Saussurea

Journal de la Société botanique de Genève

47

Société fondée en 1875

2018

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Date de parution : Septembre 2018

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A multidisciplinary study of the doum palms (*Hyphaene* Gaertn.): origin of the project, current advances and future perspectives

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Abstract

Stauffer F. et al. (2018). A multidisciplinary study of the doum palms (*Hyphaene* Gaertn.): origin of the project, current advances and future perspectives. *Saussurea*, 47, p. 83-101.

Here we describe the origins, current advances and future perspectives of the "Hyphaene Project", a multidisciplinary research effort aiming to expand our knowledge of the enigmatic genus Hyphaene (8 species), which ranks among the most economically important, yet extremely poorly known coryphoid palm genera. The genus was monographed about one century ago by the celebrated Florentine botanist Odoardo Beccari and since then only few efforts have been carried out towards its better understanding. Since 2015 the Hyphaene project has become one of the key research activities of the palm group at the Conservatory and Botanical Garden of the City of Geneva (CJBG) towards the study of continental African palms.

Résumé

Stauffer F. et al. (2018). Etude multidisciplinaire des palmiers doum (*Hyphaene* Gaertn.): origine du projet, progrès actuels et perspectives futures. *Saussurea*, 47, p. 83-101.

Nous décrivons dans cet article les origines, ainsi que les avancées, présentes et futures, du "Projet *Hyphaene*", un travail de recherches multidisciplinaires qui a pour but d'améliorer les connaissances de l'énigmatique genre *Hyphaene* (8 espèces). C'est le moins connu parmi les genres des Arecaceae coryphoïdes, mais aussi l'un des plus importants sur le plan économique. La monographie du genre a été établie il y a environ un siècle par le célèbre botaniste florentin Odoardo Beccari. Depuis lors, seules quelques tentatives ont été menées à bien pour mieux comprendre la taxonomie du genre. Depuis 2015, le "Projet *Hyphaene*" est devenu une des activités de recherche majeure du groupe "Palmiers" des Conservatoire et Jardin botaniques de la Ville de Genève (CJBG) dans le cadre de l'étude des palmiers africains continentaux.

Keywords

Taxonomy floristics ethnobotany ecology conservation Africa palms Arecaceae

Mots-clés

Taxonomie floristique ethnobotanique écologie conservation Afrique palmiers Arecaceae

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Introduction

Working in the Conservatory and Botanical Garden of Geneva offers several advantages for palm research, not only because of the presence of a rich herbarium and outstanding bibliographic collections representing all continents, but more importantly because of the possibility to interact with colleagues working in a wide range of disciplines. Nowadays, our institution hosts several high ranking researchers developing activities in domains ranging from population genetics, phylogeography and phylogeny to ethnobotany, conservation, and indooroutdoor plant cultivation. From all perspectives it was clear that a scantly known palm group would benefit from the expertise of such a team.

Identifying a palm group in need of a modern taxonomic revision has become a challenging task due to the fact that palm monographic research has literally exploded in the past 20 years. Unlike other monocotyledon families, an amazing number of taxonomic revisions, particularly from Neotropical groups, have been published. These major efforts have been not only lead by renowned palm authorities based in large botanical institutions but also by the consolidation of a new generation of prolific research teams based in small to mid-sized institutions simultaneously working in different countries. Luckily, palm monographic work is still available for those interested to do it, as some of the 185 palm genera reported by Dransfield et al. (2008) still remain imperfectly understood and require taxonomic revisions. The reasons that may explain why some palm groups have remained poorly understood are multi-factorial; however some hints may help us to understand the main reasons. First of all we may suggest "internal" factors associated to the palms themselves, as complex biological organism to collect and study, and secondly we may propose "external" factors, mostly related to difficult conditions linked to the access of wild populations where the palms thrive. Our experience working on South American palms and more recently studying palms in West Africa allows us to propose a simplified table depicting the main factors hindering palm collecting and consequently palm taxonomic studies (Fig. 1). These factors at least partially explain why a given group of palms is severely undercollected by taxonomists, and its specific boundaries extremely unclear, while for others, sampling is abundant and thorough systematic studies throughout time have enabled the proposal of infra-specific taxonomic ranks.

By the first months of 2015 we planned to launch a multidisciplinary palm project and after careful study of the different palm genera requiring urgent taxonomic revision we ended up targeting one of the most fascinating palm groups: the palm genus Hyphaene (Fig. 2), also widely known as "doum palms" (www.hyphaene.org), displaying highly unusual dichotomous branching. No surprise that this genus perfectly filled out all the requirements of a challenging palm group as depicted in our table (Fig. 1). Also in 2015 we were granted with generous funding by the Fonds A. Lombard of the Société de Physique et d'Histoire Naturelle de Genève and this became the main trigger enabling the launch of our ambitious project. From the beginning it was also clear that major efforts would have to be spent towards the study and collection of Hyphaene wild populations throughout Africa, a continent where field work is often challenging and requires very special logistic efforts.

Complexities associated to the palm

Dioecy (double collection effort)

Armed with spines (stems, petioles, rachis, blades, inflorescence bracts)

Large growth form, massive stems and leaves:

Bulky and difficult collections (often skipped by most botanists)

Few specimens (in comparison with small, understory palms)

Type material fragmented and often uninformative

Genera with large distribution range

Complexities associated to the access of wild populations

Rough environmental conditions (i.e. high temperatures, arid environment)

Lack of reliable local partners

Access to local populations limited by:

Lack of accessible roads



Ethnic / Political instability



Precarious health conditions in the region (malaria, ebola, etc)

Fig. 1. Main complexities hindering taxonomic studies in certain palm groups.





Fig. 2. Growth habit of *Hyphaene* in its natural habitat.

A. *Hyphaene compressa* growing in the Samburu National Park (Kenya). Photo: Ronald S. Phillips;

B. *Hyphaene thebaica* growing in rocky landscape in western Djibouti.

The main objectives of our project include: 1) the production of a modern taxonomic revision of the genus, including the study of the structural biology (morphology, histology, and anatomy) of vegetative and reproductive organs; 2) Next Generation Sequencing (NGS)-based phylogenetic reconstruction, including analyses of the age and divergence time between lineages and population genetic studies on closely related species; 3) phytogeographical and ecological characterization, including the sampling of information on the conservation status of the taxa studied with the aim to deliver this information to the International Union for the Conservation of Nature (IUCN); and 4) ethnobotanical inventory and collection of artefacts, uses, and common names associated with all taxa.

Current state of taxonomic knowledge of the palm genus Hyphaene

Hyphaene palms have been studied by several renowned palm experts. The first species in this group was described by the German physician and botanist Joseph Gaertner (1732-1791) in his publication De Fructibus et Seminibus Plantarum (GAERTNER, 1788), who recognized Hyphaene coriacea as the single taxon within the genus. Important contributions in the systematics of the group were carried out by two German botanists in palm science. The first one is the Bavarian palm expert Carl Friedrich Philipp von Martius (1794-1868) who contributed with the description of several taxa in Hyphaene (i.e. H. petersiana) and made some generic transfers in particular from the genus Corypha. The botanist and also gardener Hermann Wendland (1825-1923) described many distinctive taxa in the group (i.e. H. compressa, H. macrosperma). The last taxonomic treatment of Hyphaene was published more than one century ago by the celebrated Florentine botanist Odoardo Beccari (BECCARI, 1908, 1924), who interestingly probably saw these palms in the frame of the only travel he made to Africa (he visited Ethiopia, East Sudan, and Eritrea in 1870). Taxonomical notes for some species were proposed by the Indian botanist Caetano Xavier Furtado (Furtado 1967, 1970a, 1970b, 1970c), who most probably only saw H. dichotoma in India. Working almost exclusively with herbarium material, in particular fruits, these authors proposed more than 2/3 of the taxonomic names associated to the genus, most of these to the ranks of species and subspecies.

Some notes on the West African species were proposed by the French botanist August Chevalier (Chevalier & Dubois, 1938), and a thorough treatment for the genus including the East African species was published by the English palm expert John Dransfield (Dransfield, 1986). A complete revision of *H. guineensis* in Central West Africa was undertaken by the Dutch botanist Johan van Valkenburg (Van Valkenburg & Dransfield, 2004), clarifying the taxonomic identity of this palm and providing data on its ecology and distribution. A recent publication by Fred Stauffer and collaborators (Stauffer *et al.*, 2014) briefly updated our knowledge of the tropical African taxa.

Our current understanding of *Hyphaene* recognizes eight species, with at least two of them (*H. macrosperma*, *H. reptans*) extremely poorly known and for which type material is either very scant or completely lacking. Meanwhile, the highly polymorphic nature of the fruits in *Hyphaene* (extensively used in the early taxonomic works in the group) has enabled a proliferation of names. The number of currently recognized synonyms for some taxa is impressive (33 in *H. compressa*; 24 in *H. coriacea*; 21 in *H. petersiana*, and 17 in *H. thebaica*), reflecting the tortuous taxonomic and nomenclatural history of the genus and highlighting the urgent need of a modern and integrative taxonomic approach.

Field work efforts

Current efforts have strongly concentrated in field work missions (Figs. 3, 4) as this has allowed us to study wild populations and also to sample informative specimens for herbarium, anatomical, and molecular studies. Here we briefly describe the last field trips to Africa and living collections in the United States in order to collect living material of *Hyphaene*.

Ivory Coast, Ghana, Togo, and Benin (Fig. 3)

Palms in these countries were collected in several field missions between 2011 and 2015 in the frame of the Master projects at the University of Geneva of Doudjo Ouattara, Simona da Giau, and Loïc Michon. All these projects were carried out as a contribution to the palm inventory that the Conservatory and Botanical Garden of Geneva is undertaking in West Africa (STAUFFER et al., 2017). In these countries herbarium and DNA material of the two native species in the region (H. guineensis, H. thebaica) were sampled. Only few ethnobotanical objects were gathered in the frame of these collection efforts. No populations of the incompletely known species H. macrosperma could be observed by Loïc Michon in northern Benin, known to be the area where the original collections apparently came from (see details in STAUFFER et al., 2014).

Djibouti (Fig. 3)

Djibouti, a relatively small country located in the Horn of Africa, was visited in the search of populations of Hyphaene thebaica. The country is bordered by Eritrea in the north, Ethiopia in the west and south, and Somalia in the southeast. The remaining border is formed by the Red Sea and the Gulf of Aden at the east. In December 2015, Loïc Michon, Doudjo Ouattara, and Fred Stauffer spent more than a week studying and sampling wild and cultivated populations of the doum palm in this country. With the great support of Dr Abdourahman Daher (Director of the Institut des Sciences de la Vie, Centre d'Etude et de Recherche de Djibouti - CERD), Mrs Sabira Abdoulkader (researcher at CERD) and Mr Omar Osman (Researcher at CERD-FAO) we sampled in many regions of this country. We visited Hyphaene thebaica palm groves in the regions of Tadjoura, Dikhil,

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Fig. 3. Field missions to Ghana and Djibouti.

- A. Growth habit of *Hyphaene guineensis* showing basal dichotomous branching;
- B. Doudjo Ouattara in front of a juvenile of the same species in the surroundings of Agbozume (southern Ghana);
- C. Panoramic view of a compact population of *H. thebaica* growing in a salty desert of western Djibouti;
- D. Threatened population of *H. thebaica* in the coastal region of Djibouti (shores of the Red Sea);
- E. Collection of *H. thebaica* with unusual, but effective techniques.



Fig. 4. Field missions to Tanzania and South Africa.

- A. Impressive dichotomously branched individual of *Hyphaene compressa* in the coastal region of Tanzania;
- B. Intensive sampling of the same species in the foothills of the Western Usambara Mountain Range (Tanzania);
- C. Population of $H.\ petersiana$ on the shores of the Lake Eyasi (Tanzania);
- D-F. Growth habit and sampling of the widely distributed species *H. coriacea* (Kwazulu-Natal, South Africa).

and Arta, in a travel of more than 2000 km. One of the most outstanding findings of this mission was the identification of monoecious individuals of *H. thebaica*, meaning that male inflorescences and infructescences were simultaneously observed in individuals of some populations. As *Hyphaene* has been always considered as a dioecious palm genus, our finding opens several important questions about the development and evolution of reproductive syndromes in the group.

Tanzania (Fig. 4)

Tanzania is the largest country of East Africa and it is bordered by Kenya and Uganda to the north, Rwanda, Burundi, and the Democratic Republic of the Congo to the west, and Zambia, Malawi, and Mozambique to the south. The Indian Ocean is present to the east. Between August and September 2016 Didier Roguet, Loïc Michon, Adama Bakayoko, and Fred Stauffer spent more than 10 days studying and sampling wild populations of Hyphaene coriacea, H. compressa and H. petersiana in this country. Based on the treatment of John Dransfield on the palms of Tropical East Africa (DRANSFIELD, 1986) we learnt that these species were present in Tanzania and that apparently complex hybridization zones were present in the coastal savannas north to Dar es Salaam. With the great support of the botanist Frank Mbago (Head of the herbarium of the University of Dar es Salaam) we collected in several regions in this country, in particular in the coastal areas and in the central part. Long driving along more than 2500 km let us visit the regions of Pwani, Tanga, Kilimanjaro, Manyara, Arusha, Singida, Dodoma, and Morogoro. Many interesting samples of the three species reported in the country (H. coriacea, H. compressa, H. petersiana) were gathered and a great selection of ethnobotanical objects was purchased. Moreover, several audiovisual materials were recorded in the frame of our mission.

South Africa (Fig. 4)

The most recent trip of our project was done by Didier Roguet and Fred Stauffer to South Africa in September 2017. The main objective of this mission was to sample wild populations of Hyphaene coriacea and Hyphaene petersiana in the regions of Kwazulu-Natal and Mpumalanga. This travel was kindly supported by Mkhipheni Ngwenya (scientific officer) and Yashica Singh (Herbarium curator at the herbarium of the South African National Biodiversity Institute in Kwazulu-Natal), both from the South African National Biodiversity Institute (SANBI). Several important collections, including one corresponding to a possible new species in the genus (showing some morphological affinities with H. petersiana), were carried out throughout this mission. Abundant ethnobotanical data and objects were gathered in all the regions visited. Thus, a representative selection of basketry produced by the Zulu people (Bantu ethnic group) is now represented in the ethnobotanical collection of the Conservatory of Geneva.

Madagascar

In the frame of several field missions of Iacopo Luino, Laurent Gautier, and Yamama Naciri to Eastern and Southwestern Madagascar between 2013 and 2017, several populations of H. coriacea were sampled and will be represented in our analysis. The representation of these populations is extremely important as early studies have suggested that Madagascar may host a truly endemic Hyphaene species (H. shatan Bojer ex Dammer). Our project will test the hypothesis whether the latter species may be regarded as an independent taxonomic entity or whether its status as synonym of H. coriacea, as proposed by several authors (DRANSFIELD, 1986; Dransfield & Beentje, 1995), is further supported. In particular the botanist Iacopo Luino sampled abundant herbarium material and DNA samples for the molecular phylogenetic analyses.

Visit to living collections

In the frame of our study we have benefited from well-curated living palm collections from which we carried out intensive sampling and on site observations (Fig. 5). On January 2017 we studied the *Hyphaene* collections hosted by the Montgomery Botanical Center (MBC) and the Fairchild Tropical Botanical Garden (FTG), both in Miami (Florida, United States of America). During almost 10 days we carefully studied and sampled the four species therein cultivated (*H. compressa*, *H. coriacea*, *H. petersiana*, and *H. thebaica*).

For one male individual of Hyphaene coriacea cultivated at MBC, we dissected the apical region of the stem in order to understand on site the construction of Schoute's branching model. This type of branching model was proposed for Hyphaene thebaica by Schoute (1908), who argued that the benchmark dichotomous branching present in this palm was a consequence of the division of the apical meristem. By dissecting the apical meristem of palms reared at MBC we wanted to find gross morphologic evidence of this unique process (Fig. 6). Although our observations could not detect the earliest developmental stages suggesting division of the apical meristem, this field study allowed us to better understand leaf and inflorescence development in Hyphaene. Sampling in these well-curated gardens included material for anatomical studies, leaf fragments for DNA analyses and dry leaves that will be incorporated in a current project carried out by Gaspar Morcote, Lauren Raz (Universidad Nacional de Colombia) and Fred Stauffer on phytolits in extant continental African palm genera.





Fig. 5. Living palm collections in Florida (USA).

A. *Hyphaene coriacea* originally collected in Madagascar cultivated at the Montgomery Botanical Center (MBC);

B. Massive individuals of *H. dichotoma*, the only non-African species in the genus *Hyphaene*, cultivated at Fairchild Tropical Botanic Garden (FTG).



Fig. 6. Structural study of dichotomy in *Hyphaene coriacea* cultivated at the Montgomery Botanical Center (MBC).

- A. Plant dissected for the study of division of the apical meristem (Schoute's model); B. Step 0, all leaf sheaths present in the crownshaft;
- C. 15 leaf sheaths removed;
- D. 20 leaf sheaths removed;
- E. 25 leaf sheaths removed;
- F. 30 leaf sheaths removed;
- G. All leaf sheaths removed; point of dichotomous branching.

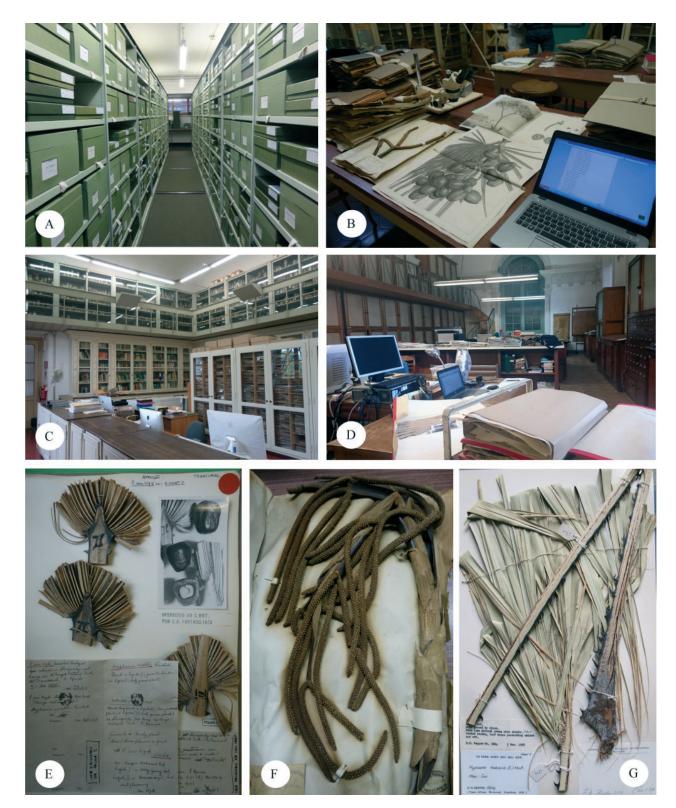


Fig. 7. Historical collections storing *Hyphaene* specimens. **A.** Herbarium of the Royal Botanic Gardens, Kew (K);

- B-C. Herbarium of the Natural History Museum of Florence (FI);
- D. Herbarium of the University of Coimbra (COI);
- E-G. Different herbarium specimens of *Hyphaene* studied in the frame of our project.

Herbarium studies on general collections and type specimens

Although the study of wild populations is fundamental for our comprehension of the different taxa composing Hyphaene, the study of type specimens associated to all the original names published in the genus remains important in order to establish taxonomic boundaries. We have focused our efforts on the herbaria storing important holdings of original material (Fig. 7). Here we briefly describe the visit to seven of these major repositories of Hyphaene historical and modern specimens.

The herbarium of the Royal Botanic Gardens, Kew (K)

On October 2014 we visited the herbarium of Kew, probably hosting one of the largest palm collections in the world. In the case of the genus Hyphaene we found 92 collections, most of them stored in cardboard boxes. There are specimens gathered from many different countries from Africa, in particular from Somalia where at least specimens from H. coriacea, H. compressa, H. reptans, and H. thebaica could be identified. Our visit to this herbarium confirmed the importance of Somalia as a critical country for the comprehensive taxonomic study of the doum palms. Some specimens from Kenya (S.A. Robertson 4429-K) report possible intermediate features between H. compressa and H. coriacea, and this has been also observed by us in the frame of our study of the coastal populations in Tanzania (Region of Tanga).

The Belgian Herbaria (BR, BRLU)

The very rich herbarium of Meise (BR) and the one of the Free University of Brussels (BRLU) were visited in May 2015. These renowned institutions store interesting collections of Hyphaene gathered in several countries of tropical Africa (i.e. Gabon, Kenya, Mali, Ethiopia, Niger, Senegal, Tanzania, and Tchad), but most specimens were clearly collected in the Democratic Republic of the Congo during the times of the Belgian colonial rule. In particular from this country we could study specimens from the native species *H. guineensis* and *H. thebaica*.

The herbarium of Florence (FI)

The main aim of our visit to the Florence herbarium in November 2016 was to study the type material associated to all the names described by the celebrated Florentine botanist Odoardo Beccari (1843-1920). Beccari studied several palm genera of the subfamily Coryphoideae and wrote the most comprehensive taxonomic treatment on the genus Hyphaene (BECCARI, 1908). Although he probably saw these palms when visiting Ethiopia, East Sudan, and Eritrea in 1870, Beccari had many correspondents and received abundant specimens from all over Africa. Although most of the species are only based on fruit material, additional hand-writing notes and drawings by Beccari often offered important hints about the taxonomic entity of the material.



- Shelter, protection, religion, traditions
- Wood, construction



- Roofing, fencing, animal feeding
- Weaving (leaves) -> baskets, mats, rows, hats sandals



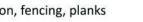
- Construction (rachis)
- Weaving (petiole) -> baskets

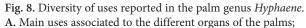


- Food (fruits)
- Sap (fermentation -> palm wine)
- · Animal feeding (fruits)
- Flowers -> associated to honey production
- Fruit -> vegetal ivory (jewelry, decoration)



- Folk medicine: Medicinal sap (Hypertension)
- · Stem-> construction, fencing, planks





B. Leaf packing of H. coriacea for transport and handicraft purposes (Kwazulu-Natal, South Africa).



The herbaria of Lisbon (LISC, LISU) and Coimbra (COI)

On March 2017 we visited the herbaria of Lisbon (LISC, LISU) and the herbarium of Coimbra (COI), all of them hosting important *Hyphaene* collections from former Portuguese colonies in Africa, in particular material sampled in Angola, Guinea Bissau, and Mozambique (also some collections from Sao Tomé and Principe). During the last years of the '70s, the Indian botanist Caetano Xavier Furtado (1897-1980), based his monographic study on *Hyphaene* stored in the Lisbon herbaria. All the *Hyphaene* material stored in this herbarium is particularly interesting from a taxonomic point of view as it was identified by Furtado himself.

Ethnobotanical studies

As already reported for other African palms, almost all species of *Hyphaene*, particularly *H. thebaica*, display a wide range of uses (Fig. 8) and this has been thoroughly documented in several ethnobotanical publications (e.g. Burkill, 1997; Amwatta, 2004, Kahn & Luxereau, 2008; Régis *et al.*, 2008; Aboshora *et al.*, 2014). In the frame of our field missions objects made of *Hyphaene* leaf blades, petioles, or fruits/seeds have been sampled and interviews with local craft weavers were recorded (Figs. 9, 10). Particular efforts gathering ethnobotanical data were made in Ghana, Djibouti, Tanzania, and more recently in the Kwazulu-Natal region (South Africa) (e.g. Zulu handcraft work), where abundant material was acquired for the ethnobotanical collection of the Conservatory and Botanical Garden of Geneva.

Our collection is now represented by more than 70 ethnobotanical objects, including brooms, brushes, mats, ropes, fans, baskets, toys, hats, dry mesocarps for tea preparation (Egypt), and hand bags, among others. These objects have been fully documented with associated data that include: locality, date and price of purchase, uses, common names, and ethnic group (when information is available). Short films were additionally recorded in Djibouti, Tanzania, and South Africa with the aim to characterize the different weaving techniques for the construction of baskets and mats. Gathering all these objects in a small database underlines the great local economic importance that inhabitants give to the palm, frequently in parallel with the uncontrolled extraction of raw material that put in danger the wild populations. For example, the variability of the waving techniques (6-7 different types) is just amazing and shows the cultural importance of these doum-made materials.

Past and current molecular phylogenetic studies

The placement of the genus *Hyphaene* in the palm subfamily Coryphoideae was first proposed by UHL & DRANSFIELD (1987) and supported strongly in later studies relying on molecular phylogenetics (DRANSFIELD *et al.*, 2008). Modern molecular phylogenies confirm *Hyphaene* as a highly supported monophyletic group,

with moderate to high support for a sister-relationship to the monotypic Medemia, endemic to the Egyptian Nubian Desert and north-eastern Sudan (ASMUSSEN et al., 2006; Baker et al., 2009). Although the monophyly of Hyphaene is not controversial, the interspecific relationships within the genus have been little explored using molecular phylogenetic approaches. Our project aims to define these relationships and proposes a monographic revision based, when possible, on monophyletic taxa. this aim, species delimitations will be assessed using molecular markers under a coalescent framework. As soon as reliable species boundaries will be found, a dated phylogeny benefiting from an accurately identified fossil (Hyphaene kapelmanii, PAN et al., 2006) will enable us to infer the age and divergence time between the different taxa. Moreover, whenever signs of hybridization have been found (such as between H. compressa, H. coriacea and H. petersiana), a more precise analysis will be conducted to evaluate the level of admixture between species.

A close collaboration with the laboratory of Phylogeny and Molecular Genetics of the Conservatory and Botanical Garden of Geneva will allow us to undertake this study (Fig. 11), relying on a promising molecular technique called Next Generation Sequencing (NGS). This method enables quick and always cheaper sequencing of thousands of loci and appears as an ideal solution for resolving genetic relationships at a low taxonomic level within Hyphaene. Among several NGS methods, one of the most commonly used in Angiosperms is the one called "targeted-sequence capture", which consists in sequencing hundreds to thousands targeted loci of an organism's genomic DNA (BARRETT et al., 2016). Practically, this method consists of the following steps: 1) sonication of the DNA samples to shear the genomic DNA in small fragments (usually 200-800 bp), 2) ligation to these fragments of "barcode" sequences that are specific to each sample allowing the pooling of many individual samples in one single reaction, 3) hybridization of the genomic DNA libraries with the baits to enrich them with the targeted loci, and 4) sequencing the enriched libraries with, for example, an illumina machine. Using this method and specific probes for palms designed by HEYDUK et al. (2015), Angela Cano could demonstrate that this approach dramatically improved the resolution and support of phylogenetic relationships among Neotropical palms (CANO, 2018), in comparison with previous studies relying on traditional sequencing techniques (i.e., Sanger's method). Based on this success, we decided to apply this strategy to our Hyphaene project. So far, we have initiated the analysis of 66 DNA samples representing 6 species of Hyphaene from more than 12 African countries, plus representative species of several related groups of palm (Medemia and other taxa of Borasseae i.e. Borassus, Bismarkia) and other Coryphoideae. We are confident that the results generated by this study will considerably improve the knowledge of the systematics and evolution of Hyphaene.

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Fig. 9. Ethnobotanical research in the palm genus *Hyphaene*. **A.** Interview carried out by Didier Roguet in a handicraft market (surroundings of Saint Lucia, South Africa);

- B. Different leaf fibers of *H. petersiana* are used in Tanzania;
- C. Old Afar woman weaving a mat made from leaves of *H. thebaica* in western Djibouti; D. Mat weaved with leaf fibers of *H. petersiana* (Lake Eyasi, Tanzania);
- E. Handicraft market in the surroundings of Saint Lucia (South Africa).

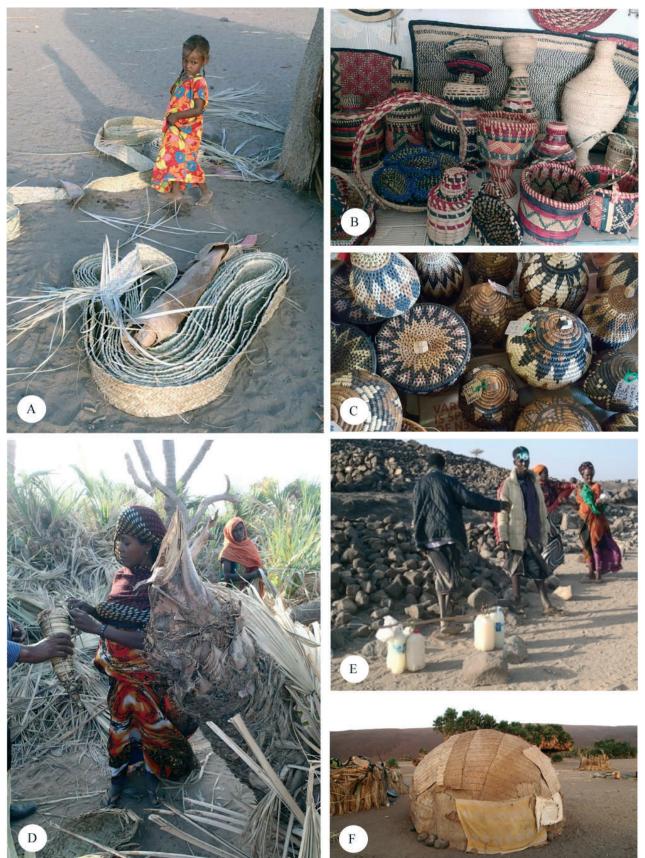


Fig. 10. Ethnobotanical research in the palm genus Hyphaene.

- A. Weaved ribbons of leaves of *H. thebaica* are used to make Afar traditional mats (western Djibouti);
- **B.** Different kind of baskets made of leaf fibers from *H. thebaica* are sold in a traditional handicraft market in the coastal city of Tadjoura (Djibouti);
- C. Representative baskets of the Zulu ethnic group made of leaf fibers from H. coriacea (Kwazulu-Natal, South Africa);
- D-E. Palm wine tapping from *H. thebaica* is a traditional Afar activity in western Djibouti;
- F. Nomadic Afar house made from leaves of *H. thebaica* in western Djibouti.

Conservation status assessments in Hyphaene

Our project actively contributed with a recent study by Cosiaux et al. (2018) on the conservation of continental African palms. In this publication it was pointed out that fewer than 10% of the 66 species native from continental Africa were threatened and within the Threatened category, one species was assessed as Critically Endangered, three as Endangered, and two as Vulnerable. Based on available data the authors identified an overall low extinction risk for African palms in the immediate future and proposed links to the generally large distribution patterns of African palm species, the broad ecological amplitudes of most species and their good representation inside the African protected areas network. Most species in Hyphaene (H. coriacea, H. compressa, H. guineensis, H. petersiana, H. thebaica) correspond to these criteria and the category of Least Concern (LC) has been proposed, whereas others (H. macrosperma, H. reptans) are part of the about 15% African palm species lacking sufficient data for an accurate assessment. Our field missions in Africa during the last 8 years have enabled us to confirm that at local scales Hyphaene populations steadily decline (Fig. 12) due to increasing drought events and clearing for agriculture (Nigeria), replacement for crops or eradicated as a weed (Ivory Coast), severe palm wine tapping of adults and juveniles (Djibouti), and uncontrolled overexploitation of leaves for craft making (South Africa) (Fig. 12). At a more global scale the general increase of world temperature will have direct consequences on the survival of species such as H. petersiana, an economic and ecological important species present in south-east Africa (BLACH-OVERGAARD

& Balsley, 2009). With respect to the Indian doum palm, *H. dichotoma*, it should be indicated that Johnson (1998) proposed the category of Near Threatened (NT) for this palm. The field observations made by our project on the conservation of the African species of *Hyphaene* were used for the assessments that are currently available on-line at the web site of the IUCN.

Future perspectives of the project

Field work remains a priority for the project, in order to study and sample wild populations of Hyphaene. Intensive field work can also highlight the presence of hybridization zones that could be further explored with population genetic studies. The missions carried out have demonstrated that the taxonomic treatment of the genus will be only possible after assessing the wide morphological diversity of the vegetative and reproductive organs. This diversity is apparently less important in taxa such as H. compressa, H. guineensis, and H. petersiana, which display well defined characters easy to recognize in the field. Other species such as H. coriacea and at some point H. thebaica require further study as they show some important polymorphism. In eastern Africa, countries such as Kenya (lake Turkana region), Somalia, Eritrea, and Ethiopia require further study as they host a large diversity of species in Hyphaene (H. coriacea, H. compressa, H. reptans, and H. thebaica). Unfortunately, political instability and insecurity are major concerns in these countries and organizing any mission there would be, at least nowadays, extremely risky. In south-central Africa, countries such as Angola

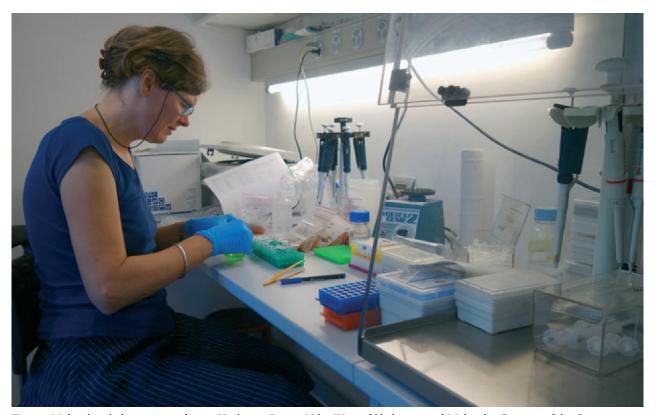


Fig. 11. Molecular phylogenetic studies in *Hyphaene*. Regine Niba (Unit of Phylogeny and Molecular Genetics of the Conservatory and Botanic Garden of Geneva) carefully undertakes the first lab manipulations aiming to extract DNA from the more than 60 samples gathered in the field and the living collections visited.





Fig. 12. Conservation of wild populations of Hyphaene.

- A. Goats hinder the regeneration of wild populations of *H. thebaica* in Djibouti as they feed on the tender leaves of juvenile individuals;
- B-C. Palm wine tapping is severely threatening adult and juvenile individuals of the same species (western Djibouti);
- D. Overharvesting of leaves affects the normal development of populations of *H. coriacea* (Kwazulu-Natal, South Africa);
- E. The leaves of this species are used for handcraft making in several South African regions; objects are commercialized in two markets visited in Durban.

and Namibia require further studies in order to explore the morphological and genetic diversity of *H. petersiana*, the single *Hyphaene* species reported in the region. In 2019 a field mission is planned to India in order to collect the only Asian species of the genus (*H. dichotoma*). Wild populations of this palm have been reported to grow along the seasonal water-courses, coastal sand dunes, and flat areas of Gujarat, Goa, and Maharashtra, along with the union territories of Daman, Diu, and Dadra. Some of these areas will be included in our sampling trip.

The study of general and type specimens will also go on in the next months. Some important collections such as the ones deposited in the herbarium of Paris (P) and Leiden (L) will be consequently visited in order to study their rich holdings of *Hyphaene* specimens. Although in most cases herbarium specimens of *Hyphaene* are incomplete and poorly informative, the associated data in the labels is often rich and particularly useful when geographical coordinates are present or can be easily deduced. Hence, they play a major role when constructing distribution maps at the species level.

The morphological data already available, in particular the one issued from the Master's project of Loïc Michon (MICHON, 2016), has already settled solid bases towards our comprehension of the structural botany in *Hyphaene*. In the frame of this study, leaf and flower anatomy (male and female) were explored employing classical methods for histological studies and Scanning Electronic Microscopy. A preliminary analysis of these data has shown that the structure of vegetative and reproductive organs in Hyphaene is rather homogeneous, in general in concordance with the observations made by Tomlinson et al. (2011) with respect to the leaf blade structure. Nonetheless, our project already offers important morphological data supporting clear differentiation of Hyphaene from its sister group Medemia, endemic to the Nubian Desert. Structural data remain important for the general characterization of the doum palms and new researches with respect to their structure will also include the roots and the fruits.

By the end of January 2018 many advances have been made in order to propose an accurate species-level molecular phylogeny of *Hyphaene*. The team of the Unit of Phylogeny and Molecular Genetics of the CJBG is now improving and testing protocols that will allow us to get the first species delimitation analyses as well as the first phylogeny of the group based on the NGS-sequence capture method. Further taxonomic and phylogeographic analyses in *Hyphaene* will have to wait the first general results coming from this lab.

Finally, as already done from its launch by the end of 2015, the website of the project (www.hyphaene.org) will be permanently updated and the coming field missions and main results will be made available to the scientific

community and the wider public. Current statistics confirm that the website has become an important tool for spreading our scientific knowledge on this palm group. Between 200 and 600 unique visitors every month, coming from countries such as USA, France, India, Brazil, or Germany, consult our website and download the documents available therein.

Acknowledgments

This project could not have been possible without the generous support of the A. Lombard Extraordinary Grant 2015, Société de Physique et d'Histoire Naturelle de Genève (SPHN), in particular through the unconditional interest of Dr. Michel Grenon. Field work in Africa has been scientifically and logistically supported by the following colleagues: Gabriel Ameka, Patrick Ekpe and Prosper Avecor (University of Accra, Ghana); Adama Bakayoko (Nangui Abrogoua University and Swiss Center of Scientific Research, Ivory Coast); Zoul-Kifouli Adeoti (University of Benin); Koffi Koudouvo (University of Togo); Abdourahman Daher (Centre d'étude et de recherche de Djibouti); Sabira Abdoulkader (Centre d'étude et de recherche de Djibouti); Omar Osman (Researcher at CERD-FAO, Djibouti); Frank Mbago (University of Dar es Salaam, Tanzania); Mkhipheni Ngwenya (Herbarium NH, South Africa); and Yashica Singh (Herbarium NH, South Africa). The Montgomery Botanical Center (Patrick Griffith, Larry Noblick, Joanna Tucker Lima, Tracy Magellan, Claudia Calonje, Jessica Sparks, Jason, Christina) and the Fairchild Tropical Garden (Carl Lewis, Chad Husby) are greatly thanked for their support collecting material in these living collections. The following colleagues kindly made accessible the study of herbarium collections: William Baker (K), Piet Stoffelen (BR), Geoffrey Fadeur (BRLU), Chiara Nepi (FI) and Riccardo Baldini (FT), Cristina Duarte, Luis Catarino and Paula Branco (LISC), Ana Isabel de Vasconcelos Correia and Alexandra Lucas (LISU), and Fátima Sales and Joaquim Santos (COI). Iacopo Luino, Yamama Naciri, and Laurent Gautier (CJBG) are thanked for important sampling (herbarium, DNA) of Hyphaene populations in Madagascar. Matthieu Grillet (CJBG) took care of all the germination tests at the Botanical Garden of Geneva based on seed sampled in the field. Régine Niba is also acknowledged for her help with lab work and libraries construction. Finally, John Dransfield (Kew Gardens) is thanked for inspiring us in the study of the enigmatic doum palms, in particular providing extremely valuable data concerning Hyphaene in East Africa and making us aware of the complexity of their study.

Except otherwise indicated, photos were taken by Eric Stauffer and Didier Roguet.

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ISSN-: 0373-2525

47:1-242 (2018) ISBN:978-2-8278-0050-6

