The *herophile* species group of *Calisto* (Lepidoptera : Nymphalidae : Satyrinae), new taxa and historical biogeography

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Abstract.   The genus *Calisto* is endemic to the West Indies and the only representative there of the Satyrinae. Here we reconstruct the evolutionary relationships of the *herophile* group and describe five new species from Cuba: *Calisto gundlachi* sp. nov., *Calisto siguanensis* sp. nov., *Calisto disjunctus* sp. nov., *Calisto sharkeyae* sp. nov. and *Calisto lastrai* sp. nov. We employ one mitochondrial and four nuclear markers to assess the phylogenetic position, Maximum Likelihood and Bayesian Inference approaches, of the new taxa. Our phylogenetic trees yielded two strongly supported main clades with four of the new species included within them and *C. sharkeyae* as sister group to the rest of the major main clade. We conduct time-divergence estimations and ancestral area reconstructions using BEAST and BioGeoBEARS. The group originated 12.15 million years ago during the middle Miocene in north-eastern Cuba, Nipe-Sagua-Baracoa Massif. After 6 million years of *in situ* evolution most lineages started to colonise other Cuban territories and the Bahamas. This scenario is consistent with key geological events, including the closure of the western Havana–Matanzas channel 8–6 million years ago, the uplift of the Sierra Maestra 6–5 million years ago, and the land connections among Cuban regions during the Miocene–Pleistocene sea level drops. Dispersal and vicariance processes may have occurred, with populations surviving floodings on the major and minor mountain ranges, which remained as 'islands'.

http://zoobank.org/urn:lsid:zoobank.org:act:03690F79-F938-42A0-B234-4A228D5C1913

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Introduction

The Greater Antillean archipelago includes the largest islands of the West Indies. The region is considered a biodiversity hotspot due to the large number of flora and fauna endemics (Myers *et al.* 2000; Ricklefs and Bermingham 2008). A significant part of the present Greater Antilles has been emerged since the Eocene (Donnelly 1988; Iturralde-Vinent 2006). Since then, the islands have suffered complex geological changes including extensive and repeated flooding, the creation and disappearance of land connections, and the creation of water channels between islands (Mann *et al.* 2002; Iturralde-Vinent 2006). Thus, at present the islands' biota is the product of a long history of dispersal and vicariant processes and species exchanges with neighbouring continental masses, resulting in a high proportion of endemism

(Ricklefs and Bermingham 2008). Some spectacular radiations have taken place, including *Anolis* lizards (Losos and Schluter 2000), *Eleutherodactylus* frogs (Hedges 1989) and *Anastraphia* and *Dendropemon* flowering plants (Acevedo-Rodríguez and Strong 2012) with several dozen endemic species described.

Within the insect order Lepidoptera, some endemic genera (e.g. *Choranthus*, *Burca*, *Atlantea*, *Mulona*) have speciated on the Greater Antilles, but only in low numbers (Field 1952; Smith *et al.* 1994). The exception is *Calisto* Hübner, the richest genus in the archipelago with 47 described species (Matos-Maraví *et al.* 2014; Pérez-Asso *et al.* 2016; Núñez *et al.* 2017*a*, 2017*b*). The genus is the only representative of the subfamily Satyrinae (Nymphalidae) on the islands, a highly diversified group in the Neotropics (Lamas 2004; Peña and

Wahlberg 2008). *Calisto* has no known close relatives, with studies showing its divergence from other lineages ~30 million years ago (Ma) (Peña *et al*. 2011; Matos-Maraví *et al*. 2014). Its species inhabit the four Greater Antilles, the Bahamas and Anegada, the easternmost of the British Virgin Islands (Smith *et al*. 1994). Though their dispersal capabilities have not been studied, these butterflies seem to be poor over-water dispersers since most species are restricted to a single island. In addition, on the larger islands many species are found in unique mountain ranges (Schwartz 1989; Smith *et al*. 1994; Núñez *et al*. 2012, 2013; Pérez-Asso *et al*. 2016).

The *herophile* group includes all the Cuban and Bahaman species of *Calisto* (Bates 1935; Matos-Maraví *et al*. 2014). Of the 11 named species, most inhabit the Cuban archipelago, whereas two occur on some of the Bahamas islands (Núñez *et al*. 2013; Matos-Maraví *et al*. 2014). Most of the Cuban species are restricted to a single mountain range except for the widespread *Calisto herophile* Hübner and *C. aquilum* Núñez from the central and western mountain ranges (Núñez *et al*. 2012, 2013).

Cuba has a complex geological history and a peculiar geography. Being more than 1100 km long, several parts of the archipelago differ in their origin, soil type and floristic composition (Borhidi 1996; Iturralde-Vinent 2006). There are four major Cuban mountain ranges, maximum altitudes between 700 and 1972 m: the Guaniguanico range in the west, the Guamuhaya massif in the centre, the south-eastern Sierra Maestra and the Nipe-Sagua-Baracoa (NSB) range in the north-east (CNNG 2000). These mountains are separated from each other by ~300 km, except for the last two, which are separated only by the narrow Guantanamo Valley. The rest of Cuba's landscape is dominated by lowlands though several smaller lower ranges, 300–500 m high, stand out among the major ones (CNNG 2000).

The historical biogeography of *Calisto* was recently reconstructed (Matos-Maraví *et al*. 2014). With 36 of the 44 described species sampled and using a six-gene dataset, all described species of the *herophile* group were included. The previous work divided the geographical range of the group into three areas: eastern Cuba, western Cuba and the Bahamas. Though practical when analysing the whole genus, this approach doesn't accurately represent the distribution of each species, restricted to a single mountain range in most cases. The discovery of several new Cuban species, described herein, represents a new opportunity to reconstruct the evolutionary relationships of the *herophile* group. In the present work, we also infer the group's historical biogeography at a finer scale, considering mountain ranges as 'islands' within the Cuban archipelago. This approach has been used for species in habitats surrounded by a matrix of unsuitable or hostile habitats with examples including isolated ponds or dunes, caves systems and mountain tops (Gillespie and Clauge 2009; Esposito *et al*. 2015; Van Dam and Matzke 2016).

Material and methods

Species description and taxonomic reassessment

The morphological characters used were those traditionally employed in the taxonomic literature on species of the *herophile* group (Bates 1935; Núñez 2009; Núñez *et al*. 2012, 2013). These characters are: the colour of the upper surface of the wings, the distinctiveness of the male androconial patch, the relative size and position of the red patch on the discal cell on the forewing (FW) under surface, the hue and intensity of pale scaling on the external side of the discal and postdiscal lines, and the number and relative size of white dots on the hindwing (HW) under surface. Since the group's species have a very similar morphology (Núñez *et al*. 2012, 2013), we included only these key features in the diagnosis and descriptions, avoiding the unnecessary repetition of shared characters. In the same way, both sexes' genitalia are very similar, with variation limited to features such as the degree of sclerotisation, the size of the uncus in relation to the tegumen, the outline of the valvae and aedeagus, and the relative size of the ductus and corpus bursae. Thus, instead of describing them, we only refer to diagnostic features in the genitalic structures of both sexes. To counteract the lack of morphological variation, diagnostic positions in the COI barcodes of the new described species are included in the diagnosis.

Abdomens were soaked in hot 10% potassium hydroxide before dissection. The structures were then dehydrated in an alcoholic series and mounted on slides using Euparal. Type specimens are deposited at the Zoologische Staatssammlung München, Munich (ZSM), at the Douglas M. Fernández Research Collection, Camagüey (DFRC), and at the Museo Nacional de Historia Natural (MNHN), the Museo Felipe Poey (MFP), and the Entomological Collection of the Instituto de Ecología y Sistemática (CZACC), all in Havana.

Taxon sampling, DNA extraction, amplification and sequencing

Our multilocus dataset is based on 30 specimens representing all of the species of the *herophile* group, including the five newly described here. Two outgroup taxa from Hispaniola, *Calisto clydoniata* and *C. chrysaoros galii*, were also included.

DNA was extracted from leg tissue of dried specimens using the DNeasy kit (Qiagen, Hilden, Germany). Primer pair sequences and PCR protocols employed to obtain new sequences are shown in Supplementary Table S1.1. A part of the COI barcode sequencing was performed at the Canadian Centre for DNA Barcoding (CCDB) and at Advances Identification Services facilities (AIM–Advanced Identification Methods GmbH, Munich, Germany, www.aimethods-lab.com) following standard protocols (CCDB 2013). DNA vouchers are deposited at the institutions mentioned in the previous sections. Sequence assembling and editing was performed using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were submitted to GenBank.

We retrieved 114 sequences belonging to 26 specimens from GenBank comprising the gene fragments cytochrome oxidase subunit I (COI) and the nuclear gene regions elongation factor 1α (EF1α), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosomal protein S5, and wingless (Supplementary Table S1.2). We also obtained 14 new sequences of COI, EF1α, and GAPDH belonging to the newly described species for the multilocus dataset (Supplementary Table S1.2). Although other specimens with available nuclear sequences were

listed in GenBank we excluded specimens with fewer markers to reduce the amount of missing data in our dataset. Specimens with single or fewer sequences were included only if no others were available.

We also prepared a dataset containing 93 COI barcodes including 31 new sequences obtained by us (Supplementary Table S1.3).

Phylogenetic analyses

All sequences were aligned manually in Bioedit 7.0.5.2 (Hall 1999). The best-fitting nucleotide substitution models were determined by the corrected Akaike information criterion implemented in Partition Finder (Lanfear *et al.* 2012) using a scheme with one partition for each marker.

We used the Bayesian Inference (BI) and Maximum Likelihood (ML) methods to reconstruct the phylogenetic relationships using the five-loci dataset, 3615 bp long. BI was implemented in MrBayes 3.2.6 (Ronquist *et al.* 2012). We ran the analysis for 20 million generations and sampled every 2000 generations. Stationarity and convergence of runs and estimates of burn-in were checked using Tracer 1.6 (Rambaut *et al.* 2013). Bayesian posterior probabilities were calculated for the majority consensus tree of all sampled trees after confirming that the average standard deviation of split frequency estimates was lower than 0.01 and discarding trees sampled within the burn-in phase (5 million generations). The ML analysis was carried out in RAxML 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway 3.3 (Miller *et al.* 2010). We used 1000 bootstrap replicates, defining the partitions per marker, and performing a search for the best-scoring tree. The results from both methods were visualised in FigTree 1.4.3 (Rambaut 2016).

Divergence time estimation

A reduced dataset with a single terminal per species was used to estimate divergence times. We included two specimens of *C. aquilum* and one of the newly described species to estimate the date of the split between their disjunct populations. The analyses were performed in BEAST 1.8.2 (Drummond *et al.* 2012). The dataset was partitioned per marker and the HKY model was set for each partition. The tree prior was set to the Birth Death process and the ucld mean prior was set with 0–10.0 limits with a uniform prior distribution. All other priors were left to defaults. The branch lengths were allowed to vary under a relaxed clock model with an uncorrelated lognormal distribution (Drummond *et al.* 2006). We ran the analyses for 20 million generations with sampling every 2000th generation. Since there are no known fossils of *Calisto* or close relatives, we used a secondary calibration point from the only previous age estimation for the whole genus (Matos-Maraví *et al.* 2014). We calibrated our tree at the root that marks the split between the *herophile* group ancestors and their Hispaniolan relatives, 21 ± 4 Ma. Convergence, effective sample sizes, and divergence times with upper and lower 95% HPD bounds were assessed in Tracer 1.6. The initial 10% of trees were discarded as burn-in. A maximum clade credibility tree with mean node heights was generated from the remaining trees using Tree Annotator 1.8.2 (Drummond *et al.* 2012). The generated tree was visualised and edited in FigTree 1.4.3.

Ancestral area estimates

The geographical distribution through time of the *herophile* group was estimated with the R package 'BioGeoBEARS' (Matzke 2013, 2014). The range of the group and the outgroup was subdivided into eight geographical areas following the distribution on the mountain ranges or islands in the case of Isla de La Juventud and the Bahamas: A, Hispaniola; B, NSB Massif; C, Sierra Maestra; D, Guamuhaya Massif; E, Galindo Hills (Habana-Matanzas Heights); F, Guaniguanico Cordillera; G, Isla de La Juventud; and H, the Bahamas (Supplementary Material Appendix S2). 'BioGeoBEARS' implements the LAGRANGE DEC model (Ree and Smith 2008), 'DIVALIKE' and 'BAYAREALIKE' within a maximum likelihood framework (Matzke 2013). Ancestral area probability was inferred using the divergence time tree obtained in BEAST.

The maximum number of areas was set to six. We conducted analyses using a non-time-stratified approach and different dispersal rates across the time slice. The non-time-stratified analysis was conducted under default settings. We used a single time slice, dividing the phylogeny at 6 Ma. Dispersal rate matrices were constructed according to the palaeogeographical configuration on each time slice (Supplementary Material Appendix S2). Though the dispersal capabilities of *Calisto* species remain unstudied, most species seem to be sedentary, being restricted to a single island or even to a single mountain range within an island. Probabilities of dispersal were set to 1 when two areas were adjacent, to 0.5 when two montane areas were separated by a lowland area less than 300 km wide (e.g. Guamuhaya Massif–Galindo Hills, Guaniguanico Cordillera–Isla de La Juventud), and to 0.01 when separated by two or more lowland areas larger than 300 km (e.g. Sierra Maestra–Guamuhaya Massif, NSB Massif–Guaniguanico Cordillera).

The dispersal probabilities among most areas were set to 0.01 before 6 Ma for several reasons. The Havana–Matanzas channel, separating western and central Cuba, was still open; most of the islands of the Bahamas were still under water and the Sierra Maestra was still submerged (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). Only the exchange for north-eastern Cuba (NSB) and Hispaniola had higher probabilities. The geological evidence implies that both territories were part of the same land block until 14–17 Ma (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006).

For the most recent period, 6 Ma to the present, dispersal across water and land were equally weighted. Extant *Calisto* apparently are incapable of dispersing over water since most species are restricted to single islands. However, throughout the Plio–Pleistocene epochs, periods of low sea level (Haq *et al.* 1987; Müller *et al.* 2008) allowed connections between islands on both the Bahaman and Cuban archipelagos (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006) (Fig. 1), allowing their colonisation. The few known non-single-island endemics are species that may have reached several islands of the Cuban and Bahaman archipelagos during the last glaciations.

Non-time-stratified and stratified approaches of all six models were compared using likelihood values (Matzke 2013).
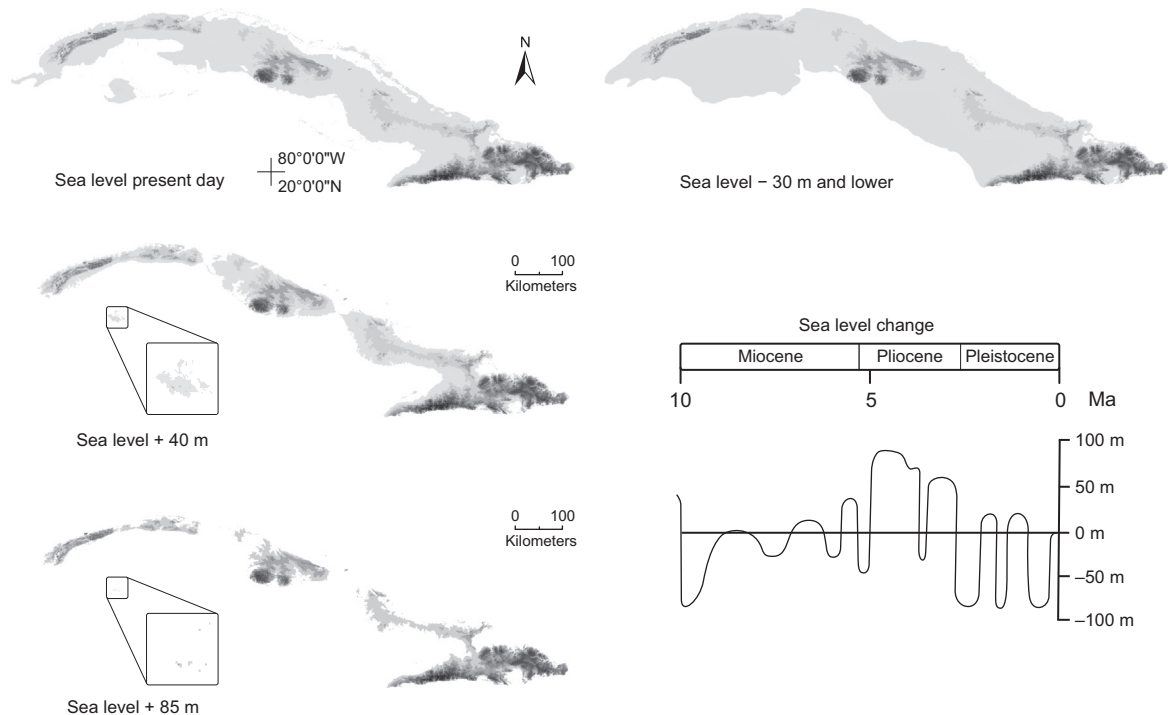
Fig. 1. Geographic and palaeogeographic maps of the Cuban archipelago for the present day and during the Pliocene–Pleistocene sea level changes. Reconstructions used a digital elevation map and follow Iturralde-Vinent (2003, 2006) reconstructions and represent high stands of the sea level at +40 m and +80 m and drops of −30 m or more. Curves represent the global sea level changes for the last 10 million years (modified from Haq *et al*. 1987; Müller *et al*. 2008).

Results

Description of new species

Calisto gundlachi **Núñez & Barro**, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:2B6BD2CB-0680-431E-80F7-72E4C2B38E84

Types

Holotype (Fig. 2*A, B*) (ZSM): male from Cuabales de Galindo, Matanzas Province, Cuba, 3.vi.2013, a dry-pinned specimen with red HOLOTYPE label. Paratypes (Fig. 2*C, D*) (ZSM, MFP): five dry-pinned specimens (three males, two females with the same data as the holotype) with red PARATYPE labels and DNA voucher labels AB-001, AB-002 and AB-003.

Diagnosis

Similar to *C. bruneri* Michener (17.2–19.9 mm, *n* = 13, male; 17.8–21.6 mm, *n* = 14, female) but smaller and with the HW lacking the pear shape of the ocellus. They are similar in the suffusion of grayish scaling on HW under surface, and the possession of two small white dots at the M2–M3 and M3–Cu1 HW cells, occasionally a third tiny white dot at M1–M2. Genitalic differences with *C. bruneri* are limited to the shape of the uncus, its broader digitiform projection in the valvae, and the more acute angle between saccus and vinculum. Allopatric from all other species of the group except *C. herophile*. COI barcode diagnostic characters are shown in Table 1.

Description

Forewing length 14.7–15.7 mm, *n* = 6, males; 17.5–17.6 mm, *n* = 2, females. Upper surface very dark brown, almost black,

androconial patch concealed by the coloration of the same hue. Under surface dark brown suffused with grey scaling, heavily at HW. FW under surface red patch occupying the central two-fifths of the discal cell. External edge of the HW postdiscal line and area around the subterminal lines with pale brown scaling. Two small white dots at the M2–M3 and M3–Cu1 HW cells, occasionally a third tiny white dot at M1–M2. HW ocellus slightly pear shaped. Genitalia moderately sclerotised, tegumen about two-thirds the length of uncus, flat; uncus tapering gradually from base towards apex, base posteriorly straight; valvae with digitiform projection tapering gradually from base towards apex with straight ventral margin (Fig. 3*A*). Female genitalia with dorsal crown tall; corpus bursae somewhat broad, near equal in length to ductus bursae (Fig. 4*A*).

Distribution

Known only from the type locality, Cuabales de Galindo, isolated serpentine hills surrounded by limestone, in the western Cuban province of Matanzas.

Habitat

Xeromorphic thorny thickets on serpentinite, locally called 'cuabales'.

Remarks

The distribution of *C. gundlachi* and its sister species, *C. bruneri*, is allopatric. Their morphologies have subtle, but constant, differences and the minimum difference among the available barcodes is 3.53%. Both inhabit thickets on serpentinite sites,

Fig. 2.   Types of the new species belonging to the *herophile* group of *Calisto*. *A–D*, *C. gundlachi*: *A*, male HOLOTYPE, upper surface; *B*, male HOLOTYPE, under surface; *C*, female PARATYPE, upper surface; *D*, female PARATYPE, under surface. *E–H*, *C. siguanensis*: *E*, male HOLOTYPE, upper surface; *F*, male HOLOTYPE, under surface; *G*, female HOLOTYPE, upper surface; *H*, female HOLOTYPE, under surface. *I–L*, *C. disjunctus*: *I*, male HOLOTYPE, upper surface; *J*, male HOLOTYPE, under surface; *K*, male PARATYPE, upper surface; *L*, male PARATYPE, under surface. *M–P*, *C. sharkeyae*: *M*, female HOLOTYPE, upper surface; *N*, female HOLOTYPE, under surface; *O*, male PARATYPE, upper surface; *P*, male PARATYPE, under surface. *Q–T*, *C. lastrai*: *Q*, female HOLOTYPE, upper surface; *R*, female HOLOTYPE, under surface; *S*, male PARATYPE, upper surface; *T*, male PARATYPE, under surface. Scale bars, 10 mm.

cuabales and *charrascales* respectively, habitats that differ in several ways including annual rainfall and floristic composition.

Etymology

Named after Johannes Christopher Gundlach, German naturalist who worked for decades in Cuba during the nineteenth century, founder of the first Cuban Natural History Museum.

Calisto siguanensis **Núñez & Barro**, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:2FE4F3A8-2501-4191-85DD-55B7643F145D

Types

Holotype (Fig. 2E, F) (ZSM): male from Siguanea, Isla de La Juventud, Cuba, 6.viii.2013, a dry-pinned specimen with red HOLOTYPE label and DNA voucher labels AIMSEqn 29032017-A02. Paratypes (Fig. 2G, H) (ZSM, MFP): three dry-pinned specimens (one male and two females with the same data as the holotype) with red PARATYPE labels and DNA voucher labels AB-008 and AIMSEqn 29032017-B04.

Diagnosis

Recognised by the combination of the dark brown upper surface of its wings, the possession of three white dots on M1–Cu1 interspaces with the central one enlarged, and by its enlarged HW ocellus. COI barcode diagnostic characters are shown in Table 1.

Description

Forewing length 14.8–16.3 mm, $n = 2$, males; 15.7–17.9 mm, $n = 2$, females. Upper surface dark brown, androconial patch concealed by the coloration of the same hue. Males and females with distinctly paler outer third and half of FW, respectively. Under surface brown, FW with red patch occupying the central

Table 1. Diagnostic positions, shaded in bold, at the COI barcode sequence of species on the *herophile* group of *Calisto*

Number of sequences per species is shown in parentheses

Note: This table is printed rotated 90° on the page. It comprises two stacked blocks, each listing the same 18 taxa against a set of diagnostic nucleotide positions of the COI barcode. Owing to the very dense rotated layout, the full position-by-position nucleotide matrix is reproduced below in summary form.

Taxa/Sequence positions — taxa (with number of sequences per species):

Taxa/Sequence positions
C. israeli (3)
C. smintheus (4)
C. dissimulatum (6)
C. aquilum (8)
C. sharkeyae (2)
C. bruneri (6)
C. gundlachi (3)
C. sibylla (2)
C. brochei (2)
C. occulta (5)
C. muripetens (3)
C. lastrai (4)
C. siguanensis
C. herophile (22)
C. apollinis (6)
C. torrei (4)
C. bradleyi (3)
C. disjunctus (4)

third of the discal cell. External edge of the HW postdiscal line heavily scaled with a broad band of pale yellow scaling. Three white dots from M1–M2 to M3–Cu1 HW cells, the one at M2–M3 noticeably larger. HW ocellus greatly enlarged. Genitalia moderately sclerotised, tegumen about two-thirds the length of uncus, flat; uncus tapering abruptly at the apical third, base posteriorly straight; valvae with digitiform projection tapering gradually from base towards apex, ventral margin straight (Fig. 3*B*). Female genitalia with dorsal crown tall; corpus bursae somewhat narrow, about two-thirds the length of ductus bursae (Fig. 4*B*).

Distribution

Known only from the type locality, Siguanea, near the west central coast of Isla de La Juventud.
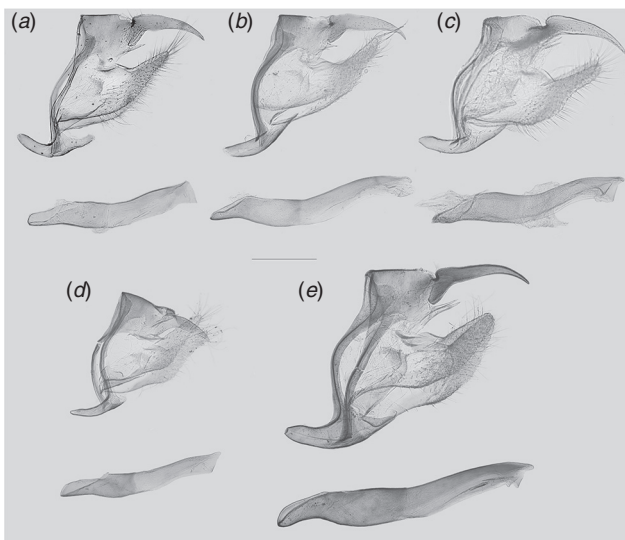
Fig. 3. Male genitalia of the new species belonging to the *herophile* group of *Calisto*, lateral view. *A*, *C. gundlachi*; *B*, *C. siguanensis*; *C*, *C. disjunctus*; *D*, *C. sharkeyae*; *E*, *C. lastrai*. Scale bar, 10 mm.

Habitat

White sand savannas.

Remarks

C. siguanensis is the only species of the genus known from white sand savannas, a particular habitat of extreme conditions restricted to only a few areas in Cuba. This species may have occasional contact with the widespread *C. herophile*, which is present in disturbed areas near the type locality and has been sequenced. The minimum pairwise distance to other members of the *herophile* group is 3.92% to *C. herophile*.

Etymology

The name is derived from the name of the type locality, Siguanea.

Calisto disjunctus **Núñez & Barro**, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:075EBEE6-DEF3-4A0A-8A32-E2A3A4B5E41

Types

Holotype (Fig. 2*I*, *J*) (ZSM): male from Estero de las Piedras, Isla de La Juventud, Cuba, 6.viii.2013, a dry-pinned specimen with red HOLOTYPE label. Paratypes (Fig. 2*K*, *L*) (ZSM, MFP, CZAC): three dry-pinned specimens (two males with the same data as the holotype; the third from El Taburete, Sierra del Rosario, Artemisa province, Cuba, 9.x.2011) with red PARATYPE labels and DNA voucher labels PM07-06, AB-005 and AIMSEqn 29032017-A10.

Diagnosis

Similar to *C. herophile* but darker, lacking the white dot at Rs–M1 and with the androconial patch well defined. Similar but smaller than *C. torrei* Núñez (19.7–23 mm, *n* = 10, male) and *C. bradleyi* Munroe (18–21.2 mm, *n* = 15, male) and lacking the shining bluish scales on the anal lobe of the latter. COI barcode diagnostic characters are shown in Table 1.
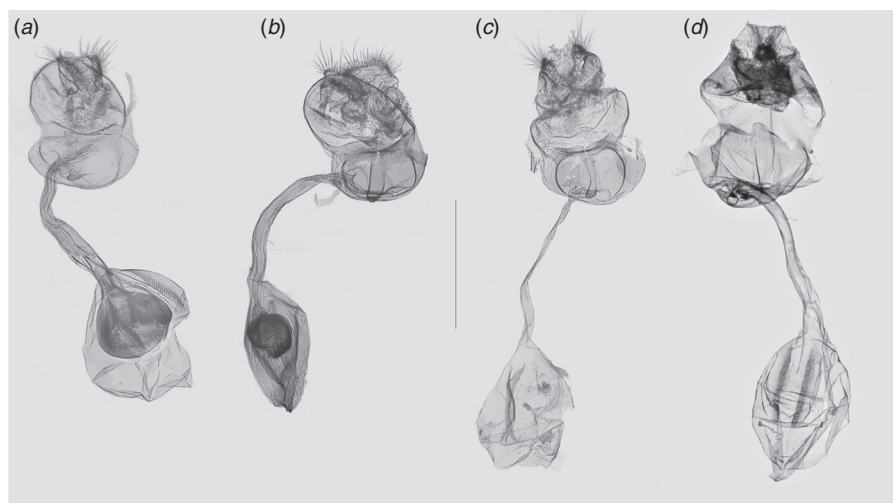

Fig. 4. Female genitalia of the new species belonging to the *herophile* group of *Calisto*, lateral view. *A*, *C. gundlachi*; *B*, *C. siguanensis*; *C*, *C. sharkeyae*; *D*, *C. lastrai*. Scale bar, 10 mm.

Description

Forewing length 16.8–17.9 mm, *n* = 4, males. Upper surface dark brown, androconial patch distinctly darker and well defined. Under surface brown, FW with a red patch occupying the central third of the discal cell. External edge of the HW postdiscal line distinctly (Isla de la Juventud) or faintly (Guaniguanico Cordillera) scaled with pale yellow. Three white dots from M1–M2 to M3–Cu1 HW cells, the one at M2–M3 distinctly larger. HW under surface heavily (Isla de la Juventud) or slightly (Guaniguanico Cordillera) suffused with pale yellow scaling. Genitalia slightly sclerotised, tegumen about two-thirds the length of uncus, slightly convex with a shallow, broad notch at the middle; uncus tapering gradually from base towards apex, base rounded protuberant; valvae with digitiform projection narrow, ventral margin slightly concave (Fig. 3C). Female unknown.

Distribution

Known from the extreme east of Guaniguanico Cordillera and the south portion of the Isla de La Juventud.

Habitat

Semideciduous forest.

Remarks

The single known specimen from the Guaniguanico Cordillera has a less contrasted under surface pattern compared with those from Isla de La Juventud. Differing also in its barcode sequence by 0.9% from Isla de la Juventud ones, this specimen may represent a different subspecies. However, in the absence of more specimens we prefer to wait to formally describe it. There are three diagnostic positions at the COI barcode sequence to recognise Guaniguanico–Isla de La Juventud populations: 307 G/A, 370 A/G and 523 C/T. The species is parapatric with *C. herophile* at both localities and with *C. bradleyi* and *C. aquilum occidentalis* at the Guaniguanico Cordillera. Minimum pairwise distances to its closest relatives, *C. torrei* and *C. bradleyi*, are above 3.3% (Table 2).

Etymology

The species name is derived from the Latin word *disjunctus* (separated), in reference to the species' distribution on two widely separated areas.

Calisto sharkeyae Núñez, Minno & Fernández, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:1F59167C-97CA-4DD5-AFE1-E00F8A73DB59

Types

Holotype (Fig. 2M, N) (DFRC): female from La Mensura, Pinares de Mayarí, Holguín Province, Cuba, 8.xi.2013, a dry-pinned specimen with HOLOTYPE label and DNA voucher label DMF-014. Paratypes (Fig. 2O, P) (DFRC): one dry-pinned specimen (one male with the same data as the holotype) with PARATYPE label and DNA voucher label DMF-015.

Diagnosis

One of the most distinctive species of the *herophile* group. Easily distinguished by the combination of the dark brown background under surface and the whitish yellow external edges of the discal and postdiscal lines, instead of yellow as in the rest of the group. In addition, wings are more rounded and the HW discal line is straighter than in the remaining species of the group. COI barcode diagnostic characters are shown in Table 1.

Description

Forewing length 15.7 mm, *n* = 1, male; 18.5 mm, *n* = 1, female. Upper surface very dark brown, almost black, androconial patch concealed by the darker basal coloration, outer two-fifths paler, also at the HW in females. Under surface brown, FW with a red patch occupying the central third of the discal cell. External side of red patch and postdiscal line edge with pale yellow scaling. HW discal line straight, only slightly inwardly bent on the inner margin. External edge of HW discal and postdiscal lines and part of the inner edges of both subterminal lines edged with whitish yellow scaling. Three small white dots at the M2–M3 and M3–Cu1 HW cells. Genitalia moderately sclerotised, tegumen equal in length to uncus, slightly curved; uncus tapering gradually towards apex, not distinctly pointed; valvae with enlarged digitiform projection, tapering gradually towards apex, ventral margin straight; saccus distinctly narrow (Fig. 3D). Female genitalia with dorsal crown short; corpus bursae somewhat broad, about two-thirds the length of ductus bursae, ductus very thin (Figs 3D, 4C).

Habitat

Xeromorphic subthorny thickets on serpentinite, locally called 'charrascales'. The climbing bamboo *Arthrostylidium farctum* (Aubl.) Soderstr. & Lourteig. (Poaceae: Bambusoideae) is dominant at the type locality. The vegetation is a dense thicket of shrubs and *A. farctum* growing on rocky hillsides and surrounded by pine forest. Sympatric with *C. bruneri*.

Distribution

Known only from the type locality, 2.6 km south-west of the entrance road to the Hotel Pinares de Mayarí, at La Mensura Piloto National Park, Pinares de Mayarí, at the Nipe plateau, the westernmost portion of the NSB range. The altitude is ~680 m.

Remarks

After *Calisto israeli* Torre, this is the most distinctive species of the *herophile* group. Its dark background colour together with the contrasting whitish edge of the discal and postdiscal lines of the HW under surface resembles the pattern of the Hispaniolan *hysius* group. However, its DNA and male genital morphology place *C. sharkeyae* within the *herophile* group. Part of the habitat at the type locality has been destroyed by mining. *C. bruneri* and *C. herophile* also occur in this area. The two individuals of the type series of *C. sharkeyae* were perching in a small opening adjacent to the pine forest. They flew close to the ground into and under the *A. farctum* foliage when disturbed.

Table 2. COI intraspecific K2P distances and minimum pairwise distances among species in the *herophile* group of *Calisto*

Distances corresponding to the species newly described in this paper are shown in bold

	israeli	smintheus	dissimulatum	aquilum	sharkeyae	bruneri	gundlachi	sibylla	brochei	occulta	muripetens	lastrai	siguanensis	herophile	apollinis	torrei	bradleyi	disjunctus
israeli	0.00																	
smintheus	9.53	0.33																
dissimulatum	9.71	5.59	0.31															
aquilum	8.75	5.94	3.93	1.64														
sharkeyae	**10.55**	**8.33**	**8.78**	**8.42**	**0.00**													
bruneri	10.09	8.76	7.72	7.72	**7.59**	1.22												
gundlachi	**9.24**	**7.24**	**6.87**	**6.69**	**5.99**	**3.53**	**0.33**											
sibylla	8.74	7.32	8.04	7.50	**6.16**	5.99	**5.91**	0.15										
brochei	7.58	6.67	7.63	7.06	**7.30**	6.14	**5.30**	4.82	0.52									
occulta	7.58	7.38	6.93	6.26	**5.56**	5.34	**4.92**	4.66	4.03	1.23								
muripetens	8.34	7.76	7.95	6.11	**5.89**	5.50	**5.18**	4.60	4.45	2.71	0.00							
lastrai	**8.55**	**8.09**	**8.27**	**7.79**	**7.44**	**6.75**	**6.17**	**5.67**	**5.20**	**4.20**	**3.69**	**0.00**						
siguanensis	**8.73**	**8.08**	**8.60**	**7.78**	**8.12**	**5.23**	**5.98**	**5.83**	**5.00**	**4.58**	**4.06**	**4.77**	**0.61**					
herophile	8.35	6.85	6.89	6.21	**6.05**	5.90	**5.44**	5.09	5.00	2.89	3.33	**3.39**	**3.92**	1.38				
apollinis	8.74	7.23	7.16	6.63	**6.06**	6.06	**5.55**	5.48	5.00	3.63	3.88	**4.43**	**4.10**	1.51	2.03			
torrei	9.02	7.39	8.19	7.98	**7.62**	6.62	**6.96**	5.24	5.82	4.47	4.67	**5.28**	**4.47**	4.29	3.90	0.36		
bradleyi	9.05	6.70	8.16	7.67	**7.50**	6.72	**7.08**	5.78	5.02	3.93	4.38	**5.79**	**5.60**	4.12	4.31	4.51	0.00	
disjunctus	**8.17**	**7.21**	**6.71**	**7.04**	**5.88**	**5.40**	**4.91**	**4.98**	**4.92**	**2.89**	**2.79**	**3.13**	**4.10**	**2.70**	**2.65**	**3.33**	**3.74**	**0.92**

Calisto lastrai Núñez, sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:11F3AF66-A433-43ED-87DB-3CAE54E05154

Types

Holotype (Fig. 2*Q, R*) (ZSM): female from Río Potosí, Moa, Holguín Province, Cuba, 4.xi.2013, a dry-pinned specimen with red HOLOTYPE label and DNA voucher label JL-016. Paratypes (Fig. 2*S, T*) (MNHN): three dry-pinned specimens (all males with the same data as the holotype) with red PARATYPE labels and DNA voucher labels JL-014, JL-017 and JL-018.

Diagnosis

Similar to *C. brochei* Torre (20.1–22.1 mm, *n* = 9, male; 20.7–22.0 mm, *n* = 5, female), *C. occulta* Núñez (18.7–22.2 mm, *n* = 7, male; 18.9–23.2 mm, *n* = 2, female) and *C. muripetens* Bates (19.4–22.4 mm, *n* = 8, male; 21.7–21.8 mm, *n* = 2, female), but smaller, with a less evident androconial patch on the FW upperside and paler on the underside. Differs also from *C. brochei* by lacking the white dot on Rs–M1 and having the yellow scaling on the outer edge of HW postdiscal line broader and paler. Red patch at FW under surface distinctly pointed on its outer edge, a feature shared only with *C. brochei*, *C. occulta* and *C. muripetens* in the *herophile* group. COI barcode diagnostic characters are shown in Table 1.

Description

Forewing length 16.8–17.4 mm, *n* = 3, males; 20.4 mm, *n* = 1, female. Upper surface dark brown, androconial patch distinctly darker. Males and females with distinctly paler outer third and half of FW, respectively. Under surface brown, FW with red patch occupying the central three-fifths of the discal cell. External edge of the HW postdiscal line heavily scaled with a broad band of pale yellow scaling. Three white dots from M1–M2 to M3–Cu1 HW cells, the one at M2–M3 larger and slightly displaced towards the postdiscal line. Genitalia sclerotised, tegumen about two-thirds the length of uncus, flat; uncus tapering gradually towards apex from middle, distinctly pointed; valvae with enlarged digitiform projection, tapering gradually towards apex, ventral margin straight; saccus distinctly enlarged (Fig. 3*E*). Female genitalia with corpus bursae somewhat broad, about two-thirds the length of ductus bursae (Fig. 4*D*).

Distribution

Known only from the type locality, near the mouth of the Potosí River, east Moa, Holguín province, at the northern foothills of the NSB Massif. Sympatric with *C. occulta* and *C. bruneri*.

Habitat

Xeromorphic subthorny thickets on serpentinite, locally called 'charrascales'.

Remarks

C. lastrai has an allopatric distribution with respect to other *Calisto* species with similar morphology, *C. brochei* and *C. muripetens*. However, these species are forest inhabitants whereas *C. lastrai* lives in *charrascales*. COI barcodes of *C. lastrai* differ by 5.2 and 3.69% from them respectively (Table 2). The other similar species, *C. occulta*, is sympatric and shares its habitat. Their barcodes differ by 4.2%. The minimum pairwise distance to another congener is 3.13% to *C. disjunctus* from western Cuba.

COI barcode analysis

We searched for diagnostic nucleotide positions for each species of the *herophile* group (Table 1). All species have at least one diagnostic position not shared with other species of the group, and up to 15 positions in the case of *C. israeli*. Closely related species such as *C. apollinis*–*C. herophile* and *C. muripetens*–*C. occulta*, having only one or two diagnostic positions, also bear positions shared with distant congeners, but diagnostic among them. The first species pair possesses diagnostic positions at 530 T/C, 550 G/A, 565 G/A and 616 C/T, respectively, and the second pair at 106 T/C, 200 A/G, 250 C/T, 355 T/C, 477 A/G, 484 T/C, 533 C/T, 544 C/T and 649 G/A.

The K2P (Kimura 2 Parameters) distances among specimens within each cluster ranged from 0.00 in several taxa to 2.03% in *C. apollinis* (Table 2). The minimum pairwise distances among specimens from different clusters were between 1.51 (*C. herophile*–*C. apollinis*) and 10.55% (*C. sharkeyae*–*C. israeli*). Minimum pairwise distances among specimens of the five new species described herein and other members of the group were between 2.65% (*C. disjunctus*–*C. apollinis*) and 10.55% (*C. sharkeyae*–*C. israeli*).

Phylogeny

Our BI and ML trees yielded similar, but not identical, topologies (Fig. 5). The differences were related to the placement of *Calisto sibylla*, a Bahaman taxon, and *C. siguanensis*, a new species from Isla de la Juventud. Several deeper nodes were weakly supported; however, the two main clades and the shallower clades were recovered mostly with strong support by both analyses (Fig. 5). In our phylogenies, only NSB taxa were positioned as sister groups to the inhabitants of other Cuban territories in all clades.

Regarding the new species herein described, *C. sharkeyae*, NSB, was placed as sister group to all other species in the major of the two main clades with strong support (Fig. 5). *C. lastrai*, also from NSB, is the sister group to a clade comprising taxa from the central and western major mountain ranges, Isla de La Juventud and the Bahamas. The strongly supported sister group relationship between *C. gundlachi* and *C. bruneri* is noteworthy since their populations are separated by 700 km. *C. disjunctus* is most closely related to *C. bradleyi*, a sympatric taxon at the Guaniguanico range. In the case of *C. siguanensis*, an Isla de La Juventud endemic, neither BI nor ML analyses recovered a strongly supported position.

Separate analyses of the mitochondrial and concatenated nuclear markers only partially matched the topologies
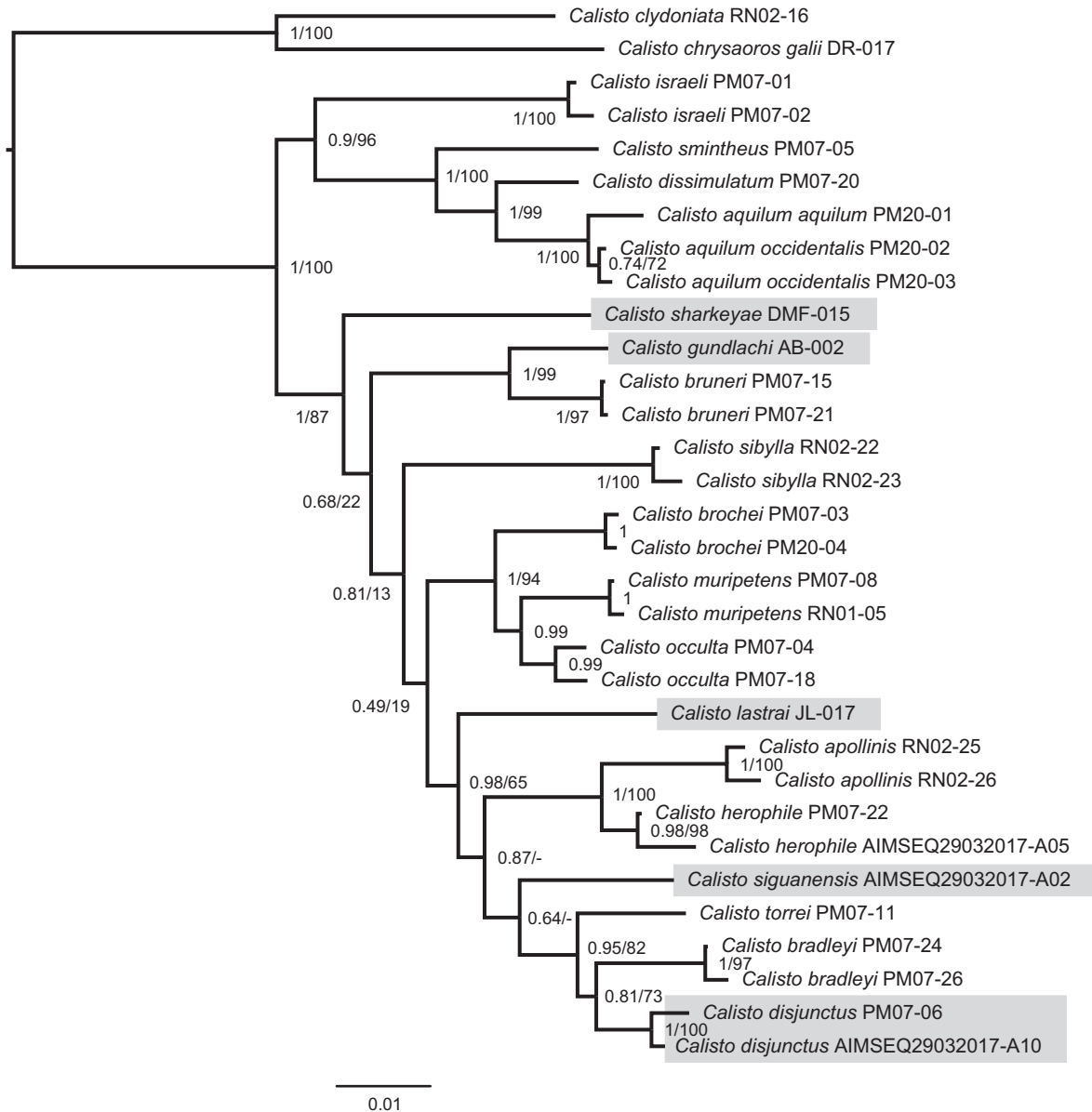
Fig. 5.   Bayesian Inference phylogenetic tree of the *herophile* group of *Calisto*. Numbers at nodes correspond to posterior probabilities and bootstrap values of the Maximum Likelihood phylogeny. Species names are accompanied by their voucher code. Gray boxes highlight the new species described in the present study.

obtained with the concatenated dataset (Supplementary Figs S3.1 and S3.2). Compared with the latter, most relationships among the major clades were weakly supported, but near the tips the same species pairs were recovered in most cases. The only strongly supported different relationship was the position of *C. israeli* as sister group to the rest of the group in the mitochondrial tree. The trees that were reconstructed using individual nuclear markers obtained even weaker topologies (results not shown).

Divergence times and biogeographical reconstruction

The topology of our chronogram is mostly in accordance with the BI phylogeny except for the position of *C. sibylla* (Fig. 6).

The Bahaman taxon was placed with maximum support as sister group to the clade *C. brochei*(*C. occulta–C. muripetens*). Overall support was stronger than in the BI tree, with all deeper nodes strongly supported. There were only two weakly supported nodes, both within the clade forming the sister group of *C. lastrai*. The crown age of the *herophile* group was estimated as 12.15 Ma (Fig. 6, Supplementary Figure S3.3).

Our ancestral area reconstruction favoured the DIVALIKE model (AICc weight: 96.7%) in the time-stratified analyses (Table 3). The same model was the best-fitting in the non-time-stratified analyses, scoring 98.4%. However, the latter case supports a widespread origin for all early-diverged taxa, including areas submerged before 6 Ma (e.g. Sierra
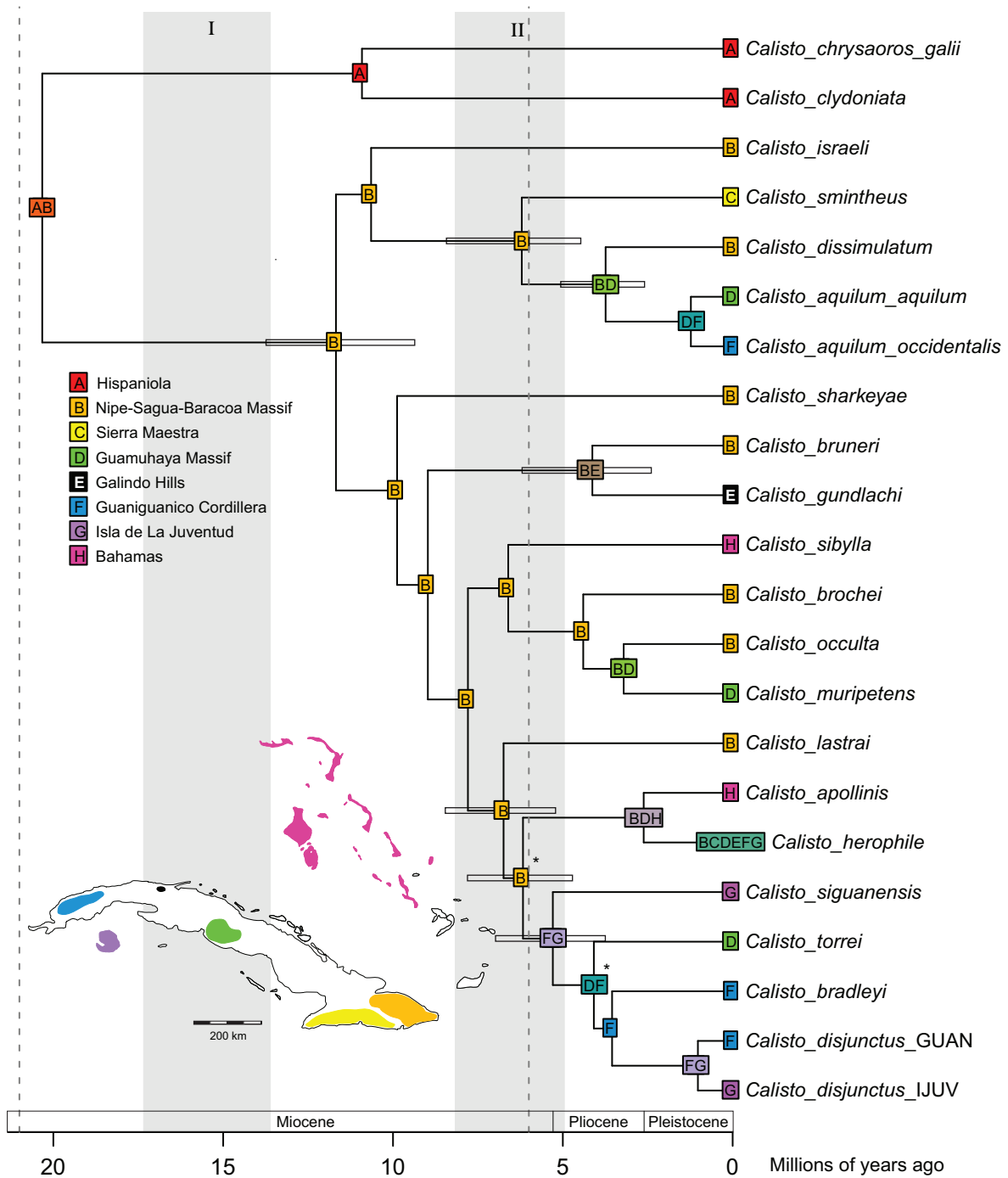
Fig. 6. Ancestral range estimates using the time-stratified approach for the DIVALIKE model on the *herophile* group of *Calisto*. The single most probable ancestral range is given. Bars at nodes indicate 95% highest posterior density (HPD) intervals of node ages. Asterisks indicate nodes with support values below 0.95. Pie charts with the probabilities for each node are shown in Supplementary Figure S.3.3. Geographical areas: A, Hispaniola; B, Nipe-Sagua-Baracoa Massif; C, Sierra Maestra; D, Guamuhaya Massif; E, Galindo Hills; F, Guaniguanico Cordillera; G, Isla de la Juventud; H, Bahamas. Gray bars indicate major geological events (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006): I, estimated age, 14–17 Ma, for the split between north-eastern Cuba and north central Hispaniola; II, estimated ages for the closure of the Havana–Matanzas channel, 8–6 Ma; the uplift of Sierra Maestra, 6–5 Ma; land connections among different Cuban regions established during late Miocene sea level drops (see Fig. 1).

Table 3.   BIOGEOBEARS model comparison for the *herophile* group species of *Calisto*

LnL, log-likelihood; *N*, no. of parameters; d, rate of dispersal; e, rate of extinction; AICc, Akaike information criterion corrected for sample size; AICc_wt, AICc weight; % AICc, percentage of AICc_wt. The best model of stratified and non-stratified analyses is shown in bold

	LnL	*N*	d	e	AICc	% AICc_wt
Time-stratified analyses						
DEC	−70.7	2	0.0766	0.0418	146.0	3.3
DIVALIKE	**−67.3**	**2**	**0.0768**	**0.0302**	**139.2**	**96.7**
BAYAREALIKE	−79.5	2	0.0729	0.1391	163.5	0.0
Non-time-stratified analyses						
DEC	−72.6	2	0.012	0.0220	149.9	1.6
DIVALIKE	**−68.5**	**2**	**0.013**	**2.00E-08**	**141.7**	**98.4**
BAYAREALIKE	−82.3	2	0.017	0.1783	169.3	0.0

Maestra) or without a land connection with north-eastern Cuba or northern–central Hispaniola, an unlikely hypothesis that we reject.

According to the chosen model, the biogeographic history of the *herophile* group is mainly characterised by intermountain and interisland dispersals (Fig. 6). In our reconstruction, the diversification of the group started around 12 Ma and is still ongoing. During the first 6 million years its evolution occurred only at NSB. From 6 Ma to the present most lineages started the colonisation of all other Cuban areas and the Bahamas (Fig. 6).

In the smaller main clade, NSB ancestors reached the southern Sierra Maestra, apparently in a single colonisation event. They also reached the central and western mountains, the Guamuhaya and Guaniguanico ranges respectively, but not the Bahamas or Isla de La Juventud (Fig. 6).

In the larger main clade, after initial diversification *in situ* most NSB lineages colonised all other territories except the Sierra Maestra. In a 700-km-long step, a lineage reached the western Galindo Hills, in the smaller Havana–Matanzas range (Fig. 6). Another lineage reached the Bahamas and the central Cuban mountains, Guamuhaya. A third lineage reached all territories west of NSB, including the Bahamas for the second time. Both Isla de La Juventud endemics evolved in this clade.

Discussion

The new species of the herophile group

The genus *Calisto* is another example of the growing phenomena of cryptic species revealed by molecular studies (Bickford *et al.* 2007; Pfenninger and Schwenk 2007; Minelli 2015). Almost lacking morphological variation, cryptic species are being unveiled across the tree of life, including butterflies (Janzen *et al.* 2009; Voda *et al.* 2014; Lavinia *et al.* 2017) and within them the Satyrinae (Núñez *et al.* 2012, 2013, 2017*a*; Pérez-Asso *et al.* 2016; Pyrcz *et al.* 2018; Nakahara *et al.* 2018).

Allopatry and the similar morphology of many *Calisto* populations may raise confusion about their status as species or subspecies. This led in the past to the ranking of taxa mainly as subspecies in Cuba (Brown and Heineman 1972; Smith *et al.* 1994; Lamas 2004). With few exceptions, wing pattern and genitalic morphology are very similar within each species

group of *Calisto* (Sourakov and Zakharov 2011; Núñez *et al.* 2012, 2013, 2017*a*; Pérez-Asso *et al.* 2016). We used morphology, distribution, habitat, COI barcodes and the phylogenetic position of each taxon as lines of evidence applying the unified species concept of De Queiroz (2007) when assessing the taxonomic status of each population.

Before comparing morphological features among allopatric populations, we first assessed the degree of difference between sympatric species (Mayr 1969) within the *herophile* group. *C. brochei* Torre and *C. dissimulatum* Núñez are sympatric at the NSB mountains whereas *C. muripetens* Bates and *C. torrei* Núñez fly together at the Guamuhaya Massif (Núñez *et al.* 2013; unpubl. data). Adults of the first pair differ only in size and in the outer edge of their androconial patches whereas those of the second pair differ in the size of their FW upperside androconial patches and red patches on the FW underside (Núñez *et al.* 2013). Members of both species pairs show distinctive features in their immature stages, differ in their barcodes and are distant from each other in the group phylogeny (Núñez *et al.* 2012, 2013; present work). The lack of morphological differences among adults of *Calisto* is also found in other species groups. *C. raburni* Gali and *C. mariposa* Pérez-Asso, Núñez & Genaro and *C. schwartzi* Gali and *C. victori* Pérez-Asso, Núñez & Genaro are examples of sympatric species with few morphological differences within the *lycieus* species group of Hispaniola (Pérez-Asso *et al.* 2016). Likewise, within the Hipsaniolan *hysius* group several sympatric species pairs occur. The best known are *C. obscura* Michener and *C. confusa* Lathy, both widespread across the island (Schwartz 1989; Smith *et al.* 1994). In the three species groups there are also several allopatric populations exhibiting equivalent small differences in their phenotypes. When comparing the new species described here with other allopatric populations, similar morphological differences arose in most cases: FW length, overall background colour, size of the red patch at FW underside, number and size of HW postdiscal dots plus a few other features. Genitalic differences are reduced to the proportional sizes of the tegumen and uncus, the shape of the tegumen and the valvae digitiform projections and the proportional length of the ductus and corpus bursae. These differences are of the same degree as those within the whole *herophile* group, with *C. israeli* and *C. sharkeyae* being the most distinctive species.

Morphology of the immature stages has proven useful to confirm the species status of previously known species (Sourakov 1996, 1999; Núñez *et al.* 2012) and when describing new taxa (Núñez *et al.* 2012, 2013). However, the immature stages remain unknown for about two-thirds of *Calisto* species, including the new ones named here.

Species ecology can be used as an additional source of data in assessing the status of the different populations. For *Calisto*, several ecological aspects have been published, but are useless for taxonomic purposes or are known for only a few species (Shelley and Ludwig 1985; Schwartz 1989; Núñez *et al.* 2012, 2013). Only distribution and habitat remain as valuable data sources when comparing different populations. Sympatric species of the *herophile* group share the same habitats, e.g. *C. israeli*, *C. brochei* and *C. occulta* inhabit NSB rain and evergreen forests (Núñez *et al.* 2012). At a more local scale, some sympatric taxa have a parapatric distribution with respect to

their habitat preferences. *C. herophile*, the only widespread Cuban species, prefers disturbed vegetation: roadsides, gardens, anthropogenic savannas, etc., whereas all other species are restricted to one or a few well preserved natural habitats: thickets on serpentine soil, rainforests, pine forests, etc. (Núñez *et al*. 2012, 2013). Of the new species described here, *C. siguanensis* is the sole inhabitant of white sands savannas. *C. lastrai* flies in *charrascales* whereas its close relatives prefer forests or disturbed lands. Likewise, *C. gundlachi* lives in *cuabales*, which are drier and differ in floristic composition from *charrascales*, the habitat of its sister taxon, *C. bruneri*. *C. disjunctus* inhabits semideciduous forests like its sister taxon, *C. bradleyi*, though in the latter case the habitat is associated with the *mogote* (karstic hills with vertical walls) vegetation complex (Núñez *et al*. 2012).

Regarding DNA, COI barcodes have been shown to be useful for recognition of the previously named species and for uncovering cryptic species in *Calisto* (Sourakov and Zakharov 2011; Núñez *et al*. 2012, 2013, 2017*a*; Pérez-Asso *et al*. 2016). Additionally, they also have supported the synonymic status of several taxa (Sourakov and Zakharov 2011; Núñez *et al*. 2017*a*, 2017*b*). COI barcodes are available for 46 of the 54 named *Calisto* species, including the ones described herein. The available barcodes allow the identification of all these species, grouping them in well defined monophyletic clusters. Intraspecific distances are usually below 1%, with only six cases where values are between 1.2 and 2.03% (Pérez-Asso *et al*. 2016; Núñez *et al*. 2017*a*, 2017*b*; present work). Interspecific distances are between 2.3 and 12.47%. Values for the new taxa described here are between 2.65 and 10.55%. The most problematic case in the genus involves *C. grannus* from Hispaniola with eight subspecies recognised as valid species in the past (Schwartz 1989; Smith *et al*. 1994). Bearing slight morphological differences, COI barcodes show that these populations apparently are of recent origin and that introgression has occurred among them (Sourakov and Zakharov 2011; Núñez *et al*. 2017*a*). The pairwise COI genetic distances among sequences, *n* = 58, of these populations are below 1.2% (Núñez *et al*. 2017*a*).

The phylogenetic relationships also shed some light on the taxonomic status of populations. *C. sharkeyae*, *C. lastrai* and *C. siguanensis* are distant from other *herophile* group members, supporting their species statuses. In the last case, although its phylogenetic position lacks support in the complete dataset (Fig. 5), probably due to a higher proportion of ambiguous data, its position is strongly supported in the time-calibrated tree obtained with the reduced dataset (Fig. 6). In the other two cases, *C. disjunctus* and *C. gundlachi*, the new taxa are sister groups to their closest relatives.

The new species described herein show that the Cuban diversity of *Calisto* is still far from being well known. Previously, all but one Cuban species of the *herophile* group were restricted to one or two of the four major mountain ranges (Núñez *et al*. 2012, 2013). In the present work, three of the five new species are inhabitants of areas outside these mountains. This finding implies that some lineages also survived at minor elevations during the Plio–Pleistocene floodings (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006).

Calisto siguanensis and *C. disjunctus* are known only from areas below 60 m at Isla de La Juventud. The available palaeogeographic reconstructions of the Cuban archipelago show that areas of the Isla de La Juventud have remained emerged since the late Eocene (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). *C. siguanensis* seems to be an old lineage; however, more molecular data (only COI barcodes were available) and information on its distribution and ecology are needed for a better assessment of its evolutionary history. Like the next species, *C. siguanensis* perhaps also inhabits south-western Cuba, where similar vegetation types are present on hills (Borhidi 1996). *C. disjunctus* seems to be a recent lineage, its presence at higher elevations in the western mountains of Cuba may have allowed its survival during past periods of high sea levels.

In the case of *C. gundlachi*, the most intriguing fact is the 700-km separation from its sister species, *C. bruneri*, at NSB. The restriction of both species to habitats on serpentines, also known as metamorphic or ultramaphic soil is noteworthy. All seven NSB endemics are confined to habitats on serpentine soil, whereas the rest of the Cuban species live in ecosystems on other soil types. The exceptions are the western *C. bradleyi* and the newly described *C. gundlachi*. In Cuba, dozens of serpentine patches, ranging from a few to 800 km^2, are outcropped across the island, many of them at low elevations of ~300 m (Iturralde-Vinent 1990; Borhidi 1996). These areas are isolated by other soil types, limestone in most cases, harbouring different ecosystems characterised by high plant endemism and extreme xeric conditions. There are scarce records of *Calisto* species from serpentine areas outside the major mountains, but there is also a large sampling bias. All these elements suggest that the search for potential relatives of *C. bruneri* and *C. gundlachi*, and perhaps all NSB lineages, at other Cuban serpentine areas would be worthwhile.

The biogeographical history of the herophile group and the role of the Cuban mountains and Plio–Pleistocene sea level changes

Our study is one of very few studies targeting an 'islands within islands' evolutionary process for the West Indies. Our results match those of studies on true island biotas (Shaw *et al*. 2015; Berger *et al*. 2016; Tänzler *et al*. 2016; Zhang *et al*. 2017) where dispersal has been found to be a critical process in their evolution.

Our findings indicate that *in situ* diversification played a major role in the evolution of the *herophile* group, but only during its initial stage at the NSB Massif. Similar patterns for island lineages have been found in other arthropod groups such as leafhoppers (Bennett and O'Grady 2013) and weevils (Zhang *et al*. 2017). The north-eastern Cuban region, together with northern Hispaniola and Puerto Rico, was part of a large block that split apart in the Miocene (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). NSB is also the Cuban region closest to Hispaniola, the homeland of the sister taxon of the *herophile* group (Matos-Maraví *et al*. 2014). NSB was separated by water from other proto-Cuban land masses during the mid to late Miocene (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006), thus the ancestors of the group

probably evolved there in isolation. This would explain the highest diversity of the group at NSB, with seven endemic species, whereas other mountain ranges or areas harbour only one to three species.

By contrast, the second half of the evolutionary history of the *herophile* group, from ~6 Ma to the present, has been characterised by dispersal and vicariance processes. Most dispersal events departed from NSB starting around 6 Ma, with all lineages, except *C. sharkeyae*, colonising other territories (Fig. 6). Since *Calisto* species apparently are incapable of dispersal over water, their arrival in other territories was possible only when new land emerged. Thus, our reconstruction matches key events in the geological history of Cuba: the closure of the western Havana–Matanzas channel 8–6 Ma, the uplift of the south-eastern Sierra Maestra 6–5 Ma, and the land connections among different Cuban regions during the late Miocene–Pleistocene sea-level drops (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). Alternating cycles of low and high sea level may have allowed dispersal and vicariant events respectively. Our palaeogeographic maps of Cuba (Fig. 1) are conservative since the average altitude of Cuba was lower at least during the early Pleistocene (Iturralde-Vinent 2003). Populations may have survived the floodings at both major and minor mountain ranges that remained as 'islands' (Fig. 1). Thus, the recent evolution of the *herophile* group on the Cuban archipelago seems to have occurred much like in other arthropod groups on true islands (Toussaint and Balke 2016; Zhang *et al.* 2017), but also in caves both on the mainland (Faille *et al.* 2015) and on islands (Esposito *et al.* 2015).

Further work on Calisto

Future taxonomic work on *Calisto* should focus on the less sampled larger islands, Cuba and Hispaniola, especially the Haitian mountains, the eastern half of the Cuban NSB Massif, and isolated extreme habitats and lower elevations. The clarification of the taxonomic status of populations like the ones belonging to the *grannus* complex on Hispaniola is another priority (Sourakov and Zakharov, 2011; Núñez *et al.* 2017a). Field work should also address species ecology and biology. Immature stages are known for only about one-third of the species (Sourakov and Emmel, 1995; Sourakov 1996, 1999; Núñez *et al.* 2012, 2013).

The evolutionary relationships and historical biogeography should be revisited soon. Recent attempts have only partially resolved these (Matos-Maraví *et al.* 2014) and there are still several taxa missing from the analyses, including recently described species (Pérez-Asso *et al.* 2016; Núñez *et al.* 2017b). Future studies should apply next-generation sequencing techniques for two main reasons. First, several species are very rare and known from just a few aging specimens (only the types in some cases), reducing the success of Sanger-based techniques. Second, it seems that *Calisto* is a relatively old taxon, 24–31 million years (Matos-Maraví *et al.* 2014; Chazot *et al.* 2019; Espeland *et al.* 2018), thus a NGS approach with a much larger amount of data would be preferable to successfully resolve the relationships to other Satyrinae.

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