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Junonia agnesberenyiae sp. nov. (Lepidoptera, Nymphalidae, Nymphalinae) from the Nimba Mountains (Guinea), West Africa

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Abstract: A new taxon of *Junonia* near *ansorgei* was collected by the author during a butterfly survey in the Nimba Mountains, Guinea in 2017. Careful comparison with a series of *J. ansorgei* from various localities throughout its distribution, including examination of the COI gene of both taxa revealed that the two are not conspecific and the new species is described as *Junonia agnesberenyiae* sp.nov. *J. agnesberenyiae* inhabits the montane slope and gallery forests of the Nimba Mountains above 1500 m (asl.) and is probably endemic to the montane zone of the Guinea Highlands and thus the new species is of conservation concern.

Key words: Lepidoptera, Nymphalidae, new species, endemism, montane forest, Guinea Highlands

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INTRODUCTION

During a butterfly survey in the Nimba Mountains, Guinea in May-June 2017 the author and his team captured a small series of a Nymphalidae, closely resembling the Eastern-Central African *Junonia ansorgei* (Rothschild, 1899) but with visibly more extensive metallic green on the upper surface including a sub-apical patch with a characteristically dentated inner edge. The extreme disjunction from the nearest known occurrences and morphological differences indicated that the Nimba population represents an undescribed taxon near *J. ansorgei* but the differences of wing pattern alone were not sufficiently pronounced to allow a decision on its taxonomic status. It was therefore logical to involve dissections of male genitalia and extraction of the COI section of mitochondrial DNA for comparison, which, along with a biogeographical overview of the known populations, allowed recognition of a new species, which is described below.

MATERIALS AND METHODS

Extensive *J. ansorgei* material was examined in the Natural History Museum (NHM), London (44 specimens from Cameroon, Democratic Republic of Congo (DRC), Uganda, Ethiopia, Tanzania and Kenya) and the African Butterfly Research Institute (120 specimens from Cameroon, DRC – mainly Kivu, Uganda, Tanzania, Zambia and Ethiopia) to justify the recognition of the new species, as well as illustrations of specimens from various populations, including those collected in North-western Zambia.

Images of the published literature were also studied (Berger, 1981; Heath *et al.*, 2002; Larsen, 1991; d'Abrera, 1997) and high resolution images of two voucher specimens (catalogue numbers: YPM ENT 412395, YPM ENT 412397) on the homepage of Yale, Peabody Museum of Natural History (collections.peadody.yale.edu) were viewed online. The available distribution records were also used in the biogeographic overview of the species.

The dissection techniques and the digital process of images follow Pyrcz & Sáfián (2018), the author uses the simplified "English" or numerical system in reference for venation described by Miller (1970).

To obtain COI sequences, DNA was extracted using NucleoSpin®Tissue (Macherey-Nagel) according to manufacturer's instruction. Primers HybLCO and HybHCO were used for amplification of *COI* mitochondrial marker. They are universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) adjusted with a T7/T3 universal primer pair (Wahlberg & Wheat, 2008). PCR was performed with a following cycle: 95ºC – 5 minutes, 40x(94ºC – 30 seconds, 50ºC – 30 seconds, 72ºC – 90 seconds), 72ºC – 10 minutes. PCR results were checked by agarose gel electrophoresis with Midori Green DNA Stain. Purification and sequencing was performed by Macrogen (Netherlands). The sequence was compared with the available voucher sample of *J. ansorgei* (NW 139-1), collected by Freerk Molleman in Uganda and sequenced by Niklas Wahlberg: (http://www.nymphalidae.net/story.php?code=NW139-1).

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Abbreviations

ANHRT: African Natural History Research Trust, Leominster, United Kingdom.
ABRI: African Butterfly Research Institute, Nairobi, Kenya.
MZUJ: Nature Education Centre, Jagiellonian University, Kraków, Poland.

DESCRIPTION OF NEW SPECIES

Genus *Junonia* Hübner, [1819]

In: Hübner, [1816-[1826]. *Verzeichniss bekannter Schmettlinge* 34 (432 + 72 pp.). Augsburg. Type-species: *Papilio lavinia* Cramer, by subsequent designation (Scudder, 1872 *Report of the Peabody Academy of Science* **1871**: 43 (24-82).

***Junonia agnesberenyiae* sp. nov.** (Figs 1A, 1D; 2A, 2D; 3A, 3D; 4A, 4D)
urn:lsid:zoobank.org:act: 2BFA12F4-D7F6-4B76-821B-A9AE17B5F1BE

Holotype: ♀ Guinea, Nimba Mountains (Monts Nimba) Mont Pierre Richeaud 01-13.VI.2017. Leg.: Sáfián, Sz. Coordinates: 7°39'49.93"N, 8°22'22.19"W. Elevation: 1500 m. Depository: African Natural History Research Trust (ANHRT), Leominster, UK. ANHRT code: ANHRT00029115.

Paratypes: 2 ♂; 3 ♀ as for Holotype, deposited in ANHRT, ABRI and MZUJ.

Facies
Holotype – female (Figs 1A, 1D):
Wingspan: 57.5 mm. Forewing length: 33.5 mm, mean 21.2 mm. Antennae: 13 mm.
Wing shape unique dead-leaf-mimicking with pointy forewing apex and strongly concave outer margin. Hindwing edge strongly rounded, prolonged into a narrow tail-like tornal projection at end of vein 1. Ground colour black, overlaid with metallic green on basal two-thirds of both wings. Green colour extends into apical area on forewings, separated by black band of zigzagging outer and diffuse inner edge. Green sheen is also present as bands along margins of both wings and submarginal lines. Marginal area also shows blue sheen in artificial illumination, stronger on hindwing. Hindwing sub-tornal area has dark-red, black eyespot with blue central dot. Underside dark, almost blackish-brown with dark olive-green pattern and dark cross-bands present on both wings: narrow one on forewing extending from vein 1 to vein 5 on, broader one on hindwing, extending from tip of tornal projection to costa. Entire underside shows metallic reflection of various colours from green to deep purple in artificial illumination. Both wings also speckled with tiny green dots-scales. Eyespot present but smaller also on underside. Body dark brown, densely covered with hairs with metallic sheen on dorsal and deep purple on ventral side. Legs dark grey, antennae are black on dorsal, pale yellowish-grey on ventral side. Clubs black with yellow tip.

Paratype male (Figs 2A, 2D):
The appearance of male *J. agnesberenyiae* is very similar to females, showing virtually no dimorphism between sexes, except the smaller size and the even more acute apex of males. Apical green patch might be disconnected from main green forewing patch.

Genitalia

Male (Figs 3A, 3D)
Compact, cylindrical and very strongly sclerotised. Uncus long, bird beak-like in lateral view, pointy, triangular-shaped in dorsoventral view. Tegumen curving down into triangular saccus, Valvae specialized, crescent-shaped, ending in elongate strongly toothed fist-like process and an additional thumb-like lobe present below tip. Sacculus bold at base with spine like posterior saccular process. Tip ends in hairy saccular extension. Aedeagus lanceolate, gently bent with spiny tip.

Differential diagnosis

J. agnesberenyiae is readily recognisable from its metallic green patch with strongly toothed inner edge in the sub-apex of forewing in both sexes (attached to the main green area with a line in most females), which feature is missing from all other populations of *J. ansorgei*. Beside this instantly recognisable difference, other diagnostic features are present, including the significantly acuter forewing apex and more drawn out (concave) outer margin of both sexes in *J. agnesberenyiae*. They also differ in key features in the male genitalia, particularly in the tip of the terminal process of valvae, which is very bold and more strongly toothed in *J. agnesberenyiae*.

Etymology

The author names this beautiful butterfly in honour of Ágnes Berényi of Balatonfüred, Hungary, ex-university friend of the author in Sopron.

DISCUSSION

The case of *J. agnesberenyiae* is borderline, where the main question is whether the population in the Nimba Mountains really differs sufficiently from *J. ansorgei* to be recognised as a distinct species rather than as a subspecies of the latter. Superficially, the two are morphologically so similar that they could easily be considered conspecific. Even the only partially extracted COI sequence (658 bp) shows a borderline case, where *J. agnesberenyiae* differs in the COI sequence from the publicly available sequence of *J. ansorgei* by 2%, while the currently recognised threshold in Nymphalidae is approximately 4% (Aduse-Poku, pers. comm.). However, its biogeographic position and habitat speciation support that *J. agnesberenyiae* deserves recognition as a full species.

Biogeography

The nearest records of *J. ansorgei* are from the Bamenda Highlands near Sabga (2 specimens) approximately 2000

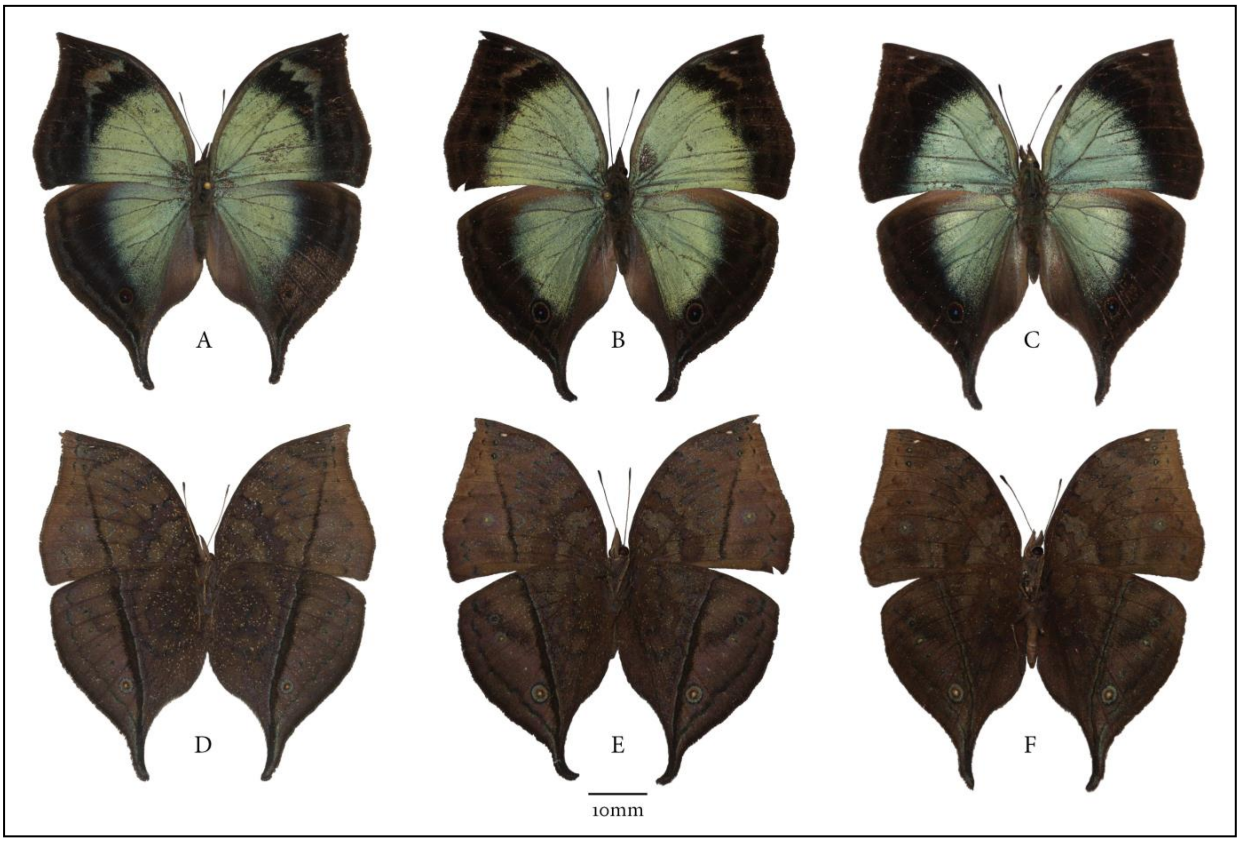

Figure 1 – Females. *J. agnesberenyiae* sp. nov. (holotype) A – recto, D – verso; *J. ansorgei* (Kivu, DRC) B – recto, E – verso; *J. ansorgei* (Mount Tabenken, Cameroon) C – recto, F – verso.

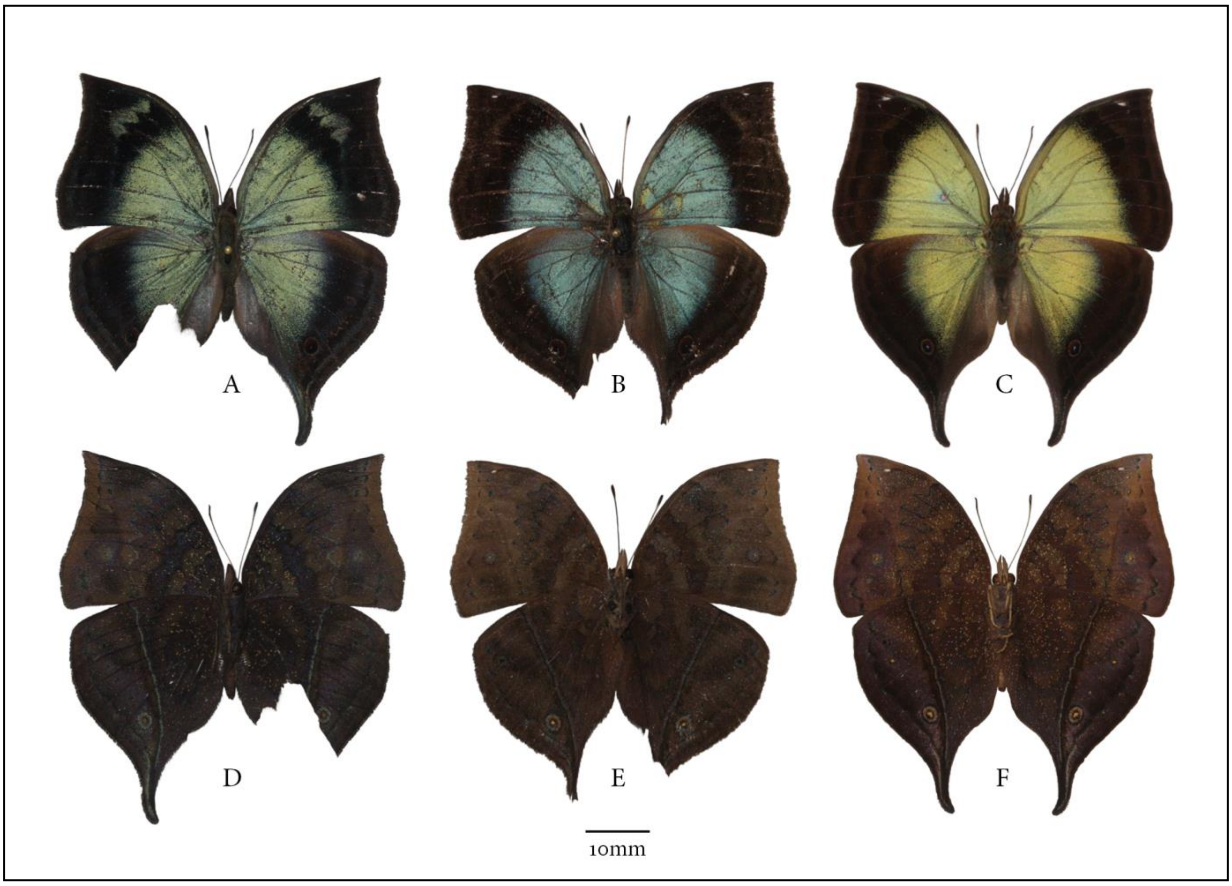

Figure 2 – Males. *J. agnesberenyiae* sp. nov. (paratype) A – recto, D – verso; *J. ansorgei* (Mount Tabenken, Cameroon) A – recto, E – verso; *J. ansorgei* (Semliki, Uganda) C – recto, F – verso.

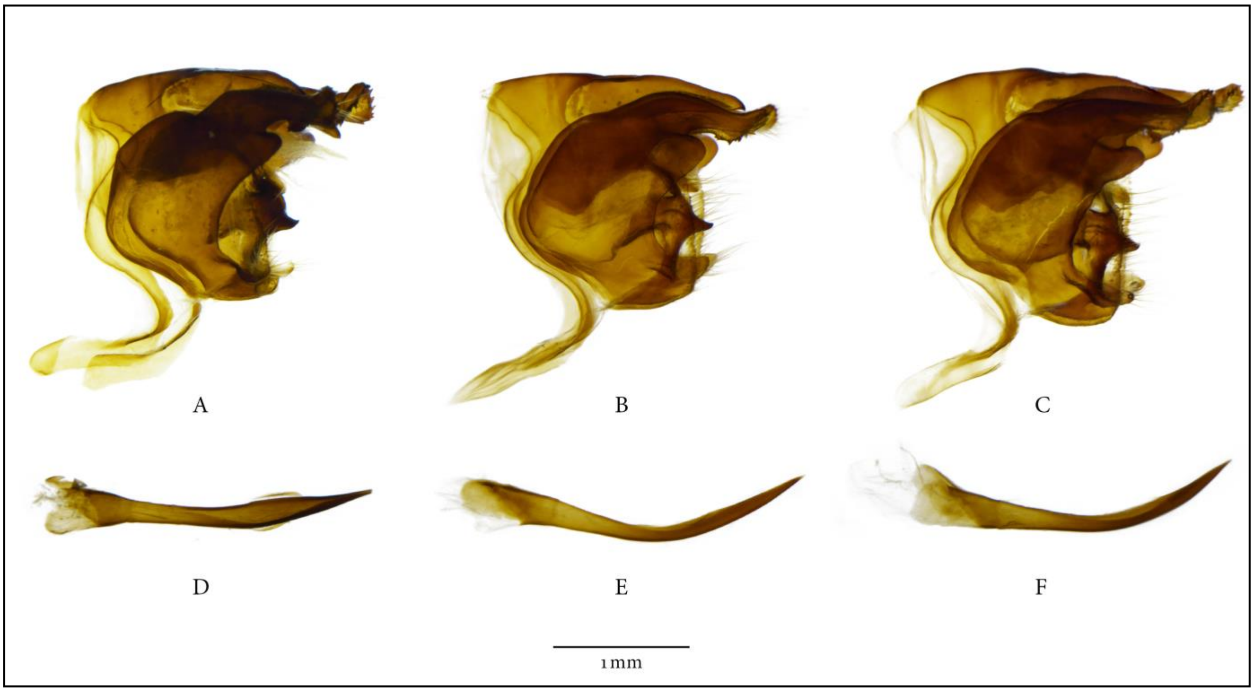

Figure 3 – Male genitalia (lateral view) and separated aedeagi of *J. agnesberenyiae* sp. nov. (paratype) – A, D; *J. ansorgei* (Mount Tabenken, Cameroon) – B, E and *J. ansorgei* (Semliki, Uganda) – C, F.

Figure 4 – Male genitalia (posterior and dorsal views) of *J. agnesberenyiae* sp. nov. (paratype) – A, D; *J. ansorgei* (Mount Tabenken, Cameroon) – B, E and *J. ansorgei* (Semliki, Uganda) – C, F.

km away from the Nimba Mountains, where the species was collected at about 1300 m asl. Further Cameroonian material from Bamboutos Mountains, Mount Mbam, and Mount Tabenken was found in the ABRI collection. These specimens were examined, and they could not morphologically be considered distinct from the nominate subspecies, with no or only minimal metallic green scaling in the apex. The extreme disjunction of *J. agnesberenyiae* and adaptation to montane forest habitat has certainly allowed significant divergence of the taxa. The differences in wing pattern show stability over the type series and the many examined specimens of *J. ansorgei*. Male genitalia also show readily recognisable differences. It must be stressed that virtually no similar montane habitats exist between the Cameroon Highlands and the Nimba Mountains.

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Figure 5 – Freshly hatched female *J. agnesberenyiae*, sunning along a forest edge at the type locality, Nimba Mountains, Guinea.

Figure 6 – Type locality and habitat of *J. agnesberenyiae* – montane forest in the Nimba Mountains (600 Forest, Mont Pierre Richeaud).

The only mountain range that reaches over 1200 m a.s.l. are found in the Jos Plateau area in Northern Nigeria, but the entire plateau is now too dry to maintain sub-montane or montane forest habitats. This applies also to the scattered mountains in Northern Ivory Coast, where such habitats could have existed during period of the last forest maximum of the Guineo-Congolian forest zone, which ended approximately 6000 years ago (Hamilton & Taylor, 1991), but since the butterfly is much more specialised on sub-montane and montane forests, it is also very sedentary and does not seem to fly much spontaneously, it is more likely that the possible common ancestor was rather widely distributed during one of the wetter interglacial periods or even before the first northern hemispheric glaciation.

Similar biogeographic pattern is not unusual in Africa, with even ecologically more tolerant species separated by the well-recognised biogeographic boundaries, such as the Dahomey Gap. One of the more striking examples is *Euphaedra ruspina* (Hewitson, 1865), which is separated from its very different relative *E. perseis* (Drury, 1773) only by the River Volta and a narrow stretch of savannah land.

Using COI gene sequences, it was recently confirmed that Libert (2005) made a correct reinstatement of *H. decira* (Plötz, 1880) for the populations formerly treated as *Heteropsis peitho* (Plötz, 1880) west of the Dahomey Gap. Although the difference in COI barcodes between these two species exceeded 4% (Aduse-Poku *et al.*, 2016), they show no significant morphological differences.

Several other species were recognised recently with similar distribution patterns, including the Liberian sub-region endemic *Cephetola wingae*, *Stempfferia katikae*, and *Pilodeudorix mano* (Sáfián, 2015a; 2015b). The closest case is that of *Uranothauma belcastroi* Larsen, 1997. It differs little from *U. frederikkae* Libert, 1993, which occurs only in the sub-montane, montane zone of the Nigeria-Cameroon Highlands. In analogy to *J. agnesberenyiae*, *U. belcastroi* is very localised and found only in a few mountains in the Guinea Highlands, but sometimes also at lower altitude compared to *J. agnesberenyiae* (Larsen, 2005).

Conservation

J. agnesberenyiae seems to be a very localised species, inhabiting sub-montane and montane forests above 1500 m asl. in the Nimba Mountains, and possibly also other high mountains in the Guinea Highlands. The extent of such montane habitats is usually restricted to gullies on the higher slopes and small flatter plateaus or depressions along the ridge of the Nimba Mountains and even the extent of potential habitat is probably less than about 1000 hectares. Currently, *J. agnesberenyiae* is known to occur in a single locality, with a possible unconfirmed sighting in the Simandou Mountains, further northwest in Guinea in similar habitat. Other localised populations could potentially still occur in West African mountains reaching above 1500 m asl., including the montane forests of the Tingi and Loma Mountains in Sierra Leone but probably excluding the already drier highest peaks of the Fouta Djalon area in North-western Guinea. Using IUCN criteria to assess its conservation status, *J. agnesberenyiae* currently falls in the category of Critically Endangered (CR B2a) with only a single population existing and an area of occupancy of <10 km^2. Potential threats are identified as natural landslides, human induced fire combined with extreme climatic events (drought), human caused climate change and potential mining operation in the close vicinity of the habitats (IUCN, 2012).

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