

## Revision of the genus *Neptis* Fabricius, 1807 (Lepidoptera: Nymphalidae) in the Afrotropical Region, Part 3: A new species from Mt Mabu, Moçambique

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**Abstract:** The revision of the genus *Neptis*, Richardson (2019), showed that many new species are revealed by barcoding and one of these species, a close relative of *Neptis rogersi* Eltringham, 1921, is formally described here. The new species from Mt Mabu is *Neptis collinsi* sp. nov. and is easily distinguished from *Neptis rogersi* by both the facies and barcode.

**Key words:** Barcode, Neighbour Joining, Morphology, Genitalia, Phylogeny, Nysiades Group, *Neptis*.

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### INTRODUCTION

Between October 2005 and June 2009 several expeditions were made to selected mountains in the Zambezia and Niassa Provinces of Moçambique and southern Malawi as part of a Darwin Initiative "Monitoring and Managing Biodiversity Loss on South-East Africa's Montane Ecosystems" (Congdon *et al.*, 2010). Six visits were made to Mt Mabu yielding a list of 200 butterfly species including 3 new species, (Bayliss *et al.*, 2014). Mt Mabu is regarded as the largest medium-altitude rainforest in southern Africa. Further expeditions were undertaken under the auspices of the African Butterfly Research Institute (ABRI) between 2010 and 2019 bringing the total number of new species discovered across all the montane localities to 30 (Bayliss *et al.*, 2019).

Whilst examining the very large number of *Neptis* specimens in the ABRI collection, two females from Mt Mabu stood out as not matching any known species but with facies showing clear affinity to *N. rogersi* Eltringham, 1921. These had been captured in November 2010 on one of the first expeditions organised by ABRI. The specimens yielded identical full length barcodes and were allocated the provisional identifier species54. This species is described here as *N. collinsi* sp. nov.

In the revision of the genus *Neptis* (Richardson, 2019), *N. rogersi* was allocated to the *Nysiades* group with some uncertainty about this placing because the *Neptis* phylogeny determined from barcodes alone showed *N. rogersi*, along with, *N. seeldrayersi* Aurivillius, 1895 and *N. alta* Overlaet, 1955, separate from the *Nysiades* group. Continuing work on the *Neptis* phylogeny at the City University of New York (CUNY), Lohman's Lab, divides the Afrotropical *Neptis* species into four clades

based on the analysis of 13 genes including the barcode. The largest of these clades, in terms of the number of species, corresponds to the *Nysiades* group as defined in Richardson (2019), and the three species *N. rogersi*, *N. seeldrayersi* and *N. alta* form a subgroup within this clade. Fig. 1 shows the barcode only phylogeny modified to place this subgroup within the clade or *Nysiades* group in line with the latest results from the CUNY research.

All four of the species *N. rogersi*, *N. collinsi*, *N. seeldrayersi* and *N. alta* have a spotted forewing cell, whereas this pattern of markings is not found in the cell of other species in the *Nysiades* group.

### MATERIAL AND METHODS

The methodology for this aspect of the study is as set out in Richardson (2019). The salient points are restated below. Note that the notation for wing markings in the *Neptis* introduced in Richardson (2019) is used in the sections below on the Description of the facies and in the Diagnosis.

#### Specimen photography

Dorsal and ventral aspects of all specimens for DNA sequencing were photographed using the technique described in Richardson (2019). The accession numbers of photographed specimens are stated in the figure captions and a shortened version of the ABRI accession number is used. For example, ABRI-2018-1234 becomes ABRI-181234.

#### DNA sequencing

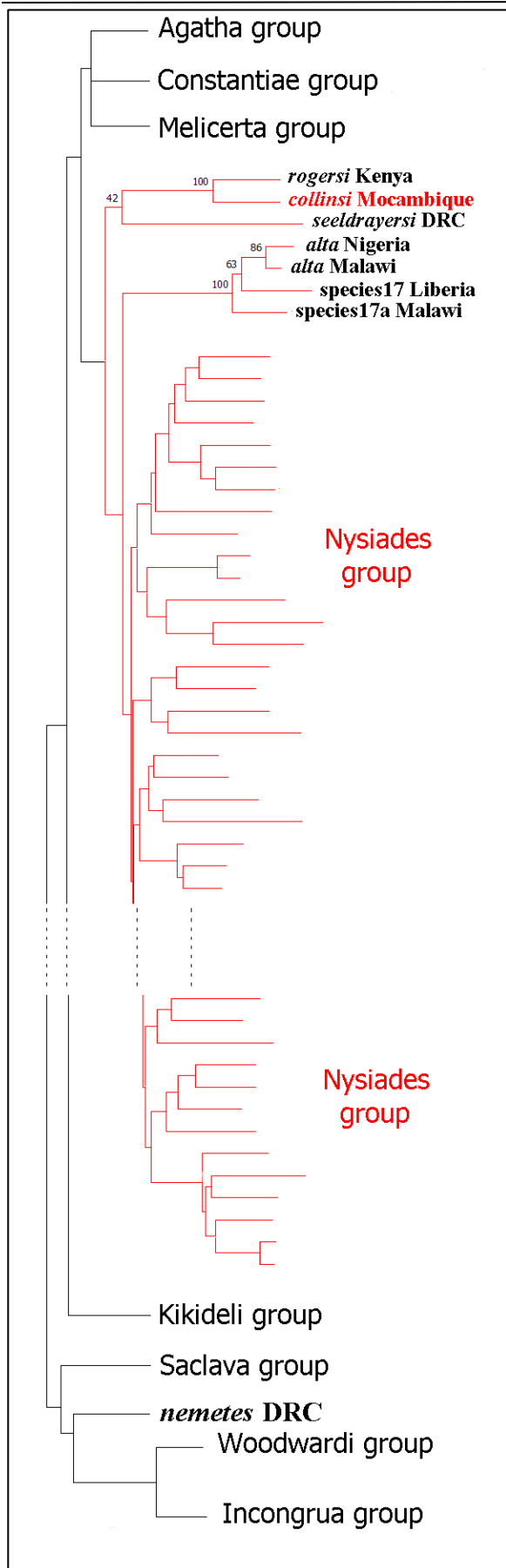
A single leg was removed from each selected specimen and submitted to the CCDB (Canadian Centre for DNA Barcoding), Guelph, Canada, along with an image of the dorsal and ventral aspects of the set specimen.

The methods for inferring phylogenetic trees available in MEGA7 (Kumar *et al.*, 2016), were used to generate a phylogenetic tree using the neighbour joining method. Additionally, a distance chart or Klee diagram was

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**Figure 1** – A schematic diagram of the *Neptis* phylogeny derived from barcodes showing the position of *N. collinsi* in a subgroup of the Nysiades group. Individual species are not named within the Nysiades group and the central section of the Nysiades group is omitted. The entire Nysiades group tree with species names can be found in Richardson (2019).

constructed showing the K2P (Kimura 2 Parameter) corrected pwd (pairwise difference) between all barcodes.

### Genitalic dissection

The techniques employed to dissect out and photograph the genitalia of the female specimens are described in detail in Richardson (2019). Note that the internal organs of the females are not distinctive in *Neptis*, only the form of the 7th and 8th sternites may be helpful. All the images of genitalia are shown with a 1 mm scale bar.

### Distribution Map

A map of Africa covering the localities for the new species was developed from the website <https://maps-for-free.com/>. The map shows terrain altitude, the principle rivers and lakes and international boundaries. The map is used as the background for an Excel chart in a workbook with formulae to transform specimen capture latitude and longitude to the map coordinates. The symbols for each species indicate the status of the specimen as follows:

- Holotype: red fill
- Paratype: yellow fill
- Other specimens: white fill
- Barcoded specimens: black outline
- Specimens not barcoded: grey outline

The latitude, longitude and altitude of the localities mentioned in this paper are listed in the Gazetteer (p. 138).

### DESCRIPTION OF NEW SPECIES

#### Genus *Neptis* Fabricius, 1807

Illiger, K., *Magazin für Insektenkunde* **6**: 282 (277–289). Type-species: *Papilio aceris* Esper, by subsequent designation (Crotch, 1872. *Cistula Entomologica* **1**: 66 (59–71)).

#### *Neptis collinsi* sp. nov. (Fig. 2)

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Only two female specimens of *N. collinsi* are known to date, both from Mt Mabu in Moçambique. The specimens are in the ABRI collection and the specimen in better condition, ABRI-1502888, is selected as the holotype. The two specimens are shown in Figure 2 along with a female specimen of *N. rogersi* for comparison. The specimens were collected in November 2010 by members of the team Stephen Georgiadis, Steve Collins, Julian Bayliss and Colin Congdon.

**Holotype** ♀: Mt Mabu, Moçambique, 16°17'52.7"S, 16°17'52.7"S, 800 to 1500 m; 1–5.xi.2010; ABRI collection.

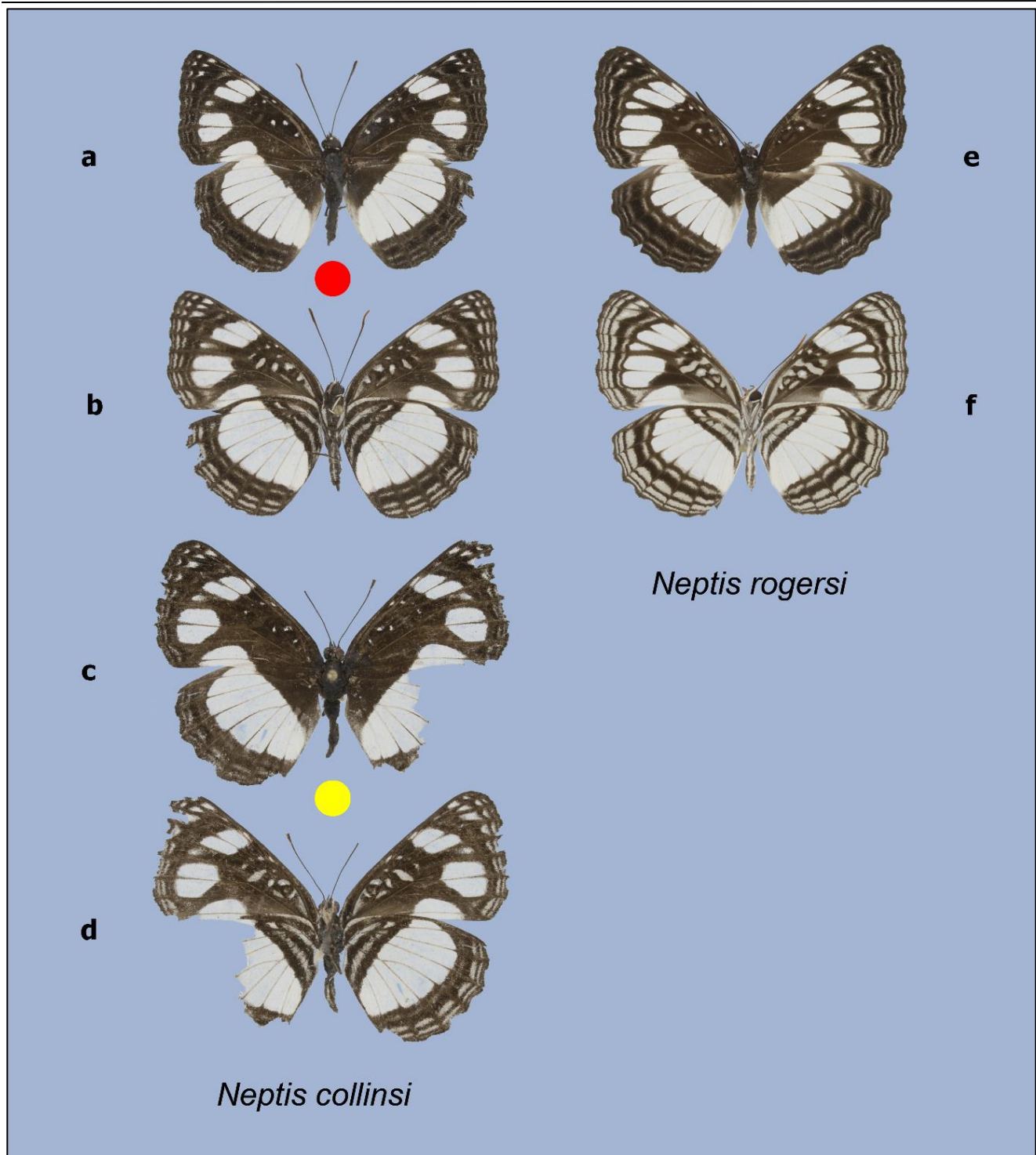
**Paratypes**: 1♀, same data as holotype.

#### Description of facies

**Holotype Female** ABRI-152888 (Fig. 2 a to d)

Wingspan: 4.6 cm. Forewing length: 2.5 cm. Antenna- wing ratio: 0.48.

**Head** White spots at the base of each antenna, a pair of white spots between the eyes at the junction of the head and thorax and an elongated white mark along the posterior distal edge of each eye; palps white distad and greyish-brown proximad; antennae black at the root,



**Figure 2** – *Neptis collinsi* sp. nov. and *Neptis rogersi* Eltringham, 1921. *N. collinsi* holotype ♀: ABRI-152888; Mt Mabui, Moçambique; 1–5.xi.2010; a) dorsal, b) ventral. *N. collinsi* paratype ♀: ABRI-152889; Mt Mabui, Moçambique; 1–5.xi.2010; c) dorsal, d) ventral. *N. rogersi* ♀: ABRI-152890; Sekoke Arabuko Forest, Kenya; ii 2007; S.C. Collins; e) dorsal, f) ventral.

grading progressively to dark orange brown at the tip on the underside, on the upper side the gradation less apparent.

**Thorax** Upper side dark grey, underside with broad whitish bands either side convergent posteriad, but not meeting. Legs (as far as can be determined from the two specimens available), fore legs whitish distally, mid legs pale greyish, hind legs darker greyish.

**Abdomen** Upper side dark grey, a faint continuous lighter line along each side, a continuous white ventral line.

**Wings** Forewing, upper side, background colour dark

blackish-brown. Cell with three well defined circular white marks, a further three fainter white marks at the distal end of the cell and a fourth in line in space Sc. A long discal band mark in space 2A (fd1) rounded proximad and tapering slightly distad, mark in space Cu2 (fd2) shorter and broader proximad, broadly separated from the mark in space Cu1 (fd3). The marks in spaces Cu1 and M3 (fd3 & fd4) separated narrowly by ground colour along vein Cu1, fd3 tapering to a point proximad. A small mark in space M2 (fd5) fading into ground colour proximad. Roughly rectangular marks in spaces M1 and R5 (fd6 & fd7) narrowly separated by ground colour along

vein M1, followed by a long narrow line in space R3 (fd8) also clearly separated from fd7 by ground colour along vein R4. A weakly defined post-discal band with linear marks in spaces Cu2 to R5. Two submarginal bands of wavy pale marks in spaces Cu2 to M1, these being rather ill defined. The anterior part of the submarginal bands comprising a further three well defined spots in spaces R5 to R3. The outer margin is very worn in both the holotype and paratype. However, it is to be expected that the outer margin will be scalloped with short black tufts of cilia at the end of the veins and white cilia in between as in *N. rogersi*. Forewing underside, background colour slightly lighter than on the upper side. Cell more heavily marked than on the upper side. A short white line along the proximal anterior edge of the cell (fcr) terminating in a well-defined white spot (fcr.1), a further large, detached spot (fct1.1) distal and a small spot (fct2.1) further distad. Two large white spots (fct1.2 & fct2.2) on the posterior side of the cell, fct2.2 being more linear than the other spots, and a linear mark proximad from these two marks. Four white spots at the distal end of the cell as on the upper side more clearly defined than the corresponding spots on the upper side. Discal band markings as on the upper side, but less clearly broken by ground colour at the veins. Post-discal band indistinct as on the upper side. Submarginal bands (fsm1 & fsm2) comprising linear marks separated at the veins, more clearly marked than on the upper side, in spaces Cu2 to M1 and continuing with spots in spaces R5 to R3, those in fsm1 being more triangular tapering proximad. Hindwing upper side, base of wing ground coloured without any markings. Discal band (hd) broad at more than 49% of the length of the hindwing at the broadest in space M2, indented at the veins distad, individual marks flat ended distad and comprising marks hd1 in space 2A to hd8 in space Sc+R1. Post-discal band indistinctly marked lighter brown. Three submarginal bands (hsm1 to hsm3) indistinctly marked and comprising long dashes between the veins, distal band (hsm3) more weakly marked. Hindwing underside, narrow whitish band (hb1) along the costa extending from the base roughly one third of the way to the apex. Two further narrow bands, proximal band (hb2) extending from the inner margin and curving distally to almost parallel the costa, distal band (hb3) extending from the inner margin curving to follow the proximal band and fragmented distally to form two indistinct spots and a short linear mark. Discal band as upper side. Post-discal band a series of six pale linear marks extending from space Cu2 to Rs. Three submarginal bands well defined, proximal band (hsm1) comprising six white dashes in spaces Cu2 to Rs, middle band (hsm2) comprising similar marks in the same spaces, distal submarginal band (hsm3) again comprising six dashes narrower than those in hsm1 and hsm2. Outer margin assumed to be scalloped, based on the closely related *N. rogersi*, with white cilia between the veins interrupted by broad black tufts at the veins.

**Sclerotisation of the abdominal exoskeleton** ♀ (Fig. 3) The 8th sternite comprises only narrow sclerotisation around the anterior edge of the ductus bursae with slight broadening before narrowing to a point on both sides. The 7th sternite is not sclerotised on the posterior edge.

### Barcoding

The two specimens of *N. collinsi* yielded identical

barcodes, 658 base pairs, that are allocated to BIN (Barcode Index Number) BOLD (Barcode of Life Data System) :ACU3870. The nearest neighbour is a single specimen of *N. rogersi* with barcode, 658 base pairs, allocated to BIN BOLD:ADM8815. The barcode pwd between *N. collinsi* and *N. rogersi* is 3.3%. The two species are distant from any other species, no species being nearer than 7.7% apwd (average pairwise difference).



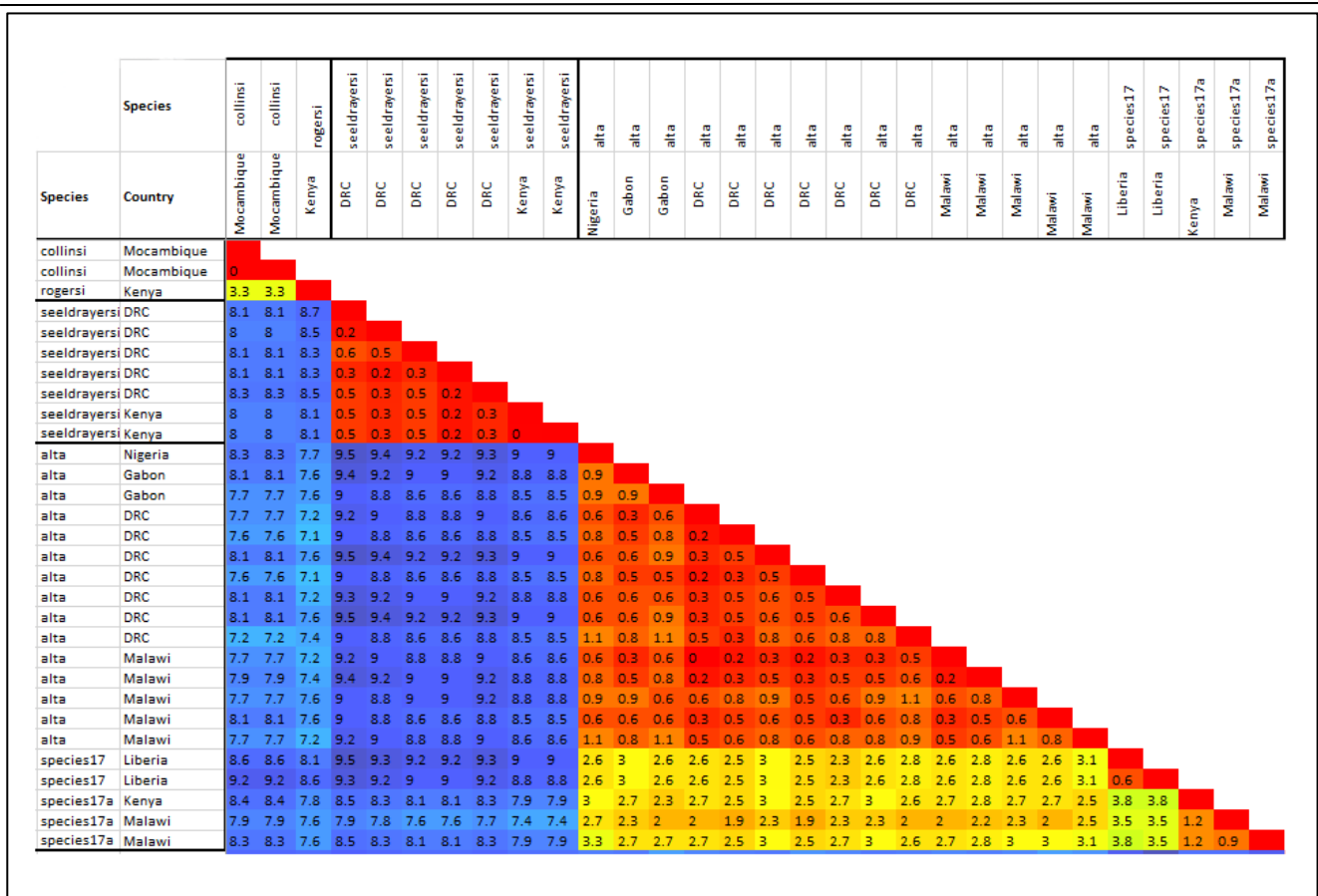
**Figure 3** – 7<sup>th</sup> and 8<sup>th</sup> sternites of the female abdomen of *N. collinsi* and *N. rogersi*. a) *N. collinsi* ♀: ABRI-152888; Mt Mabu, Moçambique; 1–5.xi.2010; b) *N. rogersi* ♀: ABRI-152887; Sekoke Arabuko Forest, Kenya; i.2008; S.C. Collins.

The relationships within the subgroup, within the Nysiades group, comprising *N. rogersi*, *N. collinsi*, *N. seeldrayersi* and *N. alta* are illustrated in the phylogenetic tree (Fig. 1). They can also be visualised using a Klee diagram of pairwise differences (Fig. 4). This shows the barcode differences between all the pairs of barcodes currently available. Specimen data, species and country of capture, are listed down the left hand side of the diagram and across the top. The barcode pairwise difference is written at the intersection of each specimen pair. The diagonal comprises specimens paired with themselves and so the pairwise difference is zero (red). Larger pwd values are colour coded through orange, yellow, green and blue in order of increasing pairwise difference and the precise value of pwd is shown in each cell. Populations with distinct barcodes form red triangles along the diagonal.

The *N. rogersi* – *N. collinsi* species pair forms the small red and yellow triangle at the top left of the diagram and shows a large pwd (coloured blue) with respect to the other two species. Only one barcode has been obtained for *N. rogersi* and two for *N. collinsi*, and so the spread of barcodes within each species cannot be assessed at this stage.

Seven barcodes for *N. seeldrayersi* form a well separated group (red triangle) with a large pwd (coloured blue) with respect to both *N. alta* and the *N. rogersi* – *N. collinsi* pair.

*Neptis alta* forms a large red triangle towards the bottom right of the diagram, 15 specimens having been barcoded. The barcode pwd values for *N. alta* show significant variance with a maximum pwd of 1.1%. Two closely related populations that may constitute separate species, species17 and species17a, form smaller triangles below *N. alta*.



**Figure 4** – Klee diagram showing barcode pairwise differences within the subgroup of species *N. collinsi*, *N. rogersi*, *N. seeldrayersi* and *N. alta*.

### Etymology

I am pleased to name the species for Steve Collins who initiated the *Neptis* revision and has provided support throughout the project by means of the ABRI collection and from his very extensive knowledge of African butterflies and their habitats.

### Material examined and distribution

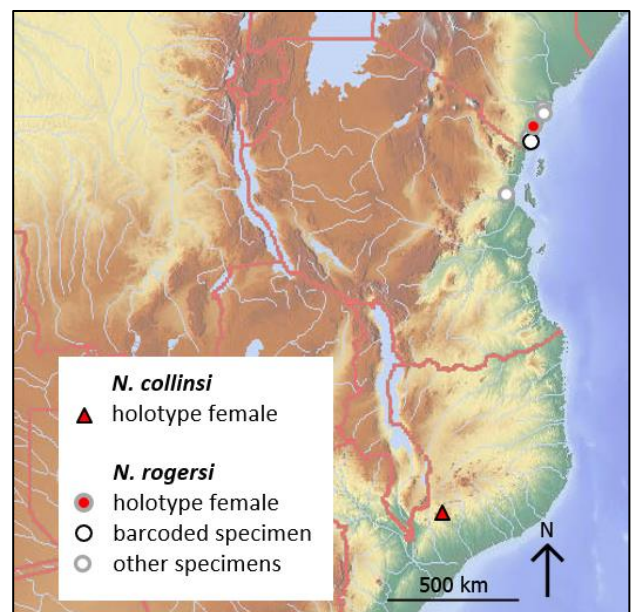
The species *N. collinsi* is only known from two females captured on Mt Mabu in Moçambique. These specimens, holotype and paratype, are in the ABRI collection in Nairobi. The capture locality for these specimens is plotted on the map (Fig. 5) along with localities for *N. rogersi* obtained from the data for specimens in the ABRI collection and additional localities given in Larsen (1991) and Kielland (1990).

### DIAGNOSIS

The option of placing this new taxon as a subspecies of *N. rogersi* is ruled out by the significant barcode separation of 3.3%.

The two species *N. collinsi* and *N. rogersi* are separable on the facies, although the large geographical separation, 1140 km, between their known habitats leaves little room for confusion. *Neptis collinsi* gives the impression of a darker butterfly than *N. rogersi*, having more reduced white markings on the forewing. On the hindwing, however, the discal band is broader in *N. collinsi* having a width, at the broadest point, of 49.1% (N=2) of the wing

length compared with 40.7% (N=4) in *N. rogersi*. The widths are based on the hindwing length from root to the end of vein M2 and the length of the discal band mark hd5 in space M2, both measurements being made on the underside of the wing.



**Figure 5** – Map showing capture localities for *N. collinsi* and *N. rogersi*.

The post discal bands are very faintly marked in *N. collinsi*, whilst they are more clearly marked in *N. rogersi*, particularly on the underside.

The submarginal bands are faintly marked on the upper side of *N. collinsi* and comprise a series of narrow, light grey dashes on the underside, broadly separated at the veins. In *N. rogersi* the submarginal bands are clearly marked on the upper side and boldly marked on the underside, narrowly separated at the veins. The proximal submarginal band on both wings (fsm1 and hsm1) is the same width as the next (fsm2 and hsm2) in *N. collinsi*, whereas the proximal submarginal band is much broader in *N. rogersi*, particularly on the hind wing. The third, distal, submarginal band (fsm3 and hsm3) is only faintly marked in *N. collinsi*, whilst it is clearly marked in *N. rogersi*.

In the forewing cell on the underside of *N. collinsi*, the spot fct2.1 is indistinct and fct2.2 is more or less linear. In *N. rogersi* these two spots are often coalesced into a single linear mark stretching across the width of the cell.

In *N. collinsi*, the 7<sup>th</sup> sternite lacks sclerotisation and the 8<sup>th</sup> sternite comprises a simple sclerotised band around the ductus bursae (Fig. 3), as in *N. rogersi*. The form of the sclerotisation of the abdominal exoskeleton of the female does not therefore distinguish the two species.

## DISCUSSION

Congdon et al, 2010, provides a biogeographical analysis of the butterflies recorded at 5 montane sites in Northern Mozambique including Mt. Mabu. An observation is that several species show significant coastal forest links. *Neptis collinsi* provides another example, the link being to *N. rogersi* from the Kenya and Tanzania coast.

Larsen (1991) and Kielland (1990) describe the coastal region of Kenya and Tanzania and identify several low to medium altitude forests from Sokoke-Arabuko in Kenya south to the Konde plateau close to the border with Mozambique. *Neptis rogersi*, though, has not been captured south of the Kiono forest in Tanzania on the same latitude as Zanzibar island. These forests benefit from two rainy seasons, in April–June and again in November–December as the Intertropical Convergence zone oscillates between the North and the South. Larsen states that the dry seasons in these areas are not as pronounced as they are even 20 to 30 km inland.

Congdon et al. (2010) states that Mt Mabu has a single rainy season between November and April when the Intertropical Convergence zone covers the area. The potentially long dry season is moderated by “relief rainfall as moist air from the Indian Ocean is swept up the mountain by the prevailing South East Trade Winds in May and June”. Julian Bayliss, personal communication, states that the Mt Mabu forests are significantly more humid than the coastal forests of Kenya and Tanzania.

A close relationship between the two species is implied by the low barcode pwd of 3.3%. Papadopoulou et al. (2010) review the mitochondrial (CO1) clock estimates of a number of authors, which are remarkably similar for

different insect lineages; Coleoptera, Orthoptera, Odonata, Hemiptera and Lepidoptera (*Papilio*) averaging out at about 3% My<sup>-1</sup> or 2.7% My<sup>-1</sup> for Lepidoptera. These barcode clock rates imply species divergence for *N. collinsi* and *N. rogersi* roughly 1.1 Mya.

## ACKNOWLEDGEMENTS

Without the attention given to the, often overlooked, *Neptis* genus by Julian Bayliss, Steve Collins, Stephen Giorgiadis and Colin Congdon in their expeditions to Mt Mabu, this new species would not have been discovered. I am grateful also to Julian Bayliss for reviewing the manuscript of this paper and adding helpful insight into the work carried on the mountains of northern Mozambique. Finally, my thanks to the Metamorphosis editorial team for undertaking the difficult task of formatting the document and incorporating the figures and tables.

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**GAZETTEER**

Where altitude is not specified on the specimen labels, it has been deduced using Google Earth. The gazetteer includes localities for *N. rogersi* taken from specimen labels and from the literature, although these are not all specifically mentioned in the text above.

Locality	Country	Latitude (deg°min'sec'')	Longitude (deg°min'sec'')	Altitude (m)
Gongoni Forest	Kenya	04°25'S	39°28'E	50
Kilifi	Kenya	3°30'40"S	39°54'30"E	20
Mabu Mt.	Moçambique	16°17'52.7"S	36°23'41.8"E	800–1 500
Kiono Forest Reserve	Tanzania	06°10'S	38°37'E	200
Rabai	Kenya	03°55'S	39°33'E	170
Sekoke Arabuko Forest	Kenya	03°20'S	39°52'E	100
Shimba Hills	Kenya	04°16'S	39°23'E	200–400