RESEARCH ARTICLE

Conservation genetics of two threatened frogs from the Mambilla highlands, Nigeria

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Abstract

Amphibians are the vertebrate group with the highest number of species threatened with extinction, and habitat loss and fragmentation are considered to be among the leading causes of their declines and extinctions. Little is known of the population biology of amphibian species inhabiting montane forests in Central and West Africa, where anthropogenic activities such as farming and cattle raising are major threats to native biodiversity. We used Amplified Fragment Length Polymorphisms (AFLPs) to assess the population genetic structure of two poorly known species, Cardioglossa schioetzi and Leptodactylodon bicolor (both in the Arthroleptidae), in and around Ngel Nyaki Forest Reserve on the Mambilla Plateau in eastern Nigeria. The landscape comprises continuous forest on steep slopes and small riparian forest fragments in a grassland matrix. While increased fragmentation is well documented for these and other forests in the mountains of Cameroon and Nigeria over the past century, there are no previous assessments of the impact of forest fragmentation on montane amphibian populations in this region. Our estimates of genetic diversity are similar across populations within each species with levels of heterozygosity values consistent with local population declines. Except for a pair of populations (C. schioetzi) we did not observe genetic differentiation between forest and riparian forest fragment populations, nor across sites within continuous forest (L. bicolor). Our results demonstrate recent gene flow between forest fragments and the adjacent protected forests and suggest that small forest corridors connecting these may lessen the genetic consequences of at least 30 years of intense and severe fragmentation in Ngel Nyaki.

Introduction

Globally, amphibians face the most extreme population declines of all major vertebrate groups [1–3]. Contributing factors include climate change, disease, and habitat fragmentation [4–6]. The severity of amphibian declines vary across geographic regions [3] and despite evidence for population declines in Africa [7], the influence of habitat fragmentation on these declines remains unknown [7, 8]. Even less is known about the impact of habitat fragmentation on population genetics of amphibian species in Africa [9,10].
As is the case with many other taxa, habitat fragmentation likely has long-term effects on the genetic viability of amphibian populations because of the combined effects of reduced population size and increased isolation [11]. Increased isolation among populations may lead to reduced dispersal and gene flow, increased levels of inbreeding, smaller effective population sizes, and loss of genetic variation [12–14]. Consequently, assessing the population genetics of a species can provide valuable information for conservation much more quickly than longitudinal demographic studies [15]. Such studies have already been widely used to inform management strategies to halt or slow down amphibian decline [10,16–22].

To begin to redress the paucity of population genetics studies of African amphibians, we focused on populations of frogs within the forests of the Nigerian Highlands of the Cameroon Volcanic Line [23]. This is one of Africa’s biodiversity hotspots [24] and hosts a rich diversity of amphibian species [25]. Despite recognition as a center of biodiversity [26,27], the diversity and ecology of amphibians in Nigeria’s mountains remain understudied [25]. The montane forests on the Mambilla Plateau have been fragmented for a long time, hundreds of years at least. However, even 30 years ago there was more connectivity among the fragments [28] and more likely the streamside forests were acting as corridors between larger forests. Within the last 30 years (HC, personal observation) there has been a dramatic increase in cattle, which first arrived on the Plateau in the 1950’s [29]. In addition to overgrazing by cattle, other threats to these forests include grass burning and land clearance for farming [30]. We used this heavily modified landscape in Nigeria’s mountains as a context for studying how forest fragmentation and associated degradation impact population genetic structure in forest-associated frog species.

Based on previous surveys of Ngel Nyaki amphibiens [25,31,32], we selected two species from the anuran family Arthroleptidae that is endemic to sub-Saharan Africa [33–37]. Both species, Cardioglossa schioetzi and Leptodactylodon bicolor, are endemic to the montane forests of Cameroon and Nigeria, they are small (<30 mm snout–vent length), have stream-adapted tadpoles, and live in leaf litter and rocky areas [37–39]. Little information exists on the population biology of these species, such as for example, the distances over which individuals disperse. Cardioglossa schioetzi occurs within small degraded riparian forests close to, but outside Ngel Nyaki Forest Reserve, as well as along the edge habitat of the continuous Ngel Nyaki forest (Fig 1). In contrast, L. bicolor is common along rocky streams within the continuous forest, but is rarely found within riparian fragments (Fig 1; [32]). Both species are on the IUCN Threatened species list (C. schioetzi—Endangered; L. bicolor—Vulnerable [40]) because of habitat degradation and the decline of remaining forest habitats on the mountains of Cameroon and Nigeria.

We aimed to determine levels of genetic diversity within and among populations of each species and use these data to assess levels of gene flow and connectivity among the populations within each species, in part to determine the extent to which grasslands act as a dispersal barrier for these species. Because its populations occur in fragments surrounded by heavily grazed grassland, we hypothesized that C. schioetzi would have less connectivity among populations and less genetic diversity than in L. bicolor.

**Material and methods**

**Study area and field sampling**

The Mambilla Plateau in Taraba State Nigeria (Fig 1) is located on the margins of the Cameroon Highlands Forest ecoregion that is well known for its rich flora and fauna, which are among the most diverse in Africa [41]. A high proportion of taxa are regional endemics [27,28]. Ngel Nyaki Forest Reserve (7°30’N, 11°30’E) is part of a network of sub-montane/
montane forests and forest fragments at elevations up to 2300 m, with a mean annual rainfall of approximately 1800 mm and mean monthly temperatures of 13–26 °C and 16–23 °C for the wet and dry seasons, respectively [30]. The reserve, on the western escarpment of the Mambilla Plateau covers approximately 4600 ha and comprises a mosaic of overgrazed montane grasslands, degraded streamside forest/shrubland strips and 720 ha of dense sub-montane forest [28,30]. We refer to these riparian fragments as ’fragments’ and Ngel Nyaki forest as ’continuous forest’. The continuous forest has a rich floristic composition with over 146 vascular plant species, many of which are endemic to Afromontane regions, including four IUCN Red Data Listed species [28]. The fragments comprise a subset of the forest species and are typically more open and disturbed than the forest.

Between July and October 2012, we searched for *C. schioetzi* and *L. bicolor* in both forest and fragments (Fig 1, Table 1). We used a combination of visual and acoustic techniques [32] to find the frogs, searching for at least four hours during the day and another four hours by the night. After two weeks of searching, we identified sites with sufficient numbers of individuals
to meet our minimum sample size of \( N = 15 \) for characterizing a population [32]: four sites within the forest: Main Forest I (MFI), Main Forest II (MFII), Main Forest III (MFIII), and Main Forest IV (MFIV); and four fragments from outside of the reserve: Fragment 1 (F1), Fragment 2 (F2), Fragment 3 (F3), and Fragment 4 (F4). In total, we collected 69 adult toe clips and 11 tadpole tail tips for \( C. \) schioetzi and for \( L. \) bicolor one toe clip and 117 tadpole tail tips. Tissue samples were preserved in microtubes with 95% ethanol and genetic analyses were performed at the University of Canterbury, New Zealand. This study was conducted with approval of the Animal Ethics Committee of the University of Canterbury (permit Ref: 2012/24R); the field permit was granted for field work in Ngel Nyaki Forest Reserve.

### AFLP profiling and data analysis

We chose to use AFLPs in our study because, while we are well aware of their limitations [42] and the fact that hypervariable genetic markers would have been preferable [15,43], we are working on species without genomic resources to refer to (e.g. [18,44]). AFLPs have been shown to be a cost-effective and rapid tool for generating many polymorphic loci useful for inferring population genetic structure of species [45–47]. While we may not have achieved the precision we could have using more sensitive molecular markers, we believe our data to be important and a significant contribution to what is known about amphibian population genetic diversity in West Africa. Moreover, an added advantage of using AFLPs is that it allowed us to make direct comparisons with other African studies [18,44,46,48,49].

We extracted genomic DNA from tissue samples using a modified CTAB (cetyltrimethylammonium bromide) protocol [50] and developed AFLPs based on Vos et al. [51] with minor modifications. We used the two enzymes EcoRI and MseI to conduct digestion of genomic DNA. We then ligated fragments to double-stranded adaptors with T4 DNA ligase. For pre-selective PCR, we used primers complementary to the Eco RI with no added nucleotides and MseI+A. For the selective PCR, we used two pairs primers: ESP1B / MSP3 and ESP1B / MSP6 (Table 2). The EcoRI selective primer, ESP1B, was labelled with a fluorescent dye (either 6-FAM or VIC, Applied Biosystems) and contained three selective bases, whereas MSP3 and MSP6 (MseI selective primers) contained four bases of extension each (Table 2). We ran the selectively amplified fragments on an ABI 3130xl Genetic Analyzer (Applied Biosystems) with Gene-Scan LIZ size standard (Applied Biosystems). Finally, we visualized AFLP fragments with GENEMAPPER 4 (Applied Biosystems) in which peaks were called using the default settings except for the peak height detection which was set at 100 Relative Fluorescent Units (RFU). It was assumed that each peak represents a different locus. The threshold intensity for a

| Species          | Locality Code | Latitude     | Longitude    | Elevation (m) | N  | PPL     | \( H_E \)  |
|------------------|---------------|--------------|--------------|---------------|----|---------|------------|
| \( C. \) schioetzi | MFI           | 7° 5.117'    | 11° 3.918'   | 1543          | 13 | 66.4    | 0.221(0.019) |
|                  | F1            | 7° 4.737'    | 11° 3.934'   | 1643          | 26 | 84      | 0.227(0.016) |
|                  | F2            | 7° 4.939'    | 11° 3.218'   | 1647          | 20 | 71.20   | 0.199(0.017) |
|                  | F3            | 7° 4.573'    | 11° 3.382'   | 1655          | 21 | 77.60   | 0.229(0.016) |
| \( L. \) bicolor  | MFI           | 7° 5.179'    | 11° 3.894'   | 1505          | 31 | 76.27   | 0.217(0.017) |
|                  | MFII          | 7° 4.859'    | 11° 3.465'   | 1641          | 20 | 65.25   | 0.198(0.018) |
|                  | MFIII         | 7° 5.368'    | 11° 3.771'   | 1599          | 19 | 74.58   | 0.238(0.018) |
|                  | MFIV          | 7° 4.498'    | 11° 3.382'   | 1561          | 25 | 66.10   | 0.208(0.018) |
|                  | F4            | 7° 6.092'    | 11° 3.613'   | 1601          | 23 | 66.95   | 0.184(0.017) |

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peak being considered a locus was 100 RFU with a length at least 100 bp. Above 300 bp the resolution was such that it was not possible to accurately identify peaks, so that only peaks between 100-300bp were included in the analysis. The presence or absence of all fragments was confirmed manually. Following Stötting et al. [52] markers were scored for all the individuals in the same analysis session to prevent scoring errors when analyzing several groups of samples.

We conducted reproducibility tests by replicating samples, as suggested by Bonin et al. [53] and Meudt and Clarke [54]. As recommended, we replicated analyses for a subset of samples (ideally 5–10% of the total number of samples) to detect fragments producing erratic patterns. To do so, we randomly selected 5% of the samples for each preferred primer combination for duplication and then assessed the error rate.

Due to the fact that mitochondrial 16S DNA sequences of selected individuals from Ngel Nyaki for both *C. schioetzi* and *L. bicolor* are less than 2% divergent from populations on Mount Oku in the Cameroonian mountains (unpublished data), similar to *Arthroleptis palava* [31], we are confident that genetic differences observed on the Mambilla Plateau are due to population-level differences rather than cryptic species.

**Genetic diversity.** We used GenAlEx v6.5 [55] to perform frequency and distance-based analyses. Allelic frequencies were estimated following the method of Lynch and Milligan [56], assuming independent nuclear loci and Hardy–Weinberg equilibrium within populations. The expected Heterozygosity ($H_E$) was calculated as $2(p)(q)$ implemented for diploid binary data (dominant markers) and assuming random mating, where $q = (1—Band Frequency)^0.5$ and $p = 1—q$.

We converted genetic data into a pairwise individual-by-individual genetic distance matrix and used this to assess genetic structure within populations. This is a true Euclidean metric [see 55] as required for the subsequent analysis of molecular variance.

**Evaluating the population genetic structure.** We performed an Analysis of Molecular Variance (AMOVA) to investigate the hierarchical partitioning of genetic variation among populations and estimate the population genetic differentiation via $\Phi_{PT}$. To do this, each of the sampling sites were treated as populations (other hierarchical analysis such as different groups of fragments or regions were not performed). $\Phi_{PT}$ is analogous to $F_{ST}$ and used for dominant markers such as AFLP [57]. This measure was calculated as $V_{AP}/(V_{AP} + V_{WP})$, where $V_{AP}$ is the variance among populations and $V_{WP}$ the variance within populations. We tested for statistical significance of the $\Phi_{PT}$ values using a nonparametric permutation method [58] with the number of permutations set to 999. By testing significance of the variance components and $\Phi$...
statistic through a permutational approach removes the assumption of normality, which is unsuitable for molecular data [58]. Significance levels following corrections for multiple comparisons via Bonferroni tests [59] were conducted.

Last, we investigated population genetic structure using the Bayesian model-based clustering algorithms implemented in STRUCTURE v2.3.3 [60]. STRUCTURE uses a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into populations based on their genotypes. It generates posterior probabilities of assignment of individuals to each of a given number of K groups or populations regardless of their site of origin [60]. For each species, we first ran STRUCTURE on all populations across the entire study area using the admixture model (no prior information) with correlated allele frequencies. We then used the population data (sampling sites) as prior information (LOCPRIOR) to assist the clustering as recommended when the signal of structure is weak [60,61]. For both species, a range of K values from 1 to 10 was performed. We ran batches of five independent runs with a burn-in of 100,000 and 500,000 iterations for each of the K values. We calculated the ‘optimal’ K (the number of genetic clusters that best fits these data) using the web version of STRUCTURE HARVESTER [62]. Below, we report estimates for K using two methods: (1) the log posterior probability of the data Ln(K) given K genetic clusters [60] and (2) ΔK [63], which is based on the rate of change in the log probability of data between successive K values.

To test for migration within each species, we used STRUCTURE to identify immigrants or individuals with recent immigrant ancestry in the last two generations. The model for this analysis uses population information with correlated allele frequencies. The model was set to GENSBACK = 2 and MIGRPRIOR = 0.001, as suggested by Falush et al. [64]. Thus, the prior probability that an individual has pure ancestry from its predefined population is 0.999.

To examine the pattern of isolation by distance, we tested for correlation between matrices of geographic and genetic distances ($F_{PT}$ pairwise) using a Mantel test as implemented in GenAlEx v6.5.

Although both GenAlEx and STRUCTURE can accommodate missing data, we removed missing genotypes from the analyses for the two preferred combinations of primers for both species in all analyses. Missing data may be problematic for some pairwise distance-based analyses implemented in GenAlEx [65]. While GenAlEx provides an option for interpolating the genetic distance for missing loci (by inserting the average genetic distances for each population level pairwise contrast, that is, within a population, or between two populations), we prevent any possible bias by eliminating the missing data from our analyses [see 55].

Results

The two species had different distributions. Cardioglossa schioetzi was recorded most often near streams in riparian forests outside the boundary of Ngel Nyaki Forest Reserve and sometimes on the edge of the continuous forest within the reserve. Specimens of this species were common in F1, but rare in F2 and F3 (see Fig 1). In contrast, L. bicolor was common along the streams within the forest, but encountered in only one riparian forest outside of the reserve.

The two preferred primer combinations, ESP1B/MSP3 and ESP1B/MSP6, together yielded 275 loci for the 198 samples representing both species (S1 Table). Of these 275 loci, 243 (88.3%) were polymorphic. We estimated the genotyping error by comparing the presence-absence matrices of repeated profiles. The genotyping error rate per locus was calculated as the ratio between the number of single-locus mismatches ($ml$) and the number of replicated loci ($nt$) [66]. The estimated genotyping error per locus was 2.9% (SD = 3.9) for ESP1B/MSP3 and 5.2% (SD = 4.3) for ESP1B/MSP6. We obtained an average error rate of 4% by taking into account the two pairs of primers.
Genetic diversity

Similar values of genetic diversity were obtained for both the four populations of *C. schioetzi* and the five populations of *L. bicolor* (Table 1). The average percentage of polymorphic loci for the four populations of *C. schioetzi* was 74.8% (Standard Error SE = 3.8). The average of genetic diversity (*H*~E~) across these populations and loci was 0.219 (SE = 0.008). We did not detect significant differences in average heterozygosity between populations (F(3,496) = 0.65, P = 0.58). The average percentage of polymorphic loci for *L. bicolor* was 69.8% (SE = 2.3), and the average value of genetic diversity was *H*~E~ = 0.209 (SE = 0.008). Average heterozygosity was not significantly different among the five populations of *L. bicolor* (F (4,585) = 1.28, P = 0.28).

Population structure

According to the overall Φ~PT~ (calculated via AMOVA) no significant genetic differentiation was detected among the populations of *C. schioetzi* (Φ~PT~ = 0.018, p = 0.097). After Bonferroni correction, pairwise estimates of Φ~PT~ among populations (Table 3) showed differences only among the population from the forest MFI with the riparian fragments F3. In contrast, no significant difference was recorded among the three riparian fragments. For *L. bicolor* no genetic differences were detected among the five populations following either the overall Φ~PT~ (Φ~PT~ = 0.026, p = 0.020) or the Φ~PT~ pairwise matrix (Table 4).

The clustering analyses implemented in STRUCTURE (both with and without prior information) revealed multiple groups for both species. For *C. schioetzi*, the analysis using all the sites across the study area without prior location information (S1 Fig) found an optimal K (based on the Ln(K)) of 5, and ΔK = 2 based on Evanno’s method. When using prior information (Fig 2), K = 8 and ΔK = 4 were recognized as the best K for each of the methods, respectively. For *L. bicolor*, without prior location information (S2 Fig), the optimal K (based on the Ln(K)) was 7, and ΔK = 2. Based on the Ln(K) estimation, with prior location information, eight genetic groups (K = 8) were detected, whereas the Evanno’s estimation detected five clusters (ΔK = 5) (Fig 3).

Migration test

The test for migration revealed that 53 of 80 individuals of *C. schioetzi* were immigrants or had ancestry in other populations in the past two generations. Migration between forest and

### Table 3. Pairwise matrix for *Cardioglossa schioetzi* of Φ~PT~. Φ~PT~ values below diagonal, and above it probability values (significant values in bold, Bonferroni correction).

|       | MF1  | F1    | F2    | F3    |
|-------|------|-------|-------|-------|
| MF1   | -    | 0.026 | 0.042 | **0.005** |
| F1    | 0.051| -     | 0.424 | 0.598 |
| F2    | 0.061| 0.000 | -     | 0.371 |
| F3    | 0.101| 0.000 | 0.000 | -     |

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### Table 4. Pairwise matrix for *Leptodactylodon bicolor* of Φ~PT~. Φ~PT~ values below diagonal, and above it probability values.

|       | MF1  | MFII  | MFIII | MFIV  | F4    |
|-------|------|-------|-------|-------|-------|
| MF1   | -    | 0.103 | 0.260 | 0.280 | 0.013 |
| MFII  | 0.021| -     | 0.112 | 0.366 | 0.007 |
| MFIII | 0.007| 0.029 | -     | 0.224 | 0.103 |
| MFIV  | 0.003| 0.011 | 0.011 | -     | 0.055 |
| F4    | 0.054| 0.073 | 0.027 | 0.039 | -     |

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Fig 2. Structure assignments for *Cardioglossa schioetzii* using sampling location information. (A) $K = 8$ clusters based on the $\text{Ln}(K)$, and (B) following Evanno’s method shows the assignment into $\Delta K = 4$ clusters. The bar plots at the bottom show (C) $K = 8$ and (D) $\Delta K = 4$. Each vertical bar represents an individual for which is shown the proportional genetic assignment.

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Fig 3. Structure estimates of K groups for *Leptodactylodon bicolor* using sampling location information. Graph (A) and bar plot (C) show K = 8 based on the Ln(K) estimation. (B) and (D) represent respectively the graph and plot of the best K following Evanno’s approach ΔK = 5. Each vertical bar represents an individual for which is shown the proportional genetic assignment.

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Within-population genetic variation

We estimated genetic diversity based on the proportion of polymorphic loci and expected heterozygosity; measures which are commonly used in studies based on dominant markers such as AFLP’s. This allowed us to directly compare our results with previous AFLP studies on amphibians undergoing local population declines. We found that our observed levels of genetic diversity for both *C. schioetzi* and *L. bicolor* are within the range reported from such previous studies (Table 5). For example, Curtis and Taylor [69] evaluated the impact of forest clearcuts on the population structure of *Dicamptodon tenebrosus* in southwestern British Columbia and reported heterozygosity (\(H_E\)) ranging from 0.192 to 0.285 in recently clearcut sites.

Genetic differentiation and genetic structure of populations

A common cause of genetic differentiation among populations is geographic distance so that in continuous habitats, poor dispersal ability and large distances between individuals may...
drive genetic differentiation \[70,71\]. However, the results of our Mantel test on the continuous forest populations of *L. bicolor* showed no evidence for significant genetic differences among them, regardless of distance. Within the continuous forest habitat dispersal occurs over at least 1.85 km (between MFIII and MFIV), the greatest distances between any of our continuous forest sites. The fact that estimates of $F_{ST}$ from AFLP’s are likely to be over estimates \[72,73\] gives us confidence that little, if any genetic differentiation exists among forest populations.

Habitat fragmentation, which often isolates populations and reduces their size \[72\] is another cause of population genetic differentiation at small geographic scales, e.g. less than five km \[72,73\]. We found evidence for this in *C. schioetzi*, where a population from the riparian forest fragment (F3) is distinct from the forest population (MFI). While F3 and MFI are in close geographic proximity (less than two km), they are separated by heavily overgrazed and annually burnt grassland, so that movement between habitats would likely be difficult. However, because AFLPs have a lower mutation rate than microsatellites \[74,75\] it is important to be aware that our estimates of $F_{ST}$ will likely be higher than if we had used microsatellites \[69,75\].

Similar findings have been reported by Dixo et al. \[76\] who compared genetic diversity of the toad *Rhinella ornata* among small and medium forest fragments that were either isolated or connected to large forest areas by corridors. They found a weak but significant $F_{ST}$ between small fragments and continuous forest, but no significant genetic differentiation among continuous forest sites.

The extent of gene flow among local populations determines their potential for genetic differentiation \[77\]. Thus, at our study site, current gene flow may explain the lack of genetic differentiation among populations within the forest (*L. bicolor*) and among the riparian forest fragments (*C. schioetzi*). This is supported by our tests for migration, which indicated that in both species, populations are weakly linked by gene flow, both current and within the past two generations. In the case of *C. schioetzi*, the test revealed that population F3 contained immigrants from the other two riparian fragments as well as from the forest population MFI. In this case, individuals may be dispersing via a stepping-stone dispersal model \[78\], moving, for example, from MFI to F3 through F1, most probably in the rainy season when grass is tall and there is no burning. Moreover, at this time of year streamflow is higher so that streams within fragments which are isolated from other in the dry season are able to join up, connecting fragments (see Fig 1). Despite this potential homogenizing effect of the wet season, significant genetic difference was detected among some population pairs. In the case of *L. bicolor*, the single riparian fragment population F4, contained immigrants from all forest populations except MFII, from which it was significantly genetically different. A possible explanation for this could be non-random gene flow, i.e. gene flow which is not constant over time, nor the same level between every population pair. Such variation could reduce the homogenizing effect of gene flow and instead promote genetic differentiation \[79,80\]. Two studies we are aware of on Parus major have demonstrated that non-random dispersal (such as we describe above) can contribute to genetic differentiation at a fine scale \[79,80\]. For example Garant et al. \[79\] found that non random dispersal influenced diversifying effects on body mass variation and Postma and van Noordwijk \[80\] observed that small-scale genetic difference in clutch size was produced by different levels of gene flow.

For both species, the results of clustering analyses in STRUCTURE differ based on the estimator used (Ln($K$) and $\Delta K$) and groupings were not consistent with sampling location. While the precise number of genetic clusters is not critical to our study, the finding of multiple population groups using both estimators supports our interpretation of population genetic structure.
A synthesis of our results suggest they are similar to the findings of a similar study on *Phrynobatrachus guineensis* in Taï National Park (TNP), Ivory Coast [81]. As in our study, and in contrast to expectations, Sandberger et al. [81] detected only a slight significant genetic differentiation among populations of this leaf litter frog and no correlation between the geographic and genetic distances (isolation by distance). Their Bayesian clustering revealed no genetic substructure. Originally *P. guineensis* was thought to be weakly mobile and highly specialized, however, high intra- and possibly inter-patch migration events explained the lack of population structure. Thus, individuals of this species are more able to disperse than was expected. We cannot rule out that this is not the case in our study and future work is needed to test this idea.

Another potential contributing factor to genetic differentiation in fragmented populations is that in fragmented landscapes species often persist as metapopulations [82]. This may well be the case for the two target species in this study [83]. If so, then the persistence of genetic variation within and among populations would depend on the factors discussed above and the ability of the species to form a metapopulation [84].

While we lack information on dispersal distances for our focal species, some examples are available for other amphibian species. For the salamander *Plethodon cinereus*, Cabe et al. [85] found clear genetic differences among plots that increased with distance (200m to 2 km). Geographic distances at larger scales (> 2 km) contributed to differentiation among populations of the European tree frog *Hyla arborea* in a fragmented landscape Angelone et al. [86]. Because the likelihood of detecting isolation by distance increases with the number of populations sampled [87], the lack of correlation in our study between geographic distance and genetic differentiation may be due to the relatively small number of sample sites included. However, sampling more sites at greater distances is difficult in this system as Ngel Nyaki Forest Reserve comprises only ~5.3 km² of forest (with no straight-line distances within continuous forest exceeding 5 km) and most of the few remaining forest fragments are relatively near to the reserve.

**Implications for conservation**

Our results contribute to the relatively limited body of knowledge of dispersal in modified landscapes for African amphibians [e.g., 18,81]. Our results suggest that despite considerable habitat degradation (especially in the riparian fragments) gene flow is still occurring (or occurred recently) among forest patches in two Afromontane frog species. This illustrates the importance of these degraded riparian forest fragments to amphibian communities.

In Nigeria and all along the Cameroon Volcanic Line [88,89], as elsewhere in the tropics [90,91], the survival of riparian forest fragments is under threat. Often fragments are not given the official protection garnered by larger patches of continuous forest [92] with disastrous consequences [93]. Evidence that these fragments not only harbour biodiversity but also have on-going gene-flow with neighboring continuous forest may provide added leverage to conservation practitioners aiming to protect isolated populations in forest fragments. On the Mambilla Plateau of eastern Nigeria, conservation efforts should focus not only on the existing Ngel Nyaki and Kurmin Danko Forest Reserves but also the small riparian forest fragments on the periphery of these forests as well as the many more like them across the mountains of Cameroon and Nigeria.

**Supporting information**

S1 Fig. Outcomes from structure without using population data for *Cardioglossa schioetzi*. Graph A) and bar plot C) depict the optimal K based on the Ln(K) K = 5, whereas graph B)
and bar plot D) show the better $K$ based on Evanno’s method $\Delta K = 2$. Each vertical bar represents an individual for which is shown the proportional genetic assignment to each cluster.

(TIF)

**S2 Fig. Outcomes from structure without using population data for *Leptodactylodon bicolor*.** Graph (A) and bar plot (C) depict the optimal $K$ based on the $\text{Ln}(K) K = 7$, whereas graph (B) and bar plot (D) show the better $K$ based on Evanno’s method $\Delta K = 2$. Each vertical bar represents an individual for which is shown the proportional genetic assignment to each cluster.

(TIF)

**S1 Table. Raw data of Cardioglossa schioetzi and Leptodactylodon bicolor.** Together, the two preferred primer combinations ESP1B/MSP3 and ESP1B/MSP6 yielded 275 loci for the 198 samples representing both species.

(XLSX)

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**References**

1. Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, et al. Status and trends of amphibian declines and extinctions worldwide. Science. 2004; 306: 1783–1786. https://doi.org/10.1126/science.1103538 PMID: 15486254

2. Beebee TJC, Griffiths RA. The amphibian decline crisis: A watershed for conservation biology?. Biol Conserv. 2005; 125: 271–285. https://doi.org/10.1016/J.BIOCON.2005.04.009

3. Hof C, Araújo MB, Jetz W, Rahbek C. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature. 2011; 480: 516–519. https://doi.org/10.1038/nature10658 PMID: 22089134

4. Houlnahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. Quantitative evidence for global amphibian population declines. Nature. 2000; 404: 752–755. https://doi.org/10.1038/35008052 PMID: 10783886

5. Lips KR, Reeve JD, Witters LR. Ecological traits predicting amphibian population declines in Central America. Conserv Biol. 2003; 17: 1078–1086. https://doi.org/10.1046/j.1523-1739.2003.01623.x

6. Cushman SA. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biol Conserv. 2006; 128: 231–240. https://doi.org/10.1016/J.BIOCON.2005.09.031
7. Hirschfeld M, Blackburn DC, Doherty-Bone TM, Gonwouo LN, Ghose S, Rödel M-O. Dramatic declines of montane frogs in a central african biodiversity hotspot. PLoS One. 2016; 11: e0155129. https://doi.org/10.1371/journal.pone.0155129 PMID: 27149624

8. Hillers A, Veith M, Rödel M-O. Effects of forest fragmentation and habitat degradation on West African leaf-litter frogs. Conserv Biol. 2008; 22: 762–772. https://doi.org/10.1111/j.1523-1739.2008.00920.x PMID: 18410401

9. La Sorte FA, Jetz W. Projected range contractions of montane biodiversity under global warming. Proceedings Biol Sci. 2010; 277: 3401–3410. https://doi.org/10.1098/rspb.2010.0612 PMID: 20534610

10. Emel SL, Storfer A. A decade of amphibian population genetic studies: synthesis and recommendations. Conserv Genet. 2012; 13: 1685–1689. https://doi.org/10.1007/s10592-012-0407-1

11. Young AG, Clarke GM. Genetics, demography and viability of fragmented populations. New York: Cambridge University Press; 2000.

12. Lacy RC, Lindenmayer DB. A simulation study of the impacts of population subdivision on the mountain brushtail possum Trichosurus caninus Ogilby (Phalangeridae: Marsupialia), in south-eastern Australia. II. Loss of genetic variation within and between subpopulations. Biol Conserv. 1995; 73: 131–142. https://doi.org/10.1016/0006-3207(95)90037-3

13. Frankham R, Briscoe DA, Ballou JD. Introduction to conservation genetics. Cambridge: Cambridge University Press; 2002.

14. Waldman B, Tocher M. Behavioral ecology, genetic diversity, and declining amphibian populations. In: Caro T, editor. Behavioral ecology and conservation biology. New York: Oxford University Press; 1998. pp. 394–443.

15. Storfer A, Eastman JM, Spear SF. Modern molecular methods for amphibian conservation. Bioscience. 2009; 59: 559–571. https://doi.org/10.1525/bio.2009.59.7.7

16. Epps CW, Wehausen JD, Bleich VC, Torres SG, Brashares JS. Optimizing dispersal and corridor models using landscape genetics. J Appl Ecol. 2007; 44: 714–724. https://doi.org/10.1111/j.1365-2664.2007.01325.x

17. Baguette M, Blanchet S, Legrand D, Stevens VM, Turlure C. Individual dispersal, landscape connectivity and ecological networks. Biol Rev. 2013; 88: 310–326. https://doi.org/10.1111/brv.12000 PMID: 23176626

18. Measey GJ, Galbusera P, Breyne P, Matthyssen E. Gene flow in a direct-developing, leaf litter frog between isolated mountains in the Taita Hills, Kenya. Conserv Genet. 2007; 8: 1177–1188. https://doi.org/10.1007/s10592-006-9272-0

19. Allentoft M, O’Brien J. Global amphibian declines, loss of genetic diversity and fitness: A review. Diversity. 2010; 2: 47–116. https://doi.org/10.3390/d2010047

20. Storfer A. Amphibian declines: Future directions. Divers Distrib. 2003; 9: 151–163. https://doi.org/10.1046/j.1472-4642.2003.00014.x

21. Monsen KJ, Blouin MS. Extreme isolation by distance in a montane frog Rana cascadae. Conserv Genet. 2004; 5: 827–835. https://doi.org/10.1007/s10592-004-1981-z

22. Blouin MS, Phillipsen IC, Monsen KJ. Population structure and conservation genetics of the Oregon spotted frog, Rana pretiosa. Conserv Genet. 2010; 11: 2179–2194. https://doi.org/10.1007/s10592-010-0104-x

23. Déruelle B, Moreau C, Nikoumbou C, Kambou R, Lissom J, Njonfang E, et al. The Cameroon Line: A Review. In: Kampunzu AB, Lubala RT, editors. Magmatism in extensional structural settings. Berlin, Heidelberg: Springer Berlin Heidelberg; 1991. pp. 274–327. https://doi.org/10.1007/978-3-642-73966-8_12

24. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000; 403: 853–858. https://doi.org/10.1038/35002501 PMID: 10706275

25. Blackburn DC. A new puddle frog (Phrynobatrachidae: Phrynobatrachus) from the Mambilla Plateau in eastern Nigeria. African J Herpetol. 2010; 59: 33–52. https://doi.org/10.1080/04416651003742160

26. Stuart SN. Conservation of Cameroon Montane forests: report of the ICBP Cameroon Montane Forest Survey, November 1983-April 1984. ICBP Cameroon Montane Forest Survey., editor. International Council for Bird Preservation; 1986.

27. Burgess ND, D’Amico Hales J, Underwood E, Dinerstein E. Terrestrial ecoregions of Africa and Madagascar: a conservation assessment. Washington, DC: Island Press; 2004.

28. Chapman JD, Chapman HM. The forests of Taraba and Adamawa states, Nigeria: an ecological account and plant species checklist. Christchurch, New Zealand: WWF, DFID, University of Canterbury; 2001.
29. Bawden MG, Tuley P. The land resources of southern Sardauna and southern Adamawa provinces, northern Nigeria: (with a short study of the high altitude grasslands). Tolworth: Land Resources Division, Directorate of Overseas Surveys; 1966.

30. Chapman HM, Olson SM, Trumm D. An assessment of changes in the montane forests of Taraba State, Nigeria, over the past 30 years. Oryx. 2004; 38: 282–290. https://doi.org/10.1017/S0030605304000151

31. Blackburn DC, Gvoždík V, Leaché AD. A New Squeaker Frog (Arthroleptidae: *Arthroleptis*) from the Mountains of Cameroon and Nigeria. Herpetologica. 2010; 66: 335–348. https://doi.org/10.1655/HERPETOLOGICA-D-10-00015.1

32. Arroyo-Lambear D. Conserving amphibian diversity: a species inventory and gene flow studies in fragmented montane forest, Mambilla Plateau, Nigeria. University of Canterbury, School of Biological Sciences. 2015.

33. Amiet JL. *Leptodactylodon* nouveaux du Cameroun (Amphibiens Anoures). Ann la Fac des Sci du Cameroun Yaoundé. 1971; 7–8: 141–172.

34. Amiet JL. Une nouvelle *Cardioglossa* orophile de la dorsale camerounaise: *C. schioetzi* nov. sp. (Amphibia, Anura, Arthroleptinae). Ann la Fac des Sci du Cameroun Yaoundé. 1981; 28: 117–131.

35. Blackburn DC. Geographic distribution: *Cardioglossa schioetzi*. Herpetol Rev. 2006; 37: 486.

36. Channing A, Rödel M-O, Channing J. Tadpoles of Africa: the biology and identification of all known tadpoles in sub-Saharan Africa. Frankfurt, Germany: Chimaira; 2012.

37. Mapouyat L, Hirschfeld M, Rödel MO, Liedtke HC, Loader SP, Gonwouo LN, et al. The tadpoles of nine Cameroonian *Leptodactylus* species (Amphibia, Anura, Arthroleptidae). Zootaxa. 2014; 3765: 29–53. https://doi.org/10.11646/zootaxa.3765.1.2 PMID: 24870883

38. Gartshore ME. The status of the montane herpetofauna of the Cameroon Highlands. In: Stuart SN, editor. Conservation of Cameroon montane forests. Cambridge: International Council for Bird Preservation; 1986. pp. 204–240.

39. McDiamid RW, Altig R. Tadpoles: the biology of anuran larvae. Chicago, Illinois: University of Chicago Press; 1998.

40. The IUCN Red List of Threatened Species. Version 2017–3. <http://www.iucnredlist.org>. 2018 [cited 28 May 2018] [Internet].

41. Olson DM, Dinerstein E. The Global 200: A representation approach to conserving the Earth’s most biologically valuable ecoregions. Conserv Biol. 1998; 12: 502–515. https://doi.org/10.1046/j.1523-1739.1998.012003502.x

42. Bensch S, Akesson M. Ten years of AFLP in ecology and evolution: why so few animals? Mol Ecol. 2005; 14: 2899–2914. https://doi.org/10.1111/j.1365-294X.2005.02655.x PMID: 16101761

43. Jehle R, Arntzen J. Microsatellite markers in amphibian conservation genetics. Herpetol J. 2002; 12: 1–9.

44. Zancolli G, Rödel MO, Steffan-Dewenter I, Storfer A. Comparative landscape genetics of two river frog species occurring at different elevations on Mount Kilimanjaro. Mol Ecol. 2014; 23: 4989–5002. https://doi.org/10.1111/mec.12921 PMID: 25230017

45. Garoia F, Guarniero I, Grifoni D, Marzola S, Tinti F. Comparative analysis of AFLPs and SSRs efficiency in resolving population genetic structure of Mediterranean *Solea vulgaris*. Mol Ecol. 2007; 16: 1377–1387. https://doi.org/10.1111/j.1365-294X.2007.03247.x PMID: 17391263

46. Milá B, Carranza S, Guillaume O, Clobert J. Marked genetic structuring and extreme dispersal limitation in the Pyrenean brook newt *Calosticton asper* (Amphibia: Salamandridae) revealed by genome-wide AFLP but not mtDNA. Mol Ecol. 2010; 19: 108–120. https://doi.org/10.1111/j.1365-294X.2009.04441.x PMID: 19943891

47. Rogell B, Thörngren H, Palm S, Laurila A, Höglund J. Genetic structure in peripheral populations of the natterjack toad, *Bufo calamita*, as revealed by AFLP. Conserv Genet. 2010; 11: 173–181. https://doi.org/10.1007/s10529-009-9232-2

48. Makowsky R, Chesser R, Rissler LJ. A striking lack of genetic diversity across the wide-ranging amphibian *Gastrophryne carolinensis* (Anura: Microhyliidae). Genetica. 2009; 135: 169–183. https://doi.org/10.1007/s10709-008-9267-5 PMID: 18392940

49. Lucas LK, Fries JN, Gabor CR, Nice CC. Genetic variation and structure in *Eurycea nana*, a federally threatened salamander endemic to the San Marcos springs. J Herpetol. 2009; 43: 220–227. https://doi.org/10.1670/0022-1511-43.2.220

50. Weising K, Nybom H, Wolff K, Meyer W. DNA fingerprinting in plants and fungi. Boca Raton: CRC Press; 1995.
51. Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Horne M, et al. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 1995; 23: 4407–4414. https://doi.org/10.1093/nar/23.21.4407 PMID: 7501463

52. Stöllting KN, Clarke AC, Meudt HM, Blankenhorn WU, Wilson AB. Cost-effective fluorescent amplified fragment length polymorphism (AFLP) analyses using a three primer system. Mol Ecol Resour. 2011; 11: 494–502. https://doi.org/10.1111/j.1755-0998.2010.02957.x PMID: 21481207

53. Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P. How to track and assess genotyping errors in population genetics studies. Mol Ecol. 2004; 13: 3261–3273. https://doi.org/10.1111/j.1365-294X.2004.02346.x PMID: 15487987

54. Meudt HM, Clarke AC. Almost Forgotten or Latest Practice? AFLP applications, analyses and advances. Trends Plant Sci. 2007; 12: 106–117. https://doi.org/10.1016/j.tplants.2007.02.001 PMID: 17303467

55. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 2012; 28: 2537–9. https://doi.org/10.1093/bioinformatics/bts460 PMID: 22820204

56. Lynch M, Milligan BG. Analysis of population genetic structure with RAPD markers. Mol Ecol. 1994; 3: 91–99. https://doi.org/10.1111/j.1365-294X.1994.tb00109.x PMID: 8019690

57. Peakall R, Smouse PE, Huff DR. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss Buchloë dactyloides. Mol Ecol. 1995; 4: 135–148. https://doi.org/10.1111/j.1365-294X.1995.tb00203.x

58. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992; 131: 479–91. PMID: 1644282

59. Rice WR. Analyzing tables of statistical tests. Evolution. 1989; 43: 223–225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x PMID: 28568501

60. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945–959. https://doi.org/10.1111/j.1365-294X.2000.01758.x PMID: 10835412

61. Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour. 2009; 9: 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x PMID: 21564903

62. Earl DA, VonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012; 4: 359–361. https://doi.org/10.1007/s12686-011-9548-7

63. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. 2005; 14: 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x PMID: 15969739

64. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes. 2007; 7: 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x PMID: 18784791

65. Peakall R, Smouse PE. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006; 6: 288–295. https://doi.org/10.1111/j.1471-8286.2006.01155.x

66. Pompanon F, Bonin A, Bellemain E, Taberlet P. Genotyping errors: causes, consequences and solutions. Nat Rev Genet. 2005; 6: 847–859. https://doi.org/10.1038/nrg1707 PMID: 16304600

67. White F. The vegetation of Africa. A descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa. Switzerland: Unesco. 1983.

68. Adie H, Kotze DJ, Lawes MJ. Small fire refugia in the grassy matrix and the persistence of Afrotemperate forest in the Drakensberg mountains. Sci Rep. 2017; 7: 6549. https://doi.org/10.1038/s41598-017-06747-2 PMID: 28747738

69. Curtis JMR, Taylor EB. The genetic structure of coastal giant salamanders (Dicamptodon tenebrosus) in a managed forest. Biol Conserv. 2003; 115: 45–54. https://doi.org/10.1016/S0006-3207(03)00092-2

70. Wright S. Isolation by Distance. Genetics. 1943; 28: 114–138. PMID: 17247074

71. Slatkin M. Gene flow and the geographic structure of natural populations. Science. 1987; 236: 787–792. https://doi.org/10.2307/1699930 PMID: 3576198

72. Andersen LW, Fog K, Damgaard C. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (Hyla arborea). Proc Biol Sci. 2004; 271: 1293–1302. https://doi.org/10.1098/rspb.2004.2720 PMID: 15306354
73. Kraaijeveld-Smit FJL, Beebee TJC, Griffiths RA, Moore RD, Schley L. Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad *Alytes muletensis*. Mol Ecol. 2005; 14: 3307–3315. https://doi.org/10.1111/j.1365-294X.2005.02614.x PMID: 16156804

74. Mariette S, Chagné D, Léziz C, Pastuszka P, Raffin A, Plomion C, et al. Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. Heredity. 2001; 86: 469. https://doi.org/10.1046/j.1365-294X.2001.00852.x PMID: 11520347

75. Gaudeul M, Till-Bottraud I, Barjon F, Manel S. Genetic diversity and differentiation in *Eryngium alpinum* L. (Apiaceae): comparison of AFLP and microsatellite markers. Heredity. 2004; 92: 508–518. https://doi.org/10.1038/sj.hdy.6800443 PMID: 15014426

76. Dixo M, Metzger JP, Morgante JS, Zamudio KR. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. Biol Conserv. 2009; 142: 1560–1569. https://doi.org/10.1016/j.biocon.2008.11.016

77. Slatkin M. Rare alleles as indicators of gene flow. Evolution. 1985; 39: 53–65. https://doi.org/10.1111/j.1558-5646.1985.tb04079.x PMID: 28563643

78. Hedrick PW. Genetics of populations. Sudbury, Massachusetts: Jones and Bartlett Publishers; 2011.

79. Garant D, Kruuk LEB, Wilkin TA, McCleery RH, Sheldon BC. Evolution driven by differential dispersal within a wild bird population. Nature. Nature Publishing Group; 2005; 433: 60–65. https://doi.org/10.1038/nature03051 PMID: 15635409

80. Postma E, van Noordwijk AJ. Gene flow maintains a large genetic difference in clutch size at a small spatial scale. Nature. 2005; 433: 65–68. https://doi.org/10.1038/nature03083 PMID: 15635410

81. Sandberger L, Feldhaar H, Lampert KP, Lamatsch DK, Rödel MO. Small, specialised and highly mobile? the tree-hole breeding frog, *Phrynobatrachus guineensis*, lacks fine-scale population structure. African J Herpetol. 2010; 59: 79–94. https://doi.org/10.1080/04416651003788619

82. Hastings A, Harrison S. Metapopulation dynamics and genetics. Annu Rev Ecol Syst. 1994; 25: 167–188. https://doi.org/10.1146/annurev.es.25.110194.001123

83. Hanski I. Single-species metapopulation dynamics: concepts, models and observations. Biol J Linn Soc. 1991; 42: 17–38. https://doi.org/10.1111/j.1095-8312.1991.tb00549.x

84. Hanski I, Gilpin ME. Metapopulation biology: ecology, genetics, and evolution. San Diego, CA: Academic Press; 1997.

85. Cabe PR, Page RB, Hanlon TJ, Aldrich ME, Connors L, Marsh DM. Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. Heredity. 2007; 98: 53–60. https://doi.org/10.1038/sj.hdy.6800905 PMID: 17006531

86. Angelone S, Kienast F, Holderegger R. Where movement happens: Scale-dependent landscape effects on genetic differentiation in the European tree frog. Ecography. 2011; 34: 714–722. https://doi.org/10.1111/j.1600-0587.2010.06494.x

87. Peterson MA, Denno RF. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. Am Nat. 1998; 152: 428–446. https://doi.org/10.1086/286180 PMID: 18811450

88. Cheek M, Onana JM, Pollard BJ. The plants of Mount Oku and the Ijim ridge, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew; 2000.

89. Cheek M, Pollard BJ, Darbyshire I, Onana JM, Wild C. The plants of Kupe, Mwanenga and the Bakossi Mountains, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew; 2004.

90. Laurance WF, Camargo JLC, Lüö RCC, Laurance SG, Pimm SL, Bruna EM, et al. The fate of Amazonian forest fragments: A 32-year investigation. Biol Conserv. 2011; 144: 56–67. https://doi.org/10.1016/J.BIOCON.2010.09.021

91. McLennan MR, Plumptre AJ. Protected apes, unprotected forest: composition, structure and diversity of riverine forest fragments and their conservation value in Uganda. Trop Conserv Sci. 2012; 5: 79–103.

92. Fagan ME, DeFries RS, Sweeney SE, Arroyo-Mora JP, Chazdon RL. Targeted reforestation could reverse declines in connectivity for understory birds in a tropical habitat corridor. Ecol Appl. 2016; 26: 1456–1474. https://doi.org/10.1890/14-2188 PMID: 27755750

93. Seiferling IS, Proulx R, Peres-Neto PR, Fahrig L, Messier C. Measuring protected-area isolation and correlations of isolation with land-use intensity and protection status. Conserv Biol. 2012; 26: 610–618. https://doi.org/10.1111/j.1523-1739.2011.01674.x PMID: 21488956