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EVOLUTION ET ADAPTATION FONCTIONNELLE DES ARBRES TROPICAUX : LE CAS DU GENRE *GUIBOURTIA* BENN.

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EVOLUTION ET ADAPTATION FONCTIONNELLE DES ARBRES TROPICAUX : LE CAS DU GENRE *GUIBOURTIA* BENN.

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Co-Promoteurs : Prof. Jean-Louis Doucet et Dr. Olivier J. Hardy

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A mes chers parents, Etienne M. Tosso et Jeanne Codjo

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Résumé

La présente thèse s'intéresse aux mécanismes à l'origine de la diversification des espèces d'arbres tropicaux. Elle utilise le genre *Guibourtia* Benn. (Fabaceae-Detarioideae) comme modèle biologique afin de comprendre les mécanismes historiques, biologiques et environnementaux, à l'origine de la diversité de ce genre aux niveaux interspécifique et intraspécifique. Plus particulièrement, elle vise à : (1) étudier au niveau interspécifique, le rôle relatif des forces évolutives neutres et de sélection dans la diversification du genre *Guibourtia* en combinant une phylogénie moléculaire avec la caractérisation des traits et les niches écologiques des espèces ; et (2) au niveau intraspécifique, questionner les causes de la différenciation des populations de trois espèces de *Guibourtia*.

Au niveau interspécifique, la phylogénie datée basée sur le séquençage du génome chloroplastique complet a globalement confirmé la taxonomie actuelle (espèces généralement monophylétiques). Elle a montré une diversification au milieu du Miocène en trois clades qui sont décrits aujourd'hui comme des sous-genres (*Guibourtia*, *Gorskia* et *Pseudocopaiva*). Elle démontre en outre que deux espèces américaines sont issues d'une migration de l'Afrique vers l'Amérique à la fin du Miocène. Il est également apparu que certains traits morphologiques ont été sélectionnés de manière convergente au sein des différents clades du genre *Guibourtia* en fonction des niches climatiques des espèces. Ce dernier résultat a été consolidé au moyen d'une expérimentation écophysiologique prouvant que la lumière constitue un important facteur de sélection et de différenciation adaptative entre trois espèces (*G. ehie*, *G. coleosperma* et *G. tessmannii*).

Au niveau intraspécifique, une étude de phylogéographie de deux espèces a permis de mettre en évidence que les barrières biogéographiques chez *G. ehie* et les gradients climatiques chez *G. coleosperma*, auraient contribué à la différenciation génétique des populations. En outre, cette étude montre une forte différenciation entre les populations de *G. ehie* d'Afrique de l'Ouest et d'Afrique centrale en lien avec quelques traits morphologiques, ce qui préjuge de l'existence d'une nouvelle espèce. Enfin, chez *G. tessmannii*, espèce aux fruits déhiscents et graines arillées, nous avons identifié les principaux disperseurs dont les calaos (*Ceratogymna atrata*) qui pourraient contribuer à une dispersion à longue distance, influençant la structure spatiale de la variation génétique des populations.

Cette thèse en utilisant le genre *Guibourtia* comme modèle d'étude a apporté de nouveaux éléments à la compréhension des mécanismes qui génèrent la diversité au sein des espèces d'arbres. Elle fournit en outre des connaissances originales sur des espèces inscrites en annexe II de la CITES.

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Abstract

This thesis studies the diversification processes of tropical tree species. It uses the genus *Guibourtia* Benn. as a biological model (Leguminosae-Detarioideae) in order to understand historical, biological and environmental mechanisms that are at the origin of the diversity of the genus. In particular, this PhD aims to: (1) understand at the interspecific level the relative role of neutral and selection forces in the diversification of *Guibourtia* species by combining molecular phylogeny with the characterization of functional traits and ecological niches; and (2) at the intraspecific level, question the causes of population differentiation of three *Guibourtia* species.

At the interspecific level, the molecular phylogeny dating based on whole plastid genome sequencing was congruent with the current taxonomy (species were generally monophyletic). It showed a diversification in the middle of the Miocene in three clades corresponding with the subgenera (*Guibourtia*, *Gorskia* and *Pseudocopaiva*). It also demonstrated that two Neotropical species resulted from a migration from Africa to South America during the late Miocene. Considering climate niches, it was also demonstrated that some morphological traits have been selected in a convergent way within the different clades. This result was confirmed by an ecophysiological experiment, which highlighted that light was an important factor of selection and adaptive differentiation between three species (*G. ehie*, *G. coleosperma* and *G. tessmannii*).

At the intraspecific level, a phylogeographic study of two *Guibourtia* species revealed that the biogeographic barriers in *G. ehie* and the climatic gradients in *G. coleosperma*, would have contributed to the genetic differentiation of populations. In addition, this study questions the delimitation of *G. ehie* since its populations showed a strong genetic differentiation between West and Central Africa. This differentiation was associated with diagnostic morphological features. Finally, in *G. tessmannii*, a species with dehiscent fruits and arillate seeds, we identified the main dispersal species, including hornbills (*Ceratogymna atrata*), which may have contributed to long-distance gene flow, influencing the spatial structure of genetic variation among populations.

This thesis, using the *Guibourtia* genus as a biological model brings new elements helping in the understanding of mechanisms generating diversity within tropical tree species. It also provides important information on species listed on Appendix II of CITES.

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Introduction générale

1. Végétation africaine : diversité et fluctuations climatiques majeures ayant façonné sa physionomie actuelle

1.1. Genèse du continent africain

La compréhension de la végétation africaine ainsi que la diversité spécifique qu'elle héberge passe par la maitrise de l'histoire de son origine. Il y a 200 millions d'années avant notre ère (Ma BP) au début du Paléozoïque, les continents formaient un unique « supercontinent » centré sur l'équateur, appelé Pangaea (Holzman, 2008). Vers 152 Ma BP durant le Jurassique moyen (Figure 0.1a), les blocs continentaux qui formaient le noyau de l'Asie du Sud-Est se sont séparés du supercontinent. La masse continentale méridionale regroupant l'Afrique, l'Inde, Madagascar, l'Amérique du Sud, l'Australie et l'Antarctique, nommée Gondwana, est demeurée positionnée le long de l'équateur, jouissant du soleil, d'une chaleur élevée avec d'abondantes précipitations (conditions idéales pour l'évolution de la forêt tropicale humide). Pendant le Crétacé (vers 94 Ma, Figure 0.1b), Madagascar et l'Inde se sont détachés progressivement du Gondwana et ont commencé à se déplacer respectivement vers l'Est et légèrement vers le Nord. L'Afrique est donc restée isolée durant une longue période du Crétacé et au début du Tertiaire, ce qui se serait traduit par un fort endémisme en grands mammifères. La configuration des continents a commencé à ressembler à celle prévalant actuellement il y a 50 Ma (Figures 0.1c, d, e) et chaque continent affiche aujourd'hui des spécificités floristiques et fauniques en fonction de son environnement abiotique et de la durée de son isolement géographique.

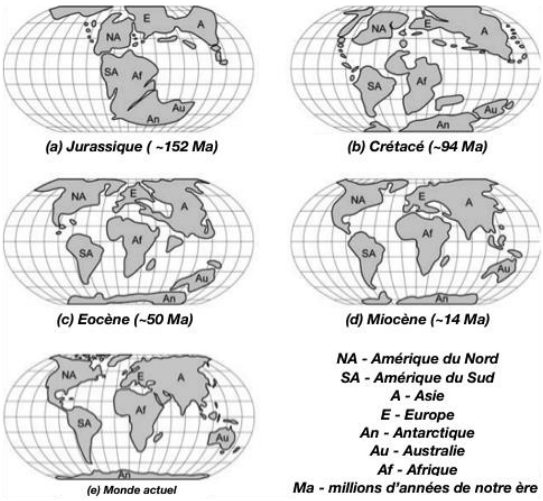

Figure 0.1. Historique des mouvements des plaques terrestres depuis le jurassique à nos jours (adaptée de Holzman (2008))

1.2. Diversité floristique des forêts tropicales d'Afrique en lien avec les fluctuations climatiques passées

L'Afrique est le continent qui abrite le plus grand nombre de biomes terrestres différents (9 sur 14 ; (Olson et al., 2001)). Les biomes tropicaux africains sont localisés entre le tropique du Cancer et le tropique du Capricorne respectivement à 23°N et 23°S (Figure 0.2). Au sein de ces biomes africains, quatre principales physionomies végétales peuvent être distinguées : les forêts denses humides, les forêts sèches/claires, les savanes et les déserts. Les deux premières désignent les forêts tropicales d'Afrique qui abritent la biodiversité la plus riche du continent, quoique la plus faible à l'échelle mondiale comparativement aux forêts tropicales d'Amérique et d'Asie (~4.500 à 6.000 espèces d'arbres africaines vs. ~18.500 à 25.000 espèces sur chacun des autres continents) (Slik et al., 2015).

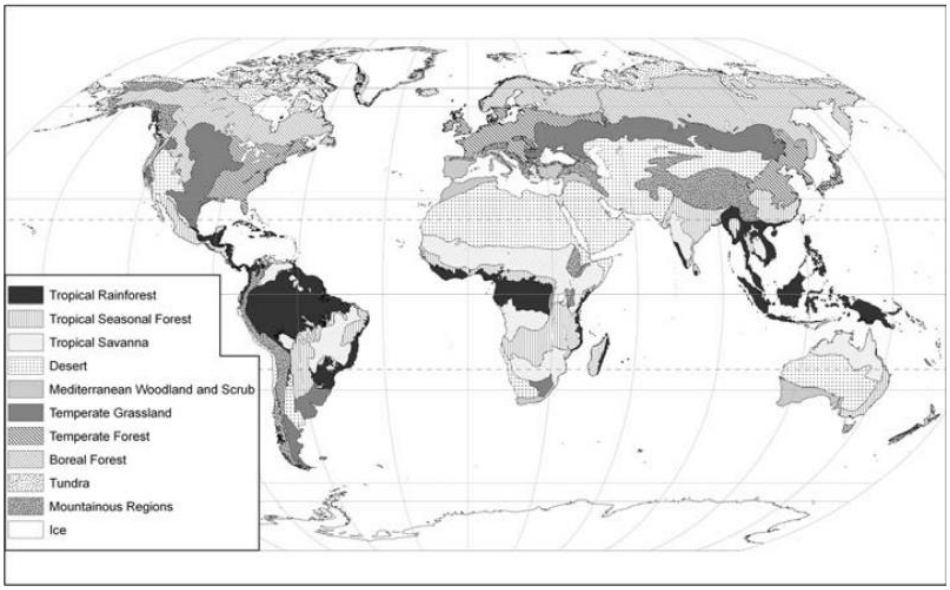

Figure 0.2. Les biomes du monde d'après Holzman (2008)

Cette faible richesse spécifique en Afrique serait majoritairement due aux évènements répétitifs d'extinctions sévères survenus depuis l'Oligocène (Corlett et Primack, 2011). Les oscillations climatiques passées, en particulier celles du Pléistocène n'ont pas autant affecté les autres continents (cf Anhuf et al. (2006) illustrant le cas de l'Afrique et l'Amérique du sud). Ces oscillations qui ont occasionné des pertes et fragmentations de surfaces forestières, auraient particulièrement contribué à l'appauvrissement de la flore Africaine (Plana, 2004). La phase de refroidissement survenue durant le Miocène (~15 Ma) aurait favorisé une régression des forêts tropicales suivie d'une aridification prononcée (Bush et al., 2007). Après cette période, au début du Pliocène (5 – 3,5 Ma) un climat plus humide, favorable à l'expansion des forêts tropicales, s'est installé. A cette période

d'expansion des forêts a succédé plusieurs cycles glaciaires et interglaciaires. De tels cycles se sont traduits en Afrique successivement par des phases froides et sèches, chaudes et humides (Plana, 2004). L'époque la plus documentée reste le dernier maximum glaciaire (24.000 – 12.000 ans) qui a fragmenté et réduit drastiquement les forêts tropicales, qui se seraient uniquement maintenues dans des îlots forestiers aujourd'hui nommés refuges forestiers (Figure 0.3 ; Maley, 1996). Ces refuges ont pu jouer à la fois les rôles de conservation et de foyer de diversification des taxons (Lovett et Friis 1996). Le début de l'holocène (il y a 10.000 ans), devint chaud et humide permettant donc à nouveau une expansion des forêts qui ont reformé un bloc presque continu (Maley, 1989).

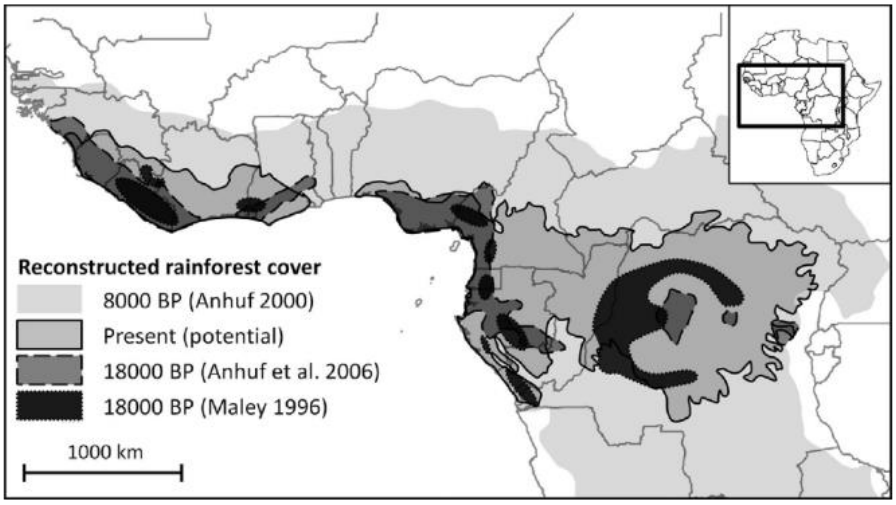

Figure 0.3. Localisation de refuges forestiers, en noir, sur base de la distribution des richesses spécifiques et des taux d'endémisme de plusieurs espèces. Le domaine du centre Guinéo-Congolais est représenté en gris foncé. En gris clair, la distribution des extensions maximales hypothétiques des forêts durant l'Holocène humide (Hardy et al., 2013, adapté de Maley (1996) et Anhuf et al. (2006)).

La cassure actuelle du bloc forestier de l'holocène humide par le corridor dénommé « Dahomey Gap », serait survenue vers 4000 et 3000 ans environ. A cette époque, une grande partie de l'Afrique de l'Ouest et du Centre affichait un régime de précipitations saisonnier et généralement plus sec (Malhi et al., 2013). En effet, il y a 2500 ans, une phase de régression forestière associée à une résurgence des graminées a été notée au niveau de l'Afrique Centrale (Vincens et al., 1999). Cette modification frappante de la végétation a été particulièrement remarquable sur les sites de Barombi Mbo (Cameroun) et Kitina (Sud-Congo) (Figures 0.4a et b). Il y a entre 1000 et 500 ans de notre ère, une reforestation progressive a été mise en exergue par (Malhi et al., 2013). Les deux blocs forestiers, séparés par le Dahomey Gap, forment ce qui est aujourd'hui appelé le centre d'endémisme Guinéo-Congolais (White, 1986).

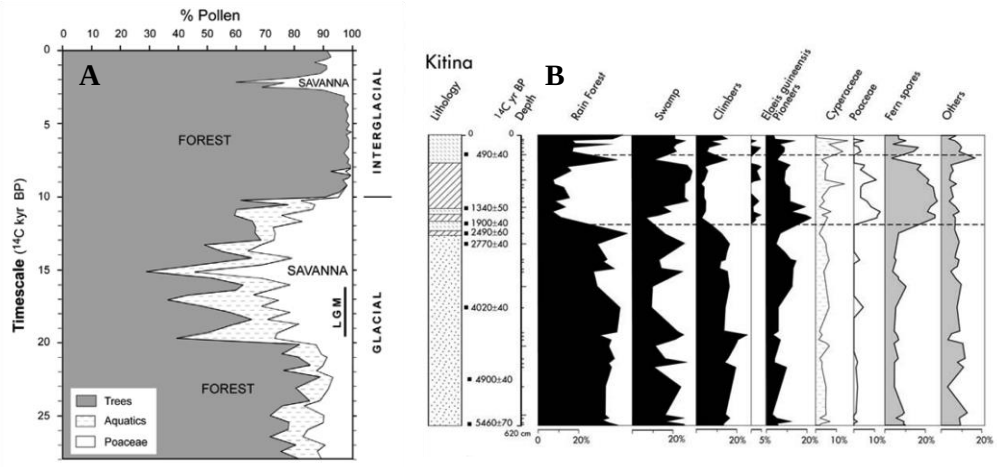

Figure 0.4. Synthèse du diagramme pollinique (A) du lac Barombi Mbo au Cameroun (Maley et Brenac, 1998), et (B) du lac Kitina au Congo (Elenga et al., 1996). Les deux figures présentent en abscisse le pourcentage de pollen en fonction du temps.

Le centre régional d'endémisme Guinéo-Congolais est subdivisé en trois domaines floristiques : domaines Haut-Guinéen, Bas-Guinéen et Congolais (White, 1979). Le Haut-Guinéen va de la Siéra-Léone au Ghana. Le Bas-Guinéen couvre le sud-est du Nigéria, le Cameroun, la Guinée Equatoriale, le Gabon, la République du Congo et le sud de la République Centrafricaine. Quant au domaine Congolais, il couvre la zone de forêt dense de la République Démocratique du Congo et une portion du sud-ouest de la République Centrafricaine. Le centre Guinéo-Congolais est bordé de zones de transition faisant la jonction avec les centres d'endémisme Soudanien et Zambézien jouissant d'un climat sec et caractérisés par une végétation plus ouverte dominée par les forêts claires et les savanes (White, 1983 ; Figure 0.5).

En plus des variations climatiques qui ont laissé des traces visibles sur la flore actuelle, les activités humaines passées ont également joué un rôle significatif (Malhi et al., 2013).

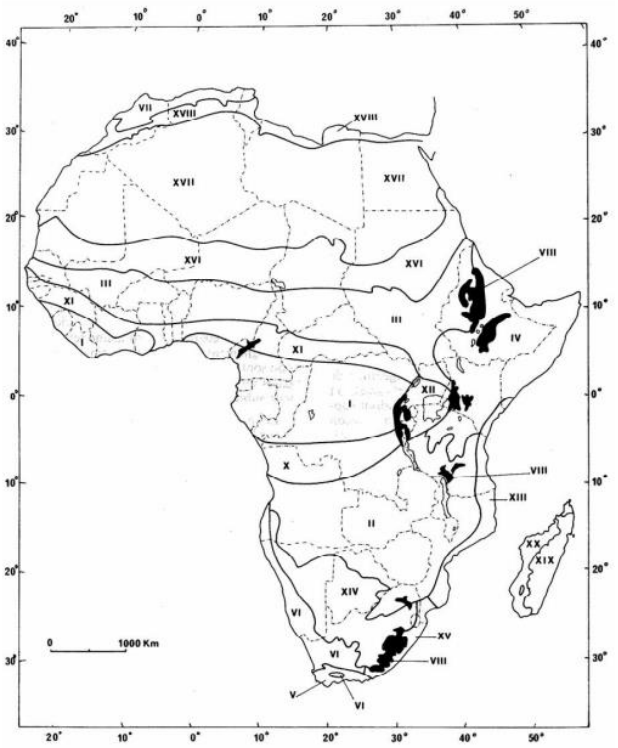

Figure 0.5. Principaux centres d'endémisme en Afrique selon White (1986) : I. centre régional d'endémisme guinéo-congolais, II. Centre régional d'endémisme zambézien, III. Centre régional d'endémisme soudanien, VIII. Centre régional d'endémisme morcelé afromontagnard, X. Zone de transition régionale Guinéo-congolaise/zambézienne, XI zone de transition régionale guinéo-congolaise/soudanienne. XIII. Mosaïque régionale de ZanzibarInhambane. XIV. Zone de transition régionale Kalahari-Highveld.

2. Impact de l'homme sur la végétation forestière d'Afrique

2.1. Impact des activités historiques

Les travaux de Oslisly et al. (2013) ont entre autres élucidé les variations historiques de démographie humaine au sein du Bassin du Congo. Hormis les peuples pygmées présents dans cette région depuis environ 60.000 ans, la colonisation des forêts d'Afrique centrale date de la fin de l'âge de la pierre (entre 40.000 et 3.500 ans BP) où l'homme du paléolithique chasseur-cueilleur a cohabité avec les agriculteurs du néolithique (Oslisly et al., 2013). Il y a environ 3.500 ans BP, ces derniers venus du Sahel et s'adonnant à une agriculture rudimentaire sur brûlis auraient profité du climat plus sec (Willis et al., 2013) et de la fragmentation consécutive de la forêt pour pénétrer dans le bloc forestier. A l'âge du fer ancien (il

y environ 2.500 ans BP), le développement de la métallurgie (qui nécessite du charbon), associé à une croissance démographique et à l'explosion de l'agriculture extensive sur brulis, notamment par les populations probablement Bantous ont profondément marqué les forêts denses africaines. Cette époque aurait atteint son apogée il y a environ 1.900 ans BP et aurait été suivie par une mystérieuse extinction ou forte réduction de la population (probablement associée aux maladies épidémiologiques) entre 1.600 et 1.000 ans BP. Plus récemment, il y a environ cinq siècles, une deuxième vague d'arrivée des populations Bantou dans le bloc forestier a été notée. Celle-ci a connu à nouveau un déclin qui a probablement favorisé l'expansion des surfaces forestières. Selon Morin-Rivat et al. (2014), cette dernière vague de migration aurait eu un impact substantiel sur la structure et la composition actuelle des forêts d'Afrique centrale. En effet, l'impact de l'ensemble de ces activités humaines passées sur les populations d'arbres, en particulier les espèces héliophiles, a été largement démontré par plusieurs auteurs (White et Oates, 1999; van Gemerden et al., 2003; Vleminckx et al., 2014; Biwolé et al., 2015; Morin, 2017).

2.2. Menaces actuelles sur la biodiversité végétale africaine

Les menaces majeures qui pèsent actuellement sur les forêts tropicales d'Afrique, plus précisément sur les forêts denses humides sont liées à la perte d'habitats due à la destruction des milieux naturels du fait de l'explosion démographique et l'urbanisation, la progression de l'agriculture, l'exploitation illégale du bois, la récolte abusive de parties de plantes sollicitées comme produits forestiers non ligneux et les changements climatiques (Bradshaw et al., 2009). Souvent, ces menaces se développent si rapidement et à une telle échelle que les efforts fournis pour sauver certaines espèces forestières se révèlent inefficaces (Corlett et Primack, 2011). En somme, la croissance démographique et ses corollaires, et le changement climatique dû à l'ère industrielle constituent aujourd'hui les principales menaces pesant sur la biodiversité des forêts tropicales (Bourque, 2000).

2.2.1. Croissance démographique

Jusqu'au 19ème siècle, la croissance démographique mondiale était faible avec des taux de natalité qui dépassaient les taux de mortalité de 50 pour 1000 (Brasseur, 1981). A partir de 1850, un boom démographique a été noté et la population humaine est passée de 1,2 à 6,8 milliards en 2010 (Primack, 2010). Aujourd'hui, les pays en voie de développement sont les plus concernés par ce boom démographique (Asongu et Jingwa, 2012). Selon l'ONU Worlds Prospects (2009), la population africaine de 2009 est appelée à doubler d'ici 2036. Dans les pays concernés, les populations pauvres et vivant avec moins d'un dollar par jour, sont dépendantes de l'agriculture itinérante sur brûlis, de la chasse et de l'élevage (Corlett et Primack, 2011). Ces activités, combinées avec le développement de l'agrobusiness (palmier à huile, soja, cacao, etc.) et des agro-industries soutenus par les intérêts commerciaux, détruisent ou dégradent d'importantes surfaces forestières (Sheldon et al., 2010).

2.2.2. Changement climatique global de l'ère industrielle

Les changements climatiques d'origine anthropique constituent une autre menace pour les forêts tropicales d'Afrique. Les trois dernières décennies ont été sans doute les plus chaudes sur terre depuis 1850 avec une tendance linéaire montrant un réchauffement de 0,85° C (0,65 à 1,06) sur la période allant de 1880 à 2012 (Figure 0.6). L'augmentation inquiétante résulte des émissions de gaz à effet de serre depuis la période préindustrielle qui est étroitement liée à la croissance économique et démographique (IPCC, 2014). Outre l'augmentation de température appelée à se maintenir dans le futur, plusieurs modèles climatiques prédisent une aridité accrue sur toute l'Afrique à partir du 21ème siècle (Serdeczny, 2017). Par contre, d'autres modèles prédisent plus de précipitations sur une bonne partie de l'Afrique (Monerie et Roucou, 2015). Entre 1951 et 2010, Spinoni et al. (2014) ont prouvé une augmentation de la fréquence des évènements de sécheresse et de coups de chaleur. Selon les prévisions, il est fort probable qu'ils soient plus fréquents avec le réchauffement global (IPCC, 2014). Plusieurs études ont relié une recrudescence de la mortalité d'arbres à la sécheresse et aux coups de chaleur (Wright, 2005 ; Wasseige et al., 2012). Le réchauffement climatique aura donc sans doute des conséquences négatives sur les écosystèmes forestiers tropicaux d'Afrique (Collins, 2011).

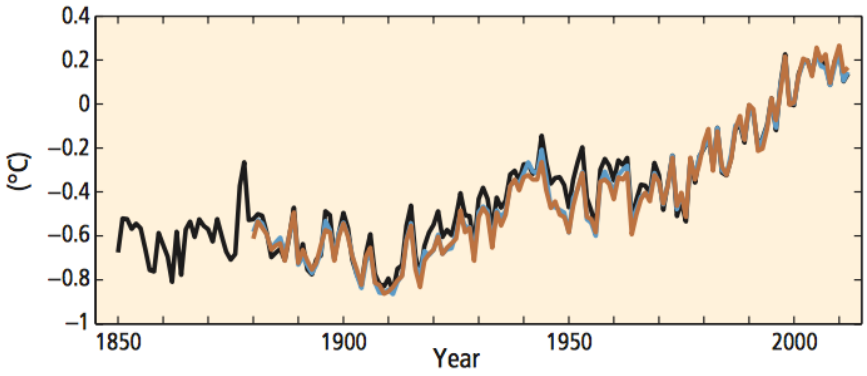

Figure 0.6. Evolution des anomalies globales annuelles de la température terrestre sur une période allant de 1850 à 2012 .

Il est en conséquence nécessaire de mieux connaître le passé de ces forêts en relation avec les climats anciens, afin de mieux prédire leur dynamique future sur la base des projections climatiques. La clarification du statut taxonomique des espèces présentes dans les forêts tropicales d'Afrique reste un préalable essentiel pour une meilleur connaissance de ces forêts.

3. Problématique de la délimitation des espèces

Il est admis que la notion d'espèce est fondamentale en biologie quoique sa définition demeure problématique (de Queiroz, 2005; Hey, 2006). Pourtant, les espèces représentent une des unités fondamentales en biologie dont les individus

présentent une forte similarité au niveau des cellules, gènes, etc. (exemple, Mayr, 1982; de Queiroz, 2005). La complexité de la définition de l'espèce provient notamment du fait qu'il existe des niveaux d'organisation biologique qui peuvent aller au-delà de la simple perception humaine (De Queiroz, 2007). Cette complexité s'est amplifiée durant ces 50 dernières années avec la prolifération de concepts qui ne font pas toujours l'unanimité au sein des naturalistes (Harrison, 1998). Traditionnellement, les espèces ont été décrites suivant le concept morphologique qui se base sur les différences morphologiques et qui reste le concept le plus généralement utilisé en pratique. Aujourd'hui, deux concepts sont particulièrement utilisés pour la délimitation des espèces : (1) le concept biologique d'espèce et (2) le concept phylogénétique d'espèce.

Le concept biologique de l'espèce a été développé par Mayr (1942) et Dobzhansky (1950) et est basée sur le principe de l'isolement reproductif. Selon ce concept, une espèce est une population ou un ensemble de populations dont les individus sont capables de se reproduire entre eux et engendrer une descendance viable et féconde, dans des conditions naturelles. Ainsi, les individus d'une même espèce sont donc génétiquement isolés d'autres ensembles équivalents du point de vue reproductif (Neil et Reece, 2007). Quant au concept phylogénétique, il a été développé par Hennig (1965) qui considère que seule l'identification de groupes monophylétiques représentant tous les descendants d'un même ancêtre et définis par des états dérivés de caractères partagés, permet de l'appréhender l'évolution des organismes et de les classer. Ainsi, selon ce concept, une espèce est définie comme un groupe monophylétique d'individus partageant une relation d'ascendance et de descendance (Ruse, 1998). L'approche actuellement utilisée par des botanistes regroupés sous le sigle APG (*Angiosperm Phylogeny Group*) est similaire à celle adoptée dans le concept phylogénétique. L'APG s'occupe de la classification au niveau supraspécifique en visant effectivement des groupes (familles, genres, etc.) monophylétiques. Il met régulièrement à jour la classification phylogénétique des plantes à fleurs (Angiospermes) depuis 1998. Les classifications APG font office de référence en matière de systématique des Angiospermes dans la communauté scientifique. La quatrième (APG IV) datant de 2016, de même que les précédentes, repose sur l'utilisation de séquences d'ADN présents dans les cellules des plantes.

La problématique de la position taxonomique des espèces végétales et en particulier des espèces sœurs et morphologiquement similaires, est particulièrement d'actualité, au vu des résultats des données moléculaires et génomiques, qui remettent en cause plusieurs postulats en systématique (Funk et Omland, 2003). De nombreuses études révèlent les insuffisances de l'utilisation unique de la taxonomie classique basée sur les caractères physiques : morphologie et anatomie (Schlick-Steiner et al., 2010). Selon Kew (2016), plus de 2.000 nouvelles espèces sont découvertes chaque année dans le monde en partie à cause de l'existence de nombreux cas d'espèces cryptiques (espèces isolées reproductivement mais difficilement différenciables morphologiquement (Carstens et al., 2013). Une fois ces espèces cryptiques identifiées au moyen d'outils génétiques, les études morphologiques, anatomiques, écologiques, etc. permettent ensuite d'identifier des différences physiques qui n'avaient pas été observées auparavant. En Afrique, il existerait de nombreuses espèces cryptiques notamment dans les forêts tropicales.

En effet, les découvertes récentes (*Milicia* (Daïnou et al., 2016), *Afzelia* (Donkpegan et al., 2017), *Santiria* (Ikabanga et al., 2017) remettraient même en cause les estimations de la richesse spécifique des forêts denses humides tropicales. Or de telles estimations sont des préalables à l'élaboration de stratégies de conservation et de gestion durable des arbres tropicaux, particulièrement ceux exploités (Bickford et al., 2007).

Selon la nature de la distribution des espèces (sympatrique ou parapatrique), la délimitation d'espèces pourrait aussi avoir divers degrés de complexité. Si les différences phénotypiques sont nettes, quelle que soit la répartition des espèces, le travail de délimitation est assez aisé. Par contre, si ces différences phénotypiques sont ténues, le recours à la reconstruction phylogénétique à l'aide de marqueurs génétiques est nécessaire. En cas de monophylie des groupes, le statut d'espèces séparées est facilement confirmable. Par contre, en cas de paraphylie (taxon regroupant une espèce ancestrale et une partie seulement de ses descendants, (Crisp et Chandler, 1996)), l'utilisation des méthodes de la génétique des populations (pour identifier les barrières reproductives) intégrée à la phylogéographie s'avère utile. En outre, si des groupes avec des différences phénotypiques ténues sont distribués en allopatrie, il est difficile de se prononcer même en ayant recours à la génétique des populations, tant qu'ils ne sont pas en contact (artificiellement ou naturellement) afin de vérifier les barrières reproductives. Il est donc beaucoup plus aisé de délimiter les espèces distribuées en sympatrie (car il est possible d'évaluer les flux de gènes intergroupes et donc statuer sur leur taxonomie) que celles en allopatrie (Fujita et al., 2012).

Aujourd'hui, un nombre croissant de chercheurs en biologie évolutive adopte cette «taxonomie intégrative» (Yeates et al., 2011) qui se fonde sur l'utilisation de diverses sources de données (par exemple, moléculaires, morphologiques, et / ou écologiques) pour délimiter les espèces (Daïnou et al., 2016; Sites et Marshall, 2004; Wiens et Penkrot, 2002). L'approche basée sur la taxonomie intégrative peut être complétée par la prise en compte des traits fonctionnels qui sont informatifs pour évaluer les adaptations des taxons (Ackerly et al., 2000). Dans tous les cas, les approches génétiques occupent une place centrale dans la délimitation des espèces d'arbres tropicaux. Cette dernière est fondamentale pour la compréhension des forces évolutives impliquées dans la diversification.

4. Forces évolutives et diversification

4.1. Mutations, base de l'évolution

4.1.1. ADN : support de l'information génétique

Chez les plantes, l'ADN (une molécule contenant l'entièreté de l'information génétique) se répartit, dans le noyau des cellules, en ADN nucléaire (ADNn), ADN chloroplastique (ADNcp) et mitochondrial (ADNmt). Le premier (ADNn) représente globalement la plus grande partie du génome des plantes. Il est transmis de manière biparentale et est également de plus en plus utilisé parce qu'il est approprié pour

résoudre les questions de délimitation taxonomique (Hare, 2001). L'ADNn comprend l'ADN ribosomique (ADNr) qui représente une partie hautement répétitive de d'ADNn qui est transcrite en ARN ribosomique (ARNr). Les ribosomes sont des complexes de protéines et d'ARNr au sein desquels se déroule la traduction des ARN messagers au cours de la biosynthèse des protéines. Les gènes de l'ARNr et leurs régions espaceur externe transcrite (ETS) et espaceur interne transcrite (ITS) sont souvent utilisées comme informations de base pour les analyses phylogénétiques (Poczai et Hyvönen, 2010). Le deuxième (ADNcp) est généralement transmis maternellement chez les angiospermes, donc dispersé via les graines (Matsushima et al., 2008). Le génome ADNcp est également connu sous le nom de plastome qui représente un matériel génétique localisé dans les plastes (organites présents dans les cellules des eucaryotes chlorophylliens). Selon Clegg et al. (1991), ce génome est largement utilisé en phylogénie et phylogéographie du fait qu'il est non-recombinant et suffisamment conservé chez les angiospermes que pour définir les marqueurs universels. Quant à l'ADN mitochondrial, il est également décrit comme à transmission uniparentale (de type maternel). Il est tout de même plus utilisé en biologie moléculaire chez les animaux que chez les plantes (Freeland et al., 2011).

Aujourd'hui, une grande attention est portée sur l'utilisation de l'ADN chloroplastique et nucléaire pour les reconstructions phylogénétiques (Li et al., 2008). Plus particulièrement, le développement extrêmement rapide des nouvelles techniques de séquençage massif d'ADN, connues sous l'appellation de « *Next Generation Sequencing*, NGS », a offert d'importantes possibilités pour mieux comprendre les scénarios évolutifs qui étaient peu élucidés en utilisant seulement quelques gènes (Novo et al., 2016).

4.1.2. Mutations et dérive génétique comme forces évolutives

Une mutation correspond à toute modification de la séquence nucléotidique d'un gène, qu'elle touche les exons, introns et séquences transcrites non traduites ou les séquences portant les signaux de contrôle de l'expression (Samouelian et al., 2009). Classiquement, on distingue quatre types de mutations : la substitution, l'insertion, la délétion et l'inversion. Ces mutations sont causées par plusieurs facteurs : les erreurs de réplication de l'ADN, l'instabilité chimique des bases nucléotidiques ou de la liaison N-glycosidique entre base et le désoxyribose, l'agression par les facteurs de l'environnement (facteurs d'ordre chimique ou physique) provoquant des lésions au niveau de la séquence d'ADN. La fréquence d'apparition spontanée de ces mutations est faible (10^{-5} – 10^{-8} par génération et par gène) (Kovalchuk et al., 2000). En fait, il s'agit d'une petite fraction de ces mutations qui, lorsqu'elles affectent une région codante, peuvent conduire à l'apparition d'un caractère phénotypique différent de celui de l'organisme de départ (Samouelian et al., 2009).

Certaines mutations peuvent s'avérer bénéfiques en conférant aux individus qui les portent une vigueur supérieure, notamment en regard des caractères de reproduction. De telles mutations sont alors sélectionnées et transmises progressivement à un grand nombre de descendants. C'est le principe qui gouverne la sélection naturelle. L'idée de la sélection naturelle est plus ancienne que les écrits de Charles Darwin et

remonterait au temps de Jean-Baptiste Lamarck (Wool, 2006). La sélection naturelle est en effet le processus par lequel les espèces s'adaptent à leur environnement (Michael, 2015). Elle conduit à un changement évolutif lorsque des individus ayant certaines caractéristiques génétiques ont (i) un plus grand taux de survie et de reproduction que d'autres dans une population et (ii) transmettent ces caractéristiques génétiques d'une génération à l'autre (Williams et Burt, 1997). Le rôle de la sélection naturelle est donc central pour expliquer les processus qui façonnent la morphologie et la physiologie des plantes (Lenski, 2017).

Outre ces mutations qui conduisent à la synthèse de protéines, il y a des mutations neutres qui se déroulent dans des régions codantes si le nucléotide d'un codon est modifié sans que l'acide aminé associé le soit. Les mutations peuvent s'accumuler différemment au sein de groupes séparés depuis suffisamment longtemps, contribuant à la structuration génétique. Elles sont associées au phénomène connu sous le nom de dérive génétique (Wright, 1931). La dérive génétique se définit comme étant un changement aléatoire de fréquences alléliques dans une population de taille finie (Boddy, 2016). En particulier, la dérive génétique est davantage perceptible dans des populations affectées par un goulot étranglement ou un effet fondateur. Un goulot d'étranglement (*bottleneck*) correspond à la réduction brusque de la taille de la population (Lippé, 2006). Il en résulte une perte de diversité avec une diminution notable des allèles rares ainsi qu'une baisse lente de l'hétérozygotie (Piry et al., 1999). L'effet de fondation est une forme de goulot d'étranglement et se produit lorsque quelques individus migrent hors d'une population d'origine pour coloniser un site inoccupé et former une nouvelle sous-population (Douglas, 2000). La dérive génétique accompagne cet effet de fondation en réduisant la diversité par rapport à la population initiale. Dans la nouvelle population, cet effet fondateur peut augmenter rapidement la fréquence d'un allèle qui était très faible dans la population initiale. Ce phénomène conduit généralement à une différenciation génétique entre populations, du moins si les flux géniques sont suffisamment limités que pour ne pas homogénéiser les fréquences alléliques (Poutasso, 2009).

4.2. Apport des méthodes de phylogéographie et de génétique des populations pour l'étude de l'organisation spatiale des lignées génétiques

Le terme phylogéographie a été introduit en 1987 à la suite des travaux de Avise et al. (1987) qui cherchaient à unir les spécialistes en biologie évolutive, alors dispersés dans divers domaines de la phylogénie et de la génétique des populations. La phylogéographie est définie par Avise (2000) comme l'étude des principes et processus gouvernant la distribution géographique des lignées généalogiques, particulièrement au sein des espèces et entre espèces proches afin de mettre en évidence les principaux facteurs qui ont influencé la distribution de la diversité génétique. Son but est donc de comprendre dans quelle mesure les événements historiques ont façonné les patrons de distribution géographique des populations, espèces et allèles (Freeland et al., 2011) en utilisant les principes de génétique des populations. Plusieurs approches statistiques ont été développées en phylogéographie. Initialement, les toutes premières approches d'analyse en

phylogéographie s'interessaient à établir de simples inférences qualitatives de l'histoire démographique des taxons étudiés, par surperposition de l'arbre haplotypique (généalogie des différents variants d'un locus non recombinant) avec la géographie de l'échantillonnage des haplotypes. Cette première approche développées par Avise et al. (1987) est associée à des techniques statistiques peu développées. La deuxième approche qui est basée sur une analyse des groupements imbriqués ou « *Nested Clade Phylogeography Analysis* » a été proposée par Templeton et al., (1995). Cette approche est basée sur une méthode cladistique destinée à analyser un arbre d'haplotypes obtenu par une étude phylogéographique. Cette méthode cherche à discriminer, selon une approche statistique, les processus historiques qui ont potentiellement influencé la distribution géographique des haplotypes. La non prise en compte du caractère stochastique des processus génétiques pour les inférences démographiques, par les deux approches mentionnées précédemment, a conduit à l'émergence de nombreuses méthodes statistiques pour réaliser des inférences phylogéographiques, basées sur la théorie de la coalescence (modèle rétrospectif de génétique des populations). En effet, deux principales approches statistiques basées sur la théorie de la coalescence sont utilisées : l'approche du maximum de vraisemblance et l'approche bayésienne (Nielsen et Beaumont, 2009). L'objectif de ces deux méthodes est d'estimer certains paramètres démographiques ou génétiques sur base du polymorphisme observé au seins des populations. Aujourd'hui, l'approche bayésienne est la plus utilisée pour identifier les pools génétiques et inférer l'histoire démographique des populations (Beerli et Palczewski, 2010; Heled et Drummond, 2009; Hey, 2009). Deux sources d'information sont donc utilisées dans cette approche : (i) une information apportée par les données génétiques et (ii) une information *a priori* sur les paramètres démographiques (p.ex. taille efficace des population) et génétiques (p.ex. taux et mode de mutation) dans le but d'obtenir la distribution *a posteriori* des paramètres à estimer (Nielsen et Beaumont, 2009). Les informations *a priori* peuvent provenir de données paléo-environnementales permettant d'obtenir des indications, non seulement sur les phénomènes historiques qui auraient pu affecter une population (glaciation, pertubation d'habitat, orogenèse), mais aussi sur l'époque de la survenance de ces différents évènements.

Les études utilisant les méthodes de phylogéographie et de génétique des populations nécessitent l'utilisation de marqueurs moléculaires composés de fragments d'ADN qui servent de repères pour suivre la transmission d'un segment de chromosome d'une génération à l'autre. De nos jours, les plus utilisés sont les marqueurs moléculaire microsatellites (SSRs) et les *single-nucleotide polymorphisms* (SNPs). Définis comme étant une séquence d'ADN formée par une répétition continue d'un motif composé de 1 à 4 nucléotides, les marqueurs moléculaires microsatellites sont situés à la fois dans le génome chloroplastique et nucléaire. Au cours des deux dernières décennies, ils ont été largement utilisés pour plusieurs raisons : ils sont (i) très polymorphes (nombreux allèles), (ii) codominants et (iii) généralement transférables entre des espèces apparentées (Mason, 2015). Ces SSRs sont particulièrement utiles non seulement pour (i) mesurer la diversité génétique au sein des populations d'organismes, (ii) caractériser la diversité génétique spatiale des organismes à large échelle, (iii) étudier les flux de gènes et

(iv) détecter les formes et zones hybrides en espèces proches (Vieira et al., 2016). Outre les marqueurs moléculaires microsatellites, les SNPs (*single-nucleotide polymorphism* ou polymorphismes d'un seul nucléotide) permettent de détecter la variation (polymorphisme) d'une seule paire de bases du génome, entre individus d'une même espèce et sont devenus des marqueurs de choix utilisés en phylogéographie et en génétique des populations (Rafalski, 2002). Aujourd'hui, le développement des techniques NGS et bioinformatiques facilitent le développement de ces marqueurs moléculaires.

En Afrique tropicale, plusieurs études phylogéographiques ont été initiées ces dernières années sur les espèces d'arbres. Ces études ont fourni des informations précieuses sur l'histoire de la végétation africaine. On sait aujourd'hui que les oscillations passées du climat et certaines barrières physiques (chaînes de montage) ont certainement influencé les patrons de distribution spatiale de la diversité génétique au sein de plusieurs espèces forestières (Hardy et al., 2013). Elles ont également permis de confirmer les résultats d'études paléo-botaniques démontrant que les périodes interglaciaires chaudes et humides ont été favorables à l'expansion des populations d'espèces forestières (Demenou et al., 2016; Donkpegan, 2017; Faye, et al., 2016; Hardy et al., 2013; Heuertz et al., 2014; Ley et al., 2017). Ces études restent en concordance avec les hypothèses de refuges et soulignent une structuration de la diversité génétique au sein des espèces d'arbres (Figure 0.7). Par contre, il existe peu d'études sur les espèces rencontrées hors de la région Guinéo-Congolaise, notamment celles de forêts denses sèches ou de savanes. Les rares études suggèrent une expansion au cours du dernier maximum glaciaire pour certains taxons et quelques signaux de fragmentation probablement en lien avec les barrières de types climatiques ou géologiques [*Acacia nilotica* (Wardill et al., 2005), *Adansonia digitata* (Tsy et al., 2009 ; Kyndt et al., 2009), *Acacia senegal* (Odee et al., 2012), *Vitellaria paradoxa* (Allal et al., 2011), *Khaya senegalensis* (Sexton et al., 2015) , *Afzelia africana* et *Afzelia quanzensis* (Donkpegan, 2017)].

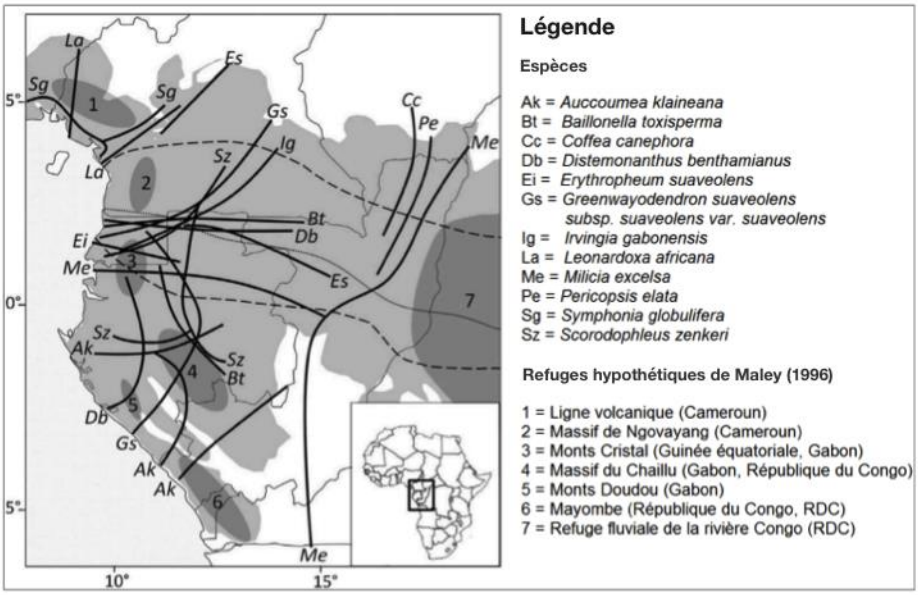

Figure 0.7. Positions approximatives des discontinuités génétiques identifiées chez 13 espèces d'arbres des forêts pluviales du Bas-Guinéen. Les refuges forestiers hypothétiques du dernier maximum glaciaire sont représentés par les aires grisées (modifié à partir de Maley, 1996 et Colyn et al., 1991). Les codes lettrés désignent des taxons. Chaque ligne noire foncée correspond à une discontinuité génétique ou limite géographique entre pools géniques parapatriques, identifiés à l'aide de microsatellites nucléaires, excepté pour Ig (données RAPD) et La (limites entre clades du génome chloroplastique). La plupart des espèces n'ayant pas été échantillonnées dans la partie Est de la carte (excepté Cc, Me et Pe), le peu de discontinuités rapportées dans cette zone est à confirmer. Les deux lignes en pointillé délimitent approximativement la zone de climat équatorial à deux saisons humides et deux saisons sèches, ces dernières étant d'intensité égale (en terme de pluviométrie) le long de la charnière climatique (ligne continue en gris)
(tiré de Hardy et al., 2013).

4.3. Délimitation des espèces par des approches phylogénétiques

Inventé par Haeckel (1866), le terme phylogénie décrit les relations entre les espèces de manière à refléter leur histoire évolutive (Darlu et Tassy, 1993). La phylogénie applique une combinaison de techniques moléculaires et statistiques pour inférer des relations évolutives et de parenté entre des organismes à partir de séquences génomiques (Galtier et Daubin, 2008). Selon la théorie de l'évolution de Darwin (1859), tous les organismes ont des ancêtres communs. Les caractères héritables sont donc transmis des ancêtres aux descendants le long des branches de l'arbre phylogénétique en subissant, ou non, des évolutions (Liu et al., 2009). Ainsi, durant l'évolution, les lignées se forment et peuvent devenir plus ou moins génétiquement isolées les unes des autres suite à une accumulation de mutations et à un flux de gènes intergroupes limité. Si les lignées restent isolées pendant de

longues périodes et dans des environnements différents, des différences morphologiques et fonctionnelles apparaitront et favoriseront l'isolement reproductif entre elles : des mutations non-silencieuses permettent une adaptation et une sélection de caractères en relation avec l'environnement.

En utilisant les séquences d'ADN (quelle que soit leur nature), les analyses phylogénétiques permettent de représenter les voies évolutives, les longueurs de branches représentant le temps ou le nombre de mutations entre les individus, gènes ou autres entités utilisées pour la construction de l'arbre phylogénétique (Figure 0.8) (De Bruyn et al., 2014). Avec les progrès récents en biologie moléculaire et le développement des techniques de séquençage, les données macromoléculaires peuvent être interprétées comme une chaîne linéaire de caractères multi-états (avec quatre états possibles (nucléotides) pour les séquences d'ADN ou 20 pour les séquences d'acides aminés). Il convient de mentionner que ces données macromoléculaires servent à réaliser des phylogénies de gènes et non d'espèces. L'intégration de l'information sur la taille efficace des populations est importante pour obtenir la phylogénie d'espèces (Rannala et Yang, 2003).

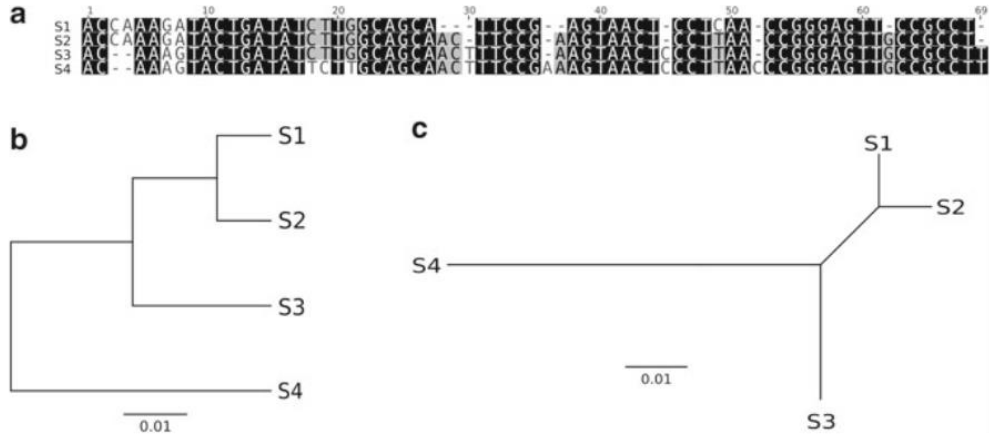

Figure 0.8. (a) Alignement de quatre séquences S1, S2, S3 et S4 avec différentes nuances de gris représentant des sites polymorphes. (b) et (c), illustrent respectivement, arbre phylogénétique enraciné et non enraciné des quatre séquences présentées dans l'alignement, barre d'échelle correspondant au nombre de substitutions par site (adapté de De Bruyn et al., 2014).

Les reconstructions phylogénétiques à partir des séquences d'ADN peuvent rencontrer différents problèmes : (i) le manque de polymorphisme au niveau des gènes utilisés, (ii) le séquençage d'un nombre insuffisant de gènes, (iii) la capture cytoplasmique généralement au sein des taxons proches (Doyle et Gaut, 2000) et (iv) le tri de lignée incomplet (*incomplete lineage sorting*) du fait d'une dérive génétique insuffisante depuis la spéciation (Hobolth et al., 2011). Ces problèmes se traduisent généralement par des cas de paraphylie et de polyphylie qui sont fréquents dans la littérature et qui ne permettent de trancher sur le statut et le nombre d'espèces sans

l'apport de données de génétique de populations (Daïnou et al., 2016; Funk et Omland, 2003; Zachos et al., 2013).

Par ailleurs, les molécules d'ADN sont également utilisées pour estimer le temps de divergence. Les mutations génétiques s'accumulent dans un génome à une vitesse souvent inconstante, ce qui nécessite des corrections pour en tenir compte. Zuckerkandl et Pauling (1965) eurent l'idée de convertir le degré de divergence moléculaire entre deux espèces en âge de la période géologique à laquelle vivait leur ancêtre commun le plus récent. Ainsi est née la datation moléculaire qui consiste à coupler un arbre phylogénétique décrivant les relations de parenté entre espèces, reconstruit à partir de séquences d'ADN ou de protéines, avec des points de calibration paléontologiques afin d'estimer les âges absolus de divergence entre organismes (Douzery et al., 2006).

4.4. Méthodes d'évaluation de l'adaptation dans la diversification

4.4.1.Etude de la plasticité phénotypique

4.4.1.1. Réaction ponctuelle à l'environnement, méthodes et déterminisme de la plasticité phénotypique

Les variations environnementales peuvent induire des modifications morphologiques, physiologiques et génétiques (Shaw and Etterson, 2012). La capacité des espèces à répondre aux changements environnementaux dépend non seulement de la diversité génétique au sein des populations mais aussi de leur plasticité phénotypique. La plasticité phénotypique est définie comme la capacité d'un même génotype à changer de forme ou de physiologie en fonction de l'environnement (Bradshaw, 1965). Elle peut être appréhendée au moyen des traits fonctionnels, souvent utilisés pour apprécier les adaptations des espèces (Perez-Harguindeguy et al., 2013). En écologie évolutive, la plasticité phénotypique pourrait faciliter l'expression de phénotypes relativement bien adaptés lorsque de nouvelles conditions environnementales apparaissent (Gratani, 2014). Elle a le potentiel d'expliquer une multitude de processus évolutifs (Van Kleunen et Fischer, 2005). Deux grandes hypothèses peuvent expliquer le déterminisme génétique de la plasticité. La première est l'hypothèse de la pléiotropie qui soutient qu'un même gène peut engendrer différents phénotypes (Bradshaw, 1965). La deuxième est l'hypothèse épistatique qui fait intervenir deux types de gènes : (i) les gènes responsables de la formation du caractère, et (ii) les gènes régulateurs qui répondent directement à des stimuli environnementaux spécifiques à même de provoquer des changements phénotypes et même génétique spécifiques (Schlichting et Pigliucci, 1998).

Dans la littérature, deux approches sont utilisées pour étudier la plasticité phénotypique : (1) l'approche basée sur l'état d'un caractère « *character state approach* » (Via et al., 1985 ; Tienderen et al., 1991) et (2) celle basée sur la norme de réaction « *reaction norm approach* » (Tienderen et al., 1991 ; Jong, 1995). Les

deux approches sont proches : dans le premier cas, on mesure la valeur moyenne d'un trait dans différents environnements tandis que dans le second, la plasticité est appréciée au moyen d'une fonction polynomiale et on exploite les paramètres de cette fonction comme variables dont on étudie les variations (ces variables étant utilisées pour établir des comparaisons entre génotypes, Figure 0.9).

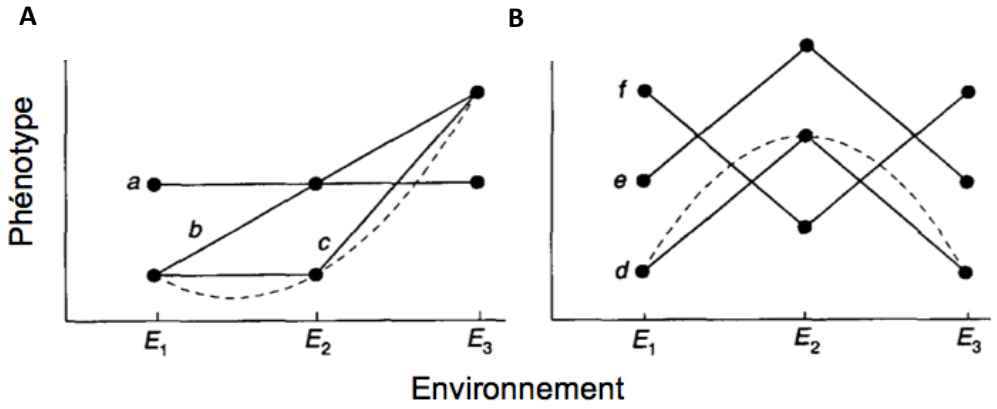

Figure 0.9. Les figures A et B illustrent différents patrons de réponses des génotypes a, b, c, d, e et f dans les environnements E1, E2 et E3. Chaque point représente la valeur moyenne du phénotype considéré et les traits pleins qui relient les points pour un même génotype montrent grossièrement une norme de réaction dans un environnement continu. L'approche liée à la norme de réaction est illustrée au niveau des génotypes c et d avec les traits en pointillé qui modélise avec une fonction polynomiale la réponse de ces génotypes dans les différents environnements considérés. Par exemple, sur la figure A, le génotype a n'est pas plastique comparativement au b et c. Ce dernier l'est uniquement entre les environnements E2 et E3. Sur la figure B, e et d illustre un effet additif du génotype contrairement au génotype f (adapté de Via et al. (1995)).

Plusieurs indices existent pour calculer la plasticité phénotypique. Selon Valladares (2006), il existe 17 indices qui permettent de comparer les plasticités des traits ou caractères entre différents génotypes ou espèces. Après avoir analysé les forces et faiblesses de chacun de ces 17 indices, il développa un nouvel indice statistiquement plus robuste : la RDPI (*Relative Distance Plasticity Index*). La formule mathématique de la RDPI est la suivante : $RDPI = \sum |(d_{ij \rightarrow i'j'}/(x_{i'j'} + x_{ij}))|/n$, où $d_{ij \rightarrow i'j'} = |x_{i'j'} - x_{ij}|$, n est le nombre total de combinaison des traitements et $d_{ij \rightarrow i'j'}/(x_{i'j'} + x_{ij})$ représente une distance relative, $d_{ij \rightarrow i'j'}$ étant la valeur absolue de la distance entre les individus j and j' soumis aux traitements i and i', respectivement; x_{ij} est la valeur du trait de l'individu j soumis au traitement i, et $x_{i'j'}$ est la valeur du trait de l'individu j' soumis au traitement i'. La valeur du RDPI est comprise entre 0 (absence de plasticité) et 1 (plasticité maximum). Cet indice semble faire l'unanimité au sein de la communauté scientifique et permet de distinguer les génotypes ou espèces en fonction de leur plasticité. En outre, l'utilisation de ces

indices de plasticité apporte des connaissances fondamentales utiles dans l'exploration des mécanismes d'adaptation ainsi que les implications au niveau de l'évolution de ces différences (Bresson, 2011).

4.4.1.2. Facteurs de l'environnement impliqués dans la plasticité phénotypique au sein des espèces d'arbres

Plusieurs facteurs de l'environnement sont utilisés pour tester la réponse des espèces d'arbres tropicaux à leur environnement: les caractéristiques pédologiques (Baltzer et al., 2005), le stress hydrique (Gebrekirstos et al., 2006; Lenski, 2017; Zhang et al., 2004), la température (Aspinwall et al., 2017), la lumière (Agyeman et al., 1999 ; Poorter, 1999 ; Markesteijn and Poorter, 2009 ; Biwolé et al., 2015 ; Lestari and Nichols, 2016 ; Barros et al., 2012; Valladares et al., 2002; Valladares et Guzmán, 2006; Valladares et al., 2016; Valladares et al., 2000), etc. Pour les espèces forestières tropicales, la lumière et le stress hydrique sont les deux facteurs clés utilisés dans les études expérimentales (Markesteijn et Poorter, 2009). Si l'étude de la réponse des espèces forestières à la lumière est un outil précieux pour définir leur tempérament, la réponse au stress hydrique quant à elle permet d'anticiper sur les problèmes majeurs auxquels ces espèces seront confrontées dans un contexte de changement climatique.

4.4.1.3. Tests comparatifs entre taxons d'espèces d'arbres proches

Les tests comparatifs au moyen d'un dispositif statistique rigoureux permettent d'appréhender la réponse adaptative des espèces aux facteurs environnementaux et de mettre en évidence l'effet des forces de sélection qui ont modelés les patrons de distribution spatiale actuellement observés. Ces tests sont adéquats pour comparer les performances de différentes provenances (Nanson, 2004). Globalement, deux approches sont utilisées afin d'identifier les différences génétiques entre provenances et caractériser les patrons géographiques de variation. La première regroupe des études expérimentales de courte durée en condition contrôlée où les graines collectées de différentes provenances sont semées dans le but d'obtenir des plantules sur lesquelles un ensemble de traits fonctionnels sont mesurés. La deuxième prend en compte les études incluant des graines de différentes provenances qui sont utilisées dans une ou plusieurs expérimentation(s) *in situ* où les traits des individus issus de la germination des graines sont mesurés sur une durée plus ou moins longue (Adam et al., 2007). Ces tests ont été largement effectués sur plusieurs espèces d'arbres en Europe (Exemple : *Fagus sylvatica*, *Picea abies*, *Abies alba*, *Quercus* spp. (Ohlemüller et al., 2006, Geßler et al., 2007)), en Amérique (Exemple : *Tsuga heterophylla*, *Pseudotsuga mensiesii*, *Pinus taeda* (Adam et al., 2007), Australie (Exemple : *Eucalyptus camaldulensis* ; Adam et al., 2007) et en Afrique (Exemple : *Acacia nilotica*, *Acacia tortilis* (Raebild, 2003), *Lophira alata* (Biwolé et al., 2015)) etc.

Outre les tests de provenance, il y a également les expérimentations de transplantations réciproques qui consistent à transplanter chaque population à tester dans tous les environnements dont sont issues ces populations. De telles

expérimentations offrent également la possibilité de tester l'adaptation locale d'une population, en comparant ses performances dans son environnement d'origine par rapport à la performance des populations issues d'environnements différents. Ici, l'adaptation locale est prouvée lorsque les performances en termes de valeur sélective d'une population dans son environnement d'origine sont meilleures que celles de n'importe quelle autre population transplantée dans ce même environnement (Kawecki et Ebert, 2004). Il existe dans la littérature quelques exemples de transplantations réciproques au sein des espèces d'arbre en Afrique (Cordeiro et al., 2009), Amérique du Sud (Fine et al., 2004) et en Europe (Dent et Burslem, 2009).

4.4.2. Méthodes statistiques de mise en relation de la phylogénie, des traits fonctionnels et de la niche écologique

Les traits fonctionnels regroupent les caractéristiques morphologiques, physiologiques, anatomiques ou phénologiques d'un organisme mesurés à l'échelle des individus et qui affectent leurs performances individuelles (Violle, 2007). Ils permettent de comprendre l'efficacité avec laquelle les organismes réalisent leurs différentes fonctions et stratégies d'allocation des ressources. Chez les plantes, plusieurs traits fonctionnels peuvent être mesurés et répertoriés (Perez-Harguindeguy et al., 2013).

La niche écologique peut être définie comme la position occupée par une espèce dans un écosystème. Il est crucial de distinguer la niche fondamentale de la niche réalisée. La niche fondamentale est l'ensemble des conditions environnementales permettant à une espèce de se reproduire en l'absence d'interactions biotiques. Contrairement à cette dernière, la niche réalisée intègre l'effet des interactions biotiques ainsi que des capacités de dispersion. Selon Naimi et Araùjo (2016), il existe plusieurs algorithmes de modélisation de niche réalisée qui utilisent les données environnementales des aires où l'organisme considéré est rencontré : (i) Generalized Linear Model (GLM ; McCullagh and Nelder, 1989), (ii) Generalized Boosted Regression Modeling (GBM ; Elith et Leathwick, 2016), (iii) Mixture Discriminant Analysis (MDA = FDA ; Thuiller, 2003), (iv) Random forests (RF ; Breiman, 2001), (v) MaxEnt (Phillips et al., 2006). Selon Reiss et al. (2011), ces différentes méthodes permettent d'estimer la distribution potentielle des organismes. Toutefois, s'ils fonctionnent bien pour les taxons pris isolément, ils n'offrent pas de possibilité de comparaison statistique multi-espèce de la niche réalisée. Afin de combler ce vide, une approche statistique multivariée a été développée par Broennimann et al. (2012), offre une multitude de possibilités pour tester de nombreuses hypothèses en écologie évolutive.

Dans une approche comparative, au niveau des taxons proches, lorsque les informations de traits fonctionnels, de niche écologique et de relations de parenté sont disponibles, il existe plusieurs méthodes pour apprécier les liens existants entre ces trois principales informations afin d'étudier l'évolution corrélée des traits (qu'ils soient fonctionnels ou écologiques) qui peut ou non mettre en évidence des phénomènes d'adaptation. Dans un premier temps, il existe des méthodes qui permettent de traiter les données de traits du point de vue phylogénétique. Ces méthodes utilisent plusieurs statistiques (Pagel, 1999 ; Blomberg, 2003 ; Verdù,

2006) qui permettent de détecter l'existence d'un signal phylogénétique (tendance pour les taxons proches phylogénétiquement d'avoir des caractères proches, (Losos, 2008)). Cette même relation peut être également mise en évidence via un test de Mantel (Legendre, 2000) qui établit une relation entre la matrice de distance phylogénétique et celle de traits ou même la relation entre les traits fonctionnels et traits de la niche écologique. Dans le dernier cas, il existe un risque d'établir une relation entre traits fonctionnels et niche écologique qui ne résulte pas d'une adaptation mais du fait que les espèces n'aient pas toutes évolué indépendamment (inertie phylogénétique).

Deux types de méthodes permettent rigoureusement de mettre en évidence les processus d'adaptation au sein d'un ensemble d'espèces proches en testant si des traits fonctionnels de ces espèces sont associés à des conditions environnementales particulières, tout en tenant compte des relations phylogénétiques entre espèces. Il y a les méthodes basées sur les modèles évolutifs tels que: la méthode des contrastes phylogénétiques indépendants (PIC ; Felsenstein, 1985) ; la régression phylogénétique des moindres carrés généralisés (PGLS ; Martins, 1994) et le modèle phylogénétique mixte (PMM ; Housworth et al., 2004). Il y a aussi des méthodes à base purement statistique à savoir : la méthode autorégressive (ARM ; Cheverud et al., 1985) et la régression sur les vecteurs propres phylogénétiques (PVR ; Diniz-Filho et al., 1998). La méthode la plus utilisée aujourd'hui est celle basée sur les contrastes phylogénétiques indépendants (PIC) basée sur une matrice de variance-covariance. Toutefois, l'utilisation de celle-ci suppose que les longueurs des branches au niveau de la phylogénie des taxons étudiés soient connues et que les traits étudiés qui ne seraient pas sélectionnés suivent un modèle d'évolution de type Brownien (aléatoire) permettant de tester si les associations entre traits (par exemple niche écologique et traits fonctionnels) le long de la phylogénie sont le fait de la dérive génétique ou de la sélection naturelle. La méthode PIC nécessite donc une phylogénie bien résolue et idéalement des traits quantitatifs. Cette approche qui est une alternative aux tests expérimentaux étudiant l'adaptation reste très peu utilisée au niveau des espèces d'arbres tropicaux (Yang et al., 2014 ; Grotkopp et Rejmánek, 2007 ; Poorter et al., 2010) et n'a quasi jamais été testée sur les arbres tropicaux d'Afrique.

4.4.3. Nécessité d'une approche pluridisciplinaire dans la compréhension des mécanismes d'adaptation au sein des complexes d'espèces d'arbres tropicaux

La pluridisciplinarité suggère une combinaison de disciplines pour aborder un sujet d'étude (Sanson, 2006). Même si les écologues sont conscients de l'importance des processus historiques (l'évolution), ils ont souvent tendance à expliquer la variabilité observée par des effets environnementaux qui peuvent être d'ordre biotique ou abiotique. Depuis les cinquante dernières années, une nouvelle discipline reliant écologie et évolution est née : l'écologie évolutive « *evolutionary ecology* ». Cette discipline intègre à la fois les influences historiques et contemporaines sur les patrons de variabilité observés. Elle étudie cette variation à différentes échelles (de l'individu jusqu'aux communautés) ou niveaux taxonomiques (Pianka, 2011). L'écologie évolutive incorpore donc directement les résultats issus des travaux sur la

génétique (phylogénie, phyloéographie, génétique de populations, etc.) et l'adaptation. Selon Cox et al. (2016), elle permet de comprendre les phénomènes biogéographiques passés grâce à la combinaison de plusieurs disciplines scientifiques à savoir : évolution, taxonomie, écologie, géologie, paléontologie, climatologie, etc.

De nos jours, l'écologie évolutive profite de l'utilisation des avancées fulgurantes en génétique moléculaire, informatique et modélisation afin de tester les hypothèses en lien avec l'adaptation tout en prenant en compte la dimension des contingences historiques. Malheureusement, très peu de travaux de recherche sur les forêts tropicales adoptent cette approche afin d'expliquer les mécanismes d'adaptation au sein des complexes d'espèces d'arbres tropicaux. Le présent travail de doctorat s'inscrit dans cette dynamique afin de mieux comprendre le passé, l'évolution historique ainsi que les moteurs qui gouvernent la diversité des taxons ligneux des forêts tropicales d'Afrique. Cette compréhension est essentielle car elle constitue un moyen efficace pour prédire la dynamique future de ces écosytèmes et proposer des modalités de gestion adaptées.

5. Objectifs, questions et structuration de la thèse

5.1. Objectifs

L'évolution et la diversification des espèces d'arbres tropicaux sont souvent interprétées comme la résultante des changements environnementaux passés et surtout climatiques (Linder, 2014). En phylogéographie, les marqueurs génomiques neutres ont généralement mis en exergue des pools génétiques témoignant de l'impact des changements climatiques majeurs, notamment les périodes glaciaires : la diversification découlerait surtout dans ce cas de l'isolement des populations et de la dérive génétique (Hardy et al., 2013). Le recours aux marqueurs génomiques neutres, quoique fondamental dans l'analyse des processus d'évolution, de différenciation des populations et de diversification, ne rend toutefois pas pleinement compte de la complexité des mécanismes à l'origine de la diversité intra et interspécifique (Avise et al., 1987). Les organismes vivants, y compris les arbres, doivent s'adapter à des environnements changeants. Les processus de sélection agissant sur des populations établies dans différents environnements peuvent ainsi être moteurs de la diversification (McKenna et Farrell, 2006). Cette sélection naturelle peut même être observée dans des groupes en sympatrie, causant dans des cas extrêmes une spéciation dite sympatrique (Bolnick et Fitzpatrick, 2007).

En dehors des espèces modèles ayant fait l'objet de séquençage de leur génome entier, il est très difficile d'utiliser des méthodes moléculaires pour évaluer l'impact de la sélection sur la diversité des organismes vivants : des « *outlier loci* » ou des gènes codants devraient être disponibles et associés aux traits identifiés comme étant sous sélection (Narum et Hess, 2011). La comparaison de paramètres ou traits fonctionnels (morphologiques, physiologiques ou anatomiques) et des niches écologiques, en tenant compte de la parenté (relation phylogénétique) entre taxons,

est une approche en plein essor pour évaluer l'influence de l'environnement dans l'adaptation et la diversification des espèces (Eiserhardt et al., 2017).

Le présent travail de doctorat utilise le genre *Guibourtia* Benn. comme modèle biologique (Leguminosae-Detarioideae) afin de comprendre des mécanismes historiques, biologiques et environnementaux, à l'origine de la diversité des écosystèmes forestiers tropicaux. Ce modèle biologique est idéal pour atteindre cet objectif global parce qu'il comprend 13 espèces d'arbres proches, distribués au sein des biomes tropicaux d'Afrique, à caractéristiques écologiques plus ou moins contrastées. Six sont rencontrées en forêt sèche : *G. carrissoana* (M.A.Exell) J.Leonard, *G. coleosperma* (Benth.) Leonard, *G. conjugata* (Bolle) J.Leonard, *G. copallifera* Benn., *G. schliebenii* (Harms) J.Leonard, *G. sousae* J.Leonard et sept en forêts denses humides : *G. arnoldiana* (De Wild. & T. Durand) J.Leonard, *G. demeusei* (Harms) J.Leonard, *G. dinklagei* (Harms) J.Leonard, *G. ehie* (A.Chev.) J.Leonard, *G. leonensis* J.Leonard, *G. pellegriniana* Leonard, et *G. tessmannii* (Harms) J.Leonard. Ce genre regroupant des espèces occupant des milieux contrastés, avec certaines distribuées en parapatrie ou en sympatrie, est intéressant pour tester l'effet des variations environnementales sur la diversité des espèces d'arbres tropicaux. En outre, la problématique soulevée par la gestion de ce genre est diverse : (1) certaines de ces espèces subissent des menaces locales importantes (exploitation illégale du bois) ; (2) les densités de population de certaines espèces de forêts denses humides d'Afrique Centrale sont souvent très faibles (0.02 ind/ha - 0,04 ind/ha [dbh $\geq$ 20 cm] pour *G. tessmannii* et *G. pellegriniana*; Doucet, 2003); (3) certaines espèces ont une aire de répartition extrêmement réduite ; (4) les informations sur leurs capacités de régénération font défaut. Enfin, il est difficile pour les exploitants industriels de différencier certaines espèces distribuées en parapatrie ou sympatrie, et très similaires morphologiquement, ce qui rend les falsifications assez fréquentes. Ces raisons ont motivé l'inscription de trois espèces du genre sur l'annexe II de la CITES (espèces menacées d'extinction ; (Koumba Pambo et al., 2016) : *G. tessmannii*, *G. pellegriniana* et *G. demeusei*.

Ce travail de doctorat vise par une approche d'écologie évolutive, à comprendre les mécanismes intervenant dans la spéciation des taxons ligneux des forêts et savannes africains. Il ambitionne plus particulièrement les objectifs suivants: (1) au niveau interspécifique, à étudier le rôle relatif des forces évolutives neutres et de sélection dans la diversification des espèces de *Guibourtia*; et (2) au niveau intraspécifique, à questionner les causes de la différenciation des populations de quelques espèces de *Guibourtia*.

5.2. Questions de recherche

Elles sont présentées en fonction des objectifs susmentionnés :

Objectif 1

1. Quelle est la fiabilité de la délimitation des espèces au sein du genre *Guibourtia* d'après les données de la littérature ?
2. Quelles sont les relations phylogénétiques entre espèces et le timing de leur diversification ?

3. Y a-t-il des convergences évolutives dans les traits morphologiques distinguant les espèces qui s'expliquent par leurs niches écologiques ?
4. Les plantules des espèces montrent-elles des comportements écophysiologiques différenciés dans un gradient lumineux qui résulterait d'adaptations à leurs habitats naturels ?

Objectif 2

5. Quel est le patron de différenciation génétique pour des marqueurs microsatellites entre populations d'espèces de *Guibourtia* et quelles en sont les causes ?
6. L'identification des animaux impliqués dans la dispersion des graines d'espèces zoochores du genre *Guibourtia* suggère-t-il des flux de gènes importants?

5.3. Structuration de la thèse

Ce travail de doctorat débute par la présente introduction générale. Ensuite, il est organisé en deux parties, chacune d'elle est subdivisée en quatres chapitres. Chaque chapitre est rédigé sous forme d'article publié, soumis ou en préparation. Les différentes parties se déclinent comme suit :

Partie 1. Diversification des espèces au sein du genre *Guibourtia*

Chapitre 1. Le genre *Guibourtia* Benn., un taxon à haute valeur commerciale et sociétale (synthèse bibliographique) – publié dans *Biotechnologie, agronomie, société et environnement*

Le chapitre 1 répond à la première question. Il propose une synthèse de l'ensemble des connaissances existantes sur le genre *Guibourtia* : histoire taxonomique, description botanique, écologie, statut de conservation. Il souligne en outre le manque d'informations écologiques et génétiques précises sur les différentes espèces du genre, ce qui ne permet pas de conclure sur les statuts de conservation. Ce chapitre démontre la difficulté de différencier certaines espèces morphologiquement proches. Il conclut sur l'importance d'utiliser la génétique moléculaire pour clarifier les positions taxonomiques des espèces au sein du genre.

Chapitre 2. Evolution in the Amphi-Atlantic genus *Guibourtia* (Fabaceae, Detarioideae), combining NGS phylogeny and morphology – publié dans *Molecular Phylogenetics and Evolution*

Le chapitre 2 répond à la question 2 . Ici, une phylogénie à haute résolution a été réalisée en utilisant une approche nécessitant le séquençage massif (NGS) des génomes chloroplastiques. La monophylie de la plupart des espèces du genre *Guibourtia* appuie la taxonomie actuelle. L'arbre phylogénétique montre une diversification durant le Miocène qui a conduit à trois clades correspondant à des entités morphologiques distinctes, précédemment décrites comme des sous-genres. Un évènement de migration d'Afrique à l'Amérique est également mis en évidence à la fin du Miocène.

Chapitre 3. Phylogenetic patterns of diversification across ecological niches in the African tree genus *Guibourtia* support convergent evolution of morphological traits along a climatic gradient

Le chapitre 3 répond à la questions 3. Ce chapitre analyse les traits morphologiques et les niches environnementales des espèces africaines de *Guibourtia*. Il démontre un signal phylogénétique tant dans les traits morphologiques que les niches climatiques des espèces. Outre l'inertie phylogénétique, il met en évidence une évolution convergente de certains traits morphologiques en réponse aux gradients environnementaux (forêts humides - forêts sèches/savanes). Ici, les résultats soulignent clairement l'effet de la sélection naturelle montrant que certains caractères morphologiques ont été sélectionnés dans la même direction au sein de différentes lignées.

Chapitre 4. When ecophysiology reveals the adaptive capacities within African tropical congeneric tree seedlings: case study of genus *Guibourtia* Benn.

Le chapitre 4 répond à la question 4 au moyen d'une approche expérimentale. En suivant la croissances et les paramètres écophysiologiques de plantules de trois espèces cultivées sous différentes intensités lumineuses, ce chapitre montre les différences de réponses fonctionnelles entre espèces de forêt dense humide et de forêt sèche. Ici, l'hypothèse selon laquelle les espèces de forêts denses humides ont une plasticité phénotypique plus élevée dans les faibles niveaux de lumière, comparativement aux espèces de forêts sèches, est confirmée. Les résultats de ce chapitre renforcent l'idée selon laquelle la sélection naturelle a joué un rôle majeur dans la diversification des espèces du genre *Guibourtia*.

Partie 2. Causes de la différenciation génétique des populations de *Guibourtia*

Chapitre 5. Microsatellite development for the genus *Guibourtia* (Fabaceae, Caesalpinioideae) reveals diploid and polyploid species – publié dans *Applications in Plant Sciences*

Le chapitre 5 développe des outils nécessaires pour répondre à la question 5. Il présente les marqueurs moléculaires microsatellites polymorphes développés à partir de *G. tessmannii*. Un test de transférabilité a été effectué avec succès uniquement sur les espèces appartenant à la même lignée que *G. tessmannii* : *G. pellegriniana*, *G. leonensis* et *G. coleosperma*. Les profils des microsatellites montrent que le genre *Guibourtia* contient des espèces diploïdes et polyploïdes, ce qui fut confirmé par une étude de cytométrie en flux.

Chapitre 6. Characterization of 19 microsatellites markers in the African tropical tree species *Guibourtia ehie* (Fabaceae, Detarioideae) – Publié dans *Applications in Plant Sciences*

Dans le chapitre 6, à la suite du chapitre précédent, il a été procédé au développement d'un second jeu de 19 marqueurs moléculaires microsatellites polymorphes, à partir d'une autre espèce du genre, *Guibourtia ehie*. Le test de transférabilité a été positif qu'avec les espèces de la même section que *G. ehie* : *G. arnoldiana*, *G. schliebenii*, *G. conjugata*, *G. dinklagei*. Les résultats de ce chapitre combinés avec ceux du chapitre 5 confirment la divergence génétique profonde entre espèces de différents sous-genres.

Chapitre 7. Phylogeographic pattern in two congeneric African tree species found in contrasting environments : *Guibourtia ehie* (A. Chev.) J. Léonard and *Guibourtia coleosperma* (Benth.) J. Léonard

Le chapitre 7 utilise une espèce de forêt sèche et une de forêt dense humide pour répondre à la question 5. Chez chaque espèce on observe une discontinuité nette de la variation génétique coincidant avec une barrière biogéographique (intervalle du Dahomey) chez l'espèce de forêt dense humide (*G. ehie*) et un gradient climatique chez l'espèce de forêt sèche (*G. coleosperma*).

Chapitre 8. Characterization of animal communities involved in seed dispersal and predation of *Guibourtia tessmannii* (Harms) J.Léonard, a species newly listed on Appendix II of CITES – article publié dans *African Journal of Ecology*

Dans ce dernier chapitre, la réponse à la question 6 a été explicitée en utilisant *Guibourtia tessmannii*, une espèce représentative des espèces zoochores du sous-genre *Pseudocopaiva*. Au moyen d'observations directes et indirectes, les animaux impliqués dans la dispersion des graines de *G. tessmannii* ont été identifiés et incluent notamment des calaos qui pourraient contribuer aux flux de gènes à longue distance.

Enfin, la présente thèse se termine par une discussion générale qui fait la synthèse de l'ensemble des résultats obtenus et discute des implications pour la gestion durable et la conservation des espèces de *Guibourtia*. Elle met aussi en exergue les potentiels axes d'investigations pour les recherches futures.

Partie 1

Diversification des espèces au sein du genre *Guibourtia*

Chapitre 1

Le genre *Guibourtia* Benn., un taxon à haute valeur commerciale et sociétale (synthèse bibliographique)

Au cours d'un séjour au British Museum Natural History, aux Royal Botanic Gardens de Kew ainsi qu'à l'Imperial Forestry Institute de l'Université d'Oxford, le botaniste belge Jean Joseph Gustave Léonard (1920 - 2013) a eu l'occasion d'examiner les collections d'un genre taxonomique tout particulier qui marquera sa carrière : *Guibourtia* Benn. Les découvertes interessantes qu'il fit ont captivé notre attention. Le présent chapitre fait le point sur ses découvertes et sur l'ensemble des connaissances sur le genre *Guibourtia*. Il est adapté de :

Tosso, F., Daïnou, K., Hardy, O. J., Sinsin, B., & Doucet, J. L. (2015). Le genre *Guibourtia* Benn., un taxon à haute valeur commerciale et sociétale (synthèse bibliographique). Biotechnologie, agronomie, société et environnement, 19(1), 71-88.

Depuis la publication de cet article, le genre *Guibourtia* a été reclassé dans la sous-famille des Detarioideae, famille des Fabaceae, suite aux travaux de LPWG (2017).

1. Le genre *Guibourtia* Benn., un taxon à haute valeur commerciale et sociétale (synthèse bibliographique)

Félicien Tosso, Kasso Daïnou, Olivier J. Hardy, Brice Sinsin et Jean-Louis Doucet

Résumé

Introduction. Connu comme étant un genre de grande importance socio-culturelle et économique, *Guibourtia* Benn. regroupe des espèces sœurs multi-usages à forte ressemblance morphologique inféodées à des climats et sols variés. Les densités des populations de ces espèces sont faibles, et il urge qu'une attention particulière leur soit portée en raison de surexploitations locales.

Littérature. Cet article basé sur une ample revue bibliographique, résume les informations disponibles sur les espèces du genre *Guibourtia* notamment en botanique, écologie, génétique, sylviculture et ethnobotanique. Il démontre (i) le caractère lacunaire des connaissances écologiques et sylvicoles sur le genre et (ii) la difficulté de différencier des espèces sœurs morphologiquement semblables. En plus de la synthèse bibliographique, une nouvelle clé de détermination des espèces du genre *Guibourtia* a été proposée.

Conclusion. Considérant le manque actuel d'informations, il est impossible de conclure sur le statut de conservation des différentes espèces et de proposer des mesures de gestion adaptées. Par ailleurs, la diversité spécifique au sein de ce genre ainsi que sa distribution au sein des biomes tropicaux en font un excellent modèle biologique permettant de comprendre les mécanismes historiques, biologiques et environnementaux, à l'origine de la diversité des écosystèmes forestiers tropicaux.

Mots-clés. *Guibourtia*, autoécologie, taxonomie, ethnobotanique, dynamique des populations, conservation, exploitation forestière, génétique, Afrique.

1.1. Introduction

De la famille des Fabaceae (sous-famille des Caesalpinioideae), le genre *Guibourtia* Benn. compte 13 espèces en Afrique dont certaines à forte valeur culturelle (arbre sacré chez les Pygmées par exemple) et commerciale (Benoit, 2011). Reconnus pour la qualité exceptionnelle de leur bois, les taxons d'Afrique Centrale font l'objet d'un important commerce (ATIBT, 2010). Actuellement, il est toutefois difficile pour les exploitants forestiers de différencier certaines espèces, très similaires morphologiquement et dont les aires de distribution se chevauchent, ce qui facilite le commerce illégal (Betti, 2012). Le problème est en outre exacerbé par une importante demande des pays asiatiques, des densités très faibles et des aires de distribution souvent réduites. Actuellement, le manque de données sur l'écologie des espèces de *Guibourtia* semble être un handicap majeur à la définition de stratégies de gestion des populations et d'identification de statuts de conservation adéquats (*IUCN Red List*).

Le présent article dresse une synthèse des connaissances scientifiques actuelles sur le genre *Guibourtia* et met en exergue les aspects méritant davantage d'investigations scientifiques. Les recherches ont été menées en utilisant (i) la base de données Scopus, (ii) Google scholar et (iii) les ressources documentaires disponibles dans les bibliothèques de Gembloux Agro Bio-Tech (Université de Liège) et du Jardin botanique national de Belgique. Les mots clés suivants en français – et leur équivalent en anglais – ont été utilisés : *Guibourtia*, bubinga, taxonomie, écologie, ethnobotanique, exploitation forestière, génétique, technologie du bois, commerce.

1.2. Origine et taxonomie du genre *Guibourtia*

1.2.1. Histoire taxonomique

L'histoire du genre *Guibourtia* remonte à 1762 quand Linné décrit le genre *Copaifera* et désigne comme espèce-type *Copaifera officinalis* L. originaire du Brésil et à feuilles multifoliolées. En 1833, Moricand décrit *Copaifera hymenaefolia* Moric., un *Copaifera* américain à feuilles bifoliolées. En 1857, un nouveau genre, *Guibourtia*, est décrit en Afrique par John Joseph Bennett qui y rattache *Copaifera copallina* Baill. originaire de Sierra Leone et dont il fait l'espèce-type *Guibourtia copallifera* Benn. Le nom *Guibourtia* donné à ce genre est inspiré du nom du pharmacien français Nicolas Jean-Baptiste Gaston Guibourt (1790-1867) qui a mené des recherches sur le copal, une substance résineuse produite par certaines espèces de *Guibourtia*, utilisée en médecine et dans l'art (Glen, 2004). La légitimité taxonomique de ce nouveau genre fut contestée par Bentham (1865) dans une étude sur les légumineuses. Il estimait que la taille, la persistance des bractéoles et le nombre de folioles n'étaient pas suffisamment pertinents pour autoriser une subdivision du genre. Ainsi, Bentham (1865) et Kuntze (1891) élargirent le genre *Copaifera* en y incluant les espèces bifoliolées africaines. Par la suite, plusieurs espèces bifoliolées furent décrites tant en Amérique qu'en Afrique.

Plus d'un demi-siècle après, toute la nomenclature des *Guibourtia* et *Copaifera* fut revisitée par Léonard. En 1949, cet auteur mena une étude taxonomique approfondie des espèces africaines du genre *Copaifera* et décida de restaurer le genre *Guibourtia*. Il montra qu'aux caractères, aisément observables relatifs au nombre et à la forme des folioles, s'ajoutent d'autres, non signalés par les travaux précédents, beaucoup plus constants et de valeurs taxonomiques manifestes, notamment la préfloraison du calice, la disposition des fleurs et l'anatomie du bois. En effet, il montra clairement que la préfloraison du calice est nettement imbriquée chez les espèces uni- et bifoliolées africaines alors qu'elle est subvalvaire chez les espèces multifoliolées (Léonard, 1949). Quant à la disposition des fleurs le long de l'axe floral, elle consiste en deux rangs opposés avec de très jeunes inflorescences comprimées chez *Copaifera* et en plus de deux rangs avec de très jeunes inflorescences strobiliformes cylindriques chez *Guibourtia* (figure 1.1). Le tableau 1 présente la liste des noms actuels validés et/ou reclassés des espèces du genre *Guibourtia* présentes en Afrique tropicale.

En se basant sur les travaux de Normand (1948), Léonard (1949) nota que les *Copaifera* ont un bois avec les canaux sécréteurs verticaux disposés en zones plus ou moins concentriques tandis que celui des *Guibourtia* en est dépourvu. Enfin, la répartition géographique des espèces fournit à Léonard (1949) quelques informations utiles. Du point de vue chorologique, il définit *Copaifera* comme étant un genre tropical surtout américain regroupant 35 espèces américaines et quatre espèces africaines, et *Guibourtia* comme un genre tropical principalement africain avec 13 espèces africaines et quatre espèces américaines.

Par la suite, Léonard (1950) proposa de regrouper également sous *Guibourtia* les espèces des genres *Gorskia* (Bolle) et *Pseudocopaiva* (Britton & Wilson) qui ont été abaissés au même titre que *Guibourtia* au rang de sous genre, le tout chapeauté par le genre *Guibourtia*. Cette classification en trois sous-genres *Guibourtia, Gorskia* et *Pseudocopaiva* était basée sur des caractères ayant trait aux fruits, graines, plantules et à l'anatomie du bois, mais elle restera peu utilisée (tableau 1.1). Ce dernier travail de révision ne sembla pas satisfaire Dwyer (1951) qui estima que les *Guibourtia* d'origine américaine devaient appartenir au genre *Copaifera*, une hypothèse contredite par Voorhoeve (1965) et Normand et Paquis (1976). Finalement, Van der Maesen et al. (1996) considèrent que la systématique du genre mérite des réflexions complémentaires. Par exemple, ces auteurs ont noté que Léonard souhaitait reclasser certaines espèces – *G. coleosperma* (Benth.) J.Léonard, *G. leonensis* J.Léonard, *G. pellegriniana* J.Léonard et *G. tessmannii* (Harms) J.Léonard – dans le genre *Pseudocopaiva* Britton. Mais l'absence de caractères distinctifs au niveau des fleurs (texture, pubescence et coloration des sépales, longueur du style) et des organes végétatifs (nombre et forme des folioles) l'ont fait hésiter et renoncer finalement à cette réorganisation.

Tableau 1.1. Liste des espèces du genre *Guibourtia* présentes en Afrique - List of species of the genus *Guibourtia* found in Africa

Sous-genre selon Léonard (1950)	Dénominations actuelles acceptées des espèces	Synonymies selon Léonard (1949) et « The Plant List (2013) » (www.theplantlist.org)	Pays de collecte du spécimen-type	Phytochories selon White (1983)
Guibourtia	*Guibourtia copallifera* Benn. (1857)	*Copaifera guibourtiana* Benth. (1865) *Copaifera copallina* Baill. (1870) *Copaiba copallifera* Kuntze (1891) *Copaiva guibourtiana* (Benth.) A.Lyons (1907) *Copaifera vuilletiana* A.Chev. (1917) *Copaifera vuilletii* A.Chev. (1920) *Copaifera copallifera* (Benn.) Milne-Redh. (1934)	Sierra Leone	I, III, XI
	Guibourtia carrissoana (M.A.Exell) J.Leonard (1949)	*Copaifera carrissoana* M.A.Exell ex Gossw. & Mendonça (1939) *Copaifera gossweileri* M.A.Exell ex Gossw. & Mendonça (1939)	Angola (Luanda)	II
	Guibourtia demeusei (Harms) J.Leonard (1949)	*Copaifera demeusei* Harms (1897) *Copaifera laurentii* De Wild. (1907)	RDC (Lac Léopold II)	I
	Guibourtia sousae J.Leonard (1950)	-	Mozambique (Maueele)	XIII
Gorskia (Bolle)	*Guibourtia dinklagei* (Harms) J.Leonard (1949)	*Copaifera dinklagei* Harms (1899) *Guibourtia liberiensis* J.Leonard (1950)	Liberia (Grand Bassa)	I
	Guibourtia conjugata (Bolle) J.Leonard (1949)	*Gorskia conjugata Bolle* (1862) *Copaifera gorskiana* Benth. (1865) *Copaifera gorskia* Schinz (1889) *Copaiba conjugata* Kuntze (1891) *Copaifera conjugata* (Bolle) Milne-Redh. (1934)	Mozambique (environs de Sena et Tette)	II, XIII
	Guibourtia arnoldiana (De Wild. & T.Durand) J.Leonard (1949)	*Copaiba arnoldiana* De Wild. & T.Durand (1900) *Copaifera arnoldiana* Th. & H.Dur. (1909) *Copaifera ehie* A.Chev. (1917)	RDC (Mayumbe)	I
	Guibourtia schliebenii (Harms) J.Leonard (1949)	*Copaifera schliebenii* Harms (1936)	RDC (Tanganyika)	II, XIII
	Guibourtia ehie (A.Chev.) J.Leonard (1949)	*Copaifera ehie* A.Chev. (1917)	Côte d'Ivoire (Bangouanou)	I
Pseudocopaiva (Britton & Wilson)	*Guibourtia coleosperma* (Benth.) Leonard (1949)	*Copaifera coleosperma* Benth. (1865) *Copaiba coleosperma* Kuntze (1891) *Copaiva coleosperma* Britton (1930)	Rhodésie du Nord (Batoka. Highlands)	II
	Guibourtia leonensis J.Leonard (1950)	-	Libéria	I
	Guibourtia tessmannii (Harms) J.Leonard (1949)	*Copaifera tessmannii* Harms (1910)	Guinée équatoriale	I
	Guibourtia pellegriniana Leonard (1949)	*Copaifera coleosperma* Benth. (1865)	Gabon (Mayumbe)	I

I: centre d'endémisme guinéo-congolais; II: centre régional d'endémisme zambézien; III: centre régional d'endémisme soudanien; XI: Zone de transition régionale guinéo-congolaise/soudanienne; XIII: Mosaïque régionale de Zanzibar-Inhambane – I : guineo-congolian regional centre of endemism ; II : zambezian regional centre of endemism ; III : soudanian regional centre of endemism ; XI : guineo-congolian/soudanian transition zone ; XIII : zanzibar-Inhambane Regional Mosaic

Figure 1.1. Morphologie de l'inflorescence des genres *Guibourtia* et *Copaifera* (Adaptée de Léonard , 1949). a) *G. demeusei*; b) *Copaifera salikounda* Heckel; c) *Copaifera mildbraedii* Harms - Inflorescence morphology of the genus *Guibourtia* and *Copaifera* (Adapted from Leonard ,1949) *G. demeusei*; b) *Copaifera salikounda* Heckel; c) *Copaifera mildbraedii* Harms

1.2.2. Les nouveaux apports de la génétique

Selon Mangenot et Mangenot (1957), le genre *Guibourtia* est diploïde avec 2n = 24 chromosomes. Il est phylogénétiquement proche des genres *Hymenaea* et *Peltogyne*, qui ensemble forment un clade bien résolu (Fougere-Danezan, 2006). Leur ancêtre commun serait le genre purement africain *Daniellia* Benn. (Fougere-Danezan et al. 2003). *Guibourtia* daterait d'environ 20 Ma tandis que *Hymenaea*, *Peltogyne* et *Daniellia* dateraient d'environ 25 MA, 33 MA et 40 MA respectivement (Fougere-Danezan, 2006). Les travaux de Fougere-Danezan (2006) montrent également que le genre n'est monophylétique que si l'espèce *G. ehie* en est exclue. Cependant, l'auteur insiste sur la nécessité d'études phylogénétiques complémentaires. En dépit du fait qu'il ait eu recours à plusieurs séquences chloroplastiques et nucléaires des régions *trnL*, *trnF* et *ITS*, la phylogénie mériterait d'être vérifiée en utilisant les séquences des régions *trnC* et *psbA*, également recommandées en « barcoding » (Gonzalez et al. 2009 ; Tripathi et al. 2013).

Finalement, *Guibourtia hymenaeifolia* serait la seule espèce américaine (Fougere-Danezan, 2006) et serait une espèce sœur des *Guibourtia* africains (Crawford, communication personnelle). La séparation Afrique / Amérique au sein du genre daterait de 12 MA environ (Crawford, communication personnelle). Elle pourrait être issue d'événements de dispersion sur une longue distance par les courants marins puisqu'elle est nettement postérieure à la fragmentation du Gondwana qui date de plus de 100 Ma (Thorne, 1973). Des études biogéographiques menées par Fougere-Danezan (2006) semblent confirmer cette hypothèse démontrant que les fruits ou graines de certaines des espèces de *Guibourtia* ont la capacité de flotter ou peuvent être associés à des ensembles flottants. En conclusion, le genre serait bien d'origine africaine (Léonard, 1949; Fougere-Danezan, 2006 ; Ulibarri, 2008) où son aire de répartition s'étendrait sur 22 pays, du Sénégal jusqu'en Mozambique (figure 1.2).

Selon la classification phytogéographique de White (1983), le genre *Guibourtia* s'étend de la zone soudanienne à la Mosaïque régionale de Zanzibar-Inhambane (tableau 1.1)

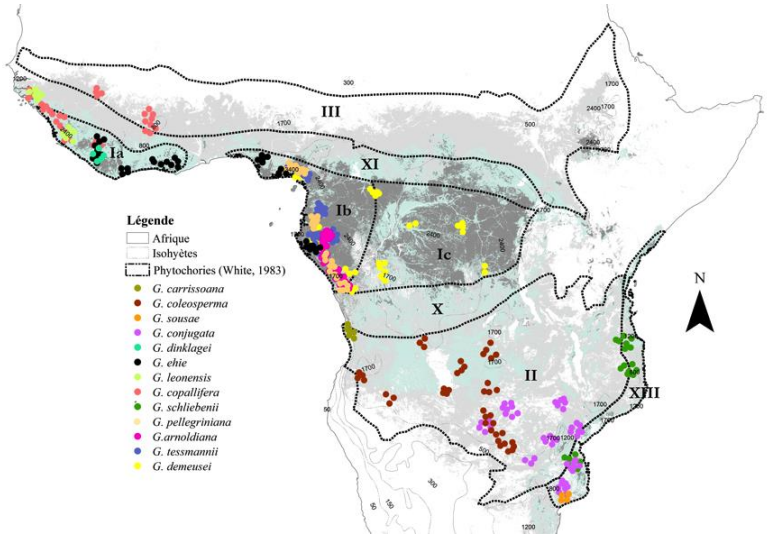

Figure 1.2. Carte de répartition des espèces du genre *Guibourtia* (adaptée d'après la base de données du Conservatoire des Jardins Botaniques de Genève (CJBG), consulté le 09/12/2013 et la carte de Mayaux et al. (2004)) - *Distribution range of Guibourtia species (map adapted from the database of the Conservatory Botanical Gardens of Geneva (CJBG), accessed 09/12 / 2013, and Mayaux et al. (2004))*

1.3. Description botanique des espèces africaines

Les espèces africaines du genre *Guibourtia* sont des arbres ou des arbustes avec un bois dépourvu de canaux sécréteurs (Léonard, 1949). Les feuilles sont alternes, généralement composées de deux folioles falciformes opposées, acuminées, entières, souvent parsemées de ponctuations translucides, à stipules petites et stipelles nulles (Léonard, 1949 ; 1950). Les inflorescences sont des panicules. Les fleurs hermaphrodites ont un réceptacle discifère sans pétales, à quatre sépales inégaux, à préfloraison nettement imbriquée avec des étamines libres dont le nombre varie de huit à dix et de un à deux (rarement quatre) ovules (Watson et Dallwitz, 1993). Quant aux fruits, ils appartiennent à trois types distincts à savoir : (i) indéhiscents, coriaces, à graines sans arille ; (ii) indéhiscents, membraneux, à graines sans arille et (iii) déhiscents, épais, coriaces, à graines avec arille.

Selon Léonard (1950), les espèces de ce genre, quoique bien distinctes pour un œil averti, demeurent morphologiquement très proches. Selon Taylor (1960), ces affinités morphologiques limiteraient considérablement les possibilités de détermination botanique sur base d'échantillons stériles.

1.3.1. Différenciation des espèces du genre Guibourtia

L'**annexe 1** résume les caractéristiques botaniques pouvant servir à distinguer les espèces africaines du genre *Guibourtia*. Cette distinction peut se faire sur base de deux types de traits qualitatifs et quantitatifs.

La clé présentée ci-après permet de différencier les espèces en partant des caractéristiques chorologiques et végétatives. Pour les espèces les plus difficiles à distinguer sur base de ces seuls critères, sont ajoutés les particularités des fleurs et des fruits. La **figure 1.3** présente les folioles, les fleurs et les fruits.

1	a. Espèce guinéo-congolaise et / ou soudanienne (I et / ou XI, figure 2)	2
	b. Espèce zambézienne (II, figure 2)	10
2.	a. Espèce du sous-centre guinéen supérieur et / ou soudanienne (Ia et / ou XI, figure 2)	3
	b. Espèce du sous-centre guinéen inférieur et /ou congolais (Ib et / ou Ic, figure 2)	6
3.	a. Une seule foliole ; 7 paires de nervures secondaires ; rapport L/l du limbe = 1,5	*G. dinklagei*
	b. Deux paires de folioles ; 5 – 12 paires de nervures secondaires ; rapport L/l du limbe compris entre 1 et 2,6	4
4.	a. 8 à 12 paires de nervures secondaires ; rapport L/l du limbe ≈1. Espèce de forêt ou de savane	*G. copallifera*
	b. Moins de 8 paires de nervures secondaires ; rapport L/l du limbe supérieur à 1. Espèce de forêt exclusivement	5
5.	a. 5 paires de nervures secondaires ; pétiole de 1,2 à 3 cm ; 6 étamines ; stipules caduques ; fruit obovale de 2,6-2,8 cm x 1,7-1,8 cm	*G. leonensis*
	b. Autres caractéristiques	8
6.	a. Limbe criblé de points translucides	7
	b. Limbe dépourvu de points translucides	8
7.	a. Espèce endémique du Mayombe ; écorce rougeâtre ; essentiellement sur terrain calcaire. Pétiole de 0,4 – 0,8 cm ; bractéoles caduques ; fruit membraneux ; obovale de 4-5 cm x 2,5-3 cm.	*G. arnoldiana*
	b. Espèce inféodée aux sols hydromorphes ; écorce non rougeâtre. Pétiole de 1,5 – 3 cm ; bractéoles persistantes ; fruit coriace ; orbiculaire de 4-5 x 3-4 cm	*G. demeusii*
8.	a. Pétiole > 1 cm ; stipules très caduques	9
	b. Pétiole < 1 cm ; stipules foliacées persistantes	*G. ehie*
9.	a. Folioles pétiolulées (1-3 mm) ; base externe du limbe arrondie ; axe d'inflorescence grêle ; ovaire stipité et glabre ; gousse déhiscente ellipsoïde de 2-2,5 cm x 1-1,5 cm ; arille rouge. Espèce inféodée aux forêts côtières sempervirentes	*G. pellegriniana*
	b. Folioles sessiles ; base externe du limbe cunéiforme ; axe de l'inflorescence très épais ; ovaire subsessile et hirsute ; gousse déhiscente ellipsoïde de 3-4 cm x 2-2,5 cm ; arille orange-rouge. Espèce non exclusivement inféodée aux forêts côtières	*G. tessmannii*
10.	a. Pétiole < 1 cm ; 6 à 7 paires de nervures secondaires	*G. schliebenii*
	b. Pétiole > 1 cm ; 5 à 12 paires de nervures secondaires	11
11.	a. Rapport L/l du limbe ≈ 1,5. Espèce endémique du Mozambique	G. sousae
	b. Rapport L/l du limbe ≥ 2	12
12.	a. > 7 paires de nervures secondaires ; rapport L/l du limbe ≈ 2,5 ; taille du pétiole comprise entre 1,4 et 3 cm ; gousse orbiculaire déhiscente de 2-3,5 cm x 1,5-2 cm; arille rouge	*G. coleosperma*
	b. ≤ 7 paires de nervures secondaires ; rapport L/l du limbe ≈ 2 ; taille du pétiole comprise entre 1 et 1,8 cm ; gousse indéhiscente ; graine non arillée	13
13.	a. Espèce endémique à l'Angola. Arbuste de 6 m de hauteur maximal. Fruit orbiculaire de 2,5-3 cm x 2 cm.	*G. carrissoana*
	b. Espèce dont l'aire de distribution s'étend de la Zambie au Mozambique. Arbre ou arbuste pouvant atteindre 18 m de hauteur maximale. Fruit ovale-orbiculaire de 3-4 cm x de 2,5 cm	*G. conjugata*

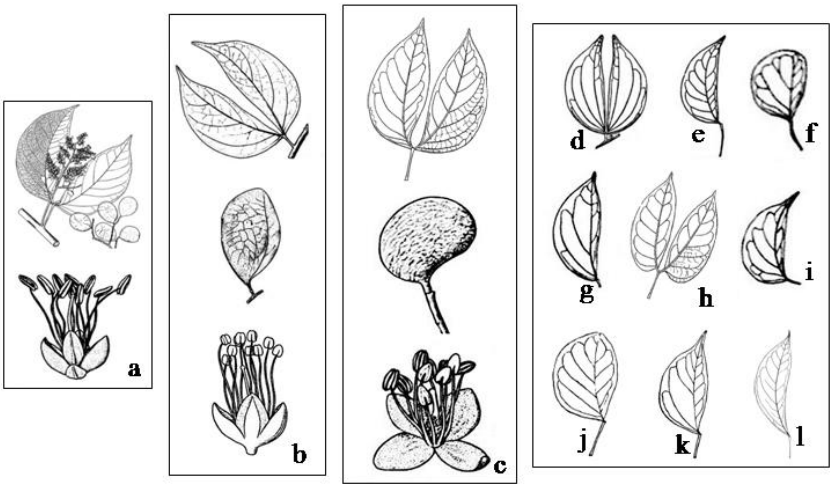

Figure 1.3. Morphologie des folioles, fleurs et graines du genre *Guibourtia* (adaptée de Léonard, 1950, Aubréville, 1968). a) *G. demeusei*; b) *G. arnoldiana*; c) *G. tessmannii*; d) *G. copallifera*; e) *G. coleosperma*; f) *G. conjugata*; g) *G. ehie*; h) *G. pellegriniana*; i) *G. schliebenii*; j) *G. sousae*; k) *G. carrissoana*; l) *G. leonensis* - Morphology of leaflets, flowers and seeds of the genus *Guibourtia* (adapted from Leonard, 1950 Aubréville, 1968) a) *G. demeusei*; b) *G. arnoldiana*; c) *G. tessmannii*; d) *G. copallifera*; e) *G. coleosperma*; f) *G. conjugata*; g) *G. ehie*; h) *G. pellegriniana*; i) *G. schliebenii*; j) *G. sousae*; k) *G. carrissoana*; l) *G. leonensis*

1.3.2. Description des plantules

Chez de nombreux arbres tropicaux, la morphologie foliaire des plantules diffère nettement de celle des adultes, et il importe donc d'y consacrer une attention particulière. Les individus du genre *Guibourtia* sont issus de plantules à germination épigée avec des cotylédons qui s'épanouissent au- dessus du sol et dont les deux premières feuilles sont alternes (Léonard, 1994). Elles se différencient des *Copaifera* par la présence (1) d'une petite pointe entre les deux folioles de la première feuille des plantules, (2) d'une seconde petite pointe en face de ces deux folioles et (3) d'un bourgeon à l'aisselle de la première feuille (figure 1.4). Une telle structure commune à toutes les espèces des sous-genres décrits par Léonard (1957) n'a été rencontrée jusqu'à présent que chez les *Trachylobium*. Toutefois, les plantules des sous-genres *Guibourtia* et *Gorskia* restent différentes de celles du sous-genre *Pseudocopaiva* (Léonard, 1957). En effet, les premières possèdent un appendice typique au collet contrairement aux deuxièmes qui en sont dépourvues (Léonard 1957 et 1994). Le tableau 1.2 fait la synthèse de la description des plantules de sept espèces du genre *Guibourtia* étudiées par Léonard.

Tableau 1.2. Description des plantules de quelques espèces du genre *Guibourtia* (Léonard, 1957) - *Seedlings description of some species of the genus* Guibourtia *(Leonard, 1957)*

Espèces	Sous-genres	Longueur et aspect de l'hypocotyle	Longueur et aspect de l'épicotyle	Aspect de la première feuille	Aspect du 1er entre-nœud	Stipules
Guibourtia copallifera	***Guibourtia***	5-8 cm pubérulent et glabrescent	2-3 mm pubescent, dépourvu d'écailles	Sessile, bifoliolée à folioles suborbiculaires	Densément pubérulent, dépourvu d'écailles	Libres (de part et d'autre du pétiole), persistantes
Guibourtia demeusei		5-13 cm	2-5 mm	Bifoliolée à folioles ovales	Généralement muni vers la base d'une écaille caduque	Libres, très caduques
Guibourtia conjugata	***Gorskia***	2-3,5 cm pubérulent, tardivement glabrescent	3-6 mm pubescent à glabrescent	Bifoliolée à folioles suborbiculaires	Dépourvu d'écailles	Libres, foliacées, subpersistantes
Guibourtia arnoldiana		3-6 cm pubérulent, glabrescent à la base	3-6 mm, pubescent	Bifoliolée à folioles suborbiculaires	Assez souvent muni d'une écaille caduque	Libres, très caduques
Guibourtia ehie		6-8 cm pubescent à glabre	2-3 mm pubescent	Bifoliolée à folioles suborbiculaires	-	Libres, foliacées, subpersistantes
Guibourtia coleosperma	***Pseudocopaiva***	3-6 cm glabre ou un peu pubescent à la partie supérieure	3-7 mm glabre et dépourvu d'écailles	Sessile, bifoliolée à folioles suborbiculaires	Glabre dépourvu d'écailles	Plus ou moins connées en une pièce axillaire allongée, foliacée, caduque
Guibourtia tessmannii		8-9 cm entièrement pubérulent	4-6 mm un peu pubescent dépourvu d'écailles	Sessile, bifoliolée à folioles oblongues	Glabre, dépourvu d'écailles	-

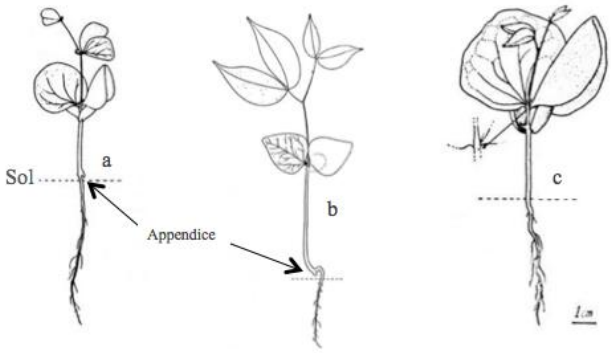

Figure 1.4. Structure des plantules des espèces du genre *Guibourtia* (adaptée de Léonard, 1957). a) *G. conjugata*, b) *G. demeusei*, c) *G. coleosperma* - Structure of seedling species of the genus *Guibourtia* (adapted from Leonard, 1957).
a) *G. conjugata* b) *G. demeusei* c) *G. coleosperma*

1.4. Ecologie du genre *Guibourtia*

1.4.1. Exigences climatiques, topographiques, pédologiques et optimum de végétation

En zone soudanienne, *G. copallifera* est la seule espèce rencontrée. Elle est établie sur des sols variés gréseux à argilo-limoneux (Aubréville, 1950 ; Burkill, 1995).

En région guinéo-congolaise, *G. demeusei* est présente sur des sols hydromorphes (Léonard, 1950 ; Gillet, 2013) des forêts périodiquement inondées et marécageuses (Léonard, 1952 ; Vivien et Faure, 1985). *G. pellegriniana*, *G. tessmannii* et *G. ehie* sont des espèces de forêts denses humides sempervirentes (Vivien et Faure 1985; Laird et al., 1996). *G. pellegriniana* est toutefois limitée aux forêts littorales du Cameroun et du Gabon (Souane, 1985). *G. arnoldiana* se rencontre sur des sols bien drainés jusqu'à 200 m d'altitude (Vivien et Faure 1985). Elle est inféodée aux sols calcaires du versant du Mayumbe (Léonard, 1950) et vit dans l'écotone forêt-savane (Monteiro, 1962). *G. leonensis* et *G. dinklagei* sont des espèces de forêt dense sèche (Wilczek et al., 1952; Burkill, 1995).

Les espèces de *Guibourtia* de la zone zambézienne et de la mosaïque régionale de Zanzibar-Inhambane sont localisées dans les formations décidues (Wilczek et al., 1952 ; Burke, 2006). *G. carrissoana* est un arbuste des zones littorales sèches semi-arides en Angola (Aubreville, 1970). *G. coleosperma* est une espèce xérophile retrouvée sur les sols sablonneux profonds (Giess, 1998), à une altitude allant de 750 à 1400 m). Elle préfère des zones où la pluviométrie annuelle est comprise entre 650 et 1100 mm et dont la température annuelle moyenne oscille entre 20 et 28 °C (Storrs, 1979). *G. conjugata* est un arbre de forêts claires ou de forêts sèches sur sols rocailleux, du niveau de la mer jusqu'à 1500 m d'altitude (Aubreville, 1970). *G. schliebenii* et *G. sousae* sont également des espèces xérophiles mais rencontrées au sud de la mosaïque régionale de Zanzibar-Inhambane à une pluviométrie inférieure à 750 mm et sur sol brun eutrophe (Bullock, 1995). Enfin, *G. schliebenii* préfère des forêts côtières (Léonard, 1950).

Les espèces de *Guibourtia* ont des sensibilités variées au feu. Par exemple, *G. ehie* y est très sensible (Vivien et Faure, 1985), contrairement à *G. coleosperma* (Storrs, 1979).

1.4.2. Tempérament des espèces et structure des populations

G. copallifera est une espèce héliophile sempervirente (White, 1983). *G. tessmannii*, *G. pellegriniana* et *G. ehie* sont vraisemblablement des espèces dont les exigences en lumière sont intermédiaires (Doucet, 2003). Pour Hawthorne (1995), ces espèces sont dites héliophiles non-pionnières. *G. demeusei* serait considérée comme une espèce sempervirente et tolérante à l'ombrage (Gillet, 2013). *G. leonensis* et *G. arnoldiana* seraient probablement des espèces héliophiles sempervirentes (Monteiro, 1962 ; Voorhoeve, 1965). Les *Guibourtia* de la zone zambézienne et de la mosaïque régionale de Zanzibar-Inhambane, *G. carrissoana*, *G. coleosperma*, *G. conjugata*, *G. schliebenii* et *G. sousae*, seraient également des espèces héliophiles sempervirentes (Léonard, 1950 ; Brummitt et al., 2007).

Les *Guibourtia* des forêts denses humides tropicales de terre ferme sont généralement très disséminés avec des densités faibles. Selon les données de plan d'aménagement forestier de certaines concessions forestières du Gabon et du Cameroun, les densités de *G. tessmannii* varient respectivement entre 0,035 à 0,231 tiges/ha et 0,001 à 0,12 tiges/ha pour les individus de DBH ≥ 20 cm (Doucet, communication personnelle). De telles informations ne sont pas disponibles pour les autres espèces.

Ces faibles densités expliquent la difficulté de dresser la structure de population de ces taxons. La figure 5 donne néanmoins quelques exemples. Les trois structures globalement décroissantes indiquent a priori une bonne régénération puisque les tiges de faibles diamètres sont abondantes (figures 1.5a, 1.5b et 1.5c).

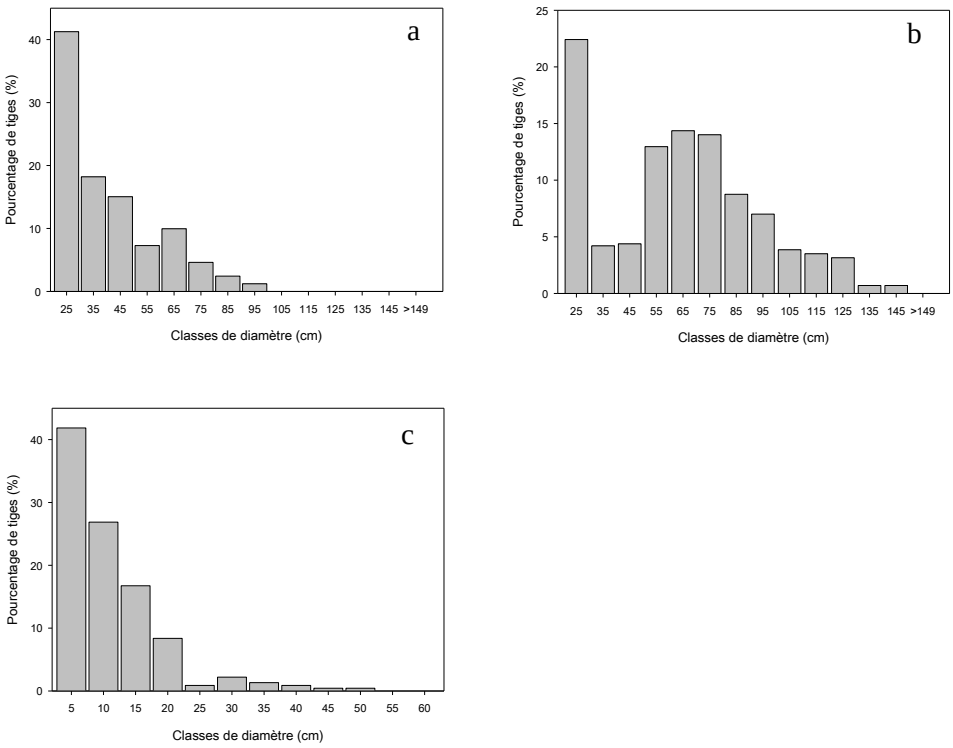

Figure 1.5. Structures diamétriques de (a) *G. demeusei* au Nord-Congo (296.000 ha), (b) *G. tessmannii* au Centre-Est du Gabon (150.000 ha) et (c) *G. copallifera* (adapté de Gnoumou et al., 2012) au Sud-Ouest du Burkina-Faso (2 ha) - Diametric structures of (a) *G. demeusei* (296,000 ha), (b) *G. tessmannii* Centre-East of Gabon (150,000 ha) and (c) *G. copallifera* (adapted from Gnoumou et al., 2012) in South-West Burkina-Faso (2 ha)

1.4.3. Phénologie et dispersion

Peu d'auteurs se sont intéressés à ces aspects. On sait toutefois que *G. copallifera* fleurit en fin de saison des pluies, de janvier à février (Arbonnier, 2004). Selon Aubréville (1968) au Gabon, *G. demeusei* et *G. arnoldiana* fleurissent pendant la grande saison des pluies (septembre à décembre). Les travaux de Doucet (2003) ont décrit la phénologie de *G. ehie*. Il en ressort que le pic de floraison pour cette espèce s'observe vers la fin de la saison sèche (septembre à décembre). Au Libéria et en Côte d'Ivoire, les arbres de *G. ehie* fleurissent au début de la saison sèche (novembre à décembre) et les fruits mûrissent en saison pluvieuse, entre janvier et février (Aubréville, 1970). Il existerait donc probablement un conservatisme de ces caractères de floraison et de fructification sur l'aire de répartition de cette espèce. En ce qui concerne *G. tessmannii* et *G. pellegriniana*, la fructification est étendue de la petite saison des pluies à la grande saison sèche au Gabon, de décembre à juillet (Aubréville, 1968). Au Cameroun, *G. tessmannii* fructifie pendant la saison pluvieuse (août) (Aubréville, 1970). La floraison de *G. leonensis* s'effectue à la fin de la saison des pluies (septembre à décembre) et la fructification démarre pendant la saison sèche, de Janvier à mars (Savill et Fox, 1967).

Contrairement aux espèces d'Amérique, dont certains agents responsables de la pollinisation sont bien identifiés, ceux qui participent à la pollinisation des espèces d'Afrique restent peu connus. *Guibourtia hymenaeifolia* est en effet pollinisé par l'abeille *Apis mellifer*a *adansonii* qui est capable d'assurer le transport du pollen sur plusieurs kilomètres (Ojeda-Camacho et al., 2013). Il est probable que les espèces africaines de *Guibourtia* soient aussi pollinisées par des Apidae (Motte-Florac, 1980).

Concernant la dispersion des diaspores, les connaissances sont tout aussi lacunaires. On peut supposer que le vent intervient dans la dispersion des gousses coriaces indéhiscentes de *G. copallifera* et *G. ehie* (Burkill, 1995). Par contre, lorsque les gousses sont arillées, on peut supposer une dispersion de type zoochore (Gautier-Hion et al., 1985).

1.5. Qualités technologiques et sylviculture des espèces de *Guibourtia*

1.5.1. Qualités technologiques

L'anatomie des *Guibourtia* a été relativement bien décrite par Bamford (2005). Les essences principales productrices de bois sont *G. arnoldiana*, *G. coleosperma*, *G. ehie*, *G. tessmannii* et *G. pellegriniana* (Lemmens et al., 2012). Le tableau 1.3 présente les caractéristiques anatomiques et technologiques les plus importantes de leur bois. Celui-ci est en général stable, lourd, dense, dur, résistant aux champignons et aux termites. Il sèche bien, à condition que l'opération soit faite lentement, après quoi, il acquiert une bonne stabilité (CTFT, 1983). L'aubier est blanchâtre, plutôt épais et possède des grains qui varient de fin à moyen (Benoit, 2011). Selon cette dernière source, les utilisations du bois varient d'une espèce à une autre. Globalement, les espèces de *Guibourtia* sont sollicitées en menuiserie, ébénisterie de

luxe, artisanat, construction navale et chemins de fer mais, aussi en fabrication des instruments de musique (guitare, harpe, flûte, tambour, etc.) (Léonard, 1950; CIRAD, 2008). Il existe une différence assez nette du point de vue texture et couleur du bois entre espèces (Simpson et TenWolde, 1999).

Tableau 1.3 : Caractéristiques anatomiques et technologiques des bois provenant des principales espèces exploitées du genre *Guibourtia* - *Wood anatomical and technological characteristics of the main exploited species of Guibourtia*

Caractères	***G. arnoldiana***	***G. coleosperma***	***G. ehie***	***G. pellegriniana***	***G. tessmannii***	**Références**
Noms commerciaux	Benge, Mutenye	Copalier	Ovengkol, Amazakoué, Hyedua,	Bubinga, Kevazingo	Bubinga, Kevazingo, Oveng	Léonard, 1950 ; Raponda-Walker et Sillans,1961 ; Bekker, 2006
Densité du bois à 12 % d'humidité (g/cm^3)	0,78-0,96	0,85	0,73–0,90	0,94	0,80 – 0,95	Monteiro, 1962 ; CIRAD 2008
Retrait radial (%)	4,6-6	2-3.7	3,4-5,5	8,2	5,2-8,1	Bolza et Keating, 1972
Retrait tangentiel (%)	8,7–10,3	3,2–5,7	6,8-10,7	9,9	6,3-10,5	Monteiro, 1962 ; CIRAD, 2008
Module de rupture (N/mm²)	138–202	85–142	127–210	-	166–195	Bolza et Keating, 1972
Module d'élasticité (1000*N/mm²)	14-21,4	9,2	13,9-21,5	16,3	15,1	Monteiro, 1962 ; CIRAD, 2008
Contrainte de rupture en compression (N/nm²)	72-84	51-58	57–81	80	66–73	Bolza et Keating, 1972
Contrainte de cisaillement (N/nm²)	8-13	14-16	8-15	-	9,5	Monteiro, 1962 ; CIRAD, 2008
Dureté Chalais-Meudon	4,5-8,3	8,8-9	5,4-11,3	-	7,9-9,0	Bamford, 2005
Diamètre des vaisseaux (µm)	120	117	120	185	110	Bamford, 2005
Nombre de vaisseaux par mm^2	13	7	6	4	3	Bamford, 2005
Largeur des cellules (µm)	2-3	5-10	2	5-10	3-4	Bamford, 2005
Hauteur des cellules (µm)	301-500	301-500	501	1000	501	Bamford, 2005
Pays producteurs	Congo et Gabon	Angola, Botswana, Namibie, Zambie et Zimbabwe	Ghana, Côte d'Ivoire, Nigéria, Gabon, Guinée-Bissau, Guinée, Sierra Leone et Libéria	Gabon, Cameroun et Nigéria	Cameroun et Gabon	Lock, 1989 ; Bamford, 2005

5.2. Sylviculture

L'exploitation forestière en Afrique Centrale et de l'Ouest est sujette à une série de mesures légales censées assurer une gestion durable. Sur le plan technique, l'une des contraintes imposées aux exploitants est la fixation d'un Diamètre Minimum d'Exploitation (DME). Le tableau 1.4 donne les DME en fonction des espèces et des pays. Ces diamètres doivent être revus à la hausse par les exploitants s'ils ne permettent pas une reconstitution suffisante après une première rotation. Mais le principal défaut de ces valeurs est qu'elles ne sont généralement pas établies sur une base scientifique. Il faudrait effectivement que les diamètres de fructification soient pris en compte afin de garantir le maintien de semenciers. Malheureusement, de telles données font défaut pour les espèces du genre *Guibourtia*.

La sylviculture des espèces du genre en est à ses balbutiements. Des essais de reboisement ont été effectués avec *G. ehie* à l'Ouest du Gabon. Après six années d'observation, Zaou et al. (1998) ont montré que les plants avaient un accroissement moyen en hauteur de 1,42 m/an dans le sous-bois et 1,45 m/an en plein éclairement. Quant à l'accroissement moyen en diamètre, il était de 1,15 m/an et 1,05 m/an respectivement dans le sous-bois et en plein éclairement. Des travaux similaires sont en cours dans le centre du Gabon (Doucet, communication personnelle).

Tableau 1.4. Le Diamètre Minimum d'Exploitation (DME) des espèces de *Guibourtia* exploitées par pays - *The Guibourtia species Minimum Exploitation Diameter (MED) by country*

Espèce	Pays	DME (cm)	Sources
G. arnoldiana	Gabon	70	Loi n°016/01 (PR, 2001)
	Congo	60	Décret 2002-437 (SGG, 2002)
G. coleosperma	Zimbabwe	50	Burke, 2006
G. demeusei	Congo	80	Décret 2002-437 (SGG, 2002)
	Gabon	70	Loi n°016/01 (PR, 2001)
	RDC	60	Arrêté n°036/CAB/MIN/ECN-EF (MIN/ECN-EF, 2006)
	RCA	60	Loi n°08.022 (PR, 2008)
G. ehie	Ghana	90	Burkill, 1995
	Gabon	70	Loi n°016/01 (PR, 2001)
	Libéria	60	Oteng-Amoako, 2006
	Côte d'Ivoire	60	Burkill, 1995
G. tessmannii	Gabon	90	Loi n°016/01 (PR, 2001)
	Cameroun	80	Arrêté n°0222/A/MINEF (MINEF, 2001)
	Congo	80	Décret 2002-437 (SGG, 2002)
G. pellegriniana	Gabon	90	Loi n°016/01 (PR, 2001)
	Cameroun	80	Arrêté n°0222/A/MINEF (MINEF, 2001)

1.6. Le commerce international du bois de *Guibourtia*

Reconnus pour la qualité exceptionnelle de leur bois d'œuvre, les *Guibourtia* sont commercialisés sous les noms de *Bubinga*, *Kevazingo*, *Ovengkol*, *Amazakoue* ou *African rosewood.* Ils font l'objet d'un important commerce. Selon ATIBT (2010), l'exportateur le plus important est le Gabon. Il commercialise ses bois vers 10 pays principaux: Chine, Hong-Kong, Japon, Italie, Belgique, Turquie, Etats-Unis, Portugal, Espagne, Angleterre (figure 1.6). Il est à noter que le continent asiatique est le principal importateur des bois de *Guibourtia*. Le Gabon en a exporté près de 90.000 m^3/an sous forme de grumes de 2007 à 2010. Suite à l'interdiction d'exporter du bois non transformé, instaurée dans ce pays en 2010, seuls des bois sciés ont été exportés entre 2011 et 2012, à raison été de 11.000 m^3/an. Au cours de cette même période, le Cameroun, la Guinée Equatoriale et la RDC n'ont chacun exporté que de 350 à 1580 m^3/an de bois sciés (ATIBT, 2008 ; 2009 ; 2010; 2012). En Côte d'Ivoire, *G. ehie* fut un bois très recherché et sa production entre 1970 et 1974 est estimée à 36.000 m^3 de bois ronds (Aubréville, 1959). Aujourd'hui, le bois de *G. ehie* a été hissé au rang de bois d'exportation de première qualité au Ghana (Lemmens et al., 2012).

Les autres espèces sont actuellement moins valorisées qu'elles ne le furent par le passé. En 1960, 6.000 m^3 de bois de *G. arnoldiana* ont été exportés par le Congo et en 1983, 10.000 m^3 par le Gabon (Normand et Paquis, 1976). Le bois de cette espèce

possède des propriétés technologiques proches de celles de l'iroko (*Milicia excelsa* (Welw.) C.C.Berg) et du teck (*Tectona grandis* L.f) (Fouarge et al., 1970).

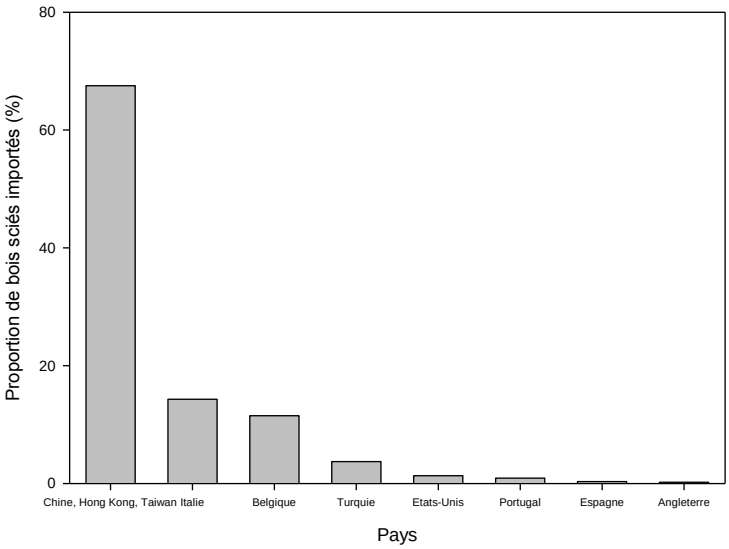

Figure 1.6. Principaux pays importateurs du bois de *Guibourtia* et proportion de bois sciés annuellement importés - *Countries that import timber of* Guibourtia *according to their importance in terms of annual imported proportion* (Source : Données ATIBT (2012))

1.7. Utilisations traditionnelles

Toutes les espèces de *Guibourtia* sont productrices de copal, une résine fraîche, translucide, de couleur ambrée ou jaune clair, à odeur parfumée (Léonard, 1950). Il s'agit d'une substance résineuse appelée « *Guibourtacacidin* » qui est localisée dans le cœur du bois des *Guibourtia* (Roux, 1959). Léonard (1950) a utilisé le terme de copal pour désigner ces résines dont les propriétés (dureté, solubilité, etc.) sont favorables, entre autres, à la production de laques et de vernis. L'utilisation de ce copal est avérée depuis plusieurs millénaires. Pendant l'époque du Moyen Empire (2022 à 1786 avant J.C.), les Egyptiens l'ont utilisé pour la momification des morts (Aufrere, 1983). A l'époque coloniale, les pygmées collectaient le copal pour les essarteurs qui le revendaient aux colons. Ce produit était utilisé dans l'industrie des vernis, mais a été finalement remplacé par des résines synthétiques (Gillet, 2013). Aujourd'hui, il pourrait présenter divers intérêts pour les industries pharmaceutiques, cosmétiques et surtout pour l'artisanat (Gillet, 2013).

Le *Bubinga*, dénomination courante des *Guibourtia* des forêts d'Afrique Centrale, surnommé le géant de la forêt, est un arbre fétiche ou sacré pour les pygmées (Bahuchet, 1985). Diverses parties (écorce, racines, sève et fruits) sont utilisées pour

divers usages (Adjanohoun, 1984 ; Fuendjiep et al., 2002 ; Ihenyen et al., 2009). Le tableau 1.5 fait la synthèse de ces différentes utilisations traditionnelles.

1.8. Quel statut de conservation pour les espèces du genre *Guibourtia* ?

Le manque de données écologiques sur les *Guibourtia* est la cause principale du faible niveau d'évaluation des taxons de ce genre par l'UICN. En effet, à part *G. ehie* (catégorie IUCN : *Least concern*) et *G. schliebenni* (catégorie IUCN : *Vulnerable*), les autres espèces n'ont pas fait l'objet d'une évaluation de leur statut de conservation (Contu, 2012). Pourtant, des menaces localement importantes existent. Ainsi, *G. copallifera* est menacé par les feux de végétation, la surexploitation du bois et de la résine au Burkina Faso (Gnoumou et al., 2012). L'effet combiné de ces facteurs en fait une espèce à haut risque d'extinction (Begon et al., 2006). En Afrique Centrale, plus particulièrement au Cameroun, l'exploitation illégale du bois, exporté vers l'Asie, fait subir aux *Bubinga* une pression considérable dont l'ampleur nécessiterait d'être précisée.

1.9. Conclusion

Il ressort de cette synthèse bibliographique que les connaissances restent globalement limitées sur les espèces du genre *Guibourtia*. Si les propriétés technologiques de certaines espèces, du fait qu'elles sont exploitées pour leur bois d'œuvre, sont assez bien documentées, la présente synthèse démontre que la littérature relative aux aspects écologiques est très pauvre.

On retiendra particulièrement que le genre *Guibourtia* (i) comporte plusieurs espèces sœurs morphologiquement semblables, (ii) rassemble des espèces rencontrées dans des formations végétales contrastées (forêt et savane) et (iii) regroupe des espèces inféodées à des sols différents (sableux, argilo-limoneux, calcaire, hydromorphe). Ces caractéristiques, parfois très différentes, font de ce genre un modèle particulièrement intéressant pour aborder les mécanismes de spéciation et d'adaptation écologique.

Par ailleurs, les caractères botaniques pouvant servir sur le terrain à distinguer des espèces en sympatrie sont soit rares, soit difficilement observables, quand leur pouvoir de discrimination n'est pas simplement limité. Par exemple, il n'est pas possible d'avoir à disposition en tout temps les fleurs de *G. tessmannii* et de *G. pellegriniana* pour les différencier. Il est donc important de compléter les descriptions botaniques. A tout le moins, il importerait d'apporter des éléments scientifiques nouveaux relatifs à la délimitation botanique des *Guibourtia* de forêt, grâce aux facilités désormais offertes par la génétique moléculaire ou la physiologie. Dans un second temps, sur base des données environnementales (climatiques, édaphiques) et de modèles de distribution passée et présente des populations, il serait possible d'évaluer la dynamique probable d'espèces de ce genre dans un contexte d'exploitation forestière et de changement climatique. Ces informations sont les

préalables à toute volonté de gérer durablement les populations et de statuer sur les statuts de conservation des espèces.

Tableau 1.5. Utilisations traditionnelles des espèces du genre *Guibourtia* en Afrique - *Traditional uses of Guibourtia species*

Espèces	Usages	Parties concernées	Références
Guibourtia arnoldiana	Usage médicinal : gale	Feuilles	Léonard, 1950 ; Raponda-Walker et Sillans, 1961
	Autre usage : combustible pour l'éclairage domestique	Copal	
Guibourtia carrissoana	Autres usages : combustible pour l'éclairage domestique	Copal	Léonard, 1950
Guibourtia coleosperma	Usage alimentaire : consommation de l'arille	Graine et arille	Léonard, 1950 ; Kamini, 2003 ; Bekker et al., 2006
	Autres utilisations : cosmétique, combustible pour l'éclairage domestique	Copal	
Guibourtia conjugata	Utilisations médicinales : douleurs stomacales et intestinales, de fièvres	Feuilles, écorce	Léonard, 1950 ; Ribeiro et al., 2010
	Autres usages : Valeurs mystiques, combustible pour l'éclairage domestique	Copal et arbre vivant	
Guibourtia copallifera	Usages médicinaux : dysenterie et paludisme	Feuilles	Léonard, 1950 ; Odugbemi, 2008
	Autres usages : bois de service et de chauffe, combustible pour l'éclairage domestique	Bois et copal	
Guibourtia demeusei	Usages médicinaux : dysenterie, poux	Feuilles et graines	Léonard, 1950 ; Raponda-Walker et Sillans, 1961 ; Lolke, 1989
	Autres usages : pesticides en agriculture, encens à l'église et combustible pour l'éclairage domestique	Copal	
Guibourtia dinklagei	Autres usages : combustible pour l'éclairage domestique	Copal	Léonard, 1950
Guibourtia ehie	Usage alimentaire : consommation des fruits	Fruits	Léonard, 1950 ; Noamesi et al., 1994
	Usages médicinaux : problèmes gastro-intestinaux, ulcère d'estomac	Feuilles, racines, écorce	
	Usage culturel : chasse les mauvais esprits	Copal	
	Autres usages : cosmétique, pesticides en agriculture et combustible pour l'éclairage domestique	Copal	
Guibourtia leonensis	Autres usages : poison de pêche et combustible pour l'éclairage domestique	Ecorce Copal	Léonard, 1950 ; Burkill, 1995
Guibourtia pellegriniana	Usages médicinaux : paludisme	Feuilles	Léonard, 1950 ; Bolza et Keating, 1972
	Autres usages : combustible pour l'éclairage domestique	Copal	
Guibourtia sousae	Autres usages : combustible pour l'éclairage domestique	Copal	Léonard, 1950
Guibourtia schliebenii	Autres usages : combustible pour l'éclairage domestique	Copal	Léonard, 1950
Guibourtia tessmannii	Usages médicinaux : aphrodisiaque, maladies cardiovasculaires, cancer, gonorrhée, blennorragie, maux de dos, hypertension, paludisme, hémorroïde etc.	Feuilles	Léonard, 1950 ; Raponda-Walker et Sillans, 1961 ; Jiofack et al., 2010
	Autres usages : protection contre des mauvais sorts, pesticides en agriculture et combustible pour l'éclairage domestique	Ecorce; copal	

Chapitre 2

Evolution in the Amphi-Atlantic genus *Guibourtia* (Fabaceae, Detarioideae), combining NGS phylogeny and morphology

La synthèse bibliographique présentée au chapitre 1 a soulevé des problèmes de délimitation taxonomique au sein du genre *Guibourtia*. Le présente chapitre a donc tenté de démêler les relations de parenté existant entre les espèces du genre. A travers une approche récente de séquençage complet du génome chloroplastique, il clarifie la position taxonomique de chaque espèce du genre *Guibourtia*. La reconstruction phylogénétique de ce genre montre une diversification durant le Miocène avec un évènement de migration d'Afrique vers l'Amérique à la fin du Miocène. Ce chapitre est adapté de :

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2. Evolution in the Amphi-Atlantic genus *Guibourtia* (Fabaceae, Detarioideae), combining NGS phylogeny and morphology

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Abstract

Tropical rain forests support a remarkable diversity of tree species, questioning how and when this diversity arose. The genus *Guibourtia* (Fabaceae, Detarioideae), characterized by two South American and 13 African tree species growing in various tropical biomes, is an interesting model to address the role of biogeographic processes and adaptation to contrasted environments on species diversification. Combining whole plastid genome sequencing and morphological characters analysis, we studied the timing of speciation and diversification processes in *Guibourtia* through molecular dating and ancestral biome reconstruction. All species except *G. demeusei* and *G. copallifera* appear monophyletic. Dispersal from Africa to America across the Atlantic Ocean is the most plausible hypothesis to explain the occurrence of Neotropical *Guibourtia* species, which diverged *ca.* 11.8 Ma from their closest African relatives. The diversification of the three main clades of African *Guibourtia* is concomitant to Miocene global climate changes, highlighting pre-Quaternary speciation events. These clades differ by their reproductive characters, which validates the three subgenera previously described: *Pseudocopaiva*, *Guibourtia* and *Gorskia*. Within most monophyletic species, plastid lineages start diverging from each other during the Pliocene or early Pleistocene, suggesting that these species already arose during this period. The multiple transitions between rain forests and dry forests/savannas inferred here through the plastid phylogeny in each *Guibourtia* subgenus address thus new questions about the role of phylogenetic relationships in shaping ecological niche and morphological similarity among taxa.

Keywords: plastid genomes captures, *Guibourtia*, molecular phylogeny, morphology, Next Generation Sequencing, speciation

2.1. Introduction

Despite the remarkable species richness of the tropical biomes and conservation issues, one critical question remains how and when rain forest diversity arose (e.g. Plana, 2004; Couvreur et al., 2011a; Couvreur, 2015). Biodiversity is unequally distributed across tropical continents (Slik et al., 2015) and we are still far from having a synthetic explanation about the lower apparent species diversity in African rain forests when compared to the Neotropics or South-East Asia (Couvreur, 2015). Dated molecular phylogenies are thus needed to reconstruct the history and evolution of taxa and understand the current biogeographical dynamics of tropical ecosystems at the inter- and intra-continental scales.

At an inter-continental scale, many plant families characteristic of rain forest ecosystems show a pantropical distribution in the three major tropical regions (America, Africa and Asia) and many of their taxa (e.g. genera, sometimes even species) are found in two or three of these regions (Couvreur et al., 2011b). Several hypotheses have been proposed to explain such disjunct distributions: (i) the break-up of the Gondwanan supercontinent (e.g. Raven and Axelrod, 1974; Conti et al., 2002), (ii) the degradation of boreotropical flora and the end of a northern mid-latitude migration corridor (e.g. Davis et al., 2002; Zerega et al. 2005; Muellner et al., 2006), and (iii) long-distance dispersal (e.g. Renner et al., 2001). In many plant taxa, when molecular data succeed in rejecting the vicariance hypotheses, they tend to confirm long distance dispersal (Pennington et al., 2006; Christenhusz and Chase, 2013; Baker and Couvreur, 2013; Armstrong et al., 2014). However, new molecular-dated inter-continental phylogenies are still needed to provide a more reliable view of the age and geographical origin of taxa showing a disjunct distribution and whether the timing and tempo of speciation on each continent coincides with geoclimatic events (Couvreur, 2011a; Richardson et al., 2015).

At the intra-continental scale, a main challenge is to validate species delineations and to infer the most putative evolutionary history of target taxa. However, there is still some limitations when applying the phylogenetic concept of species due to heterogeneous evolutionary rates between genetic markers and incomplete lineage sorting, the latter being especially important for trees with long generation time and large effective size (Daïnou et al., 2014, 2016). For Central African trees, the process of species diversification remains poorly documented, despite the emergence of some global trends. Firstly, among the few attempts to characterize the evolutionary history of rain forest trees, it appears that Miocene speciation events are not incompatible with incomplete lineage sorting and scarce morphological differences (e.g., Daïnou et al., 2014; Donkpegan et al., 2017). Secondly, studies highlighted that the most recent Pleistocene glacial/interglacial climatic oscillations promoted intraspecific diversification, but not speciation (e.g. Couvreur et al., 2011a; Duminil et al., 2015; Pineiro et al., 2017). Finally, both congruent phylogeographical patterns and idiosyncratic histories are detected among African rain forest species, especially for explaining both the role of gene flow barriers and refuge areas (e.g. Hardy et al., 2013; Dauby et al., 2014).

Here, we focus on the genus *Guibourtia* Benn. (Fabaceae, Detarioideae) because it is characterized by two South American and 13 African species (Léonard, 1949;

Tosso et al., 2015) whereas the Neotropical flora is globally richer than the African one (Couvreur, 2015). In addition, the 13 African species of *Guibourtia* occupy contrasted forest habitat types (rain forest to dry forest and savanna biomes) and appear well suited to infer the imprints left by past environmental changes on rain forest evolution. Besides, to date, botanists did not fully agree on species boundaries in this genus due to the existence of morphologically close species found in sympatry in Africa (Tosso et al., 2015). Using whole plastid genome sequencing by enrichment/hybridization/capture (McPherson et al., 2013; Mariac et al., 2014), the present study aims to better understand the evolutionary history of speciation and diversification in the genus *Guibourtia*. Modern high-throughput sequencing techniques and their applications in phylogeny have become essential to understand complex evolutionary scenarios that could not have been resolved before by sequencing a few genes (Faye et al., 2016). Indeed, they improve substantially the resolution of phylogenetic inferences even at low taxonomic levels or where recent divergence, rapid speciation or slow genome evolution occurred (Malé et al., 2014; Williams et al., 2016). This should allow a higher reliability of both phylogenetic relationships and molecular dating, benefiting systematics.

Specifically, we will address the following questions in this study:

- (i) What are the phylogenetic relationships between Neotropical and African *Guibourtia* species? How and when did *Guibourtia* occupy an Amphi-Atlantic distribution (vicariance *versus* long-distance dispersal)? For these questions, we will analyze the relative position of American and African taxa in the phylogeny, examining the temporal window of speciation and migration between continents.

- (ii) Is the current species delimitation of African *Guibourtia* supported by the phylogeny of their plastomes? How far does phylogenetic divergence reflect morphological differentiation among *Guibourtia* species? When and in which direction(s) did biome transition(s) occur and can the diversification of the genus in Africa be explained by the climatic history of the continent? Here, we will verify whether species represented by multiple samples appear monophylic, and study the phylogenetic relationships between African *Guibourtia* taxa in the light of their morphologic features and biome preferences.

2.2. Materials and methods

2.2.1. Biological model, sampling strategy

The study was conducted on all the 15 species of the genus *Guibourtia* (Fabaceae, Detarioideae), all found in tropical regions. The two neotropical species studied were *G. chodatiana* (Hassl.) J.Léonard and *G. hymenaefolia* (Moric.) J.Léonard which occur in South American rain forests. The 13 African species of *Guibourtia* include seven tropical rain forest species, *G. arnoldiana* (De Wild. & T.Durand) J.Léonard, *G. demeusei* (Harms) J.Léonard, *G. dinklagei* (Harms) J.Léonard, *G. ehie* (A.Chev.) J.Léonard, *G. leonensis* J.Léonard, *G. pellegriniana* J.Léonard, *G. tessmannii* (Harms) J.Léonard, and six dry forest or savanna species, *G. carrissoana* (M.A.Exell) J.Léonard, *G. coleosperma* (Benth.) J.Léonard, *G.*

conjugata (Bolle) J.Léonard, *G. copallifera* Benn., *G. schliebenii* (Harms) J.Léonard, and *G. sousae* J.Léonard.

The whole plastid genome was captured and sequenced for each species using one to five individuals per species (n = 40; Supplementary material Appendix 2.4). In addition, five individuals from highly divergent species (based on known morphological differentiation) were selected for genomic libraries sequencing without enrichment. Two genomic libraries were sequenced for two outgroup taxa among the closest known sister taxa of *Guibourtia*: *Crudia harmsiana* De Wild and *Daniellia pilosa* (J.Léonard) Estrella (Fougère-Danezan, 2005). Samples were collected in herbaria (Botanic Garden Meise "BR", Natural History Museum Paris "P") and from field samples from which leaf material was directly dried in silica gel.

2.2.2. DNA library construction and whole plastid genome capture

Total genomic DNA was extracted from dried leaves using the CTAB protocol (Doyle, 1987) coupled with QIAquick purification kits (Qiagen, Venlo, Netherlands), and followed by Qubit® 2.0 Fluorometer quantification (Life Technologies, Invitrogen, Foster City, USA) and by QIAxcel (Qiagen) DNA quality control.

Library preparation for multiplexed individuals was based on the protocol of Rohland and Reich (2012) but with the inclusion of additional steps published by Mariac et al. (2014) for the plastid enrichment procedure (biotinylated probes capture). A 5-10 µg of DNA aliquot per sample was sheared by sonication using a Bioruptor® Pico (Diagenode SA., Liège, Belgium) to a mean fragment size of *ca.* 400 bp. Sheared DNA was sized by dual fragment size selection using AMPure XP magnetic beads (Agencourt, Beckman Coulter, Brea, USA) to remove remaining larger (> 600 bp) and shorter (< 200 bp) DNA fragments. The next steps included blunting and 5'-phosphorylation using the Fast DNA End Repair Kit, ligation of tagged adapters using T4 DNA ligase, and a nick fill-in step performed with Bst DNA polymerase (New England Biolabs Inc. NEB, Beverly, USA). Tagging was made with multiplex 6-bp indexed adapters to enable pooling of libraries for sequencing, following the protocol published by Rohland and Reich (2012). A real-time PCR was then performed to extend Illumina adaptors and enrich library fragments using the KAPA HiFi RT PCR library amplification kit (KAPA Biosystems, Boston, USA), on a StepOnePlus analyzer (Applied Biosystems, Foster City, USA). Most of the steps were followed by AMPure XP bead-based sample clean-up steps and a check of DNA quality using a QIAxcel (Qiagen).

To enrich genomic libraries in plastid sequences, we applied the sequence capture using PCR probes (SCPP) method (Peñalba et al., 2014) adapted to plastid DNA by Mariac et al. (2014). Biotinylated probes were produced in the laboratory from one sample of *Guibourtia tessmannii* collected in the field, using 18 long-range universal PCR primers combinations (Uribe-Convers et al., 2014) with the LongAmp® Taq PCR Kit (NEB). These probes were sheared and sized, targeting 400 bp in length, as previously done for genomic libraries. Probes biotinylation was done with a 5' TEG-biotinylated linker using a Phusion® High-Fidelity PCR Master

Mix (Invitrogen, Carlsbad, USA). In solution capture of plastid DNA was done by hybridization of biotinylated probes to genomic libraries during 48 h, with then immobilization of target DNA by streptavidin beads (Dynabeads C1, Invitrogen). A prehybridization has been undertaken before enrichment with Phusion Taq to increase the concentration of DNA aliquots.

The plastid genome enrichment phase does not guarantee that all parts of the plastid genome will be captured, thus the protocol of genomic libraries preparation was also applied without enrichment to reconstruct a reference plastid genome on one sample among five species tested (highest quality library conserved), using *ca.* 1/20th of the MiSeq run versus *ca.* 1/100th of the run for each plastid genome enriched sample. Independent genomic libraries were also sequenced for the outgroup taxa (without enrichment). All these steps are summarized in Figure 2.1.

Libraries were titrated for sequencing using the KAPA Library Quantification Kit (Kapa Biosystems, Boston, USA) and fragment size distributions checked using the QIAxcel, before pooling libraries. Paired-end sequencing (2 × 150 bp) was performed on an Illumina MiSeq with reagent kit V2 at the GIGA platform (Liège, Belgium).

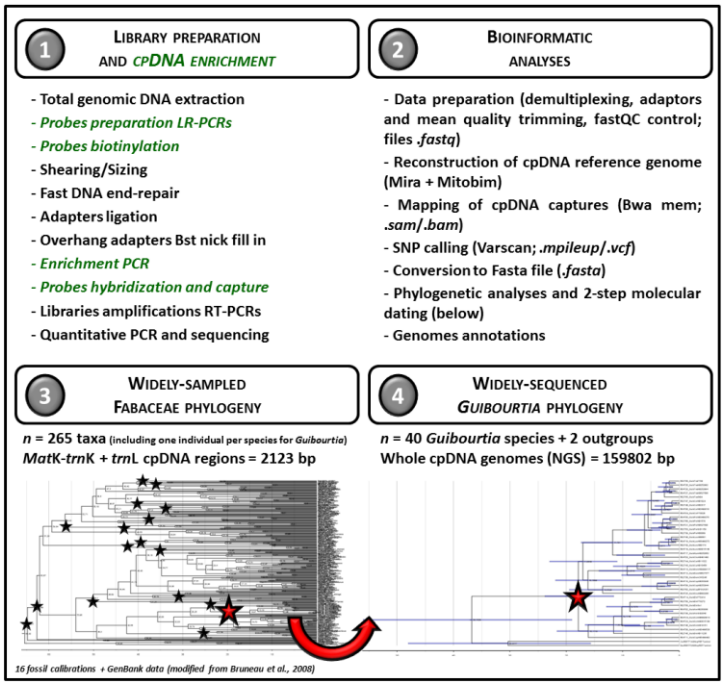

Figure 2.1. Main laboratory and bioinformatic steps followed for reconstructing the phylogeny of the genus *Guibourtia*

2.2.3. Plastid genomes reconstruction

Standard Illumina pipeline software (v 1.7 or later) was used for base-calling and quality filtering, with standard chastity settings used to produce fastq data files whose quality was checked with FastQC. Demultiplexing based on the 6-bp barcodes was performed using the freely available python script Demultadapt (https://github.com/Maillol/demultadapt), using a 0-mismatch threshold. Adapters and low-quality bases were trimmed using Cutadapt 1.2.1 (Martin, 2011) with the following options: quality cut-off = 20, minimum overlap = 7 and minimum length = 35. Reads with a mean quality lower than 30 were discarded afterwards using a freely available perl script (https://github.com/SouthGreenPlatform/arcad-hts/blob/master/scripts/arcad_hts_2_Filter_Fastq_On_Mean_Quality.pl).

The software Mitobim 1.5 (Hahn et al., 2013) was used to reconstruct the plastid genome of *Guibourtia*, assembling the reads from the non-enriched genomic DNA library. Mitobim mapped these reads using MIRA 3.4.1.1 (Chevreux et al., 1999) to highly conserved regions of the closely related reference genomes available in GenBank; *Tamarindus indica* (Fabaceae, Detarioideae; KJ468103) was retained after comparison with other genomes. The GenBank genome used to initiate the process of reference plastid genome reconstruction is not assumed to have the correct linear order and the resolving process specifically detects rearrangements and inversions, and corrects these.

The mapping of plastid DNA (pDNA) enriched libraries was performed using Bwa Mem 0.7.5a-r405 (Li and Durbin, 2009) with -M and -B 4 options, using the plastid reference genomes reconstructed in the previous steps with the reference genomic library of *Guibourtia*. Samtools 1.1 (Li and Durbin, 2009) was used to generate a mpileup file with option -B, following recommendations of Scarcelli et al. (2016). Finally, we used Varscan 2.3.7 (Koboldt et al., 2012) to call SNPs (option –min-var-freq set to 50%) before converting it to a fasta multi-alignment file (bioinformatic pipeline summarized in Figure 2.1).

The final complete plastid genomes were annotated using Geneious v. 7.1.3 (Kearse et al., 2012) and through comparison to published complete plastid genome sequences available in GenBank. The newly generated sequences are available in GenBank (see Appendix 2.4 for accession numbers) and bam files are available on request to authors.

2.2.4. Phylogenetic analyses and molecular dating

A two-step molecular dating approach was used. The first step used a large taxonomic coverage of Fabaceae and several fossil calibrated nodes to estimate the crown age of the genus *Guibourtia* using mostly published sequences for a limited number of genes. The second step used our whole plastid genomes of *Guibourtia* samples to estimate the nodes ages of the *Guibourtia* phylogeny (Figure 2.1). The phylogenies were reconstructed with the Bayesian MCMC analysis implemented in BEAST 1.8.2 (Bayesian Evolutionary Analysis by Sampling Trees; Drummond and Rambaut, 2007).

For the first step, we extracted the *mat*K/*trn*K (analyzed separately) and *trn*L plastid DNA regions from one plastid genome sequenced for each *Guibourtia*

species (n = 15), and the NGS genomic libraries of *Daniellia pilosa* and *Crudia harmsiana*. These data were combined to the same plastid DNA regions sequenced for 250 Fabaceae species (including only taxa with data available for these three plastid regions) by Bruneau et al. (2008) and available in GenBank. Sequences were aligned in MAFFT 7 (Multiple alignment program for amino acid or nucleotide sequences: *http://mafft.cbrc.jp/alignment/software/*). The models GTR+G, GTR+G and HKY+G were chosen using jModelTest 2.1.7 (Darriba et al., 2012) for *mat*K, *trn*K, and *trn*L alignments, respectively. Sixteen fossil calibration points were used (see Appendix 2.5) to anchor the phylogeny, following Bruneau et al. (2008), where all these fossils are described in detail. Each calibration point was parameterized as minimum age with a normal, lognormal or gamma distribution (Appendix 2.5). These nodes and those of strongly supported clades in the Fabaceae (Bruneau et al., 2008) were constrained as monophyletic.

For the second step (Figure 2.1), we anchored the phylogeny of the plastid genomes of 40 *Guibourtia* samples by setting the age of the crown node of *Guibourtia* taxa as a normal distributed random variable (mean = 19.41, StDev = 3.0) with a 95% interval between 14.48 and 24.35 Ma, as obtained through the first step. The model GTR+G was applied.

At each molecular dating step, an XML file was generated using BEAUti (Bayesian Evolutionary Analysis Utility). An uncorrelated lognormal relaxed clock model and a Yule process of speciation were applied. One MCMC analysis was run for 100 million generations each, sampling trees at 10,000 step intervals. We used Tracer 1.4 (Drummond and Rambaut, 2007) to assess convergence, estimate Effective Sample Sizes (ESS), and examine the posteriors of all the parameters. Mean heights were taken in TreeAnnotator 1.4.8 (Drummond and Rambaut, 2007), and trees were plotted in FigTree 1.1.2 (http://tree.bio.ed.ac.uk/software/figtree/).

In parallel, a phylogenetic network of the 40 *Guibourtia* plastomes was established from a Neighbor-Net analysis (Bryant and Moulton, 2004) using SplitsTree 4.14.2 (Huson and Bryant, 2006). This phylogenetic Neighbor-Joining distance-based method works quickly by agglomerating clusters and tends to construct resolved haplotype networks representing both groupings in the data and evolutionary distances between taxa.

2.2.5. Ancestral characters reconstruction

Ancestral states reconstructions were performed for habitat types (rain forest *versus* dry forest/savanna), using a maximum likelihood approach implemented in the R package phytools (Revell, 2012). We also applied maximum parsimony principles to infer biome transitions, assuming that species can be adapted to only one type of biome.

2.2.6. Morphological data

In total, 45 descriptors were scored for the 40 individuals used for whole plastome sequencing. These descriptors were sorted into vegetative and reproductive traits

(Appendix 2.6). Specimens were collected between 1889 and 2010 and kept in the National Museum of Natural History in Paris P (France) and the Botanic Garden Meise BR (Belgium). Only the specimens for which the determination has been validated by botanists specialized in Fabaceae (most often by J. Leonard, J. Wieringa, M. Fougère-Danezan or R. Letouzey) were taken into account.

The morphological characters used were those listed in the determination keys of Léonard (1949), Aubréville (1970), Fougère-Danezan et al. (2010), and Tosso et al. (2015) (Appendix 2.6). We added characters potentially displaying high interspecific variation as the presence of glands on the leaflets, the shape of the apex of leaflets, the persistence of stipules, and the type of fruit (Appendix 2.6). After being removed, floral organs to be measured were rehydrated in boiling water at 90°C for 3 minutes. The flowers (1-3) were then dissected and observed. The floral pieces were measured with a micrometer incorporated into a binocular microscope (Nachet GLI 154), magnification x 10-40. Microscopy was also used to check for the presence/absence of glands and the hairiness on the leaflets, on the flowers or fruits. The other qualitative characteristics (form of leaflets, position of the median veins, and secondary veins, etc.) were directly observed.

To describe the morphological similarity between species, a matrix of morphometric distances between the 40 analysed specimens was constructed by calculating the Gower distance (Gower, 1971) for (i) all morphometric characters, (ii) reproductive characters only, and (iii) vegetative characters only, using the R package FD (Laliberté et al., 2010). Hill and Smith analysis (Hill and Smith, 1976; Kiers, 1991) allowed to ordinate both qualitative and quantitative morphometric variables. Then, we considered the scree plot (Cattell, 1966) and Kaiser criterion (Kaiser, 1960) to assess the number of axes that represent enough the ordination patterns after a Principal Coordinate Analysis (PCoA), using the R package ade4 (Chessel et al., 2012).

2.3. Results

2.3.1. NGS data

The non-enriched genomic library of *G. leonensis* used for reconstructing the reference plastid genome (size of 159,802 bp) contained 1,454,120 R1-R2 paired reads, of which 2.29% appeared to be of plastid origin, making an average depth of 27X. The genomic libraries of *Daniellia pilosa* and *Crudia harmsiana* used for reconstructing outgroup plastid genomes (sizes of 160,314 bp and 159,664 bp, respectively) contained 1,307,116 and 1,638,114 R1-R2 paired reads, of which 4.5% and 0.9% were of plastid origin, respectively.

For the 40 pDNA enriched libraries of *Guibourtia*, on average, 332,974 R1-R2 paired reads (standard deviation, stdev 272,188 R1-R2 paired reads) were obtained for each NGS plastid genome capture, of which 96.7% (stdev 1.8%) appeared to be of plastid origin on average, illustrating a good yield for pDNA enrichment (Appendix 2.4). The average depth was 285X (mean stdev 223) and the mean coverage at ≥10X was around 84.4% (stdev 7.5%). After SNP (Single Nucleotide

Polymorphic) calling, 6,300 SNPs and 1,412 indels were retained and used for reconstructing fasta files of complete plastid genomes.

2.3.2. Phylogenetic relationships and molecular dating from NGS plastid genomes

Integrating our plastid genomes for one individual per species within the Fabaceae phylogeny published by Bruneau et al. (2008) for the *mat*K-*trn*K and *trn*L plastid DNA regions, the genus *Guibourtia* was well supported as monophyletic (posterior probability of 1; Appendix 2.5). The genus *Hymenaea* is sister to *Guibourtia*, and *Peltogyne* is sister to both genera. In this Fabaceae phylogeny anchored by 16 fossil calibration points, the crown node of *Guibourtia* was dated to 19.41 Ma, with a 95% higher posterior density interval ranging between 14.50 and 24.26 Ma (Appendix 2.1). These divergence values were then implemented in our intra-*Guibourtia* phylogeny exclusively based on complete plastid genomes. For each molecular dating step, most of the parameters had reached stationarity with ESS values over 200, thus deemed reliable (all the parameters have reached at least 100).

The Bayesian Maximum Clade Credibility phylogeny provided by BEAST at the intra-*Guibourtia* level (40 *Guibourtia* taxa + 2 outgroups) showed strong overall support for each node (posterior probabilities mainly of 1 or 0.99 for recent divergences; Appendix 2.2). Three clades diverged around 17.08-17.86 Ma (95% HPD of 10.98-22.98 and 11.57-23.80 Ma, respectively between clades 1/2-3 and clades 2/3; Figure 2.2). The clade 1 is composed of *G. arnoldiana*, *G. ehie*, *G. conjugata*, *G. schliebenii*, *G. dinklagei* and one of the four samples of *G. copallifera* from Sierra Leone. The clade 2 included *G. demeusei*, *G. carrissoana*, *G. sousae*, and three samples of *G. copallifera* collected in Burkina Faso, Mali, and Congo. The clade 3 is composed of *G. tessmannii*, *G. pellegriniana*, *G. leonensis*, and *G. coleosperma*. The Neotropical taxa *G. chodatiana* and *G. hymenaefolia* are included in the clade 3, but diverged from other African *Guibourtia* around 11.78 Ma (95% HPD: 6.52-17.38 Ma). When several samples were analysed per species (10/15 species), the monophyly of the species was always well supported except for *G. demeusei* and *G. copallifera*.

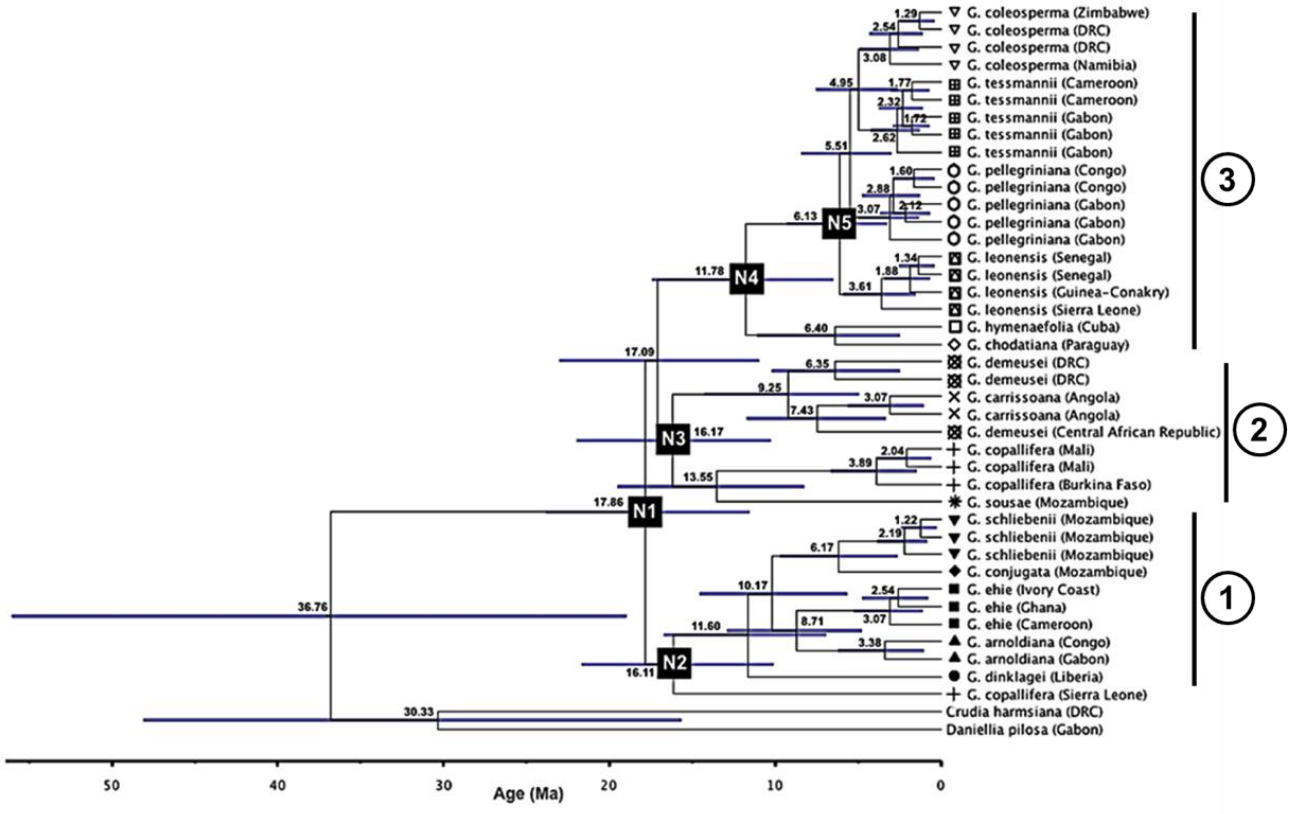

Figure 2.2. Divergence time chronogram of the whole plastid genome sequenced for *Guibourtia* and two outgroup taxa, using BEAST software (Bayesian maximum clade credibility tree). Numbers on nodes indicate the mean divergence time estimate (in Ma), with bars referring to the 95% highest posterior density intervals around node ages. Nodes N1 to N5 are discussed in the text (see Appendix 2.5). All except two nodes are supported at posterior probabilities of at least 0.99 (see Appendix 2.2).

The NeighborNet analysis (Figure 2.3) is congruent with the result of the BEAST analyses, with the same three clades identified. The divergence between taxa belonging to clade 1 is relatively more recent than that observed within the other clades. Especially, the NeighborNet tree revealed an obvious star-like topology for the clade 1.

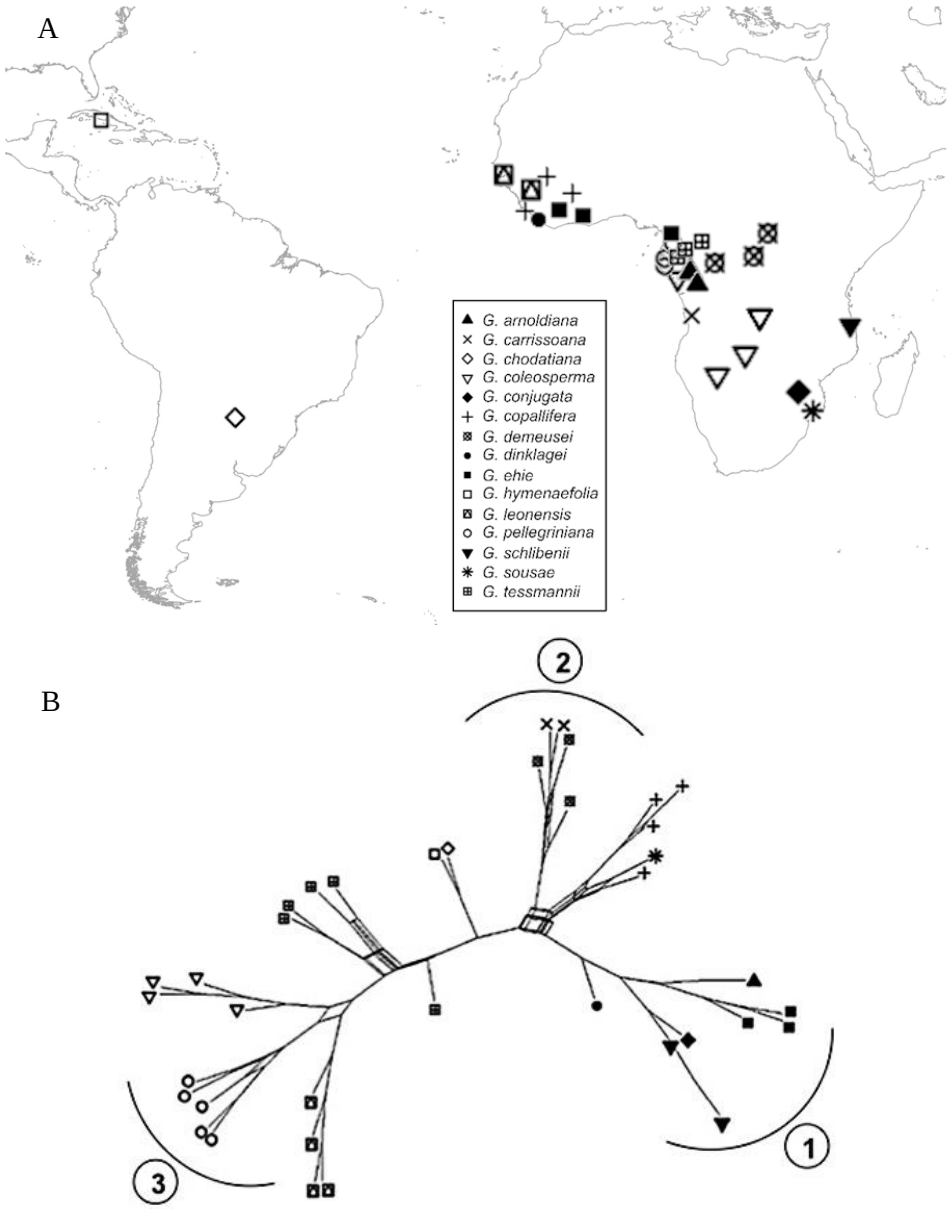

Figure 2.3. Distribution map of sequenced samples of *Guibourtia* species (A) and Neighbor-Net tree of whole plastid genomes (B)

2.3.3. Biome transitions

Each of the three clades contains rain forest (RF) species and dry forest/savanna (DFS) species (Figure 2.4). By using maximum likelihood approach for reconstructing ancestral states, the most recent common ancestor of *Guibourtia* species was difficult to assign to a specific biome, the scaled likelihood at the root reaching 57% for RF and 43% for DFS (Figure 2.4). Applying maximum parsimony principles, if we assume a RF ancestral state, at least four biome transitions are needed, either four RF to DFS transitions, or three RF to DFS and one DFS to RF transitions. If we assume a DFS ancestral state, at least five biome transitions are needed, either four DFS to RF and one RF to DFS transitions, or three DFS to RF and two RF to DFS transitions. The only transition common to all these scenarios is the RF to DFS transition leading to *G. coleosperma*, which would have occurred between 3 and 5 Ma, thus during the Pliocene.

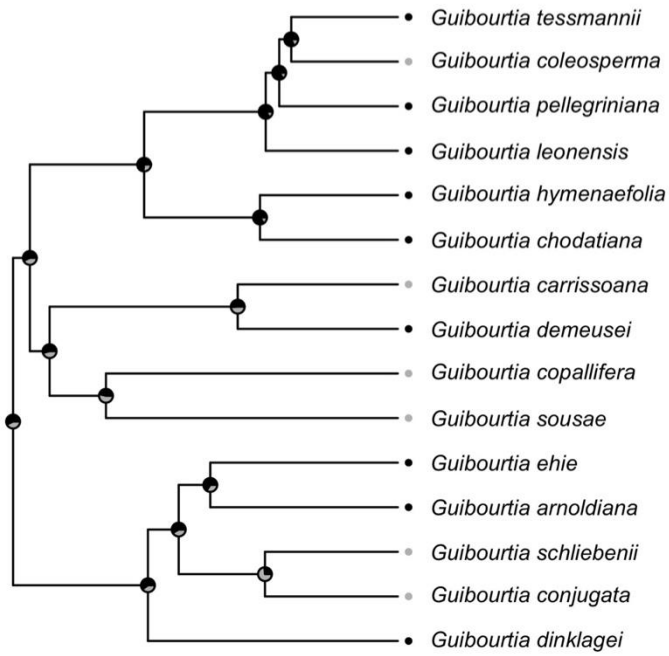

Figure 2.4. Reconstruction of ancestral biomes using the ancestral state reconstruction method (maximum likelihood) implemented in the R package phytools (Revell, 2012). Pie-charts represent the probabilities that an ancestor is adapted to rain forests (black; scaled likelihood at the root: 56.79%) or to dry forests/savannas (grey; scaled likelihood at the root: 43.20%).

2.3.4. Morphometric delineation in Guibourtia *species*

To disentangle morphological units, the first two components of the Principal Coordinate Analysis (PCoA) explained 33.75% and 19.38% of the total morphometric variation. The PCoA analysis scatter plot showed three morpho-groups (Figure 2.5A). The first group is composed of *G. copallifera*, *G. demeusei*, *G. carrissoana*, and *G. sousae*. The second group included *G. arnoldiana*, *G. ehie*, *G. conjugata*, *G. schliebenii*, and *G. dinklagei*. The third group is composed of *G. tessmannii*, *G. pellegriniana*, *G. leonensis*, *G. coleosperma*, *G. chodatiana*, and *G. hymenaefolia*. Hence, morphometric groups and phylogenetic clades are highly congruent.

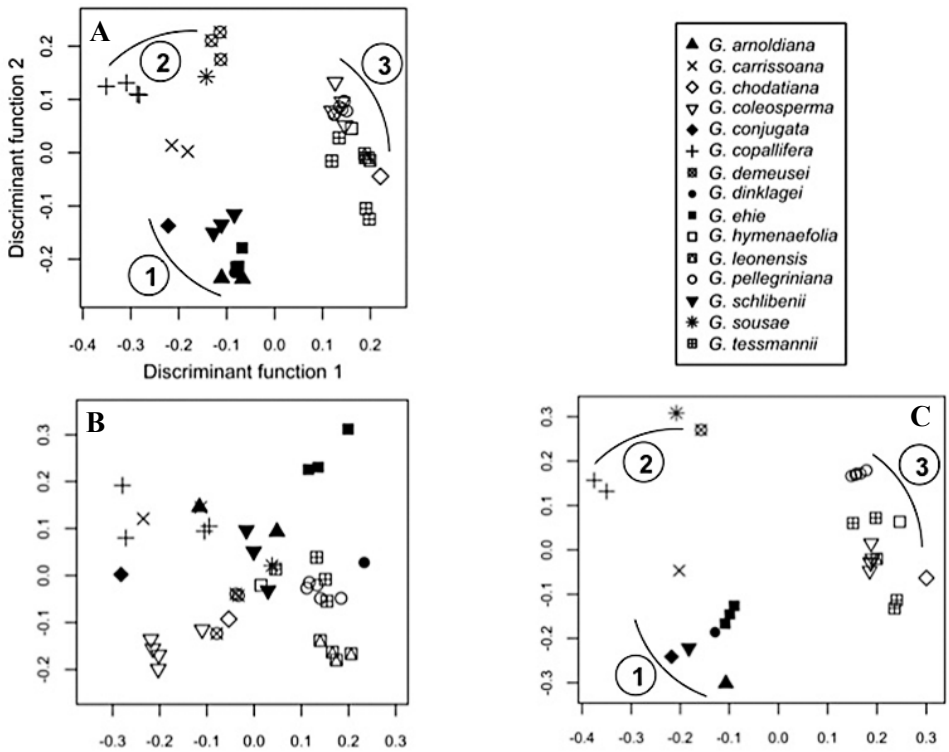

Figure 2.5. Morphometric Principal Coordinate Analysis considering all *Guibourtia* species, and either all tested morphological characters (A), or only vegetative characters (B), or only reproductive characters (C). See Table 1 for the list of characters included.

The first two components of the PCoA based on reproductive morphometric characters explained 40.27% and 25.56% of the total variance. The corresponding scatter plot (Figure 2.5B) displayed the same three distinct morpho-groups as described above. However, the PCoA scatter plot based on vegetative characters did

not separate the above-mentioned morpho-groups (43.19% of the total variance explained by the first two components; Figure 2.5C).

Through Hill and Smith's analysis on both all morphometric characters and then reproductive characters, it appears that the first morpho-group can be characterized by the following combination of characters: secondary leaf veins basilar, marginal venation, persistent or sessile stipules, persistent bracteoles, glabrous sepals and non arrilate seeds, no venation on the fruit and non arillate seeds. The second morpho-group was characterized by coriaceous limb, apex of leaflets obtuse, persistent stipule, deciduous bracteole, axillary and terminal inflorescence, globular bud and presence of venation on the fruit. The third group showed the following features: deciduous bracteole, axillary and terminal inflorescence, dehiscent fruit with long and stipitate stipe, no gland on the fruit and arillate seeds.

2.4. Discussion

2.4.1. Origin of the Amphi-Atlantic tropical distribution of Guibourtia

The pantropical distribution of 59 families and 334 genera of seed plants is one of the most important plant biogeographic patterns of intercontinental range disjunction (Thorne, 1973). Given that the origin of many families and genera postdates the progressive fragmentation of the Gondwana continent which started *ca.* 180 Ma (McLoughlin, 2001), transcontinental dispersal must have occurred. Three pathways can be seen as alternative hypotheses for explaining the biogeographical connections between Afrotropical and Neotropical floras (Appendix 2.3): (1) the Boreotropical North Atlantic Land Bridge (NALB) between Europe and North America in the early Tertiary (mainly *ca.* 35-54 Ma), (2) the Bering Land Bridge (BLB or Beringia) between Asia and North America from the early Palaeogene into the late Miocene/early Pliocene, and (3) the direct Atlantic dispersal between Africa and Neotropics. This third pathway was characterized successively by land connection (until *ca.* 96 Ma), potential insular stepping stones (short-distance overseas dispersal via island chains of the Sierra Leone Rise and/or the Rio Grande Rise–Walvis Ridge), and oceanic long-distance dispersal of diaspores by wind or oceanic currents (Renner, 2004; Erkens et al., 2009). Phylogenetic trees, as developed here for the genus *Guibourtia* can thus be used to test the likelihood of these different hypotheses, especially to infer the source and time of entry of taxa into continents (Donoghue and Moore, 2003).

Only two *Guibourtia* forest species are growing in the Neotropics, compared to the 13 African ones, suggesting that Africa is probably the center of origin of this genus. In addition, Neotropical *Guibourtia* are not early branching but included in a clade of otherwise African species. This is supported by our highly resolved plastid phylogeny: a single transatlantic migration event is necessary if the ancestor of all *Guibourtia* occurred in Africa, while three transatlantic migration events in the reverse direction should be assumed if the ancestor of *Guibourtia* occurred in America. Nevertheless, it is interesting to note that *Hymenaea* and *Peltogyne*, the

two genera most related to *Guibourtia*, occur in the Neotropics (except *H. verrucosa* which is East-African), suggesting that transoceanic migration was not so exceptional. Among the Neotropical taxa, *G. hymenaefolia* is a deciduous tree mostly found in dry forests in Brazil, Bolivia and Paraguay (Jardim et al., 2003) while the deciduous *G. chodatiana* grows in gallery forests in North and South of America (Cuba, Brazil, etc.; Lorenzi, 1998). Although their taxonomic status has been debated (one species instead of two according to Barneby, 1996), the age of divergence between the two Neotropical samples was estimated around 6.40 Ma (95% HPD between 1.59 and 11.24 Ma), and the Neotropical clade diverged from the closest African samples around 11.78 Ma (95% HPD between 6.52 and 17.38 Ma), during the Miocene. Hence, assuming a single migration event, the latter must have occurred between these two time estimates, which are far more recent than the age of the Gondwanan breakup (*ca*. 96-105 Ma; Morley, 2003) and of the Boreotropics land bridge (*ca*. 35-54 Ma; Tiffney, 1985; Davis et al., 2002). Regarding the Bering Land Bridge, which connected Asia and North America from the early Palaeogene until its closure between 7.4 and 4.8 Ma (Tiffney and Manchester, 2001), no *Guibourtia* taxa has been described in fossil layers in Eurasia and northern America that could validate this pathway of migration. In addition, the BLB route, which was probably too far north, was less frequently used by tropical taxa than the NALB route, due to the fact that daylight might have been a limiting factor for migration of megathermal plant taxa, such as *Guibourtia* (Tiffney and Manchester, 2001; Morley, 2003).

The most plausible explanation for the Amphi-Atlantic *Guibourtia* distribution appears to be long-distance (oceanic) dispersal from Africa to South America. This is in line with an increasing number of molecular dating studies which reveal that such dispersal events occurred, especially from Africa to South America during the Miocene: Atherospermataceae (Renner et al., 2000), *Symphonia globulifera* (Clusiaceae; Dick et al., 2003), *Gossypium* (Malvaceae; Cronn and Wendel, 2004), Myrtaceae, Vochysiaceae (Sytsma et al., 2004), *Manilkara* (Armstrong et al., 2014), *Barleria oenotheroides* (Acanthaceae; Martín-Bravo and Daniel, 2016) (see the review in Appendix 2.3). Other cases of transatlantic dispersal were summarized by Renner (2004) and De Queiroz (2005), who also suggested possible mechanisms for these events. Since some seeds or fruits of Detarioideae have good ability to float, Fougère-Danezan (2005) assumed that dispersal by oceanic long-distance dispersal is plausible. In addition, transoceanic dispersal by floating seeds or by seeds associated to large mats of vegetation was estimated to occur in less than two weeks during the Miocene (Houle, 1998). This case exemplifies once again that oceans do not constitute an insurmountable barrier for plants over geological time, and disjunct continental floras are more connected than had been thought previously when vicariance was the main hypothesis for the occurrence of Amphi-Atlantic taxa (De Queiroz, 2005).

2.4.2. Diversification of Guibourtia *in Africa*

Our results based on whole plastid genome sequencing highlight that the genus *Guibourtia* is well supported as monophyletic (Appendix 2.1), as suggested by Fougère-Danezan (2005) who used only seven *Guibourtia* species. Among the 13 African species, eight of the 10 species represented by more than one individual appeared monophyletic for the plastome (Appendix 2.2). Our plastome phylogeny gives therefore some support to the current species delimitation. It must be noted that incongruence between species delimitation and plastome phylogeny is not uncommon and could result from incomplete lineage sorting and/or chloroplast captures, even if species are correctly delineated (e.g. Duminil et al., 2012; Daïnou et al., 2014; Cavender-Bares et al., 2011; Neophytou et al., 2011; Acosta and Premoli, 2010; Premoli et al., 2012). The globally good congruence in the case of *Guibourtia* suggests that the plastome phylogeny should be a good proxy of the species phylogeny. The two exceptions showing paraphyly (*G. copallifera* and *G. demeusei*) might thus result from incomplete lineage sorting or chloroplast captures. Nevertheless, to delineate more reliably *Guibourtia* species and identify reproductively isolated groups, nuclear markers would be needed.

The whole-plastid-genome-based chronogram revealed that the genus *Guibourtia* diverged during the Miocene (*ca.* 14.5-24 Ma; node N1, Figure 2.2) and continued until Late Miocene and Early Pliocene, as confirmed by Fougère-Danezan (2005). This period coincided with the second major environmental changes that occurred from the Early to the Mid-Miocene (15-23 Ma): the African continent moved northwards, the Equator down positioned, humid vegetation disappeared in the Sahara, and thus the rain forest belt shifted southwards (Maley, 1996; Jacobs, 2004). An increasingly number of genetic studies tends to confirm the crucial role of Miocene events in the African flora composition, especially for: *Inga* (Fabaceae, Mimosoideae; Richardson et al., 2001), *Afromomum* (Zingiberaceae; Auvrey et al., 2010), *Isolona* and *Monodora* (Annonaceae; Couvreur et al., 2011), *Erythrophleum* (Fabaceae, Detarioideae; Duminil et al., 2013), *Manilkara* (Sapotaceae; Armstrong et al., 2014), *Milicia* (Moraceae; Daïnou et al., 2014), the African rattans palms *Ancistrophyllinae* (Arecaceae; Faye et al., 2016b), *Afzelia* (Fabaceae, Detarioideae; Donkpegan et al., 2017). The three main clades identified in our phylogeny (as described below; nodes N2-4, Figure 2.2) diverged around the middle Miocene climatic transition between *ca.* 14.8-16 Ma (Flower and Kennett, 1994), when continuous rain forest has split, due to renewed cooling and aridity across Africa (Davis et al., 2002; Couvreur et al., 2008). Especially, this major environmental change may have caused biome shift and then species divergence (Losos et al., 1997; Reznick et al., 1997), promoting speciation within many taxa (Smith et al., 1997; Schluter, 1998). Finally, if speciation largely predates the Quaternary, Pleistocene glacial/interglacial climatic oscillations (from 2.7 Ma, and mainly 1.8 Ma) might promote the recent divergence within taxa belonging to the clade 1: *G. coleosperma*, *G. tessmannii*, *G. pellegriniana* and *G. leonensis* (node N5, Figure 2.2). It is important to note that these divergence events seem congruent to some major environmental changes but we cannot establish reliable causal links based on the outcomes of the present study.

Inferring ancestral states through the plastid phylogeny, the genus *Guibourtia* is characterized by multiple biome shifts, with at least four transitions, mainly between rain forests and dry forest/savannas and at least one occurring during the Pliocene. These multiple transitions differ from the unique transition revealed for *Afzelia* species, where Quaternary climatic oscillations could have favoured the biome shift between rain and dry forests combined with the phenomenon of polyploidization (Donkpegan et al., 2017).

2.4.3. New phylogenetic and morphological insights for systematics of Guibourtia

A significant contribution of floral and fruit traits emerged for the morphological delimitation of *Guibourtia* species. This can be explained by some literature statements which assume that reproductive characters evolve faster than vegetative ones in taxa with specialist pollinators, which should be the case for *Guibourtia* species. Plants with generalist pollinators or wind pollination could develop more specific vegetative traits whereas their reproductive characters could remain very similar, as observed in the genus *Milicia* (Daïnou et al., 2014). Besides, Cardoso et al. (2012) highlighted that reproductive characters cannot be used to predict phylogenetic relationships in the early branches of Papilionoid legumes, hypothesizing that reproductive characters might be better at shallow phylogenetic time scale.

Fundamentally, we distinguish three main clades in the phylogeny, which are congruent with the three subgenera described by Léonard (1949) and confirmed by our morphometric analyses (Figure 2.5). The clade 1 encompasses *G. tessmannii*, *G. pellegriniana*, *G. leonensis*, *G. coleosperma*, *G. chodatiana* and *G. hymenaefolia*, and was described as the subgenus *Pseudocopaiva* (Britton & Wilson) by Léonard (1949). The clade 2, with *G. copallifera*, *G. demeusei*, *G. carrissoana* and *G. sousae*, corresponds to the subgenus *Guibourtia*, and the clade 3, including *G. arnoldiana*, *G. ehie*, *G. conjugata*, *G. schliebenii* and *G. dinklagei*, belongs to the subgenus *Gorskia* (Bolle).

The subgenus *Pseudocopaiva* is different from the two other ones by the type of fruit and seed (dehiscent fruit and arillate seeds for one and indehiscent fruits and not arillate seeds for others). The difference between the subgenus *Guibourtia* and the subgenus *Gorskia* can be linked to the fundamental difference regarding (i) the persistence of bracteoles (the bracteoles of the first are deciduous while those of the second are persistent) and (ii) the thickness of the seeds (membranous for the first and thick for the second).

2.5. Conclusions

In this paper, we highlighted the important role of dispersal across the Atlantic Ocean, which promoted the diversification of *Guibourtia* into the Neotropics. The majority of species appeared monophyletic and the crown age of the plastomes sampled within each monophyletic species was usually anchored in the Pliocene or

early Pleistocene (2.6-6 Ma). Besides, the divergence between the plastomes of sister monophyletic species was usually dated in the late Miocene or Pliocene (5-13 Ma). Finally, in each clade, morphologically supported by reproductive characters, we can assume at least one biome transition, mainly from rain forest to dry forest/savanna.

The next step, under progress, will be to use the genus *Guibourtia* as a model to address questions about niche conservatism and convergent evolution. Targeting the ecological amplitude of African *Guibourtia*'s taxa, we could now rely on this new highly resolved plastid DNA phylogeny to examine how far ecological niche influences organism morphology among related species.

Chapitre 3

Phylogenetic patterns of diversification across ecological niches in the African tree genus *Guibourtia* support convergent evolution of morphological traits along a climatic gradient

Ce chapitre combine la phylogénie moléculaire établie dans le chapitre 2 avec une analyse des traits morphologiques et de la niche environnementale des espèces africaines du genre *Guibourtia*. Il examine au moyen d'une approche pluridisciplinaire l'effet de la dérive génétique et de la sélection naturelle dans la diversification des espèces du genre *Guibourtia*. Le présent chapitre montre que certains traits morphologiques ont été sélectionnés de manière convergente au sein des différents clades de ce genre en fonction des niches climatiques des espèces.

3. Phylogenetic patterns of diversification across ecological niches in the African tree genus *Guibourtia* support convergent evolution of morphological traits along a climatic gradient

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Abstract

Adaptive evolution is thought to be a major driver of organism diversification but the link between phenotypic traits and environmental niche remains little documented in tropical trees. Moreover, the respective roles of phylogenetic inertia and convergent evolution in shaping environmental niche and phenotypic trait similarity among related plant taxa is not well understood. Indeed, a correlation between species traits and species environmental niche among a sample of species may result from (1) convergent evolution if different environmental conditions have selected different sets of traits, or (2) phylogenetic inertia if niche and morphological differences between species are simply function of their phylogenetic divergence, in which case the trait-niche correlation does not imply any direct causal link. The aim of this study is to understand the relationships between environmental niche divergence and morphological divergence among congeneric species while accounting for phylogenetic inertia. This issue was addressed with the timber tree genus *Guibourtia* Benn. (Leguminosae, Detarioideae) which contains 13 African species occupying various forest habitat types, from rain forest to dry woodlands, with different climate and soil conditions. To this end, we combined morphological data with recent ecological niche modelling and used a highly resolved plastid phylogeny of the 13 African *Guibourtia* species. First, we demonstrated phylogenetic signals in both morphological traits (Mantel test between phylogenetic and morphological distances between species: r=0.24, p=0.031) and environmental niches (Mantel test between phylogenetic and niche distances between species: r=0.23, p=0.025). Second, we found a significant correlation between morphology and niche, at least between some of their respective dimensions (Mantel's r=0.32, p=0.013), even after accounting for phylogenetic inertia (Phylogenetic Independent Contrast: r=0.69, p=0.018). This correlation occurred between some leaflet and flower traits and solar radiation, relative humidity, precipitations and temperature range. Our results demonstrate the convergent evolution of some morphological traits in response to climatic factors in congeneric tree species and highlight the action of selective forces, along with neutral ones, in shaping the divergence between tropical plants.

Keywords: *Guibourtia*, taxonomy, niche comparison, phenotypic adaptation, phylogenetic signal, speciation, Phylogenetic Independent Contrast

3.1. Introduction

To date, the mechanisms underlying plant species diversification and the evolution of their morphological traits in tropical forests remain little known. Moreover, the role of environmental variation as a potential driver of adaptive morphological evolution (Ortiz-Medrano et al., 2016) has received little attention in African tropical tree species. Understanding these mechanisms can provide important insights into the evolution of African biomes and guide strategies for their conservation (Linder, 2014).

If natural selection by abiotic factors is a main driver of the evolution of species traits, a close relationship between species traits and the environmental conditions can be expected (Ricklefs, 1987; Schluter, 1988). However this statement remains to be demonstrated, especially for congeneric tropical tree species, because morphologically similar tree species can occur in contrasted environments, e.g. from dense rain forests to dry woodlands/savannas, so that the relative importance of adaptive processes in shaping the morphological traits that discriminate them remains unclear. To assess whether particular traits are selected by particular environmental conditions one can study the correlation between trait variation and environmental variation among a set of species. However, it is important to distinguish morphology-niche associations due to convergent evolution from that related to phylogenetic inertia.

Convergent evolution is the independent evolution of a set of similar phenotypic characteristics in organisms from different lineages subject to similar abiotic or biotic agents of natural selection (Cody and Mooney, 1978). It is considered as an evidence of adaptation (McLennan and Brooks, 1993; Pagel, 1994) and implies causal morphology-niche relationships. One classical example of convergent evolution occurs between Cactaceae (cactus) and Euphorbiaceae (euphorbs), two phylogenetically distant families (McDowell, 1991) that have evolved independently succulent (water-storing) stems to adapt to desert conditions. In contrast, phylogenetic inertia is the tendency for traits to resist evolutionary change despite environmental perturbations (Edwards and Naeem, 1993). An apparent morphology-niche association in a set of species may thus occur if the most phylogenetically related species tend to share both similar traits and similar environmental niches due to slow trait and niche evolution, a situation that does not imply the selection of particular traits by particular environmental conditions. Hence, it is crucial to have a well resolved phylogeny of the species of the study clade to distinguish convergent evolution from spurious morphology-niche association due to phylogenetic inertia, and to assess the role of adaptation in the diversification process (Giarla and Esselstyn, 2015).

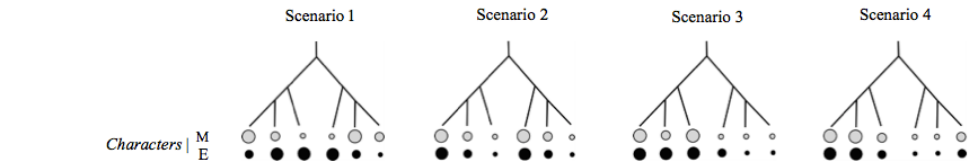
The ecomorphological approach was developed to study the relationship between morphology and environmental factors (Motta & Kotrschal, 1991). It requires a characterization of the morphological traits and of the environment of species in a comparative framework (Wiens and Graham, 2005). For the later one, Broennimann et al. (2012) proposed multivariate techniques to quantitatively compare the environmental niches between pairs of species (e.g. indices of niche overlap, niche equivalency and niche similarity). These new niche modelling tools combined with

phylogenetic information (i) help to assess how the realized niches (see Soberon, 2007) of closely related species have evolved over time (e.g., Guisan & Thuiller, 2005; Ortiz-Medrano et al., 2016), and (ii) are essential to explain the observed biogeographical patterns and to test ecological hypotheses (Rato et al., 2015).

To factor out the impact of phylogenetic inertia when studying the correlation between different species characteristics, Felsenstein (1985) developed the method of phylogenetic independent contrasts (PIC). Alternatively, partial mantel tests have also been proposed, whereby the phylogenetic distance between species is used as a covariate to test the association between the distance matrices of the two species characteristics (Legendre, 2000). Partial Mantel tests can be used with multivariable distances but they must be interpreted with caution because they tend to be liberal (excess of false positives; Guillot et Rousset, 2013).

The present work aimed to understand the relationships between environmental niche and morphological (dis)similarity among congeneric tropical tree species while accounting for phylogenetic inertia. More specifically we addressed the following questions: (i) do species morphological traits and environmental niches display phylogenetic signals?, (ii) are morphological and environmental (dis)similarity between species correlated?, (iii) are morphological and environmental (dis)similarity still correlated after factoring out the impact of phylogenetic inertia, suggesting convergent evolution? By answering these questions, we tested whether the association between morphological traits and environmental niche within congeneric species supports one of the four scenarios detailed in Figure 3.1.

We addressed this issue using the genus *Guibourtia* Benn. (Leguminosae, Detarioideae) which includes 13 African tree species (Tosso et al., 2015). This genus represents a good model for our questions because (i) its species occur over a wide range of habitat types (rain forests, dry forests/savannas), and (ii) a well-resolved phylogeny of this genus is now available (Tosso et al., 2018) and shows three clades, each including species occupying contrasting environments.



	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Correlation M-P (Phylogenetic signal in M)	No	No	Yes	Yes
Correlation E-P (Phylogenetic signal in E)	No	No	Yes	Yes
Correlation M-E	No	Yes	Yes	Yes
Correlation M-E | P (PIC: Phylogenetic Independant Contrast)	No	Yes	No	Yes

Figure 3.1. Four scenarios (Sc1 to Sc4) regarding the association between a morphological trait (M) and a dimension of the environmental niche (E) within six closely related species of known phylogeny (P). The trait and niche values are represented by the size of each grey (M) or black (E) circle. Under Sc1, species morphological traits and environmental niches evolved quickly and independently, showing no phylogenetic signal and no M-E correlation. Under Sc2, morphological trait and environmental niche evolved quickly but show strong M-E correlation due to convergent evolution (same M traits selected in similar E niches). Under Sc3, both morphological trait and environmental niche evolve slowly, leading to phylogenetic signals, and resulting in a M-E correlation, but there is no remaining M-E correlation when applying a PIC (Phylogenetic Independent Contrast) analysis factoring out the correlation due to phylogenetic dependence. Under Sc4, a strong M-E correlation is due both to phylogenetic inertia and convergent evolution (significant PIC analysis). We expect the M-E correlation to be stronger than the M-P and E-P correlations in scenarios 2 and 4 implying convergent evolution, and to be less strong than the M-P and E-P correlations in scenario 3 implying only phylogenetic dependence.

3.2. Methods

3.2.1. Study species

The African *Guibourtia* species occur in a variety of vegetation types, across the Sudano-Sahelian Region, the Guineo-congolian Region and the Zanzibar-Inhambane regional Mosaic (Tosso et al., 2015). The 13 species can be roughly categorized into species inhabiting relatively dry and seasonal climates harbouring tropical dry forests (dense dry forests and woodlands) or wooded savannas, hereafter identified by the # symbol, and species inhabiting wet/moist and weakly seasonal climates favouring dense wet forests (species with * symbol). Six species are associated to dry and seasonal climates: *G. carrissoana*[#] (M.A.Exell) J.Leonard, *G. coleosperma*[#] (Benth.) Leonard, *G. conjugata*[#] (Bolle) J.Leonard, *G. copallifera*[#] Benn., *G. schliebenii*[#] (Harms) J.Leonard, *G. sousae*[#] J.Leonard; and seven species

to wet/moist climates : *G. arnoldiana*[*] (De Wild. & T. Durand) J.Leonard, *G. demeusei*[*] (Harms) J.Leonard, *G. dinklagei*[*] (Harms) J.Leonard, *G. ehie*[*] (A.Chev.) J.Leonard, *G. leonensis*[*] J.Leonard, *G. pellegriniana*[*] Leonard, and *G. tessmannii*[*] (Harms) J.Leonard (Appendix 3.1)**.**

3.2.2. Morphological traits

A total of 281 georeferenced herbarium samples were used for the morphological analyses. They were collected between 1889 and 2010, and conserved in museums and botanical gardens (the National Museum of Natural History in France, the Meise Botanic Garden in Belgium, the Royal Botanic Garden of Edinburg in UK, the Nairobi University Herbarium in Kenya, and the Herbarium of Maputo in Mozambique). 57% of these samples were fertile (with flowers, fruits and/or seeds) and only the specimens for which the determination was validated by experienced botanists in Leguminosae (mostly J. Leonard, J. Wieringa, Fourgère-Danezan or R. Letouzey) were taken into account. A list of 45 morphological characters were measured on all (or only fertile) samples, considering the determination keys of Léonard (1950), Aubréville (1970), Tosso et al. (2015) and Fougère-Danezan et al. (2010) (Table 1). Concerning flowers, once removed, the parts to be measured were rehydrated in boiling water at 90 °C for 3 min. The flowers (1-3) were then dissected and observed. The floral pieces were measured with a micrometer incorporated into a binocular microscope (Nachet GLI 154), at magnification x 10-40. The microscope was also used to check for the presence of glands and hairs on the leaflets, flowers and fruits (Table 3.1).

3.2.3. Environmental data

Environmental data corresponding to occurrence points of the 13 *Guibourtia* species were used to model the species environmental niches. To this end, in addition to the geographical coordinates of the 281 specimens used for the morphological characterization, we extracted georeferenced data of reliably determined specimens from Kew herbarium (K) and Naturalis herbarium (L), giving us a total of 401-presence records after removing duplicates. Climate and soil data were extracted from the Climatic Research Unit (CRU) of the University of East Anglia (New et al., 1999; Mitchell and Jones, 2005) and "FAO Digital Soil Map of the World, version 3.6". Both climatic and soil data were interpolated at 0.5° spatial resolution. To avoid redundancy in environmental information, a Principal Component Analysis (PCA) was used as a data reduction technique (Heikkinen et al. 2006). We chose the least correlated variables that best explain the distribution of *Guibourtia* species: monthly means of temperature (°C), precipitation (mm), solar radiation (w/m^2), relative humidity (%), temperature range (°C), Potential evapotranspiration (mm), wind speed (m/s) and soil potential Hydrogen (pH).

Table 3.1. The 45 morphological characters (including 15 vegetative, and 30 reproductive characters) considered for characterizing *Guibourtia* individuals

Morphological traits	**Character states or measurement unit in case of quantitative variables**
Vegetative characters	
Position of primary leaf veins	submarginal, marginal, median
Apex leaflets	obtuse, acuminate
Glands on the abaxial side of the limb	absent, present
Limb	membranous, coriaceous, (sub)coriaceous
Petiole hairiness	glabrous, pilous
Gland on petioles	absent, present
Stipule	obsolete, persistent
Size of stipules	absent, tiny, foliaceous
Number of leaflets per leaf	one leaflet, two leaflets
Number of secondary leaf veins supra basilar	1, 2, 3, 4, 5
Number of secondary leaf veins basilar	1, 2, 3
Length of leaflets	cm
Width of leaflets	cm
Length of acumen	cm
Petiole length	cm
Reproductive characters	
Inflorescence position	axillary, axillary and terminal, terminal
Type of inflorescence	panicle => cob, cluster
Pedicel	absent, present
Bracts	obsolete, persistent
Bracts hairiness	absent, present
Gland on bracts	absent, present
Bracteoles	obsolete, persistent
Shape of bracts	orbicular, linear
Hairiness of the external face of the bracts	absent, present
Shape of flower bud	cylindrical, globular, ellipsoid
Hairiness bud on the external face of the sepals	absent, present
Hairiness on the inner side of the sepals	absent, present
Gland on sepals	absent, present
Hairiness of disc	absent, present
Pilosity of ovary	glabrous, pilous
Stipe of the ovary	sessile, stiped
Hairiness of the stipe's ovary	glabrous, pilous
Type of fruit	indehiscent fruit, dehiscent fruit
Gland on fruit	absent, present
Veins on the fruit outer surface	absent, present
Stipe of the fruit	absent, present
Arillus on the seed	absent, present
Length of sepals	mm
Width of sepals	mm
Length of stipe of the ovary	mm
Length of the fruit	cm
Width of the fruit	cm
Thickness of the fruit	mm
Length of the fruit stipe	cm
Number of seeds per fruit	1,2

3.2.4. Phylogenetic data

In a previous study, we built a high-resolution phylogeny of the genus *Guibourtia* using the whole chloroplast genome (Appendix 3.2; Tosso et al., 2018). We reconstructed the phylogeny and performed molecular dating for the 13 African species using a Bayesian MCMC analysis implemented in BEAST v1.8.2 (Bayesian Evolutionary Analysis by Sampling Trees; Drummond and Rambaut, 2007).

3.2.3. Analyses

3.2.3.1. Morphological similarity

To describe the morphological similarity between species, we proceeded in the following steps. First, a morphometric distance matrix between the 281 analysed specimens was obtained by calculating the Gower distance (Gower 1971) using the R package "FD" (Laliberté et al., 2010). Second, we applied a PCoA (namely PCoA-based morphological distance) on this distance matrix to synthesize morphological variation on four axes that explained 85.19% of the total variation. Pearson's correlation coefficients between original morphological variables and axis scores were used to interpret each PCoA axis in terms of morphological gradients. Third, we computed the means and standard errors (SE) of the PCoA scores of the specimens for each species along each axis. Finally, we computed the Euclidean distance between the mean species scores along the first four PCoA axes to describe morphological distances between species.

3.2.3.2. Environmental niche comparison

The environmental niche of species was described using a Principal Coordinates Analysis, as done for morphological data, to facilitate the analysis of the morphology-niche relationship. Here we considered the environmental variables extracted from the 281 specimens for which morphological data were available. We then computed the Gower distance between specimens to perform a PCoA and kept the three first axes explaining 90.10% of the total variation in environmental variables. The mean species scores along these axes were used to describe the environmental niche of the species, and the Euclidean distance between mean species scores along the three axes provided niche distances between species.

We conducted complementary environmental niche comparison analyses using the 401-presence records to further describe the degree of niche similarity and niche overlap between species, applying the approaches developed by Broennimann et al. (2012). First, to determine the limits of the area occupied by the 401-presence records (Burgman and Fox 2003), we applied the α-hull polygons technique using the R package Alphahull (Pateiro-López and Rodriguez-Casal, 2010). We then obtained the background data (Warren et al., 2010) for the 13 species taken together by using a buffer zone of 2,000 km around this area (except in the ocean) because this gave the best models (MaxEnt approach "results not shown") based on preliminary values of the Area Under the Curve (AUC; from 0.93 to 0.99). This background was used to perform a smoothing technique described in Broennimann et al. (2012) which implemented an ordination technique (PCA-ent) that divides the environmental space in cells and applied a kernel density function to determine the 'smoothed' density of each species occurrence in each cell.

To determine the degree of shared environmental niche between a pair of species, we calculated an index of niche overlap. This index quantifies the proportion of niche shared by the considered couple of species, and can be computed by means of the Schoener's D statistic ($D = 1 - 0.5 \sum |p_{X,i} - p_{Y,i}|$ where $p_{X,i}$ and $p_{Y,i}$ stand for the

probability assigned by the environmental niche modelling for species X and Y, respectively, to cell i; and the sum is taken over all the cells of the bidimensional environmental space; Schoener, 1968; Warren et al., 2008). D varies from 0 (complete disjunction) to 1 (fully overlapping niches).

The Schoener's D statistic was then used to perform two tests of niche differentiation: niche similarity and niche equivalency tests as detailed in Broennimann et al. (2012). The comparison of niche similarity and niche equivalency tests is given in supplementary material (Appendix 3.S1). All analyses were performed in the R platform (R Development Core Team, 2013).

3.2.3.3. Testing phylogenetic signal in morphology and environmental niche

We inferred pairwise phylogenetic distance between species [P] (patristic distance; function "cophenetic.phylo" in R package "ape"; Paradis et al., 2004) based on the phylogeny of the *Guibourtia* species. To test the existence of phylogenetic signal (Losos, 2008), we used two methods. First, for univariate data, we computed Blomberg's K (Blomberg et al., 2003) using the function "phylosig" (R package "phytools") for environmental niche and morphology separately using (i) species means of each quantitative morphometric and environmental variable, (ii) mean species scores along each morphological and niche PCoA axis. To increase the power of the test, we integrated also the estimated standard errors (SE) of the mean values for each species following Ives et al. (2007). Second, for pairwise distances, we performed Mantel tests (Mantel, 1967) between phylogenetic distance and PCoA-based morphological or niche distances. We increased the power of the test using the square root of phylogenetic distance matrix ($P^{1/2}$) as suggested by Hardy and Pavoine (2012).

3.2.3.4. Assessing the morphology - environmental niche relationship

To assess whether environmental niche divergence (PCoA-based niche distance) and environmental niche overlap (D statistic) were related to morphological divergence (PCoA-based morphological distance), we used Mantel tests. To further assess the morphology-niche relationship among *Guibourtia* species, we applied two-block partial least squares (2B-PLS, Rohlf and Corti, 2000) analysis to explore patterns of covariation between the mean species scores along the same number of PCoA morphological and PCoA environmental niche axes. We retained ten axes to summarize 100% of the total variation in the two datasets. The *Rv* coefficient (Robert and Escouffier, 1976) was used to summarize the amount of covariance in each dataset that is accounted for the other dataset. The significance of this statistics was assessed via permutation of the rows of each dataset.

3.2.3.5. Morphological adaptation and niche divergence accounting for phylogenetic inertia

Furthermore, to verify if the morphology-niche relationship is not simply due to phylogenetic inertia, we performed two types of analyses. First, partial Mantel test examined whether environmental niche was still related to morphological traits after

accounting for phylogenetic relationships (using a matrix of phylogenetic raw distance as co-variate). Second, we employed the Phylogenetically Independent Contrasts (PIC) approach (Felsenstein, 1985) to test the correlation between species scores along each of the first two morphological PCoA axes with each of the first two niche PCoA axes. PIC analyses were also done on each morphological trait weighting in the first two niche axes in order to identify the specific morphological traits selected by each niche axis. The null hypothesis for PIC test assumes no evolutionary link between two traits (evolution by purely random genetic drift without selection). All the analyses were performed in the R platform (R Development Core Team, 2013), using the functions "pic" (R package "ape") and "mantel.partial" (R package "vegan").

3.3. Results

3.3.1. Morphological variation among species and phylogenetic signal

The first two components of the PCoA-based morphological distance explained 32.50% and 26.40% of the total variation, respectively (Figure 3.2). The results showed that species associated to wet/moist climates (*G. ehie* and *G. arnoldiana* excepted) were characterized by the combination of the following main characters: large and long leaflets exhibiting marginal venation, subcoriace limb, apex of leaflet acuminate, axillary inflorescence, persistent and pilous bracteoles, no pedicels and no venation on the fruit (Appendix 3.3). By contrast, the species associated to dry and seasonal climates showed small leaflets with submarginal venation, coriace limb, apex of leaflet obtus, axillary and terminal inflorescence, obsolete and glabrous bracteoles, and venation on the fruit.

We detected an overall phylogenetic signal for some morphological traits (Appendix 3.7). Morphological similarity was significantly correlated to phylogenetic distance (Mantel test: r=0.24, p=0.031). This trend was also confirmed when Blomberg's K test was performed on PCoA-based morphological axes (K=1.2, p=0.017; K=0.80, p=0.130 for the first two axes). Among the 11 quantitative morphological traits, only four displayed significant K values (number of secondary leaf veins supra basilar, length of leaflets, petiole length and width of sepals; Appendix 3.3).

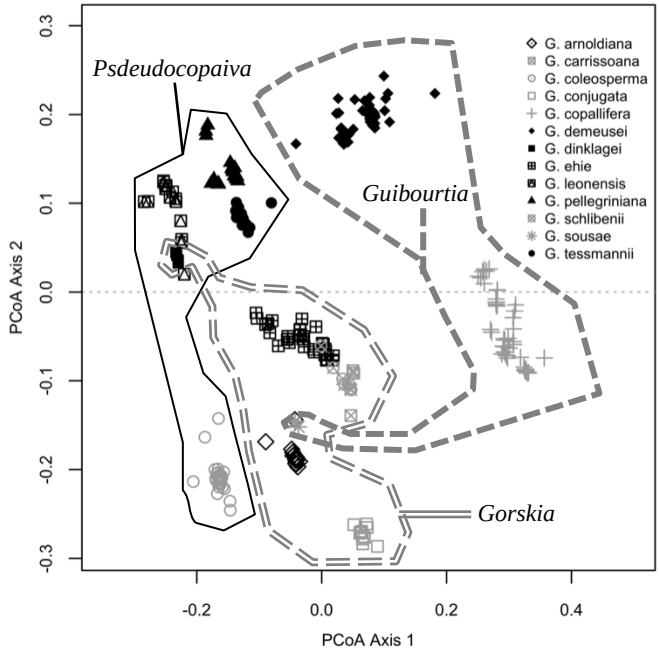


Figure 3.2. Principal Coordinate Analysis on 45 morphological traits measured on 281 *Guibourtia* individuals. The symbols in black represent species associated to wet/moist climates whereas those in grey illustrate species associated to dry and seasonal climates. The appartenence of species to three monophyletic subgenera (*Pseudocopaiva*, *Guibourtia* and *Gorskia*) is also shown. It is worth noting that the first PCoA axis is associated to these phylogenetic groups while the second axis is associated to wet/dry climate preferences.

3.3.2. Pattern of environmental niche evolution

The Principal Component Analysis (PCA-ent) of environmental niche properties applied on 401 occurrences data gave a first axis explaining 51.7% of the total variance generated by the eight tested variables and mainly loaded by monthly mean thermal amplitude and monthly mean solar radiation towards positive values, and monthly mean potential evapotranspiration and monthly mean precipitation towards negative values (Figure 3.3; Appendix. 3.4). The second axis explained about 19.9% of the variation and was mainly loaded by monthly mean wind speed and pH of the soil towards positive values, and monthly mean temperature towards negative values. All rain forest species showed a distribution in the environmental space with very low values on both PCA-ent axis 1 (around -4) and axis 2 (around -1, except for *G. leonensis*), which indicate high precipitation and low solar radiation. Dry forest and woodland species generally displayed highest density at less negative values along axis 1 (between -3 and 0) and tended to differentiate along axis 2 (highest density between -1 and 1 depending on species). The right part of axis 1 (scores > 0) would correspond to the desert zones where *Guibourtia* is absent.

Similarly, for axis 2 scores greater than 2 along axis 2 may correspond to low-temperature areas), inappropriate for the survival and growth of *Guibourtia* species.

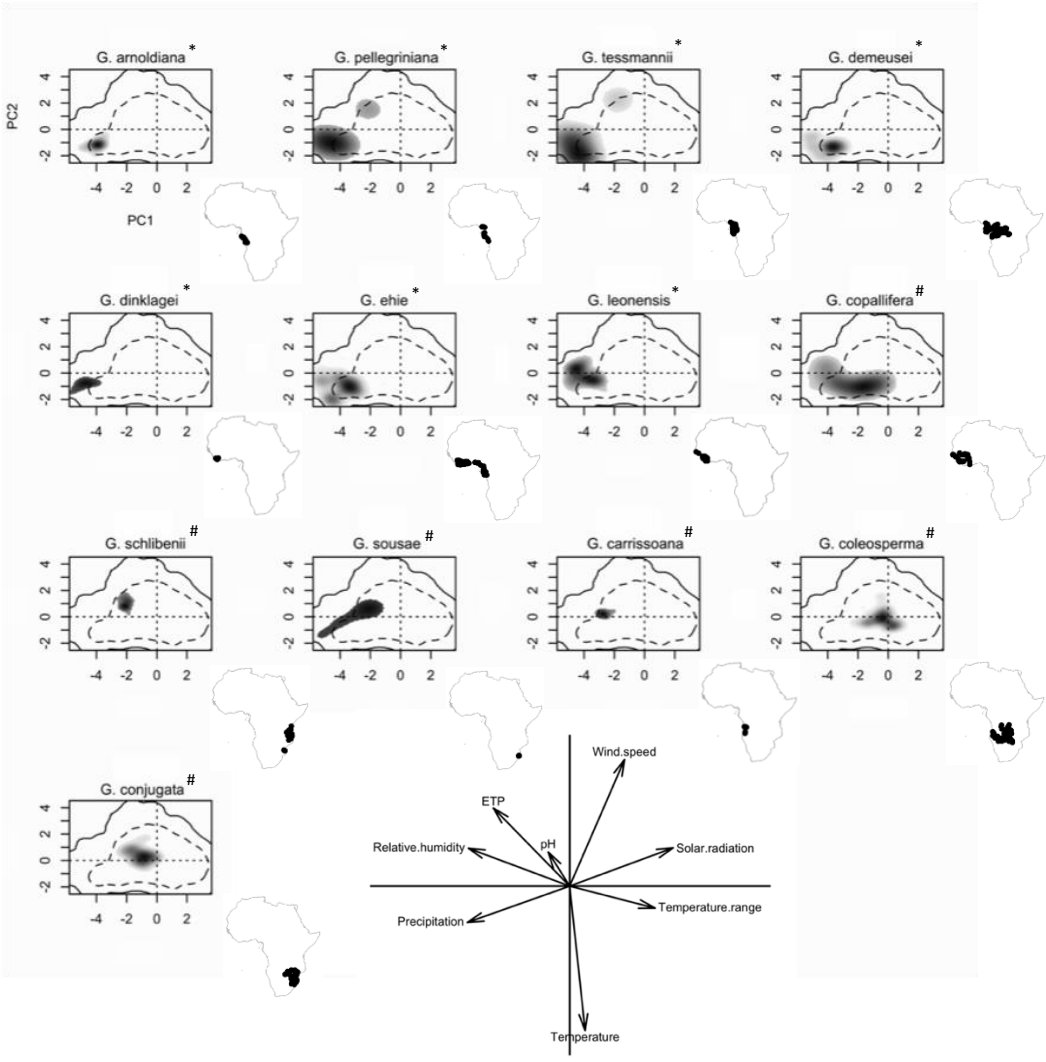


Figure 3.3. Environmental niches of the 13 African *Guibourtia* species in two main environmental axes produced by the principal component analysis (PCA-ent) applied on 401 occurrences. For each species, the grey-to-black shading represents the grid cell density of the species' occurrences (black being the highest density). The dashed and solid lines delimit respectively 50% and 100% of the available environment conditions in the study area. The last panel presents the contribution of variables for loading the main PCA-ent axes and the percentage of inertia explained by axes one and two. The geographical distribution of each species is presented below each PCA-ent. *: rain forest species. #: dry forest/savanna species.

As expected, niche overlap values showed that most pairs of rain forest vs dry forest and woodland species occupy different environmental niches (Figure 3.3, Appendix 3.S2). The results highlighted a more marked overlap between the niches of species associated to wet/moist climates than between species associated to dry and seasonal climates as confirmed by the results of niche equivalency (strict niche identity) and niche similarity tests detailed in supplementary materiel (Appendix 3.S3).

We detected a phylogenetic signal for species environmental niche through the correlation between phylogenetic distance and PCoA-based niche distance (Mantel test: r=0.16, p=0.04) or niche overlap (Mantel test: r=-0.23, p=0.025). However, the univariate Blomberg's K tests seemed to lack power (K=0.99, p=0.139; K=0.76, p=0.895 for the first two niche PCoA based niche distance axes, Figure 4) and when testing each environmental variable in turn, only monthly mean precipitation showed a marginally significant signal (Blomberg's K test: K=0.87, p=0.08) (Appendix 3.4).

3.3.3. Relationships between morphology and environmental niche without accounting for phylogenetic inertia

We found statistically significant correlations between species morphological similarity and niche overlap (Mantel test: r=-0.44, p<0.001) or PCoA-based niche distance (Mantel test: r=0.67, p=0.001). The 2B-PLS analysis confirmed the strong association between morphology and environment variation: 40 % of the covariance in morphology is explained by environmental conditions (*Rv*=0.40, p<0.001).

3.3.4. Morphological similarity and environmental niche resemblance accounting for phylogenetic inertia

The partial Mantel test between PCoA-based niche distance and morphological similarity using phylogenetic distances as a third matrix of covariates was statistically significant (r=0.24, p<0.030; Figure 3.4). Interestingly, when using only quantitative morphological traits which displayed no significant phylogenetic signal (length of stipe of the ovary, thickness of the fruit, stipe of the ovary, length of sepals, length of acumen and number of secondary leaf veins basilar, Appendix 3.6), the relationship between morphological similarity and PCoA-based niche distance was stronger (Mantel test between species: r=0.28, p=0.039) than using only traits that exhibited phylogenetic signal (Mantel test between species: r=0.12, p=0.787).

However, PIC analysis of the four pairwise comparisons between the two first PCoA-based environmental niche axes and morphological axes was significant only between Axis 2-morphology and Axis 1- environmental niche and marginally significant between Axis 1-morphology and Axis 2- environmental niche (Table 3.2). The morphological PCoA axis 2 gathered principally flower traits (inflorescence position, type of inflorescence, pedicel, bracts, bracts hairiness and pilosity of flower bud on the external face of the sepals), some leaflets and fruit traits (leaflets dimension and venation, veins on the fruit surface while axis 1 essentially summarized fruit traits (type of fruit, thickness of the fruit, gland on fruit, stipe of the fruit), seeds (arillus on the seed) and some leaflets and flowers traits

(number of leaflet veins, aspect of bracteoles and sepals; Appendixes 3.3, 3.5 and 3.8). Concerning the environmental niche axes, niche PCoA axis 1 was correlated with solar radiation, relative humidity, precipitations and temperature range. This axis exhibits rain forest-dry woodland gradient whereas axis 2 was related to wind speed and potential evapotranspiration.

Table 3.3. Correlation between morphological and environmental niches along the first two PCoA axes by phylogenetically independent contrasts

Niche optimum / Morphology	Axis 1 (*solar radiation, relative humidity, precipitations and temperature range*)	Axis 2 (*wind speed and potential evapotranspiration*)
Axis 1 (*fruit traits, seeds, leaflets and flowers traits*)	r=-0.33; p=0.290	r=-0.53; p=0.075
Axis 2 (*flower, leaflets and fruit traits*)	**r=0.69; p=0.018**	r=-0.44; p=0.144

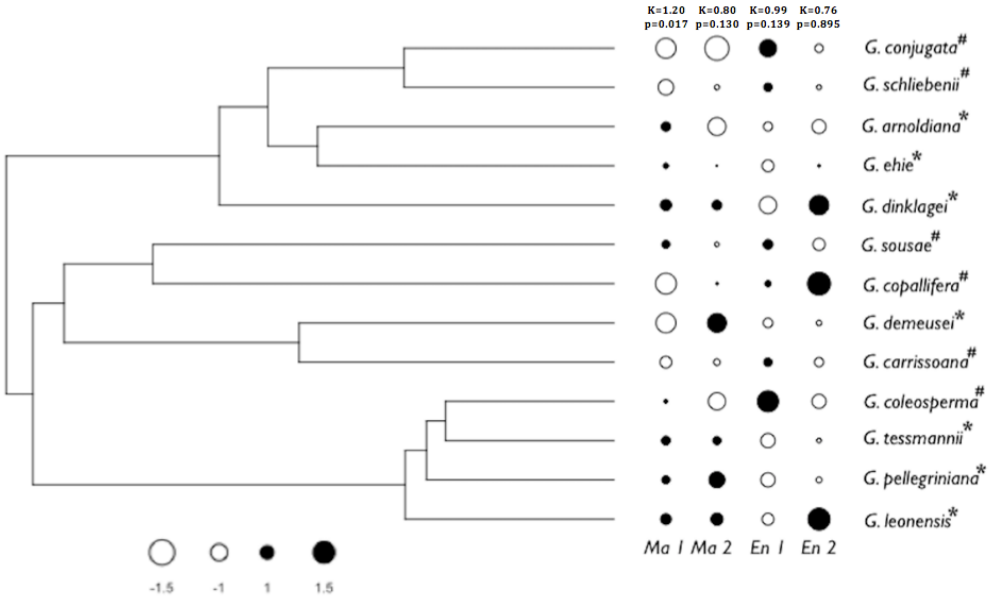


Figure 3.4. Phylogeny of African *Guibourtia* species with species scores along the first two PCoA axes of morphological data (Ma1, Ma2) and environmental niche data (En1, En2). *: rain forest species. #: dry forest/savanna species.

3.4. Discussion

In this study we demonstrated that morphological differences between African *Guibourtia* species are significantly associated with niche divergence, even after factoring out the impact of phylogenetic inertia. This trend was particularly evident when floral traits and leaf dimensions were considered along a rain forest – dry woodland gradient. These findings validate a set of scenarios highlighting (i) both phylogenetic inertia and convergent evolution (scenario 4) and (ii) an independent evolution of some morphological traits and environmental niche (scenario 1). They suggest that both selection forces and neutral ones contributed to the morphological divergences and similarities among *Guibourtia* species.

3.4.1. Niche overlap, equivalency and similarity

A comparison of niches of pairs of African *Guibourtia* species showed low and high values of niche overlap that can be used to interpret the morphological differentiation patterns. The low values of pairwise niche overlap could be explained when the spatial distributions for pairs of rain forest vs dry woodland species are compared. As explained by Acevedo et al. (2014), if the rain forest species cannot adapt to suboptimal environmental conditions of dry woodland and savanna, then the barrier will continue to prevent gene flow between these species, and they will inevitably continue to diverge according to the allopatric speciation model, although regular gene flow may occur in the contact zones (Kozak and Wiens, 2006). We observed that 52.38% of pairs of rain forest species occupied niches which exhibited some kind of similarity according to similarity test (23.81% according to niche equivalency test), while only 26.67% of pairs of dry forest and woodland species occupied similar niches according to similarity test (0% according to equivalency test). This result suggests that dry forests and woodlands could have a larger diversity of habitat types and environmental constraints than in rain forests (Favier et al., 2012).

Our results also indicated very few cases of species pairs exhibiting equivalent niches (three pairs of rain forest species). Niche equivalency and niche similarity tests are not always congruent because niche equivalency implies niche similarity but not the reverse. Niche similarity means that two species are more similar than a random expectation, not that they are strictly equivalent. This finding is congruent with what is expected for environmental niches of closely related species that will not be equivalent but more similar (Warren et al., 2014). Moreover, niche equivalency test is more conservative than the niche similarity test (Aguirre-Gutiérrez et al., 2015) since the former is more sensitive to the geographic location of species pairs.

3.4.2. Speciation and evolutionary hypotheses within the genus **Guibourtia**

Considering that geographical and ecological factors could contribute to species divergence, two main hypotheses could be proposed to further understand species evolution within the genus *Guibourtia*. The first hypothesis is disruptive ecological adaptation (Knouft et al., 2006), which assumes that ecological niches tend to diverge in near relatives, reducing inter-specific competition (Losos et al., 2003). The second one deals with the fragmentation of favourable habitat leading to related species (Acevedo et al. 2014). This hypothesis supposes that the ancestor of related species occupied a large range that became fragmented in different allopatric refuges following past climatic fluctuations. Tosso et al. (2018) demonstrated that *Guibourtia* started to diversify ~14-24 Ma and continued until Late Miocene and Early Pliocene as confirmed by Fourgère-Danezan et al (2005). The two first divergence events that occurred ~14.8 – 16 Ma (Tosso et al., 2018) ultimately lead to three clades corresponding to the subgenera *Gorskia*, *Guibourtia* and *Pseudocopaiva* described by Léonard (1949). This period coincides with the second major environmental perturbation that occurred from the Early to the Mid-Miocene (23–15mya): humid vegetation disappeared in the Sahara, the continent moved northwards, down positioning the Equator, the rain forest belt shifted southwards (Maley, 1996). During the Late Miocene and Early Pliocene, and especially with the onset of the glacial–interglacial cycles of the Quaternary, the African rain forest probably became fragmented while drier ecosystem types (dry dense forest, woodland, savanna) expanded (Dupont et al., 2013). Our results, in combination with the above-mentioned work, could help hypothesize that the common ancestor of *Guibourtia* species probably occupied a large range in Africa. However, the common ancestor may also have had a restricted area and speciation occurred mainly during geographical expansions.

In addition to these two main hypotheses mentioned previously, it's important to underline three important points. First, each subgenus (Appendix 3.2) presents a wide distribution from West Africa to Southern Africa via Central Africa. Thus there is no biogeographic signal between these three clades. Second, there is a diversity of configurations between sister species: (i) there are cases of parapatric distribution along environmental gradients that may suggest ecological speciation (*G. demeusei* vs *G. carrissoana*, *G. coleosperma* vs *G. tessmannii*), (ii) also cases of parapatric to sympatric distribution without strong divergence of niche suggesting allopatric speciation with secondary contact (*G. conjugata – G. schliebenii*; *G. arnoldiana – G. ehie*) and (iii) a case of allopatric distribution, West Africa vs Southern Africa, without strong niche divergence (*G. sousae* vs *G. copallifera*, but which diverged formerly). Third, it seems that biome transitions occured as well from dry environments to moist forest environments (case of *G. demeusei* based on the phylogeny and the dry environment occupied by *G. carrissoana, G. copallifera and G. sousae* in the subgenus *Guibourtia*) as in the opposite direction (case of *G. coleosperma* in subgenus *Pseudocopaiva*, and of the clade formed by *G. conjugata* and *G. schliebenii* in the subgenus *Gorskia*). The reconstitution of

ancestral habitat types (presented in chapter 2; Figure 2.4) seems in favor of a transition scenario from moist forest environment to dry environment, between 3 and 5 Ma during the Pliocene. This result of ancestral biome reconstruction is congruent with several others studies that reported that Pleistocene climatic fluctuations had strongly affected species diversity through subsequent transitions between forest and savanna ecosystems (Maley, 2000; Bonnefille, 2011). According to Morley (2000), the successive rain forest expansions and fragmentations that occurred during the Pliocene would have generated the geographical isolation of plant species. In the genus *Guibourtia*, we found multiple biome transitions. Multiple biome transitions have also been detected during this period in herbaceous plants, for instance in the genera *Coccinia* (Holstein and Renner, 2011) and *Begonia* (Plana et al., 2004). This is explained by the fact that they have faster rates of molecular evolution when compared to tree taxa (Smith and Donoghue, 2008). However, this signal of biome transition is not always observed in African tree species. In the genus *Afzelia*, Donkpegan (2017) supposed only one biome shift between dry and moist environments, combined with polyploid speciation during the Miocene. In genera *Isolona* and *Monodora*, the origin of species were dated before or at the beginning of the Pleistocene suggesting that the Pleistocene climatic fluctuations was not a major driver of speciation (Couvreur et al., 2008; Couvreur et al. 2011a). Additionnal studies are thus needed to assess the frequency of biome transition patterns on African tree species during the Pleistocene climatic fluctuations.

Based on all these observations, it is difficult with the current data to favor a particular mode of speciation within African *Guibourtia* taxa although convergent evolution was observed in certain morphological traits.

3.4.3. Relationship between morphology, phylogeny and environmental niche: evidence of adaptive forces

The recent incorporation of phylogenetic and functional information into biogeographical analyses provides a more complete understanding of evolutionary and ecological processes (Molina-Venegas et al., 2015). In this study, we highlighted that morphological traits could show a phylogenetic signal. This finding is consistent with works of Lee and Collins (2001), Blomberg et al. (2003) and Valverde-Barrantes et al. (2015) who found that similarity in morphometric forms largely reflects genealogical relationships.

We proved that *Guibourtia* morphological traits were correlated with environmental features. This correlation was not only due to phylogenetic inertia as shown by: (i) the results of PIC analysis for two particular M and E axes (PIC: $r=0.69$, $p=0.018$); (ii) the mantel correlation between environmental niche and morphology traits (Mantel test $r=-0.44$, $p<0.001$) which was stronger than the one between the morphology and phylogeny (Mantel test $r= 0.24$, $p=0.031$) but also between the phylogeny and environmental niche (Mantel test $r= 0.16$, $p=0.04$); (iii) the correlation morphology-environmental niche always present when the morphological traits exhibiting no phylogenetic signal were considered (Mantel test $r= 0.31$, $p=0.008$).

The overall signal between morphology and environmental niche was significant in rain forest species especially when only the reproductive traits were considered. It was also significant for woodland species when all the morphological characters were taken into account together. Among species having dehiscent fruits and arillate seeds dispersed by animals, three are found in rain forests (*G. tessmannii*, *G. pellegriniana* and *G. leonensis*) and one in dry forests (*G. coleosperma*). Except the latter one, dry forest and woodland species have indehiscent fruits with non arillate seeds dispersed either by wind or water. This finding was highlighted by Turner (2001) and Chazdon et al. (2003) and confirms the domination of animal dispersed plants in rain forest.

Besides, the relation between PCoA-based niche distance and morphological dissimilarities was stronger for the PCoA axes considering principally flower traits and leaflets dimension along the rain forest-dry woodland gradient, which evokes selection forces acting on these phenotypic characters. Rain forest species (e.g. *G. tessmannii*[*], *G. pellegriniana*[*], *G. leonensis*[*] etc.) have longer and larger leaflets than woodland species (*G. sousae*[#], *G. conjugata*[#], *G. carrissoana*[#] etc.). As leaf area index is among the best indicator to assess the adaptation of species to light conditions for photosynthesis (Hoffmann et al., 2005), a relationship between leave size and environmental niche is expected. Species living in rain forests invest much more in leaf area (adaptation for a competitive light environment) in comparison to species of more open woodlands, while the latter invest more in root development to improve their capture of soil water during the dry season (adaptation to water stress, Hoffmann and Franco, 2003). We also observed that floral traits could be selected. Rain forest *Guibourtia* species have axillary inflorescences while the woodland species have axillary and terminal inflorescences. Herrera (1996) explained this phenomenon by an adaptation to insect pollinators. This kind of adaptation deserves to be more deeply investigated in African biomes.

Even though it is known that each species has specific morphological responses depending on the environmental conditions to which it is subjected to (Gratani et al., 2003), our results are among the few in Africa that prove that part of the morphological differences between congeneric tree species result from environmental adaptation. This is in line with some studies conducted within various genera which have found the same association (Fontanella et al., 2012; Cicero and Koo, 2012; Fort et al., 2015). Ribeiro et al. (2014) also demonstrated that niches differences among species are a evolutionary force shaping diversification but studies did not clearly demonstrate a direct correlation between morphology and environmental niche. However, these results contradict those of Couvreur et al. (2011a) who have proven that adaptation to climatic differences between sister species has not been a major driver of speciation in trees of African tropical rain forests.

However, as shown in our results, the relationship between morphology and environmental niche was not evident for all morphological traits. When some leaflets and flowers traits (number of leaflet veins, aspect of bracteoles and sepals) are considered along the rain forest-woodland gradient, no significant link was

detected between morphology and environmental niche. This result underlines the possible action of genetic drift as an evolutionary force, although one cannot rule out the hypothesis that selection acted again but in response to other factors and climate (Felsenstein, 1985).

3.5. Conclusion

This study is the first in Africa that clearly demonstrated that similar phenotypes evolve independently in different lineages by combining new genomic data set and environmental niche modelling techniques with morphological characterisation using herbaria collections. We characterized the main environmental variables that constrain the potential distribution of African *Guibourtia* species. We also assessed the diversity and similarity of the environmental niches of these species and demonstrated phylogenetic signals of environmental niche, at some morphological traits and significant correlation between niche divergence and morphological divergence, even after accounting for phylogenetic inertia, at least for some of their respective dimensions. These results suggest that convergent evolution has occurred. The significant differences in environmental niches spaces also reflect the morphological distances within African *Guibourtia* species due both to neutral processes (e.g. drift) and selection forces at a certain level.

The link demonstrated between morphology and the environmental niche for African *Guibourtia* species could also serve as a basis for predicting long-term phenotypic changes at the species level in the genus.

Chapitre 4

When ecophysiology reveals the adaptive capacities within African tropical congeneric tree seedlings: case study of the genus *Guibourtia* Benn.

Le chapitre 4 examine l'effet de la variation environnementale sur la survie et la plasticité phénotypique des traits fonctionnels de trois espèces du genre *Guibourtia* (*G. ehie*, *G. coleosperma* et *G. tessmannii*). Par une approche expérimentale, il étudie la croissance et les traits fonctionnels de plantules de ces trois espèces cultivées dans sept environnements d'éclairement relatif croissants de 1% à 100%. A la suite du chapitre 3, il questionne également sur le rôle de la sélection naturelle dans la diversification du genre. Ce chapitre met en exergue que la lumière constitue un important facteur de différenciation adaptative entre ces trois espèces.

4. When ecophysiology reveals the adaptive capacities within African tropical congeneric tree seedlings: case study of genus *Guibourtia* Benn.

Félicien Tosso, Olivier J. Hardy, Kasso Daïnou, Adeline Fayolle, Marc Aubinet, Christine Moureaux and Jean-Louis Doucet

Abstract

Understanding how light heterogeneity affects phenotypic plasticity of tropical tree species allows to predict the effect of environmental change on their fitness. In this paper, we used growth, biomass allocation, morphological and photosynthesis traits in a comparative and experimental framework to assess the phenotypic plasticity in response to light of congeneric species belonging to the genus *Guibourtia* Benn. (Fabaceae-Detarioideae). During 22 months, the development of seedlings of three *Guibourtia* species (*G. ehie*, *G. tessmannii* and *G. coleosperma*) was assessed under controlled conditions, at seven different light levels (1%, 4%, 10%, 25%, 40%, 60% and full sunlight) simulating natural light gradient from shaded forest understory to large canopy opening. *Guibourtia tessmannii* (rain forest species) and *G. coleosperma* (dry forest/woodland species) belong to the same subgenus while *G. ehie* (rain forest species) belongs to another subgenus. These three species were used to check to what extent two sister species occurring in contrasting environments conserve their traits more than two species living in similar environments but genetically more distant. The results showed that *G. tessmannii and G. ehie* turned out to be non-pioneer light demanding and deciduous species while *G. coleosperma* was a light demanding and evergreen species. The two rain forest species exhibited high phenotypic plasticity in low light level in comparison to dry forest species. This suggests that *G. coleosperma* is a poor competitor in shaded environment and probably evolved when rain forest retracted during Pliocene climatic oscillations. Implications for the sustainable management of the populations of these three species (including one listed on appendix II of CITES) are discussed.

Key words: Africa, tropical forests, phenotypic plasticity, *Guibourtia*, light response, functional traits

4.1. Introduction

Plants display phenotypic plasticity in reponse to temporal and spatial environmental heterogeneity through physiological, molecular and genetic responses (Reyer et al., 2013, Shaw and Etterson, 2012). In particular, phenotypic plasticity may be essential for tree species survival in heterogeneous and variable environments (Bradshaw, 1965, Sultan, 1992). In a context of global change, studying phenotypic plasticity helps to understand how tree species will respond to new climatic scenarios and what will be the functional processes involved (Van Kleunen and Fischer, 2007, Matesanz et al., 2010).

The phenotypic plasticity may be complementary to the evolutionary specialization which poses that genotypes are strongly selected by environment, leading in specialized ecotypes (Lortie and Aarssen, 1996). Valladares et al. (2000) associated specialization in a favourable environment with an increase of phenotypic plasticity that can lead, at community scale, to an increase biological diversity (Valladares et al., 2000). To date, most research focused on abiotic factors, such as light and water, to understand the phenotypic plasticity of plant species (Markesteijn and Poorter, 2009). In forests and woodlands, light varies in quality and quantity beneath plant canopies, between gap and understory locations (Valladares et al., 2016). Species developed specific functional adaptations to cope with light heterogeneity. For instance, the ability of tree species to colonize low light environment is associated with enhanced survival as assumed by the conservative resource-use strategy (Veneklaas and Poorter, 1998, Walters and Reich, 1999). The difference in light heterogeneity can lead to interspecific differences in the capacity of tree species to tolerate shade or to compete for light (Valladares et al., 2016).

Several experimental researches targeting tropical forest species have demonstrated that tree species respond to light in different ways, in terms of morphological, physiological and biomass allocation traits (Agyeman et al., 1999, Poorter, 1999, Markesteijn and Poorter, 2009, Biwolé et al., 2015, Lestari and Nichols, 2016). Most of these studies are consistent with the results of Laanisto and Niinemets (2015) who assumed that shade tolerance is at the centre of many ecological processes at population and ecosystem levels. But the interpretation of such experiments could depend on the type of the traits measured (Gratani, 2014). Most studies focussed on traits related to strategies of resources assimilation, biomass allocation and growth but physiological traits such as photosynthetic traits have often been neglected while they are very useful to fully interpret survival strategies (Evans, 1989).

Furthermore, the comparison of phenotypic plasticity between congeneric species is little addressed [but see (Valladares et al., 2000, Barros et al., 2012)], while it may provide important information to further understand species diversification and evolutionary patterns (Rutherford et al., 2017). Several studies done in southern America confirm that phylogenetically related tree species from savanna and forest ecosystems represent two distinct functional types (Barros et al., 2012). However, the case of African plant taxa has received little attention.

Here, we explore the phenotypic plasticity of species of the African genus *Guibourtia* Benn. Belonging to the Fabaceae-Detarioideae, the genus *Guibourtia*

comprises 13 species found in contrasting environments (rain forests, dry forests/savannas; Tosso et al., 2015), and it is subdivided in three monophyletic subgenera (Tosso et al., 2018). In the present study, we used standardized experimental conditions simulating different light environments as described by Agyeman et al. (1999) to study the phenotypic response of seedlings. Three species were used: *G. ehie* (A.Chev.) J.Léonard (1949), *G. tessmannii* (Harms) J.Léonard (1949) and *G. coleosperma* (Benth.) J.Léonard (1949). The first two are found in evergreen and semi-deciduous rain forests and are sympatric in Cameroon and Gabon (Tosso et al., 2015) while *G. coleosperma* is a dry forest/woodland species (Tosso et al., 2015). *Guibourtia tessmannii* and *G. coleosperma* belong the same subgenus (Tosso et al., 2018) but occur in different biomes, making them ideal to understand how evolution changed traits and their plasticity evolved. *Guibourtia tessmannii* and *G. ehie* are rain forest trees species occurring locally in sympatry but they do not belong to the same subgenus. These two species are then also ideal to check whether the same response to light was selected or if they show niche differentiation in shared habitat.

In this study, we used growth, biomass allocation, morphological and photosynthesis traits to assess the phenotypic plasticity in response to light of the seedlings of these three congeneric species. We addressed in this study the following questions: (1) How the studied traits change according to light intensity? (2) To what extent two sister species occurring in contrasting environments conserve their traits more than two genetically more distant species living in similar environments? (3) Are the rain forest species more plastic than dry forest / woodland species in low light conditions? As we used in this study seedlings from seeds collected from geographically very close mother trees, we assume that the genotypic variability between seedlings of the same species will be low, so that the response of seedlings of each species in a novel environment can be considered as phenotypic plasticity in the sense of Bradshaw (1965).

4.2. Materials and methods

4.2.1. Study species

The target tree species of the present study were: *G. coleosperma, G. ehie* and *G. tessmannii*. *Guibourtia coleosperma* is a light-demanding and evergreen species living in woodlands and distributed from DRC to Zimbabwe (Bamford *et al.*, 2005; Tosso *et al.*, 2015). *Guibourtia tessmannii* is a deciduous tree species having intermediate light requirements and found in evergreen rain forests from Cameroon to Gabon (Doucet, 2003; Tosso et al., 2015). *Guibourtia ehie* may have similar light requirements as *G. tessmannii* and is a tree species frequently distributed in evergreen and semi-deciduous rain forests from Liberia to Gabon (Doucet, 2003; Tosso *et al.*, 2015). They are all timber species of economic importance.

4.2.2. Study site

The experiment was carried out during two years (starting in January 2015) at Ma'an (2°15'N, 11°20'E) in south-western Cameroon. The climate is equatorial and characterized by an annual temperature of 23.8°C, a mean annual rainfall of 1,686 mm with two distinct rainy seasons: March-June and September-November (Olivry, 1986). The elevation is around 600 m and the soils are classified as Ferrasols (Jones et al., 2013). The vegetation in the region is classified by Letouzey (1985) as the Atlantic Biafran forest dominated by Fabaceae and other coastal indicators (Biwole et al., 2015).

4.2.3. Light treatments

The seedlings were grown at seven different light levels: 1%, 4%, 10%, 25%, 40%, 60% of unshaded irradiance, and 100% corresponding to full sunlight. The light levels 1-60% were obtained in six shadehouses spaced 3 m away from each other in an open area and positioned perpendicularly to the daily track of sun. Each shadehouse had a height of 1.8 m, and a basal area of 2 m x 3 m. They were obtained by varying the number and spacing between slats of *Raphia* sp. fixed on the sides and roof of the shadehouses. The seventh light level consisted of a platform completely exposed to full light (100 % of incident light). In each shadehouse, Photosynthetically Active Radiation (PAR) sensors (PAR/CBE 80, Solem S.A., Palaiseau, France), which measure photosynthetic photon flux density (*PPFD*) were used to calibrate the light level. Measurement campaigns over seven days were organized in March, August and November 2015, February and July 2016 and March 2017. These measurement campaigns helped to adjust *Raphia* slats when needed. The temperature (*T*) and relative humidity (*RH*) were recorded using data logger Ebro EBI 20-TH1 during the last four campaigns. The temperature and relative humidity data were used to compute vapour pressure deficit (*VPD*; Murray, 1966). This climatic variable, measuring the "drying power" of air, plays an important role in determining the relative rates of growth and transpiration of plants (Monteith and Unsworth, 2007).

The six light measurement campaigns confirmed that the initial light level within each shadehouse was maintained over the experimental period (Appendix 4.1) and showed a larger inter-treatment variation than intra-treatment variation. In the November 2015 (dry season) measurement campaign, we noticed great variation among shadehouses in minimum and maximum values of temperature, relative humidity and vapour pressure deficit. For the other measurement campaigns, there was no significant difference between shadehouses. The climatic variables in each shadehouse (temperature, humidity and vapour pressure deficit) were generally strongly correlated with light and could probably slightly alter the impact of light: light was positively correlated with temperature and vapour pressure deficit while it was negatively correlated with relative humidity (Appendix 4.2).

4.2.4. Experimental design

Seeds of *G. ehie*, *G. coleosperma* and *G. tessmannii* were collected in Ivory Coast (6°12'N and 4°3'W), Namibia (17°51'N and 19°40'E) and Cameroon (2°13'N and 10°22E), respectively. The seeds of each studied species were collected from five to eight individuals of known geographic populations. The seeds were sown in 5-litre polystyrene bags (35 cm high, 15 cm diameter) containing: (i) one-third forest top soil that provides a substrate with natural nutrients essential for seedling growth, (ii) two-thirds river sand that was appropriate to facilitate the daily watering and the harvest of the whole root system. The composition in macro and micronutrients of the soil used to fill the bags is described in Appendix 4.3. The seeds were germinated in a nursery having 30% of light level (*PPFD*, 599 ± 52 µmol/m^2/s) and the seedlings were maintained in this environment until the cotyledon leaves of seedlings were fallen off as suggested by Kitajima (1996). At the end of the corresponding 32 day period, 20 seedlings of *G. ehie*, 13 of *G. coleosperma* and 27 of *G. tessmannii* were installed in each shadehouse. This sample size variation among species resulted from the limited plant material available. In each light treatment, we used an experimental design constituted by individuals of the three species, which were installed in the number mentioned above. Seedlings were watered every day without restriction.

4.2.5. Functional traits measurements

4.2.5.1. Photosynthesis measurements

Measurements of gas exchange were performed in January 2017 using the LICOR 6400 portable photosynthesis system (LI-Cor, Lincoln, NE, USA). The photosynthesis measurements were done on 10 seedlings randomly chosen per species and light treatment. Two complete leaves were sampled per individual for the measurements which were done from 7:00 to 13:00 on well-watered seedlings to avoid the effect of photoinhibition (Kenzo et al., 2007, Mengistu et al., 2011). We then constructed light response curves, which represent a function of photosynthetic photon flux density (*PPFD*, µmol/m^2/s) and net photosynthesis (*A*, µmol CO_2/m^2/s). These light response curves (Appendix 4.4) were used to generate the set of photosynthetic parameters. A light curve program was used to automatically vary the LI-6400's LED light source levels starting from 2500, 1500, 1000, 500, 200, 100, 50, 20 to 0 µmol/m^2/s. A duration of 3-5 min at each light level was selected thanks to preliminarily observations showing that this time was enough to reach stable photosynthetic rate during the measurement at each light level. Inside of the LI-6400 IRGA leaf chamber environment, the temperature and the CO_2 concentration were maintained at 27 °C and 400 µmol/m^2/s (current CO_2 concentration in the atmosphere), respectively. This temperature is close to that of these species in their natural habitat (Stors, 1979 and Tosso et al., 2015).

To generate the set of photosynthetic parameters with the light response curves, equation 1 was used.

$$A = \frac{A_{qe} \times I \times A_{max}}{\sqrt{A_{qe}^2 \times I^2 + A_{max}^2}} - R_d \text{ (eq. 1)}$$

This equation (eq.1) was derived from Thornley et al. (1976), Marshall et al. (1980) and Ye et al. (2007). *A* represents the net photosynthesis (µmol $CO_2/m^2/s$), *I* the light (photosynthetic photon flux density (PPFD)), A_{qe} the apparent quantum efficiency (initial slope of the curve, (µmol $CO_2/m^2/s$)/(µmol (PPFD)/m^2/s)), A_{max} the maximum of net photosynthesis (the asymptote of photosynthesis, µmol $CO_2/m^2/s$) and R_d the dark respiration rate (the y-intercept of the light response curve, µmol $CO_2/m^2/s$). The parameters were used as photosynthesis traits in the next analyses in order to appreciate the photosynthetic capacities of each species in the different light treatments (Table 4.1).

4.2.5.2. Morphological and biomass allocation measurements

During 22 months of experiment (from April 2015 to January 2017) the following parameters were measured: the stem length, the stem collar diameter, the number of leaves, the number of branches and the mortality. At the end of the experiment, these values were used to calculate the following growth variables: height relative growth rate (*RGRh*, related to shade avoidance strategy, Poorter, 1999) and diameter relative growth rate (*RGRd*, estimator of plant growth performance, Poorter, 1999) (Table 4.1).

Other functional traits were measured at the end of the experiment in order to determine variables related to light capture at the leaf level. We computed: the Specific Leaf Area (*SLA*; Perez-Harguindeguy et al., 2013), the Leaf Shrinkage (*SL*; Hiziroglu, 2007), the Leaf Moisture Content (*LMC*; Behm et al., 2004) and the Leaf Dry Matter Content (*LDMC*; Perez-Harguindeguy et al., 2013). We used three samples of leaves per plant (including leaves used for photosynthetic measurements). We then measured leaf area (including petiole) for each fresh leaf using an electronic scanner (HP ScanJet 200) and ImageJ 1.51 (Wayne Rasband, National Institues of Health, U.S.A., http://rsb.info.nih.gov/ij). The same samples of leaves were oven dried for 72 hours at 70°C and leaf area was again measured as well as the weight. We also measured leaf thickness (mean of leaflet thickness related to physical strength of leaves, Perez-Harguindeguy et al., 2013) for the same leaves using digital callipers at two different positions. We paid attention to take into account only the leaf blade located between the leaf nerves.

To calculate the biomass allocation, we weighed leaves, stem and roots separately for the 10 individuals (those used for the photosynthesis analyses) after oven drying them as explained above. The root length for each individual was measured using a ruler. The total area of leaves was also determined as explained for the determination of leaf area. The mass and leaf area of the leaves mentioned above (used for *SLA* calculation) were taken into account. We used the protocol described in Perez-Harguindeguy et al. (2013) to compute : the Leaf Mass Ratio (*LMR*), the Stem Mass Ratio (*SMR*), the Root Mass Ratio (*RMR*), the Leaf Area Ratio (*LAR*), the Leaf Area Root Mass Ratio (*LAMR*) and the Specific Root Length (*SRL*). The

first three variables are the indictor of strategies of resource allocation in plants and describe how plants cope with environmental constraints (Dent and Burslem, 2009). *LAR* and *LAMR* are related to light capture at the leaf level and resources assimilation (Perez-Harguindeguy et al., 2013) and *SRL* to drought resistance (Comas and Eissenstat, 2004).

Table 4.1. Synthesis of functional traits used in this study

Funtional traits (Abbreviation)	Calculation	Units
Plant size		
Seedlings total biomass	-	g
Height	-	cm
Height Relative Growth Rate (*RGRh*)	$[\ln(h_2)-\ln(h_1)]/t_2-t_1$	yr^{-1}
Diameter Relative Growth Rate (*RGRd*)	$[\ln(d_2)-\ln(d_1)]/t_2-t_1$	yr^{-1}
Biomass allocation		
Leaf Mass Ratio (*LMR*)	Leaf mass/ total plant mass	$g\ g^{-1}$
Stem Mass Ratio (*SMR*)	Stem mass/ total plant mass	$g\ g^{-1}$
Root Mass Ratio (*RMR*)	Root mass/ total plant mass	$g\ g^{-1}$
Morphology		
Number of leaves	-	-
Number of branches	-	-
Leaflet thickness	-	mm
Specific Leaf Area (*SLA*)	Leaf area/leaf mass	$cm^2\ g^{-1}$
Leaf Shrinkage (*SL*)	(Fresh leaf area-dry leaf area)/fresh leaf area	$cm^2\ cm^{-2}$
Leaf Moisture Content (*LMC*)	Fresh leaf instant mass-oven dry mass)×100/ovendry leaf mass	$g\ g^{-1}$
Leaf Dry Matter Content (*LDMC*)	Fresh leaf mass/oven dry leaf	$g\ g^{-1}$
Leaf Area Ratio (*LAR*)	Leaf area/total plant mass	$g\ g^{-1}$
Leaf Area Root Mass Ratio (*LAMR*)	Total leaf area/oven dry root mass	$cm^2\ g^{-1}$
Specific Root Length (*SRL*)	Root length/oven dry root mass	$cm\ g^{-1}$
Photosynthesis		
Maximum of net photosynthesis (A_{max})	Asymptote of the light response curve	$\mu mol\ CO_2/m^2/s$
Dark respiration rate (Rd)	y-intercept of the light response curve	$\mu mol\ CO_2/m^2/s$
Apparent quantum efficiency (A_{qe})	Initial slope of the light response curve	$\mu mol\ CO_2)/(\mu mol$ (PPFD))

* For *RGRh* and *RGRd*, h_1/d_1 and h_2/d_2 represent respectively seedling stem length (*h*) or stem diameter (*d*) respectively, at times t_1 (first measurement in April 2015) and t_2 (last measurement in January 2017).

4.2.6. Statistical analyses

To quantify species survival in light treatments, the survival function *S(t)* was estimated over time using the non-parametric Kaplan-Meier estimator (Harrell, 2015). The survival function *S(t)* measures the probability that an individual will survive beyond time *t*, the time since the beginning of the experiment (eq. 2):

$$S(t) = \prod i | ti \leq t \ \left(1 - \frac{di}{ni}\right) \text{ (eq. 2)}$$

Where *ti* corresponds to time interval, *di* is the number of deaths recorded in the interval time *ti*, *ni* represents the number of seedlings that are alive at the end of interval-censored *ti*, and Π describes the product of all cases less than or equal to *t*. The survival curves were compared using G-rho family of tests (Harrington and Fleming 1982).

To evaluate the association among seedling traits in the three species, we used principal component analysis (PCA). The 20 traits (Table 1), except *SMR* (in order to avoid collinearity with biomass allocation variables), were used as continuous variables after normalization

As suggested by Markesteijn and Poorter (2009), proportional functional traits were arcsine-transformed (*LMR*, *SMR*, *RMR*, A_{qe}) and the other parameters (Table 4.1) were $\log_{10}$-transformed before the analyses to improve normality and reduce heteroscedasticity. In order to test the effect of light, species and seedlings biomass on traits, we used two-way nested ANCOVA, with light and species as fixed effects and seedling biomass as covariate. The equivalent of R^2 was calculated and symbolized by η^2. It represents the amount of variation explained by light, species and seedlings biomass and was calculated by dividing the sum of squares of the effect by the total sums of squares of the model ($\eta^2 \times 100$). As the model showed that there was not strong effect of seedling biomass on most traits, we did not correct for ontogenetic effect. All statistical analyses were done using R.2.15.0 (Team, R., 2013).

4.2.7. Phenotypic plasticity

To test the hypothesis that rain forest species are more plastic under low light level in comparison to woodland species, we estimated phenotypic plasticity for each studied trait (Table 4.1) in each species using the relative distance phenotypic index (*RDPI*) described by Valladares et al. (2006) (eq. 3):

$$RDPI = \sum [\ |x_{i'j'} - x_{ij}| / (x_{i'j'} + x_{ij})\ ] / n \text{ (eq. 3)}$$

Where for a pair of individuals (j and j') grown under different light conditions (I and i'), x_{ij} is the trait value of individual j in light condition i, and $x_{i'j'}$ the trait

value of individual j' in light condition i', n is the total number of possible ij-i'j' combinations and the sum is taken over all these combinations. Initially, the range of *RDPI* is from 0 (no plasticity) to 1 (maximum plasticity, when the trait is 0 in one environment). In the present work, we calculated the modified *RDPI* (*RDPIm*) in the same way with the difference that we did not use the absolute value of the relative distance $|x_{i'j'} - x_{ij}| / (x_{i'j'} + x_{ij})$ but the real difference with the condition that i' is superior to i (i'>i):

$$RDPIm = \sum [(x_{i'j'} - x_{ij}) / (x_{i'j'} + x_{ij})] / n \quad \text{(eq. 4)}$$

The *RDPIm* ranges from -1 to 1 and has the advantage of showing the direction of the phenotypic plasticity (negative or positive), which is important to interpret the positive and negative effect of light variation on seedlings functional traits. In order to compare the phenotypic plasticity under different ranges of light conditions, plasticity was estimated within three ranges of light: low light [1% - 10 %], middle light [25% - 40%] and high light [60% - 100%]. The univariate t-test was used to test the hypothesis that the *RDPIm* of each rain forest species (*G. ehie* and *G. tessmannii*) was higher under low light level (1%-10%) in comparison to dry forest species (*G. coleosperma*). In addition, a Tukey test after performing an ANOVA was also used to compare *RDIPm* between pairs of species for each functional trait.

4.3. Results

4.3.1. Plant growth and mortality rate

The diameter relative growth rate (*RGRd*) was highest at 25% and 60 % light for rain forest and dry forest species, respectively (Figure 4.1). The same trend was observed in *G. coleosperma* for *RGRh* (shade avoidance strategy), whereas *G. ehie* and *G. tessmannii* attained high *RGRh* at 10% light.

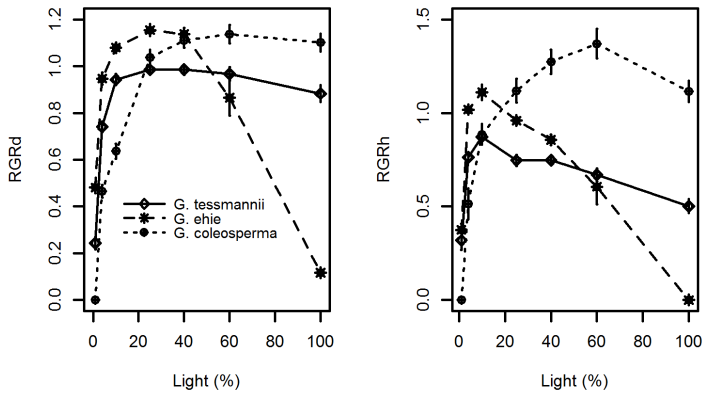


Figure 4.1. Relative growth rates in diameter and height (*RGRd* and *RGRh*) of seedlings in reponse to a range of light environments (1% to 100%) for three *Guibourtia* species.

Guibourtia ehie exhibited a significant difference in the survival probability among light treatments (Figure 4.2, log-rank test X^2=151, p<0.001). No mortality occurred under the first four low light treatments (1%, 4%, 10% and 25%) while the mean survival probability dropped to 94.74%, 73.68% and 0% under 40%, 60% and 100% of full sunlight, respectively. Light treatments also affected the survival of *Guibourtia tessmannii* seedlings (Figure 1, log-rank test X^2=223, p<0.001) but here no mortality occurred except under 1% light treatment where only 17.86 % of the individuals survived. The same trend was also observed in *G. coleosperma* (Figure 4.2, log-rank test X^2=135, p<0.001) where no individuals survived under 1% light against 100% surviving individuals in other light treatments.

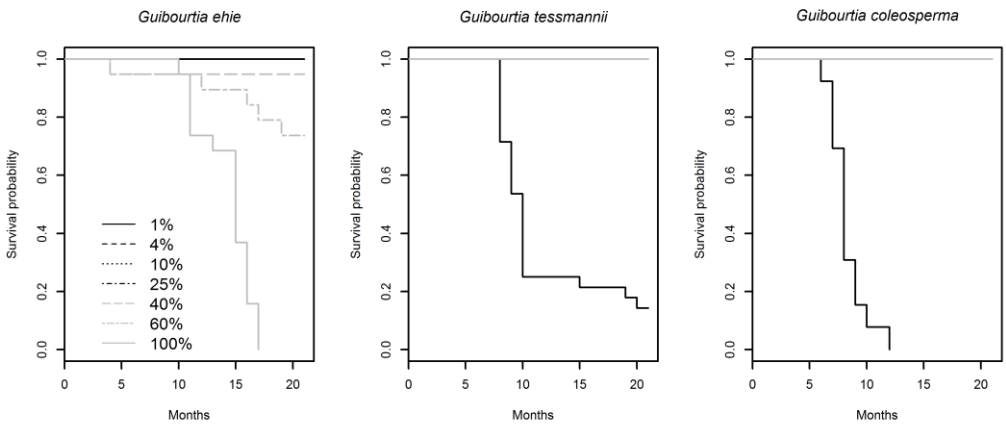


Figure 4.2. Survival probability of *G. ehie*, *G. tessmannii* and *G. coleosperma* in each light treatment. For *G. tessmannii* and *G. coleosperma*, the survival probability was 100% in all shadehouses from 4 to 100% irradiance.

4.3.2. Traits association and ordination of the seedlings

Association among traits were analysed through a principal component analysis (PCA, Figure 4.3). The first two PCA axes together explained 56.46% of the total variation. The first PCA axis explained 35.69% of the variation and was strongly correlated with the light gradient. Species invested more in light capture strategy at the leaf level and allocated less biomass to roots (*LAMR*, *SLA*, *LMR* and *SRL*) between 1% to 4% light while between 4% to 10% light, they invested more in shade avoidance strategy (*RGRh* and height). In contrast, between 25% to 100% light, we observed an increasing strategy of biomass allocation, light capture and photosynthetic activities by producing more total seedling biomass, *RGRd*, *RMR* with high number of branches and number of leaves and higher values of leaf thickness, *LDMC*, *LAR*, *LMC*, *Amax* and *Rd*.

The second axis explained 20.77% of total variation. This axis was correlated with the species environmental preferences (rain forest vs dry woodland). At the bottom

of this axis, we have evergreen and semi-deciduous rain forest species with the highest values of total seedling biomass, height and *SL* (more biomass production and light interception). In contrast, at the top of the same axis, we find the dry forest species with strategy related to drought resistance and a fundamental trade-off between a rapid production of biomass and an efficient conservation of nutrients (high values of *LMC*, *LMR*, *LMDC* and *SRL*).

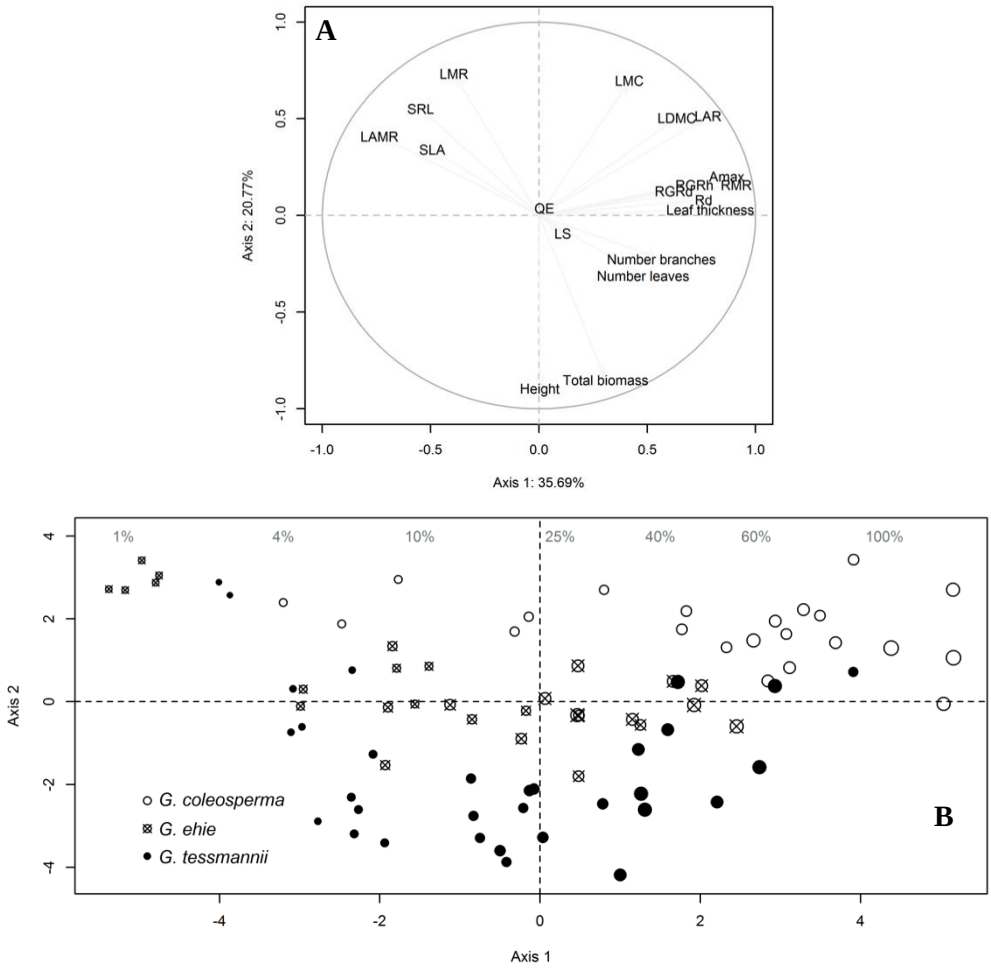


Figure 4.3. Principal component analysis (PCA) based on 19 functional traits measured on *Guibourtia ehie*, *G. coleosperma* and *G. tessmannii* seedlings grown under a range of light intensities in shadehouses. Panel (A) represents the correlation circle of the 19 traits and panel (B) shows the scores of the individuals. The symbol for each species is indicated in the legend, and the size of each symbol reflects light intensities (from 1% to 100% of full sun light).

4.3.3. Effect of light levels on species functional traits

All the studied traits turned out to be species dependent as they differed significantly among species (Table 4.2). In particular, some of plant size and biomass allocation traits (total seedling biomass, *RGRd*, *SMR* and *RMR*), morphological traits (number of branches, leaf thickness, *SLA*, *SL* and *SRL*) and all photosynthetic traits displayed strong species effects (Table 4.2) with the amount of explained variation varying from 16.33% to 64.21%. Light also affected the responses of all the traits but the light x species interaction was significant for 14 out of 20 variables, such as total seedling biomass, *RGRd* and some morphological traits (number of branches, *SL* and *LDMC*). The results also showed a significant species x biomass interaction effect on four traits (height, *RGRd*, number of branches and *SRL*), but the magnitude of this interaction effect was small (2.13 – 9.47%). This significant species x biomass interaction effect for traits implied that species followed different ontogenetic trajectories.

4.3.4. Effect of light on developmental strategies

4.3.4.1. Plant biomass allocation

Rain forest species (*G. ehie* and *G. tessmannii*) attained the highest total seedling biomass under intermediate light levels (25%) whereas dry forest species (*G. coleosperma*) reached their own highest biomass at high light (Fig. 4.4). For all species, *LMR* (biomass allocation to leaves) decreased with light whereas *SMR* and *RMR* (biomass allocation to stem and roots) increased with light with the exception of *G. coleosperma* (dry forest species) for which it decreased from 40 % to 100 % light (Figure 4.4).

4.3.4.2. Plant strategies for resources assimilation

Regarding functional traits associated to light interception and resources assimilation (namely morphological traits), the results showed an increase of the number of leaves up to 10% and 40% for rain forest species and the woodland species, respectively (Figure 4.3). The number of branches increased with light for *G. coleosperma* and *G. ehie*, whereas the peak for *G. tessmannii* was attained at 25% light. Considering the efficiency of plants to display their leaves to intercept light and to assimilate resources, for both rain forest and woodland species, *SLA*, *LAR*, *LMDC* and *LAMR* decreased with light, whereas leaf thickness (physical strength of leaves) increased with light. Regarding the plant water status and drought resistance, for both rain and woodland forest species, *LMC* decreased with light in contrast to *SL*, which decreased with light except for the woodland species (Figure 4.4).

Table 4.2. Two-way ANCOVA with light (n=7) and species (n=3) as fixed effects and $\log_{10}$ (total biomass) as a covariable. F-values, significance levels (ns; *P < 0.05; **P < 0.01; ***P < 0.001) and the amount of explained variation (η^2) of the effects are given. η^2 represents the amount of variation explained by light, species and seedlings biomass and was calculated by dividing the sum of squares of the effect by the total sums of squares of the model ($\eta^2 \times 100$). F-values of factors having the largest effect on plant variable are given in bold.

		Light			species			Light * species			Biomass			Biomass*species		
Group	**Variable**	F	P	η^2	F	P	η^2	F	P	η^2	F	P	η^2	F	P	η^2
Plant size	Total seedling biomass	177	***	32.32	**393.65**	***	43.77	40.53	***	16.64						
	Height	13.49	***	12.99	**193.83**	***	62.17	2.18	*	1.3	87.7	***	14.07	4.09	*	9.47
	RGRd	**32.57**	***	61.96	7.52	**	4.77	4.8	***	15.23	29.98	***	9.5	13.46	***	8.53
	RGRh	26.98	***	46.73	21.38	***	12.34	2.71	**	7.83	**52.87**	***	15.26	1.39	ns	0.8
Biomass allocation	*LMR*	**107**	***	73.65	36.32	***	8.29	9.63	***	10.99	0.62	ns	0.07	1.04	ns	0.23
	SMR	10.2	***	19.56	**83.87**	***	53.6	2.49	*	7.95	0.03	ns	0	0.03	ns	0.02
	RMR	31.83	***	54.06	**37.31**	***	21.11	2.75	**	7.86	0.12	ns	0.04	0.55	ns	0.31
Morphology	Number of leaves	14	***	26.93	26.37	***	16.9	2.29	*	7.32	**91.24**	***	29.24	1.08	ns	0.69
	Number of branches	15.24	***	39.07	**21.8**	***	18.63	3.03	**	12.94	4.74	*	2.03	2.49	*	2.13
	Leaf thickness	13.79	***	41.31	**20.56**	***	20.53	1.29	ns	6.47	1.25	ns	0.62	1.61	ns	1.61
	SLA	33.35	***	62.44	**40.33**	***	17.05	1.82	*	4.64	0.11	ns	0.03	1.45	ns	0.75
	SL	3.59	**	5.7	**121.54**	***	64.21	4	***	10.58	13.92	***	3.68	0.46	ns	0.24
	LMC	**14.13**	***	51.81	3.45	*	4.22	1.08	ns	6.58	0.21	ns	0.13	0.99	ns	1.21
	LDMC	**7.26**	***	35.76	1.94	*	3.19	1.27	ns	10.45	0.77	ns	0.64	0.91	ns	1.49
	LAR	**14.25**	***	51.93	3.49	*	4.23	1.1	ns	6.7	0.48	ns	0.29	0.82	ns	0.99
	LAMR	**78.28**	***	81.12	2.86	*	0.99	3.69	***	6.36	7.05	*	1.22	0.34	ns	0.12
	SRL	143.38	***	65.89	**192.78**	***	16.33	10.78	***	6.14	106.03	***	6.03	19.6	***	2.23
Physiology	*Amax*	59.74	***	56.77	**70.99**	***	22.48	4.17	***	6.6	30.05	***	4.76	0.11	ns	0.03
	Rd	12.18	***	39.81	**16.02**	***	17.46	1.43	ns	7.77	4.62	*	2.52	0.29	ns	0.31
	QE	1.61	ns	11.46	**2.14**	*	3.51	1.4	ns	8.28	10.47	**	6.23	0.96	ns	0.51

4.3.4.3. Photosynthetic responses to light

The maximum net assimilation (*Amax*) follows the same pattern as total seedling biomass. The higher value of *Amax* was reached at 100% light for the woodland species whereas the rain forest species showed the highest value of Amax under 25% light. For the three species, dark respiration (*Rd*) increased with light (Figure 4.4).

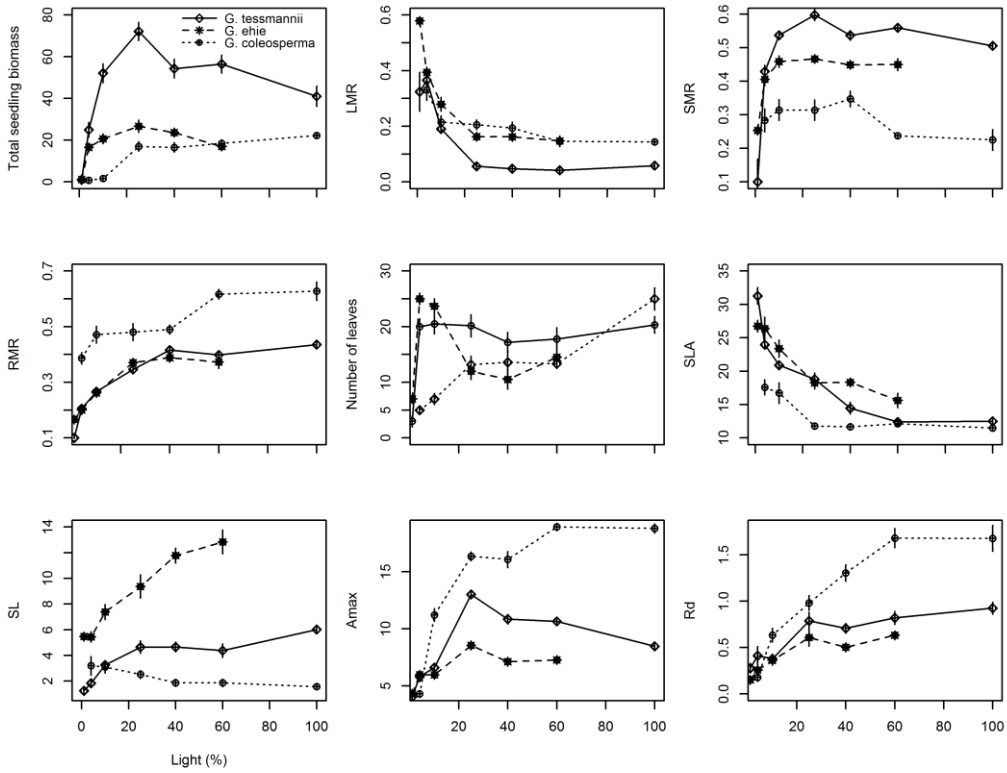


Figure 4.4. Allocation, assimilation and photosynthetic trait responses to light for three congeneric species. Figures from first at right to last at left refer to total biomass, Leaf Mass Ratio (*LMR*), Stem Mass Ratio (*SMR*), Root Mass Ratio (*RMR*), number of leaves, Specific Leaf Area (*SLA*), leaf shrinkage, Maximum of net photosynthesis (*Amax*) and Dark respiration rate (*Rd*).

4.3.5. Phenotypic plasticity

There was a significant difference of traits phenotypic plasticity between species and light levels (Table 4.4). The hypothesis that rain forest species (*G. ehie* and *G. tessmannii*) displayed higher phenotypic plasticity at low light level [1% - 10%] compared to dry forest species (*G. coleosperma*) was confirmed for traits mainly related to plant size. At [1% -10%] light, most studied traits were highly plastic but the observed values were higher for the rain forest species *G. ehie* and *G. tessmannii*. The highest values were observed in some plant size (total seedling biomass, height), biomass allocation (*SLR*) and morphology (number of leaves) traits for these two species. The high phenotypic plasticity for these traits tended to decrease under higher irradiances. At [25% - 40%] light, the morphology trait *LAMR* was the most plastic trait for both rain forest species. In contrast, the total seedling biomass exhibited the highest value of phenotypic plasticity in *G. coleosperma*. At [60% - 100%] light, some traits related to plant size (total seedling biomass, height, *RGRd*, *RGRh*) and morphology (number of leaves, leaf thickness, *SLA*, *LMC*, *SRL*) exhibited highest value of phenotypic plasticity in the dry forest species *G. coleosperma* while rain forest species (*G. ehie* and *G. tessmannii*) displayed lower phenotypic plasticity at this light level.

Table 4.4. The means ± standard errors of the modified *RDPI* (*RDPIm*) computed for the 20 traits and three species in three ranges of light treatments [1% - 10%]; [25% - 40%] and [60% to 100%]. The alphabetic letters represent the results of Turkey test after ANOVA comparing *RDIPm* between species for each trait. The same letter means that there is no difference between species for the trait considered and different letters indicate a significant difference. In the last column, we presented results of the univariate t-test to test the hypothesis that the *RDPIm* of rain forest species (*G. ehie* and *G. tessmannii*) is higher under low light level [1% - 10%] in comparison to dry forest species (*G. coleosperma*). The values in bold indicate the highest *RDPIm* in the light level considered.

Traits	**Species**		**Light**			**Hypothesis at [1% - 10 %] light level**
	2	*1*	*[1% - 10 %]*	*[25% - 40%]*	*[60% - 100%]*	*µ1>µ2*
Total seedling biomass	*G. coleosperma*		-0.35±0.09a	**-0.8±0.04a**	**0.81±0.03a**	
		G. ehie	**0.87±0.02b**	0.25±0.06b	0.14±0.05b	t=9.76***
		G. tessmannii	**0.96±0b**	0.02±0.04b	-0.15±0.07b	t=12.08***
Height	*G. coleosperma*		0.12±0.03a	0.34±0.03a	**-0.93±0a**	
		G. ehie	**0.42±0.02b**	-0.15±0.01b	0.07±0.01b	t=7.24***
		G. tessmannii	**0.56±0.01c**	-0.08±0.01b	-0.33±0.01c	t=12.48***
RGRd	*G. coleosperma*		0.21±0.03a	0.24±0.01a	**0.88±0.01a**	
		G. ehie	**0.31±0.01b**	0.03±0.01b	0±0b	t=3.18**
		G. tessmannii	**0.64±0.01c**	0.02±0.01b	-0.08±0.03b	t=13.53***
RGRh	*G. coleosperma*		0.17±0.01a	0.15±0.03a	**0.87±0.01a**	
		G. ehie	0.14±0.01a	-0.13±0.02b	0.03±0.02b	t=-2.19ns
		G. tessmannii	0.45±0.04b	-0.03±0.01b	-0.42±0.04c	t=6.22***
LMR	*G. coleosperma*		-0.19±0.09a	0.06±0.11a	0.04±0.12a	
		G. ehie	-0.36±0.04b	-0.25±0.02b	-0.07±0.06a	t=-2.35ns
		G. tessmannii	-0.25±0.03a	-0.63±0.07c	0.09±0.1a	t=-2.82ns
SMR	*G. coleosperma*		-0.06±0.06a	-0.06±0.05a	-0.01±0.05a	
		G. ehie	0.29±0.02b	-0.01±0.01a	0±0.02a	t=2.90**
		G. tessmannii	0.19±0.03c	0±0.01a	-0.03±0.01a	t=2.14*
RMR	*G. coleosperma*		-0.1±0.03a	0.01±0.04a	0.01±0.04a	
		G. ehie	0.22±0.03b	0.2±0.01b	-0.03±0.03a	t=1.58*
		G. tessmannii	-0.07±0a	0.22±0.02b	0.02±0.01a	t=-0.79ns
Number of leaves	*G. coleosperma*		0.04±0.03a	0.4±0.04a	**-0.79±0.01a**	
		G. ehie	**0.51±0.01b**	-0.38±0.07b	0.18±0.04b	t=15.27***
		G. tessmannii	**0.72±0c**	-0.07±0.03b	-0.03±0.06b	t=25.14***
Number of branches	*G. coleosperma*		-0.1±0.05a	0.46±0.05a	-0.3±0.02a	
		G. ehie	0.28±0.02b	0.3±0.05a	0.04±0.01b	t=6.75***
		G. tessmannii	0.45±0c	-0.11±0.03b	0.36±0.06c	t=10.87***
Leaf tickness	*G. coleosperma*		0.04±0.01a	0.19±0.02a	**0.68±0.01a**	
		G. ehie	0.05±0.02a	0.07±0.02b	-0.01±0.02b	t=0.39ns
		G. tessmannii	0.22±0b	0.14±0.05b	-0.16±0.03b	t=30.03***
SLA	*G. coleosperma*		-0.08±0a	-0.18±0.01a	**-0.77±0.01a**	
		G. ehie	-0.07±0.02a	-0.11±0.01a	-0.08±0.02b	t=0.33ns
		G. tessmannii	-0.21±0b	-0.17±0.02a	-0.1±0.03b	t=-23.28ns
SL	*G. coleosperma*		0.04±0.06a	-0.28±0.09a	-0.03±0.02a	
		G. ehie	0±0.04a	0.21±0.02b	0.06±0.03a	t=-0.54ns
		G. tessmannii	0.14±0b	0.14±0.03b	0.14±0.02b	t=1.66*
LMC	*G. coleosperma*		-0.1±0.02a	-0.16±0.01a	**-0.98±0a**	
		G. ehie	0.01±0.02b	-0.08±0.02b	-0.05±0.02b	t=3.92***
		G. tessmannii	0.07±0b	-0.1±0.01b	-0.18±0.01c	t=10.62***
LDMC	*G. coleosperma*		-0.15±0.02a	-0.09±0.01a	-0.23±0.02a	
		G. ehie	-0.06±0.04a	0.02±0.02b	-0.06±0b	t=1.91*
		G. tessmannii	0.05±0b	-0.05±0.01a	-0.1±0.01c	t=10.93***
LAR	*G. coleosperma*		-0.07±0.01a	-0.1±0.01a	-0.23±0.02a	
		G. ehie	0±0.01b	-0.05±0.01b	-0.03±0.01b	t=3.96***
		G. tessmannii	0.04±0b	-0.06±0.01b	-0.1±0.01c	t=9.87***
LAMR	*G. coleosperma*		-0.45±0.05a	-0.39±0.15a	0.07±0.02a	
		G. ehie	**-0.52±0.05a**	**-0.85±0.06b**	0.22±0.06b	t=-1.08ns
		G. tessmannii	**-0.64±0b**	**-0.89±0.04b**	0.52±0.04c	t=-4.16ns
SRL	*G. coleosperma*		-0.85±0.02a	0.07±0a	**-0.64±0.01a**	
		G. ehie	**-0.86±0.02a**	-0.19±0b	0.24±0b	t=-0.29ns
		G. tessmannii	**-0.86±0a**	-0.06±0.03c	0.33±0.1b	t=-0.69ns
Amax	*G. coleosperma*		0.44±0.02a	0.19±0.02a	0.09±0.01a	
		G. ehie	0.16±0.01b	0.04±0.03b	0.05±0.01a	t=-11.89ns
		G. tessmannii	0.24±0.03b	0.22±0.03a	-0.12±0.01b	t=-5.72ns
Rd	*G. coleosperma*		0.58±0.04a	0.32±0.04a	0.09±0.05a	
		G. ehie	0.52±0.03a	0.12±0.04	0.14±0.03a	t=-1.22ns
		G. tessmannii	0.17±0.08b	0.29±0.04a	0.13±0.04a	t=-4.61ns
EQ	*G. coleosperma*		0.24±0.03a	0.05±0.03a	-0.04±0.02a	
		G. ehie	-0.18±0.09b	-0.07±0.03b	-0.01±0.02a	t=-4.11ns
		G. tessmannii	0.11±0.08a	-0.06±0.02b	-0.08±0.04ab	t=-1.43ns

4.4. Discussion

4.4.1. Does light intensity influence functional traits within Guibourtia *congeneric tropical tree species?*

This work mainly aimed to show how the studied traits change according to light intensity. The results highlight strong interspecific differences in (i) survival and (ii) plant size (total biomass and height), biomass allocation (*SMR* and *RMR*), morphological (leaf thickness, *SLA*, leaf shrinkage, number of branches and *SRL*) and photosynthetic (*Amax* and *Rd*) traits along the light gradient.

Regarding survival and plant size traits, our results showed that under 1% light, all *G. ehie* individuals and about 20 % of *G. tessmannii* individuals (both rain forest species) survived while no *G. coleosperma* (dry forest species) survived. In contrast, under full sun light (100%), no *G. ehie* individual survived, contrary to *G. tessmannii* and *G. coleosperma* individuals that all survived. Hence, the rain forest species *G. ehie* seems to be a shade tolerant species able to survive in shaded understory for years without significant growth (Canham, 1988, Kitajima, 1994). On the other hand, our results showed that *G. ehie* and *G. tessmannii* maintain positive values of *RGRd* for individuals under 1% light, suggesting that these two rain forest species could not be pioneer according to Agyeman et al. (1999). Hence, as they attend high *RGRd* and total biomass at 25% light, this behaviour suggests that *G. ehie* and *G. tessmannii* could be Non Pioneer Light Demanding (NPLD) species according to Hawthorne (1995). Since *G. ehie* did not exhibit high *RGRd* and total biomass at 10%, it could not be considered as a shade tolerant species. In contrast, *G. coleosperma* (dry forest species) appears to be a shade-intolerant species at 1% light with negative *RGRd* at this light level. But it survives above 1% light level and reaches high relative growth rate and total biomass under 60% and 100% light respectively. *Guibourtia coleosperma* is a light demanding species that survives and maintain positive growth rates only in high light environments (Poorter, 1999).

The light responses for seedling morphology and biomass allocation traits also differ along the light gradient between *Guibourtia* species. The results showed a difference among species in *SMR*, *RMR* and *SRL*. The allocation to stem growth (*SMR*) is higher for *G. ehie* and *G. tessmannii* than for *G. coleosperma*, and inversely for the allocation to roots (*RMR* and *SRL*). These results are coherent with those of Walters and Reich (1996) who affirmed that species which colonize low light environment reduce biomass turnover and increase storage allocation (i.e., structural biomass: stems and thick roots). Moreover, the strategy of dry forest species is to enhance water uptake via a higher root length (increasing of *RMR*) in order to forage more efficiently in deeper soil layers where more water is available (Engelbrecht et al., 2005). The trends we observed for *SLA* are congruent with Sánchez-Gómez et al. (2006) but in contradiction with the conservative resource-use hypothesis (Reich et al., 2003, Sánchez-Gómez et al., 2006, Veneklaas and Poorter, 1998, Walters and Reich, 1999) that predicts that shade tolerant species exhibit thick leaves with low *SLA*. In our results, we found that *G. ehie* and *G. tessmannii* exhibited lower *SLA* than *G. coleosperma* suggesting that the two rain forest species could not be considered as shade tolerant species. The same trend was also observed in leaf

thickness and leaf shrinkage (*SL*). However, there are some trait differences between *G. tessmannii* and *G. ehie*. In fact, the leaves of the former are thicker with higher values of *SL* than the latter one, suggesting that *G. ehie* is more shade-tolerant than *G. tessmannii* according to Fahn (1982). The leaf shrinkage (*SL*) explains the ability of leaf cells to lose turgor after a water loss under drought conditions (Bussotti et al., 2002). Our results are well explained by Niinemets (2001) who proved that species having dense leaf tissue with thick cells and high capacity to avoid desiccation are dominant under climates with higher mean temperatures and greater solar radiation.

Concerning physiological traits, a significant species effect was also detected. The dry forest species exhibited higher photosynthesis and respiration rates than rain forest species. This result is consistent with Thompson et al. (1992), Dusenge et al. (2015) and Lestari and Nichols (2016). In addition to changes observed for plant size, biomass allocation and morphological traits in *G. tessmannii* and *G. ehie*, the the level of light aslo caused physiological changes. This corroborates the idea that *G. ehie* and *G. tessmannii* are NPLD species since, according to Chazdon et al. (1996) and Montgomery and Chazdon (2002), shade tolerant species generally use morphological changes rather than altering photosynthetic capacity as response to light intensity changes. Similar patterns were found by Veneklaas and Poorter (1998) throughout a literature review of seedling growth in 120 tropical tree species.

When all the information above are combined with the classification of Oldeman and van Dijk (1991) it appears that *G. ehie* and *G. tessmannii* should be classified as NPLD species (struggling gamblers) and *G. coleosperma* as a light demanding species (hard gamblers).

4.4.2. Functional trait conservatism between Guibourtia *species*

By testing the effect of light on the growth of congeneric plants, we finally noticed in our experimental setup a potentially confounding effect of water stress under high light treatments (as shown by our results on climatic monitoring, Appendix 4.2). In fact, high light can be associated to water stress that leads plants to increase the leaf temperature and water vapour concentration inside the leaf and therefore increase water loss by transpiration (Chapin III et al., 2011). To verify the effect of drought in the experiment, we used the biomass allocation to roots versus leaves, which represents one of the best predictors of trade-off between drought and shade tolerance (Smith and Huston, 1989). The trade-off between drought and shade tolerance is found between *LMR* and *RMR* ($r=-0.65$, $P<0.001$). These results dispel all doubt and underline evidence of biomass allocation trade-off in line of the Smith and Huston (1989) assumption. This trade-off between drought and shade tolerance helps to fully interpret the second PCA axis (Figure 2). In fact, this axis represents the deciduousness axis and clearly shows separation between persistent and deciduous species. *Guibourtia coleosperma* seems to be persistent whereas *G. ehie* and *G. tessmannii* appear be deciduous. This result is consistent with Lemmens et al. (2012) who also confirmed that *G. coleosperma* is persistent and confirm the findings of Poorter (2004) and (Doucet, personal communication) who assumed that *G. ehie* and *G. tessmannii* are deciduous species. These species are found in evergreen forests (Lemmens et al., 2012) but *G. ehie* from West Africa (which is

potentially a species different from the *G. ehie* from Central Africa, see chapter 7) can occur in semi-deciduous forests (Poorter et al., 2004). In our results, *G. ehie* and *G. tessmannii* enhance total biomass, number of leaves, invest more biomass in stem and are associated with grow taller. In contrast *G. coleosperma* was linked with resources assimilation, plant water status, biomass allocation to roots, drought tolerance and photosynthetic activities (high *LMC*, *LMR*, *SRL*, *LDMC*, *LAR*, *Amax* and *RMR*). According to Ackerly (2004), Markesteijn and Poorter (2011) and Poorter and Markesteijn (2008), persistent species have tough and resistant leaves contrary to deciduous species that shed their leaves to avoid drought. Persistent species adopt a conservative strategy by allocating more biomass in root and leaf tissue whereas deciduous species (here also rain forest species) follow acquisition resource strategy that allow them to maximize resource uptake (light, nutrients etc.) to be more competitive in wet microhabitats (Markesteijn and Poorter, 2011). In addition, the low biomass investment in leaves (low *LMR*) by deciduous species can be compensated by having high *SRL* and realizing large leaves per unit of biomass invested (high *SLA*) throughout the formation of thin (low thickness) or low density leaves [low *LMDC*; (Witkowski and Lamont, 1991)].

Finally, it appears that *G. coleosperma and G. tessmannii* (two sister species) adopt different strategies to cope with light and drought. The former is persistent and light demanding while the latter is deciduous and NPLD species. This two sister species do not conserve their traits in contrary to what is observed between *G. ehie* and *G. tessmannii* (congeneric but not closely related species) that share similar characteristics. Hence, the pattern observed between *G. coleosperma* and *G. tessmannii* could suggest an adaptive response to light heterogeneity that could be reflected in the study of their phenotypic plasticity.

4.4.3. Phenotypic plasticity: evidence of adaptation within the genus Guibourtia

As shown above, plant species have different strategies to cope with light heterogeneity. This difference between strategies leads plant species to (i) specialize to one end of this environmental gradient or (ii) increase their ability to accommodate their phenotypes to the light available in each particular environment (Valladares et al., 2002). According to Bazzaz (1996), plant evolution in heterogeneous environment leads to one of two alternatives mentioned above. Our results are congruent with the theoretical expectations on species adaptation and specialization: rain forest species (as demonstrated above) performed better in term of growth performance than dry forest / woodland species in low light treatment, and inversely. For the following functional traits related to biomass production, growth performance, resources assimilation and light interception such as: total seedling biomass, height, *RGRd*, *RGRh*, leaf moisture content, *LDMC*, *SMR*, number of leaves, number of branches and *LAR*, *G. ehie* and *G. tessmannii* are more plastic phenotypically than *G. coleosperma*. This confirms our hypothesis that rain forest species are more plastic in low light environment than dry forest species. In our results, nearly all rain forest plant size variables displayed higher plasticity

under low light. These findings are in the same line as Valladares et al. (2000) and highlight that specialization strategies developed by rain forest species to a low-resource environment seem to start with modification in key variables related to growth. In turn, *G. coleosperma* appears more plastic under high light treatment for the traits related to biomass production, growth performance, light capture and drought resistance such as total seedling biomass, height, *RGRd*, *RGRh*, number of leaves, leaf thickness, *SLA*, *LMC*, *SRL* as confirmed by Kitajima (1994) for shade intolerant species. The performance of *G. coleosperma* is also consistent with the specialization hypothesis and confirmed once again its capacity to survive and grow under intense solar radiation (Valladares et al., 2002).

4.4.4. Contribution of phenotypic plasticity in the understanding of the evolutionary history within the genus Guibourtia*: evidence of natural selection forces*

The higher plasticity of several traits under low light level [1-10%] for *G. ehie* and *G. tessmannii* compared to *G. coleosperma* proves that the latter is a poor competitor for light, limiting its expansion in rain forests. In fact, the rain forest is a highly competitive environment (Barros et al., 2012). During the Quaternary, the glacial/interglacial climatic oscillations (from 2.7 Ma, and mainly 1.8 Ma) induced expansion and retraction of savanna and forest biomes and may have promoted the diversification within *Guibourtia* subgenus *Pseudocopaiva*, which contains *G. coleosperma*, *G. tessmannii*, *G. pellegriniana* and *G. leonensis* (Tosso et al., 2018). It is important to note that these divergence events seem congruent to some major environmental changes but we cannot establish reliable causal links based on the outcomes of the present study. Poumot (1989) and Morley and Richards (1993) assumed that the first glacial advance around 2.5 million years ago deeply reduced the area of rain forest in favour of savannas, and other glacial advances in the quaternary had similar effects. As *G. tessmannii* and *G. coleosperma* are two closely related species (Tosso et al., 2018), the changes in Africa past climate have probably favoured speciation between these two species.

Nowadays, comparative ecophysiological studies of species with a reliable phylogenetic hypothesis might elucidate important evolutionary questions and help to understand the main forces responsible for changes in plasticity (Monson, 1996; Valladares et al., 2000). In this study, we note a completely opposite strategy between *G. coleosperma* and *G. tessmannii* in terms of plasticity regarding plant performance and resources assimilation traits, suggesting the action of natural selection forces in the differentiation between these two taxa. We can hypothesize that *G. tessmannii* occupied a large range habitat, part of which would have been affected by drought during the Quaternary climatic oscillations. Some *G. tessmannii* individuals in this new dry habitat have adapted and became over time a new species, today called *G. coleosperma*.

4.5. Conclusion

This study showed the important role of functional trait variation in response to light in African tree species. It highlights that species occurring in the same biome have more similar functional traits than more closely related species that occur in contrasted biomes, suggesting that natural selection contributed to trait divergence between *Guibourtia* species. The understanding of phenotypic plasticity in congeneric species provides additional information to understand the evolutionary history of taxa in particular when it is combined with phylogenetic tree. Finally, such studies give tangible tools for the forest management and restoration ecology as they help to properly define the ecological profile and light requirements of each species, especially for *G. tessmannii*, which represents an important timber tree species newly listed in Appendix II of CITES list.

Partie 2

Causes de la différenciation génétique des populations de *Guibourtia*

Chapitre 5

Microsatellite development for the genus *Guibourtia* (Fabaceae, Detarioideae) reveals diploid and polyploid species

Le chapitre 2 a souligné une forte divergence entre les trois clades du genre *Guibourtia*. Dans ce chapitre 5, nous avons utilisé les marqueurs moléculaires neutres afin de mieux apprécier ces niveaux de divergence. Un premier jeu de 16 marqueurs moléculaires microsatellites polymorphes a été développé sur *G. tessmannii* et des tests de transférabilité ont été réalisés sur les autres espèces du genre. Ce chapitre est adapté de :

Tosso, F., Doucet, J. L., Kaymak, E., Daïnou, K., Duminil, J., & Hardy, O. J. (2016). Microsatellite development for the genus *Guibourtia* (Fabaceae, Caesalpinioideae) reveals diploid and polyploid species. Applications in plant sciences, 4(7), 1600029.

5. Microsatellite development for the genus *Guibourtia* (Fabaceae, Detarioideae) reveals diploid and polyploid species

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ABSTRACT

• ***Premise of the study:*** Nuclear microsatellites (nSSRs) were designed for *Guibourtia tessmannii* (Leguminosae), a highly exploited African timber tree, to study population genetic structure and gene flow

• ***Methods and Results:*** We developed 16 polymorphic nSSRs from a genomic library tested in two populations of *G. tessmannii* and three populations *G. coleosperma*, respectively. These nSSRs display three to 14 alleles per locus (mean= 8.94) in *G. tessmannii*. Cross-amplification tests in nine congeneric species demonstrated that the genus *Guibourtia* contains diploid and polyploid species. Flow cytometry results combined with nSSRs profiles suggest that *G. tessmannii* is octoploid.

• ***Conclusions:*** nSSRs revealed that African *Guibourtia* species include both diploid and polyploid species. These markers will provide information on the mating system, patterns of gene flow and genetic structure of African *Guibourtia* species.

Key words: flow cytometry; *Guibourtia;* Leguminosae; microsatellites; next-generation sequencing; polyploidy.

5.1. Introduction

The African tree *Guibourtia tessmannii* (Harms) J.Leonard is a hermaphrodite rain forest species distributed from Cameroon to Gabon (Fougère-Danezan, 2007; Tosso et al., 2015). Known as "bubinga" or "kevazingo", it has high commercial and social importance but undergoes significant threats due to illegal logging. The genus *Guibourtia* belongs to the Leguminosae and includes 13 African species distributed from Senegal to Mozambique in forest or savanna habitats. The genus was divided by Leonard (1949) in three main subgenera: (i) *Pseudocopaiva*: *G. tessmannii*, *G. pellegriniana* Leonard, *G. coleosperma* (Benth.) Leonard, *G. leonensis* J.Leonard; (ii) *Guibourtia*: *G. carrissoana* (M.A.Exell) J.Leonard, *G. copallifera* Benn., *G. demeusei* (Harms) J.Leonard, *G. sousae* J.Leonard; (iii) *Gorskia*: *G. arnoldiana* (De Wild. & T.Durand) J.Leonard, *G. conjugate* (Bolle) J.Leonard, *G. dinklagei* (Harms) J.Leonard, *G. ehie* (A.Chev.) J.Leonard, *G. schliebenii* (Harms) J.Leonard. We developed polymorphic microsatellite markers for *G. tessmannii* and tested them on nine African congeneric species to verify species delimitation and document population genetic structure and gene flow patterns. As microsatellite typing suggested that some species were polyploid, we used flow cytometry to compare the ploidy levels of two related species for which appropriate fresh material was available.

5.2. Methods and results

5.2.1. Microsatellite development

We extracted total DNA from 30 mg of dry leaf of *G. tessmannii* (FT0001; Appendix 5.1) using a cetyltrimethylammonium bromide (CTAB) method (Fu et al., 2005). We prepared a non-enriched DNA genomic library, following Mariac et al. (2014), and generated 150bp long paired-end reads on an Illumina MiSeq platform (San Diego, USA). We assembled the resulting 78279 reads by pair with PANDAseq (Masella et al., 2012). Using the software QDD (Meglécz et al., 2014), we detected 2483 microsatellite loci. Of these, 149 had at least 8 repeats and flanking regions appropriate to define pairs of PCR primers. We developed primers for 48 loci with at least eight di-, tri- or tetranucleotide repeats, and primers regions at least 20 bp distant from the microsatellite region. We added one of four possible linkers (Q1–Q4; Micheneau et al., 2011) to the 5' end of the forward primer of each locus to label PCR products with fluorochromes FAM, NED, VIC, and PET (Table 5.1).

We tested 48 primers pairs using two samples of *G. tessmannii*: FT0002 and FT0003 (Appendix 5.1). PCR reactions (total volume of 15 µL) used 1.5 µL buffer (10×), 0.6 µL $MgCl_2$ (25 mM), 0.45 µL dNTPs (10 mM each), 0.3 µL of each primer (0.2 µM), 0.08 µL TopTaq DNA Polymerase (5 U/µL; QIAGEN, Venlo, The Netherlands), 1.5 µL of Coral Load, 1 µL of template DNA (of ca. 10–50 ng/µL), and 9.27 µl water. PCR conditions were: 94°C (4 min); 30 cycles of 94°C (30 s), 55°C (45 s), and 72°C (1 min); and a final extension at 72°C (10 min). We

visualized PCR products stained with SYBR Safe (Invitrogen, Merelbeke, Belgium) on a 1% agarose gel. Forty-two loci amplified consistently.

Table 5.1. Characterization of 16 polymorphic and one monomorphic nuclear microsatellite loci isolated from *G. tessmannii*

Primers[a]	Primers sequences (5'-3')	Labeled primer[b]	Repeat motif	Ta (°C)	Allele range size (bp)	GenBank accession no.
R12-Seq10*	F: AGGACTTAAGAATGGTGATGCAA	Q1-6-FAM	$(AT)_{10}$	60	150-200	KX086193
	R: TTTGGCTTCCCTCTCTTCCT					
R12-Seq15*	F: CCTGATTGGAGTTACACCACC	Q1-6-FAM	$(AG)_{13}$	60	98-124	KX086194
	R: AGGACAAGCTTGAGCGACAT					
R12-Seq21*	F: TTTCATTCAAACAAACCGCA	Q2-NED	$(AAT)_{11}$	60	176-218	KX086197
	R: CTGACACACAAACACAGCCA					
R12-Seq35*	F: GACACTCCTCAGGTGGTTTCA	Q3-VIC	$(AAT)_{20}$	60	123-165	KX086204
	R: GAGGTTAGATTCCAACATGTGC					
R12-Seq29*	F: CCAAATTGCAGACGATGAAA	Q3-VIC	$(AAG)_{11}$	60	205-247	KX086201
	R: AATTCGGACTTGAAGTTGCAG					
R12-Seq08**	F: AACATGCATACTTTAACCGCAA	Q4-PET	$(AAAG)_{9}$	60	148-172	KX086191
	R: TTTCAATCAACACTTATCCTTGG					
R12-Seq06**	F: ATCTCCGCTTGTATCTGCGT	Q1-6-FAM	$(AG)_{8}$	60	187-203	KX086190
	R: AATCAAGCCTCCGTAAAGCA					
R12-Seq26**	F: CACAATACTAGAGCTGAAGAAACATGA	Q2-NED	$(AAG)_{13}$	60	153-186	KX086200
	R: CACGAGAAAGGGAGGAAATG					
R12-Seq34**	F: GACACTCCTCAGGTGGTTTCA	Q3-VIC	$(AAT)_{13}$	60	150-186	KX086203
	R: GAGGTTAGATTCCAACATGTGC					
R12-Seq16**	F: CCCATAATCAGCCTACAAACC	Q2-NED	$(AG)_{11}$	60	226-262	KX086195
	R: CAGATGAGGTAGACATTGTGGG					
R12-Seq09***	F: ACCTACGTTTGTGATTATGAATGG	Q1-6-FAM	$(AG)_{8}$	60	166-196	KX086192
	R: TTTGGGTGATCTTTATGCTTTC					
R12-Seq20***	F: AAATCCGGAGGAGAGGAAGA	Q2-NED	$(AG)_{8}$	60	194-218	KX086196
	R: CTGACTCTGGCTTGACCCAT					
R12-Seq22***	F: TTATGATGCGTGTCCCAAA	Q2-NED	$(AT)_{21}$	60	157-177	KX086198
	R: GAATTGAATGCAGGGAGGAC					
R12-Seq01****	F: CCTCATCATAACAATTCAAGTGC	Q1-6-FAM	$(AT)_{20}$	60	201-241	KX086189
	R: GATGCCATGACTCTGGCTAAA					
R12-Seq25****	F: CATAGACTTGGAGGGAGCCA	Q2-NED	$(AG)_{9}$	60	174-196	KX086199
	R: TTGCTTCCTTGTATCTTTAACAATTT					
R12-Seq31****	F: ATTCCTATCAGATGAACAGATTATCA	Q3-VIC	$(AT)_{8}$	60	221-245	KX086202
	R: AGCTTGTTGCAAATTGGATTG					
R12-Seq43**	F: GGCAGAATTTCCAGAAGCAA	Q4-PET	$(AT)_{23}$	60	143	KX086205
	R: ACACAACCTTCCTTTCCTGC					

Note : Ta = annealing temperature ;

[a] *=Multiplex Mix 1, ** = Mix 2, ***= Mix 3, ***= Mix 4.

[b] Q1 = TGTAAAACGACGGCCAGT (Schuelke, 2000); Q2 = TAGGAGTGCAGCAAGCAT; Q3 = CACTGCTTAGAGCGATGC;
Q4 =CTAGTTATTGCTCAGCGGT (Q2–Q4, after Culley et al., 2008).

We assessed polymorphism on seven *G. tessmannii* individuals from Cameroon and Gabon (Appendix 5.1). We used fluorescent labeling by PCR amplification in a total volume of 15 µL, combining: 0.15 µL of the reverse and 0.1 µL of the forward (0.2 µM for both) microsatellite primers, 0.15 µL of Q1–Q4 labeled primers (0.2 µM each), 3 µL of Type-it Microsatellite PCR Kit (QIAGEN), H_2O, and 1 µL of DNA. PCR conditions were: 5-min initial denaturation at 95°C, followed by 30 cycles of (95°C for 30 s, 60°C for 90 s, 72°C for 1 min), 10 cycles of (95°C for 30s, 55°C for 45 s, 72°C for 60 s, 72°C for 1 min) and a final elongation step at 60°C for 30 min. We mixed to 1.1 µL of each PCR product with 12 µL of Hi-Di Formamide (Life Technologies, Carlsbad, California, USA) and 0.3 µL of MapMarker 500 labeled with DY-632 (Eurogentec, Seraing, Belgium). The preparation was genotyped on an ABI3730 sequencer (Applied Biosystems, Lennik, The Netherlands).

After excluding loci that did not amplify consistently or were unreadable, we combined 16 polymorphic loci (one locus "R12-Seq43" was monomorphic) in 4 multiplexed reactions (Table 1) using Multiplex Manager 1.0 software (Holleley and Geerts, 2009). Preliminary population genetic analyses were performed on three populations of *G. tessmannii* (35-58 individuals per population; Table 2 and Appendix 5.1). Multiplexed PCRs were as above except that 3 µL of the 5x Q-solution of the Type-it Microsatellite PCR Kit were added. The individuals of *G. tessmannii* studied revealed a high degree of polymorphism, with more than two alleles per individual, suggesting a polyploid genome (Table 5.2).

5.2.2. Microsatellites markers data analysis in G. tessmannii *and* G. coleosperma

The three populations of *G. tessmannii* (Table 5.2 and Appendix 5.1) presented three to 14 alleles per locus (mean of 8.94 alleles per locus, Table 2). Single-locus genotypes had one to eight alleles (2.35±0.94 alleles per locus) and no fixed heterozygosity, suggesting an autopolyploid.

For *G. coleosperma*, the diploid species where cross-amplification was the most successful (see later), we considered two populations (Table 5.2). For each of the 10 amplifiable loci, we calculated allele size range, number of alleles (A) per locus, observed (Ho) and expected (He) heterozygosities, inbreeding coefficient (F) and null allele frequency (r) with INEst 1.0 (Chybicki and Burczyk, 2009). Deviation from Hardy–Weinberg equilibrium (HWE) was tested for each locus with SPAGeDi (Hardy and Vekemans, 2002). Loci exhibited one to 14 alleles (mean=4.5) with Ho (mean ± SE) = 0.28 ± 0.09 and He = 0.41 ± 0.11 for Democratic Republic of Congo (DRC) population and one to 10 alleles (mean=3.67) with Ho = 0.17 ± 0.05 and He = 0.36 ± 0.10 for Namibia population. Significant deviation from HWE was observed in at least one population for four primer pairs. Locus R12-Seq20 and R12-Seq22 for DRC population exhibited significant deficit of heterozygotes due to the presence of null alleles (Table 5.2).

Table 5.2. Results of initial primer screening of 17 nuclear microsatellite loci developed in *G. tessmannii* (three populations) and 10 which cross-amplified in *G. coleosperma* (two populations)

Locus	*G. tessmannii* (octoploid)									*G. coleosperma* (diploid)											
	Gabon (Makokou; N=35)			Gabon (Bambidie; N=58)			Cameroon (Ma'an; N=38)			DRC (Dilolo; N=20)						Namibia (Rundu; N=13)					
	A	Rn	Mn	A	Rn	Mn	A	Rn	Mn	N	A	Ho	He	Fa	r	N	A	Ho	He	Fa	r
R12-Seq15	4	1-3	1.13	11	1-3	1.5	6	1-3	1.51	20	4	0.11	0.26	0.31	0.09±0.08	12	2	0.08	0.08	0***	0.11±0.09
R12-Seq10	14	1-5	2.34	18	1-4	2.02	20	1-6	2.13	18	14	0.67	0.91	0.23	0.07±0.05	13	10	0.62	0.92	0.33	0.07±0.05
R12-Seq35	6	1-4	1.85	13	1-5	2.69	8	1-3	2.12	20	4	0.5	0.71	0.12	0.06±0.05	13	4	0.46	0.7	0.35	0.10±0.07
R12-Seq08	6	1-4	2.45	5	1-3	1.28	5	1-3	1.89	20	2	0	0.09	1*	0.11±0.09	13	1	-	-	-	0.12±0.10
R12-Seq26	7	1-5	2.56	11	1-4	1.91	9	1-3	1.65	20	1	-	-	-	0.12±0.09	13	1	-	-	-	0.13±0.10
R12-Seq34	7	1-6	4.48	5	1-4	2	5	2-3	2.08	20	7	0.67	0.67	0	0.05±0.04	13	5	0.15	0.73	0.79	0.20±0.11
R12-Seq16	14	1-8	5.38	16	2-8	4.72	13	2-8	4.22	19	3	0.5	0.39	-0.07	0.05±0.04	13	1	-	-	-	0.12±0.12
R12-Seq09	10	1-5	2.95	12	2-6	3.55	12	2-6	4.06	19	2	0.06	0.11	0.49	0.29±0.09	12	2	0	0.51	1	0.72±0.11
R12-Seq20	9	1-5	2.63	5	1-3	1.51	2	1-2	1.46	20	3	0	0.3	1**	0.20±0.36	12	-	-	-	-	1±0.00
R12-Seq22	3	1-3	1.42	9	1-3	1.22	4	1	1	20	6	0.34	0.77	0.49**	0.16±0.07	13	7	0.23	0.87	0.74	0.29±0.11
R12-Seq01	11	1-6	2.83	14	1-7	2.98	17	1-7	2.94												
R12-Seq25	7	1-4	2.03	7	1-4	2.25	7	1-4	2.26												
R12-Seq31	13	1-5	2.31	10	1-4	2.26	7	1-6	2.88												
R12-Seq21	10	1-4	2.44	12	1-3	2.07	11	1-3	1.68												
R12-Seq29	6	1-4	2.16	4	1-3	1.76	10	1-3	1.94												
R12-Seq06	5	1-3	2.03	4	1-4	2.14	5	1-3	2.11												
R12-Seq43	1	1	1	1	1	1	1	1	1												

Note: -= not applicable; N=number of individuals sampled; A = number of alleles; Rn= range of number of alleles per individual; Mn=mean number of alleles per individual; F = fixation index; He = expected heterozygosity; Ho = observed heterozygosity; r = frequency of null alleles;

[a] For F= significance of deviation from Hardy–Weinberg equilibrium: *P<0.05; ** P < 0.01; ***P<0.001.

5.2.3. Flow cytometry

We used flow cytometry to confirm the ploidy level of *G. tessmannii* and compare its genome size with *G. coleosperma*. We used fresh material from seeds collected in Central Gabon (*G. tessmannii*) and north Namibia (*G. coleosperma*) (Appendix 5.1). From 1 cm^2 pieces of fresh leaves, we obtained suspensions of leaf cells nuclei by chopping them in a buffer solution using the CyStain UV Precise P kit (Partec GmbH) with DAPI (4',6-diamidino-2-phenylindole, dilactate). We ran samples with Ploidy Analyser equipment (Partec GmbH, Münster, Germany). We used tomato as an internal standard (*Solanum lycopersicum* L. "Montfavet 63-5" [2C = 1.99 pg, 40.0% GC; Marie and Brown, 1993]). Under the assumption that the GC content of our samples and the standard were similar, the genome size of *G. coleosperma* ranged from 3.20 to 3.70 pg (N=3) and *G. tessmannii* from 11.87 to 15.78 pg (N=3). Although these estimates should be considered with caution in the absence of information on the GC content, the genome size of *G. tessmannii* is nearly four times larger than the one of *G. coleosperma*. As the later species displays microsatellites profiles typical of diploids, the flow cytometry results confirm that *G. tessmannii* is an octoploid species.

5.2.4. Cross-amplification in congeneric species and ploidy determination

Among the 17 loci selected from *G. tessmannii*, a majority successfully amplified in two other species from the subgenus *Pseudocopaiva* (Table 5.3). Less than six loci amplified in the other species, most of which belong to other subgenera (Table 5.3). In *G. pellegriniana* all loci were polymorphic and the genotypes showed up to eight alleles per individual and locus, suggesting octoploid genome. By contrast, in the other species individuals did not display more than two alleles per locus, suggesting diploid genomes.

5.3. Conclusion

We developed 16 polymorphic microsatellite markers in *G. tessmannii* and that amplify to varying degrees in nine congeneric species. The microsatellites and flow cytometry results showed for the first time that the genus *Guibourtia* includes diploid and polyploid species. These markers will be useful to assess the mating system and genetic structure of *Guibourtia* species.

Table 5.3. Results of cross-amplification (allele size ranges) of microsatellite loci isolated from *G. tessmannii* and tested in nine additional taxa

Subgenera	*Pseudocoapiva*			*Guibourtia*			*Gorskia*		
Species	*G. pellegriniana*, N=14	*G. leonensis*, N=3	*G. coleosperma*, N=33	*G. carrissoana*, N=2	*G. copallifera*, N=7	*G. demeusei*, N=9	*G. arnoldiana*, N=2	*G. ehie*, N=20	*G. dinklagei*, N=1
R12-Seq10	156-170	148-150	148-186	-	-	-	-	-	-
R12-Seq15	108-124	-	108-122	-	-	-	-	-	-
R12-Seq21	182-212	-	-	-	-	-	-	-	-
R12-Seq35	129-159	141-150	136-154	-	-	-	-	-	-
R12-Seq29	199-223	-	-	-	-	217	-	-	-
R12-Seq08	136-168	-	152-156	142-148	-	-	-	136-208	-
R12-Seq06	194-198	194	-	-	-	-	-	-	-
R12-Seq26	156-180	158-160	158*	-	-	158	-	138-198	-
R12-Seq34	150-174	150	150-160	150	-	-	-	150-174	154
R12-Seq16	226-250	202	224-266	236	226	228-252	232	232-252	-
R12-Seq09	200	-	168-170	-	-	-	-	-	-
R12-Seq20	205	-	203-223	-	203-205	203-207	-	-	-
R12-Seq22	169-173	-	168-172	-	164-172	162-166	-	-	-
R12-Seq01	205-231	-	-	-	-	-	-	-	-
R12-Seq25	180-190	-	-	-	-	-	-	180-192	-
R12-Seq31	221-231	-	-	-	-	-	-	-	-
R12-Seq43	143*	143*	-	-	-	-	-	143*	-

Note: * monomorphic loci

Chapitre 6

Characterization of 19 microsatellite markers in the African tropical tree species *Guibourtia ehie* (Fabaceae, Detarioideae)

Dans le chapitre 6, nous avons développé un jeu de 19 marqueurs moléculaires neutres sur *G. ehie*, une espèce appartenant à un clade différent de celui de *G. tessmannii* (chapitre 5). Des tests de transférabilité ont été également réalisés sur les autres espèces du genre afin de consolider nos résultats quant au niveau de divergence entre espèces de différents sous-genres. Le présent chapitre est adapté de :

Tosso, F., Doucet, J. L., Migliore, J., Daïnou, K., Kaymak, E., Kameni, F. S. M., & Hardy, O. J. (2017). Characterization of microsatellite markers in the African tropical tree species *Guibourtia ehie* (Fabaceae, Detarioideae). Applications in plant sciences, 5(7), 1700023.

6. Characterization of 19 microsatellites markers in the African tropical tree species *Guibourtia* ehie (Fabaceae, Detarioideae)

Félicien Tosso, Jean-Louis Doucet, Jérémy Migliore, Kasso Daïnou, Esra Kaymak et Franck Monthe and Olivier J. Hardy

ABSTRACT

• ***Premise of the study****:* Microsatellites primers (SSRs) were developed in *Guibourtia ehie* (Fabaceae, Caesalpinioideae), to study population genetic structure and the history of African vegetation.

• ***Methods and Results****:* We isolated 18 polymorphic SSRs from a non-enriched genomic library. This set of primer pairs were tested on four populations and the results showed two to 16 alleles per locus with mean observed and expected heterozygosities ranging from 0.27 ± 0.05 to 0.57 ± 0.05. Cross-amplification tests in 13 congeneric species were successful for the four taxa belonging to the subgenus *Gorskia*.

• ***Conclusions****:* This set of microsatellite markers will be useful to investigate the phylogeography and population genetics of *G. ehie*, a key representative of African semi-deciduous moist forests.

Key words: *Guibourtia ehie;* microsatellites; next-generation sequencing.

6.1. Introduction

Guibourtia ehie (A.Chev.) (Fabaceae, Caesalpinioideae) is a timber species found in evergreen and semi-deciduous moist forests from Liberia to Gabon (Tosso et al., 2015). It is distributed on both sides of the Dahomey Gap, a portion of forest-savanna mosaic separating the Upper and the Lower Guinean rain forest blocks (Salzmann and Hoelzmann, 2005). *Guibourtia ehie* is an insect-pollinated and wind-dispersed species (Tosso et al., 2015) exhibiting an abundant natural regeneration around the mother plant (Lemmens et al., 2008). Known as Ovengkol in Gabon and Amazakoué in Ivory Coast, it produces wood of high economic value. The major threat on this species (registered as vulnerable on the IUCN Red List) is logging which causes local population declines (Hawthorne, 1995). *Guibourtia ehie* is therefore a good candidate to assess the impact of logging on gene flow (pollen and seed dispersal) and to study spatial genetic diversity issues before considering conservation plans. In addition, the wide spatial distribution of this species seems interesting to better understand the history of African vegetation and the role of the Dahomey gap, in relation to successive past environmental changes. As only few microsatellites (SSRs) developed for *Guibourtia tessmannii* (Harms) J. Léonard (a central African species) cross-amplified in *G. ehie* (Tosso et al., 2016), we developed here a new set of polymorphic SSRs.

6.2. Methods and results

6.2.1. Development of microsatellite primers

To identify and characterize SSRs, total genomic DNA was extracted (from *G. ehie* dry leaf "FT0272"; Appendix 6.1) following the cetyltrimethylammonium bromide (CTAB) protocol described in Fu et al. (2005). We used Illumina MiSeq platform (GIGA platform, Liège, Belgium) to construct a non-enriched genomic DNA library following Mariac et al. (2014), and to generate 255460 paired-end reads of 145±3 bp long which were pair-assembled with PANDAseq (Masella et al., 2012). The software QDD with the default settings (Meglécz et al., 2014) was used to identify 3597 microsatellites loci following the three classical steps: (i) SSRs detection, (ii) elimination of similar sequences, and (iii) primer design. Among them, we selected a subset of 64 loci: (i) having at least eight di- or trinucleotide repeats, (ii) with primers located at least 20 bp from the SSR motif, and (iii) characterized by PCR products from 130 to 300 bp-long. To have a good distribution of loci sizes and to facilitate multiplexing in the next steps, we then selected 48 loci for amplification tests. Each locus was labeled with fluorochromes FAM, NED, VIC, and PET by adding one of four possible linkers (Q1–Q4; Micheneau et al., 2011) to the 5' end of the forward primer (Table 6.1).

6.2.2. Microsatellite tests

Amplification tests of 48 primer pairs were performed using two individuals of *G. ehie*: FT0288 and FT0478 (Appendix 6.1) in 15 µL of PCR reactions with the

following conditions: 1.5 µL buffer (10×), 0.6 µL $MgCl_2$ (25 mM), 0.45 µL dNTPs (10 mM each), 0.3 µL of each primer (0.2 µM), 0.08 µL TopTaq DNA Polymerase (5 U/µL; QIAGEN, Venlo, The Netherlands), 1.5 µL of Coral Load, 1 µL of template DNA (of ca. 10–50 ng/µL), and 9.27 µL water. PCR conditions were: 94°C (4 min); 30 cycles of 94°C (30 s), 57°C (45 s), and 72°C (1 min); and a final extension at 72°C (10 min). Amplification products stained with 9 µL of TE 1X were examined using the QIAxcel DNA Screening Kit (method AL420; alignment marker 15-5000 bp; size marker 100-2500 bp; Qiagen, Venlo, Netherlands). Thirty loci amplified the expected target fragments out of the 48 primer pairs selected for initial trial.

These 30 SSR primer pairs were further tested in eight individuals from Ghana and Cameroon (Appendix 6.1). PCR reactions were performed for each of the 30 SSR primer pairs in 15 µL total volumes: 0.15 µL of the reverse and 0.1 µL of the forward (0.2 µM for both) microsatellite primers, 0.15 µL of Q1–Q4 labeled primers (0.2 µM each), 7.5 µL of Type-it Microsatellite PCR Kit (QIAGEN), 3 µL of the 5x Q-solution, 3.1 µL of H_2O and 1 µL of DNA. PCR conditions were: 5-min initial denaturation at 95°C, followed by 25 cycles of 95°C for 30 s, 57°C for 90 s, 72°C for 1 min; 10 cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 60 s; and a final elongation step at 60°C for 30 min. All individuals were genotyped on an ABI3730 sequencer (Applied Biosystems, Lennik, The Netherlands; ULB-EBE platform) using 1.1 µL of each PCR product, 12 µL of Hi-Di Formamide (Life Technologies, Carlsbad, California, USA) and 0.3 µL of MapMarker 500 labeled with DY-632 (Eurogentec, Seraing, Belgium). We selected 19 primer pairs exhibiting clear chromatograms with no ambiguity in allele size determination. Eighteen primer pairs were polymorphic and one locus was monomorphic (GuiE-ssr-04).

These loci were included in four multiplexed reactions (Table 1) using Multiplex Manager 1.0 software (Holleley and Geerts, 2009). To assess their polymorphism level, we genotyped between 15 to 23 individuals in each of four populations from Ghana, Ivory Coast, Liberia and Cameroon, totaling 78 samples (Table 6.2 and Appendix 6.1). We conducted multiplexed PCR reactions and conditions as previously described, except that we readjusted the quantity of H_2O to obtain a total volume of 15 µL.

Table 6.1. Characteristics of 19 nuclear microsatellite markers designed for *Guibourtia ehie*

Primers[a]	Primers sequences (5'-3')	Labeled primer[b]	Repeat motif	Allele range size (bp)	GenBank accession no.
Multiplex 1					
GuiE-ssr39	F: CACTGCTTAGAGCGATGCTCGGTTAGTGAAATGGTTGTTG R: ATTAGTTCCATGATCATTACTTCAAA	Q3-VIC	$(AT)_{14}$	132-156	KY929303
GuiE-ssr34	F: TAGGAGTGCAGCAAGCATGCATTGTTGGAATGATAAATTCAC R: GGATTCAACTATGAAAGGAAACA	Q2-NED	$(AT)_{10}$	152-180	KY929300
GuiE-ssr18	F: TAGGAGTGCAGCAAGCATCAAGTGGGTTCATTGCGTTA R: CGTTGGCTGTGAAGAGAAAGT	Q2-NED	$(AG)_{14}$	180-190	KY929294
GuiE-ssr05	F: TGTAAAACGACGGCCAGTTGACCCAACATATAGAGCATGAG R: CCCTATGGGTGATTGTATGC	Q1-6-FAM	$(TC)_{9}$	262-264	KY929289
GuiE-ssr33	F: TGTAAAACGACGGCCAGTTTAAACCCTAAAGCACAATCAAA R: CCAAGGCATCCACATGAACTA	Q1-6-FAM	$(AG)_{11}$	142-153	KY929299
Multiplex 2					
GuiE-ssr36	F: TAGGAGTGCAGCAAGCATCAAAGGACCTTCCTGCAACT R: TCAAGTACGATCCTCAGAAATCTT	Q2-NED	$(CT)_{13}$	147-163	KY929301
GuiE-ssr03	F: CTAGTTATTGCTCAGCGGTAATGAGGCAGCTTGCATTG R: CGCTAATTAGTTGATACTATGCTCG	Q4-PET	$(TG)_{13}$	219-283	KY929287
GuiE-ssr02	F: CACTGCTTAGAGCGATGCTTAGTAGCTGAATTTCTCATGCAA R: CGCTAATTAGTTGATACTATGCTCG	Q3-VIC	$(ATT)_{10}$	262-294	KY929286
GuiE-ssr06	F: CACTGCTTAGAGCGATGCCCTAAAGCCAAGTGATCCCA R: GAATTGAAGAATGAGATGCCAA	Q3-VIC	$(TA)_{14}$	232-294	KY929290
GuiE-ssr31	F: TGTAAAACGACGGCCAGTTTAAACCCTAAAGCACAATCAAA R: CCAAGGCATCCACATGAAC	Q1-6-FAM	$(AG)_{11}$	143-153	KY929298
Multiplex 3					
GuiE-ssr01	F: TGTAAAACGACGGCCAGTCATGGATCACAAACCCGTTA R: GTGCTAAATTCCTATTTGGTCTTACTG	Q1-6-FAM	$(AG)_{11}$	308-316	KY929285
GuiE-ssr04[c]	F: CTAGTTATTGCTCAGCGGTAATGCCTAATGGGTAAAGCC R: TCCAAGTTTAGAACTTTAAATAGGTTG	Q4-PET	$(CAT)_{8}$	267	KY929288
GuiE-ssr15	F: CACTGCTTAGAGCGATGCTGGACTCAGATGATCCATTGTT R: TGCATACACTAGGAAGGAACGA	Q3-VIC	$(CT)_{14}$	200-230	KY929293
GuiE-ssr21	F: TGTAAAACGACGGCCAGTCCCACGAGTGAGAGTGAAGG R: TCACATTACCAGTCTTCCTTGTC	Q1-6-FAM	$(TC)_{22}$	141-189	KY929295
GuiE-ssr38	F: TAGGAGTGCAGCAAGCATTGTGCCAAAGATACTCCCAA R: TGTGGTAGTCAAGCACCCAA	Q2-NED	$(AG)_{10}$	143-152	KY929302
Multiplex 4					
GuiE-ssr08	F: CTAGTTATTGCTCAGCGGTACATGGCTTCTGGTGACGTT R: GAACGGCCTTACAAACGAAA	Q4-PET	$(TA)_{21}$	222-260	KY929291
GuiE-ssr11	F: CTAGTTATTGCTCAGCGGTTTCTCACGTCTACTTTCAAATCATTG R: CAAATTGCCTGTTGGGTTCT	Q3-VIC	$(AT)_{14}$	205-245	KY929292
GuiE-ssr28	F: CTAGTTATTGCTCAGCGGTTTCTCACGTCTACTTTCAAATCATTG R: AAACAAATTTAGTTAAAGGAAGAGGG	Q4-PET	$(TA)_{10}$	159-167	KY929296
GuiE-ssr30	F: TAGGAGTGCAGCAAGCATATTGATTTCGACAAACACAACA R: CTGAGATATCCTTTGCACATCG	Q2-NED	$(AG)_{14}$	145-157	KY929297

[a] Optimal annealing temperature was 57°C and 53°C respectively for cycles 1 and 2.
[b] The linkers (Q1, Q2, Q3, Q4) attached to the forward primers are underlined.

[c] Monomorphic locus.

6.2.2. Data analysis

We used INEst 1.0 (Chybicki and Burczyk, 2009) to calculate the following indices on each of the four populations: number of alleles per locus (*A*), observed (*Ho*) and expected (*He*) heterozygosities, and inbreeding coefficient (*F*). We also tested deviation from Hardy–Weinberg equilibrium (HWE) for each locus with SPAGeDi (Hardy and Vekemans, 2002).

Table 6.2. Results of 19 SSRs in four populations of *Guibourtia ehie*

	Ghana, *N*=20				Ivory Coast, *N*=23				Cameroon, *N*=15				Liberia, *N*=20			
Locus	*A*	*Ho*	*He*	*F*[a]	*A*	*Ho*	*He*	*F*[a]	*A*	*Ho*	*He*	*F*[a]	*A*	*Ho*	*He*	*F*[a]
Multiplex 1																
GuiE-ssr39	2	0.1	0.19	0.47	2	0.04	0.33	0.87	2	0.07	0.3	0.78	7	0.65	0.77	0.16
GuiE-ssr34	3	0.45	0.66	0.32	3	0.09	0.64	0.86***	5	0.07	0.74	0.91***	3	0.3	0.55	0.46**
GuiE-ssr18	3	0	0.68	1***	3	0.39	0.75	0.48	3	0.67	0.69	0.03	2	0.05	0.5	0.90***
GuiE-ssr05	1	0	0	1	1	0	0.23	1	1	0	0.24	1	2	0.05	0.14	0.65
GuiE-ssr33	1	0	0	1	1	0	0.09	1	1	0	0	1	1	0	0	1
Multiplex 2																
GuiE-ssr36	3	0.1	0.53	0.81***	7	0.22	0.72	0.70***	8	0.67	0.71	0.07	4	0.1	0.38	0.74
GuiE-ssr03	3	0.5	0.47	-0.07	7	0.26	0.65	0.60**	8	0.67	0.87	0.23	3	0.1	0.41	0.75
GuiE-ssr02	5	0.15	0.49	0.69***	2	0	0.54	1***	0	0	0	1	1	0	0.43	1
GuiE-ssr06	9	0.6	0.88	0.32***	7	0.26	0.7	0.63**	7	0.33	0.86	0.61***	7	0.5	0.84	0.41
GuiE-ssr31	1	0	0	1	3	0.09	0.44	0.80**	1	0	0	1	1	0	0.18	1
Multiplex 3																
GuiE-ssr01	2	0.05	0.05	0	1	0	0.43	1	1	0	0.24	1	1	0	0.26	1
GuiE-ssr04	1	0	0	1	1	0	0.5	1	1	0	0.24	1	1	0	0.26	1
GuiE-ssr15	1	0	0	1	2	0	0.51	1	4	0.2	0.31	0.35	2	0	0.19	1*
GuiE-ssr21	5	0.85	0.78	-0.09	8	0.48	0.86	0.45	4	0.13	0.36	0.63*	4	0.4	0.57	0.3
GuiE-ssr38	2	0.25	0.22	-0.11	2	0.26	0.65	0.6	3	0	0.68	1***	2	0.4	0.58	0.31
Multiplex 4																
GuiE-ssr08	11	0.75	0.92	0.18*	11	0.39	0.88	0.55***	2	0	0.57	1**	7	0.5	0.82	0.39
GuiE-ssr11	8	0.4	0.72	0.44***	10	0.43	0.84	0.48	10	0.27	0.88	0.69***	3	0.2	0.72	0.72*
GuiE-ssr28	4	0.5	0.48	-0.03	3	0.26	0.68	0.61	1	0	0.24	1	2	0.45	0.67	0.33
GuiE-ssr30	3	0.65	0.66	0.01	3	0.26	0.75	0.65**	5	0.53	0.77	0.31	4	0.45	0.77	0.41

Note: -= not applicable; *N*=number of individuals sampled; *A* = number of alleles; *He* = expected heterozygosity; *Ho* = observed heterozygosity; *F* = fixation index; [a] For *F*= significance of deviation from Hardy–Weinberg equilibrium: * $P<0.05$; ** $P < 0.01$; *** $P<0.001$.

The mean number of alleles per locus among the four populations was 7 (range 1-16). The observed heterozygosity (mean ± SE) was 0.28±0.10 (range 0-0.85), 0.18±0.17 (range 0–0.48), 0.19±0.09 (range 0–0.67) and 0.22±0.07 (range 0-0.50) for Ghana, Ivory Coast, Cameroon and Liberia populations respectively. The expected heterozygosity was 0.41±0.11 (range 0–0.92), 0.59±0.07 (range 0–0.88), 0.46±0.10 (range 0–0.88) and 0.48±0.08 (range 0–0.84) for Ghana, Ivory Coast, Cameroon and Liberia populations respectively. Four loci exhibited significant deviations from HWE in the Ghana population only (GuiE-ssr03, GuiE-ssr21, GuiE-ssr38 and GuiE-ssr28) due to the presence of null alleles (Table 6.2). All these SSR sequences have been deposited in GenBank and Biosample (Table 6.1).

6.2.3. Cross-amplification in other Guibourtia *species*

We tested the 19 loci on 13 congeneric species using the PCR conditions described above. Three to eight of the 19 loci successfully amplified in two other species from the subgenus *Gorskia* (to which *G. ehie* belongs) whereas two to six amplified for subgenus *Guibourtia* and two to three for subgenus *Pseudocopaiva* (Table 6.3).

The locus GuiE-ssr15 was shared by all species. The limited transferability of *G. ehie* SSRs, also observed for *G. tessmannii* SSRs (Tosso et al., 2016), indicates a rather deep molecular divergence among *Guibourtia* species.

6.3. Conclusion

In this study, we developed 18 polymorphic microsatellite markers in *Guibourtia ehie*. These microsatellite markers will be useful to study intraspecific diversity and gene flow. They are also suitable to study the demographic history of *G. ehie* and provide insights into the past changes in African moist forest cover.

Table 6.3. Cross-amplification results of 19 microsatellite markers isolated from *G. ehie* and tested in 13 congeneric species belonging to three subgenera.

	Subgenus *Gorskia*				Subgenus *Pseudocopaiva*					Subgenus *Guibourtia*			
Species	*G. arnoldiana* (*N* = 3)	*G. schliebenii* (*N* = 3)	*G. conjugata* (*N* = 1)	*G. dinklagei* (*N* = 1)	*G. tessmannii* (*N* = 10)	*G. pellegriniana* (*N* = 7)	*G. coleosperma* (*N* = 6)	*G. leonensis* (*N* = 1)	*G. hymenaefolia* (*N* = 1)	*G. carrissoana* (*N* = 2)	*G. copallifera* (*N* = 6)	*G. demeusei* (*N* = 6)	*G. sousae* (*N* = 1)
Multiplex 1													
GuiE-ssr39	122_136	130	154-156	-	130	130-136	-	-	-	118	-	118	132
GuiE-ssr34	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr18	180	-	196-198	-	-	-	-	-	-	-	-	-	-
GuiE-ssr05	268-274	-	-	248-266	-	262-270	-	-	-	-	-	-	-
GuiE-ssr33	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
Multiplex 2	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr36	153-155	136-154	-	-	148-172	154-156	-	-	-	-	144-156	182-206	-
GuiE-ssr03	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr02	278-280	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr06	200	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr31	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
Multiplex 3	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr01	314	-	266-272	-	-	-	-	-	-	-	-	-	-
GuiE-ssr04	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr15	242	200-266	200	210-224	204	194-206	174-206	204	214	208	160	208-240	206
GuiE-ssr21	-	146	148	156-168	141-146	141	-	-	144	-	-	-	-
GuiE-ssr38	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
Multiplex 4	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr08	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr11	-	-	-	-	-	174-182	182	182	-	-	-	-	-
GuiE-ssr28	-	-	-	-	-	-	-	-	-	-	-	-	-
GuiE-ssr30	-	150-160	-	-	-	-	-	-	-	-	157	-	-

Chapitre 7

Phylogeographic patterns in two congeneric African tree species found in contrasting environment: *Guibourtia ehie* (A.Chev.) J.Léonard and *Guibourtia coleosperma* (Benth.) J.Léonard

Le chapitre 7 a utilisé les deux jeux de marqueurs moléculaires neutres développés dans les chapitres 5 et 6 afin d'examiner si les barrières géographiques et/ou les gradients climatiques ont contribué à des différenciations génétiques entre populations de *Guibourtia*. Deux espèces vivant dans des écosystèmes constrastés ont été étudiées dans ce chapitre : *Guibourtia ehie* et *Guibourtia coleosperma*. En outre, les résultats de ce chapitre préjugent de la présence d'une nouvelle espèce au sein du genre *Guibourtia*.

7. Phylogeographic patterns in two congeneric African tree species found in contrasting environments: *Guibourtia ehie* (A.Chev.) J.Léonard and *Guibourtia coleosperma* (Benth.) J.Léonard

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Abstract

Biogeographic barriers as well as ecological gradients can be the drivers of genetic differentiation among populations, possibly leading to speciation. In African tropical forests, the relative contributions of these two factors remain to be demonstrated. In the present paper we used variation in nuclear microsatellites (nSSRs) to explore how geographical barriers or an ecological gradient can influence genetic patterns of diversity, or even speciation, in trees from the Guineo-Congolian and Zambezian regions. By using two congeneric tree species, the moist forest species *Guibourtia ehie* and the woodland species *Guibourtia coleosperma*, we detected four genetic clusters in the former and two in the latter. These genetics clusters were geographically coherent. In *G. ehie* the strong genetic differentiation combined with morphological differences between West African and Central African clusters suggests that this taxon may contain two distinct species (F_{ST} varying from 0.47 to 0.55) with unassigned individuals found between Southern Nigeria and southwest Cameroon. In *G. coleosperma*, two clusters were well differentiated (F_{ST}=0.06) along a north-south axis corresponding to a rainfall gradient. By studying the phylogeography of rain forest species found on both sides of the Dahomey Gap, this work contribute to the debate about the history of African vegetation. Moreover, our results highlight the contribution of population genetic tools to an integrative taxonomy by uncovering hidden species.

Key words: Detarioideae, phylogeography, microsatellite markers, population genetics, cryptic species

7.1. Introduction

Although less diversified than tropical America and Asia (Slik et al., 2015), the plant diversity in tropical Africa is remarkable. This diversity, mainly located in the Guineo-Congolian rain forests according to White (1983), has been impacted by climatic oscillations during the last million years (Dupont et al., 2001, Miller and Gosling, 2014). Phylogeographic and population genetic studies of African plants, especially tree species (characterized by low speciation rate), have been interpreted in the light of paleo-vegetation data (Maley and Brénac, 1987, Daïnou, 2012; Ewédjè and Hardy, 2012; Duminil et al., 2013; Hardy et al., 2013, Demenou et al., 2016; Donkpegan, 2017; Ley et al., 2017). These studies gave evidence of geographical discontinuities in genetic variation in many forest species, indicating past population isolation, probably due to past climate oscillations and/or barriers due to inhospitable habitat (mountain chains, dry areas, etc.). Moreover, such phylogeographic studies revealed cryptic species occuring in sympatry or parapatry in several tree taxa (Daïnou et al., 2016; Donkpegan, 2017; Ikabanga et al., 2017). Hence, they contribute to understand the history of the African vegetation, which is less documented than in temperate regions (Hardy et al., 2013). Nevertheless, there are still many knowledge gaps : (i) studies in Zambezian drylands and savannas are few, (ii) there is a limited number of phylogeographic studies covering the Upper and Lower Guinean phytogeographic regions with sampling in Nigeria, allowing to assess the boundary between these biogeographical areas, a subject still debated.

According to Koffi et al. (2011) the Atlantic ocean and the Dahomey Gap (a portion of forest-savanna mosaic separating the Upper and the Lower Guinean rain forest blocks, Salzmann and Hoelzmann, 2005) could represent barriers to seed dispersal between West Africa and Central Africa. At the same time, the works of Daïnou (2012) and Duminil et al. (2013) found that the limit between the most adjacent West African and Central African genetic clusters of two tree species could be situated 500 to 1000 km east of the Dahomey Gap, in Nigeria (for *Milicia excelsa*, Daïnou, 2012) and near the forest-savanna limit in northern Cameroon (for *Erythrophleum suaveolens*, Duminil et al., 2013). These limits are congruent with the extraordinary floristic and faunal diversity in the region between the Cross River (in Southeastern Nigeria) and the Cameroonian volcanic line (in Southwestern Cameroon), suggesting that this region could be considered as a major vegetation divide between West and Central Africa (Udo, 1970; Pilbrow, 2010; Nicolas et al., 2012). However, the conclusion of Daïnou (2012) and Duminil (2013) may result from the ecological characteristics of the species studied. *Milicia excelsa* and *Erythrophleum suaveolens* have relatively large ecological amplitude as they are found both in dense moist forest and in forest galleries of dry areas (Aubréville, 1985; Hawthorne, 1995). It is therefore interesting to refine our knowledge about the history of African rain forests by studying species more dependent on rain forest environment.

Beside the important contribution of phylogeny, phylogeography and population genetics to the understanding of the African vegetation history, these disciplines are also valuable to address species delineation and evaluate hybridization rates in closely related taxa (Daïnou et al., 2016). Population genetic methods using nuclear

microsatellite markers (SSRs) allows to assess reproductive isolation, which is the basis of the biological species concept (Yeates et al., 2011). These nuclear microsatellite markers, when combined with morphological descriptions, have revealed the existence of cryptic species among African tropical tree species (e.g. in *Milicia*, Daïnou et al., 2016 ; *Erythrophleum*, Duminil et al., 2013 ; *Afzelia*, Donkpegan, 2017 ; *Santiria*, Ikabanga et al., 2017). Population genetics thus contribute to an integrative taxonomy by combining morphological description of identified genetic clusters in order to highlight the diagnostic characteristics that can differentiate species.

In this paper, we focused on the genus *Guibourtia* Benn. (Ceasalpinioideae, Detarioideae) which is characterized by a large geographical distribution and, in Africa, comprises 13 timber species growing in various tropical biomes, from tropical moist forests to dry forests and savannas (Tosso et al., 2015). In particular, we considered two *Guibourtia* species found in contrasting environments to study the geographical distribution of their genetic diversity. *Guibourtia ehie* (A.Chev.) J.Léonard (1949) is a moist forest tree species distributed from Upper Guinea to Lower Guinea, with an interruption in the Dahomey Gap. It is a good model to assess the impact of biogeographical breaks by assessing the degree of divergence between these two Guineo-Congolian phytogeographic regions, locate the exact position of the break (closer to the Dahomey Gap or the Cameroonian Volcanic line), and check whether their populations really belong to the same species. *Guibourtia coleosperma* (Benth.) J.Léonard (1949) is a species occurring in Zambezian dry forests and woodlands but embedded in a clade of Guineo-Congolian moist forest species (Tosso et al., 2018), suggesting that it has relatively recently adapted to drier conditions. It is thus a good model to investigate the possible impact of a shift of ecological niche on the phylogeographic pattern. The aim of this paper is to use microsatellites to investigate how geographical barriers or ecological gradients can influence genetic patterns of diversity, or even speciation, within *Guibourtia ehie* and *Guibourtia coleosperma*.

7.2. Material and Methods

7.2.1. Study species

Guibourtia ehie (A.Chev.) J.Léonard (1949) is a non pioneer light demanding deciduous tree found in evergreen and semi-deciduous rain forests from Liberia to Gabon (Doucet, 2003; chapitre 4), hence it is distributed on both sides of the Dahomey Gap. *G. ehie* is insect pollinated with seeds that are wind-dispersed. It produces wood of high economic value, known as ovengkol in Gabon and amazakoué in Ivory Coast (Tosso et al. 2015, Tosso et al. 2017). *Guibourtia coleosperma* (Benth.) J.Léonard (1949) is a light demanding and evergreen species adapted to dry forests and woodlands, distributed from DRC to Zimbabwe (Bamford *et al.*, 2005; Tosso *et al.*, 2015). It is also insect pollinated and has seeds mainly dispersed by avian frugivores including hornbills (Calvert, 1984).

7.2.2. Sampling and SSR genotyping

We used georeferenced (GPS) samples (silica-dried leaf or cambium) of 160 *G. ehie* individuals from six different countries and 30 *G. coleosperma* individuals. The sampling of each species was representative of their respective distribution ranges. DNA was extracted using the NucleoSpin plant kit (Macherey-Nagel, Duren, Germany) following the manufacturer's instructions. Following the protocols described in Tosso et al. (2016, 2017), 19 microsatellites loci (GuiE-ssr39, GuiE-ssr34, GuiE-ssr18, GuiE-ssr05, GuiE-ssr23, GuiE-ssr36, GuiE-ssr03, GuiE-ssr02, GuiE-ssr06, GuiE-ssr31, GuiE-ssr01, GuiE-ssr04, GuiE-ssr15, GuiE-ssr21, GuiE-ssr38, GuiE-ssr08, GuiE-ssr11, GuiE-ssr28 and GuiE-ssr30; (Tosso et al., 2017) and 10 microsatellites loci (R12-seq15, R12-seq10, R12-seq35, R12-seq08, R12-seq26, R12-seq34, R12-seq16, R12-seq09, R12-seq20 and R12-seq22; (Tosso et al., 2016) were used to amplify each individual of *G. ehie* and *G. coleosperma*, respectively. Twelve *G. ehie* and three *G. coleosperma* individuals that showed less than 50% amplified loci were removed from the dataset so that 148 *G. ehie* and 27 *G. coleosperma* individuals were used for the next analyses.

7.2.3. Genetic structure in G. ehie *and* G. coleosperma

For each species, we used TESS 2.3.1 (Chen et al., 2007) and STRUCTURE 2.3.3 (Pritchard et al., 2000) to identify genetic clusters. With TESS, we fixed the maximum number of clusters (K_{max}) from 2 to 10 and chose the admixture model. We set the interaction parameter ψ to 0 (spatial information not taken into account to identify genetic clusters). Ten runs were executed for each value of K_{max} and each run was based on 100,000 MCMC iterations with a 10,000 burn-in period. To identify the most likely number of clusters, we plotted the values of the deviance information criterion (DIC) against K_{max} to identify the asymptote. Once the number of clusters (K_{max}) was inferred, the four best runs (i.e. with lowest DIC) out of the ten were used to compute the average cluster membership (q) of each individual using the program CLUMPP (Jakobsson and Rosenberg, 2007). In the first step the individuals were assigned to a given cluster when $q > 0.9$ to explore genetic structure pattern. STRUCTURE 2.3.3 was also used under the admixture model with allele frequencies independent among clusters to compare its results with those obtained with TESS. We ran the simulation for values of K from 1 to 10 clusters, with 10 repetitions per each value of K, setting the number of iterations to 100,000 and the burn-in period to 10,000. We used STRUCTURE Harvester (online platform: http://taylor0.biology.ucla.edu/structureHarvester/) to compute the posterior log likelihood of the data, LnP(D|K) for each independent run per K. The best value of K was inferred by identifying the one with LnP(D|K) approaching an asymptote with least variation between independent runs (Chen et al., 2007). Once, the value of K was identified, the program CLUMP was again used as described above and the assignment of the individuals was carried out in the same way as described with TESS. The concordance rates between TESS and STRUCTURE results were evaluated. In the case of *G. ehie*, as two genetic clusters highly differentiated were detected, the same approach (described above) was used within each major genetic cluster in order to identify possible minor genetic clusters. To disentangling the effect of Isolation-By-Distance (IBD) from barriers to gene flow in

the genetic structures of *G. ehie* and *G. coleosperma*, we further analysed how the kinship coefficient (F_{ij}) between pairs of individuals *i* an *j* evolved with spatial distance when *i* and *j* belong (1) to the same cluster or (2) to different genetic clusters. Under IBD we expect that the between cluster F_{ij} curve decays with distance and is globally aligned to the within cluster F_{ij} curves. By contrast, if clusters result from a long-standing barrier to gene flow, the between cluster F_{ij} curve is expected to be independent from distance (horizontal) with values much lower than for the within cluster F_{ij} curves. F_{ij} was estimated as a correlation coefficient between allelic states, following the estimator of J. Nason (Loiselle et al. 1995), and computed using SPAGeDi 1.5 (Hardy and Vekemans, 2015). Using the same reference population (the entire sample for each species) we tested the significance of the Spatial Genetic Structure (SGS) pattern within and among genetic cluster (see results) by comparing the observed regression slope (b_{LD}) of F_{ij} on logarithmic distance d_{ij} with its expected distribution obtained from 10,000 permutations of rows and columns of the inter-individual distance matrix (Mantel test).

7.2.4. Genetic differentiation and diversity between and within clusters

For each genetic cluster we computed the following multilocus genetic diversity parameters with SPAGeDi 1.5 (Hardy and Vekemans, 2015): total number of alleles (Ao), allelic richness (R_S), expected heterozygosity (He). We excluded from these analyses the loci (GuiE-ssr15, GuiE-ssr28, GuiE-ssr38 for *G. ehie* and R12-seq09, R12-seq22 and R12-seq34 for *G. coleosperma*) exhibiting either excess of homozygotes or fixed heterozygosity. We used INEst 1.0 (Chybicki and Burczyk, 2009) to estimate the corrected inbreeding coefficient (*Fi*) in each genetic cluster after excluding, in addition to loci mentioned above, monomorphic loci. The proportion of the private alleles in each genetic cluster was also computed. The global differentiation and differentiation between pairs of genetic clusters was determined using F_{ST} and R_{ST}. The latter is useful to test phylogeographical signal dependent of allele size as it is expected to be larger than F_{ST} if stepwise mutations have contributed to the differentiation (Hardy et al., 2003). Nei's standard genetic distance with sample size correction (D_S) was also computed between genetic clusters as it is expected to better reflect divergence time than F_{ST}, which depends much on genetic drift (Daïnou et al., 2016). These differentiation parameters were tested with permutation tests in SPAGeDi 1.5 (Hardy and Vekemans, 2015).

7.3. Results

7.3.1. Bayesian clustering analyses: inferring spatial population structure

In *G. ehie*, TESS analyses indicated that the DIC reached an asymptote at K_{max}=3. This result was confirmed by STRUCTURE analyses that also showed a plateau of ln P(D|K) at K=3 (Appendixes 7.1A and 7.1B). The results of TESS and STRUCTURE were concordant at 96.59 %. The values of cluster membership

coefficients showed an abrupt progression. The following three genetic clusters were obtained using a cluster membership coefficient q > 0.6: the first genetic cluster is located in Upper Guinea [UG], the second is in Lower Guinea [LG]) and the third one included only three individuals found between Nigeria and southwest Cameroon. With a cluster membership coefficient q > 0.9, two genetic clusters corresponding exactly to UG and LG (Figure 7.1A and 7.1D) were obtained with three unassigned individuals (a cluster membership coefficient less or equal to 0.9 ($q \leq 0.9$) at $K_{max}=2$). These unassigned individuals were those constituting the third genetic cluster mentioned above. Hereafter we focused on the two genetic clusters UG and LG since the number of unassigned individuals were too few to go further.

The two genetic clusters (UG and UL) were much differentiated ($F_{ST}=0.46\pm0.09$), so that we ran again TESS within each 'major' genetic cluster to verify whether additional subdivision occurred. Again, two 'minor' genetic clusters were inferred in both UG and LG major clusters. In Upper Guinea, a western cluster (UGw) included mostly individuals from Liberia and Ivory Coast whereas an eastern cluster (UGe) was centred on Ghana (Figure 7.1B and 7.1D). Some unassigned individuals (using $q<0.6$) were found between UGw and UGe. In Lower Guinea, a large northern genetic cluster (LGn) stretched from Nigeria to western Gabon, while a southern genetic cluster (LGs) covered southern Gabon (Figure 7.1C and 7.1D).

Regarding *G. coleosperma*, STRUCTURE and TESS analyses were 96.67 % concordant, showing an asymptote reached at $K_{max}=2$. Contrary to what was observed with *G. ehie*, the distribution of assignment coefficient values was more progressive (Figure 7.2A et 7.2B). A northern genetic cluster (CGn) was located in southern DRC and a southern cluster (CGs) was found in northern Namibia, northern Botswana, southern Angola and southern Zambia (Figure 7.2A et 7.2B). Two individuals located in southern DRC were unassigned.

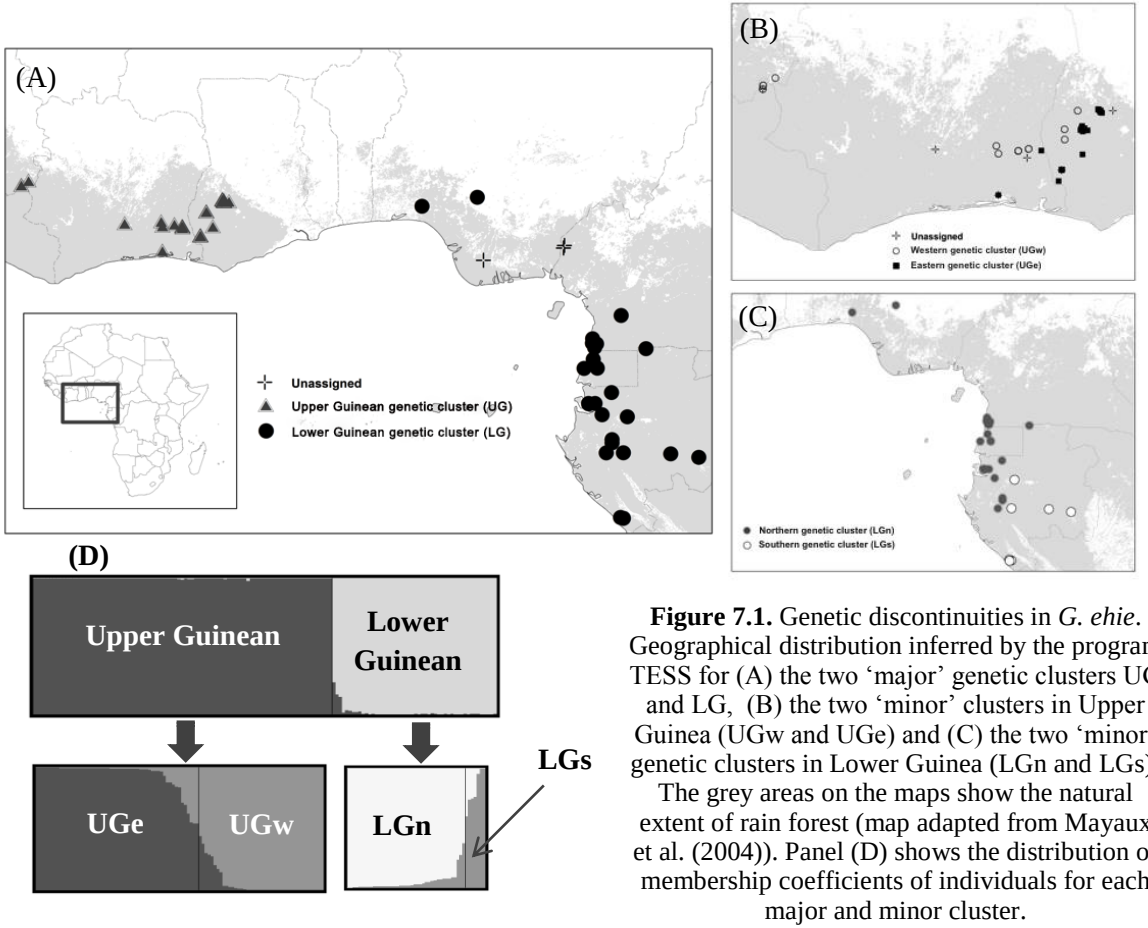

Figure 7.1. Genetic discontinuities in *G. ehie*. Geographical distribution inferred by the program TESS for (A) the two 'major' genetic clusters UG and LG, (B) the two 'minor' clusters in Upper Guinea (UGw and UGe) and (C) the two 'minor' genetic clusters in Lower Guinea (LGn and LGs). The grey areas on the maps show the natural extent of rain forest (map adapted from Mayaux et al. (2004)). Panel (D) shows the distribution of membership coefficients of individuals for each major and minor cluster.

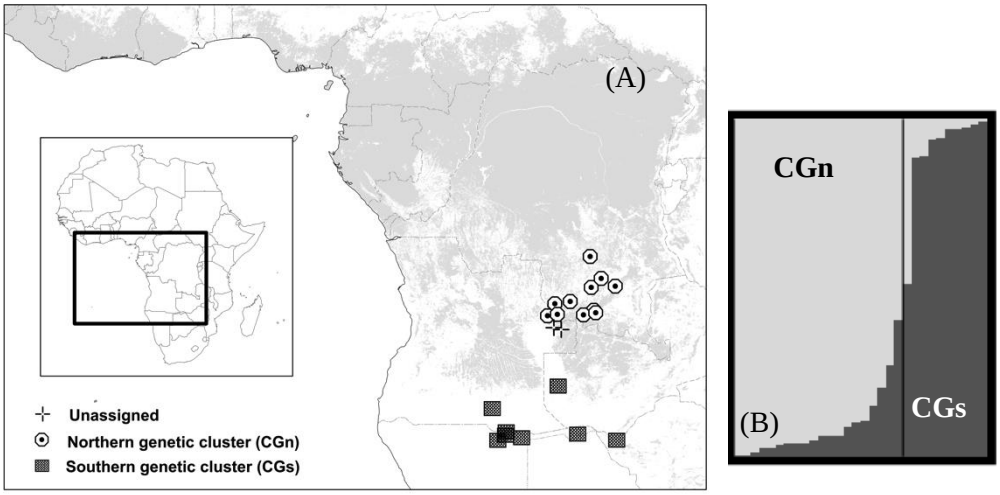

Figure 7.2. A) Geographical distribution of the two genetic clusters (CGn and CGs) in *G. coleosperma* inferred by the program TESS. The grey areas on the maps show the natural extent of rain forest and dry forest vegetation and white the savannas (map adapted from Mayaux et al. (2004)). and B) membership coefficient of *G. coleosperma* individuals

7.3.2. Testing Isolation-By-Distance within and between genetic clusters

In *G. ehie* we detected IBD within UG and within LG genetic clusters (b_{LD}=-0.015±0.004, p<0.0001 and b_{LD}=-0.021±0.017, p<0.001 respectively), but not when considering pairs of individuals from UG and LG genetic cluster (b_{LD}=0.06±0.11). IBD was not detected within and between CGn and CGs genetic clusters of *G. coleosperma* (b_{LD}=-0.012±0.014^{ns}, b_{LD}=-0.021±0.017^{ns} and b_{LD}=0.074±0.08, p<0.0001 respectively within CGn and CGs genetic clusters and among the two genetic clusters) but the low sample sizes probably limited the testing power.

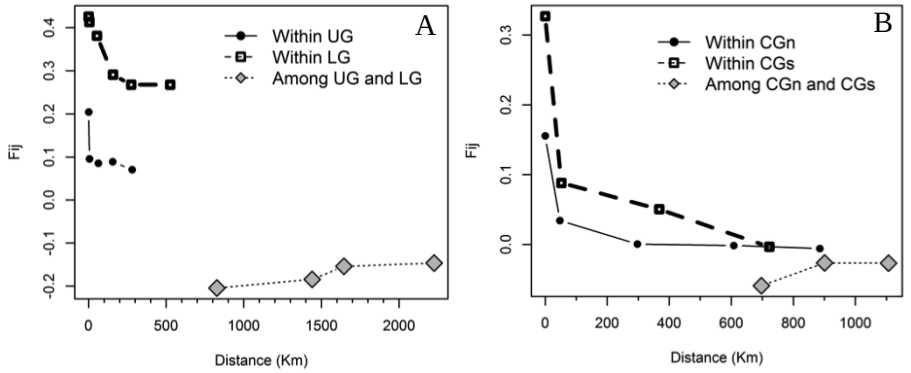

Figure 7.3. Mean kinship coefficient between pairs of (A) *G. ehie* and (B) *G. coleosperma* individuals within and between genetic clusters plotted against distance in the whole sample of each species.

7.3.3. Genetic diversity and differentiation in G. ehie

Pairwise F_{ST} among the four genetic clusters reveal a deep differentiation between UG and LG minor genetic clusters (F_{ST} varying from 0.47 to 0.55, Table 1) while the differentiation between minor genetic clusters of UG and LG was much lower (F_{ST} from 0.06 to 0.11, Table 7.1). As the profound differentiation was observed between UG and LG minor genetic clusters, the percentages of private alleles reached 25.64% in UG and 36.41% in LG (Appendix 7.3). The Lower Guinean minor genetic clusters (LGn and LGs) displayed higher genetic diversity (Rs ranging from 3.25 to 3.27, He from 0.49 to 0.50) than the UGw and UGe genetic clusters (Rs ranging from 2.34 to 2.79, He from 0.35 to 0.46).

Within each major genetic cluster, the minor clusters from Upper Guinea were relatively well differentiated (F_{ST}=0.11 between UGw and UGe) whereas LGn and LGs clusters showed low differentiation (F_{ST}=0.06). R_{ST} values, that take into account microsatellite allele sizes, showed the same trends as F_{ST} and with similar values, suggesting the absence of phylogeographic signals between all pairs of genetic clusters (alleles size permutation tests not significant; Hardy et al. 2003), except between UGe and LGs genetic clusters where significant phylogeographic signal was detected (R_{ST}=xx and F_{ST}=xx). In the same line, patterns of pairwise Nei's Ds distances among pairs of clusters were congruent with those of F_{ST} and R_{ST} (Appendix 7.2).

Our results showed a low inbreeding coefficient for Lower Guinean genetic clusters (LGn and LGs; Fi close to zero). However, we noted a high or overestimated inbreeding coefficient for Upper Guinean genetic clusters (Fi=0.15 in UGw and Fi=0.11 in UGe) suggesting a selfing mating system in these genetic clusters or a spatial genetic structure within them causing biparental inbreeding.

Table 7.1. Genetic characteristics of the four genetic clusters (only individuals with q>0.6) in *G. ehie* and matrix of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal)

Genetic clusters	Differentiation parameters R_{ST}|F_{ST}				Diversity parameters					
	UG_w	UG_e	LG_n	LG_s	N	A_o	R_s	He	Fi	Pa
UG_w		0.116	0.493	0.549	26	63	2.34	0.354	0.1542	25.64%
UG_e	0.108		0.429	0.447	61	92	2.79	0.464	0.1151	
LG_n	0.288	0.468		0.064	41	114	3.27	0.503	0.0003	36.41%
LGs	0.502	0.589*	0.098		7	62	3.25	0.492	0.0000	
All clusters	R_{ST}=0.379			F_{ST}=0.383	135	331	3.55	0.637		

UGw: western genetic cluster in Upper Guinea, UGe: eastern genetic cluster in Upper Guinea, LGn: northern genetic cluster in Lower Guinea, LGs: southern genetic cluster in Lower Guinea, N: number of individuals, Ao: number of alleles, Rs: allelic richness, He: expected heterozygosity corrected for sample size, Fi: inbreeding coefficient estimated by INEst, Pa: percentage of private alleles for UG and LG.

7.3.4. Genetic diversity and differentiation in G. coleosperma

In *G. coleosperma*, the pattern of genetic differentiation showed a moderate differentiation between the northern and southern genetic clusters (F_{ST}=0.06 and pairwise Ds=0.17). We detected a significant phylogeographical signal (R_{ST}=0.32, p<0.001). The genetic diversity of the two clusters was quite similar (Rs=3.74, He=0.47 in CGn, Rs=3.40, He=0.44 in CGs; Table 7.2). However, 22 % of private alleles were detected in CGn (see details for each locus in Appendix 7.4) while none was found in GCs. After accounting for the effect of null alleles, we found high value of inbreeding coefficient suggesting that selfing can occur in *G. coleosperma* or that there is substantial biparental inbreeding, as observed in UGw and UGe genetic clusters of *G. ehie*.

Table 7.2. Genetic characteristics of the two genetic clusters (only individuals with q>0.6) in *G. coleosperma* and matrix of pairwise F_{ST} (above diagonal) and R_{ST} (below diagonal)

Genetic clusters	Differentiation parameters R_{ST}|F_{ST}		Diversity parameters					
	CG_n	CG_s	N	A_o	R_s	He	Fi	Pa
CG_n		0.064	19	33	3.74	0.474	0.1812	22%
CG_s	0.322***		9	25	3.40	0.439	0.2589	0%
All clusters			28	58	3.82	0.466		

CGn: northern genetic cluster, CGs: southern genetic cluster, N: number of individuals, Ao: number of alleles, Rs: allelic richness, He: expected heterozygosity corrected for sample size, Fi: inbreeding coefficient estimated by INEst, Pa: percentage of private alleles.

7.5. Discussion

7.5.1. Phylogeography in G. ehie

We found four geographically coherent genetic clusters within *G. ehie*, suggesting the presence of past barriers to gene flow. Genetic differentiation was particularly important between UG and LG (F_{ST}=0.47 to 0.55). These values represent the highest pairwise genetic differentiation recorded among UG and LG tree populations. In others studies that used SSRs, the F_{ST} between populations of UG and LG was 0.07 for *Terminalia superba* (Demenou et al., 2017), 0.09 for *Erythrophleum ivorense* (Duminil et al., 2013), 0.20 for *Milicia excelsa* (Daïnou et al., 2014), 0.26 for *Pentadesma butyracea* (Ewédjè, 2012) and 0.35 for *Distemonanthus benthamianus* (Demenou et al., 2016). The biogeographic separation between Upper and Lower Guinea is reflected in patterns of both plant and animal diversity (White, 1979; Mayr and O'Hara, 1986; Linder, 2001). Among the hypotheses that can explain genetic differentiation between centres of endemism (Hardy et al., 2013), it is plausible that the populations of *G. ehie* might be largely distributed during forest expansion periods but was isolated in the two forest blocks (UG and LG) when the forest has been split. Two situations can explain the genetic differentiation between *G. ehie* UG and LG populations: (S_1) the populations of *G. ehie* might be largely distributed during forest expansion periods but were isolated in the two forest blocks (UG and LG) when the forest was fragmented, or (S_2) the spatial genetic structure observed is due to isolation by distance which poses that two individuals are more likely to resemble genetically each other if they are geographically close due to limited gene dispersal (Loveless and Hamrick, 1984). If S_2 was true, one would expect IBD among UG and LG genetic clusters. In this study, we did not observe the pattern described in S_2 since there was no IBD among these two genetic clusters (Figure 7.3A).

This pattern suggests therefore a strong discontinuity between UG and LG highlighting that the split between UG and LG can be presumably caused by the separations of both forest blocks since the end of the Pliocene. Considering that Dahomey Gap probably lost forest during Pleistocene (at least during the glacial period), it is plausible that populations of UG and LG were isolated during this period. The works of Koffi et al. (2011), Ewèdjè (2012) and Demenou et al. (2016) have also found that the boundaries between the genetic pools correspond almost to the geographic boundaries of the Dahomey-Gap (Figure 7.4).

However, the three unassigned individuals (considering a membership coefficient q>0.9) could represent hybrids resulting from crossing between UG and LG individuals. If this hypothesis is true, we could imagine that there would be barriers to gene flow between Nigeria and Cameroon. But genotyping more individuals from Nigeria is needed to test this hypothesis. The location of these three unassigned individuals (Figure 7.1A) tends to suggest that the Cross River and the Cameroonian volcanic line may be a barrier to gene flow as suggested Daïnou et al. (2014), Duminil et al. (2013) and Hardy et al. (2013) who assumed that the limit of most adjacent west African and central African gene pools is located in Nigeria and north western Cameroon rather than in Dahomey Gap (Figure 7.4). Other phylogeographic studies on tree species (*Pentadesma butyracea*, Ewèdjè, 2012; *Anthonotha*

macrophylla, Demenou, 2018; *Terminalia superba*, Demenou et al., 2017; Figure 7.4) showed also a genetic discontinuity in the same region but located in Nigeria. However, it is important to highlight a lack of sampling in the heart of Nigeria, as in our case, and this clearly obscure the actual location of any common genetic cline in that country. Better sampling effort in Nigeria is necessary to properly identify the floristic and phylogeographic divides between Upper and Lower Guinea.

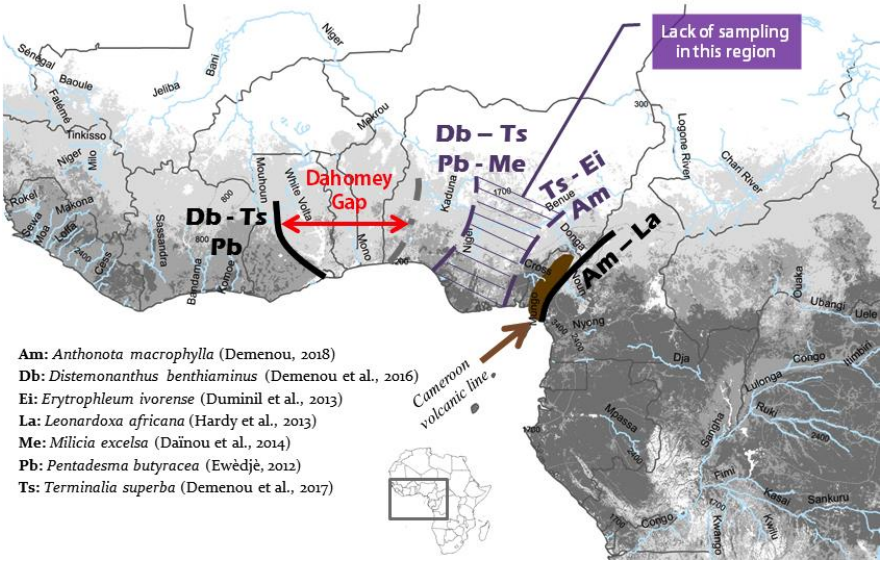

Figure 7.4. Genetic discontinuities between Upper and Lower Guinean for some rain forest tree species. Each species is represented by a code defined in the legend.

Genetic diversity seems lower in Upper Guinean clusters (He: 0.35 – 0.46) than in Lower Guinean clusters (He: 0.49 – 0.50). This trend was also observed in *Milicia* (Daïnou et al., 2016) and *Distemonanthus* (Demenou et al., 2016) although the difference of values was not pronounced. In addition, *G. ehie* populations in Upper Guinea are found in semi-deciduous rain forests whereas those in Lower Guinea are found in moist evergreen forests. Thus, they could have particular alleles selected in individuals of populations from each of the two regions, contributing to the difference observed. Samples of *G. ehie* in the Upper Guinean region were divided into two clusters with a F_{ST}=0.11, unlike other species that did not show a subdivision in the Upper Guinean region [*Pentadesma butyracea* (Ewédjè and Hardy, 2012), *Milicia excelsa* (Daïnou et al., 2016) and *Distemonanthus benthamianus* (Demenou et al., 2016)]. This underlines once again the importance of multiplying such studies on more tree species in order to better understand the dynamics of tree populations in this region.

We obtained a low F_{ST} value between the two genetic clusters identified in the Lower Guinea phytochory (F_{ST}=0.09). Such relatively reduced genetic differentiation were also obtained in *Erythrophleum suaveolens* (Duminil et al., 2013), *Milicia excelsa* (Daïnou et al., 2016) and *Distemonanthus benthamianus*

(Demenou et al., 2016). A phylogeographic signal was noted in *G. ehie* only between UGe and LGs.

7.5.3. Does G. ehie *represents a single species?*

In addition to the high F_{ST} value observed between the Upper Guinean and the Lower Guinean regions in *G. ehie* populations (F_{ST}=0.46 Figure 7.1a), the high proportion of private alleles between the two Upper Guinean and Lower Guinean clusters (25.64% in UG and 36.41% in LG) leads one to wonder if *G. ehie* is really a single species.

When we analysed the botanical characteristics of *G. ehie* leaves on individuals from Cameroon, Gabon, Ivory Coast and Ghana we noted some difference that could be explained by the existence of two species, one in Upper Guinea, the other in Lower Guinea. First, it has been described that individuals of *G. ehie* from Ivory Coast and Ghana have leaflets with translucent dots (Hawthorne and Gyakari, 2001, Léonard, 1950), which is not the case in Cameroon (individual from Douala and Campo region, Aubréville, 1970) and Gabon (individuals from Nkogo region, Aubréeville, 1968). This difference is consistent with our microscopic observations of leaflets on 15 samples from Upper and Lower Guinean phytochories: we found many translucent dots on inner face of leaflets for Upper Guinean samples whereas they were absent or very scarce in Lower Guinean samples (see appendix 7.5). Second, Aubréville (1959) and Hawthorne and Gyakari (2001) observed that West African individuals produce solidified copal, which could be collected at the base of the tree, while this seems not to be the case in Central Africa (at least, this has never been mentioned in any Central African flora consulted). We further observed that the inner margin of individual leaflets from Upper Guinean is straight whereas it is curved in individuals from Lower Guinean. Moreover, the base of the leaflets is stripped for the former and oval for the later (Appendix 7.6). These morphological differences combined with a strong genetic differentiation lead us to suspect that the individuals from West Africa and Central Africa could be two different species, an hypothesis to test further. Furthermore, a particular effort might be made in the contact zone (between southern Nigeria and southwest Cameroon where the presence of hybrids is suspected) to properly clarify this issue. Anyhow, these results reveal once again the power of population genetics approaches to detect the hidden genetic diversity within taxa exhibiting slight phenotypical difference or having disjunct spatial distributions (Liu et al., 2011, Daïnou et al., 2016).

7.5.2. Phylogeography in G. coleosperma

We found a relatively low level of diversity within CGn and CGs genetic clusters (He=0.43 to 0.47). Such low diversity was reported in other dry forest/woodland species : *Afzelia quanzensis* (He = 0.45) (Jinga and Ashley, 2018), *Firmiana danxiaensis* (He = 0.364) (Chen et al. 2014), and *Fontainea picrosperma* (He = 0.407) (Lamont et al. 2016). This low genetic diversity was mainly interpreted as the consequence of small and isolated populations (Chen et al. 2014; Lamont et al. 2016).

Guibourtia coleosperma was divided along a north-south axis in two differentiated genetic clusters (F_{ST}=0.06; R_{ST}=0.32) with a significant phylogeographic signal ($R_{ST} > F_{ST}$). The relatively low F_{ST} suggests that, contrary to the case of *G. ehie,* no strong barrier to gene flow between the CGn and CGs regions affected *G. coleosperma* populations, either because the isolation time has been too short or because sufficient gene flow persists to limit genetic differentiation. However, this is not consistent with the significant phylogeographic signal within *G. coleosperma* genetic clusters indicating ancient divergence where mutations contributed to the cluster differentiation (Hardy et al., 2003). The small sample sizes (N=9 and 19) might limit the precision of our estimates so that these results must be interpreted with caution. Considering that the southern cluster (CGs) does not present private alleles contrary to the northern cluster (CGn), we can hypothesize that CGs differentiated from the CGn cluster by adapting to drier conditions across the climatic gradient (dry forest for CGn, woodland for CGs) assuming that the species originate from the CGn area. This evolutionary trend may even have started earlier, given that the sister species of *G. coleosperma* in Central Africa (*G. tessmannii*) is located in rain forest (between Cameroon and Congo, Tosso et al., 2018) and belong to a clade dominated by rain forest species (subgenus *Pseudocopaiva*), so that *G. coleosperma*, and then its GCs cluster, may have evolved by adapting to ever drier climatic conditions. The results of Jinga and Ashley (2018) on *Afzelia quanzensis* showed a similar pattern in the same region of Africa.

7.6. Conclusion

This study demonstrated the high genetic structuration within *G. ehie* and *G. coleosperma*. Our results highlighted that climatic gradients in *G. coleosperma* and biogeographical barriers (Dahomey Gap or/and Cross River and the Camerounian volcanic line) in *G. ehie* may favour genetic differentiation of their populations. In addition, our population genetics approach combined with morphological observations give evidence that *G. ehie* individuals from Upper and Lower Guinea could potentially belong to two distinct species.

Chapitre 8

Characterization of animal communities involved in seed dispersal and predation of *Guibourtia tessmannii* (Harms) J.Léonard, a species newly listed on Appendix II of CITES

Ce dernier chapitre décrit au moyen d'observations directes et indirectes les animaux impliqués dans la dispersion des graines de *G. tessmannii*, une espèce représentative des espèces zoochores du genre *Guibourtia*. Il montre en particulier que les calaos participent à la dispersion des graines de *G. tessmannii* et pourraient potentiellement contribuer aux flux de gènes à longue distance. Ce chapitre est adapté de :

Tosso, F., Cherchye, G., Hardy, O. J., Daïnou, K., Lognay, G., Tagg, N., Haurez, B., Souza, A., Heuskin, S., & Doucet, J. L. (2017). Characterization of animal communities involved in seed dispersal and predation of *Guibourtia tessmannii* (Harms) J. Léonard, a species newly listed on Appendix II of CITES. African Journal of Ecology. DOI. 10.1111/aje.12480.

8. **Characterization of animal communities involved in seed dispersal and predation of *Guibourtia tessmannii* (Harms) J.Léonard, a species newly listed on Appendix II of CITES**

Felicien Tosso, Gauthier Cherchye, Olivier J. Hardy, Kasso Daïınou, Georges Lognay, Nikki Tagg, Barbara Haurez, Alain Souza, Stephanie Heuskin and Jean-Louis Doucet

Abstract

Characterization of the ecology of endangered timber species is a crucial step in any forest management strategy. In this study, we described the animal communities involved in seed dispersal and predation of a high-value timber species *Guibourtia tessmannii* (Fabaceae; Detarioideae), which is newly listed on Appendix II of CITES. We compared the animal communities between two forest sites (Bambidie in Gabon and Ma'an in Cameroon). A total of 101 hours of direct observations and 355 days of camera trapping revealed that a primate (*Cercopithecus nictitans nictitans*) and a hornbill (*Ceratogymna atrata*) were important seed dispersers in Gabon. Conversely, a greater presence of a rodent (*Cricetomys emini*), which could act both as predator and disperser, was observed in Cameroon. This study suggests that animal communities involved in seed dispersal of *G. tessmannii* may vary depending on environmental conditions and anthropogenic impacts. However, further studies are needed to properly identify the factors involved in seed dispersal and predation of *G. tessmannii*.

Keywords: Detarioideae; birds; primates; seed dispersal; seed predation; tropical rain forest

8.1. Introduction

A large proportion of plants depend upon animals for seed dispersal (Herrera and Pellmyr, 2009). In tropical forests, it has been estimated that between 50 and 80% of tree species produce fruits that are dispersed by mammals and birds (Howe et Smallwood, 1982). Seed dispersal is a critical step in the biological cycle of plants and a key process for the maintenance of tropical forest biodiversity at different scales (Wang et Smith, 2002).

At a large scale, the composition and behavior of dispersal agents may vary both geographically and temporally (Gautier-Hion et al., 1993). These variations might be due to (i) tight co-evolutionary relationships between plants and seed dispersers (Chapman et al., 1992) and (ii) plant community structure and composition (Dennis, 2007). The latter is influenced both by controlling factors (e.g. climate and geologic materials) and dependent factors (vegetation, consumers, decomposers and transformers, soil and microclimate) (Lewis, 1969). Anthropogenic pressures can also have a significant influence: For example, land conversion and defaunation caused by hunting modify the disperser community (Cordeiro and Howe, 2003). In Africa, Effiom et al. (2014) showed that hunting pressure might have a local direct negative effect on large mammals and in turn may have direct and indirect effects on other trophic levels. It is therefore crucial to investigate seed dispersers of a particular plant species across various habitats (Blake et al., 2012).

The seeds and fruits produced by forest tree species contain important nutrients for frugivore and granivore communities (Guindon, 1997; Whitney et al., 1998). Moreover, seed and fruit traits, such as size and shape, influence these plant–animal interactions (Gautier-Hion et al., 1985; Rodríguez et al., 2013; Vander Wall, 1990). The digestion processes as well as animal size also play crucial roles (Stiles, 2000). There is thus a strong relationship between fruiting plant species and their dispersal agents, that can affect both plant and animal communities in a forest ecosystem (Cordeiro et Howe, 2003; Santos et Tellería, 1994)

As well as the relationships between fruiting plant species and their dispersal agents, the mechanisms of seed dispersal are also complex, in that many biotic processes—such as predation, pathogen attack and secondary dispersal—can affect the fate of seeds between primary dispersal and germination (Andresen et al., 2005). Primary dispersal is the removal of a fruit from a tree and the deposition of its seeds in an establishment area (Stoner et Henry, 2009). Secondary dispersal is defined as any significant movement of seeds after primary dispersal from plants (Vander Wall et al., 2005). Rodents play a significant role in this process, for example through scatter hoarding (Forget and Vander Wall, 2001), which is defined as a strategy when animals disperse seed reserves in small caches in many different locations (Brodin, 2010).

The behavior of seed dispersers can affect the genetically effective size of plant populations and the extent of gene flow between populations. Identifying the vectors involved in both primary and secondary seed dispersal processes might provide essential and practical information for forest management (Daïnou et al., 2012).

Guibourtia tessmannii (Harms) J.Léonard is an important African tree species, newly listed on Appendix II of CITES (Koumba Pambo et al., 2016). Known as “bubinga” or “kévazingo”, it has high commercial and social importance and is

currently under significant threat due to illegal logging (Tosso et al., 2015). Its seeds are surrounded by a red aril, a typical characteristic of seeds primarily dispersed by primates and large birds (Gautier-Hion et al., 1985). However, knowledge regarding its ecology and more precisely its dispersal and regeneration is lacking. The aim of this study was to characterize the community of consumers of *G. tessmannii* fruits and seeds in contrasting environmental conditions.

8.2. Materials and Methods

8.2.1. Study species

Guibourtia tessmannii (Fabaceae; Detarioideae) is a large tree reaching 40 m in height (Doucet, 2003b) distributed in rain forests from Cameroon to Gabon (Tosso et al., 2015). *Guibourtia tessmannii* is hermaphrodite and polyploid (Tosso et al., 2016a). Its fruit is a pod (mean size ± standard deviation: 3.32±0.65 cm long and 2.23±0.59 cm wide) which contains a single grain (mean size ± standard deviation: 2.3±0.14 cm long and 1.15±0.21 cm wide) covered by a red aril, and its fruiting period extends from December to March (Meunier et al., 2015; Tosso et al., 2016a).The species is classified as non-pioneer light demanding (Doucet, 2003b). Commercially known as bubinga or kévazingo, the wood has a very high commercial value; in addition, its bark is used by local communities for its medicinal properties (Tosso et al., 2015).

8.2.2. Study area

The study was carried out in two evergreen forest concessions, both managed by Forest Stewardship Council (FSC)-certified logging companies. The two sites exhibit quite different environmental conditions. “Wijma” concession (Cameroon) is located at Ma’an (2°30’N, 10°30’E) (Figure 8.1a); the forest is dominated by Fabaceae-Cesalpinioïdeae (36.67%), Meliaceae (13.33%), Sapotaceae (13.33%) and Sterculiaceae (5%) (Table 8.1). The climate is equatorial (1 686 mm of annual rainfall and an annual average temperature of 23.8°C) and the soil is yellow lateritic on acid rocks (Olivry, 1986). The density of *G. tessmannii* is an average of 0.02 ind.ha^{-1} (dbh ≥ 20 cm) (Figure 8.1b). “Precious woods” concession (Gabon) is located at Bambidie (0°30’S, 12°30’E) (Figure 8.1a) and the forest is dominated by Fabaceae-Cesalpinioïdeae (31.71%), Meliaceae (17.07%), Burseraceae (12.20%) and Myristicaceae (7.32%) (Table 1). The climate is equatorial (1 700 mm of annual rainfall and an annual average temperature of 25.3°C) and the soil is ferralitic on Francevillien sandstone (Carrière, 1999; Olivry, 1986). The density of *G. tessmannii* is an average of 0.04 ind.ha^{-1} (dbh ≥ 20 cm). While both logging companies currently implement anti-poaching activities, the two sites have contrasted hunting histories. Wijma is surrounded by villages and the bushmeat trade is developed towards Equatorial Guinea, whereas in Precious woods, human density is low and hunting activities are poorly developed (Haurez, 2015).

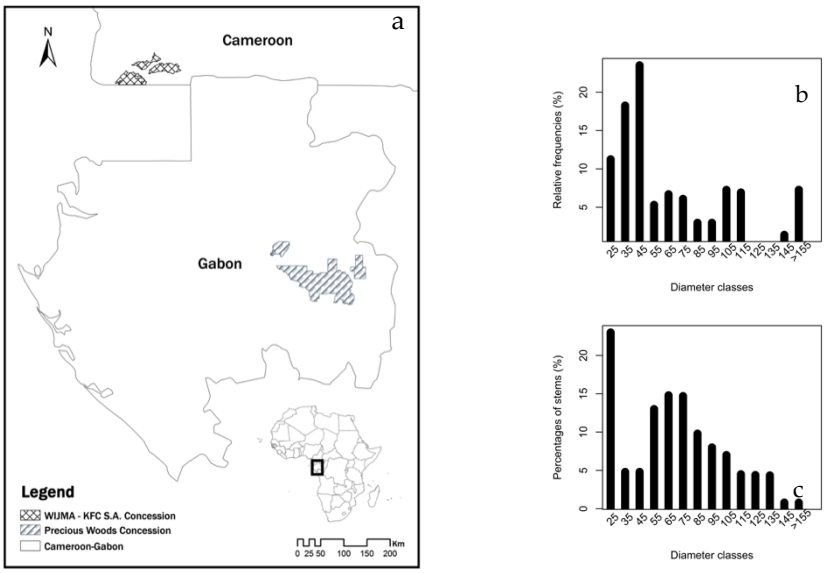

Figure 8.1. a) Location of the studied logging concessions in Cameroon and Gabon – b) Diametric structure of *G. tessmannii* in the "Wijma" forest management units where observations were performed (Cameroon, 109,757 ha) – c) Diametric structure of *G. tessmannii* in the Precious woods logging concession (Gabon, 200,000 ha)

Table 8.1. Percentage of each dominating plant families and tree species in "Wijma" concession (Cameroon) and "Precious woods" concession (Gabon)

Families	Pourcentage	Main species
"Wijma" concession (Cameroon)		
Fabaceae - Caesalpinioïdeae	36.67%	*Erythrophleum ivorense* A.Chev.; *Distemonanthus benthamianus* Baill.; *Erythrophleum suaveolens* (Guill. & Perr.) Brenan; *Brachystegia cynometroides* Harms
Meliaceae	13.33%	*Leplaea thompsonii* E.J.M. Koenen & J. J. de Wilde ; *Lovoa trichilioides* Harms ; *Khaya ivorensis* A.Chev., *Entandrophragma candollei* Harms
Sapotaceae	13.33%	*Aningeria robusta* (A. Chev.) Aubrév.& Pellegr ; *Gambeya beguei* (Aubrév. & Pellegr.) Aubrév. & Pellegr.; *Aningeria altissima* (A.Chev.) Aubrév. & Pellegr.
Sterculiaceae	5.00%	*Pterygota bequaertii* De Wild. ; *Nesogordonia kabingaensis* (K. Schum.) Capuron ex R. Germ., ; *Eribroma oblongum* (Mast.) Pierre ex A. Chev.
"Precious woods" concession (Gabon)		
Fabaceae - Caesalpinioïdeae	31.71%	*Julbernardia pellegriniana* Troupin ; *Distemonanthus benthamianus* Baill. ; *Bikinia letestui* (Pellegr.) Wieringa ; *Tetraberlinia bifoliolata* (Harms) Hauman
Meliaceae	17.07%	*Lovoa trichilioides* Harms ; *Leplaea cedrata* (A. Chev.) E.J.M. Koenen & J. J. de Wilde ; *Khaya ivorensis* A. Chev. ; *Entandrophragma utile* (Dawe & Sprague) Sprague
Burseraceae	12.20%	*Aucoumea klaineana* Pierre ; *Dacryodes buettneri* (Engl.) H.J. Lam. ; *Dacryodes normandii* Aubrév. & Pellegr.
Fabaceae - Faboïdeae	7.32%	*Pterocarpus soyauxii* Taub. ; *Millettia laurentii* De Wild. ; *Bobgunnia fistuloides* (Harms) J.H. Kirkbr. & Wiersema
Myristicaceae	7.32%	*Staudtia kamerunensis* Warb. ; *Pycnanthus angolensis* (Welw.) Warb. ; *Coelocaryon preussii* Warb.

8.2.3. Identification of dispersers and predators of Guibourtia tessmannii

Camera traps and direct observational methods were used to identify seed (and aril) consumers and to characterize their behavior (Tobler et al., 2008; Yumoto, 1999). Direct observations allowed the identification of animals visiting tree crowns while camera traps targeted both diurnal and nocturnal terrestrial eaters without disturbing them (Babweteera et Brown, 2010). Studies were conducted according to the species specific fruiting period in each country: January–March 2015 in Cameroon and December 2015 in Gabon, over 65 and 10 days, respectively.

As the densities of *G. tessmannii* in the study areas were very low as mentioned above, only seven focal fruiting trees were found and selected between 2 to 20 km apart (to avoid spatial autocorrelation). Five fruiting trees in Cameroon and two in Gabon were monitored with camera traps. We observed each tree for at least 10 days and monitored cameras weekly. Automatic Moultrie M-990i camera traps were triggered by movement within the range of the detector to take photographs and videos (Seufert et Fischer, 2010), capturing four photographs per minute as well as a 30-s video for each trigger. To estimate time spent by seed (and aril) consumers beneath focal trees, camera traps automatically recorded the date and time of each observation. In order to obtain a good observation of animal activities around the seed tree, the cameras were installed nearby and facing in the direction of the seed tree base. In front of each camera we placed a small pile of fresh seeds with arils (10 to 50 units) collected from the base of the tree (adapted from Kitamura et al. (2006), Babweteera et Brown (2009), Seufert et Fischer (2010), Moupela et al. (2014)). We defined independent visits as (i) consecutive records of individuals of different species, (ii) non-consecutive records of individuals of the same species, and (iii) consecutive records of individuals of the same species taken more than 30 min apart (O'Brien et al., 2003). We recorded the following data for all independent visits: animal species, feeding behavior when applicable, time and duration of visits, and the number of animals detected (Chapman et Chapman, 1996; Kitamura et al., 2006). Sampling effort was defined as the number of operating days (24 h) multiplied by the number of trap days, i.e. the number of active cameras.

Furthermore, five adult *G. tessmannii* trees (3 in Cameroon and 2 in Gabon) were directly observed during four- to five-hour sessions between 06h00 and 16h00 over the space of several days, totalling 101 hours (51 h in Cameroon and 50 h in Gabon).

8.2.4. Data analysis

All photographed or directly-observed species were identified to the lowest possible taxonomic level using Kingdon (2015) for mammals and Serle et al. (1993) for birds.

The duration of visits involving consumption was calculated using the difference between times of arrival and departure of animals directly observed, or the difference between time of first and last photographs in a sequence for camera-trap data. According to Schupp (1993), fruit consumption of an animal represents a component of dispersal when it exceeds a certain threshold, and is influenced by the

frequency of visits and their duration. We then assessed the effectiveness of seed/aril consumption for each animal species by calculating the 'index of seed consumption' effort (I_{SCE}), adapted from (Moupela et al., 2014) and (Haurez et al., 2015). I_{SCE} was computed as follows:

$$Isce = (\frac{Niobs.cons \times D}{\sum_{i=1}^{n} Niobs.cons \times D}) \times 100$$

With Niobs.cons = number of individuals of a given species involved in seed/aril consumption, D = mean length of visit involving seed consumption, n = number of identified species involved in seed/aril consumption.

Seed dispersal/predation functions of different animal species were determined using (i) information provided by pictures and videos obtained through the present study and (ii) from existing literature. Animals were classified into three categories ((Gautier-Hion et al., 1985), (Moupela et al., 2014) and (Haurez et al., 2015)): (1) predator: those that destroy seeds; (2) disperser: those that transport seeds from the vicinity of the parent trees and consume arils while leaving seeds intact; (3) neutral: those that show no interest in *G. tessmannii* fruits/seeds. Finally, we compared animal species identified in Gabon with those identified in Cameroon.

8.3. Results

8.3.1. Identification of seed dispersers and predators of Guibourtia tessmannii

For a total of 355 camera-trap days in both study areas, 213 vertebrate detection events occurred (95.67 % involving mammals and 4.33 % birds).

In Gabon, we identified two animal species through direct observations: an arboreal primate, the putty-nosed monkey (*Cercopithecus nictitans nictitans* (Linnaeus, 1766)), and the black-casqued wattled hornbill (*Ceratogymna atrata* (Temminck, 1835)) with an I_{SCE} of 8.80 % and 72.43 %, respectively. Based on camera-trap data only, the nocturnal Emin's pouched rat (*Cricetomys emini* (Wroughton, 1910)) was identified (I_{SCE} = 18.77 %).

In Cameroon, four animals were observed with camera traps: (i) the Emin's pouched rat, with a high value of I_{SCE} (I_{SCE} = 95.55 %) during the night, (ii) the Thomas's rope squirrel (*Funisciurus anerythrus* (Thomas, 1890)) (I_{SCE} = 2.58 %), (iii) the mandrill (*Mandrillus sphinx* (Linnaeus, 1758)) and (iv) the black guineafowl (*Agelastes niger* (Cassin, 1857)), both with I_{SCE} of zero (Table 8.2, Figure 8.2). Direct observations revealed the African pied hornbill (*Tockus fasciatus* (Shaw, 1811), I_{SCE} = 1.79 %), the great blue turaco (*Corythaeola cristata* (Vieillot, 1816), I_{SCE} = 0.08 %) and the presence of Thomas's rope squirrel (I_{SCE} = 2.58 %).

Visits by *C. n. nictitans* involved more than eight individuals whereas visits by *C. cristata*, *C. atrata*, and *T. fasciatus* involved fewer than four individuals.

Cercopithecus nictitans nictitans came in groups and broke the branches bearing fruits in order to eat the seed arils. Once the arils were eaten, the seeds were spat out onto the ground, often at the foot of the fruiting tree. We observed many broken branches carrying empty pods at the foot of the fruiting trees used for direct

observations. In addition, many seeds devoid of their arils were observed on the ground. Regarding *C. atrata*, *T. fasciatus* and *C. cristata*, individuals stayed several minutes in the canopy extracting seeds from the pods. They often carried off a seed when they took flight. *Cricetomys emini* ate the arils of *G. tessmannii* seeds but many were also observed, via camera traps, putting dozens of seeds in their mouths and carrying them away. Their role was similar to that of *Funisciurus anerythrus* except that they store their seeds in trees (often the same *G. tessmannii* fruiting tree). The mandrills and the black guineafowl observed were not involved in any act of consumption of seeds or arils.

Figure 8.2. Some *G. tessmannii* seeds consumers; A) *Agelastes niger*, B) *Cricetomys emini*, C) *Funisciurus anerythrus*, D) *Mandrillus sphinx*, E) *Cricetomys emini* attracted by sulcatone.

Table 8.2. Animal species observed during direct and indirect observations of *Guibourtia tessmannii* trees in logging concessions in Cameroon and Gabon. Nobs = total number of observations, Nind/obs (SD) = number of individuals of a specific species per observation (standard deviation), Nobs.cons = number of observations of a given species involving consumption of seeds, D = mean length of visit involving consumption of fruits, and I_{SCE} = index of seed consumption effort

Animal species	Common name	Nobs	Nind/obs (SD)	Nobs.cons	D (min)	I_{SCE}	Period of activity	Role
GABON								
Mammals								
Cercopithecus nictitans nictitans (Linnaeus, 1766)[a]	Putty-nosed monkey	3	8 (0.57)	3	7.5	8.80	Day	Disperser
Cricetomys emini (Wroughton, 1910)[b]	Emin's pouched rat	8	1	8	6	18.77	Night	Predator, disperser (occasional, through scatter-hoarding or dropping during transport)
Birds								
Ceratogymna atrata (Temminck, 1835)[a]	Black-casqued wattled hornbill	19	1.57 (0.16)	13	14.25	72.43	Day	Disperser
CAMEROON								
Mammals								
Funisciurus anerythrus (Thomas, 1890)[a&b]	Thomas's rope squirrel	13	1	11	6.5	2.58	Day and Night	Predator, disperser (occasional, through scatter-hoarding or dropping during transport)
Mandrillus sphinx (Linnaeus, 1758)[b]	Mandrill	1	3	0	0.5	0	Day	Uncertain
Cricetomys emini (Wroughton, 1910)[b]	Emin's pouched rat	54	1	54	49	95.55	Night	Predator, disperser (occasional, through scatter-hoarding or dropping during transport)
Birds								
Agelastes niger (Cassin, 1857)[b]	Black guineafowl	1	4	0	8	0	Day	Uncertain
Corythaeola cristata (Vieillot, 1816)[a]	Great blue turaco	3	2.67 (0.33)	1	2.25	0.08	Day	Disperser
Tockus fasciatus (Shaw, 1811)[a]	African pied hornbill	11	1.80 (0.25)	6	8.25	1.79	Day	Disperser

Note: a) direct observations, b) indirect observations (using camera traps)

8.4. Discussion

8.4.1. Guibourtia tessmannii *seed dispersers and predotors*

Both direct and indirect observations allowed for the identification of five main animal species involved in the seed dispersal of *G. tessmannii*: *Cricetomys emini*, *Ceratogymna atrata*, *Cercopithecus nictitans nictitans*, *Tockus fasciatus* and *Funisciurus anerythrus*. These results are in concordance with the zoochorous "syndrome" proposed by Gautier-Hion et al. (1985) who assumes that dehiscent fruits with brightly arillate and poorly protected seeds are dispersed by large birds, monkeys and rodents.

Cricetomys emini exhibited the highest value of I_{SCE} in the Cameroon site. Rodents are generally described as seed predators (Beaune et al., 2013; Haurez et al., 2015; Moupela et al., 2014) or secondary dispersers by scatter hoarding (Brodin, 2010). Based on evidence from our photographic and video observations, rodents ate the arils on site and could also transport around 30 seeds in their mouths. In fact, many researchers have previously documented the essential role of scatter hoarding by

rodents in the regeneration of large-seeded tree species (Hulme et Kollmann, 2004) in Australia (Theimer, 2001), Asia (M. Yasuda et al., 2000), America (Forget, 1996; Jansen et al., 2004) and Africa (Bationo et al., 2002; Debroux, 1998; Forget et Vander Wall, 2001). In particular, *Cricetomys kivuensis*, a species phylogenetically close to *C. emini*, has been shown to play an important role in the secondary dispersal of large seeds in Afro-tropical forests (Nyiramana et al., 2011). Scatter hoarding rodents remove and disperse nutrient-rich seeds that have either fallen directly beneath the parent tree or have been deposited by other animals (Vander Wall, Forget, Lambert, et Hulme, 2005). Since in the Cameroon site, rodents exhibit (i) a high frequency of visitation of fruiting *G. tessmannii* trees, (ii) a long visit duration and (iii) the removal of a large number of seeds at each visit, they are likely to contribute to the seed dispersal of this species. The role of Thomas's rope squirrels (*F. anerythrus*) in *G. tessmannii* seed dispersal is probably much less important than that of *C. emini* (Emmons, 1980) as they store their seeds in trees (often the same *G. tessmannii* fruiting tree).

Guibourtia coleosperma (Benth.) J.Léonard shares the same fruit and seed characteristics as *G. tessmannii* and is mainly dispersed by avian frugivores including hornbills (Calvert, 1984). In this study, the black-casqued wattled hornbill (*Ceratogymna atrata*) and the African pied hornbill (*Tockus fasciatus*) found in Gabon and Cameroon, respectively, have previously been described as *G. tessmannii* seed dispersers (Jensch et Ellenberg, 1999; Mbelli, 2002). In African tropical forests, hornbills are among the principal seed-eaters and are essential for seed dispersal (Kemp et Woodcock, 1995): *Ceratogymna* species have been described to disperse 22 % of the known tree flora in Africa, including many economically important species (Whitney et Smith, 1998).

In this study, the next most important *G. tessmannii* seed or aril consumer was the putty-nosed monkey (*C. n. nictitans*), which was exclusively observed in Gabon. Medium- and small-sized primates are known to disperse seeds through defecation and spitting (Poulsen et al., 2001; Cooke et McGraw, 2007 ; Russo et Chapman, 2011). In the present study, we observed this species breaking fruit-bearing branches, consuming arils and spitting out the naked seed (at the foot of *G. tessmannii* fruiting tree), thus highlighting its role in *G. tessmannii* seed dispersal, though probably only for short distances.

8.4.2. Differences in Guibourtia tessmannii *consumer assemblages in Cameroon and Gabon sites*

Our results showed that *G. tessmannii* seed consumers vary geographically, as proposed by Rey et Manzaneda (2007). The different geographic locations could explain the difference observed in *G. tessmannii* consumer assemblages between sites. The two study sites were distant from each other and located at both sides of the equator: one fairly littoral (Cameroon) and the other inland (Gabon). The abiotic conditions (mainly climate and soil) could directly influence forest composition (Fayolle et al., 2014), food diversity and fruit availability, which in turn may affect the frugivore and granivore community compositions (Morin, 2009). *G. tessmannii* is a rare species; the difference observed might also be due to the sample size. As the

two sites exhibited contrasting hunting activities, additional data are needed to test if the difference in *G. tessmannii* consumer assemblages in Cameroon and Gabon sites could be explained by varying levels of hunting pressure. It could be particularly interesting to examine if the rarefaction of large mammals influences the density of rodents by a competition release mechanism (Fa et Brown, 2009).

8.5. Conclusions and perspectives

In this study, we characterized the animal communities involved in seed dispersal and predation of *G. tessmannii*. Our results showed that the assemblages of *G. tessmannii* consumers are different between sites. This difference in seed disperser assemblage for *G. tessmannii* might be related to the differing environmental conditions or to differences in forest disturbance. However, more extensive studies are required across the range of this tree species, both in Gabon and Cameroon, in order to confirm and explain our findings. More specifically, disentangling the role of environmental conditions and hunting pressure would be necessary for the implementation of conservation strategies of this high-value timber species.

Discussion générale

Discussion générale

La présente recherche doctorale ambitionnait de comprendre les mécanismes à l'origine de la diversification des espèces ligneuses africaines et de la différenciation de leurs populations. Le genre *Guibourtia* a été utilisé comme modèle biologique en combinant des analyses innovantes aux échelles interspécifique et intraspécifique, ainsi que des approches expérimentales. Il ressort de ce travail que les patrons de diversification au sein du genre sont complexes, impliquant à la fois des forces neutres et de sélection. Le travail met notamment en évidence que les barrières biogéographiques, les gradients climatiques ou même la dispersion des graines par les animaux pourraient influencer la différenciation génétique des populations. Il propose des perspectives de recherche sur les aspects méritant davantage d'investigations. Il se termine par les implications pour la gestion durable et la conservation des populations d'espèces du genre *Guibourtia*.

1. Que retenir de la taxonomie du genre *Guibourtia* ?

Les chapitres 1 à 4 ont été consacrés à l'analyse de la proximité génétique entre les taxons du genre *Guibourtia*. Elles font écho au débat sur la taxonomie des espèces de *Guibourtia* soulevé par Léonard en 1949, en combinant plusieurs approches intégrant des études morphologiques et génétiques.

Les informations obtenues nous permettent de mettre en évidence que ce sont les caractères morphologiques reproductifs qui sont les plus utiles pour séparer les espèces étudiées (chapitre 2). Cette observation *a priori* triviale va toutefois à l'encontre des résultats obtenus par Donkpegan (2017) sur le genre *Afzelia* (Fabaceae, Detarioideae) qui comme le nôtre, regroupe de nombreuses espèces sœurs, diploïdes et tétraploïdes vivant aussi bien dans des savanes que dans des forêts denses humides. La classification basée sur ces caractères morphologiques reproductifs est en parfaite cohérence avec les patrons phylogénétiques définis en utilisant le plastome et des gènes nucléaires. Ils permettent de confirmer qu'il existe bien trois sous-genres au sein du genre *Guibourtia* tels que proposés par Léonard (1949) : *Guibourtia*, *Gorskia* et *Pseudocopaiva*. Le premier sous-genre comprend *G. copallifera*, *G. demeusei*, *G. carrissoana* et *G. sousae*, le deuxième est composé de *G. arnoldiana*, *G. ehie*, *G. conjugata*, *G. schliebenii* et *G. dinklagei* et le troisième sous-genre regroupe *G. tessmannii*, *G. pellegriniana*, *G. leonensis* et *G. coleosperma*. Le critère principal distinctif entre ces trois sous-genres est le type de fruit qui est indéhiscent avec une graine non arillée pour les sous-genres *Guibourtia* (fruit indéhiscent épais) et *Gorskia* (fruit indéhiscent mince) tandis que dans le sous-genre *Pseudocopaiva*, le fruit est déhiscent avec une graine arillée. Cette différence majeure au niveau du type de fruit concorde avec les résultats de transférabilité de marqueurs microsatellites présentés dans les chapitres 5 et 6 : les marqueurs microsatellites développés sur *G. tessmannii* ne sont transférables que sur les espèces appartenant au sous-genre *Pseudocopaiva* et ceux développés sur *G. ehie* ne sont transférables qu'aux espèces appartenant au sous-genre *Gorskia*. Il convient toutefois de noter qu'il existe de un à quatre loci sur 19 qui sont transférables sur les

espèces appartenant à d'autres sous-genres que celui de l'espèce cible des marqueurs microsatellites développés (Tosso et al., 2015 ; Tosso et al., 2016).

Si nos travaux ont globalement confirmé la taxonomie actuellement décrite chez *Guibourtia*, ils questionnent toutefois sur le nombre réel d'espèces africaines. Ils suggèrent l'existence de taxons cryptiques car tous les groupes morphologiques ne sont pas parfaitement monophylétiques avec les séquences chloroplastiques. En effet, les séquences chloroplastiques n'ont pas confirmé la monophylie de *G. demeusei* et *G. copallifera*. Ceci pourrait s'expliquer par (i) une capture chloroplastique ou (ii) un tri de lignées incomplet du fait probablement d'une série d'événements de spéciation ou (iii) des espèces différentes.

Le débat sur les espèces cryptiques remonte à Darlington (1940) et Mayr (1942). Les considérant comme des espèces difficiles à différencier morphologiquement, Mayr (1966) prôna le concept biologique de l'espèce. En sus de la biologie moléculaire, la phylogéographie et la génétique des populations s'avèrent nécessaires pour tester l'isolement reproductif et évaluer les taux d'hybridation entre les taxons (Daïnou et al., 2016). Cette approche intégrative a permis de suspecter la présence de nouvelles espèces au sein des genres *Milicia* (Daïnou et al., 2016), *Afzelia* (Donkpegan, 2017) et *Santiria* (Ikabanga et al., 2017). Le nombre d'espèces d'arbres en Afrique tropicale pourrait donc être beaucoup plus important que les chiffres actuels ne le laissent présager.

Le cas de *G. ehie* présenté au chapitre 7 suggère une fois de plus l'existence d'espèces cachées au sein de taxons bien connus. En effet, nous avons mis en évidence que les individus de *G. ehie* d'Afrique de l'Ouest et ceux d'Afrique centrale pourraient constituer deux espèces différentes. Nous avons noté que la face inférieure des folioles des individus d'Afrique de l'Ouest est parsemée de points translucides, contrairement à ceux d'Afrique Centrale qui en sont dépourvus. Ce caractère morphologique est connu pour être distinctif de certaines espèces du genre *Guibourtia* (Aubreville, 1968 ; Aubreville, 1970 ; Léonard, 1949). Nous n'avons malheureusement pas pu observer des échantillons fertiles de *G. ehie*, lesquels sont indispensables pour la description complète des espèces. La monophylie observée chez *G. ehie* dans le chapitre 2 sur base des séquences chloroplastiques pourrait être liée au fait que nous n'avons utilisé (Figure 2.2) que des échantillons provenant d'Afrique de l'Ouest et du Sud-Ouest Cameroun (probablement une zone de contact, Figure 7.1). De plus, une phylogénie est généralement basée sur un nombre restreint d'échantillons par espèce.

En outre, une phylogénie réalisée avec des gènes nucléaires (travaux complémentaires à cette thèse) capturés et séquencés grâce aux sondes nucléaires développés à partir des transcriptomes d'espèces de Detarioideae, permettent d'obtenir une topologie concordante avec celle obtenue avec les séquences chloroplastiques bien qu'elle n'ait été réalisée qu'avec un individu par espèce (sauf pour *G. tessmannii* et *G. pellegriniana*). Toutefois, (1) une phylogénie utilisant un plus grand nombre d'échantillons par espèce et couvrant toute l'aire de distribution et (2) l'utilisation d'outils démontrés efficaces pour la délimitation comme les *haplowebs* (Flot et al., 2010) seraient nécessaire pour affiner la délimitation au sein du genre *Guibourtia*. En résumé, sur base de nos résultats, nous

suspectons au moins 14 espèces au sein du genre *Guibourtia* dont deux (*G. tessmannii* et *G. pellegriniana*) semblent être octoploïdes.

2. Origine et histoire des populations du genre *Guibourtia*

La présente thèse a démontré que l'origine du genre *Guibourtia* est africaine (chapitre 2) comme la plupart des Detarioideae (Estrella et al., 2017). Les deux genres les plus proches sont les genres néotropicaux *Hymenaea* et *Peltogyne*. Si l'hypothèse de migration transocéanique via *Hymenaea verrucosa* (espèce rencontrée en Afrique de l'Est) a été évoquée par Fougère-Danezan (2005) pour expliquer l'origine du genre *Guibourtia*, notre recherche n'a pas pu approfondir cette question. Nos résultats d'estimation de divergence de gènes au sein du genre ont indiqué une origine des *Guibourtia* qui daterait du Miocène (~14,5-24 Ma) avec des patrons de spéciations différents. En comparant nos dates avec celles des deux autres genres, il ressort que les genres *Hymenaea* (~ 23,7–25,6 Ma) et *Peltogyne* (~ 31,4–33 Ma) seraient probablement plus anciens que le genre *Guibourtia* (Fougere-Danezan, 2005), ce qui tend à confirmer l'hypothèse de dispersion à longue distance. La migration de l'Afrique vers l'Amérique des *Guibourtia* néotropicaux remonterait vers 11,78 Ma environ. Concernant les espèces africaines du genre *Guibourtia* et sur base de nos résultats, il est difficile d'affirmer qu'un mode de spéciation particulier a été le moteur de la diversification. Toutefois, plusieurs hypothèses peuvent être faites quant aux modes de spéciation faisant intervenir les facteurs biogéographiques, environnementaux et la polyploïdisation.

Le concept de spéciation allopatrique a été introduit pour la première fois par Wallace en 1855. Il suppose la formation d'espèces par isolement géographique de populations conduisant à des sous-espèces puis des espèces distinctes. Ce processus requiert un temps long notamment pour les espèces d'arbres à longue durée de vie (Couvreur et al., 2008). Le mode de spéciation allopatrique a été largement mis en évidence chez de nombreux taxons africains, et souvent mis en lien avec les refuges forestiers (Maley, 1996 ; Robbrecht, 1996 ; Moritz et al., 2000 ; Linder, 2001 ; Couvreur et al., 2008). La distribution spatiale de certaines paires d'espèces appartenant au même clade (ex. *G. tessmannii – G. leonensis*) supporte ce mode de spéciation.

La spéciation parapatrique suggère que les populations en divergence ne sont pas totalement isolées géographiquement mais possèdent une zone de contact étroite (Gavrilets et al., 2000). Ce mode de spéciation pourrait potentiellement concerner les paires d'espèces *G. conjugata – G. schliebenii* et *G. arnoldiana – G. ehie* dans le sous-genre Gorskia. Des cas d'hybrides ayant été prouvés au sein d'espèces ligneuses vivant en parapatrie (cas du genre *Milicia*, Daïnou et al. 2017), il serait intéressant que des travaux futurs puissent investiguer les flux de gènes entre paires d'espèces dans les zones de contact.

La spéciation écologique est définie comme la sélection de traits adaptatifs à des niches écologiques différentes entraînant un isolement reproducteur des populations (Dajoz, 2012). Même s'il y a de la sélection, il est toutefois difficile de prouver

qu'elle seule soit à l'origne de la spéciation car une spéciation allopatrique suivie d'une adaptation différentielle ne peut pas être exclue. L'impact de la sélection à des climats différents a été clairement mis en évidence dans le chapitre 3, montrant dans chaque sous-genre (clade) des convergences adaptatives de traits morphologiques en réponse au gradient climatique entre forêt dense humide - forêt sèche/savane (exemple des paires d'espèces *G. tessmannii* - *G. coleosperma* et *G. demeusei* – *G. carrissoana*). Ce type de démonstration nécessitant une approche pluridisciplinaire est, à l'heure actuelle, assez peu utilisé, notamment sur les taxons d'arbres tropicaux d'Afrique. Pour ce faire, nous avons utilisé uniquement des variables environnementales abiotiques afin de caractériser la niche des espèces du genre *Guibourtia*. Or, il est connu que la niche écologique au sens large se réfère non seulement aux éléments abiotiques du milieu (exemples : climat, sol, ressources), mais aussi aux composantes biotiques, y compris les interactions intra et interspécifiques (exemples : compétition, prédation, mutualisme) (Rundle et Nosil, 2005; Schluter, 2009). De nouvelles approches de modélisation de niche pouvant intégrer le maximum d'éléments afin de caractériser l'écologie des espèces d'arbres sont nécessaires pour affiner nos conclusions, et afin de capter les spécificités de certaines espèces comme par exemple *G. demeusei* (espèce inféodée aux bords de cours d'eau) ou *G. arnoldiana* (espèce rencontrée sur les sols calcaires). Plus particulièrement, les résultats du chapitre 4 démontrant, au moyen d'une approche expérimentale, que *G. tessmannii* (espèce de forêt dense sempervirente) a une plasticité phénotypique plus élevée dans les faibles niveaux d'éclairement comparativement à *G. coleosperma* (espèce de forêt sèche et de savane), sont congruents avec l'hypothèse de la sélection par le milieu qui aurait conduit à une plus grande plasticité phénotypique dans les conditions où chacune des deux espèces est plus adaptée. Ces résultats soulignent l'importance de combiner les informations génétiques avec les expérimentations écophysiologiques afin de mieux comprendre l'histoire évolutive des taxons d'arbres tropicaux (Barros et al., 2012).

Quant au mode de spéciation de type polyploïde, il a été observé au niveau du sous-genre *Pseudocopaiva*. La polyploïdie est un état héréditaire de la possession de plus de deux ensembles complets de chromosomes (Comai, 2005). Dans le chapitre 5 de cette thèse, nous avons mis en évidence que *G. tessmannii* est octoploïde avec une taille de génome similaire à celle de *G. pellegriniana*. Cependant, les études de caryologie mises en œuvre afin de confirmer ce niveau de ploïdie n'ont pu être achevées. Pour *G. leonensis* (espèce de forêt dense sempervirente), les résultats du même chapitre indiquent que les profils des marqueurs microsatellites sont similaires à ceux d'espèces diploïdes mais nous n'avons malheureusement pas obtenu d'échantillons frais pour réaliser les études de cytométrie en flux ni de caryologie pour confirmation. Des travaux futurs sont souhaitables sur cet aspect afin de statuer sur la garniture chromosomique de toutes les espèces du genre *Guibourtia* en général, et celle du sous-genre *Pseudocopaiva* en particulier. Nous pouvons affirmer qu'au sein de ce sous-genre, *G. coleosperma* (espèce de forêt sèche et de savane) est diploïde tandis que *G. tessmannii* et *G. pellegriniana* (espèces de forêt denses sempervirentes) sont des espèces polyploïdes. Ce patron est similaire à celui observé par Donkpegan (2017) dans le genre *Afzelia* où les espèces forestières sont tétraploïdes tandis que celles de forêts sèches ou de savanes sont

diploïdes. Le mode de spéciation de type polyploïde est un processus évolutif très fréquent au sein des plantes (Soltis et Soltis, 1993,1999) et probablement sous-estimé au sein des espèces d'arbres tropicaux. Généralement, l'apparition de ce type de spéciation est un processus complexe qui peut impliquer des divergences écologiques entre les lignées de différentes ploïdies et/ou un mélange génétique avec des espèces parentales (Soltis et Soltis, 2000 ; Soltis et al., 2007). Dans le cas du sous-genre *Pseudocopaiva*, d'autres travaux sont nécessaires afin de démêler les causes à l'origine de la spéciation par polyploïdie dans ce sous-genre. En réalité, les forêts tropicales d'Afrique contiendraient des espèces végétales qui sont dans 85 à 90 % des cas des paléopolyploïdes c'est-à-dire des anciens polyploïdes dont le génome a été diploïdisé (Mangenot et Mangenot, 1962).

Ces différents mécanismes de spéciation observés ou suspectés dans le genre *Guibourtia* illustrent parfaitement la complexité des patrons de diversification au sein des taxons d'arbres tropicaux d'Afrique.

3. Contribution des forces neutres et de sélection dans les mécanismes de diversification au sein du genre *Guibourtia* : importance de l'étude des traits fonctionnels

Les travaux de Fisher (1930) et Wright (1931) démontrent bien que le débat sur la contribution relative des forces neutres et de sélection à la diversification des taxons est ancien: « *a central controversy among biologists is the relative importance of natural selection and genetic drift as creative forces shaping biological diversification* » (Fisher, 1930). Aujourd'hui, il y a une unanimité quant au rôle combiné des forces neutres et de sélection dans la diversification des organismes. Toutefois, l'importance relative de ces deux types de mécanisme a été peu documenté pour les arbres tropicaux (Eiserhardt et al., 2017).

Dans le chapitre 4, nous avons montré via une approche expérimentale l'effet de la sélection naturelle, en étudiant la réponse des plantules d'espèces génétiquement proches (mais occupant des environnements contrastés) à un gradient d'éclairement. Ces approches intégrant des espèces sœurs ne sont pas nouvelles. Elles ont été testées sur les espèces d'arbres d'Amérique du sud (Valladares et al., 2006 ; Barros et al., 2012) et d'Amérique du nord (Stacy et al., 2017). Elles offrent la possibilité d'appréhender les adaptations des taxons grâce aux traits fonctionnels. A part les traits liés à la photosynthèse, les traits fonctionnels étudiés dans ce travail ont déjà été étudiés sur d'autres espèces (Agyeman et al., 1999, Biwolé et al., 2015). C'est toutefois pour la première fois, à notre connaissance, qu'ils sont testés sur un complexe d'espèces ligneuses sœurs. Les données de photosynthèse (en particulier l'assimilation maximale nette) ont aidé à mieux comprendre la réponse des espèces, et il serait souhaitable qu'elles soient intégrées dans les expérimentations écophysiologiques incluant les espèces ligneuses tropicales africaines.

Aujourd'hui, de plus en plus de chercheurs intègrent dans leurs travaux l'épigénétique (variation héritable de l'expression des gènes en fonction de l'environnement), qui peut fournir des informations précieuses sur les réactions physiologiques induites par des conditions environnementales contrastées

(Passow et al., 2017). Parallèlement, avec le développement des techniques et méthodes de biologie moléculaire, il est aussi possible de tester l'effet de la sélection naturelle. En effet, l'identification croissante de locus, initialement supposés neutres, mais spécifiques aux populations d'un environnement donné (*outlier loci*), témoigne indirectement du rôle de la sélection dans la différenciation des populations (Beaumont et al., 2004 ; Foll et al., 2008 ; Excoffier et al., 2009 ; Rajora et al., 2016). Le développement de SNPs impliqués dans le photopériodisme développés sur *Populus tremula* (Ma et al., 2010) ainsi que les marqueurs microsatellites (SSRs) et SNPs impliqués dans les réponses adaptatives aux changements climatiques et développés sur *Pinus strobus* (Rajora et al., 2016) sont des illustrations des possibilités de combiner les marqueurs moléculaires neutres et les *outlier loci* pour appréhender les moteurs gouvernant la diversification de taxons.

4. De la différenciation de populations d'espèces à la spéciation au sein du genre *Guibourtia* : quels sont les facteurs en présence ?

La différenciation des populations d'une espèce donnée est un préalable à la diversification et la spéciation (Harvey et al., 2017). Dans les chapitres 7 et 8 de cette thèse, nous avons identifié quelques facteurs pouvant contribuer à la différenciation des populations de certaines espèces du genre *Guibourtia*. L'étude de phylogéographie de *G. ehie* (chapitre 7) a permis de mettre en évidence que les barrières biogéographiques semblent participer à la différenciation des populations de ce taxon, voire à leur spéciation. Depuis longtemps, les barrières biogéographiques sont connues pour agir comme moteurs de la diversification biologique au sein des plantes (Satler et Carstens, 2017) et leur rôle a été mis en évidence au niveau de certaines espèces ligneuses africaines dont *Erythrophleum ivorense* (Duminil et al., 2013) et *Distemonenthus benthamianus* (Demenou et al., 2016). Toujours dans le chapitre 7, l'étude de la phylogéographie de *G. coleosperma* a révélé une différenciation des populations de cette espèce le long d'un gradient climatique (Figure 7.2). La synthèse de Hardy et al. (2013) confirme une structuration de la diversité génétique le long de gradients environnementaux pour plusieurs taxons (*Erythrophleum suaveolens*, *Baillonella toxisperma*, *Distemonanthus benthamianus*, *Greenwayodendron suaveolens*, *Scorodophloeus zenkeri*).

Par ailleurs, cette thèse a permis d'identifier dans le chapitre 8 les agents disperseurs de graines de *G. tessmannii* (espèce ayant des fruits déhiscents et des graines arillées, représentative du sous-genre *Pseudocopaiva*). Il est connu que les mécanismes de dispersion ont un impact direct sur la structure spatiale de la variation génétique des populations de plantes (Bossart et Prowell, 1998). Les espèces dont les graines sont dispersées à proximité de la plante mère ont souvent une structure génétique spatiale plus forte que celles dont les graines sont dispersées à de grandes distances (Hamrick et al., 1993). Dans le chapitre 8, deux principaux disperseurs de *G. tessmannii* ont été identifiés : le cercopithèque hocheur (*Cercopithecus nictitans nictitans*) et le calao (*Ceratogymna atrata*). La littérature

nous renseigne que *Cercopithecus ascanius* (une espèce arboricole sœur du cercopithèque hocheur) a une courte distance de dispersion (quelques dizaines de mètres ; Lambert, 2001). Nos observations directes indiquent que les cercopithèques hocheurs viennent en groupe de huit individus en moyenne, cassent les branches contenant les fruits de *G. tessmannii*, consomment l'arille et crachent la graine nue au pied de l'arbre. Par ailleurs, certaines branches portant les fruits peuvent être transportées, donc dispersés sur quelques dizaines de mètres avant consommation de l'arille par ces cercopithèques hocheurs. Selon Holbrook et al. (2000), la distance de dispersion de *Ceratogymna atrata* peut être beaucoup plus grande et pourrait varier entre 3,5 et 7 km. Considérant la différence en termes de distance de dispersion pour ces deux disperseurs de *G. tessmannii*, la structure génétique spatiale des populations de cette espèce ligneuse dépendrait donc de la présence et de l'importance de chacun de ces deux disperseurs dans les biotopes où les populations de *G. tessmannii* sont rencontrées. Il est toutefois important de nuancer l'impact des vecteurs de dispersion de graines ; en effet, le syndrome de pollinisation ainsi que les mécanismes de dissémination du pollen peuvent également affecter significativement la structure génétique spatiale des populations, en contrebalançant par exemple une faible dispersion des graines.

5. Implications des résultats dans la gestion durable et la conservation des populations d'espèces d'arbres exploités du genre *Guibourtia*

Cette thèse de doctorat a permis d'apporter des éléments pertinents sur l'histoire évolutive et la taxonomie du genre *Guibourtia*. Elle a également permis de comprendre comment les espèces d'arbres tropicaux d'Afrique peuvent s'adapter à leur environnement. Nos travaux ont des implications d'ordre pratique pouvant aider à la conservation et la gestion durable de certaines espèces du genre *Guibourtia*, plus particulièrement celles qui sont exploitées pour le bois d'œuvre.

Premièrement, la clarification de la position taxonomique des taxons exploités pour leur bois d'œuvre est essentielle pour la mise en place de stratégies de conservation efficace (Daïnou et al., 2016). A titre d'illustration, *G. tessmannii* et *G. pellegriniana* très proches morphologiquement, sont exploités indistinctement sous la même appellation (*Bubinga* au Cameroun et *Kévazingo* au Gabon) (Betti, 2012). Or, ces espèces affichent des densités de population très faibles et des aires de distribution souvent réduites (Tosso et al., 2015). Les résultats de la présente thèse prouvent que *G. tessmannii* et *G. pellegriniana* sont deux espèces différentes devant bénéficier de stratégies de conservation spécifiques. Nos travaux complémentaires de ceux présentés dans cette thèse révèlent que *G. tessmannii* et *G. pellegriniana* pourraient être distinguées par la texture de la gousse et le détachement de l'écorce (Figure 9.1). Ces résultats ont été importants dans la décision de l'inscription de ces deux espèces, en plus de *G. demeusei* (par principe de précaution du fait de la similarité de leur bois) sur l'Annexe 2 de la CITES (Pambo et al., 2016). Cette inscription sur l'Annexe 2 de la CITES pourra limiter les menaces sur ces espèces. En raison du risque élevé d'extinction à l'état sauvage des

populations (critères A1d et A3 de l'UICN (2001)) de *G. tessmannii* et *G. pellegriniana*, nous proposons que ces deux espèces soient également inscrites sur la liste rouge de l'UICN dans la catégorie EN (en danger). Quant à *G. demeusei*, nous suggérons qu'elle soit classée dans la catégorie DD (données insuffisantes) en raison du manque de données disponibles indispensables pour statuer sur les risques d'extinction sur cette espèce.

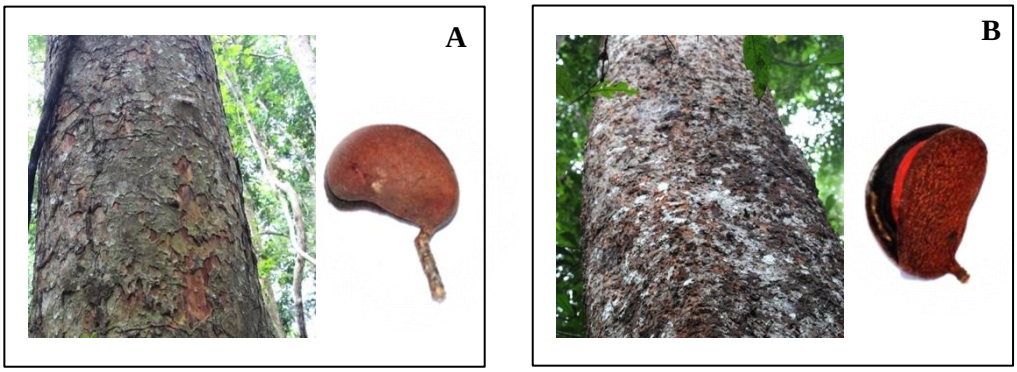

Figure 9.1. Ecorce (à gauche) et gousse (à droite) de A) *Guibourtia pellegriniana* et B) *Guibourtia tessmannii*.

Deuxièmement, nous avons également mis en évidence que les individus de *G. ehie* d'Afrique centrale et d'Afrique de l'Ouest sont génétiquement et morphologiquement différents et appartiennent très probablement à deux espèces distinctes. Cette découverte est une avancée notable pour la conservation et la gestion durable des populations de cette essence qui est commercialisée pour son bois d'œuvre sous le nom de *Amazakoué* en Afrique de l'Ouest et *Ovengkol* en Afrique Centrale. *G. ehie* est classée vulnérable sur la liste rouge de l'UICN (Hawthorne, 1995). Mais nous pensons, au regard de notre expérience de terrain, que le taxon d'Afrique de l'Ouest serait le plus menacé. Sur base de nos résultats, qui devront être confirmés par des études de description botanique à large échelle, nous proposons que les populations des deux taxons de *G. ehie* soient réévaluées. En considérant le manque de données disponibles nécessaires pour évaluer actuellement le risque d'extinction en fonction de sa distribution et/ou de l'état des populations de *G. ehie* d'Afrique Centrale, nous proposons actuellement qu'elles soient classées dans la catégorie DD (données insuffisantes). Par contre, nous suggérons que les populations de *G. ehie* d'Afrique de l'Ouest soient maintenues dans la catégorie VU (vulnérable) à cause des densités faibles associées aux pressions exercées sur les ressources forestières du fait de l'expansion continue des superficies agricoles.

Troisièmement, notre approche expérimentale a permis de conclure que *G. tessmannii* et *G. ehie* (d'Afrique de l'Ouest) seraient des semi-héliophiles tandis que *G. coleosperma* serait un héliophile strict. Sachant que l'étude de la réponse à la lumière des essences forestières est un outil précieux pour le gestionnaire forestier

(Doucet et al., 2009), nos résultats permettront d'optimiser les méthodes de régénération assistée. En particulier, ils pourront permettre aux concessions forestières, impliquées dans la certification forestière FSC, d'optimiser les stratégies de régénération assistée de ces espèces. Les plantules de ces deux espèces forestières devraient être installées dans les trouées d'abattage ou sur des parcs-forêt de moyenne taille afin de leur assurer une croissance optimale. Concernant *G. ehie* d'Afrique Centrale (non prise en compte dans le chapitre 4), de telles expérimentations sont attendues afin de préciser son tempérament et identifier les interventions sylvicoles adaptées à cette espèce.

Enfin, les résultats du chapitre 8 ont souligné que l'absence de disperseurs de graines dans le biotope de *G. tessmannii* au Sud-Cameroun, probablement en lien avec le braconnage, pourrait avoir influencé négativement le potentiel de régénération de l'espèce. Ces résultats mettent en évidence l'impact de la defaunation sur la dynamique des populations d'espèces appartenant au sous-genre *Pseudocopaiva*. Des stratégies de conservation de la faune sont donc nécessaires afin d'assurer une conservation des espèces appartenant à ce sous-genre.

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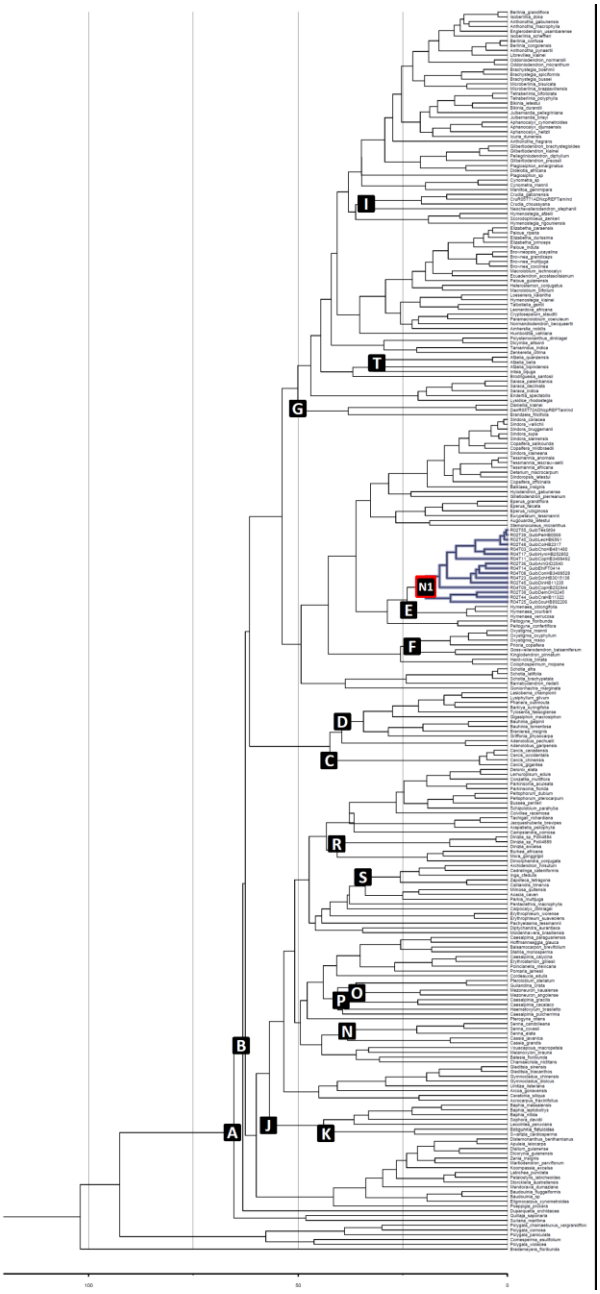
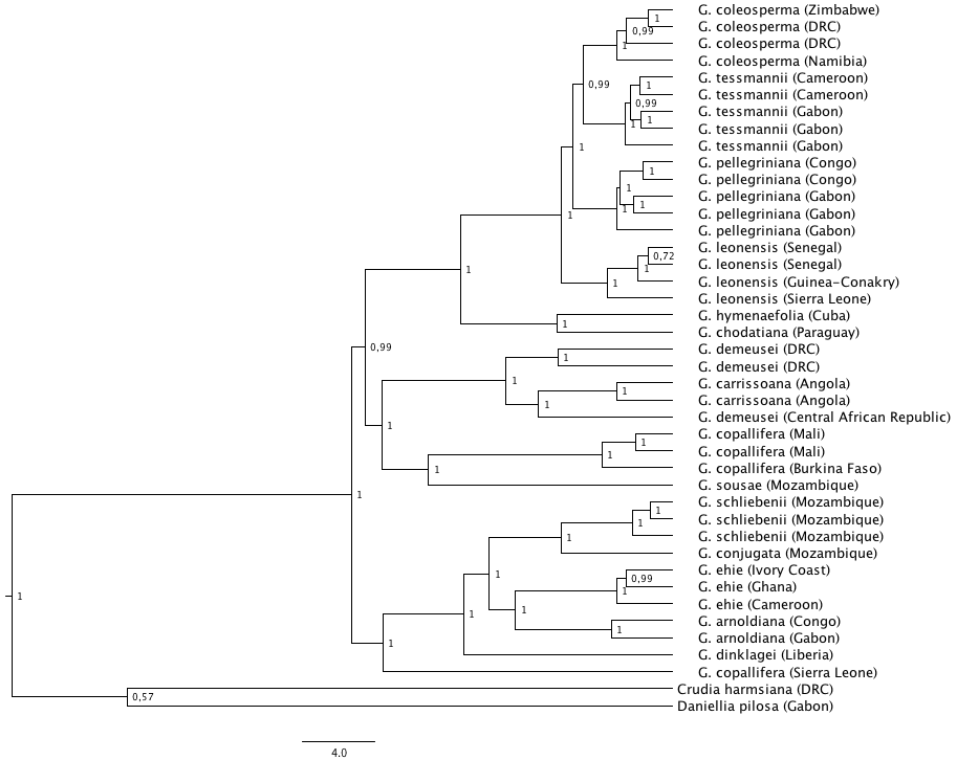
Annexes

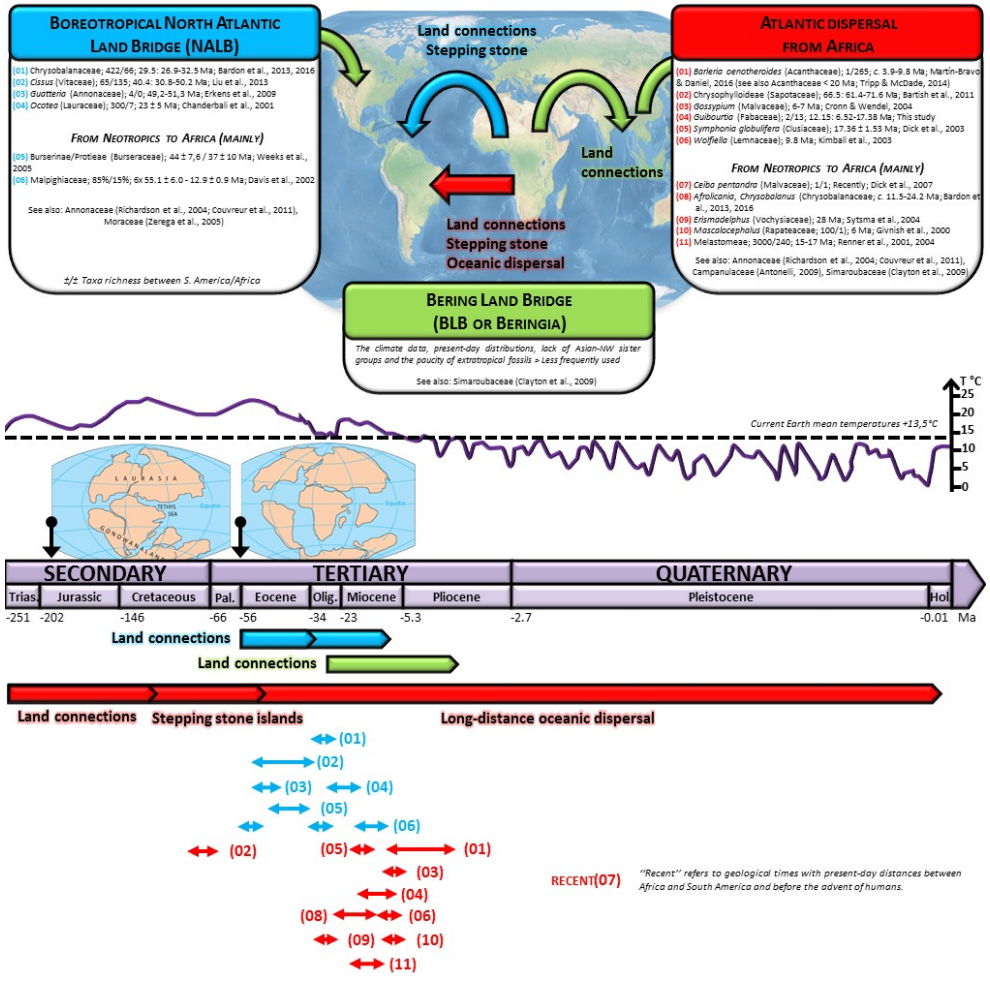
Appendix 2.1. Divergence time chronogram of the *Mat*K-*trn*K (analyzed separately) and *trn*L chloroplast DNA regions sequenced for 250 Caesalpinioideae downloaded from Bruneau et al. 2008) and our 15 *Guibourtia* taxa, using BEAST software (Bayesian maximum clade credibility tree). Letters on nodes refer to mean divergence time estimates (in Ma) detailed in Appendix 2.5.



Appendix 2.2. *Guibourtia* phylogeny with clades posterior probabilities using BEAST 1.8.2.



Appendix 2.3. Alternative hypotheses for the *ex situ* origin of elements of Neotropical diversity. according to scientific literature combining phylogenetics and molecular dating.



Appendix 2.4. Samples collection of *Guibourtia* analyzed by whole plastid genome sequencing and morphometric measurements.

Taxa	DNA Ids	Countries	Localities	Collection dates	Altitudes	Latitudes	Longitudes	Nb reads R1+R2 paired	Percentage mapped reads	Percentage Coverage at 1X	Percentage Coverage at 3X	Percentage Coverage at 10X	Average depth	StDev depth	Morphological measurements / GenBank
Guibourtia arnoldiana	B12-GID2040	Gabon	Mayumbe. West village Biboura. on the road to RDC	23/03/2012	312	-3.40982	11.4185	531378	98.33	94.56	91.74	87.73	501.71	396.56	Yes
Guibourtia arnoldiana	B26-HB253056	Congo	Road Guena-Boubissi. km 7 (Meise Botanic Garden in Belgium)	14/02/1961	/	-4.5	12.23	183312	97.90	86.80	80.38	72.66	140.76	147.97	Yes
Guibourtia carrissoana	B19-HB10458	Angola	Herbarium JB Teixeira et al n°10458 (Meise Botanic Garden in Belgium)	8/06/1966	/	-8.934193	13.186471	298734	98.46	89.16	82.52	74.51	249.26	261.91	Yes
Guibourtia carrissoana	B20-HB11322	Angola	Herbarium LA Grandvux Bonbosa n°11322 (Meise Botanic Garden in Belgium)	30/03/1967	/	-8.836048	13.2593647	702254	97.92	94.19	89.63	84.70	633.50	573.38	Yes
Guibourtia chodatiana	B28-HB481480	Paraguay	(National Museum of Natural History in France)	1/12/1976	/	-25.28402	-57.631351	402838	98.30	96.15	91.75	84.51	313.72	300.11	Yes
Guibourtia coleosperma	B24-HB1024	Zimbabwe	Herbarium A. Angus n°1024 (Meise Botanic Garden in Belgium)	25/12/1952	/	-15.0333	22.8833	622194	97.67	98.45	96.54	93.71	578.15	428.17	Yes
Guibourtia coleosperma	B25-HB2317	DRCongo	Herbarium M Shaijes n°2317 (Meise Botanic Garden in Belgium)	17/06/1984	1050	-10.4	25.28	660260	96.17	98.63	96.90	94.02	609.24	462.78	Yes
Guibourtia coleosperma	B29-FT0028	Namibie	Rundu	17/07/2014	/	-17.84989	19.67261	114960	94.73	98.52	96.55	92.81	99.59	59.25	Yes
Guibourtia coleosperma	B31-HB3499516	Angola	Huila (Meise Botanic Garden in Belgium)	22/11/1963	613	-14.92806	14.658782	548788	98.67	96.60	93.57	89.38	461.99	379.87	Yes
Guibourtia conjugata	B33-HB3499528	Mozambique	Suldo save guyja-Laniçado (Meise Botanic Garden in Belgium)	18/06/1905	756	-23.6548	32.174605	102984	95.49	85.39	79.03	71.12	80.22	78.37	Yes
Guibourtia copallifera	B09-ABFGUIC61	Burkina Faso	Bamfora	12/01/2014	237	9.76002	-4.59009	274420	98.10	94.03	90.82	86.86	259.91	197.91	Yes
Guibourtia copallifera	B34-HB252944	Mali	Bamako (National Museum of Natural History in France)	9/10/1907	883	12.639232	-8.0028892	291758	96.78	94.19	90.61	86.43	253.54	196.64	Yes
Guibourtia copallifera	B35-HB252945	Mali	Mts Mandingues vers Sibi (National Museum of Natural History in France)	4/03/1973	609	12.377685	-8.32664	170422	94.41	91.46	87.09	81.59	144.81	114.47	Yes
Guibourtia copallifera	B37-HB3499492	Sierra Leone	Mano (National Museum of Natural History in France)	16/10/1939	34	6.9294578	-11.500654	247320	98.37	88.35	81.78	75.02	184.80	188.70	Yes
Guibourtia demeusei	B14A-OH3245	CongoK	Mabali	17/06/2013	/	-0.88319	18.1235	191740	95.55	93.05	89.60	83.69	174.98	139.44	Yes
Guibourtia demeusei	B15-HB0069	Gabon	Ogoué-Mante / Herbier JC Monandza Mbembo n°69 (Meise Botanic Garden in Belgium)	8/04/2004	/	-2.248708	9.592977	217316	93.40	97.70	95.88	91.30	195.11	138.15	Yes

Taxa	DNA Ids	Countries	Localities	Collection dates	Altitudes	Latitudes	Longitudes	Nb reads R1+R2 paired	Percentage mapped reads	Percentage Coverage at 1X	Percentage Coverage at 3X	Percentage Coverage at 10X	Average depth	StDev depth	Morphological measurements / GenBank
Guibourtia demeusei	B38-HB527577	DRCongo	Yangambi (Meise Botanic Garden in Belgium)	23/06/1938	256	0.8070691	24.452979	125256	97.05	79.69	74.32	67.55	90.52	99.37	Yes
Guibourtia demeusei	B39-HB2999117	Central African Republic	Yassaba (Meise Botanic Garden in Belgium)	21/04/2005	633	/	/	180162	94.70	86.25	80.65	73.79	143.71	136.68	Yes
Guibourtia dinklagei	B21-HB11235	Liberia	Grand Bassin / Herbarium JT Baldwin n°11235 (Meise Botanic Garden in Belgium)	7/03/1948	/	6.279089	-10.76031	1221892	97.84	88.76	82.88	76.44	950.28	1147.66	Yes
Guibourtia ehie	B04-Ge1	Cameroon	Mamfé	/	/	5.741667	10.101389	91602	96.21	94.13	91.63	86.43	84.70	57.30	Yes
Guibourtia ehie	B05-FT0272	Ghana	Yamfo	11/08/2014	321	7.09241	2.11953	231164	98.00	93.20	89.97	86.17	218.61	167.89	Yes
Guibourtia ehie	B41-FT0414	Ivory Coast	Besso	22/07/2014	/	6.21915	-3.42273	148462	97.35	93.11	90.90	87.20	131.75	89.68	Yes
Guibourtia hymenaefolia	B44-HB252852	Cuba	(National Museum of Natural History in France)	14/05/1905	62	22.131599	-80.338226	374120	98.15	96.44	94.46	90.85	331.76	246.88	Yes
Guibourtia leonensis	B16-HB6561	Senegal	Herbarium RP Berhaut n°6561 (National Museum of Natural History in France)	21/11/1963	/	12.485	-16.546	351594	96.05	98.39	96.91	94.20	326.20	227.60	Yes
Guibourtia leonensis	B18-HB0174	Guinea-Conakry	Praia Varela / Herbarium J D'Orey n°174 (National Museum of Natural History in France)	13/01/1954	/	12.401944	-16.200556	475500	96.21	97.08	94.27	89.11	412.72	356.45	Yes
Guibourtia leonensis	B45-HB3015140	Sierra Leone	Sula mountains south (National Museum of Natural History in France)	4/08/2010	367	8.98528	-11.71694	161808	96.49	98.39	97.05	93.74	142.86	87.59	Yes
Guibourtia leonensis	B46-HB3499272	Senegal	Basse Casamance. Region Oussouye. Dianthème (National Museum of Natural History in France)	1/08/1963	387	12.488449	-16.543676	197010	97.92	94.99	90.62	83.91	165.91	141.10	Yes
Guibourtia pellegriniana	B30-HB3499270	DRCongo	Kouilou inférieur (Meise Botanic Garden in Belgium)	/	/	-4.142841	11.889172	249132	96.22	95.67	91.81	84.69	200.66	171.59	Yes
Guibourtia pellegriniana	B10-GID1159	Gabon	Camp WWF. Sette Cama.	21/09/2009	/	-2.534444	9.769999	136734	96.09	97.51	95.16	89.63	126.58	86.58	Yes
Guibourtia pellegriniana	B11-HB1578	Congo	Konilon / Herbarium Dowsett-Lemaire n°1578 (Meise Botanic Garden in Belgium)		/	-1.94472	9.86578	266718	96.19	97.10	94.50	88.35	238.92	192.50	Yes
Guibourtia pellegriniana	B47-HB252994	Gabon	Ipassa. 10 km S Makokou. chablis XI (Meise Botanic Garden in Belgium)	26/04/1979	493.4	0.5633173	12.857236	146448	97.61	96.29	93.78	86.86	127.81	94.48	Yes
Guibourtia pellegriniana	B48-HB527590	Gabon	Panga (Meise Botanic Garden in Belgium)	4/09/1914	599.8	-3.3	10.616667	612186	97.59	95.20	91.80	86.26	484.12	459.60	Yes

Taxa	DNA Ids	Countries	Localities	Collection dates	Altitudes	Latitudes	Longitudes	Nb reads R1+R2 paired	Percentage mapped reads	Percentage Coverage at 1X	Percentage Coverage at 3X	Percentage Coverage at 10X	Average depth	StDev depth	Morphological measurements / GenBank
Guibourtia schliebenii	B23-HB10151	Mozambique	Herbarium Luke WRQ Kibune O Nacamo E n°10151 (Meise Botanic Garden in Belgium)	15/12/2003	/	-11.15296	39.7343379	1243126	98.67	91.27	85.32	79.20	979.63	1153.36	Yes
Guibourtia schliebenii	B50-HB3015138	Mozambique	N (FZ). Cabo Delgado Province. Quiterajo. edge of escarpment (Meise Botanic Garden in Belgium)	29/11/2008	/	-11.8175	40.34167	277256	98.62	94.44	91.59	87.37	247.28	187.21	Yes
Guibourtia schliebenii	B51-HB6893814	Mozambique	N (FZ). Cabo Delgado Province. Nangade district. Aldea Rovuma village on Pundanhar to Nangade road (Meise Botanic Garden in Belgium)	6/11/2009	/	-11.00056	39.73583	210336	97.37	92.17	88.78	84.39	185.28	147.04	Yes
Guibourtia sousae	B52-HB892206	Mozambique	Maueele (Meise Botanic Garden in Belgium)	1/12/1936	/	-24.6254	33.95793395	524176	97.83	94.56	90.57	84.89	443.25	387.75	Yes
Guibourtia tessmannii	B53-HB252982	Cameroon	Forest of Koumou (Meise Botanic Garden in Belgium)	1950-8	/	1.6666669	16.04999	121356	94.09	86.23	80.32	72.59	92.55	94.42	Yes
Guibourtia tessmannii	B54-HB527596	Gabon	Ogooué-Ivindo. E. Lopé-Okanda Reserve. along roads S of SEG lumber camp. W. Offoué River (Meise Botanic Garden in Belgium)	15/05/1992	/	0.46444	11.75583	62608	90.87	86.74	79.86	69.84	46.32	42.75	Yes
Guibourtia tessmannii	B01-Gt4	Gabon	Ogooué-Lolo. Gabon	/	/	-0.42658	12.58337	301402	98.04	99.00	98.37	96.90	287.23	165.48	Yes
Guibourtia tessmannii	B02-FT09	Cameroon	Village Ma'an. Manioc field	24/03/2014	/	2.372383	10.632217	48216	93.13	97.08	94.95	86.17	43.51	26.51	Yes
OG *Crudia harmsiana*	B56_FT0691	DRCongo	Mpili. river Lombambon South Lake Tumba	23/01/2007	330	-1.01667	17.96667	1638114	/	/	/	/	/	/	No GenBank To come
OG *Daniellia pilosa*	B57_FT0692	Gabon	CEB	23/08/1992	500	-0.68333	12.93333	1307116	/	/	/	/	/	/	No GenBank To come
Guibourtia leonensis (genomic library non enriched)	B16_HB6561	Senegal	Herbarium RP Berhaut n°6561 (National Museum of Natural History in France)	21/11/1963	/	12.485	-16.546	1454120	2.29	99.62	99.29	94.80	27.03	15.59	No GenBank To come

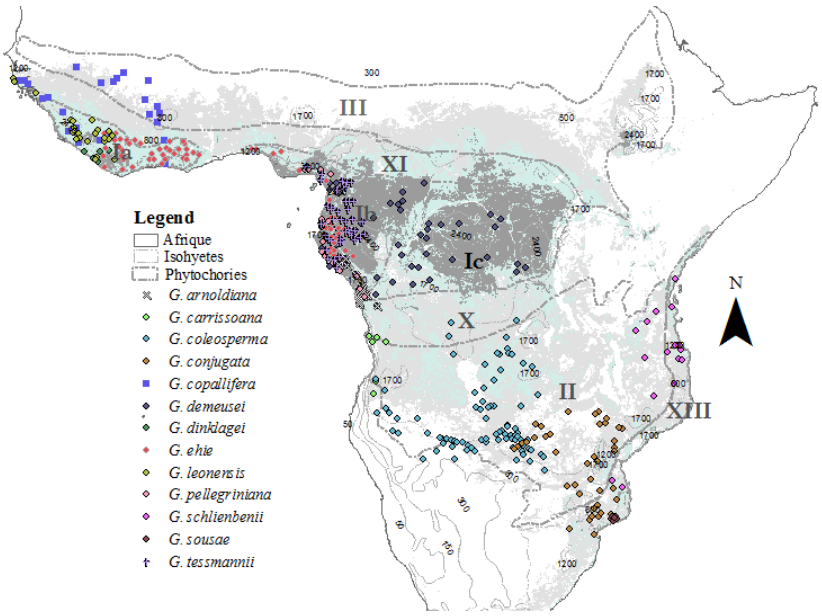
Appendix 2.5. Estimated node ages and their 95% highest posterior density (HPD) for the main nodes defined in the Fabaceae (Appendix 2.1) and *Guibourtia* (Figure 2.2) phylogenies, using BEAST 1.8.2.

Node characteristics		Constraint age (Ma)	Mean divergence time estimate (Ma)	95% higher posterior density	Posterior probabilities
A	Fabaceae stem node	65 (Fixed, Normal)	65.39	64.38-66.30	1.00
B	Fabaceae crown node	60 (Minimum, LogNormal)	62.58	60.48-64.56	1.00
C	*Cercis* stem node	34 (Minimum, LogNormal)	41.37	34.77-48.33	1.00
D	*Bauhinia s.l.* stem node	46 (Minimum, LogNormal)	39.89	34.23-46.04	0.91
E	*Hymenaea* stem node	24 (Minimum, LogNormal)	23.94	20.07-27.91	1.00
F	MRCA *Prioria* and *Oxystigma* stem node	24 (Minimum, LogNormal)	25.67	21.10-30.28	1.00
G	*Daniellia* and *Brandzeia* clade stem node	53 (Minimum, LogNormal)	50.35	45.92-54.86	0.90
I	*Crudia* crown node	45 (Minimum, LogNormal)	35.16	30.65-40.35	1.00
J	Papilionoideae stem node	55 (Minimum, LogNormal)	56.82	53.29-60.35	1.00
K	*Swartzia* stem node	45 (Minimum, LogNormal)	43.37	37.26-49.53	1.00
N	*Senna* stem node	45 (Minimum, LogNormal)	38.08	32.23-43.68	1.00
O	*Mezoneuron* stem node	45 (Minimum, LogNormal)	36.22	31.56-41.13	1.00
P	*Caesalpinia s. s.* stem node	45 (Minimum, LogNormal)	39.46	34.83-44.39	0.64
R	*Dinizia* stem node	45 (Minimum, LogNormal)	40.63	34.39-46.47	0.70
S	*Ingeae* stem node	45 (Minimum, LogNormal)	33.81	29.27-38.40	0.99
T	*Afzelia* crown node	27.32 (Minimum, Gamma)	30.57	27.95-33.61	1.00
N1	*Guibourtia* crown node	19.41 (14.48-24.35) (Fixed, Normal)	17.86	11.57-23.80	1.00
N2	*Guibourtia* Clade 1 crown node	/	16.11	10.08-21.67	1.00
N3	*Guibourtia* Clade 2 crown node	/	16.17	10.29-21.94	1.00
N4	Neotropical *Guibourtia* crown node	/	11.78	6.52-17.38	1.00
N5	*Guibourtia* Clade 3 crown node	/	6.13	3.28-9.28	1.00

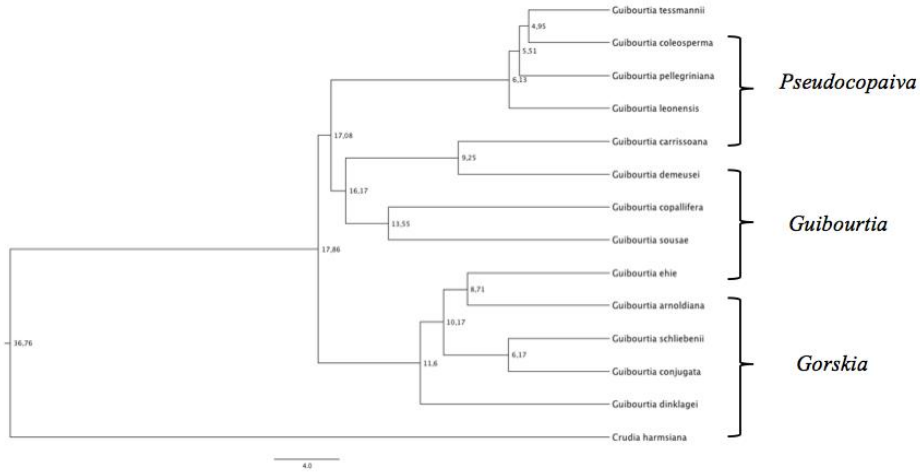
Appendix 2.6. Morphological (vegetative and reproductive) characters considered for describing *Guibourtia* individuals.

Characters (morphological variables)	Character states, values or units
Vegetative characters	
Position of primary leaf veins	submarginal, marginal, median
Apex leaflets	obtuse, acuminate
Glands on the abaxial side of the limb	absent, present
Limb	membranous, coriaceous, (sub)coriaceous
Petiole hairiness	glabrous, pilous
Gland on petioles	absent, present
Stipule	deciduous, persistent
Size of stipules	absent, tiny, foliaceous
Number of leaflets per leaf	one leaflet, two leaflets
Number of secondary leaf veins supra basilar	1, 2, 3, 4, 5
Number of secondary leaf veins basilar	1, 2, 3
Length of leaflets	cm
Width of leaflets	cm
Length of acumen	cm
Petiole length	cm
Reproductive characters	
Inflorescence position	axillary, axillary and terminal, terminal
Type of inflorescence	panicle => cob, panicle=> cluster
Pedicel	absent, present
Bracts	deciduous, persistent
Bracts hairiness	absent, present
Gland on bracts	absent, present
Bracteoles	deciduous, persistent
Shape of bracts	orbicular, linear
Hairiness of the external face of the bracts	absent, present
Shape of flower bud	cylindrical, globular, ellipsoid
Flower hairiness bud on the external face of the sepals	absent, present
Calyx hairiness inner side of the sepals	absent, present
Gland on sepals	absent, present
Hairiness of disc	absent, present
Pilosity of ovary	glabrous, pilous
Stipe of the ovary	sessile, stiped
Hairiness of the stipe's ovary	glabrous, pilous
Type of fruit	indehiscent fruit, dehiscent fruit
Gland on fruit	absent, present
Veins on the fruit outer surface	absent, present
Stipe of the fruit	absent, present
Aril on the seed	absent, present
Length of sepals	mm
Width of sepals	mm
Length of stipe of the ovary	mm
Length of the fruit	cm
Width of the fruit	cm
Thickness of the fruit	mm
Length of the fruit stipe	cm
Number of seeds	1,2

Appendix 3.1. Map of distribution range of African *Guibourtia* species



Appendix 3.2. High-resolution phylogeny (whole plastic genome sequenced) of African *Guibourtia* species (Tosso et al., submitted) with the subdivisions into subgenera according to Leonard(1949)



Appendix 3.3. Correlation of morphological variables with two principal morphology PCoA axes, phylogenetic signal of quantitative morphological traits (Blomber's K and associated test) and phylogenetic signal test using Maddison and Slatkin (1991) method

Morphological traits	Axe 1	Axe 2	Blomberg's K	p-value	p-value (phylogenetic signal test; Maddison and Slatkin, 1991)
Number of leaflets per leaf	-0.11	-0.05	-	-	-
Number of secondary leaf veins supra basilar	**0.56**	0.28	**0.94**	**0.020**	-
Number of secondary leaf veins basilar	**-0.88**	0.17	0.53	0.239	-
Length of leaflets	0.1	**0.68**	**0.73**	**0.053**	-
Width of leaflets	0.07	**0.52**	0.64	0.174	-
Apex leaflets	-0.27	**-0.67**	-	-	0.63
Glands on the underside of the limb	**-0.58**	-0.33	-	-	0.165
Length of acumen	0.06	0.33	0.62	0.338	-
Petiole length	0.41	0.47	**1.71**	**0.003**	-
Petiole hairiness	-0.21	-0.28	-	-	0.397
Stipule	-0.4	-0.12	-	-	-
Inflorescence position	-0.36	**-0.82**	-	-	0.846
Type of inflorescence	0.18	**-0.63**	-	-	0.196
Pedicel	0.25	**-0.75**	-	-	0.42
Bracts	**-0.51**	**0.66**	-	-	0.365
Bracts hairiness	-0.48	**0.66**	-	-	-
Shape of flower bud	0.06	-0.48	-	-	**0.037**
Pilosity of flower bud on the external face of the sepals	-0.34	**0.58**	-	-	0.999
Pilosity of calyx on inner side of the sepals	**0.73**	0.2	-	-	0.999
Gland on sepals	**-0.74**	-0.37	-	-	0.999
Length of sepals	-0.32	0.14	0.55	0.428	-
Width of sepals	0.13	0.54	**0.76**	**0.042**	-
Pilosity of disc	**0.54**	-0.08	-	-	0.999
Pilosity of ovary	**0.72**	0.01	-	-	0.635
Pilosity of the stipe's ovary	**0.91**	-0.44	-	-	0.212
Type of fruit	**-0.75**	-0.03	-	-	**0.003**
Stipe of the ovary	0.18	0.16	0.37	0.643	-
Gland on fruit	**-0.55**	-0.39	-	-	0.401
Thickness of the fruit	**0.55**	0.2	0.62	0.21	-
Veins on the the fruit surface	-0.02	**-0.8**	-	-	0.34
Stipe of the fruit	**0.82**	-0.21	-	-	0.389
Length of stipe of the ovary	**0.67**	-0.1	0.38	0.749	-
Arillus on the seed	**-0.69**	-0.06	-	-	0.999
Leaf veins_mediane	-0.11	-0.05	-	-	0.999
Leaf veins_marginale	-0.17	**0.7**	-	-	0.999
Leaf veins_marginale	0.15	**-0.71**	-	-	0.999
Limb_coriace	0.15	0.44	-	-	0.133
Limb_membranous	0.3	-0.19	-	-	0.424
Limb_subcoriace	-0.11	**0.54**	-	-	0.999
Stipule size_absente	-0.4	-0.12	-	-	0.999
Stipule size_tiny	-0.01	-0.04	-	-	0.999
Stipule size_foliaceous	**-0.52**	-0.12	-	-	0.999

Appendix 3.4. Correlation of environmental variables with two principal ecological niche optimum axes

	Axis 1	Axis 2	Blomberg's K	p
Wind speed	-0.222	**-0.917**	0.70	0.386
Temperature	0.251	-0.199	0.71	0.300
Solar radiation	**-0.957**	-0.152	0.77	0.197
Relative Humidity	**0.929**	0.052	0.77	0.161
Precipitations	**0.787**	-0.335	**0.87**	**0.085**
Potential evapotranspiration	0.014	**-0.958**	0.63	0.494
Temperature range	**-0.935**	0.075	0.61	0.630
pH	-0.323	0.290	0.63	0.348

Appendix 3.5. Correlation of morphological variables with the first ecological niche axis

Morphological characters	r correlation (Niche ~ Morphometry)	r-pvalue
Mediane venation	0.25	0.403
marginal venation	0.60	0.030
submarginal venation	-0.67	0.012
Round inner margin	-0.09	0.770
Linear inner margin	0.09	0.770
Apex acuminate leaflet	0.63	0.022
Apex obtuse leaflet	-0.63	0.022
Leaflet glands absent	0.67	0.013
Leaflet glands present	-0.58	0.036
Length petiole	-0.03	0.923
Membranous lamina	-0.17	0.574
Sub-tough limb	0.39	0.184
Petiole glabrous	0.39	0.183
Petiole Pilose	-0.41	0.167
Stipulates obsolete	0.03	0.928
Stipulates persistent	-0.03	0.928
Stipules absent	0.03	0.928
Stipules foliaceous	0.00	0.991
Stipules tiny	-0.05	0.881
Inflorescence axillary	0.84	0.000
Inflorescence terminal and axillary	-0.84	0.000
Cob inflorescence	0.62	0.025
Cluster inflorescence	-0.62	0.025
Pedicel absent	0.76	0.003
Pedicel present	-0.76	0.003
BRAT.caduque	-0.50	0.084
BRAT.persistants	0.58	0.038
Ellipsoid bud	0.41	0.169
Globular bud	-0.41	0.169
Glabrous bud	-0.16	0.608
Pilous bud	0.25	0.409
Glabrous sepal	-0.05	0.881
Pikous sepal	0.22	0.480
Glabrous ovary	0.06	0.842
Pilous ovary	-0.10	0.736
Dehiscent fruit	0.32	0.280
Indehiscent fruit	-0.32	0.293
Glands on fruits absent	0.58	0.038

Morphological characters	**r correlation (Niche ~ Morphometry)**	**r-pvalue**
Glands on fruits present	-0.49	0.093
Stipule absent	0.20	0.512
Stipule present	-0.17	0.578
Gland sepal absent	0.47	0.103
Gland sepal present	-0.40	0.181
Gland sepal absent	-0.25	0.403
Number of secondary leaf veins supra basilar	0.24	0.420
Number of secondary leaf veins basilar	-0.15	0.618
Length of leaflets	0.80	0.002
Width of leaflets	0.84	0.001
Length of acumen	0.31	0.308
Length of sepals	0.40	0.218
Width of sepals	0.66	0.028
Fruit form	0.37	0.298
Thickness of the fruit	0.75	0.031

Appendix 3.6. Phylogenetically independent contrasts tests on quantitative morphological variables with the two first ecological niche axes

Morphological traits	**Phylogenetically independent contrasts**			
	Niche Axis 1		Niche Axis 2	
	r	p-value	r	p-value
Number of secondary leaf veins supra basilar	-0.346	0.270	0.449	0.143
Number of secondary leaf veins basilar	0.162	0.616	0.020	0.952
Length of leaflets	0.495	0.540	0.257	0.420
Width of leaflets	0.747	**0.005**	-0.653	**0.021**
Length of acumen	0.800	**0.002**	-0.640	**0.025**
Length of sepals	0.485	0.110	-0.634	**0.027**
Width of sepals	0.440	0.153	-0.372	0.234
Fruit form	0.630	**0.028**	-0.554	**0.062**
Thickness of the fruit	0.253	0.428	-0.275	0.387

Appendix 3.S1. Comparison niche equivalency and niche similarity tests

The niche equivalency test was performed by comparing the observed niche overlap value (*D*), using the occurrence of the target species pair, to a null distribution of 100 overlap values obtained after random permutation of the occurrence records between species. We declared non-equivalence of ecological niches if the observed niche overlap value was significantly lower than the null distribution ($P \leq 0.05$; Broennimann et al., 2012). Niche equivalency test is a strict test of niche identity (Kirchheimer et al., 2016), assessing whether the environmental ranges of species are interchangeable. Besides, the niche similarity test differs from niche equivalency test since it assesses whether the observed niche overlap value between target species differs from simulated niche overlaps where the geographical distribution of one of the two species is shifted in the background area while keeping the same shape and size (Warren et al., 2010; Broennimann et al., 2012). This asymmetric test compares the niche overlap of one range randomly distributed over its background keeping

the range of the other species unchanged (a->b), and then does the reciprocal comparison (a<-b) (Silva et al., 2014). We repeated each randomization 100 times, producing a null distribution of overlap values to which the observed score was compared. This test aims to discern niche differentiation caused by spatial autocorrelation of environmental data and limited species distributions from true ecological niche differences (Broennimann et al., 2012; Hu et al., 2016).

Appendix 3.S2. Environmental niche comparisons for African *Guibourtia* species. Niche overlap values are presented for the comparisons of niche similarity and equivalency of species a with species b.

***Guibourtia* species**		**Niche Overlap**	**Niche similarity**		**Niche Equivalency**
a	***b***		***a* -----> *b***	***b* -----> *a***	
G. arnoldiana	*G. pellegriniana*	0.17	Similar*	ns	Different*
	G. tessmannii	0.18	ns	ns	Different*
	G. demeusei	0.22	ns	ns	Different*
	G. dinklagei	0.14	ns	ns	Different*
	G. ehie	0.53	Similar*	ns	Different*
	G. leonensis	0.16	ns	ns	Different*
	G. copallifera	0.26	Similar*	ns	Different*
	G. schliebenii	0.3	ns	ns	Different*
	G. sousae	0.13	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0.1	ns	ns	Different*
	G. conjugata	0.33	Similar*	ns	Different*
G. pellegriniana	*G. tessmannii*	0.83	Similar*	Similar*	**Equivalent**
	G. demeusei	0.47	Similar*	ns	Different*
	G. dinklagei	0.56	Similar*	Similar*	**Equivalent**
	G. ehie	0.32	ns	ns	Different*
	G. leonensis	0.72	ns	ns	Different*
	G. copallifera	0.18	ns	ns	Different*
	G. schliebenii	0.01	ns	ns	Different*
	G. sousae	0.01	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0	ns	ns	Different*
G. tessmannii	*G. demeusei*	0.52	ns	Similar*	Different*
	G. dinklagei	0.24	ns	ns	Different*
	G. ehie	0.37	ns	ns	Different*
	G. leonensis	0.65	Similar*	Similar*	Different*
	G. copallifera	0.13	ns	ns	Different*
	G. schliebenii	0	ns	ns	Different*
	G. sousae	0.01	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0	ns	ns	Different*
G. demeusei	*G. dinklagei*	0.45	ns	Similar*	Different*
	G. ehie	0.6	ns	ns	Different*
	G. leonensis	0.22	ns	ns	Different*
	G. copallifera	0.52	Similar*	Similar*	Different*
	G. schliebenii	0	ns	ns	Different*
	G. sousae	0	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0	ns	ns	Different*
G. dinklagei	*G. ehie*	0.63	Similar*	Similar*	Different*
	G. leonensis	0.68	Similar*	Similar*	**Equivalent**
	G. copallifera	0.46	ns	Similar*	Different*
	G. schliebenii	0	ns	ns	Different*
	G. sousae	0	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0	ns	ns	Different*

Guibourtia species		Niche Overlap	Niche similarity		Niche Equivalency
a	***b***		***a -----> b***	***b -----> a***	
G. ehie	*G. leonensis*	0.36	ns	Similar*	Different*
	G. copallifera	0.32	ns	ns	Different*
	G. schliebenii	0.1	ns	ns	Different*
	G. sousae	0	ns	ns	Different*
	G. carrissoana	0.33	Similar*	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0.2	ns	ns	Different*
G. leonensis	*G. copallifera*	0.27	Similar*	ns	Different*
	G. schliebenii	0	ns	ns	Different*
	G. sousae	0	ns	ns	Different*
	G. carrissoana	0	ns	ns	Different*
	G. coleosperma	0	ns	ns	Different*
	G. conjugata	0	ns	ns	Different*
G. copallifera	*G. schliebenii*	0.1	ns	ns	Different*
	G. sousae	0.11	ns	ns	Different*
	G. carrissoana	0.11	ns	ns	Different*
	G. coleosperma	0.16	ns	ns	Different*
	G. conjugata	0.13	ns	ns	Different*
G. schliebenii	*G. sousae*	0.58	Similar*	Similar*	Different*
	G. carrissoana	0.15	ns	ns	Different*
	G. coleosperma	0.09	ns	ns	Different*
	G. conjugata	0.67	Similar*	ns	Different*
G. sousae	*G. carrissoana*	0.33	Similar*	ns	Different*
	G. coleosperma	0.11	ns	ns	Different*
	G. conjugata	0.53	ns	ns	Different*
G. carrissoana	*G. coleosperma*	0.13	ns	ns	Different*
	G. conjugata	0.39	Similar*	ns	Different*
G. coleosperma	*G. conjugata*	0.43	Similar*	Similar*	Different*

ns, not similar.

*The ecological niches are significantly ($P < 0.05$) more similar than expected by random.

Appendix 3.S3. Environmental niche comparison

For all pairwise comparisons, the null hypothesis of niche equivalency (strict niche identity) was rejected except for the following pairs of rain forest species: *G. pellegriniana*[*] - *G. dinklagei*[*], *G. tessmannii*[*] - *G. dinklagei*[*], *G. tessmannii*[*] - *G. ehie*[*] and *G. dinklagei*[*] – *G. leonensis*[*] (Table 2). On the other hand, analyses of niche similarity (Table 2) pointed out significant similarity both for rain forest species pairs (e.g. *G. pellegriniana*[*] - *G. tessmannii*[*], *G. pellegriniana*[*] - *G. leonensis*[*], *G. tessmannii*[*] - *G. ehie*[*], and *G. dinklagei*[*] - *G. leonensis*[*]) and for dry forest and woodland pairs of species (e.g. *G. sousae*[#] - *G. conjugata*[#], *G. schliebenii*[#] - *G. sousae*[#]). Some pairs (both rain forest and dry forest and woodland pairs of species) shared niche spaces that were more similar than expected by chance but only in one direction (e.g., *G. arnoldiana*[*] - *G. pellegriniana*[*], and *G. schliebenii*[#] - *G. conjugata*[#]), exhibiting for instance that the niche space of *G. arnoldiana* is included in the one of *G. pellegriniana* but not the reverse because the latter species has a large niche amplitude.

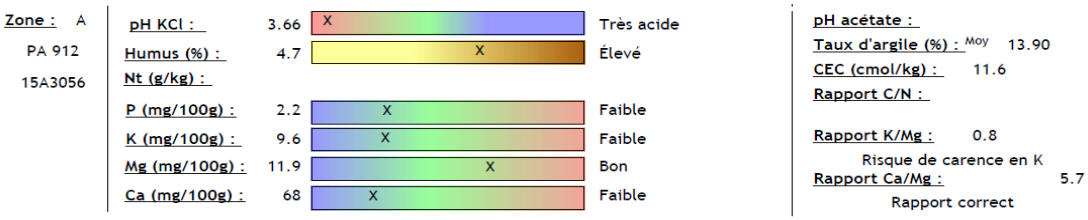
Appendix 4.1. Light intensity in six shadehouses for six dates in percentage of the light intensity under full sun light (given in µmol/m^2/s). For each month, measurements were performed during seven days.

Shadehouse	Date						Mean light intensity	Mean %
	March 2015	August 2015	November 2015	February 2016	July 2016	March 2017		
I	0.98%	1.05%	0.60%	1.51%	1.31%	1.80%	18.43	1.22%
II	3.52%	4.04%	2.96%	4.21%	3.92%	4.18%	57.59	3.82%
III	10.06%	12.33%	8.54%	12.84%	9.89%	9.91%	158.81	10.54%
IV	22.02%	26.03%	22.75%	27.88%	22.73%	23.94%	365.30	24.24%
V	39.58%	40.48%	33.20%	48.20%	37.78%	37.59%	596.22	39.56%
VI	56.15%	61.64%	61.34%	65.01%	60.99%	56.67%	904.61	60.02%
Light intensity under full sun	2016±71.91	1088±33.8	1521±55.86	1589±47.51	1224±41.26	1604±21.9	1507	100.00%

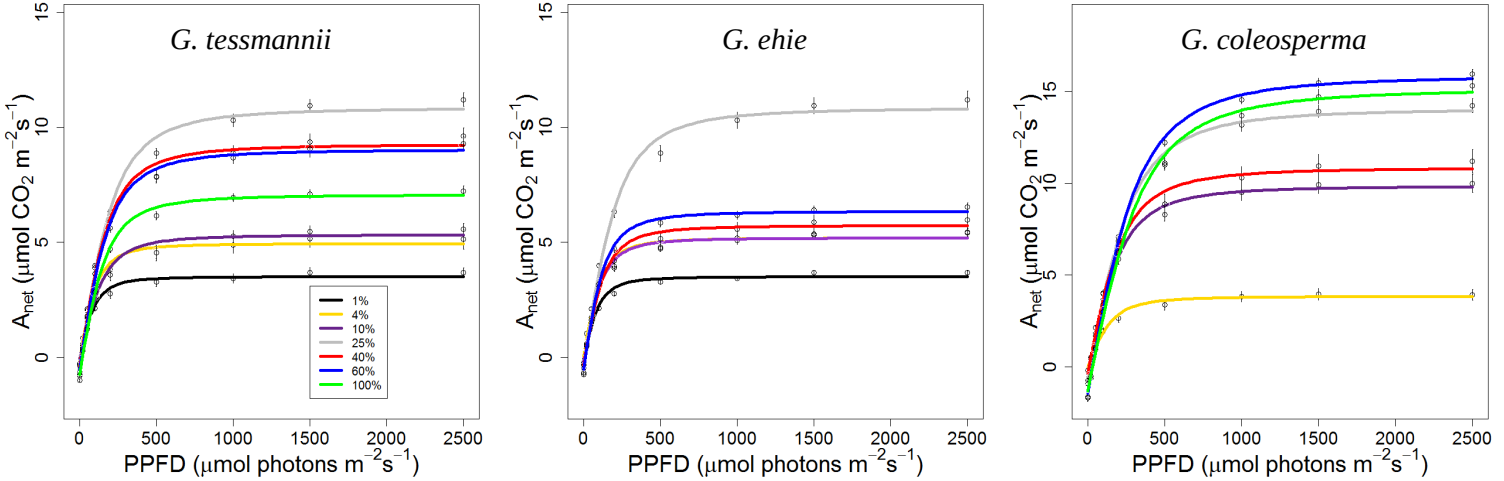
Appendix 4.2. Minimum and maximum (7-day period) for temperature (T), relative humidity (RH) and vapour pressure deficit (VPD) in each shadehouse and correlation between mean of each climatic variables with light

	November 2015						February 2016						July 2016						March 2017					
	T (°C)		HR (%)		VPD (kPa)		T (°C)		HR (%)		VPD (kPa)		T (°C)		HR (%)		VPD (kPa)		T (°C)		HR (%)		VPD (kPa)	
Shade house	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
I	21	33.1	61.1	99.5	0.012	1.531	21.1	38.4	41.9	98.5	0.018	1.005	20.8	31.5	57.5	97.1	0.033	0.623	19.9	34.6	48.7	98.5	0.018	0.805
II	21.1	39.2	43.3	99.6	0.009	2.986	20.9	37.7	42.2	98.8	0.014	0.984	19.3	29.9	61.1	96.4	0.040	0.548	19.9	36.1	45.8	98.9	0.013	0.880
III	20.7	34.5	57.1	99.8	0.004	1.718	20.7	37.8	41.4	97.3	0.032	1.000	19.1	29.5	62.5	96.6	0.037	0.522	19.2	36.1	47.4	99.2	0.009	0.844
IV	20.7	38.3	47.7	99.9	0.002	2.614	20.7	37.6	42.6	97.1	0.036	1.055	18.8	30.1	61.4	97.1	0.032	0.544	19.8	36.6	44.6	99	0.011	0.908
V	20.9	42.8	47.3	99.2	0.023	3.592	20.5	38.5	40.4	99	0.012	1.031	18.8	31	56.3	97	0.033	0.634	19.2	36.8	43.7	99	0.011	0.929
VI	22.2	46.3	48.1	81.7	0.536	4.396	20.5	38.6	39.3	99	0.012	1.051	20.6	33	49.8	97.5	0.028	0.764	19.8	37	42.8	98.3	0.019	0.953
Full exposed	24.3	48.5	39.6	82	0.377	5.845	20.9	35.3	38.5	98.7	0.015	1.082	18.9	35.2	52	98.2	0.019	0.763	19	38.8	43.4	99	0.020	0.982
Correlation with light: Pearson's r (p_value)	0.96 (p<0.0001)		-0.96 (p<0.001)		0.97 (p<0.0001)		0.76 p<0.05		-0.76 p<0.05		0.78 p<0.05		0.46 p>0.05		-0.72 p>0.05		0.84 p>0.05		0.17 p>0.05		-0.17 p>0.05		0.89 p<0.05	

Appendix 4.3. Composition in macro and micronutrients of the soil



Appendix 4.4. Light response curves per species and light treatment



Appendix 5.1. Voucher information for the samples used in this study [a]

Species	n	Voucher N°	Country	Latitude	Longitude
Guibourtia tessmannii[b]	1	FT0001	Gabon	1.4286	11.5886
Guibourtia tessmannii[c]	3	FT0002, FT0635-FT0636	Cameroon	2.2236	10.3793
Guibourtia tessmannii[c]	4	FT0003, FT0800-FT0802	Gabon	-0.3802	12.5649
Guibourtia tessmannii[d]	35	FT0540-FT0545, FT0572-FT0600	Gabon	0.36	13.10
Guibourtia tessmannii[d]	58	FT0800-FT0849, FT0851-FT0856, FT0900-FT0902	Gabon	0.76	12.9
Guibourtia tessmannii[d]	38	FT0605-FT0636, OH4675, OH4679, OH4682, OH4683, OH4684, OH4685	Cameroon	2.37	10.63
Guibourtia pellegriniana[d]	14	FT0641-FT0654	Gabon	-2.53	9.77
Guibourtia coleosperma[d]	20	FT0698-FT0717	DRC	-10.48	22.45
Guibourtia coleosperma[d]	13	FT0021-FT0024, FT0028-FT0031, FT0722-FT0726	Namibia	-18.05	19.62
Guibourtia leonensis[d]	3	BR0000013186371[f], BR0000013186401[f], BR0000013186388[f]	Liberia	7.66	-10.02
Guibourtia demeusei[d]	9	FT0873 – FT0879, OH3245, BR0000009459977[f]	DRC	-0.88	18.12
Guibourtia ehie[d]	10	FT0335-FT0344	Ivory Coast	6,28	-3,68
Guibourtia ehie[d]	10	FT0163-FT0172	Ghana	7,02	-2,05
Guibourtia carrissoana[d]	2	BR0000013186210[f], BR0000013186418[f]	Angola	-8.83	13.25
Guibourtia copallifera[d]	7	FT0880 - FT0886	Burkina-Faso	9.95	-4.67
Guibourtia arnoldiana[d]	2	FT0638, GID2040	Gabon	-3.4098	11.4185
Guibourtia dinklagei[d]	1	BR0000013186265[f]	Liberia	6.23084	-9.81249
Guibourtia tessmannii[e]	3	FT0007, FT006, FT008	Gabon	-0.42	12.58
Guibourtia coleosperma[e]	3	FT0020, FT0024, FT0028	Namibia	-17.99	24.09

Note : n = number of individuals

[a] Vouchers are deposited at the Herbarium of the Université Libre de Bruxelles, Belgium (BRLU), silica gel collection of Dr. Olivier Hardy.

[b] Individual used for DNA bank enriched in microsatellite markers.

[c] Individual used for tests of amplification and polymorphism.

[d] Individuals used for cross amplification.

[e] Individuals used for flow cytometry (code for the mother tree).

[f] Codes of specimen on which sample was collected in Botanic Garden Meise (BR) in Belgium

Appendix 6.1. Voucher information for the samples used in this study [a]

Species	*N*	Voucher no.	Country	Latitude	Longitude
Guibourtia ehie[b]	1	FT0272	Ghana	7.09241	-2.11953
Guibourtia ehie[c]	1	FT0288	Ghana	7.08999	-2.11845
Guibourtia ehie[c]	1	FT0478	Ivory Coast	6.30892	-5.28866
Guibourtia ehie[d]	5	FT0497, FT0491,FT0515, FT0510, FT0521	Ivory Coast	6.21	-3.41
Guibourtia ehie[d]	3	FT0241, FT0261, FT0241	Ghana	7.07	-2.08
Guibourtia ehie[d]	8	OH4661-OH4668	Cameroon	2.31	9.96
Guibourtia ehie[d]	20	FT0029, FT0038, FT0059, FT0078, FT0087, FT0095, FT0102, FT0104, FT0115, FT0125, FT0137, FT0146, FT0158, FT0163, FT0169, FT0180, FT0192, FT0192a,FT0193,FT0197	Ghana	7.06	-2.08
Guibourtia ehie[d]	23	FT0398-FT0400, FT0336, FT0355, FT0363, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859	Ivory Coast	6.21	-2.42
Guibourtia ehie[d]	15	FT0398, FT0336, FT0355, FT0363, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859	Cameroon	2.44	9.92
Guibourtia ehie[d]	20	NB116, NB389, NB391, NB395, NB399, NB401, NB402, NB403, NB405, NB408, NB413, NB414, NB415, NB417, NB418, NB419, NB423, NB424, NB425, NB91	Liberia	7.56	-8.64
Guibourtia arnoldiana (De Wild. & T. Durand) J.Leonard[e]	3	HB00527556	Gabon	-1.3465	9.7232
		HB00253056	Congo	-4.5	12.23
		GiD2040	Gabon	-3.4098	11.4185
Guibourtia schliebenii (Harms) J.Leonard[e]	3	B23-HB10151	Mozambique	-11.1529	39.7343
		B50-HB3015138	Mozambique	-11.8175	40.34167
		B51-HB6893814	Mozambique	-11.0005	39.7358
Guibourtia conjugate (Bolle) J.Leonard[e]	1	B33-HB3499528	Mozambique	-23.6548	32.1746
Guibourtia dinklagei (Harms) J.Leonard[e]	1	B21-HB11235	Liberia	6.279	-10.7603
Guibourtia tessmannii (Harms) J.Leonard[e]	10	FT0607-FT0613, FT0635-FT0636	Cameroon	2.2236	10.3793
		FT0001	Gabon	1.4286	11.5886
Guibourtia pellegriniana Leonard[e]	7	B11-HB1578	Congo	-1.94472	9.86578
		FT0641-FT0646	Gabon	-2.53	9.77
Guibourtia coleosperma (Benth.) Leonard[e]	6	FT0021-FT0025, FT0028	Namibia	-17.85	19.67
Guibourtia leonensis J.Leonard[e]	1	B45-HB3015140	Sierra Leone	8.9852	-11.7169
Guibourtia hymenaefolia (Moric.) J.Léonard[e]	1	B44-HB252852	Cuba	22.1315	-80.3382
Guibourtia carrissoana (M.A.Exell) J.Leonard[e]	2	B19-HB10458	Angola	-8.9341	13.1864
		B20-HB11322	Angola	-8.836	13.2593
Guibourtia copallifera Benn.[e]	5	FT0880-FT0884	Burkina-Faso	9.95	-4.67
Guibourtia demeusei (Harms) J.Leonard[e]	6	FT0873-FT0875, OH3245	Congo	-0.8831	18.123
		B15-HB0069	Gabon	-2.2487	9.5929
		B38-HB527577	DRC	0.807	24.4529
Guibourtia sousae J.Leonard[e]	1	B52-HB892206	Mozambique	-24.6254	33.9579

Note: *N* = number of individuals

[a] Vouchers are deposited at the Herbarium of the Université Libre de Bruxelles, Belgium (BRLU), silica gel collection of Dr. Olivier Hardy.

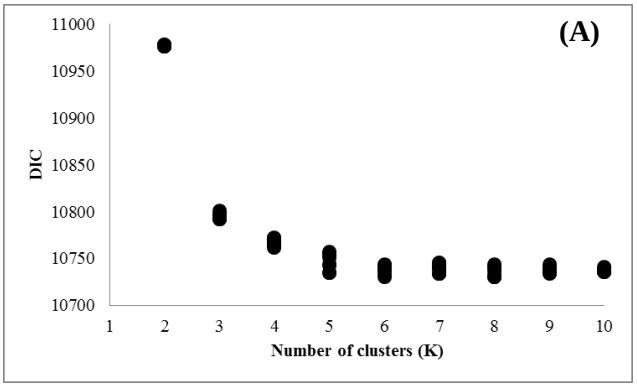
[b] Individual used for genomic library.

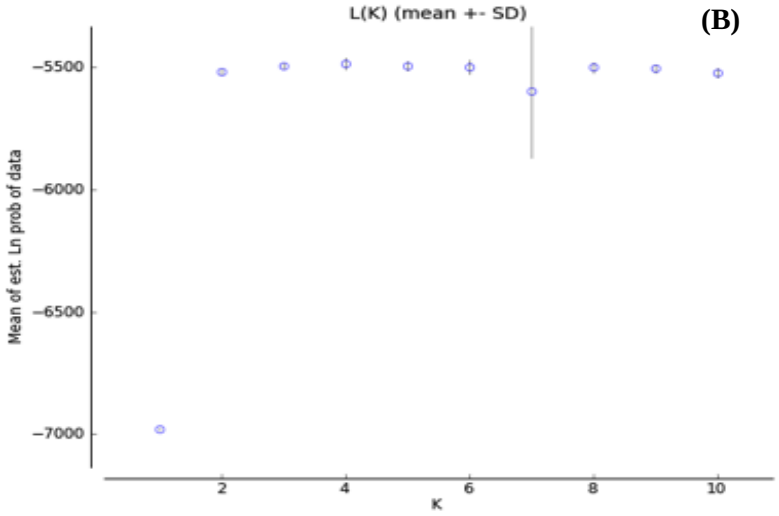
[c] Individuals used for tests of amplification.

[d] Individuals used for tests of polymorphism.

[e] Individuals used for cross amplification tests.

Appendix 7.1. (A) variation in the DIC as a function of number of *G. ehie* genetic cluster (K). (B) variation in means of Ln (likelihood) of the data as a function of the number of hypothetical genetic clusters (K) in *G. ehie*





Appendix 7.2. Nei pairwise among *G. ehie* genetic clusters

	UG1	UG2	LG1	LG2
UG1				
UG2	0.101			
LG1	1.266	1.315		
LG2	1.674	1.462	0.08	

Appendix 7.3. *Guibourtia ehie* alleles frequencies in UG and LG major genetic clusters

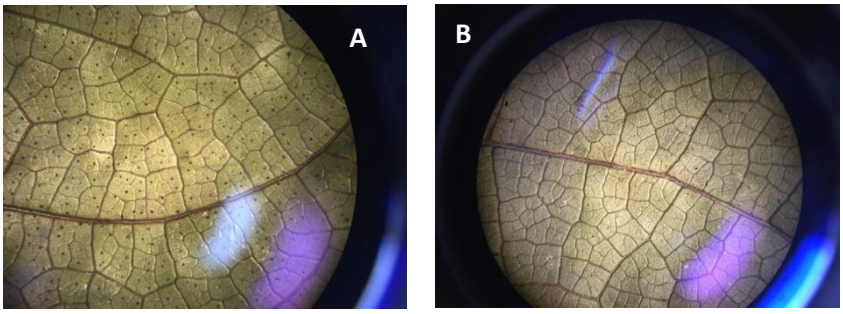
Locus	Alleles	UG	LG	Locus	Alleles	UG	LG
Seq33	143	0.00	0.98	Seq3	219	0.012	0.024
	145	0.00	0.02		253	0.006	0.000
	153	1.00	0.00		263	0.673	0.000
Seq5	258	0.01	0.00		265	0.036	0.286
	260	0.00	0.03		267	0.012	0.131
	262	0.02	0.90		269	0.024	0.155
	264	0.96	0.07		271	0.006	0.119
	266	0.01	0.00		273	0.006	0.060
Seq34	152	0.01	0.07		275	0.214	0.107
	154	0.35	0.05		277	0.012	0.012
	156	0.54	0.13		279	0.000	0.012
	158	0.09	0.08		283	0.000	0.024
	160	0.01	0.19		289	0.000	0.012
	162	0.00	0.13		291	0.000	0.024
	164	0.00	0.11		293	0.000	0.012
	166	0.00	0.07		295	0.000	0.012
	167	0.00	0.02		297	0.000	0.012
	168	0.00	0.02	Seq21	155	0.000	0.010
	170	0.00	0.02		163	0.371	0.563
	180	0.00	0.11		165	0.000	0.073
Seq18	180	0.01	0.45		167	0.000	0.146
Seq39	182	0.00	0.02		169	0.011	0.052
	184	0.00	0.02		171	0.226	0.073
	186	0.45	0.35		173	0.000	0.021
	188	0.44	0.16		175	0.000	0.021
	190	0.10	0.00		177	0.000	0.010
	118	0.00	0.08		179	0.016	0.021
	122	0.00	0.02		181	0.070	0.000
	130	0.00	0.02		183	0.177	0.010
	132	0.79	0.07		185	0.011	0.000
	134	0.02	0.01		187	0.075	0.000
	136	0.00	0.02		189	0.027	0.000
	138	0.00	0.01		191	0.016	0.000
	140	0.03	0.01	Seq4	255	0.000	0.011
	142	0.00	0.61		267	1.000	0.989
	144	0.01	0.03	Seq15	200	0.000	0.865
	146	0.06	0.03		204	0.011	0.021
	148	0.01	0.02		208	0.000	0.031
	150	0.00	0.02		216	0.989	0.000
	152	0.05	0.02		224	0.000	0.010
	154	0.03	0.01		226	0.000	0.052
	156	0.01	0.01		228	0.000	0.010
Seq6	232	0.03	0.00		230	0.000	0.010
	250	0.01	0.00	Seq1	308	0.000	0.979
	256	0.01	0.07		314	0.978	0.021
	258	0.03	0.08		316	0.022	0.000
	260	0.01	0.04	Seq38	143	0.679	0.077
	262	0.10	0.08		144	0.011	0.000
	263	0.01	0.00		145	0.310	0.026
	264	0.28	0.09		148	0.000	0.103
	266	0.20	0.24		149	0.000	0.718
	268	0.15	0.14		151	0.000	0.051
	270	0.04	0.08		153	0.000	0.026
	272	0.03	0.08	Seq30	141	0.000	0.012
	274	0.02	0.01		145	0.000	0.134
	275	0.01	0.00		151	0.000	0.061
	276	0.01	0.05		153	0.401	0.671
	278	0.01	0.00		155	0.157	0.085

	290	0.04	0.00		157	0.442	0.037
	292	0.03	0.00	Seq28	159	0.304	1.000
	306	0.00	0.03		161	0.571	0.000
Seq2	262	0.02	0.00		163	0.065	0.000
	264	0.05	0.00		165	0.018	0.000
	268	0.01	0.00		167	0.036	0.000
	292	0.81	0.06		225	0.006	0.000
	294	0.06	0.14	Seq11	201	0.000	0.013
	296	0.04	0.14		203	0.000	0.051
	298	0.00	0.02		205	0.000	0.038
	300	0.00	0.14		209	0.000	0.038
	302	0.01	0.12		211	0.000	0.051
	304	0.00	0.06		213	0.000	0.051
	306	0.00	0.06		217	0.006	0.077
	308	0.00	0.04		219	0.013	0.141
	310	0.00	0.10		221	0.058	0.090
	312	0.00	0.06		223	0.135	0.026
	316	0.01	0.00		225	0.128	0.000
	322	0.00	0.04		227	0.276	0.026
	396	0.00	0.02		229	0.083	0.038
Seq36	143	0.00	0.01		231	0.064	0.051
	147	0.00	0.01		233	0.160	0.115
	149	0.23	0.01		235	0.032	0.051
	151	0.55	0.04		237	0.019	0.000
	153	0.11	0.17		239	0.026	0.051
	155	0.00	0.09		241	0.000	0.026
	157	0.03	0.24		243	0.000	0.038
	159	0.07	0.41		245	0.000	0.026
	161	0.01	0.01	Seq8	220	0.006	0.000
	167	0.01	0.00		222	0.000	0.947
	171	0.01	0.00		226	0.006	0.000
					228	0.006	0.000
					230	0.142	0.000
					232	0.068	0.000
					234	0.167	0.000
					236	0.049	0.000
					238	0.099	0.000
					242	0.043	0.000
					244	0.099	0.000
					246	0.025	0.000
					248	0.062	0.000
					250	0.123	0.053
					252	0.056	0.000
					254	0.025	0.000
					256	0.019	0.000
					260	0.006	0.000

Appendix 7.4. *Guibourtia coleosperma* private alleles in CGn and CGs genetic clusters.

Locus	Alleles	CGn	CGs
Seq10	178	0.0555556	0
	192	0.037037	0
	194	0.037037	0
	208	0.037037	0
Seq16	232	0.0714286	0
	240	0.0714286	0
	248	0.0178571	0
Seq09	202	0.75	0
	204	0.25	0
Seq20	211	0.02	0
	217	0.04	0
	227	0.04	0

Appendix 7.5. Difference in Upper Guinean (A) and Lower Guinean (B) *Guibourtia ehie* individual leaflets. The pictures show presence of translucent dots on the inner face of leaflets in A whereas they are absent in B.



Appendix 7.6. Differences in leaflets of *G. ehie* individuals form Upper Guinean (A) and Lower Guinean (B). The two individuals are taken from the MNHN (Paris) database.