

A new species of *Pareuptychia* Forster, 1964 (Lepidoptera, Nymphalidae, Satyrinae) from the northwestern Amazon basin with characterisation of two potential synapomorphies for the genus

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Abstract: A new species of *Pareuptychia* Forster, 1964, *P. milleri* n. sp., is described based on male specimens from the northwestern Amazon basin. Male specimens can be distinguished from other *Pareuptychia* species by the reduced white dorsal wing markings and darker ventral wing coloration. Two male genitalia characters are identified as likely synapomorphies for *Pareuptychia*. Morphological characters including male genitalia are illustrated and a distribution map is provided. The female of *P. milleri* n. sp. is unknown.

Key words: Euptychiina, taxonomy, Ecuador, Peru

Resumen. Se describe una nueva especie de *Pareuptychia* Forster, 1964, *P. milleri* n. sp., basada en machos de la cuenca noroccidental del Amazonas. Los machos pueden ser distinguidos de otras especies de *Pareuptychia* por la reducción de las marcas blancas en la parte dorsal y tener en la parte ventral del ala un patrón de coloración más oscuro. Dos caracteres de la genitalia del macho son identificados como probables sinapomorfias para *Pareuptychia*. Los caracteres morfológicos incluyendo la genitalia masculina son ilustrados y además se presenta un mapa con la distribución de la especie. La hembra de *P. milleri* n. sp. es desconocida.

Palabras clave: Euptychiina, taxonomía, Ecuador, Perú

INTRODUCTION

Pareuptychia Forster, 1964 is a genus of Neotropical butterflies widely distributed from Mexico to northern Argentina, occurring from sea level to approximately 2000 m elevation. Species in this genus are common in butterfly assemblages, being some of the most frequently represented taxa in diversity studies (e.g. Francini *et al.*, 2011; Giovenardi *et al.*, 2013; Lamas *et al.*, 1996; Ríos-Málaver, 2007; Salinas-Gutiérrez *et al.*, 2009). Adults fly close to the ground, generally below 1.5 m, and are frequently encountered in old secondary growth or along forest edges (e.g. Barbosa, 2013; DeVries, 1987).

Despite the relatively large number of taxa in the genus, including at least nine species and over a dozen subspecies (Lamas, 2004; Brévignon, 2005), all have a characteristic wing pattern, most notably with the ventral surface having distinctive longitudinal white and brown bands. This confusing similarity has stimulated suggestions that *Pareuptychia* might represent a complex of cryptic species (Marín *et al.*, 2011) similar to that of *Hermeuptychia* Forster, 1964 or *Ypthimoides* Forster, 1964 (Barbosa *et al.*, 2015; Cong & Grishin, 2014; Seraphim *et al.*, 2014), and might contain a number of undescribed species. Integrative taxonomy can be used as a tool to help solve the difficulties presented by such complexes, with demonstrable effectiveness in species identification in cryptic species

complexes with limited numbers of specimens available for study (Melville *et al.*, 2014). Recent studies in Euptychiina have incorporated such an integrative taxonomic approach, proving its usefulness in the description of new species (e.g. Barbosa *et al.*, 2015; Cong and Grishin, 2014; Freitas *et al.*, 2015).

The present paper describes a new species of *Pareuptychia* from the northwest of the upper Amazon basin, based on an integrative taxonomic approach (Padial *et al.*, 2010; Pante *et al.*, 2015; Schlick-Steiner *et al.*, 2010) using both morphological and molecular data.

MATERIALS AND METHODS

Morphology: Legs, labial palpi, and abdomens were soaked in hot KOH solution for 5-10 minutes, dissected, and subsequently stored in glycerine. Drawings of labial palpi and foreleg were done using a camera lucida attached to a Leica MZ 16 stereomicroscope. Wing scales were removed with ethanol to examine and study wing venation. Terminology for wing venation follows the Comstock-Needham system described in Miller (1970: 44) and terminology for wing pattern elements follows Peña & Lamas (2005). Nomenclature of genitalia mostly follows Klots (1956), however, we follow Peña & Lamas (2005) for the term aedeagus. We also follow Muschamp (1915) for the term 'brachia'. Finally, we follow Austin & Mielke (2008) in referring to the part of the genitalia

typically termed the 'vinculum' as the 'combined ventral arms of tegumen and dorsal arms of saccus' (see Nakahara *et al.*, 2015, for discussion).

Specimens were studied in the following collections:

BMNH	The Natural History Museum, London, UK
DATR	David Trembath collection, Dorking, UK
HAWA	Haydon Warren-Gash collection, London, UK
MECN	Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador
MGCL	McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, USA
MUSM	Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru
ZMHU	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany
ZUEC	Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil

Molecular data: DNA was extracted from two legs of specimens of the new species using the DNeasy blood & tissue kit (Qiagen, Valencia, CA, USA). Part of the Cytochrome Oxidase (COI) gene (the 'barcode' region) was amplified using the following primers: LCO_nym (forward, TTTCTACAAATCATAAAGATATTGG) and HCO_nym (reverse, TAAACTTCAGGGGTGACCAAAAAATCA). PCR products were prepared in 25 µl reactions using the Omega 2x Taq Mastermix (Omega Bio-tek, Norcross, GA, USA) and reaction conditions were as follows: 1 min at 94°C followed by 5 cycles of 30 s at 94°C, 40 s at 45°C, 1 min at 72°C, followed by 34 cycles of 30 s at 94°C, 40 s at 51°C, 1 min at 72°C, followed by 5 min at 72°C. PCR products were checked by 1.2% agarose gels stained with ethidium bromide. Purification and sequencing were done at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida.

In Brazil, genomic DNA was extracted from other *Pareuptychia* specimens using the DNeasy Tissue Kit (Qiagen). Amplification of the DNA barcode region was performed using primers LCO (5'GGTCAACAAATCATAAAGATATTGG) and NANCY (5'CCTGGTAAATATAAATATAAACTTC) (Simons *et al.*, 1994). PCR reactions were performed in a final volume of 20 µl containing 2 µl of genomic DNA, 50 mM KCl, 3 mM MgCl₂, 0.5 mg BSA/mL, 0.2 mM of each primer, 4 mM of each nucleotide and 1 U of Taq polymerase (Fermentas, Lithuania). PCR products were purified using the selective precipitation method with polyethylene glycol 6000 (PEG6000) (Schmitz and Riesner, 2006) and sequenced on ABI Prism BigDye kit sequencer 3730XL (Applied Biosystems) using the service LaCTAD in Universidade Estadual de Campinas in São Paulo. Sequences were uploaded to Genbank (Table 1). Sequence quality was analyzed with the program FinchTV v. 1.3.1 (Geospiza Inc.) and aligned in the program MUSCLE (Edgar, 2004) with sequences obtained previously and available from Genbank (Table 1). A Maximum Likelihood (ML) phylogenetic analysis was conducted to study the position of the new species inside the genus with the model GTR + G and bootstrap resampling of 1000 replicates, in the program MEGA 5.0 (Tamura *et al.*, 2011).

Table 1. List of GenBank accession numbers for sequences used in this study.

Genus	Species	Genbank number	Locality
<i>Atlanteuptychia</i>	<i>ernestina</i>	DQ338793	Brazil – São Paulo
<i>Cissia</i>	<i>penelope</i>	AY508530	Ecuador – Napo
<i>Cissia</i>	<i>penelope</i>	GU205833	Peru – Junín
<i>Chloreuptychia</i>	<i>arnaca</i>	KP848784	Panama – Barro Colorado
<i>Erichthodes</i>	<i>julia</i>	GU205834	Peru – Junín
<i>Neonympha</i>	<i>mitchellii</i>	KC112171	USA
<i>Satyrotaygetis</i>	<i>satyrina</i>	AY508575	Costa Rica – Puntarenas
<i>Megeuptychia</i>	<i>antonoe</i>	JQ548139	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>binocula</i>	GU205858	Peru – Madre de Dios
<i>Pareuptychia</i>	<i>binocula</i>	KX087108	French Guiana
<i>Pareuptychia</i>	<i>hesionides</i>	AY508567	Ecuador – Napo
<i>Pareuptychia</i>	<i>hesionides</i>	KX087109	Colombia – Meta
<i>Pareuptychia</i>	<i>metaleuca</i>	KX087110	Colombia – Antioquia
<i>Pareuptychia</i>	<i>metaleuca</i>	KX087111	Colombia – Antioquia
<i>Pareuptychia</i>	<i>metaleuca</i>	GU334284	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>metaleuca</i>	HM406614	Panama – Barro Colorado
<i>Pareuptychia</i>	<i>metaleuca</i>	HM406615	Panama – Barro Colorado
<i>Pareuptychia</i>	<i>metaleuca</i>	HM905330	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>metaleuca</i>	JQ578340	Costa Rica – Guanacaste
<i>Pareuptychia</i>	sp	KX087112	Colombia – Meta
<i>Pareuptychia</i>	sp	KX087113	Colombia – Meta
<i>Pareuptychia</i>	<i>milleri</i>	KX087107	Ecuador – Pastaza
<i>Pareuptychia</i>	<i>ocirrhoe</i>	AY508568	Ecuador – Napo
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087114	Colombia – Chocó
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087115	Colombia – Chocó
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087116	Colombia – Antioquia
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087117	Colombia – Antioquia
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087118	Colombia – Antioquia
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU205859	Brazil – São Paulo
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU334288	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU658936	Mexico – Campeche
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU658937	Mexico – Campeche
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU658938	Mexico – Quintana Roo
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU658942	Mexico – Quintana Roo
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU666815	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>ocirrhoe</i>	GU675312	Costa Rica – Guanacaste
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087119	Colombia – Amazonas
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087120	Colombia – Amazonas
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087121	Brazil – Mato Grosso do Sul
<i>Pareuptychia</i>	<i>ocirrhoe</i>	KX087122	Brazil – Mato Grosso do Sul

RESULTS

Relationships: The Maximum Likelihood (ML) tree (Fig. 1) indicates that there are two well supported clades (*P. metaleuca* (Boisduval, 1870) + *P. binocula* (Butler, 1867) + *Pareuptychia* sp.) (bs = 100) and [*P. ocirrhoe* (Fabricius, 1776) + *P. hesionides* Forster, 1964]) (bs = 98). *Pareuptychia milleri* is recovered as sister to the *P. metaleuca* + *P. binocula* + *Pareuptychia* sp. clade, although with low support (bs = 68).

Pareuptychia milleri Nakahara, Marín & Neild, new species (Figs. 2-5)

Diagnosis. This species can be distinguished from other male *Pareuptychia* species by the melanic coloration of its ventral surface, and by the reduced white coloration of its dorsal surface, where it is restricted to forewing cells Cu₂ and 2A. The male genitalia can be distinguished from that of many other *Pareuptychia* species by having a less developed hump on the dorsal margin of the valva. The female is unknown.

Description.

MALE. Forewing length 20.0 mm - 23.5 mm (n=5) (holotype: 22.0 mm).

Head. Eyes brown, posteriorly darker, sparsely hairy, hairs relatively short and somewhat golden; labial palpi (Fig. 4) with first segment short, second segment covered with white and black scales laterally, black hair-like scales dorsally, as long as segment width, ventrally adorned with long black and white hair-like scales 3-4 times as long as second segment width, second segment about as long as eye diameter, third segment dorsally and ventrally covered with black

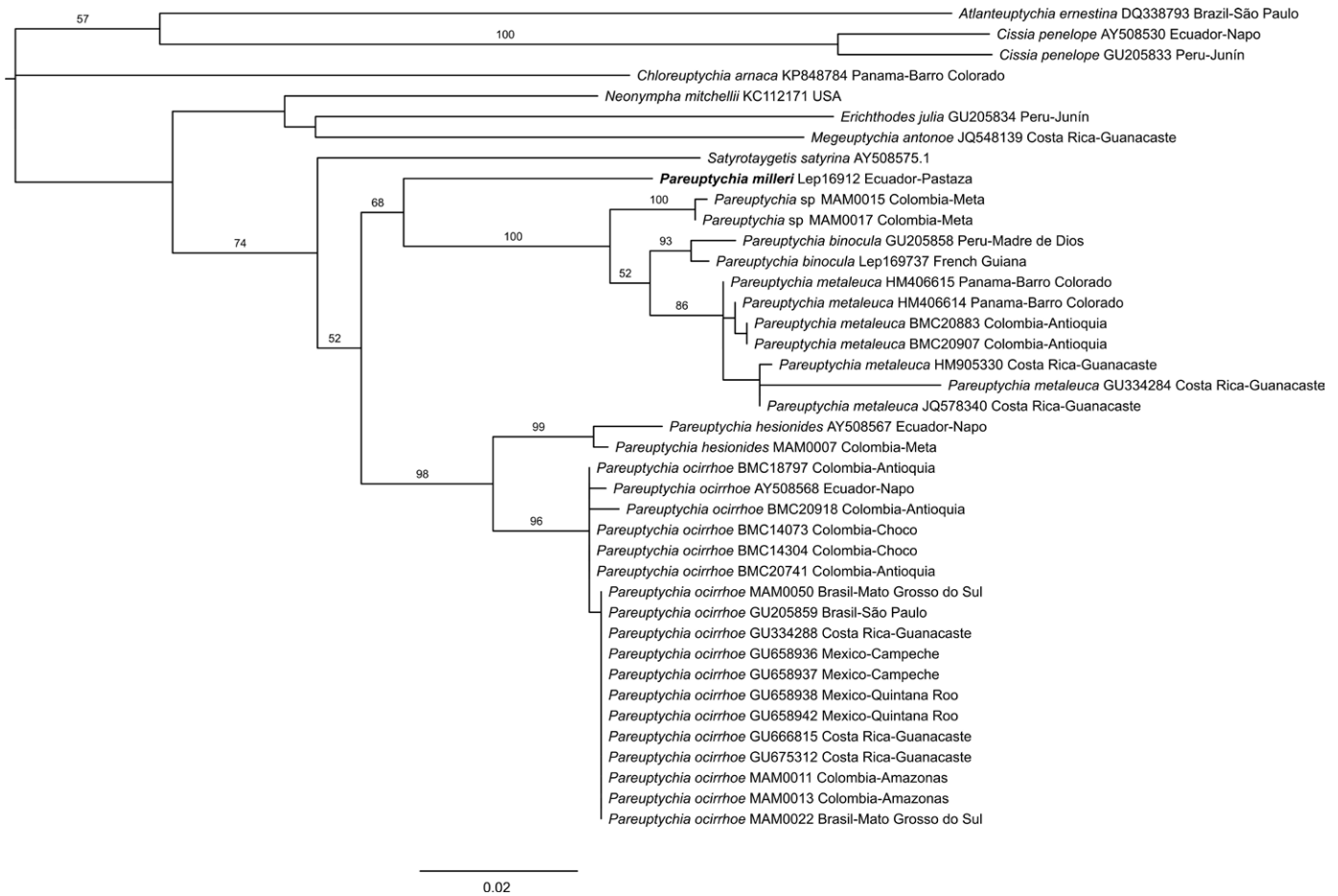


Fig. 1. Maximum likelihood tree based on COI barcode region. Bootstrap support values are provided for each node.

scales, a line of white scales laterally, one-third of second segment in length; antennae approximately two-fifths of forewing costa in length, with ca. 36 segments ($n=1$), pedicel about half as long as scape, flagellum lighter than scape and pedicel, with distal 13-15 segments composing club.

Thorax. Black scales scattered dorsally, elsewhere with brownish hair-like scales.

Legs. Foreleg (Fig. 4) tarsus and tibia about same length, femur slightly longer; tarsus and tibia of pterothoracic legs adorned with spines ventrally, pair of relatively short tibial spurs present at distal end of tibia.

Abdomen. (Fig. 3d, e): Eighth tergite membranous, anterior end sclerotized, posterior end weakly sclerotized; eighth sternite appears as two separated sclerotized regions on ventral side of eighth abdominal segment.

Male genitalia. (Fig. 3): Tegumen dorsal margin convex, ventral margin flattened, somewhat shield-shaped in lateral view; uncus relatively thick, slightly longer than tegumen in lateral view, two lumps present in lateral view, both dorsally convex, posterior lump bifid in dorsal view; brachia narrow, almost same as uncus in length, slightly arched upwards in lateral view, apex slightly hooked inwards in dorsal view; ventral arms of tegumen fused to posterior ventral margin of tegumen, middle section roughly straight; appendices angulares present; saccus almost same as uncus in length, dorsal arms of saccus combined with ventral arms of tegumen; valva wide at anterior side and tapers towards posterior end, ventral margin extending anteriorly beyond dorsal anterior margin by about same distance as dorso-ventral width of mid valva, middle section of ventral margin slightly convex in lateral view, middle section of dorsal margin slightly concave in lateral view, anterior one-fifth narrow, tapered towards end, slightly hooked,

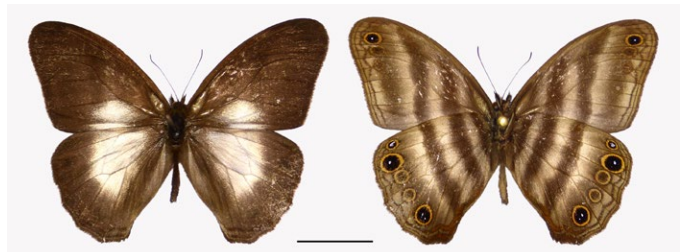


Fig. 2. Male holotype of *P. milleri*, left dorsal, right ventral. Scale bar = 10mm

curved inwards approximately 50 degrees in dorsal view; aedeagus relatively straight, slightly longer than tegumen plus uncus in length, two cornuti present.

Wing venation. (Fig. 4): Forewing recurrent vein absent, forewing subcostal vein swollen at base, forewing cubital vein with basal region inflated; hindwing humeral vein present.

Wing shape. (Figs. 2, 4): Forewing somewhat triangular, mid outer margin of forewing slightly concave; hindwing rounded, slightly concave between tornus and mid anal margin.

Wing pattern. (Fig. 2): Dorsal forewing ground colour brownish (greyish-brown in fresh specimens), distally slightly darker, especially apex; white discal patch cell Cu_2 and 2A with edges dusted with brown scales, reduced or nearly absent in some specimens, slightly translucent, thus subtly revealing ventral bands; dorsal hindwing ground colour same as forewing, distally slightly darker; white patch present in discal cell with edges dusted with brown scales, extending into basal areas of cells from veins Rs to Cu_2 and present also in some specimens as a dusting of white scales in cells Sc + R_1 , Cu_2 , and 2A). Ventral forewing

and hindwing pattern typical of *Pareuptychia* except ground colour somewhat melanic, thus basal, discal, postdiscal, submarginal and marginal brown bands darker than typical *Pareuptychia*, and white areas between these bands also melanic, dusted with brown scales.

FEMALE: Unknown.

Types: **HOLOTYPE** ♂: **ECUADOR:** *Napo:* Finca Alvarez, km 12 via Pto Napo-Ahuano [1°3'17.60"S, 77°40'39.76"W] Alt. 475 m 23.08.[20]10 No. 22236 D. Trembath/A. Neild// (DATR, to be deposited in MECN).

PARATYPES: **ECUADOR:** *Orellana:* 1♂: Rio Coca, 300 m. vii.1971 R. de Lafebre// A. C. Allyn Acc. 1971-41// Allyn Museum photo No. 09177514// Genitalia vial SN-15-089 S. Nakahara// (MGCL); *Napo:* 1♂: Rio Shinquipino 550 m.; xi.1973 R. de Lafebre// A. C. Allyn Acc. 1974-7// Genitalia vial SN-14-196 S. Nakahara// (MGCL); 1♂: Finca Alvarez, km 12 via Pto Napo-Ahuano [1° 3'17.60"S, 77°40'39.76"W] Alt. 475 m 23.08.[20]10 No. 22235 D. Trembath/A. Neild// (DATR); 1♂: El Capricho ridge 800 m Tena - Puyo Rd 13.9.2011 H. WARREN-GASH// (HAWA); *Pastaza:* 1♂: Yutsuntsa, Rio Yutsuntsa 2°21'4"S, 76°27'14"W 190-250 m. 11 Jul 2014 leg. S. Nakahara & S. Padrón (MGCL); *Morona-Santiago:* 2♂♂: Wuapula (approximately 5 km N of Tres Marias), 17-18 January 2016, 1360 m, -2.126813, -78.089803, leg. S. Nakahara (MGCL, one male to be deposited in BMNH). **PERU:** *Loreto:* 1♂: Rio Sucusari, 140 m Explorapmapo-ACEER 03°14'S, 72°55'W 12 Sept 1995 leg. J. Grados// (MUSM); *Rioja:* 1♂: bei Lydia Cr. (Stgr.)// genitalia vial M-9053♂ Lee D. Miller// *Euptychia* sp. ? (God[man]// Rioja Peru [18]87 Garl.// (ZMHU).

Additional data (sight records by A. Neild): **ECUADOR:** *Napo:* 2♂♂: stream located approximately 2 km NE of Apuya (Oliverio Velastegui's finca), 22 October 2015, 480 m, 1° 5'38.56"S, 77°46'35.49"W; 2♂♂: Apuya, just off main vehicular track, on east side of river bridge, 19 October 2015, 475 m, 1° 6'21.49"S, 77°47'28.73"W.

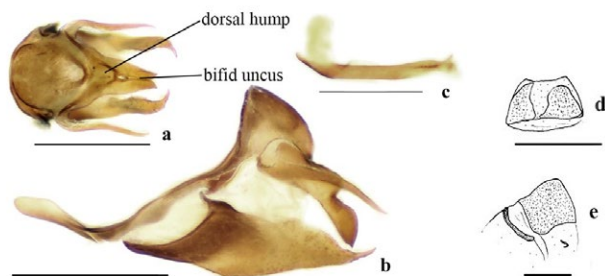


Fig. 3. Male genitalia of *P. milleri*: **a.** dorsal view of tegumen, brachia and uncus; **b.** lateral view; **c.** aedeagus; **d.** ventral view of eighth sternite; **e.** lateral view of seventh and eighth tergite (Genitalia vial: SN-15-089) Scale bar = 1 mm.

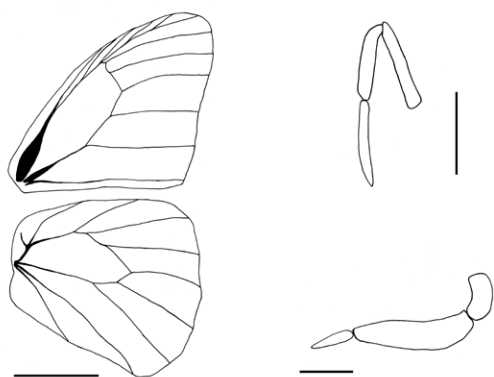


Fig. 4. Morphology of *P. milleri*: left wing venation (scale bar = 10 mm); top right male foreleg, bottom right labial palp (scale bars = 1 mm).

Etymology. The specific epithet is coined in honour of Lee D. Miller for his pioneering studies of Satyrinae, including the euptychiine butterflies. He also examined and dissected this species at the ZMHU, which perhaps represents the first collected specimen of this species.

Distribution (Fig. 5): This species is known from the northwestern Amazon basin in Ecuador and Peru.

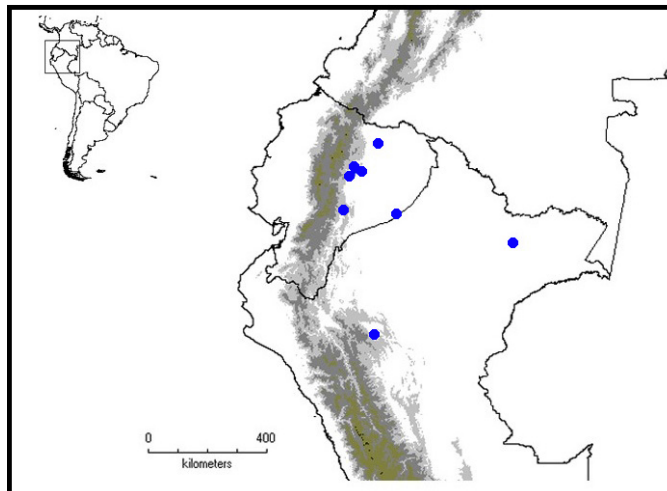


Fig. 5. Map showing collecting/observation localities for *P. milleri* (blue dots).

Biology (Figs. 6-8): All except one observation of this species in the field are from habitat along the banks of streams. AN encountered this species in eastern Ecuador (Napo province) in 2010, where his attention was drawn to an unusual white-patched brown butterfly patrolling from 3 to 7 m above the banks of a shallow and slow-flowing stream, about 4 m wide, and perching on leaves overhanging the water at about 7 m above the ground. His initial thoughts when observing it from a distance were that it might be a species of *Euselasia* Hübner, [1819] (Riodinidae), but he was surprised upon closer inspection to see that it appeared to be an aberrant specimen of *Pareuptychia*. However, a second specimen was collected soon after, also behaving in a manner most atypical for the genus (species of which generally fly in the forest understorey), and it then seemed more likely that this phenotype could represent an undescribed species. AN observed similar behaviour in nearly identical habitat in 2015 at two sites near Apuya (also in Napo province, Ecuador; see “Additional data” below the type series data for further details), along the steep banks of shallow, slow-flowing, and wide streams (approximately 3-4 m in width) in dense tropical lowland evergreen forest. On all of these occasions AN observed a perching male chase off an intruder of the same species. When perching (Fig. 6), males sit on leaf tips facing the stream, and remain stationary for many minutes, before flying off to occupy a different perch. AN observed patrolling behaviour, with males ranging up to 50 m from one perch to the next. These 2015 observations were made from 09:30 hrs to at least midday, after which time AN was unable to continue his observations. SN encountered this taxon in July, 2014 flying approximately 1 m above the ground, around thick



Fig. 6. Perching male of *P. milleri* beside the bridge over the Río Apuya (Napó, Ecuador).

vegetation along the Río Yutsuntsa, near the Achuar community of Yutsuntsa in southeastern Ecuador. SN made additional observations of two males of this species in January 2016 in Morona-Santiago province, Ecuador, where individuals were found perching about 3 m above ground along a narrow stream. These 2016 observations were both made around 10:30-11:00 hrs on two consecutive days in cloud forest at 1360 m, the latter in contrast to the majority of records which are from lowland tropical evergreen forest from about 140-800 m elevation.

DISCUSSION

This species is placed in the genus *Pareuptychia* based on the presence of a bifid uncus with a dorsal hump (see Fig. 3a); both characters are likely synapomorphies for *Pareuptychia* (pers. obs.). Forster (1964) observed that *Pareuptychia* is a well-defined genus differentiated from other Euptychiina by the peculiar shape of the uncus. Although he did not define this character, it is likely that he was referring to at least one of these male genitalic characters. *Pareuptychia lydia* (Cramer, 1777) does not possess these characters, but the historical inclusion of this taxon in *Pareuptychia* is unsubstantiated and its generic placement requires further study.

Despite the fact that many euptychiine genera were recovered as polyphyletic or paraphyletic, Peña *et al.* (2010) recovered *Pareuptychia* as a monophyletic group, with four species included in that analysis. In the same paper, *Pareuptychia* was recovered as sister to [*Megeuptychia* Forster, 1964 + *Neonympha* Hübner, 1818], and given that these two likely synapomorphies are not present in *Megeuptychia* and *Neonympha* species (Willmott & Hall, 1995; Benmesbah, 2015; pers. obs.), *Pareuptychia* is apparently one of the best defined euptychiine genera.

A principal reason for conducting the molecular analysis was to exclude the possibility that the melanic phenotype of *P. milleri* represents an aberrant form of a described *Pareuptychia* species. Although the male genitalia seem to be informative, we were unable to examine these for all *Pareuptychia* species,



Fig. 7. Typical riverine habitat of male *P. milleri* (Finca Álvarez, Napó, Ecuador).



Fig. 8. Typical riverine habitat of male *P. milleri* (near Apuya, Napo, Ecuador).

and due to the relative similarity of genitalia within the genus, we considered the molecular analysis to be especially useful for species-level diagnoses. The ML tree (Fig. 1) dispelled the possibility of *P. milleri* representing aberrant individuals of another species, instead supporting *P. milleri* as a distinct species. However, the placement of this taxon as sister to the clade [*P. metaleuca* + *P. binocula* + *Pareuptychia* sp.] has only moderate support (bs = 68), even though this clade itself is highly supported (bs = 100). The other well-supported clade (bs = 98) consists of *P. ocirrhoe* and *P. hesionides*. Although the systematic placement of *P. milleri* remains to be resolved, the existence of two well-supported clades within *Pareuptychia* indicate its distinct specific status, and it is possible *P. milleri* will prove not to belong in either.

The male of *P. milleri* appears to be uncommon based on the small number of specimens known to us. However, AN encountered four males near Apuya, Napo, Ecuador within a four day period (see ‘Additional data’), and SN encountered two males within a two-day period in Morona-Santiago, Ecuador (see ‘Types’), suggesting (in the absence of evidence of seasonality) that it may be a relatively common species that is overlooked because of its restricted habitat which is not often frequented by collectors. The female of *P. milleri* is not known to us, perhaps due to its greater rarity in the field than the male sex. However, there is also the possibility that the female is very similar to females of one of the known *Pareuptychia* species, and thus has been overlooked by lepidopterists.

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