Orange fringes, crenulate hindwings and genomic DNA identify a new species of *Jonaspyge* from Honduras (Hesperiidae: Pyrrhopyginae)

Robert J. Gallardo[1] and Nick V. Grishin[2]

1. La Unión Suyapa, Las Vegas, Santa Barbará, Honduras; email: rgallardo32@gmail.com
2. 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

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Abstract: *Jonaspyge elizabethae* **n. sp.** is described from southwestern Honduras. It is similar to the other two *Jonaspyge* O. Mielke, 2002 species in having metallic dark-blue wings with purple sheen, crenulate hindwing outer margin, and black body with orange palpi and an orange abdomen tip. It is diagnosed by bright-orange (instead of white) fringes and dark (instead of orange) cheeks. Genomic sequence analysis of *Jonaspyge* reveals that it is a close relative of *Jonaspyge jonas* (C. Felder & R. Felder, 1859) and *Jonaspyge tzotzili* (H. Freeman, 1969), differing from them by 5.3% in the COI DNA barcode. This new, third species of *Jonaspyge* is the most divergent member of the genus.

Resumen: *Jonaspyge elizabethae* **n. sp.** se describe desde el suroeste de Honduras. Es parecida a las otras dos especies de *Jonaspyge* O. Mielke, 2002 por tener alas de color azul oscuro metálico con brillo púrpura, un margen exterior crenulado de las alas traseras y un cuerpo negro con palpos y punto del abdomen anaranjados. Se diagnostica con franjas de color anaranjada brillante (en lugar de blancas) y mejillas oscuras (en vez de anaranjadas). El análisis de la secuencia genómica de *Jonaspyge* revela que es un pariente cercano de *Jonaspyge jonas* (C. Felder & R. Felder, 1859) y *Jonaspyge tzotzili* (H. Freeman, 1969), diferenciando entre ellos en un 5,3% en el código de barras de ADN COI. Esta nueva tercera especie de *Jonaspyge* es el miembro más divergente del género.

Key words: biodiversity, firetip skipper butterflies, genomics, Neotropics, taxonomy.

INTRODUCTION

In 2018, a large dark skipper was observed on *Fuchsia paniculata* Lindl. (Onagraceae) flowers in southwestern Honduras. Its appearance unmistakably suggested the hesperiid firetip subfamily (Pyrrhopyginae) (Mielke, 2005), with its robust body, shiny-purple wings of characteristic shape, slightly curved antennae with a stout and blunt club, and orange end of abdomen. However, its precise identity was unclear, with a combination of bright-orange fringes on both wings with a prominently crenulate hindwing not known for any species of firetips (see photographs in Warren *et al.* (2017)). Among North American firetip species, both forewing and hindwing fringes are orange only in *Chalypyge chalybea* (Scudder, 1872), while a number of South American species of *Pyrrhopyge*, such as those of the "Amyclas group" and "Hadassa group" of Evans (1951: 20, 23), have orange fringes. However, the hindwing is not crenulate in any of these species, whereas crenulation is characteristic of *Jonaspyge* O. Mielke, 2002, a genus of two closely related species with white fringes (Zhang *et al*., 2019). Furthermore, *Apyrrothrix aesculapus* (Staudinger, 1876), formerly placed in *Jonaspyge* largely due to this crenulation and recently transferred to *Apyrrothrix* Lindsey, 1921 because it proved not to be monophyletic with *Jonaspyge* in genomic DNA analysis (Zhang *et al.*, 2019), is characterized by dark forewing fringes, while the hindwing fringes are orange. Some *A. aesculapus* specimens have patches of orange scales in the forewing fringe, so the possibility remained that the Honduran specimens were *A. aesculapus* with more extensive orange coloration of the fringes. However, the orange palpi of the Honduran firetip suggested a species-level taxon different from *A. aesculapus*, which is characterized by black palpi. Here, we undertook a study to determine the taxonomic status (species vs. subspecies) and generic placement (*Jonaspyge*, *Apyrrothrix*, or a new genus) of the Honduran firetip.

MATERIALS AND METHODS

Standard entomological techniques were used for dissection (Robbins, 1991): the distal part of the abdomen was broken off, soaked for 40 minutes (or until cleared) 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 follow Steinhauser (1981), except that cucullus is called harpe in this work, and we use the term "cheek" in the sense of Evans (1951) to refer to the dense clump of hair-like

Figure 1. The holotype of *Jonaspyge elizabethae* **n. sp.** Data in text. Dorsal and ventral views are on the left and right respectively.

scales immediately below the eye. Photographs of specimens and habitat were taken by RJG with a Canon Powershot SX50 camera; dissected genitalia were photographed by NVG in glycerol with a Nikon D200 camera without the lens and through microscope at about 1x magnification. Genitalia photographs were taken in several focus slices and stacked in Photoshop to increase depth of field. Images were assembled and edited in Photoshop CS5.1. DNA sequencing and analysis techniques were the same as reported by Zhang *et al*. (2019).

RESULTS AND DISCUSSION

We analyzed the Honduran firetip specimens in terms of facies, genitalia and DNA, and concluded that they represented a new species of *Jonaspyge*, which we describe here.

***Jonaspyge elizabethae*, new species**

http://www.zoobank.org/FB67DE84-3980-4CE2-BCC5-9487A743CD55

(Figs. 1-6)

Description. Male (n=8, Figs. 1-2): right forewing length = 27 mm in holotype (mean 27.9 mm, standard deviation 1.1). Both sides of wings nearly black, metallic dark-blue with purple sheen; forewing outer margin nearly same length as inner margin, outer margin convex near apex and tornus, nearly straight between veins M_2 and CuA_2; hindwing almost triangular with rounded apex, crenulate along outer margin. Fringes on both wings bright-orange above and below, except fringes black at forewing apex by costa, along costal margin to $Sc+R_1$ vein on hindwing, and along inner margin of both wings to tornus. Only fringe scales orange, no orange scales on remainder of wings. Head mostly black, with some orange scales between eyes and near palpi above; palpi orange, same color as fringes; cheeks black; antennae nearly black, except brown nudum and beige area around club ventrally. Body black, including patagia, tegulae and legs; end of abdomen orange, with orange tuft. Male genitalia (Fig. 3): tegumen as long as wide, about same length as uncus arms, two short projections (half of tegumen length) directed posteriad at base of uncus; uncus divided, arms widely separated from each other, about three times longer than wide at base; gnathos small, barely developed, upturned, spiculose on its surfaces caudad, widely separated from uncus; distance from gnathos ventral side to base of uncus dorsally is about same as length of uncus arms; saccus as long as wide, triangular and somewhat pointed anteriad; valva as broad as long, costa with a broad bump; harpe 1.5 times longer than valva, at its base broader than valva, narrowing towards middle, and narrowly rounded caudad, with irregular broad teeth (asymmetrically placed on valvae) with serrated dorsal edge curving inwards towards other valva, and narrow spike-like projection as long as costa at base directed cephalad; sacculus with a toothed basal edge ending with a larger tooth in middle; penis short, same length as uncus with tegumen, slightly wider than uncus arms at base, phallobase about 1/3 of penis length, aedeagus terminally asymmetrical, ostium narrowly triangular basad, extending to left, ostium keel protruding on left side and with fine teeth around ostium, vesica without cornuti. **Female** unknown.

Barcode sequence of the holotype. Genbank Accession MZ046695, voucher NVG-18038D01, 658 base pairs:

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAATTGGAACTTCTTTA
AGAATATTAATTCGAACTGAACTAGGAACCCCAGGATCTTTGATTGGAGATG
ATCAAATTTATAATACCATCGTAACAGCTCATGCATTTATTATAATTTTTTT
TATGGTAATACCAATTATAATTGGAGGTTTCGGAAATTGATTAGTACCTCTA
ATATTAGGAGCTCCTGATATAGCCTTTCCTCGAATAAATAATATAAGATTTT
GATTATTGCCCCCATCTTTAACACTTCTTATTTCTAGAAGCATCGTAGAAAA
TGGAGCTGGAACTGGATGAACTGTCTACCCCCCCTCTTTCTTCTAATATTGCC
CATCAAGGAGCTTCTGTTGACTTAGCAATTTTTTCTTTGCATCTAGCTGGAA
TTTCATCAATTTTAGGAGCTATTAATTTTATTACTACAATTATTAATATACG
AATTAGAAATTTATCATTTGATCAAATACCCCTTTTTGTATGAGCAGTTGGA
ATTACAGC ATTATTATTATTACTATCGTTACCTGTTTTAGCAGGAGCCATT
ACTATACTTTTAACTGACCGAAACCTTAATACTTCTTTTTTTGATCCTGCAG
GAGGAGGAGATCCTATTTTATATCAACACCTATTT

Sequence of the paratype NVG-18038D02 (Genbank Accession MZ046696) is identical.

Type material. Holotype ♂ (Figs. 1, 3) has the following 4 rectangular labels: white printed: || HONDURAS: La Paz Dpt. | Municipality of Opatoro | 11.4 air mi E of Marcala | along road V-493, el. 2000 m | 14°08'35.1"N, 87°51'56.8"W | 29 March 2018 | leg. Robert J. Gallardo ||; white printed: || DNA sample ID: | NVG-18038D01 | c/o Nick V. Grishin ||; white printed: | genitalia | NVG200302-01 | Nick V. Grishin||; red printed || HOLOTYPE ♂ | *Jonaspyge* | *elizabethae* ||. **Paratypes**: 8 ♂♂, all from the same locality as the holotype, collected in 2019 on 29 March (2 ♂♂), 4 April (1 ♂) and 5 April (5 ♂♂). The holotype and 2 paratypes to be deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Type locality. HONDURAS: La Paz Department, Municipality of Opatoro, 11.4 air mi E of Marcala, along road V-493, GPS: approx. 14°08'35.1"N, 87°51'56.8"W, elevation 2000 m (Fig. 4). It is a roadside with rich multi-story vegetation and abundant flowers of *Fuchsia paniculata*.

Etymology. This species is named in honor of the late Elizabeth M. Thompson of Tallahassee, Florida, whose genuine appreciation of the natural world instilled in those around her a deep reverence for nature and the importance of protecting it. We suggest "Elizabeth's Firetip" as an English name.

Figure 2. *Jonaspyge elizabethae* **n. sp.** adults nectaring on wildflowers. Photos on *Fuchsia paniculata* (red flowers, left) by Roger Medina (used with permission), from the type locality. Photos on *Viburnum sp.* (white flowers, right) by RJG, from La Esperanza, Intibucá.

Figure 3. Male genitalia of *Jonaspyge elizabethae* **n. sp.** The genital capsule of the holotype is shown in left lateral view (left) and dorsal view (right). The penis is positioned to be visible (not hidden inside the capsule), on the left.

Distribution and phenology. Currently, this species is known only from three sites in southwestern Honduras (Fig. 6). (1) The species was first collected at the type locality in the Department of La Paz, Municipality of Opatoro in late March and early April, and individuals were subsequently observed in same area in June 2019. (2) One individual was photographed on 12 September 2018 by Edwin Miranda in the Department of Lempira, Municipality of San Manuel Colohete in the southern portion of Celaque National Park, GPS: 14°27'57.49"N, 88°39'56.57"W, elevation 1825 m. (3) Several individuals were also observed and photographed (by RJG) in the Department of Intibucá, Municipality of La Esperanza on September 20, 2018, GPS: 14°21'28.57"N, 88°08'16.27"W, elevation 1875 m.

Diagnosis. This species is placed in *Jonaspyge* because it has all the traits of the genus as given in the Evans identification key for the "Jonas group", except that fringes are not white and cheeks are not orange (Evans, 1951: 8), and as defined by Mielke (2002). In particular, broad forewings and crenulate hindwings are characteristic of *Jonaspyge*. In the male genitalia, the new species differs from its congeners by the harpe being much broader at the base, and by having smaller projections at the base of uncus and a longer projection at the base of harpe. The COI DNA barcode sequence is 5.3% (35 base pairs) different from both *Jonaspyge jonas* (C. Felder & R. Felder, 1859) and *J. tzotzili* (H. Freeman, 1969), which differ from each other by 4.6% (30 base pairs), suggesting a congeneric relationship and

Figure 4. The type locality showing the highland habitat of *Jonaspyge elizabethae* **n. sp.**

Figure 5. Collecting of *Jonaspyge elizabethae* **n. sp.** from a truck. Photographs by Roger Medina, used with permission.

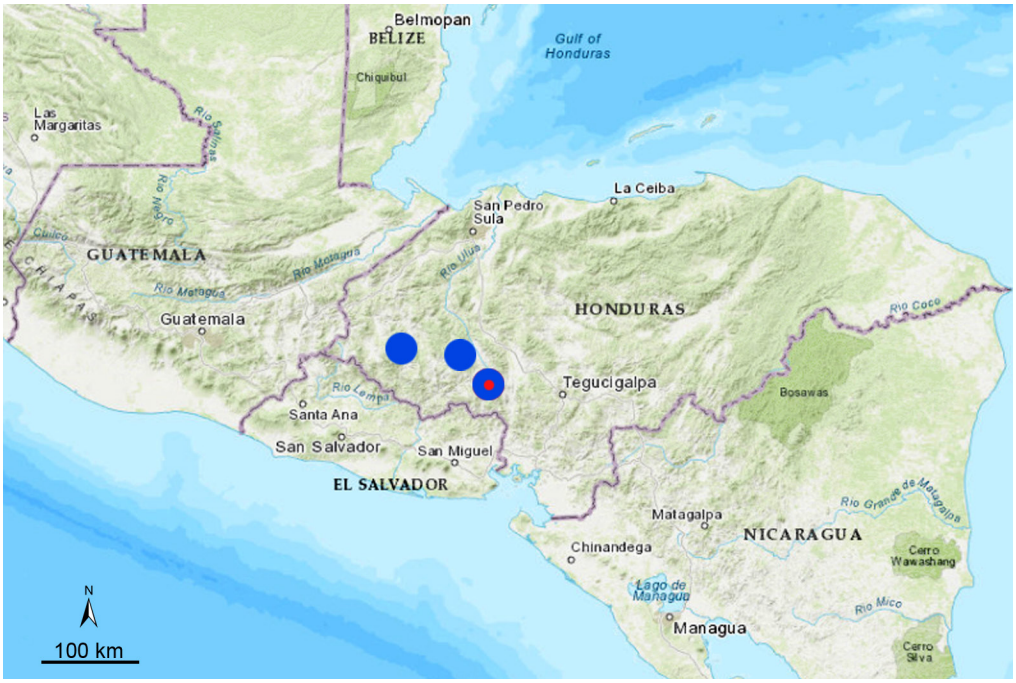

Figure 6. Map with localities for *Jonaspyge elizabethae* **n. sp.** shown as blue circles, the type locality is marked by a red dot.

yet distinction as species. The new species is quite distant from the phenotypically similar *Apyrrothrix aesculapus* and differs from it by 8.4% (55 base pairs) in the barcode. The new species is distinguished from all similar species by the combination of shiny dark-blue wings with bright-orange fringes on both wings, crenulate hindwing, and black body with orange palpi and orange abdomen tip.

Notes on habitat and behavior. *Jonaspyge elizabethae* has been found only at high elevations where it typically inhabits montane broadleaf forest as well as mixed broadleaf and pine-oak habitats. In these habitats, individuals were observed primarily along forest edges, flying from 09:00 to 15:00, where they searched for nectar sources. Numerous individuals were also flying and perching at canopy height of 20 m or higher. On one occasion, however, one individual was encountered in a peach orchard in the outskirts of La Esperanza. These skippers nectared frequently on *Fuchsia paniculata*, *Viburnum* sp. (Adoxaceae) and *Salvia* sp. (Lamiaceae). In the La Paz site they were observed feeding on flowers mostly in the upper reaches of very large, tree-sized *Fuchsia*. No male-male interactions were noticed, even at *Fuchsia* plants where numerous males were present in close proximity.

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