METAMORPHOSIS

LEPIDOPTERISTS' SOCIETY OF AFRICA

Volume 32: 60–66

ISSN 1018–6490 (PRINT)
ISSN 2307–5031 (ONLINE)

Phylogenetic relationships, distribution and abundance of *Charaxes mtuiae* Collins Congdon and Bampton, 2017 (Papilionoidea: Nymphalidae: Charaxinae) and its host plant in the Udzungwa mountain forest in southern Tanzania

Published online: 8 November 2021
DOI: https://dx.doi.org/10.4314/met.v32i1.12

Devolent Mtui*[1], Karina S. Bringas[2], Erik Ciaccio[2], Luc Leblanc[2], Raymond Okick[1], Deusdedith Bwenge[1], & Chris A. Hamilton[2]
[1] Tanzania Wildlife Research Institute, Arusha, Tanzania
[2] University of Idaho, Department of Entomology, Plant Pathology and Nematology, Moscow, Idaho.
Emails: deovolentem@gmail.com; karinas@uidaho.edu; ciac1074@vandals.uidaho.edu; leblancl@uidaho.edu; raymond.okick@tawiri.or.tz; deusfidelis2014@gmail.com; hamiltonlab@uidaho.edu.
*corresponding author

Abstract: *Charaxes mtuiae* Collins Congdon and Bampton, 2017 was discovered in 2005 in the Udzungwa Mountains of Tanzania, where the caterpillars feed exclusively on *Diospyros natalensis.* This study was aimed at determining the abundance and spatial distribution of *C. mtuiae* and its host plant, as well as its evolutionary history. Field surveys were conducted between April 2017 and December 2018. Stems of the host plant were counted in sampling plots and their diameter at breast height measured, and categorised as mature if the diameter was ≥ 12 cm, otherwise as recruits. *Charaxes mtuiae* was sampled by searching for immatures on the leaves of the host plant and capturing adults using traps. DNA material of *C. mtuiae* was extracted, sequenced, and aligned with 63 other species of Charaxes. A total of 1,173 stems of the host plant including 1,064 recruits and 102 mature stems were recorded. One specimen of *C. mtuiae* found at caterpillar stage was raised to adulthood, and three empty pupa cases of *C. mtuiae* were recorded. The phylogenetic relationship of *C. mtuiae* and its sister lineages was concordant with previous descriptions, based on morphology. Our results indicate that *C. mtuiae* is rare, despite the high abundance of its host plant. Continued research and monitoring of *C. mtuiae* population to understand its ecological requirements; and expansion of surveys into other parts of the country where the host plant occurs to establish its distribution country-wide are recommended.

Key words: Butterflies, Kihansi, *Diospyros natalensis*

Citation: Mtui, D., Bringas, K.S., Ciaccio, E., Leblanc, L. Okick, R. Bwenge , D. & Hamilton, C.A. 2021. Phylogenetic relationships, distribution, and abundance of *Charaxes mtuiae* Collins et al., 2017 (Papilionoidea: Nymphalidae: Charaxinae) and its host plant in the Udzungwa mountain forest in southern Tanzania. *Metamorphosis* **32**: 60–66.

INTRODUCTION

The Kihansi Charaxes, *Charaxes mtuiae* (Collins *et al*. 2017), was discovered in October 2005 in the forested Udzungwa Mountains in southern-central Tanzania. It is viewed as a close relative of *C. mutinondo* Collins, Congdon, and Bampton, 2017 (Zambia), *C. gallagheri* van Son, 1962 (Zimbabwe), *C. martini* van Son, 1966 (Mount Mulanje, Malawi) and *C. martini helenae* Henning, 1982 (Mount Zomba, Malawi) (Collins *et al*., 2017). The caterpillars of these five taxa are monophagous, feeding exclusively on *Diospyros natalensis* Harv. Brenan (Ebenaceae) (Collins *et al*., 2017), an evergreen tree widespread along the eastern side of African mainland from Northern Kenya to South Africa, and from near sea level to 1525 m elevation, growing on rocky areas along streams and riverbanks (White, 1988). In Tanzania, *D. natalensis* is known to occur in the Wala River Forest Reserve (Tanzania central), the western Usambara mountains, the Shume Forest Reserve (Eastern Arc mountains), and the Selous Game Reserve (coastal land) (White, 1988). Despite widespread host availability, the presence of *C. mtuiae* has only been reported in the Udzungwa mountains, possibly because there have not been attempts to locate it in other parts of the country.

It is known that distribution and abundance of butterflies are constrained by the availability of host plant (Yamamoto *et al*., 2007) and species traits (Curtis *et al*., 2015). Species with narrow diets, narrow niche breadths, low mobility, and habitat specialists have a strong relationship with host-plant abundance (Curtis *et al*., 2015). Monophagous species tend to have smaller range sizes than polyphagous species, and their individuals tend to concentrate in areas with larger numbers of host plants (Quinn *et al*., 2017).

The taxonomic description of *C. mtuiae* (Collins *et al*., 2017) was based on morphological features and life history. However, accurate delimitation of closely related species requires both morphological and molecular information, to help elucidate the evolutionary history and relationships among species (Samways *et al*. 2010). By producing molecular data for *C. mtuiae*, we aimed to confirm the previous morphological taxonomic hypothesis.

Since the discovery of *C. mtuiae*, quantitative information on the abundance and spatial distribution of this butterfly and its host plant, as well as its evolutionary history have been lacking.

Received: 4 July 2021
Accepted: 31 October 2021

Such information is necessary for the conservation of *C. mtuiae* in the Kihansi Gorge Forest. In this paper we determine the size class, distribution and abundance of its host plant, *D. natalensis*; map the spatial distribution of *C. mtuiae* and relate its abundance to the availability of its host plant; and determine the phylogenetic placement of *C. mtuiae* in relation to other *Charaxes* species.

METHODS AND MATERIALS

Study site

The Kihansi Gorge Forest is located between latitudes 8.61º and 8.59 º South, and longitudes 35.82º and 35.86º East, in south-central Tanzania (Fig. 1). The gorge lies along the Kihansi River, covering an area of about 400 ha at elevations of 300–1100 m. The gorge consists of four vegetation types: moist forest, spray wetland vegetation, deciduous woodland, and submontane secondary vegetation (Lovett *et al.*, 1997). The montane and submontane vegetation types are common at mid and high altitudes (above 580 m), probably representing areas of forest or woodland formerly cleared for agriculture and by fire (Lovett *et al*., 1997). *Diospyros natalensis* is present in the upper part of the gorge (within an area of about 200 ha) at an elevation of 950–1100 m (Fig. 1c). Mean monthly temperatures range from 16°C (June and July) to 31°C (January and February). The annual rainfall pattern is bimodal where short rains fall from November to January and long rains from March to May.

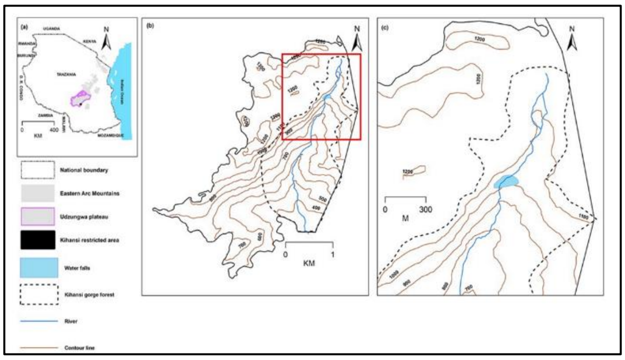

Figure 1 – Location of the study site in Tanzania (a), the restricted area owned by Tanzania Electrical Company (b), and the portion of the Kihansi Gorge and sampling area, to which *Charaxes mtuiae* and its food plant are restricted (c).

Taxa selection for genomic analysis

The genus *Charaxes* Ochsenheimer, 1816, consists of 187 Afrotropical species (Williams, 2018), of which 40% (75 species) occur in Tanzania (Kielland, 1990), and 40% (29 species) of the Tanzanian species occur in the Kihansi Gorge forest (Mtui *et al*., 2019a). While this genus has five subgenera (Aduse-Poku *et al*., 2009; Williams, 2019), for phylogenetic analysis, we chose to focus on subgenus *Eriboea* Hübner, 1819, and its six species-groups (*anticlea*, *etheocles, etesipe, eupale, hildebrandti* and *jahlusa*); and subgenus *Charaxes* (species group: *jasius* and *tiridates*) which all occur in the Afrotropical region. DNA sequences of the animal barcode gene, mitochondrial cytochrome oxidase I (COI) (Hebert *et al*., 2004) for 63 representative species of each species-group were downloaded (Table S1) from the Barcode of Life Data Systems (BOLD) and the National Center for Biotechnology Information (NCBI) GenBank database (Ratnasingham & Hebert, 2007).

DNA analysis

DNA was extracted from the thoracic muscle tissue of an adult specimen of *C. mtuiae* collected in October 2018, using a modified G-Biosciences Omniprep^{TM} kit protocol. The DNA was amplified using GoTaq Green PCR Master mix (Promega 2017; 2018) and Lepidoptera COI primers including LEP- F1, 5' –ATTCAACCAATCATAAAGAT AT-3' and LEP-R1, 5'-TAAACTTCGTCCAAAAA-3' (Hebert *et al*. 2004). The Polymerase Chain Reaction (PCR) products were then cleaned using ExoSAP-I (Bell 2008) and inspected using gel electrophoresis. The DNA extraction and PCR procedures were carried out in Hamilton's Arthropod Molecular Systematics Laboratory in the Department of Entomology, Plant Pathology and Nematology (University of Idaho), and sent to GENEWIZ, LLC for DNA sequencing. Base calling of the 616 bp *C. mtuiae* sequence, and alignment with sequences from the 63 other species of *Charaxes* (subgenus *Eriboea*), were performed using Geneious 2021.1 (Kearse *et al*., 2012). Phylogenetic reconstruction was performed using maximum-likelihood in IQ-TREE 2.1.2 (Nguyen *et al*., 2015) following nucleotide site substitution modeling using MODELFINDER (Kalyaanamoorthy *et al*. 2017). Node support was generated from 1000 replicates of the ultrafast bootstrap approach (Hoang *et al*., 2018).

Sampling and data collection for *Diospyros natalensis*

Field surveys were conducted over a period of 20 months (April 2017 to December 2018), in the upper portion of Kihansi gorge forest occupied by *D. natalensis* (Fig. 1c). In a preliminary survey in 2017, we mapped general areas of occurrence of the food plant in the study area – see Mtui *et al*., 2019b, in which we selected fifteen 20 x 20 m sampling plots (Fig. S1-b) at five different sites. The number of plots per site ranged between two and five. The four corners of each plot were georeferenced and marked with flagging tape for future monitoring. All woody plant stems of ≥ 12 cm diameter (categorized as mature) were measured at breast height (CBH) using measuring tape, or individually counted if below the prescribed CBH (categorized as recruits). The abundance of *D. natalensis* relative to other woody plants was compared to assess the commonness or rarity of the species. To prevent inadvertent repetition, each stem measured or counted was marked by spraying paint on its bark. For trees with multiple stems, only the main stem was measured. Dead and regenerating (coppicing) stems were also recorded.

Sampling approach and data collection for *Charaxes mtuiae*

The sampling approach for *C. mtuiae* combined systematic and opportunistic searching for eggs, caterpillars, and pupae on the leaves of mature branches of *D. natalensis*. Adults were captured as they were sighted, and by maintaining 26 Van Someren traps baited with fermented banana (Fig. S1c). Since monophagous butterflies tend to prefer feeding on young leaves of their host-plants (Cates, 1980), we targeted our searches for eggs/larvae and pupae on young leaves on the branches of the host plant. The numbers of *Charaxes* species caught in the study area, from both systematic and opportunistic methods, were combined to estimate relative abundance of each species and estimate the commonness or rarity of *C. mtuiae* relative to other species (Tables 1 & S2). Species with fewer than 10 recorded individuals were categorized as rare (less abundant), 10 to 50 individuals as moderately abundant, and over 50 individuals as commonly abundant.

RESULTS

Abundance of *C. mtuiae* and other species of *Charaxes*

A total effort of 28,092 person-hours were spent searching and trapping butterflies (Table S3). From this effort, only one individual of *C. mtuiae* was recorded as a caterpillar; and three as empty pupa cases (Table 1, Figs 2 & 3) which according to Colin Congdon and Steve Collins (from African Butterfly Research Institute), who have over 30 years' experience on collecting and rearing *Charaxes*, are very likely are of *C. mtuiae* because there are no any other *Charaxes* that feed on *D. natalensis*, and even if there were *Charaxes* larvae wandering around they would not have pupated on *D. natalensis*. The caterpillar and the three pupal cases of *C. mtuiae* were observed through opportunistic searching of leaves on *D. natalensis* branches, and all were found on tree branches that were about 5 to 10 m from the river course (Fig. 3). The caterpillar was found on the last leaf (at the tip) of a mature tree about 7.5 m tall, and three pupal cases were found on branches of different mature trees, about 3 m from the ground.

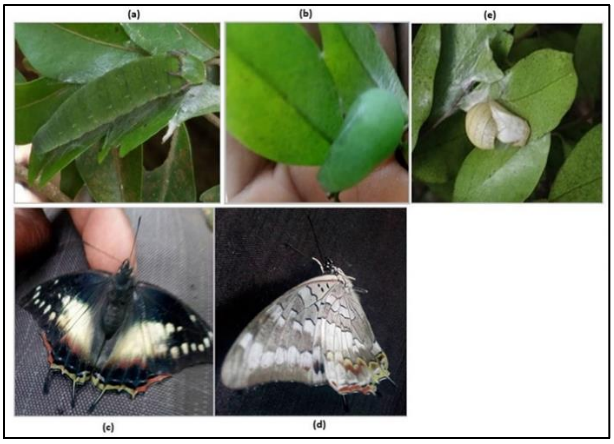

Figure 2 – Individuals of *Charaxes mtuiae* recorded in Kihansi Forest through searching on leaves of *Diospyros natalensis*: (a) final instar caterpillar observed on 23.ix.2018; that developed to a pupa on 06.x.2018 (b); then emerged as an adult on 27.x.2018 (c) dorsal side and (d) ventral side; (e) One of three pupa cases observed on 24.ix.2018.

The caterpillar of *C. mtuiae* (Fig. 2-a) was reared to adult in the field, by enclosing the caterpillar on a branch of its host plant in a mesh bag. This specimen, deposited at the University of Idaho's William F. Barr Entomological Museum, was used for DNA extraction. We also recorded 27 other species of *Charaxes* (326 individuals) and 48 other species of butterflies (593 individuals) (Tables 1 & S2), all caught in the baited traps.

Of the 28 species of *Charaxes* recorded, 16 of these, including *C. mtuiae*, were determined to be least abundant, with the remaining 12 species moderately or commonly abundant (Table 1). Among the least abundant species, 31% were monophagous (*C. mtuiae*, *C. congdoni*, *C. mccleeryi*, *C. aubyni*, and *C. tiberius*), 38% were oligophagous (*C. baumanni*, *C. wakefieldi*, *C. achaemenes*, *C. bohemani*, *C. guderiana*, and *C. howarthi*), 25% were polyphagous (*C. etheocles*, *C. castor*, *C. dilutus dilutus* and *C. saturnus*), and the host plant for the remaining 6% (*C. lucyae*) is unknown (Table S4). Of the five subgenera and 24 species groups documented within the genus *Charaxes* (Aduse-Poku *et al.*, 2009; Williams, 2018), this study comprises 28 recorded species from three subgenera and 11 species groups (Table 1).

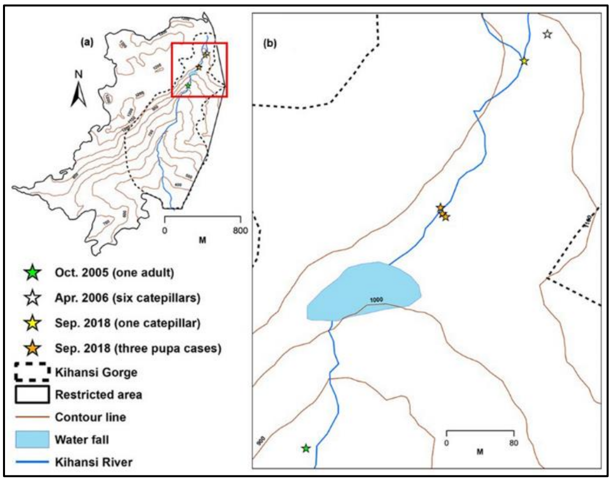

Figure 3 – Locations where *Charaxes mtuiae* has been observed between 2005 and 2018 in the Kihansi gorge forest (South-central Tanzania) [(a) insert map and (b) detail of area in red box (a).

Table 1 – Relative abundance of 28 species of *Charaxes* recorded in the study area. Relative abundance of the 48 other species of butterflies collected in the area is included on supplementary Table S2.

No.	Species name	Species-group	N	% of Total
1	*C. pollux pollux* (Cramer)	*jasius*	101	30.9
2	*C. cithaeron kennethi* Poulton	*tiridates*	47	14.4
3	*C. acuminatus acuminatus* Thurau	*varanes*	26	8.0
4	*C. varanes vologeses* (Mabille)	*varanes*	25	7.6
5	*C. violetta melloni* Fox	*tiridates*	18	5.5
6	*C. candiope candiope* (Godart)	*candiope*	13	4.0
7	*C. druceanus proximans* Joicey & Talbot	*jasius*	12	3.7
8	*C. ethalion littoralis* van Someren	*etheocles*	12	3.7
9	*C. macclounii* Butler	*cynthia*	12	3.7
10	*C. brutus alcyone* Stoneham	*jasius*	11	3.4
11	*C. protoclea azota* (Hewitson)	*cynthia*	11	3.4
12	*C. jahlusa argynnides* Westwood	*jahlusa*	10	3.1
13	*C. aubyni aubyni* van Someren & Jackson	*etheocles*	6	1.8
14	*C. tiberius tiberius* (Grose-Smith)	*exanthe*	4	1.2
15	*C. baumanni baumanni* (Rogenhofer)	*anticlea*	3	0.9
16	*C. congdoni* Collins	*etheocles*	2	0.6
17	*C. etheocles carpenteri* van Someren & Jackson	*etheocles*	2	0.6
18	*C. wakefieldi* (Ward)	*euxanthe*	2	0.6
19	*C. mtuiae* Collins, Congdon & Bampton	*etheocles*	1	0.3
20	*C. achaemenes achaemenes* Felder & Felder	*etesipe*	1	0.3
21	*C. bohemani* Felder & Felder	*tiridates*	1	0.3
22	*C. castor castor* (Cramer)	*jasius*	1	0.3
23	*C. dilutus dilutus* Rothschild	*eupale*	1	0.3
24	*C. guderiana* (Dewitz)	*etheocles*	1	0.3
25	*C. howarthi* Minig	*etheocles*	1	0.3
26	*C. lucyae lucyae* van Someren	*jasius*	1	0.3
27	*C. saturnus* Butler	*jasius*	1	0.3
28	*C. mccleeryi iringae* Kielland	*etheocles*	1	0.3
	TOTALS		**327**	**100**

Phylogenetic placement of *C. mtuiae*

The position of *C. mtuiae* in our phylogenetic reconstruction agrees with previous descriptions and previously hypothesised species relationships from species across Zambia, Malawi, and Zimbabwe, provided by Collins, Congdon, and Bampton (2017). Our topology shows that the *C. mtuiae* belongs to species-group *etheocles* as was expected, and strongly supports *C. martini* as a sister species with moderate support for *C. gallagheri* as the sister lineage to these taxa (Fig. 4).

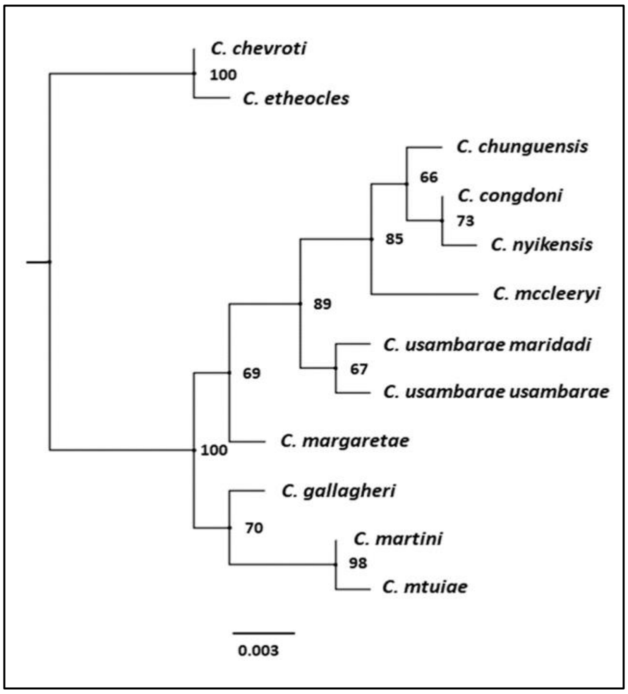

Figure 4 – Maximum likelihood tree based on COI sequences of *Charaxes mtuiae* and several of its phylogenetically closest neighbors. Bootstrap branch support is indicated on the respective branches.

Abundance of *Diospyros natalensis* and other woody plants

We recorded a total of 9,415 stems of woody plants belonging to 129 taxa (107 identified to species level and 22 to genus level). Of all recorded stems, 12.5% were of *D. natalensis* (11.3% recruits and 1.2% mature) (Table 2 and Table S5).

DISCUSSION

Our results indicate that *C. mtuiae* is an extremely rare species (Table 1), despite the high abundance of its host plant, *D. natalensis*, across the Udzungwa Mountains (Table 2). This finding is contrary to studies that reported a positive relationship between abundance of butterflies and resource availability (Curtis *et al.*, 2015; Yamamoto *et al.*, 2007; Döring & Hoffmann, 2004). Our two years of extensive surveys for *C. mtuiae* revealed only 4 individuals. Such difficulties in finding the target species are not uncommon for rare butterfly species. In a four-year extensive search for *Pseudaletis leonis* Staudinger, 1888 – a rare species of forest lycaenid in Sierra Leone – and for *P. agrippina agrippina* Druce, 1888 (synonym: *P. ugandae* Riley, 1928) in Uganda, Owen (1971) found only one specimen of each species in each respective area. Similarly, researchers in Guatemala recorded only six individuals of a rare hesperiid (*Inglorius mediocris* Austin, 1997) over 20 years of surveys (Haddad, 2019). Owen (1991) suggested three possible explanations for species rarity: first, the species exists in populations that extend over small areas and, as a consequence, are rarely discovered and the few known specimens may be strays from substantial populations; second, species are hard to access, as known for a number of forest species, where they may be spending most of their time in the canopy, hence rarely sighted from ground; third, most of the time the population exists in low density and only occasionally builds in numbers. In all these cases, discovery is a matter of chance or opportunity. Based on our field observations, the three explanations above may explain the rareness of *C. mtuiae*. This species has limited known distribution range, yet its discovery was by chance. The first specimen of *C. mtuiae* ever sighted was found standing on a concrete wall of a water intake, and probably briefly flew down from the canopy to drink water or seep minerals. Most species of *Charaxes* are known to be canopy dwellers (Larsen, 1999).

According to Owen (1979), rare butterfly species are far more likely to appear during the wet season, rather than the dry, though he did not specify whether the seasonal appearance refers to mature and/or immature stages of butterflies. We are therefore not able to directly relate our findings to Owen (1979) because the few mature and immature stage specimens of *C. mtuiae* were observed during both wet and dry seasons. We found the pupa cases (Fig. 3) during the dry season (September 2018), from which butterflies probably emerged a week or two earlier; and we found a final instar caterpillar during the same season, from which the adult emerged in October, one week before the start of the short rainy season. It was also in October that we caught the adult *C. mtuiae* in 2005. Additionally, Congdon and Bampton (pers. comm.) collected six final instars in 2006; all but one of which, a

Table 2 – Abundance of stems of *Diospyros natalensis* relative to other woody plants measured in five sampling plots. The "Taxa" column indicates the number of recorded taxa, with those identified to genus level in brackets. The percentage inside the brackets under the last three columns are relative abundance of stems per size class per block and in each sampling site.

Taxon	Plot	(m^2)	Taxa	Stems		Mature		Recruits	
				No.	%	No.	%	No.	%
Diospyros natalensis	A	1600	1	158	1.7	17	0.2	141	1.5
	B	2000	1	190	2.0	24	0.3	166	1.8
	C	800	1	360	3.8	29	0.3	331	3.5
	D	1200	1	446	4.7	31	0.3	415	4.4
	E	400	1	19	0.2	8	0.1	11	0.1
SUB TOTALS		**6000**	**1**	**1173**	**12.5**	**109**	**1.2**	1064	11.3
Other woody plants	A	1600	47 (6)	1606	17.1	122	1.3	1484	15.8
	B	2000	72 (13)	2531	26.9	202	2.1	2326	24.7
	C	800	38 (9)	1107	11.8	113	1.2	994	1.06
	D	1200	43 (7)	2546	27.8	136	1.4	2410	25.6
	E	400	52 (2)	455	4.8	61	0.6	394	4.2
SUB TOTALS		**6000**	**129**	**8242**	**87.5**	**634**	**6.7**	**7608**	**80.8**
TOTALS		**6000**	**130**	**9415**	**100**	**743**	**7.9**	**8672**	**92.1**

female, were parasitised. She eclosed in the first week of November 2006. In April 2006, during the heavy rainy season, we found six first and second instar caterpillars, and all were again parasitized. In the same season of April 2007, Congdon and Bampton (pers. comm.) collected a final instar larva which emerged as an adult male. From this chronology it appears that caterpillars of *C. mtuiae* are vulnerable to parasitoids and this no doubt contributed to their rareness as adult individuals, as not many immatures survive to adult stage. The main questions that remain to be answered are how many generations does *C. mtuiae* produce in a single year and when do they lay eggs? Answering these questions will be useful in determining the conservation status of this butterfly.

The phylogenetic structural position of *C. mtuiae* appears to be consistent with descriptions made based on morphology (Collins *et al*., 2017). These results provide strong evidence that the *C. mtuiae* is different from *C. martini martini* and *C. ghalagheri*. However, *C. mtuiae* has two other two siblings, C. *mutinondo* and *C. martini helenae*, for which we were unable find genetic information, and their separation and relationships cannot yet be adequately confirmed and resolved.

Recommendations for conservation measures

Charaxes mtuiae appears to be a rare species, endemic to the Kihansi River gorge within the vicinity of its host plant (200 ha), restricted along the riverbanks. *Diospyros natalensis* also appears important to other species in the ecosystem, such as *Euphaedra* sp. (Lepidoptera: Nymphalidae), *Euptera kinugnana* Grose-Smith (Lepidoptera: Nymphalidae), one unidentified species of hawkmoth (Lepidoptera: Sphingidae), and one unidentified species of stink bug (Hemiptera: Pentatomoidea) (Fig. 5).

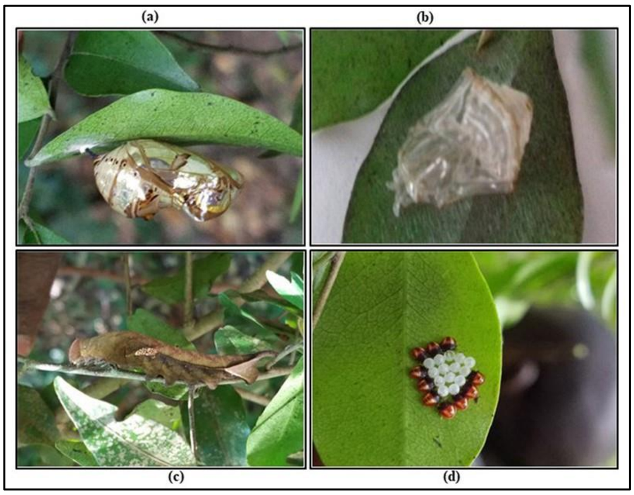

Figure 5 – Other species of insects hosted by *Diospyros natalensis* in Kihansi gorge: (a) pupae of *Amauris* sp.; (b) pupal case of *Euptera kinugnana*, (c) hawkmoth larva and (d) freshly hatched stink bug nymphs.

Habitat degradation and habitat loss are a major cause of butterfly population decline and loss worldwide. In Europe, one-fifth of butterfly species are Threatened or Near Threatened due to habitat degradation, habitat loss, or reduction of habitat connectivity (Van Swaay *et al*. 2010; Warren *et al*. 2020). The survival of *D. natalensis* in the Kihansi gorge forest is crucial for the maintenance of *C. mtuiae* populations and other species that depend on this plant. The long-term survival of *C. mtuiae* may be threatened by habitat destruction, especially by frequent wild fires which have been observed in the area (Mtui *et al*., 2019), notably in 2016 when fire killed over 50 stems of *D. natalensis* (Fig. S2). According to Nzunda *et al*. (2008), *D. natalensis* has a poor re-sprouting ability when severely disturbed. The limited ability of *D. natalensis* to regenerate may result in habitat size reduction and habitat fragmentation, endangering the survival of the *C. mtuiae* population. Another potential threat is drought, which may occur if the Kihansi River flow is not maintained, given the fact that the host plant is documented as occurring along the stream/riverbanks (White, 1988).

Charaxes mtuiae is the third endemic species discovered in the Kihansi gorge, others being the Kihansi spray toad *Nectophyrnoides asperginis* (Poynton *et al*., 1999) and Kihansi wild coffee *Coffea kihansiensis* (Davis & Mvungi, 2004). The toad has since gone extinct in the wild due to *Chytridiomycosis* disease (Weldon *et al*., 2020), and habitat alterations resulting from establishment of Kihansi hydropower dam, causing a drastic reduction in the Kihansi river flow (Williams, 2009).

The long-term survival of *C. mtuiae* will therefore require good management of its habitat, both by establishing and maintaining firebreaks to prevent wildfires from entering the evergreen forest, and maintaining continuity of water flow in the river. We recommend continued research and monitoring of the population of this species to understand its ecological requirements, the number of generations that *C. mtuiae* has in one year, when eggs are laid, and which chemical compounds within *D. natalensis* attract or are phagostimulants for *C. mtuiae*. Lastly, discovering where in the country *C. mtuiae* also occurs (additional *D. natalensis* areas), will guide conservation measures of *C. mtuiae* in the future.

ACKNOWLEDGEMENTS

We thank the Global Environmental Facility, World Bank and National Environmental Council for financial support. We are also grateful to Dr. Edwin Lewis at the University of Idaho, Department of Entomology, Plant Pathology and Nematology for hosting D. Mtui in the William F. Barr Entomological Museum during her sabbatical and to Andrea Noble Stuen, Hamilton’s lab manager. Leonard Haule, Goodluck Massawe, Amberson Kalenga, Bakari Lupia, Parson Kalenga, Martin Nziku, Sunday Njogoro, Juma Kanyeka, Sylvester Bernad and Theodora Venance are thanked for their support in field and the laboratory support. Henry Ndangalasi for identification and summarising the woody plants. Colin Congdon and Steve Collins are acknowledged for identification of butterfly specimens, and for their comments to improve the manuscript. Tanzania Wildlife Research Institute and the Tanzania Commission for Science and Technology gave permission to conduct the study. Richard Vane-Wright and Ian Richardson kindly and thoroughly reviewed our manuscript, and made many useful comments to improve it.

LITERATURE CITED

ADUSE-POKU, K., VINGERHOEDT, E., & WAHLBERG, N. 2009. Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetics and Evolution*, **53(2)**: 463–478. https://doi.org/10.1016/j.ympev.2009.06.021.

BELL, J.R. 2008. A simple way to treat PCR products prior to sequencing using ExoSAP-IT®. *BioTechniques* **44(6)**: 834. https://doi.org/10.2144/000112890.

CATES, R.G. 1980. Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: The effect of resource abundance and plant chemistry. *Oecologia* **46(1)**: 22–31. https://doi.org/10.1007/BF00346961.

COLLINS S., CONGDON C., & BAMPTON, I. 2017. Review of the *Charaxes gallagheri* complex resulting in two new species and undescribed morphs of *Charaxes gallagheri* van Son, 1962 (Lepidoptera, Nymphalidae, Charaxinae). *Entomologia Africana* **22(1)**: 19–30.

CURTIS, R.J., BRERETON, T.M., DENNIS, R.L.H., CARBONE, C., & ISAAC, N.J.B. 2015. Butterfly abundance is determined by food availability and is mediated by species traits. *Journal of Applied Ecology* **52(6)**: 1676–1684. https://doi.org/10.1111/1365-2664.12523.

DAVIS, A.P., & MVUNGI, E.F. 2004. Two new and endangered species of *Coffea* (Rubiaceae) from the Eastern Arc Mountains (Tanzania) and notes on associated conservation issues. *Botanical Journal of the Linnean Society* **146(2)**: 237–245. https://doi.org/10.1111/j.1095-8339.2004.00328.x.

DÖRING, E., & HOFFMANN, M.H. 2004. Distribution analysis of monophagous butterflies (Lepido - ptera) and their host plants in the western Palaearctic. *Schlechtendalia* **12**: 85–93.

HADDAD, N. 2019. *The last butterflies: A scientist's quest to save a rare ad vanishing creature*. Princeton University Press.

HEBERT, P.D., PENTON, E.H., BURNS, J.M., JANZEN, D.H., & HALLWACHS, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences* **101(41)**: 14812–14817. https://doi.org/10.1073/pnas.0406166101.

HOANG, D.T., CHERNOMOR, O., VON HAESELER, A., MINH, B.Q., & VINH, L.S. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35(2)**: 518–522. https://doi.org/10.1093/molbev/msx281.

KALYAANAMOORTHY, S., MINH, B. Q., WONG, T.K.F., VON HAESELER, A., & JERMIIN, L.S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* **14(6)**: 587–589. https://doi.org/10.1038/nmeth.4285.

KEARSE, M., STURROCK, S., & MEINTJES, P. 2012. *The Geneious 6.0.3 read mapper*. http://assets.geneious.com/documentation/geneious/GeneiousReadMapper.pdf.

KIELLAND, J. 1990. *Butterflies of Tanzania*. Hill House Publishers.

LARSEN T.B. 1991. *The butterflies of Kenya and their natural history*. New York: Oxford University Press.

LOVETT, J.C., HATTON, J., MWASUMBI, L.B., & GERSTLE, J.H. 1997. Assessment of the impact of the lower Kihansi hydropower project on the forests of Kihansi Gorge, Tanzania. *Biodiversity and Conservation* **6(7)**: 915–933. https://doi.org/Doi 10.1023/A:1018307412267.

MTUI, D., KOHI, E., OKICK, R., DEUSDEDITH, B., & LEONARD, H. 2019. *Status and distribution of Kihansi Charaxes and its food plant in the proposed Kihansi Conservation Area. Technical report.* Submitted to National Environmental Council (NEMC) June 2019.

MTUI, D., CONGDON, C., BAMPTON, I., KALENGA, P., & LEONARD, H. 2019. Altitudinal Distribution and Monthly Occurrence of Butterflies in the Kihansi Gorge Forest , Tanzania , with a Checklist of Species. *Tanzania Journal of Science* **45(4)**: 543–558.

NGUYEN, L.T., SCHMIDT, H.A., VON HAESELER, A., & MINH, B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32(1)**: 268–274. https://doi.org/10.1093/molbev/msu300.

NZUNDA, E.F., GRIFFITHS, M.E., & LAWES, M.J. 2008. Sprouting by remobilization of above-ground resources ensures persistence after disturbance of coastal dune forest trees. *Functional Ecology* **22(4)**: 577–582. https://doi.org/10.1111/j.1365-2435.2008.01405.x

OWEN, D.F. 1991. *Pseudaletis leonis*: a rare mimetic butterfly in a West African rain forest (Lepidoptera : Lycaenidae). *Tropical Lepidoptera* **2(2)**: 111–113.

OWEN, D.F. 1971. *Tropical butterflies : the ecology and behaviour of butterflies in the tropics with special reference to African species*. Clarendon Press, Oxford.

POYNTON, J.C., HOWELL, K.M., CLARKE B.T. & LOVETT, J.C. 1999. A critically endangered new species of *Nectophrynoides* (*Anura*: Bufonidae) from the Kihansi Gorge, Udzungwa Mountains, Tanzania. *African Journal of Herpetology* **47**: 59–67.

PROMEGA. 2017. *GoTaq ® Long PCR GoTaq ® Long PCR Master Mix Instructions for use of product M4021: Technical manual*. www.promega.com/protocols/

PROMEGA. 2018. *GoTaq® Green Master Mix - DNA Polymerase Chain Reaction: Protocol*. Guidelines for Amplification by PCR. https://doi.org/10.1007/978-3-642-58362-9_35.

QUINN, R.M., GASTON, K.J., & ROY, D.B. 2017. Nordic Society Oikos Coincidence in the Distributions of Butterflies and Their Foodplants. *Oikos* **21(3)**: 279–288.

RATNASINGHAM, S., & HEBERT, P.D.N. 2007. BOLD: The Barcode of Life Data System: Barcoding. *Molecular Ecology Notes* **7(3)**: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x.

SAMWAYS, M.J., MCGEOCH, M.A. & NEW, T.R. 2010. *Insect conservation: a handbook of approaches and methods.* Sutherland, W.J. (ed.) Oxford University Press.

VAN SWAAY, C., CUTTELOD, A., COLLINS, S., MAES, D., LÓPEZ MUNGUIRA, M., ŠAŠIĆ, M., SETTELE, J., VEROVNIK, R., VERSTRAEL, T.,

WARREN, M., WIEMERS, M., & WYNHOF, I. 2010. European red list of butterflies. In *Publications Office of the European Union*. https://doi.org/10.2779/83897.

WARREN, M.S., MAES, D., VAN SWAAY, C.A.M., GOFFART, P., VAN DYCK, H., BOURN, N.A.D., WYNHOFF, I., HOARE, D., & ELLIS, S. 2021. The decline of butterflies in Europe: problems, significance, and possible solutions. *Proceedings of the National Academy of Science of the United States of America* **118(2)**: 1–10. https://doi.org/10.1073/pnas.2002551117.

WELDON, C., CHANNING, A., MISINZO, G., & CUNNINGHAM, A.A. 2020. Disease driven extinction in the wild of the Kihansi spray toad, *Nectophrynoides asperginis*. *African Journal of Herpetology* **69(2)**: 151–164.

WHITE, F. 1988. The Taxonomy , Ecology and Chorology of African Ebenaceae II . The Non-Guineo- Congolian Species of *Diospyros* (Excluding sect. *Royena*). *Bulletin Du Jardin Botanique National de Belgique* **58(3)**: 325–448.

WILLIAMS, M.C. 2021. Afrotropical Butterflies: Genus *Charaxes* Ochsenheimer, 1816. https://www.metamorphosis.org.za/articlesPDF/1278/390%20Genus%20Charaxes%20Ochsenheimer.pdf

WILLIAMS, N. 2009. Red list species update fears. *Current Biology* **19(22)**: 1013–1014. https://doi.org/10.1016/j.cub.2009.11.007.

YAMAMOTO, N., YOKOYAMA, J., & KAWATA, M. 2007. Relative resource abundance explains butterfly biodiversity in island communities. *Proceedings of the National Academy of Sciences of the United States of America*, **104(25)**: 10524–10529. https://doi.org/10.1073/pnas.0701583104.