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Systematyka i filogeneza afrykańskich motyli z rodzaju *Apisa*.

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Rozprawa doktorska

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Department of Invertebrate Zoology

Systematic phylogeny in African moths of the genus *Apisa* (Lepidoptera: Arctiinae).

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Doctoral thesis

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Podziękowania

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SPIS TREŚCI

Streszczenie

Rodzaj *Apisa* Walker, 1855 należy do rzędu Lepidoptera, rodziny Erebidae, podrodziny Arctiinae, plemienia Syntomini i zawiera 13 znanych gatunków (aktualny katalog w: **Paśnik & Przybyłowicz, manuskrypt**). Zasięg występowania tego rodzaju obejmuje środkową i południową część Afryki oraz Półwysep Arabski (Przybyłowicz, 2009). Jeden gatunek *A. manetti* Turati, 1924 znany jest z Libii, ale jego rzeczywisty zasięg występowania jest niemożliwy do zweryfikowania przez brak dostępności do materiału z tych obszarów.

Obiekt badań stanowią średniej wielkości motyle nocne o rozpiętości skrzydeł od 2-4,5 cm, bardzo jednolitym szaro-ochrowym ubarwieniu i rysunku zredukowanym do słabo widocznych ciemniejszych linii wzdłuż żyłek skrzydeł.

Przedstawiona rozprawa doktorska składa się ze spójnej tematycznie serii trzech artykułów (**Przystałkowska** [=Paśnik]**, 2022**; Zootaxa); (**Paśnik i in., 2023**; Arthropod Systematics & Phylogeny); (**Paśnik & Przybyłowicz, manuskrypt**). Weryfikuje hipotezy o istnieniu w obrębie rodzaju szeregu gatunków kryptycznych, a co za tym idzie większej liczby taksonów niż dotychczas opisano. Na podstawie danych morfologicznych i molekularnych potwierdza także przynależność wszystkich gatunków do rodzaju *Apisa*.

W badaniach molekularnych użyto sekwencji trzech markerów – jednego mitochondrialnego i dwóch jądrowych: pierwszej podjednostki oksydazy cytochromowej (COI), genu *wingless* (WG) i rybosomalnego białka S5 (RpS5).

Badane okazy sfotografowano, zmierzono długość i rozpiętość skrzydeł, a także wykonano preparaty z aparatów genitalnych samców oraz samic. W sumie morfologicznie przeanalizowano 300 okazów, sporządzając przy tym 238 preparatów. Badania potwierdziły monofiletyczność opracowywanej grupy motyli. Wykryto, opisano i zilustrowano przy pomocy SEM autapomorfię rodzaju występującą u wszystkich przedstawicieli, którą jest brak arolium pomiędzy pazurkami stóp. Wskazano, że ten stan cechy wyróżnia rodzaj *Apisa* od wszystkich innych rodzajów Syntomini pozostających z nim w bliskich relacjach filogenetycznych.

Dzięki kompleksowej analizie morfo-genetycznej udało się dokonać redeskrypcji części gatunków i opisać trzy nowe (*A. atrovenosa*, *A. asipa*, *A. diversa* **sp. nov.**) potwierdzając hipotezę, że rodzaj *Apisa* stanowi kompleks gatunków kryptycznych.

Udowodniono także, że obecny podział na trzy podrodzaje *Apisa* s. str., *Parapisa* Kiriakoff, 1952 i *Dufraneella* Kiriakoff, 1953, opierający się na różnicach w budowie morfologicznej unkusa i wyrostka wewnętrznej części walwy, jest sztuczny, a cechy te dywersyfikowały w sposób niezależny u różnych grup gatunków. Przeprowadzona analiza wyznaczania gatunków (ASAP) wskazała na występowanie 18 hipotetycznych gatunków w obrębie badanej grupy okazów.

W konkluzjach podkreślono istotną kwestię, że w przypadku grup tak jednorodnych morfologicznie, a jednocześnie cechujących się znacznym stopniem zmienności morfologicznej, samo zastosowanie podejścia integratywnego nie gwarantuje wyjaśnienia problemów systematyczno-filogenetycznych.

Przedstawione badania stanowią spójną całość, która poszerza i porządkuje obecny stan wiedzy na temat systematyki i filogenezy rodzaju *Apisa*. Wyniki stanowią solidną podstawę do przeprowadzenia dalszych badań z wykorzystaniem nowoczesnych technik analitycznych.

Szczególnie intrygujące, a nierozwiązane kwestie, stanowią biologia, behawior czy badania feromonów, których poznanie na pewno przyczyni się do jeszcze lepszego poznania tak jednolitej morfologicznie grupy motyli jaką jest rodzaj *Apisa*.

Summary

The genus *Apisa* Walker, 1855 belongs to the order Lepidoptera, family Erebidae and subfamily Arctiinae, tribe Syntomini. It includes 13 known species (the newest catalog in **Paśnik & Przybyłowicz, manuscript**). The distribution range of the genus includes central and southern Africa and the Arabian Peninsula. One species, *A. manetti*, is known from Libya but its actual range is impossible to scarcity of material.

The study organisms are medium-sized nocturnal lepidopterans with a wingspan of 2-4.5 cm, a very uniform gray-ochre coloration and a pattern reduced to faintly visible darker lines along the wing veins.

The present dissertation consists of a thematically coherent series of three articles (**Przystałkowska** [=Paśnik]**, 2022;** Zootaxa); (**Paśnik i in., 2023**; Arthropod Systematics & Phylogeny); (**Paśnik & Przybyłowicz, manuscript**). It examines the hypothesis of a number of cryptic species occurring within *Apisa*, some taxa of which are undescribed. The results of analysis of morphological and molecular data confirm also that all examined species belong to the genus *Apisa*.

For molecular studies, three markers were used, one mitochondrial and two nuclear: the first subunit of cytochrome oxidase (COI), the wingless gene (WG) and ribosomal protein S5 (RpS5).

Altogether 300 specimens were morphologically analyzed. Prior to more detailed analyses, they were photographed and the wing length and wingspan were measured. 238 genital preparations of males and females were made. The morphological study confirmed the monophyly of *Apisa.* An autapomorphy of the genus that is present in all its representatives is the absence of an arolium between the tarsal claws. This is described and illustrated using SEM photography. It is emphasized that absence of arolium distinguishes the genus *Apisa* from all other genera of Syntomini that are phylogenetically closely related.

By comprehensive morpho-genetic analysis, it was possible to redescribe several species and describe three new ones (*A. atrovenosa*, *A. asipa*, *A. diversa* **sp. nov.**), confirming the hypothesis that the genus *Apisa* is indeed a complex of cryptic species.

The study proves that the current division into three subgenera *Apisa* s. str., *Parapisa* Kiriakoff, 1952 and *Dufraneella* Kiriakoff, 1953, based on the morphological structure of the uncus and the length of the process of valva, is artificial and these features diversified

independently in different groups of species. A species delimitation analysis (ASAP) indicates the presence of 18 hypothetical species within the studied group of specimens.

The conclusions emphasized the important point that in the case of a group as morphologically homogeneous as this and yet still characterized by a significant degree of morphological variation, the application of an integrative approach alone does not solve all systematic and phylogenetic problems.

The research presented here represents a coherent revision that expands and organizes the current state of knowledge on the systematics and phylogeny of the genus *Apisa*. The results provide a foundation for further research using more sophisticated analytical techniques.

Particularly intriguing and unresolved issues are the biology and behavior of the different species as well as the studies of pheromones, which would certainly contribute to an even better understanding of such a morphologically homogeneous group of lepidopterans as is represented by the genus *Apisa*.

Publikacje wchodzące w skład rozprawy doktorskiej

Przystałkowska A. [=**Paśnik**] (2022). A new species of *Apisa* Walker, 1855 (Lepidoptera: Erebidae: Arctiinae) from Uganda with remarks on the apomorphies of the genus. Zootaxa, 5128(1), 91-106. DOI: 10.11646/zootaxa.5128.1.5

Zootaxa, IF (1.026), 5-letni Impact Factor (1.073), MEiN: 70

Wkład w publikację: *Zaplanowałam i zaprojektowałam eksperyment, wykonałam preparaty i przeprowadziłam analizy morfologiczne, wykonałam prace laboratoryjne, analizy molekularne, przygotowałam tabele i ryciny, interpretowałam wyniki, przygotowałam treść manuskryptu.* **Wkład szacowany na 100%.**

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

Arthropod Systematics & Phylogeny, IF (2.262), 5-letni Impact Factor (2.262), MEiN: 100

Wkład w publikację: *Przygotowałam preparaty do analiz morfologicznych (aparaty genitalne, preparaty z łusek), przeprowadziłam prace laboratoryjne i analizę wyników molekularnych, przygotowałam ryciny i tabele. Przejrzałam i zatwierdziłam ostateczny manuskrypt, miałam współudział w analizie danych, napisaniu manuskryptu.* **Wkład szacowany na 70%.**

Paśnik A, Przybyłowicz Ł (manuskrypt) Systematics and phylogeny of *Apisa* (Lepidoptera: Erebidae: Syntomini) – current stage of knowledge and perspectives.

Manuskrypt wysłany do Organism Diversity and Evolution, IF (2.663), 5-letni Impact Factor (2.741), MEiN: 100

Wkład w publikację: *Wykonałam prace laboratoryjne i preparaty genitalne, przeprowadziłam analizy filogenetyczne. Przygotowałam tekst i prowadziłam konsultacje zagraniczne. Miałam współudział w interpretowaniu wyników.* **Wkład szacowany na 75%.**

Wstęp

Nasz stan wiedzy o różnorodności gatunkowej cały czas wzrasta lecz, jak wskazuje liczba rokrocznie opisywanych nowych gatunków organizmów, wciąż jest niewystarczający. Przez dziesięciolecia opisy nowych taksonów owadów, a w szczególności motyli, bazowały wyłącznie na cechach morfologii zewnętrznej (kolor, wzór na skrzydłach). Znacznie później standardową metodą stała się szczegółowa analiza aparatów rozrodczych zarówno samców jak i samic. Rozwój technik molekularnych sprawił, że obecnie istotnym elementem opisu jest również analiza genetyczna. Obecnie powszechnie akceptowane jest, że najlepszym podejściem dla naukowego stawiania hipotez jakimi są opisy nowych gatunków jest połączenie dostępnych technik badawczych zwane taksonomią integratywną. Połączenie metod morfologicznych i genetycznych umożliwia najpełniejszą i najdokładniejszą charakterystykę dostępnych do badań okazów. Nowoczesna morfologia korzysta również z różnorodnych metod obrazowania, z których w taksonomii szczególnie użyteczne jest obrazowanie SEM, umożliwiające nie tylko analizę drobnych mikrostruktur, ale również uzyskanie bardziej naturalnego niż w mikroskopach świetlnych, trójwymiarowego obrazu obiektu. W badaniach genetycznych motyli standardową cechą diagnostyczną jest marker mitochondrialny genu COI, traktowany jako swoisty kod kreskowy danego organizmu (Karmazina i in., 2020).

Gatunki są podstawowymi, cennymi i ważnymi jednostkami różnorodności biologicznej, dlatego błędy w wyznaczaniu granic gatunków mogą mieć poważne konsekwencje (Chenuil i in., 2019). Procesu opisywania, a w konsekwencji późniejszego oznaczania okazów, nie ułatwiają gatunki kryptyczne, które mogą stanowić znaczną część różnorodności biologicznej. Problem w niespójnej definicji gatunku dodatkowo utrudnia oszacowanie ich udziału w bioróżnorodności, ekologii i procesach ewolucyjnych (Struck i in., 2018).

Rodzaj *Apisa* Walker, 1855 będący przedmiotem niniejszych badań należy do plemienia Syntomini Herrich-Schäffer, 1846 jednego z czterech w obrębie Arctiinae Leach, 1815. Podrodzina ta to najliczniejsza i najbardziej różnorodna morfologicznie grupa rodziny Erebidae Leach, 1815 wchodzącej w skład nadrodziny sówek Noctuoidea. Cała podrodzina niedźwiedziówkowatych (Arctiinae) stanowi grupę kosmopolityczną i dość dobrze zbadaną w porównaniu do wielu innych rodzin tzw. motyli drobnych (Weller i in., 2009). Przynależność *Apisa* do Syntomini jest potwierdzona przez dwie cechy morfologiczne wspólne dla wszystkich przedstawicieli plemienia: całkowite zlanie żyłek Sc, Rs i M1 w skrzydle tylnym (Holloway,

1988) oraz obecność dobrze rozwiniętych, parzystych gruczołów feromonowych na odwłoku samicy (Jacobson & Weller 2002). Rodzaj *Apisa* Walker, 1855, będący przedmiotem rozprawy, został zaproponowany dla gatunku *Apisa canescens* Walker, 1855. W pierwotnym opisie nie zostały podane wyraźne cechy diagnostyczne nie tylko rodzaju, ale również jedynego, zaliczonego do niego gatunku. Krótki opis zawiera jedynie lakoniczne informacje odnośnie kolorystyki, długości skrzydeł i pokroju ciała. Mimo, że w późniejszych publikacjach zawierających opisy kolejnych gatunków, rodzaj *Apisa* traktowany był jako spójna morfologicznie grupa, nigdy nie doczekał się właściwej diagnozy pozwalającej na jednoznaczne określenie jego odmienności od innych przedstawicieli Syntomini. Kwestia ta staje się coraz bardziej istotna w kontekście coraz liczniejszych, ogólnych prac filogenetycznych dotyczących relacji ewolucyjnych w obrębie całych Arctiinae (nawet Erebidae, czy Noctuoidea). Dodatkowo badania nowych materiałów pochodzących z różnych obszarów Afryki stopniowo powiększające zarówno liczbę gatunków jak i wiedzę o zmienności morfologicznej wskazują na pilną potrzebę jasnej diagnozy rodzaju.

W obecnej klasyfikacji rodzaj dzieli się na trzy podrodzaje: *Apisa* s. str. Walker, 1855; *Parapisa* Kiriakoff, 1952; *Dufraneella* Kiriakoff, 1953 wyróżniane na podstawie cech aparatów kopulacyjnych samców. Jeden gatunek *A. manetti* Turati, 1924 znany z Libii, nie jest obecnie zaliczany do żadnego podrodzaju ze względu na niedostępność materiału typowego, a zwłaszcza braku informacji na temat budowy aparatów genitalnych żadnej z płci.

Podrodzaj nominatywny wyróżnialny jest przez obecność długiego wyrostka na wewnętrznej powierzchni walwy. Należą do niego gatunki: *A. canescens* Walker, 1855 i *A. arabica* Warnecke, 1935, chociaż ten ostatni przez część badaczy uważany jest za podgatunek *A. canescens*.

Cechą diagnostyczną podrodzaju *Parapisa* zaproponowaną przez Kiriakoffa (1952) jest wyraźnie rozdwojona końcowa część unkusa stanowiącego dystalną część aparatu kopulacyjnego samca. Do podrodzaju należą trzy gatunki: *A. cinereocostata* Holland, 1892 z bardzo szerokim rozstawem ramion unkusa i dużej zmienności tej cechy (gatunek polimorficzny), *A. subargentea* Joice & Talbot, 1921, u którego płytko wcięte ramiona unkusa są wąsko rozdzielone a łuski na skrzydłach posiadają srebrny połysk, oraz *A. asipa* **Paśnik, Tarcz & Przybyłowicz, 2023**, u którego rozdwojenie jest wąskie, trójkątne, ale bardzo głębokie. Pomimo ogólnego podobieństwa, w każdym przypadku dwudzielny unkus ma wyraźnie odmienną architekturę, a dane genetyczne zawarte w pracy **Paśnik i in.,** (2023) poddają w wątpliwość monofiletyzm grupy.

Z kolei rodzaj *Dufraneella* ma wyróżniać się według Kiriakoffa (1953) wyraźnym skróceniem wydłużonego u *Apisa* s. str. wyrostka wewnętrznej części walwy. Cecha zaproponowana przez autora nie jest jednoznaczna i nie pozwala na obiektywne zaklasyfikowanie taksonów o krótszym i dłuższym wyrostku odpowiednio do podrodzajów *Dufraneella* i *Apisa* s. str. Wątpliwości te potwierdza przeniesienie przez Przybyłowicza (2009) gatunku *A. hildae* Kiriakoff, 1961, opisanego pierwotnie w podrodzaju nominatywnym, do *Dufraneella*. *Dufraneella* obejmuje aż sześć gatunków. Dwa opisane zostały przez Rothschilda już w 1910 roku (*A. rendalli* i *A. subcanescens*). Kolejny *Metarctia grisescens* Dufrane, 1945 został przeniesiony do *Apisa* w 1953 przez Kiriakoffa. Następne dwa gatunki opisane przez Kiriakoffa to *A. fontainei* w 1959 i *A. hildae* w 1961 roku. W 2022 opisany został szósty gatunek z tego podrodzaju *A. atrovenosa* **Przystałkowska, 2022**. W związku z wątpliwościami co do właściwej klasyfikacji przedstawicieli każdego z podrodzajów rodzi się pytanie nie tylko o wartość wyróżniających je cech diagnostycznych, ale również o wspólną ewolucyjną przeszłość zaliczanych do nich gatunków.

Przedstawiciele rodzaju *Apisa* zasiedlają Afrykę Subsaharyjską wraz z Półwyspem Arabskim, a jeden gatunek *A. manetti* został opisany z wybrzeża Libii (Cyrenaika). Ze względu na jednolitość morfologiczną powodującą trudności w oznaczaniu dane dotyczące rozmieszczenia poszczególnych taksonów są rzadko publikowane (Przybyłowicz i in., 2019, Seizmair, 2017), a część z nich budzi poważne wątpliwości co do poprawności oznaczeń ze względu na zaniechanie analizy aparatów kopulacyjnych (np. Ochse, 2017). W rezultacie znane dotychczas zasięgi bazują na pojedynczych obserwacjach i uniemożliwiają szerszą analizę biogeograficzną w odniesieniu do całego rodzaju.

Jednolitość morfologiczna opisanych dotychczas taksonów w porównaniu z ich znaczną zmiennością powoduje znaczne trudności w jednoznacznym przyporządkowaniu do poziomu gatunku. Z tego też powodu zarówno systematyka jak i filogeneza rodzaju jest tylko częściowo rozwiązana i brak jest kompleksowych rewizji opartych o różne metody badawcze i liczny materiał porównawczy. Publikacja Przybyłowicza (2009) oparta jedynie na analizie morfologicznej typów deskrypcyjnych umożliwiła tylko częściowe uporządkowanie synonimiki bez odniesienia do kwestii zasadności i ewolucyjnej spójności wydzielonych wcześniej podrodzajów.

Cele i hipotezy badawcze

Cele badań

Celem przedstawionego cyklu trzech artykułów było określenie powiązań filogenetycznych, wyjaśnienie problemów taksonomicznych oraz określenie granic zmienności wewnątrz i międzygatunkowej w obrębie niezwykle jednolitego morfologicznie rodzaju *Apisa* z wykorzystaniem zarówno danych morfologicznych (struktury i narządy zewnętrzne i wewnętrzne) jak i molekularnych (zróżnicowanie sekwencji nukleotydów fragmentów DNA mitochondrialnego (mtDNA) i jądrowego (rDNA)).

Cel rozprawy doktorskiej realizowano w formie następujących zadań badawczych:

1. Ocena ukrytej różnorodności przy pomocy rozwiązań systematyki konwencjonalnej i skonfrontowanie wyników z molekularną delimitacją gatunków **(Paśnik & Przybyłowicz, manuskrypt)**.
2. Określenie apomorfii rodzaju *Apisa* oraz weryfikacja zasadności jego podziału na podrodzaje **(Przystałkowska, 2021**).
3. Syntetyczne opracowanie systematyczne rodzaju *Apisa* wraz z kluczami do oznaczania oraz ilustracją cech diagnostycznych **(Paśnik i in., 2023, Paśnik & Przybyłowicz, manuskrypt).**

Hipotezy badawcze

W publikacjach zweryfikowano następujące hipotezy badawcze:

1. Grupa stanowi najprawdopodobniej zbiór gatunków kryptycznych i liczy więcej taksonów niż dotychczas opisano (**Przystałkowska, 2022; Paśnik i in., 2023; Paśnik & Przybyłowicz, manuskrypt**).
2. Rodzaj *Apisa* stanowi grupę monofiletyczną. (**Paśnik i in., 2023**; **Paśnik & Przybyłowicz manuskrypt**).
3. Dotychczasowy podział rodzaju na podrodzaje *Apisa* s. str., *Parapisa* i *Dufraneella* jest sztuczny i nie odzwierciedla ewolucyjnych pokrewieństw pomiędzy taksonami (**Paśnik & Przybyłowicz, manuskrypt)**.
4. Podejście integratywne pozwala na wyjaśnienie problemów systematycznych i filogenetycznych w obrębie rodzaju *Apisa* (**Paśnik i in., 2023; Paśnik & Przybyłowicz, manuskrypt**).

Materiały i metody

Materiał badawczy

Analizy przeprowadzone zostały na materiale muzealnym. Obiekt badań stanowiły osobniki dorosłe motyli nocnych z rodzaju *Apisa*. Są to średniej wielkości motyle nocne o rozpiętości skrzydeł od 2 do 4,5 cm.

Ubarwienie ciała jest stosunkowo jednolite, osobniki wszystkich gatunków są jasne, szaro-kremowe. Strona grzbietowa ubarwiona podobnie do brzusznej. Głowa tego samego koloru co tułów (jasno beżowo-szara). Czułki pierzaste, brak wyraźnego dymorfizmu płciowego. Ubarwienie odwłoka jednolite nie odcinające się od ubarwienia reszty ciała.

Łuski pokrywające powierzchnie skrzydeł ułożone dachówkowato, cała powierzchnia skrzydeł pokryta lekko lśniącymi łuskami u *A. subargentea*, u innych rozmieszczone nieco luźniej, z wyjątkiem gatunku *A. asipa*, gdzie łuski są wyraźnie wydłużone i bardzo wąskie z wyraźną skulpturą w postaci podłużnych żeberek (Fig. 7, 13, **Paśnik i in., 2023**).

Badania przedstawione w artykułach wchodzących w skład rozprawy doktorskiej zostały przeprowadzone w Instytucie Systematyki i Ewolucji Zwierząt Polskiej Akademii Nauk w Krakowie oraz na Uniwersytecie w Lund. Materiał do badań morfologicznych i molekularnych pochodził z szeregu europejskich muzeów, kolekcji prywatnych i materiałów zgromadzonych w zbiorach Lepidopterologicznych ISEZ PAN (**Przystałkowska, 2022**; **Paśnik i in., 2023; Paśnik i Przybyłowicz, manuskrypt**). Z dostępnych materiałów opisano 3 nowe gatunki (*A. atrovenosa* **Przystałkowska, 2022**; *A. asipa* **Paśnik, Tarcz & Przybyłowicz, 2023**, *A. diversa* **Paśnik & Przybyłowicz, manuskrypt**).

Analiza morfologiczna

Do analiz morfologicznych wykorzystano 300 osobników. Wszystkie zostały przebadane i oznaczone początkowo do poziomu morfogatunku na podstawie zewnętrznych cech morfologicznych. Osobniki zostały rozdzielone na płci, a każdy z osobników został sfotografowany aparatem cyfrowym Canon 70D i 6D z obiektywem makro EF 50 mm lub makro EF 100 mm na statywie, w namiocie bezcieniowym. Wykonano zdjęcia strony grzbietowej, brzusznej i zdjęcia etykiet. Utworzono bazę z danymi o osobnikach wykorzystanych do badań. Aby przyporządkować osobniki do rangi gatunkowej wykonano 238 preparatów genitalnych.

Preparaty mikroskopowe zostały wykonane wykonano według standardowej procedury preparowania Macrolepidoptera. Oderwane odwłoki macerowane były w kąpieli wodnej 10% roztworu wodorotlenku potasu (KOH). Czas maceracji zależny był od płci, stopnia zniszczenia okazów i wahał się od kilkunastu do kilkudziesięciu minut. Przed przystąpieniem do preparowania odwłoki przepłukane zostały w wodzie destylowanej w celu usunięcia resztek wodorotlenku. Aparaty genitalne oczyszczono z niepotrzebnych tkanek i przeniesiono na szkiełko podstawowe do kropli gliceryny. W celu uwidocznienia delikatnych struktur (torebka kopulacyjna u samic, wezyka w aedeagusie u samców) genitalia wybarwiono czernią chlorazolową. Preparaty przechowywane są w glicerynie i stanowią bazę materiału porównawczego. Po zakończeniu badań materiał utrwalany jest Euparalem na szkiełkach podstawowych, zaetykietowany i włączony do kolekcji ISEZ PAN lub odesłany do instytucji skąd został wypożyczony. Zdjęcia preparatów zostały wykonane przy użyciu aparatu zintegrowanego z lupą Leica S9i. Wykonane fotografie zostały poddane post produkcji przy wykorzystaniu programów Adobe Photoshop CC, Corel Photo Paint i Aphinity Designer.

Przygotowano preparaty do zdjęć SEM ze stóp trzech gatunków z rodzaju *Apisa* sp. i trzech gatunków do porównań *Balacra rattrayi* (Rothschild, 1910), *Anapisa holobrunnea* (Talbot, 1932), *Amata phegea* (Linnaeus, 1758) (Fig. 4, **Przystałkowska, 2022**). Z gatunku *A. asipa*, *A. subargentea*, *A. cinereocostata* wykonano preparaty z łusek pierwszej pary skrzydeł. Zdjęcia preparatów mikroskopowych (Fig. 7, **Paśnik i in., 2023**) wykonane zostały na stereomikroskopie Nikon SMZ1000 z podpiętym aparatem Canon 70D. Zdjęcia spod mikroskopu SEM (Fig. 13, **Paśnik i in., 2023**) zostały wykonane przy użyciu mikroskopu JEOL JSM5410.

Naświetlanie światłem ultrafioletowym może wpływać za zachowanie żywych osobników. Naświetlanie materiałów muzealnych może uwidocznić u niektórych gatunków rysunki na skrzydłach, niewidoczne w świetle widzialnym. U niektórych przedstawicieli Lepidoptera występują wzorce wewnątrzgatunkowe jak i międzygatunkowe. Często są one charakterystyczne dla danego gatunku i mogą przybierać skomplikowane wzory. Potencjalną ich funkcją może być sygnalizacja drapieżnikom o toksyczności lub niejadalności. Być może obecność wzorów jest sygnałem dla potencjalnych partnerów. Technika stosowana na okazach muzealnych ma swoje ograniczenia, ponieważ nie ma możliwości badania wzorów w ruchu i w naturalnym środowisku. Sprawdzono przedstawicieli rodzaju *Apisa*, ale po naświetlaniu światłem ultrafioletowym nie zaobserwowano żadnych wzorów na skrzydłach.

Metody laboratoryjne

Analizy morfologiczne i molekularne przeprowadzone zostały na materiałach muzealnych (kolekcje suche). Od wybranych do badań molekularnych okazów oderwano po dwa odnóża, umieszczono je w sterylnych probówkach typu „eppendorf". Każdą probówkę opatrzono odpowiednią etykietą, którą również podpięto pod okaz. Do przeprowadzenia izolacji DNA użyto zestawu NucleoSpin Tissue Kit (Macherey-Nagel, Niemcy) wykorzystując standardową procedurę. Amplifikację przeprowadzono w oparciu o trzy markery: mitochondrialny pierwszej podjednostki oksydazy cytochromowej (COI), jądrowe białko rybosomalne S5 (RpS5) i wingless (WG) (**Przystałkowska, 2022**). Użyte startery dla genu COI (startery specyficzne dla rzędu Lepidoptera) wymienione zostały w publikacji (**Przystałkowska, 2022**, **Paśnik i in., 2023**), dla genów RpS5 i WG (**Paśnik i in., 2023)**. Wszystkie zmiany w objętości i odczynnikach zamieszczono w tabeli 3 w manuskrypcie **Paśnik & Przybyłowicz**.

Otrzymane sekwencje oczyszczono i przeprowadzono reakcję sekwencjonowania. Odczyty wykonano częściowo w firmie zewnętrznej Macrgoen (Amsterdam, Holandia), Genomed (Warszawa, Polska) oraz w Pracowni Technik Molekularnych ISEZ PAN z użyciem sekwenatora ABI3130xl. Otrzymane sekwencje (ang. *forward* i *reverse*) przed przystąpieniem do dalszych analiz były sprawdzane i przyrównane w programie BioEdit (Hall, 2004). Obliczono odległości genetyczne pomiędzy gatunkami w rodzaju *Apisa* przy użyciu programu MEGA 11 (Tamura i in, 2021) (Tabela 1, **Przystałkowska, 2022**; Tabela S3, **Paśnik i in., 2023;** Tabela S1 **Paśnik & Przybyłowicz, manuskrypt**).

Analiza filogenetyczna

Podczas analiz powiązań filogenetycznych w obrębie rodzaju zastosowano metodę największej wiarygodności (Maximum Likelihood ML) i wnioskowanie bayesowskie (Bayesian Interference BI). Najlepszy model ewolucji wyliczono w programach MEGA 11 i RAxML (Stamatakis A, 2014) z użyciem najmniejszego Bayesowskiego kryterium informacyjnego Schwartza (BIC). Analizę wnioskowania bayesowskiego wykonano w programie MrBayes 3.2.7 (Roniquist i in., 2012). Wnioskowanie przeprowadzono na 6 milionach pokoleń (**Przystałkowska, 2022**) i 4 milionach pokoleń (**Paśnik i in., 2023**; **Paśnik & Przybyłowicz, manuskrypt**) z zapisem drzew co tysiąc pokoleń. W każdym przypadku odrzucono pierwsze 25% drzew przed uzyskaniem drzewa konsensusowego. Metodę ML przeprowadzono z użycie programu MEGA 11, w celu oszacowania rzetelności drzewa zastosowano samopróbkowanie (bootstrap) co 1000 powtórzeń. Jako grup zewnętrznych użyto *Anapisa hollobrunea* (MO76629)*, Tervurenia eloumdeni* (OM523179). W Paśnik & Przybyłowicz (manuskrypt) podano tabelę z wybranymi grupami zewnętrznymi tabela 4. Otrzymane sekwencje zostały włączone do bazy GenBank i opatrzone konkretnym numerem dostępu (GenBank specimen voucher). Część sekwencji okazów z grupy zewnętrznej pochodzi z dostępnych w bazie danych. Nowo opisane gatunki zostały umieszczone w internetowej wersji Oficjalnego Rejestru Nomenklatury Zoologicznej (ZooBank).

Sieci haplotypów zostały wygenerowane metodą minimalnego łączenia (minimum joining) dla mitochondrialnego markera pierwszej podjednostki oksydazy cytochromowej (COI) w programie DnaSP v5.10.01 (Librado i Rozas, 2009) a następnie opracowane i zwizualizowane w PopArt v1.7 (Leigh i Bryant 2015). Sieci haplotypów wygenerowane zostały dla 51 sekwencji polomorficznego gatunku *A*. *cinereocostata*.

Analiza wyznaczania granic gatunków

Do wyznaczenia granic gatunków (species delimitation) wykorzystano internetową wersję programu ASAP (Assemble Species by Automatic Partitioning) (Puillandre i in., 2021). Do analiz użyto drzewa filogenetycznego opartego na sekwencjach genów COI, WG, RpS5 bez grup zewnętrznych (**Paśnik i in., 2023**). Dzięki automatycznemu grupowaniu osobników na podstawie podobieństw sekwencji algorytm wykorzystuje takie metody jak analiza klastrowa, sieci filogenetyczne, analiza odległości genetycznych, aby dokonać oceny podobieństw między sekwencjami i przyporządkować osobniki do różnych grup reprezentujących oddzielne gatunki.

Wyniki

Wyniki pierwszego opracowania (**Przystałkowska, 2022**) potwierdzają słabe poznanie zmienności morfologicznej wewnątrz i międzygatunkowej w obrębie rodzaju. Morfologia narządów rozrodczych osobników z rodzaju *Apisa* nie była do tej pory kompleksowo opracowana, a wciąż dla niektórych taksonów jak *A. arabica* Warnecke, 1934 i *A. manetti* Turati, 1924 cechy diagnostyczne nie są dobrze zdefiniowane (**Przystałkowska, 2022**). Rozwój technik obrazowania takich jak mikroskopia skaningowa dostarcza dodatkowych narzędzi do identyfikacji gatunków kryptycznych poprzez analizę detali anatomicznych. W pracy po raz pierwszy zilustrowano istotną cechę rodzajową w obrębie Syntominni. Rodzaj *Apisa* charakteryzuje się brakiem arolium (struktury znajdującej się pomiędzy pazurkami na stopie) (Fig. 4, **Przystałkowska, 2022**). Zazwyczaj arolium ma postać miękkiej tkanki kutikularnej wypełnionej hemolimfą i najpewniej pełniącej funkcję pomocniczą przy poruszaniu poprzez przyleganie do powierzchni.

Z dostępnego materiału udało się odróżnić od znanych gatunków osobniki o wyraźnie ciemnym użyłkowaniu skrzydeł. Po wykonaniu preparatów z aparatów genitalnych i dokładnych badaniach morfologicznych i molekularnych opisano nowy gatunek. Nowy gatunek, *Apisa atrovenosa* **Przystałkowska, 2022** ze stanowisk z Ugandy i Gabonu ze względu na cechy morfologiczne umieszczony został w podrodzaju *Dufraneella*.
Rozwój technologii molekularnych, analiz genetycznych i technik obrazowania przyczynia się do lepszego zrozumienia i identyfikacji gatunków kryptycznych. To z kolei przekłada się na działania związanie z prowadzeniem badań nad ewolucją i ekologią gatunków.

Wyniki drugiego opracowania (**Paśnik i in., 2023)** umożliwiły stworzenie klucza do oznaczenia przedstawicieli podrodzaju *Parapisa* (klucz na podstawie zewnętrznych cech morfologicznych, budowy aparatów genitalnych samców i samic). W publikacji dokonano redeskrypcji znanych gatunków *A. subargentea* Joicey & Talbot, 1921, *A. cinereocostata* Holland, 1893 oraz zawarto opis *A. asipa* **Paśnik, Tarcz & Przybyłowicz, 2023**. Opisany gatunek znany jest z obszaru Gór Kameruńskich stanowiących duże centrum różnorodności gatunkowej w zachodniej Afryce oraz z jednego stanowiska w Nigerii. Opisano zmienność łusek przedniego skrzydła w polu pomiędzy żyłkami CuA1 i CuA2 a krawędzią komórki DC. Łuski zobrazowano na zdjęciach spod binokularu, mikroskopu świetlnego (Fig. 7, **Paśnik i in., 2023**) i mikroskopu SEM (Fig. 13, **Paśnik i in., 2023**).

Dzięki możliwości analizy dużej liczby okazów i wykonanych preparatów genitalnych wykazano i szczegółowo przeanalizowano zmienność wewnątrzgatunkową *A*. *cinereocostata*. Gatunek ten charakteryzuje się znacznym polimorfizmem zarówno ubarwienia skrzydeł (morfotyp ciemny i jasny) jak i budowy unkusa (Fig. 12, **Paśnik i in., 2023**). Analiza molekularna (COI) potwierdziła, przynależność wszystkich analizowanych morfotypów do jednego taksonu. Na potwierdzenie zmienności genetycznej *A*. *cinereocostata* wygenerowano sieci haplotypów z 51 otrzymanych sekwencji genu COI. Otrzymano 26 różnych haplotypów, które można podzielić na dwie ogólne grupy: A liczącą 28 i B 23 okazy. Na sieciach zaznaczono kraj odłowu osobników (Fig. 3A, **Paśnik i in., 2023**) gdzie okazy z Mali (N=6) charakteryzują się zestawem 5 różnych haplotypów unikalny dla tego kraju. Największe zróżnicowanie ubarwienia skrzydeł (Fig. 3B, **Paśnik i in., 2023**) zaobserwowano w Liberii skąd przeanalizowano największą liczbę okazów. Zaobserwowano, że okazy charakteryzujące się ciemnym morfotypem ubarwienia skrzydeł zlokalizowane są w dużej haplogrupie B.

Wyniki trzeciego opracowania (**Paśnik & Przybyłowicz, manuskrypt)** oparte zostały na analizie filogenetycznej z wykorzystaniem trzech markerów mitochondrialnego COI i jądrowych WG i RpS5. Do potwierdzenia wyznaczonych morfologicznie kladów wykorzystano zaawansowane narzędzie używane w genetyce populacyjnej jak również w taksonomii molekularnej do delimitacji gatunków. Metoda ASAP (Assemble Species by Automatic Partioning) wykorzystując dane genetyczne, takie jak sekwencje DNA (wykorzystano geny COI, WG i RpS5), ocenia różnice między osobnikami i klasyfikuje je jako ten sam lub różny gatunek. Metoda ta automatycznie partycjonuje osobniki na podstawie podobieństw sekwencji genetycznych. Założeniem jest, że osobniki należące do tego samego gatunku mają większe podobieństwo genetyczne między sobą niż z osobnikami należącymi do innych gatunków. Metoda ta jest szczególnie przydatna w przypadku, kiedy tradycyjne metody oparte na morfologii czy ekologii mogą nie być wystarczające lub trudne do zastosowania. Uwzględniona zostaje genetyczna zmienność wewnątrzgatunkowa i międzygatunkowa umożliwiając określić granice pomiędzy gatunkami. Wykorzystane zostaje podejście Bayesowskie do estymacji parametrów modelu i modelowanie różnic między fragmentami sekwencji. W oparciu o tą metodę wydzielono 18 grup potencjalnych gatunków (Fig. 2, **Paśnik & Przybyłowicz, manuskrypt)**. Opisany został także wpływ i rola feromonów u gatunków kryptycznych. Często samce i samice wydzielają różne feromony, które są odbierane przez osobniki płci przeciwnej. Dzięki temu przekazywana jest informacja o płodności czy gotowości do rozrodu. Gatunki nieodróżnialne przy pomocy dostępnych

technik, a różniące się molekularnie mogą posiadać barierę zapobiegającą krzyżowaniu i umożliwiające dobór partnerów na podstawie sekwencji feromonowej. Aby zbadać to zjawisko, niezbędne jest wykorzystanie różnych metod badawczych, uwzględnienie kontekstu biologicznego i specyficznych cech charakteryzujących badany organizm.

Jednym z rezultatów powyższej pracy było stworzenie klucza do oznaczania gatunków z rodzaju *Apisa* opartego o cechy morfologiczne. Uaktualniliśmy katalog z gatunkami uwzględniając nowo opisane taksony rezygnując jednocześnie z podziału na podrodzaje. Zdefiniowano na nowo zasięgi występowania i omówiono aspekt zoogeograficzny w kontekście całego rodzaju. Opisano jeden nowy dla wiedzy gatunek oraz dokonano redeskrypcji 7 kolejnych. Do analiz molekularnych użyto 57 próbek (obejmujących reprezentację 8 gatunków). Przeprowadzone badania i otrzymane wyniki sfalsyfikowały hipotezę o występowaniu trzech podrodzajów *Apisa* s. str, *Parapisa*, *Dufraneella*. Prawdopodobnie wszystkie modyfikacje morfologiczne są wynikiem niezależnej ewolucji cech i ich kombinacji u poszczególnych gatunków. Udowodniono, że długość wyrostka walwy różni się nie tylko pomiędzy gatunkami, ale także w obrębie jednego taksonu.

Wyniki przeprowadzonych analiz potwierdziły duże zróżnicowanie w obrębie rodzaju *Apisa*. W przypadku okazów z długim wyrostkiem na walwie obserwowane cechy i zmienność morfologiczna nie mogą zostać jednoznacznie zinterpretowana na podstawie dostępnego do badań materiału. Można przypuszczać, że część cech może charakteryzować nowe taksony ale zdecydowano się nie opisywać ich bez dalszych dokładniejszych badań aby nie przyczyniać się do tzw. inflacji taksonomicznej poprzez sztuczne zawyżanie liczby nieprecyzyjnie zdiagnozowanych taksonów. Na podstawie badań genetycznych można wysnuć hipotezę, że Wschodnia Afryka lub Półwysep Arabski mogą stanowić centrum dywersyfikacji rodzaju.

Dyskusja

Rodzaj *Apisa* wydaje się być niezwykle trudną taksonomicznie grupą Lepidoptera. Charakteryzuje się zewnętrzną jednorodnością połączoną ze znaczną zmiennością obserwowalnych cech morfologicznych, a także uproszczeniem morfologii genitaliów.

Przeprowadzone badania stanowią pierwszą kompleksową analizę rodzaju w oparciu o rozbudowane dane morfologiczne i molekularne. Wyniki molekularne przeprowadzone zostały w oparciu o trzy geny (COI, WG i RpS5). Dzięki temu uzyskane wyniki mogą stanowić podstawę do bardziej rozbudowanych badań nad rodzajem. Wciąż nieznana jest biologia, ekologia, rośliny żywicielskie i postaci larwalne. Rozpoznanie i dokładna identyfikacja gatunków kryptycznych jest istotna zarówno dla ochrony różnorodności biologicznej, jak i dla zrozumienia procesów ewolucyjnych. Wraz z postępem technologii molekularnych i coraz bardziej precyzyjnymi metodami analizy genetycznej, możliwe staje się lepsze ich zrozumienie i rozpoznawanie (Struck i in., 2018). Odkrycie i właściwe zidentyfikowanie tych gatunków ma znaczenie dla ochrony różnorodności biologicznej i zarządzania ekosystemami. Niestety przez brak dostatecznych badań wiele gatunków kryptycznych wciąż pozostaje nierozpoznanych. Często przez zbliżone cechy morfologiczne takie jak kształt skrzydeł, ubarwienie czy rozmiar ciała trudno je zdiagnozować co może prowadzić do błędnych identyfikacji. To prowadzi do trudności i niewystarczalności tradycyjnych metod morfologicznych (MacLeod i in., 2022). Koniecznością jest zastosowanie innych technik np. takich jak analiza genetyczna, która pomaga w odkryciu różnic w sekwencjach odpowiednich genów i sugerować istnienie nierozpoznanych wcześniej gatunków. Przez porównanie otrzymanych sekwencji możemy określić stopień podobieństw jak i różnic genetycznych między osobnikami (Hajibabaei i in., 2006).

Każda dodatkowa metoda np. różne techniki obrazowania jak mikroskopia skaningowa może dostarczać dodatkowych narzędzi do identyfikacji gatunków kryptycznych, poprzez dokładną analizę detali anatomicznych i cech mikroskopowych (**Przystałkowska, 2022**). Dodatkowo zastosowana metoda - analiza wyznaczania granic gatunków przeprowadzona w oparciu o metodę ASAP wykazała osiemnaście potencjalnych gatunków w obrębie badanych okazów rodzaju *Apisa*. Część z nich koresponduje z wynikami molekularnymi i morfologicznymi (**Paśnik & Przybyłowicz 2023, manuskrypt** Fig. 1, Fig. 2). Metoda ASAP jako jedna z wielu dostępnych metod delimitacji gatunków opartych na danych molekularnych jest szczególnie przydatna w przypadku, gdy tradycyjne metody oparte na samej morfologii czy

ekologii mogą być niewystarczające lub trudne do zastosowania (Ortiz i in., 2023). Dzięki takim możliwościom i kompleksowemu podejściu można w sposób bardziej obiektywny identyfikować nowe gatunki, analizować populacje czy wreszcie na podstawie tych danych rekonstruować relacje filogenetyczne. Występowanie polimorfizmu i stosunkowo małych różnic międzygatunkowych może sugerować na występowanie gatunków kryptycznych, których bez większej ilości materiału oraz innych dokładniejszych technik nie jesteśmy w stanie identyfikować.

Chociaż uzyskano wiele nowych informacji genetycznych i morfologicznych, nadal nie jest możliwe zinterpretowanie relacji i pełne zrozumienie procesów specjacyjnych w obrębie rodzaju *Apisa*. Połączenie podejścia morfologicznego z molekularnym obejmującym trzy wybrane geny COI, WG, RpS5 nie rozwiązało wszystkich problemów systematycznych w obrębie badanego rodzaju. Rodzaj ten jest z pewnością grupą monofiletyczną, co potwierdzają metody genetyczne i morfologiczne. Jednak relacje w obrębie rodzaju między gatunkami pozostają nadal nierozwiązane. Wykonane badania pokazały, że zróżnicowanie między i wewnątrzgatunkowe w obrębie rodzaju jest znacznie większe niż zakładano na początku.

Kwestią limitującą możliwości analiz, szczególnie molekularnych to ograniczony dostęp do świeżo zbieranego materiału. W większości dysponowałam materiałem suchym, muzealnym zbieranym wiele lat temu, a to przekłada się na liczbę uzyskanych dobrej jakości izolatów DNA. Niekiedy materiał świeży także nie nadaje się do badań molekularnych ze względu na warunki w jakich jest przechowywany i konserwowany. Zdarza się to wtedy, gdy okazy poddawane są procesowi szybkiego nagrzewania podczas suszenia lub wielokrotnemu rozwilżaniu w celu kolejnego przepreparowania.

Dzięki drobnym cechom morfologii (kolor skrzydeł, obecność widocznych, ciemnych żyłek, kształt łusek), informatywnym cechom zlokalizowanym na aparatach genitalnych oraz dzięki zastosowaniu technik molekularnych udało się wyznaczyć kilka grup gatunków wyraźnie wyróżniających się na tle innych. Cechy te zostały potraktowane jako diagnostyczne i stanowiły podstawę do formalnego opisu nowych taksonów.

Wiadomo, że geny mogą mieć własną niezależną historię genealogiczną, a łączenie danych nie zawsze rozwiązuje problem, jeśli chodzi o relacje między gatunkami (Wahlberg i in., 2009) Wiadomo również, że dobre gatunki mogą nadal wymieniać materiał genetyczny poprzez hybrydyzację (introgresję). W wielu badaniach udowodniono, że spokrewnione gatunki wykazują introgresję nie wszystkich genów, ale tylko niektórych z nich. Najczęstszym z nich jest odziedziczony po matce chloroplastowy lub mitochondrialny DNA

(Chan i in., 2005). W przypadku młodych ewolucyjnie gatunków podobieństwo w morfologii może nie być oczywiste i może zająć więcej czasu, aby wizualnie zaobserwować różnice między poszczególnymi taksonami.

Apisa jest przykładem stosunkowo młodej i morfologicznie niezwykle jednorodnej grupy. Pomimo sygnału molekularnego, który silnie sugeruje wysoką różnorodność systematyczną z licznymi, nieopisanymi taksonami, nie znaleziono wyraźnych cech diagnostycznych, uzasadniających opisanie tak wielu nowych taksonów. Dlatego też postawiono hipotezę, że prawdopodobnie mogą istnieć inne, niemorfologiczne i znacznie subtelniejsze czynniki odgrywające główną rolę w tej dywersyfikacji. Przykładem może być zróżnicowanie pod względem składu chemicznego feromonów, różnice w rozmieszczeniu poszczególnych populacji (allopatryczne występowanie), inne rośliny żywicielskie, lub wreszcie sezonowe zastępowanie się gatunków.

Napotkane trudności pokazują niezwykłość rodzaju *Apisa* jako intrygującego obiektu do przyszłych badań taksonomicznych i filogenetycznych z uwzględnieniem bardziej wyrafinowanych metod genetycznych. Wskazane będzie zastosowanie sekwencjonowania nowej generacji (NGS) a także szczegółowa analiza dużych serii okazów pochodzących w tych samych regionów kontynentu.

Podsumowanie

Uzyskane wyniki pozwalają na wysunięcie następujących wniosków na temat systematyki i filogenezy badanych motyli z rodzaju *Apisa*:

1. Rodzaj jest grupą monofiletyczną co znajduje potwierdzenie zarówno w analizach genetycznych jak i w oparciu o cechy morfologiczne. Wykryta i szczegółowo opisana autapomorfia (brak arolium) jest obecna u wszystkich przedstawicieli rodzaju i jednoznacznie wyróżnia go spośród bliskich filogenetycznie rodzajów Syntomini. Biologiczne korzyści z zaniku tej struktury pozostają jednak całkowicie nieznane.
2. Wewnętrzne relacje taksonów w obrębie *Apisa* pozostają niewyjaśnione, jednak badania jednoznacznie wskazały na sztuczność dotychczasowego podziału na trzy podrodzaje *Apisa* s. str., *Parapisa* i *Dufraneella* w oparciu o morfologię unkusa oraz wyrostek walwy. Cechy te ewoluowały niezależnie u różnych gatunków i nie mogą stanowić podstaw podziału rodzaju na mniejsze monofiletyczne grupy. W szczególności przypisywanie wartości diagnostycznej obecności/brakowi oraz długości wyrostka wewnętrznej powierzchni walwy jest subiektywne i nie odzwierciedla ewolucyjnych pokrewieństw pomiędzy taksonami. W związku z tym sztuczny podział na mało liczne podrodzaje jest błędny i zbyteczny.
3. Rodzaj *Apisa* liczy znacznie więcej gatunków niż dotychczas sądzono. Część z nich została opisana w toku badań i szczegółowo zdiagnozowana. Jednak w wielu przypadkach zaobserwowana różnorodność morfogenetyczna jest niemożliwa do jednoznacznej interpretacji na podstawie posiadanego materiału badawczego i jest jedynie sygnałem świadczącym o prawdopodobnym istnieniu licznych gatunków kryptycznych.
4. Badania genetyczne wskazują wschodnią Afrykę jako hipotetyczny obszar powstania badanego rodzaju i jednocześnie jego centrum bioróżnorodności.
5. Rodzaj *Apisa* jest grupą bardzo trudną taksonomicznie. Cechuje się wyjątkową jednolitością morfologiczną cech zewnętrznych (wzór, ubarwienie) połączoną ze znacznym uproszczeniem budowy narządów genitalnych obu płci. Jest to jednocześnie powiązane ze znaczną zmiennością stanów cech, która (przy ograniczonym materiale badawczym) uniemożliwia ich obiektywną interpretację.

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A new species of *Apisa* Walker, 1855 (Lepidoptera: Erebidae: Arctiinae) from Uganda with remarks on the apomorphies of the genus

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Abstract

A new species of *Apisa* Walker, 1855 from Uganda (East Africa) and Gabon (Central Africa) is described according to morphological characters and DNA barcode information. A diagnosis, detailed description, distribution, and illustrations of specimens and the male genitalia are provided. Diagnostic characters are found in wing shape, strongly marked wing venation and details of the male genital apparatus.

The DNA barcode sequences of twelve specimens of *A. atrovenosa* **sp. n.** are compared with other members of *Apisa*. Diagnostic characters of the genus are summarised and discussed for the first time with an emphasis on the lack of arolium as the obvious autapomorphy. This character is shown by SEM photographs.

Key words: Africa, description, taxonomy, tiger moths, arolium, Syntomini, Arctiinae

Introduction

Apisa Walker, 1855 is a small genus of Syntomini moths belonging to the family Erebidae, subfamily Arctiinae. Walker introduced this new generic name for the single species described by him in 1855 as *Apisa canescens*. The basic descriptive characters were the external morphology with measurement of body and wing length.

Unfortunately, although the original work mentions four syntypes, only two specimens have been found in Walker's collection deposited in the Natural History Museum in London. One of them was designated as a lectotype by Przybyłowicz (2009) to stabilize the current concept of the name. Following the original description, subsequent authors proposed 16 additional new taxa which are now ascribed to 10 valid species. The genus is currently divided into three subgenera: *Apisa* s. str., *Dufraneella* Kiriakoff, 1953 and *Parapisa* Kiriakoff, 1952. The last one is easily separated from the remaining two subgenera by the deeply bifid uncus which is sharply pointed in its terminal portion in *Apisa* s. str. and *Dufraneella*. The subgenus *Parapisa* includes two species: *A. cinereocostata* Holland, 1892 with a broadly divided uncus, and *A. subargentea* Joicey & Tabot, 1921 in which the slit in the uncus is very narrow. The differences between *Apisa* s. str. and *Dufraneella* are less prominent and are expressed mostly by the presence (*Apisa*) or absence (*Dufraneella*) of an elongate, sclerotized process on the inner portion of the valva. The nominate subgenus includes two species: *A. canescens* Walker, 1855 and *A. arabica* Warnecke, 1934. Some researchers suggest that *A. arabica*, whose range is limited to the Arabian Peninsula, is only a subspecies of *A. canescens* (Hacker 1999; El-Hawagry *et al.* 2013), while others treat it as a valid species (Wiltshire 1980a, 1980b, 1990, Przybyłowicz 2009). The least known and most mysterious member of the genus is *A. manetti* Turati, 1924 known only from five syntypes collected in Libya (Cyrenaica). The genital apparatus of both male and female of the species is unknown. Unfortunately, the original description is not detailed enough to place it in one of the three subgenera (Przybyłowicz 2009). This is the only member of the genus recorded north of the Sahara Desert. Moreover, the type locality is separated by a distance of at least 2500 km from the nearest known records of *Apisa* in sub-Saharan Africa.

Dufraneella is the largest subgenus of *Apisa* which includes five species. *Apisa rendalli* and *A. subcanescens* were described by Rothschild in 1910. *Apisa subcanescens* has the largest wingspan in the subgenus whilst, *A. rendalli* is relatively small with dark brown wings which makes it similar to remaining members of the subgenus.

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Over thirty years later, Dufrane (1945) described *Metarctia grisescens* which was subsequently transferred to the genus *Apisa* by Kiriakoff (1953). Later, Kiriakoff (1959) described *A. fontainei* and then, in 1961, *A. hildae*.

Until now, the genus has never been revised. As a result, the taxonomic status of subgenera and most of the species remains uncertain. The only published work containing comprehensive but still limited information on the whole genus is the illustrated catalogue of Thyretina by Przybyłowicz (2009). Extensive material examined in the preparation of the phylogenetic revision of the genus resulted in the discovery of an unknown taxon clearly separated from all known species, which is described in the present paper.

A precise morphological characterization of the genus has never been published and the ascription of subsequent taxa relied on superficial similarities to already described species. Detailed morphological examination of specimens representing all members of *Apisa* yielded the discovery of an unknown yet but the well-recognizable character which is here described as an obvious autapomorphy of the studied group.

The aim of this paper is to describe a new species of *Apisa* and to provide clear diagnostic characters separating the genus from the remaining genera of Syntomini. SEM images of the generic trait are presented to better visualise this diagnostic structure. A phylogenetic tree is also presented to show the putative relationships of the new species with congeners.

Materials and methods

Morphological studies

Dry, pinned material was examined using a stereoscopic microscope Nikon SMZ1000.

The photos of specimens were taken with a Canon EOS 70D digital camera with compact-macro lens EF 50mm.

From selected specimens, the abdomen was removed by forceps and placed in a test tube with 10% potassium hydroxide solution. The tube was placed in a glass beaker in a water bath at 100 ˚C and macerated for 15–20 minutes. After maceration, the abdomen was placed in a Petri dish containing water and detergent to remove KOH, scales and soft tissues. Genital apparatus was isolated from the abdomen by forceps and entomological pins. Cleaned genitalia were stained with the use of chlorazol black (Merck, Darmstadt, Germany). Permanent genitalia slides were mounted in Euparal. The genitalia photos were taken using a stereoscopic microscope Leica S9i. Obtained images were adjusted with the Adobe Photoshope 6C program. The terminology for the genitalia is based on Kôda (1987).

Scanning electron microscope images of five samples were taken. Specimens with well-preserved structures were selected for photography, glued to the holder and sputter coated with gold using an Ion Sprayer JEOL JFC-1100E. The photos were taken at the Jagiellonian University, Krakow, Poland. A scanning electron microscope JEOL JSM5410 with tungsten cathode was used. The map showing distribution was prepared with QGIS 3.10.

Molecular studies

Twelve individuals representing all known specimens of the new taxon were used for molecular studies. For DNA extraction, two legs of the mounted collection specimen were removed (the oldest ones collected in 2014). Every specimen was tagged with a label "number DNA", after removing a leg. The DNA was extracted using the NucleoSpin® Tissue (MACHEREY-NAGEL). The amplified product comprised a fragment of the mitochondrial COI (the standard DNA barcode COI 5' region).

In many cases, the DNA barcode can resolve genetic diversity to the species level (Wahlberg & Zimmermann 2000, Kirchenko *et al.* 2015). The sequence was amplified using primers designed for Lepidoptera (LEP-F1, LEP-R2) (Hebert *et al.* 2004). The PCR reaction was performed in 10 µl volume (1 µl of template, 2 µl of B PCR buffer, 0,2 µl LEP-F1, 0,2 µl LEP-R2, 0,2 dNTP, 6,3 H_2O, 0,1 µl Taq polymerase). The unpurified PCR reaction product was checked on 1% agarose gel. PCR product was sequenced in both directions using the same primers as for the PCR reaction (LEP-F1/LEP-R1). The amplified products were electrophoresed in 1% agarose gel for 30 min at 100 V and visualized under UV. The PCR products were purified with the Exo-BAP kit. After sequencing in both forward and reverse direction, the chromatograms obtained from an outside company (Genomed) were managed and aligned using BioEdit version 7.0.9.0 (Hall 2004) and checked manually. The two methods were used to established phylogenetic relationships. The Maximum likelihood tree was constructed in MEGA 7.0.9 (Tamura *et al.* 2007)

and then redesigned using Corel Photo Paint. The tree was obtained automatically by applying BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and the topology was selected with the superior log likelihood value. Model of sequence evolution T92+G was selected with MEGA 7.0.9 (Tamura *et al*. 2007). To obtain BI tree the MrBayes was used (Ronquist 2012). For the COI data the best fit was the symmetrical gamma distribution model. The posterior probabilities were based on two Markov chain Monte Carlo (MCMC) runs, composed of tree heated and one cold chain. Markov chains were run for 6 milion generations with sampling every 1000 generations. Branch supports were calculated by using Bootstrap values. The default 25% burn-in was applied before obtaining the consensus tree. The analysis involved 16 specimens and all three positions excluding gaps and missing data. There were a total of 658 base pairs in the final dataset.

Anapisa holobrunnea (Talbot, 1932) was selected as an outgroup to root the tree. Previously obtained sequences of other species of the genus *Apisa* were used to create the phylogenetic tree.

Abbreviations used

ISEA PAS—Institute of Systematics and Evolution of Animals Polish Academy of Sciences (Kraków, Poland)
GS—genital slide
MO—private collection of Michael Ochse (Weisenheim am Berg, Germany)
ZSM—the Bavarian State Collection of Zoology (Zoologische Staatssammlung München, Munich, Germany)
ANHRTUK—African Natural History Research Trust (Leominster, the UK)
IZ JU—Institute of Zoology of the Jagiellonian University (Kraków, Poland)

Type deposition
The holotype is deposited in ISEA PAS. All paratypes come from the private collection of Michael Ochse and will be finally deposited in ZSM.

Results

Apisa Walker, 1855

Apisa Walker, 1855: 916
Type species: *Apisa canescens* Walker, 1855: 916 (designated by monotypy).

Diagnosis. Genus designated by monotypy. It can be clearly distinguished from other syntomines by its uniform colouration and the markedly reduced wing pattern characteristic for all species. Contrary to the other genera, the entire body is grey or ochraceous, varying from pale to relatively dark depending on the species. The weakly expressed pattern, if traceable, is restricted to the darker veins of wings. However, without exception, the same background colouration is visible on wings and remaining parts of the body. Most similar to *Apisa* are members of *Lempkeella* Kiriakoff, 1953, *Tervurenia* Przybyłowicz, Ochse, 2017, *Daphaenisca* Kiriakoff, 1953 and *Anapisa holobrunnea*. The first three genera contain species with rather dark, uniformly coloured wings which may resemble the darkest species of *Apisa*. They differ by a contrasting pattern of red, orange or yellow markings on abdomen or thorax, which is never developed in *Apisa* (for ilustrations see Przybyłowicz & Ochse 2017). *Anapisa holobrunnea* is similar to small, dark *Apisa* species as *A. grisescens* or *A. rendalli* but differs in distinctly elongate hindwings which are proportionally shorter and wider in *Apisa* and ochraceous-yellow tarsomeres never similarly coloured in *Apisa* (for ilustrations see Przybyłowicz & Bąkowski 2011).

The new and very obvious autapomorphy of *Apisa* described in this paper is the lack of arolium. This structure was checked and observed in all other members of former Thyretini (sensu Przybyłowicz 2009). This group, with exception of *Thyretes* Boisduvan, 1847 and *Pseudothyretes* Dufrane, 1945 which both possess arolium is later confirmed to form a monophyletic lineage within Syntomini (Przybyłowicz et al. 2019). The clear position of *Apisa* within a thyretine clade corroborates the statement that the lack of arolium is a diagnostic and discrete autapomorphy of the genus.

Male genitalia does not provide the discrete diagnostic characters distinguishing the genus from other syntomines. However, the combination of a shortened, terminally concave valva and narrow, elongated saccus can provisionally be considered as the supplementary distinguishing features of *Apisa*.

The genitalia of females are too poorly known to be used in more general comparisons.

***Apisa* (*Dufraneella*) *atrovenosa* sp. n.**
http://zoobank.org/ F9D99276-1628-4D27-9CCF-687033184C9A
(Figs 1–3, 7)

Material examined

Type material. Holotype: ♂, Uganda 4 km W of Mpigi Mpanga Forest Camp 1250m, 27.10.2014 (at light), 0°12'23''N 32°18'06''E leg. Łukasz Przybyłowicz; GS UG8_22_04_2016, ISEZ_13 [ISEA PAS].

Paratypes (11♂♂): ♂**,** Uganda, Central Region Mpigi District, 4km west of Mpigi, Mpanga Forest, 0°12'23.19''N, 32°18'8.5''E 1216 m, light attraction October 26th, 2014 leg. Michael Ochse; GS 01_07_06_2019, OM523165; ♂**,** as above but October 18th, 2015; GS 02_12_02_2019, OM523166; ♂**,** as above but October 30th, 2015; GS 03_07_06_2019, OM523168; ♂**,** as above but January 27th, 2015; GS 03_12_02_2019, OM523169; ♂**,** Uganda, Western Regin Kasese Province, Kibale National Park, Makerere Field Station, Kanyawara Camp 0°33'53.69''N, 30°21'22.21''E 1557 m, light attraction October 24th, 2014, leg. Michael Ochse; GS 02_07_06_2019, OM523167; ♂ Gabun, Provinz Ogooué Ivindo, 43km SW Makokou, Wasserfälle, 473m, 0° 17' 24,649''N, 12° 34' 21,664''E, 22.11.2017, Lichtfang, leg. Michael Ochse; GS 07_12_02_2019, OM523170 [ISEA PAS]; ♂, Gabon, Mikongo (Rougier), Monts de Cristal (Secondary forest), 430m 0°29'47''N, 11°10'42''E, 28.vii-12.viii.2019 Actinic Light, Albert, J-L,. Aristophanous, M., Bie Mba, J., Dérozier, V., Moretto, P. Leg. ANHRT:2019.17; ANHRTUK 00204595, OM523178; ♂, as above but ANHRTUK 00137937, OM523175; ♂, as above but ANHRTUK 00208616, OM523174; ♂, as above but ANHRTUK 00137938, OM523176; GS 03_21_12_2021; ♂, as above but ANHRTUK 00137939, OM523177; GS 03_15_12_2021

Diagnosis. The new taxon is easily distinguishable externally from all other *Apisa* species by the unique pattern of the wings with veins strongly marked by much darker scales whereas other congeners have uniform dark or pale ochraceous wings with a darker forewing costa. The male genitalia provide additional discrete characters. The pointed uncus and presence of short and apically blunt process located on the ventral portion of valva excludes *A. atrovenosa* **sp. n.** from the subgenera *Apisa* (pointed uncus, elongate process) and *Parapisa* (bifid uncus, short process) and places it in the subgenus *Dufraneella*. Within the subgenus, *A. atrovenosa* **sp. n.** differs from *A. subcanescens* and *A. hildae* in the lack of a distinctive, short, spine-like sclerotisation at the distal termination of aedeagus. The remaining three species of the subgenus differ from *A. atrovenosa* **sp. n.** in the shape of the process of the valva which is atrophied in *A. rendalii*, reduced to small tubercle in *A. grisescens*, and narrow, needle-like and apically pointed in *A. fontainei*, whereas the process of *A. atrovenosa* **sp. n.** is finger-like with wide, blunt apex. The female genitalia remain unknown.

FIGURE 1. *Apisa atrovenosa* **sp. n.**, holotype upperside, underside with labels.

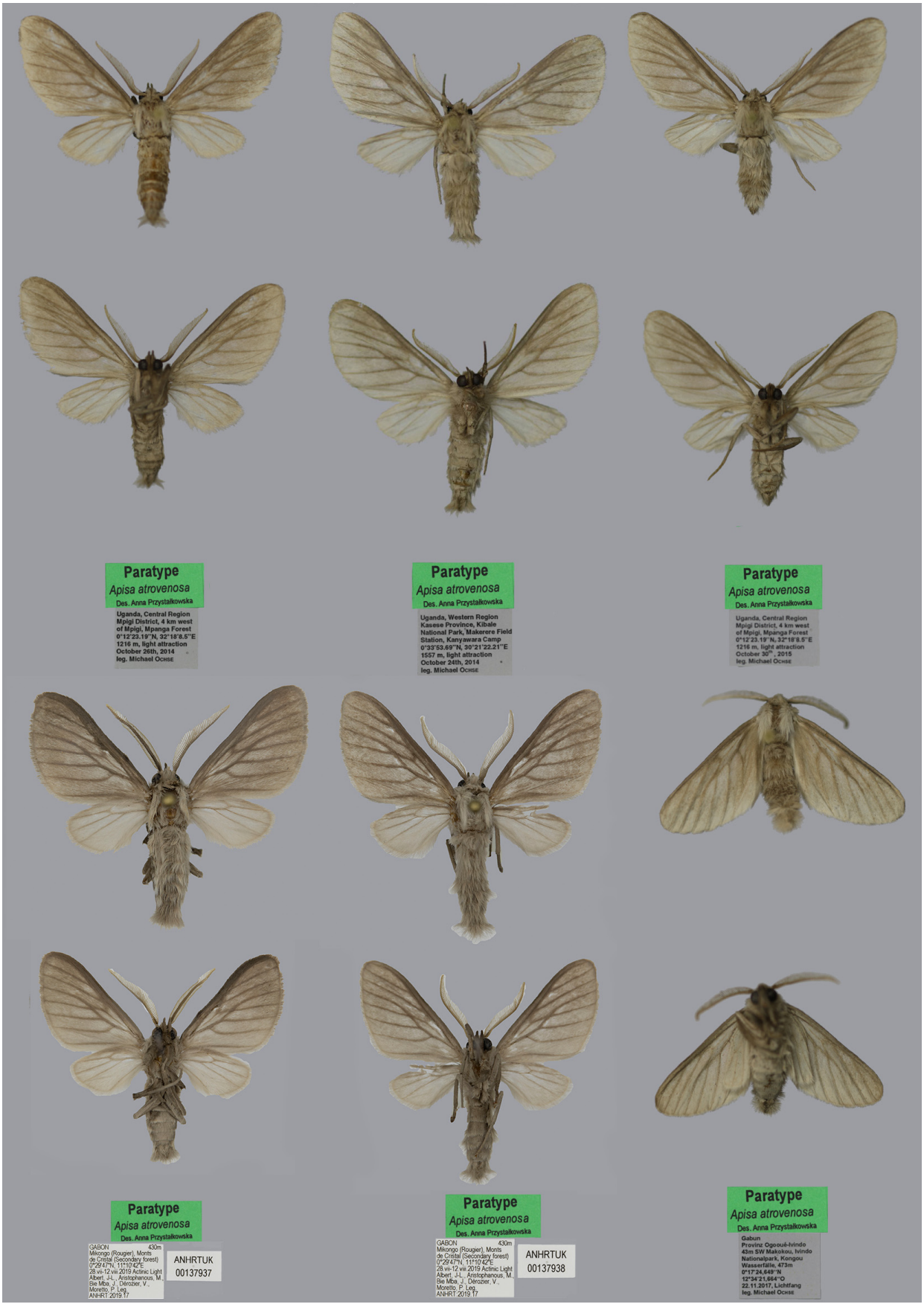

FIGURE 2. *Apisa atrovenosa* **sp. n.**, paratypes upperside, underside with labels. Upper row represents specimens from Uganda while lower row specimens from Gabon.

Description

Head. Frons and vertex covered with dense, protruding, elongate scales of uniformly pale beige colour. Antenna bipectinate, cilia pale creamy, scapus plain beige, concolorous with head. Labial palp elongate, straight with terminal segment directed downwards, colouration slightly darker than head. Palpi brown, straight, the lengths of head covered with hair. Proboscis absent Eye large, naked, convex.

Thorax. Uniformly pale greyish, entirely covered with long hair-like scales.

Wings. Wingspan 21.0–25.0 mm (n = 7). Forewing length about 12–14 mm. Wing colour and pattern of the new species resemble the other members of the genus. Ground colour solid beige, matt, slightly darker than body with strongly marked, darker costa. Postdiscal portion delicately suffused with sparse dark scales. All veins covered with distinctly darker blackish scales. Cilia beige. Hindwing paler than forewing, with contrast between pale background and dark veins less distinct (although always present). Underside of both wings paler than upper side but with same pattern. Retinaculum narrow, pointing towards the underside of front wing

Legs. Uniformly pale greyish. Epiphysis stout, reaching up to ¾ length of fore tibia. 3rd tibia with short terminal pair of spurs, of which outer one longer and narrower. Additional tooth on claw absent. Arolium absent.

Abdomen. Plain beige. Scales on abdomen long and hair-like. Terminal hair pencil absent.

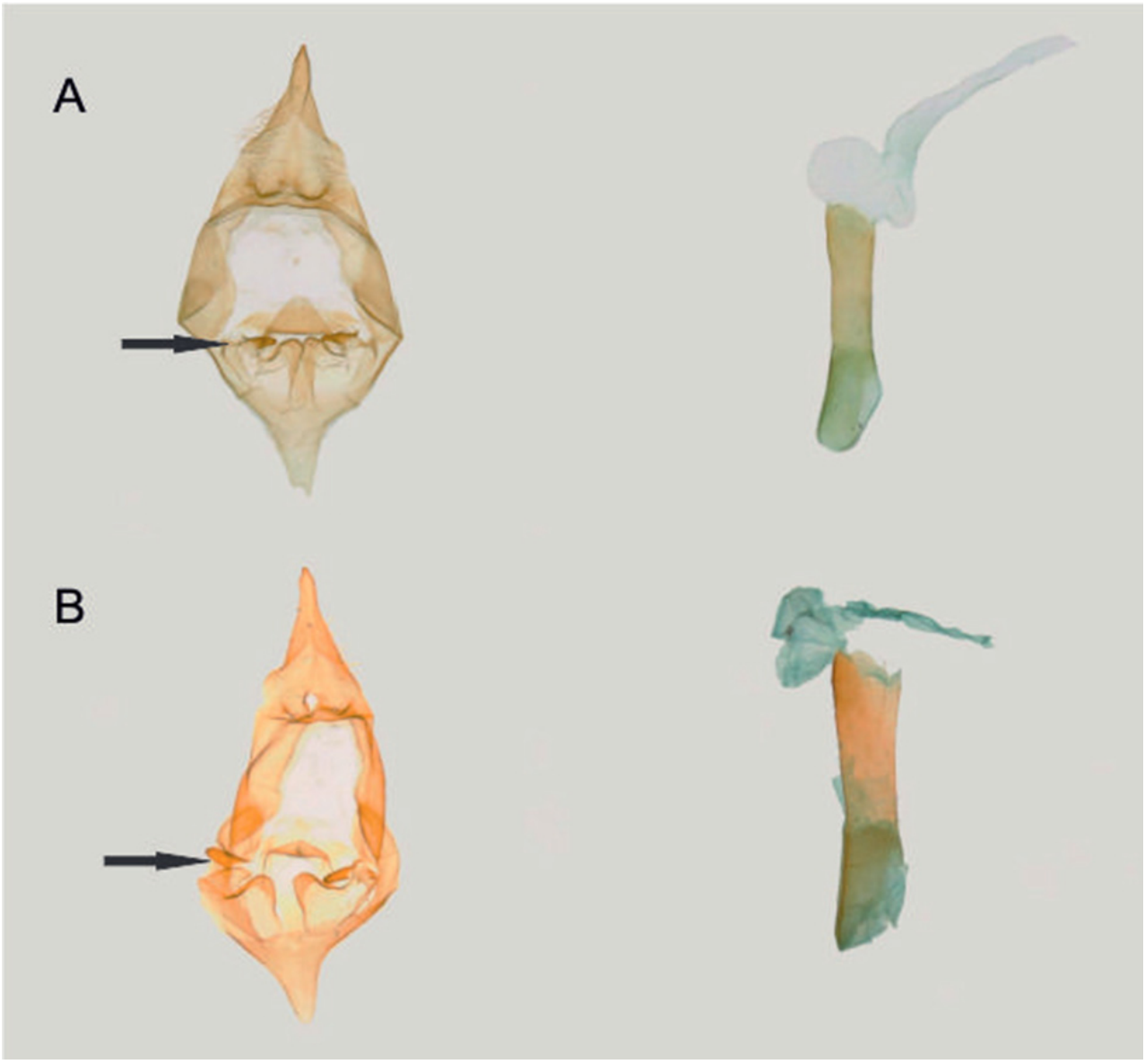

FIGURE 3. *Apisa atrovenosa* **sp. n.**, holotype (A) and one of paratypes (B). Male genitalia, aedeagus with everted vesica. The arrows marks the process located on the valva.

Male genitalia (Fig. 3). Genitalic capsule relatively small, similar in size to other members of the genus. Tegumen narrow with sparse, short erect setae, uncus sclerotised, elongate, subtriangular. Saccus wide at the base, blunt ended. Valva small, reduced, subsquare, sclerotised at the apical part with U-shaped notch, softly ended, equipped with a sparse, protruding setae along margin; distinctly, sclerotized, apically blunt process in basal, subcostal zone. Juxta plate-shaped, moderately sclerotised. Vinculum wide extended into elongate, subtriangular saccus. Aedeagus straight and short, well sclerotised. Vesica membranous, without sclerotisation and cornuti, dilated in the basal portion.

Female genitalia. Unknown.

Etymology. The name *atrovenosa* is a combination of a Latin adjective *atro* meaning "black" and *venosa* meaning "full of veins" and reflects the characteristic dark suffusion of veins contrasting with a much paler background.

Distribution (Fig. 7). Until now, *A. atrovenosa* **sp. n.** is known only from Uganda and Gabon.

Host plant. The host plant remains unknown.

Remarks. The intensity of the blackish vein lines may vary slightly among individuals of *A. atrovenosa*. To the contrary, the darker expression of wing veins visible on specimens of other *Apisa* species depicted in Przybyłowicz (2009) cannot be interpreted as the presence of blackish scales along veins. This is just an artificial colouration of chitinous veins magnified by the light used while taking photos. The scale covering wings of all species except the new one is almost uniformly monochromatic.

Individuals of *A. atrovenosa* from Gabon display neither external, morphological nor genitalic differences from the Ugandean specimens therefore they are confidently included in the type series of a single taxon. Specimens representing Ugandean and Gabonese localities show no genetic differences within the population. However, the small differences in the COI barcode region (1.1-1.2%) are observed between Ugandean and Gabonese populations. The only exception is a single specimen from SW of Makoku (Gabon) differing by 1.2% and 1.1% from Ugandean and Gabonese populations respectively. It can be hypothesized that it represents a separate population as this locality is about 230 km apart from the collecting place of the remaining specimens from Gabon. It is highly probable that the large gap between eastern and central African populations is the result of underexploration of this region of continent but not a natural break in the range of newly described taxon.

Discussion

Systematic overview

Morphology of reproductive organs of *Apisa* is not well elaborated. Even considering males for which genitalia are described for most taxa (except *A. arabica* and *A. manetti*), the diagnostic characters are not properly assessed. Intra- and interspecific morphological variation is unclear, which makes difficult to interpret illustrations or descriptions correctly. The quality of genitalia slides of several types is so poor that the detection or interpretation of given characters is tough. Given the external similarity of all taxa, the proper interpretation of observed variability is very difficult. In case of females, our knowledge is restricted to only two species: *A. canescens* Walker, 1855 and *A. subargentea* Joicey & Tabot, 1921, of which genitalia are illustrated in Przybyłowicz (2009).

Members of the genus *Apisa* are widely distributed in sub-Saharan Africa. They are known from both western and eastern regions of the continent and are also common in South Africa. Two taxa extend beyond this range, notably *A. manetti* known only from northern Libya and *A. arabica* restricted to southern regions of the Arabian Peninsula. Unfortunately, these exceptionally distributed taxa have an unclear systematic position and relationships with other species. They are known from a few individuals, in case of *A. manetti* exclusively the old ones. Present faunistic data do not allow for a precise and reliable definition of the range of each species but reflect merely the distribution of localities.

Morphological analysis

The entire genus *Apisa*, unusually, lacks the arolium that can be recognised as the major diagnostic character distinguishing this lineage from other genera of Syntomini.

The arolium is a structure that enables hexapods to attach to various surfaces. It has evolved independently in few lineages of hexapods. In insect evolution, the arolium appeared probably for the first time in Plecoptera, Dictyoptera and some Orthoptera. Now it is present in many insects groups (Eberhard *et al.* 2009) and is also common in

Lepidoptera (Gorb & Beutel 2001). The arolium is usually a soft cuticular tissue filled with hemolymph. However, its detailed anatomical structure differs depending on the group of insects (Frantsevich & Gorb 2002; Eberhard *et al*., 2009). For example, it is composed of a slightly sclerotized structure in *Spodoptera littoralis* (Boisduval, 1833) (El Degwi & Gabarty 2015). The arolium is mobile and can be extended or retracted not only actively (via contraction of muscle) but also passively (Ferderle & Endlein 2004). To adhere to the surface, there must be a contraction of the unguitractor muscle mediating the bending of the claws (Ferderle *et al.* 2001). This can make it easier to adhere to smooth, vertical surfaces, but it also allows for a more stable grip during windy weather or copulation. Most research conducted on the arolium refers to Hymenoptera and it has been best studied in this order of insects, despite that, the details of how the locomotory attachment functions is unclear (Beutel *et al.* 2020).

The absence of an arolium between the tarsal claws was found to be an autapomorphic character state of the genus *Apisa* (Fig. 4 AB). For this study, the presence of this structure was checked and confirmed for all genera clustered in the lineage containing former Thyretina (sensu Przybyłowicz 2009) (Fig.4 C). Additionally, the presence of the arolium was confirmed for the morphologically similar species *Anapisa holobrunnea* (Fig. 4 D) and a member of genus *Amata* (Fig. 4 E) as well as *Balacra rattrayi* (Fig. 4 C) as summarized by Kristensen (2003), the presence of the arolium is a plesiomorphic condition widespread among most Lepidoptera including Arctiinae. To my knowledge, there is no case of arolium reduction/loss previously reported for the superfamily Noctuoidea. Therefore, the discovery of the complete atrophy of the arolium in *Apisa* is the first such case reported in such a diverse lineage of erebids as the subfamily Arctiinae, or such reduction resulting in entire absence of this structure was otherwise reported in several other unrelated families such as Hepialidae (Nielsen & Robinson 1983), Papilionidae (Miller 1987) and Nymphalidae (Zamre *et al.* 2020). In Sphingidae, the presence or absence of an arolium is a feature that allows identification of several genera within the subfamilies Sphinginae and Smerinthinae (Hundsdoerfer 2017).

The factors responsible for losing of the arolium in *Apisa* are unknown. Almost nothing is known about the biology and behaviour of individuals of this genus. Also, the host plants or the microhabitat where they most commonly live are uncertain. We can speculate that during resting, the moths belonging to genus *Apisa* do not sit on stems so that an adhesive function is unnecessary. Perhaps if they do attach to surfaces, they choose very rough ones and use the well-developed claws on their feet to attach. Instead of a well-developed arolium, there is an ungutractor with an empodium of variable length developed in all species of the genus *Apisa*. This last structure is analogous to the bristle-like one reported for many dipterans (Friedemann *et al.* 2014), perhaps serving as an additional sensory organ with the length depending on the environment inhabited. These structures are localised where the arolium usually develops—between the claws on the legs. The SEM photo of legs are provided (Fig. 4).

A new species of *Apisa* is described based on six males from Uganda and six malesfrom Gabon. Photographs of the whole individuals and male genital structures are provided to illustrate the morphological features essential for the designation of the new species. The presence of an process on the valva differing strongly from other similar valva shapes of the genitalic apparatus is a stable diagnostic character. The new species was discovered by integrating several sources of data: external morphology, comparative analysis of genital structures and molecular data. Preliminary results show the need for a complex revision of the whole genus to systemise informative features because of the extraordinary morphological uniformity, so that specimens can be incorrectly determined. With such a high morphological uniformity characterising the whole genus, a more precise study is needed to finally resolve the systematics of the group.

Molecular analyses

For molecular comparative analyses, all twelve specimens from the type series of *A. atrovenosa* **sp. n.** were sampled. The p-distance between barcode sequences of six specimens from Uganda does not show any variability and are uniform over the DNA barcode region (Tab. 1). This is despite the fact that they come from two different localities separated by a distance of approximately 200 km in a straight line. The specimens from Gabon differs from them by 1.1–1.2%. This difference can be explained as a typical intraspecific variation within a single taxon. Overall, the DNA barcode homogeneity of the sample studied corroborates the morphological data and the hypothesis that the specimens represent a single, until now unknown species.

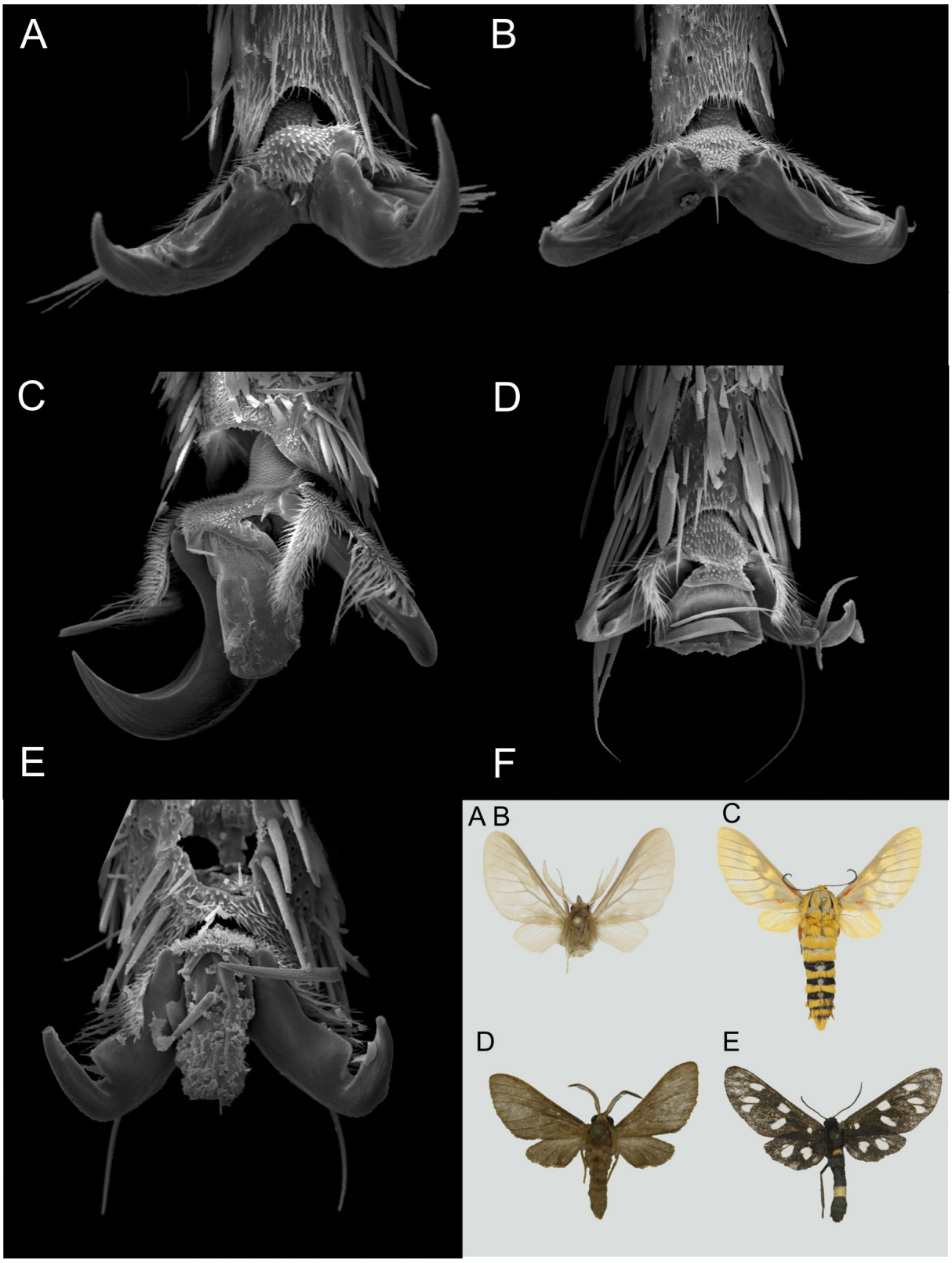

FIGURE 4. SEM photo of leg without and with arolium. A, B *Apisa* sp., C *Balacra rattrayi,* D *Anapisa holobrunnea,* E *Amata phegea.* Photo F shows the imago used for SEM photography. Each specimen is marked with a label corresponding to the SEM image of the tarsus

TABLE 1. Pairwise distances between DNA barcode sequences of species of *Apisa* and *Anapisa* species. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model in Mega 7.0.9 The analysis involved 16 specimens and all three positions. There were a total of 658 positions in the final dataset. Ugandan specimens highlighted in light grey, Gabonese in dark grey.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523164)																
2. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523165)	0,000															
3. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523166)	0,000	0,000														
4. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523167)	0,000	0,000	0,000													
5. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523168)	0,000	0,000	0,000	0,000												
6. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523169)	0,000	0,000	0,000	0,000	0,000											
7. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523170)	0,012	0,012	0,012	0,012	0,012	0,012										
8. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523174)	0,011	0,011	0,011	0,011	0,011	0,011	0,011									
9. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523175)	0,011	0,011	0,011	0,011	0,011	0,011	0,011	0,000								
10. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523176)	0,011	0,011	0,011	0,011	0,011	0,011	0,011	0,000	0,000							
11. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523177)	0,011	0,011	0,011	0,011	0,011	0,011	0,011	0,000	0,000	0,000						
12. *A.* (*Dufraneella*) *atrovenosa* **sp. n.** (OM523178)	0,011	0,011	0,011	0,011	0,011	0,011	0,011	0,000	0,000	0,000	0,000					
13. *A.* (*Parapisa*) sp. (OM523171)	0,087	0,087	0,087	0,087	0,087	0,087	0,076	0,075	0,075	0,075	0,075	0,075				
14. *A.* (*Dufraneella*) sp. (OM523172)	0,083	0,083	0,083	0,083	0,083	0,083	0,069	0,071	0,071	0,071	0,071	0,071	0,037			
15. *A.* (*Apisa*) s. str. (OM523173)	0,082	0,082	0,082	0,082	0,082	0,082	0,067	0,073	0,073	0,073	0,073	0,073	0,040	0,045		
16. *Anapisa holobrunnea* (OM523179)	0,133	0,133	0,133	0,133	0,133	0,133	0,135	0,131	0,131	0,131	0,131	0,131	0,137	0,130	0,127	

Apisa atrovenosa **sp. n.** shows a significant genetic distance from three other members of genus *Apisa* representing all known subgenera. The pairwise genetic distance between them and *A. atrovenosa* **sp. n.**, according to the T92+G model, varies from 6.7–8.7%. The distance to *Anapisa holobrunnea* is 13.3% for specimens from Uganda and 13.1–13.5% for specimens from Gabon. In this case, the broad genetic gap between the new taxon and all other *Apisa* members may be interpreted as an argument for its elevation as a separate subgenus. Also, the preliminary phylogenetic tree generated based on the DNA barcode region does not locate *A. atrovenosa* **sp. n.** within any known subgenus of *Apisa* (Fig. 5, 6). However, morphological characters of male genitalia clearly ascribe *A. atrovenosa* **sp. n.** to the subgenus *Dufranella*. More generally, ongoing revisionary study on *Apisa* with inclusion a near comprehensive set of taxa and more genetic markers should clarify this issue, leading to redefining subgenera or even their rejection.

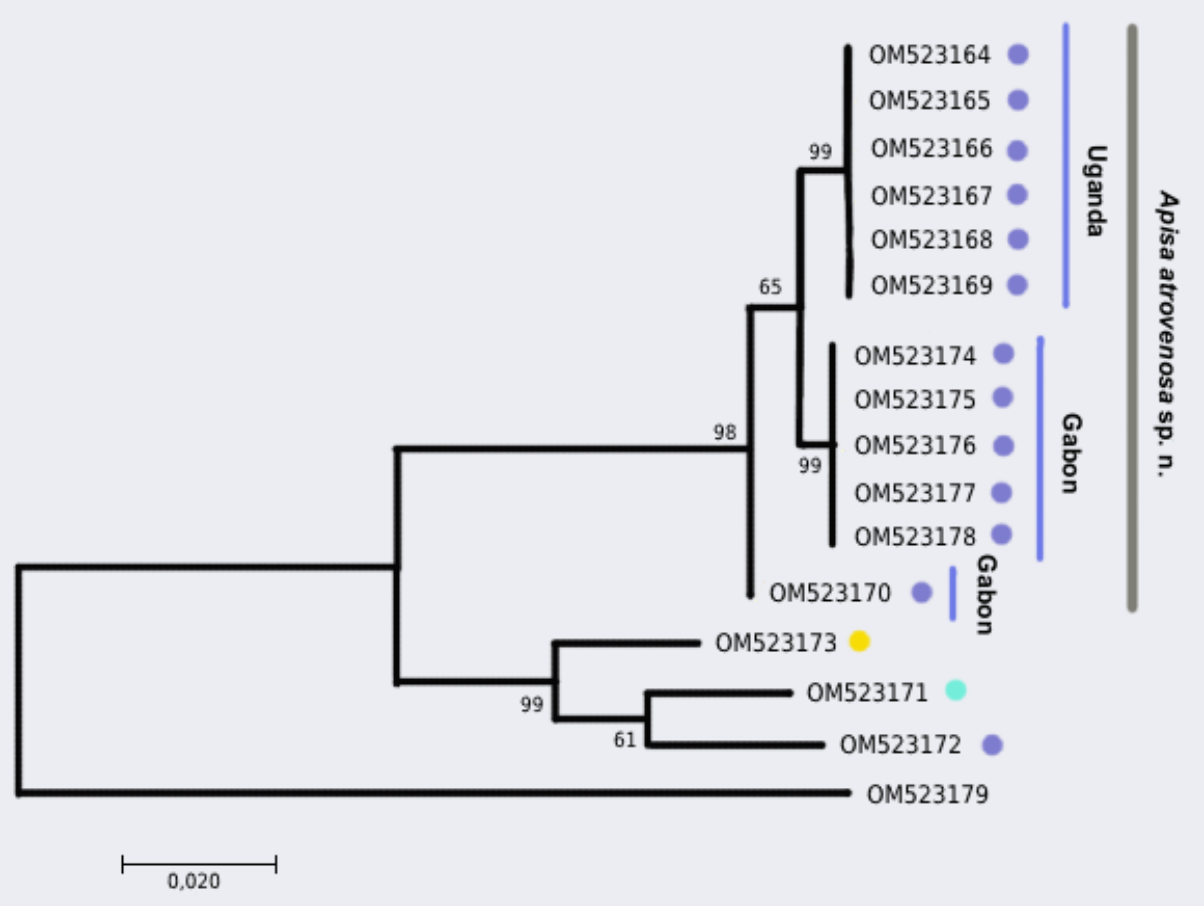

FIGURE 5. Molecular Phylogenetic analysis by the Maximum Likelihood method. Bootstrap support values are indicated at nodes. The tree shows all type specimens of *A. atrovenosa* **sp. n.** belonging to *Dufraneella* subgenus (purple dot). One specimen of each subgenus is also shown (yellow dot—*Apisa* s. str., blue dot—*Parapisa*). *Anapisa holobrunnea* was used as an outgroup. Scale bar—number of substitutions per site.

Conclusions

The examination of the unstudied material of *Apisa* from equatorial Africa allowed for description of a new taxon remarkably different from already known species. The detailed morphological analysis revealed a very obvious, albeit not easily visible character separating *Apisa* from all other Syntomini. The study confirmed that the genus is morphologically uniform and is in need of extensive revision with use of modern taxonomic methods. It is very likely that the present division for subgenera will be significantly modified as a result of future studies.

TABLE 2. Table of sequence vouchers included in the GenBank with species name.

Number	GenBank Accession Number	Specie
1.	OM523164	*Apisa (Dufraneella) atrovenosa* **sp. n.**
2.	OM523165	*Apisa (Dufraneella) atrovenosa* **sp. n.**
3.	OM523166	*Apisa (Dufraneella) atrovenosa* **sp. n.**
4.	OM523167	*Apisa (Dufraneella) atrovenosa* **sp. n.**
5.	OM523168	*Apisa (Dufraneella) atrovenosa* **sp. n.**
6.	OM523169	*Apisa (Dufraneella) atrovenosa* **sp. n.**
7.	OM523170	*Apisa (Dufraneella) atrovenosa* **sp. n.**
8.	OM523171	*Apisa (Parapisa)* sp.
9.	OM523172	*Apisa (Dufraneella)* sp.
10.	OM523173	*Apisa (Apisa)* sp.
11.	OM523174	*Apisa (Dufraneella) atrovenosa* **sp. n.**
12.	OM523175	*Apisa (Dufraneella) atrovenosa* **sp. n.**
13.	OM523176	*Apisa (Dufraneella) atrovenosa* **sp. n.**
14.	OM523177	*Apisa (Dufraneella) atrovenosa* **sp. n.**
15.	OM523178	*Apisa (Dufraneella) atrovenosa* **sp. n.**
16.	OM523179	*Anapisa holobrunnea* (Talbot, 1932)

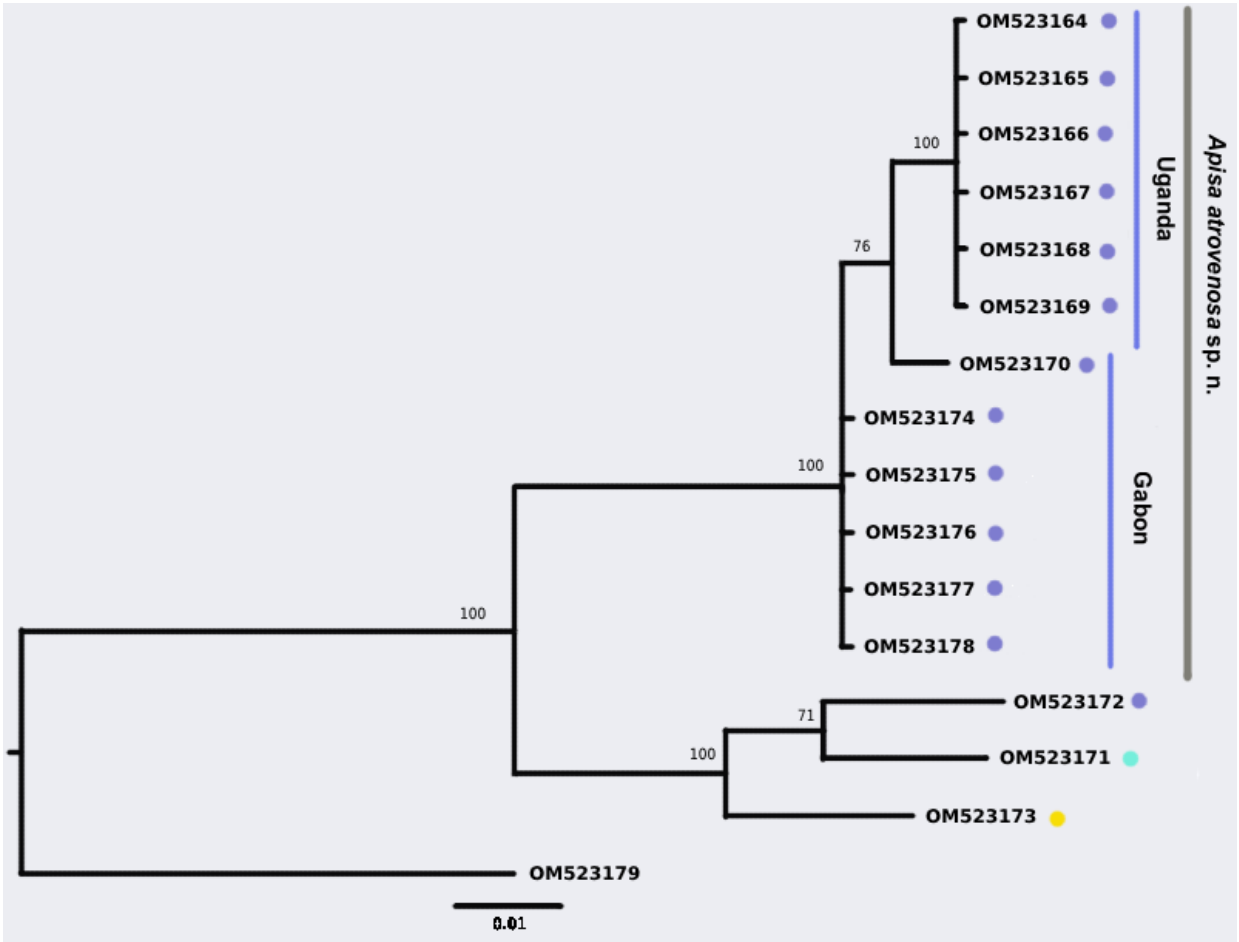

FIGURE 6. Phylogenetic tree based on Bayesian inference method including COI sequences. Values at nodes correspond to posterior probability support. Tree showing a new species of *A. atrovenosa* **sp. n.** (purple dot), representatives of other subgenus (yellow dot—*Apisa* s. str., blue dot—*Parapisa*) and outgroup (*Anapisa holobrunnea*), detailed species description in Table 2.

FIGURE 7. Distribution of the new species. Red dots represent Ugandean specimens, yellow one paratypes from Gabon.

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

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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 glycerin. Mounted microscope slides were made using Marc Andre 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.

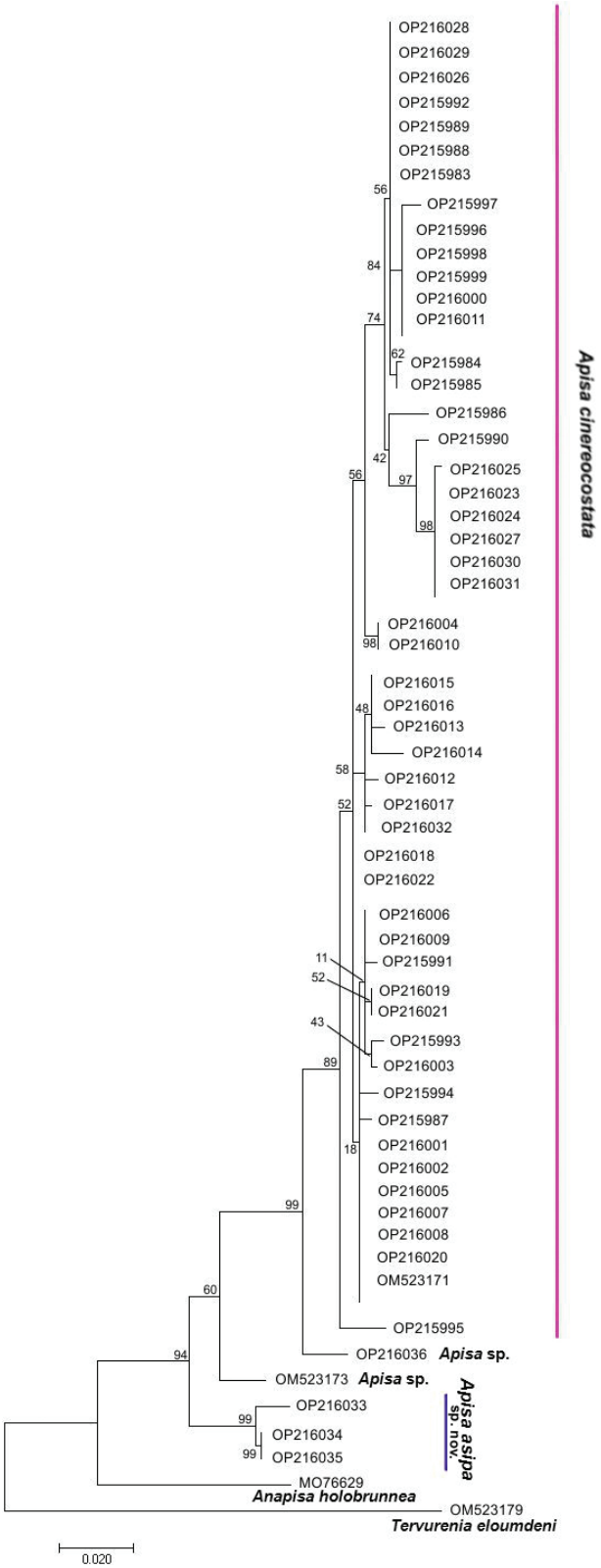

Figure 1. Phylogenetic tree showing the variation of *A.* (*P.*) *cinereocostata,* with two species *Tervurenia eloumdeni* and *Anapisa holobrunnea* as the outgroup. The sequences of two representatives of subgenus *Apisa* are given and also the sequences of the new species *A.* (*P.*) *asipa* **sp. 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.

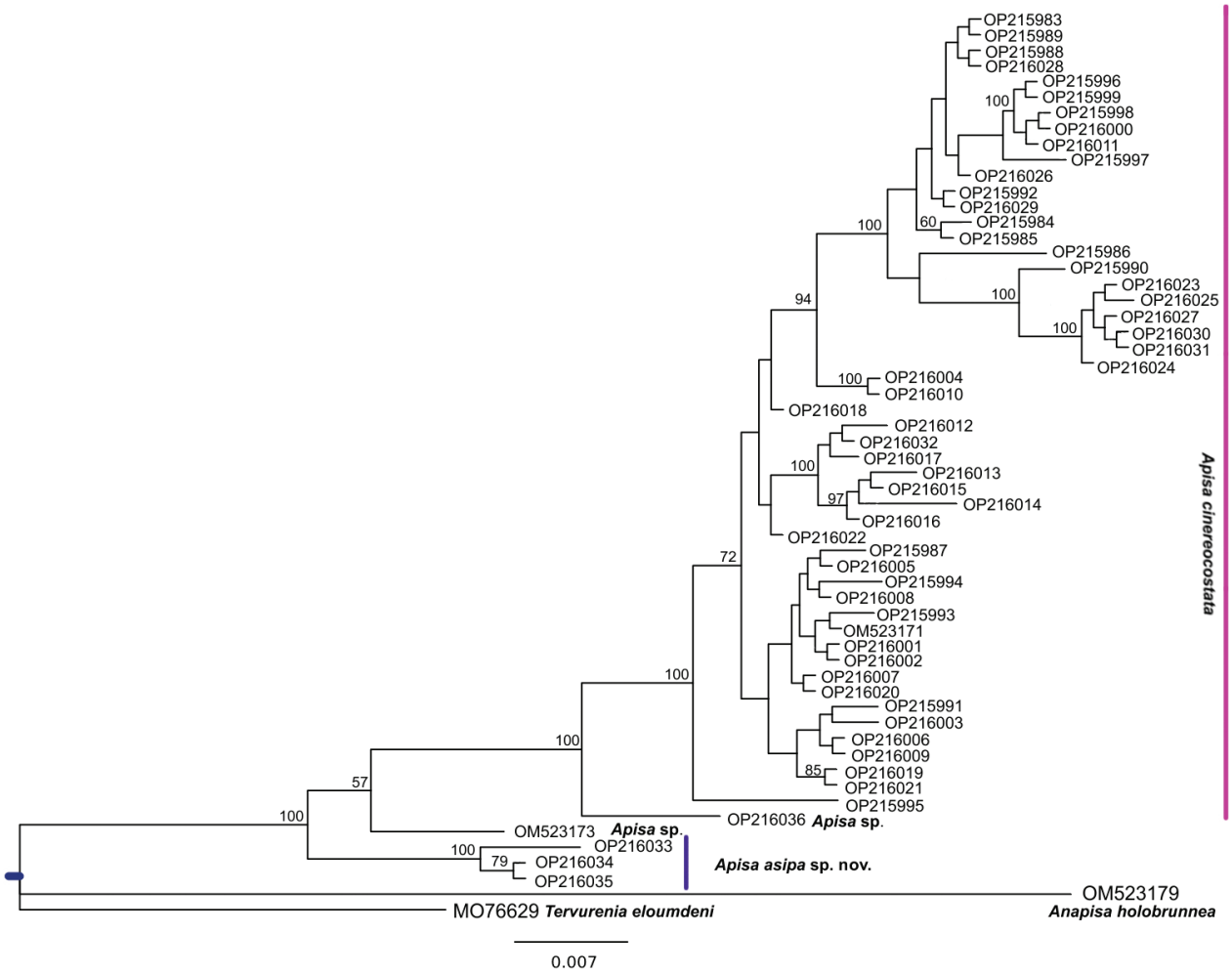

Figure 2. Phylogenetic tree based on Bayesian interference method including COI sequences. Values on nodes correspond to posterior probability support. The analysis included representatives of two subgenera *Apisa* (*Apisa*) (samples *Apisa* sp.) and *Apisa* (*Parapisa*) (samples *cinereocostata*, *asipa*) and the outgroup taxa *T. eloumdeni* and *A. holobrunnea.*

3. Results

3.1. Determination keys

3.1.1. Determination key to males based on the external morphology (Figs 7–9, 13–14)

1 Forewing silver-grey, opaque, never semi-transparent (Fig. 14B); background distinctively shiny; scales between CuA1 and CuA2 near DC dense, flattened, adjacent to wing membrane, with smoothly rounded termination (Figs 7A, 13A) ..... ***A.* (*P.*) *subargentea***

– Forewing greyish-ochraceous, always at least semi-transparent, matt, or indistinctly shiny (Fig. 14A, C, D); scales between CuA1 and CuA2 near DC needle-like or with triangular concavity at termination **2**

2 Forewing semi-transparent (Fig. 14C, D); scales between CuA1 and CuA2 near DC always straight, of two forms very narrow, needle-like and flattened with concave termination (Figs 7B, 13B); 1A+2A markedly convex towards DC in one-third of its length (Fig. 14C, D)..... ***A.* (*P.*) *cinereocostata***

– Forewing almost transparent (Figs 8, 9, 14A); scales between CuA1 and CuA2 near DC minute, sparse, needle-like, always arc-shaped (Figs 7C, 13C); 1A+2A almost straight, without distinct curve in one third of its length (Figs 7–8, 14A) ..... ***A.* (*P.*) *asipa* sp. nov.**

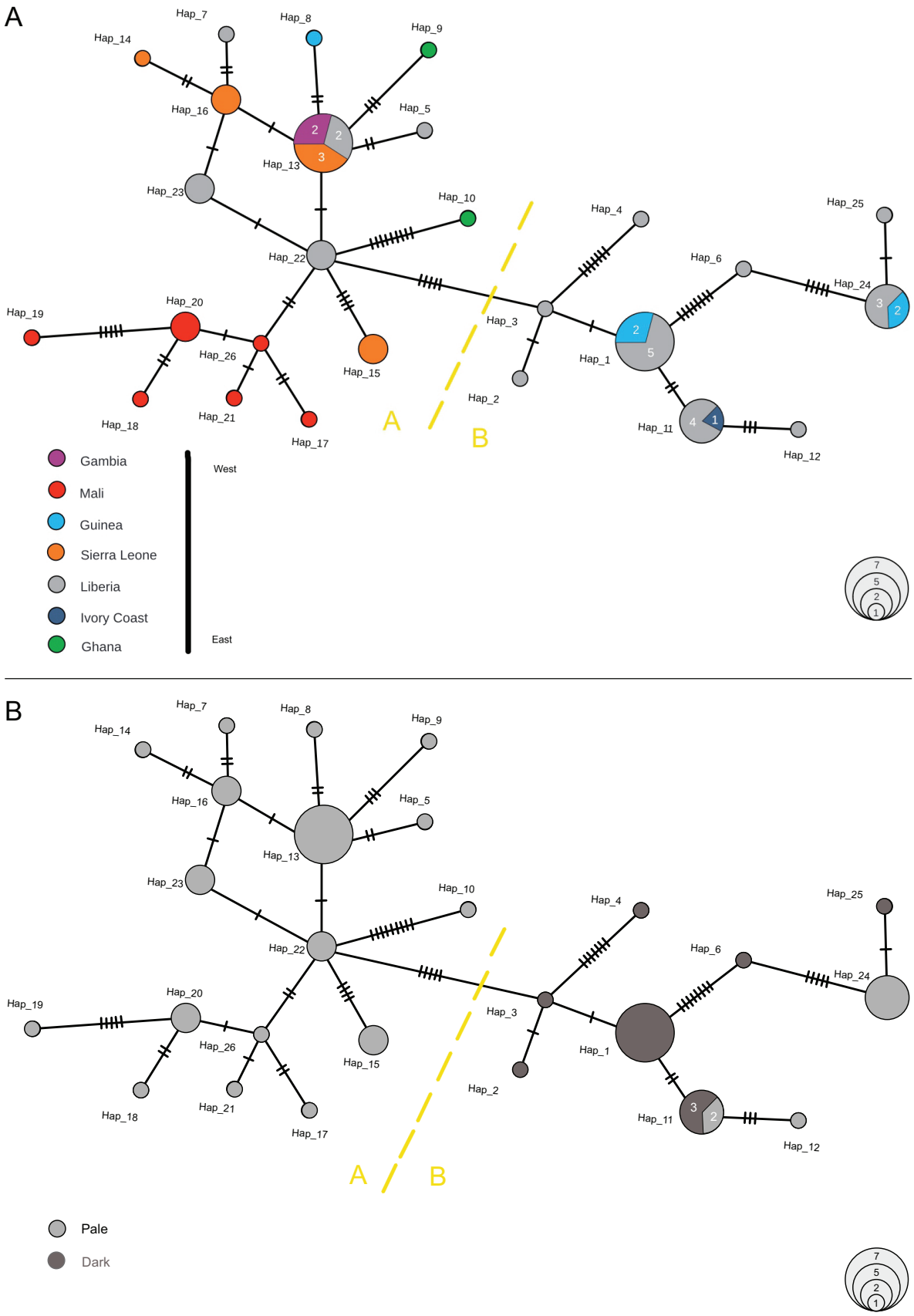

Figure 3. Haplotype network of *A.* (*P.*) *cinereocostata*. Constructed using the 51 mitochondrial cytrochrome c oxidase subunit I (COI) sequences obtained. The size of the circles is proportional to the frequency of the haplotype (on the legend in the bottom right one, two, five and seven individuals). 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***

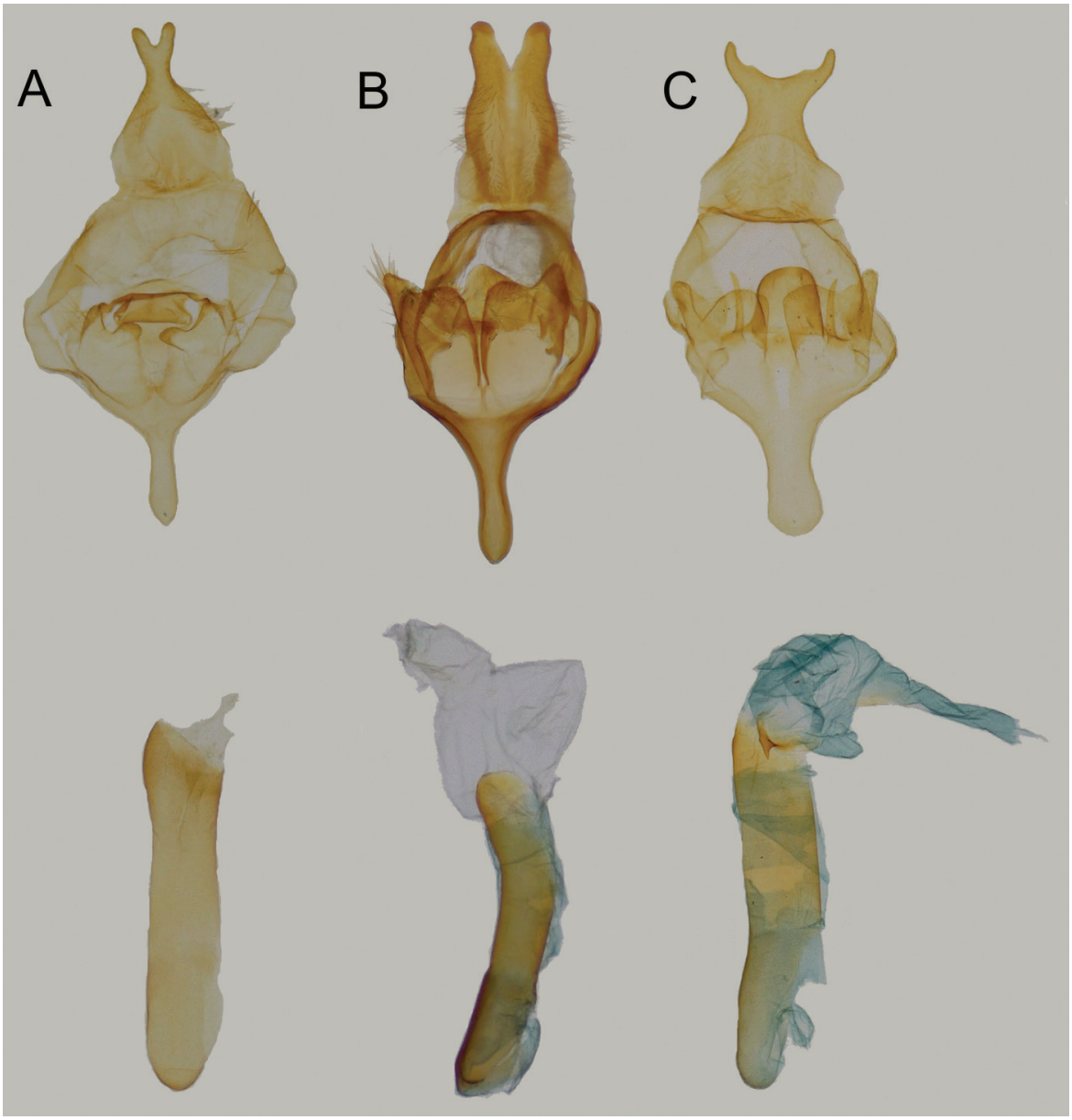

Figure 4. Overview of the genital apparatus of males *A.* (*P.*) *subargentea* (**A**), *A.* (*P.*) *asipa* **sp. nov.** (**B**) and *Apisa* (*P.*) *cinereocostata* (**C**).

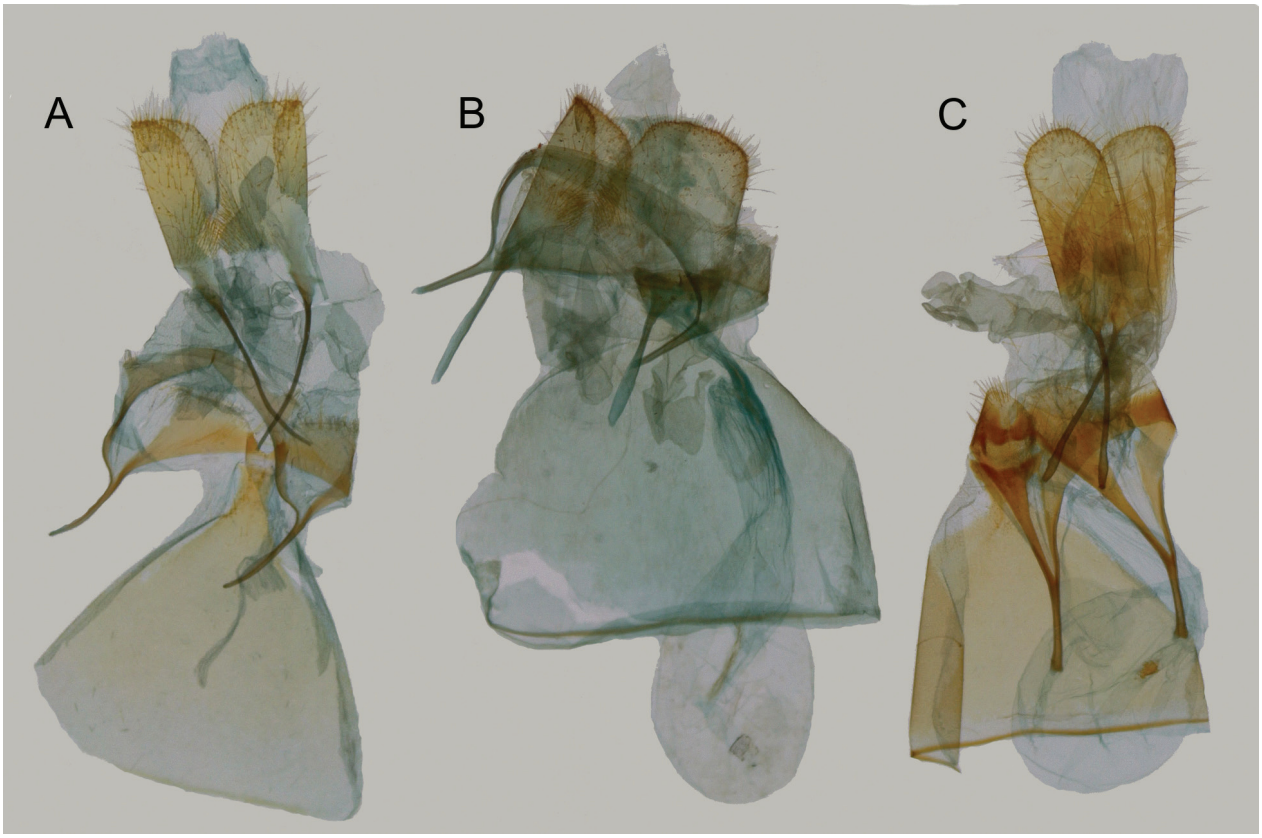

Figure 5. Compilation of female genital organs *A.* (*P.*) *asipa* **sp. nov.** (**A**), *A.* (*P.*) *cinereocostata* (**B**), *A.* (*P.*) *subargentea* (**C**).

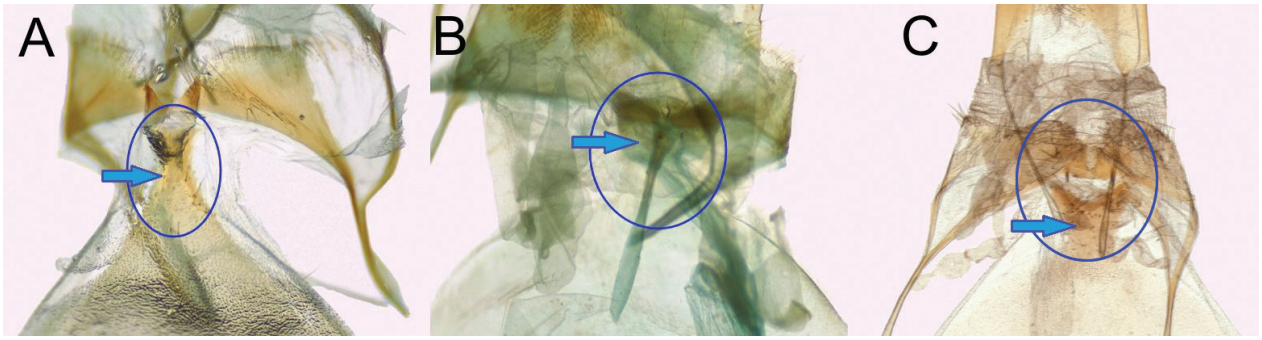

Figure 6. Details of female genitalia with both a marked antrum and a sclerotization of VII sternite. The arrow marks the location and sclerotization of the sternite. From left *A.* (*P.*) *asipa* **sp. nov.** (**A**), *A.* (*P.*) *subargentea* (**B**), *A.* (*P.*) *cinereocostata* (**C**).

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

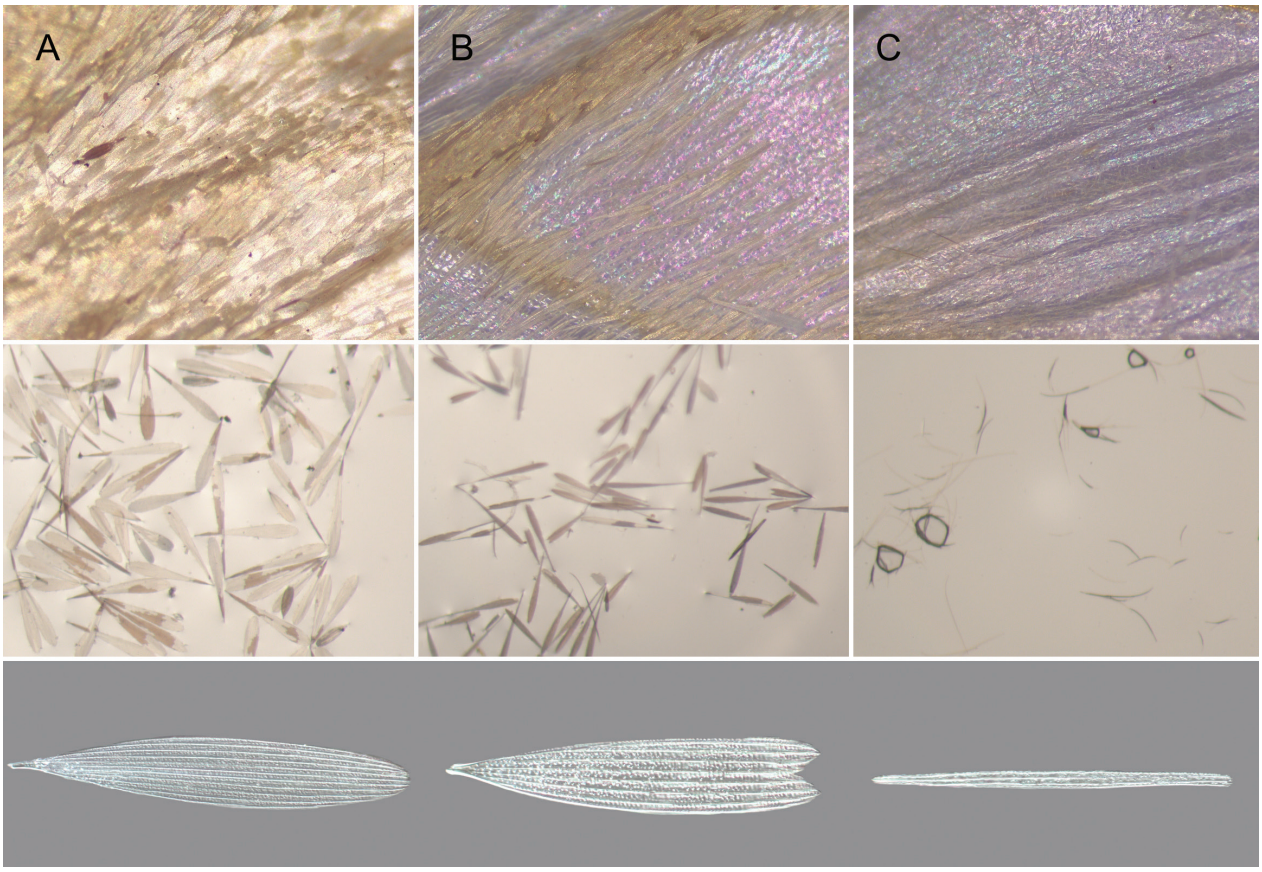

Figure 7. Differences in the scale morphology of wing region between CuA1 and CuA2 near DC of *A. subargentea* (**A**) (tightly fitting), *Apisa cinereocostata* (**B**) (loosely fitting) and *A. asipa* **sp. nov.** (**C**) (sparse).

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. Nigeria Kaduna, 10.4.70, leg. Dr. Politzar; Genitalpraparat, Heterocera, Nr. 32.331, Museum Witt München; ex coll. Politzar; ♂ as above but 13.4.70, 1♂ as above but Genitalpraparat, 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; subdorsally 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, submedal 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.

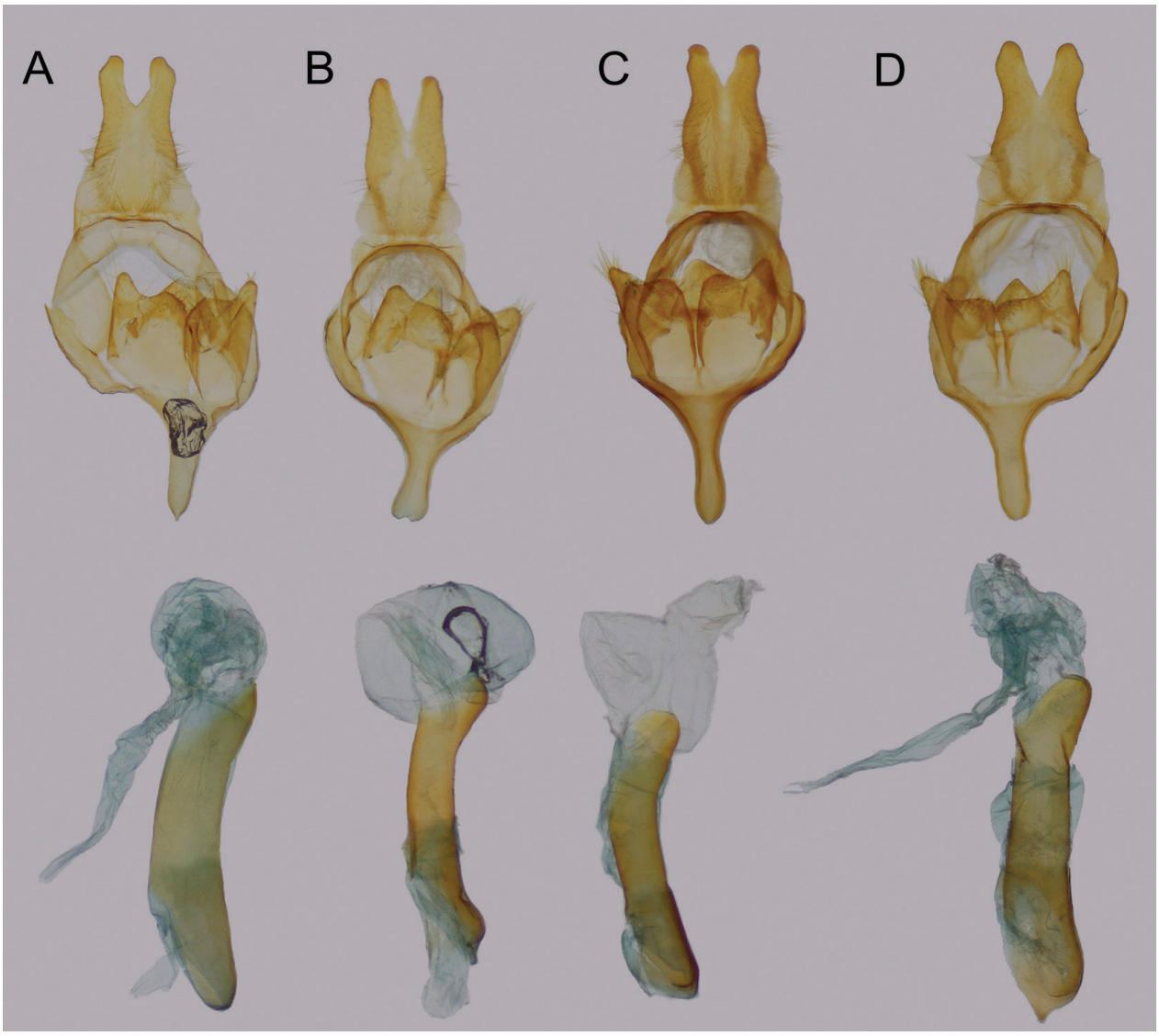

Figure 10. *Apisa* (*P.*) *asipa* **sp. nov.** holotype (**A**), paratype (**B**–**D**). Male genitalia, phallus with everted vesica.

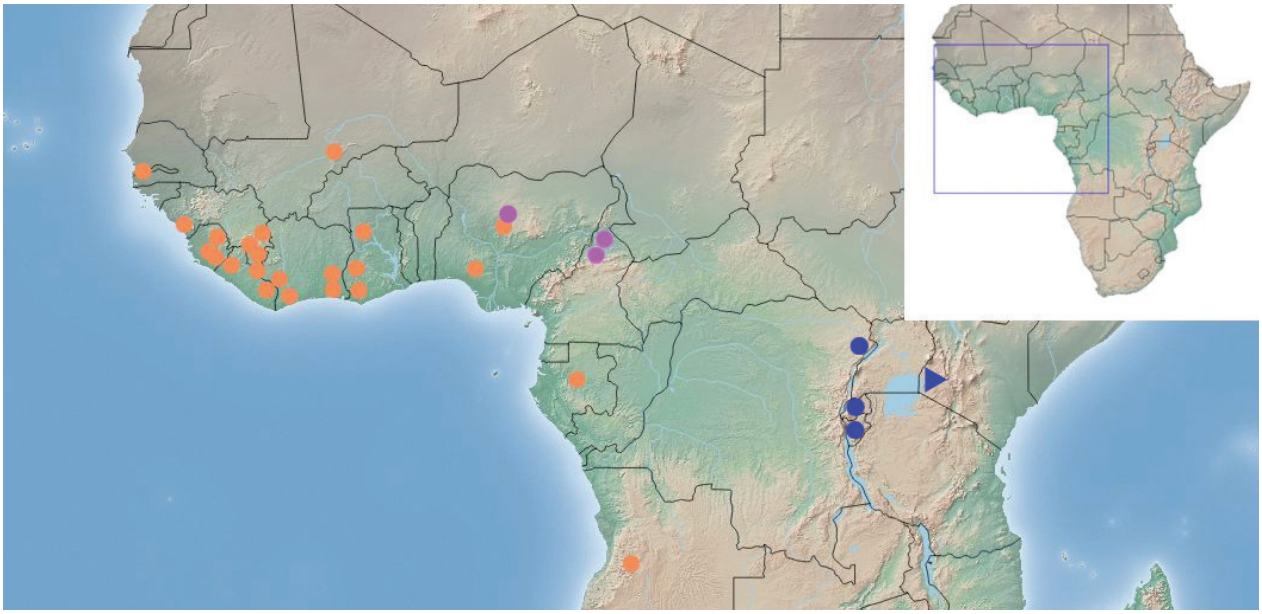

Figure 11. Distribution of *Apisa* (*P.*) *cinereocostata* and *Apisa* (*P.*) *asipa* **sp. nov. Orange dots** represent the localities of *A.* (*P.*) *cinereocostata*, **purple dots** *A.* (*P.*) *asipa* **sp. nov.**, **blue dots** *A.* (*P.*) *subargentea*. The blue triangles indicate the literature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

be detected. Genitalia differ in the shape of elongate saccus 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 residental 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 Forestiére, 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 Guineé) Mont Pierre Richeaud (montane forest), 7°39'49.31" N, 8°22'20.06"W 21–30.viii.2017 Light trap (250w blened 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 Guineé) 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: Jomoro, 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., Sáfian, 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, Stsslg 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. Polltzar; 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 membraneous 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-

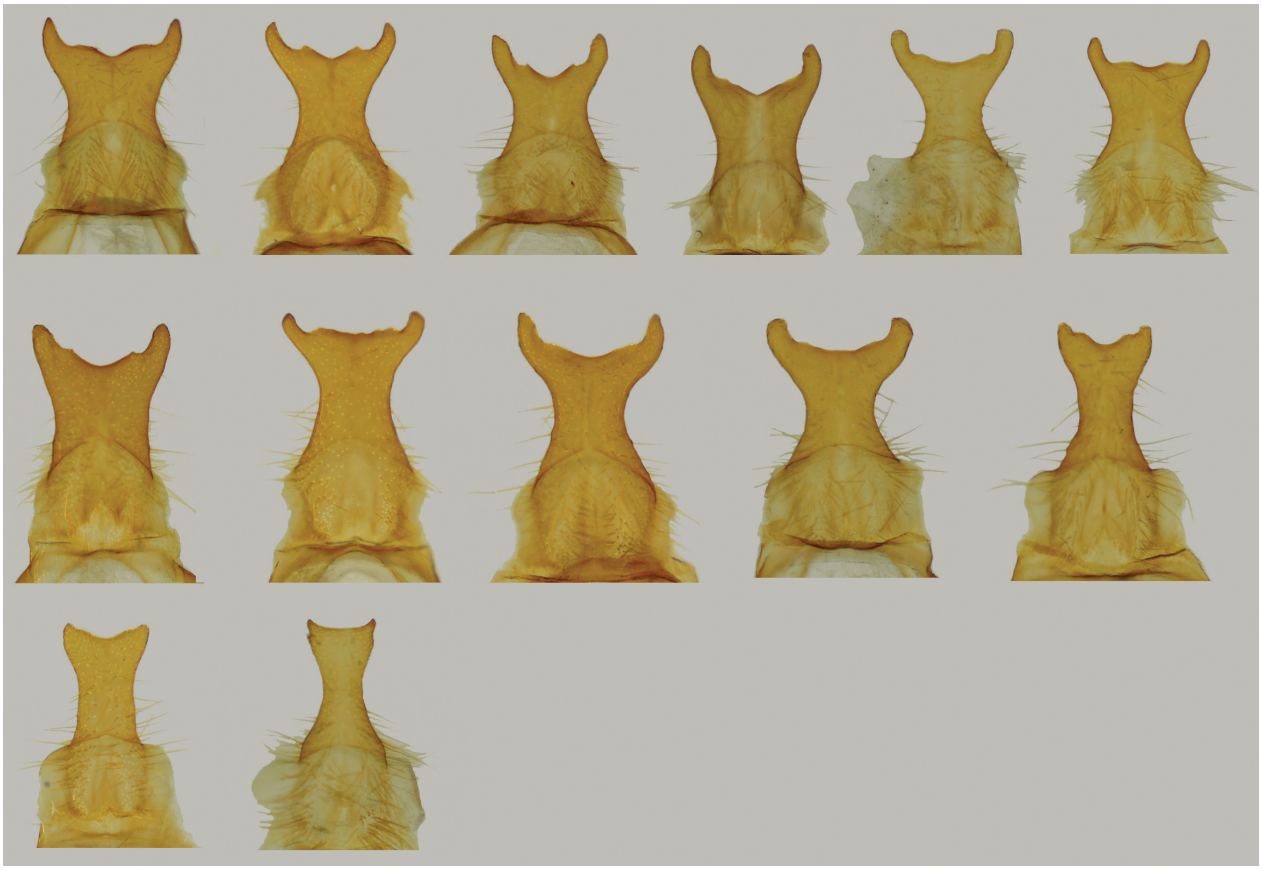

Figure 12. Overview of variation in the bifid termination of uncus in *A.* (*P.*) *cinereocostata*. Arranged from the broadest forms, with well developed lateral arms towards the dwarf forms.

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 grisescens 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, ramii 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, narrow dully ended; dorsal pheromone glands in form of two separate, elongate, membraneous, 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. grisescens*, 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 (*Tervurenia 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 *Hystrichophora* (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 costal 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

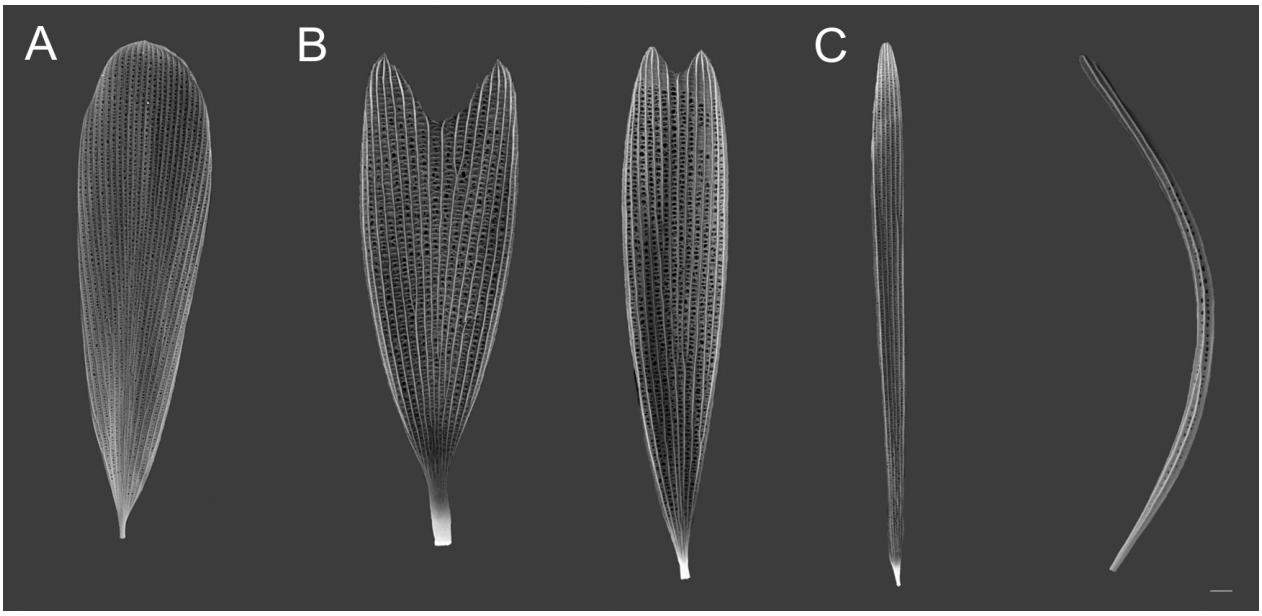

Figure 13. SEM image showing the differences between scale shape of *Apisa* (*P.*) *subargentea* (**A**) (flattened, convex termination), *A.* (*P.*) *cinereocostata* (**B**) (flattened, concave termination) and *A.* (*P.*) *asipa* **sp. nov.** (**C**) (narrow, needle-like).

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).

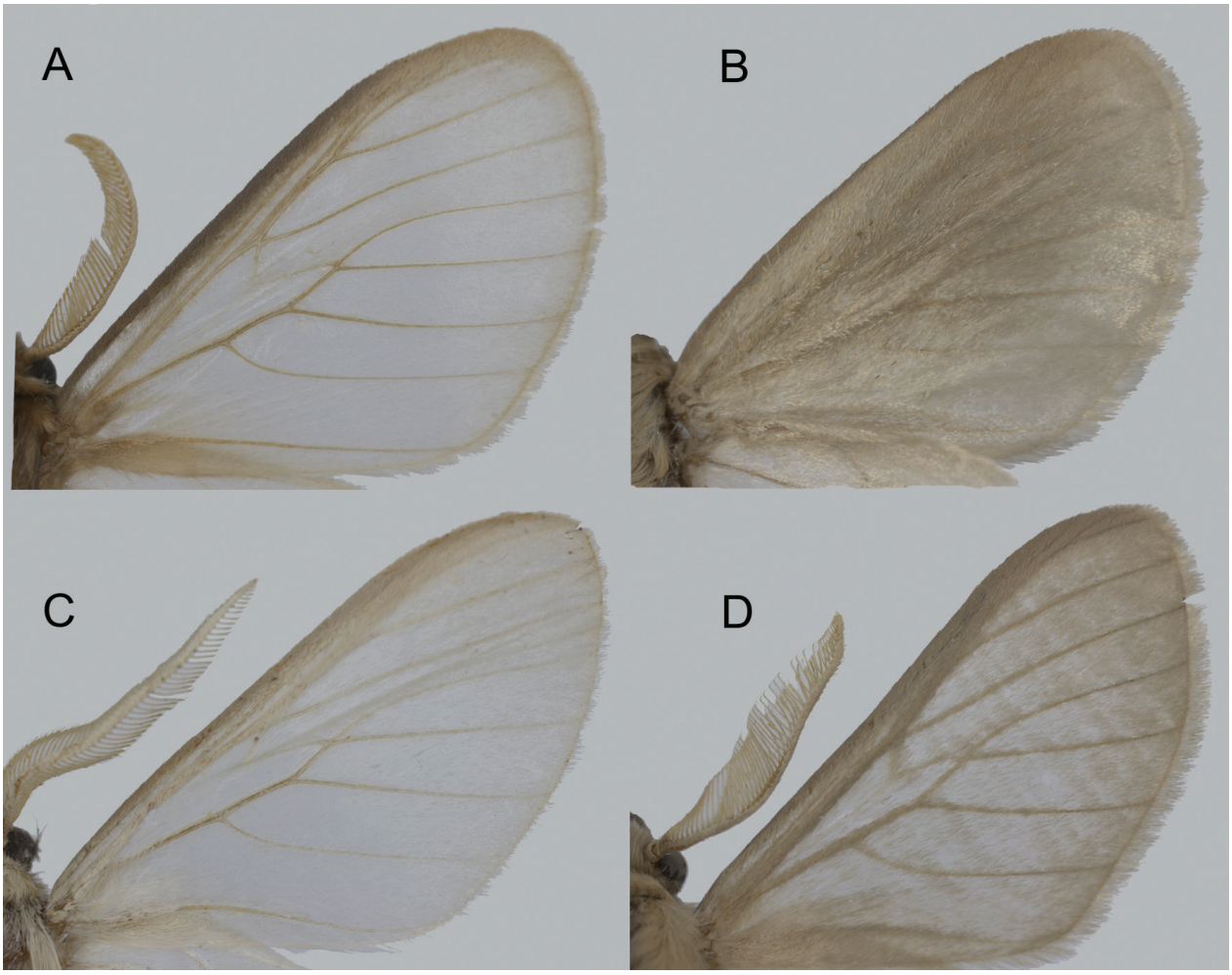

Figure 14. Differences in the forewing transparency. *Apisa* (*P.*) *asipa* **sp. nov.** (**A**), wing transparent; *A.* (*P.*) *subargentea* (**B**), wing opaque; *A.* (*P.*) *cinereocostata* (**C**, **D**), wing semitransparent.

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.

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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*.

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.

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..

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

ARTYKUŁ 3

Systematics and phylogeny of *Apisa* (Lepidoptera: Erebidae: Syntomini) – current stage of knowledge and perspectives.

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ABSTRACT

The genus *Apisa* Walker, 1855 is reviewed on the basis of morphological and molecular information. Both methods gave largely conflicting results. Molecular analysis, based on three markers, COI, wingless (WG) and RpS5, does not resolve the evolutionary relationships between species. However, it clearly shows the artificial nature of the current subgeneric division. There is also lack of signal in the morphological study. As a result, the present system is considered uninformative and artificial from an evolutionary point of view. Therefore, the subgenera *Dufraneella* Kiriakoff, 1953 **(syn. nov.)** and *Parapisa* Kiriakoff, 1952 **(syn. nov.)** are here formally synonymised with *Apisa* Walker, 1855. Based on new diagnostic characters, *A. diversa* (**sp. nov**.) is described from Gabon and the Democratic Republic of the Congo. *A. subcanescens* Rotschild, 1910 and *A. arabica* Warnecke, 1934 are redescribed with the first description of the female and the male genitalia of the respective taxa. New country records are provided for several species. A revised checklist of the genus and a key based on male genital characters are provided. The current state of knowledge of *Apisa* is discussed in details.

KEY WORDS

Taxonomy, morphology, molecular, revised checklist, determination key

INTRODUCTION

Species are valuable and important units of biodiversity, therefore errors in their delimitation can have serious consequences for any further analysis, synthesis or conservation policy (Chenuil et al., 2019; Piemontese et al., 2020). Cryptic species may represent a significant proportion of global biodiversity. However, the inconsistent definition of species and the large parameter space relevant to species delimitation make it difficult to estimate biodiversity at species level, including for the analysis of ecological and evolutionary processes (Carstens et al., 2013). Species provide an important link between many areas of taxonomy, phylogeny and ecosystem functioning (Struck et al., 2018).

An important tool for helping to resolving these ambiguities is the combination of traditional morphological methods with molecular analyses. Phylogenetics is increasingly playing a central role in uncovering the relationships between taxa and thus helping to unravel the processes behind speciation. However, studies of species-level relationships using only molecular methods should not be based on just a single gene. On the contrary, most proposed species-level phylogenies have been based only on the so-called “DNA barcode” fragment of the mitochondrial genome, mainly because of its ease of amplification. Such an approach, based on a single gene tree may lead to a different interpretation of evolutionary history than one that combines multiple genes (Wahlberg et al., 2009).

The erebid subfamily Arctiinae (also known as woolly bears or tiger moths) is a large cosmopolitan group of often medium sized lepidopterans. Like other Macroheterocera, they have been recognised as "quite well studied" compared to many other groups especially the so-called micromoths (Weller et al., 2009). However, not all tiger moths are well studied. A fascinating example of the intricate relationships developing within a cryptic assemblage of species is the entire afrotropical genus *Apisa*, which includes species that are morphologically very similar to each other. *Apisa* is one of the most taxonomically enigmatic groups within the Arctiinae. Very little is known about their biology, ecology and the host plants remain unknown. Almost all taxa lack any obvious pattern and many of them are prone to exceptional variability in morphology of genitalia (Paśnik et al., 2023). Such characteristics of *Apisa* imply that the taxonomic status of some of the already described species remains controversial. Conversely, the high level of observed morphological variation becomes a significant challenge when hypothesising the species-level distinctiveness of given morphotypes.

The extent to which morphological variation can affect the precision of species differentiation has not been studied in detail, despite its potential impact on our ability to use genital morphology to reliably identify species (Shtinkov et al., 2016).

A key tool for species-level taxonomy is the diversity and differences in the structure of male and female genitalia. This makes the informative features of these internal reproductive structures of great value and importance in species identification (Mutanen & Kaitala, 2006). It is important to remember that the process of evolutionary diversification of genitalia is quite rapid (Eberhard, 1985). Differences in genital shape may be caused by changes during life history or may be an adaptive trait to aspects of life history. Often these characters provide a good basis for distinguishing between closely related sibling species. The most variable part of the male genital apparatus is often considered to be the distal and dorsal part of the valva (Goulson, 1993). Very often, where morphological similarities are strong, differences in the structure of the genitalia provide important diagnostic details even in closely related taxa (Hosken & Stockley, 2004).

From a different point of view, the knowledge of genital polymorphism in insects is poor (Mutanen & Kaitala, 2006). Most information on polymorphism applies to groups such as arachnids and molluscs (Huber & Gonzalez, 2001; Jocque, 2002).

This study aims to clarify the taxonomy of the genus *Apisa* and to test whether the existing subgeneric divisions reflect the evolutionary relationships between its members. It also aims to compare the utility of morphological and molecular methods for species delimitation and for diagnosing different morphospecies and phylogenetic lineages. For the first time a determination key for all known species is constructed and the male genitalia are extensively illustrated with particular emphasis on their variability. Finally, the current limitations of the integrative approach to resolving the taxonomy of *Apisa* are discussed in detail, and suggestions are made for future studies.

MATERIAL AND METHODS

All analyses were carried out on museum material, properly mounted on entomological pins and stored in cabinets. A digital docket was created for each specimen . Material was then selected for genetic testing.

Morphological studies

Photos of the individuals were taken using a Canon 6D camera with a 100mm macro lens. Raw images were processed using Adobe Photoshop CC. Wings measurement were taken using a digital caliper from the base to the apex of the wing.

Preparations of genitalia were made from selected specimens to confirm species identity. To analyse this structure, the abdomen was detached and macerated in a 10% potassium hydroxide solution in a water bath for about 30 to 40 minutes. The time varied depending on the size of the abdomen and the degree of deterioration. Scales, unwanted membranes and remains of the digestive tract were removed from the specimens. Membranous structures (vesica in males and copulatory bursa in females) were stained with chlorazol black and then embedded in euparal, labelled accordingly and added to the collection.

Photographs of the genitalia were taken using a Leica S9i stereomicroscope. Images were adjusted using Adobe Photoshop CC software. The terminology for the genitalia is based on Kôda (1987) and morphology terminology follows Miller (1991).

Morphological characters were not used to resolve the phylogeny, either as a separate analysis or in combination with the molecular data. This approach was dictated by the very small number of morphological characters that could be coded in the form of a matrix. In fact, only 3 characters (shape of uncus, presence/absence of cornutus, development of valva process) could be coded with some degree of objectivity in discrete character states. Considering the number of taxa (12 species), this number is far too small to obtain interpretable information from a resulting tree. The remaining morphological characters available for study and evaluation fall into two categories, neither of which can be useful in revealing phylogenetic aspects. The first – much smaller - group contains autapomorphies of the entire genus (absence of arolium) or of individual taxa (e.g. modified scales, veins covered with dark scales, elongated uncus). Such characters are by nature diagnostic for individual taxa but do not help to resolve phylogenies. The second group consists of characters that could not be assigned to discrete states with even moderate confidence. Within *Apisa,* such characters can be extracted from both the external morphology and reproductive organs. Such types of variable and continuous characters are: forewing length varying from about 10 to 40 mm, overall colouration varying continuously from dark ochraceous to almost whitish, darker costa contrasting to varying degrees with paler wing background, variable degree of development and shape of termination of the valva process, variable shape of elongated saccus, phallus generally tubular, straight of variable length and width. Finding proper,

potentially phylogenetically sound characters in *Apisa* is further complicated by the fact that the females of most species remain unknown, so female genitalia cannot be used to search for additional characters. Male genitalia are also very simplified. This second tendency is observed in many evolutionarily young groups of Lepidoptera including the Arctiines. In *Apisa,* the valva is much reduced in length, generally subovoid without obvious sclerotization (except for variably developed ventral processes), and the vesica lacks any cornuti (except for a single basal one in some species), scobinations or sclerotized zones.

All of the above mentioned characteristics of *Apisa* made a morphologically based phylogeny uninformative and even impossible to analyse objectively. Therefore, in the present study, molecular analyses were used to investigate the phylogenetic relationships within *Apisa* and between related genera. The morphological data were prioritised to characterise the variability detected for smaller sets of specimens, and this approach was not always congruent with the molecular results.

Molecular studies

For molecular studies, two legs were removed from each of the designated individuals. The DNA was extracted using the NucleoSpin Tissue Kit (Macherey-Nagel) according to their protocol. Three genetic markers were selected one mitochondrial; cytochrome c oxidase subunit I (COI) 658 bp, and two nuclear; wingless (WG) 400 bp, and RpS5 611 bp. Amplification of the mitochondrial gene followed the protocol provided in Hebert (2004). The nuclear genes were amplified according to the protocols in Wahlberg and Wheat (2008). The exact primers and references are given Table 1.

Marker	Primer	5'-3' primer sequence
COI	Lep-F1	ATTCAACCAATCATAAAGATAT
	Lep-R1	TAAACTTCTGGATGTCCAAAAA
WG	HybLepWG1	TAA TAC GAC TCA CTA TAG GGG ART GYA ART GYC AYG GYA TGT CTG G
	HybLepWG2	ATT AAC CCT CAC TAA AGG GA CTI CGC ARC ACC ART GGA ATG TRC A
RpS5	HybrpS5degF	TAA TAC GAC TCA CTA TAG GGa tgg cng arg ara ayt gga ayg a
	HybrpS5degR	ATT AAC CCT CAC TAA AGG Gc ggt trg ayt trg caa cac g

Table 1. *Primers for COI (after Hebert et al., 2004), WG and RpS5 (after Wahlberg & Wheat, 2008). Note the use of universal tails on both the forward and reverse primers, which facilitate sequencing reactions (see Wahlberg and Wheat 2008 for details).*

The unpurified PCR reaction products were checked on a 1% TBE agarose gel for 30 min at 100 V, and then visualised under the UV. The products were then purified using Exo-BAP mix or EURx Agarose-Out DNA Purification Kit (EURx, Poland) following the standard protocol. The BrilliantDye v3.1 Terminator Cycle Sequencing Kit (NimaGen, the Netherlands) was used for sequencing. The resulting products were sequenced in two directions. The PCR products were sequenced either in the Institute of Systematics and Evolution of Animals Polish Academy of Sciences, using an ABI Prism 3130xl sequencing machine or in an external company (Genomed, Macrogen). The obtained chromatograms were manually checked and aligned with a reference sequences by using the BioEdit software version 7.0.9.0 (Hall, 2004).

The exact PCR recipe and the PCR protocol are provided in Tables 2 and 3.

PCR steps	COI	WG	RpS5
1. Initial activation	1 min – 94°C	5 min – 95°C	5 min – 95°C
2. Denaturation	1 min – 94°C	30 sec – 94°C	30 sec – 94°C
3. Annealing	1 min 30 sec – 45°C	30 sec – 50°C	30 sec – 55°C
4. Elongation	1 min 15 sec – 72°C	1 min 30 sec – 72°C	1 min 30 sec – 72°C
5. Final elongation	5 min – 72°C	10 min – 72°C	10 min – 72°C
Number of cycles (2-4)	6 cycles (45°C) 36 cycles (51°C)	40 cycles	40 cycles

Table 2. *PCR protocol for COI, WG, RpS5.*

Primer	Reagent	Volume
COI	dH2O – 6,3µl Primer 1 – 0,2 µl Primer 2 – 0,2 µl Taq – 0,1 µl DNA extract – 1,0 µl dNTPs – 0,2 µl Buffer – 2 µl	10 µl
WG	dH2O – 2,5µl Primer 1 – 0,4 µl Primer 2 – 0,4 µl Supreme NZYTaq II 2x Green Master Mix – 5 µl DNA extract – 2,0 µl	10,3 µl
RpS5	dH2O - 4µl Primer 1 – 0,625 µl Primer 2 – 0,625 µl Bioline 2x My Taq HS Red Mix – 6,25 µl DNA extract – 1,0 µl	12,5 µl

Table 3. *Exact recipe for PCR reaction.*

All laboratory work was carried out at the Laboratory of Molecular Techniques in ISEA PAS, except for the RpS5 gene, where part of the work was carried out at Lund University.

Bayesian inference (BI) analyses were performed using MrBayes ver. 3.2.7 (Ronquist et al., 2012). The BI analyses used four independent runs each with four Metropolis-coupled chains with default heating parameters (one cold and three heated). Chains were sampled once every thousand generations for 2 million generations, with the first 25% of samples were discarded as burn-in. A mixed model of sequence evolution was chosen. The analysis was run

four times, each time with a random starting tree. All analyses converged to an average standard deviation of split frequencies below 0.01.
The outgroups and the GenBank accession numbers are given in Table 4.

Species	GenBank acces number
Neophemula vitrine	LN009
Pseudomelisa sp.	LN071
Balacra (Daphenisca) affinis	LN043
Lempkeella cf. *vanoyei*	LN064
Tervurenia eloumdeni	MO76629
Balacra rubricincta	LN006
Balacra furva	LN010
Balacra pulchra	LN040
Balacra herona	LN045
Balacra flavimaculata	LN042
Balacra preussi	LN046
Balacra rattrayi	LN044
Balacra compsa	LN041
Paramelisa dollmani	LN013
Balacra rubrostriata	LN008
Metarctia sg. *Thyretarctia*	LN072
Anapisa holobrunnea	OM523179

Table 4. *List of species used as outgroups for analysis*.

Clade robustness was estimated by posterior probabilities. All trees obtained were visualised using FigTree 1.4.3 (Rambaut, 2009) and graphically processed using Affinity Designer. Pairwise sequence divergence was calculated in the MEGA 11 (Tamura et al., 2021). The matrix is shown in the Table. S1.

Sequence based species delimitation test

To objectively assess the number of potential species obtained on the phylogenetic tree, the Assemble Species by Automatic Partitioning (ASAP) method was used (Puillandre et al., 2021). This method uses the pairwise distance for partition sequences into potential species. It is based on the detection of DNA barcode gaps in the variation between putative groups (Puillandre et al., 2012). Different models were calculated and selected using AIC. The JC69 substitution model was selected.

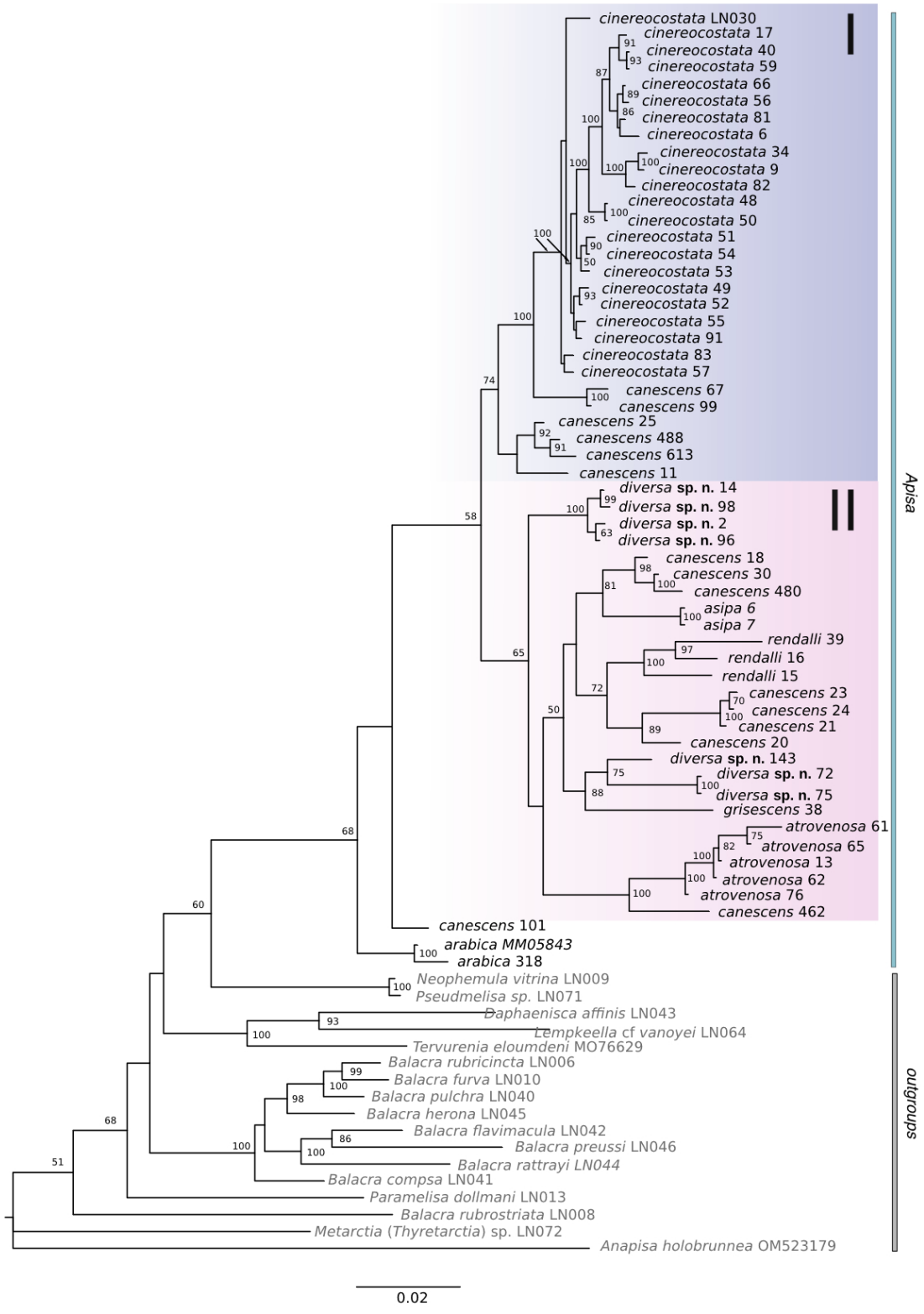

Figure 1**.** *Phylogenetic tree based on Bayesian interference method including COI, wg and RpS5 sequences. Values on nodes correspond to posterior probability suport. Values below 50% are not included in the tree. Outgroups are marked in gray (more imformation in table 4). Known species and morphologically separated species are marked on the right side. The numbers correspond to the DNA samples and the LNXXX, OMXXX are the GenBank accesion numer. Scale bar unit: expected substitutions per site.*

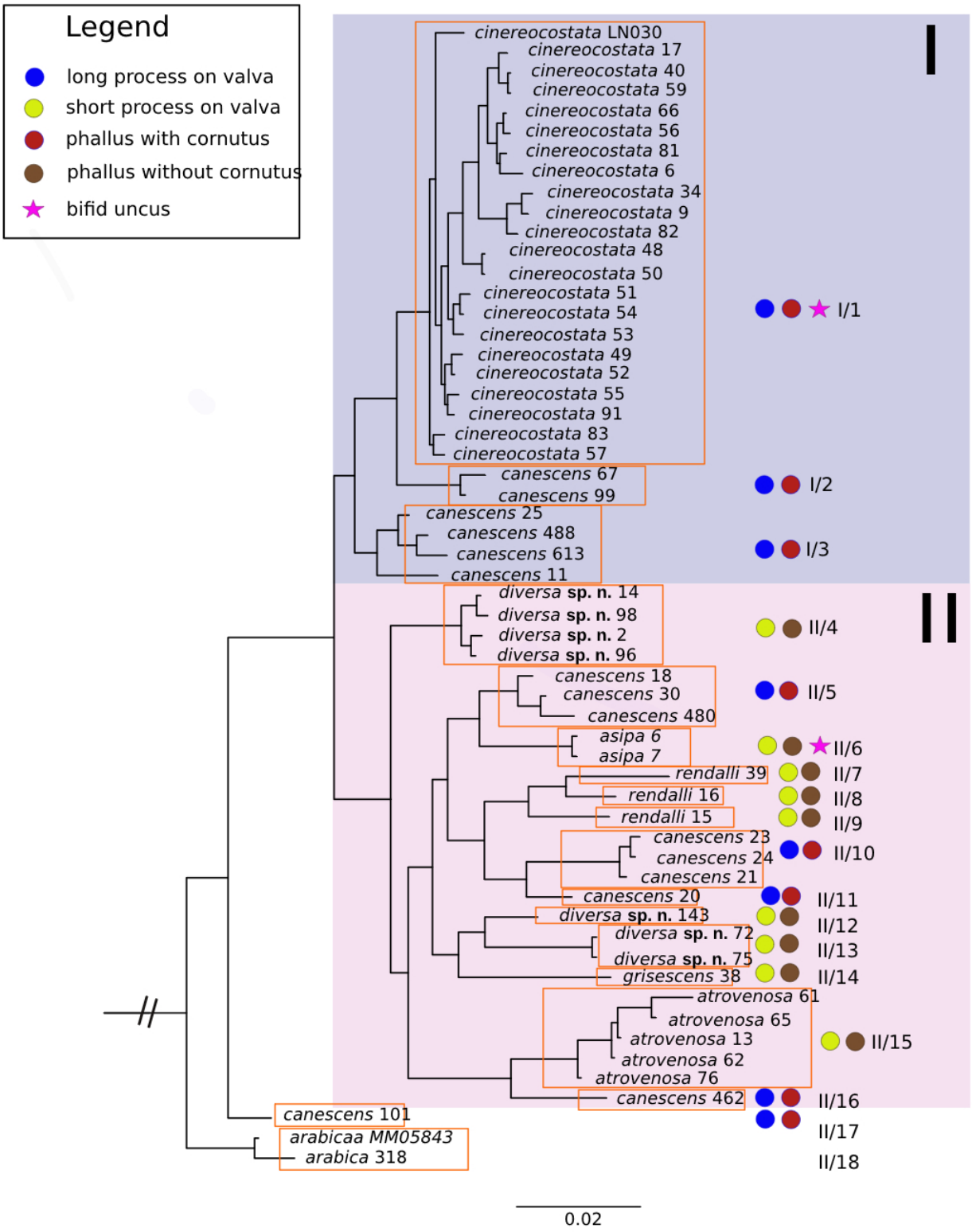

Figure 2. *Tree based on Bayesian inference including COI, WG and RpS5 sequences. The tree does not include outgroups and shows only specimens from the genus Apisa. Orange frames and numbers (e.g. I/1, I/2….) show grouping from the ASAP (Assemble Species by Automatic Partitioning) delimitation approach. Coloured dots reflect morphological characters (see legend for details). On the right, species that fit the delimitation are marked.*

Acronyms of institutions and collections

ANHRTUK – African Natural History Research Trust (Leominster, UK);

DEIB – Deutsches Entomologisches Institut Berlin;

DRC – Democratic Republic of Congo;

ISEA PAS – Institute of Systematics and Evolution of Animals Polish Academy of Sciences, Kraków, Poland;

KBIN – Royal Belgian Institure of Natural Sciences, Brussels, Belgium;

KL – Knud Larsen private collection, Dennmark;

MCSG (MCSNG) – Museo Civico di Storia Nturale di Genova, Italy;

MO – private collection of Michael Ochse (Weisenheim am Berg, Germany), later ZSM;

NHMUK (formerly BMNH) – The Natural History Museum, London UK;

MWM – Museum Thomas Witt, Munich, Germany;

MZLU – Zoological Museum of Institute of the University of Lund;

NHMW – Naturhistorisches Museum Wien; Vienna, Austia;

RBINS – Royal Belgian Institute of Natural Sciences, Brussels, Belgium;

RMCA – Royal Museum for Central Africa, Tervuren, Belgium;

RMNH – Naturalis Biodiversity Center, Leiden Netherlands;

SMNS – Staatliches Museum fur Naturkinde, Stuttgart, Germany;

USMB – Ubersee Museum, Bremen, Germany;

ZMUC – Zoological Museum, University of Copenhagen, Denmark;

ZMS - Zoologische Sammlung des Bayerischen Staates, Munich, Germany;

coll. R. Fiebig – private collection of Ralf Fiebig;

coll. T. Baron – private collection of Thomas Baron;

coll. D. Stadie – private collection of Dirk Stadie;

Other abbreviations

GS – genital slide;

RESULTS

Genetic analysis of *Apisa*

The genus *Apisa* belongs to the informal "*Balacra* clade" of Syntomini. Based on our phylogenetic studies *Apisa* is recognised as a monophyletic lineage, but without support (PP = 60%). In this respect the results are congruent with those results published in a previous phylogenetic study of the tribe Syntomini (Przybyłowicz et al., 2019), which included two species (*A. canescens* and *A. cinereocoststa*). The sister clade to *Apisa* was found to be the *Neophemula* + *Pseudmelisa* clade. All three genera are sister to the ((*Daphaenisca* + *Lempkeella*) + *Tervurenia*) clade, although this position is not supported (PP = 30%).

Our molecular analysis includes 57 specimens. Interestingly the two most basal lineages represent samples that originated from North East Africa and the Arabian Peninsula (Oman). These samples are all characterised by a single uncus and an elongate process of the valva. All remaining *Apisa* are placed in two major clades (hereafter referred to as clades I and II), although without support (PP = 58%). Each of them contains morphotypes with single/forked uncus, elongate/short valva process and present/absent thorn like cornutus.

The core portion of the clade I forms a monophyletic lineage (I/1) representing a single taxon *A. cinereocostata* (22 specimens), which is unique for its unusual polymorphism in the shape of uncus in the male genitalia. The large number of specimens shows that the species is monophyletic, and allows the assessment of genetic variability within a species of *Apisa*. The results obtained confirm the previous hypothesis that regardless of the degree of development of the paired processes of uncus all specimens are genetically coherent and represent a single well-separated and strongly supported clade with a PP value of 100%.

The remaining specimens of clade I form two smaller clades I/2 (2 specimens) and I/3 (4 specimens), I/2 being sister to *A. cinereocostata* and I/3 being sister to these two. Clade I/2 is represented by relatively small specimens from West Africa (Liberia, Ghana) while I/3 consists of slightly larger specimens from South Africa (Angola, RSA, Mozambique). All these 6 specimens are very different from *A. cinereocostata* in the morphology of male genitalia, and can be classified as widespread and polymorphic *A. canescens* on the basis of current knowledge.

Clade II is much more heterogeneous both morphologically, and in the number of terminal clades. This clade also contains all molecularly analysed samples characterised by the absence of a single thorn-like cornutus in the vesica and a reduced (not elongate and sharpened at tip) process of the ventral portion of valva. The tree topology suggests 6 or 7 distinct clades. The species delimitation analysis multiplies the number of putative species-

level categories to as much as 13 (further on referred as clades II/4 to II/16). In contrast, the morphological approach through the examination of many more specimens does not perfectly fit any of the molecular based resolutions but also suggests a lower number of taxa.

The basal position of II/4 is not supported (PP = 65%), but the morphological characters clearly distinguish the specimens from already known taxa of *Apisa*. On the basis of both genetic and morphological distinctiveness, it is hypothesised here that they belong to a new, hitherto unknown taxon, formally described in this paper as *Apisa diversa* **(sp. nov.).** It is also interesting to note that three other specimens, representing the same morphotype, form a different clade II/12-13. To avoid any future nomenclatural complications, the holotype has been designated for the specimen with the known haplotype and the type series contains only the specimens belonging to the same clade. The specimens belonging to the other clade (II/12-13) are not included in the type series. They are considered as morphologically indistinguishable in this study, but their true taxonomic status will have to be tested in further studies using additional material.

Clade II/15 contains all 5 samples representing in our study *A. atrovenosa*. Surprisingly it is sister to a single specimen (II/16) which, again based on morphology, can be classified as the polymorphic species *A. canescens*. At this stage it can only be speculated that the position of this particular specimen does not reflect the true evolutionary relationships with *A. atrovenosa* and, in a wider context, the coherence of the *canescens* morphotype. The remaining clades form a morphologically rather heterogeneous assemblage, including small separate clades characterised by different states of the same morphological structures. The assemblage also includes exceptionally distinctive *A. asipa,* which possesses both highly modified wing scales, a forked uncus, a short valva process and an absent cornutus.

Much more difficult is the interpretation of the intrageneric variability of *Apisa*. The results clearly indicate the high level of cryptic diversity within the genus, in particular suggesting the existence of several separate taxa currently classified as a single, common species *A. canescens*. The samples assigned to this name on the basis of current knowledge are distributed throughout the tree, forming small clades I/2, I/3, II/5, II/10-11, II/16 and II/17. Based on the morphology of the male genitalia, this assemblage is characterised by a sharply terminated uncus, the presence of a thorn like cornutus and a very long, narrow process of the valva, which far exceeds the length of the valva. Apart from these key genital characters, examination of the collected material did not allow the discovery of any reliable discrete characters, that would allow a proper diagnosis of members of each of the separate *A. canescens* clades. The situation becomes even more complicated when the morphological

analysis includes numerous specimens that have not been genetically examined. The clear boundaries of given characters (long/short, convex/concave) are blurred by a wide range of transition states. In order to avoid any speculation, unsupported interpretations and nomenclatural burden, we have refrained from describing new taxa in the complex of *A. canescens*.

The generated molecular tree also highlights that *A. cinereocostata* and *A. asipa*, both characterised by the forked tip of the uncus, are not closely related within *Apisa*. This suggests that the current division of the genus into three separate subgenera does not reflect natural evolutionary affinities, but was proposed on the basis of superficial similarities and unclear diagnostic characters. As far as the presence/absence of the forked uncus is a discrete character, the division of subgenus *Dufraneella* based on the partial reduction of the valva process is much more subjective and unclear after examination of more specimens from different regions of Africa. Another morphological character that undermines the legitimacy of the current subdivision of *Apisa* into subgenera is the presence or absence of the thorn like cornutus. The cornutus is present in all typical *canescens*-like morphotypes with a single uncus, but also in *A. cinereocostata* with a widely forked tip of the uncus, whereas it is absent in *A. subargentea* and *A. asipa*. *Apisa cinereocostata* also has a relatively long and pointed process of the valva, which differs drastically from the small, reduced one typical for *A. subargentea* and *A. asipa*. All of the above strongly suggests that neither of the two subgenera forms a monophyletic lineage characterised by clear autapomorphy, and that the division of *Apisa* into subgenera (at least in the present sense) should therefore be revised. As a result, the subgenera *Dufraneella* Kiriakoff, 1953 **(syn. nov.)** and *Parapisa* Kiriakoff, 1952 **(syn. nov.)** are here formally synonymised with *Apisa* Walker, 1855.

ASAP (Assemble Species by Automatic Partitioning)

As a result of the ASAP analysis the distance gap approach identified between 11 to 48 hypothetical species. We chose the first ASAP score (1.50) which provides the best scenario with 18 hypothetical species.

Determination key to the males of genus *Apisa* based on the male genitalic characters. Comments on the key.

Apisa manetti is not included because its male genitalia are still unknown. However, it can be easily determined from its distribution, as it is the only *Apisa* species known from North Africa (Libya: Cyrenaica). *Apisa arabica* and *A. canescens* cannot be separated on the

basis of differences in male genitalia mainly due to the unresolved taxonomic status of many variable morphotypes now treated collectively as *A. canescens*. The entire key should be treated as preliminary and the results should always be compared with the habitus. Due to the external similarity of all *Apisa* species, it is impossible to prepare such a key constructed for the habitus characters. However, some species or group of species possess certain characters which, when combined with genitalia, are very useful for their determination. These include general colouration, morphology of scales, indistinct pattern of fore and hind wing. It is advisable to consult the diagnosis or description of the relevant taxa whenever the key below gives an uncertain solution.

1. Uncus bifid ……2
-. Uncus undivided, sharply terminated…….…….4
2. Vesica with a single, well produced, thorn-like cornutus; process of valva elongate, the length of valva; paired, terminal processes of uncus widely separated so bifurcation U-shaped…….…..… *A. cinereocostata*
-. Vesica without cornuti; process of valva invisible or in form of minute tubercle; paired, terminal processes of uncus narrowly separated so bifurcation V-shaped……..……..…3
3. Uncus constricted before terminal bifurcation; terminal lobes shorter than one quarter of the length of uncus ..... *A. subargentea*
-. Uncus with parallel margins not constricted before terminal bifurcation; terminal lobes approximately the half the length of uncus.....*A. asipa*
4. Vesica with a single, well produced, thorn-like cornutus…….……..5
-. Vesica without cornuti…….……8
5. Process of valva shorter than the length of valva…….…….....6
-. Process of valva much longer than the length of valva……..……7
6. Uncus more than two times as long as its width at base counted from the ventral side; uncus distinctly lanceolate widened before sharp termination; phallus no less than six times longer as its width at middle…….…….*A. subcanescens*
-. Uncus approximately two times as long as its width at base counted from the ventral side; uncus without distinct lanceolate widening before sharp termination; phallus no more than five times longer as its width at middle…….…….*A. hildae*
7. No clear diagnostic characters, distributed in Arabian Peninsula……..……..*A. canescens*
-. No clear diagnostic characters, distributed in sub-Saharan Africa……..…… *A. arabica*
8. Distal, sclerotized margin of phallus evenly terminated……..…..……..……9

-. Distal, sclerotized margin of phallus slightly distended and opened sublaterally……..…..10

9. Uncus evenly subtriangular when examined from ventral side……...*A. grisescens*

-. Uncus narrowed in half the length, then with almost parallel margins until sharp termination…….……...*A. atrovenosa*

10. Process of valva atrophied or in form of indistinct tubercle……..…...*A. diversa* **sp. nov.**

-. Process of valva short but well developed, usually sharply terminated……..11

11. Process of valva usually spatulate, at least three times longer than wide at the base; phallus on average no less than four times longer than wide ……..……..*A. fontainei*

-. Process of valva usually subtriangular, at most three times longer than wide at the base; phallus on average no more than four times longer than wide……..…….*A. rendalli*

Revised catalogue of *Apisa*

***Apisa* Walker, 1855b: 916-917.**

Type species: *Apisa canescens* Walker, 1855 (by monotypy).

Dufraneella Kiriakoff, 1953: 14-15. **[syn. nov.]**

Type species: *Metarctia grisescens* Dufrane, 1945 (by original designation).

Parapisa Kiriakoff, 1952: 174. **[syn. nov.]**

Type species: *Parapisa bourgognei* Kiriakoff, 1952d (by original designation).

1. *arabica* Warnecke, 1934: 63 (*Apisa*). Syntypes [Yemen] “San’a” [ZIMH].

lippensi Kiriakoff, 1960a: 4 (*Apisa canescens* ssp.). Holotype [Saudi Arabia] “Habne (ville)” [KBIN – not located, Ł. Przybyłowicz].

2. *asipa* Paśnik, Przybyłowicz & Tarcz, 2023: 379-383 (*Apisa*). Holotype [Cameroon] "North Region, Wack (La Falaise)"[ANHRT]

3. *atrovenosa* Przystałkowska, 2022: 94-97 (*Apisa*). Holotype [Uganda] "W of Mpigi, Mpanga Forest Camp" [ISEA PAS]

4. *canescens* Walker, 1855: 917 (*Apisa*). Lectotype “South Africa” [BMNH].

pallata Pl ötz, 1880: 78 (*Psychotoe*). Holotype [Congo] “West-Africa, Abo” [UGD].

cana Holland, 1893: 394-395 (*Apisa*). Syntypes [Gabon] “Valley of the Ogove River” [CMNH].

microcanescens Berio, 1935: 59 (*Apisa canescens* ssp.). Holotype [Somalia] „Giuba, Belet Amin” [MCSNG].

tamsi Kiriakoff, 1957: 122 (*Apisa*). Holotype [Burundi] „Kasengi” [RMCA].

5. *cinereocostata* Holland, 1893: 394 (*Apisa*). Holotype [Gabon] “Valley of the Ogove River” [CMNH].

bourgognei Kiriakoff, 1952: 173-174 (*Apisa*). Holotype [Ivory Coast] “Bingerville” [MNHN].

6. *diversa* Paśnik & Przybyłowicz **[sp. nov.]**

7. *fontainei* Kiriakoff, 1959: 25 (*Apisa*). Holotype [Rwanda] “Kisenyi” [RMCA].

8. *grisescens* Dufrane, 1945: 131 (*Metarctia*). Holotype [DRC] „Kamituga” [KBIN].

9. *hildae* Kiriakoff, 1961: 96-97 (*Apisa*). Holotype [Namibia] „Okahandja“ [ZSM].

10. *manettii* Turati, 1924: 46-49 (*Apisa*). Syntypes [Libya] “Cyrenaica: Bengasi-Fuehat, giardino Vella ad El Berca” [MRSN].

11. *rendalli* Rothschild, 1910: 441-442 (*Apisa*). Lectotype [Malawi] “Zomba, Upper Shire River” [BMNH].

nyasae Kiriakoff, 1957: 95 (*Apisa grisescens* ssp.). Holotype [Malawi] „Nyasaland, near Mlanje“ [BMNH].

12. *subargentea* Joice & Talbot, 1921: 158 (*Apisa*). Holotype [Rwanda] “Lake Tshohoa” [BMNH].

13. *subcanescens* Rothschild, 1910: 442 (*Apisa*). Lectotype [Senegal] “Casamance” [BMNH].

Taxonomic part

All known taxa of *Apisa* are listed in alphabetical order and are extensively commented. References to relevant publications are given for the species described in Przystałkowska (2022) and Paśnik et. all., (2023).

Apisa arabica Warnecke, 1934

Material examined: (5 ♂♂)

♂ Sultanate of Oman, Prov. Dhofar 28 km westlich Al Mughsayl 23.-26.12.2009 LF/TF, N16°50'27,4"E 53°40'78,5" 730m, leg. Bittner, Lehmann & Stadie, GS 03_04_11_2019, DNA 139; ♂ same but GS 01_04_11_2019, DNA 140; ♂ Sultanate of Oman, Prov. Dhofar 25 km westlich, N16°50'45"E53°41'11" 05./08.08.2010 LF 690m, leg. Stadie, Lehmann & Bittner, GS 03_25_10_2019, DNA 138; ♂ Sultanate of Oman, Prov. Dhofar 20 km westlich Al Mughsayl 23.-26.12.2009 LF/TF, N16°51'99,3"E 53°42'91,1" 100m, leg. Bittner, Lehmann & Stadie, GS 02_04_11_2019, DNA 137 (coll. D. Stadie); ♂ 64 Yemen Prov. Sana'a, 13°45'N, 44°10'E, road, Ta'izz-Ibb, 5 km s Nagdal Ahmar, 2280m, 7.III.2000, leg. F. Aulombard, M. Fibiger, H. Hacker & H.P. Schreier, GS 02_17_10_2019, DNA 136 (ISEA PAS)

Morphology.

Head. Frons, vertex covered with dense ochraceous hairy scales, only scapus with distinctly paler, short, flattened scales; labial palpus the same colour, three segmented with second segment the longest; antenna bipectinate, flagellum ochraceous; eye convex, indistinctly ovoid, naked; proboscis absent.

Thorax and abdomen. Dorsal portion uniformly ochraceous, concolorous to head, ventral portion pale ochraceous concolorous to the hindwing.

Wings. Length 14-16 mm (n= 5♂); forewing ochraceous, indistinctly semi-transparent in central portion, with slightly darker costa, apex rounded; underside of the same colour, retinaculum well developed; hindwing pale ochraceous, gradually darken from costa towards the dorsum, underside concolorous.

Male genitalia (first description). Tegumen relatively narrow, of even width; uncus narrowing towards the sharp tip, slightly widened before termination, dorsal surface covered with dense stiff setae; vinculum arms narrow, well sclerotized not fused with tegumen; valva shortened, subsquare, with shallowly concave terminal margin; ventral process of valva prominent, elongate, ¾ the lengths of uncus, sharply pointed with slightly bent termination, in

general shape needle-like; saccus twice as long as wide, with round termination; juxta in form of small subovate plate; transtilla prominent, heavily sclerotized, with rounded medio-distal margin; phallus straight, even weight, with basal portion slightly bent; vesical with a single thorn like cornutus at laterobasal portion.

Distribution: Oman, Yemen, (Saudi Arabia by Przybyłowicz 2009).

Comments. The species is very similar to *A. canescens*. There are no reliable diagnostic characters on the male genitalia. This is not due to the objective lack of such minute differences but rather to the still unresolved taxonomy of extreme (as for *Apisa*) polymorphism of specimens now treated as *A. canescens*. This external variability of *A. canescens* is also significant in northeastern Africa, but the specimens for the Arabian Peninsula are surprisingly much more homogeneous, both in terms of genital morphology and also in terms of size and overall colouration. In all 5 specimens examined, the male genitalia differ only indistinctly in the degree of deflection of the distal part of valva process. Additional material from both sides of the Red Sea is needed to assess the taxonomic status of the Arabian populations. To avoid the premature synonymisation of two distinct taxa, we retain the Arabian populations as a separate species. It should be notes that the type of *A. canescens* is from RSA and may not necessarily represent the same entity as *A. arabica*. An additional argument for not synonymising the two taxa is the fact that the most basal clade on the molecular tree is composed exclusively of samples from the Arabian Peninsula (Oman), indicating the significant genetic distinctiveness of the Arabian populations.

Genetic information. Within the clade the genetic divergence range is equal 0.0%. The nearest neighbour is *A. canescens (canescens* 101) with 2-3% pairwise distance. The *A. canescens* shows remarkably high infraspecific genetic divergence, which will be discussed below.

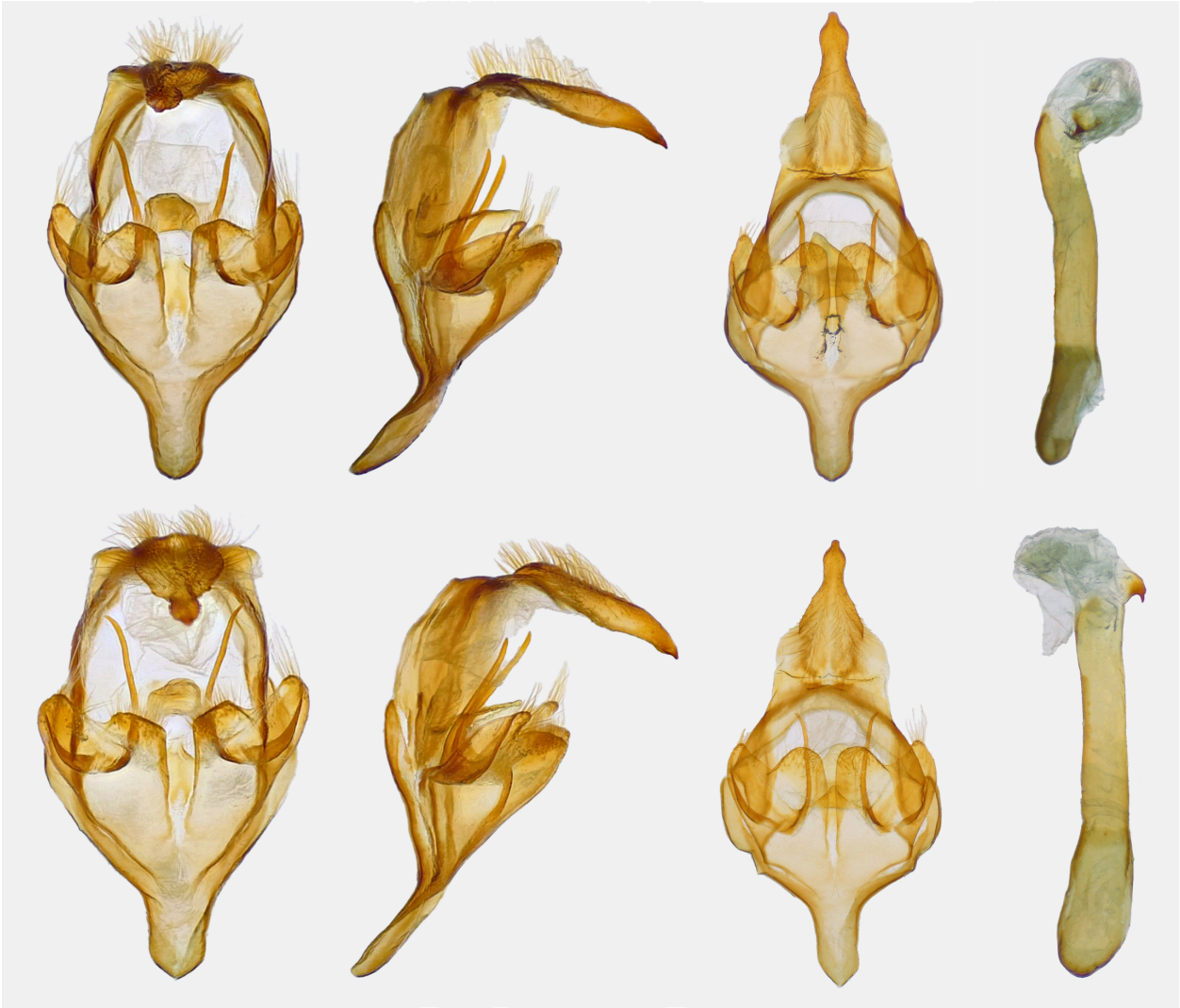

Figure 3. *Apisa Arabica, male genitalia. Frontal view, lateral view, genital slide, phallus.*

Figure 4. *Apisa arabica, imago. Upper side, under side, the labels.*

Apisa asipa Paśnik, Przybyłowicz & Tarcz, 2023

Comments. Taxon described in the paper by Paśnik, Przybyłowicz, Tarcz (2023) and elaborated in details therein.

Apisa atrovenosa Przystałkowska, 2022

Comments. Taxon described in the paper by Przystałkowska (2022) and elaborated in details therein.

Apisa canescens Walker, 1855

Material examined: *Lectotype*: ♂ S. Africa (BMNH).

Additional material: (♂♂129): ♂ Coll. Mus. Congo Kasenyi VIII-1937 (J. Brido) [Holotype. *Apisa tamsi*] (RMCA); ♂ Somalia IT., Belet Amin. (Giuba) VIII.1934 [Paratype *Apisa canescens microcanescens*]; ♂ same but VIII.1934.29; GS 05_15_02_2023; ♂ same but 10.VIII.1934 Patrizi; ♂ same but VIII.1934 Patrizi; ♂ same but GS 01_20_01_06 (MCSNG); ♂ Eritrea A.O.I Elaberet 12.XI.1938 F. Vaccaro, Museo Civico di Genowa, ex coll. Berio acquist. 1983; Typus, GS 03_15_02_2023 (MCSG); ♂ Angola Huambo Prov. Rd. Huambo-Caconada E Catata, 1667m, 13º23'58.6" S; 15º26'54.1"E, 25.XI.2017, leg S. Naumann, E. Ott & H. Sulak; Museum Witt; GS 06_22_05_19; DNA 119 (MWM); ♂ Angola; Dundo 1-8-1933; GS 02_11_01_2022 (RBINS); ♂ Angola Huila Prov. 2 km SWW Negola, 14º8'56.1" S; 14º28'20.5"E, 1606m, 16.XI.2017, leg S. Naumann, E. Ott & H. Sulak; Coll. Stefan Naumann; GS 02_27_02_2023; DNA 613 (ISEA PAS); ♂ Angola, prov. Uige, Sierra Uige 7o36'31.1"S 14o57'43.2"E, 850m 03.ii.2014, at light, leg. M. Nuss; GS 01_27_02_2023; DNA 11 (BMNH); ♂ Congo (DRC) Equateur Envinon de Lukolela 19 X 1993 leg. Ph. Oremans; GS 04_23_07_2018 (ISEA PAS); ♂ Cote D'Ivoire Gouedie 25km NW Man Plantagen in Regenwaldfragmenten 7º32' n. Br. 7º45' e. L. ca. 620 mNN 20.VIII.1997 LF (125 w HQL) T. Karisch legit; GS 03_02_03_2023 (ZMUC); ♂ Eritrea Adi-Abuna; VI-VII.1939 Cap. A.Richini; Museo Civico di Genova; ex coll. Berio acquist. 1983; GS 04_15_02_2023; ♂ Eritrea A.O.I. 23.8.1938 F.Vaccaro, Museo Civico di Genova; GS 04_05_12_2018; ♂ Eritrea A.O.I 29.9.1938 F. Vaccaro; Museo Civico di Genowa; GS 01_03_03_2023; ♂ same but GS 02_21_02_2023; DNA 134; ♂ Eritrea A.O.I Dorfu 20.X.1938 F. Vaccaro; Museo Civico di Genova; GS 06_06_03_2023 (MCSG); ♂ Ethiopia Southern Province ca. 125 km SW Addis Abeba 5 km N Abelti, at Omo River, 2000m, 28 October 2010 leg. De Freina, Museum Witt; GS 03_26_04_2019; DNA 107 (MWM); ♂ Ghana. Western: Bia Forest, 250m, 6km. W. Adwufia 12.-13.x.2007 Knud Larsen; GS

01_01_04_2019; DNA 97; ♂ same but GS 04_01_04_2019; DNA 104; ♂ Ghana. Ashanti: Bobiri 240m 4. Km. N. Kubeasi 9.-12.iii.2010 Knud Larsen & Wojciech Kubasik; GS 01_20_03_2019; DNA 99 (KL); ♂ W-Africa, Guinea Konakri, Macenta Prefecture Ziama Forest 550m, 250 watt; April 2017 leg. Petrányi G; Muller GC; Kravchenko VD et al., Thomas Witt Stiftung; GS 01_23_05_2019; DNA 120; ♂ same as but GS 02_26_04_2019; DNA 106 (MWM); ♂ Kenya, Western Prov. Kakamega Forest N.R. sec. forest 1600m 11.ix.2002 Lichtfalle (2) 0.21,31N; 34.51,82E leg. L. Kühne (SMNS); ♂ Kenia Malindi 77, Schiller; ex coll. H. Schiller; GS 05_02_03_2023 (ISEA PAS); ♂ Kenia – Nairobi V-1946 A. Maggi; Museo Civico di Genova; GS 03_05_12_18 (MCSG); ♂ Kenya, Prov. E-Rift Valley, zw. Kajiado & Mamnga, UMG. Sira IA Surawa, S01°56.559 E36°43.311, 7-8.v.2010- 1740M – Lux leg. J. Cave & T.A Newton-Chance; Museum Witt; GS 02_24_05_2019; DNA 125 (MWM); ♂ Kenya. Central P.: Castle Forest Lodge, 6km. N Kimunye 0°22’43.53”S 37°18’32.29” 22.-29.x.2013 2075 m D. Agassiz, S. Beavan, R. Heckford & K. Larsen; GS 01_19_03_2019; DNA 101 (KL); ♂ S Kenya crossing between Nguruman Escarpment-Lake Magadi truck and Ewaso Nigro River 31.07.1998 leg. Ł. Przybyłowicz; GS S102; ♂ same but GS 05_03_03_23; ♂ same but GS 08_20_02_23; ♂ same but GS S73; ♂ same but GS 06_12_01_2022; DNA 131; ♂ same but GS 01_10_07_2018; ♂ same but GS 09_20_02_2023 (ISEA PAS); ♂ Liberia, Grand Gedeh County, Putu Range 19-31.XII.2010 leg.: Sáfián, Sz., Zakar, E.; GS 01_02_07_2018; DNA 192 (ISEA PAS); ♂ Liberia 230m, Lofa County, Zuwulor Village School 7°54’52”N, 9°31’08”W 8.xi.2017 Light Trap (blended bulb 250W) Aristophanous, M., Sáfián, Sz., Simonics, G., & Smith, L., leg. ANHRT: 2017.33; ANHRT 00201628; GS 03_14_12_2021; DNA 159 (ANHRT); ♂ Westafrica, Liberia Nimba County, Nimba-Berge 4 km südostlich Yekepa 7°33’39.31”N, 8°30’23.67”W 622 m, Lichtfang 13. Juni 2013 leg. Michael Ochse; GS 04_12_02_2019; DNA 67 (coll. Ochse later ZSM); ♂ Malawi NE of Mulanie village Mulanje Mt. above 2000m arassland. June 2016 local collectors; GS 02_23_10_2019; DNA 146 (coll. R. Fiebig); ♂ same but GS 04_26_04_2019; DNA 108 (ANHRT); ♂ H.S.Staude 10/12/82 Zomba Plateau Malawi; GS 05_12_01_2022; DNA 129 (ISEA PAS); ♂ Tananta Magarotto Hill Forest Edge 14.viii.1994 Leg. Frontier Coll. ZMUC, GS 01_23_07_2018 (ZMUC); ♂ Mozambique 22m Maputo Special Reserve West Gate (San Thicket) 26°30’14.2”S 32°42’59.6”E 9-17.ii.2018 Actinic Light Trap Laszlo, G., Mulvaney, J., Smith, L. Leg. ANHTRT: 2018.2; ANHRTUK 00209529; GS 01_15_12_2021; DNA 163 (ANHRT); ♂ Mozambique, Sofala Prov. Gorongosa N.P. Chitengo Camp., -XE40.1897934047; 34.35213983 28.03-15.04.2016, Marek Bąkowski leg.; GS 01.27.07.2018; DNA 25 (ISEA PAS); ♂ Namibia, Khomas Region 22°32’51,3”S

17º16’39,2”E Trans Kalahari Inn at light 14.12.2014 1918 m, 22 km E of Winthoek leg. R. Dobosz & D. Chłond; 5959/74398 coll. Upper Silesian Museum (USMB) Bytom, Poland; GS 04_02_03_2023; DNA 152; ♂ same but 5959/77257, GS 07_06_03_2023; DNA 151 (USMB); ♂ Namibia; Erongo Mts., N of Usakos 21º47’17”S 15º37’38”E 14.01.2016 Ameib Range Lodge, 1045m leg. Ł. Przybyłowicz al light; NA D5; GS UG7 22-04 2016; DNA 18; ♀ Namibia; E of Mariental 24o20’20”S 18o24’12”E Kalahari Farmhouse 07.01.2016 1170m, at light leg. Ł. Przybyłowicz; NA B10; GS 02_10_07_2018; DNA 30; ♂ Namibia; 22 km E of Windhoek 22º32’51”S 17º16’39”E 1900m Trans Kalahari inn 4.01.2016, leg. Ł. Przybyłowicz at light; DNA 23; ♂ same but DNA 24; ♂ same but GS 04_16_07_2018; DNA 21; ♂ same but DNA 22; ♂ same but GS UG 6. 22-04 2016; ♂ Namibia; W of Rehoboth 23º19’25”S 17º00’58”E Lake Oanob Resort Camp 5.01.2016, leg. Ł. Przybyłowicz; GS 02_12_07_2018; DNA 20 (ISEA PAS); ♂ Namibia, Waterberg 22.11.1993 Touritericamp leg. Mey & Ebert; GS 01_12_07_2018; ♂ Namibia, 8.XI.1999 Popa Falls, LF leg. W.Mey; GS 05_16_07_2018 (DEIB); ♂ Namibia Otjozondjupa Region Paradise Rest Camp, 800 m S 19.087504/ E 18.600036 4.12.2016, leg. H. Sulak, A. Prozorov & R. Yakovlev Museum Witt; GS 01_09.05_2019; DNA 114; ♂ Nord- Namibia Westl. Caprivi Umg. Bagani Mahango-Wildreservat 1000-1100m 28.-29.1.1998 leg. De Freina; GS 01_22_05_2019; ♂ Nordost-Namibia Westl. Caprivi Okavango – Ufer 30km SE, der Popa-Wasserfalle 1050m 27.-29.1.1998 leg. De Freina; GS 04_24_05_2019 (MWM); ♂ Nigeria Bendel State Okomu Forest Res. 20.05.1984. leg. J. Wojtusiak; GS 02_13_01_2022; ♂ same but GS S100; ♂ Nigeria Anambra State Nsukka F. Res. 28.09.1982 leg. J. Wojtusiak; GS 03_21_02_2023 (ISEA PAS); ♂ Nigeria, leg. J. Birket-Smith; 5.11.60 1m H9; ♂ same (ZMUC); ♂ RSA, Eastern Cape Asante Sana, light- trap, 11.XI.2012 leg. W. Mey; GS 02_29_01_2018; DNA 488; ♂ RSA, North Cape N. Hanover, Dwaal-fonterin, 21.1.2012 leg. W. Mey, LF; GS 01_2018_29_01; DNA 480; ♂ RSA, West Cape Villiersdorp, Wolf-Kloof, 26.2.2014 leg. W. Mey; GS 01_02_03_2023; DNA 462; ♂ RSA, Cape Prov. De Hoop N.R. 16.-18.11.1993 leg. Mey & Ebert; GS 03_12_07_2018 (DEIB); ♂ South Africa/Limpopo Pr Krüger NP./Punda Maria Camp 24.1.2015 460m leg. Th. Baron; GS 01_27_12_2019; DNA 150 (Coll. T. Baron); ♂ Südafrica Limpopo Pr. Nylstroem (Modimolle) Bela Bela ca. 6km 3.4.06 leg. Th. Baron; GS 02_27_12_2019; DNA 149; (coll. T. Baron); ♂ Südafrica Provinz Limpopo 8km S Louis Trichard Ben Lavin Nature Reserwe 1000m 21.-28.12.2008 leg. Et coll. De Freina Museum Witt, München; GS 05_24_05_2019; DNA 128; ♂ South Africa Kwazulu-Natal, Ramsgate Butterfly Sanctuary 30º53S, 30º20E, 45 m, 12-14.IV.2007 leg. Gurkovich & Zolotuhin Museum Witt; GS 01_24_05_2019; DNA 124 (MWM); ♂ Republic South Africa

Provinc Limpopo Waterberg mountain Valwater 29 km NW Moonriver Bush Bungalow S 24º13’ / E 28º23’ 1520m ü.NN 12.-15.11.2015 LF leg. Fiebig, Schellhorn & Stadie; BC RSA 1417; GS 03_23_10_2019; DNA 142 (Coll. R. Fiebig); ♂ RSA Prov. Limpopo Waterberg mts. Valwater 29 km NW Moonr. Bush Bung S24º13’/E28º23’ 1520m 12.15.11.2015LF leg. Fiebig Schellhorn & Stadie; GS 01_17_10_2019; DNA 135 (Coll. D. Stadie); ♂ South Africa 660m Kwazulu Natal, Pietermaritzburg, Cumberland Nature Reserve 28o30’50”S, 30o30’17”E 15-16.ii.2018 R.V. Yakovlev & V. Kovtunovich, leg.; ANHRTUK 00135084; African Natular History Research Trust ANHRT:2018.34; GS 06_21_12_2021; DNA 186 (ANHRT); ♂ Rep Of South Africa, Ukhahlamba-Drakensberg Park Catedral Peak – Main Gate 28º56’S 29º14’E 1360 m at light 1.12.2004 leg. Ł. Przybyłowicz; GS 02_18_07_2018; DNA 19 (ISEA PAS); ♂ E. Blaauw Pretoria can. 1900; GS 01_18_07_2018 (RNHM); ♂ SW Afr. Kaokoveld 5 miles SE Kowares 90 miles SE Ohopoho. 2.VI.51. No. 322; Swedish South Africa Expedition 1950-1951 Brinck-Rudebeck, At light in the evening; MZLU 2020 010; MZLU-LEP 00008919; GS 02_04_03_2020; DNA 153; ♂ same but MZLU 2020 011; MZLU-LEP 00008920; GS 02_12_01_2022; DNA 154; ♂ same but MZLU 2020 014; MZLU-LEP 00008923; GS 03_04_03_2020; DNA 157 (MZLU); ♂ S. Africa 1952; GS 04_12_07_201 (ZMUC); ♂ Nelspruit 11.1917 H. G. Breijer; GS 03_29_06_2018 (ZSM); ♂ Tanzania, 1520 m W Usambara Mts. Mazumbai U.F.S., 1985.01.24. Nr. 29. Leg. L. Peregovits; Usambaras Rain Forest Res. Project; GS 07_24_07_2018; ♂ same but 1985. 02.01. Nr. 48, GS 03_23_07_2018 (ISEA PAS); ♂ Bagamoyo VI.92; GS 03_20_07_2018; ♂ 28.VI.1988 Tanzania, Mt. Meru untare Primarwaldzone nahe Mgaresero Mt. Lodge leg. E.M.u.M. Lodl; Tanzania Expedition 1988 E.M.u.M. Lodge M. u. E. Arenberger; GS 01_13_01_2022; ♂ Tanganyika Terr. Naitivi b. Lindi II.-VII.’29, F. Zimmer; GS 02_23_07_2018 (NHMW); ♂ Tanzanie: Morogoro Region, Mikesse Hills, 378 m, 06º40.478’ S 037º58.315; E., 15-IV-2006 (Ph. Darge); GS 03_17_02_2023; DNA 127; ex coll. Darge ZSM (ZSM); ♂ Tanzania, Arusha suburb light trap, 160W, No.159 MV lamp, Hung. Sci. Africa Exp. “Teleki” 13. Feb. 1988 leg. A. Vojnits; GS 04_06_03_2023 (ISEA PAS); ♂ Tanganyika B.E. Africa Arusha, 1960.III.3 Dr Szunyoghy; GS 06_21_02_2023; ♂ Africa, Tanzania Lake Manyara 3150 ft. 27.V.1965 leg. Dr. J. Szunyoghy, GS 08_06_03_2023; ♂ Africa, Tanzania USA River 3900 Ft 1965 leg. D. Szunyogny J., Lighttrap; GS 05_06_03_2023; ♂ same but GS 01_12_01_2022; ♂ Africa, Tanzania USA River 3900 ft. IX-II.1965-66 leg. Dr. J. Szunyoghy; GS 04_03_03_2023; ♂ same but GS 06_02_03_2023; ♂ Africa, Tanzania Lake Serei 3150 ft. 6.IX.1965 leg: Dr. J. Szunyoghy; GS 05_21_02_2023; ♂ same but GS 06_10_07_2018; ♂ same but 14.VIII.1965, GS 03_12_01_2022; ♂ same but

17.VIII.1965, GS 03_16_07_2018; ♂ same but 20.VIII.1965, GS 01_21_02_2023; ♂ same but 24.VIII.1956, GS 01_16_07_2018; ♂ same but 26.IX.1965, GS 02_05_07_2018; ♂ same but 26.VIII.1956, GS 02_16_07_2018; DNA 133 (ISEA PAS); ♂ Uganda, Kampala 15.02.1976 Leg. K. Strzałka; GS 02_20_02_23; DNA 132 (ISEA PAS); ♂ Zambia 1205m, Zambezi Rapids (Miombo/Riverine forest mosaic) 11º7'30"S, 24º11'6"E, 4-9.xi.2018 LepiLED Light Trap, Aristophanus, M., Derozier, V., Laszlo, G., Oram, D., leg., ANHRT:2018.40; ANHRTUK 00073837; GS 07_21_02_2023; DNA 209; ♂ same but ANHRTUK 00067858; GS 04_17_02_2023; DNA 189; ♂ Zambia 1280m, Kalungu, north of Isoka S9º40'52", E32º42'50" 5-8.iii.2017 Light Trap MV leg. Oram, D., Miles, W., Smith, L. ANHRT:2017.24; ANHRTUK 00160179; GS 06_17_02_2023; DNA 200; ♂ Zambia 1300m, Nyangombe Falla (Miombo/Riverine forest mosaic) 11º48'25"S, 24º32'12"E, 17-23.xi.2019 Actinic Light Trap, Bashford, M., Miles, W., Mulvaney, L., Smith, R., leg., ANHRT:2019.25; ANHRTUK 00111499; GS 08_21_02_2023; DNA 204; ♂ same but ANHRTUK 00141761; GS 05_17_02_2023 ; DNA 199; ♂ Zambia 1340m, Jiwundu Swamp (Miombo/Riverine forest mosaic) 11º51'54"S, 25º33'20"E, 29.x.-4.xi.2018 LepiLE, Aristophanus, M., Derozier, V., Laszlo, G., Oram, D., leg., ANHRT:2018.40; ANHRTUK 00073851; GS 07_15_12_2021; DNA 193; ♂ same but ANHRTUK 00073850; GS 03_07_12_2021; DNA 208; ♂ Zambia 1400m, Hillwood, Ikelenge (Miombo/Riverine forest mosaic) 11º16'02"S, 24º18'59"E, 23-30.xi.2019 Actinic Light Trap, Bashford, M., Miles, W., Mulvaney, L., Smith, R., leg., ANHRT:2019.25; ANHRTUK 00122750; GS 07_20_02_2023; DNA 194; ♂ same but ANHRTUK 00122359; GS 06_15_12_2021; DNA 195; ♂ Zambia 1684m, Danger Hill, 30km North of Mpika, Muchinga Province 11º37'38"S, 31º33'56"E, 27-30.iv.2019 Actinic Light Trap Aristophanus, M., Derozier, V., Laszlo, G., Miles, W. leg., ANHRT:2019.12; ANHRTUK 00094811; GS 04_21_12_2021; DNA 202 (ANHRT); ♂ Zambia Northern Zambia, Mutinondo, 1390m, Wet Miombo 26.xii.2010 S12º23'30.9" E31º19'23.8" light trap, J Lenz legit; BC RSA 2078; Genitalpraparat Heterocera Nr. 30.986 Museum Witt München; DNA 145 (MWM); ♂ Zimbabwe Mashonaland E, Goromonzi, 1588m Miombo, 26.ii.2011, S 17º53'40" E 31º19'13" light trap J.Lenz legit; BC Eth 1261; Genitalpraparat Nr. 121.2016 coll. R. Fiebig; DNA 148 (coll. R. Fiebig); ♂ Zimbabwe, Masvingo Kyle Nat. Park 1.-4.12.1993, leg. Mey & Ebert; GS 07_12_01_2022 (DEIB); ♂ Musee du Congo Lulua: Kapanga XI-1933 Overlaet; GS 04_21_02_2023 (RMCA); ♂ ? Kivi, 11.XI.51 ex larva; GS 03_05_07_2018 (RBINS); ♂ localitas ? dubiosa; vide No. 863-029.1955.; GS 02_03_03_2023 (RMCA); ♂ Museum Leiden verzameling M. Führbringer; GS

03_03_03_2023 (RMNH); ♂ Coll A. Mocho 6.IV.16 Ghinda; GS 01_06_03_2023 (ISEA PAS).

Comments. This taxon is certainly a conglomerate of more than a single species. However, the present study based on an integrative methodology does not provide a clear systematic picture of this assemblage of forms distributed throughout sub-Saharan Africa. As with many studies of this type, the lack of available material, particularly fresh specimens suitable for the genetic part of the project, appeared to be the main factor. Despite the examination of 139 specimens, the dissection of 110 males and 1 female and the extraction of DNA from 58 samples representing specimens collected in 19 countries, the results do not allow for a clear taxonomic elaboration of the assemblage of specimens characterised by the process of valva longer than the length of valva. Conversely, the study revealed unexpected genetic and morphological diversity within the group.

The lack of clear diagnostic characters resulted in no formal proposal of the new species to be described. As a result, all specimens examined that could be unambiguously assigned to another species are listed as members of *A. canescens* in the Material examined section.

The morphological examination of the type specimen (habitus and male genitalia) and its comparison with morphotypes for which the molecular data are available indicate that the type is best placed among the specimens of clade I/3. The male genitalia of all four specimens in this clade are almost identical to those of the lectotype. The specimens are also characterised by the size comparable to the type, in contrary to the representatives of the clades II/10-11 which are much smaller on average. In addition, the hairy scales covering the thorax are slightly darker than the background of the wings in members of clade I/3, whereas they are less of the same colour in members of clade II/5..

Genetic information. *Apisa canescens* is a highly polymorphic taxon in the present interpretation.. On the tree (Figs 1-2), the specimens morphologically assigned to this species form six, rather unrelated clades (I/2, I/3, II/5, II/10-11, II/16, II/17).

Between two samples in clade I/2 the value of p-distance is 0.5%. Between this clade and representatives of clade I/1 (*A. cinereocostata*) the smallest distance is 2.5% with sample *cinereocostata* 49 and the largest is 4.2% with *cinereocostata* 34 and *cinereocostata* 9. Within the clade I/3, which includes specimens morphologically closest to the lectotype of *A. canescens* the distances between individuals vary between 0.6-1.3%. In the separate clade II/5 sister to II/6 representing *A. asipa* the distance ranges from 2.5-3.3%. Similarly the single sample forming clade II/16 differs from clade II/15 *atrovenosa* by 3.7-4.7 %.

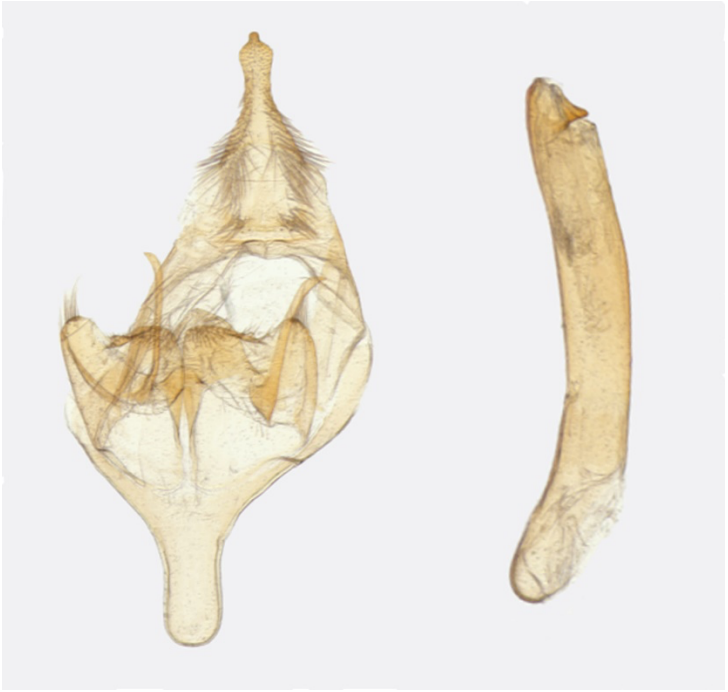

Figure 5. *Apisa canescens. Lectotype, male genitalia.*

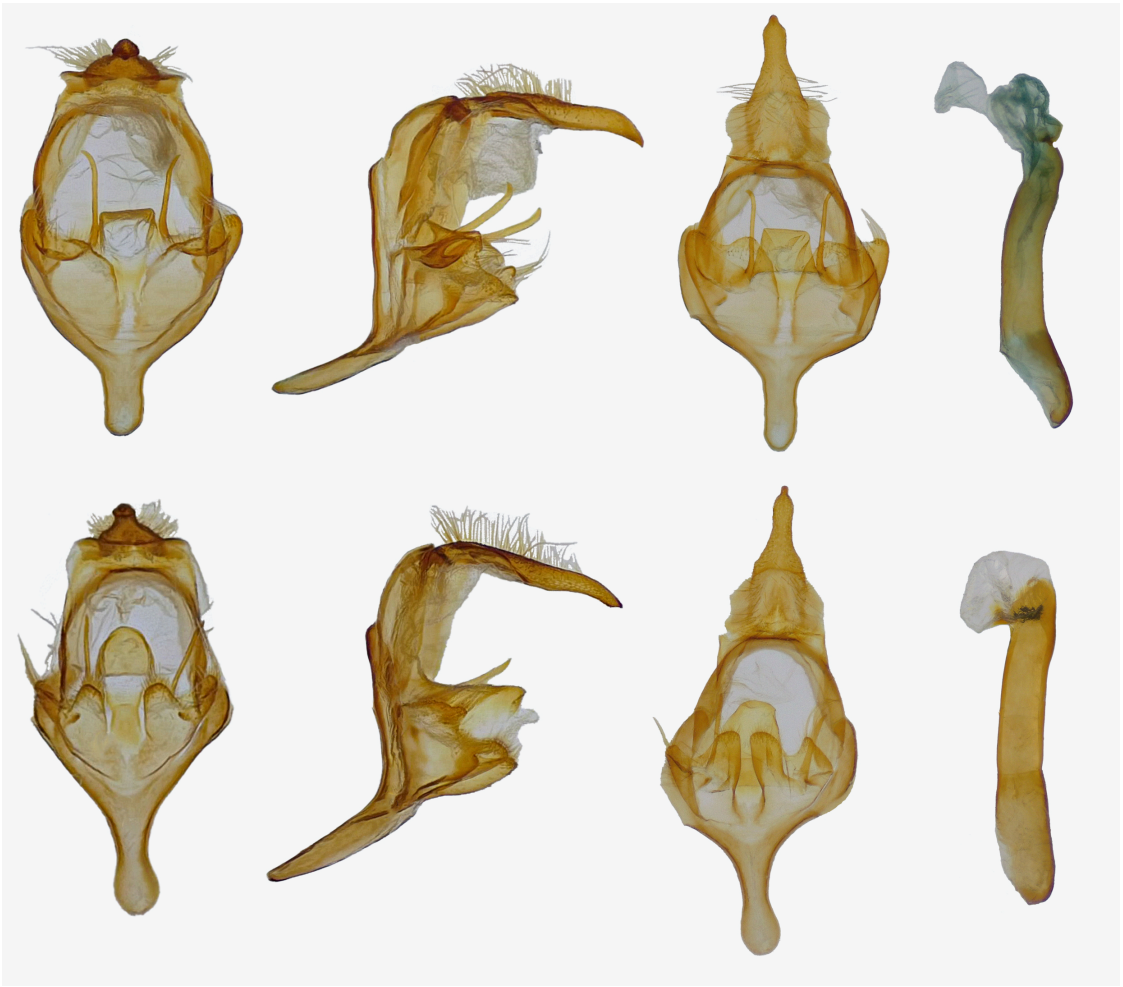

Figure 6. *Apisa canescens, variability in morphology of male genitalia.*

Figure 7. *Apisa canescens, imago upper side, under side, the labels.*

Apisa cinereocostata Holland, 1893:

Comments. Taxon redescribed and elaborated in details in the paper by Paśnik, Przybyłowicz, Tarcz (2023).

Apisa diversa **sp. nov.**

Material examined:

Holotype: ♂ DRC Congo. Orientale: Yangambi 450 m., 14.-23.v.2012, Knud Larsen, GS 06_24_07_2018, DNA 2 (ISEA PAS)

Paratypes: (3♂♂) ♂ DRC Congo. Orientale: Yangambi 450 m., 14.-23.v.2012, Knud Larsen, GS 03_19_03_2019, DNA 98; ♂ same but GS 02_20_03_2019, DNA 96 (KL); ♂ DR Congo, Province Orientale, T shopo, Yangambi Biosphere Reserve, 460 m, 0º45'N 24º20'E, 20.v.2012, leg. J & W. De Prins, GS 03_27_02_2023, DNA 14 (RMCA)

Additional material: (6♂♂). Specimens studied molecularly:

♂ Gabun Provinz Ogooué-Ivindo, 7km SW Makokou, Ivindo Nationalpark, Ipassa, Forschungslager, 519m, 0º30'43,973"N 12º48'13,253"O, 20.11.2017, Lichtfang, leg. Michael Ochse, GS 08_12_02_2019, DNA 72; same but ♂ 18.11.2017, GS 02_10_06_2019, DNA 75 (coll. Ochse later ZSM)

Specimens studied only morphologically:

♂ DRC/Congo, Salonga National Park, S002°45’22.79”, E020°18’55.56”, Ekongo Camp January 2017 VD Kravchenko & GC Muller, GS 01_25_10_2019, DNA 143 (coll. R. Fiebig); ♂ Coll. Mus. Congo, Uele: Pailis, 27.II.1957, Dr M. Fontaine, GS 1_05_04_2019 (RMCA); ♂ Gabun Provinz Ogooué-Ivindo, 7km SW Makokou, Ivindo Nationalpark, 600m W Ipassa Forschungslager, 549m, 0°30’30,480”N 12°47’53,220”O 21.11.2017, Lichtfang leg. Michael Ochse, GS 01_10_06_2019, DNA 88 (coll. Ochse later ZSM); ♂ Gabun Parc National Ivindo, Station de recherché de l’passa, 7,5 km SW Makokou. ca. 520 m, Villa No 5, 0°30”41.9”N 12°48’10.9”E, 27.VI.2016 LF 250 W ML, Klaus-Rüdiger BECK light, GS 02_20_07_2018, DNA ISEZ 443 (ISEA PAS).

Diagnosis. The new taxon differs from all other members of *Apisa* by combination of three characters which are present in all examined specimens but altogether are not present in any other taxon. There are: a) elongate forewing with sharp angle of apex, b) creamy-white patagia and tegulae, c) creamy-white hindwing contrasting with distinctly darker, grayish forewing. In other species patagia and tegulae are concolorous with forewing background or are darker, hindwing are also of the same tone as forewing. *Apisa diversa* is the only *Apisa* with so sharp apex which is gently rounded in the other species. Male genitalia differs from most of other species by single, sharply terminated termination of uncus and lack of cornuti. Combination of these two characters is present also in *A. grisescens, A. atrovenosa, A. fontainei* and *A. rendalli*. From this four, the new species differs by its entirely atrophied or rudimentary process of valva, which in the above species is shorter than the length of valva but always well developed and at least longer then its width.

Description.

Head. Frons and vertex creamy-white; labial palpus distinctly darker, ochraceous, three segmented, densely covered with narrow scales; antenna bipectinate, flagellum pale ochraceous; eye convex, indistinctly ovoid, naked; proboscis absent.

Thorax. Uniformly creamy-white both dorsally and ventrally including patagia and tegule, concolorous to the second pair of wings; in some specimens with admixture of indistinctly darker scales along dorso-median line.

Abdomen. Upperside and underside uniformly concolorous with thorax, covered with elongate, hair-like scales.

Wings. Forewing length 9-13 mm (n = 10♂); apex acute not widely rounded; background pale ochraceous, noticeably darker than the second pair of wings; wings covered with matte, elongate scales, only slightly semitransparent; underside paler then upper side slightly shiny; hindwing uniformly creamy white.

Male genitalia. Tegumen narrow, rounded, lateral arms well developed; uncus at the tegumen slightly tapered, sclerotized, relatively long, apically pointed and gently bent ventrally; vinculum, narrow, distinctly elongate and overlapping the terminal portion of tegumen arms; valva, shortened, subquadrate with a delicate U-shaped concavity along terminal margin; irregular, elongate, stiff setae from the outer subterminal portion of valva; ventral process of valva rudimentary or completely atrophied; saccus elongate, narrow, rounded at tip, approximately the length of valva; phallus strait, short and slim with sublateral opening of vesical; vesical pouch like, without cornuti.

Female genitalia. Unknown.

Etymology. The species name *diversa* refers to the variation observed in male genitalia.

Variation. Visible in the structure of the genital apparatus of males.

Remarks. Some of the genital apparatuses well sclerotized and some membranous with less clearly visible features.

Distribution: DRC, Gabon.

Comments. Some of the specimens examined are not included in the type series. Despite the fact that no morphological differences can be seen, the two specimens from which the barcode region have been obtained formed a separate clade on the tree. We have therefore, decided to consider these specimens as additional material until the more detailed studies can be carried out using more sophisticated methods and based on a larger set of specimens.

Genetic information. Within the clade containing the holotype of *A. diversa* **sp. nov.**, pairwise distance values range from 0.0-0.5% and between other specimens of this species, differences range from 0.2 up to 6.2%. The entire clade II/4 in which the holotype occur, is a sister group to clades II/5-II/16. Despite the fact that the species are morphologically uniform, the genetics of the species we include in the *diversa* **sp. nov.** show that there is little relationship between them. P-distances and species delimitation analysis show this. Between clades II/12 and II/13 (*A. diversa* **sp. nov.**) and the species *A. grisescens,* which is the nearest neighbour (II/14), the values vary between 4.9-5%.

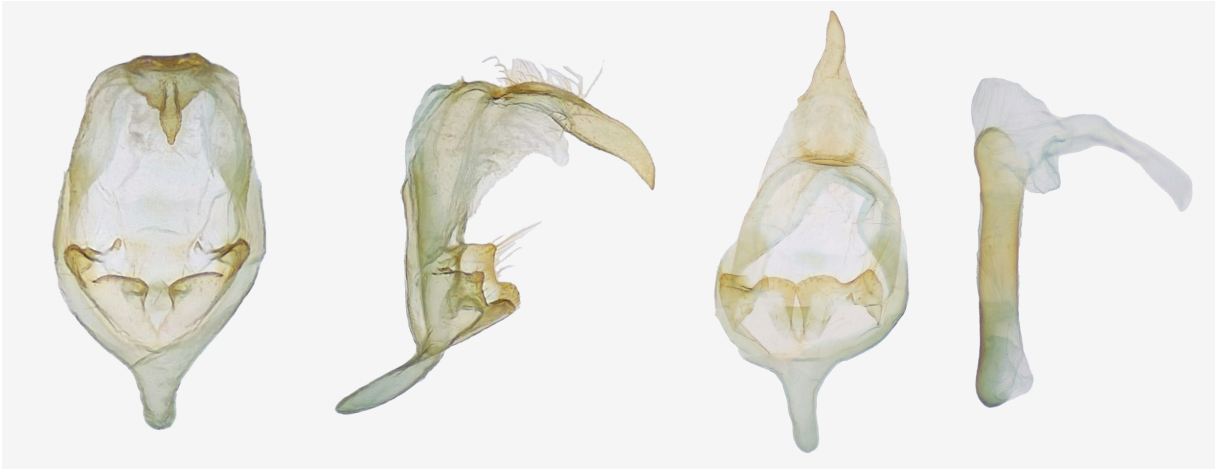

Figure 8. *Apisa diversa* ***sp. nov****. Holotype. Male genitalia, phallus with everted vesica.*

Figure 9. *Apisa diversa* ***sp. nov****. variation of the male genital apparatus with a visible appendage on the valva and reduced to the form of a secleritised spot. The blue circle indicates the variability of the character.*

Figure 10. *Apisa diversa* ***sp. nov****., imago, upper side, under side, the labels.*

Apisa fontainei Kiriakoff, 1959

Material examined.

Holotype: ♂ Coll. Mus. Congo Ruanda: Kisenyi 26-IV-1957 Dr M. Fontaine; GS nr P77 (RMCA).

Additional material: (6 ♂♂): ♂ Bukava (Kivu) 15.XII.51 ex larva ?, GS 03_06_03_2023 (RMCA); ♂ Coll. Mus. Congo Kibali-Ituri: Nioka 3-VI-1953 J. Hecq, GS 04_05_07_2018 (RMCA); ♂ Kenya, Western Prov. Kakamega Forest N.R. sec. forest 1600m 19.ii.2002 Lichtfalle (2) 0.21,1N; 34.51E leg. F.N. Namu, GS 01_29_06_2018, DNA 12; same but ♂ GS 02_02_03_2023, DNA 10 (SMNS); ♂ Ruanda: Kisenyi 26-IV-1957 Dr M. Fontaine; Apisa3 Rozt., GS 01_20_02_2023 (RMCA); ♂ Urundi: Kitega 21-XII-1968? Dr M. Fontaine, GS 02_06_03_2023 (RMCA).

Diagnosis. The species cannot be separated from other *Apisa* based on the external characteristic. The only reliable characters can be found in male genitalia. The most similar species in this respect is *A. rendalli*. Both species differ in the shape and size of the process of ventral zone of valva. In *A. fontainei* it is spatulate and at least three times longer than wide at the base while in *A. rendalli* it is subtriangular, at most three times longer than wide at the base. Additionally, the phallus of *A. fontainei* is on average no less than four times longer than wide while it is on average no more than four times longer than wide in *A. rendalli*. This pair of species is also very different in general coloration. *A. fontainei* is pale ochraceous with distinctively darker costa, while *A. rendalli* is uniformly dark ochraceous (the darkest of all *Apisa* species). This distinction can be treated as the diagnostic character only for this pair of species after previous preliminary determination based on the male genitalia because other forms also express colouristic similarity to *A. fontainei* and *A. rendalli*.

Distribution. DRC, Kenya, Ruanda, Burundi.

Comments. *Apisa fontainei* appears to be another East African species restricted to the region East of the Congo Basin but not reaching regions South of the East African highlands.

Genetic information. Despite the material collected for genetic studies, no satisfactory results could be achieved. Species not included in the molecular analyses.

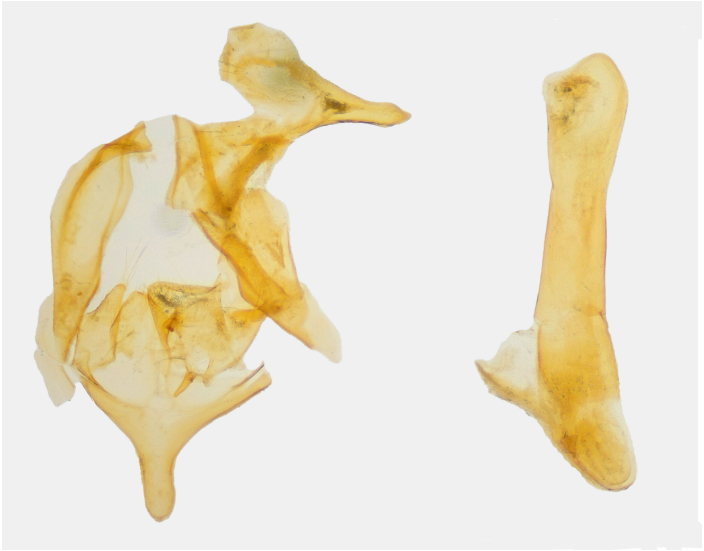

Figure 11. *Apisa fontainei, holotype, male genitalia.*

Figure 12. *Apisa fontainei, male genitalia.*

Figure 13. *Apisa fontainei, imago, upper side, under side, the labels.*

Apisa grisescens Dufrane, 1945

Material examined:

Holotype: ♂ Kamituga (Kigu)1-1-40 Coll. Dufrane; GS 1 23-12, (KBIN).

Additional material: (5 ♂♂):

♂ Kamituga 18.12.39 Dufrane. Coll. Mus Congo, *Metarctia grisescens*, paratype (RMCA); ♂ Zambia 1340m Kambishi, Jiwundu S 11º54'39", E 25º29'05" 18-19.x.14 Light Trap leg. Smith, Takano & Oram, ANHRTUK 00051006, GS 04_22_01_2019, DNA 38; ♂ Zambia 1400m Hillwood, Ikelenge S11º16'02" E24º18'59" 21-28.x.13 Light Trap, leg. Smith, R., Tanako, H., Chmurova, L. & Smith, L., ANHRT 00013, ANHRTUK 00051019, GS 04_14_01_2019, DNA 60; ♂ Zambia 1300m Nyangombe Falls (Mimbo/Riverine forest mosaic) 11º48'25"S, 24º32'12"E; 17-23.xi.2019 Actinic Light Trap Bashford, M., Miles, W., Mulvaney., L. Smith, R. Leg. ANHRT 2019.25, ANHRTUK 00111498, GS 02_23_12_2021, DNA 197; ♂ same but ANHRT 2019.25, ANHRTUK 00124327, GS 03_23_12_2021, DNA 205; ♂ same but ANHRT 2019.25, ANHRTUK 00202045, GS 02_22_12_2021, DNA 201 (ANHRT).

Diagnosis. *Apisa grisescens* belongs to the group of species characterized by single (not forked) uncus and lack of cornuti in vesica. In this group it forms a pair of genitalicly similar taxa with *A. atrovenosa*. Both are distinguished by relatively short, broad phallus which terminate evenly onto wide opening of vesica. Within this pair *A. grisescens* is unique in the structure of uncus which is rather short and subtriangular, without narrowing before termination. Presence or absence of such narrowing is always easy to evaluate when genitalia are observed from the ventral direction.

Distribution. Zambia (DRC, Malawi, Tanzania by Przybyłowicz 2009).

Comments. The male genitalia of the holotype are a very good state of preservation, allowing the reliable comparison with other specimens included in the study. This comparison has allowed the known distribution of the species to be extended to another country, Zambia, although the known range of the species is still restricted to the semidry zone of East Africa, south from the equator.

Genetic information. Species and distance between nearest neighbours discussed at *A. diversa* **sp. nov.**

Figure 14. *Apisa grisescens, holotype, male genitalia.*

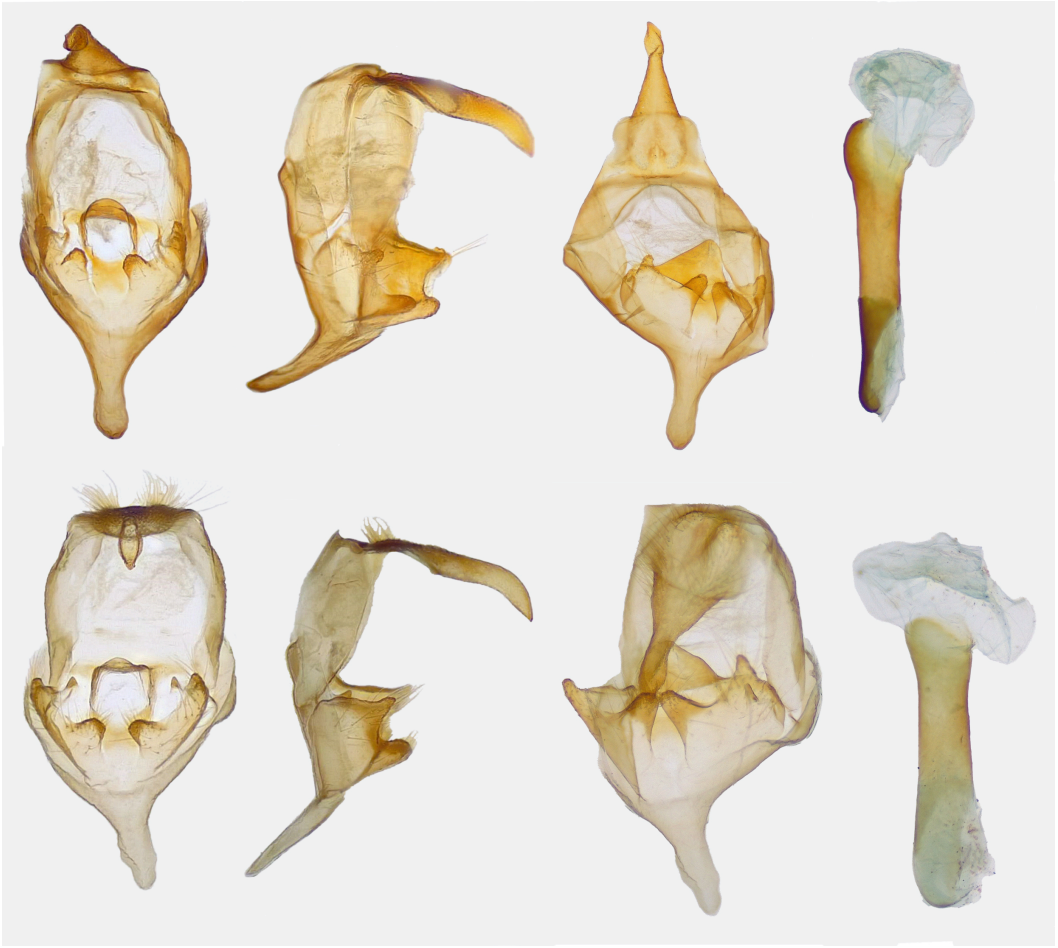

Figure 15. *Apisa grisescens, male genitalia.*

Figure 16. *Apisa grisescens, habitus, upper side, under side, the labels.*

Apisa hildae Kiriakoff, 1961

Material examined:

Holotype: ♂ S. W. Africa, Okahandja 1.IV.56 leg. F. Gaerdes Staatssamml. München; GS P76 (ZSM)

Diagnosis. *Apisa hildae* is very similar to *A. rendalli*, but differs by the paler colouration which is "honey" rather than dark ochraceous, and the presence of well developed thorn-like cornutus which is absent in the type and all other examined specimens of *A. rendalli*.

Distribution. Namibia.

Comments. A species is currently known from a single specimen. Apart from the differences mentioned above, *A. hildae* is very similar to *A. rendalli* and the additional specimens are needed to assess the stability of the diagnostic characters. It is well known that the tone of the background may be a subject to individual variation or may be depend on the habitat. Typically, populations from more open and dry areas tend to be paler than those from more wooded and humid areas. In this particular case, Namibia is generally semi-desert whereas Malawi and Mozambique are dominated by savannah and dry forest, and it can be hypothesised that these differences may be responsible for the paler colouration of the Namibian specimen. The presence of the prominent cornutus is much more stable character in *Apisa*, although some variation in its degree of development can be observed. In the view of the above, it can be concluded that the additional comparative material is needed to obtain more objective data on the taxonomic status of *A. hildae*.

Genetic information. Due to the lack of material, it was not possible to include this species for molecular studies.

Figure 17. *Apisa hildae, holotype, male genitalia.*

Figure 18. *Apisa hildae, imago, upper side, under side, the labels.*

Apisa manettii Turati, 1924: 46-49

Comments. No samples available for study. Despite repeated efforts, neither the type series nor the new materials could be examined. The types are housed in the Museum Turin (Italy). However, in the absence of Lepidoptera curator or someone who might be able to locate the specimens, the types have not been studied. The type series was collected in Cyrenaica (north Libya). Over the years, various attempts have been made to obtain new specimens or even to contact a collector in Libya who might be able to collect in Cyrenaica, but without success. Therefore, the taxon remains unrevised since its description.

Genetic information. As the species is not amenable to any analysis, it has not been included in the analyses.

Apisa rendalli Rothschild, 1910

Material examined: (20 ♂♂): ♂ Malawi Ruo Gorge, Ruo Valley 09.II.2004. Leg. L. Arvik coll. M. Fibiger, GS UG 9. 22-04-2016, DNA 16 (ISEA PAS); ♂ Eritrea Asmara 9-IX-1968 L. Barbera; Mus Genova ex coll. L. Barbera; GS 04_11_01_2022; ♂ same but 12-IX-1968; GS 03_11_01_2022; ♂ same but 18-IX-1968; GS 01_05_12_2018; ♂ same but GS 02_05_12_2018 (MCSG); ♂ Kenya centr. Mount Kenya Castle Forest Lodge S0º22'47 E37º18'35 29./31.03.2019LF 1900-2100m leg. Stadie, Fiebig & Schellh (coll. D. Stadie).; BC KEN 138; GS 02_17_02_2023; ♂ Mozambique 630m, Manica Province, Chimanimani, National Reserve, Moribane Forest. Ndzou Camp (Moist Forest) 19º44'01.4"S, 33º20'15.1"E, 3-5.viii.2018 MV Light Trap; Laszlo, G., Miles, W., Vetina, A leg. ANHRT:2018.30, GS 01_14_01_2019, DNA 39, ANHRTUK 00046906; ♂ same but GS 03_16_02_2023, DNA 177, ANHRTUK 00096034; ♂ same but GS 01_16_02_2023, DNA 176, ANHRTUK 00047823; ♂ same but GS 01_17_02_23, DNA 175, ANHRTUK 00047824; ♂ same but GS 02_07_12_2021, DNA 172, ANHRTUK 00094421; ♂ same but GS 02_16_02_2023, DNA 173, ANHRT 00094422; ♂ same but GS 04_14_12_2021, DNA 174, ANHRTUK 00094478 (ANHRT); ♂ Mozambique, Sofala Prov. Coutada 12, Nyago hunting camp (18º39.642S; 035º27.334E 09-16.04.2016 Marek Bąkowski leg, GS 4_2016_10_03, DNA 310 (ISEA PAS); ♂ Tanganyika – Terr., Matengo Hochland, wsw. Von Songea, 21.-31.III.'36. Zerny; Mbinga 13-1400 m, GS 04_12_01_2022 (NHMW); ♂ Tanzania Muheza Distr.: Amani 900-950m 11.XII.1992 leg. L. Aarvik, GS 09_06_03_2023 (NHMO); ♂ Tanzania Somanga (SM) 25.vii.1992 Frontier leg. Coll., GS 10.VI 2; ♂ Tanzania 37*48'E, 7*01'S, Yuvu River Kimboza Forest Res. 9.ii.1994 leg. Frontier, GS 03_13_01_2022 (ZMUC); ♂ Tanzanie: Mts Uluguru Kimboza for. Heliophile alt. 600m 24-30/VII/71, Coll. Mus. Tervuren, Mission Mts. Uluguru, L.Berger, N. Leleup, J. Debecker V/VIII/71, GS Doct 056 (RMCA); ♂ Tanzania: Morongo Region: Kilombero District: Udzungwa Mts Nat. Park, Mang'ula 4-6.xii.2006, 550 m S07º50.705' E036º52.643' M. Fibiger & L. Arvik, GS 01_20_07_2018, DNA 15.

Diagnosis. Species differing from all other taxa except *A. hildae* by the elongate, hairy scales covering entire wings. Such protruding scales are especially densely packed in the costal area of wing making it thick, and dowdy. In other species even if the hair-like scales are extensively spread on the wing membrane there is always an admixture (especially in the costal area) of typically developed, flattened, adjacent scales. *Apisa rendalli* differs also from *A. hildae* (known only from the type) by dark ochraceous coloration, which is honey in another species. In male genitalia *A. rendali* lacks cornutus of vesica while in *A. hildae* it is well developed. Some specimens of *A. canescens* from East Africa (like Erythrea, Kenya) are

very dark and similar to *A. rendalli*. However, their genitalia are typical for *A. canescens* and the scales of the basalo-costal portion of a wing are not very long and hairy but although elongate, they are always flattened and rather adhering to the surface of the wing.

Distribution. Malawi, Mozambique, Tanzania (DRC by Przybyłowicz 2009).

Comments. *Apisa rendalli* remained one of the least known taxa of *Apisa*. Closer examination of the additional material revealed a very important and unique structure of the wing scales, which are hair-like in this species. This character allowed the superficial separation of *A. rendali* from other dark *Apisa* species. As a result, the species was recorded for the first time from two additional countries, Tanzania and Mozambique. The new localities do not significantly extend the known range of the species, but rather confirm its restriction to the East Africa, south of the equator. In this distribution pattern, *A. rendalli* resembles another rare species, *A. grisescens*. It is also interesting to note that neither of these two species extends further south than the Limpopo River valley. They are not known from the RSA, Botswana or Namibia, so they cannot be considered true South African elements.

Genetic information. Within the species (clades II/7-II/9), the distance varies from 4.4 to 5.7%. Clade II/7 shows the smallest distance with clade II/11 and it is 4.4%, the largest we can see between individual *canescens* 24 of clade II/10 and it is 5.9%.
Between clades II/10 and II/11 with individuals of the species *A. canescens* the smallest value is 3.5% between *rendalli* 16 and *canescens* 20 of clade II/11. The highest value is between *rendalli* 16 and *canescens* 24 and is 5.4%.

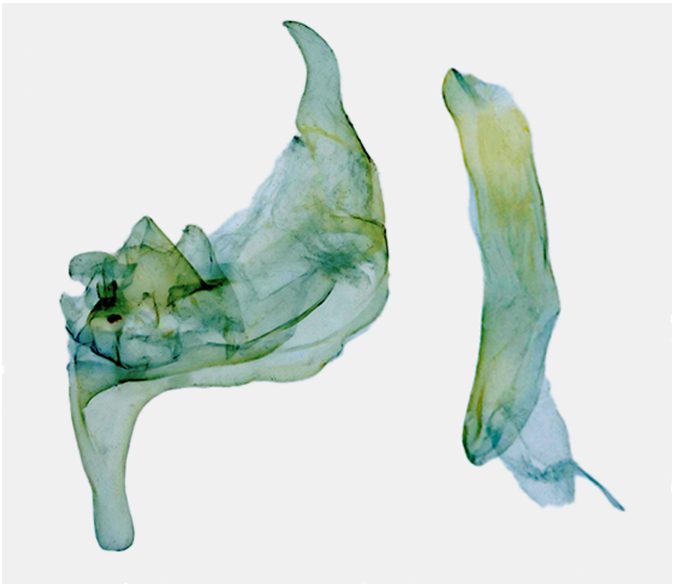

Figure 19. *Apisa rendalli, lectotype, male genitalia.*

Figure 20. *Apisa rendalli, male genitalia.*

Figure 21. *Apisa rendalii, imago, upper side, under side, the labels.*

Apisa subargentea Joicey & Talbot, 1921

Comments. Taxon redescribed and elaborated in details in the paper by Paśnik, Przybyłowicz, Tarcz (2023).

Apisa subcanescens Rotschild, 1910

Material examinated.

Lectotype: Senegal „Casamance, Senegambia, E. Laglaize" [BMNH].

Additional material: (5♂♂, 2♀♀)

♀ Ivory Coast 481m, Dolla Ranch (tree savannah), 07°58'7.7"N, 07°34'35.7"W, 27.v-5.vi.2018 Actinic Light Trap, Aristophanous, M., Miles, W., Moretto, P., Outtara, Y. leg., ANHRT:2018.28, ANHRTUK 00103588, GS 02_01_03_2023; ♀ Senegal 30m, Simenti, Niokolo-Koba NP, 13°1'33"N, 13°17'4"W, 3-16.vi.2019 MV Light Trap, Aristohpanous, M, Moretto, P., Mulvaney, L. leg., ANHRT:2019.14, ANHRTUK 00210006, GS 01_01_03_2023 (ANHRT); ♂ Ivory Coast 479m, Denguélé Classified Forest, 09°30'0.6"N, 07°40'51.1"W, 11-18.xi.2019 Black-Light Trap Aristophanous, M., Dérozier, V., Moretto, P., Ouattara, S. Leg. ANHRTUK 00223432, GS 03_20_02_2023, DNA 158; ♂ same but 6-14.vi.2018 Actinic Light Trap Aristophanous, M., Miles, W., Moretto, P., Outtara, Y. leg. ANHRT: 2018.28, ANHRTUK 00209129, GS 05_20_02_2023, DNA 168; ♂ Ivory Coast 481m, Dolla Ranch (tree savannah), 07°58'7"N, 07°34'35.7"W, 27.v-5.vi.2018 Actinic Light Trap, Aristophanous, M., Miles, W., Moretto, P., Outtara, Y. leg., ANHRT:2018.28, ANHRTUK 00103589, GS 06_20_02_2023, DNA 169; ♂ Senegal 200m, Dindefelo Camp, 12o22'43"N, 12o19'27"W, 27.v-2.vi.2019 Actinic Light, Aristophanous, M., Moretto, P. Mulvaney, L., leg. ANHRT:2019.14, ANHRTUK 00215863, GS 01_07_12_2021, DNA 165; ♂ same but ANHRTUK 00215862, GS 02_15_02_2023, DNA 190.

Diagnosis. The species cannot be separated from the remaining two *Apisa* species inhabiting Western Africa. It resembles *A. canescens* and *A. cinereocostata* by sharing with them pale ochraceous colouration of the entire body and somewhat darker costa of forewing. From both above taxa it can be easily separated based on male genitalia. In *A. cinereocostata* uncus is bifid, while in *A. canescens* and *A. subcanescens* it is terminated in a single, sharp tip. The diagnostic difference between these two refers to the length of ventral process of valva which is much shorter than the length of valva in *A. subcanescens* while much longer in *A. canescens*.

Redescription.

The redescription is based on the photographs of the lectotype and supplementary material. There is no doubt that the types and the material represent the same species due to the overall similarity in all morphological characters including the body colour and size but especially the structure of the genital apparatus. Female is here described for the first time.

Head. Frons and vertex pale ochraceous; antenna bipectinate, lengths up to 2/3 of the costa of the fore-wing, concolorous with head; labial palpus three segmented, straight, covered with darker ochraceous scales; proboscis absent; eye convex and large.

Thorax and abdomen. Covered with dense, long, straight hair-like scales, uniformly pale ochraceous from above, underpart slightly paler, the same tone as hindwing.

Wings. 11-16 mm (n= 7; 5♂, 2♀,); fore and hindwing semi-transparent, covered with short scales, forewing indistinctly darker than hindwing, but general colouration pale ochraceous; costa of the fore wing slightly darker.

Male genitalia. Tegumen of even width, lateral arms well developed; uncus prominent, elongate, of lanceolate shape, with parallel margins in the basal half, then widened and sharply narrowed into a pointed tip; vinculum sclerotized, overlapping the terminal portion of tegumen arms but separated from tegumen; valva, shortened, subquadrate with a broad U-shaped concavity along terminal margin; irregular, zones of short setae on terminal costal and saccular portion of valva; ventral process of valva shorten than valva but well develops, sharply pointed; saccus broad, at most two times as long as broad, rounded at tip; phallus slim, strait except delicate bent in proximal portion, terminal end with sublateral opening of vesica; vesica pouch like, with a single thorn-like cornutus at base.

Female genitalia. Papillae anales subquadrate, covered with sparse, protruding setae forming more dense zone at the basodorsal margin; posterior apophyses of similar length as papillae anales, about two times longer than anterior apophyses; pheromone glands in form of membraneous, elongate, irregular shape pouches, dorsal separate, ventral with single broad, shallow opening; ductus bursae membraneous, narrow in basal third then broadened from the opening of slender ductus seminalis; bursa copulatrix delicate, membranous, without distinct plicae; signum located in medial portion of bursa, longitudinal, granulate, sclerotized, in form of elongate, irregular plate about four times as long as broad; ostium small, rounded, with heavily sclerotized lateral walls.

Sexual dimorphism. There is no clear difference between females and males. Female is slightly larger than male and has shorter rami of antenna which are longer than in male.

Distribution. Ivory Coast, Senegal.

Comments. The female was collected on the same day and in the same location as one of the males, so both specimens are treated as representing the same species. It is known from the labels that the specimens were caught in the Sudanese forest and tree savannah. All specimens were attracted to the light trap and actinic light. No differences were observed between Senegalese and Ivory Coast individuals. The male genitalia of the lectotype corresponded very well to the genitalia of all 5 specimens examined, therefore the details no visible in illustration of the type are from these specimens.

Genetic information. No molecular data could be obtained.

Figure 22. *Apisa subcanescens, male genitalia.*

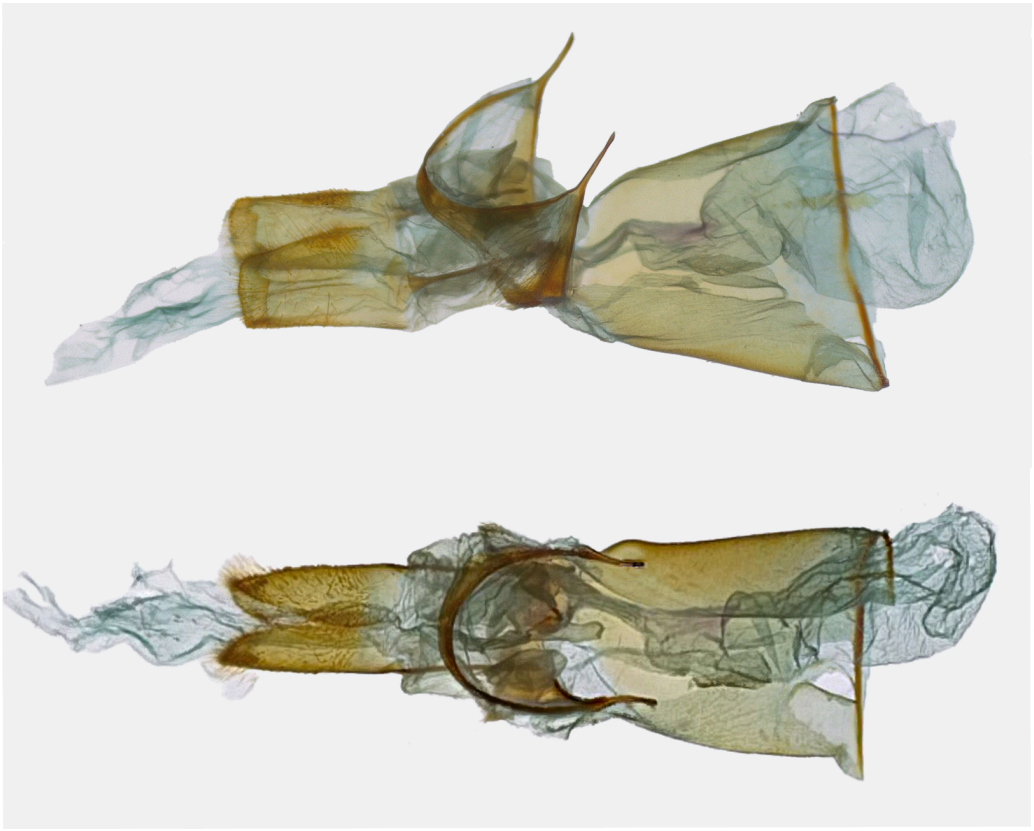

Figure 23. *Apisa subcanescens female genitalia.*

Figure 24. *Apisa subcanescens, imago, upper side, under side, the labels.*

DISCUSSION

Zoogeographical account.

The genus *Apisa* is distributed throughout sub-Saharan Africa, including the southern part of the Arabian Peninsula. In addition, a species (*A. manetti*) is described from the Mediterranean basin, extending the range of the genus to the Palaearctic. The specimens available for study come both from the interior and also from the extremities of the continent: Senegal and Gambia in the west, Somalia and Eritrea in the east, and RSA in the south. The number of the taxa, as well as the general variability of different forms, is unevenly distributed across this large range of *Apisa, with* species richness increasing significantly from the west to the east of the continent. The vast area of West Africa east to Nigeria is occupied by only three species: *A. cinereocostata*, *A. canescens* and *A. subcanescens*. All are indistinguishable externally, but are easy to separate by the morphology of the male genitalia. *A. cinereocostata* and *A. canescens* are widespread taxa, although the former appears to be absent from the eastern and southern parts of the continent. Only *A. subcanescens* is restricted to the westernmost fringes of Africa. Further east is another very remarkable species, *A. asipa*, recently described from the highlands of Cameroon and Nigeria. It shares a forked uncus with *A. cinereocostata* and *A. subargentea,* but otherwise does not appear to be closely related to either of these taxa. Furthermore, its scale morphology places it on a separate position within *Apisa*. Central, equatorial Africa is not particularly species-rich. The only endemic species is *A. diversa* **sp. nov.**, described here from Gabon and western DRC. In addition, *A. atrovenosa*, an East African element, is known from an isolated population in Gabon.

The eastern and southern parts of the continent are much more diverse in terms of the number of taxa. Besides the extremely variable *A. canescens* there are three other species known only from South Africa, namely *A. hildae*, *A. rendalli* and *A. grisescens*. Further north, *A. fontainei* and the strange, silvery opalescent *A. subargentea* are found in Kenya, Rwanda, Burundi and eastern DRC. They share a reduced valva process (except *A. hildae* known from the holotype only) and a vesica without of cornuti. On the contrary, they are very different in colouration from the deep dark ochraceous *A. rendalli* to the very pale *A. fontainei*. It is interesting to note that the greatest variability in colour and size of *A. canescens* is also observed in the dry habitats of eastern Africa from Ethiopia to Tanzania. This is in contrast to the rather uniform structure of the male genitalia and the narrow and elongated process of the

valva. Such a phenomenon may indicate the existence of undescribed, yet cryptic taxa or imply dynamic evolutionary processes within the widespread *A. canescens*. Finally, a separate taxon, *A. arabica*, closely related to *A. canescens,* is known to occur in semi-desert areas of the Arabian Peninsula. Given the above, East Africa could be the speculative centre of origin of the genus. Such a hypothesis is supported by the high number of species in the region and the high morphological diversity at both interspecific and intraspecific levels. Genetic data also support such a hypothesis, as a small clade consisting of two members of *A. arabica* is located in a basal position within the genus.

Since the DNA barcodes of the species show limited nucleotide variation, the separation must have been very recent. Rapid evolution by sexual selection is a possibility that deserves further investigation, including testis genomic data (Alipanah et al., 2022). It is known that genes can have their own independent history, and combining data is not always informative when it comes to relationships between species (Wahlberg et al., 2009). It is commonly assumed that well-described species are genetically isolated from each other, allowing an accurate and correct phylogeny to be constructed. However, it is becoming increasingly clear that good species can still exchange genetic material through hybridisation (introgression). Many studies have shown that related species do not introgress of their genes, but only some of them. The most commonly introgressed genes are maternally inherited chloroplast or mitochondrial DNA (Chan et al., 2005). In this case, some of the species may be sister taxa or members of a species complex in which evolutionary divergence is sometimes so recent that significant morphological differences have not had time to accumulate. Therefore, for very young species, morphological similarity in may not be unexpected and more divergence time may be required before differences between taxa can be visually observed (Struck et al., 2018).

Species delimitation has been identified as a method that does not always bring us closer to a solution and sometimes causes confusion on almost every aspect of the definition of the "species" level (Stanton et al., 2019). Despite all efforts, phylogenetic diversity has been shown not to reliably capture functional diversity (Mazel et al. 2018).

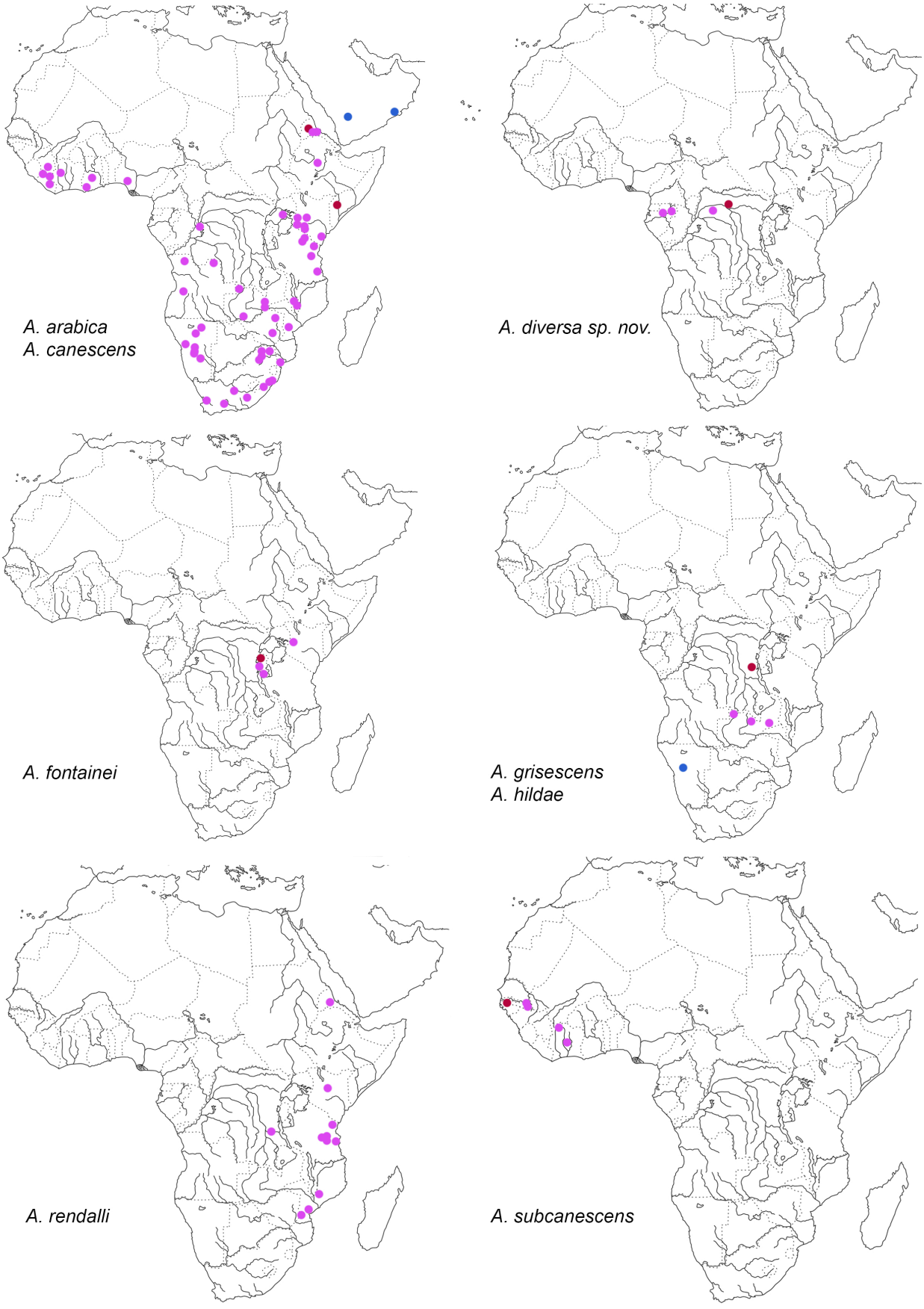

Figure 25. *Distribution of species of the genus Apisa. Dark pink color indicates type material, blue color indicates A. arabica and A. hildae.*

Integrative taxonomy in the context of uncovering *Apisa* systematics

The present study clearly shows that an integrative taxonomic approach is not always a sufficient tool for exploring and explaining complicated and largely unknown patterns and processes that shape the current biodiversity of a given group of organisms. The genus *Apisa* is an excellent example of a taxonomically challenging group of moths that remains elusive. Despite examination of more than 186 specimens (167 genitalia slides) representing populations from almost all of Africa and the Arabian Peninsula, and despite attempts at molecular analysis of as many as 94 specimens, the core evolutionary relationships within *Apisa* remain obscure. In contrast to many similar studies of generic-level lineages, where well-resolved internal nodes contrast with weakly supported terminal assemblages of two or more morphogroups, in this case the opposite picture was obtained. The generated tree provides weak support for major lineages, while most of the terminal smaller clades can be relatively well characterised on the basis of morphology, which does not necessarily mean that they can be assigned to or recognized as distinct species.) In the light of the present results and the difficulties encountered, a number of key factors can be identified which may significantly improve future results.

The first and central factor is access to the freshly collected material. This deficiency was clearly evident during the present study. The overall large number of specimens did not translate into a sufficient number of good quality genetic extracts due to the age of specimens available. Even freshly collected specimens, from different collections or collectors, with potentially high value for genetic study, can be severely affected by their previous treatment. Apparently good quality specimens appeared to have been subjected to rapid heating in the drying process or repeated remoistening, and in some cases were almost legless. Even the morphological study suffered from the lack of sufficient series of specimens representing the same populations. This lack of sufficient material is particularly problematic in a group as homogeneous in terms of external morphology as *Apisa*.

Apisa is a case of a relatively young and morphologically unusually homogeneous group. Despite the molecular signal which strongly suggesting high systematic diversity with numerous undescribed taxa, no clear diagnostic characters were found that could be used to test this hypothesis, as it relates to the description of numerous new taxa. Therefore, it is further hypothesised that probably there may be other, non-morphological or subtle factors that play the major role in this diversification. Below we discuss the putative importance of each of these factors in the evolution of *Apisa* variability in the light of the relevant literature.

UV light. Difference in the wing pattern visible under the UV light are well-known characters used to separate several taxa in *Colias* (Pieridae) (eg. Rutowski et al., 2007; Stella et al., 2018) and in *Pieris napi* (Stella et al., 2016). It was checked whether the different morphotypes, especially those forming separate clades on our tree, show reflectance in UV light and express any variation in this respect. In particular, *A. subargentea*, which shows a degree of opalescence unusual in *Apisa,* was expected to be highly reflective. However, despite a thorough examination of all body parts (upper wing, underwing, head, abdomen, legs) we did not observe any signs of reflectance or iridescence in the examined specimens. Therefore, we postulate that UV reflectance cannot be considered as a driver responsible for the hidden diversity within *Apisa*.

Pheromone diversity. Pheromones are chemical substances that organisms emit to communicate with other individuals of the same species. They play an important role in the process of matching sexual partners, including sexual selection in animals (Nandagopal et al., 2008). Differences in pheromones between the sexes may be due to differences in physiology, hormones and genetics. In many animal species, males and females secrete different pheromones, that are detected by individuals of the opposite sex. These pheromones can convey information about fertility, reproductive availability, health or genotype. Thus, sexual selection can occur based on the detection of and response to specific pheromones (Wyatt, 2017). For species that humans cannot distinguish morphologically, species isolation processes may occur of at the chemical level (Hillis, 1987).
Much scientific research has focused on identifying and understanding pheromones in cryptic species. Pheromones in cryptic species can have a variety of functions, depending on the specific needs and reproductive strategies of a species.

Pheromone testing involves a variety of methods that allow scientists to identify, analyse and understand these chemicals (Cerkowniak et al., 2013). In moths, female pheromones tend to be more important and better studied than in other insects.
In Arctiinae, however, male structures such as coremata often play a prominent role (Boppre & Schneider, 1989).

The females of *Apisa* are much less known than the males. They have only been described for *A. apisa, A. cinereocostata, A. subargentea* and *A. subcanescens*. In all four cases the female genitalia are equipped with very well developed paired ventral and dorsal pheromone glands. They bear large, membranous, elongated pouches of equal length. The dorsal pheromone glands occur in two separate openings, while the ventral glands merge into a single broad structure. Such prominent glands suggest that pheromone communication plays

an important role in courtship and may therefore be responsible for the differences between taxa. Pheromones can play an important role in the process of recognition and communication between individuals (Fernández, 2013). They can be used to identify sexual partners, mark territory, lure partners to copulate, and convey other information important for survival and reproductive success (Johansson, 2005; Whittaker, 2022). Females often secrete pheromones that attract males to copulate, and males may secrete pheromones that indicate their readiness to breed (Wedell, 2005).

To test this, a direct comparison of the chemical composition of the pheromones would be necessary, as similarly developed pheromone glands have been observed in other Syntomini, such as *Balacra*.

Allopatric distribution. Allopatric distribution occurs when two or more species occur in different geographical areas without their ranges of occurrence overlapping. This means that the species are geographically separated by some form of barrier that prevents them from freely interbreeding and exchanging genes (Losos et al., 2003). It is possible that *Apisa* is in the early stages of speciation. Here we do not yet observe clear features of morphological lineages and the selection and species barrier is not apparent to us. This isolation should prevent free interbreeding and an evolutionary separation of populations that would allow the formation of new species (Mayr, 1959). Allopatric dispersal is one of the most important processes leading to speciation, the formation of new species. It can be the result of a long-term evolutionary process that occurs over many thousands or millions of years (Wahlberg et al., 2011).

Host plant differences. In the case of butterflies, cryptic species can have very similar wing patterns and colours, making them difficult to distinguish, even for experienced entomologists. One way to distinguish cryptic species is to study the host plant preferences of these butterflies. Different species of butterflies often prefer different types of plants as egg-laying and feeding sites, so studying their host plants can help identify species (Shashank et al., 2014). It is also worth noting that some butterfly species may show variation in host plant preference depending on circumstances, such as food availability and environmental conditions. Therefore, a single host plant is not always sufficient to discriminate between two butterfly species (Nylin et al., 2000). Host-plant shifts can also be a driver that accelerates speciation and is involved in the existence of cryptic species (Hernández-Roldán J-L et al., 2016).

Identifying such uniform species can be a difficult task, especially when cryptic species are involved. The use of different methods, such as the study of host plant

preferences, morphological characteristics and molecular techniques, can help to identify and distinguish between such species. In the case of *Apisa*, many unknowns remain: the life cycle, habitat preferences, larval forms and host plants are completely unknown, so this potential factor cannot yet be assessed.

Seasonal replacement. Seasonal replacement, also known as allochronic speciation (Alexander, Bigelow 1960), can be a barrier to interbreeding between morphologically similar species, because lepidopterans often lay eggs on specific host plants that ocure at certain times of the year. Therefore, individuals may be limited in their ability to interbreed with individuals with different host preferences or that occur at different times. Seasonal changes in the availability of host plants and changes in environmental conditions contribute to the maintenance of species distinctiveness.

In the case of *Apisa,* this factor cannot be studied because the available material, especially of *A. canescens*, is too fragmentary to allow reliable comparisons. On the one hand, the different morphotypes (size, colouration) come from different regions with different climatic conditions. On the other hand, there are too few specimens coming from the same or not so distant localities to be able to assess whether they replace each other seasonally.

CONCLUSIONS

1) Although we have incorporated a great deal of new genetic and morphological information, it is still not possible to interpret relationships and fully understand speciation processes within *Apisa*. The genus is certainly a monophyletic group as confirmed by genetic analysis and morphological methods. However, the interspecific relationships within the genus remain unresolved.
2) The present study falsifies the hypothesis of the existence of three distinct evolutionary groups (respectively with a bifid uncus, with a short valva process and with a long valva process, respectively) within *Apisa*, which were previously treated as separate subgenera *Apisa* s. str, *Parapisa* and *Dufraneella.* On the contrary, it is hypothesised that these morphological modifications evolved independently and in different combinations in given species. In particular, the length of the ventral process of the valva can vary not only between species but also between specimens of a single taxon. As a result, a formal synonymy of all subgenera with *Apisa* is postulated.
3) This study shows that *Apisa* is not much more diverse than was expected. Some of the observed variation can be objectively assessed in terms of the discrete diagnostic

characters, that we hypothesise characterise the new taxa. For others, we were able to provide a new diagnosis. However, in the case of many specimens, particulary those characterised by elongate valva process, the morphological variability observed cannot be interpreted unequivocally on the basis of the material available. The existence of such a high degree of polymorphism strongly suggests the existence of numerous, as yet undescribed, cryptic species.

4) The genetic part of this study suggests that East Africa or even the Arabian Peninsula could be the hypothetical centre of diversification of the genus. This is suggested by the basal position of the samples from Oman on the tree, by the much higher diversity of taxa in East Africa and by the much higher colour polymorphism of the *canescens*-like specimens across the northern zone of East Africa (from Eritrea to Tanzania).
5) The genus *Apisa* appears to be a taxonomically extremely difficult group of Lepidoptera, characterised by external uniformity mixed with significant variation in observable morphological characters and also simplification of genital morphology. Even a combination of the morphological approach with the study of three selected genetic markers (COI, WG, RpS5) did not give a clear picture of the classification of *Apisa* and the evolutionary relationships between taxa. These difficulties make *Apisa* a fascinating object of future scientific investigation with the application of more sophisticated genetic methods such as next generation sequencing (NGS) or anchored hybrid enrichment (AHE), allowing older material to be sequenced, but also with the use of much more extensive research material.

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Table S1. GenBank access numbers, locality and the date of collection.

Species	Locality and the date of collection	Genbank access number
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 1.6.2017	OP215983
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 1.6.2017	OP215984
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 1.6.2017	OP215985
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 31.5.2017	OP215986
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 31.5.2017	OP215987
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 2.6.2017	OP215988
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 2.6.2017	OP215989
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 2.6.2017	OP215990
Apisa cinereocostata	Grand Cape Mount Couty, Gola Mational Forest, Iseral; 18.10.2012	OP215991
Apisa cinereocostata	Gbarpolu County, Gola National Forest, Kungbor, Nordrand; 31.5.2017	OP215992
Apisa cinereocostata	Haute Guinée, 8 km, nordlich Konsankoro; 4 Juni 2013	OP215993
Apisa cinereocostata	Volta Region, Likpe Bakua; 05-06.IX.2010	OP215994
Apisa cinereocostata	Mole; 16.iii.2010	OP215995
Apisa cinereocostata	Bunso; 31.X.2009	OP215996
Apisa cinereocostata	Bunso; 21.-23.iii.2010	OP215997
Apisa cinereocostata	Bunso Arboretum; X.2011	OP215998
Apisa cinereocostata	Central Region, Rainforest Lodge, Kakum National Park; XII.2011	OP215999
Apisa cinereocostata	Western Region, Visitor Centre, Ankasa National Park; 27-30.XI.2011	OP216000
Apisa cinereocostata	Kuloro	OP216001
Apisa cinereocostata	Abuko	OP216002
Apisa cinereocostata	Tiwai Island, Moa River; 17-22.vi.2016	OP216003
Apisa cinereocostata	Loma Mountains, farmland/forest mosaic; 11-15.vi.2016	OP216004

Apisa cinereocostata	Baoma, Goderich	OP216005
Apisa cinereocostata	Baoma, Goderich	OP216006
Apisa cinereocostata	Baoma, Goderich	OP216007
Apisa cinereocostata	Baoma, Goderich	OP216008
Apisa cinereocostata	Baoma, Goderich	OP216009
Apisa cinereocostata	Loma Mountains farmland/forest mosaic; 11-15.vi.2016	OP216010
Apisa cinereocostata	Tai NP., Tai Research Station (SRET); 25.iii-17.iv.2017	OP216011
Apisa cinereocostata	Mopti region, Dogon Plateau, Bandiagara; 28.07.2017	OP216012
Apisa cinereocostata	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger; 27.07.2017	OP216013
Apisa cinereocostata	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger; 28.07.2017	OP216014
Apisa cinereocostata	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger; 28.07.2017	OP216015
Apisa cinereocostata	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger; 28.07.2017	OP216016
Apisa cinereocostata	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger; 28.07.2017	OP216017
Apisa cinereocostata	Gbarpolu County, Gola National Forest Kungbor, Nordrand; 1.6.2017	OP216018
Apisa cinereocostata	Gbarpolu County, Gola National Forest Kungbor, Nordrand; 1.6.2017	OP216019
Apisa cinereocostata	Gbarpolu County, Gola National Forest Kungbor, Nordrand; 1.6.2017	OP216020
Apisa cinereocostata	Gbarpolu County, Gola National Forest Kungbor, Nordrand; 6.6.2017	OP216021
Apisa cinereocostata	Gbarpolu County, Gola National Forest Kungbor, Nordrand; 30.5.2017	OP216022
Apisa cinereocostata	Nimba Mountains, Mount Gangra summit; 17-25.III.2017	OP216023
Apisa cinereocostata	Nimba Mountains, Mount Gangra western slope; 16-17.III.2017	OP216024
Apisa cinereocostata	Nimba Mountains, Mount Gangra western slope; 16-17.III.2017	OP216025
Apisa cinereocostata	Nimba Mountains, Mount Gangra western slope; 16-17.III.2017	OP216026
Apisa cinereocostata	Nimba Mts., Cellcom Rd.; 10-24.iii.2017	OP216027
Apisa cinereocostata	Guinée Forestiére, 27 km südlich Bounouma, Foret Classée du Dieké; 7 Juni 2013	OP216028

Apisa cinereocostata	Nimba Mts, 600 forest SMFG, concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest); 21-30.viii.2017	OP216029
Apisa cinereocostata	Nimba Mts, 600 forest SMFG concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest); 21-30.viii.2017	OP216030
Apisa cinereocostata	Nimba Mts, 600 forest SMFG concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest); 21-30.viii.2017	OP216031
Apisa cinereocostata	80 km SW of Bamako, near Kenieroba river Niger; December 2015	OP216032
Apisa cinereocostata	80 km SW of Bamako, near Kenieroba river Niger December 2015	OM523171
Apisa sp.	-	OM523173
Apisa sp.	-	OP216036
Apisa asipa sp. nov.	North Region, Wack; 2-21.x.2018	OP216033
Apisa asipa sp. nov.	North Region, Wack; 2-21.x.2018	OP216034
Apisa asipa sp. nov.	North Region, Wack; 2-21.x.2018	OP216035

Table. S2. Specimens used for analysis with locations, numbers of genital slides, access to GenBank database.

Genus	Species	Country	Locality	Altitude level	Coordinates	Data	Collector	Sex	Genital slide	DNA	GenBank
Apisa	*asipa*	Cameroon	North Region, Wack	900	07°40'16,5"N 13°33'18,4''E	2-21.x.2018	Safian Sz., Simonics G.	♂	04_15_12_2021	206_03_11_2021	OP216035
Apisa	*asipa*	Cameroon	North Region, Wack	900	07°40'16,5"N 13°33'18,4''E	2-21.x.2018	Safian Sz., Simonics G.	♂	04_07_12_2021	198_03_11_2021	OP216033
Apisa	*asipa*	Cameroon	North Region, Wack	900	07°40'16,5"N 13°33'18,4''E	2-21.x.2018	Safian Sz., Simonics G.	♂	05_15_12_2021	192_03_11_2021	OP216034
Apisa	*asipa*	Cameroon	North Region, Wack	900	07°40'16,5"N 13°33'18,4''E	2-21.x.2018	Safian Sz., Simonics G.	♂	01_23_12_2021	207_03_11_2021	
Apisa	*asipa*	Cameroon	Adamaua Poli	500		8.V.37	A. Weidhols	♂	01_11_01_2022		
Apisa	*asipa*	Cameroon	Adamaua Poli	500		8.V.37	A. Weidhols	♀	01_27_01_2022		
Apisa	*asipa*	Nigeria	Kaduna			10.4.70	Dr Politzar	♂			
Apisa	*asipa*	Nigeria	Kaduna			10.4.70	Dr Politzar	♂			
Apisa	*asipa*	Nigeria	Kaduna			10.4.70	Dr Politzar	♂			
Apisa	*subargentea*	Democratic Republic of Congo	Kibali-Ituri Nioka			7.VI.1953	J. Hecq	♂	05_10_07_2018		
Apisa	*subargentea*	Democratic Republic of Congo	Kibali-Ituri Nioka			27.XI.1953	J. Hecq	♂	1.10.IV.		
Apisa	*subargentea*	Burundi	Gitega			13.III.1967	Dr M. Fontaine	♀			
Apisa	*subargentea*	Democratic Republic of Congo	Kibali-Ituri Nioka			31.V.1954	J. Hecq	♀			
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂		81_02_02_2019	OP215983
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂	01_26_03_2019	78_02_02_2019	OP215984
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂	05_27_03_2019	77_02_02_2019	OP215985
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	31.5.2017	Michael Ochse	♂	04_27_03_2019	80_02_02_2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	31.5.2017	Michael Ochse	♂		85.02.02.2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	31.5.2017	Michael Ochse	♂	02_26_03_2019	89_02_02_2019	OP215986
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	31.5.2017	Michael Ochse	♂		84_02_02_2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	31.5.2017	Michael Ochse	♂		90_02_02_2019	OP215987
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	2.6.2017	Michael Ochse	♂		74_02_02_2019	OP215988
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	2.6.2017	Michael Ochse	♂		73_02_02_2019	OP215989
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Radiostation	460	7°38'53,212"N, 10°34'26,907"W	4.6.2017	Michael Ochse	♂		87_02_02_2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°39'35,399"N, 10°33'51,459"W	2.6.2017	Michael Ochse	♂	02_27_03_2019	82_02_02_2019	OP215990
Apisa	*cinereocostata*	Liberia	Grand Cape Mount Couty, Gola Mational Forest, Iseral	232	7°22'52.65"N, 10°51'8.73"W	18.10.2012	Michael Ochse	♂		71_02_02_2019	OP215991
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest, Kungbor, Nordrand	401	7°38'54.683"N, 10°34'35.154"W	31.5.2017	Michael Ochse	♂		79_02_02_2019	OP215992

Genus	Species	Country	Locality	Altitude level	Coordinates	Data	Collector	Sex	Genital slide	DNA	GenBank
Apisa	*cinereocostata*	Liberia	Nimba County, Nimba-Berge, 4km südostlich Yekepa	622	7°33'39.31"N, 8°30'23.67"W	13. Juni 2013	Michael Ochse	♂	04_12_02_2019	67_02_02_2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂	03_26_03_2019	95_02_02_2019	OP216018
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂	01_12_02_2019	83_02_02_2019	OP216019
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	6.6.2017	Michael Ochse	♂	04_26_03_2019	86_02_02_2019	
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	1.6.2017	Michael Ochse	♂	06_27_03_2019	93_02_02_2019	OP216020
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	6.6.2017	Michael Ochse	♂	06_12_02_2019	94_02_02_2019	OP216021
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'54,683"N, 10°34'35,154"W	30.5.2017	Michael Ochse	♂	03_27_03_2019	92_02_02_2019	OP216022
Apisa	*cinereocostata*	Liberia	Gbarpolu County, Gola National Forest Kungbor, Nordrand	401	7°38'38,504"N, 10°34'47,423"W	29.5.2017	Michael Ochse	♂	01_27_03_2019	91_02_02_2019	
Apisa	*cinereocostata*	Liberia	Nimba Mountains, Mount Gangra summit		7°32'45.82"N, 8°38'9.36"W	17-25.III.2017	Szafián, Sz., Simonics, G	♂	01_05_07_2018	5_27_06_2018	OP216023
Apisa	*cinereocostata*	Liberia	Nimba Mountains, Mount Gangra western slope		7°33'29.73"N, 8°38'16.40"W	16-17.III.2017	Szafián, Sz., Simonics, G	♂		9_27_06_2018	OP216024
Apisa	*cinereocostata*	Liberia	Nimba Mountains, Mount Gangra western slope		7°33'29.73"N, 8°38'16.40"W	16-17.III.2017	Szafián, Sz., Simonics, G	♀		7_27_06_2018	OP246025
Apisa	*cinereocostata*	Liberia	Nimba Mountains, Mount Gangra western slope		7°33'29.73"N, 8°38'16.40"W	16-17.III.2017	Szafián, Sz., Simonics, G	♀		6_27_06_2018	OP216026
Apisa	*cinereocostata*	Liberia	Grand Gedeh, County, Putu Range			19-31.XII.2010	Sáfián, Sz., Zakar, E	♂	02_02_07_2018	ISEZ DNA 163	
Apisa	*cinereocostata*	Liberia	Nimba Mts., Cellcom Rd.	700	7°32'47.5"N, 8°32'1.33"W	10-24.iii.2017	Sáfián, Sz., Simonics, G	♂	04_15_01_2019	34_13_12_2018	OP216027
Apisa	*cinereocostata*	Liberia	Nimba Mts., Cellcom Rd.	1000-1100	7°32'45.9"N, 8°31'21"W	12-16.iii.2017	Sáfián, Sz., Simonics, G	♂	03_15_01_2019	58_13_12_2018	
Apisa	*cinereocostata*	Liberia	Nimba County, Yekepa residental area	508	7°34'26.3"N, 8°32'31.6"W	10-31.iii.2017	Sáfián, Sz.	♂	03_30_01_2019	57_13_12_2018	
Apisa	*cinereocostata*	Guinea	Guinée Forestiére, 27 km südlich Bounouma, Foret Classée du Dieké	482	7°27'52.20" N, 8°50'36.60"W	7 Juni 2013	Michael Ochse	♂		66_02_02_2019	OP216028
Apisa	*cinereocostata*	Guinea	Haute Guinée, 8 km nördlich Konsankoro	575	9°6'18.99" N, 8°0'42.06"W	7 Juni 2013	Michael Ochse	♂		68_02_02_2019	
Apisa	*cinereocostata*	Guinea	Konakri, Macenta Prefecture, Ziama Forest	550		April 2017	Petányi G; Muller GC; Kravchenko VD et al.	♂	01_23_10_2019	141_15_10_2019	
Apisa	*cinereocostata*	Guinea	Nimba Mts, 600 forest SMFG, concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest)	1536	7°39'49.31" N, 8°22'20.06"W	21-30.viii.2017	Sáfián, Sz., Simonics, G	♂	02_30_01_2019	37_13_12_2018	OP216029
Apisa	*cinereocostata*	Guinea	Nimba Mts, SMFG concession area (Société des Mines de Fer de Guineé)	700	7°42'2.83" N, 8°23'58.60"W	16-25.vii.2017	Sáfián, Sz.	♂	01_15_01_2019	56_13_12_2018	
Apisa	*cinereocostata*	Guinea	Nimba Mts, 600 forest SMFG concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest)	1536	7°39'49.31" N, 8°22'20.06"W	21-30.viii.2017	Sáfián, Sz., Simonics, G., leg.	♂		35_13_12_2018	OP216030
Apisa	*cinereocostata*	Guinea	Nimba Mts, 600 forest SMFG concession area (Société des Mines de Fer de Guineé) Mont Pierre Richeaud (montane forest)	1536	7°39'49.31" N, 8°22'20.06"W	21-30.viii.2017	Sáfián, Sz., Simonics, G.	♂		36_13_12_2018	OP216031
Apisa	*cinereocostata*	Guinea	Coyah			1963.VIII.22	K. Ferencz	♂			
Apisa	*cinereocostata*	Guinea	Konakri, Macenta Prefecture Ziama Forest	550		17.11-01.12.2016	Petrányi G; Muller, GC; Kravchenko VD et al.	♂	02_25_10_2019	144_15_10_2019	
Apisa	*cinereocostata*	Guinea	Haute Guinée, 8 km, nordlich Konsankoro	575	9°6'18.99" N, 9°0'42.06"W	4 Juni 2013	Michael Ochse	♂	03_10_06_2049	70_02_02_2019	
Apisa	*cinereocostata*	Guinea	Haute Guinée, 8 km, nordlich Konsankoro	575	9°6'18.99" N, 9°0'42.06"W	4 Juni 2013	Michael Ochse	♂	05_12_02_2019	69_02_02_2019	OP215993

Genus	Species	Country	Locality	Altitude level	Coordinates	Data	Collector	Sex	Genital slide	DNA	GenBank
Apisa	*cinereocostata*	Guinea	Konakri, Macenta Prefecture, Ziama Forest	550		2017	GC Muller VD Kravchenko & G Petranyi	♂	01_15_11_2019	147_15_10_2019	
Apisa	*cinereocostata*	Ghana	Volta Region, Likpe Bakua			05-06.IX.2010	Dall'Astra, U., Dall'Astra A. & Sáfián, Sz.	♂		27_01_08_2018	OP215994
Apisa	*cinereocostata*	Ghana	Bia Forest	250		12-13.x.2007	Knud Larsen	♂	02_19_03_2019	100_18_03_2019	
Apisa	*cinereocostata*	Ghana	Mole	150		16.iii.2010	Knud Larsen & Wojciech Kubasik	♂	03_01_04_2019	103_18_03_2019	
Apisa	*cinereocostata*	Ghana	Mole	150		16.iii.2010	Knud Larsen & Wojciech Kubasik	♂		03_27_06_2018	OP215995
Apisa	*cinereocostata*	Ghana	Ashanti: Bobiri	240		9.-12.iii.2010	Knud Larsen & Wojciech Kubasik	♂	01_20_03_2019	99_18_03_2019	
Apisa	*cinereocostata*	Ghana	Jomoro, Ankasa	90		2-3.v.2007	Knud Larsen	♂	04_10_07_2018	01_27_06_2018	
Apisa	*cinereocostata*	Ghana	Bunso			31.X.2009	Sz. Sáfián	♂		28_01_08_2018	OP215996
Apisa	*cinereocostata*	Ghana	Bunso	300		21.-23.iii.2010	Knud Larsen & Wojciech Kubasik	♂	02_01_04_2019	102_18_03_2019	OP215997
Apisa	*cinereocostata*	Ghana	Bunso Arboretum			X.2011	Sáfián, Sz.	♀		29_01_08_2018	OP215998
Apisa	*cinereocostata*	Ghana	Central Region, Rainforest Lodge, Kakum National Park			XII.2011	Sáfián, Sz.	♂		26_01_08_2018	OP215999
Apisa	*cinereocostata*	Ghana	Western Region, Visitor Centre, Ankasa National Park			27-30.XI.2011	Dall'Astra, U., Sáfian, Sz., Ochse, M	♂		LN030	
Apisa	*cinereocostata*	Ghana	Western Region, Visitor Centre, Ankasa National Park			27-30.XI.2011	Dall'Astra, U., Sáfian, Sz., Ochse, M.	♂	08_24_07_2018	17_01_08_2018	OP216000
Apisa	*cinereocostata*	Gambia	Kotu		13°27'22" N, 16°14'23"W		R.W.Goff	♂		44_13_12_2018	
Apisa	*cinereocostata*	Gambia	Tanji		13°22'52" N, 16°46'83"W		R.W.Goff	♂		41_13_12_2018	
Apisa	*cinereocostata*	Gambia	Tanji		13°22' N, 16°46'W		R.W.Goff	♂		47_13_12_2018	
Apisa	*cinereocostata*	Gambia	Kuloro		13°17'54" N, 16°34'10"W		R.W.Goff	♂		43_12_12_2018	OP216001
Apisa	*cinereocostata*	Gambia	Abuko		13°23'41" N, 16°38'45"W		R.W.Goff	♂		46_13_12_2018	OP216002
Apisa	*cinereocostata*	Sierra Leone	Tiwai Island, Moa River	120	N 07°33'00" W 11°21'09"	17-22.vi.2016	Takano Miles & Goff	♂		49_13_12_2018	OP216003
Apisa	*cinereocostata*	Sierra Leone	Loma Mountains, farmland/forest mosaic	420	N 09°07'47" W 11°05'24"	11-15.vi.2016	Takano, Miles & Goff	♂	02_15_01_2019	50_13_12_2018	OP216004
Apisa	*cinereocostata*	Sierra Leone	Baoma, Goderich		8o25'41"N 13o15'47"W		R.W.Goff	♂		54_13_12_2018	OP216005
Apisa	*cinereocostata*	Sierra Leone	Baoma, Goderich		8°25'41"N 13°15'47"W		R.W.Goff	♂	01_22_01_2019	55_13_12_2018	OP216006
Apisa	*cinereocostata*	Sierra Leone	Baoma, Goderich		8°25'41"N 13°15'47"W		R.W.Goff	♂	02_22_01_2019	53_13_12_2018	OP216007
Apisa	*cinereocostata*	Sierra Leone	Baoma, Goderich		8°25'41"N 13°15'47"W		R.W.Goff	♂	01_30_01_2019	52_13_12_2018	OP216008
Apisa	*cinereocostata*	Sierra Leone	Baoma, Goderich		8°25'41"N 13°15'47"W		R.W.Goff	♂	02_14_01_2019	51_13_12_2018	OP216009
Apisa	*cinereocostata*	Sierra Leone	Loma Mountains farmland/forest mosaic	420	N09°07'47" W11°05'24"	11-15.vi.2016	Takano, Miles & Goff	♂	03_22_01_2019	48_13_12_2018	OP216010
Apisa	*cinereocostata*	Nigeria	Kaduna			14.X.1971	H. Polotzar	♂			

Genus	Species	Country	Locality	Altitude level	Coordinates	Data	Collector	Sex	Genital slide	DNA	GenBank
Apisa	*cinereocostata*	Nigeria	Bendel State, Okomu F. Res.			27.05.1984	J. Wojtusiak	♂			
Apisa	*cinereocostata*	Nigeria	Owena			18.6.60		♂			
Apisa	*cinereocostata*	Nigeria	Bendel State, Okomu F. Res.			19.05.1984	J. Wojtusiak	♂			
Apisa	*cinereocostata*	Ivory Coast	Tai NP., Tai Research Station (SRET)	174	05°50'00"N 07°20'32.0"W	25.iii-17.iv.2017	Aristophanus, A., Aristophanus, M., Geiser, M.,	♂		40_13_12_2018	OP216011
Apisa	*cinereocostata*	Ivory Coast	Ayamé II, Barrage de la Bia			6/9-V-1964	Griveaud et Piart	♂			
Apisa	*cinereocostata*	Ivory Coast	Bingerville			13.04.1914	Melou G.	♂			
Apisa	*cinereocostata*	Ivory Coast	Grand Besebl			12.-14.3.86	Dr. Polltzar	♂	03_22_05_2019		
Apisa	*cinereocostata*	Ivory Coast	Ayamé II, Barrage de la Bia			9/12-I-1964	Griveaud et Piart	♂	02_29_06_2018		
Apisa	*cinereocostata*	Ivory Coast	Bingerville			13.04.1914	Melou G.	♂	03_10_07_2018		
Apisa	*cinereocostata*	Ivory Coast	Tai Research Station (SRET)	174	05°50'00"N 07°20'32.0"W	25.iii-17.iv.2017	Aristophanus, A., Aristophanus, M., Geiser, M.,	♂	03_14_01_2019	59_13_12_2018	
Apisa	*cinereocostata*	Gambia	Abuko, Nature Reerve		13°22'22"N 16°38'55"W		R. Goff	♂		45_13_12_2018	
Apisa	*cinereocostata*	Gambia	Abuko, Nature Reerve		13°22'22"N 16°38'55"W		R. Goff	♂		42_13_12_2018	
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger	360		28.07.2017	Muller, K. Kravchenko, M. Traore & al.	♂	01_08_05_2019	112_15_04_2019	OP216017
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger	360		28.07.2018	Muller, K. Kravchenko, M. Traore & al.	♂	02_08_05_2019	111_15_04_2019	OP216016
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger	360		28.07.2018	Muller, K. Kravchenko, M. Traore & al.	♂	03_09_05_2019	115_15_04_2019	OP216015
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger	360		28.07.2018	Muller, K. Kravchenko, M. Traore & al.	♂	03_23_05_2019	122_16_04_2019	OP216014
Apisa	*cinereocostata*	Mali	Mopti region, Dogon Plateau, Bandagara	450-850		January 2013	Muller & K. Kravchenko	♂	04_08_05_2019	113_15_04_2019	
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, 360 m, a. s. l., Near Kenieroba, River Niger	360		27.07.2017	Muller, K. Kravchenko, M. Traore & al.	♂	02_09_05_2019	116_16_04_2019	OP216013
Apisa	*cinereocostata*	Mali	Mopti region, Dogon Plateau, Bandiagara	450-470		28.07.2017	Muller, K. Kravchenko, M. Traore & al.	♂	02_23_05_2019	121_16_04_2019	OP216012
Apisa	*cinereocostata*	Mali	Mopti region, Dogon Plareau, Bandiagara	450-470		November 2015	Muller, K. Kravchenko, M. Traore & al.	♂	03_08_05_2019	109_15_04_2019	
Apisa	*cinereocostata*	Mali	80 km SW of Bamako, near Kenieroba river Niger	360		December 2015	Muller, K. Kravchenko, M. Traore & al.	♀	04_23_05_2019	123_16_04_2019	OP216032
Apisa	*cinereocostata*	Angola	Huambo Prov., rd. Huambo – Caconada, E Catata	1667	13°23'58.6"S 15°26'54.1"E	25.XI.2017	S. Naumann, E, Ott & H. Sylak	♂	04_22_05_2019	117_16_04_2019	
Apisa	*cinereocostata*	Gabon	Kangwé, Ogové Riv.				A. C. Good		3.22.12		

Table. S3. 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.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
A. cinereocostata	OP215983																																																											
A. cinereocostata	OP215984	0,003																																																										
A. cinereocostata	OP215985	0,002	0,002																																																									
A. cinereocostata	OP215986	0,012	0,012	0,011																																																								
A. cinereocostata	OP215987	0,014	0,014	0,012	0,020																																																							
A. cinereocostata	OP215988	0,000	0,003	0,002	0,012	0,014																																																						
A. cinereocostata	OP215989	0,000	0,003	0,002	0,012	0,014	0,000																																																					
A. cinereocostata	OP215990	0,012	0,015	0,014	0,019	0,023	0,012	0,012																																																				
A. cinereocostata	OP215991	0,015	0,015	0,014	0,022	0,008	0,015	0,015	0,025																																																		
A. cinereocostata	OP215992	0,000	0,003	0,002	0,012	0,014	0,000	0,000	0,012	0,015																																																
A. cinereocostata	OP215993	0,014	0,014	0,012	0,020	0,006	0,014	0,014	0,023	0,008	0,014																																														
A. cinereocostata	OP215994	0,015	0,015	0,014	0,019	0,008	0,015	0,015	0,025	0,009	0,015	0,008																																														
A. cinereocostata	OP215995	0,023	0,023	0,022	0,030	0,015	0,023	0,023	0,033	0,020	0,023	0,019	0,017																																														
A. cinereocostata	OP215996	0,003	0,006	0,005	0,015	0,017	0,003	0,003	0,015	0,019	0,003	0,017	0,019	0,027																																													
A. cinereocostata	OP215997	0,008	0,011	0,009	0,020	0,022	0,008	0,008	0,020	0,023	0,008	0,022	0,023	0,028	0,005																																												
A. cinereocostata	OP215998	0,003	0,006	0,005	0,015	0,017	0,003	0,003	0,015	0,019	0,003	0,017	0,019	0,027	0,000	0,005																																											
A. cinereocostata	OP215999	0,003	0,006	0,005	0,015	0,017	0,003	0,003	0,015	0,019	0,003	0,017	0,019	0,027	0,000	0,005	0,000																																										
A. cinereocostata	OP216000	0,003	0,006	0,005	0,015	0,017	0,003	0,003	0,015	0,019	0,003	0,017	0,019	0,027	0,000	0,005	0,000	0,000																																										
A. cinereocostata	OP216001	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014																																										
A. cinereocostata	OP216002	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000																																									
A. cinereocostata	OP216003	0,015	0,015	0,014	0,022	0,008	0,015	0,015	0,025	0,006	0,015	0,005	0,009	0,017	0,019	0,023	0,019	0,019	0,019	0,005	0,005																																									
A. cinereocostata	OP216004	0,009	0,012	0,011	0,019	0,011	0,009	0,009	0,019	0,012	0,009	0,011	0,012	0,020	0,012	0,017	0,012	0,012	0,012	0,008	0,008	0,012																																								
A. cinereocostata	OP216005	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000	0,000	0,005	0,008																																								
A. cinereocostata	OP216006	0,012	0,012	0,011	0,019	0,005	0,012	0,012	0,022	0,003	0,012	0,005	0,006	0,017	0,015	0,020	0,015	0,015	0,015	0,002	0,002	0,003	0,009	0,002																																							
A. cinereocostata	OP216007	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000	0,000	0,005	0,008	0,000	0,002																																							
A. cinereocostata	OP216008	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000	0,000	0,005	0,008	0,000	0,002	0,000																																							
A. cinereocostata	OP216009	0,012	0,012	0,011	0,019	0,005	0,012	0,012	0,022	0,003	0,012	0,005	0,006	0,017	0,015	0,020	0,015	0,015	0,015	0,002	0,002	0,003	0,009	0,002	0,000	0,002	0,002																																							
A. cinereocostata	OP216010	0,009	0,012	0,011	0,019	0,011	0,009	0,009	0,019	0,012	0,009	0,011	0,012	0,020	0,012	0,017	0,012	0,012	0,012	0,008	0,008	0,012	0,000	0,008	0,009	0,008	0,008	0,009																																							
A. cinereocostata	OP216011	0,003	0,006	0,005	0,015	0,017	0,003	0,003	0,015	0,019	0,003	0,017	0,019	0,027	0,000	0,005	0,000	0,000	0,000	0,014	0,014	0,019	0,012	0,014	0,015	0,014	0,014	0,015	0,012																																							
A. cinereocostata	OP216012	0,015	0,015	0,014	0,022	0,011	0,015	0,015	0,025	0,012	0,015	0,011	0,012	0,017	0,019	0,023	0,019	0,019	0,019	0,008	0,008	0,012	0,012	0,008	0,009	0,008	0,008	0,009	0,012	0,019																																						
A. cinereocostata	OP216013	0,017	0,017	0,015	0,023	0,012	0,017	0,017	0,026	0,014	0,017	0,012	0,014	0,022	0,020	0,025	0,020	0,020	0,020	0,009	0,009	0,014	0,014	0,009	0,011	0,009	0,009	0,011	0,014	0,020	0,008																																					
A. cinereocostata	OP216014	0,022	0,022	0,020	0,028	0,014	0,022	0,022	0,031	0,015	0,022	0,014	0,015	0,020	0,025	0,030	0,025	0,025	0,025	0,011	0,011	0,012	0,019	0,011	0,012	0,011	0,011	0,012	0,019	0,025	0,012	0,011																																					
A. cinereocostata	OP216015	0,014	0,014	0,012	0,020	0,009	0,014	0,014	0,023	0,011	0,014	0,009	0,011	0,019	0,017	0,022	0,017	0,017	0,017	0,006	0,006	0,011	0,011	0,006	0,008	0,006	0,006	0,008	0,011	0,017	0,005	0,003	0,008																																					
A. cinereocostata	OP216016	0,014	0,014	0,012	0,020	0,009	0,014	0,014	0,023	0,011	0,014	0,009	0,011	0,019	0,017	0,022	0,017	0,017	0,017	0,006	0,006	0,011	0,011	0,006	0,008	0,006	0,006	0,008	0,011	0,017	0,005	0,003	0,008	0,000																																					
A. cinereocostata	OP216017	0,014	0,014	0,012	0,020	0,009	0,014	0,014	0,023	0,011	0,014	0,009	0,011	0,019	0,017	0,022	0,017	0,017	0,017	0,006	0,006	0,011	0,011	0,006	0,008	0,006	0,006	0,008	0,011	0,017	0,005	0,006	0,011	0,003	0,003																																					
A. cinereocostata	OP216018	0,009	0,009	0,008	0,015	0,005	0,009	0,009	0,019	0,006	0,009	0,005	0,006	0,014	0,012	0,017	0,012	0,012	0,012	0,002	0,002	0,006	0,006	0,002	0,003	0,002	0,002	0,003	0,006	0,012	0,006	0,008	0,012	0,005	0,005	0,005																																					
A. cinereocostata	OP216019	0,011	0,011	0,009	0,017	0,006	0,011	0,011	0,020	0,005	0,011	0,006	0,008	0,015	0,014	0,019	0,014	0,014	0,014	0,003	0,003	0,005	0,008	0,003	0,002	0,003	0,003	0,002	0,008	0,014	0,008	0,009	0,014	0,006	0,006	0,006	0,002																																					
A. cinereocostata	OP216020	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000	0,000	0,005	0,008	0,000	0,002	0,000	0,000	0,002	0,008	0,014	0,008	0,009	0,011	0,006	0,006	0,006	0,002	0,003																																					
A. cinereocostata	OP216021	0,011	0,011	0,009	0,017	0,006	0,011	0,011	0,020	0,005	0,011	0,006	0,008	0,015	0,014	0,019	0,014	0,014	0,014	0,003	0,003	0,005	0,008	0,003	0,002	0,003	0,003	0,002	0,008	0,014	0,008	0,009	0,014	0,006	0,006	0,006	0,002	0,000	0,003																																					
A. cinereocostata	OP216022	0,009	0,009	0,008	0,015	0,005	0,009	0,009	0,019	0,006	0,009	0,005	0,006	0,014	0,012	0,017	0,012	0,012	0,012	0,002	0,002	0,006	0,006	0,002	0,003	0,002	0,002	0,003	0,006	0,012	0,006	0,008	0,012	0,005	0,005	0,005	0,000	0,002	0,002	0,002																																					
A. cinereocostata	OM523171	0,011	0,011	0,009	0,017	0,003	0,011	0,011	0,020	0,005	0,011	0,003	0,005	0,015	0,014	0,019	0,014	0,014	0,014	0,000	0,000	0,005	0,008	0,000	0,002	0,000	0,000	0,002	0,008	0,014	0,008	0,009	0,011	0,006	0,006	0,006	0,002	0,003	0,000	0,003	0,002																																					
A. cinereocostata	OP216023	0,014	0,017	0,015	0,020	0,025	0,014	0,014	0,008	0,026	0,014	0,025	0,026	0,035	0,017	0,019	0,017	0,017	0,017	0,022	0,022	0,027	0,020	0,022	0,023	0,022	0,022	0,023	0,020	0,017	0,026	0,028	0,033	0,025	0,025	0,025	0,020	0,022	0,022	0,022	0,020	0,022																																					
A. cinereocostata	OP216024	0,014	0,017	0,015	0,020	0,025	0,014	0,014	0,008	0,026	0,014	0,025	0,026	0,035	0,017	0,019	0,017	0,017	0,017	0,022	0,022	0,027	0,020	0,022	0,023	0,022	0,022	0,023	0,020	0,017	0,026	0,028	0,033	0,025	0,025	0,025	0,020	0,022	0,022	0,022	0,020	0,022	0,000																																					
A. cinereocostata	OP216025	0,015	0,019	0,017	0,022	0,026	0,015	0,015	0,009	0,028	0,015	0,026	0,028	0,036	0,019	0,020	0,019	0,019	0,019	0,023	0,023	0,028	0,022	0,023	0,025	0,023	0,023	0,025	0,022	0,019	0,028	0,030	0,034	0,026	0,026	0,026	0,022	0,023	0,023	0,023	0,022	0,023	0,002	0,002																																					
A. cinereocostata	OP216026	0,000	0,003	0,002	0,012	0,014	0,000	0,000	0,012	0,015	0,000	0,014	0,015	0,023	0,003	0,008	0,003	0,003	0,003	0,011	0,011	0,015	0,009	0,011	0,012	0,011	0,011	0,012	0,009	0,003	0,015	0,017	0,022	0,014	0,014	0,014	0,009	0,011	0,011	0,011	0,009	0,011	0,014	0,014	0,015																																					
A. cinereocostata	OP216027	0,014	0,017	0,015	0,020	0,025	0,014	0,014	0,008	0,026	0,014	0,025	0,026	0,035	0,017	0,019	0,017	0,017	0,017	0,022	0,022	0,027	0,020	0,022	0,023	0,022	0,022	0,023	0,020	0,017	0,026	0,028	0,033	0,025	0,025	0,025	0,020	0,022	0,022	0,022	0,020	0,022	0,000	0,000	0,002	0,014																																					
A. cinereocostata	OP216028	0,000	0,003	0,002	0,012	0,014	0,000	0,000	0,012	0,015	0,000	0,014	0,015	0,023	0,003	0,008	0,003	0,003	0,003	0,011	0,011	0,015	0,009	0,011	0,012	0,011	0,011	0,012	0,009	0,003	0,015	0,017	0,022	0,014	0,014	0,014	0,009	0,011	0,011	0,011	0,009	0,011	0,014	0,014	0,015	0,000	0,014																																					
A. cinereocostata	OP216029	0,000	0,003	0,002	0,012	0,014	0,000	0,000	0,012	0,015	0,000	0,014	0,015	0,023	0,003	0,008	0,003	0,003	0,003	0,011	0,011	0,015	0,009	0,011	0,012	0,011	0,011	0,012	0,009	0,003	0,015	0,017	0,022	0,014	0,014	0,014	0,009	0,011	0,011	0,011	0,009	0,011	0,014	0,014	0,015	0,000	0,014	0,000																																					
A. cinereocostata	OP216030	0,014	0,017	0,015	0,020	0,025	0,014	0,014	0,008	0,026	0,014	0,025	0,026	0,035	0,017	0,019	0,017	0,017	0,017	0,022	0,022	0,027	0,020	0,022	0,023	0,022	0,022	0,023	0,020	0,017	0,026	0,028	0,033	0,025	0,025	0,025	0,020	0,022	0,022	0,022	0,020	0,022	0,000	0,000	0,002	0,014	0,000	0,014	0,014																																					
A. cinereocostata	OP216031	0,014	0,017	0,015	0,020	0,025	0,014	0,014	0,008	0,026	0,014	0,025	0,026	0,035	0,017	0,019	0,017	0,017	0,017	0,022	0,022	0,027	0,020	0,022	0,023	0,022	0,022	0,023	0,020	0,017	0,026	0,028	0,033	0,025	0,025	0,025	0,020	0,022	0,022	0,022	0,020	0,022	0,000	0,000	0,002	0,014	0,000	0,014	0,014	0,000																																					
A. cinereocostata	OP216032	0,012	0,012	0,011	0,019	0,008	0,012	0,012	0,022	0,009	0,012	0,008	0,009	0,017	0,015	0,020	0,015	0,015	0,015	0,005	0,005	0,009	0,009	0,005	0,006	0,005	0,005	0,006	0,009	0,015	0,003	0,005	0,005	0,005	0,005	0,005	0,003	0,005	0,005	0,005	0,003	0,005	0,023	0,023	0,025	0,012	0,023	0,012	0,012	0,023	0,023																																					
A. asipa sp. nov.	OP216033	0,054	0,054	0,052	0,056	0,052	0,054	0,054	0,054	0,052	0,054	0,049	0,051	0,054	0,056	0,057	0,056	0,056	0,056	0,049	0,049	0,049	0,054	0,049	0,049	0,049	0,049	0,049	0,054	0,056	0,051	0,052	0,057	0,049	0,049	0,049	0,047	0,047	0,049	0,047	0,047	0,049	0,049	0,049	0,051	0,054	0,049	0,054	0,054	0,049	0,049	0,047																																					
A. asipa sp. nov.	OP216034	0,051	0,051	0,049	0,052	0,049	0,051	0,051	0,047	0,049	0,051	0,046	0,051	0,054	0,052	0,054	0,052	0,052	0,052	0,046	0,046	0,046	0,051	0,046	0,046	0,046	0,046	0,046	0,051	0,052	0,049	0,051	0,055	0,047	0,047	0,047	0,044	0,044	0,046	0,044	0,044	0,046	0,046	0,046	0,047	0,051	0,046	0,046	0,046	0,046	0,046	0,009																																					
A. asipa sp. nov.	OP216035	0,051	0,051	0,049	0,052	0,049	0,051	0,051	0,047	0,049	0,051	0,046	0,051	0,054	0,052	0,054	0,052	0,052	0,052	0,046	0,046	0,046	0,051	0,046	0,046	0,046	0,046	0,046	0,051	0,052	0,049	0,051	0,055	0,047	0,047	0,047	0,044	0,044	0,046	0,044	0,044	0,046	0,046	0,046	0,047	0,051	0,046	0,046	0,046	0,046	0,046	0,009	0,000																																					
A. s. str.	OM523173	0,044	0,044	0,043	0,049	0,039	0,044	0,044	0,044	0,043	0,044	0,039	0,044	0,041	0,048	0,049	0,048	0,048	0,048	0,039	0,039	0,039	0,044	0,039	0,039	0,039	0,039	0,039	0,044	0,048	0,041	0,046	0,051	0,043	0,043	0,039	0,038	0,038	0,039	0,038	0,038	0,039	0,046	0,046	0,048	0,044	0,046	0,044	0,044	0,046	0,046	0,041	0,038	0,031	0,031																																					
A. sp.	OP216036	0,028	0,028	0,027	0,031	0,026	0,028	0,028	0,038	0,028	0,028	0,023	0,028	0,028	0,028	0,033	0,028	0,028	0,028	0,023	0,023	0,025	0,028	0,023	0,025	0,023	0,023	0,025	0,028	0,028	0,025	0,030	0,034	0,026	0,026	0,027	0,022	0,023	0,023	0,023	0,022	0,023	0,039	0,039	0,041	0,028	0,039	0,028	0,028	0,039	0,039	0,025	0,049	0,046	0,046	0,036																																					
T. eloumdeni	MO76629	0,086	0,086	0,084	0,086	0,086	0,086	0,086	0,084	0,089	0,086	0,086	0,090	0,088	0,090	0,088	0,090	0,090	0,090	0,086	0,086	0,086	0,090	0,086	0,086	0,086	0,086	0,086	0,090	0,090	0,084	0,089	0,095	0,086	0,086	0,086	0,084	0,084	0,086	0,084	0,084	0,086	0,084	0,084	0,086	0,086	0,084	0,086	0,084	0,084	0,084	0,071	0,069	0,069	0,062	0,084																																					
A. holobrunnea	OM523179	0,119	0,121	0,119	0,123	0,125	0,119	0,119	0,123	0,123	0,119	0,125	0,117	0,117	0,121	0,119	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,121	0,119	0,125	0,123	0,121	0,121	0,121	0,119	0,119	0,121	0,119	0,119	0,121	0,121	0,121	0,123	0,119	0,121	0,119	0,119	0,121	0,121	0,119	0,112	0,110	0,110	0,114	0,114	0,120

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OŚWIADCZENIE

Oświadczam, że w pracy **Paśnik A, Przybyłowicz Ł (2023) Systematics and phylogeny of *Apisa* (Lepidoptera: Erebidae: Syntomini) – current stage of knowledge and perspectives.**
Mój udział polegał na: pomocy w opracowaniu koncepcji badań i interpretacji wyników, konsultacji części dyskusyjnej oraz weryfikacji spójności merytorycznej tekstu manuskryptu. Swój wkład w przygotowanie publikacji oceniam na 25%.

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OŚWIADCZENIE

Przyjmuję rozprawę doktorską wykonaną pod moim promotorstwem przez panią mgr Annę Paśnik ust. pt. „Systematyka i filogeneza afrykańskich motyli z rodzaju *Apisa*."
W mojej ocenie rozprawa ta spełnia kryteria ustawowe określone w art. 1 ustawy z dnia 20 lipca 2018r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018r. poz. 1668).

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OŚWIADCZENIE

Oświadczam, że w pracy **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**
mój udział polegał na pomocy w opracowaniu koncepcji badań, interpretacji wyników i konsultacji tekstu manuskryptu. Swój wkład w przygotowanie publikacji oceniam na 15%.

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