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A new species of the genus *Amerila* Walker, 1855 from Tanzania (Lepidoptera: Erebidae: Arctiinae: Amerilini)


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Abstract

In this paper, we describe a new species, *Amerila francesae* Ignatev & László, **sp. n.**, from Tanzania, East Africa. Considering the distinctive characters of external and genital morphology corroborated by genetic data retrieved from mitochondrial COI-5P gene, we assign this new species and *A. mulleri* Häuser & Boppré, 1997 with the *Amerila magnifica* Rothschild, 1910 species complex. We illustrate the habitus, the male and female genitalia, the distribution of the new species and its closest allies.

Key words: Integrative taxonomy, systematics, genitalia morphology, DNA barcode, COI-5P, Afrotropics.

Introduction

Amerila Walker, 1855 is a species-rich genus of tiger moths (Erebidae: Arctiinae: Amerilini) comprising more than 90 described species distributed in the Old World tropics (Häuser 1993). De Prins & de Prins (2021) lists 43 valid species-group names from Sub-Saharan Africa, including its offshore islands, Madagascar and the Mascarene Archipelago. The genus has been attracting an increased attention of collectors as well as researchers since a monograph of the Afrotropical *Amerila* by Häuser & Boppré (1997) was published. This revision underlaid several more recent studies regarding the genus (e.g., Dubatolov (2009), Przybyłowicz *et al.* (2019b, c)), and highlighted some morphological, taxonomic and phylogenetic gaps still in need to be filled.

Detailed morphological and molecular studies of the extensive *Amerila* materials in the holdings of the African Natural History Research Trust (Leominster, United Kingdom), the Museum Witt (Weiden in der Oberpfalz, Germany), and the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, (Kraków, Poland) enabled us to contribute to the taxonomic and phylogenetic knowledge of *Amerila* in general and led to the discovery of a new species which is described in this paper: *A. francesae* **sp. n.** Additionally, we discuss the potential affinities of the new species to *A. magnifica* Rothschild, 1910 and *A. mulleri* Häuser & Boppré, 1997. Systematic studies conducted by Zahiri *et al.* (2012), Zaspel *et al.* (2014), and Przybyłowicz *et al.* (2019a) suggested that *Amerila* forms a distinct lineage highly diverging from other Arctiinae clades leading to the delineation of the genus in a separate tribe (Dubatolov 2010). This concept seems to be well supported by recent molecular and morphological studies (Ignatev *in prep.*) and it is followed in this paper.

Material and methods

Abbreviations of the depositories used:

ANHRT – African Natural History Research Trust, Leominster, United Kingdom;

MWW – Museum Witt, Weiden in der Oberpfalz, Germany;

NECJU – Nature Education Centre of Jagiellonian University, Krakow, Poland;

ZSM – Bavarian State Collection of Zoology/Zoologische Staatssammlung München, Munich, Germany.

Other abbreviations used:

CCDB – Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph;

DRC – Democratic Republic of the Congo;

LG – genitalia slides prepared by Gyula M. László.

Morphological studies

The genital apparatuses were dissected, stained with Evans Blue or Eosin Red and embedded in Euparal on microscope slides applying standard methods of preparation (Lafontaine & Mikkola 1987). Photos of adults were taken using a Nikon D90 SLR camera equipped with Nikkor AF Micro 60 mm lens. Genitalia were photographed either by using a Leica DFC450 camera mounted on a stereo microscope or a Canon EOS 700D camera mounted on a Wild M7A stereo microscope.

Forewing length was measured from the base to the apex of the wings, along the wing costa.

Terminology of genital morphology follows Kôda (1987).

The map of distribution for *A. magnifica* species complex was created by using <https://www.simplemappr.net/> website.

Data of material examined

Amerila magnifica (Rothschild, 1910): Mozambique. 1 ♂, Maputo Special Reserve, Ponta Milibangalala, Dune Grassland, 15m, 26°26'58.6"S, 32°55'29.8"E, 25-30.V.2017, MV Light Trap, Aristophanous, M., László, G., Miles, W., Vetina, A. leg.; 1 ♂, Maputo Special Reserve, West Gate, Sand Thicket, 22m, 26°30'14.2"S, 32°42'59.6"E, 21-30.XI.2016, Light Trap, Aristophanous, M., Cristovaõ, J., László, G., Miles, W. leg. DNA Barcode/BOLD process id. ANHRTUK-0006175/ANLMN3308-21; 1 ♂, same data, DNA Barcode/BOLD process id. ANHRTUK-0006176/ANLMN3309-21, gen. slide No. LG 5500 (ANHRT).

Amerila mulleri Häuser & Boppré, 1997: Tanzania. 1 ♂, Mount Meru, Arusha NP, 1679m, S03°14'51", E36°50'38", 18-24.VII.2012, Light Trap, leg. Smith, R. & Takano, H., gen. slide No. LG 4521 (ANHRT). DRC. 1 ♀, Cameroon, Mt. Cameroon (SW slope), Elephant camp (1850 m asl) 4°08'43.2"N, 9°05'13.2"E, 2014.11.24, lgt. V. Maicher, Sz. Safian, S. Janecek, R. Tropek, genitalia structures are in the glycerine in a vial attached to the specimen (NECJU).

Amerila francesae **sp. n.**: data are listed under the description of the new species.

Molecular analysis

MWW specimen of *A. francesae* **sp. n.** (No. NI141): Total genomic DNA was extracted from leg muscle tissue of dried specimen preserved in pure ethanol using the Genomic DNA Mini Kit (Tissue) (Geneaid,

Taiwan). The cytochrome c oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.* 1994). PCR were performed in 11.5 µl reaction volume using 6.25 µl, 2 x Bioline MyTaq HS Red Mix, 4 µl dH₂O (PCR H₂O), 0,625 µl of each primer and 2 µl of the genomic DNA. The PCR profile consisted of an initial denaturation step at 94°C for 5 min, 92°C for 1 min, followed by 40 cycles at 50°C for 1 min, 72°C for 1 min and a final extension step of 5 min at 72°C. Successful PCR products (2 µl each) were purified using the mixture of 135 µl FastAP + 85 µl Exo I, and sequenced in both directions.

ANHRT specimens of *A. magnifica* (n = 2) and *A. francesae* **sp. n.** (n = 1): DNA barcodes were obtained by removing tarsal segments from dry specimens and submitted to the CCBD. Sequences were obtained using Single Molecule Real-Time sequencing through the Sequel (PacBio) pipeline at CCDB (Hebert *et al.* 2018).

The COI barcode sequences for *A. mulleri*, *Miltochrista miniata* (Förster, 1771), and *Catocala semirelictica hippolyta* Gall & Hawks, 2010 (Table 1), were obtained from BOLD (<http://www.boldsystems.org>) and GenBank (<https://www.ncbi.nlm.nih.gov/>) databases. All analysed sequences were edited and assembled in Bioedit 7.2. Informer Technologies, Inc and in Geneious Prime 2021.2.2. ML analyses were carried out in IQTree (Nguyen *et al.* 2015). Bootstrap support was calculated using 1000 replicates. BI analyses were performed in MrBayes (Ronquist & Huelsenbeck 2003) and Geneious Prime 2021.2.2 with four independent runs, each having three heated and one cold chain. Analyses were run for 6 million generations with trees sampled every 1000 generations. The first 25% of each run was discarded as burn-in. Sequences of *Miltochrista miniata* and *Catocala semirelictica hippolyta* were used as outgroups. Tree was visualized using Geneious Prime 2021.2.2. Pairwise sequence divergence was calculated using Kimura's two parameter (K2P) distance model (Kimura 1980) in MEGA version (Kumar *et al.* 2018).

Table 1. Taxonomic information and GenBank COI accession numbers of the taxa included in this study.

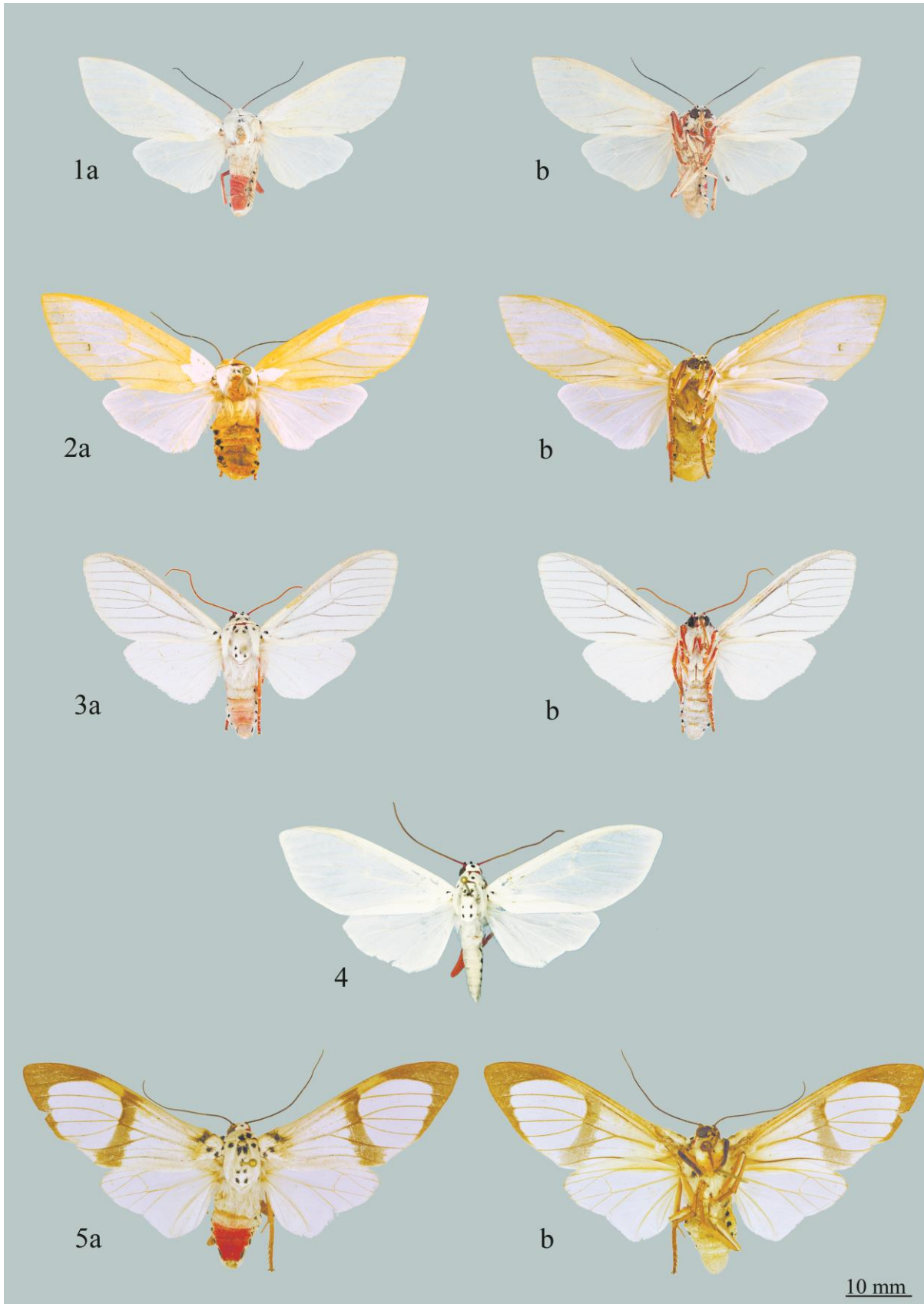
Species	Specimen	Origin	GenBank COI Acc. №	References
<i>Amerila mulleri</i>	CAM_101	Cameroon	MG596292	Przybylowicz <i>et al.</i> 2019a
	CAM_102	Cameroon	MG596293	Przybylowicz <i>et al.</i> 2019a
	CAM_158	Cameroon	MG596294	Przybylowicz <i>et al.</i> 2019a
	CAM_159	Cameroon	MG596295	Przybylowicz <i>et al.</i> 2019a
<i>Amerila magnifica</i>	ANLMN3308-21	Mozambique	OM158448	This study
	ANLMN3309-21	Mozambique	OM158446	This study
<i>Amerila francesae</i> sp.n.	NII141	Tanzania	OM169367	This study
	ANLMN3427-21	Tanzania	OM158447	This study
<i>Catocala semirelictica</i> <i>hippolyta</i>	ABCNA001-06	USA, California	MF126536	Zahiri <i>et al.</i> 2017
<i>Miltochrista miniata</i>	ABOLA123-14.COI-5P	Austria	MG522231	Huemer <i>et al.</i> 2018

Description of the new species

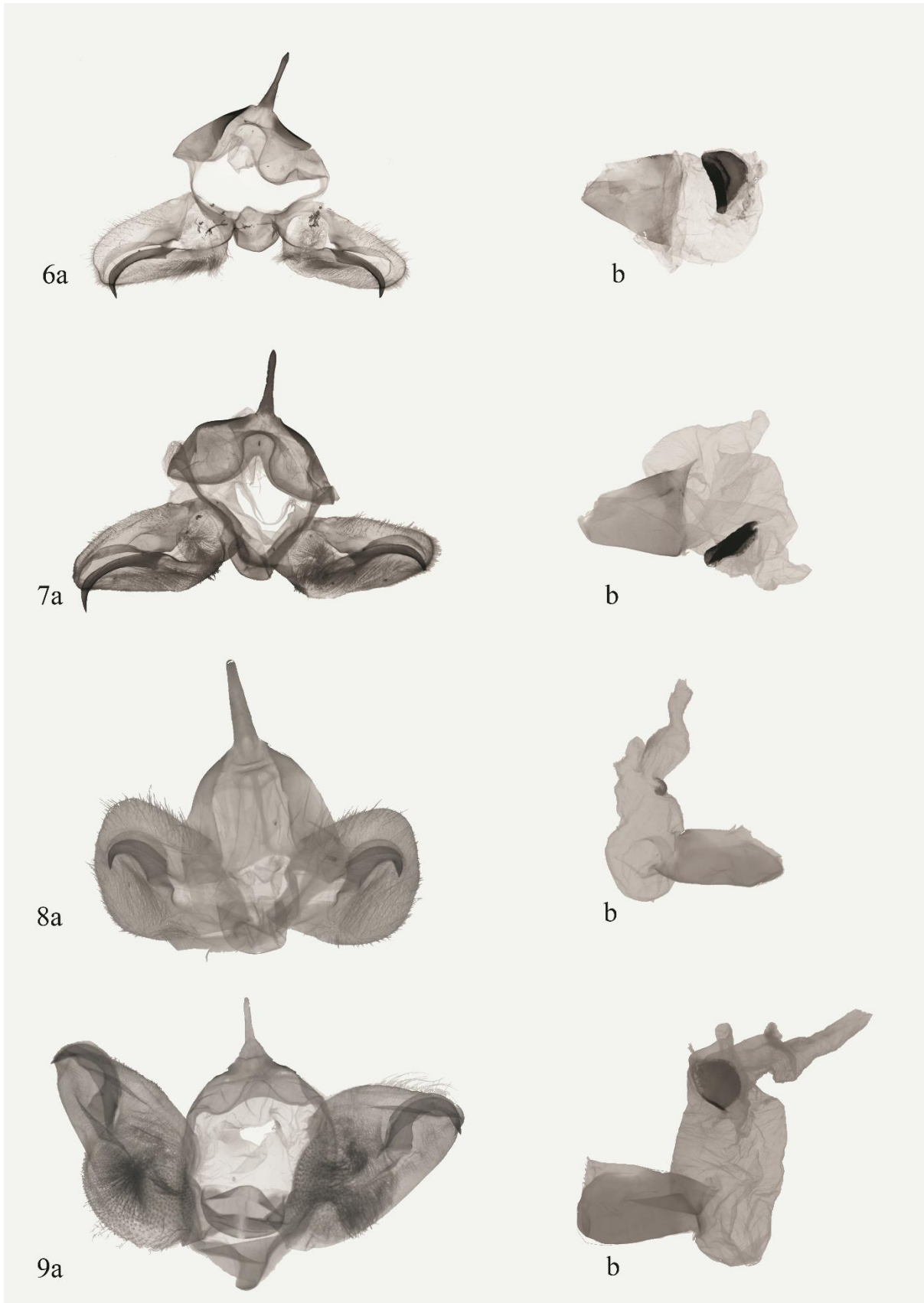
Amerila francesae Ignatev & László, **sp. n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:BFC3933A-50F0-401B-9871-321462F445F3>
(Figs 1, 2, 6, 7, 10, 12, 13)

Holotype. ♂, “Tanzania Tanga, Usambara Mountains, 17km NW Lushoto, Magamba 28. II. – 1.III. 2003, 1900m. leg. M. Fibiger, H. Hacker, K. Larsen, H.-P. Schreier.”, Gen. slide No.-33.477, DNA process id. NII141 (MWW).



Figures 1-5. Adults of *Amerila magnifica* Rtsch. 1910 species complex. 1 – holotype ♂ of *A. francesae* **sp. n.** dorsal view (a), ventral view (b) Tanzania (MWW); 2 – paratype ♀ of *A. francesae* **sp. n.** dorsal view (a), ventral view (b) Tanzania (MWW); 3 – ♂ of *A. mulleri* dorsal view (a), ventral view (b) Tanzania (ANHRT); 4 – ♀ of *A. mulleri* dorsal view Cameroon (NECJU); 5 – ♂ of *A. magnifica* dorsal view (a), ventral view (b) Mozambique (ANHRT).



Figures 6-9. Male genitalia of *Amerila magnifica* species complex. 6 – *A. francesae* **sp. n.** holotype ventral view (a), aedeagus (b) Gen. slide No. 33.477, Tanzania (MWW); 7 – *A. francesae* **sp. n.** paratype ventral view (a), aedeagus (b) Gen. slide No. LG 4522, Tanzania (ANHRT); 8 – *A. mulleri* ventral view (a), aedeagus (b) Gen. slide No. LG 4521, Tanzania (ANHRT); 9 – *A. magnifica* ventral view (a), aedeagus (b) Gen. slide No. LG 5500, Mozambique (ANHRT).

Paratypes (9 ♂, 2 ♀ in total). 5 ♂ and 2 ♀, with the same data as in the holotype, Gen. slide No. 36.320 (♀) (MWW); 1 ♂, same data, (coll. Günter Müller, Freising, Germany / Bamako, Mali); 1 ♂, Iringa region, S Njombe, Nundu Forest, 2045m, 09°26.048'S, 034°49.846'E, 6-IV-2007, (Ph. Darge), Gen. slide No. 36.318 (ZSM); 1 ♂, Tegetero, Uluguru Mountains, 1100m, S06°55'03", E37°43'16", 30.VI-3.VII.2010, Light trap, leg. Smith, R. & Takano, H., Gen. slide No. LG 4522, unique id. ANHRTUK 00194266, DNA barcode/BOLD process id. ANHRTUK-00194266/ANLMN3427-21; 1 ♂, Maskati, Nguru Mountains, 1759m, S06°03'29.3", E37°29'08.4", 4-7.VII.2010 Light trap, leg. Smith, R. & Takano, H., Gen. slide No. LG 5488, unique id. ANHRTUK 00194065 (ANHRT).

Diagnosis. The new species is confusingly similar to *A. mulleri* Häuser & Boppré, 1997 (Figs 3, 4, 8, 11) but distinguished in the shape of the forewing, where the apex in *A. francesae* **sp. n.** is narrower and more pointed (Figs 1, 2, 3, 4). The configuration of the male genitalia of the two species differs in the following features: *A. francesae* has a somewhat narrower uncus, markedly shorter and broader tegumen, more elongate valva and much longer and narrower harpe. The aedeagus and the vesica of the new species is considerably thicker, with much larger, rod-like cornutus bearing two short longitudinal lobes, which is a small, round, scobinated bulge in the allied species (Figs 6, 7, 8, 9). The structure of female genitalia (Figs 10, 11) should be used for safe identification, where signum bursae represented by a tight band of sclerotized patches and dashes, is specific for *A. francesae* **sp. n.** and is absent in *A. mulleri*.

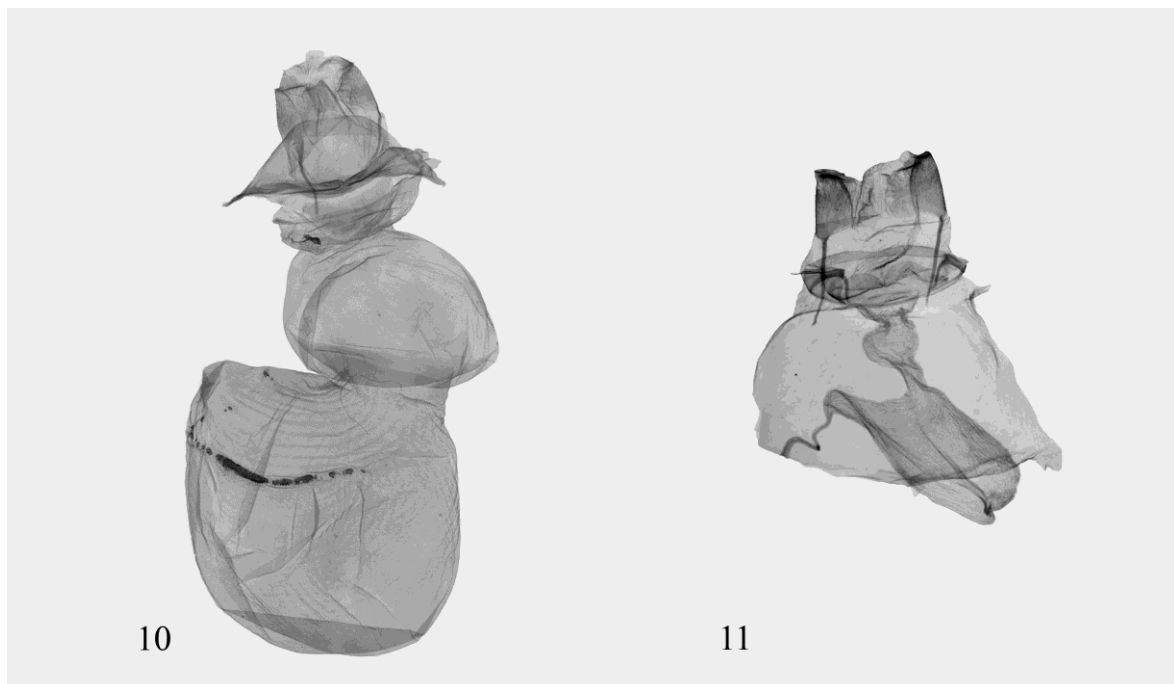
Description. Male (Fig. 1). Wingspan: 48-54 mm (holotype 52 mm); forewing length: 24-26 mm (holotype 25 mm). Head. Antenna filiform, dark blackish-brown; scapus and pedicellus barrel-shaped, brownish-red; flagellum with very thin and short ciliation, first 10-11 segments of flagellum covered in brownish-red scales dorsally. Frons lightly bulged, white, with belt of black scales basally; vertex thickly covered by white scales, with large black sub-quadrate central mark posteriorly. Compound eye very large, globular; ocellus distinct; proboscis well developed. Labial palp upcurved, rounded apically, covered by red and white scales, distally bearing a belt of dark brown scales; mid segment slightly longer than basal segment; distal segment half as long as mid segment, with blackish-brown apex. Thorax. Patagium, tegula, mesoscutum, mesoscutellum white; patagium with a small, dark brown lateral dot, tegula with a similar posterior dot, size and intensity of dots may vary. Foreleg. Coxa white, with dark brown blotch subbasally; femur pale red, with narrow field of white scales in inner side, distally with a blotch of dark brown scales; tibia white with narrow field of pale red scales in inner side; tarsus covered by white, red and pale brown scales. Mid- and hindlegs similar in coloration. Forewing. Uniformly snow-white, relatively narrow, triangular, apically rounded, costal margin straight in its proximal two-thirds, then gently arcuate; ventral margin straight. Underside as upperside. Hindwing. Uniformly white, triangular, with rounded apex; costal margin slightly curved; outer margin slightly concave medially; ventral margin almost straight. Abdomen. Both sides of segments 1-4 covered in long white scales; segments 5-8 dorsally covered in short red scales, ventral side of all segments white with an admixture of pale yellow scales; lateral side bearing two lines of black blotches, one along the lateral margin of tergites and another subspiracular.

Male genitalia (Figs 6, 7). Uncus long, narrow, distally slightly tapered, apically pointed. Tegumen short, rather broad, dome-shaped, deeply notched antero-medially; vinculum short, broadly rounded; saccus well developed, rounded. Valva broad, ovoid, dorsal margin gently arcuate, ventral margin almost straight, apex broadly rounded; valva surface with long, thin setae along ventral and dorsal margins. Harpe long, slim, gradually tapered distally, curved ventrally, pointed apically, reaching outer margin of valva. Corema small, spherical, covered in fine, long setae. Juxta membranous, unmodified. Aedeagus very short, strongly dilating posteriorly, weakly sclerotized; vesica relatively short, very thick, with a large, inflated basal diverticulum; cornutus situated distally, heavily sclerotized, elongate, thick, rod-like with two short, longitudinal lobes.

Description. Female (Fig. 2). Wingspan: 51-57 mm; forewing length: 25-28 mm. Head. Antenna filiform, dark blackish-brown; scapus and pedicellus barrel-shaped and brownish-red; flagellum with very thin and short ciliation, first 6-8 segments of flagellum covered by brownish-red scales, with white scales in inner side. Frons slightly bulged, white, with a belt of black scales basally; vertex thickly covered in white scales, with large black sub-quadrate central marking posteriorly. Compound eye very large, globular; ocellus distinct; proboscis well developed. Labial palp, upcurved, rounded apically, covered by red and white scales, distally bearing a belt of dark brown scales; mid segment slightly longer than basal segment; distal segment half as long as mid segment, with blackish-brown apex. Thorax. Patagium, tegula, mesoscutum,

mesoscutellum white; patagium laterally with a blotch of black scales; mesoscutum with two round black blotches. Foreleg. Coxa white, with dark brown blotch subbasally; femur pale red, with narrow field of white scales in inner side, distally with a blotch of dark brown scales; tibia white with narrow field of pale red scales in inner side; tarsus covered by white, red and pale brown scales. Mid- and hindlegs similar in coloration. Forewing. Uniformly snow-white with a black basal spot, relatively narrow and elongate, apically pointed; costal margin gently arcuate; ventral margin slightly curved in its basal third. Underside as upperside. Hindwing. Uniformly white, triangular, with rounded apex; costal margin slightly curved; outer margin slightly concave; ventral margin almost straight.

Female genitalia (Fig. 10). Papilla analis short and narrow, apically rounded and covered sparsely with short, fine setae. Posterior apophysis moderately long and rather thin. Eighth tergite short, broad-based trapezoidal, weakly sclerotized; anterior apophysis nearly three times shorter than posterior one, wedge-shaped. Ostium bursae very broad, antrum short, funnel-like, weakly sclerotized. Ductus bursae short, conspicuously swollen, membranous. Corpus bursae nearly spherical, membranous, weakly rugose in distal half; signum bursae represented by a narrow, interrupted band of heavily sclerotized, spinulose patches and dashes.



Figures 10-11. Female genitalia of *Amerila magnifica* species complex. 10 – *A. francesae* sp. n. paratype ventral view. Gen. slide No. 36.320, Tanzania (MWW); 11 – *A. mulleri* ventral view, Cameroon (NECJU)

Etymology. It is with great pleasure to dedicate this new species to Ms Frances Witt, daughter of Dr Alessa Witt (one of the co-founders of the world-famous research museum – “Museum WITT”).

Discussion

In their 1997 revision of the Afrotropical *Amerila*, Häuser & Boppré recognized shared characters in the male genitalia structures of *A. mulleri* and *A. magnifica* (Figs 5, 8, 9) suggesting that these species “probably represent a well-differentiated subgroup within the genus” (Häuser & Boppré 1997, p. 30). This well corresponds with our observations of the distinctive male genitalia features namely the conspicuously short aedeagus and the vesica bearing a single, flattened, plate-like sclerotization, rather than a spike-shaped cornutus, and the elongate, undivided uncus which is ca. 5 times longer than in the other known *Amerila* species. It is worth mentioning that besides the shared genitalia features, the males of these species have also the diagnostic semi-red/semi-white dorsal coloration of the abdomen. This set of morphological characters well supports a distinct lineage within *Amerila* comprising *A. magnifica*, *A. mulleri*, and the herewith described *A. francesae*.

NEW SPECIES OF AMERILA FROM TANZANIA

Table 2. Inter- and intraspecific K2P distances of COI-5P sequences in the *A. magnifica* species complex.

COI-5P	<i>A. magnifica</i> ANLMN3308-21	<i>A. magnifica</i> ANLMN3309-21	<i>A. mulleri</i> CAM101	<i>A. mulleri</i> CAM158	<i>A. mulleri</i> CAM102	<i>A. mulleri</i> CAM159	<i>A. francesae</i> sp. n. NI141	<i>A. francesae</i> sp. n. ANLMN3427-21
<i>A. magnifica</i> ANLMN3308-21								
<i>A. magnifica</i> ANLMN3309-21	0,00%							
<i>A. mulleri</i> CAM101	9,36%	9,36%						
<i>A. mulleri</i> CAM158	9,36%	9,36%	0,00%					
<i>A. mulleri</i> CAM102	9,18%	9,18%	0,15%	0,15%				
<i>A. mulleri</i> CAM159	9,18%	9,18%	0,15%	0,15%	0,00%			
<i>A. francesae</i> sp. n. NI141	7,97%	7,97%	4,02%	4,02%	4,37%	4,37%		
<i>A. francesae</i> sp. n. ANLMN3427-21	8,33%	8,33%	6,09%	6,09%	6,26%	6,26%	0,65%	

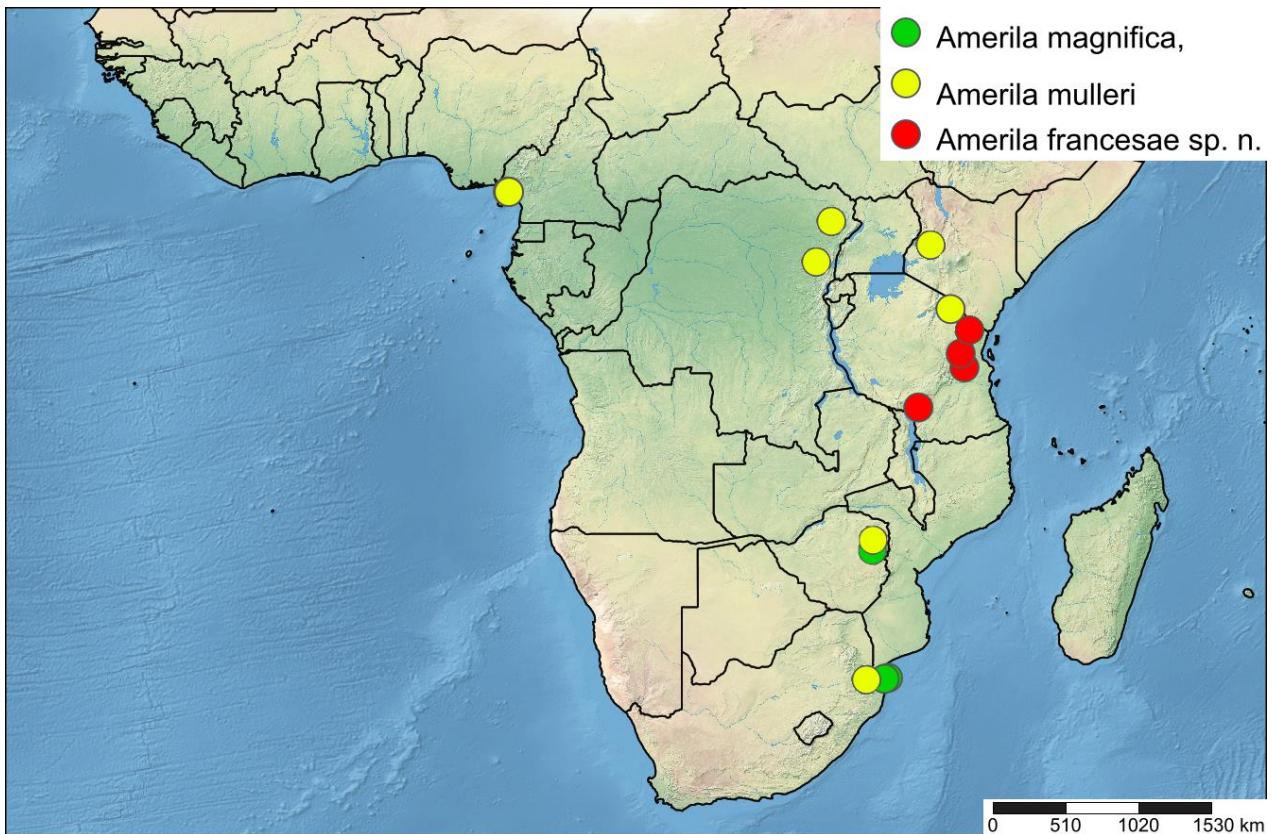


Figure 12. Distribution of the *A. magnifica* species complex.

In total, eight specimens of the *Amerila magnifica* species complex and an outgroup (Table 1., Fig. 13) were included in the molecular analysis. The phylogenetic trees were built using Bayesian and maximum likelihood methods resulting in identical topologies, thus only the Bayesian tree is illustrated in this paper (Fig. 13). The Bayesian posterior probability values for the nodes were, in total, higher than the bootstrap values. This leads to the conclusion, that the taxa of the *A. magnifica* species complex are evidently separated from other *Amerila* lineages forming a monophyletic group. The phylogenetic analysis divides the complex into three sub-lineages in which *A. francesae* is recovered as a sister species to *A. magnifica*, while *A. mulleri* forms a separate unit. The calculated pairwise distances (K2P) of mtDNA are remarkably large within the *A. magnifica* species complex falling in the range of 4.02 – 9.36% (Table 2). The genetic divergence values between *A. magnifica* and *A. mulleri* are 9.18-9.36%, between *A. magnifica* and *A. francesae* are 7.97-8.33%. The new species differs from *A. mulleri* by 4.02-6.26%. The intraspecific divergence within *A. magnifica*, *A. mulleri* and *A. francesae* are 0.00%, 0.15% and 0.65%, respectively.

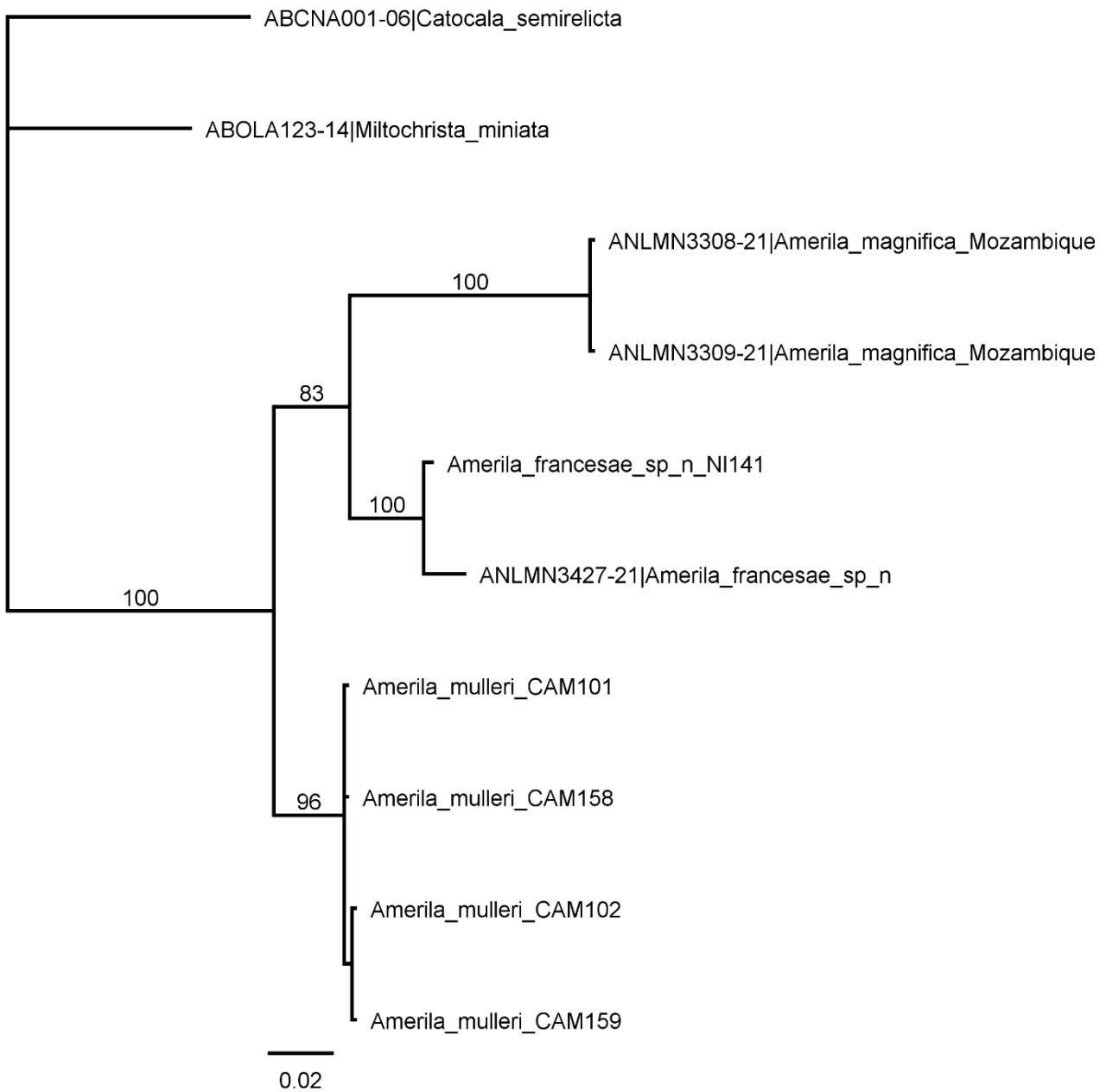


Figure 13. Bayesian phylogenetic tree of the *A. magnifica* species complex based on sequences of mitochondrial cytochrome c oxidase subunit I (COI-5P).

Based on the very few accessed specimens, the species complex shows a peculiar distribution pattern (Fig. 12). *Amerila magnifica* has a southeast African coastal distribution with sparse records from Kenya (the type locality is not specified in the original description), the Vumba Mountains in East Zimbabwe and the Maputoland in South Mozambique which latter locality represents a new distributional record. *Amerila mulleri* was described from the Vumba Mountains in Zimbabwe and reported from Ituri in North Kivu (DRC), the Kakamega Forest in Kenya and Swaziland (Häuser & Boppré 1997). The species has later been recorded from the Mount Cameroon (Przybyłowicz *et al.* 2019b) representing a population being geographically rather remote from the East African main range. *Amerila francesae* seems to have a more limited distribution with scattered records from the Eastern Arc Mountains in Tanzania. However, it cannot be ruled out that the species will be recorded in other mountain systems, as well as that further, yet unknown members of the species group may be present in the isolated mountains of East Africa.

Acknowledgements

We are indebted to Dr Verena Witt & Dr Alessa Witt (MWW), Dr Axel Hausmann (ZSM) and Dr Günter Müller (Freising, Germany / Bamako, Mali) for providing us access to study the Lepidoptera collections under their care. We are grateful for the improvements suggested by the anonymous reviewers.

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