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GENITALIA, DNA BARCODES, LARVAL FACIES, AND FOODPLANTS PLACE THE MIMETIC SPECIES *NEOXENIADES MOLION* IN *RHINTHON* (HESPERIIDAE: HESPERIINAE)

JOHN M. BURNS

Department of Entomology, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC 127, room E-515, Washington, DC 20013-7012, USA; email: burns@si.edu

DANIEL H. JANZEN

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

WINNIE HALLWACHS

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

MEHRDAD HAJIBABAEI

Department of Integrative Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1; email: mhajibab@uoguelph.ca

AND

PAUL D. N. HEBERT

Department of Integrative Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1; email: phebert@uoguelph.ca

ABSTRACT. Facies can fool taxonomists as well as predators. *Neoxeniades molion* (Godman)—one of the many, large neotropical skippers in the major mimicry group that includes all the cryptic species of the *Astrartes fuligator* complex—is misclassified. It really relates to a species of *Rhinthon* that differs greatly from it in facies and size. In both sexes, the genitalia of *Rhinthon molion*, **new combination**, and *R. osca* (Plötz) are nearly identical, even down to a peculiar titillator on the left side of the aedeagus of the male. Males also share a secondary sex character along vein 2A of the forewing. DNA barcodes unite *R. osca* and *R. molion* in a tight, two-taxon cluster: their sequence divergence is about 3.5%. Caterpillars of the two species are alike but distinguishable, and, in Costa Rica, they have been found feeding on various native species of Marantaceae—seven of which are the same—and also on one and the same introduced species of Cannaceae (these are closely related plant families grouped in the order Zingiberales). Because *Rhinthon* is widespread and relatively speciose in Central and South America, it can no longer be considered a genus primarily of the Greater Antilles. DNA barcodes, which are useful for identifying known species and for indicating possible cryptic species, are useful in this study (in combination with other, more traditional, taxonomic characters) for pulling supposedly unrelated species together into the same genus.

Additional key words: mimicry, *Rhinthon molion*, new combination, secondary sex character, Zingiberales, Marantaceae

Genitalia and barcodes are once again prime movers. This was true when they catalyzed the shift of the hesperiine skipper *Telles arcalaus* (Stoll) to *Thracides* and, as a consequence, synonymized the former genus with the latter (Burns *et al.* 2009). *Telles* was in the K Group of Evans (1955) whereas *Thracides* is in his O Group. What male and female genitalia and DNA barcodes say now is that *Neoxeniades molion* (Godman) is a species of *Rhinthon*. Both the general appearance of caterpillars and what they eat confirm this statement. Owing to the independence of these weighty characters, their mutual reinforcement is especially significant.

Data in this study stem from large-scale rearings of wild-caught caterpillars in Area de Conservación Guanacaste (ACG), in northwestern Costa Rica (Janzen & Hallwachs 2008).

RESULTS

Adult Facies and Mimicry (Figs. 1–14). Adult facies mislead: although both *Rhinthon molion*, **new combination**, and *R. osca* (Plötz) are basically brown with prominent hyaline spots in the central forewing, *R. osca* is a medium-sized skipper with ochreous alar overscaling dorsally and a brownish body ventrally (Figs. 1–4) whereas *R. molion* is a large skipper, with blue dorsal overscaling and a yellow ventral body (Figs. 5–8). These divergent species share the following forewing hyaline spots: a large one in Cu_1 – Cu_2 , an adjacent, smaller, double one in the cell, and tiny ones in R_3 – R_4 and R_4 – R_5 . (In *R. osca*, the two parts of the double cell spot are slightly separate, resembling an = sign, in males; but they are more or less fused in females.) *Rhinthon osca* always has a tiny, distally displaced,

hyaline spot in R_5-M_1 , as well as a small spot in M_3-Cu_1 , neither of which appears in *R. molion*. (One deviant female out of 95 reared specimens of *R. osca* even has tiny hyaline spots in M_1-M_2 and M_2-M_3 .) Tiny, opaque, dorsal and ventral hindwing spots in *R. osca* are lacking in *R. molion*. On the other hand, only *R. molion* has a white costal margin on the ventral forewing and, especially, on the ventral hindwing.

It is this conspicuous, proximal, white costal margin of the ventral hindwing, plus the yellow ventral body color, the blue dorsal overscaling, the absence of the forewing spot in M_3-Cu_1 , and the absence of all hindwing spots, that place *R. molion* (Figs. 5–8) within a large, widespread, neotropical mimicry complex (28 of whose member species appear, in dorsal view, in Janzen *et al.* 2009: fig. 4). *Rhinthon molion*, a hesperiine, is exceedingly similar to the eudamine *Astraptus YESENN* (Figs. 9–12), which is one of 11 recently discovered, and provisionally named, cryptic species in the *Astraptus fulgerator* species complex (Hebert *et al.* 2004; Janzen *et al.* 2009). Mimetic convergence of *R. osca* and *A. YESENN* even includes distal development of opaque white scaling on the ventral forewing in Cu_2-2A (Figs. 6, 8, 10, 12).

Except for the degree of white scale development in Cu_2-2A , which is greater in females than it is in males, neither of these two mimetic species is sexually dimorphic in color pattern (Figs. 5–12). All three species in Figs. 1–12 express the usual hesperiid sexual dimorphism in wing shape, wherein the wings of females are broader and more apically rounded than are those of males.

Males of *R. osca* and *R. molion* share a distinctive secondary sex character, a brand comprising specialized scales (presumably for disseminating pheromone[s]) near the posterior edge of the dorsal forewing, along vein 2A, about halfway between the outer margin and the base of the wing (Figs. 13, 14). This brand is paler and more noticeable in *R. osca* than it is in *R. molion*.

Male Genitalia (Figs. 15–18). The genitalia of the *Rhinthon* males (including even their paired cornuti) look remarkably similar. When individual variation is taken into account, the genitalia are nearly identical (number of dissections compared: 6 *R. osca* and 5 *R. molion*). However, there is a distinct and consistent difference in the anterior outline of the juxta in lateral view: rounded in *R. osca* (Fig. 16) but pointed in *R. molion* (Fig. 18).

These *Rhinthon* males have two peculiar genitalic features. The aedeagus is slightly bifurcate at its ventrodiscal end, and the left bifurcation sports a long, lightly sclerotized, delicate, dentate titillator that extends posteriad and curves dorsad (Figs. 15–18). The

uncus, in a more or less posterior view (Figs. 15, 17), is hollowed out in such a way as to suggest a pitched roof above the gnathos, although, in lateral view (Figs. 16, 18), it appears to be massive and solid at its distal end. The views of the genitalia in Figs. 15 and 17 are purposely oblique, mainly to show both the rooflike form of the uncus (something that the usual dorsal view does not do) and the bifurcation at the distal end of the aedeagus, but also to convey more about the dentation at the distal ends of the symmetrical valvae than is evident in lateral view.

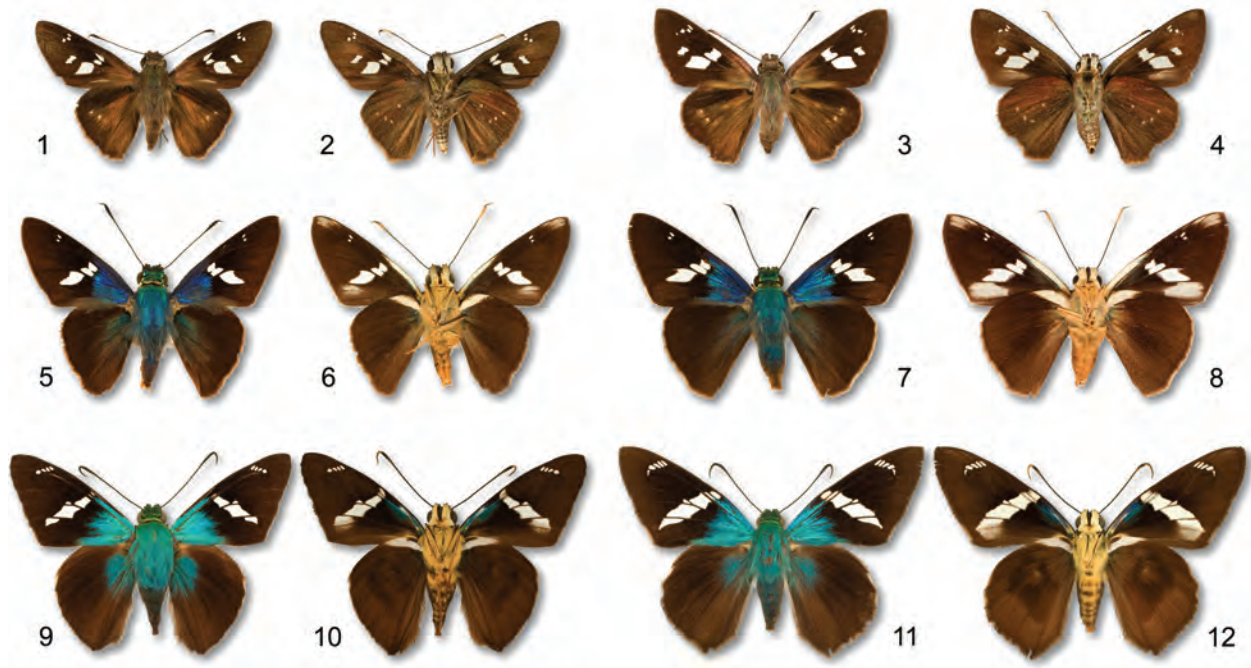
The steeper pitch of the uncus roof in Fig. 15 as opposed to Fig. 17 is not an interspecific difference. It merely reflects slightly different angles of observation and the fact that the uncus of the individual in Fig. 17 happens to be less laterally compressed. These figures are not strictly comparable in some other respects as well, e.g., the right valva of the male in Fig. 17 is, by chance, somewhat splayed.

Female Genitalia (Figs. 19–21). The female genitalia are also similar (number of dissections compared: 6 *R. osca* and 5 *R. molion*). A notable common feature is the greatly reduced (and lightly sclerotized) lamella antevaginalis, ventral to the ostium bursae. A more or less distinguishing (though variable) feature is the shape of the posterior edge of the lamella postvaginalis, on either side of its prominent, midventral, V-shaped notch: in outline, that edge is less rounded in *R. osca* (Fig. 19) than it is in *R. molion* (Fig. 21).

Because female genitalia are often less fully and less heavily sclerotized than are those of the male, they can be more individually variable. As always, in comparing genitalia in order to distinguish between individual and interspecific variation, the state of the dissection and the viewpoint of the observer are critical. Seeming interspecific differences in the genitalia shown in Figs. 19 and 21 are mostly artifactual. For example, the lamella postvaginalis is, by chance, splayed in Fig. 19 but not in Fig. 21 (moreover, as a result, overlying tergum VIII appears in Fig. 19 but not in Fig. 21). Orientation of dissection X-6399 so as to give a good view of the sterigma (Fig. 21) foreshortens some other features, especially the ovipositor lobes (whose real length is evident in Fig. 20).

The ovipositor lobes, in lateral view (Fig. 20), have a straight distal edge; and their large setae are peripheral rather than generally distributed (Figs. 19–21). Together, the ovipositor lobes present to the outer world a flat face, ringed with long setae.

Larval Facies (Figs. 22–27). Adults of *R. osca* (Figs. 1–4) and *R. molion* (Figs. 5–8), with their disparate color patterns, come from caterpillars so

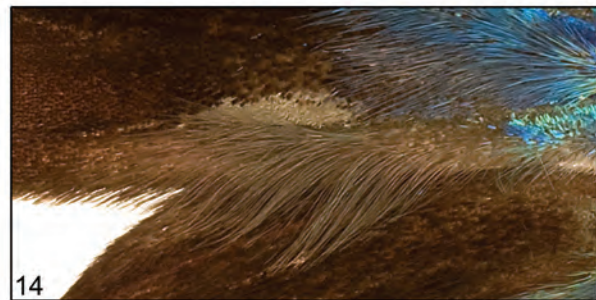


FIGS. 1–12. Reared adults of two species of *Rhinthon* (Hesperiinae) and one species of *Astraptes* (Eudaminae) from Area de Conservación Guanacaste, Costa Rica. Males in the two left columns, females in the two right columns; dorsal views odd-numbered, ventral views even-numbered. Circa 3/4 natural size. Specimens (with unique voucher codes) housed in USNM. **1–4**, *R. osca* (♂ 03-SRNP-20996, ♀ 00-SRNP-10191). **5–8**, *R. molion* (♂ 00-SRNP-10634, ♀ 01-SRNP-21511). **9–12**, *A. YESENN* (♂ 03-SRNP-15877, ♀ 02-SRNP-30211).

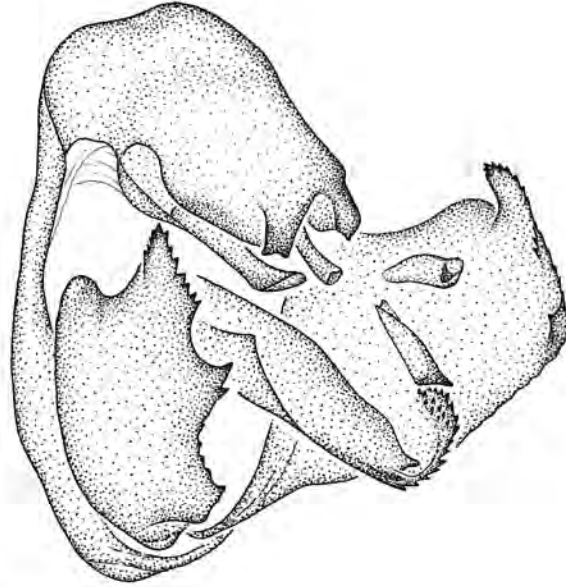
similar to each other that they are often confused in the wild, even by experienced collectors. However, grown caterpillars are distinguishable (Figs. 22–27). Basic features of the color pattern shared by last-instar caterpillars are a black nubbly head whose outer edges, in frontal view, present a pair of broad, highly contrasting, light yellow, dorsoventral stripes; and a body that is finely and densely dotted with green. On each side, the black head of *R. osca* has a second yellow, dorsoventral stripe (Fig. 24), which is so posterolateral in position as to be hidden in most views. Lacking this stripe, *R. molion* is solidly black in the same area (Fig. 25). In *R. osca*, but not *R. molion*, the yellow frontal

stripe is actually light brown along most of its inner margin (Figs. 22, 24, 26); and the lateral adfrontal suture (Stehr 1987: 290, fig. 26.1) is also brown (Fig. 26), not black, as it is in *R. molion* (Fig. 27). The green dots on the body are more prominent in *R. molion* than they are in *R. osca*. But laterally, in *R. osca*, some green dots merge into narrow, irregular strips of solid green that collectively form two wavy, somewhat discontinuous, longitudinal stripes along each side (Fig. 22).

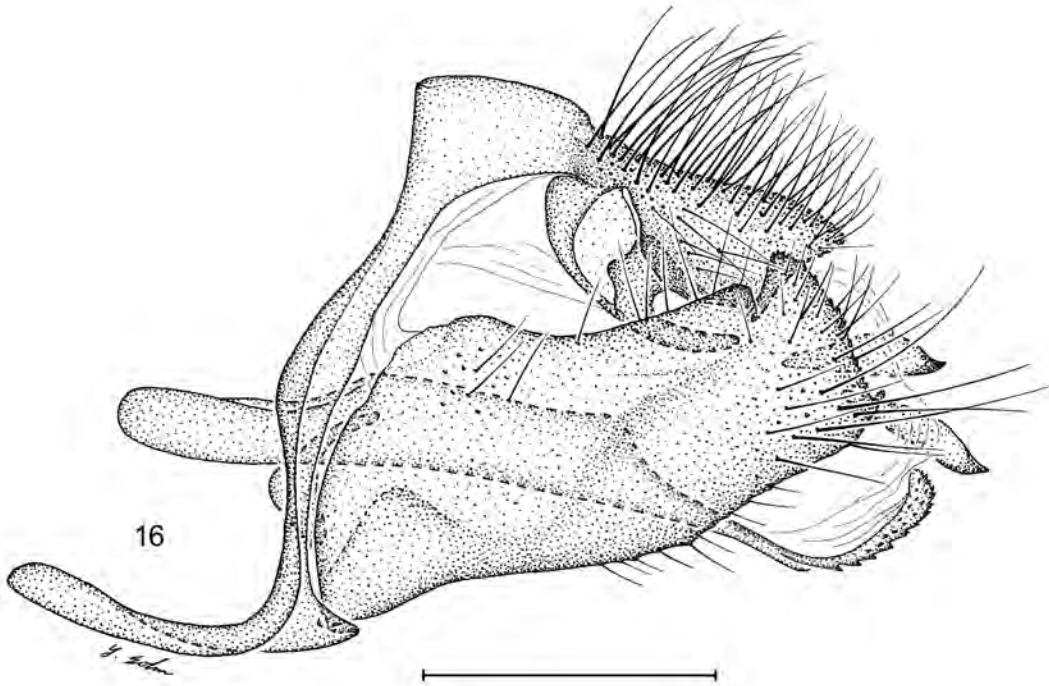
The most notable features of a *Rhinthon* pupa (Figs. 28, 29) are the long proboscis sheath that extends almost to the tip of the cremaster and the pair of rusty to



FIGS. 13, 14. Male secondary sex character (brand) above vein 2A on dorsal surface of left forewing in two species of *Rhinthon*. **13**, *R. osca* (04-SRNP-48123). **14**, *R. molion* (06-SRNP-56965).

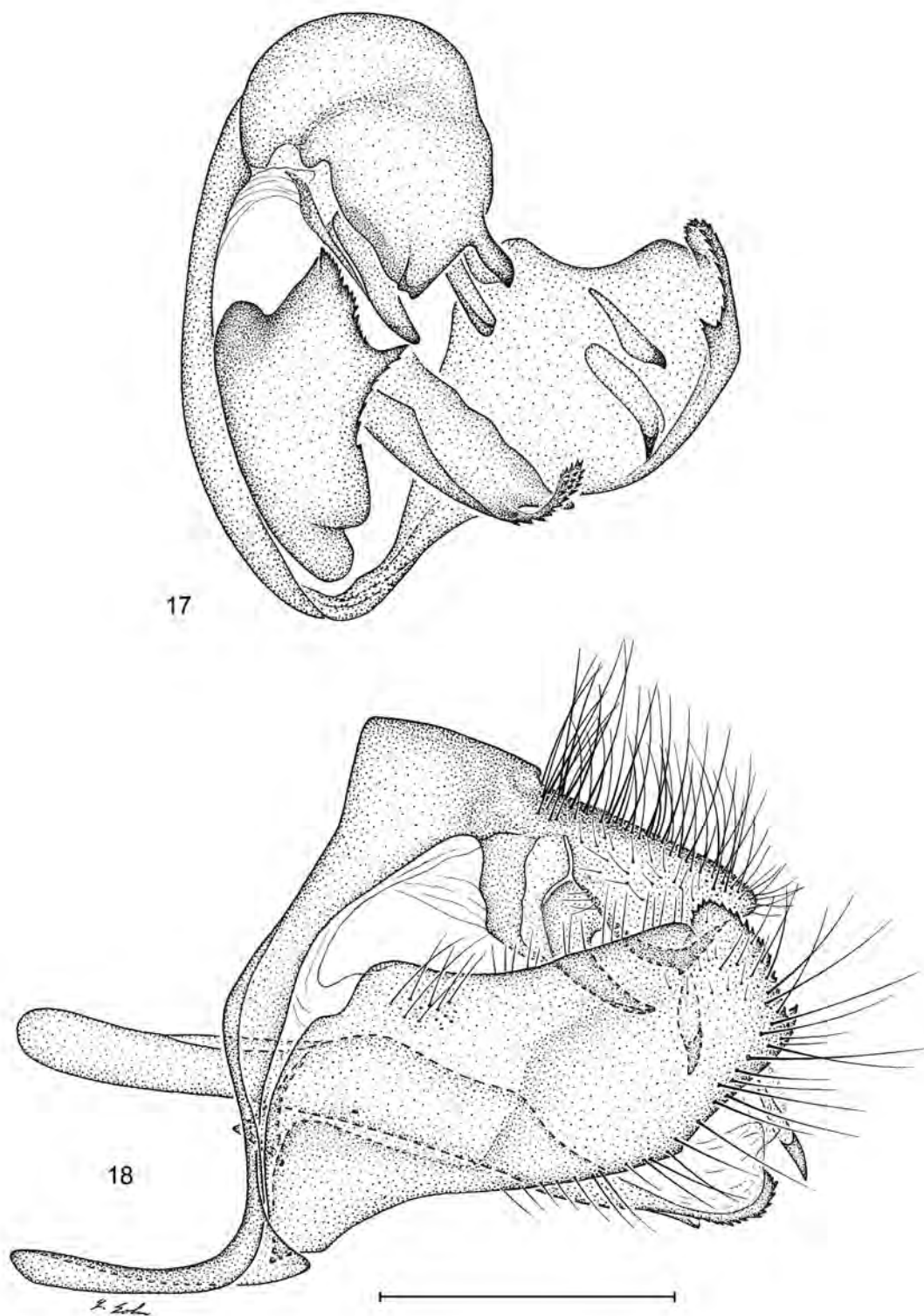


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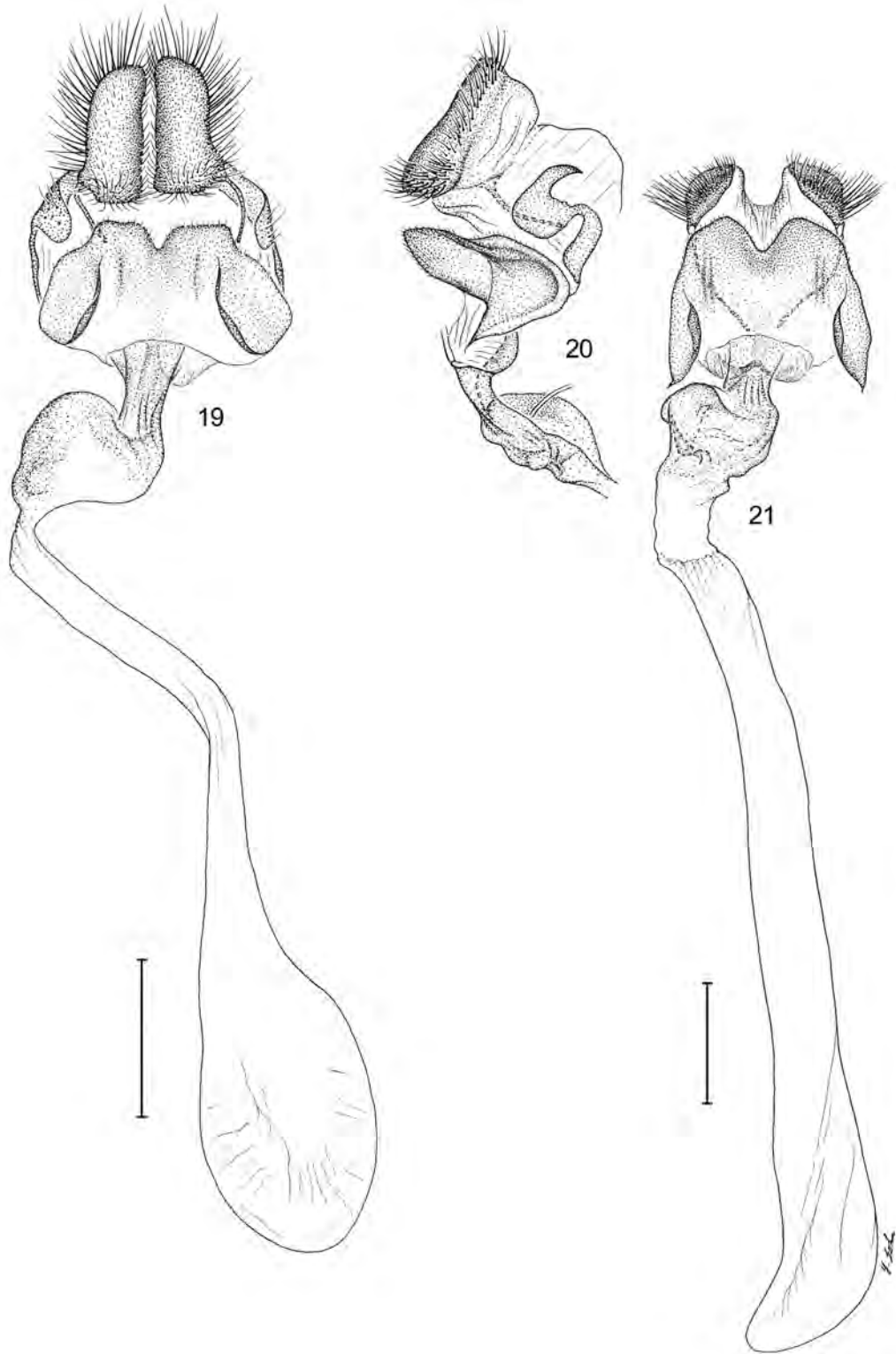


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FIGS. 15, 16. Male genitalia of *Rhinthon osca* (Burns genitalia dissection no. X-4211, voucher code 95-SRNP-6882; scale = 1 mm). **15**, Left posterodorsolateral view. **16**, Left lateral view.



FIGS. 17, 18. Male genitalia of *Rhinthon molion* (X-5802, 01-SRNP-21512; scale = 1 mm). **17**, Left posterodorsolateral view. **18**, Left lateral view.



FIGS. 19-21. Female genitalia of two species of *Rhinthon* (scale = 1 mm). **19**, *R. osca* in ventral view (X-6712, 03-SRNP-3184). **20, 21**, *R. molion* in right lateral and ventral views (X-6399, 05-SRNP-4892).

reddish mesothoracic spiracle covers (MacNeill 1964: 201, fig. 8) that contrast with their surroundings enough to suggest eyes, particularly in frontal view. The pupa and the interior of its shelter are at least partly coated with a flocculent white wax.

Foodplants (Table 1). *Rhinthon* caterpillars in ACG feed only on plants in closely related families of the order Zingiberales: Marantaceae, Cannaceae, Zingiberaceae, and Heliconiaceae. Most records, by far, are in Marantaceae, and within that family, in genus *Calathea*. *Rhinthon osca* and *R. molion* have similar tastes: they eat the same species of *Calathea* and, much less often, the same species of *Pleiostachya* (and both skippers have expanded their diet by using the same introduced species of *Canna*). The main difference is that *R. osca* is frequently found on *Maranta arundinacea*, a plant on which *R. molion* has never been found. Rearing records for *R. osca*, but not *R. molion*, include three more genera of Marantaceae, as well as one species of Zingiberaceae and one of Heliconiaceae. This may merely reflect the fact that the records for *R. osca* outnumber those for *R. molion* by 5 to 1.

Rhinthon cubana (Herrich-Schäffer), which some treat as just subspecifically distinct from *R. osca* (see Mielke 2005: 1269–1270), inhabits the Greater Antilles. Scant foodplant records agree with two from ACG, Costa Rica: in Cuba, Gundlach (1881) found *R. cubana* feeding on *Canna*, and Fernández (2001) found it feeding on *M. arundinacea*.

DNA Barcodes. Neighbor-joining trees derived from DNA barcodes of reared ACG hesperiids have consistently grouped *R. osca* and *R. molion* (under the names *Rhinthon cubana* and *Neoxeniades molion*) in a tight, two-taxon cluster: interspecific sequence divergence is about 3.5%. To date, 13 adults of *R. osca* and 16 of *R. molion* have been barcoded. These *Rhinthon* species tree far from species that are true *Neoxeniades* (see Appendix SII in the online version of Janzen *et al.* 2009).

DISCUSSION

With the addition of *R. molion*, *Rhinthon* can no longer be called a “primarily West Indian genus” (Smith *et al.* 1994). *Rhinthon molion* ranges from Mexico to Peru, and *R. osca* ranges even more widely, from southern Texas and Mexico to Colombia, Ecuador, and Venezuela, plus Trinidad and Tobago, whereas *R. cubana* occurs sporadically only in Cuba, Jamaica, Hispaniola, and Puerto Rico. A supposed West Indian congener can be discounted: the singular facies of the Hispaniolan skipper *R. bushi* Watson, which was described from one male from the Dominican Republic, does not appear to fit even the expanded *Rhinthon* mold (or, indeed, that of other hesperine

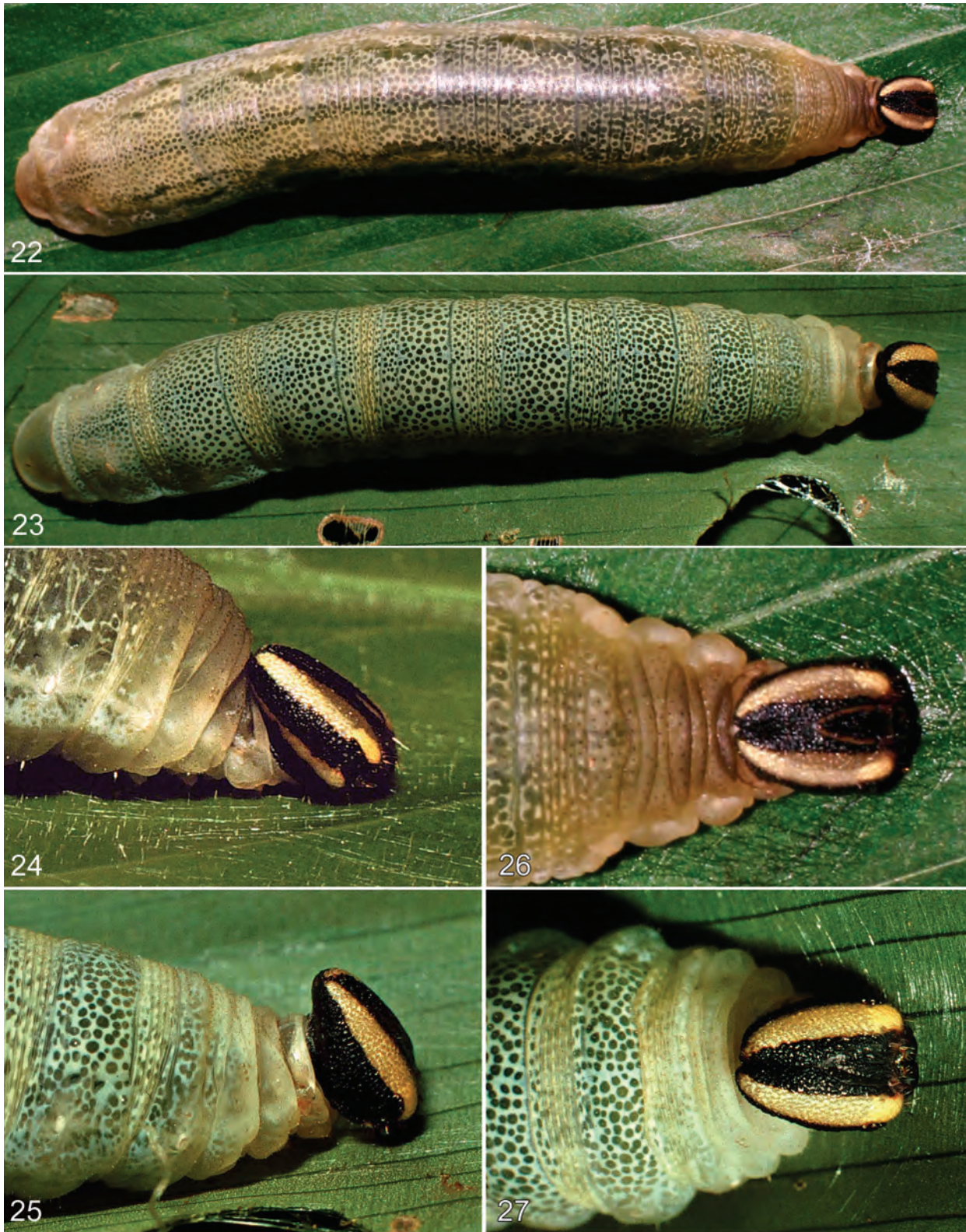
TABLE 1. Larval foodplants of *Rhinthon* in Area de Conservación Guanacaste, northwestern Costa Rica, and number of rearing records for each species of plant.

<i>Rhinthon molion</i>		
Cannaceae		
	<i>Canna indica</i> (introduced)	14
Marantaceae		
	<i>Calathea crotalifera</i>	23
	<i>Calathea insignis</i>	3
	<i>Calathea lasiostachya</i>	1
	<i>Calathea lutea</i>	15
	<i>Calathea macrosepala</i>	4
	<i>Calathea marantifolia</i>	1
	<i>Pleiostachya leiostachya</i>	9
	<i>Pleiostachya pruinosa</i>	2
<i>Rhinthon osca</i>		
Cannaceae		
	<i>Canna indica</i> (introduced)	15
	<i>Canna tuerckheimii</i>	3
Heliconiaceae		
	<i>Heliconia latispatha</i>	5
Marantaceae		
	<i>Calathea crotalifera</i>	23
	<i>Calathea lasiostachya</i>	1
	<i>Calathea lutea</i>	53
	<i>Calathea macrosepala</i>	47
	<i>Calathea marantifolia</i>	64
	<i>Calathea villosa</i>	4
	<i>Hylaeantho hoffmannii</i>	24
	<i>Ischnosiphon elegans</i>	1
	<i>Maranta arundinacea</i>	96
	<i>Pleiostachya leiostachya</i>	3
	<i>Pleiostachya pruinosa</i>	5
	<i>Stromanthe tonckat</i>	8
Zingiberaceae		
	<i>Renalmia cernua</i>	3

genera). Moreover, “further examination of the genitalia of this insect suggests that its placing in *Rhinthon* was incorrect . . .” (Smith *et al.* 1994).

Now that it includes *R. molion*, *Rhinthon* emerges as yet another neotropical genus with one or more species whose adults converge on the flashy color pattern shown in Figs. 5–12. This presumably mimetic convergence is all the more notable because *Rhinthon* and *Astraptus* are in different subfamilies (as are some of the other genera involved in this mimicry complex).

Despite the removal of *R. molion*, *Neoxeniades* still includes species such as *N. luda* (Hewitson) and *N. pluviasilva* Burns with the mimetic color pattern (Burns *et al.* 2007: figs. 12–15, 24–27; Janzen *et al.* 2009: fig. 4, photos 23, 24), although it is less exact (especially ventrally) than the resemblance between *R. molion* and the species of the *A. fulgurator* complex (Janzen *et al.* 2009: fig. 4, photos 1–11). But despite the removal of *R. molion*, *Neoxeniades* is still polyphyletic (Burns, in prep.).



FIGS. 22–27. Last-instar caterpillars of *Rhinthon osca* (even-numbered) and *R. molion* (odd-numbered). 22, 23, Entire caterpillar in dorsal view (06-SRNP-46342 above, 98-SRNP-3076 below). 24, 25, Anterior end in lateral view (93-SRNP-2443 above, 98-SRNP-3076 below). 26, 27, Anterior end in dorsal view (06-SRNP-46342 above, 98-SRNP-3076 below).



FIGS. 28, 29. Pupa of *Rhinthon molion* in lateral and ventral views (02-SRNP-8146).

The genitalia do not suffer the same selection pressures as do facies, and by themselves provide enough information to justify the shift of *molion* from *Neoxeniades* to *Rhinthon*. The genitalia of *R. molion* mirror those of *R. osca*, but differ conspicuously, in all of their parts, from those of true *Neoxeniades* (compare Figs. 15–18 with Burns *et al.* 2007: figs. 38–41).

The diverse covarying characters—DNA barcodes, larval color pattern, and larval diet—that support the genitalic evidence are no doubt genetically independent of one another; and so, in general, they are potentially of great taxonomic value. In the case of *Rhinthon*, all three of these characters are strong. In some cases, however, DNA barcodes are the most useful of these three characters for low-level grouping. Consider, for example, the phylogenetically compact *A. fulgerator* complex of 11 species (which has been analyzed with specimens from ACG, although the complex ranges far more widely and includes still more species). The *A. fulgerator* species complex constitutes a distinct cluster in a neighbor-joining tree derived from barcodes; but the caterpillars of the various species do not (despite some shared basic elements) conform to a single color

pattern (Hebert *et al.* 2004: fig. 2; Janzen *et al.* 2009: fig. 5, photos 1–11), nor do they all eat plants that are phylogenetically close to each other.

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