



A review of the subgenus *Parapisa* of *Apisa* (Lepidoptera: Erebidae: Arctiinae) with description of a remarkable species from Cameroonian Highlands

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<https://zoobank.org/D013BE81-D73D-4CE9-A5EE-593976C945D0>

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Received 14 October 2022

Accepted 15 March 2023

Published 6 April 2023

Academic Editors Andreas Zwick, Martin Wiemers

Citation: Paśnik A, Tarcz S, Przybyłowicz Ł (2023) A review of the subgenus *Parapisa* of *Apisa* (Lepidoptera: Erebidae: Arctiinae) with description of a remarkable species from Cameroonian Highlands. *Arthropod Systematics & Phylogeny* 81: 371–394. <https://doi.org/10.3897/asp.81.e96319>

Abstract

The subgenus *Parapisa* of the genus *Apisa* is reviewed based on the examination of 104 specimens. *Apisa* (*P.*) *cinereocostata* and *A.* (*P.*) *subargentea* are redescribed and their intraspecific variation is analysed in detail. A new species *A.* (*P.*) *asipa*, similar in the general coloration to other *Apisa* taxa, but very distinctive in the male genital morphology and the shape of the wing scales, is described from Cameroon and Nigeria. *Apisa* (*P.*) *cinereocostata* is hypothesized to be a widespread, but highly polymorphic taxon with significant variation in body size, intensity of grey coloration, and the proportions and shape of certain morphostructures of male genitalia. Determination keys and extensive illustrations of the variation are provided to enable proper identification of specimens.

Keywords

Africa, genital slides, molecular analysis, moths, new species, Syntomini, taxonomy

1. Introduction

For some time now, there has been an increasing number of in-depth studies of African Lepidoptera (e.g. Coache et al. 2018; Hacker et al. 2012; Mey and Krüger 2019). Nevertheless, there is still a large knowledge gap remaining, especially in Equatorial Africa, which is underexplored in almost every aspect of its biodiversity (Beck et al. 2017). Despite its high conservation value, the real composition and diversity of Central African insects remains poorly studied, thus every new taxonomic publication brings significant, often unexpected discoveries. There are many unexplored families, not only of tiny, inconspicuous moths but also of eye-catching and colourful butterflies,

and several new taxa have been recently described from the area e.g. in Cossidae (Yakovlev and Witt 2017), Lecithoceridae (Park et al. 2019), Lycaenidae (Sáfián and Collins 2022) and Nymphalidae (Nakahara et al. 2022).

Besides purely α -taxonomy publications, more comprehensive revisionary studies of species groups and genera have been published recently, including those devoted to tiger moths (Durante and Apinda-Legnouo 2022, Durante et al. 2021).

A unique example of a large scale ecological project is the one focused on the study of Mount Cameroon, which is an active volcano, on southwestern flanks covered with

primary tropical rainforest. It represents one of the well-known biodiversity hotspots, with an exceptionally high number of recorded taxa (Mertens et al. 2021; Larsen 2005). As a result of the study, it turned out that the cyclic occurrence of the dry season and the rainy season has a significant impact on the food source for caterpillars and imagines (Maicher et al. 2018). Research associated with elevational patterns of the species richness during the dry season and the wet seasons were conducted on Mt Cameroon making it one of the better studied sites. The peak of species richness underwent seasonal shifts where it decreased during the rainy season with the exception of the subfamily Arctiinae which recorded an increase (Maicher et al. 2019).

One of the groups of moths from this region deserving to be studied in detail is genus *Apisa* Walker, 1855 (Lepidoptera: Erebidae: Arctiinae), and especially its subgenus *A. (Parapisa)* Kiriakoff, 1952, restricted to equatorial areas of Africa. The genus *Apisa* consists of greyish-ochraceous, inconspicuous, and superficially uniform moth species, diagnosis based on the shape of male genital apparatus and the lack of arolium (for details see Przystałkowska 2022). Thus, they have never been taxonomically studied in detail and therefore remained a taxonomic “Gordian knot”. Taking into account their high intra- and interspecific variation, they need to be tackled based on large series of specimens. This is luckily possible, as individuals representing this subgenus are usually easily collected and thus housed in reasonable numbers in many scientific collections.

Currently, three subgenera are recognized within the genus: *Apisa (Apisa)*, *Apisa (Dufraneella)* Kiriakoff, 1953, and *Apisa (Parapisa)* Kiriakoff, 1952. The latter is the easiest to identify because of the bifurcate tip of its uncus. So far, two species of this subgenus have been known. They differ from each other in the shape of genital apparatus, and particularly in the details of the tip of uncus: *A. (P.) cinereocostata* Holland, 1893 has widely separated terminal protrusions (however variable, indicating intraspecific polymorphism), and *A. (P.) subargentea* Joicey and Tabot, 1921 has a very narrow concavity below the tip (Przybyłowicz 2009).

The biology, including the food plant(s) of *A. (Parapisa)*, as generally of the entire genus *Apisa* remains unknown. The only published information is that some species of the genus *Cosmos* (Asteraceae) might be the food plant for the species *A. (A.) canescens* Walker, 1855 (Sevastopulo 1975).

A fairly large problem is the abovementioned morphological uniformity across the genus, resulting in the small number of well-defined distinguishing characters that may be used to separate the species. Thus, a reliable determination is only possible with large series and access to reference specimens, best with the combination of morphological and molecular methods. This relatively new approach, known as integrative taxonomy, is based on the idea that results of different methods should be combined to strengthen the taxonomic hypotheses (Yeates et al. 2011). By using this method we can combine “traditional” comparative morphological studies with more

modern ones like molecular, biogeographical and ecological studies or advanced statistical methods (Gebiola et al. 2012). Nowadays combining these two approaches becomes more and more easy, as the DNA data are constantly accumulated in public databases.

The aim of this paper is to revise the subgenus *A. (Parapisa)* using the integrative approach. The extensive study of long series of specimens ascribed provisionally to taxon *A. (P.) cinereocostata* is undertaken to explain its unusual morphological and genetic variability. For the first time, the determination keys based on external and genital characters of both males and females are constructed, together with a description of a remarkable new species. The morphology of wing scales, which turned out to be an important diagnostic character is also analyzed and illustrated by means of light and SEM microscopy.

2. Material and methods

Analysed specimens of the genus *Apisa* were collected in Liberia, Guinea, Ghana, Gambia, Sierra Leone, Nigeria, Ivory Coast, Mali, and Angola. In total, 104 specimens were analysed.

2.1. Morphology

Each specimen was photographed using a Canon 70D digital camera with a macro lens EF 50 mm. Genital slides were made from 70 individuals (64 males and 6 females). Abdomens were detached from selected specimens and macerated in 10% KOH solution in a water bath for about 30 minutes. Next, each abdomen was transferred to a petri dish with distilled water and a drop of liquid reducing surface tension. Scales were removed from the abdomen with a fine and thin brush. The cleaned abdomen was transferred to a new petri dish and unnecessary soft tissues were removed with entomological pins. Soft membranes, i.e. parts of aedeagus and female preparations were stained with chlorazol black. If possible, vesica was everted from the aedeagus. After the preparations were made, the specimens were labeled and the preparations were stored on basal slides in glycerin. When the comparative analyses are completed, the slides will be permanently encapsulated in Euparal (Agar Scientific, Essex, UK) and included in the collection. Pictures of the slides were taken using a stereoscopic microscope Leica S9i system. Images were adjusted with the Adobe Photoshop CC program. The morphology terminology follows Miller (1991), and for the genitalia we refer to Koda (1987).

For wing scales examination one specimen was selected from each species: *A. (P.) subargentea*, *A. (P.) cinereocostata*, and *A. (P.) asipa* **sp. nov.** A stereoscopic microscope Nikon SMZ1000 with mounted camera Canon 70D was used to take magnification photographs of scales and

to prepare them for permanent preparations. Photographs of scales were taken on a basal slide in a drop of glycerol.

From the surface of the wings, the scales were scraped into a dish with alcohol using a moistened entomological pin. Permanent slide preparations were made using Marc André II (Daghighi et al. 2016) mounting medium. The scales and their sculpture were carefully examined using a Zeiss Axio Imager A2 contrast phase microscope. Photographs were made using a camera Canon 70D mounted on the microscope. The received photos were stacked using Helicon Focus 7.7.4. The final photos were processed in the Adobe Photoshop CC program.

The differences in scales have been visualized by use of the scanning electron microscope. The specimens were selected, one specimen from each of the three species. Using a binocular microscope, scales were gently scraped from the wing fragments with an entomological pin. From the scales, three types of preparations were made, one on a basic slide where the material was embedded in glycerol. Mounted microscope slides were made using Marc André II mounting medium. A separate preparation has been made for SEM images. The scales were glued onto carbon glue holders and covered with gold using an Ion Sprayer JEOL JFC-1100E. For taking photos a scanning microscope JEOL JSM5410 with tungsten cathode was used. The images were taken at the Institute of Geological Sciences at the Jagiellonian University, Kraków, Poland.

2.2. Molecular laboratory procedure

Specimens collected not earlier than about 10 years ago were selected for DNA isolation. From each dried specimen one or two legs were sampled. The isolation of DNA was done with the NucleoSpin Tissue kit (Machery-Nagel, Germany), following the manufacturer's protocol.

Sequence of the barcode part of the mitochondrial gene cytochrome c oxidase subunit I (COI) was obtained with the use of the primer pair LEP-F1 (5'-ATT CAA CCA ATC ATA AAG ATA T-3'), and LEP-R1 (5'-TAA ACT TCT GGA TGT CCA AAA A-3') (Hebert et al. 2004).

The ready hot-start PCR mix (StartWARM HS-PCR Mix, A&A Biotechnology, Poland) was used. PCR reactions were performed in a total volume of 10 µl. The amplified products were electrophoresed in 1% TBE agarose gel for 30 min at 100 V and visualized under UV. PCR products were purified with Exo-BAP mix (EURx, Poland), following the standard protocol. Then successful PCR products were sequenced in both directions using the same primers as for PCR reaction (LEP-F1/LEP-R1). For sequencing BrilliantDye v3.1 Terminator Cycle Sequencing Kit (NimaGen, the Netherlands) was used. Sequence reading was done with the use of an ABI Prism 3130xl sequencing machine in the Laboratory of Molecular Techniques at ISEA PAS. Obtained sequences were compared with chromatograms and aligned manually with a reference sequence in BioEdit software version 7.0.9.0 (Hall 2004).

For old, historic specimens of *A. (P.) subargentea* DNA was isolated from the legs using the GeneMATRIX Bio-Trace DNA Purification kit (EURx, Poland), following the standard protocol for tissue with a modification, the incubation time of the material was increased to overnight.

For these samples primers LEP-F1/LEP-R1 failed, and additional PCR reaction was carried out using primers ZBJ-ArtF1c (5'-AGA TAT TGG AAC WTT ATA TTT TAT TTT TGG-3') and ZBJ-ArtR2c (5'-WAC TAA TCA ATT WCC AAA TCC TCC-3') for a short fragment of the COI gene (150 bp) (Zeale et al. 2011).

2.3. Phylogenetic analyses

DNA sequences generated during this study are deposited in the GenBank database, and the accession numbers are provided in Table S1, Table S2.

The p-distance (Table S3) between barcode sequences was calculated in MEGA11 (Tamura et al. 2021). Two methods were used to reconstruct the phylogenetic relationships: maximum likelihood (ML) and Bayesian interference (BI). The best-fitting model for ML analyses were selected using the minimum Bayesian Information Criterion (BIC) (Schwarz 1978).

The ML analyses were carried out in MEGA11 (Tamura et al. 2021). Bootstrap support was calculated using 1000 replicates. The T92+G model was selected. The DNA sequences of the taxa selected as the outgroup were extracted from the GenBank Database.

The BI analyses were carried out using MrBayes ver. 3.2.7 (Ronquist et al. 2012), with four independent runs, each with four Metropolis-coupled chains with default heating parameters (one cold and three heated). The chains were sampled once every thousand generations for 4 million generations and the first 25% of samples were discarded as burn-in. The general time reversible (GTR) model of sequence evolution was selected as the most universal, neutral, independent and applicable model.

The discrete gamma distribution of rates among sites was applied (GTR + G). The analysis was run four times, each with a random starting tree. All analyses converged to an average standard deviation of split frequencies below 0.01. Clade robustness was estimated by posterior probabilities.

All obtained trees were visualized with FigTree 1.4.3 (Rambaut 2009) and graphically edited using CorelDraw Graphic Suite 2017.

2.4. Haplotypes

The analysis of haplotype diversity (Hd) and nucleotide diversity (π) was carried out using DnaSP v5.10.01 (Librado 2009). Haplotype networks were constructed using the Minimum Joining method (Bandelt 1999) implemented in the PopART v1.7 software (Leigh 2015).

2.5. Abbreviations

ANHRT – African Natural History Research Trust, Leominster, UK; **CMNH** – Carnegie Museum of Natural History, Pittsburgh, USA; **DRC** – Democratic Republic of Congo; **ISEA PAS** – Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland; **KL** – Knud Larsen private collection, Denmark; **MWM** – Museum Thomas Witt, Munich, Germany; **NHMUK** – Natural History Museum, London, UK; **NHMW** – Naturhistorisches Museum Wien, Austria; **RMCA** – Royal Museum for Central Africa, Tervuren, Belgium; **ZIN** – Zoological Institute St. Petersburg, Russian Federation; **ZSM** – Zoologische Sammlung des Bayerischen Staates, Munich, Germany.

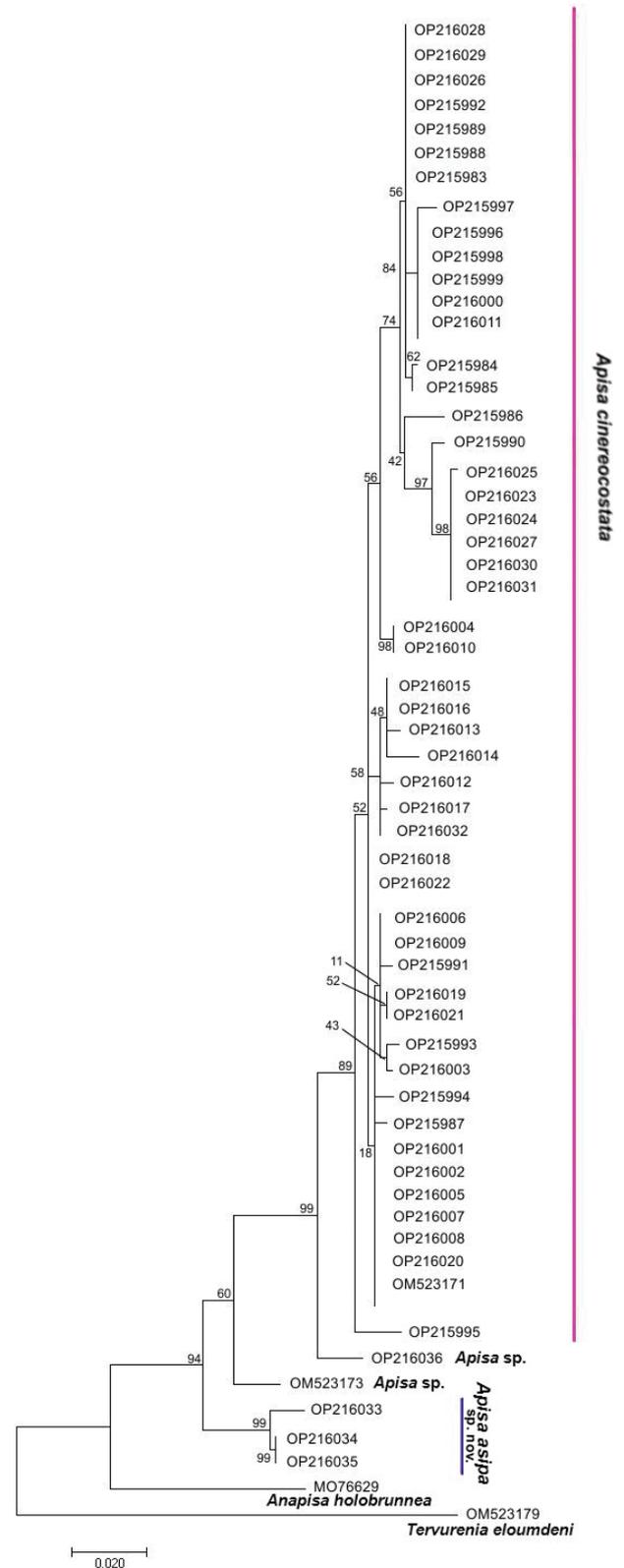


Figure 1. Phylogenetic tree showing the variation of *A. (P.) cinereocostata*, with two species *Tervurenia eloumdeni* and *Anapisa holobrunnea* as the outgroup. The sequences of two representatives of subgenus *Apisa* are given and also the sequences of the new species *A. (P.) asipa* sp. nov. The tree was constructed with sequences of the mitochondrial cytochrome c oxidase subunit I (COI) fragment using the maximum-likelihood method. Bootstrap values are presented. All positions containing gaps and missing data were eliminated. Phylogenetic analyses were conducted using MEGA 11.

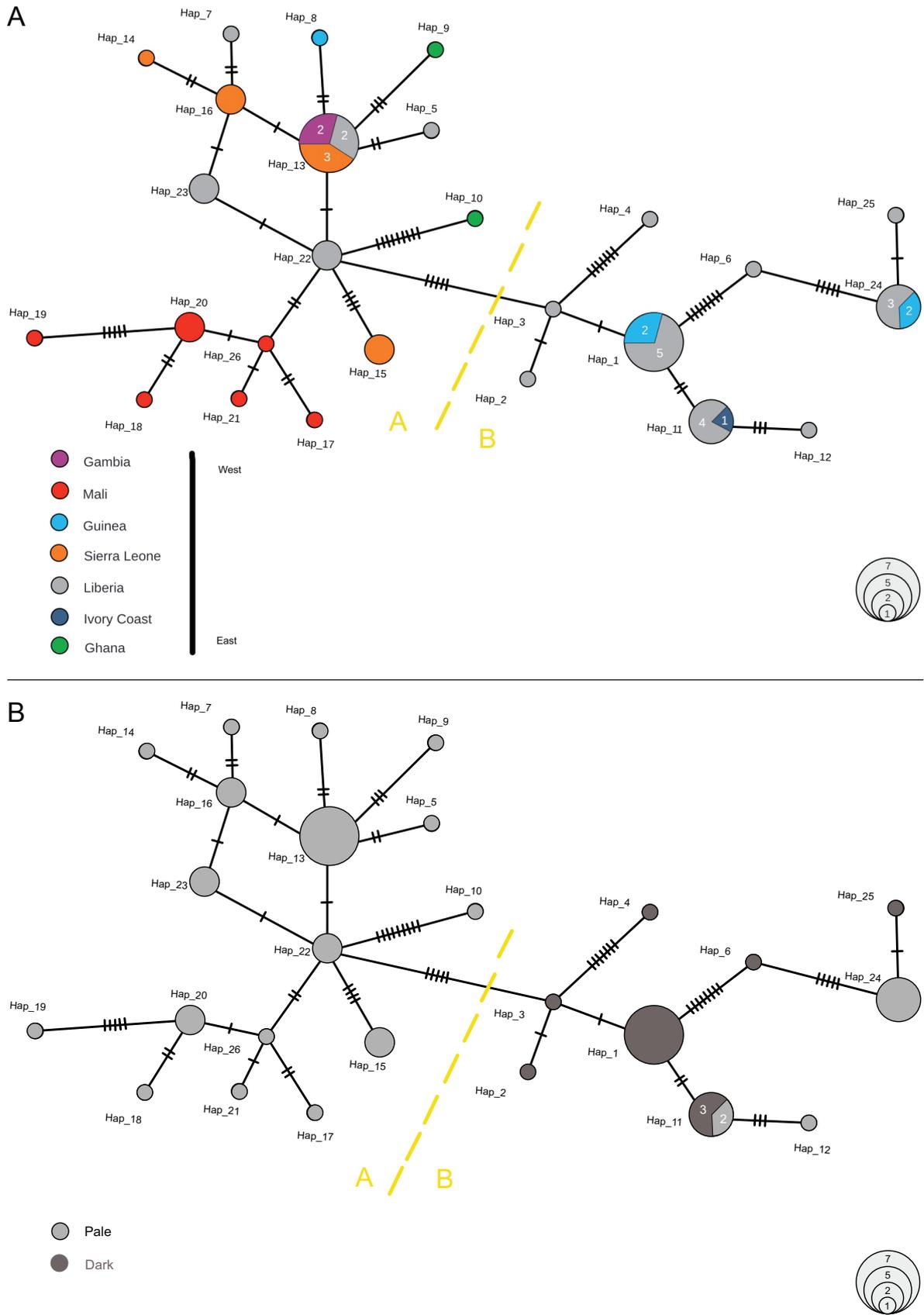


Figure 3. Haplotype network of *A. (P.) cinereocostata*. Constructed using the 51 mitochondrial cytochrome c oxidase subunit I (COI) sequences obtained. The size of the circles is proportional to the frequency of the haplotype (on the legend in the bottom right one, two, five and seven individuals). The legend on the left represents the seven countries from which the individuals came. The black dashes on particular branches represent nucleotide substitutions between particular haplotypes. The yellow dashed line separates the two larger haplotype groups A and B. Analyses were conducted with Minimum joining in PopART v1.7 software. **A** Including countries. Each country is marked with a different color in the legend. The countries are arranged from west to east. **B** Including two color forms. The gray color indicates the pale morphotype and the dark gray indicates the dark morphotype.

3.1.2. Determination key to males based on the genitalia (Figs 4, 10)

- 1 Process of valva elongate, narrow; vesica with single, well developed cornutus (Fig. 4C)....*A. (P.) cinereocostata*
- Process of valva invisible or in form of minute tubercle, vesica without cornuti.....2
- 2 Uncus constricted before terminal bifurcation; terminal lobes shorter than one quarter of the length of uncus (Fig. 4A).....*A. (P.) subargentea*
- Uncus with parallel margins not constricted before terminal bifurcation; terminal lobes approximately the half the length of uncus (Figs 4B, 10).....*A. (P.) asipa sp. nov.*

3.1.3. Determination key to females based on the genitalia (female of *A. (P.) asipa* is known from a single female with damaged bursa copulatrix) (Figs 5, 6)

- 1 Distal, submedial sclerotization of VII sternite Y-shape, significantly longer than wide (Fig. 6A)*A. (P.) asipa sp. nov.*
- Distal, submedial sclerotization of VII sternite Y-shape, approximately as long as wide (Fig. 6B, C).....2
- 2 Signum heavily sclerotized, irregular, suboval, at most twice as long as wide (Fig. 5C).....*A. (P.) subargentea*
- Signum sclerotized, irregular, elongate, at least twice as long as wide (Fig. 5B).....*A. (P.) cinereocostata*

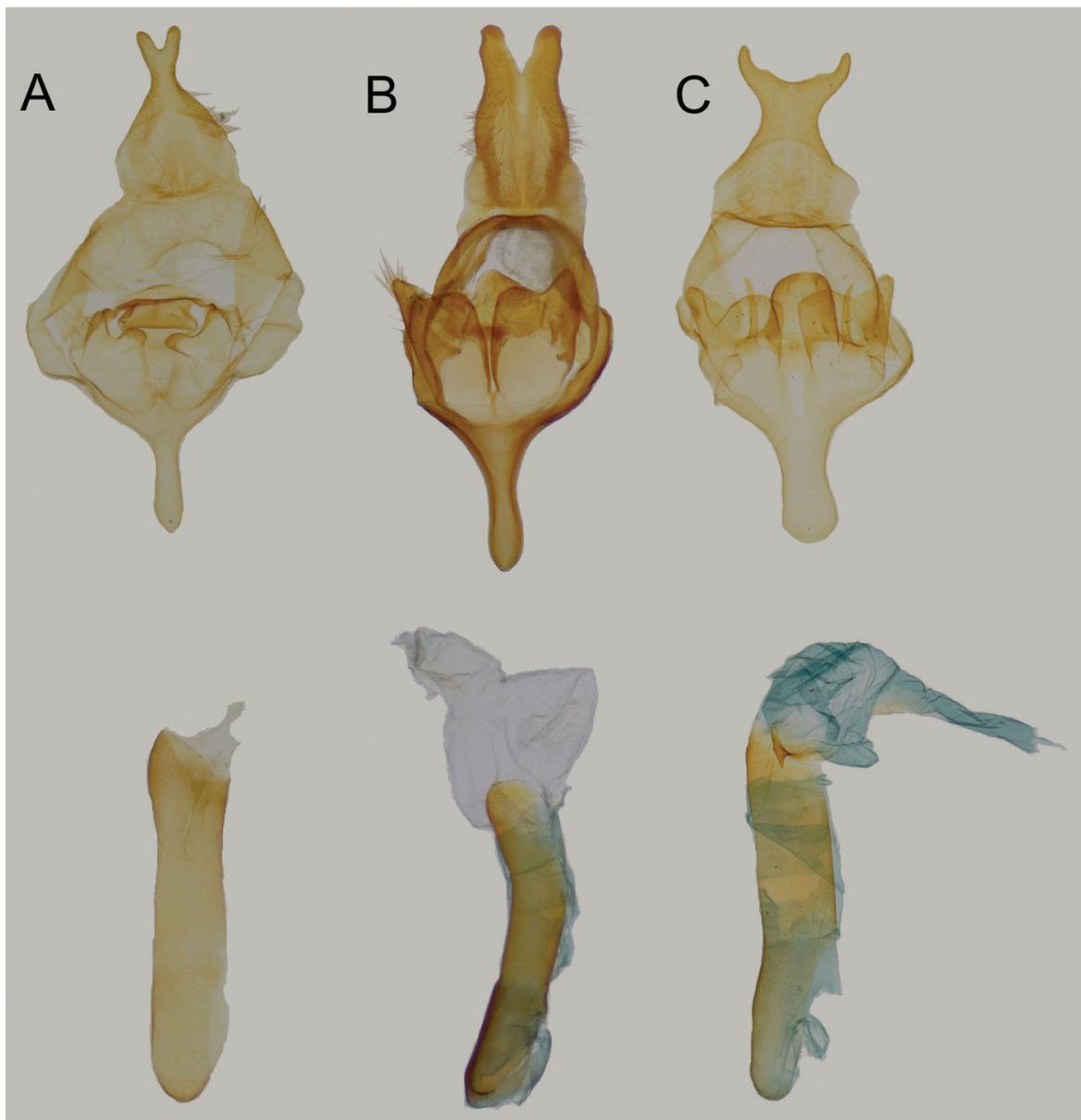


Figure 4. Overview of the genital apparatus of males *A. (P.) subargentea* (A), *A. (P.) asipa sp. nov.* (B) and *Apisa (P.) cinereocostata* (C).

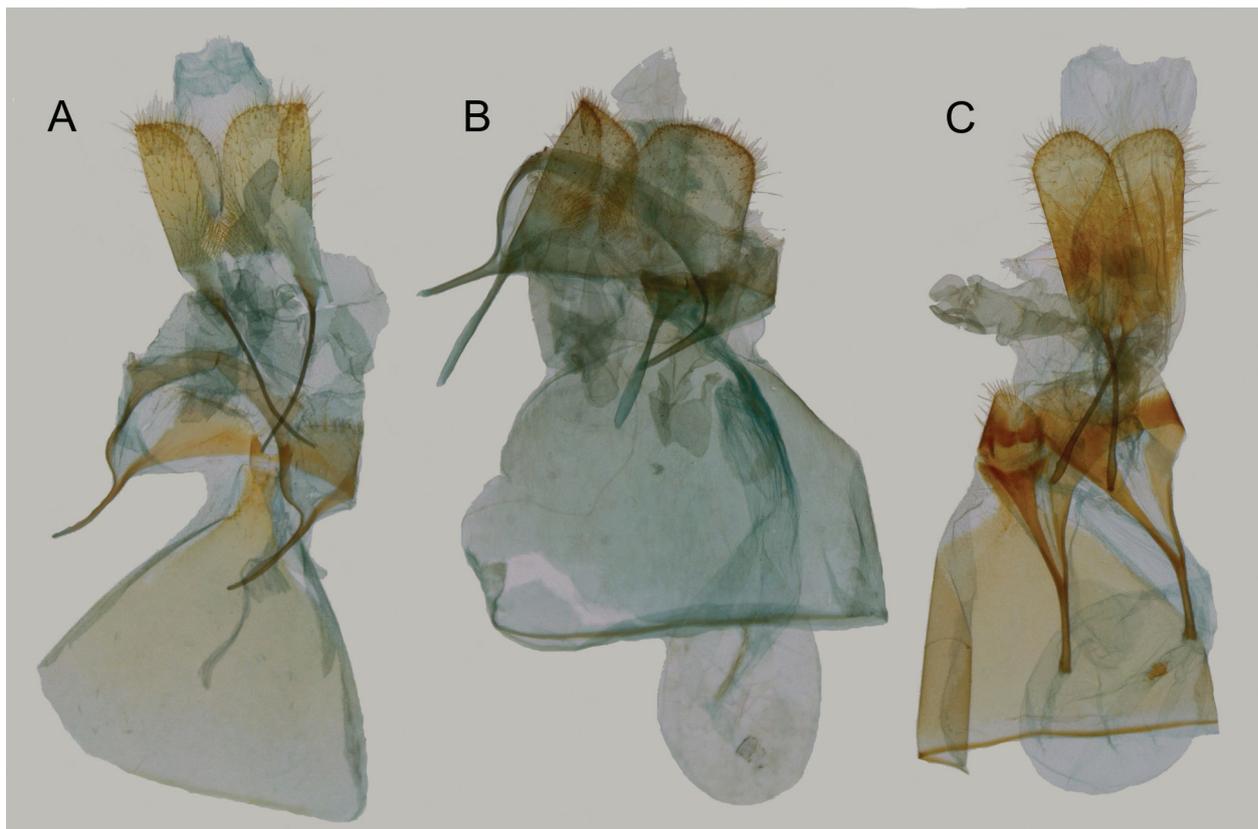


Figure 5. Compilation of female genital organs *A. (P.) asipa* sp. nov. (A), *A. (P.) cinereocostata* (B), *A. (P.) subargentea* (C).

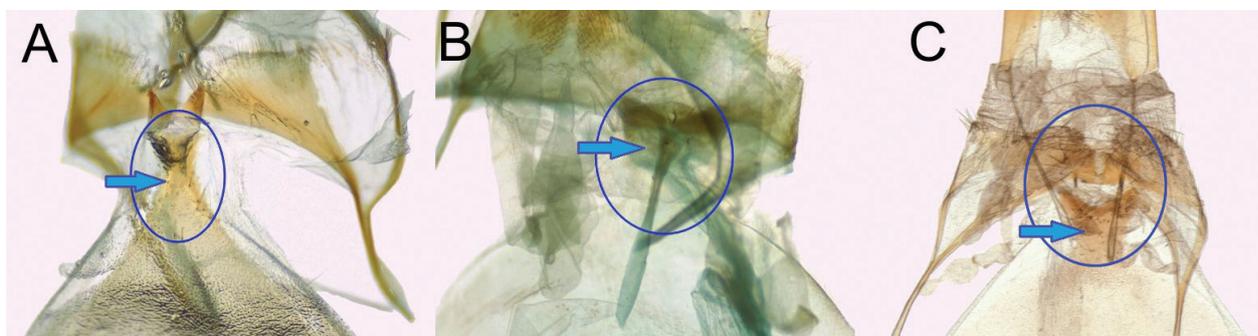


Figure 6. Details of female genitalia with both a marked antrum and a sclerotization of VII sternite. The arrow marks the location and sclerotization of the sternite. From left *A. (P.) asipa* sp. nov. (A), *A. (P.) subargentea* (B), *A. (P.) cinereocostata* (C).

3.2. Species taxonomy

Apisa Walker, 1855

Subgenus *Parapisa* Kiriakoff, 1952

Type species. *Apisa (Parapisa) bourgognei* Kiriakoff, 1952: 173–175 (by original designation)

Diagnosis. The subgenus differs from the two remaining subgenera viz. *Apisa* s. str. and *Dufraneella* Kiriakoff, 1953 by bifid uncus, which in the other subgenera is simple and sharply pointed.

Comments. Subgenus *A. (Parapisa)* currently comprises three species, including the newly described one. *Apisa (P.) subargentea* Joicey and Talbot, 1921 was described from a single female, hence its subgeneric placement for a long time has been impossible to indicate, until male specimens collected in Kenya allowed for the correct allocation of this taxon to *A. (Parapisa)* (Przybyłowicz and Kühne 2008). Also the genitalia of *A. (P.) cinereocostata* Holland, 1893 remained unknown for a long time, and only relatively recently this taxon was identified as a member of the subgenus *A. (Parapisa)*. Simultaneously, the genital and external similarities between *A. (P.) cinereocostata* and *A. bourgognei* lead to the conclusion that they represent the same taxon, which

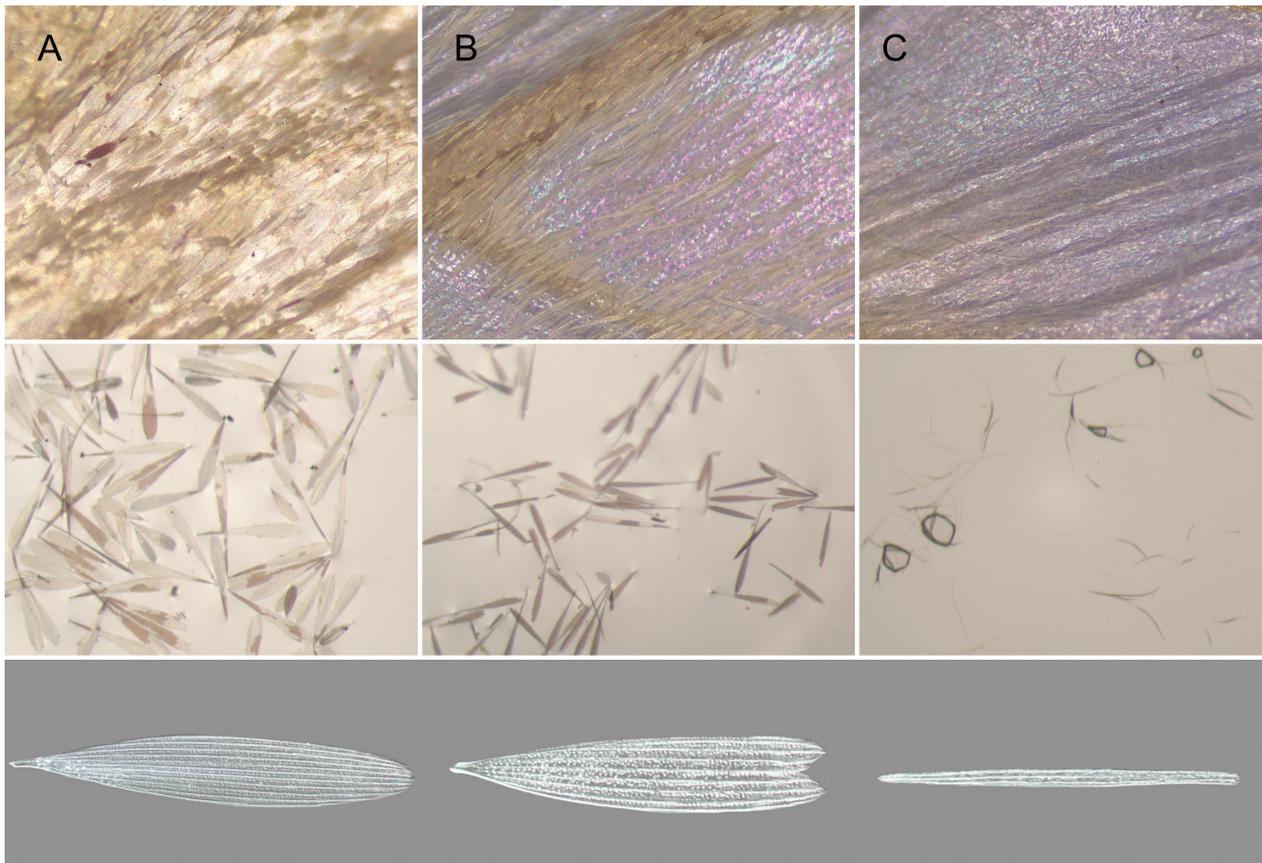


Figure 7. Differences in the scale morphology of wing region between CuA1 and CuA2 near DC of *A. subargentea* (A) (tightly fitting), *Apisa cinereocostata* (B) (loosely fitting) and *A. asipa* sp. nov. (C) (sparse).

should bear the name *A. (P.) cinereocostata* (Przybyłowicz 2009).

Taking into account lack of well-defined pattern on wings and body which might serve as a source of diagnostic characters, the differentiations between darker and paler coloration used in descriptions should be treated with reservation. The degree of colour saturation varies significantly depending on illumination, which combined with the overall uniformity of specimen coloration makes it very difficult to objectively compare darker and paler structures and the intensity of the differences.

Apisa (P.) asipa sp. nov.

<https://zoobank.org/AA48B114-FBC8-4838-B547-6296F38514B0>

Figs 4B, 5A, 6A, 7C, 8–10, 13C, 14A

Material examined. *Holotype*: ♂ Cameroon 900m, North Region, Wack (La Falaise), 07°40'16,5"N 13°33'18,4"E, 2–21.x.2018 Cold Cathode UV, Light Trap, leg. Safian, Sz., Simonics, G., ANHRT:2018.36; ANHRTUK 00071898; GS P322; OP216034; (ANHRT). — *Paratypes*: (6♂♂, 1♀) ♂ as above but ANHRT:2018.36; ANHRTUK 00071900; GS P323; OP216033; ♂ as above but ANHRT:2018.36; ANHRTUK 00113541; GS P324; OP216035; ♂ as above but ANHRT:2018.36; ANHRTUK 00060240; GS P325; (ANHRT); ♂ Adamaua Poli (500 m) b. Garua, A. Weidhols 8.V.37; GS P326; ♀ as above but GS P327; (NHMW); ♂ N. Ni-

geria Kaduna, 10.4.70, leg. Dr. Politzar; Genitalpreparat, Heterocera, Nr. 32.331, Museum Witt München; ex coll. Politzar; ♂ as above but 13.4.70, 1♂ as above but Genitalpreparat, Heterocera, Nr. 32.332 (ZSM).

Diagnosis. Due to the extreme similarity of all members of *Apisa* and the fact that subgenera are separated by genital morphology only, the diagnosis of the new taxon in part referring to external characters does not differentiate the subgenera. *Apisa (P.) asipa* sp. nov. is externally very similar to other uniformly coloured, ochraceous members of the genus *Apisa*. This overall similarity is enhanced by extreme general colour homogeneity of *Apisa* combined with intraspecific variability of the background tint and what is important is the degree of fading of specimens in collections. However, the clear and discrete diagnostic character for the new taxon is the morphological structure of scales covering wings. For the objective and unambiguous separation of the new taxon from all remaining *Apisa* the zone between veins, CuA1 and CuA2 near DC (Fig. 7C) should be examined as the reference character. Uniquely for *A. (P.) asipa* sp. nov. it is covered by moderately dense minute, narrow, arc-shaped, needle-like scales making the wing semitransparent pale ochraceous. In none of the available specimens of the new taxon (including the single female), any straight (flat or needle-like) scales were observed. In all remaining species of *Apisa*, the same zone is opaque and covered with densely overlapping flat scales or semi-transparent ones, but with numerous elongate, straight, needle-like scales.



Figure 8. *Apisa asipa* sp. nov. holotype upperside, underside with labels.

Male genitalia allow for an easy separation of *A. (P.) asipa* sp. nov. Bifid, instead of single pointed uncus locates it within the subgenus *Parapisa*. It is separated from the two other taxa allocated there by a narrow and deep, V-shaped slit of terminal lobes and not distinctly narrowed, lateral margins of uncus. Both characters are very obvious and easy to observe.

Female genitalia examined are partly damaged and incomplete. Additionally, they are unknown for several other *Apisa* species, hence do not allow for a confident diagnosis of the new taxon.

Description. Head. Frons and vertex pale ochraceous; labial palpus darker, three segmented of which the second is the longest and the last directed downwards, densely covered with narrow scales; scapus pale ochraceous; flagellum bipectinate, concolorous with scapus; flagellomeres honey; eye convex, indistinctly ovoid. — **Thorax.** Vestiture unicolorous pale ochraceous expressing darker or lighter tint depending on the illumination; external portion of coxa, femur, and tibia of foreleg and to less extent the middle and distal leg darker than the internal portion (closer to body when legs suppressed); epiphysis stout reaching 4/5 of the foretibia length; mid and hind tibia with a pair of short, terminal spurs. — **Abdomen.** Entirely pale ochraceous, concolorous with the rest of the body. Upperside similar to underside. — **Forewing.** Semi-transparent, uniformly pale ochraceous, except for area along costa which is distinctly darker and the same colour as labial palpus and external portion of leg; veins well visible, pale honey; cilia pale cream; R1–R2 separated from R3–R5; M2–M3 from one point; distances between M3–CuA1 and CuA1–CuA2 similar; 1A+2A almost straight, without a distinct curve in one third of its length; coloration of underside similar to upperside, retinaculum present. — **Hindwing.** Coloration somewhat paler than in forewing; cilia almost white, Rs–M1 on a long stalk of more than half of their length.

Male genitalia. (Fig. 10) Tegumen rather narrow, slightly broadened laterally. Vinculum much narrower, widely fused with lateral arms of tegumen. Uncus well developed, broad, subparallel margins not tapering towards termination but indistinctly narrowed in mid-length; sub-

dorsally in form of a pair of longitudinal swellings separated by a submedian concavity and divided terminally into deep, rather narrow subtriangular slit; each swelling with a group of protruding setae in its basal half; terminal tips gently bent subventrally. Valva subsquare, much shorter than uncus, terminal margin concave medially; distal portion of costa and sacculus provided with elongate, stiff setae; costa subbasally with a thorn-like short, acute process surrounded by membranous zone. Juxta in form of longitudinal, submedial plate. Transtilla lateral arms weakly sclerotized, almost invisible, submedial portion enlarged, fused with juxta, and forming sclerotized anellus. Saccus about three times as long as broad terminating into an elongate, sclerotized process gradually narrowed towards sharp tip. Phallus straight and short. Vesica membranous, oval extended without cornuti and sclerotisation.

Female genitalia. (Figs 5A, 6A) Partly damaged. Papillae anales longer than broad, sparsely covered with protruding setae, denser towards terminal portion; apophyses posteriores almost twice as long as papillae anales, narrow, needle-like; apophyses anteriores somewhat shorter than papillae anales, narrow with lateral, membranous ridge in distal portion; dorsal pheromone glands in form of separate, broad, deep pouches; the membranous basal zone of ventral pheromone glands partly damaged so the morphology of opening impossible to describe otherwise (based on single gland) similar to dorsal pheromone glands but much narrower and smaller, somewhat finger-shaped; ostium small, rounder, antevaginal plate medially concave; weakly expressed, almost membranous; ductus bursae slender, membranous, straight; ductus seminalis slender originating from the base of corpus bursae; corpus bursae entirely damaged (absent); sternite VII subtriangular, distinctly narrowed and more sclerotized in distal portion, terminal zone narrow provided with longitudinal submedian protrusion and Y-shaped.

Variation. Difficult to assess. Three males come from the same sampling (place, time), the fourth one is much older, slightly damaged and faded. Within the three males from Wack, only some very indistinct variation in the intensity of the ochraceous coloration of the wings and body can



Figure 9. *Apisa (P.) asipa* sp. nov. paratypes upperside, underside with labels.

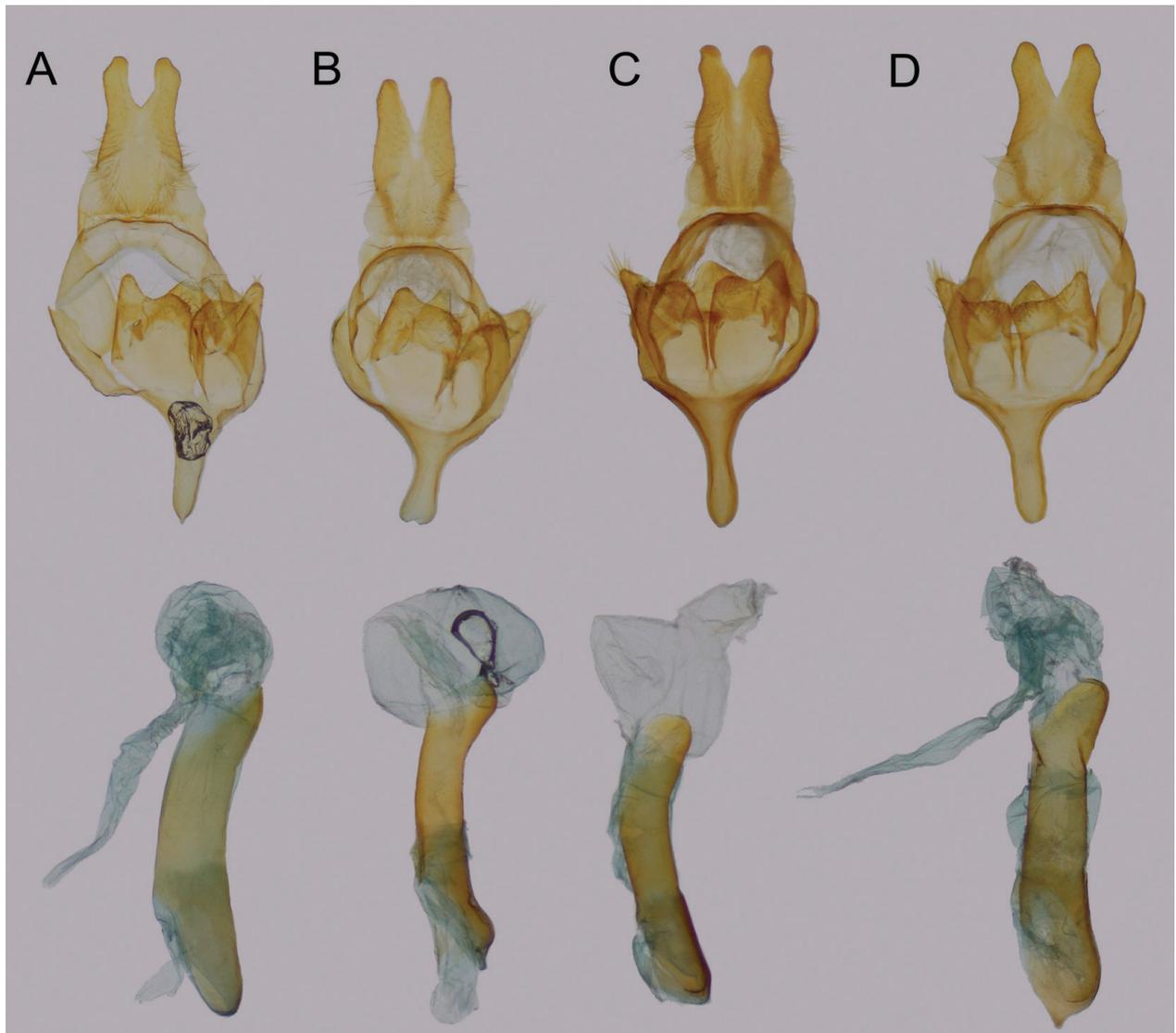


Figure 10. *Apisa (P.) asipa* sp. nov. holotype (A), paratype (B–D). Male genitalia, phallus with everted vesica.

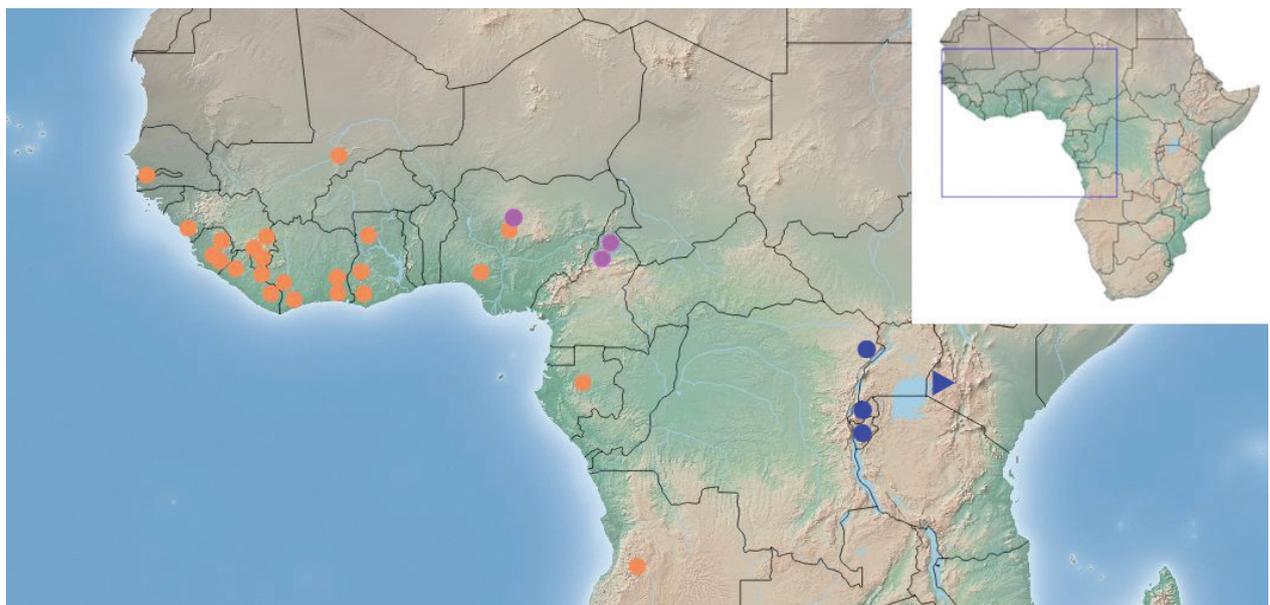


Figure 11. Distribution of *Apisa (P.) cinereocostata* and *Apisa (P.) asipa* sp. nov. Orange dots represent the localities of *A. (P.) cinereocostata*, purple dots *A. (P.) asipa* sp. nov., blue dots *A. (P.) subargentea*. The blue triangles indicate the literature location of *A. (P.) subargentea*.

be detected. Genitalia differ in the shape of elongate sacculus which may have parallel or slightly concave lateral margins and rounded or triangular termination.

Sexual dimorphism. Weakly expressed and in available material reliably visible only in the length of rami of antenna which in female are approximately three times shorter than in male counted at central portion of antenna. Male from Adamaua has M2–M3 of forewing on a short stalk.

Distribution. (Fig. 11) Known only from two localities in the northern region of Cameroon and one in Nigeria.

Etymology. The specific epithet “*asipa*” is the anagram of word *Apisa*, the name of the genus, which the new taxon belongs to.

Apisa (P.) cinereocostata Holland, 1893

Figs 4C, 5B, 6C, 7B, 12, 13B, 14C–D

Apisa cinereo-costata Holland, 1893, Psyche, 6: 394 t. typica: Valley of the Ogove River

Apisa bourgognei Kiriakoff, 1952: 173–175; synonymized by Przybyłowicz (2009)

Material examined. Holotype ♂: Gabon: “Kangwé, Ogové Riv., W. Africa [leg.] A. C. Good”, GS P374 [CMNH].

Other material. (84 ♂♂, 4 ♀♀) ♂ West Africa, Liberia, Gbarpolu County, Gola National Forest, Kungbor, Nordrand, 401m, 7°38'54.683"N, 10°34'35.154"W, Lichtfang, 1.6.2017, leg. Michael Ochse; ♂ same but OP215983, ♂ same but OP215984, GS P328; ♂ same but OP215985, GS P329; ♂ same but 31.5.2017, GS P330; ♂ same but OP215986, GS P331; ♂ same but OP215987; ♂ same but OP215992; ♂ same but 1.6.2017, OP216018, GS P332; ♂ same but OP216019, GS P333; ♂ same but OP216020, GS P334; ♂ same but 6.6.2017, GS P335; ♂ same but OP216021, GS P336; ♂ same but 30.5.2017, OP216022, GS P337; ♂ same but 29.5.2017, OM523171, GS P338; ♂ same but 2.6.2017, OP215988; ♂ same but OP215989; ♂ same but OP215990, GS P339; ♂ same but Radiostation, 460m, 7°38'53.212"N, 10°34'26.907"W, Lichtfang, 4.6.2017, leg. Michael Ochse; (coll. Ochse later ZSM); ♂ West Africa, Liberia, Grand Cape Mount County, Gola National Forest, Iseral, 7°22'52.65"N, 10°51'8.73"W, 232m, Light attraction, October 18th, 2012, leg. Michael Ochse; BC ZSM LeP76644, OP215991; (coll. Ochse later ZSM); ♂ Liberia Nimba Mountains, Mount Gangra summit, 7°32'45.82"N, 8°38'9.36"W, 17–25.III.2017, Leg.: Sáfián, Sz., Simonics, G., OP216023, GS P375; ♂ Liberia Nimba Mountains, Mount Gangra western slope, 7°33'29.73"N, 8°38'16.40"W, 16–17.III.2017, Leg.: Sáfián, Sz., Simonics, G., OP216024; ♀ same but OP216025; GS P376; ♀ same but OP216026; (ANHRT); ♂ Liberia, Grand Gedeh, County, Putu Range, 19–31.XII.2010, Leg.: Sáfián, Sz., Zakar, E., GS S469; (ISEA PAS); ♂ Liberia 700m, ENNR, Nimba Mts., Cellcom Rd., 7°32'47.5"N, 8°32'1.33"W, 10–24.III.2017 Light trap (250w blended bulb) & cold cathode UV light bucket trap (8w), Sáfián, Sz., Simonics, G. Leg., ANHRT: 2017.36, ANHRTUK 00051002, OP216027, GS P340; ♂ Liberia 1000–1100m, ENNR, Nimba Mts., Cellcom Rd., 7°32'45.9"N,

8°31'21"W, 12–16.III.2017 Light trap (250w blended bulb) & cold cathode UV light bucket trap (8w), Sáfián, Sz., Simonics, G. Leg.; ANHRTUK 00022983, Ex. Coll. Sz. Sáfián, ANHRT: 2017.36, GS P341; ♂ Liberia 508m, Nimba County, Yekepa residential area, 7°34'26.3"N, 8°32'31.6"W, ex-Pupa hatched on: 10–31.III.2017, Sáfián, Sz., leg.; ANHRTUK 00023404; Ex. Coll. Sz. Sáfián, ANHRT: 2017.36, GS P342; (ANHRT); ♂ Westafrika, Guinea, Guinée Forestière, 27 km südlich Bounouma, Foret Classée du Dieké, 7°27'52.20" N, 8°50'36.60"W, 482m, Lichtfang, 7. Juni 2013, leg. Michael Ochse, Falter für Publikation fotografiert, OP216028; ♂ Westafrika, Guinea, Haute Guinée, 8 km nördlich Konsankoro, 9°6'18.99" N, 8°0'42.06"W, 575m, Lichtfang, 7 Juni 2013, leg. Michael Ochse; (coll. Ochse later ZSM); ♂ W-Africa, Guinea, Konakri, Macenta Prefecture, Ziama Forest, 550m, 250 watt, April 2017, leg. Petányi G; Muller GC; Kravchenko VD et al., Thomas Witt Stiftung, GS P343; (Coll. R. Fiebig later ZSM); ♂ Guinea 1536m, Nimba Mts, 600 forest SMFG, concession area (Société des Mines de Fer de Guinée) Mont Pierre Richeaud (montane forest), 7°39'49.31" N, 8°22'20.06"W 21–30.VIII.2017 Light trap (250w blended bulb) & cold cathode UV light bucket trap (8w), Sáfián, Sz., Simonics, G, leg, ANHRT: 2017.36; ANHRTUK 00051005, OP216029, GS P344; ♂ same but ANHRTUK 00051003, OP216030; ♂ ANHRTUK 00051004, OP216031; (ANHRT); ♂ Guinea 700m, Nimba Mts, SMFG concession area (Société des Mines de Fer de Guinée) Cité 1, 7°42'2.83" N, 8°23'58.60"W, 16–25.VII.2017 General coll at Light Sáfián, Sz. Leg., ANHRT: 2017.36; ANHRTUK 00050212; Ex. Coll. Sz. Sáfián, ANHRT: 2017.36, GS P345; (ANHRT); ♂ Afrika, Guinée, Coyah, 1963.VIII.22., Dr. K.Ferencz (ISEA PAS); ♂ West Africa, Guinea Konakri, Macenta Prefecture Ziama Forest, 550m, 17.11–01.12.2016 Generator 250 Watt, Leg. Petrányi G; Muller, GC; Kravchenko VD et al., GS P346; (Coll. R. Fiebig later ZSM); ♂ Westafrika, Guinea Haute Guinée, 8 km, nördlich Konsankoro, 9°6'18.99" N, 9°0'42.06"W, 575m, Lichtfang, 4. Juni 2013, leg. Michael Ochse, GS P347; ♂ same but OP215993, GS P348; (Coll. Ochse later ZSM); ♂ Guinea Konakri, Macenta Prefecture, Ziama Forest 550m, Mt Nimba, Mav. 2017, leg. GC Muller VD Kravchenko & G Petranyi, GS P349; (Coll. R. Fiebig later ZSM); ♂ Ghana, Volta Region, Likpe Bakua, 05–06.IX.2010, Leg.: Dall'Astra, U., Dall'Astra A. & Sáfián, Sz., OP215994; (ISEA PAS); ♂ Ghana. Western: Bia Forest, 250m, 6km. W. Adwufia, 12–13.X.2007, Knud Larsen, GS P377; ♂ Ghana. Northern: Mole 150m. 8 km, N. of Gate, 16.III.2010, Knud Larsen & Wojciech Kubasik, GS P378; (Coll. KL); ♂ Ghana. Northern: Mole 150m. 8 km, N. of Gate, 16.III.2010 Wojciech Kubasik, OP215995; (ISEA PAS); ♂ Ghana. Western: Jomor, Ankasa, 90m. 2–3.V.2007, leg. Knud Larsen, GS P379; (Coll. KL); ♂ Ghana, Bunso, 2009.X.31, leg.: Sz. Sáfián, OP215996; (ISEA PAS); ♂ Ghana. Eastern: Bunso 4 km. S. 300m, 21.–23.III.2010, Knud Larsen & Wojciech Kubasik, OP215997, GS P380; (Coll. KL); ♀ Ghana, Eastern Region, Bunso Arboretum X.2011, leg.: Sáfián, Sz., OP215998; GS S475; ♂ Ghana, Central Region, Rainforest Lodge, Kakum National Park, XII.2011 Leg.: Sáfián, Sz., OP215999; (ISEA PAS); ♂ Ghana, Western Region, Visitor Centre, Ankasa National Park, 27–30.XI.2011, Leg.: Dall'Astra, U., Sáfián, Sz., Ochse, M.; ♂ same but OP216000, GS P350; (Coll. Ochse later ZSM); ♂ R.W.Goff Kotu Gambia 13°27'22" N, 16°14'23"W, ANHRTUK 00051010; ♂ R.W.Goff Tanji Gambia 13°22'52" N, 16°46'83"W, ANHRTUK 00051007; ♂ same but 13°22' N, 16°46' W, ANHRTUK 00051013; ♂ R.W.Goff Kuloro Gambia 13°17'54" N, 16°34'10"W, ANHRTUK 00051009, OP216001; ♂ R.W.Goff Abuko Gambia 13°23'41" N, 16°38'45"W, ANHRTUK 00051012, OP216002; ♂ Sierra Leone 120m, Tiwai Island, Moa River, N 07°33'00" W 11°21'09", 17–22.VI.2016 Light trap, leg. Takano Miles & Goff, ANHRT 2017.18, ANHRTUK 00020750, OP216003; ♂ Sierra Leone 420m, Loma Mountains, farm-

land/forest mosaic, N 09°07'47" W 11°05'24", 11–15.vi.2016 Light Trap, leg. Takano, Miles & Goff, ANHRT: 2017.18, ANHRTUK 00017145, OP216004, GS P351; ♂ same but ANHRTUK 00017144; OP216010; GS P352; ♂ R.W.Goff. Baoma, Goderich, Sierra Leone, 8°25'41"N 13°15'47"W, ANHRTUK 00051017, OP216005; ♂ same but ANHRTUK 00051018, OP216006, GS P352; ♂ same but ANHRTUK 00051016, OP216007, GS P353; ♂ same but ANHRTUK 00051015, OP216008, GS P354; ♂ same but ANHRTUK 00051014, OP216009, GS P355; (ANHRT); ♂ Nigeria, Kaduna, 14.X.1971, leg. H. Politzar, Stsllg München; (ZSM); ♂ Nigeria, Bendel State, Okomu F. Res., 27.05.1984, leg. J. Wojtusiak; ♂ same but 19.05.1984; (ISEA PAS); ♂ Nigeria Owena, 18.6.60, m H9; (ISEA PAS); ♂ Ivory Coast, 174m, Tai NP., Tai Research Station (SRET), 05°50'00"N 07°20'32.0"W, 25.iii–17. iv.2017, MV light, Aristophanus, A., Aristophanus, M., Geiser, M., Moretto, P., leg., ANHRT: 2017.25, ANHRTUK 00046056, OP216011; ♂ same but ANHRTUK 00047970, GS P356; (ANHRT); ♂ Afrique occ. Fr. Cote d'Ivoire, Bingerville, Melou G. 13.04.1914; ♂ same but GS P357; (ZIN); ♂ Ivory Coast, Grand Besebl, 12.–14.3.86, leg. Dr. Politzar; ex coll. Politzar, GS P358; (ZSM); ♂ Cote d'Ivoire, Ayamé II, Barrage de la Bia, 6/9-V-1964, Griveaud et Piart; ♂ same but 9–12.I.1964; GS P359; (RMCA); ♂ R. Goff. Abuko, Nature Reerve, Nr Compound. 13°22'22"N 16°38'55"W, ANHRTUK 00051011; ♂ same but ANHRTUK 00051008; (ANHRT); ♂ Southern Mali, 80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger, 28.07.2017, leg. Muller, K. Kravchenko, M. Traore & al., Museum Witt; ♂ same but OP216017, GS P360; ♂ same but OP216016, GS P361; ♂ same but OP216015, GS P362; ♂ same but OP216014, GS P363; ♂ same but OP216013, GS P364; (MWM); ♂ Northern Mali, Mopti region, Dogon Plateau, Bandiagara, 450–750m, 28.07.2017, leg. Muller, K. Kravchenko, M. Traore & al., Museum Witt, OP216012, GS P365; (MWM); ♂ Mali, Mopti region, Dogon Plateau, Bandagara, 450–850 m, January 2013, leg. Muller & K. Kravchenko, Museum Witt, GS P366; ♂ same but 450–750 m, November 2015, GS P367; (MWM); ♀ Southern Mali, 80 km SW of Bamako, near Kenieroba river Niger, 360m, December 2015, leg. Muller, K. Kravchenko, M. Traore & al. Museum Witt, OP216032, GS P368; (MWM); ♂ Angola, Huambo Prov., rd. Huambo – Caconada, E Catata, 1667 m, 13°23'58.6"S 15°26'54.1"E, 25.XI.2017, leg. S. Naumann, E. Ott & H. Sulak, Museum Witt, GS P369; (MWM)

Redescription (based on a male HT). Forewing length 12 mm. — **Head.** Frons and vertex creamy white; labial palpus darker, three segmented of which second is the longest and the third directed downwards, covered with short scales broader than those covering head; scapus creamy white; flagellum bipectinate, concolorous with scapus; flagellomeres honey, ramii in medial part four times as long as antenna width; eye convex, indistinctly ovoid. — **Thorax.** Vestiture unicolorous creamy white expressing darker or lighter tint depending on the illumination; legs of the same uniform coloration; epiphysis reaching 2/3 of the foretibia length; mid and hind tibia with a pair of short, terminal spurs. — **Abdomen.** Entirely creamy white, concolorous with the rest of the body. Upperside similar to underside. — **Forewing.** Dull, subhyaline especially in middle zone, almost opaque along margins and in the outer third of the wing length, covered with creamy white, intermixed scales of two different shapes, elongate needle-like and flattened with distinctly triangle-concaved terminal margin; veins and subcostal zone up to DC slightly darker, costa ochraceous; cilia creamy

white; R1 separated from R2–R5 but glued-like to R stem for most of its length; M2–M3 from one point; distances between M3–CuA1 and CuA1–CuA2 similar; 1A+2A markedly convex towards DC in one third of its length; coloration of underside similar to upperside, retinaculum present. — **Hindwing.** Coloration somewhat paler than in forewing; cilia almost white, Rs–M1 completely fused.

Male genitalia. (Figs 4C, 12) Tegumen rather narrow, slightly broadened laterally. Vinculum much narrower than tegumen, widely fused with its lateral arms. Uncus well developed, provided dorsally and sublaterally with numerous setae, gradually narrowing up to 3/4 of its length then widened forming forked termination almost as broad as base of uncus; terminal concavity broad, widely U-shaped with a pair of indistinct teeth on distal margin. Valva subsquare, approximately half length of uncus, terminal margin concave medially; outer portion of costa provided with several rather short, stiff setae; costa subbasally with a sword-like, prominent, sclerotized protrusion about 4–5 times longer than its width at base. Juxta in form of weakly sclerotized, submedial plate fused in the subventral margin of valva. Transtilla lateral arms weakly sclerotized, almost invisible, submedian portion enlarged, fused with juxta and forming prominent, tubular, sclerotized anellus. Saccus terminating into a sclerotized process widely rounded at tip, about twice as long as broad. Phallus straight and short. Vesica membranous with a single thorn-like cornutus in basal portion.

Female genitalia. (Figs 5B, 6C) Papillae anales longer than broad, sparsely covered with protruding setae denser towards terminal portion; apophyses posteriores of similar length as papillae anales, narrow, dully ended; apophyses anteriores shorter than papillae anales, narrow; dorsal pheromone glands in form of two separate, elongate, membranous, irregular shape pouches; ventral pheromone glands with single opening then separated into finger-like, irregular pouches narrower than dorsal pheromone glands; ductus bursae membranous, straight, subbasal portion narrower then widened in one third of the length; ductus seminalis slender originating from the widening; corpus bursae subsquare, delicate; signum located in proximal portion of corpus bursae, longitudinal, sclerotized, granulate, in form of elongate irregular plate at least twice as long as broad; sternite VII subrectangular with laterodistal zone membranous and submedial portion partly surrounding ostium sclerotized and Y-shaped, Y-shaped emargination wider than long.

Variation. Very variable species in the intensity of dark tint of the body. The holotype male represents the pale, almost 'whitish' colour morph while some specimens can be much darker up to almost completely ochraceous with all intermediate forms. The darker, indistinct pattern can be also observed in the pale specimens especially on different portions of head as frons or vertex. The male genitalia also express significant variation in morphology of uncus and especially the development and perspicui-

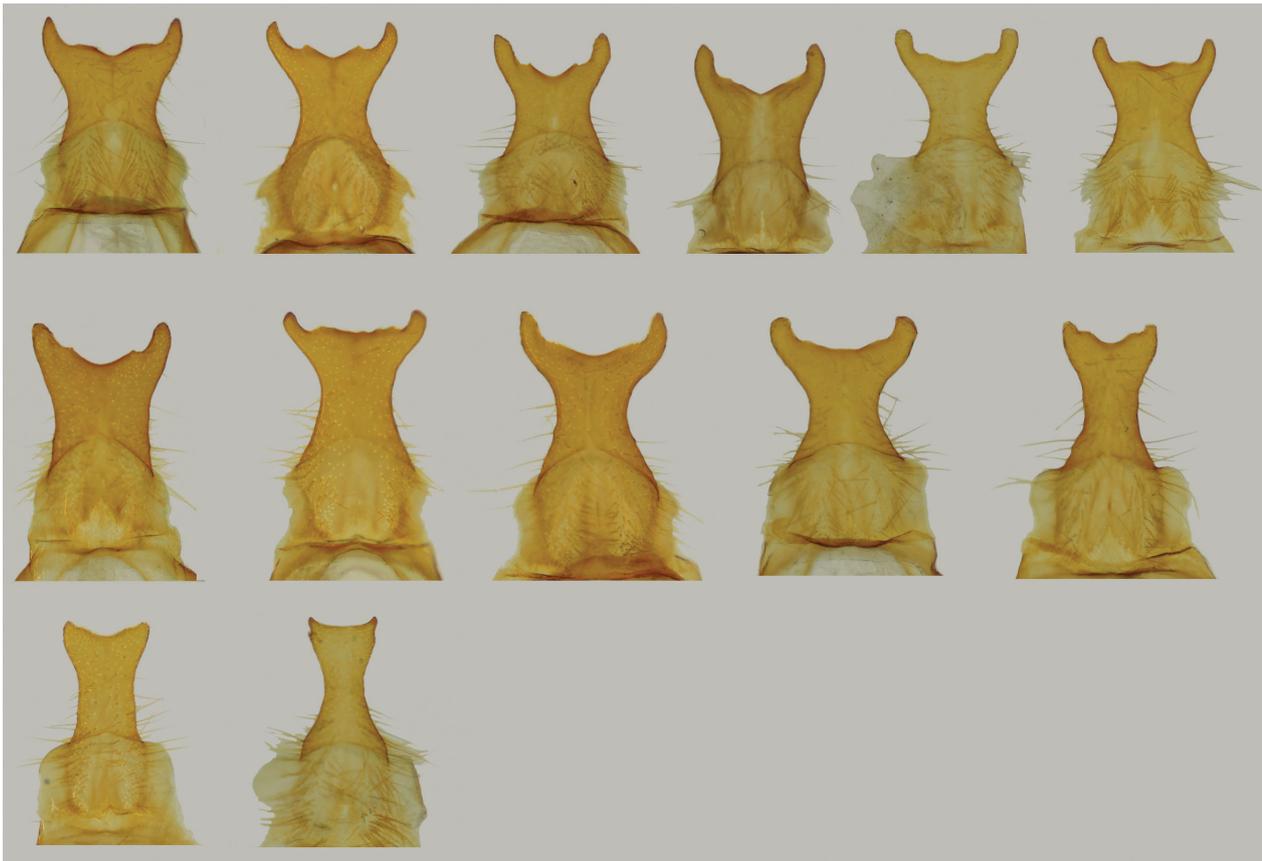


Figure 12. Overview of variation in the bifid termination of uncus in *A. (P.) cinereocostata*. Arranged from the broadest forms, with well developed lateral arms towards the dwarf forms.

ty of its forked termination. The detailed examination of detected variability is elaborated in the discussion part of the article.

Sexual dimorphism. The female differs from the male by much shorter rami of antenna which are twice as long as the width of the antenna and by shorter epiphysis reaching at most 2/3 of the foretibia length.

Distribution. (Fig. 11) Angola, Gambia, Ghana, Guinea, Ivory Coast, Liberia, Mali, Sierra Leone.

Comments. The details of the holotype locality are extracted from the label and were not published together with the original description.

Apisa (P.) subargentea Joicey and Talbot, 1921

Figs 4A, 5C, 6B, 7A, 13A, 14B

Apisa subargentea Joicey & Talbot 1921, Bull. of the Hill. Mus., 1(1): 158 [*A. subargentea*] t. typica: Lake Tshohoa, Ruanda District.

Material examined. Holotype: ♀ “Lake Tshohoa, Ruanda Dist., Cent. Afr. Aug. '19, T.A. Barns; Joicey Bequest. Brit. Mus. 1934–120”; g.s. ARCT 5795 [NHMUK]

Other material. ♂ *Apisa griseocens subargentea*, Joicey and Talbot; Coll. Mus. Congo, Kibali-Ituri Nioka, 7.VI.1953, J.Hecq; GS P373; ♂ Mus. Congo, Kibali-Ituri Nioka, 27.XI.1953, J.Hecq; GS P372; ♀ Burundi Gitega, 13.III.1967, Dr M. Fontaine; Coll Museum Tervuren; GS P671; ♀ Coll. Mus. Congo, Kibali-Ituri: Nioka, 31.V.1954, J. Hecq; GS P670; (RMCA)

Description of male (based on a specimen from Kibali-Ituri, Nioka collected 7.VI.1953). — **Head.** Frons and vertex pale ochraceous; labial palpus darker, three segmented of which second is the longest and the third directed downwards, covered with short scales broader than those covering head; scapus pale ochraceous; flagellum bipectinate, concolorous with scapus; flagellomeres honey, rami in medial part four times as long as antenna width; eye convex, indistinctly ovoid. — **Thorax.** Vestiture unicolorous pale ochraceous expressing darker or lighter tint depending on the illumination; legs of the same uniform coloration; epiphysis stout reaching 4/5 of the foretibia length; mid and hind tibia with a pair of short, terminal spurs. — **Abdomen.** Entirely pale ochraceous, concolorous with the rest of the body. Upperside similar to underside. — **Forewing.** Opaque, densely covered by flattened scales with distinct, clearly visible shine on the entire surface of the wing; scales suboval, moderately elongate, with rounded terminal margin, pale ochraceous, slightly darker along veins, with admixture of white-creamy ones in areas between them; subcostal zone up to DC indistinctly darker than remaining part of wing; veins

covered by scales; cilia pale cream; R1 separated from R2–R5; M2–M3 narrowly separated; distances between M3–CuA1 and CuA1–CuA2 similar; 1A+2A almost straight, without distinct curve in one third of its length; coloration of underside similar to upperside, retinaculum present. — **Hindwing.** Coloration somewhat paler than in forewing; cilia almost white, Rs–M1 completely fused.

Male genitalia. (Fig. 4A) Tegumen rather narrow, slightly broadened laterally, provided with a few stout, elongate, protruding setae in dorsolateral zone. Vinculum much narrower, widely connected but not completely fused with lateral arms of tegumen. Uncus well developed, but basal margin not as broad as in *cinereocostata* and *asipa*; distinctly narrowing until the 3/4 of its length then slightly widened forming forked termination; both tips of forks and bottom of concavity smoothly rounded; subbasal and dorsolateral portions with hairy setae. Valva subsquare, much shorter than uncus, terminal margin concave medially; outer portion of costa provided with several elongate, stiff setae; costa subbasally with a short, bulbous, sclerotized protrusion. Juxta in form of longitudinal, submedial plate fused in the subventral margin of valva. Transtilla lateral arms weakly sclerotized, almost invisible, submedian portion enlarged, fused with juxta, and forming sclerotized anellus. Saccus terminating into a lanceolate, sclerotized process about three times as long as broad. Phallus straight and short. Very similar to *A. (P.) asipa* **sp. nov.** Vesica membranous without cornuti and any sclerotization.

Female genitalia. (Figs 5C, 6B) Papillae anales longer than broad, sparsely covered with protruding setae slightly denser towards terminal portion; apophyses posteriores at least as long as papillae anales, narrow, needle-like; apophyses anteriores shorter than papillae anales, narrowly ended; dorsal pheromone glands in form of two separate, elongate, membranous, irregular shape pouches; ventral pheromone glands with single, broad, shallow opening then separated into finger-like, irregular pouches much narrower than dorsal pheromone glands; ostium small, rounder, antevaginal plate membranous; postvaginal plate well developed, sclerotized, with a pair of anterolateral extensions towards antevaginal zone; ductus bursae membranous, straight, subbasal portion narrower than widened in one third of the length; ductus seminalis slender originating from the widening; corpus bursae subsquare, delicate; signum distinctly sclerotized, granulate, in form of small, irregular plate at most twice as long as broad; sternite VII subtriangular, gradually narrowed towards distal portion, terminal zone more sclerotized, Y-shaped.

Variation. The limited number of specimens does not allow for a proper detection of individual variation. Among the examined females it is expressed by differences in forewing length and intensity of ochraceous coloration, which may be more or less pale. Additionally, in some specimens, both males and females, the fused Rs–M1 can be forked before the termination.

Sexual dimorphism. The female differs from the male by much shorter rami of antenna which are twice as long as the width of antenna and by shorter epiphysis reaching at most 2/3 of the foretibia length.

Distribution. (Fig. 11) Known from west DRC, Rwanda, Burundi and Kenya.

Comments. The detailed description of *A. (P.) subargentea* was never published. The original, very short and superficial description refers to the female (Joicey and Talbot 1921). Already in 1960 the taxon was regarded as a synonym of *A. griseescens*, albeit with a question mark and no argumentation for such an action. The discovery of the male resulted in revision of the taxonomic status of the taxon and ascription to the proper subgenus (Przybyłowicz and Kuhne 2008). Male and female genitalia were illustrated in Przybyłowicz (2009), however with only a short summary of the diagnostic characters. Given the unusual homogeneity of all members of *Apisa*, it is reasonable to provide a detailed redescription of both sexes amended with illustrations of the newly discovered key characters.

3.3. Morphological variability

The taxonomic interpretation of 89 specimens of *A. (P.) cinereocostata* takes into consideration its polymorphic nature, regarding both the external morphology and male genitalia. One of the variable characters is general coloration of the entire body. Within the series of specimens available for examination, there are both very pale, almost whitish specimens and dark ochraceous ones, and all intermediate forms. To test if this variation may depend on the geographic distribution of the specimens, we ordered them following the respective countries from the west (Gambia) to the east (Angola). Although this approach is highly subjective due to the inaccuracy in ascription of the intermediate forms to dark or pale category, there is no clear signal that the colour forms may express any clinal variation. Instead, they are randomly dispersed within samples originating from different countries. Even assumption that some populations from neighbouring countries are so closely located that they represent in fact a single population does not change the picture of the rather random distribution of this polymorphism. Similar results are obtained by comparison of the morphology of uncus of 83 males. Forms with the wide and narrow tips are likewise randomly distributed across the entire range of the species.

The remaining two species are represented in our study in too few specimens to observe any clear morphological variability, except the most common referring to the indistinct differences in wing length and intensity of coloration. Much larger sets of specimens are necessary to investigate this aspect, however it is unlikely that any of the two taxa is as polymorphic as is *A. (P.) cinereocostata*.

3.4. Molecular analysis and the haplotypes

To carry the phylogenetic analyses two methods were used – ML and BI (Figs 1, 2). For the molecular analyses 84 specimens were selected, from which 50 sequences of *A. (P.) cinereocostata* and 3 sequences of *A. (P.) asipa* **sp. nov.** were obtained. Unfortunately, it was not possible to obtain sequences from the species *A. (P.) subargentea*, most likely due to the age and possible contamination of the material – only 4 specimens, collected more than 50 years ago were available to us. The length of the final alignment of the COI sequences equals 658 bp. Sequences obtained from the species *A. (P.) cinereocostata* are characterized by a relatively low intraspecific variation. Molecular analyses confirm morphological results from 50 genital preparations of males of the *A. (P.) cinereocostata* species.

The ML and BI phylogenetic trees based on the COI gene show similar, but not identical topology (Figs 1, 2). Both the analyses show the distinctiveness of the polymorphic species *A. (P.) cinereocostata* from the newly described *A. (P.) asipa* **sp. nov.** and two other representatives of the genus, with high support values.

3.4.1. P-distances within *A. (P.) cinereocostata*

To assess the intraspecific genetic variability of *A. (P.) cinereocostata*, the p-distance between barcode sequences of 51 specimens was calculated. For a comparison also samples representing *A. (P.) asipa* **sp. nov.** as a representative of the same subgenus and other *Apisa* species were included (Tab. S3). The p-distances within *A. (P.) cinereocostata* vary between 0.0 and 3.6%. The values above 3% are scored for just 9 pairs of specimens and most of the genetic variability is lower than 2%. This variability is independent of the morphotypes. The two darkest and two palest specimens were compared in this respect. The p-distances between palest-darkest specimens varies between 1.4–1.9%, while the distance between the pairs of two palest and two darkest specimens equals respectively 1.1% and 0.3%. All these values fall within a genetic variability typical for a single taxon.

The number of specimens available for this genetic study was limited to three specimens collected in the same locality. The p-distance between them varies between 0.0 and 0.9%.

The interspecific p-distance between *A. (P.) cinereocostata* and other members of *Apisa* varies from 3.8% to 5.7%, but the lowest value is scored only for four specimens. For *A. (P.) asipa* **sp. nov.** the lowest distance from *A. (P.) cinereocostata* is 4.4%, while the difference to members of subgenus *Apisa* s. str. despite the significant morphological differences is on average lower and varies from 3.1 to 3.8%.

The interspecific distance between *A. (P.) cinereocostata* or *A. (P.) asipa* **sp. nov.**, and *A. (A.) canescens* vary between 3.1% and 5.7%, respectively. Finally, the

two members of other genera used as the outgroup (*Ter-vurenia eloumdeni* and *Anapisa holobrunnea*) differ from *Apisa* by a p-distance of 6.2% to 12.5%.

3.4.2. Haplotype network

Haplotypes were obtained for 51 sequences of *A. (P.) cinereocostata*. The haplotype network was prepared for the specimens representing the polymorphic taxon *A. (P.) cinereocostata* (N = 51). For the remaining taxa too few specimens were available to construct separate networks or include them into the network of *A. (P.) cinereocostata*. Altogether 26 different haplotypes were recognized, and they can be divided into two general haplogroups A (N = 28) and B (N = 23). For the further analysis of this genetic diversity, information on the countries of collecting and colour forms is included (Fig. 14A). The haplogroups A and B are not discrete as concern their geographic pattern. Specimens representing the haplogroup A are distributed across all countries where the analysed *Apisa* specimens were collected, with exception of the Ivory Coast. The analysis of the network reveals that only specimens from Mali (N = 6) are characterized by a set of 5 different haplotypes, unique for this country. They are clustered into a single assemblage that is most similar, but still distant by 3 substitutions, to Hap_22 expressed by part of specimens from Liberia). Although the haplogroup B is represented by specimens only from three countries: Guinea, Liberia and Ivory Coast, these countries cover the central, extensive part of the entire range of *A. (P.) cinereocostata*. A weak signal suggesting some distinctiveness of the Easternmost populations from Ghana is visualized by the existence of unique haplotypes Hap_9 and Hap_10, but only for two specimens. The most common haplotype, Hap_13, was found in 7 specimens from: Gambia (2 specimens), Sierra Leone (3), and Liberia (2). Another common haplotype is Hap_1, characteristic for 7 specimens from Guinea (2) and Liberia (5). As many as 17 haplotypes are found only once. Liberia, the country from which the highest number of specimens was analyzed, shows also the highest variation in the haplotype pattern with 14 different and 10 unique of them. Considering the dark and pale specimens, the latter are clustered exclusively in the haplogroup B (Fig. 3B). However, this division is not complete because three haplotypes: Hap_11, Hap_12 and Hap_24 from this subgroup are represented only (in two cases) or in part by the pale morphotype.

4. Discussion

4.1. Concept of subgenus *Apisa* (*Parapisa*)

The concept of dividing *Apisa* into three subgenera is based on key morphological differences in the male genitalia: *A. (Parapisa)* – uncus bifid; *A. (Apisa)* – uncus single, the process of valva long; *A. (Dufraneella)* – uncus single, the process of valva short (Kiriakoff 1952b, 1953).

Our genetic data are only based on the COI barcode fragment, and the phylogenetic analyses do not reflect the obvious morphological division indicated by the uncus. In the molecular phylogenetic tree, two samples that don't represent *A. (Parapisa)* based on morphology are located between *A. (P.) cinereocostata* and *A. (P.) asipa*, which both, from a morphological perspective, represent unambiguously the subgenus *A. (Parapisa)*. However, it should be stressed that due to the limitations of using only a single gene and lacking DNA sequence data for *A. (P.) subargentea*, we don't aim to produce a robust phylogeny of *A. (Parapisa)* or even the entire genus *Apisa*. Instead, we consider our genetic analysis results a confirmation of the evolutionary distinctiveness of both the genital-polymorphic *A. (P.) cinereocostata* and the peculiar *A. (P.) asipa*. Considering that the genetic signal does not correlate with the morphological data, we propose to maintain the distinction of subgenus *A. (Parapisa)* until new data and evidence will become available to test this hypothesis.

The study and revision of the remaining taxa of the genus *Apisa* are in progress. Therefore, we refrain from the precise determination of *Apisa* samples not representing the subgenus *A. (Parapisa)* at this stage. However, the examination of the uncus (bifid vs single) allows to associate any given sample with or outside of *A. (Parapisa)*. Based on these morphological differences the two undetermined *Apisa* species do not belong to *A. (Parapisa)* and, as we argue above, their position in the molecular phylogeny between the two representatives of *A. (Parapisa)* may be artificial and might not depict the true phylogenetic relationships.

4.2. Variability of *A. (P.) cinereocostata*

Our study of specimens of *A. (P.) cinereocostata* confirms species affiliation and high variability. Descriptions of characters and genetic analysis (COI mtDNA) supporting this polymorphism are provided for adult males and females of pale and dark morphotypes of the species.

The DNA barcodes obtained from museum specimens are very useful to resolve taxonomic uncertainty with the type species and some cryptic species, especially if morphological data on its own is insufficient (Hernandez-Triana 2014). Often the species of interest had been collected long before the DNA sequencing started to be a frequently used technique in biodiversity studies and describing new species (Mitchell et al. 2015). However, barcoding of museum specimens may be challenging because the degree of DNA degradation depends on how the material has been stored. Sometimes also contaminations with other genetic material may occur during inappropriate storage and preservation (Hernandez-Triana 2014).

The range of the species *A. (P.) cinereocostata* covers the western part of the African continent, the collected material comes from countries quite close to each other. It is impossible to distinguish some isolated populations, morphologically or genetically distinct, to treat

them as separate species, which shows how high diversity and variability exists in *A. (P.) cinereocostata*. The available material and almost complete lack of data on the non-morphological characteristic of the taxon makes impossible any speculation on the biological drivers favouring the existence of different colour forms and so significant plasticity in morphology of the male genitalia. The observed pattern is especially intriguing, because whereas colour polymorphism is not rare in Lepidoptera, the morphology of the male genitalia is usually very stable within a species and this attribute is widely used to separate similar species (Mutanen and Kaitala 2006, but see Fibiger et al. 2009).

The most well-known examples of polymorphism are: sexual dimorphism, polyphenism, color polymorphism, and geographic polymorphism (Grados 2019). There are known species with a continuous variation in the genital apparatus of males, both within and between populations. For example, in *Pammene luedersiana* (Tortricidae) some geographic variability without a systematic shape variation was observed (Mutanen et al. 2007). In some species exhibiting variation in the structure of the male apparatus, slight modifications are possible in those parts of the genitalia that do not play a key role during copulation. In the genus *Hystriochophora* (Tortricidae), an evolutionary process appears to lead towards greater intraspecific differentiation (Gilligan et al. 2008). An example of a polymorphic species belonging to the tribe Arctiini is *Watsonidia fulgida* Grados, 2019. Within the species both males and females represent separate morphotypes with continuous variation in the male genitalia (Grados 2019). Many cases of polymorphism are observed within the family Geometridae, for example in *Alcis repandata*, *Idaea aversata*, or *Angerona prunaria* (Ford 1953).

Also, we cannot rule out a hypothesis that the observed polymorphism is a result of existence of several (at least two) closely related cryptic species. However, getting a clearer picture favouring or falsifying this hypothesis would require access to a much larger set of specimens, covering more or less evenly the entire range of the taxon in question. Finally, the observed variability may be a result of an ongoing diversification process, although this hypothesis is unlikely given the fact that none of the forms is geographically restricted or can be linked with any environmental factor like altitude or type of vegetation.

It can be also assumed that the high morphological variability is maintained within populations as the response for the wide distribution and the utilization of the very different microhabitats on the large area stretching from almost sub-Saharan western Africa, through the coastal equatorial areas along the Guinea Bay up to the again semiarid uplands of Angola.

4.3. Morphology of scales

Scales and their structures are one of the most studied photonic structures for a long time (Mouchet et al. 2018). Detailed study of the *A. (Parapisa)* morphology revealed

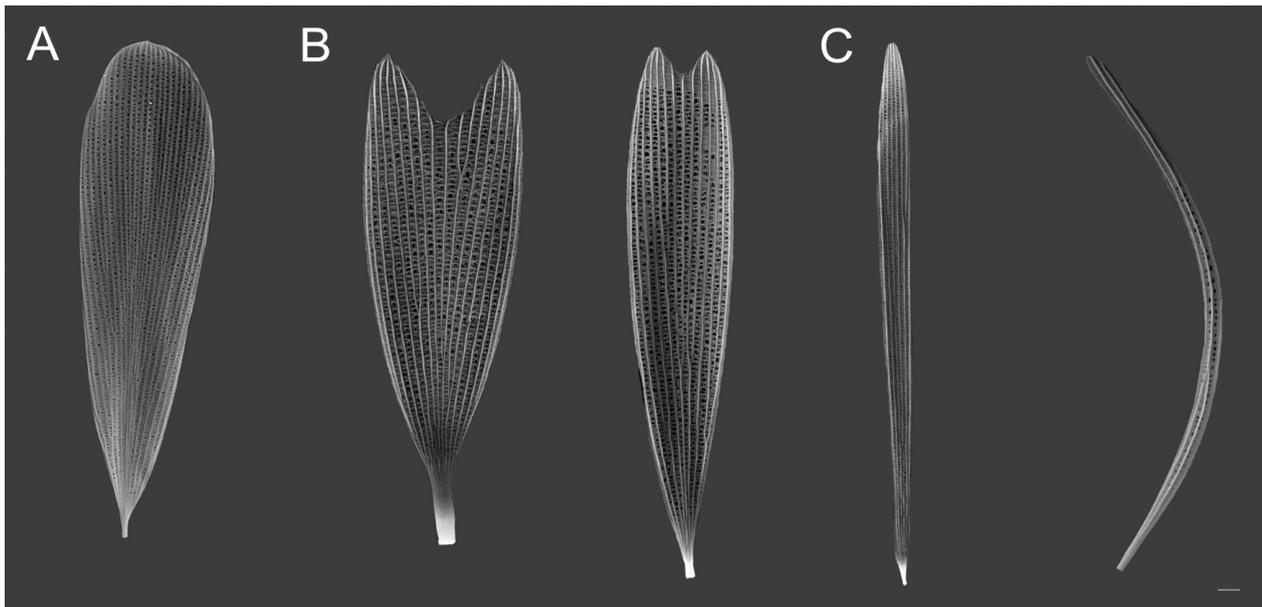


Figure 13. SEM image showing the differences between scale shape of *Apisa (P.) subargentea* (A) (flattened, convex termination), *A. (P.) cinereocostata* (B) (flattened, concave termination) and *A. (P.) asipa* **sp. nov.** (C) (narrow, needle-like).

an unusual interspecific variability in the wing scale morphology. That exoskeletal feature occurs in very broad range of structural and functional diversity (Boppré et al. 2019). Among three examined species there is a gradual series of modifications which is most obvious when observed on the central, upper portion of the forewing. In *A. (P.) subargentea* all scales are typically flattened (Fig. 13A) and tightly fitting to each other. In *A. (P.) cinereocostata* (Fig. 13B) despite variation in coloration the respective zone of a wing is covered by similar in shape, relatively loosely flattened scales with distinctly concave termination. The most modified, extremely narrow scales are characteristic for *A. (P.) asipa* **sp. nov.** (Figs 13C, 14). With their upraised position and rather sparse arrangement they make the wing almost transparent. The gradual narrowing of scales may suggest that in *A. (P.) subargentea* they reflect the most plesiomorphic condition. However, the situation is more complicated because *A. (P.) subargentea* is the only taxon not only in *A. (Parapisa)* but of the entire genus *Apisa* characterized by distinctive, silver shine of wing which is one of the most obvious diagnostic characters of the species. Visual signals are one of the most important communication channels for the Lepidoptera, where the photonic nanostructures play a large role – they are very specific for a given species (Kertész et al. 2021). This indicates the complicated morphological structure of scale and the silver being the structural colour may be linked with the courtship behaviour of *A. (P.) subargentea*.

4.4. Biogeographic aspects

The range of the subgenus *A. (Parapisa)* covers a large area in the subequatorial zone of Africa. However, each of the three taxa is characterized by a very distinctive type of distribution. *Apisa (P.) subargentea* is known

from a relatively small area within east equatorial Africa with a wide distributional gap of about 1500 km, separating it from the remaining two species. It also occupies the highest altitudes of all taxa, with no known records from low elevations. Despite the small number of known specimens, this taxon seems to be linked with the East African Highlands. Such pattern of distribution is somewhat unusual assuming the common evolutionary history of *A. (Parapisa)*. In contrary, *A. (P.) asipa* **sp. nov.** is restricted to the upland regions of central Africa with very few known localities in Adamawa and Jos Plateaus. Despite the fact that both regions are not dramatically different from the surrounding areas, they depict some degree of uniqueness in their flora and fauna. Jos Plateau, and in particular Amurum Forest Reserve is an Important Bird Area (IBA) of Nigeria with at least 300 known bird species, including many endemics (Agaldo 2020). It is a place where *Gallinago media* occurs and migrates (Ezealor 2011). The topography of the area, with surrounding lowland plateau make it an excellent habitat for xerophytes. A parasitic angiosperm which is endemic to Africa occurs here. In 2018 there was the first report of *Hydnoria* in that ecological zone (Agyeno et al. 2018).

The location and biome of the Cameroon's mountains, including Adamawa, make it one of West Africa's biggest hot spots (Myers et al. 2000). The region has more than 200 plant species considered endangered, of which more than 80 are endemic (Sainge et al. 2017). The entire mountain region of Cameroon is one of the most important African sites in terms of its level of endemism and species richness. Moreover, these sites form peculiar ecoregions with stable environments that are refugia for many species (Blackburn 2008). A few years ago, as many as 114 plants endemic to Cameroon were known, with 29 species known from the well-studied Campo-Ma'an region (Tchouto et al. 2006).



Figure 14. Differences in the forewing transparency. *Apisa (P.) asipa* sp. nov. (A), wing transparent; *A. (P.) subargentea* (B), wing opaque; *A. (P.) cinereocostata* (C, D), wing semitransparent.

Contrary to the abovementioned species *Apisa (P.) cinereocostata* is a really widespread taxon known from several countries in Central and west Africa. Accumulation of fresh material and detailed examination of museum collections allowed for the significant widening of its known range which now much better depicts the real distribution. The taxon is not restricted to the subcostal zone of Guinea Gulf. The new record from Angola stretches the range more to the south, while numerous specimens from Mali indicate its presence in the Sub-Saharan zone. These records are very interesting zoogeographically, because they constitute the first such distributional data on the occurrence of *Apisa* so far to the north in western Africa. Until now only eastern African records from Ethiopia and even the Arabian Peninsula are known. Taking into account also the old records from Libya, it seems very probable that *Apisa* was once distributed across the whole of Africa north of the equator, before the Sahara formation which took place about 6,000 years ago, this is the time when great changes in biome took place in this youngest desert (Hänninen 2021). Eastern Africa is therefore not necessarily the only passage from tropics to more temperate regions but due to the existence of land masses stretching further north and lack of wide barriers such as the Mediterranean Sea the descendants of this distribution can nowadays be

detected in subarid habitats of the Arabian Peninsula. On the contrary the earlier western distributional gains were totally obliterated by desertification reaching the Atlantic coast and spreading into huge areas of West Africa. *Apisa* population discovered in Mali may be the witness of the much wider previous distribution of the genus.

To sum up the biogeographic aspects, it should be noted that only members of the subgenus *A. (Parapisa)* (two in West Africa, one in East Africa) are separated by a wide geographic gap. As a whole, the genus *Apisa* is distributed evenly across the entire extent of Sub-Saharan Africa without a gap in the central part of the continent. However, the detailed distribution of every taxon (especially the most common *A. (A.) canescens*) is not elaborated in detail, and it is not clear if Central Africa is inhabited by a single, widespread species or if it is home to more taxa.

5. Conclusions

We revised the subgenus *A. (Parapisa)* which is one of the three subgroups of *Apisa*. It is distributed in the wide areas of subequatorial Africa stretching from the Atlan-

tic to the Indian Ocean coasts. Three taxa are recognized with very different distribution. The newly described *A. (P.) asipa* sp. nov. is restricted to uplands of central Africa, while the least known *A. (P.) subargentea* inhabits eastern Africa. Based on the examination of more than 80 specimens we concluded that *A. (P.) cinereocostata* is a widespread, highly polymorphic taxon with regard its overall coloration and the male genitalia morphology. We were not able to link this variability with any orographic or ecological factors. Therefore we recommend in-depth studies on the life-history requirements of each of the two colour morphs of the species. Additionally, more sophisticated molecular methods should be applied after gathering numerous and freshly collected specimens from different areas in search of possible explanation of this phenomenon.

The new species unexpectedly appeared to be unique among all other known *Apisa* in its morphology of wing scales that are exceptionally narrow. The nature of this modification remains unresolved. More detailed field study is desired to assess if this is really an endemic of Central African Highlands.

Finally we would like to stress that *A. (P.) subargentea* despite its description already in the XIXth century still remains the least known *A. (Parapisa)*, known from a few specimens only. This is also the only *Apisa* species with silvery opalescent wings indicating their complicated, structural morphology, yet another aspect which should be a focus of future study.

6. Acknowledgements

We are indebted to Gyula László (African Natural Research Trust), Michael Ochse, Sáfán Szaboles, Thomas Witt, Knud Larsen for providing access to Lepidoptera collections and for lending materials for research, Nikolai Ignatev for providing photos and genital slides for the type series and for the productive, substantive email conversations, Irena Brunarska (Institute of Geological Sciences at the Jagiellonian University, Kraków, Poland) for taking the SEM photos, David Agassiz for the language consultation and comments on the manuscript. The third author is grateful to Alberto Zilli for his help during work at NHMUK collection.

7. References

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Supplementary material 1

Table S1

Authors: Paśnik A, Tarcz S, Przybyłowicz Ł (2023)

Data type: .docx

Explanation note: Index of GenBank access numbers with the specie names *A. (P.) cinereocostata*, *A. (P.) asipa* **sp. nov.**, *T. eloumdeni*, *A. holobrunnea*.

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Link: <https://doi.org/10.3897/asp.81.e96319.suppl1>

Supplementary material 2

Table S2

Authors: Paśnik A, Tarcz S, Przybyłowicz Ł (2023)

Data type: .docx

Explanation note: Specimens used for analysis with locations, numbers of genital slides, access to GenBank database.

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Link: <https://doi.org/10.3897/asp.81.e96319.suppl2>

Supplementary material 3

Table S3

Authors: Paśnik A, Tarcz S, Przybyłowicz Ł (2023)

Data type: .docx

Explanation note: Pairwise distances between DNA barcode sequences of species of *Apisa (P.) cinereocostata*, *A. (P.) asipa* **sp. nov.**, *Apisa* s. str., *Tervurenia eloumdeni*, *Anapisa holobrunnea*. The number of base substitutions per site between sequences are shown. The analysis involved 58 nucleotide sequences. All positions containing gaps and missing data were eliminated. In the final dataset, there were a total of 658 positions. Analyses were conducted using the Tamura 3-parameter model in Mega 7.0.9. The light blue color indicates representatives of the subgenus *Apisa*, the green color *Apisa asipa* **sp. nov.** The last two individuals of *Tervurenia eloumdeni* and *Anapisa holobrunnea* were selected as outgroups. Dark gray indicates two representatives of the dark morphotype and light gray indicates the light morphotype of the species *A. (P.) cinereocostata*. Light gray in the table indicates individuals with a distance greater than 2.8%. The intersections of light and dark individuals and the distance between them are marked in bright yellow..

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Link: <https://doi.org/10.3897/asp.81.e96319.suppl3>