Rozprawa doktorska

ZACHOWANIE FLORY WSCHODNIOAFRYKAŃSKIEJ: ZNACZENIE OBSZARÓW CHRONIONYCH

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Poznań 2024

Doctoral thesis

CONSERVATION OF THE EASTERN AFRICAN FLORA:
THE IMPORTANCE OF PROTECTED AREAS

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Poznań 2024

Many people have made it possible for me to achieve this work:

I thank my parents, Roy F. and Clarine B. Gereau, who gave me the love of knowledge and their example of the hard work necessary to make a positive impact on the world.

Professor Janice M. Glime of Michigan Technological University instilled in me an unquenchable thirst for understanding of the natural world and helped me develop the tools to exercise it, for which I am eternally grateful.

The late Dr. Alwyn H. Gentry honed my plant identification skills and gave me the confidence to start describing and publishing new species, then left us far too soon – he will be forever missed.

Professor Rogasian L.A. Mahunnah, my first Tanzanian friend and host in my formative years, will always have a place in my heart.

The late Leonard B. Mwasumbi was my botanical mentor and field companion during my early years in Tanzania and has left an indelible impression on my memory.

Dr. Porter P. Lowry II and Dr. Tariq Stévart have provided administrative help and guidance and given me the room to develop the Missouri Botanical Garden's Tanzania program, without which none of this work would have been possible.

I am especially grateful to Prof. UAM dr. hab. Justyna Wiland-Szymańska, who believed in me when I did not, and has guided this work through to a successful conclusion.

I dedicate this work to Peter H. Raven for his encouragement and guidance that have been an inspiration and a lifeline to me for more than four decades; to W.R. Quentin Luke, without whose untiring leadership as Chair of the Eastern African Plant Red List Authority there would be no extinction risk assessments on which to base our conservation priorities; and above all to Charlotte M. Taylor, my wife, colleague, and life partner in all things, *sine qua nihil perfecissem.*

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List of publications included in the doctoral thesis

1. Raven, P.H., **R.E. Gereau**, P.B. Phillipson, C. Chatelain, C.N. Jenkins & C. Ulloa Ulloa. 2020. The distribution of biodiversity richness in the tropics. *Science Advances* 09 Sep 2020: Vol. 6, no. 37. DOI: 10.1126/sciadv.abc6228.

2. Ract, C., N.D. Burgess, L. Dinesen, P. Sumbi, I. Malugu, J. Latham, L. Anderson, **R.E. Gereau**, M. Gonçalves de Lima, A. Akida, E. Nashanda, Z. Shabani, S. Tango, S. Mteleka, Dos Santos Silayo, J. Mwangi, G. Lyatuu, P.J. Platts & F. Rovero. 2024. Nature Forest Reserves in Tanzania and their importance for conservation. *PLoS ONE* 19(2): 1-18. e0281408. https://doi.org/10.1371/journal.pone.0281408

3. Linan, A.G., **R.E. Gereau**, R. Sucher, F.H. Mashimba, B. Bassüner, A. Wyatt & C.E. Edwards. 2024. Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas*. *Appl. Pl. Sci.* 12(3): e11589: pp. 1-14

4. Gavin-Smyth, N. & **R.E. Gereau**. 2022. Two new species of *Impatiens* (Balsaminaceae) from the Eastern Arc Mountains of Tanzania. *Novon* 30: 122-127.

5. **Gereau, R.E.**, S. Kativu, P. Meerts, L. Merrett, J. Osborne & K. Vollesen. 2023. *Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania. *Novon* 31: 181-190.

6. Cheek, M., **R.E. Gereau** & J. Kalema. 2024. *Afrothismia ugandensis* nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda. *Kew Bull.* DOI: 10.1007/s12225-024-10212-5 pp. 1-11.

The total IF coefficient of publications included in the doctoral thesis is 21.5. The total number of points for publications included in the doctoral thesis according to the list of scored journals of the Ministry of Science and Higher Education is 460. The value of the IF coefficient was given according to the year of publication.

List of abbreviations used in the work

CFR – Central Forest Reserve

CR – Critically Endangered (Red List category)

EACF – Eastern Arc and Coastal Forests of Tanzania and Kenya

EAPRLA – Eastern African Plant Red List Authority

EN – Endangered (Red List category)

FR – Forest Reserve

IBA – Important Bird Area

IUCN – International Union for Conservation of Nature

KBA – Key Biodiversity Area

LC – Least Concern (Red List category)

LC (PA) – Least Concern (Protected Area Dependent) (proposed Red List designation)

LR/cd – Least Concern (conservation dependent) (former Red List designation)

MBG – Missouri Botanical Garden

METT – Management Effectiveness Tracking Tool

NFR – Nature Forest Reserve

NT – Near Threatened (Red List category)

PA – protected area

TFS – Tanzania Forestry Services Agency

TIPA – Tropical Important Plant Area

VU – Vulnerable (Red List category)

Abstract

Biodiversity inventories, extinction risk assessments, and protected areas (PAs) are essential elements for the development of effective and sustainable nature conservation. Biological inventories are needed to delineate the units of biodiversity and their geographic distribution on scales varying from global to continental to regional to very local. These inventories provide the context for understanding patterns of species distribution and a taxonomic baseline for conservation analysis and planning. Extinction risk assessments gather and analyze the data on threats to the survival of species and their habitats. The IUCN Red List process is the most widely accepted global standard for assessment of extinction risk based on a uniform set of categories and criteria. Some refinements to the Red List assessment process are needed to deal more accurately with the status of species wholly dependent on current conservation measures. PAs are designed and managed to mitigate threats to species in a stable and sustainable regulatory system. Global Biodiversity Hotspots are defined in terms of endemic plant species richness and degree of historical habitat loss, and provide another platform for organizing the information needed for conservation prioritization and planning. Key Biodiversity Areas (KBAs), identified as sites of global importance for the persistence of biodiversity, do not necessarily establish conservation priorities, but they are highly valuable for informing systematic conservation planning and priority setting when used to guide the delineation and expansion of PAs. The utility of PAs is greatly enhanced by the use of the Management Effectiveness Tracking Tool (METT). Because PAs of various categories are present in all eastern African countries and they occupy approximately one-third of Tanzania’s land area, this part of the world is an appropriate region for the study of the role of PAs in nature conservation.

The first hypothesis of this thesis is that PAs constitute the primary component and organizing principle of practical and sustainable nature conservation in eastern Africa, and the

second objective is to investigate the use of existing PAs in the furtherance of *in-situ* and *ex-situ* biodiversity conservation in Tanzania. To fulfill these tasks, a review article by Raven et al. (2020) is proposed as an introduction for estimating the global position of tropical Africa in terms of biodiversity richness. The paper combines the numbers of vascular plant species in the Afrotropical Region, Latin America, and Southeast Asia with species numbers for insects and major vertebrate animal groups to produce reliable estimates of total biodiversity values for these three regions. The results demonstrate the dominance of Latin America in world biodiversity richness, with at least a third of the world's species occurring there, while the Afrotropical Region has proportionally far fewer species per unit area than the geographically much smaller Southeast Asia. This paper is followed by the presentation of an analysis of Tanzania's network of 22 Nature Forest Reserves (NFRs), recognized as forest areas of exceptionally high importance for globally unique biodiversity (Ract et al., 2024). It concludes that the 22 NFRs cover ca. 10% of Tanzania's total land area, and are home to nearly 20% of the vascular plant species in the country. Thus, the NFR network has captured approximately twice the amount of the national vascular plant species biodiversity than would be predicted from its size alone. The next step is to propose a case study, which in this thesis is an original research article by Linan et al. (2024) analyzing the genetic diversity of *Karomia gigas* (Lamiaceae). This tree species is currently known from only 43 wild individuals in two small forest reserves in southeastern Tanzania. With fewer than 50 mature individuals, *K. gigas* has been assessed as Critically Endangered under Red List criterion D. The species is threatened by habitat loss and low seed viability due to fungal infestation, and could certainly not survive in the wild without the *in-situ* protection afforded by these two FRs. Because of these factors, *K. gigas* has been the object of a collaborative propagation program between the Tanzania Forest Services Agency (TFS) and the Missouri Botanical Garden (MBG). The resulting *ex-situ* populations at TFS and MBG have allowed DNA extraction and sequencing to determine the genetic diversity of the wild and *ex-situ* populations. Analyses have discerned definite genetic structure within and between the populations and identified some gaps and inefficiencies in the conservation of genetic diversity in *K. gigas*, but also suggest strategies to improve conservation of genetic diversity. *Karomia gigas* shows greater genetic diversity in its wild populations than in its cultivated populations. Thus, continued *in-situ* conservation of the wild populations is needed to maintain a level of genetic diversity capable of responding to changing environmental conditions, with the *ex-situ* cultivated populations serving as a living gene bank.

To show the importance of PAs for species conservation, three papers deal with taxa new for science whose survival is dependent on these areas. Two new species of *Impatiens* (Balsaminaceae) were described from the Eastern Arc Mountains of Tanzania and Kenya, with discussion of their distributions, habitats, and relationships, and a provisional conservation status was proposed for each species. Each of these two new species is strictly endemic to a single Tanzanian NFR, and both are provisionally assessed as Least Concern (Protected Area Dependent): LC (PA) (Gavin-Smyth & Gereau, 2022). The new species *Chlorophytum vespertinum* (Asparagaceae tribe Anthericeae) was described from southwestern Tanzania and central to northern Zambia, with a discussion of its distribution, habitats, and relationships, and a provisional conservation status was proposed. The new species is known from seven locations in Tanzania and Zambia, three of which appear to benefit from some level of effective protection. In Tanzania, the new species grows within the Kalambo NFR. The species may be considered well protected there and at two of its locations in Zambia, with its other four locations apparently unprotected, leading to a provisional conservation status of Vulnerable, VU B1ab(iii)+2ab(iii) (Gereau et al., 2023). In the final paper, Cheek et al. (2024) reconsider the status of *Afrothismia winkleri* var. *budongensis* (Afrothismiaceae), raising it to the rank of species and renaming it as *A. ugandensis* based on a suite of eight diagnostic morphological characters. *A. ugandensis* is apparently very rare, being known only from two specimens collected in 1940 and 1998 in the Budongo Central Forest Reserve in western Uganda. Due to its very small population size, *A. ugandensis* is provisionally assessed as Critically Endangered under IUCN Red List criterion D.

Although tropical east Africa is less rich in biodiversity than other tropical areas of the world, it contains part or all of four of the world’s 36 Global Biodiversity Hotspots. Tanzania, with the largest vascular plant flora in eastern Africa and home to part of two of those Hotspots, is among the botanically best-explored countries in tropical Africa. Much of the land area of tropical eastern Africa is covered by various categories of PAs. A time series analysis of METT data shows that NFR management effectiveness increased between 2015 and 2021; this further reinforces the primacy of the NFR network in Tanzanian nature conservation. One of the first decisions in conservation planning is whether taxa can be effectively conserved *in-situ* in their natural habitats, or if *ex-situ* conservation measures are needed. Ten of Tanzania’s NFRs are home to taxa assessed as LC (PA) by the Eastern African Plant Red List Authority (EAPRLA). These taxa may be considered amenable to strictly *in-situ* conservation, with measures for their continued protection to be included in the NFR management plans and periodically checked by means of the METT. Both Global Biodiversity Hotspots and Key Biodiversity Areas depend on the relative stability and regulatory framework provided by PAs to achieve their long-term conservation goals. This evidence supports the hypothesis that protected areas constitute the primary component and organizing principle of practical and sustainable nature conservation in eastern Africa.

Decisions as to whether any given taxon can be effectively conserved *in-situ* or if *ex-situ* conservation measures are needed can be based on its distribution and threats and information about PA effectiveness. Based on these criteria, the 48 plant taxa occurring in NFRs that are assessed as LC (PA) can be considered amenable to *in-situ* conservation;

Karomia gigas needs both *in-situ* and *ex-situ* conservation measures; *Chlorophytum vespertinum* can benefit from both *in-situ* and *ex-situ* measures; and *Afrothismia ugandensis* can only be conserved *in-situ*. The development of this methodology fulfills the second objective of this thesis: to investigate the use of existing PAs in the furtherance of *in-situ* and *ex-situ* biodiversity conservation in Tanzania.

In conclusion, existing biodiversity inventories show tropical eastern Africa to be a globally important target area for effective nature conservation, with a high level of knowledge of threats to the survival of species and their habitats in the form of Red List assessments and a robust network of protected areas (PAs), with Tanzania's system of Nature Forest Reserves (NFRs) providing effective protection to a large proportion of the country's vascular plant flora. Global Biodiversity Hotspots and Key Biodiversity Areas provide valuable means of organizing conservation priorities and planning, but depend on the regulatory stability of PAs to provide tangible conservation benefits. Decisions about *in-situ* and *ex-situ* conservation priorities are central to conservation planning and can be based on species' known distribution and threats and information about the PAs in which they occur. In Tanzania, these data are available from known and tested sources, enabling these decisions to be made in the furtherance of effective and sustainable nature conservation.

Abstract in Polish

Inwentaryzacje bioróżnorodności, oceny ryzyka wyginięcia i obszary chronione (ang. *protected areas*, PA) są niezbędnymi elementami rozwoju skutecznej i zrównoważonej ochrony przyrody. Inwentaryzacje biologiczne są potrzebne do określenia jednostek bioróżnorodności i ich geograficznego rozmieszczenia w skalach od globalnej, przez kontynentalną, regionalną do zupełnie lokalnej. Inwentaryzacje te zapewniają kontekst do zrozumienia wzorców rozmieszczenia gatunków i taksonomiczną podstawę do analizy i planowania ich ochrony. Oceny ryzyka wyginięcia gromadzą i analizują dane dotyczące zagrożeń dla przetrwania gatunków i ich siedlisk. Założenia Czerwonej Listy IUCN są najszerzej akceptowanym globalnym standardem oceny ryzyka wyginięcia w oparciu o jednolity zestaw kategorii i kryteriów. Niektóre udoskonalenia procesu oceny Czerwonej Listy są potrzebne, aby dokładniej oceniać status gatunków całkowicie zależnych od bieżących zabiegów ochronnych. Obszary chronione są projektowane i zarządzane w celu łagodzenia zagrożeń dla gatunków w systemie uregulowanym, stabilnym i zrównoważonym. Centra różnorodności biologicznej (Global Biodiversity Hotspots) są definiowane w kategoriach bogactwa gatunków roślin endemicznych i stopnia utraty siedlisk historycznych i stanowią kolejną platformę do ujednolicania informacji potrzebnych do ustalania priorytetów i planowania ochrony. Kluczowe obszary bioróżnorodności (Key Biodiversity Areas, KBA), zidentyfikowane jako miejsca o globalnym znaczeniu dla trwałości bioróżnorodności, niekoniecznie ustalają priorytety ochrony, ale są bardzo cenne dla informacji o systematycznym planowaniu ochrony i ustalaniu priorytetów, gdy są wykorzystywane do kierowania wytyczaniem i rozszerzaniem obszarów chronionych. Efektywność działania tych ostatnich jest znacznie poprawiona dzięki użytkowaniu narzędzia do śledzenia skuteczności zarządzania Management Effectiveness Tracking Tool (METT). Ponieważ obszary chronione różnych kategorii są obecne we wszystkich krajach Afryki Wschodniej i zajmują około jednej

trzeciej powierzchni Tanzanii, ta część świata jest odpowiednim regionem do badania ich roli w ochronie przyrody.

Niniejsza praca doktorska testuje hipotezę, że obszary chronione (PA) są podstawowymi jednostkami organizującymi praktyczną i zrównoważoną ochronę przyrody we wschodniej Afryce. Równocześnie, jej celem jest zbadanie wykorzystania istniejących PA w celu promowania ochrony bioróżnorodności *in situ* i *ex situ* w Tanzanii. Aby zrealizować te zadania, artykuł przeglądowy autorstwa Raven i in. (2020) jest proponowany jako wstęp do oszacowania globalnej pozycji tropikalnej Afryki pod względem bogactwa bioróżnorodności. W artykule połączono liczbę gatunków roślin naczyniowych w regionie afrotropikalnym, Ameryce Łacińskiej i Azji Południowo-Wschodniej z liczbą gatunków owadów i głównych grup kręgowców, aby uzyskać wiarygodne szacunki całkowitych wartości bioróżnorodności dla tych trzech regionów. Wyniki pokazują dominację Ameryki Łacińskiej w światowym bogactwie bioróżnorodności, przy czym występuje tam co najmniej jedna trzecia gatunków świata, podczas gdy region afrotropikalny ma proporcjonalnie znacznie mniej gatunków na jednostkę powierzchni niż geograficznie znacznie mniejsza Azja Południowo-Wschodnia. W kolejnej pracy przedstawiono analizę sieci 22 leśnych rezerwatów przyrody (Nature Forest Reserves, NFR) Tanzanii, uznanych za obszary leśne o wyjątkowo dużym znaczeniu dla światowo unikalnej bioróżnorodności (Ract i in., 2024). Wniosek jest taki, że 22 leśne rezerwaty przyrody obejmują ok. 10% całkowitej powierzchni Tanzanii i są domem dla prawie 20% gatunków roślin naczyniowych w tym kraju. Powoduje to, że to sieć NFR zawiera około dwukrotnie większą liczbę krajowej różnorodności gatunków roślin naczyniowych, niż można by przewidzieć na podstawie samych jej rozmiarów. Następnym krokiem było zaproponowanie studium przypadku, które w tej rozprawie jest oryginalnym artykułem badawczym Linana i in. (2024), analizującym różnorodność genetyczną *Karomia gigas* (Lamiaceae). Ten gatunek drzewa jest obecnie znany tylko z 43 dzikich osobników w dwóch małych rezerwatach leśnych z południowo-wschodniej Tanzanii. Z mniej niż 50 dojrzałymi osobnikami, gatunek *K. gigas* został oceniony jako krytycznie zagrożony na podstawie kryterium D Czerwonej Listy. Gatunek ten jest zagrożony utratą siedlisk i niską żywotnością nasion z powodu inwazji grzybów i z pewnością nie mógłby przetrwać w naturze bez ochrony *in situ* zapewnianej przez te dwa rezerwaty leśne. Z powodu tych czynników *K. gigas* stał się przedmiotem wspólnego programu propagacji między Tanzania Forest Services Agency (TFS) i Missouri Botanical Garden (MBG). Powstałe populacje *ex-situ* w TFS i MBG umożliwiły ekstrakcję DNA i jego zsekwencjonowanie w celu określenia różnorodności genetycznej populacji dzikiej i *ex-situ*. Analizy pozwoliły na wyodrębnienie określonej struktury genetycznej w obrębie populacji i między nimi oraz zidentyfikowanie pewnych luk i nieefektywności w zachowaniu różnorodności genetycznej *K. gigas*. Sugerują one strategie mające na celu poprawę zachowania różnorodności genetycznej tego gatunku. *Karomia gigas* wykazuje większą różnorodność genetyczną w swoich dzikich populacjach niż w populacjach uprawnych. Dlatego też konieczna jest dalsza ochrona dzikich populacji *in situ* w celu utrzymania poziomu różnorodności genetycznej wystarczającej do reagowania na zmieniające się warunki środowiskowe, przy czym populacje uprawiane *ex situ* służą jako żywy bank genów.

Aby wykazać znaczenie obszarów chronionych dla ochrony gatunkowej, opublikowano trzy artykuły dotyczące taksonów nowych dla nauki, których przetrwanie zależy od takich jednostek. Opisano dwa nowe gatunki *Impatiens* (Balsaminaceae) z Gór Łuku Wschodniego w Tanzanii i Kenii, omawiając ich rozmieszczenie, siedliska i relacje, a dla każdego gatunku zaproponowano tymczasowy status jego ochrony. Każdy z tych dwóch nowych gatunków jest ściśle endemiczny dla pojedynczego tanzańskiego leśnego rezerwatu przyrody, a oba są tymczasowo oceniane jako najmniejszej troski (zależne od obszaru chronionego): LC (PA) (Gavin-Smyth & Gereau, 2022). Nowy gatunek *Chlorophytum* *vespertinum* (Asparagaceae, plemię Anthericeae) został opisany z południowo-zachodniej Tanzanii i od środkowej do północnej Zambii, z omówieniem jego rozmieszczenia, siedlisk i powiązań, a także zaproponowano jego tymczasowy status ochrony. Nowy gatunek jest znany z siedmiu stanowisk w Tanzanii i Zambii, z których trzy wydają się korzystać z pewnego poziomu skutecznej ochrony. W Tanzanii nowy gatunek rośnie w obrębie Kalambo NFR. Gatunek ten można uznać za dobrze chroniony tam i w dwóch lokalizacjach w Zambii, podczas gdy jego pozostałe cztery miejsca najwyraźniej nie są chronione, co prowadzi do tymczasowego statusu ochrony jako narażony, VU B1ab(iii)+2ab(iii) (Gereau i in., 2023). W ostatniej pracy Cheek i in. (2024) ponownie rozważają status taksonu *Afrothismia winkleri* var. *budongensis* (Afrothismiaceae), podnosząc go do rangi gatunku i zmieniając nazwę na *A. ugandensis* na podstawie zestawu ośmiu morfologicznych cech diagnostycznych. *A. ugandensis* jest najwyraźniej bardzo rzadki, znany tylko z dwóch okazów zebranych w 1940 i 1998 roku w rezerwacie leśnym Budongo Central w zachodniej Ugandzie. Ze względu na bardzo małą liczebność populacji *A. ugandensis* jest tymczasowo oceniany jako gatunek krytycznie zagrożony w ramach kryterium D Czerwonej Listy IUCN.

Chociaż Afryka tropikalna jest mniej bogata pod względem różnorodności biologicznej niż inne tropikalne obszary świata, zawiera ona w sobie część lub całość czterech z 36 światowych centrów różnorodności biologicznej. Tanzania, jako kraj zawierający dwa z tych centrów, z największą florą roślin naczyniowych we wschodniej Afryce, należy do państw najlepiej poznanych pod względem botanicznym w Afryce tropikalnej. Znaczna część areału lądowego tropikalnej Afryki Wschodniej jest objęta obszarami ochronnymi o różnych kategoriach. Analiza danych METT w seriach czasowych pokazuje, że skuteczność zarządzania leśnymi rezerwatami przyrody wzrosła w latach 2015–2021; dodatkowo wskazuje to na istotność sieci NFR w ochronie przyrody w Tanzanii. Jedną z pierwszych decyzji dotyczących planowania ochrony jest to, czy taksony mogą być skutecznie zachowane *in situ* na ich naturalnych siedliskach, czy też potrzebne są działania *ex situ*. Dziesięć NFR w Tanzanii jest domem dla taksonów ocenianych jako LC (PA) przez Wschodnioafrykański Komitet ds. Czerwonej Listy Roślin (Eastern African Plant Red List Authority, EAPRLA). Taksony te można uznać za możliwe do ochrony wyłącznie *in situ*, przy czym środki ich ciągłej ochrony zostaną uwzględnione w planach zarządzania NFR i okresowo sprawdzane za pomocą METT. Zarówno centra globalnej różnorodności biologicznej, jak i kluczowe obszary różnorodności biologicznej zależą od względnej stabilności i ram regulacyjnych zapewnianych przez obszary chronione, w celu osiągnięcia ich długoterminowych celów ochrony. Dowody te potwierdzają hipotezę, że obszary chronione stanowią główny komponent zrównoważonej ochrony przyrody w Afryce Wschodniej i organizują ją od strony praktycznej.

Decyzje dotyczące tego, czy dany takson może być skutecznie chroniony *in situ*, czy też potrzebne są działania *ex situ*, mogą opierać się na informacjach o jego rozmieszczeniu, zagrożeniach oraz o skuteczności działań ochronnych w obszarach chronionych. Na podstawie tych kryteriów 48 taksonów roślinnych występujących w leśnymi rezerwatami przyrody, które są oceniane jako LC (PA), można uznać za podatne na ochronę *in situ*; *Karomia gigas* wymaga zarówno środków ochrony *in situ*, jak i *ex situ*; *Chlorophytum vespertinum* może skorzystać zarówno z działań *in situ*, jak i *ex situ*; a *Afrothismia ugandensis* można zachować jedynie *in situ*. Opracowanie tej metodologii spełnia drugi cel tej pracy: zbadanie zastosowania istniejących obszarów chronionych w ramach ochrony różnorodności biologicznej *in situ* i *ex situ* w Tanzanii

Podsumowując, istniejące spisy różnorodności biologicznej wykazują, że tropikalna Afryka Wschodnia jest istotnym globalnie obszarem docelowym dla efektywnej ochrony przyrody, z wysokim poziomem wiedzy o zagrożeniach dla przetrwania gatunków i ich siedlisk w postaci ocen Czerwonej Listy i rozbudowanej sieci obszarów chronionych, z systemem leśnych rezerwatów przyrody w Tanzanii, zapewniając skuteczną ochronę dużej części flory naczyniowej w kraju. Globalne centra różnorodności biologicznej i kluczowe obszary różnorodności biologicznej zapewniają cenne sposoby organizowania priorytetów ochrony i planowania, ale zależą od stabilności regulacyjnej obszarów chronionych w celu zapewnienia namacalnych korzyści dla ochrony. Decyzje dotyczące priorytetów ochrony *in situ* i *ex situ* są kluczowe dla planowania ochrony i mogą opierać się na znanym rozmieszczeniu i zagrożeniach gatunków oraz informacjach o obszarach chronionych, w których występują. W Tanzanii dane te są dostępne ze znanych i zaufanych źródeł, umożliwiając podejmowanie tych decyzji w ramach skutecznej i zrównoważonej ochrony przyrody.

1. **Introduction**

Effective nature conservation requires an accurate and comprehensive knowledge of: 1) the units of biodiversity, their distribution on the landscape, and their environmental needs and habitat tolerances; 2) biotic and abiotic threats to their continued survival; and 3) the regulatory measures already in place to abate those threats. Biological inventories provide the baseline data to fulfill the first of these prerequisites for conservation on a global to a very local scale. Extinction risk assessments gather and analyze the data on threats to the survival of species and their habitats; and protected areas (PAs) are designed and managed to mitigate those threats in a stable and sustainable regulatory system.

1.1. Biological inventories

Global enumerations of biodiversity richness (Raven et al., 2020; Gereau & Ulloa, 2021) provide the context for understanding differential patterns of distribution of continental floras and faunas. The continuously updated *African Plant Database* (2024) is a comprehensive and reliable source of nomenclatural information and currently accepted names for continental Africa and Madagascar, greatly facilitating scientific communication between regions with differing floristic and taxonomic traditions. On the scale of a single continent, Sosef et al. (2017) fulfill a fundamental prerequisite for effective conservation and sustainable utilization of biodiversity by presenting an analysis of the patterns of plant diversity in tropical Africa, based on the RAINBIO mega-database of tropical African vascular plant species distributions (Dauby et al., 2016). They conclude that in terms of sampling completeness, Tanzania is among the botanically best-explored countries in tropical Africa, putting it in a relatively good position to make scientifically well-informed decisions about conservation priorities.

On a regional scale, the *Flora of Tropical East Africa*, published between 1952 and 2012 (see Beentje & Smith, 2001; Gereau et al., 2012: 9), provides a taxonomic baseline for the entire vascular plant flora of Kenya, Tanzania, and Uganda. Floristic inventories, including vegetation and phytogeographic studies of individual sites (e.g. Gereau et al., 1994) and primary exploration leading to area checklists (e.g. Gereau et al., 2012), refine this biodiversity information on a subregional scale; while field guides (e.g. Lovett et al., 2006), taxonomic overviews of individual families (e.g. Couvreur et al., 2006), and descriptions of individual new species (e.g. Gereau & Walters, 2003; Gereau & Bodine, 2005; Lantz & Gereau, 2005; Dawson & Gereau, 2010; Kenfack et al., 2015; Gavin-Smyth & Gereau, 2022; Gereau et al., 2023) provide updates to the published flora treatments.

1.2. Extinction risk assessments

The IUCN Red List of Threatened Species (IUCN, 2024) provides a global standard for assessment of extinction risk based on its uniform set of Categories and Criteria (IUCN, 2012) and its Guidelines (IUCN Standards and Petitions Committee, 2024). The Eastern African Plant Red List Authority (EAPRLA: Gereau et al., 2009) is a group of 34 volunteer botanists charged with the Red List assessment of plants in 11 eastern African countries. Since 2006, the EAPRLA has conducted assessments of 2,950 vascular plant taxa (species, subspecies, and varieties) in a series of 21 workshops. Geographic distributions and other primary data for those assessments are derived from comprehensive herbarium specimen label data gathered from all available sources and stored on the Missouri Botanical Garden's Tropicos® database (Tropicos, 2024), providing a rich source of comprehensive data on these taxa for further analyses. The Red List assessments and spatial analyses reported in this thesis are based on those sources.

A taxon in any of the three threatened Red List categories – Vulnerable (VU = facing a high risk of extinction in the wild), Endangered (EN = facing a very high risk of extinction in the wild), or Critically Endangered (CR = facing an extremely high risk of extinction in the wild) – is clearly of special conservation concern, while a Near Threatened (NT) taxon is one that does not meet the criteria for a threatened category at the present time, but is close to qualifying for or is likely to qualify for a threatened category in the near future. A taxon is Least Concern (LC) when it has been evaluated against the criteria and does not qualify for NT or any of the threatened categories. The EAPRLA has proposed the designation of Least Concern (Protected Area Dependent) [LC (PA)] for taxa that do not meet the threshold for NT or any threatened category under IUCN Categories and Criteria version 3.1 (IUCN, 2012), but are entirely dependent on the effective conservation measures provided by one or more PAs to maintain that status. The "PA" qualifier (or "tag") is not currently recognized by IUCN for use with the category Least Concern and is not included in the primary display of an assessment on the current IUCN Red List website (IUCN, 2024), but this information can be found in the text of the assessment under "Assessment information in detail: Justification" (e.g. de Kok et al., 2019). The EAPRLA has recommended (Luke et al., 2014) that this tag be adopted to distinguish these cases from an unqualified assessment of LC, the latter indicating that the taxon simply does not meet the threshold for any threatened category regardless of conservation intervention. Under the previous IUCN Categories version 2.3 (IUCN, 1994), the designation "Lower Risk/conservation dependent" (LR/cd) was available for a taxon that did not meet the threshold for any threatened category due to ongoing conservation measures, but only if those measures were explicitly designed to protect the taxon being assessed. A descriptor is clearly needed for any LC taxon entirely dependent on protection of its habitat within one or more PAs, whether or not it was targeted during the design of those measures.

1.3. Protected areas

The International Union for Conservation of Nature (IUCN) defines a Protected Area (PA) as "An area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and natural and associated cultural resources, and managed through legal or other effective means" (Dudley, 2008; Stolton et al., 2013). Approximately one-third of Tanzania's land area is covered by PAs of various categories, including Nature Forest

Reserves (NFRs: Doggart et al., 2017), National Parks, Forest Reserves, and Game Reserves (Gereau et al., 2022). Together with the network of NFRs discussed by Ract et al. (2024), the National Forest Policy of 1998 and the Forest Act of 2002 (United Republic of Tanzania, 1998, 2002) designed a system of national and local authority forest reserves for the preservation of their biodiversity and sustainable use of natural resources and habitats, with varying degrees of restriction on human activities (Burgess et al., 2007b). Although often smaller than the NFRs and with a more variable degree of management effectiveness, these forest reserves play an important role in the conservation of taxa not protected by the NFR network.

The utility of PAs is greatly enhanced by the ability to monitor and adjust the effectiveness of their conservation measures through the use of the Management Effectiveness Tracking Tool (METT; Hockings et al., 2006). Burgess et al. (2012, 2016) used the METT to assess the management effectiveness of 115 sites in the Tanzanian coastal forest zone between 2003 and 2015 and found that National Parks and government-run Game Reserves had the highest scores, followed by village-managed Forest Reserves, with central government-managed Forest Reserves having the poorest scores. BirdLife International (2013) and Gereau et al. (2014) reported the results of the application of the METT to 397 forest sites within the Eastern Arc and Coastal Forests Region, with widely varying results, but with private forests in the Eastern Arc Mountains of Tanzania having generally higher scores than government Forest Reserves and village forests.

Of course, PAs are not the only lens through which to view conservation priorities and planning. Global Biodiversity Hotspots are defined as areas having at least 1,500 endemic plant species and having lost at least 70% of their original primary vegetation. As originally defined (Myers et al., 2000), continental Africa contained part or all of 5 of the world’s 25 designated Hotspots, with only one in eastern Africa: the Eastern Arc and Coastal Forests of

Tanzania and Kenya (EACF). Mittermeier et al. (2004) subsequently separated the Eastern Arc Mountains as part of a newly recognized Eastern Afromontane Hotspot and included the Coastal Forests of Kenya and Tanzania in an expanded Coastal Forests of Eastern Africa Hotspot. However, the EACF Hotspot/Region continued to be a focus of biodiversity and conservation research and investment for more than a decade after the hotspot redefinition of 2004.

The Critical Ecosystem Partnership Fund (2003a,b) produced an Ecosystem Profile and an Outcomes Database of the EACF Hotspot, the latter listing 333 globally threatened species of plants and animals. Periodic status reports (Ndang'ang'a, 2008; BirdLife International, 2013; Gereau et al., 2014) updated the Ecosystem Profile and the Outcomes Database, and a thorough review of the IUCN Red List (IUCN, 2015) revealed a total of 793 globally threatened species of amphibians, birds, gastropods, insects, freshwater crabs, mammals, reptiles, and plants (Gereau et al., 2016), a 140% increase over the 2003 total. The 165 sites of the EACF Region that were surveyed for the study (Gereau et al., 2016: Appendix 2) are extremely variable in size and composition, and were not primarily selected because they were coextensive with existing PAs, with some exceptions including Magombera Forest Reserve (now Nature Forest Reserve: UNEP-WCMC, 2019), Selous Game Reserve, and Udzungwa Mountains National Park. On the other hand, 48 of the 165 surveyed sites had previously been designated as Important Bird Areas (IBAs: BirdLife International, 2024), while 145 had been recognized as Key Biodiversity Areas (KBAs), although not by the global KBA Standard now in use. The KBA Standard (IUCN, 2016) employs a set of criteria and quantitative thresholds conceptually similar to the IUCN Red List Categories and Criteria (IUCN, 2012) to identify sites of global importance for the persistence of biodiversity, and although KBAs do not necessarily establish conservation priorities, they are highly valuable for informing systematic conservation planning and priority setting.

The interplay between KBA designations and the PA network is well illustrated by two case studies in northern Tanzania. Hanang Forest Reserve was upgraded to the status of Mount Hanang Nature Forest Reserve in 2016 (Doggart et al., 2017). The Missouri Botanical Garden worked with the Tanzania Forest Service and the World Conservation Monitoring Centre to upgrade the biological priority status of the previously designated Mt. Hanang KBA based on “trigger species” of both plants and animals (Gereau, 2019a). Simultaneously, the same partners undertook a study to determine whether the nearby Nou Forest Reserve (subsequently proposed for upgrade to Nou NFR; Ract et al., 2024) qualifies as a new KBA within the Eastern Afromontane Biodiversity Hotspot (Gereau, 2019b). Collectively, the two sites are home to five potential trigger species of plants, four species of insects, three of mammals, and one of reptiles. The recently described rodent species *Fukomys hanangensis* Faulkes et al. (2017; family Bathyergidae Waterhouse) is endemic to the area of the two Reserves and some of the unprotected land between them and has subsequently been assessed as Endangered under IUCN Red List criteria A, B, C, and D (Faulkes et al., 2020). Along with two shared potential trigger species of plants, this range-restricted, threatened species links the two Reserves and raises the question of whether they would be more appropriately designated as separate KBAs, or collectively considered a single KBA including the unprotected corridor between them; this subject is currently under discussion with the Eastern and Central Africa KBA Regional Focal Point. This is an excellent example of how governments can utilize KBA sites and their supporting biodiversity data to guide the expansion of PAs, a process that has been very well developed in Gabon (Texier et al., 2024).

2. Objectives

The two objectives of this work are:

2.1. To test the hypothesis that protected areas (PAs) constitute the primary component and organizing principle of practical and sustainable nature conservation in eastern Africa

2.2. To investigate the use of existing PAs in the furtherance of *in-situ* and *ex-situ* biodiversity conservation in Tanzania

3. Comments on publications

3.1. Publication 1

This review article (Raven et al., 2020) provides the global context for understanding eastern African biodiversity by combining the numbers of indigenous vascular plant species in three major tropical regions of the world (Afrotropical, Latin America, and Southeast Asia) with species numbers for insects, birds, amphibians, reptiles, and mammals to produce reliable estimates of total biodiversity values for these three regions. Vascular plant species numbers for the Afrotropical Region (sub-Saharan Africa plus Madagascar) and Latin America (Mexico and the Antilles to southern South America) were enumerated accurately from regularly updated floristic databases with standardized taxonomy (Phillipson et al., 2017; African Plant Database, 2020; Ulloa et al., 2020), while those for Southeast Asia (excluding Bangladesh, India, and Bhutan, but including from Myanmar and Thailand west to the Philippines and New Guinea) were derived from a review of floras then in preparation (Middleton et al., 2019; Joyce et al., 2020). Species numbers for the various animal groups were derived from the available literature and databases (see references cited in Raven et al., 2000) and consultation with taxonomic experts. The results more precisely quantify previous observations of the dominance of Latin America in world biodiversity richness, with at least a third of the world's species occurring there. In terms of global biodiversity richness, the Afrotropical Region is the "odd man out" (Couvreur, 2014), with proportionally far fewer species per unit area than the geographically much smaller Southeast Asia.

3.2. Publication 2

This original research article (Ract et al., 2024) presents an analysis of Tanzania's network of Nature Forest Reserves (NFRs; Doggart et al., 2017), which were recognized as forest areas of exceptionally high importance for globally unique biodiversity under the National Forest Policy of 1998 and the Forest Act of 2002 (United Republic of Tanzania, 1998, 2002). Lists of vascular plant taxa (species, subspecies, and varieties) in each NFR were created using data from the Missouri Botanical Garden's Tropicos database, augmented with records from the GBIF portal and the IUCN Red List (see Ract et al., 2024: Online Table S1, Tanzania NFR Plants_data.xlsx). The 22 NFRs gazetted by 2023 cover an area of 9,475 km^2, ca. 10% of Tanzania's total land area, and are home to 2,289 plant species (2,804 total taxa), nearly 20% of the estimated 11,500 species in the country. Thus, the NFR network has captured approximately twice the amount of the national vascular plant species biodiversity than would be predicted from its size alone.

3.3. Publication 3

This original research article (Linan et al., 2024) analyzes the genetic diversity of *Karomia gigas* (Faden) Verdc. (Lamiaceae Martinov), a tree species first described from the coast of Kenya (as *Holmskioldia gigas* Faden; Faden, 1988) and reported as possibly extinct (Verdcourt, 1992; Beentje, 1994), then later rediscovered in southern coastal Tanzania, more than 600 km south of the original locality (Clarke et al., 2011). The species is currently known from only 43 wild individuals (21 mature and 22 juvenile) in two small national authority forest reserves, Mitundumbea FR (87 km^2) in Kilwa District and Litipo FR (10 km^2) in Lindi Rural District. With fewer than 50 mature individuals, *K. gigas* has been assessed as Critically Endangered under Red List criterion D (Mashimba & Shaw, 2022). The species is threatened by low seed viability due to fungal infestation, habitat loss due to agricultural expansion, residential and touristic development, and logging, and could certainly not survive in the wild without the *in-situ* protection afforded by these two FRs.

Because of its Critically Endangered status and apparently total lack of natural regeneration in the wild, *Karomia gigas* has been the object of a collaborative propagation program between the Tanzania Forest Services Agency (TFS) and the Missouri Botanical Garden (MBG). After extensive propagation trials to overcome the very low seed viability, these efforts have resulted in an *ex-situ* population of six individuals at TFS and 28 individuals at MBG. Collection of leaf tissue samples from all known living individuals of *K. gigas*, both wild and *ex-situ*, has allowed DNA extraction and sequencing to determine the genetic diversity of the wild and *ex-situ* populations. Wild populations were found to have greater levels of genetic diversity than *ex-situ* populations and fewer than one-third of wild individuals are represented *ex-situ*, identifying some gaps and inefficiencies in the conservation of genetic diversity in *K. gigas*, but also suggesting strategies to improve conservation of genetic diversity *ex-situ*. Analysis of relatedness between pairs of samples can help to generate a captive breeding strategy to maintain genetic diversity for eventual repatriation to the wild, including the type locality in Kenya.

In addition to the study of its genetic diversity, the *ex-situ* population of *Karomia gigas* at MBG has enabled the study of its previously unknown floral morphology and verification of its systematic position (Gereau et al., 2024). When Faden (1988) first described the species in the genus *Holmskioldia* Retz. (Lamiaceae subfamily Scutellarioideae), its flowers were unknown, and its floral morphology remained unknown when Verdcourt (1992) transferred it to the genus *Karomia* Dop (Lamiaceae subfamily Ajugoideae). The first documented flowering of *K. gigas* was in early 2021 on saplings in the MBG greenhouses, allowing verification of its placement in *Karomia* rather than *Holmskioldia* (Dop ,1932; Fernandes, 1988).

3.4. Publication 4

This original taxonomic article (Gavin-Smyth & Gereau, 2022) describes two new species of *Impatiens* Riv. ex L. (Balsaminaceae A. Rich.) from the Eastern Arc Mountains of Tanzania and Kenya (Burgess et al., 2007a; Platts et al., 2011), discusses their distributions, habitats, and relationships, and provides a provisional conservation status for each species. Each of these two new species is strictly endemic to a single Tanzanian NFR, and both are provisionally assessed as Least Concern (Protected Area Dependent).

3.5 Publication 5

This original taxonomic article (Gereau et al., 2023) publishes the new species *Chlorophytum vespertinum* Vollesen (Asparagaceae Juss. tribe Anthericeae Bartling) from southwestern Tanzania and central to northern Zambia, discusses its distribution, habitats, and relationships, and provides a provisional conservation status for the new species. The new species is known from seven distinct threat-defined “locations” (sensu IUCN, 2012) in Tanzania and Zambia, three of which appear to benefit from some level of effective protection. In Tanzania, the new species grows within the Kalambo NFR, which was declared in 2019 comprising an area of 465 km^2 previously occupied by the Kalambo River and Kalambo Fall Territorial Forest Reserves, both declared in 1957 (Government of Tanzania, 2019). The species may be considered well protected there and at two of its locations in Zambia, one a government forest reserve and the other a privately owned reserve, with the two other locations in Tanzania and two in Zambia apparently unprotected. With these considerations in mind, the calculated Extent of Occurrence of 8,846 km^2, and an Area of Occupancy of 28 km^2, the provisional conservation status of the species is Vulnerable, VU B1ab(iii)+2ab(iii).

3.6. Publication 6

Afrothismiaceae Cheek & Soto Gomez is a family of achlorophyllous mycoheterotrophic herbs with a single genus, *Afrothismia* (Engl.) Schltr., restricted to continental tropical Africa (Cheek et al., 2023). This original taxonomic article (Cheek et al., 2024) reconsiders the status of *Afrothismia winkleri* (Engl.) Schltr. var. *budongensis* Cowley, described from a single specimen collected in 1940 in the Budongo Central Forest Reserve in western Uganda (Cowley, 1988) and rediscovered in the same forest reserve in 1998 by R. Gereau. A suite of eight diagnostic morphological characters was deemed to justify the recognition of *A. winkleri* var. *budongensis* as a species distinct from *A. winkleri* sensu stricto, which grows ca. 2,300 km to the west in Cameroon and Gabon and in a different forest type. *A. winkleri* var. *budongensis* was therefore raised to the rank of species and renamed as *A. ugandensis* Cheek, nom. nov.

Afrothismia winkleri var. *budongensis* was previously assessed as Data Deficient (DD) due to doubt about its taxonomic status and lack of knowledge of its host plant(s) (IUCN SSC East African Plants Red List Authority, 2013a). After extensive exploration of the area around the 1998 collecting site and incorporation of current information about the conservation status of the forest, Cheek et al. (2004) cited a continuing decline in habitat quality at the single location of *A. ugandensis* and a probable population size of far fewer than 50 mature individuals to justify a provisional Red List assessment of Critically Endangered: CR B2ab(iii); D [as ‘D1’].

4. Discussion

Even though an analysis of global biodiversity (Raven et al., 2020) indicates that tropical Africa is less rich in biodiversity than other tropical areas of the world, it contains part or all of four of the world’s 36 Global Biodiversity Hotspots (Mittermeier et al., 2004), giving it a unique and irreplaceable position in the conservation of the world's natural resources. An analysis of the patterns of plant diversity in tropical Africa (Sosef et al., 2017) shows that Tanzania, with the largest vascular plant flora in eastern Africa and home to part of two Global Biodiversity Hotspots, is among the botanically best-explored countries in tropical Africa, putting it in a relatively good position to make scientifically well-informed decisions about conservation priorities.

A large proportion of the land area of tropical eastern Africa is covered by protected areas (PAs) of various categories, including Nature Forest Reserves, National Parks, Forest Reserves, Forest Plantations, Village Forests, Game Reserves, Wildlife Management Areas, and Game Controlled Areas (UNEP WCMC & IUCN, 2024). A time series analysis of Management Effectiveness Tracking Tool (METT) data shows that NFR management effectiveness increased between 2015 and 2021, especially where donor funds have been available; this further reinforces the primacy of the NFR network in Tanzanian nature conservation (Ract et al. 2024).

One of the first decisions that must be made in conservation planning is whether the targeted taxa can be effectively conserved *in-situ* in their natural habitats, or if some kind of *ex-situ* conservation measures are needed to assure their survival (IUCN/SSC, 2008; McGowan et al., 2017). Table 1 (page 32) presents a summary showing that the NFR network is home to many plant taxa of high conservation value, with large numbers of taxa in the threatened and Near Threatened IUCN categories (Ract et al. 2024). Of particular interest are the ten NFRs with taxa assessed as Least Concern (Protected Area Dependent): LC (PA). These taxa are clearly appropriate subjects for *in-situ* conservation efforts in these areas where they are already receiving effective and sustainable protection under Tanzanian law, and may therefore be considered amenable to strictly *in-situ* conservation, with measures for their continued protection to be included in the NFR management plans and periodically checked by means of the METT.

Table 1. Tanzania's 22 Nature Forest Reserves, with land area of each, total number of vascular plant taxa, number of taxa strictly endemic to NFR, number of threatened taxa (VU, EN, or CR), number of Near Threatened (NT) taxa, and number of taxa assessed as Least Concern, Protected Area Dependent [LC (PA)]; summarized from Ract et al., 2024: Online Table S1

NFR name	**area in km^2**	**total taxa**	**NFR endemics**	**threatened**	**NT**	**LC (PA)**
Amani	83.8	427	8	104	22	7
Chome	142.8	474	4	45	5	7
Essimingor	60.7	158		2		
Hassama	49.0	1				
Itulu Hill	3,885.1	1				
Kalambo	419.6	106		2	1	
Kilombero	1,345.1	485	1	67	9	12
Magamba	92.8	87		31	3	4
Magombera	26.2	109		11	3	
Minziro	257.2	503		4	4	
Mkingu	264.3	258	9	52	10	10
Mt. Hanang	58.7	127	2	5		4
Mt. Rungwe	136.5	354	3	27	3	1
Mwambesi	1,129.0					
Nilo	60.3	128		23	3	1
Nou	289.4	106		3		
Pindiro	122.5	25		3	2	
Pugu-Kazimzumbwi	89.7	280	1	48	14	
Rondo	117.4	243	3	44	10	
Uluguru	241.2	480	45	156	13	9
Uzigua	276.6					
Uzungwa Scarp	327.6	231	3	44	4	4
Total	9,475.4		79			

Global Biodiversity Hotspots (Myers et al., 2000; Mittermeier et al., 2004) provide a greater understanding of the uneven distribution of worldwide biodiversity richness and a useful and illuminating framework for the organization and funding of biodiversity and conservation research, but provide no conservation benefits in and of themselves. Key Biodiversity Areas (IUCN, 2016) and more taxon-delimited entities such as Important

Bird Areas (BirdLife International, 2014) and Tropical Important Plant Areas (TIPAs; Darbyshire et al., 2017) identify sites of global importance for the survival of biodiversity, but depend on the relative stability and regulatory framework provided by PAs to achieve the long-term conservation of their biological taxa and ecosystems. This evidence supports the hypothesis that protected areas constitute the primary component and organizing principle of practical and sustainable nature conservation in eastern Africa.

It is important not only to conserve areas rich in biodiversity, but also to assess the genetic variability of the species protected there. The Critically Endangered Tanzanian tree species *Karomia gigas* shows greater genetic diversity in its wild populations (43 individuals) than in its cultivated populations (34 individuals), even though the number of individuals in each group is similar (Linan et al., 2024). Thus, continued *in-situ* conservation of the two small PAs in which the wild populations live is needed to maintain a level of genetic diversity capable of responding successfully to ever-changing environmental conditions, with the *ex-situ* cultivated populations serving as a living gene bank and source of material for reinforcement plantings.

Even though the vascular plant flora of eastern Africa is comparatively well studied, new taxa are still described from there. In the scope of this thesis three new species were described and one formerly recognized variety was raised to species rank, all with provisional Red List assessments. All of them are found in protected areas.

Impatiens butu Gavin-Sm. (Gavin-Smyth & Gereau, 2022) is endemic to Chome NFR in the South Pare Mountains, and is provisionally assessed as LC (PA). Chome NFR is home to the seven taxa assessed as LC (PA) shown in Table 2 (page 34). *Impatiens butu* and one other LC (PA) taxon, *Chamaepentas hindsioides* var. *parensis*, are strictly endemic to Chome NFR; four other taxa are endemic to the Eastern Arc Mountains; and one taxon, *Leucas volkensii* var. *parviflora*, is recorded mostly from protected areas in central Kenya, with one southern outlier in the South Pare Mountains. *Impatiens ndovu* Gavin-Sm. (Gavin-Smith & Gereau, 2022) is endemic to Mkingu NFR in the Nguru Mountains, and is also assessed as LC (PA). Mkingu NFR is home to the ten taxa assessed as LC (PA) shown in Table 3 (page 35). Four taxa including *I. ndovu* are strictly endemic to Mkingu NFR; three other taxa are endemic to the Nguru Mountains but not restricted to Mkingu NFR; and the other three taxa are all endemic to the Eastern Arc Mountains. Thus, both Chome and Mkingu NFRs play a significant role in the *in-situ* conservation of elements of the local and regional flora that would have a very limited chance of survival without the protected area network.

Table 2. Vascular plant taxa in Chome Nature Forest Reserve assessed as LC (PA)

Family	**Taxon**	**Documentation**	**Distribution**
Balsaminaceae	*Impatiens butu* Gavin-Sm.	Gavin-Smyth & Gereau, 2022	endemic to Chome NFR
Lamiaceae	*Leucas volkensii* Gürke var. *parviflora* Sebald	EAPRLA Workshop 6, 2012 (unpublished)	Chome NFR and central Kenya
Melastomataceae	*Memecylon deminutum* Brenan	IUCN SSC East African Plants Red List Authority, 2020b	endemic to Eastern Arc
Rubiaceae	*Chamaepentas hindsioides* (K. Schum.) Kårehed & B. Bremer var. *parensis* (Verdc.) Kårehed & B. Bremer	EAPRLA Workshop 9, 2014 (unpublished)	endemic to Chome NFR
Rubiaceae	*Ixora albersii* K. Schum.	EAPRLA Workshop 10, 2015 (unpublished)	endemic to Eastern Arc
Rubiaceae	*Mitriostigma usambarense Verdc.*	IUCN SSC East African Plants Red List Authority, 2019	endemic to Eastern Arc
Rubiaceae	*Polysphaeria macrantha Brenan*	Amano et al., 2022c	endemic to Eastern Arc

Chlorophytum vespertinum Vollesen (Gereau et al., 2023) is known from seven distinct collecting localities in southwestern Tanzania and central to northern Zambia, including the Kalambo NFR in Tanzania and two protected localities in Zambia, one a government forest reserve and the other a privately owned reserve. It lacks any form of effective protection in its four other known localities, and is provisionally assessed as Vulnerable, VU B1ab(iii)+2ab(iii). Kalambo NFR has not been the subject of intensive botanical inventory, with only 106 vascular plant taxa reported within its area of 465 km^2. Of these 106 taxa, only three are of elevated conservation interest: *Dichaetanthera erici-rosenii* (R.E. Fr.) A. Fern. & R. Fern. (Endangered; Hills, 2023), *Chlorophytum vespertinum* (Vulnerable; Gereau et al., 2023), and *Helichrysum kalandanum* Lisowski (Near Threatened; Howard et al., 2020). Thus, even at this modest level, Kalambo NFR is contributing to the *in-situ* conservation of the threatened and near threatened regional flora.

Table 3. Vascular plant taxa in Mkingu Nature Forest Reserve assessed as LC (PA)

Family	**Taxon**	**Documentation**	**Distribution**
Balsaminaceae	*Impatiens ndovu* Gavin-Sm.	Gavin-Smyth & Gereau, 2022	endemic to Mkingu NFR
Cyatheaceae	*Cyathea humilis* Hieron. var. *pycnophylla* Holttum	EAPRLA Workshop 9, 2014 (unpublished)	endemic to Eastern Arc
Lauraceae	*Beilschmiedia kweo* (Mildbr.) Robyns & R. Wilczek	de Kok et al., 2019	endemic to Eastern Arc
Melastomataceae	*Dissotis dichaetantheroides* Wickens	IUCN SSC East African Plants Red List Authority, 2020a	endemic to Nguru Mts.
Melastomataceae	*Medinilla engleri* Gilg	IUCN SSC East African Plants Red List Authority, 2013c	endemic to Eastern Arc
Polypodiaceae	*Stenogrammitis rupestris* (Parris) Labiak	IUCN SSC East African Plants Red List Authority, 2013b	endemic to Mkingu NFR
Rubiaceae	*Chassalia bonifacei* Thulin & S. Mankt.	EAPRLA Workshop 9, 2014 (unpublished)	endemic to Mkingu NFR
Rubiaceae	*Chassalia christineae* Thulin & S. Mankt.	EAPRLA Workshop 9, 2014 (unpublished)	endemic to Mkingu NFR
Rubiaceae	*Pavetta axillipara* Bremek.	Amano et al., 2022a	endemic to Nguru Mts.
Rubiaceae	*Pavetta coelophlebia* Bremek.	Amano et al., 2022b	endemic to Nguru Mts.

Afrothismia ugandensis Cheek is newly recognized at the rank of species, having been formerly known as *A. winkleri* var. *budongensis* (Cheek et al., 2024). It is known only from

Budongo Central Forest Reserve in Uganda and is apparently very rare, having been observed only twice, once in 1940 and once in 1998. It was provisionally assessed (Cheek et al., 2024) as Critically Endangered: CR B2ab(iii); D. However, the authors who proposed this assessment have subsequently concluded that the minimal threats currently affecting the Strict Nature Reserve of Nyakafunjo, the area within Budongo Forest where the 1998 collection was made, do not justify the assertion of continuing decline in habitat quality and have proposed a revised assessment of CR D (Darbyshire et al., unpubl.), i.e., Critically Endangered based only on small population size.

To some extent, the situation of *Afrothismia ugandensis* resembles that of *Karomia gigas* in that the deciding factor for an assessment of Critically Endangered is the small number of mature individuals. Under criterion B, the currently active threats to the two threat-defined locations of *K. gigas* might justify an assessment of EN B2ab(iii); and the plausible future threat from increased encroachment into Nyakafunjo Reserve due to high population pressures along the boundaries of Budongo CFR would justify an assessment of VU D2 for *A. ugandensis*. In both cases, the protection afforded by one or more forest reserves is nearly certainly the reason that either species survives today; it is almost unimaginable that *K. gigas* would have survived to the present without the *in-situ* protection of the two small forest reserves in which it occurs, or that *A. ugandensis* would have persisted in its habitat from 1940 until 1998 without the *in-situ* protection of Budongo CFR, or that it would persist there into the future if that protection were removed.

The decisions as to whether any given taxon can be effectively conserved *in-situ* in its natural habitat or if some kind of *ex-situ* conservation measures are needed to assure survival can be based on the taxon’s distribution and threats using data from sources such as the IUCN Red List (2024) and supporting databases such as Tropicos, and information about PA effectiveness from various sources including the World Database on Protected Areas (UNEP

WCMC & IUCN, 2024), METT scores, and the experience of local experts including members of the EAPRLA. The 48 vascular plant taxa occurring in Tanzanian NFRs that have been assessed as LC (PA) can all be considered amenable to *in-situ* conservation, with monitoring of the conservation effectiveness in those NFRs needed to assure their continued protection. The Critically Endangered species *Karomia gigas* is in need of *ex-situ* conservation to assure its survival in the face of known threats and a very low population size, and of *in-situ* conservation to maintain the genetic diversity of its wild populations. The Vulnerable species *Chlorophytum vespertinum* benefits from ongoing *in-situ* conservation in Kalambo NFR and the two Zambian protectected areas where it occurs, but could also benefit from *ex-situ* measures as insurance against population reduction and habitat degradation in its unprotected populations. Although *Afrothismia ugandensis* might hypothetically benefit from *ex-situ* conservation measures, its extreme rarity and the nearly unsurmountable difficulty of propagating and cultivating an achlorophyllous mycoheterotroph dictate that only *in-situ* conservation is feasible; similar considerations apply to extremely rare and recalcitrant species in Tanzania. The development of this methodology fulfills the second objective of this thesis: to investigate the use of existing PAs in the furtherance of *in-situ* and *ex-situ* biodiversity conservation in Tanzania. Tanzania's many other plant taxa of special conservation concern are in need of similar analyses to determine their suitability for *in-situ* or *ex-situ* conservation according to the localities in which they occur.

5. Conclusions

The most important conclusions resulting from this study are:

1. The primary elements in the design and execution of effective nature conservation are biodiversity inventories, extinction risk assessments, and a robust network of protected areas (PAs).

2. Tropical eastern Africa possesses an extensive network of PAs, with Tanzania's system of Nature Forest Reserves (NFRs) providing effective protection to a large proportion of the country's vascular plant flora, including many species of high conservation value.
3. Global Biodiversity Hotspots, Key Biodiversity Areas, Important Bird Areas, and Tropical Important Plant Areas provide valuable means of organizing conservation priorities and planning, but provide no conservation benefits in and of themselves.
4. The regulatory stability of PAs and the ability to monitor their effectiveness over time confer special advantages on them that confirm the hypothesis (the first objective of this study) that PAs constitute the primary component and organizing principle of practical and sustainable nature conservation in eastern Africa.
5. Decisions about *in-situ* and *ex-situ* conservation priorities are central to conservation planning.
6. In Tanzania, these decisions can be based on the species' known distribution and threats and information about the PAs in which they occur, data that are available from known and tested sources.
    a. Species occurring exclusively in effectively conserved PAs, i.e. those assessed as Least Concern (Protected Area Dependent) by the Eastern African Plant Red List Authority, are good candidates for *in-situ* conservation.
    b. Some rare and highly threatened species may be in need of *ex-situ* conservation to assure their survival in the face of known threats, and of *in-situ* conservation to maintain their wild population numbers and genetic diversity.
    c. Some very rare and highly threatened species may not be amenable to *ex-situ* conservation due to inherent difficulties of locating or propagating them, and so can only be conserved *in-situ*.

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7. Publication 1

Raven, P.H., R.E. Gereau, P.B. Phillipson, C. Chatelain, C.N. Jenkins & C. Ulloa Ulloa. The distribution of biodiversity richness in the tropics. 2020. Science Advances 09 Sep 2020: Vol. 6, no. 37. DOI: 10.1126/sciadv.abc6228.

ECOLOGY

The distribution of biodiversity richness in the tropics

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We compare the numbers of vascular plant species in the three major tropical areas. The Afrotropical Region (Africa south of the Sahara Desert plus Madagascar), roughly equal in size to the Latin American Region (Mexico southward), has only 56,451 recorded species (about 170 being added annually), as compared with 118,308 recorded species (about 750 being added annually) in Latin America. Southeast Asia, only a quarter the size of the other two tropical areas, has approximately 50,000 recorded species, with an average of 364 being added annually. Thus, Tropical Asia is likely to be proportionately richest in plant diversity, and for biodiversity in general, for its size. In the animal groups we reviewed, the patterns of species diversity were mostly similar except for mammals and butterflies. Judged from these relationships, Latin America may be home to at least a third of global biodiversity.

Detailed studies over the past several decades have made it possible to compare accurately the numbers of species of indigenous vascular plants in Africa, Latin America, and Southeast Asia, and the results are unexpected. In summary, the Afrotropical Region (Africa south of the Sahara with Madagascar), with an area of 22,657,000 km^2, is home to 56,451 plant species. These were enumerated accurately following the development of the regularly updated African Plant Database (*1*) and the Madagascar Catalogue *(2)*, both regional nomenclature repositories with standardized taxonomy. For Latin America (Mexico south, including the West Indies), with 19,197,000 km^2, a similar effort (*3*) has recorded 118,308 known species. These registers are kept updated; the numbers provided here are current. Thus, Latin America is home to more than twice as many plant species as the Afrotropical Region in an area only about 85% of its size. Tropical Africa is comparatively even poorer in species, since 9582 of the species recorded in the Afrotropical Region occur only in Madagascar (587,000 km^2), and 16,405 of the species occur only in Southern Africa (2,704,000 km^2), with 41 species shared uniquely between Madagascar and Southern Africa. There are also 4330 plant species shared uniquely between Tropical and Southern Africa. These figures demonstrate the well-known floristic richness of Southern Africa. If we subtract the plant species found only in Madagascar and Southern Africa from the total, Tropical Africa, with an area of 19,366,000 km^2, is home to only 30,423 plant species in an area essentially the same size as Latin America, which has some 3.8 times as many species of plants—the difference between the two areas is growing rapidly due to the higher rate of discovery of new species in Latin America. Although Latin America is ecologically more diverse than mainland tropical Africa, the latter includes rainforest, coastal forest, extensive dry plains, and the mountains of East and Southern Africa. There is no area in Africa, however, nearly as rich in plant species as the Andes—a true major area of endemism that remains relatively poorly known biologically [see also (*4*)].

A recent review of floras that are currently being prepared for Southeast Asia (excluding Bangladesh, India, and Bhutan but including from Myanmar and Thailand west to the Philippines and New Guinea), with an area of 5,708,000 km^2, shows that this region is home to approximately 50,000 indigenous species of vascular plants (*5*, *6*). In contrast, the entire Afrotropical Region, with an area almost four times as large, has only 56,451 recorded species and is much better known than its Asian counterpart.

Before analyzing the reasons for these remarkable disparities, it will be useful to compare the figures for vascular plants (*1*, *3*, *5*–*7*) with those for selected groups of animals, ones that are well known enough to make possible meaningful comparisons. To obtain the most accurate figures possible for these groups, we consulted the available literature (*8*–*19*) and then discussed each group with leading global experts. By doing so, we have been able to present the most up-to-date figures for each of them that are available anywhere.

Current estimates of the species present in each area mentioned are summarized in Table 1 and illustrated in Fig. 1. The number of Afrotropical species that occur only in Madagascar (i.e., are not shared with either tropical or Southern Africa) is listed separately. In general, the regional abundances of species of breeding birds, lizards, and snakes are roughly in line with those for vascular plants. However, there are some notable differences between the patterns we reviewed for the groups listed.

For Madagascar, there are fewer species of butterflies recorded than would be expected from the number in the Afrotropical region, but the ratio for moths resembles that in other groups (28,529 regionally, 4681 in Madagascar = 17.2%). There are many more species of amphibians in the Neotropics than one would estimate from their numbers in other tropical regions. Significantly, there are relatively more African mammals compared with their Neotropical counterparts. While rodents resemble other groups in this respect [870 species (Latin America) versus 430 species (Afrotropical region)], primates (144 versus 192) and, especially, hoofed animals, Cetartiodactyla (27 versus 89), present unusual distribution patterns.

Part of the difference in mammalian species diversity between Latin America and Tropical Africa (cf. Cetartiodactyla) certainly can be attributed to the extermination of most of the large mammals of the Neotropical region by the humans who arrived there some

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Table 1. Estimated total numbers of described species for main taxonomic groups across selected tropical regions (*1*, *3*, *5*–*19*).

Taxonomic group	Latin America*	Afrotropical Region†	Afrotrop spp. only in Madagascar	Percentage Mad/Afrotrop‡	Southeast Asia
Vascular plants	118,308	56,451	9582	17%	50,000
Butterflies	9000	4426	320	7%	4000
Breeding birds	4626	2134	263	12%	2660
Ants	3917	3343	867	26%	3663
Amphibians	3398	1178	341	29%	1085
Lizards	2225	1409	337	24%	1129
Mammals	1793	1421	246	17%	1305
Snakes	1573	681	109	16%	751

*Mexico southward through all of South America, including the West Indies. †That is, including Madagascar. ‡Percentage contribution of Madagascar to Afrotropical Region species total, rounded to the nearest percentage.

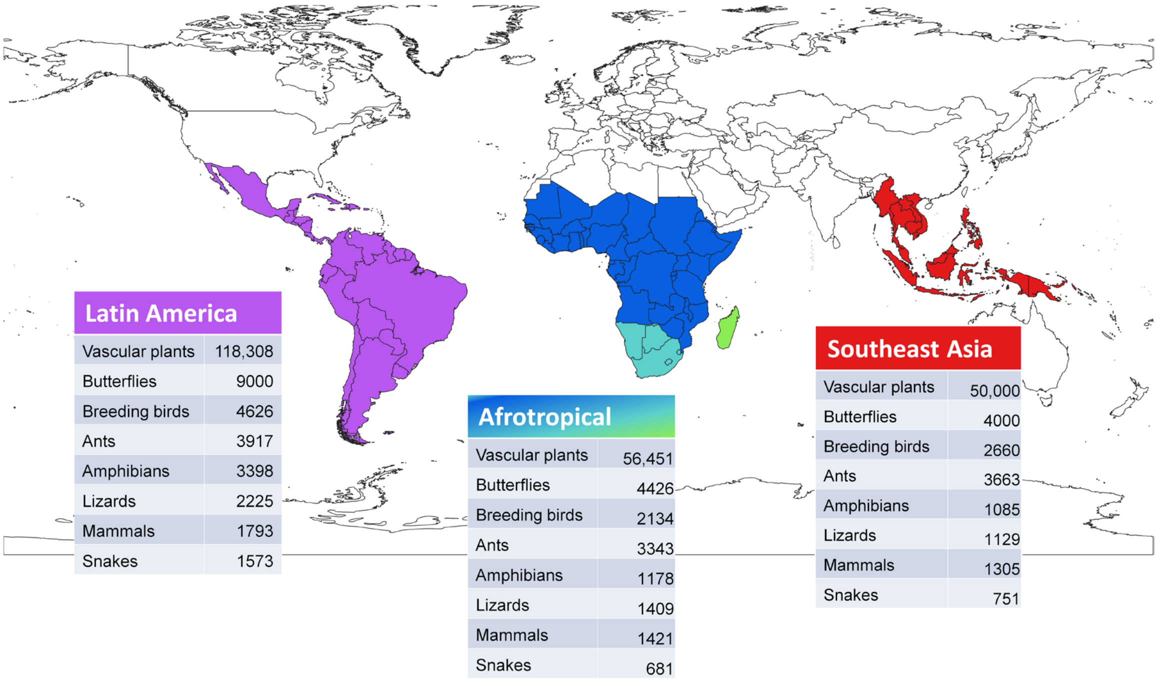

Fig. 1. Map of the world showing the three tropical major regions with a summary of the biodiversity richness for each, with the three floristically distinctive subunits of the Afrotropical Region: Tropical Africa (dark blue), Southern Africa (pale blue), and Madagascar (green).

10,000 years ago (*20*). In Africa, the entire course of evolution of hominids over the past 6 to 8 million years took place in the presence of large mammals, many of which survive to the present. In addition, North American mammals originally invaded South America following the elevation of the Panama land bridge some 3.5 million years ago, with uplands developing about 2.2 million years ago (*21*). North American mammals invaded South America in far greater numbers than Eurasian ones invaded Africa, possibly another factor in reducing the diversity of the surviving South American mammals. In contrast, the reason that fewer ant species are recorded in the Neotropics than would be expected may well be that they remain understudied there.

For every group of organisms, except butterflies, Madagascar has remarkably many species relative to the rest of the Afrotropical Region, of which it constitutes just over 1/40th the size. Considering that there are probably at least 3000 additional species of vascular plants to be discovered and named in Madagascar (*2*), Madagascar's contribution to the vascular plant flora of the Afrotropical Region is probably actually more than 20%.

Considering that a rapidly growing 118,308 species of vascular plants are known from Latin America and that the global total now is about 384,000 species (*22*), it seems likely that a third of more or all existing vascular plant species occur in Latin America and that the same may hold true for biodiversity in general. How many species that would imply depends on estimates for the total existing number of eukaryotes and so cannot be made with any degree of precision at this point.

It is important now to turn to a consideration of how well known vascular plants are in each of the three tropical regions we have analyzed [Table 2 (*1*–*3*, *7*, *23*, *24*) and Fig. 2]. On average, 173 newly described species have been added to the flora of the Afrotropical Region each year since 2008, with 54 of these from Madagascar [Table 2 (*1*, *7*)]. An average of 749 new species has been described annually from Latin America for the past 25 years, with no demonstrable tendency to level off [Table 2 (*23*)]. The number of newly described plant species added for Southeast Asia (5,708,000 km^2) has been growing at an average of 364/year for the past 12 years, more than twice as many as for the entire Afrotropical Region (22,657,000 km^2), which is almost four times as large. This is a clear indication of how very rich in plant species and relatively poorly known Southeast Asia remains [see also (*6*)].

In recent years, the publication of new species of vascular plants by scientists living in Latin American countries has increased markedly. These increases have become possible because of freely available botanical online resources (e.g., Tropicos, The International Plant Name Index, and Biodiversity Heritage Library), low publication costs, and rapid electronic publication. At the same time, traditional studies of tropical plants have continued in Europe, North America, and Asia, as they have been increasing locally within the tropical countries themselves.

Since Latin America is somewhat smaller than the Afrotropical Region, the reasons for its far greater species richness deserve analysis. Comparing continental areas of rainforest, forest occupies about 5,500,000 km^2 in the Amazon Basin, with additional extensive areas in the Chocó of Northwest South America, the Mata Atlântica of Brazil (originally 1,500,000 km^2, but with only about 7% of the original area remaining), and especially the extraordinarily rich forests and highland vegetation of the Andes [e.g., (*25*), for butterflies]. In contrast, all of the rainforests in Africa occupy only about 3,900,000 km^2. Most of the 5,700,000 km^2 area of Southeast Asia was originally rainforest, so that species number comparisons with Africa seem reasonable. Overall, considering that, with respect to their biodiversity, tropical forests are the richest habitats on Earth, their regional representation obviously has an effect on the overall numbers of species found in the major regions considered here.

Although Latin America is somewhat smaller than the Afrotropical Region, it is home to more than twice as many species of vascular plants and of several groups of animals as well. A review of the geological and climatic history of Africa and South America provides some reasons for this major discrepancy (*26*). When these continents separated from their original positions as parts of Gondwanaland, about 88 million years ago, their floras, judged from the fossil record, were similar. The continents moved apart gradually, so that by the start of the Tertiary Era, 66 million years ago, they were only about 800 km apart, with islands scattered between them. Africa and Eurasia became relatively close by the middle Miocene Period, about 17 to 18 million years ago, with biotic exchange between them still limited but increasing from that time onward (*27*).

At the end of the Cretaceous Period, Africa and South America were low-lying and level, with moist subtropical forests extending from coast to coast. To the north, across the Tethys Sea, the plants and animals of Eurasia and North America were markedly different from their tropical counterparts. By the Eocene Period (56 to 34 million years ago), the plants and animals of the two southern continents had become significantly different (*28*), and they have continued to diversify further to the present. Subsequently, with the formation of the Rift Valley system of East Africa, the eastern and southern regions of the continent were gradually uplifted to their present heights, some of the mountains (e.g., Mt. Kilimanjaro, 5900 m) within the past 1 million years. The mountains of South Africa, which is topographically quite rugged, range up to 3450 m in height. Mostly subsea 17 million years ago, East Africa rose relatively rapidly to its present elevation, especially during the most recent 5 to 6 million years. As this process continued, the African mountains were attaining their present heights, and the contours of the modern Rift Valley were taking shape (*29*).

This extensive orogeny profoundly affected regional climates and hence the nature of local ecosystems (*26*). In Sub-Saharan Africa, savannas and dry woodlands replaced many of the moist forests that had originally occupied about half the total area (*30*). Although still biologically rich, the drier habitats have proportionately many

Table 2. Number of newly described species of vascular plants for Latin America, Southeast Asia, and specified portions of Africa, by year, 2008–2019 (*1*–*3*, *7*, *23*, *24*).

Year	Latin America	Southeast Asia	Afrotropical Region	Tropical Africa	Southern Africa	Madagascar
2008	697	298	190	94	46	50
2009	704	291	223	116	54	53
2010	807	291	172	103	38	31
2011	647	246	155	63	65	27
2012	633	401	134	65	27	42
2013	729	317	166	77	44	45
2014	707	374	153	40	41	72
2015	873	453	212	88	47	77
2016	833	404	178	71	22	85
2017	917	448	152	42	31	79
2018	728	450	126	56	34	55
2019	714	395	169	122	21	36
12-year mean	749	364	173	78	41	54

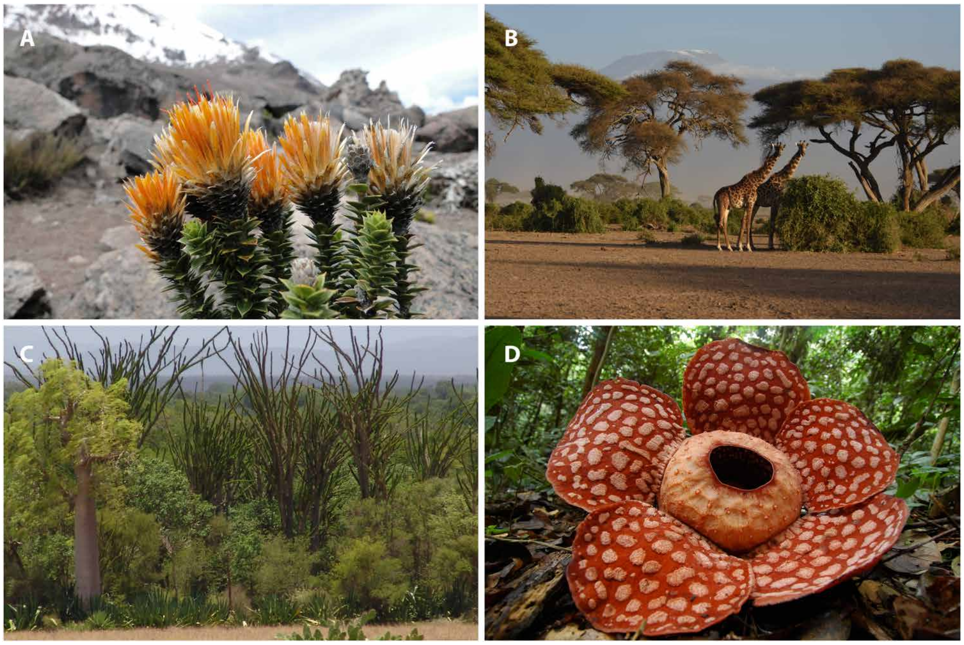

Fig. 2. Plant species from the Andes Mountains, East Africa, Madagascar, and Malaysia. (**A**) *Chuquiraga jussieui* at the base of volcano Chimborazo, Ecuador. Photo credit: Flora of The World. (**B**) *Vachellia tortilis (Acacia tortilis),* grazed by giraffes at the foot of Mt. Kilimanjaro. Photo credit: David C. Western. (**C**) *Alluaudia procera* spiny thicket, Berenty, Madagascar. The family Didieriaceae is highly characteristic of Madagascar. *Moringa drouhardii* on the left, and noxious introduced *Opuntia stricta* in the foreground. Photo credit: Flora of the World. (**D**) *Rafflesia cantleyi*, a unique parasitic plant, northern Malaysia rainforest. Photo credit: Charles Davis.

fewer species than do tropical moist forests. As they expanded, they greatly reduced Africa's moist forests and left many of them as separate patches [e.g., (*31*)]. The separation of South America from Antarctica about 55 million years ago, eventually leading to the formation of ice sheets in the south, strongly cooled the Benguela Current, running up the west coast of Africa, and caused the spread of arid climates there too. The original rainforest, now much smaller and fragmented, also became much poorer in species of plants and animals than it had been originally (*26*).

The events that led to the formation of the modern biota of Africa were quite distinct from those that took place in Latin America and in Southeast Asia. In Latin America, the elevation of the Andes over the past 6 to 10 million years has protected most of South America from spreading aridity, with the effects of the cold, northward-flowing Humboldt Current mainly confined to the relatively narrow strip of level lands west of the Andes. In Southeast Asia, the predominant insularity and moisture have preserved the original biological richness, which has also been accentuated by extensive regional orogeny. Because of the factors discussed, the cool periods during Pleistocene affected Africa much more severely than they did the other two major tropical regions. The well-known and extensive biological interchange between North and South America following the establishment of their Pleistocene connection and of the nearly continuous mountains now linking them has enriched the biota of the Andes greatly. Rapid speciation in these mountains, partly based on the northern immigrants, has significantly added to the overall biological richness of most groups of South American organisms (*27*, *28*, *32*).

UNRESOLVED QUESTIONS

The destruction of ecosystems is taking place so rapidly throughout the tropics that it will be difficult to obtain additional information bearing on the matters reviewed here. With the global population currently 7.8 billion, projected to increase to nearly 10 billion over the next 30 years, and our current consumption of natural productivity having reached around 175% of what is available sustainably (www.footprintnetwork.org), the future for most natural ecosystems looks dim. In Sub-Saharan Africa, the situation is especially dire; the 1.2 billion people there are estimated to double by mid-century and quadruple by the end of this century. Few predict that there will be many areas of tropical forest left by the end of the century. Therefore, to find answers to the obvious questions of how many species are there, how their ecosystems function, and so forth, we will need to do the best we can as soon as we can. Thus far, we have named no more than 10% of tropical plants and animals, and at current rates of discovery, most are likely to be long gone before we become aware of their existence. A division of effort between strengthening our knowledge of well-known groups, similar to those reviewed here, to reveal general patterns, and gaining some knowledge of the lesser-known groups by carefully designed sampling approaches, is apparently the best that we can do. But it is well worth the effort to do so, for its own sake and to provide the best possible basis for conservation.

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Acknowledgments: We are grateful to the following people for the invaluable discussion on data for selected groups of animals that allow for comparison with our data with plants: A. Bauer (reptiles) and B. Fisher (ants); lepidopteran data were discussed with S. Collins, J. Dobson, J. D. Holloway, D. Lees, R. K. Rollins, R. Vane-Wright, and M. Williams. We are also grateful to D. Middleton, National Parks Board, Singapore, for the valuable discussions on Southeast Asia and to S. Miller, U.S. National Museum, Smithsonian Institution, for additional discussions. We thank C. Davidson and S. Christoph (Flora of The World), C. Davis, and D. C. Western for permitting use of their photographs. **Funding:** No grants were received for any aspect of this study. **Author contributions:** P.H.R., R.E.G., and P.B.P. contributed equally to the article, C.C. to the Afrotropical analysis, C.U.U. to the Latin American analysis, and C.N.J. to the analysis of vertebrates. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** Data used in the analyses are publicly available in the online databases cited in the main text.

Submitted 4 May 2020
Accepted 28 July 2020
Published 9 September 2020
10.1126/sciadv.abc6228

Citation: P. H. Raven, R. E. Gereau, P. B. Phillipson, C. Chatelain, C. N. Jenkins, C. Ulloa Ulloa, The distribution of biodiversity richness in the tropics. *Sci. Adv.* **6**, eabc6228 (2020).

8. Authorship statements for Publication 1

AUTHOR'S STATEMENT PUBLICATION 1

I hereby declare that the article:

"The distribution of biodiversity richness in the tropics" by Raven, P.H.*, **Gereau, R.E.***, Phillipson, P.B.*, Chatelain, C., Jenkins, C.N. & Ulloa Ulloa, C. (2020) *Science Advances* 09 Sep 2020: Vol. 6, no. 37. DOI: 10.1126/sciadv.abc6228
*co-first authors

is part of my PhD thesis. I am a co-first author of this article. I performed part of the analysis of vascular plant species numbers for the Afrotropical Region and checked the consistency and accuracy of species numbers for all major taxon groups and geographic regions. My results are shown as parts of Table 1 and Figure 1. I helped to conceptualize the article, contributed to writing the manuscript, and participated in responding to review comments and the final review process.

Roy E. Gereau

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AUTHORSHIP CONTRIBUTION STATEMENT

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Date	9 October 2024
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Date	8Oct2024
Signature	

9. Publication 2

Ract, C., N.D. Burgess, L. Dinesen, P. Sumbi, I. Malugu, J. Latham, L. Anderson, R.E. Gereau, M. Gonçalves de Lima, A. Akida, E. Nashanda, Z. Shabani, S. Tango, S. Mteleka, Dos Santos Silayo, J. Mwangi, G. Lyatuu, P.J. Platts & F. Rovero. 2024. Nature Forest Reserves in Tanzania and their importance for conservation. PLoS ONE 19(2): 1-18. e0281408. https://doi.org/10.1371/journal.pone.0281408

PLOS ONE

RESEARCH ARTICLE

Nature Forest Reserves in Tanzania and their importance for conservation

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OPEN ACCESS

Citation: Ract C, Burgess ND, Dinesen L, Sumbi P, Malugu I, Latham J, et al. (2024) Nature Forest Reserves in Tanzania and their importance for conservation. PLoS ONE 19(2): e0281408. https://doi.org/10.1371/journal.pone.0281408

Editor: Stephanie S. Romanach, U.S. Geological Survey, UNITED STATES

Received: January 21, 2023

Accepted: October 10, 2023

Published: February 5, 2024

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0281408

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Abstract

Since 1997 Tanzania has undertaken a process to identify and declare a network of Nature Forest Reserves (NFRs) with high biodiversity values, from within its existing portfolio of national Forest Reserves, with 16 new NFRs declared since 2015. The current network of 22 gazetted NFRs covered 948,871 hectares in 2023. NFRs now cover a range of Tanzanian habitat types, including all main forest types—wet, seasonal, and dry—as well as wetlands and grasslands. NFRs contain at least 178 of Tanzania's 242 endemic vertebrate species, of which at least 50% are threatened with extinction, and 553 Tanzanian endemic plant taxa (species, subspecies, and varieties), of which at least 50% are threatened. NFRs also support 41 single-site endemic vertebrate species and 76 single-site endemic plant taxa. Time series analysis of management effectiveness tracking tool (METT) data shows that NFR management effectiveness is increasing, especially where donor funds have been available. Improved management and investment have resulted in measurable reductions of some critical threats in NFRs. Still, ongoing challenges remain to fully contain issues of illegal logging, charcoal production, firewood, pole-cutting, illegal hunting and snaring of birds and mammals, fire, wildlife trade, and the unpredictable impacts of climate change. Increased tourism, diversified revenue generation and investment schemes, involving communities in management, and stepping up control measures for remaining threats are all required to create a network of economically self-sustaining NFRs able to conserve critical biodiversity values.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Protected areas are essential for conserving biodiversity and maintaining flows of ecosystem services, including storing carbon and providing regular water flows. They are also an important buffer against climate change [1, 2]. The International Union for Conservation of Nature (IUCN) defines a Protected Area (PA) as "An area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and natural and associated cultural resources, and managed through legal or other effective means" [1].

The creation of protected forest areas in Tanzania has a long history stretching back to the German colonial period in the late 1800s. The 'forest reserves' system expanded over time, first during the German and subsequent British colonial periods and since Tanzanian independence in 1961 [3–6]. Initially, management aims of these areas included reserves established to produce natural forest resources (timber and charcoal), protection of natural forests (water catchment reserves and for the prevention of landslides and erosion), and the establishment of plantation forestry using exotic species.

After the implementation of the 'new' Forest Policy in 1998 [7] and the Forest Act in 2002 [8], national and local authority forest reserves were designed for the preservation of their biodiversity and sustainable use of natural resources and habitats, and human activities were restricted [4, 5]. Under this legislative framework, Nature Forest Reserves (NFRs) were recognised as forest areas of exceptionally high importance for globally unique biodiversity and managed in most cases with solid protection and are recognised as IUCN Category 1b. The first phase of declaring NFRs was in the Eastern Arc Mountains ecoregion [9] during 1997–2009, starting with Amani Nature Reserve in the East Usambara Mountains in 1997.

Over the past 25 years, the Tanzanian government has continued identifying and upgrading other biologically important reserves to become NFRs. These sites initially fell under the ownership and management of the Forestry and Beekeeping Division of the Ministry of Natural Resources and Tourism and (since 2011) the Tanzania Forest Services (TFS) Agency. After the first phase of declaring NFRs, the network expanded to cover all the different forest types in the country (including the Coastal forests, the Northern Volcanics, the Southern Highlands, Guineo-Congolian lowland forests, Miombo woodland and Miombo-Acacia woodland). The current network of NFRs in Tanzania contains 22 reserves with 4 additional reserves being proposed in 2022 (Fig 1). However, our analyses contain only 21 reserves declared, as the raw data is not available yet for (i) the proposed sites (except for Nou NFR) and (ii) one recently declared reserve ((Uvinza), gazetted beginning of the year 2022). Uplifting the existing reserves to the status of NFR was undertaken to facilitate the development of income-generating activities like tourism, to address threats facing some of the reserves that were being managed as part of the network of reserves at regional and district levels, and to recognise the exceptional biological values of sites outside the network of national parks and other reserves managed primarily for large mammal conservation in Tanzania.

We describe and assess the conservation value of Tanzania's NFR network for the first time by (i) evaluating the development of the NFR network and assessing its coverage of Tanzanian biodiversity, focusing on endemic species; (ii) assessing the coverage by the NFR network and other protected areas across Tanzania of endemic and rare species (iii) assessing the effectiveness of NFRs and whether management resources have been deployed to the most important sites to achieve conservation goals. We provide current insights into the management challenges in the NFR and discuss how these might be addressed in the future.

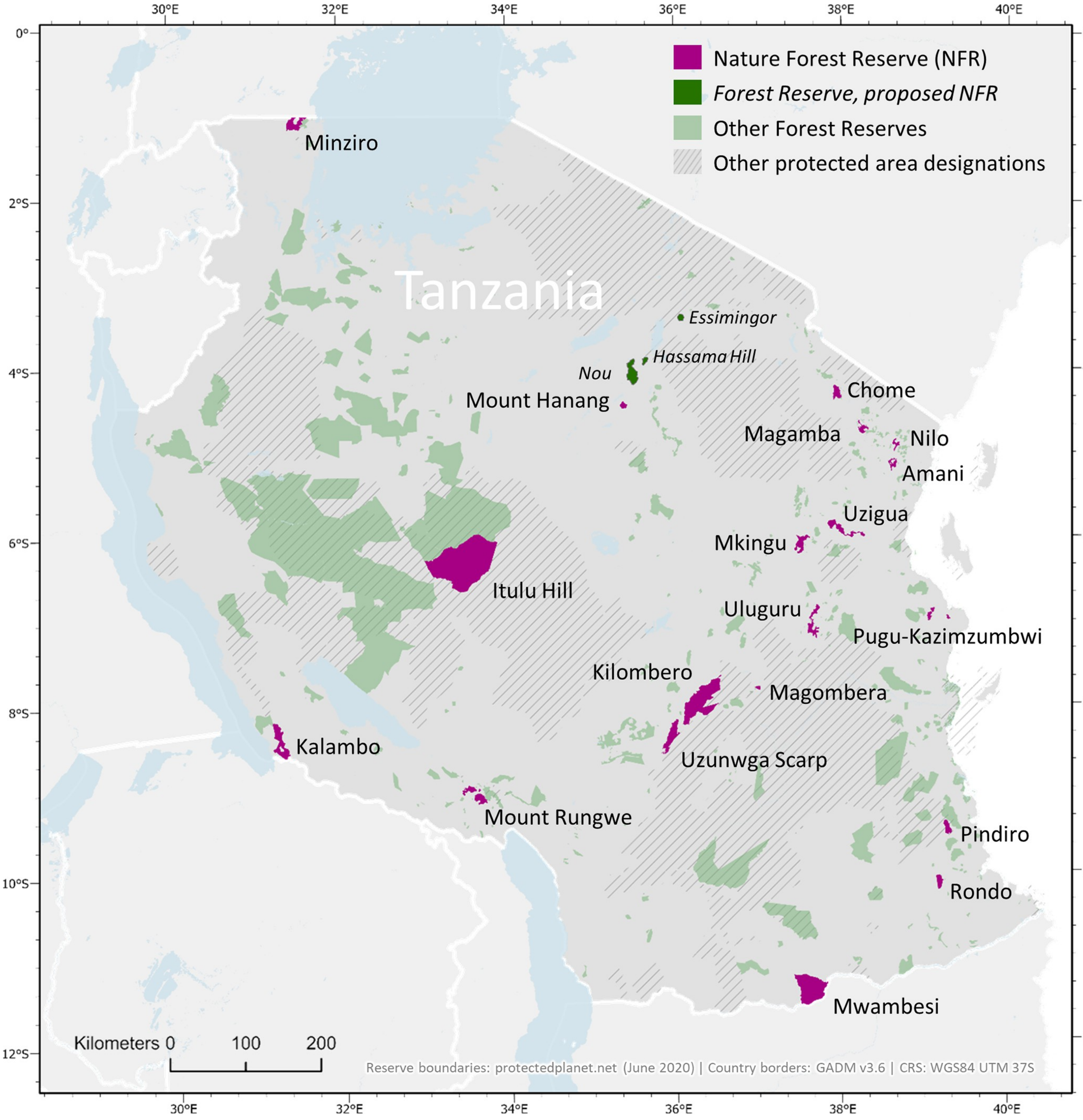

Fig 1. Map of Tanzania showing Nature Forest Reserves (pink), proposed Forest Reserves (dark green), other Forest Reserves (green) and other kinds of protected areas (shaded).

https://doi.org/10.1371/journal.pone.0281408.g001

Methods

Data collection

We collected available published and unpublished data on the Tanzanian NFRs (summarised in S1 Table). Data NFRs include declaration details, biological features, management information, and threats. Management data are available for 18 sites as the five were too recently declared (Pugu-Kazimzumbwi, Uzigua, Essimingor, Hassama Hill and Nou).

Site level species data

Lists of vertebrate species and plant taxa in each NFR were built upon existing lists of vertebrates for the Eastern Arc Mountains [6, 10–12] and coastal forests [13] and the addition of data from other sites by the authors. Lists of plant taxa in each FNR were created using data from the Missouri Botanical Garden, augmented with records from the GBIF portal (accessed in July 2020) and the IUCN red list. The designation "taxa" is used throughout the text to mean "terminal taxa" for plants, including species, subspecies, and varieties. The unit of analysis for vertebrates is species. Taxa or species occurring in each of the NFRs were categorised as endemic (both endemic to Tanzania or to the NFR itself) and threatened with extinction according to IUCN Red List categories: Critically Endangered (CR), Endangered (EN), and Vulnerable (VU).

Tanzania wide species data

Vertebrate species distribution data for the whole of Tanzania were compiled from existing GIS data of potential distributions: birds [14], amphibians and mammals [15], and reptiles [16]. Species occurrences within NFR were calculated using GIS analysis of species range distributions overlaid on NFR shapefiles from the Word Database on Protected Areas (https://www.protectedplanet.net/en).

Management data

Declaration date and area for each NFR were gathered from Tanzanian government records. Assessments of NFR management effectiveness were done using the Management Effectiveness Tracking Tool (METT) [17–19]. METT assessments were applied four times for 11 NFR; in 2015, 2017, and 2019—and for 17 NFR in 2021. To assess management effectiveness further, we used data on forest disturbance collected for four NFRs (Amani, Kilombero, Mkingu, and Uluguru) where baseline (2001—pre NFR creation) and more recent (2019) survey information exists. Disturbance data were gathered using 10 m wide by 600 m long transects within the forest (a total length surveyed of 3.3km; [20]). Data on living, naturally dead, or cut stems of trees were collected. Repeating disturbance transects before and after reserve declaration aimed to determine whether the declaration of NFR status is correlated with reduced habitat degradation.

Data on income generation and tourism trends were collated from the managers of each NFR, reserve management plans, and from unpublished baseline, mid-term, and end-line NFR surveys undertaken as part of a Global Environment Facility (GEF) project "*Enhancing the Forest Nature Reserves Network for Biodiversity Conservation in Tanzania*'. In 2019, compiled data were checked by managers of all 17 NFRs and the staff of UNDP-Tanzania and the Tanzania Forest Service [21]. Income was standardised to US dollars across the years assessed. Some further updates on tourism and funding were provided by Tanzania Forest Service staff in June 2023 based on July 2022-June 2023 data.

Data analysis

We assessed the biodiversity value of individual NFRs as the number of both endemic and threatened taxa in each reserve using our database of species-per-reserve. Arranging reserves in chronological order of declaration facilitated an assessment of how biodiversity value has been added through the declaration of new NFRs. The correlation between the number of endemic and threatened taxa per NFR and the declaration date was tested using the non-parametric Spearman's rank correlation coefficient.

We assessed Tanzania endemic species coverage by the NFR network using range data from the IUCN Red List for amphibians, reptiles, birds, and mammals overlaid onto GIS data for NFRs. NFRs were grouped according to the date they were established, resulting in eight groups/time frames (as several reserves were declared the same year). For each endemic species, the percent of its range covered by the sites was determined and classified into different categories: (1) gap species: 0% of an endemic species' range covered by the sites, (2) poorly covered species: between 0–2% of an endemic species' range covered by the sites, (3) 2–5% of an endemic species' range covered by the sites, (4) 6–10% of an endemic species' range covered by the sites, (5) between 11–20%, (6) between 21–50%, and (7) more than 50% of an endemic species' range covered by the sites. The proportion of endemic species was calculated in each of these categories. We also mapped the number of endemic species not included in the NFR network as it was developed over time.

In addition, we used reserve polygons from the World Database on Protected Areas (accessed in April 2022) to determine if there are other types of protected and conserved areas (National Parks, Game Reserves, Forest Reserves, Conservation Areas, Village Land Forest Reserves, and Game Controlled Areas) that overlap with the ranges of gap/poorly covered species once species covered by the 23 NFRs are excluded. The percent species range covered by different protected area categories was calculated, as was the proportion of species in each of the classification categories. Analyses were carried out using the statistical software R (version 4.0.5) [22], the spatial Geographic Information System QGIS (version 3.16.14), and Microsoft Excel.

For management effectiveness and other aspects of reserve management, possible correlations among the covariates were tested using Spearman rank correlation, Friedman test and the post hoc pairwise Wilcoxon rank sum test in R [22].

Methodological limitations

The data and analyses used here are subject to several limitations.

Firstly, for the management effectiveness analyses there is limited independent verification of the METT data from reserve managers, and past METT data on recently declared NFRs were missing. Earlier studies have shown that METT data are valuable but need to be interpreted with care when done using self-assessment [17, 23], especially older METT versions, i.e., METT 1–3. To obtain the best outcome from the METT assessment process, studies suggest that METT assessment is done by a group of people to reduce bias [19, 24]. In addition, METT 4 requires that local communities are involved in the assessment. Our METT data used different versions of the tool which we combined. Management effectiveness assessment benefited from field surveys of forest disturbance, but these were only available for four NFRs.

Secondly, for the species gap analyses, we used range data only, without considering life history or population data. This means that the viability of populations within a NFR or more broadly is not examined. Species range data may also be biased, especially for areas that have been poorly sampled [25].

Thirdly, undertaking analyses using current species occurrence data does not consider possible range changes due to climate change. A study based on modelling the impact of climate change on Tanzanian forests demonstrated that most forest ecosystems, especially montane forests in the Eastern Arc Mountains will be affected with habitat losses of more than 40% under the optimistic RCP4.5 scenario by 2055 [26].

Results

Biodiversity values of NFRs using site level data

Analysis of our species-by-sites database shows that older NFR sites supported more threatened taxa than recently declared sites (Fig 2A and S2 Table). Amani, Kilombero, and Uluguru

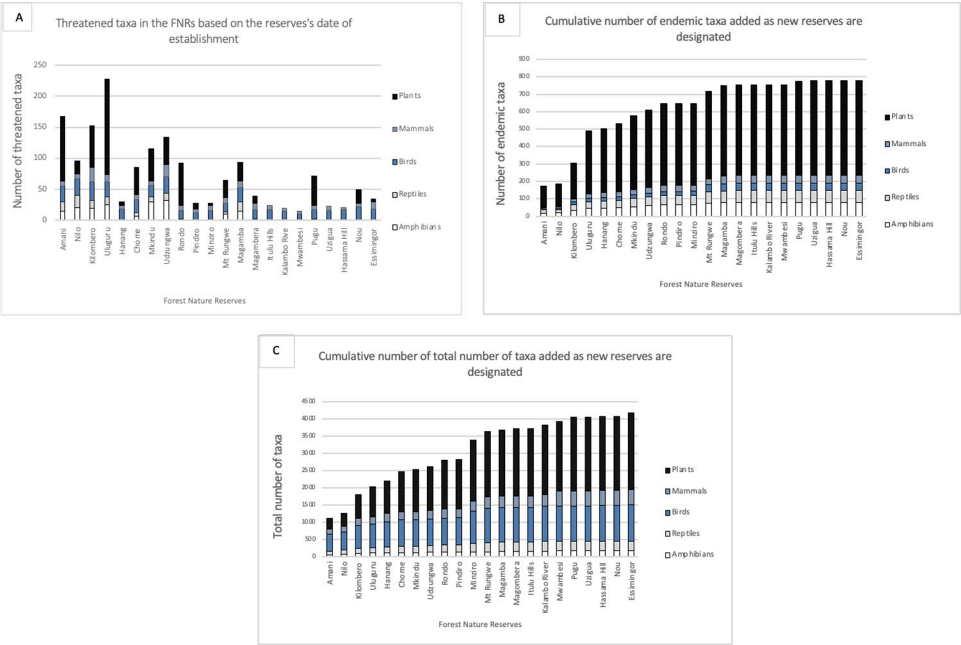

Fig 2. Importance of the network of NFRs for Tanzanian endemics and globally threatened taxa since 1977. A: Number of threatened taxa in the NFRs. B: Cumulative number of endemic taxa. C: Cumulative number of total taxa (including endemic, threatened, and other taxa). Reserves are ordered according to the year of their designation. The stacked bars are separated depending on the taxa: black represent plants, light blue is for the mammals, dark blue represent the birds, grey is the reptiles and white symbolize the amphibians.

https://doi.org/10.1371/journal.pone.0281408.g002

NFRs had the highest number of threatened taxa, followed by Nilo, Chome, Mkingu, Uzungwa Scarp, Rondo, and Magamba. Many of the threatened species were also endemic to Tanzania (see Fig 2B, Amani to Mangula). As expected, as new reserves were added to the network there was a gradual increase in the number taxa in the NFR network, but this was found to asymptote for all reserves declared since 2019 (Fig 2C). The cumulative number of endemic taxa was correlated with total taxa and date of declaration of the reserves (Fig 2, $r_s = 0.97$, $p < 0.001$ for all taxon groups). This significant increase is also found in each taxon group separately ($r_s = 0.95$, $p < 0.001$ for each taxon group).

Coverage by NFRs of Tanzanian endemic and threatened species using range map data

Our GIS analysis using IUCN species range data, shows that as the number of NFRs increased, the number of Tanzania endemic vertebrate species covered by reserves also increased, and the number of gap species declined. For the 26 Tanzanian endemic bird species, all species now have part of their range covered by NFRs (Fig 3A). Of the 26 endemic mammals, the number of endemic gap species declined to 5% when all sites were included (Fig 3B). More than 25% of 65 Tanzanian endemic amphibian species ranges are partially covered by the NFRs (corresponding to more than 50% of the species range covered) (Fig 3C). However, amphibians contained the highest proportion of gap species for all sites combined (Figs 3C and 4C). The proportion of 63 endemic reptile gap species declined to 10% when all NFR were added (Fig 3D).

The richness of endemic gap species declined as more NFRs were added to the network. However, the number of poorly covered endemic species increases, as more species are

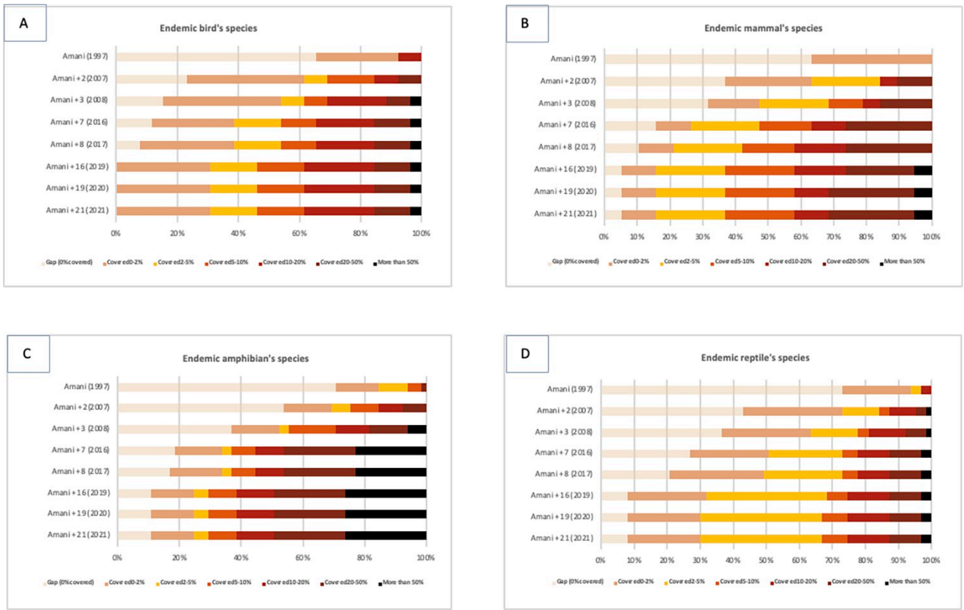

Fig 3. Proportion of Tanzanian endemic vertebrate species ranges covered by NFRs over time. Years when the reserves were gazetted are in parentheses, and the number of reserves declared in each time frame is indicated after "Amani". The light bars refer to the endemic gap species, where 0 per cent of their range is covered. Other colours show the percentage of the endemic species range covered by the reserves. It goes form light brown, between 0 and 2 percent is covered, yellow between 2 to 5 percent covered, orange between 5 and 10 percent, dark red, between 10 and 20 percent, dark brown, between 20 and 50 percent, and black, where more than 50 percent of the species range is covered by the reserves.

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included with only a small part of the range covered by NFRs (Fig 4D–4F). The most notable remaining coverage gaps after all 23 NFRs are included are for endemic reptile species in south-eastern Tanzania (Rondo and Pindiro areas) and central areas of the country. The most notable remaining gap for amphibians was a species of frog (*Hyperolius puncticulatus*), endemic to the island of Unguja (often known as Zanzibar) (Fig 4C).

If all types of protected areas present in Tanzania are included in the analysis, the proportion of gap and poorly covered endemic species declines further (Fig 5). For birds, there are no poorly covered species across all protected areas, compared to 30% poorly covered bird species in NFRs (Table 1). However, for amphibians, almost 7% of endemic amphibian species remain unprotected even when all protected areas are considered, but this is reduced from 11% for just NFRs (Table 1).

Effectiveness of NFR management in achieving conservation goals

The effectiveness of management of the NFR network, as assessed using METT data, increased over time. For the 11 NFRs present in 2015 the METT percent was 55%, 69% in 2017 for 12 NFRs, 69% in 2019 for 17 NFRs assessed, and in 2021 increased to a mean score of 87% for the 17 NFRs evaluated (Fig 6A). Management effectiveness has improved in recent years, and each year was statistically significant different from the other (Friedman's test, chi-squared value = 37.789, df = 3 and p value = 3.133e-08) (Fig 6A). Furthermore, the statistically significant difference of METT scores was the greatest between the year 2015 and 2021, following our initial hypothesis that the average management effectiveness has improved over the years (Pairwise Wilcoxon rank sum test, p value between the year 2015 and 2021 = 2.6e-05). Total tourist numbers (mainly international visitors) increased steadily in most NFRs, from 1,698 tourists in 2016 to 4,097 tourists in 2019 (Fig 6C; rs = 0.95, all p values < 0.0123, N = 17). New data post the COVID-19 Pandemic shows that in 2022 a total of 242,824 tourists visited NFRs

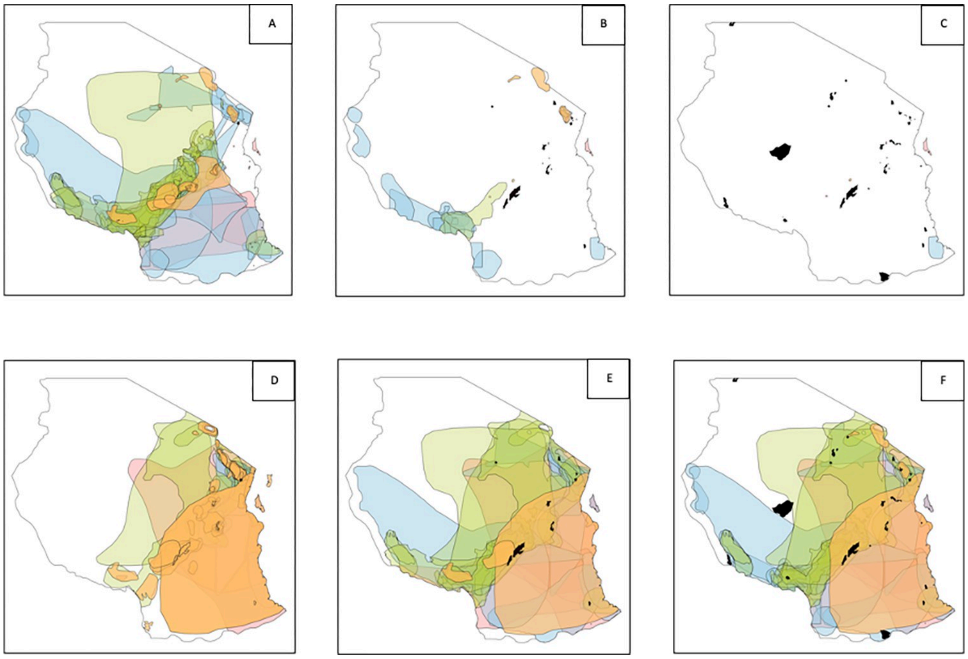

Fig 4. Coverage of the range of Tanzanian endemic 'gap and poorly covered' endemic species in 1997, 2017 and 2022. Coverage of the range of Tanzanian endemic 'gap' species (panels A, B, C) and poorly covered endemic species (panels D, E, F). Panels A and D show endemic species coverage in 1997 when the first reserve was added to the network. Panels B and E show the endemic species coverage in 2017, with 9 reserves in the network. Panels C and F represent the species coverage in 2022, with 19 NFRs and 3 proposed NFRs included in the network. The orange coverage range displays endemic mammal species, blue reptile species, green bird species, and light pink amphibian species. The NFRs are indicated as black polygons.

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and brought in a total of TZS 1.360,940,965 (about 550,000 USD). Income generated decreased slightly from 2017 to 2019, and again in 2021, due the effects of COVID-19 Pandemic (Friedman's test, chi-squared value = 10.478, df = 3 and p value = 0.01491) (Fig 6D). However, the results from the post hoc tests showed no statistical difference of the income generated between the four years (it should be noted that our dataset contained a lot of unavailable data, especially for the more recent sites).

Management capacity, including the number of buildings, transport, and equipment present in each NFR increased between 2015 and 2019, but the number of staff and rangers declined in 2019 (Fig 6E). Only office equipment (corresponding to the number of computers, photocopier scanners, printers, GPS units, solar batteries, and hard drives) increased between 2015 and 2019.

Analysis of forest disturbance transect data from four NFRs in 2001 (before these NFRs were declared—except for Amani) and 2019 shows that numbers of cut trees and cut poles per hectare have declined since the reserves were declared (except for Uluguru and Kilombero NFRs) (Fig 7). The number of live trees was stable (in Uluguru NFR) or decreased (in Kilombero and Mkingu NFRs). In contrast, the number of live poles increased (except for Kilombero NFR). However, none of the results were significant.

Discussion

How well do NFRs capture Tanzanian biodiversity values?

The existing system of NFRs was not formally designed using strategic conservation planning approaches, but was based on a combination of expert knowledge, the framework of

Fig 5. Coverage of the ranges of gap and poorly covered endemic Tanzanian vertebrate species outside all the types of protected areas in the country. Orange shows endemic mammal species, blue reptiles, and green represents endemic amphibian species. There are no gap species of birds. Protected areas are indicated as grey polygons.

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ecoregions, and picking the 'best' remaining sites owned by the current management body–Tanzania Forest Service. We have shown that the first designated NFR sites, in the Eastern Arc Mountains or Coastal Forests ecoregions, have higher biodiversity values in terms of endemic and threatened species than more recently declared sites. The first declared NFRs were known to have globally important biodiversity values [10, 27, 28] and had received more research funding and survey efforts [10, 29]. Many of the newer NFRs have received little or no biodiversity survey attention, so we expect their known values to increase (see e.g., Minziro NFR, [30]). However, the more recent sites also add value and together the NFRs cover the majority of Tanzanian endemic and threatened species (Fig 2B and 2C). However, the current NFR coverage might be moving towards saturation even if our analysis also showed that there are more species endemic to Tanzania that could be capture in the enhanced network.

Table 1. Comparison of the proportion of the range of gap and poorly covered endemic amphibian, reptile, bird, and mammal species, when all protected areas are included compared to only the NFRs.

	All types of protected areas		All NFRs	
	% gap species	**% poorly covered species**	**% gap species**	**% poorly covered species**
Amphibian species	6.52	0	10.77	13.85
Reptile species	6.52	6.52	7.94	22.22
Bird species	0	0	0	30.76
Mammal species	2.17	0	5.26	10.53

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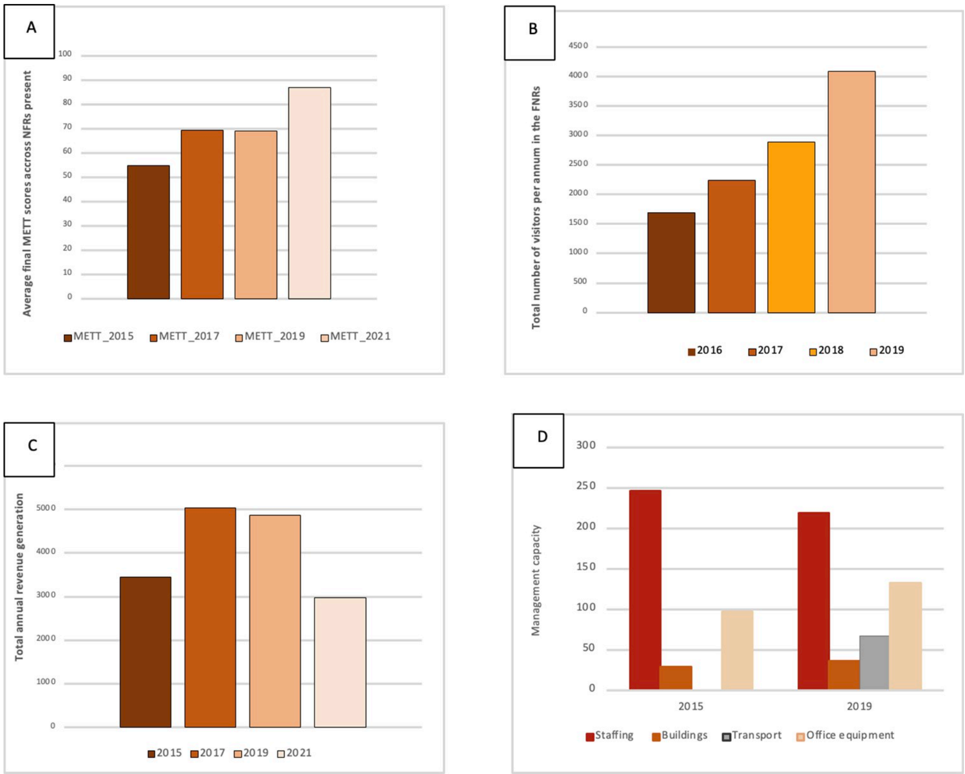

Fig 6. Changes in management scores from 2015–2021 for A) mean METT score across the sites; B) the annual number of tourists in all sites; C) average total revenue generated in the reserves (US dollars); D) sum of the management capacity in 2015 and 2019 comprising staffing (staff and rangers), buildings (offices and ranger posts), transport (vehicles), and office equipment.

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What gaps remain in the NFR network?

The NFR network in Tanzania compliments its world-renowned national parks system that mainly protects non-forest or drier forest habitats, with the exceptions of Udzungwa Mountains, Mahale, and Kilimanjaro. As more NFRs have been created, the proportion of Tanzanian endemic gap species has decreased for all species groups (Fig 3). This is especially true for endemic mammal and bird species, which respectively have 5% and 0% of gap species remaining. The size of the NFRs compared with the large range of some Tanzanian endemic species means that not all species are adequately covered. However, if we also include other protected areas in Tanzania then a greater proportion of ranges are included within protected lands (Fig 5).

The largest number of NFR gap species were from coastal forests (Figs 4 and 5). Even after adding all the types of protected areas in the country, the protection gap in the southeastern region of the country remains. There are a few, relatively small, forest reserves present in that region. There may be an opportunity to expand reserves, such as Rondo NFR or Matapwa Forest Reserve, to cover a part of the ranges of current gap species.

Assessment of the management of the Nature Forest Reserves

Development of the NFRs has been funded by a combination of the Tanzanian government (through the Tanzania Forest Service) and donor funding including the Global Environment Facility, the European Union, the Eastern Arc Mountains Conservation Endowment Fund,

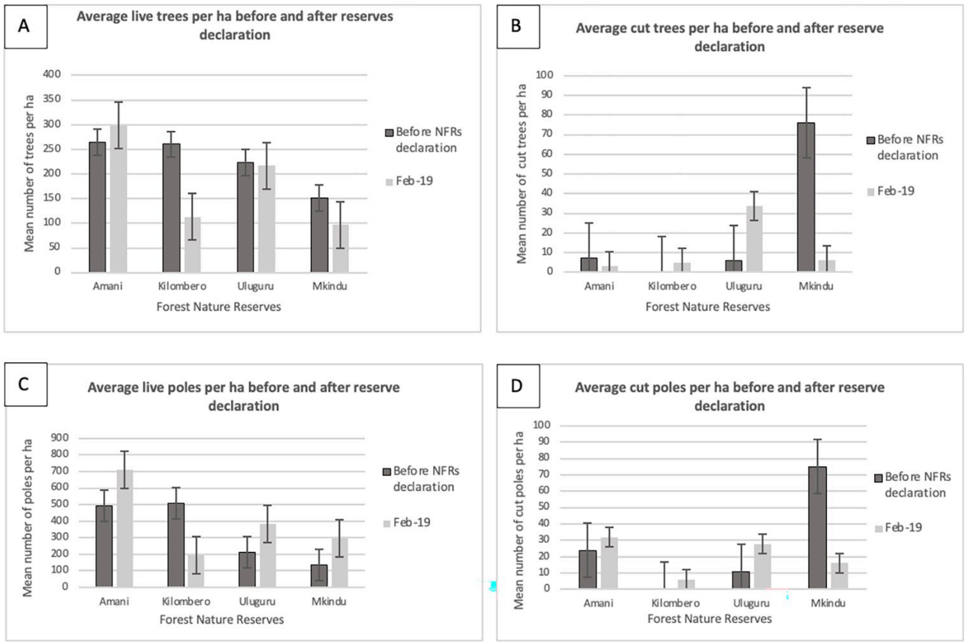

Fig 7. Changes in forest disturbance over time A) average living trees (>20 cm dbh) per ha, B) average cut trees per ha, C) average living poles per ha (<20 cm dbh), and D) average cut poles per ha in Amani, Kilombero, Uluguru, and Mkingu Nature Forest Reserves before reserve declaration and in February 2019.

https://doi.org/10.1371/journal.pone.0281408.g007

and private donors such as the Aage V. Jensen Foundation, as well as local and international NGOs. This support has been critical to creating the current system of NFRs and has led to the observed improvement in management effectiveness (METT scores) from 2015 to 2021 (Fig 6A).

Sustainable funding of the NFR network is critical to long term management and retention of biodiversity values. The number of tourists visiting the sites increased from 2015 to 2019, but their contribution to management costs remained small (Fig 6B and 6C). Reserves such as Chome, Magamba, and Amani, with better infrastructure, accessibility, and proximity to the northern tourism circuit, had the largest growth in tourism numbers. International tourism was heavily impacted by the COVID-19 pandemic. Tourism numbers in the July-June financial year straddling 2022–2023 were greatly improved with over 240,000 tourists visiting the sites and bringing in over 550,000 USD. There remains potential for further improving local and international tourists visiting the NFRs and increasing revenue collection, but the management costs far exceed the income generation so far and care must be taken not to develop facilities that remain underused.

Forest disturbance (using the proxy of cut trees and poles) generally declined from 2001 to 2019 in three of the four studied NFRs, suggesting improved management at these sites. However, this improvement could be due to fewer suitable trees and poles to harvest: indeed, our analyses showed that the live trees density are globally decreasing in three sites of the four sites studied. In addition, evidence of harvesting could be demonstrated with an increase in the density of live poles, as these are coming back in areas where the trees have been cut. The increase in the live poles density was observed in our study in three of the four sites studied. However, this assessment is only available for four reserves, and we have no overview of how this condition may have changed in other sites. Anecdotal evidence suggests that some other reserves are experiencing considerable disturbance, including hunting pressure, that is not captured in the available data. This is the case in Uzungwa Scarp NFR, where decades of poor management of the former Forest Reserve have led to a dramatic loss of wildlife [31, 32], and snares set to catch mammals and birds remain prevalent in 2022 (field researchers pers com). However, since its upgrading to NFR status in 2016, increased management efforts supported by local and international agencies and implemented in close partnership with TFS have led to a considerable reduction in some other threats to this reserve, like tree cutting and farmland encroachment [33]. Hunting pressure and long-term forest encroachment may have extirpated the Udzungwa forest partridge species, *Xenoperdix udzungwensis*, from the Nyumbanitu forest within the Kilombero NFR, where it was still at least until 2016 [34]. On ground insights from all other NFR would likely also contain important stories of success and remaining challenges for NFR management.

Recommendations

Our study has shown that for a more effective coverage and protection of endemic gap species, the TFS should urgently consider further expanding the current network. This can be done both by creating new NFRs and re-gazetting existing sites to higher-level categories of conservation management. This primarily relates to south-eastern Tanzania.

To improve the management effectiveness of NFRs, we suggest that future management plans should emphasise enforcing hunting and logging regulations at all sites, raising awareness of conservation issues in local villages, and providing local people with viable alternatives to activities that impact the forests and their biodiversity.

More biodiversity monitoring, including participatory biodiversity monitoring, should be carried out to compare against management effectiveness scores. This is crucial for current and future sites for the NFRs to be effective in their purposes. Baseline biodiversity surveys are also required for sites with no or little biodiversity data (e.g., Hassama, Itulu Hill, Mwambesi, Pindiro, and Uzigua NFRs). Additional surveys of species/taxa distribution patterns are needed, and future research is needed to detect range shifts due to global warming [35] and to assess how to better prepare and manage future climate risks. Furthermore, life history information, species abundance and human uses should be added to future analyses [36].

For the main management authorities, a realistic, inflation-linked budget is necessary to afford new equipment, facilities and infrastructure, and to maintain patrols in the NFR network. To increase the budget for reserve management, we suggest the following potential revenue streams: capturing revenue from ecosystem services (such as water catchment and carbon stocks [37–39]); soliciting additional investment or donations from philanthropic donors, and increasing the revenue generated from touristic activities [10].

Supporting information

S1 Fig. Painting 1: Fischer's turaco (*Tauraco fischeri*) and white-starred robin (*Pogonocichla stellata*) found in the sub-montane forests of the Nature Reserves in the East Usambara and Uluguru Mountains. Painting 2: The narrowly endemic Udzungwa forest partridge (*Xenoperdix udzungwensis*), and grey-faced sengi (*Rhynchocyon udzungwensis*) encountering each other on the forest floor in the Kilombero Nature Reserve in the Udzungwa Mountains within the Eastern Arc mountains region.
(ZIP)

S1 Table. Online data sources used in this study: Biodiversity (species lists for plants, birds, mammals, reptiles, and amphibians per site); management (revenue generation, tourist numbers, management capacity, and forest disturbance); geospatial data (species

range maps, protected areas, etc.) used for the spatial analysis.
(TIF)

S2 Table. Basic attributes of 21 declared and one proposed Tanzanian Nature Forest Reserves.
(ZIP)

Acknowledgments

We thank the many staff of the Tanzania Forest Service and key donors, including the Global Environment Facility (via UNDP and the World Bank), the Critical Ecosystem Partnership Fund, the European Union, Governments of Norway, Finland and Denmark, and facilitation by several NGOs: WWF, Tanzania Forest Conservation Group, CARE International, IUCN, WCS, BirdLife International, and African Wildlife Foundation. We also want to thank Jon Fjeldså for his two paintings on some of the species occurring in the Nature Forest Reserves in Tanzania.

Dedication: To those who put their heart and soul into creating a network of Tanzanian Nature Reserves and died too early along the way: Alan Rodgers, John Mejissa, Corodius Sawe, Gerald Kamwenda, Peter Sumbi.

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10. Authorship statements for Publication 2

AUTHOR'S STATEMENT PUBLICATION 2

I hereby declare that the article :

"Nature Forest Reserves in Tanzania and their importance for conservation" by Ract, C., Burgess, N.D., Dinesen, L., Sumbi, P., Malugu, I., Latham, J., Anderson, L., **Gereau, R.E.**, Gonçalves de Lima, M., Akida, A., Nashanda, E., Shabani, Z., Tango, S., Mteleka, S., Silayo, D.S., Mwangi, J., Lyatuu, G., Platts, P.J. & Rovero, F. (2024) *PLoS ONE* 19(2): e0281408. Pp. 1-18. https://doi.org/10.1371/journal.pone.0281408

is a part of my PhD thesis. I participated in the conceptualization of the article, compiled the vascular plant data from the Missouri Botanical Garden's Tropicos database enhanced by data from the GBIF Portal and the IUCN Red List, analyzed the floristic composition of each Nature Forest Reserve, and reviewed all aspects of the manuscript relevant to plants. I contributed Online Table S1: Tanzania NFR Plants_data.xlsx. I participated in final revision process.

Roy E. Gereau

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Prof. UAM dr. hab. Justyna Wiland-Szymańska
Supervisor of PhD candidate

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article “**Nature Forest Reserves in Tanzania and their importance for conservation**” by Ract, C., Burgess, N.D., Dinesen, L., Sumbi, P., Malugu, I., Latham, J., Anderson, L., Gereau, R.E., Gonçalves de Lima, M., Akida, A., Nashanda, E., Shabani, Z., Tango, S., Mteleka, S., Silayo, D.S., Mwangi, J., Lyatuu, G., Platts, P.J. & Rovero, F. (2024) PLoS ONE 19(2): e0281408. Pp. 1-18. https://doi.org/10.1371/journal.pone.0281408.

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Contribution	Conceived the paper and did much of the writing, with the final push to get it published by Claire Ract based on her masters project on this issue
Date	9 October 2024
Signature	Neil B

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Date	09/10/2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

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Date	9 October 2024
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AUTHORSHIP CONTRIBUTION STATEMENT

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AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "**Nature Forest Reserves in Tanzania and their importance for conservation**" by Ract, C., Burgess, N.D., Dinesen, L., Sumbi, P., Malugu, I., Latham, J., Anderson, L., Gereau, R.E., Gonçalves de Lima, M., Akida, A., Nashanda, E., Shabani, Z., Tango, S., Mteleka, S., Silayo, D.S., Mwangi, J., Lyatuu, G., Platts, P.J. & Rovero, F. (2024) PLoS ONE 19(2): e0281408. Pp. 1-18. https://doi.org/10.1371/journal.pone.0281408.

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Date	10 October 2024
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11. Publication 3

Linan, A.G., R.E. Gereau, R. Sucher, F.H. Mashimba, B. Bassüner, A. Wyatt & C.E. Edwards. 2024. Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in Karomia gigas. Appl. Pl. Sci. 12(3): e11589: pp. 1-14

Received: 13 October 2023 | Accepted: 23 February 2024

DOI: 10.1002/aps3.11589

Applications in Plant Sciences

APPLICATION ARTICLE

Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas*

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This article is part of the special issue "From Theory to Practice: New Innovations and Their Application in Conservation Biology."

Abstract

Premise: Although ex situ collections of threatened plants are most useful when they contain maximal genetic variation, the conservation and maintenance of genetic diversity in collections are often poorly known. We present a case study using population genomic analyses of an ex situ collection of *Karomia gigas*, a critically endangered tropical tree from Tanzania. Only ~43 individuals are known in two wild populations, and ex situ collections containing 34 individuals were established in two sites from wild-collected seed. The study aimed to understand how much diversity is represented in the collection, analyze the parentage of ex situ individuals, and identify efficient strategies to capture and maintain genetic diversity.

Methods: We genotyped all known individuals using a 2b-RADseq approach, compared genetic diversity in wild populations and ex situ collections, and conducted parentage analysis of the collections.

Results: Wild populations were found to have greater levels of genetic diversity than ex situ populations as measured by number of private alleles, number of polymorphic sites, observed and expected heterozygosity, nucleotide diversity, and allelic richness. In addition, only 32.6% of wild individuals are represented ex situ and many individuals were found to be the product of selfing by a single wild individual.

Discussion: Population genomic analyses provided important insights into the conservation of genetic diversity in *K. gigas*, identifying gaps and inefficiencies, but also highlighting strategies to conserve genetic diversity ex situ. Genomic analyses provide essential information to ensure that collections effectively conserve genetic diversity in threatened tropical trees.

KEYWORDS

botanic garden, conservation genetics, ex situ, genetic diversity, *Karomia*, parentage analysis, Tanzania

Biodiversity is experiencing a global crisis. Although extinction is a natural process that occurs across all branches of life, a recent study found that the current extinction rate in plants is up to 500 times greater than the background rate before 1900 (Humphreys et al., 2019), with the greatest rate found in tropical and subtropical shrubs and trees. Nic Lughadha et al. (2020) found that around 42.8% of plant species are threatened with extinction, primarily due to anthropogenic threats such as habitat loss due to urbanization and commercial activities, direct exploitation, competition with invasive species, and climate change. As a result of these pressures, some plant species have been reduced to just a few individuals, requiring urgent conservation action to prevent their extinction.

Ideally, the conservation of a threatened plant species occurs in its natural habitat with protection and proper management; however, ex situ conservation (e.g., conserving germplasm off-site in a protected area, seed bank, or botanical garden) is a complementary approach commonly used in conjunction with in situ conservation efforts. The primary goals of ex situ conservation are to safeguard

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against the loss of individuals and populations of a threatened species and to provide source material for population augmentations and reintroductions (Cohen et al., 1991; Falk and Holsinger, 1991; Kramer et al., 2011; Guerrant et al., 2014; Center for Plant Conservation, 2019). Ex situ conservation is particularly important for safeguarding species experiencing threats in their native habitat, including widespread habitat conversion, adverse land use practices, or reproductive failure (Pritchard et al., 2012). For critically endangered species with only a small number of remaining individuals, ex situ conservation is critical for safeguarding against catastrophic, irrecoverable losses of these individuals, which represent most or all of the genetic diversity remaining in the species.

Most ex situ plant collections (hereafter referred to as collections) consist of either living plants or banked germplasm, the latter usually consisting of seeds stored in seed banks specifically for conservation purposes (Kramer et al., 2011). However, some species do not reliably produce seeds or have seeds with low viability, in which case propagation through tissue culture or other approaches may be the only way to develop a collection. Furthermore, some seeds do not remain viable using traditional storage techniques (i.e., recalcitrant or exceptional species), including those of many tropical species, and must be germinated shortly after field collection and maintained as living plants in collections. Collections of whole living plants can be useful for captive breeding programs to generate new plants for augmentations and (re)introductions. However, whole living plants are resource-intensive to maintain, and approaches to ensure efficiency in the resources invested in these collections are of considerable interest to the plant conservation community.

It is widely recognized that collections of plants are most valuable when they encompass as much of a species' genetic and geographic variation as possible (Schoen and Brown, 1993; Petit et al., 1998; De Souza et al., 2015). Because genetic diversity is important for maintaining the resiliency and adaptive potential of a species, the goal of ex situ conservation efforts for critically endangered plant species with only a few remaining individuals is often to safeguard all of the remaining genetic diversity (e.g., Mashburn et al., 2023). However, collections may not represent the full genetic diversity for a variety of technical reasons. Often, ex situ conservation efforts occur in response to a crisis, forcing conservationists to collect whatever tissue is available. Conservationists may lack permission or be physically unable to access some individuals (Griffith et al., 2020). Some plants may not produce sufficient seed or seeds may have low germination rates, limiting the ability to represent some individuals in collections when vegetative propagation or tissue culture is not possible. Although some plants that do not produce seeds may contribute to an ex situ collection by serving as pollen donors, the paternal parents of seeds in collections are rarely known, limiting our knowledge of which individuals are represented. Thus, analyses that help clarify parentage of collections can provide important insight into the wild individuals represented ex situ and those that should be targets for additional collection efforts, when possible.

Another concern related to ex situ collections is that if they are founded with only a few individuals, mating within the population can lead to declines in genetic diversity over time due to inbreeding and genetic drift (Lacy, 1987; Willoughby et al., 2015; Foster et al., 2022). This issue has been of particular interest for collections of animals in zoos because they are often founded with only a few individuals and there may be a limited ability to source new individuals from wild populations. Previous studies found that one of the most effective strategies to maintain genetic diversity in a closed, captive animal population is to maintain as large an effective population size as possible by selecting breeding individuals that are the most genetically distant from the rest of the population (Lacy, 2012). This is accomplished by selecting individuals with the lowest mean kinship (Fernandez et al., 2004), which is the probability that an allele sampled from an individual is identical by descent with an allele at the same locus sampled at random from the population (Willoughby et al., 2015). Mean kinship in zoos is frequently assessed and managed across multiple sites as a unit (i.e., metapopulations) through careful pedigree tracking or by employing genome-wide molecular marker data (Weir and Goudet, 2017; Goudet et al., 2018), which are used to coordinate breeding efforts across institutions. Collections of critically endangered plants may be susceptible to declines in genetic diversity due to small founder population sizes, inbreeding, and drift (e.g., Foster et al., 2022; Diaz-Martin et al., 2023), but most do not have established ex situ breeding programs to manage their genetic diversity.

Given the importance of maintaining genetic diversity in collections and the fact that most ex situ conservation efforts of critically endangered plant species are hindered by a lack of insight into how much diversity is conserved, additional work is needed to ensure that collections effectively and efficiently conserve genetic diversity. One important way to do so is to use population genomic approaches to compare the genetic diversity present in wild populations and ex situ collections and assess the parentage of ex situ individuals, which can then be used to develop a strategy to ensure that as much as possible of the wild diversity is conserved (e.g., Diaz-Martin et al., 2023; Mashburn et al., 2023). Population genomic data can also be used to assess mean kinship among individuals in a collection to develop breeding strategies to maintain genetic diversity. However, the use of genomic approaches to conserve and manage genetic variation in collections of critically endangered plants is not commonplace, even though this information is crucial for ensuring that essential genetic diversity is safeguarded against irrecoverable losses.

In this study, we used an ex situ conservation program for *Karomia gigas* (Faden) Verdc. (Lamiaceae) as a case study to explore the use of population genomic approaches to evaluate and manage genetic diversity in a critically endangered, tropical tree species. *Karomia gigas* is a large tree that occurs in two widely separated coastal forest reserves in Tanzania (ca. 100 km apart), where it is threatened by land conversion to agriculture, logging, and charcoal operations. In 2020, only 43 known wild individuals remained in two populations (Litipo and Mitundumbea). Due to the small number of individuals, the threat of habitat loss, and the risk of logging, *K. gigas* was recently listed by the International Union for Conservation of Nature (IUCN) as critically endangered (Mashimba and Shaw, 2022). Given its particularly high risk of extinction, safeguarding all of the remaining genetic diversity of the species is crucial for maintaining its viability. Thus, beginning in 2017, the Tanzania Forest Service (TFS) and Missouri Botanical Garden (MBG) began working together to establish an ex situ collection of *K. gigas*. In 2018, attempts to propagate the species from seed and cuttings resulted in successful seed germination. The seedlings were used to establish two collections: one at MBG in St. Louis, Missouri, USA, containing 28 individuals, and one at TFS in Morogoro, Tanzania, containing six individuals. However, the amount of genetic diversity remaining in the wild, the degree to which the wild diversity is safeguarded in collections, and the optimal strategy to develop and maintain genetic diversity in collections in the future are unknown.

Here, we used a population genomic approach to genotype all known individuals of *K. gigas*. We compared genetic diversity between wild populations and collections, conducted parentage analysis of the individuals in the collections, and analyzed mean kinship within the collections. Our goals were: (1) to understand how much genetic diversity is represented in the collections relative to the wild populations, (2) to analyze the contribution of wild individuals to the parentage of ex situ individuals, and (3) to devise a strategy to conduct genetically informed controlled crosses among ex situ individuals to maintain genetic diversity in the collections. We discuss the implications of the results for devising strategies to conserve maximal genetic diversity in *K. gigas* and to conduct genetically informed breeding to maintain levels of genetic diversity in the collections.

METHODS

Study species

Karomia gigas is a large tropical tree with small purple and white zygomorphic flowers and large, oval, papery fruits (Figure 1). It was first discovered in Kenya in 1977, at which time only one mature tree and one sapling were found. When the species was described (as *Holmskioldia gigas* Faden), Faden (1988) reported that the two original plants had been cut down and speculated that the species may be extinct. Intensive

FIGURE 1 Images of *Karomia gigas* depicting (A) Tanzania Forest Service staff collecting fruits from a mature, wild individual of *K. gigas* for ex situ propagation, (B) flower and flower buds, (C) developing fruits, (D) seeds, and (E) ex situ individuals grown at the Missouri Botanical Garden. Photo credits: Fandey Mashimba (A, C); Rebeca Sucher (B); Andrew Wyatt (D); Christine Edwards (E).

searches in coastal Kenya in the late 1980s and early 1990s failed to locate additional plants, and the species was again reported to be possibly extinct (Verdcourt, 1992; Beentje, 1994). Unexpectedly, a survey conducted by the Frontier-Tanzania Coastal Forest Research Programme discovered a single fruit of *K. gigas* at the edge of Ngarama Forest Reserve in Tanzania, over 600 km away from its type locality, which was verified using the type and one other herbarium specimen from the type locality (Clarke et al., 2011). Additional searches for *K. gigas* identified two populations occurring in seasonally dry tropical forests within Mitundumbea and Litipo Forest Reserves in southern coastal Tanzania (Clarke et al., 2011; Gereau et al., 2022) (Figure 2). In their published IUCN Red List assessment, Mashimba and Shaw (2022) reported only 43 known wild individuals, including 21 mature trees and 22 juveniles (i.e., young trees 5–9 m tall; no wild seedlings have been observed). The calculated area of occupancy of 16 km^2 (using 2 × 2-km grid cells) is probably an overestimate given the small number of individuals in each subpopulation. Litipo Forest Reserve has a surface area of only 10 km^2, whereas Mitundumbea, with its 15 individuals grouped into three geographically distinct clusters, has an area of 87 km^2.

Propagation of ex situ collections

We attempted to propagate *K. gigas* through seed, cuttings, air layering, and tissue culture. Although most attempts were

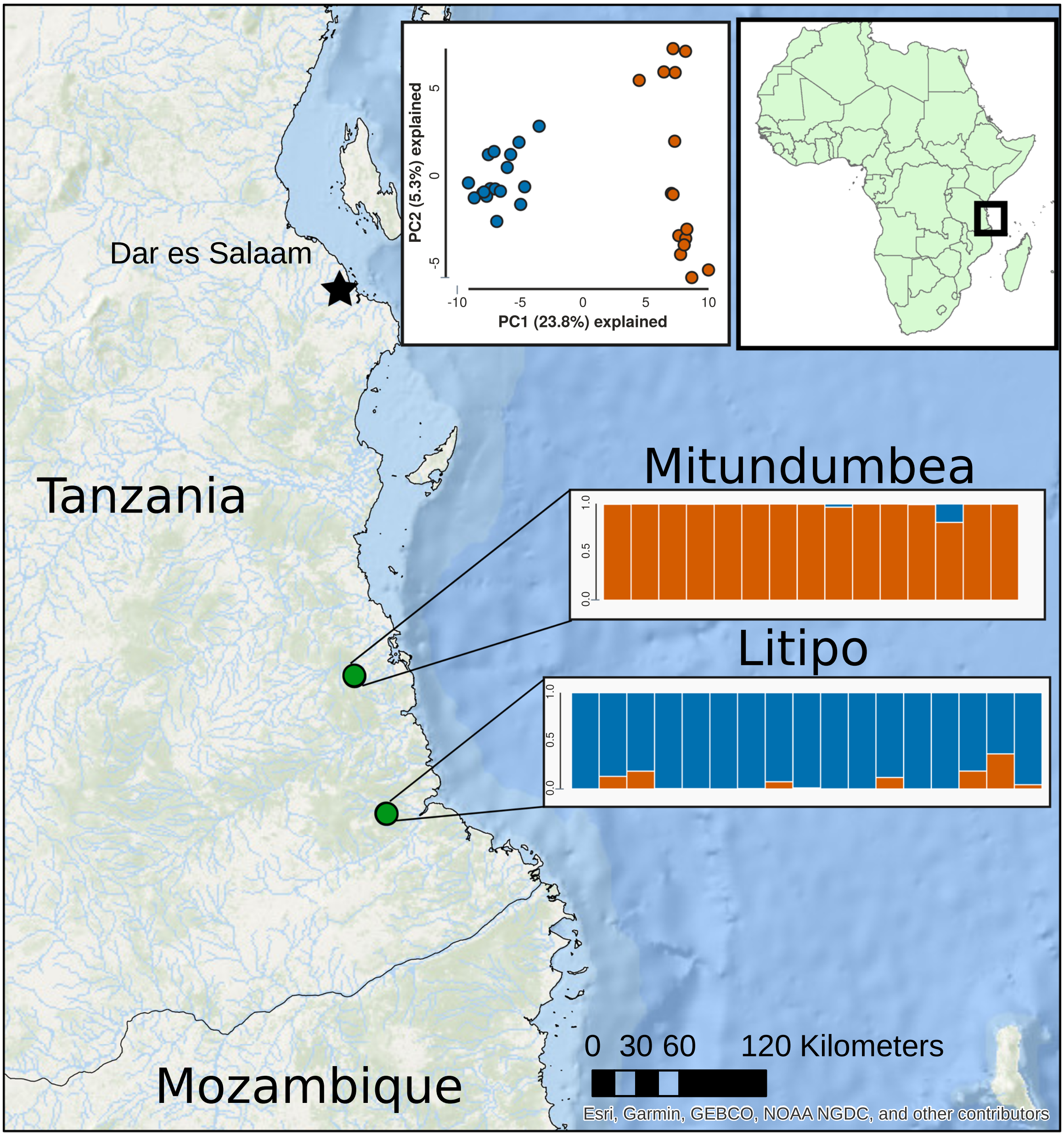

FIGURE 2 Collection localities and genetic structure of wild populations of *Karomia gigas*. Barplots depict admixture proportions inferred by STRUCTURE ($K = 2$). PCA plot of the two wild populations, Litipo (blue) and Mitundumbea (orange).

unsuccessful, propagation through seed achieved some success (Appendix S1; see Supporting Information for detailed treatments). For the MBG collection, fruits of *K. gigas* were collected from six wild maternal individuals found at Litipo and Mitundumbea. Fruits were directly collected from each individual by tree climbers. Of the more than 24,000 seeds received at MBG in 2018, only 133 appeared to be potentially viable; of these, 29 germinated and one individual died post-germination, resulting in 28 individuals at MBG. For the TFS collection, fruits of *K. gigas* were collected from two wild maternal individuals. The number of seeds used for germination attempts at TFS was not recorded but resulted in a collection of six individuals.

Sampling for genetic analyses

We collected leaf tissue for genetic analysis in 2020–2021 from a total of 77 individuals, representing all known living individuals of *K. gigas*. For the wild populations, we collected 28 individuals from Litipo Forest Reserve and 15 from Mitundumbea Forest Reserve in 2020 (Figure 2). Ex situ samples included six individuals planted outside the TFS office in Tanzania and 28 individuals from MBG's collection in the United States (collected in 2021). Each of the ex situ plants in MBG's living collections is associated with the 9-digit accession number of the wild maternal trees from which seeds were collected as documented in MBG's Living Collections Management System (https://www.livingcollections.org). Voucher specimens for the two wild populations are stored at the University of Dar es Salaam herbarium (DSM). All leaf tissue was dried and preserved in silica gel at room temperature until DNA extraction.

DNA extraction, library preparation, and sequencing

DNA extraction and high-throughput DNA sequencing library preparation were conducted in the Conservation Genetics lab at MBG. DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol with an additional sorbitol wash (Doyle and Doyle, 1987; Štorchová et al., 2000). The DNA concentration of each sample was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Libraries were prepared using a 2b-RADseq approach (Wang et al., 2012) to generate high-quality single-nucleotide polymorphism (SNP) data across the genome following the protocol described in Linan et al. (2021). We digested 250–500 ng of DNA with the type-IIb restriction enzyme *Bcg*I (New England Biolabs, Ipswich, Massachusetts, USA), which generates 36-bp DNA fragments. Unique adapters were ligated to samples in each column of a 96-well plate. Ligated DNA samples were amplified using High Fidelity Phusion PCR mix (New England Biolabs) for 14 PCR cycles, while incorporating a uniquely barcoded PCR primer for each row, resulting in 77 uniquely dual-indexed barcoded samples per plate. The final PCR was amplified for 15 cycles, and amplification was checked using agarose gel electrophoresis. The resulting bands were excised and purified using a MinElute Gel Extraction Kit (QIAGEN, Hilden, Germany). The amount of DNA in each excised band was quantified, normalized, and pooled into one sample with a final concentration of 10 nM, which was sequenced at Northwestern University Center for Genetic Medicine on one lane of an Illumina HiSeq 4000 (Illumina, San Diego, California, USA) using 1 × 50-bp reads.

Locus assembly

Raw reads were inspected with FASTQC version 0.11.5 (Andrews, 2010), demultiplexed using a custom script from the Matz lab (https://github.com/z0on/2bRAD_denovo), and quality filtered using FASTX toolkit version 0.0.14 (options: -Q 33 -p 90). Demultiplexed reads were assembled de novo using STACKS version 2.60 (Catchen et al., 2013). Following Paris et al. (2017), we determined optimal STACKS settings for the minimum stack depth parameter (-m) and maximum distance between stacks (-M) using the R package RADstackshelpR version 0.1.0 (DeRaad, 2021). The final parameters were $m = 3$, $M = 1$, and a maximum distance between catalog loci of $n = 3$ with gapped alignments disabled. We removed loci that were absent in >50% of samples along with those with heterozygosity >0.75 to remove potentially paralogous loci (Paris et al., 2017) and retained one random SNP per locus (to avoid linkage among loci). The resulting data set that contained all 77 samples was used to analyze population genetic diversity, parentage, and internal relatedness (IR; see description of analyses below). For STRUCTURE and principal component analysis (PCA; see below), which are sensitive to missing data, we created a reduced data set in which we removed samples with >40% missing data, resulting in the removal of 14 samples, leaving 63 samples.

Genetic diversity of wild and ex situ populations

Genetic diversity was examined by grouping samples in two ways. First, we compared genetic diversity measures between individuals grouped into wild (containing both Litipo and Mitundumbea samples; $n = 43$) and ex situ (containing both MBG and TFS samples; $n = 34$) populations. We then repeated the analysis, grouping individuals into the four populations: Litipo ($n = 28$), Mitundumbea ($n = 15$), MBG ($n = 28$), and TFS ($n = 6$). Using STACKS, we calculated observed heterozygosity (H_o), expected heterozygosity (H_e), nucleotide diversity (π), private alleles, and Wright's inbreeding coefficient (F_{IS}; Wright, 1922) based on the SNP data set, which contains only variable sites. Although nucleotide diversity is traditionally analyzed using entire loci containing both invariant and variant sites, our analyses of nucleotide diversity were based on SNP data and were used to compare genetic diversity among wild and ex situ populations in this study; thus, the estimates contained in the present study should not be used to compare genetic diversity with other taxa. To correct for differences in population sample size when comparing estimates of allelic diversity, we calculated rarified allelic richness (A_R) using HP-Rare version 1.1 (Kalinowski, 2005). Finally, we calculated Tajima's D statistic (Tajima, 1989) for wild *K. gigas* populations to test for signatures of a genetic bottleneck using VCFtools version 0.1.15 (Danecek et al., 2011) as implemented in the R package r2vcftools version 0.0.0.9 (https://rdrr.io/github/nspope/r2vcftools/), with 1000 simulations of allele frequency spectrum under the neutral model to test for significance.

Assessing the genetic representation of wild individuals in the ex situ collection

We performed parentage analysis in Cervus version 3.0.7 to identify the parents of each ex situ individual (Marshall et al., 1998; Kalinowski et al., 2007). All wild individuals were used as candidate parents in a simulation of parentage analysis using the "parent pair, sexes unknown" option in Cervus, with the following settings: 10,000 offspring simulated, 43 candidate parents (wild populations), "Proportion sampled" = 1, "Proportion loci typed" = 0.5, "Minimum typed loci" set to 50% of all loci, and all other parameters set to the defaults. Parentage analysis was conducted using the "Parent pair, sexes unknown" option using default parameters, including "the two most likely parents" for each offspring from the list of candidate parents. This analysis can select the same candidate parent twice if the individual is the result of selfing (or biparental inbreeding). Confidence in parentage assignment was assessed via the likelihood ratio of each candidate parent (taking account of possible typing errors), in the form of a natural log of the likelihood ratio (LOD) score. We also inspected the results to identify samples with a large number of mismatching loci and non-significant tri-loci scores compared to their most likely parents, which may represent individuals in which one parent may not be in the database, potentially indicating undiscovered wild individuals.

To confirm the status of progeny identified as being the product of selfing/biparental inbreeding, we calculated IR, a measure of the proportion of homozygous loci in an individual, using the R function GENHET version 3.1 (Coulon, 2010). Any sample resulting from a selfing event is expected to exhibit a significant increase in homozygosity compared to parental individuals. To test for differences in IR among selfed collections and wild populations, we conducted one-way ANOVA analyses with post-hoc Tukey honest significant difference (HSD) pairwise comparisons. The results of parentage and IR analysis were used to generate lists of wild individuals serving as parents contributing to each population and those not represented in collections. To assess the contribution of the parental genotypes to the collections populations, we: (1) calculated the proportion of each population derived from each pair of parents (i.e., full-sib groups), and (2) calculated the proportion of each population derived from each unique genotype, where a selfed individual is a contribution of 1 from the parental genotype, and an outcrossed individual is a contribution of 0.5 from each parental genotype.

To validate results of parentage analysis and assess how genetic variation is structured within and among wild populations and collections, we analyzed the data using PCA and Bayesian clustering in STRUCTURE version 2.3.4 (Pritchard et al., 2000). For both analyses, we analyzed patterns of genetic structure in two data sets: (1) only wild individuals, and (2) both wild and ex situ individuals. PCA and STRUCTURE were performed and visualized using the analysis toolkit in iPyrad version 0.9.92 (Eaton and Overcast, 2020). In STRUCTURE, samples were assigned to genetic clusters, with assignments to two or more clusters indicating admixture between genetic clusters. We ran five separate runs at each K from 1 to 4 using an admixture model and correlated allele frequencies, with a burn-in of 100,000 generations and a run length of 300,000 generations. We used both delta K and plots of the -Ln likelihood values at each K in STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to estimate the optimal number of clusters in the data set.

To help guide decisions about controlled crosses in the collections, we estimated pairwise kinship (coancestry) between all sampled individuals using $\hat{\beta}_{jj'}$ ("beta"; Weir and Goudet, 2017), which estimates the kinship coefficient between a pair of samples relative to the average kinship of all pairs of samples. The kinship coefficient was calculated using the "beta.dosage" function in the R package HIERFSTAT version 0.5-11 (Goudet, 2005).

RESULTS

Sequencing and locus assembly

Sequencing returned 235,118,486 total reads with a Phred score greater than 33, with each sample averaging 3,014,340 reads (range 133,082 to 8,923,992 reads per sample). The STACKS assembly produced a matrix containing 77 individuals genotyped at 1183 loci with 16.7% missing data. For STRUCTURE and PCA, which are sensitive to missing data, we created a reduced data set in which we removed 14 samples with >40% missing data (11 samples from Litipo, one from TFS, and two from MBG), leaving 63 individuals.

Genetic diversity measures across wild and ex situ populations

When comparing genetic diversity between all wild populations (Litipo + Mitundumbea) and ex situ (MBG + TFS) collections, wild populations displayed greater levels of

TABLE 1 Genetic diversity metrics calculated for each *Karomia gigas* population and for populations combined into "wild" and "ex situ" groups.

Population	Private alleles	A_R	Sites	Polymorphic sites	% Polymorphic loci	H_o	H_e	π	F_{IS}
Litipo	184	1.35	1182	739	62.5	0.157	0.149	0.155	0.008
Mitundumbea	144	1.36	1182	745	63.0	0.160	0.156	0.163	0.018
MBG	78	1.34	1182	757	64.0	0.128	0.136	0.139	0.054
TFS	15	1.28	1177	301	25.6	0.143	0.099	0.123	−0.037
Ex situ (combined)	95	1.61	1182	812	68.7	0.128	0.139	0.142	0.057
Wild (combined)	370	1.73	1182	1087	92.0	0.154	0.167	0.170	0.068

Note: A_R = allelic richness; H_o = observed heterozygosity; H_e = expected heterozygosity; π = nucleotide diversity; F_{IS} = inbreeding coefficient; MBG = Missouri Botanical Garden; TFS = Tanzania Forest Service.

diversity across all metrics (Table 1). Notably, wild populations had a greater number of private alleles (370 vs. 95), polymorphic sites (1087 vs. 812), H_o (0.154 vs. 0.128), H_e (0.167 vs. 0.139), π (0.170 vs. 0.142), and A_R (1.73 vs. 1.61) than collections, respectively. When comparing genetic diversity metrics among populations, the two wild populations had nearly identical values for A_R, polymorphic loci, H_o, H_e, π, and F_{IS}, although Litipo had a greater number of private alleles than Mitundumbea (184 vs. 144; Table 1), possibly due to Litipo's larger population size. MBG's collection had a greater number of polymorphic loci than Litipo or Mitundumbea (757 vs. 739 and 745, respectively), higher F_{IS} values (0.054 vs. 0.008 and 0.018), similar values of A_R (1.34 vs. 1.35 and 1.36), and lower H_o (0.128 vs. 0.157 and 0.160) and H_e (0.136 vs. 0.149 and 0.156) values than Litipo or Mitundumbea, respectively (Table 1). TFS's collection had the lowest diversity across all genetic diversity metrics, likely due to its small number of individuals (six), but also had the lowest inbreeding coefficient (F_{IS}; Table 1). Finally, Tajima's D was 0.15 ($P = 0.004$) for wild populations (Litipo + Mitundumbea), indicating a population bottleneck.

Parentage analysis of ex situ individuals

We successfully assigned parents to 33 of the 34 ex situ individuals; the sole individual for which parentage analysis was unsuccessful had >50% missing data. However, three additional individuals, all from the same accession and mother tree (LC_2018_1457_1_MT1, LC_2018_1457_2_MT1, LC_2018_1457_3_MT1), had non-significant LOD scores, indicating lower confidence in the inference of their parents (Table 2). Because parentage was not confidently assigned, we removed these individuals from all subsequent analyses. Of the 30 individuals with confident parentage assignments, 29 were inferred to be the result of reproduction within the same population and only one individual was inferred to be a cross between wild populations (Table 2, Figure 3), indicating little gene flow between the wild populations.

Results of parentage analysis showed that a large proportion of individuals in the collection were the result of self-fertilization. Of the 30 individuals with confident matches, parentage analysis indicated that 16 ex situ individuals (53.3%) were the result of outcrossing, whereas 14 (46.7%) were the result of self-fertilization (selfed). Thirteen of the 14 selfed ex situ individuals occurred in the MBG population and originated from seed collected from one individual at Mitundumbea (MT03), whereas only one TFS individual was produced via selfing and originated from Litipo (Table 2). To validate the results of parentage, we compared the IR of individuals that were found to be selfed to that of wild individuals and outcrossed individuals in the collection. As expected, the average IR of selfed individuals (average −0.078) was significantly greater (i.e., more homozygous) than that of wild individuals (average −0.167) (Table 2; Appendices S2, S3).

Parentage analyses showed that only a small proportion of wild individuals served as parents and that one parent contributed disproportionately to each of the two collections. In the 30 ex situ individuals with confident parentage matches, only 14 of the 43 wild individuals (32.6%) served as parents, whereas 29 (67.4%) had no offspring represented in the collections (Appendix S2). Of the 14 parents, 10 occur in Litipo and four occur in Mitundumbea (Figure 3). In the MBG collection, individuals originated from parents in both Litipo and Mitundumbea (Table 2, Figure 3). Eight wild genotypes served as parents to the 24 MBG ex situ individuals, forming 11 unique full-sib groups (Figure 3). In MBG, the majority of individuals (13 of 24) were derived from selfing by MT03, such that genotype MT03 was by far the most common parent, serving as a parent for 58% of the MBG population (Figure 3). Based on the maternal individuals identified by parentage analysis, the MBG ex situ collection likely originated from just four maternal trees, even though collection records stated that the seeds originated from six. The TFS population originated only from parents in Litipo. Five wild genotypes served as a parent to the six TFS ex situ individuals, forming five unique full-sib groups (Figure 3). However, genotype LT01 was over-represented, serving as a parent in 50% of the TFS population (Figure 3).

We next assessed the extent to which the parent reported in collection records corresponded to the results of parentage

TABLE 2 Results of parentage analysis for individuals in the ex situ collections at Missouri Botanical Garden (MBG) and the Tanzania Forest Service (TFS), along with internal relatedness (IR) measures.

Sample ID[a]	Population	Inferred Parent 1	Inferred Parent 2	Fertilization method	Trio loci compared[b]	Trio loci mismatching[b]	Trio LOD score[c]	IR
LC_2018_1449_LT1	MBG	LT1	LT21	outcrossed	1580	31	97.6*	−0.127
LC_2018_1450_1_LT7	MBG	LT1	LT6	outcrossed	444	12	13.7*	0.007
LC_2018_1450_2_LT7	MBG	LT1	LT8	outcrossed	1544	26	84.9*	−0.007
LC_2018_1450_3_LT7	MBG	LT1	LT21	outcrossed	1425	17	107.8*	−0.176
LC_2018_1452_3_MT4	MBG	MT3	MT8	outcrossed	1641	43	25.2*	−0.078
LC_2018_1452_4_MT4	MBG	MT3	MT3	selfed	1426	33	90.9*	−0.039
LC_2018_1452_5_MT4	MBG	MT3	MT3	selfed	1399	40	68.4*	−0.136
LC_2018_1452_6_MT4	MBG	MT3	MT3	selfed	1517	45	75.3*	−0.026
LC_2018_1452_7_MT4	MBG	MT3	MT3	selfed	1371	30	126.4*	−0.044
LC_2018_1452_8_MT4	MBG	MT3	MT3	selfed	1473	40	78.6*	−0.089
LC_2018_1452_9_MT4	MBG	MT3	MT3	selfed	1447	34	134.0*	−0.107
LC_2018_1452_10_MT4	MBG	MT3	MT3	selfed	1476	42	71.2*8	−0.24
LC_2018_1452_11_MT4	MBG	MT3	MT3	selfed	1355	41	90.6*	−0.013
LC_2018_1452_12_MT4	MBG	MT3	MT3	selfed	1331	30	104.1*	−0.168
LC_2018_1454_1_MT2	MBG	MT2	MT6	outcrossed	1629	36	125.5*	−0.067
LC_2018_1454_2_MT2	MBG	LT18	MT2	outcrossed	1471	27	36.0*	−0.112
LC_2018_1454_3_MT2	MBG	MT2	MT8	outcrossed	1668	52	3.7*	−0.182
LC_2018_1455_1_MT1	MBG	LT1	LT17	outcrossed	1255	18	116.0*	−0.215
LC_2018_1455_2_MT1	MBG	LT1	LT14	outcrossed	847	15	36.1*	−0.076
LC_2018_1456_1_MT3	MBG	MT3	MT3	selfed	1146	27	99.9*	0.021
LC_2018_1456_2_MT3	MBG	MT2	MT3	outcrossed	1488	32	35.2*	−0.07
LC_2018_1457_1_MT1	MBG	MT1	MT9	outcrossed	1392	51	−24.5	−0.208
LC_2018_1457_2_MT1	MBG	LT18	MT1	outcrossed	1307	39	−81.3	−0.221
LC_2018_1457_3_MT1	MBG	LT18	MT1	outcrossed	1202	35	−57.9	−0.202
LC_2018_1458_1_MT3	MBG	MT3	MT3	selfed	1436	29	129.2*	−0.067
LC_2018_1458_2_MT3	MBG	MT3	MT3	selfed	1258	40	18.4*	−0.038
LC_2018_1458_3_MT3	MBG	MT3	MT3	selfed	1040	31	38.9*	−0.096
SD57_FM46	TFS	LT1	LT2	outcrossed	1299	44	22.1*	−0.173
SD58_FM47	TFS	LT1	LT20	outcrossed	770	15	42.2*	−0.018
SD59_FM46	TFS	LT2	LT27	outcrossed	1384	39	49.3*	−0.124
SD60_FM47	TFS	LT1	LT1	selfed	1125	22	124.3*	−0.045
SD61_FM48	TFS	LT1	LT6	outcrossed	555	23	18.4*	−0.043
SD77_FM48	TFS	LT1	LT6	outcrossed	933	17	86.3*	−0.058

Note: LOD = natural log of the likelihood ratio.

[a] Sample LC_2018_1452_1_MT4 was not genotyped at enough loci to infer parents and was excluded from parentage analysis. Individuals that did not show high confidence in parentage analysis are indicated in italics.

[b] “Trio loci compared” are the number of shared loci that were analyzed between the sample (Sample ID) and inferred parents, while “Trio loci mismatching” are number of mismatches in loci between inferred parents and offspring (Sample ID).

[c] Significant LOD scores are indicated by an asterisk.

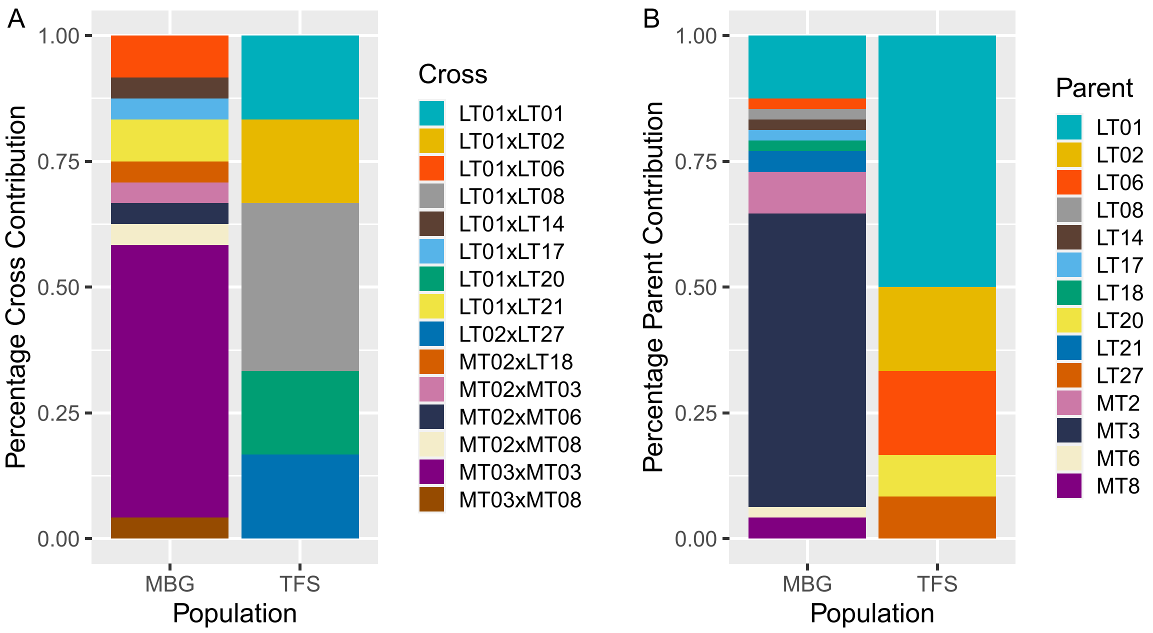

FIGURE 3 Genotypic and parental contribution to the ex situ collection. The relative contribution of (A) each full-sib cross and (B) each parent to the Missouri Botanical Garden (MBG) and Tanzania Forest Service (TFS) ex situ populations.

analysis for the ex situ individuals from MBG, with the assumption that at least one of the genetically inferred parents should match the maternal individual reported in collection records. Of the 24 individuals in the collection at MBG for which we were able to assign parentage, 15 showed a mismatch between the recorded maternal parent in collection records and the parents identified through parentage analysis. Thirteen of the 15 mismatches correctly identified the population of origin but misidentified the maternal individual that produced the seeds within the population; for example, seeds recorded as originating from MT4 were likely collected from MT3 based on parentage analysis. Both the source population and the maternal plant were misidentified for two trees (LC_2018_1455_1_MT1 and LC_2018_1455_2_MT1) that were recorded as originating from Mitundumbea but for which both of the most likely parents occur in Litipo.

Analyses of genetic structure

The results of the STRUCTURE analysis for the wild populations (Litipo and Mitundumbea) suggested an optimal K of 2 based on both the Evanno method (ΔK) and the K value at which log likelihood values ($\ln \Pr[X|K]$) reached a plateau (Appendix S4). The two populations each formed distinct genetic clusters both in STRUCTURE and in PCA, with little admixture inferred between them, although the Litipo population contained a few individuals that showed a small amount of ancestry from the Mitundumbea genetic cluster (Figure 2). In the PCA, PC1 explained 23.8% of the variation and divided samples into two distinct groups corresponding to the Litipo and Mitundumbea populations. PC2, which explained 5.3% of the variation, separated Mitundumbea into two groups (Figure 2).

The STRUCTURE analysis including both wild and ex situ individuals (Figure 4) also showed $K = 2$ as the optimal value of K, with wild individuals grouped by population of origin as described above. The placement of ex situ individuals into STRUCTURE clusters generally agreed with the results of parentage analysis. Ex situ individuals clustered with one of the two wild populations, indicating their origin; the MBG population had individuals that clustered with both wild populations, whereas the TFS population clustered only with Litipo individuals. The assignment of ex situ individuals to source populations largely agreed with collection records except for two individuals at MBG in which the source population was likely mislabeled (LC_2018_1455_1_MT1 and LC_2018_1455_2_MT1). The placement of ex situ individuals in the PCA analysis also corresponded closely with the results of parentage analysis; the ex situ individuals identified as being the result of self-fertilization were placed in close proximity to the parent identified by parentage analysis (e.g., the tight group of ex situ individuals clustering with one wild individual in the lower left corner of Figure 4B), whereas outcrossed individuals were generally placed in an intermediate position between the two parents identified by parentage analysis (e.g., the ex situ individuals in the center-left of Figure 4B).

Analysis of relatedness for captive breeding

Finally, we analyzed kinship coefficients, as measured by beta, between pairs of samples to generate strategies for a captive breeding strategy to cross the most genetically

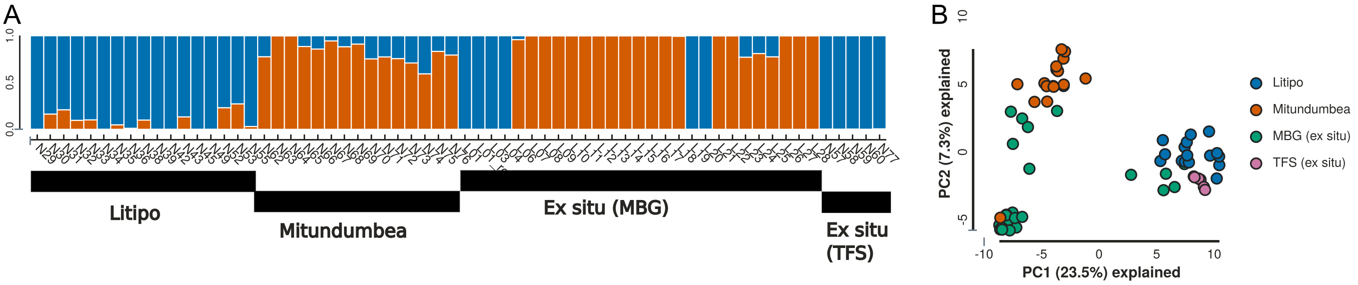

FIGURE 4 Analysis of genetic structure in *Karomia gigas* wild and ex situ populations. (A) STRUCTURE plot ($K = 2$), organized by population. (B) PCA plot depicting the four populations assessed. Note that the large cluster of individuals in the extreme lower left corner of the PCA shows the numerous selfed offspring in MBG collections that originated from a wild individual from Mitundumbea.

divergent individuals to maintain genetic diversity. As expected, the mean kinship coefficients within each of the four populations (MBG, TFS, Mitundumbea, and Litipo) showed similarly positive values ranging from 0.417 (TFS) to 0.483 (MBG), whereas comparisons between individuals from different populations were lower, reflecting lower kinship (Appendix S5). When comparing individuals within the MBG population, we found the lowest pairwise kinship coefficients between individuals originating from different wild populations (−0.2 to 0.08), indicating low kinship.

DISCUSSION

Here, we used the ex situ conservation program for *Karomia gigas* as a case study to evaluate the utility of population genomic assessments for managing the genetic diversity in collections of critically endangered tropical trees. To identify inefficiencies in collections and pinpoint priorities for increasing the ex situ representation of wild individuals, we compared genetic diversity between wild populations and ex situ collections and conducted parentage analysis to understand the contribution of wild individuals to the ex situ collection. We also analyzed kinship among individuals in collections to develop a captive breeding program. The study revealed insights into the genetic composition of an ex situ collection, with important implications for ensuring that extant genetic diversity is conserved and maintained in ex situ collections through a captive breeding program.

One goal of our study was to understand how much genetic diversity is represented in the collections of *K. gigas* relative to wild populations. The wild populations showed a signature of a genetic bottleneck, which was expected given the large reduction in the species' range. The two wild populations exhibited comparable levels of diversity despite differences in population size, but the two collections exhibited lower diversity than either wild population (Table 1). When comparing all wild vs. all ex situ individuals, the wild populations displayed slightly higher levels of genetic diversity than the collections, as has been reported in previous studies (Hoban et al., 2020; Diaz-Martin et al., 2023; Mashburn et al., 2023). This is unsurprising given that the collections of *K. gigas* were established from seed collected from only a few wild individuals. However, despite having lower diversity, we found that the collections conserved about 75% of the polymorphic loci found in the wild population (Table 1), suggesting that a fair amount of the wild genetic diversity is represented in the collection as a whole. Two factors that have likely facilitated the maintenance of genetic diversity in the ex situ conservation efforts are that both wild populations are represented in the collections and that more than 50% of the individuals were produced from outcrossing. Interestingly, another recent study also showed only a small reduction in genetic diversity in a collection of an open-pollinated tropical tree established from seed (Diaz-Martin et al., 2023). These results suggest that establishing collections from seed can be an effective way to conserve a moderate amount of the genetic diversity of open-pollinated tropical trees, even when they are established from a subset of wild individuals.

For critically endangered species, however, the goal for ex situ collections is often to represent all of the genetic diversity found in known wild individuals; we therefore conducted parentage analysis to identify which wild individuals contributed to the collection. Parentage analysis revealed that individuals from both Litipo and Mitundumbea (wild populations) are currently represented in collections, but only a small subset (32.6%) of the total known wild individuals of *K. gigas* are represented ex situ. The small proportion of wild individuals represented in collections is likely due to issues such as a lack of reproductive maturity for some individuals (which represents ca. 50% of the wild population) and difficulty collecting fruits from a large canopy tree (Figure 1). To increase the representation of wild individuals and increase genetic diversity in collections, we recommend prioritizing for additional seed collection efforts any mature individuals that have not previously served as a parent for the collections, as well as those with the lowest kinship coefficients with the existing parents of the collections (Appendix S5). Monitoring of the wild juvenile trees for reproductive maturity will also be necessary so that their seeds can be added to the ex situ collections as they begin to produce fruit.

Because a collection of large tropical trees is difficult to maintain in most botanical gardens due to space constraints, and particularly so when the botanical garden is located in a temperate region and the collection must be maintained in greenhouses, we investigated how to maximize genetic diversity in the most efficient way possible in *K. gigas*. Parentage analysis in *K. gigas* revealed a disproportionately large contribution of some wild parents to the ex situ collection; for example, the majority of individuals in the MBG population (13 of 24) were produced through selfing by MT3, and one individual in TFS was derived from selfing by LT1. Because MT3 and LT1 were also parents to other outcrossed individuals in the collections, these selfed individuals add nothing in terms of representing wild parents or adding unique genetic diversity, revealing some inefficiency in the collections. Given the few individuals remaining in the species, these selfed individuals are still valuable, but some could be distributed to other botanical gardens located in tropical areas where they would be easier to maintain, which would also serve as an additional safeguard against the extinction of the species. Furthermore, over the long term, given the large size of *K. gigas* trees, we will likely need to use a metacollection strategy paired with tissue culture to maintain the genetic diversity of *K gigas* in ex situ collections. These results illustrate how genetic analysis can help identify inefficiencies in an ex situ collection, potentially saving space, time, and money that could be used to improve conservation capacity. Although previous studies employed a resampling analysis to understand whether a subset of individuals in collections can capture maximal wild genetic diversity in the most efficient way possible (i.e., following Namoff et al., 2010; Griffith et al., 2015, 2021; Wei and Jiang, 2020), this is not currently needed in *K. gigas* given the small number of individuals currently in the collections. However, such analyses may be necessary in the future if additional ex situ individuals are added to the collection.

Another interesting result is the possibility that at least one unknown mature tree may have contributed to the ex situ collection. Parentage could not be confidently assigned to three individuals at MBG, all originating from the same maternal individual (MT1) and fruit. The genetic mismatch between these individuals and their most likely parents suggests that one parent may be an unknown or unsampled wild individual. Supporting this hypothesis is the relatively large number of private alleles in the MBG population given its status as a collection (Table 1), which may be attributable to the unique alleles found in the collection that were not sampled in the wild. Additional searches are necessary to identify additional wild individuals of *K. gigas*, illustrating how parentage analysis of collections can provide unexpected insights into wild populations, such as identifying the possible existence of unknown wild individuals.

Another goal of the study was to determine how to conduct genetically informed controlled crosses among ex situ individuals to maximize genetic diversity in subsequent generations. A recent study found that because trees take a long time to reach reproductive maturity and individuals in collections may not become reproductively mature at the same time, open pollination in collections of slow-growing, self-compatible tropical trees can lead to high rates of selfing, causing dramatic declines in genetic diversity and a high risk of inbreeding depression in the next generation (i.e., the captive-born generation; Diaz-Martin et al., 2023). The collection of *K. gigas* currently contains many individuals produced by selfing and many individuals that are half or full sibs (Figure 3); thus, allowing passive, open pollination would likely lead to high inbreeding and declines in genetic diversity in subsequent generations, as was found previously in ex situ collections of other tropical plants (Foster et al., 2022; Diaz-Martin et al., 2023). Careful planning of suitable crosses is therefore necessary to avoid inbreeding. We calculated pairwise kinship among all *K. gigas* individuals (Appendix S5); as ex situ individuals become reproductively mature, they will be crossed with those with the lowest kinship to help maintain genetic diversity and minimize inbreeding. At MBG, captive breeding efforts have already begun and, so far, have involved crossing individuals originating from different wild populations. For TFS, whose collection originated only from Litipo, captive breeding may include hand pollinations with pollen collected from individuals at Mitundumbea. Given the limited gene flow between wild populations, any offspring resulting from these crosses could be used for augmentations to increase genetic diversity in natural populations ("assisted gene flow"; Sgrò et al., 2011; Aitken and Whitlock, 2013).

However, one concern with the approach of minimizing kinship between individuals is that crossing individuals from different source populations could result in outbreeding depression, which can occur when two locally adapted individuals cross and produce offspring that are poorly adapted to both parental environments. However, trees in general are predominantly outcrossing and have several features that facilitate high rates of gene flow (Petit and Hampe, 2006), making outbreeding depression somewhat unlikely. Regardless, it will be useful in the future to conduct crosses between individuals with the lowest kinship originating both from the same source population and from different source populations to see if either shows lower fitness. Monitoring the fitness of offspring derived from all of the types of crosses will be important to help fine-tune captive breeding and reintroduction efforts in the future.

Finally, results from parentage analysis highlight why using provenance alone (without genetic analysis) may be problematic for devising a captive breeding strategy for plants. In the present study, we found a mismatch between the recorded maternal individual and the parents identified using parentage analysis for a significant proportion of the collection. One of the main causes for these mismatches likely arose due to issues with relocating individuals between collecting trips to sample seeds and DNA. When the seed was collected in 2018, the locations of the individuals from which seeds were collected were recorded with a low-resolution GPS, but individuals were not physically tagged in the field. When researchers returned to collect DNA samples in 2020, the low resolution of the GPS coordinates led to difficulty in understanding which tree corresponded to the previous GPS points, such that researchers did their best to assign a name to each tree while sampling. In 2020, the GPS coordinates were re-recorded along with the inferred identity of the tree. However, our results suggest that the identity of several individuals was misassigned in 2020. Because the GPS coordinates taken in 2020 for DNA allow us to relocate each tree and the parentage results allow us to identify which wild individuals gave rise to the collection, we will update the parents recorded in our collections management system to match those identified by parentage analysis. Generally, however, these issues with provenance data are not uncommon (e.g., Diaz-Martin et al., 2023) and highlight why genetic analysis is so important for informing conservation efforts of critically endangered species.

Furthermore, even in cases where the maternal parent is accurately identified, we recommend conducting parentage analysis to devise captive breeding strategies for ex situ collections because the pollen donor is usually unknown, such that it is unknown whether ex situ individuals are the result of selfing or outcrossing (Diaz-Martin et al., 2023). Because making crosses based on erroneous or incomplete source data could result in inbreeding, we therefore advocate the use of marker-based kinship estimators to inform crosses (Goudet et al., 2018), which provides all necessary information to ensure that captive breeding efforts in collections of critically endangered trees maintain genetic diversity.

CONCLUSIONS

This case study provides important insights into why population genomics and parentage analysis are important for managing genetic diversity in collections of long-lived tropical trees. Parentage analysis based on population genomic data can help identify individuals that are not currently represented ex situ, and therefore become targets for future seed collection efforts. It may also identify individuals with a parent not yet identified in the wild, providing information about whether searches for additional wild individuals may be necessary. Parentage analysis can also identify individuals that are the product of selfing or those that represent the same full-sib groups, which could be used to streamline collections, improving the efficiency of ex situ conservation efforts. Finally, the information provided by population genomic data regarding both the parentage and the kinship of individuals in a collection can provide important insights into the most suitable crosses to make in a captive breeding program to maintain genetic diversity. Because of the small number of *K. gigas* individuals, our genomic analysis was not especially expensive, but the genetic approach used here significantly improves our ability to conserve genetic diversity in this critically endangered species; we therefore recommend its use for improving the efficiency and effectiveness of conserving genetic diversity in ex situ conservation programs for a wide range of critically endangered plant species.

AUTHOR CONTRIBUTIONS

C.E.E., A.W., R.S., F.H.M., and R.E.G. planned and designed the research. F.H.M., R.S., A.W., and R.E.G. developed the ex situ collection. B.B. generated the genomic data, and A.G.L. and C.E.E. analyzed the data and wrote the first draft. All authors edited and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Botanic Gardens Conservation International (BGCI) and Kirsty Shaw for organizing initial fieldwork; the staff of the Tanzania Forestry Service (TFS), including Lenin Festo, for facilitating the Material Transfer Agreement between Tanzania and the Missouri Botanical Garden (MBG); Yahya Abeid for assistance in fieldwork; and Mzee Jangwa for monitoring the phenology of individuals in the Mitundumbea population. The Conservation Genetics Program at MBG is supported by private donations from Phillip and Sima Needleman, Stephen and Camilla Brauer, and the Bellwether Foundation.

DATA AVAILABILITY STATEMENT

Data are available on the National Center for Biotechnology Information (NCBI)'s Sequence Read Archive under BioProject ID PRJNA1027492.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Protocols used for seed germination of *Karomia gigas*.

Appendix S2. List of each individual included in the study, including its origin, sample ID, whether missing data exceeded the 40% threshold, whether the individual served as a parent in the ex situ collection, and its internal relatedness.

Appendix S3. Comparison of internal relatedness (IR) values between selfed and outcrossed collections and wild populations as inferred by parentage analyses. Boxplots with different letters denote statistically significant differences from one another based on Tukey's HSD comparison of means ($\alpha = 0.05$).

Appendix S4. Analysis of K estimation, showing the delta K (blue) and the probability of the data (red).

Appendix S5. Pairwise kinship matrix of all individuals in the study as measured by beta, calculated using the "beta.dosage" function in the R package HIERFSTAT v.0.5-11.

How to cite this article: Linan, A. G., R. E. Gereau, R. Sucher, F. H. Mashimba, B. Bassuner, A. Wyatt, and C. E. Edwards. 2024. Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas*. *Applications in Plant Sciences* 12(3): e11589. https://doi.org/10.1002/aps3.11589

12. Authorship statements for Publication 3

AUTHOR'S STATEMENT PUBLICATION 3

I hereby declare that the article:

"Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas*" by Linan, A.G., **Gereau, R.E.**, Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14

is a part of my PhD thesis. I participated in the original fieldwork, helped to negotiate the material transfer agreement that authorized the shipment of seeds of this rare species from Tanzania to the US for propagation and research, and provided logistical and communication support for collaborative efforts between the American and Tanzanian members of the project. I participated in the conceptualization of the article and contributed to writing the manuscript, particularly the sections about the morphology of the species, its discovery and taxonomic history, and the two small protected areas where it grows. I participated in the fianl revision process and response to reviewers' comments.

Roy E. Gereau

Roy E. Gereau

JWiland. Szymańska

Prof. UAM dr. hab. Justyna Wiland-Szymańska
Supervisor of PhD candidate

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article “**Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas***” by Linan, A.G., Gereau, R.E., Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14.

Co-Author Name	**Christine E. Edwards**
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Contribution	Genetic study experimental design, supervision of genetic work, genetic data analysis, writing of initial draft, manuscript revisons
Date	09/10/24
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "**Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas***" by Linan, A.G., Gereau, R.E., Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14.

Co-Author Name	**Alexander G. Linan**
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Contribution	Conducted analyses of genomic data (PCA, STRUCTURE, diversity metrics, etc.), generated figures, and led the writing of the manuscript.
Date	October 9, 2024
Signature	Alexander Linan

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "**Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas***" by Linan, A.G., Gereau, R.E., Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14.

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Affiliation	Tanzania Forest Service Agency, Directorate of Tree Seed Production, Misitu House 21 JPM Street P.O. Box 2228 **Itega, Dodoma,** Tanzania
Contribution	I hereby declare that the article indicated above: • Has been jointly written by the aforementioned authors above. I participated in the original sample collection fieldwork, curation of samples, filling of forms and all necessary credentials, coordinated the signing of the material transfer agreement that authorized the shipment of seeds of this rare species from Tanzania to the US for propagation and research and acted as a focal person for providing logistical and communication support for collaborative efforts between the Tanzanian and American and members of the project. • I contributed to writing the manuscript, the ecology of the tree, sample collection methods, seed biology and germination protocols and general perspectives of botanical survey.
Date	9/10/2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the work in the article "**Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas***" by Linan, A.G., Gereau, R.E., Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14, of which I am a co-author, has been included in the doctoral thesis of Roy E. Gereau

Co-Author Name	**Rebecca Sucher**
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Date	9/10/2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am aware that the work in the article "**Capturing and managing genetic diversity in ex situ collections of threatened tropical trees: A case study in *Karomia gigas***" by Linan, A.G., Gereau, R.E., Sucher, R., Mashimba, F.H., Bassüner, B., Wyatt, A. & Edwards, C.E. (2024) *Applications in Plant Sciences* 12(3): e11589: pp. 1-14, of which I am a co-author, has been included in the doctoral thesis of Roy E. Gereau

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Contribution	Conceptual and editing
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13. Publication 4

Gavin-Smyth, N. & R.E. Gereau. 2022. Two new species of Impatiens (Balsaminaceae) from the Eastern Arc Mountains of Tanzania. Novon 30: 122-127.

Two New Species of *Impatiens* (Balsaminaceae) from the Eastern Arc Mountains of Tanzania

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Abstract. We describe and illustrate the new species *Impatiens butu* Gavin-Sm. (Balsaminaceae) from the South Pare Mountains and *I. ndovu* Gavin-Sm. from the Nguru Mountains of Tanzania and compare them with morphologically similar and sympatric species of *Impatiens* L. Both localities are within the Eastern Arc Mountains of Kenya and Tanzania, a well-known center of plant species richness and high endemism.

Key words: Balsaminaceae, Eastern Arc Mountains, *Impatiens*, Nguru Mountains, South Pare Mountains, Tanzania.

Impatiens L. (Balsaminaceae) is one of the world's largest plant genera, containing at least 1200 species (Yuan et al., 2004; Janssens, 2009; Fischer et al., 2021). The family Balsaminaceae is characterized by zygomorphic flowers with a modified lower sepal that is typically tapered or constricted into a nectary-tipped spur. The unmodified lateral sepals are usually two, less typically four, and the five petals are differentiated into a single dorsal petal and four lateral petals. The five stamens are connate into a ring surrounding the stigma and fall off in a single piece before the stigma is receptive. *Impatiens* is further distinguished within the family by the explosive fusiform capsules. *Impatiens* species have great diversity in morphology and habit: extensive variation in floral form including lower sepals with poorly defined or no spurs, and species that are terrestrial herbs, epiphytes, or woody shrubs. The genus is mainly distributed in tropical and subtropical Asia, Africa, and Madagascar, with a few species in temperate North America and Eurasia.

About 130 *Impatiens* species occur in tropical Africa (Grey-Wilson, 1980, 1982; Fischer et al., 2021). At least 27 species (including the two species described here and one more yet to be described) are endemic to the Eastern Arc Mountains of Tanzania and Kenya (Burgess et al., 2007; Platts et al., 2011), and more undescribed species of this genus are expected to be found in this species-rich region.

1. **Impatiens butu** Gavin-Sm., sp. nov. TYPE: Tanzania. Kilimanjaro Region: Same District, Chome Forest Reserve, Gonja Ward, SW of Kanza Village in forest dominated by *Syzygium* and *Ocotea usambarensis* near Heghambu, 04°20′38″S 37°58′38″E, 1850 m, 26 Feb. 2001, *J. A. Mlangwa 1400* (holotype, MO!; isotypes, F!, K!, NHT!, WAG!).

Diagnosis. Haec species quoad staturam, foliorum formam marginesque ac magnitudinem relativam petalorum lateralium superiorum cum inferioribus *Impatienti pseudoviolae* Gilg simillima, sed ab ea foliis angustioribus, pedunculo breviore, bractea longiore, petalo superiore minore atque floribus semper albis (nec violaceo-rosaceis usque purpurascentibus) distinguitur; ab omnibus congeneris Africae continentalis sepalo inferiore calcari omnino carente differt.

Procumbent glabrous herb to 25 cm tall, rooting at nodes. Leaves spirally arranged, becoming congested toward stem apex; petiole 0.7–1.1 cm, without fimbriae or occasionally with 1 or 2 inconspicuous fimbriae near base; blade ovate, 1.5–2.5 × 1–1.2 cm, asymmetrically attenuate into petiole at base, acute and apiculate at apex; lateral veins 3 or 4 pairs; margin crenate, glandular-fimbriate. Inflorescence a 1-flowered reduced raceme (as implied by presence of bract); peduncle 1.2–1.6 cm; bract 5–7 mm, linear-lanceolate; pedicel 9–13 mm, slender. Lateral sepals 1 pair, ca. 6 mm, subulate; lower sepal 6–8 mm, navicular; spur absent; petals white; dorsal petal 7–8 × 6–7 mm, suborbicular with a narrow crest terminating in an acute point; lateral united petals entire, ca. 9 mm with the upper of each pair ca. 8 × 2.5–3 mm, oblong, the lower ca. 9 × 2.5 mm, elliptic; upper and lower petals about equal in size; ovary glabrous. Fruit ca. 12 × 2 mm, narrowly fusiform; seeds unknown. Figure 1.

doi: 10.3417/2022782

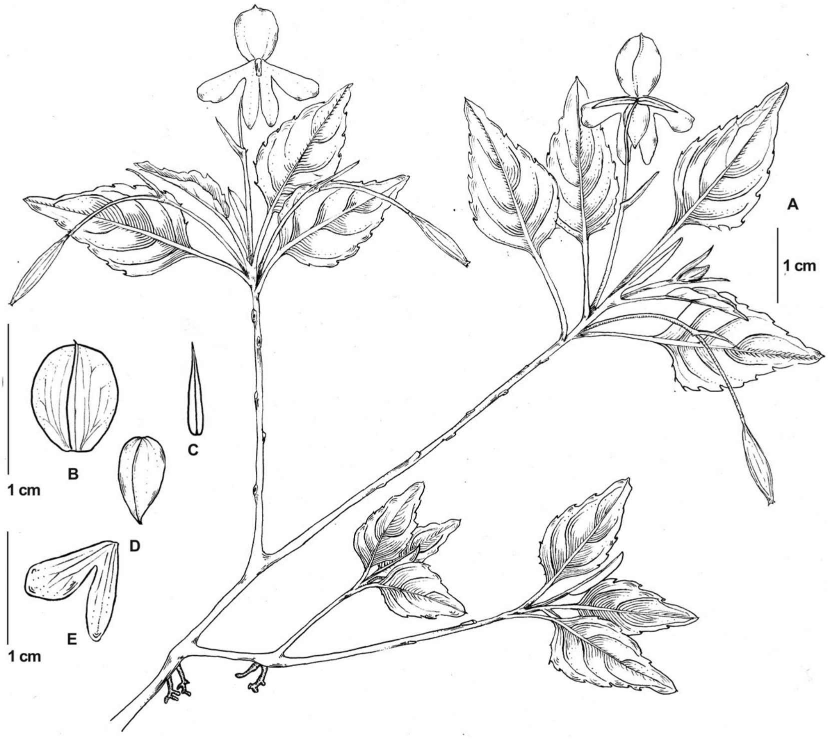

Figure 1. *Impatiens butu* Gavin-Sm. —A. Habit of flowering plant with immature fruit. —B. Dorsal petal, posterior view. —C. Lateral sepal. —D. Lower sepal, posterior view. —E. Lateral united petals, one pair. All based on *J. A. Mlangwa 1400* (F, K, MO, NHT, WAG). Drawn by N. Gavin-Smyth.

Distribution and habitat. This new species is known only from the type collection in the South Pare Mountains (Fig. 2), one of the 13 mountain blocs of the Eastern Arc Mountains of Kenya and Tanzania (Platts et al., 2011). The South Pare Mountains are home to 50 of the 552 vascular plant species known to be endemic to the Eastern Arc Mountains (Gereau, unpublished data), and are also home to significant numbers of globally threatened species of birds, amphibians, mammals, and reptiles (Gereau et al., 2016).

In 2001, the type locality was within Chome Forest Reserve, re-gazetted in 2016 as Chome Nature Forest Reserve (NFR) (Doggart et al., 2017). The Reserve covers an elevational range of 1250–2463 m. *Impatiens butu* was collected at 1850 m elevation in wet montane forest dominated by *Ocotea usambarensis* Engl. This vegetation type is characteristic of the middle to upper slopes of Chome NFR between ca. 1800 and 2300 m elevation, and is the habitat of a very high proportion of the 473 total taxa (species, subspecies, and varieties) of vascular plants known from the Reserve (Gereau, unpublished data). Three previously described vascular plant taxa are known to be strictly endemic to Chome NFR: *Chamaepentas hindsioides* var. *parensis* (Verdc.) Kårehed & B. Bremer (Rubiaceae), *Pimpinella silvicola* Hemp (Apiaceae), and *Streptocarpus parensis* B. L. Burtt (Gesneriaceae). However, these all inhabit moss-covered upper montane forest, elfin forest, and rocky open areas at higher elevations (2000–2460 m) than *I. butu*.

Provisional conservation status. *Impatiens butu* is known from a single collection site in a well-managed protected area, and nothing is known of its population size or trend. Thus, only IUCN Red List criterion B (IUCN, 2012) can be applied to assess its conservation status. The extent of occurrence (EOO) cannot be calculated from a single point, and using the required grid cell size of 2 × 2 km (IUCN Standards and Petitions Committee, 2022), the area of occupancy (AOO) is 4 km^2, falling below the threshold value for Critically Endangered under criterion B2. However, there are no known threats to the species in its single locality, and therefore no “location” can be defined, and the species fails to meet the conditions for any threatened category under criterion B. Furthermore, the effective and continued protection provided by the legal status of an NFR (the highest protection status afforded in Tanzania) pre-

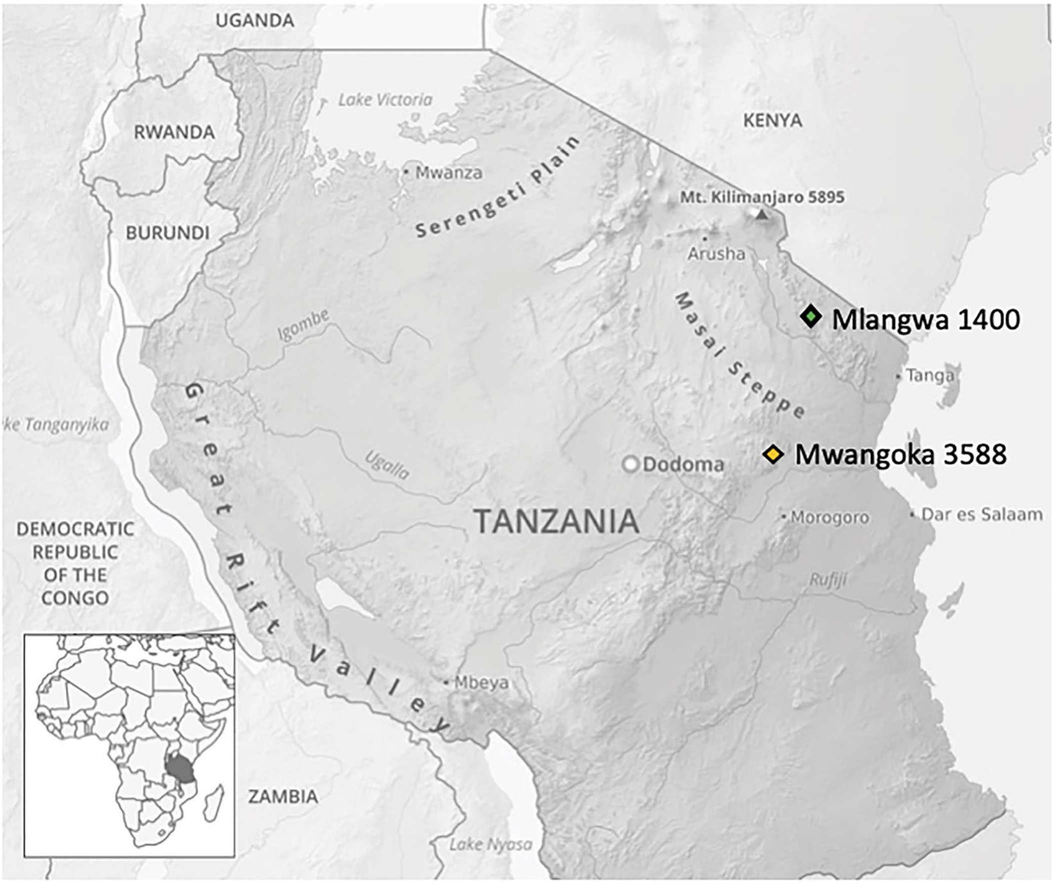

Figure 2. Map of Tanzania (https://www.freeworldmaps.net/africa/tanzania/tanzania-physical-map.jpg). Green diamond = type collection of *Impatiens butu* Gavin-Sm.; orange diamond = type collection of *I. ndovu* Gavin-Sm. © http://www.freeworldmaps.net/

cludes even a plausible threat that could justify an assessment of Vulnerable D2. Therefore, the Red List status of *I. butu* is provisionally assessed as Least Concern (LC), with the proviso that this status is entirely dependent on the continued, effective conservation of Chome NFR.

Etymology. *Impatiens butu* is named for the Swahili word "butu," meaning "blunt," which refers to the complete lack of a spur.

Notes. Other *Impatiens* species lacking the spur that is distinctive of the Balsaminaceae include *I. ecornuta* Gerry Moore, Zika & Rushworth of North America and the large *Impatiens* subg. *Trimorphopetalum* (Baker) Eb. Fisch., endemic to Madagascar and with well-supported monophyly (Rahelivololona et al., 2018); however, none of these spurless species show other strong similarities to *I. butu*. The evolution of this flower type has been linked to a loss in pollinator dependency, with associated characteristics such as decreased nectar production, flower display size, and pollen and ovule number (Abrahamczyk et al., 2021).

In Africa, *Impatiens joachimii* G. M. Schulze, *I. elachistocentra* G. M. Schulze ex Schlieben, *I. rosulata* Grey-Wilson, and *I. quadrisepala* R. Wilczek & G. M. Schulze have extremely reduced spurs; however, their lower sepals are bucciniform, whereas the lower sepal of *I. butu* is navicular. Otherwise, these taxa have few strong similarities.

Rather, *Impatiens butu* shows strongest similarity to *I. pseudoviola* Gilg (see Table 1), and some similarity to *I. humifusa* G. M. Schulze in relative size and shape of the upper and lower lateral petals. However, the flowers and leaves of *I. butu* are both smaller than those of *I. pseudoviola* and *I. humifusa*. *Impatiens butu* and *I. pseudoviola* have similar stature and leaf shape and margins. While the flowers of *I. pseudoviola* are

Table 1. Key morphological traits of *Impatiens pseudoviola* Gilg and *I. butu* Gavin-Sm.

	I. pseudoviola	*I. butu*
Leaf blade	1.2–5.3(–7) × 1.4–3.5(–4) cm	1.5–2.5 × 1–1.2 cm
Peduncle	1.8–2.2(–4) cm	1.2–1.6 cm
Bract	2–4 mm	5–7 mm
Flower color	violet, pink, purplish, rarely white; lower lateral petals with dark pink stripe	white
Lateral sepals	2.5–3.5 mm	6 mm, subulate
Dorsal petal	8–11 × 10–12 mm	7–8 × 6–7 mm
Upper lateral petal	8–11 × 4–7 mm	8 × 2.5–3 mm
Lower lateral petal	7–10 × 2.5–5 mm	9 × 2.5 mm
Spur	1.2–3.6 cm	without spur

typically violet-pink to purplish, rarely white morphs have been observed (e.g., *Agnew & Coe 8771*, MO; "flowers white or palest pink"). Although *I. butu* and *I. pseudoviola* are clearly morphologically similar in the characters noted above and sympatric at the type locality of *I. butu*, the complete lack of a spur in *I. butu* quickly distinguishes them as distinct species.

2. Impatiens ndovu Gavin-Sm., sp. nov. TYPE: Tanzania. Morogoro Region: Mvomero Distr., Nguru South Forest Reserve, grassland in permanent swamp area surrounded by *Osyris lanceolata*, *Rubus*, *Morella salicifolia*, and *Olinia rochetiana*, 06°03′58″S 37°29′52″E, 2006 m, 1 Nov. 2004, *M. A. Mwangoka 3588* (holotype, MO!; isotypes, F!, K!, NHT!, WAG!).

Diagnosis. Haec species quoad folia late ovata spiraliter disposita, sepala lateralia in pari unico ac ovarium glabrum *Impatienti gesneroideae* Gilg simillima, sed ab ea planta praeter bracteas sepalaque mox glabrescente, petiolo longiore, pedicello breviore, sepali inferioris calcari ad apicem 2- vel 3-lobo atque petalis lateralibus unitis brevioribus pari inferiore suborbiculari distinguitur.

Succulent herb ca. 150 cm tall. Leaves spirally arranged; petiole (2.2–)5–6 cm, pubescent when young, glabrescent with age; blade ovate, 4.5–7 × 2.5–4 cm, asymmetrically attenuate into petiole at base, acuminate and acute at apex; lateral veins 5 to 7 pairs with fine pubescence below on midrib and veins when young; margin crenulate-serrate with few basal fimbriae. Inflorescence a 2- to 4-flowered subumbellate raceme; peduncle 2–4 cm; bracts 4–5 mm, pubescent, narrowly lanceolate; pedicels 15–17 mm. Flowers red; lateral sepals 1 pair, ca. 4 mm, with sparse trichomes, ovate-lanceolate, attenuate; lower sepal 5–9 mm, narrowly bucciniform with few sparse trichomes around upper margin, tapering into a 1.7–2.2 cm spur, slightly incurved, ending in widened tip with 2 or 3 pouch-like lobes ca. 2 mm; dorsal petal ca. 5 × 7 mm, shallowly cucullate with a narrow and sparsely pubescent crest terminating in an acute point ca. 1 mm; lateral united petals entire, ca. 7.5 mm, not extending beyond upper margin of lower sepal, with upper petal of each pair 4.5–5.5 × 3–3.5 mm, lower ca. 3 × 3–3.5 mm; ovary glabrous. Fruit 8–10 × 1.5–3.5 mm, fusiform, glabrous. Figure 3.

Distribution and habitat. This new species is known only from the type collection from the Nguru Mountains (Fig. 2), one of the 13 mountain blocs of the Eastern Arc Mountains of Kenya and Tanzania (Platts et al., 2011). The Nguru Mountains are home to 131 of the 552 vascular plant species known to be endemic to the Eastern Arc Mountains (Gereau, unpublished data), and are also home to significant numbers of globally threatened species of amphibians, birds, insects, mammals, and reptiles (Gereau et al., 2016). In 2004, the type locality was within Nguru South Forest Reserve, which was combined with the former Mkindo Forest Reserve and re-gazetted in 2016 as Mkingu Nature Forest Reserve (Doggart et al., 2017). The Reserve covers an elevational range of 300–2400 m, with seven vegetation types, varying from lowland wet forest at 300–900 m in valleys of the eastern slopes to montane heath on the upper ridges above 2000 m. The type locality is at 2006 m elevation in grassland in a permanent swamp area, surrounded by the drier montane forest type of the western slopes above Maskati Mission. Of the 257 total taxa (species, subspecies, and varieties) of previously described vascular plants known from the Reserve (Gereau, unpublished data), eight taxa are known to be strictly endemic to the Reserve. *Impatiens nguruensis* Pócs (Balsaminaceae) has been collected quite near to the type locality of *I. ndovu* and at generally similar elevations (1900–2260 m), but in "mossy montane rainforest" and tall montane forest rather than in grassland or swamp. The seven other endemic taxa occur at elevations of 1400–2000 m in mostly forested habitats. Thus, the north-central and western parts of Mkingu NFR have already yielded a

Figure 3. *Impatiens ndovu* Gavin-Sm. —A. Habit of flowering plant with mature fruit. —B. Lower sepal and spur, lateral view. —C. Leaf margin. —D. Dorsal petal, lateral view. —E. Lateral united petals, one pair. —F. Lateral sepal. All based on *M. A. Mwangoka 3588* (F, K, MO, NHT, WAG). Drawn by N. Gavin-Smyth.

substantial number of narrowly endemic taxa, including *I. ndovu.* This would seem to warrant a good deal of further botanical inventory, especially given the relatively large size of the Reserve (264 km^2) and its only moderate level of past collecting.

Provisional conservation status. *Impatiens ndovu* is known from a single collection site in a well-managed protected area, and nothing is known of its population size or trend. Thus, only IUCN Red List criterion B (IUCN, 2012) can be applied to assess its conservation status. The EOO cannot be calculated from a single point, and using the required grid cell size of 2 × 2 km (IUCN Standards and Petitions Committee, 2022), the AOO is 4 km^2, falling below the threshold value for Critically Endangered under criterion B2. However, there are no known threats to the species in its single locality, and therefore no "location" can be defined, and the species fails to meet the conditions for any threatened category under criterion B. Furthermore, the effective and continued protection provided by the legal status of an NFR (the highest protection status afforded in Tanzania) precludes even a plausible threat that could justify an assessment of Vulnerable D2. Therefore, the Red List status of *I. ndovu* is provisionally assessed as Least Concern (LC), with the proviso that this status is entirely dependent on the continued, effective conservation of Mkingu NFR.

Etymology. *Impatiens ndovu* is named for a Swahili word meaning "elephant." *Ndovu* refers to its broad spur with sac-like lobes developing on either side of the tip, resembling an elephant's trunk.

Notes. Few other African *Impatiens* species have the unusual character of a bilobed spur: the spur tip of *I. digitata* Warb. divides into four to six lobes, that of *I. tricaudata* G. M. Schulze divides digitately into three lobes, while some specimens of *I. polhillii* Grey-Wilson can have widened or bulging spur tips. This interesting new species from the Nguru Mountains in Tanzania has two or three shallow saccate lobes, but otherwise shows few similarities to the other African species with digitately lobed spurs. Rather, the characters of spiral leaf arrangement and pedunculate inflorescences ally *I. ndovu* with *I. fischeri* Warb., *I. gesneroidea* Gilg, and *I. superglabra* (Grey-Wilson) Eb. Fisch., Abrah., Holstein & S. B. Janssens. However, *I. ndovu* has markedly shorter peduncles than the Kenyan endemic *I. fischeri*, and has lower lateral petals that do not extend beyond the upper margin of the lower sepal. Importantly, *I. ndovu* has only one pair of lateral sepals, while *I. fischeri* has two pairs, a trait that distinguishes the Tuberosae clade of *Impatiens* (Yu et al., 2016) from the rest of African *Impatiens*, indicating that these species are not likely to be close relatives. The lateral united petals of *I. ndovu* are similar to those of *I. gesneroidea* and *I. superglabra* in size and shape. However, in contrast to *I. ndovu*, *I. gesneroidea* is pubescent on most parts; in *I. superglabra* the ovary is pubescent and the pedicels, peduncle, and petioles are comparatively short. *Impaties ndovu* shows similarities to *I. ulugurensis* Warb. and *I. kilimanjari* Oliv. in that they occupy upland grasslands and have bucciniform red flowers with lateral united petals that do not extend far beyond the upper margin of the lower lateral sepal. However, these species have opposite or verticillate leaf arrangement in contrast to the spiral leaf arrangement of *I. ndovu.*

Acknowledgments. We thank John Amani Mlangwa and Moses Anyelwisye Mwangoka for collecting the excellent type material of these two new species, the National Herbarium of Tanzania (NHT) for coordinating and facilitating the processing and shipment of these and many other specimens, and the Tanzania Forest Conservation Group for supporting the fieldwork leading to the collection of *Impatiens ndovu*.

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14. Authorship statements for Publication 4

AUTHOR'S STATEMENT PUBLICATION 4

I hereby declare that the article:

"Two new species of *Impatiens* (Balsaminaceae) from the Eastern Arc Mountains of Tanzania" by Gavin-Smyth, N. & **Gereau, R.E.** (2022) *Novon* 30: 122-127. doi: 10.3417/2022782

is a part of my PhD thesis. I helped to conceptualize the article, provided the Latin diagnoses of the two new species, analyzed the floristic composition of the protected areas in which they occur, and produced the provisional Red List assessments. I contributed to writing the manuscript, particularly the distribution and habitat sections for both species, and participated in the final review process and response to reviewers' comments.

Roy E. Gereau

Roy E. Gereau

JWiland. Szymańska

Prof. UAM dr. hab. Justyna Wiland-Szymańska
Supervisor of PhD candidate

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "**Two new species of *Impatiens* (Balsaminaceae) from the Eastern Arc Mountains of Tanzania**" by Gavin-Smyth, N. & Gereau, R.E. (2022) *Novon* 30: 122-127. doi: 10.3417/2022782.

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Contribution	I wrote the introduction, the English descriptions, the etymology, and the notes sections. I examined the herbarium specimens, determined that they were new species, and I drew the illustrations. I made the map and I responded to reviewer comments.
Date	October 9, 2024
Signature	

15. Publication 5

Gereau, R.E., S. Kativu, P. Meerts, L. Merrett, J. Osborne & K. Vollesen. 2023. Chlorophytum vespertinum (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania. Novon 31: 181-190.

Chlorophytum vespertinum (Asparagaceae, Anthericeae), a New Species from Zambia and Tanzania

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Abstract. *Chlorophytum vespertinum* Vollesen from Zambia and Tanzania is described and illustrated. The species exhibits characters unique in *Chlorophytum* Ker Gawl.: pendulous flowers and fruits, flowers that open late in the afternoon and close before midnight, and leaves with a long-extended reflexed or recoiled tip. Its affinities are discussed in the context of recent taxonomic work on the genus. The species is distributed from central northern Zambia to southwestern Tanzania and usually grows on termite mounds in *Brachystegia* Benth. woodland or in surrounding areas. *Chlorophytum vespertinum* is assigned a preliminary IUCN Red List status of Vulnerable.

Key words: *Brachystegia* woodland, distribution, ecology, IUCN conservation status, morphology, taxonomy, termite mounds.

Chlorophytum Ker Gawl. (Asparagaceae: Agavoideae, Anthericeae) is a large genus containing approximately 150 species (Stevens, 2002). It is distributed throughout the Old World tropics but is particularly diverse in eastern and southern central Africa. Fifty-two species have been recorded from the Flora of Tropical East Africa area (Nordal et al., 1997), 56 in the Flora Zambesiaca area (Kativu et al., 2008), 49 in the Flore d'Afrique Centrale area (Meerts, 2015), and 46 from Angola (Figueiredo & Smith, 2008). Outside this core area, numbers fall away quickly with 31 species in South Africa (Germishuizen et al., 2006), 23 in Ethiopia and Eritrea (Nordal, 1997), and 35 in the whole of the Flora of West Tropical Africa area (Hepper, 1968). Meerts and Bjorå (2012) point to Katanga and northern Zambia as a center of diversity for *Chlorophytum.*

Since the publication of the Flora of Tropical East Africa and Flora Zambesiaca, two additional species have been described from Tanzania (Bjorå, 2008; Meerts, 2011), one from Zambia (Osborne et al., 2022) and one from Namibia (Kativu & Bjorå, 2016).

During fieldwork in the Mutinondo Wilderness Area in Zambia from 2010 to 2022, a *Chlorophytum* species with unusual morphological characters was found to be quite common on old established termite mounds in *Brachystegia* woodland and occasionally on edges of riverine forest. Searches in the herbaria at Meise (BR), Kew (K), and Missouri Botanical Garden (MO) found four earlier specimens of the same taxon from the Kalambo area in northernmost Zambia, one from near Kasama, and three from the Sumbawanga area in neighboring Tanzania. Where detailed ecology is recorded, these collections also indicated that the species grows on termite mounds in woodland or on the edges of riverine forest. Detailed examination of this material at Kew failed to reveal any match with known species of similar morphology and ecology or from the same geographical area (northern Zambia, southeastern Congo [Katanga], and southwestern Tanzania).

In Kativu et al. (2008: 77), the three *Hoell & Nordal* specimens cited below are mentioned in the discussion under *Chlorophytum amplexicaule* Baker as representing a taxon that "might deserve taxonomic recognition." These specimens and the others mentioned herein represent a heretofore undescribed species here named *C. vespertinum*.

doi: 10.3417/2023857

MATERIALS AND METHODS

Living plants were collected and studied by S. Bidgood, L. Merrett, and K. Vollesen at Mutinondo Wilderness Area and extensively photographed by L. Merrett. Preserved specimens from Mutinondo as well as older collections were studied in the herbaria of the Royal Botanic Gardens, Kew (K), Missouri Botanical Garden (MO) by Roy Gereau, and at Meise Botanic Garden (BR) by Pierre Meerts.

The conservation status of *Chlorophytum vespertinum* was assessed according to the IUCN Red List categories and criteria (IUCN, 2012). Georeferences were taken or estimated from the herbarium collections, and potential threats at each locality were assessed using the authors' knowledge of the locality or by using Google Earth imagery (Google Earth, 2022). Extent of occurrence (EOO) was calculated from a minimum convex polygon using the online GeoCAT tool (Bachman et al., 2011). Area of occupancy (AOO) was estimated using a 4 km^2 (2 × 2 km) cell, as required by the IUCN Red List guidelines (IUCN Standards and Petitions Committee, 2022).

TAXONOMIC TREATMENT

Chlorophytum vespertinum Vollesen, sp. nov. TYPE: Zambia, Muchinga Prov., Mpika Distr., Mutinondo Wilderness Area, Chipundu Dambo [12°27′S 31°18′E], 7 Dec. 2019, *Bidgood, Merrett & Vollesen 9858* (holotype, K [barcode] K001328473!; isotypes, BR!, K!, MO!, UZL!).

Diagnosis. Haec species inter congeneros in eadem regione crescentes quoad capsulam longiorem quam latiorem *Chlorophyto blepharophyllo* Schweinf. ex Baker simillima, sed ab eo foliis ad marginem semper glabris, inflorescentia pedicelloque papillosos atque seminibus minoribus distinguitur; ab omnibus congeneris apice foliari elongato recurvato, pedicellis per anthesin recurvatis et florescentia vespertina bene distincta.

Erect *perennial herb* with single scape from a small, flattened discoid corm. *Corm* without fibrous remnants of old leaf bases. *Roots* numerous, to 15 cm, white, of 2 types: thinner (ca. 0.5 mm diam.), wiry ones without tubers and thicker (ca. 1 mm diam.), fleshy ones with terminal fusiform tubers to 2.7 × 1.5 cm. Leaf sheaths clasping stem, forming a basal "pseudostem" 4–10 cm. *Cataphylls* (basal leaves with reduced lamina) 4 to 6, green, reflexed at apex, to 4(–6) × 3 cm, base clasping "pseudostem," distinctly auriculate, glabrous, gradually larger upward, uppermost with small lamina. Upper *leaves* 2 to 5, spirally arranged (i.e., not distichous), narrowly ovate to elliptic, uniformly pale to bright green, erect when young, eventually spreading to reflexed, glabrous, 10–25 × 1.5–4 cm on flowering specimens, to 45 × 6.5 cm on fruiting specimens; apex drawn out into a long filiform straight (when young) to recurved or recoiled tip; base of lower leaves auriculate, of upper hidden by sheathing bases of lower leaves; midrib and lateral veins inconspicuous, lateral veins to 20 per side, transverse veins present, inconspicuous; margin glabrous, straight (not undulate), narrowly membranous. *Inflorescences* appearing with young leaves, longer than leaves in flower, shorter in fruit, erect in flower, erect or eventually decumbent in fruit, unbranched or with 1 to 4 branches from near base; peduncle 10–25 cm, terete, glabrous, floriferous part 2–17 cm, rachis moderately to densely papillose; internodes 0.5–1.5(–2) cm; *bracts* supporting lateral branches glabrous, thinly membranous, 1–2.5 cm, with a broad ovate basal part 4–10 mm and a long filiform apical part 6–15 mm, not clasping stem; floral bracts similar, basal bracts to 12 mm with filiform part to 9 mm; apical bracts 3–5 mm with filiform part 2–3 mm. *Flowers* open and star-shaped at anthesis, 2 to 5 per bract at base of inflorescence, solitary upward; pedicel 1–4(–6) mm, not elongating in fruit, spreading in young bud but recurved at anthesis, with a distinct articulation at or slightly above middle, below articulation papillose-glandular, above articulation glabrous; *tepals* spreading or reflexed, glabrous, with pale green central part and white membranous lateral parts, with 3 inconspicuous longitudinal veins, ovate-elliptic, 3 outer ones 5–7 × 1.5–2 mm, 3 inner ones 4–6 × ca. 1.5 mm, extending to 9 and 8 mm, respectively, after flowering; *filaments* spreading, smooth, flattened, white, 3–6 mm; *anthers* bright yellow, linear-lanceolate, ca. 1.5 mm, papillose; *ovary* sessile, subglobose, dark green, 1–2 mm diam., glabrous; *style* soon deciduous, filiform, 2–3 mm at anthesis, extending to 5 mm; *stigma* truncate. *Capsule* pendulous, brown, 3-locular, oblong to slightly obovoid, triangular in lateral view with prominent angles, valves with faint transverse ribs, 6–8 × 4–6 mm, glabrous; apex retuse. *Seeds* 5 or more, black, glossy, irregularly cup-shaped, 1–1.5 mm diam,; intercellular furrows vertical, 10–15 µm deep, sharp-edged; periclinal walls of testa cells 10–30 µm diam., mostly flat, or occasionally with a tiny papilla in the middle. Figures 1–4.

Phenology. Flowering specimens of *Chlorophytum vespertinum* have been seen or collected at the beginning of the rainy season from late October to mid-December. Mature fruit and seeds have been collected during the main rainy season from late January to mid-March.

Distribution. *Chlorophytum vespertinum* is primarily known from two restricted areas: one in the extreme northeastern corner of Zambia (Mbala District) and neighboring southwestern Tanzania (Sumbawanga Rural District) and another in central northern Zambia (Mpika

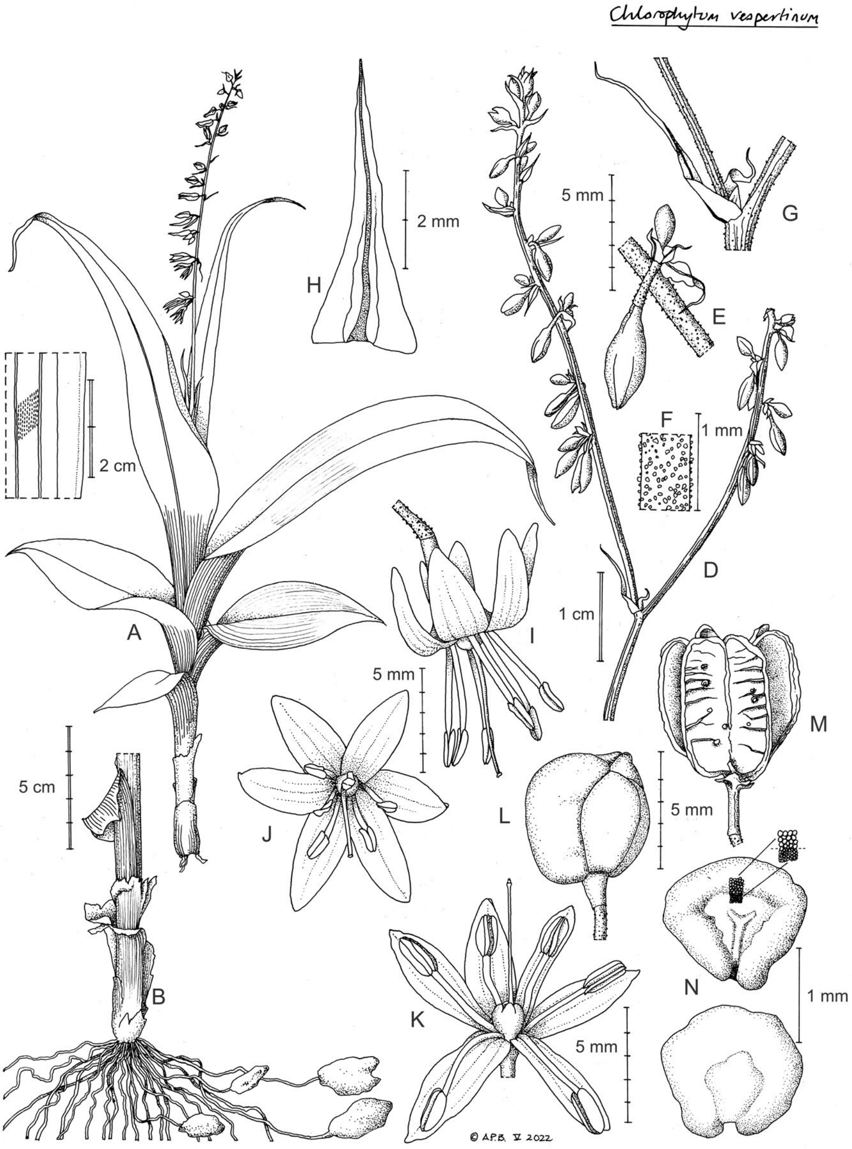

Figure 1. *Chlorophytum vespertinum* Vollesen. —A. Habit. —B. Stem base with roots and tubers. —C. Part of upper leaf surface. —D. Inflorescence. —E. Inflorescence axis, pedicel, and flower bud. —F. Enlarged part of inflorescence axis. —G. Inflorescence node with bracts. —H. Bract. —I, J, K. Open flowers. —L. Ovary. —M. Mature capsule. —N. Mature seed, upper with detail of surface ornamentation. A–C, K–L *Bidgood et al. 9858*. D–H *Gereau et al. 7074*. I, J photos by Lari Merrett. M, N *Merrett 2507*. Drawn by Andrew Brown.

District). There is also one collection from near Kasama, about equidistant between these two areas. The habitat (termite mounds) of *C. vespertinum* is widespread throughout central Africa. It is therefore very likely that the species also occurs in neighboring areas of southern Congo (Katanga), and it will probably also have a wider distribution in northern Zambia and southwestern Tanzania. Figure 5.

Habitat. *Chlorophytum vespertinum* has been collected at elevations from 1150 to 1800 m. At Mutinondo, *C. vespertinum* commonly grows on mature, settled *Macrotermes* termite mounds in *Brachystegia* woodland or in the border zones between termite mounds and woodland or seasonally wet grassland or riverine forest. These mounds can be substantial in size, when fully developed usually 5–10 m in diameter

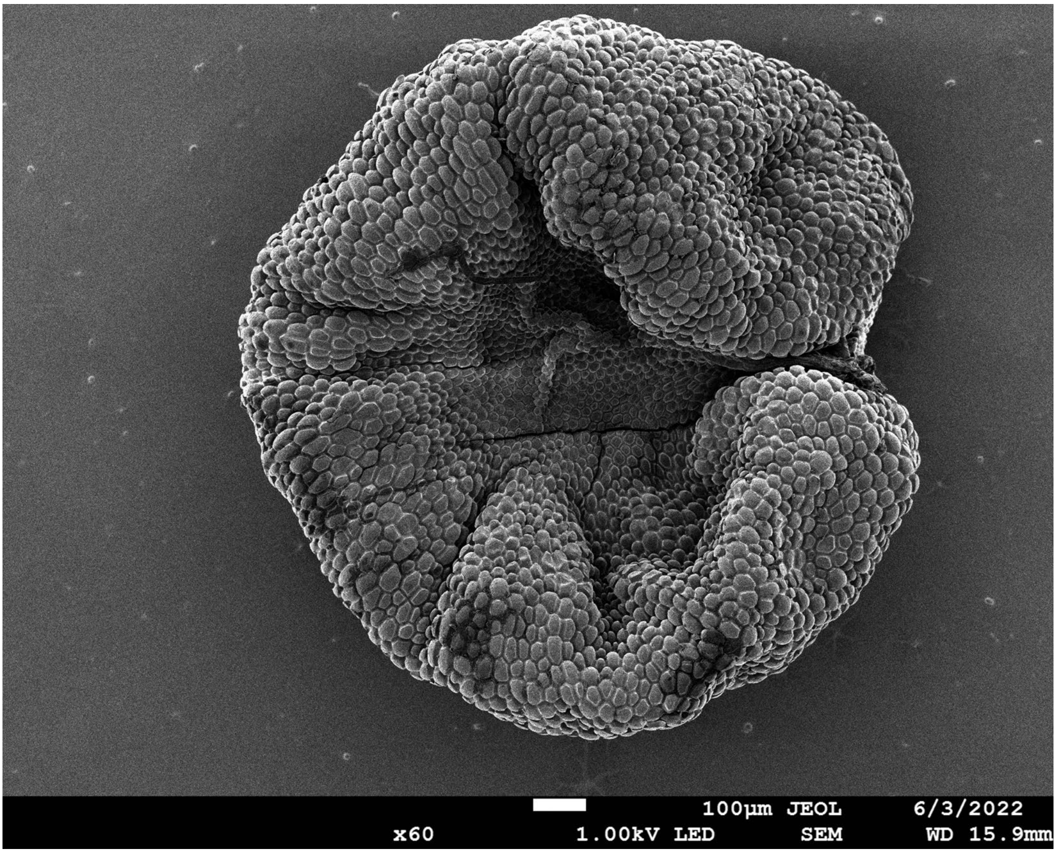

Figure 2. Surface structure of mature seeds of *Chlorophytum vespertinum* Vollesen. (SEM photographs by Iris van der Beeten, Botanic Garden, Meise.) Whole seed, ventral view.

and up to 3 m in height. The soil is a heavy, gray clay loam that is considerably richer in base cations than the soil of the surrounding vegetation. The vegetation on the mounds is therefore usually quite different from and denser than the surrounding woodland. Trees and shrubs characteristically or exclusively growing on the termite mounds at Mutinondo include *Allophylus africanus* P. Beauv., *Apodytes dimidiata* E. Mey. ex Arn., *Bersama abyssinica* Fresen., *Combretum platypetalum* Welw. ex M. A. Lawson, *Diospyros mespiliformis* Hochst. ex A. DC., *Ficus thonningii* Blume, *F. glumosa* Delile, *Grewia pachycalyx* K. Schum., *Grewia schinzii* K. Schum., *Gymnosporia putterlickioides* Loes., *Hibiscus ovalifolius* (Forssk.) Vahl, *Maerua triphylla* A. Rich., *Oncoba spinosa* Forssk., *Searsia quartiniana* (A. Rich.) A. J. Mill., *Shirakiopsis elliptica* (Hochst.) Esser, *Gymnanthemum amygdalinum* (Delile) Sch. Bip. ex Walp., and *Ziziphus mucronata* Willd. The herb layer is usually sparse with no, or very little, grass cover. Characteristic common species in the herb layer include *Costus spectabilis* (Fenzl) K. Schum., *Kniphofia reynoldsii* Codd, and the present new species. A number of these species are quite fire tender and would not be able to survive the annual bushfires if growing in the woodland proper, but the large, well-established termite mounds with their denser tree and shrub cover, sparse herb layer, and no or hardly any grass cover often avoid destructive burning.

While some of the older collections of *Chlorophytum vespertinum* were also recorded as growing on termite mounds (*Gereau et al. 7074*, *Harder et al. 1288*, *Hoell & Nordal 135*), others (*Bredo 6185*, *Harder et al. 1225*, *Hoell & Nordal 131*, *Hoell & Nordal 142*, *E. A. Robinson 4103*) give the habitat as base of rocky hills, edges of riverine forest, or woodland. All these collections only have broad, generalized vegetation descriptions. It is thus impossible to ascertain whether the actual habitats for some of these were forest or woodland floor proper and not in border zones toward or on old, decayed termite mounds.

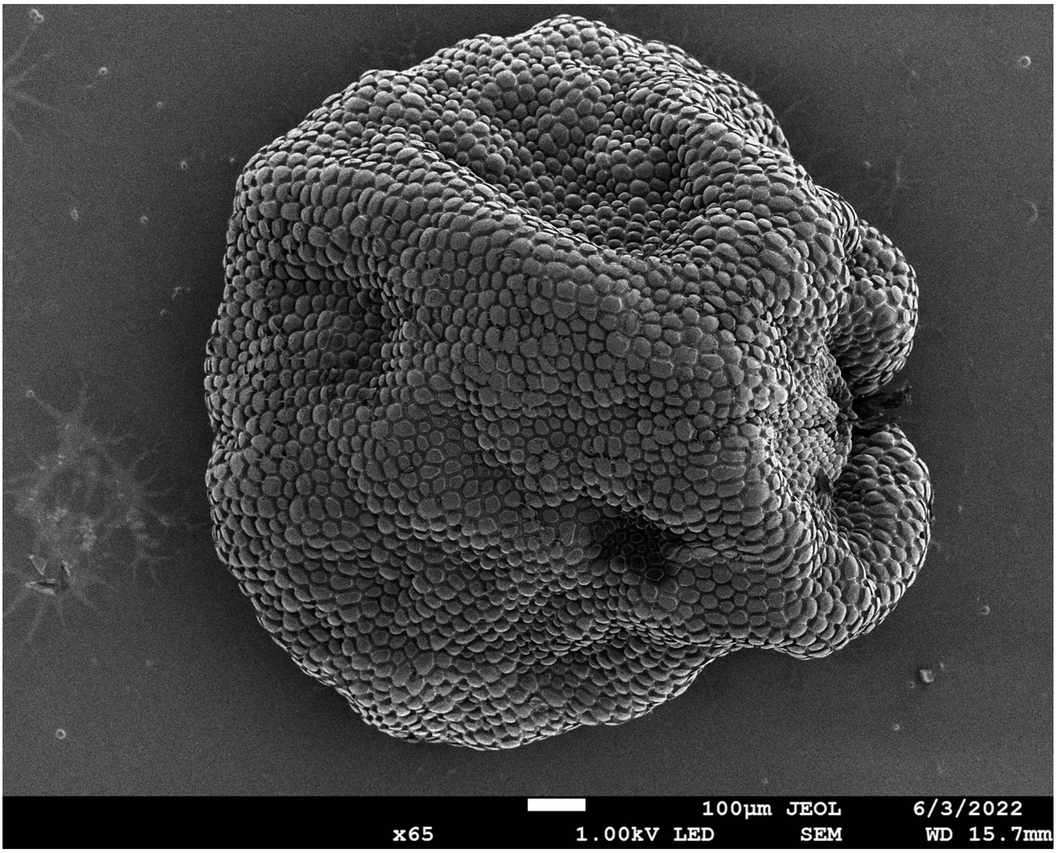

Figure 3. Surface structure of mature seeds of *Chlorophytum vespertinum* Vollesen. (SEM photographs by Iris van der Beeten, Botanic Garden, Meise.) Whole seed, dorsal view.

Pollination and seed dispersal. Two questions immediately arise from the general description of *Chlorophytum vespertinum*. Firstly, what type of insect pollinates the flowers? Evening-flowering species (e.g., in Acanthaceae) usually have long-tubed, strongly scented flowers and are pollinated by large moths. *Chlorophytum vespertinum* has an open flower with a spreading to reflexed perianth and has no discernible scent. This type of flower is normally pollinated during the day by bees, flies, or beetles. This of course also may be the case here, but we have not noticed any insect activity around the opened flowers in the evening. Another more interesting possibility—also considering the habitat—would be termite pollination. Secondly, how are the seeds dispersed? The capsule is large, nodding, and contains numerous seeds that are small in comparison with the seeds of other species of *Chlorophytum*. The glossy black seeds are strongly water repellent. This may indicate that the seeds are long lived and adapted to prolonged storage.

IUCN Red List category. *Chlorophytum vespertinum* is known from 12 collections representing seven distinct localities in Tanzania and Zambia. Three of the seven collecting sites appear to benefit from some level of protection.

From Tanzania, *Harder et al. 1288* (MO) is within the boundaries of the Kalambo River Nature Forest Reserve, which was declared in 2019 comprising an area of 465 km^2 previously occupied by the Kalambo River and Kalambo Fall Territorial Forest Reserves, both declared in 1957 (Government of Tanzania, 2019). Although the species was recorded as "rare" at the time of collection (October 1992), the area has been under continuous protection for 65 years and now enjoys the highest level of protection afforded by the Tanzanian government, so the species may be considered well protected there. The northernmost Zambian collection, *Bredo 6185* (BR), was recorded as coming from "Ulungu, Sizye Village," and maps within the boundaries of the Lungu Forest Reserve, which was desig-

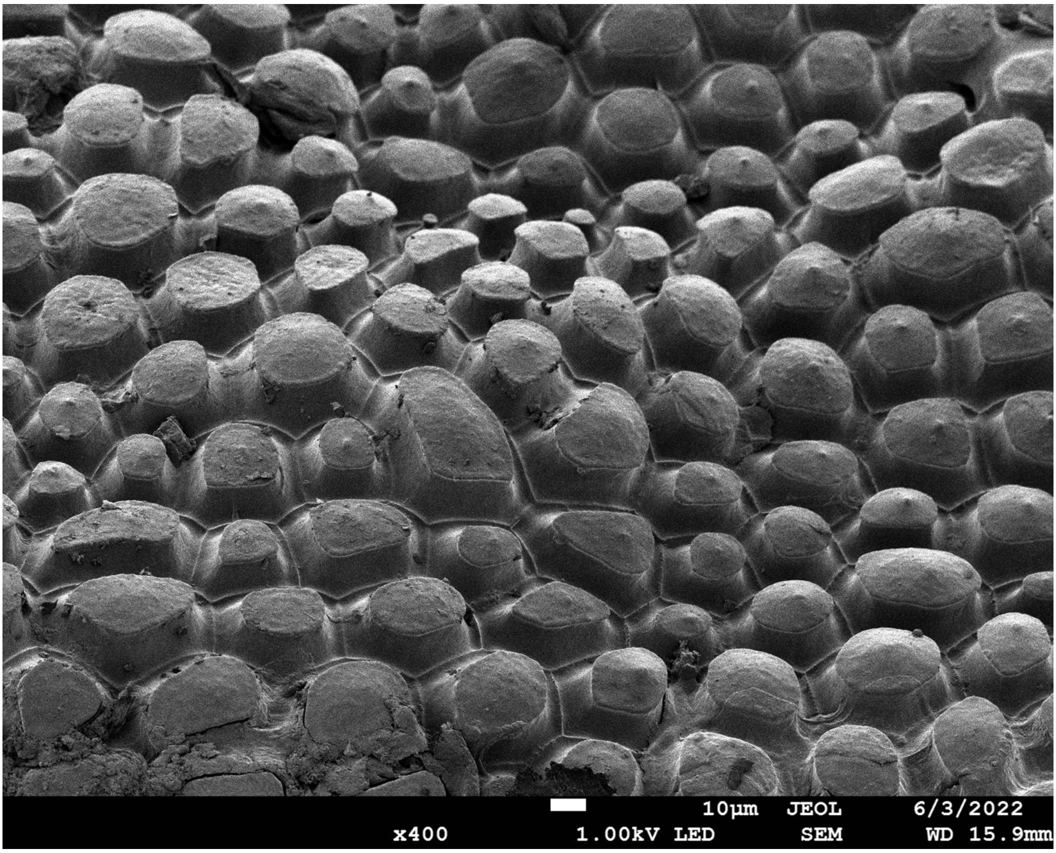

Figure 4. Surface structure of mature seeds of *Chlorophytum vespertinum* Vollesen. (SEM photographs by Iris van der Beeten, Botanic Garden, Meise.) Detail of surface ornamentation.

nated in 1964 and has an area of 189 km^2 (<https://www.protectedplanet.net/26957>). Little other information is available about this protected area. From the Mutinondo Wilderness Area in central northern Zambia there are four recent collections from 2017 to 2020. At Mutinondo, *C. vespertinum* has been observed and counted on a number of termite mounds (Vollesen, pers. obs. 2017–2019). Extrapolating from the sampled termite mounds to the total area of ca. 250 km^2, the total Mutinondo subpopulation would almost certainly amount to several hundred individuals. The Mutinondo Wilderness Area is a privately owned reserve that is managed solely for ecotourism and conservation purposes, and no agriculture or harvesting of woodland products takes place within the area. There are no known current or future threats to the species at this locality. The species does not have a local name at Mutinondo, and none have been recorded at any of the other localities. It is therefore unlikely that it is used for medicinal or any other purposes.

The other four collecting sites lack any known governmental or private protection. The northernmost Tanzanian collection, *Gereau et al. 7074* (K, MO), was collected at a Wildlife Conservation Society research camp on village land, with no formal protection but in relatively intact vegetation, which still looks intact in Google Earth imagery from 2017; the species was reported as "abundant" there. The other unprotected Tanzanian site, "11.0 km SSW of Moravian mission at Tatanda (Livingstone Memorial Mission) on Sumbawanga - Mbala (Zambia) highway," *Harder et al. 1225* (MO), is in an intense agricultural area adjacent to a main highway and can be assumed to be subject to at least some decline in the quality of its habitat. In Zambia, the site of the three collections from Kalombo Farm in 2006 appears to have intact woodland vegetation in Google Earth imagery from 2022, but the site at 28 km southeast of Kasama, *E. A. Robinson 4103* (K), is in an area that is highly impacted and partially denuded by agriculture and settlements and must certainly have lost much of the species' habitat since the collection in 1960. Therefore, at least two of the seven

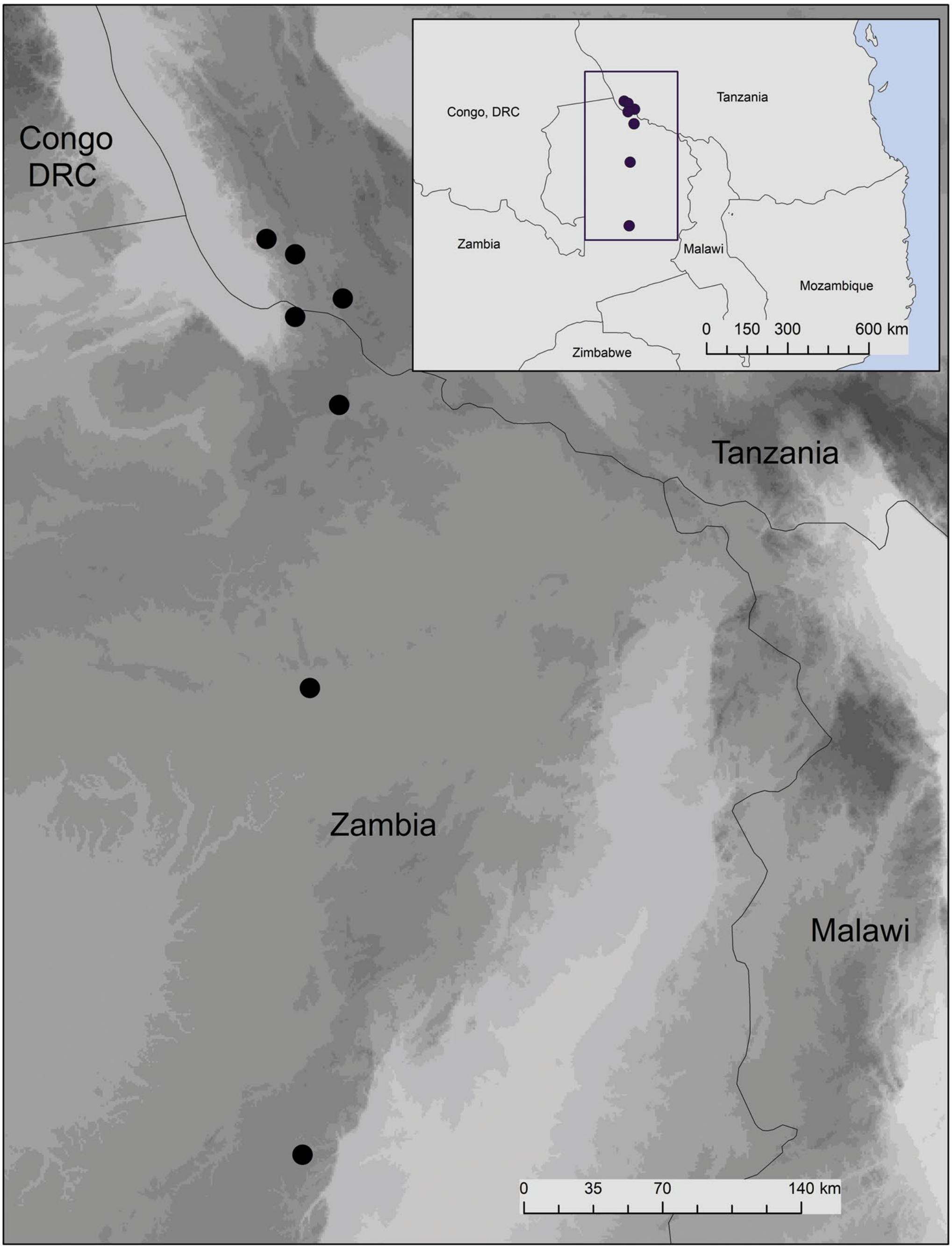

Figure 5. Distribution of *Chlorophytum vespertinum* Vollesen (black dots) in Tanzania and Zambia.

collecting sites are subject to the threat of agricultural land conversion.

Information on population size is not precise enough to permit the application of IUCN Red List criterion C or D, and no information on population reduction that would enable the use of criterion A is available, leaving only criterion B (Geographic range) available for the preliminary conservation assessment of this species. From the specimen coordinates, the EOO is 8846 km^2, within the limits for Vulnerable (VU) under criterion B1; the AOO is 28 km^2, within the limits for Endangered under criterion B2 (IUCN, 2012). Given the scale

Table 1. Matrix of taxonomically important characters comparing *Chlorophytum vespertinum* Vollesen with closely related species in the same region and similar habitats.

	C. blepharophyllum var. *amplexicaule*	*C. blepharophyllum* var. *blepharophyllum*	*C. brachystachyum*	*C. gallabatense* var. *floribundum*	*C. gallabatense* var. *gallabatense*	*C. vespertinum*
Flower and fruit orientation	erect	erect	erect	erect	erect	pendulous
Inflorescence axis and pedicel	glabrous	glabrous	glabrous	papillose	glabrous	papillose
Pedicel, articulation	near middle	near middle to apical	near apex	near apex	near middle to apical	near middle
Capsule	ca. 8 × 6 mm, longer than wide, erect	8–12(–15) × 6–12 mm, longer than wide, erect	3–5 × 4–6 mm, wider than long, erect	6–8 × 5–7 mm, longer than wide, erect	3–5 × 6–7 mm, wider than long, erect	6–8 × 4–6 mm, longer than wide, pendulous
Seeds, number per locule, size	not seen	discoid, 5 or more, 2.5–3 mm diam.	cup-shaped, 1 or 2, ca. 2 mm diam.	irregularly cup-shaped, 5 or more, ca. 2 mm diam.	discoid, 1 or 2, 2–3 mm diam.	irregularly cup-shaped, 5 or more, 1–1.5 mm diam.
Leaf margin	ciliate	ciliate (rarely glabrous)	ciliate	glabrous	glabrous	glabrous

of the principal threat, each of the three protected sites and each of the four unprotected sites may be considered a "location" (sensu IUCN, 2012 and IUCN Standards and Petitions Committee, 2022). Given the EOO and AOO, the existence of seven threat-defined locations, and a continuing decline of at least the quality of habitat at two locations, the Red List status of *Chlorophytum vespertinum* is here assessed as Vulnerable [VU B1ab(iii)+2ab(iii)].

Notes. *Chlorophytum vespertinum* exhibits some unique characters in *Chlorophytum*. The evening flowering habit, the long, recurved to recoiled leaf apex, and the recurved pedicels and pendulous fruits have not been observed in any other species. Recurved pedicels are occasionally seen in other species in the bud stage but never in open flowers. Other similar taxa with leaf bases forming a conspicuous "pseudostem" are *C. blepharophyllum* Schweinf. ex Baker var. *amplexicaule* (Baker) Meerts, *C. blepharophyllum* var. *blepharophyllum*, *C. gallabatense* Schweinf. ex Baker var. *floribundum* (Baker) Meerts, and *C. brachystachyum* Baker. Apart from these, *C. gallabatense* var. *gallabatense* and *C. pauper* Poelln. occasionally develop an indistinct "pseudostem."

Table 1 summarizes key features that distinguish these taxa. *Chlorophytum blepharophyllum* (both varieties) and *C. brachystachyum* have a ciliate leaf margin, while *C. gallabatense* (both varieties) has a glabrous leaf margin. In the flowering state, *C. gallabatense* var. *floribundum* has a papillose inflorescence axis, while *C. blepharophyllum*, *C. brachystachyum*, and *C. gallabatense* var. *gallabatense* all have a glabrous inflorescence axis. The pedicel is articulated near the apex in *C. brachystachyum* and *C. gallabatense* var. *floribundum*, but near the middle in *C. blepharophyllum* and. *C. gallabatense* var. *gallabatense*. In fruit, *C. blepharophyllum* and *C. gallabatense* var. *floribundum* have a capsule that is longer than wide (6–12[–15] × 5–7[–12] mm) with many-seeded locules, while *C. brachystachyum* and *C. gallabatense* var. *gallabatense* have a smaller capsule that is slightly wider than long (3–6 × 4–7 mm) with 1- or 2-seeded locules. In *C. pauper*, the rhizome and leaf bases are covered with fibrous remains of old leaves, a character not seen in any of the other related species.

Of these, *Chlorophytum vespertinum* is most similar to *C. gallabatense* var. *floribundum* in having a papillate inflorescence axis and pedicels. In addition to its long, recurved to coiled leaf apices, it differs from that taxon in its pendulous flowers and fruits, the pedicels articulated near the middle, and smaller seeds.

Paratypes. TANZANIA. **Rukwa Region:** Sumbawanga Rural Distr., 11 km SW of Tatanda Mission, 8°34′S 31°29′E, 24 Oct. 1992, *Harder et al. 1225* (MO); 35 km W of Matai on Matai–Kasanga rd., Lwasu Hills, 8°22′S 31°16′E, 28 Oct. 1992, *Harder et al. 1288* (MO); Loasi River Valley, Nawinga Stream, Kafukaka Village, 8°17′S 31°08′E, 12 Nov. 2009, *Gereau et al. 7074* (K, MO). ZAMBIA. **Northern Province:** Mbala Distr., Ulungu, Sizye Village, 8°39′S 31°16′E, 6 Nov. 1948, *Bredo 6185* (BR); Kalombo Farm, 9°03′S 31°28′E, 4 Dec. 2006, *Hoell & Nordal 131* (K!, O); Kalombo Farm, 9°03′S 31°28′E, 4 Dec. 2006, *Hoell & Nordal 135* (K, O not seen); Kalombo Farm, 9°03′S 31°28′E, 5 Dec. 2006, *Hoell & Nordal 142* (K, O not seen); Kasama Distr., 28 km SE of Kasama, 10°20′S 31°20′E, 22 Nov. 1960, *E. A. Robinson 4103* (K). **Muchinga Province:** Mpika Distr., Mutinondo Wilderness Area, Chipundu Dambo, 12°27′S 31°18′E, 20 Feb. 2017, *Bidgood et al. 9377* (K); Mpika Distr., Mutinondo Wilderness Area, Chipundu Dambo, 12°27′S 31°18′E, 7 Dec. 2019, *Merrett & Vollesen* in *Bidgood 9858* (K); Mpika Distr., Mutinondo Wilderness Area, Chipundu Dambo, 12°27′S 31°18′E, 23 Jan. 2020, *Merrett 2507* (K); Mpika Distr., Mutinondo Wilderness Area, Chipundu Dambo, 12°27′S 31°18′E, 13 Mar. 2020, *Merrett 2527* (K).

Acknowledgments. Our sincere thanks go to the owners of Mutinondo Wilderness Ltd., Mpika, Zambia, for allowing Sally Bidgood and Kaj Vollesen to use their excellent facilities. We would also like to thank the curator of the herbarium in Meise (BR) for allowing us to study their collections. We are also grateful to Iris van der Beeten (Meise Botanic Garden) for the excellent SEM pictures (Fig. 2) and to Charlotte Taylor (MO) for critical reading of the manuscript. The beautiful illustration (Fig. 1) was prepared by Andrew Brown, the final electronic file of the illustration was prepared by Mike Blomberg, and the distribution map (Fig. 5) was prepared by Burgund Bassüner.

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ISSN 1055-3177 (PRINT); ISSN 1945-6174 (ONLINE)

16. Authorship statements for Publication 5

AUTHOR'S STATEMENT PUBLICATION 5

I hereby declare that the article:

"*Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania" by **Gereau, R.E.**, Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190

is a part of my PhD thesis. I was the first collector or a co-collector of all of the cited specimens of the new species from Tanzania, participated in the conceptualization of the article, studied older specimens in the Missouri Botanical Garden herbarium (MO), provided the Latin diagnoses of the new species, produced the provisional Red List assessment, and contributed to the morphological analysis in Table 1. I contributed to writing the manuscript, served as the author for correspondence during its production, and was responsible for the final revision process and response to reviewers' comments.

Roy E. Gereau

Roy E. Gereau

J Wiland-Szymańska

Prof. UAM dr. hab. Justyna Wiland-Szymańska
Supervisor of PhD candidate

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article “***Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania**” by Gereau, R.E., Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190.

Co-Author Name	**Shakkie Kativu**
Affiliation	Department of Biological Sciences, University of Zimbabwe, Box MP167, Mt. Pleasant, Harare, Zimbabwe
Contribution	I checked the type material against all recorded species from central and southern African, and confirmed its unique characteristics. I revised the manuscript, and verified the Latin diagnosis and Red List assessment.
Date	09/10/2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "*Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and **Tanzania**" by Gereau, R.E., Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190.

Co-Author Name	**Pierre Meerts**
Affiliation	Meise Botanic Garden, Nieuwelaan 38, BE-1860 Meise, Belgium; and Université Libre de Bruxelles, Av. F.D. Roosevelt 50, CP 244, BE-1050, Brussels, Belgium
Contribution	I looked for material of the new species in the collections of Meise Botanic Garden (BR) and Université Libre de Bruxelles (BRLU) and found one specimen. I also participated to commenting a first draft.
Date	9 Oct 2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "*Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and **Tanzania**" by Gereau, R.E., Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190.

Co-Author Name	**Lari Merrett**
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Contribution	Photographs & on site observation
Date	9th October 2024
Signature	L. Merrett

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "***Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania**" by Gereau, R.E., Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190.

Co-Author Name	**Jo Osborne**
Affiliation	Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K.
Contribution	As a co-author of this article, I contributed data for mapping and comments on the manuscript.
Date	9/10/2024
Signature	

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "***Chlorophytum vespertinum* (Asparagaceae, Anthericeae), a new species from Zambia and Tanzania**" by Gereau, R.E., Kativu, S., Meerts, P., Merrett, L., Osborne, J. & Vollesen, K. (2023) *Novon* 31: 181-190.

Co-Author Name	**Kaj Vollesen**
Affiliation	Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K.
Contribution	Co-author
Date	9 October 2024
Signature	Kaj Vollesen

17. Publication 6

Cheek, M., R.E. Gereau & J. Kalema. 2024. Afrothismia ugandensis nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda. Kew Bull. DOI: 10.1007/s12225-024-10212-5 pp. 1-11.

KEW BULLETIN
DOI 10.1007/S12225-024-10212-5

ISSN 1874-933X (print)
ISSN 0075-5974 (electronic)

Afrothismia ugandensis nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda

Martin Cheek[1], Roy E. Gereau[2] & James Kalema[3]

Summary. The fully mycotrophic (or mycoheterophic) *Afrothismia ugandensis* Cheek nom. nov. (Afrothismiaceae), formerly described as *A. winkleri* (Engl.) Schltr. var. *budongensis* Cowley from the Budongo Forest in Western Uganda, is renamed, redescribed and illustrated. This change in status is supported by eight newly elucidated, qualitative, morphological, diagnostic characters despite the overall similarity with *A. winkleri,* a species restricted to Cameroon and Gabon. *Afrothismia ugandensis* is remarkable in the genus for occurring in semi-deciduous (not evergreen) forest and for having ellipsoid or ovoid (vs globose) root bulbils. It has only been recorded twice, first in August 1940, and most recently in June 1998, despite targeted searches in recent years. In both 1940 and 1998, only single individuals appear to have been detected. A single site for the species is known with certainty. It is here assessed as Critically Endangered [CR B2ab(iii); D1] using the IUCN categories and criteria. *A. ugandensis* is threatened by forest degradation and clearance due to illegal, selective, small-holder logging for firewood and charcoal, timber and limited agriculture.

Key Words. Achlorophyllous mycotroph, Conservation, Glomerales, Glomeromycota, Mutualism, TIPAs programme.

Introduction

Fully mycotrophic heterotrophs, often known as achlorophyllous mycotrophic plants, mycoheterophs (Merckx 2013) or saprophytes, are remarkable for lacking all chlorophyll and being completely dependent on fungi for their survival. In continental Africa, individual species or entire genera that are achlorophyllous mycotrophs occur in the families Orchidaceae, Gentianaceae and Burmanniaceae, while all members of Afrothismiaceae, Triuridaceae and Thismiaceae are fully mycotrophic (Cheek & Ndam 1996; Cheek & Williams 1999; Cheek 2006; Cheek *et al.* 2023a).

Although some earlier authors (Jonker 1938; Maas-van de Kamer 1998) placed *Afrothismia* (Engl.) Schltr. and associated genera as a tribe Thismieae within the family Burmanniaceae sensu lato, molecular phylogenetic data (e.g. Merckx *et al.* 2006) strongly indicate that Thismiaceae are best placed as a separate family (Cheek *et al.* 2018a). Further, subsequent relatively well-sampled analyses place Afrothismiaceae as sister to Taccaceae + Thismiaceae, in a different subclade of Dioscoreales from Burmanniaceae sensu stricto (Merckx *et al.* 2009; Lin *et al.* 2022). The families are separated from each other by numerous morphological characters and Afrothismiaceae was formally recognised recently (Cheek *et al.* 2023a).

Afrothismiaceae, with a single genus, *Afrothismia,* are confined to tropical continental Africa (not a single species is known from Madagascar, the Comores nor the Mascarenes). *Afrothismia* differs from the genera of Thismiaceae by the annulus inserted deep inside the perianth tube; stamens inserted below the annulus; anthers usually adnate to stigma, but see notes below for *Afrothismia ugandensis* and note that anthers adnate to stigmas have recently been reported in the new Thismiaceae genus *Relictithismia* Suetsugu & Tagane (Suetsugu *et al.* 2024); rhizomes with clusters of usually spherical tubers and being confined to tropical equatorial central and eastern Africa (Cheek *et al.* 2018a). Sixteen species have been formally described, now 17 with this paper, but there appear to be seven additional undescribed species (Cheek *et al.* 2023a).

The genus *Afrothismia* was erected by Schlechter (1906), based on *Thismia winkleri* Engl. which had been placed in *Thismia* sect. *Afrothismia* Engl. (Engler 1905). He described a second species, *A. pachyantha* Schltr., that he had collected in the then German colony Kamerun, now Cameroon. The range of the genus was formally extended to East Africa by Cowley (1988), with *A. insignis* Cowley from Tanzania and *A. winkleri* var. *budongensis* Cowley from Uganda. By 2003, the original two species had increased in number to

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Accepted for publication 1 July 2024.

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four, including the Cameroonian species, *A. gesnerioides* H.Maas (Maas-van de Kamer 2003).

In the ensuing 15 years, the number of formally described new species quadrupled to 16, with discoveries from Gabon (Dauby *et al.* 2008), Kenya (Cheek 2004), Malawi (Cheek 2009) and Tanzania (Cheek & Jannerup 2006); but the largest number of discoveries by far has been made in Cameroon. Here, eight new species of *Afrothismia* have been published, one extending to Nigeria (Franke 2004; Franke *et al.* 2004; Sainge & Franke 2005; Sainge *et al.* 2005, 2013; Cheek 2007; Cheek *et al.* 2019). Most of the Cameroonian species fall within the Cross-Sanaga interval (Cheek *et al.* 2001), which holds the highest flowering plant species (Barthlott *et al.* 1996) and generic (Dagallier *et al.* 2020) diversity per degree square in Tropical Africa, and which is the site of many new discoveries, including genera new to science (Litt & Cheek 2002; Cheek *et al.* 2003, 2018b). Many of the Cameroonian *Afrothismia* species feature in the *Red Data Book of Cameroon Plants* (Onana & Cheek 2011) and most species of the genus are Critically Endangered (Cheek *et al.* 2023a) including the type species, *A. winkleri* (Engler) Schltr., until now considered to include the new Ugandan taxon (IUCN 2013) described here. One Cameroonian species is considered extinct after targeted searches (Cheek *et al.* 2019), while several others have not been recorded alive in several decades and so may also be extinct, e.g. *A. zambesiaca* Cheek (Cheek 2009).

Although species niche modelling has indicated that *Afrothismia* might occur in West Africa, west of the Dahomey gap (Sainge *et al.* 2017), recent targeted expert surveys there have failed to find any species of the genus, although they have produced other new species of fully mycotrophic Dioscoreales (Cheek & van der Burgt 2010; Cheek *et al.* 2023b).

Lacking any green tissue, *Afrothismia* spp. depend on vesicular arbuscular mycorrhizal fungi for sustenance. The genus *Rhizophagus* P.A.Dang (Glomerales, Glomeromycota) has been implicated as the fungal partner of the genus (Franke *et al.* 2006) and delayed co-speciation between *Afrothismia* and the fungal partner has been demonstrated (Merckx 2008; Merckx & Bidartondo 2008). However, the autotrophic partners of these fungi remain unknown.

The new taxon of *Afrothismia* reported in this paper is a result of the Uganda Tropical Important Plant Areas programme (TIPAs, see Darbyshire *et al.* 2017; Richards *et al.* 2024). Cowley (1988) recognised the Ugandan taxon as different at varietal rank from *Afrothismia winkleri*, based on quantitative characters and a difference in ovary colour. Here we show that there are eight additional, qualitative diagnostic characters which are more than adequate to support separation of the Ugandan material from *Afrothismia winkleri* at full species level. Therefore, we here formally describe the available material as *Afrothismia ugandensis*.

Materials & Methods

Nomenclatural changes were made according to the *Shenzhen Code* (Turland *et al.* 2018). Names of species and authors follow IPNI (2024, continuously updated), family assignments follow POWO (2024, continuously updated). Herbarium material was examined with a Leica Wild M8 dissecting binocular microscope fitted with an eyepiece graticule measuring in units of 0.025 mm at maximum magnification. The drawing was made with the same equipment with a Leica 308,700 camera lucida attachment. The following herbaria were inspected for specimens: B, BM, EA, K, MHU, SRGH, YA and WAG. Herbarium codes follow *Index Herbariorum* (Thiers 2024, continuously updated).

The use of technical terms follows Beentje & Cheek (2003) and the format of the description follows those in other papers describing new species in *Afrothismia*, e.g. Cheek *et al.* (2004), Cheek & Jannerup (2006), Cheek *et al.* (2019). The specimens cited have all been seen by one or other of the co-authors. The conservation assessment follows the IUCN (2012) Red List of Threatened Species categories and criteria.

Results

The species described in this paper as *Afrothismia ugandensis* Cheek was first encountered, collected and preserved in spirit from the Budongo Forest of Uganda, in August 1940, by Eggeling (*Eggeling* 4041, K) who made a watercolour sketch of it (Fig. 1). The specimen was initially determined at Kew as *A. winkleri,* but included in the *Flora of Tropical East Africa* Burmanniaceae account as *A. winkleri* var. *budongensis* Cowley (Cowley 1988). The taxon was differentiated from *A. winkleri* based on quantitative characters and a difference in colour of the ovary (Cowley 1988). However, the two taxa are separated geographically by c. 2300 km. They can also be separated morphologically, using the eight additional, qualitative characters indicated in Table 1 and in the recognition section. This evidence is more than adequate for species level rather than varietal status. *A. ugandensis* is remarkable for being the only published species of the genus from Uganda and the westernmost member of the family recorded in East Africa (Cheek *et al.* 2023a).

Afrothismia ugandensis *Cheek* **nom. nov.** Type: Uganda, Budongo Forest, "Tiny saprophyte 1½ to 2 inches high, growing among dead leaves in dense shade, on the floor of the Budongo Forest, Uganda. Stem and scale leaves pale brown. Perianth with 6 long filiform appendages (straight in life). Perianth partly yellow, partly claret, partly colourless and more or less transparent, as shown in the attached sketch.", fl., Aug.

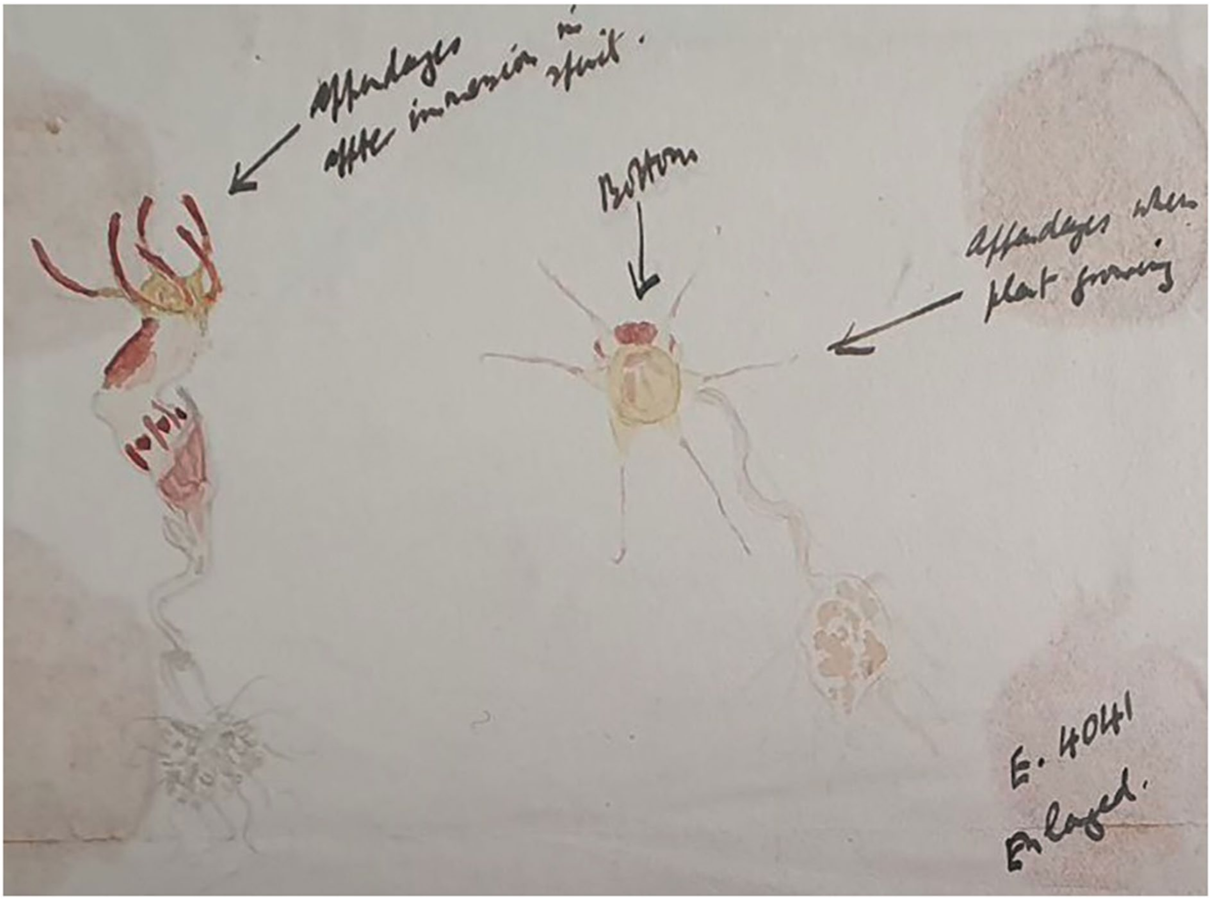

Fig. 1. *Afrothismia ugandensis* Cheek. Water coloured field sketch of *Eggeling* 4041 (K, spirit collection number 3669). Left, flowering plant, showing flower in side view annotated presumably by Eggeling "appendages after immersion in spirit". Right, plan view "appendages when plant growing". Mounted on the herbarium sheet cross-referenced to the spirit (and only surviving) specimen. DRAWN BY W. J. EGGELING

Table 1. The characters separating *Afrothismia ugandensis* from *Afrothismia winkleri.* Data for the second species including from Engler (1905)

	Afrothismia ugandensis	*Afrothismia winkleri*
Tepal lobes	base hastate, with flanking pair of subsidiary triangular lobes	base entire, lacking any lobes
Corona surface	densely papillate	smooth
Annulus surface	densely puberulent	glabrous
Annulus shape	entire, constant in width, continuous	divided into 6 broadly triangular lobes
Anther connective distal appendage, attached to stigma?	absent, stamen not attached to stigma (possibly an artefact)	present, ovate, attached to stigma
Style surface	glabrous	densely puberulent
Outer (abaxial) stigma surface	glabrous	sparsely puberulent
Stigma shape and lobing	divided nearly to the base into three triangular/oblong lobes, each lobe itself bifid	shallow bowl, minutely 6-lobed at margin
Geographic range	Uganda	Cameroon and Gabon

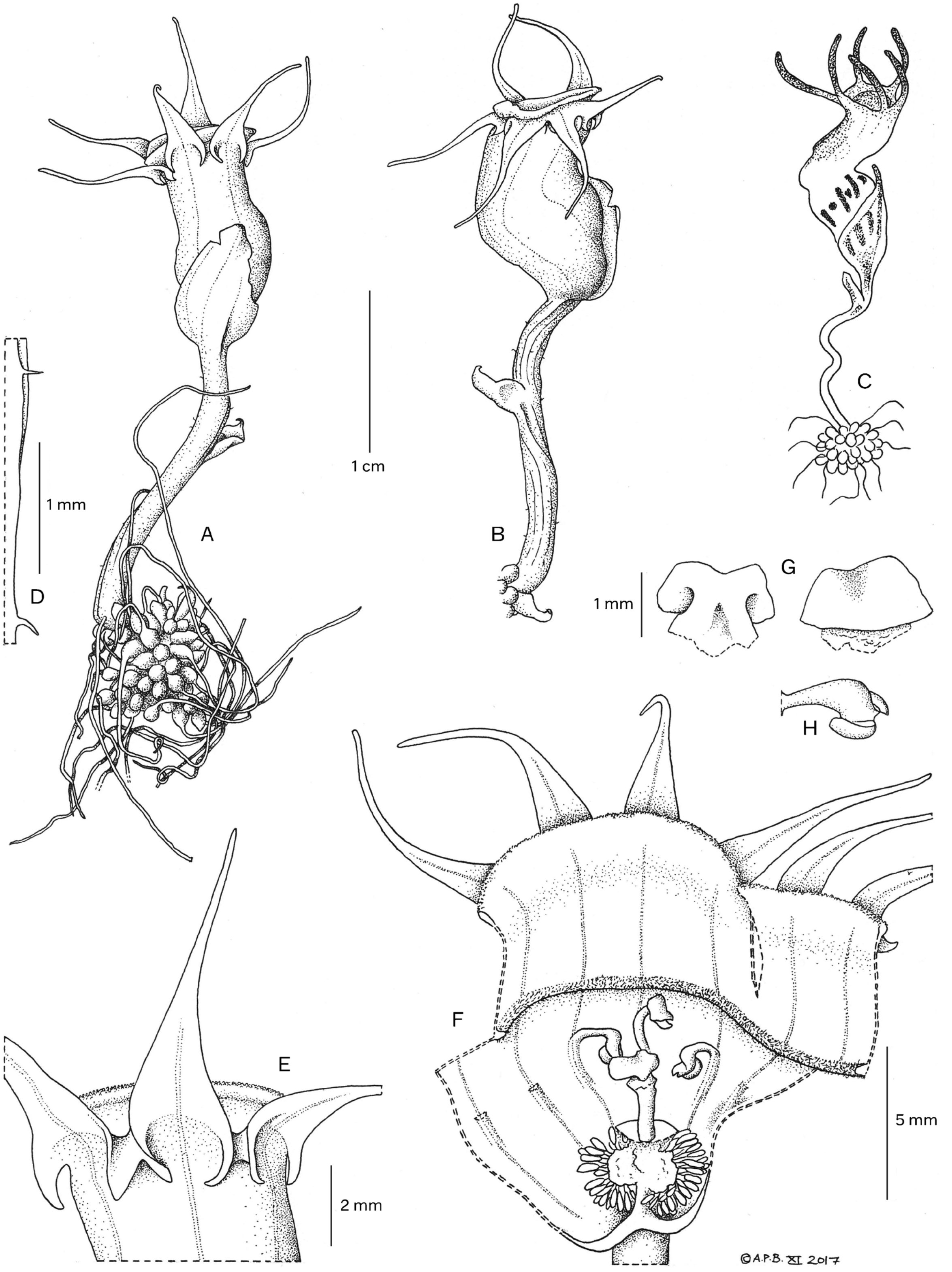

Fig. 2. *Afrothismia ugandensis* Cheek. **A** habit, whole plant, showing flower in dorsal view; **B** as A, but showing flower in side view; **C** copy of habit painting by Eggeling (no scale bar was given), presumably of the plant in **A** & **B**; **D** detail of stem showing hairs; **E** upper perianth tube showing connation of the tepal lobe bases; **F** dissected damaged flower (only 3 of 6 stamens remain intact); **G** stigma lobe, lower surface (left) and upper (right); **H** stamen viewed in situ. From *Eggeling* 4041 (K, spirit collection number 3669). DRAWN BY ANDREW BROWN.

1940, *Eggeling* 4041 (holotype K spirit collection number 3669 [K000593368]). Figs 1, 2.

http://www.ipni.org/urn:lsid:ipni.org:names:77345995-1

Afrothismia winkleri (Engler) Schltr. var. *budongensis* Cowley, *Fl. Trop. E. Africa, Burmann.* (1988: 7) **replaced synonym**.

Achlorophyllous mycotrophic herb with only the flower emerging above the leaf-litter. *Bulbil cluster* completely covering the short, naked (scale leaves absent) rhizome, c. 9 – 10×8 mm; bulbils ellipsoid or ovoid, each c. 1 – 2.5×1 mm, with an apical rootlet up to 3.5 cm long, 0.2 mm in diam. (Fig. 2A). *Stem* (peduncle), succulent, concealed in substrate, vertical, unbranched, terete with 5 – 6 inconspicuous longitudinal ridges, c. 20 – 30 mm long, 2 mm in diam.; hairs very sparse, simple, patent, 0.2 mm long; scale-leaf (bract) single, inserted approx. midlength, ovate-triangular, c. 0.4×0.2 mm; axillary bud globose; internodes c. 10 mm long. *Inflorescence* 1-flowered; flower-subtending bract sheathing the base of the flower, translucent when live (field painting), ovate, 7 – 8×c. 5.5 mm, apex acuminate (Fig. 2C). *Flowers* slightly asymmetric, c. 1.5 cm long and 2 cm in diam. including the perianth lobes (preserved material). *Perianth tube* directed vertically, L–shaped, the axis of the lower perianth tube angled at c. 30 – 40 degrees away from the vertical, the upper tube angled from the lower tube at 50 – 60 degrees in the opposite direction (towards the vertical), tube c. 8 mm long, 5 mm wide at midpoint (junction of lower and upper tubes). Lower (proximal) tube funnel-shaped, c. 5 mm wide at junction with ovary, widening to c. 6.5 mm before constricting to c. 5 mm at junction with upper tube; outer surface translucent or white with 6 longitudinal purple lines, each separated by a purple spot on the lower tube and ovary. Upper tube cylindrical, c. 4×5 mm, translucent or white with a purple patch in the distal dorsal half, glabrous and lacking projections. Inner surface of perianth tube with an internal flange (annulus) at junction of lower and upper tube, above the insertion of the staminal filaments, flange continuous, unlobed, projecting into the tube c. 0.3 mm, 0.3 mm thick at the base, densely hairy, hairs patent, simple, 0.1 mm long; inner perianth surface otherwise glabrous and lacking ornamentation. Corona yellow, slightly funnel-shaped, projecting 1 – 1.25 mm above the insertion of the perianth lobes, the mouth orbicular in plan view, c. 7 mm in diam., the rim thickened, c. 0.25 mm diam., minutely papillose (Fig. 2F). *Tepals* six, monomorphic (equal), patent in life (field painting), (±forward-directed in spirit preserved material), purple, narrowly triangular, flattened, 8 – 9×c. 2 mm wide at base, tapering gradually to the minutely rounded apex; with narrowly triangular basal lobes, c. 1.5×0.3 – 0.5 mm, reflexed, curved under the main lobe and connate with the basal lobes of adjoining tepals (Fig. 2E), unornamented, glabrous. *Stamens* six, staminal filaments joined to perianth tube for c. 2.5 mm, appearing as purple lines on exterior tube surface, distal part free, terete 1.8 – 2 mm long, c. 0.25 mm diam., arching inward 180 degrees towards the stigma, distal part swollen abaxially, convex, c. 0.5 mm wide, confluent with the flat expanded connective, together shaped like a duck's head (Fig. 2F); anthers ellipsoid, c. 0.8×0.6 mm, 2 thecae c. 0.1 – 0.2 mm wide, separated by the connective (Fig. 2H); connective c. 0.5×0.5 mm, not extended as a distal appendage nor adhering to the stigma surface (Fig. 2F). *Ovary* cup shaped, c. 3 mm long, c. 5 mm wide at junction with perianth; placentation axile (placenta attached at base and apex), the ovules inserted on a globose placenta held on a stalk c. 1 mm long (Fig. 2F); ovules ellipsoid c. 1.25 mm long, funicle c. 1 mm long. Style cylindrical, c. 1.5 mm long, 0.5 mm in diam. glabrous and unornamented; stigma 3-lobed nearly to the base, lobes flat, patent, smooth, glabrous, oblong triangular in outline, c. 1×2 mm, each bilobed, the subsidiary lobes lateral, reflexed (Fig. 2G). *Fruit and seed* not seen.

RECOGNITION. *Afrothismia ugandensis* Cheek differs from *A. winkleri* in the tepal lobes appearing hastate with a pair of subsidiary triangular lobes (vs no lobes in *A. winkleri*); the corona rim densely papillate (vs glabrous); the annulus entire, continuous, unlobed, densely puberulent (vs lobed, glabrous); the style and abaxial stigma glabrous (vs puberulent) and the stigmas deeply 3-lobed with bifid lobes (vs. stigma forming a shallow bowl with 6 equal, minute lobes).

DISTRIBUTION & ECOLOGY. Semideciduous submontane forest in Budongo Central Forest Reserve; 1040 – 1100 m above sea-level. No additional associated achlorophyllous species are recorded as co-occurring. Associated taxa (collected with the paratype under ATBP numbers 635 to 655) include *Caloncoba crepiniana* (De Wild. & T.Durand) Gilg (Achariaceae), *Acalypha neptunica* Müll.Arg. var. *neptunica* (Euphorbiaceae), *Ritchiea albersii* Gilg (Capparaceae), *Strombosia scheffleri* Engl. (Olacaceae), *Elatostema monticola* Hook.f. (Urticaceae), *Peperomia fernandopoiana* C.DC. (Piperaceae), *Brachystephanus africanus* S.Moore and *Brillantaisia owariensis* P.Beauv. (Acanthaceae), *Celtis gomphophylla* Baker (Cannabaceae), *Geophila uniflora* Hiern and *Hymenocoleus hirsutus* (Benth.) Robbr. (Rubiaceae), *Microlepia speluncae* (L.) T.Moore (Dennstaedtiaceae), *Cyclosorus interruptus* (Willd.) H.Itô (Thelypteridaceae), *Campylospermum densiflorum* (De Wild. & T.Durand) Farron (Ochnaceae), *Afromorus mesozygia* (Stapf) E.M.Gardner (Moraceae), *Marantochloa leucantha* (K.Schum.) Milne-Redh. (Marantaceae), *Trichilia rubescens* Oliv. (Meliaceae), *Pellaea dura* (Willd.) Hook. (Pteridaceae), *Heterotis rotundifolia* (Sm.) Jacq.-Fél. (Melastomataceae) and *Grewia flavescens* Juss. (Grewiaceae/Malvaceae: Grewoideae).

ADDITIONAL SPECIMENS EXAMINED. UGANDA. U2 (see Map in Beentje & Cheek 2003) Masindi Distr.;

Budongo Forest Reserve, Nyakafunjo Nature Reserve, unlogged forest, 01°42′40"N, 031°31′32"E, 1040 m elev., fl., 24 June 1998, *S. M. Maishanu* ATBP (African Tropical Biodiversity Programme) 653, Tropicos specimen ID 1282593, specimen missing, see notes below. Field note: "Rare saprophyte with a deep floral tube. Leaflets with root swelling. Tube mouth bright yellow with purple patch on upper part of floral tube. Base of floral tube marked with purple streaks."

This gathering was made by S. M. Maishanu (Saidu Muhammed Maishanu, most recently at the Sokoto Energy Research Centre, Usmanu Danfodiyo University, Sokoto, Nigeria) as part of a training exercise run by the ATBP (https://tropical-biology.org/conservation-projects/) in Budongo. The second author, RG, was with Maishanu at the time and assisted writing the field notes, and RG also searched at that time without success for a second individual of the *Afrothismia*, walking 200 m in each direction searching in the dense herbaceous vegetation along the forest trail. The find was not the result of a targeted search, in fact there was a delay before the specimen was identified. The specimen was identified by RG in 2000 from memory and label notes. Like all unicate specimens from the ATBP collections, the single specimen was left at MHU, however, it has not been relocated there. There is no doubt about the identification, since the description of the flower is a perfect match with that of the type specimen (MC pers. obs.).

Some effort has since been made to find more material but with no success yet. During a recent fieldwork expedition to Budongo Central Forest Reserve, the Makerere TIPAs team consisting of four botanists led by JK was joined by two experienced field station parabotanists. They conducted surveys of the forest in mid-October 2023, which was a wet part of the year (ideal for finding *Afrothismia* in flower), and searched for IPA trigger species, including *Afrothismia*. The survey covered the area surrounding the Field Station within the Nyakafunjo (N3) compartment, where the 1998 collection of *A. ugandensis* was made. This is the forest tract considered by the managers to be the most intact (undisturbed): the Strict Nature Reserve (SNR). The forest canopy was indeed thick and closed, while the forest floor was generally open, with sparse ground vegetation cover. The survey was extended to cover areas beyond the SNR up to c. 3.5 km from the station, and lasted four days, but did not yield any sign of *Afrothismia*. It is to be hoped that the species will be re-found one day, with more targeted surveys particularly during the June – August window.

CONSERVATION STATUS. Only two collections, each of a single individual, made nearly 60 years apart (Aug. 1940 and June 1998), are known of *Afrothismia ugandensis*, both from the Budongo forest. The earliest collection has no indication of where within the forest it was collected, while the second has geographical coordinates and detailed notes. Using the precautionary principle, therefore, it cannot be ruled out that the species is only known at the single, precisely recorded site. Many other species of the genus are known from a single collection at single sites within larger forest areas (see introduction references). The Area of Occupancy of *Afrothismia ugandensis* is estimated as 4 km^2, using the IUCN (2024) guidelines of 2 km × 2 km cell size, and Extent of Occurrence as slightly larger, as required by IUCN (2012, 2024).

This site is probably one of the most sampled sites for plants in Budongo, since it is only 2.45 km from the field station and along a trail. Although the plants of *Afrothismia ugandensis* are small and the flower is held at ground level, they are not completely inconspicuous due to the yellow and purple flower colour. Studies of *Afrothismia* species in the field in Cameroon (Cheek 2006; Cheek *et al.* 2004) have found that flowering can be continuous over several weeks or even months at sites but that species can be absent from apparently suitable habitat despite targeted searches (e.g. Cheek *et al.* 2000, 2010, 2011; Harvey *et al.* 2004, 2010). The few collections (and only two individuals recorded) at Budongo suggest that the species is genuinely highly range restricted and infrequent. The sole recorded site is close to the southern boundary of the forest where there appear to be fronts of forest clearance approaching about 800 m to the SW and 1.45 km to the SE (Google Earth Pro imagery dated 25 June 2017, Maxar Technologies). However, the observations from 2023 fieldwork suggest the quality of this area is stable with one less dense canopy area actually increasing its canopy cover. The presence of chimpanzees particularly bolsters the conservation of this forest. However, although the forest in Nyakafunjo Nature Reserve was said to be unlogged, not all species recorded at the site are characteristic of deep, closed forest: e.g., *Grewia platyclada* K.Schum, *Afromorus mesozygia*, *Celtis gomphophylla*, *Ritchiea albersii* (RG pers. obs.). We know nothing about the fungal symbiont or its requirements which adds to the uncertainty of the requirements of this *Afrothismia*. Even small, very local levels of disturbance may affect this very rare species.

Budongo Forest and many of its species are currently under threat from many sources: population pressure from immigration due to wars and civil unrest in neighbouring countries means cropland is scarce and the forest edges are encroached with burning and clearing; illegal pitsawing of the trees for fuelwood, charcoal and timber is well-organised; agribusiness employs outreach farmers who extend cultivation beyond forest boundaries (Budongo Field Station

2024, continuously updated) and illegal tree felling. Together, these qualify *Afrothismia ugandensis* as from a single location whose habitat quality is declining. With only two individuals known (well below the threshold of 50), the species also satisfies the requirements for the Critically Endangered category under Criterion D. The conservation status of *A. ugandensis* is, therefore, assessed provisionally here as Critically Endangered [CR B2ab(iii); D1] using the IUCN (2012) categories and criteria.

Budongo Central Forest Reserve covers an area of 822 km^2. It qualifies as an Important Plant Area under criterion A. Sub-criterion A(i) is triggered by the presence of one Critically Endangered, three Endangered and 16 Vulnerable species, while A(iii) is triggered by the presence of highly restricted endemics, *Afrothismia winkleri* var. *budongensis* [DD] and *Uvariopsis* sp. nov. 1 Uganda (IPA data sheet, Richards & Darbyshire 2024). Further detailed and up-to-date botanical data characterising the Budongo Central Forest Reserve is given in this reference.

ETYMOLOGY. Named for Uganda, where it is both endemic and the only known species of the genus in the country.

NOTES. *Afrothismia ugandensis* is remarkable in the genus for occurring in semi-deciduous (vs evergreen) forest and for having ellipsoid or ovoid (vs globose) root bulbils. The non-attachment of the stamens to the stigma is also unique in the genus, but this observation comes with the caveat that it is based on material damaged by a previous dissection (e.g. 3 of the 6 stamens are missing) and needs to be confirmed if and when new material becomes available to be sure that it is not an artefact of damage.

That *Afrothismia ugandensis* has been considered a variety of *A. winkleri* is unsurprising given their overall similarities and they may well share a recent common ancestor. The Budongo forest is considered amongst the easternmost examples of the Congolian phytogeographic domain (White 1983). Until recent geological times, the forest habitat of *Afrothismia* extended continuously across the Congo basin to the Cameroon home of *A. winkleri,* but this forest was only intermittently present in much of the Pleistocene, being replaced with grasslands in colder and drier periods (Kingdon 1989; Maley 2001). It is conceivable that the common ancestor of the two taxa once extended across the basin in wetter periods, but that separation in dry periods resulted in the two species diverging. *A. ugandensis* has little similarity with the Kenyan *A. baerae* Cheek in which the lower and upper perianth tube share the same vertical axis and the corona mainly occludes the mouth. The Ugandan species shares more similarities with the Tanzanian Eastern Arc species *A. mhoroana* Cheek and *A. insignis* but lacks the ± globose lower tube axis and verrucate perianth of those species.

The collector and illustrator of the type and only specimen seen for this paper, Joe (William Julius) Eggeling (1909 – 1994) joined the British Colonial Forestry Service in 1931 and was first posted to Uganda where his inventory and management plan of the Budongo Forest in Bunyoro was considered a masterpiece in tropical forestry (Polhill & Polhill 2015). Thirty-one plant names on IPNI (2024, continuously updated) are named in his honour, including the genus *Eggelingia* Summerh. (Orchidaceae). These are mainly Ugandan and Tanzanian species that he brought to light from among the c. 3800 herbarium specimens he collected and deposited at BM and K. He became Chief Conservator of Ugandan forests in 1945 and in his retirement in his native Scotland led many conservation initiatives that led to the creation of areas protected for biodiversity conservation (Dalyell 1994; Wikipedia, continuously updated).

Many important aspects of the biology of this fascinating genus remain imperfectly or completely unknown, such as pollination biology (only observed in one species), microsporogenesis, cytology, and seed dispersal. Budongo Forest offers the possibility of filling in these gaps in knowledge for the genus in general, as well as for *Afrothismia ugandensis* in particular.

Discussion

The case of *Afrothismia ugandensis,* formerly included in the apparently greatly disjunct Cameroon and Gabon species *A. winkleri* (albeit recognised as varietally different), suggests that it might repay effort to reexamine other such cases of apparently widely disjunct species with this range. A recent re-assessment of another such disjunct species (western Uganda and Cameroon) species, *Keetia purseglovei* Bridson (Rubiaceae), showed that the Cameroon material was a separate species from the Ugandan (Cheek & Onana 2024). A further example of an apparently widespread and Cameroon and Ugandan disjunct taxon, *Dovyalis spinosissima* Gilg (Salicaceae) also led to the recognition of distinct species in both countries after further taxonomic study (Cheek & Ngolan 2007). This taxonomic work matters because range restricted and nationally endemic species can otherwise be overlooked and not properly prioritised for conservation, increasing their risk of global extinction. Until species are documented, described and known to science, it is difficult to assess them for their IUCN Red List conservation status, and, therefore, the possibility of conserving them is reduced (Cheek *et al.* 2020). An additional example of a narrowly endemic threatened species in Uganda is *Encephalartos whitelockii* P.J.H.Hurter (Zamiaceae), which is also Critically Endangered (Kalema 2010). It is endemic to the Mpanga Gorge, where at least one other potentially threatened species is in the course of elucidation (e.g. Mucaleque *et al.* 2024).

Perhaps the highest priority for such range restricted threatened species is to conserve them in their natural habitats (e.g., by including them in Important Plant Areas, Darbyshire *et al.* 2017) and to develop species conservation action plans to improve the likelihood of their survival (e.g. Couch *et al.* 2022). Both Budongo and Mpanga Gorge are already evidenced as IPAs. Conservation in situ is crucial for fully mycotrophic species such as *Afrothismia ugandensis* since ex situ conservation is currently impossible when the autotrophic partner(s) of the relationship is unknown. Global extinction is a high possibility with highly range restricted, infrequent, single site endemic species such as *Afrothismia ugandensis*. Effort is needed if it is not to follow other African fully mycotrophic species that are already well documented as extinct (Onana & Cheek 2011; Cheek & Onana 2011; Cheek *et al.* 2018a, 2019).

Acknowledgements

We thank Melissa Bavington for her care of the spirit collection at K, including the specimen on which this paper is based. We thank two anonymous reviewers for constructive comments on an earlier version of this manuscript.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

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Publisher's Note

18. Authorship statements for Publication 6

AUTHOR'S STATEMENT PUBLICATION 6

I hereby declare that the article:

"*Afrothismia ugandensis* nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda" by Cheek, M., **Gereau, R.E.** & Kalema, J. (2024) *Kew Bulletin*, DOI: 10.1007/s12225-024-10212-5, pp. 1-11

is a part of my PhD thesis. I performed part of the original fieldwork on which the rediscovery of this species was based, participated in the conceptualization of the article, provided notes on the species' morphology and habitat, identified the associated species, and participated in researching the nomenclatural history of the genus *Afrothismia.* I contributed to writing the manuscript, particularly the sections on the protected area in which the species is found and the provisional conservation assessment. I participated in the final review process and response to reviewers' comments

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Supervisor of PhD candidate

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article "***Afrothismia ugandensis* nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda**" by Cheek, M., Gereau, R.E. & Kalema, J. (2024) *Kew Bulletin*, DOI: 10.1007/s12225-024-10212-5, pp. 1-11.

Co-Author Name	**Martin Cheek**
Affiliation	Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK
Contribution	I wrote the description of the species and contributed to the rest of the paper, and I commissioned the figures
Date	9 Oct 2024
Signature	Martin Cheek

AUTHORSHIP CONTRIBUTION STATEMENT

I hereby declare that I am a co-author of the article “***Afrothismia ugandensis* nom. nov. (Afrothismiaceae), Critically Endangered and endemic to Budongo Central Forest Reserve, Uganda**” by Cheek, M., Gereau, R.E. & Kalema, J. (2024) *Kew Bulletin*, DOI: 10.1007/s12225-024-10212-5, pp. 1-11.

Co-Author Name	**James Kalema**
Affiliation	Makerere University Herbarium, Department of Plant Sciences, Microbiology & Biotechnology, P.O. Box 7062, Kampala, Uganda
Contribution	Writing of the article; its preliminary editing; recent effort to find more material in Budongo forest (albeit with no success); provisional assessment of its conservation status against the IUCN criteria (yet to be published)
Date	9 October 2024
Signature	

19. Supplementary materials for Publication 2

Painting 1. Fischer's turaco (*Tauraco fischeri*) and white-starred robin (*Pogonocichla stellata*) found in the sub-montane forests of the NatureReserves in the East Usambara and Uluguru Mountains.

Painting 2: The narrowly endemic Udzungwa forest partridge (*Xenoperdix udzungwensis*), and grey-faced sengi (*Rhynchocyon udzungwensis*) encountering each other on the forest floor in the Kilombero Nature Reserve in the Udzungwa Mountains within the Eastern Arc mountains region.

Table S1: Online data sources used in this study: biodiversity (species lists for plants, birds, mammals, reptiles, and amphibians per site); management (revenue generation, tourist numbers, management capacity, and forest disturbance); geospatial data (species range maps, protected areas, etc.) used for the spatial analysis.

Data	Links (DOI)
Raw taxonomic list per site	https://doi.org/10.6084/m9.figshare.24311656.v1
Data on management of the Forest Nature Reserves	https://doi.org/10.6084/m9.figshare.24312466.v1
Raw geospatial data	https://doi.org/10.6084/m9.figshare.24311632.v1

Table S2: Basic attributes of 21 declared and one proposed Tanzanian Nature Forest Reserves

NFR NAME	ZONE	REGION	DISTRICT	AREA (Ha)	YEAR GAZETTED	TOTAL TANZANIAN ENDEMIC PLANT TAXA	TOTAL TANZANIAN ENDEMIC VERTEBRATE SPECIES	TOTAL NUMBER OF SINGLE SITE ENDEMIC VERTEBRATE SPECIES	TOTAL THREATENED TAXA
Amani	North	Tanga	Korogwe & Muheza	8,380	1997	122	53	10	162
Chome	North	Kilimanjaro	Same	14,607	2016	48	14	8	87
Itulu hills	West	Tabora	Sikonge	403,625.62	2019	0	2	0	24
Kalambo	SH	Rukwa	Kalambo	46539.739	2019	1	0	0	19
Kilombero	Southern Highlands	Iringa & Morogoro	Kilolo & Kilombero	134,511	2007	94	69	1	151
Magamba	North	Tanga	Lushoto	9,284	2016	34	32	4	92
Magombera	East	Morogoro	Kilombero	2,615.32	2019	9	4	0	39
Minziro	Lake	Kagera	Misenyi	25,716.65	2016	0	0	1	28
Mkingu	East	Morogoro	Mvomero	26,334	2016	77	51	15	114
Mount Hanang	Central	Manyara	Hanang	5,871	2016	8	5	1	29
Rungwe	SH	Mbeya	Mbeya Rural & Rungwe	24,680.6	2009	39	61	3	64
Mwambesi	South	Ruvuma	Tunduru	112,901	2019	0	0	0	15
Nilo	North	Tanga	Korogwe & Muheza	6,025	2007	33	51	0	97
Pindiro	South	Lindi	Kilwa	12,249	2019	1	0	0	21
Rondo	South	Lindi	Lindi Rural & Ruangwa	11,742	2007	26	10	3	80
Uluguru	East	Morogoro	Morogoro Rural, Morogoro Urban & Mvomero	24,495.4	2008	198	51	14	224
Uzungwa Scarp	SH	Iringa & Morogoro	Kilombero, Kilolo & Mufindi	32,763	2016	58	72	5	133
Pugu-Kazimzumbwi	East	Dar es Salaam & Pwani	Ilala, Kinondoni, Kisarawe & Mkuranga	8,965.30	2020	27	5	0	72
Uzigua	East	Pwani & Tanga	Chalinze, Handeni & Kilindi	27654.58	2020	0	0	0	23
Essimingor	North	Arusha	Monduli	6100	2022	1	0	0	35
Hassama Hill	Central	Manyara	Babati & Mbulu	3,815.9	2022	0	0	0	27
Nou	Central	Manyara	Babati & Mbulu	29,812.23	PROPOSED	2	2	0	28

20. Supplementary materials for Publication 3

Appendix S1. Protocols used for seed germination of *Karomia gigas*.

For the Tanzania Forest Service (TFS) ex situ population (ExSP), fruits of *Karomia gigas* were collected from two wild maternal individuals at Litipo and soaked in cold water (at room temperature) until the mesocarp imbibed. The mesocarps were removed from the seeds using sterilized tweezers. Seeds were cleaned by rinsing them with deionized water (DI), spraying them with disinfectant (one part 5.25% sodium hypochlorite in 10 parts water), and then rinsing again with DI. Seeds were sown into 4-inch potting tubes containing a sterilized potting soil containing a 1:2:1 mixture of sand, forest topsoil, and organic manure to mimic soil characteristics of natural populations. This resulted in six plants that are now planted at the TFS offices in Tanzania.

For the Missouri Botanical Garden (MBG) ExSP, fruits of *K. gigas* were collected from six wild maternal individuals found at Litipo and Mitundumbea, resulting in 131 potentially viable seeds. Several approaches were used to maximize the chance of seed germination. All seeds were pretreated with Captan (fungicide), followed by: (a) soaking overnight in 50 mL of water with a smoke paper disk, (b) soaking in water for 18 hours, or (c) no additional pretreatment. Seeds were sown in one of three substrates: (a) in Bulb Mix and covered with 1/8 inch of trap rock, (b) in Ball Perennial Mix, or (c) on a damp paper towel in a sealed plastic bag. Partial germination was successful under each protocol. Of the 131 initial seeds received, 29 germinated and one individual died post-germination, resulting in 28 individuals at MBG.

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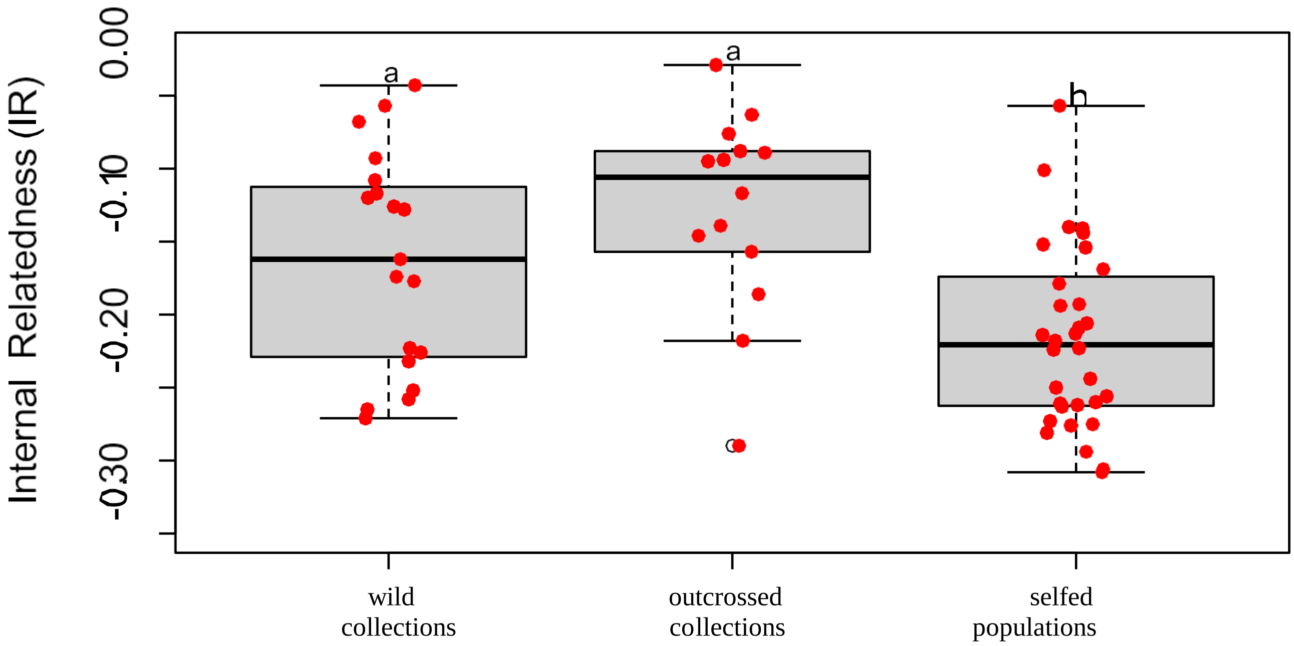
DOI 10.1002/aps3.11589

Appendix S2. List of each individual included in the study

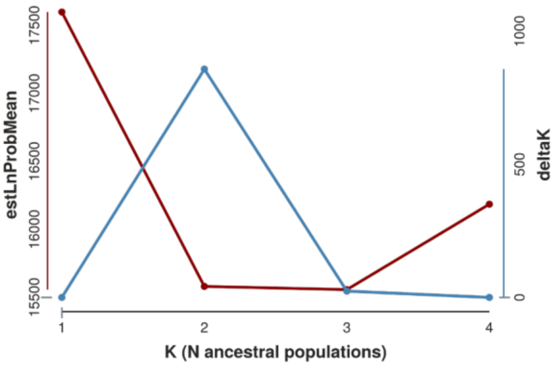
Population	Source	missing data	sample ID	Parent of ex situ individual?	Internal relatedness
MBG	ex situ		LC_2018_1449_	N/A	-0.127
MBG	ex situ	>40%	018_1450_1	N/A	0.007
MBG	ex situ		LC_2018_1450_2	N/A	-0.007
MBG	ex situ		LC_2018_1450_3	N/A	-0.176
MBG	ex situ	>40%	018_1452_1	N/A	-0.026
MBG	ex situ		LC_2018_1452_10	N/A	-0.044
MBG	ex situ		LC_2018_1452_11	N/A	-0.089
MBG	ex situ		LC_2018_1452_12	N/A	-0.107
MBG	ex situ		LC_2018_1452_3	N/A	-0.24
MBG	ex situ		LC_2018_1452_4	N/A	-0.013
MBG	ex situ		LC_2018_1452_5	N/A	-0.168
MBG	ex situ		LC_2018_1452_6	N/A	-0.078
MBG	ex situ		LC_2018_1452_7	N/A	-0.039
MBG	ex situ		LC_2018_1452_8	N/A	-0.136
MBG	ex situ		LC_2018_1452_9	N/A	-0.067
MBG	ex situ		LC_2018_1454_1	N/A	-0.112
MBG	ex situ		LC_2018_1454_2	N/A	-0.182
MBG	ex situ		LC_2018_1454_3	N/A	-0.215
MBG	ex situ		LC_2018_1455_1	N/A	-0.076
MBG	ex situ		LC_2018_1455_2	N/A	0.021
MBG	ex situ		LC_2018_1456_1	N/A	-0.07
MBG	ex situ		LC_2018_1456_2	N/A	-0.208
MBG	ex situ		LC_2018_1457_1	N/A	-0.233
MBG	ex situ		LC_2018_1457_2	N/A	-0.221
MBG	ex situ		LC_2018_1457_3	N/A	-0.202
MBG	ex situ		LC_2018_1458_1	N/A	-0.067
MBG	ex situ		LC_2018_1458_2	N/A	-0.038
MBG	ex situ		LC_2018_1458_3	N/A	-0.096
Litipo	wild		LT1	yes	-0.144
Litipo	wild		LT10	no	-0.104
Litipo	wild		LT11	no	-0.129
Litipo	wild	>40%	LT12	no	-0.144
Litipo	wild	>40%	LT13	no	-0.164
Litipo	wild		LT14	yes	-0.051
Litipo	wild		LT15	no	-0.09

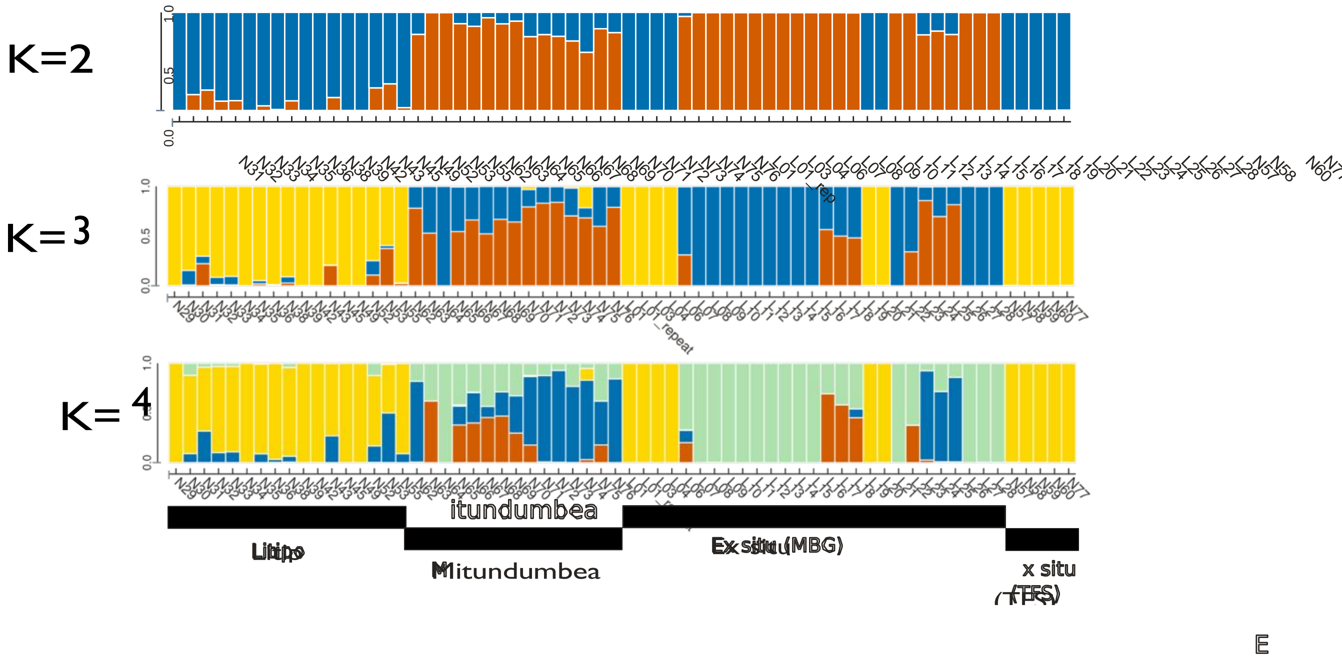
Litipo	wild	>40%	LT18	yes	-0.078
Litipo	wild	>40%	LT16	no	0.021
Litipo	wild		LT17	yes	-0.007
Litipo	wild	>40%	LT19	no	0.174
Litipo	wild		LT2	yes	-0.163
Litipo	wild	>40%	LT20	yes	-0.017
Litipo	wild		LT21	yes	-0.206
Litipo	wild	>40%	LT22	no	-0.081
Litipo	wild	>40%	LT23	no	0.223
Litipo	wild		LT24	no	-0.094
Litipo	wild		LT25	no	-0.159
Litipo	wild	>40%	LT26	no	-0.12
Litipo	wild		LT27	yes	-0.2
Litipo	wild	>40%	LT28	no	0.021
Litipo	wild		LT3	no	-0.119
Litipo	wild		LT4	no	-0.102
Litipo	wild		LT5	no	-0.226
Litipo	wild		LT6	yes	-0.173
Litipo	wild		LT7	no	-0.091
Litipo	wild		LT8	yes	-0.225
Litipo	wild	>40%	LT9	no	0.175
Mitundumbea	wild		MT1	yes	-0.212
Mitundumbea	wild		MT10	no	-0.164
Mitundumbea	wild		MT11	no	-0.168
Mitundumbea	wild		MT12	no	-0.244
Mitundumbea	wild		MT13	no	-0.156
Mitundumbea	wild		MT14	no	-0.211
Mitundumbea	wild		MT15	no	-0.143
Mitundumbea	wild		MT2	yes	-0.213
Mitundumbea	wild		MT3	yes	-0.231
Mitundumbea	wild		MT4	no	-0.21
Mitundumbea	wild		MT5	no	-0.258
Mitundumbea	wild		MT6	yes	-0.256
Mitundumbea	wild		MT7	no	-0.174
Mitundumbea	wild		MT8	yes	-0.194
Mitundumbea	wild		MT9	yes	-0.223
TFS	ex situ		SD57_FM46	N/A	-0.173
TFS	ex situ		SD58_FM47	N/A	-0.018
TFS	ex situ		SD59_FM46	N/A	-0.124
TFS	ex situ		SD60_FM47	N/A	-0.045
TFS	ex situ	>40%	SD61_FM48	N/A	-0.043
TFS	ex situ		SD77_FM48	N/A	-0.058

Appendix S3. Comparison of internal relatedness (IR) values between selfed and outcrossed collections and wild populations as inferred by parentage analyses. Boxplots with different letters denote statistically significant differences from one another based on Tukey’s HSD comparison of means (α = 0.05).



Appendix S4. Analysis of *K* estimation, showing the delta *K* (blue) and the probability of the data (red)





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DOI 10.1002/aps3.11589

Appendix S5. Pairwise kinship matrix of all individuals in the study as measured by beta, calculated usin

	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1
LC_2018_1	-0.25	0.35	0.14	0.16	0.16	0.02	-0.12	-0.12
LC_2018_1	0.35	-0.18	0.16	0.15	0.18	0.01	-0.12	-0.1
LC_2018_1	0.14	0.16	0.04	0.27	0.31	-0.12	0.03	0.02
LC_2018_1	0.16	0.15	0.27	-0.02	0.2	-0.09	-0.09	-0.06
LC_2018_1	0.16	0.18	0.31	0.2	-0.17	0.03	-0.03	-0.02
LC_2018_1	0.02	0.01	-0.12	-0.09	0.03	0.07	0.24	0.38
LC_2018_1	-0.12	-0.12	0.03	-0.09	-0.03	0.24	-0.2	0.25
LC_2018_1	-0.12	-0.1	0.02	-0.06	-0.02	0.38	0.25	0.07
LC_2018_1	-0.14	-0.11	-0.03	-0.08	-0.07	0.46	0.19	0.33
LC_2018_1	-0.17	-0.15	-0.17	-0.14	-0.11	0.35	0.16	0.33
LC_2018_1	-0.17	-0.14	-0.05	-0.12	-0.09	0.38	0.2	0.36
LC_2018_1	-0.12	-0.11	-0.02	-0.06	-0.05	0.42	0.23	0.36
LC_2018_1	-0.19	-0.17	-0.06	-0.12	-0.11	0.39	0.2	0.33
LC_2018_1	-0.14	-0.13	0.08	-0.08	-0.06	0.44	0.2	0.37
LC_2018_1	-0.18	-0.15	-0.03	-0.13	-0.12	0.36	0.2	0.32
LC_2018_1	-0.11	-0.11	0.03	-0.06	-0.06	0.55	0.22	0.39
LC_2018_1	-0.19	-0.2	-0.09	-0.16	-0.14	0.15	0.08	0.13
LC_2018_1	-0.15	-0.17	-0.11	-0.11	-0.1	0.2	0.1	0.17
LC_2018_1	-0.18	-0.17	-0.07	-0.13	-0.11	0.13	0.14	0.17
LC_2018_1	0.18	0.16	0.37	0.37	0.21	0.13	-0.06	-0.03
LC_2018_1	0.25	0.23	0.38	0.35	0.31	0.08	0.05	0.03
LC_2018_1	-0.14	-0.13	0.04	-0.09	-0.04	0.39	0.24	0.38
LC_2018_1	-0.13	-0.14	-0.03	-0.11	-0.09	0.23	0.15	0.24
LC_2018_1	-0.15	-0.15	-0.12	-0.14	-0.1	0.07	0.02	0
LC_2018_1	-0.11	-0.12	-0.17	-0.06	-0.09	0.12	0.07	0.13
LC_2018_1	-0.12	-0.14	-0.01	-0.11	-0.1	0.07	0.02	0.07
LC_2018_1	-0.13	-0.13	-0.01	-0.09	-0.05	0.27	0.2	0.37
LC_2018_1	-0.07	-0.07	0.08	-0.07	-0.02	0.35	0.23	0.38
LC_2018_1	-0.05	-0.06	0.12	-0.01	-0.03	0.42	0.23	0.33
LT1	0.23	0.2	0.43	0.34	0.29	-0.02	-0.04	0
LT2	-0.06	-0.07	-0.05	0.03	0	0.08	-0.05	0.03
LT3	0.07	0.06	-0.02	0.08	0.11	0.04	-0.09	-0.06
LT4	0.07	0.07	0.19	0.06	0.18	-0.18	-0.07	-0.05
LT5	0	0.04	0.12	-0.02	0.13	-0.05	-0.07	-0.07
LT6	0.16	0.18	0.33	0.18	0.18	0.3	-0.1	-0.08
LT7	0.09	0.1	0.22	0.22	0.19	-0.03	-0.1	-0.07
LT8	0.04	0.03	0.12	0.15	0.13	-0.01	-0.05	-0.04
LT9	0.31	0.34	0.45	0.39	0.53	0.15	0.22	0.05
LT10	0.09	0.1	0.12	0.07	0.13	0.21	-0.03	-0.01
LT11	0.15	0.16	0.22	0.15	0.21	-0.06	-0.11	-0.08
LT12	-0.07	0.07	0.16	-0.28	-0.05	-0.09	-0.1	-0.29
LT13	0.14	0.16	0.2	0.02	0.15	-0.21	-0.21	-0.4
LT14	0.23	0.21	0.27	0.2	0.29	0.07	-0.07	-0.07

LT15	0.06	0.07	0.15	-0.02	0.08	0.08	-0.08	-0.1
LT16	0.14	0.13	0.39	0.02	0.13	-0.22	-0.09	-0.12
LT17	0.24	0.23	0.27	0.24	0.23	-0.1	-0.09	-0.13
LT18	0.12	0.05	0.09	0.17	0.22	0.11	0.01	0.01
LT19	-0.1	0.09	0.15	-0.03	0.17	-0.33	-0.11	-0.05
LT20	0.24	0.27	0.32	0.21	0.34	0.02	0.04	0.01
LT21	0.16	0.17	0.02	0.01	0.18	0.08	-0.13	-0.1
LT22	0.01	-0.05	0.04	-0.08	-0.05	-0.05	-0.19	-0.16
LT23	0.45	0.25	0.08	0.28	0.52	-0.55	0.28	-0.61
LT24	0	0.02	0.14	0.04	0.09	0.05	0.01	0.01
LT25	-0.05	-0.05	0.08	0.03	0.03	0	-0.06	0.02
LT26	-0.01	0.05	0.17	0.11	0.12	-0.41	-0.38	-0.37
LT27	0.02	0.02	0.09	0.12	0.1	0.05	-0.09	-0.08
LT28	0.09	0.11	0.22	0.08	0.1	0.05	0.08	-0.1
SD57_FM4	0.02	0.03	0.06	0.17	0.09	-0.05	-0.11	-0.08
SD58_FM4	0.22	0.2	0.35	0.36	0.25	0.15	0.05	0.01
SD59_FM4	0.04	0.05	0.02	0.18	0.1	0.05	-0.06	-0.04
SD60_FM4	0.18	0.18	0.38	0.33	0.25	0.22	0.02	0.03
SD61_FM4	0.18	0.17	0.42	0.31	0.25	-0.18	-0.07	-0.12
MT1	-0.09	-0.11	-0.06	-0.1	-0.03	0.1	0.05	0.07
MT2	-0.15	-0.17	-0.08	-0.13	-0.11	0.2	0.1	0.16
MT3	-0.19	-0.17	-0.06	-0.12	-0.1	0.35	0.21	0.38
MT4	-0.17	-0.18	-0.12	-0.13	-0.1	0.12	0.04	0.11
MT5	-0.18	-0.16	-0.15	-0.11	-0.15	0.25	0.01	0.06
MT6	-0.16	-0.16	-0.12	-0.13	-0.11	0.14	0.08	0.14
MT7	-0.12	-0.12	-0.02	-0.04	-0.05	0.12	0.1	0.12
MT8	-0.12	-0.12	-0.08	-0.11	-0.07	0.07	0.21	0.15
MT9	-0.12	-0.13	-0.08	-0.09	-0.09	0.1	0.08	0.09
MT10	-0.19	-0.14	-0.09	-0.13	-0.13	0.15	0.02	0.04
MT11	-0.14	-0.13	-0.16	-0.09	-0.1	0.22	0.02	0.05
MT12	-0.12	-0.09	-0.02	-0.07	-0.07	0.28	0.04	0.08
MT13	-0.05	-0.06	-0.03	-0.05	-0.05	0.15	0	0
MT14	-0.14	-0.12	-0.09	-0.07	-0.08	0.22	0.1	0.15
MT15	-0.1	-0.1	-0.08	-0.08	-0.12	0.08	-0.01	0.08
SD77_FM4	0.19	0.16	0.44	0.36	0.29	0.03	0	-0.02

g the “beta.dosage” function in the R package HIERFSTAT v.0.5-11.

LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1
-0.14	-0.17	-0.17	-0.12	-0.19	-0.14	-0.18	-0.11	-0.19
-0.11	-0.15	-0.14	-0.11	-0.17	-0.13	-0.15	-0.11	-0.2
-0.03	-0.17	-0.05	-0.02	-0.06	0.08	-0.03	0.03	-0.09
-0.08	-0.14	-0.12	-0.06	-0.12	-0.08	-0.13	-0.06	-0.16
-0.07	-0.11	-0.09	-0.05	-0.11	-0.06	-0.12	-0.06	-0.14
0.46	0.35	0.38	0.42	0.39	0.44	0.36	0.55	0.15
0.19	0.16	0.2	0.23	0.2	0.2	0.2	0.22	0.08
0.33	0.33	0.36	0.36	0.33	0.37	0.32	0.39	0.13
-0.12	0.29	0.27	0.32	0.3	0.31	0.33	0.35	0.1
0.29	-0.07	0.36	0.29	0.32	0.33	0.31	0.35	0.08
0.27	0.36	0	0.35	0.33	0.34	0.33	0.38	0.07
0.32	0.29	0.35	-0.07	0.33	0.32	0.34	0.37	0.07
0.3	0.32	0.33	0.33	-0.03	0.3	0.35	0.36	0.07
0.31	0.33	0.34	0.32	0.3	0	0.34	0.38	0.13
0.33	0.31	0.33	0.34	0.35	0.34	-0.05	0.39	0.04
0.35	0.35	0.38	0.37	0.36	0.38	0.39	-0.01	0.1
0.1	0.08	0.07	0.07	0.07	0.13	0.04	0.1	-0.16
0.14	0.11	0.11	0.12	0.1	0.15	0.11	0.15	0.24
0.11	0.11	0.13	0.16	0.13	0.15	0.13	0.18	0.15
-0.07	-0.14	-0.12	-0.04	-0.11	-0.06	-0.12	-0.04	-0.17
0.05	-0.03	-0.01	0.01	-0.1	0.01	-0.07	0.03	-0.09
0.33	0.33	0.36	0.37	0.37	0.37	0.35	0.39	0.08
0.17	0.18	0.19	0.2	0.17	0.19	0.17	0.23	0.19
-0.02	-0.04	-0.04	-0.03	-0.06	-0.01	-0.03	0	-0.08
0.03	0.04	0.03	0.07	0.05	0.06	0.05	0.08	0.01
-0.01	-0.05	-0.03	0.03	-0.05	0	-0.03	0.04	-0.09
0.31	0.36	0.38	0.35	0.39	0.35	0.36	0.37	0.11
0.34	0.29	0.32	0.35	0.34	0.34	0.36	0.39	0.12
0.34	0.32	0.32	0.35	0.35	0.33	0.35	0.41	0.09
-0.03	-0.11	-0.1	-0.02	-0.12	-0.05	-0.11	-0.03	-0.15
0.02	-0.03	0	0	-0.03	-0.03	-0.01	0.04	-0.18
-0.07	-0.13	-0.07	-0.07	-0.09	-0.11	-0.15	-0.08	-0.16
-0.06	-0.13	-0.11	-0.09	-0.12	-0.13	-0.12	-0.04	-0.21
-0.06	-0.17	-0.16	-0.15	-0.11	-0.13	-0.09	-0.08	-0.15
-0.1	-0.16	-0.13	-0.08	-0.15	-0.13	-0.15	-0.1	-0.18
-0.09	-0.13	-0.12	-0.07	-0.11	-0.1	-0.13	-0.07	-0.16
-0.07	-0.1	-0.08	-0.05	-0.1	-0.08	-0.09	-0.05	-0.14
0.08	-0.21	-0.09	0.03	-0.3	0	-0.06	-0.09	-0.12
-0.03	-0.09	-0.05	-0.02	-0.09	-0.04	-0.08	-0.03	-0.14
-0.1	-0.19	-0.14	-0.08	-0.15	-0.12	-0.15	-0.09	-0.23
-0.32	-0.3	-0.31	-0.26	-0.37	-0.14	-0.2	-0.19	-0.32
-0.24	-0.5	-0.55	-0.49	-0.43	-0.19	-0.22	-0.26	-0.15
-0.08	-0.15	-0.15	-0.1	-0.16	-0.12	-0.15	-0.11	-0.14

-0.08	-0.16	-0.15	-0.12	-0.14	-0.09	-0.16	-0.11	-0.15
-0.08	-0.35	-0.23	-0.12	-0.23	-0.24	-0.21	-0.16	-0.15
-0.08	-0.23	-0.2	-0.1	-0.21	-0.15	-0.17	-0.12	-0.29
0.02	-0.05	-0.05	-0.01	-0.06	-0.03	-0.02	0.01	-0.11
-0.25	-0.27	-0.38	-0.12	-0.54	-0.33	-0.27	-0.09	-0.31
0.01	-0.09	-0.07	0.01	-0.11	0.01	-0.07	-0.01	-0.13
-0.13	-0.19	-0.14	-0.11	-0.16	-0.17	-0.17	-0.12	-0.2
-0.04	-0.2	-0.18	-0.08	-0.2	-0.1	-0.08	0.02	-0.17
-0.13	-0.55	-0.57	-0.35	-0.51	-0.41	0.02	-0.44	-0.24
0.01	-0.05	-0.03	0.01	-0.05	-0.01	-0.03	0.02	-0.12
-0.07	-0.08	-0.05	-0.04	-0.07	-0.03	-0.07	-0.02	-0.16
-0.32	-0.32	-0.25	-0.19	-0.45	-0.32	-0.22	-0.26	-0.36
-0.1	-0.12	-0.1	-0.11	-0.14	-0.11	-0.13	-0.06	-0.18
-0.01	-0.26	-0.13	-0.08	-0.17	-0.1	-0.08	-0.09	-0.15
-0.12	-0.14	-0.11	-0.09	-0.14	-0.12	-0.14	-0.07	-0.18
0	-0.04	-0.02	0.07	-0.06	0.01	-0.08	0.01	-0.09
-0.07	-0.09	-0.07	-0.06	-0.08	-0.06	-0.08	0	-0.16
-0.02	-0.06	-0.03	0.02	-0.04	0.01	-0.03	0.04	-0.09
-0.14	-0.18	-0.13	-0.08	-0.19	-0.13	-0.17	-0.1	-0.16
0.02	0	0.01	0.02	-0.01	0.06	0.02	0.05	-0.01
0.13	0.08	0.1	0.11	0.12	0.14	0.1	0.16	0.3
0.33	0.32	0.36	0.35	0.35	0.34	0.36	0.4	0.09
0.09	0.05	0.11	0.12	0.06	0.06	0.1	0.14	0.08
0	-0.03	0.02	0.03	0.02	0.02	0	0.08	0.07
0.08	0.06	0.1	0.13	0.11	0.1	0.09	0.16	0.15
0.04	0.01	0.04	0.08	0.06	0.06	0.02	0.09	0.05
0.08	0.05	0.11	0.12	0.1	0.1	0.09	0.13	0.02
0.02	0.01	0.06	0.09	0.02	0.05	0.03	0.08	-0.01
-0.02	0	-0.02	0.01	0	-0.05	-0.03	0.04	-0.05
-0.01	0	-0.01	0.03	-0.01	0	-0.02	0.05	-0.04
0.01	0.01	0.02	0.05	0.02	0.03	0	0.06	-0.02
-0.07	-0.05	-0.06	-0.03	-0.04	-0.05	-0.05	0	-0.08
0.12	0.05	0.09	0.13	0.06	0.07	0.05	0.13	0.02
0.03	0.01	0.02	0.03	0.02	0.05	0	0.06	0
0	-0.1	-0.06	0	-0.12	-0.02	-0.09	-0.02	-0.13

LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1	LC_2018_1
-0.15	-0.18	0.18	0.25	-0.14	-0.13	-0.15	-0.11	-0.12
-0.17	-0.17	0.16	0.23	-0.13	-0.14	-0.15	-0.12	-0.14
-0.11	-0.07	0.37	0.38	0.04	-0.03	-0.12	-0.17	-0.01
-0.11	-0.13	0.37	0.35	-0.09	-0.11	-0.14	-0.06	-0.11
-0.1	-0.11	0.21	0.31	-0.04	-0.09	-0.1	-0.09	-0.1
0.2	0.13	0.13	0.08	0.39	0.23	0.07	0.12	0.07
0.1	0.14	-0.06	0.05	0.24	0.15	0.02	0.07	0.02
0.17	0.17	-0.03	0.03	0.38	0.24	0	0.13	0.07
0.14	0.11	-0.07	0.05	0.33	0.17	-0.02	0.03	-0.01
0.11	0.11	-0.14	-0.03	0.33	0.18	-0.04	0.04	-0.05
0.11	0.13	-0.12	-0.01	0.36	0.19	-0.04	0.03	-0.03
0.12	0.16	-0.04	0.01	0.37	0.2	-0.03	0.07	0.03
0.1	0.13	-0.11	-0.1	0.37	0.17	-0.06	0.05	-0.05
0.15	0.15	-0.06	0.01	0.37	0.19	-0.01	0.06	0
0.11	0.13	-0.12	-0.07	0.35	0.17	-0.03	0.05	-0.03
0.15	0.18	-0.04	0.03	0.39	0.23	0	0.08	0.04
0.24	0.15	-0.17	-0.09	0.08	0.19	-0.08	0.01	-0.09
-0.2	0.17	-0.12	-0.05	0.13	0.2	-0.04	0.02	-0.03
0.17	-0.23	-0.13	-0.01	0.18	0.17	0.02	0.05	0
-0.12	-0.13	-0.07	0.38	-0.06	-0.09	-0.12	-0.08	-0.07
-0.05	-0.01	0.38	0.09	0.02	-0.06	0.01	-0.04	0.04
0.13	0.18	-0.06	0.02	0	0.23	-0.01	0.08	-0.01
0.2	0.17	-0.09	-0.06	0.23	-0.2	-0.03	0.03	0
-0.04	0.02	-0.12	0.01	-0.01	-0.03	-0.32	0.09	0.15
0.02	0.05	-0.08	-0.04	0.08	0.03	0.09	-0.25	0.05
-0.03	0	-0.07	0.04	-0.01	0	0.15	0.05	-0.28
0.16	0.17	-0.09	-0.04	0.35	0.23	-0.03	0.05	-0.02
0.18	0.17	-0.04	0.03	0.38	0.21	-0.03	0.06	-0.01
0.14	0.2	0	0.07	0.36	0.2	-0.02	0.1	0.07
-0.14	-0.14	0.37	0.36	-0.06	-0.09	-0.08	-0.04	-0.05
-0.09	-0.1	0.04	0.05	0.04	-0.12	-0.15	-0.06	-0.12
-0.12	-0.15	0.1	0.07	-0.11	-0.07	-0.17	-0.09	-0.1
-0.16	-0.16	0.11	0.15	-0.07	-0.17	-0.17	-0.1	-0.15
-0.13	-0.14	0.07	0.13	-0.08	-0.14	-0.22	-0.11	-0.16
-0.17	-0.17	0.19	0.22	-0.12	-0.15	-0.2	-0.1	-0.16
-0.12	-0.15	0.21	0.23	-0.05	-0.14	-0.17	-0.1	-0.12
-0.15	-0.12	0.15	0.19	-0.1	-0.12	-0.16	-0.09	-0.12
-0.09	-0.13	0.36	0.43	-0.17	-0.05	0	-0.1	0.27
-0.11	-0.1	0.1	0.14	-0.03	-0.09	-0.15	-0.1	-0.09
-0.18	-0.2	0.21	0.22	-0.1	-0.16	-0.18	-0.16	-0.2
-0.36	-0.31	-0.07	-0.09	-0.15	-0.27	-0.4	-0.32	-0.13
-0.19	-0.1	0.17	0.21	-0.27	-0.1	-0.19	-0.17	-0.14
-0.16	-0.15	0.28	0.3	-0.13	-0.12	-0.13	-0.13	-0.11

-0.17	-0.18	0.07	0.04	-0.15	-0.12	-0.18	-0.16	-0.13
-0.25	-0.1	0.14	0.12	-0.11	-0.23	-0.14	-0.23	-0.14
-0.22	-0.19	0.29	0.19	-0.14	-0.2	-0.19	-0.2	-0.16
-0.06	-0.07	0.19	0.21	0.01	-0.09	-0.06	-0.11	-0.04
-0.33	-0.17	0.26	0.12	-0.2	-0.38	-0.38	-0.4	-0.06
-0.15	-0.09	0.28	0.28	-0.06	-0.03	-0.06	-0.1	-0.03
-0.19	-0.21	0.03	0.08	-0.15	-0.14	-0.2	-0.16	-0.18
-0.08	-0.18	-0.05	0.02	-0.09	-0.24	-0.29	-0.24	-0.18
-0.49	-0.46	0.39	0.25	-0.08	-0.39	-0.17	-0.1	-0.5
-0.07	-0.07	0.09	0.13	-0.03	-0.09	-0.07	-0.05	-0.07
-0.07	-0.09	0.03	0.09	-0.06	-0.07	-0.14	-0.07	-0.1
-0.35	-0.33	0.11	0.06	-0.22	-0.49	-0.41	-0.46	-0.32
-0.1	-0.13	0.12	0.19	-0.07	-0.12	-0.15	-0.08	-0.12
-0.15	-0.13	0.19	0.22	0	-0.28	-0.19	-0.26	-0.19
-0.13	-0.11	0.13	0.2	-0.09	-0.14	-0.14	-0.11	-0.13
-0.06	-0.05	0.43	0.41	-0.01	0.05	-0.01	-0.03	-0.02
-0.12	-0.08	0.16	0.2	-0.02	-0.09	-0.1	-0.07	-0.11
-0.06	-0.06	0.38	0.4	0	-0.02	-0.05	-0.03	0
-0.17	-0.16	0.3	0.36	-0.11	-0.15	-0.17	-0.17	-0.2
0.04	0.02	-0.06	0.06	0.04	0.03	0.17	0.17	0.16
0.27	0.17	-0.13	-0.08	0.14	0.21	-0.02	0.03	-0.03
0.11	0.13	-0.1	-0.07	0.38	0.19	-0.05	0.03	0
0.07	0.05	-0.11	-0.05	0.08	0.1	-0.05	-0.01	-0.04
0.06	0.03	-0.12	0.04	0.03	0.02	-0.05	0	-0.04
0.13	0.11	-0.09	-0.03	0.1	0.09	-0.02	0.03	-0.01
0.08	0.09	-0.04	0.09	0.04	0.05	0.01	0.09	-0.02
0	0.16	-0.07	0.03	0.14	0.06	0.05	0.04	0.03
0.02	0.05	-0.05	0.11	0.06	0.06	0.07	0.04	0.05
-0.01	0.03	-0.1	0.01	-0.03	0.02	0.02	0.05	0
0	0.01	-0.11	0	0.02	-0.03	-0.01	0.03	-0.05
0	0.02	-0.07	-0.01	0.05	0.01	-0.02	0.02	-0.08
-0.04	-0.06	-0.02	0.05	-0.02	-0.02	-0.08	0.04	-0.08
0.08	0.05	-0.07	0.06	0.09	0.06	0.01	0.02	-0.01
0	0.01	-0.1	0	0.02	0.06	0.06	0.14	0.06
-0.13	-0.16	0.36	0.35	-0.05	-0.06	-0.04	-0.06	-0.08

LC_2018_1	LC_2018_1	LC_2018_1	LT1	LT2	LT3	LT4	LT5	LT6
-0.13	-0.07	-0.05	0.23	-0.06	0.07	0.07	0	0.16
-0.13	-0.07	-0.06	0.2	-0.07	0.06	0.07	0.04	0.18
-0.01	0.08	0.12	0.43	-0.05	-0.02	0.19	0.12	0.33
-0.09	-0.07	-0.01	0.34	0.03	0.08	0.06	-0.02	0.18
-0.05	-0.02	-0.03	0.29	0	0.11	0.18	0.13	0.18
0.27	0.35	0.42	-0.02	0.08	0.04	-0.18	-0.05	0.3
0.2	0.23	0.23	-0.04	-0.05	-0.09	-0.07	-0.07	-0.1
0.37	0.38	0.33	0	0.03	-0.06	-0.05	-0.07	-0.08
0.31	0.34	0.34	-0.03	0.02	-0.07	-0.06	-0.06	-0.1
0.36	0.29	0.32	-0.11	-0.03	-0.13	-0.13	-0.17	-0.16
0.38	0.32	0.32	-0.1	0	-0.07	-0.11	-0.16	-0.13
0.35	0.35	0.35	-0.02	0	-0.07	-0.09	-0.15	-0.08
0.39	0.34	0.35	-0.12	-0.03	-0.09	-0.12	-0.11	-0.15
0.35	0.34	0.33	-0.05	-0.03	-0.11	-0.13	-0.13	-0.13
0.36	0.36	0.35	-0.11	-0.01	-0.15	-0.12	-0.09	-0.15
0.37	0.39	0.41	-0.03	0.04	-0.08	-0.04	-0.08	-0.1
0.11	0.12	0.09	-0.15	-0.18	-0.16	-0.21	-0.15	-0.18
0.16	0.18	0.14	-0.14	-0.09	-0.12	-0.16	-0.13	-0.17
0.17	0.17	0.2	-0.14	-0.1	-0.15	-0.16	-0.14	-0.17
-0.09	-0.04	0	0.37	0.04	0.1	0.11	0.07	0.19
-0.04	0.03	0.07	0.36	0.05	0.07	0.15	0.13	0.22
0.35	0.38	0.36	-0.06	0.04	-0.11	-0.07	-0.08	-0.12
0.23	0.21	0.2	-0.09	-0.12	-0.07	-0.17	-0.14	-0.15
-0.03	-0.03	-0.02	-0.08	-0.15	-0.17	-0.17	-0.22	-0.2
0.05	0.06	0.1	-0.04	-0.06	-0.09	-0.1	-0.11	-0.1
-0.02	-0.01	0.07	-0.05	-0.12	-0.1	-0.15	-0.16	-0.16
0	0.34	0.33	-0.09	0.01	-0.06	-0.07	-0.13	-0.11
0.34	0.03	0.37	-0.04	0.01	-0.08	-0.07	-0.08	-0.1
0.33	0.37	-0.01	0.01	-0.01	-0.06	-0.06	-0.02	-0.1
-0.09	-0.04	0.01	-0.11	0.05	0.09	0.08	0.08	0.26
0.01	0.01	-0.01	0.05	-0.2	0.02	-0.02	-0.05	-0.05
-0.06	-0.08	-0.06	0.09	0.02	-0.17	0.03	0.03	0.07
-0.07	-0.07	-0.06	0.08	-0.02	0.03	-0.16	0.3	0.06
-0.13	-0.08	-0.02	0.08	-0.05	0.03	0.3	-0.32	0.05
-0.11	-0.1	-0.1	0.26	-0.05	0.07	0.06	0.05	-0.22
-0.09	-0.07	-0.04	0.26	0	0.22	0.06	0.05	0.13
-0.06	-0.06	-0.06	0.21	0.16	0.02	-0.03	-0.07	0.08
-0.14	0.09	-0.01	0.35	0.08	0.11	0.26	0.21	0.42
0	0.02	0	0.14	0.05	-0.02	0.09	0.02	0.13
-0.11	-0.05	-0.06	0.25	0	0.05	0.14	0.09	0.17
-0.19	-0.21	-0.11	-0.03	-0.12	-0.25	0.01	0.1	0.09
-0.38	-0.28	-0.12	0.08	-0.28	-0.2	-0.06	0.09	0.26
-0.13	-0.05	-0.09	0.23	0	0.08	0.11	0.15	0.28

-0.07	-0.06	-0.12	0.07	-0.02	-0.02	0.03	0.07	0.11
-0.17	-0.16	-0.01	0.11	-0.1	-0.22	0.03	0.12	0.22
-0.14	-0.07	-0.06	0.28	-0.03	0	0.09	0.12	0.26
-0.04	0.07	0.02	0.2	0.15	0.03	0.12	0.2	0.22
-0.25	-0.23	0.17	0.01	0.13	0.09	0.14	0.12	0
0	0.05	0.05	0.3	0.04	0.01	0.2	0.2	0.27
-0.11	-0.08	-0.12	0.09	-0.07	0.03	0.12	0.06	0.16
-0.16	-0.06	-0.06	-0.04	-0.03	-0.28	-0.13	-0.16	0.04
-0.3	-0.02	0.12	0.42	0.49	0.17	0.01	0.01	0.46
0.06	0.01	0.01	0.12	0.11	-0.01	0.08	0.01	0.03
-0.04	0.01	-0.01	-0.01	-0.08	-0.04	-0.05	-0.11	-0.03
-0.22	-0.12	-0.03	0.11	0.01	-0.05	0.05	-0.12	0.02
-0.08	-0.06	-0.03	0.15	0.14	0.02	-0.01	-0.06	0.01
-0.19	0	-0.05	0.22	0.05	0.04	-0.06	-0.02	0.23
-0.08	-0.06	-0.03	0.2	0.16	0	-0.05	-0.13	0.04
-0.03	0.01	0.06	0.4	0.02	0.07	0.05	0.1	0.27
-0.04	-0.02	0.02	0.21	0.19	0.01	-0.05	-0.06	0.04
-0.01	0.03	0.04	0.43	0.09	0.1	0.09	0.06	0.24
-0.17	-0.11	-0.11	0.37	-0.03	-0.03	0.05	0.07	0.27
0.02	0	0.05	-0.04	-0.15	-0.1	-0.15	-0.15	-0.12
0.14	0.14	0.14	-0.15	-0.13	-0.14	-0.19	-0.12	-0.15
0.37	0.34	0.37	-0.09	-0.02	-0.11	-0.1	-0.13	-0.17
0.11	0.1	0.09	-0.14	-0.1	-0.09	-0.17	-0.2	-0.15
0.07	0.06	0.04	-0.14	-0.11	-0.13	-0.13	-0.23	-0.16
0.11	0.11	0.13	-0.11	-0.11	-0.15	-0.14	-0.12	-0.12
0.05	0.08	0.09	-0.04	-0.09	-0.09	-0.13	-0.11	-0.12
0.1	0.12	0.14	-0.03	-0.07	-0.13	-0.11	-0.1	-0.11
0.04	0.04	0.07	-0.06	-0.08	-0.12	-0.12	-0.15	-0.14
0.03	-0.02	0	-0.12	-0.08	-0.1	-0.12	-0.15	-0.15
0.03	0.01	0.02	-0.09	-0.09	-0.11	-0.11	-0.16	-0.14
0.06	0.07	0.07	-0.05	-0.03	-0.11	-0.1	-0.15	-0.11
-0.04	-0.04	-0.04	-0.04	-0.03	-0.07	-0.1	-0.11	-0.07
0.09	0.11	0.08	-0.05	-0.06	-0.12	-0.11	-0.13	-0.11
0.03	0.01	0.02	-0.08	-0.12	-0.12	-0.18	-0.18	-0.11
-0.09	-0.02	0	0.39	-0.03	0.02	0.05	0.11	0.31

LT7	LT8	LT9	LT10	LT11	LT12	LT13	LT14	LT15
0.09	0.04	0.31	0.09	0.15	-0.07	0.14	0.23	0.06
0.1	0.03	0.34	0.1	0.16	0.07	0.16	0.21	0.07
0.22	0.12	0.45	0.12	0.22	0.16	0.2	0.27	0.15
0.22	0.15	0.39	0.07	0.15	-0.28	0.02	0.2	-0.02
0.19	0.13	0.53	0.13	0.21	-0.05	0.15	0.29	0.08
-0.03	-0.01	0.15	0.21	-0.06	-0.09	-0.21	0.07	0.08
-0.1	-0.05	0.22	-0.03	-0.11	-0.1	-0.21	-0.07	-0.08
-0.07	-0.04	0.05	-0.01	-0.08	-0.29	-0.4	-0.07	-0.1
-0.09	-0.07	0.08	-0.03	-0.1	-0.32	-0.24	-0.08	-0.08
-0.13	-0.1	-0.21	-0.09	-0.19	-0.3	-0.5	-0.15	-0.16
-0.12	-0.08	-0.09	-0.05	-0.14	-0.31	-0.55	-0.15	-0.15
-0.07	-0.05	0.03	-0.02	-0.08	-0.26	-0.49	-0.1	-0.12
-0.11	-0.1	-0.3	-0.09	-0.15	-0.37	-0.43	-0.16	-0.14
-0.1	-0.08	0	-0.04	-0.12	-0.14	-0.19	-0.12	-0.09
-0.13	-0.09	-0.06	-0.08	-0.15	-0.2	-0.22	-0.15	-0.16
-0.07	-0.05	-0.09	-0.03	-0.09	-0.19	-0.26	-0.11	-0.11
-0.16	-0.14	-0.12	-0.14	-0.23	-0.32	-0.15	-0.14	-0.15
-0.12	-0.15	-0.09	-0.11	-0.18	-0.36	-0.19	-0.16	-0.17
-0.15	-0.12	-0.13	-0.1	-0.2	-0.31	-0.1	-0.15	-0.18
0.21	0.15	0.36	0.1	0.21	-0.07	0.17	0.28	0.07
0.23	0.19	0.43	0.14	0.22	-0.09	0.21	0.3	0.04
-0.05	-0.1	-0.17	-0.03	-0.1	-0.15	-0.27	-0.13	-0.15
-0.14	-0.12	-0.05	-0.09	-0.16	-0.27	-0.1	-0.12	-0.12
-0.17	-0.16	0	-0.15	-0.18	-0.4	-0.19	-0.13	-0.18
-0.1	-0.09	-0.1	-0.1	-0.16	-0.32	-0.17	-0.13	-0.16
-0.12	-0.12	0.27	-0.09	-0.2	-0.13	-0.14	-0.11	-0.13
-0.09	-0.06	-0.14	0	-0.11	-0.19	-0.38	-0.13	-0.07
-0.07	-0.06	0.09	0.02	-0.05	-0.21	-0.28	-0.05	-0.06
-0.04	-0.06	-0.01	0	-0.06	-0.11	-0.12	-0.09	-0.12
0.26	0.21	0.35	0.14	0.25	-0.03	0.08	0.23	0.07
0	0.16	0.08	0.05	0	-0.12	-0.28	0	-0.02
0.22	0.02	0.11	-0.02	0.05	-0.25	-0.2	0.08	-0.02
0.06	-0.03	0.26	0.09	0.14	0.01	-0.06	0.11	0.03
0.05	-0.07	0.21	0.02	0.09	0.1	0.09	0.15	0.07
0.13	0.08	0.42	0.13	0.17	0.09	0.26	0.28	0.11
-0.12	0.1	0.28	0.06	0.09	-0.23	0.02	0.13	0.04
0.1	-0.27	0.35	0.05	0.05	-0.2	-0.09	0.11	-0.04
0.28	0.35	0.27	0.32	0.37	0.13	0.38	0.53	0.26
0.06	0.05	0.32	-0.13	0.12	-0.06	0.04	0.14	0.03
0.09	0.05	0.37	0.12	-0.17	0.03	-0.02	0.25	0.14
-0.23	-0.2	0.13	-0.06	0.03	-0.37	-0.07	0.18	0.22
0.02	-0.09	0.38	0.04	-0.02	-0.07	-0.35	0.27	0.12
0.13	0.11	0.53	0.14	0.25	0.18	0.27	-0.06	0.11

0.04	-0.04	0.26	0.03	0.14	0.22	0.12	0.11	-0.16
0.01	-0.02	0.17	-0.01	0.14	-0.03	0.15	0.2	0.25
0.18	0.08	0.39	0.16	0.25	0.14	0.3	0.23	0.11
0.13	0.17	0.37	0.13	0.15	-0.05	0.07	0.24	0.07
0.38	-0.09	0.62	-0.15	0.08	0.03	0.1	0.37	0.45
0.22	0.12	0.38	0.22	0.31	0.01	0.18	0.28	0.16
0.05	-0.04	0.4	0.13	0.17	0.12	0.14	0.21	0.15
-0.1	-0.1	0.18	-0.06	-0.11	0.14	0.04	-0.07	-0.12
0.57	0.05	0.36	0.25	0.15	0.12	0.03	0.33	0.54
0.03	0.07	0.24	0.19	0.07	0.11	-0.25	0.06	-0.02
0.02	-0.01	0.3	0.04	-0.02	-0.17	-0.09	-0.01	-0.07
0.08	0.08	0.46	0.01	0.12	-0.07	-0.17	0.15	0.08
0.07	0.11	0.31	0.06	0.03	-0.27	-0.08	0.13	-0.08
0.12	0.14	0.24	0.2	0.05	0.05	-0.27	0.19	0.1
0.06	0.14	0.26	-0.02	0.06	-0.03	-0.26	0.12	-0.08
0.24	0.23	0.38	0.16	0.27	0	0.18	0.3	0.09
0.1	0.17	0.22	0.02	0.07	-0.07	-0.25	0.13	-0.06
0.25	0.18	0.51	0.12	0.21	0.12	0.12	0.27	0.08
0.19	0.13	0.39	0.13	0.28	-0.05	-0.05	0.16	-0.05
-0.08	-0.13	0.12	-0.15	-0.16	-0.06	-0.07	-0.1	-0.15
-0.13	-0.14	-0.09	-0.07	-0.19	-0.14	-0.15	-0.15	-0.12
-0.12	-0.11	-0.06	-0.07	-0.13	-0.19	-0.46	-0.14	-0.14
-0.13	-0.12	-0.13	-0.09	-0.15	-0.32	-0.14	-0.12	-0.13
-0.16	-0.15	-0.23	-0.16	-0.2	-0.46	-0.24	-0.15	-0.19
-0.12	-0.12	-0.12	-0.1	-0.15	-0.26	-0.28	-0.1	-0.13
-0.08	-0.06	0.06	-0.09	-0.12	-0.19	-0.21	-0.02	-0.09
-0.11	-0.09	0.21	-0.04	-0.13	-0.08	-0.24	-0.08	-0.08
-0.14	-0.04	0.06	-0.1	-0.16	-0.25	-0.13	-0.07	-0.12
-0.11	-0.09	-0.1	-0.12	-0.17	-0.14	-0.18	-0.15	-0.12
-0.1	-0.09	-0.09	-0.14	-0.16	-0.26	-0.25	-0.11	-0.13
-0.08	-0.11	-0.12	-0.08	-0.11	-0.2	-0.42	-0.07	-0.14
-0.05	-0.07	-0.04	-0.03	-0.08	-0.33	-0.18	-0.04	-0.1
-0.13	-0.09	0.02	-0.06	-0.09	-0.26	-0.24	-0.08	-0.12
-0.08	-0.13	-0.21	-0.12	-0.17	-0.39	-0.32	-0.11	-0.19
0.22	0.16	0.6	0.17	0.27	0.08	0.18	0.22	0.02

LT16	LT17	LT18	LT19	LT20	LT21	LT22	LT23	LT24
0.14	0.24	0.12	-0.1	0.24	0.16	0.01	0.45	0
0.13	0.23	0.05	0.09	0.27	0.17	-0.05	0.25	0.02
0.39	0.27	0.09	0.15	0.32	0.02	0.04	0.08	0.14
0.02	0.24	0.17	-0.03	0.21	0.01	-0.08	0.28	0.04
0.13	0.23	0.22	0.17	0.34	0.18	-0.05	0.52	0.09
-0.22	-0.1	0.11	-0.33	0.02	0.08	-0.05	-0.55	0.05
-0.09	-0.09	0.01	-0.11	0.04	-0.13	-0.19	0.28	0.01
-0.12	-0.13	0.01	-0.05	0.01	-0.1	-0.16	-0.61	0.01
-0.08	-0.08	0.02	-0.25	0.01	-0.13	-0.04	-0.13	0.01
-0.35	-0.23	-0.05	-0.27	-0.09	-0.19	-0.2	-0.55	-0.05
-0.23	-0.2	-0.05	-0.38	-0.07	-0.14	-0.18	-0.57	-0.03
-0.12	-0.1	-0.01	-0.12	0.01	-0.11	-0.08	-0.35	0.01
-0.23	-0.21	-0.06	-0.54	-0.11	-0.16	-0.2	-0.51	-0.05
-0.24	-0.15	-0.03	-0.33	0.01	-0.17	-0.1	-0.41	-0.01
-0.21	-0.17	-0.02	-0.27	-0.07	-0.17	-0.08	0.02	-0.03
-0.16	-0.12	0.01	-0.09	-0.01	-0.12	0.02	-0.44	0.02
-0.15	-0.29	-0.11	-0.31	-0.13	-0.2	-0.17	-0.24	-0.12
-0.25	-0.22	-0.06	-0.33	-0.15	-0.19	-0.08	-0.49	-0.07
-0.1	-0.19	-0.07	-0.17	-0.09	-0.21	-0.18	-0.46	-0.07
0.14	0.29	0.19	0.26	0.28	0.03	-0.05	0.39	0.09
0.12	0.19	0.21	0.12	0.28	0.08	0.02	0.25	0.13
-0.11	-0.14	0.01	-0.2	-0.06	-0.15	-0.09	-0.08	-0.03
-0.23	-0.2	-0.09	-0.38	-0.03	-0.14	-0.24	-0.39	-0.09
-0.14	-0.19	-0.06	-0.38	-0.06	-0.2	-0.29	-0.17	-0.07
-0.23	-0.2	-0.11	-0.4	-0.1	-0.16	-0.24	-0.1	-0.05
-0.14	-0.16	-0.04	-0.06	-0.03	-0.18	-0.18	-0.5	-0.07
-0.17	-0.14	-0.04	-0.25	0	-0.11	-0.16	-0.3	0.06
-0.16	-0.07	0.07	-0.23	0.05	-0.08	-0.06	-0.02	0.01
-0.01	-0.06	0.02	0.17	0.05	-0.12	-0.06	0.12	0.01
0.11	0.28	0.2	0.01	0.3	0.09	-0.04	0.42	0.12
-0.1	-0.03	0.15	0.13	0.04	-0.07	-0.03	0.49	0.11
-0.22	0	0.03	0.09	0.01	0.03	-0.28	0.17	-0.01
0.03	0.09	0.12	0.14	0.2	0.12	-0.13	0.01	0.08
0.12	0.12	0.2	0.12	0.2	0.06	-0.16	0.01	0.01
0.22	0.26	0.22	0	0.27	0.16	0.04	0.46	0.03
0.01	0.18	0.13	0.38	0.22	0.05	-0.1	0.57	0.03
-0.02	0.08	0.17	-0.09	0.12	-0.04	-0.1	0.05	0.07
0.17	0.39	0.37	0.62	0.38	0.4	0.18	0.36	0.24
-0.01	0.16	0.13	-0.15	0.22	0.13	-0.06	0.25	0.19
0.14	0.25	0.15	0.08	0.31	0.17	-0.11	0.15	0.07
-0.03	0.14	-0.05	0.03	0.01	0.12	0.14	0.12	0.11
0.15	0.3	0.07	0.1	0.18	0.14	0.04	0.03	-0.25
0.2	0.23	0.24	0.37	0.28	0.21	-0.07	0.33	0.06

0.25	0.11	0.07	0.45	0.16	0.15	-0.12	0.54	-0.02
-0.06	0.27	0.08	0.11	0.23	0.1	0.05	-0.17	-0.03
0.27	-0.05	0.17	0.08	0.28	0.22	-0.09	0.31	0.02
0.08	0.17	-0.06	0.11	0.28	0.19	0.03	0.58	0.1
0.11	0.08	0.11	0.06	0.29	0.12	0.15	-0.02	0.18
0.23	0.28	0.28	0.29	0.03	0.29	-0.03	0.2	0.09
0.1	0.22	0.19	0.12	0.29	-0.3	0	0.44	0.03
0.05	-0.09	0.03	0.15	-0.03	0	-0.25	-0.11	0.06
-0.17	0.31	0.58	-0.02	0.2	0.44	-0.11	0.17	0.32
-0.03	0.02	0.1	0.18	0.09	0.03	0.06	0.32	-0.1
-0.18	-0.06	-0.03	0.01	-0.03	-0.06	-0.18	-0.3	0
-0.05	0.17	0.13	0.05	0.19	0.14	0.01	0.07	-0.03
-0.11	0.05	0.14	0.08	0.1	-0.06	-0.19	0.04	0.05
0.2	0.14	0.16	0.28	0.05	0	0.08	0.46	0.15
-0.06	0.13	0.14	0.24	0.12	-0.04	0.04	0.17	0.07
0.2	0.25	0.28	0.27	0.33	0.09	0.04	0.24	0.03
-0.02	0.09	0.18	0.15	0.12	-0.05	0.03	0.13	0.07
0.16	0.28	0.23	0.14	0.31	0.07	-0.01	0.42	0.1
0.19	0.2	0.05	0.27	0.2	0.07	0.04	0.4	-0.01
-0.18	-0.13	-0.07	-0.05	0.03	-0.17	-0.22	-0.23	-0.07
-0.26	-0.23	-0.13	-0.19	-0.1	-0.2	-0.16	-0.37	-0.07
-0.22	-0.19	-0.08	-0.23	-0.06	-0.17	-0.2	-0.34	-0.01
-0.23	-0.22	-0.14	-0.37	-0.14	-0.15	-0.16	-0.6	-0.05
-0.25	-0.21	-0.05	-0.44	-0.08	-0.19	-0.22	-0.51	-0.09
-0.11	-0.21	-0.14	-0.38	-0.11	-0.17	-0.19	-0.37	-0.08
-0.07	-0.16	-0.08	-0.21	-0.06	-0.17	-0.11	-0.34	-0.04
-0.12	-0.1	-0.03	-0.07	-0.02	-0.13	-0.2	0.03	0.02
-0.04	-0.15	-0.07	-0.27	-0.09	-0.2	-0.43	-0.64	-0.03
-0.18	-0.17	-0.08	-0.23	-0.1	-0.18	-0.26	-0.27	-0.02
-0.21	-0.22	-0.04	-0.29	-0.11	-0.17	-0.19	-0.5	-0.06
-0.05	-0.13	0	-0.08	-0.03	-0.14	-0.15	-0.15	0
-0.08	-0.07	-0.06	-0.29	-0.08	-0.11	-0.21	-0.21	0
-0.18	-0.11	-0.06	-0.07	-0.06	-0.15	-0.32	-0.54	-0.05
-0.24	-0.22	-0.06	-0.49	-0.09	-0.16	-0.36	-0.76	-0.09
0.12	0.22	0.16	0.23	0.28	0.09	-0.02	0.35	0.02

LT25	LT26	LT27	LT28	SD57_FM4	SD58_FM4	SD59_FM4	SD60_FM4	SD61_FM4
-0.05	-0.01	0.02	0.09	0.02	0.22	0.04	0.18	0.18
-0.05	0.05	0.02	0.11	0.03	0.2	0.05	0.18	0.17
0.08	0.17	0.09	0.22	0.06	0.35	0.02	0.38	0.42
0.03	0.11	0.12	0.08	0.17	0.36	0.18	0.33	0.31
0.03	0.12	0.1	0.1	0.09	0.25	0.1	0.25	0.25
0	-0.41	0.05	0.05	-0.05	0.15	0.05	0.22	-0.18
-0.06	-0.38	-0.09	0.08	-0.11	0.05	-0.06	0.02	-0.07
0.02	-0.37	-0.08	-0.1	-0.08	0.01	-0.04	0.03	-0.12
-0.07	-0.32	-0.1	-0.01	-0.12	0	-0.07	-0.02	-0.14
-0.08	-0.32	-0.12	-0.26	-0.14	-0.04	-0.09	-0.06	-0.18
-0.05	-0.25	-0.1	-0.13	-0.11	-0.02	-0.07	-0.03	-0.13
-0.04	-0.19	-0.11	-0.08	-0.09	0.07	-0.06	0.02	-0.08
-0.07	-0.45	-0.14	-0.17	-0.14	-0.06	-0.08	-0.04	-0.19
-0.03	-0.32	-0.11	-0.1	-0.12	0.01	-0.06	0.01	-0.13
-0.07	-0.22	-0.13	-0.08	-0.14	-0.08	-0.08	-0.03	-0.17
-0.02	-0.26	-0.06	-0.09	-0.07	0.01	0	0.04	-0.1
-0.16	-0.36	-0.18	-0.15	-0.18	-0.09	-0.16	-0.09	-0.16
-0.07	-0.35	-0.1	-0.15	-0.13	-0.06	-0.12	-0.06	-0.17
-0.09	-0.33	-0.13	-0.13	-0.11	-0.05	-0.08	-0.06	-0.16
0.03	0.11	0.12	0.19	0.13	0.43	0.16	0.38	0.3
0.09	0.06	0.19	0.22	0.2	0.41	0.2	0.4	0.36
-0.06	-0.22	-0.07	0	-0.09	-0.01	-0.02	0	-0.11
-0.07	-0.49	-0.12	-0.28	-0.14	0.05	-0.09	-0.02	-0.15
-0.14	-0.41	-0.15	-0.19	-0.14	-0.01	-0.1	-0.05	-0.17
-0.07	-0.46	-0.08	-0.26	-0.11	-0.03	-0.07	-0.03	-0.17
-0.1	-0.32	-0.12	-0.19	-0.13	-0.02	-0.11	0	-0.2
-0.04	-0.22	-0.08	-0.19	-0.08	-0.03	-0.04	-0.01	-0.17
0.01	-0.12	-0.06	0	-0.06	0.01	-0.02	0.03	-0.11
-0.01	-0.03	-0.03	-0.05	-0.03	0.06	0.02	0.04	-0.11
-0.01	0.11	0.15	0.22	0.2	0.4	0.21	0.43	0.37
-0.08	0.01	0.14	0.05	0.16	0.02	0.19	0.09	-0.03
-0.04	-0.05	0.02	0.04	0	0.07	0.01	0.1	-0.03
-0.05	0.05	-0.01	-0.06	-0.05	0.05	-0.05	0.09	0.05
-0.11	-0.12	-0.06	-0.02	-0.13	0.1	-0.06	0.06	0.07
-0.03	0.02	0.01	0.23	0.04	0.27	0.04	0.24	0.27
0.02	0.08	0.07	0.12	0.06	0.24	0.1	0.25	0.19
-0.01	0.08	0.11	0.14	0.14	0.23	0.17	0.18	0.13
0.3	0.46	0.31	0.24	0.26	0.38	0.22	0.51	0.39
0.04	0.01	0.06	0.2	-0.02	0.16	0.02	0.12	0.13
-0.02	0.12	0.03	0.05	0.06	0.27	0.07	0.21	0.28
-0.17	-0.07	-0.27	0.05	-0.03	0	-0.07	0.12	-0.05
-0.09	-0.17	-0.08	-0.27	-0.26	0.18	-0.25	0.12	-0.05
-0.01	0.15	0.13	0.19	0.12	0.3	0.13	0.27	0.16

-0.07	0.08	-0.08	0.1	-0.08	0.09	-0.06	0.08	-0.05
-0.18	-0.05	-0.11	0.2	-0.06	0.2	-0.02	0.16	0.19
-0.06	0.17	0.05	0.14	0.13	0.25	0.09	0.28	0.2
-0.03	0.13	0.14	0.16	0.14	0.28	0.18	0.23	0.05
0.01	0.05	0.08	0.28	0.24	0.27	0.15	0.14	0.27
-0.03	0.19	0.1	0.05	0.12	0.33	0.12	0.31	0.2
-0.06	0.14	-0.06	0	-0.04	0.09	-0.05	0.07	0.07
-0.18	0.01	-0.19	0.08	0.04	0.04	0.03	-0.01	0.04
-0.3	0.07	0.04	0.46	0.17	0.24	0.13	0.42	0.4
0	-0.03	0.05	0.15	0.07	0.03	0.07	0.1	-0.01
-0.22	-0.05	-0.03	0	-0.05	0.08	-0.05	0.05	-0.11
-0.05	-0.32	-0.15	0.01	0.07	0.21	0.04	0.11	0.14
-0.03	-0.15	-0.26	0.02	0.13	0.13	0.16	0.16	0.06
0	0.01	0.02	-0.03	0.14	0.26	0.14	0.26	0.11
-0.05	0.07	0.13	0.14	-0.23	0.21	0.34	0.17	0.22
0.08	0.21	0.13	0.26	0.21	0.05	0.21	0.47	0.32
-0.05	0.04	0.16	0.14	0.34	0.21	-0.14	0.19	0.2
0.05	0.11	0.16	0.26	0.17	0.47	0.19	0.01	0.36
-0.11	0.14	0.06	0.11	0.22	0.32	0.2	0.36	-0.07
-0.08	-0.34	-0.13	-0.15	-0.12	0.05	-0.08	0.03	-0.07
-0.1	-0.28	-0.16	-0.22	-0.15	-0.03	-0.13	-0.06	-0.14
-0.07	-0.27	-0.1	-0.16	-0.12	-0.03	-0.08	-0.04	-0.19
-0.12	-0.28	-0.15	-0.3	-0.17	-0.09	-0.12	-0.09	-0.23
-0.14	-0.6	-0.15	-0.24	-0.13	-0.03	-0.1	-0.06	-0.19
-0.1	-0.32	-0.16	-0.11	-0.12	-0.09	-0.09	-0.05	-0.17
-0.04	-0.43	-0.08	-0.02	-0.08	0.02	-0.04	0.02	-0.1
-0.11	-0.3	-0.14	-0.13	-0.14	-0.04	-0.07	0	-0.14
-0.1	-0.28	-0.05	-0.12	-0.09	0	-0.06	-0.04	-0.2
-0.06	-0.24	-0.13	-0.11	-0.15	-0.01	-0.11	-0.06	-0.14
-0.08	-0.25	-0.09	-0.13	-0.12	-0.02	-0.08	-0.05	-0.15
-0.07	-0.25	-0.08	0.04	-0.1	-0.01	-0.08	-0.03	-0.12
-0.07	-0.4	-0.05	-0.09	-0.09	0.03	-0.04	-0.02	-0.11
-0.07	-0.29	-0.09	-0.06	-0.09	-0.01	-0.06	-0.04	-0.17
-0.11	-0.39	-0.14	-0.3	-0.14	-0.05	-0.12	-0.03	-0.18
-0.02	0.14	0.06	0.24	0.18	0.38	0.17	0.36	0.37

MT1	MT2	MT3	MT4	MT5	MT6	MT7	MT8	MT9
-0.09	-0.15	-0.19	-0.17	-0.18	-0.16	-0.12	-0.12	-0.12
-0.11	-0.17	-0.17	-0.18	-0.16	-0.16	-0.12	-0.12	-0.13
-0.06	-0.08	-0.06	-0.12	-0.15	-0.12	-0.02	-0.08	-0.08
-0.1	-0.13	-0.12	-0.13	-0.11	-0.13	-0.04	-0.11	-0.09
-0.03	-0.11	-0.1	-0.1	-0.15	-0.11	-0.05	-0.07	-0.09
0.1	0.2	0.35	0.12	0.25	0.14	0.12	0.07	0.1
0.05	0.1	0.21	0.04	0.01	0.08	0.1	0.21	0.08
0.07	0.16	0.38	0.11	0.06	0.14	0.12	0.15	0.09
0.02	0.13	0.33	0.09	0	0.08	0.04	0.08	0.02
0	0.08	0.32	0.05	-0.03	0.06	0.01	0.05	0.01
0.01	0.1	0.36	0.11	0.02	0.1	0.04	0.11	0.06
0.02	0.11	0.35	0.12	0.03	0.13	0.08	0.12	0.09
-0.01	0.12	0.35	0.06	0.02	0.11	0.06	0.1	0.02
0.06	0.14	0.34	0.06	0.02	0.1	0.06	0.1	0.05
0.02	0.1	0.36	0.1	0	0.09	0.02	0.09	0.03
0.05	0.16	0.4	0.14	0.08	0.16	0.09	0.13	0.08
-0.01	0.3	0.09	0.08	0.07	0.15	0.05	0.02	-0.01
0.04	0.27	0.11	0.07	0.06	0.13	0.08	0	0.02
0.02	0.17	0.13	0.05	0.03	0.11	0.09	0.16	0.05
-0.06	-0.13	-0.1	-0.11	-0.12	-0.09	-0.04	-0.07	-0.05
0.06	-0.08	-0.07	-0.05	0.04	-0.03	0.09	0.03	0.11
0.04	0.14	0.38	0.08	0.03	0.1	0.04	0.14	0.06
0.03	0.21	0.19	0.1	0.02	0.09	0.05	0.06	0.06
0.17	-0.02	-0.05	-0.05	-0.05	-0.02	0.01	0.05	0.07
0.17	0.03	0.03	-0.01	0	0.03	0.09	0.04	0.04
0.16	-0.03	0	-0.04	-0.04	-0.01	-0.02	0.03	0.05
0.02	0.14	0.37	0.11	0.07	0.11	0.05	0.1	0.04
0	0.14	0.34	0.1	0.06	0.11	0.08	0.12	0.04
0.05	0.14	0.37	0.09	0.04	0.13	0.09	0.14	0.07
-0.04	-0.15	-0.09	-0.14	-0.14	-0.11	-0.04	-0.03	-0.06
-0.15	-0.13	-0.02	-0.1	-0.11	-0.11	-0.09	-0.07	-0.08
-0.1	-0.14	-0.11	-0.09	-0.13	-0.15	-0.09	-0.13	-0.12
-0.15	-0.19	-0.1	-0.17	-0.13	-0.14	-0.13	-0.11	-0.12
-0.15	-0.12	-0.13	-0.2	-0.23	-0.12	-0.11	-0.1	-0.15
-0.12	-0.15	-0.17	-0.15	-0.16	-0.12	-0.12	-0.11	-0.14
-0.08	-0.13	-0.12	-0.13	-0.16	-0.12	-0.08	-0.11	-0.14
-0.13	-0.14	-0.11	-0.12	-0.15	-0.12	-0.06	-0.09	-0.04
0.12	-0.09	-0.06	-0.13	-0.23	-0.12	0.06	0.21	0.06
-0.15	-0.07	-0.07	-0.09	-0.16	-0.1	-0.09	-0.04	-0.1
-0.16	-0.19	-0.13	-0.15	-0.2	-0.15	-0.12	-0.13	-0.16
-0.06	-0.14	-0.19	-0.32	-0.46	-0.26	-0.19	-0.08	-0.25
-0.07	-0.15	-0.46	-0.14	-0.24	-0.28	-0.21	-0.24	-0.13
-0.1	-0.15	-0.14	-0.12	-0.15	-0.1	-0.02	-0.08	-0.07

-0.15	-0.12	-0.14	-0.13	-0.19	-0.13	-0.09	-0.08	-0.12
-0.18	-0.26	-0.22	-0.23	-0.25	-0.11	-0.07	-0.12	-0.04
-0.13	-0.23	-0.19	-0.22	-0.21	-0.21	-0.16	-0.1	-0.15
-0.07	-0.13	-0.08	-0.14	-0.05	-0.14	-0.08	-0.03	-0.07
-0.05	-0.19	-0.23	-0.37	-0.44	-0.38	-0.21	-0.07	-0.27
0.03	-0.1	-0.06	-0.14	-0.08	-0.11	-0.06	-0.02	-0.09
-0.17	-0.2	-0.17	-0.15	-0.19	-0.17	-0.17	-0.13	-0.2
-0.22	-0.16	-0.2	-0.16	-0.22	-0.19	-0.11	-0.2	-0.43
-0.23	-0.37	-0.34	-0.6	-0.51	-0.37	-0.34	0.03	-0.64
-0.07	-0.07	-0.01	-0.05	-0.09	-0.08	-0.04	0.02	-0.03
-0.08	-0.1	-0.07	-0.12	-0.14	-0.1	-0.04	-0.11	-0.1
-0.34	-0.28	-0.27	-0.28	-0.6	-0.32	-0.43	-0.3	-0.28
-0.13	-0.16	-0.1	-0.15	-0.15	-0.16	-0.08	-0.14	-0.05
-0.15	-0.22	-0.16	-0.3	-0.24	-0.11	-0.02	-0.13	-0.12
-0.12	-0.15	-0.12	-0.17	-0.13	-0.12	-0.08	-0.14	-0.09
0.05	-0.03	-0.03	-0.09	-0.03	-0.09	0.02	-0.04	0
-0.08	-0.13	-0.08	-0.12	-0.1	-0.09	-0.04	-0.07	-0.06
0.03	-0.06	-0.04	-0.09	-0.06	-0.05	0.02	0	-0.04
-0.07	-0.14	-0.19	-0.23	-0.19	-0.17	-0.1	-0.14	-0.2
-0.25	0.05	0.01	-0.05	-0.04	0.02	0.03	0.03	0
0.05	-0.23	0.12	0.09	0.1	0.16	0.06	0.03	0
0.01	0.12	-0.19	0.07	0.01	0.11	0.03	0.09	0.01
-0.05	0.09	0.07	-0.27	-0.02	0.08	0.02	0.07	0
-0.04	0.1	0.01	-0.02	-0.33	0.05	0.08	0	0.01
0.02	0.16	0.11	0.08	0.05	-0.28	0.18	0.07	0.05
0.03	0.06	0.03	0.02	0.08	0.18	-0.19	0.1	0.11
0.03	0.03	0.09	0.07	0	0.07	0.1	-0.21	0.13
0	0	0.01	0	0.01	0.05	0.11	0.13	-0.26
0.05	-0.02	-0.03	-0.02	-0.02	-0.03	0.06	0.04	0.06
0	-0.01	-0.01	-0.05	0	-0.03	0.02	0	0.02
-0.04	0	0.01	-0.04	0.01	0	0.03	-0.02	-0.02
-0.04	-0.04	-0.08	-0.06	-0.05	-0.06	0.01	-0.02	-0.04
0.01	0.09	0.07	0.03	0.07	0.07	0.04	0.04	0.09
0.14	0.04	0.01	-0.03	-0.03	0	0	0	-0.02
0.01	-0.11	-0.09	-0.14	-0.05	-0.1	-0.02	-0.06	-0.05

MT10	MT11	MT12	MT13	MT14	MT15	SD77_FM48
-0.19	-0.14	-0.12	-0.05	-0.14	-0.1	0.19
-0.14	-0.13	-0.09	-0.06	-0.12	-0.1	0.16
-0.09	-0.16	-0.02	-0.03	-0.09	-0.08	0.44
-0.13	-0.09	-0.07	-0.05	-0.07	-0.08	0.36
-0.13	-0.1	-0.07	-0.05	-0.08	-0.12	0.29
0.15	0.22	0.28	0.15	0.22	0.08	0.03
0.02	0.02	0.04	0	0.1	-0.01	0
0.04	0.05	0.08	0	0.15	0.08	-0.02
-0.02	-0.01	0.01	-0.07	0.12	0.03	0
0	0	0.01	-0.05	0.05	0.01	-0.1
-0.02	-0.01	0.02	-0.06	0.09	0.02	-0.06
0.01	0.03	0.05	-0.03	0.13	0.03	0
0	-0.01	0.02	-0.04	0.06	0.02	-0.12
-0.05	0	0.03	-0.05	0.07	0.05	-0.02
-0.03	-0.02	0	-0.05	0.05	0	-0.09
0.04	0.05	0.06	0	0.13	0.06	-0.02
-0.05	-0.04	-0.02	-0.08	0.02	0	-0.13
-0.01	0	0	-0.04	0.08	0	-0.13
0.03	0.01	0.02	-0.06	0.05	0.01	-0.16
-0.1	-0.11	-0.07	-0.02	-0.07	-0.1	0.36
0.01	0	-0.01	0.05	0.06	0	0.35
-0.03	0.02	0.05	-0.02	0.09	0.02	-0.05
0.02	-0.03	0.01	-0.02	0.06	0.06	-0.06
0.02	-0.01	-0.02	-0.08	0.01	0.06	-0.04
0.05	0.03	0.02	0.04	0.02	0.14	-0.06
0	-0.05	-0.08	-0.08	-0.01	0.06	-0.08
0.03	0.03	0.06	-0.04	0.09	0.03	-0.09
-0.02	0.01	0.07	-0.04	0.11	0.01	-0.02
0	0.02	0.07	-0.04	0.08	0.02	0
-0.12	-0.09	-0.05	-0.04	-0.05	-0.08	0.39
-0.08	-0.09	-0.03	-0.03	-0.06	-0.12	-0.03
-0.1	-0.11	-0.11	-0.07	-0.12	-0.12	0.02
-0.12	-0.11	-0.1	-0.1	-0.11	-0.18	0.05
-0.15	-0.16	-0.15	-0.11	-0.13	-0.18	0.11
-0.15	-0.14	-0.11	-0.07	-0.11	-0.11	0.31
-0.11	-0.1	-0.08	-0.05	-0.13	-0.08	0.22
-0.09	-0.09	-0.11	-0.07	-0.09	-0.13	0.16
-0.1	-0.09	-0.12	-0.04	0.02	-0.21	0.6
-0.12	-0.14	-0.08	-0.03	-0.06	-0.12	0.17
-0.17	-0.16	-0.11	-0.08	-0.09	-0.17	0.27
-0.14	-0.26	-0.2	-0.33	-0.26	-0.39	0.08
-0.18	-0.25	-0.42	-0.18	-0.24	-0.32	0.18
-0.15	-0.11	-0.07	-0.04	-0.08	-0.11	0.22

-0.12	-0.13	-0.14	-0.1	-0.12	-0.19	0.02
-0.18	-0.21	-0.05	-0.08	-0.18	-0.24	0.12
-0.17	-0.22	-0.13	-0.07	-0.11	-0.22	0.22
-0.08	-0.04	0	-0.06	-0.06	-0.06	0.16
-0.23	-0.29	-0.08	-0.29	-0.07	-0.49	0.23
-0.1	-0.11	-0.03	-0.08	-0.06	-0.09	0.28
-0.18	-0.17	-0.14	-0.11	-0.15	-0.16	0.09
-0.26	-0.19	-0.15	-0.21	-0.32	-0.36	-0.02
-0.27	-0.5	-0.15	-0.21	-0.54	-0.76	0.35
-0.02	-0.06	0	0	-0.05	-0.09	0.02
-0.06	-0.08	-0.07	-0.07	-0.07	-0.11	-0.02
-0.24	-0.25	-0.25	-0.4	-0.29	-0.39	0.14
-0.13	-0.09	-0.08	-0.05	-0.09	-0.14	0.06
-0.11	-0.13	0.04	-0.09	-0.06	-0.3	0.24
-0.15	-0.12	-0.1	-0.09	-0.09	-0.14	0.18
-0.01	-0.02	-0.01	0.03	-0.01	-0.05	0.38
-0.11	-0.08	-0.08	-0.04	-0.06	-0.12	0.17
-0.06	-0.05	-0.03	-0.02	-0.04	-0.03	0.36
-0.14	-0.15	-0.12	-0.11	-0.17	-0.18	0.37
0.05	0	-0.04	-0.04	0.01	0.14	0.01
-0.02	-0.01	0	-0.04	0.09	0.04	-0.11
-0.03	-0.01	0.01	-0.08	0.07	0.01	-0.09
-0.02	-0.05	-0.04	-0.06	0.03	-0.03	-0.14
-0.02	0	0.01	-0.05	0.07	-0.03	-0.05
-0.03	-0.03	0	-0.06	0.07	0	-0.1
0.06	0.02	0.03	0.01	0.04	0	-0.02
0.04	0	-0.02	-0.02	0.04	0	-0.06
0.06	0.02	-0.02	-0.04	0.09	-0.02	-0.05
-0.22	0.14	0.02	0.06	0.06	0.02	-0.1
0.14	-0.22	0.18	0.03	0.04	0.08	-0.08
0.02	0.18	-0.28	0.04	0.01	0.12	0
0.06	0.03	0.04	-0.21	0	0.01	-0.03
0.06	0.04	0.01	0	-0.23	-0.03	-0.02
0.02	0.08	0.12	0.01	-0.03	-0.19	-0.09
-0.1	-0.08	0	-0.03	-0.02	-0.09	-0.03