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Genomics reveals a new genus and species from a single female specimen (Lepidoptera: Hesperiidae: Hesperiinae: Hesperiini: Moncina)

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Abstract

New taxa in Hesperiidae (Lepidoptera: Papilionoidea) are traditionally proposed after inspection of male genitalia, which largely form the basis for Hesperiidae taxonomy. However, with genomic DNA sequencing, even a single female specimen can be placed in a phylogenetic context of existing classification and taxonomically assigned with confidence. Genomic sequencing of an unusually patterned Hesperiidae female from San Martin, Peru, characterized by pearly spots outlining an inverted heart pattern on the rust-colored ventral hindwing, reveals that it represents an undescribed genus and species named here as *Gemmia buechei* Brockmann and Grishin, **new genus** and **new species**.

Keywords

Skipper butterflies; South America; biodiversity; female genitalia; museomics

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ZooBank registration. https://zoobank.org/2FA538FA-7D65-4097-9BBA-71CD1B2795E5

Introduction

Neotropical regions are rich in undescribed species. While many of them may be cryptic and evade recognition by visual inspection (Hebert et al. 2004), others are distinctive and can be recognized as new at the first glance (Turland et al. 2012). However, it may be a challenge to place such distinctive species within a taxonomic hierarchy, in particular when only a single specimen is known. Hesperiidae taxonomy relies heavily on the analysis of male genitalia, and descriptions of genus-group taxa traditionally report the structure of male genitalia. However, genera are defined as monophyletic groups of species, and a confident phylogeny that includes all close relatives is a reliable way to define them (Cong et al. 2019; Li et al. 2019; Zhang et al. 2019, 2020, 2022). Here, we illustrate this approach and propose a new Hesperiidae genus based on a single distinctively patterned female specimen. We believe that bringing a new species and the new genus to the attention of researchers has advantages over waiting to find more of its specimens, in particular males. The genomics-based approach that we use puts the phylogenetic placement of this new taxon on a strong footing.

Materials and Methods

Specimens used in this study were from the following collections: American Museum of Natural History, New York, NY, USA (AMNH); Los Angeles County Museum of Natural History, Los Angeles, CA, USA (LACM); Museum für Naturkunde, Berlin, Germany (MFNB); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); and the private research collection of Ernst Brockmann (EBC). Standard entomological techniques were used for dissection (Robbins 1991), i.e., the distal part of the abdomen was broken off, soaked for 45 minutes in 10% KOH at 60°C, dissected, and subsequently stored in a small glycerol-filled vial on the pin under the specimen. Genitalia and wing venation terminology follows Steinhauser (1981). Length measurements are in metric units and were made from photographs of specimens taken next to a scale and magnified on a computer screen. Photographs of specimens were taken with a Panasonic Lumix DMC-FZ50 with Macro converter Panasonic DMW-LC55E, following Häuser et al. (2005); dissected genitalia were photographed in glycerol with the Nikon D200 camera without a lens and through a microscope at 2x and 4.5x magnification. Images were assembled and edited in Photoshop CS5. Genitalia photographs were taken in several focus slices and stacked in Photoshop to increase depth of field. Genomic DNA was extracted from a leg, and libraries were prepared and sequenced as previously reported (Li et al. 2019). Phylogenetic tree construction was performed following Zhang et al. (2022). In brief, the maximum-likelihood tree was constructed from protein-coding regions in the Z chromosome using IQ-TREE v1.6.12 under a GTR+GAMMA model (Nguyen et al. 2015). To estimate the confidence of each node, we generated 100 replicates of 10,000 codons randomly sampled from the total set of codons in the Z chromosome and constructed maximum-likelihood trees for each replicate. The support values of each node were summarized from these replicate trees using the sumtrees routine in the DendroPy package (Sukumaran and Holder 2010). The method to find diagnostic DNA characters is described in Cong et al. (2019) and we used the *Lerema (Lerema) accius* (J. E. Smith, 1797) genome assembly as a reference (Cong et al. 2015).

Results

An unusually patterned female specimen of Hesperiidae was noticed among the material given to the first author by Michael Büche (Fig. 1a, b). In wing pattern, it did not match any known species and was believed to be undescribed. To place this probably new species among Hesperiidae, we obtained its whole genome and constructed genomic phylogenetic trees of representative species. According to our phylogeny, the species is confidently placed in the *Phlebodes* Hübner, [1819] subgroup of related genera (marked by blue branches in Fig. 1d) within subtribe Moncina A. Warren, 2008. The new species is sister to a clade of *Phlebodes* and *Dubia* Grishin, 2019, not closely allied to any existing genera, and therefore belongs to a new genus, described here, together with this species.

Gemmia buechei Brockmann and Grishin, new genus and new species

https://zoobank.org/89315B0A-D839-453A-B9A7-81439D67C0F7

https://zoobank.org/66B847C1-C51D-450B-939B-0938D7AE7A5B

Diagnosis of the new genus.—Differs from close relatives, such as *Phlebodes* and *Dubia*, by ventral hindwing pale spots in cells M_1-M_2 and M_2-M_3 being offset towards wing base, not aligned with other spots in postdiscal row; in female genitalia, ductus bursae with bursa copulatrix longer, reaching thorax, sterigma longer, and more expanded in its anterior portion, with rounder sides. Due to the lack of males and monotypic composition of the genus, it is best diagnosed by a combination of the following DNA characters: lac704.5.28:T96C, lac7147.4.1:C46A, lac884.3.3:A590T, lac221.9.2:A233A (not G), lac886.16.8:C464C (not A), lac49.46.10:G109G (not A), lac357.30.3:G1355G (not A), lac1895.5.35:T37T (not A), lac1416.2.1:A449A (not G), lac580.72.1:T75T (not C), lac3441.2.1:A436A (not G), and lac886.16.8:T1215T (not C). See Appendix 1 for abbreviations and sequences.

Description of the new genus.—We hypothesize that the following characters may be shared among species of the genus. Medium-sized, forewing length around 20 mm. Body brown, abdominal sternites with a median dark line, paler on either side of this line, especially in the distal half where they are overscaled with cream-colored scales. Antennae about half of forewing length, dark-brown, ventrally pale near the club, nudum brown. Wings brown, could be with pale spots, ventral side brighter colored, with rusty, orange, and yellow scales and white or pearly spots. In female genitalia (Fig. 1c), ductus bursae with bursa copulatrix very long, spanning the entire abdomen up to thorax, no signa, ductus gradually thickening towards bursa; ductus bursae ventrally sclerotized by sterigma, ductus seminalis connecting near sterigma, around the sclerotized area; sterigma expanded, nearly rectangular with concave sides in ventral view and on each side with a lateral ridge rounded caudad and protruding past lamella antevaginalis; lamella postvaginalis notched in the middle, on the sides connected through the expanded ridge to mostly convex, straight in the middle lamella antevaginalis.

Type species.—*Gemmia buechei* Brockmann and Grishin, **new species.**

Species included in the genus.—Only the type species.

Parent taxon for the genus.—Subtribe Moncina A. Warren, 2008.

Diagnosis of the new species.—This mostly brown species is identified by nine or ten pearly spots on the ventral hindwing outlining an inverted heart pattern on a rusty-colored background.

Description of the new species.—Female: (n=1, Fig. 1a,b) right forewing length (wing base to apex) 19 mm in holotype. Palpi missing in the holotype. Fringes brown. Dorsally, wings dark chocolate brown, forewing with traces of pale subapical spots, costal area of hindwing paler. Ventrally, rust-brown to chestnut colored, with darker, nearly black-brown discal forewing area and paler, yellowish submarginal areas of wing and planer brown areas towards inner margins of both wings (except hindwing cell 3A rust-brown); forewing with 3 subapical pearly spots in a straight line and thickening from costa towards outer margin from a dash to nearly round, and a faint spot (patch of pearly scales) in the middle of cell M_3-CuA_1; hindwing with ten large, mostly elongated pearly spots arranged to outline an inverted heart pattern: triplet of spots towards base, weakest (nearly missing on left hindwing) in cell CuA_2-1A+2A, and heptad in postdiscal area arranged to form “3” on left wing, pattern alternatively described as two spot (one near base and one in outer half) in each of cells Sc+R_1-RS and CuA_2-1A+2A, and one spot in each of cells: discal (in distal, posterior area), RS-M_1, M_1-M_2, M_2-M_3, M_3-CuA_1, and CuA_1-CuA_2. For genitalia description (Fig. 1c) see description of the genus above, because we hypothesize that they would be mostly similar for all species in this genus. Male: unknown or unrecognized.

Barcode.—The COI barcode sequence of the holotype (sample NVG-18066C02, GenBank accession ON256154, 658 base pairs) is:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATATTAGGAACATCCTTAAGTTTATTAATTCGTACAGAATTAG
GAAATCCAGGTTCTTTAATTGGCGATGATCAAATTTATAATACTATCGTAACAGCTCATGCATTTATTATAATTTTT
TTTATAGTAATACCTATTATAATTGGGGGATTTGGTAATTGATTAGTTCCATTAATATTAGGAGCCCCTGATATAGC
TTTCCCCCGAATAAATAACATAAGATTTTGAATATTACCCCCATCCTTAATATTATTAATCTCAAGAAGAATTGTAG
AAAATGGTGCAGGAACAGGATGAACTGTATATCCCCCTCTTTCCTCTAATATTGCTCATCAAGGAGCATCTGTTGAC
TTAGCAATTTTTTCCTTACATTTAGCTGGAATTTCTTCTATTTTAGGAGCTATTAACTTTATTACTACAATTATCAA
TATACGAATTAGAAATTTAGCATTCGATCAAATACCTTTATTTGTCTGATCAGTAGGTATTACTGCATTATTATTAC
TTTTATCTTTACCTGTATTAGCTGGAGCTATTACTATATTATTAACTGATCGAAATTTAAATACATCATTTTTTGAT
CCTGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

Types.—Holotype ♀ (Fig. 1). presently in the research collection of Ernst Brockmann (Lich, Germany), to be deposited in the collection of the Museo de Historia Natural, Universidad Mayor de San Marcos, Lima, Peru, bears the following three rectangular labels: white printed and hand-printed [Peru IV.2012 | Dept. San Martin | 1500-1800 m | ex coll. Michael Büche], white printed [DNA sample ID: | NVG-18066C02 | c/o Nick V. Grishin], red printed [HOLOTYPE ♀ | *Gemmia buechei* | Brockmann & Grishin].

Type locality.—Peru: San Martin, elevation 1500–1800 m.

Etymology.—The genus name *Gemmia* is a feminine noun in the nominative singular, given for the gem-like pearly spots on the ventral hindwing of the type species. The specific epithet *buechei* is named to honor its collector, Michael Büche, who generously gave many interesting specimens to the first author. The species epithet is a noun in the genitive case.

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Appendix 1

DNA sequences of *Lerema accius* (J. E. Smith, 1797) exons with diagnostic characters of *Gemmia* Brockmann and Grishin, **new genus**. Sequences of *Lerema accius* nuclear exons (NCBI genomic assembly accession GCA_001278395.1) (Cong et al. 2015) with diagnostic characters for *Gemmia* are provided below. The position used as a character state is shown in bold italic font. Base pair in this position is the one present in the *L. accius* reference genome, and may not correspond to the actual base pair in *Gemmia*. The characters are given as abbreviations in the lines starting from the > symbol, e.g., lac704.5.28:T96C means position 96 in exon 28 of gene 5 from scaffold 704 of the *L. accius* reference genome is C, changed from T in the ancestor; (not G) means that this position is not G in *Gemmia*. If the function of a corresponding protein is known or can be predicted, it is stated after the | symbol.

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>lac704.5.28:T96C | Predicted: Down syndrome cell adhesion molecule-like
protein Dscam2

TGTTACTTCGAATTTTCTTTCTGTTTATTTAACCGTGGTTCTTTTACGTACCGATCTTTTATTCCAGTCCCCCCAAA
CATTCTGCCATTTTCGTT***T***GGCGAAAAACCAGCAAATGTTGGCGAATATTTACAAGCATCGTGCACCGTTAATCTCG
GTGATCTTCCGCTTACGATCGCTTGGACGTTCAATGGGCACTTGATATCACAACGAAACAGTGACTATTCTATGAGC
AACTCTAAAAGAAGTAGCCTCCTTATAATCGAATCGGTCGACGCTAAACACGCTGGTTCCTATACTTGCGTCGGTGA
AAATCGCGCGGGAAAATCGACATACTCCTCGGATTTAGTT

>lac7147.4.1:C46A

ATGACAACGAAAGTAAAGAAAATCGTTGGAAATGATGCATTCCAA***C***GAATTAACTTCCTATATCAG

>lac884.3.3:A590T | uncharacterized protein LOC101737811 isoform X1

ACAATCCAACGAATGTCTTCCACGAACGTTGTTATTAGGATATGGTGTCACGTCCTCACTTCACCGTGTAAGGATGG
CGAGGACAAGTGTGATAGGGTCCAAGACGAAATTATTCAGACTTGGGGAGTGTATTTTAGCGGTTTGAGGTACATTG
GGGCCAACCTCGCGTTAAACTTCACGTCAGATTGCTTCAAGAGCAACACGCTGGTTTTCCAAATGCACGGCGGATAC
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TTCTGCTCGTACAAAAGCCTTAAATTAGACATTATACCACCAGAAAATGAAATAGAACATGGATCTCTGTCTTCGGA
CTCTTCCGGAAAAACTCACGTGTCAAAAATAACATTAGAGTCTCGCGTTTTGCAAAACTACAGACATTTCAAAGAAG
GAAGGTCTATATCACCTAGTAAAATTTCAAGGAAGTCGGATAAGGAGTATTCTAAAAGCCATAGTCCAGTGGAGAAA
GGCTTTAAACAGAACTTCCCAAGTTTAAAAGCTTCTGCGAGTTTGAACATGGCATTGAATCATAGGGAAGGCGTAAG
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TTAAAGAATTCACGACCAGTGATGAAAACTGTGATATATCAAAAATCGAGTCACAAGAGGAATTATCCGCCAGCAAA
ATAGACTTAAGTGAAACCGCAGAGAGTTACGATTTGAGAAACGAATCTGATGGAATGCCTAAATATCGGTACTTGGC
GTTGAGTTTTCTTAAATCGGAAATCCGGCCAAGTTACAACGCGTCCAAGCTGCAGAGCTTGCATTTGCTTCAATACT
CAATAAAGAAGAAGCAAGAAGCAGTCCAAGAGATTAAAGAAAGAATCTATCAGAAGTCTGCTTTAGCGAATGAGTCT
TGGACTTTACCGGAGAATGCTTTCGAAATCAAACACAAGTCCAGGAGTAGATCTCAGAGGAATCTTTTCTCGCCGGA
AGAGAATGAAAGGTTTCAGAAGGAACGGTCGTCGTCTCAGAGTGATTTGAGCAAGAATGGAAGAATCAAAGATGACA
AAACACCCATTTCGAATGGTCCAAGACTCACTTTAAAGTTGAACGATCTGTTGTCTTAC

>lac221.9.2:A233A (not G) | protein rhomboid

ATGGAGCAATTAAACGAAGAAGATGGATCGCCGGTGGCGGTGCATCAGGGGAGAAGAATCCTCCCGGACGTGGTGCC
GCCGGCCCGGGCCTGGGTGGAGCACGAGGAGTCGGAGCGACTGACCGATAGCAGGACGAACAGTCCCGGGGAGAACA
GGCGGCTGCTCAGCCCCGACGTCCACAAACAGACTCGTCTCCAAGCTGGACTCACTTTAGGAGTCAAGGATATAAAG
A***A***ACATACGAAATGTCCTCAAATTGCGAAAAAAGTGAGACCTACAGTGAAATCGAAGTTCCAGAAAAGGAAGGAGAA
ACTTATCGAAATTCTAAAGCCGCCTTACTTCATCATCACCATGATAATAATTATA

>lac886.16.8:C464C (not A) | uncharacterized protein LOC101745102

GTTGATTCTTTTACCGTGAACCAAAAACTAAGTGGTAATTCGTCGTGCATGAAAGTATACGACGTGTCTCAACATAG
ATCAACTCATACAAGCCAACATTCTGAACCAATGAGTACATCCTCTACTGAAAACCAAACTTCTTCAGACAAAAGCC
ACCCAAAGTCATTTAATTTGGTAAAACTATTTATGAAACAGAAAAGTATTAGTAATGACGGAATTCATGGTATGGAT
CAATTAGATAGATCGGAATGTTGGCCTTCTAGTTCAGGTGGTGAGAGTGGTGAATCAATGGGAGAGCAAAAACTTGG
GGACTCAAAATCTATATCAACAGATAGACCCCAAGCCGAATTACCATTAGATGCTGATTATAATAGCTCGGCTGTCT
ATGATGAAGTCCGATCAGTTAATCCATTTAATAGAGTATACGACGAAGTTATTGAAGAAGAAGACAATCTTGAAACG
A
CCAAATTAAGCGGCAGCGAAACGAATTTATATGCTGTTGTTAACAAACCGCACATTAAAAGAGCCAATATAAATATAAATAACAGCCCATGTAAAA

>lac49.46.10:G109G (not A) | importin subunit alpha-7

GGTCTCGACAAAATAGAATTCCTGCAGTCGCATGAGAACCTTGATATATATCAGAAATCATTTGAAATAATTGAGAA
TTACTTTGGATCGGAAGGAGAGGATAATAGA***G***TAGCGCCCGAAGTGTCTTCCACAACTGATCAGTACCAATTTAACA
CCGACCAATCAGTGCCTATGGGTGGTTACCAGTTC

>lac357.30.3:G1355G (not A) | A-kinase anchor protein 10, mitochondrial
isoform X1

CAACCGAACATATTTGAAAAGAAATCTAAATTGTCTCTAACGTTACATGACATACTTGTTGACAAAAAGTCTATTAA
GTATTTTATCTTATTTATGGAAACAATTGGTAAGCAATTGTTAATAAGATGTTGGATGGAACTAGACAATTTTAAGA
TGCAAGTTTTATTTAATAGTAAGTTAAATGAAGATGATAAGCGTCTACAGTTAAGTCGTACCAAAAGCTTTGATGTT
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CCCAATAAATTAATATTACCCGATGAGCTGAGGAAGTCAGTGATAAGTAATATATGTCAACCAGAAGGTCAAATAAA
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GAAGAAGAGATGGCATGGCAGATAGCACATATGATTGTTAAAGATGTAACTGACTTAACCATGGCACCTCCTGATAA
TGAAAGTGATGATGTA

>lac1895.5.35:T37T (not A) | Down syndrome cell adhesion molecule-like protein Dscam2

AACTATCCGTCGTTCAACGATCACATTCATCCGCCG***T***CGCGATTCTCCGACGACACGGAGTGCGAGGGTCACCATCG
GCGGAAACGCAGCGGTCACGCGGCCCACGCGGCTCACTTGGCCCACTCGGCCCACTCCGAGCCTAAAGTGACTCGCG
AAGCCTTCCAAATCAACGTC

>lac1416.2.1:A449A (not G) | mRNA cap guanine-N7 methyltransferase

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AGACTGAACATCAACGCTTAGGTACTATGAGCAAGAGTGAATGGGAAGTTGCA

>lac580.72.1:T75T (not C) | glycylpeptide N-tetradecanoyltransferase 2

ATGGAAGATAATAAGGCAAACCAACCCGGCGGATATCAAAATACACATGAAGATAAACAGCCAAAGAGGAAAAA
TAAAAACAAGAAAAAGCGAAGTGGCGGGGACGGGGACATTGAGGGTTCGGGGTCAGACCTAAAAACGGACATTGTAACCATTCCTACTGGTGATAT

>lac3441.2.1:A436A (not G) | cap-specific mRNA (nucleoside-2′-O-)-methyltransferase 2

ATGTTTCATAAACCAGATAGTTATTTCCGACAGAAATATTATAAAGAAGATTTTCAGGACGAGCTGAACGACTTATT
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GTTCCTGACACAAGCCTGGACCAAATTCTATGAATGCGCTTGCACATACAATGTTGTGCCACTGGAAGCTATTGCAG
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>lac886.16.8:T1215T (not C) | uncharacterized protein LOC101745102

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ACTGCCAAAATATGGATGAATGGGATCAAAATCAAGTA

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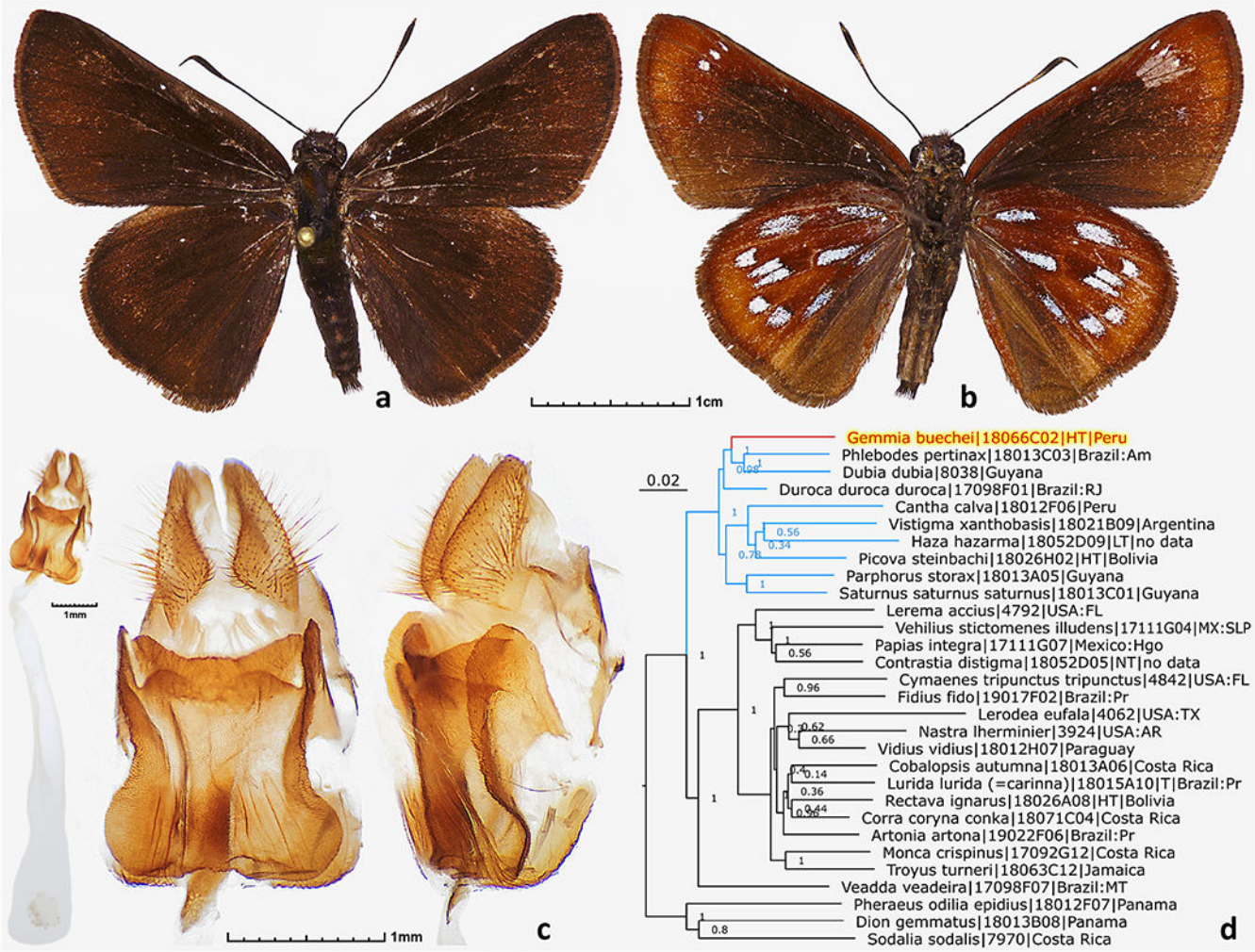

Figure 1.

The holotype and phylogenetic placement of *Gemmia buechei* Brockmann and Grishin, gen. n., sp. n. **a**) Dorsal and **b**) Ventral views of the holotype, female, NVG-18066C02, from Peru: San Martin, additional data are in the text. **c**) Genitalia in ventral view, enlarged sterigma in ventral and right ventrolateral views: left, middle and right, respectively. **d**) Phylogenetic tree constructed from protein-coding regions of the Z chromosome: statistical support values (bootstrap fractions) are shown by the nodes, numbers after species names refer to DNA samples. Scale bars are shown by the images. For the tree, the unit is the estimated number of base pair substitutions per position (i.e., the scale bar corresponds to 2 substitutions per 100 positions). We sequenced a syntype of *Megistias carinna* Schaus, 1902, a name currently considered a junior subjective synonym of *Lurida lurida* (Herrich-Schäffer, 1869), thus the notation *Lurida lurida (=carinna)*.