El Colegio de la Frontera Sur

Estatus de *Biblis hyperia aganisa* (Papilionoidea: Nymphalidae) en la península de Yucatán

Tesis
presentada como requisito parcial para optar al grado de
Maestro en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en Sistemática y Ecología

Por

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Dedicatoria

A mi familia: madre, padre, hermanas, hermanos, que de alguna forma han puesto su granito de arena con sus charlas, comprensión y cariño.

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Resumen

El uso de código de barras es una herramienta eficaz para reconocer especies cripticas, que son taxones similares en su apariencia externa y, que en muchas ocasiones han sido reportados bajo un mismo nombre y que a través de métodos moleculares se reconocen las diferencias genéticas. El género *Biblis* Fabricius, 1807 se consideró monotípico, pero su especie tiene 6 subespecies. Sin embargo, estudios recientes con el uso de COI, un gen de origen mitocondrial, ha evidenciado que se trata de un complejo de especies crípticas. Para probar estos resultados, se estudiaron especímenes de la península de Yucatán y áreas colindantes, para evidenciar la presencia de más de una especie en lo que se reconoce en México como *Biblis hyperia aganisa* Boisduval, 1836 con el uso de tres genes, uno mitocondrial (COI) y dos nucleares (MDH y DAPDH); así como un análisis morfológico que incluye caracteres cualitativos y cuantitativos. En total se analizaron 214 secuencias del gen COI, de éstas 206 corresponden a *Biblis* y ocho al grupo externo, 171 secuencias son de México, 22 de Costa Rica, nueve de Argentina, una de Estados Unidos, y una de la isla de Guana, Islas Vírgenes Británicas. Se formaron tres grupos de México en clados distintos, ECO 01+DHJ02, ECO 02+DHJ01, y ECO 03. Se evaluaron caracteres cuantitativos aplicando un análisis discriminante que fue eficiente para reconocer *Biblis aganisa* y dos especies indescritas. Los caracteres que contribuyeron significativamente al reconocimiento de estas especies fueron la longitud alar, longitud del margen anal y la distancia de la banda al margen externo. Para los genitales en machos fue el ángulo del tegumento, uncus y la longitud del hipandrio. Mientras que en hembras fue la anteapófisis y la longitud del abdomen. También se evaluaron y codificaron 34 caracteres y se obtuvo un árbol de máxima parsimonia donde 16 caracteres fueron informativos, y se obtuvieron 6 apomorfías en ECO 01, dos en ECO 02 y cinco en ECO 03. Se confirma la existencia de tres especies crípticas de *Biblis* en México, dos para la península de Yucatán, una de estas ya descrita que corresponde a *Biblis aganisa* y otra aun por describir. Y la tercera para el norte de Oaxaca y Sinaloa, también sin describir.

Palabras clave: especies crípticas, análisis morfológico, inferencia bayesiana, COI, análisis discriminante.

Introducción

La identificación de especies a través del código de barras de ADN (COI), ha resultado ser una herramienta eficaz para la distinción y el descubrimiento de especies nuevas (Blaxter 2003; Hallwachs et al. 2008; Janzen et al. 2017). Esta técnica se basa en la premisa de que la diversidad de la secuencia dentro de un segmento corto y estandarizado del genoma, puede proporcionar un código de barras biológico único, lo que permite identificaciones a nivel especie (Hebert et al. 2003; Hajibabaei et al. 2006). El COI es de origen mitocondrial (ADNmt) y presenta una tasa de mutación rápida, lo que resulta en variación significativa en las secuencias entre las especies y una variación baja dentro de la misma especie (Spelding 2003). De tal forma, es una herramienta útil para un reconocimiento expedito, donde el análisis de los linajes muestra una divergencia profunda (mayor al 2% ordinariamente en vertebrados y 3% en Lepidoptera) (Hebert et al. 2003). Sin embargo, esto no es sustituto de un estudio taxonómico completo, debido a que el uso de esta técnica debe ser combinada con datos morfológicos y ecológicos para que los resultados sean robustos para el reconocimiento final de las especies (Hebert et al. 2004b). Es decir, la divergencia genética puede ser una guía útil para el reconocimiento de los linajes, cuando las divergencias sean inusualmente bajas o altas, pero deben analizarse en combinación con otros caracteres, como el patrón de las alas, morfología genital e historia de vida y donde las poblaciones se conecten entre sí (Spelding 2003).

Estudios que usan el código de barras en la clase Insecta han puesto al descubierto un gran número de especies crípticas (e. g., Hajibabaei et al. 2006; Hallwachs et al. 2008; Li et al. 2010; Nieukerken et al. 2012). Las especies crípticas, son taxones morfológicamente similares que en ocasiones han sido determinadas bajo un único nombre y que difieren en diversos atributos biológicos, incluyendo la especificidad del hospedero, sistema de apareamiento, la susceptibilidad a los

diferentes parasitoides, y el comportamiento del parasitoide (Hebert et al. 2004a; Bickford et al. 2007).

Un ejemplo excelente del uso del COI en estudios sistemáticos es el del género *Hermeuptychia* Forster, 1964. Seraphim et al. (2014), estudiaron la variabilidad y los límites de las especies de este género y evidenciaron los conflictos entre los genes mitocondriales y los enfoques morfológicos clásicos para identificar y delimitar especies dentro de este género, y concluyen que existe una diversidad criptica dentro del género. Más tarde, varios trabajos describieron especies nuevas basados en morfología de genitales y análisis genéticos (Cong y Grishin 2014; Cong et al. 2021; Nakahara et al. 2017). Janzen et al. (2017) confirmaron la existencia de especies cripticas dentro del género *Udranomia* A. Butler, 1870 de la familia Hesperiidae (Lepidoptera), habiendo una concordancia de la secuenciación completa del genoma nuclear con el código de barras, distribución ecológica, historia natural, y la variación del color en los adultos.

Burns et al. (2008), con base en información genética y la relación de sus plantas hospederas, analizaron un complejo de especies crípticas del noroeste de Costa Rica del género *Perichares* Scudder, 1872 (Hesperiidae: Hesperiinae) y encontraron que la información de las plantas hospederas constituyen caracteres ecológicos distintivos y complementarios a los morfológicos, al existir preferencias de oviposición de las hembras. Por otro lado, Jasso-Martínez et al. (2016) resolvieron un problema taxonómico con el complejo *Enantia jethys* (Pieridae) de México usando caracteres moleculares. Ellos confirmaron tres especies reportadas en la literatura y evidenciaron una potencial especie críptica no descrita. Más tarde, Jasso-Martínez et al. (2018) demostraron eventos de hibridación en este complejo utilizando diferentes genes de origen mitocondrial y nuclear, cuando se encuentran en simpatría parcial.

El género *Biblis* Fabricius, 1807 es monotípico, cuya especie presenta seis subespecies, una de las cuales aún no se encuentra descrita de Perú (Lamas 2004). Las localidades tipo de estas subespecies son las siguientes: para *B. hyperia*

hyperia (Cramer, 1779), de St. Thomas, Islas Vírgenes, Britanicas; *B*. *h. laticlavia* (Thieme, 1904) en Rio Napo, Ecuador; *B*. *h. nectanabis* (Fruhstorfer, 1909) de Rio Grande del Sur, Brasil; *B*. *h. pacifica* (A. Hall, 1928) de Huigra, Ecuador; y *B*. *hyperia aganisa* Boisduval, 1836, de "Java"; sin embargo, Godman y Salvín (1893) mencionan que se trata de un error la localidad de "Java" y que el ejemplar tipo es de México, como lo muestra la etiqueta del tipo (Mexique). Como también se conocen varias sinonimias, cuyos tipos provienen de Oaxaca, México.

Biblis aganisa Boisduval, 1836 fue descrita como especie. Más tarde su estatus cambio, y se colocó como una subespecie de *Biblis hyperia* (Cramer) sin reunir pruebas suficientes para justificar este cambio (Lamas 2004). Lo mismo ocurrió con *Biblis laticlavia* que se describió como especie, no obstante, es fácilmente reconocible por su amplia banda submarginal en el dorso del ala anterior, muy diferente en esta especie en tamaño y forma con las demás del género; además de las diferencias considerables en los genitales masculinos. Dos integrantes más de este género *B*. *h*. *pacifica* (Hall, 1928) y *B*. *h*. *nectanabis* (Fruhstorfer, 1909) fueron descritas como subespecies de *Didonis biblis* Fabricius, 1807 (ahora es sinónimo de *B*. *hyperia*) y posteriormente movidas como subespecies de *B*. *hyperia,* que exhiben diferencias muy sutiles en la banda submarginal del ala posterior, en tamaño y coloración.

Prado et al. (2011) en un estudio sobre la diversidad de larvas y adultos de especies de la familia Nymphalidae (Papilionoidea) de la Península de Yucatán usando Citocromo C Oxidasa I (COI), encontraron porcentajes de divergencia mayor a 2 % en secuencias de dos grupos reconocidos como *Biblis hyperia aganisa,* con un promedio de divergencia de 4.6 %, que corresponden al taxón conocido para México y otro taxón aun no descrito y que no ha sido estudiado con técnicas complementarias, como un análisis morfológico, morfométrico, y con muestras de diferentes zonas del área de distribución de la especie.

A la fecha, ningún estudio detallado donde se incluya un análisis con caracteres moleculares y morfológicos que verifiquen el estatus de las subespecies de *Biblis hyperia* se ha publicado. Zhang et al. (2021) proponen que *Biblis aganisa* es una especie válida, por lo que su estatus de especie fue restablecido. Aunque en su estudio, excluyen los caracteres morfológicos y utilizan la secuencia de solo un espécimen como representante de *B*. *aganisa*; además que la secuencia del espécimen no es del país de origen de la localidad tipo, y ni misma zona biogeográfica. Según este estudio, *Biblis* y *Vila* Kirby, con un porcentaje de separación del 7 % (46 pb) son subgéneros de *Biblis,* y las dos especies que incluyeron del subgenero *Biblis* difieren en distancia genética del 4.6% (30 pb), por lo que no es monotípico*,* ya que estaría compuesto de las especies de ambos subgéneros, en caso de *Vila,* se consideran tres especies válidas de las cuales dos de éstas fueron consideradas en su estudio (*V*. *azeca* (E. Doubleday, [1848]) y *V*. *eudiformis* Joicey & Talbot, 1918).

Un caso similar ocurre para *Biblis aganisa,* proviene de la base de datos pública en BOLD (http://www.boldsystems.org/index.php), donde Janzen y Hajibabaei (2009) registraron secuencias de ejemplares de Costa Rica, las cuales fueron previamente comparadas con el índice de código de barras (BIN), que son secuencias asignadas a una Unidad Taxonómica Operativa (OTU), y que muchas veces corresponden a especies conocidas (Ratnasingham y Hebert 2013). Sin embargo, cuando el OTU no corresponden con un BIN de la base, este grupo de secuencias probablemente no pertenece a una especie conocida o la especie no se ha secuenciado. Los porcentajes entre OTU son superiores al 2% de distancia genética, por lo que estos autores asignaron un nombre de OTU diferente a los tres grupos de secuencias: *Biblis* sp. *aganisa*DHJ01 (n = 5), *Biblis* sp. *aganisa*DHJ02 (n = 4), y *Biblis* sp. *aganisa*DHJ03 (n = 14) (ver Anexo).

De acuerdo a los datos anteriores y al porcentaje de 4.6% de separación entre los dos grupos de la península de Yucatán en México encontrado por Prado et al. (2001), existen dos posibles especies cripticas en México y tres especies

potenciales en Costa Rica. Los grupos de México fueron determinados por unas pocas secuencias de COI (11 ejemplares: *Biblis hyperia*ECO 01 (n = 1) y *Biblis hyperia*ECO 02 (n = 10)).

Zhang et al. (2021) evidencian que la especie mexicana es una especie distinta a *Biblis hyperia*, por lo que seguir considerando los grupos de México como *Biblis hyperia* no sería adecuado. La especie mexicana nombrada con especímenes tipo de Oaxaca y México es *Biblis aganisa*, que fue descrita como especie. A partir de aquí, los grupos de México son analizados como *Biblis aganisa,* y se conserva el grupo alfanumérico original para simplificar el nombre: ECO 01, ECO 02, como fueron nombrados por Prado et al. (2011).

Para el reconocimiento de la biodiversidad, así como para cualquier estudio ecológico, de manejo y conservación de especies, es necesario tener clara la identidad de los taxones que se quieren estudiar. Resolver si *Biblis aganisa* es un complejo de especies es importante para la realización de estudios posteriores y un primer avance del estudio sistemático del género *Biblis*

Hipótesis: Las diferencias genéticas halladas en *B. aganisa* de México previamente mediante marcador COI se debe a que ésta constituye un complejo de especies crípticas.

Predicción: Si este es un complejo críptico, entonces cada grupo genético tendrá caracteres morfológicos que soporten dichos conjuntos. Por lo que la pregunta es: ¿Cuáles son esos caracteres morfológicos congruentes que soportan las diferentes especies dentro del complejo?

Por lo que el objetivo general de la presente investigación es demostrar la existencia de especies cripticas, evaluando caracteres genéticos y que puedan ser reconocidas morfológicamente.

“New complex of cryptic species discovered in genus *Biblis* (Papilionoidea: Nymphalidae: Biblidinae) in Mexico”

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New complex of cryptic species discovered in genus Biblis (Papilionoidea: Nymphalidae: Biblidinae) in Mexico

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Abstract:	Our research focuses on demonstrating the existence of cryptic species named under Biblis aganisa. We used COI sequences to delimit Biblis species for Mexico and examine phylogenetic relationships, with sequences from Mexico, Costa Rica, Argentina, USA, and Guana Island using a Bayesian inference tree. Additionally, we used 17 concatenated sequences of the MDH and GAPDH nuclear genes for Mexico, and a Bayesian inference tree and a neighbor-joining tree were obtained. We performed a discriminant analysis with quantitative traits using female and male wing and genitalia; and a tree of maximum parsimony based on 39 qualitative characters of parts of the wings, head, and male genitalia. The results were congruent in the three analyses; three groups were formed based on DNA, ECO 01 + DHJ02, ECO 02 + DHJ01, and ECO 03. The wings characters that contributed more than 50 % in the separation: wing length, anal margin length, and distance from the band to the outer margin. In the male genitalia, it was the angle of the integument, uncus, and the length of the hypandron. While in females, it was the angle of the antepophysis and the length of the abdomen. For the analysis of qualitative characters, a tree of maximum parsimony was obtained where 20 characters were informative. We confirmed the existence of three cryptic Biblis species in Mexico, two not yet described, and one corresponds to Biblis aganisa = ECO 02, this one is in sympatry in Oaxaca and Sinaloa (ECO 03) and Peninsula de Yucatán (ECO 01)

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New complex of cryptic species discovered in genus *Biblis* (Papilionoidea: Nymphalidae: Biblidinae) in Mexico

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hugo Álvarez García and Carmen Pozo. The first draft of the manuscript was written by Hugo Álvarez García and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest. The authors declare that they have no conflicts of interest.

Competing Interests. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Abstract

Our research focuses on demonstrating the existence of cryptic species named under *Biblis aganisa*. We used COI sequences to delimit *Biblis* species for Mexico and examine phylogenetic relationships, with sequences from Mexico, Costa Rica, Argentina, USA, and Guana Island using a Bayesian inference tree. Additionally, we used 17 concatenated sequences of the MDH and GAPDH nuclear genes for Mexico, and a Bayesian inference tree and a neighbor-joining tree were obtained. We performed a discriminant analysis with quantitative traits using female and male wing and genitalia; and a tree of maximum parsimony based on 39 qualitative characters of parts of the wings, head, and male genitalia. The results were congruent in the three analyses; three groups were formed based on DNA, ECO 01 + DHJ02, ECO 02 + DHJ01, and ECO 03. The wings characters that contributed more than 50 % in the separation: wing length, anal margin length, and distance from the band to the outer margin. In the male genitalia, it was the angle of the integument, uncus, and the length of the hypandrium. While in females, it was the angle of the anteapophysis and the length of the abdomen. For the analysis of qualitative characters, a tree of maximum parsimony was obtained where 20 characters were informative. We confirmed the existence of three cryptic *Biblis* species in Mexico, two not yet described, and one corresponds to *Biblis aganisa* = ECO 02, this one is in sympatry in Oaxaca and Sinaloa (ECO 03) and Peninsula de Yucatán (ECO 01)

Keywords: *Biblis aganisa***,** COI, MDH and GAPDH nuclear genes, Yucatan peninsula

Introduction

Identifying species based on the DNA barcode has proven to help distinguish and discover new species (Blaxter 2003; Hallwachs et al. 2008; Janzen et al. 2017; Gaytán et al. 2020; D'ercole et al. 2021). The premise is that sequence diversity within a short, standardized segment of the mitochondrial genome (mtDNA) can provide a unique biological barcode, allowing species-level identifications, it presents a rapid mutation rate, a significant variation in sequences between different species, and a low variation within the same species (Hebert et al. 2003; Hajibabaei et al. 2006). A barcode system analyzes lineages whose species may present genetic divergence (greater than 2%, ordinarily in vertebrates and 3% in Lepidoptera: Hebert et al. 2003; Sperling 2003). However, this is not a substitute for a complete taxonomic study, and the use of barcodes must be combined with morphological and ecological data for the results to be decisive for the final recognition of the species (Sperling 2003; Hebert et al. 2004b).

Different studies have used the barcode in the Insecta class and have uncovered many cryptic species (e. g., Hallwachs et al. 2008; Nieukerken et al. 2012; Seraphim et al. al. 2014; Kim et al. 2020; Moraes et al. 2021). Cryptic species are morphologically similar taxa determined under a single name (Bickford et al. 2007; Gill et al. 2016). They may differ in biological attributes, like host specificity, mating system, and susceptibility to different parasitoids, among others (Hebert et al. 2004a; Bickford et al. 2007; Pfenninger and Schwenk 2007; Trontelj and Fier 2009).

An excellent example of the usefulness of the COI, as a tool in molecular systematics, developed in the genus *Hermeuptychya* (Fabricius, 1775) from the first publication with the use of this gene in this genus (Seraphim et al. 2014), where the unknown cryptic diversity within this genus was exposed and from which it led to the discovery of new and corroborated species with morphological analysis of genitalia (Cong and Grishin 2014; Nakahara et al. 2017; Cong et al. 2021). On the other hand, in some cases, it has worked to detect cryptic species of *Astraptes fulgerator* (Walch, 1775) (Hebert et al. 2004), but not enough evidence has been presented to confirm this (Brower 2006). In another study, Janzen et al. (2017) confirmed the existence of cryptic species within the species previously known as *Udranomia kikkawai* (Weeks, 1906) of the Hesperiidae family, registered from Mexico to Brazil. They find two more species within it using complete genome sequencing and

barcode (COI). They concluded showing a subtle difference in adult color variation, size, ecological distribution, and natural history.

On the other hand, Jasso-Martínez et al. (2016) solved the taxonomic problem about the number of species within the *Enantia jethys* complex (Pieridae) of the Mexican species, using COI as a molecular character. They confirmed the three species reported in the literature and a fourth potential cryptic species not yet described. Later Jasso-Martínez et al. (2018) demonstrated hybridization events in three species of the genus *Enantia* that occur in partial sympatry using different genes. Burns et al. (2008) analyzed a complex of cryptic species of the genus *Perichares* (Hesperiidae: Hesperiinae) from northwestern Costa Rica based on genetic information and their host plants´ relationship. They found that information on the host plants of this genus constitutes an ecological character, which helps distinguish oviposition preferences of the females of each species.

The genus *Biblis* Fabricius, 1807 (Lepidoptera: Nymphalidae) is monotypic, whose species has six subspecies in America, one of which has not been described from Peru (Lamas 2004) yet. The type localities of these subspecies are the following: *Biblis hyperia hyperia* (Cramer, [1779]) from St. Thomas, “Indies Occidentales”; *B. h. laticlavia* (Thieme, 1904) from Rio Napo in Ecuador; *B. h. nectanabis* (Fruhstorfer, 1909) from Rio Grande do Sul, Brazil; *B. h. pacifica* (A. Hall, 1928) from Huigra, Ecuador; *B. h. aganisa* Boisduval, 1836, from "Java", however, Godman and Salvin (1893) mention that it is an error about the locality of "Java" and that the type specimen comes from Mexico, without mentioning the possible locality within Mexico, and on the other hand the known specimen and that it is a well-known synonym comes from [Oaxaca]; finally, *B. hyperia* nov. ssp., from Peru (Lamas 2004).

Biblis aganisa Boisduval, 1836, was described as a species. Later the status was changed to a subspecies of *Biblis hyperia* (Cramer, 1779) without gathering sufficient evidence to justify this change (Lamas 2004). The same step with *Biblis laticlavia* (Thieme, 1904), described as a species easily recognized by its broad submarginal band of the posterior wing on the back and the marked difference in the size of each of the spots that make up this band, very different in this species with the others of the group. In addition to exhibiting considerable differences in male genitalia to consider it a species. Two more members, *B. h. pacifica* (Hall,

1928) and *B. h. nectanabis* (Fruhstorfer, 1909) of this genus were described as subspecies of *B. hyperia*, the second later placed in this species whose differences are very subtle in the size and coloration of the submarginal band.

Prado et al. (2011) examined the diversity of larvae and adults of the Nymphalidae (Papilionoidea) family from the Yucatan Peninsula using Cytochrome C Oxidase I (COI), where *Biblis hyperia* was also included, that had divergence percentages above 3% in the sequences in individuals from the same geographic area. What suggests the presence of new species. *Biblis hyperia* split into two separate groups, with an average divergence of 4.6%, and were named with a provisional OTU: *Biblis hyperia*ECO 01 and *Biblis hyperia*ECO 02, and as antecedent only a single known group present in Mexico.

However, no detailed studies include molecular characters combined with morphological data to verify status of subspecies of *Biblis hyperia*. Recently, Zhang et al. (2021) proposed that *Biblis aganisa* is a valid species and will rise again in species status it a distinct species from *Biblis hyperia*. However, morphological data are not included, and the only specimen used as a representative of *B. aganisa* does not even come from Mexico (country of origin of the type specimen, different biogeographic region). According to this study, the genus *Biblis* would be composed of two subgenera *Biblis* and *Vila* Kirby, with a separation percentage of 7% (46 bp), between both subgenera and 4.6% (30 bp) between species in the subgenera *Biblis*, which should be considered non-monotypic, since it would be composed of the species of each of these subgenera. In the case of *Vila*, three valid species are known; two of these were considered in his study (*V. azeca* (E. Doubleday, [1848]) and *V. euidiformis* Joicey & Talbot, 1918).

A similar case is found in the public database in GenBank/Bold System where Janzen and Hajibabaei (21-Dec-2009) registered sequences of *Biblis* specimens from Costa Rica, which were assigned to three BINs (BOLD: AAC6005, BOLD: ABY4876 and BOLD: AAC0692). BINs are a group of sequence assigned to an operational taxonomic unit (OTU), that often correspond to species (Ratnasingham and Hebert 2013). Janzen and Hajibabaei assigned a name to each group of sequences. *Biblis* sp. *aganisa*DHJ01 (n = 5), *Biblis* sp. *aganisa*DHJ02 (n = 4) and *Biblis* sp. *aganisa*DHJ03 (n = 14). Out of a total of four BINs registrations in

BoldSystem (http://www.boldsystems.org/index.php/Public_BarcodeIndexNumber_Home), two *Biblis* BINs share Mexico and Costa Rica.

According to the 4.6% separation percentage between the two groups of the Yucatan peninsula in Mexico, there are two possible cryptic species (Prado et al. 2011). Furthermore, possibly three cryptic species in *Biblis* from Costa Rica (according to the OTUS of Janzen and Hajibabaei on GenBank/BOLD, not yet thoroughly analyzed). The groups from Mexico were determined by a few specimens (11 specimens): *Biblis hyperia*ECO 01 (1) and *Biblis hyperia*ECO 02 (10) and by a single gene fragment (COI).

The study by Prado et al. 2011 was prior to that of Zhang et al. (2021), where it is shown that the Mexican species is different from *Biblis hyperia*, so continuing to consider the groups from Mexico as B. *hyperia* would not be correct. The Mexican species named with type specimens from Oaxaca and Mexico is *Biblis aganisa*, which was described as a species, and its species status has re-emerged. From here on, we will refer to the *Biblis* of Mexico groups, temporarily as *Biblis aganisa*.

There is no study includes an analysis of morphological characters and enough samples from different sites in Mexico to verify if there are cryptic species named as *B. aganisa*. Therefore, the evidence is insufficient to confirm whether two or more cryptic *Biblis* species occur in Mexico. Consequently, it is crucial to carry out more detailed morphological and genetic studies to confirm the potential new cryptic species inside the genus *Biblis* in the Yucatan peninsula, Mexico.

According to the above, the present study ample the molecular analyzes using COI and also two nuclear genes. And also, a morphological study that verifies the existence of cryptic species in *Biblis aganisa*. Considering the following questions more specifically: (1) Are there morphological characters of the adults that support separating two or more cryptic species of *Biblis*? (2) There is a congruence of the same groups formed with mtDNA, and nuclear genes (nDNA) with the morphometric analysis of adults. (3) The information from points 1 and 2 permits to identify how many cryptic *Biblis* species occur in Mexico? (4) If so, what are the geographic area where they are in sympatry? And finally (5) How are the groups formed?

Methods

Biological material

A total of 171 specimens of *Biblis aganisa* of Campeche, Quintana Roo, Yucatan, Tabasco, Oaxaca, Queretaro and Zacatecas deposited in the Lepidoptera Collection of the Museo de Zoología of ECOSUR-Chetumal (ECOCH-L) and in the Lepidoptera Collection from the Museo de Zoología de la Facultad de Ciencias (MZFC) of the Universidad Nacional Autónoma de Mexico (UNAM) have been selected for morphological and genetic analyses. In addition, we searched COI gene sequences of the *Biblis* genus in GenBank (Benson et. al 2014): eleven sequences used by Prado et al. (2011) were downloaded, 22 sequences from Costa Rica and nine sequences from Argentina have also included and four *Biblis* (two from the sugenera *Biblis* and two from the *Vila*) sequences used by Zhang et al. (2021) and one from *Vila azeca* (GQ864818.1) not used by these authors. Five sequences from five Nymphalidae species of GenBank were taken as outgroup (*Colobura annulata* Willmott, Constantino & J. Hall, 2001, *Archimestra teleboas* (Ménétriés, 1832), *Mestra amymone* (Ménétriés, 1857), *Mestra hersilia* (Fabricius, 1776) and *Mestra dorcas* (Fabricius, 1775)) for molecular analyses, already used in other studies on systematic (Walhlberg et a. 2005a, 2005b, 2009; Chazot et al. 2020) (complete data in Supplementary Information, excel sheet). For the mitogenome sequences of *Biblis hyperia* (NVG-19094E05), *B. aganisa* (NVG-17117F03), *Vila azeca* (NVG-19095B04), *V. eueidiformis* (NVG-19095B05) of the project (PRJNA731937) (Zhang et. al. 2021), we did the treatment to obtain COI. The cleaning of the adapters carried out with the Trim using BBDuk tool in Geneious (version 2020.2.3), afterwards, once the readings were cleaned, we made an assembly or COI mapping of these four species with the reference sequence used by Zhang et al. (2021), for later, the consensus sequences were included in the alignment.

Genetic processes and phylogenetic analyses

The extraction, amplification and sequencing of the specimens of the *Biblis* genus from Mexico (N = 160), were made considering the protocols from Hajibabaei et al. (2005) and Ivanova et al. (2006). Sequencing was carried out using COI primers with demonstrated efficacy in butterfly studies (Vodă et al. 2015; Jasso-Martínez et al. 2016), amplifying a ~ 657 bp fragment: LepR1(5´TAAACTTCTGGATGTCCAAAAAATCA-3') and LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3'). Additionally, two nuclear genes were sequenced (for N = 17 specimens of *Biblis* of Mexico) that have been effective for phylogenetic studies with butterflies (Wahlberg and

Wheat 2008; Wahlberg et al. 2016): 1) GAPDH (HybFrigga 5'-TAATACGACTCACTATAGGGAARGCTGGRGCTGAATATG t-3') and HybBurre 5'-ATTAACCCTCACTAAAGGGGWTTGAATGTACTTGATRAG RTC-3') and 2) MDH (HybMDHF 5'-TAATACGACTCACTATAGGGGAYATNGCNCCNATGATGGGNGT-3') and MDHmidR 5'-ATTAACCCTCACTAAAGGGAAYTGNGTRGATGARTGRTTNCC-3'). The samples from the other countries were not sequenced, however the same markers (LepF1/ LepR1) were used to sequence COI. The sequences were downloaded from the GENBANK of specimens from Costa Rica (N = 22), and Argentina (N= 9), and the five outgroups.

A total of four alignments were realized consisting of one for each gene separately (COI, MDH and GAPDH), and a concatenated alignment (COI+MDH+GAPDH) with 17 Mexican specimens for which we have the three genes sequenced plus one specimen of *Biblis hyperia* from GeneBank (voucher number NW106-3). All alignments were made in the Geneious Prime 2020.2.3 program (Kearse et al. 2012) using the Muscle 3.8.425 option and a refined alignment (Robert 2004; Edgar and Batzoglou 2006). The Mega X program (Kumar et al. 2018) was used for selecting the evolutionary model, which selected the GTR + G + I model with a lower BIC value (Huelsenbeck and Rannala 2004; Lecocq et al. 2013). Two methods were used to build the trees: Bayesian inference (Huelsenbeck and Ronquist 2001; Holder and Lewis 2003) and Neighbor Joining (NJ) (Saitou and Nei 1987). Trees under the Bayesian inference were constructed for the COI gene and the concatenated alignment of the three genes dataset using MrBayes (Huelsenbeck y Ronquist 2001). Analyses considered a gamma distribution with five categories and a percentage of invariant sites; MCMCMC chains ran for 5 million generations, and 25% of the trees were discarded (burned). A consensus tree was calculated after the burns. The posterior probabilities summarized in the MrBayes consensus tree were drawn on the clade maximum credibility tree as recommended by García-Sandoval (2014) for better visualization. The NJ consensus tree was constructed with the concatenated alignment of the three genes using the Geneious Prime (2020. version 2.3) program, considering the Tamura Nei model, with 1000 replicas, and collapsing the branches from 50% of the support. Sequence divergences were estimated using the Kimura two-parameter (K2P) distance model (Kimura 1980) in MEGA X (Tamura et al. 2011). We calculated the mean intraspecific and interspecific sequence divergence.

Morphological analyses

Based on our results of the genetic analysis, we have done morphological measurements on 148 specimens (85 males and 63 females) organized into three groups (ECO 01 (N = 4), ECO 02 (N = 137) and ECO 03 (N = 7)). From each specimen of both sexes, quantitative and qualitative characters were taken from the dorsal and the ventral view of the wings and for male and female genitalia. For the genitalia analysis, abdomens were removed and placed in labeled glass jars with a 10% sodium hydroxide (KOH) solution for one or two days (depending on how dry the abdomens are). Genitalia was removed using a stereoscope to remove fat and tissue with forceps, fine-tip dissecting needles, and a fine-bristle brush to remove scales. Clean genitalia (including hypandrium) were placed on concave slides with 70% ethanol or glycerol. Lateral, dorsal, and ventral view photographs were taken using a millimeter scale to obtain later measurements and comparisons (Zubek et al. 2015). Image J software was used for the measurements, calibrated with the millimeter scale each time a new photograph was used (Abramoff et al. 2004; Collins 2007).

Quantitative characters of *wings*. The following twelve dorsal (D) and ventral (V) measurements were taken with a digital vernier, following the nomenclature of the wings according to Miller 1970. An alphanumeric key was assigned as described below: wing length (D1), distance from band to disc cell (D2), band distance to outer margin (D3), spot length in cell M3-CuA1 (D4), spot length in Rs-M1 (D5), spot length in cell CuA2-1A + 2A (D6), band to disc cell distance (V1), distance from band to outer margin (V2), spot length in cell M3-CuA1 (V3), length of the spot in cell Rs-M1 (V4), spot length in CuA2-1A + 2A (V5), anal margin length (V6) (Supplementary Information: Fig. 1).

Quantitative characters of genitalia. The following ten male genitalia measurements were annotated: aedeagus length (EL), valvae length (VL), tegument length (TL), uncus length (UL), tegumen angle (TA), tegumen to saccus distance (TSD), uncus angle (UA), saccus length (SL), valvae width (VA), and androchonial patch length (APL) (Supplementary Information: Fig. 2a). Additionally, measurements on the hypandrium were made: hypandrium length (HL), hypandrium width (HW), and hypandrium angle (HA) (Supleppentary Information: Fig 2b). For the female genitalia: abdomen length (AL), width of ostium bursae (A), shortest distance between anteapophysis (B), longest distance between anteapophysis (C), length of anteapophysis (D), height of ostium

bursae (E) (Supplementary Information: Fig. 2c), length of copus bursae (F), approximate length of ductus bursae (G), width of papilla analis (H), and angle of anteapophysis (I) (Supplementary Information: Fig. 2d).

Four discriminant analyses were made based on the female wings, male wings, female genitalia, and male genitalia looking for groups formation of *Biblis hyperia aganisa*. Additionally, we performed a similarity percentage analysis (SIMPER) considering Euclidian distances as dissimilarity measure for female and male wings and genitalia separately to evaluate the contribution of the different measures. These analyses were processed with PAST version 4.03 (Hammer et al. 2001).

Qualitative characters of wings, head, body and genitalia. A total of 34 characters were taken, six from the dorsal view and six from the female and male ventral view (Supplementary Information: Figs: 3-5). A total of sixteen characters from male genitalia and four of the head (Labial palps) and antennas and two from the body were encoded (Supplementary Information: Fig. 3-10) (see table 1 and 2 in Supplementary information). We used the matrix generated and captured in the Mezquite program version 2.75 (Maddison 2021) and exported it in Nexus format for analysis. From the qualitative characters of wings of male and female, male genitalia, and body, head and antennas of male, the first tree of phylogenetic relationship based on maximum parsimony was obtained using PAUP V4.0a (Swofford 2001). All the characters were treated as disordered, and an exhaustive search was used, applying 1000 replicas. The optimization was with Accelerated Transformation (ACCTRAN). *Mestra amymone* (Ménétriés, 1857) was used as an external group (Freitas and Brown 2004). Then with the same matrix in WInclada Asado version 1.7 (Nixon, 2004), the characters were mapped, and two trees were built with heuristic search. For the first one, the complete matrix with all the characters was used, and for the second tree, all non-informative characters were excluded so as not to inflate the length of the tree (L) and the consistency index (CI) (Goloboff, 1993, 1995). A table of diagnostic characters was built from the tree map with the informative characters.

Geographical distribution

Using the coordinates of the 160 Mexican specimens belonging to the three groups (ECO 01 (4), ECO 02 (149), ECO 03 (7)) a distributional map was elaborated with the ArcGis program version 10.2.1

Results

Phylogenetic analyses

The Bayesian phylogeny constructed using COI gene dataset (Fig. 1; 214 sequences: 22 from Costa Rica, nine from Argentina, 171 from Mexico, one from EU, and one from the British Virgin Islands: Guana Island) revealed two sister clades well supported: 1) the first clade is confirmed by the majority of Costa Rica samples (DHJ03, DHJ02), samples from Argentina (*Biblis nectanabis*), and four samples from Mexico belonging to the ECO 01. The only sample of *Biblis hyperia* was combined with DHJ03. Remarkably, these four Mexican samples are pooled with some Costa Rica samples (DHJ02) and they are sister to Argentina samples; 2) a second clade is formed by all other Mexican samples separating very well the ECO3 from ECO2 samples. Some Costa Rica samples (DHJ01) and *Biblis aganisa* are pooled with ECO2 samples from Mexico in this clade.

The percentages of identity between the groups of the first clade made up of Mexico, Costa Rica, Argentina, and Island Guana. ECO 01, which was combined with Costa Rica (DHJ02), the percentage was 99.22%. In contrast, ECO 01 with Costa Rica (DHJ03) was 97.48%, with Argentina (*Biblis nectanabis*) 97.67%, and with the only sample of *Biblis hyperia* from Guana Island (95.54%). In the other clade composed of the groups from Mexico (ECO 02 and ECO 03), the percentage of identity was 96.12%. Some samples from Costa Rica (DHJ01) were combined with ECO 02 and with the only sample from Hidalgo (Texas, USA) by *Biblis aganisa* and their identity percentage was 100% (Table 1).

For the concatenated analysis, we obtained a final alignment of 1,440 bp (516 bp of COI + 344 bp for MDH + 580 bp for GAPDH). Both analyses, Bayesian (Fig. 2A) and NJ (Fig. 2B), permit to observe a separation between the three Mexican groups: ECO1 (blue in figures), ECO2 (green in figures), and ECO3 (pink in figures). Nevertheless, the Bayesian analysis presents a polytomy at the node between the three Mexican groups which does not resolve the separation. On the contrary, the NJ tree permits resolving the separation between the three Mexican groups with low to very high support.

The genetic divergence values were obtained in the groups of the first clade, made up of Costa Rica, Argentina, Guana Island, and Mexico. In ECO 01 combined with Costa Rica (DHJ02) and NW106-3, the mean

intraspecific distance was 0.42%. However, in ECO 01 and Costa Rica (DHJ03), the interspecific distance was 2.80%, and with Argentina (*Biblis nectanabis*) was 2.44%. For the second clade composed of the majority from Mexico and some samples from Costa Rica, the divergence between ECO 02 and ECO 03 was 4.12%. ECO 02 was combined with some samples from Costa Rica (DHJ01) and a *Biblis aganisa* from Hidalgo (Texas, USA), and the intraspecific divergence was 0.22%. The most considerable divergence between the groups from Mexico (ECO 01 and ECO 03) was 5.85% (Table 2).

Morphological analyses

Quantitative characters

The discriminant analysis based on male wings' characters' permits separating the three groups (Fig. 3A; ECO 01 in blue, ECO 02 in green, and ECO 03 in fuchsia). ECO 01 is well separated from the two remaining groups, while a slight overlap is noted between ECO 02 and ECO 03. The SIMPER analysis based on male wing measurements (Fig 10A, Supplementary Information) indicates that three variables could explain 50% of the separation between groups: wing length (D1), anal margin length (V6), and band to outer margin (V2).

The Discriminant analysis, considering the characters of females´ wings (Fig 3B), also permits separating the three groups. In this case, ECO 03 (fuchsia on Fig.3B) is well separate from the two other groups, while a slight overlap is observed between ECO 02 (green on Fig. 3B) and ECO 01 (blue on Fig.3B). The SIMPER analysis (Fig 10B Supplementary Information) revealed that the same characters of wing females explain 50% of the separation between groups: wing length (D1), anal margin length (V6), and band to outer margin (V2).

The discriminant analysis based on male genitalia characters (Fig 3C) permits to separate very well the three groups (ECO 01, ECO 02, and ECO 03) without any overlap. The SIMPER analysis (Fig 10C, Supplementary Information) indicates that only two characters explain 90% of the separation of the three groups: tegument angle (TA) and uncus angle (UA).

The discriminant analysis based on female genitalia characters (Fig3D) permits to separate also very well the three groups (ECO 01, ECO 02, and ECO 03) without any overlap. The SIMPER analysis (figure 3D, Supplementary Information) indicates that only two characters explain 90% of the separation of the three groups: angle anteapophysis (I) and length abdomen (AL).

Qualitative characters

We use a matrix of 34 characters (12 from the wings, six from the head, antennas, body and 16 from male genitalia (see table 1 in the Supplementary information) for the Maximum parsimony analysis, resulting in a single more parsimonious tree of 58 steps with a consistency index (CI) equal to 0.9138 and a retention index (RI) equal to 0.7222 and reporting 18 non-informative characters. The obtained tree has two clades, one with three terminal branches: *Biblis* sp n. 1(ECO 01), *Biblis aganisa* (ECO 02), and *Biblis* sp. n. 2 (ECO 03), and two terminal branches in a different clade: *Biblis hyperia* and *B. h. laticlavia* (Fig 12 and 13, Supplementary information). On the other side, the analysis with exhaustive and heuristic methods produced similar results using all the characters or just using the informative characters, for these last one 16 characters were informative, and the length of the tree was (L = 32), CI = 84, RI = 72 (see Fig 13, Supplementary information). The informative characters present only in the Mexican species were spot color in cell Sc+R1, basal point size, tegumen in lateral view, valvae in ventral view (size), and aedeagus (size) (see table 1 and Figs 3-10, Supplementary Information). We select the characters with the best CI fit and apomorphies for every three terminal branches of the first clade. Resulting for ECO 1, the size of the spot in the cell Sc + R1 is quasi absent to small (which one may be hardly visible or maybe small), the valvae in ventral view is slender, and the size of the aedeagus is medium; for ECO 02 the size of the basal points can range from small to large and in ECO 03, the integument may be slightly more arched. No more informative characters were found. A comparison of these diagnostic characters of the groups can be seen in more detail in Table 3 (supplementary information)

Geographical distribution

The map shows the records of the three groups: ECO 01, ECO 02 and ECO 03 in Mexico (Fig. 4), shows a broad distribution for *B. aganisa* ECO 02 (circles in green) mainly in the Southwest region with records in the Yucatán Peninsula (Quintana Roo, Yucatán and Campeche states), Chiapas, Oaxaca and only one record from Zacatecas. Meanwhile, the records for the *B. aganisa* ECO 01 (circles in blue) have a restricted distribution in

the South of the Yucatan Peninsula (Quintana Roo and Campeche states), near the border of Belize and Guatemala. Finally, *B. aganisa* ECO 03 (circles in pink) presents a restricted distribution in the North of Oaxaca and Sinaloa.

The map permits observing some sympatric area between groups. The ECO 01 and ECO 02 groups present a sympatric distribution in the south part of the Yucatan peninsula, more precisely in the Calakmul region of Campeche. On the other hand, ECO 02 and ECO 03 are in sympatry in the north of Oaxaca (Fig. 4).

Discussion

Previous studies with molecular traits using COI in *Biblis hyperia* have documented the separation of *Biblis hyperia*, establishing the possibility that it is a complex of cryptic species. Prado et al. (2011) proposed the division into two species when analyzing the specimens in the Yucatan Peninsula. Later, Zhang et al (2021) recognized that the typical subspecies is different at a specific level from the taxon of North and Central America, renaming it *Biblis aganisa*. Our study confirms the result of Zhang et al. (2021) when comparing all our samples with the *Biblis aganisa* and *Biblis hyperia* sequences, which are grouped into different clades. Moreover, all the samples of the ECO 02 group are the same as *Biblis aganisa*. In our study, what is established by Prado et al (2011) is confirmed by studying a more significant number of samples; in addition, a third lineage (ECO 03) is recognized (figure 1). On the other hand, in GenBank / BoldSystem, Janzen and Hajibabaei (2009), recognize three lineages (DHJ01, DHJ02 and DHJ03), two of which are genetically close to the lineages of Mexico (ECO 01 = DHJ02 and ECO 02 / DHJ01) (figure 1). The three lineages discovered with specimens from Mexico are strongly supported by the COI and two nuclear genes that have proven effective for phylogenetic studies (Wahlberg and Wheat 2008; Whalberg et al 2016). In addition, the resulting clades in the trees (Bayesian Inference IB / Neighbor-Joining NJ) present the same groupings and a similar topology, where these three groups were located, which supports that there is sufficient support (fig 2). The genetic distances according to the Kimura model (Kimura 1980) between groups or species, found both by Prado et al (2011) and Zhang et al. (2021) establish that there is a valid difference at a specific level, having a threshold above 4% with COI for *Biblis* and above 3% for Lepidoptera as suggested by Hebert et al. (2003). In our study, genetic distances between ECO 01 / ECO 02 were similar to Prado et al (2011). On the other hand, the new group (ECO

03) presents a distance of 5.87% for ECO 01 and 4.12% for ECO 02; this is congruent and not surprising since their biogeographic areas are different from their evolutionary history (Fig 4).

The use of discriminant analysis in other groups of Lepidoptera using measurements of parts of the wings and structures of the male genitalia has been proper to delimit species (Kolev 2005; Hernández-Roldán and Munguira 2008; Prieto et al. 2009; Nuñez et al. 2021) and even to confirm cryptic species (Dincă et al. 2011). According to the discriminant analyses in which linear measurements were used in wing characters (figs 3A and 3B), the results were congruent with the IB and NJ trees (figs 1 and 2). This method turned out to be effective for species discrimination, as there was no overlap between the three groups. On the other hand, the male genitalia of the three groups are similar, but when applying this analysis, the difference is clear, as there is no overlap between the groups (Fig. 3C and 3D). Hypandrium examination has been helpful for reliable diagnosis in the delimitation of species within Biblidinae, despite only shape and proportions being used (Jenkins 1990; Zubek et al. 2015; Leite et al. 2017). In our study, the length and angle of the hypandrium were two fundamental measures to support the separation. The impossibility of differentiation by a simple observation, means that these three species must be considerate cryptic species.

Phylogenetic studies where molecular and morphological characters are included are crucial to resolve relationships between species, particularly in unresolved nodes that become more robust when more characters are added to the analysis (Wahlberg and Nylin 2003). On the other hand, if there is a good selection of characters, the results may be consistent with genetic analyzes (Shi et al. 2015). According to the cladistics analysis where we included 34 morphological characters (qualitative characteristics) of head, body, wings and genitalia, with a single more parsimonious tree, this turned out to be in accordance with the molecular phylogeny since the same relationships were recovered between the three groups (ECO 01 (ECO 02, ECO 03)) (Fig 11, Supplementary Information).

Some of the characters that contribute to the formation of these groups are, for example, in the ECO 02 group, which is the most variable in its coloring pattern, it presents an exclusive characteristic that coincides with that presented in the type specimen of *Biblis aganisa* described by Boisduval of “Java” in 1836, that in ventral view

the submarginal band is thin and red scales predominate in it. However, this unique feature is rare since it was present in only 8% of all individuals. This variant of the state of band pattern characters in the analysis, did not give as an informative character, but an apomorphy. In addition, this group presents another variant of the submarginal band, which is wide clear, with few red scales, which resembles the type specimen of *Didonis pasira* Doubleday, [1848], which is currently considered synonymous with *B. aganisa*. So, we suggest that ECO 02 is the group that represents *B. aganisa*. For presenting two variants of the character status of the band pattern, a combination that does not occur in the other two groups. Besides that, the ECO 02 group has these characteristics. However, this form of the clear broad submarginal band is not exclusive to the group since it is also observed in individuals of ECO 03.

On the other hand, the ECO 01 group shares with both groups the pattern of the intermediate submarginal band (character 7, intermediate band to white) (see Fig 3, Supplementary Information), but the pinkish scales are absent in the submarginal area of the anterior wing in ventral view, a characteristic that is also shared with the other two groups. On the other hand, we observed a characteristic that not all the specimens of the ECO 03 group present, but that is only present in this group and is a red spot in the CuA1 cell of the forewings in dorsal view; however, not all the individuals present it, for this reason it was not included as a character. When examining the qualitative characteristics of the three groups by themselves, we consider it is difficult to identify any group since they share several characteristics with the other groups, so it is not very reliable to use only qualitative characters to discriminate species.

The data on the distributions of the sequenced specimens was necessary to detect sympatry areas and delimitation of the groups. One specimen (NW106-3) is related to ECO 01 and from which it is not known where the sample comes from, but it may come from between Mexico and Costa Rica. Our results on the actual distributions supported ECO 01 as a different group and further away from ECO 03, also because it did not present areas of sympatry and was in a different biogeographical area with its history (Fig 4). Also, there was no overlap of the groups using quantitative characteristics in both wings and genitalia (fig 3). There are other groups of Lepidoptera that do not extend their distribution beyond the Yucatan peninsula (Llorente et al. 2006), just to mention a few examples: *Heraclides rogeri rogeri* (Boisduval, 1836), *Hamadryas julitta* (Fruhstorfer,

1914). Menwhile, ECO 02 and ECO 03 that have broader distributions in Mexico, ECO 02, it is likely that their distribution is towards the Gulf slope and on the contrary in ECO 03 it exists towards the Mexican Pacific slope, with the zone of sympatry in both groups in northern Oaxaca, as demonstrated in this study and what occurs in other groups of Lepidoptera (e. g. *Lasaia*, Arellano-Covarrubias et al. 2019).

Now, it is demonstrated by this work that the genus *Biblis* is more diverse in Mexico than it was supposed in recent times. Also, we confirm the proposal of Zhang et al (2021) to reinstall the Boisduval nomination of *Biblis aganisa*; and reported the existence of two new species in the Mexican territory in sympatry with *B. aganisa*. Finally, we consider these species as cryptic species.

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Table 1 Means of identity with COI gene. Numbers in black are groups that present similarity above 98% in their bases and that are within the same clade.

	*Biblis. hyperia**	DHJ03	*Biblis nectanabis**	ECO 01	NW106-3	DHJ02	ECO 03	*Biblis aganisa*	ECO 02	DHJ01
B. hyperia	-									
DHJ03	**99.03**	-								
*B.nectanabis**	97.09	97.29	-							
ECO 01	95.54	97.48	97.67	-						
NW106-3	96.90	97.29	97.48	**99.81**	-					
DHJ02	96.32	96.71	97.67	**99.22**	**99.42**	-				
ECO 03	94.57	94.38	94.38	94.38	94.19	94.38	-			
B. aganisa	95.35	95.54	96.12	95.54	95.35	95.54	96.12	-		
ECO 02	95.35	95.54	96.12	95.54	95.35	95.54	96.12	**100**	-	
DHJ01	95.35	95.54	96.12	95.54	95.35	95.54	96.12	**100**	**100**	-

* With only one sequence taken from Zhang et al. 2021

Table 2 Distance matrix of the Kimura model (gen COI) shown under the diagonal, all them above 2%. The percent in parentheses correspond to mean values of intraspecific sequence divergence.

Group	*Biblis hyperia** / DHJ03 (0.52 %)	*Biblis aganisa** / ECO 02 / DHJ01 (0.22 %)	ECO 01 / NW106-3 / DHJ02 (0.42 %)	*Biblis nectanabis* (0.57 %)	ECO 03 (0.06 %)
Biblis hyperia * / DHJ03	-				
Biblis aganisa * / ECO 02/DHJ01	4.67 %	-			
ECO 01 / NW106-3 / DHJ02	2.80 %	4.55 %	-		
Biblis nectanabis	2.67 %	3.88 %	2.44 %	-	
ECO 03	5.90 %	4.12 %	5.85 %	5.78 %	-

* With only one sequence taken from Zhang et al. 2021

Figure Captions

Fig. 1 Bayesian phylogenetic relationship based on COI gene for the species of *Biblis* (Nymphalidae: Biblidinae). The numbers in the clades correspond to the posterior probability values, as support of the clade

Fig. 2 A. Phylogenetic tree using Bayesian inference showing the relationship between *Biblis* (Nymphalidae: Biblidinae) groups from Mexico using three genes (COI, MDH and GAPDH). The number next to the nodes indicates the posterior probability for Bayesian analysis as a supporting measure. B. Neighbor-Joining consensus tree using an alignment of 20 concatenated sequences of three genes (COI- MDH- GAPDH). The numbers in the nodes represent the support of the clades in percentage. Blue = *Biblis aganisa*ECO 01; green = *Biblis aganisa*ECO 02 and pink = *Biblis aganisa*ECO 03

Fig. 3 Discriminant analysis with morphometric measurements of the wings and genitalia of the male and female. A: Male wings and B: Female wings. C: genitalia in male and D: genitalia in female. Filled blue squares: *Biblis aganisa*ECO 01, filled green triangles: *Biblis aganisa*ECO 02 and pink filled diamonds: *Biblis aganisa*ECO 03

Fig. 4 Location of *Biblis* groups: blue circles = ECO 01, green circles = ECO 02 and pink circles = ECO 03. The areas where the groups overlap are zones of sympatry

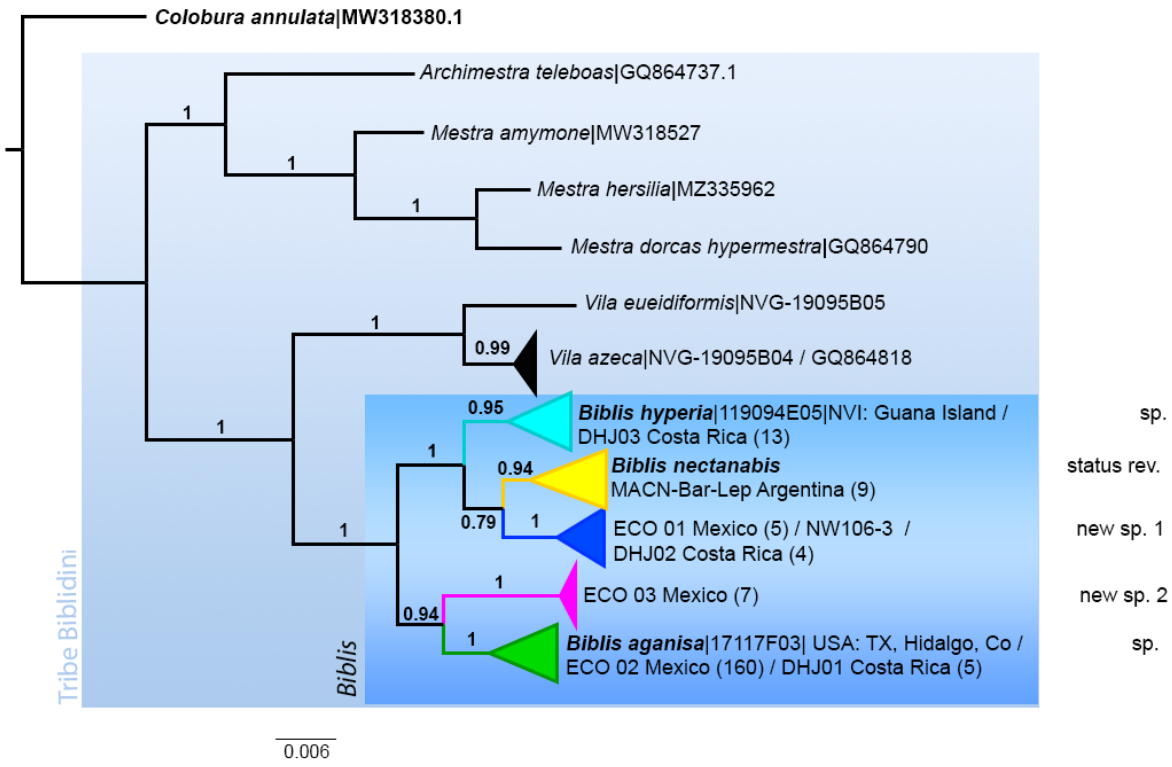
Colobura annulata|MW318380.1
Archimestra teleboas|GQ864737.1
Mestra amymone|MW318527
Mestra hersilia|MZ335962
Mestra dorcas hypermestra|GQ864790
Vila eueidiformis|NVG-19095B05
Vila azeca|NVG-19095B04 / GQ864818
Biblis hyperia|119094E05|NVI: Guana Island / DHJ03 Costa Rica (13)
Biblis nectanabis MACN-Bar-Lep Argentina (9)
ECO 01 Mexico (5) / NW106-3 / DHJ02 Costa Rica (4)
ECO 03 Mexico (7)
Biblis aganisa|17117F03| USA: TX, Hidalgo, Co / ECO 02 Mexico (160) / DHJ01 Costa Rica (5)
sp.
status rev.
new sp. 1
new sp. 2
sp.
Tribe Biblidini
Biblis
0.006

Fig. 1

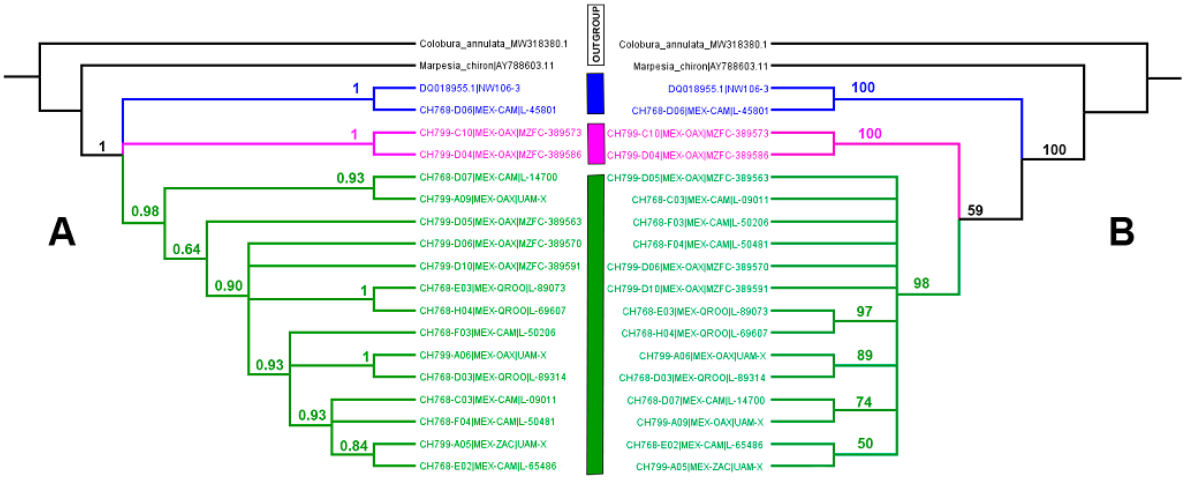
A
B
OUTGROUP
Colobura_annulata_MW318380.1
Marpesia_chiron|AY788603.11
DQ018955.1|NW106-3
CH768-D06|MEX-CAM|L-45801
CH799-C10|MEX-OAX|MZFC-389573
CH799-D04|MEX-OAX|MZFC-389586
CH768-D07|MEX-CAM|L-14700
CH799-A09|MEX-OAX|UAM-X
CH799-D05|MEX-OAX|MZFC-389563
CH799-D06|MEX-OAX|MZFC-389570
CH799-D10|MEX-OAX|MZFC-389591
CH768-E03|MEX-QROO|L-89073
CH768-H04|MEX-QROO|L-69607
CH768-F03|MEX-CAM|L-50206
CH799-A06|MEX-OAX|UAM-X
CH768-D03|MEX-QROO|L-89314
CH768-C03|MEX-CAM|L-09011
CH768-F04|MEX-CAM|L-50481
CH799-A05|MEX-ZAC|UAM-X
CH768-E02|MEX-CAM|L-65486

Fig. 2

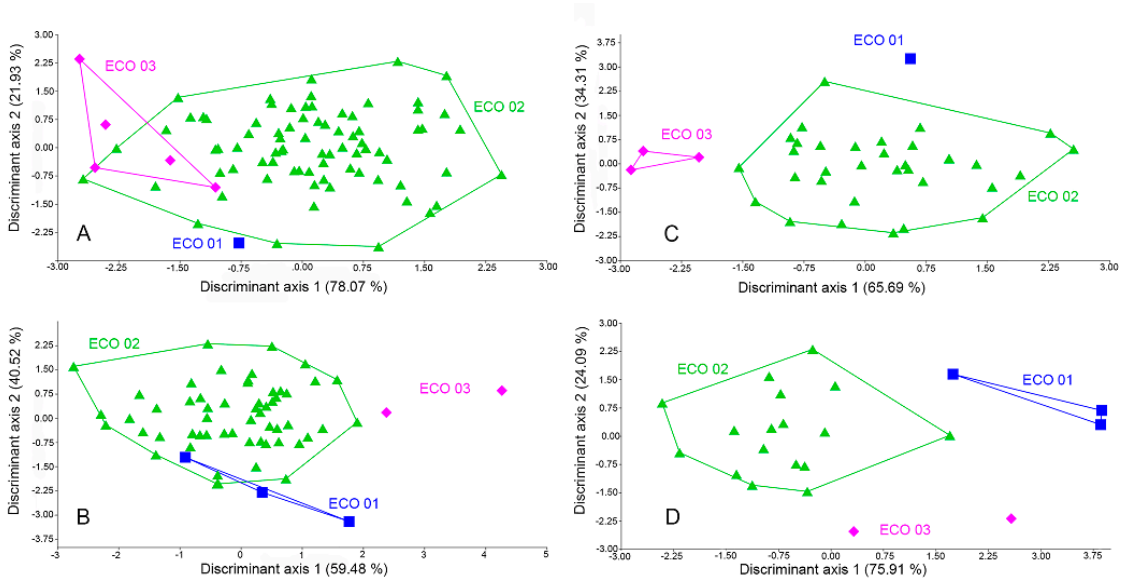
ECO 03
ECO 02
ECO 01
A
Discriminant axis 2 (21.93 %)
Discriminant axis 1 (78.07 %)
ECO 01
ECO 03
ECO 02
C
Discriminant axis 2 (34.31 %)
Discriminant axis 1 (65.69 %)
ECO 02
ECO 03
ECO 01
B
Discriminant axis 2 (40.52 %)
Discriminant axis 1 (59.48 %)
ECO 02
ECO 01
ECO 03
D
Discriminant axis 2 (24.09 %)
Discriminant axis 1 (75.91 %)

Fig. 3

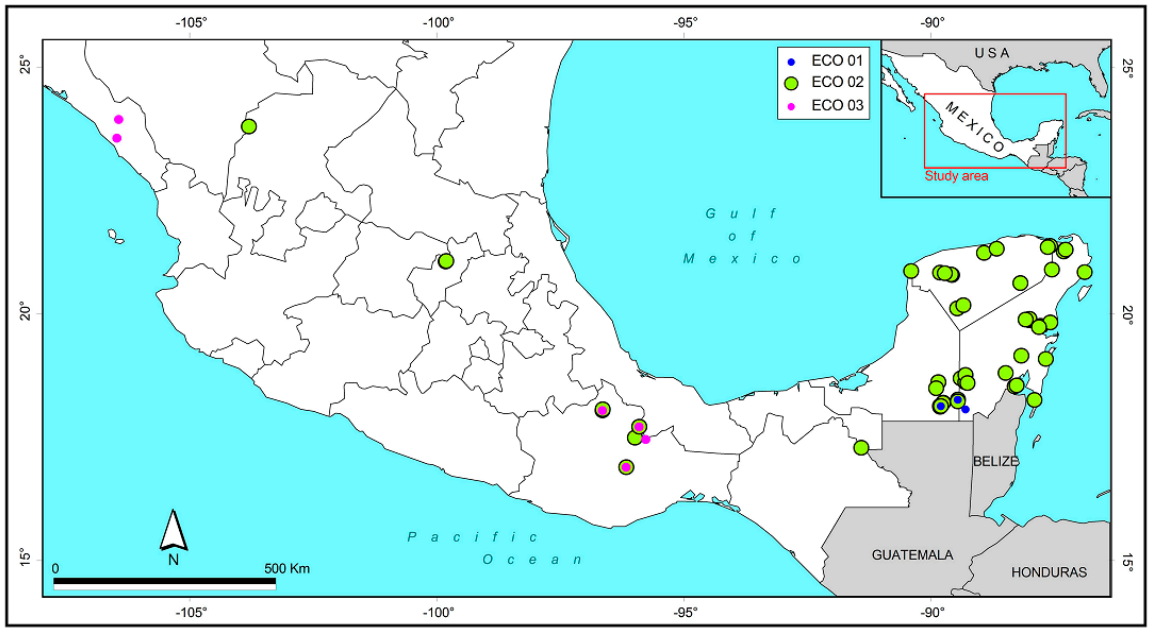
-105°
-100°
-95°
-90°
25°
20°
15°
ECO 01
ECO 02
ECO 03
USA
MEXICO
Study area
G u l f
o f
M e x i c o
BELIZE
GUATEMALA
HONDURAS
P a c i f i c
O c e a n
N
0
500 Km

Fig. 4

Supplementary Information (SI)

New complex of cryptic species discovered in genus *Biblis* (Papilionoidea: Nymphalidae: Biblidinae) in Mexico

Neotropical Entomology

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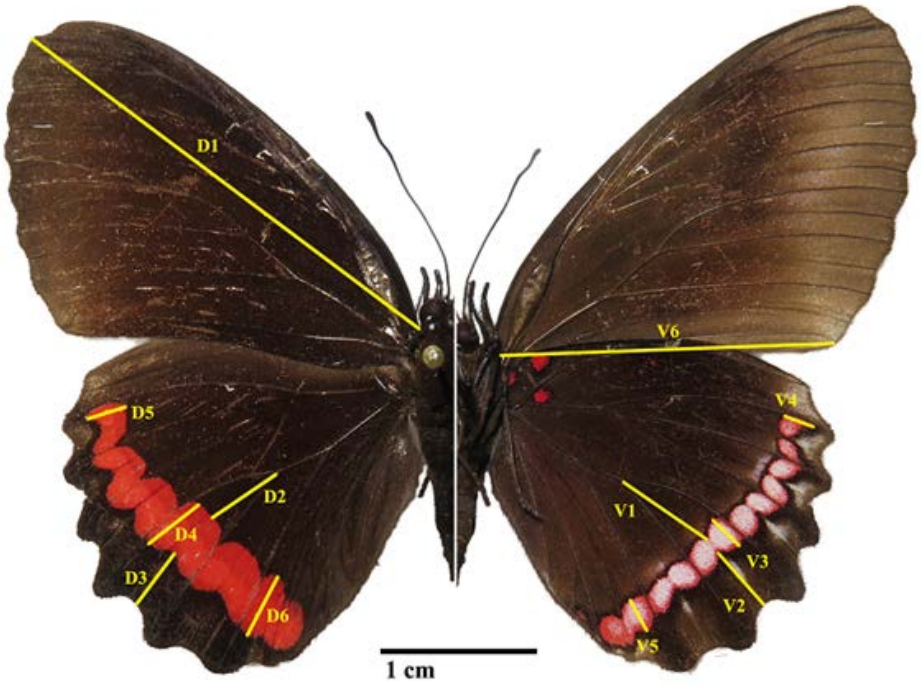

Fig. 1 Measurements on wings. Dorsal and ventral view. Wing length (D1), Distance from band to disc cell (D2), Band distance to outer margin (D3), Spot length in cell M3-CuA1 (D4), Spot length in Rs-M1 (D5), Spot length in cell CuA2-1A + 2A (D6), Band to discal cell distance (V1), Band to outer margin distance (V2), Spot length in cell M3-CuA1 (V3), Length of spot in cell Rs-M1 (V4), Spot length in CuA2-1A + 2A (V5), Anal margin length (V6)

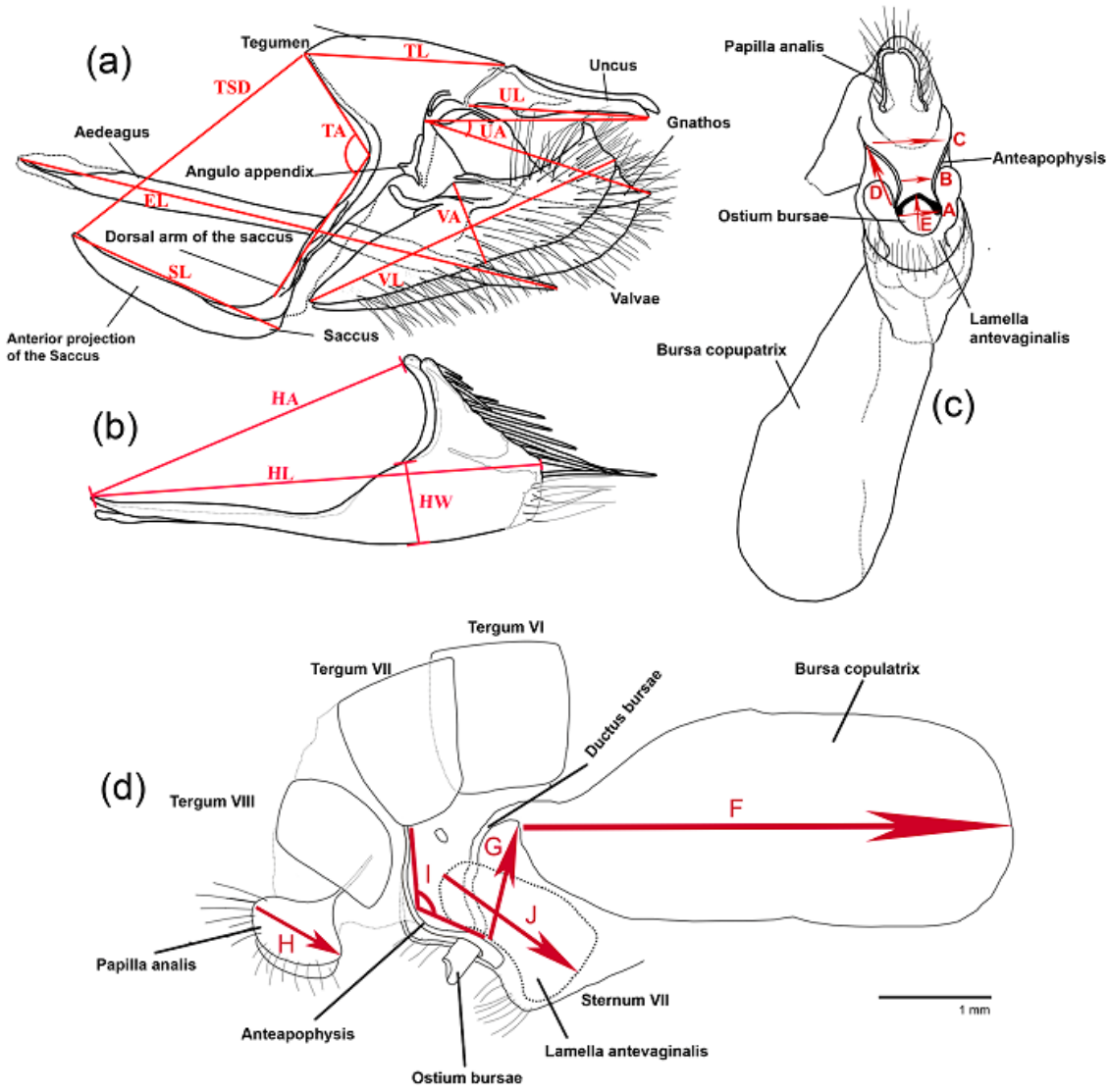

Fig. 2 (a) Measurements in male genitalia. Were measured: edeagus length (EL), valvae length (VL), tegumen length (TL), uncus length (UL), tegumen angle (TA), tegumen to saccus distance (TSD), Uncus angle (UA), saccus length (SL), valvae width (VA) and androchondrial patch length (APL). **(b)** Measurements in male hypandrium. length (HL), width (HW), angle (HA) were measured. **(c)** width of ostium bursae, B = shortest distance between anteapophysis, C = longest distance between anteapophysis, D = length of anteapophysis, E = height of ostium bursae. **(d)** F = length of copus bursae, G = approximate length of ductus bursae, H = width of papilla analis, and I = angle of anteapophysis

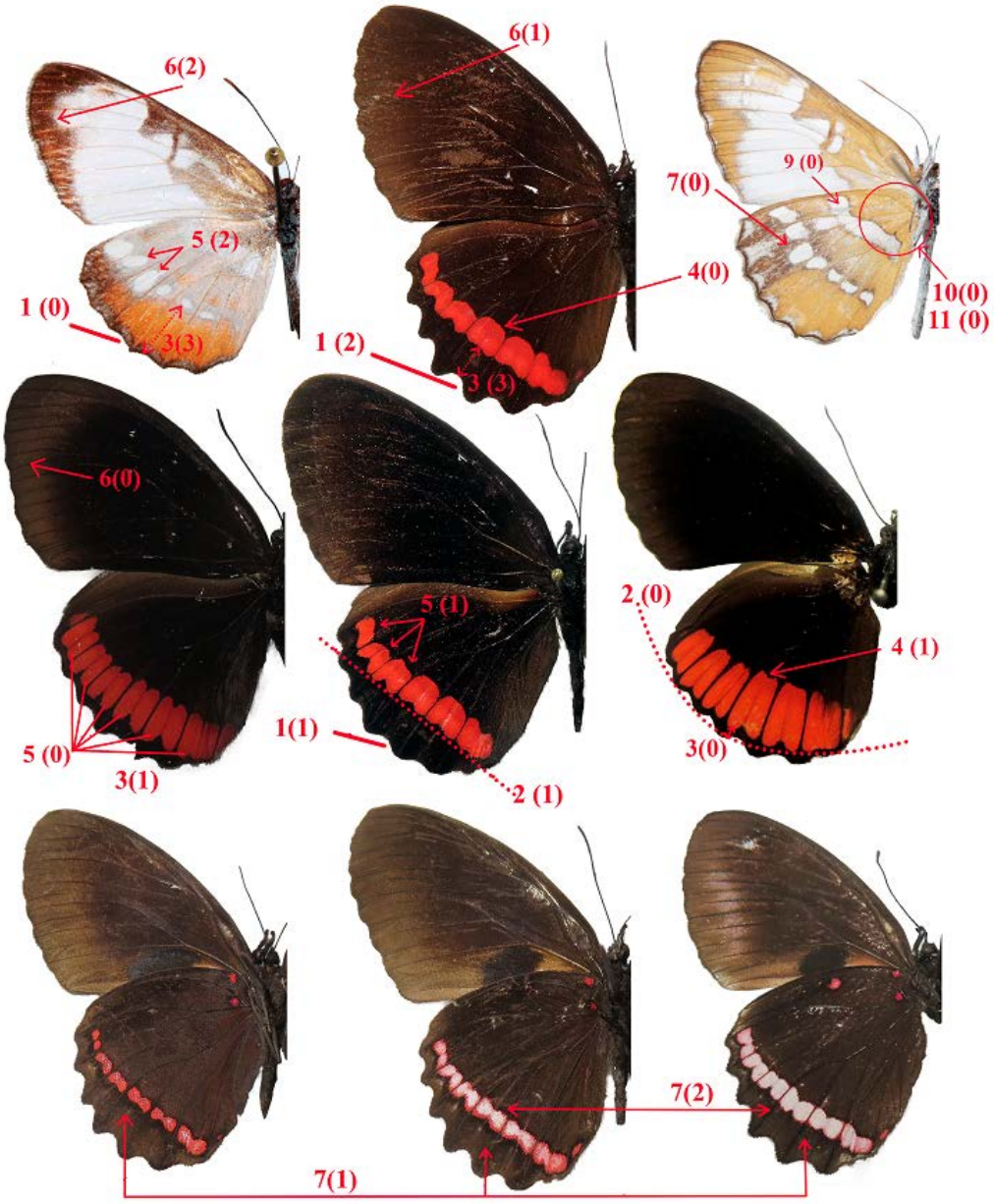

Fig. 3 Characters in wings: (dorsal: 1, 2, 3, 4; ventral: 7). Character 1: 1(0) weakly wavy; 1(1) moderately wavy; 1(2) strongly wavy. Character 2. Band shape: 2(0) convex; 2(1) straight. Character 3. Submarginal band (distance): 3(0) near the outer margin; 3(1) moderately close; 3(2) away from the outer margin (2)., Character 4. Submarginal band (size): 4(0) Short spots; 4(1) long spots. Character 5. Postdiscal spots: 5(0) same cell length RsM1 to Cu1Cu2; 5(1) spots in cells Rs-M1 to M2-M3 shorter than in cells M3-Cu1 to Cu1-Cu2; 5(2) spots in longer Rs-M1 and M1-M2 cells. Character 6. Submarginal area of forewing in dorsal view: 6(0) very clear that it is the rest of the wing; 6(1) slightly lighter than the rest of the wing (1); 6(2) darker than the rest of the wing (2). Character 7. Band pattern: 7(0) white band; 7(1) narrow red band to white wide; 7 (2) intermediate band to white wide

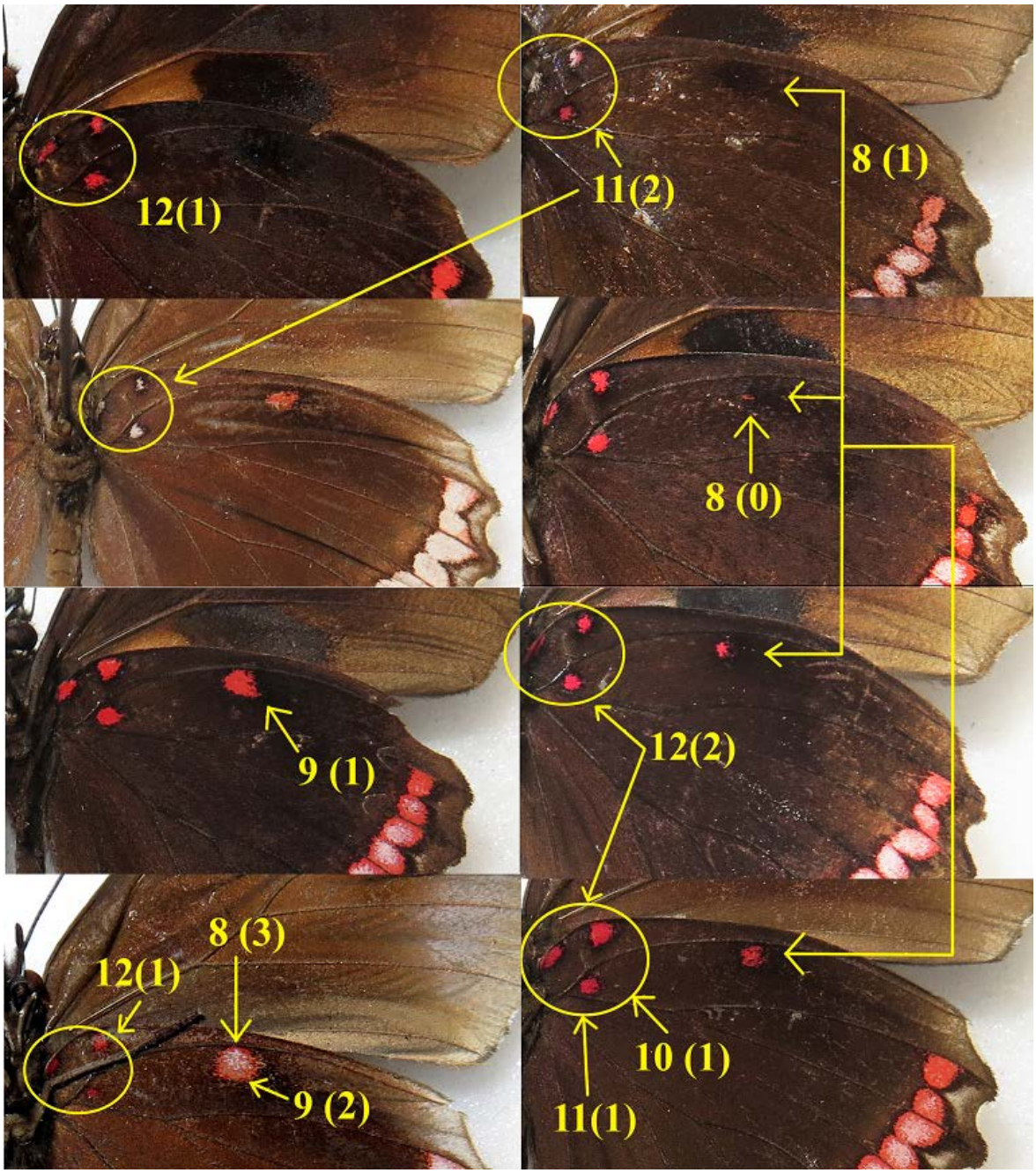

Fig. 4 Ventral characters. Character 8. Spot size in cell Sc+R1: 8(0) quasi absent; 8(1) quasi absent to small; 8(2) quasi absent to large; 8(3) big. Character 9. Spot color in cell Sc + R1: 9(0) white; 9(1) red; 9(2) red or red with white. Character 10. Basal area ventral view of the posterior wing: 10(0) base without spots; 10(1) spotted base. Character 11. Basal zone: 11(0) clear without spots; 11(1) dark with red spots; 11(2) dark with red spots with white. Character 12. Basal point size: 12(0) lost; 12(1) small to big; 12(2) medium to big

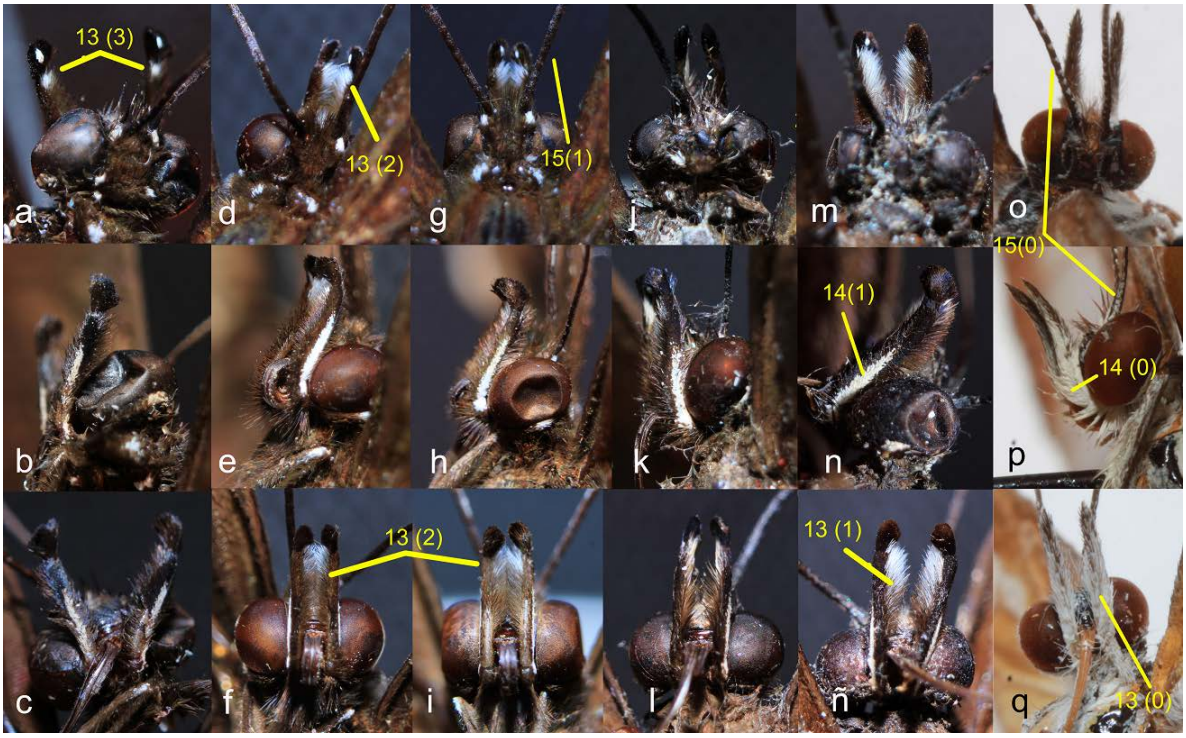

Fig. 5 Character 13. Labial palps (medium segment, internal zone): 13 (0) all the internal part; 13(1) abundant white cilia; 13(2) medium with white cilia; 13(3) few white cilia. a, b, c: ECO 01; d, e, f: ECO 02; g, h, i: ECO 03; j, k, l: *Biblis hyperia*; m, n, ñ: *Biblis hyperia laticlavia* and o, p, q: *Mestra amymone*

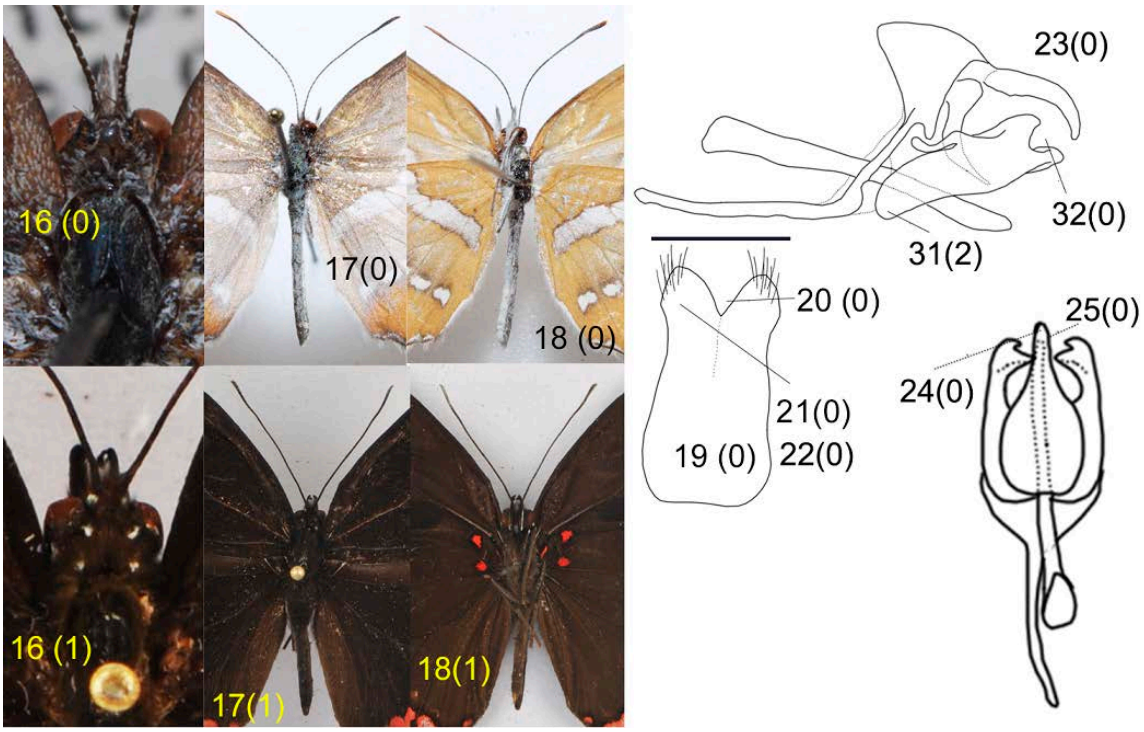

Fig. 6 Character 16. Head in dorsal view: 16(0) dotted with white scales surface; 16 (1) brown surface with six white spots. Character 17. Thorax and abdomen (dorsal view): 17(0) surface, abundant white scales; 17(1) brown, no white scales. Character 18. Thorax and abdomen (ventral view): 18(0) surface, abundant white scales; 18 (1) dark brown. Character 23. Proximal portion of the uncus lateral view: 23(0) not narrow, more sclerosed (0); Character 24. Distal portion of the uncus in dorsal view: 24(1): not bifurcated (0). Character 31. Valvae in lateral view, proximal portion: 31(2) round. Character 32. Valvae in lateral view, distal portion: 32(0) bifurcated

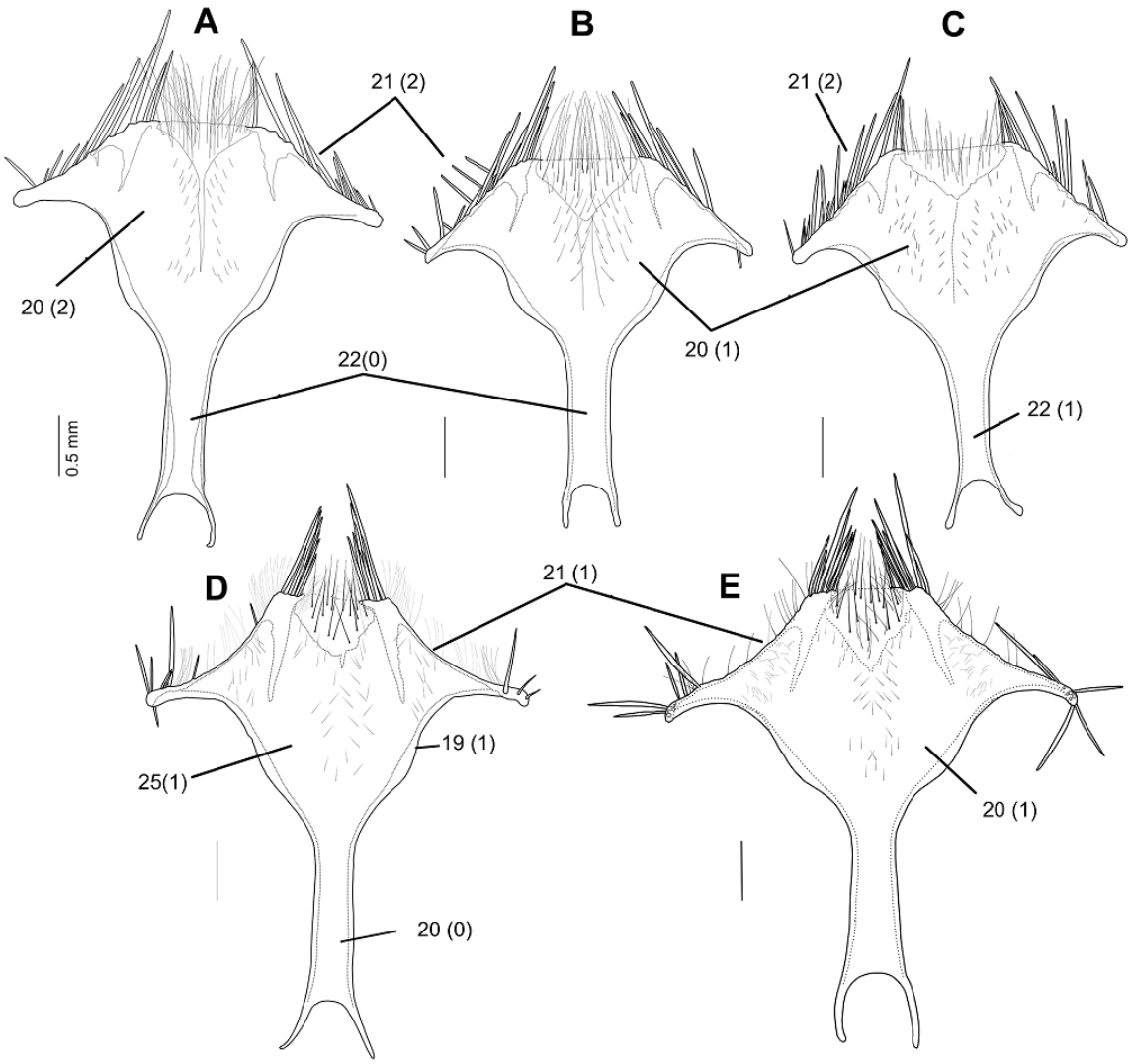

Fig. 7 Hypandrium in ventral view (extended). Character 19. Hypandrium: 19(0) quadrangular shape; 19(1) cup shape (1). Character 20. Distal portion of hypandrium: 20(0) concave, absence of lateral projections; 20(1) semi square, with lateral projections; 20(2) semi rectangular¸ with lateral projections. Character 22. Proximal portion of hypandrium: 22(0) similar to the width of the distal part (0); 22(1) same width in center; 22(2) slightly narrower in the center (2). Character 25. Distal portion of the uncus in dorsal view: 25(0) single horn; 25(1) horns closed; 25(2) open horned open. a: ECO 01, b: ECO 02, c. ECO 03, d: *Biblis hyperia*, e: *Biblis hyperia laticlavia*

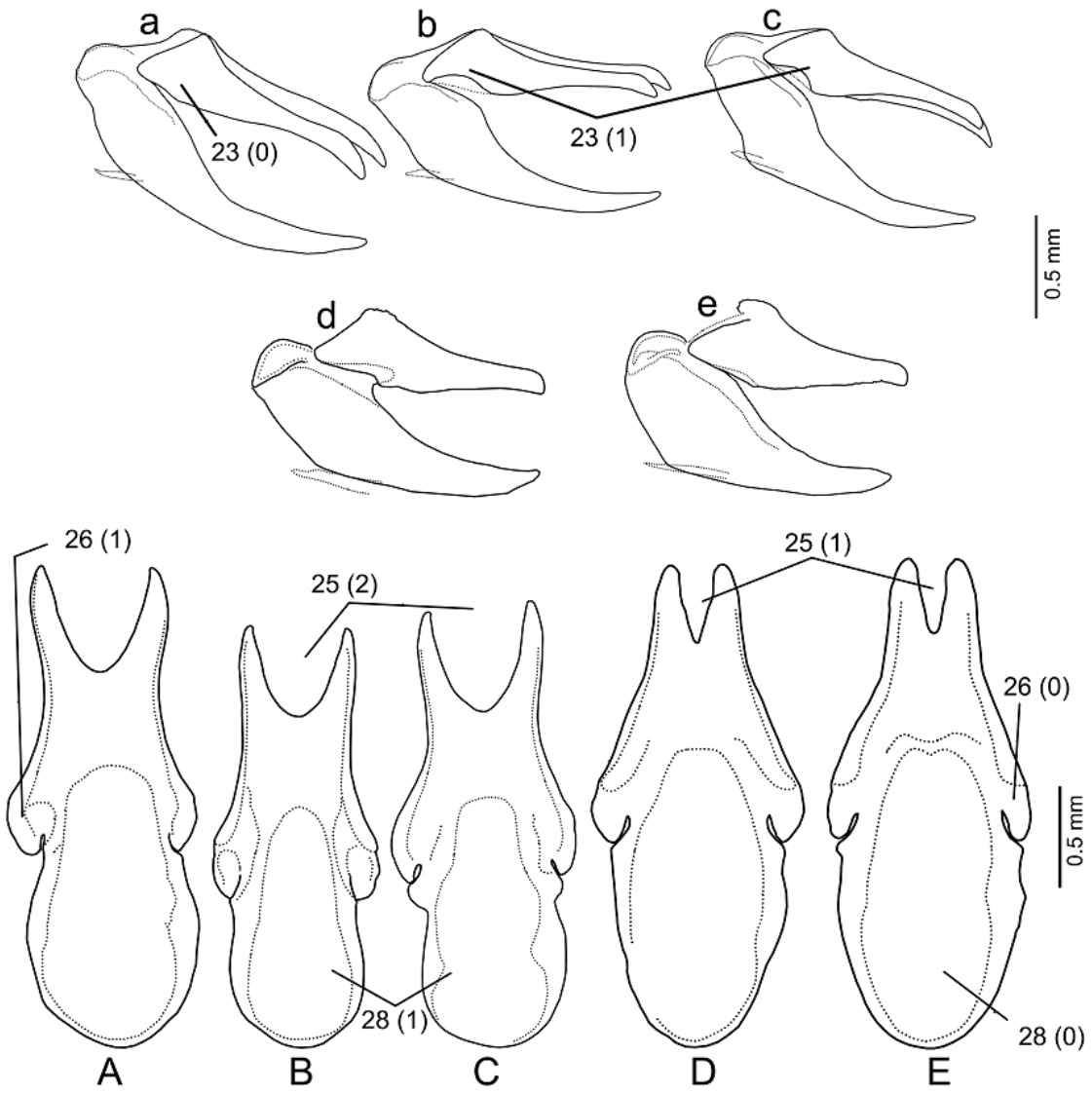

Fig. 8 Uncus and tegumen (side: lowercase letter and dorsal: lowercase letter). Character 23. Proximal portion of the uncus lateral view: 23(0) not narrow, more sclerosed; 23(1) narrow, less sclerosed. Character 25. Distal portion of the uncus in dorsal view: 25(0) single horn; 25(1) horns closed; 25 (2) open horned open. Character 28. Tegumen in dorsal view: 28(0) oval; 28(1) round. a/A: ECO 01, b/B: ECO 02, c/C. ECO 03, d/D: *Biblis hyperia*, e/E: *Biblis hyperia laticlavia*

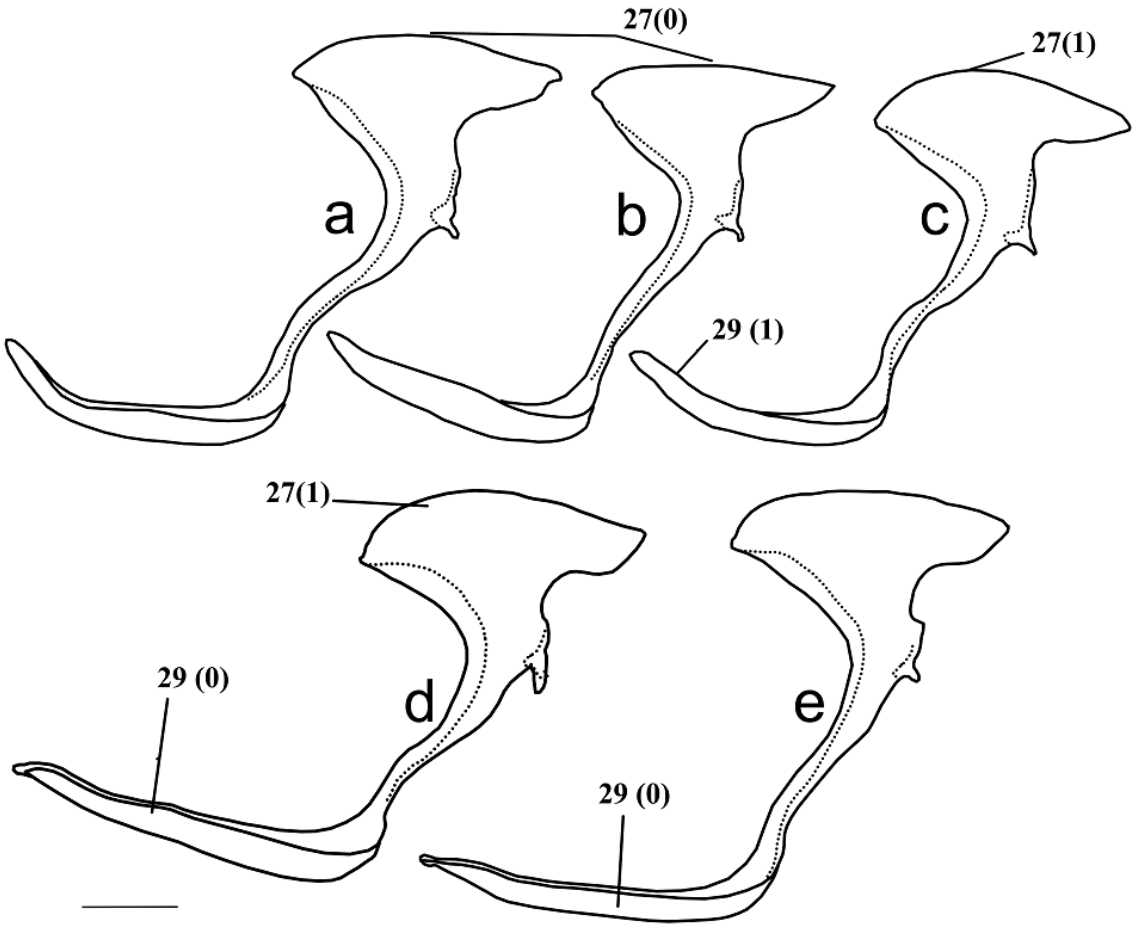

Fig. 9 Tegumen in lateral view. Character 31 (1): Tegumen in lateral view: 27(0) Stooped; 27(1) slightly stooped. Character 29. Saccus in lateral view (size): 29(0) long and straight; 29(1) short and curved. a: ECO 01, b: ECO 02, c: ECO 03, d: *Biblis hyperia*, e: *Biblis hyperia laticlavia*

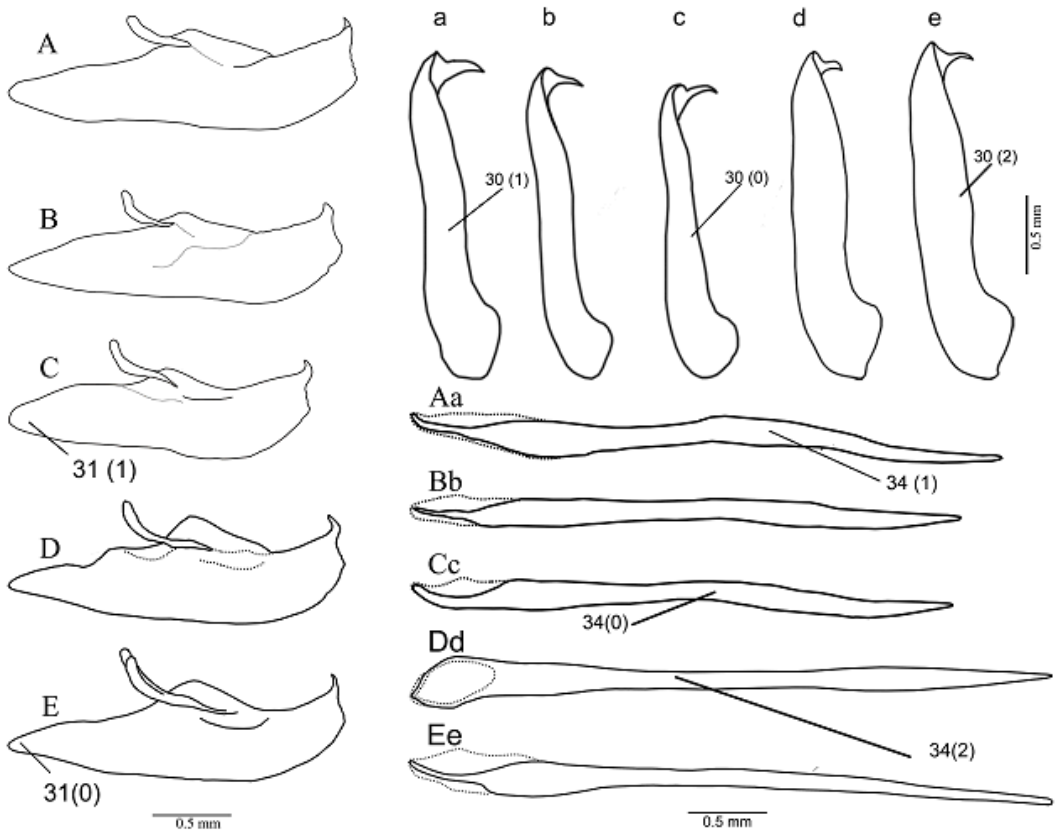

Fig. 10 Capital letters valvae in lateral view and valvae in ventral view lowercase letters. The uppercase and lowercase letters the aedeagus in lateral view. Character 30. Valvae in ventral view (size): 30(0) small, 30(1) slender, 30(2) robust. Character 31. Valvae in lateral view, proximal portion: 31(0) pointed; 31(1) slightly round; 31(2) round. Character 34. Aedeagus: 34(0) short; 34(1) medium; 34(2) long

Table 1 Character qualitative in wings, head and genitalia.

Character of the wings (dorsal view)
Character 1. Outer margin (hindwings): weakly wavy (0); moderately wavy (1); strongly wavy (2)
Character 2. Band shape: convex (0); straight (1)
Character 3. Submarginal band (distance): near the outer margin (0); moderately close (1); away from the outer margin (2)
Character 4. Submarginal band (size): Short spots (0); long spots (1)
Character 5. Postdiscal spots: same cell length RsM1 to Cu1Cu2 (0); spots in cells Rs-M1 to M2-M3 shorter than in cells M3-Cu1 to Cu1-Cu2 (1); spots in longer Rs-M1 and M1-M2 cells (2)
Character 6. Submarginal area of forewing in dorsal view: very clear that it is the rest of the wing (0); slightly lighter than the rest of the wing (1); darker than the rest of the wing (2)
Character of the wings (ventral view)
Character 7. Band pattern: white band (0); narrow red band to white wide (1); intermediate band to white wide (2)
Character 8. Spot size in cell Sc+R1: quasi absent (0); quasi absent to small (1); quasi absent to large (2); big (3)
Character 9. Spot color in cell Sc + R1: white (0); red (1); red or red with white (2)
Character 10. Basal area ventral view of the posterior wing: base without spots (0); spotted base (1)
Character 11. Basal zone: clear without spots (0); dark with red spots (1); dark with red spots with white (2)
Character 12. Basal point size: lost (0); small to big (1); medium to big (2)
Character of the head and body
Character 13. Labial palps (medium segment, internal zone): all the internal part (0); abundant white cilia (1); medium with white cilia (2), few white cilia (3)
Character 14. Palps in lateral view: scales all over the palp (0); with scales only in the center (1)
Character 15. Antennae: Brown, with white scales between segments (0); completely dark brown (1)
Character 16. Head in dorsal view: “glazed” surface (0); brown surface with six white spots (1)
Character of thorax and abdomen
Character 17. Thorax and abdomen (dorsal view): surface, abundant white scales (0); brown, no white scales (1)
Character 18. Thorax and abdomen (ventral view): surface, abundant white scales (0); dark brown (1)
Character of the genitalia male
Character 19. Hypandrium: quadrangular shape (0); cup shape (1)
Character 20. Distal portion of hypandrium: concave, absence of lateral projections (0); semi square, with lateral projections (1); semi rectangular, with lateral projections (2)
Character 21. Spines on the distal part of the hypandrium: not spines, with fine bristles (0); only shoulder and hands (1); on the entire arm, y bristles in projections (2)
Character 22. Proximal portion of hypandrium: similar to the width of the distal part (0); same width in center (1); slightly narrower in the center (2)
Character 23. Proximal portion of the uncus lateral view: not narrow, more sclerosed (0); narrow, less sclerosed (1)
Character 24. Distal portion of the uncus: not bifurcated (0); bifurcated (1)
Character 25. Distal portion of the uncus in dorsal view: single horn (0); horns closed (1); open horned open (2)
Character 26. Proximal portion of the uncus in dorsal view: deflated (0); inflated on the sides (1)
Character 27. Tegumen in lateral view: slightly stooped (0); Stooped (1)
Character 28. Tegumen in dorsal view: oval (0); round (1)
Character 29. Saccus in lateral view (size): long and straight (0); short and curved (1)
Character 30. Valvae in ventral view (size): small (0), slender (1), robust (2)
Character 31. Valvae in lateral view, proximal portion: pointed (0); slightly round (1); round (2)
Character 32. Valvae in lateral view, distal portion: bifurcated (0); not bifurcated (1)
Character 33. Valvae in lateral view of the distal portion: less than half the width of the valvae (0); more than half the width of the valvae (1)
Character 34. Aedeagus: short (0); medium (1); long (2)

Table 1 Qualitative character matrix of the wings, head and genitalia, based on table 1.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Mestra amymone	0	1	2	0	2	2	0	3	0	0	0	0	0	0	0
***Biblis* sp. n.1 (ECO 01)**	2	1	2	0	1	1	2	1	1	1	2	2	3	1	1
***Biblis aganisa* (ECO 02)**	2	1	2	0	1	1	1	2	2	1	2	1	2	1	1
***Biblis* sp. n. 2 (ECO 03)**	2	1	2	0	1	1	2	3	2	1	1	2	2	1	1
Biblis hyperia	1	0	1	0	0	0	2	2	1	1	1	1	2	1	1
Biblis hyperia laticlavia	1	0	0	1	1	1	2	0	1	1	1	2	1	1	1

	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**
Mestra amymone	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0
***Biblis* sp. n.1 (ECO 01)**	1	1	1	1	2	2	1	0	1	2	0	0	1	1	1	0	1	1	1
***Biblis aganisa* (ECO 02)**	1	1	1	1	1	2	1	1	1	2	1	0	1	1	0	0	1	0	0
***Biblis* sp. n. 2 (ECO 03)**	1	1	1	1	1	2	2	1	1	2	1	1	1	1	0	1	1	0	0
Biblis hyperia	1	1	1	1	2	1	1	0	1	1	0	0	0	0	2	0	1	0	2
Biblis hyperia laticlavia	1	1	1	1	1	1	1	0	1	1	0	0	0	0	2	0	1	0	2

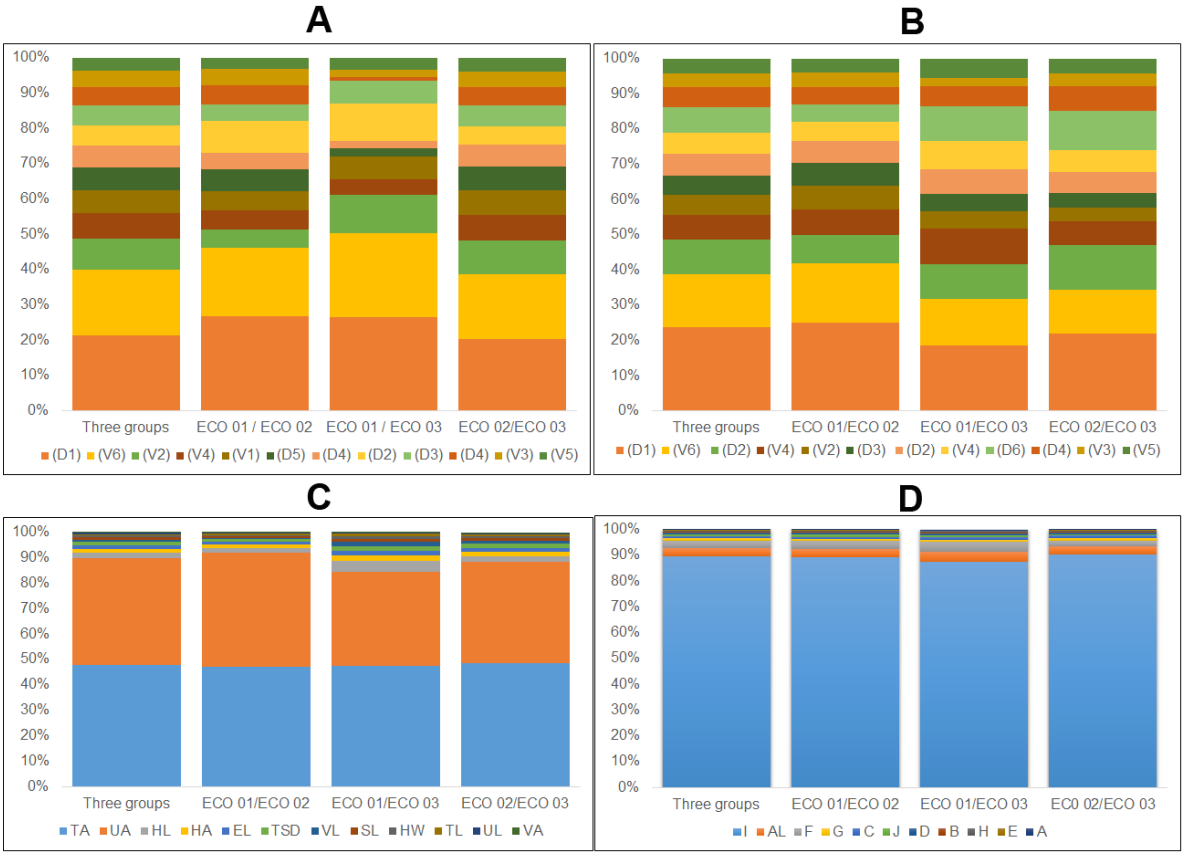

Fig. 11 Simper of morphometric measurements of wings and genitalia. **A** y **C:** males, **B** y **D**: females. In both sexes are measurements on wings, the initial with D is dorsal and initial with V is ventral. Wing length (D1), distance from band to disc cell (D2), band distance to outer margin (D3), spot length in cell M3-CuA1 (D4), spot length in Rs-M1 (D5), spot length in cell CuA2-1A + 2A (D6), band to disc cell distance (V1), distance band to outer margin (V2), spot length in cell M3-CuA1 (V3), length of spot in cell Rs-M1 (V4), spot length in CuA2-1A + 2A (V5), anal margin length (V6). **C:** in structures of the male genitalia: Hipandrium length (HL), hipandrium width (HW), hipandrium angle (HA) were measured; edeagus length (EL), valvae length (VL), Tegumen length (TL), Uncus length (UL), tegumen angle (TA), tegumen to sacus distance (TSD), Uncus angle (UA), Sacus length (SL), and valvae width (VA). **D:** in structures of the female genitalia: AL= length of abdomen, A = width of ostium bursae, B = shortest distance between anteapophysis, C = longest distance between anteapophysis, D = length of anteapophysis, E = height of ostium bursae, F = length of copus bursae, G = approximate length of ductus bursae, H = width of papilla analis, and I = angle of anteapophysis.

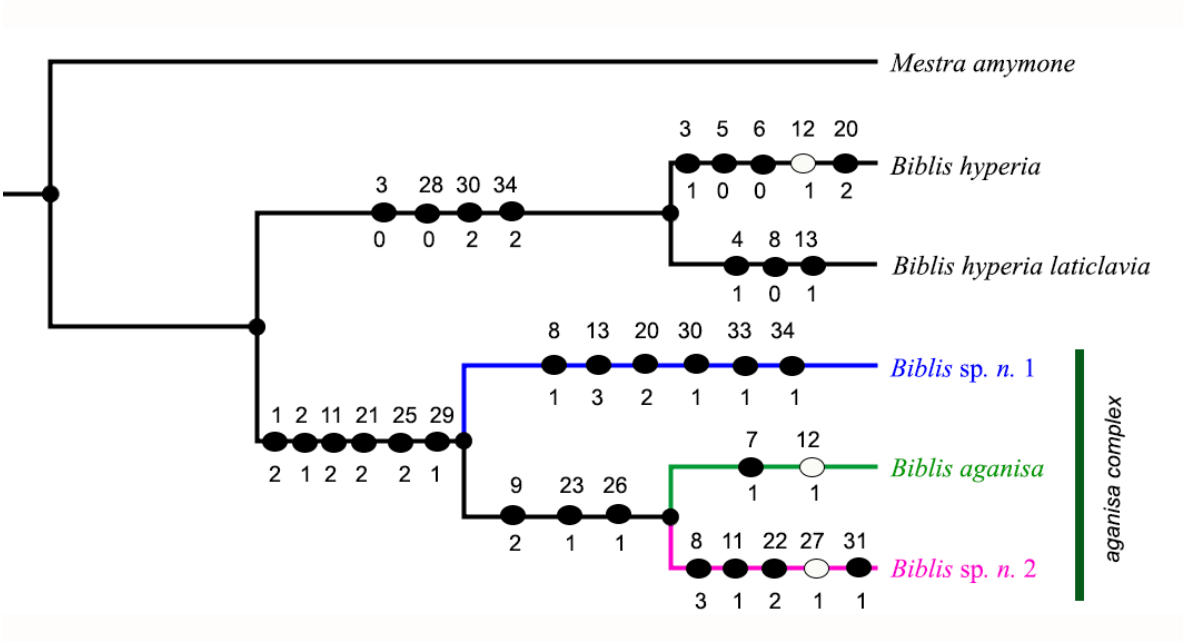

Fig. 12 Tree of maximum parsimony, with exhaustive search in PAUP based on qualitative characters of the wings, head and genitalia. Tree length = 58, CI = 0.9138/0.8333 excluding uninformative characters; HI = 0.0862/0.1563 excluding uninformative characters; RI = 0.7222 and RC= 0.6600. Apomorphies are indicated by character numbers and ovals included in the analysis.

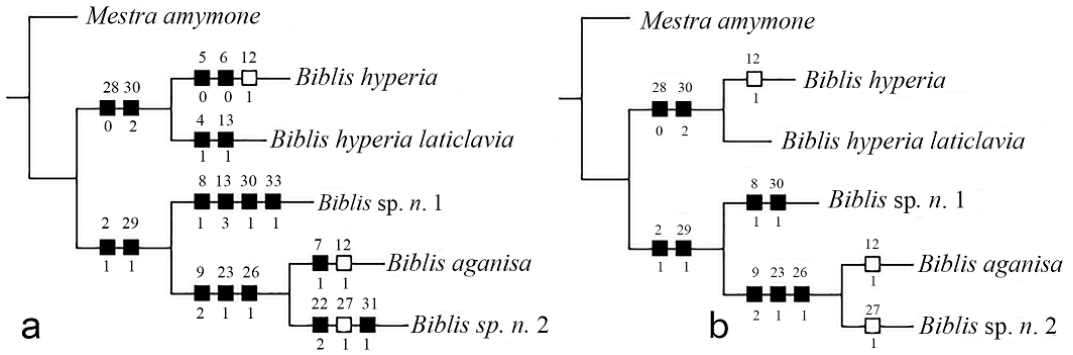

Fig. 13. Phylogenetic hypothesis for the genus *Biblis* Fabricius, 1807, based on morphological characters. Black circles: synapomorphies and autapomorphies; White squares are: multistate characters that do not represent synapomorphies. Numbers above the squares correspond to the character and under the squares to the state of the character. **a**. with non-informative characters (L = 58, CI = 91, RI = 72), **b.** with only informative characters (L = 32, CI = 84, RI = 72)

Table 3 List of diagnostic characters of *Biblis* de Mexico.

number	Character	***Biblis* sp. n. 1**	***Biblis aganisa***	***Biblis* sp. n. 2**
7	Band pattern		narrow red band to white wide	
8	Spot size in cell Sc+R1	***quasi absent to small**		big
12	Basal point size		***Small to big**	
13	Labial palps (medium segment, internal zone)	few white cilia		
22	Proximal portion of hypandrium			slightly narrower in the center
27	Tegumen in lateral view			***Stooped**
30	Valvae in ventral view (size):	***slender**		
31	Valvae in lateral view, proximal portion		slightly round	
33	Valvae in lateral view of the distal portion	more than half the width of the valvae		
34	Aedeagus (size)	***medium**		

*In bold font: Informative characters

Conclusión

El estudio molecular con COI, MDH y DAPDH confirmó la separación de *Biblis aganisa* en tres grupos con un fuerte soporte de los clados, para el árbol filogenético de inferencia Bayesiana, y el Neighbor-joining. Las distancias genéticas de acuerdo al modelo de Kimura para *Biblis* sp. n.1 (ECO 01) / *Biblis aganisa*(ECO 02) fueron de 4.55%, *Biblis* sp. n.1(ECO 01)/ *Biblis* sp. n.2(ECO 03) fueron de 5.85% y *Biblis aganisa* (ECO 02)/*Biblis* sp.n.2(ECO 03) de 4.12%.

El estudio morfológico con caracteres cuantitativos en alas y genitales en ambos sexos, reveló su eficiencia para reconocer las especies crípticas de *Biblis* de México. Las medidas en las alas que contribuyeron a reconocer esta separación fueron la longitud alar, la longitud del margen anal, y la distancia de la banda al margen externo. Las medidas en los genitales en los machos fueron el ángulo del tegumento, ángulo del uncus, y la longitud del hipandrio. Mientras que en las hembras el ángulo o curvatura del anteapófisis y la longitud del abdomen.

El estudio morfológico reveló que los caracteres morfológicos cualitativos están compartidos por las especies crípticas, por lo que el uso exclusivo de los mismos no funciona eficientemente para separar las especies mexicanas. Sin embargo, se encontraron caracteres no informativos que permiten la separación de los taxones *v. gr.* coloración de los palpos *Biblis* sp. n.1 patrón de banda submarginal delgada roja en vista ventral *Biblis aganisa* macha roja en el ala anterior en vista dorsal *Biblis* sp. n.2.

Este estudio permitió comprobar la existencia de un complejo de especies crípticas del género *Biblis* (Nymphalidae: Biblidinae) en México, compuesto de tres especies. Una especie ya descrita, que corresponde a *Biblis aganisa* Boisduval, 1836. Dos especies no descritas, una para la península de Yucatán y una para la sierra norte de Oaxaca hacia el occidente de México hasta el sur de Sinaloa.

Biblis sp n.1 y *Biblis aganisa* son simpátricos en la península de Yucatán, mientras que *Biblis aganisa* y *Biblis* sp n.2) son simpátricos en la sierra norte de Oaxaca.

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Anexo.

Lista de secuencias tomadas de GenBank (https://www.ncbi.nlm.nih.gov/genbank/)

TAXÓN	GENBANK	ID-MUSEO	PAÍS	ESTADO	COI	MDH	DAPDH
Colobura annulata	MW318380.1	PM01_16	ND	ND	SI	SI	SI
Marpesia chiron	AY788603.1	NW115-4	Brasil	Rondonia, Ariquemes	SI	SI	SI
Mestra amymone	MW318527.1	NW162_2	ND	ND	SI	No	No
Mestra hersilia	MZ335962.1	MACN-Bar-Lep-ct 07597	ND	ND	SI	No	No
Mestra dorcas hypermestra	NW129-22	GQ864790	ND	ND	Si	No	No
Biblis	DQ018955.1	NW106-3	ND	ND	Si	Si	Si
Biblis hyperia	SAMN19303179	NVG-19094E05	Islas Vírgenes, Británicas	Isla Guana	Si	No	No
Biblis aganisa	SAMN19303178	NVG-17117F03	USA	TX, Hidalgo Co.	Si	No	No
Archimestra teleboas	GQ864737.1	NW152-8	ND	ND	Si	No	No
Vila azeca	SAMN19303180	NVG-19095B04	Bolivia	ND	Si	No	No
Vila azeca	GQ864818.1	NW129-18	-	ND	Si	No	No
Vila eueidiformis	SAMN19303181	NVG-19095B05	Perú	ND	Si	No	No
*Biblis aganisa*DHJ01	GU156933.1	02-SRNP-27761	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblis aganisa*DHJ01	GU156934.1	02-SRNP-31542	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblis aganisa*DHJ01	GU156935.1	03-SRNP-18474	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblis aganisa*DHJ01	GU333778.1	04-SRNP-46916	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblis aganisa*DHJ01	GU333779.1	04-SRNP-46910	Costa Rica	Área de Conservación Guanacaste	Si	No	No
Biblis sp. nov DHJ02	GU156936.1	04-SRNP-26174	Costa Rica	Área de Conservación Guanacaste	Si	No	No
Biblis sp. nov DHJ02	GU156937.1	04-SRNP-26165	Costa Rica	Área de Conservación Guanacaste	Si	No	No
Biblis sp. nov DHJ02	JQ578342.1	ND	Costa Rica	Área de Conservación Guanacaste	Si	No	No
Biblis sp. nov DHJ02	GU333780.1	02-SRNP-31578	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156938.1	05-SRNP-46316	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156939.1	04-SRNP-26428	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156941.1	04-SRNP-34211	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156942.1	04-SRNP-26245	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156943.1	04-SRNP-26057	Costa Rica	Área de Conservación Guanacaste	Si	No	No

*Biblishyperia*DHJ03	GU156944.1	04-SRNP-26250	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156945.1	04-SRNP-26164	Costa Rica	Área de Conservación Guanacaste	SI	No	No
*Biblishyperia*DHJ03	GU156946.1	02-SRNP-31543	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156947.1	02-SRNP-31541	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156948.1	03-SRNP-17816	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU156949.1	03-SRNP-18621	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU333781.1	02-SRNP-33079	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblishyperia*DHJ03	GU333782.1	04-SRNP-46915	Costa Rica	Área de Conservación Guanacaste	Si	No	No
*Biblis aganisa*ECO 02	GU659951.1	L-43781	México	Quintana Roo	Si	No	No
*Biblis aganisa*ECO 02	GU659952.1	L-86661	México	Yucatán	Si	No	No
*Biblis aganisa*ECO 02	GU659953.1	L-87067	México	Yucatán	Si	No	No
*Biblis aganisa*ECO 02	GU659955.1	L-65754	México	Campeche	Si	No	No
*Biblis aganisa*ECO 02	GU659956.1	L-28372	México	Campeche	Si	No	No
*Biblis aganisa*ECO 02	GU659957.1	L-28376	México	Campeche	Si	No	No
Biblis sp. nov. ECO 01	GU659958.1	L-40210	México	Campeche	Si	No	No
*Biblis aganisa*ECO 02	GU659960.1	L-71014	México	Yucatán	Si	No	No
*Biblis aganisa*ECO 02	GU659961.1	L-70547	México	Yucatán	Si	No	No
*Biblis aganisa*ECO 02	GU659962.1	L-02366	México	Quintana Roo	Si	No	No
*Biblis aganisa*ECO 02	JN201249.1	DNA-02240	México	Yucatán	Si	No	No
Biblis nectanabis	MF545479.1	MACN-Bar-Lep 01483	Argentina	Entre Rios	Si	No	No
Biblis nectanabis	MF545510.1	MACN-Bar-Lep 01893	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF545807.1	MACN-Bar-Lep 00831	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF545834.1	MACN-Bar-Lep 01147	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF546498.1	MACN-Bar-Lep 02164	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF546511.1	MACN-Bar-Lep 00799	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF547102.1	MACN-Bar-Lep 00833	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF547135.1	MACN-Bar-Lep 01026	Argentina	Misiones	Si	No	No
Biblis nectanabis	MF547355.1	MACN-Bar-Lep 02209	Argentina	Misiones	Si	No	No