

# A DISTINCTIVE NEW SUBSPECIES OF *CATASTICTA POUJADEI* FROM THE CORDILLERA DEL CÓNDROR IN EASTERN ECUADOR (LEPIDOPTERA: PIERIDAE: PIERINAE)

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**Abstract-** A new subspecies, *Catasticta poujadei condor* Radford & Willmott, **n. ssp.**, is described from the isolated Cordillera del Cóndror in Ecuador. Despite marked differences in size and wing pattern in comparison with the nominate subspecies, a 636 bp sequence from the mitochondrial gene *Cytochrome Oxidase I* (COI), also known as the “barcode” region for animals, showed little difference in comparison with *C. poujadei poujadei* from the adjacent main Andean cordillera. Coupled with a study of geographic variation within the *C. poujadei* from the Peruvian Andes, these data suggest that the new taxon is best treated as a subspecies. The taxon is currently known only from the type locality and its distinctiveness suggests that further endemic butterfly taxa remain to be discovered in the Cordillera del Cóndror.

**Resumen-** Se describe una nueva subespecie, *Catasticta poujadei condor* Radford y Willmott, **n. ssp.**, de la aislada Cordillera del Cóndror en Ecuador. A pesar de marcadas diferencias en el tamaño y patrón de las alas en comparación con la subespecie nominal, una secuencia de 636 bp del gen mitocondrial *citocromo oxidasa I* (COI), también conocida como la región de “código de barras” para los animales, mostró escasa diferencia en comparación con *C. poujadei poujadei* de la vecina Cordillera Andina. Junto a un estudio de la variación geográfica de *C. poujadei* de los Andes peruanos, estos datos sugieren que el nuevo taxón es mejor tratado como una subespecie. Este taxón se conoce solamente de la localidad tipo y es tan distinto que parece probable que otros taxones endémicos de mariposas serán descubiertos en la Cordillera del Cóndror.

## INTRODUCTION

The pierine genus *Catasticta* Butler, 1870 currently contains 97 recognized species (Lamas, 2004; Bollino & Costa, 2007; Bollino, 2008; Bollino & Boyer, 2008) distributed in montane areas throughout the neotropical region. It is particularly diverse at elevations from 1500-2000m in the eastern Andes and approximately 50 species have been recorded from Ecuador alone (Willmott & Hall, unpublished data). However, despite a number of previous studies (e.g., Reissinger, 1972; Racheli, 1996) and extensive material housed in various public collections, its taxonomy is not completely understood (Bollino, 2008). In particular, *Catasticta* species often show geographic variation, have few or no genitalic characters of use in species delimitation, and may have extremely similar wing patterns, complicating the taxonomic association of allopatric taxa. Fortunately, DNA sequence data now provide a new set of characters for use in Lepidoptera species taxonomy in situations where more traditional characters are not conclusive (e.g. Brower, 1996; Prudic *et al.*, 2008; Nazari *et al.*, 2010, 2011; Bonebrake *et al.*, 2011; Sourakov & Zakharov, 2011), as we illustrate here. A further problem is the lack of knowledge of the distribution of many high elevation species, which often occur in restricted elevational bands. Recent efforts to find and intensively sample hilltop localities where males, and occasionally females, congregate have revealed cases of unrecognized sibling species (e.g., Bollino & Boyer, 2008; Willmott, unpublished data), in addition to resulting in the discovery of new taxa (Lamas & Bollino, 2004), as in the case of this paper.

The new subspecies described herein was discovered in the Ecuadorian Cordillera del Cóndror during the recent Cambridge University Lepidoptera Expedition to the ‘Tercera Cordillera’ (hereafter, CULEPEX), led by the first author. This partially isolated chain of mountains to the east of the Andes lies within the most diverse of the Earth’s ‘biodiversity hotspots’, the Tropical Andes (Myers *et al.*, 2000). Within it, the

Cordillera del Cóndror stands out as having “the richest flora of any area this size in the New World” (Schulenberg & Awbrey, 1997). Distinctive flat-topped ‘tepui’s indicate the unusual sedimentary geology of the Cordillera, and these host numerous plants not found in the neighbouring basaltic Andes, including a number of disjunct taxa previously thought to be endemic to the Guiana Shield, hundreds of kilometres to the north (Neill, 2007). Large areas of primary habitat do remain intact, particularly at higher elevations, in part due to landmines laid during the series of border conflicts between Ecuador and Perú, the most recent of which occurred in 1995. Although recent successes in establishing protected areas in both Ecuador and Perú are promising (Guayasamin & Bonaccorso, 2011; Alcalde *et al.*, 2005), there is increasing pressure in the Cordillera del Cóndror from deforestation, grazing and mineral exploitation, making it a priority for scientific investigation. It should also be noted that none of the Cordillera del Cóndror is formally protected above 2500m in Ecuador, despite evidence that it represents a globally unique habitat (Schulenberg & Awbrey, 1997). A number of new butterfly taxa were discovered during expeditions to the Peruvian part of the Cordillera in 1987 and 1994, and during an ornithological expedition to the Ecuadorian side in 1993 (Lamas, 1997), but since those expeditions, no further concerted attempts have been made to sample the butterfly fauna of the region. In addition, the butterfly fauna of the Cordillera del Cóndror occurring above 2500m has remained virtually unexplored, so that surveying these habitats was an important goal of CULEPEX. This paper thus represents the first in a series describing the fauna and new taxa encountered during CULEPEX.

## METHODS

Field surveys were conducted at two main sites in the Cordillera del Cóndror in August and September 2010. A maximized sampling strategy (Brown, 1972) was used by expedition members to sample as large an area and as broad a diversity

of habitats and taxa as possible. For more details about the expedition, see Radford *et al.* (2012). Voucher specimens were collected using a standard butterfly net and extendable handle (up to 3m) and stored in glassine envelopes preserved in boxes containing silica gel.

A large number of collections in Europe, USA and South America have been visited by the second author during research for a book on the butterflies of Ecuador (Willmott & Hall, in preparation), to examine types, record distribution data and study geographic variation. The following acronyms and abbreviations are used in the text: FLMNH: McGuire Center for Lepidoptera at the Florida Museum of Natural History, Gainesville, FL, USA; MECN: Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador; DFW: Dorsal forewing; VFW: Ventral forewing; DHW: Dorsal hindwing; VHW: Ventral hindwing.

We used the synonymic checklist of Lamas (2004) to identify relevant described names, and examined associated type specimens in museums, in Abadjiev (2006) and via the Butterflies of America website ([www.butterfliesofamerica.com](http://www.butterfliesofamerica.com)). Dissections were made using standard techniques, male abdomens being soaked in hot 10% KOH solution for approximately 15 mins and subsequently stored in glycerol. Drawings were made using a Leica MZ12.5 stereomicroscope at 50x magnification and a camera lucida. Morphological terms for genitalia largely follow Klots (1956), and terminology for wing venation follows the Comstock & Needham (1918) system (wing cells are referred to by their bounding veins).

We extracted genomic DNA from two legs removed from dried, papered specimens of the new taxon and related taxa using Qiagen's DNeasy Blood & Tissue Kit following the manufacturer's protocol, incubating samples overnight (20-24hr) and using a final elution volume of 100ul. We amplified the first half of the mitochondrial gene *cytochrome oxidase I* (COI), also known as the barcode region for animals (Hebert *et al.*, 2003), using the primers LepF1 (forward, ATTCAACCAATCATAAAGATAT) and LepR1 (reverse, TAAACTTCTGGATGTCCAAAAA) (Hebert *et al.*, 2004), or LCO (forward, GGTCAACAAATCATAAAGATATTGG) and HCO (reverse, TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al.*, 1994). All PCR reactions were conducted in a 20ul volume comprising 2ul DNA, 0.8ul MgCl<sub>2</sub> (50mM), 13.4 ddH<sub>2</sub>O, 2ul buffer (10X), 0.8ul dNTPs (10mM), 0.4ul of each primer (10uM), and 0.2 ul Platinum® Taq DNA Polymerase (5U/ul). 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 35 cycles of 30 s at 94°C, 40 s at 51°C, 1 min at 72°C, followed by 5 min at 72°C. Single strands of PCR products were sequenced by University of Florida's Interdisciplinary Center for Biotechnology Research Sanger Sequencing Group using the same primers as in the PCR. Sequences were aligned using BioEdit v. 7.1.3 (Hall, 1999) and by eye. We conducted both a neighbor-joining (NJ) analysis and a maximum parsimony (MP) analysis on the DNA sequence data. Despite criticism of NJ analyses (Goldstein & DeSalle, 2011), they remain a simple way to graphically

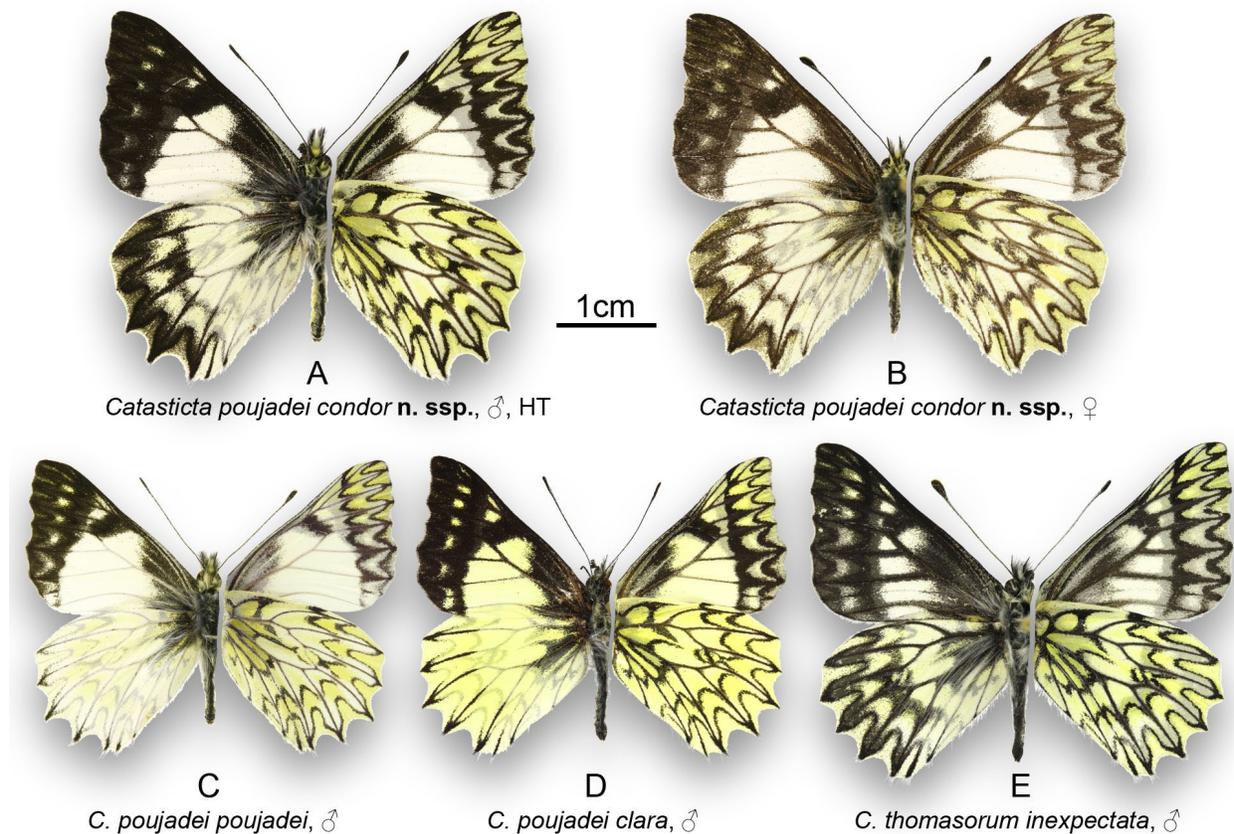


Fig. 1. Adults of the new taxon, *C. poujadei* and *C. thomasorum*. A) *Catasticta poujadei condor* n. ssp., PAN128; B) *Catasticta poujadei condor* n. ssp., PAN190; C) *Catasticta poujadei poujadei*, Ecuador, FLMNH; D) *Catasticta poujadei clara*, Peru, FLMNH; E) *Catasticta thomasorum thomasorum* FLMNH-145430, Ecuador, FLMNH.

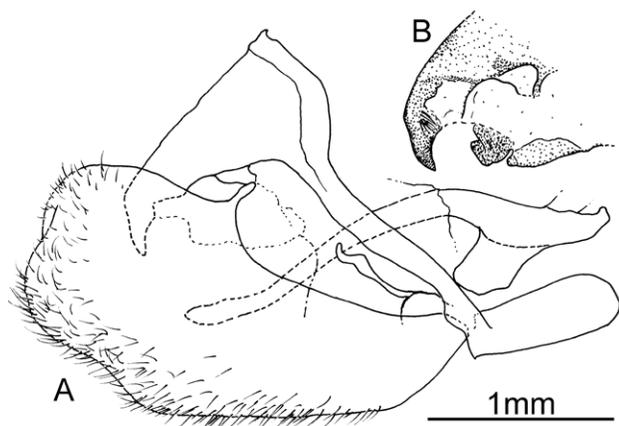


Fig. 2. *Catasticta poujadei condor*, n. ssp., male genitalia (KW-12-115). A) lateral view; B) lateral view uncus, tegumen and gnathos with valva removed.

represent similarity among taxa and hence decide the most appropriate taxonomic rank, still a necessarily subjective step in almost all studies. MP analysis complements NJ by allowing the identification of putative nucleotide autapomorphies for species, which may be tested by inclusion of additional individuals. All analyses were conducted using MEGA 5.05 (Tamura *et al.*, 2011), with the following options selected: for NJ, we used the Kimura 2-parameter substitution model with pairwise deletion of sites with missing data, and other default settings, while for MP analysis we used the Close-Neighbor-Interchange heuristic search with 100 random addition starting trees, evaluating branch support with 100 bootstrap replicates. Trees were rooted with *Catasticta semiramis semiramis* (Lucas, 1852). All new sequences are deposited in GenBank.

## RESULTS AND DISCUSSION

### Relationships among *Catasticta* taxa

The final aligned sequences were 636 bp in length, of which, within the ingroup, 62 were variable and 59 parsimony informative. The MP analysis found a single shortest tree of length 158 steps, which recovered the same clades (*C. p. poujadei* (Dognin, 1887), *C. p. condor* Radford & Willmott, n. ssp. and *C. thomasorum* Jasinski, 1998) as the NJ tree (Fig. 4).

### *Catasticta poujadei condor* Radford & Willmott, new subspecies; Figs. 1A,B, 2, 3, 4

**Diagnosis and identification:** *Catasticta poujadei condor* n. ssp. (Fig. 1A,B) does not closely resemble any other *C. poujadei* taxa (e.g., Fig. 1C,D), nor indeed the probably closely related *C. thomasorum* (Fig. 1E). In size, *C. p. condor* is the largest subspecies, with a mean FW length of 25.0 mm (n=4, 24-26 mm) compared with 20.8 mm (n=14, 20.5-22.5 mm) for Ecuadorian *C. p. poujadei* (Fig. 1C). The DFW is most similar to *C. p. poujadei*, but the distal edge of the white discal band is straight, rather than convex, and overall it is narrower, with the white submarginal spots less pronounced in *C. p. condor*. The DHW, however, is very distinct from *C. p. poujadei*, with black postdiscal markings in M3-M1 and a broad black distal marginal band, thus more closely resembling *C. poujadei eximia* Röber, 1909, although the pale markings of *C. p. condor* are a lighter creamy-white than the yellow of *C. p. eximia*. The combination of a broad, anteriorly tapering white DFW discal band, with the broad black margin of the DHW, distinguishes *C. p. condor* from all four subspecies of *C. poujadei* and both of *C. thomasorum*.

### Description:

**MALE** (Fig. 1A): Forewing length 25 mm.

**Wing shape:** Wings triangular; dentate FW apex with indentation at subapical margin in cells Cu1-M2; HW distal margin increasingly scalloped at vein ends from apex to tornus.

**Dorsal surface:** Forewing ground colour black with broad white band extending from discal and postdiscal anal margin to posterior half of discal cell and base of cell Cu1-M3; basal edge of white band extends furthest basally in cells 2A-Cu2 and discal cell, and is suffused with black scales; black veins clearly visible across white band; complete series of small white submarginal spots suffused with black scales, spot in cell M1-R5 is elongate, not circular; postdiscal white spot at costa in cell M2-M1 and white subcostal streak in cell R4-R3; costal margin has diffuse white scales from base to two-thirds distance to discal cell end. Hindwing ground colour black with broad, whitish-cream discal band covering middle of wing, extending from anal margin to costa to leave black in basal half of discal cell and Rs-Sc+R1; black postdiscal scaling in cells Cu2-M3, forming a broad black square in cell M3-M2 and narrower curved dash in cell M2-M1, separates discal band from submarginal band of whitish-cream blocks, these almost fused with discal band towards costa and anal margin; broad black marginal border except for cream triangular marginal internervular markings suffused with black scales, bordered by a solid black submarginal line; fringe white. **Ventral surface:** In general pattern similar to nominate subspecies, except black markings all more pronounced. Forewing pale yellow submarginal markings notably chevron-shaped rather than almost triangular as in *C. p. poujadei*. Darker medial postdiscal region where black markings on dorsal surface show through. Black streak protruding upwards into discal cell almost connects to semi-circular black mark at distal end of cell, whereas there is a clear gap in all other subspecies of both *C. poujadei* and *C. thomasorum*. **Head:** Eyes chestnut; labial palpi cream with long cream and black hairs protruding ventrally; antennae black. **Body:** Thorax black covered with tufts of black and cream hairs dorsally and ventrally; abdomen black dorsally with fine white hairs on upper half, creamy-white ventrally with no hairs; legs

Table 1. Voucher information for *Catasticta* DNA sequence data.

Taxon	Locality	DNA voucher number	GenBank number
<i>C. semiramis semiramis</i>	Colombia: Cundinamarca: P.N. Chingaza	LEP-04031	KF753881
<i>C. poujadei condor</i> n. ssp.	Ecuador: Zamora-Chinchi: Destacamento Paquisha Alto (-3.898, -78.484)	LEP-03879	KF753895
<i>C. poujadei condor</i> n. ssp.	Ecuador: Zamora-Chinchi: Destacamento Paquisha Alto (-3.897, -78.48)	LEP-03880	KF753894
<i>C. poujadei condor</i> n. ssp.	Ecuador: Zamora-Chinchi: Destacamento Paquisha Alto (-3.898, -78.484)	LEP-03881	KF753893
<i>C. poujadei condor</i> n. ssp.	Ecuador: Zamora-Chinchi: Destacamento Paquisha Alto (-3.898, -78.484)	LEP-03882	KF753892
<i>C. poujadei condor</i> n. ssp.	Ecuador: Zamora-Chinchi: Destacamento Paquisha Alto (-3.898, -78.484)	LEP-03883	KF753896
<i>C. poujadei poujadei</i>	Ecuador: Zamora-Chinchi: San Francisco, casa de Arcoiris (-3.988, -79.095)	LEP-00207	KF753890
<i>C. poujadei poujadei</i>	Ecuador: Zamora-Chinchi: San Francisco, casa de Arcoiris (-3.988, -79.095)	LEP-00208	KF753891
<i>C. poujadei poujadei</i>	Ecuador: Loja: Cajanuma (-4.117, -79.172)	LEP-00209	KF753888
<i>C. poujadei poujadei</i>	Ecuador: Azuay: Ñuñurco (-2.759, -78.67)	LEP-00315	KF753887
<i>C. poujadei poujadei</i>	Ecuador: Azuay: Ñuñurco (-2.759, -78.67)	LEP-00316	KF753889
<i>C. poujadei poujadei</i>	Ecuador: Azuay: Ñuñurco (-2.759, -78.67)	KW-071009-72	KF753885
<i>C. poujadei poujadei</i>	Ecuador: Azuay: Ñuñurco (-2.759, -78.67)	KW-071009-73	KF753886
<i>C. thomasorum inexpectata</i>	Ecuador: Azuay: km 20 Gima-Gualaquiza rd. (-3.271, -78.902)	LEP-03877	KF753882
<i>C. thomasorum inexpectata</i>	Ecuador: Azuay: Maylas, km 16 Gualaceo-Limón rd. (-2.958, -78.7)	LEP-00317	KF753883
<i>C. thomasorum thomasorum</i>	Ecuador: Loja: Hito Quingo (-4.724, -79.437)	LEP-03878	KF753884

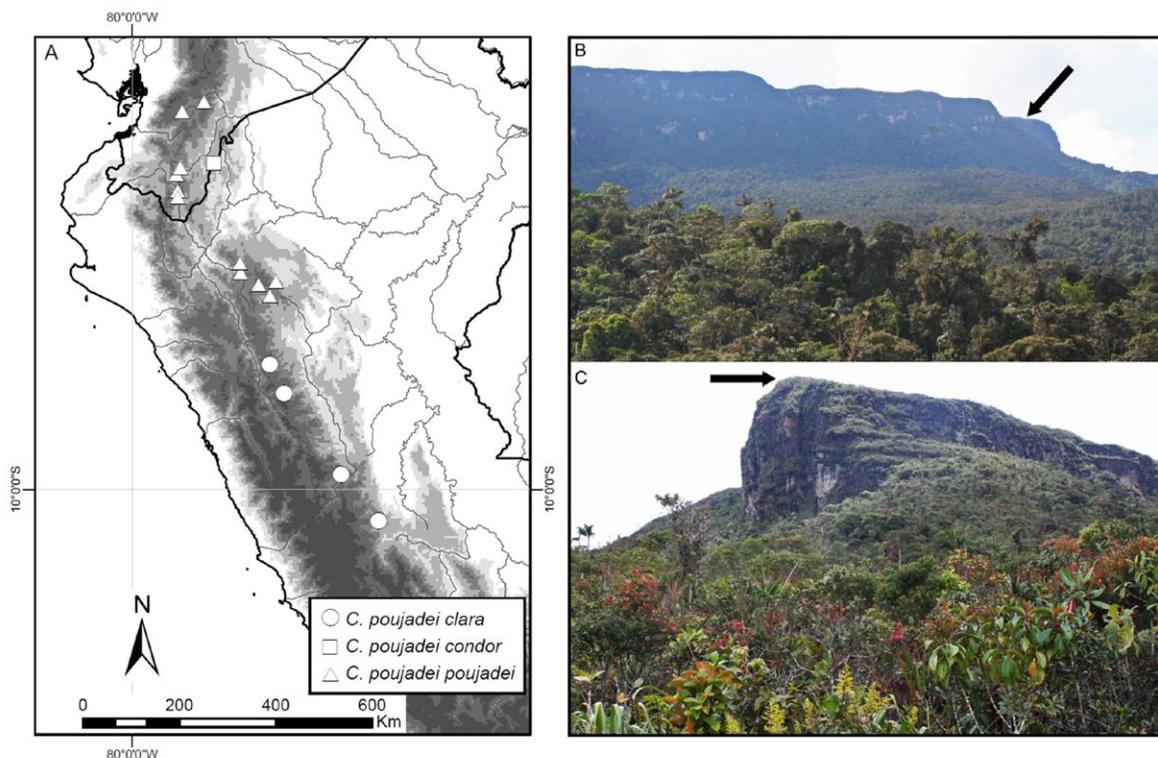


Fig. 3. A) Distribution of *C. poujadei* subspecies. B) Type locality of *C. p. condor* viewed from northeast, from road approaching Destacamento Paquisha Alto. C) Hilltop where *C. p. condor* was collected, seen from the trail at the top of the plateau.

black. *Genitalia* (Fig. 2A,B): saccus short, broad, similar in length to tegumen; tegumen also short, broad, uncus short and curved ventrally in middle, ending in a blunt point, with small tuft of setae laterally in middle; gnathos weakly sclerotized with slight bumps on surface (Fig. 2B); valva large, straight ventral edge, broad rounded distal tip, without ornamentation; aedeagus narrow, curved ventrally, with ventral keel-like process in middle of anterior portion; juxta tall, narrow and slightly undulate in lateral view.

**FEMALE** (Fig. 1B): Forewing length 26 mm. Externally similar in all respects to male, except wings slightly broader, dorsal dark markings slightly paler, ventral yellow markings slightly less intense. *Genitalia*: not examined.

**Types**: HOLOTYPE ♂: **ECUADOR**: *Zamora-Chinchipe*: Destacamento Paquisha Alto, [3°53'50"S, 78°28'49"W], 2425m, (Jamie Radford), 4 September 2010, [PAN128], (FLMNH, to be deposited in MECN) (CULEPEX Expedition, 2010).

PARATYPES (3 ♂, 1 ♀): **ECUADOR**: *Zamora-Chinchipe*: Destacamento Paquisha Alto, [3°53'53"S, 78°29'2"W], 2444m, (Jamie Radford), 7 September 2010, 1 ♂ [PAN191], 1 ♂ [PAN192], 1 ♀ [PAN190], 8 September 2010, 1 ♂ [PAN212], (FLMNH) (CULEPEX Expedition, 2010).

**Etymology**: This subspecies is named after the Cordillera del Cóndor where it was discovered, an incredibly biodiverse mountain range spanning Ecuador and Peru, which is coming under increasing threat from mineral exploitation (see Discussion).

**Natural history and distribution**: *Catasticta poujadei condor* is currently known from a single flat-topped mountain or 'tepui' in the Cordillera del Cóndor, southeastern Ecuador (Fig. 3A-C). This tepui, also known as Cerro Machinaza in Peru (Schulenberg & Awbrey, 1997), was accessed from the Ecuadorian military base Destacamento Paquisha Alto, near the village of El Blanco. No other collections have been made to date in this mountain range at the elevation where the new subspecies was collected. All male individuals were collected 'hilltopping' at one of the two highest points on the plateau, a 'hito' (border marker) on the main path across the tepuis and the exposed bluff shown in Fig. 3C.

## DISCUSSION

At first glance, this new taxon appeared to be phenotypically intermediate between *C. poujadei poujadei* and *C. thomassorum*, and we initially considered it likely to represent a distinct, allopatric species. However, for a number of reasons we believe subspecies status is the most appropriate until further data suggest otherwise, including perhaps the collection of typical *C. p. poujadei* in the Cordillera del Cóndor. Despite the marked differences in size and wing pattern between *C. p. condor* and *C. p. poujadei*, which seem surprising given the proximity of *C. p. condor* to *C. p. poujadei* in the adjacent Andes (nearest known localities c. 70 km apart) and lack of differentiation in other butterfly taxa with which they occur, the molecular data strongly support subspecies status. Out of the 636 bp COI sequence obtained, 59 sites were parsimony informative, but just a single nucleotide (G, rather than A, at position 11935 from alignment with *Bombyx mori* (Linnaeus, 1758) mitochondrion genome, NCBI Reference Sequence NC\_002355.1) was an apparent autapomorphy for *C. p. condor*. By contrast, specimens of *C. p. poujadei* from Loja and Zamora-Chinchipe differed at 4 positions in comparison with those from Azuay. The estimated evolutionary divergence (using the Kimura 2-parameter model) between northern and southern Ecuadorian *C. poujadei poujadei* was 0.011, while that between *C. p. condor* and *C. p. poujadei* was 0.009. Thus, there was greater genetic divergence between phenotypically indistinguishable specimens of *C. poujadei* in the Andes than between *C. p. poujadei* and *C. p. condor*. *Catasticta. p. condor* and *C. p. poujadei* also share the inclined distal edge of the white DFW discal band and are ecologically similar; *C. p. poujadei* occurs at the same elevation in the Andes as *C. p. condor* and can similarly be found hilltopping on hills covered

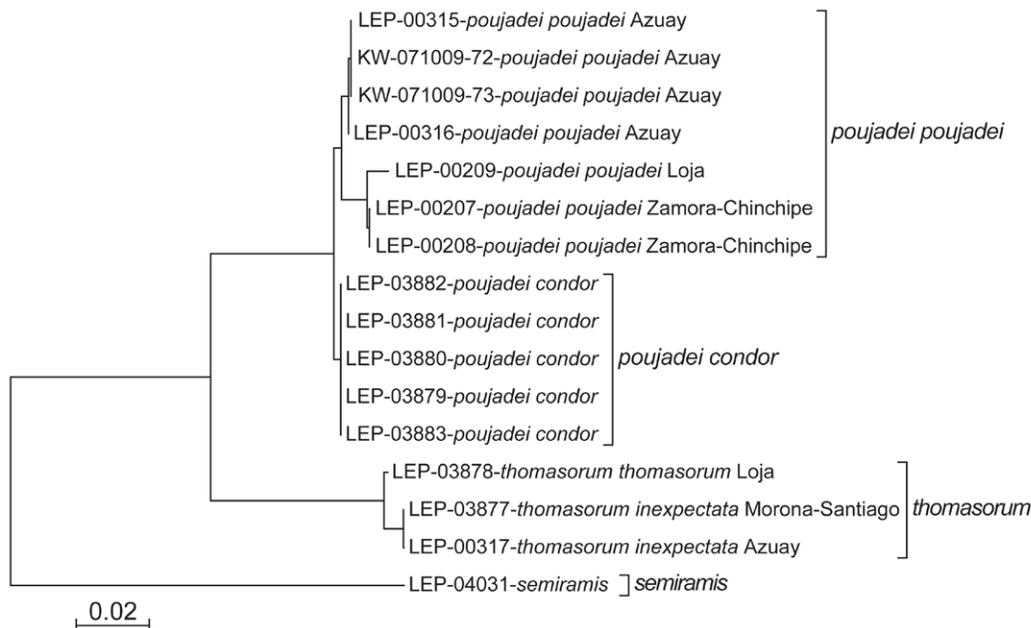


Fig. 4. Neighbor-joining tree (Kimura 2-parameter) for *Catasticta poujadei* and *C. thomasorum* based on 636 bp of COI (barcode region).

with low vegetation. Finally, an apparently undescribed taxon from central Peru (P. Boyer, pers. comm.) is similar to *C. p. condor* in size (FW length 25mm) and approaches it in wing pattern, having more extensive black dorsally than nominate *C. p. poujadei*. This Peruvian taxon seems very likely to be a subspecies of *C. poujadei*, thus supporting treatment of *C. p. condor* as a subspecies.

The high-altitude plateaus of the Cordillera del Cóndor are a globally-unique, well preserved and highly diverse group of habitats that should be studied as a matter of urgency. In addition to the new taxon described here, CULEPEX discovered a further seven possible new butterfly taxa and recent work has yielded further botanical discoveries (Neill, 2007), new species of frogs (Terán & Guayasamín, 2010) and the identification of 14 globally threatened or near-threatened bird species (Freile & Santander, 2005). Despite these discoveries, the vast majority of the Cordillera del Cóndor has no formal protection and is coming under increasing pressure from mineral exploitation since its identification as “the most prospective & under-developed Copper-Gold belt” in the world (Dorato Resources Inc, 2010), and the renewal of mining activities in the area following approval of Ecuador’s Mining Law in 2009. Work is ongoing to establish the Abiseo-Cóndor-Kutukú Conservation Corridor (CCACK), stretching across approximately 13 million hectares from Sangay National Park in Ecuador to the Cordillera Azul National Park in Perú. Key achievements include the creation of the Parque Nacional Ichigkat Muja – Cordillera del Cóndor in 2007 which covers almost 90,000 ha, and the Concesión de Conservación Alto Huayabamba protecting 143,000 ha (ITTO *et al.*, 2009), both on the Peruvian side of the range. However, there is limited protection of the Cordillera del Cóndor within Ecuador, and most of the highest parts of the range with the greatest potential endemism which have been included in many of these protected areas also overlap with existing mining concessions (Acción Ecológica, 2013).

The current status of knowledge of the Lepidoptera fauna of the higher elevations of the Cordillera del Cóndor is

limited, consisting of T. A. Parker’s collection on the Ecuadorian side during an ornithological expedition in 1993, Conservation International’s RAP7 expedition to the Peruvian side in 1994 (Lamas, 1997), a single day’s collection made by the second author in 2009, several field trips by S. Padrón (pers. comm.) to a small area over the last five years, and CULEPEX. A total of 107 butterfly species were recorded for the first time in the Cordillera del Cóndor by CULEPEX, including 14 species previously recorded from three or fewer sites in Ecuador, taking the total number of Lepidoptera species recorded in the Cordillera del Cóndor to 539. This undoubtedly represents only a small fraction of its true diversity and further studies are recommended to support efforts to conserve this unique environment.

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