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Authors: Hernández Vázquez, Ana M., Torres, Javier, Amador De La Cruz, Angélica M., Holodick, Nichol, Webster, Sarah E., et al.

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Ana M. Hernández Vázquez[1, 2, *], Javier Torres[3], Angélica M. Amador de la Cruz[1, 4], Nichol Holodick[5, 6], Sarah E. Webster[5, 7], Alina Rodríguez-Mallon[8], Georgina Espinosa López[1, 9], and Alejandro Barro Cañamero[10]

[1]*Department of Biochemistry, Faculty of Biology, University of Havana, Havana, Cuba*
[2]*https://orcid.org/0000-0003-2111-1677; ana.hernandez@fbio.uh.cu*
[3]*School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska, U. S. A.; https://orcid.org/0000-0002-7873-3250; metalofis@gmail.com*
[4]*https://orcid.org/0009-0009-8279-322X; angelicamador4122@gmail.com*
[5]*Center for Immunobiology and the Department of Investigative Medicine, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, Michigan, U. S. A.*
[6]*https://orcid.org/0000-0002-2671-6918; nichol.holodick@wmed.edu*
[7]*https://orcid.org/0000-0003-0884-9458; sarah.webster@wmed.edu*
[8]*Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology, Havana, Cuba; https://orcid.org/0000-0002-6950-6793; alina.rodriguez@cigb.edu.cu*
[9]*https://orcid.org/0000-0003-0064-7464; georgina@fbio.uh.cu*
[10]*Department of Animal and Human Biology, Faculty of Biology, University of Havana, Havana, Cuba; https://orcid.org/0000-0003-2509-8852; abarro@fbio.uh.cu*

**Corresponding author*

Abstract—The butterfly family Riodinidae Grote, 1895 is a megadiverse Neotropical group comprising 133 genera and over 1,300 species, yet the Antilles only hosts the monotypic genus *Dianesia* Harvey & Clench, 1980, represented by *D. carteri*. This species includes two subspecies: *D. carteri carteri* (Holland, 1902) from the Bahamas and north-central Cuba, and *D. carteri ramsdeni* (Skinner, 1912), endemic to south-central and eastern Cuba. Recently, a disjunct population of *Dianesia* was discovered in western Cuba, marking the first record of the genus in this region, and raising questions about its taxonomic status. Here, we tested whether this geographically isolated population represents a distinct species by integrating phylogenetic, genetic distance, and phenotypic analyses. Genetic analyses using nuclear and mitochondrial markers revealed no shared haplotypes between populations, minimal effective gene flow, and significantly high F_{st} values, supporting strong genetic differentiation. Pairwise genetic distances showed a clear 'barcode gap,' with maximum intrapopulation divergence falling below minimum interpopulation divergence. Phylogenetic reconstruction recovered reciprocal monophyly between eastern and western populations, with robust bootstrap support. Morphologically, the western population is diagnosable by unique wing patterning, including whitish transverse bands and orange scale lines absent from the eastern subspecies, as well as a row of orange spots extending from the ocellus to the costal margin in both males and females. These results collectively support that the western population is a distinct species. Based on these lines of evidence, we formally describe the western population as a new species, *Dianesia galindoensis* Barro, Hernández & Torres sp. nov., with Lomas de Galindo in Mayabeque province as the type locality. Additionally, we recognize *D. carteri ramsdeni* and *D. carteri carteri* at the rank of species—*D. ramsdeni* (Skinner, 1912) stat. nov., and *D. carteri* (Holland, 1902).

RESUMEN—La familia de mariposas Riodinidae Grote, 1895 es un grupo neotropical megadiverso que comprende 133 géneros y más de 1,300 especies; sin embargo, las Antillas solo albergan el género monotípico *Dianesia* Harvey & Clench, 1980, representado por *D. carteri*. Esta especie incluye dos subespecies: *D. carteri carteri* (Holland, 1902) de las Bahamas y el centro-norte de Cuba, y *D. carteri ramsdeni* (Skinner, 1912), endémica del centro-sur y del oriente de Cuba. Recientemente, se descubrió una población disyunta de *Dianesia* en el occidente de Cuba, marcando el primer registro del género en esta región y generando preguntas sobre su estatus taxonómico. En este estudio, evaluamos si esta población geográficamente aislada representa una especie distinta integrando análisis filogenéticos, de distancia genética y fenotípicos. Los análisis genéticos utilizando marcadores nucleares y mitocondriales no revelaron haplotipos compartidos entre las poblaciones, un flujo genético efectivo mínimo y valores de F_{st} significativamente altos, lo que indica una fuerte diferenciación genética. Las distancias genéticas por pares mostraron una clara 'brecha de código de barras', con una divergencia intrapoblacional máxima inferior a la divergencia interpoblacional mínima. La reconstrucción filogenética mostró monofilia recíproca entre las poblaciones oriental y occidental, con un sólido respaldo de bootstrap. Morfológicamente, la población occidental es diagnosticable por un patrón único en las alas, que incluye bandas transversales blanquecinas y líneas de escamas anaranjadas ausentes en la subespecie oriental, así como una fila de manchas anaranjadas que se extiende desde el ocelo hasta el margen costal en ambos sexos. Estos resultados en conjunto respaldan que la población occidental constituye una especie distinta. Con base en estas evidencias, describimos formalmente la población occidental como una nueva especie, *Dianesia galindoensis* Barro, Hernández & Torres sp. nov., con Lomas de Galindo en la provincia de Mayabeque como localidad tipo. Además, reconocemos a *D. carteri ramsdeni* y a *D. carteri carteri* en el rango de especie—*D. ramsdeni* (Skinner, 1912) stat. nov. y *D. carteri* (Holland, 1902).

The Neotropical realm harbors a staggering wealth of butterfly diversity, yet the full extent of this richness remains unknown given limited exploration—particularly in remote island systems where biogeographic isolation tends to increase endemic biodiversity (Whittaker and Fernández-Palacios 2007; Gross et al. 2025). The family Riodinidae Grote, 1895 is a diverse and understudied group of butterflies, comprising more than 8% of all known butterfly species. Approximately 93% of these species are found in the Neotropics, distributed across 133 genera and over 1,300 species (Espeland et al. 2015; Seraphim 2018). Despite the high diversity of Riodinidae in the region, only one genus—*Dianesia* Harvey & Clench, 1980—occurs in the Antilles (Espeland et al. 2015).

The nomenclatural history of *Dianesia carteri* (Holland, 1902) started when Holland (1902) described *Charis carteri* Holland, 1902 from specimens collected in New Providence Island, Bahamas. *Charis carteri* was later transferred to *Apodemia* C. Felder & R. Felder, 1865 (Stichel 1911). Independently, Skinner (1912) described a species from a different genus—*Mesosemia ramsdeni* Skinner, 1912—from Monte Iberia, Holguín province, eastern Cuba. Bates (1935), in a revision of Cuban butterflies, concluded that the Bahamian *A. carteri* (Holland, 1902) and the Cuban *M. ramsdeni* were the same species, but distinct subspecies, and reclassified the Cuban taxon as *Apodemia carteri ramsdeni,* although without providing details for this taxonomic movement. Bates (1935) acknowledged that he could not compare these populations, and that the status of the names should be regarded as uncertain. *Apodemia carteri* was later transferred to *Dianesia* Harvey and Clench, 1980 (Harvey and Clench 1980), which is the present-day treatment. The subspecific rank for the Cuban and Bahamian populations was not challenged (Riley 1975; Harvey and Clench 1980), despite the presence of phenotypic differences between them.

Currently, *Dianesia* is represented by a single species, *Dianesia carteri*, which has two subspecies. The nominate subspecies—*D. carteri carteri* (Holland, 1902)—is distributed across the Bahamas and cays of the Sabana-Camagüey Archipelago in north-central Cuba (Aborrezo-Pérez 2006; Núñez et al. 2020), while a second subspecies—*D. carteri ramsdeni* (Skinner, 1912)—has been sporadically reported from a few localities in south-central and eastern Cuba (Alayo and Hernández 1987; Hernández et al. 1998; Núñez and Barro 2016; Álvarez et al. 2024; GBIF Occurrence Download: https://doi.org/10.15468/dl.6a5t47; Fig. 1).

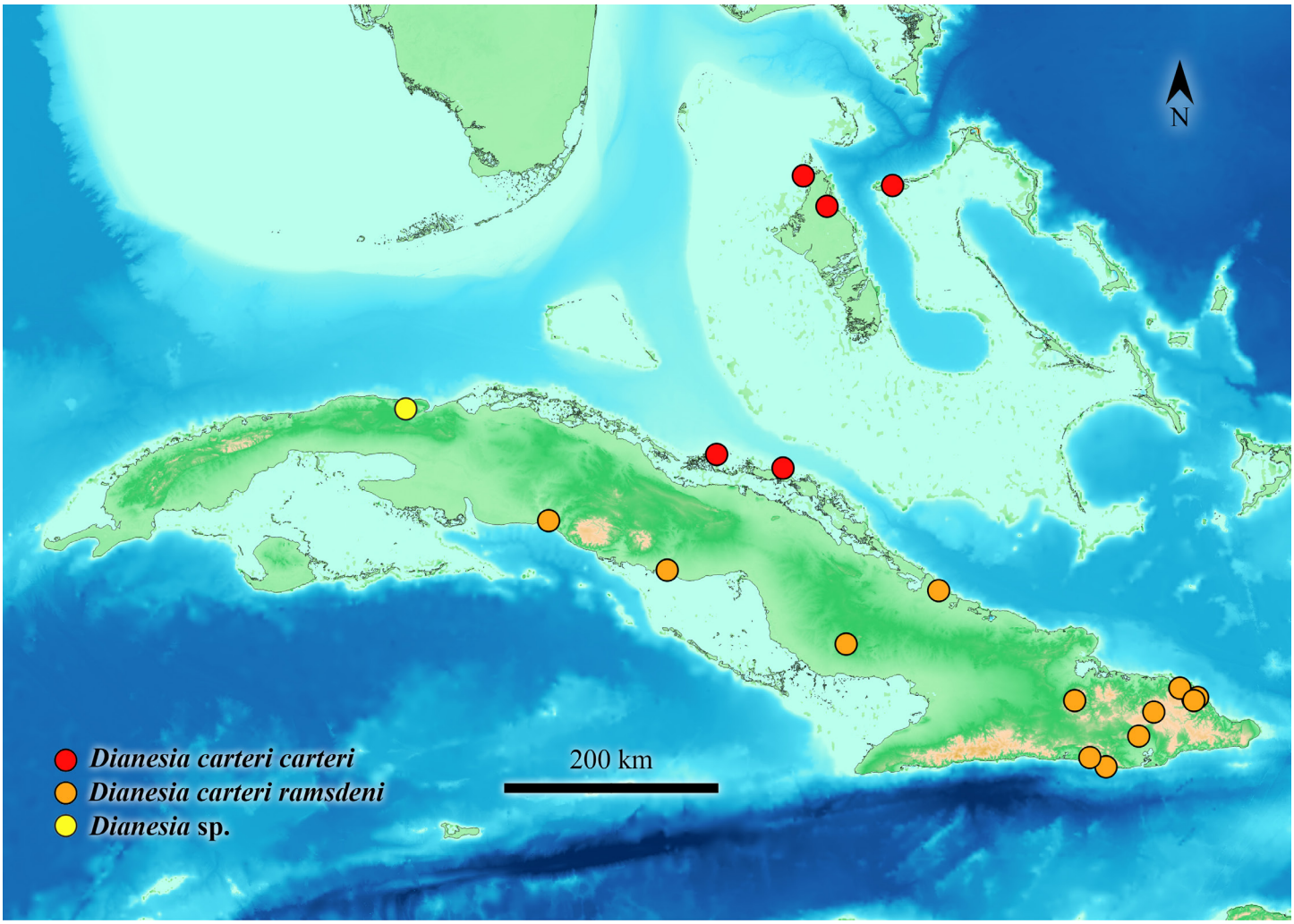

FIGURE 1. Distribution of the butterflies *Dianesia* sp., *D. carteri carteri* (Holland, 1902), and *D. carteri ramsdeni* (Skinner, 1912).

During field expeditions conducted by our research group in western Cuba at Lomas de Galindo (Álvarez and Barro 2025), a serpentine area located at Santa Cruz del Norte municipality, Mayabeque province (Fig. 2), we observed butterflies of the genus *Dianesia* (Fig. 3). On a first inspection, the specimens of this population seemed lighter than *D. carteri ramsdeni*. Additionally, their distribution is disjunct, with Lomas de Galindo being approximately 170 km from Cienfuegos Bay—the closest *D. carteri ramsdeni* record—and 780 km from Monte Iberia—its type locality. Due to superficial phenotypic distinctness of the Loma de Galindo's *Dianesia* population, and its apparent geographic separation from populations of *D. carteri ramsdeni*, we wondered whether our newly discovered population was a distinct species.

Genetic analyses are a crucial tool to test for evolutionary independence of populations, especially those with subtle phenotypic differentiation or lack of it (Schwartz and Waples 2006). Several studies have addressed the phylogeny of Riodinidae, utilizing both nuclear and mitochondrial markers, but the genus *Dianesia* has not been addressed in much detail (Campbell et al. 2000; Campbell and Pierce 2003; Espeland et al. 2015; Seraphin et al. 2018). In this study, we use a multilocus molecular approach, based on traditional mitochondrial and nuclear markers, in combination with a phenotypic assessment to test the hypothesis that the newly discovered population of *Dianesia* is a distinct species.

MATERIALS AND METHODS

To test our distinct species hypothesis, we followed the General Lineage species concept (Frost and Hillis 1990; de Queiroz 1999, 2005, 2007; Coyne and Orr 2004). For the genetic data we expected independent evolutionary history and genetic differentiation, and for the phenotypic data we expected diagnosis.

FIGURE 2. Habitat of *Dianesia* sp. at Lomas de Galindo, Mayabeque province, western Cuba. Photos by Ana M. Hernández Vázquez.

FIGURE 3. *Dianesia* sp. in its natural habitat at Lomas de Galindo, Mayabeque province, western Cuba. Note the typical riodinid behavior of resting on the under side of leaves with the wings outstretched. Photos by Ana M. Hernández Vázquez.

Specimens used

For type specimen designation and phenotypic comparisons, we used specimens of *Dianesia* spp., which were not identified beyond genus, previously deposited at the entomological collection of the Faculty of Biology, University of Havana, Cuba. We used five specimens, one female and four males, collected at Lomas de Galindo, in western Cuba (23.0661, -81.7341), and three specimens, one female and two males of *D. carteri ramsdeni* from Monte Iberia, in eastern Cuba (20.4828, -74.7231). The datum for all coordinates provided in this study is WGS84. For genetic analyses, we used three specimens of each of the two localities.

Evaluating independent evolutionary history and genetic differentiation

We extracted DNA from a single leg taken from each of three specimens from each locality using the DNeasy kit (Qiagen, New York, U. S. A.), following the manufacturer's instructions. We amplified the mitochondrial Cytochrome Oxidase I (COI) given that Hebert et al. (2003) proposed that analyzing the 5′ re-

TABLE 1. List of primers used for amplification and sequencing of the genes Cytochrome Oxidase I (COI), Elongation factor 1 alpha (Ef-1a), and Wingless (Wg).

Gene	Primer name	Direction	Sequence 5'–3'
COI	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG
COI	COI Nancy	Reverse	CCCGGTAAAATTAAAATATAAACTTC
Ef-1a	M51.9	Forward	CARGACGTATACAAAATCGG
Ef-1a	rcM4	Reverse	ACAGCVACKGTYTGYCTCATRTC
Wg	Wg1	Forward	GARTGYAARTGYCAYGGYATGTCTGG
Wg	Wg2	Reverse	ACTICGCRCACCARTGGAATGTRCA

gion of the mitochondrial COI gene could serve as a universal DNA barcode for identifying species and assessing cryptic diversity. However, some studies have also shown that the sole use of COI as a barcode was not always enough to identify all the species of butterflies evaluated, and that adding a fragment of a nuclear gene to the analysis resolved issues in some of the problematic species (Elias et al. 2007). Therefore, we also amplified the nuclear genes Elongation factor 1 alpha (Ef-1a) and Wingless (Wg). All the amplifications were conducted using primers proven to be successful for Riodinidae (Espeland et al. 2015; Table 1). We conducted polymerase chain reactions in a final volume of 25 µL using the GoTaq Green Master Mix (Promega, Wisconsin, U. S. A.), with the following cycling conditions: an initial denaturation at 94° C for 2 minutes, followed by 35 cycles of denaturation at 94° C for 1 minute, annealing at 50° C for 40 seconds, and extension at 72° C for 1 minute. We added another extension step at 72° C for 5 minutes, and afterwards the reaction was cooled to 4° C. To confirm the presence of the corresponding amplicons, we visualized PCR products in 1.8% agarose gel electrophoresis. We performed Sanger sequencing, both forward and reverse, directly from the PCR products, and the service was outsourced to Genewiz from Azenta Life Science (New Jersey, U. S. A.).

Besides our original sequences, we also downloaded sequences from GenBank (Appendix 1) to root the phylogenetic tree (see below). To obtain an error-free dataset, we manually reviewed our sequences by comparing forward and reverse chromatograms using MEGA6 (Kumar et al. 2016). Then, we aligned the resulting consensus sequences via the Muscle algorithm (Zambrano et al. 2017).

To test for genetic differentiation, we calculated a series of key genetic indices that provide insights into variability, differentiation, and gene flow, using the program DNAsp v5.10.01 (Librado and Rozas 2009). We obtained the number of haplotypes (h), the haplotype diversity (Hd), nucleotide diversity (π), the number of polymorphic sites, and the total number of mutations. These indices identify genetic differences among populations, if present. We also calculated the effective number of migrants (Nm), indicative of genetic flow between the populations, and the fixation index (F_{st}). F_{st} measures the amount of genetic variation that can be explained by the population genetic structure; thus, it quantifies the genetic differentiation between populations (Wright 1969). Its values range between 0 and 1, indicative of minimal and maximal differentiation, respectively. $F_{st} < 0.05$ indicates low genetic differentiation, $0.05 \leq F_{st} < 0.15$ suggests moderate differentiation, while $F_{st} \geq 0.15$ suggests high genetic differentiation (Wright 1969; Shane 2005).

A barcode gap refers to a clear separation between the maximum genetic distance observed within a species (intraspecific variation) and the minimum genetic distance observed between distinct species (interspecific variation) (Hebert et al. 2003). A barcoding gap is considered to exists when the average interspecific distance is at least 10 times greater than the average intraspecific genetic distance (Hebert et al. 2004), providing a threshold for species delimitation. To detect discontinuous genetic clusters or barcoding gaps, we calculated intra- and inter-population genetic distances using COI sequences with MEGA6 (Kumar et al. 2016). We conducted the analyses using uncorrected p-distances, the Kimura 2-parameter model, widely used for detecting barcoding gaps across species (Attiná et al. 2020; Gaytán et al. 2021) and the Tamura 3-parameter + G model with gamma-distributed rate variation. The Kimura 2-parameter (K2P) model distinguishes

TABLE 2. Genetic diversity indices for two populations across three genes: Cytochrome Oxidase I (COI), Elongation factor 1 alpha (Ef-1a), and Wingless (Wg). The table shows number of polymorphic sites, number of mutations, nucleotide diversity (π), haplotype diversity (Hd), fixation index (F_{st}), and effective number of migrants (Nm) for each gene and population (considering both populations merged and separated).

Gene	Population	n	polymorphisms	mutations	π	H	Hd	F_{st}	Nm
COI	Merged	6	27	28	0.02419	5	0.93	0.9	0.02
	Dianesia sp.	3	3	2	0.00317	2	0.67		
	D. carteri ramsdeni	3	2	3	0.00211	3	1		
Ef-1a	Merged	6	3	3	0.0037	2	0.6	1	0
	Dianesia sp.	3	0	0	0	1	0		
	D. carteri ramsdeni	3	0	0	0	1	0		
Wg	Merged	6	1	1	0.0018	2	0.6	1	0
	Dianesia sp.	3	0	0	0	1	0		
	D. carteri ramsdeni	3	0	0	0	1	0		

between transitions and transversions, it assumes that all four nucleotides occur at equal frequencies and that transitions occur at higher rate than transversions. By accounting for these two substitution rates separately, the model corrects for multiple substitutions at the same site and provides a more accurate estimate of genetic distance between sequences compared to simpler models. However, it does not consider differences in base composition or variation in substitution rates among sites (Kimura 1980). On the other hand, Tamura 3-parameter model, which was the model MEGA6 software determined to best fit our data, corrects for multiple hits by explicitly accounting for differences between transitional and transversional substitution rates as well as G+C-content bias, reflecting unequal base composition among sequences. The model incorporates a discrete gamma distribution (+G) to model evolutionary rate heterogeneity among sites, allowing for variable substitution rates across nucleotide positions (Tamura 1992). Since the results are very similar, we show only the ones corresponding to Tamura 3-parameter + G model.

Last, we reconstructed phylogenetic trees to test whether *Dianesia* sp. from Lomas de Galindo and *D. carteri ramsdeni* are distinct clades (as expected for different species), using *Napaea actoris* Hewitson, 1875 as an outgroup. We generated Maximum Likelihood (ML) phylograms using IQ-TREE v1.6 (Nguyen et al. 2015) with a concatenated dataset from all three markers. We determined the best-fit substitution model via ModelFinder, implemented within IQ-TREE (Kalyaanamoorthy et al. 2017), partitioned by codon position and by marker. We calculated branch support with 10,000 replicates for the Ultrafast Bootstrapping algorithm (Hoang et al. 2018).

Evaluating phenotypic distinctness

To assess phenotypic diagnosis, we compared the male genitalia and specimens' coloration using a stereomicroscope (Novel, China). To extract the genitalia, we dissected the abdomens and immersed them in a 10% KOH solution heated to 60–90°C for 5–15 minutes. Next, we thoroughly rinsed the specimens with distilled water to remove residual KOH and neutralized them with 5% lactic acid. We then dehydrated the cleared genitalia sequentially in ethanol solutions of increasing concentrations (70%, 96%, and 100%), with each step lasting approximately 10 minutes. Last, we stored the genitalia in glycerol.

RESULTS

Evaluating independent evolutionary history and genetic differentiation

We calculated several genetic diversity indices to evaluate whether *Dianesia* sp. and *D. carteri ramsdeni* were genetically distinct (Table 2). The analysis of the merged populations showed that of the 631 sites retrieved for COI, 27 were polymorphic, where 22 were parsimony-informative with two variants. Ef-1a presented three polymorphic sites, and Wg presented only one, in both cases parsimony-informative sites with two variants (Appendix 2). For all the genes, the nucleotide diversity within each locality was very low,

TABLE 3. Estimates of Evolutionary Divergence over Sequence Pairs within and between different Riodinidae species and genera. The genetic distances between groups are shown below the diagonal and within groups at the diagonal. Standard error estimates are shown, between groups above the diagonal and within group between parenthesis at the diagonal. There is no value corresponding with intra specie genetic distance for *Dianesia carteri carteri* because there was only one sequence available.

	Dianesia sp.	*Di. carteri ramsdeni*	*Di. carteri carteri*	*Do. deodata*	*Do. elvira*	*M. asa*	*M. carissima*
Dianesia sp.	0.0035 (0.002)	0.009	0.010	0.017	0.017	0.018	0.018
Di. carteri ramsdeni	0.042	0.0023 (0.002)	0.009	0.016	0.017	0.017	0.018
Di. carteri carteri	0.049	0.044	—	0.015	0.016	0.017	0.018
Dodona deodata	0.128	0.122	0.114	0 (0)	0.012	0.019	0.019
Dodona elvira	0.111	0.115	0.104	0.073	0 (0)	0.019	0.018
Mesosemia asa	0.130	0.126	0.128	0.150	0.145	0.007 (0.003)	0.014
Mesosemia carissima	0.135	0.136	0.131	0.145	0.140	0.094	0 (0)

and an order of magnitude lower than that of the full set of individuals studied. Haplotype diversity was high for COI, with five haplotypes detected—two in *Dianesia* sp. and three in *D. carteri ramsdeni*, that were not shared between localities. For Ef-1a and Wg, haplotype diversity was zero; only one haplotype was identified for each of these genes, which was not shared between localities. Consistent with this, the effective number of migrants was extremely low and values were very high. These results suggest that the genetic flow between the populations is very limited and that the populations are genetically differentiated.

Next, we evaluated the existence of barcoding gaps between the study populations using COI sequences. If *Dianesia* sp. and *D. carteri ramsdeni* are grouped together, the genetic distance value is 0.0259. However, assuming two different populations, the intrapopulation genetic distances obtained were an order of magnitude lower, 0.0035 for *Dianesia* sp. and 0.0023 for *D. carteri ramsdeni*, and 12 and 18.3 times lower, respectively, than the genetic distance between the populations (0.042), supporting the existence of a barcoding gap (Table 3).

The genetic distance between *D. carteri carteri* of Bahamas and both Cuban populations were also more than an order the magnitude higher than the intraspecific distances (0.049 for *Dianesia* sp. and 0.044 for *D. carteri ramsdeni*). Then, we compared the genetic distances obtained between our study populations with the values calculated for different Riodinidae species from the same and different genera, confirming the genetic distance between our two populations were in the same order of magnitude than the ones obtained between different Riodinidae species from the same genera (Appendix 1).

Phylogenetic reconstruction using Maximum Likelihood (ML) strongly support a sister relationship and reciprocal monophyly of *D. carteri ramsdeni* from eastern Cuba, and *Dianesia* sp. from Lomas de Galindo, western Cuba. These two lineages form a well-supported clade together with an accession of *D. carteri carteri* from the Bahamas (Fig. 4).

TAXONOMIC TREATMENT

Due to geographical isolation, genetic differentiation, and evolutionary independence, we found support for the hypothesis that *Dianesia* sp. from Lomas de Galindo, western Cuba is a distinct species, which we describe below. Phenotypic, geographic, and molecular data support that the western and eastern populations of Cuban *Dianesia* are distinct species. This poses a problem for *Dianesia* taxonomy, because the recognition of *D. galindoensis* sp. nov. makes *D. carteri*, inclusive of *D. carteri carteri* and *D. carteri ramsdeni*, paraphyletic

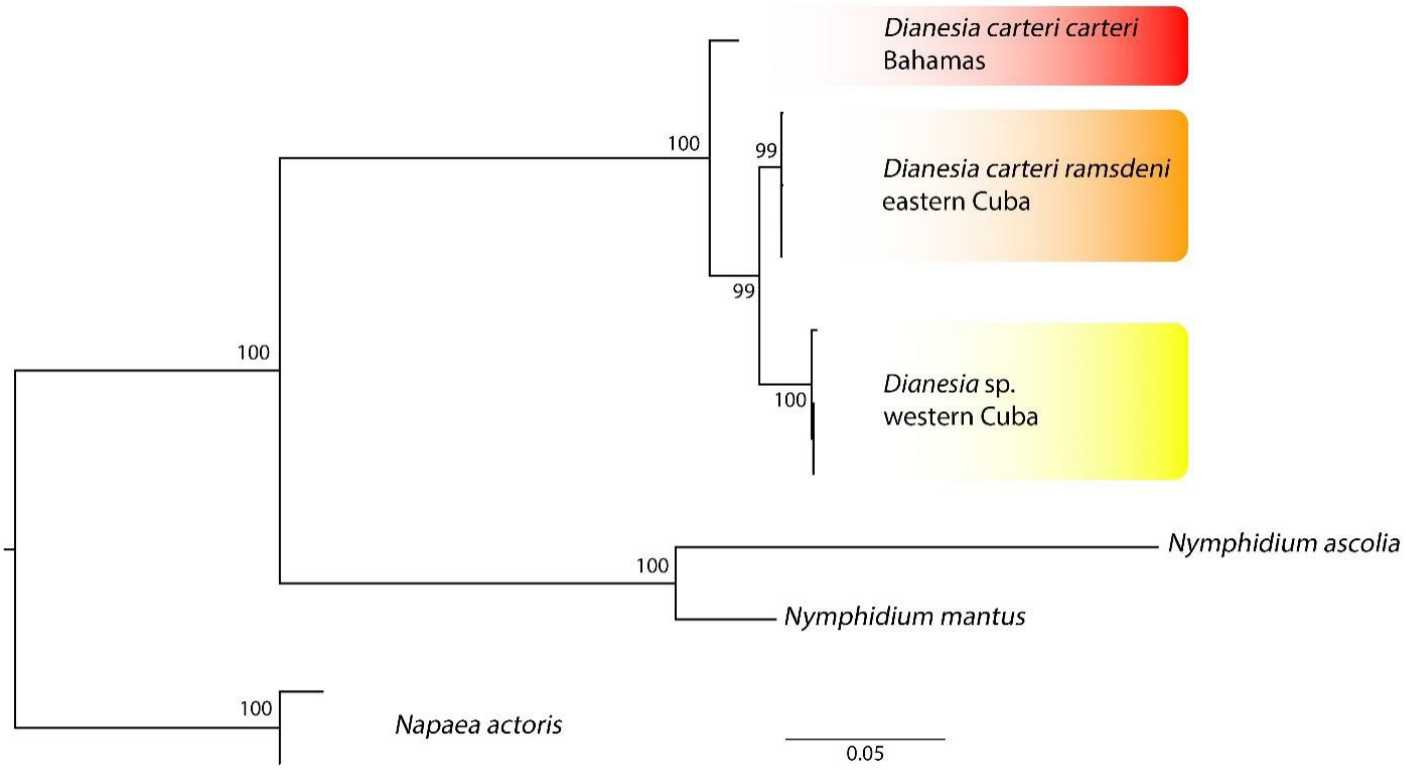

FIGURE 4. Maximum Likelihood consensus tree inferred using IQ-TREE v1.6 from a concatenated alignment of 1,448 bp, comprising the mitochondrial gene Cytochrome Oxidase I (COI) and the nuclear genes Elongation factor 1 alpha (Ef-1a) and Wingless (Wg). Nodal support values correspond to 10,000 ultrafast bootstrap replicates. The tree shows strong support for the reciprocal monophyly of *Dianesia carteri ramsdeni* (eastern Cuba) and the new *Dianesia* species from western Cuba. *Dianesia carteri carteri* is represented by a single accession from the Bahamas, and is recovered sister to the other two clades within *Dianesia*. *Napaea actoris* was used as the outgroup. Scale bar indicates the number of substitutions per site.

(Fig. 4). However, all three taxa are geographically isolated and are phenotypically and molecularly diagnosable. Thus, we propose the elevation of the subspecific taxon *Dianesia carteri ramsdeni* to *D. ramsdeni* stat. nov.—restricted to eastern and south-central mainland Cuba. *Dianesia carteri*, from Bahamas and some keys in north-central Cuba, is treated at the rank of species.

Dianesia galindoensis Barro, Hernández & Torres sp. nov.

(Figs. 3, 5, 6)

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HOLOTYPE: Adult male. Cuba, Mayabeque, Santa Cruz del Norte, Lomas de Galindo; 150 m a.s.l., 23.0633° N, 81.6561° W, 30/VI/2014, A. Barro y A. Serrano, deposited at the Entomological Collection of the Faculty of Biology (FB), catalog number 13.15009 (FB 13.15009.)

PARATYPES: 3 males, 1 female. Cuba, Mayabeque, Santa Cruz del Norte, Lomas de Galindo; 150 m a.s.l., 23.0633° N, 81.6561° W, 30/VI/2014, A. Barro y A. Serrano, FB 13.15010 (1 male); VII/2018, A. Barro, FB 13.15011 (1 male); 15/VII/2022, A. Barro, FB 13.15012 (1 male); IX/2021, A. Barro, FB 13.15013 (1 female). Same coordinates as holotype.

Description of holotype

Head: Brown, with orange scales forming a line near the cervix and encircling the base of the antennae. Antennae black, with bluish-white scales on each segment that give the antennae a segmented appearance. The eyes are green.

Forewings: Forewing length (FWL) = 16 mm. Pale brown with two white spots near the costa, located two-thirds of the distance from the wing base to the apex; a white fascia crosses the wing from these spots to the inner margin; whitish bands with lines of orange scales

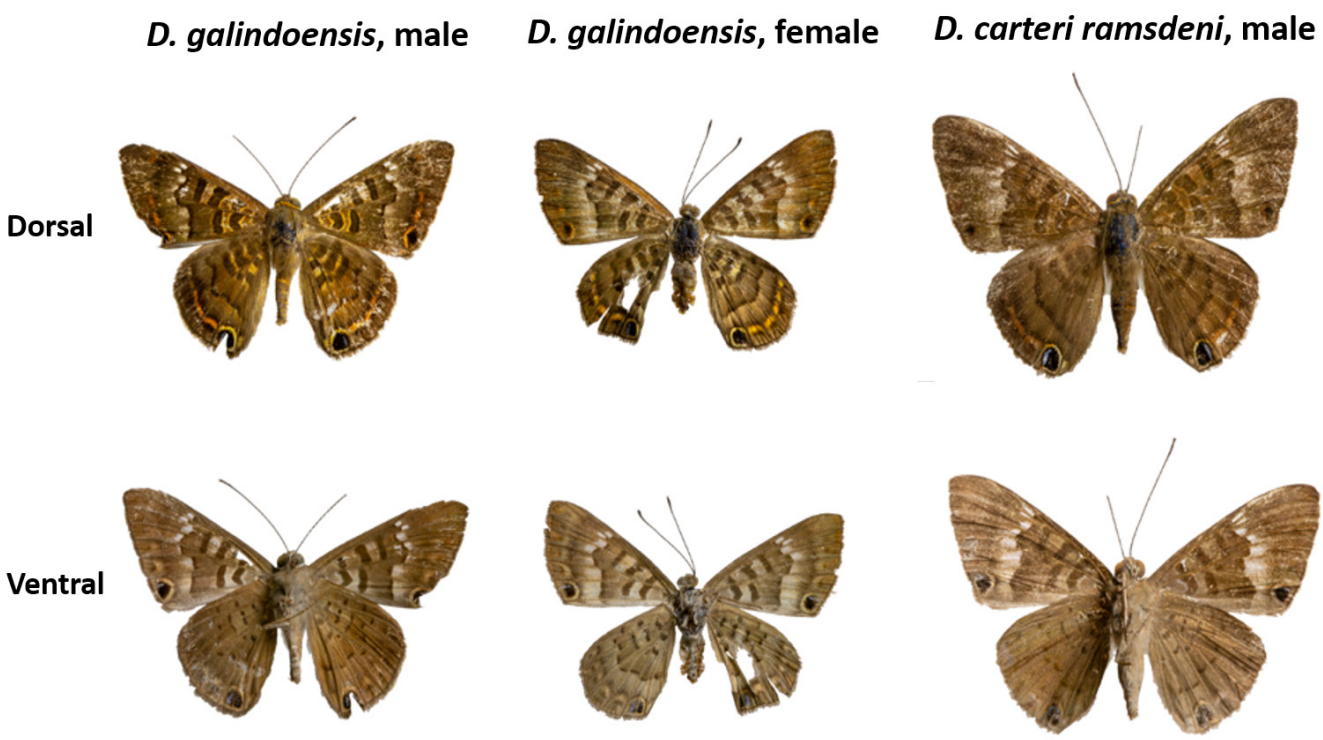

FIGURE 5. Dorsal and ventral views of Cuban *Dianesia* lineages. From left to right: *D. galindoensis* sp. nov., holotype, male (FB 13.15009); *D. galindoensis* sp. nov., paratype, female (FB 13.15013); *D. carteri ramsdeni*, male (FB13.15277). All specimens are from their respective type localities: Lomas de Galindo, Mayabeque (*D. galindoensis* sp. nov.), and Monte Iberia, Guantánamo (*D. carteri ramsdeni*). Photos by Ana M. Hernández Vázquez.

alternate with four dark brown lunular spots that traverse both pairs of wings; a black ocellus at the anal angle is surrounded by orange scales along the inner margin.

Hindwings: Pale brown with three dark brown lunular spots alternating with light orange spots in the basal third; a dark brown line crosses the wing; a black ocellus—more prominent than that of the forewings—at the anal angle is bordered by a thin line of yellow scales and blue scales along the outer margin, forming an arch; a row of orange spots extends from the ocellus to the costal margin.

Male genitalia: Uncus lobed, with one tooth on each lobe; vinculum abruptly curved in its upper half; valvae simple, with a slightly sclerotized posterior edge and membranous toward their junction with the vinculum; aedeagus long and slender, slightly curved (Fig. 6).

Abdomen: brown, with segments delimited by light orange dorsal thin bands.

Diagnosis

Dianesia galindoensis sp. nov. differs from *D. carteri ramsdeni* in that the former is lighter in coloration. Additionally, *D. galindoensis* sp. nov. exhibits whitish transverse bands combined with lines of orange scales on its wings, whereas *D. carteri ramsdeni* displays only a single white fascia on the forewing. In both sexes of *D. galindoensis* sp. nov., a row of orange spots extends from the ocellus to the costal margin of the wing, a feature observed in the females but not the males of *D. carteri ramsdeni*. The molecular diagnosis of *D. galindoensis* sp. nov. is based on 27 nucleotide differences *with D. carteri ramsdeni*, including 23 in COI, three in Ef-1a, and one in Wg (Appendix 2).

Variability: Male paratypes FWL were 14–17 mm (mean 15 mm, n = 3). The rest of the characters are very similar to the holotype. Female paratype (Fig. 5) FWL = 14 mm, phenotypically similar to the male.

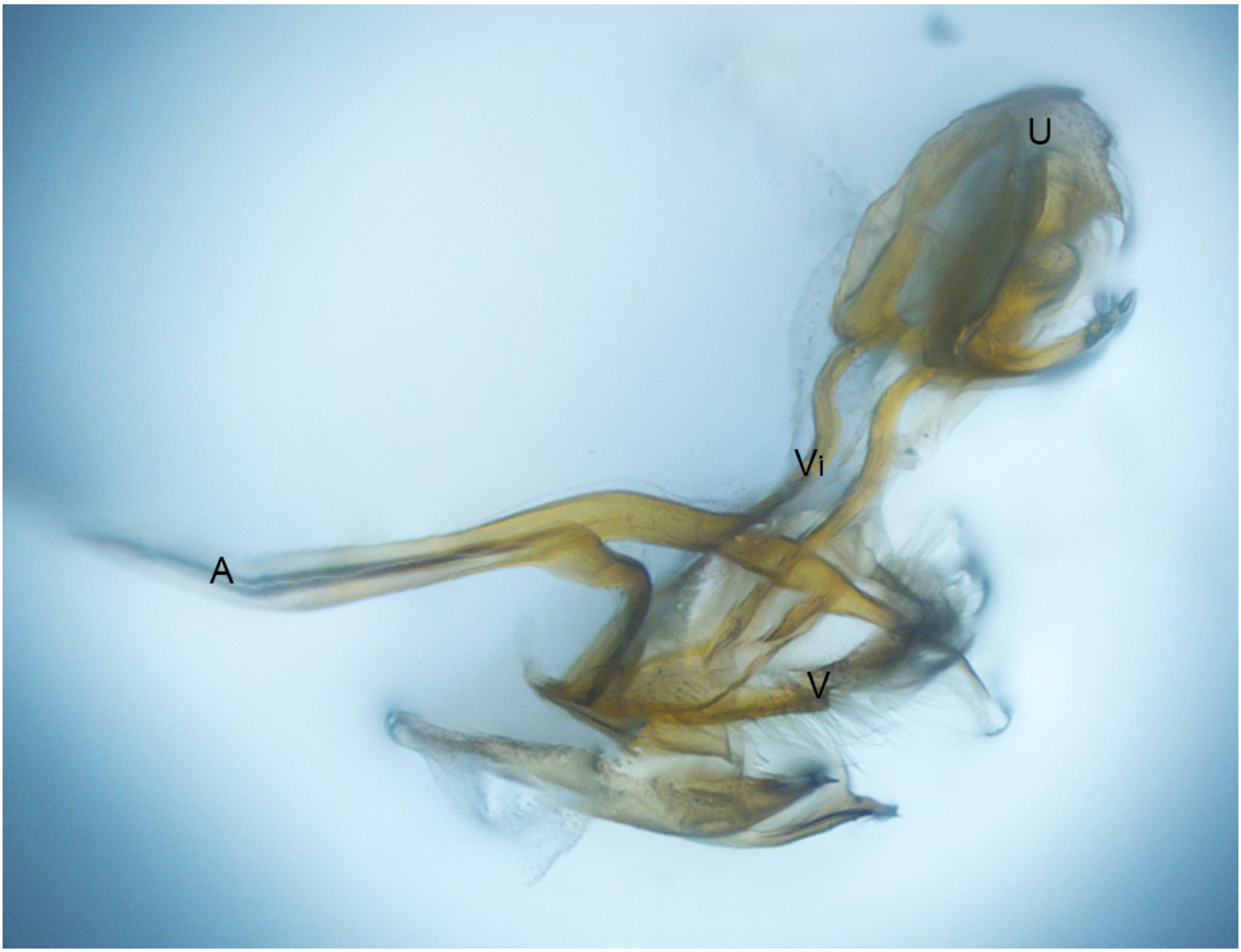

FIGURE 6. Genitalia of male *Dianesia galindoensis* sp. nov., paratype (FB 13.15012). U: uncus; A: aedeagus; V: valva; Vi: vinculum. Olympus microscope, 40×.

Natural history

Dianesia galindoensis sp. nov. inhabits dry lowland serpentine vegetation (locally known as "cuabal") at elevations around 150 m a.s.l. in the Alturas Havana-Matanzas region (Fig. 1). It flies in open areas and perches on shrubs with its head downwards (Fig. 2), avoiding the high temperatures of sun-exposed zones. In June 2014, ABC observed more than 20 individuals flying around and feeding from a flowering *Bourreria havanensis* Miers (Boraginaceae) and on *Phyllanthus orbicularis* Kunth (Phyllanthaceae).

Etymology

The specific epithet "galindoensis" refers to the type locality, Lomas de Galindo, Santa Cruz del Norte municipality, Mayabeque province, Cuba.

Dianesia ramsdeni (Skinner, 1912) stat. nov.

urn:lsid:zoobank.org:act:DC2CA268-D240-49C6-8591-BDD0DBF4A0E4

Mesosemia ramsdeni Skinner, 1912 in Skinner (1912): 126 (original description)

(Fig. 5)

DISCUSSION

In concordance with genetic differentiation between different species, *Dianesia galindoensis* sp. nov. and *D. ramsdeni* did not share haplotypes for any of the genes studied. The effective number of migrants was extremely low, indicating the lack of gene flow between populations. F_{st} values were very high, supporting the genetic distinctness of the two populations.

Several studies on the order Lepidoptera have

demonstrated the utility of evaluating the emergence of barcoding gaps to determine if the studied populations reached species-level thresholds of genetic divergence (Hausmann et al. 2013; Dincă et al. 2015; Lavinia et al. 2017a). Pairwise analyses of the mean genetic distances for each population, showed that the maximum intraspecific distance was more than an order of magnitude lower than the minimum distance between the populations, suggesting the presence of a barcode gap. These genetic distances are in concordance with the ones obtained by Attiná et al. (2021), that determined that the average intra-species genetic distance of 156 species (110 genera) of neotropical butterflies was 0.0029 (0.002–0.0037) and mean divergence to the nearest neighbor species was nearly 13× the average distance to the furthest conspecific. Furthermore, the genetic distance obtained in our study for *D. galindoensis* sp. nov. and *D. ramsdeni*, are within the interval of the ones we calculated for different stablished species of Riodinidae.

From the phylogenetic point of view, distinct species are expected to exhibit reciprocal monophyly, reflecting the accumulation of genetic differences due to restricted gene flow and independent evolutionary trajectories (Frost and Hillis 1990; de Queiroz 1999, 2005, 2007; Coyne and Orr 2004). Our phylogenetic reconstruction strongly supported a reciprocal monophyly of *Dianesia ramsdeni*, *D. carteri*, and *D. galindoensis* sp. nov. (Fig. 4).

Dianesia galindoensis sp. nov. can be distinguished in the field because exhibits a lighter brown color, whitish transverse bands combined with lines of orange scales on its wings, as well as a row of orange spots from the ocellus to the costal margin of the wing both in females and males. With this nomenclatural update, the genus *Dianesia* includes three species, distributed in Bahamas and Cuba.

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APPENDIX 1. Sequences used in this study. The molecular markers are Cytochrome Oxidase I (COI), Elongation factor 1 alpha (Ef-1a), and Wingless (Wg). Accessions that begin with PV- are newly generated in this study.

Species	COI	Ef-1a	Wg	Source	Purpose
Dianesia carteri	KT286509	KT286204	KT286031	GenBank	Phylogeny reconstruction
Napaea actoris	KT286540	KT286233	KT286058	GenBank	Phylogeny reconstruction
Napaea actoris	KT286444	KT286233	KT285977	GenBank	Phylogeny reconstruction
Nymphidium ascolia	MG607846	MG607515	MG608555	GenBank	Phylogeny reconstruction
Nymphidium ascolia	MG607847	MG607516	MG608558	GenBank	Phylogeny reconstruction
Nymphidium mantus	MG607851	MG607518	MG608558	GenBank	Phylogeny reconstruction
Dodona deodata	OR939342	-	-	GenBank	Calculate p-distances
Dodona deodata	OR939340	-	-	GenBank	Calculate p-distancess
Dodona elvira	OK340919	-	-	GenBank	Calculate p-distances
Dodona elvira	OK340919	-	-	GenBank	Calculate p-distancess
Mesosemia asa	JQ569529	-	-	GenBank	Calculate p-distancess
Mesosemia asa	JQ569587	-	-	GenBank	Calculate p-distances
Mesosemia carissima	GU153488	-	-	GenBank	Calculate p-distances
Mesosemia carissima	GU153487	-	-	GenBank	Calculate p-distances
Mesosemia carissima	GU153486	-	-	GenBank	Calculate p-distances
Dianesia galindoensis	PV655153	PV656470	PV656476	This study	Genetic diversity, phylogenetics
Dianesia galindoensis	PV655154	PV656471	PV656477	This study	Genetic diversity, phylogenetics

Dianesia galindoensis	PV655155	PV656472	PV656478	This study	Genetic diversity, phylogenetics
Dianesia ramsdeni	PV655156	PV656473	PV656479	This study	Genetic diversity, phylogenetics
Dianesia ramsdeni	PV655157	PV656474	PV656480	This study	Genetic diversity, phylogenetics
Dianesia ramsdeni	PV655158	PV656475	PV656481	This study	Genetic diversity, phylogenetics

APPENDIX 2. Molecular diagnosis among *Dianesia* lineages. *D. g.* = *D. galindoensis*, *D. r.* = *D. ramsdeni*, and *D. c.* = *D. carteri*.

Gene	Site	*D. g.*	*D. r.*	*D. c.*
COI	45	A	A	T
COI	63	A	A	T
COI	69	A	C	C
COI	84	T	T	C
COI	111	C	T	T
COI	132	C	C	T
COI	138	A	A	G
COI	144	G	A	A
COI	156	T	T	C
COI	180	C	A	C
COI	190	T	T	C
COI	198	C	T	T
COI	216	C	C	T
COI	241	T	C	T
COI	249	C	T	T
COI	279	T	T	C
COI	297	T	A	A
COI	312	A	T	A
COI	318	C	T	C
COI	354	C	T	A
COI	378	A	G	C
COI	379	C	C	T
COI	402	C	T	T
COI	405	T	C	T
COI	429	A	C	C
COI	435	C	T	T
COI	462	T	A	A
COI	486	A	G	A
COI	504	T	C	T
COI	516	T	T	A
COI	525	T	T	A
COI	567	T	C	T
COI	582	T	C	T
COI	585	C	C	A
COI	600	A	T	A
COI	609	T	A	A
Ef-1a	73	A	G	G
Ef-1a	85	T	C	C
Ef-1a	127	T	T	A
Ef-1a	217	A	A	T
Ef-1a	253	A	A	T
Ef-1a	280	C	C	T
Ef-1a	311	T	T	C
Ef-1a	457	T	C	C
Wg	26	A	A	T
Wg	107	A	A	T
Wg	176	A	T	T