



Description of a new *Cymothoe* Hübner, 1819 from northern Mozambique (Lepidoptera: Nymphalidae: Limenitidinae)

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Robin van Velzen^{1,2}, Steve C. Collins³, Oskar Brattström⁴, and T. Colin E. Congdon³

¹ Biosystematics Group, Wageningen University, Wageningen, The Netherlands. E-mail: robin.vanvelzen@wur.nl

² Netherlands Centre for Biodiversity Naturalis (section NHN – Wageningen branch), Wageningen University, Wageningen, The Netherlands.

³ African Butterfly Research Institute, P.O. Box 14308, 0800 Nairobi, Kenya. E-mail: collinsabri@gmail.com (corresponding authors).

⁴ Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom. E-mail: ob269@cam.ac.uk

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Abstract: A new species and two subspecies of *Cymothoe* from three forested mountains in northern Mozambique are described. It was first discovered on Mt. Namuli in November 2007, and subsequently on Mt. Mabu in 2008 and Mt. Inago in 2009. Ova were found on *Rawsonia lucida* Harw. & Sond. (Achariaceae), and larvae from all three mountains were successfully reared to the adult stage. Montane *Cymothoe* characteristically reside in forest clearings; they are relatively sedentary and generally do not move between forest patches if separated by a significant distance. The new taxa are far from all other known East African montane species, probably diverging in the Pliocene c. 4 million years ago. DNA barcode analysis suggests genetic divergence between individuals from Mt Mabu and those from the other two populations. An IUCN Red list status is allocated based on our current knowledge from the three field sites where it is known to occur. A conservation strategy is required to conserve this species, especially the relict population to be found on Mt. Inago. If action is not taken soon this local Mt. Inago population will disappear.

Key words: *Cymothoe*, *Rawsonia*, Mabu, Namuli, Inago, Mozambique.

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INTRODUCTION

The genus *Cymothoe* Hübner, 1819 (Nymphalidae, Limenitidinae) is endemic to forested regions in Africa. Most species occur from Sierra Leone (Gola forest) in the west to western Kenya (Kakamega forest) and Tanzania (Lake Tanganyika) in the east and north-western Zambia (Mwinilunga) in the south. Geographically widespread species such as *C. herminia* Grose-Smith, 1889, *C. beckeri* Herrich-Schaeffer, 1858, and *C. fumana* Westwood, 1850 occur almost throughout this entire region, suggesting its biological coherence, although many subregions exist with a composition of endemic *Cymothoe* species (van Velzen *et al.*, 2009, McBride *et al.*, 2009). Besides this major area there are two additional disjunct distributions: The first is on Madagascar with the endemic *C. lambertoni* Oberthür, 1923. The second is in eastern and southern Africa with the most widespread species being *C. coranus* Grose-Smith, 1889, occurring from south-east Kenya and eastern Tanzania to the Eastern Cape in South Africa

(Beaurain 1988), *C. alcimeda* Godart, 1824 occurs in South Africa and eastern Zimbabwe, and is the southern species group (*alcimeda* species group) of a clade (AUR) of species related to *C. aurivillii* Staudinger, 1899 (Van Velzen *et al.*, 2013). The other species group of this clade (*aurivillii* species group) are montane species occurring east of the Albertine Rift, in the north from the Taita Hills in Kenya (*C. teita* van Someren, 1939), through Kilimanjaro (*C. collinsi* Rydon, 1980), the Pare mountains, Usambaras and Ngurus (*C. magambae* and *C. amaniensis* Rydon, 1980), to the Uluguru, Rubeho and Udzungwa mountains (*C. aurivillii*) and the southern highlands of Tanzania and the Nyika Plateau of Malawi (*C. cottrelli* Rydon, 1980), the Zomba Plateau (*C. zombana* Bethune-Baker, 1926) and the Mulanje Massif (*C. melanjae* Bethune-Baker 1926) in southern Malawi to the Vumba mountains on the Zimbabwe-Mozambique border (*C. vumbui* Bethune-Baker, 1926) in the south. Nearly taxa within the *aurivillii* species group are allopatric, although *C. vumbui* is sympatric with *C. alcimeda* in Zimbabwe.

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The species described below was first collected by J. Bayliss on Mt. Namuli in November 2007 (Bayliss, 2008; Timberlake *et al.*, 2009), Mt. Mabu in September 2008 (Congdon & Bampton, 2009; Bayliss *et al.*, 2014), and Mt. Inago in 2009 (Bayliss *et al.*, 2010). Males were found more commonly but the females proved elusive.

The males from Mt. Mabu are paler yellow than those from Mts Inago and Namuli, and the yellow areas of the Namuli males are heavily dusted with black. Nevertheless it appears that they are all races of the same species. Montane *Cymothoe* of this species group characteristically reside in forest clearings; they are relatively sedentary and generally do not move between forest patches if separated by a significant distance. They are shade loving, living below the forest canopy. Males perch on the edge of small clearings in the forest, and come down to bask in sunny patches on the forest floor. Females seldom leave the shade.

MATERIALS AND METHODS

All specimens of the new *Cymothoe* were collected opportunistically with a range of butterfly nets on a number of visits to Mts Namuli, Mabu and Inago by members of the African Butterfly Research Institute (ABRI) based in Nairobi. In addition, eggs and larvae from all three mountains were raised to the adult stage.

Within the framework of a DNA barcoding project on the genus *Cymothoe*, DNA sequences of the mitochondrial Cytochrome Oxidase I gene were extracted for several individuals from Mts Mabu, Namuli, and Inago as well as from the closely related species within the species group *C. teita*, *C. aurivillii*, *C. cottrelli*, using *C. alcimeda* as outgroup. After alignment with MAFFT (Katoh & Standley, 2013), a phylogenetic tree was reconstructed using the RAxML algorithm (Stamatakis, 2014) with bootstrapping to assess node support.

In order to prepare the genitalia, abdominal tissue was first placed in an individual glass vial containing about 500 µl of 10% KOH solution heated to just below boiling point. After 10 min. the tissue was removed from the vial and placed in 70% ethanol solution. The genitalia were then cleaned from soft tissue under a stereomicroscope using a pair of micro forceps. Images of the genitalia were taken using a Leica DFC495 digital camera coupled to a Leica M125 stereomicroscope (Fig. 2). To improve the depth of field for the images, focus-stacking software provided with the microscope was used (Leica Application Software version 3.8.0), and images were later cleaned up in Adobe Photoshop CS4/CS5, only neutralizing the background and balancing the contrast across samples. The structure itself was not edited. The genitalia were photographed whilst submerged in 70% ethanol (Fig. 2).

SYSTEMATICS

Cymothoe baylissi van Velzen, Collins, Brattström & Congdon, sp. nov. (Plate 1: Figs 1, 4, 7, 10)

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Type material:

Holotype: ♂ Mozambique, Mt. Namuli, 15°23'56"S, 37°02'25"E, 1,758 m, 24.xi.2008. TCEC/MH (ABRI).

Allotype: ♀ same data but 21.i.2009 (eclosed). JB/IB/TCEC/MH (ABRI). **Paratypes:** 3 ♂ 4 ♀ same data except 20–30.xi.2008.

Description

Male (Plate 1 – Figs 1, 4)

Forewing length 28.6 mm (n = 40); with convex costa, rounded apex with most distal point at M1, concave termen with most proximal point between M3 and CuA₁, rounded tornus, and convex dorsum. Hind wing rounded with drawn-out tornus. Margins slightly scalloped.

Upper side: ground colour creamy white or yellowish white. All wings divided into i) light basal half with postbasal and discal areas in ground colour, and ii) dark distal band with postdiscal and submarginal areas in blackish brown. Cilia brown, with white dashes at vein ends.

Forewing: Base up to roughly the intersection of veins CuA₁ and CuA₂ dusted dark greyish-brown. Light basal half with five fine black lines; irregular basal line in the cell from Sc+R to CuA, irregular postbasal line from R through the cell and intersection of veins CuA₁ and CuA₂ to 2A two fifths from base, slightly convex postbasal line in cell from R to CuA₁+M3. Discal loop in cell from base of M2 turning just before intersection of veins M3 and CuA₁, and discal line from M1 to 2A, concave at each interspace, running from one tenth of M1, one fifth of M2, one fifth of M3, one tenth of CuA₁, one third of CuA₂ and three fifths of 2A. Intersections between veins R1, R2, M1 and M2 marked blackish brown. Dark distal band covers roughly two fifths of M1, two fifths of M2, quarter of M3, fifth of CuA₁, two fifths of CuA₂, and three fifths of A2. Two rows of six sagittate marks in interspaces between R5 and 2A: postdiscal row with marks in ground colour equally long and wide and adjoining submarginal row with marks in black half as long as wide; sagittate marks in interspace R5–M1 smallest in both rows, two between M3 and CuA₂ largest and those in CuA₂–2A with two points.

Hindwing: Base up to roughly one sixth of Rs dusted dark greyish-brown or black. Light basal half with fine black line running from two fifths Sc+R1 concave and then convex to one sixth of Rs and then again concavely to one tenth of M1. This line extends through one fifth of M2, intersection of veins M3 and CuA₁, one third of CuA₂, and three fifths of A2 in some specimens. A discal fine black line makes smaller irregular semi-circular shape in cell. Dark distal band starts from roughly two thirds of costa, half of Rs, M1 and M2, one third of M3 and CuA₁, half of CuA₂, and three quarters of 2A. The two rows of sagittate marks extend to hind wing with another seven coupled marks in interspaces between Sc+R1 and 2A. First and/or last postdiscal marks in ground colour may be small or absent in so

Underside: Ground colour creamy or ochreous white. All wings divided into light basal half, with postbasal and discal areas in ground colour, and darker distal band, with postdiscal and submarginal areas in greyer version of ground colour. Transverse

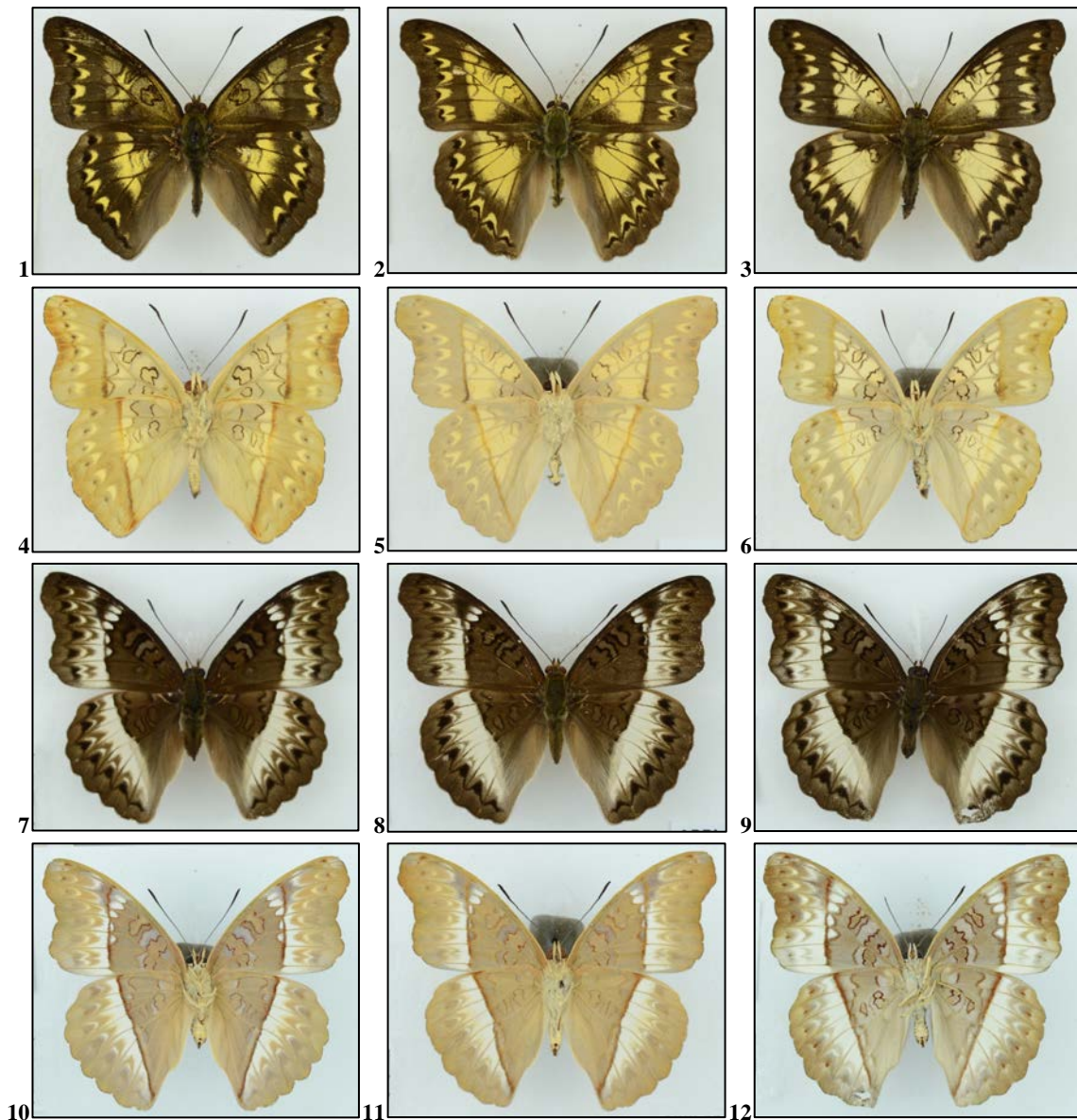


PLATE 1

Figures 1, 4 – *Cymothoe baylissi baylissi* Holotype ♂, recto, verso. Mt Namuli, Mozambique, 24.xi.2008, TCEC/MH, in ABRI, Nairobi.

Figures 2, 5 – *Cymothoe baylissi monicae* Holotype ♂, recto, verso. Mt Inago, Mozambique, 15.i.2009 (bred), TCEC/MH/IB, in ABRI, Nairobi.

Figures 3, 6 – *Cymothoe baylissi poppyana* Holotype ♂, recto, verso. Mt Mabu, Mozambique, 11.xi.2008 (bred), TCEC/IB/MH, in ABRI, Nairobi.

Figures 7, 10 – *Cymothoe baylissi baylissi* Allotype ♀, recto, verso. Mt Namuli, Mozambique, 21.i.2009 (bred) JB/IB/TCEC/MH, in ABRI, Nairobi.

Figures 8, 11 – *Cymothoe baylissi monicae* ♀, recto, verso. Mt Inago, Mozambique, 10.i.2009 (bred), TCEC/IB/MH/JB, in ABRI, Nairobi.

Figures 9, 12 – *Cymothoe baylissi poppyana* ♀, recto, verso. Mt Mabu, Mozambique, 10.xi.2008 (bred), TCEC/IB/MH, in ABRI, Nairobi.

Photography: Teresa Di Micco de Santo

line separates light basal half and darker distal band. Wing margins lined with ochreous band widest on forewing between R5 and CuA₁.

Forewing: Light basal half with same irregular fine lines as on dorsal surface in brown to black. Transverse line brown or ochreous, usually darker towards dorsum and fading towards costa in some individuals, running straight from roughly two thirds of costa to three fifths of A2. Darker distal band with two rows of marks: postdiscal row consisting of sagittate marks in ground

colour equally long and wide similarly as on dorsal surface, and submarginal row consisting of small black dots in interspaces between R3 and 2A usually connected with very thin black zigzag lines.

Hindwing: Light basal half with fine black line running concave from one quarter of Sc+R1 to intersection of veins R1 and M1; another fine black line running from Sc+R1 to M1, similar to dorsal surface, but continuing convex from M1 to M2 just before transverse line and then to M3 just after intersection with vein CuA₁. Area

between Sc+R1, M1 and two fine black lines same colour as darker distal band. Cell with postbasal black line of irregular shape resembling two fused semicircles and discal black line making smaller irregular semi-circular shape similar to dorsal surface. Transverse line ochreous, darkest towards costa and dorsum and usually faded between veins M1 and M3, running straight from roughly half of costa to four fifths of dorsum. Darker distal band with two rows of marks similar to forewing.

Female (Plate 1 – Figs 7, 10)

Forewing length 32.1 mm (n = 11); wing shape similar to male.

Upper side: Ground colour dark brown. All wings have broad white postdiscal band with narrow zigzag band in ground colour over its distal edge, bordered by submarginal row of blackish brown sagittate marks.

Forewing: Base up to white postdiscal band dark brown ground colour. Cell with five fine black lines: irregular basal line from Sc+R to CuA similar to male, one pair of roughly parallel irregular wavy lines from R to CuA₁+M3 and second pair of irregular lines from M1 to intersection of CuA₁ and M3. The space between each pair of lines usually lighter tone of greyish brown. Discal band of ovate white patches in interspaces between veins R3+4+5 and CuA₂ with those between R3+4+5 and M2 largest and those between CuA₁ and CuA₂ reduced in some individuals. White postdiscal band from roughly half of R3, half of M1, two fifths of M2, a third of M3, a quarter of CuA₁, one third of CuA₂, and half of A2. Submarginal row of six blackish brown sagittate marks equally long and wide in interspaces between R5 and 2A; two marks between M3 and CuA₂ largest and those in CuA₂–2A with two points.

Hind wing: Base up to white postdiscal band in dark brown ground colour with series of fine black lines similar to male hind wing underside. Fine black line running concave from one quarter of Sc+R1 to intersection of veins R1 and M1; another black line from two fifths of Sc+R1 concave, then convex to one sixth of Rs, then again concavely to one tenth of M1 and continuing convex from M1 to M2 just before the white postdiscal band and then to M3 just after intersection with vein CuA₁. Cell has postbasal black line of irregular shape resembling two fused semicircles and discal black line making smaller irregular semi-circular shape. White postdiscal band from roughly half of costa, one third of Rs, one quarter of M1 and M2, one tenth of M3 and CuA₁, half of CuA₂, and five sixths of 2A. Submarginal row of seven blackish brown sagittate marks in interspaces between Sc+R1 and 2A; mark between CuA₂ and 2A is smallest.

Underside: Ground colour ochreous. All wings with dark ochreous brown discal transverse line and broad white postdiscal band. Wing margins lined with ochreous band widest on forewing between R5 and CuA₁, similar to male.

Forewing: Base up to discal transverse line in ochreous ground colour. Cell with same five fine black lines as on dorsal surface, but basal line and pair of postbasal lines extend from CuA to 2A. Space between base and

basal line and pair of postbasal lines usually lighter tone of grey. Discal band of ovate white patches in interspaces between veins R3+4+5 and CuA₂ similar to upperside. Transverse line straight from roughly two thirds of costa to three fifths of A2, similar to male. Submarginal row of marks absent or consisting of small black dots in interspaces between R5 and 2A. Apex with light grey or white patch between R3 and R5.

Hind wing: Base up to discal transverse line in ochreous ground colour with series of fine black lines similar to upperside. Transverse line straight from roughly half of costa to four fifths of dorsum, similar to male. Submarginal row of marks absent or consisting of small black dots in interspaces between Sc+R1 and 2A.

Male genitalia (Fig. 13)

The main differences in relative proportions between the genitalia of *C. alcimeda* (smaller) *C. baylissi* and *C. teita* are described below, ignoring differences in absolute size.

The uncus of all species have a pronounced beak-like projection in a ventral direction at its terminal end, but this shape is less pronounced and also relatively thinner in *C. alcimeda*. All three species have thick bases of the gnathos that initially projects ventrally. In *C. baylissi* and *C. teita* the gnathos is then angled sharply at about two third of its length extending in a posterior direction forming an almost straight angle on the anterior ventral edge. In *C. alcimeda* the gnathos instead gradually becomes thinner after about half of its length and extends further ventrally forming a gradual gentle curve towards the posterior angle. The anterior edge is evenly curved throughout the entire gnathos.

The anterior part of the tegumen is prominently extended in *C. baylissi*, meeting the vinculum in a right angle, while in *C. teita* the vinculum is tilted forward in a dorsal posterior angle. This gives the genitalia of *C. baylissi* a more upright impression. *C. alcimeda* is somewhat intermediate between the other two species. The saccus of *C. baylissi* is thinner than in the other two species getting gradually thinner at its anterior end, and has a more marked waist where it connects to the vinculum.

The valvae of *C. alcimeda* are relatively straight, getting gradually thinner along almost their entire length with the ventral edge curving abruptly at the terminal end so that it forms a sharp point where it meets the dorsal edge. In *C. baylissi* the valvae are boomerang shaped tilting in a posterior angle and thickest at about half of their length. The tips are evenly rounded. In *C. teita* the dorsal and ventral edge of the valvae run almost in parallel for most of their length, but with a gradual tapering at the posterior end. The dorsal edge is then turned down at the terminal end meeting the ventral edge in a sharp angle pointing posteriorly. The aedeagus of *C. alcimeda* is slender compared to the other two species.



Figure 13 – Genitalia of *Cymothoe* species: **A** *C. baylissi* sp. nov.; **B** *C. teita*; **C** *C. alcimeda*. Scale bar = 2 mm

Systematic placement and diagnosis

C. baylissi sp. nov. is a member of the montane *Cymothoe aurivillii* species group. The male is morphologically similar to the Kenyan endemic *C. teita*, because of the large extent of the lightly coloured creamy area on the forewing and the regular submarginal row of creamy sagittate marks of nearly the same size on the hind wing. Other members of the species group have a large black band across the forewing upper side, reducing the lightly coloured area on the forewing and a more irregular submarginal row of creamy sagittate marks that differ much more in size. Males of *C. baylissi* can be differentiated from those of *C. teita* by the even larger extent of the lightly coloured creamy area on the forewing and by the absence of the orangey red submarginal spots in *C. teita*. The female is very similar to other members of the species group but may be differentiated by the lighter, more yellow ground colour of the underside.

Early stages: Eggs were found on *Rawsonia lucida* Harv. & Sond. (Achariaceae), and on Mt Mabu females were observed ovipositing on this tree. Larvae were raised to the adult stage on all three mountains (Congdon *et al.*, 2010). Eggs are laid in batches, and on emergence, the young larvae eat the egg shells (Fig. 14), and go on to eat any infertile eggs in the batch, a behaviour not previously observed by the authors. Larvae are gregarious in the early instars (Fig. 15), becoming solitary in the third instar (Fig. 16), after which they rest on the upper surface of a leaf of the host plant. The fully mature larva (Figs 17–18) moves to the underside of a leaf to pupate (Figs 19–20).



Figure 14 – *C. baylissi* sp. nov. ova (left) and 1st instar larvae (right)



Figure 15 – 2nd instar larvae (left) and final instar larva head shield (right).



Figure 16 – 3rd instar larvae



Figure 17 – Final instar larva, dorso-lateral view



Figure 18 – Final instar larva dorsal view

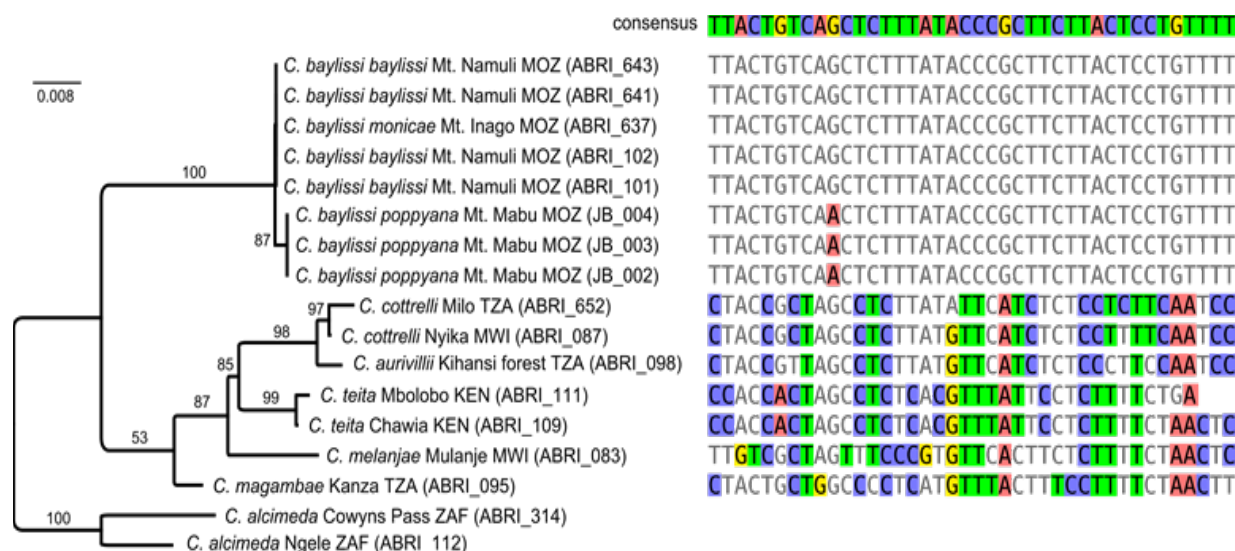


Figure 20 – Left: phylogenetic tree for *C. baylissi* and related species based on DNA barcode sequences. Node values represent bootstrap support values; scale bar indicates substitutions per site. Right: Alignment of DNA barcode sequences showing only variable sites; differences from consensus are highlighted.



Figure 19 – Pupa, dorsal view (left) and lateral view (right)

DNA analysis

DNA CO1 sequences suggest that *C. baylissi* sp. nov. is well diverged from *C. teita* and other closely related species (Fig. 20). All sequences from *C. baylissi* individuals were identical, except that those from Mt Mabu all have one adenine where all individuals from Mts Namuli and Inago have a guanine (Fig. 14). Based on our limited sampling, it thus seems that there is genetic divergence between *C. baylissi poppyana* from Mt. Mabu versus *C. baylissi baylissi* and *C. baylissi monicae* from the other two mountains, and these populations are accordingly given subspecific status:

***Cymothoe baylissi monicae* ssp. nov.** (Plate 1: Figs 2, 5, 8, 11)

Type material:

Holotype: ♂ Mozambique, Mt. Inago, 15°04'48"S, 37°23'24"E, 1478m, 15.i.2009, TCEC/ IB/MH/JB (ABRI). **Allotype:** ♀ same data. **Paratypes:** 2 ♂, 3 ♀ same data.

Males from Mt. Inago have less dark dusting than the nominate subspecies, and are a deeper yellow than the Mt. Mabu males. Females are similar to those from Mt. Namuli.

***Cymothoe baylissi poppyana* ssp. nov.** (Plate 1: Figs 3, 6, 9, 12)

Type material:

Holotype: ♂ Mozambique, Mt. Mabu 16°17'10"S, 36°24'01"E, 980 m, 11.xi.2008, TCEC/IB/MH. (ABRI). **Allotype:** ♀ same data but x.2008. **Paratypes:** 3 ♂, 3 ♀ same data.

Males from Mt. Mabu have a paler yellow ground colour than the nominate subspecies, and are less heavily dusted with brown. They are otherwise similar. Females are similar to those from Mt. Namuli.

Etymology: The species *C. baylissi* is named for Julian Bayliss, who was the first to collect it on Mt. Namuli in November 2007. Over the course of his career, he has put great effort into the conservation of species in Africa.

Subspecies *monicae* is named in honour of his mother, Monica Bayliss; and subspecies *poppyana* for his daughter Poppy.

DISCUSSION

Distribution

Cymothoe baylissi has been found only in mid-altitude montane forests on three sites in northern Mozambique (Fig. 21). Nevertheless, it seems to prefer altitudes below 1600 m, suggesting that its original range included forests at lower elevations in areas that are now under cultivation (Congdon, *et al.*, 2010; Bayliss *et al.*, 2014).

On Mt. Namuli the species is found at altitudes above 1500 m. However, the original forest extent at this site extended to altitudes below this (1000 m) and it is thought that the species is now found at its higher altitude range limit as a result.

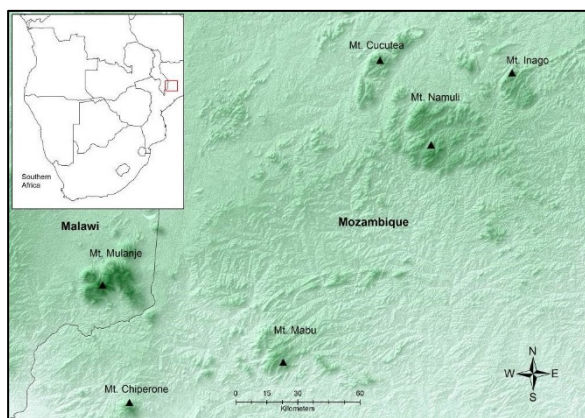


Figure 21 – Map showing study area between Malawi and Mozambique, and location of Mts. Mabu, Namuli and Inago in relation to neighbouring mountains above 1500 m.

On Mt. Mabu it was locally common around the main forest camp at an altitude of 950 m. As with Namuli the species is found up to altitudes of 1500 m. The evidence of its occurrence on Mabu at these altitudes supports the theory that mid-altitude forest at 1000–1500 m is its natural altitude range. On Mt. Inago it was restricted to small patches of refuge forest (<5 ha) at altitudes of approximately 1500 m (Bayliss *et al.*, 2010), however the original forest extent would have been much greater, as on Mts. Namuli and Mabu. It is probable that the species also occurs on neighbouring Mt. Cucutei. On Mts. Mabanje and Chiperone it is replaced by *C. melanjae*, and on Mt. Zomba in southern Malawi by *C. zombana*.

Larval host-plants

Rawsonia species are larval host-plants for the related species *C. amaniensis*, *C. aurivillii*, *C. vumbui*, *C. cottrelli* and *C. teita*, as well as for the Achariaceae specialists *C. coranus*, *C. caenis*, and *C. herminia*. Achariaceae are known to be cyanogenic and *Rawsonia lucida* contains cyanogenic glycosides cyclopentenylglycine and gynocardin (Andersen *et al.*, 2001), which are hypothesised to act as oviposition stimulant for *Cymothoe* (Clausen *et al.*, 2002).

Evolution

The genus *Cymothoe* is considered an example of a rapid radiation, with an elevated rate of species diversification during the last 7 million years (van Velzen *et al.*, 2013). Within this radiation, *C. baylissii* was estimated to have diverged from closely related *C. teita* and *C. alcimeda* roughly 4 million years ago (van Velzen *et al.*, 2013). This suggests that it represents a relatively old lineage within the montane species group – which is in line with our results based on DNA barcode sequences.

Conservation Status

The species is highly threatened on Mt. Inago as a result of severe deforestation (Bayliss *et al.*, 2010; Fishpool & Bayliss, 2010). It is currently struggling to survive in these remnant forest patches, along with other sedentary forest species such as the new species

of pygmy chameleon also found in these forest patches (Branch *et al.*, 2014). It is in imminent danger of extinction at Mt. Inago unless conservation action is taken. At Mt. Namuli it is also threatened due to deforestation as a result of potato farming in the remaining forests. At Mt. Mabu the population is stable and the forest area is large and the current threat to the forest is low. However this situation needs to be monitored as commercial deforestation is an ongoing threat and active in the area (<20 km) surrounding Mabu forest (Bayliss *et al.*, 2014).

Consequently, the species is given a Red List status of Vulnerable (VU) applying the IUCN (2010) criteria A2, B2a, C2ai, D2, based on our current knowledge from the three field sites where it is known to occur.

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