Hiding Behind Gaudy Looks, a New Central American Species of Phareas (Hesperiidae: Eudaminae)

Authors: Grishin, Nick V., Janzen, Daniel H., and Hallwachs, Winnie

Source: The Journal of the Lepidopterists' Society, 67(3) : 161-174

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.v67i3.a3

Journal of the Lepidopterists' Society
67(3), 2013, 161–174

HIDING BEHIND GAUDY LOOKS, A NEW CENTRAL AMERICAN SPECIES OF *PHAREAS* (HESPERIIDAE: EUDAMINAE)

NICK V. GRISHIN

Howard Hughes Medical Institute and Departments of Biophysics and Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, USA 75390-9050; email: grishin@chop.swmed.edu

DANIEL H. JANZEN

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; email: djanzen@sas.upenn.edu

AND

WINNIE HALLWACHS

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; email: whallwac@sas.upenn.edu

ABSTRACT. A new species of *Phareas* is described from Costa Rica and Panama. *Phareas burnsi* Grishin, **sp. nov.** differs from its South American sister species, *Phareas coeleste* Westwood, 1852, by wing patterns, which were quantified and revealed two clusters with a profound hiatus between them; male secondary sexual characters on hindwing; female genitalia, mostly in the depth of distal notch on lamella postvaginalis; and mitochondrial DNA sequences showing about 4.5% divergence in the COI barcode. Unexpectedly large individual variation in the shape of the *Phareas* male genitalic valvae is illustrated.

Additional key words: Area de Conservación Guanacaste, biodiversity, caterpillars, cryptic species, skipper butterflies

A question of what constitutes a species and how to define boundaries between species is in the core of biological sciences. Insects, and butterflies in particular, are valuable model organisms to address such problems due to their easily observed diversity of shapes and patterns. Recent advances in molecular techniques enable researchers to add molecules into the mix of characters used in delineation of species. Comparisons of short, 654 nucleotide sequences of mitochondrial DNA encoding the C-terminal segment of cytochrome oxidase subunit 1 (COI), dubbed DNA barcodes, were able to flush out a number of unsuspected cryptic species in many groups of organisms, but in particular in the Eudaminae, a subfamily of skipper butterflies (Hesperiidae) (Janzen et al. 2011). When combined with traditionally used characters such as wing patterns and genitalia, DNA barcodes have been shown to be a useful taxonomic tool for detecting cryptic species, as well as for straight identification of them (Janzen et al. 2009).

Species differ in their ranges and variability. Some Eudaminae are known from both Americas and maintain constant phenotypes and barcodes throughout, e.g. *Urbanus dorantes dorantes* (Stoll 1790) has been recorded from USA to Argentina, and barcode sequences are identical in specimens from Arizona and Paraguay, as may be seen from the BOLD database (www.boldsystems.org, Ratnasingham & Hebert 2007). Others have more limited distributions and elevated diversity in barcodes, e.g. *Astraptes fulgerator* (Walch 1775) group taxa, which all look very similar and may not even be consistently separable by any adult characters except their DNA barcodes; they are, however, readily separable by the combination of their caterpillar color patterns and food plants (Hebert et al. 2004). There are all imaginable cases in between these two extremes. Thus, Central and South American skippers that look similar may in fact be distinct biological species and should be carefully examined. Many neotropical skippers have been described from South American specimens, mostly from Suriname and Brazil (e.g. Cramer 1775–1780, Westwood 1852), thus leaving their Central American and North American sibling species without names.

Here, we analyze one of the most beautiful or gaudy skippers, depending on your viewpoint. The well-known neotropical *Phareas coeleste* Westwood, 1852 displays a white smiley face on its metallic blue forewing and an astonishingly different ventral hindwing that is orange bordered with black shining purple. Armed with a dozen Central American specimens reared from Area de Conservación Guanacaste (Costa Rica) and backed up by the analysis of wing shapes and patterns, genitalia and barcodes, we conclude that the Costa Rican and Panamanian populations are an undescribed species, distinct from South American *P. coeleste*.

MATERIALS AND METHODS

Adult specimens used in this study are in the following collections: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); Museum für Naturkunde, Berlin, Germany (ZMHB); and Natural History Museum, London, UK (BMNH). Standard entomological techniques were used for dissection (Robbins 1991), i.e. the adult abdomen was soaked for 24 hours in 10% KOH at room temperature, dissected and subsequently stored in a small glycerol vial pinned under the specimen. Genitalia and wing venation terminology follow Klots (1970) and Comstock (1918), respectively. Length measurements are in metric units and were made from photographs of specimens taken with a scale and magnified on a computer screen. Photographs of whole specimens were taken with Nikon D200 camera through a 105mm f/2.8G AF-S VR Micro- Nikkor lens; genitalia were photographed through a microscope. DNA sequences were downloaded from GenBank http://www.genbank.gov/, aligned by hand since insertions or deletions were absent, and analyzed using the Phylogeny.fr server at http://www.phylogeny.fr/ with default parameters (Dereeper et al. 2008). The majority of these DNA barcodes have been reported in Janzen et al. (2011) and photos and collateral data of the reared Costa Rican specimens are available from the on-line database (Janzen & Hallwachs 2012) for the caterpillar inventory of Area de Conservación Guanacaste (ACG) and the BOLD database (Ratnasingham & Hebert 2007), to confirm identifications.

RESULTS AND DISCUSSION

Mass rearing and collecting of ACG moths, butterflies and their caterpillars (Janzen et al. 2011) has produced rich datasets of specimens and their DNA barcode sequences. Many of these species are very rare in collections made from net-caught or trap-caught free-flying adults. A number of common South American species were recorded from Costa Rica, or sizable Central American series of them were obtained for the first time. These allow meaningful comparison and analysis. Close inspection of large series of specimens and their DNA barcodes often leads to the discovery of cryptic sibling species hiding within series of named well-known, brightly colored and uniquely recognizable phenotypes (Janzen et al. 2009: 20-21).

Phareas coeleste described by indication (illustration only, text followed a couple of months later) by Westwood (1852) from a single female (Figs. 5, 21) is unmistakable in its appearance. In fact, it is so unique and distinctive, that no synonyms have been coined for it by subsequent researchers, not considering misspellings, and it has been mostly residing in a monotypic genus (either *Phareas* Westwood, 1852, or its junior objective synonym *Grynopsis* Watson, 1893). *Phareas coeleste* is widespread in South America, and recorded from Colombia to Bolivia and Brazil (Evans 1952). A series of several dozen South American specimens is curated in the Natural History Museum, London (BMNH), but there are no specimens north of Colombia. Very few collections house *Phareas* sp. from Costa Rica and Panama. Not counting ACG reared material, there are four Panamanian specimens (1 male and 3 females, curated as *P. coeleste*) in USNM and one Costa Rican specimen in Ichiro Nakamura collection. These numbers are so few, and the looks of *Phareas* are so inimitable that it might be hard to imagine that there is a species different from *P. coeleste* in this genus.

Rearing caterpillars by the ACG inventory produced a series of this skipper, totaling 12 adult specimens, which were compared to Panamanian and South American specimens. Interesting wing shape and pattern similarities to Panamanian specimens, and differences from South American specimens prompted further investigation, which, being supported by morphometric analysis of wing patterns and comparison of DNA barcodes and genitalia, led to the conclusion that Central American specimens represented a species very similar to, but consistently distinct from *P. coeleste*. This undescribed species is named here.

Phareas burnsi Grishin, **new species**

(Figs. 1–4, 9–12, 17–20, 25–28, 33 part, 34a–c, 35a–k, 36a–d,i–q, 37a–r, 38 part)

Description: *Female* (n=11, Figs. 1–4, 17–20) – holotype forewing length 28 mm (paratypes 27 to 29 mm); wings broad and rounded, forewing termen slightly convex, hindwing almost elliptical in shape, with a broad but shallow lobe in the posterior half from just before vein M_3 to tornus, termen scalloped at veins anteriad of the lobe; dorsal forewing metallic dark teal blue with purple gloss at the base and apex, six white partly opaque spots: discal cell spot quadrate, slightly smaller than Cu_1–Cu_2 cell, almost pentagonal spot near the base of Cu_1, elongated oval, medially constrained spot by the tornus in Cu_2–2A cell, elongated rhomboid-shaped spot from the base of M_3 vein to the middle of Cu_1 vein, a triplet of smaller subapical spots in R_5–M_1, M_1–M_2 and M_2–M_3 cells, central spot offset distad, and a doublet of aligned small spots in R_3–R_4 and R_4–R_5 cells; dorsal hindwing monochrome metallic dark teal blue with purple sheen; ventral forewing darker shade of blue than dorsal side, brown-gray by the base, basal half of Cu_2–2A cell and 2A cell, slightly overscaled with orange near the base towards costa, dorsal pattern of spots repeated; ventral hindwing orange with broad dark purple blue marginal band narrowing from costa to the middle of Cu_1–Cu_2 cell, stair-step edges of the band along veins $Sc+R_1$, Rs, and M_1 within basal 1/3 of these cells, edge continuous posteriad, bleeding blue scales into orange areas, dark scales along and posteriad of vein 2A and marginally along veins Cu_1 and Cu_2, at the base of humeral lobe dark area diffuse, not forming a defined spot; fringes dark brown above and below, forewing with some white scales between the veins above, and longer narrow

areas of white scales below, hindwing with three white areas between veins $Sc+R_1$, Rs, M_1 and M_3, a speckle of dark scales next to M_2 and some white scales below along the margin between veins within the lobe; head dark-brown above, ridges of pale scales near palpi and near collar, white spots above and behind the eyes, below white, narrowly dark brown posteriad of white cheeks, collar dark-brown, eyes brown, palpi with the third segment stout and spatulate, placed near the outer edge of the second segment, palpi brown with patches of pale scales above, white centrally below and on the sides; antennae dark brown, near the club pale orange laterally posteriad and ventrally half-ringed with pale orange along distal segment edges, nudum paler brown, 22–25 segments (n=5); thorax dark brown above, orange below; legs mostly orange with darker scales dorsally, more at the bases, hind tibiae with 1 pair of spurs; abdomen dark-brown above, cream to pale orange centrally below. *Female genitalia* (Fig. 36a–d, i–q): lamella postvaginalis broader than long, distal margin with a central deep triangular notch, almost straight, very slightly concave on both sides of the notch, lamella bulged anteriad of the notch, the notch as deep as the anterioposterior bulge length; lamella antevaginalis very narrow, fully sclerotized, margin undulated; antrum sclerotized, matching the notch length in diameter; ductus with corpus bursae about 2.5 times sterigma width.

Male (n=6, Figs. 9–12, 25–28, 34a–c) – forewing lengths 26 to 28 mm, similar to female, nudum comprised of 22–24 segments (n=5), but with costal fold on forewing for about half of its length and a shallow fold along 2A vein for most of its length on hindwing, scales inside the fold brown, concolorous with the background, tufts of dark hair-like scales along the sides of 2A hindwing fold and inside it, and on sides of abdomen; white spot in Cu_2–2A more elongated basad, its basal end reaches the level of the middle of the Cu_1–Cu_2 white spot. Pecten on hindleg tarsus, concolorous with leg scales. *Male genitalia* (Fig. 35a–k): tegumen a bit longer than wide, rounded; uncus slightly shorter than tegumen, undivided, beak-like, terminally bifid in lateral view, with 2 small side lobules; gnathos widely separated from uncus, prominently sculptured; saccus triangular in ventral view, as long as wide; valva without processes and projections, cucullus bending inwards dorsad and expanding into a broad tooth directed anteriodorsad and a lobe directed posteriad, the lobe mostly short, equal to the tooth in length, but longer in some specimens, cucullus abnormally underdeveloped in one specimen (Fig. 35k, voucher code 05-SRNP-30469); aedeagus slightly longer than tegumen together with uncus, but shorter than valva, with a row of medium-sized (length about equal to aedeagus diameter) cornuti.

Barcode sequence of the holotype: GenBank accession GU149831, 658 bp :

AACTTTATATTTTATTTTTGGAATTTGAGCTGGAATATTAGG
TACTTCATTAAGATTACTAATTCGAACAGAATTGGGAACCCCA
GGATCTTTAATTGGAGATGACCAAATTTATAATACAATTGTAAC
AGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATA
ATTGGAGGATTTGGAAATTGACTTGTTCCTTTAATATTAGGTG
CCCCCGATATAGCCTTCCCACGAATAAACAACATAAGTTTTTG
ATTACTACCCCCATCATTAACTTTATTAATTTCTAGAAGTATTGT
AGAAAATGGTGCAGGAACTGGATGAACAGTATATCCCCCTTTA
TCAGCAAACATTGCACACCAAGGATCATCTGTAGATTTAGCAA
TTTTCTCTTTACATTTAGCAGGAATTTCATCTATTTTAGGAGCT
ATTAATTTTATTACTACAATTATTAATATACGAATTAGAAATTTAT
CCTTTGATCAAATACCTTTATTTGTTTGAGCAGTAGGAATTACA
GCATTATTACTTCTTCTATCTCTTCCAGTTTTAGCTGGAGCTAT
TACTATATTACTTACTGATCGAAATTTAAATACATCATTCTTTGA
TCCAGCAGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

Types: *Holotype* female has the following labels: white printed & hand-printed - / Voucher: D.H.Janzen & W.Hallwachs / DB: http://janzen.sas.upenn.edu / Area de Conservación Guanacaste, / COSTA RICA. / 05-SRNP-30644 /; yellow printed - / LEGS AWAY / FOR DNA /; red printed - /HOLOTYPE ♀ / *Phareas burnsi* / Grishin /. Holotype data: Costa Rica: Guanacaste Province, Area de Conservación Guanacaste, Sector Pitilla, Pasmompa, 11.01926° -85.40997°, 440m, collected on 14-II-2005 as first instar feeding on buds and young leaves of a sapling of the rain forest tree *Ormosia coccinea* (Fabaceae) by the parataxonomist Manuel Ríos, caterpillar prepupal date: 16-III-2005, adult eclosion date: 07-IV-2005. Paratypes: 6♂♂ and 10♀♀. **Costa Rica:** 1♀ Limón Prov., Tortuguero, 2 km N of the village, 10-V-2005; Guanacaste Prov., Area de Conservación Guanacaste, Sector Pitilla: 1♂1♀ Pasmompa, 11.01926° -85.40997°, 440m, reared in 1999, food plant *O. coccinea*, voucher codes 99-CALI-790 and 99-CALI-787, respectively; 1♂ Pasmompa, 11.01926° -85.40997°, 440m, collected on 05-II-2005 as antepenultimate instar, adult eclosed on 27-III-2005, food plant *O. coccinea*, voucher code 05-SRNP-30469; 1♂ Pasmompa, 11.01926° -85.40997°, 440m, collected on 05-II-2005 as instar before antepenultimate, adult eclosed on 20-III-2005, food plant *O. coccinea*, voucher code 05-SRNP-30577; 2♂♂ Estacion Pitilla, 10.98931° -85.42581°, 675m, reared in 1999, voucher codes 02-SRNP-35231 and 02-SRNP-35232; 1♀ Pasmompa, 11.01926° -85.40997°, 440m, collected on 14-II-2005 as penultimate instar, adult eclosed on 17-III-2005, food plant *O. coccinea*, voucher code 05-SRNP-30643; 1♀ Pasmompa, 11.01926° -85.40997°, 440m, collected on 05-II-2005 as last instar, adult eclosed on 10-III-2005, food plant *O. coccinea*, voucher code 05-SRNP-30576; 1♀ Pasmompa, 11.01926° -85.40997°, 440m, collected on 14-II-2005 as first instar, adult eclosed on 31-III-2005, food plant *O. coccinea*, voucher code 05-SRNP-30642; 1♀ Sendero Evangelista, 10.98680° -85.42083°, 660m, collected on 27-III-2004 as last instar, adult eclosed on 24-IV-2004, food plant *O. panamensis*, voucher code 04-SRNP-31520; 1♀ Sendero Cuestona, 10.99455° -85.41461°, 640m, collected on 09-XI-2004 as last instar, adult eclosed on 12-XII-2004, food plant *O. coccinea*, voucher code 04-SRNP-56226. **Panama:** 1♂ Canal Zone, Summit, 2-V-1964, genitalia NVG120207-05; 1♀ Canal Zone, Gamboa, X-1968; 1♀ Canal Zone, Gamboa, 9° 07'N 79° 41'W, 28-X-1978, leg. G. B. Small, genitalia NVG120207-06; 1♀ Darién Province, Cerro Pirre, ca. 0m, 15-IV-1976, leg. G. B. Small. Additional data for ACG specimens (voucher codes with -SRNP-) are at http://janzen.sas.upenn.edu/caterpillars/database.lasso (Janzen & Hallwachs 2012).

Deposition of types: Holotype is in the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). Two paratypes are depoisted in the Natural History Museum, London, UK (BMNH) (02-SRNP-35231 & 05-SRNP-30642), two paratypes are deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL (MGCL) (02-SRNP-35232 & 04-SRNP-56226), one paratype from Costa Rica: Limón Province is in the Ichiro Nakamura collection, and all other paratypes remain in USNM.

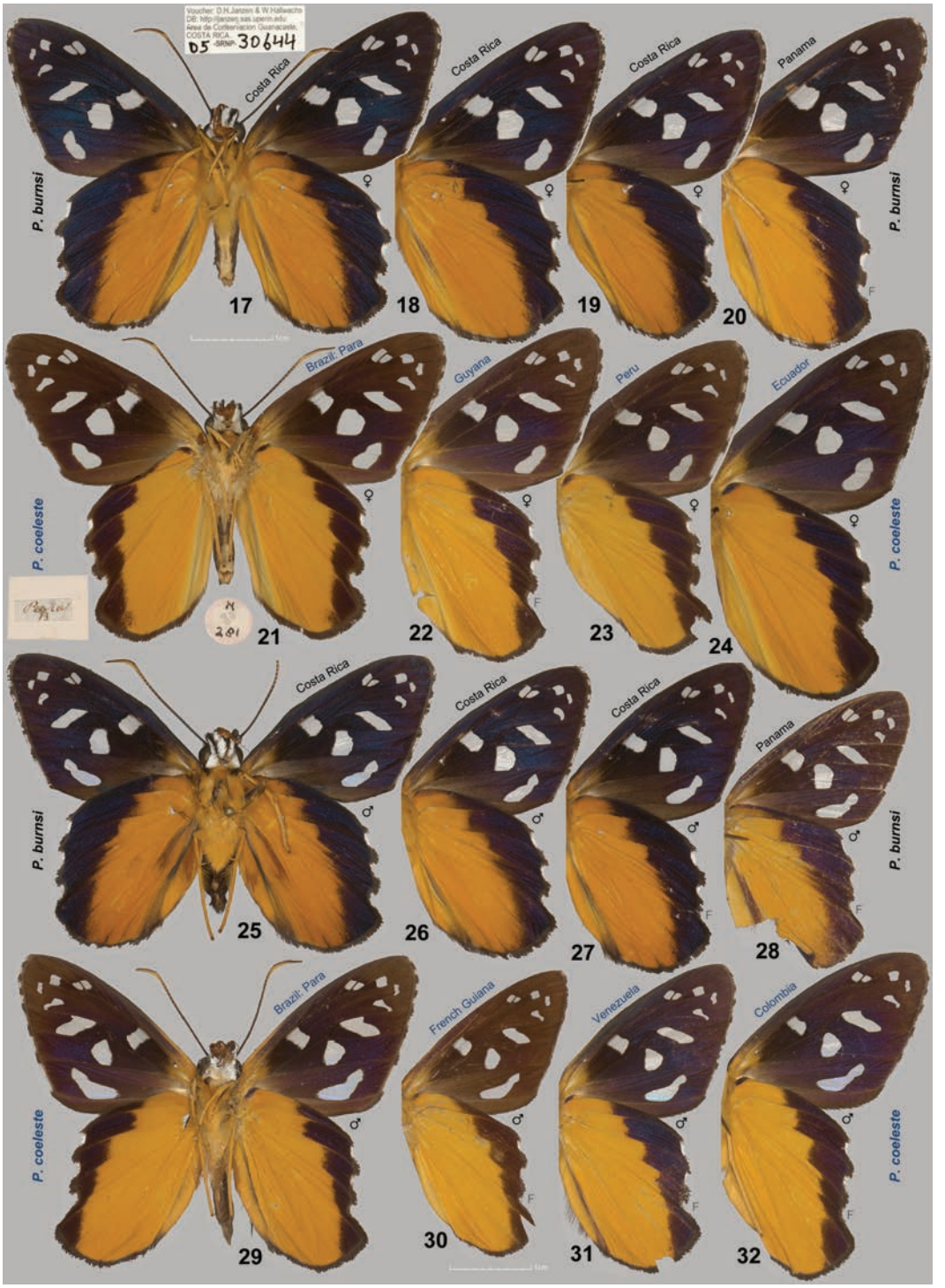
Type locality: COSTA RICA: Guanacaste Province, Area de Conservación Guanacaste, Sector Pitilla, Pasmompa, 11.01926° -85.40997°, 440m.

Etymology: *Phareas burnsi* is named in honor of Dr. John M. Burns, Curator of Lepidoptera (emeritus) Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, who has identified and curated over 17,000 reared and DNA barcoded ACG inventory specimens of Hesperiidae in which this species and these specimens are embedded. John's extraordinary skill of combining science with poetry to craft a flowing prose of entertaining taxonomic texts ("entertaining" and "taxonomic" could be sympatric!) remains unmatched and can hardly, if ever, be repeated. His meticulous attention to details, keen eye for the differences and hard, diligent work are behind a myriad of exciting discoveries about skippers, their genitalia and evolution, many of which are yet to come. The name is a masculine noun in the genitive case.

Distribution and phenology: Currently, the species is known from Costa Rica and Panama. In Costa Rica, it has been collected in May and reared to eclose in March, April and December (Janzen & Hallwachs 2012). In Panama, collection dates are from April, May and October.

Diagnosis: The new species belongs to *Phareas* as defined by Evans (1952) because: (a) it possesses palpi with the third segment being stout and spatulate and positioned near the outer edge of the second segment, not in the center of it; (b) antennae are bent at the

FIGS. 1-32. Adults. 1–16. dorsal views and **17–32.** ventral views of the same specimens. **1–4, 17–20.** *P. burnsi* ♀♀, **9–12, 25–28.** *P. burnsi* ♂♂; **5–8, 21–24.** *P. coeleste* ♀♀, **13–16, 29–32.** *P. coeleste* ♂♂; **1, 17 & 5, 21.** are holotypes of *P. burnsi* (voucher code 05-SRNP-30644, data in text) & *P. coeleste* (Brazil: Pará, Hewitson collection, specimen number BMNH(E) #808437), respectively; **2–4, 18–20, 9–12, 25–28.** paratypes. All specimens in USNM collection, except as indicated in brackets, and except *P. coeleste* holotype (**5, 21**.) which is in BMNH; copyright of its photos: Trustees of the Natural History Museum, London; used with permission. Voucher codes for Costa Rica, ACG paratypes: **2, 18.** 04-SRNP-56226 [MGCL]; **3, 19.** 05-SRNP-30576; **9, 25.** 05-SRNP-30577; **10, 26.** 02-SRNP-35232 [MGCL]; **11, 27.** 05-SRNP-30469; data in text. Data for others: **4, 20.** Panama: Canal Zone, Gamboa, X-1968; **6, 22.** Guyana: Acarai Mountains, Sipu River, 1° 21.3'N 58° 57.4'W 2000–2500' {4–10}-XI-2000, leg. S. Fratello et al. USNM ENT 00179859; **7, 23.** Peru: near Iquitos, 23-I-1932;



FIGS. 1-32 (continued from previous page). 8, 24. Ecuador: Sucumbíos, ridge between Río Ushaue and Río Puchuchoa, km. 4 Lumbaquí - La Bonita rd., 0° 05'N 77° 17'W, 850m, {12–14}-III-2004, leg. I. Aldas; **12, 28.** Panama: Canal Zone, Summit, 2-V-1964, genitalia NVG120207-05 (genitalia Fig. 35g); **13, 29.** Brazil: Pará, Belem, IV-1960, leg. J. Kesselring, genitalia NVG120207-18; **14, 30.** French Guiana: St. Jean, Maroni, IV-1904, collection Wm. Schaus (genitalia Figs 35t); **15, 31.** Venezuela: T. F. Amaz. Cerro de la Neblina Basecamp, 0° 50'N 66° 10'W, 140m, 26-I-1985, at black light on bank of Rio Baria, leg. P. J. & P. M. Spangler, R. A. Faitoute & W. E. Steiner; **16, 32.** Colombia: Caquetá Department, Florencia, 1300', 22-I-1971, leg. S. S. Nicolay (genitalia Fig. 35q). F to the right of the tornal lobe indicates mirror image (left-right inverted). Labels for primary type specimens (**1, 5, 17, 21**) are shown near each specimen. Species names for each row, general locations and sexes are shown. All images are to scale except labels of *P. burnsi* holotype, which are 0.75 times the scale (reduced).

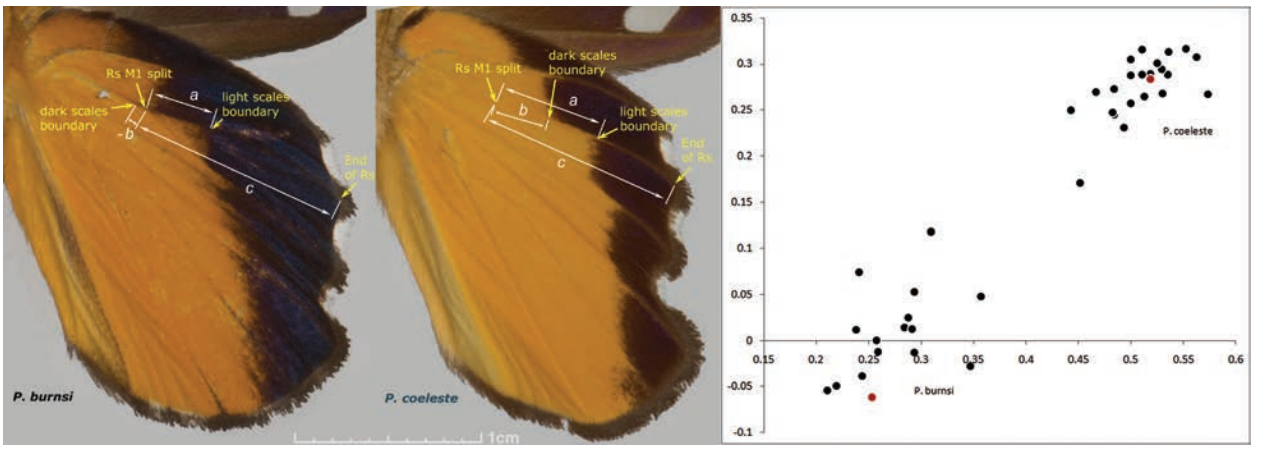


FIG. 33. Measurements used to distinguish *P. burnsi* and *P. coeleste*. Hindwings ventrals of *P. burnsi* holotype (left) and *P. coeleste* holotype (right) are shown. Distances measured are: ***a*** – between the split of Rs and M_1 veins and the boundary of light scales in Rs – M_1 cell along Rs vein; ***b*** – between the split of Rs and M_1 veins and the boundary of dark scales in $Sc+R_1$ – Rs cell along Rs vein; ***c*** – between the split of Rs and M_1 veins and the distal end of Rs vein. Negative values of b correspond to dark scales intruding basally of the Rs and M_1 split. **Morphometric differences** between *P. burnsi* (lower left cluster of points) and *P. coeleste* (upper right cluster of points) are shown on the graph. Horizontal axis is the ratio ***a/c*** of the distance between the split of Rs and M_1 veins and the boundary of light scales in Rs – M_1 cell along Rs vein (***a***) to the distance between the split of Rs and M_1 veins and the distal end of Rs vein (***c***) on hindwing ventral side. Vertical axis is the ratio ***b/c*** of the distance between the split of Rs and M_1 veins and the boundary of dark scales in $Sc+R_1$ – Rs cell along Rs vein (***b***) to the distance between the split of Rs and M_1 veins and the distal end of Rs vein (***c***) on hindwing ventral side. Points corresponding to holotypes of both taxa are shown in red.

beginning of the nudum with 22–25 segments, not after the nudum; (**c**) distance between forewing veins Cu_1 and M_3 at the origin is about twice the distance between veins M_3 and M_2, not equal to it; (**d**) hindwing lobed from vein M_3 to the tornus; (**e**) hind tibiae with 1 pair of spurs, not 2; (**f**) males with a fold along hindwing vein 2A surrounded by tufts of dark long hair-like scales and short tufts on both sides of abdomen. In all these and other characters the new species is very close to South American *P. coeleste* and is reasonably considered to be its Central American counterpart; however, there is no way to know, with the data in hand, which is the ancestor of which, or neither. The following characters consistently set *P. burnsi* **n. sp.** apart: **1**) the marginal dark band on the ventral hindwing is broader, as quantified on Figs. 33, and is more diffuse along the inner edge with dark scales bleeding into the orange area; while the stair-step scalloping breaks at the veins of *P. coeleste* are noticeable in cells near the costa, the inner edge of the dark band is straighter, not wavy towards tornus (Figs. 17–32); **2**) the hindwing is rounder, less elongated and the tornal lobe, while similarly broad, is shallower and less developed (Figs. 1–32); **3**) *P. coeleste* has a prominent patch of dark scales, some of which are hair-like, at the base near and on the humeral lobe on the ventral hindwing, but in *P. burnsi* it is reduced to a diffuse patch of dark scales and does not stand out (Figs. 17–32); **4**) the fold along the vein 2A on dorsal hindwing in males is less developed in *P. burnsi* than in *P. coeleste*, covered with brownish scales concolorous with the background and anal fold scales, but not prominently cream-colored as in *P. coeleste* (Fig. 34, however, when the fold is closed, its pale, cream colors may not be clearly visible in *P. coeleste*, e.g. in Fig 13, fold is open on the left wing and is closed on the right wing, fold is closed in Fig. 14, fully open in Fig. 15, and partly open (basad) in Fig. 16; additionally, a tuft of darker hair-like scales inside the fold may obscure its pale lining), tufts of hair-like scales around 2A hindwing vein seem to be darker colored in *P. burnsi* than in *P. coeleste* (Fig. 34); **5**) the central notch in the lamella postvaginalis is deeper, about equal to the anteriordorsal length of the bulge anterior of the notch, but is only about half of the bulge length in *P. coeleste* (Fig. 36); **6**) the DNA barcode sequence differs from *P. coeleste* by about 4.5% (Fig. 38). Interestingly, morphological and wing pattern characters 1–3 are similarly expressed in both sexes, making unambiguous association of sexes possible in the absence of barcode data, and female genitalia offer a more consistent separation between the two species than do the male genitalia, which are very variable in the shape of cucullus (Fig. 35).

Immatures and food plants: All ACG caterpillars of *P. burnsi* (Fig. 37a–m) have usually been found feeding on buds and very young, flimsy and expanding leaves in the crowns of 1–3 m tall saplings of *Ormosia coccinea* (Aubl.) Jacks. (Fabaceae), with the single exception being a caterpillar feeding on the very young foliage of *O. panamensis* Benth. The habitat of these

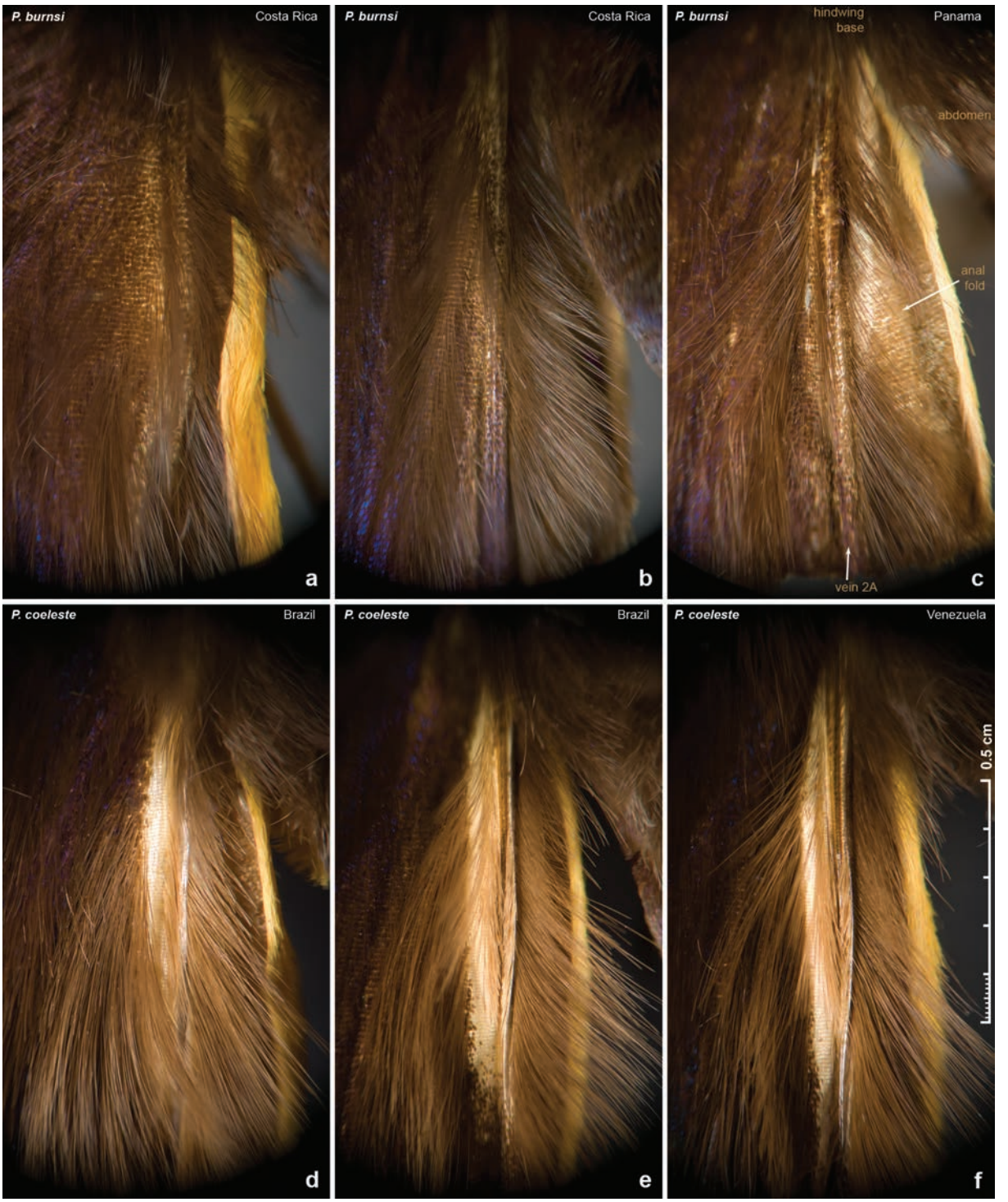


FIG. 34. Dorsal view of left hindwing area around vein 2A in males. a–c. *P. burnsi* paratypes, **d–f.** *P. coeleste*. Base of hindwing is above, tornus below (but is out of view). Part of abdomen base is visible in the right upper corner. Yellow area in **a.** by the anal margin is a part of ventral surface of anal fold that it bent over. All specimens in USNM collection, except as indicated in brackets. **a, b.** Costa Rica: ACG, voucher codes 02-SRNP-35231 [BMNH] (genitalia Figs. 35i) and 02-SRNP-35232 [MGCL] (wings Figs. 10, 26), respectively; **c.** Panama: Canal Zone, Summit, 2-V-1964, genitalia NVG120207-05 (wings Figs. 12, 28, genitalia Fig. 35g); **d.** Brazil: Pará, Belem, V-1962; **e.** Brazil: Rondônia, 62km S Ariquemes, Fazenda Rancho Grande, 10° 32'S 62° 48'W, 165m, 29-IX - 10-XI-1991, leg. B. P. Harris (genitalia Fig. 35v); **f.** Venezuela: T. F. Amaz. Cerro de la Neblina Basecamp, 0° 50'N 66° 10'W, 140m, 26-I-1985, at black light on bank of Rio Baria, leg. P. J. & P. M. Spangler, R. A. Faitoute & W. E. Steiner (wings Figs. 15, 31). All images are to scale.

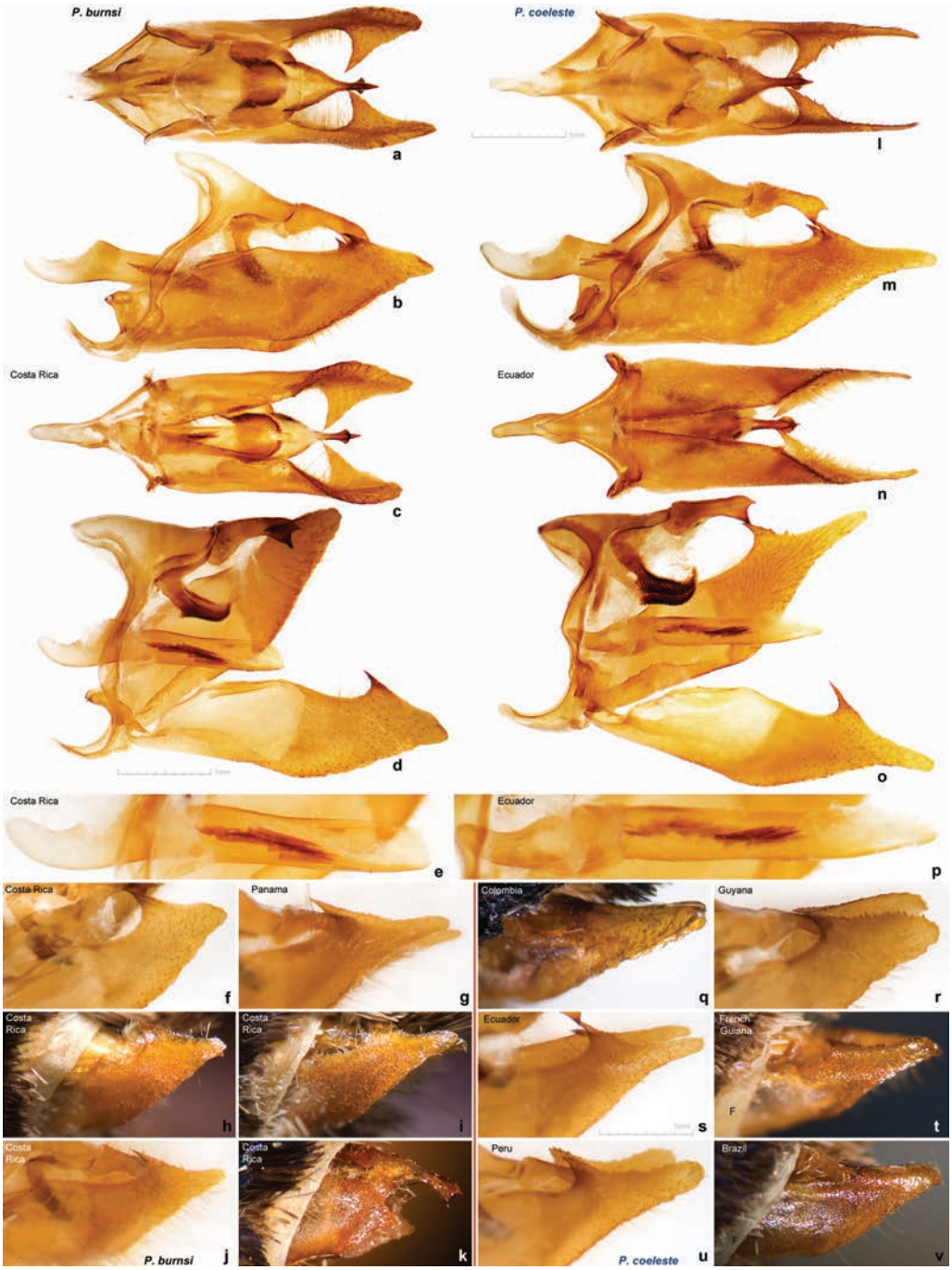


FIG. 35. Male genitalia. a–k. *P. burnsi*; **l–v.** *P. coeleste*. **a–d, l–o.** complete genital capsule; **e, p.** enlarged right lateral view of penis; **f–k, q–v.** lateral view of distal parts, mostly valvae, **h, i, k, q, t, v.** photographed in situ. Views: **a, l.** dorsal; **b, d, m, o.** right lateral, in **d, o.** valvae are pushed apart; **c, n.** ventral. All specimens in USNM collection, except as indicated in brackets. Specimen data: **a–f.** Costa Rica: ACG, voucher code 05-SRNP-30577, genitalia NVG120513-05; **g.** Panama: Canal Zone, Summit, 2-V-1964, genitalia NVG120207-05 (specimen Figs. 12, 28); **h.** Costa Rica: ACG, voucher code 02-SRNP-35323; **i.** Costa Rica: ACG, voucher code 02-SRNP-35231 [BMNH]; **j.** Costa Rica: ACG, voucher code 99-CALI-790, genitalia NVG120207-07; **k.** Costa Rica: ACG, voucher code 05-SRNP-30469; **l-p, s.** Ecuador: Esmeraldas, El Durango, km. 40, Lita-San Lorenzo Rd., 1° 02'45"N 78° 38'06"W, 300m, {25,27}-VIII-2002, J.P.W. Hall & M.A. Solis, genitalia NVG120513-04; **q.** Colombia: Caquetá Department, Florencia, 1300', 22-I-1971, leg. S. S. Nicolay (specimen Figs. 16, 32.); **r.** Guyana: Mazaruni-Potaro, Kaieteur Falls, 5° 14'N 59° 33'W, 200–450m, 26-XII-1989 - 1-I-1990, leg. S. Fratello, genitalia NVG120207-08; **t.** French Guiana: St. Jean, Maroni, IV-1904, collection Wm. Schaus (specimen Figs. 14, 30); **u.** Peru: Tingo Maria, VIII-1979, genitalia NVG120513-03; **v.** Brazil: Rondônia, 62km S Ariquemes, Fazenda Rancho Grande, 10° 32'S 62° 48'W, 165m, 29-IX - 10-XI-1991, leg. B. P. Harris. F indicates mirror image (left–right inverted). All images are to scale except **e.** and **p.**, which are 1.7 and 1.5 times the scale (enlarged) respectively.

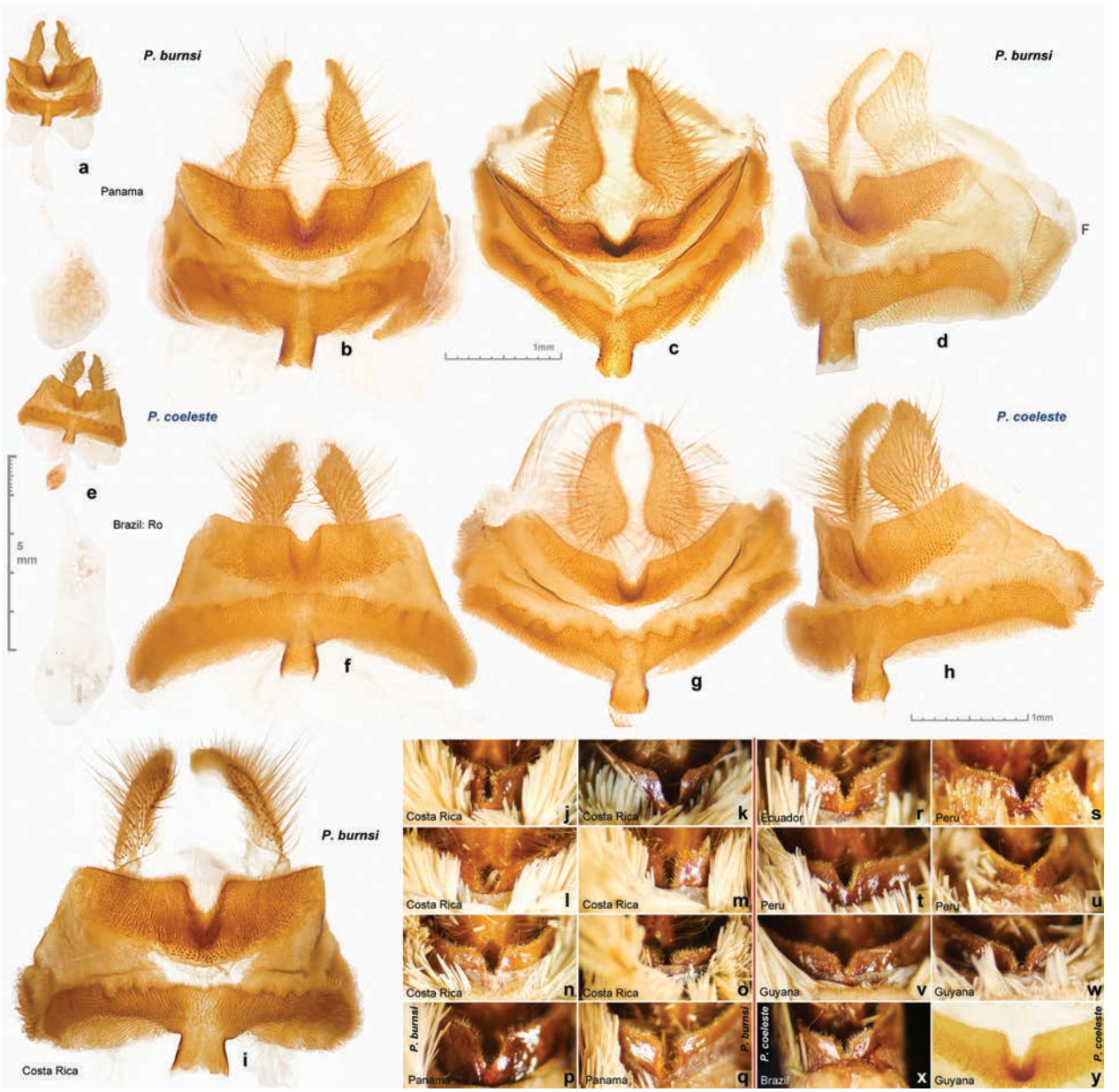


FIG. 36. Female genitalia. a–d, i–q. *P. burnsi*; **e–h, r–y.** *P. coeleste*. **a. e.** complete genitalia, **b–d, f–i.** enlarged sterigma and ovipositor lobes, **j–y.** median part of lamella postvaginalis, **j–x, y.** photographed in situ. All specimens in USNM collection, except as indicated in brackets. All images are to scale, except **a.** and **e.**, which are smaller and the scale for them is indicated on the left. **a–d.** paratype, Panama: Canal Zone, Gamboa, 9° 07'N 79° 41'W, 28-X-1978, leg. G. B. Small, genitalia NVG120207-06; **e–h.** Brazil: Rondônia, vic. Caucalandia, 10° 32'S 62° 48'W, 160-350m 22-X-1991, leg. J. Kemner, genitalia NVG120207-19; **i, k–q.** paratypes, **j.** holotype, **i–o.** Costa Rica, ACG, voucher codes: **i.** 05-SRNP-30642, genitalia NVG120513-01 [BMNH]; **j.** 05-SRNP-30644; **k.** 05-SRNP-30576; **l.** 04-SRNP-56226 [MGCL]; **m.** 05-SRNP-30643 ; **n.** 04-SRNP-31520; **o.** 99-CALI-787; data in text. Data for others: **p.** Panama: Darién Province, Cerro Pirre, ca. 0m, 15-IV-1976, leg. G. B. Small; **q.** Panama: Canal Zone, Gamboa, X-1968; **r.** Ecuador: Sucumbíos, ridge between Río Ushaue and Río Puchuchoa, km. 4 Lumbaquí - La Bonita rd., 0° 05'N 77° 17'W, 850m, {12-14}-III-2004, leg. I. Aldas; **s.** Peru: Loreto Province, Rio Amazonas, 200m, Explorama Inn, 25mi E Iquitos; 9-12 & 17-21-IX-1990, leg. R. Leuschner; **t.** Peru: Huanaco Department, Upper Huallaga Valley, X-1990; **u.** Peru: near Iquitos, 23-I-1932; **v.** Guyana: Acarai Mountains, Sipu River, 1° 21.3'N 58° 57.4'W 2000–2500' {4-10}-XI-2000, leg. S. Fratello et al. USNM ENT 00179859; **w.** Guyana: Mazaruni-Potaro, Kaieteur Falls, 5° 14'N 59° 33'W, 200–450m, 18-XII - 25-XII-1989, leg. S. Fratello; **x.** Brazil, J. C. Hopfinger Collection 1962; **y.** Guyana, Omai, genitalia NVG120513-02. F indicates mirror image (left–right inverted).

FIG. 37. *Phareas* immatures. a–r *P. burnsi* - Costa Rica: ACG; **s, t** *P. coeleste* - French Guiana: Galion. **a–m, s** caterpillars: **a-l, s** last instar, **j–m** penultimate instar. **o–r, t** pupae. Voucher codes for *P. burnsi* immatures: **a–c** 95-SRNP-576; **d–i** 01-SRNP-9030; **j–n** 04-SRNP-31795; **o** 95-SRNP-577; **p–r** 04-SRNP-31800. The lengths of caterpillars 01-SRNP-9030, 04-SRNP-31795, and *P. coeleste* are 48, 50, and 42mm, respectively. All *P. burnsi* immatures shown did not produce adults and were either parasitized or died of disease. For instance, in addition to white spots, white *Winthemia* Wood28 (Tachinidae) eggs are glued to the cuticle of the last two segments of the caterpillar in **e** and **i**; the diseased and dead pupa in **o** is dark with little pattern, the other pupal images are normal in coloration, except that one wing case of the pupa shown on **p** is darker than the rest of the pupa, possibly due to disease. *P. coeleste* immatures **s** and **t** photographed on 29-IV-1991 and 9-V-1991 respectively, eclosed on 22-V-1991; F indicates mirror image (left-right inverted). Photographs of *P. coeleste* are by Christian Brévignon.

species of *Ormosia* Jacks. is natural and anthropogenic rain forest margins at intermediate elevations (400–700 m). This degree of specialization needs to be considered in an ecosystem containing at least 4,000 species of plants, more than 2,000 species of which have been surveyed for caterpillars feeding on them. Considering that more than 350 species of Hesperiidae caterpillars (ca. 102,000 specimens) have been found by the rain forest portion of the ACG caterpillar inventory, we feel comfortable viewing *P. burnsi* as highly specialized to feed on very young *Ormosia* foliage. Whether or not the caterpillars also occur in the new foliage of the crowns of 20–40 m tall adult *Ormosia* remains to be seen.

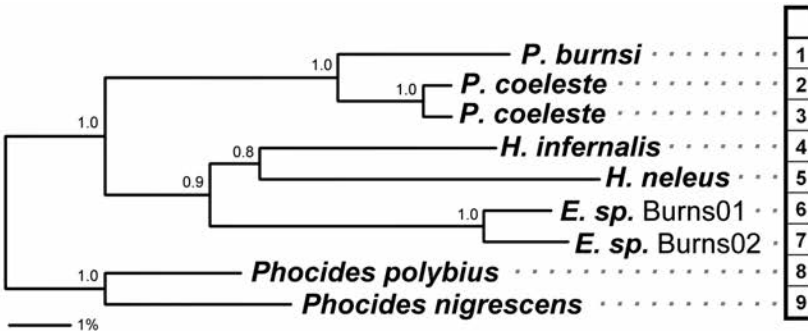
The newly hatched caterpillar takes about 30 days to develop into a prepupa, which is at the fast end of the Hesperiidae development process for such a large skipper caterpillar. Other skipper species of similar high body weight often use as much as twice as long for caterpillar development; however, they generally feed on mature foliage as well as very young foliage, while *P. burnsi* unambiguously prefers very young foliage (whose chemical defenses may be poorly developed, and nutrient content high). It folds a leaf over itself and lightly silks the two portions together. When the nest is ripped open, as a bird might do, instead of retreating or vacating the nest, it turns its head toward the opening and thrusts it out at the intruder, rendering its "face" (Fig. 37c,f,h) part of a very large array of ACG caterpillar species with face-like color patterns on wings, and on caterpillar and pupal heads. The selective force generating these false eyes could be that of small birds, and perhaps some mammals, in that it fits a general pattern that suggests a snake or other predator from which the caterpillar predator does best if it flees (Janzen et al. 2010). Pupae (Fig. 37o–r) are cream-colored with black stripes and spots, and possess an unusually expanded and rounded compartment on the head anterioventrad and between glazed eye-pieces (Mosher 1916).

Interestingly, both caterpillar and pupa of *P. coeleste* (Fig. 37s–t) show apparent differences from *P. burnsi* (Fig. 37a–r), however it remains unclear if these differences hold in a series larger than one individual. A single caterpillar of *P. coeleste* from French Guiana, found as an ultimate instar, was reared by Christian Brévignon (unpublished). Prominent white spots of *P. burnsi* caterpillar are either missing or residual in *P. coeleste* caterpillar. However, the yellow "headlights" on the head are similar, and behavior of disturbed caterpillar pointing the "face" at intruder is similar as well (C. Brévignon, pers. comm.). The shape and pattern of protruding compartment on the head seem to differ in pupae (Fig. 37r,t), being more rounded and with the dark lateral stripe being further from its distal end in *P. coeleste*.

Discussion

The monotypic genus *Phareas* was proposed by Westwood (1852) for *P. coeleste*, newly described from a single female. This genus (or its synonym *Grynopsis*) has been used for *P. coeleste* by most authors (Mielke 2005) who stress the uniqueness of this skipper. *Phareas coeleste* is set far apart from many other skippers not only by the metallic-blue white spotted, and orange with black and white dorsal and ventral wing patterns and peculiar hindwing shape with a very wide tornal lobe, but also by male secondary sexual characters on the hindwing and abdomen, consisting of a fold along the vein 2A surrounded by tufts of long scales and smaller tufts on the abdomen. This uniqueness masks potential existence of other species with similar appearance. It is hard to suspect diversity when the prototype South American species is very different from all other skippers.

The paucity of specimens in collections is another obstacle to assessing intraspecific variation. *Phareas coeleste* is widely distributed in South America. Large series of it exist in collections worldwide, and it is commonly observed in the field in South America by butterfly watchers and photographers. In contrast, *P. burnsi* is known only from Costa Rica and Panama, is exceedingly rare in collections (e.g. there were none in the Natural History Museum, London) and we have never seen an adult in the wild in ACG. Without the special barcoding, ecological and morphological attention that the ACG specimens receive, it is not likely that this species would have been noticed, at least not any time soon. A small series of four specimens from Panama in the USNM collection, which look like *P. coeleste* and were curated among it, were apparently insufficient to notice phenotypic differences. Only with a series of a dozen ACG specimens did meaningful analysis of variation become possible, and consistent differences in the width of the ventral hindwing black band were detected. DNA barcoding did not reveal *P. burnsi* because the ACG inventory had no barcoded South American specimens with which to compare it. To assess statistical significance of these apparent differences, they were quantified (Fig. 33) in all 16 *P. burnsi* specimens and a similar size sample of *P. coeleste* specimens, together with the holotype, from all parts the range, including Colombia, Ecuador, Peru, Guyana, British Guiana and Brazil. The analysis resulted in two well-separated clusters of points with a definitive hiatus between them. Costa Rican and Panamanian specimens formed one cluster, and all South American specimens,



	1	2	3	4	5	6	7	8	9
1	–	31	28	73	89	84	82	67	76
2	**4.7**	–	6	64	81	80	78	65	73
3	**4.3**	**0.9**	–	64	83	79	79	65	74
4	11.2	9.8	9.8	–	57	63	66	68	75
5	13.6	12.4	12.7	**8.7**	–	66	70	84	85
6	12.8	12.2	12.1	9.6	10.1	–	16	72	73
7	12.5	11.9	12.1	10.1	10.7	**2.4**	–	75	78
8	10.2	9.9	9.9	10.4	12.8	11.0	11.5	–	33
9	11.6	11.2	11.3	11.5	13.0	11.2	11.9	**5.0**	–

FIG. 38. DNA-derived data. Distance tree obtained with BioNJ method (www.phylogeny.fr, Dereeper et al. 2008) is shown on the left and distance matrix is shown on the right. Bootstrap support values are shown by each node in the tree. The scale bar corresponds to about 1% difference. All specimens are from Costa Rica, Area de Conservación Guanacaste, unless stated otherwise. ACG voucher codes where available (with -SRNP-, Janzen & Hallwachs 2012) and GenBank accessions (two letters followed by 6 digits, http://genbank.gov) for each of the nine sequences are: **1.** *Phareas burnsi* holotype – 05-SRNP-30644, GU149831; **2.** *P. coeleste* – Peru: San Martin, Juanjui, -7.1° -76.44°, 280m, 25-X-2003, F. Koenig, JF851944; **3.** *P. coeleste* – French Guiana: Nouragues Research Station, 4.09831° -52.6804°, 300m, 25-I-2010, leg. C. Lopez-Vaamonde, HQ989371; **4.** *Hyalothyrus infernalis* (Möschler, 1877) – French Guiana: Nouragues Research Station, 4.09831° -52.6804°, 300m, 16-I-2010, leg. C. Lopez-Vaamonde, HQ989294; **5.** *H. neleus* (Linnaeus, 1758) – 00-SRNP-2796, JF752878; **6.** *Entheus* Burns01 – 05-SRNP-30012, DQ292436; **7.** *Entheus* Burns02 – 05-SRNP-31934, GU150401; **8.** *Phocides polybius lilea* (Reakirt, [1867]) – 05-SRNP-45012, GU150688; **9.** *Phocides nigrescens* E. Bell, 1938 – 04-SRNP-23796, GU161792. The two *Entheus* species are currently undescribed and bear the interim names of "Burns01" and "Burns02" as in Janzen et al. (2011). Percent difference and the number of different nucleotides are shown below and above the diagonal in the distance matrix, respectively. For congeners, percent difference values are shown in bold font.

including a specimen from Colombia, formed another cluster. Assuming Gaussian distribution of points in each morphometric cluster from Fig. 33, out of ten million *Phareas* specimens, only one is expected to cross from one cluster to the next and thus be identified incorrectly (P-value $9.76 \cdot 10^{-8}$). This statistic gives high confidence in reliability of wing pattern characters in telling *P. burnsi* and *P. coeleste* apart, though once it was realized that there are two species, DNA barcodes reliably distinguish them as well (see below).

Analysis of genitalia revealed interesting differences among females and unexpectedly large variability in male genitalia. As stated in the diagnosis, the depth of the notch in lamella postvaginalis seems to distinguish the two species (Fig. 36). This difference might be noticeable *in situ* upon brushing the scales off the end of the abdomen. While it is not possible to relate the depth of the notch to the length of the bulge anterior to the notch without full dissection, because the bulge is covered by the last sternite (Fig. 36), a simple measurement of the notch depth is usually sufficient. Despite differences in sizes of these specimens, for 10 females of *P. burnsi* and 9 females of *P. coeleste*, the notch was less than 0.24mm deep in *P. coeleste* (Mean: 0.196mm, SD: 0.025mm), and more than 0.24mm deep in *P. burnsi* (Mean: 0.287mm, SD: 0.036mm). While some measurements in pairs of species are close to each other and thus using them might not offer reliable separation for a large *P. coeleste* (with possibly larger notch) and a smaller *P. burnsi* (with expectedly smaller notch), they are indicative of potential female genitalic differences between the two species. Due to these differences in females, variability of male genitalia, definitive sex associations of reared *P. burnsi* specimens backed up not only by phenotypic characters (1 to 3 in the diagnosis above), but also by identical barcodes, female specimen was chosen as the holotype of *P. burnsi*. While mostly male specimens are selected as holotypes nowadays, we decided that a choice of a female is particularly fitting in this case to facilitate comparisons with *P. coeleste*, largely because *P. coeleste* holotype (by monotypy) is also a female.

Male genitalia, on the contrary, revealed a wide array of phenotypes. When a single typical individual is taken to illustrate genitalia (Fig. 35a–e, l–o) the most noticeable difference is in length and shape of the distal portion of valvae (cucullus). The distal part of the cucullus is shorter and broader in the *P. burnsi* specimen (Fig. 35a–e), while being longer and narrower in the *P. coeleste* specimen (Fig. 35l–o). Although this general trend seems to hold up in many specimens (Fig. 35), there are interesting exceptions. Even for reared ACG specimens, variation in cucullus is appreciable (e.g. Fig. 35hi), and one specimen had highly aberrant genitalia (Fig. 35k) with an underdeveloped cucullus reduced to a doublet of small terminal projections. All ACG specimens possess 100% identical barcodes and we have no cause to think that they are anything but a single species. Interestingly, one *P. coeleste* specimen from Guyana had an unexpectedly broad cucullus (Fig.

35r), and one *P. burnsi* specimen from Panama had a longer and narrower cucullus (Fig. 35g). We suggest that these levels of differences in cucullus shape are intraspecific individual variation. We hypothesize that the distal end of the cucullus is not fully formed in a pupa, but expands upon eclosion. Conversely, it is also conceivable that male genitalic variation may reflect additional cryptic species to be discovered, but many more specimens, dissections and DNA sequences would be required to support such a view. Careful examination of other characters in genitalia failed to reveal differences beyond those expected from individual variation. It is interesting that in some Hesperiidae, like *Phareas*, female genitalia might show more taxonomically useful differences than male genitalia.

DNA barcodes are available for nine ACG *P. burnsi* specimens and they are all identical in sequence. DNA barcodes are available for two *P. coeleste* specimens, one from Peru, the other one from French Guiana, and they show 0.9% difference between the two (Fig. 38), but 4.3% and 4.7% difference from *P. burnsi*. Differences within 1% in specimens from distant localities are expected as intraspecific variation, however barcode differences of as little as 0.2% are known between morphologically and ecologically different (but very similar) Hesperiidae (Burns et al. 2007). Importantly, we are not aware of any Hesperiidae species showing 4.5% barcode difference within a species. Such a difference is highly indicative of an interspecific divergence, consistent with morphological differentiation in wing shapes, patterns and genitalia. Interestingly, this 4.5% difference is significantly larger than the 2.4% difference between the two undescribed *Entheus* species from ACG (Fig. 38), which are sympatric and distinct in their biology (Janzen et al. 2011).

Although 654 base pairs of barcode sequence are usually too few for statistically sound phylogenetic inference, which also could be hindered by hybridization and introgression (Zakharov et al. 2009), we observe confident bootstrap statistics close to 1 for all nodes in a NJ tree of selected taxa that were viewed as being evolutionarily close to *Phareas* (Evans 1952) (Fig. 38). Also, other phylogenetic methods offered by phylogeny.fr web-server (Dereeper et al. 2008), such as PhyML, MrBayes and TNT produce the same tree (data not shown), increasing our confidence in the results. The three genera, in addition to *Phareas*, selected for the tree were *Hyalothyrus* Mabille, 1878, *Entheus* Hübner, [1819] and *Phocides* Hübner, [1819]. *Hyalothyrus* and *Entheus* are the genera placed next to *Phareas* in Evans (1952) key and are expected to be evolutionarily closest to it. *Phocides* Hübner, [1819] was taken as a genus from the same "Group B." of Evans (1952), but with different palpi and suggested to be close to *Nascus* E. Watson, 1893 in a comprehensive phylogenetic analysis (Warren et al. 2008, 2009). Thus, *Phocides* seemed to be a reasonable choice for an outgroup for the tree. In the Evans key, which frequently reflects phylogenetic arrangements, *Phareas* is grouped with *Hyalothyrus* by the ratio of distances between origins of certain forewing veins, and *Entheus* is set apart. In the barcode tree, *Hyalothyrus* and *Entheus* are sister taxa, but *Phareas* is positioned away from both of them, and it is closer to *Phocides* by DNA distance (Fig. 38). While the closeness of *Hyalothyrus* and *Entheus* is not particularly surprising, especially taking into account closeness of female wing patterns between certain species of these genera (images in Warren et al. 2013) coupled with synapomorphic similarities in palpi and antennae (Evans 1952), smaller than expected distances between *Phareas* and *Phocides* are interesting and require further analysis. It is possible that peculiar palpi shared by certain genera in the Evans' "Group B" are synapomorphic, and *Phareas* is a true member of the group, but its pupa (Fig. 37o–r) appears very different from any of the other ACG species of Hesperiidae in the Evans' "Group B", with a protruding compartment on the head, and is superficially more similar to such genera as *Nicephellus* Austin, 2008 and *Dyscophellus* Godman & Salvin, 1893 than to *Entheus* and *Phocides* (Janzen & Hallwachs 2012, Warren et al. 2013). Clearly, more data are needed to assess the phylogenetic position of *Phareas*.

In summary, consistency of differences in wing shapes and patterns, female and to lesser extent male genitalia and DNA barcodes strongly argue for *P. burnsi* being a distinct biological species, which is a Central American relative of the widespread and more common South American *P. coeleste*. In wing patterns, *P. burnsi* can be most easily distinguished from *P. coeleste* by a broader and more diffuse dark marginal band on the ventral hindwing. Careful analysis of Central American specimens of other species described from South America may similarly lead to further discoveries of undescribed species.

ACKNOWLEDGEMENTS

We are grateful to Robert K. Robbins, John M. Burns and Brian Harris (National Museum of Natural History, Smithsonian Institution, Washington DC), Wolfram Mey (Museum für Naturkunde, Berlin, Germany), David Lees and Blanca Huertas (Natural History Museum, London, UK), for granting access to the collections under their care and stimulating discussions; Bernard Hermier for many enlightening discussions, critical reading of the manuscript and numerous suggestions; Christian Brévignon for his records and photographs of *P. coeleste* immatures and kind persmission to use them in this work; Andy War-

ren and Ichiro Nakamura for providing information about the Limón Province Costa Rican specimen and encouragement; Ernst Brockmann for DNA barcode of *P. coeleste* and discussions; and anonymous reviewers for helpful comments, corrections and edits. The study has been supported by U.S. National Science Foundation grants BSR 9024770 and DEB 9306296, 9400829, 9705072, 0072730, 0515699, and grants from the Wege Foundation, International Conservation Fund of Canada, Jessie B. Cox Charitable Trust, Blue Moon Fund, Guanacaste Dry Forest Conservation Fund, Area de Conservación Guanacaste, and University of Pennsylvania (DHJ).

LITERATURE CITED

BURNS, J.M., JANZEN, D.H., HAJIBABAEI, M., HALLWACHS, W., & 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. Lepid. Soc. 61: 138–153.

COMSTOCK, J. H. 1918. The wings of insects. The Comstock Publishing Company, Ithaca. xviii+430 pp., 10 pls.

CRAMER, P. 1775–1780. De uitlandische Kapellen voorkomende in de drie Waereld-Deelen Asia, Africa en America. Papillons exotiques des trois parties du monde l'Asie, l'Afrique et l'Amérique. Amsteldam, S. J. Baalde; Utrecht, Barthelemy Wild and J. Van Schoonhoven & Comp. 4 Volumes.

DEREEPER, A., V. GUIGNON, G. BLANC, S. AUDIC, S. BUFFET, F. CHEVENET, J. F. DUFAYARD, S. GUINDON, V. LEFORT, M. LESCOT, J. M. CLAVERIE, & O. GASCUEL. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36(Web Server issue): W465–W469.

EVANS, W. H. 1952. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part II (Groups B, C, D) Pyrginae. Section I. London, British Museum (Natural History). v + 178 pp., pls. 10–25.

HEBERT, P. D. N., PENTON, E. H., BURNS, J. M., JANZEN, D. H. & W. HALLWACHS. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Nat. Acad. Sci. 101: 14812–14817.

JANZEN, D. H. & W. HALLWACHS. 2012. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservación Guanacaste (ACG), northwestern Costa Rica <http://janzen.sas.upenn.edu>.

JANZEN, D. H., W. HALLWACHS & J. M. BURNS. 2010. A tropical horde of counterfeit predator eyes. Proc. Nat. Acad. Sci. 107: 11659–11665.

JANZEN D. H., W. HALLWACHS, J. M. BURNS, M. HAJIBABAEI, C. BERTRAND & P. D. N. HEBERT. 2011. Reading the Complex Skipper Butterfly Fauna of One Tropical Place. PLoS ONE 6: e19874.

JANZEN D. H., W. HALLWACHS, P. BLANDIN, J. M. BURNS, J.-M. CADIOU, I. A. CHACÓN, T. DAPKEY, A. R. DEANS, M. E. EPSTEIN, B. ESPINOZA, J. G. FRANCLEMONT, W. A. HABER, M. HAJIBABAEI, J. P. W. HALL, P. D. N. HEBERT, I. D. GAULD, D. J. HARVEY, A. HAUSMANN, I. KITCHING, J. D. LAFONTAINE, J.-F. LANDRY, C. LEMAIRE, J. Y. MILLER, J. S. MILLER, L. D. MILLER, S. E. MILLER, J. J. MONTERO, E. G. MUNROE, S. RAB GREEN, S. RATNASINGHAM, J. E. RAWLINS, R. K. ROBBINS, J. J. RODRIGUEZ, R. ROUGERIE, M. J. SHARKEY, M. A. SMITH, M. A. SOLIS, J. B. SULLIVAN, P. THIAUCOURT, D. B. WAHL, S. J. WELLER, J. B. WHITFIELD, K. R. WILLMOTT, D. M. WOOD, N. E. WOODLEY & J. J. WILSON. 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. Mol. Ecol. Res. 9 (Supplement 1): 1–26.

KLOTS, A. B. 1970. Lepidoptera, pp. 115–130. *In* Tuxen S. L. (ed.), Taxonomist's glossary of genitalia in insects. Munksgaard, Copenhagen.

MIELKE, O. H. H. 2005. Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera). Sociedade Brasileira de Zoologia, Curitiba, Paraná, Brazil, xiii + 1536 pp.

MOSHER, E. 1916. A classification of the Lepidoptera based on characters of the pupa. Bull. Illinois State Lab. Nat. Hist. 12: 17–159.

RATNASINGHAM, S. & P. D. N. HEBERT. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Not. 7: 355–364.

ROBBINS, R. K. 1991. Evolution, comparative morphology, and identification of the Eumaeine butterfly genus *Rekoa* Kaye (Lycaenidae: Theclinae). Smith. Contr. Zoo. #498, 64 pp.

WARREN, A. D., K. J. DAVIS, E. M. STANGELAND, J. P. PELHAM & N. V. GRISHIN. 2013. Illustrated Lists of American Butterflies. [20-VII-2013] <http://www.butterfliesofamerica.com>.

WARREN, A. D., J. R. OGAWA & A. V. Z. BROWER. 2008. Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics 24: 642–676, 2 figs., 5 tabs.

WARREN, A. D., J. R. OGAWA & A. V. Z. BROWER. 2009. Revised classification of the family Hesperiidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. System. Entomol. 34: 467–523, 4 figs., 1 tab.

WESTWOOD, J. O. 1852. *In*: Doubleday, E., The genera of diurnal Lepidoptera: comprising their generic characters, a notice of their habits and transformations, and a catalogue of the species of each genus. London, Longman, Brown, Green & Longmans. 2, pl. 78, fig. 4 (d).

ZAKHAROV E. V., N. F. LOBO, C. NOWAK & J. J. HELLMANN. 2009. Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity 102: 590–599.

Submitted for publication 24 October 2012; revised and accepted 6 December 2012.