



Morphology and COI barcodes reveal four new species in the *lyceius* group of *Calisto* (Lepidoptera, Nymphalidae, Satyrinae)

ANTONIO R. PÉREZ-ASSO¹, RAYNER NÚÑEZ-AGUILA² & JULIO A. GENARO³

¹Research Associate, National Museum of Natural History, Santo Domingo, Dominican Republic.: 7620 SW 142 Avenue, Miami, FL 33183. E-mail: perezasso@hotmail.com.

²Departamento de Colecciones Zoológicas y Sistemática, Instituto de Ecología y Sistemática, Carretera de Varona km 3. 5, Capdevila, Boyeros, CP 11900. La Habana, Cuba. Current address: Zoologische Staatssammlung Muenchen, Münchenhausenstraße 21, 81247 München. E-mail: raynernunez75@gmail.com

³Research Associate, Florida State Collection of Arthropods, Division of Plant Industry P.O. Box 147100/1911, SW 34 St., Gainesville, FL 32614-7100, U.S.A. E-mail: polimita@hotmail.com

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Abstract

The predominantly Greater Antillean endemic genus *Calisto* Hübner, 1823 is highly diversified on several islands being more species rich on Hispaniola. We conducted expeditions during five years in the Dominican Republic resulting in new findings related with *lyceius* species group. Material belonging to this group was examined following the traditional morphological characters employed in genus taxonomy, and the COI barcode sequences obtained were analyzed through different approaches: Neighbor Joining clustering, ABGD, Maximum Likelihood (ML), and Bayesian Inference (BI). Analysis yielded 12 groups representing putative species: eight corresponding to previously named ones and four new species which are described in the present work: *C. mariposa* sp. nov., *C. azua* sp. nov., *C. victori* sp. nov., and *C. samana* sp. nov. The results also confirmed a single taxonomic entity within *C. pulchella* Lathy and the conspecific nature of *C. franciscoi* Gali and *C. hendersoni*. A dichotomic key for identification of species within the group is also given. Both phylogenetic reconstruction methods (ML and BI) employing molecular data achieved similar results with the relationships

among the majority of taxa being supported by some ecological and morphological features. The exceptions were *C. zangis* Fabricius, *C. raburni* Gali, and *C. pulchella*, grouped together in a weakly supported clade. These species possess a highly differentiated adult and immature morphology which indicates an earlier divergence.

Key words: Greater Antilles, Hispaniola, Jamaica, COI, ABGD, barcoding, Bayesian Inference, Maximum Likelihood, new species, phylogeny, monophyly, diagnostic sites, divergent morphology

Introduction

Sourakov & Zakharov (2011) properly named the members of the genus *Calisto* Hübner, 1823 as Darwin's butterflies. These butterflies today exhibit an astonishing radiation at their home range, the Greater Antilles. The genus is endemic to the islands and the sole representative of Satyrinae (Lamas 2004). Most of their 44 known species are endemic from one of the Greater Antilles, the Bahamas or Anegada Island at the British Virgin Islands (Matos-Maraví *et al.* 2014). The largest portion of this diversity corresponds to Hispaniola with 28 species (Sourakov & Zakharov 2011, Matos-Maraví *et al.* 2014). The genus has received great attention since the end of nineteenth century with dozens of works published including an increasing number after the arrival of molecular studies (Sourakov & Zakharov 2011, Núñez *et al.* 2012, 213, Matos-Maraví *et al.* 2014).

The first arrangement in species groups for the genus was performed by Bates (1935). This author artificially created “sections” and “groups” based on some morphological characters, most of them related to wing venation, androconial patch, and male genitalia. *Calisto lyceius*, a species described from a single pair collected on Isla Saona, off the southeastern Hispaniolan coast (Bates 1935) and discovered across the strait in Boca de Yuma on the island of Hispaniola in 2002 (Sourakov, 2007) was placed in the *hysius* group together with *C. hysius* (Godart 1823), *C. confusa* 1899 and *C. tragi* Bates 1935. The *lyceius* species group of *Calisto* was created by Gali (1985) who included *C. lyceius* and five Hispaniolan species described by him: *C. crypta*, *C. franciscoi*, *C. hendersoni*, *C. raburni*, and *C. schwartzi*. *Calisto hendersoni* was later synonymized with *C. franciscoi* by Sourakov (2000) based on the lack of morphological divergence. The main character used for group identification was the dense reddish suffusion on the ventral surface of wings. Another species similar to *C. schwartzi*, but from another mountain range, *C. tasajera* Gonzalez, Schwartz & Wetherbee 1991, was added few years later by González *et al.* (1991), and Hedges and Johnson who independently collected this species in another locality, published a taxonomic key to the *lyceius* group, which they viewed as a Hispaniolan bunch grass-feeding butterflies with diffused red underside coloration (Hedges and Johnson, 1994).

The evolutionary history of the group in the context of the entire Caribbean was first proposed by Sourakov (2000), who included into the group the Puerto Rica/Anegada complex (*C. nubila*, *C. anegadensis*), and suggested affinity of the complex to *C. pulchella* based on a single synapomorphy (suparstematal seta in the first instar larva). It was proved by the molecular study of Sourakov & Zakharov (2011) who employed the barcode region of the mitochondrial gen COI. However, the group was monophyletic only with the inclusion of two more taxa: the Hispaniolan *C. pulchella* and the Jamaican *C. zangis* Fabricius 1775. This relationship was recently confirmed by Matos-Maraví *et al.* (2014) employing six genes in an evolutionary reconstruction that included the first divergence time estimates for the genus.

Recent intensive sampling at Hispaniola has resulted in the discovery of new species belonging to the *lyceius* group. Four new species belonging to the *lyceius* species group are described herein. Despite the recent phylogenetic reconstruction by Matos-Maraví *et al.* (2014), we performed it again to obtain hypothesis on the phylogenetic relationships of all species within the group including the newly described in the present work. Diagnosis and color illustrations of both pinned and living adults and male and female genitalia are provided for most species. A taxonomic key is also provided.

Materials and methods

Specimens' collection. The field work involved was conducted in 2010-2014 across the Dominican Republic and included visit to the type localities summarized by Gali (1985) and Schwartz (1989) (see Material examined and distribution maps for details).

Morphological characters and species diagnosis. Taxonomic characters employed in the present study were those usually employed in works on the *lyceius* group (e. g. Gali 1985, Schwartz 1989, Gonzalez *et al.* 1991, Hedges & Johnson, 1994, Sourakov (1997, 2000), Pycz (2010)). Regarding genitalia terminology we followed Johnson *et al.* (1987), Hedges & Johnson, 1994, Johnson & Hedges (1998) and Sourakov (1997, 2000).

Since the group shows a relatively unvariable external morphology: dark brown upper surface and mostly reddish undersurface of wings varying only in size and tonality, we decide to include only detailed comparative diagnosis for all species including the newly described herein to avoid the unnecessary repetition of shared characters across the work. In the same way, only key features are mentioned at the “Male genitalia” and “Female genitalia” sections below each species. For all species exclusive diagnostic fixed states and their position at the COI barcode sequence are also mentioned at the Diagnosis section.

Molecular protocols, sequence editing and sequence characterization. Data acquisition and analysis DNA extraction, PCR amplification, and sequencing of the COI barcode region were performed at the Canadian Centre for DNA Barcoding (CCDB) and followed standard protocols (CCDB 2013). PCR and sequencing used a single pair of primers: LepF1 (ATTCAACCAATCATAAAGATATTGG) and LepR1 (TAAACTTCTGGATGTCCAAAA AATCA) (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 editing and alignment were done manually using BioEdit v7.0.9 (Hall 1999). DNA sequences have been submitted to GenBank (see Table 1 for accession numbers). DNA voucher specimens are deposited at the Victor Gonzalez Research Collection, Puerto Rico.

Additional DNA sequences from *lyceius* group members were downloaded from GenBank, <http://genbank.gov/> (Benson *et al.* 2014). We have only downloaded sequences from species of well known distribution, since many GenBank sequences have imprecise distribution data, for example, an entire province or country as the collecting place (Table 1).

Molecular diversity indices were calculated with DnaSP 5.10.01 (Librado & Rozas 2009).

DNA barcoding study. The classic DNA barcoding (Hebert *et al.* 2003) calculates a genetic distance between specimens using Kimura's 2-parameter, K2P, distance (Kimura 1980) to reveal a barcode gap or the break in the distribution among genetic distances of specimens belonging to the same species and these of specimens from different species. However, uncorrected genetic distances, uncorrected p-distance, have also been used since K2P could be inappropriate when employing it for closely related taxa (Srivathsan & Meier 2011). The genetic distances were calculated using the program MEGA5.1 (Tamura *et al.* 2011). We calculated both uncorrected p-distance and the K2P since the later have been traditionally used in all the previous work on *Calisto* (all distances in the text are uncorrected p-distance, K2P values appear only in Table 4). We employed ExcaliBAR 1.0 (Aliabadian *et al.* 2014) to determinate the barcoding gap between intraspecific and interspecific sequences based on uncorrected p-distance matrix originally computed by MEGA.

Alternatively we also conducted a character base approach of the COI sequences to identify the presence or absence of discrete nucleotide substitutions, character states. These substitutions potentially allow the identification of species or even populations (Rach *et al.* 2008, Tavares & Baker, 2008, Brower 2010). After the Neighbor Joining implementation, we arranged the sequences first by each hypothetical species and then by localities. Then this fasta file was visually inspected in MEGA looking for unique substitutions at each site within hypothetical species and also within populations.

Species delineation by ABGD. The Automatic Barcode Gap Discovery method (Puillandre *et al.* 2011, 2012) was used to sort the available 139 sequences into genetic clusters or hypothetical species. This algorithm automatically finds the inflection point in the frequency distribution of ranked pairwise genetic distances between aligned homologous sequences, and does so recursively to get the finest partition of the data set into candidate species (Puillandre *et al.* 2011, 2012). A matrix of pairwise uncorrected p-distances in MEGA excluding all ambiguous positions between each pair of sequences was calculated. We used the ABGD web-interface available at: <http://www.wabi.snv.jussieu.fr/public/abgd/> using the default values for all parameters except the relative gap width (X) that was set to 1.1 when higher values (1.5 is the default) failed in detect more than one group. The analysis was performed employing the three implemented models (Jukes-Cantor, K2P, and Simple Distance).

Phylogenetic placement. Though COI was the only marker included in this work and the trees obtained may not necessarily represent the actual relationships, we performed a phylogenetic reconstruction. Our main goals were to look for the closest relatives of the new species described and to compare our result with those obtained in the most recent multigene study on *Calisto* by Matos-Maraví *et al.* (2014).

TABLE 1. List of specimens with COI barcode sequences analyzed and their corresponding collection and repository data and GenBank accession numbers. Voucher codes are unique identifiers referring to specimens at repositories and to individual records in the Barcode of Life Datasystems (www.boldsystems.org). Collection acronyms: MGCL—McGuire Center for Lepidoptera, USA, NSG—Nymphalidae Systematics Group, Lund, VGRC—Victor González Research Collection, Puerto Rico.

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto eleleus</i>	WI-JAG-537	JN300013	Dominican Republic, Independencia, S. Bahoruco, caseta 2	VGRC
<i>Calisto eleleus</i>	WI-JAG-538	JN300014	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto nubila</i>	WI-JAG-484	KX536775	Puerto Rico, Real-Anon, Hacienda Paraiso	VGRC
<i>Calisto nubila</i>	WI-JAG-489	KX736711	Puerto Rico, Quebradillas, Guajataca	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-785	KX536717	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-786	KX536722	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-787	KX536740	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-788	KX536732	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-975	KX536699	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-976	KX536742	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-983	KX536785	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-1028	KX536779	Dominican Republic, Azua, El Número	VGRC
<i>Calisto azua</i> (= <i>Calisto</i> sp. 4)	WI-JAG-1029	KX536720	Dominican Republic, Azua, El Número	VGRC
<i>Calisto crypta</i>	McGuire09-CAL-Sat29	JN197336	Dominican Republic, Monte Cristi	MGCL
<i>Calisto crypta</i>	McGuire09-CAL-Sat30	JN197335	Dominican Republic, Monte Cristi	MGCL
<i>Calisto crypta</i>	McGuire09-CAL-Sat31	JN197334	Dominican Republic, Monte Cristi	MGCL
<i>Calisto crypta</i>	McGuire09-CAL-Sat32	JN197333	Dominican Republic, Monte Cristi	MGCL
<i>Calisto crypta</i>	McGuire09-CAL-Sat33	JN197332	Dominican Republic, Monte Cristi	MGCL
<i>Calisto crypta</i>	WI-JAG-863	KX536776	Dominican Republic, Monte Cristi	VGRC
<i>Calisto crypta</i>	WI-JAG-864	KX536695	Dominican Republic, Monte Cristi	VGRC

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TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto crypta</i>	WI-JAG-865	KX536724	Dominican Republic, Monte Cristi	VGRC
<i>Calisto crypta</i>	WI-JAG-866	KX536712	Dominican Republic, Monte Cristi	VGRC
<i>Calisto crypta</i>	WI-JAG-867	KX536795	Dominican Republic, Monte Cristi	VGRC
<i>Calisto franciscoi</i>	WI-JAG-773	KX536704	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-774	KX536794	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-775	KX536793	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-776	KX536701	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-777	KX536791	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-784	KX536765	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-948	KX536689	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-984	KX536799	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-985	KX536781	Dominican Republic, Azua, El Número	VGRC
<i>Calisto franciscoi</i>	WI-JAG-778	KX536746	Dominican Republic, Independencia, Duverge, Laguna El Limon	VGRC
<i>Calisto franciscoi</i>	WI-JAG-779	KX536725	Dominican Republic, Independencia, Duverge, Laguna El Limon	VGRC
<i>Calisto franciscoi</i>	WI-JAG-780	KX536758	Dominican Republic, Independencia, Duverge, Laguna El Limon	VGRC
<i>Calisto franciscoi</i>	WI-JAG-1022	KX536686	Dominican Republic, Pedernales, Bahía de las Águilas	VGRC
<i>Calisto franciscoi</i>	WI-JAG-1023	KX536757	Dominican Republic, Pedernales, Bahía de las Águilas	VGRC
<i>Calisto franciscoi</i>	WI-JAG-1024	KX536730	Dominican Republic, Pedernales, Bahía de las Águilas	VGRC
<i>Calisto franciscoi</i>	WI-JAG-1025	KX536702	Dominican Republic, Pedernales, Bahía de las Águilas	VGRC
<i>Calisto franciscoi</i>	WI-JAG-1026	KX536705	Dominican Republic, Pedernales, Bahía de las Águilas	VGRC
<i>Calisto lyceus</i>	McGuire09-CAL-Sat97	JN197379	Dominican Republic, La Altagracia	MGCL

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TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto lyceius</i>	McGuire09-CAL-Sat98	JN197378	Dominican Republic, La Altagracia	MGCL
<i>Calisto lyceius</i>	McGuire09-CAL-Sat99	JN197377	Dominican Republic, La Altagracia	MGCL
<i>Calisto lyceius</i>	McGuire09-CAL-Sat100	JN197376	Dominican Republic, La Altagracia	MGCL
<i>Calisto lyceius</i>	WI-JAG-858	KX536751	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-859	KX536710	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-860	KX536688	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-861	KX536703	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-862	KX536718	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-1012	KX536767	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-1013	KX536766	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto lyceius</i>	WI-JAG-1014	KX536753	Dominican Republic, La Altagracia, Boca de Yuma	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-875	KX536762	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-876	KX536764	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-957	KX536769	Dominican Republic, Independencia, S. Bahoruco, caseta 1	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-958	KX536797	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-959	KX536719	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-1005	KX536691	Dominican Republic, Pedernales, S. Bahoruco, Entre caseta 1 y Puerto Escondido	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-1030	KX536736	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto mariposa</i> (= <i>Calisto</i> sp. 1)	WI-JAG-1031	KX536731	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto pulchella</i>	DR003	GQ357225	Dominican Republic, Puerto Plata	NSG
<i>Calisto pulchella</i>	DR015	KF054317	Dominican Republic, La Vega, La Ciénaga	NSG

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TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto pulchella</i>	McGuire09-CAL-Sat48	JN197394	Dominican Republic, La Vega	MGCL
<i>Calisto pulchella</i>	McGuire09-CAL-Sat49	JN197393	Dominican Republic, La Vega	MGCL
<i>Calisto pulchella</i>	WI-JAG-540	JN300016	Dominican Republic, Santiago, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-552	JN300028	Dominican Republic, Santiago, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-553	JN300029	Dominican Republic, Santiago, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-554	JN300030	Dominican Republic, Santiago, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-555	JN300031	Dominican Republic, salto La Demajagua	VGRC
<i>Calisto pulchella</i>	WI-JAG-878	KX536728	Dominican Republic, La Altagracia, San Rafael de Yuma	VGRC
<i>Calisto pulchella</i>	WI-JAG-879	KX536752	Dominican Republic, La Altagracia, San Rafael de Yuma	VGRC
<i>Calisto pulchella</i>	WI-JAG-880	KX536734	Dominican Republic, La Altagracia, San Rafael de Yuma	VGRC
<i>Calisto pulchella</i>	WI-JAG-881	KX536782	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-882	KX53674	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-883	KX536796	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-884	KX536783	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-885	KX536756	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-886	KX536754	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-887	KX536768	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-888	KX536713	Dominican Republic, La Vega, La Ciénaga	VGRC
<i>Calisto pulchella</i>	WI-JAG-889	KX536735	Dominican Republic, La Vega, Ebano Verde, El Arroyazo	VGRC
<i>Calisto pulchella</i>	WI-JAG-890	KX536786	Dominican Republic, La Vega, Ebano Verde, El Arroyazo	VGRC
<i>Calisto pulchella</i>	WI-JAG-891	KX536741	Dominican Republic, La Vega, Ebano Verde, El Arroyazo	VGRC

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TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto pulchella</i>	WI-JAG-960	KX536759	Dominican Republic, La Altagracia, San Rafael de Yuma	VGRC
<i>Calisto pulchella</i>	WI-JAG-961	KX536801	Dominican Republic, La Altagracia, San Rafael de Yuma	VGRC
<i>Calisto raburni</i>	WI-JAG-790	KX536777	Dominican Republic, Independencia, S. Bahoruco, El Aguacate	VGRC
<i>Calisto raburni</i>	WI-JAG-874	KX536788	Dominican Republic, Independencia, S. Bahoruco, caseta 1	VGRC
<i>Calisto raburni</i>	WI-JAG-877	KX536737	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto raburni</i>	WI-JAG-947	KX536755	Dominican Republic, Independencia, S. Bahoruco, El Aguacate	VGRC
<i>Calisto raburni</i>	WI-JAG-955	KX536697	Dominican Republic, Independencia, S. Bahoruco, caseta 1	VGRC
<i>Calisto raburni</i>	WI-JAG-956	KX536750	Dominican Republic, Independencia, S. Bahoruco, caseta 1	VGRC
<i>Calisto raburni</i>	WI-JAG-978	KX536745	Dominican Republic, Pedernales, S. Bahoruco, Aceitillar	VGRC
<i>Calisto raburni</i>	WI-JAG-1004	KX536715	Dominican Republic, Pedernales, S. Bahoruco, Entre caseta 1 y Puerto Escondido	VGRC
<i>Calisto raburni</i>	WI-JAG-1006	KX536770	Dominican Rep., Pedernales, S. Bahoruco, Entre caseta 1 y Puerto Escondido	VGRC
<i>Calisto raburni</i>	WI-JAG-1034	KX536709	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto raburni</i>	WI-JAG-1035	KX536690	Dominican Republic, Sierra Prieta, Distrito Nacional	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-971	KX536700	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-972	KX536748	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-973	KX536698	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-974	KX536789	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-1020	KX536787	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto samana</i> (= <i>Calisto</i> sp. 2)	WI-JAG-1021	KX536763	Dominican Republic, Samaná, Cueva del Agua	VGRC
<i>Calisto schwartzii</i>	WI-JAG-490	JN299967	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC

..... continued on the next page

TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto schwartzii</i>	WI-JAG-491	JN299968	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-541	JN300017	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-781	KX536803	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-782	KX536733	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-783	KX536721	Dominican Republic, Pedernales, S. Bahoruco, Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-996	KX536760	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-997	KX536739	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-998	KX536687	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-999	KX536802	Dominican Rep., Pedernales, S. Bahoruco, Entre Acetitillar y Las Abejas	VGRC
<i>Calisto schwartzii</i>	WI-JAG-1040	KX536784	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-1041	KX536716	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-1042	KX536773	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto schwartzii</i>	WI-JAG-1043	KX536708	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto tasajera</i>	WI-JAG-868	KX536747	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-869	KX536800	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-870	KX536729	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-871	KX536744	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-872	KX536696	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-873	KX536790	Dominican Republic, San Juan, Valle de Bao	VGRC
<i>Calisto tasajera</i>	WI-JAG-1008	KX536706	Dominican Republic, La Vega, Valle Nuevo, Entre Destacamento Militar y La Piramide	VGRC

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TABLE 1. (Continued)

Species	Voucher code	Accession number	Locality	Specimen repository
<i>Calisto tasajera</i>	WI-JAG-1009	KX536707	Dominican Republic, La Vega, Valle Nuevo, Entre Destacamento Militar y La Piramide	VGRC
<i>Calisto tasajera</i>	WI-JAG-1010	KX536694	Dominican Republic, La Vega, Valle Nuevo, Entre Destacamento Militar y La Piramide	VGRC
<i>Calisto tasajera</i>	WI-JAG-1011	KX536780	Dominican Republic, La Vega, Valle Nuevo, Entre Destacamento Militar y La Piramide	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-789	KX536798	Dominican Republic, Independencia, S. Bahoruco, caseta 2	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-964	KX536692	Dominican Republic, Independencia, S. Bahoruco, caseta 2	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-977	KX536772	Dominican Republic, Independencia, S. Bahoruco, Aceitillar	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-990	KX536726	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-991	KX536778	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-992	KX536792	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-993	KX536738	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-994	KX536761	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-995	KX536743	Dominican Rep., Pedernales, S. Bahoruco, Entre Aceitillar y Las Abejas	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-1000	KX536727	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-1001	KX536714	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-1002	KX536749	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-1032	KX536723	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto victori</i> (= <i>Calisto</i> sp. 3)	WI-JAG-1033	KX536771	Dominican Rep., Independencia, S. Bahoruco, Entre caseta 2 y caseta 1	VGRC
<i>Calisto zangis</i>	McGuire09-CAL-Sat1	JN197406	Jamaica	MGCL
<i>Calisto zangis</i>	RN02-12	KF054338	Jamaica	MGCL
<i>Calisto zangis</i>	RN02-13	KF054339	Jamaica	MGCL

We performed analyses following two methods: Maximum Likelihood (ML) and Bayesian Inference (BI). In both cases *Calisto nubila* (Puerto Rico) and *Calisto eleleus* (Hispaniola) were used as outgroups since they seem to be two among the earliest divergent species within the genus (Matos-Maraví *et al.*, 2014). The ML analyses were also conducted online on the CIPRES Portal using the RaxML algorithm with the default GTRGAMMA model (Stamatakis *et al.* 2008). The reliability of the internal branches in the ML tree was assessed by 1000 bootstrap replicates.

In the BI approach, we infer the best-fitting model of molecular evolution and partition scheme to apply using PartitionFinder 1.0.1 (Lanfear *et al.* 2012). The best-fitting partition/substitution model scheme, as determined by the AICc, was implemented in a Bayesian inference analysis with MrBayes 3.2 (Ronquist *et al.* 2012). Two independent MCMC analyses with four simultaneous chains (one cold and three heated) for each analysis were run for 20 million generations and the sampling of trees and parameters was set to every 1000 generations. Convergence of the two runs was determined by the stationary distribution plot of the log likelihood values against number of generations and confirmed by the average standard deviation of split frequencies which in all the cases were lower than 0.05. We discarded the first 5 million generations as burn-in and trees were summarized under the 50 percent majority rule method.

Abbreviations and acronyms:

ABGD	Automatic Barcode Gap Discovery
AMNH	American Museum of Natural History, New York, U.S.A.
bp	base pair(s)
BS	Maximum Likelihood Bootstrap
BMNH	British Museum of Natural History. London, UK
CZACC	Instituto de Ecología y Sistemática, Havana, Cuba
FW	forewing
HW	hindwing
K2P	Kimura 2 parameter
MCZ	Museum of Comparative Zoology, Harvard, Boston, U.S.A.
MGCL	McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, U.S.A.
MNHNSD	Museo Nacional de Historia Natural de Santo Domingo, Santo Domingo, Dominican Republic
NJ	Neighbor Joining
PP	Bayes posterior probability
UN	underside of wings
UNFW	underside of forewing
UNHW	underside of hindwing
UPFW	upper side of forewing
UPHW	upper side of hindwing
VGRC	Victor González Research Collection, Puerto Rico

Results

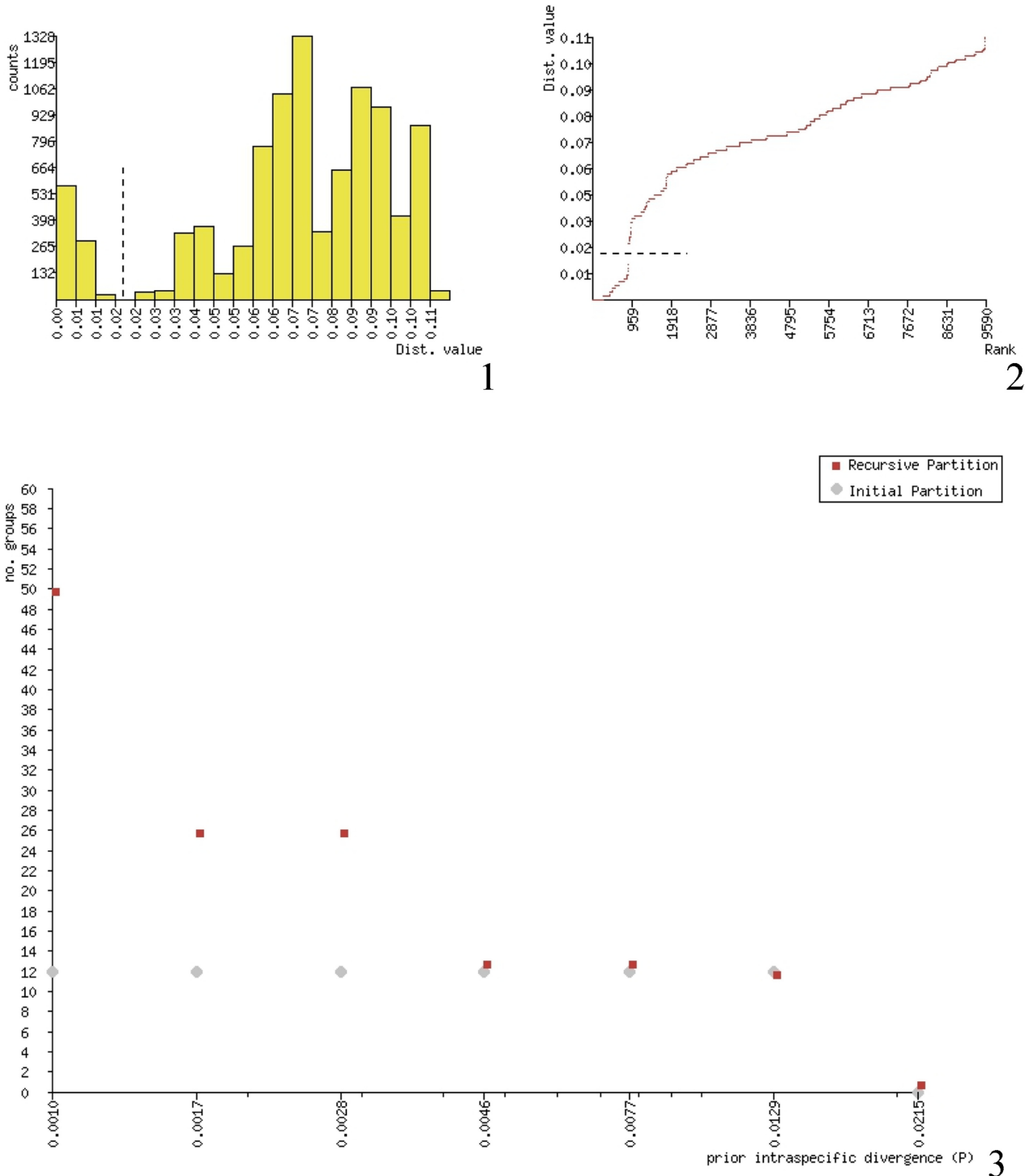
The *lyceius* species group of *Calisto*

The *lyceius* group comprises 12 species, four of them newly described in present work. The external morphology of group members could be generalized as follows: medium to large size species according to genus standards (FW length: 15.0–26.3 mm ♂, 15.6–28.9 mm ♀); UP of wings dark brown, females with reddish blotches present on the HW or both on FW and HW; males with a black or yellow and grey androconial patch located below the discal cell or at its apex; UN of wings with extensive areas covered with brick reddish color; UNFW ocellus with two white pupils; UNHW usually with a single ocellus with a white pupil basally displaced and two to four white dots in the post discal row above the ocellus.

Regarding genitalia both female and male genitalia shares most features with the remaining species of *Calisto*

and do not show exclusive characters that serve to the definition of the *lyceius* group. The few species with particular diagnostic features will be commented and discussed separately case by case.

DNA barcoding. The Neighbor–Joining algorithm obtained 12 clusters, eight corresponding to the previous known species and other four entities referred here as *Calisto* sp. 1 to 4 (Appendix 1). Intra cluster distances values were among 0 and 1.54/1.52% (K2P/p–distance) whereas inter cluster values were among 2.49/2.43 and 12.47/11.4% evidencing the existence of a gap.



FIGURES 1–3. Output of the Automatic Barcode Gap Discovery (ABGD) web-interface (<http://www.wabi.snv.jussieu.fr/public/abgd/>) after the input of distance data belonging to 139 available COI sequences of the *lyceius* species group of *Calisto*. 1—Histogram of distances. 2—Ranked distances. 3—Initial and recursive partitions. Dotted line in 1 and 2 signaling approximate position of gap center.

Species delineation by ABGD. The ABGD method also identified a barcode gap centered around 1.8% of divergence between the available COI sequences. The analysis defined the existence of 13 to 12 hypothetical species in all recursive partitions with prior intraspecific genetic divergence values between 0.46% and 1.29%, a result we considered more likely than 26 or more species with intraspecific divergence values below 0.28% or as a single species with intraspecific divergence values greater than 2.15% (Figs. 1–3, Appendix 2). The 13 species hypothesis obtained twice by the analysis was due to a single sequence, coded as WI-JAG-990 *Calisto* sp3, which differs 0.76 to 1.06% or 5 to 7 bp from other *Calisto* sp3 sequences. In both 12 and 13 partitions, the method grouped the same sequences clustered by Neighbor-Joining except for the above mentioned sequence.

Taxonomic treatment

Calisto zangis (Fabricius, 1775)

Papilio zangis Fabricius, 1775: 486

Papilio agnes Cramer, 1782: 73

Hipparchia zangis: Hübner, 1816: 57

Satyrus zangis: Godart, 1823: 525

Calisto zangis: Doubleday *et al.*, 1851: 399; Butler, 1868: 97; Kirby, 1871: 103; Möschler, 1886: 27; Staudingern, 1888: 232; Lathy, 1899: 222; Weymer, 1911 in Seitz: 240; Kaye, 1926: 477; Gaede, 1931: 480; Bates, 1935: 245; Michener, 1943: 6; Munroe, 1951: 232; Brown & Heineman, 1972: 51; Smith *et al.*, 1994: 47; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Material examined. SYNTYPES, 2 ♂, TL: “Carolina” (error). Photograph examined, available at http://www.butterfliesofamerica.com/L/calisto_zangis_types.htm.

Diagnosis (Figs. 4–7). FW length: 22 mm ♂,♀. Males can be easily differentiated from any other *Calisto* by the nearly round androconial patch covered by greyish and brown scales and located on the distal extreme of discal cell. Females can be recognized by the reddish suffusion over the brown background on both wings. The spines on dorsal edge of valvae at the male genitalia are unique among all *Calisto*. The species may be differentiated from all other members of the *lyceius* group by five unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 75). As illustrated. The toothed dorsal edge of the valvae is a unique feature among all known *Calisto*. Gnathi absent.

Female genitalia. Unavailable for present study.

Distribution. Restricted to Jamaica where is widespread (not showed).

Biology. Species inhabits forests paths and open areas with bushes from the sea level to 1500–1800 m high (Munroe 1951, Brown & Heineman 1972, Smith *et al.* 1994). The immature stages were described by Turner in Brown & Heineman (1972). Larvae feed on *Axinopus compressus* and *Cynodon dactylon*.

Molecular characterization. The intraspecific haplotype diversity in the available sequences (n=3) was $H_d = 1$. Nucleotide diversity amounted to $\pi = 0.00795$. The average nucleotide composition is A = 28.6, T = 41.4, C = 15.3, and G = 14.7. The mean sequence divergence and the haplotype and nucleotide diversity values for each of the *lyceius* group species are showed in Table 3. Overall mean distance among the three available sequences is 0.81%, differing from each other by 2 to 7 bp. The lowest overall mean distance to other member of the *lyceius* group is 7.31% or 46 bp, *C. raburni* (Table 4).

Remarks. Lathy (1899) noted considerably variation in the amount of reddish scaling on the dorsal surface of the hindwings and the richness of marking at the ventral surface. He found that specimens above 1500 m at the Blue Mountains were darker and less bright than those found below 300 m in the western slopes. Further research will be addressed to determinate if these differences are taxonomically relevant.

Calisto pulchella Lathy, 1899

Calisto pulchella Lathy, 1899: 225; Weymer, 1911 in Seitz: 240; Hall, 1925: 165; Gaede, 1931: 480; Bates, 1935: 245; Michener, 1943: 6

Calisto pulchella pulchella: Munroe, 1951: 232; Brown & Heineman, 1972: 51; Schwartz, 1989: 446; Smith *et al.*, 1994: 48; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Calisto pulchella darlingtoni Bates, 1939, **new syn.** Bates, 1939: 50 (as *C. pulchella*); Clench, 1943: 28; Munroe, 1951: 232; Brown & Heineman, 1972: 51; Schwartz, 1989: 450; Smith *et al.*, 1994: 48; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Material examined. SYNTYPES 2 ♂, HAITI. Priddyi. (BMNH). Photographs examined, available at http://www.butterfliesofamerica.com/L/calisto_p_pulchella_types.htm. A cotype ♀ with the same data represents *Calisto pulchella* aberration *tenebrosa* also described by Lathy and was reviewed too. The exact number of cotypes is not clear in the original description.

Calisto pulchella darlingtoni Type material. HOLOTYPE ♂, DOMINICAN REPUBLIC: Constanza, August 1938, Darlington, 3–4000 ft, M. C. Z. Type 25918 (MCZ). Photographs examined, available at: http://www.butterfliesofamerica.com/L/calisto_pulchella_darlingtoni_types.htm

Other material (24 ♂, 7 ♀). REPÚBLICA DOMINICANA: PROVINCIA PUERTO PLATA, Imbert, Salto La Demajagua, 15.xii.07, A. R. Pérez-Asso & A. López, en cañaveral, 2 ♂ (DNA voucher JAGWI-555); PROVINCIA LA VEGA, Parque Nacional Alejandro Bermúdez, La Ciénaga, 3.xii.08, J. A. Genaro, 2 ♂ (DNA vouchers JAGWI-540, 552–554); same data as anterior except 28.xi.11, A. R. Pérez-Asso & A. L. Sánchez, en bosque latifoliado, 11 ♂ 4 ♀ (DNA vouchers JAGWI-881–883, 887, 888); Parque Nacional Ébano Verde, El Arroyazo, 4.vii.12, A. R. Pérez-Asso & A. López, en pinar antropizado, 3 ♂ (DNA vouchers JAGWI-889–891); Parque Nacional Valle Nuevo, La Nuéz, 2050 m, 3.xii.08, A. López & A. R. Pérez-Asso, en pinar; PROVINCIA SANTIAGO, Parque Nacional Alejandro Bermúdez, Loma del Oro-Arroyo Malo, 19.vi.11, A. López & A. R. Pérez-Asso, en pinar, 2 ♀; PROVINCIA LA ALTAGRACIA, San Rafael de Yuma, 25.vii.12, A. R. Pérez-Asso & A. López, en cañaveral, 4 ♂ 1 ♀ (DNA vouchers JAGWI-879–879, 960, 961) (VGRC).

Diagnosis (Figs. 8–13, 54, 55). FW length: 23.5–26.3 mm ♂, 26.2–28.9 mm ♀. *Calisto pulchella* may be easily separated from all other species of the genus by its larger size and some elements of wing pattern. UNFW is brown with slight dark reddish tinge usually restricted to cell. UNHW is dark brown with orange at base and at least the entire posterior half of wing reaching the external subterminal line. The females have straighter FW margins and distinctive paler background with specimens showing an orange post discal patch on UP. There is some variation in the size of UNHW ocellus, larger and broader in males than in females, and in the tone, orangish or reddish, at UNHW. The species may be differentiated from all other members of the *lyceius* group by fourteen unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 76). As illustrated. The rounded bulbous tegumen and the long and slender curved uncus as well as the shape of the short are very distinctive. Gnathi absent.

Female genitalia (Fig. 87). As illustrated. Sclerotized ring absent. Dorsal crown gradually tapering toward sides, divided at the middle where the enlarged internal loop running anteriorly (down in ventral view) through the posterior genital plate ending in a highly sclerotized “cup”. Posterior genital plate slightly sclerotized at both sides of loop. Anterior genital plate absent.

Distribution. The species is widespread in both Haiti and Dominican Republic inhabiting both lowlands and high mountains up to 2074 m (Schwartz 1989, Smith *et al.* 1994) (Fig. 97). Although its original distribution will never be known, the species was almost surely favored by the introduction of exotic sugar cane at Hispaniola by Spaniards during colonial times.

Biology. *Calisto pulchella* inhabits numerous natural and modified situations including rocky coastal vegetation (Fig. 101), xeric forests, mesic forests, pine forests, floodplains, open ravines, marshy meadows, sugar cane fields, and cocoa and coffee plantations (Schwartz 1989). The species received a great attention since became a sugar cane pest. Its life cycle was first described by Wollcott (1922) and posteriorly detailed and illustrated in color by Sourakov (1996).

Schwartz (1989) offered numerous details on species natural history including distribution, altitudinal distribution, abundance, habitat preference, flight time, and nectar sources.

Molecular characterization. We obtained sequences from additional 15 highland and 6 lowland specimens, most of them from the same localities of the three previous sequences, plus three sequences from GenBank. The nucleotide composition were A = 29.3, T = 39.3, C = 16.9, and G = 14.5, characterization values are showed in Table 3. Overall mean distance among the available sequences is 0.57%, differing from each other by 0 to 7 bp. Overall mean distance within our highland and lowland specimens groups was 0.51 and 0.05% (3 and 1 bp) respectively, whereas the mean divergence between them was 0.63% or 4 bp. Comparison of our highland and lowland groups with two previous highland sequences resulted in little divergence ranging from 0.71 to 0.81% or 4

to 5 bp. The lowest genetic distance of *C. pulchella* COI sequences to other member of the *lyceius* group is 8.45% or 54 bp to *C. zangis* (Table 4).

Remarks. A highland subspecies, *C. pulchella darlingtoni*, was described from Cordillera Central by Clench (1943). Its validity was supported by Wisor & Schwartz (1985) and Schwartz (1989) arguing differences in some characters such as more fulvous UNHW and smaller HW ocellus in highland specimens. However, Smith *et al.* (1994) mentioned the existence of intergrades that prevent a clear separation of the two morphs. Sourakov & Zakharov (2011) commented that the genetic divergence (3.5%) between these taxa was that of good species. They compared two sequences from highland specimens, JN197393 and JN197394, against one obtained from a lowland specimen, GQ357225. The later differs 3.63 and 3.24% or 19 and 21 bp from our highland and lowland groups respectively. The only explanation for these differences is an error during the process to obtain that sequence. This hypothesis is reinforced when comparisons with an additional sequence we found in GenBank, KF054317, are made. The sequence has divergences of 2.13 and 2.21% or 11 to 12 bp respect to our highland and lowland groups but also of 3.4% or 18 bp to GQ357225. The later sequence apparently also suffers from the same kind of problem. Pairwise distances among all available sequences are showed in Table 4. Moreover, neither Neighbor Joining clusters nor Bayesian Inference or Maximum Likelihood trees showed a dichotomy evidencing two groups representing two distinct taxa (Appendix 1, Fig. 107), all available sequences group together in a single clade.

Concluding, if previously there was some evidence of the existence of two allopatric taxa, present evidence from COI sequences doesn't support it and even less the existence of a third taxon. Taking account all elements above given we propose consider *C. pulchella darlingtoni* a synonym of *C. pulchella*.

***Calisto raburni* Gali, 1985**

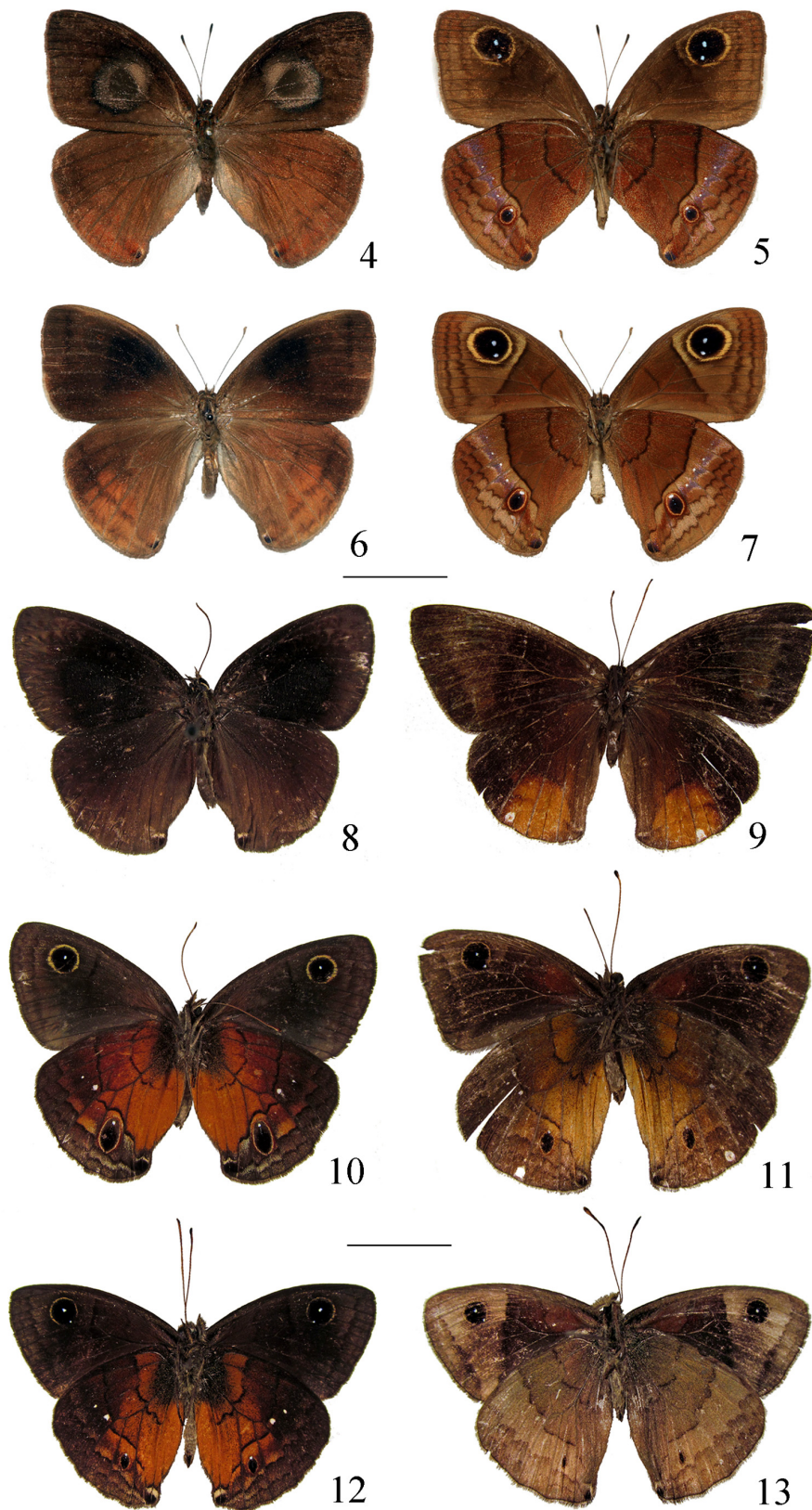
Calisto raburni Gali, 1985: 8; Schwartz, 1989: 434; Smith *et al.*, 1994: 53; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Material examined. HOLOTYPE ♂, REPÚBLICA DOMINICANA: Provincia de Independencia, 7 km NE El Aguacate, 519 m, 6.vii.1983 A. Schwartz (AMNH). Photographs examined, available at:

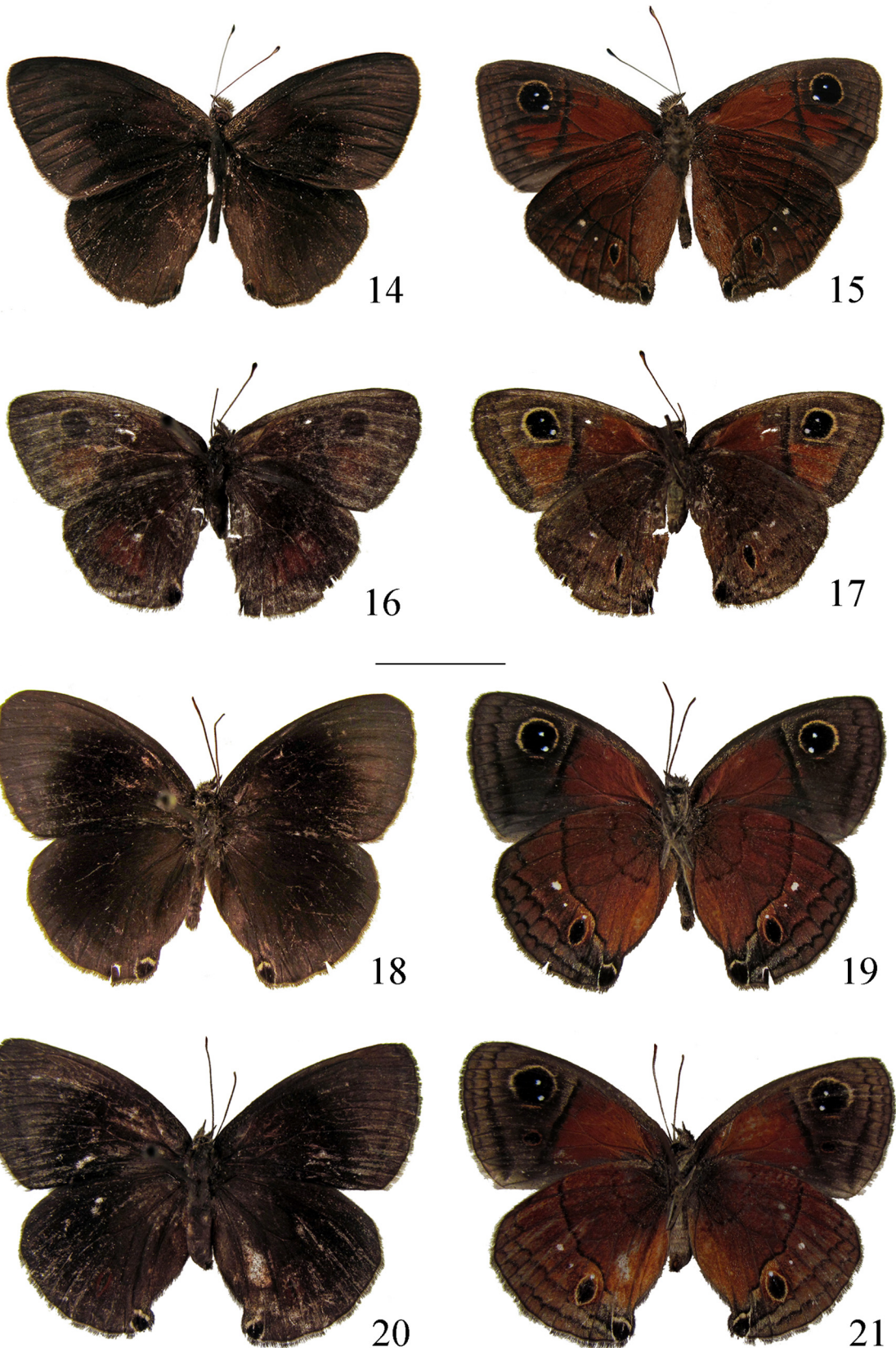
http://www.butterfliesofamerica.com/L/calisto_raburni_types.htm.

Other material (13 ♂, 2 ♀). REPÚBLICA DOMINICANA: PROVINCIA INDEPENDENCIA, Parque Nacional Sierra de Bahoruco, El Aguacate, 1055 m, 9.vi.11, A. López & A. R. Pérez-Asso, en pinar, 1 ♂ (DNA voucher JAGWI-790); Parque Nacional Sierra de Bahoruco, camino entre Caseta 1 y Puerto Escondido, 24.xi.11, A. R. Pérez-Asso & A. López, en bosque seco con Tibisí (*Arthrostygidium* spp.), 1 ♂ (DNA voucher JAGWI-874); same data as anterior except 16.viii.12, 2 ♂ (DNA vouchers JAGWI-955, 956); same data as anterior except 24.vi.13, 2 ♂ (DNA vouchers JAGWI-1004, 1006); PROVINCIA PEDERNALES, Parque Nacional Sierra de Bahoruco, Aceitillar, 14.vii.12, A. R. Pérez-Asso & A. López, en pinar, 1 ♂ (DNA voucher JAGWI-978); DISTRITO NACIONAL, Santo Domingo, Sierra Prieta, 26.vii.12, A. R. Pérez-Asso & A. López, en bosque seco con Tibisí (*Arthrostygidium* spp.), 2 ♂ (DNA voucher JAGWI-877); same data as anterior except 26.viii.14, 4 ♂ 2 ♀ (DNA vouchers JAGWI-1034, 1035) (VGRC).

Diagnosis (Figs. 14–17, 56, 57). FW length: 14.9–17 mm ♂, 15.6–16.9 mm ♀. *Calisto raburni* needs to be compared with three new species of the *lyceius* group described in present work: *C. mariposa* sp. nov., *C. victori* sp. nov., and *C. azua* sp. nov. All four species are reddish, but having various tones, and have two white dots at UNHW: one enlarged dot between M2–M3 veins and another distinctly smaller between M3–CU1. *Calisto raburni* is smaller on the average compared to the other three taxa and possesses a very elongated HW ocellus with an acute distal end whereas in the others it is usually broader with the distal end rounded or barely pointed in the case of *C. mariposa*. *Calisto raburni* can be also separated from *C. mariposa* and *C. victori* by the extension in most specimens of the reddish scaling beyond the post discal line reaching the first subterminal line at UNFW. Females have the UN similar, including the whitish scaling, but with the reddish areas have an orangish tone. The females of *C. victori* are also orangish but lack the whitish scaling and those of *C. mariposa* and *C. azua* are redder and browner. The species may be differentiated from all other members of the *lyceius* group by fifteen unique fixed states of its DNA barcode (Table 2).



FIGURES 4–13. Adults of the *lyceius* species group of *Calisto*. 4—*C. zangis* male upperside, Crown Lands N of Troy, Trelawny. 5—*C. zangis* male underside, same data. 6—*C. zangis* female upperside, Bog Walk. 7—*C. zangis* female underside, same data. 8—*C. pulchella* male upperside, San Rafael de Yuma, La Altagracia. 9—*C. pulchella* female upperside, La Ciénaga, Cordillera Central. 10—*C. pulchella* male underside, same data as 9. 11—*C. pulchella* female underside, same data as 10. 12—*C. pulchella* male underside, same data as 9. 13—*C. pulchella* female underside, Loma del Oro, Santiago. Images 4–7 reproduced under permission of A. D. Warren and colleagues.



FIGURES 14–21. Adults of the *lyceius* species group of *Calisto* (cont.). 14—*C. raburni* male upperside, camino de Caseta 1 a Puerto Escondido, Sierra de Bahoruco. 15—*C. raburni* male underside, same data. 16—*C. raburni* female upperside, Sierra Prieta, Sierra de Yamasá. 17—*C. raburni* female underside, same data. 18—*C. mariposa* sp. nov. HOLOTYPE male upperside, Sierra Prieta, Sierra de Yamasá. 19—*C. mariposa* sp. nov. HOLOTYPE male underside, same data. 20—*C. mariposa* sp. nov. PARATYPE female upperside, same data. 21—*C. mariposa* sp. nov. PARATYPE female underside, same data.

Male genitalia (Fig. 77). As illustrated. The flattened short hairy uncus and the compressed stout adeagus are unique among all *Calisto* species. The gnathi are present as small acute processes.

Female genitalia (Fig. 88). As illustrated. The incomplete sclerotized ring and the sclerite of the posterior genital plate are unique among all known *Calisto*.

Distribution. The species was described from one locality, 7 km NE El Aguacate (Independencia province), near the Haitian–Dominican border at the northern slope of the Sierra de Bahoruco range (Fig. 98). Sourakov (2000) extended its range 50 km to the southeast, Barahona province. Here we record it from few more localities near the type locality and for the first time from the southern slope of the Sierra de Bahoruco, at Aceitillar (Pedernales province). More remarkable is the presence of *C. raburni* also 150 km to the east, out from the Bahoruco range. This new locality, Sierra Prieta, lies few kilometers to the north of Santo Domingo and is a small hill satellite of the south of Sierra de Yamasá, which is separated by a narrow valley from the Cordillera Central (Fig. 98).

Biology. Only few details of species habitat were commented by Schwartz (1989) that mentioned a transition from xeric through transitional forests. Sourakov (2000) recorded it from a heavily coffee planted valley near Polo town, Barahona province. We observed the species in xeric forest with climbing grass, *Arthrotylidium* spp. (Fig. 102) and also inhabiting pine forests. There are no other data on the species natural history.

Molecular characterization. The nucleotide composition were A = 29.8, T = 39.2, C = 16.2, and G=14.7, characterization values are showed in Table 3. Overall mean distance among the available sequences is 0.52%, differing from each other by 0 to 5 bp. Mean distances of the COI sequences between Sierra de Bahoruco (n=8) and Sierra Prieta (n=3) populations is 0.72% or 5 bp, whereas the overall mean variation within each population is 0.42% or 3 bp at Sierra de Bahoruco and 0 at Sierra Prieta. With the available sequences, each population can be recognized by the following two substitutions: 220 T (Sierra Prieta)/C (Sierra de Bahoruco), and 403 G/A respectively. The lowest genetic distance to a relative of the *lyceius* group is 7.31% or 46 bp to *C. zangis* (Table 4).

Remarks. Specimens from Sierra Prieta have UNHW ocellus distinctly larger than specimens from Sierra de Bahoruco.

The absence of *Uniola virgata* (Poir.) Griseb., a tussock grass, was noted at most localities except at the type locality (Schwartz 1989). An association with *U. virgata* has been proposed for most members of the *lyceius* group, except *C. schwartzi* and *C. raburni* (Schwartz 1989, Smith *et al.* 1994). During field work previous to present paper it was noted at several localities, including the new ones, the abundance of a climbing grass, *Arthrotylidium* spp. (Fig. 102).

The species' presence out of Sierra de Bahoruco is noteworthy. It opens the possibility to look for *C. raburni* at other areas lying in the 150 km that separate the eastern limit of Bahoruco and Sierra Prieta, mainly the southern slope of the Cordillera Central.

***Calisto mariposa* Pérez–Asso, Núñez & Genaro sp. nov.**

Holotype. ♂, REPÚBLICA DOMINICANA: DISTRITO NACIONAL, Santo Domingo, Sierra Prieta, 26.viii.14, A. R. Pérez–Asso & A. López, en bosque seco con Tibisí (*Arthrotylidium* spp.), DNA voucher JAGWI–1030 (VGRC).

Paratypes. 19 ♂, 6 ♀. Same data as holotype, 11 ♂ 6 ♀; same data as holotype except 26.vii.2012, 11 ♂, DNA vouchers JAGWI–875, 876, 958, 959; PROVINCIA INDEPENDENCIA, Parque Nacional Sierra de Bahoruco, camino entre Caseta 1 y Puerto Escondido, 24.vi.2013, A. López & A. R. Pérez–Asso, 3 ♂, DNA vouchers JAGWI–957, 1003, 1005 (CZACC, MGCL, MNNSD, VGRC).

Diagnosis (Figs. 18–21, 58, 59). FW length: 17.6–19.1 mm ♂, 18.5–20.8 mm ♀. *Calisto mariposa* needs to be compared with syntopic *C. raburni*, and with *C. victori* sp. nov. and *C. azua* sp. nov., two new species also described in this work. All four species are reddish, but having various tones, and have two white dots at UNHW: one enlarged dot between M2–M3 veins and another distinctly smaller between M3–CU1. *Calisto mariposa* has the UNHW background mostly reddish (males) or orangish (females) without scattered pale yellowish or whitish scaling as occurs at the other three taxa. From *C. victori* and *C. azua* the species also differs by the usually acute distal end of the UNHW ocellus, the latter is also acute in *C. raburni* but its ocellus is much more elongated, the later species is smaller on the average. The species can be also separated from *C. azua* and *C. raburni* by lacking the reddish color beyond the post discal line at UNFW in both sexes, only few specimens show the suffusion at the outer edge line whereas that color is more extended toward the area below the ocellus or even reaching the

subterminal line. Males can be also recognized by having the UPFW area anterior to androconial patch also blackish whereas in the other three is distinctly paler as the remaining FW surface. *Calisto victori* sp. nov. bears pear shaped UNHW ocellus and its males have the UN background mixed with dark reddish scaling. *Calisto mariposa* sp. nov. maybe also differentiated from all other members of the *lyceius* group by five unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 78). As illustrated. Greatly enlarged regarding animal size. Tegumen large and rounded. Uncus rounded at the base, straight only slightly curved at the tip. Adeagus caecum strongly curved to the right in dorsal view.

Female genitalia (Fig. 89). As illustrated. This and all following species share the possession of a complete sclerotized ring with lateral “horns” pointing dorsal.

Distribution. The species known distribution is disjunct, as in *C. raburni*, but it has been recorded only from two localities (Fig. 98). One at the northern slope of Sierra de Bahoruco and the other at Sierra Prieta, 150 km to the east, a small hill satellite of the south of Sierra de Yamasá, which is separated by a narrow valley from the Cordillera Central (Fig. 98).

Biology. The only available information is on species habitats: xeric forests with climbing grass, *Arthrostylidium* spp. (Fig. 102).

Etymology. This species is named for the Mirabal sisters, Dominican Republic national heroines who fought against the Trujillo’s dictatorship being assassinated in 1960. They were and are still called “mariposas”, the Spanish word for butterflies, by its people.

Molecular characterization. The nucleotide composition were A = 29.4, T = 40.4, C = 15.7, and G = 14.5, characterization values are showed in Table 3. Overall mean distance among the available sequences is 0.23%, differing from each other by 0 to 3 bp. Variation of COI sequences within both populations is 0 whereas overall mean distance between both populations from Sierra de Bahoruco (n=3) and Sierra Prieta (n=6) is 0.46%, or 3 bp. With the available sequences, each population can be recognized by the following three substitutions: 43 C (Sierra Prieta)/T (Sierra de Bahoruco), 358 T/C, and 415 G/A respectively. The lowest genetic distance to other member of the *lyceius* group is 6.72% or 43 bp to *C. franciscoi* (Table 4).

Remarks. *Calisto mariposa* sp. nov. specimens were originally included in the cluster named *Calisto* sp. 1 during the sequence analysis. The species disjunction regarding distribution is remarkable and coincides with that of *C. raburni*. The latter and the superficial resemblance among them are the most probably reasons the species remained overlooked until present work. It probably also inhabits places with xeric habitats at the southeastern slope of the Cordillera Central. The high divergence COI from other group members together with its larger size regarding the syntopic *C. raburni* led us to the discovery of this species.

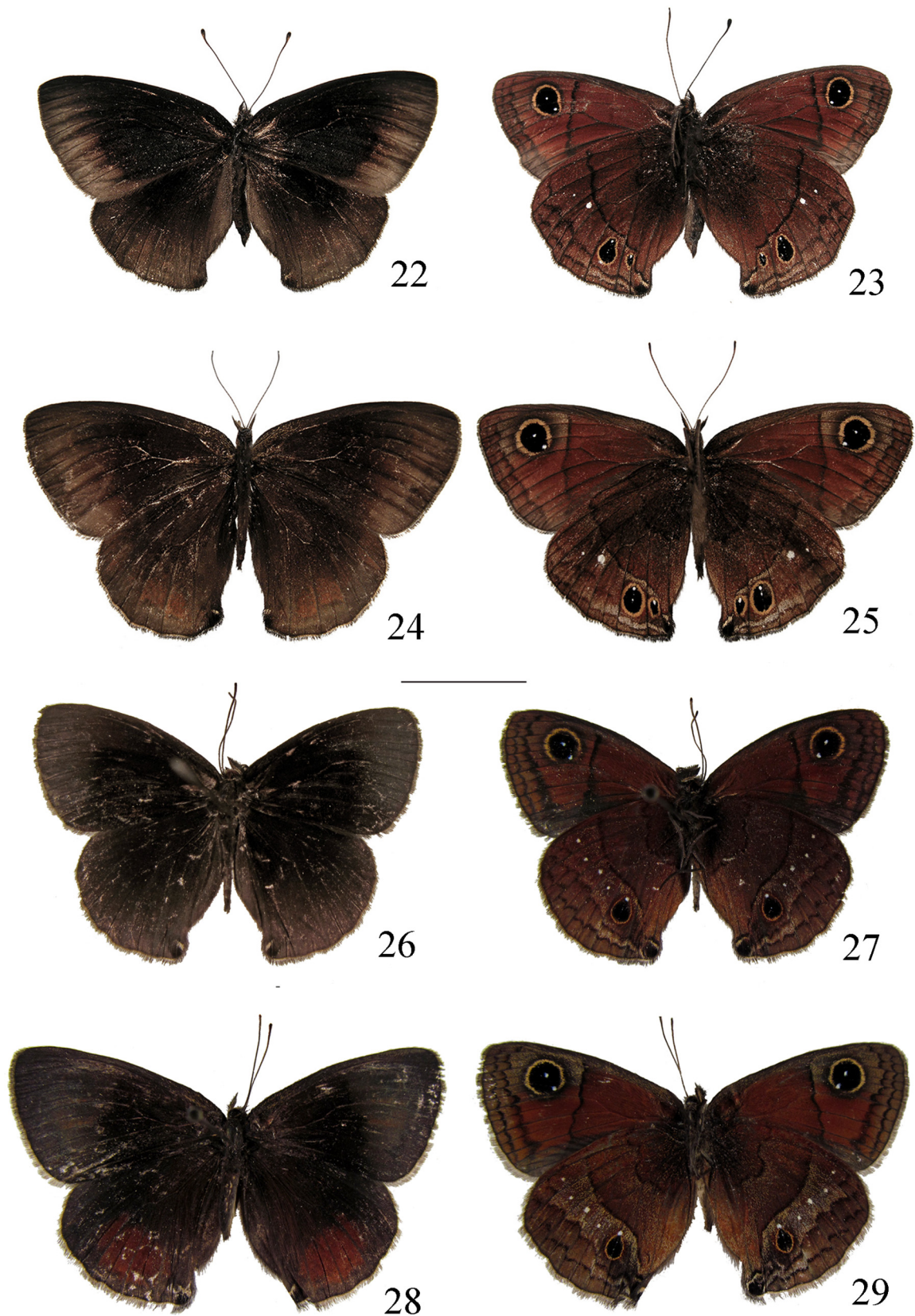
***Calisto tasajera* Gonzalez, Schwartz & Wetherbee, 1991**

Calisto tasajera Gonzalez, Schwartz & Wetherbee, 1991: 1; Smith *et al.*, 1994: 54; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Material examined. Type series not examined.

Other material (12 ♂, 6 ♀). REPÚBLICA DOMINICANA: PROVINCIA SANTIAGO, Cordillera Central, Parque Nacional Alejandro Bermúdez, Valle de Bao, 12.vii.11, A. López & A. R. Pérez–Asso, en pinar con Pajón (*Danthonia dominguensis*), 8 ♂ 5 ♀ (DNA vouchers JAGWI–868–873); PROVINCIA LA VEGA, Parque Nacional Valle Nuevo, camino a 5 km de Destacamento Militar hacia La Pirámide, 27.vi.13, A. López & A. R. Pérez–Asso, en pinar con Pajón (*Danthonia dominguensis*), 4 ♂ 1 ♀ (DNA vouchers JAGWI–1008–1011) (VGRC).

Diagnosis (Figs. 22–25, 60, 61). FW length: 21.4–23.2 mm ♂, 23.9–24.5 mm ♀. *Calisto tasajera* is one of species most easily to diagnose within the *lyceius* group. Together with its large size, the possession of two ocelli at UNHW (there is one additional ocellus at Cu2–2A in most specimens), and the presence of two white dots at M1–M2 (smaller) and M2–M3 (larger and basally displaced) are also diagnostic. Other species with two white dots within the *lyceius* group have them different located, between M2–M3 and M3–Cu1 vein pairs. In addition *C. tasajera* is the only species of its group inhabiting above 1800 m and reaching 2800 m. The species may be also differentiated from all other members of the *lyceius* group by eight unique fixed states of its DNA barcode (Table 2).



FIGURES 22–29. Adults of the *lyceius* species group of *Calisto* (cont.). 22—*C. tasajera* male upperside, Valle de Bao, Cordillera Central. 23—*C. tasajera* male underside, same data. 24—*C. tasajera* female upperside, same data. 25—*C. tasajera* female underside, same data. 26—*C. schwartzi* male upperside, camino desde Caseta 2 hasta Caseta 1, Sierra de Bahoruco. 27—*C. schwartzi* male underside, same data. 28—*C. schwartzi* female underside, same data. 29—*C. schwartzi* female underside, same data.

Male genitalia (Fig. 79). As illustrated. Greatly reduced regarding animal size. Valvae apexes acute and curved back. Gnathi very long and thin, acute, slightly curved upward.

Female genitalia (Fig. 90). As illustrated. The shape of the anterior genital plate, an obtuse triangle with concave sides, is diagnostic.

Distribution. The species is known from several high altitude localities of the Cordillera Central, all from the La Vega, San Juan and Santiago provinces (Fig. 99).

Biology. *Calisto tasajera* inhabits flooded places covered by sedges, ferns, pines, and a different species of bunch grass, *Danthonia domingensis* Hack. & Pilg. (Gonzalez *et al.* 1991, Hedges & Johnson 1994) (Fig. 103). Sourakov (2000) noted the association among *C. tasajera* and *D. domingensis*. The species lives considerably higher than any other member of the *lyceius* group, among 2000 and 2800 m. Its life cycle was partially described and illustrated by Sourakov (2000).

Molecular characterization. The nucleotide composition were A=30.1, T=39.0, C=16.6, and G=14.3, characterization values are showed in Table 3. Overall mean distance among the available sequences is 0.34%, differing from each other by 0 to 5 bp. Variation of COI sequences between Valle de Bao specimens (n=6) respect to Valle Nuevo (n=4) is 0.51% or 2 to 5 bp, with the variation within each population 0.2% or 0 to 1 bp. With the available sequences, each population can be recognized by the following two substitutions: 166 A (Valle de Bao)/G (Valle Nuevo), and 403 A/G. The lowest overall mean distance to a relative of the *lyceius* group is 5.92% or 39 bp, to *C. victori* (Table 4).

***Calisto schwartzi* Gali, 1985**

Calisto schwartzi Gali, 1985: 7; Schwartz, 1989: 436; Smith *et al.*, 1994: 53; Lamas, 2004: 207

Material examined. Holotype ♂, Republica Dominicana: Prov. de Pedernales: 1 km N Aceitillar, 1281 m; 6.x.1983, A. Schwartz (AMNH). Photographs examined, available at http://www.butterfliesofamerica.com/L/calisto_schwartzi_types.htm.

Other material (22 ♂, 17 ♀). REPÚBLICA DOMINICANA: PROVINCIA PEDERNALES, Parque Nacional Sierra de Bahoruco, Las Abejas, 1345 m, 23.xi.08, J. A. Genaro & A. R. Pérez-Asso, 1 ♀; same data as anterior except 22.viii.10, A. López, A. R. Pérez-Asso & J. A. Genaro, 2 ♂, 1 ♀ (DNA vouchers JAGWI-490, 491, 541); same data as anterior except 6.vii.11, A. López & A. R. Pérez-Asso, en pinar, 2 ♂ 3 ♀ (DNA vouchers JAGWI-781-783); Parque Nacional Sierra de Bahoruco, Aceitillar, 1637m, 6.xi.11, A. López & A. R. Pérez-Asso, en pinar, 1 ♂ 3 ♀; same data as anterior except 14.vii.12, A. R. Pérez-Asso & A. López, 3 ♀; Parque Nacional Sierra de Bahoruco, Entre Aceitillar y Las Abejas, 19.v.13, A. R. Pérez-Asso & A. López, 1 ♂ (DNA voucher JAGWI-999); PROVINCIA INDEPENDENCIA, Parque Nacional Sierra de Bahoruco, Caseta 2, 5.vii.12, A. R. Pérez-Asso & A. López, en pinar, 1 ♂; camino desde Caseta 2 hasta Caseta 1, 22.vi.13, A. López & A. R. Pérez-Asso, en pinar, 4 ♂ 1 ♀ (DNA vouchers JAGWI-996-998); same data as anterior except 7.viii.14, A. López & A. R. Pérez-Asso, 11 ♂ 5 ♀ (VGRC).

Diagnosis (Figs. 26–29, 62, 63). FW length: 17.8–20.8 mm ♂, 22.4–24.5 mm ♀. *Calisto schwartzi* shares the possession of a four white dots at UNHW with *C. lyceius*, *C. crypta*, *C. franciscoi*, and a new species described herein, *C. samana* **sp. nov.** From all these *C. schwartzi* differs by its larger size, a distinctly darker reddish tone at UN of both wings, and by have basally displaced the white dot at the interspace between M2–M3 veins. The syntopic *C. victori* **sp. nov.** exhibits a similar UN background dark reddish color but differs by having only two white dots at UNHW and by its distinctly smaller females. Another distinguishing feature is the pear shape of the UNHW ocellus, a characteristic shared only by *C. victori*, *C. pulchella*, and *C. tasajera*. *Calisto schwartzi* may be also differentiated from all other members of the *lyceius* group by four unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 80). As illustrated. Tegumen moderately flat with a shallow groove about the middle. Uncus hairy at base, gradually tapering towards apex. Adeagus strongly arched with a short subapical broad tooth.

Female genitalia (Fig. 91). As illustrated. The shape of the anterior genital plate resembling a triangle with slightly convex sides is diagnostic.

Distribution. The species was originally described from a single locality, 1 km N Aceitillar at Pedernales

province in the southern slope of the Sierra de Bahoruco range (Gali 1985). Schwartz (1989) recorded it from few more localities very close to the original one all also at the southern half of the Sierra de Bahoruco (Fig. 99). Here we record it for the first time from Las Abejas, also at the southern slope of the Sierra de Bahoruco range, and expand its range to the northern slope where we find it at two localities (Fig. 99).

Biology. The species inhabits only xeric pine forests above 1000 m where an unidentified tussock grass, not *Uniola virgata*, grows (Fig. 104). Gali (1985) and Schwartz (1989) informed on few nectar sources, altitudinal distribution, collection months, and copula data.

Molecular characterization. The nucleotide composition were A=29.8, T=40.2, C= 15.1, and G=14.9, characterization values are showed in Table 3. Variation of the 14 available COI sequences is 0.12% or 1 bp. The same distance values were obtained when comparing specimens from the northern (n=7) and southern (n=7) slopes of Sierra de Bahoruco. There are not substitutions that allow the recognition of the populations on both North and South slopes of the Sierra de Bahoruco range. The lowest overall mean distance of *C. schwartzi* to other member of the *lyceius* group is 4.67% or 31 bp, to *C. azua* (Table 4).

***Calisto azua* Pérez–Asso, Núñez & Genaro sp. nov.**

Holotype: ♂, REPÚBLICA DOMINICANA: PROVINCIA AZUA, Hatillo, El Número, 24.viii.14, A. R. Pérez–Asso & A. López, DNA voucher JAGWI–1028 (VGRC).

Paratypes: 11 ♂, 1 ♀. Same data as holotype, 9 ♂, DNA vouchers JAGWI–785–788, 1029; same data as holotype except 14.vi.13, 1 ♂, DNA voucher JAGWI–983; same data as holotype except 23.vi.13, 1 ♂ 1 ♀, DNA vouchers JAGWI–975, 976 (CZACC, MGCL, MNNSD, VGRC).

Diagnosis (Figs. 30–33, 64, 65). FW length: 17.3–18.7 mm ♂, 23.4 mm ♀. *Calisto azua* needs to be compared with *C. raburni*, *C. mariposa* sp. nov., and *C. victori* sp. nov. All four species are reddish, but having various tones, and have two white dots at UNHW: one enlarged dot between M2–M3 veins and another distinctly smaller between M3–CU1. From all *C. azua* differs by having small reddish orange areas at UPHW of males around the position of UN ocellus, by its browner tone at UNHW, and by possessing pale yellow scaling along the external side of post discal line at both wings. From *C. mariposa* sp. nov. and *C. victori* sp. nov. can be also separated by the extension in most specimens of the reddish scaling beyond the post discal line reaching the first subterminal line at UNFW. Females are distinctly larger and darker than males. The species may be differentiated from all other members of the *lyceius* group by two unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 81). As illustrated. Large. Uncus gradually tapering towards apex. Gnathi long and thin, slightly curved upward. Adeagus strongly arched.

Female genitalia. Unable to dissect the genitalia because of the damaged abdomen.

Distribution. The species is known from a single locality, El Número, near Hatillo town, Azua province, at the southern slope of the Cordillera Central eastern extreme (Fig. 100).

Biology. The species inhabits only *Acacia* scrub and forest with *Uniola virgata* stands (Fig. 105).

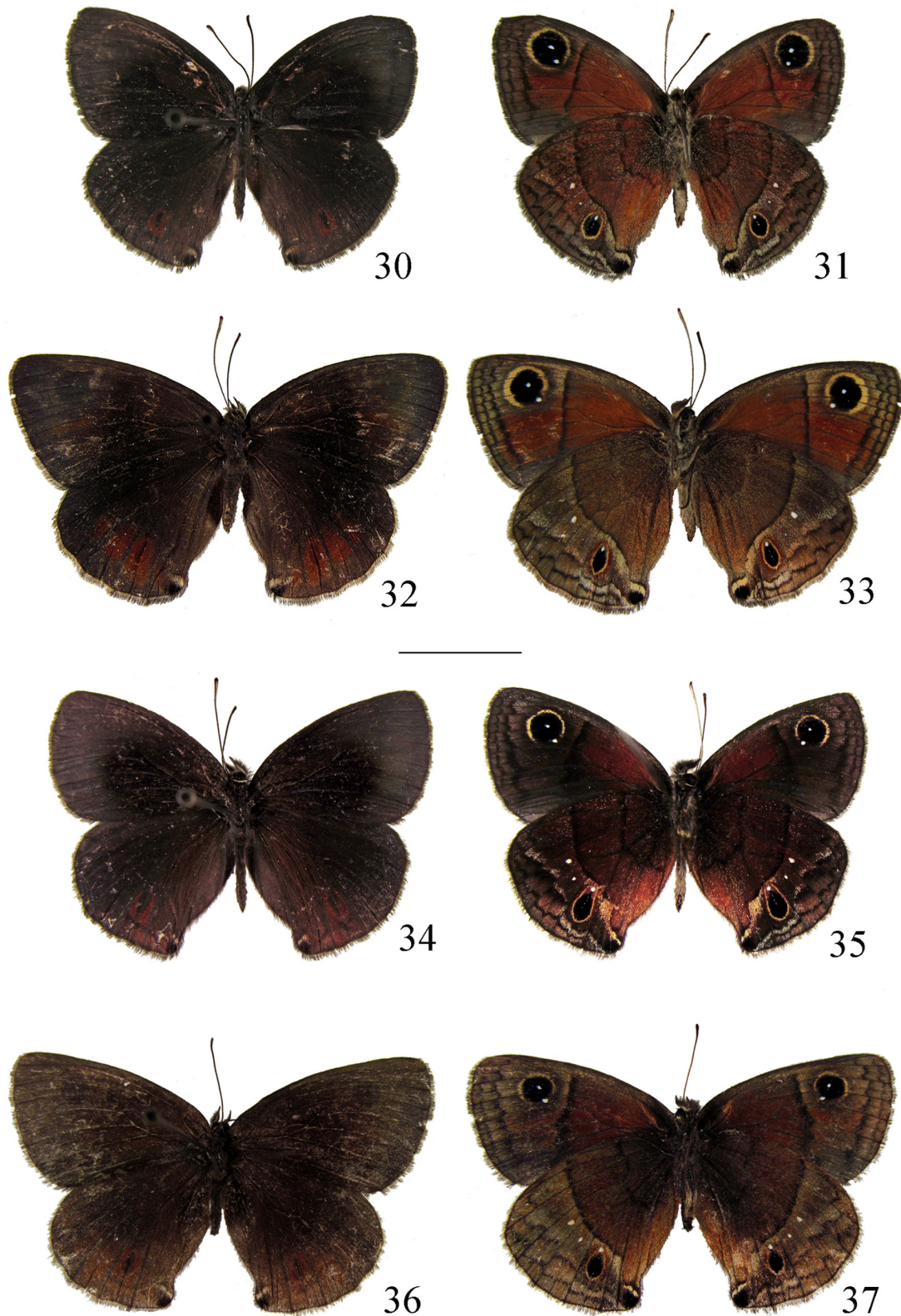
Etymology. The specific name of this species refers to the Dominican Republic province where its type located is placed: Azua.

Molecular characterization. The nucleotide composition were A=29.5, T=39.9, C= 15.9, and G=14.7, characterization values are showed in Table 3. Overall mean distance of the 9 available COI sequences is 0.15% or 1 bp. The lowest distance of *C. azua* to any member of the *lyceius* group is 3.7% or 24 bp to *C. victori* (Table 4).

Remarks. The larger size and scarce reddish suffusion at underside of wings in both sexes led us to discover and name *C. azua*. *Calisto azua* sp. nov. specimens were originally included in the cluster named *Calisto* sp. 4 during the sequence analysis. The only other member of the *lyceius* group at the type locality and its vicinity is the distinctive smaller and redder *C. franciscoi*.

***Calisto victori* Pérez–Asso, Núñez & Genaro sp. nov.**

Holotype: ♂, REPÚBLICA DOMINICANA: PROVINCIA INDEPENDENCIA, Parque Nacional Sierra de Bahoruco, camino entre Caseta 2 y Caseta 1, 7.viii.14, A. R. Pérez–Asso & A. López, en pinar, DNA voucher JAGWI–1032 (VGRC).



FIGURES 30–37. Adults of the *lyceius* species group of *Calisto* (cont.). 30—*C. azua* sp. nov. HOLOTYPE male upperside, El Número, Hatillo, Azua. 31—*C. azua* sp. nov. HOLOTYPE male underside, same data. 32—*C. azua* sp. nov. PARATYPE female upperside, same data. 33—*C. azua* sp. nov. PARATYPE female underside, same data. 34—*C. victori* sp. nov. HOLOTYPE male upperside, camino desde Caseta 2 hasta Caseta 1, Sierra de Bahoruco. 35—*C. victori* sp. nov. HOLOTYPE male underside, same data. 36—*C. victori* sp. nov. PARATYPE female upperside, same data. 37—*C. victori* sp. nov. PARATYPE female underside, same data.

Paratypes: 29 ♂, 3 ♀. Same data as holotype, 17 ♂ 3 ♀, DNA voucher JAGWI–1033; same data as holotype except 22–24.v.2013, 11 ♂, DNA vouchers JAGWI–1000–1002; PROVINCIA INDEPENDENCIA, Parque Nacional Sierra de Bahoruco, Caseta 2, 7.vi.11, A. López & A. R. Pérez–Asso, 1 ♂, DNA voucher JAGWI–789; same data as anterior except 6.vii.11, 1 ♂, DNA voucher JAGWI–964; PROVINCIA PEDERNALES, Parque Nacional Sierra de Bahoruco, Aceitillar, 14.vii.12, A. R. Pérez–Asso & A. López, 1 ♂, DNA voucher JAGWI–977; Parque Nacional Sierra de Bahoruco, camino de Aceitillar hasta el cruce de Las Abejas y Caseta 2, 19.vi.13, A. López & A. R. Pérez–Asso, en pinar, 6 ♂ 1 ♀, DNA vouchers JAGWI–990–995 (CZACC, MGCL, MNNSD, VGRC).

Other material (10 ♂, 2 ♀). Same data as holotype, unpinned.

Diagnosis (Figs. 34–37, 66, 67). FW length: 17.2–19.4 mm ♂, 20.5 mm ♀. *Calisto victori* **sp. nov.** needs to be compared with *C. raburni*, *C. mariposa* **sp. nov.**, and *C. azua* **sp. nov.** All four species are reddish, but having various tones, and have two white dots at UNHW: one enlarged dot between M2–M3 veins and another distinctly smaller between M3–CU1. From the three species *C. victori* **sp. nov.** differs by pear shape of its UNHW ocellus, and from the first two by having a reddish suffusion at UPHW around ocellus in males. Its reddish tone is very similar to that of syntopic *C. schwartzi* males that also possess pear shaped ocellus at UNHW but the latter has four white dots of similar size at UNHW instead of two as occurs in *C. victori* **sp. nov.**. The species can be also separated from *C. azua* and *C. raburni* by lacking of reddish color beyond the post discal line at UNFW in both sexes, only few specimens show the suffusion at the outer edge line whereas that color is more extended toward the area below the ocellus or even reaching the subterminal line. The species may be differentiated from all other members of the *lyceius* group by four unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 82). As illustrated. Uncus basal two thirds gradually tapering, apex abruptly tapered downwards. Aedeagus strongly arched with a short subapical broad tooth, transverse sclerotized process just before apex.

Female genitalia (Fig. 92). As illustrated. The shape of the anterior genital plate, approximately rounded but anteriorly dilated (top in ventral view) is diagnostic.

Distribution. The species is restricted to the Sierra de Bahoruco range where is present on both southern and northern slopes (Fig. 99).

Biology. The only known habitat type is pine forest (Fig. 104).

Etymology. This species is named on behalf of Victor González a nature lover and our main sponsor during this work.

Molecular characterization. The nucleotide composition were A=29.4, T=39.7, C= 16.4, and G=14.5, characterization values are showed in Table 3. Overall mean distance among the available sequences is 0.19%, differing from each other by 0 to 7 bp. The same distance values were obtained when specimens from the northern (n=7) and southern (n=7) slopes of Sierra de Bahoruco were compared. There are not substitutions that allow the recognition of the populations on both North and South slopes of the Sierra de Bahoruco range. The lowest distance of *C. victori* **sp. nov.** to any member of the *lyceius* group is 3.7% or 24 bp to *C. azua* (Table 4).

Remarks. *Calisto victori* **sp. nov.** specimens were originally included in the cluster named *Calisto* sp. 3 during the sequence analysis. Some specimens bear a tiny white dot at M1–M2 veins interspace. The species probably was overlooked due its resemblance with the syntopic *C. schwartzi* being the absence of two white dots, occasionally one, surely attributed to *C. schwartzi*'s variability. *Calisto schwartzi* and *C. victori* **sp. nov.** present slight genitalic differences and possess distinctive COI barcodes that serve for a clear separation.

Calisto lyceius Bates, 1935

Calisto lyceius Bates, 1935: 240; Michener, 1943: 6; Munroe, 1951: 224; Brown & Heineman, 1972: 51; Schwartz, 1989: 426; Smith *et al.*, 1994: 52; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202
Calisto lyceia: Gali, 1985: 2

Material examined. Holotype ♂, Dominican Republic, Saona Island, 21.i.1932, Armour Exp., M.C.Z. Type 21988 (MCZ). Photographs examined, available at: <http://insects.oeb.harvard.edu/mcz/index.htm>

Other material (11 ♂, 2 ♀). REPÚBLICA DOMINICANA: PROVINCIA LA ALTAGRACIA, Parque Nacional del Este, Boca de Yuma, 24.vii.12, A. López & A. R. Pérez–Asso, bosque seco, en costa rocosa con Espartillo (*Uniola*

virgata), 7 ♂ 2 ♀ (DNA vouchers JAGWI–858–862); same data as anterior except 10.vi.13, 4 ♂ (DNA vouchers JAGWI–1012–1014) (VGRC).

Diagnosis (Figs. 38–41, 68, 69). FW length: 15.8–16.6 mm ♂, 18.2–18.7 mm ♀. *Calisto lyceius* requires comparisons with *C. crypta*, *C. franciscoi* and *C. samana* **sp. nov.** All four species are characterized by their large reddish orange areas at UN of both wings and a row of four white dots at the post discal area at UNHW. From *C. crypta*, *C. lyceius* differs only by its smaller size whereas its males can be separated from *C. franciscoi* ones by their androconial patches which contrast with surrounding paler background. In the latter species the androconial patch is concealed by the darker surrounding background. There are no external differences between *C. lyceius* and *C. samana* males; however, their females are different since these of the latter lack the reddish orange at UNHW having a brown background. *Calisto lyceius* may be also differentiated from all other members of the *lyceius* group by six unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 83). As illustrated. Tegumen moderately rounded with a deep groove. Valvae with a smooth dorsal keel, broader just before apex. Adeagus caecum strongly curved to the left in dorsal view.

Female genitalia (Fig. 93). As illustrated. This and the following species share the possession of an elliptic anterior genital plate.

Distribution. *Calisto lyceius* was described from Saona Island, about 1 km off the southwestern coast of Hispaniola (Bates 1935). Half century later the species was found by Gali (1985) in a nearby second satellite island, Catalina, also very close to the south coast of the main island (Gali 1985). The species was found in 2002 at the Boca de Yuma, La Altagracia province by Sourakov who, on the same occasion, tried unsuccessfully to find it on Isla Saona and Isla Catalina (Sourakov, 2007) (Fig. 100).

Biology. Only known from *Acacia* scrubs and forests both coastal and inner areas (Schwartz 1989, Sourakov 2007, Sourakov & Zakharov 2011), where it flies in close association with *Uniola virgata* bunch grass (Fig. 101).

Molecular characterization. The nucleotide composition were A=29.0, T=40.7, C= 15.5, and G=14.7, characterization values are showed in Table 3. Overall mean distance of the 12 available COI sequences is 0.03% or 0 to 1 bp. The lowest divergence of *C. lyceius* to other member of the groups is 4.42% or 29 bp to *C. franciscoi* (Table 4).

Remarks. *Calisto lyceius* form a tight subgroup within the *lyceius* group together with *C. crypta*, *C. franciscoi* and *C. samana*. They have little morphological differentiation and share the possession large reddish orange areas at UN of wings, of a row of four white dots at the post discal area at UNHW, and very similar male and female genitalia.

The assignment of the main island population to *C. lyceius* needs to be confirmed since the species was described from specimens collected on Saona Island. Schwartz (1989) commented that identity of specimens found on Catalina Island also needed confirmation. However, there are no other works addressing this matter. Since no material was available to us for the molecular study, we followed Sourakov & Zakharov (2011) who assigned the Hispaniolan population (Boca de Yuma, La Altagracia) near both islands to *C. lyceius*.

***Calisto franciscoi* Gali, 1985**

Calisto franciscoi Gali, 1985: 4; Smith *et al.*, 1994: 52; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Calisto hendersoni Gali, 1985: 6; Sourakov 2000: 73; Lamas, 2004: 207

Material examined. Holotype ♂, Republica Dominicana: Prov. de Azua, Tabara Abajo, 5.iv.1984, A. Schwartz, AMNH. Photographs examined, available at http://www.butterfliesofamerica.com/L/calisto_franciscoi_types.htm.

Calisto hendersoni Gali, 1985. Holotype ♂, Republica Dominicana: Prov. de Independencia: 4 km E El Limón, 2.iv.1984 A. Schwartz, (AMNH). Photographs examined, available at http://butterfliesofamerica.com/L/calisto_hendersoni_types.htm.

Other material (25 ♂, 21 ♀). REPÚBLICA DOMINICANA: PROVINCIA PEDERNALES, Bahía de las Águilas, 11.viii.14, A. R. Pérez–Asso & A. López, en bosque seco con Espartillo (*Uniola virgata*), 5 ♂ 5 ♀ (DNA vouchers JAGWI–1022–1027); PROVINCIA INDEPENDENCIA, Duverge, El Limón, 13.vi.11, A. López & A. R. Pérez–Asso, en bosque seco con Espartillo, 1 ♂ 2 ♀ (DNA vouchers JAGWI–778–780); PROVINCIA AZUA, Hatillo, El Número, 23.vi.11, A. López & A. R. Pérez–Asso, en bosque seco con Espartillo (*Uniola virgata*), 5 ♂ 6 ♀ (DNA vouchers JAGWI–784–948); same data as anterior except 14.vi.13, 2 ♂ (DNA vouchers JAGWI–984, 985); same data as

anterior except 11.xi.13, 1 ♂ 2 ♀; same data as anterior except 24.viii.14, 8 ♂ 5 ♀; Playa Monte Rio, 19.xi.13, A. R. Pérez–Asso, en bosque seco con Espartillo, 3 ♂ 1 ♀. (VGRC).

Diagnosis (Figs. 42–45, 70, 71). FW length: 15.0–17.5 mm ♂, 16.6–18.8 mm ♀. *Calisto franciscoi* requires comparisons with *C. crypta*, *C. lyceius* and *C. samana* **sp. nov.** All four species are characterized by their large reddish orange areas at UN of both wings and a row of four white dots at the post discal area at UNHW. From the other three species, *C. franciscoi* differs by the darker two thirds of the UPFW of males which mask the androconial patch. In the others the androconial patch is distinctly darker than the basal two thirds of UPFW. Few other external differences are *C. franciscoi* smaller size when compared to *C. crypta*, and the possession of reddish orange color by females at UNHW which is lacking in *C. samana* ones. *Calisto franciscoi* may be also differentiated from all other members of the *lyceius* group by two fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 84). As illustrated. Tegumen flat. Uncus base rounded, expanded backward. Valvae with a smooth dorsal keel, a broad tooth pointing upward just before apex.

Female genitalia (Fig. 94). As illustrated.

Distribution. The species is known from several localities of southwestern Dominican Republic ranging from the west of the Peravia province to the border with Haiti (Fig. 100). After *C. pulchella*, *C. franciscoi* is the second most broadly distributed member of the *lyceius* group.

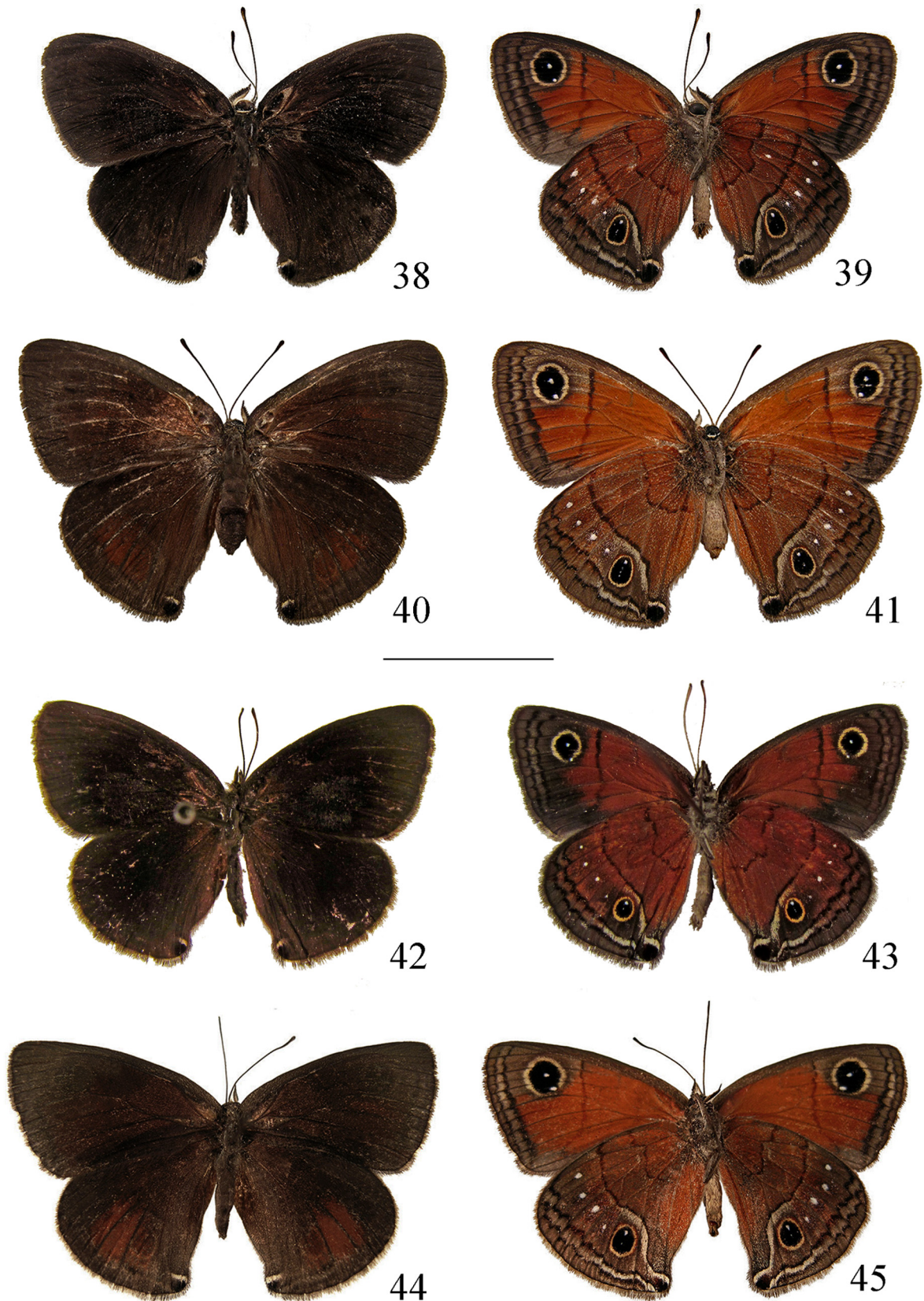
Biology. The species inhabits only *Acacia* scrubs and forest with *Uniola virgata* stands at the coast and low elevations up to 400 m (Gali 1985, Schwartz 1989) (Fig. 105). Gali (1985) and Schwartz (1989) also provide information on nectar sources and phenology.

Molecular characterization. The nucleotide composition were A=29.6, T=39.9, C= 16.2, and G =14.3, characterization values are showed in Table 3. We obtained 18 COI sequences from three localities: three from specimens captured nearby El Limón (type locality of *C. hendersoni*), nine from El Número, Azua province, and six specimens from Bahía de Las Aguilas, Pedernales province, being the latter two known localities for *C. franciscoi* (Schwartz 1989). Overall mean distance among all sequences is 0.66% or 4 bp. Variation within each locality is 0.1% (1 bp), 0.24% (2 bp), and 0.2% (1 bp) respectively. Overall mean distances of the sequences from the first locality respect to the second and third ones are 0.33% or 2 bp and 1.11% or 7 bp respectively whereas the latter two differ by 1.16% or 7 bp. With the available sequences, the Bahía de Las Aguilas population can be recognized from the remainder two by the following substitutions: 1 A (Bahía de Las Aguilas)/G (El Limón & El Número), 136 T/C, 217 G/A, 226 C/T, 557 T/C, and 640 T/C respectively. The fixed states 226 C of the Bahía de Las Aguilas population and 136C and 557C of the El Limón & El Número ones are unique among all members of the *lyceius* group. The lowest overall mean distance of *C. franciscoi* to other member of the *lyceius* group is 3.57% or 23 bp, to *C. crypta* (Table 4).

Remarks. The synonymy of *Calisto hendersoni* Gali under *C. franciscoi* Gali suggested by Sourakov (2000) and followed by Lamas (2004) is confirmed here. Sourakov (2000) compared the members of the *lyceius* group and referred them as “*C. franciscoi/hendersoni*” and found no differences between their male and female genitalia.

The extension of reddish orange at UNFW was the character employed by Gali (1985) to separate the two species: up to the basal subterminal line in *C. hendersoni* and only to the ocellus in *C. franciscoi*. The same feature was used by Schwartz (1989) and Hedges & Johnson (1994). However, it seems due to phenotypic variability of males. Our male specimens exhibit reddish areas varying in extension but in all the females available to us the reddish color reaches the basal subterminal line. Even the distribution points to the conspecific nature of the population at El Limón, the type and only known locality of *C. hendersoni*. This locality constitutes the northwestern limit of the *C. franciscoi* distribution, with other populations present 20 km or fewer both to the east and south from El Limón.

Based on the COI data, the Bahía de Las Aguilas population shows an incipient differentiation respect the two other sampled. As *C. franciscoi* inhabits low elevation, probably de Sierra de Bahoruco is acting as a barrier preventing gene flow between north and southern populations. However, since morphology didn't provide any additional support for a split we prefer wait until additional evidence become available to propose a different status for the Bahía de Las Aguilas population.



FIGURES 38–45. Adults of the *lyceius* species group of *Calisto* (cont.). 38—*C. lyceius* male upperside, Boca de Yuma, La Altagracia. 39—*C. lyceius* male underside, same data. 40—*C. lyceius* female upperside, same data. 41—*C. lyceius* female underside, same data. 42—*C. franciscoi* male upperside, El Número, Hatillo, Azua. 43—*C. franciscoi* male underside, same data. 44—*C. franciscoi* female underside, same data. 45—*C. franciscoi* female underside, same data.

Calisto crypta Gali, 1985

Calisto crypta Gali, 1985: 3; Smith *et al.*, 1994: 52; Lamas, 2004: 207; Sourakov & Zakharov, 2011: 202

Material examined. Holotype ♂, Republica Dominicana: Provincia de Monte Cristi, near Monte Cristi, 13.iii.1931, A.L. Stillman. AMNH, photographs examined, available at http://www.butterfliesofamerica.com/L/calisto_crypta_types.htm.

Other material (8 ♂, 3 ♀). REPÚBLICA DOMINICANA: PROVINCIA MONTE CRISTI, Monte Cristi, Salinas de Jicaquito, 10.vii.12, A. R. Pérez–Asso & A. López, en bosque seco con Espartillo (*Uniola virgata*), 8 ♂ 1 ♀ (DNA vouchers JAGWI–863–867); same data as anterior except 16.xi.13, 2 ♀. (VGRC)

Diagnosis (Figs. 46–49, 72). FW length: 16.5–20.2 mm ♂, 19.2–20.8 mm ♀. *Calisto crypta* requires comparisons with *C. franciscoi*, *C. lyceius* and *C. samana* **sp. nov.** All four species are characterized by their large reddish orange areas at UN of both wings and a row of four white dots at the post discal area at UNHW. From *C. lyceius*, *C. crypta* differs only by its larger size whereas its males can be separated from *C. franciscoi* ones by their androconial patches which contrast with surrounding paler background. In the latter species the androconial patch is concealed by the darker surrounding background. There are no external differences between *C. crypta* and *C. samana* males; however, their females are different since these of the latter lack the reddish at UNHW having a brown background. *Calisto crypta* may be also differentiated from all other members of the *lyceius* group by one unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 85). As illustrated. Tegumen moderately flat. Valvae with a smooth dorsal keel.

Female genitalia (Fig. 95). As illustrated.

Distribution. *Calisto crypta* is restricted to a relatively small area around Monte Cristi, at the northwestern portion of the Dominican Republic (Gali 1985, Schwartz 1989) (Fig. 100). Most known localities are near the coast; however, some specimens have been found southward up to 40 km, in the foothills of the northwestern portion of the Cordillera Central (Schwartz 1989).

Biology. Like the previous species, *C. crypta* inhabits only *Acacia* scrubs and forests with *Uniola virgata* stands from the coast to low elevations up to 366 m (Michener 1943, Schwartz 1989) (Fig. 105). Gali (1985) and Schwartz (1989) informed feeding on *Croton linearis* (*Croton cascarilla* (L.) L.) as well collection months. Sourakov (2000) noted the very close association of *C. crypta* with *Uniola virgata*. He reared the species to the third larval instar using *Stenotaphrum secundatum*, St. Augustine grass, as substitute food. The head capsule of the first instar is unique by having a characteristic frontal “x” spot and other dark elements over a beige background (Sourakov 2000). All other known first instars of *Calisto* possess unpatterned unicolor heads (orange, brown or black).

Molecular characterization. The nucleotide composition were A = 29.8, T = 39.2, C = 16.7, and G = 14.3, characterization values are showed in Table 3. Overall mean distance of the 10 available sequences is 0.16% or 1 bp. The lowest overall mean distance to any member of the *lyceius* group is 2.75% or 17 bp to *C. samana* **sp. nov.** (Table 4).

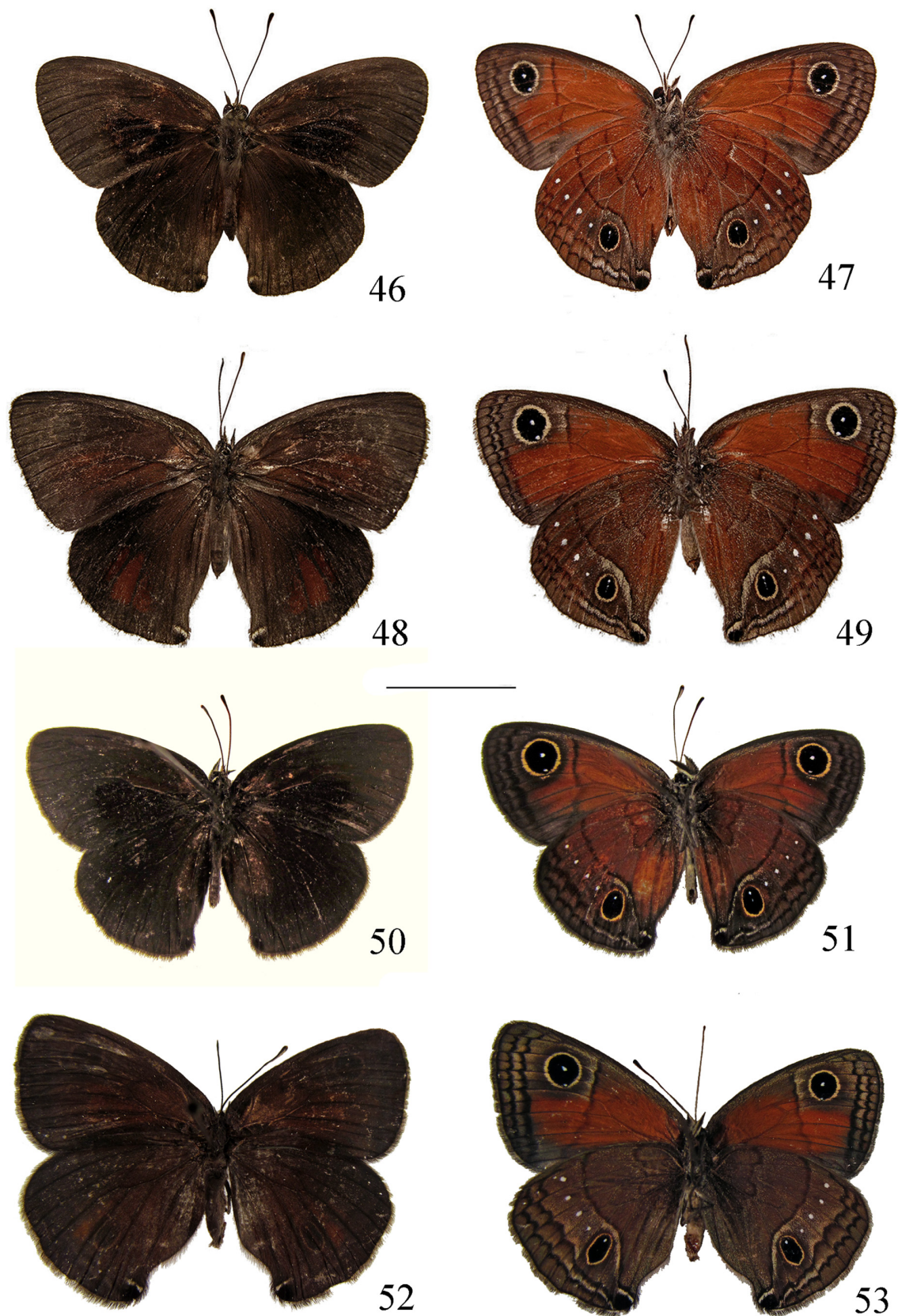
Calisto samana Pérez–Asso, Núñez & Genaro **sp. nov.**

Calisto sp. Gali, 1985: 10; Schwartz, 1989: 428

Holotype: ♂, REPÚBLICA DOMINICANA: PROVINCIA SAMANÁ, Cueva del Agua, 14.viii.14, A. R. Pérez–Asso & A. López, en costa rocosa con Espartillo (*Uniola virgata*), DNA voucher JAGWI–1020 (VGRC).

Paratypes: 24 ♂, 6 ♀. Same data as holotype, 14 ♂ 3 ♀, DNA voucher JAGWI–1021; same data as holotype except 6.xii.07, 2 ♂ 2 ♀, DNA vouchers JAGWI–971–974; same data as holotype except 13.xi.13, 8 ♂ 1 ♀ (CZACC, MGCL, MNNSD, VGRC).

Diagnosis (Figs. 50–53, 73, 74). FW length: 15.9–17.4 mm ♂, 16.1–19.2 mm ♀. *Calisto samana* **sp. nov.** requires comparisons with *C. franciscoi*, *C. lyceius* and *C. crypta*. All four species are characterized by their large reddish orange areas at UN of both wings and a row of four white dots at the post discal area at UNHW. From *C. franciscoi* whose males have the androconial patch concealed by the darker surrounding background at UPFW, *C. samana* males differ by the distinctly paler brown background. There are no external differences among *C. samana*



FIGURES 46–53. Adults of the *lyceius* species group of *Calisto* (cont.). 46—*C. crypta* male upperside, Salinas de Jicaquito, Monte Cristi. 47—*C. crypta* male underside, same data. 48—*C. crypta* female upperside, same data. 49—*C. crypta* female underside, same data. 50—*C. samana* sp. nov. HOLOTYPE male upperside, Cueva del Agua, Samaná. 51—*C. samana* sp. nov. HOLOTYPE male underside, same data. 52—*C. samana* sp. nov. PARATYPE female upperside, same data. 53—*C. samana* sp. nov. PARATYPE female underside, same data.



FIGURES 54–57. Live adults of the *lyceius* species group of *Calisto*. 54—*C. pulchella* male, La Ciénaga, Cordillera Central. 55—*C. pulchella* female, same data. 56—*C. raburni* male, camino de Caseta 1 a Puerto Escondido, Sierra de Bahoruco. 57—*C. raburni* female, same data.

males and those of *C. crypta* and *C. lyceius*; however, their females differ in the browner UNHW of the former. *Calisto samana* may be also differentiated from all other members of the *lyceius* group by three unique fixed states of its DNA barcode (Table 2).

Male genitalia (Fig. 86). As illustrated. Similar to that of *C. crypta* but tegumen with a broad shallow groove and heavier valvae.

Female genitalia (Fig. 96). As illustrated.

Distribution. All our specimens are from a single locality, Cueva del Agua, Samaná peninsula, Samaná province (Fig. 100). Gali (1985) and Schwartz (1989) informed on two specimens of the *lyceius* group deposited at MNHNSD from Puerto Francés Viejo, between Samaná and Las Galeras (see comments at Remarks).

Biology. The only available information is on habitat, rocky coastal vegetation with *Cocos* grove and *Uniola virgata* stands (Fig. 106).

Etymology. The specific name of this species refers to both the Dominican Republic province and peninsula where its population was located: Samaná.

Molecular characterization. The nucleotide composition were A = 29.1, T = 39.3, C = 16.7, and G = 15.0, characterization values are showed in Table 3. Overall mean distance of the 6 available COI sequences is 0.11% or 1 bp. The lowest distance to any member of the *lyceius* group is 2.83% or 17 bp, to *C. crypta* (Table 4).

Remarks. *Calisto samana* **sp. nov.** specimens were originally included in the cluster named *Calisto* sp. 2 during the sequence analysis. The specimens of the *lyceius* group from Samaná mentioned by Gali (1985) and Schwartz (1989) almost surely represents this species since there are no other members of the group known from the Samaná peninsula. Schwartz and Raburn found the area “too mesic” with no “appropriate” habitat for *lyceius* group members; however, we found the species there in the same habitat they describe: coastal *Cocos* grove. The presence of the population in isolation and the fading of reddish suffusion in the females together with distinctive COI barcodes allowed the recognition of this new taxon.

Key to species of the *lyceius* group

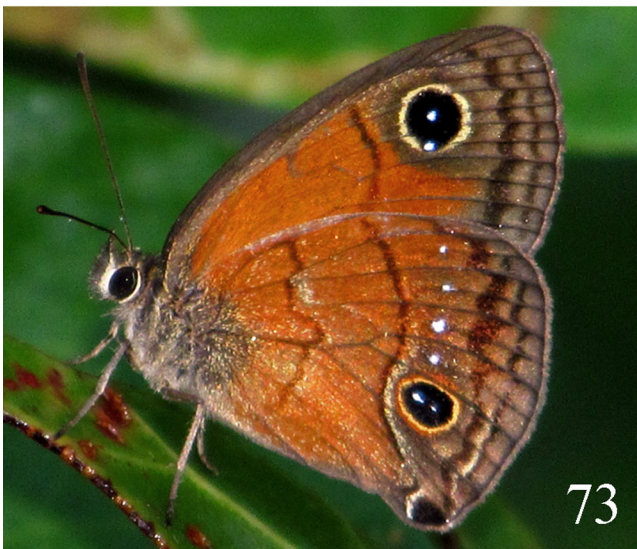
1. Males with a nearly round greyish androconial patch on distal end of discal cell; females with suffusion of reddish scaling over brown background on both wings; restricted to Jamaica (Figs. 4, 7) *C. zangis*
- Males with an ellipsoidal dark brown androconial patch on area below discal cell; females with suffusion of reddish scaling over brown background only on hindwing, when present; restricted to Hispaniola. 2
2. Ventral surface of hindwing with a postdiscal row of white three or less dots above the ocellus 3
- Ventral surface of hindwing with a postdiscal row of four white dots above the ocellus 8
3. Ventral surface of forewing uniform brown in males and brown with slight reddish suffusion at discal cell in females; ventral surface of hindwing with a distinct bright orange patch in males; FW length: 23.5–26.3 mm ♂, 26.2–28.9 mm ♀; widespread distribution (Figs. 10–13, 54) *C. pulchella*
- UN of both wings brown with reddish suffusion in both sexes although its extent varies at each taxon; FW length smaller in both sexes; restricted distribution 4
4. UNHW with white dots in the post discal areas at interspaces between M1–M2 and M2–M3 veins, rarely with a third tiny dot at M3–Cu1; usually with a second smaller ocellus in the Cu2–2A interspace at UHHW; populations above 1800 m (Figs. 23, 25, 60, 61) *Calisto tasajera*
- UNHW with white dots in the post discal areas at interspaces between M2–M3 and M3–Cu1 veins, rarely with a third tiny dot at M1–M2; without a second ocellus at UNHW; populations below 1800 m 5
5. UNHW distinctly browner only with slight trace of orangish near the anal margin; post discal at UN of both wings and UNHW subterminal lines distinctly edged with pale yellow scaling (more evident in females); males with reddish suffusion at UPHW around ocellus; restricted to areas nearby El Número (Azua province) (Figs. 30, 31, 33, 64, 65) *Calisto azua* **sp. nov.**
- UNHW no distinctly browner, reddish or orangish areas more extended in both sexes; post discal at UN of both wings and UNHW subterminal lines without pale yellow scaling edge or only with a trace of it; males with or without reddish suffusion at UPHW around ocellus; no distributed at areas nearby El Número (Azua province) 6
6. Males with area anterior to androconial patch paler than patch; UNHW ocellus very elongated, thin and ovoid, with the distal end acute; UNFW with the reddish areas reaching at least the area below ocellus (Figs. 15, 17, 56, 57) *Calisto raburni*
- Males with area anterior to androconial patch as dark as patch; UNHW ocellus broad, ovoid or pear shaped, with the distal end rounded or only slightly acute; UNFW with the reddish areas only to post discal line or surpassing it slightly but not reaching the area below ocellus 7
7. UN of wings reddish areas distinctly darker with a rusty tone in males; UNHW post discal and subterminal lines edged by pale yellow scaling (Figs. 35, 37, 66, 67) *Calisto victori* **sp. nov.**
- UN of wings reddish areas not distinctly darker with an orangish tone in males; UNHW post discal and subterminal lines not edged by pale yellow scaling (Figs. 19, 21, 58, 59) *Calisto mariposa* **sp. nov.**
8. UN of wings rusty red in males; white dot at M2–M3 outside the row, basally displaced; UNHW ocellus pear shaped; above 1000 m (Figs. 27, 29, 62, 63) *Calisto schwartzi*
- UN of wings orange red; white dot at M2–M3 in the row, not basally displaced; UNHW ocellus ovoid; below 600 m 9
9. Basal two thirds of UNFW as black as androconial patch (males); distributed in southwestern Dominican Republic (Peravia, Azua, Bahoruco, Barahona, Independencia, and Pedernales provinces) (Figs. 38, 101) *Calisto franciscoi*
- Basal two thirds of UNFW paler than androconial patch (males); not distributed in southwestern Dominican Republic 10
10. Females lacking almost all trace of orange reddish at UNHW, background distinctive pale brown; distributed in the Samana peninsula (Figs. 53, 74, 101) *Calisto samana* **sp. nov.**
- Females with slight orange reddish tint at UNHW, background pale brown orangish; not distributed in the Samana peninsula 11
11. FW length: 15.8–16.6 mm ♂, 18.2–18.7 mm ♀; distributed in southeastern Dominican Republic, Saona and Catalina Islands, and around Boca de Yuma, La Altagracia province (Fig. 101) *Calisto lyceius*
- FW length: 16.5–20.2 mm ♂, 19.2–20.8 mm ♀; distributed in northwestern Dominican Republic, mostly at the Monte Cristi province (Fig. 101) *Calisto crypta*



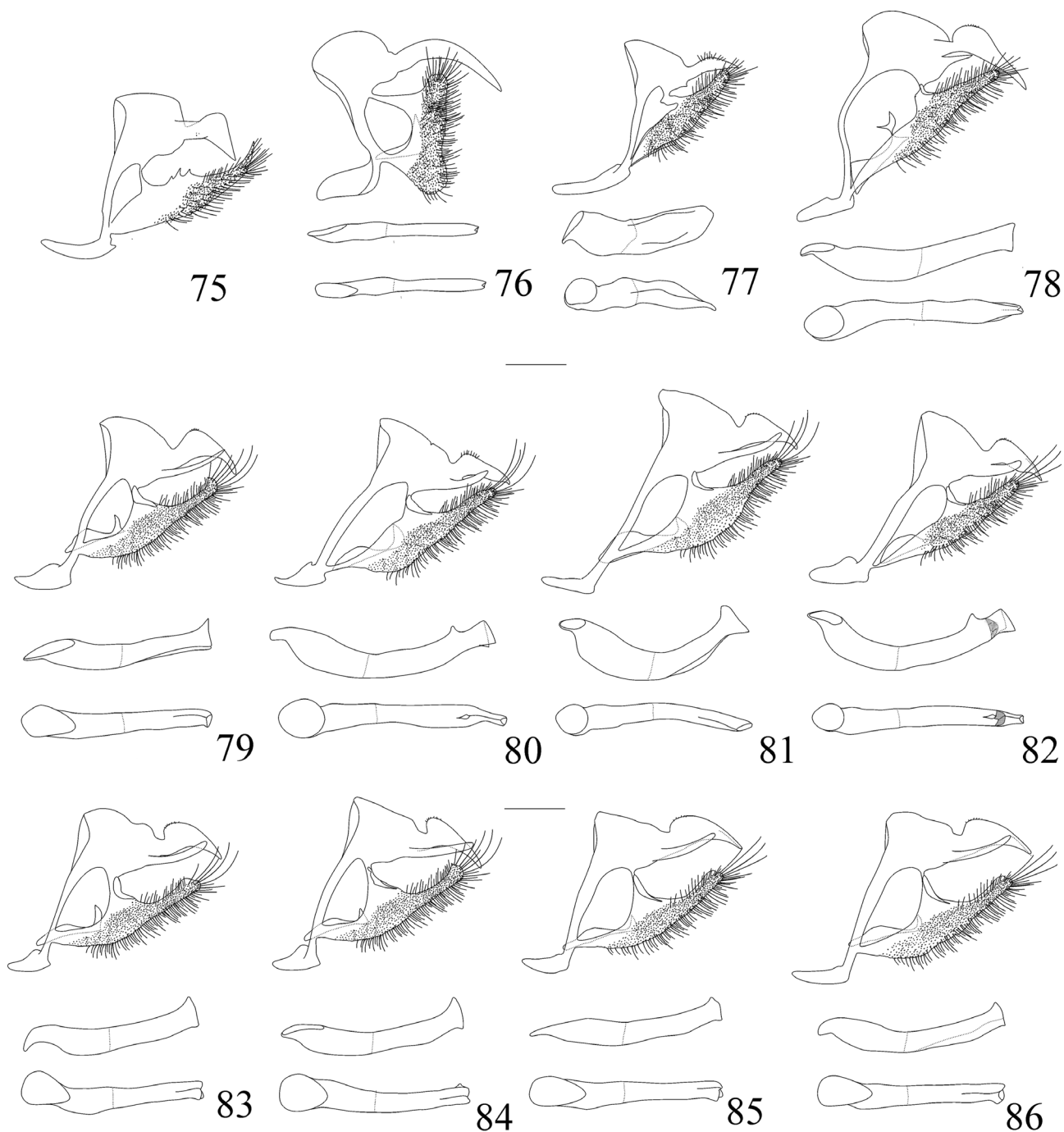
FIGURES 58–63. Live adults of the *lyceius* species group of *Calisto*. 58—*C. mariposa* sp. nov. male, Sierra Prieta, Sierra de Yamasá. 59—*C. mariposa* sp. nov. female, same data. 60—*C. tasajera* male, Valle de Bao, Cordillera Central. 61—*C. tasajera* female, same data. 62—*C. schwartzi* male, camino desde Caseta 2 hasta Caseta 1, Sierra de Bahoruco. 63—*C. schwartzi* female, same data.



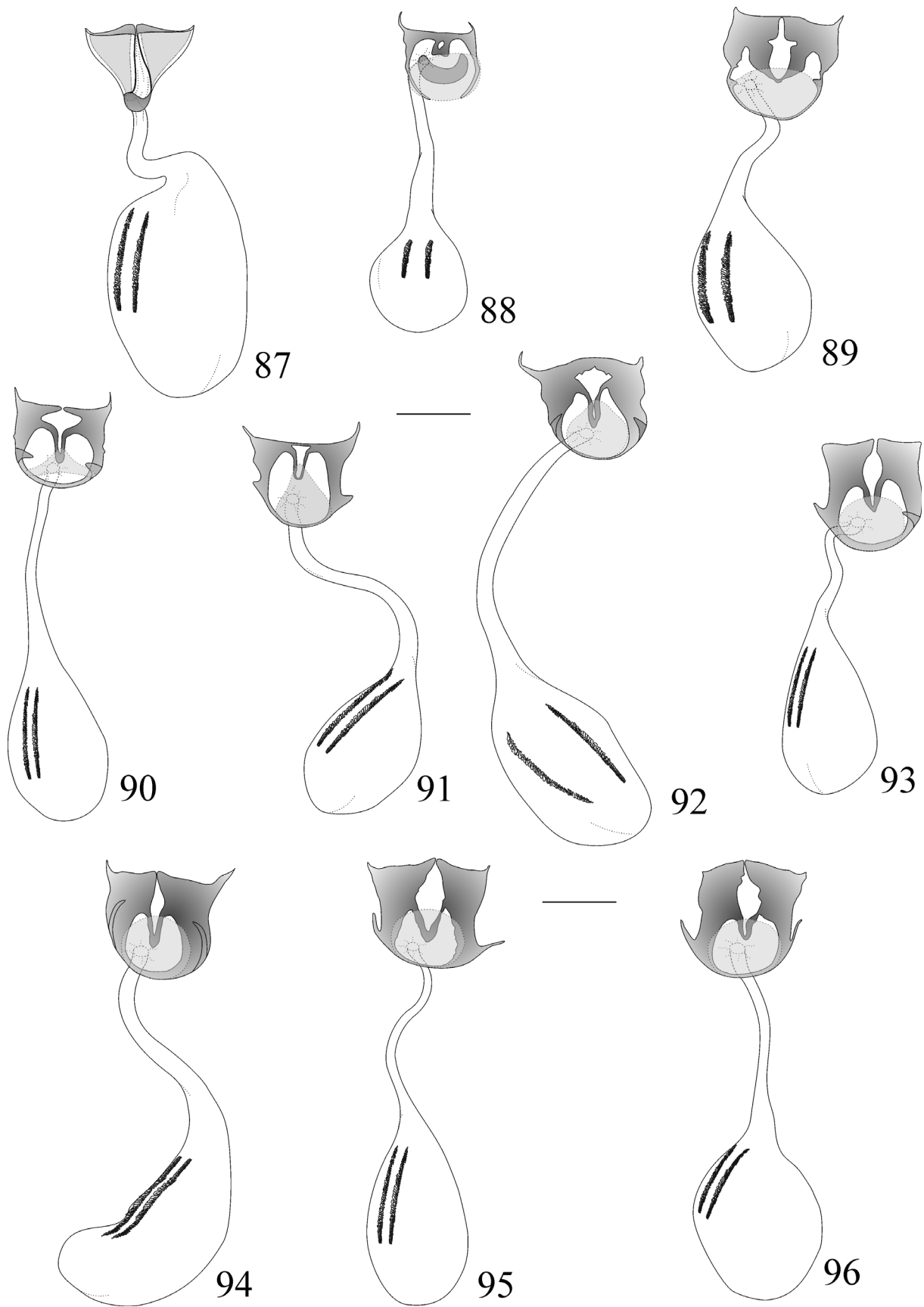
FIGURES 64–69. Live adults of the *lyceius* species group of *Calisto*. 64—*C. azua* sp. nov. male, El Número, Hatillo, Azua. 65—*C. auza* sp. nov. female, same data. 66—*C. victori* sp. nov. male, camino desde Caseta 2 hasta Caseta 1, Sierra de Bahoruco. 67—*C. victori* sp. nov. female, same data. 68—*C. lyceius* male, Boca de Yuma, La Altagracia. 69—*C. lyceius* female, same data.



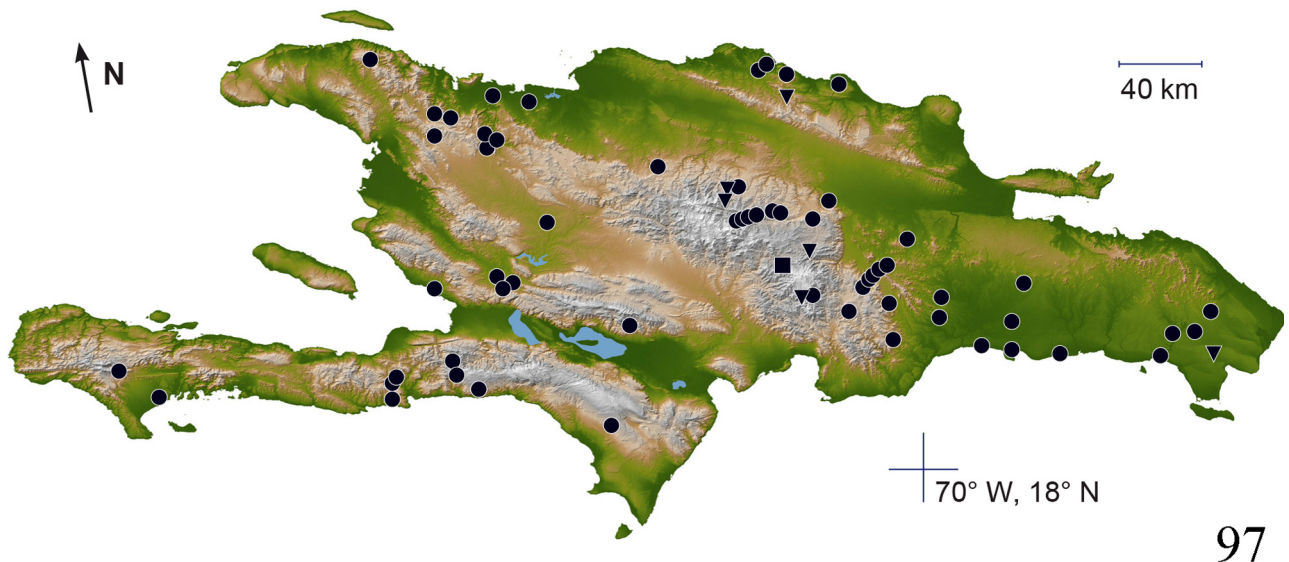
FIGURES 70–74. Live adults of the *lyceius* species group of *Calisto*. 70—*C. franciscoi* male, Playa Monte Rio, Azua. 71—*C. franciscoi* female, Laguna El Limón, Sierra de Bahoruco. 72—*C. crypta* female, Salinas de Jicaquito, Monte Cristi. 73—*C. samana* sp. nov. male, Cueva del Agua, Samaná. 74—*C. samana* sp. nov. female, same data.



FIGURES 75–86. Male genitalia of the *lyceius* species group of *Calisto* (top: main body in lateral view, middle aedeagus in lateral view, aedeagus in dorsal view). 75—*C. zangis*, modified from Bates (1935). 76—*C. pulchella*. 77—*C. raburni*. 78—*C. mariposa*. 79—*C. tasajera*. 80—*C. schwartzi*. 81—*C. azua* **sp. nov.** 82—*C. victori* **sp. nov.** 83—*C. lyceius*. 84—*C. franciscoi*. 85—*C. crypta*. 86—*C. samana* **sp. nov.** Scale bar 0.5 mm.



FIGURES 87–96. Female genitalia of the *lyceius* species group of *Calisto* (ventral view). 87—*C. pulchella*. 88—*C. raburni*. 89—*C. mariposa* **sp. nov.** 90—*C. tasajera*. 91—*C. schwartzi*. 92—*C. victori* **sp. nov.** 93—*C. lyceius*. 94—*C. franciscoi*. 95—*C. crypta*. 96—*C. samana* **sp. nov.** Scale bar 1 mm.



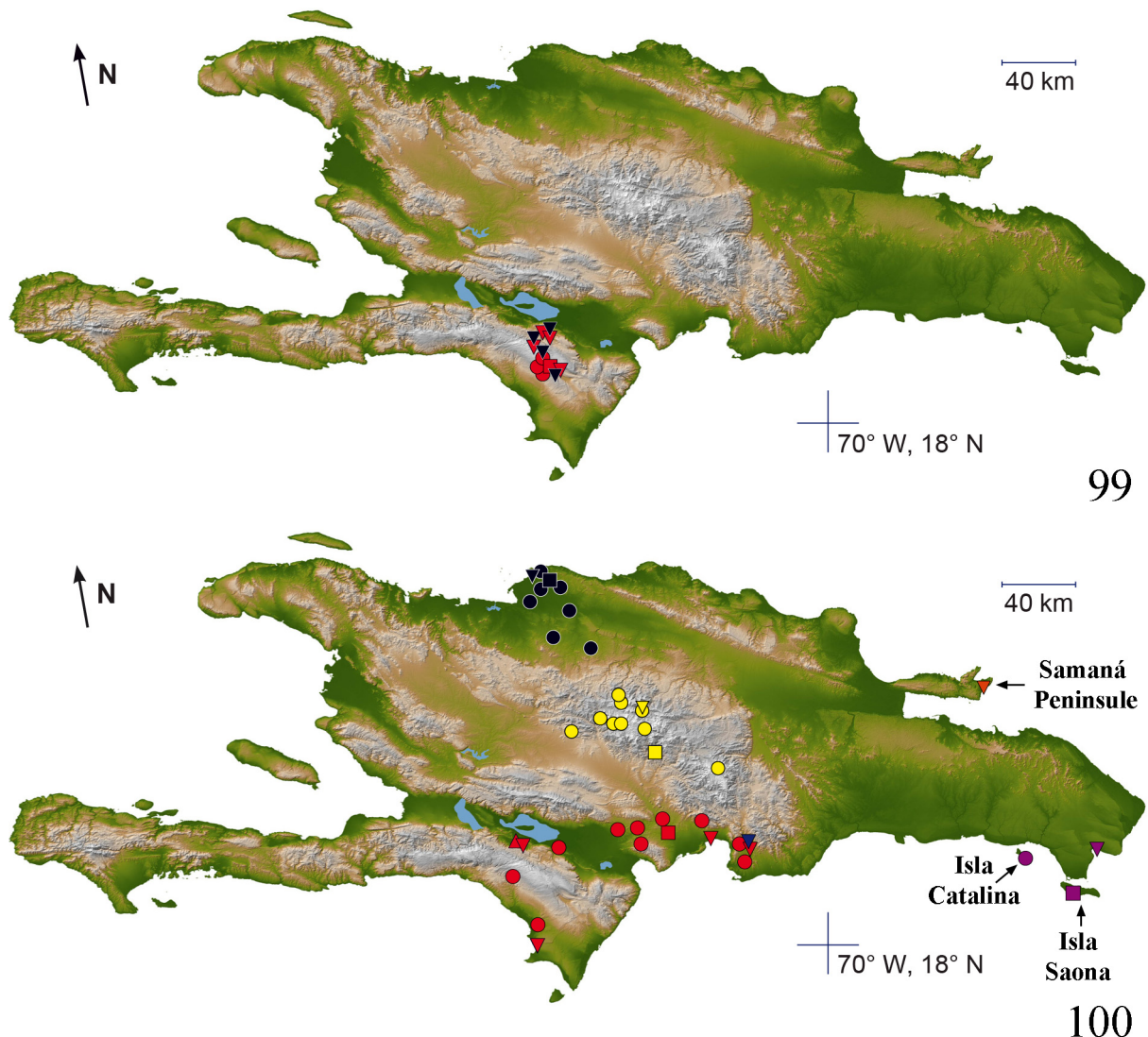
FIGURES 97–98. Geographical distribution of the *lyceius* species group of *Calisto* (triangles represent localities from where DNA barcodes were obtained; squares represent the type locality for species previously described). 97—*C. pulchella*. 98—*C. raburni* (red symbols) and *C. mariposa* **sp. nov.** (black symbols).

Checklist of *lyceius* group species

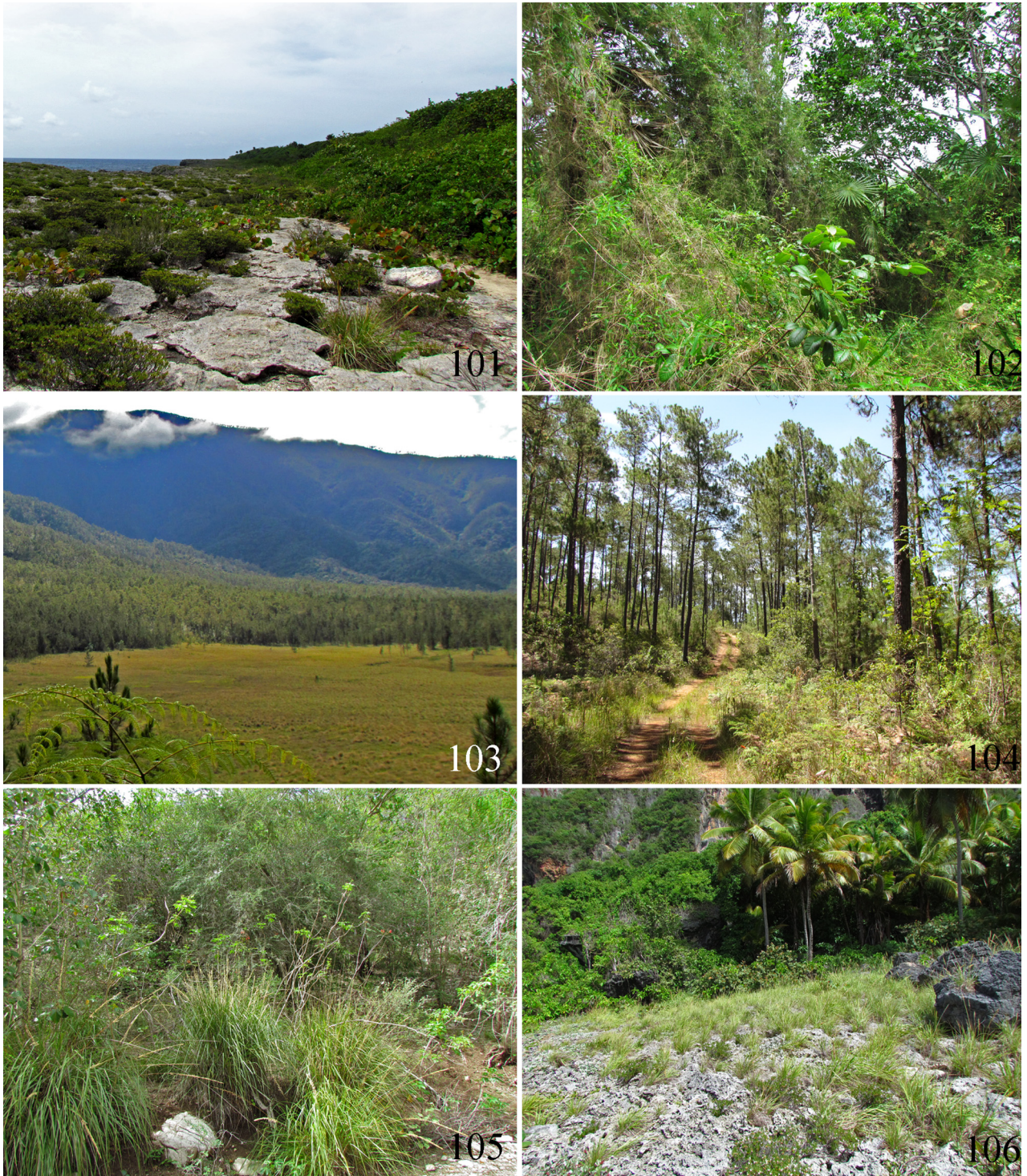
- Calisto zangis* (Fabricius, 1775)
- Calisto pulchella* Lathy, 1899
- Calisto pulchella pulchella*: Munroe, 1951
- Calisto pulchella darlingtoni* Bates, 1939, **syn. nov.**
- Calisto raburni* Gali, 1985
- Calisto mariposa* Pérez–Asso, Núñez & Genaro **sp. nov.**
- Calisto tasajera* Gonzalez, Schwartz & Wetherbee, 1991
- Calisto schwartzi* Gali, 1985
- Calisto azua* Pérez–Asso, Núñez & Genaro **sp. nov.**
- Calisto victori* Pérez–Asso, Núñez & Genaro **sp. nov.**
- Calisto lyceius* Bates, 1935
- Calisto franciscoi* Gali, 1985

Phylogenetic relationships among the members of the *lyceius* species group. The phylogenetic reconstruction recovered a monophyletic *lyceius* group with strong support (PP/BS =1/73) and the inclusion of all 12 species (Fig. 107). Both BI and ML methods resulted in the same topology. The *lyceius* group was divided in a clade including *zangis-raburni-pulchella* weakly supported, 0.59/51, with *C. raburni* and *C. pulchella* positioned as sister species, 0.65/68 (Fig. 107)

All other *lyceius* group species were included in a larger clade, 0.99/78 where *Calisto mariposa* **sp. nov.** (= *Calisto* sp. 1) was positioned as the sister of the remaining species, 1/94 (Fig. 107). The clade is divided in two clades each containing four species. In the first, *Calisto tasajera* is placed as sister, 0.90/72, of a more inclusive moderately or strongly supported clade depending the method, 0.91/95, where *C. schwartzi* is sister of a pair of species newly described in present paper: *C. azua* **sp. nov.** (= *Calisto* sp. 4) and *C. victori* **sp. nov.** (= *Calisto* sp. 3), 1/96 (Fig. 107). The second clade, 1/99, was splitted in two clades containing each a pair of sister species, *C. lyceius*–*C. franciscoi*, recovered with weak support 0.39/28, and the strongly supported *C. crypta*–*C. samana* **sp. nov.** (= *Calisto* sp. 2), 0.99/99 (Fig. 107).



FIGURES 99–100. Geographical distribution of the *lyceius* species group of *Calisto* (triangles represent localities from where DNA barcodes were obtained; squares represent the type locality for species previously described). 99—*C. schwartzi* (red symbols) and *C. victori* **sp. nov.** (black symbols). 100—*C. tasajera* (yellow symbols), *C. azua* **sp. nov.** (blue symbol), *C. lyceius* (purple symbols), *C. franciscoi* (red symbols, inverted triangle type locality of *C. hendersoni*), *C. crypta* (black symbols), and *C. samana* **sp. nov.** (orange symbol).



FIGURES 101–106. Habitats of the *lyceius* species group of *Calisto*. 101- Rocky coastal vegetation at Boca de Yuma, La Altagracia province: *C. pulchella* and *C. lyceius*. 102—Xeric forest with climbing grass at Sierra Prieta, Sierra de Yamasá: *C. raburni* and *C. mariposa* **sp. nov.** 103—Flooded plains and pine forest at Valle de Bao, Cordillera Central: *C. tasajera*. 104—Pine forest at the road between Caseta 1 and Caseta 2, Sierra de Bahoruco: *C. schwartzi* and *C. victori* **sp. nov.** 105—Xeric forest with *Uniola virgata* stands at El Número, Hatillo, Azua province: *C. azua* **sp. nov.** and *C. franciscoi*. 106—Rocky coastal vegetation with *Cocos* grove and *Uniola virgata* stands at Cueva del Agua, Samaná peninsula: *C. samana* **sp. nov.**

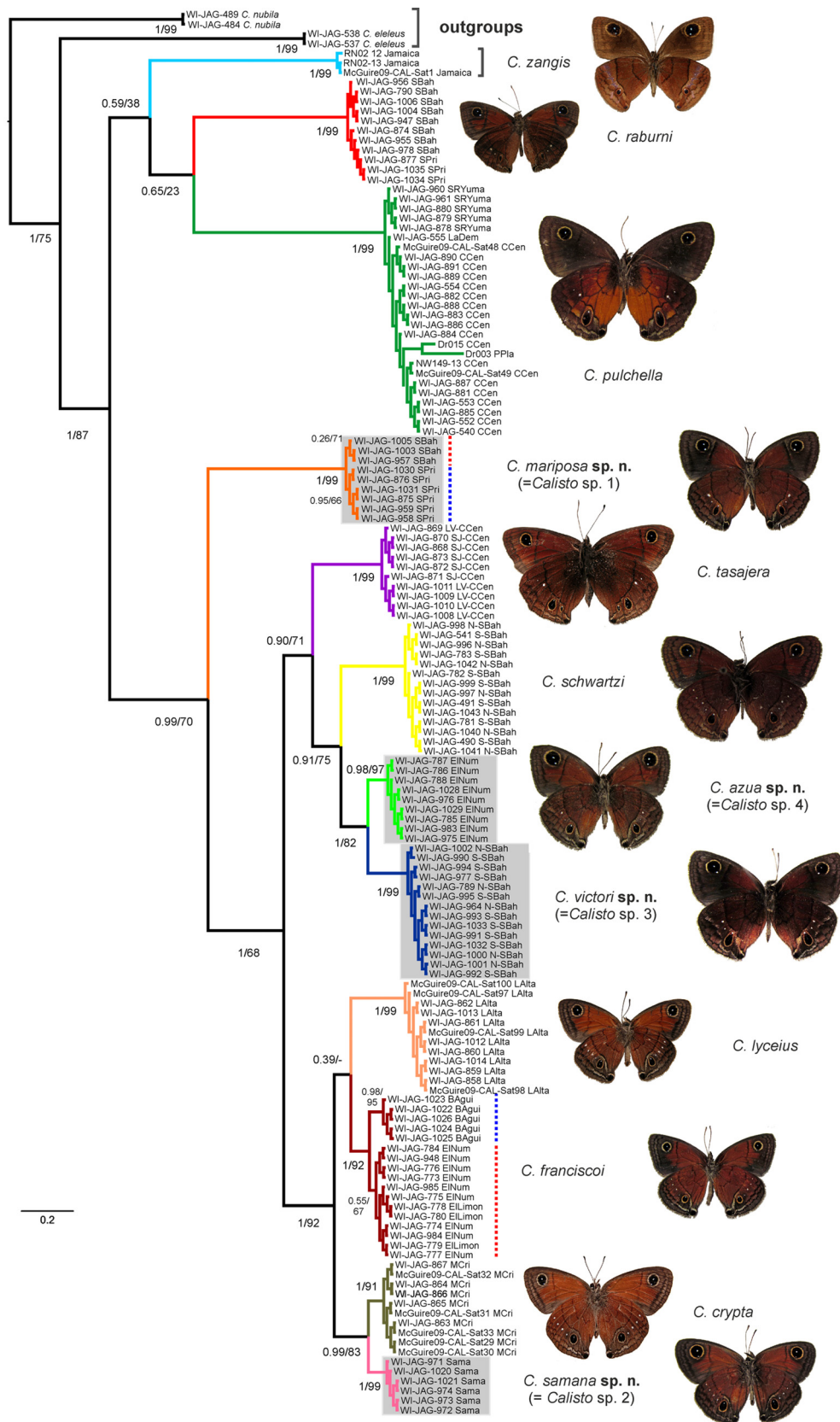


FIGURE 107. Phylogenetic tree obtained using the available COI barcode sequences of the *lyceius* species group of *Calisto*. The tree shown is 50% majority-rule consensus tree inferred from Bayesian analysis. Numbers at the nodes indicate posterior probabilities and bootstrap values (PP/BS). Branch colors identify species and pale gray boxes indicate the four new species described in present article. Topologies differences by the two reconstruction methods employed in the bottom clade are detailed in the text.

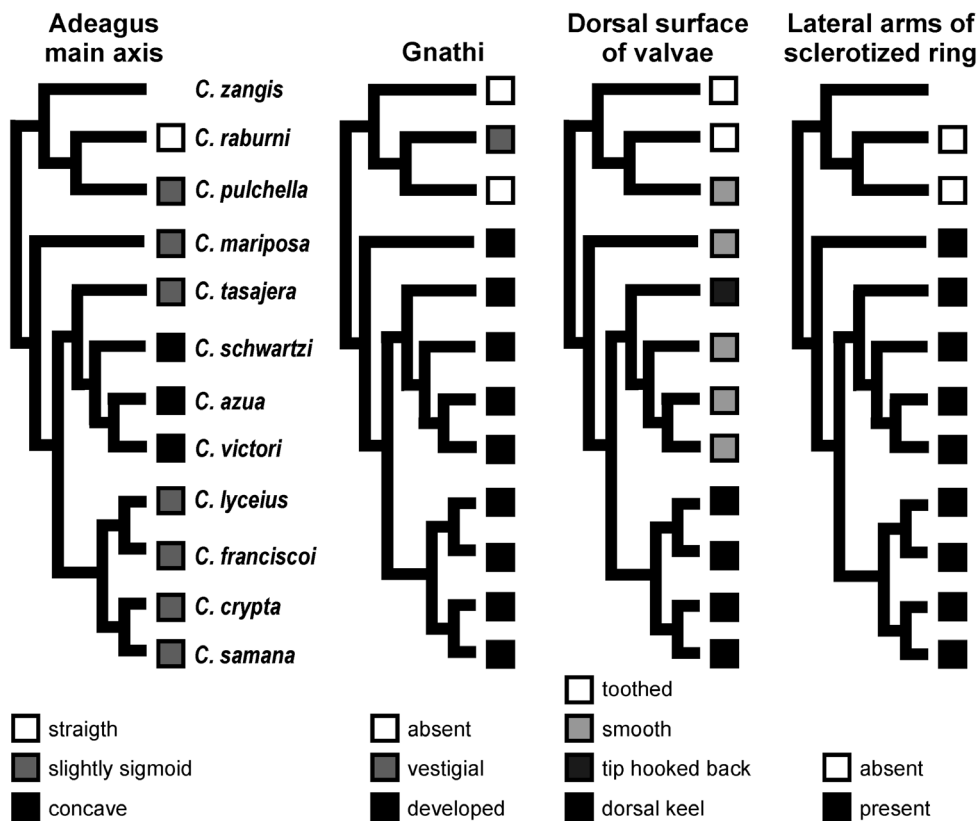
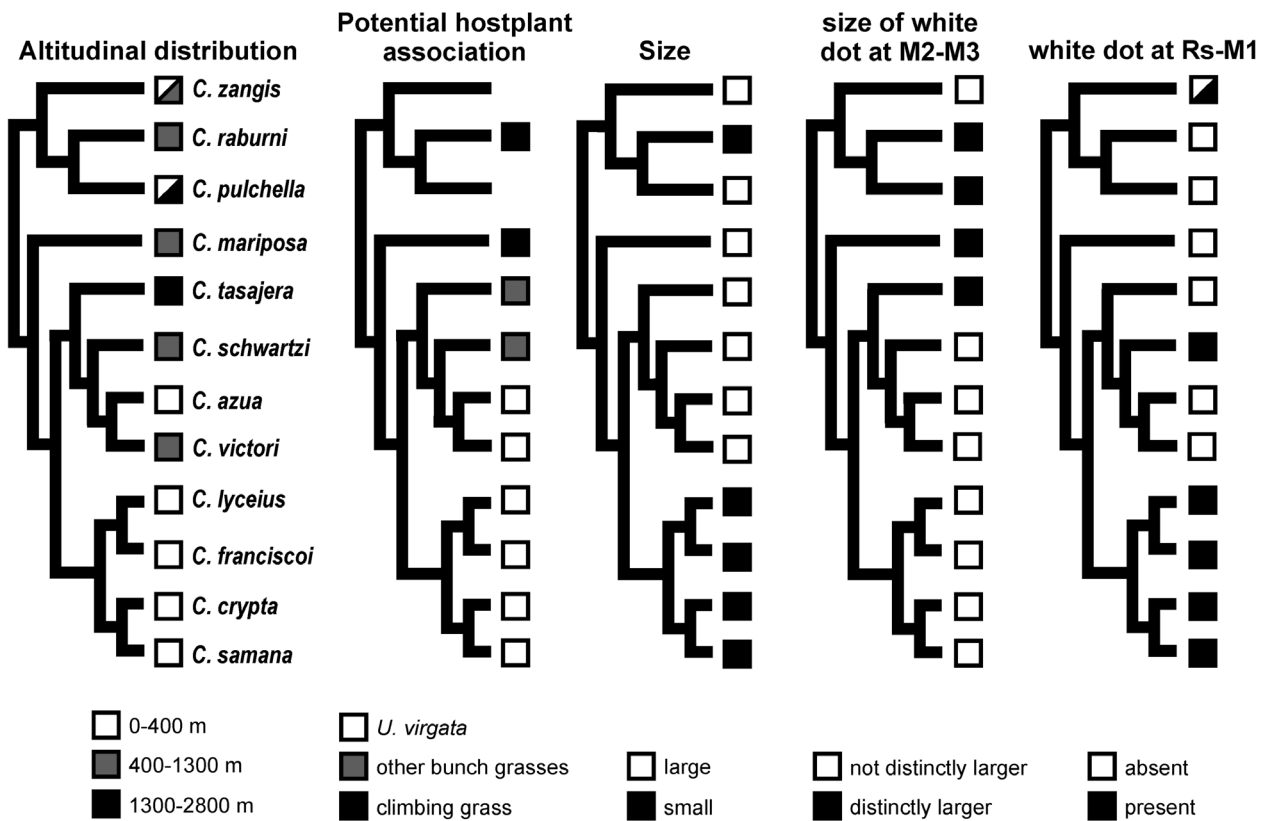


FIGURE 108. Ecological and morphological characters of the *lyceius* species group of *Calisto* mapped onto the ingroup tree simplified from figure 107.

TABLE 2. (continued) .

Species (no. sequences)/ Sites	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	TOTAL	
<i>Calisto crypta</i> (n=10)	T	T	A	T	T	A	A	T	T	T	A	T	A	G	A	T	A	C	T	A	C	T	T	T	T	A	T	A	T	T	1
<i>Calistofrancisco</i> (n=18)	T	T	A	T	T	A	A	T	T	T	A	A	G	A	T	A	C	T	A	T	C	T	T	T	T	A	T	A	T	T	2
<i>Calistolyceius</i> (n=12)	T	C	A	T	C	T	A	A	T	T	T	T	T	T	G	A	T	T	C	T	A	T	T	T	T	T	A	C	T	T	6
<i>Calisto pulchella</i> (n=27)	A	T	A	T	T	C	T	T	T	C	T	A	G	A	A	A	A	A	A	C	A	A	T	T	T	T	T	T	C	T	14
<i>Calistoraburni</i> (n=11)	C	T	A	T	A	C	A	A	C	A	C	T	T	A	G	A	A	T	T	T	T	C	T	C	T	T	C	T	T	T	13
<i>Calisto zangis</i> (n=3)	C	T	A	T	T	A	A	T	T	T	T	A	A	A	G	A	A	T	T	T	C	T	C	T	T	C	T	A	T	T	5
<i>Calisosehwartzi</i> (n=14)	C	T	A	T	T	A	A	T	T	T	T	A	A	G	A	T	A	T	A	T	T	A	T	C	T	A	A	T	T	T	5
<i>Calisto</i> sp. 3 (n=14) <i>C. victori</i> sp. nov.	C	T	G	T	T	A	A	T	T	T	T	A	G	A	A	A	T	A	T	T	T	A	T	T	T	T	A	T	T	T	4
<i>Calisto</i> sp. 4 (n=9) <i>C. azua</i> sp. nov.	C	T	A	T	T	A	A	T	T	T	T	A	G	A	G	A	A	T	A	T	T	A	T	T	T	A	T	A	T	T	2
<i>Calisto</i> sp. 2 (n=6) <i>C. samana</i> sp. nov.	T	T	A	T	T	A	A	T	T	T	T	A	G	A	T	A	C	T	A	T	C	T	T	T	T	A	T	A	T	T	3
<i>Calisto</i> sp. 1 (n=9) <i>Calisto mariposa</i> sp. nov.	T	T	A	C	A	T	A	A	T	T	G	A	G	A	A	A	A	A	T	T	A	T	A	T	T	T	T	T	T	T	5
<i>Calistotaxajera</i> (n=10)	C	T	A	T	T	A	A	T	T	T	T	A	G	A	C	A	T	T	A	T	T	C	T	C	T	T	A	T	T	C	8

TABLE 3. Summary of molecular diversity indices of COI barcode sequences of species within the *lyceius* group of *Calisto* as calculated with DnaSP 5.10.01. SD= standard deviation, SE= standard error.

Species	Number of sequences	Number of haplotypes, h	Overall mean distance, d (S.E.)	Haplotype diversity, Hd (SD)	Nucleotide diversity, π
<i>Calisto zangis</i>	3	3	0.0081 (0.0034)	1.000 (0.272)	0.00795
<i>Calisto raburni</i>	11	5	0.0052 (0.0017)	0.764 (0.107)	0.00537
<i>Calisto pulchella</i>	23	11	0.0057 (0.0015)	0.884 (0.038)	0.00579
<i>Calisto</i> sp. 1 (<i>Calisto mariposa</i> sp. nov.)	9	2	0.0023 (0.0013)	0.500 (0.128)	0.00228
<i>Calisto tasjiera</i>	10	4	0.0034 (0.0015)	0.733 (0.101)	0.00322
<i>Calisto schwartzi</i>	14	4	0.0012 (0.0008)	0.626 (0.110)	0.00124
<i>Calisto</i> sp. 4 (<i>Calisto azua</i> sp. nov.)	9	3	0.0015 (0.0009)	0.417 (0.191)	0.0152
<i>Calisto</i> sp. 3 (<i>Calisto victori</i> sp. nov.)	14	5	0.0019 (0.0007)	0.505 (0.158)	0.00192
<i>Calisto lyceius</i>	12	2	0.0003 (0.0002)	0.167 (0.134)	0.00025
<i>Calisto franciscoi</i>	18	8	0.0064 (0.0027)	0.882 (0.052)	0.00644
<i>Calisto crypta</i>	10	3	0.0016 (0.0011)	0.711 (0.086)	0.00162
<i>Calisto</i> sp. 2 (<i>Calisto samana</i> sp. nov.)	6	3	0.0011 (0.0008)	0.600 (0.215)	0.00122

TABLE 4. Genetic distances, p-distance at upper right and Kimura 2 parameters at lower left, of COI barcode sequences among *lyceius* group members. Intraspecific distances showed in the text below each species.

	<i>C. crypta</i> n=10	<i>C. raburni</i> n=11	<i>C. schwartzi</i> n=14	<i>Calisto</i> sp. 2 (<i>C. samana</i> sp. n.) n=6	<i>C. franciscoi</i> (<i>C. azua</i> sp. n.) n=18	<i>Calisto</i> sp. 4 (<i>C. azua</i> sp. n.) n=9	<i>Calisto</i> sp.1 (<i>C. mariposa</i> sp. n.) n=9	<i>Calisto</i> sp.3 (<i>C. victori</i> sp. n.) n=14	<i>C. lyceius</i> n=12	<i>C. pulchella</i> n=24	<i>C. zangis</i> n=3	<i>C. tasajera</i> n=10
<i>C. crypta</i>		8,88	6,63	2,75	3,57	6,49	7,09	7,33	4,68	9,41	8,01	6,06
<i>C. raburni</i>	9,54		9,22	9,05	8,15	8,49	7,79	9,20	9,08	8,59	7,31	8,70
<i>C. schwartzi</i>	7,03	9,89		7,56	6,94	4,67	7,17	5,58	6,98	10,41	7,53	5,98
<i>Calisto</i> sp.2 (<i>C. samana</i> sp. n.)	2,83	9,74	8,11		3,95	6,59	8,34	7,30	5,61	10,13	8,51	6,78
<i>C. franciscoi</i>	3,70	8,68	7,38	4,11		6,16	6,72	6,48	4,42	9,25	7,48	5,95
<i>Calisto</i> sp.4 (<i>C. azua</i> sp. n.)	6,86	9,04	4,87	6,98	6,49		7,60	3,70	7,17	9,43	7,67	6,04
<i>Calisto</i> sp.1 (<i>C. mariposa</i> sp. n.)	7,51	8,29	7,58	8,94	7,08	8,08		8,09	7,08	9,05	8,06	7,14
<i>Calisto</i> sp.3 (<i>C. victori</i> sp. n.)	7,81	9,86	5,86	7,77	6,85	3,84	8,63		6,86	10,68	9,01	5,92
<i>C. lyceius</i>	4,90	9,75	7,42	5,93	4,61	7,62	7,48	7,26		10,37	7,85	6,84
<i>C. pulchella</i>	10,10	9,21	11,28	10,96	9,91	10,12	9,69	11,60	11,23		8,45	10,29
<i>C. zangis</i>	8,53	7,74	7,97	9,12	7,94	8,12	8,56	9,68	8,34	9,04		8,19
<i>C. tasajera</i>	6,41	9,31	6,32	7,23	6,28	6,38	7,55	6,23	7,28	11,14	8,74	

TABLE 5. Some highly divergent morphological features of *Calisto zangis*, *C. raburni*, and *C. pulchella* compared to other congeners. Sources: Bates (1935), Turner in Brown & Heineman (1972), Johnson *et al.* (1987), Sourakov (1996, 2000), present work.

Features/Taxa	<i>C. zangis</i>	<i>C. raburni</i>	<i>C. pulchella</i>	other <i>Calisto</i> *
MALE FOREWING				
androconial patch	rounded, grayish, located on distal end of FW cell	elliptic or triangular, black, located between posterior margin of cell and the posterior margin of wing		
MALE GENITALIA				
adeagus main axis (lateral view)	-	sigmoid, broad and strongly compressed	straight and cylindrical	sigmoid or concave, relatively slender and slightly compressed
tegumen dorsal shape (lateral view)	slightly rounded	flat to slightly rounded	heavily rounded, bulbous	flat to moderately rounded
uncus shape (lateral view)	beak like, gradually tapered towards apex	flattened, gradually tapered towards apex	beak like but long and slender	beak like, gradually tapered towards apex
general outline of valvae	toothed dorsally	digitiform	short, very broad base abruptly tapering	digitiform
FEMALE GENITALIA				
sclerotized ring	-	incomplete	absent	complete
anterior genital plate	-	present	absent	present
sclerite on posterior genital plate	-	present	absent	
IMMATURE STAGES				
small horns on top of head at first larval instar	present	-	absent	present
X-mark on frons of head at final larval instar	absent	-	present	
overall head shape at final larval instar	subtriangular	-	moderately rounded	subquadrate
body tegument vestiture at final larval instar	-	-	setae and minute spines	setae

*excludes other species with divergent morphology outside *lyceius*, groups *nubila*, *eleleus*, and *chrysaoros*

Discussion

The only morphological feature that presently defines the *lyceius* group is the possession of reddish color covering most of the UN wing surface. It was the single character also used by Gali (1985) and still remains as the only way to demarcate the group. Gali (1985) had some doubts in his definition of the group due to possession of reddish areas by members of *hysius* group. Nevertheless, the wings UN is predominantly brown in all known *hysius* species with the reddish zones occupying relatively smaller areas of FW. The above mentioned feature is also present in *Calisto nubila* Lathy 1899, a species outside the *lyceius* group and sister of all living *Calisto* (Matos-Maraví *et al.* 2014). In lesser extent, *C. anegadensis* Smith, Miller & McKenzie 1991 also shows a reddish suffusion at UN but it probably groups apart and together with *C. nubila* as pointed by their unique genital morphology (Smith *et al.* 1991, Sourakov 1997).

All other adult or immature characters of most species within *lyceius* group are shared with species belonging to other groups or are lacking in at least one of its members. For example, the off line position of the white dot between M2–M3 veins is not present in all *lyceius*' group species (Bates 1935, Gali 1985, Gonzalez *et al.* 1991, present work). The shape of the uncus at the male genitalia of many species is practically the same to that of *C. confusa* and *C. hysius* from the *hysius* group (Gali 1985, Johnson & Hedges 1998, Sourakov 2000).

In the female genitalia, the internal loop of the sclerotized ring is also present in members of the *hysius* and *herophile* groups whereas the anterior genital plate is shared with members of the *herophile* group (Johnson & Hedges 1998, Sourakov 2000, Núñez *et al.* 2012, 2013). Ecologically, although several species (*C. crypta*, *C. lyceius*, *C. franciscoi*, *C. samana*, *C. azua*) are restricted to xeric habitats others are ecologically widespread (*C. pulchella*, *C. zangis*) or are inhabitants of middle to high mountain vegetation types (*C. schwartzi*, *C. tasajera*) (Gali 1985, Schwartz 1989, Gonzalez *et al.* 1991, Smith *et al.* 1994).

The early divergence of the *Calisto pulchella*, *C. zangis* and *C. raburni* ancestors is probably the main reason preventing the successful diagnosis of the *lyceius* group. All three taxa exhibit a highly differentiated morphology in most cases unique among all living *Calisto* which are summarized in Table 5. These features led to some past researchers to hypothesize a different generic placement for some of them (Smith *et al.* 1994, Sourakov 1996, Johnson in Sourakov 2000), but the authorities on satyrine morphology find sufficient evidence to consider them a single genus within Pronophilini, though recognizing it as a separate clade - Calistina (Pyrz 2010). The molecular studies have recovered *Calisto* as monophyletic and distant to any other Satyrinae (Peña *et al.* 2011, Matos-Maraví *et al.* 2014). These works seem to indicate that the genus is very old with the employed molecular markers showing higher molecular variability than within other genera. All reconstructed phylogenies evidenced the lack of phylogenetic signal to resolve some deeper nodes and the placement of *C. zangis* and *C. raburni* as well species outside the *lyceius* group having also divergent morphologies: *Calisto arcas* Bates 1939, *C. eleleus* Bates 1935, and the members of the *chrysaoros* group. So, while the genus is undoubtedly highly unusual in its old age and morphological divergence, we, like researchers before us, find it a correct course to preserve it as a single taxonomic entity.

Despite employing a single gen, our phylogenetic reconstruction of the *lyceius* group agrees with the major findings of Sourakov & Zakharov (2011), also using only COI barcodes, and the multigene work by Matos-Maraví *et al.* (2014). Some morphological features seem to support the reconstructed relationships.

The *zangis-pulchella-raburni* clade was also recovered by Sourakov & Zakharov (2011) and Matos-Maraví *et al.* (2014) although the relationships among them differ. The only strongly supported arrangement, *C. pulchella* as sister of *C. zangis-C. raburni*, was obtained by Matos-Maraví *et al.* (2014). As stated in previous paragraphs the highly differentiated morphology of these taxa seems to support their early divergence (Table 5). They and the earlier diverged species in the next clade, *C. mariposa* and *C. tasajera* also share besides its larger size, the possession of only two or three white dots at the M1 to Cu1 veins interspaces with that one at M2–M3 enlarged, and lack the dot at Rs-M1 interspace (Fig. 108). The only exceptions is *C. zangis*, which bears three to four tiny dots, and *C. raburni* which is distinctly smaller (Fig. 108).

Calisto mariposa was recovered as sister of the remaining members of *lyceius*. This position is also supported by similarities of their male and female genitalia including the possession of gnathi and lateral upward arms in the sclerotized ring respectively (Fig. 108). The later feature is unique among *Calisto*. Other two new species, *C. azua* and *C. victori*, were grouped as sisters in a clade together with *C. tasajera* and *C. schwartzi*. They share a larger size, a darker reddish tone and possession of fewer white dots at UN with the exception of the latter which bears

four (Fig. 108). The aedeagus of *C. shwartzi*, *C. azua* and *C. victori* males has a concave main axis (lateral view) which is slightly sigmoidal in the rest (Fig. 108). This the only major change regarding the relationships compared with Matos–Maraví *et al.* (2014) where *C. tasajera* was placed as sister of all other taxa in the second larger clade obtained by them.

Calisto samana seems to be sister of *C. crypta* being both inhabitants of northern Hispaniolan dry coastal areas. These species form a compact strongly supported clade together with *C. franciscoi* and *C. lyceius*, which occurs in similar habitats at the south coast also with *U. virgata* stands (Fig. 108). All share the possession of four white dots, a small size, and the presence of a dorsal keel on the valvae distal half in the male genitalia (Fig. 108).

Each species of the *lyceius* group, including the newly described, is supported by distinct morphological features. Both NJ and ABGD methods converged towards similar cluster numbers supported the existence of four undescribed species. The fifth hypothetical new species obtained in two partitions of the ABGD implementation seems to be due to an artifact since differences with other sequences of its own cluster are below the gap between intraspecific and interspecific distances. More divergent sequences within other NJ clusters, for example within *C. franciscoi*, remained on the same partition at ABGD. The consistency and congruence of both methods have been assessed in many recent works across a high number of animal groups (Paz & Crawford 2012, Puillandre *et al.* 2012, Hamilton *et al.* 2013, Ratnasingham & Hebert 2013). In addition, the NJ and ABGD results were also congruent with these of the Maximum Likelihood and Bayesian Inference phylogenetic reconstruction methods.

While after decades of studies focused on *Calisto*, the discovery of several new species seems unlikely, there are several reasons that could explain it. First, the high number of species within the *lyceius* group could be a consequence of its antiquity. The group seems to contain the oldest of all Hispaniolan *Calisto* (Matos–Maraví *et al.* 2014) having thus a longer time lapse to evolve. Second, until now collecting efforts were probably inadequate which allowed some species inhabiting Hispaniolan localities with xeric habitats to remain undetected. Adult emergence and breeding seems to be correlated with the sporadic rains at these habitats (Schwartz 1989, Sourakov 2000). Examples are the scarcity of *C. crypta* in our trips that was also noted by Schwartz (1989), Sourakov (2000), and the unsuccessful trips by A. Schwartz and J. W. Raburn to find the Samana's species (Schwartz, 1989). Third, as for many other butterflies groups, the advent of DNA studies has revealed the existence of hidden cryptic species within *Calisto*. These species once discovered and thoroughly studied showed small but constant morphological differences as evidenced in Cuban species (Núñez *et al.* 2012, 2013).

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