EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **120**: 324–337, 2023
doi: 10.14411/eje.2023.034
ORIGINAL ARTICLE

Diversity and relationships between Andean shrubland puna butterflies in the genus *Punargentus* (Lepidoptera: Nymphalidae: Satyrinae)

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Key words. Taxonomy, new species, male genitalia, COI barcodes, target enrichment, Cordillera Negra, Peru

Abstract. Two new species and two new subspecies of the satyrine butterfly genus *Punargentus* Heimlich, *P. atusparia* sp. n., *P. heimlichi* sp. n., *P. blanchardi libertas* ssp. n. and *P. atusparia yupania* ssp. n., are described from north-central Peru (Ancash, Huánuco and La Libertad). The affinities of the new taxa are evaluated based on molecular data obtained using target enrichment and COI barcoding. *Punargentus* is fully supported and consists of two branches, the *P. lamna* clade with four species and *P. blanchardi* clade with three species, two of which are described here. Species in the *P. blanchardi* clade occur in Andean shrubland, at altitudes between 2800–3600 m, whereas those in the *P. lamna* clade occur in puna grassland at 3600–4600 m, with occasional overlaps. Based on their habitat preferences, none of the newly described species are in need of immediate conservation measures. The genus *Etcheverrius* Herrera is reinstated, with *E. chiliensis* (Guérin-Méneville) and *E. tandilensis* (Köhler), comb. n.

ZooBank Article Registration: https://zoobank.org/urn:lsid:zoobank.org:pub:E5E918F3-47F0-44EE-8C48-FBA1D36993F6

INTRODUCTION

The Andes is the longest mountain chain in the world with an impressive mosaic of ecosystems and the highest biodiversity (Pennington et al., 2010). The true magnitude of the diversity, however, still remains to be revealed despite much research and doubtless progress in this area (Comer et al., 2022). Research efforts are, however, geographically biased, with emphasis on particular areas with high biotic potentials and those that are easily accessible. In terms of Lepidoptera, a good example is the valley of Cosñipata in the department of Cuzco (Qosqo) in Peru, an area, which over the last few decades has been visited by many collectors and researchers (Lamas et al., 2021). At the other extreme there are the remote, xeric Inter-Andean valleys and isolated ridges. Recent studies carried out by the staff of the Jagiellonian University and Peruvian co-workers revealed the biotic potential of such areas. Although the species richness in these areas may not be particularly high the incidence of endemicity is and is frequently associated with unusual behaviour, morphological adaptations and intricate geographic patterns (Pyrcz & Wojtusiak, 2010; Cerdeña et al., 2014).

Among the butterflies specific to these habitats is the satyrine genus *Punargentus* Heimlich, 1963, which belongs to the Neotropical subtribe Pronophilina and is a representative of the infratribe Neosatyriti (sensu Matz &

Brower, 2016), which occurs in temperate, sub-Antarctic and high altitude grasslands in the southern portion of South America. *Punargentus* was synonymized with the genus *Argyrophorus* Blanchard, 1852 by Pyrcz (2010), a decision upheld by Pyrcz et al. (2016). It was, however, reinstated by Matz & Brower (2016), who also synonymized *Etcheverrius* Herrera (1965) and *Palmaris* Herrera (1965) with *Punargentus*, by including their respective type species *Satyrus chiliensis* Guérin-Méneville (1830) and *Hipparchia monticolens* Butler (1881). *Punargentus* is the only, nearly Peruvian endemic genus in the infratribe Neosatyriti, with only *P. lamna* and *P. angusta* partly occurring in northern Bolivia (Cerdeña et al., 2014). Recently, two species of *Punargentus* were described (in *Argyrophorus*) by Pyrcz et al. (2019), but subsequent field work in the Andean shrubland in north-central Peru, in the departments of Ancash, Huánuco, La Libertad and Cajamarca, revealed that the species richness of *Punargentus* is even higher than previously thought. The aim of this paper is to describe new species and subspecies of *Punargentus* and evaluate their relatedness using molecular and morphological characters.

MATERIAL AND METHODS

Morphological studies

The butterflies were collected using entomological nets during field work in the departments of Huánuco, Ancash and La Libertad (Peru). Comparative material is deposited in the Museo de Historia Natural de la Universidad Nacional de San Agustín, Arequipa, Peru (MUSA), Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM) and Nature Education Centre (former Zoological Museum), Jagiellonian University, Kraków, Poland (CEP-UJ), as well as in the collection of Pierre Boyer (Le Puy Sainte Reparade, France) (PBF), which will be incorporated into CEP-UJ. Adults were photographed using Panasonic Lumix GH5 camera and colour plates were composed in Adobe Photoshop 11. Genitalia were dissected using standard procedures. Terminal parts of male and female abdomens were removed and soaked in hot 10% KOH solution for 5–10 min and then cleaned of soft tissue in distilled water and transferred to glycerol. Female genitalia were additionally stained with chlorazol black. Genitalia were photographed using a Nikon digital camera DS-Fi1 and the images were stacked in Combine ZP and edited in Corel PHOTO-PAINT X3 programs to enhance focus and improve clarity. The genitalia were kept in glycerol vials pinned under each specimen. The terminology for the genitalia mostly follows Razowski (1996). The following abbreviations are used in the text: FW – forewing; HW – hindwing; D – dorsum; V – venter.

DNA extraction, amplification and sequencing

Legs (in most cases two, meso and metathoracic per specimen) of the butterflies were used for DNA extraction using a DNeasy® Blood & Tissue Kit (Qiagen, Germany) according to the attached protocol. A PCR reaction was performed to obtain a fragment of the mitochondrial gene, cytochrome oxidase subunit I (mtDNA, COI). For this purpose, universal primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) were used (Folmer et al., 1994). For some samples that did not yield a positive PCR result, two primer combinations: LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) plus K699 (5′-WGGGGGGTAAACTGTTCATCC-3′) and TegF4 (5′-AGAATYGTAGAAAATGGAGCAGG-3′) plus Nancy (5′-CCTGGTAAAATTAAAATATAAACTTC-3′) were used to obtain COI in two fragments. A PCR reaction was carried out in a 30 µl mixture, consisting of 2 µl of template DNA, 20.72 µl of ddH_2O, 2.8 µl of 10× Pol buffer C (on Taq DNA Polymerase "hot start", EUR_X, Poland), 1.96 µl of 25 mM $MgCl_2$ (on Taq DNA Polymerase "hot start", EUR_X, Poland), 0.84 µl of each primer, 0.56 µl of 10 mM dNTP (ThermoFisher Scientific, USA) and 0.28 µl of Taq-Polymerase (on Taq DNA Polymerase "hot start", EUR_X, Poland). The PCR conditions consisted of 5 min of initial denaturation at 95°C; 30 s of denaturation at 94°C, followed by 30 s of annealing at 50°C and 90 s elongation at 72°C for 40 cycles; followed by a final elongation step for 10 min at 72°C. A 3 µl sample of the PCR product was run on a 1.5% agarose gel for 30 min at 100 V to verify DNA quality. PCR products were cleaned and sequenced by Genomed (Warsaw, Poland). A total of 21 sequences were deposited in the GenBank database (Table 1).

Table 1. Accession codes of COI sequences used in this study. CEP-UJ DNA – label of DNA-sampled specimen in the CEP-UJ collection.

Genus	Species/subspecies	Country	Locality	Collector	CEP-UJ DNA	GenBank Code
Punargentus	*heimlichi*	Peru	San Marcos	T. Pyrcz	2230	OR102506
Punargentus	*heimlichi*	Peru	San Marcos	P. Pyrcz	3699	OR102507
Punargentus	*heimlichi*	Peru	San Marcos	T. Pyrcz	3700	OR102508
Punargentus	*heimlichi*	Peru	Huacaspata	T. Pyrcz	5414	OR102509
Punargentus	*blanchardi blanchardi*	Peru	Aylambo	T. Pyrcz	3701	OR102510
Punargentus	*blanchardi blanchardi*	Peru	Aylambo	T. Pyrcz	3702	OR102511
Punargentus	*blanchardi blanchardi*	Peru	Aylambo	T. Pyrcz	1810	OR102512
Punargentus	*idealis idealis*	Peru	Orcos	T. Pyrcz	3703	OR102513
Punargentus	*idealis idealis*	Peru	Orcos	T. Pyrcz	3704	OR102514
Punargentus	*rubrostriata* ssp.	Peru	Pastoruri	T. Pyrcz	3705	OR102515
Punargentus	*rubrostriata* ssp.	Peru	Pastoruri	T. Pyrcz	3707	OR102516
Punargentus	*atusparia atusparia*	Peru	Pastoruri	T. Pyrcz	3709	OR102517
Punargentus	*atusparia atusparia*	Peru	Recuay	T. Pyrcz	3710	OR102518
Punargentus	*atusparia atusparia*	Peru	Huacllan	T. Pyrcz	3713	OR102519
Punargentus	*atusparia atusparia*	Peru	Curcuy	T. Pyrcz	3714	OR102520
Punargentus	*atusparia atusparia*	Peru	Curcuy	T. Pyrcz	3715	OR102521
Punargentus	*atusparia yupania*	Peru	Yupan	T. Pyrcz	5444	OR102522
Punargentus	*atusparia yupania*	Peru	Yupan	T. Pyrcz	5445	OR102523
Punargentus	*atusparia yupania*	Peru	Yupan	T. Pyrcz	5446	OR102524
Punargentus	*angusta* ssp.	Peru	La Oroya	T. Pyrcz	2872	OR102525
Punargentus	*lamna* ssp.	Peru	Huanuco	T. Pyrcz	2228	OR102526

Table 2. Locality information and NCBI Accession numbers of Target Enrichment sequences used in this study. Data for the sample in **bold** were taken from Pyrcz et al. (2023).

Genus	Species/subspecies	Country	Locality	Collector	Extraction code	NCBI SRA Number
Nelia	*ureta*	Chile	Volcan Calbuco	D. Benyamini	DLC-643	SAMN37475567
Auca	*barrosi*	Chile	Ibacache	T. Pyrcz	DLC-626	SAMN37475568
Argyrophorus	*argenteus*	Chile	Liucura	P. Boyer	DLC-621	SAMN37475569
Palmaris	*antarcticus*	Chile	El Chalten	P. Boyer	DLC-644	SAMN37475570
Palmaris	*monticolens monticolens*	Chile	Pino Hachado	P. Boyer	DLC-623	SAMN37475571
Palmaris	*gustavi gustavi*	Chile	Parinacota	P. Boyer	DLC-622	SAMN37475572
Pampasatyrus	*gyrtone biezankoi*	Brazil	Urupema	T. Pyrcz	DLC-637	SAMN37475573
Pampasatyrus	*gorkyi*	Peru	Espinar	T. Pyrcz	DLC-600	SAMN37475574
Pampasatyrus	*nilesi*	Argentina	El Molle	P. Boyer	DLC-641	SAMN37475575
Pampasatyrus	*quies*	Brazil	Urubici	T. Pyrcz	DLC-642	**SAMN29835187**
Pampasatyrus	*glaucope boenninghauseni*	Brazil	Campos do Jordao	T. Pyrcz	DLC-636	SAMN37475576
Pampasatyrus	*reticulata reticulata*	Brazil	S.J. dos Ausentes	T. Pyrcz	DLC-638	SAMN37475577
Pampasatyrus	*edmondsii*	Argentina	Villavicencio	P. Boyer	DLC-630	SAMN37475578
Etcheverrius	*tandilensis*	Argentina	La Ventana	P. Boyer	DLC-614	SAMN37475579
Etcheverrius	*chiliensis elwesi*	Chile	Chillan	T. Pyrcz	DLC-595	SAMN37475580
Punargentus	*angusta* ssp.	Peru	La Oroya	T. Pyrcz	DLC-625	SAMN37475581
Punargentus	*lamna* ssp.	Peru	Huanuco	T. Pyrcz	DLC-604	SAMN37475582
Punargentus	*heimlichi*	Peru	San Marcos	T. Pyrcz	DLC-605	SAMN37475583
Punargentus	*blanchardi blanchardi*	Peru	Aylambo	T. Pyrcz	DLC-602	SAMN37475584

For target enrichment (TE) samples, DNA was extracted using the Qiagen Blood & Tissue kit with the modifications given in Mayer et al. (2021). We used the TE probe kit from Mayer et al. (2021) and the molecular work followed the protocols of that study. Raw sequence data was processed using the TEenriAn workflow (Mayer et al., 2021) as modified in Pyrcz et al. (2023). The raw sequence files were submitted to the NCBI Sequence Read Archive with accession numbers SAMN37475567–584 (see Table 2).

Sequence alignment and phylogenetic analyses

Target enrichment data for five *Punargentus* samples and 15 outgroups, selected from among other genera of the infratribe Neosatyriti (Matz & Brower, 2016) of the subtribe Pronophilina, were processed (Table 2). In addition to the 19 cytochrome C oxidase I (COI) sequences generated for this study, COI sequences for three *Punargentus angusta* specimens (Record ID: FAHAA1338-18, FAHAA1339-18, and FAHAA1340-18 from Huancayo, Junín, Peru) and three *P. lamna* specimens (Record ID: GBLN3084-10, GBLN3085-10, and GBLN3086-10 from Pachacayo, Junín, Peru) were downloaded from the BOLD database (http://boldsystems.org). The Sanger COI data were aligned with the target enrichment COI data using MAFFT v.7.490 with the -add option (Katoh & Standley, 2013). All loci were concatenated using AMAS (Borowiec, 2016), and the full dataset contained COI + 620 nuclear loci (248,285 bp) and 42 samples.

The dataset was partitioned according to locus and models selected using ModelFinder (Kalyaanammorthy et al., 2017) in IQ-Tree (Minh et al., 2020). A tree was inferred using maximum likelihood (ML) in IQ-Tree. Twenty tree searches were performed to assess topological stability. Ultrafast bootstrap with the -bnni option to reduce the effect of model violation (UB, Hoang et al., 2018) and the SH-like approximate likelihood ratio test (ALRT, Guindon et al., 2010) were calculated as measures of branch support. The final tree was prepared using the R package ggtree (Yu et al., 2017).

The alignments, selected models and the tree file can be found in the supplementary data on Zotero: 10.5281/zenodo.8214754

RESULTS

Genus *Punargentus* Heimlich, 1963

Punargentus Heimlich, 1963: 78. Type species was designated by Heimlich as *Argyrophorus lamna* Thieme, 1904, but Heimlich actually examined a male of *Argyrophorus lamna* form *angusta* (Weymer, 1911), which is a separate species. Therefore, the correct generic type species is *Punargentus angusta* (Weymer).

Punargentus Heimlich: Pyrcz, 2010: 214 (as syn. of *Argyrophorus* Blanchard, 1852); Matz & Brower, 2016: 60.

There are hardly any distinctive, external morphological features for separating the genus *Punargentus* from other genera of the infratribe Neosatyriti (some putative features were pointed out by Matz & Brower, 2016), which is why, among others, its representatives were previously associated with, for example *Argyrophorus* (Pyrcz, 2010). In fact, the five species in the *Punargentus lamna* clade (i.e., *P. lamna, P. angusta, P. rubrostriata* Pyrcz, Cerdeña & Florczyk, 2019, and *P. idealis* Pyrcz & Cerdeña, 2019) share with *Argyrophorus* a conspicuous silver patch covering a large portion of the FWV, and sometimes also the HWV. The male genitalia are very simple and differ little from some other genera of the Neosatyriti, in particular *Argyrophorus, Etcheverrius* Herrera, 1965*, Palmaris* Herrera, 1965 and *Pampasatyrus* Hayward, 1953, the latter of which is widely sympatric with *Punargentus*. The species of *Punargentus* can be distinguished from the above mentioned four other genera by the stout and short uncus, widened laterally and, in most species, also bulged ventrally in the middle, in contrast to *Etcheverrius* which also has a stout but shorter, gradually narrowing, tubular uncus. *Punargentus* shares with *Etcheverrius* another, otherwise unique character in the female genitalia, the papillae anales are covered with dense and very long hair. *Punargentus* is distributed from northern Bolivia (Yungas de La Paz) to northern Peru (southern Cajamarca). Before this study,

Fig. 1. Photographs of adults of the *Punargentus blanchardi* clade. A – *P. atusparia atusparia* sp. n., male, dorsal view (Laguna Curcuy); B – idem, ventral view; C – *P. atusparia atusparia* sp. n., female, dorsal view (Laguna Curcuy); D – idem, ventral view; E – *P. atusparia yupania* ssp. n., male, dorsal view (above Yupán); F – idem, ventral view; G – *P. atusparia yupania* ssp. n., female, dorsal view (above Yupán); H – idem, ventral view; I – *P. blanchardi libertas* ssp. n., male, dorsal view (Chongos); J – idem, ventral view; K – *P. blanchardi libertas* ssp. n., female, dorsal view (Chongos); L – idem ventral view; M – *P. blanchardi blanchardi*, male, dorsal view (Aylambo); N – idem, ventral view; O – *P. blanchardi celendini*, male, dorsal view (Sucre); P – idem, ventral view; R – *P. heimlichi* sp. n., male, dorsal view (San Marcos); S – idem, ventral view; T – *P. heimlichi* sp. n., female, dorsal view (Chavín de Huántar); U – idem, ventral view.

four species were recognized, *Punargentus lamna*, which is distributed throughout the geographic range of the genus, *P. angusta* found in south-central Peru, and two species restricted to the department of Ancash in central Peru, *P. rubrostriata* and *P. idealis*.

Key to the identification of species

1 FWD with a silver patch ..... species of the *Punargentus lamna* clade
– FWD without any silver markings (species of the *Punargentus blanchardi* clade) 2
2 FWV with a brick red or orange suffusion from base to median area ..... *P. atusparia* sp. n.
– FWV all brown or only slightly suffused with chestnut in basal and post basal area 3
3 Hindwing roughly triangular ..... *P. heimlichi* sp. n.
– Hindwing oval ..... *P. blanchardi*

Descriptions of new taxa

Punargentus atusparia Pyrcz & Boyer, sp. n.

ZooBank taxon LSID:
883D1E57-DDB8-478D-B0A0-C9EE9F6B137F

Punargentus sp.: Matz & Brower, 2016: 10, 79, fig. 1, tab. 2.

Diagnosis

This species is most similar to the northern allopatric, *P. blanchardi* (Pyrcz & Wojtusiak, 2010), with a similar size, wing shape and HWV pattern of transverse lines. It differs in having a dark, blackish brown upper surface, whereas in *P. blanchardi* it is chestnut, with shorter, although quite variable, FWD subapical and submarginal yellow streaks, in some individuals almost absent, whereas well-marked streaks, usually four or, in some individuals, five in *P. blanchardi*; more contrasting HWV colour, with

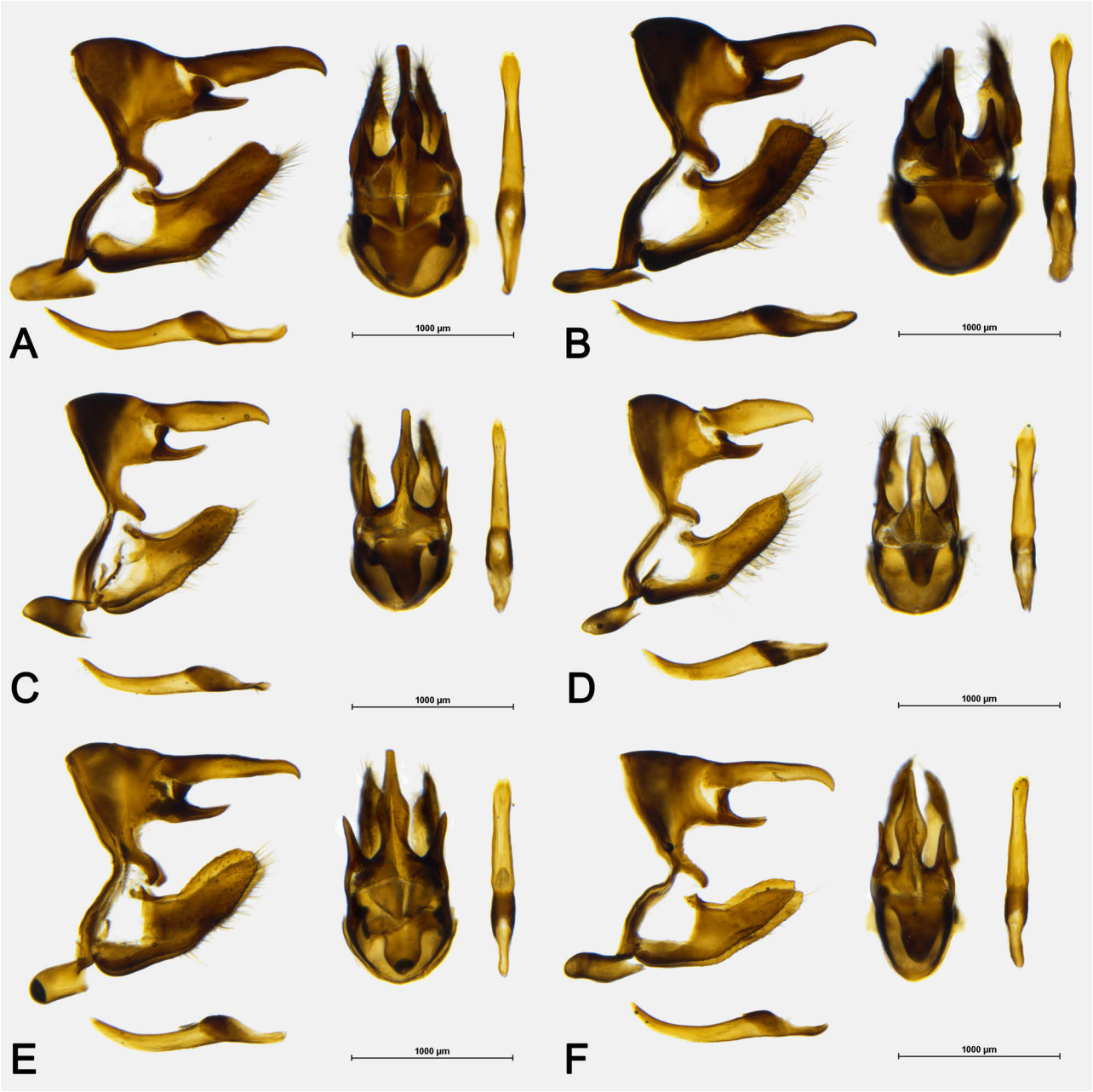

Fig. 2. Male genitalia of species in the *Punargentus blanchardi* clade (lateral view: left and dorsal view: right): A – *P. blanchardi libertas* ssp. n. (Huancaspata); B – *P. blanchardi libertas* ssp. n. (Huanapampa); C – *P. atusparia yupania* ssp. n. (Yupán); D – *P. heimlichi* sp. n. (San Marcos); E – *P. atusparia atusparia* sp. n. (Pastoruri); F – *P. atusparia atusparia* sp. n. (Recuay).

a marked steely whitish overcast and regular submarginal line, whereas it is irregular and interrupted in *P. blanchardi;* also in having a larger FWV subapical double ocellus, but most of all by the presence of a large crimson red FWV patch extending from the base of the wing to the postdiscal area, without reaching the subapical ocellus.

Description

Male (Figs 1A, B): **Head**: eyes dark brown, naked; antennae reaching 2/5 of the length of the costa, with large, spatulate clubs. **Wings**: FW average length 18,8 mm (n = 19), triangular with a subacute apex and slightly concave margin and long fringes, mostly dark brown or sandy yellow in subapical and apical area. FWD shiny walnut brown, gradually becoming darker distally; three to five inter-venal yolk yellow stripes extending from postdiscal to submarginal area in R_5-M_1, M_1-M_2, M_2-M_3, M_3-CuA_1 and CuA_1-CuA_2, enclosing a blackish brown ocellus in the middle, except in the shorter R_5-M_1 and M_3-CuA_1. FWV ground colour blackish brown with a brick red suffusion, variable in size and intensity, but in most of the individuals examined extending to postdiscal area from costa to vein CuA_1; two large, joined sub marginal black ocelli with white pupils, in M_1-M_2 and M_2-M_3, circled with yellow, occasionally a small yellow dot on CuA_1-CuA_2, subapical and apical area suffused with light grey and magenta, along with a smooth, black marginal line. HW oval with a smooth outer margin; fringes long, grey brown. HWD

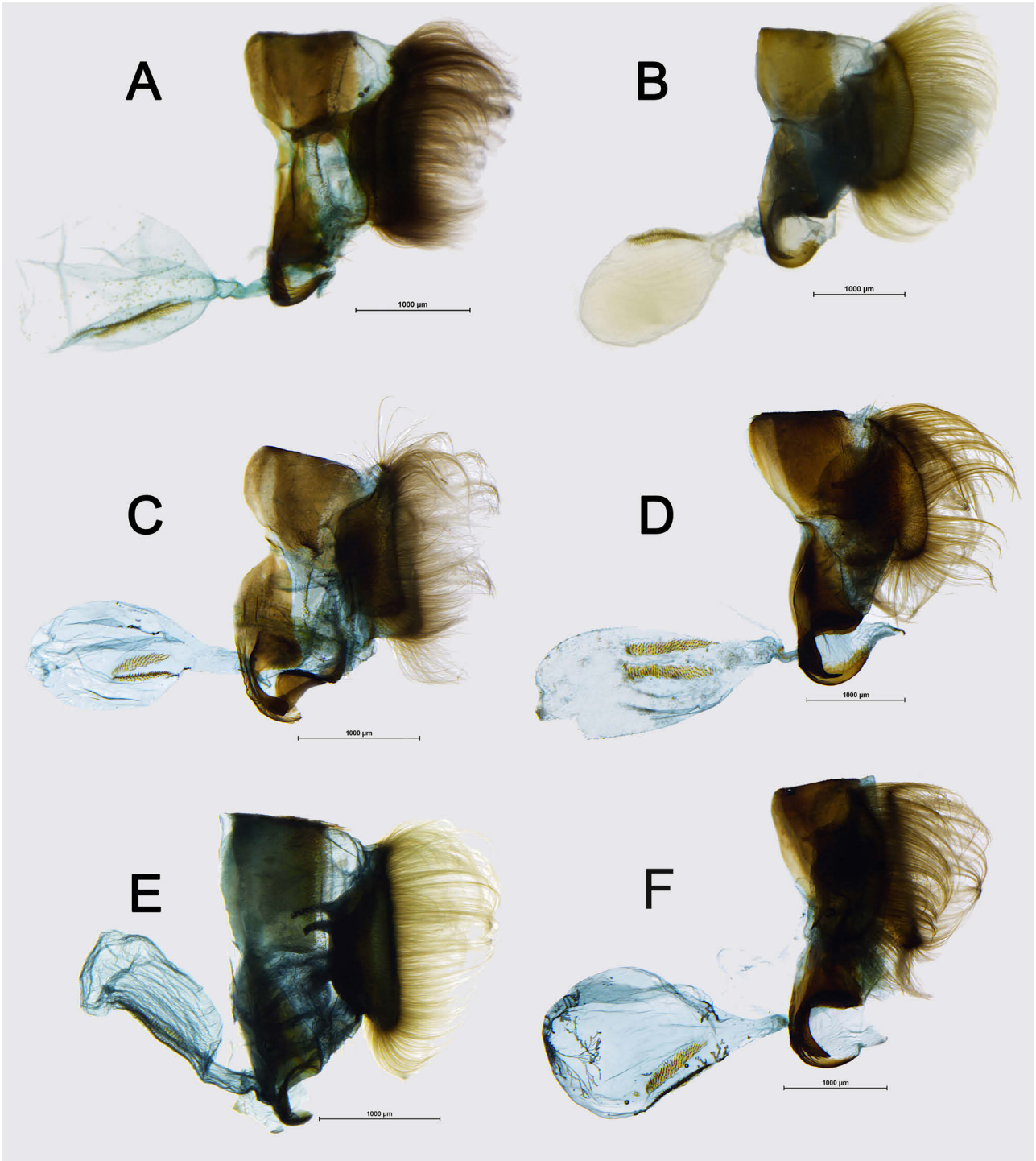

Fig. 3. Female genitalia of species in the *Punargentus blanchardi* clade (in lateral view). A – *P. atusparia atusparia* sp. n., prep. genit. 1481 (Curcuy); B – *P. blanchardi blanchardi*, prep. genit. H_71 (Aylambo); C – *P. atusparia yupania* ssp. n., prep. genit 10252 (Yupan – Bamba); D – *P. blanchardi libertas* ssp. n., prep. genit. 10254 (Chongos); E – *P. heimlichi* sp. n., prep. genit. 12853 (San Marcos).

colour same as FW, walnut brown, homogenous. HWV ground colour cedar brown heavily dusted with lighter brown and white scales, slightly denser in postdiscal and submarginal areas; a series of black narrow bands extending from costa towards anal margin, one, extremely irregularly, from post basal area, across mid discal cell, into the space CuA_2-1A/1B, the second, postdiscal, equally irregular, sharply displaced along the veins, reaching both costal and anal margin, a submarginal zigzagging, and smooth marginal veins, interrupted by veins with whitish and magenta scales; a series of sandy-yellow inter-venal stripes extending from the postdiscal to the submarginal bands, one in each cell, except for CuA_1-1A/1B. **Genitalia** (Figs 2E, 2F): Tegumen stout, with a flat dorsum, uncus aligned with dorsum of tegumen (unlike *P. blanchardi* where the dorsum of the tegumen is convex and the uncus is raised), one and a half times its length (longer and thinner than in *P. atusparia yupania* ssp. n.), slightly flattened dorso-ven-

trally and wider in the middle (in contrast to *P. heimlichi* sp. n. where the uncus is considerably stouter, raised but not wider in the middle), with a short tip curved downwards, subunci aligned with the uncus, half the length of the tegumen, with a wide base and a sharp tip; pedunculus long with a blunt tip, slightly curved; sacculus wide with a blunt extremity, the valvae with a blunt tip and a delicately serrated dorsum, approximately one and a half times the length of the uncus (longer than in *P. atusparia yupania* ssp. n.), aedeagus tubular, the same length as the valvae, with a slightly upcurved tip, approximately the same width throughout, except for the narrower proximal opening.

Female (Figs 1C, D): Sexual dimorphism slight (FW average length 19,2 mm; n = 4), expressed in terms of oval wings, more conspicuous light orange markings on upper surface and a slightly lighter HWV ground colour. **Genitalia** (Fig. 3A): Wide, puffy papillae anales (same as in *P. heimlichi* sp. n. and *P. blanchardi*) with prominent posterior apophysis (wider than in *P. atusparia yupania* ssp. n., *P. heimlichi* sp. n. and *P. blanchardi*); post vaginal plate wide, pocket-like, strongly sclerotized, with a smooth surface (similar to *P. heimlichi* sp. n. and *P. blanchardi*); ductus bursae slightly sclerotized only basally, moderately long, opening gradually into a large (bigger than in *P. heimlichi* sp. n.), balloon-like corpus bursae with a smooth surface, with two, parallel signa, approximately two-thirds the length of the bursa (longer than in *P. atusparia yupania* ssp. n. and *P. heimlichi* sp. n.).

Type material. Peru, Ancash. Holotype ♂: "Peru, Departamento Ancash, Route 109, Recuay-Huarmey, eastern slopes, S09°43′14″ W77°28′50″, 3800 m, 21.vi.2021, P. Boyer leg.", currently in CEP-UJ (to be deposited in MUSM). Paratypes (161♂ and 78♀): 9♂ and 2♀: same data as the holotype, PBF; 22♂ and 5♀: Carretera a Pastoruri km 2 depuis Route 3N, S09°51′13″ W77°23′57″, 3600 m, 18.vi.2021, P. Boyer leg., PBF; 27♂ and 9♀: Laguna Curcuy, 3 km Est de Huaylas, S08°52′33″ W77°52′26″, 2850 m, 19.vi.2021, P. Boyer leg., PBF; 2♂ and 7♀: Route 109, Huarmey vers Recuay km 87, versant occidental, S09°48′7″ W77°38′15″, 3040 m, 21.vi.2021, P. Boyer leg., PBF; 4♂ and 2♀: Tuctu, S Huaraz, S09°42′15″ W77°27′38″, 3380 m, 11.vi.2022, P. Boyer leg., PBF; 4♂: AN1225, km 1 à 6, Utcuyacu, S09°50′31″ W77°26′09″, 3600–3900 m, 11.vi.2022, P. Boyer leg., PBF; 10♀: 3 km E Huaylas, Laguna Curcuy, 08°52.68′S; 77°52.49′W; 2880–2980 m, 19.06.2021; T. Pyrcz leg. CEP-UJ; 29♂ and 6♀: 6 km S Cátac, Via Pastoruri, 09°51.20′S; 77°24.08′W, 3730–3760 m, 18.06.2021, T. Pyrcz leg., CEP-UJ; 4♂ and 7♀: Huarmey-Recuay 2 km E Huacllán, 09°47.89′S 77°39.48′W, 3040–3050 m, 21.06.2021, T. Pyrcz leg., CEP-UJ; 10♂ and 1♀: Cordillera Negra, 3 km W Recuay, 09°43.55′S, 77°29.11′W, 3800–3850 m, 21.06.2021, T. Pyrcz leg., CEP-UJ; 30♂: 3 km E Huaylas, Laguna Curcuy, 08°52.68′S, 77°52.49′W, 2880–2980 m, 20.06.2021, T. Pyrcz leg., CEP-UJ; 18♂ and 2♀: Recuay-Tuctu; 9°41′5″S; 77°28′25″W; 3350–3400 m; 11.VI.2022; T. Pyrcz leg., CEP-UJ; 10♂, 2♀: Cerro Amancaes, cerca (= near) Santo Toribio, 3000 m, [08°50′S, 77°54′W], 22.v.[19]80, G. Lamas leg., MUSM; 3♂, 2♀: Huaylas, [Laguna] Curcuy, 2900 m, [08°53′S, 77°52′W], 19.v.[19]81, P. Hocking leg., MUSM; 2♀: cerca (= near) de Cajacay, 3000 m, 10°08′S, 77°24′W, 16.vii.2006, C. Peña leg., MUSM; 3♂: Recuay, Utcuyacu, S 09°49′29″ W 77°25′19″, 3620 m, 11.vi.2022, J. Farfán leg., MUSA; 1♀: same data, J. Cerdeña leg., MUSA; 2♂: Recuay, 2 Km N Recuay, S 09°42′15″ W 77°27′37″, 3380 m, 11.vi.2022, J. Farfán leg., MUSA; 3♂ and 1♀: Aija, ruta Huarmey – Aija, S 09°48′01″ W 77°38′21″, 3100 m, 21.vi.2021, J. Cerdeña leg., MUSA; 4♂ and 2♀: Recuay, ruta Recuay – Aija, S 09°43′24″ W 77°28′29″, 3100 m, 21.vi.2021, J. Cerdeña leg., MUSA; 2♂: Huaylas, 2 Km SE Laguna Curcuy, S 08°52′38″ W 77°52′21″, 2800 m, 20.vi.2021, J. Cerdeña leg., MUSA; 10♂ and 4♀: Huaylas, 2 Km E Huaylas, S 08°52′35″ W 77°52′29″ , 2800 m, 19.vi.2021, J. Cerdeña leg., MUSA; 5♂ and 5♀: same data, J. Farfán leg., MUSA; 10♂ and 3♀: Catac, 7 Km S Catac, S 09°51′12″ W 77°23′59″, 3700 m, 18.vi.2021, J. Farfán leg., MUSA; 8♂ and 7♀: same data, J. Cerdeña leg., MUSA.

Type locality. Peru, Ancash, Recuay – Huarmey, road 109.

Etymology. This species is named after Pedro Pablo Atusparia (1840–1887), born in Huaraz, the leader of the largest local rebellion of indigenous people in the region of Ancash, which took place in 1885, in the context of the 1884–1885 Peruvian civil war, which affected the entire area where this species occurs.

Distribution and habitat

This species is reported only in the department of Ancash where it occurs throughout the so-called Callejón de Huaylas, the middle part of the valley of the Río Santa, between the parallel ranges of the Cordillera Blanca to the east and the Cordillera Negra to the west (Fig. 6) and also locally on the Pacific slopes of the latter. The most likely northern limit to its distribution is the deep valley where the Santa River turns abruptly westwards towards the Pacific coast, the so-called Cañón del Pato and in the south in the canyon of the Marca River. It is not reported further south in the well-sampled valley of Ocros. It is recorded from 2800 m near Caraz to 3800 m in the valley below the Nevado Pastoruri. *Punargentus atusparia atusparia* sp. n. is widespread in the Callejón de Huaylas wherever suitable *matorral puna* habitats occur and *Stipa* bunch-grasses are abundant (larval host-plants of *Punargentus*) (Fig. 5). It tolerates disturbance and occurs in secondary pine and eucalyptus forests if there is grass in the understory. Even though its distribution area is a heavily populated and intensively cultivated region where natural puna has been replaced by crops and its habitat is certainly shrinking, no species protection measures are currently needed. There are some morphological differences between the various populations with those from Pastoruri the darkest and those from the western slopes below Aija more patterned and lighter in colour, and those from Caraz the smallest. However, there is also considerable individual variation within each population and all the colour pattern forms are present, but their frequency varies.

Punargentus atusparia yupania **Pyrcz & Cerdeña, ssp. n.**

ZooBank taxon LSID:
34F95B98-F121-4D35-BF28-15D47E5AB2F0

Diagnosis and description

Male (Figs 1E, F): This subspecies is considerably smaller than the nominotypical subspecies (FW average length: 15 mm; n = 10), the colour of the upper surface of the wings is lighter, beige with a marked steely flush, the subapical black dots are fainter as are the yellowish streaks, when apparent; in some specimens there is only

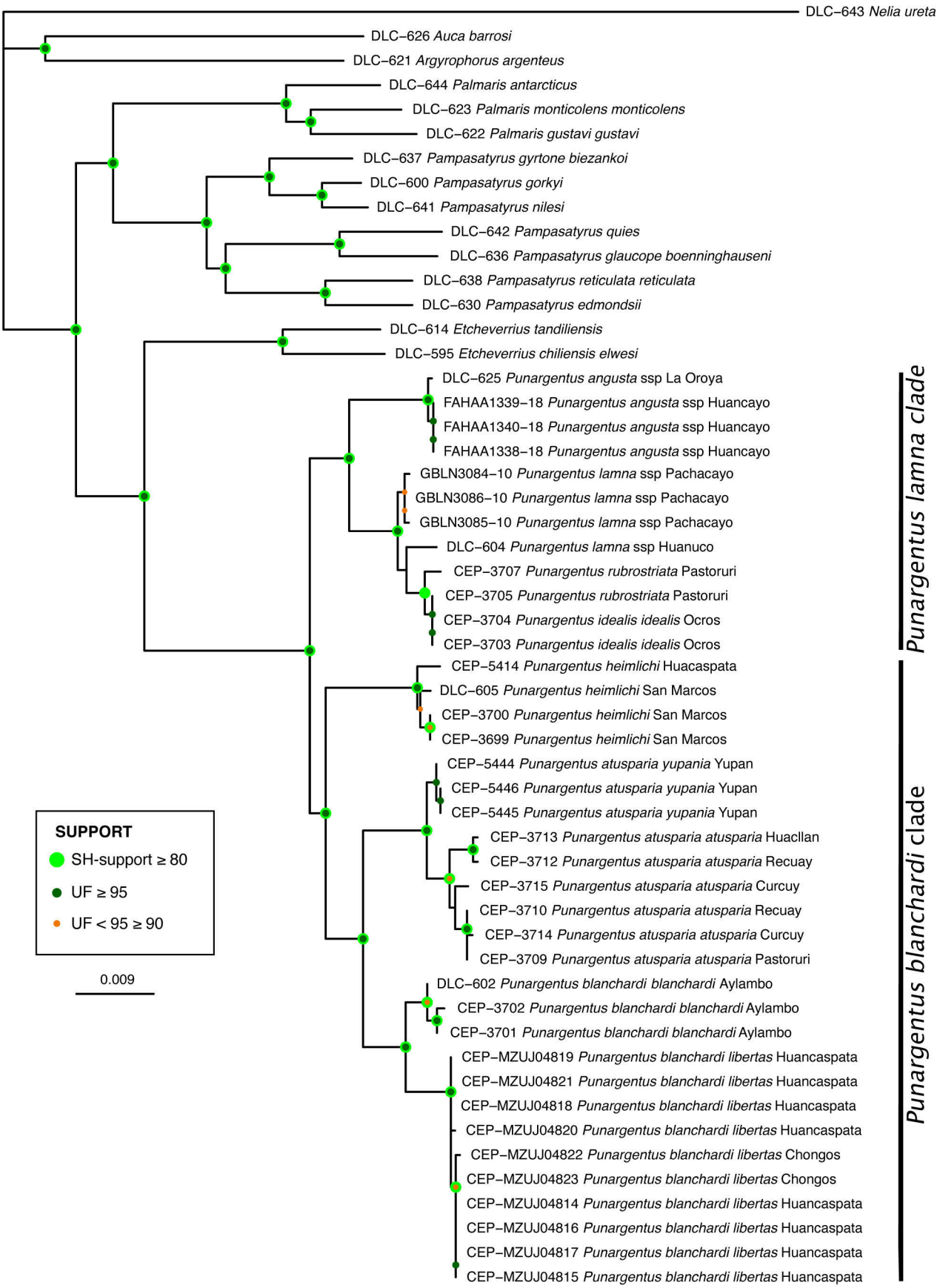

Fig. 4. Maximum likelihood tree based on both the Sanger and target enrichment data as inferred in IQ-tree. Explanation of the support values are in the box at the left of the base of the tree.

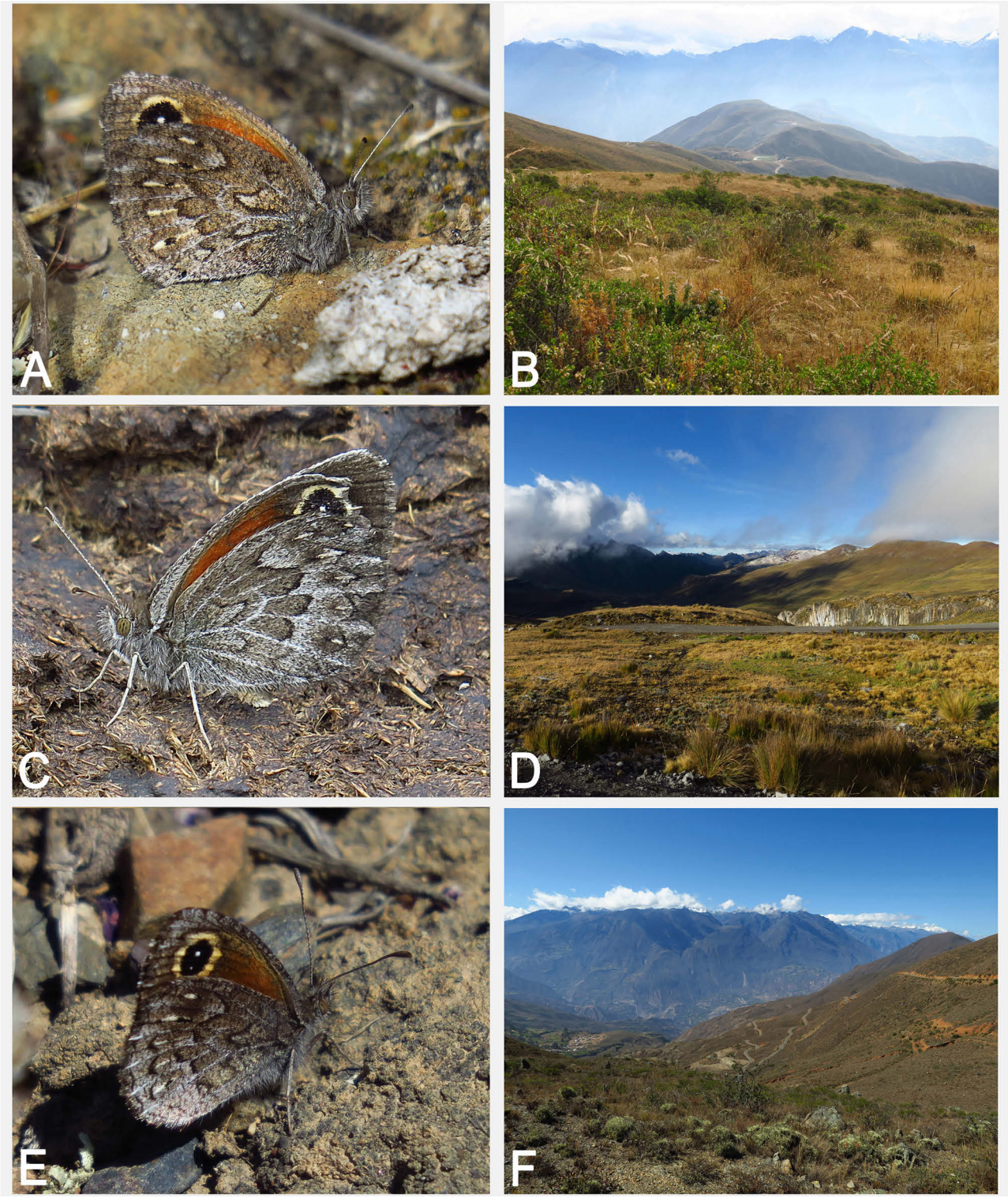

Fig. 5. Photographs of subspecies of *Punargentus atusparia* in the field and of their natural habitats. A – *P. atusparia atusparia* sp. n., male, Laguna Curcuy; B – Andean shrubland near Laguna Curcuy above Huaylas, 3000 m; C – *P. atusparia atusparia* sp. n., male, valley of Pastoruri; D – puna vegetation in the valley of Pastoruri, 3600 m; E – *P. atusparia yupania* ssp. n., male, above Yupán. F – dry Andean shrubland between Yupán and Bambas, 3000 m (photographs taken by P. Boyer).

a faint russet suffusion at the base of the under surface of the forewing and the discal cell, but in most cases there is none and the ground colour of the hindwing is dull grey, considerably less contrasting than in the nominotypical subspecies. **Genitalia** (Fig. 2C): They differ mostly in that the uncus is shorter and bulkier and valvae shorter in *P. atusparia yupania*.

Female (Fig. 1G, H): Sexual dimorphism is slight (FW average length: 16,6 mm; n = 3), with the female a light beige colour with an even more prominent golden-steely

flush and more prominent forewing with yellow markings on the subapical part of the upper surface. **Genitalia** (Fig. 3C): Do not differ from those the nominotypical subspecies.

Type material. Peru, Ancash: Holotype ♂: "Peru, Departamento Ancash, Yupán – Bambas; 8°36′44″S/77°59′54″W; 3100–3120 m; 12.vi.2022; T. Pyrcz leg.", currently in CEP-UJ (to be deposited in MUSM); Paratypes (55♂ and 9♀): 6♂ and 2♀: Route 3N, Yupán vers Llapo, S08°37′17″ W77°58′55″, 3200 m, 12.vi.2022, P. Boyer leg., PBF; 5♂ and 1♀: Bambas, Route 3N, Yupán vers Llapo, S08°36′03″ W77°59′55″, 3600 m, 12.vi.2022, P. Boyer leg., PBF; 3♂: Tauca vers Bambas, S08°32′39″ W78°02′15″, 3300 m, 13.vi.2022, P. Boyer leg., PBF; 23♂ and 1♀: Yupán – Bambas; 8°36′44″S/77°59′54″W; 3100–3120 m; 12.vi.2022; T. Pyrcz leg., CEP-UJ; 4♂ and 1♀: 2 km N Bambas; 8°35′0″S/77°59′59″W; 2850–2860 m; 13.vi.2022; T. Pyrcz leg., CEP-UJ; 1♂: Yánac, 2860 m, 08°37′S, 77°52′W, 21.vii.2006, C. Peña leg., MUSM; 7♂ and 4♀: Yupán, 2 Km O ruta hacia Bambas, S 08°37′19″ W 77°59′00″, 3210 m, 12.vi.2022, J. Farfán leg., MUSA; 3♂: same data, J. Cerdeña leg., MUSA; 1♂: Yupán, 3 Km O ruta hacia Bambas, S 08°36′45″ W 77°59′55″, 3200 m, 12.vi.2022, J. Farfán leg., MUSA; 1♂: Yupán, 2.5 Km O ruta hacia Bambas, S 08°36′53″ W 77°59′32″, 3380 m, 12.vi.2022, J. Farfán leg., MUSA; 1♂: Bambas, ruta hacia Tauca, S 08°35′19″ W 78°00′16″, 2910 m, 12.vi.2022, J. Farfán leg., MUSA.

Type locality. Peru, Ancash, Yupán – Bambas.

Etymology. This subspecies is named after the town of Yupán, just above which it was first discovered.

Distribution and habitat

This subspecies occurs in the upper valley of the Chuquicara River, a tributary of the Santa River (Fig. 6). The geographical barrier between the areas of distribution of the two subspecies is the extremely narrow Cañón del Pato. *P. a. yupania* occurs in several places between Yupán, Bambas and Conamires at 3000–3400 m in both open puna grassland and shrubby *matorral* puna, which is slightly drier than the usual habitat of the nominotypical subspecies (Fig. 5). Individuals are active in late morning and behave in a similar way to other congeners of the *P. blanchardi* group, flying low above the vegetation, feeding on nectar of mostly yellow Asteraceae and basking on rocks and sandy ground. When the temperature is high they seek refuge in shaded places and become inactive. Other interesting species of butterflies found in the same habitat include *Phulia phantasma* Lamas, Boyer & Willmott, 2022, *Eldoradina cyanea* (Baletto, 1993), *Nabokovia faga* (Dognin, 1895), *Strymon heodes* (Druce, 1909), *Itylos* sp. or *Mathania* sp. *P. a. yupania* ssp. n. is common in its habitat, and since the areas where it occurs, rocky or sandy dry slopes, are not suitable for agriculture, it is not currently threatened.

Punargentus blanchardi libertas Pyrcz & Farfán, ssp. n.

ZooBank taxon LSID:
093911CF-03BC-493B-8C54-2D886CCCDA67

Diagnosis and description

Male (Figs 1I, J): This subspecies differs from the nominotypical subspecies (Figs 1M, N) and *P. b. celendini* (Pyrcz & Wojtusiak, 2010) (Figs 1O, P) by the darker

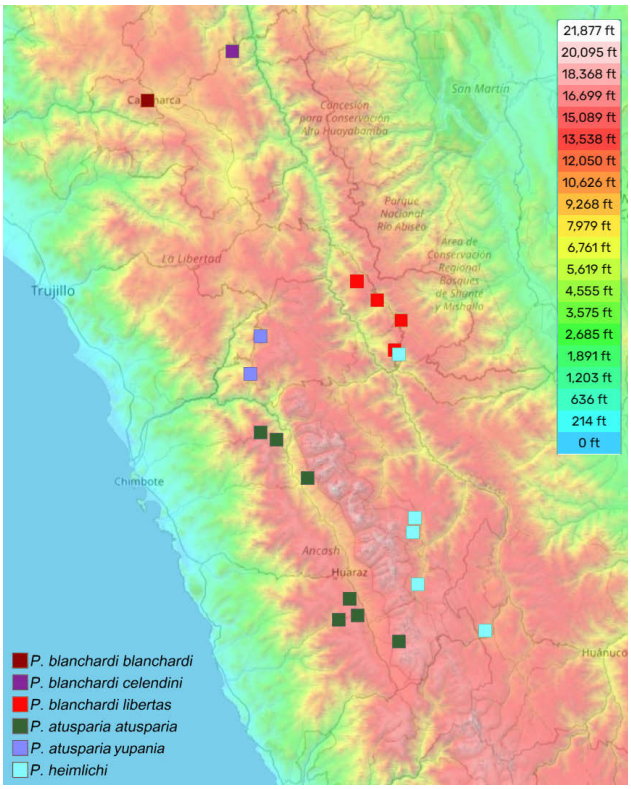

Fig. 6. Map showing the distributions of the species of the *Punargentus blanchardi* clade (en-us.topographic-map.com).

brown upper- and under surfaces of the forewing and a more contrasting, blackish brown with white dusting, than the dull brown of *P. b. celendini*, with veins outlined in white like the nominotypical subspecies, which however have a darker whitish colour. The three subspecies differ in the arrangement of the blackish transverse lines on the hindwing, with the submarginal line in the nominotypical sub species more clearly defined and extending further from the distal margin than in the other two, the postdiscal line consists of half-moons in the nominotypical subspecies and *P. b. libertas* ssp. n. and is irregular in *P. b. celendini*. In addition, there are usually two subapical dots on the under surface of the forewing in *P. b. libertas* ssp. n., and only one in the other two. The size of *P. b. libertas* ssp. n. is very variable but on average it is smaller than *celendini* and slightly larger than the nominotypical subspecies. There are appreciable differences in the colour pattern of populations of *P. b. celendini* at Huanapampa, Huancaspata and Chongos, but the recognition of further subspecies would be premature. **Genitalia** (Figs 2A, B): Tegumen strong, with a domed dorsum (similar to *P. heimlichi* sp. n. but unlike the flat dorsum in *P. atusparia* sp. n.), uncus higher in relation to the dorsum of the tegumen (unlike *P. atusparia* sp. n. whose uncus is in alignment with the dorsum of the tegumen, one and a half times its length (considerably longer than in *P. heimlichi* sp. n.), slightly flattened dorso-ventrally and wider in the middle (unlike in *P. heimlichi* sp. n. whose uncus is not wider in the middle), with a sharp tip curved downwards (more noticeably than in *P. atusparia* sp. n.), subunci aligned with the uncus for

approximately one-fourth the length of the uncus (much shorter than in *P. atusparia* sp. n.) with a sharp tip, the gap between uncus and subunci is narrow (as in *P. heimlichi* sp. n. but unlike in *P. atusparia* sp. n. where the gap is wider); pedunculus long with a blunt tip, slightly curved downwards; the sacculus is wide but differs between populations, the valvae with a wide, blunt and irregular tip (unlike *P. atusparia* sp. n. and *P. heimlichi* sp. n. where the tips are sharp), a smooth dorsum, approximately one and a half times the length of the uncus (as in *P. atusparia* sp. n.), aedeagus tubular, slightly longer than the valvae, with a slightly upcurved tip, approximately the same width throughout, except for the narrower proximal opening.

Female (Figs 1K, L): Sexual dimorphism is slight, with the female a lighter brown colour and with more prominent yellow markings on the upper surfaces of the forewings. **Genitalia**: As illustrated (Fig. 3D). Those of the nominate subspecies (Fig. 3B) are similar to *P. atusparia* sp. n., differ from *P. heimlichi* sp. n., which has a larger corpus bursae and signa, and from the nominate *P. atusparia* sp. n. in having a less prominent posterior apophysis.

Type material. Peru, La Libertad. Holotype ♂: "Peru, Departamento La Libertad, Chongos; 8°13′38″S/77°19′54″W; 3150–3180 m; 19.vi.2022; T. Pyrcz., leg.", currently in CEP-UJ (to be deposited in MUSM); Paratypes (59♂ and 26♀): 5♂ and 3♀: Tayabamba – Utcubamba, Route 129 km 7, S08′15′05″ W77°17′21″, 3400 m, 16.vi.2022, P. Boyer leg., PBF; 14♂ and 9♀: Chongos, vers Huancaspata, S08′28′11″ W77°17′02″, 3200 m, 15.vi.2022, P. Boyer leg., PBF; 15♂ and 10♀: Chongos, 8°13′38″S/77°19′54″W; 3150–3180 m, 19.vi.2022; T. Pyrcz., leg., CEP-UJ; 7♂: Route 12A, Sihuas, T. Pyrcz leg., CEP-UJ; 10♂ and 1♀: 1,5 km W Huanapampa, 8°15′17″S 77°17′21″W; 3400–3420 m, 16.vi.2022; T. Pyrcz leg., CEP-UJ; 4♂: 2 km SW Huancaspata, 8°28′12″S/77°17′3″W, 3150–3250 m, 15.vi.2022, T. Pyrcz leg., CEP-UJ; 2♂ and 2♀: Tayabamba, ruta Tayabamba – Ongón, S 08°15′04″ W 77°17′21″, 3419 m, 16.vi.2022, J. Cerdeña leg., MUSA; 1♂: Tayabamba, 2 Km SO Tayabamba, S 08°17′17″ W 77°18′17″, 3550 m, 17.vi.2022, J. Cerdeña leg., MUSA; 1♂: Tayabamba, Chongos, S 08°13′36″ W 77°19′54″, 3178 m, 19.vi.2022, J. Cerdeña leg., MUSA.

Type locality. Peru, La Libertad, Chongos.

Etymology. This subspecies is named after the department where it occurs, La Libertad, *libertas* in Latin, a feminine noun.

Distribution and habitat

P. b. libertas ssp. n. occurs in the upper valley of the Parcoy River, a tributary of the Marañón River (Fig. 6). Its range, however, may well extend further south and west. It is quite common in puna *matorral*, somewhat denser than that of *P. atusparia* sp. n. which is restricted to patches of grassland vegetation. This area of Peru is under a less severe anthropogenic pressure than the Callejón de Huaylas, therefore suitable habitats are still abundant. As with *P. atusparia* sp. n., this subspecies survives where eucalyptus or pines grow provided there are *Stipa* grasses in the undergrowth. It is found at altitudes between 3000– 3400 m. Other interesting butterflies occurring in the same type of habitat are *Catasticta atahuallpa* (Eitschberger & Racheli, 1998) and *Thespieus peruviae* Lindsey, 1925.

***Punargentus heimlichi* Pyrcz, sp. n.**

ZooBank taxon LSID:
E0441713-DD8A-4477-AC0F-3EF6758860E5

Diagnosis

This species externally most closely resembles *P. blanchardi*, but it can be distinguished from other species by its more compact hindwings, triangular in outline rather than oval, with a slightly produced tornus, a pale reddish suffusion on the FWV, reaching further distally than in *P. atusparia* sp. n., a third, small ocellus on the FWV in M_3-CuA_1, is invariably present, a similar colour pattern on its HWV as *P. atusparia* sp. n., a markedly steely whitish overcast, unlike the mostly brownish in *P. blanchardi*, the pattern of transverse lines is slightly different, with more prominent incisions in the postdiscal line and yellowish submarginal streaks much longer than in both congeners.

Description

Male (Figs 1R, S): Head: eyes chocolate brown, naked; antennae reaching 2/5 the length of the costa, with large, spatulate clubs. Wings: FW (average length 19,6 mm; n = 9) triangular with a blunt apex and straight outer margin and long, dark brown (except in apical area where they are intermittent and sandy yellow) fringes. FWD walnut brown, glossy, slightly darker on distal half; four intervenal yolk yellow stripes extending from postdiscal to sub marginal area on M_1-M_2, M_2-M_3, M_3-CuA_1 and CuA_1-CuA_2, enclosing a blackish brown ocellus in the middle, except for the shorter one on M_3-CuA_1. FWV ground colour wood brown with a golden yellow suffusion, variable in size and intensity but in most individuals covering most of the discal cells and the postdiscal area from costa to CuA_1, three large, submarginal black ocelli with white pupils, those on M_1-M_2, the largest, and M_2-M_3 combined and CuA_1-CuA_2 the smallest, and usually blind, enclosed in an irregular yellow patch with pointed outer edge ending in black stripes extending distally, subapical and apical area with a dusting of whitish and light brown scales, a smooth, black marginal line, interrupted with short, whitish, intervenal streaks. HW roughly triangular with a smooth outer margin, slightly incised along inner margin below tornus; fringes long, grey brown. HWD colour same as FW, walnut brown, in most individuals with thin, orangey inter-venal, postdiscal to submarginal stripes, one in each cell, in some individuals obsolete, fringes dark brown. HWV ground colour cedar brown heavily dusted with lighter brown scales that are pale and whitish along the distal edge of the postdiscal area; a series of black narrow bands extending from costa towards the anal margin; one, extremely irregular, from the post-basal area, across mid discal cell, and into CuA_2-1A/1B along the vein towards the wing base, the second, postdiscal, equally irregular, sharply displaced along the veins, reaching both the costa and anal margins, submarginal zigzagging and a smooth marginal ones, all interrupted by veins with whitish scales; a series of slightly yellow inter-venal stripes extending from the postdiscal to the submarginal bands, one in each cell, generally between four and five of them enclosing a central, elongated black

Table 3. Genetic distance between the taxa in *Punargentus* and selected outgroups based on COI barcodes.

		1	2	3	4	5	6	7	8	9	10	11	12
1	*P. heimlichi*	–											
2	*P. blanchardi blanchardi*	**6.5%**	–										
3	*P. blanchardi libertas*	**6.7%**	**2.4%**	–									
4	*P. idealis idealis*	**5.2%**	**5.0%**	**5.6%**	–								
5	*P. rubrostriata*	**5.0%**	**4.9%**	**5.5%**	**0.2%**	–							
6	*P. atusparia atusparia*	**6.0%**	**5.2%**	**5.3%**	**5.6%**	**5.2%**	–						
7	*P. atusparia yupania*	**5.9%**	**4.6%**	**5.2%**	**5.5%**	**5.3%**	**1.7%**	–					
8	*P. angusta*	**5.2%**	**6.1%**	**5.9%**	**3.7%**	**3.7%**	**6.0%**	**5.7%**	–				
9	*P. lamna*	**4.9%**	**6.0%**	**6.1%**	**1.3%**	**1.2%**	**5.5%**	**5.7%**	**3.7%**	–			
10	*Pampasatyrus gorkyi*	10.8%	10.7%	11.2%	9.8%	9.9%	11.0%	10.2%	9.1%	10.3%	–		
11	*Argyrophorus argenteus*	11.3%	11.9%	12.2%	10.9%	11.1%	11.9%	11.1%	10.3%	10.9%	8.9%	–	
12	*Etcheverius chiliensis*	7.7%	8.2%	8.5%	7.7%	7.8%	8.6%	8.3%	7.7%	7.7%	8.2%	9.2%	–

dot; outer margin light-greyish brown. Abdomen: dorsally covered with blackish brown, ventrally with thick, whitish scales. **Genitalia** (Fig. 2D): Tegumen thick, with a domed dorsum (similar to *P. blanchardi* but unlike the flat dorsum of *P. atusparia* sp. n.), uncus very large (compared to *P. atusparia* and *P. blanchardi*) raised in relation to the dorsum of the tegumen (unlike *P. atusparia* sp. n. whose uncus is aligned with the dorsum of the tegumen), one and a half times its length (slightly shorter than in *P. blanchardi*), compressed laterally and not wider in the middle (in contrast to *P. blanchardi* or *P. atusparia* sp. n.), with a sharp tip curved downwards (more noticeable than in *P. atusparia* sp. n.), subunci aligned with the uncus approximately one-third the length of the uncus (much shorter than in *P. atusparia* sp. n., about the same length as in *P. blanchardi*) with a sharp tip, the gap between uncus and subunci narrow (as in *P. blanchardi,* but unlike in *P. atusparia* sp. n. where it is wider); pedunculus long with a blunt tip, slightly curved downwards; sacculus broad but shorter than in *P. blanchardi*, valvae with the apex tapering to a narrow tip (same as in *P. atusparia* sp. n.), and a smooth dorsum, approximately one and a half times the length of the uncus (about the same length as in *P. atusparia yupania* ssp. n.), aedeagus tubular, the same length as the valvae (shorter than in *P. blanchardi*), with a slightly upcurved tip, approximately the same width throughout, except for the narrower proximal opening.

Female (Figs 1T, U): Sexual dimorphism is slight, although the hindwings of the female are more oval, as in the other two congeners, but the underside is lighter in colour than in *P. blanchardi* and *P. atusparia* sp. n. **Genitalia** (Fig. 3E): Similar to *P. atusparia* sp. n. and *P. blanchardi* except for the smaller corpus bursa and shorter signa, posterior apophysis longer than in *P. a. yupania* ssp. n., but similar to *P. blanchardi*.

Type material. Peru. Holotype ♂: "Peru, Depto. Ancash; 10 km N San Marcos; Puente Jaucán; 2720–2780 m; 15.vi.2019; T. Pyrcz leg.", currently in CEP-UJ (to be deposited in MUSM); Paratypes (36♂ and 4♀): Ancash: 9♂: Puente Jaucán, NE San Marcos, S09°25′34 W77°10′03, 2750 m, 15.vi.2019, PB Leg., PB; 10♂: 10 km N San Marcos; Puente Jaucán; 2720–2780 m; 15.vi.2019; T. Pyrcz leg., CEP-UJ; 1♀: Chavín de Huántar; Via El Túnel; 3300–3350 m; 29.vii.2003; T. & J. Pyrcz leg.; 1♂: Río Carash, San Marcos, 2960 m, [09°31′S, 77°09′W], 29.v.[19]84, J.E. Pérez leg., MUSM; 2♂: cerca (= near) de Purhuay, 3500 m, 09°19′S, 77°12′W, 19.vii.2006, C. Peña leg., MUSM; 1♂: same data but G. Lamas leg., MUSM; 4♂: Culluchaca, ruta Culluchaca – Yunguilla, 09°18′02″ W 77°00′46″, 2429 m, 15.vi.2019, J. Farfán leg., MUSA; 1♂: same data, J. Cerdeña leg., MUSA; La Libertad: 1♂: 2 km SW Huancaspata; 8°28′12″S/77°17′3″W; 3150–3250 m; 15.vi.2022; T. Pyrcz leg., CEP-UJ; 1♂ and 2♀: Huancaspata, 2 Km S Huancaspata, S 08°28′26″ W 77°17′41″, 3165 m, 15.vi.2022, J. Farfán leg., MUSA; 3♂: same data, J. Cerdeña leg., MUSA; Huánuco: 2♂: La Unión, 3200 m, [09°51′S, 76°50′W], 29.vi.[19]65, P. Hocking leg., MUSM; 1♂: same data but 24.vii.[19]65, MUSM; 1♀: Quivilla, 2700 m, [09°36′S, 76°44′W], 28.vi.[19]65, P. Hocking MUSM.

Type locality. Peru, Ancash, 10 km N San Marcos, Puente Jaucán.

Etymology. This species is named after Wilhelm Heimlich, the author of the description of the genus *Punargentus* in 1963.

Distribution

This species occurs on the eastern slopes of the Cordillera Blanca in valleys of the tributaries of the upper Marañón River, such as the Vizcarra (= Urqumayu) River in the departments of La Libertad, Ancash and Huánuco, as far south as La Unión. The northernmost known locality is Huancaspata, where it occurs sympatrically with *P. blanchardi libertas* ssp. n.

Relationships

The species of *Punargentus* form a fully supported clade sister to the genus *Etcheverrius*. Members of *Punargentus* form two fully supported clades, the *P. lamna* and the *P. blanchardi* clades (Fig. 4), which is in accordance with the morphological characters described in the Introduction. The *blanchardi* clade includes three fully supported clades that correspond with *P. heimlichi* sp. n., *P. atusparia* sp. n. and *P. blanchardi*. On the other hand, the *lamna* clade includes three out of four samples of *P. angusta* in a sister position to an unresolved clade that includes the three remaining species, *P. lamna, P. rubrostriata* (Pyrcz, Cerdeña & Florczyk, 2019) and *P. idealis* (Pyrcz & Cerdeña, 2019) (Pyrcz et al., 2019) and one sample of *P. angusta*.

Based on the molecular analysis, the species originally described as *Cosmosatyrus tandilensis* Köhler, 1935 and more recently associated with the genus *Punargentus* by Matz & Brower (2016) is moved to the reinstated genus *Etcheverrius* Herrera, 1965 as *E. tandilensis* comb. nov.

together with the type species *E. chiliensis* comb. reinst. (Fig. 4). Also, accordingly, the genus *Palmaris* appears to be more distantly related to *Punargentus*, contrary to the opinion of Matz & Brower (2016), and sister to *Pampasatyrus*. However, its formal status will be discussed in a separate paper (Pyrcz et al., in prep.).

Mean genetic distances based on COI (Table 3) between the species of the *P. blanchardi* clade vary between 4.6–6.7%. The subspecies of *P. atusparia* and *P. blanchardi* differ respectively by 1.7% and 2.4%. Genetic distances between the species in the *P. lamna* clade vary from 0.2% between *P. idealis* and *P. rubrostriata* to 3.7% between *P. idealis* and *P. angusta*.

DISCUSSION

Based on research in north-central Peru, the number of species of *Punargentus* increased from three in 2010 (Pyrcz & Wojtusiak, 2010), five in 2019 (Pyrcz et al., 2019) (all new species were described in the genus *Argyrophorus*) to seven currently, which makes it among the most diverse genera in the infratribe Neosatyriti. The catalogue of species of *Punargentus* is by no means complete. In particular, *P. lamna* is highly polytypic with several (at least four) new subspecies to be described when more comprehensive material becomes available, especially molecular data on the *lamna* clade. Also, vast areas of the western Cordillera in La Libertad and Cajamarca, as well as the upper drainage area of the Marañón River have not been sampled for puna butterflies, an area likely to harbour more new taxa of *Punargentus*.

Molecular data based on target enrichment and Sanger sequencing data convincingly demonstrate that *Punargentus* is monophyletic and a sister genus to *Etcheverrius*, which occurs from the southern Pacific coast in Chile to the northern Atlantic coast in Argentina in temperate grassland habitats. It also shows that *Punargentus* clusters in two clades, in accordance with striking differences in their morphological characters (i.e. upper surface of wings with silver patches in all the species of the *P. lamna* clade and all brown wings in the *P. blanchardi* clade). Current molecular data does not support the separate specific status of some of the species in the *lamna* clade, in particular, *P. rubrostriata* and *P. idealis*, for which the genetic distances based on COI sequences are below 1%, but nevertheless they are morphologically very easily identifiable (Pyrcz et al., 2019). On the other hand, morphological differences between the species in the *blanchardi* clade are less evident, whereas molecular data fully support their separate specific status, with important genetic distances, well above the barcode specific threshold. In particular, *P. heimlichi* sp. n., despite its close superficial resemblance to *P. blanchardi*, is more distantly related to the other two species.

Interestingly, the pairs *P. blanchardi* – *P. lamna* ssp., *P. atusparia* sp. n. – *P. rubrostriata* and *P. heimlichi* sp. n. – *P. lamna* ssp. clearly replace each other parapatrically with increase in altitude. The species of the *P. blanchardi* clade occur at low altitudes, from 2800 m to 3400 m and only sporadically at 3800 m, which is the highest altitude at which they were recorded. When they do so, they can be syntopic with species of the *P. lamna* clade, as in the Pastoruri valley, where *P. atusparia* sp. n. and *P. rubrostriata* are syntopic and equally abundant at about 3600 m. The species of the *P. lamna* clade usually occur at, or slightly above, 4000 m, but exceptionally can be found as high as 4700 m at Altiplano or down to 3000 m near Huánuco. However, the species in the *P. lamna* clade are much more widespread and occur as far south as northern Bolivia, whereas those of the *P. blanchardi* clade are restricted to north-central Peru.

ACKNOWLEDGEMENTS. This research was supported by an Internal Research Grant of the Institute of Zoology and Biomedical Research of the Jagiellonian University, N18/DBS/000016, and an NCN grant Harmonia-10 2018/30/M/NZ8/00293 "Evolutionary biogeography and diversification of the predominantly Andean butterfly subtribe Pronophilina (Nymphalidae, Satyrinae) based on phylogenetic data generated using modern molecular methods". We would like to thank K. Florczyk (CEP-UJ) for dissecting the male genitalia of *Punargentus atusparia atusparia* and N. Wahlberg (Lund) for additional data on *Punargentus lamna*.

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Received March 30, 2023; revised and accepted August 23, 2023
Published online November 8, 2023