

# A uniquely patterned new species of *Emesis* from Honduras (Riodinidae)

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**Abstract:** *Emesis eleanorae* Gallardo & Grishin n. sp. is described from western Honduras. It differs from other species of *Emesis* Fabricius, 1807 in having a row of prominent iron-gray crescent-shaped postdiscal spots on both wings above, outlined by paler areas basad and mirrored as merlot-colored spots below, with the largest by the forewing costa, and in its females being bright golden-orange in color. Genomic sequence analysis of *Emesis* reveals that the new species belongs to the subgenus *Aphacitis* Hübner, [1819] and is sister to the clade containing *Emesis diogenia* Prittwitz, 1865 and *Emesis heteroclita* Stichel, 1929, and the clade of these three species is sister to *Emesis vulpina* Godman & Salvin, 1886.

**Resumen:** *Emesis eleanorae* Gallardo & Grishin n. sp. se describe desde el Occidente de Honduras. Se diferencia de otras especies de *Emesis* Fabricius, 1807 por tener una fila de prominentes puntos postdiscales en forma de media luna, color gris-hierro en la parte de arriba de ambas alas, delineadas por áreas más pálidas y reflejadas debajo como manchas de color merlot, las cuales son más grandes en la costa delantera, y en las hembras son de color dorado-anaranjado brillante. El análisis de la secuencia genómica de *Emesis* revela que la nueva especie pertenece al subgénero *Aphacitis* Hübner, [1819] y es pariente del clado de *Emesis diogenia* Prittwitz, 1865 y *Emesis heteroclita* Stichel, 1929, y el clado de estas tres especies son parientes de *Emesis vulpina* Godman y Salvin, 1886.

**Key words:** Biodiversity, Emerald Valley, genomics, Neotropics, metalmark butterflies, taxonomy.

## INTRODUCTION

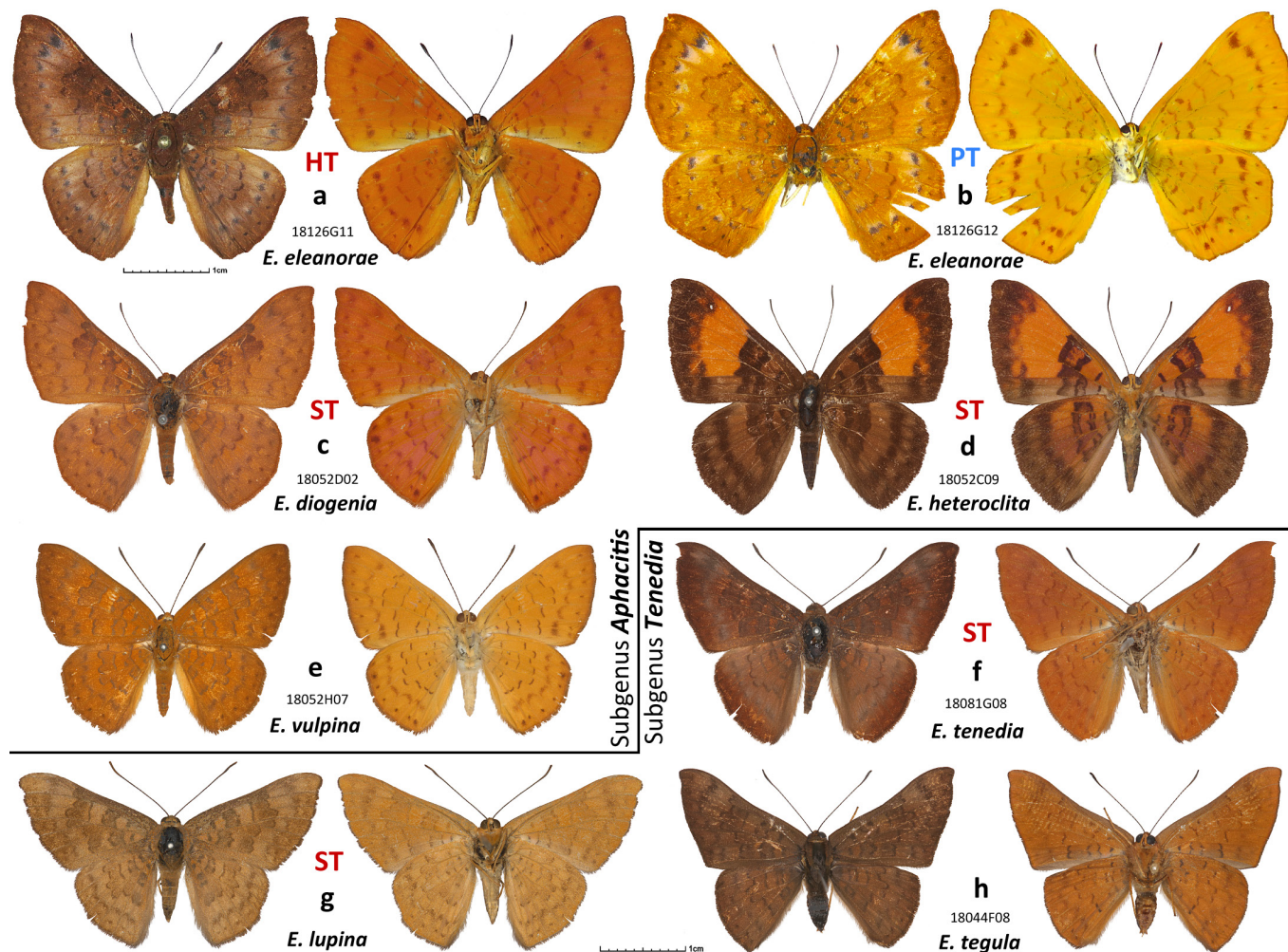
Extensive surveys of the butterfly fauna have been conducted continuously since August 2017 by RJG and Maria Olivia Diaz on their property in western Honduras. The property is named “Emerald Valley” (Valle Esmeralda) after the Emerald Toucanet, *Aulacorhynchus prasinus* (Gould, 1833) (Ramphastidae), a common, small, green toucan that resides there, and the valley and surrounding low mountains are densely covered by evergreen tropical forest, further justifying the name. Emerald Valley currently contains 16.7 hectares and is located at the juncture between the Departments of Santa Bárbara and Cortés, near the northwestern edge of the Lake Yojoa.

A large and showy species of *Emesis* Fabricius, 1807 (Riodinidae) was encountered by RJG nectaring on *Chromolaena odorata* (Asteraceae) flowers in the gardens by their residence. In contrast to the other seven species of *Emesis* known from Emerald Valley, this one stood out by its large size (next to *Emesis furor* Butler & H. Druce, 1872) and unique patterns and colors, and could not be easily assigned to a known species. Here, we carried out morphological and genomic analyses of this species and compared it with other species of *Emesis*.

*Emesis* is a genus of 39 known species that has recently been reviewed from a genomic perspective (Zhang *et al.*, 2019). The taxonomy in that study was based on Callaghan & Lamas (2004) and revised using genomic sequence data. The genome-level phylogeny partitions *Emesis* into six subgenera, which are mostly in agreement with phenotypic data, but due to possible mimicry and convergence, some species that belong to different subgenera may appear similar; *Emesis* are frequently difficult to identify to species due to these phenotypic similarities. To solve these problems in this study, we place the unidentified Honduran *Emesis* species in a phylogenetic framework using its genomic DNA sequence and describe this species as new.

## MATERIALS AND METHODS

Standard entomological techniques were used for dissection (Robbins, 1991), with the distal part of the abdomen broken off, soaked for 40 minutes (or until cleared) in 10% KOH at 60°C, dissected, and subsequently stored in a small glycerol-filled vial on the pin under the specimen. Genitalia and wing venation terminology follow Steinhauser (1981), except that cucullus is called harpe in this work. Photographs of specimens (Fig. 1a, b), live adults and habitat were taken by RJG with a Canon Powershot SX50 camera and of all other specimens by NVG



**Figure 1.** *Emesis* specimens. **a.** *E. eleanorae* n. sp. holotype ♂, NVG-18126G11 (also illustrated in Fig. 2a); **b.** *E. eleanorae* n. sp. paratype ♀, NVG-18126G12; (also illustrated in Fig. 2b); **c.** *E. diogenia* syntype ♂, Brazil: Rio de Janeiro, NVG-18052D02 [ZMHB]; **d.** *E. heteroclita* syntype ♂, Peru, NVG-18052C09 [ZMHB]; **e.** *E. vulpina* ♂, Mexico, NVG-18052H07 [ZMHB]; **f.** *E. tenedia* syntype ♂, Venezuela, NVG-18081G08 [NHMUK]; **g.** *E. lupina* syntype ♂ [NHMUK]; **h.** *E. tegula* ♂, Costa Rica, 12-SRNP-20247, NVG-18044F08 [USNM]. Dorsal and ventral views are on the left and right respectively. DNA sample numbers (where available) and species names are given. HT, ST and PT denote holotype, syntype and paratype, respectively. Species from subgenera *Aphacitis* and *Tenedia* are shown above and below the line, respectively. Copyright (©) for f and g: Trustees of the Natural History Museum, London (used with permission).

with Nikon D800 cameras through a 105 mm f/2.8G AF-S VR Micro-Nikkor lens; dissected genitalia were photographed by NVG in glycerol with a Nikon D200 camera without the lens and through microscope at about 4.5x magnification. Genitalia photographs were taken in several focus slices and stacked in Photoshop to increase depth of field. Images were assembled and edited in Photoshop CS5.1. DNA sequencing and analysis techniques were the same as reported previously (Zhang *et al.*, 2019). The following collection abbreviations are used in this paper : USNM: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ZMHB: Museum für Naturkunde, Berlin, Germany; NHMUK: Natural History Museum, London, UK; CSUC: Colorado State University Collection, Fort Collins, CO, USA; BMUW: The Burke Museum of Natural History and Culture, Seattle, WA, USA. Data for sequenced specimens are in the supplemental file to Zhang *et al.* (2019). Additional specimens studied here were: NVG-18052H07, *E. vulpina*, Mexico, *leg.* Radlich, coll. H. Stichel [ZMHB] (Fig. 1e); NVG-18044C12, *E. aurinna*,

Panama, Darién, Cana, 400 m, *leg.* G. B. Small, 20 Sep 1982 [USNM]; NVG-18044G10, *E. fatimella fatimella*, Peru, Madre De Dios, Parque Manu, Pakitza, 340 m, 11°55' 48"S, 71°15' 18"W, *leg.* G. Lamas 15 Oct 1991 [USNM]; NVG-19044G03, *E. lupina*, Guatemala, Departamento de Alta Verapaz, Municipio San Cristóbal Verapaz, Baleu, above 1350 m, *leg.* E. C. Welling, 26 May 1966 [AMNH]; NVG-18126H01, *E. tegula*, Honduras, Santa Bárbara Department, Emerald Valley, *leg.* Robert J. Gallardo, 3 Jan 2018; NVG-18044H11, *E. tenedia*, Colombia, Valle del Cauca, Calima Dam, 1000 m, 3°53'N, 76°34'W, *leg.* J. Bolling Sullivan, 11 Jan 1992 [USNM].

## RESULTS AND DISCUSSION

We analyzed phenotypes of all *Emesis* species and genomic sequences of all but two (*Emesis sinuata* Hewitson, 1877 and *Emesis toltec* Reakirt, 1866), and concluded that the Honduran specimens belong to a new species, which we describe here.





**Figure 2.** *Emesis* adults nectaring and resting. a–c. *E. eleanorae* n. sp.: a. holotype ♂ (also illustrated in Fig. 1a); b. paratype ♀ (also illustrated in Fig. 1b); c. paratype ♂; d. *E. tenedia* ♀; e. *E. tegula* ♂; f. *E. brimo vimena* ♂. All from Emerald Valley, Honduras.

***Emesis eleanorae* Gallardo and Grishin, new species**

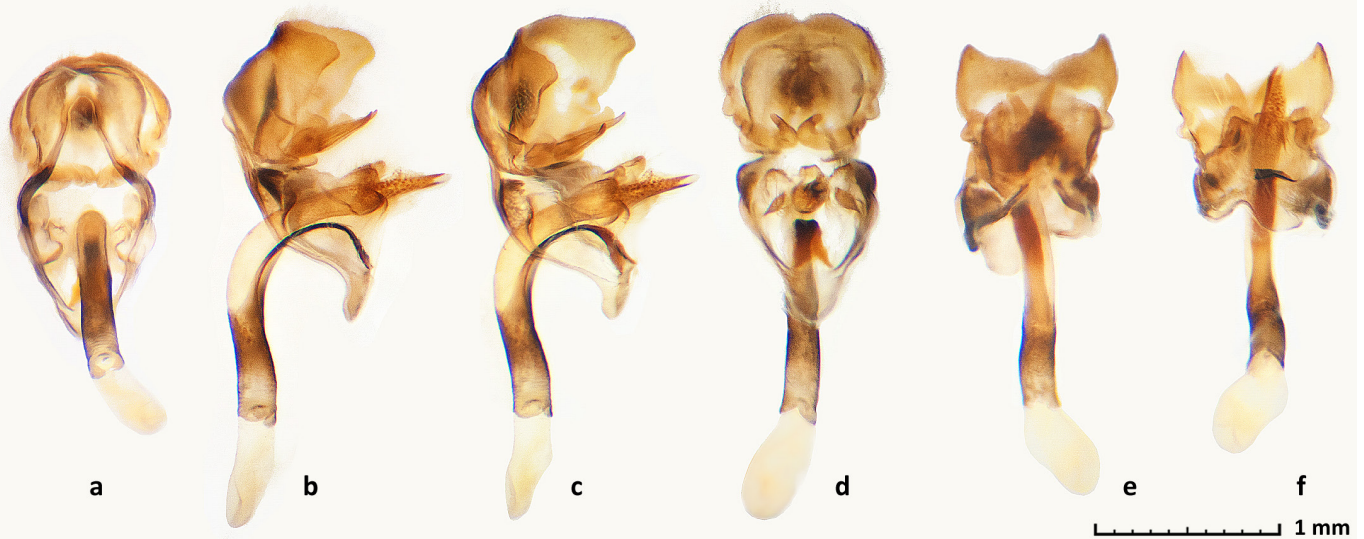
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(Figs. 1–6)

**Description. Male** (n=2, Figs. 1a, 2a, c): right forewing length = 19.5 mm in holotype and 18.5 mm in paratype. Wings nearly triangular, forewing produced, slightly hooked at apex, costa slightly concave in middle, outer margin convex from vein  $M_1$  to tornus, concave at vein  $M_1$ ; dorsal wing ground color chestnut-brown to dark orange, with prominent postmarginal row of iron-gray crescents in every forewing cell from  $R_2-M_1$  (largest) to  $CuA_2-1A+2A$  (double spots) and more rounded spots in hindwing cells from  $Rs-M_1$  to  $CuA_2-1A+2A$ , flanked by paler patches or areas of scales basad, a submarginal row of smaller round spots on both wings, discal curved row of faint streaks, and several iron-gray spots in basal half of wing placed in a pattern typical for *Emesis*, patches of pale scales in between some of these spots, in particular on hindwing; spot pattern repeated on mostly rusty-orange ventral side, spots merlot-colored and

particularly prominent in postdiscal row, submarginal spots weak or absent, area by forewing inner margin yellow; fringes brown. Head, thorax and abdomen chestnut-brown above and yellow-orange (including legs) below, eyes pale olive-green in live individuals, fading to brown in pinned specimens; antennae gray, paler at segment junctions. Male genitalia (Fig. 3): tegumen as long as wide; uncus short and wide, half-dome shaped, strongly bilobed, each lobe narrows to acute angle; gnathos arms as long as uncus, horn-like, converge in middle and then slightly diverge; saccus shorter than gnathos, rounded at tip, valva wider than long, about same length as gnathos arms, bilobed towards end, lobes nearly equal, dorsal lobe slightly longer than ventral; transtilla small, bump-like with a notch at end; pedicel strongly sclerotized, long and curved to fit aedeagus; aedeagus boomerang-shaped, smoothly bent nearly to right angle near its middle, gradually narrower towards tip, length as genital capsule height, vesica with numerous small cornuti. Female (n=2, Figs. 1b, 2b): right forewing lengths = 20 mm. Larger than male, with rounder wings, golden-orange in color above and golden-yellow below, similar to male in pattern, but most spots paler-brown, postdiscal spots iron-gray.





**Figure 3.** Male genitalia of *Emesis eleanorae* n. sp. (paratype NVG-18126G10) shown in several views: **a.** anterior; **b.** left lateral; **c.** left posterior lateral; **d.** posterior; **e.** dorsal, slightly tilted to the left to reveal saccus; **f.** ventral.

**Barcode sequence of the holotype.** Genbank Accession MZ047078, voucher NVG-18126G11, 658 base pairs:

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AACATTATATTTATTTTGGGAATTTGGTCAGGAATAGTAGGTACATCATT
AGTTTATTAATTCGAATAGAATTAGGAACCTCAGGTTCAATTAATGGAGATG
ATCAAATTTATAATACTATCGTAACTGCCCATGCTTTTATTATAATTTCTT
TATAGTTATACCTATTATAATGGAGGATTTGGTAATGATTAGTTCCATTA
ATATTAGGAGCCCCTGATATAGCTTTCCCGCAATAAATAATATAAGATTTT
GACTATTACCTCCATCACTATTCTTATTAATTTCAAGAAGAATCGTAGAAAA
TGGAGCAGGAACAGGATGAACAGTGTACCCCCACTTTCTCCAATATTGCT
CATGGAGGATCTTCAGTTGATTAGCTATTTTCTCATTACATTTAGCTGGTA
TTTCTTCAATTTTAGGAGCAATTAATTTTATTACTACTATTATTAATATACG
TATTAATAATATATCTTTTGATCAAATACCATTATTTGCTGATCTGTTGGA
ATACAGCTCTTTTATTATTATATATCACTTCCCTGTTTTAGCTGGAGCTATTA
CTATATTACTAACAGACCGTAATTTAAATACTTCAATTTTTGATCCTGCAGG
AGGAGGAGATCCAATTTTATATCAACATTTATTT
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Sequences of the paratypes NVG-18126G10 (Genbank Accession MZ047077) and NVG-18126G12 (Genbank Accession MZ047079) are identical to each other and differ from the holotype in two positions: C154T and T412G.

**Type material and other individuals examined.** **Holotype** ♂ (Figs. 1a, 2a) has the following 4 rectangular labels: white printed: || HONDURAS: Santa Bárbara Dpt. | Municipality of Las Vegas | 3.8 air miles NNW of Las Vegas | 0.8 mi W of Lake Yojoa, el. 815 m | 14°55'31.37"N, 88°02'57.64"W | 1 January 2019 | leg. Robert J. Gallardo ||; white printed: || DNA sample ID: | NVG-18126G11 | c/o Nick V. Grishin ||; white printed: | genitalia | NVG200320-01 | Nick V. Grishin||; red printed || HOLOTYPE ♂ | *Emesis eleanorae* | Gallardo & Grishin|. **Paratypes:** 3 ♂ (January 5, 2019, Fig. 2c and two specimens on February 4, 2021), and 1 ♀, (January 13, 2019, Figs. 1b, 2b), from the same locality as the holotype. The holotype is to be deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM). Two additional females of this species were observed at the same locality on 11 June 2019 (neither collected nor photographed) and on 29 January 2021 (photographed, but not collected).

**Type locality.** HONDURAS: Santa Bárbara Department, Municipality of Las Vegas, 3.8 air miles NNW of Las Vegas, 0.8 mi W of Lake Yojoa, GPS: 14°55'31.37"N, 88°02'57.64"W, elevation 815 m (Figs. 5, 6). The site is located along the western flank of Santa Barbara National Park.

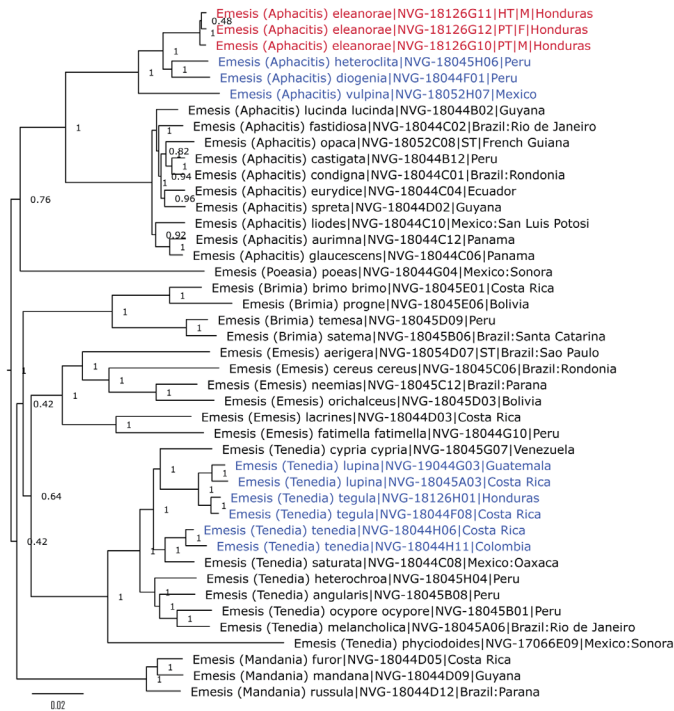
**Etymology.** This species is named in honor of Eleanor Gallardo, RJG's mother. She fostered Robert's interest in butterflies from the age of 11 when he began to study and collect butterflies in California. This passion led him to install the first of many butterfly rearing facilities in Honduras, carry out continuing butterfly surveys across Honduras that led to the discovery of

a number of new species, including the one described here, and work on the forthcoming book *Guide to the Butterflies of Honduras*.

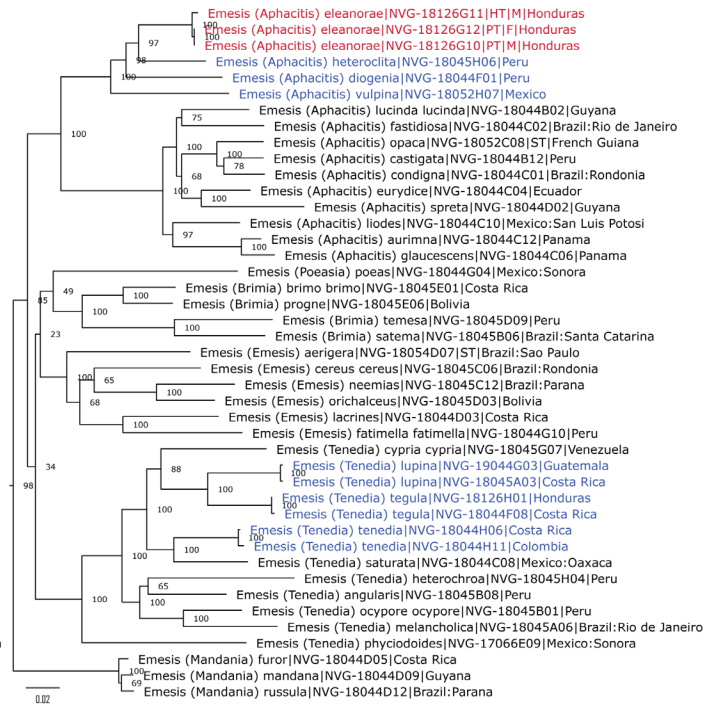
**Distribution and phenology.** This species is known only from Emerald Valley in western Honduras. Despite the continuous, ongoing surveys at Emerald Valley, only seven individuals of this species have been detected; the four collected or observed in 2019, and two males that subsequently were collected (4 February 2021) and one female photographed (29 January 2021). One male and the female were observed feeding on Bitter Vine (*Mikania micrantha*, Asteraceae). Six of the eight species of *Emesis* that occur at Emerald Valley are considered rare to uncommon (Gallardo, unpublished data) with *E. brimo* having been recorded for there only once. The low frequency of observations for some *Emesis* species that occur there, including *E. eleanorae* n. sp., may be attributed to such factors as these species primarily inhabiting the forest canopy, not actually breeding at that site, the low density of their larval food plants, or a combination of these factors.

**Diagnosis and phylogeny.** This species can be distinguished from all other *Emesis* species by a row of prominent iron-gray crescent-shaped postdisical spots on both wings above, outlined by paler areas basad and mirrored as merlot-colored spots below, with the largest by the forewing costa (Figs. 1a, b, 2a–c). Its females are bright golden-orange with forewings that are more broadly hooked at the apex, distinguishing them from other *Emesis* species with similarly colored females, which have narrower wings with more rounded forewing apex. Males are chestnut-brown in color and thus are similar to *E. tedia* (Fig. 1f) and *E. tegula* (Figs. 1h, 2e), but are larger in size and are identifiable by the crescent-shaped postdisical spots. Similarly shaped spots are present in the species's close relative, the South American *E. diogenia* Prittwitz, 1865 (Fig. 1c), which is more uniformly colored, and is orange-brown rather than chestnut-brown. Another close relative, *E.*

## a nuclear genome tree



## b mitochondrial genome tree



**Figure 4.** Phylogenetic trees constructed from protein-coding regions in **a.** nuclear and **b.** mitochondrial genomes. The trees were rooted with *Curvie emesia* (Hewitson, 1867) (NVG-5245, USA: Texas, Hidalgo Co.), *Apodemia duryi* (W. H. Edwards, 1882) (NVG-700, USA: Texas, Brewster Co.), and *Apodemia ares* (W. H. Edwards, 1882) (NVG-17114H01, USA: New Mexico, Hidalgo Co.), not shown in the trees for compactness. Names in red font are for *E. eleanorae* n. sp. and those in blue font are for other species illustrated in Fig. 1.

*heteroclita* Stichel, 1929, has a rather different wing pattern that is assumed to result from mimicry with other butterflies (Fig. 1d). These relatives, including also *E. vulpina* Godman & Salvin, 1886, were identified by phylogenetic analysis of nuclear and mitochondrial genomic sequences (Fig. 4). The phylogeny shows that *E. eleanorae* n. sp. is distinct from related species and confidently places it in the subgenus *Aphacitis* Hübner, [1819] (in the *diogenia* species group), while other species that are similar in appearance (Fig. 1) are in the subgenus *Tenedia* Grishin, 2019. Our phylogenetic analysis includes all but two *Emesis* species, namely the Ecuadorian *Emesis sinuata* Hewitson, 1877, which differs from all *Emesis* by its unique wing shape with a tooth in the middle of each outer margin, and the Mexican *Emesis toltec* Reakirt, 1866, the identity of which remains unclear due to the type specimens being lost. No known *Emesis* species matches closely the original description of *E. toltec*, which, among other things, states “Underneath ... a large patch of that [ferruginous] color at the apex of the primaries, and another across their middle” (Reakirt, 1866). Such patches or their hints are not present in *E. eleanorae* and therefore this species is not *E. toltec*.

**Notes on habitat and behavior.** *Emesis eleanorae* n. sp. has been found only at the type locality, Emerald Valley in Honduras. The valley floor sits at 815 m elevation, with surrounding peaks about 100 m higher. The geology of the property and immediate surroundings is primarily karstic in origin, and as a result there are relatively fewer creeks or rivers in the area, and the property itself contains no surface

water except for a small vernal pool that forms during the rainy season. The upper slopes and ridgeline of Emerald Valley are covered with outcroppings of large limestone boulders, and approximately 75% of the property is at a slope of 40° or more. Most of the site is covered with broadleaf evergreen forest, 90% of which is mature second growth and the remainder of which is young second growth. Some of the more common tree species that occur there include Spanish Cedar (*Cedrela odorata*) (Meliaceae), “Mozote” (*Helicarpus appendiculatus*) (Malvaceae), *Cecropia peltata* (Urticaceae), *Alchornea* sp. (Euphorbiaceae) and Gumbo Limbo (*Bursera simaruba*) (Burseraceae). Shrubs include numerous species of Piperaceae, Rubiaceae and Melastomataceae. The mature second growth forest comprises a wide assortment of tree species, many reaching 25 m or higher. The understory is dense and dark, and contains plants such as *Dieffenbachia* (Araceae), *Heliconia* (Heliconiaceae) and numerous Marantaceae. A prominent understory palm occurring there is “Pacaya” (*Chamaedorea tepejilote*) (Arecaceae). A noticeable plant that grows along the forest edges, upper slopes and ridgeline is a native, fine-leaved bamboo (*Rhipidocladum racemiflorum*) (Poaceae).

The annual blooming of *Chromolaena odorata* lasts approximately 4-6 weeks and has provided some intriguing initial data on nectaring Lepidoptera. It is the only plant noted on the property that attracts species from all six butterfly families, including those that almost exclusively inhabit the forest interior, as well as the canopy (Gallardo, pers. obs.). A whole suite of species has been observed utilizing these flowers that have virtually never been seen during the remainder of the





**Figure 5.** Type locality and habitat of *Emesis eleanorae* n. sp. **a.** overview of Emerald Valley, Honduras; **b.** photo from a drone that includes upper ridgeline; **c.** the type locality is around the RJG residence (house in the middle), GPS 14°55'31.37"N, 88°02'57.64"W, drone photo; **d.** a patch of *Chromolaena odorata* at the type locality; **e.** female paratype nectaring on *C. odorata*.

year. The two *Chromolaena* patches adjacent to their residence have been monitored closely for three consecutive years, with three of the four sightings of *E. eleanorae* having been noted during one of the blooming cycles.

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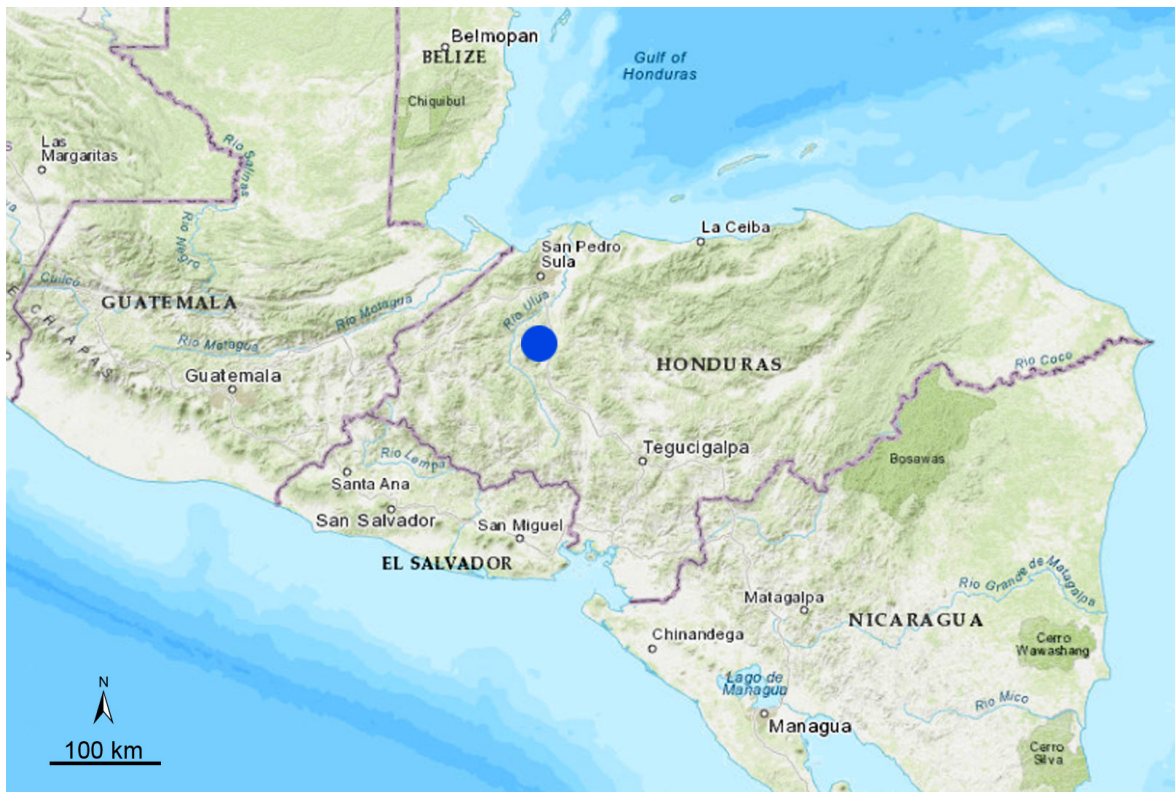


Figure 6. Map with type and only known locality for *Emesis eleanorae* n. sp. marked as a blue circle.

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