



Research paper

Integrative data helps the assessment of a butterfly within the *Udranomia kikkawai* species complex (Lepidoptera: Hesperiiidae): Immature stages, natural history, and molecular evidence



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ABSTRACT

The immature stages of the skipper butterfly *Udranomia kikkawai* from Brazil are described, including information on host plant use in cerrado savanna, shelter building behavior, and barcode sequences. The caterpillars feed on young leaves of two host food plants, *Ouratea spectabilis* and *Ouratea hexasperma* (Ochnaceae). The duration from egg hatching to adult eclosion averaged 30 days. Immature stages of *U. kikkawai* morphologically resemble those of other Hesperiiidae by lacking head horns and complete lack of body scoli, and having a fusiform pupa with uniform color (except for mimetic false eye spots) and no projections. The young-leaf color of *Udranomia* caterpillars contrasts with that observed in the sister genus *Drephalys*, whose larvae are conspicuously colored. The food plant of *Udranomia* is also very uniform: all records are Ochnaceae across the distribution range of *Udranomia*. Based on molecular data, Brazilian populations correspond to the true *U. kikkawai*, which is part of a species complex that includes at least two additional undescribed species from Costa Rica. Our paper shows that free access to exchangeable database platforms can raise natural history studies to an integrative level that helps clarify patterns of diversity within taxonomically problematic groups.

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1. Introduction

Skipper butterflies (Lepidoptera: Hesperiiidae) present remarkable diversity among the butterfly families, especially in the Neotropics (Beccaloni and Gaston, 1995). Many genera in this family have taxonomic problems, due to the homogeneity of external morphology and color patterns, coupled with great species

richness. However, in the past few years several cryptic species complexes have been revealed based on integrative taxonomy (e.g., Hebert et al., 2004; Burns et al., 2008; Janzen et al., 2009, 2011; Grishin et al., 2013; Bertrand et al., 2014). In addition, robust phylogenetic hypotheses have been proposed so that higher systematics of Hesperiiidae is now better understood (Warren et al., 2008, 2009).

The genus *Udranomia* Butler, 1870 is morphologically related to *Phanus* Hübner, 1819, *Drephalys* Watson, 1893 and *Augiades* Hübner, 1819, in the *Augiades* group of Evans (Evans, 1952), a relationship recently confirmed by molecular data (Warren et al., 2009). Currently, four species are described in *Udranomia* (Mielke 2005): *Udranomia eurus* (Mabille and Boulet, 1919), *Udranomia kikkawai* (Weeks, 1906) *Udranomia orcinus* (C. Felder & R. Felder, 1867), all

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Fig. 1. Unidentified species of *Udranomia kikkawai* species group in Tambopata Research Center, Peru. Photo by Kim Garwood.

2.2. Morphology

Measurements were taken and general aspects of morphology were observed using a stereomicroscope equipped with a metric scale. Egg ‘size’ is recorded as height and diameter. The total length of larvae and pupae was measured in dorsal view. The head capsule width of larvae is the distance between the most external parts (as in Bächtold et al., 2012). We recorded the duration of larval and pupal stages and also the days on which the head capsule was changed. Color patterns of immature stages *in vivo* were photographed with a digital camera. The terminology for descriptions of early stages follows Hinton (1981) for eggs, Stehr (1987) for general morphology of larvae, and Mosher (1916) for pupae.

2.3. Molecular systematics

Total genomic DNA was isolated from eight individuals using Invisorb® Spin Tissue Mini Kit (STRATEC Molecular, Germany), including three individuals of *U. spitzii* (the only *Udranomia* species not yet sequenced) and five individuals of *U. kikkawai* representing three different Brazilian populations (Table 2). The barcode region proposed by Hebert et al. (2003), which is the 5′ portion of the mitochondrial DNA (mtDNA) gene cytochrome oxidase subunit I (COI, 658 bp), was sequenced according to published protocols (Wahlberg and Wheat, 2008). Sequences were aligned with those of other *Udranomia* from GenBank (Table 2) and BOLD, comprising all four described species within the genus *Udranomia*.

The mean genetic distances between and within groups were calculated using MEGA v. 6.0 (Tamura et al., 2013), under Kimura-two-Parameter (K2P) model of nucleotide substitution (Kimura, 1980). Each group was defined for each species of *Udranomia* and four groups comprising *U. kikkawai*: three different groups corresponding to the *U. kikkawai* complex found in Costa Rica and one group matching with the specimens from Brazil.

The final matrix comprised 103 individuals of *Udranomia* and 32 outgroups (Table 2). Bayesian analyses (BI) were carried out using the program MrBayes 3.2 (Ronquist and Huelsenbeck, 2003) on the CIPRES portal (Miller et al., 2010). The model-jumping feature of the program was utilized; therefore, all the possible GTR submodels were sampled according to their posterior probability (Ronquist et al., 2012). The gamma parameter was also included to allow site rate variation. Four simultaneous chains were run for 10×10^6 generations for two runs, sampling trees every 1000 cycles. The first 2500 trees were discarded as “burn in” based on when the runs had converged and reached equilibrium. The convergence of the likelihood traces of the independent runs was assessed with TRACER v1.5, and the ESS (effective sample size) values were verified to be above 300 for all parameters, which indicates that they were sufficiently sampled to estimate their posterior distributions (Drummond et al., 2006). The maximum-likelihood analyses (ML) were run with RAXML (Stamatakis, 2014) modeled according to the GTR+G model with 1000 bootstrap replicates and a search for the maximum likelihood topology on the CIPRES portal.

3. Results

3.1. Natural history of *Udranomia kikkawai*

Two larval food plants occur in the study site: *Ouratea hexasperma* and *Ouratea spectabilis* (Ochnaceae). Eggs were found only on young reddish newly expanding leaves. Eleven eggs of *Udranomia kikkawai* were found on *O. spectabilis* and three on *O. hexasperma*. Both species of *Ouratea* are common in Cerrado in southeast Brazil; they bear extrafloral nectaries that attract a rich ant fauna (Oliveira et al., 1995; Oliveira and Freitas, 2004; Byk and Del-Claro, 2010).

widely distributed in the Neotropics, and *Udranomia spitzii* (Hayward, 1942), endemic to the savanna formations of South America (Mielke, 2005). Recently, studies based on mitochondrial sequences (DNA barcodes) suggest that *U. kikkawai* (Fig. 1) forms a complex of cryptic species with at least three sympatric and parapatric taxa in Costa Rica (Janzen et al., 2009, 2011). The barcode clusters named by the authors *Udranomia kikkawai*DHJ01, *Udranomia kikkawai*DHJ02, and *Udranomia kikkawai*DHJ03, respectively, do not show obvious differences in larval and adult morphology (wings and genitalia), but differ in food plant use and occupy two different ecosystems and the intergrade between them as if it was a third ecosystem (Janzen et al., 2011; D.H. Janzen, unpubl. data).

The purpose of this study was to describe for the first time the immature stages of *U. kikkawai*, including information about natural history and morphology for populations from southeast Brazil. We also evaluated the systematic position of Brazilian populations in relation to other species of the genus and the *U. kikkawai* complex in Costa Rica. Moreover, information about food plants is revised, morphology and natural history are compared, and relations to other Eudaminae species are discussed.

2. Materials and methods

2.1. Study sites, collection and rearing

Eggs were collected from *Ouratea spectabilis* (Ochnaceae) new and expanding leaves during December 2011 in an area of Cerrado *sensu stricto* in the “Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia”, CCPIU (18°59′S, 48°18′W), Uberlândia, Brazil (see Assunção et al., 2014 for study site characterization). Caterpillars were reared from eggs collected in the field. Additional data on immature stages and behavior were obtained in three additional Cerrado areas in the municipalities of Itirapina and Mogi Guaçu (both in São Paulo state, SE Brazil) and in Brasília (Federal District, Central Brazil). All immatures were placed individually in 500 ml plastic containers with young leaves of *O. spectabilis* that were offered *ad libitum* and larvae were checked daily to replace food and clean. Food plant use and behavior of *U. kikkawai* caterpillars were observed in the field. Head capsules were collected and kept for measurements. The duration of instars and pupae development was recorded. Adults, capsules and pupal skins have been deposited in the entomological collection of the Universidade Estadual de Campinas (Museu de Zoologia “Adão José Cardoso, ZUEC).

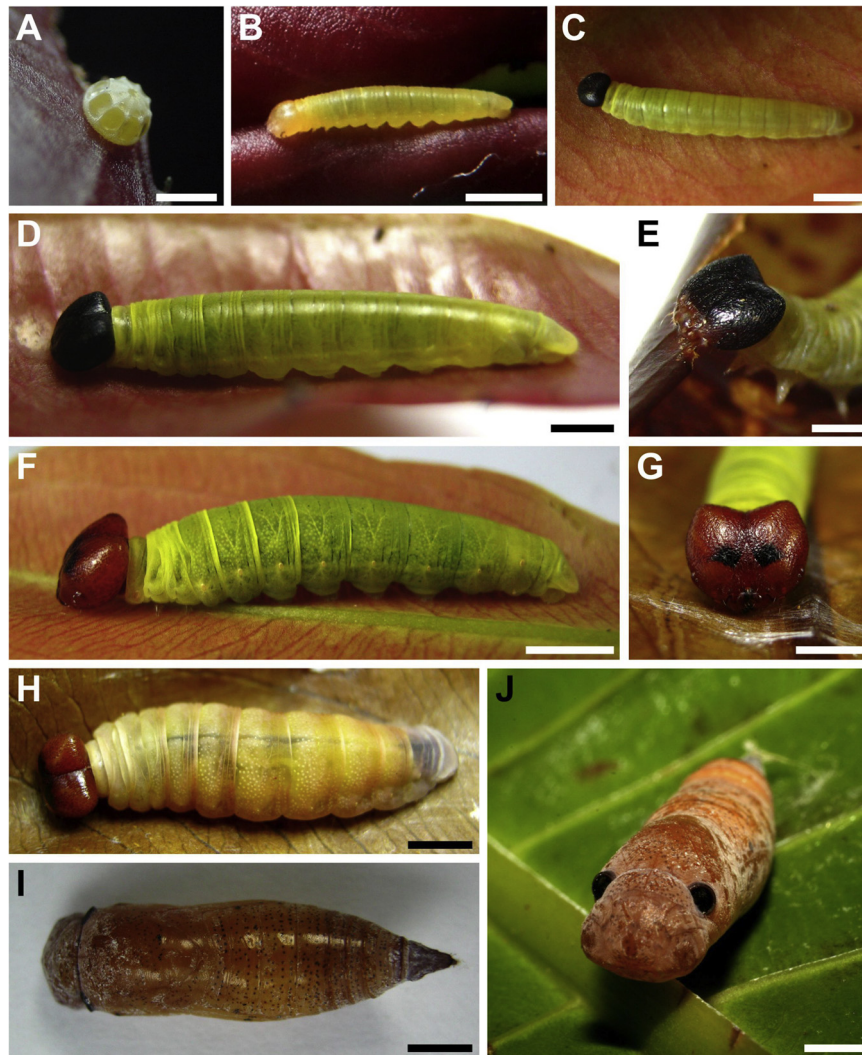


Fig. 2. Immature stages of *Udranomia kikkawai* on *Ouratea spectabilis* in areas of cerrado savanna in southeast Brazil (A–H) and Area de Conservación Guanacaste, Costa Rica (J). A, egg; B, first instar; C, second instar; D, third instar; E, head capsule of third instar in lateral view; F, fifth (last) instar; G, head capsule of fifth instar in frontal view; H, prepupa; I, pupa; J, pupa in frontal view showing the false eyes (Janzen et al., 2010), voucher code 07-SRNP-41327-DHJ422344. Scales = 0.5 mm (A and E), 1.5 mm (B–D, G), and 3.0 mm (F, H–J), respectively. Photo (J) by Daniel Janzen. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

The larvae feed and build leaf shelters on young expanding leaves of both species of plants, and each shelter is occupied by a single larva. Two different types of shelters are built by the larvae (shelter types *sensu* Greeney, 2009): (1) first and second instar larvae build shelters by rolling young leaves (type 1 or “no-cut shelter”), and (2) third to last instar larvae make two cuts on the leaves towards the midrib, and then the two parts of the leaf are folded and glued with silk (shelter type 5). The duration from egg hatching to adult eclosion averages 30 days.

3.2. Immature-stage morphology of *Udranomia kikkawai*

Egg (Fig. 2A). Hemispheric, light yellow in color, with 8–10 vertical ribs enlarged near micropylar area; height 0.41–0.45 mm, diameter 0.79–0.85 mm ($N=07$).

First instar (Fig. 2B). Entirely yellowish after hatching; after feeding, the body becomes light greenish and head capsule pale yellow. Head and body tegument smooth, without scoli or projections. Head capsule width: 0.41–0.45 mm, maximum body length 5.1 mm. Duration: 2 days ($N=07$).

Second instar (Fig. 2C). Yellowish body, black head. Intestinal contents dark green in color and visible. Head capsule width: 0.63–0.77 mm, maximum body length 8.1 mm. Duration: 2 days ($N=07$).

Third instar (Fig. 2D). Similar to second instar. Head capsule width: 1.24–1.45 mm, maximum body length 12.6 mm. Duration: 1–2 days ($N=07$).

Fourth instar (Fig. 2E). Yellow greenish body; black head with mouth parts dark reddish brown. Head capsule width: 2.28–2.43 mm ($N=06$), maximum length 20.5 mm. Duration: 1–2 days ($N=07$).

Fifth (last) instar (Fig. 2F–H). Very similar to previous instar, but with dark brown head and diffusely black spots extended laterally in the adfrontal area (Fig. 2G). Head capsule width: 4.16–4.5 mm ($N=07$), maximum length 31.1 mm. Duration: 7–10 days ($N=06$) of which 2–3 days corresponded to the prepupal time (Fig. 2H) ($N=06$).

Pupa (Fig. 2I–J). General profile elongated, without spines or appendages. General color light orange with black scattered spots, mesothoracic spiracles colored as conspicuous black false eyes

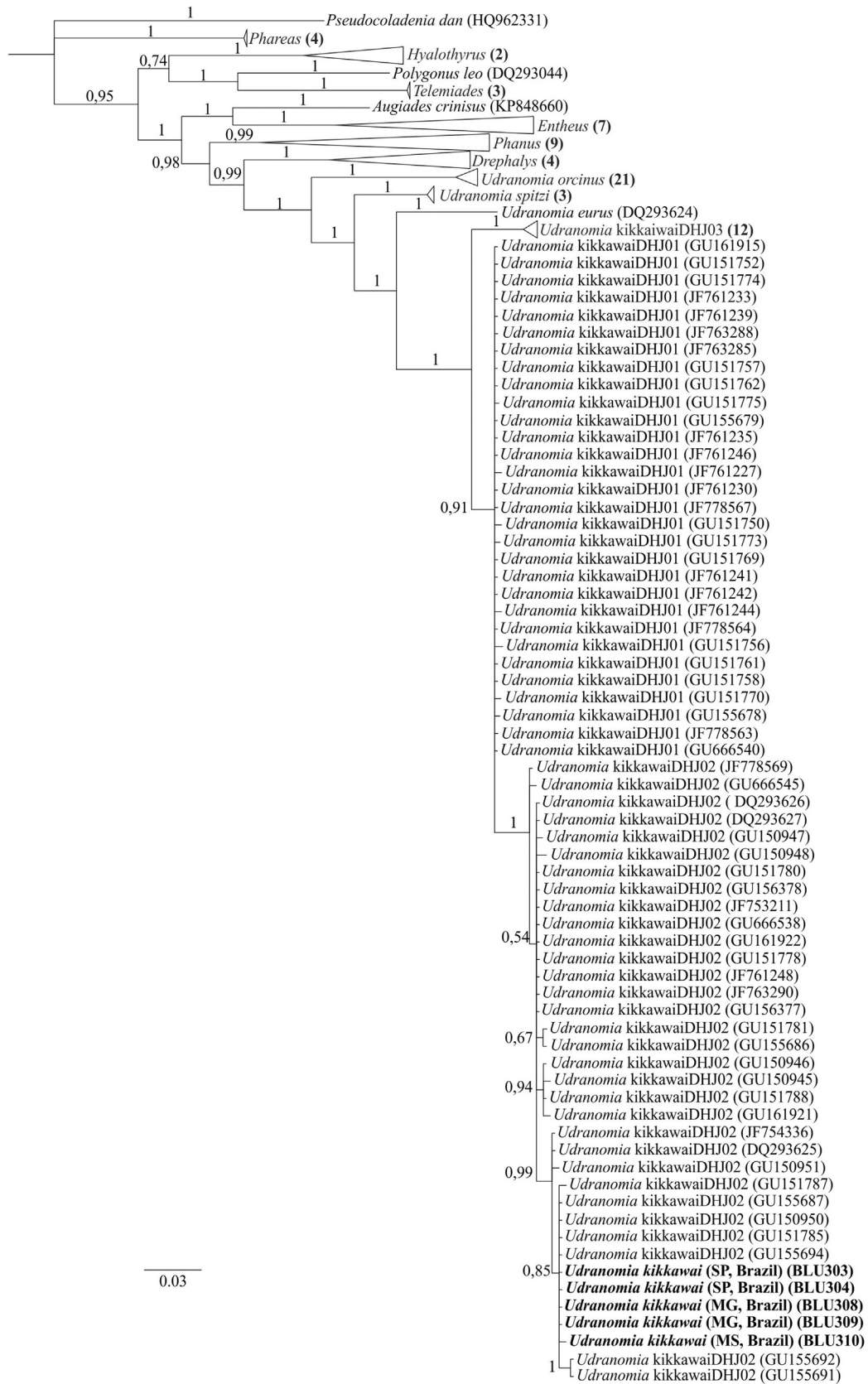


Fig. 3. Bayesian Inference tree for all *Udranomia* species and available Eudaminae as outgroup. Brazilian populations of *U. kikkawai* in bold. For voucher details and genetic distances see Tables 2 and 3.

Table 1
Summary of food plant records for *Udranomía* species.

Host plant (Ochnaceae)	Localities	References
<i>Udranomía eurus</i>		
<i>Cespedesia spathulata</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Udranomía kikkawai</i>		
<i>C. spathulata</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Ouratea lucens</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>O. hexasperma</i>	Brazil (DF, MG, SP)	Diniz et al. (2001), Present study
<i>O. spectabilis</i>	Brazil (MG)	Present study
<i>Quiina amazonica</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Q. schippii</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Udranomía orcinus</i>		
<i>Cespedesia spathulata</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Lacunaria panamensis</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>O. lucens</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>O. promines</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>O. purdieana</i>	Trinidad	Cock and Alston-Smith (2013)
<i>O. subscens</i>	Brazil (PA)	Moss (1949)
<i>Q. amazonica</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Q. macrophylla</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Q. schippii</i>	Costa Rica	Janzen and Hallwachs (2015)
<i>Udranomía spitzzi</i>		
<i>O. hexasperma</i>	Brazil (DF, MG)	Diniz and Morais (1995), Diniz et al. (2001), Bächtold et al. (2012, 2014)
<i>O. spectabilis</i>	Brazil (MG)	Bächtold et al. (2012, 2014)

Brazilian States: DF, Distrito Federal; MG, Minas Gerais; SP, São Paulo; PA, Pará.

(Janzen et al., 2010) in frontal view. Maximum length 19.41 mm, width at A3 5.35 mm. Duration: 12–14 days ($N = 06$).

3.3. Systematic position

Based on the Bayesian tree (Fig. 3), the genus *Udranomía* is monophyletic, with the Brazilian *U. kikkawai* nested within the clade *U. kikkawai*DHJ02, which corresponds to the true *U. kikkawai* as based on the sequences of the type specimen (N. Grishin and D.H. Janzen, unpubl. data). The genus *Udranomía* is sister to *Drephalys*, in a clade that also includes *Augiades*, *Entheus* Hübner, 1819 and *Phanus*, all in the *Augiades* section of Evans (1952). Other two genera of the *Augiades* section, namely *Phareas* Westwood, 1852 and *Hyalothyryus* Mabille, 1878 grouped together with the Pyrginae outgroups, making the subfamily Eudaminae appear to be non-monophyletic if only these genes are considered.

4. Discussion

In general, the immature stages of *U. kikkawai* morphologically resemble those of other HesperIIDae by lacking head horns and completely lacking body scoli, and by having a fusiform pupa with uniform color and without any type of projections (see Scoble, 1995; Greeney and Warren, 2003, 2004; Silva et al., 2012; Moraes et al., 2012). This uniformity is related to HesperIIDae caterpillars and pupae spending most of their lives inside leaf shelters, where projections are non-adaptive. Most of the reported variation is in the body and head color pattern, or in species that are mimetic of aposematic caterpillars (e.g., Hebert et al., 2004; Janzen et al., 2009, 2010; Janzen and Hallwachs 2015).

The immature stages of all known species of *Udranomía* are very uniform: Eggs are hemispherical with well-marked vertical ribs and a micropilar area centered on the top surface; the larval tegument is smooth with yellowish-green color pattern, without thorns and/or projections; the pupae are brownish and lack projections (see Bächtold et al., 2012; Cock and Alston-Smith, 2013; Janzen and Hallwachs, 2015). There are some differences in head capsule color and shape between *Udranomía* species, but not within *U. kikkawai* complex. The conspicuous cases are that *U. kikkawai* species has a dark brown head capsule in the second and third instar, while

these are light brown in *U. spitzzi*, and the *U. kikkawai* cephalodor-sal area has two acuminate ends (Fig. 2G) while these are rounded in *U. spitzzi* (Bächtold et al., 2012). The leaf-colored cryptic body of *Udranomía* larvae contrasts with that of its sister genus *Drephalys*, whose larvae are ringed black and white mimics of aposematic caterpillars (Burns and Janzen, 1999; Janzen and Hallwachs, 2015). In coloration, larvae and pupae of *Udranomía* are similar to those of *Phanus* and *Entheus* (Janzen and Hallwachs, 2015), both of which are specialists at feeding on very young and pale expanding leaves.

Food plant use by *Udranomía* is also very uniform; the food plants are Ochnaceae across the distribution range of the hesperiid-s (Table 1). The species of Ochnaceae vary geographically and *Udranomía* species appear to feed only on Ochnaceae wherever the butterfly occurs. In addition to this species-level oligophagy, the larvae are also very specialized in that they eat only new leaves. In the Cerrado savanna, which has two distinct seasons (dry and wet), the availability of resources (new leaves) is markedly seasonal (Muniz et al., 2012; Bächtold et al., 2014). Studies conducted in the cerrado with *U. spitzzi* have reported a relationship between preference and performance, with better larval development achieved on the food plant most used, which is *O. spectabilis* (Bächtold et al., 2014).

Our molecular results showed that populations of *U. kikkawai* of cerrado savanna in southeastern Brazil are part of the same *U. kikkawai* species complex as occurs in Costa Rica, and related to the barcode cluster *U. kikkawai*DHJ02, which is the true *U. kikkawai* (D.H. Janzen, N. Grishin, and J. Burns, in prep.) (Fig. 3). It is clear that sympatric and parapatric species of skipper butterflies may differ by as few as 1–2 base pairs in their COI barcodes (e.g., Burns et al., 2007; Janzen et al., 2011). Data available for all three clusters in the “Dynamic database for the macrocaterpillar fauna in Costa Rica” (Janzen and Hallwachs, 2015) show consistent differences in food plant use and microgeographic distribution between all three species (D.H. Janzen, unpubl. data).

In recent years, the use of the DNA barcode had a strong impact on the way species are revealed, thus uncovering a hitherto unknown biodiversity (Hebert et al., 2004; Silva-Brandão et al., 2009; Janzen et al., 2009, 2011). Our paper shows that free access to exchangeable database platforms can raise natural history studies

Table 2
Specimens of sequenced Eudaminae for the evolutionary history inference of *Udranomia kikkawai* with code, sampling sites data, and GenBank accession numbers. Accession numbers will be available after acceptance.

Species	Individuals	Locality	Genbank codes
<i>Augiades crinitus</i>	1	Barro Colorado Is, Panama	KP848660
<i>Drephalys alcmon</i>	1	Area de Conservación Guanacaste, Costa Rica	JF752622
<i>Drephalys kidonoi</i>	1	Area de Conservación Guanacaste, Costa Rica	GU155933
<i>Drephalys oria</i>	1	Area de Conservación Guanacaste, Costa Rica	HQ934277
<i>Drephalys Burns01</i>	1	Area de Conservación Guanacaste, Costa Rica	GU149637
<i>Entheus Burns01</i>	2	Area de Conservación Guanacaste, Costa Rica	DQ292404, JF777900
<i>Entheus Burns02</i>	1	Area de Conservación Guanacaste, Costa Rica	HM885842
<i>Entheus Burns03</i>	1	Area de Conservación Guanacaste, Costa Rica	GU666452
<i>Entheus huertasae</i>	1	Barro Colorado Is, Panama	KF921081
<i>Entheus matho</i>	2	Barro Colorado Is, Panama	KP848878, KP848881
<i>Hyalothyryus neleus</i>	1	Area de Conservación Guanacaste, Costa Rica	DQ292564
<i>Hyalothyryus sp. 1YB</i>	1	Barro Colorado Is, Panama	KP849006
<i>Phanus albiapicalis</i>	1	Barro Colorado Is, Panama	HM406627
<i>Phanus ecitonorum</i>	1	Barro Colorado Is, Panama	KP849244
<i>Phanus marshalliDHJ01</i>	1	Barro Colorado Is, Panama	KP849252
<i>Phanus marshalliDHJ02</i>	1	Barro Colorado Is, Panama	KP849251
<i>Phanus marshalliDHJ02</i>	1	Area de Conservación Guanacaste, Costa Rica	JQ578341
<i>Phanus Janzen01</i>	1	Area de Conservación Guanacaste, Costa Rica	JQ529036
<i>Phanus vitreusDHJ01</i>	1	Area de Conservación Guanacaste, Costa Rica	HQ933602
<i>Phanus vitreusDHJ02</i>	1	Area de Conservación Guanacaste, Costa Rica	JF754062
<i>Phanus vitreusDHJ03</i>	1	Area de Conservación Guanacaste, Costa Rica	JF753050
<i>Phareas burnsi</i>	4	Area de Conservación Guanacaste, Costa Rica	GU149832–GU149834, GU161783
<i>Polygonus leo</i>	1	Area de Conservación Guanacaste, Costa Rica	DQ293044
<i>Pseudocoladenia dan</i>	1	Trang: Khaobantot Mountains, Thailand	HQ962331
<i>Telemiades fides</i>	3	Area de Conservación Guanacaste, Costa Rica	DQ293541–DQ293543
<i>Udranomia erus</i>	1	Area de Conservación Guanacaste, Costa Rica	DQ293624
<i>Udranomia kikkawai</i>	BLU-303	Pirassununga (SP), Brazil	
<i>Udranomia kikkawai</i>	BLU-304	Pirassununga (SP), Brazil	
<i>Udranomia kikkawai</i>	BLU-308	Uberlândia (MG), Brazil	
<i>Udranomia kikkawai</i>	BLU-309	Uberlândia (MG), Brazil	
<i>Udranomia kikkawai</i>	BLU-310	Três Lagoas (MS), Brazil	
<i>Udranomia kikkawaiDHJ01</i>	30	Area de Conservación Guanacaste, Costa Rica	GU151750, GU151752, GU151756–GU151758, GU151761, GU151762, GU151769, GU151770, GU151773–GU151775, GU155678, GU155679, GU161915, GU666540, JF761227, JF761230, JF761233, JF761235, JF761239, JF761241, JF761242, JF761244, JF761246, JF763285, JF763288, JF778563, JF778564, JF778567
<i>Udranomia kikkawaiDHJ02</i>	31	Area de Conservación Guanacaste, Costa Rica	DQ293625–DQ293627, GU150945–GU150948, GU150950, GU150951, GU151778, GU151780, GU151781, GU151785, GU151787, GU151788, GU155686, GU155687, GU155691, GU155692, GU155694, GU156377, GU156378, GU161921, GU161922, GU666538, GU666545, JF753211, JF754336, JF761248, JF763290, JF778569
<i>Udranomia kikkawaiDHJ03</i>	12	Area de Conservación Guanacaste, Costa Rica	GU151793, GU151794, GU151799, GU151800, GU155695, JF754341, JF761249, JF761251, JF761252, JF761254, JF763293, JF778572
<i>Udranomia orcinus</i>	21	Area de Conservación Guanacaste, Costa Rica	DQ293628, DQ293629, GU150952, GU150954–GU150956, GU151801, GU155696, GU156379, GU161925–GU161927, GU161929, GU666539, HM409482, JF753212, JF754347, JF754352, JF761259, JF763294, JF763296
<i>Udranomia spitzzi</i>	BLU-302	Pirassununga (SP), Brazil	
<i>Udranomia spitzzi</i>	BLU-311	Uberlândia (MG), Brazil	
<i>Udranomia spitzzi</i>	BLU-312	Uberlândia (MG), Brazil	

MG, Minas Gerais; MS, Mato Grosso do Sul; SP, São Paulo.

Table 3
Comparison of inter- and intraspecific divergences (%) for the DNA “barcodes” of *Udranomia* species (number of individuals in parentheses). Values of divergence below 2 percent are in bold; (–) data not available.

	<i>U. orcinus</i>	<i>U. erus</i>	<i>U. kikkawaiDHJ01</i>	<i>U. kikkawaiDHJ02</i>	<i>U. kikkawaiDHJ03</i>	<i>U. kikkawai (Brazil)</i>	<i>U. spitzzi</i>
<i>U. orcinus</i> (21)	0						
<i>U. erus</i> (1)	11.72	–					
<i>U. kikkawaiDHJ01</i> (30)	13.94	6.35	0.08				
<i>U. kikkawaiDHJ02</i> (31)	14.64	6.98	1.28	0.31			
<i>U. kikkawaiDHJ03</i> (12)	15.24	8.26	3.78	4.22	0.20		
<i>U. kikkawai</i> Brazil (5)	14.61	6.96	1.26	0.43	3.75	0	
<i>U. spitzzi</i> (3)	8.52	7.83	6.76	7.40	8.05	7.38	0.40

to an integrative level that helps clarify patterns of diversity within taxonomically problematic groups.

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References

- Assunção, M.A., Torezan-Silingardi, H.M., Del-Claro, K., 2014. Do ant visitors to extrafloral nectaries of plants repel pollinators and cause an indirect cost of mutualism? *Flora Morphol. Distrib. Funct. Ecol. Plants* 209, 244–249. <http://dx.doi.org/10.1016/j.flora.2014.03.003>.
- Bächtold, A., Del-Claro, K., Kaminski, L.A., Freitas, A.V.L., Oliveira, P.S., 2012. Natural history of an ant–plant–butterfly interaction in a Neotropical savanna. *J. Nat. Hist.* 46, 943–954. <http://dx.doi.org/10.1080/00222933.2011.651649>.
- Bächtold, A., Lange, D., Del-Claro, K., 2014. Influence, or the lack thereof, of host phenology, architecture and climate on the occurrence of *Udranomia spizii* (Hesperiidae: Lepidoptera). *Entomol. Sci.* 17, 66–74. <http://dx.doi.org/10.1111/ens.12038>.
- Beccaloni, G.W., Gaston, K.J., 1995. Predicting species richness of Neotropical forest butterflies: Ithomiinae (Lepidoptera: Nymphalidae) as indicators. *Biol. Conserv.* 71, 77–86. [http://dx.doi.org/10.1016/0006-3207\(94\)00023-J](http://dx.doi.org/10.1016/0006-3207(94)00023-J).
- Bertrand, C., Janzen, D.H., Hallwachs, W., Burns, J.M., Gibson, J.F., Shokralla, S., Hajibabaei, M., 2014. Mitochondrial and nuclear phylogenetic analysis with Sanger and next-generation sequencing shows that, in Área de Conservación Guanacaste, northwestern Costa Rica, the skipper butterfly named *Urbanus belli* (family Hesperiidae) comprises three morphologica. *BMC Evol. Biol.* 14, 153. <http://dx.doi.org/10.1186/1471-2148-14-153>.
- Burns, J.M., Janzen, D.H., 1999. *Drephalys*: division of this showy Neotropical genus, plus a new species and the immatures and food plants of two species from Costa Rican dry forest (Hesperiidae: Pyrginae). *J. Lepid. Soc.* 53, 77–89.
- Burns, J.M., Janzen, D.H., Hajibabaei, M., Hallwachs, W., Hebert, P.D.N., 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.
- Burns, J.M., Janzen, D.H., Hajibabaei, M., Hallwachs, W., Hebert, P.D.N., 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Área de Conservación Guanacaste, Costa Rica. *Proc. Natl. Acad. Sci. U. S. A.* 105, 6350–6355. <http://dx.doi.org/10.1073/pnas.0712181105>.
- Byk, J., Del-Claro, K., 2010. Ant-plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Popul. Ecol.* 53, 327–332. <http://dx.doi.org/10.1007/s10144-010-0240-7>.
- Cock, M.J.W., Alston-Smith, S., 2013. *Udranomia* spp. (Lepidoptera: Hesperiidae: Eudaminae) in Trinidad, West Indies. *Living World* 2013, 29–31.
- Diniz, I.R., Morais, H.C., 1995. Larvas de Lepidoptera e suas plantas hospedeiras em um cerrado de Brasília, DF, Brasil. *Rev. Bras. Entomol.* 39, 755–770.
- Diniz, I.R., Morais, H.C., Camargo, A.J.A., 2001. Host plants of lepidopteran caterpillars in the cerrado of the Distrito Federal, Brazil. *Rev. Bras. Entomol.* 45, 107–122.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, e88. <http://dx.doi.org/10.1371/journal.pbio.0040088>.
- Evans, W.H., 1952. A Catalogue of the American Hesperiidae Indicating the Classification and Nomenclature Adopted in the British Museum (Natural History). Part II. Pyrginae. Section I. British Museum, London.
- Greeney, H.F., Warren, A.D., 2003. Notes on the life history of *Eantis thraso* (Hesperiidae: Pyrginae) in Ecuador. *J. Lepid. Soc.* 57, 43–46.
- Greeney, H.F., Warren, A.D., 2004. The life history of *Noctuana haematospila* (Hesperiidae: Pyrginae) in Ecuador. *J. Lepid. Soc.* 59, 6–9.
- Greeney, H.F., 2009. A revised classification scheme for larval hesperiid shelters, with comments on shelter diversity in the Pyrginae. *J. Res. Lepidoptera* 41, 53–59.
- Grishin, N.V., Janzen, D.H., Hallwachs, W., 2013. Hiding behind gaudy looks, a new Central American species of *Phareas* (Hesperiidae: Eudaminae). *J. Lepid. Soc.* 67, 161–174. <http://dx.doi.org/10.18473/lepi.v67i3.a3>.
- Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B* 270, S96–S99. <http://dx.doi.org/10.1098/rsbl.2003.0025>.
- Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., Hallwachs, W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrartes fulgurator*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 14812–14817. <http://dx.doi.org/10.1073/pnas.0406166101>.
- Hinton, H.E., 1981. *Biology of Insect Eggs*. Pergamon Press, London.
- Janzen, D.H., Hallwachs, W., 2015. Dynamic Database for an Inventory of the Macrocaractillar Fauna, and its Food Plants and Parasitoids, of the Área de Conservación Guanacaste (acg), Northwestern Costa Rica [internet]. University of Pennsylvania, Philadelphia (PA) [cited 14 January 2016]. Available from: <http://janzen.sas.upenn.edu>.
- Janzen, D.H., Hallwachs, W., Blandin, P., Burns, J.M., Cadiou, J.-M., 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., Gaud, I.D., Harvey, D.J., Hausmann, A., Kitching, J., Lafontaine, D., Landry, J.-F., Lemaire, C., Miller, J.Y., Miller, J.S., Miller, L., Miller, S.E., Montero, J., Munroe, E., Green, S.R., 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., Wilson, J.J., 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Mol. Ecol. Resour.* 9 (Suppl. s1), 1–26. <http://dx.doi.org/10.1111/j.1755-0998.2009.02628.x>.
- Janzen, D.H., Hallwachs, W., Burns, J.M., 2010. A tropical horde of counterfeit predator eyes. *Proc. Natl. Acad. Sci. U. S. A.* 107, 11659–11665. <http://dx.doi.org/10.1073/pnas.0912122107>.
- Janzen, D.H., Hallwachs, W., Burns, J.M., Hajibabaei, M., Bertrand, C., Hebert, P.D.N., 2011. Reading the complex skipper butterfly fauna of one tropical place. *PLoS One* 6, e19874. <http://dx.doi.org/10.1371/journal.pone.0019874>.
- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- Mielke, O.H.H., 2005. *Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera)*, Vols. 1–6. Sociedade Brasileira de Zoologia, Curitiba.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE), IEEE, pp. 1–8. <http://dx.doi.org/10.1109/GCE.2010.5676129>.
- Moraes, A.R., Greeney, H.F., Oliveira, P.S., Barbosa, E.P., Freitas, A.V.L., 2012. Morphology and behavior of the early stages of the skipper, *Urbanus emeraldus*, on *Ureca baccifera*, an ant-visited host plant. *J. Insect Sci.* 12, 52. <http://dx.doi.org/10.1673/031.012.5201>.
- Mosher, E., 1916. A classification of the Lepidoptera based on characters of the pupa. *Bull. Illinois State Lab. Nat. Hist.* 12, 1–165.
- Moss, A.M., 1949. Biological notes on some Hesperiidae of Pará and the Amazon (Lep. Rhop.). *Acta Zool. Lilloana* 7, 27–80.
- Muniz, D.G., Freitas, A.V.L., Oliveira, P.S., 2012. Phenological relationships of *Eunica bechina* (Lepidoptera: Nymphalidae) and its host plant, *Caryocar brasiliense* (Caryocaraceae), in a Neotropical savanna. *Stud. Neotrop. Fauna Environ.* 47, 111–118. <http://dx.doi.org/10.1080/01650521.2012.698932>.
- Oliveira, P.S., Freitas, A.V.L., 2004. Ant-plant-herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften* 91, 557–570. <http://dx.doi.org/10.1007/s00114-004-0585-x>.
- Oliveira, P.S., Klitzke, C., Vieira, E., 1995. The ant fauna associated with the extrafloral nectaries of *Ouretea hexasperma* (Ochnaceae) in an area of cerrado vegetation in Central Brazil. *Entomol. Mon. Mag.* 131, 77–82.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574. <http://dx.doi.org/10.1093/bioinformatics/btg180>.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. <http://dx.doi.org/10.1093/sysbio/sys029>.
- Scoble, M.J., 1995. *The Lepidoptera. In: Form, Function and Diversity. The Natural History Museum & Oxford University Press, London.*
- Silva, N.P., Araujo, E.B., Mielke, O.H., Morais, H.C., 2012. Aspectos biológicos de *Heliopetes arsalte* (Linnaeus, 1758) (Hesperiidae, Pyrginae) em Cerrado do Distrito Federal. *Biosci. J.* 28, 114–117.
- Silva-Brandão, K.L., Lyra, M.L., Freitas, A.V.L., 2009. Barcoding lepidoptera: current situation and perspectives on the usefulness of a contentious technique. *Neotrop. Entomol.* 38, 441–451. <http://dx.doi.org/10.1590/S1519-566X2009000400001>.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. <http://dx.doi.org/10.1093/bioinformatics/btu033>.
- Stehr, F.W., 1987. Order Lepidoptera. In: Stehr, F.W. (Ed.), *Immature Insects, Vol. I. Kendall/Hunt Publishing, Dubuque*, pp. 293–294.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. <http://dx.doi.org/10.1093/molbev/mst197>.
- Wahlberg, N., Wheat, C.W., 2008. Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of

- lepidoptera. *Syst. Biol.* 57, 231–242, <http://dx.doi.org/10.1080/10635150802033006>.
- Warren, A.D., Ogawa, J.R., Brower, A.V.Z., 2008. Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperidae (Lepidoptera: Hesperioidea). *Cladistics* 24, 642–676, <http://dx.doi.org/10.1111/j.1096-0031.2008.00218.x>.
- Warren, A.D., Ogawa, J.R., Brower, A.V.Z., 2009. Revised classification of the family Hesperidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. *Syst. Entomol.* 34, 467–523, <http://dx.doi.org/10.1111/j.1365-3113.2008.00463.x>.