Illegal Wildlife Trade (IWT) Challenge Fund Evidence Annual Report

To be completed with reference to the "Project Reporting Information Note": (https://iwt.challengefund.org.uk/resources/information-notes/)

It is expected that this report will be a **maximum of 20 pages** in length, excluding annexes)

Submission Deadline: 30th April 2024

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1. IWT Challenge Fund (IWTCF) Project Information

Project reference	IWTEV013
Project title	Unlocking DNA barcoding to identify illegal timber
Country/ies	Gabon, Democratic Republic of the Congo, United Kingdom
Lead Partner	Royal Botanic Gardens, Kew (RBGK)
Project partner(s)	University of Kisangani (UNIKIS), Democratic Republic of the Congo National Center for Scientific and Technology Research (CENAREST), Gabon World Forest ID (WFID), USA
IWTCF grant value	
Start/end dates of project	01/04/2023-31/03/2024 originally, **an extension until 30/06/2024 was approved**
Reporting period (e.g. April 2023-Mar 2024) and number (e.g. Annual Report 1, 2, 3)	April 2023-Mar 2024, Annual Report 1
Project Leader name	Victor Deklerck, Sidonie Bellot
Project website/blog/social media	This is in development on the worldforestid.org website.
Report author(s) and date	Sidonie Bellot, Victor Deklerck, Dyana Bourobou and Janvier Lisingo - 30 April 2024.

2. Project summary

The illegal timber trade (ITT) is the most profitable natural-resource crime, costing 50-152 billion USD/year [1,2]. Up to 90% of tropical timber may be illegally sourced [3]. ITT drives about half of forest loss, with devastating consequences including loss of biodiversity, natural capital, and long-term income for local communities and producer countries [4]. In addition, the illegal logging pressure on highly valued wood species is significant, leading to vulnerability, exhaustion or even near-extinction of the species. A key obstacle to ITT regulation are look-alike species (with similar visual/macroscopic /microscopic features) that are often intermixed in trade. To enforce timber

regulations through local and international frameworks (UKTR, EUTR, Lacey Act, CITES), cost-effective high-throughput tools are required to identify traded timber species in producer and consumer countries.

Currently, timber species identification relies on wood anatomy and mass spectrometry, which require costly equipment and specialist expertise. A more accurate and cheaper method is species identification using DNA regions ("barcodes"). However, studies are lacking to identify the timber trade points (e.g. exploitation, distribution, consumer) and conditions at which DNA barcoding will be most useful, and the degraded nature of timber DNA raises technical challenges that remain to be fully addressed.

This project will provide the knowledge and scientific evidence required for better monitoring and regulation of ITT through the wide and novel implementation of DNA barcoding. This is being done by performing research along three axes: 1) identifying the needs and the conditions for successful implementation of timber DNA barcoding in two focus countries, 2) assessing the potential and limitations of timber DNA for DNA barcoding, and 3) developing DNA barcoding methods applicable to key timber species.

This project is performed in collaboration with researchers from the focus countries, i.e. Democratic Republic of the Congo (DRC) and Gabon. These countries have been selected because they are ITT and illegal deforestation hotspots [6] and because the species studied as part of this project are multi-purpose trees that fulfil numerous domestic and health needs of local indigenous populations in forest, rural and urban areas of Gabon and DRC. The exploitation of forests, centred on the removal of mature trees, results in a significant loss for human forest communities dependent on the ecosystem services provided by these multi-purpose trees, notably pygmies, rural Bantu and, to a lesser extent, urban Bantu communities. For these reasons, researchers at local institutes (CENAREST in Gabon, UNIKIS in DRC) are very keen on developing the DNA analysis capacity of their labs to support the conservation of local wildlife, including through an improved monitoring of the timber trade and of its impacts on forest regeneration.

The timber species we are focusing on are mahogany look-alike species, which include protected and threatened species known to be difficult to monitor in trade (see [5]). Specifically, we are developing DNA barcodes for as many species as possible from genera *Entandrophragma*, *Khaya*, *Lovoa* and *Swietenia*, with special focus on the following species: *Entandrophragma angolense* (Tiama, IUCN Red list: NT), *Entandrophragma candollei* (Kosipo, IUCN Red list:VU), *Entandrophragma cylindricum* (Sapelli, IUCN Red list: VU), *Entandrophragma utile* (Sipo, IUCN Red list: VU), *Lovoa trichilioides* (Dibétou, IUCN Red list: LC), *Khaya anthotheca* (Acajou d'Afrique, IUCN Red list: VU), *Khaya ivorensis* (Acajou d'Afrique, IUCN Red list: VU) *Swietenia macrophylla*, *Swietenia mahagoni* and *Swietenia humilis* (Mahogany, CITES Appendix II, IUCN Red list: VU, NT, EN respectively).

To provide evidence relating to the need and conditions for successful DNA-based timber trade monitoring (research axis 1), we are undertaking stakeholder consultations in the focus countries and in selected importing countries (Belgium, UK) via the use of a questionnaire and through in person and online conversations. To understand the limitations of timber DNA (research axis 2), we are reviewing and testing different DNA extraction protocols on multiple timber samples from the focus species. To develop DNA barcoding methods adapted to the focus species and countries (research axis 3), we are building a DNA reference database for all species in the focus genera, identifying a few DNA regions that can distinguish between the focus species on their own, and testing the best (cost-efficient) way to sequence these regions (i.e. the barcodes) from different types of wood from the focus species.

All the DNA data, DNA barcodes and protocols will be made publicly available, which will enable people in the focus countries and anywhere else to develop timber DNA barcoding for Meliaceae without having to go through the generation of another reference database or the design of new barcodes. The results of the stakeholders consultations and timber DNA extraction protocol testing will be used to decide at what points and in what conditions DNA barcoding may best be used in the focus countries. This will form the foundation for the development of wider projects and funding applications to support the deployment of timber DNA barcoding where it was identified to be the most relevant, feasible and cost-efficient.

Ultimately, this project will enhance in-country DNA analysis capacity, and strengthen law-enforcement tools that can protect endangered timber species and their ecosystem services to local communities and beyond by deterring their over-exploitation.

3. Project stakeholders/partners

The project was originally formally led by V. Deklerck (at the time employed by RBGK), and leadership has been extended to S. Bellot (RBGK) following V. Deklerck's move to be Director of Science of WFID at Meise Botanic Garden. The two other main project partners, D. Bourobou and J. Lisingo, have been invited to co-design the project since the beginning. V. Deklerck and D. Bourobou already knew each-other and were aware of their respective interest in DNA barcoding and wood traceability. From early discussions, it became clear that it would be interesting to expand the project to a country like DRC where illegal timber trade is a big issue but logistical challenges may be more prominent than in Gabon. It was then logical to first look if someone in DRC would indeed be interested in the matter, which is when J. Lisingo was approached and confirmed his interest in developing a lab at the University of Kisangani that could be used for DNA analyses supporting plant research and conservation.

Early conversations between all project partners have been instrumental as they enabled to highlight the need to generate further evidence about the conditions under which DNA barcoding could be used to monitor the timber trade in Gabon and DRC, which resulted in the decision to start with a small, Evidence, project instead of large, Main, project as originally planned. Since then, regular meetings and email exchanges (sometimes easier than meetings) as well as in person meetings (with D. Bourobou in Gabon and with J. Lisingo at RBGK) have enabled D. Bourobou and J. Lisingo to contribute to the shaping of the project. For instance D. Bourobou organised the workshop in Gabon and was the main author of the resulting report (summary in Annex 4.3), and conversations with her and with stakeholders in Gabon (Annex 4.1) are shaping the current project in terms of DNA extraction protocol organisation and are laying the foundations of future collaborative projects involving more stakeholders from Gabon. In DRC, J. Lisingo has provided insights regarding how the current project relates to poverty alleviation, he has co-designed DNA barcode regions with S. Bellot, and his visit at RBGK has been organised based on the training and networking needs he expressed. Moreover, J. Lisingo has made links with other stakeholders in DRC (Annex 4.1) who may be involved in future larger projects and who already informed this project by providing feedback on the relevance and challenges of implementing DNA barcoding in the country (see list of stakeholders in Annex 4.1). Although a large part of the labwork taking place at RBGK was done without much input from them because it mainly involved sampling specimens from the Kew herbarium, D. Bourobou and J. Lisingo will be strongly involved in the interpretation and publishing of the DNA reference dataset and DNA protocols. For instance, final curation of the DNA reference dataset requires to double check the identity of samples for which the data behaves unexpectedly, a task that can best be achieved by J. Lisingo given his botanical expertise, and the expertise of both D. Bourobou and J. Lisingo will be necessary to put the results into context from a botanical and societal perspective. The redaction of Article 1 will therefore involve them heavily. Moreover, their insights will also be instrumental in designing future projects, which will build on the feedback received from all consulted stakeholders. The visit of D. Bourobou at RBGK is intended to facilitate co-redaction of such a future project and of Article 1, while remote interactions will remain crucial so that J. Lisingo (and V. Deklerck) can remain strongly involved. Evidence for this will be provided in the final project report.

Two main challenges to smooth collaboration were identified in this project. This first one was obtaining visas for D. Bourobou and J. Lisingo to visit RBGK. The coup and confusion around new procedures in Gabon delayed the visit of D. Bourobou so that it still has not happened. Moreover, the complexity of the procedure in DRC together with the need to articulate it with already-planned travels and the need to postpone the Gabon workshop due to the coup made it so that J. Lisingo could not attend the workshop in Gabon due to a visa appointment that exact day and had to visit RBGK before 19 April 2024, preventing an overlap between his visit and that of D. Bourobou. All these issues prevented the in-person meetings in Gabon and the UK to

be as productive and fruitful as they could have been by enabling in-person ideas and knowledge exchange between all main project partners. The issues are being mitigated through online meetings reuniting all partners, and in-person discussions between S. Bellot and each partner separately. However, a lesson taken forward is that future projects need to accommodate the likelihood of very long delays in organising the in person visits and therefore should not last less than two or three years, especially if more stakeholders need to travel. The second challenge was that D. Bourobou and J. Lisingo are not fully comfortable with the use of English, meaning that the written contribution of D. Bourobou and J. Lisingo to the project application, project reports and future publications cannot be direct. This is mitigated by the fact that S. Bellot and V. Deklerck speak and write French fluently, so meetings can still be very productive and oral or written French input can still be incorporated in the project outputs in English, which can then be circulated back to the partners who understand written English.

Although S. Bellot has the DNA barcoding expertise and V. Deklerck has the timber traceability expertise, the project aims and resulting outputs would have risked being completely irrelevant for the focus countries without the constant input of the project partners from Gabon and DRC and, through them, of dozens of other stakeholders from these countries. For instance, we originally thought that the biggest limitation may be logistic but it turned out that functional labs are already available in Gabon and that such a lab may be possible to build in DRC with less means than originally expected. It was also unclear at the beginning what the training needs could be in each focus country, whereas we now have a clearer idea of this. Moreover, discussions with multiple stakeholders have also revealed the importance of DNA tests at stages in the supply chain where leaves or sapwood are still available, removing some of the need of getting workable DNA from heartwood, which was originally viewed as a major obstacle. Finally, the collaborative nature of the project was also appreciated in the focus countries. For instance, bringing many different stakeholders in the same room during the Gabon workshop was perceived by all participants as a novel and exciting step forward with huge potential to help them in their respective professional endeavours and to further coordinate illegal timber trade monitoring and law enforcement.

4. Project progress

4.1 Progress in carrying out project Activities

Activities contributing to Output 1 have all been undertaken, even though stakeholder consultation via a questionnaire and a workshop in Gabon had to be delayed by more than two months following the coup in Gabon at the end of August 2023.

Activities contributing to Output 2 have all been completed or are ongoing (activities 2.4 and 2.5 relating to testing the DNA extraction protocols on a wide diversity of samples). We may not be able to test the protocol on species outside of our focus genera (optional activity 2.5) due to time constraints.

Activities contributing to Output 3 have all been completed or are ongoing (activities 3.8, 3.9, 3.10, relating to testing new DNA barcodes together with partners from Gabon and DRC), even though the completion of activities relating to the production and analysis of the DNA reference dataset (activities 3.5, 3.6, 3.7) had been delayed by 2 months due to unforeseen circumstances (see approved change request).

4.2 Progress towards project Outputs

Output 1: New data and knowledge providing a better understanding of the requirements for DNA barcoding implementation at key points of the timber trade supply chain

Following a review of the timber trade literature (activity 1.1) and in collaboration with our partners in Gabon and the DRC, a questionnaire (19 questions) was developed. This questionnaire aims at better understanding the current capacity and knowledge on timber tracing techniques within different stages of the timber supply chain. The questionnaire was sent out to stakeholders in Gabon and the DRC, to the UK Office for Product Safety and Standards (which is responsible for timber shipment testing in the UK) and to the timber forensic institute "ENFORCE" in Belgium (which performs scientific testing for timber species identification). So far, we have received 8 responses (and a wealth of additional informal feedback through a workshop - see below) from Gabonese stakeholders, 1 response from UK OPSS, 1 response from the Belgian ENFORCE center, and in-person feedback from 19 DRC stakeholders (activity 1.2). Once those results are in, we will prepare a report (Report 1) presenting the different stakeholders, the questionnaire and discussions, and supply chain points at which DNA barcoding may be the most relevant (indicator 1.1). For the moment, a list of the stakeholders consulted is available in Annex 4.1, and the questionnaire addressed to stakeholders is available in Annex 4.2.

A stakeholder consultation workshop was held in Gabon on 13 December 2023 (activity 1.3). There were 36 participants from 22 scientific, governmental and non-governmental organisations, mainly from Gabon (Ministère des Eaux et Forêts du Gabon, Direction Régionale des Douanes du Gabon, Ecole des Eaux et Forets, Centre National de la Recherche Scientifique et Technologique - CENAREST - including IRAF, IRET, IRT, Herbier National and IPHAMETRA, Laboratoire de Recherche et de Valorisation du Matériau Bois, Direction Generale des Industries, du Commerce du Bois et de la Valorisation des Produits Forestiers, Wave Gabon, Brainforest, Tracer-Gabon, Université Internationale de Libreville, Meise Botanic Gardens, World Forest ID, University of Wageningen, RBG Kew). The workshop was organised by D. Bourobou with input from S. Bellot. It was a hybrid workshop, allowing some scientists from European organisations (including V. Deklerck) to contribute online, but most participants were present in person. The workshop was covered by the national Gabonese newspaper and television. A list of the workshop participants and a summary of the main recommendations agreed upon by all participants at the end of the workshop are provided in Annex 4.3, together with evidence for media coverage (the full report will be provided with the final report).

D. Bourobou also organised lab visits on 11 December 2023 during which S. Bellot, C. Quintero-Berns and D. Bourobou herself could assess equipment available and talk to the lab directors of two main labs that could be used for timber DNA barcoding (see Output 3 and corresponding Annex 4.6 for details).

Preliminary insights from the stakeholders consultations (questionnaires and workshop) on the pros and cons of implementing DNA barcoding and on conditions for implementation (indicator 1.2) indicate different levels of knowledge of the scientific techniques necessary to deploy DNA barcoding among the scientists and other stakeholders consulted in Gabon and DRC. Scientists have a basic knowledge of the principles and some have had some hands-on experience with DNA barcoding in local or foreign labs, while non-scientists tend to lack the basic understanding required to make informed decisions about deploying or not DNA barcoding to serve their interests. Regardless, interest was high among all stakeholders, including policy makers and law enforcers. Logistic issues appear to be more prominent in DRC, especially outside Kinshasa, than in Gabon, but in-depth conversation among project partners during the visit of J. Lisingo to RBG Kew in April 2024 and follow up conversation between J. Lisingo and his colleagues at University of Kisangani have started to reveal how logistical challenges could be overcome in the near future. Motivation is high to start building scientific and logistic capacity for the implementation of DNA barcoding in both countries. Specifically, there is a consensus among consulted stakeholders in Gabon that it would be useful to have a DNA field test so that species can be identified within concession, that a species certificate can travel with the timber (at least in Gabon), and that further occasional DNA tests could be conducted to support controls before the wood is exported, assuming they could be cheap and quick so that wood shipments do not need to be held for a long time. This would open doors to enable full species traceability along the supply chain and until secondary markets. Importantly, feedback from stakeholders suggests that DNA barcoding may be useful

at points of the supply chain where leaves, bark and/or sapwood would still be available, which makes it less essential for the approach to work on heartwood, from which DNA is much more difficult to extract, at least in the focus countries. More details are currently still being collected and will be published in the final report and associated supporting evidence, together with final conclusions.

Output 2. A better understanding of timber DNA properties and of DNA extraction techniques that can improve its quality

We reviewed 36 research publications discussing different DNA extraction protocols (activity 2.1). In addition, we held meetings with several international experts to discuss the application of published DNA extraction protocols. Meetings were held with Steven Janssens (Meise Botanic Garden, Belgium), Samuel Vanden Abeele (University of Cambridge, United Kingdom) and Céline Blanc Jolivet (Thünen Centre of Competence, Germany). The protocol optimisation work was also informed by lab visits and discussions during the workshop in Gabon, as it became clear following these visits that at least two labs in Gabon have already the necessary equipment to perform pilot plant DNA barcoding, even though further equipment and infrastructure may be needed for a large scale implementation. Based on these literature and scientific consultations, a baseline protocol was written up, and key parameters to test and adjust were identified (indicator 2.1, activity 2.2). These parameters were systematically tested using four samples from three species through performing 192 DNA extractions (activities 2.3-2.6). Comparisons of outputs enabled us to identify best values for each of the key parameters, and to write the definitive protocol. A few other parameters are also being tested but under a less rigorous approach due to time constraints. The protocol is now being used on 18 samples of 8 species, and will be used on more samples if time allows, both to confirm its performance across the focus species and possibly beyond (indicator 2.2) and to generate wood DNA extractions that can be used to test the DNA barcodes developed as part of Output 3. A summary of our findings and of the protocol optimisation framework is provided in Annex 4.4, together with a list of the samples on which the protocol has been tested so far. The outcome from our literature review and discussions will be synthesised and published together with the final protocols in Report 2 and part of it will be used in Article 1 to fuel discussion about the feasibility of timber DNA barcoding.

Output 3. A better understanding of the DNA barcodes and methods that can be used to monitor the trade of a key timber group

A reference DNA dataset has been generated, comprising the sequence of up to 317 nuclear and plastid genes (median: 268 genes) for one to 19 samples for 23 species (indicator 3.1), i.e. all the species from all the focus genera (*Entandrophragma, Khaya, Lovoa, Swietenia*). For each species, samples from a wide geographic range have been selected (activities 3.1-3.3). To ensure reliable sample authentication and enable back checking, samples were mainly obtained from the Kew herbarium, although a few samples were taken from WFID collections. DNA was extracted from each sample, Illumina DNA libraries were prepared from each DNA extract (activity 3.4), and the libraries were then split. One half was submitted directly to Illumina sequencing (activity 3.5) while the other half underwent hybridisation with baits targeting 353 genes known for their phylogenetic performance across angiosperms. The libraries enriched in the 353 target genes were then submitted to Illumina sequencing too (activity 3.6). The Illumina data was cleaned and clean sequencing reads were then assembled into genes. For each gene, sequences were compared (aligned) across samples and a phylogenetic tree was produced. A summary tree was then produced using all the gene trees. The tree shows that most samples group according to their species, except for a few, which tend to be samples with low data. The reference DNA dataset is currently being cleaned to make sure only samples with good data and correct name assignment are included. A list of the samples sequenced and of the amount of data generated for each sample is provided in Annex 4.5

By comparing the information contained in 343 genes for each of the genera, we identified 3 genes that had a lot of information, and no signs of being present in multiple copies in any of the species. For each of these genes, we identified potential barcodes, i.e. regions variable across species flanked by regions conserved across species (activity 3.7). This way, a total of 10 DNA barcodes were identified, each between 200 and 400 bp-long (Indicator 3.2). Primers were designed for each DNA barcode, and their amplification by PCR and sequencing using Sanger sequencing is currently tested at RBGK (activities 3.8-3.10). The information necessary to sequence the DNA barcodes will be provided in Report 2 together with the final project report and published in Article 1.

Based on discussions with D. Bourobou and J. Lisingo, lab visits in Gabon and protocol tests, a classification of what methods and barcodes can be used in different contexts (field concession, customs, local labs, foreign labs) is starting to emerge (indicator 3.3). A summary of the visits and relevant conversations is provided in Annex 4.6. Conclusions on the matter will be synthesised following further discussion between all partners and selected external stakeholders to be held in June 2024, and published as part of Report 2 together with the final project report.

4.3 Progress towards the project Outcome

The goal of this project was to generate **a body of evidence and** to create **an international network that will support improved monitoring and regulation of the timber trade through the use of DNA barcoding** (Outcome statement). This is necessary before any attempt to widely deploy DNA barcoding tools to monitor the timber trade because technical and logistical challenges first need to be identified and addressed.

A first challenge was to clarify in what conditions would DNA barcoding truly help monitoring illegal timber trade and to understand the positioning of timber trade monitoring stakeholders regarding this approach in the focus countries. Before the project, the knowledge of different stakeholders about DNA barcoding was unclear but thought by the local partners to be scarce, and many stakeholders did not have a global view of the existing infrastructure in place to monitor the timber trade in their country and of the remaining gaps or issues in this infrastructure, which prevented them to design solutions, especially DNA-based solutions. To address this issue, we aimed to ***identify stakeholders, and requirements for implementation of DNA barcoding at key points of the timber trade*** (Indicator 0.1). So far, stakeholders have been identified in terms of their category (government agencies, scientists, non-governmental organisations, law enforcement etc.; Annex 4.1), and people representing all these categories have either taken part in a workshop in Gabon in December 2023 (Annex 4.3) or been contacted informally by D. Bourbon (Gabon) and J. Lisingo (DRC) or their colleagues to discuss (and increase) their knowledge of DNA barcoding and how they thought the approach may be useful and applied to their context. The discussions were guided but not restricted to a questionnaire that we designed (Annex 4.2). The feedback provided by stakeholders is currently being analysed and, following discussions between main project partners planned for end of May and beginning of June 2024, it will be fully transcribed and consolidated into Report 1, which will be published online at the end of the project.

A second challenge was to make sure that the scientific conditions to use DNA barcoding were reunited. This included ***creating and making available a high quality reference dataset for the focus species and their look-alikes and close relatives (ca. 22 species), comprising multiple samples per species and hundreds of nuclear and plastid genes for each sample*** (Indicator 0.2). While some of these species have been genetically studied in the past and the data is publicly available, DNA barcoding requires a reference dataset as complete as possible in terms of species, and with as many samples as possible per species, to reduce the risk of false positives and false negatives during sample identification. Such comprehensive reference did not exist before, but we have now created it using hundreds of genes that can either be studied together or separately (see Annex 4.5) , providing flexibility in the choice of DNA barcodes and DNA barcode sequencing approaches. This flexibility will allow users with

different means and different types of material (e.g. leaves, sapwood, heartwood, processed wood) to use different approaches but the same reference database. An important aspect of this flexibility was to enable users with limited means and/or time to obtain a reliable sample identification by sequencing only a few DNA barcodes and matching them to the corresponding subset of genes in the reference database. To do this, we aimed to ***identify DNA regions that, on their own, could allow species identification in our focus group of look-alikes*** (Indicator 0.3). Although standard plant DNA barcodes exist, they are not always as performant as custom-designed DNA barcodes. We therefore looked in our newly generated reference dataset, which also includes standard DNA barcodes, for new regions that varied consistently between species and had properties that could facilitate their sequencing in a cost-efficient manner. We found ten regions (divided in 3 genes), and we are now testing the performance of these regions in the lab, and comparing it with that of standard barcodes (evidence and details to be provided in the final project report).

Beyond performance at distinguishing species, an important aspect of DNA barcode performance is the facility and cost-efficiency with which they can be sequenced from the DNA obtained from the products to identify. This has long been a challenge for DNA barcoding applications to timber monitoring because the DNA extracted from timber can be very degraded or even absent, and the more degraded it is, the more difficult and costly it is to obtain reliable data from it. The uncertainty to be able to use DNA from timber, and the lack of clarity regarding the conditions in which DNA barcoding may be desirable along the timber supply chain have so far restricted the use of the technique to isolated court cases, by opposition to it being used as a routine law enforcement tool. To address this, we wanted to ***characterise the range of DNA qualities that can be obtained from timber products of the focus species, and protocol modifications that can improve DNA quality*** (Indicator 0.4), and we also aimed to clearly put this in relation with stakeholders feedback to ***identify what DNA barcodes and DNA sequencing approaches could fit logistic and economic requirements for DNA barcoding implementation at key points of the timber trade*** (Indicator 0.5). We optimised a protocol that shows good performance on sapwood, and that can also provide DNA from heartwood but at a lower quality and yield. Based on this, it appears that cheap DNA barcode sequencing can be performed for sapwood samples but we are still experimenting to determine if cheap approaches can be used on heartwood samples and if not, if expensive approaches are able to provide results, which could make them worthwhile in selected cases. These experimentations are ongoing and results will be provided in the final report. Meanwhile, discussions with stakeholders and between project partners have made clear that in the focus countries, leaf and sapwood samples would often be available and therefore that cheap DNA barcoding approaches may be feasible and valuable, even if they cannot be applied to heartwood or processed wood.

A final challenge to deploy DNA barcoding in the focus countries is the availability of people with the necessary skills and knowledge to use the approach and interpret the results. For this reason, a last aim is that, by the end of this project, ***at least one person from each of the focus countries has the labwork and bioinformatic skills required to perform DNA barcoding*** (Indicator 0.6), acknowledging that actually deploying the approach may require further, larger, funding applications to build the local capacity in terms of equipment and infrastructure. Although project partners from both countries had already some theoretical knowledge of plant DNA barcoding and had already had some hands-on experience in foreign labs, neither had seen or taken part to the development of DNA barcoding tools from beginning to end, and both were keen to be further trained so that they could confidently start implementing the approach and train others to do so in local labs. Due to delays in the visa application process, so far only one partner (J. Lisingo) could visit RBGK, while the visit of D. Bourobou is planned for the beginning of June 2024. Although J. Lisingo could only stay at RBGK for 2 weeks due to a passport validity issue, he was trained in designing primers to sequence DNA barcodes and then designed the primers for two of the regions on his own. He was then trained in extracting DNA from wood and performed 60 DNA extractions on his own, and he was also trained in amplifying DNA barcodes from the extracted DNA while performing 32 PCR amplifications on his own. J. Lisingo also discussed at length with S. Bellot about how the DNA database had been generated and about how DNA data could then be matched to the database to identify samples. He left with the confidence that he could apply these techniques

in his own lab. Additional training will be organised in June online and during the visit of D. Bourobou to RBGK.

In summary, the indicators were helpful in that, by delivering under each indicator, we demonstrate the creation and existence of a new body of evidence about the timber supply chain and about timber DNA barcoding as far as the focus countries and focus species are concerned. This evidence is valuable beyond the focus countries, for application in exporting countries with similar challenges, but also to inform how importing countries may be able to use DNA barcoding, as the approaches and reference database remain the same. Creating this body of evidence involved engaging with multiple actors in the focus countries and beyond (including Belgium, UK and the USA), which has initiated the creation of a network of people willing to collaborate and to explore the use of DNA barcoding for timber trade monitoring. Going forward, in the next couple of months and post-project, it will be important to further strengthen our body of evidence by continuing to test the performance of our new DNA barcodes on different types of wood material, from the focus species and beyond, and to further strengthen our network by designing larger projects involving more directly the different categories of stakeholders.

4.4 Monitoring of assumptions

Assumption 1: We are able to identify key implementation challenges and incentives. This assumption proved verified in that we were able to identify challenges to the implementation of DNA barcoding in Gabon (lack of knowledge about the technique and its costs and advantages among non-scientists stakeholders) and DRC (same as in Gabon with additional gaps in training and lab capacity), as well as incentives (complementarity with existing traceability system and suitability to the needs of law enforcers in Gabon; opportunities to foster research and education in the field of plant genetics in both countries). See preliminary evidence in the summary of the workshop in Gabon (Annex 4.3); full evidence will be provided with the final report. However, our knowledge is deeper about Gabon than DRC given that we had longer discussions with more stakeholders from the former country, and further stakeholder consultations remain needed to support DNA barcoding implementation in both countries. For this reason, networking and gathering further evidence will remain an important axis of future projects even though existing evidence and networks are already sufficient to start pilot tests in both countries.

Assumption 2: The DNA quality obtained from wood samples will enable the use of cheap DNA barcoding methods. This assumption is still under test, but from our first results, it is partially verified. Indeed, the DNA that we extracted from some wood types (bark, sapwood) is of sufficient quality to use cheap methods (i.e. PCR amplification and Sanger sequencing) and feedback from stakeholders shows that it would be valuable to apply DNA barcoding at stages of the supply chain where such type of material is available (see Annex 4.4 and 4.6 for preliminary evidence; full evidence to be provided with the final report). However, so far, we do not have evidence that the DNA obtained from heartwood will be of sufficient quality to be submitted to cheap DNA barcoding methods. This is still under test and development. If sufficient quality cannot be obtained from heartwood of the focus species, it will be a useful outcome to communicate to stakeholders and interested scientists, and it will mainly affect the control of timber outside of exporting countries, where other, more expensive methods, are more easily available.

Assumption 3: The DNA regions targeted to build the reference dataset vary sufficiently to enable the identification of DNA barcodes characteristic of each species. This assumption has now been verified as we could select 3 individual genes that group samples according to their species and excluding samples from other species. However, tests are still ongoing to see if the 10 subregions chosen to serve as DNA barcodes in the three genes can

provide a reliable identification for all the species, and to evaluate if all subregions need to be used or if one or a few are enough. Evidence will be provided in the final report.

4.5 Impact: achievement of positive impact on illegal wildlife trade and poverty reduction

This project is enabling us to develop DNA barcoding approaches suitable to rapidly and cost-efficiently identify key timber species within the Meliaceae family in both Gabon and the DRC. Although routine timber checks will not be implemented in the focus countries by the end of this project, the intended impact of the project, which was to lay the scientific foundation and establish the partnerships required to do so in the future, will be achieved. Indeed, the stakeholder consultation demonstrated that there is a high interest in seeing this come to fruition in the near future and brought together the necessary stakeholders behind this common goal (Annex 4.3), while the literature review and labwork have generated the evidence, barcode regions and reference data needed to produce a suitable DNA barcoding toolkit for accurate and robust species identification (Lowe et al., 2011; Iawa J. 32(2):251–62) in any export or import country that deals with timber species from the Meliacea family. Ultimately, this evidence project will enable larger projects to improve the monitoring and control of illegal timber trade through DNA-based tracking. By doing so, the project will facilitate a more sustainable management of forests. This will reduce the pressure on the threatened species that constitute the forests and decrease the risk that local communities lose important sources of livelihood when those species become rarer or extinct (see Sections 5 and 6 for further details).

5. Thematic focus

Our project mainly aligns with the following themes: (2) Ensuring effective legal frameworks and deterrents and (3) Strengthening law enforcement by providing the scientific evidence (Outputs 2 and 3) and context evaluation (Output 1) necessary to put in place an approach that will reliably and cost-effectively enable law enforcers to gather the evidence they need (timber species identification) to enforce the law. Moreover the project also less directly aligns to themes (1) Reducing demand for IWT products because the availability of a cost-efficient mean to certify that timber products do not come from protected species will encourage a switch of consumers towards products of which the legality can be ensured and thereby also protect the legal market. Finally, long term contributions to theme (4) Developing sustainable livelihoods to benefit people directly affected by IWT are explained in Section 6 below.

6. Impact on species in focus

The species targeted in this grant were identified by World Forest ID, based on a survey with 22 enforcement agencies (available upon request to WFID), as being the priority to collect reference data on to allow effective species identification and timber tracing. In particular, *Khaya* species will be included soon in the CITES Appendix II, and as such it will receive a higher protection status and its trade will need to be monitored, increasing the need for DNA barcodes able to distinguish these species from look-alikes. By generating a DNA reference dataset comprising hundreds of nuclear and plastid genes for multiple samples (Indicator 3.1) representing the global genetic diversity of all focus species and their look-alikes and close relatives this project will facilitate fundamental research on the demographics and regeneration of these species. By publicly releasing DNA barcodes (Indicator 3.2) and protocols (Indicator 2.2) designed specifically for these species together with the reference dataset, the project will enable accurate identification of samples from these species collected anywhere in the world.

In a context where in-country actors are keen on making the most of these outputs (Indicator 1.2), this will inform and improve the conservation management and sustainable exploitation of the species.

7. Project support for multidimensional poverty reduction

This project is intended to contribute to the reduction of the illegal timber trade by deterring fraud through timber identification tests using DNA-based techniques at the level of the timber traceability chain in the Guinean-Congolese basin forest sub-region, with focus on Gabon and DRC. Although Gabon is considered an Upper Middle Income Country, it was crucial to include Gabon in the project to provide a baseline for forensic research in the Guinean-Congolese basin. The knowledge exchange between Gabonese and DRC experts is instrumental to the development of DNA capacity (both in terms of experience and necessary equipment) in the DRC.

The species on which the project focuses are used as timber, firewood, or in cosmetics (oil) and their organs (roots, bark, seeds, leaves) are used for the preparation of decoctions or powders for the local pharmacopoeia. Moreover, the high intensity exploitation of these species and the expansion of forest concessions by the timber industry leads to a reduction in the density of trees that can provide food for elephants, resulting in food scarcity and a lack of food diversity in the territory of elephants. Consequently, preventing the removal of these multi-uses trees from forest areas, including elephant-inhabited areas, will (1) help to reduce the intensity of the human-elephant conflict (transgression of elephants into farmers' plantations in search of food) and (2) help to ensure that forest and rural communities can continue to meet their basic domestic and health needs through the sale or the use of raw or semi-processed harvested products (bark, leaves, roots, seeds, oils, etc.) from forest areas to rural and urban areas. Indeed, it should be remembered that one of the causes of the rural exodus in Gabon is the lack of basic income and access to healthcare.

Similarly, in the DRC, local communities, i.e. the farmers who live next to forest concessions, and more particularly indigenous peoples, are the most affected by the exploitation of the focus species. For example, *Entandrophragma cylindricum* is a tree that hosts edible caterpillars (*Cirina forda*) that are highly prized by local communities and represent an important source of proteins and revenue, especially in periods during which hunting is forbidden (Lisingo et al. 2010; Geo-Eco-Trop 34:139-146). Intensive logging of this tree species deprives communities of edible caterpillars, sometimes leading to conflicts between loggers and local communities. In the long term, the conservation or restoration of forest areas with species of trees that host edible caterpillars could therefore support the fight against global warming and poverty.

In summary, sustainable forest management measures, backed up by effective action to reduce the illegal timber trade, are essential to preserve the forest ecosystems and their services to people in Gabon and DRC and beyond. This includes people whose livelihoods depend directly on the forest and its products, but also people that are dependent on income generated by the legal timber trade, because this income is undercut by the illegal trade and prices. In the long term, this project and the future, larger, projects enabled by its outcome will help combating the illegal exploitation of timber, which will likely increase the value of exported timber and boost government revenue while increasing the sustainability of forest resources management and contributing to protect the livelihoods of communities who depend on these resources.

8. Gender Equality and Social Inclusion

Please quantify the proportion of women on the Project Board[1].	50% (two women, two men)
Please quantify the proportion of project partners that are led by women, or which have a senior leadership team consisting of at least 50% women[2].	0% (The scientific departments of RBGK, WFID, CENAREST and University of Kisangani are all directed by men). However, they all have women among their senior leadership teams, including partner D. Bourobou, and the CEO of WFID is a woman).

GESI Scale	Description	Put X where you think your project is on the scale
Not yet sensitive	The GESI context may have been considered but the project isn't quite meeting the requirements of a 'sensitive' approach	X
Sensitive	The GESI context has been considered and project activities take this into account in their design and implementation. The project addresses basic needs and vulnerabilities of women and marginalised groups and the project will not contribute to or create further inequalities.	
Empowering	The project has all the characteristics of a 'sensitive' approach whilst also increasing equal access to assets, resources and capabilities for women and marginalised groups	
Transformative	The project has all the characteristics of an 'empowering' approach whilst also addressing unequal power relationships and seeking institutional and societal change	

We assessed ourselves as "not yet sensitive" because the "vulnerabilities and basic needs of women and marginalised groups" were not explicitly accounted for during project design, mainly because the project did not involve many partners or activities. Nevertheless, main project partners were chosen for their relevance to the project while considering gender balance, resulting in gender balance among the two project PIs (V. Deklerck: M; S. Bellot: F), the two research assistants / lab technicians (C. Quintero-Berns: F; L. Csiba: M) and the two partners from Gabon and DRC (D. Bourobou: F; J. Lisingo: M). Moreover, gender balance was also a consideration during the organisation of the workshop in Gabon (Annex 4.3) where 32% of the attendants and 53% of the people who made an oral presentation were women. In the medium term, the deployment of genetic tools to identify timber products in the focus countries as part of future, larger projects enabled by this first project will provide opportunities for young women to take part in wood traceability training courses and research projects at universities

[1] A Project Board has overall authority for the project, is accountable for its success or failure, and supports the senior project manager to successfully deliver the project.

[2] Partners that have formal governance role in the project, and a formal relationship with the project that may involve staff costs and/or budget management responsibilities.

and Grandes Ecoles in Gabon and DRC. Finally, the improvement of timber traceability using genetic tools will ultimately contribute to the sedentarisation and maintenance of the autonomy of women in forest and rural areas, thanks to the income derived from the sale of products from the gathering of non-wood forest products.

9. Monitoring and evaluation

Monitoring and Evaluation was done collaboratively via weekly meetings between Deklerck, Bellot and the Research Assistant and monthly meetings between all project partners. Meeting notes were saved in a shared folder accessible to all partners. When one of the partners would not attend, a report of what was discussed was emailed to them in the most convenient language for them (English for UK and Belgium-based partners, French for Gabon and DRC partners) and their approval or input was sought so that major monitoring and decision making always involved all partners. A GANNT chart aligned to the outputs and indicators was also produced and shared between all partners.

As demonstrated in Section 3.3, indicators and associated activities are suitable to deliver on the project Outcome and there are sufficient tangible indicators of achievement to monitor progress, notably a list of stakeholders to consult, a questionnaire, questionnaire replies and other feedback notes and workshop reports, lists of samples to perform labwork on, DNA data generated from the samples, lists of articles reviewed, baseline and final protocols, primer sequences of the newly designed DNA barcodes, DNA concentration and size measurements, DNA barcode sequences, syntheses of the stakeholder consultation activities (Report 1) and of the lab results (Report 2), and an article presenting the DNA barcoding toolkit for the focus species and its relevance and applicability in the focus countries (Article 1). All these indicators have now been created or are in the process of being created or finalised (see Annexes 4.1-4.6 for preliminary evidence).

The M&E plan has not changed during the project, but some meetings have been replaced by emails as and when needed because it was difficult to find dates of meetings suitable for all partners. In future, longer projects, it will be important to find ways to maintain regular whole-team meetings as they enable us to reveal and discuss in depth unforeseen points. Nevertheless, we found that regular emailing was sometimes more efficient and preferred by the partners in focus countries given the many meetings they already have to attend, as long as communication regarding all aspects of the project was maintained between all partners, which was the case. Full team meetings are planned for May and June, in order to plan final output delivery and post-project activities.

10. Lessons learnt

As already reflected upon in other sections, a main lesson learnt has been that developing new international partnerships in countries from which the citizens are submitted to complex visa procedures to enter the UK, and/or in countries with unstable political situations requires all partners to invest significant time and money in administrative procedures, which can put a strain on the delivery of other project outputs and even jeopardise the building of the partnership itself (by preventing travel), especially in the context of one-year projects. Another lesson was that the recruiting of new staff has to be accounted for as part of the project given that there is not much time between the notice that the project is funded and the start of the project. This can lead to suboptimal recruitment and/or to a lower productivity than originally planned, especially for one-year projects. Given that this is a one year project, we requested an extension of 3 months to accommodate for these issues and other unforeseeable ones (sick

leave) and we are now back on track to deliver on the Outcome by the newly approved project end date (30 June 2024). Learning from this, **if a similar project had to be repeated in the future, we would either considerably reduce the goals in terms of labwork and in-person visits, or frame it as a 2-year project,** depending on what is possible given the funding instruments available.

Globally, we found that the **team work necessary for efficient stakeholder consultation and for delivering insightful evidence has worked very well** despite the four main partners being each in different countries. This likely stems from efforts to involve partners since the conception of the project and throughout it, and the short in-person visits have strengthened links and cooperation by enabling all partners to truly give and receive and for the expertise of each partner to be valued. This clearly showed the importance of in-person visits and convinced us that longer (>3-week) visits would be ideal so that new partnerships can achieve their full potential in terms of taking a project to the next level.

Finally, although it has not significantly affected the delivery of the project, our **note taking and achievements monitoring has at times been suboptimal**, with some notes being taken by multiple partners and other notes being insufficiently detailed, resulting in some time being lost in tracking down past decision making. This likely stemmed from a lack of clarity in terms of task sharing, possibly stemming from the fact that the project first had 1 lead (V. Deklerck) and then two (S. Bellot joined in when V. Deklerck left RBGK). This has been discussed and task sharing and the way to take notes will be more precisely agreed on when designing and initiating future projects.

11. Actions taken in response to previous reviews (if applicable)

Not applicable as this is a 1-year project (please see Half-Year report for how we addressed feedback provided when the project was funded)

12. Risk Management

This was originally a one-year project. The main risks not accounted for were the possibility of a coup in Gabon (which was not considered likely by anyone), and the fact that a RBGK staff member responsible for sending samples to the sequencing company would go in sick leave while the project partners responsible to oversee the sample sequencing were in annual leave. The coup in Gabon led to a replacement of stakeholders and a closure of borders which delayed the stakeholder consultation and the workshop and Gabon lab visits by two months. It also added a lot of administrative load on the local project partner (D. Bourobou), which delayed her visa application, itself complicated by the lack of political stability and by a lack of clarity regarding new visa application procedures. This led to the impossibility for D. Bourobou to visit RBGK before the originally-agreed end of the project (March 2024). The delay in sending samples for sequencing in turn delayed the data analysis by more than a month. To make sure all outputs could be delivered despite these issues, we therefore requested a 3-months extension of the project.

13. Sustainability and legacy

The outputs of the project will consist in two reports, genomic data for hundreds of samples, and an article. All these outputs will be published online in open access by the time the final project report is due (September 2024).

The workshop in Gabon and satellite stakeholder consultations in country have generated interest among local botanists, law enforcers, policy makers, scientists, teachers, non-governmental and governmental organisations and private companies. This is demonstrated by

how keen these actors were to follow up with further actions and by their interventions in the local media (Annex 4.3). The theme of the project (timber trade monitoring) is of interest to the general public in the focus countries, as demonstrated by the nation-wide media coverage of the workshop in Gabon (Annex 4.3).

Capacity has been increased in Gabon by ensuring that all previously-mentioned actors attending the workshop received a basic explanation of the scientific principles and associated advantages and drawbacks of timber DNA barcoding and by strengthening the links between local scientists and non-scientist stakeholders. Capacity has also been increased in DRC via the training in DNA barcoding data generation and analysis received by J. Lisingo during his visit to RBGK, which J. Lisingo is planning to transmit to future students with the help of S. Bellot and other partners. The added capacity in DRC is further illustrated by the fact that J. Lisingo has now the confidence and knowledge to start building a genetic lab at the University of Kisangani, as demonstrated by the fact that he recently initiated discussions with colleagues at his university to establish a list of the equipment and budget needed for this. The list will be provided with the final report and used to apply for funding. Meise Botanic Garden and the Royal Museum for Central Africa (Belgium) have shown interest to help support the building of a genetic lab at the University of Kisangani.

Capacity has also been increased in the UK/RBGK through the explanations about forestry and timber trade challenges in the focus countries given to S. Bellot by D. Bourobou, J. Lisingo and other local stakeholders, and through the added experience in timber DNA analyses developed by S. Bellot and C. Quintero-Berns during the project. Finally, capacity has also increased for V. Deklerck (in Belgium) who now has a better understanding of DNA-based timber traceability.

A long term goal of the project is to set the stage for future extended capacity for in-country timber trade monitoring and DNA-based plant identification in general, and for such capacity to ultimately foster the creation of new jobs in the environment management sector and the accession to these jobs by populations currently limited in their career choices due to poverty and political instability. Increasing the education and job opportunities in the region of Kisangani via the establishment of a new lab and of environmental monitoring projects has the potential to deliver this long term goal, as does the deployment of DNA-based timber identification in Gabon, especially if it is supported by an updated education curriculum including a strong genetic and botanical component, which was seen as desirable by many workshop attendants.

14. IWT Challenge Fund identity

This project was initially developed as part of the wider efforts of World Forest ID to develop timber tracking but the project has a clear identity as a project funded by the IWT Challenge Fund in which WFID plays a role.

Focus countries were not very familiar with the IWT Challenge Fund but this project has changed this and partners in focus countries are willing to explore how the fund may support future projects.

The IWT Challenge Fund has so far been promoted during the workshop in Gabon, where presentations from project partners and communications (e.g. invitations) mentioned it, although we note that the logo itself has not been used as often as it could have, which is an area for future improvement. The Fund was also promoted internally at RBGK when Research Assistant C. Quintero-Berns presented the project to the Trait and Function department (>100 people) and when the obtention of the grant was announced through various RBGK Science-wide channels (directed to ca. 300 scientists). V. Deklerck also promoted the IWT Challenge Fund and displayed its logo when mentioning the project at various WFID meetings involving partners from multiple countries.

We have updated the Office for Product Safety and Standards (OPSS) on the project, IWT CF and what DNA barcoding could mean for timber tracing and for species screening on import in the UK. Nick Whittle (OPSS) also filled in our questionnaire.

The project has been publicised through Twitter/X and Linkedin [redacted] and further social media communications will happen when the outputs are delivered, and they will link back to the IWT Challenge Fund social media channels.

15. Safeguarding

Has your Safeguarding Policy been updated in the past 12 months?		No
Have any concerns been reported in the past 12 months		No
Does your project have a Safeguarding focal point?	No	
Has the focal point attended any formal training in the last 12 months?	Not applicable	
What proportion (and number) of project staff have received formal training on Safeguarding?		Past: 100% (4) Planned: 0%
Has there been any lessons learnt or challenges on Safeguarding in the past 12 months? Please ensure no sensitive data is included within responses. Nothing to report		
Does the project have any developments or activities planned around Safeguarding in the coming 12 months? If so please specify. Nothing planned		
Please describe any community sensitisation that has taken place over the past 12 months; include topics covered and number of participants. Nothing to report		
Have there been any concerns around Health, Safety and Security of your project over the past year? If yes, please outline how this was resolved. A coup in Gabon raised safety concerns as a trip there was planned when the coup happened (end of August 2023). The trip to Gabon was postponed and project-related activities of the local partner were kept to a minimum (only desk-based activities) until after the situation had settled (i.e. until December 2023).		

16. Project expenditure

Table 1: Project expenditure during the reporting period (April 2023-March 2024)

Project spend (indicative) since last Annual Report	2023/24 Grant (£)	2023/24 Total actual IWTCF Costs (£)	Variance %	Comments (please explain significant variances)
Staff costs (see below)	[redacted]			
Consultancy costs				
Overhead Costs				Late recruitment of Research Assistant reduced overhead charge
Travel and subsistence				
Operating Costs				
Capital items (see below)				Computer equipment lower than budget
Others (see below)				
TOTAL	**78,240.12**	**75,603.44**		

Table 2: Project mobilised or matched funding during the reporting period (1 April 2023 – 31 March 2024)

	Secured to date	Expected by end of project	Sources
Matched funding leveraged by the partners to deliver the project (£)	[redacted] WFID expeditions and sample collections for the key timber species group we focussed on. RBGK (UK, [redacted] Accessioning, curation, and custodial care of physical WFID samples at Kew.	NA	NA
Total additional finance mobilised for new activities occurring outside of the project, building on evidence, best	NA	NA	NA

practices and the project (£)			

17. Other comments on progress not covered elsewhere

Difficulties encountered during the year have been discussed in Sections 9 and 11. Following the workshop in Gabon, it was clear that further conversations between UK, Belgium, Gabon and DRC stakeholders will be helpful to design the next projects, so the possibility for a follow-up online meeting with selected stakeholders before the end of the project is currently being explored.

The Royal Museum for Central Africa (Belgium) is building a wood science laboratory in Kisangani in the DRC. We are actively exploring the option to add a DNA laboratory to this plan, in collaboration with UNIKIS (partner in this grant). We are currently identifying the needed funding to achieve this.

18. OPTIONAL: Outstanding achievements or progress of your project so far (300-400 words maximum). This section may be used for publicity purposes.

I agree for the Biodiversity Challenge Funds to edit and use the following for various promotional purposes (please leave this line in to indicate your agreement to use any material you provide here).

This section will be filled in the final project report.

19. Annex 1: Report of progress and achievements against logframe for Financial Year 2023-2024

Project summary	Progress and Achievements April 2023 - March 2024	Actions required/planned for next period
Impact Our original project did not require an impact statement	The development of robust and efficient timber identification tools in West Central Africa has been challenging due to limited resources and complex on-the-ground situations. In this project, we performed round table multi-stakeholder discussions to determine at which stage of the supply chain these tools can be most effective, trained local scientists in DNA barcoding techniques and developed a DNA timber barcoding protocol which can be implemented in the focus countries. Within Gabon, all conditions appear to be reunited so that timber samples could be rapidly and routinely checked for their species identity. A crucial aspect here is the buy-in from government and timber industry stakeholders, which was confirmed during a workshop in Gabon. In the DRC more investment on material capacity is necessary and we are actively looking into procuring funding to build a DNA barcoding laboratory in the country. Actively protecting Africa's natural resources is crucial towards maintaining and ensuring the livelihood of local communities (see Sections 3.5, 5 and 6).	
Outcome A body of evidence and international network that will support improved monitoring and regulation of the timber trade through the use of DNA barcoding.		
Outcome indicator 0.1 We have identified stakeholders, and requirements for implementation of DNA barcoding at key points of the timber trade.	Stakeholders have been identified and consulted. The feedback has provided information on the relevance of DNA barcoding for monitoring timber trade in the focus countries. Evidence is being written up in Report 1, which will be provided with the final report. See partial evidence under Output 1.	Consolidate the feedback from DRC, finish to write Report 1
Outcome indicator 0.2 We have created and made available a reference DNA dataset for ca. 20 timber species, including multiple samples per species and comprising hundreds of nuclear and plastid genes for each sample.	The reference DNA dataset has been generated and is currently being analysed and curated for public release, evidence under the form of a link to the online repository containing the reference database will be provided with the final report. See partial evidence under Output 3.	Submit the DNA data to Genbank

Outcome indicator 0.3 Using the reference dataset, we have identified DNA regions that can allow species identification in the group of look -alikes.	We have identified 3 main DNA regions with high potential to serve as barcodes, and we have designed primers to sequence these regions. Tests are ongoing in the lab to assess the most cost-efficient way to use these new DNA barcodes. The regions and protocols to sequence them are being written up in Report 2, which will be provided with the final report. See partial evidence under Output 3.	Continue tests. Finish to write Report 2 and Article 1
Outcome indicator 0.4 We have characterised the range of DNA q ualities that can be obtained from timber products of the focus species, and protocol modifications that can improve DNA quality.	Tests have been performed to establish what protocol works better on different types of wood material. The final protocol will be written up in Report 2, which will be provided with the final report. See partial evidence under Output 2.	Finish to write Report 2 and Article 1
Outcome indicator 0.5 We have identified what DNA barcodes and DNA sequencing approaches could fit logistic and economic requirements for DNA barcoding implementation at key points of the timber trade.	Lab visits in Gabon and conversations with partners have clarified what approaches are feasible and desirable in the focus countries. The preferred approach is that of PCR and Sanger sequencing so tests of this approach are ongoing. This is being written up in Reports 1 and 2, which will be provided with the final report. See partial evidence under Output 3.	More conversations to be help between partners to identify next steps necessary for the deployment of such methods post-project
Outcome indicator 0.6 At least one person from each of the focus countries has the labwork and bioinformatic skills required to perform DNA barcoding.	Training of partner J. Lisingo in the lab and in computer-based DNA barcode design has taken place during his visit at RBG Kew. The visit of partner D. Bourobou was delayed and is now planned for June 2024. See partial evidence under Output 3.	Training of second partner, reinforcement and verification of of acquired skills to be organised
Output 1 New data and knowledge providing a better understanding of the requirements for DNA barcoding implementation at key points of the timber trade supply chain		
Output indicator 1.1 Key supply chain stages, timber processing steps and stakeholders have been identified, including key locations in the supply chain for DNA barcoding implementation, and relevant questions to ask to stakeholders have been agreed upon.	A flowchart presenting the different actors and supply chain points is being made. A list of the stakeholders consulted is available in Annex 4.1, and the questionnaire addressed to stakeholders is available in Annex 4.2.	Outputs to be consolidated in a single report (Report 1), and selectively used to frame Article 1 as needed
Output indicator 1.2	So far 10 out of 55 questionnaires have been sent back but informal responses have been gathered from 2 additional European enforcement (UK and Belgium) stakeholders. Answers are currently being analysed. A workshop took place in Gabon in December 2023, with 32 participants who provided further feedback on	Further conversations to be held between partners and selected stakeholders.

For these key steps in the chain, DNA barcoding pros and cons and conditions for implementation have been identified.	the potential and relevance of using DNA barcoding for timber trade monitoring. A full report of the workshop has been written and circulated to all participants. A summary of the main recommendations from the workshop is provided in Annex 4.3 (the full report will be provided with the final report).	Synthesis and next steps to be then written up as part of Report 1 and used as a basis for future projects.
Output 2. A better understanding of timber DNA properties and of DNA extraction techniques that can improve its quality		
Output indicator 2.1. Promising existing protocols for extracting DNA from timber have been identified and an experimental framework for protocol optimisation has been designed	Literature was reviewed and two protocols were identified as particularly promising. A framework to test and, if relevant, optimise these protocols was designed. A summary of our findings and of the protocol optimisation framework is provided in Annex 4.4.	Finish to write the procedure and final protocols in Report 2 and Article 1
Output indicator 2.2. Protocols have been tested and optimised for the focus species and a few other timber species	The final protocol has been agreed upon based on the tests. This protocol is currently tested on the focus species before being definitively written up. A list of samples so far used to test the final protocol is provided in Annex 4.4.	Do further tests on different types of wood and different samples. Possibly on different species if time allows.
Output 3. A better understanding of the DNA barcodes and methods that can be used to monitor the trade of a key timber group		
A reference DNA dataset has been generated for the focus group, including multiple samples per species and comprising hundreds of nuclear and plastid genes for each sample	The reference DNA data have been generated for 172 samples representing the focus species. Up to 317 nuclear and plastid genes sequences have been generated for each sample. The dataset is currently being analysed for publication. A list of the samples sequenced and of the amount of data generated for each sample is provided in Annex 4.5	Release the data online and use them in Article 1
A set of candidate DNA barcodes adapted to different DNA analysis methods has been identified for the focus group	The sequences of 343 genes obtained when doing the reference dataset have been compared across the focus species and genera, leading to the identification of 3 genes with the highest potential for DNA barcoding. 10 sub-regions have been chosen inside these 3 genes to serve as DNA barcodes. Primers for sequencing these 10 barcodes have been designed and are now being tested on DNA extracted from wood from the focus species. Barcode regions and primers are available upon request and will be published in Report 2 and Article 1.	Continue tests of the selected barcodes and write up results in Report 2 and Article 1
Methods and barcodes have been classified depending on if they can or cannot be used at key points of the supply chain, due to logistic/economic requirements	Conversations between partners have been held about this now that partners are better aware of the requirements for each method. This has been facilitated by the visit of labs in Gabon in December 2023 and by the visit of J. Lisingo to Kew in April 2024 (see summary of the visits and relevant conversations in Annex 4.6).	Continue conversations during visit of D. Bourobou at Kew in June 2024, and online Write up conclusions as part of Report 2 and possibly also in

		Article 1 if relevant given the framing of the article.

20. Annex 2: Project's full current logframe as presented in the application form (unless changes have been agreed)

Project summary	SMART Indicators	Means of verification	Important Assumptions
Impact:			
Outcome: A body of evidence and international network that will support improved monitoring and regulation of the timber trade through the use of DNA barcoding.	1. Q2 YR1 We have identified stakeholders, and requirements for implementation of DNA barcoding at key points of the timber trade. 2. Q2 YR1 We have created and made available a reference DNA dataset for ca. 20 timber species, including multiple samples per species and comprising hundreds of nuclear and plastid genes for each sample. 3. Q3 YR1 Using the reference dataset, we have identified DNA regions that can allow species identification in the group of look-alikes. 4. Q3 YR1 We have characterised the range of DNA qualities that can be obtained from timber products of the focus species, and protocol modifications that can improve DNA quality. 5. Q4 YR1 We have identified what DNA barcodes and DNA sequencing approaches could fit logistic and economic requirements for DNA barcoding implementation at key points of the timber trade. 6. Q4 YR1 At least one person from each of the focus countries has the labwork and bioinformatic s kills	1. A publicly accessible report presenting our findings is available on the World Forest ID at RBGK webpage (Report 1). 2. The genetic data are available online (Treegenes and/or GenBank databases) 3, and 4. A publicly accessible report presenting the reference dataset, the DNA extraction protocols and DNA quality assessment results and the DNA regions (barcodes) is available on the World Forest ID at RBGK webpage (Report 2) 5. At least one article presenting all our results and conclusions drawn from combining both the supply chain analysis (Report 1) and DNA analysis (Report 2) lines of evidence has been submitted to a peer-reviewed journal and the draft is available on the World Forest ID at RBGK webpage (Article 1). 6. After the workshop at Kew, the international collaborators are able to independently perform DNA extraction and sequencing.	

Project summary	SMART Indicators	Means of verification	Important Assumptions
	required to perform DNA barcoding.		
Output 1 New data and know ledge providing a better understanding of the requirements for DNA barcoding implementation at key points of the timber trade supply chain	1.Q2 YR1 Key supply chain stages, timber processing steps and stakeholders have been identified, including key locations in the supply chain for DNA barcoding implementation, and relevant questions to ask to stakeholders have been agreed upon. 2. Q4 YR1 For these key steps in the chain, DNA barcoding pros and cons and conditions for implementation have been identified.	1. Flowchart presenting the results, list of relevant stakeholders to consult, and list of questions to ask the stakeholders are available on the World Forest ID at RBGK webpage as part of Report 1. 2. Written synthesis on DNA barcoding applicability and pathways towards implementation available on the World Forest ID at RBGK webpage as part of Report 1	We are able to identify key implementation challenges and incentives. Mitigation: we are well implanted in the timber trade sector.
Output 2 A better understanding of timber DNA properties and of DNA extraction techniques that can improve its quality	1. Q1 YR1 Promising existing protocols for extracting DNA from timber have been identified and an experimental framework for protocol optimisation has been designed 2. Q3 YR1 Protocols have been tested and optimised for the focus species and a few other timber species	1. Written synthesis of promising protocols and experimental design available on the World Forest ID at RBGK webpage as part of Report 2. 2. Optimised D NA extraction protocol for the focus group + recommendations for timber in general are available on the World Forest ID at RBGK webpage as part of Report 2.	The DNA quality obtained from wood samples will enable the use of cheap DNA barcoding methods. Miti gation: multiple “mini -barcodes” can be used in combination [21], and some actors of the supply chain could accommodate expensive approaches working on degraded DNA.
Output 3 A better understanding of the DNA barcodes and methods that can be used to monitor the trade of a key timber group	1. Q2 YR1 A reference DNA dataset has been generated for the focus group, including multiple samples per species and comprising hundreds of nuclear and plastid genes for each sample. 2. Q3-Q4 YR1 A set of candidate DNA barcodes adapted to different DNA analysis methods has been identified for the focus group	1. The genetic data are available online (Treegenes and/or GenBank databases) 2 & 3. The barcodes and conclusions on barcodes and methods adapted to implementation at different points of the supply chain are presented in Report 2 available on the World Forest ID at RBGK webpage, and published in full in Article 1.	The DNA regions targeted to build the reference dataset vary sufficiently to enable the i dentification of DNA barcodes characteristic of each species Mitigation: these regions have been shown to vary between closely -related species in many plant families [16,17,18].

Project summary	SMART Indicators	Means of verification	Important Assumptions
	3. Q4 YR1 Methods and barcodes have been classified depending on if they can or cannot be used at key points of the supply chain, due to logistic/economic requirements		

Activities (each activity is numbered according to the output that it will contribute towards, for example 1.1, 1.2 and 1.3 are contributing to Output 1)

1.1: We will review the literature on timber trade and traceability and leverage our network of international par tners.
1.2: We will hold meetings with timber suppliers and traders, law enforcers (e.g. BEIS -OPSS) and policy makers, focusing on DRC and Gabon
1.3: A 3-day workshop will be organised in one of the focus countries, including participants from all project partner organisations.

2.1: We will review existing protocols, identify protocol steps to optimise (reagents utilised, amount of starting material, DNA purification strategy).
2.2: Improvement options will be tested keeping in mind the logistic/economic c onstraints in the focus countries (DRC and Gabon), following discussion among the partners.
2.3: We will apply the protocol optimisation plan to leaf (positive control), cambium, heartwood and processed/old heartwood samples of a species of each of the gen era.
2.4: At least 3 replicates per treatment per sample type per species will be performed for up to ca. 500 DNA extractions.
2.5: Depending on resources and time, the optimised protocol may then be tested on representative timber species of a few add itio nal key genera such as Dalbergia and Pterocarpus.
2.6: Labwork will be performed by RBGK Research Assistant in collaboration with local researchers from DRC and Gabon who will be invited at Kew.

3.1: To generate the reference DNA dataset, we will sample min 3 individuals from ca. 22 species (focus group + congeneric sp ecies)
3.2: Samples will be selected based on their well identified voucher specimen and their georeference information. Airdried l eaf samples will be prioritized.
3.3: Wood samples from WFID collection and xylaria will be used to test the use of wood samples for making DNA reference data sets.
3.4: Samples will be submitted to DNA extraction and DNA library preparation (see the following 2 activities).
3.5: Part will be submitted to shotgun illumina sequencing to recover ribosomal DNA, plastid and mitochondrial genes.
3.6: Rest will be submitted to target sequence capture with Angiosperms -353 probe kit + Illumina sequencing, allowing sequencing the same 353 nuclear re gions in all samples.
3.7: To identify candidate DNA barcodes adapted to different DNA analysis methods, reference data will be analysed together w ith other existing data for the focus species.
3.8: Amplification and sequencing of some of these candidate b arcodes will be tested on heartwood DNA samples of the focus species.
3.9: Different methods will be tested to establish what combination of barcodes -method could be applied under logistic/economic constraints identified for different supply chain stages and countries.
3.10: Labwork will be performed by RBGK Research Assistant in collaboration with local researchers from DRC and Gabon who wil l be invited at Kew.

21. Annex 3 Standard Indicators

Table 1 Project Standard Indicators

There were no standard indicators in the original application

IWTCF Indicator number	Name of indicator	Units	Disaggregation	Year 1 Total	Year 2 Total	Year 3 Total	Total to date	Total planned during the project

Table 2 Publications

Title	Type (e.g. journals, best practice manual, blog post, online videos, podcasts, CDs)	Detail (authors, year)	Gender of Lead Author	Nationality of Lead Author	Publishers (name, city)	Available from (e.g. weblink or publisher if not available online)

No publications so far

23. Checklist for submission

	Check
Different reporting templates have different questions, and it is important you use the correct one. Have you checked you have used the **correct template** (checking fund, type of report (i.e. Annual or Final), and year) and **deleted the blue guidance text** before submission?	x
Is the report less than 10MB? If so, please email to BCF-Reports@niras.com putting the project number in the subject line.	x
Is your report more than 10MB? If so, please discuss with BCF-Reports@niras.com about the best way to deliver the report, putting the project number in the subject line.	x
Have you included means of verification? You should not submit every project document, but the main outputs and a selection of the others would strengthen the report.	x
If you are submitting photos for publicity purposes, do these meet the outlined requirements (see section 17)?	x
Have you involved your partners in preparation of the report and named the main contributors	x
Have you completed the Project Expenditure table fully?	x
Do not include claim forms or other communications with this report.	