HERBAXYLAREDD
Interdisciplinary exploitation of the federal Herbarium and Xylarium for tropical forest management

Contract - BR/143/A3/HERBAXYLAREDD

SUMMARY

In order to renew, complement and strengthen the reference value of both the Herbarium (Meise Botanic Garden) and Xylarium (Royal Museum for Central Africa, Tervuren), HERBAXYLAREDD aimed at generating knowledge, through multidisciplinary and integrative analysis of specimens' traits and meta-data, on Central African forest ecology and management as well as on forest products, especially wood. The study of plant traits, wood anatomy and technology, molecular and genetic affinities of plant and wood samples allowed exploring species distribution and functional strategies, growth performance of trees, and technology of lesser used timber species, with implications for carbon stocks estimations of Central African forests, energy content of woody species and to combat the illegal timber trade.

The databases of the Xylarium and Herbarium are characterised by a specific organisation and database arrangement. In order to match both unique collections, the African herbarium collection containing over 850,000 dried specimens was screened for matches with the Xylarium. In total over 5,000 matches were found between both databases allowing us to link various metadata on morphology, phenology and wood anatomy. Of those species, a subset was genetically fingerprinted to create a reference database of African tree species in which specimens are linked with the DNA barcode genes *matK* and *rbcL*. The newly generated *rbcL* and *matK* sequences from curated herbarium vouchers stored at Meise Botanic Garden were applied in a total evidence methodology in which the newly generated barcode sequences were added to a large phylogeny of woody African rain forest taxa embedded in an evolutionary framework of angiosperms. This *matK* and *rbcL* based phylogeny of over 36,000 different angiosperm species provides interesting insights in the evolution of plants at the species level or higher and is used as tool for general ecological and evolutionary studies on angiosperms as well as on African taxa. An additional element in botanical research that has been investigated in the current project is what the impact is of wrongly identified specimens on further ecological and evolutionary analyses. For this, the botanical field identifications of 756 woody plants from permanently monitored plots in the Yangambi Biosphere Reserve were verified. Here we showed that at species level, only 56% of the individuals appeared to be correctly identified. Approximately 78% of the individuals were correctly identified at the genus level, while family identification was correct for approximately 90% of the individuals. These results clearly show that caution is needed when using specimens for which the botanical identity has not been validated after the initial identification in the field. Despite the large number of wrongly identified species not all

further analyses suffered from these misidentifications. Whereas evolutionary and ecological studies (e.g. biogeography, trait evolution) are for example greatly impacted by misidentification, biodiversity assessment studies are not significantly affected.

In order to obtain well resolved phylogenetic networks classical SSR as well as modern high-throughput sequencing techniques such as targeted genome capture and Genome skimming by shotgun sequencing was applied. Within Meliaceae, the genera *Entandrophragma* and *Khaya* (commonly known as African mahogany) were studied, whereas within the Myristicaceae, the genus *Staudtia* was investigated. For Leguminosae, the genera *Scorodophleus* and *Prioria* were assessed. The results of these in depth population genetic analyses were used to prevent genetic erosion, to make proper assessments for conservation management and to verify the geographical origin of traded logs. Moreover, these new methods can be used for rapid species and provenance identification in order to more intensively combat illegal logging activities.

Leaf and seed traits were determined using specimens from the Herbarium. Wood trait measurements on the other hand, were obtained from wood samples stored in the Xylarium (Tw collection). The Xylarium was screened for wood technological traits, namely the dimensional stability (resistance against swelling or shrinkage) and the natural durability (resistance against fungal decay) in search of lesser-used or alternative timber species. The developed methodologies allow determining these traits and the Xylarium can now be screened in the search for these timbers. The difference in behavior of several species in terms of dimensional stability and wood density was explained via wood anatomical differences. Over 2,000 DART TOFMS spectra (> 900 specimens, 55 species) were collected from Tervuren Wood Collection specimens, of which a part was added to the ForeST© database (U.S. Fish and Wildlife Forensic Service Laboratory). Xylarium specimens are the ideal reference material as DART-TOFMS for timber identification only requires a small wood sliver to obtain the chemical fingerprint which is the basis for the identification**.** An automated protocol is proposed to determine the pre-processing parameters leading to the highest classification accuracy and several species within look-a-like groups are discerned. We also give the first indication towards the use of chemical fingerprints from tropical tree rings to determine the geographical provenance.

The calorific value and ash content of branches, stem, regrowth and the remaining stump have been determined for several species from the Congo Basin. Functional traits at the seedling stage and architectural traits on larger trees were measured in the field. For the *Erythrophleum* genus, the integrated framework was applied, combining wood and leaf traits and ecophysiological measurements on branches. Georeferenced collections were used to delineate species distribution and examine niche evolution. The results showed that ecological speciation through climate has played a key role in the evolution of the *Erythrophleum* species. The differential distribution and climatic niche of the species indicated adaptive divergence along rainfall gradients, that have probably been boosted by past climate fluctuations. Furthermore**,** the distribution of the forest and savanna biomes across Africa were studied and modeled, using floristic information. Two major environmental gradients were identified across Africa, with strong overlap between forests and

savannas on the precipitation/aridity gradient, and strong partition of northern and southern savannas on the temperature/elevation gradient. While some tree genera were found to transcend the two biomes, the distribution of forest and savanna specialists, and of generalists, moreover confirmed the floristic specificity of the forest and savanna biomes, and demonstrated for the first time the mix composition of the bistability area, with only few generalist species.

Furthermore, georeferenced herbarium samples were used to derive phenological data, and examine large-scale variation in flowering. Herbarium derived phenology was first validated using field observations available for timber species, monitored in several sites across Central Africa, mostly in logging concessions, but also from historical collections such as in Luki, Democratic Republic of the Congo. Extensive collections, archived scientific data and well directed field work allowed an informative analysis of 150 rainforest species, including their increment assessed by different means. Additionally, a large-scale dated phylogenetic tree of angiosperms was constructed. Such a framework is used to assess the evolutionary relationships among species, but it also provides a useful tool to optimize the evolution of specific morphological, anatomical or physiological traits (e.g., vessel size of wood), resolve eco-evolutionary queries, or predict putative trait characteristics based on the position of a species within a certain lineage or group (most recent common ancestor approach).

The Tervuren Xylarium and the Meise Herbarium are the result of more than a century of intense sampling in Central Africa. They are now by far the two most important botanical reference collections in the world for tropical Africa. The collections played a crucial role during the exploration of Africa and formed the cradle of Central African botany, agronomy and forestry. Both collections witnessed in the last decades a substantially strengthened international interest because of their acknowledged relevance for climate change research, economic development and enforcement of nature conservation laws and regulations. As such they represent unique data sources, given their vastness, the spatial extent they cover and the taxonomical diversity they contain. Their value is still growing in an actual context of the huge logistical difficulties of collecting new study material in Africa. They are relevant for two prominent political issues: climate change and wildlife traffic where timber represents the bulkiest commodity.

Although the collections are physically separated and curated in two different institutions, they are naturally clustered because of thousands of shared specimens, for which there is wood in the Tervuren Xylarium and leaves, flowers and fruits in the Meise Herbarium. The HERBAXYLAREDD project showed that the information content is very complementary and that linking the collections strengthens substantially their respective scientific value in an actual context of dire need for data on the changing Central African forests.

KEYWORDS

Xylarium, Herbarium, tropical forest ecology, tropical forest management, wood technology, scientific heritage