



Oxynetra: Facies and DNA Barcodes Point to a New Species from Costa Rica (Hesperiidae: Pyrginae: Pyrrhopygini)

Authors: Grishin, Nick V., Burns, John M., Janzen, Daniel H., Hallwachs, Winnie, and Hajibabaei, Mehrdad

Source: The Journal of the Lepidopterists' Society, 67(1) : 1-14

Published By: The Lepidopterists' Society

URL: <https://doi.org/10.18473/lepi.v67i1.a1>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Journal of the Lepidopterists' Society
67(1), 2013, 1–14

OXYNETRA: FACIES AND DNA BARCODES POINT TO A NEW SPECIES FROM COSTA RICA
(HESPERIIDAE: PYRGINAE: PYRRHOPYGINI)

NICK V. GRISHIN

Howard Hughes Medical Institute and Departments of Biophysics and Biochemistry, University of Texas Southwestern, Dallas, TX 75390;
email: grishin@chop.swmed.edu

JOHN M. BURNS

Department of Entomology, National Museum of Natural History, Smithsonian Institution, Box 37012, Washington, DC 20013-7012;
email: burnsj@si.edu

DANIEL H. JANZEN

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

WINNIE HALLWACHS

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

AND

MEHRDAD HAJIBABAEI

Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G2W1, Canada;
email: mhajibab@uoguelph.ca

ABSTRACT. *Oxyetra stangelandi* Grishin & Burns, new species, from high elevations of Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica, is most similar to *Oxyetra hopfferi* Staudinger, known from mountains of central and southern Costa Rica and western Panama. These hesperiid species differ mainly in body color pattern and in DNA barcodes. We compare their barcodes, nucleotide by nucleotide, together with barcodes of a congener and a species of the related genus *Olafia*, and use the barcode data to show phylogenetic relationships. We describe the new species, its discovery, its male and female genitalia, and its life history as a cloud forest herbivore of *Prunus annularis* (Rosaceae). In ACG, no other skippers feed on this plant species, and no other skippers of the tribe Pyrrhopygini feed on plants in the family Rosaceae. Various stages of *O. stangelandi* belong to mimicry complexes. Although our adults, which are reared from wild-caught caterpillars, are split between the sexes (4 males, 6 females), there are scarcely any females of *Oxyetra* in the world's museums.

Additional key words: cryptic species, sexual dimorphism, genitalia (male and female), mimicry, *Prunus annularis*.

Some butterflies depart from the usual lepidopteran show of opaque scales by evolving wings that are partly to fully transparent. Among skipper butterflies of the tribe Pyrrhopygini, many of which are gaudy, certain members of the genus *Oxyetra* caught our attention owing not only to extensive alar transparency but also to a troubling lack of taxonomic transparency.

The neotropical genus *Oxyetra* C. & R. Felder, 1862—proposed for *O. semihyalina* C. & R. Felder, 1862—currently comprises three species: *O. semihyalina*, *O. confusa* Staudinger, 1888, and *O. hopfferi* Staudinger, 1888. Both sexes of *O. semihyalina*, and especially of *O. confusa*, have extensively transparent wings, with forewings whose hyalinity is primarily medial but also subapical. In males of *O. hopfferi*, the medial hyalinity is reduced and the subapical hyalinity is lacking; but in females, there is

none at all—the wings are entirely black. Given such extreme sexual dimorphism, it is not surprising that the female was originally described as a distinct species in its own genus, *Dis annulatus* Mabille, 1889. However, Godman and Salvin (1893) correctly synonymized genus *Dis* with *Oxyetra* and argued that the female of *O. annulatus* might prove to be that of *O. hopfferi*. Evans (1951) formally synonymized *O. annulatus* with *O. hopfferi*, an action that prevails (Mielke 2005).

Unlike its congeners, *O. hopfferi* is rare in collections, as are females of *Oxyetra* generally (e.g., for BMNH, Evans [1951] records 99 males of *O. semihyalina* and 44 males of *O. confusa*, mainly from Bolivia and especially Peru, but no females of either species). After 22 years of intensive continuous collecting and rearing in Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica (Burns & Janzen 2001, Janzen et al. 2009), the first

O. hopfferi-like specimen surfaced in July 2001, too late to appear in the detailed account (then in press) of pyrrophygine skippers of ACG (Burns & Janzen 2001). This unforeseen species adds a new larval foodplant, *Prunus annularis* Koehne, and a new family, Rosaceae, to those eaten by Pyrrophygini in ACG (Janzen & Hallwachs 2012).

That 2001 specimen, a male (voucher code 01-SRNP-6995), has—in contrast to the multi-orange-banded abdominal pattern of male *O. hopfferi*—a single orange band on abdominal tergum III. However, one (02-SRNP-23283) of two males reared in 2002, has, in addition to the bold orange band on tergum III, some sparse orange scaling on tergum IV; and the male (03-SRNP-3638) reared in 2003 has a scattering of 23 orange scales there. Despite such individual variation, the general effect is one band.

Multiple rearings of phenotypically similar wild-caught caterpillars unequivocally associate the dimorphic sexes, and DNA barcoding confirms the association. Barcodes of all 10 ACG specimens are identical (Fig. S1 of Janzen et al. 2011).

In early 2011, when Mike Stangeland began preparing photographs of reared *Oxyntera* males for the Butterflies of America (BoA), website <http://butterfliesofamerica.com> (Warren et al. 2012), he noticed that they differed from photographs of *O. hopfferi* males in other collections and raised the possibility of a new taxon. Subsequent investigation, assisted by many collaborators, indicates that the northwestern Costa Rica specimens represent an undescribed species, closely related to, but distinct from, *O. hopfferi*.

Oxyntera stangelandi Grishin & Burns, new species (Figs. 1a, b, 2a–h, 3a, 5, 7, 8)

Description. *Size:* Male smaller than female. Forewing length (mm) of four males: 17.1, 18.1, 18.2, 18.3; of six females: 20.2, 20.7, 21.1, 21.5, 22.2, 22.6. (These measurements will average less than those of wild-caught adults, because conditions and duration of rearing often stunt growth.)

Nudum of antennal club: Fewer segments in male than in female. Number of segments in left nudum/right nudum of four males: 20/20, 20/20, ?/20, 22/22; of five females: 24/23, 23/25, 25/25, ?/25, 26/25. (Number of nudum segments not correlated with wing length.)

Facies: Pronounced sexual dimorphism:

Facies, male: Hindwing (HW) narrow and elongate; forewing (FW) extending well beyond it. Outer wing margin very slightly concave at cell $CuA_2-1A+2A$ of FW and at cells between veins M_1 and M_3 of HW. Dorsal and ventral FW (including fringe) brownish-black with blue/purple sheen (except for some whitish fringe scales at cell $CuA_2-1A+2A$ in three of four males), and with bold, median, wide, straight-edged, hyaline band from anterior edge of discal cell to vein $1A+2A$; band divided into three large, aligned parts by dark-scaled veins CuA_1 and CuA_2 ; its outer edge evened by tiny, triangular, hyaline wedge at very base of cell M_3-CuA_1 . Dorsal HW concolorous with FW (except fringe around tornus white), with two median, large, roughly rectangular, aligned, hyaline spots (in cell $Sc+R_1-R_2$ and discal cell), separated by vein R_2 , and suggesting continuation of FW band; three

of four males with small, postmedian pair of hyaline spots in proximal ends of cells M_3-CuA_1 and CuA_2-CuA_3 (these spots expressed only ventrally in fourth male). Ventral HW similar to dorsal, but with wing base white and with two small, opaque, white spots in cell $CuA_2-1A+2A$, one median and one submarginal (the latter spot looking more or less dual). Scaled antenna black; nudum medium brown. Head and body primarily brownish-black with aqua to blue/purple sheen, but marked as follows: two tiny white spots at base of antenna and one small spot at dorsoposterior margin of eye; first and second segments of palpus ventrally white, with white continuing, without interruption, in midventral strip as far as base of foreleg, and thereafter in separate midventral patches between bases of midlegs, of hindlegs, and at posterior margin of each sternite (some abdominal patches resembling transverse dashes and progressively decreasing in size). Large orange spot on anterior 40% of tegula. Orange band (with slight middorsal notch in anterior margin) across tergum III.

Facies, female: Remarkably different from male. HW relatively broad and rounded. Both wings uniformly brownish-black, above and below, with strong, purplish, metallic sheen (imparting “greasy” appearance). HW fringe narrowly white, FW fringe variably so (never at apex). White head and body markings of male greatly reduced or not expressed: spot at dorsoposterior margin of eye present, but only one white spot at base of antenna (on anterior side); ventral white of basal segments of palpus narrow, and not continued posteriorly, except for short, vestigial, midventral dashes at posterior margin of abdominal segments. Tegula all black. Conspicuous orange band across tergum III, as in male, except for narrow middorsal break.

Genitalia, male (Fig. 3a): Tegumen about twice as wide as high and about twice as long as uncus; supported ventrally, on each side, about halfway along its length; distal end with small middorsal knob, flanked by pair of distally directed, blunt, rudimentary projections. Uncus narrower than tegumen and tapering slightly toward distal end; distal end with medial V-shaped notch; short fat prong on either side of notch curved ventrad, terminating in blunt point. Valva with body prominently humped and appearing triangular in lateral view; distal division narrowed, tapered, curved dorsad, and dentate along anterior edge; ventral inner side of valva giving rise to two dorsal projections: proximal one low, less obvious, and wide, with uneven, finely dentate dorsal edge; distal one long, narrow, curved mediad, dentate, and sharply pointed. Saccus very short. Penis more or less uniform in diameter and conspicuously humped near distal end; no cornuti.

Genitalia, female (Fig. 5): Lamella postvaginalis—lying just below, and extending well distad of, ovipositor lobes—shaped like wide flat paddle, expanding somewhat in thickness and width along its length; distal end rounded, with slight medial notch. Lamella antevaginalis narrowly extending dorsad, from dorsolateral rim of ostium bursae, almost as far as dorsal edge of ovipositor lobes; its surface indented and appearing wavy at some angles. Ostium bursae remarkably large, elliptical, and well-sclerotized, forming posteriorly directed cup. Ductus bursae membranous, initially about half diameter of ostium bursae, then abruptly decreasing in diameter, changing direction, and leaving blind pocket at anterior end of large-diameter portion of ductus bursae. Ductus seminalis joining ductus bursae middorsally, slightly anterior of sclerotized ostium bursae cup. Corpus bursae membranous.

Holotype (Fig. 1a): Male, voucher code 02-SRNP-23284, Sendero Derrumbe, 1220 m, Sector Cacao, Area de Conservacion Guanacaste, Costa Rica, lat 10.92918, long -85.46426, collected in penultimate larval instar by Harry Ramirez [deposited in USNM]. Yellow label reads “LEGS AWAY/FOR DNA.”

DNA barcode (633 bp) of holotype:

```
AACTTTATATTTTATATTTTGGAAATTTGACGAGGAATAATTGGAA
CTTCATTAAGATTACTAATTCGAACTGAATTAAGTACCCCCGG
ATCTTTAATTGGAAATGACCAAATTTATAACACTATTGTAACAG
CTCATGCAATTTATTAATTTTTTTTATAGTTATACCTATTATAA
TTGGAGGATTTGGAAATTCATTAGTTCCCTTTTAATATTAGGAGC
TCCAGATATAGCTTTCCCTCGAATAAATAATATAAGATTTTGAT
TATTACCTCCATCTTTAACTCTTTTAATTTCAAGAAGAATTGTA
GAAACCGGTGCTGGAAGTGGATGAACACTTTCCCTCTCTCTCT
TTCTTCTAATATTGCCCATCAAGGAACCTTCGATTGATTAGCT
```

ATTTTCTCTTCATTTAGCTGGAATTTCTTCAATTTAGGGG
 CTATTAATTTTATTACAACAATTATTAAATATACGAATTAATAAATT
 TATCTTTTGACCAAAATACCTCTTTTGTGTGAGCCGTAGGAAT
 TACTGCATTTATTATTATTATCTTTACCTGTATTAGCAGGTG
 CTATTACTATACTTTTAAACAGACCGAAATATTAATACTTCCTTT
 TTTGATCCTGCAGGAGGAGGAGA

Paratypes (Figs. 1b, 2a–h): 3 males (voucher codes 01-SRNP-6995, 02-SRNP-23283, 03-SRNP-3638), 6 females (02-SRNP-23109, -23110, -23285, -23286, -23540, -24529) from Sendero Toma Agua, 1140 m; Estacion Cacao, 1150 m; and Sendero Derrumbe, 1220 m—all in Sector Cacao, Area de Conservacion Guanacaste, Costa Rica [deposited in USNM]. For further details, see Janzen & Hallwachs 2012.

Larval habitat; elevation range: Cloud forest. Type specimens collected at 1140, 1150, and 1220 m.

Foodplant: *Prunus annularis* (Rosaceae).

Etymology: Named in honor of Mike Stangeland—a research associate of the McGuire Center for Lepidoptera and Biodiversity at the Florida Museum of Natural History, University of Florida, and the photo editor and administrator of BoA—who has prepared >100,000 butterfly images for online display.

Diagnosis. Males of *O. stangelandi*, with one orange band across tergum III, differ from males of sister species *O. hopfferi*, with their five-banded abdomen (and even from hesperiids generally [except for *O. semihyalina*, which has a single, pale orange band]). In both sexes, the orange of the abdominal bands is redder in *O. stangelandi* than it is in *O. hopfferi*. Males of *O. stangelandi* lack *O. hopfferi*'s narrow streak of whitish hairlike scales on the dorsal HW near the middle of cell 1A+2A–A3 and parallel to its bounding veins. The number of nudum segments in *O. stangelandi* (ranging in four males from 20 to 22) may exceed that of *O. hopfferi* (whose three inspected males have only 17, 18, and 19 segments).

In females of *O. stangelandi*, as opposed to those of *O. hopfferi*, the ventral white on palpal segments one and two is relatively restricted and narrow, and the adjacent thoracic venter is black instead of white. The black middorsal break in the orange band across tergum III appears slightly narrower in *O. stangelandi* than in *O. hopfferi*. Wing fringes are predominantly white in *O. stangelandi* but, with one exception, not in *O. hopfferi*.

The mitochondrial nucleotide sequence in the C-terminal segment of COI (i.e., the DNA barcode) of *O. stangelandi* is unique; and it differs from those of *O. hopfferi* by 2.6% to 3.2%. This critical distinction is analyzed in detail below, where it is also treated in a broader context.

DISCUSSION

Genitalia. Do male genitalia distinguish *O. stangelandi* from *O. hopfferi*? Close, side by side comparison of the dissections that appear in Fig. 3, as well as another, reveals various small differences. These include a larger middorsal knob at the distal end of the tegumen, a more massive, more dentate distal division of the valva, a longer, more dentate pointed projection

from the inner side of the valva, and a longer penile hump in *O. hopfferi* than in *O. stangelandi*. Judging from over half a century of perusing countless skipper genitalia, Burns cautions that these apparent differences may reflect nothing more than individual variation. No matter how simple their form, genitalia vary appreciably within a population; and individual variation generally becomes more evident as genitalic complexity increases. Given our minimal genitalia samples—two complete dissections of *O. stangelandi* and one of *O. hopfferi*, plus photographs of valval exteriors (in situ, and partly exposed by dusting) of two other specimens of *O. hopfferi*—we cannot identify interspecific differences with certainty. A difference we once considered potentially valuable in telling *O. stangelandi* and *O. hopfferi* apart involves the dorsal margin of the valval body, distal to its pronounced hump: in lateral view, this margin makes an even, concave curve down to a slightly upturned, rounded, protruding base in *O. hopfferi* (Fig. 3b) but a straight line to a sharp, wide, basal angle in *O. stangelandi* (Fig. 3a). No such difference marks the second example of *O. stangelandi*.

However, relatively minor genitalic differences may mean more in *Oxyetra* than they do in many other genera, because the genitalia of all four species are conservative variations on a single complex theme. Even though the facies of *O. semihyalina* and *O. confusa* (Fig. 6) differ considerably from those of *O. hopfferi* and *O. stangelandi* (Figs. 1, 2), their genitalia (Fig. 4) do not. Features showing interspecific variation between *O. semihyalina* and *O. confusa* include the distal division of the valva and the penile hump, which are features noted above that may distinguish *O. stangelandi* from *O. hopfferi*. The differences in the distal division of their valvae (compare the oblique views of genitalia in Figs. 3a and 3b) look especially promising for species discrimination. (For an example of intraspecific variation in genitalic form, compare photos of the male genitalia of *O. confusa* in Orellana 2008:fig. 212 with Fig. 4b.)

Barcode differences and intrageneric phylogeny.

We obtained barcode sequences for the entire type series of *O. stangelandi* and for three *O. hopfferi* specimens. All 10 of our *O. stangelandi* sequences are identical within their sequenced length, and all specimens are from the same locality. Sequences of the three *O. hopfferi* specimens differ from each other by 2 or 4 nucleotides, which amounts to 0.3% and 0.6% intraspecific differences (Fig. 7a); and these three specimens are from different localities within Costa Rica. The *O. stangelandi* sequence differs from those of *O. hopfferi* by 17, 19, and 21 nucleotides (2.6%, 2.9%, 3.2% differences). Differences of this magnitude are



FIG. 1. *Oxynetra* type specimens. **a**, *O. stangelandi* holotype, male, ACG, Costa Rica, voucher code 02-SRNP-23284. **b**, paratype, female, ACG, Costa Rica, 02-SRNP-23286. **c**, *O. hopfferi* holotype, male, Chiriqui, Panama, leg. Ribbe, Staudinger Collection [ZMHB]; with specimen labels. **d**, *Dis annulatus* holotype, female, Chiriqui, Panama, leg. Trötsch, Staudinger Collection [ZMHB]; with specimen labels. Each specimen in dorsal (above) and ventral (below) view.



FIG. 2. *Oxynetra* adults. **a-h**, *O. stangelandi*. **i-q**, *O. hopfferi*. **f-l, n-o, q**, males (banded wings). **a-e, m, p**, females (unmarked wings). **Subscripts:** **v**, ventral view; **l**, live individual; **a**, magnified portion of dorsal hindwing near anal fold. **i-k**, from Puntarenas, COSTA RICA. **l-q**, from Chiriqui, PANAMA. **n**, illustration of *O. hopfferi* holotype from original description (Staudinger 1888). **o-p**, illustrations of *O. hopfferi* and *Dis annulatus* holotypes from Godman & Salvin (1893). **q**, illustration of *O. hopfferi* holotype from Draudt (1921), apparently copied from Staudinger illustration (n) instead of drawn from specimen. Voucher codes of Costa Rican paratypes: **a**, 02-SRNP-23540; **b**, 02-SRNP-23109; **c**, 02-SRNP-23285; **d**, 02-SRNP-23110; **e**, 02-SRNP-24529; **f**, 03-SRNP-3638; **g**, 01-SRNP-6995; **h**, 02-SRNP-23283. **i**, Coto Brus, Sabalito, Cotoncito, 1500 m, 8.943, -82.787, Puntarenas, COSTA RICA, 14 September 2006, R. Gonzalez [INBio]; **j**, Coto Brus, Sabalito, Estacion Biologica Las Alturas, 1500 m, 8.949, -82.836, Puntarenas, COSTA RICA, 31 January 1992, M. Zumbado [INBio]; **k**, 97-ZFuentes-055: Peñas Blancas, Monteverde, Puntarenas, COSTA RICA, 1997, Z. Fuentes [USNM].

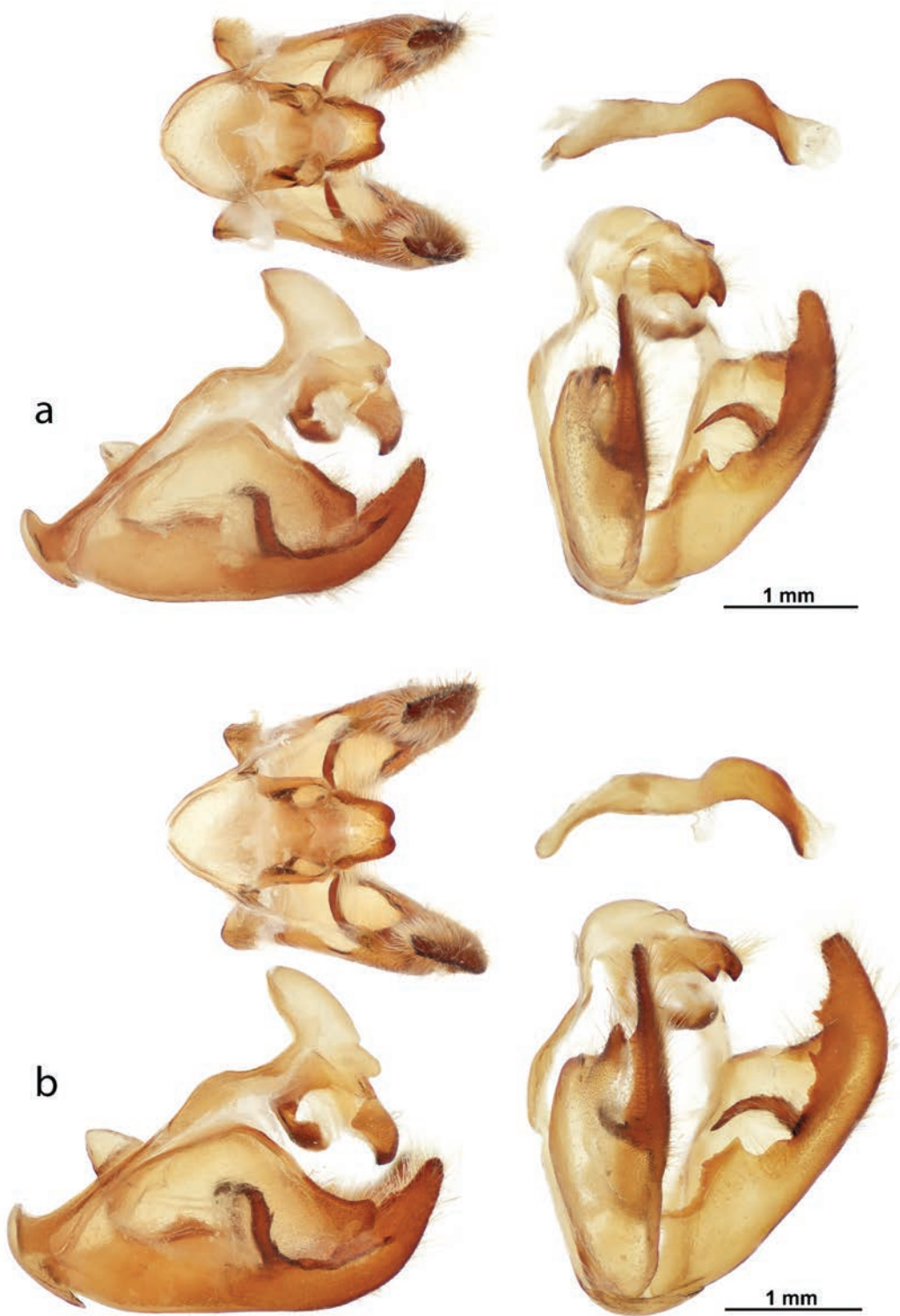


FIG. 3. *Oxyetra* male genitalia. **a**, *O. stangelandi*, dissection code X-6958. ACG, COSTA RICA, voucher code 02-SRNP-23283 [USNM]. **b**, *O. hopfferi*, dissection code X-6959. Peñas Blancas, Monteverde, Puntarenas, COSTA RICA, 1997, Z. Fuentes, voucher code 97-ZFuentes-055 [USNM]. Left side: genitalia (minus penis) in dorsal and left lateral views. Right side: penis in left lateral view, genitalia (minus penis) in oblique view.

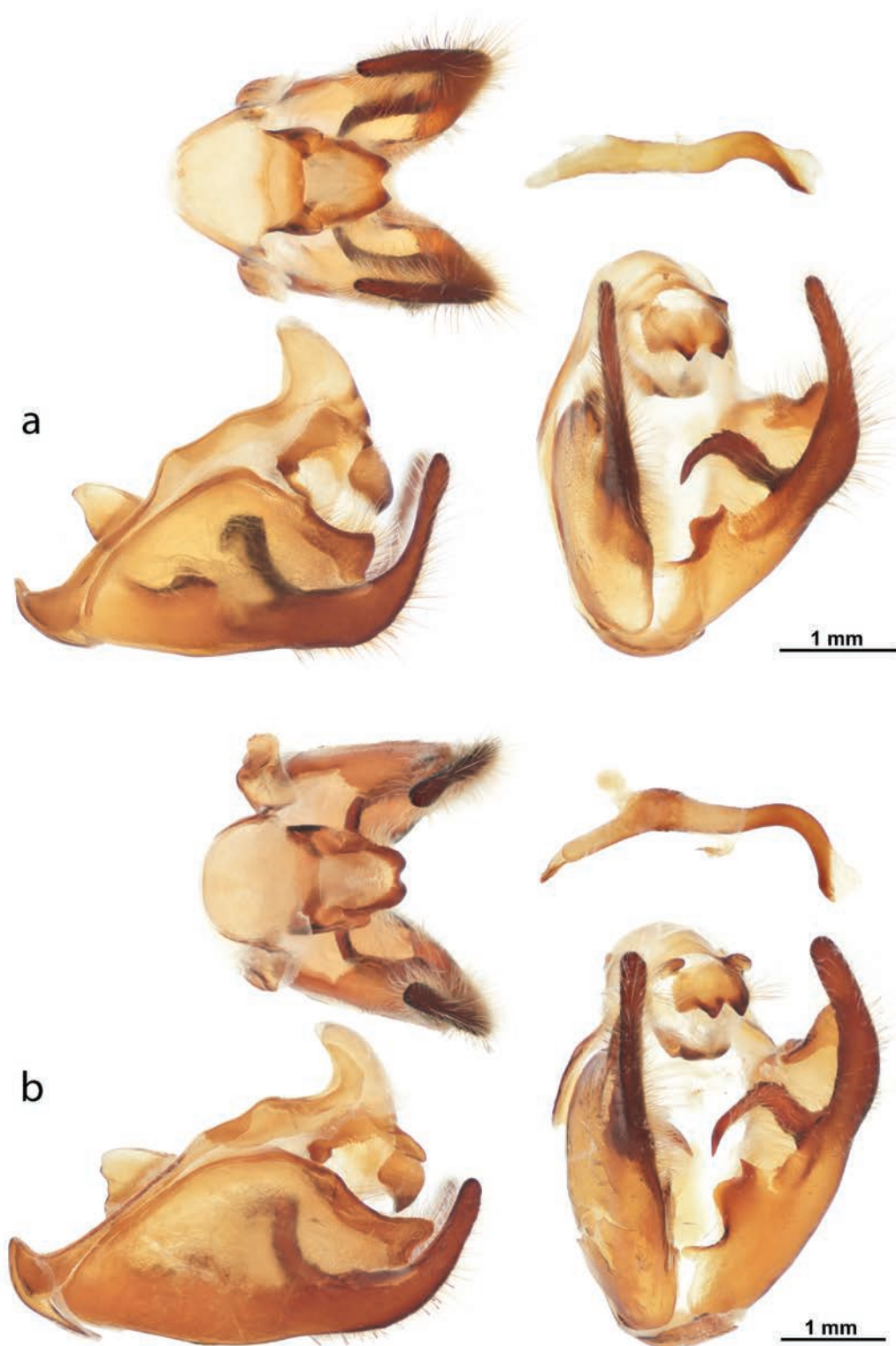


FIG. 4. *Oxyetra* male genitalia. **a**, *O. semihyalina*, dissection code X-6976. Tingo Maria, 300 m, Huanuco, PERU, 24 June 1982, S. S. Nicolay [USNM]. **b**, *O. confusa*, dissection code X-6977. Carpish, 3000 m, 9° 42' S, 76° 04' W, Huanuco Dept., PERU, February 1992 [USNM]. Left side: genitalia (minus penis) in dorsal and left lateral views. Right side: penis in left lateral view, genitalia (minus penis) in oblique view.

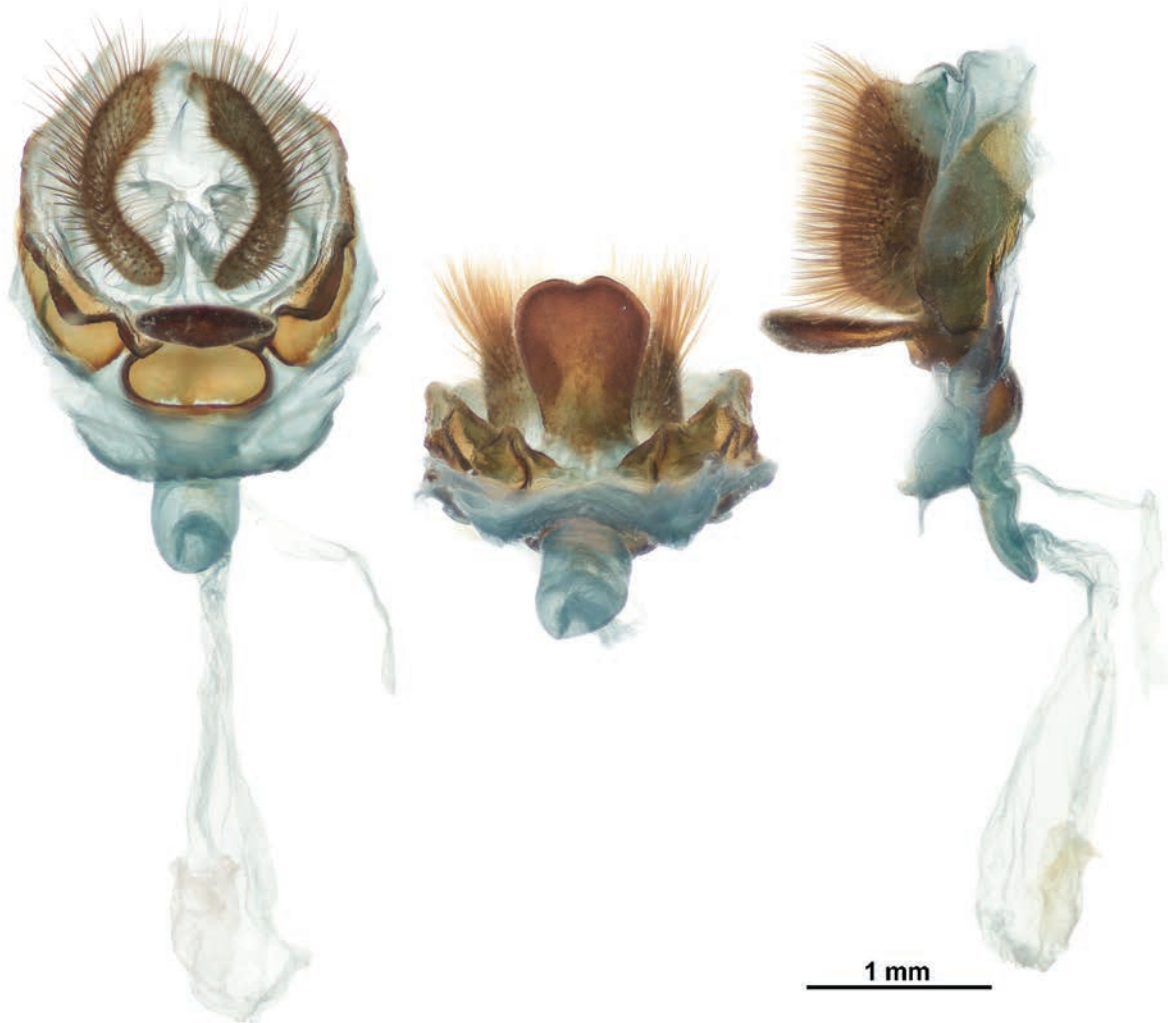


FIG. 5. *Oxynetra stangelandi* female genitalia. Dissection code X-6960. ACG, COSTA RICA, voucher code 02-SRNP-23285 [USNM]. Left: posterior view. Middle: ventral view. Right: right lateral view.

consistent with interspecific distances found in other similar sets of hesperiid species. Moreover, in some unquestionably valid cryptic species, interspecific distances are much smaller: in large ACG samples of *Perichares*, for example, *P. adela* (Hewitson) differs from *P. prestoeaphaga* Burns by about 0.6%, from *P. geomaphaga* Burns by about 0.8%, and *P. prestoeaphaga* from *P. geomaphaga* by about 0.7% (Burns et al. 2008). Indeed, some closely related skipper species may differ by only 1 to 3 nucleotides (Burns et al. 2007).

To compare the differences between *O. stangelandi* and *O. hopfferi* to differences between them and

species that morphologically appear related but more distant, we queried BLAST search <<http://www.ncbi.nlm.nih.gov/blast>> for publically available sequences most similar to that of *O. stangelandi* and obtained barcodes of *O. confusa* and *Olafia rosci* (Hopffer, 1874) (Fig. 7a). *Olafia* is the genus most similar to *Oxynetra*; and its sole species, *Ol. rosci*, was previously treated as *Oxynetra* (Evans 1951). The *O. stangelandi* sequence differs from sequences of *O. confusa* and *Ol. rosci* by 35 and 36 nucleotides, respectively (around 5.5%). Though smaller, the barcode distances between *O. stangelandi* and *O. hopfferi* are still comparable to those between *O.*

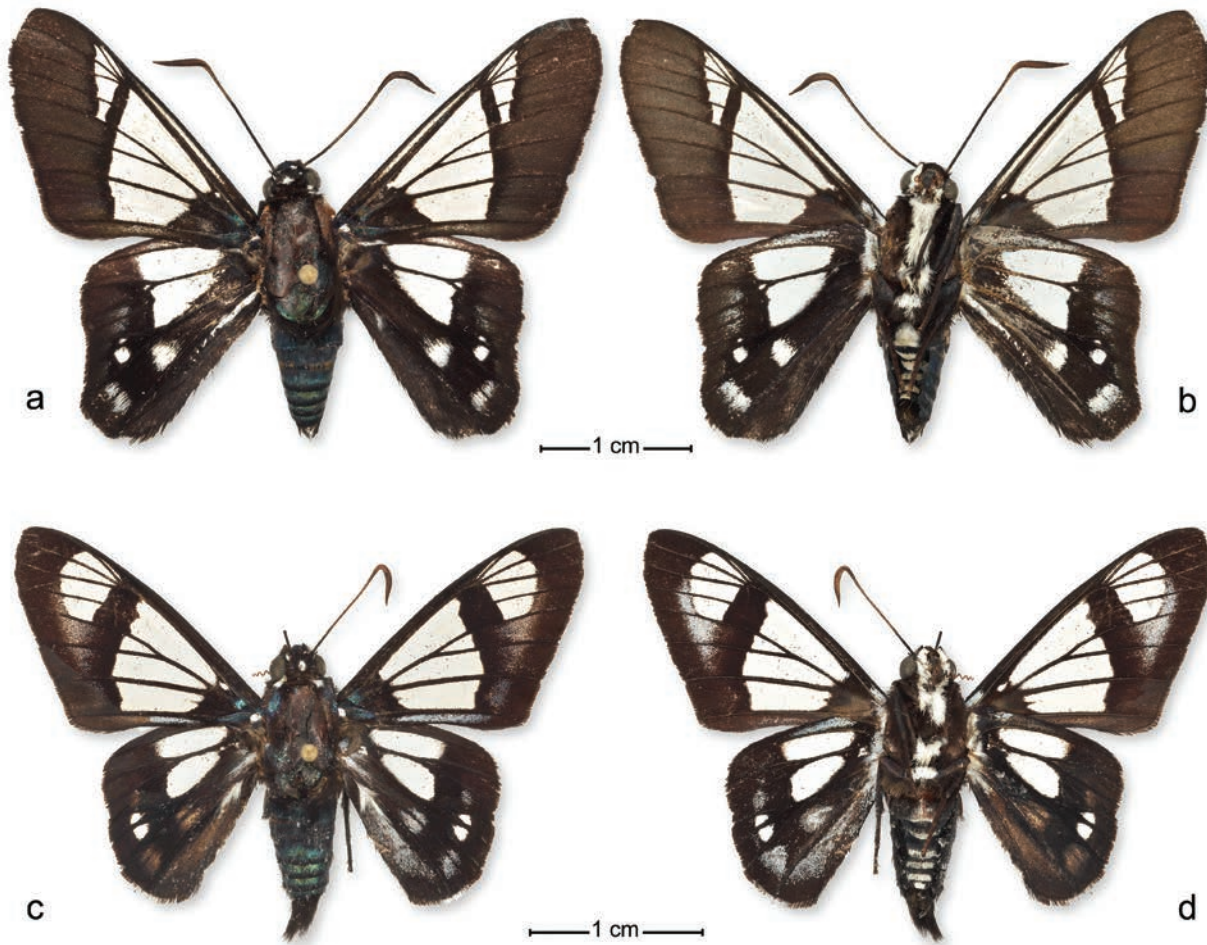


FIG. 6. *Oxynetra* adult males. **a–b**, *O. semihyalina*, Carpish, PERU, May 1992 [USNM]. **c–d**, *O. confusa*, Carpish, 3000 m, 9° 42' S, 76° 04' W, Huanuco Dept., PERU, February 1992 [USNM]. **a, c**, dorsal view. **b, d**, ventral view.

stangelandi and *O. confusa*, and even to those between *Oxynetra* and *Olafia*.

Although the 654 base pair sequence is generally too short for reliable phylogenetic inference, we applied four standard phylogenetic reconstruction programs offered at <http://www.phylogeny.fr> to our genetic data. The resulting trees (Fig. 7b–e) are topologically identical and show *O. stangelandi* and *O. hopfferi* as sister species, in agreement with the phenotypic data. Branch lengths in the trees in Fig. 7b, c, and d are to scale and graphically show the extent of intraspecific variation in *O. hopfferi* and how it scales to interspecific differences.

Distribution, ecology, life history, behavior, and mimicry. So far, *O. stangelandi* is known only from the 1,000–1,500 m zone of cloud forest covering the top of Volcan Cacao (centered by latitude 10.93328, longitude -85.45729) in central ACG. The enormous rearing project in ACG, which is often coupled with mass barcoding of the reared specimens, has led to the description of many new insect species solely from ACG material; but this does not mean that these species are extremely limited in their geographic distribution.

The shrub-treelet foodplant, *Prunus annularis* (Rosaceae), occurs throughout the understory and edges of this dense forest and reaches a height of 5 m.



FIG. 8. *Oxynetra stangelandi* immatures. a–d, f–m, caterpillars. e, n–z, pupae. Voucher codes: a–d, f–h, n–r, 02-SRNP-23109; e, x, 02-SRNP-23283; i–m, 02-SRNP-23110; s–w, y–z, 02-SRNP-23284 (holotype).

Caterpillars of *O. stangelandi* have been found feeding only on this species; and since almost all other species of plants in this habitat have been intensively searched for over 20 years (Janzen & Hallwachs 2012) for any and all caterpillars (>30,000 records), we assume that *O. stangelandi* is monophagous. Because it is not known to share this foodplant with other species of Hesperidae, we infer that empty hesperiid larval shelters on *P. annularis* belong to this species.

The first individual of *O. stangelandi* (01-SRNP-6995) was found as a pupa (2 July 2001; eclosed 26 July 2001) in a leaf shelter it had made in the top of an unknown 60 cm tall understory sapling below a 3–4 m tall *P. annularis*. Skipper caterpillars often construct pupal shelters on plants adjacent to, but different from, their foodplant. A year later, various instars of *O. stangelandi* were found on *P. annularis* in the general vicinity (Janzen & Hallwachs 2012), although at least 10 years of search of this foodplant prior to 2001 had not turned up any hesperiid larval shelters. Continued explicit and haphazard search of *P. annularis* foliage in the same general area from 2003–2011 has yielded only one more caterpillar (03-SRNP-3638), plus one parasitized pupa on a non-foodplant. *Oxyntera stangelandi* is clearly a very low density species in the 1–3 m height range easily searched by parataxonomists; however, the specimens encountered may represent only the bottom of a higher height distribution of the caterpillars. Dates of adult eclosion range from late July to late December, but the sample is too small to estimate annual phenology. A female caterpillar (02-SRNP-23285) found as second instar on 19 July 2002 and reared in a protective net placed over the foodplant, pupated 30 August 2002 and eclosed 19 September 2002 (at 1220 m elevation).

Oxyntera stangelandi caterpillars have the hairy, strikingly yellow- and black-banded body and hairy head (Fig. 8a–d, f–m) characteristic of many pyrhopygine skipper caterpillars (Burns & Janzen 2001; Janzen et al. 2009). They are no doubt part of a generally aposematic mimicry system involving hundreds of other ACG caterpillars. Likewise, orange eyespots on the black head of *O. stangelandi* caterpillars place them in a mimicry complex that comprises hundreds of species of ACG caterpillars exhibiting a great diversity of false eyespot patterns (Janzen et al. 2010). Moreover, the mature pupa is basically white (with small orange and black spots, Fig. 8e, s–z), resembling the white pupae of many other shelter-inhabiting ACG skipper species that mimic pupae attacked by fungi. Pupae that are fungal victims usually become white, highly inedible, and even gustatorially dangerous (see images in Janzen & Hallwachs 2012). In the newly molted *O. stangelandi*

pupa, the head, pronotum, and appendages, including wings, look more or less orange and contrast with dull maroon dorsolateral bands on the cream to tan ground color of the rest of the body (Fig. 8n–r). After about two days, a white waxy layer is exuded over the surface, giving the pupa the white appearance that remains until eclosion.

Although no adult *O. stangelandi* has been observed in the field, its behavior, when newly eclosed in a large rearing container, is unusual. Newly eclosed ACG hesperiids, in many thousands of cases and across more than 400 species, hold their wings together over their back during hardening, and then usually keep them in that position while walking and perching in the rearing containers. However, both sexes of *O. stangelandi* harden their wings in the position shown in Fig. 2h₁ and hold them that way while walking with the characteristic gait and slight up-and-down wing motion of a large ctenuchine arctiid moth (many species of which are diurnal, brightly colored, transparent-winged, and widely believed to be chemistry-based aposematic or Hymenoptera mimics). Though aware that the male in Fig. 2h₁ must be a hesperid that he had photographed repeatedly as a developing larva and pupa, Janzen had to look twice to reassure himself that it was not a contaminating arctiid whose cocoon had inadvertently come in with food foliage. While the striking colors of the male may serve in courtship and the black color of the female in heat absorption in cold cloud forest, these colors also function in at least two mimicry rings.

History of discovery. We initially addressed Stangeland's suggestion that the reared ACG material might not be *O. hopfferi* by procuring a specimen whose facies closely match those of the holotype (male) of *O. hopfferi* (Fig. 1c). The new specimen (voucher code 97-ZFuentes-055 [deposited in USNM]) is a male that eclosed from a pupa collected in 1997 in Peñas Blancas, Monteverde, Puntarenas Province, Costa Rica, which is about 100 km southeast of ACG and considerably closer to the *O. hopfferi* type locality (Chiriqui). This male has the characteristic *O. hopfferi* abdomen with five orange bands, as well as a hindwing with a short, narrow white streak in cell 1A+2A–3A. One of its legs, sacrificed to DNA sequencing, yielded a barcode that differed by 2.6% from the uniform barcodes previously obtained for all ten ACG specimens. Subsequent comparison of genitalia gave the uncertain result discussed above.

Our need of a larger sample of *O. hopfferi* to assess variation was frustrated by the rarity of this species in collections. In addition to the Monteverde specimen (Fig. 2k) and the holotypes of *O. hopfferi* (Fig. 1c) and *D. annulatus* (Fig. 1d), we inspected four male

specimens, all from Chiriqui, Panama (1, BMNH; 2, ZMHB; 1, MTD). With the help of Kim Garwood and Isidro Chacón, we obtained photographs of four more specimens: three males (from Chiriqui, Panama [OM-DZUP], Fig. 2l; and Puntarenas, Costa Rica [INBio], Fig. 2i–j) and one female (from Chiriqui, Panama [OM-DZUP], Fig. 2m). The facies of our sample of nine males and two females of *O. hopfferi* from southern Costa Rica and western Panama do not vary significantly (except that fringe color is dark in the *Dis annulatus* holotype and largely white in the other female).

Oxyntera annulatus, originally described (in genus *Dis*) from an almost black female with one orange band on her abdomen, has been synonymized with *O. hopfferi*. Were *O. annulatus* the female of the ACG entity, the latter would assume that name. The holotype (Fig. 1d) exhibits a white prothoracic venter devoid of dark scales. The females of *O. stangelandi* have a fully black thoracic venter, concolorous with the rest of the body (Figs. 1b, 2a_v–e_v). Moreover, they have narrower white areas on the palpi below and a narrower dark middorsal break in the abdominal orange band, which is redder than in the *O. annulatus* holotype; and they have white wing fringes, in contrast to the mostly dark fringes of the *O. annulatus* holotype. Holotypes of both *O. hopfferi* and *O. annulatus* are from Chiriqui, Panama. We agree that *O. annulatus* is indeed a female of *O. hopfferi*. Even without knowing the locality, it is possible to associate the *O. annulatus* female with the *O. hopfferi* male, as they both exhibit prominently developed white areas on the venter, contrasting to more restricted distribution of white in both sexes of *O. stangelandi*; and orange bands on the abdomen, differing from redder bands in both sexes of *O. stangelandi*. These differences hold up well in available samples of the two species: 9 males, 2 females of *O. hopfferi*, and 4 males, 6 females of *O. stangelandi*.

Oxyntera illustrations in historic works are illuminating. Illustrations in Godman and Salvin (1879–1901) are usually more accurate than are those in Draudt (1921). However, for *O. hopfferi*, the white streak near the anal fold of the hindwing of the holotype does not appear in Godman and Salvin (vol. 3, pl. 74, fig. 18, reproduced here, Fig. 2o). Although the Draudt illustration (1921: pl. 165, row b, reproduced here, Fig. 2q) of apparently the same specimen depicts this streak clearly, it lacks the small, transparent, triangular wedge at the base of forewing cell M_3 – CuA_1 and shows 7 orange bands (6 major and 1 minor) instead of “5 bright orange-red transverse band[s] on the dorsum of the abdomen” as specified in the text (Draudt 1921:849). The Draudt illustration was probably drawn from the

illustration in the original description (Staudinger 1888: pl. 99, as *felderi*, reproduced here, Fig. 2n), because the Staudinger illustration also shows 7 orange bands.

Collaboration among researchers from five countries has resulted in rearing and recognition of a new species of *Oxyntera* that differs from its sister species, *O. hopfferi*, in body pattern and perhaps in number of antennal nudum segments and in male genitalia. Barcode differences (2.6% to 3.2%) clearly separate the two species. The male and female associations made through rearing and DNA sequencing of *O. stangelandi*, and similar sexual dimorphism in the facies of both *O. stangelandi* and *O. hopfferi*, indirectly support the synonymy of *O. annulatus* (described from a female) with *O. hopfferi* (described from a male).

ACRONYMS OF MUSEUMS

BMNH The Natural History Museum, London, England
 INBio Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica
 MTD Senckenberg Museum für Tierkunde, Dresden, Germany
 OM-DZUP O. H. H. Mielke, Curitiba, Paraná, Brazil, together with the collection of Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil
 USNM National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
 ZMHB Zoologisches Museum, Humboldt Universität, Berlin, Germany

ACKNOWLEDGEMENTS

We are grateful to Mike Stangeland for bringing phenotypic differences of the new species to our attention; Blanca Huertas and David Lees (BMNH), Wolfram Mey (ZMHB), and Matthias Nuss (MTD) for providing unrestricted access to specimens under their care and help in the collections; Gerardo Lamas and Bernard Hermier for sharing images of *Oxyntera* type specimens; Kim Garwood and Isidro A. Chacón for providing photographs of *Oxyntera hopfferi* specimens; Ernst Brockmann for barcode sequences of *Oxyntera confusa* and *Olafia roscius*; Donald Harvey for dissecting genitalia; Karie Darrow for photographing genitalia of four *Oxyntera* species, plus adults of the two South American species; Zobeida Fuentes and ACG parataxonomists Harry Ramirez, Mariano Pereira, Dunia Garcia, Manuel Pereira, and Freddy Quesada for finding and rearing *Oxyntera* caterpillars and for identifying their foodplant; Isidro A. Chacón for a specimen of *O. hopfferi*; many of the above as well as Andy Warren for discussing various aspects of this study; and two anonymous reviewers for helpful comments. 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 Conservacion Guanacaste, and University of Pennsylvania (DHJ); by the Government of Canada through Genome Canada and the Ontario Genomics Institute, and by NSERC Canada (MH).

LITERATURE CITED

- BURNS, J. M. & D. H. JANZEN. 2001. Biodiversity of pyrrhopygine skipper butterflies (Hesperiidae) in the Area de Conservación Guanacaste, Costa Rica. *J. Lepid. Soc.* 55:15–43.
- BURNS, J. M., D. H. JANZEN, M. HAJIBABAEI, W. HALLWACHS & 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.
- . 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *Proc. Natl. Acad. Sci. USA* 105:6350–6355. doi/10.1073/pnas.0712181105
- DRAUDT, M. 1921. Family: Hesperidae, Skippers, pp. 836–1011, pls. 160–193. *In* Seitz, A. (ed.), *The Macrolepidoptera of the world*, vol. 5, *The American Rhopalocera*. Alfred Kernen Verlag, Stuttgart.
- EVANS, W. H. 1951. A catalogue of the American Hesperioidea indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A, Pyrrhopyginae. *British Museum (Natural History)*, London. x + 92 pp., pls. 1–9.
- GODMAN, F. D. & O. SALVIN. 1879–1901. *Biologia Centrali-Americana. Insecta. Lepidoptera-Rhopalocera*. Vol. 2, 782 pp. Vol. 3, 113 pls.
- 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, P. BLANDIN, J. M. BURNS, J. CADIOU, I. CHACON, 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, D. LAFONTAINE, J. LANDRY, C. LEMAIRE, J. Y. MILLER, J. S. MILLER, L. MILLER, S. E. MILLER, J. MONTERO, E. 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. *Molecular Ecol. Resources* 9 (Supplement 1):1–26. doi:10.1111/j.1755-0998.2009.02628.x
- JANZEN, D. H., W. HALLWACHS & J. M. BURNS. 2010. A tropical horde of counterfeit predator eyes. *Proc. Natl. Acad. Sci. USA* 107:11659–11665. doi:10.1073/pnas.0912122107
- 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(8): e19874. doi:10.1371/journal.pone.0019874
- MIELKE, O. H. H. 2005. Catalogue of the American Hesperioidea: Hesperioidea (Lepidoptera). Vol. 1. Complementary and supplementary parts to the checklist of the neotropical region. Hesperioidea: Hesperioidea: Pyrrhopyginae. *Sociedade Brasileira de Zoologia, Curitiba, Paraná, Brazil*. XIII + 125 pp.
- ORELLANA, A. 2008. Pyrrhopyginae de Venezuela (Lepidoptera: Hesperioidea: Hesperioidea). *Entomotropica* 23:177–291.
- STAUDINGER, O. 1888. I. Theil. Exotische Tagfalter in Systematischer Reihenfolge mit Berücksichtigung neuer Arten. *In* Staudinger, O. & E. Schatz, 1884–1892, *Exotische Schmetterlinge*. Fürth, G. Löwensohn. (21): 1–2, 271–333 + [10] pp., pls. 99–100.
- WARREN, A. D., K. J. DAVIS, E. M. STANGELAND, J. P. PELHAM & N. V. GRISHIN. 2012. Illustrated lists of American Butterflies. [17–VIII–12] <<http://www.butterfliesofamerica.com>>

Received for publication 18 May 2012; revised and accepted 24 August 2012.