Clinging to survival: Critically Endangered Chapman's pygmy chameleon *Rhampholeon chapmanorum* persists in shrinking forest patches

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Abstract The Critically Endangered Chapman's pygmy chameleon *Rhampholeon chapmanorum* is endemic to the low elevation rainforest of the Malawi Hills in southern Malawi. Much of this forest has been converted to agriculture and it was uncertain whether chameleon populations have persisted. We used current and historical satellite imagery to identify remaining forest patches and assess deforestation. We then surveyed forest patches for the presence of this chameleon, and assessed its genetic diversity and structure. We estimated that 80% of the forest has been destroyed since 1984, although we found extant populations of the chameleon in each of the patches surveyed. Differentiation of genetic structure was strong between populations, suggesting that gene flow has been impaired. Genetic diversity was not low, but this could be the result of a temporal lag as well as lack of sensitivity in the mitochondrial marker used. Overall, the impact of forest loss is assumed to have led to a large demographic decline, with forest fragmentation preventing gene flow.

Keywords Africa, Chapman's pygmy chameleon, extinction, habitat loss, Malawi, reptile, *Rhampholeon chapmanorum*, threatened species

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Introduction

Overall extinction risk for chameleons is substantially higher than the global average of c. 15% for squamate reptiles (Böhm et al., 2013), with 34% of species categorized as threatened and 18% as Near Threatened (IUCN, 2019). Most threatened species are forest specialists, with habitat loss and degradation being the primary threat (Tolley et al., 2016). Given that forest chameleons are intolerant of transformed habitats, as forest is lost chameleon populations decline and become locally extinct. There are five Critically Endangered forest-living chameleon species in mainland Africa, all threatened by forest loss (IUCN, 2019). They inhabit small rainforest patches on mountain inselbergs that tend to have high endemicity (Menegon et al., 2008; Conradie et al., 2016; Gereau et al., 2016; Lyakurwa et al., 2019). Thus, the loss of forest patches results in the extinction of endemic species (e.g. Betts et al., 2017), including chameleons.

The Critically Endangered *Rhampholeon chapmanorum* is a small (3.5–5.5 cm body length), terrestrial chameleon endemic to the low elevation rainforest of the Malawi Hills near Nsanje in southern Malawi (Fig. 1, Plate 1). These mountains rise to c. 900 m and were once covered by dense rainforest, primarily on the eastern slopes between 700–900 m (Dowsett-Lemaire et al., 2001). At the time of the description of *R. champanorum* there were indications that substantial areas of forest were being lost (Tilbury, 1992). To safeguard the species, 37 individuals (24 females and 13 males) from the type locality in the Malawi Hills were released in 1998 in a forest patch at Mikundi, Malawi, c. 95 km to the north (Dowsett-Lemaire et al., 2001; Tilbury, 2018). Follow-up surveys at the release site in 2001 and 2012 confirmed the population was present (C.R. Tilbury, unpubl. data). However, given the apparent loss of forest in the species' natural range, it was uncertain whether the remaining forest fragments still contained viable populations (Tolley et al., 2014).

Rhampholeon chapmanorum is unlikely to inhabit transformed areas, and therefore forest degradation, fragmentation and loss would be expected to cause an overall demographic decline (e.g. Andrén, 1994), leading to local extinctions. Furthermore, the loss of connectivity between populations affects ecosystem functioning, disrupts metapopulations, reduces gene flow and increases genetic population structure even over small spatial scales. Thus, the
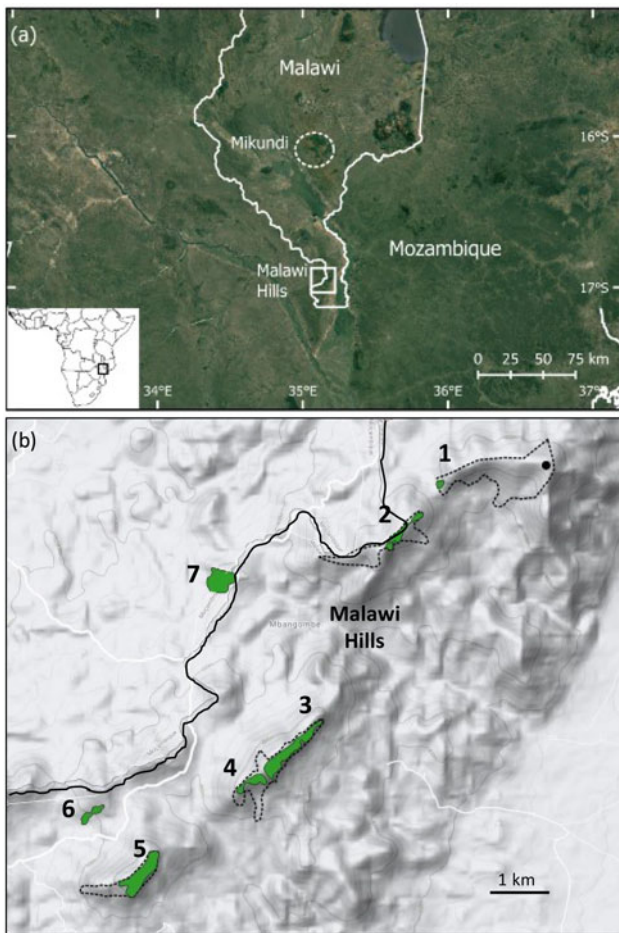
FIG. 1 (a) Overview map of sites searched for *Rhampholeon chapmanorum*. Broken circle shows the locality for Mikundi (exact locality obscured) and the square shows the Malawi Hills locality, and (b) forest patches in the Malawi Hills superimposed on a topographic map with contour lines. The present extent of forest patches is shaded, with the estimated extent in 1984/1985 shown by the dotted lines. Type locality is indicated by the black dot. Forest patches are numbered as in Table 1, except patches 6 and 7, which were not surveyed.

PLATE 1 Male *Rhampholeon chapmanorum* in Mabunga forest.

effect of habitat loss on the population is both direct (via population decline) and indirect because genetic diversity and gene flow are expected to diminish over time, reducing the adaptive potential of the population (Haddad et al., 2015; Leigh et al., 2019). The latter effects can be amplified in small populations because genetic drift is greater, leading to strong differentiation in genetic structure between populations, and inbreeding depression through fixation of deleterious alleles and elevated homozygosity, reducing resilience (Hanski, 2011). Information on population size, spatial genetic structure and diversity is therefore required to understand fully the extinction risk for *R. chapmanorum*.

To investigate these issues, we surveyed forest patches in both Malawi Hills and Mikundi, to assess whether *R. chapmanorum* populations are extant, quantified the extent of forest loss in the Malawi Hills using recent and historical satellite imagery as a proxy to gauge demographic decline, and examined the genetic structure and diversity of the populations. Because there are no historical baseline estimates of population size or genetic structure/diversity, we assume that demographic decline is proportional to forest loss. We predicted that fragmentation has disrupted gene flow, leading to strong differentiation of genetic structure and low genetic diversity.

Methods

We estimated the extent of forest loss using historical (1984/1985) and recent (2019) satellite imagery of Malawi Hills from *Google Earth* (Google, Mountain View, USA). Historical satellite images were of poor quality, and therefore images from both 1984 and 1985 were used. Polygons were created around each of the forest patches for these time periods, exported as kmz files, mapped in *QGIS 2.18* (QGIS Development Team, 2016) and their areas quantified. The 700 m contour was used as a guide to assess where low elevation rainforest might have originally occurred (e.g. Dowsett-Lemaire et al., 2001).

Logistical constraints did not allow us to survey all the forest patches, but we were able to access two forest patches in the Malawi Hills and one at Mikundi, during 13–16 March 2016 (Fig. 1). We walked forest trails at night to record chameleons, using torchlight. Transects were defined by presence of trails and were therefore neither randomly chosen nor of a set distance or time. The location of each chameleon encountered was recorded (± 3 m) and a tissue sample was taken from a subset of adult chameleons (c. 2 mm tail clip) for genetic analysis. All individuals were returned to the same perch on which they were found.

To assess the genetic diversity within and between populations from each forest patch, an 845 bp fragment of the ND2 mitochondrial gene was sequenced for 19 individuals. Genomic DNA was extracted using salt extraction (MacManes, 2008). ND2 was amplified in a PCR reaction

TABLE 1 Forest patch areas in the Malawi Hills in 1984/1985 and 2019, with estimated extent lost. Patch numbers (in parentheses) correspond to the numbers in Fig. 1b.

Forest patch (no.)	1984/1985 (ha)	2019 (ha)	% lost
Type locality/Chief's ancestors (1)	88.5	1.2	98.6
Patch 2	35.0	6.0	82.9
Mwanambweli (3 & 4)	44.7	16.5 (patch 3), 5.25 (patch 4)	51.3
Mabunga (5)	28.3	16.6	41.3
Total	196.5	45.55	76.8

volume of 25 μl with 0.4 μl of 10 mM dNTPs, 2.5 μl of 25 mM MgCl$_2$, 0.3 μl of 10 pmol forward (vMet2) and reverse (vTrp) primer (Cunningham & Cherry, 2004), 2.5 μl of Mg^{2+} free buffer solution, 0.15 μl Taq polymerase, and 1–2 μl of 25 ng/μl DNA template. Thermal cycling included initial denaturation for 4 min at 95 °C followed by 35 cycles denaturation for 45 s at 94 °C, annealing for 45 s at 52 °C, extension for 60 s at 72 °C, and final extension for 10 min at 72 °C. PCR products were run on a 1% agarose gel and visualized under a UV light to verify amplification. Amplicons were sequenced using the forward primer at Macrogen Inc. (Amsterdam, The Netherlands). Sequences were edited and aligned in *Geneious R11* (Geneious, 2021) and deposited in GenBank (accession numbers: MT779023–MT779042). In addition, sequence from an individual originally sampled in 1998 from the type locality was available on GenBank (AY524919) for comparison.

Haplotype (*h*) and nucleotide (*π*) diversity for each locality was estimated (Tamura–Nei model of substitution, α = 0.01) using *Arlequin 3.5* (Excoffier & Lischer, 2010). A haplotype network was constructed using *Network 5.0.1.1* (Bandelt et al., 1999; Polzin & Daneschmand, 2003) to assess shared haplotypes between localities, including the two GenBank sequences from the type locality. To examine genetic structure between forest patches, an analysis of molecular variance (AMOVA) was run, estimating pairwise Φ_{ST} in *Arlequin*.

Results

The satellite imagery showed that the forest extent in the Malawi Hills may have been c. 196 ha in 1984, in three patches (Table 1). As of 2019, the patches were much reduced, with two of the three patches fragmented into smaller patches totaling c. 40 ha, and the forest at the type locality completely cleared (Fig. 1). Currently, Mabunga forest is the largest intact patch (16.6 ha), having been reduced from 35 ha since 1984. Overall, we estimate c. 80% forest loss during 1984–2019. Given that the original extent (pre-1984) is not known, the magnitude of loss could have been greater as the original forest extent may have reached the lower slopes (e.g. Dowsett-Lemaire et al. 2001). There are also two additional low forest patches (at 550–620 m) on the western slopes of the Malawi Hills (13 and 3.9 ha; patches 6 and 7, respectively, in Fig. 1), but it is unknown if they provide suitable habitat for chameleons.

We surveyed two of the five rainforest forest patches that were furthest apart. The first patch (1.19 ha) is c. 1.8 km south-west of the type locality (Fig. 1; patch 1). Local community leaders indicated that the Chief's ancestors are buried in the forest, and it is considered sacred ground. We were therefore only permitted to survey along a footpath that skirted just inside the forest, where we recorded seven adult chameleons (six females, one male) during one evening. Although we were not permitted further inside this forest our observations of chameleons along the footpath suggest there is a population within the sacred forest, despite the small size of this patch. The second, Mabunga forest (Fig. 1; patch 5; 16.6 ha), is c. 7 km to the south-west of the first. We did not observe chameleons on the forest edge, but we recorded 10 individuals inside the forest along a footpath (seven females, three males) during one evening. Midway between the Chief's ancestors and Mabunga forests there are additional patches (Fig. 1; patches 2, 3 & 4; the latter two at Mwanambweli forest) that we were not able to access, but given their location and extent, we assume there are chameleon populations present. Finally, we surveyed the forest patch at Mikundi (Fig. 1) where chameleons had been released in 1998. We recorded 21 adult chameleons, plus 11 juveniles and hatchlings (body size 1.5–2.0 cm) during one evening, but we did not observe chameleons on the forest edge or outside the forest.

Estimates of genetic diversity for all populations were within the range considered normal compared to other chameleon and small-bodied reptile species (Table 2; cf. Hague & Routman, 2016; Main et al., 2018; Rutherford et al., 2019). There were no shared haplotypes between forest patches, including those previously sequenced from the original at the type locality (Fig. 2). Similarly, the AMOVA showed a significant difference between each of the forest patches (Φ_{ST} = 0.23, P < 0.001) suggesting strong differentiation of genetic structure between populations and reduced gene flow across the relatively small distances between the patches surveyed in the natural range (Table 3).

Discussion

Although populations of *R. chapmanorum* persist in the Malawi Hills, the forest extent has been significantly reduced to a total of c. 40 ha. We can confirm there are extant

TABLE 2 Genetic diversity estimates (± SD) for *Rhampholeon chapmanorum* from three forest patches, showing haplotype diversity *h*, nucleotide diversity π and number of haplotypes *H*. Patch numbers (in parentheses) correspond to the numbers in Fig. 1b.

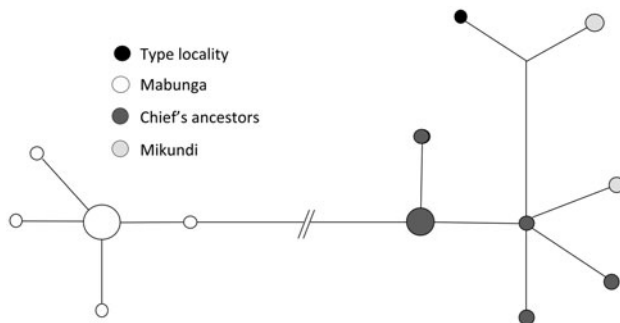| Forest patch (no.)	n	$h \pm$ SD	$\pi \pm$ SD	H
Chief's ancestors forest (1)	6	0.800 ± 0.172	0.003 ± 0.0021	4
Mabunga (5)	9	0.722 ± 0.159	0.002 ± 0.0014	5
Mikundi (released population)	4	0.833 ± 0.222	0.014 ± 0.0098	3

FIG. 2 Haplotype network for *R. chapmanorum* with haplotypes shaded according to locality where chameleons were sampled. Branch lengths are proportional to the number of mutations and circle size is proportional to the number of individuals with that haplotype.

TABLE 3 Pairwise analysis of molecular variance for *R. chapmanorum* from three forest patches, with Φ_{ST} values (bottom matrix) and P values (top matrix). Patch numbers (in parentheses) correspond to the numbers in Fig. 1b.

	Chief's ancestors (1)	Mabunga (5)	Mikundi
Chief's ancestors (1)		< 0.010	< 0.001
Mabunga (5)	0.088		< 0.001
Mikundi	0.032	0.083	

populations in at least two of the five forest patches at higher elevations, and we assume the other higher elevation patches also hold populations. There are two patches at lower elevation that we did not survey, and we cannot assume that *R. chapmanorum* populations are present there, given these are below 700 m and low elevation rainforest was not considered to have occurred there (Dowsett-Lemaire et al., 2001). Chameleons were not observed outside forest although observations at the sacred forest were made at slightly degraded forest edge. *Rhampholeon* species are forest specialists (Branch et al., 2014) and cannot tolerate transformed habitats, so where forest has been destroyed, the populations will have gone locally extinct (as in the type locality). Conversely, chameleons appear to be abundant at the Mikundi release site, with chameleons of all age classes observed.

From aerial photography, the extent of the Malawi Hills forest was previously estimated to be c. 400 ha (Dowsett-Lemaire & Dowsett, 1988). This is approximately double our estimate of 196 ha for 1984, but the date of the aerial photographs used was not indicated. If the low elevation rainforest covered the mountain down to 700 m (Dowsett-Lemaire et al., 2001) the forest extent could have been 1,050 ha historically. Although speculative, this could mean that 96% of the original forest has been lost. Our estimate of a minimum of 80% forest loss since the mid 1980s is likely to have resulted in a similar decline of *R. chapmanorum*, or at least a dramatic population decline over at least 35–40 years. Despite this, genetic diversity is not low, but this can be explained by the lack of a linear relationship between genetic diversity and population declines that result from habitat loss (Pflüger et al., 2019). Erosion of genetic diversity at detectable levels has a long time lag that is influenced by the effective population size, the number of generations and the degree of habitat loss and fragmentation (e.g. Tilman et al., 1994; Krauss et al., 2010; Hoban et al., 2014; Wu et al., 2020). Conversely, genetic structure responds more rapidly to habitat fragmentation (Keyghobadi et al., 2005; Pflüger et al., 2019) because gene flow is impeded by isolation of local patches (Balkenhol et al., 2013). Populations of *R. chapmanorum* do not have reduced genetic diversity but they do have strong differentiation of genetic structure, with no shared haplotypes despite the short distance between them. This indicates that gene flow has been disrupted over a scale of just a few kilometres and that the metapopulation is not intact, increasing extinction risk.

Individuals from the released population at Mikundi are in the same haplotype group as those from the type locality and the Chief's ancestors sacred forest, although the haplotypes occur at different frequencies. Given that the original released population was small, the observed difference is probably the result of a founder effect in which the released individuals were not a random sample of the range of haplotypes in the original population. This can result in haplotype frequency differences between the new and source populations, and the effect can be magnified over time as genetic drift changes the proportions of haplotypes. In this case, assuming the generation time of *Rhampholeon* is 1–1.5 years, there would have been 15–20 generations since the release, which is sufficient time for genetic drift to have differentiated the populations given the small founding population.

Overall, our findings indicate there has been a severe demographic decline with disruption of gene flow as a result

of extreme forest loss. The effects of this decline have yet to be fully manifested in the genetic composition, as genetic diversity and differentiation can continue to be negatively affected for many generations after habitat loss is halted, even with subsequent population size increases (Pflüger et al., 2019). The genetic differentiation shows that gene flow has already been disrupted, and the forest loss requires immediate attention to prevent further loss of genetic diversity. Urgent conservation action is needed, including halting of forest destruction and recovery of habitat to promote connectivity. Although part of the Malawi Hills falls within a Key Biodiversity Area (Matandwe Forest Reserve) most of the forest falls outside the reserve boundary (c.f. BirdLife International, 2020), and the effectiveness of the forest reserve is questionable, given that most of the destruction has been within its boundaries. Although extending the reserve to encompass all the forest patches would be a first step, measures are needed to avert the destruction of the remaining patches. In addition to *R. chapmanorum*, other endemic species could occur there, which would add weight to the need to conserve the remaining patches. Unfortunately, few surveys have been carried out in the area, with only widespread species of plants, birds and butterflies recorded (Dowsett-Lemaire et al., 2001).

A species action plan that defines conservation activities is urgently needed for *R. chapmanorum*. However, both the planning and the recommended actions require strong leadership, personnel, stakeholder engagement, including with government departments, and sufficient funding to ensure success. Many international organizations support the production and execution of species action plans (e.g. IUCN Species Survival Commission, WWF) and the involvement of these or analogous organizations will be essential to prevent the extinction of this species in the wild.

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