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A new species of the *hysius* species-group of *Calisto* Hübner (Lepidoptera, Nymphalidae, Satyrinae) and insights into the status of different populations currently attributed to *C. grannus* Bates

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Abstract

A species belonging to the *hysius* group of *Calisto* is newly described. *Calisto bahoruco* **new species** inhabits the eastern-most area of the southern Hispaniola Sierra de Bahoruco. The species is closely related to *C. hysius* Godart, another endemic from the southern mountains. The two species differ in average forewing length (larger in *C. hysius*), the relative size of ocelli (larger in *C. bahoruco*), and in the darker ground color with more contrasted paler edges of lines at underside of wings in *C. bahoruco* compared to *C. hysius*. Their male genitalia differ in the shape of the uncus and in the heavier sclerotization in *C. bahoruco*. COI barcodes bear a minimum of 15 differences between the two species. All species delimitation methods applied to the COI dataset, ABGD, BIN, bPTP, mPTP, and PTP yielded 8 species. The exception was the GMYC that failed to recognize the new taxon and oversplitted several other taxa. COI gene tree obtained by Maximum Likelihood and Bayesian have identical topologies agreeing with a multilocus phylogeny reconstructed in a previous work on *Calisto* and placing the new taxon as sister of *C. hysius*. Additionally, we provide new evidence of the conspecific nature of all named populations within *Calisto grannus* group.

Key words: Greater Antilles, Hispaniola, COI, ABGD, barcoding, Bayesian Inference, BIN, character base, diagnostic sites, GMYC, haplotype network, Maximum Likelihood, monophyly, new species, gen tree, phylogeny, PTP

Introduction

The genus *Calisto* Hübner is the only representative of the Satyrinae in the Greater Antilles with 47 known species, most of them endemic from a single island (Matos–Maravi *et al.* 2014, Pérez–Asso *et al.* 2016, Núñez *et al.* in press). The island of Hispaniola possesses the highest species richness with several species groups commonly referred by authors in the rich bibliography on the genus (eg. Bates 1935, Munroe 1950, Gali 1985).

Intensive collecting efforts during the last decade together with the increasing number of molecular studies are bringing light into the genus taxonomy and phylogenetic relationships (Sourakov & Zakharov 2011, Núñez *et al.* 2012, 2013, in press, Matos–Maravi *et al.* 2014, Pérez–Asso *et al.* 2016). Currently, 39 species, 83% of the total known, have at least one gene sequenced.

The *hysius* species group, originally defined by Bates (1935), includes ten species. *Calisto confusa* Lathy, *C. obscura* Michener and *C. batesi* Michener are widespread across Hispaniola (Schwartz 1989, Smith et al. 1994). The remainder are known from a single mountain range or are restricted to one or few localities.

In the present work we describe the eleventh species of the group, a taxon closely related to *C. hysius* Godart. In addition, we test the species boundaries within the *hysius* group using 215 COI barcode sequences available from eight species applying several species delimitation methods, and provide additional evidence that populations within *Calisto grannus* group are conspecific.

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Material and methods

Specimen collections. The field work involved collecting trips from 2010 to 2014 across the Dominican Republic including the visit to the type localities, or their vicinity, for all species previously described, mentioned in detail by Schwartz (1989).

Morphological characters and species diagnosis. We investigated those taxonomic characters traditionally used in the work on the *hysius* group (Bates 1935, Michener 1943, Gonzalez 1987). As a starting point, we followed the species definition for each taxon of the *hysius* species group proposed by the latest authors (Johnson & Hedges 1998, Sourakov & Zakharov 2011).

Molecular protocols and sequence editing. 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 (TAAACTTCTGGATGTCCAAAAAATCA) (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 *hysius* group members were downloaded from GenBank, http://genbank.gov/ (Benson *et al.* 2014). However, we only used these from specimens with precise locality data, since some GenBank sequences have imprecise distribution data (Table 1).

Phylogenetic reconstruction. The phylogenetic reconstruction of the *hysius* species group was performed following Maximum Likelihood (ML) and Bayesian Inference (BI) methods. In both cases *Calisto eleleus* Bates was used as outgroup being one of the earliest divergent species of the genus (Matos– Maraví *et al.*, 2014). Our main goal was to assess the position of the new species described herein.

For the ML analysis, we used RAXML (Stamatakis *et al.* 2008) with 1000 rapid bootstrap replicates and a search for the maximum likelihood topology on the CIPRES portal (Miller *et al.* 2010). The data were modeled according to the GTR + G model.

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). The independent MCMC analyses were run for 20 million generations and the sampling of trees and parameters was set to every 1000 generations at CIPRES. 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.

Species delimitation methods. The species delimitation methods Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), General Mixed Yule Coalescent model (GMYC) (Pons *et al.* 2006, Monaghan *et al.* 2009) and Poisson Tree Processes (PTP) (Zhang *et al.* 2013) were used to test the boundaries of species within the *hysius* groups as currently recognized.

The Automatic Barcode Gap Discovery method was used (Puillandre *et al.* 2011, 2012) to sort the available 215 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://wwwabi.snv.jussieu.fr/public/abgd/ using the default values for all parameters. The analysis was performed employing the three implemented models (Jukes–Cantor, K2P, and Simple Distance).

The Generalized Mixed Yule Coalescent (GMYC) method (Fujisawa & Barraclough 2013, Pons *et al.* 2006) is a likelihood method for delimiting independently evolving species. The GMYC method requires an ultrametric tree without identical sequences to avoid zero length terminal branches that hamper the likelihood estimation. The ultrametric tree was obtained in an analysis performed using BEAST v.1.8.2. (Drummond *et al.* 2012) at CIPRES

server with 100 million generations and sampling every 10000 generations, under a HKY substitution model. The GMYC was run as implemented in the GMYC web server (http://species.h–its.org/gmyc/).

The Poisson tree processes (PTP) is a new model that can delimit species using non ultrametric phylogenies (Zhang *et al.* 2013). The fundamental assumption of this method is that the number of substitutions is significantly higher between species than within species (Zhang *et al.* 2013). There are three variants of this method available in web servers and all were implemented. bPTP is an version of the original maximum likelihood PTP (maximum likelihood PTP search result is part of the bPTP results) that adds Bayesian support (BS) values to delimited species on the input tree. Higher BS value on a node indicates all descendants from this node are more likely to be from one species (Zhang *et al.* 2013). Analysis used the tree resulting in newick format from the BI analysis as input for the bPTP server, http://species.h–its.org/ (Zhang *et al.* 2013). We also applied the Multi-rate PTP method, Kapli *et al.* (2016), using the ML analysis tree as input for the two variants, mPTP and PTP, available at http://mptp.h–its.org/#/tree.

We also compared the results with the output of the BIN system from BOLD. This system employs a two-stage algorithm (Refined Single Linkage) that couples single linkage and Markov clustering to assign sequences to a sequence cluster that is subsequently assigned a unique identifier termed a Barcode Index Number (Ratnasingham & Hebert 2013). The Refined Single Linkage algorithm matches the taxonomic performance of competing approaches, but couples this with protocols that are simple enough to allow the automated assignment of all new barcode records to a BIN (Ratnasingham & Hebert 2013).

Character base. We inspected visually the COI alignment 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). We arranged the clustered sequences of each hypothetical species and then by localities in a single fasta file. Then this file was visually inspected in MEGA looking for unique substitutions at each site within hypothetical species as well as within populations.

Abbreviations and acronyms:

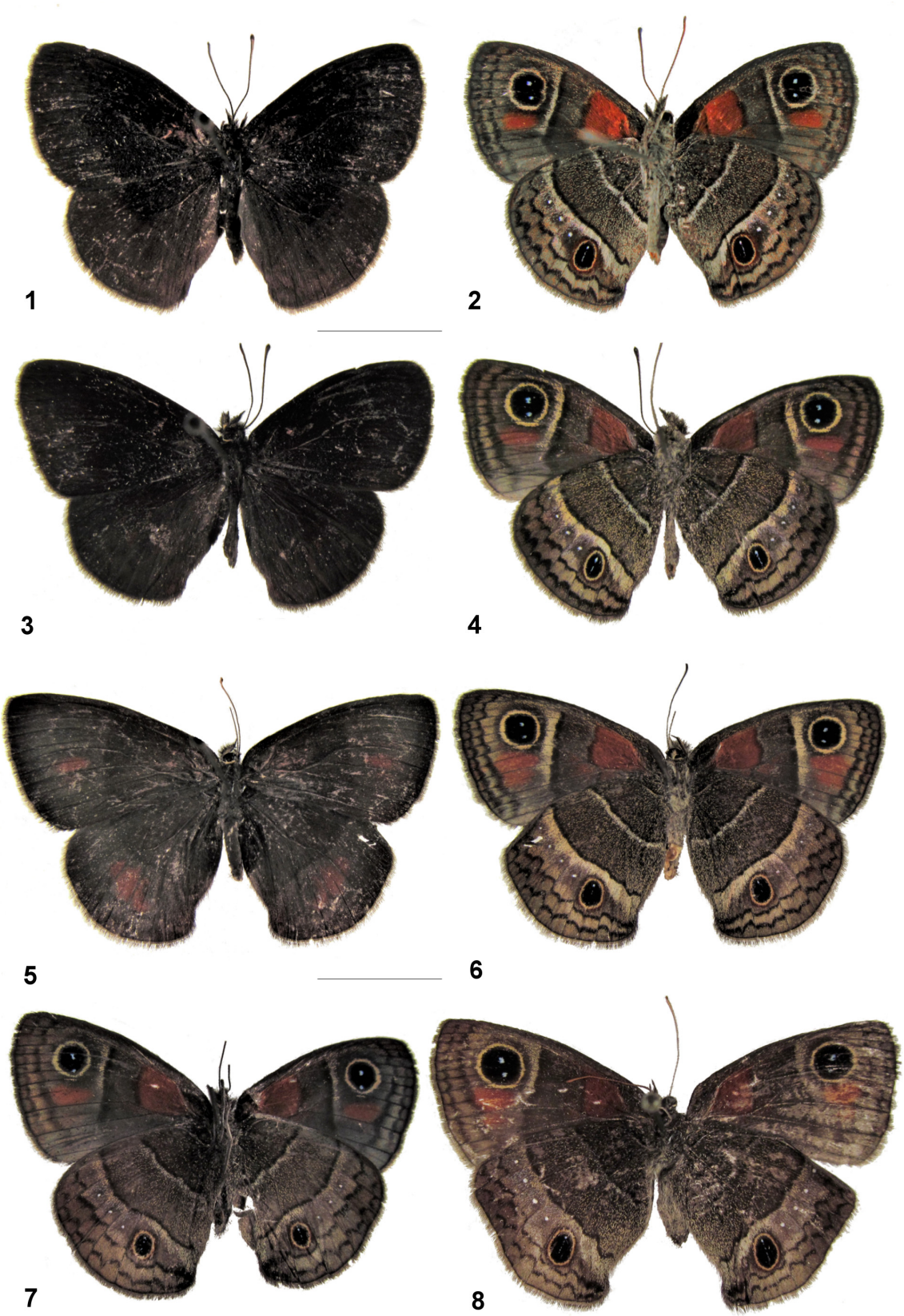
ABGD	Automatic Barcode Gap Discovery
BIN	Barcode Index Number
BS	Maximum Likelihood Bootstrap
FW	forewing
FWL	forewing length
GMYC	Generalize Mixed Yule Coalescent
HW	hindwing
K2P	Kimura 2 parameter
PP	Bayes posterior probability
PTP	Poisson Tree Process
VGRC	Victor González Research Collection, San Juan, Puerto Rico
ZSM	Zoologische Staatssammlung Muenchen, Munich, Germany

Results

Taxonomy

***Calisto bahoruco* Pérez–Asso, Núñez & Genaro, new species**
Figures 1–6, 9–10

Diagnosis. *Calisto bahoruco* **n. sp.** requires comparison with its closest relative *C. hysius*. Both have a similar color pattern but differ in several characters. Average FWL is smaller in *C. bahoruco,* 15 mm in males (n=5) and 16.8 mm females (n=5), than in *C. hysius*, 16.5 mm in males (n=7), 18.3 mm in females (n=4). Ocelli are distinctly larger in *C. bahoruco* with a median of the ratio ocellus largest diameter/wing length of 0.20 at FW and 0.18 at HW. Values in *C. hysius* are 0.17 and 0.14 at FW and HW respectively. Wing pattern of *C. bahoruco* shows a darker background on the under surface, the discal of the HW is straighter and smooth, the overall pale scaling on



FIGURES 1–8. Adults of the *hysius* species group of *Calisto*. 1–2 *C. bahoruco* **new species**, male holotype, Villa Nizao, Paraíso, Barahona, República Dominicana: 1—upper surface, 2—under surface. 3–4 *C. bahoruco* **new species**, male paratype, same data: 3—upper surface, 4—under surface. 5–6 *C. bahoruco* **new species**, female paratype, same data: 5—upper surface, 6—under surface. 7–8 Under surface *C. hysius*, Los Arroyos, Pedernales, Sierra de Bahoruco, República Dominicana: 7—male, 8—female. Scale bar 10 mm. Pictures by Antonio R. Pérez–Asso.

FIGURES 9–12. Living adults of the *hysius* species group of *Calisto*. 9–10 *C. bahoruco* **new species**, Villa Nizao, Paraíso, Barahona, República Dominicana. 11–12 *C. hysius*, Los Arroyos, Pedernales, Sierra de Bahoruco, República Dominicana. Pictures by Pérez–Asso.

the distal edge of lines is much more contrasting compared to the equivalent in *C. hysius* which shows a paler ground colour, less contrasting distal edges of lines and the discal line more irregular than that in *C. bahoruco*. Genitalia are very similar in both sexes, however, the tegumen of *C. hysius* male broadly protrudes backwards compared to that of *C. bahoruco* (Figs. 13, 14), and in addition the male structure of the latter is notably more sclerotized (Fig. 13). The minimum pairwise K2P genetic distance among *C. bahoruco* **n. sp.** and a representative of the *hysius* group is to *C. hysius*, 2.3%, with intraspecific genetic distances averaging 0.14 and 0.05% respectively (Table 2). Comparison of COI barcodes belonging to both species showed *C. hysius* has a cytosine at positions 187 and 517, characters absent from any other *hysius* group member including *C. bahoruco* **n. sp.** (Table 3). The barcode of the latter species bears a thymine at position 220 whereas *C. hysius* possesses cytosine. However, this character is not exclusive since it is present in two sequences of *C. batesi*, all remaining 207 analyzed sequences bear a cytosine in that position. Additional non exclusive nucleotide positions distinguishing the COI barcodes of both species are mentioned in the Species delimitation methods section.

Description. Male (Figs 1–4): FWL 14.2–16.2 mm. Upper surface blackish brown, FW with triangular androconial patch from base to the cell on the area below the latter. Underside dark brown, paler beyond the post discal line on the FW. Discal cell with a red spot outwardly edged by a transverse blackish brown line. FW post discal line with a narrow pale yellow band on its outer edge. FW ocellus moderately large, circular, with two white pupils and edged below by a red spot. HW dark brown, background color formed by a mix of dark brown and pale yellow scales. HW discal line moderately straight outwardly edged by a narrow but distinctive band of white scales. HW ocellus elliptical, with a single basal white pupil and a trace of white scales along its larger axis; area

around external ring rusty colored. Area above ocellus with four tiny white dots being the one at Sc–Rs interspace the smaller. Post discal and subterminal lines edged by pale yellow contrasting scales on the outer and inner sides respectively.

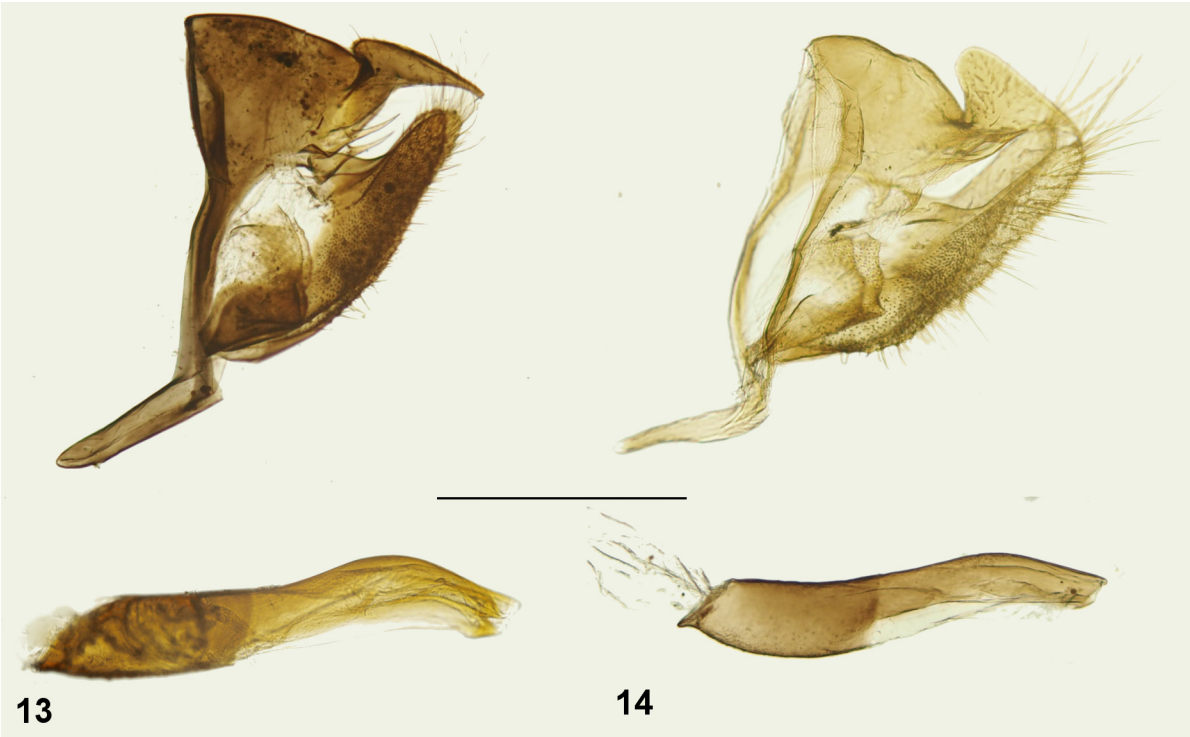
Genitalia (Fig. 13). As illustrated.

Female (Figs 5, 6): FWL 15.7–17.9 mm. Similar to male except: Upperside of wings brown, outer half of wings paler. Outer third of the four wings with a rusty spot at central position.

Genitalia (Fig. 15). As illustrated.

HOLOTYPE. Male. Villa Nizao, Paraíso, Barahona, República Dominicana, 5–VIII–2014, A. R. Pérez–Asso & A. López coll., DNA voucher code JAGWI–1018 (VGRC).

PARATYPES. 4♂, 5♀, same data as holotype except DNA voucher codes JAGWI–1015, 1016, 1018, R–114, R–115 (VGRC). Genitalia slide preparations ♂: Rh1745, Rh1752, and ♀: Rh1747 (ZSM).



FIGURES 13–14. Male genitalia of the *hysius* species group of *Calisto*, lateral view 13—*C. bahoruco*, **new species**. 14—*C. hysius*. Scale bar 0.5 mm.

Distribution (Fig. 17). Only known from the type locality, Villa Nizao, Barahona province, at the foothills of eastern extreme of Sierra de Bahoruco, Dominican Republic. Apparently the species also inhabits several localities around the type locality, all at Barahona province (see Discussion).

Natural history. Unknown. The type series was collected only at the type locality, a secondary mesic forest mixed with coffee plantations.

Comparative examined material. *Calisto hysius*:

Type. *Satyrus hyisus* 68, Godart (RSM). Reviewed through pictures available at Butterflies of America website (Warren *et al.* 2015).

Additional material (9♂, 4♀): camino de Los Arroyos a Ojo de Agua, Pedernales, República Dominicana, 28–VII–2010, A. López, A. R. Pérez–Asso & J. A. Genaro colls., DNA voucher codes WI–JAG–493–497 (5 ♂). Same data as anterior except July 2010, J. A. Genaro & A. R. Pérez–Asso colls., DNA voucher code WI–JAG–549 (♂). El Aguacate, 1055 m, pinar, Sierra de Bahoruco, Pedernales, República Dominicana, 9–Jun–2011, A. López & A. R. Pérez–Asso colls. (3 ♀). Zapoten, 1545 m, pinar, Sierra de Bahoruco, Pedernales, República Dominicana, 11–Jun–2011, A. López & A. R. Pérez–Asso colls. (1 ♂, 1 ♀). Las Abejas, pinar–latifoliado, PN Sierra de Bahoruco, Pedernales, República Dominicana, 20–11–2011, A. R. Pérez–Asso & A. López colls. (2 ♂). (VGRC).
Genitalia slide preparations ♂: Rh1743, Rh1746, and ♀: Rh1739, Rh1744 (ZSM).

FIGURES 15–16. Female genitalia of the *hysius* species group of *Calisto*, ventral view. 15—*C. bahoruco*, **new species**. 16—*C. hysius*. Scale bar 1 mm.

FIGURE 17. Geographic distribution of the *hysius* species group of *Calisto*. Black rhombus—type locality of *C. bahoruco*, **new species**; question marks—potential distribution of *C. bahoruco* following information by Schwartz (1989) and Warren et al. (2015); white rhombus—type locality of *C. hysius*, after Johnson & Hedges (1998); white circles—distribution of *C. hysius*, after Schwartz (1989).

COI barcodes of the *hysius* group species

We obtained 150 COI barcodes and downloaded 59 additional sequences from GenBank. The 209 sequences in total were 570 to 658 bp long representing eight of the eleven species of the hysius group, including *C. bahoruco*. Only *C. loxias* Bates, *C. pauli* Johnson & Hedges and *C. thomasi* Johnson & Hedges lack sequences.

Barcodes had 166 variable sites, 25.2%, of which 153 were informative, 23.3%. Intraspecific variation ranged from 0.04 to 0.77% whereas minimum pairwise distances from 2.3 to 12.4%, or 15 to 65 differences (Table 2).

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 (BOLD, www.boldsystems.org).

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto grannus amazona	WI-JAG-857	Dominican Republic, San Juan, Comparticion, Cordillera Central	MF172153
Calisto grannus amazona	WI-JAG-856	Dominican Republic, San Juan, Comparticion, Cordillera Central	MF172119
Calisto grannus amazona	WI-JAG-855	Dominican Republic, San Juan, Comparticion, Cordillera Central	MF172167
Calisto grannus amazona	WI-JAG-854	Dominican Republic, San Juan, Comparticion, Cordillera Central	MF172179
Calisto grannus amazona	WI-JAG-853	Dominican Republic, San Juan, Comparticion, Cordillera Central	MF172144
Calisto grannus amazona	WI-JAG-852	Dominican Republic, San Juan, Valle de Bao, Cordillera Central	MF172209
Calisto grannus amazona	WI-JAG-851	Dominican Republic, San Juan, Valle de Bao, Cordillera Central	MF172173
Calisto grannus amazona	WI-JAG-850	Dominican Republic, San Juan, Valle de Bao, Cordillera Central	MF172160
Calisto grannus amazona	WI-JAG-849	Dominican Republic, San Juan, Valle de Bao, Cordillera Central	MF172102
Calisto grannus amazona	WI-JAG-848	Dominican Republic, San Juan, Valle de Bao, Cordillera Central	MF172104
Calisto bahoruco	WI-JAG-1019	Dominican Republic, Barahona, Villa Nazao, Paraiso	MF172195
Calisto bahoruco	WI-JAG-1018	Dominican Republic, Barahona, Villa Nazao, Paraiso	MF172133
Calisto bahoruco	WI-JAG-1016	Dominican Republic, Barahona, Villa Nizao, Paraiso	MF172180
Calisto bahoruco	WI-JAG-1015	Dominican Republic, Barahona, Villa Nizao, Paraiso	MF172161
Calisto bahoruco	R-114	Dominican Republic, Barahona, Villa Nizao, Paraiso	MF172252
Calisto bahoruco	R-115	Dominican Republic, Barahona, Villa Nizao, Paraiso	MF172253
Calisto batesi	WI-JAG-517	Dominican Republic, Pico Diego de Ocampo	JN299994
Calisto batesi	WI-JAG-981	Dominican Republic, La Vega, Jarabacoa	MF172171
Calisto batesi	WI-JAG-515	Dominican Republic, camino a Playa Fronton, Samana	JN299992
Calisto batesi	WI-JAG-513	Dominican Republic, Puerto Plata, Pico Isabel de Torres	JN299990
Calisto batesi	WI-JAG-512	Dominican Republic, Las Abejas, Sierra Bahoruco	JN299989
Calisto batesi	WI-JAG-548	Dominican Republic, Las Abejas, Sierra Bahoruco	JN300024
Calisto batesi	WI-JAG-516	Dominican Republic, Pico Diego de Ocampo	JN299993
Calisto batesi	WI-JAG-514	Dominican Republic, camino a Playa Fronton, Samana	JN299991

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto batesi	WI-JAG-911	Dominican Republic, Santiago Rodriguez, La Leonor	MF172192
Calisto batesi	WI-JAG-910	Dominican Republic, Santiago Rodriguez, La Leonor	MF172188
Calisto batesi	WI-JAG-909	Dominican Republic, Santiago Rodriguez, La Leonor	MF172156
Calisto batesi	WI-JAG-908	Dominican Republic, Santiago Rodriguez, La Leonor	MF172165
Calisto batesi	WI-JAG-907	Dominican Republic, Santiago Rodriguez, La Leonor	MF172189
Calisto batesi	WI-JAG-906	Dominican Republic, Santiago Rodriguez, La Leonor	MF172154
Calisto batesi	WI-JAG-905	Dominican Republic, Santiago Rodriguez, La Leonor	MF172185
Calisto batesi	WI-JAG-904	Dominican Republic, Santiago Rodriguez, La Leonor	MF172169
Calisto batesi	WI-JAG-903	Dominican Republic, Santiago Rodriguez, La Leonor	MF172113
Calisto batesi	WI-JAG-902	Dominican Republic, Santiago Rodriguez, La Leonor	MF172118
Calisto batesi	WI-JAG-901	Dominican Republic, Santiago, Pico El Rubio	MF172120
Calisto batesi	WI-JAG-900	Dominican Republic, Santiago, Pico El Rubio	MF172106
Calisto batesi	WI-JAG-899	Dominican Republic, Santiago, Pico El Rubio	MF172108
Calisto batesi	WI-JAG-898	Dominican Republic, Santiago Rodriguez, La Leonor	MF172124
Calisto batesi	WI-JAG-897	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172174
Calisto batesi	WI-JAG-896	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172105
Calisto batesi	WI-JAG-895	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172099
Calisto batesi	WI-JAG-894	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172182
Calisto batesi	WI-JAG-893	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172208
Calisto batesi	WI-JAG-892	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172181
Calisto batesi	McGuire09-CAL-Sat6	Dominican Republic, Santiago	JN197311
Calisto batesi	McGuire09-CAL-Sat7	Dominican Republic, Santiago	JN197312
Calisto batesi	McGuire09-CAL-Sat38	Dominican Republic, Elias Pina	JN197308
Calisto batesi	McGuire09-CAL-Sat64	Dominican Republic, Elias Pina	JN197307
Calisto batesi	McGuire09-CAL-Sat78	Dominican Republic, La Vega	JN197306

.....continued on the next page

TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto confusa	WI-JAG-942	Dominican Republic, La Vega, El Aroyazo, Ebano Verde	MF172205
Calisto confusa	WI-JAG-939	Dominican Republic, Santiago, Loma del Toro	MF172159
Calisto confusa	WI-JAG-938	Dominican Republic, Santiago Rodriguez, La Leonor	MF172121
Calisto confusa	WI-JAG-937	Dominican Republic, Santiago Rodriguez, La Leonor	MF172135
Calisto confusa	WI-JAG-1017	Dominican Republic, Barahona, Villa Nizao, Paraiso	MF172101
Calisto confusa	WI-JAG-543	Dominican Republic, Mirador de Paraiso, Barahona	JN300019
Calisto confusa	WI-JAG-969	Dominican Republic, Independencia, Zapoten, Sierra de Bahoruco	MF172206
Calisto confusa	WI-JAG-967	Dominican Republic, La Vega, PN Armando Bermudez, La Cienaga	MF172177
Calisto confusa	WI-JAG-940	Dominican Republic, Santiago, Loma del Toro	MF172139
Calisto confusa	WI-JAG-511	Dominican Republic, salto La Demajagua	JN299988
Calisto confusa	WI-JAG-510	Dominican Republic, salto La Demajagua	JN299987
Calisto confusa	WI-JAG-508	Dominican Republic, camino a Playa Fronton, Samana	JN299985
Calisto confusa	WI-JAG-970	Dominican Republic, Independencia, Las Abejas, Sierra de Bahoruco	MF172157
Calisto confusa	NW149-18	Dominican Republic	KF054329
Calisto confusa	DR016	Dominican Republic	JN881879
Calisto confusa	DR032	Dominican Republic	KF054319
Calisto confusa	McGuire09-CAL-Sat35	Dominican Republic, Puerto Plata	JN197331
Calisto confusa	McGuire09-CAL-Sat45	Dominican Republic, La Vega	JN197330
Calisto confusa	McGuire09-CAL-Sat46	Dominican Republic, La Vega	JN197329
Calisto confusa	McGuire09-CAL-Sat58	Dominican Republic, Elias Pina	JN197328
Calisto confusa	McGuire09-CAL-Sat113	Dominican Republic, Sierra de Bahoruco	JN197327
Calisto debarriera	WI-JAG-979	Dominican Republic, Santiago, La Leonor, Moncion	MF172107
Calisto debarriera	WI-JAG-944	Dominican Republic, La Vega, El Aroyazo, Ebano Verde	MF172112
Calisto debarriera	WI-JAG-968	Dominican Republic, La Vega, La Nuez, Valle Nuevo	MF172176
Calisto debarriera	WI-JAG-963	Dominican Republic, La Vega, La Cienaga	MF172142

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto debarriera	WI-JAG-962	Dominican Republic, La Vega, La Cienaga	MF172200
Calisto debarriera	WI-JAG-533	Dominican Republic, Puerto Plata, Pico Isabel de Torres	JN300009
Calisto debarriera	WI-JAG-945	Dominican Republic, La Vega, El Aroyazo, Ebano Verde	MF172170
Calisto debarriera	WI-JAG-943	Dominican Republic, La Vega, El Aroyazo, Ebano Verde	MF172109
Calisto debarriera	WI-JAG-941	Dominican Republic, La Vega, El Aroyazo, Ebano Verde	MF172098
Calisto debarriera	WI-JAG-509	Dominican Republic, Puerto Plata, Pico Isabel de Torres	JN299986
Calisto debarriera	WI-JAG-507	Dominican Republic, Parque Nacional A Bermudez, La Cienaga	JN299984
Calisto debarriera	WI-JAG-818	Dominican Republic, Puesto Piramide 204, Sierra de Neiba	MF172125
Calisto debarriera	McGuire09-CAL-Sat5	Dominican Republic, Santiago	JN197341
Calisto debarriera	McGuire09-CAL-Sat50	Dominican Republic, La Vega	JN197340
Calisto debarriera	McGuire09-CAL-Sat51	Dominican Republic, La Vega	JN197339
Calisto debarriera	McGuire09-CAL-Sat59	Dominican Republic, Elias Pina	JN197338
Calisto debarriera	McGuire09-CAL-Sat75	Dominican Republic, La Vega	JN197337
Calisto debarriera	DR019	Dominican Republic	KF054318
Calisto debarriera	McGuire09-CAL-Sat112	Dominican Republic, Sierra de Bahoruco	JN197380
Calisto grannus dilemma	WI-JAG-837	Dominican Republic, La Vega, 8 km colonia japanesa, Valle Nuevo	MF172150
Calisto grannus dilemma	WI-JAG-836	Dominican Republic, La Vega, 8 km colonia japanesa, Valle Nuevo	MF172175
Calisto grannus dilemma	WI-JAG-835	Dominican Republic, La Vega, 8 km colonia japanesa, Valle Nuevo	MF172134
Calisto grannus dilemma	WI-JAG-834	Dominican Republic, La Vega, 8 km colonia japanesa, Valle Nuevo	MF172122
Calisto grannus dilemma	WI-JAG-833	Dominican Republic, La Vega, 8 km colonia japanesa, Valle Nuevo	MF172132
Calisto grannus dilemma	McGuire09-CAL-Sat2	Dominican Republic, Santiago	JN197365
Calisto grannus dilemma	McGuire09-CAL-Sat4	Dominican Republic, Santiago	JN197364
Calisto grannus dilemma	McGuire09-CAL-Sat8	Dominican Republic, Santiago	JN197363
Calisto grannus dilemma	McGuire09-CAL-Sat37	Dominican Republic, Elias Pina	JN197362
Calisto grannus dilemma	McGuire09-CAL-Sat39	Dominican Republic, Elias Pina	JN197361

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto grannus dilemma	McGuire09-CAL-Sat42	Dominican Republic, Elias Pina	JN197360
Calisto grannus dilemma	McGuire09-CAL-Sat43	Dominican Republic, Elias Pina	JN197359
Calisto grannus dilemma	McGuire09-CAL-Sat44	Dominican Republic, Elias Pina	JN197358
Calisto grannus dilemma	McGuire09-CAL-Sat74	Dominican Republic, La Vega	JN197357
Calisto grannus dilemma	McGuire09-CAL-Sat76	Dominican Republic, La Vega	JN197356
Calisto grannus dilemma	McGuire09-CAL-Sat77	Dominican Republic, La Vega	JN197355
Calisto grannus dilemma	McGuire09-CAL-Sat79	Dominican Republic, La Vega	JN197354
Calisto grannus dilemma	McGuire09-CAL-Sat80	Dominican Republic, La Vega	JN197353
Calisto grannus dilemma	McGuire09-CAL-Sat82	Dominican Republic, Elias Pina	JN197352
Calisto grannus dystacta	WI-JAG-847	Dominican Republic, Santiago, La Leonor	MF172168
Calisto grannus dystacta	WI-JAG-846	Dominican Republic, Santiago, La Leonor	MF172163
Calisto grannus dystacta	WI-JAG-845	Dominican Republic, Santiago, La Leonor	MF172187
Calisto grannus dystacta	WI-JAG-844	Dominican Republic, Santiago, La Leonor	MF172136
Calisto grannus grannus	WI-JAG-551	Dominican Republic, Valle Nuevo, Constanza	JN300027
Calisto grannus grannus	WI-JAG-946	Dominican Republic, La Vega, 8 km colonia japonesa, Valle Nuevo	MF172184
Calisto grannus grannus	WI-JAG-814	Dominican Republic, La Vega, La Nuez, Valle Nuevo	MF172114
Calisto grannus grannus	WI-JAG-813	Dominican Republic, La Vega, La Nuez, Valle Nuevo	MF172207
Calisto grannus grannus	WI-JAG-812	Dominican Republic, La Vega, La Nuez, Valle Nuevo	MF172097
Calisto grannus grannus	WI-JAG-811	Dominican Republic, La Vega, La Piramide, Valle Nuevo	MF172115
Calisto grannus grannus	WI-JAG-810	Dominican Republic, La Vega, La Piramide, Valle Nuevo	MF172155
Calisto grannus grannus	McGuire09-CAL-Sat27	Dominican Republic, La Vega	JN197351
Calisto grannus grannus	McGuire09-CAL-Sat	Dominican Republic, La Vega	JN197350
Calisto grannus grannus	McGuire09-CAL-Sat67	Dominican Republic, La Vega	JN197349
Calisto grannus grannus	McGuire09-CAL-Sat69	Dominican Republic, La Vega	JN197348
Calisto grannus grannus	McGuire09-CAL-Sat70	Dominican Republic, La Vega	JN197347

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto grannus grannus	McGuire09-CAL-Sat95	Dominican Republic, La Vega	JN197346
Calisto grannus grannus	McGuire09-CAL-Sat96	Dominican Republic, La Vega	JN197345
Calisto grannus grannus	McGuire09-CAL-Sat47	Dominican Republic, La Vega	JN197366
Calisto hysius	WI-JAG-497	Dominican Republic, Pedernales, camino de Los Arroyos a Ojo de Agua	JN299974
Calisto hysius	WI-JAG-496	Dominican Republic, Pedernales, camino de Los Arroyos a Ojo de Agua	JN299973
Calisto hysius	WI-JAG-495	Dominican Republic, Pedernales, camino de Los Arroyos a Ojo de Agua	JN299972
Calisto hysius	WI-JAG-494	Dominican Republic, Pedernales, camino de Los Arroyos a Ojo de Agua	JN299971
Calisto hysius	WI-JAG-493	Dominican Republic, Pedernales, camino de Los Arroyos a Ojo de Agua	JN299970
Calisto hysius	WI-JAG-549	Dominican Republic, camino de Los Arroyos a Ojo de Agua, Sierra de Bahoruco	JN300025
Calisto hysius	McGuire09-CAL-Sat83	Dominican Republic, Pedernales	JN197375
Calisto hysius	McGuire09-CAL-Sat84	Dominican Republic, Pedernales	JN197374
Calisto grannus micheneri	WI-JAG-832	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172141
Calisto grannus micheneri	WI-JAG-831	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172172
Calisto grannus micheneri	WI-JAG-830	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172197
Calisto grannus micheneri	WI-JAG-829	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172117
Calisto grannus micheneri	WI-JAG-828	Dominican Republic, Santiago, Loma del Toro, Cordillera Central	MF172100
Calisto grannus micrommata	WI-JAG-822	Dominican Republic, Puesto Piramide 204, Sierra de Neiba	MF172193
Calisto grannus micrommata	WI-JAG-821	Dominican Republic, Puesto Piramide 204, Sierra de Neiba	MF172127
Calisto grannus micrommata	WI-JAG-820	Dominican Republic, Puesto Piramide 204, Sierra de Neiba	MF172166
Calisto grannus micrommata	WI-JAG-819	Dominican Republic, Puesto Piramide 204, Sierra de Neiba	MF172190
Calisto obscura	WI-JAG-936	Dominican Republic, Santiago, Pico El Rubio	MF172151
Calisto obscura	WI-JAG-935	Dominican Republic, Santiago, Pico El Rubio	MF172191
Calisto obscura	WI-JAG-934	Dominican Republic, Santiago, Pico El Rubio	MF172130
Calisto obscura	WI-JAG-933	Dominican Republic, Santiago, Pico El Rubio	MF172186

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto obscura	WI-JAG-932	Dominican Republic, Santiago, Pico El Rubio	MF172138
Calisto obscura	WI-JAG-544	Dominican Republic, Las Mercedes, Pedernales,	JN300020
Calisto obscura	WI-JAG-542	Dominican Republic, Las Abejas, Sierra Bahoruco	JN300018
Calisto obscura	WI-JAG-532	Dominican Republic, Fondo Paradi, Jaragua	JN300008
Calisto obscura	WI-JAG-531	Dominican Republic, Mirador de Paraiso, Barahona	JN300007
Calisto obscura	WI-JAG-530	Dominican Republic, Mirador de Paraiso, Barahona	JN300006
Calisto obscura	WI-JAG-529	Dominican Republic, camino de Pedernales, a Ojo de Agua, Sierra Bahoruco	JN300005
Calisto obscura	WI-JAG-522	Dominican Republic, Las Abejas, Sierra Bahoruco	JN299999
Calisto obscura	WI-JAG-521	Dominican Republic, Las Abejas, Sierra Bahoruco	JN299998
Calisto obscura	WI-JAG-520	Dominican Republic, Las Abejas, Sierra Bahoruco	JN299997
Calisto obscura	WI-JAG-519	Dominican Republic, Las Abejas, Sierra Bahoruco	JN299996
Calisto obscura	WI-JAG-772	Dominican Republic, Pedernales, Mapioro, Oviedo	MF172199
Calisto obscura	WI-JAG-771	Dominican Republic, Pedernales, Fondo Paradi, Oviedo	MF172198
Calisto obscura	WI-JAG-770	Dominican Republic, Pedernales, Aceitillar, Sierra de Bahoruco	MF172183
Calisto obscura	WI-JAG-769	Dominican Republic, Pedernales, Caseta 1, Sierra de Bahoruco	MF172162
Calisto obscura	WI-JAG-768	Dominican Republic, Pedernales, Las Abejas Sierra de Bahoruco	MF172147
Calisto obscura	WI-JAG-767	Dominican Republic, Pedernales, Las Abejas Sierra de Bahoruco	MF172103
Calisto obscura	WI-JAG-766	Dominican Republic, Pedernales, Las Abejas Sierra de Bahoruco	MF172210
Calisto obscura	McGuire09-CAL-Sat9	Dominican Republic, Santiago	JN197392
Calisto obscura	McGuire09-CAL-Sat10	Dominican Republic, Santiago	JN197391
Calisto obscura	McGuire09-CAL-Sat13	Dominican Republic, Santiago	JN197390
Calisto obscura	McGuire09-CAL-Sat34	Dominican Republic, La Altagracia	JN197389
Calisto obscura	McGuire09-CAL-Sat40	Dominican Republic, Elias Pina	JN197388
Calisto obscura	McGuire09-CAL-Sat122	Dominican Republic, Pedernales	JN197386

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto obscura	NW149-12	Dominican Republic	KF054324
Calisto obscura	NW150-1	Dominican Republic	KF054330
Calisto grannus phoinix	WI-JAG-843	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172129
Calisto grannus phoinix	WI-JAG-842	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172145
Calisto grannus phoinix	WI-JAG-841	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172196
Calisto grannus phoinix	WI-JAG-840	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172146
Calisto grannus phoinix	WI-JAG-839	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172158
Calisto grannus phoinix	WI-JAG-838	Dominican Republic, La Vega, El Arroyazo, Ebano Verde	MF172194
Calisto sommeri	WI-JAG-550	Dominican Republic, camino entre Las Abejas y caseta 2, Sierra Bahoruco	JN300026
Calisto sommeri	WI-JAG-506	Dominican Republic, pinar entre Las Abejas y caseta 2, Sierra Bahoruco	JN299983
Calisto sommeri	WI-JAG-505	Dominican Republic, pinar entre Las Abejas y caseta 2, Sierra Bahoruco	JN299982
Calisto sommeri	WI-JAG-504	Dominican Republic, pinar entre Las Abejas y caseta 2, Sierra Bahoruco	JN299981
Calisto sommeri	WI-JAG-503	Dominican Republic, Pedernales, Los Arroyos, Sierra Bahoruco	JN299980
Calisto sommeri	WI-JAG-502	Dominican Republic, Pedernales, Los Arroyos, Sierra Bahoruco	JN299979
Calisto sommeri	WI-JAG-501	Dominican Republic, Pedernales, Los Arroyos, Sierra Bahoruco	JN299978
Calisto sommeri	WI-JAG-492	Dominican Republic, Pedernales, Los Arroyos, Sierra Bahoruco	JN299969
Calisto sommeri	WI-JAG-827	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172148
Calisto sommeri	WI-JAG-817	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172201
Calisto sommeri	WI-JAG-816	Dominican Republic, Pedernales, Aceitillar, Sierra de Bahoruco	MF172149
Calisto sommeri	WI-JAG-815	Dominican Republic, Pedernales, caseta 2, Sierra de Bahoruco	MF172137
Calisto sommeri	McGuire09-CAL-Sat52	Dominican Republic, Pedernales	JN197401
Calisto sommeri	McGuire09-CAL-Sat53	Dominican Republic, Pedernales	JN197400
Calisto sommeri	McGuire09-CAL-Sat54	Dominican Republic, Pedernales	JN197399
Calisto sommeri	McGuire09-CAL-Sat55	Dominican Republic, Pedernales	JN197398
Calisto sommeri	McGuire09-CAL-Sat93	Dominican Republic, Pedernales	JN197397

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TABLE 1. (Continued)

Taxa	Voucher code	Locality	Genbank Accession Number
Calisto sommeri	McGuire09-CAL-Sat94	Dominican Republic, Pedernales	JN197396
Calisto tragius	WI-JAG-989	Dominican Republic, Independencia, caseta 3, Bahoruco	MF172126
Calisto tragius	WI-JAG-988	Dominican Republic, Independencia, caseta 3, Bahoruco	MF172111
Calisto tragius	WI-JAG-987	Dominican Republic, Independencia, caseta 3, Bahoruco	MF172110
Calisto tragius	WI-JAG-986	Dominican Republic, Independencia, caseta 3, Bahoruco	MF172140
Calisto tragius	WI-JAG-982	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172123
Calisto tragius	WI-JAG-826	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172131
Calisto tragius	WI-JAG-825	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172204
Calisto tragius	WI-JAG-824	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172152
Calisto tragius	WI-JAG-823	Dominican Republic, Pedernales, Loma El Toro, Sierra de Bahoruco	MF172178
Calisto tragius	RN02-18	Dominican Republic	KF054342
Calisto eleleus	WI-JAG-538	Dominican Republic	JN300014

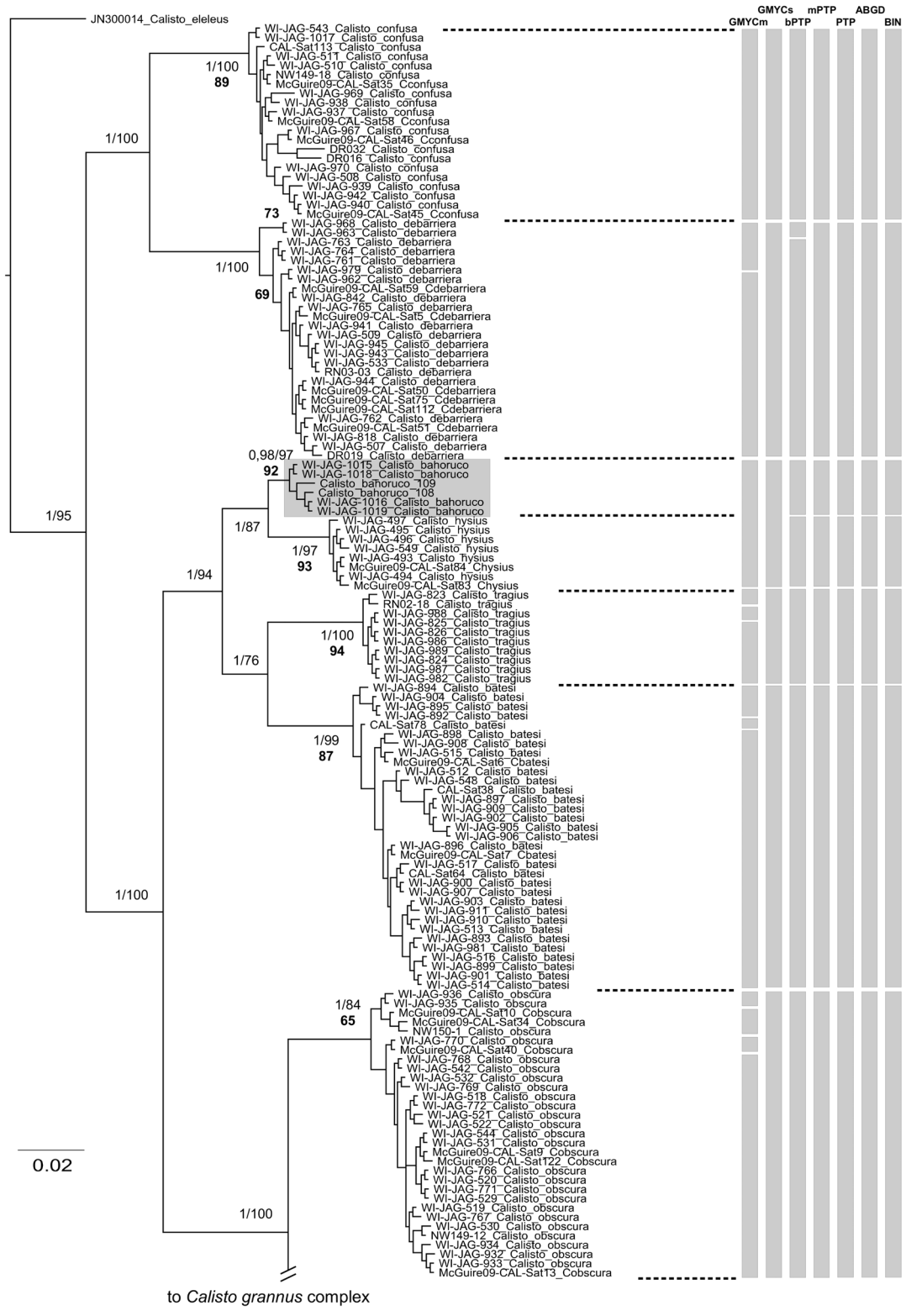
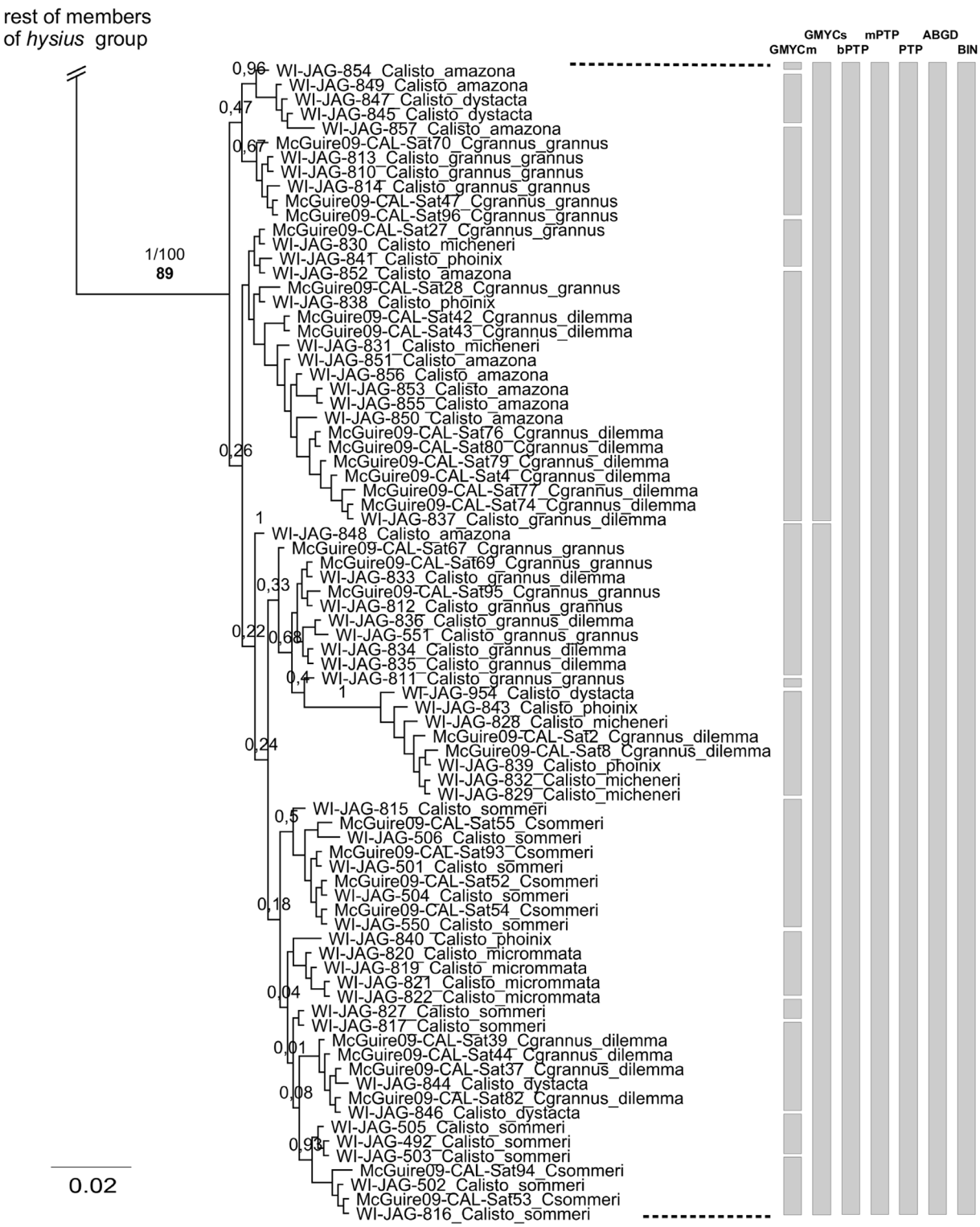


FIGURE 18. Bayesian COI barcodes gen tree reconstructed by MrBayes showing the relationships among taxa belonging to the *hysius* species group of *Calisto*. Numbers above branches represent the posterior probalities / boostrap values of Bayesian Inference / Maximum Likelihood. Bold numbers at species clades represent the support values for each species calculated by the Bayesian Poisson Tree Process (bPTP) species delimitation method. Gray box indicating the position of the species described herein, *C. bahoruco*. Gray bars at the right side of tree showing the species hypothesis obtained by the different species delimitation methods applied: Generalize Mixed Yule Coalescent single and multiple threshold (GMYCs, GMYCm), Poisson Tree Process, PTP, variants: Bayesian (bPTP), Maximum Likelihood (mPTP), and standard (PTP), Automatic Barcode Gap Discovery (ABGD), and Barcode Index Number (BIN).


rest of members
of *hysius* group
GMYCs
GMYCm
mPTP
bPTP
ABGD
PTP
BIN
1/100
89
0,96
0,47
0,67
0,26
1
0,33
0,22
0,68
0,4
1
0,24
0,5
0,18
0,04
0,01
0,08
0,93
WI-JAG-854_Calisto_amazona
WI-JAG-849_Calisto_amazona
WI-JAG-847_Calisto_dystacta
WI-JAG-845_Calisto_dystacta
WI-JAG-857_Calisto_amazona
McGuire09-CAL-Sat70_Cgrannus_grannus
WI-JAG-813_Calisto_grannus_grannus
WI-JAG-810_Calisto_grannus_grannus
WI-JAG-814_Calisto_grannus_grannus
McGuire09-CAL-Sat47_Cgrannus_grannus
McGuire09-CAL-Sat96_Cgrannus_grannus
McGuire09-CAL-Sat27_Cgrannus_grannus
WI-JAG-830_Calisto_micheneri
WI-JAG-841_Calisto_phoinix
WI-JAG-852_Calisto_amazona
McGuire09-CAL-Sat28_Cgrannus_grannus
WI-JAG-838_Calisto_phoinix
McGuire09-CAL-Sat42_Cgrannus_dilemma
McGuire09-CAL-Sat43_Cgrannus_dilemma
WI-JAG-831_Calisto_micheneri
WI-JAG-851_Calisto_amazona
WI-JAG-856_Calisto_amazona
WI-JAG-853_Calisto_amazona
WI-JAG-855_Calisto_amazona
WI-JAG-850_Calisto_amazona
McGuire09-CAL-Sat76_Cgrannus_dilemma
McGuire09-CAL-Sat80_Cgrannus_dilemma
McGuire09-CAL-Sat79_Cgrannus_dilemma
McGuire09-CAL-Sat4_Cgrannus_dilemma
McGuire09-CAL-Sat77_Cgrannus_dilemma
McGuire09-CAL-Sat74_Cgrannus_dilemma
WI-JAG-837_Calisto_grannus_dilemma
WI-JAG-848_Calisto_amazona
McGuire09-CAL-Sat67_Cgrannus_grannus
McGuire09-CAL-Sat69_Cgrannus_grannus
WI-JAG-833_Calisto_grannus_dilemma
McGuire09-CAL-Sat95_Cgrannus_grannus
WI-JAG-812_Calisto_grannus_grannus
WI-JAG-836_Calisto_grannus_dilemma
WI-JAG-551_Calisto_grannus_grannus
WI-JAG-834_Calisto_grannus_dilemma
WI-JAG-835_Calisto_grannus_dilemma
WI-JAG-811_Calisto_grannus_grannus
WI-JAG-954_Calisto_dystacta
WI-JAG-843_Calisto_phoinix
WI-JAG-828_Calisto_micheneri
McGuire09-CAL-Sat2_Cgrannus_dilemma
McGuire09-CAL-Sat8_Cgrannus_dilemma
WI-JAG-839_Calisto_phoinix
WI-JAG-832_Calisto_micheneri
WI-JAG-829_Calisto_micheneri
WI-JAG-815_Calisto_sommeri
McGuire09-CAL-Sat55_Csommeri
WI-JAG-506_Calisto_sommeri
McGuire09-CAL-Sat93_Csommeri
WI-JAG-501_Calisto_sommeri
McGuire09-CAL-Sat52_Csommeri
WI-JAG-504_Calisto_sommeri
McGuire09-CAL-Sat54_Csommeri
WI-JAG-550_Calisto_sommeri
WI-JAG-840_Calisto_phoinix
WI-JAG-820_Calisto_micrommata
WI-JAG-819_Calisto_micrommata
WI-JAG-821_Calisto_micrommata
WI-JAG-822_Calisto_micrommata
WI-JAG-827_Calisto_sommeri
WI-JAG-817_Calisto_sommeri
McGuire09-CAL-Sat39_Cgrannus_dilemma
McGuire09-CAL-Sat44_Cgrannus_dilemma
McGuire09-CAL-Sat37_Cgrannus_dilemma
WI-JAG-844_Calisto_dystacta
McGuire09-CAL-Sat82_Cgrannus_dilemma
WI-JAG-846_Calisto_dystacta
WI-JAG-505_Calisto_sommeri
WI-JAG-492_Calisto_sommeri
WI-JAG-503_Calisto_sommeri
McGuire09-CAL-Sat94_Csommeri
WI-JAG-502_Calisto_sommeri
McGuire09-CAL-Sat53_Csommeri
WI-JAG-816_Calisto_sommeri
0.02

18 (cont.)

Table 2. COI genetic distance values among taxa of the *hysius* group. Bottom right Kimura 2- parameters (K2P) minimum pairwise distances, upper left minium number of differences. Columns heading showing below each taxon the number sequences (n) and average K2P intraspecific distance.

	C. batesi n=33, 0.59	*C. confusa* n=21, 0.46	*C. debarriera* n=26, 0.23	*C. bahoruco* **n. sp.** n=6, 0.14	*C. hysius* n=8, 0.05	*C. obscura* n=31, *C. grannus* n=80, 0.47	0.77	*C. tragius* n=10, 0.04
C. batesi		56	47	32	38	48	49	31
C. confusa	9.8		33	49	49	64	65	48
C. debarriera	8.9	6.4		50	54	64	60	58
C. bahoruco **n. sp.**	5.1	8.5	9.5		15	48	50	35
C. hysius	6.1	8.5	10.3	2.3		47	54	35
C. obscura	8.5	12.1	12.4	8.7	8.3		29	55
C. grannus	8.8	11.9	12.4	9	9.8	5.4		52
C. tragius	5.7	9.8	11.5	6.5	6.5	10.9	11.1	

Table 3. Nucleotide sites with unique fixed states present in COI barcodes of species belonging to the *hysius* group of *Calisto*.

Taxa/Positions	**10**	**25**	**38**	**49**	**67**	**100**	**127**	**187**	**208**	**214**	**238**	**256**	**281**	**319**	**325**	**328**	**334**	**346**	**352**	**367**	**373**	**385**
C. confusa n=21	C	T	G	T	A	A	T	T	T	A	A	T	A	T	A	A	T	T	T	T	C	T
C. batesi n=33	T	C	G	T	T	A	A	T	A	A	T	T	A/G	T	A	T	T	C	A	T	A	T
C. debarriera n=26	T	T	A	C	A/G	T	T	T	A	A	C	A	C	T	A	A	C	T	C	T	A	C
C. obscura n=31	T	T	G	T	A	A	C	T	A	C	T	T	A	T	A	T	T	G	A	T	A	T
C. hysius n=8	T	T	G	T	A	A	A	C	A	A	C	T	A	T	A	T	T	T	A	T	A	T
C. grannus n=80	T	T	G	T	A/G	A/G	T	T	A	T	C	T	A/G	C	A	T	G	A	A	T	A/G	T
C. tragius n=10	T	T	G	T	A	A	A	T	A	A	T	T	G	T	T	C	T	T	A	C	A	T
C. bahoruco **n. sp.** n=6	T	T	G	T	A	A	A	T	A	A	C	T	A	T	A	T	T	T	A	T	A	T

(cont.)	**397**	**400**	**412**	**421**	**436**	**446**	**482**	**499**	**500**	**505**	**517**	**520**	**529**	**539**	**542**	**544**	**556**	**557**	**559**	**604**	**637**	**653**
C. confusa n=21	T	A	C	A	T	C	T	C	C	T	T	A	C	T	C	T	A	T	A	T	T	T
C. batesi n=33	T	C	T	A	T	T	T	A	T	T	A	A	T	T	C	T	A	T	G	T	C	T
C. debarriera n=26	T	C	A	A	T	T	T	T	T	C	T	A	G/T	T	C	T	A	T	A	T	T	C
C. obscura n=31	T	T	A	T	T	T	T	A/G	T	T	A	A	A	C	C	T	A/C	C	A	T	T	T
C. hysius n=8	T	T	T	A	T	T	T	A	T	T	C	A	A	T	C	T	A	T	A	T	T	T
C. grannus n=80	C	C	A	A	C	T	A/G	A	T	T	A	A	A	T	T	A/G	A	T	A	C	T	T
C. tragius n=10	T	T	T	C	T	T	T	A	T	T	T	T	A	T	C	T	T	T	A	T	T	T
C. bahoruco **n. sp.** n=6	T	C	T	A	T	T	T	A	T	T	T	A	A	T	C	T	A	T	A	T	T	T

Phylogenetic reconstruction with COI

Both phylogenetic methods recovered the same topology with all relationships strongly supported (Fig. 18). The only differences were evidenced within some species clades. *Calisto confusa* and *C. debarriera* were placed as sister species (PP= 1, BS=100) and the sister of all other *hysius* group species (1, 95). All other species were grouped in two clades (1, 100). *Calisto obscura* and *C. grannus* are sister species (1, 100) while the remaining four species were included in another clade (1, 94). The latter cluster is composed by other two species pairs: *C. hysius* and *C. bahoruco* (1, 87) and *C. tragius* and *C. batesi* (1, 76).

Species delimitation methods in the hysius group

Most species delimitation methods yielded results matching the current accepted species within the *hysius* group (Fig. 18, Appendix). The exception was the GMYC, mainly the multiple threshold variant that splitted all species except *C. confusa.* Both GMYC variants failed to recognize the new species here described, *C. bahoruco*. The new taxon was delimited by all other methods employed (Fig. 18, Appendix).

The character base approach revealed the existence of 2 to 11 diagnostic fixed nucleotide positions in the barcodes of each species belonging to the *hysius* group except *C. bahoruco* (Table 3). However, COI barcodes of the latter can be easily separated from these of its sister species, *C. hysius.* They differ by the change on nucleotide positions 187 and 517, which are diagnostic for *C. hysius* among all group members, but also in other 13 positions (*C. hysius*/*C. bahoruco*): 82 (T/C), 118 (C/T), 166 (G/A), 184 (G/A), 202 (T/C), 220 (C/T), 271 (C/T), 349 (C/T), 355 (C/T), 364 (T/C), 400 (T/C), 562 (A/G) and 634 (T/C). Within *C. grannus* the only diagnosable population is *C. grannus micrommata*, Sierra de Neiba, which bears a thymine at position 622 whereas specimens from all other populations have an adenine.

The *C. grannus* populations

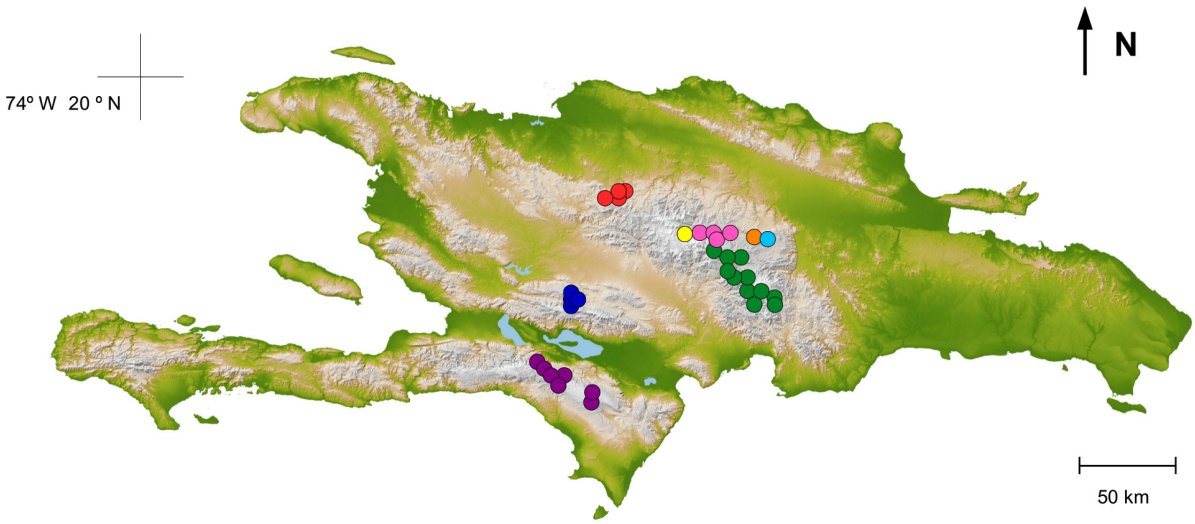
We analyzed 58 sequences belonging to the eight taxa included in the former *C. grannus* complex or species group due its genitalic morphology and the possession of two circled ocelli with central pupils at the under surface of HW (Schwartz & Gali 1984, Gonzalez 1987). Some were originally described as separate species from localities separated a few kilometers at the major mountain range of Hispaniola, the Cordillera Central (*C. grannus grannus* M. Bates, 1939, *C. grannus dilemma* González, 1987, *C. grannus phoinix* González, 1987, *C. grannus amazona* González, 1987, *C. grannus micheneri* Clench, 1944) whereas others were discovered at more distant places of the C. Central (*C. grannus dystacta* González, 1987), the middle Sierra de Neiba (*C. grannus micrommata* Schwartz & Gali, 1984) or the southern Sierra de Bahoruco (*C. grannus sommeri* Schwartz & Gali, 1984) (Fig. 19). Sourakov & Zakharov (2011), based on COI barcode sequences obtained from 28 specimens, lowered the status of most of these taxa from species to subspecies level using mitochondrial DNA as evidence of low divergence level between them. We did not include sequences from the latter study in our analysis as they lacked precise geographical data, with the exception of six sequences representing *C. grannus sommeri*, which we included in our dataset.

No Bayesian, Maximum Likelihood or Neighbor Joining produced a single cluster formed only by specimens from the same locality or belonging to a single taxon with most clades weakly supported (Fig. 18). K2P pairwise distances values were between 0 and 2.1%, or 0 to 12 differences, with intra-taxon minimum pairwise distances ranging from 0 (*C. grannus micrommata*) to 1.1% (*C. grannus phoinix*) and intertaxa minimum pairwise distances from 0.3 (*C. grannus micrommata– C. grannus sommeri*) to 1.2% (*C. grannus amazona– C. grannus micheneri*).

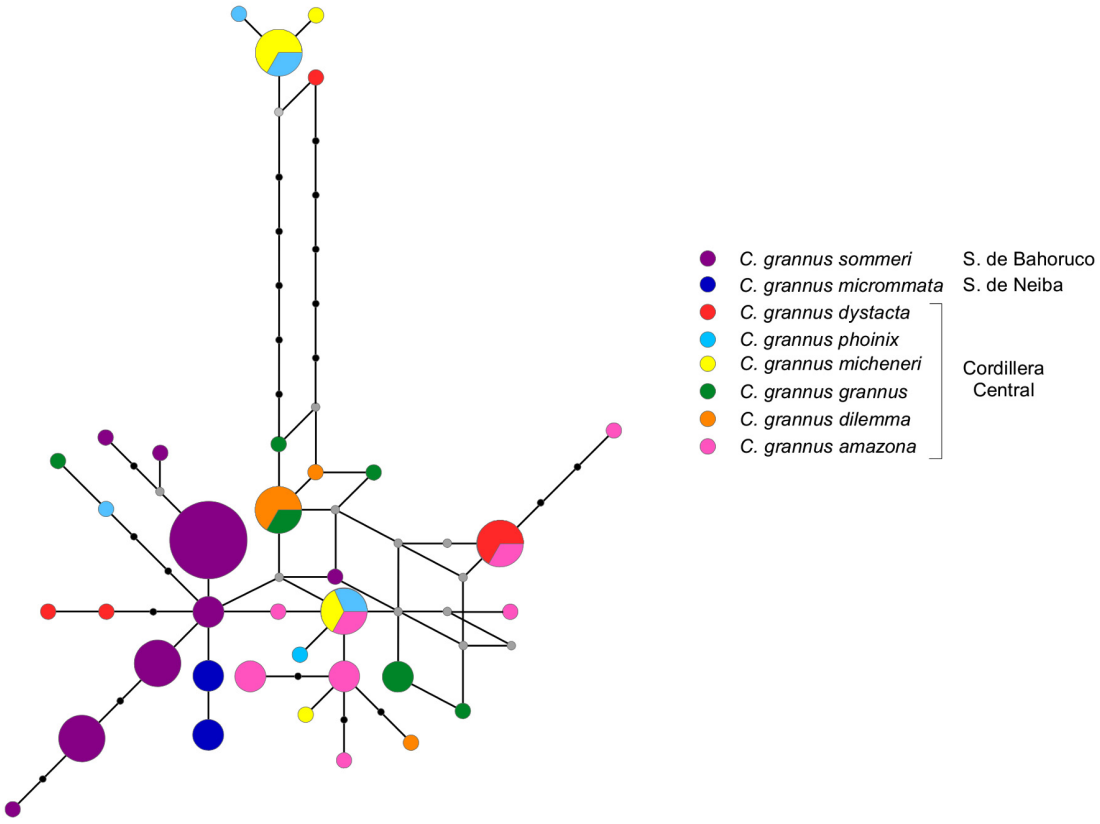
The species identification methods recovered a single entity (all PTP variants, ABGD, BIN) or two and as much as 14 entities, GMYC single and multiple threshold respectively (Fig. 18). The later method also mixed specimens from different named populations mixed within the delimited entities.

The Median Joining haplotype network shed some light on the relationships of these populations (Fig. 20). The analysis identified 36 haplotypes. *Calisto grannus sommeri* and *C. grannus micrommata* were represented by 8, 18 sequences, and 2, 4 sequences, haplotypes respectively. These haplotypes are unique from their respective

geographic ranges, Sierra de Bahoruco and Sierra de Neiba. Populations of the northern Cordillera Central included 26 haplotypes, 36 sequences, 19 of them represented by unique sequences. Four haplotypes of these populations are shared by two or three named populations (Fig. 20).



19



20

FIGURES 19–20. 19—Geographic distribution of *C. grannus* populations, nomenclature follows Sourakov & Zakharov (2011). 20—Median Joining Haplotype network obtained in Network v. 5.0; circles size proportional to number of sequences; gray circles are hypothetical haplotypes; black circles represent changes; colors represent subspecies sensu Sourakov & Zakharov (2011).

Discussion

More information about the new species described herein and its sister *C. hysius* is needed to assess how their ancestral populations splitted in a seemingly continuous mountain habitat. Needed data include additional molecular markers, habitat, immature stages, and distribution. Several markers besides COI were sequenced by Matos–Maravi et al (2014) for two *C. hysius* specimens but at present only COI barcodes are available for *C. bahoruco*. In the same way, the immature stages of *C. hysius* were described by Sourakov (1996) but those of *C. bahoruco* remain unknown.

Regarding distribution, although we found *C. bahoruco* at a single locality we suspect the species inhabits other low to middle elevation localities at the Barahona province depicted by Schwartz (1989) for *C. hysius*. All these localities are at the extreme eastern area of Sierra de Bahoruco. *Calisto hysius* range seems to occupy most of the southern Hispaniolan mountain range west of Barahona, from Pedernales and Independencia provinces of Dominican Republic along the Tiburon Peninsule to the Massif de la Hotte, Haiti. Our main clue to assert this is the fact that *C. bahoruco* type specimens match three specimens identified as *C. hysius* from two other localities of the Barahona province illustrated on the Butterflies of America website (Warren *et al.* 2016). In the website there is another specimen with a different color pattern also attributed to *C. hysius*. We think it probably represents the northern subspecies of *C. hysius*, *C. hysius aleucosticha* Correa & Schwartz, a taxon for which both sequences and specimens are lacking for present study. One *C. hysius* adult from 1.5 km S Los Arroyos, Pedernales province, illustrated by Sourakov (1996) match our *C. hysius* specimens from a nearby locality. In addition, the male genitalia of our *C. hysius* from Los Arroyos match better the drawing of Haitian specimens by Johnson & Hedges (1998) than the male structures of *C. bahoruco* type series, mainly the prominent shape of the tegumen base. Evidently the species were confused in the past due to their similar wing pattern and close distribution.

Phylogenetic relationships reconstructed by both methods concur with the topology resulting from six genes obtained by Matos–Maraví *et al.* (2014). *Calisto confusa* and *C. debarriera* species pair is sister of all other species. The rest splits in two, with *C. grannus* and *C. obscura* being sister of the remaining species. Despite using COI barcodes, there was a good phylogenetic signal. The only change is the position of *C. bahoruco* **n. sp.** as sister species of *C. hysius*.

Concerning *Calisto grannus*, there is no doubt that all known populations are monophyletic, descendants from a common ancestor. According to the COI data, populations within *C. grannus* seem to have an ongoing gene exchange or it continued until recently. The latter is evident at Cordillera Central where several haplotypes can be shared between two or even three populations and where individuals of the same population may have different haplotypes suggesting introgression. We found cases of such genetic introgression among populations of *C. g. phoinix*, *C. g. micheneri* and *C. g. dystacta*, the latter being moderately isolated from all other C. Central populations. In fact, all C. Central populations except the westernmost *C.g. dystacta*, are so close to each other that the gene exchange almost certainly is ongoing. Haplotypes from Sierra de Neiba and Sierra de Bahoruco are found, as could be expected, exclusively in their respective ranges, suggesting a prolonged geographic isolation. These results are also supported by observations on the immature stages: Sourakov & Emmel (1995) and Sourakov (1996) described immature stages of *C. grannus grannus*, *C. g. dilemma* and *C. g. sommeri,* and found that while the first two taxa share morphology, *C. g. sommeri* exhibited a spotted form of caterpillars not found in C. Central subspecies.

While our DNA analysis overall confirms the results obtained by Sourakov & Zakharov (2011), our study significantly increases the dataset and provides a more precise locations data for the studied populations. Nevertheless, we feel that only with further morphological studies and DNA analyses involving additional markers will we be able to reassess the taxonomic status of *C. grannus* populations.

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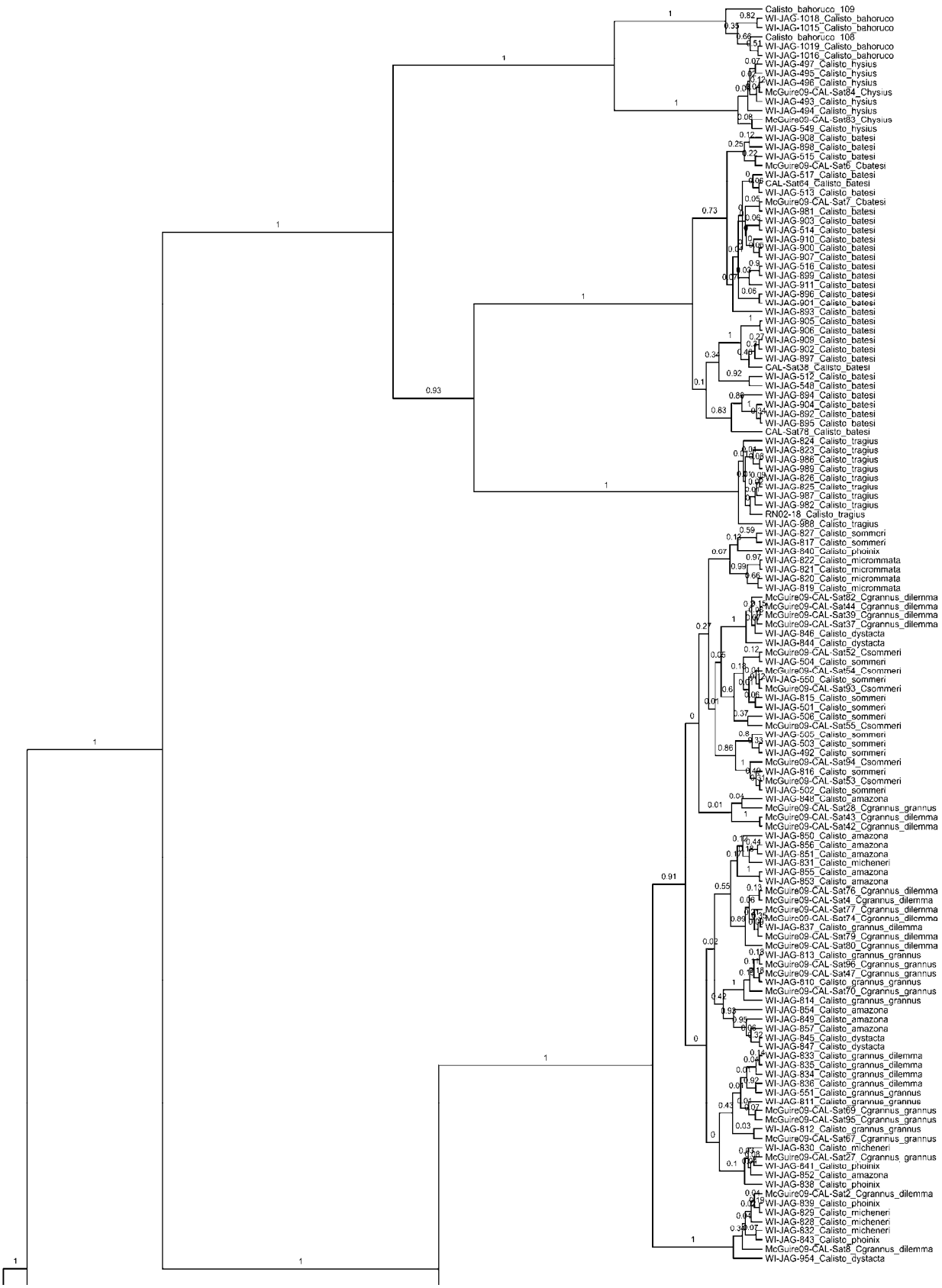
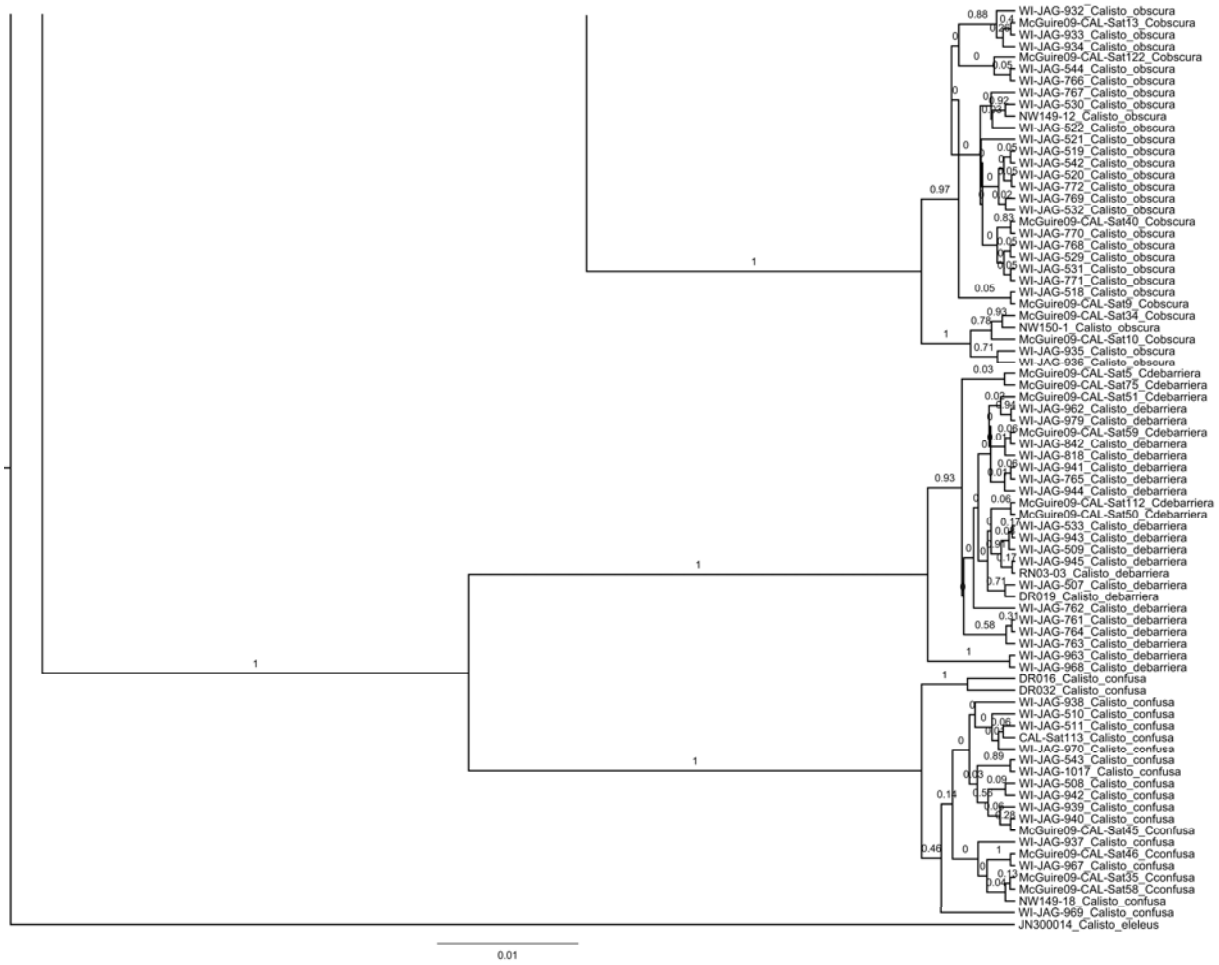
APPENDIX 1. Output of the species delimitation methods applied using 214 COI barcodes beloging to eight species form *hysius* group of *Calisto*.

Generalized Mixed Yule Coalescent

Ultrametric starting tree obtained in BEAST software v.1.8.2

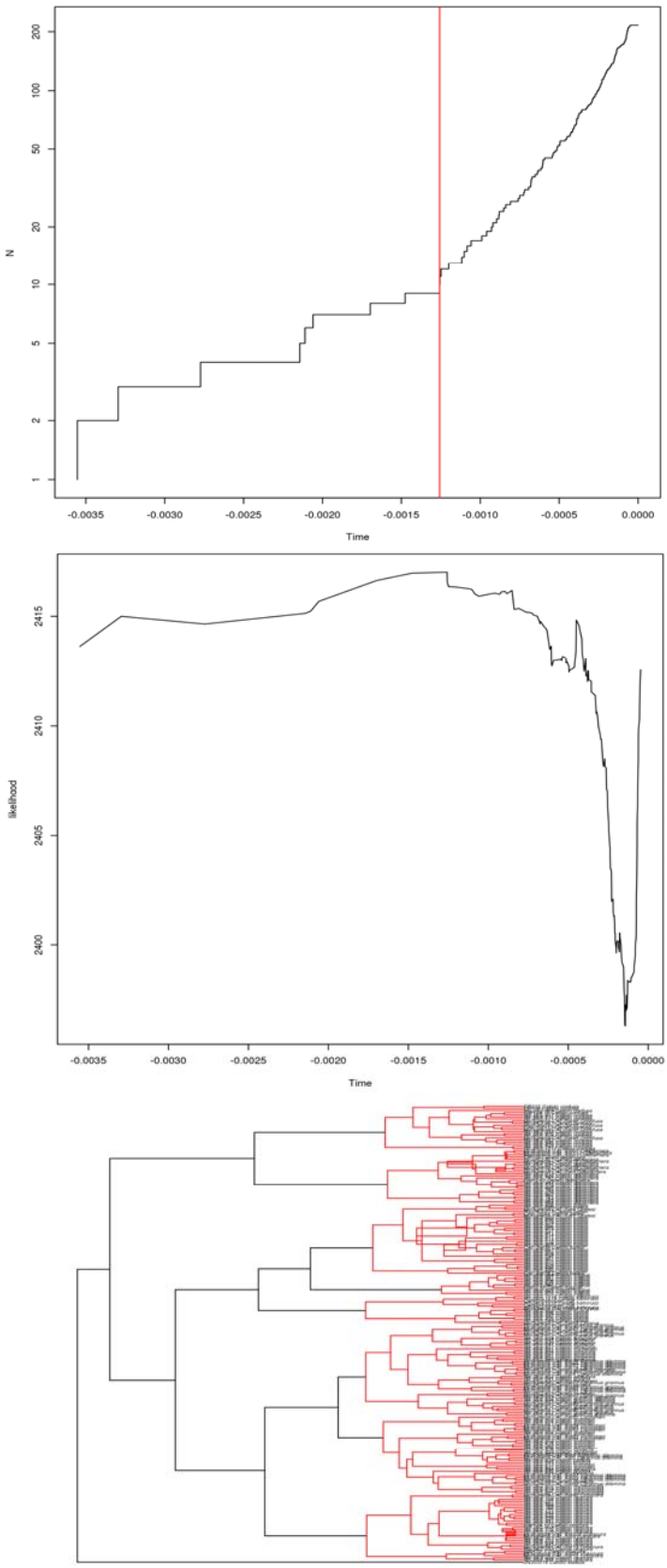
Parameters output of the GMYC single threshold method:





method: single
likelihood of null model: 2413.628
maximum likelihood of GMYC model: 2417.009
likelihood ratio: 6.762109
result of LR test: 0.03401157*
number of ML clusters: 8
confidence interval: 3-30
number of ML entities: 9
confidence interval: 4-30
threshold time: -0.001257703

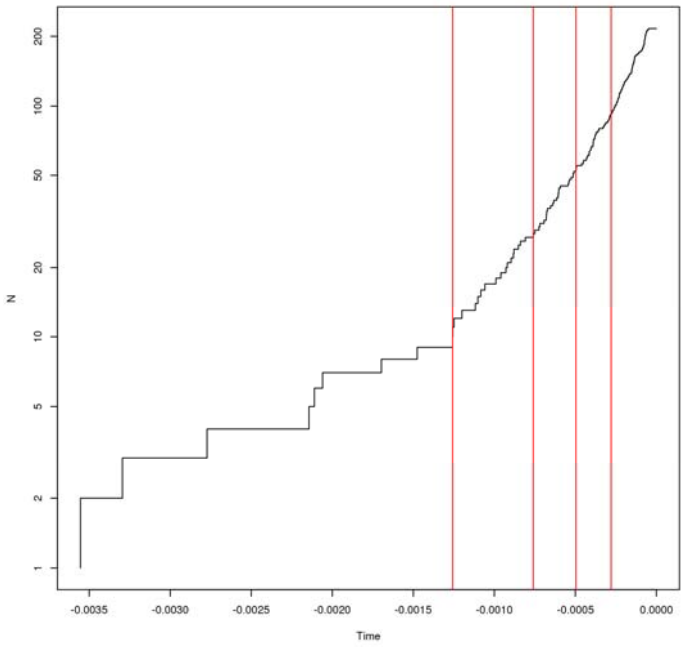
Graphic output of the GMYC single threshold method:

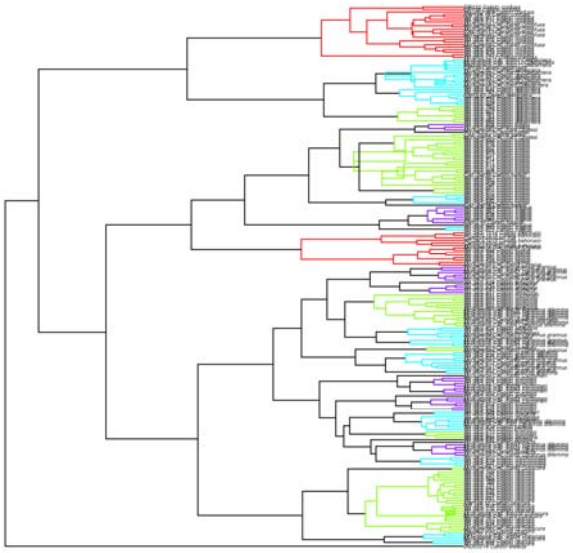


Parameters output of the GMYC multiple threshold method:

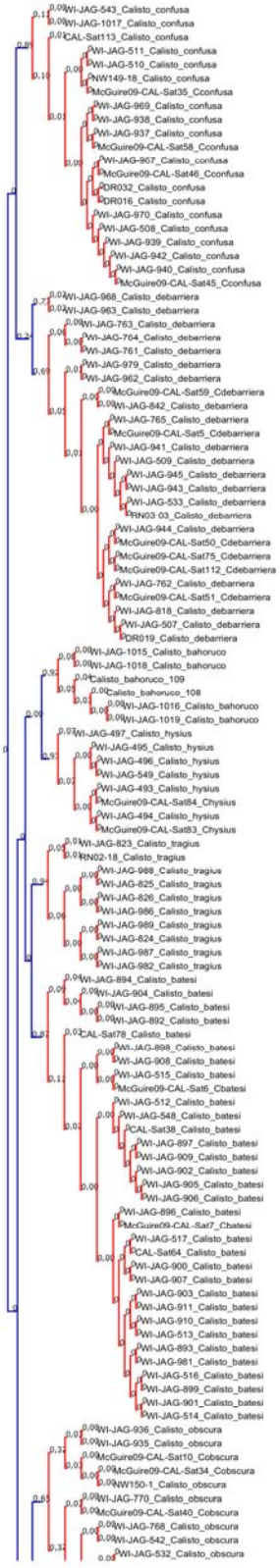
method: multiple
likelihood of null model: 2413.628
maximum likelihood of GMYC model: 2423.836
likelihood ratio: 20.41686
result of LR test: 3.68583e-05***
number of ML clusters: 33
confidence interval: 29-44
number of ML entities: 42
confidence interval: 34-57
threshold time: -0.001257703 -0.0007598622 -0.0004966197 -0.0002791752

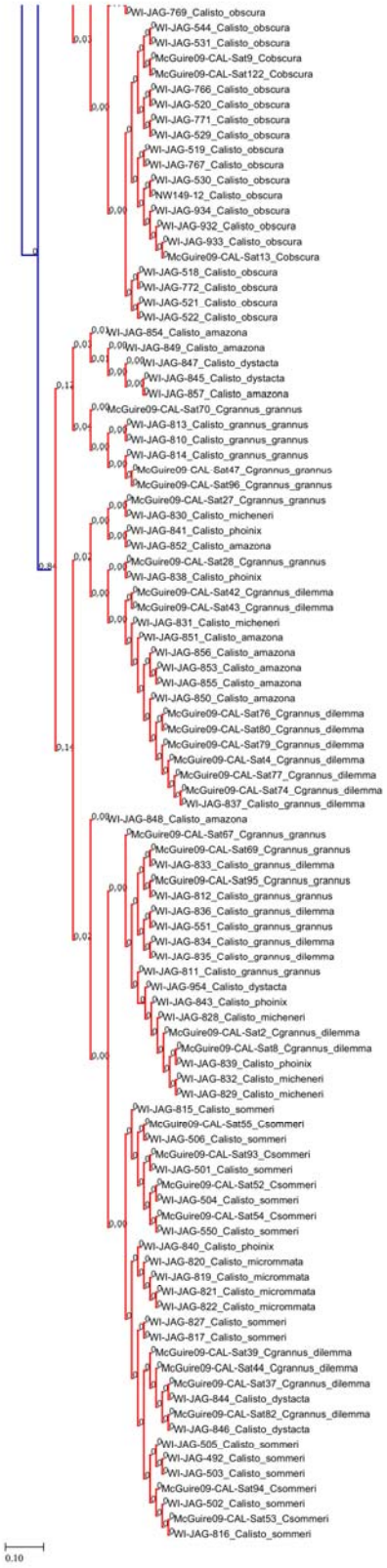
Graphic output of the GMYC multiple threshold method:





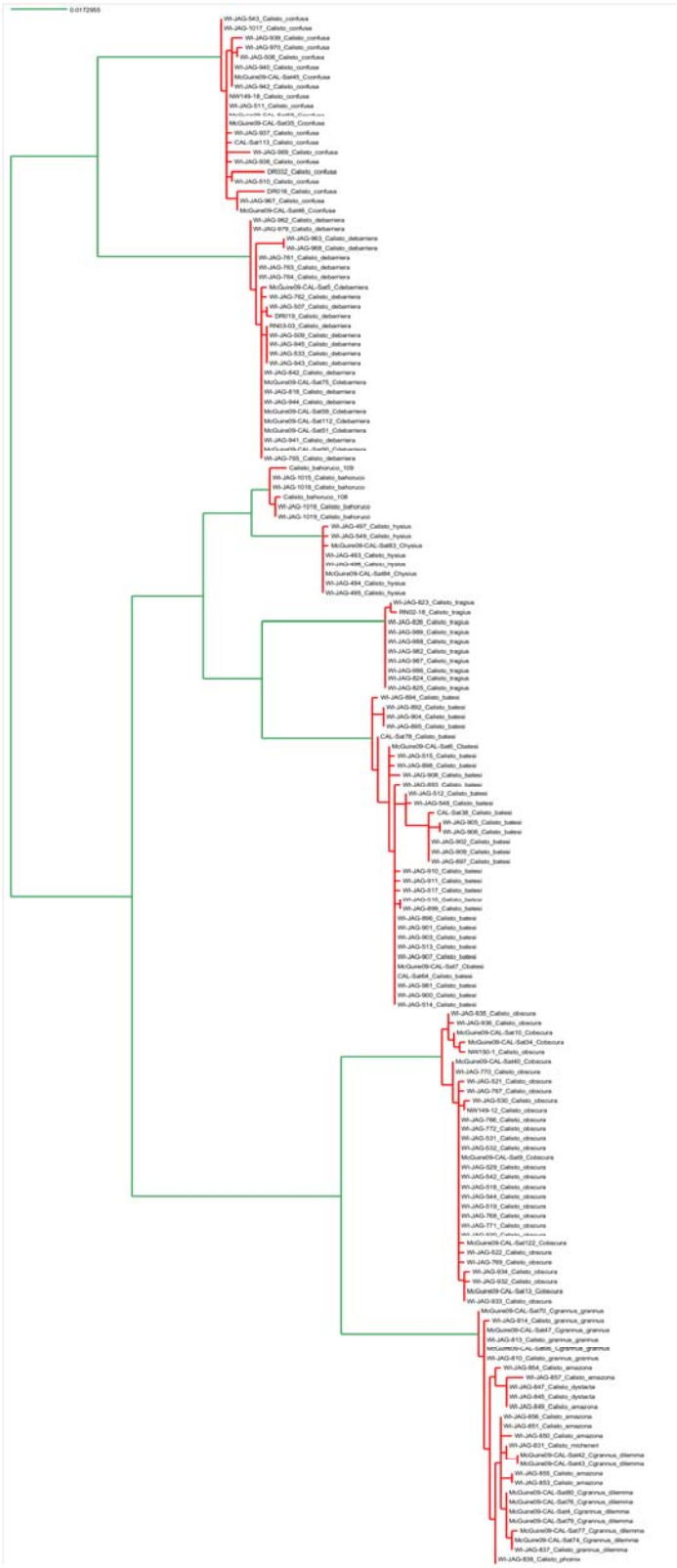
Bayesian species delimitaton using the Poisson Tree Process (bPTP) based on the distribution of nucleotide substitutions on the Bayesian Inference tree of hysius group species. Terminal branches in blue indicate lineages that stand as separate species and the clades in red are lumped into single species.

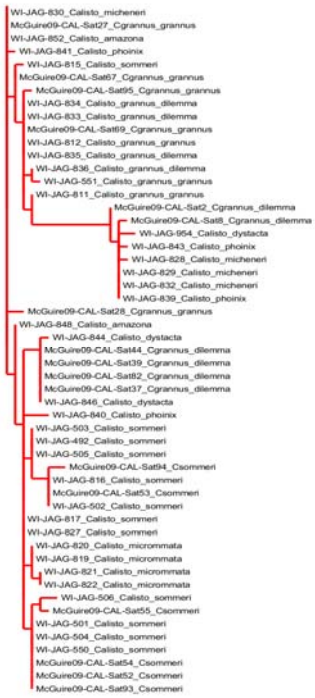

WI-JAG-543_Calisto_confusa
WI-JAG-1017_Calisto_confusa
CAL-Sat113_Calisto_confusa
WI-JAG-511_Calisto_confusa
WI-JAG-510_Calisto_confusa
NW149-18_Calisto_confusa
McGuire09-CAL-Sat35_Cconfusa
WI-JAG-969_Calisto_confusa
WI-JAG-938_Calisto_confusa
WI-JAG-937_Calisto_confusa
McGuire09-CAL-Sat58_Cconfusa
WI-JAG-967_Calisto_confusa
McGuire09-CAL-Sat46_Cconfusa
DR032_Calisto_confusa
DR016_Calisto_confusa
WI-JAG-970_Calisto_confusa
WI-JAG-508_Calisto_confusa
WI-JAG-939_Calisto_confusa
WI-JAG-942_Calisto_confusa
WI-JAG-940_Calisto_confusa
McGuire09-CAL-Sat45_Cconfusa
WI-JAG-968_Calisto_debarriera
WI-JAG-963_Calisto_debarriera
WI-JAG-763_Calisto_debarriera
WI-JAG-764_Calisto_debarriera
WI-JAG-761_Calisto_debarriera
WI-JAG-979_Calisto_debarriera
WI-JAG-962_Calisto_debarriera
McGuire09-CAL-Sat59_Cdebarriera
WI-JAG-842_Calisto_debarriera
WI-JAG-765_Calisto_debarriera
McGuire09-CAL-Sat5_Cdebarriera
WI-JAG-941_Calisto_debarriera
WI-JAG-509_Calisto_debarriera
WI-JAG-945_Calisto_debarriera
WI-JAG-943_Calisto_debarriera
WI-JAG-533_Calisto_debarriera
RN03-03_Calisto_debarriera
WI-JAG-944_Calisto_debarriera
McGuire09-CAL-Sat50_Cdebarriera
McGuire09-CAL-Sat75_Cdebarriera
McGuire09-CAL-Sat112_Cdebarriera
WI-JAG-762_Calisto_debarriera
McGuire09-CAL-Sat51_Cdebarriera
WI-JAG-818_Calisto_debarriera
WI-JAG-507_Calisto_debarriera
DR019_Calisto_debarriera
WI-JAG-1015_Calisto_bahoruco
WI-JAG-1018_Calisto_bahoruco
Calisto_bahoruco_109
Calisto_bahoruco_108
WI-JAG-1016_Calisto_bahoruco
WI-JAG-1019_Calisto_bahoruco
WI-JAG-497_Calisto_hysius
WI-JAG-495_Calisto_hysius
WI-JAG-496_Calisto_hysius
WI-JAG-549_Calisto_hysius
WI-JAG-493_Calisto_hysius
McGuire09-CAL-Sat84_Chysius
WI-JAG-494_Calisto_hysius
McGuire09-CAL-Sat83_Chysius
WI-JAG-823_Calisto_tragius
RN02-18_Calisto_tragius
WI-JAG-988_Calisto_tragius
WI-JAG-825_Calisto_tragius
WI-JAG-826_Calisto_tragius
WI-JAG-986_Calisto_tragius
WI-JAG-989_Calisto_tragius
WI-JAG-824_Calisto_tragius
WI-JAG-987_Calisto_tragius
WI-JAG-982_Calisto_tragius
WI-JAG-894_Calisto_batesi
WI-JAG-904_Calisto_batesi
WI-JAG-895_Calisto_batesi
WI-JAG-892_Calisto_batesi
CAL-Sat78_Calisto_batesi
WI-JAG-898_Calisto_batesi
WI-JAG-908_Calisto_batesi
WI-JAG-515_Calisto_batesi
McGuire09-CAL-Sat6_Cbatesi
WI-JAG-512_Calisto_batesi
WI-JAG-548_Calisto_batesi
CAL-Sat38_Calisto_batesi
WI-JAG-897_Calisto_batesi
WI-JAG-909_Calisto_batesi
WI-JAG-902_Calisto_batesi
WI-JAG-905_Calisto_batesi
WI-JAG-906_Calisto_batesi
WI-JAG-896_Calisto_batesi
McGuire09-CAL-Sat7_Cbatesi
WI-JAG-517_Calisto_batesi
CAL-Sat64_Calisto_batesi
WI-JAG-900_Calisto_batesi
WI-JAG-907_Calisto_batesi
WI-JAG-903_Calisto_batesi
WI-JAG-911_Calisto_batesi
WI-JAG-910_Calisto_batesi
WI-JAG-513_Calisto_batesi
WI-JAG-893_Calisto_batesi
WI-JAG-981_Calisto_batesi
WI-JAG-516_Calisto_batesi
WI-JAG-899_Calisto_batesi
WI-JAG-901_Calisto_batesi
WI-JAG-514_Calisto_batesi
WI-JAG-936_Calisto_obscura
WI-JAG-935_Calisto_obscura
McGuire09-CAL-Sat10_Cobscura
McGuire09-CAL-Sat34_Cobscura
NW150-1_Calisto_obscura
WI-JAG-770_Calisto_obscura
McGuire09-CAL-Sat40_Cobscura
WI-JAG-768_Calisto_obscura
WI-JAG-542_Calisto_obscura
WI-JAG-532_Calisto_obscura



Multi-rate Poisson Tree Process (mPTP) delimitation. Terminal branches in red indicate lineages that stand as separate species and the clades in red are lumped into single species.

Graphic output





Output:

Command: ../bin/mptp mptp --ml --multi --outgroup JN300014_Calisto_eleleus --outgroup_crop --svg_width 1366 --svg_marginleft 14 --svg_marginright 14 --tree_file ../uploads/f75l6e8f2cit9sdur47eankbl2.newick --output_file ../uploads/f75l6e8f2cit9sdur47eankbl2.1
Number of edges greater than minimum branch length: 124 / 428
Null-model score: 516.549719
Best score for multi coalescent rate: 516.549719
Number of delimited species: 8

Species 1:
WI-JAG-543_Calisto_confusa
WI-JAG-1017_Calisto_confusa
WI-JAG-939_Calisto_confusa
WI-JAG-970_Calisto_confusa
WI-JAG-508_Calisto_confusa
WI-JAG-940_Calisto_confusa
McGuire09-CAL-Sat45_Cconfusa
WI-JAG-942_Calisto_confusa
NW149-18_Calisto_confusa
WI-JAG-511_Calisto_confusa
McGuire09-CAL-Sat58_Cconfusa
McGuire09-CAL-Sat35_Cconfusa
WI-JAG-937_Calisto_confusa
CAL-Sat113_Calisto_confusa
WI-JAG-969_Calisto_confusa
WI-JAG-938_Calisto_confusa
DR032_Calisto_confusa
WI-JAG-510_Calisto_confusa

DR016_Calisto_confusa
WI-JAG-967_Calisto_confusa
McGuire09-CAL-Sat46_Cconfusa

Species 2:
WI-JAG-962_Calisto_debarriera
WI-JAG-979_Calisto_debarriera
WI-JAG-963_Calisto_debarriera
WI-JAG-968_Calisto_debarriera
WI-JAG-761_Calisto_debarriera
WI-JAG-763_Calisto_debarriera
WI-JAG-764_Calisto_debarriera
McGuire09-CAL-Sat5_Cdebarriera
WI-JAG-762_Calisto_debarriera
WI-JAG-507_Calisto_debarriera
DR019_Calisto_debarriera
RN03-03_Calisto_debarriera
WI-JAG-509_Calisto_debarriera
WI-JAG-945_Calisto_debarriera
WI-JAG-533_Calisto_debarriera
WI-JAG-943_Calisto_debarriera
WI-JAG-842_Calisto_debarriera
McGuire09-CAL-Sat75_Cdebarriera
WI-JAG-818_Calisto_debarriera
WI-JAG-944_Calisto_debarriera
McGuire09-CAL-Sat59_Cdebarriera
McGuire09-CAL-Sat112_Cdebarriera
McGuire09-CAL-Sat51_Cdebarriera
WI-JAG-941_Calisto_debarriera
McGuire09-CAL-Sat50_Cdebarriera
WI-JAG-765_Calisto_debarriera

Species 3:
Calisto_bahoruco_109
WI-JAG-1015_Calisto_bahoruco
WI-JAG-1018_Calisto_bahoruco
Calisto_bahoruco_108
WI-JAG-1016_Calisto_bahoruco
WI-JAG-1019_Calisto_bahoruco

Species 4:
WI-JAG-497_Calisto_hysius
WI-JAG-549_Calisto_hysius
McGuire09-CAL-Sat83_Chysius
WI-JAG-493_Calisto_hysius
WI-JAG-496_Calisto_hysius
McGuire09-CAL-Sat84_Chysius
WI-JAG-494_Calisto_hysius
WI-JAG-495_Calisto_hysius

Species 5:
WI-JAG-823_Calisto_tragius
RN02-18_Calisto_tragius
WI-JAG-826_Calisto_tragius
WI-JAG-989_Calisto_tragius
WI-JAG-988_Calisto_tragius

WI-JAG-982_Calisto_tragius
WI-JAG-987_Calisto_tragius
WI-JAG-986_Calisto_tragius
WI-JAG-824_Calisto_tragius
WI-JAG-825_Calisto_tragius

Species 6:
WI-JAG-894_Calisto_batesi
WI-JAG-892_Calisto_batesi
WI-JAG-904_Calisto_batesi
WI-JAG-895_Calisto_batesi
CAL-Sat78_Calisto_batesi
McGuire09-CAL-Sat6_Cbatesi
WI-JAG-515_Calisto_batesi
WI-JAG-898_Calisto_batesi
WI-JAG-908_Calisto_batesi
WI-JAG-893_Calisto_batesi
WI-JAG-512_Calisto_batesi
WI-JAG-548_Calisto_batesi
CAL-Sat38_Calisto_batesi
WI-JAG-905_Calisto_batesi
WI-JAG-906_Calisto_batesi
WI-JAG-902_Calisto_batesi
WI-JAG-909_Calisto_batesi
WI-JAG-897_Calisto_batesi
WI-JAG-910_Calisto_batesi
WI-JAG-911_Calisto_batesi
WI-JAG-517_Calisto_batesi
WI-JAG-516_Calisto_batesi
WI-JAG-899_Calisto_batesi
WI-JAG-896_Calisto_batesi
WI-JAG-901_Calisto_batesi
WI-JAG-903_Calisto_batesi
WI-JAG-513_Calisto_batesi
WI-JAG-907_Calisto_batesi
McGuire09-CAL-Sat7_Cbatesi
CAL-Sat64_Calisto_batesi
WI-JAG-981_Calisto_batesi
WI-JAG-900_Calisto_batesi
WI-JAG-514_Calisto_batesi

Species 7:
WI-JAG-935_Calisto_obscura
WI-JAG-936_Calisto_obscura
McGuire09-CAL-Sat10_Cobscura
McGuire09-CAL-Sat34_Cobscura
NW150-1_Calisto_obscura
McGuire09-CAL-Sat40_Cobscura
WI-JAG-770_Calisto_obscura
WI-JAG-521_Calisto_obscura
WI-JAG-767_Calisto_obscura
WI-JAG-530_Calisto_obscura
NW149-12_Calisto_obscura
WI-JAG-766_Calisto_obscura
WI-JAG-772_Calisto_obscura
WI-JAG-531_Calisto_obscura

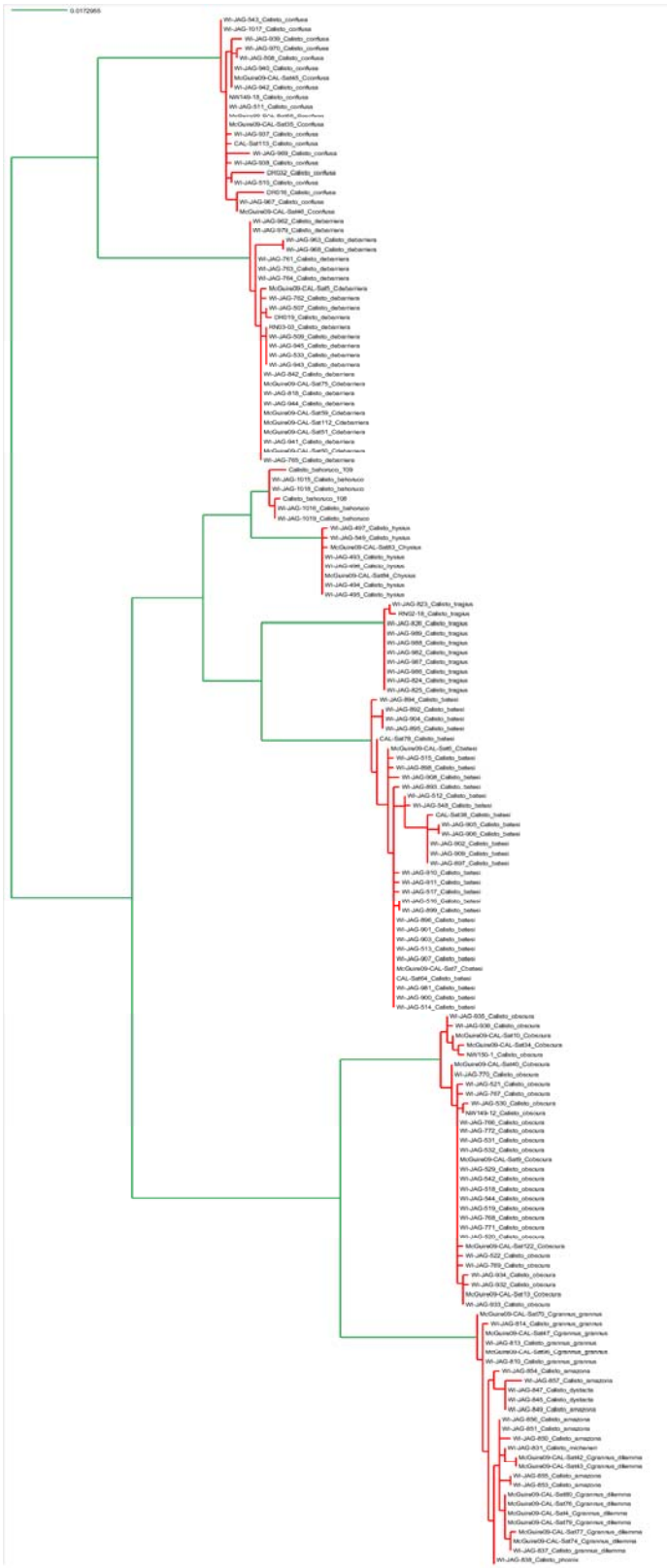
WI-JAG-532_Calisto_obscura
McGuire09-CAL-Sat9_Cobscura
WI-JAG-529_Calisto_obscura
WI-JAG-542_Calisto_obscura
WI-JAG-518_Calisto_obscura
WI-JAG-544_Calisto_obscura
WI-JAG-519_Calisto_obscura
WI-JAG-768_Calisto_obscura
WI-JAG-771_Calisto_obscura
WI-JAG-520_Calisto_obscura
McGuire09-CAL-Sat122_Cobscura
WI-JAG-522_Calisto_obscura
WI-JAG-769_Calisto_obscura
WI-JAG-934_Calisto_obscura
WI-JAG-932_Calisto_obscura
McGuire09-CAL-Sat13_Cobscura
WI-JAG-933_Calisto_obscura

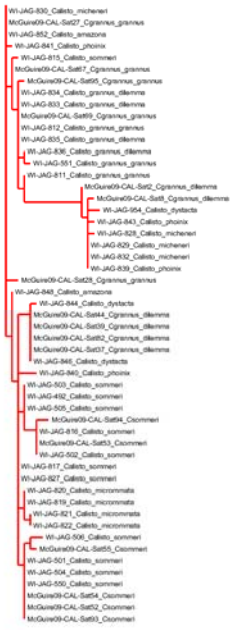
Species 8:
McGuire09-CAL-Sat70_Cgrannus_grannus
WI-JAG-814_Calisto_grannus_grannus
McGuire09-CAL-Sat47_Cgrannus_grannus
WI-JAG-813_Calisto_grannus_grannus
McGuire09-CAL-Sat96_Cgrannus_grannus
WI-JAG-810_Calisto_grannus_grannus
WI-JAG-854_Calisto_amazona
WI-JAG-857_Calisto_amazona
WI-JAG-847_Calisto_dystacta
WI-JAG-845_Calisto_dystacta
WI-JAG-849_Calisto_amazona
WI-JAG-856_Calisto_amazona
WI-JAG-851_Calisto_amazona
WI-JAG-850_Calisto_amazona
WI-JAG-831_Calisto_micheneri
McGuire09-CAL-Sat42_Cgrannus_dilemma
McGuire09-CAL-Sat43_Cgrannus_dilemma
WI-JAG-855_Calisto_amazona
WI-JAG-853_Calisto_amazona
McGuire09-CAL-Sat80_Cgrannus_dilemma
McGuire09-CAL-Sat76_Cgrannus_dilemma
McGuire09-CAL-Sat4_Cgrannus_dilemma
McGuire09-CAL-Sat79_Cgrannus_dilemma
McGuire09-CAL-Sat77_Cgrannus_dilemma
McGuire09-CAL-Sat74_Cgrannus_dilemma
WI-JAG-837_Calisto_grannus_dilemma
WI-JAG-838_Calisto_phoinix
WI-JAG-830_Calisto_micheneri
McGuire09-CAL-Sat27_Cgrannus_grannus
WI-JAG-852_Calisto_amazona
WI-JAG-841_Calisto_phoinix
WI-JAG-815_Calisto_sommeri
McGuire09-CAL-Sat67_Cgrannus_grannus
McGuire09-CAL-Sat95_Cgrannus_grannus
WI-JAG-834_Calisto_grannus_dilemma
WI-JAG-833_Calisto_grannus_dilemma
McGuire09-CAL-Sat69_Cgrannus_grannus

WI-JAG-812_Calisto_grannus_grannus
WI-JAG-835_Calisto_grannus_dilemma
WI-JAG-836_Calisto_grannus_dilemma
WI-JAG-551_Calisto_grannus_grannus
WI-JAG-811_Calisto_grannus_grannus
McGuire09-CAL-Sat2_Cgrannus_dilemma
McGuire09-CAL-Sat8_Cgrannus_dilemma
WI-JAG-954_Calisto_dystacta
WI-JAG-843_Calisto_phoinix
WI-JAG-828_Calisto_micheneri
WI-JAG-829_Calisto_micheneri
WI-JAG-832_Calisto_micheneri
WI-JAG-839_Calisto_phoinix
McGuire09-CAL-Sat28_Cgrannus_grannus
WI-JAG-848_Calisto_amazona
WI-JAG-844_Calisto_dystacta
McGuire09-CAL-Sat44_Cgrannus_dilemma
McGuire09-CAL-Sat39_Cgrannus_dilemma
McGuire09-CAL-Sat82_Cgrannus_dilemma
McGuire09-CAL-Sat37_Cgrannus_dilemma
WI-JAG-846_Calisto_dystacta
WI-JAG-840_Calisto_phoinix
WI-JAG-503_Calisto_sommeri
WI-JAG-492_Calisto_sommeri
WI-JAG-505_Calisto_sommeri
McGuire09-CAL-Sat94_Csommeri
WI-JAG-816_Calisto_sommeri
McGuire09-CAL-Sat53_Csommeri
WI-JAG-502_Calisto_sommeri
WI-JAG-817_Calisto_sommeri
WI-JAG-827_Calisto_sommeri
WI-JAG-820_Calisto_micrommata
WI-JAG-819_Calisto_micrommata
WI-JAG-821_Calisto_micrommata
WI-JAG-822_Calisto_micrommata
WI-JAG-506_Calisto_sommeri
McGuire09-CAL-Sat55_Csommeri
WI-JAG-501_Calisto_sommeri
WI-JAG-504_Calisto_sommeri
WI-JAG-550_Calisto_sommeri
McGuire09-CAL-Sat54_Csommeri
McGuire09-CAL-Sat52_Csommeri
McGuire09-CAL-Sat93_Csommeri

Poisson Tree Process (PTP) delimitation. Terminal branches in red indicate lineages that stand as separate species and the clades in red are lumped into single species.

Graphic output





Output:

Command: ../bin/mptp mptp --ml --single --pvalue 0.001 --outgroup JN300014_Calisto_eleleus --outgroup_crop --svg_width 1366 --svg_marginleft 14 --svg_marginright 14 --tree_file ../uploads/f75l6e8f2cit9sdur47eankbl2.newick --output_file ../uploads/f75l6e8f2cit9sdur47eankbl2.2
Number of edges greater than minimum branch length: 124 / 428
Null-model score: 516.549719
Best score for single coalescent rate: 584.466328
Number of delimited species: 8

Species 1:
WI-JAG-543_Calisto_confusa
WI-JAG-1017_Calisto_confusa
WI-JAG-939_Calisto_confusa
WI-JAG-970_Calisto_confusa
WI-JAG-508_Calisto_confusa
WI-JAG-940_Calisto_confusa
McGuire09-CAL-Sat45_Cconfusa
WI-JAG-942_Calisto_confusa
NW149-18_Calisto_confusa
WI-JAG-511_Calisto_confusa
McGuire09-CAL-Sat58_Cconfusa
McGuire09-CAL-Sat35_Cconfusa
WI-JAG-937_Calisto_confusa
CAL-Sat113_Calisto_confusa
WI-JAG-969_Calisto_confusa
WI-JAG-938_Calisto_confusa
DR032_Calisto_confusa
WI-JAG-510_Calisto_confusa
DR016_Calisto_confusa
WI-JAG-967_Calisto_confusa

McGuire09-CAL-Sat46_Cconfusa

Species 2:
WI-JAG-962_Calisto_debarriera
WI-JAG-979_Calisto_debarriera
WI-JAG-963_Calisto_debarriera
WI-JAG-968_Calisto_debarriera
WI-JAG-761_Calisto_debarriera
WI-JAG-763_Calisto_debarriera
WI-JAG-764_Calisto_debarriera
McGuire09-CAL-Sat5_Cdebarriera
WI-JAG-762_Calisto_debarriera
WI-JAG-507_Calisto_debarriera
DR019_Calisto_debarriera
RN03-03_Calisto_debarriera
WI-JAG-509_Calisto_debarriera
WI-JAG-945_Calisto_debarriera
WI-JAG-533_Calisto_debarriera
WI-JAG-943_Calisto_debarriera
WI-JAG-842_Calisto_debarriera
McGuire09-CAL-Sat75_Cdebarriera
WI-JAG-818_Calisto_debarriera
WI-JAG-944_Calisto_debarriera
McGuire09-CAL-Sat59_Cdebarriera
McGuire09-CAL-Sat112_Cdebarriera
McGuire09-CAL-Sat51_Cdebarriera
WI-JAG-941_Calisto_debarriera
McGuire09-CAL-Sat50_Cdebarriera
WI-JAG-765_Calisto_debarriera

Species 3:
Calisto_bahoruco_109
WI-JAG-1015_Calisto_bahoruco
WI-JAG-1018_Calisto_bahoruco
Calisto_bahoruco_108
WI-JAG-1016_Calisto_bahoruco
WI-JAG-1019_Calisto_bahoruco

Species 4:
WI-JAG-497_Calisto_hysius
WI-JAG-549_Calisto_hysius
McGuire09-CAL-Sat83_Chysius
WI-JAG-493_Calisto_hysius
WI-JAG-496_Calisto_hysius
McGuire09-CAL-Sat84_Chysius
WI-JAG-494_Calisto_hysius
WI-JAG-495_Calisto_hysius

Species 5:
WI-JAG-823_Calisto_tragius
RN02-18_Calisto_tragius
WI-JAG-826_Calisto_tragius
WI-JAG-989_Calisto_tragius
WI-JAG-988_Calisto_tragius
WI-JAG-982_Calisto_tragius
WI-JAG-987_Calisto_tragius

WI-JAG-986_Calisto_tragius
WI-JAG-824_Calisto_tragius
WI-JAG-825_Calisto_tragius

Species 6:
WI-JAG-894_Calisto_batesi
WI-JAG-892_Calisto_batesi
WI-JAG-904_Calisto_batesi
WI-JAG-895_Calisto_batesi
CAL-Sat78_Calisto_batesi
McGuire09-CAL-Sat6_Cbatesi
WI-JAG-515_Calisto_batesi
WI-JAG-898_Calisto_batesi
WI-JAG-908_Calisto_batesi
WI-JAG-893_Calisto_batesi
WI-JAG-512_Calisto_batesi
WI-JAG-548_Calisto_batesi
CAL-Sat38_Calisto_batesi
WI-JAG-905_Calisto_batesi
WI-JAG-906_Calisto_batesi
WI-JAG-902_Calisto_batesi
WI-JAG-909_Calisto_batesi
WI-JAG-897_Calisto_batesi
WI-JAG-910_Calisto_batesi
WI-JAG-911_Calisto_batesi
WI-JAG-517_Calisto_batesi
WI-JAG-516_Calisto_batesi
WI-JAG-899_Calisto_batesi
WI-JAG-896_Calisto_batesi
WI-JAG-901_Calisto_batesi
WI-JAG-903_Calisto_batesi
WI-JAG-513_Calisto_batesi
WI-JAG-907_Calisto_batesi
McGuire09-CAL-Sat7_Cbatesi
CAL-Sat64_Calisto_batesi
WI-JAG-981_Calisto_batesi
WI-JAG-900_Calisto_batesi
WI-JAG-514_Calisto_batesi

Species 7:
WI-JAG-935_Calisto_obscura
WI-JAG-936_Calisto_obscura
McGuire09-CAL-Sat10_Cobscura
McGuire09-CAL-Sat34_Cobscura
NW150-1_Calisto_obscura
McGuire09-CAL-Sat40_Cobscura
WI-JAG-770_Calisto_obscura
WI-JAG-521_Calisto_obscura
WI-JAG-767_Calisto_obscura
WI-JAG-530_Calisto_obscura
NW149-12_Calisto_obscura
WI-JAG-766_Calisto_obscura
WI-JAG-772_Calisto_obscura
WI-JAG-531_Calisto_obscura
WI-JAG-532_Calisto_obscura
McGuire09-CAL-Sat9_Cobscura

WI-JAG-529_Calisto_obscura
WI-JAG-542_Calisto_obscura
WI-JAG-518_Calisto_obscura
WI-JAG-544_Calisto_obscura
WI-JAG-519_Calisto_obscura
WI-JAG-768_Calisto_obscura
WI-JAG-771_Calisto_obscura
WI-JAG-520_Calisto_obscura
McGuire09-CAL-Sat122_Cobscura
WI-JAG-522_Calisto_obscura
WI-JAG-769_Calisto_obscura
WI-JAG-934_Calisto_obscura
WI-JAG-932_Calisto_obscura
McGuire09-CAL-Sat13_Cobscura
WI-JAG-933_Calisto_obscura

Species 8:
McGuire09-CAL-Sat70_Cgrannus_grannus
WI-JAG-814_Calisto_grannus_grannus
McGuire09-CAL-Sat47_Cgrannus_grannus
WI-JAG-813_Calisto_grannus_grannus
McGuire09-CAL-Sat96_Cgrannus_grannus
WI-JAG-810_Calisto_grannus_grannus
WI-JAG-854_Calisto_amazona
WI-JAG-857_Calisto_amazona
WI-JAG-847_Calisto_dystacta
WI-JAG-845_Calisto_dystacta
WI-JAG-849_Calisto_amazona
WI-JAG-856_Calisto_amazona
WI-JAG-851_Calisto_amazona
WI-JAG-850_Calisto_amazona
WI-JAG-831_Calisto_micheneri
McGuire09-CAL-Sat42_Cgrannus_dilemma
McGuire09-CAL-Sat43_Cgrannus_dilemma
WI-JAG-855_Calisto_amazona
WI-JAG-853_Calisto_amazona
McGuire09-CAL-Sat80_Cgrannus_dilemma
McGuire09-CAL-Sat76_Cgrannus_dilemma
McGuire09-CAL-Sat4_Cgrannus_dilemma
McGuire09-CAL-Sat79_Cgrannus_dilemma
McGuire09-CAL-Sat77_Cgrannus_dilemma
McGuire09-CAL-Sat74_Cgrannus_dilemma
WI-JAG-837_Calisto_grannus_dilemma
WI-JAG-838_Calisto_phoinix
WI-JAG-830_Calisto_micheneri
McGuire09-CAL-Sat27_Cgrannus_grannus
WI-JAG-852_Calisto_amazona
WI-JAG-841_Calisto_phoinix
WI-JAG-815_Calisto_sommeri
McGuire09-CAL-Sat67_Cgrannus_grannus
McGuire09-CAL-Sat95_Cgrannus_grannus
WI-JAG-834_Calisto_grannus_dilemma
WI-JAG-833_Calisto_grannus_dilemma
McGuire09-CAL-Sat69_Cgrannus_grannus
WI-JAG-812_Calisto_grannus_grannus
WI-JAG-835_Calisto_grannus_dilemma

WI-JAG-836_Calisto_grannus_dilemma
WI-JAG-551_Calisto_grannus_grannus
WI-JAG-811_Calisto_grannus_grannus
McGuire09-CAL-Sat2_Cgrannus_dilemma
McGuire09-CAL-Sat8_Cgrannus_dilemma
WI-JAG-954_Calisto_dystacta
WI-JAG-843_Calisto_phoinix
WI-JAG-828_Calisto_micheneri
WI-JAG-829_Calisto_micheneri
WI-JAG-832_Calisto_micheneri
WI-JAG-839_Calisto_phoinix
McGuire09-CAL-Sat28_Cgrannus_grannus
WI-JAG-848_Calisto_amazona
WI-JAG-844_Calisto_dystacta
McGuire09-CAL-Sat44_Cgrannus_dilemma
McGuire09-CAL-Sat39_Cgrannus_dilemma
McGuire09-CAL-Sat82_Cgrannus_dilemma
McGuire09-CAL-Sat37_Cgrannus_dilemma
WI-JAG-846_Calisto_dystacta
WI-JAG-840_Calisto_phoinix
WI-JAG-503_Calisto_sommeri
WI-JAG-492_Calisto_sommeri
WI-JAG-505_Calisto_sommeri
McGuire09-CAL-Sat94_Csommeri
WI-JAG-816_Calisto_sommeri
McGuire09-CAL-Sat53_Csommeri
WI-JAG-502_Calisto_sommeri
WI-JAG-817_Calisto_sommeri
WI-JAG-827_Calisto_sommeri
WI-JAG-820_Calisto_micrommata
WI-JAG-819_Calisto_micrommata
WI-JAG-821_Calisto_micrommata
WI-JAG-822_Calisto_micrommata
WI-JAG-506_Calisto_sommeri
McGuire09-CAL-Sat55_Csommeri
WI-JAG-501_Calisto_sommeri
WI-JAG-504_Calisto_sommeri
WI-JAG-550_Calisto_sommeri
McGuire09-CAL-Sat54_Csommeri
McGuire09-CAL-Sat52_Csommeri
McGuire09-CAL-Sat93_Csommeri

Ouputs generated by the ABGD web- interface (available at: http://wwwabi.snv.jussieu.fr/public/abgd/) for the available COI sequences of the hysius species group of *Calisto.*