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Identification of Apocynaceae climbers in the Sangha Trinational, Central Africa

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August 2020

Thesis submitted in partial fulfilment for the MSc in the Biodiversity and Taxonomy of Plants

Abstract

Apocynaceae climbers are easy to distinguish from other plant families due to the white latex and the opposite leaves. Identification at lower taxon levels, however, is more difficult as fertile material is rarely available in the field. This study aimed to facilitate the identification of Apocynaceae climbers in the Sangha Trinational, Central Africa. For this, a morphological study on 42 species was conducted by examining digital specimens from across their distribution. An identification key to the species based on sterile characters is presented. Results showed that Apocynaceae climbers can be grouped based on sterile traits, however, for a few species, delimitation without fertile characters has proved to be difficult. The key is the first of that kind to be produced for central African Apocynaceae climbers, yet it needs to be subject to further revision and testing.

Secondly, this study aimed to assess the utility of *rbcL* as barcode marker for plants of the Sangha Trinational. For this, 87 *rbcL* sequences of 50 species in 28 families were subjected to two BLAST searches. Results showed that *rbcL* performs well at discriminating families (97 % identification matches) and is satisfactory at genus-level (77 %). Potentials and perils of DNA barcoding for identification are discussed.

Keywords: Apocynaceae, identification key, DNA barcoding, *rbcL*, Sangha Trinational

Acknowledgements

I would first like to thank my principal supervisor Dr David Harris for his invaluable support, guidance and motivating words throughout the project. I would also like to thank my supervisors Dr Laura Forrest and Dr Michelle Hart for their expertise and input on DNA barcoding.

Thanks to Hannah Atkins and Dr Colin Pendry for testing the identification key. Special thanks to my fellow students at RBGE for their friendship throughout the year. And last but not least, thanks to my family for their support during my studies and beyond.

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cf. compare

DD decimal degree

e.g. for example

et al. et alii, and others

i.e. in other words

Fig. Figure

1 Introduction

1.1 Introduction to Apocynaceae Jussieu

Apocynaceae Jussieu (Gentianales) consists of 366 genera with around 4,500 species and forms one of the largest angiosperm families (APG IV, 2016; Endress *et al*., 2014; Fishbein *et al*., 2018). The family includes trees, shrubs, herbs, vines and climbers. They are found across the world with the main distribution and greatest diversity in the tropics and subtropics. A characteristic trait of the family is the usually white latex (Endress & Bruyns, 2000).

Endress & Bruyns (2000) moved the former Asclepiadaceae s. str. to Apocynaceae, after molecular studies have shown that taxa of the subfamily Periplocoideae are more closely related to Apocynoideae than to other Asclepiadaceae (Endress *et al*., 2014). The most recent classification by Endress *et al*. (2014) recognizes five subfamilies within Apocynaceae: Rauvolfioideae, Apocynoideae, which were traditionally placed in Apocynaceae, and Asclepiadoideae, Periplocoideae and Secamonoideae, which were formerly placed within Asclepiadaceae. This study follows the classification by Endress *et al.* (2014), yet in some literature used Apocynaceae and Asclepiadaceae are treated as separate families (*e.g.* Hawthorne & Jongkind, 2006).

1.2 Introduction to tropical climbers

For this study, climbers are defined as rooted woody and non-woody plants with climbing mechanisms which rely on the support of other plants for growth. Climbers are abundant in the tropical forests, usually constituting up to 30 % of the species diversity (Schnitzer & Bongers, 2002; Swaine *et al.*, 2017). In tropical forests with low annual rainfall or during dry season, they are considered to be advantaged over trees (Schnitzer *et al*., 2005) due to their diverse biomechanical adaptations (Paul & Yavitt, 2011).

Climbers contribute to various aspects of forest ecology. They influence the forest structure and function, play an important role for nutrient cycling and provide food and habitats (*e.g.* as pathways) for animals. Apart from their ecological function, climbers provide service to people for medicine, food, art or construction (Bongers *et al*., 2002; Parthasarathy *et al*., 2015). Examples of Apocynaceae climbers with economic uses are *Strophanthus* (drugs, arrow poison) (Beentje, 1982) and *Landolphia* (rubber, bird lime) (Persoon *et al*., 1992). The diverse aspects of climbing plants have attracted the interest of researchers in the past, yet climbers remain an understudied habit (Bongers *et al*., 2002).

1.3 Identification of Apocynaceae climbers

1.3.1 Morphological identification

Out in the tropics, Apocynaceae climbers are quite easy to distinguish from other families, with their opposite leaves and characteristic white latex. However, when it comes to determining an Apocynaceae specimen to genus or species level, identification gets more difficult. As flowering seasons of plants vary and make up a short time of the year, there is often only sterile material available. Due to great heights reached by the tallest climbers, it is sometimes even hard to get leaves. Moreover, there is a lack of identification tools for climbers – most identification keys available for tropical plants focus on trees and are based on reproductive traits. For example, the identification key for Apocynaceae in the *Flora of West Tropical Africa* (Hutchinson *et al*., 1963) covers climbers, yet the leads can only be followed if fruits and flowers are present. There are however, exceptional modern identification tools on tropical plants for some regions such as the book published by Hawthorne & Jongkind (2006). It allows identification by sterile characters and includes trees, shrubs and climbers.

1.3.2 Molecular identification: DNA barcoding

Beside morphological identification, molecular identification through DNA barcoding (see chapter 4.1.1) has been in the focus of plant researchers throughout the past 15 years. Promises of DNA barcoding include a fast and easy identification of species which can assist where morphological determinations are not possible. Yet, DNA barcoding is a comparatively young technique and the way of application is variable for different plant groups. Hence, a lot of studies nowadays focus on testing the usefulness of different barcode markers for specific genera or families of different floras. An elaborate DNA barcode study on tropical trees was published by Parmentier *et al.* (2013). Two further studies have focussed on the effectiveness of DNA barcoding for Apocynaceae (*e.g.* Cabelin & Alejandro, 2016; Selvaraj *et al*., 2015). However, no study has yet been conducted on tropical climbers.

1.4 Aims of the study

As only little research has been done on Apocynaceae climbers, this study will focus on increasing the knowledge of this plant group, and on facilitating their identification. The geographic area of interest is the Sangha Trinational in tropical Central Africa (see chapter 2.1.1), which has been intensively studied by Dr. David Harris over the past two decades.

The main objectives of this research are:

i) To assemble data and information on the Apocynaceae climbers in the Sangha Trinational.

ii) To assess which Apocynaceae climbers may be expected to occur in the Sangha Trinational, that have not yet been collected in the area.

iii) To provide an identification key for Apocynaceae climbers in the Sangha Trinational based on sterile characters.

iv) To make an assessment on the utility of *rbcL* as barcode marker for plants of the Sangha Trinational, and climbers in particular.

2 Assembling data and information on Apocynaceae climbers in the Sangha Trinational

2.1 Materials and Methods

2.1.1 Study area

The Sangha Trinational is a transnational protected area complex located in the north-western Congo Basin. It is called 'Trinational de la Sangha' (TNS) in French and includes three adjoining national parks: the Nouabalé-Ndoki National Park (Republic of the Congo), the Lobéké National Park (Cameroon) and the Dzanga-Ndoki National Park (Central African Republic) which in total comprise an area of 746,309 ha (UNESCO World Heritage Centre, 2020). For this research, the study site is defined by a rectangle (1.90 – 3.10 °N x 15.3 – 17.2 °E, in DD) covering the Sangha Trinational (Fig. 1).

The monthly average temperature in the study area varies between 25.4 °C (December) and 29.3 °C (April). The mean annual precipitation ranges from 1500 to 1600 mm, with the highest rainfall in September and October. The driest months are December, January and February with usually less than 50 mm rainfall per month (Harris, 2002).

Harris (2002) defined seven vegetation types present in the study site: (1) Mixed species *terra firma* forest, (2) *Gilbertiodendron dewevrei* forest, (3) Riparian forest, (4) Open swamp forest, including *Raphia* swamp, (5) Seasonally flooded forest along the Sangha River, (6) Cyperaceae dominated meadows along streams –"*bais*", (7) Savanna.

The Sangha Trinational was described as "one of the least well collected areas of Africa" by White (1979). However, the knowledge about the Sangha Trinational has increased since then: around 19,900 herbarium collections are recorded in the Sangha Trinational database and

various papers on plant families within the area have been published (*e.g.* Harris, 2002; Lachenaud & Harris, 2010; Ndolo Ebika *et al.*, 2018).

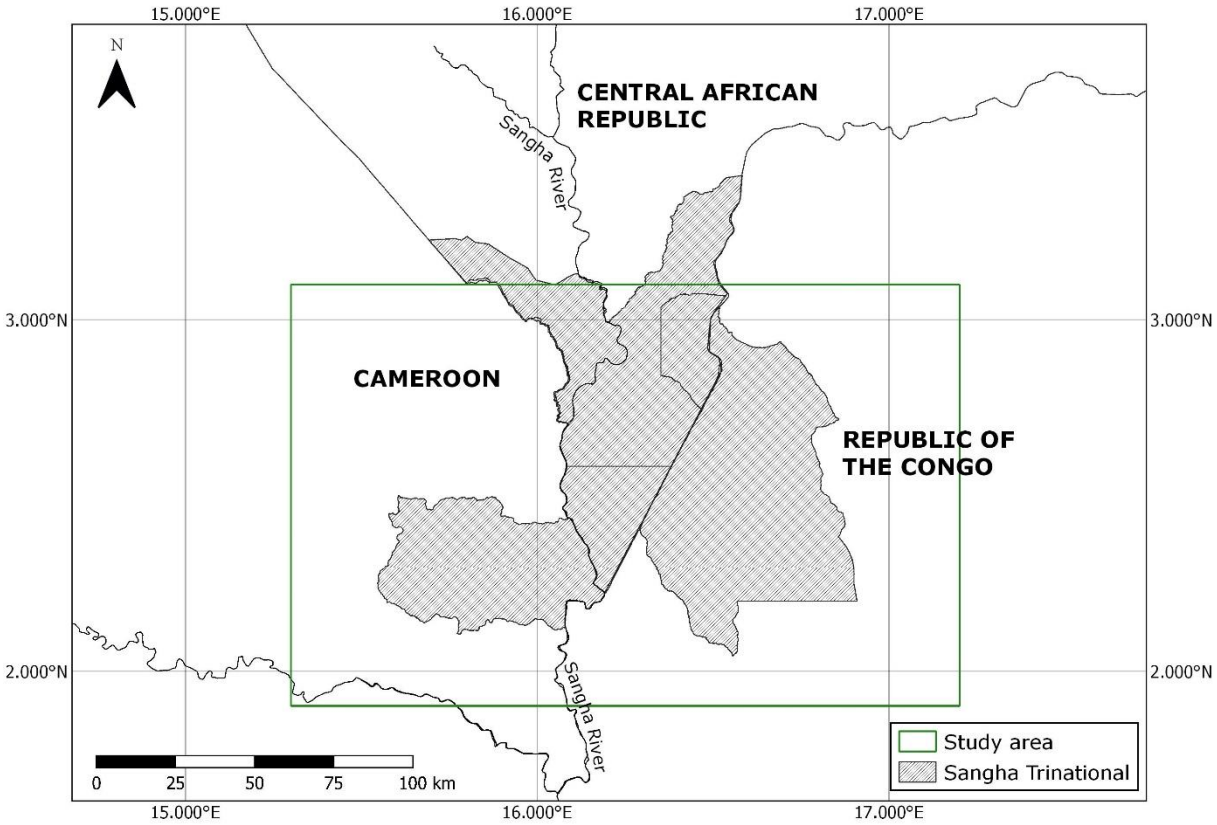


Figure 1. Study area in the Sangha Trinational.

2.1.2 Sangha Trinational dataset

In the period from 1971 to April 2019, principal collectors Moukassa, Nzolani Silaho, Harris, Ndolo Ebika, Medjibe, Fay, Carroll, Ndoundou Hockemba, Koni, Gentry, Remis, Goldsmith, Fangounda, Kuroda, Mbani, Wraber, Madzoké Bola, Iyenguet, Thomas, Schlott, Letouzey, Mbani, Kami and Schmidt collected 19,909 specimens of 149 vascular plant families as a botanical inventory of the Sangha Trinational. A large part of the collections made by Harris were sterile vouchers. All species collected were documented in a checklist by Harris (2002), each with specimen citations, and databased in the Botanical Research and Herbarium Management System (BRAHMS v7.9.14 available at https://herbaria.plants.ox.ac.uk/bol/). The database furthermore includes collections made after the publication of the checklist in 2002.

I primarily used the Sangha Trinational dataset to select study species, and to check the determination history of a voucher when necessary. The dataset contained 113 specimens of 47 species of Apocynaceae climbers in 28 genera. Of these, 42 species in 26 genera (102 specimens in total) have been collected within the study area and were thus selected as study taxa for this research (Table 1). Taxon names used in the Sangha Trinational dataset were searched for in

The Plant List (TPL) (http://www.theplantlist.org/) to check whether they were accepted names or synonyms.

Table 1. Apocynaceae climbers known from herbarium specimens collected in the Sangha Trinational. Data extracted from the Sangha Trinational dataset.

No.	Species	No.	Species
1.	*Alafia caudata*	22.	*Landolphia landolphioides*
2.	*Alafia multiflora*	23.	*Landolphia owariensis*
3.	*Ancylobothrys robusta*	24.	*Landolphia robustior*
4.	*Ancylobothrys scandens*	25.	*Landolphia villosa*
5.	*Anisopus efulensis*	26.	*Marsdenia magniflora*
6.	*Baissea axillaris*	27.	*Motandra guineensis*
7.	*Baissea gracillima*	28.	*Neoschumannia kamerunensis*
8.	*Baissea major*	29.	*Oncinotis glabrata*
9.	*Baissea multiflora*	30.	*Oncinotis gracilis*
10.	*Baissea subrufa*	31.	*Oncinotis hirta*
11.	*Batesanthus purpureus*	32.	*Oncinotis tenuiloba*
12.	*Clitandra cymulosa*	33.	*Orthopichonia barteri/O. schweinfurthii*[1]
13.	*Cryptolepis sanguinolenta*	34.	*Periploca nigrescens*
14.	*Cyclocotyla congolensis*	35.	*Pycnobotrya nitida*
15.	*Cylindropsis parvifolia*	36.	*Saba comorensis*
16.	*Cynanchum adalinae*	37.	*Strophanthus preussii*
17.	*Dictyophleba lucida*	38.	*Strophanthus sarmentosus*
18.	*Dictyophleba ochracea*	39.	*Strophanthus thollonii*
19.	*Gongronema latifolium*	40.	*Tabernaemontana eglandulosa*
20.	*Gymnema sylvestre*	41.	*Telosma africana*
21.	*Landolphia incerta*	42.	*Vahadenia laurentii*

Field photographs were available for five specimens of Apocynaceae climbers: *Secamone brevipes* (Moutsamboté 6082), *Tacazzea apiculata* (Kami 4372), *Tabernaemontana eglandulosa* (Harris 8576) and two unnamed specimens (Moukassa 4, Moutsamboté 6121). All these specimens were collected outside the study area. The small number of specimens with

1 Referring to specimen "Harris & Fay 1195" which is recorded as *O. barteri* and was later determined as *O. schweinfurthii*. The specimen could not be assigned to either species with certainty (see chapter 3.4.3). As a result, both species names are treated in the identification key and were scored separately during character investigation (see Appendix 2: Character spreadsheet).

photographs is due to difficult conditions in the field. For example, plant material was often out of reach in the tree canopy and hard to capture in a photograph.

Silica-gel dried leaves were sampled for 16 specimens of Apocynaceae climbers. Cambium in silica gel was sampled for four specimens (Table 2).

Table 2. Specimens of Apocynaceae climbers with silica-gel material in the Sangha Trinational dataset.

Specimen	Determination	Silica gel dried leaf	Cambium in silica gel
Harris 10255	*Landolphia*		x
Harris 10253	unknown		x
Harris 10273	unknown		x
Harris 10289	unknown		x
Harris 10193	*Cynanchum adalinae*	x	
Harris 10256	*Landolphia*	x (older leaf)	
Harris 10256	*Landolphia*	x	
Moutsamboté 6082	*Secamone brevipes*	x	
Harris 10272	*Strophanthus*	x	
Harris 10293	*Strophanthus*	x	
Harris 8576	*Tabernaemontana eglandulosa*	x	
Kami 4372	*Tacazzea apiculata*	x	
Moutsamboté 6121	unknown	x	
Harris 9847	unknown	x	
Harris 9858	unknown	x	
Harris 10199	unknown	x	
Harris 10205	unknown	x	
Harris 10228	unknown	x	
Harris 10244	unknown	x	
Harris 10251	unknown	x	
Harris 10291	unknown	x	

2.1.3 RAINBIO dataset

RAINBIO is a mega-database containing of 610,117 georeferenced records for vascular plants from sub-Saharan tropical Africa compiled from 13 public and non-public databases, including 3,571 herbarium specimen records collected from the Dzanga-Sangha region and collated by David J. Harris (Harris, 2002). The database covers 25,356 native tropical African vascular

plant species, representing 89 % of all known plant species in this area. For 91 % of the species within the RAINBIO database habit information is provided. Ten habit types are recognized: tree, shrub, shrublet, liana, vine, climber, epiphyte, herb, parasite and myco-heterotroph (Dauby *et al.*, 2016).

In this study, I used the RAINBIO database to validate the occurrence of the previously selected study taxa in the Sangha Trinational. Moreover, I used RAINBIO data to address the question, what species to expect in the study area that are not recorded in the list of species known to occur in the Sangha Trinational area, which includes specimens collected by Harris *et al*. (Harris, 2002). The RAINBIO dataset was filtered to Apocynaceae species with a climbing habit (climber, vine, liana), and with records in the three countries partly covered by the Sangha Trinational as well as in the Democratic Republic of the Congo (Congo-Kinshasa). The output was imported to BRAHMS and geographically filtered to a rectangle area including the Sangha Trinational and a buffer zone around it (0.30 – 4.45 °N x 13.2 – 19.0 °E, in DD) (Fig. 2). After filtering off all records from the Dzanga-Sangha database, the list of species occurring in the defined geographical area was compared with the list from the Sangha Trinational dataset. If a species was listed in both RAINBIO and the Sangha Trinational dataset, it was interpreted as a validation for the previous collections of Harris *et al*.

For the species which were listed in the Sangha Trinational dataset but did not appear in RAINBIO, possible explanations for their absence was assessed. To check if the species names may have changed, I checked the determination history of the specimens in the Sangha Trinational dataset in BRAHMS and looked up the specimens on Naturalis (https://bioportal.naturalis.nl) and Tropicos (https://www.tropicos.org/). If species had other records available in RAINBIO, the occurrences in Central Africa, Cameroon, Congo-Brazzaville and Congo-Kinshasa were mapped to assess their wider distribution and frequency. For species which did not have any records in these countries, the distribution was checked on the Global Biodiversity Information Facility portal (GBIF) (https://www.gbif.org/).

From the RAINBIO species output, 29 species which have not yet been recorded in the Dzanga-Sangha database were found to occur in the selected geographical rectangle. The species were mapped using DIVA-GIS v.7.5.0 (Hijmans *et al.*, 2012) to estimate whether they are likely to occur in the Sangha Trinational. A distribution in areas with rainforests similar to those in the Sangha Trinational was interpreted as an indication that the species may occur there as well. Also, occurrences near the Sangha Trinational were considered to show that the species might be expected in that area. Nineteen of the 29 species were found to be expected in the Sangha Trinational.

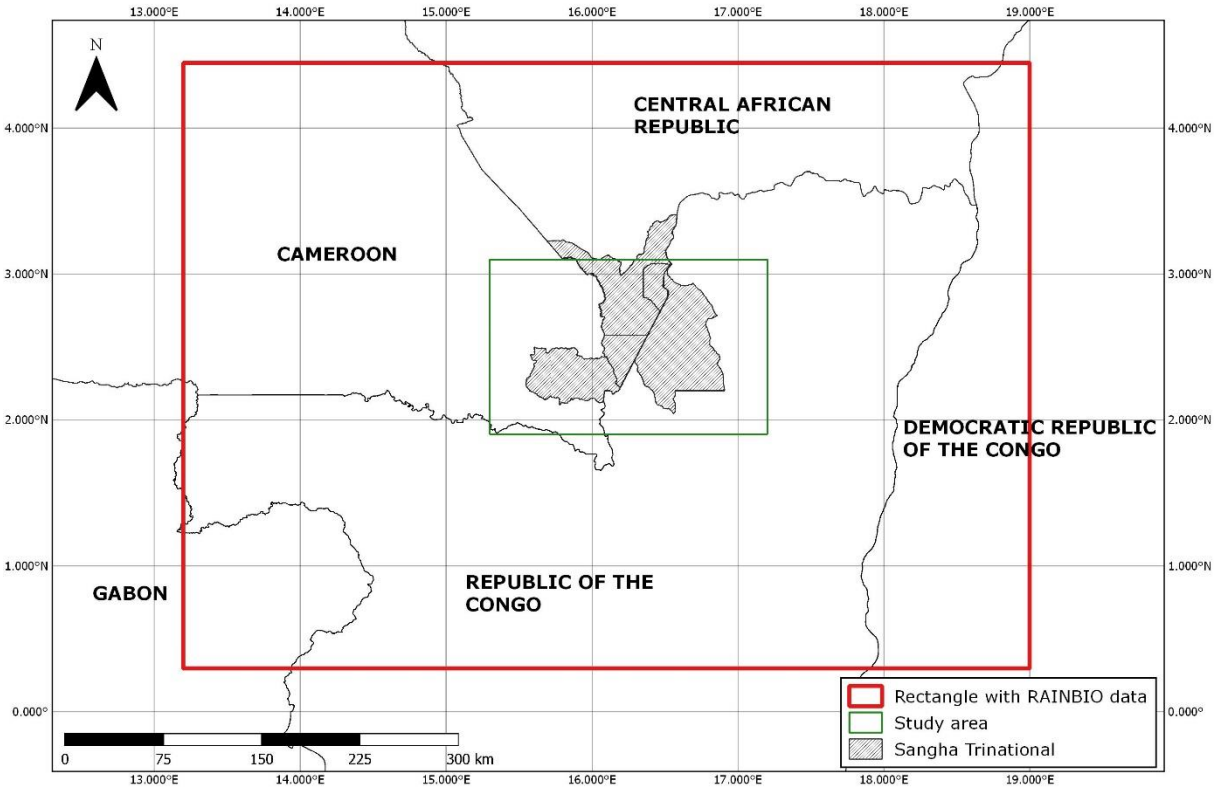


Figure 2. Geographic area used for examining RAINBIO occurrences, indicated by the red rectangle.

2.2 Results

2.2.1 Study species from the Sangha Trinational without occurrences in the RAINBIO dataset

As outlined in the methods (chapter 2.1.3), the RAINBIO database was used to validate the occurrence of the selected study species in the Sangha Trinational. Of the 42 species from the Sangha Trinational dataset, 32 were listed in the RAINBIO extract. The following 10 species did not have occurrences in the geographic area used for comparison: *Alafia caudata, Batesanthus purpureus, Dictyophleba lucida, Landolphia robustior, Landolphia villosa, Marsdenia magniflora, Neoschumannia kamerunensis, Oncinotis tenuiloba, Orthopichonia schweinfurthii, Telosma africana.*

Possible explanations for their absence from RAINBIO data in the defined rectangle are listed in Table 3. *Batesanthus purpureus, Landolphia villosa, Marsdenia magniflora, Neoschumannia kamerunensis* and *Orthopichonia schweinfurthii* seem to be rare or very rare species – across Africa and worldwide. Others are generally well collected in neighbouring countries to the study area (*e.g.* Democratic Republic of the Congo and Nigeria). Their absence in the RAINBIO extract could be explained by the fact that the other 12 institutions contributing to the RAINBIO

database do not have their research focus in and around the Sangha Trinational, and hence have fewer collections within this area.

Table 3. Apocynaceae climbers not occurring in the RAINBIO dataset within the geographic area used for validation of study species.

No.	Species	Discussion points
1.	*Alafia caudata*	Mapping all RAINBIO occurrences showed that the species is frequent in the Democratic Republic of the Congo. It was twice recorded in the south of Republic of the Congo and once in the south-west of Cameroon. Species does not seem to be rare. Four collections were made by Harris (2002) in Central African Republic, Sangha-Mbaéré. This shows that the RAINBIO examination area (red rectangle) is not very well collected. The Sangha Trinational is a well collected part within the area.
2.	*Batesanthus purpureus*	Mapping all RAINBIO occurrences of the species showed that it appears to be rare: only four occurrences were recorded in Cameroon (west and south), a country which has generally been well collected. Also, there was one record in the north-east of the Democratic Republic of the Congo.
3.	*Dictyophleba lucida*	No RAINBIO records in Cameroon, Central African Republic and Republic of the Congo. However, the species frequently occurs in the Democratic Republic of the Congo. This shows that the RAINBIO examination area is not very well collected.
4.	*Landolphia robustior*	Mapping all RAINBIO records showed frequent occurrence in the west of Cameroon and the Democratic Republic of the Congo; two occurrences were recorded in Republic of the Congo. This shows that the RAINBIO examination area is not very well collected
5.	*Landolphia villosa*	No RAINBIO records in Cameroon, Central African Republic and Republic of the Congo. Only five records across the Democratic Republic of the Congo. Species seems to be very rare, which may explain why it is not found in the RAINBIO examination area.
6.	*Marsdenia magniflora*	Only one RAINBIO occurrence listed for the species, recorded in the Democratic Republic of the Congo. Only five occurrences with coordinates are recorded across Africa on GBIF, two of which lie in Republic of the Congo and Democratic Republic of the Congo. The species appears to be very rarely collected

7.	*Neoschumannia kamerunensis*	RAINBIO database contains two occurrences of the species from Cameroon. As these are listed with the habit "tree", they did not appear in the RAINBIO subset I used for examination. Only six occurrences of the species are recorded worldwide on GBIF, two of which lie in west Cameroon, showing the rarity of the species. However, it was once collected in the Sangha Trinational ("Harris 4203").
8.	*Oncinotis tenuiloba*	Mapping all RAINBIO records of the species showed one occurrence in the west of Cameroon, and three records in the north of the Democratic Republic of the Congo. Its low number of occurrences in both countries, which are quite well collected, indicates that it is a rare species. Also, there is only one record of the species in the Sangha Trinational ("Harris 2889").
9.	*Orthopichonia schweinfurthii*	Mapping all RAINBIO records of the species showed three occurrences in the southwest of Cameroon and one record in the north of Central African Republic. The species seems to be rare, as is also shown with the few records held on GBIF. All but one occur in the Central African Republic and Cameroon. In contrast, *Orthopichonia barteri* occurs within the RAINBIO examination area. This might be an indication that the specimen held in the Sangha Trinational dataset ("Harris & Fay 1195") is indeed *O. barteri* as first determined by Harris.
10.	*Telosma africana*	Mapping all RAINBIO records of the species showed seven occurrences in Cameroon and one record at the southern border between Republic of the Congo and the Democratic Republic of the Congo. This shows that the RAINBIO examination area is not very well collected.

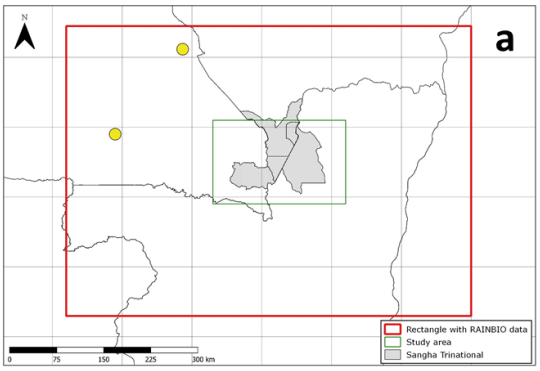
2.2.2 Expected species in the Sangha Trinational

The examination of RAINBIO occurrences revealed 19 species that may be expected in the Sangha Trinational but have not yet been collected in the area (Table 4). Their distribution within the defined geographic range is mapped in Fig. 3 a-s. In addition to assessing the proximity of records to the Sangha Trinational, the occurrence and abundance of records in similar vegetation types was evaluated.

The distribution maps of Fig. 3 show that the expected species are predominantly distributed in south-east Cameroon and in the south-west of the Central African Republic. For example, *Alafia lucida* (Fig. 3 c) and *Oncinotis pontyi* (Fig. 3 n) have been collected in south-east Cameroon six and four times, respectively, reaching the borders of the Sangha Trinational.

Table 4. Apocynaceae climbers to be expected in the Sangha Trinational. Data extracted from the RAINBIO dataset.

No.	Species	No.	Species
1.	*Alafia benthamii*	11.	*Landolphia foretiana*
2.	*Alafia erythrophthalma*	12.	*Landolphia mannii*
3.	*Alafia lucida*	13.	*Mondia whiteii*
4.	*Alafia schumannii*	14.	*Oncinotis pontyi*
5.	*Ancylobothrys amoena*	15.	*Secamone afzelii*
6.	*Anisopus mannii*	16.	*Strophanthus gratus*
7.	*Baissea leonensis*	17.	*Strophanthus hispidus*
8.	*Baissea welwitschii*	18.	*Tacazzea pedicellata*
9.	*Landolphia congolensis*	19.	*Tylophora sylvatica*
10.	*Landolphia dewevrei*		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Rectangle with RAINBIO data
Study area
Sangha Trinational
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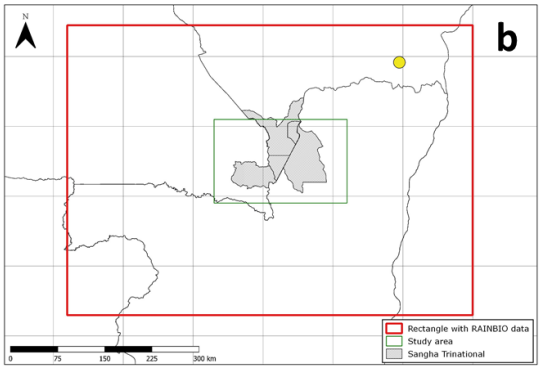
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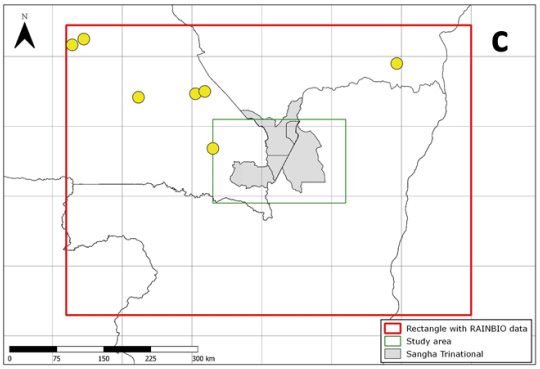
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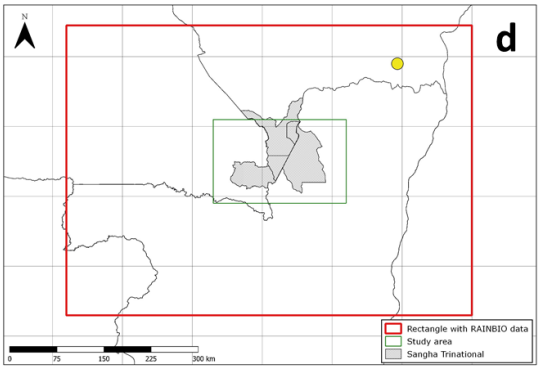
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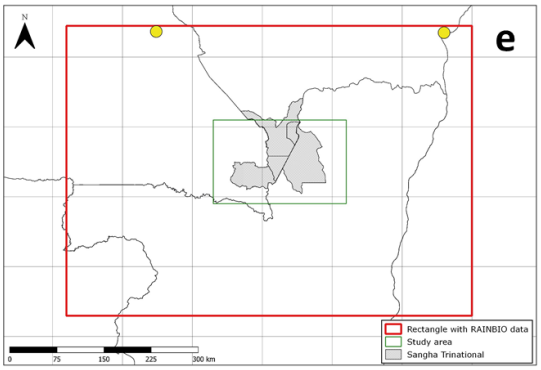
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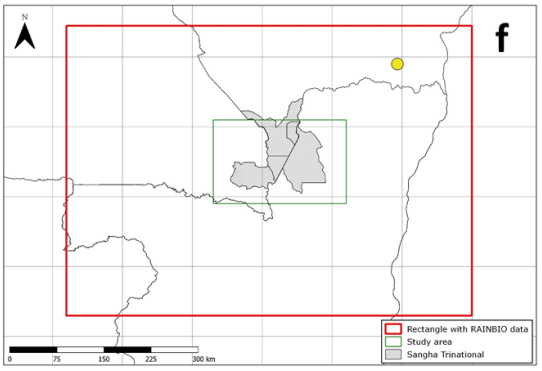
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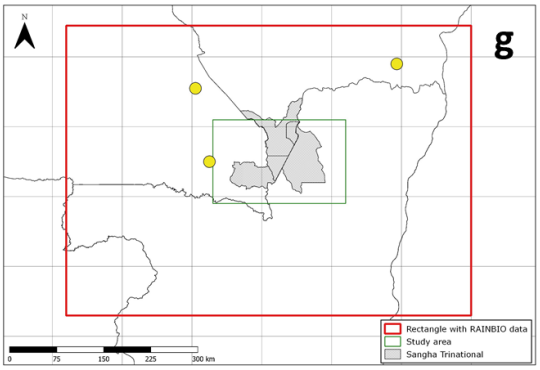
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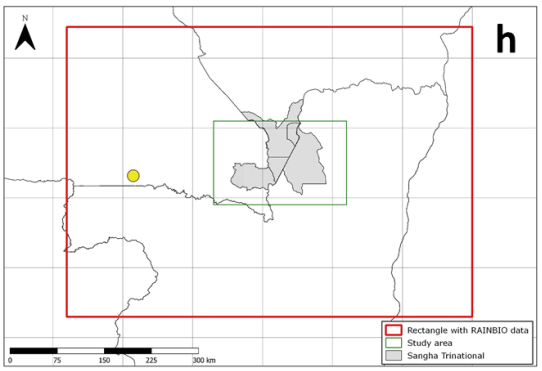
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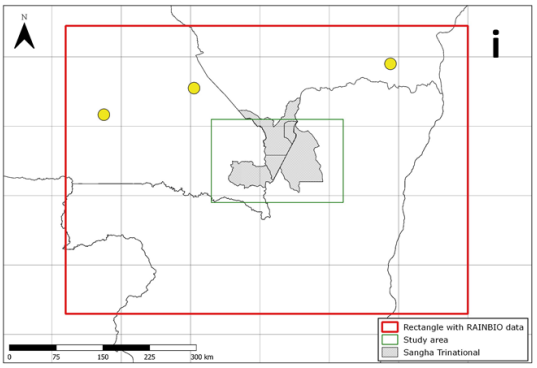
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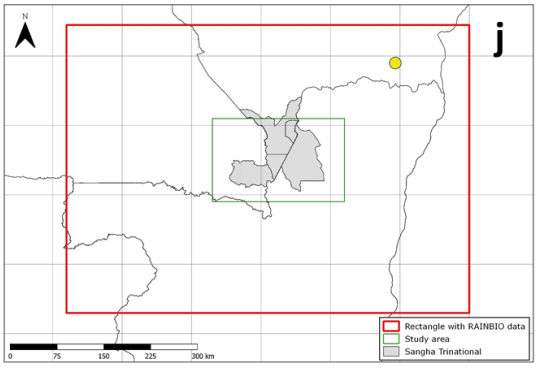
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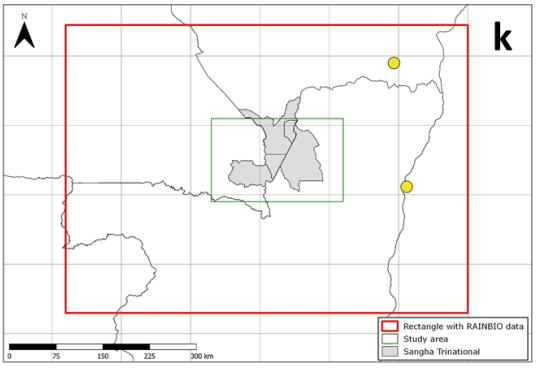
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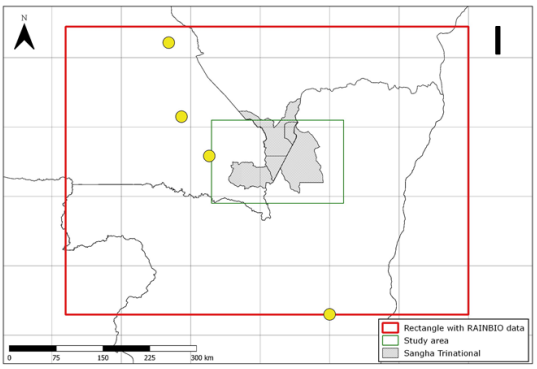
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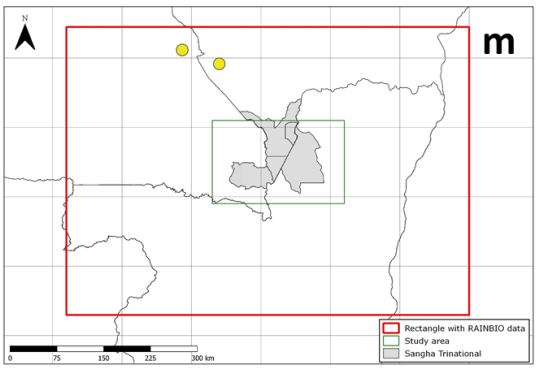
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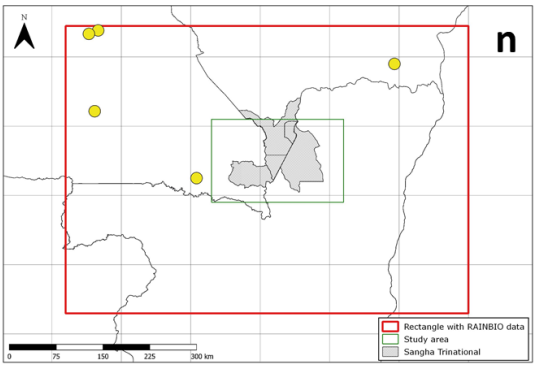
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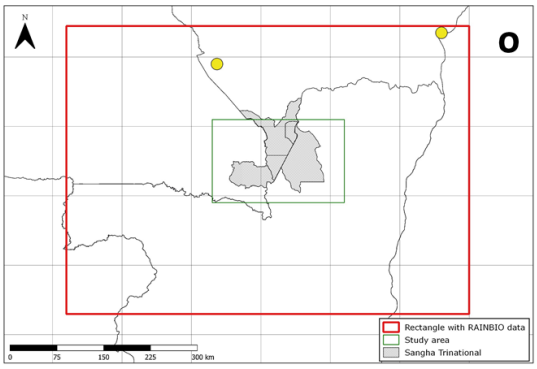
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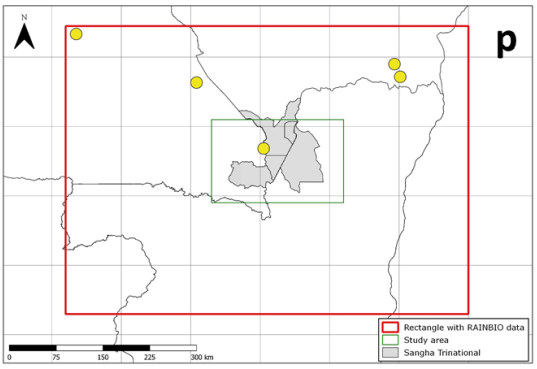
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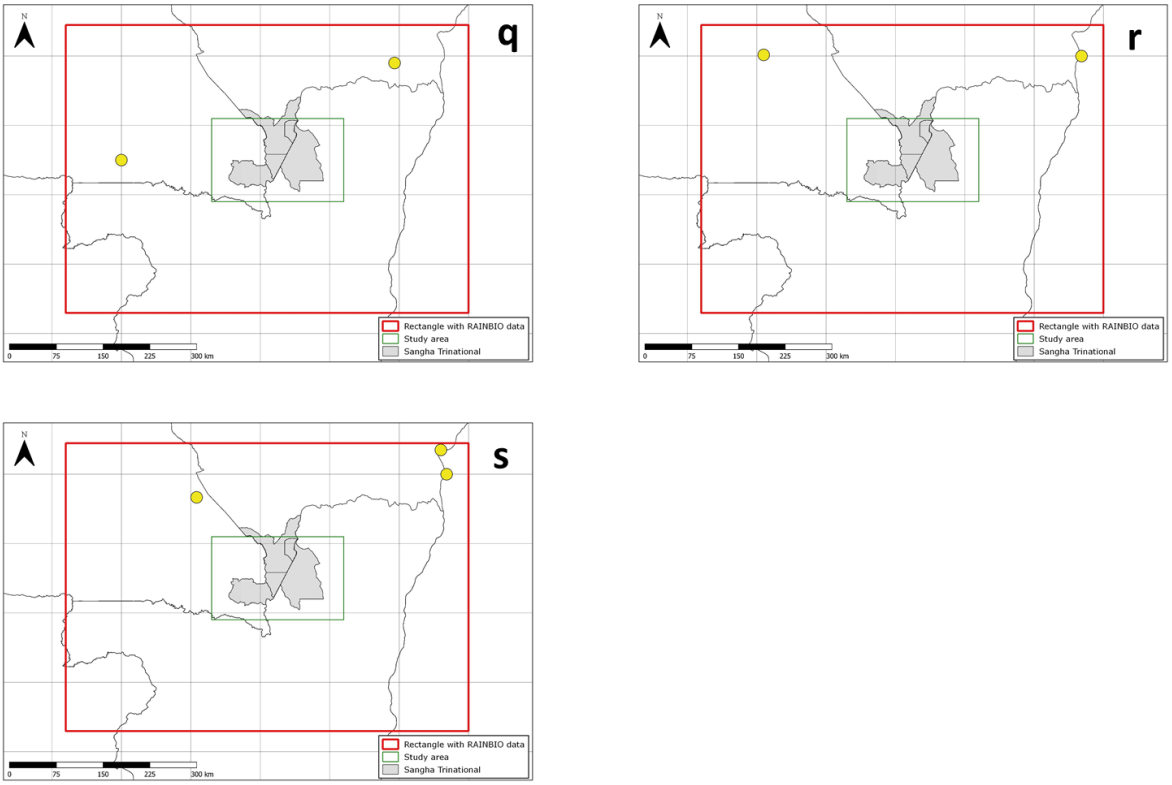


Figure 3. Occurrences of the 19 expected species within the geographic rectangle used to examine RAINBIO data, **a-s**, **a** *Alafia benthamii*, **b** *Alafia erythrophthalma*, **c** *Alafia lucida*, **d** *Alafia schumannii*, **e** *Ancylobothrys amoena*, **f** *Anisopus mannii*, **g** *Baissea leonensis*, **h** *Baissea welwitschii*, **i** *Landolphia congolensis*, **j** *Landolphia dewevrei*, **k** *Landolphia foretiana*, **l** *Landolphia mannii*, **m** *Mondia whiteii*, **n** *Oncinotis pontyi*, **o** *Secamone afzelii*, **p** *Strophanthus gratus*, **q** *Strophanthus hispidus*, **r** *Tacazzea pedicellata*, **s** *Tylophora sylvatica*.

2.3 Discussion

2.3.1 Input data

The Sangha Trinational dataset compiled data of Apocynaceae climbers with information on determination history and specimen status. The data was very valuable for this study, especially in terms of geographic data. It was found that some names in the database were not up to date. For example, one specimen was recorded under the name *Parquetina nigrescens*, which is regarded as a synonym of *Periploca nigrescens* and thus corrected to the accepted name. Also, the wrong spelling of the genus *Ancylobothrys* in the database and some other online databases – incl. the RAINBIO database – was detected and corrected (*Ancylobotrys* → *Ancylobothrys*).

The RAINBIO database currently is the only publicly available synoptic database of vascular plants in tropical Africa. It was informative by providing further reliable geographic records of the selected study species, and helpful for validating the occurrence of these species. Yet, it is to consider that despite the large amount of records compilated in the database, it does not depict the full distribution of the species across tropical Africa (as not all occurrences available

are held within RAINBIO). While checking species occurrences on GBIF, I noticed that GBIF holds species records which are missing in RAINBIO and vice versa.
Furthermore, it was found that some species names are recorded under their synonyms, as was the case for *Gymnema sylvestre* which was listed by the name of its synonym *Marsdenia sylvestris*. This requires additional caution when comparing the RAINBIO data with other datasets. A shortcoming of the database is that no information on voucher specimens of the records is given. This would have allowed to check images and obtain more collection data for further examination or comparison with own collections.

2.3.2 Selection of study species

It should be noted that one species (*Ancylobothrys amoena*) with a specimen collected very close to but outside of the defined geographical area was not included in the study species and is hence missing in the identification key. However, it is treated in the list of species expected to be found in the Sangha Trinational.

Validating the presence of study species by checking occurrence records within the RAINBIO database revealed that ten species have not been recorded within the area of examination. However, it was decided to still include them within this study for two reasons: First, the evaluation of possible reasons showed that five of the species appear rare from the low number of records across their whole range, which explains their absence from the RAINBIO data within the examined area. Second, the remaining five species have been well-collected in adjoining regions to the examined area. This implies that the examined area is a poorly collected region and does not depict actual species distributions.

2.3.3 Expected species in the Sangha Trinational

The “Guineo-Congolian regional centre of endemism” is one of 18 African phytochoria described by White (1983). It covers a large area of central African rain forest in north-east Gabon, south-east Cameroon, south-west Central African Republic and the north of Republic of the Congo. The prevailing vegetation type within the region is defined as the “mixed moist semi-evergreen rainforest” (White, 1983), and is also present in the Sangha Trinational (Harris, 2002). The majority of species in this type are widely distributed. Two further vegetation types in the phytochorion are found in the Sangha Trinational: “Single dominant moist evergreen and semi-evergreen Guineo-Congolian rain forest” and “Guineo-Congolian swamp forest and riparian forest” (White, 1983). The three subtypes reflect some of the seven vegetation types defined for the study area by Harris (2002) (see chapter 2.1.1), for example, “Seasonally flooded

forest along the Sangha River", "Mixed species *terra firma* forest" and "Riparian forest", where climbers are particularly are common (Harris, 2002).

The assessment of occurrence records of potential new species was based on the proximity of their occurrence, even from a single herbarium specimen, to the Sangha Trinational and the vegetation/forest type of the collection region. The results revealed 19 species which can be expected in the study area based on these factors. If they were to be actually found and collected, the recorded species number of Apocynaceae climbers in the Sangha Trinational would increase by almost half.

While the results can be used as a guideline for what to possibly look out for in the field and may extend options for species identification, they cannot be treated as valid predictions of species occurrences or populations. For that, other determining factors such as climate variables, soil type, competition, dispersal limitation and habitat fragmentation (McCune, 2016) had to be taken into account. Moreover, the estimations made were merely qualitative by using "expert opinion". Quantitative methods such as species distribution models (SDMs) were not applied. Depending on survey settings, these can provide strong predictions of species distribution by assessing present occurrences and suitable habitats (Elith & Leathwick, 2009).

On one hand, the RAINBIO data has ten species missing in the range of the study region, of which is known that they occur in the area (see chapters 2.1.2. and 2.2.1). On the other hand, analyses of RAINBIO records showed that another 19 species are likely to occur in the area but are missing in the Sangha Trinational dataset. Using both datasets allowed for a better interpretation of what should be expected in the study. If only one dataset had been consulted, the picture of present and expected species would have been less complete and less nuanced.

3 Morphological identification

3.1 Introduction

A morphological investigation is carried out for the 42 species of Apocynaceae climbers in the Sangha Trinational (chapter 2.1.2) using digital herbarium specimens. Based on sterile characters, an identification key to the species is created.

3.2 Materials and Methods

3.2.1 Herbarium specimens studied

Digital specimen images for examination were obtained from the Global Plants database on JSTOR (https://plants.jstor.org/), the Global Biodiversity Information Facility portal (GBIF)

(https://www.gbif.org/), and the online herbarium databases of Naturalis (https://bioportal.naturalis.nl/) and Meise Botanic Garden (http://www.botanicalcollections.be/#/en/home). Photographs examined were either from GBIF or from photographs taken by Harris *et al.* associated with specimen collections. A total of 312 digital herbarium specimens were studied for all species. A list of all specimens examined can be found in Appendix 1. Measurements were taken using the measuring tool in the image viewer of Global Plants on JSTOR.

3.2.2 Character examination

A first set of vegetative characters for examination was selected by assessing which characters are visible on digital specimen images of three genera selected for a preliminary investigation. Further characters were added after consulting literature (Endress *et al.*, 2018; Hawthorne & Jongkind, 2006; Hutchinson *et al.*, 1963) and checking for commonly used characters. Distinctive characters were primarily found in the identification guide by Hawthorne & Jongkind (2006) which contains a key to Apocynaceae species of Western African forests which is largely based on sterile characters. A list of examined characters is found in Table 5.

Data from examined specimens were recorded in an Excel spreadsheet (Appendix 2) where they were readily comparable. The terminology in the character descriptions is based on *Plant Identification Terminology: An Illustrated Glossary* (Harris & Woolf Harris, 2001).

Table 5. Qualitative and quantitative characters scored from digital herbarium specimens.

Character	
Branch/Branchlet pubescence	Leaf size
Lenticels (presence/absence)	Leaf base
Internode length	Leaf apex
Nodal scars (presence/absence)	Leaf acumen length
Tendrils (presence/absence)	Leaf margin
Stipules (presence/absence)	Leaf pubescence
Stipule position	Leaf texture
Stipule shape	Midrib channel
Petiole length	Midrib elevation
Petiole pubescence	Number of lateral vein pairs
Petiole channel	Vein pubescence
Ochrea	Tertiary venation pattern
Colleters	Leaf domatia (presence/absence
Leaf shape	Leaf domatia type

3.2.3 Key building

The key to the species was prepared using characters from the species descriptions. I chose those characters which are most distinctive to split the species. In addition, I considered characters which are mostly available and visible in the field to make the key useful to field botanists. Species were grouped according to diagnostic characters, similar to the grouping used by Hawthorne & Jongkind (2006). The key was tested using digitised and herbarium specimens at Royal Botanic Garden Edinburgh (RBGE).

3.3 Results

3.3.1 Characters examined

The morphological analysis resulted into six major morphological species groups. Indumentum, branch lenticels, number of lateral vein pairs and leaf domatia were found to be the most significant characters for creating species clusters. Characters which could not be consistently or reliably scored were not included in the key. For *Neoschumannia kamerunensis* no specimen images were available on JSTOR Global Plants, so that no measurements could be recorded for this species. Measurements used in the key were taken from the descriptions in the most recent taxonomic treatment of the species (Meve, 1995) and the Flora of West Tropical Africa (Hutchinson *et al.*, 1963).

Each morphological group is defined by a combination of characters specific for the group. Unique to the species of group 1 is the presence of domatia in the axils of the leaf nerves. The type of domatia are tuft domatia in all species (cf. Fig. 11), with the exception of *Oncinotis glabrata* which has pit domatia (Fig. 12). Species in this group never have tendrils. The tertiary venation is often transverse scalariform and colleters are often present. The group comprises nine species in three genera.

Species in group 2 are without tendrils and domatia. Their petioles have ochrea at the base or colleters in the axils. The group consists of five species in two genera.

Group 3 consists of species with more than 20 pairs of closely spaced transverse lateral veins (cf. Fig. 10). The group comprises four species in three genera.

Group 4 is characterized by species which lack domatia and have long or dense hairs on branches and/or petioles. They often have tendrils. The group comprises nine species in six genera.

Species in group 5 do not fit into groups 1-4. They are characterized by large cordate leaves with strong basal nerves which are particularly distinct (Fig. 4) and not present in any of the

other groups. The species sometimes have stipules. The group consists of five species in five genera.

Group 6 includes species which do not exhibit any of the character combinations present in groups 1-5. The species are difficult to identify without referring to the previous groups, as they are not united by the presence of specific characters. They never have domatia. The group consists of eleven species in nine genera.

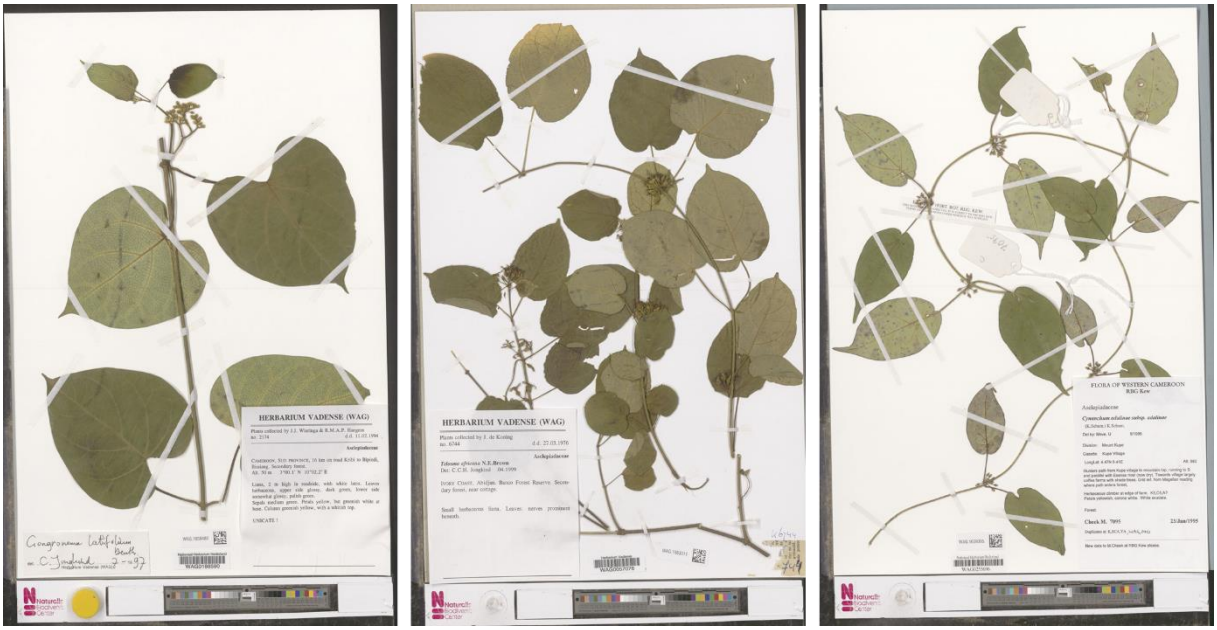


Figure 4. Large cordate leaves in *Gongronema latifolium* (left), *Telosma africana* (middle), *Cynanchum adalinae* (right). Left: Wieringa, Haegens 2174 (WAG), middle: de Koning 6744 (WAG), right: Cheek, Mensley, Simon, Daniel, Enokpa, Ameka, Buillard 7095 (WAG), accessed on *Naturalis* https://bioportal.naturalis.nl/.

Branches/branchlets

Generally, branches and branchlets were easy to describe from digital specimens. However, on some specimen images with low resolution and quality, it was difficult to distinguish lenticels from bark texture and to assess the degree of pubescence. Moreover, nodal scars on branches were not clearly visible on specimen images. Despite their importance given in literature (Hawthorne & Jongkind, 2006), they were disregarded as useful character in the key, as the interpretation of what a nodal scar looks like could lead to confusion when using the key. Internode length could be quantified reliably but did not show significant differences between species. Branch pubescence and the presence of lenticels were found to be important characters for distinguishing between species and hence used for grouping species in the identification key (*e.g.* lenticels in *Strophanthus*, cf. Fig. 5).

Figure 5. Corky branch lenticels in *Strophanthus preussii* (top), *S. thollonii* (middle), *S. sarmentosus* (bottom). Top: Welwitsch 5996 (COI), middle: Latilo 1 (FHI), bottom: Smeathman s.n. (BM), accessed on *JSTOR Global Plants* https://plants.jstor.org/.

Tendrils

For all species supposed to have tendrils according to literature, tendril-like structures could be observed on specimen images. However, these structures were highly variable between species in terms of growth origin and extent (cf. Fig. 6), which made it difficult to obtain a consistent impression of how tendrils in Apocynaceae climbers look like. In *Vahadenia laurentii*, for example, it was hard to tell whether the tendrils are short in length or were truncated during collection and mounting. Nonetheless, as tendril-like structures were present in 17 of 43 species, they were considered as a discerning character and used to cluster species into groups.

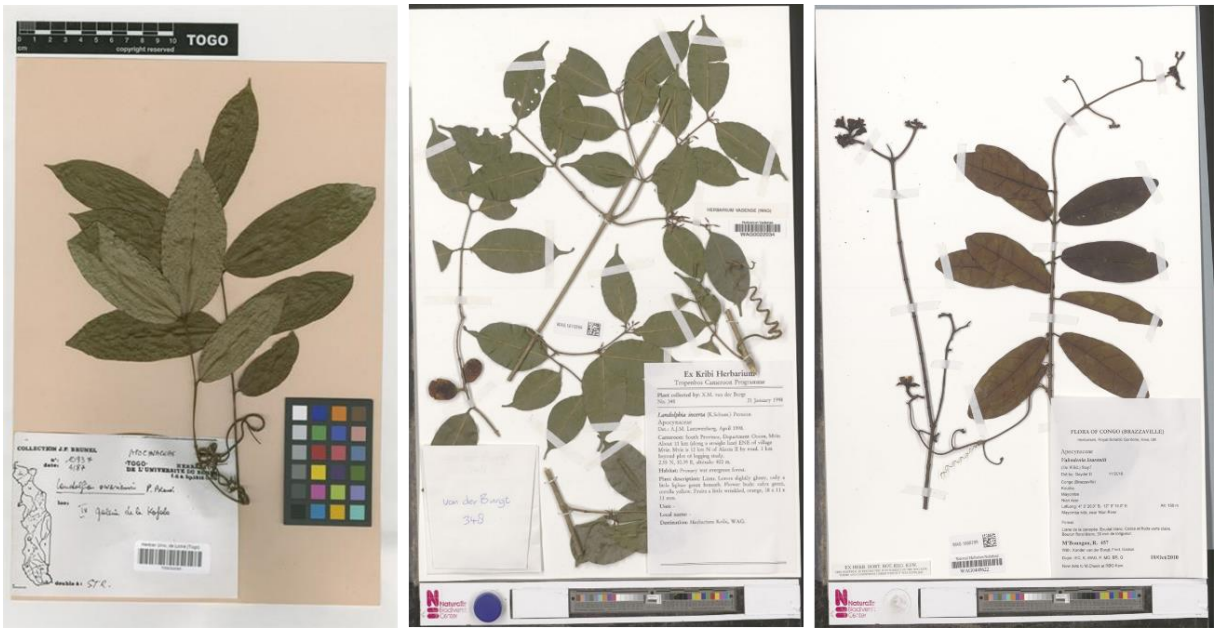


Figure 6. Tendril-like structures on *Landolphia owariensis* (left), *Landolphia incerta* (middle), V*ahadenia laurentii* (right). Left: Brunel 10937 (TOGO), accessed on *JSTOR Global Plants* https://plants.jstor.org/; middle: van der Burgt 348 (WAG), right: M'Boungou, van der Burgt, Gislain 457 (WAG), accessed on *Naturalis* https://bioportal.naturalis.nl/.

Stipules

According to literature, seven species were supposed to have stipules. For three of them (*Dictyophleba ochracea*, *Landolphia incerta* and *Neoschumannia kamerunensis*) I could not or only hardly recognize stipules on the specimens. Hence, stipules were only used as discerning character in the key where they were always absent, or to distinguish species with clearly visible stipules (cf. Fig. 7) from species with no stipules (*e.g. Dictyophleba lucida* from *Landolphia villosa*).

Figure 7. Interpetiolar stipules on *Dictyophleba lucida*. Gossweiler 6262 (COI), accessed on *JSTOR Global Plants* https://plants.jstor.org/.

Leaves

Leaf size was not useful to create large species clusters, but to split species into smaller subsets. Hence, leaf length and width were either used together or singly to create couplets within the key. Leaf shape was generally found to be uninformative, as it was scored on a range between elliptic and obovate for most species. However, variations were recognized when comparing two or three species grouped together in the key, so that leaf shape was included within descriptions to key out single species. Leaf base and apex were variable among species and recognized to be significant characters in telling species apart. Acumen length was used as backing character in some cases. Leaf margin was not significant for clustering species as it was invariably scored as 'entire', with the exception of four species with a slightly revolute margin (cf. Fig. 8). In these cases, however, it was used as descriptive supporting character in the key. Leaf texture was particularly useful for differentiating species with coriaceous leaves, such as *Strophanthus preussii*, *S. sarmentosus* var. *sarmentosus* and *S. thollonii*.

Leaf pubescence and glands were helpful characters in creating subgroups within the key or used as support in descriptions to key out single species as was the case for petiole length, pubescence, channel and ochrea. Colleters were hardly visible on specimen images, however they were included in the descriptions of Group 1 and 2, as Hawthorne & Jongkind (2006) rely on them as helpful character for differentiating these species groups.

Figure 8. Slightly revolute leaf margins in *Oncinotis gracilis* (top) and *O. tenuiloba* (bottom). Top: Millen 106 (K), bottom: Swynnerton 87 (SRGH), accessed on *JSTOR Global Plants* https://plants.jstor.org/.

The number of lateral vein pairs was found to be a particularly significant character in discerning the species. The number varied from 3-5 pairs of laterals (cf. Fig. 9) to >30 pairs (cf. Fig. 10). The midrib channel was considered as supporting character for distinguishing species. However, the width (broadly vs. narrowly channelled) could not be determined reliably on the specimen images due to poor resolution in many cases, so that it was not included as significant trait. Pubescent veins were infrequent among the species and therefore included as discerning character in the key. The presence of leaf domatia was very distinctive and rare (cf. Fig. 11-12), and therefore used as a trait to delimit the first species group.

Figure 9. Leaves of *Oncinotis tenuiloba* with up to five pairs of lateral veins. Reprinted from GBIF.org, by Troos van der Merwe, accessed on June 9, 2020 via https://www.gbif.org/occurrence/2423161709.

Figure 10. Leaf of *Pycnobotrya nitida* with many finely closely spaced lateral veins. Mann 1809 (P), accessed on *JSTOR Global Plants* https://plants.jstor.org/.

Figure 11. Tuft domatia in *Baissea gracillima*. Devred 677 (IUK), accessed on *JSTOR Global Plants* https://plants.jstor.org.

Figure 12. Pit domatia in *Oncinotis glabrata*. Welwitsch 5978 (LISU), accessed on *JSTOR Global Plants* https://plants.jstor.org.

3.3.2 Key to Apocynaceae climbers in the Sangha Trinational

An identification key to 43 species of Apocynaceae climbers in the Sangha Trinational is provided. It is based on sterile characters and divided into six morphological groups, each with a description of common characters. Each group contains a discrete, indented dichotomous key.

The key is best used by first checking which group description matches the specimen or plant at hand, starting with Group 1 and proceeding sequentially. The key under the respective group can then be used to identify the material to species level.

Group	Description
Group 1	Apocynaceae climbers **with domatia**, often with transverse 3° scalariform venation; without tendrils; often with colleters
Group 2	Apocynaceae climbers without domatia; no tendrils; petioles with **‚ochrea‘** (small structures at base which protect developing leaves in bud), or colleters in the axils
Group 3	Apocynaceae climbers with **>20 pairs of fine, closely spaced** (1-3 mm) transverse **lateral veins**; without domatia
Group 4	Apocynaceae climbers with **long or dense hairs on branches and/or petioles**, often with tendrils; without domatia
Group 5	Apocynaceae climbers not in Groups 1-4; **leaves becoming cordate at least on larger leaves**, sometimes with strong basal nerves; sometimes with stipules; without domatia
Group 6	Apocynaceae climbers different to those above; without domatia

Group 1 – Apocynaceae climbers **with domatia**, often with transverse 3° scalariform venation; without tendrils; often with colleters

Key to Species

1a. Leaves with pit domatia; leaf base rounded

2a. Branches glabrous, sometimes with brown lenticels; leaves elliptic to ovate, apex apiculate or acuminate; petioles 9-20 mm; 6-12 pairs of lateral veins..……..***Oncinotis glabrata***

1b. Leaves with tuft domatia; leaf base cuneate or rounded, sometimes cordate

2a. Petioles not channelled

3a. Tertiary venation scalariform, branches mostly without lenticels, petioles without glands

4a. Leaves ovate to obovate or oblong, base rounded to cuneate, apex acuminate; lateral veins glabrous; branches usually glabrous, sometimes sparsely pubescent, with lenticels.……..***Baissea gracillima***

4b. Leaves obovate or elliptic, sometimes orbicular to rotund when young, base rounded or subcordate, apex rounded or acuminate; lateral veins sometimes minutely pubescent below; branches pubescent, without lenticels…….***Baissea major***

4c. Leaves ovate to oblong, base cordate to rounded, apex obtuse or acute, sometimes mucronate; lateral veins glabrous; branches densely pubescent when young, without lenticels……..…….***Baissea axillaris***

3b. Tertiary venation reticulate; branches with lenticels, petioles with glands on lower side

4a. Leaves elliptic or obovate-oblong, base cordate or rounded, apex caudate, margin not revolute; branches with small orange-brown lenticels……....***Motandra guineensis***

4b. Leaves elliptic to obovate, base cuneate or rounded, apex acuminate, margin sometimes slightly revolute; branches sometimes with large lenticels….***Oncinotis gracilis***

2b. Petioles channelled

3a. Petioles with two pairs of glands on the lower side

4a. Branches glabrous, sometimes shortly pubescent; leaves obovate to oblanceolate, base cuneate, apex acuminate, margin sometimes slightly revolute, petioles pubescent……..……...***Oncinotis tenuiloba***

3b. Petioles without glands

4a. Lateral veins not arcuate; leaves elliptic to ovate or obovate, base rounded to truncate, apex acuminate, tuft domatia yellow; petioles pubescent.....***Baissea multiflora***

4b. Lateral veins arcuate; leaves elliptic or oblanceolate to oblong, base cuneate to sometimes rounded, apex acute to acuminate; tuft domatia brown; petioles very shortly pubescent to glabrous…….…….....……***Baissea subrufa***

Group 2 – Apocynaceae climbers without domatia; no tendrils; **petioles with ‚ochrea‘** (small structures at base which protect developing leaves in bud), **or colleters in the axils**

Key to Species

2a. Branches with corky lenticels; ochrea generally divided into two points pointing away from twigs at base of petiole; tertiary venation ± inconspicuous

2a. Branches glabrous; petioles with ochrea at the base; 3-14 pairs of lateral veins, laterals glabrous; tertiary venation inconspicuous.…….....***Strophanthus preussii***

2b. Branches glabrous, sometimes minutely pubescent with long hairs; petioles with ochrea at the base; 3-7 pairs of laterals veins, laterals glabrous, sometimes finely pubescent below; tertiary venation visible, reticulate below…….....***Strophanthus sarmentosus var. sarmentosus***

2c. Branches glabrous; petioles with colleters in the axils; 4-7 pairs of lateral veins, laterals glabrous; tertiary venation inconspicuous…..……..……..…....***Strophanthus thollonii***

2b. Lenticels on branches not corky, pale brown; no translucent dots on leaves; ochrea different; tertiary venation conspicuous

2a. Leaves often >10 cm long, >5 cm wide, base rounded to cordate, apex apiculate; petioles 7-13 mm long…….……...…..***Alafia multiflora***

2b. Leaves <10 cm long; <5 cm wide, base cuneate, apex caudate, sometimes acuminate; petioles 1-4 mm long……...……..……***Alafia caudata***

Group 3 – Apocynaceae climbers with **>20 pairs of fine, closely spaced (1-3 mm) transverse lateral veins;** without domatia

Key to Species

1a. Petioles channelled; with tendrils

2a. Leaves elliptic, base cuneate, apex apiculate with acumen c. 5-8 mm;. 35-50 pairs of lateral veins…….....…….….***Orthopichonia barteri***

2b. Leaves elliptic or ovate, base cuneate, leaf apex obtuse to apiculate with acumen c. 2-6 mm; >20 (c. 25-45) pairs of lateral veins…..……..***Orthopichonia schweinfurthii***

1b. Petioles not channelled; without tendrils

2a. Leaves elliptic to obovate, base cuneate, apex cuspidate; 20-40 pairs of lateral veins; branches with lenticels…….……....***Cyclocotyla congolensis***

2b. Leaves elliptic-oblong to oblanceolate, base cuneate, apex apiculate, lower leaf side with many black dots; numerous (>50) pairs of lateral veins; branches without lenticels.....…***Pycnobotrya nitida***

Group 4 – Apocynaceae climbers with **long or dense hairs on branches and/or petioles**, often with tendrils; without domatia

Key to Species

1a. Leaves not cordate, plant with dense hairs, without long spreading hairs

2a. Tertiary venation conspicuously reticulate; midrib glabrous below

3a. 9-20 pairs of lateral veins; branches densely pubescent with very short hairs; leaves glabrous; petioles 3-8 mm, pubescent....…….……***Ancylobothrys scandens***

3b. 7-13 pairs of lateral veins; branches very shortly pubescent; leaves glabrous; petioles 9-12 mm, shortly pubescent……..…..……***Ancylobothrys robusta***

2b. Tertiary venation reticulate, but not very conspicuous; midrib pubescent below

3a. Petioles pubescent; midrib finely pubescent below; 8-14 pairs of lateral veins, laterals pubescent below..…….……..***Landolphia owariensis***

3b. Petioles densely pubescent; midrib prominent and densely pubescent below; c. 5-8 pairs of lateral veins, laterals densely pubescent below…….……...…..***Oncinotis hirta***

1b. Leaf base cordate, or hairs on branches often long and spreading

2a. Branches finely pubescent or glabrous; petioles usually <13 mm

3a. Branches usually glabrous; interpetiolar stipules triangular; leaves glabrous and shiny, base cordate; petioles densely pubescent; tertiary venation conspicuously reticulate...…….……..…***Dictyophleba lucida***

3b. Branches finely pubescent with long hairs; leaves ciliate, base cuneate or rounded; petioles sparsely pubescent; tertiary venation scalariform to reticulate.....***Landolphia villosa***

2b. Branches densely pubescent; petioles up to 2.1 cm

3a. Leaves usually >15 cm long; stipules present

4a. Interpetiolar stipules triangular; leaves 15-21 cm, glabrous, base subcordate to rounded; apex acuminate or cuspidate; petioles 0.9-2 cm; 6-10 pairs of lateral veins……..…..……..…...….***Dictyophleba ochracea***

3b. Leaves <15 cm long; stipules absent

4a. Leaves 1-13 cm long, glabrous, base cordate, cuneate, or rounded, apex acuminate; 3-4 pairs of lateral veins.….....…...***Gymnema sylvestre***

4b. Leaves 4.5-12.5 cm long, finely pubescent on both sides, base cordate, apex caudate; 4-15 pairs of conspicuous lateral veins.....***Marsdenia magniflora***

Group 5 – Apocynaceae climbers not in Groups 1-4; **<u>leaves becoming cordate at least on larger leaves</u>**, sometimes with strong basal nerves; sometimes with stipules; without domatia

Key to Species

1a. Interpetiolar stipules present

2a. Leaf base cordate, apex acuminate to caudate; 6 pairs of lateral vein; tertiary venation scalariform……***Batesanthus purpureus***

1b. No stipules

2a. More than 4 pairs of lateral veins

3a. Leaves elliptic to oblong or ovate, base rounded to cordate, apex acuminate to cuspidate; petioles 2-5.5 mm; 6-10 pairs of lateral veins; drying black…***Periploca nigrescens***

3b. Leaves broadly ovate to orbicular, sometimes ovate-oblong, base cordate to truncate or cuneate; apex cuspidate; petioles 1.-2.5 mm, 4-6 pairs of lateral veins.....***Telosma africana***

2b. 3-4 pairs of lateral veins

3a. Petioles 3.8-7 cm; leaves 6-11.5 cm, ovate to cordate, base deeply cordate to subcordate; lower side with black gland dots near base; venation palmate……..***Gongronema latifolium***

3b. Petioles 0.5-3.5 cm; leaves 3.5-5 cm, ovate, base deeply cordate; 3-4 pairs of lateral veins, venation pinnate……..……...***Cynanchum adalinae***

Group 6 – Apocynaceae climbers different to those above; without domatia

Key to Species

1a. Leaves with 5-25 pairs of lateral veins

2a. Leaf apex caudate

3a. Leaves base cuneate; apex caudate with acumen 0.8-2 cm; petioles not channelled; 7-13 pairs of lateral veins……..…….……***Cylindropsis parvifolia***

3b. Leaf base cuneate to rounded; apex caudate with acumen 0.3-1.2 cm; petioles channelled; c. 10-25 pairs of lateral veins……...***Landolphia incerta***

2b. Leaf apex not caudate

3a. Leaf base cuneate; apex apiculate; lower side with black gland dots; petioles not channelled; (6)9-14 pairs of lateral veins; tertiary venation reticulate…..***Clitandra cymulosa***

3b. Leaf base rounded to cuneate; apex rounded, obtuse or acuminate; petioles not channelled; (6)7-14 pairs of lateral veins; tertiary venation scalariform-reticulate……***Saba comorensis***

3c. Leaf base cuneate; apex acuminate; petioles channelled; 8-16 pairs of lateral veins; tertiary venation reticulate…….……..***Landolphia landolphioides***

3d. Leaf base subcordate, or cuneate to rounded; apex obtuse to rounded or apiculate; petioles not channelled; 5-12 pairs of lateral veins; tertiary venation reticulate…….....***Vahadenia laurentii***

1b. Leaves with 3-8 pairs of lateral veins

2a. Branches with lenticels

3a. Internodes c. 10 cm long

4a. Leaf apex acuminate; 4-7 pairs of lateral veins, petioles 1-2.5 cm, glabrous; internodes c. 10 cm long, without tendrils…...……...…***Neoschumannia kamerunensis***

3b. Internodes < 10 cm long

4a. Leaf apex acuminate; 3-6 pairs of lateral veins, petioles 5-8 mm, glabrous; without tendrils……..……...***Cryptolepis sanguinolenta***

4b. Leaf apex caudate; 5-8 pairs of lateral veins, petioles 4-18 mm, glabrous; with tendrils…..……..…….……...…...……***Landolphia robustior***

2b. Branches without lenticels

3a. Leaves obovate to oval, base rounded to cuneate; apex acuminate to cuspidate, lower side without dots; usually 3, sometimes 4 pairs of conspicuous lateral veins; with tendrils……...***Anisopus efulensis***

3b. Leaves elliptic; base cuneate; apex acuminate, lower side with black dots; 3-6 pairs of lateral veins; tertiary venation inconspicuous; without tendrils.……...…….…...***Tabernaemontana eglandulosa***

3.3.3 Sample species descriptions of *Saba comorensis* and *Oncinotis tenuiloba*

A complete taxonomic account would usually include detailed descriptions for each species after the identification key. As no proper taxonomic revision was conducted, I deliberately refrained from including descriptions of all species at this point. However, to demonstrate how these would look like, two sample species descriptions including taxonomic citations, description of sterile characters and distribution maps are provided. *Saba comorensis* depicts a widely distributed species across Africa, whereas *Oncinotis tenuiloba* is an example for a restricted species in this region.

Information about distribution, ecology, fruiting and flowering time, and altitude refers to the whole distribution range and was obtained from Plants of the World Online (POWO, 2019), Leeuwenberg & van Dilst (1989) and De Kruif (1985).

1. ***Saba comorensis*** (Bojer ex A. DC.) Pichon, Mem. Inst. Franç. Afrique Noire 35: 303 (1953).
Homotypic synonyms: *Vahea comorensis* Bojer ex A. DC., Prodr. [A. P. de Candolle] 8: 328 (1844); *Landolphia comorensis* (Bojer ex A. DC.) K. Schum., Bot. Jahrb. Syst. 15: 402 (1892). **Type:** Mem. Inst. Franç. Afrique Noire 35: 303 (1953), Comoros, *Bojer* s.n. (Isotype: K!; Barcode K000233567)
Heterotypic synonyms: *Landolphia florida* Benth., Niger Fl. [W. J. Hooker]: 444. (1849); *Vahea florida* (Benth.) F. Muell., Extra-trop. Pl. Indian ed.: 344 (1880); *Pacouria florida* (Benth.) Hiern, Cat. Afr. Pl. (Hiern) i. 662 (1898); *Saba florida* (Benth.) Bullock, Kew Bull. 13(3): 391 (1959); *Willughbeia cordata* Klotzsch, Naturw. Reise Mossambique [Peters] 6(Bot., 1): 283 (1861); *Landolphia cordata* (Klotzsch) K. Schum., Bot. Jahrb. Syst. 15: 406 (1892); *Landolphia dubia* Lassia, Mascarenh. & Landolph. Madag.: 76 (1927); *Pacouria dubia* (Lassia) Pichon, Mém. Mus. Hist. Nat., Paris n.s., 24: 144 (1948); *Pacouria comorensis* (Bojer ex A. DC.) Roberty, Bull. Inst. Franç. Afrique Noire 15: 1427 (1953); *Landolphia mayottensis* Pierre ex Poiss., Rech. Fl. Mérid. Madagascar: 162 (1912).

   Key Reference: Leeuwenberg, A. J. M., & van Dilst, F. J. H. (1989). Saba (Pichon) Pichon Series of Revisions of Apocynaceae XXVII. Bulletin Du Jardin Botanique National de Belgique / Bulletin van de National Plantentuin van België, 59(1/2), 189.

   Illustrations: Leeuwenberg, A. J. M., & van Dilst, F. J. H. (1989). Fig. 1-2, pp. 191,193.

   Liana. Branches glabrous, with lenticels, internodes 2.7-8.5 cm; tendrils present. Leaves elliptic to ovate, sometimes narrowly elliptic, 7.7-16 X 3.8-7.5 cm, base rounded to cuneate, apex rounded, obtuse, sometimes acuminate, margin entire, glabrous on both sides; veins arching (brochidodromous), 6-13 pairs of lateral veins, tertiary venation scalariform-reticulate, domatia absent; petioles 0.8-1.4 cm, glabrous.

   Flowering time: throughout the year (from Leeuwenberg & van Dilst, 1989).
   Fruiting time: throughout the year (from Leeuwenberg & van Dilst, 1989).
   Ecology: In forests, forest edges, open woodland and near rivers.
   Altitudinal range: 0-2000 m.
   Distribution: Tropical Africa, from Senegal to Madagascar and Ethiopia to Zimbabwe (from Leeuwenberg & van Dilst, 1989).

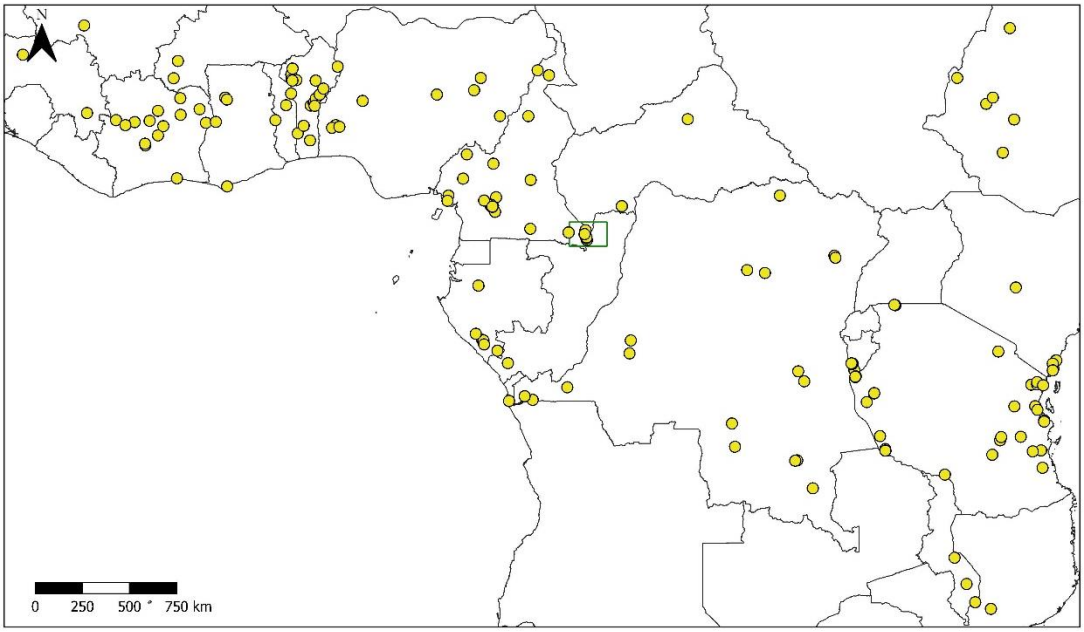


Figure 13. Distribution of *Saba comorensis* across sub-Saharan tropical Africa. Data extracted from the RAINBIO database.

Specimens examined:
ANGOLA: Cuanza Norte, Dalatando – Cazengo: Gossweiler 10296 (COI), accessed on *JSTOR Global Plants*.
CAMEROON: Rives du Dja, Mintom: Letouzey 11742 (WAG), accessed on *BioPortal Naturalis*; North Region: de Wilde, de Wilde-Duyfjes 3163 (WAG), accessed on *BioPortal Naturalis*.
CENTRAL AFRICAN REPUBLIC: Lobaye, Boukoko: Tisserant (Équipe) 1294 (WAG), accessed on *BioPortal Naturalis.*
COMOROS: insula Juarne Archipel, Comorensis: Bojer, s.n., accessed on *JSTOR Global Plants*.
DEMOCRATIC REPUBLIC OF THE CONGO: Katanga (Shaba), Vallée Lofeï (aval des chutes): Bodenghien 103 (WAG), accessed on *BioPortal Naturalis*; Ref. du Zaire (Shaba): Schaijes 3125 (BR), accessed on *Meise Botanic Garden, BR Herbarium Catalogue*.
NIGERIA: Niger Expedition: Vogel 101 (K), accessed on *JSTOR Global Plants*.
TANZANIA: Kilimanjaro, Namvi river valley, South Kilimanjaro, Hai district: Hemp 5068 (UBT), accessed on *JSTOR Global Plants*; Kilimanjaro, Karanga river: Hemp 4593 (UBT), accessed on *JSTOR Global Plants*.

REPUBLIC OF THE CONGO: Sangha: Village Bomassa: Ndolo Ebika: 929 (E)

2. ***Oncinotis tenuiloba*** Stapf, Bull. Misc. Inform. Kew 1898(143): 307 (1898).
Heterotypic synonyms: *Oncinotis inandensis* J. M. Wood & M. S. Evans, J. Bot. 37: 254 (1899); *Oncinotis chirindica* S. Moore, J. Linn. Soc., Bot. 40: 141 (1911); *Oncinotis natalensis* Stapf, Bull. Misc. Inform. Kew 1907: 52 (1907); *Motandra erlangeri* K. Schum. ex Engl., Sitzungsber. Preuss. Akad. Wiss. 1906, 742 (1906), nom. illeg.; *Motandra erlangeri* K. Schum., Bot. Jahrb. Syst. 33: 318 (1903).

Key Reference: De Kruif, A. P. M. (1985). A revision of Oncinotis Benth., series of revisions of Apocynaceae XVI. Wageningen Agricultural University Papers, 85(2), 5–45.

Type: *Oncinotis tenuiloba* Stapf Bull. Misc. Inform. Kew 1898(143): 307 (1898), Democratic Republic of the Congo, Likasa (Holotype: BR!; Barcode: BR0000008860095).

Climber. Branches glabrous, sometimes shortly pubescent, with lenticels, internodes 1.4-6.5 cm. Leaves obovate to oblanceolate, 5-12.1 X 2-4.5 cm, base cuneate, apex acuminate with acumen 4.9-11.5 mm, margin entire, sometimes slightly revolute, glabrous on both sides, midrib channelled above, 3-5 pairs of lateral veins, tertiary venation scalariform-reticulate, tuft domatia present; petioles 5.2-8 mm, finely pubescent, channelled.

Flowering time: towards the end of the dry and the beginning of the rainy seasons (from De Kruif, 1985); Sep-Oct.
Fruiting time: dry seasons (from De Kruif, 1985).
Ecology: Rain forests, swamp forests, riverine forests and secondary forests, on sand, clay and rocky outcrops (from De Kruif, 1985).
Altitudinal range: 0-1800 m (from De Kruif, 1985).
Distribution: Africa, from Western Nigeria to Ethiopia to Eastern South Africa (from De Kruif, 1985).

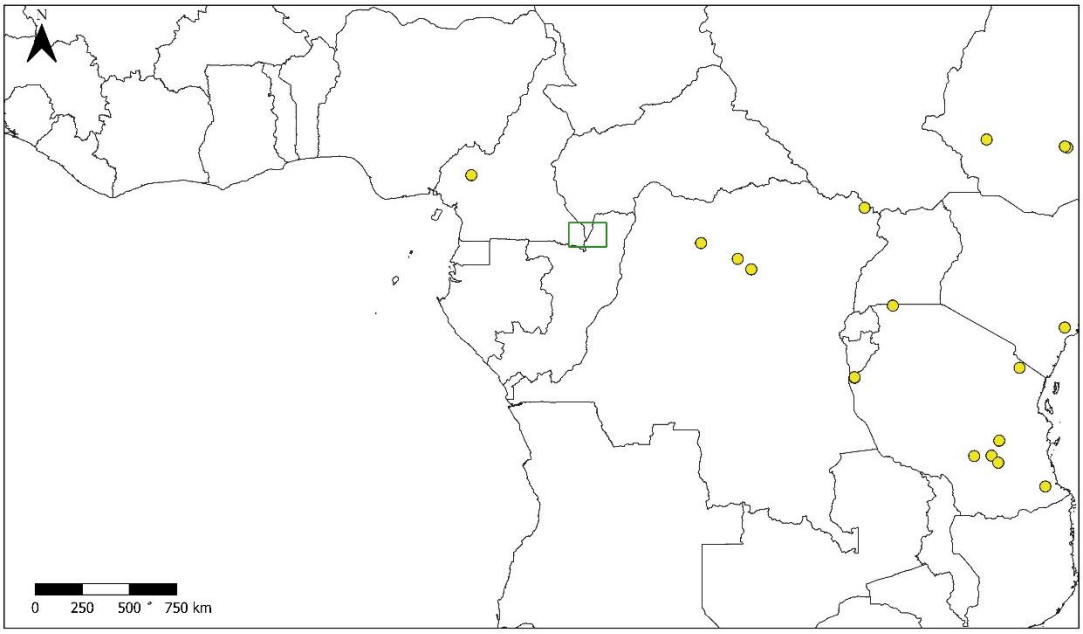


Figure 14. Distribution of *Oncinotis tenuiloba* across sub Saharan tropical Africa. Data extracted from the RAINBIO database.

Specimens examined:
CAMEROON: West Region, Bafoussam: Jacques-Félix 3017 (WAG), accessed on *BioPortal Naturalis*.
CENTRAL AFRICAN REPUBLIC: Sangha-Mbaéré: Harris 2889 (WAG), accessed on *BioPortal Naturalis*.
DEMOCRATIC REPUBLIC OF THE CONGO: locality unknown: Louis 9207 (IUK), accessed on *JSTOR Global Plants*; Ubundu: Lejoly 2933 (BR), accessed on *Meise Botanic Garden, BR Herbarium Catalogue*.
SOUTH AFRICA: Natal, Inanda: Wood 1009 (MO), accessed on *JSTOR Global Plants*.
TANZANIA: Kilimanjaro, Msaranga-Valley, Moshi District: Hemp 786 (UBT), accessed on *JSTOR Global Plants*.
ZIMBABWE: Chirinda Forest, Swynnerton 87 (SRGH), accessed on *JSTOR Global Plants*.

3.4 Discussion

3.4.1 Character scoring

Due to lack of access to herbaria, this project made use of digital specimen images to examine morphological characters of the study species. One the one hand, this allowed for a greater range of specimens for observation. On the other hand, observing specimens virtually came with some shortcomings: The majority of images were of high quality, so that most characters could be recognized easily. However, on images with poor resolution, certain traits could not be recognized with certainty as it was difficult to distinguish them from surrounding tissue (*e.g.* stipules). Also, small characters which would usually be observed using a hand lens (*e.g.* indumentum and domatia) were generally tricky to see, especially in cases where they were not very distinct. Thus, I could not score all characters to the desired standard, so that the results should be viewed as preliminary species descriptions which need to be confirmed or revised after conducting a more precise character investigation using real specimens. However, I attempted to score characters in as much detail as was possible given the circumstances. For each species, I thoroughly studied species descriptions in revisions and identification keys and selected a variety of specimens for examination to ensure a certain level of within species variation.

Despite the detailed observations, I did not develop a sense of "knowing" the specimens while scoring the characters, which I consider to be vital for taxonomic work. This may have been due to missing physical proximity, *i.e.* not being able to see three dimensional specimens laid out next to each other for comparison, or simply due to the high number of species studied.

The measuring tool on JSTOR Global Plants allowed to calibrate the ruler before measuring, so that accurate and reliable measurements could be taken. Unfortunately, other virtual herbaria used in this study did not incorporate measuring tools. It must therefore be considered that traits were only quantified on specimens available on JSTOR, which were type specimens in most cases.

3.4.2 Identification key

The first step of creating the identification key was to decide which kind of key should be used. Initially, I prepared a single-entry, bracketed dichotomous key including all 43 species. As recommended by Sosef *et al.* (2020), each couplet divided the remaining species into two groups of almost equal size. I soon found that the key was inadequate to address the aim of facilitating the identification of Apocynaceae climbers in the field: First, the key was confusing and hard to follow due to the large number of species. Second, it was difficult to trace back which species

were morphologically similar as they did not appear close to each other in the key structure. Third, and most importantly, there was a high potential of getting stuck in a dead end if the characters described in a couplet were not visible, not clearly identifiable or missing on the unknown specimen/species. Although the latter issue is not unusual when using identification keys (Brncic, 2007), I found that it could render the key useless, as the couplets did not lead to taxonomic groups, but to morphological clusters only. A key that led to genera first and then included a key to species within that genus could provide some orientation, even if characters in a couplet were disputable. However, because this study included 26 genera – most of them with one species only – this option was not considered to be suitable.

The second form of dichotomous key is the so-called indented key. In contrast to a bracketed key where both leads of a couplet are directly listed one after another, an indented key separates the two leads in space. Also, there are no numbers at the end of a couplet which direct to the next question. Instead, one follows the next question immediately below the lead that is correct (Sosef *et al.*, 2020). This has the advantage, that one does not need to look far for the next question or flip pages several times to continue identification.

The key prepared in this study is an indented key and follows the structure of the identification key used by Hawthorne & Jongkind (2006) for Apocynaceae in west Africa (see chapters 3.2.3 and 3.3.2). The grouping into morphological clusters allows to – more or less quickly – check the species at hand for a combination of characters and proceed if these are not present. This narrows down the choices of which subkey to use and may facilitate and speed up identification in the field. Additionally, in cases where a plant cannot be identified with certainty, it is helpful that similar species occur together in the key. In this way, a small selection of species can be shortlisted, and species descriptions checked afterwards to complete identification.

I chose the sets of characters defining the morphological groups by modifying the descriptions used by Hawthorne & Jongkind (2006), meaning I removed, added and adjusted traits. I considered this approach appropriate, because it based on a published key that made species level identifications without fertile characters possible and has proved to be successful in the field, even in other regions of Africa (Brncic, 2007; Harris, 2007; Van Staden, 2007). The key includes characters I could not recognize on the specimens, but which were previously found to be distinctive characters by different authors. For example, colleters and ochrea are used in the description of Group 1 and/or Group 2. As Hawthorne & Jongkind (2006) use them as major discerning characters, it is hoped that these characters are field characters and observable with

the bare eye or using a hand lens. Nevertheless, it should be noted, that the distinctness of these characters was not verified in this study.

A weak point of the key is that the placement of *Baissea subrufa* under Group 1 – "Leaves with tuft domatia" was not made with certainty. Neither could I identify from the specimens whether the domatia observed were pit or tuft domatia nor did the species description in the most recent revision (van Dilst, 1995) provide information regarding the type of domatia. Hence, there is potential for confusion if the domatia in *Baissea subrufa* are recognized as pit domatia by the identifier. However, due to the similarity with the other species in the genus, which have tuft domatia, I decided to place it within this group.

Another issue that came up while preparing the key, was the definition of tendrils. As already outlined in the results (see chapter 3.3.1) there is no consensus of what defines a tendril in Apocynaceae climbers. Furthermore, Dr. David Harris, who is a well-experienced field botanist in tropical forests, stated that he has barely seen tendrils attached to or associated with Apocynaceae climbers while being out in the field. This raised the questions whether tendrils will be useful as descriptive character in the identification key. Nevertheless, I decided to mention tendrils as supportive character in the key for three main reasons. First, I could clearly recognize "tendrils" or tendril-like structures on specimens (cf. Fig. 6), hence disregarding them would not reflect my observations. Second, tendrils have been found to be specific for certain species, so in case they are observed at an unknown species, this excludes other species and narrows down the possibilities for identification. Third, I found that including tendrils in the key may get people to look for the character and may animate the discussion of what a tendril is.

From people testing the key, I received feedback saying that the species boundaries for *Baissea gracillima*, *B. major* and *B. axillaris* in Group 1 (lead 1b-3a) were not clear enough. A suggestion was to break the three choices down into two couplets using indumentum as discerning character. Also, it was suggested to split the species *Clitandra cymulosa*, *Saba comorensis*, *Landolphia landolphioides* and *Vahadenia laurentii* in Group 6 (lead 1a-2b) into couplets. However, due to the use of digital specimen images, strong discerning characters between the species could not be found. Both parts of the key would be the first to address when revising it with the use of herbarium specimens.

3.4.3 Problematic names

In the Sangha Trinational dataset, the specimen "Harris & Fay 1195" is recorded under the species name *Orthopichonia barteri* and was determined as *Orthopichonia schweinfurthii* by

Leeuwenberg in 1992. Yet, Harris (2002) cites the specimen under the name *Orthopichonia barteri* in his checklist. On Naturalis and Tropicos, however, the specimen is listed as *Orthopichonia schweinfurthii*. Due to this ambiguity, I tried to identify which species it is by using identification keys, species descriptions and comparing it to other specimens of both species. In the most recent revision of the genus (Vonk, 1989), I found following characters to separate the two species from each other:

(1) "blade leathery when dried" (*O. schweinfurthii*) vs. "blade papery or subcoriaceous when dried" (*O. barteri*)

(2) "branchlets smooth, glabrous" (*O. schweinfurthii*) vs. "branchlets often densely pubescent at the nodes" (*O. barteri*)

Unfortunately, it was difficult to confidently make out the blade texture from digital images. In addition, I could not see "densely pubescent nodes" on any specimens of *Orthopichonia barteri,* so that this character was also not helpful for identifying the specimen. Lastly, other visible characters were overlapping and very similar in both species, so that I could not assign specimen "Harris & Fay 1195" to either species with certainty.

Further problematic names were *Periploca nigrescens* and *Parquetina nigrescens*. The Plant List (TPL) lists *Periploca nigrescens* as accepted species with *Parquetina nigrescens* being its synonym. On Plants of the World (POWO), however, both names are listed as synonyms of *Cryptolepis nigrescens,* which in turn is not recorded in The Plant List at all. The confusion about the names sometimes made it difficult to be sure online information about the species are not misleading.

4 The potential of DNA barcoding for identification

4.1 Introduction

4.1.1 The concept of DNA barcoding

In 2003, Hebert *et al.* (2003) coined the term of DNA barcoding, which is the use of short standardized DNA regions for species identification (CBOL Plant Working Group, 2009; Hebert & Gregory, 2005) and discovery (Hebert *et al.*, 2016). The term 'DNA barcode' is often explained by comparing the DNA sequence of an organism to a barcode found on a supermarket product, which is unique for that product and distinguishes it from other items. Generating barcode sequences only requires a small sample of plant material for DNA extraction, such as silica gel dried leaves or leaves from dried herbarium specimens. To identify an unknown organism, the barcode sequence is queried against a reference database containing other sequences to find

potential matches (Sosef *et al.*, 2020). DNA barcoding is considered a comparatively fast and easy way of species identification which overcomes problems of morphological identification, such as damaged or incomplete specimens and the scarcity of taxonomic specialists (CBOL Plant Working Group, 2009; Kress & Erickson, 2012).

Nevertheless, DNA barcoding is recognized as a support of taxonomy rather than a replacement of taxonomic work. It provides indications for unknown organisms and has the ability to enhance taxonomic hypotheses and phylogenies (Hebert & Gregory, 2005; Vijayan & Tsou, 2010).

The Consortium for the Barcode of Life (CBOL) is an international initiative including more than 130 organizations from 45 countries. It was established in 2004 and aims at developing DNA barcoding as a global standard for species identification. It is further dedicated to developing a DNA barcode library for all multicellular life (http://www.ibol.org/). For this purpose, the Barcode of Life Data System (BOLD) was launched in 2005. It currently contains records for more than 100,000 species (Ratnasingham & Hebert, 2007).

4.1.2 Use of DNA barcoding for plant identification

An ideal barcode should be applicable for a wide range of taxa (universal) and easy to apply (*e.g.* in the lab). Furthermore, the interspecific variation of the barcoding region should be higher than the variation within species to allow effective discrimination of taxa. This is referred to as the "barcode gap". In addition, it should be possible to sequence the ideal gene region by using a single primer pair and only performing little manual editing of the sequence traces (Vijayan & Tsou, 2010).

The mitochondrial *Cytochrome c oxidase I* (COI) is the preferred standardized sequencing region for animals (Hebert *et al.*, 2003). For plants, COI is not effective due to low substitution rates of the plant mitochondrial DNA (CBOL Plant Working Group, 2009). As alternative barcode for plants, the CBOL Plant Working Group (2009) proposed parts of the two plastid DNA regions *rbcL* and *matK*. It was later suggested to add the non-coding intergenic spacer *trnH-psbA* and the internal transcribed spacers (ITS) of the nuclear ribosomal DNA to achieve a better resolution of species discrimination (Hollingsworth, 2011; Hollingsworth *et al.*, 2011; Vijayan & Tsou, 2010). At present, most specimen-based barcoding studies use a combination of several barcode loci.

DNA barcodes are applied in a wide range of fields in plant sciences, such as plant systematics, ecology, evolutionary biology, conservation and forensics. Especially in the tropics, the use of

DNA barcoding can speed up the identification of numerous unknown plants and expand the knowledge of understudied taxa and species interactions (Kress, 2017).

4.1.3 DNA barcoding in Apocynaceae

The original project outline intended to conduct a DNA barcode study with a selection of African Apocynaceae climbers using *rbcL* and at least one other barcode marker – for instance *matK* or ITS2, which were both suggested as best barcode for the family by different authors (Cabelin & Alejandro, 2016; Selvaraj *et al.*, 2015). It would have investigated how previously created morphological species clusters correspond to molecular groups. Also, the usefulness of the selected barcode markers within this difficult group would have been assessed. However, due to restricted lab access as a result of COVID-19 and distance learning, this was no longer possible. The molecular part of this project was hence changed to an examination of already sequenced material of a wider range of tropical species, as will be explained below. Nevertheless, a short overview of the application of DNA barcodes in Apocynaceae is given.

DNA barcoding has been used in several studies of Apocynaceae. For example, Khanum *et al.* (2016) sequenced the whole plastid genomes of species of the genus *Cynanchum* for the purpose of a revision. A recent molecular phylogenetic study of Apocynaceae presented a well-supported and better resolved phylogeny of the family, using the largest number of species and molecular data analysed and sampled up until then (Fishbein *et al.*, 2018). Their results provide evidence that a large sequence reference database of plastid and complete plastome sequences with discriminative ability is available on GenBank. The number of sequences available in the nucleotide database on GenBank is relevant as the sequence data often serves as reference dataset for barcode studies.

A search on GenBank showed that 1,479 *rbcL* sequences are available for Apocynaceae in the nucleotide database (August 16, 2020, search: “ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit“ and “Apocynaceae”). Of these, 105 *rbcL* sequences are from specimens collected in Africa (https://www.ncbi.nlm.nih.gov/). As of August 19, 2020, 24 genera of Apocynaceae climbers present and expected to occur in the Sangha Trinational (chapters 2.2.1 and 2.2.2) are represented with *rbcL* sequences on GenBank.

Selvaraj *et al.* (2015) evaluated six DNA loci (*matK*, *rbcL*, *atpB*, *rpoC1*, *psbA-trnH* and ITS) for their ability to identify Apocynaceae species and genera. The study revealed nuclear ITS2 as the marker with the highest discriminative power – and hence ideal DNA barcode – in this family. A nucleotide search on GenBank revealed that 2,839 ITS2 sequences and 2,132 ITS

sequences of Apocynaceae are available in the database (August 16, 2020, search for ITS2: “Internal transcribed spacer 2“ and “Apocynaceae”; search for ITS: “Internal transcribed spacer 1“ and “Apocynaceae”). Of these, 352 ITS2 sequences and 410 ITS sequences are from specimens collected in Africa.

Another study tested the efficiency of *matK*, *rbcL*, *trnH-psbA* and *trnL-F* as barcodes to identify ethnomedicinal Apocynaceae species (Cabelin & Alejandro, 2016). Here, *matK* was suggested as the best barcode for the family, as it showed high interspecific variance and very low intraspecific variance compared to other markers. 1,960 accessions of *matK* sequences of Apocynaceae were found on GenBank (August 16, 2020, search: “maturase K“ and “Apocynaceae”). Of these, 86 sequences are from specimens collected in Africa.

4.1.4 Aim

Reviewing different papers on the effectiveness of different barcode loci showed that multilocus barcodes are often found to be more effective than the use of a single region. The *rbcL* region is rarely found to have sufficient variation at the species level for species discrimination (Kress & Erickson, 2007). Hence, it is mainly considered as suitable marker for barcoding at the species level when combined with other markers such as ITS, *matK* or *trnH-psbA* (*e.g.* Bell, Loeffler, & Brosi, 2017). Yet, *rbcL* shows variation at the genus level (Newmaster *et al.*, 2006) and is widely used due to its universality and easy amplification.

Based on existing sequence data from RBGE, this study explores whether *rbcL* can be a useful barcode marker to discriminate tropical plant taxa. Furthermore, it is asked at which the taxonomic level *rbcL* barcoding is successful. If possible, assessments will be made on the usefulness of *rbcL* for tropical climbers in particular.

4.2 Materials and Methods

4.2.1 Input data

For the purpose of a grant proposal for DNA barcoding, Dr. David Harris (Royal Botanic Garden Edinburgh, RBGE) intended to generate preliminary data for silica-gel dried cambium and leaves from species in the Republic of Congo. Hence, in 2014, attempts were made to sequence the *rbcL* region for 135 specimens collected by Harris and Ndolo-Ebika in the Republic of Congo. The specimens included tropical trees and climbers from 34 plant families.

The DNA was extracted from silica gel dried leaf material and cambium, using standard column-based methods. The primer pair rbcL-aF (Kress & Erickson, 2007) and rbcL-ajf634R (Fazekas *et al.*, 2008) was used for PCR amplification and sequencing of the plastid DNA

region *rbcL*. DNA could be successfully amplified for 87 of the silica dried leaf samples. Sequencing reactions resulted in bi-directional reads for 71 of these samples and unidirectional reads for a further 16 samples.

This study made use of these 87 *rbcL* sequences, of which 30 sequences were from tropical climbers, including two unidentified Apocynaceae species. In the field identifications by Harris & Ndolo-Ebika, all specimens have been determined to family level, 83 to genus level and 62 to species level. Based on these identifications, the 87 sequences represent 28 families, 48 genera and 50 species.

4.2.2 DNA sequence analysis

Specimens were imported into a BOLD project (Barcode of Life Data System) (Ratnasingham & Hebert, 2007) by uploading raw specimen data, and adding the associated *rbcL* trace files (chromatograms) and corresponding nucleotide sequences. To estimate the taxonomic relationship of the sequences, a neighbour joining tree was built using the Taxon ID Tree tool on BOLD. Only sequences with a length > 200 bp were included, and these were aligned using MUSCLE (Edgar, 2004). The Kimura-2-Parameter was selected as distance model and ambiguous bases were deleted pairwise.

4.2.3 *rbcL* identification

All sequences were subjected to two BLAST searches (**B**asic **L**ocal **A**lignment **S**earch **T**ool) (Altschul *et al.*, 1990) for identity assessment using the BOLD Identification Engine (https://www.boldsystems.org/index.php/IDS_OpenIdEngine) as well as the NCBI's nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi). The BOLD Identification Engine uses all published and non-published *rbcL* and *matK* sequences (> 500 bp) uploaded to BOLD as reference database for plants. Currently, this includes 95,000 *rbcL* sequences. The NCBI's blastn searches sequences on GenBank, which covered 260,815 *rbcL* sequences for plants as of August 15, 2020.

The resulting BLAST matches are ranked by their alignment score and the similarity. The alignment score reflects the mismatches and gaps of the query sequence and the reference sequence. The similarity expresses the extent to which the query sequence is related to the matching sequence in the reference database. It is based on the number of base pairs at the same position in the alignment and is often expressed as a percentage (Madden, 2011). As the alignment score varies depending on the sequence length, results of both BLAST searches were compared using the similarity value.

Using the top three hits listed in the BLAST result output for each specimen, it was recorded how many hits were in concordance with the field determinations for family, genus and species level. Also, it was checked whether *rbcL* sequences were available on GenBank for the genus and species determinations made by Harris and Ndolo-Ebika to assess whether potential mismatches could have been due to unrepresented reference sequences.

4.3 Results

4.3.1 Sequences

From the total *rbcL* amplicon of 607 bases, sequence lengths obtained here ranged from 135-607 bp. Of the 87 specimens with some sequence data, 55 (63 %) were found to be BOLD barcode compliant. For all barcoding regions, a sequence is generally defined as barcode compliant if it has a bi-directional read and a sequence length over 500 bp. For the *rbcL* primer pair used in this study, sequences with a length shorter than 607 bp are considered as incomplete sequences as the full amplicon has not been read. This dataset included 48 (55 %) incomplete sequences. A list with details on all sequences can be found in Appendix 3.

A neighbour-joining three showing the phylogenetic relationship of 81 sequences with a length > 200 bp and including the field determinations of the specimens is depicted in Fig. 15.

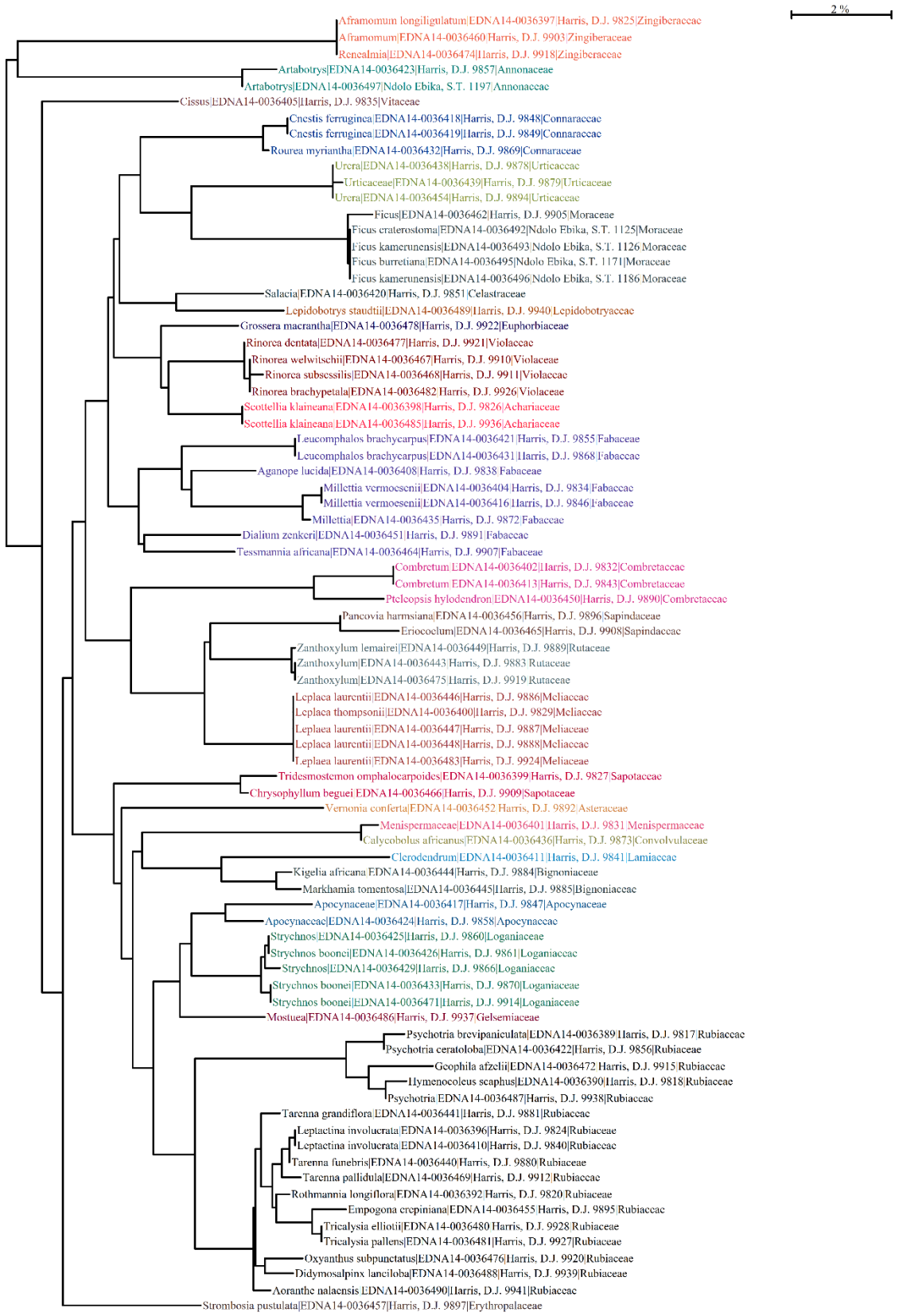


Figure 15. Neighbour-joining tree printed from BOLD for *rbcL* sequences > 200 bp, n = 81. Taxon names are field determinations by Harris & Ndolo-Ebika. Colours represent family affiliation.

4.3.2 Similarity values of BLAST results

In the BLAST results output, the top hit is the hit with the highest alignment score and the highest similarity. Similarity values of top matches ranged from 97.9 % – 100 % for both BLAST searches.
There was a 100 % similarity between the query and the subject sequence for 27 top matches from the search in the BOLD database, and for 39 top matches from the GenBank BLAST.
For 21 matches in BOLD and 31 matches in GenBank there was a similarity of 99.5 % and higher.

4.3.3 Family

The neighbour-joining tree presented in Fig. 15 shows that specimens identified as belonging to the same family are grouped together. This indicates that the field identifications based on morphological characters correspond with the grouping resulting from the *rbcL* sequence.

There was a concordance between the previous family identification and the top match of both BLAST searches for 84 specimens[2] (97 %). For one case, the family matched with the second hit in the GenBank search. For two specimens the family was different to the BLAST IDs. The specimen “Harris 9815” (*Leptaulus zenkeri*, Cardiopteridaceae) was identified as *Caloncoba flagelliflora* (Salicaceae) in the GenBank search and as *Xylotheca kraussiana* (Achariaceae) in BOLD. The specimen “Harris 9831” (species unknown, Menispermaceae) was identified as Primulaceae by the BLASTn search in GenBank and as Convolvulaceae by the search in the BOLD database.
In the identification notes of specimen “Harris 9815”, the determiner (Harris D.J.) expressed doubt about the identification, saying that the hairs on the stem and the overall look seem a little different than in *Leptaulus zenkeri*. After comparing specimen photographs with the type specimens on of *Caloncoba flagelliflora* and *Xylotheca kraussiana* JSTOR it was found that the specimen resembles *C. flagelliflora*.

For the specimen “Harris 9831” no herbarium material or photographs were found for comparison with the BLAST IDs.

4.3.4 Genus

Of the 83 records which had field determinations to genus level, the genus identified by BLAST matched for 64 specimens (77 %), representing 31 genera.

[2] BLAST results for all 87 *rbcL* sequences can be found in Appendix 3.

The BLAST search within the curated database, BOLD, resulted in 51 matches that correspond with the field determination at genus level. Five of these matches were not recognized by the GenBank search (Table 6).

The BLASTn search in GenBank resulted in 59 matches with the previously given genus name. Thirteen of these matches were not recognized by the BOLD search (Table 7).

Table 6. Identification matches unique to the BLAST search in BOLD

EDNA-Nr.	Specimen	Field identification	BOLD ID	Family
EDNA14-0036400	Harris, D.J. 9829	*Leplaea thompsonii*	*Guarea*[3] *thompsonii*	Meliaceae
EDNA14-0036446	Harris, D.J. 9886	*Leplaea laurentii*	*Guarea thompsonii*	Meliaceae
EDNA14-0036447	Harris, D.J. 9887	*Leplaea laurentii*	*Guarea thompsonii*	Meliaceae
EDNA14-0036448	Harris, D.J. 9888	*Leplaea laurentii*	*Guarea thompsonii*	Meliaceae
EDNA14-0036483	Harris, D.J. 9924	*Leplaea laurentii*	*Guarea thompsonii*	Meliaceae

Table 7. Identification matches unique to the BLASTn search in GenBank.

EDNA-Nr.	Specimen	Field identification	GenBank ID	Family
EDNA14-0036397	Harris, D.J. 9825	*Aframomum longiligulatum*	*Aframomum sp.*	Zingiberaceae
EDNA14-0036413	Harris, D.J. 9843	*Combretum*	*Combretum indicum*	Combretaceae
EDNA14-0036421	Harris, D.J. 9855	*Leucomphalos brachycarpus*	*Bowringia callicarpa*	Leguminosae
EDNA14-0036431	Harris, D.J. 9868	*Leucomphalos brachycarpus*	*Bowringia callicarpa*	Leguminosae
EDNA14-0036440	Harris, D.J. 9880	*Tarenna funebris*	*Tarenna supra-axillaris*	Rubiaceae
EDNA14-0036443	Harris, D.J. 9883	*Zanthoxylum*	*Zanthoxylum madagascariense*	Rutaceae
EDNA14-0036445	Harris, D.J. 9885	*Markhamia stipulata*	*Markhamia tomentosa*	Bignoniaceae
EDNA14-0036449	Harris, D.J. 9889	*Zanthoxylum lemairei*	*Zanthoxylum pinnatum*	Rutaceae
EDNA14-0036460	Harris, D.J. 9903	*Aframomum*	*Aframomum angustifolium*	Zingiberaceae
EDNA14-0036466	Harris, D.J. 9909	*Chrysophyllum lanceolatum*	*Chrysophyllum beguei*	Sapotaceae
EDNA14-0036469	Harris, D.J. 9912	*Tarenna pallidula*	*Tarenna lasiorhachis*	Rubiaceae
EDNA14-0036475	Harris, D.J. 9919	*Zanthoxylum*	*Zanthoxylum madagascariense*	
EDNA14-0036489	Harris, D.J. 9940	*Lepidobotrys staudtii*	*Lepidobotrys staudtii*	Lepidobotryaceae

The genera which received matches with the BLAST results included nine genera with a climbing habit (*Artabotrys*, *Clerodendrum*, *Combretum*, *Leucomphalos*, *Millettia*, *Rourea*, *Salacia*, *Strychnos* and *Urera*).

[3] All African Guarea have been moved to Leplaea (Koenen & de Wilde, 2012).

The genera for which no match with the BLAST result was found are listed in Table 8. It must be noted that for *Leplaea*, genus matches were received for other accessions with the same field identification (Table 6). Table 9 shows that for five of the genera without matches (*Leptaulus*, *Plagiostyles*, *Scottellia*, *Tessmannia* and *Tridesmostemon*) only one species in the genus had *rbcL* sequences on GenBank. The other "mismatched" genera showed a species coverage for *rbcL* ranging from 5-27 %.

Table 8. Genera of field determinations not matching identifications given from BLAST searches. Values in brackets represent the number of *rbcL* sequences in GenBank (status as of August 16, 2020). Asterisks indicate genera with a climbing habit.

No.	Genus	Family	No.	Genus	Family
1.	*Aganope** (4)	Leguminosae	10.	*Plagiostyles* (2)	Euphorbiaceae
2.	*Calycobolus** (2)	Convolvulaceae	11.	*Psychotria* (502)	Rubiaceae
3.	*Cissus** (236)	Vitaceae	12.	*Renealmia* (27)	Zingiberaceae
4.	*Cnestis** (2)	Connaraceae	13.	*Scottellia* (1)	Achariaceae
5.	*Combretum** (254)	Combretaceae	14.	*Tarenna* (23)	Rubiaceae
6.	*Empogona* (11)	Rubiaceae	15.	*Tessmannia* (1)	Leguminosae
7.	*Leplaea* (3)	Meliaceae	16.	*Tiliacora** (7)	Menispermaceae
8.	*Leptaulus* (2)	Cardiopteridaceae	17.	*Tridesmostemon* (1)	Sapotaceae
9.	*Pancovia* (4)	Sapindaceae			

All 48 genera investigated were represented with *rbcL* sequences on GenBank, yet only seven genera showed a species coverage of 50 % or more. It must be noted that these were small genera containing less than ten species (Table 9).

Table 9. Species coverage for the *rbcL* region in GenBank for genera investigated in this study (status as of August 17, 2020). Values in bold indicate a species coverage of at least 50%. Asterisks indicate genera with a climbing habit.

Genus	# of species in genus	# of species with *rbcL*	%
Aframomum	55	5	9%
*Aganope**	11	3	27%
Aoranthe	5	2	40%
*Artabotrys**	102	21	21%
*Calycobolus**	10	2	20%
Chrysophyllum	89	25	28%
*Cissus**	324	75	23%
*Clerodendrum**	308	33	11%
*Cnestis**	13	2	15%

*Combretum**	289	60	21%
Dialium	41	12	29%
Didymosalpinx	**4**	**3**	**75%**
Empogona	28	5	18%
Eriocoelum	12	3	25%
Ficus	841	222	26%
Geophila	27	7	26%
Grossera	8	2	25%
Hymenocoleus	12	1	8%
Kigelia	**1**	**1**	**100%**
Lepidobotrys	**1**	**1**	**100%**
Leplaea	7	1	14%
Leptactina	27	7	26%
Leptaulus	6	1	17%
*Leucomphalos**	4	1[4]	25%
Markhamia	**5**	**5**	**100%**
*Millettia**	202	19	9%
Mostuea	9	1	11%
Oxyanthus	34	8	24%
Pancovia	12	3	25%
Plagiostyles	**2**	**1**	**50%**
Psychotria	1,865	188	19%
Pteleopsis	10	3	39%
Renealmia	86	4	5%
*Rinorea**	164	37	23%
Rothmannia	42	18	43%
Rourea	65	9	14%
*Salacia**	132	25	19%
Scottellia	3	1	33%
Strombosia	**9**	**6**	**67%**
*Strychnos**	168	34	20%
Tarenna	192	15	8%
Tessmannia	12	1	8%
*Tiliacora**	24	5	21%
Tricalysia	79	10	13%
Tridesmostemon	**2**	**1**	**50%**

[4] Accession filed under genus Bowringia, which has been moved to Leucomphalos (Breteler, 1994).

*Urera**	43	17	40%
Vernonia	671	17	3%
Zanthoxylum	176	43	24%

4.3.5 Species

Together, both BLAST searches resulted in species level matches for seven of the 62 specimens with previous field determinations (11 %), each representing a different species (Table 10). *Kigelia africana* and *Lepidobotrys staudtii* are species in monotypic genera whereas the others are species of polytypic genera. None of these species were climbers.

The BLAST search in the BOLD database hit two more species matches than the BLASTn search in GenBank. For the species matches received from both BLAST searches, identical similarity values were reported. For five matches, the query sequence had a 100 % similarity with the corresponding sequence in the reference database (Table 10).

For 20 of the 50 species investigated, *rbcL* sequences were available in the nucleotide database on GenBank. Of the recorded species matches, five species had *rbcL* sequences on GenBank. The two species *Hymenocoleus scaphus* and *Rothmannia longiflora* were not represented for *rbcL* in the database.

Table 10. Species matches of both BLAST searches. Similarity values indicate the extent to which the query sequence is related to the corresponding sequence in the reference database.

Species	**Family**	**Similarity**	**identified by**	
			BOLD BLAST	**GenBank BLASTn**
Grossera macrantha	Euphorbiaceae	100%	x	x
Hymenocoleus scaphus	Rubiaceae	100%	x	
Kigelia africana	Bignoniaceae	99.67%	x	x
Lepidobotrys staudtii	Lepidobotryaceae	98.96%		x
Leplaea thompsonii	Meliaceae	100%	x	
Rothmannia longiflora	Rubiaceae	100%	x	
Strombosia pustulata	Olacaceae	100%	x	x

4.4 Discussion

4.4.1 Sequence quality

The quality of DNA extracted from plant material affects the success of amplification and how well a barcode sequence is received. Degradation of DNA and loss of quality is more likely when extracted from dried herbarium specimens than from fresh plant material instantly dried

in silica gel (Alsos *et al.*, 2020), as was also demonstrated by Korpelainen & Pietiläinen (2019) who found that barcoding success declines with increasing age of herbarium material.
As the DNA was analysed in this study was from silica gel dried leaves, it can be assumed that the sequences analysed in this study were of sufficient quality. Yet, it cannot be ruled out that age or condition of the preserved leaves might have still affected the concentration of DNA.
The quality of the barcode sequence is also determined by other factors such as the presence of secondary metabolites during extraction that might inhibit PCR (Friar, 2005). For example, these kinds of interfering compounds are often found in taxa of Lamiaceae (*e.g* Dodoš *et al.*, 2014). The standard barcoding protocol used for the PCR reaction uses the PCR additive CES and is considered to usually be successful for a wide range of taxa (Ralser *et al.*, 2006).

4.4.2 Reference databases

The instructions for the BOLD Identification engine in the BOLD handbook (https://www.boldsystems.org/index.php/resources/handbook) state that a successful species match is not guaranteed due to the lack of coverage of plant sequences in comparison to animal sequences. A *rbcL* sequence gap for plants also exists on GenBank as was reflected by the low species coverage of the genera examined in this study (chapter 4.3.4). Hence, a close match with a GenBank sequence does not rule out that another taxon would have reached a similar or better result if it had been sequenced.

The guidelines to the BOLD ID engine furthermore include a warning stating that the database is unvalidated and contains records without species level identification. Also, some species only have preliminary, unconfirmed names. Hence, caution must be exercised when interpreting sequence matches from BOLD. Further inspection and comparison of specimens should be conducted by experienced taxonomists to validate proposed taxon names. An example for the need of expert knowledge is specimen “Harris 9815”, which was misidentified and redetermined by the aid of species name proposed by the BLASTn search in GenBank. The BLAST identification alone would not have been convincing enough for changing the name. Due to the fact that Harris has collected *Caloncoba flagelliflora* (Salicaceae) in the Sangha Trinational once before, he could refrain from checking specimens of the other proposed species which were distributed across South Africa and South America.

The results of the BLAST search in BOLD present information for the ranks phylum, class order, family, genus, species and subspecies, if available for the accession. Moreover, there is an option to view more details on the specimen for published accessions. However, it was found that the taxonomy is not always up to date. For example, the genus matches of the five

specimens of *Leplaea* were listed with the synonym *Guarea* (see chapter 4.3.4). Without the expert knowledge that all African *Guarea* have been moved to *Leplaea*, the match would not have been recognized. A note on this issue in the results view, or built-in links to taxonomic websites such as Plants of the World online (http://powo.science.kew.org/), The Plant List (http://www.theplantlist.org/) or IPNI (https://www.ipni.org/) would help making the data system more accessible for anyone engaged or interested in DNA barcoding as intended by the platform.

Curated and annotated databases are preferred to public databases such as GenBank, which contain a large proportion of poor data. Here, wrong or misleading entries can become rampant, so that correct and validated entries lose their power as reference data (Vilgalys, 2003). Other than as required by BOLD, many sequences in public databases are not linked to a vouchered specimen but are often associated with environmental samples (Meiklejohn *et al.*, 2019). This renders further comparisons and identity checks impossible. Again, the misidentified specimen mentioned above serves as an example for the importance of specimens and photographs. Without these, the wrong field determination would not have been detected and the sequence may have been submitted to GenBank – adding a further misleading entry to the database.

Although BOLD may be more reliable than public databases in the sense that it requires certain standards for a sequence to qualify as barcode sequence to be published, the database should be used with caution. For example, many species in the curated database are represented by only one or two specimens (https://www.boldsystems.org/index.php/resources/handbook). This must be viewed critically as several samples of a species are required to reliably depict within-species variation in a reference database (Sosef *et al.*, 2020). According to them, at least ten samples per species are recommended. In an ideal barcode study, a solid reference library containing multiple specimens with validated expert identifications is built. Due to the reduced scope of the present examinations, GenBank and BOLD are used as substitute databases.

For a DNA barcode study for the 62 Apocynaceae climbers expected in the Sangha Trinational, it is proposed that five samples per species would be sufficient to construct a reliable reference database, if the species concept is accepted. This adds up to 310 samples which would need to be sequenced. This work was based on the assumption that the species concept is fine, however, if there is reason to suspect a species concept, more specimens should be sampled. For example, the species *Orthopichonia schweinfurthii* and *Orthopichonia barteri* are difficult to tell apart and the previous investigation revealed, that it is not certain which of both actually occurs in the Sangha Trinational (see chapters 2.1.2 and 3.4.3). If there was a cryptic species in the

Sangha Trinational, it is doubtful that sampling five specimens would capture it. Therefore, expanding the sampling range to areas outside the Sangha Trinational – by collecting the same number of samples in different areas across the species range – might give a mean indication of the species concept.

Creating a reference library is often stated to be the "ideal" way when using DNA barcoding for species authentication. Yet, many papers solely rely on public databases (e.g Cabelin & Alejandro, 2016; Che Husin *et al.*, 2018). This raises the question how informative and reliable these studies are and – more far-reaching – whether these approaches contribute to the proclaimed goal of the International Barcode of Life Consortium (iBOL) to assemble a global DNA barcode reference library for species identification, or rather constitute an obstacle to it.

Meiklejohn *et al.* (2019) found that GenBank performs better as a reference database than BOLD for identifying insects at species level. However, for species-level identifications of plants, the authors recorded a similar performance of the databases (~81 %). In this regard it must be noted that the BOLD database contains sequences from GenBank and conversely, sequences published on BOLD are submitted to GenBank. Thus, many sequences are stored in both databases and similar performance results are not surprising (Meiklejohn *et al.*, 2019). The overlap of sequences in both databases make it difficult to make clear statements on which one is more useful as a reference database. I encountered this issue when comparing the identifications of both BLAST searches. Each BLAST search found concordant matches with the field determination, which was not found in the other search, while at the same time many matches were identical, giving the same similarity value.

While BOLD provides a curated framework with requirements that guarantee a certain standard of barcodes, there is still a need for more validation, monitoring and sampling until it can be used as reliable global barcode reference library.

4.4.3 Limitations of BLAST

The BLAST tool used for this study is one of the most widely used public sequence analysis tools (Madden, 2011; McGinnis & Madden, 2004). The comparison of sequences allows to examine evolutionary relationships and explore the functions of a new DNA sequence (Madden, 2002; Madden, 2013). However, the BLAST algorithms are very complex (Korf *et al.*, 2003). For the NCBI's BLASTn search conducted in this study, the default settings were applied. Yet, the window allows to adjust algorithm parameters which I found hard to conceive.

Reviewing literature and reading questions in forums revealed that these settings are rather designed for advanced users such as bioinformaticians.

A more important issue encountered during this study was the interpretation of BLAST results. The results pages report five values which express the relationship of the query and the subject: maximum score, total score, query cover, expect value (e-value) and percent identity/similarity. Additionally, it is reported how many base pairs differ between sequences and a graphic summary is provided. Although in this study the similarity was used for comparing sequences, as it was perceived as most comprehensive value, the e-value is most commonly used and often considered as the statistically most significant metric (Pagni & Jongeneel, 2001). Yet, it is widely misunderstood by users as it is less intuitive and relies on many variables including database size (González-Pech *et al.*, 2019; Pagni & Jongeneel, 2001). Furthermore, a statistical measure does not necessarily reflect a biologically meaningful result (Madden, 2002).

However, a difficult aspect of the similarity value is that there is no common threshold that defines when a match is significant or can be considered as correct. Results show that many top hits showed a 100 % similarity, meaning that query and subject sequence were identical. For some specimens, a 100 % similarity was also recorded for the second and third match. As a specimen cannot have several identities this, for one thing, shows that the similarity value alone is not sufficient for ranking the hits and drawing confident conclusions on the concordance of query and subject identity. On the other hand, it may be that the selected barcode loci is not powerful enough for discrimination as will be discussed in chapter 4.4.5.

4.4.4 Family

Results confirm that the *rbcL* region is very reliable at discriminating specimens on a family level as was previously proven by studies testing the discriminatory power of different barcode loci for taxon delimitation (Braukmann *et al.*, 2017; de Vere *et al.*, 2012). From reviewing studies published on DNA barcoding, it seems that barcoding is not used to authenticate plant families. Yet, this does not reflect the use of molecular data for taxonomic classification on the family level, as DNA barcoding primarily aims at discriminating between species within a genus or a family (Kress *et al.*, 2005). For example, the Angiosperm Phylogeny Group (APG), classifies flowering plants on order and family rank mostly based on molecular data (APG, 1998; APG II, 2003; APG III, 2009; APG IV, 2016).

During a tropical biodiversity field trip as part of this study programme, we were trained to identify plant families based on vegetative characters alone. While some families – such as

Apocynaceae with the opposite leaves and white latex – were very easy to recognize, I experienced that others were more difficult to identify just from sterile characters. For example, the families Euphorbiaceae and Malvaceae share a lot of sterile traits which sometimes makes it difficult to confidently assign a specimen to either of the families. In such cases, fertile characters might be a help, however in the tropics, these are only rarely available. Also, most identification books focus on fruits and flowers and are only useful to a limited extent. Here, I believe that DNA barcoding could be a fast way to distinguish between difficult families or check whether family names given in the field were accurate. Especially for cases, where only limited material can be collected, like some single leaves, DNA barcoding could be a good starting point for identification.

4.4.5 Genus

With a proportion of 77 % matching genus names, *rbcL* region showed a reasonable ability to identify specimens to genus level when compared to success rates reached in a thematically comparable barcode study. Parmentier *et al*. (2013) tested the effectiveness of *rbcL*, *matK* and *trnH-psbA* for the identification of central African rainforest trees. At genus level, *rbcL* showed a barcoding success of 98 % at a local scale (50 ha) and 84 % at a regional scale (1 ha). The result at the local scale was similar for *trnH-psbA* but significantly better than *matK*. However, on a regional scale, *rbcL* performed worst. The authors found that the success of *rbcL* for genus identification decreased by 5 – 26 % when the database used included the genera of the query samples, but not all species (10, 20 or 50 % missing species) (Parmentier *et al.*, 2013). This brings up the question whether a higher proportion of matching genus identifications would have been obtained in the present study if more query species were represented in the databases used (see chapter 4.3.5).

The genus mismatches recorded for 17 genera might be due to a low species coverage for these genera in GenBank, and/or a small number of *rbcL* sequences in the reference database. Indeed, the results show that five of these genera were represented by only one species (Table 9). Besides, for most of the genera there were only between one and four sequences in the refence database (Table 8).

Another plausible explanation is that either the field identification or the identification of the proposed subject sequence was wrong. An example for that is specimen “Harris 9821” (EDNA14-0036393), priorly named *Leplaea*. The BLAST results assigned it to other genera (*Aglaia*/*Dysoxylum*), whereas another five specimens with the same field identification

(EDNA14-0036400, EDNA14-0036446, EDNA14-0036447, EDNA14-0036448, EDNA14-0036483) were identified “correctly”.

Furthermore, mismatches might simply be due to the fact that *rbcL* is not discriminatory enough in these groups. For Canadian plants, Braukmann *et al*. (2017) showed that the taxonomic resolution of *rbcL* at genus level varies between families. In the 25 families investigated, Asteraceae showed the lowest percentage (78 %), the best resolution was recorded for Violaceae (100 %) (Braukmann *et al*., 2017). These differences between families may be influenced by varying evolution rates and other events during evolution such as hybridization (Caetano Wyler & Naciri, 2016).

Non-monophyletic genera are another possible reason for failure at genus-level identification.

Given the satisfactory ability of *rbcL* to tell tropical climber genera apart (64 % matches), it would be interesting to use the marker for a DNA barcoding study to authenticate morphologically very similar and closely related (Endress *et al*., 2014) genera of Apocynaceae climbers (*e.g. Motandra* and *Oncinotis*). Barcoding using *rbcL* could also be useful to discriminate the difficult genera of Group 6 of the identification key created in the first part of this study, which includes understudied genera of the subfamily Asclepiadoideae (chapter 3.3.2). In this regard, one might also include the two unnamed Apocynaceae specimens (EDNA14-0036417 and EDNA14-0036424).

4.4.6 Species

Despite the fact that 40 % of the species investigated were represented with *rbcL* sequences on GenBank, only 14 % received a match by the BLAST search. Although other factors may have caused the high proportion of mismatches (86 %) (as discussed above), this result reflects the scientific consensus that *rbcL* alone is not sufficient for discrimination at species level.

Burgess *et al.* (2011) hypothesized that species are expected to be morphologically and genetically more distinctive if they are the only one in the genus. For the multiple barcode *rbcL* + *matK*, they showed that species identification was more successful for monotypic genera (100 %) than for polytypic genera (83.6 %). In the present study, the two species in monotypic genera (*Kigelia africana* and *Lepidobotrys staudtii*) were both identified correctly, however their similarity value was lower (99.67 % and 98.96 %, respectively) than for the species matches of polytypic genera (100 %) (Table 10).

When it comes to the aim of DNA barcoding, the rapid identification and discovery of species, there is a controversy among scientist about how useful DNA barcoding actually is. Regarding new species discovery, some authors argue that the actual gap in taxonomic knowledge is the low quota of formal descriptions of already discovered species (Pinheiro *et al.* 2019). Other opponents find that DNA barcoding is often "overemphasized" and poses a threat to traditional taxonomy by promoting wrong identifications and claiming a large amount of funds (Will *et al.*, 2005).

5 Communicating my research

5.1 Background

Working on a research project during times of social distancing required accessing resources remotely and through online sources. Without the innumerable amount of publicly available scientific papers and databases, I would not have been able to carry out a project and write up the dissertation to this extent. This is just one example showing how important it is to communicate own research findings to the public. Furthermore, scientific knowledge is vital for finding solutions to complex challenges in society (Davies & Horst, 2013). However, without communication to relevant actors and decision makers, research findings can hardly be included into action plans. For these reasons, I dealt with this topic throughout my project, thinking about what science communication means for myself and how I would like to publicly communicate the results of this dissertation.

5.2 What public science communication do I consume?

Thinking about this question, the first kind of science communication that crossed my mind were scientific papers and reviews published in journals and mostly linked to my studies. This includes the use of literature search engines such as Google Scholar (https://scholar.google.com/), Web of Science (https://login.webofknowledge.com/) and the UoE online library – to name a few – as well as the scientific network and database ResearchGate (https://www.researchgate.net/). However, there turned out to be a lot more platforms and channels of science communication I draw on: newspapers, wikis, blog articles, Youtube videos, tweets and podcasts are sources I actually very regularly use to get information on a wide range of topics. One example is a German podcast called *Krautnah* (https://krautnah.de/). It addresses a range of topics on plant sciences and is designed to be comprehensive for everybody.

5.3 Communication plan

My supervisor Dr David Harris proposed to me a plan of how I might communicate the findings of my thesis and experiences I made during the project:

1 - Dissertation seminar – presenting my research to external examiner and RBGE staff and students

2 - Uploading a PDF file of my thesis online

3 - Uploading PowerPoint slides of my presentation to the online open access repository Figshare (https://figshare.com/)

4 - Publishing a blog entry about my project experience on *Botanics stories – RBGE Personal & Project Stories* (https://stories.rbge.org.uk/)

5 - Drafting a *Wikipedia* entry on a genus of Apocynaceae climbers

6 - Drafting a paper with my supervisors on a key to the Apocynaceae lianas in the Sangha Trinational

5.4 Modified communication plan

After I have read some literature on the various ways of communication science (*e.g.* Smith, 2015), I modified the proposed communication plan to fit personal preferences and according to specific aims:

	Action point	**Aim**	**Audience**
1	Dissertation seminar	to present my research process and results	- external examiner - RBGE staff and students - friends and family
2	Publishing a PDF file of my thesis online, *e.g.* on ResearchGate or GRIN (https://www.grin.com/)	to make my research findings permanently available (open access) and enhance knowledge about Apocynaceae climbers	- public - scientific community
3	Uploading PowerPoint slides of my presentation to Figshare	to provide a permanently accessible, comprehensive summary of my research project	- public - students - plant scientists
4	Publishing a blog entry on RBGE *Botanics stories*	to give insights about my experience of doing a herbarium research project remotely and during COVID-19	- RBGE staff, members and students - prospective RBGE students

5	Drafting a paper with my supervisors on a key to the Apocynaceae climbers in the Sangha Trinational	to provide an identification aid on the difficult group of Apocynaceae climbers	- plant scientists (ecologists, taxonomists, etc.)
6	Giving interviews with journalists, *e.g.* for a podcast or for a press release on the RBGE homepage	to inform about the newly published identification key and to talk about digital taxonomic work	- public

5.5 Results

As I do not engage much in social media, I did a small self-experiment to try out whether I could imagine using social media for science communication. For a period of two months, I wrote weekly tweets about my progress and thoughts on the research project. I did not publish them as of now, as I do not actively use Twitter. However, the tweets summarize important contents I would include and communicate in the action points mentioned above.

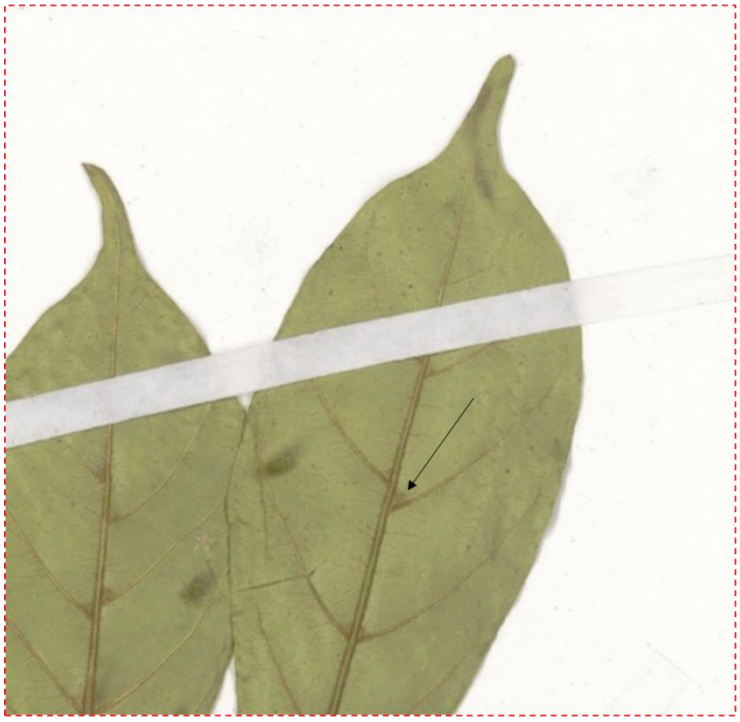
Tweets

Week 1: June 01, 2020

Due to remote working, I am virtually scoring characters on online herbarium specimens for my MSc summer project and keep asking myself: Is it possible to keep taxonomy standards in digital age? (Photo: Me still getting used to wearing a mask).

Week 2: June 08, 2020:

I had quite a few aha moments while grouping species of Apocynaceae climbers for an identification key. A discerning character within that group are domatia (sing. domatium from the Latin "domus", meaning home). Domatia are tiny chambers produced by plants that house arthropods. They are mostly found on the lower leaf surface, in the axils or forks of veins. (Specimen: *Motandra guineensis*, https://data.biodiversitydata.nl/naturalis/specimen/WAG.1607721)



Week 3: June 15, 2020:

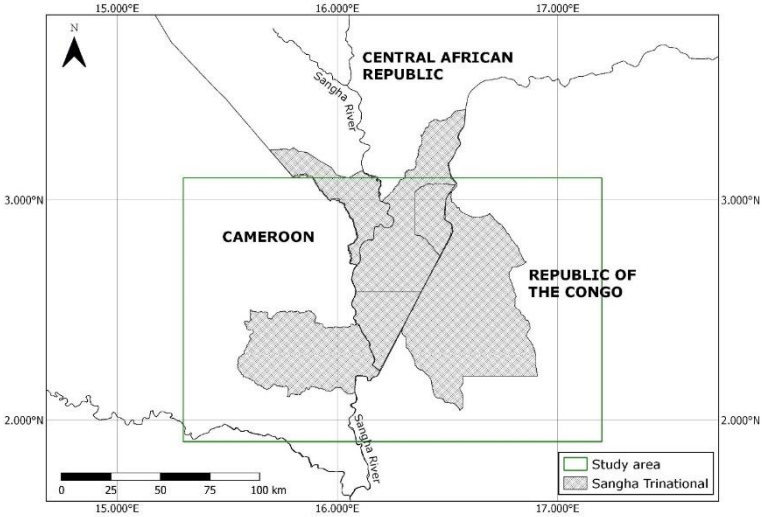
A tendril is often defined as “A slender threadlike appendage of a climbing plant, often growing in a spiral form, that stretches out and twines round any suitable support.” It is formed by modification of a part of a plant, such as a stem, a leaf or leaflet, or a stipule. Here some impressions of how tendrils in Apocynaceae climbers look like. (Specimens: *Landolphia owariensis*, https://data.biodiversitydata.nl/naturalis/specimen/WAG.1609730 and *Landolphia incerta*, https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610844)

Week 4: June 22, 2020:

I finally finished the identification key for Apocynaceae climbers in the Sangha Trinational. I am agog for it to get tested in the herbarium at RBGE by David Harris, let alone in the field after the pandemic.

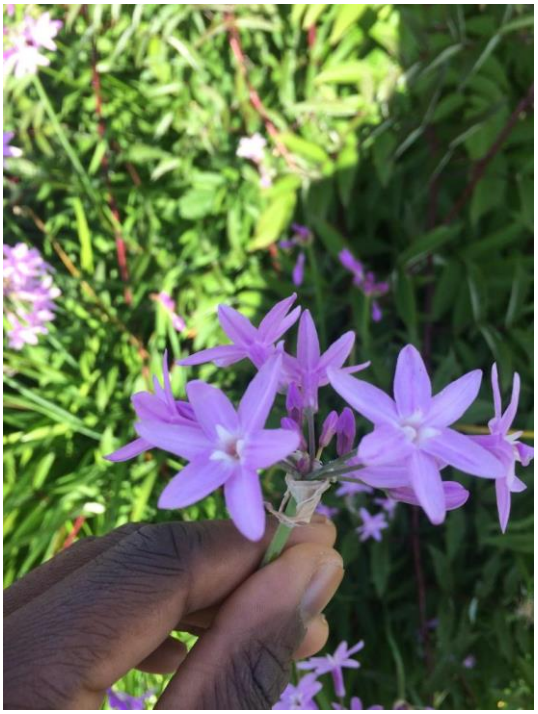
Week 5, June 29, 2020:

The Sangha Trinational is a transnational protected area complex located in the north-western Congo Basin. It includes three adjoining national parks: the Nouabalé-Ndoki National Park (Republic of the Congo), the Lobéké National Park (Cameroon) and the Dzanga-Ndoki National Park (Central African Republic).



40 years ago, it was considered as one of the least well-known areas in Africa. Over the past decades, knowledge about this site has accumulated and plenty of papers have been published. Today, it is a Natural World Heritage Site and research about the area is still carrying on. Happy to be a small part of it.

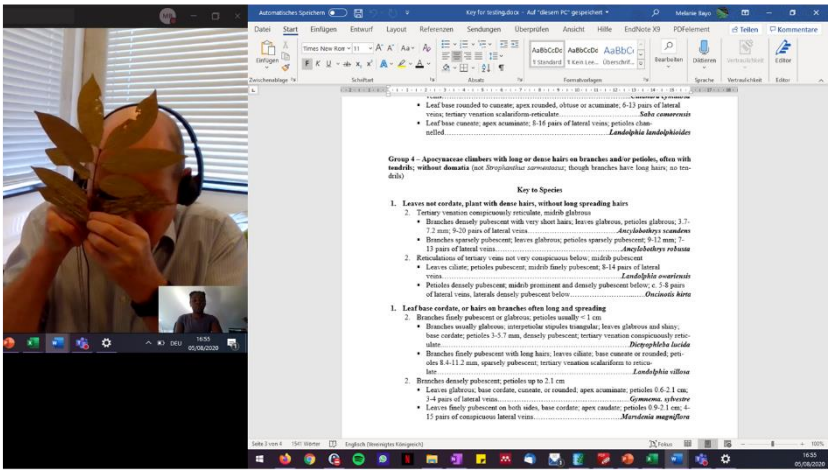
Week 6: July 06, 2020:
I'm currently in the writing process of my dissertation and reminiscing about the exceptional times I had during my studies at @RBGE. Hope I'll get to visit the Botanics again soon!! (Left: a shot of *Agapanthus africanus* (Amaryllidaceae), right: *Hoya carnosa* (Apocynaceae), both at RBGE).



Week 7: July 13, 2020:
Once again, thinking of and discussing the constraints that come with virtual taxonomy. While I am sure that real specimens cannot be replaced by digital images, I see a need for more high-quality digital specimen images to facilitate remote taxonomic work.

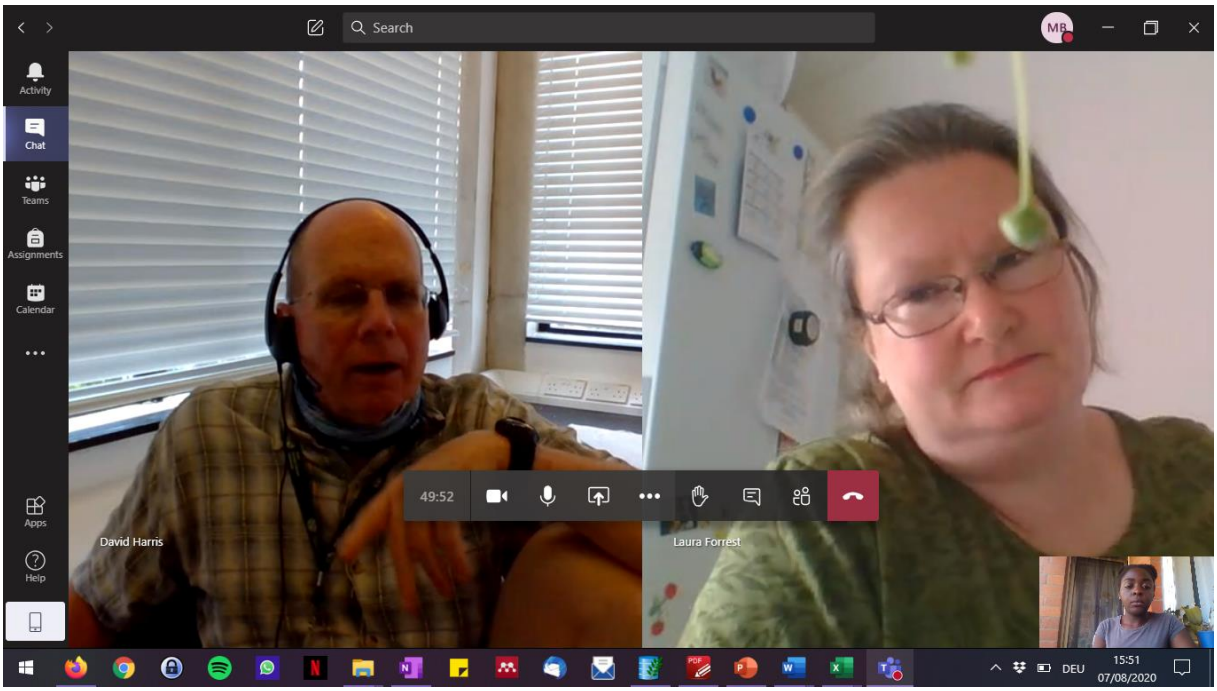
Week 8: July 27, 2020:
I'm currently exploring the tools in BOLD (Barcode of Life Data System, https://www.boldsystems.org). Plant species identification through DNA barcodes is fast and cheap; and might help to get a better understanding of the world's biodiversity.

Week 9: August 03, 2020:
Remote key testing from Edinburgh to Germany with my project supervisor and herbarium curator Dr David Harris!



Week 10: August 10, 2020:

Celebrating a small success of DNA barcoding and morphological taxonomy: A misidentified specimen was just redetermined after checking against photographs with the proposed identification from a BLAST result. (Photo: Dr David Harris, Dr Laura Forrest and myself during a Teams call on DNA barcoding)



5.6 Summary

Dealing with science communication while working on my research project taught me that there are some appealing and fun ways of informing people outside the subject area about my research. Moreover, I realised that there are plenty ways for public engagement, which go beyond publishing papers and regular posts social media. I am therefore very much looking forward to putting the plan outlined above into action.

6 Conclusion

This study aimed at facilitating the identification of Apocynaceae climbers in the Sangha Trinational by providing an identification aid based on sterile characters. The identification key presented solely uses vegetative characters and is the first of that kind to be produced for central African Apocynaceae climbers. Yet, it needs to be subject to further revision and testing, especially by actually using it in the forests of the Sangha Trinational.

Furthermore, this study sought to assess which species of Apocynaceae climbers can be expected in the study area that have not yet been recorded in the Sangha Trinational dataset. The findings reveal a significant amount of species, the collection and study of which would broaden the knowledge about the diversity and species composition of a natural World Heritage Site. In light of this, a further step would be to include these species in the created identification key.

The barcode investigation revealed that *rbcL* is a useful starting point for DNA barcoding of tropical plants. For future barcode studies on Apocynaceae climbers, it is recommended to include further barcode markers, such as *matK* and ITS2, which seem to be useful for the family. As the lack of reference sequences was shown to be a major limitation for the use of DNA barcoding, more sequence data needs to be generated and made available online. A major contribution of RBGE could be to construct a reference library for Apocynaceae climbers and upload the DNA accessions to GenBank. The in-house costs for a single attempt of sequencing *rbcL*, *matK* and ITS2 regions for Apocynaceae climbers in the Sangha Trinational would be around £7,600.

In the long term, it could be attempted to sequence all incoming plant material of Apocynaceae climbers on a regular basis, to confirm field determinations and identify unknown material. In this context, it might be worth putting effort into sequencing DNA from silica gel-dried cambium, as this might help identifying specimens for which only branch material can be obtained in the field.

Identification of *Caloncoba flagelliflora* (Salicaceae)

In conclusion, this study showed that for plant species identification, morphology and DNA are not exclusive but complement each other. The case of specimen “Harris 9815” explained in chapter 4.4.2 is a good example of how DNA sequences and specimens alone are often not sufficient to identify a plant but can be successful when used in combination.

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Appendix

Appendix 1. Links to digital specimens examined for character investigation.

Alafia caudata
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925776
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008824585
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.g00014768
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220184
5 - http://www.botanicalcollections.be/specimen/BR0000014301520
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1549128
7 - https://www.gbif.org/occurrence/1936555526

Alafia multiflora
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.gh00057397
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00088334
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0015294-0
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233917
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233918
6 - http://www.botanicalcollections.be/specimen/BR0000014720062
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1549391

Ancylobothrys robusta
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233384
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233385
3 - http://www.botanicalcollections.be/specimen/BR0000014706202
4 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1475576
5 - https://data.huh.harvard.edu/databases/specimen_search.php?mode=details&id=1402674
6 - http://www.botanicalcollections.be/specimen/BR0000014706158

Ancylobothrys scandens
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu219971
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu219972
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu219973
4 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1475604
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1475612
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1475675

Anisopus efulensis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000006419158
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000042513
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000930096
4 - http://www.botanicalcollections.be/specimen/BR0000009026230
5 - http://data.rbge.org.uk/herb/E00193181

Baissea axillaris
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000234017
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15229
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070744
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070739
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15250
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15202
7 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15230

8 - http://www.botanicalcollections.be/specimen/BR0000014724404
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1482649
10 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1482686
11 - http://www.botanicalcollections.be/specimen/BR0000014723940

Baissea gracillima
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ny00038519
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0110133
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925819
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15256
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15227
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15225
7 - http://www.botanicalcollections.be/specimen/BR0000014710872
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1482882
9 - http://data.rbge.org.uk/herb/E00607132

Baissea major
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236385
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236386
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00005340
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15213
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15219
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15228
7 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15211
8 - http://www.botanicalcollections.be/specimen/BR0000014712845
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483261
10 - http://www.botanicalcollections.be/specimen/BR0000014712395
11 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483263

Baissea multiflora
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236387
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236388
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413293
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100111
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070738
6 - http://www.botanicalcollections.be/specimen/BR0000014716942
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483299
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483296

Baissea subrufa
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15247
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15252
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15217
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15218
5 - http://www.botanicalcollections.be/specimen/BR0000014715747
6 - http://www.botanicalcollections.be/specimen/BR0000014717529
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483555
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1482857
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1483577

Batesanthus purpureus
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008861559
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000234315
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00193221
4 - http://www.botanicalcollections.be/specimen/BR0000014567049
5 - http://www.botanicalcollections.be/specimen/BR0000014567070

Clitandra cymulosa
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925654
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0060021-0
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.nu0019092-0
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan11949
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491651
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491645
7 - https://www.gbif.org/occurrence/2236070728

Cryptolepis sanguinolenta
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220194
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00077101
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220195
4 - http://www.botanicalcollections.be/specimen/BR0000005086894
5 - http://www.botanicalcollections.be/specimen/BR0000014805158
6 - http://www.botanicalcollections.be/specimen/BR0000014805172

Cyclocotyla congolensis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008825780
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008825773
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100138
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan11989
5 - http://www.botanicalcollections.be/specimen/BR0000014251634
6 - http://www.botanicalcollections.be/specimen/BR0000014252051
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491754

Cylindropsis parvifolia
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413327
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00071594
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0031796-0
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233336
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0057439-0
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491812
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491807

Cynanchum adalinae
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00069522
2 - http://www.botanicalcollections.be/specimen/BR0000008861757
3 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1659395
4 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1659376
5 - http://data.rbge.org.uk/herb/E00486114

Dictyophleba lucida
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100142
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bja326223185
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bja455048175
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bja565076365
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00071596
6 - http://www.botanicalcollections.be/specimen/BR0000014254840
7 - http://www.botanicalcollections.be/specimen/BR0000014254871
8 - https://www.gbif.org/occurrence/2235822341

Dictyophleba ochracea
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0110100
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100143
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.g00015310
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.goet005739
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233394
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00071597
7 - http://www.botanicalcollections.be/specimen/BR0000014257674
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491992
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1491983
10 - http://data.rbge.org.uk/herb/E00737309

Gongronema latifolium
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925986
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000305284
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00069497
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan23200
5 - http://www.botanicalcollections.be/specimen/BR0000014823145
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1658487
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1658488

Gymnema sylvestre
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bja139950732
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.tub003583
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.tub003584
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0175129
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0175128
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bja364081960
7 - http://www.botanicalcollections.be/specimen/BR0000014824647
8 - http://www.botanicalcollections.be/specimen/BR0000014824616

Landolphia incerta
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070668
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070666
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070665
4 - http://www.botanicalcollections.be/specimen/BR0000009019874
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610894

Landolphia landolphioides

1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.s-g-3538
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925594
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.a00078994
4 - http://www.botanicalcollections.be/specimen/BR0000014316951
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610614
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610586

Landolphia owariensis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00105705
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233472
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.togo02083
4 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1609977
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610018
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1610018
7 - https://www.gbif.org/occurrence/437964867

Landolphia robustior
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070695
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070694
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0060856-0
4 - http://www.botanicalcollections.be/specimen/BR0000015329370
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1609573
6 - https://www.gbif.org/occurrence/2235787276

Landolphia villosa
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008859501
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008859495
3 - http://www.botanicalcollections.be/specimen/BR0000015333247

Marsdenia magniflora
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000017075
2 - http://www.botanicalcollections.be/specimen/BR0000014832512
3 - http://www.botanicalcollections.be/specimen/BR0000014832338
4 - http://www.botanicalcollections.be/specimen/BR0000014832444
5 - http://www.botanicalcollections.be/specimen/BR0000014832505

Motandra guineensis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000009863453
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236383
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu236384
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-391009
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.c10003805
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lwi493734773
7 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lwi564231746
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1607744
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1607778
10 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1607751

Neoschumannia kamerunensis

1 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1657728

Oncinotis glabrata
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220173
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100218
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220174
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.lisu220176
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1622141
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1622193
7 - https://www.gbif.org/occurrence/2235744259

Oncinotis gracilis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233894
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0086046-0
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925830
4 - http://www.botanicalcollections.be/specimen/BR0000014552496
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1622043
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1622041

Oncinotis hirta
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233880
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.m0110139
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-391008
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00070723
5 - http://www.botanicalcollections.be/specimen/BR0000014553240
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621939
7 - http://www.botanicalcollections.be/specimen/BR0000014552700

Oncinotis tenuiloba
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100271
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.srgh0075889-0
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ubt0001873
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.iuk15186
5 - http://www.botanicalcollections.be/specimen/BR000001455439
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621817
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621816

Orthopichonia barteri
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233413
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413378
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413379
4 - http://www.botanicalcollections.be/specimen/BR0000014555527
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621541
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621769

Orthopichonia schweinfurthii
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233409
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233410
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233404

4 - http://www.botanicalcollections.be/specimen/BR0000014556692
5 - https://www.gbif.org/occurrence/1056751784
6 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1621586

Periploca nigrescens
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ld1226289
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925881
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan23240
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan23241
5 - http://www.botanicalcollections.be/specimen/BR0000013499150
6 - http://www.botanicalcollections.be/specimen/BR0000014560927
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1657506
8 - http://www.botanicalcollections.be/specimen/BR0000014559228

Pycnobotrya nitida
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.mo-100306
2 - https://plants.jstor.org/stable//10.5555/al.ap.specimen.gh00091783
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413393
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233940
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233939
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0057619-0
7 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan12076
8 - http://www.botanicalcollections.be/specimen/BR0000013692179
9 - http://www.botanicalcollections.be/specimen/BR0000013690908
10 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1619622
11 - http://www.botanicalcollections.be/specimen/BR0000013691462

Saba comorensis
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00071620
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233568
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233575
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ubt0001857
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ubt0001856
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ubt0001858
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1607470
8 - http://www.botanicalcollections.be/specimen/BR0000014309021
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1617536
10 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1617538

Strophanthus preussii
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413420
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925767
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.hbg502658
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00005625
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0067670-0
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.togo02126
7 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan12099
8 - http://www.botanicalcollections.be/specimen/BR0000014328299

9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1606144

Strophanthus sarmentosus
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925765
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.togo02154
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.togo02148
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00072324
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.togo02145
6 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan12202
7 - http://www.botanicalcollections.be/specimen/BR0000014325410
8 - https://www.gbif.org/occurrence/1945002276
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1605948

Strophanthus thollonii
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.fhi0040901-0
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ifan12214
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.ya0005258
4 - https://data.biodiversitydata.nl/naturalis/specimen/L.2708052
5 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1605743
6 - https://data.biodiversitydata.nl/naturalis/specimen/L.2708054

Tabernaemontana eglandulosa
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413440
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233752
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233732
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233753
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00217101
6 - http://www.botanicalcollections.be/specimen/BR0000014343230
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1674116
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1674083

Telosma africana
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.p00413440
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233752
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233732
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000233753
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00217101
6 - http://www.botanicalcollections.be/specimen/BR0000014343230
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1674116
8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1674083

Vahadenia laurentii
1 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925645
2 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.bm000925644
3 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008861603
4 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.br0000008861740
5 - https://plants.jstor.org/stable/10.5555/al.ap.specimen.coi00071629
6 - http://www.botanicalcollections.be/specimen/BR0000014357176
7 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1668794

8 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1668739
9 - https://data.biodiversitydata.nl/naturalis/specimen/WAG.1668789