



Discovery of a remarkable new species of *Lymanopoda* (Lepidoptera: Nymphalidae: Satyrinae) and considerations of its phylogenetic position: An integrative taxonomic approach

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Abstract. A new species of *Lymanopoda* Westwood, a cloud forest Neotropical genus of Satyrinae, is described from the páramo grasslands on an isolated, peripheral massif in the Colombian Central Cordillera of the Andes: *L. flammigera* Pyrcz, Prieto & Boyer, sp. n. The genus *Lymanopoda* is species-rich (approx. 65 species) and its alpha taxonomy is relatively well researched. Relationships within the genus using molecular data have also been explored. The new species is outstanding for its golden yellow colour in males, not found in any other neotropical Satyrinae. Cladograms were constructed based on COI sequences of 47 species of *Lymanopoda* (~70% of the known species) including 17 from Colombia. The new species segregates in the “tolima” clade, which comprises four other high altitude Colombian species, as well as two from Ecuador. However, it is the comparative analysis of male genitalia, in particular the superuncus and valvae, which identified its closest relatives, thus confirming that genital characters can help refine molecular phylogenies. In addition to identifying species using mitochondrial DNA (mtDNA barcodes), nucleotide sites with unique fixed states used to identify nine species of *Lymanopoda* from Colombia are also presented.

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INTRODUCTION

Colour patterns of butterfly wings are among the most outstanding expressions of evolution. Under certain conditions they are extremely plastic and even dramatic changes can be controlled by simple genetic mechanisms and quickly respond to selective pressure, for example in *Heliconius* (Kronforst & Papa, 2015). Colours have many adaptive roles, commonly in intraspecific sexual communication, warning, mimetic relations or, very frequently, crypsis. In different groups of butterflies different roles dominate. In the cosmopolitan subfamily Satyrinae, the prevailing adaptation is camouflage, and its over 2500 species are overwhelmingly dark with shadows of brown, thus their common English name (browns), with some elements enhancing their cryptic colouration, such as stripes and patches imitating the substrate, in most cases on the

undersides of their wings. In a few genera, such as *Elymnias* Hübner, 1818 and *Elymniopsis* Fruhstorfer, 1907, showy colours are, however, dominant, which is explained by their involvement in Batesian mimicry rings (Mallet & Joron, 1999). Among the few Satyrinae genera with species bearing conspicuous wing patches is the neotropical montane *Lymanopoda* Westwood. This genus can be considered as one of the best known among South American Satyrinae and there are a number of papers published, especially in the last two decades, on their taxonomy and distribution (Pyrcz, 1999, 2003, 2004, 2005, 2012; Pyrcz & Boyer, 2011; Pyrcz & Rodríguez, 2006; Pyrcz et al., 1999, 2009a,b, 2010, 2016), phylogenetics (Casner & Pyrcz, 2010; Marín et al., 2016), ecology (Pyrcz & Wojtusiak, 2002; Pyrcz & Garlac, 2012) and biology (Montero & Ortíz, 2012). Many of the more than 60 species of

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Lymanopoda have conspicuous white, blue, and reddish ground colours, which may be marked with white or green patches. The underlying evolutionary rationale for this is still unknown but there is growing evidence that some kind of mimicry is involved (Pyrce, in prep.). Yet, even in *Lymanopoda* the discovery of a species with shiny golden yellow males was extremely surprising as this kind of colouration is not only unique for the genus but also among all worldwide Satyrinae. Here we investigate its affinities within the genus *Lymanopoda* and address some questions about the adaptive role of its colour pattern.

MATERIAL AND METHODS

Morphological studies

Most of the material used in this study was obtained during field-work by C. Prieto and P. Boyer in Colombia. Specimens used for morphological studies were examined in the Nature Education Centre (formerly Zoological Museum) of the Jagiellonian University in Kraków (CEP-MZUJ). Types and additional specimens were examined in major public museums including Instituto de Ciencias Naturales de la Universidad Nacional, Bogotá, Colombia (ICN), the Natural History Museum, London, UK (NHMUK), Museo de Agronomía de la Universidad Central, Maracay, Venezuela (MIZA), Staatliches Museum für Tierkunde, Dresden, Germany (MTD) and Zoologische Museum, Humboldt Universität, Berlin, Germany (ZMHB), as well as in the collections of Pierre Boyer (PB) and Carlos Prieto (RCCP).

The terminal parts of the abdomens (including the genitalia) were removed from the specimens and soaked in 10% KOH solution for 5–10 min. Subsequently, abdomens were preliminarily cleaned using soft tissue in water in order to expose genital parts. Water was removed from dissected genitalia using 90% and 95% solutions of ethanol. A Nikon digital camera DS-Fi1 and an Olympus SZX9 stereomicroscope were used for taking pictures of the dissections, which were then processed in Adobe Photoshop 7.0 CE and Corel PHOTO-PAINT X3 programs to enhance focus and improve quality. The dissected genitalia were kept in glycerol in vials pinned under the corresponding specimens. Genital terminology largely follows Klots (1956). Adults were photographed using a Minolta E-500 digital camera. Colour plates were composed using Adobe Photoshop version 8. The following abbreviations are used in the text: FW – forewing; HW – hindwing; D – dorsum; V – venter; HDP – hindwing dorsal median patch.

Material and sampling area

Partial nucleotide sequences of mtDNA cytochrome c oxidase subunit I gene (COI) of individuals from several populations occurring in the Andes in Colombia that were previously identified morphologically, were analyzed. Tissue samples were extracted from identified pinned specimens collected in the past 10 years, as it is less likely that sequence data can be obtained from old material. Altogether 79 specimens, representing 47 species, yielded a DNA sequence of over 400 base pairs (bp) in length. Specimens with shorter sequences were excluded from the analyses.

Molecular delimitation of species and barcodes

For the DNA analyses, 79 individuals of 47 species of *Lymanopoda* were included as well as 2 individuals of two different genera as an outgroup, *Corades chelonis* Hewitson and *Lasiophila zapatoza* (Westwood). One or two legs were removed from each dried specimen and stored in individual tubes. DNA extraction, amplification and sequencing of the barcode region of the COI gene were carried out at the Canadian Centre for DNA Barcode-

ing (CCDB), Ontario, Canada, using standard high throughput protocols (Ivanova et al., 2006; deWaard et al., 2008). PCR amplification with a single pair of primers consistently recovered a 658 bp region near the 5' end of COI that included the standard 648 bp barcode region for the animal kingdom (Hebert et al., 2004). Complete specimen data including images, voucher deposition, GenBank accession numbers, GPS coordinates, sequences and trace files are accessible in the Barcode of Life Data System (BOLD) (Table 3).

Sequence divergences for the barcode region were quantified using the Kimura 2 Parameter model, employing the analytical tools in BOLD (BOLD alignment, pairwise deletion). This was done to determine whether there is a barcode gap (a break in the distribution among genetic distances of specimens belonging to the same species and those of specimens from different species), that would allow the identification of the specimens examined. Genetic distances between species are reported as minimum pairwise distances, while intraspecific variation is reported as mean and maximum pairwise distances.

Several quantitative species delimitation algorithms for molecular data have been developed over the past decade, including approaches dedicated to DNA barcodes such as Automatic Barcode Gap Discovery (ABGD) and Refined Single Linkage (RESL) Analysis algorithm (Puillandre et al., 2012; Ratnasingham & Hebert, 2013). Each specimen with a sequence longer than 500bp automatically gains a BIN (Barcode Index Number) assignment on BOLD that is based on the RESL algorithm (Ratnasingham & Hebert, 2013). BINs may be merged when genetically intermediate specimens are added, or split when new records reveal a clear sequence divergence structure. Distance-based neighbour-joining (NJ) was used to reconstruct DNA barcode gene trees. Despite certain limitations, NJ has repeatedly been shown to perform well for species identification (Huelsenbeck & Hillis, 1993; Kumar & Gadagkar, 2000; Mihaescu et al., 2009; Mutanen et al., 2016).

Phylogenetic relationships

A reconstruction of the phylogenetic relationships of species of *Lymanopoda* was done using the Maximum Likelihood (ML) method. Two species of Satyrinae were used as an outgroup: *Corades chelonis* and *Lasiophila zapatoza*. The analysis was done using the Phylogeny.fr platform (Dereeper et al., 2008, 2010) and sequences were aligned using MUSCLE (v3.8.31) and configured for highest accuracy (MUSCLE with default settings).

The phylogenetic tree was constructed using the ML method implemented in the PhyML program (v3.1/3.0 aLRT). The HKY85 substitution model was selected assuming an estimated proportion of invariant sites (of 0.601) and four gamma-distributed rate categories to account for the percentage heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma = 1.147). The reliability of internal branches was assessed using the aLRT test (SH-Like). The graphical representation and editing of the phylogenetic tree were done using TreeDyn (v198.3).

RESULTS

Taxonomy

Genus *Lymanopoda* Westwood, 1851

Lymanopoda Westwood, 1851 (May): Pl. LXVII, Figs 6, 7. Type species: *L. samius* Westwood, 1851, by monotypy.

Sarromia Westwood, 1851 (May): Pl. LXVII, Fig. 5. Type species: *S. obsoleta* Westwood, 1851, by monotypy. Synonymized by Westwood, 1851 (July): 401–402.

Zabirnia Hewitson, 1877: 92. Type species: *Z. zigomala* Hewitson, 1877, by monotypy. Synonymized by Pyrce, 2004: 463.

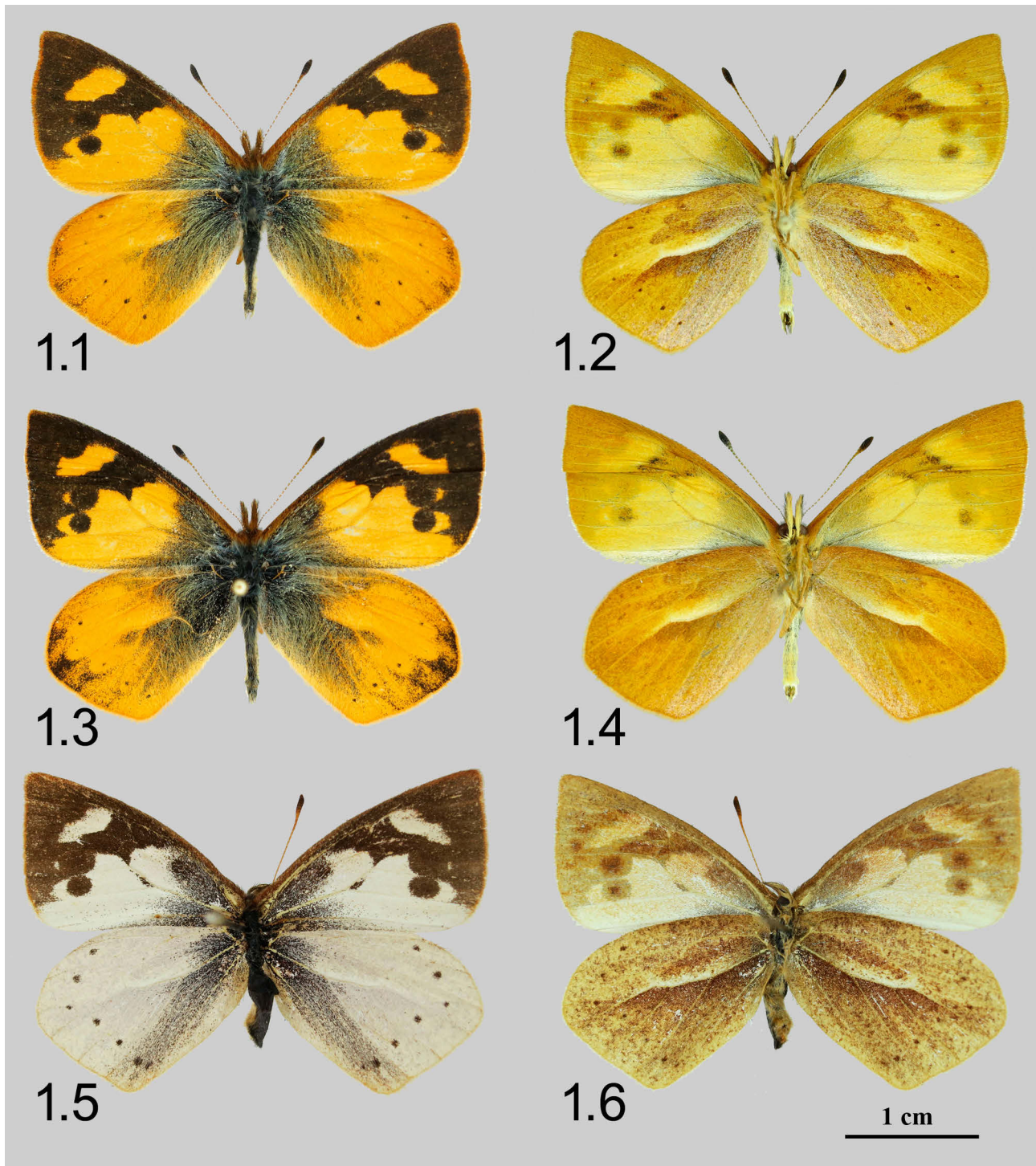


Fig. 1. Adults of *Lymanopoda flammigera* sp. n. 1.1 – male paratype (upperside); 1.2 – male paratype (underside); 1.3 – male paratype (upperside); 1.4 – male paratype (underside); 1.5 – female paratype (upperside); 1.6 – female paratype (underside).

Trophonina Röber, 1889: 222. Type species: *Lymanopoda acraeida* Butler, 1868, by monotypy. Synonymized by Pyrcz, 2004: 463.

Sabatoga Staudinger, 1897: 143. Type species: *S. mirabilis* Staudinger, 1897, by monotypy. Synonymized by Adams & Bernard, 1977: 270.

***Lymanopoda flammigera* Pyrcz, Prieto & Boyer, sp. n.**

(Figs 1.1–1.6, 2.1–2.4, 3.6)

ZooBank taxon LSID:

4B29BAB2-E924-4C1B-AFCA-AF131920FF02

Diagnosis

This species has the size, wing shape, and wing pattern similar to *L. huilana* Wreymmer, 1911 and *L. tolima* Weymer, 1890 (depicted in Fig. 5), but males differ from both these species and from any other congener by the golden yellow colour of their upper and undersides. The females are

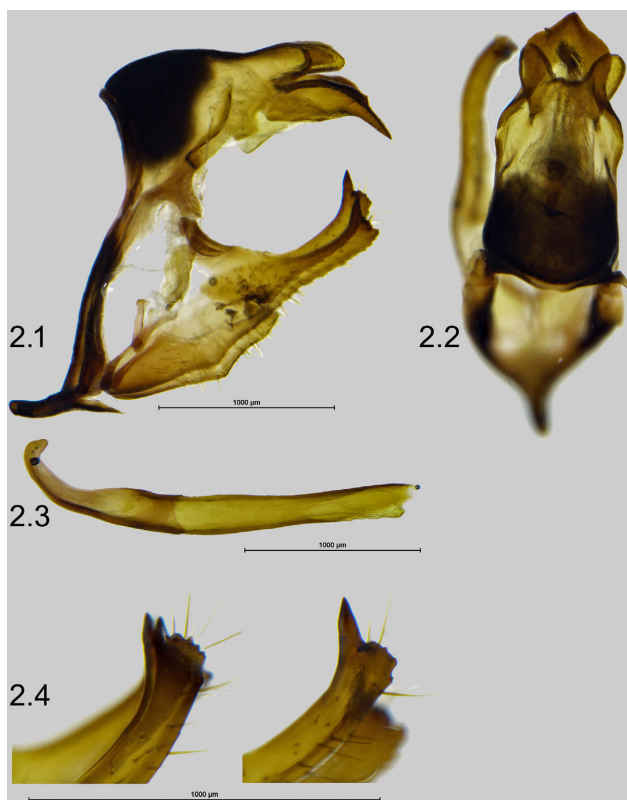


Fig. 2. Male genitalia of *Lymanopoda flammigera* sp. n. (paratype). 2.1 – lateral view; 2.2 – view from above; 2.3 – aedeagus in lateral view; 2.4 – details of the apices of the right and left valvae in lateral view.

whitish and thus nearly inseparable from the most closely related species, which are, however, not sympatric.

Description

Male. (Figs 1.1–1.4) *Head*: Eyes chestnut covered with long, black hairs; labial palps two and a half the length of head, covered with yellow and black hairs, dorsally also brown scales; frons with a tuft of brown hair; antennae reaching half length of the costa, chestnut with white scales at the base of each flagellomere, club composed of 10 segments, strongly flattened and dilated, brown, dorsally slightly lighter with a median groove. *Thorax*: Dorsally black, mostly naked, with some long but sparse silver hairs, tegulae covered with long, golden brown hairs; ventrally black but covered with long and dense yellow and white hairs; femora of second and third pair of legs black, with first pair and tibiae and tarsi yellow, densely covered with scales. *Wings*: FW (length: 20–21 mm) triangular with a pointed apex, straight outer margin and shallow tornus; HW oval with a rounded apex and straight outer margin from vein M2 to tornus where bent nearly at a right angle, anal margin straight. FWD yellow of variable shade, between pale yellow (in older individuals) and golden yellow from basal to postmedian area, except for a greyish basal suffusion and an elongated patch in subapical area; distally dark brown with sharp basal notches along the discal cell and vein Cu2A, a dark brown ocellus in space Cu1A–Cu2A. HWD varying between pale yellow and golden yellow with a greyish basal and medial suffusion and with

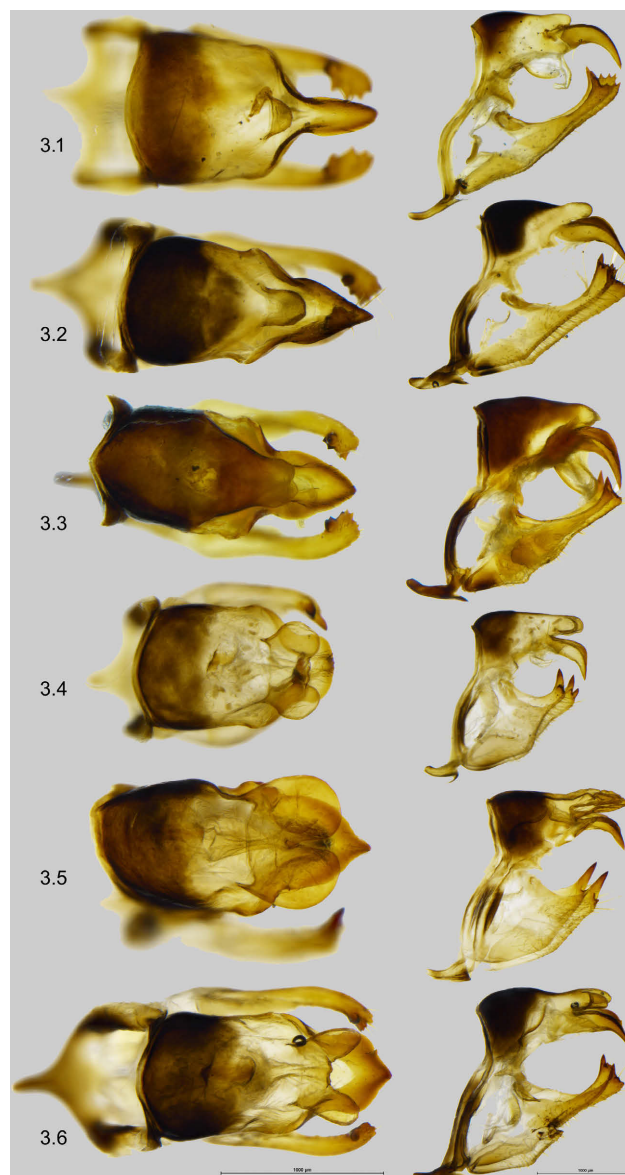


Fig. 3. Male genitalia of “tolima” clade (left: view from above, right: lateral view). 3.1 – *Lymanopoda huilana dominicae*; 3.2 – *L. huilana huilana*; 3.3 – *L. huilana salazari*; 3.4 – *L. tolima*; 3.5 – *L. cassneri*; 3.6 – *L. flammigera* sp. n.

a series of minute, sub marginal black dots (and in some specimens more or less developed marginal dark patches between tornus and apex). FWV colour pattern similar to that on the upper side, but the yellow basal area invariably lighter, and all the dark brown elements are dull and barely visible except for the darker patch in the postdiscal area. HWV light orange almost lacking a pattern except for a lighter, elongated patch in discal cell and a darker brown area immediately behind discal cell; sub marginal tiny black spots as on the upper side. *Abdomen*: Black dorsally and laterally (covered with dense, velvet black hairs and scales), ventrally with sandy yellow scales and hairs. *Genitalia* (Figs 2.1–2.4, 3.6): Tegumen strongly sclerotized with a slightly bulged dorsal surface; superuncus prominent, reaching half length of the uncus, bifurcated; uncus stout with a sharp tip pointing downwards; gnathos reduced, blunt; subscaphium small and weakly sclerotized;

Table 1. Summary of genetic distances among 47 species of *Lymanopoda*. For each species, the mean and maximum intra-specific values are compared to the nearest neighbour distance. Where the species is represented by a singleton, N/A is displayed for intra-specific values.

Species	Mean Intra-Sp	Max Intra-Sp	Nearest species	Nearest neighbour	Distance to NN
<i>Corades chelonis</i>	N/A	0	<i>Lasiophila zapatoza</i>	GWOTU985-17	10.81
<i>Lasiophila zapatoza</i>	N/A	0	<i>Corades chelonis</i>	GWOTU974-17	10.81
<i>Lymanopoda acraeida</i>	N/A	0	<i>Lymanopoda venosa</i>	GBGL8234-12	5.51
<i>Lymanopoda affineola</i>	N/A	0	<i>Lymanopoda apulia</i>	GBMIN34627-13	2.76
<i>Lymanopoda albocincta</i>	0.35	0.59	<i>Lymanopoda panacea</i>	GWOTR709-16	4.26
<i>Lymanopoda albomaculata</i>	N/A	0	<i>Lymanopoda apulia</i>	GBMIN34627-13	3.3
<i>Lymanopoda altis</i>	N/A	0	<i>Lymanopoda confusa</i>	GBMIN34624-13	2.59
<i>Lymanopoda apulia</i>	N/A	0	<i>Lymanopoda affineola</i>	GBGL8210-12	2.76
<i>Lymanopoda araneola</i>	N/A	0	<i>Lymanopoda shefteli</i>	GBGL8232-12	3.59
<i>Lymanopoda caeruleata</i>	N/A	0	<i>Lymanopoda caucana</i>	GWOTR725-16	0.77
<i>Lymanopoda caracara</i>	N/A	0	<i>Lymanopoda flammigera</i>	GWOTR859-16	6.77
<i>Lymanopoda casneri</i>	N/A	0	<i>Lymanopoda tolima</i>	GWOTR858-16	3.31
<i>Lymanopoda caucana</i>	0.14	0.21	<i>Lymanopoda caeruleata</i>	GWOTU968-17	0.77
<i>Lymanopoda confusa</i>	N/A	0	<i>Lymanopoda altis</i>	GWOTR733-16	2.59
<i>Lymanopoda dietzi</i>	N/A	0	<i>Lymanopoda altis</i>	GWOTR733-16	3.3
<i>Lymanopoda eubagioides</i>	N/A	0	<i>Lymanopoda inde</i>	GBMIN34617-13	3.86
<i>Lymanopoda euopsis</i>	N/A	0	<i>Lymanopoda venosa</i>	GBGL8234-12	5.51
<i>Lymanopoda excisa</i>	N/A	0	<i>Lymanopoda pieridina</i>	GWOTR758-16	4.39
<i>Lymanopoda ferruginosa</i>	N/A	0	<i>Lymanopoda shefteli</i>	GBGL8232-12	3.3
<i>Lymanopoda flammigera</i>	0	0	<i>Lymanopoda tolima</i>	GWOTR858-16	5.3
<i>Lymanopoda florenciensis</i>	0.18	0.19	<i>Lymanopoda affineola</i>	GBGL8210-12	4.19
<i>Lymanopoda hazelana</i>	N/A	0	<i>Lymanopoda samius</i>	GWOTR751-16	6.99
<i>Lymanopoda huilana</i>	N/A	0	<i>Lymanopoda melia</i>	GBGL8226-12	4.41
<i>Lymanopoda hyagnis</i>	N/A	0	<i>Lymanopoda umbratilis</i>	GBGL8233-12	0.39
<i>Lymanopoda inde</i>	N/A	0	<i>Lymanopoda eubagioides</i>	GBMIN34621-13	3.86
<i>Lymanopoda ionius</i>	N/A	0	<i>Lymanopoda pieridina</i>	GWOTR758-16	2.94
<i>Lymanopoda labda ssp.</i>	N/A	0	<i>Lymanopoda araneola</i>	GBGL8213-12	4.69
<i>Lymanopoda lecromi</i>	N/A	0	<i>Lymanopoda maletera</i>	GWOTR722-16	3.06
<i>Lymanopoda magna</i>	N/A	0	<i>Lymanopoda obsoleta</i>	GWOTR712-16	4.75
<i>Lymanopoda maletera</i>	0	0	<i>Lymanopoda lecromi</i>	GBGL8224-12	3.06
<i>Lymanopoda marianna</i>	N/A	0	<i>Lymanopoda lecromi</i>	GBGL8224-12	7.5
<i>Lymanopoda melia</i>	N/A	0	<i>Lymanopoda huilana</i>	GWOTR866-16	4.41
<i>Lymanopoda nadia</i>	N/A	0	<i>Lymanopoda ferruginosa</i>	GBGL8220-12	5.35
<i>Lymanopoda nevada</i>	0	0	<i>Lymanopoda paramera</i>	GWOTR874-16	5.78
<i>Lymanopoda nivea</i>	N/A	0	<i>Lymanopoda pieridina</i>	GWOTR758-16	4.21
<i>Lymanopoda obsoleta</i>	0.77	0.77	<i>Lymanopoda confusa</i>	GBMIN34624-13	2.95
<i>Lymanopoda panacea</i>	N/A	0	<i>Lymanopoda apulia</i>	GBMIN34627-13	3.31
<i>Lymanopoda paramera</i>	0	0	<i>Lymanopoda nevada</i>	GWOTR877-16	5.78
<i>Lymanopoda pieridina</i>	N/A	0	<i>Lymanopoda ionius</i>	GWOTR745-16	2.94
<i>Lymanopoda prusia</i>	N/A	0	<i>Lymanopoda tolima</i>	GWOTR858-16	9.24
<i>Lymanopoda rana</i>	N/A	0	<i>Lymanopoda umbratilis</i>	GBGL8233-12	3.94
<i>Lymanopoda samius</i>	0.1	0.15	<i>Lymanopoda hazelana</i>	GBMIN34619-13	6.99
<i>Lymanopoda shefteli</i>	N/A	0	<i>Lymanopoda hyagnis</i>	GBMIN34618-13	1.95
<i>Lymanopoda tolima</i>	0	0	<i>Lymanopoda casneri</i>	GWOTR869-16	3.31
<i>Lymanopoda umbratilis</i>	N/A	0	<i>Lymanopoda hyagnis</i>	GBMIN34618-13	0.39
<i>Lymanopoda venosa</i>	N/A	0	<i>Lymanopoda caucana</i>	GWOTR726-16	4.91
<i>Lymanopoda vivienteni</i>	N/A	0	<i>Lymanopoda ferruginosa</i>	GBGL8220-12	7.77

appendix angular, stout but short with a sharp tip; valva elongated, wide in basal half, narrower in the middle, ends with a wide serrated apex and a prominent processus pointing upwards; saccus short and flattened dorso-ventrally; aedeagus simple, tubular, the valva + saccus very slightly arched, with a smooth surface.

Female. (Figs 1.5, 1.6) Sexual dichroism prominent; yellow is replaced by white pigmentation, however the dark brown-blackish elements of the colour pattern are nearly identical, except that they are slightly larger on the FWD, entering more deeply into the discal cell. The HWD sub marginal black dots are also larger. Otherwise, the wing shape of the female differs slightly in being less elongated,

especially the hindwings (FW length: 21 mm). Female genitalia not examined.

Molecular characterization. No intraspecific haplotype diversity was found in the available sequences ($n = 5$). The lowest overall mean distance to another member of the genus is 5.3% to *L. tolima* from Nevado del Ruiz. BIN number: BOLD: ADD7260. Diagnostic fixed states and their position in the COI barcode sequence are depicted in Table 2.

Type material. Holotype male: Colombia, Valle, Tenerife, Páramo Las Domínguez, 3600 m, 29.i.2017, specimen number: i1241, sequence page in BOLD: GWOTU1004-17, C. Prieto. Deposited in ICN. Paratypes, (8♂ and 1♀): 1♀: Colombia,

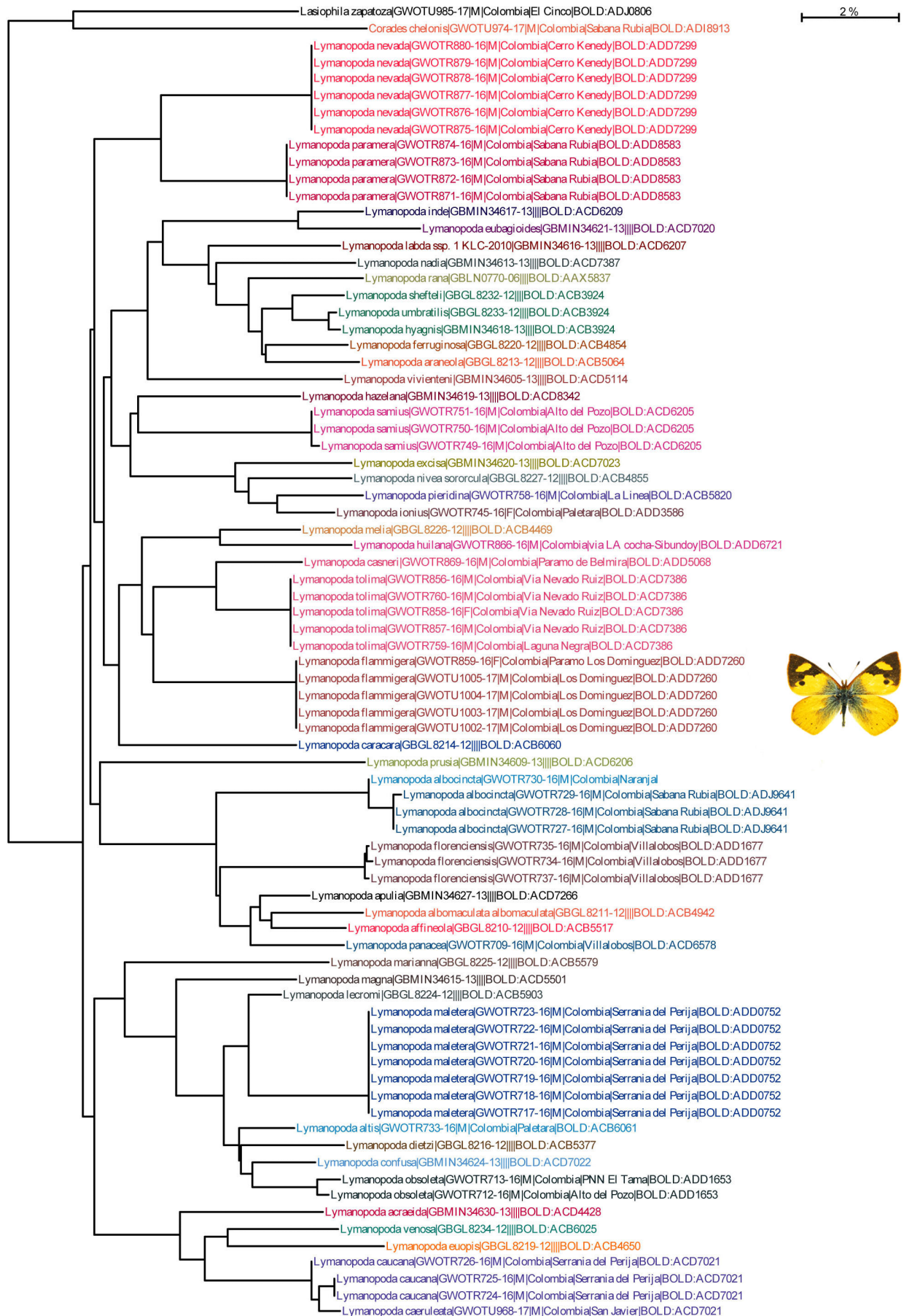


Fig. 4. Neighbour Joining (NJ) identification tree of full-length barcodes (658 bp) for 47 species of *Lymanopoda* using the K2P-parameter model. BIN (Barcode Index Number) assignment using BOLD is also depicted.

Table 2. Nucleotide sites with unique fixed states, which serve to identify nine species of *Lymanopoda* from Colombia. Only species with three or more individuals were included in the analysis. Red – diagnostic characters; orange – partially diagnostic characters.

Group Name	43	60	63	66	72	78	84	97	99	114	115	132	139	162	177	201	204	213	216
<i>Lymanopoda flammigera</i>																			
<i>Lymanopoda tolima</i>						C			C					C					
<i>Lymanopoda maletera</i>				G			G			G								A	
<i>Lymanopoda samius</i>		G	C								C				C				
<i>Lymanopoda paramera</i>	C											C							
<i>Lymanopoda nevada</i>					C														
<i>Lymanopoda albocincta</i>															A				
<i>Lymanopoda caucana</i>								T	A				A						
<i>Lymanopoda florenciense</i>																C	G		C

Group Name	222	225	231	234	237	240	261	264	279	282	285	309	312	313	318	321	327	330	333
<i>Lymanopoda flammigera</i>		G			C		C												
<i>Lymanopoda tolima</i>												G						T	
<i>Lymanopoda maletera</i>			C			G				C	C		G	C					
<i>Lymanopoda samius</i>																A			C
<i>Lymanopoda paramera</i>																			
<i>Lymanopoda nevada</i>	C			G											T				
<i>Lymanopoda albocincta</i>									A										
<i>Lymanopoda caucana</i>																T	G		
<i>Lymanopoda florenciense</i>								G											

Group Name	334	349	351	359	366	369	372	381	399	402	405	414	420	429	432	433	439	447	453
<i>Lymanopoda flammigera</i>									G										
<i>Lymanopoda tolima</i>																			
<i>Lymanopoda maletera</i>				G								C	T	G			A	C	
<i>Lymanopoda samius</i>										A						C			
<i>Lymanopoda paramera</i>																			
<i>Lymanopoda nevada</i>								G											
<i>Lymanopoda albocincta</i>										C					C				
<i>Lymanopoda caucana</i>					A	T	C												
<i>Lymanopoda florenciense</i>	C	C	A																C

Group Name	468	492	504	510	527	549	552	556	558	561	565	567	577	579	582	583	585	586	589
<i>Lymanopoda flammigera</i>				C					G										
<i>Lymanopoda tolima</i>																		C	
<i>Lymanopoda maletera</i>		C						A			A		A						
<i>Lymanopoda samius</i>	C					A										T			
<i>Lymanopoda paramera</i>					G		T								T				
<i>Lymanopoda nevada</i>			C							G		C					G		C
<i>Lymanopoda albocincta</i>																			
<i>Lymanopoda caucana</i>							G												
<i>Lymanopoda florenciense</i>								A						C					

Group Name	612	615	627	630	639	648	660	669	672	675	678	693
<i>Lymanopoda flammigera</i>	C											
<i>Lymanopoda tolima</i>			T					T	C			
<i>Lymanopoda maletera</i>							A					
<i>Lymanopoda samius</i>				T	C							
<i>Lymanopoda paramera</i>			G							T	T	
<i>Lymanopoda nevada</i>									G	G		
<i>Lymanopoda albocincta</i>												
<i>Lymanopoda caucana</i>												
<i>Lymanopoda florenciense</i>		T										

Valle, Tenerife, Páramo Las Domínguez, 3300 m, 21.vii.2013, specimen number: i918, sequence page in BOLD: GWOTR859-16, C. Prieto, RCCP; 3♂: Colombia, Valle, Tenerife, Páramo Las Domínguez, 3600 m, 29.i.2017, specimen numbers: i1239,

i1240, i1242, sequence pages in BOLD: GWOTU1002-17, GWOTU1003-17, GWOTU1005-17, C. Prieto, RCCP; 3♂ Colombia, Valle del Cauca, Páramo Las Domínguez, Tenerife, NE Palmira 03°45'N, 76°05'W, 3500–3600 m, 29.i.2017, PB; 1♂

Table 3. List of specimens, localities and BOLD accession numbers for each individual used in the molecular study.

Species	Process ID	Country	Region	Lat	Lon	Elev
<i>Lasiophila zapatoza</i>	GWOTU985-17	Colombia	Perija	10.3667	–72.95	2200
<i>Corades chelonis</i>	GWOTU974-17	Colombia	Perija	10.35	–72.9167	2800
<i>Lymanopoda nevada</i>	GWOTR880-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda nevada</i>	GWOTR879-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda nevada</i>	GWOTR878-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda nevada</i>	GWOTR877-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda nevada</i>	GWOTR876-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda paramera</i>	GWOTR874-16	Colombia	Serrania del Perija	10.35	–72.9167	2800
<i>Lymanopoda paramera</i>	GWOTR873-16	Colombia	Serrania del Perija	10.35	–72.9167	2800
<i>Lymanopoda paramera</i>	GWOTR872-16	Colombia	Serrania del Perija	10.35	–72.9167	2800
<i>Lymanopoda flammigera</i>	GWOTR859-16	Colombia	Tenerife	3.68333	–76.1	3600
<i>Lymanopoda tolima</i>	GWOTR858-16	Colombia	PNN Los Nevados	4.98333	–75.3333	3200
<i>Lymanopoda tolima</i>	GWOTR857-16	Colombia	PNN Los Nevados	4.98333	–75.3333	3200
<i>Lymanopoda tolima</i>	GWOTR856-16	Colombia	PNN Los Nevados	4.98333	–75.3333	3200
<i>Lymanopoda tolima</i>	GWOTR760-16	Colombia	PNN Los Nevados	4.98333	–75.3333	3200
<i>Lymanopoda flammigera</i>	GWOTU1005-17	Colombia	Valle, Tenerife	3.68333	–76.1	3600
<i>Lymanopoda samius</i>	GWOTR751-16	Colombia	Ocana	8.03333	–73.0167	2700
<i>Lymanopoda samius</i>	GWOTR750-16	Colombia	Ocana	8.03333	–73.0167	2700
<i>Lymanopoda caucana</i>	GWOTR726-16	Colombia	Manaure	10.35	–72.9167	1900
<i>Lymanopoda florenciensis</i>	GWOTR735-16	Colombia	Bota Caucana	1.51667	–76.3167	1550
<i>Lymanopoda florenciensis</i>	GWOTR734-16	Colombia	Bota Caucana	1.51667	–76.3167	1550
<i>Lymanopoda albocincta</i>	GWOTR730-16	Colombia	Argelia	2.36667	–77.1833	2700
<i>Lymanopoda albocincta</i>	GWOTR729-16	Colombia	Manaure	10.35	–72.9167	2500
<i>Lymanopoda albocincta</i>	GWOTR728-16	Colombia	Manaure	10.35	–72.9167	3000
<i>Lymanopoda flammigera</i>	GWOTU1004-17	Colombia	Valle, Tenerife	3.68333	–76.1	3600
<i>Lymanopoda flammigera</i>	GWOTU1003-17	Colombia	Valle, Tenerife	3.68333	–76.1	3600
<i>Lymanopoda caucana</i>	GWOTR725-16	Colombia	Manaure	10.35	–72.9167	1800
<i>Lymanopoda maletera</i>	GWOTR723-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda maletera</i>	GWOTR722-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda maletera</i>	GWOTR721-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda maletera</i>	GWOTR720-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda maletera</i>	GWOTR719-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda maletera</i>	GWOTR718-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda obsoleta</i>	GWOTR713-16	Colombia	Herran	7.41667	–72.4333	2400
<i>Lymanopoda venosa</i>	GBGL8234-12	Perú	Puno			1200
<i>Lymanopoda umbratilis</i>	GBGL8233-12	Perú	Cuzco			1200
<i>Lymanopoda shefteli</i>	GBGL8232-12	Perú	Cuzco			2500
<i>Lymanopoda rana</i>	GBLN0770-06	Perú	Pasco			2500
<i>Lymanopoda prusia</i>	GBMIN34609-13	Perú	Pasco			2800
<i>Lymanopoda nivea</i>	GBGL8227-12	Ecuador	Napo			2700
<i>Lymanopoda nadia</i>	GBMIN34613-13	Ecuador	Morona-Santiago			2800
<i>Lymanopoda melia</i>	GBGL8226-12	Ecuador	Tungurahua			3600
<i>Lymanopoda marianna</i>	GBGL8225-12	Venezuela	Merida			3100
<i>Lymanopoda magna</i>	GBMIN34615-13	Perú	Molinopampa			2870
<i>Lymanopoda lecroini</i>	GBGL8224-12	Venezuela	Tachira			2700
<i>Lymanopoda labda ssp</i>	GBMIN34616-13	Colombia	Antioquia			2700
<i>Lymanopoda inde</i>	GBMIN34617-13	Perú	Molinopampa			3200
<i>Lymanopoda hyagnis</i>	GBMIN34618-13	Perú	Cuzco			2900
<i>Lymanopoda hazelana</i>	GBMIN34619-13	Ecuador	Loja			3000
<i>Lymanopoda ferruginosa</i>	GBGL8220-12	Perú	Cuzco			2050
<i>Lymanopoda excisa</i>	GBMIN34620-13	Ecuador	Loja			3025
<i>Lymanopoda euopis</i>	GBGL8219-12	Costa Rica	Irazú			2700
<i>Lymanopoda eubagioides</i>	GBMIN34621-13	Perú	Cuzco			2600
<i>Lymanopoda dietzi</i>	GBGL8216-12	Venezuela	Tachira			2700
<i>Lymanopoda confusa</i>	GBMIN34624-13	Ecuador	Zamora-Chinchi			2100
<i>Lymanopoda araneola</i>	GBGL8213-12	Perú	Molinopampa			2870
<i>Lymanopoda apulia</i>	GBMIN34627-13	Perú	Pasco			2600
<i>Lymanopoda albomaculata</i>	GBGL8211-12	Bolivia	Cochabamba			2750
<i>Lymanopoda affineola</i>	GBGL8210-12	Perú	Puno			2700
<i>Lymanopoda acraeida</i>	GBMIN34630-13	Perú	Cuzco			1400
<i>Lymanopoda vivienteni</i>	GBMIN34605-13	Colombia	Guasca			3200
<i>Lymanopoda nevada</i>	GWOTR875-16	Colombia	Sierra Nevada	11.1	–74.0333	2800
<i>Lymanopoda paramera</i>	GWOTR871-16	Colombia	Serrania del Perija	10.35	–72.9167	2800
<i>Lymanopoda casneri</i>	GWOTR869-16	Colombia	Belmira	6.65	–75.6667	3100
<i>Lymanopoda huilana</i>	GWOTR866-16	Colombia	Sibundoy	1.13333	–77.0833	3000
<i>Lymanopoda tolima</i>	GWOTR759-16	Colombia	PNN Los Nevados	4.98333	–75.3333	3200
<i>Lymanopoda pieridina</i>	GWOTR758-16	Colombia	Calarca	4.46667	–75.55	3200
<i>Lymanopoda samius</i>	GWOTR749-16	Colombia	Ocana	8.03333	–73.0167	2700
<i>Lymanopoda ionius</i>	GWOTR745-16	Colombia	Purace	2.16667	–76.3833	3000
<i>Lymanopoda florenciensis</i>	GWOTR737-16	Colombia	Bota Caucana	1.51667	–76.3167	1400
<i>Lymanopoda altis</i>	GWOTR733-16	Colombia	PNN Purace	2.16667	–76.3833	2900
<i>Lymanopoda albocincta</i>	GWOTR727-16	Colombia	Manaure	10.35	–72.9167	3000
<i>Lymanopoda flammigera</i>	GWOTU1002-17	Colombia	Valle, Tenerife	3.68333	–76.1	3600
<i>Lymanopoda caucana</i>	GWOTR724-16	Colombia	Manaure	10.35	–72.9167	1900
<i>Lymanopoda maletera</i>	GWOTR717-16	Colombia	Villanueva	10.35	–72.9167	2600
<i>Lymanopoda obsoleta</i>	GWOTR712-16	Colombia	Ocana	8.03333	–73.0167	2700
<i>Lymanopoda panacea</i>	GWOTR709-16	Colombia	Bota Caucana	1.51667	–76.3167	1800
<i>Lymanopoda caeruleata</i>	GWOTU968-17	Colombia	Sierra Nevada	10.8833	–74.0167	1500
<i>Lymanopoda caracara</i>	GBGL8214-12	Ecuador	Napo			3600

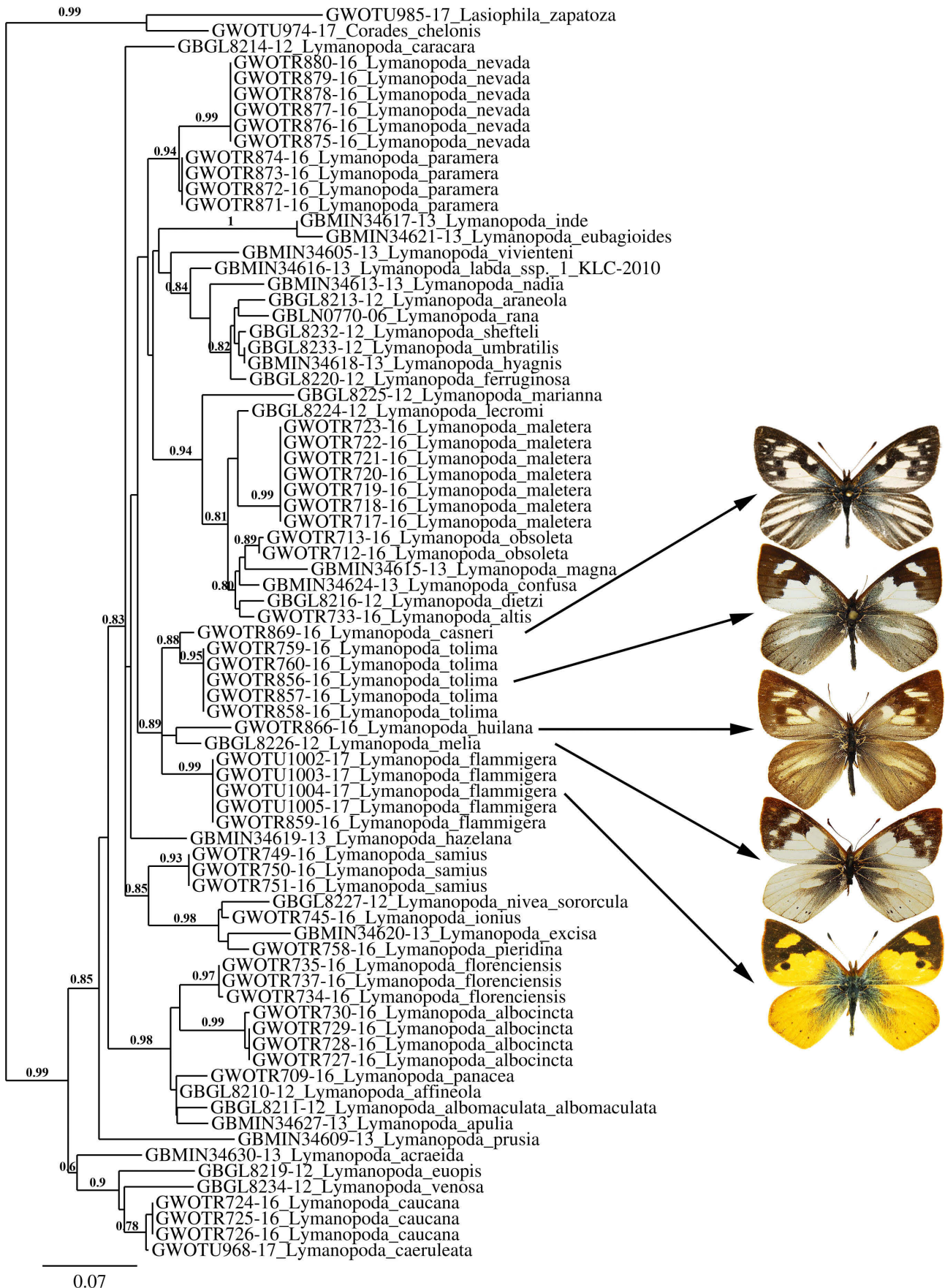


Fig. 5. Phylogenetic tree constructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT) and showing the relationships among taxa belonging to *Lymanopoda*. Numbers represent branch support values.

Colombia, Valle del Cauca, Páramo Las Domínguez, Tenerife, NE Palmira 03°45'N, 76°05'W, 3500–3600 m, 29.i.2017, P. Boyer leg., CEP-MZUJ; 1♂ Colombia, Valle del Cauca, Páramo Las Domínguez, Tenerife, NE Palmira 03°45'N, 76°05'W, 3500–3600 m, 28.i.2017, P. Boyer leg., prep. genit. 498/14.02.2017 J. Lorenc-Brudecka, CEP-MZUJ.

Type locality. Páramo de Las Domínguez (= Pan de Azúcar), Tenerife, NE Palmira, Valle del Cauca Department, 03°45'N, 76°05'W, 3500–3600 m a.s.l.

Etymology. The specific epithet “*flammigera*” is the nominative feminine singular of “*flammiger*” from the latin “*flamma*” (= flame) and -iger (gero) (= to carry, to bear), in reference to the intense orange-yellow colour of the males of this butterfly.

Bionomics. Males patrol at 1–2 m above the ground in the cloud forest – páramo ecotone. Males patrol around midday along the sunny edges of paths. The immature stages and larval food plants are unknown but are presumed to be *Chusquea* bamboo, as is the case with other species of *Lymanopoda*, common in the collecting area.

Distribution. This species is known only from the type locality, Páramo de Las Domínguez (Pan de Azúcar in some maps), an isolated massif situated west of the main Colombian Central Cordillera range of the Andes. It occurs in the páramo grassland at 3300–3600 m, just above timberline. Adults were collected in January and July.

Species delimitation based on barcode analysis

A NJ tree was generated for 47 species and 79 individuals of *Lymanopoda*. When discrepancies between the DNA-based and standard taxonomy were found, the specimen was examined to confirm that its morphological identification was correct, and the alignment and trace files were carefully re-examined. It was found that 47 morphospecies were assigned to 44 BINs (Fig. 4), therefore showing a 94% of congruence between morphospecies and BINs. The morphospecies *L. hyagnis*, *L. umbratilis* and *L. shefteli* were placed in the NJ tree under the same BIN code due to the low genetic divergence of 0.39% between *L. hyagnis* and *L. umbratilis*, and 1.9% between *L. shefteli* and *L. hyagnis*. Similarly, the genetic divergence between the morphologically very divergent *L. caeruleata* and *L. caucana* is 0.77% (Table 1). However, in all the cases, the identification of an unknown specimen by matching its sequence to those in the reference library led to correct results. Nucleotide sites with unique fixed states that were used to identify nine species (those represented by at least three specimens in our dataset) of *Lymanopoda* from Colombia are compared in Table 2.

The species of *Lymanopoda* examined have a mean intraspecific genetic distance of 0.05% (n = 78 comparisons of barcodes > 600 bp). Maximum intraspecific divergence was 0.77%. The mean interspecific genetic distance was 9.60% (n = 2848 comparisons of barcodes > 600 bp). Maximum interspecific divergence was 13.37% and minimum interspecific distance was 0.39%.

Lymanopoda phylogeny

A phylogenetic tree was constructed using the ML method for 47 species of *Lymanopoda*, including 17 from Colombia and 30 others whose COI sequences were available

in GenBank (Fig. 5), out of ~65 known, which makes up 70% of all known species. The tree presents four main clades, one of which is called here for convenience “*obsoleta*” with 14 species including the two species, *L. florenciense* Salazar, Henao & Vargas, 2004 and *L. maletera* Adams & Bernard, 1979, not sequenced before, the “*ionius*” clade with 17 species, the “*caucana*” clade with five species and “*tolima*” clade with eight species. The latter is subdivided into two clades, one of which includes two species not sequenced before, *L. nevada* Krüger, 1924 and *L. paramera* Adams & Bernard, 1979, whereas the other contains six species including *L. flammigera* sp. n. and two other species not included in the generic phylogeny produced previously (Casner & Pyrcz, 2010), *L. tolima* and *L. casneri* Pyrcz & Clavijo, 2016, the latter, however, sequenced by Marín et al. (2017). The resolution of this clade is low and presents a polytomy, therefore the position of the new species relative to other five species is not established.

DISCUSSION

Colour patterns

The new species is remarkable first of all because of its unusual golden-yellow colour of males, unique not only among other congeners but also within the entire species-rich subtribe Pronophilina (over 650 species), and arguably among all neotropical and even worldwide Satyrinae. The evolutionary basis of this outstanding colouration is unknown but the hypothesis that this colouring is somehow related to mimicry, seems unlikely. This is because the Sulphur *Colias dimera* Doubleday, 1847, which is the potential model, although generally very common in the Colombian páramos and probably obnoxious, has not been detected in the region where *L. flammigera* sp. n. occurs. Other related Colombian species, such as *L. huilana*, *L. tolima*, *L. zebra* Pyrcz & Rodríguez, 2007, *L. casneri* and *L. melia* Weymer, 1911, are predominantly white or black and white, which is certainly associated with thermoregulation and the limited solar radiation at high altitudes, and the higher absorption of UV. It could eventually also prove to be the case for *L. flammigera* although the optical qualities of its wing pigments and scales have not been investigated so far. It is worth pointing out that there are several similarly pigmented species of skippers (Hesperiidae) in the high tropical and temperate Andes within the genera *Zalomes* Bell, *Wahydra* Steinhauser, *Hylephila* Billberg, and one yet undescribed species of *Racta* Evans.

It is however puzzling why such unusual colour patterns evolved in just one isolated area whereas throughout the northern and central Andes most páramo species of *Lymanopoda* are predominantly white. On the other hand, it is true that the genus *Lymanopoda* is particularly plastic phenotypically and a number of species occurring in cloud forests or the forest-páramo ecotone have colour patterns that are unusual for the subfamily Satyrinae, for example, the blue *L. hazelana* Brown, 1943, *L. samius* Westwood, 1851 and *L. cinna* Westwood, 1889, green patched *L. marianna* Staudinger, 1897 or red *L. inaudita* Pyrcz, 2010. Some of these colour patterns are almost certainly due to

mimetic relationships, an issue currently being investigated (Pyrzcz, in prep.).

Barcoding

This study provides an initial assessment of the usefulness of DNA barcoding in *Lymanopoda*. The NJ tree analysis yielded high percentage of correct identifications in the genus *Lymanopoda*. In the tree, 94% of the morphospecies used in this study formed distinct clades and were assigned a Barcode Index Number (BIN) matching perfectly the morphology based identifications. In 6% of the cases, more than one morphospecies shares a BIN number with other species. These cases include five species in this study: *L. hyagnis*, *L. umbratilis*, *L. shefteli* cluster together and have the same BIN number; and *L. caeruleata* and *L. caucana* also have the same BIN number. The former three species belong to a complex group of morphologically similar taxa occurring allopatrically in parallel valleys in the Madre de Dios upper basin in southern Peru and northern Bolivia, whose relationships are still not fully understood, and their separate specific status is yet to be confirmed by more thorough taxonomic studies involving their spatial, geographic and altitudinal distribution patterns. *L. caeruleata* and *L. caucana* are allopatric species, morphologically easily separable by their predominantly blue (*L. caeruleata*) and brown (*L. caucana*) wing colour patterns and genital characters, so their separate specific status is strongly supported. Our results confirm that DNA barcoding is a highly efficient method for identifying species in the subfamily Satyrinae, as pointed out in another recent study on high Andean butterflies (Marín et al., 2017).

Phylogeny

The cladogram based on the COI marker produced for 47 species has to be considered as complementary relative to previous studies as it takes into consideration only one marker, compared to 40 species and 5 molecular markers (Casner & Pyrcz, 2010). We, however, chose to use only the COI marker because one of the key issues of this study was to investigate the robustness of barcoding relative to morphological traits in evaluating relationships within the genus *Lymanopoda*, in particular, between hypothetically closely related taxa. It is interesting, from this perspective, to point out that, regarding the subdivision of the genus into main monophyletic groups and, in particular, the basal position of the “*caucana*” clade comprising 5 species, the results are highly congruent with previous molecular (Casner & Pyrcz, 2010) and morphological phylogenetic hypotheses (Pyrzcz, 2001). The position of *L. prusia*, Heilmich, 1973, as a sister to the remaining species of *Lymanopoda* is, however, not confirmed.

The “*tolima* clade”, with 6 species in Casner & Pyrcz’s paper, is here restricted to 4 species, two of which were not previously examined, *L. huilana* and *L. flammigera* sp. n. This well supported clade includes all the high altitude páramo species, examined so far, distributed from north-central Colombia (Belmira) to Ecuador. Also, all of these species share a number of morphological synapomorphies, which support its monophyly. In this respect, the Peruvian

species, *L. inde* Pyrcz, 2004 and *L. eubagioides* Butler, 1873, excluded from this clade, stand apart, and their position within this clade suggested originally by Casner & Pyrcz (op. cit.) should be reconsidered. Importantly, two white páramo species, *L. nevada* and *L. paramera*, found in isolated ranges in northern Colombia, were included in the molecular analysis for the first time. Although they superficially resemble the species in the “*tolima*” clade by being predominantly white, they were placed in a separate clade, even if they still occur in the larger unit comprising the “*tolima*” clade and not in the other two large clades, “*excisa*” and “*obsoleta*”.

By combining molecular and morphological data it is possible to determine the closest relatives of *L. flammigera* sp. n. within the “*tolima*” clade. COI based analysis is inconclusive in this respect in showing a polytomy. Comparisons of male genitalia show, however, that *L. flammigera*, *L. casneri* and *L. tolima* share a unique synapomorphy, a bifurcate, dorso-ventrally flattened, prominent rounded superuncus. In *L. huilana* and *L. hazelana* the superuncus is considerably smaller and not bifurcated even if two lateral lobes are noticeable. Other characters are less evident, although the valvae of *L. casneri* and *L. tolima* are more similar, being short with a single prominent apical tooth, whereas the valvae of *L. flammigera* sp. n. are narrower in the middle and much longer, looking in this respect more like those of *L. huilana*. In *L. melia*, the sister species of *L. tolima* according to Casner & Pyrcz’s (op. cit.) phylogeny, the superuncus is short and single. These data have important phylogeographical implications. *L. tolima* diverged in the first place from *L. huilana* even though there is a continuity of páramo habitats between the areas in Quindío, Tolima and Valle del Cauca in the Central Cordillera with those in Cauca and Nariño further south where *L. huilana* occurs. On the other hand, there are currently no appropriate páramo habitats over 200 km between Quindío and the Páramo de Belmira in Antioquia where *L. casneri* is found. Apparently some more complex underlying paleoecological processes have resulted in the shaping of present day distributions of páramo *Lymanopoda* species in this part of Colombia.

Final considerations

This study highlights two important facts. It is confirmed that genitalia, in particular those of males, are extremely valuable not only in alpha-taxonomy but also phylogenetically. Here a comparative analysis refines some data obtained using molecular tools. Of course, not in all taxa are male genitalia as informative, which depends mostly on the number of modifications leading to the evolution of noticeable phenotypical traits even in closely related groups of taxa. In the genus *Lymanopoda* such traits are appreciable. Secondly, our study confirms the usefulness of the COI marker in species definition as well as in phylogenetic considerations, a role that has been questioned. Here, COI support data on 17 species of Colombian *Lymanopoda* helped refine the phylogeny of the genus, and is congruent in most aspects with the previously proposed arrangement based on five markers. In other words, COI

does work at least in the genus *Lymanopoda*, even if in some other taxa of Lepidoptera this may not necessarily be the case. This study expanded our knowledge on the evolution of the genus *Lymanopoda* by adding seven more species to its phylogeny. Data for several key species are however still missing, in Colombia in particular for *L. mirabilis* (Staudinger), a high páramo species with unusual extremely elongated wings and atypical genitalia from the southern part of the Cordillera Oriental, and *L. melendeza* Adams from the Sierra del Cocuy that has some resemblance in both genitalia and colour patterns to the Venezuelan *L. marianna* Staudinger, known so far only from the holotype.

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