



A new subspecies of *Calisto disjunctus* Núñez & Barro (Lepidoptera: Nymphalidae: Satyrinae) from Western Cuba, with a key to the Cuban members of the genus

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Abstract

Calisto disjunctus hersheyi **ssp. n.** is described from western Cuba. Additionally, a dichotomous key including all described Cuban taxa of the *herophile* species group of *Calisto* is given together with illustrations of live specimens of most taxa.

Key words: Jardines de Hershey, Lomas de Galindo, El Taburete, DNA, COI, barcoding

Introduction

The genus *Calisto* Hübner is the sole representative of the Satyrinae in the West Indies, from where it is endemic (Riley 1975; Smith *et al.* 1994; Núñez *et al.* 2012). The genus has no close relatives and has been isolated for at least 20 Ma, reaching a tremendous diversification as its species seem to be poor at dispersal over water (Matos-Maraví *et al.* 2014; Núñez *et al.* 2019). Hispaniola is the headquarters of the genus with more than half of the described species (Pérez-Asso *et al.* 2016), and Cuba has currently 16 described species (Núñez *et al.* 2019). Initially with only two accepted species and a handful of subspecies, DNA barcoding and morphological studies have led to the discovery of new species and the elevation of subspecies to specific status (Núñez *et al.* 2012; 2013; 2019). The *herophile* species group, present in Cuba and Bahamas, is a cryptic complex with very similar species differing in subtle morphological characters and in their DNA. Few of them are wide-ranging species, such as *C. herophile* Hübner, *C. aquilum* Núñez and *C. dissimulatum* Núñez, the rest being confined to a single mountain range or particular habitat (Núñez *et al.* 2013; 2019).

Calisto disjunctus Núñez & Barro is one of the most recently described species of the genus (Núñez *et al.* 2019). The type series was collected in Estero de las Piedras, Ciénaga de Lanier, on the southwestern coast of Isla de la Juventud. It is said to be common in that area (A. Barro, pers. comm.). These authors also analyzed a specimen from El Taburete, Sierra del Rosario, in the western mountains of Guaniguanico on the main island. It was present in previous analyses (Núñez *et al.* 2012; 2013) and slightly differed in its sequences from Isla de la Juventud ones. This led the authors to suggest that it could represent a different subspecies (Núñez *et al.* 2019), but in the absence of additional specimens they preferred to wait until more material become available.

During butterfly surveys in Jardines de Hershey, Santa Cruz del Norte, Mayabeque Province, Álvarez & Corso (2020) found *C. disjunctus* flying with the ubiquitous *C. herophile* in disturbed semideciduous forest patches. Additional specimens collected in Lomas de Galindo, on the borders of the provinces of Mayabeque and Matanzas, also proved to belong to this species. These findings expanded the known range of the species more than 100 km to the east from El Taburete. Furthermore, the COI barcodes of specimens from these two localities were more similar to the sequence of the specimen from El Taburete. All specimens from mainland Cuba differed in their sequences and morphology from Isla de la Juventud ones. These results supported the hypothesis of Núñez *et al.* (2019) that the specimen from Cuba might represent a different subspecies. In this work, we formally describe this new subspecies of *C. disjunctus*, including molecular and morphological characters towards its recognition, while providing infor-

mation on its biology and possible origin. Finally, we propose an updated key to all Cuban *Calisto* taxa and illustrate all but two with images of live specimens.

Materials and Methods

Taxon sampling, DNA extraction and sequencing

DNA was extracted from leg tissue of dried specimens using the DNeasy kit (Qiagen, Hilden, Germany). Polymerase chain reactions used the primer pair LepF1/LepR1 (Hebert *et al.* 2004) which recovers a 658 bp region near the 5' end of COI including the 648 bp barcode region for the animal kingdom (Hebert *et al.* 2003). Sequence assembling and editing was performed using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were submitted to GenBank and we retrieved seven from the database (Table 1).

TABLE 1. *Calisto* specimens included in the COI barcode dataset. In bold are highlighted new added sequences.

Species	COUNTRY, region	Voucher code	Voucher depository	COI
<i>Calisto disjunctus disjunctus</i>	CUBA, Isla de la Juventud	AB-005	NSG	JN881872
<i>Calisto disjunctus disjunctus</i>	CUBA, Isla de la Juventud	AIMSEQ29032017	NSG	JN881873
<i>Calisto disjunctus hersheyi</i>	CUBA, Habana-Matanzas Hills	YCH012	MFP	MW308679
<i>Calisto disjunctus hersheyi</i>	CUBA, Habana-Matanzas Hills	YCH014	MFP	MW309680
<i>Calisto disjunctus hersheyi</i>	CUBA, Habana-Matanzas Hills	RN018-022	CZACC	MW309681
<i>Calisto disjunctus hersheyi</i>	CUBA, Guaniguanico Cordillera	PM07-06	CZACC	KC896066
<i>Calisto disjunctus hersheyi</i>	CUBA, Habana-Matanzas Hills	McGuire09-CAL-Sat103	MGCL	JN197373
<i>Calisto bradleyi</i>	CUBA, Guaniguanico Cordillera	PM07-24	CZACC	JN881882
<i>Calisto bradleyi</i>	CUBA, Guaniguanico Cordillera	PM07-25	CZACC	JN881883
<i>Calisto bradleyi</i>	CUBA, Guaniguanico Cordillera	PM07-26	CZACC	JN881884

Molecular analyses

All sequences were manually aligned using BioEdit 7.0.5.2 (Hall 1999). We calculated genetic distances between both populations of *C. disjunctus* and its closer relative *C. bradleyi* Michener (see Núñez *et al.* 2019) using the p-distance model in the software MEGA X (Kumar *et al.* 2018). We inferred the relationships of our *C. disjunctus* specimens and *C. bradleyi* employing the maximum likelihood (ML) approach in IQ-TREE 1.6.12 (Nguyen *et al.* 2015). We analyzed sequences in both a non-partitioned scheme and a one partition per locus scheme. For both analyses, the best models of nucleotide substitution were determined in IQ-TREE using ModelFinder (Kalyaanamoorthy *et al.* 2017). In order to obtain nodal support, we employed the UFBoot functionality (Minh *et al.* 2013) which performs ultrafast bootstrap, and the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) (Guindon *et al.* 2010), both with 1000 replicates. We retained and show the tree with the highest ML score, visualized in Fig-Tree 1.4.3 (Rambaut 2016).

Abbreviations

FW, forewing
 HW, hindwing
 UN, under surface
 UP, upper surface
 UNFW, under side of forewing

UNHW, under side of hindwing
 UPFW, upper side of forewing
 UPHW, upper side of hindwing

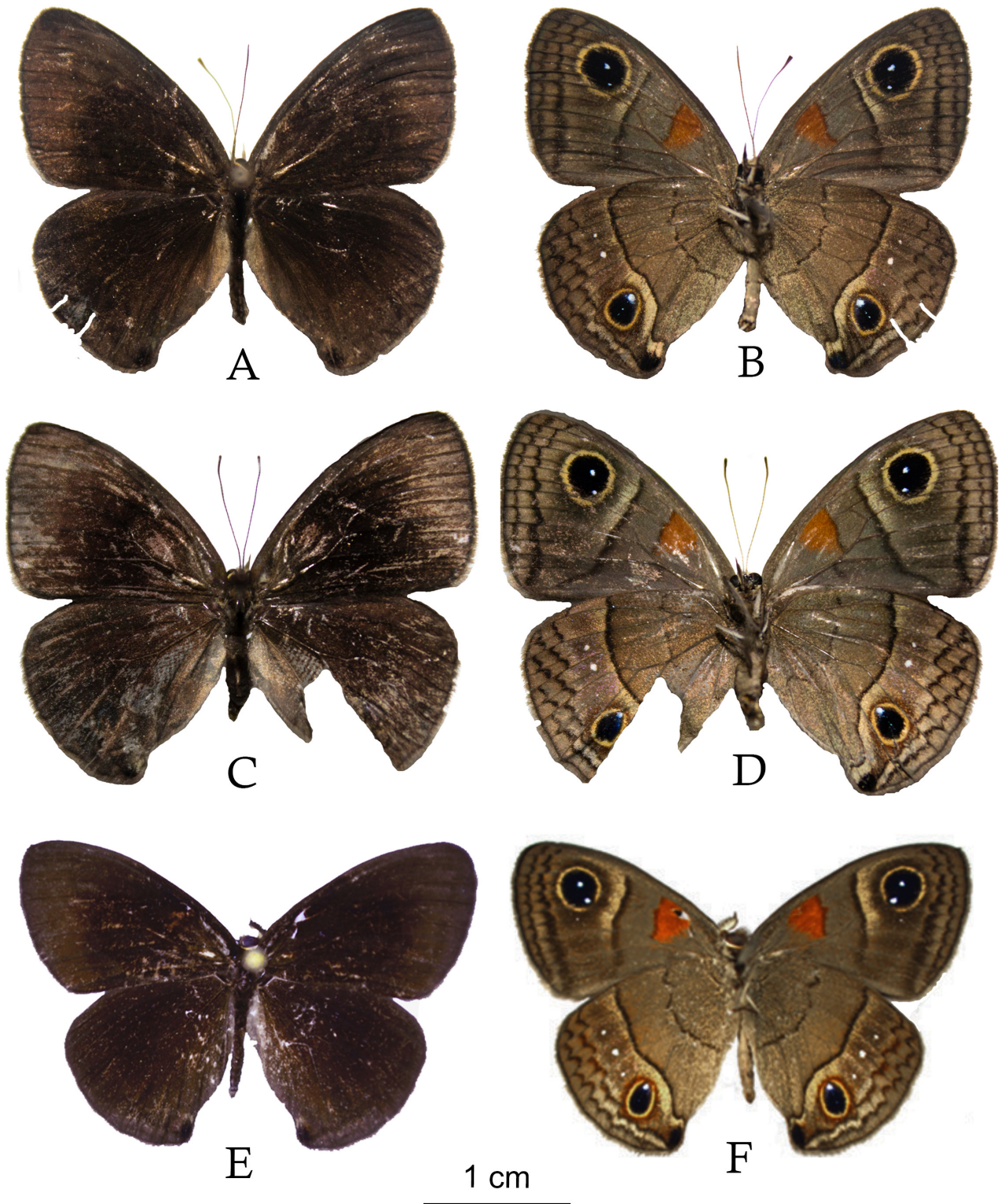


FIGURE 1. Type specimens of *Calisto disjunctus*. A: ♂ *Calisto disjunctus hersheyi* **ssp. n** holotype, dorsal view. B: ♂ *Calisto disjunctus hersheyi* **ssp. n** holotype, ventral view. C: ♀ *Calisto disjunctus hersheyi* **ssp. n** paratype, dorsal view. D: ♀ *Calisto disjunctus hersheyi* **ssp. n** paratype, ventral view. E: ♂ *Calisto d. disjunctus* Núñez & Barro holotype, dorsal view. F: ♂ *Calisto d. disjunctus* Núñez & Barro holotype, ventral view.

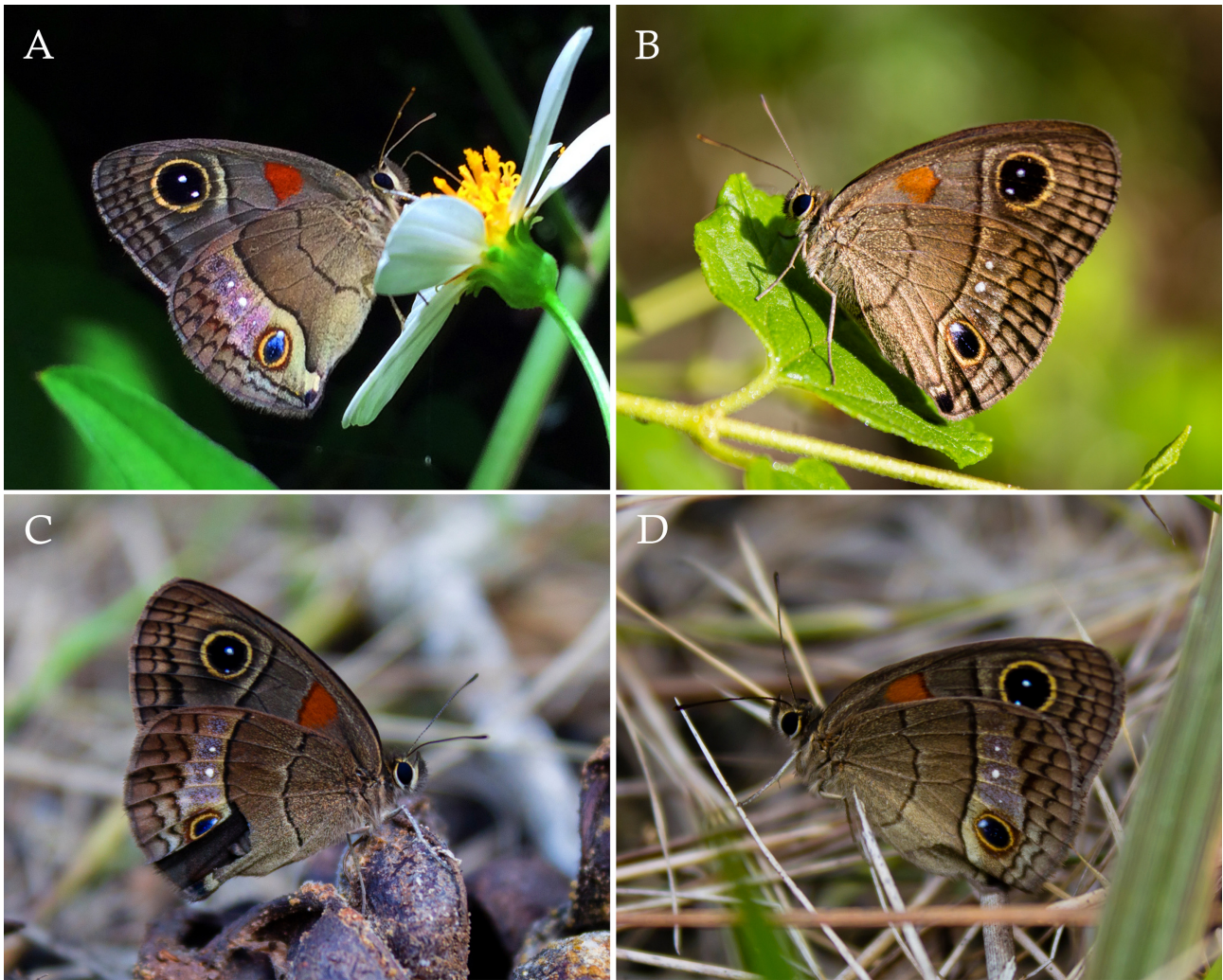


FIGURE 2. Photographs of live adults of *Calisto disjunctus hersheyi* ssp. n. A: Jardines de Hershey, 14/VIII/2018, nectaring on *Bidens pilosus* L. B: Lomas de Galindo, 4/X/2019. C: Same location, 30/XII/2019. D: Same location, 29/II/2020. Photos by Yosiel Álvarez.

Results

Calisto disjunctus hersheyi Álvarez & Núñez, ssp. n.

Figures 1A-D, 2, 3, 7D

<http://zoobank.org/urn:lsid:zoobank.org:act:12697C34-0B65-46A3-BB10-5F4AB6259F32>

Type material. Holotype (Fig. 1A-B)—♂, CUBA, Mayabeque, Santa Cruz del Norte, Jardines de Hershey, 23° 08' N, 81° 55' W, 80m, 23/VIII/2019, Col. Y. Álvarez. Deposited in Yosiel Álvarez Collection (YAC). **Paratypes** (Fig. 1C-D)—six ♂, three ♀. Of these, three ♂ with same data as for holotype, DNA vouchers YCH012 and YCH014; one ♀ with same data as for holotype except 11/XIII/2019; one ♀ with same data as for holotype except 20/I/2020. One ♂, CUBA, Artemisa, Sierra del Rosario, El Taburete, 22° 83' N, 82° 92' W, 80m, 9/X/2011, Col. R. Núñez, DNA voucher PM07-06. One ♂: CUBA, Mayabeque, Santa Cruz del Norte, Lomas de Galindo, 23° 03' N, 81° 42' W, 75m, 28/VI/2019, Col. Y. Álvarez, DNA voucher RN18-022; one ♀ with the same data as for previous exemplar except 29/VI/2019. One ♂: CUBA, Matanzas, VII/1993. Paratypes deposited in the Museo Felipe Poey (MFP), the Institute of Ecology and Systematics collection (CZACC), both in Havana, Cuba, and at the McGuire Center for Lepidoptera and Biodiversity (MGCL), Gainesville, United States.

Etymology. The subspecies is named after Jardines de Hershey, the locality in which most individuals of the type series were collected.

Diagnosis. *Calisto disjunctus hersheyi* requires comparison with several of its congeners. From typical *C. d. disjunctus*, it differs by its paler, less contrasting UN with a less cover of pale yellow scales; on the UNHW the white dot between M_2 - M_3 veins is larger and the M_3 - Cu_1 one is smaller and more displaced to the outer margin; sometimes it is greatly reduced or even absent. The male genitalia differ in the greater width of the valvae and a shorter saccus in *C. d. hersheyi*. From sympatric *C. herophile*, *C. disjunctus hersheyi* differs in its larger average size, darker UP, paler UN, on the UNHW lacking the Rs - M_1 white dot and the M_3 - Cu_1 dot smaller and displaced to the outer margin. From *C. bradleyi*, possibly a sympatric species at Guaniguanico, it differs in its smaller average size and lighter colour on the UN, a smaller area covered by red scales in the UNFW cell and the absence of the bluish scales present in front of the black spot on the tornal lobe of UNHW in *C. bradleyi*. Moreover, the base of the uncus is wider in male *C. d. hersheyi* and the ductus bursae is shorter and wider than in females of *C. bradleyi*. From *C. torrei* Núñez, it differs in its smaller average size, paler UN, a reduced area covered by red scales in the UNFW cell, HW less round and on the UNHW the white dot M_3 - Cu_1 smaller and displaced to the outer margin. Also, the base of the valvae is more curved in male *C. d. hersheyi*. COI diagnostic positions for *C. d. disjunctus* and *C. d. hersheyi* are at 307 A/G, 370 G/A and 523 T/C, respectively.

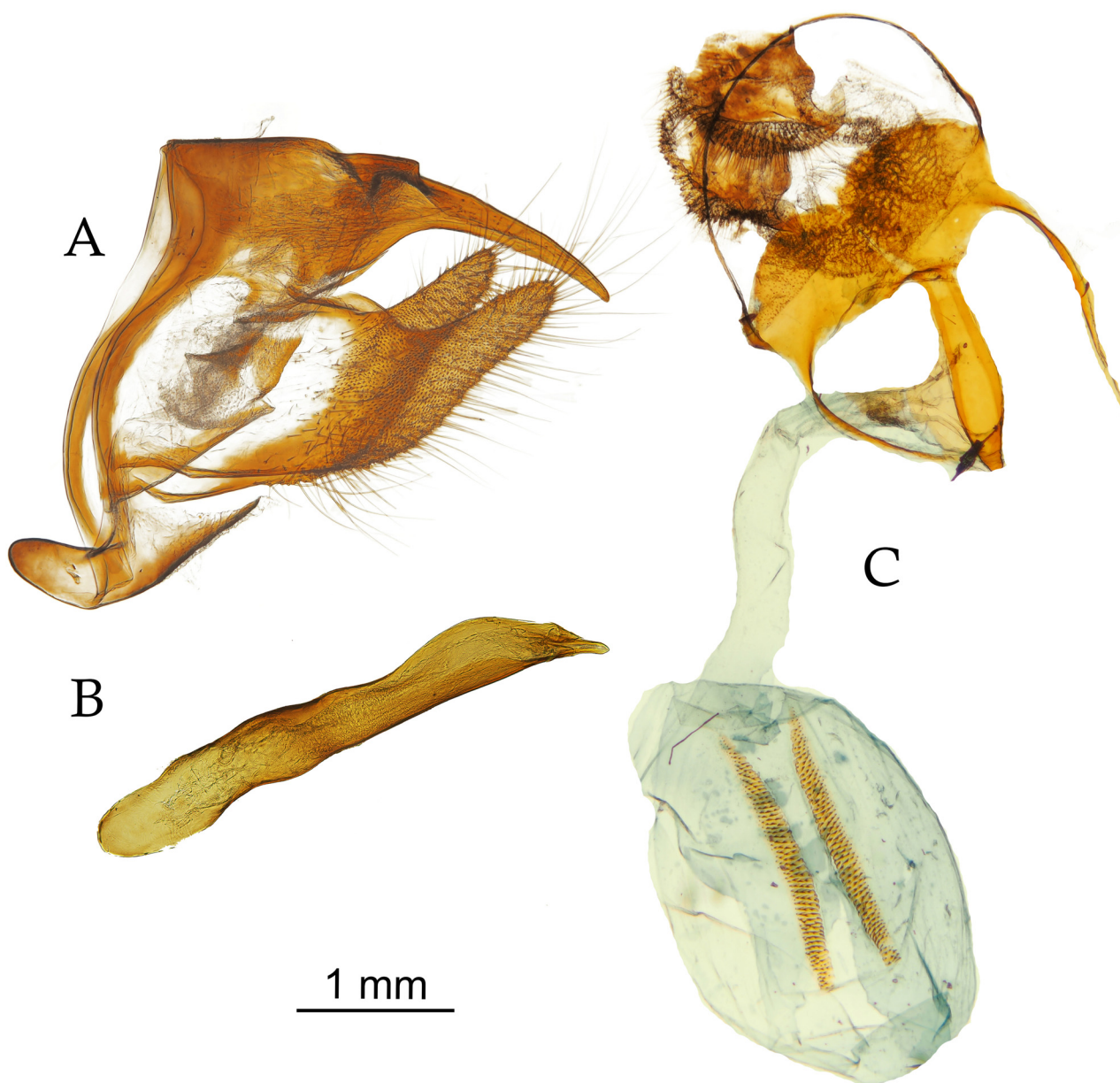


FIGURE 3. Genitalia of *Calisto disjunctus hersheyi* ssp. n. A: Male genitalia. B: Aedeagus. C: Female genitalia.

Description. FW length ♂ 16.6–18.2 mm ($n = 6$), ♀ 18.4–19.5 mm ($n = 3$). Females with broader wings and paler colour. UP dark brown, distal half of FW paler; androconial patch darker and well defined in males, not entering the discal cell. UNFW brown, the red patch occupying the central third of the discal cell, often with a straight outer edge but sometimes with a pointed outer edge. External edge of the postdiscal lines of both wings faintly scaled with pale yellow, mostly near ocelli; sometimes yellow scales slightly marked or almost absent. Basal area of UNHW poorly suffused with pale yellow scales. Three white dots from M_1 - M_2 to M_3 - Cu_1 UNHW cells, the one at M_2 - M_3 distinctly larger and the one between M_3 - Cu_1 very small and displaced to the outer margin and absent in some specimens. Area enclosing the white dots with a suffusion of pale lilac scales on fresh individuals. A well-marked black spot at the tornal lobe of the UNHW, also perceptible from the UP. Male genitalia slightly sclerotized, tegumen about two-thirds the length of the uncus, slightly convex with a shallow, broad notch at the upper middle; uncus tapering gradually from base to apex, not distinctly pointed; base rounded protuberant; valvae with digitiform projection slightly wide, ventral margin slightly concave; saccus relatively short (Fig. 3A). Female genitalia with dorsal crown tall; corpus bursae wide, as long as the ductus bursae, the latter notably wide (Fig. 3C).

Distribution. Known only from El Taburete, Sierra del Rosario, Artemisa, in the Guaniguanico mountain range, and from Jardines de Hershey and Lomas de Galindo, Santa Cruz del Norte, Mayabeque, in the Habana-Matanzas Hills. A paratype proceeded from an unknown locality within Matanzas province. There are also visual reports from Escaleras de Jaruco, Jaruco, Mayabeque, 15 km southwest from Hershey (Fig. 4). Probably *C. disjunctus hersheyi* is more widespread at the Guaniguanico and Habana-Matanzas mountain ranges. Sympatric with *C. herophile* throughout its range, and with *C. gundlachi* Núñez & Barro in Lomas de Galindo. Probably sympatric with *C. bradleyi* in the Guaniguanico mountain range.

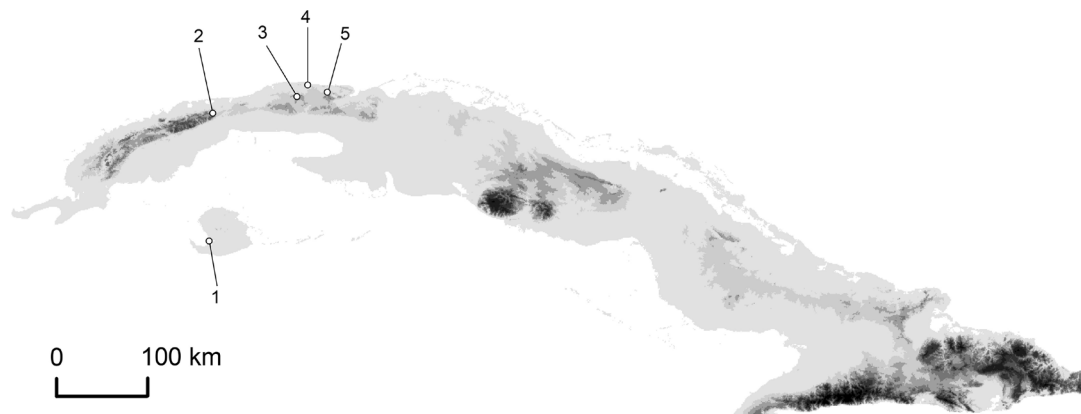


FIGURE 4. Distribution of *Calisto disjunctus*. 1: Estero de las Piedras, Isla de la Juventud (type locality of *Calisto d. disjunctus*). For *Calisto disjunctus hersheyi* **ssp. n.**: 2: El Taburete, Sierra del Rosario, Artemisa. 3: Escaleras de Jaruco, Jaruco, Mayabeque (visual record). 4: Jardines de Hershey, Santa Cruz del Norte, Mayabeque. 5: Lomas de Galindo, Santa Cruz del Norte, Mayabeque.

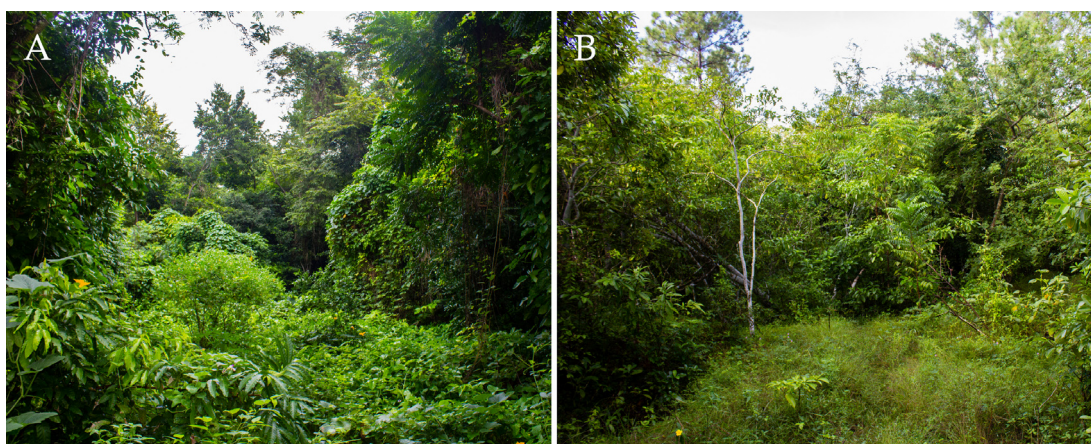


FIGURE 5. Habitat of *Calisto disjunctus hersheyi* **ssp. n.** A: Secondary forest clearing in Jardines de Hershey, Santa Cruz del Norte, Mayabeque. B: Gallery forest clearing within serpentine scrub-woodland in Lomas de Galindo, Santa Cruz del Norte, Mayabeque.

Habitat. Semideciduous forest, gallery forest and secondary forest (Fig. 5). If the species is indeed found in Escaleras de Jaruco, it is probable that it also inhabits the “mogote” vegetation complex.

Biology. In Jardines de Hershey, the subspecies seems to be more abundant in the late months of summer (Álvarez & Corso 2020), but it has been also reported from January to March. In Lomas de Galindo it has been seen flying from June to March. Adults fly low and slowly between bushes, but more actively than *C. herophile*, in forest clearings. They typically congregate around flowering bushes but isolated individuals are also likely to be found. In Galindo, stray individuals can occasionally be spotted in disturbed open areas. Adults have been seen visiting flowers of *Bidens pilosus* L. and *Melanthera nivea* (L.) Smill.

Immature stages. Unknown.

Molecular data

The p-distances between specimens of *C. d. disjunctus* and *C. d. hersheyi* ranged from 0.45% to 0.90% (Table 2), with 0.61% as mean. From the sister species *C. bradleyi*, mean minimum pairwise distances were 3.8% for *C. d. hersheyi* and 3.9% for *C. d. disjunctus*. Within individuals of *C. d. hersheyi*, mean minimum pairwise distances were 0.2%, while for the other two taxa were 0%. Both non-partitioned and partitioned approaches recovered the same topology for the tree, but the partitioned scheme obtained a greater support and thus we show that tree. *C. disjunctus* subspecies were recovered with maximum support for both measures (Fig. 6).

TABLE 2. COI distances among specimens of *Calisto disjunctus* and *C. bradleyi*, calculated using the p-distance model.

	d. disjunctus_AB-005	d. disjunctus_AIMSEQ29032017	d. hersheyi_McGuire09-CAL-Sat103	d. hersheyi_PM07-06	d. hersheyi_YCH014	d. hersheyi_YCH012	d. hersheyi_RN18-022	bradleyi_PM07-25	bradleyi_PM07-24	bradleyi_PM07-26
d. disjunctus_AIMSEQ29032017	0									
d. hersheyi_McGuire09-CAL-Sat103	0.45	0.46								
d. hersheyi_PM07-06	0.92	0.92	0.45							
d. hersheyi_YCH014	0.48	0.48	0	0.48						
d. hersheyi_YCH012	0.55	0.55	0	0.55	0					
d. hersheyi_RN18-022	0.63	0.63	0.15	0.63	0.15	0.18				
bradleyi_PM07-25	3.92	3.93	3.74	3.92	3.75	3.81	3.92			
bradleyi_PM07-24	3.94	3.95	3.76	3.94	3.77	3.81	3.94	0		
bradleyi_PM07-26	4.11	4.11	3.92	4.11	3.93	3.83	4.10	0	0	

Key to the adults of Cuban *Calisto*

Núñez *et al.* (2012) provided a key for the Cuban species of *Calisto*, using both morphological and biogeographical data. Since then, eight new species and two subspecies have been described for the Cuban fauna, and so the original key is now obsolete. Thus, we propose an updated key with all known Cuban *Calisto* taxa, including the subspecies named herein. We illustrate with live specimens all taxa except *C. siguanensis* Núñez & Barro and the typical subspecies of *C. disjunctus* (Fig. 7).

- 1 UN surface colour very dark brown; no red scales on the UNFW cell; triangle of white scales along the inner margin at the UNHW; FW length: 22–26 mm ♂, 25–27 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto israeli* Torre (Fig. 7I).

- UN surface colour not very dark brown; always with red scales on the UNFW cell; no triangle of white scales near the base on the UNHW **2.**
- 2 UNHW discal and postdiscal lines nearly straight and edged distally by pale yellowish white scales; FW length: 15.7 mm ♂, 18.5 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto sharkeyae* Núñez, Minno & Fernández (Fig. 7G).
- UNHW discal and postdiscal line waved or curved, never nearly straight, edged or not by yellow scaling **3.**
- 3 UNHW with four white dots external to the postdiscal line **4.**
- UNHW with two or three white dots external to the postdiscal line **9.**
- 4 UN surface dark rich red brown; UNHW postdiscal line edged by bright yellow scales; red patch on the UPHW tornal lobe; FW length: 19–25 mm ♂ and ♀; exclusive to Sierra Maestra mountains *Calisto smintheus* Bates (Fig. 7H).
- UN surface not rich red brown; UNHW postdiscal line edged by pale yellow scales; no red spot on the UPHW tornal lobe **5.**
- 5 UN surface dark brown; red scales covering more than half the UNFW cell and forming a patch with a pointed outer edge; UNHW ocellus rounder; FW length: 16–22 mm ♂, 20–22 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto brochei* Torre (Fig. 7M).
- UN surface not dark brown; red scales covering less than half the UNFW and forming a patch with a straight outer edge; UNHW ocellus not always rounder **6.**
- 6 UN surface distinctly paler and not heavily suffused with pale yellow scaling; UNHW postdiscal line scarcely edged by yellow scales **7.**
- UN surface strongly contrasting, heavily suffused with pale yellow scaling; UNHW postdiscal line heavily edged by yellow scales **8.**
- 7 UN surface lighter; UNFW inner submarginal line twice as wide in the tornal lobe than in apex; UNHW discal line rarely surrounded by very pale yellow scaling; UNHW white dots between M₁-M₂ and M₂-M₃ veins larger than the others; FW length: 14–19 mm ♂, 17–21 mm ♀; all throughout Cuba and Isla de la Juventud *Calisto herophile* Hübner (Fig. 7A).
- UN surface darker; UNFW inner submarginal line less than twice as wide in the tornal lobe than in apex; UNHW discal line surrounded by pale yellow scales; UNHW white dots very small and almost equal in size; FW length: 17.4–19.8 mm ♂, 17.3–18.2 mm ♀; exclusive to Guamuha mountains *Calisto a. aquilum* Núñez (Fig. 7O).
- 8 UNFW inner submarginal line twice as wide in the tornal lobe than in apex; UNHW ocellus more than half width of the UNFW ocellus; UN postdiscal lines more suffused by pale yellow scaling; FW length: 16.5–17.6 mm ♀; exclusive to Viñales mountains *Calisto aquilum occidentalis* Núñez (Fig. 7P, the image portrays a worn individual).
- UNFW inner submarginal line less than twice as wide in the tornal lobe than in apex; UNHW ocellus half width of the UNFW ocellus; UN postdiscal lines less suffused by pale yellow scaling; FW length: 16.3–18.1 mm ♂, 18.1–18.8 mm ♀; exclusive to Nipe-Sagua-Baracoa and Sierra Maestra mountains *Calisto dissimulatum* Núñez (Fig. 7N).
- 9 UN surface heavily suffused with gray scales; white dot of the UNHW ocellus always in its inner margin; UNHW white dots present between M₂-M₃ and M₃-Cu₁, sometimes dot between M₁-M₂, also present **10.**
- UN surface not heavily suffused with grey scales; white dot of the UNHW ocellus rarely in its inner margin; UNHW white dots present between M₁-M₂, M₂-M₃ and M₃-Cu₁, the latter sometimes absent **11.**
- 10 UNHW ocellus pear-shaped; FW length: 16–19 mm ♂, 18–21 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto bruneri* Michener (Fig. 7F).
- UNHW ocellus rounder, sometimes thinner near the inner white dot but never pear-shaped; FW length: 14.7–15.7 mm ♂, 17.5–17.6 mm ♀; exclusive to Lomas de Galindo *Calisto gundlachi* Núñez & Barro (Fig. 7E).
- 11 Red scales covering more than half the UNFW cell and forming a patch with a pointed outer edge **12.**
- Red scales covering less than half the UNFW cell and forming a patch with a straight or pointed outer edge **14.**
- 12 UN surface pale; UNHW postdiscal line heavily edged by pale yellow scaling; FW length: 16.8–17.4 mm ♂, 20.4 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto lastrai* Núñez (Fig. 7J).
- UN surface dark; UNHW postdiscal line faintly edged by pale yellow scaling **13.**
- 13 Androconial patch about one half the length of FW; UNHW white dot between M₁-M₂ larger than the one between M₃-Cu₁, which can be absent; FW length: 18–22 mm ♂, 20–23 mm ♀; exclusive to Guamuha mountains *Calisto muripetens* Bates (Fig. 7K).
- Androconial patch about two fifths the length of FW; UNHW white dots between M₁-M₂ and M₃-M₄ similar in size and very small, sometimes absent; FW length: 17–20 mm ♂, 18–21 mm ♀; exclusive to Nipe-Sagua-Baracoa mountains *Calisto occulta* Núñez (Fig. 7L).
- 14 UN surface dark; UNHW ocellus very large, as large as the UNFW ocellus; FW length: 14.8–16.3 mm ♂, 15.7–17.9 mm ♀; exclusive to Isla de la Juventud *Calisto siguanensis* Núñez & Barro.
- UN surface never dark, UNHW ocellus smaller than the UNFW ocellus **15.**
- 15 UN surface almost without pale yellow scaling surrounding postdiscal lines; black spot on the tornal lobe of UNHW with bluish scales in the front; FW length: 17–20 mm ♂, 20–21 mm ♀; exclusive to Guaniguanico mountains *Calisto bradleyi* Munroe (Fig. 7C).
- UN surface with pale yellow scaling surrounding postdiscal lines; black spot on the tornal lobe of UNHW without bluish scales in the front **16.**
- 16 UN surface brown; red scales covering half of the UNFW cell; UNHW white dots aligned; FW length: 19.7–23 mm ♂, 21.2–24.3 mm ♀; exclusive to Guamuha mountains *Calisto torrei* Núñez (Fig. 7B).
- UN surface pale brown; red scales covering central third of the UNHW cell; UNHW with white dot between M₃-Cu₁ distinctly displaced to the outer margin (*Calisto disjunctus*) **17.**
- 17 UN surface with postdiscal lines surrounded by heavy pale yellow scaling; UNHW white dots between M₁-M₂ and M₃-Cu₁ similar in size, the latter slightly displaced to the outer margin; FW length: 16.8–17.9 mm ♂; exclusive to Isla de la Juventud

- *Calisto d. disjunctus* Núñez & Barro.
 - UN surface whit postdiscal lines feebly surrounded by pale yellow scaling, specially FW; UNHW white dot between M₃-Cu₁ smaller than the one between M₁-M₂ and greatly displaced to the outer margin, sometimes absent; FW length: 16.6–18.2 mm ♂, 18.4–19.5 mm ♀; exclusive to Guaniguanico and Habana-Matanzas mountains . . . *Calisto disjunctus hersheyi* ssp. n (Fig. 7D).

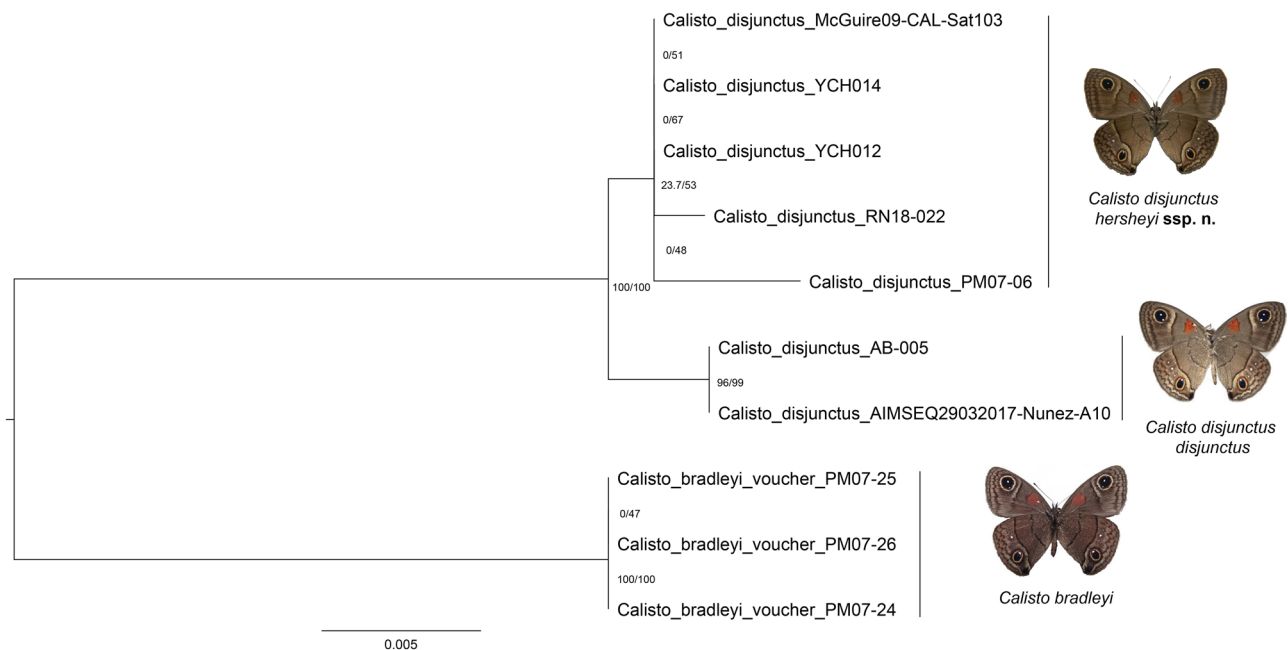


FIGURE 6. Relationships of *Calisto disjunctus* subspecies and the sister taxon *Calisto bradleyi*, recovered by ML analysis for COI barcodes performed in IQ-TREE. Nodal support represent bootstrap and SH-aLRT, respectively.

Discussion

Historically, there has been an intense debate about the species concept, and within it, subspecies concept, regarding not only the concept itself but the criteria employable in species delimitation. Several proposals have arisen, each one dealing with a different and exclusive criterion to define species boundaries (Mayr 1942; Simpson 1951; Henning 1966; Michener 1970; Van Valen 1976; de Queiroz 1998). However, more recently de Queiroz (2007) unified species concept has been widely embraced as a solution to this old dilemma. Based on this, Braby *et al.* (2012) proposed a similar solution for the subspecies issues, particularly dealing with Lepidoptera. For these authors, subspecies are an early phase of lineage divergence which have only acquired a few distinctive features and have not yet achieved full reproductive isolation and no gene flow from other populations of the same species.

Extant populations of *Calisto disjunctus* are allopatric. The species of the genus *Calisto* have been noticed as evolving in “islands within islands” (Matos-Maraví *et al.* 2014; Núñez *et al.* 2019), achieving tremendous diversification within a limited geographic area and speciating allopatrically but probably also parapatrically in different habitats of a same region. In the case of *C. disjunctus*, it seems unlikely that two allopatric populations separated by water are able to exchange genetic material, particularly for butterflies incapable of dispersal over water.

Both populations have subtle but constant morphological differences, mostly related to some aspects of wing colour and pattern. While the female of the Isla de la Juventud population is unknown, males of both populations also have minor differences in genital structure. Ecological niches of these butterflies have not been studied, but their biology suggest that they are minor pollinators and prey for some predators, as many other butterfly species (New 2013). It is unlikely that both populations of *C. disjunctus* have differences regarding this aspect; however, they do have different habitat preference. In Isla de la Juventud, the species has been detected in marsh grasslands (A. Barro, pers. comm.), a very different habitat from the forest types where the mainland Cuba population lives (Núñez *et al.* 2019; Álvarez & Corso 2020), which, though different in vegetal composition, are similar in a number of ways (see Capote & Berazaín 1984).

Finally, *C. disjunctus* subspecies differ in their COI barcodes by a mean distance of 0.61%. This divergence

degree is slightly below that usually accepted for subspecific level, between 1-2% (Rougerie *et al.* 2014; Shiraiwa *et al.* 2014); however, distances within each subspecies are below that value and the ML tree (Fig. 6) recovered both populations as well defined genetically. Thus, their reciprocal monophyly, the subtle differences in morphology and habitat use, and the allopatry of the populations of *C. disjunctus*, while not sufficient for specific status, suggest that these populations can be treated as different subspecies.



FIGURE 7. Live adults of Cuban species of *Calisto*. A: *C. herophile*. B: *C. torrei*. C: *C. bradleyi*. D: *C. disjunctus hersheyi*. E: *C. gundlachi*. F: *C. bruneri*. G: *C. sharkeyae*. H: *C. smintheus*. I: *C. israeli*. J: *C. lastrai*. K: *C. muripetens*. L: *C. occulta*. M: *C. brochei*. N: *C. dissimulatum*. O: *C. a. aquilum*. P: *C. aquilum occidentalis* (worn individual). Photos by: Andy J. Corso (A), Rayner Núñez (B-C, F-I, K-P), Yosiel Álvarez (D-E) and Douglas M. Fernández (J).

Regarding the origin of the ancestor of this species, the evidence points toward a Cuban origin. The split of *C. disjunctus* and its sister species *C. bradleyi* occurred around four million years ago (Núñez *et al.* 2019), probably after a period of high sea level when they remained isolated by water in the ranges of their respective ancestral populations. Following the view of a Cuban origin for *C. disjunctus*, the ancestor of these species could have isolated in two different mountain ranges: Guaniguanico with its two Sierras, S. de los Órganos and del Rosario; and the Habana-Matanzas hills, all separated by lowlands. Núñez *et al.* (2019) estimate the split of the populations of *C. disjunctus* probably less than a million years ago, during the mid-Pleistocene. During that time, sea level was several dozen meters below current level (Iturralde-Vinent 2006), allowing the existence of land connection between Cuba and Isla de la Juventud. During the following years sea level increased and decreased several times (Iturralde-Vinent 2006), with these populations remaining fully isolated today. The individual from El Taburete is slightly different in its sequence from the Hershey and Galindo ones, so further work could confirm its status as a distinct population or subspecies, isolated from the Habana-Matanzas one.

The existence of this species and other recently discovered taxa, such as *C. gundlachi*, on low hills outside the

largely sampled four major mountain ranges of Cuba, should encourage researchers to continue sampling middle and low elevations searching for unknown *Calisto* taxa along the more than 1000 km of the Cuban geography. Localities such as Sierra de Cubitas and Santa Clara Northern Hills, among others, remain largely unexplored, and could be home to undiscovered taxa of *Calisto*.

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