and genitalia drawings; photographs were produced using a Leica DFC450 attached to a Leica M205 C stereomicroscope and stacked using Leica Application Suite X (LAS X v.5.02). The genitalia were dissected after soaking the abdomen (broken off from the specimen at its base) in hot 10% KOH for 5–10 minutes. The wing venation terminology follows MacNeill (1964: 194, fig. 1A) and terminology for genitalia is in accordance with (Nakahara *et al.* 2018). The following museum abbreviations are used in the text: **AMNH**: American Museum of Natural History, New York, USA; **CBF**: Colección Boliviana de Fauna, Universidad Mayor San Andrés, La Paz, Bolivia; **DZUP**: Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil; **FILS**: Fåhraeus Institute, Lund, Sweden; **MCZ**: Museum of Comparative Zoology, Harvard University, Cambridge, USA; **MEM**: John R. MacDonald private collection, Mississippi Entomological Museum, Mississippi State University, Starkville, USA; **MfN**: Museum für Naturkunde, Berlin, Germany; **NHMUK**: The Natural History Museum, London, UK; **USNM**: Smithsonian National Museum of Natural History (formerly United States National Museum), Washington, DC, USA.

We morphologically examined the following male specimens to decide on applications of species-group names (label data written verbatim, separated by double slashes).

Fulvatis fulvius (Plötz, 1882) (lectotype, designated herein): Holotypus//4859//Fulvia N. Cameta Sieb.//*fulvius* Pl. Type//DNA sample ID: NVG-15031G04 c/o Nick V. Grishin//(MfN); BOLIVIA—Dept. Beni, Prov. Vaca Diez, Riberalta, Laguna San Jose, Comunidad Berlin, 136 m, 10° 52′ 51"S 65° 59′ 9"W, 14-10-2019, G. Siebel leg.//CHFC 6823//(CBF); Para. *Lower Amazon* (A. M. Moss.) *Bung. fulvius* [underside: BM 1947. 453]//991//(NHMUK).

Fulvatis scyrus (Bell, 1934) (holotype): yumbatos X-31. Peru.//Bungalotis scyrus Bell Holotype ♂//TYPE//G818//DNA sample ID: NVG-15104A06 c/o Nick V. Grishin//(AMNH); Peru, Loreto Quebrada Balina Rio nanay Alt 120 3° 56′ 30"[S] 73° 45′ 54"[W] 9-10.iv-v.2018 C. Fåhraeus DNA D911//; Peru, San Martin [Martín] Tarapoto Chazuta vic. -6.565, -76.1443 Alt 350 m 1-30.xii.2016 Miranda/Fåhraeus DNA 37087//; Peru, Loreto Distr Santa maria Alto Nanay Rio aguas negra 8-29.vi.2017 C. Fåhraeus/Santos DNA 25699//(all at FILS).

DNA extraction, library preparation, and sequencing

Genomic DNA (gDNA) was extracted from legs of four *F. regalodelcielo* specimens (molecular vouchers: MEM-SN-002, 004, 005, and 006) as well as one *Salatis canalis* specimen (MEM-SN-001) using a Qiagen DNeasy Blood & Tissue Kit. The final elution volume was 50 µl and 15 µl was used for library preparation and sequencing. Extracted gDNA was quantified by a Picogreen assay measured on a Spectramax i3 plate reader (Molecular Devices) and normalized to 4 ng prior to library preparation. Sequencing libraries were prepared using the Illumina DNA library preparation kit. The gDNA was fragmented and adapter sequences were tagged through the Illumina DNA Prep assay's on-bead tagmentation technology. Tagmented samples were enriched and indexed using 12 cycles of amplification with PCR primers, which included dual 10 bp index sequences to allow for multiplexing. Libraries were prepared using the MANTIS Liquid Handler (Formulatrix) and purified through magnetic bead-based clean-up using PCR Clean DX Beads (Aline Biosciences) on a Biomek FXP Single Arm System with Span-8 Pipettor (Beckman Coulter, A31843). Resulting libraries were assessed using a 4200 TapeStation (Agilent Technologies) and quantified by qPCR (Roche Sequencing). Libraries were pooled and sequenced on a single lane of an Illumina NovaSeq SP flow cell using single-end 100 bp reads.

Nuclear genome assembly

Raw sequencing reads were trimmed to remove low-quality reads and adapter sequences using Trimmomatic v.0.39 (Bolger *et al.* 2014) with option LEADING: 3 TRAILING: 3 SLIDINGWINDOW: 4:15 MINLEN: 36. Trimmed reads were assembled *de novo* using SPAdes v4.0.0 (Prjibelski *et al.* 2020) with *k*-mer sizes of 21, 33, and 55. The National Center for Biotechnology Information (NCBI) Foreign Contamination Screen (FCS) pipeline was used

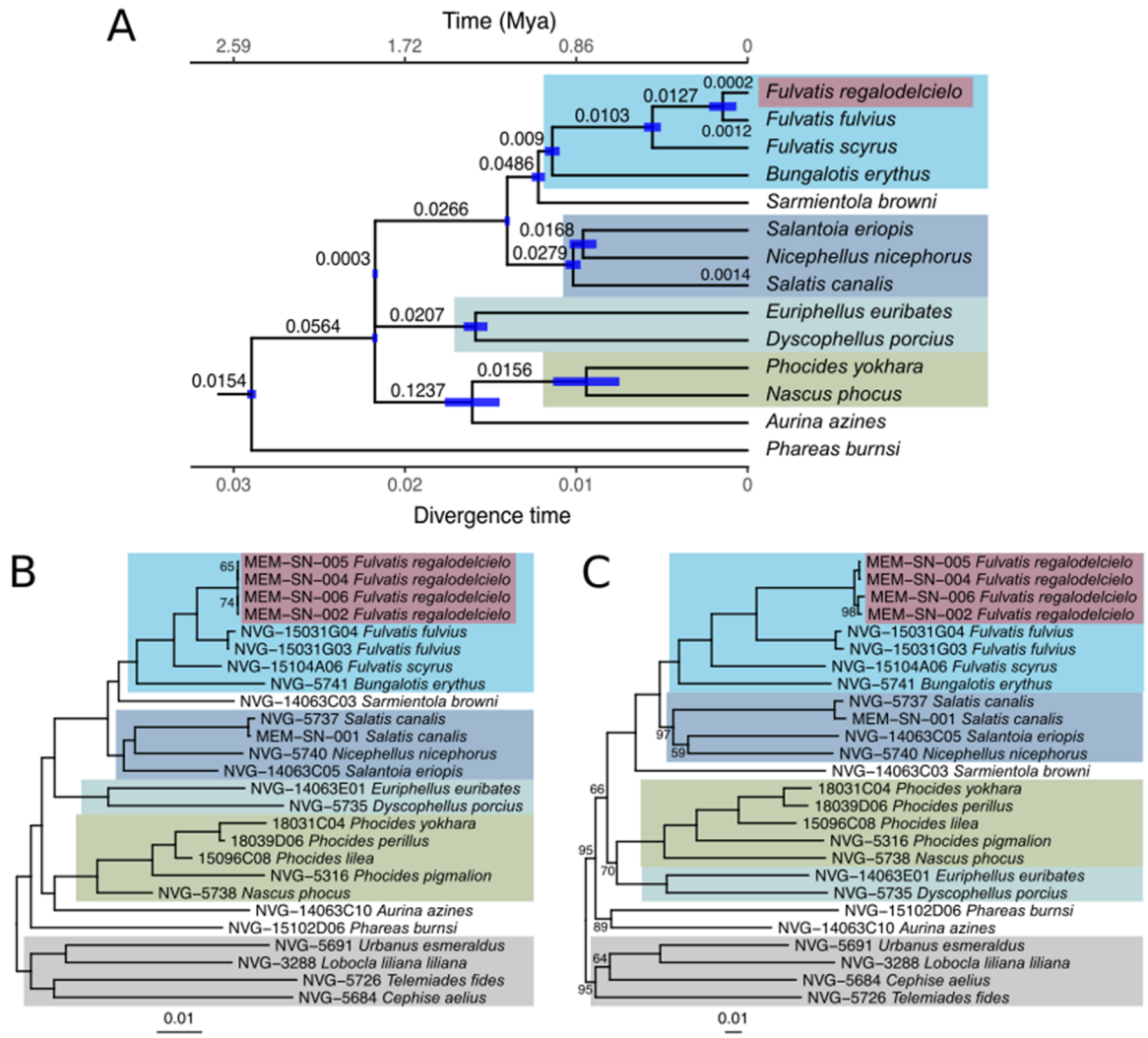

Figure 1. Phylogenies of the tribe Phocidini (Eudaminae). A, species tree with divergence times (*x*-axis) and effective population sizes (numbers above branch, with 95% highest density interval), measured in the expected number of mutations per site, inferred from nuclear genome (21803 loci) under the multispecies coalescent model using BPP with heterozygous genotypes treated as ambiguities (Supporting Information, Table S5). Only three species had more than one sampled sequence so their effective population size could be estimated. The top *x*-axis indicates absolute time in million years ago (Mya), calculated by assuming a mutation rate of 2.9×10^{-9} per site per generation and 4 generations per year (Supporting Information, Table S6; see Material and Methods). Estimates assuming 3 or 5 generations per year are in Supporting Information, Table S6. B, C, concatenated ML trees obtained from the nuclear genome (B) and mitochondrial genome (C) using IQ-TREE under the GTR+F+G4 model. Bootstrap support values are shown on branches if they are below 100%. Coloured boxes indicate stable clades among the three phylogenies. *Fulvatis regalodelcielo* is highlighted in red. The grey box indicates outgroups from the tribe Eudamini used to root the ML trees.

to remove contaminant sequences from the assembly (Astashyn *et al.* 2024). Assembly statistics were calculated using GFASTATS v.1.3.6 (Formenti *et al.* 2022) and gene completeness (BUSCO scores) was assessed against the Lepidoptera Odb10 database (5286 single-copy orthologues) using COMPLEASM v.0.2.6 (Huang and Li 2023). Results are in Supporting Information, Table S1.

Mitochondrial genome assembly

The mitochondrial genome was assembled *de novo* from trimmed reads in two steps. First, an initial assembly was created by mapping reads to the reference mitochondrial genome of *Cecropterus lyciades* (Geyer, 1832) (Hesperiidae: Eudaminae: Eudamini) at conserved regions (Shen *et al.* 2017) using MIRA v.4.0.2 (Chevreux *et al.* 1999). Then conserved regions were extended to a full assembly using MITOBIM v.1.9.1 (Hahn *et al.* 2013). MITOS2 (Donath *et al.* 2019) was used to annotate coding sequences (CDS) and tRNA genes in the mitochondrial genome while DeGeCl v1.1 (Fiedler *et al.* 2023) was used to annotate rRNA genes. All annotated features were manually validated against the reference mitochondrial genome of *C. lyciades* (NCBI Accession: NC_030602.1) using ACT v.18.2.0 (Carver *et al.* 2008). All assemblies contained all key features of a metazoan mitochondrial genome (13 CDS, 22 tRNA genes, and 2 rRNA genes).

Nuclear genome dataset

We obtained 17 genomes of species in the tribe Phocidini and four genomes of species in the tribe Eudamini from published studies (Li *et al.* 2019, Zhang *et al.* 2020, 2022) (Supporting Information, Table S2). This dataset consisted of 26 genomes from 21 species.

Trimmed reads of each sample were aligned to the *C. lyciades* reference genome (Shen *et al.* 2017) using BWA MEM v.0.7.17 (Li 2013) with options -M -T 50. Mapped reads were sorted using SAMTOOLS SORT v.1.21 and duplicate reads were masked using PICARD's MARKDUPLICATES in GATK v.4.4.0.0 (Poplin *et al.* 2018). Genotyping was performed at the species level, where multiple samples from the same species were jointly genotyped, using MPILEUP and CALLS (with -m option for multiallelic calling) modules in BCFTOOLS v.1.21 (Li *et al.* 2009). Indels were removed. Low-quality single-nucleotide variant calls were excluded if the quality-by-depth (quality score QUAL divided by allelic depth AD) was below 2 or the root mean square mapping quality (MQ) was below 40. To obtain multilocus data, genomic coordinates of coding loci were obtained from regions of the reference genome annotated as CDS (exons). At each locus, genotype data were converted to sequences using BCFTOOLS CONSENSUS. To avoid heterozygous phasing errors, heterozygous genotypes were represented using the IUPAC notation (e.g. 'R' for 'A/G' and 'G/A'). Sites that did not pass the variant quality filter above or had read depth below 5 were masked as missing using a gap character ('–'). Missing genotypes were coded as 'N'. Sequences with more than 90% missing data were excluded. Among the remaining sequences, sites with more than 50% missing data were excluded. Loci were excluded if fewer than five sequences remained or there were less than 10 sites after filtering. We obtained 53,803 coding loci in total. This dataset represented about 57% of the 94,657 exons annotated in the reference genome.

Concatenated nuclear genome phylogeny

All nuclear coding loci were included in the concatenated alignment which excluded sites with gaps in more than 80% of the sequences. The resulting alignment consisted of 26 sequences and 5,177,303 columns, with 410,248 (27%) parsimony-informative sites, and 63% missing data. A maximum-likelihood phylogeny was inferred using IQ-TREE v.2.3.6 (Minh *et al.* 2020) under the best-fit model GTR+F+G4 selected using ModelFinder in IQ-TREE (Kalyaanamoorthy *et al.* 2017). Branch supports were based on the ultrafast bootstrap approximation (Hoang *et al.* 2018) using 1,000 replicates. The tree was rooted using outgroup species in the tribe Eudamini. We also performed the analysis using an alignment which excluded sites containing more than 50% gaps. This alignment had 385,650 columns, with 7% parsimony-informative sites, and 43% missing data.

Species tree analysis

We used a full-likelihood multispecies coalescent method to infer a species tree of the tribe Phocidini from the nuclear genome dataset. To simplify the computation, we only kept one sample [*Phocides yokhara* (Butler, 1870)] from the genus *Phocides* Hübner, 1819 as a representative of this genus. While we did not necessarily include type species for polytypic genera, Zhang *et al.* (2022) suggest that those taxa selected herein do form a clade with the type species of each genus. We also excluded the outgroup species (tribe Eudamini) since rooting was done under the molecular clock assumption. Furthermore, to enrich the data for our specimens, we analysed a subset of loci that contained at least one sequence of the focal samples. This reduced dataset contained 21,803 loci from 19 specimens from 14 species (Supporting Information, Table S2). The data were analysed in blocks of 1,000 or 2,000 loci. In total, there were 22 1,000-locus blocks and 11 2,000-locus blocks. These blocks should be considered as random collections of coding loci since scaffolds of the reference genome were not assigned to chromosomes.

We first inferred likely species tree topologies by analysing blocks of loci separately. For each block, we estimated the posterior distribution of species trees under the multispecies coalescent model using BPP v.4.8.2 (Rannala and Yang 2017, Flouri *et al.* 2018). We assigned a diffuse gamma prior G(2, 200), with mean 0.01, to the root age and all effective population size parameters (θ) associated with each branch of the species tree. Other divergence times were assigned a uniform-Dirichlet distribution (Yang and Rannala 2010). Both divergence times ($\tau = T\mu$) and population size parameters ($\theta = 4N\mu$) are in the units of the expected number of mutations per site, with one unit being the expected time to accumulate one mutation per site. Here, T is the divergence time in generation, μ is the mutation rate per site and N is the effective population size. For the species tree topology, we assigned a uniform prior on rooted species trees. Heterozygous genotypes were resolved computationally in BPP using the algorithm of Gronau *et al.* (2011). Four independent runs of Markov chain Monte Carlo (MCMC) were performed for each block using different initial tree topologies. The concatenated ML tree was used as one of the initial trees. Each MCMC chain was run for 10^6 iterations after a burn-in of 10^4 iterations, with samples recorded every 100th iteration. Each run took about 120–150 hours for the 1,000-locus blocks ($22 \times 4 = 88$ runs in total) and 250–300 hours for the 2,000-locus blocks ($11 \times 4 = 44$ runs in total). Convergence was assessed by comparing the posterior species tree distributions from independent runs and non-convergent runs (if any) were discarded. The remaining runs were combined to obtain a final posterior distribution for each block. We summarized posterior distributions across all blocks as proportions of species trees that appeared as the most common tree topology, or a maximum a posteriori (MAP) tree, in at least one block.

The blockwise estimates of species trees revealed a single tree topology as the most common and highly supported across blocks of loci. We then inferred the parameters associated with this topology, namely, species divergence times and effective population sizes, using bpp v.4.8.2 (Rannala and Yang 2017, Flouri *et al.* 2018). Prior specifications were as above. All 21,803 loci were used. We performed four independent runs of MCMC, each with 8×10^5 iterations after a burn-in of 10^4 iterations and sampling every 100th iteration. Each run took about 27 days using four threads. Convergence of MCMC samples was visually inspected before the samples were pooled across runs to produce final posterior estimates of the species tree parameters. To assess the robustness of the estimates, we performed the same analysis on data subsets of four 5,000-locus blocks, with the last block having 6,803 loci. To assess the impact of genotyping errors at a low read depth cut-off (5×), we repeated the above analysis by treating each sequence as haploid and treating heterozygous sites as ambiguity (i.e. without phasing) instead of phasing each input sequence into two haploid sequences. There were 10 sets of analysis in total. Finally, species divergence times were scaled to absolute time in years by assuming a neutral mutation rate of 2.9×10^{-9} per site per generation (Keightley *et al.* 2015, Mackintosh *et al.* 2019), and three to five generations per year.

Mitochondrial genome phylogeny

We performed mitochondrial genome assembly and annotation for 21 additional samples using the same procedure as before. Thirteen protein-coding genes were extracted from annotated mitochondrial genomes of all 26 samples. Sequences of each gene were aligned according to amino acid translation with the invertebrate mitochondrial code using MACSE v.2.07 (Ranwez *et al.* 2011). Each alignment was visually inspected for correct alignment of codons and manually trimmed if needed. Trimmed alignments of all 13 genes were concatenated into a single alignment containing 11,142 sites, 29% of which were parsimony-informative. A maximum-likelihood phylogeny was inferred using the same procedure as for the nuclear genome phylogeny.

RESULTS

Fulvatis regalodelcielo MacDonald, Thurman & Nakahara, sp. nov.

(Figs. 1–4)

ZooBank LSID: urn:lsid:zoobank.org:act:0A268530-15A5-43D8-AE76-10C600521DBB.

Genome sequencing and assembly

Illumina sequencing generated about 50 million raw sequencing reads on average for each specimen (Supporting Information, Table S1). We successfully assembled two out of five nuclear genomes (MEM-SN-002 and MEM-SN-006) into scaffolds. These assemblies spanned about 300 megabases (Mb) and had a scaffold N50 length of about 1.5 kilobases (kb). In each assembly, we recovered about 66% of the BUSCO genes (5286 single-copy orthologues in Lepidoptera) as either complete genes or fragmented genes. The GC content was about 36% in both assemblies. These assemblies were not used in subsequent analyses. We successfully retrieved a full mitochondrial genome from all specimens. An annotated mitochondrial genome of the holotype is shown in Supporting Information, Figure S1.

Species tree of tribe Phocidini

To establish the placement of *F. regalodelcielo* in the tribe Phocidini (Eudaminae), we compiled a multilocus dataset comprising the five newly generated genomes as well as additional 21 genomes from previous studies from short-read sequencing data. Multispecies coalescent analysis of the nuclear genome led to a single dominant species tree (Fig. 1A). It was the most frequent MAP tree (64% of 1,000-locus blocks) and was more supported than other topologies, with the median and maximum posterior probability across blocks of 0.60 and 0.93, respectively (Supporting Information, Tables S3–S4, Figs S2–S3). This topology was also identical to the concatenated maximum-likelihood (ML) tree obtained from the nuclear genome (Fig. 1B; Supporting Information, Fig. S4). Meanwhile, the concatenated ML tree from the mitochondrial genome differed slightly from the main topology, mainly in the placement of two taxa, *Sarmientola browni* (Mielke, 1967) and *Aurina azines* (Hewitson, 1867), while the relationships among major clades agreed well (Fig. 1C).

Systematic placement and diagnosis

The phylogenetic hypothesis based on the full-likelihood coalescent method, as well as two maximum-likelihood trees generated from two independent concatenated datasets (mitochondrial genome and nuclear genome) all recovered *F. regalodelcielo* as a member of the tribe Phocidini (Eudaminae) (Fig. 1). *Fulvatis regalodelcielo* was found as sister to the type species of *Fulvatis* Grishin, 2022 (i.e. *Telegonus fulvius* Plötz, 1882) in all datasets, with *Fulvatis scyrus* recovered as sister to this sister pair. *Fulvatis* was recovered as a monophyletic group with these three species strongly supported as forming a clade. The genetic data confirmed two males and two females (MEM-SN-002, 004, 005, and 006) to be conspecific, supporting the two different habiti reflecting sexual dimorphism.

We estimated the divergence time (τ) between *F. regalodelcielo* and its sister species, *F. fulvius*, to be about 0.0015–0.0040 (Fig. 1A; Supporting Information, Fig. S5, Table S5). This translates to about 0.1–0.4 Mya assuming a neutral mutation rate (μ) of 2.9×10^{-9} per site per generation (Keightley *et al.* 2015, Mackintosh *et al.* 2019) and three to five generations per year (Supporting Information, Table S6). We also inferred that *F. regalodelcielo* has a low effective population size $\theta = 4N\mu$ of about 0.0002–0.0014 (Supporting Information, Table S5), or $N = 1.7 \times 10^4$–1.2×10^5 individuals. The variation in the estimates reflects the uncertainty in the genotyped heterozygotes, which is partly due to the use of a distant reference genome of *C. lyciades*, which belongs to the tribe Eudamini. The estimated effective population sizes of the sibling species *F. fulvius* and *F. scyrus* are about an order of magnitude larger (Supporting Information, Fig. S5, Table S5). These estimates are robust to the choice of genomic loci whether all loci or subsets of 5,000 loci were used (Supporting Information, Fig. S5, Table S5).

Due to the lack of DNA data to confirm the species identification of female specimens of two other congeneric species of *Fulvatis*, the following diagnosis is restricted to male individuals. *Fulvatis regalodelcielo* is readily distinguished from two other congeneric species by its larger and more prominent semi-transparent hyaline macules on the forewing. While the overall wing shape of *F. regalodelcielo* is similar to *F. fulvius*, *F. regalodelcielo* possessed semi-hyaline macules in forewing cells R_3, R_4, M_2, and Cu_2, whereas semi-hyaline macules were absent in these cells in *F. fulvius*. Additionally, the semi-hyaline macules in forewing cells M_3 and Cu_1 were larger and closer together in *F. regalodelcielo* than in *F. fulvius*. The darker markings on the dorsal hindwing appeared more prominent in *F. regalodelcielo* compared to *F. fulvius*. The ventral margin of the tegumen appeared straighter and longer in *F. regalodelcielo*, while a concavity is noticeable in *F. fulvius* due to the broad gnathos base and ventral arm of the tegumen in lateral view. The gnathos of *F. regalodelcielo* is narrow throughout with a slight inflation at the base in lateral view where it connected to the tegumen. The ventral margin of the uncus terminated in a blunt and rounded point at the base (near the tegumen) in *F. regalodelcielo*, whereas it tapered towards the base and terminated in a pointier manner in *F. fulvius*. The apical process of the valva appeared (comparatively) narrower and longer in *F. regalodelcielo*, whereas it is broader in *F. fulvius*. The phallus of *F. regalodelcielo* had a horn-like projection, while this structure appeared to be absent in *F. fulvius*. *Fulvatis regalodelcielo* is distinguished from *F. scyrus* by its more triangular forewing shape and the lack of costal fold, as well as the lack of a darker region of the dorsal hindwing anterior of M_1. The semi-hyaline macule characters to distinguish

F. regalodelcielo from *F. fulvius* could also be applied to separate *F. regalodelcielo* and *F. scyrus*, except for the forewing discal cell having a black smudge-like marking instead of semi-hyaline macules in *F. fulvius*. Furthermore, *F. regalodelcielo* could be distinguished from other members of Phocidini by the combination of these following characters: (i) lack of costal fold; (ii) semi-hyaline macule in cell Cu_2; (iii) presence of yellowish androconial hair-like scales inserted in the intersegmental membrane between the eighth abdominal segment and genitalia; (iv) horn-like projection accompanying the phallus.

Description

Male: Forewing length 26–27.5 mm ($N = 3$; holotype: 26 mm; Figs. 2A, B).

Head: Eyes chestnut brown with no visible interommatidial setae, light ochre scales and elongated ochre-coloured hair-like scales present at base; frons dark brown with ochre-coloured long hair-like scales and ochre-coloured to creamy white scales; white scales visible at base of labial palpi in ventral view; labial palpi overall length approximately 3 mm ($N = 1$), first segment short, with long pale ochre hair-like scales in lateral view, white scales present

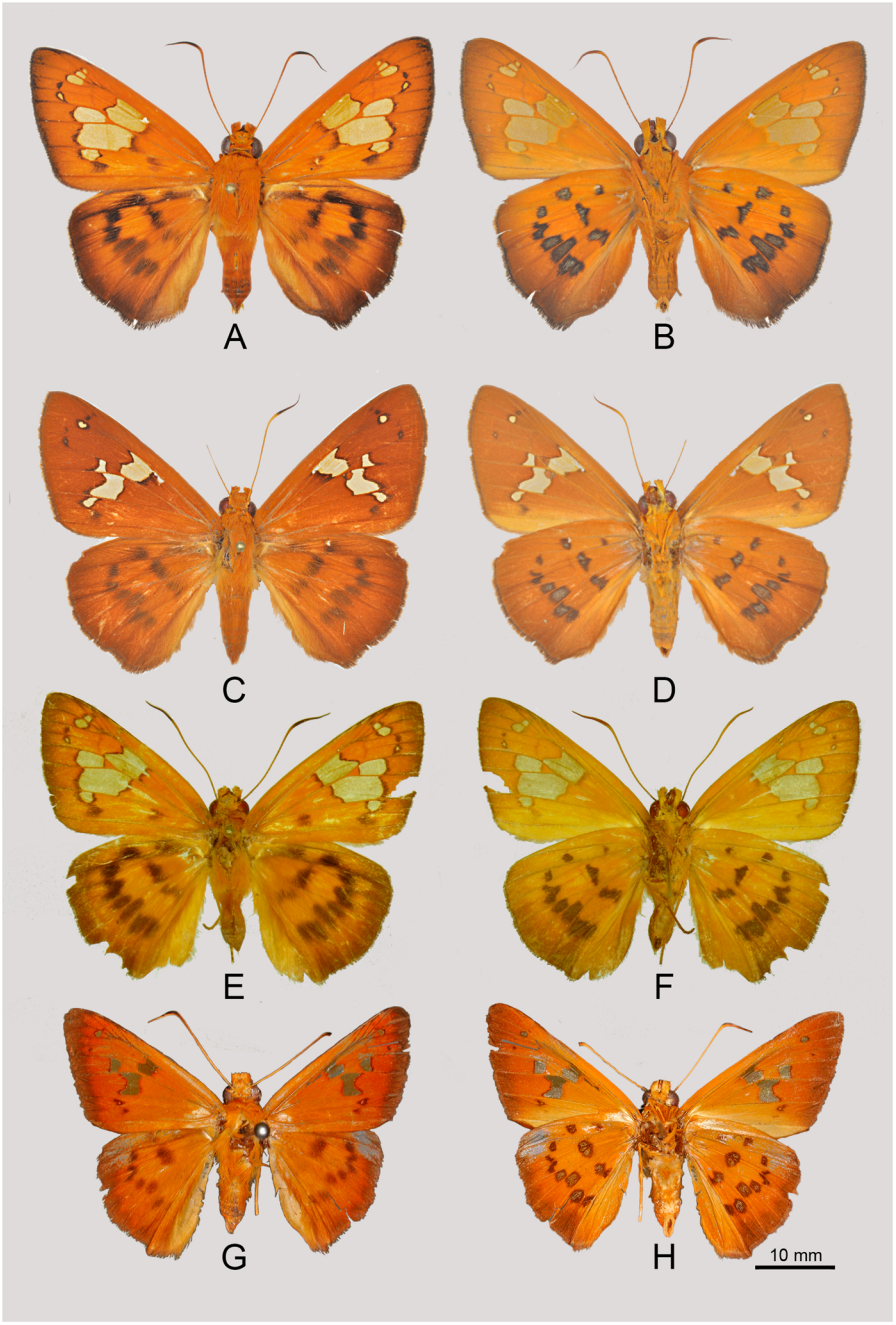

Figure 2. *Fulvatis* taxa discussed herein: *F. regalodelcielo*: (A) holotype male, dorsal view; (B) holotype male, ventral view; (C) paratype female, dorsal view; (D) paratype female, ventral view; (E) Colombian male, dorsal view; (F) Colombian male, ventral view; *F. fulvius*: (G) lectotype male, dorsal view; (H) lectotype male, ventral view.

on interior side, second segment length similar to eye diameter, dorsally with ochre-coloured scales and scales appearing more elongated and hair-like ventrally, scales darker antero-dorsally, third segment short, stout, and round-ended, with dark ochre scales and anteriorly becoming setiform, most of third segment visible in dorsal view but base appearing somewhat 'tucked in' behind anterior scales of second segment in lateral view (Fig. 4C); antenna about two-thirds of forewing in length (approximately similar in length from wing base to distal end of discal cell), flagellomeres covered with dark ochre scales, apiculus arcuate and tapering to rather sharp point, appearing darker dorsally and brownish ventrally, club gradually transforming to apiculus at flagellomeres composing nudum (nudum *c.* 35 flagellomeres; Fig. 4B). Thorax: Brown, dorsally and laterally scattered with pearl scales and orangish long hair-like scales, ventrally (i.e. below wings) with pearl scales as well as orangish long hair-like scales visible sparsely; prothoracic leg femur and tibia brown, with orange scales and similarly coloured elongated hair-like scales, epiphysis present on tibia, tarsus with orange scales, ventrally with longitudinal rows of spines except for fifth tarsal segment; mesothoracic femur brown with orange scales and similarly coloured long hair-like scales ventrally, tibia brown with orange scales, dorsally darker, adorned with rows of spines laterally (spines not noticeable ventrally), as well as pair of spurs at distal end (inner spur longer than outer spur), tarsus colour pattern similar to tibia, ventrally with three longitudinal rows of spines except for fifth tarsal segment apparently possessing two longitudinal rows of spines; metathoracic leg similar to mesothoracic leg except for presence of additional pair of tibial spurs roughly in middle of ventral tibia surface (inner spur longer than outer spur). Abdomen: Dark brownish with layers of white scales and grey scales, in addition to orange long hair-like scales apparently denser dorsally; yellowish androconial hair-like scales inserted in intersegmental membrane between eighth abdominal segment and genitalia, apparently concentrated more towards ventral side of eighth abdominal segment (Fig. 3A). Wing venation: Forewing discal cell length approximately 60% of forewing length, origin of R_s towards base compared to origin of Cu_1, otherwise as illustrated (Fig. 4A). Wing shape: Forewing overall sub-triangular to triangular and appearing elongated horizontally, costa approximately straight, no costal fold, apex rather pointed, outer margin very slightly convex, inner margin approximately straight; hindwing appearing somewhat quadrilateral, costa curved, concavity noticeable in posterior half of outer margin, influencing somewhat pronounced tornus. Wing pattern: Dorsal forewing ground colour orange, black scaling visible along outer margin; eight semi-hyaline macules decorated with black scaling in cells R_3, R_4, R_5, M_2, M_3, Cu_1, Cu_2, and discal cell, as illustrated; dark markings visible as small spot in cell M_1 (can be interpreted as a reduced semi-hyaline macule), smear in cell Cu_2 just below semi-hyaline macule, another postbasal smear in same space; fringe mixture of orangish and dark scales. Dorsal hindwing ground colour same as dorsal forewing, distally appearing darker due to smear along outer margin; dark brown smear-like markings with fuzzy edges present in cells $Sc+R_1$, R_s, M_1, M_3, Cu_1, and Cu_2, overall appearing as discal and postdiscal bands connected at $Sc+R_1$ by postdiscal marking in that cell, discal marking in same cell being displaced basad; fringe orangish anteriorly and gradually becoming darker posteriorly. Ventral forewing ground colour slightly paler than dorsal surface, appearing lighter in cells Cu_2 and 2A, creamy yellow at extreme base; semi-transparent hyaline macules as in forewing except for lacking obvious black marginal scalings; black markings absent except for small spot in cell M_1 and trace in cell Cu_2. Ventral hindwing ground colour similar to dorsal surface except for darker distal area less pronounced; dark marking largely mirroring dorsal surface but markings more defined with dark brown borders and irregular white hair-like overscaling inside each marking. Genitalia (Figs 3B–G): Tegumen slender and appearing somewhat rectangular but anteriorly broadening in lateral view; gnathos narrow, appearing as slender continuation of tegumen; uncus short and thick, apical tip ending in hooked point in lateral view, hair-like setae present on dorsal margin towards base; ventral arms of tegumen and dorsal arms of saccus broadening towards middle with weakly sclerotized rectangular plate projecting anteriorly at widest point; saccus anteriorly projecting as long and narrow process, similar or slightly longer than tegumen in length, posterior margin of saccus slightly projecting; valva with cephalic portion broad and roughly quadrate, apical process rectangular and evenly broad in lateral view, slightly curving inwards in dorsal view with no obvious inner serration; phallus roughly straight and evenly broad, length similar to valva, phallobase occupying approximately quarter of phallus, aedeagus with horn-like projection located dorsally with serrated ventral margin, cornuti as series of rather elongated spikes (vesica not everted) (Figs. 3F, G).

Female: Forewing length 28–30 mm ($N = 3$) (Figs. 2C, D).

Habitus similar to male except as follows: Forewing outer margin more convex and overall appearing rounder and less produced; hindwing outer margin smooth and tornus more pronounced; ground colour brownish; each semi-transparent hyaline macules roughly half in size, those in cells R_3, R_4, and M_1 insignificant, basal smear in cell Cu_2 absent. Genitalia: Papillae anales with apophyses posteriores about 1 mm long; lamella antevaginalis as pair of anchor-like structures accompanied with serration apparently developed from ostium bursae, enclosed by moderately sclerotized broader-than-long semi-circular plate barely separated by membranous region in middle; lamella postvaginalis rectangular with concavity in middle of distal margin in ventral view, apparently fused to lamella antevaginalis; ductus bursae sclerotized, constricted before joining with bulbous corpus bursae; ductus seminalis arising dorsally right before narrowing towards corpus bursae; corpus bursae sac-like and postero-anteriorly oblong.

Molecular data repository

Assembly statistics and BUSCO scores for four samples of *F. regalodelcielo* sequenced for the present work were tabulated and are presented in Supporting Information, Table S1. Mitochondrial genome assemblies, multilocus nuclear genome data, and concatenated alignments are available on Zenodo at https://zenodo.org/record/16610584. Raw sequencing data are available in the NCBI Sequence Read Archives (SRA) database under BioProject PRJNA1213337. BioSample accession numbers: SAMN46325588 (MEM-SN-001), SAMN46325589 (MEM-SN-002), SAMN46325590 (MEM-SN-004), SAMN46325591 (MEM-SN-005), and SAMN46325592 (MEM-SN-006). SRA accession numbers: SRR32189787–91.

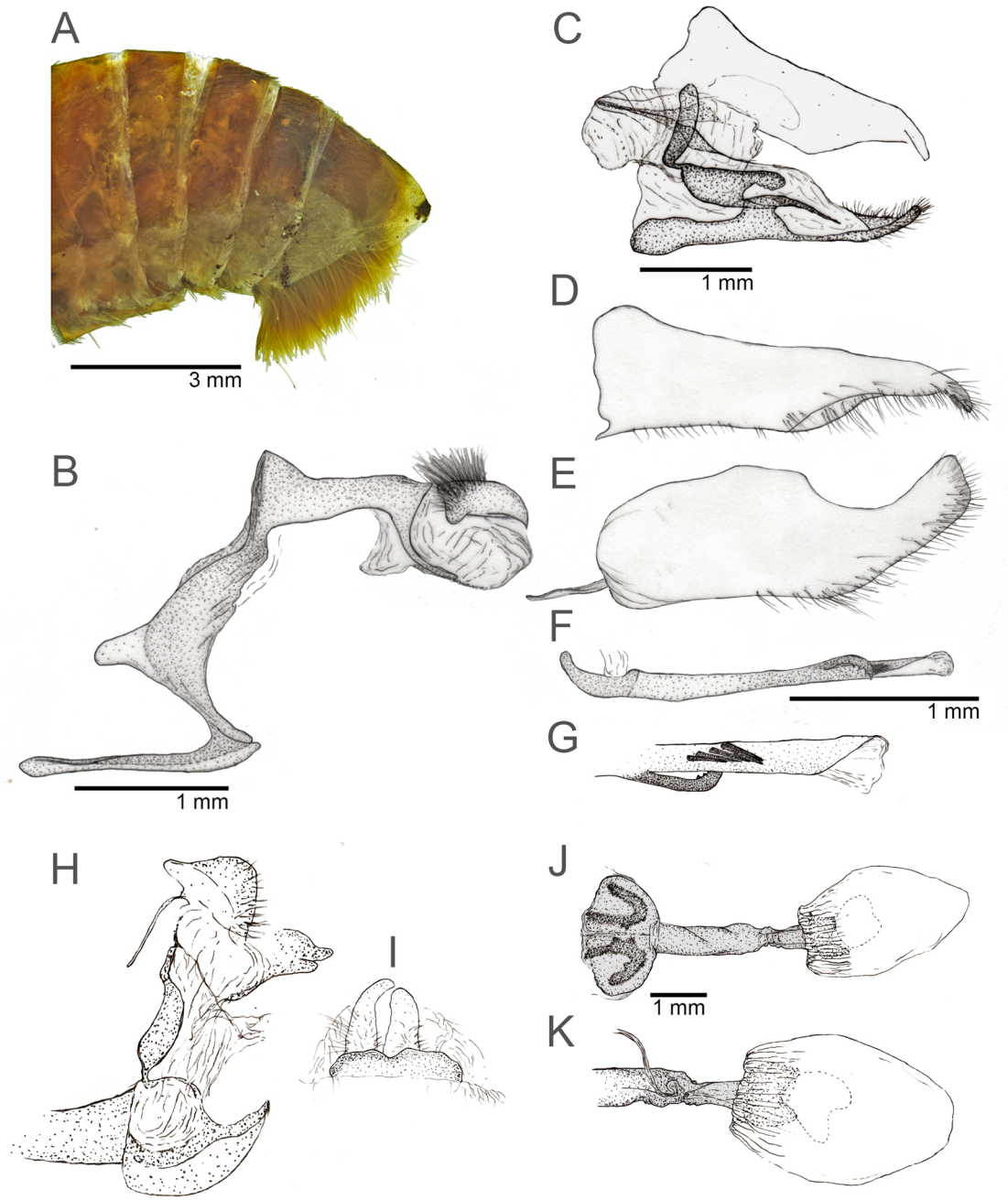

Figure 3. Male and female genitalia of *F. regalodelcielo*: (A) terminal abdominal segments of male with androconial scales at the end; (B) male genitalia without phallus and valva; (C) valvae in dorsal view (two valvae not separated); (D) valva in ventral view; (E) valva in lateral view; (F) phallus in lateral view; (G) close-up view of the terminal end of the phallus, showing cornuti and a horn-like projection; (H) Lateral view of 8th abdominal segment of the female; (I) ventral view of the papillae anales; (J) ventral view of the female genitalia; (K) dorsal view of the female genitalia showing the location of the ductus seminalis.

Types

Holotype, male with the following labels written verbatim, separated by double slashes (Figs. 2A, B): PANAMA: Darien National Park vic. Cerro Pirre, Rancho Frio, ca. 100 m., 8.0198, -77.7325 [N 08° 01′ 11.3″ W 077° 43′ 57.0″], Feb. 27, 2023 Albert Thurman – collected at mercury vapor/UV light//DNA Sample # MEM-SN-23-005//USNM ENT 01913152//(USNM).

Paratype, female with the following labels written verbatim, separated by double slashes (Figs. 2C, D): PANAMA: Darien[;] Darien National Park vic. Cerro Pirre 'Rancho Frio' ca.100m N 08° 01′ 11.3″ W 077° 43′ 57.0″ Feb. 11, 2015 Dan Bogar//collected at Mercury vapor/UV light//DNA Sample # MEM-SN-23-006//USNM ENT 01913153//(USNM).

Other Paratypes

Two males, with the following labels written verbatim, separated by double slashes: PANAMA: Darien[;] Darien National Park vic. Cerro Pirre 'Rancho Frio' c.100m N 08° 01′ 11.3″ W 077° 43′ 57.0″

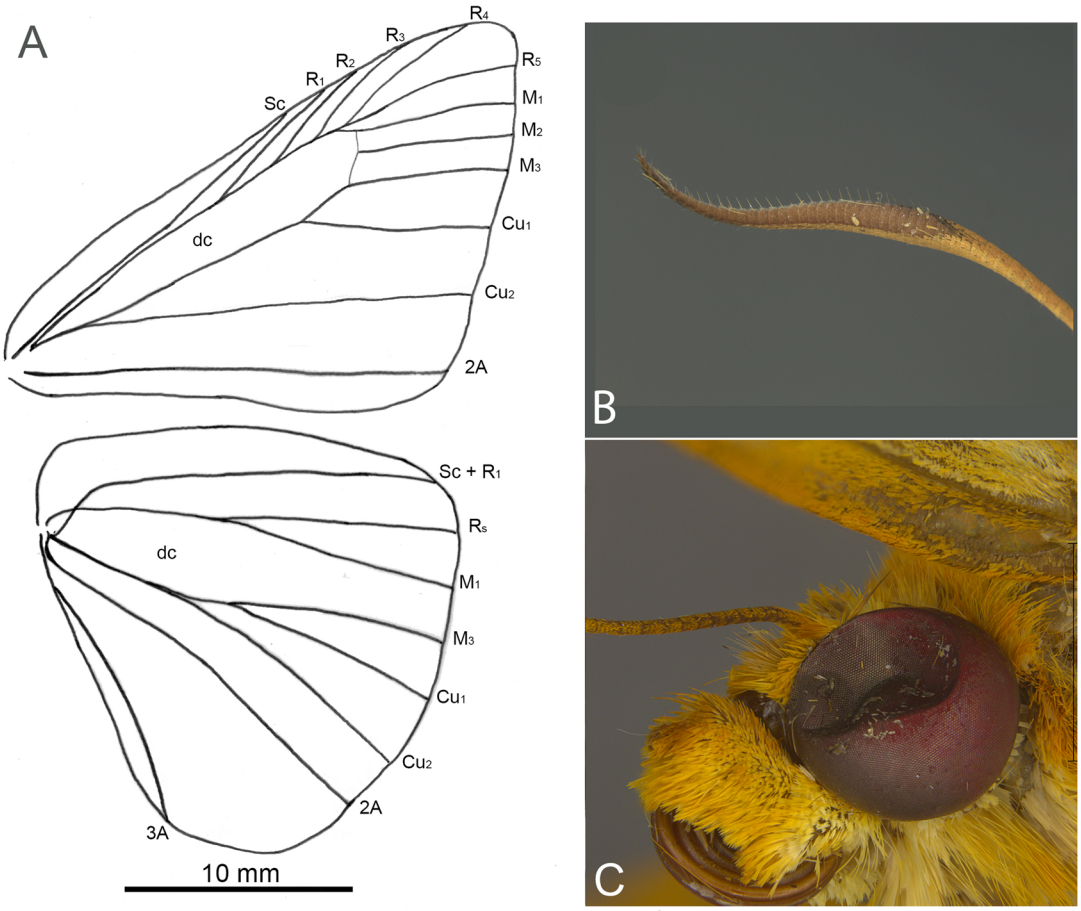

Figure 4. Morphological features of *F. regalodelcielo*: (A) male wing venation; (B) antenna with an emphasis on the nudum; (C) lateral view of the head.

Feb. 8, 2015 John R. MacDonald//Genitalic vial SN-MCZ-004//MEM-SN-004//MCZ-ENT00253967 (MCZ); PANAMA: Darien; Darien National Park; lower slope of Cerro Pirre; ca. 400 m near gps point: N 08° 00′ 40.2″; W 077° 43′ 24.5″ –landed under leaf at ca. 1 m above ground- March 2, 2019 John R. MacDonald//MEM-SN-003//(MEM). Two females, with the following labels written verbatim, separated by double slashes: PANAMA: Darien[;] Darien National Park vic. El Real/Cerro Pirre Rancho Frio c.100m N 08° 01′ 11.3″ W 077° 43′ 57.0″ Jan 10, 2016 John R. MacDonald//Genitalic vial SN-MCZ-005//MEM-SN-002//MCZ-ENT00253966 (MCZ); Panama: Darien[;] Darien National Park vic. Cerro Pirre Rancho Frio, 100 m N 08° 01′ 11.3″ W 077° 43′ 57.0″ Feb 12, 2014 J. R. MacDonald//(MEM).

Other examined specimens

A male specimen from Colombia with the following labels written verbatim, separated by double slashes (Figs. 2E, F): 22-VI [VII] -1988 Rio Claro, Antioquia [Colombia], K[eith] Brown *col. Leg.*//OM 25. 329//*Salatis fulvius* (Plötz, 1882)//(DZUP) [photograph examined]. Examination of the field notes compiled by the collector (Keith Brown) suggests this site was visited by him on July 22 1988, not June 22 as written on the label, thus the month on the label appears to be a *lapsus calami*. The site appeared in Keith Brown's handwritten note as 'Rio Claro, km 150 Autopista Medellin—Doradal', and the site can be georeferenced as follows: 5°54'21.7"N 74°51'15.3"W. The field note is accompanied with an indication of collecting two male specimens of '*Salatis salatis*?', which presumably represents *F. regalodelcielo* (Fig. 5A). The second specimen has not been located during the course of the present study, but it is likely to be housed at DZUP or at the State University of Campinas (São Paulo, Brazil). The collection at the latter institution holds Keith Brown's research material, as well as his field notes.

Derivation of the species-group name

The species-group name is based on a Spanish phrase 'regalo del cielo', meaning 'gift from heaven', an allusion to its beauty giving a serendipitous feeling of a 'gift from heaven' in the field. The specific epithet is to be treated as a noun in apposition.

Distribution

Fulvatis regalodelcielo is known to date from the type locality situated in Darién Province, Panama, in addition to a single record from Antioquia department, Colombia.

Habitat and adult biology (Fig. 6A)

The habitat for Panamanian specimens consisted of lowland tropical rainforest of the Chocó-Darien ecoregion which extends from

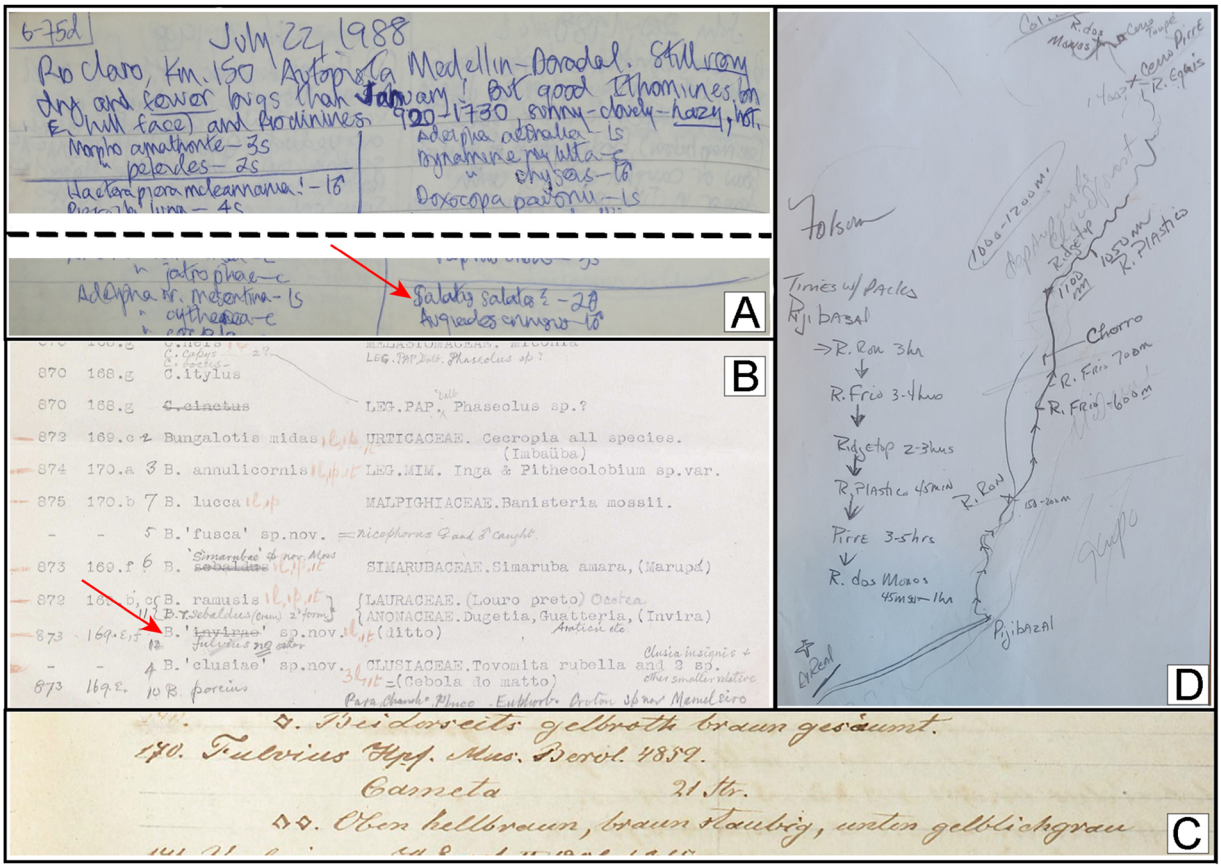

Figure 5. Archival records discussed herein: (A) Keith Brown's field notes showing collecting data relevant to the present study (red arrow indicates '*Salatis salatis*?'); (B) Arthur Moss's documentation of host plant records for skippers in Brazil, dated October 1930 (red arrow indicates records for *B. fulvius*; from the Library and Archives collections of the Natural History Museum, London); (C) Carl Plötz's manuscript showing *T. fulvius* in his keys; (D) Gordon Small's back-of-envelope notes (Smithsonian Institution Archives. Record Unit 7474, Box 1., 'Lepid. Misc' folder).

western Colombia into Panama (Fagua *et al.* 2019). The Chocó-Darien ecoregion is characterized as having high rainfall as well as being home to unique and diverse fauna and flora. Two male specimens of *F. regalodelcielo* were collected between 10 a.m. and 3 p.m. after observed landing under leaves. One specimen landed under a leaf about 0.6 m above the ground and the other landed under a leaf about 1.3 m above the ground. Some associates of *F. regalodelcielo* include: HESPERIIDAE: *Salatis canalis* (Skinner, 1920), *Bungalotis midas* (Cramer, 1775), *Porphyrogenes omphale* (Butler, 1871), *Entheus huertasae* Grishin, 2013, *Eracon paulinus* (Stoll, 1782); RIODINIDAE: *Euselasia andreae* Hall, Willmott & Busby, 1998, *Euselasia tarinta* (Schaus, 1902), *Mesosemia carderi* Druce, 1904; NYMPHALIDAE: *Napeogenes stella delgadoi* Vitale & Constantino, 2012, *Thyridia psidii aedesia* Doubleday, 1847, *Adelpha zina zina* (Hewitson, 1867); and PIERIDAE: *Dismorphia amphione beroe* (Lucas, 1852). Austin (2008) discussed crepuscular or potential nocturnal behaviour of Phocidini (Eudaminae) skippers. While *F. regalodelcielo* has never been encountered being active during dusk nor at night, there seems to be some evidence to support that this species is also one of those 'night' skippers: the holotype male specimen was attracted to the mercury vapor light around 5 a.m. (see Fig. 6B). Both mercury vapor and UV lights were run all night and occasionally checked by J.R.M. and A.T., but the holotype male was not spotted until 5:00 am, suggesting that the individual flew at the crack of dawn. During the course of conducting field work in Panama, J.R.M. and A.T. observed many other skipper taxa attracted to mercury vapor and/or UV light set-ups, with the majority of them belonging to Phocidini (Phocidini taxa marked with asterisk): *Bungalotis astylos* (Cramer, 1780)*, *Bungalotis quadratum* (Sepp, 1845)*, *Bungalotis midas* (Cramer, 1775)*, *Bungalotis erythus* (Cramer, 1775)*, *Salatis canalis* (Skinner, 1920)*, *Dyscophellus porcius* (Felder & Felder, 1862)*, *Dyscophellus ramon* (Evans, 1952)*, *Dyscophellus ramusis* (Stoll, 1781)*, *Dyscophellus phraxanor* (Hewitson, 1876)*, *Nascus phocus* (Cramer, 1777)*, *Nascus broteas* (Cramer, 1780)*, *Porphyrogenes sula* (Williams & Bell, 1940)*, *Porphyrogenes omphale* (Butler, 1871)*, *Cephise aelius* (Plötz, 1881) (Eudamini), *Carystoides* sp. (Megathymini). Additionally, there is evidence supporting that other Phocidini taxa were also attracted to light elsewhere in the Neotropical region (e.g. Mielke 1973: 28). However, it must be noted that diurnal butterfly species can also be found attracted to lights at night such as *Phoebis trite* (Linnaeus, 1758) (Pieridae), *Brevianta hyas* (Godman & Salvin, 1887) (Lycaenidae), *Euselasia regipennis* (Butler & Druce, 1872) (Riodinidae), and *Oxeoschistus hilara* (Bates, 1865) (Nymphalidae) (J.R.M. & A.T., personal observations). Positive phototaxis behaviour does not necessarily support nocturnal or crepuscular behaviour, but these numerous records of Phocidini taxa attracted to light at night may not be random.

Figure 6. Habitat pictures of the type locality of *F. regalodelcielo* (both photographed by A.T.): (A) trail behind the MiAmbiente facilities at Rancho Frio; (B) area in front of the MiAmbiente buildings at Rancho Frio where the holotype was collected.

Immature biology

The immature biology of *F. regalodelcielo* is unknown and the only source of early stage biology for the genus appears to be by Moss (1949). As stated in the introductory section of Moss's (1949: 27–28) *Biological notes on some 'Hesperiidae' of Para and the Amazon*, this work was edited by Kenneth J. Hayward (1891–1972) since Arthur M. Moss (1872–1948) died before completion. We thus examined existing manuscripts prepared by Moss to understand his observation prior to Hayward's interpretation. The manuscript entitled 'NOTES ON THE SPECIES HESPERIINAE' represents part of Moss's series of observational notes of skipper immature stages during his time in Pará, Brazil. *Fulvatis fulvius* appears on p. 19 as *Bungalotis fulvius* (Library and Archives collections of the Natural History Museum, London), although it is evident that Moss was confused about the identity of this species since it seems he regarded this taxon as an undescribed taxon ('envirae' after the host plant) for many years. The host plants were reported as '*Louro preto*', *Nectandra mollis* (both Lauraceae), '*Graviola*', *Annona muricata*, Annonaceae species, 'Araticú', '*Dugetia*', and '*Guatteria*'. This is roughly consistent with his 11-page manuscript entitled 'HESPERIDAE [*sic*] OF PARA', dated October 1930, which seems to be a compilation of host plant records for skippers, where *B. fulvius* appears on p. 3 with similar host plant records mentioned above (Fig. 5B). Although researching details of how these manuscripts were compiled and edited is beyond the scope of the present study, examination of the collection of Moss at the NHMUK confirmed that his concept of *B. fulvius* was in accordance with the present work. The discrepancy between the host plant records in this manuscript and those reported in Moss (1949) is likely due to Hayward's interpretation. Subsequently, Silva *et al.* (1968: 313) and Brown (1992: 47) reported host plant records for *F. fulvius* from Brazil by attributing to the record of Moss (1949) [Brown (1992) also referenced Silva *et al.* (1968)]. Peña *et al.* (1995) also reported the host plant record of *F. fulvius* (as *Bungalotis fulvius*) from Brazil without clear attribution, and it is likely that this is a repetition of the data of Moss, not an independent record. According to the aforementioned records, known host plants for *Fulvatis* belong to two plant

families: Lauraceae and Annonaceae. A mature larva is illustrated in Moss (1949: 53, pl. 2, fig. 13) and a pinned larva is located in his collection currently housed at the NHMUK (BMNH DES No. Rh. 5005).

DISCUSSION

Fulvatis regalodelcielo is the third known species of *Fulvatis*. While species-level taxonomy of *Fulvatis* can be considered as straightforward, generic classification of these species and closely related taxa has been in flux. The species-group name *fulvius* first appeared in a combination with *Telegonus* Hübner, [1819] (Plötz 1882), and subsequent authors did not reach a consensus as to its generic classification (e.g. Draudt 1922, Moss 1949, Evans 1952). The specific epithet *scyrus* was originally introduced in combination with *Bungalotis* Watson, 1893 by Ernst Bell (Bell 1934). Evans (1952) established *Salatis* Evans, 1952 to accommodate *B. scyrus* and *T. fulvius*, in addition to four other species. Like many other skipper genera, *Salatis* was not recovered as a monophyletic entity in the recent phylogenetic study utilizing genomic data and *Fulvatis* was established to harbour *B. scyrus* and *T. fulvius*, two taxa which do not form a clade with the type species of *Salatis* (i.e. *Papilio salatis* Stoll, 1782) (Zhang *et al.* 2022). Generic diagnosis of *Fulvatis* is provided in Zhang *et al.* (2022), which still seems to hold even with the inclusion of *F. regalodelcielo* (we did not examine the characters based on nucleotides). *Telegonus fulvius* was described by Carl Plötz (1814–1886), who referred to at least a single specimen in the original description, in a form of keys, by stating 'Mus. Berol. N. 4859' (Plötz 1882: 79). While mention of a single voucher is interpretable as an indication of a single specimen used to prepare the original description, there is room for interpretation about the number of specimens used by Plötz, as reinforced by Zhang *et al.* (2023c: 161). Therefore, following Recommendation 73F of the ICZN (1999), we consider the male specimen labelled '4859' housed at MfN to be a syntype, not a holotype fixed by monotypy. To remove any ambiguity and settle the nomenclature, we designate this specimen in MfN as the lectotype of *Telegonus fulvius* Plötz, 1882 (lectotype designation). This specimen bears these following five labels described above in the Material and Methods chapters and repeated here (label data written verbatim, separated by double slashes): Holotypus//4859//Fulvia N. Cameta Sieb.//fulvius Pl. Type//DNA sample ID: NVG-15031G04 c/o Nick V. Grishin//[photographed by B. Hermier]. This specimen comfortably fits the concept of a taxon discussed herein represented by this species-group name *T. fulvius* (Figs. 2G, H). Carl Plötz's manuscript dated 1876 suggests this lectotype numbered '4859' was used by Plötz to prepare his keys where *T. fulvius* was numbered 140 with a slight discrepancy in the wing pattern description compared to the published version (i.e. *Oben hellbraun, braun staubig, unten gelblichgrau* in the manuscript) (Fig. 5C).

Our morphological findings show *F. regalodelcielo* and *F. fulvius* share morphological characters that are not shared with *F. scyrus*: (1) lack of costal fold (costal fold is present in *F. scyrus*); (2) triangular forewing shape (rounded in *F. scyrus*); (3) presence of yellowish androconial long hair-like scales densely surrounding genitalia at eighth abdominal segment (absent in *F. scyrus*); (4) apical process of valva quadrate and broad (narrow and tapering in *F. scyrus*). Two of these characters (1 and 3) appear to be restricted to *F. regalodelcielo* and *F. fulvius* within Phocidini, thereby one can argue for establishing a monospecific genus for *B. scyrus*. Characters such as length and presence/absence of costal fold have been used to support generic classification in this group, including establishment of *Fulvatis* (Evans 1952, Austin 2008, Zhang *et al.* 2022). The state of character 3 strengthens this delimitation of *F. regalodelcielo* and *F. fulvius* from *F. scyrus* since these specialized scales found in male Lepidoptera are known to have systematic and taxonomic value (Hall and Harvey 2002). Androconial organs associated with the male genitalia have been reported in some butterfly taxa (e.g. Callaghan 1983), although documentation of such abdominal structures appear to be absent in Hesperiidae. Epstein and Corrales (2004: fig. 82) reported seemingly homologous hair-like organs in a species of *Talima* Walker, 1855 (Lepidoptera: Limacodidae), which are also loosely attached to the intersegmental membrane like *F. regalodelcielo* and *F. fulvius* (M. Epstein, personal communication). These abdominal androconial hair-like scales present in *F. regalodelcielo* and *F. fulvius* might be involved in dissemination of pheromones or scent to complement the lack of costal fold in these two species.

Despite the systematic significance of androconial organs, the generic classification discussed above can be called into question given the compact fulvous phenotype of males for the three *Fulvatis* species. On the other hand, its sister genus *Bungalotis* includes at least 11 species with somewhat varying phenotypes. Many Phocidini species are lowland taxa and adults are reported as being associated with army ants and could be captured by the so-called 'Ahrenholtz technique' (Lamas 1983, Lamas *et al.* 1993), although those species in Evans's (1952) group D of 'Pyrginae' [i.e. 'night skippers' discussed in Austin (2008)] never seem to have been recorded as such. This lack of records supports unique traits for these 'night skippers' being crepuscular and attracted to light as evidenced herein, since the 'Ahrenholtz technique' lures individuals by relying on their visual cue and will only be effective for diurnal skippers. While existing host plant records of these Phocidini taxa are scattered across several different plant families (Cock and Alston-Smith 1990, Beccaloni *et al.* 2008), evidence suggests that these skippers seem to share some uncommon biological traits. Since one purpose of the genus-group name is to allow prediction and communicate biological traits, the ecological information available for these Eudaminae skippers may support a broad generic classification by synonymizing many recently established genera in Phocidini, but reorganization of existing classification should take into account historical usage of these genus-group names to reduce confusion.

As seen from major works on Neotropical skippers (e.g. Draudt 1922, Moss 1949, Evans 1952, Mielke 2004), those genera which received species-group names currently classified in *Fulvatis* are still in use. Therefore, a point that merits discussion is whether *Bungalotis* should be used to accommodate *F. scyrus*, *F. fulvius*, and *F. regalodelcielo* since *Fulvatis* is found as a sister to *Bungalotis* (Fig. 1; Zhang *et al.* 2022). Given that some authors did classify *T. fulvius* in *Bungalotis* (e.g. Moss 1949), coupled with the fact that *Bungalotis* as currently conceived is not phenotypically compact, accommodating *Fulvatis* species in this long-standing widely

accepted genus might have been a better taxonomic decision. While we accept that generic classification involves subjectivity, we argue for a parsimonious approach of applying existing genus-group names to recognize new clades to leave the prior classification intact. Since the genus-group name has been proposed for the clade sister to *Bungalotis* by Zhang *et al.* (2022), we adopt this classification and propose a new species-group name in combination with *Fulvatis* and avoid unnecessarily changing the first part of the binomina of existing taxa.

Fulvatis regalodelcielo is only known from two different sites: Cerro Pirre (type locality) in eastern Panama and Rio Claro in northwestern Colombia. These sites are approximately 400 km apart and both fall into the trans-Andean region, resulting in another sister species pair occurring on the either side of the tropical Andes: *F. regalodelcielo* from Central America and its sister species *F. fulvius* being a predominantly Amazonian taxon. As discussed above, these two sister species are both lowland taxa, hence the Andean divide acting as a barrier to restrict their distribution to one side of the chain. In addition to their external morphological differences documented above and genomic data presented herein, this adds another layer of evidence towards their status as distinct species. A number of recent studies incorporating molecular data have revealed many instances of sister species pairs or clades of Neotropical butterflies which are separated by the tropical Andes (e.g. Elias *et al.* 2009).

Although the first known specimen for *F. regalodelcielo* is reportedly from Colombia, this specimen is excluded from the type series since we were not able to examine this specimen in person and genetic data has not been obtained from this male specimen housed at DZUP. Six Panamanian specimens constituting the type series, four of which have genomic data, were all collected at the type locality and its vicinity over the past decade or so. We note that *F. regalodelcielo* is not represented in the late Gordon Small's (1934–1989) butterfly collection housed at USNM, representing the most comprehensive butterfly collection amassed for Panama. Gordon was an outstanding Lepidopterist, who spent over two decades in Panama from the early 1960s, collecting and studying butterflies throughout the country; he is the sole collector of the entire type series for several new butterfly taxa described from Panama. Examination of Gordon's collecting data sheets and existing museum specimens suggest that he did sample butterflies at Cerro Pirre in 1976 and 1982, at different elevations. Furthermore, a back-of-envelope map of Darién drawn by Gordon illustrates how he hiked and navigated through this remote part of Panama (Fig. 5D; Smithsonian Institution Archives. Record Unit 7474, Box 1., 'Lepid. Misc' folder). While the map is oriented with North at the bottom and names of some localities not traceable, it is evident that Gordon passed through Cerro Pierre along the ridgeline. GPS points and elevations recorded by J.R.M. during the field work in the area roughly follow Gordon's handwritten map and it is likely that Gordon passed through the habitat of *F. regalodelcielo*. Despite visiting the type locality and its vicinity, one reason why *F. regalodelcielo* was not discovered by Gordon in Panama is perhaps due to Gordon's main interest being in Lycaenidae and Riodinidae, not Hesperiidae. In fact, Gordon apparently did not compile data for Hesperiidae specimens he collected, whereas data sheets for his specimens exist for other butterfly families (e.g. Nakahara *et al.* 2023: fig. 3). *Fulvatis regalodelcielo* is one of the few butterfly species described from Panama in recent years without Gordon's contribution, emphasizing and highlighting the importance of sustained field work to document Panamanian butterfly fauna by A.T., J.R.M., and colleagues.

While *F. regalodelcielo* can be identified and described based solely on external morphology, we demonstrate the taxonomic merit of incorporating genomic data in the present study. Recent drastic generic changes in Hesperiidae classification (e.g. Cong *et al.* 2019, Li *et al.* 2019, Zhang *et al.* 2019b, 2022) is largely due to a lack of a phylogenetic framework in long-standing and widely accepted classification of skippers (e.g. Evans 1951, 1952, 1953, 1955, Mielke 2004) where many genera were found to be paraphyletic or polyphyletic by genomic studies. It hardly needs stating that generic classification should ideally be supported by monophyly of the genus with its type species as part of the clade, which is typically achieved through DNA sequence data. Nevertheless, as demonstrated in the present study, dominance of genomic data in biological classification leads to recognition and naming of clades by reconciling phylogenetic hypotheses. This approach of classifying organisms strictly by delineating clades typically results in numerous taxonomic changes, some of which may be counterintuitive and/or may not be supported with morphological characters. Thus, the advent and common usage of high-throughput sequencing techniques seem to have resulted in some departure from the over 250 years of the Linnaean system of classification, which was established to find orderliness in the diversity of life. Although the Linnaean system is still intact, the practicality and information content of classification schemes aligning strictly with evolutionary history (i.e. clades) may not necessarily be far reaching. It is important to remember that taxonomy is the science of classifying and naming organisms to effectively communicate about organisms and their unique characters towards a better understanding of the natural world.

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SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

Raw sequencing data are available in the NCBI Sequence Read Archives (SRA) database under BioProject PRJNA1213337. Mitochondrial genome assemblies, multilocus nuclear genome data, and concatenated alignments are available on Zenodo at https://zenodo.org/record/16610584.

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