

BULLETIN OF THE ALLYN MUSEUM

Published By
The McGuire Center for Lepidoptera and Biodiversity
Florida Museum of Natural History
University of Florida
P. O. Box 112710
Gainesville, FL 32611-2710

Number 166

5 November 2012

ISSN-0097-3211

A NEW SPECIES OF *OPSIPHANES* DOUBLEDAY, [1849] FROM COSTA RICA (NYMPHALIDAE: MORPHINAE: BRASSOLINI), AS REVEALED BY ITS DNA BARCODES AND HABITUS

Isidro A. Chacón¹, José Montero-Ramírez¹, Daniel H. Janzen², Winnie Hallwachs²,
Patrick Blandin³, C. R. Bristow⁴, and Mehrdad Hajibabaei⁵

¹Instituto Nacional de Biodiversidad (INBio), Apdo. 22-3100, Santo Domingo, Heredia,
Costa Rica, e-mails: ichacon@inbio.ac.cr and jmontero@inbio.ac.cr

²Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mails: djanzen@sas.upenn.edu and whallwac@sas.upenn.edu

³Entomologie, Muséum national d'Histoire naturelle, 57, rue Cuvier, 75005 Paris, France
e-mail: blandin@mnhn.fr

⁴2 Sid Bank, Sid Lane, Sidmouth Devon EX10 5NX UK, e-mail devonbristows@btinternet.com

⁵Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph,
Guelph, Ontario, Canada N1G 2W1

ABSTRACT: *Opsiphanes jacobsoni* new species (Nymphalidae) is described from Costa Rica with descriptions of habitat, host plants, last instar larva and pupa. The holotype is DNA barcoded (COI), and a cladogram of the species of *Opsiphanes* present in Area Conservacion Guanacaste (ACG) is included. We review this new species in the context of the Costa Rican species of *Opsiphanes* and specifically the complex of *Opsiphanes cassina* C. Felder & R. Felder, 1862.

KEY WORDS: Area de Conservacion Guanacaste, Brassolini, caterpillar inventory, Costa Rica, Lepidoptera, new species, Nymphalidae, *Opsiphanes*, palm-eating caterpillars, rain forest, rare species.

INTRODUCTION

In the ongoing caterpillar inventory of the northwest Costa Rican low to middle-elevation forests of Area de Conservacion Guanacaste (ACG) (Janzen et al. 2009, 2011), the large caterpillars of five medium-sized species of Brassoline butterflies *Opsiphanes quiteria* (Stoll, 1780), *O. tamarindi* C. Felder & R. Felder, 1861, *O. bogotanus* Distant, 1875,

O. invirae (Hübner, [1808]), and *O. fabricii* (Boisduval, 1870) have been encountered more than 3,300 times in the past 34 years. The caterpillars of a much rarer sixth species, *O. zelotes* Hewitson, 1873, have also been encountered 16 times (Janzen and Hallwachs 2012), though in contrast to the former five species, free-flying adults of *O. zelotes* have not been encountered in nature in ACG.

However, during the intense caterpillar inventory of the mid-elevation rain forest (400-600 m) in Sector Pitilla of northeastern ACG, a seventh species of *Opsiphanes* has been encountered at very low density. The male adults are very similar to the males of the common *O. fabricii* widely called *O. cassina*, in Costa Rica (see DeVries (1987)), and designated as *O. cassina fabricii* by DeVries (1987) and Bristow (1991), but separated from the nominate *O. cassina* by Blandin (1977). The male of this seventh species was initially noticed because it has different DNA barcodes but then it was realized that its color pattern is slightly outside the range of variation of *O. fabricii* males in ACG. Upon a close scrutiny, its female is easily differentiated from its larger look-alike, the female of the Central American subspecies of *O. quiteria quirinus*. Thus, considering the present state of knowledge on the genus *Opsiphanes* (Bristow 1991; Casagrande 2004), this ACG species is new.

Here we describe this species as a new member of the well-known Costa Rican, and hence Central American, butterfly fauna, and hope it will no longer be overlooked amongst other similar species of *Opsiphanes*. Its cryptic presence also suggests the usefulness of close examination of any seemingly variable single species of *Opsiphanes*, so as to determine to what degree it might be a complexes of cryptic species (as has proven to be the case with numerous other ACG species of large and conspicuous Lepidoptera (Janzen et al. 2009, 2011)).

MATERIALS AND METHODS

Genitalia (Figs. 9-14) dissections and measurements were made with an Olympus SZ60 stereomicroscope with a calibrated ocular micrometer. The following protocol was used for the dissection of genitalia. Abdomens were prepared using a 10% solution of KOH. Mercurochrome and Eosin Y were used as stains. The genitalia and pelt were subsequently stored in glycerol for examination (70% ethanol solution, 3:1). The genitalia and the pelt were slide mounted using Euparal. Genitalic slides were photographed using a JVC 3-CCD color video camera through an Olympus SZ60 stereomicroscope, both mounted in an Olympus SZH-ILLD illumination base and by using Montage explorer software (version: 2.01.0075, Synoptics Ltd.) and Auto-Montage software (version: 4.02.0014, Synoptics Ltd.). Morphological terminology follows Klots (1970) and Kristensen (2003). Acronyms for depositories of specimens are as follows: INBio - Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica; MNHN – Muséum national d'Histoire naturelle, Paris, France; FLMNH/MGCL – McGuire Center for Lepidoptera and Biodiversity; USNM – National Museum of Natural History, Washington D.C, USA. All images here and many others for comparison are available on the ACG caterpillar inventory web site (Janzen and Hallwachs 2012).

Taxonomic Background and Methodological Considerations

This new species description is constructed on the fundamental revision of *Opsiphanes* by Bristow (1991) and Blandin (1977). We take into account all the previously described species of *Opsiphanes*, which is important because all the previously known ACG species

belong to widely distributed classical species: *Opsiphanes quiteria* and *O. invirae* exist throughout the Neotropical Region, from Central America to southeastern Brazil; the complex *O. cassina/fabricii* (see below) is distributed from Mexico to western Ecuador, western and eastern Colombia, Venezuela and the Guianas, northeastern Brazil (Para), eastern Ecuador, Peru and Bolivia; *O. tamarindi* exists in Central America, Colombia, Ecuador (on both sides of the Andes) and may be in Peru (depending on taxonomists' viewpoints); *O. bogotanus* also exists in Central America, Colombia, and western Ecuador; even the very rare *O. zelotes* is known from Central America and Colombia.

Our new species is very similar to *O. fabricii* and it appears as its sister-species in the Neighbor Joining Tree (Fig. 27). Within the ACG fauna, *O. "fabricii"* is an obvious entity and was raised to species status by Blandin (1977). In concordance with Bristow (1991), we feel that the *O. "fabricii"* ACG population is *O. cassina fabricii* and therefore now to be called *O. fabricii*, and is distinct from *O. cassina chiriquensis* by both habitus and DNA barcode. However, there are nomenclatural problems within the *cassina/fabricii* complex, that need further review. *Opsiphanes cassina* was described in 1862 from western Amazonia (Bristow 1991). Its main diagnostic character is the existence of a limited, ochre-orange patch in the forewing cell, which however does not form a band crossing the cell as in the Central American taxon *O. fabricii* (1870), which has long been considered to be a subspecies of *O. cassina* (DeVries 1987; Bristow 1991). Later, the subspecies *O. merianae* Stichel, 1902 (Surinam), *O. notanda* Stichel, 1904 (Peru), *O. numatius* Fruhstorfer, 1912 (Colombia) = *O. periphetes* Fruhstorfer, 1912 (Colombia) were described. All of these species have an orange band across the forewing cell, as in *O. fabricii*. However, in a survey of the brassoline biogeography, Blandin (1977) noted that in the MNHN, there are an *O. cassina* male and an *O. "fabricii"* male from the same exact Ecuadorian locality. He proposed to separate *O. cassina* (reduced cell patch) from the *O. "fabricii/notanda/etc."* group (with a complete cell band). This group is named *O. fabricii* according to taxonomic priority, and which we follow here. Bristow (1991) did not consider this point, and included *O. fabricii*, *O. notanda*, and other subspecies, including new ones, in *O. cassina*. There are now many specimens from northern Peru (San Martín, Amazonas) and Ecuador that confirm the sympatry between *O. cassina* and *O. "fabricii"*-like specimens (Blandin, paper in prep.). The ACG population belongs to the nominate *O. fabricii fabricii* as defined under *O. cassina fabricii* by Bristow (1991).

***Opsiphanes jacobsonum* Chacón & Montero, new species**

(Figs. 1-4, 9-14, 16, 18, 20, 21-22, 24, 26)

Diagnosis. Among known species of *Opsiphanes*, the male of *O. jacobsonum* is similar only to *O. fabricii* and to *O. cassina* in that it has yellowish-orange in the forewing cell (see images in DeVries (1987), Bristow (1991)). It is close to *O. fabricii*, but it can be distinguished by the yellowish-orange patch in the forewing cell being widely separate from the forewing diagonal band rather than touching or even merging with it as is the case with *O. fabricii* and *O. cassina*. The female is superficially similar to the females of *O. fabricii* and of *O. cassina* except that the broad diagonal band across the forewing is white instead of yellow as in *O. fabricii* and *O. cassina*. Thus, the female looks like a small female of *O. quiteria quirinus*. Compare figures 1-4 with images of ACG *O. fabricii* (Fig. 5-8) and many more displayed at <http://janzen.bio.upenn.edu/caterpillars/database.lasso>.

Description. Male – forewing length 34.70 mm (Figs. 1-2); venation typical for *Opsiphanes* (e. g., Stichel 1904).



Figures 1-4. Male holotype and female paratype of *Opsiphanes jacobsoni*: **1-2)** male (06-SRNP-34098), forewing length 34.70 mm; **3-4)** female (06-SRNP-34095), forewing length 45.50 mm.

Head: Orange-brown clubbed antennae, dark-brown between each flagellomere. Compound eyes black and rounded, surrounded by a ring of beige scales. Frons and vertex with a mix of beige and dark-brown piliform scales. Pointed labial palpi beige on dorsum, with a dark-brown line in the middle from base to apex. Dark, shiny black proboscis.

Thorax: Dorsum dark-brown with a mix of beige and black scales; anterior part with beige and bright black, piliform scales, posterior part beige; tegulae dark brown. Ventral thorax dark-brown with irregular beige patches. Forelegs beige on dorsum, with a dark-brown line in the middle; midleg and hindleg femur grey-black on dorsum; tibia and tarsus light-brown.

Abdomen: dorsal part dark-brown, ventral part light-brown, an elongate lateral cuticular patch with beige scales on abdominal segment III, 2.63 mm in length and a smaller round patch on segment IV (Fig. 14).

Wings: Dorsal forewing brown, except that from Cu_2 to anal margin, costal margin, and marginal area from Cu_1 to R_4 the wing is light-brown. Termen lightly excavated, with an irregular prominent yellowish-orange band extending from the middle of costal margin to tornus. Discal cell with an irregular yellowish-orange macula not touching or fused with the yellowish-orange band. Three small, white, subapical macules.

Ventral forewing with a complex pattern: outer margin light-brown from apex to M_2 , dark-brown from M_2 to tornus; costal margin light-brown. Two black undulate lines from M_1 to 2A. Ocellus oval with distal margin straight, located between M_1 - M_2 , black with linear white pupil (3.23 mm, ocelli measured in the mid-cell to outer edge of dark outline) and



Figures 5-8. Male and female of *Opsiphanes fabricii*: **5-6**) male (08-SRNP-30449), forewing length 63 mm; **7-8**) female (06-SRNP-18664), forewing length 73 mm.

outlined with a yellow ring surrounded by red-brown shading. Just below the ocellus there is a yellow apical dash surrounded by red-brown shading; this is followed by a black irregular postmedial band from M_2 to Cu_1 ; an oblique yellow band extends from Cu_1 to tornus. From the inner margin of discal cell to posterior margin, a dark-brown patch delimited by the yellow oblique band. Discal cell beige with an irregular pattern of dark-brown striations inside distal part of discal cell and bases of R, M_1 and M_2 ; inside the middle of discal cell, there is an irregular light-brown bar of a three cells outlined with dark-brown, followed by a dark-brown basal band.

Dorsal hindwing dark-brown; termen slightly undulate; anal margin beige and translucent due to ventral markings showing through. Dark-brown recumbent hairpencils (4.12 mm) inside discal cell just above Cu_2 and beige semi-erect hairpencils (3.38 mm) originating just caudad of the middle of vein 2A; narrow shiny area on both sides of vein 2A proximad and distad to hairpencil; anal area heavily overscaled with light brown hair-like scales. Postmedial band undulate and orange-brown, from apex to tornus.

Ventral hindwing light-brown with a complex pattern of dark-brown, black and white striations. Anal margin beige with dark-brown regular striations; costal margin grey with white striations. Large tan ocellus, located between $Sc+R_1$ to M_1 , outlined with a black ring with a crescent-shaped white pupil with yellow shading. Smaller ocellus located between Cu_1 to Cu_2 , light-brown outlined by a black ring; black pupil surrounded by a white crescent and filled with yellow scales. Satellite ocellus light brown surrounded by a dark-brown line with diffuse dark-brown pupil.

Female – Forewing length 45.50 mm (paratype) (Figs 3-4). Head, thorax and abdomen nearly identical to that of male.

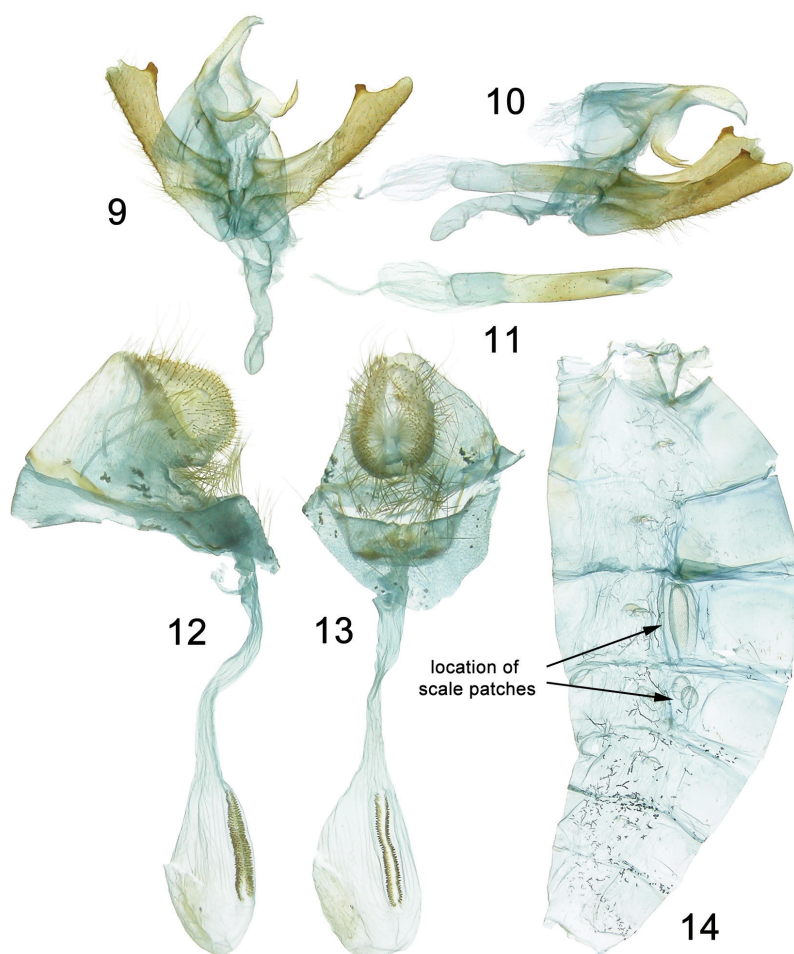
Wings: Dorsal forewing dark-brown, except from Cu_2 to anal margin and costal margin, light-brown. Termen slightly undulate, with an irregular prominent white band extending from the middle of costal margin to tornus. Discal cell with an irregular dirty white macula. Three small white subapical macules. Ventral forewing with complex pattern, nearly identical to that of male, except by the oblique band that is wider and white from costal margin to M_1 and creamy from M_1 to tornus. In the lower margin inside the discal cell a white irregular patch surrounded by a dark-brown. Dorsal hindwing light-brown; termen slightly undulate; anal margin beige and translucent with ventral markings showing through. Marginal area dark-brown and anal area heavily overscaled with light-brown hair-like scales. Postmedial band undulate and reddish-brown, from apex to tornus.

Ventral hindwing nearly identical with that of male, except for the lack of satellite ocellus.

Male genitalia: (Figs. 9-11) uncus in lateral view curved downward to sharp caudal point, with a few setae on lateral surface, sclerotized lateral and dorsal, membranous ventrally; tegumen weakly sclerotized and smooth; saccus narrow and elongate, slightly sclerotized, length similar to that of uncus and tegumen combined; gnathos in lateral view membranous and wide at base, sclerotized and curved upward and pointed at distal part; valva length, 17.15 mm; valve in lateral view broad at base, saccular margin serrate and hairy, inner surface hairy, lateral and ventral costal margin without setae; valva apex with a heavily sclerotized pointed projection on costal margin; phallus longer than valva, 24.5 mm, nearly straight, with tiny spine-like projections on phallobase; juxta heart shaped.

Female genitalia: (Figs. 12-13) sterigma broader than long, posterior section weakly sclerotized, anterior section with two arms sclerotized nearly meeting centrally in a concave depression; ductus bursae short weakly sclerotized; corpus bursae elongated, 19.6 mm x 16.2 mm, with striated surface, parallel signa, length 16.66 mm, with sclerotized triangular projections sorted in rows; papillae anales granulated, posterior half covered by several setae and anterior half sclerotized; posterior apophysis sclerotized, elongated and narrowed.

Types. HOLOTYPE ♂: Costa Rica: Guanacaste: Area de Conservación Guanacaste, Sector Pitilla, Pasmompa, 11.01926, -85.40997, 440 m, caterpillar found 11 September 2006, prepupa 12 November 2006, adult eclosed 8 December 2006, Manuel Ríos, 06-SRNP-34098 (INBio). **PARATYPES** 4 ♂ and 4 ♀ as follows: 1 ♂ Costa Rica: Guanacaste: ACG, Sector Pitilla, Sendero Cuestona, 10.99455, -85.41461, 640 m, caterpillar found 4 July 2006, adult eclosed 24 July 2006, Calixto Moraga, 06-SRNP-32525 (MNH); 1 ♂ Costa Rica: Guanacaste: ACG, Sector Pitilla, Pasmompa, 11.01926, -85.40997, 440 m, caterpillar found 25 July 2006, adult eclosed 28 September 2006, Manuel Ríos, 06-SRNP-33145 (USNM); 1 ♂ Costa Rica: Guanacaste: ACG, Sector Pitilla, Medrano, 11.01602, -85.38053, 380 m, caterpillar found 1 August 2010, adult eclosed 25 September 2010, Ricardo Calero, 10-SRNP-72530 (USNM); 1 ♂ Costa Rica: Alajuela: ACG. Sector Rincon Rain Forest, Estacion Llanura, 10.9163, -85.37869, 135 m, caterpillar found 22 March 2012, adult eclosed 29 April 2012, Cirilo Umaña, 12-SRNP-75601 (FLMNH/MGCL); 1 ♀ Costa Rica: Guanacaste: ACG, Sector Pitilla, Pasmompa, 11.01926, -85.40997, 440 m, caterpillar found 1 September 2006, adult eclosed 28 September 2006, Manuel Ríos, 06-SRNP-34044 (FLMNH/MGCL); 1 ♀ Costa Rica: Guanacaste: ACG, Sector Pitilla, Pasmompa, 11.01926, -85.40997, 440 m, caterpillar found 11 September 2006, adult eclosed 6 October 2006, Calixto Moraga, 06-SRNP-34095 (INBio); ♀ Costa Rica: Guanacaste: ACG, Sector Pitilla,



Figures 9-14. Genitalia of *Opsiphanes jacobsoni*: **9)** male (06-SRNP-34098) ventral view; **10)** same specimen, lateral view; **11)** same specimen, aedeagus; **12)** female (06-SRNP-34095 lateral view; **13)** same specimen, ventral view; **14)** male abdomen lateral view (scale reduced relative to figures 9-11).

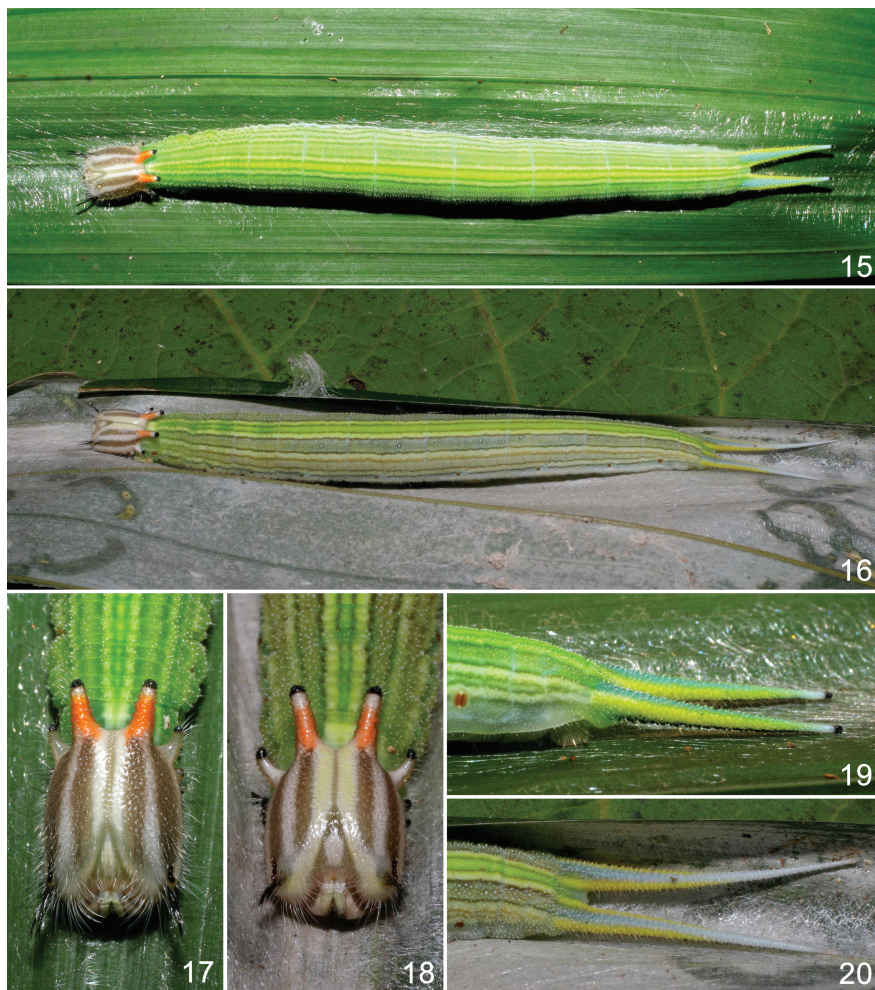
Quebradona, 10.99102, -85.39539, 475 m, caterpillar found 10 May 2010, adult eclosed 8 June 2010, Ricardo Calero, 10-SRNP-71551 (MNH); ♀ Costa Rica: Alajuela: ACG, Sector Rincon Rain Forest, Estacion Llanura, 10.9163, -85.37869, 135 m, caterpillar found 22 March 2012, adult eclosed 27 April 2012, Cirilo Umaña, 12-SRNP-75600. (USNM).

Deposition of Types. The holotype male, and a female paratype are deposited in INBio. A male and female paratype are deposited at FLMNH/MGCL, USNM, and MNHN.

DNA barcode. The DNA barcode of holotype male 06-SRNP-34098 is listed in Appendix 1.

Etymology This species is dedicated to Irwin and Joan Jacobs of San Diego, California, in recognition of their generous support for conservation of the only rain forest in which *O. jacobsonum* is known to occur.

Mature larva (Figs. 16, 18, 20) whiskered, slender and long. Head capsule with a corona of four pairs of horns of differing lengths with granulated surface and white setae. First pair of horns largest on epicranium, orange at two-thirds, apex dirty pink with black tips. Second pair small dirty pink with black tips. The two remaining pairs of horns tiny and black. Epicranial suture dark brown followed by a pair of white cream stripes from epicranial



Figures 15-20. *Opsiphanes fabricii* and *O. jacobsonum* last instar larvae for comparison: **15)** *O. fabricii* (08-SRNP-57337); **16)** *O. jacobsonum* (06-SRNP-34094); **17)** *O. fabricii* head capsule (08-SRNP-57337); **18)** *O. jacobsonum* head capsule (06-SRNP-34094); **19)** *O. fabricii* caudae (08-SRNP-57337); **20)** *O. jacobsonum* caudae (06-SRNP-34094).

notch to base of antennae. In each side of dorsolateral part of head capsule two dark brown stripes separated by two dirty pink stripes. Stemmata dark-brown and shiny. Frons dirty pink; labrum white cream; clypeus and mandibles dirty pink. Body ground color green with a mix of light brown and gray, with a pea green conspicuous dorsal midline, delimited by a green-yellowish band; laterodorsal line bluish green with several grey dots; lateral line greyish-green delimited by two narrow light grey-green bands. Spiracles reddish-brown. Prespiracular area greyish blue. Anal fork (caudae) cerulean with dirty yellow margins. The prepupa (Fig. 21) loses much of its distinct body and head patterning about the time that it is seeking a place to spin the pad of silk into which to hook itself; while the specimen in figure 21 died of disease after pupating, its sib (06-SRNP-33145) survived and is unambiguously *O. jacobsonum* by its DNA barcode (Fig. 27) and habitus.

Pupa (Figs. 22, 24, 26) stout with bifurcate head; ground color pea green with dark-brown stripes; cremaster green with dark-brown margins; ventral edge slightly concave and dorsum keel. Wing pads ventrally with two dark-brown dots, distal dots larger than proximal; lateral prominent silver macule outlined with dark-brown just caudad of its middle; below the macule a dark-brown dot; abdomen with thin dark-brown lateral line below spiracles, spiracles light-brown.



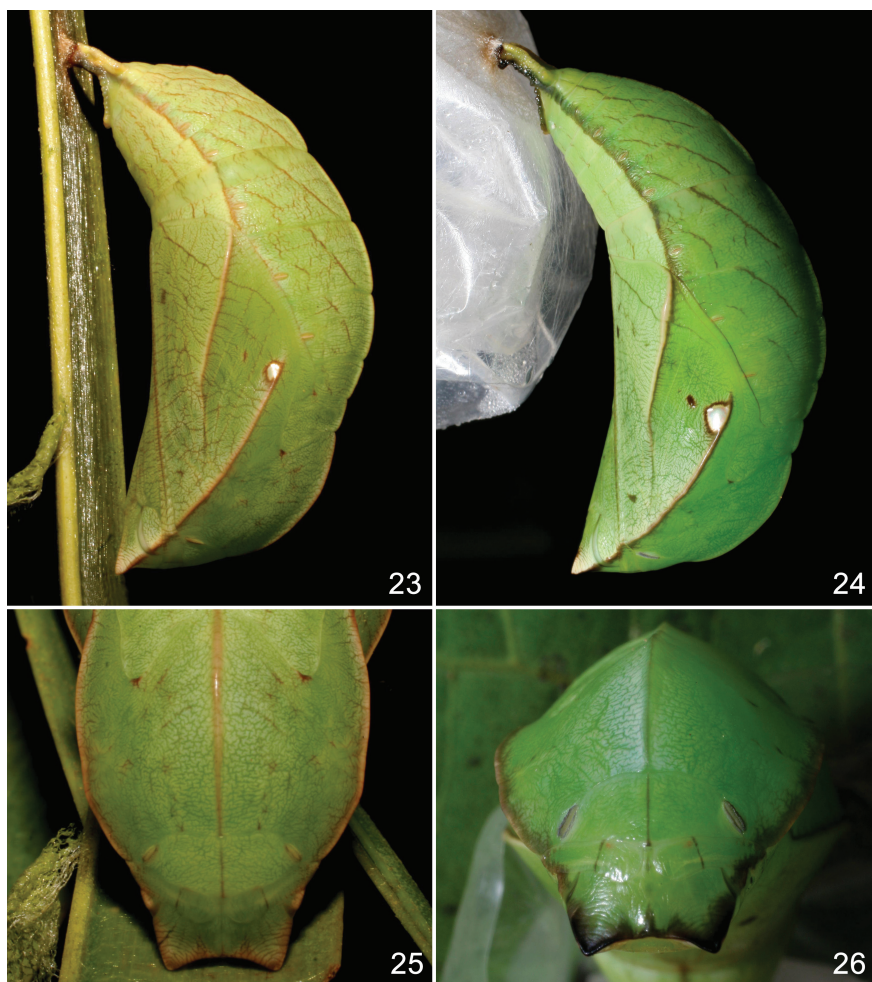
Figures 21-22. *Opsiphanes jacobsonum*: 21) head capsule of prepupa (06-SRNP-33144); 22) ventral view of pupa (06-SRNP-34044).

Comparison of Habitats, Larval Food Plants, and Early Stages

Fourteen *O. jacobsonum* caterpillars have been found to date in the Sector Pitilla rain forest understory (380-640 m elevation) (multiple blue dots in Fig. 28), while feeding on leaves of *Cryosophila warszewiczii* (n=12), *Astrocaryum alatum* (n=1) and *Prestoea decurrens* (n=1). In what appears to be the exact same rain forest understory habitat, *O. quiteria* caterpillars, the only other palm-eating *Opsiphanes* in this forest understory, have been found 76 times with the following frequencies: *Astrocaryum alatum* (n=13), *Bactris gracillior* (n=3), *Chamaedorea dammeriana* (n=4), *Chamaedorea pinnatifrons* (n=26), *Chamaedorea tepejilote* (n=4), *Euterpe precatoria* (n=1), *Geonoma congesta* (n=4), *Geonoma ferruginea* (n=2), *Geonoma interrupta* (n=5), *Prestoea decurrens* (n=13), and *Synechanthus warszewiczianus* (n=1). On a broader scale, among all ACG mid-elevation rain forest understory palm-eating *Opsiphanes*, 880 caterpillars of *O. quiteria* and 16 *O. zelotes* have been found; it is striking that the great bulk of caterpillars of *O. jacobsonum* were found on one species of palm, a species that is not used by *O. quiteria*. An additional male and female caterpillar of *O. jacobsonum* were reared in 2012 from a garden coconut sapling (*Cocos nucifera*) at Estacion Llanura (135 m) in Sector Rincon Rain Forest of ACG (the isolated blue dot in Fig. 28). These two were accompanied by two caterpillars of *O. invirae* and two of *O. fabricii*, and all were in the full sun adjacent to secondary rain forest, a far more insolated habitat than the Sector Pitilla collections of *O. jacobsonum*. While many

species of *Opsiphanes* circulate easily between insolated habitats (though they are mostly dawn and dusk fliers, in ACG, there are three that seem to stay firmly in forest understory: *O. zelotes*, *O. quiteria* and *O. jacobsonum*.

While *O. jacobsonum* caterpillars (see images at <http://janzen.bio.upenn.edu/caterpillars/database.lasso>) appear to be restricted to rain forest understory palm foliage as food plants, except for the two rearings at Estacion Llanura mentioned above, these caterpillars of the look-alike *O. fabricii* are normally encountered feeding on *Acrocomia aculeata* and *Cocos nucifera* palm foliage in isolated pasture and early secondary succession just a few hundred meters away, but never in the rain forest understory. Equally enigmatic,



Figures 23-26. *Opsiphanes fabricii* and *O. jacobsonum* pupae: **23)** *O. fabricii* lateral view (08-SRNP-40515); **24)** *O. jacobsonum* lateral view (06-SRNP-34044); **25)** *O. fabricii* dorsal view of cephalic margin, prothorax, and mesothorax (08-SRNP-40515); **26)** *O. jacobsonum* dorsal view of cephalic margin, prothorax, and mesothorax (06-SRNP-34044).

the only other ACG palm-feeding *Opsiphanes* (besides *O. quiteria*), *O. invirae*, has been found only on foliage of introduced *Cocos nucifera* growing on the edges of ACG rain forest (n=22).

In *O. jacobsonum*, the first pairs of horns on the epicranium are orange at two-thirds, apex dirty pink with black tips, while in *O. fabricii*, the pairs are orange for most of their length, with the terminal portion dirty pink with black tips. The pairs of tiny horns in *O. jacobsonum* are black and brilliant, while in *O. fabricii*, they are light pink with black tips (Figs. 17-18). In *O. jacobsonum*, the anal fork is light blue with dirty yellow margins and the apex is totally light blue, while in *O. fabricii*, the apex is totally black (Figs. 19-20). The pupal ground color of *O. jacobsonum* is pea green with dark-brown stripes, and the cremaster is green with dark-brown margins. In *O. fabricii*, the pupal ground color is green brown and the cremaster is light-brown (Figs. 23-24). In *O. jacobsonum*, the margins of the cephalic projection are black in dorsal view, while in *O. fabricii*, they are light brown (Figs. 25-26).

DISCUSSION

If only the male of *O. jacobsonum* had been collected and had not been DNA barcoded, and found to be clearly different from sympatric (ecologically parapatric) *O. fabricii*, this new species would have been viewed (and perhaps has been by other collectors) as simply a variant of the common and widespread *O. fabricii* (Figs. 5-8). However, the very distinctive white-banded female with her dark red diffuse markings on the dorsal hindwings, coupled with a distinctive DNA barcode separates *O. jacobsonum* very firmly from the complex of *O. fabricii*, *O. cassina* and other similar species in appearance (Fig. 27).

At first glance, she appears to be a somewhat dwarfed *O. quiteria* female, a similarity that may have been evolutionarily driven by the conditions of the same dark understory habitat of the mid-elevation rain forest occupied by the *O. quiteria* female.

Opsiphanes jacobsonum either has an extremely limited distribution of perhaps only several hundred km² in ACG (Fig. 28), which we suspect not to be the case, or as is the case with *Opsiphanes zelotes* in ACG, it is not attracted to butterfly traps baited with fermenting fruit - the standard way *Opsiphanes* is collected and inventoried throughout the Neotropics. The actual microgeographic and ecological distributions of both of these species in Costa Rica was found first by finding their caterpillars, and will not be even approximately known until the palm-eating caterpillar inventory is extended throughout Costa Rican mid-elevation rain forest.

From what we know, no *O. jacobsonum*-like specimen exists in the many public collections of Neotropical *Opsiphanes* that have been studied by Bristow and/or Blandin, except for one rather similar male from Quintana-Roo, Mexico, illustrated in figure 27B of Bristow (1991) and considered by the author at that time as a dark form of *O. cassina fabricii*.

The description of this new species of *Opsiphanes* increases the number of species of this genus in Mexico-Central America (Austin et al. 2007). Moreover, the cryptic presence of *O. jacobsonum* suggests the usefulness of close examination of any seemingly variable single species of *Opsiphanes*, such as *O. fabricii*, so as to determine to what degree it might be a complex of species (as has proven to be the case with numerous other ACG species of large and conspicuous Lepidoptera (Janzen et al. 2009, 2011)). DNA barcoding is particularly useful for this purpose. In this context, the relationships among the multiple taxa that have been described as subspecies of *O. cassina*, among which are the Central American *fabricii*,

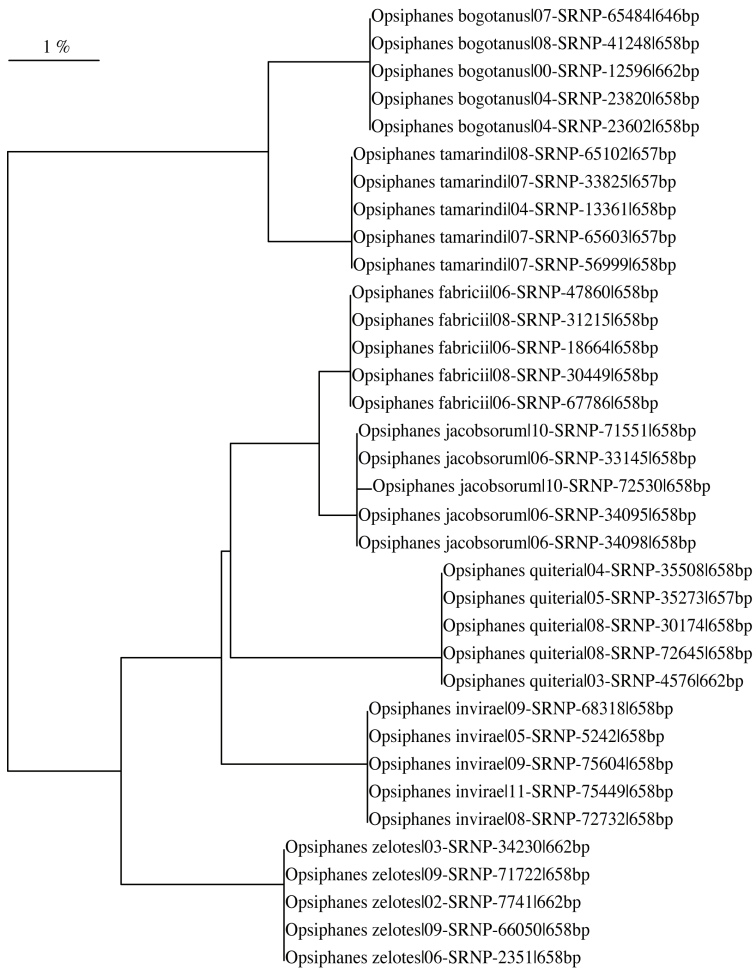


Figure 27. The *Opsiphanes* species from ACG in an NJ barcoding tree. Sample sizes are restricted to a haphazardly selected set of five specimens for each species. All specimens were reared from wild-caught caterpillars and further information on each can be found at Janzen and Hallwachs (2012).

chiriquensis, *aiellae*, and along with the trans- and cis-Andean subspecies, certainly need to be revisited. Such a complex may well contain various other locally or ecologically restricted species. The discovery of *O. jacobsoni* opens exciting perspectives on the diversity of the genus *Opsiphanes*. The presently described “morphospecies” could possibly include several genuine, biologically separated species. However, analysis of such complexes as the *cassina/fabricii* complex requires geographically extensive modern collecting of easily DNA barcoded fresh specimens, a goal made all the more reasonable following this discovery of *O. jacobsoni* in a very intensively butterfly-collected Central American country.

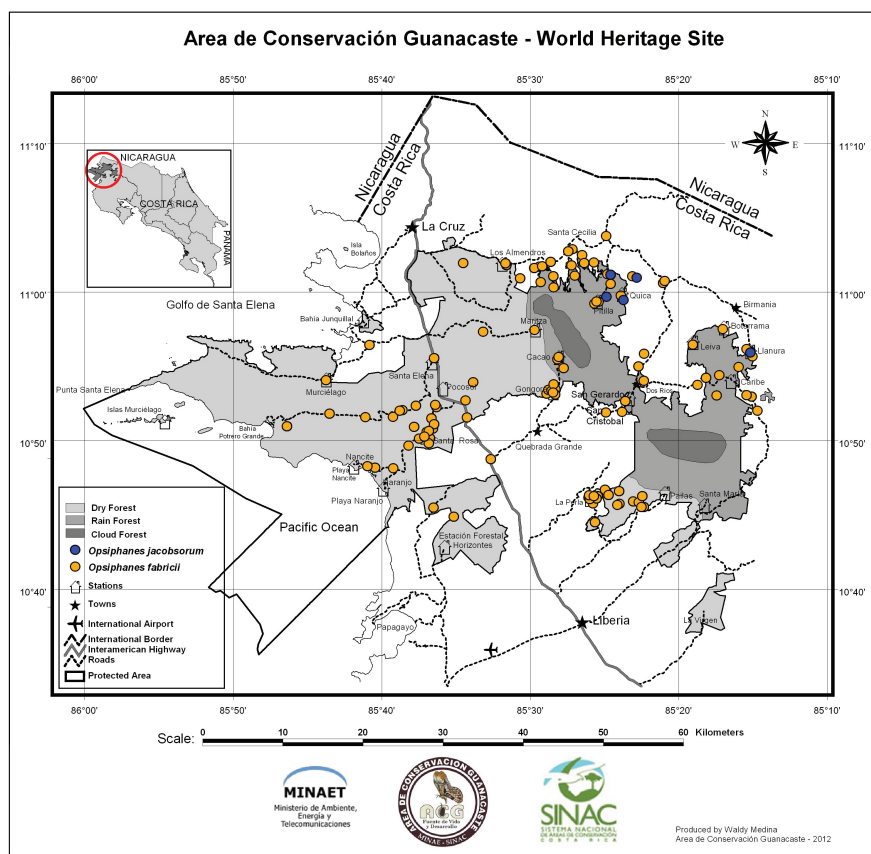


Figure 28. Localities in ACG where caterpillars of *O. jacobsoni* (blue) and *O. fabricii* (yellow) have been found and reared to adults.

ACKNOWLEDGMENTS

We emphatically and gratefully acknowledge the support of the Area de Conservación Guanacaste (ACG) for preserving the forests in which these species live, the ACG parataxonomists for finding and rearing the caterpillars, Bernardo Espinoza for genitalia images, and the Guanacaste Dry Forest Conservation Fund, the Wege Foundation, the International Conservation Fund of Canada, the JRS Biodiversity Foundation, Jessie Hill, and the University of Pennsylvania for funding portions of this research. This study was also supported by National Science Foundation DEB 0515699 to DHJ and by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to MH. Laboratory analyses of these sequences were funded by the Government of Canada through Genome Canada and the Ontario Genomics Institute (2008-0GI-ICI-03). This manuscript benefitted from two anonymous reviewers, and the publication benefitted enormously from the editing and arranging by D. Matthews and J. Y. Miller.

LITERATURE CITED

- Austin, G. T., Warren, A. D., Penz, C. M., Llorente-Bousquets, J. E., Luis-Martinez, A., and Vargas-Fernandez, I. 2007. A new species of *Opsiphanes* Doubleday, [1849] from western Mexico (Nymphalidae: Morphinae: Brassolini). *Bulletin of the Allyn Museum* 150:1-20.
- Blandin, P., 1977. La distribution géographique des Brassolinae (Lepidoptera - Satyridae). Faits et problèmes. In: Descimon, H., (Ed.), *Biogéographie et évolution en Amérique tropicale*. Publications du Laboratoire de Zoologie de l'Ecole Normale Supérieure, n°9: 161-218.
- Bristow, C. R. 1991. A revision of the brassoline genus *Opsiphanes* (Lepidoptera: Rhopalocera). *Zoological Journal of the Linnean Society* 101: 203-293.
- Casagrande, M. M. 2004. Tribe Brassolini. Pp. 201-205 in G. Lamas (ed.) *Atlas of Neotropical Lepidoptera. Checklist: Part 4A, Hesperioidea-Papilionoidea*. Gainesville, FL: Scientific Publishers. 439 pp.
- DeVries, P. J. 1987. *Butterflies of Costa Rica and Their Natural History, I. Papilionidae, Pieridae and Nymphalidae*. Princeton, NJ: Princeton Univ. Press. 327pp.
- Janzen, D.H., Hallwachs, W., Blandin, P., Burns, J.M., Cadiou, J., Chacon, I., Dapkey, T., Deans, A.R., Epstein, M.E., Espinoza, B., Franclemont, J.G., Haber, W.A., Hajibabaei, M., Hall, J.P.W., Hebert, P.D.N., Gauld, I.D., Harvey, D.J., Hausmann, A., Kitching, I., Lafontaine, D., Landry, J., Lemaire, C., Miller, J.Y., Miller, J.S., Miller, L., Miller, S.E., Montero, J. Munroe, E., Rab Green, S., Ratnasingham, S., Rawlins, J.E., Robbins, R.K., Rodriguez, J.J., Rougerie, R., Sharkey, M.J., Smith, M.A., Solis, M.A., Sullivan, J.B., Thiaucourt, P., Wahl, D.B., Weller, S.J., Whitfield, J.B., Willmott, K.R., Wood, D.M., Woodley, N.E., and Wilson, J.J. 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources* 9 (Supplement 1):1-26. doi:10.1111/j.1755-0998.2009.02628.x
- Janzen, D. H., Hallwachs, W., Burns, J. M., Hajibabaei, M., Bertrand, C., Hebert, P.D.N. 2011. Reading the complex skipper fauna of one tropical place. *PLoS ONE* 6(8): e19874. doi:10.1371/journal.pone.0019874
- Janzen, D. H. and Hallwachs, W. 2012. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plans and parasitoids, of Area de Conservacion Guanacaste (ACG), northwestern Costa Rica. <<http://janzen.sas.upenn.edu>>.
- Klots, A. B. (1970) Lepidoptera. In: Tuxen, S.L. (Ed.), *Taxonomist's glossary of genitalia in insects*. 2 ed. Munksgaard, Copenhagen, pp. 115-130.
- Kristensen, N. P. (Ed.) (2003) *Lepidoptera, Moths and Butterflies. Part 36. Vol. 2. Morphology, Physiology, and development*. Walter de Gruyter & Co., Berlin & New York, 564 pp.
- Stichel, H. 1904. Lepidoptera Rhopalocera Fam. Nymphalidae, Subfam. Brassolinae. *Genera Insectorum* 20: 1-48.
- Stichel, H. 1932. Brassolidae. In: Strand, E. (ed.), *Lepidopterorum Catalogus*, Pars 51. W. Junk, Berlin: 1-115.

APPENDIX 1

DNA barcode of holotype male 06-SRNP-34098.

MHAAC456-07|JQ548486|06-SRNP-34098|*Opsiphanes jacobsoni*|COI-5P

ACTTTATATTTTATTTTGGAAATTTGAGCAGGCATAGTAGGAACATCTTTAAGACTTAT-
CATTGGAACAGAATTAGGTAATCCAAGTTTTTTAATTGGAGATGATCAAATTTATAATA-
CAATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTG-
GAGGATTTGGAAATTGATTAGTTCCTCTAATATTAGGAGCCCCGTATATAGCTTTCCCC-
GAATAAATAATATAAGTTTCTGACTCCTTCCCCCTTCCCTAATACTTTTAATTTCAAGAAGT-
ATTGTTGAAAATGGAGCCGGAACAGGATGAACAGTGTACCCCCCACTTTCATCTA-
ATATTGCTCATGGAGGATCATCTGTTGATTTAGCTATTTTTTCTTTCATTTAGCCGG-
TATTTCTTCTATTTTAGGAGCAATTAACCTTTATTACTACAATTATTAATATACGAATTAATA-
ATATATCCTATGATCAAATACCTTTATTTGTATGAGCAGTTGGTATTACAGCTCTTTTAT-
TACTTCTTTCTTTACCAGTTTTAGCAGGTGCTATTACTATATTATTAACAGATCGTAATTTA-
AATACTTCATTTTTTGACCTGCAGGTGGAGGAGATCCTATTTTATACCAACATCTA

Numbers of the *Bulletin of the Allyn Museum* are published at irregular intervals.
Price \$ 2.35