

Description of a new species of *Pseudodebis* Forster, 1964 from Central America (Lepidoptera: Nymphalidae: Satyrinae)

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Abstract: We name and describe a new nymphalid butterfly species, *Pseudodebis hartmanni* Nakahara & MacDonald, n. sp. (Satyrinae: Satyrini) based on differences in its wing pattern and DNA from the closely related species. Phylogenetic analysis suggests that this Central American species belongs to a strongly supported clade that also includes two sympatric species from western Ecuador: *P. nakamurai* Nakahara & Willmott, 2021 and *P. pieti* Nakahara & Willmott, 2021. This whole clade is sister to a Central American species, *P. zimri* Butler, 1869. The new species described here is currently known only from several submontane and montane rainforest habitats across Costa Rica and Panama; its description raises the total number of *Pseudodebis* to 11 species and underscores the hidden genetic and taxonomic diversity within Euptychiina.

Key words: Costa Rica, Euptychiina, Panama, species description, taxonomy

INTRODUCTION

Over the past five years, genomic data has increasingly been used in the field of Lepidoptera higher-level systematics (e.g., Espeland *et al.* 2019; Li *et al.* 2019). However, DNA barcoding based on the 5' half of the *cytochrome c oxidase I* gene (Hebert *et al.* 2003) is still a cheap and fast approach to test species status hypothesis and to provide first clues for species-level classifications (e.g., Humer & Mutanen, 2015; Willmott *et al.* 2017). Here, we present a case that highlights the value of taxonomic efforts that combine multiple lines of evidence (morphological and DNA barcoding) in guiding the design of future genomic studies focused on cryptic species complexes and phylogeography. The subject of this study is the butterfly genus *Pseudodebis* Forster, 1964 (Nymphalidae: Satyrinae: Euptychiina), a Neotropical group that until this year was thought to be relatively diverse only on the eastern side of the Andes. Here, we document a new species currently known from scattered localities in Costa Rica and Panama. Our findings, together with those of Nakahara *et al.* (2021), who described two new species from western Ecuador, should encourage an in-depth genomic study of *Pseudodebis* populations in the western Andes to better understand their systematics, evolutionary history and aid in their conservation in the face of rapid biodiversity loss due to anthropogenic factors. *Pseudodebis*, as with many other genera in the nymphalid subtribe Euptychiina, has had a very complicated species-level taxonomy with several undescribed cryptic species due to morphological homogeneity across species and high intraspecific variability. Due to rather

limited phenotypic evidence to guide species identifications, DNA barcoding has contributed for over 10 years towards revealing the hidden diversity of euptychiine species across various genera (e.g., Marín *et al.* 2009; Cong & Grishin, 2014; Barbosa *et al.* 2015; Nakahara *et al.* 2018c). However, ongoing molecular and morphological work suggests that there is still a high number of undocumented species in several genera; for example, about 30 species remain undescribed in the so-called “*Taygetis* clade”, including some in the genus *Pseudodebis*. The monophyly of *Pseudodebis* has been well supported in molecular phylogenetic studies and a morphological synapomorphy of the genus has recently been identified (e.g., Matos-Maraví *et al.* 2013; Nakahara *et al.* 2021). The number of *Pseudodebis* species has increased to 10 after the description of two species from western Ecuador, *P. pieti* Nakahara & Willmott, 2021 and *P. nakamurai* Nakahara & Willmott, 2021, but current evidence suggest that several more await taxonomic treatment.

As indicated in Nakahara *et al.* (2021), during the course of reviewing the species-level classification of *Pseudodebis*, a series of unidentified specimens from Central America was noticed by the authors. At first glance, their rather large adult size and montane preference suggested that they might be conspecific with the west Andean species *P. pieti*, but lack of molecular data precluded firm identification. Subsequently, DNA barcode data obtained from several individuals indicated instead that these Central American specimens represent a divergent group and a possible different species. Furthermore, there exist rather subtle but stable wing pattern differences between these individuals and *P. pieti*. We therefore take this

opportunity to name and describe this *Pseudodebis* species, as a contribution towards a better understanding of *Pseudodebis* diversity.

MATERIALS AND METHODS

We studied the morphology of relevant *Pseudodebis* specimens following standard protocols, namely soaking abdomens for 10 minutes in 10% KOH at 80°C and performing subsequent genitalic dissection. Genitalic morphology and other external morphological characters were examined using a Leica MZ 16 stereomicroscope at various magnifications up to 100x, with drawings also prepared using a camera lucida attached

to this microscope. Genitalia, wing elements and venation terminology largely follow Nakahara *et al.* (2018b). Specimens relevant to this study were examined in public and private collections and we use these following acronyms throughout the text: Ichiro Nakamura collection, Williamsville, NY, USA (ICNA); Mississippi Entomological Museum, Mississippi State University, Mississippi state, MS, USA (MEM); McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL, USA (MGCL). The following abbreviations are also used in the text: DFW (dorsal forewing); DHW (dorsal hindwing); VFW (ventral forewing); VHW (ventral hindwing).

DNA barcode sequences were generated from relevant

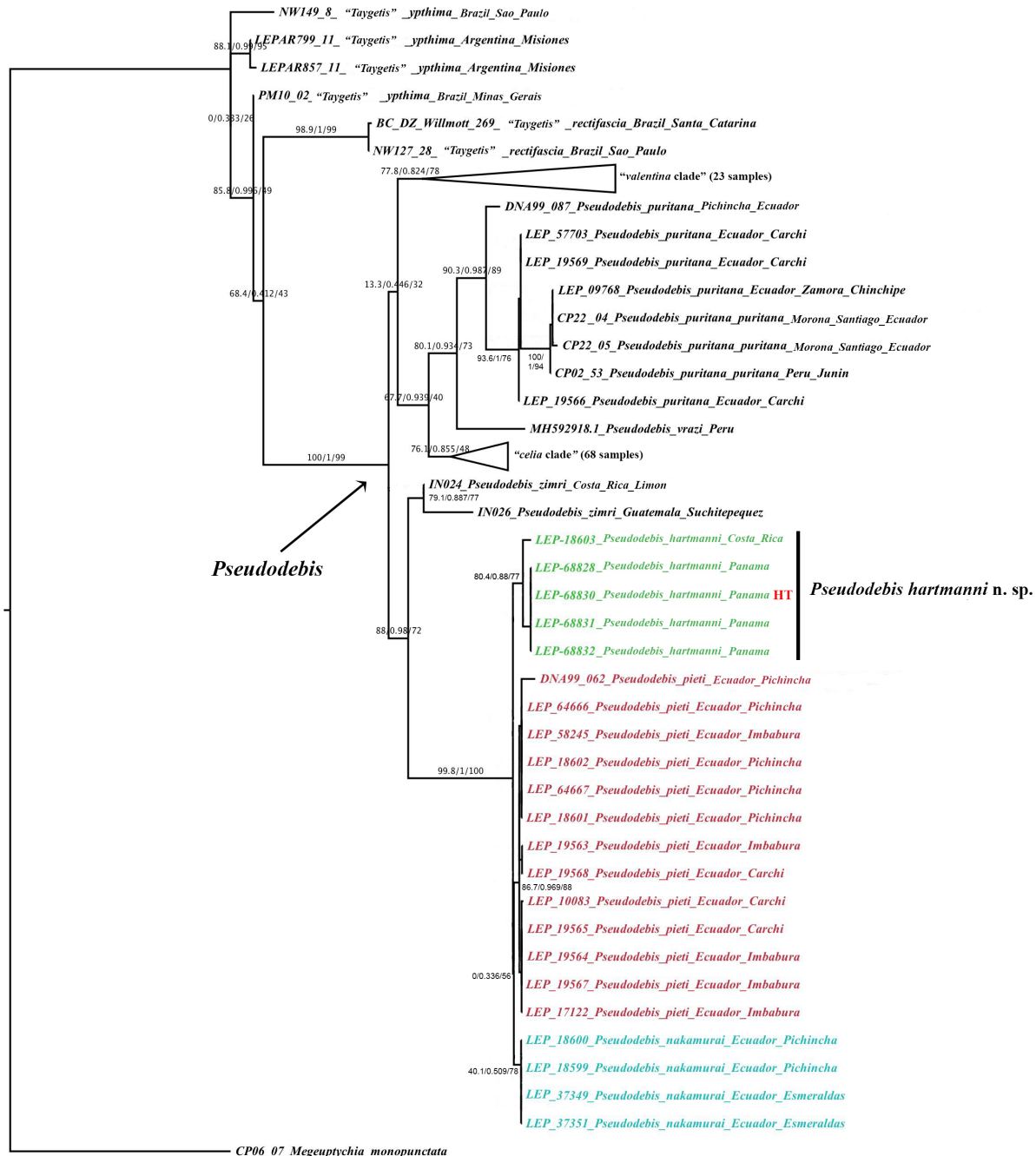


Figure 1. Maximum likelihood tree showing the relationships among species of *Pseudodebis* inferred in IQ-TREE2 (LnL= -2965.527), with branch support representing SH-aLRT/aBayes/UFBoot. HT denotes the holotype specimen.

Table 1. Accession information for new DNA sequences obtained for this study.

DNA Voucher code	Species	GenBank Accession	Locality
LEP-18603	<i>Pseudodebis hartmanni</i>	MW173396	San José, Costa Rica
LEP-68828	<i>Pseudodebis hartmanni</i>	MW173394	Chiriquí, Panama
LEP-68830	<i>Pseudodebis hartmanni</i>	MW173395	Chiriquí, Panama
LEP-68831	<i>Pseudodebis hartmanni</i>	MW173397	Chiriquí, Panama
LEP-68832	<i>Pseudodebis hartmanni</i>	MW173398	Chiriquí, Panama

specimens following the methods described in Nakahara *et al.* (2018a). GenBank accession numbers for these newly obtained sequences are listed in Table 1. These sequences were incorporated into the matrix compiled for Nakahara *et al.* (in press: Table1) and aligned using MUSCLE (v 3.8.425) (Edgar 2004) in Geneious (v 11.1.5) (Biomatters Ltd.). The matrix consists of 135 individuals and the final alignment was 657 base pairs in length. In order to graphically assess taxonomic status, phylogenetic analysis with maximum likelihood as an optimality criterion was performed based on the aforementioned dataset using IQ-TREE2 (v.2.0.6) (Minh *et al.* 2020). Based on the ModelFinder (Kalyaanamoorthy *et al.* 2017), coupled with the “merge” option, TN+F+G4 was applied to the 1st codon position, F81 was applied to model the 2nd codon position, and TIM+F+G4 to model the 3rd codon position. Confidence was assessed by approximate Bayes branch test (aBayes; Anisimova *et al.* 2011), Shimodaira-Hasegawa-like approximate Likelihood Ratio Test (Guindon *et al.*, 2010), and ultrafast bootstrap (UFBoot) (Hoang *et al.* 2018) with “bnni” option to reduce the model violation. We ran 10 independent

analyses and the tree that gave the highest log-likelihood score (LnL= -2965.527) for the above dataset was manually rooted with *Megeuptychia monopuctata* Willmott & Hall, 1995. We also calculated patristic distances employing the Tamura-Nei distance model based on a dataset consisting of four species discussed herein by producing an unrooted Neighbour-Joining tree.

SPECIES DESCRIPTION

Pseudodebis hartmanni Nakahara & MacDonald, new species
(Figs 1-4)

Systematic placement and diagnosis. *Pseudodebis hartmanni* n. sp. is recovered as a member of *Pseudodebis* based on the reduction of the brachia in male genitalia (Fig. 3a), a synapomorphy of *Pseudodebis*, and confirmed with our maximum likelihood phylogenetic hypothesis. *Pseudodebis* is represented as a strongly supported monophyletic group (SH-aLRT/aBayes/UFBoot = 100/1/99), with its type species, *Papilio valentina* Cramer, 1779, found inside the clade, and thus this new species is unequivocally placed within this genus. The partial COI sequences of *Pseudodebis hartmanni* n. sp. (n = 5) are recovered as a monophyletic group and part of a well-supported clade being sister to *P. nakamurai* + *P. pieti*, two species known from the western Andes (SH-aLRT/aBayes/UFBoot = 99.8/1/100; Fig. 1). The patristic genetic distances

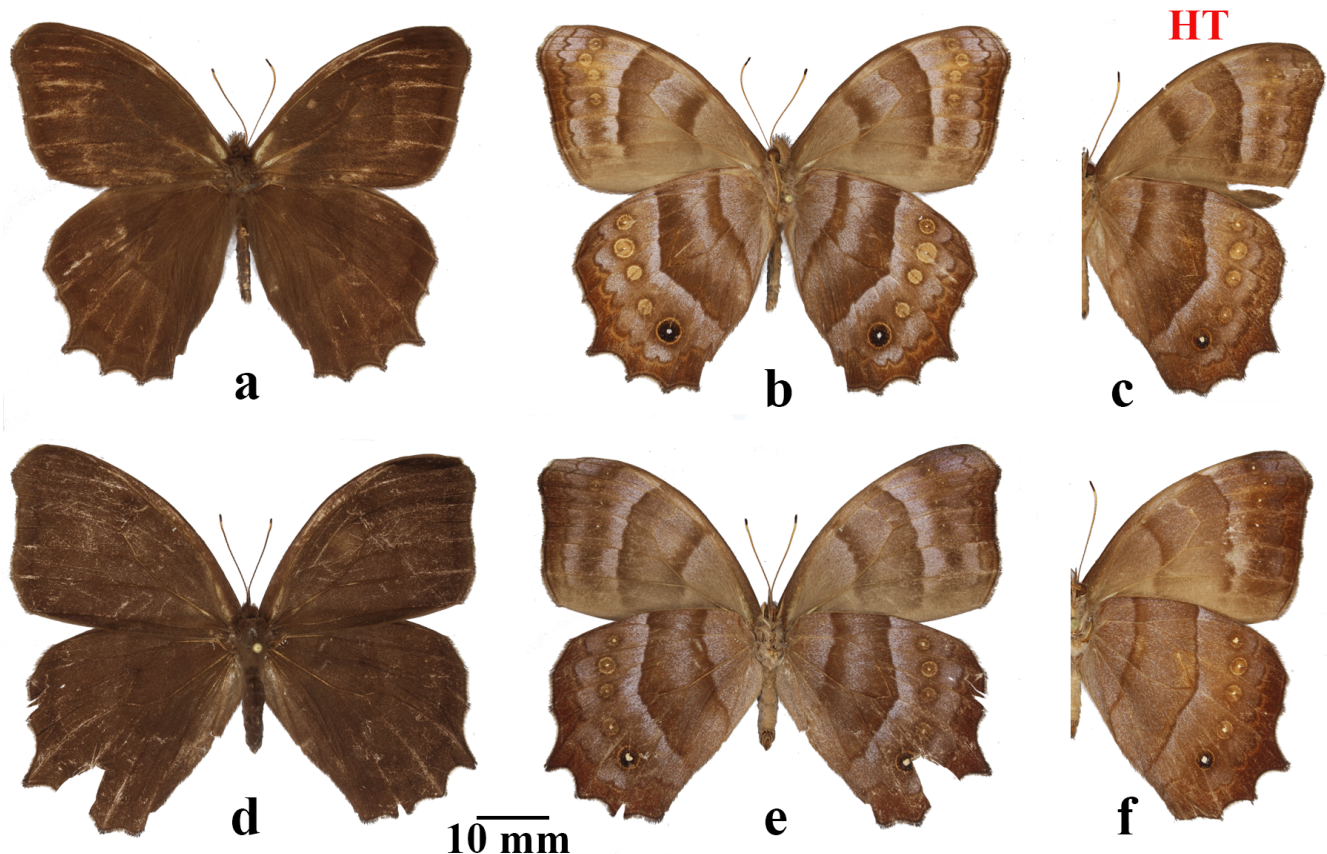


Figure 2. *Pseudodebis hartmanni* n. sp. type series: **a)** paratype male (LEP-18603), dorsal surface; **b)** ditto, ventral surface; **c)** holotype male (LEP-68830), ventral surface; **d)** paratype female (LEP-68831), dorsal surface; **e)** ditto, ventral surface; **f)** paratype female from El Valle, Panama (DNA data not obtained), ventral surface. Scale bar = 10 mm.

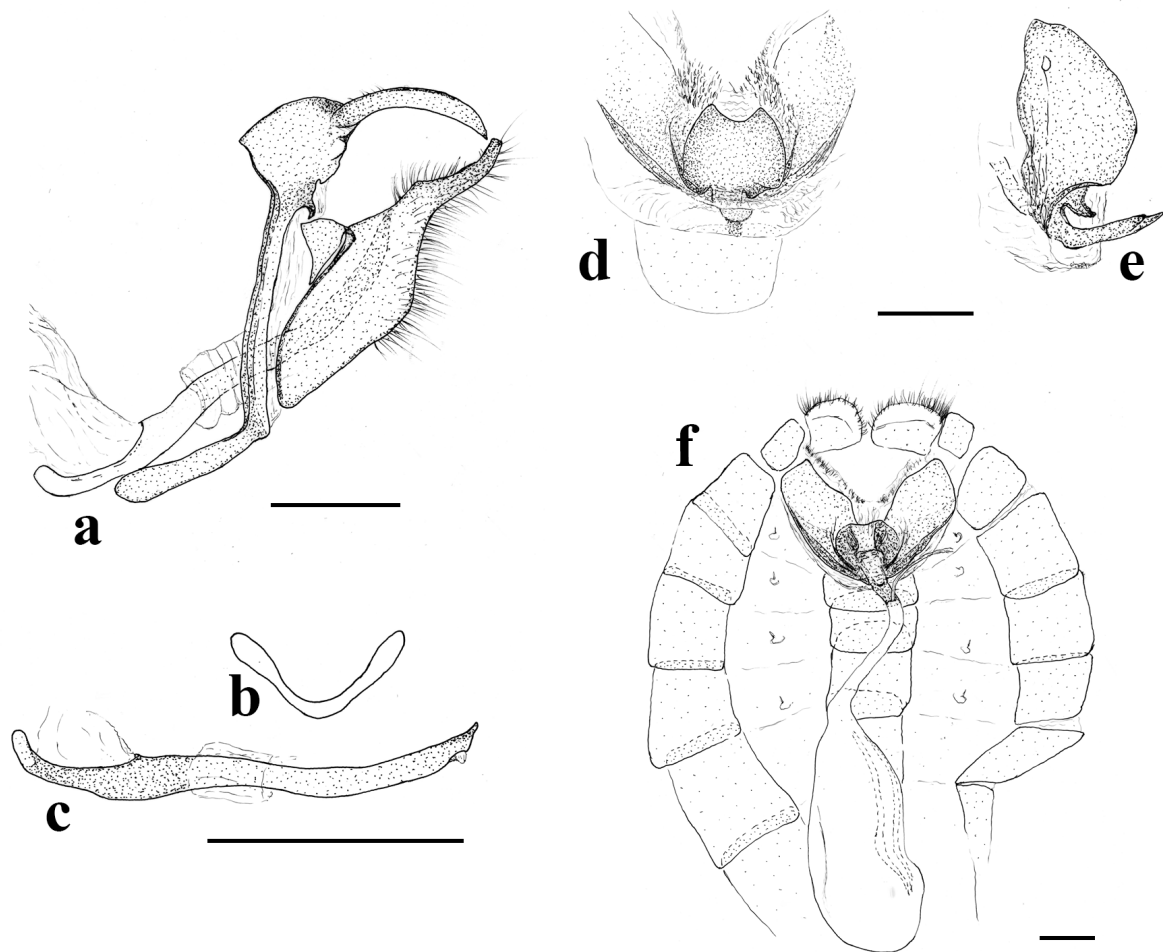


Figure 3. Male and female genitalia of *P. hartmanni* n. sp.: a) male genitalia in lateral view; b) juxta in posterior view; c) phallus in lateral view; d) lamella antevaginalis in ventral view, with inter-segmental membrane of seventh and eighth abdominal segment expanded; e) lateral view of lamella antevaginalis and the lateral plate of eighth abdominal segment; f) dorsal view of female genitalia with inter-segmental membrane of seventh and eighth abdominal segment folded. Male genitalia illustrations are based on dissection vial SN-20-41; female genitalia illustrations are based on SN-20-54. Scale bar = 1 mm.

calculated based on DNA barcodes showed low divergence between *P. hartmanni* n. sp. (n = 5) and *P. nakamurai* (n = 4), ranging from 1.4% to 1.7%; *P. hartmanni* n. sp. (n = 5) and *P. pieti* (n = 12) to range from 1.3% to 2.5%; *P. hartmanni* n. sp. (n = 5) and *P. zimiri* (n = 2) to range from 4.6 % to 5.2 %; and infra-specific divergence among five individuals of *P. hartmanni* n. sp. to range from 0% to 0.9%.

The male of *P. hartmanni* n. sp. is distinguished from *P. pieti* by the following characters, although it must be noted that due to the infra-specific variation of both species (see Nakahara *et al.* 2021), these characters are not always reliable separately and thus should be used in combination: 1) ground colour of ventral surface appearing more reddish in *P. hartmanni* n. sp. (rather greyish in *P. pieti*); 2) whitish scales distal of VFW postdiscal band and VHW postdiscal band, in addition to area basal of VFW discal band and VHW discal band, more apparent in *P. hartmanni* n. sp. (less apparent, or sometimes absent in *P. pieti*); 3) VFW discal band and VHW discal band fading distally, thus appearing somewhat wider and less defined in *P. hartmanni* n. sp. (these bands appear more narrow and defined in *P. pieti*); 4) VHW postdiscal band rather straight before

terminating at inner margin in *P. hartmanni* n. sp. (this band generally curves inwards in *P. pieti*). The same set of characters apparently can be applied for distinguishing females of these two taxa as well. Despite their close relationship, *P. hartmanni* n. sp. is less likely to be confused with *P. nakamurai*, as males, and to lesser extent females, of *P. nakamurai* have pale yellowish scaling scattered more or less evenly throughout the ventral wings, whereas *P. hartmanni* n. sp. ventrally appears more brownish, thus giving different impression of overall appearance. Furthermore, *P. hartmanni* n. sp. is distinguished from *P. nakamurai* by the following characters: 1) the forewing length of *P. hartmanni* n. sp. is greater than that of *P. nakamurai* (e.g., male forewing length mean 37 mm (n=5) for *P. hartmanni* n. sp., 29.6 mm (n=7) for *P. nakamurai*); 2) VFW and VHW of *P. hartmanni* n. sp. is overlaid with silverish scales, whereas these scales are hardly visible on the ventral side of *P. nakamurai*; 3) ocellus in the VHW cell Cu_1 is filled with black inside the ring in *P. hartmanni* n. sp., whereas this ocellus in *P. nakamurai* is usually brownish. Although not a member of this focal western Andes clade, an undescribed species known from Panama is phenotypically similar to *P. hartmanni* n. sp. and both being

largely sympatric. Nevertheless, these two species can be readily distinguished by the lack of black colouration filling the ocellus in the VHW cell Cu_1 in the undescribed species, coupled with the much broader apical process of the valva (narrow in *P. hartmanni* n. sp.) and the anterior margin of the tegumen more pronounced (rather flattened in *P. hartmanni* n. sp.). Further information and discussion regarding this undescribed species will be provided in a forthcoming paper focused on the “*celia* clade” (Nakahara *et al.* in prep.).

Description.

MALE: Forewing length 35-40 mm (mean 37 mm; n=5)

Head: Eyes with hair-like scales, white scales at base; frons dark brownish, with whitish scales and long greyish hair-like scales; first segment of labial palpi similar to second segment in width, covered with white scales and elongated scales, whitish long seti-form scales some distally appearing greyish, present ventrally, scales on exterior side overall appearing ochre-ish, second segment length similar to longitudinal eye axis, covered with whitish scales and white scales laterally, elongated hair-like scales along interior edge of dorsal surface, scales appearing darker distally, ventrally with whitish long seti-form scales and white elongated scales, some appearing distally darker, length apparently variable, some as long as third segment of labial palpi, scales on exterior side overall appearing ochre-ish, third segment roughly one-third of second segment in length, porrect, covered with dark greyish scales dorsally and ventrally, with whitish scales visible laterally; antennae slightly shorter than half of forewing length, with flagellum ca. 37 antennomeres (n=1), distal 10-11 segments composing rather insignificant club, distal 5 antennomeres (n=1) black, greyish scales and whitish scales at base of each side of antennomeres more apparent towards base. **Thorax:** Brownish, dorsally and laterally (above wings) scattered with greyish scales and dense long light brownish hair-like scales, ventrally (below wings) scattered with whitish scales and long whitish hair-like scales; foreleg reduced, with long hair-like scales, foretibia and foretarsus about same length, single small subsegment apparently present at distal end of tarsus; pterothoracic legs femur ochre dorsally and whitish ventrally, without spines, tibia and tarsus of pterothoracic legs ochre overall, dorsally darker, ventrally lighter, colour becomes darker on distal segments of legs, tibia with two longitudinal rows of spines ventrally, in addition to spines present laterally on both sides, tibial spurs present at distal end of tibia, spurs equal in length, tarsus with roughly three longitudinal rows of spines ventrally, rows of spines increase to four from distal end of tarsus and towards distally. **Abdomen:** Eighth tergite appearing as narrow band along anterior margin of eighth abdominal segment, posterior broad patch invisible. **Wing venation:** Basal half of forewing subcostal vein swollen; R_2 rising roughly at origin of discocellular vein m_1 - m_2 ; base of cubitus swollen; forewing recurrent vein absent; hindwing humeral vein developed; origin of hindwing M_2 towards M_1 than M_3 . **Wing shape and pattern:** Forewing subtriangular, apex angular, falcate, costal margin slightly convex, outer margin convex above M_3 (falcate portion), tornus rounded, inner margin almost straight, but rounded towards thorax near base; hindwing slightly elongate, rounded, costal margin slightly convex, outer margin undulating, especially distal of M_3 being most pronounced, to less extent distal of Cu_1 , and Cu_2 , tornus slightly angular, inner margin slightly concave near tornus, anal lobe convex, slightly round; **Dorsal forewing** black androconial scales present in middle of DFW, roughly mirroring area basal of VFW postdiscal band, apparently faded near costa and area around inflated portion of subcostal vein and cubitus; **Dorsal hindwing** black androconial scales present in middle of DHW, roughly mirroring area basal of VHW postdiscal band, apparently faded near base; **Ventral forewing** ground colour somewhat appearing reddish brown, overlaid with silver-ish scales (especially apparent distal of postdiscal band); discal band rather poorly defined, appearing as broad but distally fading dark brownish short band in discal cell; postdiscal band narrower but basal dark brownish shading making overall appearance somewhat broad; otherwise other elements as illustrated (Fig. 2a); **Ventral hindwing** ground colour similar to ventral forewing, similarly covered with silver-ish scales, especially area between postdiscal band and submarginal band; discal band similar to that of ventral forewing except for extending across wing from costa to inner margin; postdiscal band, similar to that of ventral forewing except for bent when crossing M_3 , not terminating in pronounced basal curve as it nears inner margin; submarginal ocellus in cell Cu_2 with single white pupil and black central area ringed in yellow to orange; otherwise other elements as illustrated (Fig. 2a). **Genitalia:** as illustrated (Fig. 3a-c); hair-like setae visible along dorsal margin of uncus; brachium reduced;

ductus ejaculatorius entering antero-dorsal opening of phallobase posteriorly, vesica exits at posterior tip of aedeagus; no obvious sclerotized cornuti (i.e., cornuti apparently absent).

FEMALE: Forewing length 33.5-38.5 mm (mean 36.7 mm; mm; n=7)

Similar to male, except as follows: five tarsomeres present on foretarsus, distally with spines ventrally; wing shape overall appearing broader and rectangular; dorsal androconial scales absent. **Female abdomen and genitalia:** as illustrated (Fig. 3d-f); intersegmental membrane of seventh and eighth abdominal segment ventrally folded to lesser extent, expandable, with weakly sclerotized region present; ductus seminalis delineates basal sclerotized region and posterior membranous region of ductus bursae; corpus bursae large, reaching basal portion of abdomen.

Types. HOLOTYPE male with the following labels written verbatim: // Panama: Chiriquí [Chiriquí] Santa Clara Finca Hartmann ca. 1,540 m. N 08° 50' 34.8" W 082° 45' 43.9" Aug. 11, 2010 J. R. MacDonald// DNA voucher LEP-68830// (MEM).

PARATYPES (7♂, 7♀; label data reproduced verbatim): **PANAMA:** 1♂: // Panama: Chiriquí [Chiriquí] Santa Clara Finca Hartmann ca. 1,500 m. N 08° 51' 41.1" W 082° 44' 35.7" Aug. 11, 2010 J. R. MacDonald// DNA voucher LEP-68833// Genitalic vial SN-20-41 S. Nakahara// (MEM); 1♀: // Panama: Chiriquí [Chiriquí] Santa Clara Finca Hartmann ca. 1,540 m. N 08° 50' 34.8" W 082° 45' 43.9" Aug. 11, 2010 J. R. MacDonald// DNA voucher LEP-68832// Genitalic vial SN-20-54 S. Nakahara// (MEM); 1♀: // Panama: Chiriquí [Chiriquí] Santa Clara Finca Hartmann ca. 1,250 m. N 08° 50' 44.3" W 082° 45' 39.7" July 16, 2018 J. R. MacDonald// DNA voucher LEP-68831// (MEM); 1♂: // PANAMA: California 27.xii. 1972 H.L. King// FLMNH-MGCL Specimen 284470// (MGCL); 1♀: // PANAMA California 27.xii. 1971 H.L. King// Allyn Museum Acc. 73-39// FLMNH-MGCL Specimen 284472// (MGCL); 1♀: // Panama: Cocle El Valle 800-850 m.// Jan. 7 1986 John R. MacDonald// LEP-68829// (MEM); **COSTA RICA:** 1♂: // COSTA RICA: Prov. Cartago 1 km N of Moravia de Chirripo Rio Moravia, 1050-1200m 12. xi. 2006 I. Nakamura leg.// (ICNA); 1♂: // COSTA RICA: Prov. Alajuela 2 km SE of Cariblanco, 750-850m 10°15'21"N, 84°10'11"W 9. vi. 2005 I. & M. Nakamura, M. Posla & J. Corrales leg.// (ICNA); 1♂: // COSTA RICA: Prov. San José Bajo la Hondura, 1150-1450m 10°03'37"N, 83°58'55"W 23. x. 2006 I. Nakamura leg.// (ICNA); 1♂: // COSTA RICA: Prov. San José Bajo la Hondura, 1150-1450m 10°03'37"N, 83°58'55"W 22. vii. 2010 I. Nakamura leg.// DNA voucher LEP-18603// (ICNA); 1♀: // COSTA RICA: Prov. San José Bajo la Hondura, 1150-1450m 10°03'37"N, 83°58'55"W 13. vi. 2005 I. Nakamura leg.// (ICNA); 1♀: // COSTA RICA: Prov. San José Bajo la Hondura, 1150-1450m 10°03'37"N, 83°58'55"W 23. x. 2006 I. Nakamura leg.// (ICNA); 1♂: // Costa Rica Puntarenas Cotoncito La Amistad 1700 mts 11-XII-83 leg. R. Hesterberg// R. Hesterberg coll. MGCL Accession # 2001-1// FLMNH-MGCL Specimen 284469// Genitalic vial SN-20-75 S. Nakahara// (MGCL); 1♀: // COSTA RICA Puntarenas Prov. Finca Las Cruces 5 km San Vito de Java 29 Sept 1986 leg G&A Austin// G T Austin coll MGCL Acc. 2004-5// FLMNH-MGCL Specimen 284471// (MGCL).

Etymology. The specific epithet is in recognition of the Señor Ratibor “Chicho” Hartmann, the patriarch of Finca Hartmann, where the holotype male was captured. The Hartmann family have been supportive of natural history studies, including the butterfly faunistic research conducted by the junior author and colleagues, on their property for more than 45 years. The name is treated as a Latinized masculine noun in the genitive case.

Variation. Like its two sister species, *P. pieti* and *P. nakamurai*, this species also exhibits phenotypic variation, including within some of the diagnostic characters mentioned above. This variation includes: 1) Silver-ish ventral scales reduced in a few specimens such as FLMNH-MGCL 284472; 2) ocellus in the VHW cell Cu_1 lacking black coloration (consequently appearing similar to submarginal ocelli in other cells) in a few specimens such as FLMNH-MGCL 284472 and 284470; 3) ventral bands appearing narrow and defined, lacking faded area

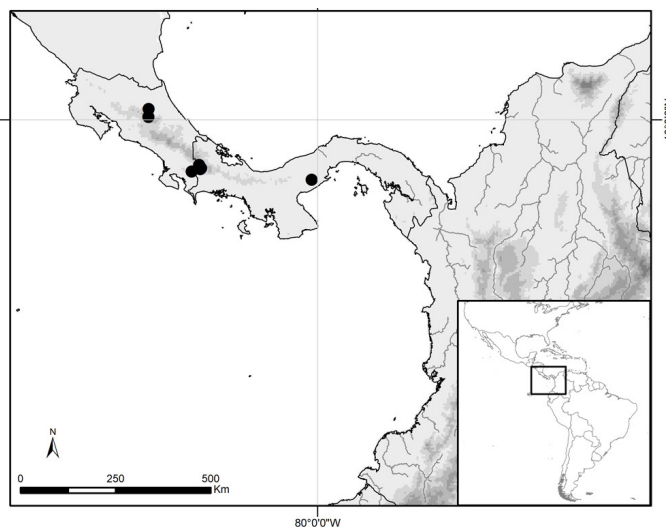


Figure 4. General view of Finca Hartmann (photo credit: Albert Thurman); distribution map of *P. hartmanni* **n. sp.** based on museum specimens.

in a few specimens such as the female from El Valle, Panama (Fig. 2f).

Distribution and natural history (Fig. 4). *Pseudodebis hartmanni* **n. sp.** has been found in sub-montane to montane rainforest in Panama and Costa Rica at elevations ranging from 750 m to 1,500 m. The most similar, closely related species is *P. pieti*, which occurs also at elevations from 600 m to 1700 m in montane cloud forest along the slopes of the western Andes. In Panama, JRM collected *P. hartmanni* individuals using fruit baits including rotten banana and mango, mixed with sugar and beer. Some noteworthy other sympatric species observed at two Panamanian sites include: *Forsterinaria neonympha* (C. Felder & R. Felder, 1867), *Hermeuptychia harmonia* (Butler, 1867), *Cyllopsis rogersi* (Godman & Salvin, 1878), *Manataria hercyna* (Hübner, [1821]), *Epiphile iblis* C. Felder & R. Felder, 1861, *Doxocopa cyane* (Latreille, [1813]) (all Nymphalidae), *Detritivora nicolayi* (Hall & Harvey, 2001) (Riodinidae), and *Celaenorrhinus aegiochus* (Hewitson, 1876) (Hesperiidae). *Forsterinaria neonympha* is also found at one site in Costa Rica, Bajo la Hondura, which appears to be a site that also supports butterfly species rarely seen elsewhere, including *Drucina leonata* Butler, 1872 and *Oxeoschistus puerta* (Westwood, 1851) (both Nymphalidae).

Remarks. A pair of specimens from Panama at the MGCL both bear a label with “California” as their locality (FLMNH-MGCL 284472 and 284470), with H [Harry]. L. King as the collector for these two individuals. There exist at least two localities which can be referred to as “California” in Panama, both in Chiriquí province: California located near Puerto Armuelles on the Pacific coast (N 08° 20’ 13.11” W 82° 52’ 04.90”; elevation approximately 30 m), and another locality, Nueva California, located on the far side of the Chiriquí River on the route to Finca Hartmann, close to the type locality (N 08° 49’ 42.60” W 82° 40’ 00.40”; elevation approximately 1,600m). With no further locality information provided on the label it is impossible to determine with certainty where these specimens were collected. Nevertheless, Nueva California seems the more likely site for

the collection of these two specimens, given its proximity to the type locality and the apparent sub-montane preference of this species. Harry L. King passed away in 1985 (J. Heppner, pers. comm.) and thus the true provenance of these specimens will remain unknown.

DISCUSSION

We here describe and name a new species of *Pseudodebis* based on rather subtle phenotypical differences compared to a closely related, allopatric species, *P. pieti*. As mentioned above, due to infra-specific variation the wing patterns of *P. hartmanni* **n. sp.** and *P. pieti* can overlap, and one might argue for their conspecificity. Nevertheless, their specific status can be justified and supported mainly by the maximum likelihood tree based on the DNA “barcode” data. The molecular data presented herein is strongly supporting reciprocal monophyly of *P. hartmanni* **n. sp.** (SH-aLRT/aBayes/UFBboot = 99.8/1/100) (Fig. 1), and being sister to *P. pieti* + *P.nakamurai*. In addition, Nakahara *et al.* (2021) showed that their reciprocal monophyly was strongly supported, although that study did not include *P. hartmanni* **n. sp.** due to the lack of sequence data then available. The partial COI sequences of *Pseudodebis hartmanni* **n. sp.** form a monophyletic group, with the four Panamanian individuals recovered as a strongly supported clade (SH-aLRT/aBayes/UFBboot = 88.4/0.966/94), while a single individual from Costa Rica is sister to this clade. The barcoding genetic distance data between *P. hartmanni* **n. sp.** and *P. pieti* is rather low but clearly delineates phylogenetic structure. In the light of these subtle but consistent morphological and molecular evidences, we propose *P. hartmanni* **n. sp.** from Costa Rica and Panama is best treated as a species different from *P. pieti*. A number of other butterfly species are also known only from the sub-montane to montane region of Costa Rica and Panama (e.g., *Eutresis dilucida* (Staudinger, 1885), *Drucina leonata* (Butler, 1872), *Adelpha demialba* (Butler, 1872) (all Nymphalidae), *Panoquina danishi* Anderson, 2014, *Dalla pulchra* (Godman, 1900), *Buzyges idothea* (Godman, 1900) (all Hesperiidae)). The clade including *P. zimri* (Butler, 1869), as shown in Fig.1,

comprises *Pseudodebis* taxa occurring in the western Andes, and illustrates the fact that *Pseudodebis* is more diverse in the western Andes rather than the eastern side, an atypical diversity pattern for euptychiines also followed mostly by the members of the so-called “*Cyllopsis* clade” (*sensu* Espeland *et al.* 2019). We would like to emphasize that our effort to document the unrecognized diversity of *Pseudodebis* in Central America and the west Andes calls for an in-depth genomic study that includes samples of at least four evolutionary units distributed in the western Andes.

We therefore update the described alpha diversity of *Pseudodebis*, which now includes 11 species, 4 of them occurring west of the tropical Andes, although few additional undescribed species of *Pseudodebis* still await description from this region. Our study calls for attention to one of the regions with high biodiversity decline in the Neotropics, where there are still several undescribed taxa in urgent need for taxonomic treatment.

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