

Flower morphological diversity in *Aframomum* (Zingiberaceae) from Africa – the importance of distinct floral types with presumably specific pollinator associations, differential habitat adaptations and allopatry in speciation and species maintenance

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Background and aims – *Aframomum* (61 species) is a tropical African genus of the family Zingiberaceae (~53 genera) of perennial rhizomatous herbs most often growing in the forest understorey. The family Zingiberaceae is known for a high diversity in floral morphology and pollination systems in Asia, however, almost nothing is known in Africa. In this study we explore the floral diversity in *Aframomum* and the potential of this diversity to mediate isolation as driving force for species divergence and maintenance in *Aframomum* in contrast to other postulated drivers such as allopatry and habitat differentiation.

Methods and key results – We conducted a survey of floral morphology in eighteen species across the genus identifying five floral types and their morphological adaptations to different pollinators. Another 38 species were subsequently attributed to one of these types based on monographic descriptions and photos. Differences in floral types were based on slight changes in the relative length and position of dorsal petal, labellum and the complex of anther and style. The mapping of floral types onto an existing phylogenetic tree indicated their repeated independent evolution. The preponderate presence of one rather uniform floral type in about 60% of all taxa (Trumpet type) suggests a low number of inferred pollinator shifts (30%).

Conclusion – The low number of inferred shifts in floral types alone cannot alone explain the diversification of this genus. Here the often small non-overlapping distribution ranges and narrow habitat preferences of species and the phylogenetic distance of sympatric species suggest the additional importance of allopatry and phylogenetic incompatibility as isolation mechanisms in maintaining species boundaries in this genus.

Key words – *Aframomum*, Africa, character mapping, floral types, mechanical isolation, phylogeny, pollination, tropical understorey, Zingiberaceae.

INTRODUCTION

The order Zingiberales is renowned for its high floral variability and great diversity of pollination systems (Kress & Specht 2005). Within this order, the eponymous family Zingiberaceae (~53 genera) contains the highest number of genera and species. Its bilateral flowers are generally showy, with three petals, only one fertile stamen and an often conspicuous petaloid structure called the labellum. Developmentally, the two androecial whorls, typical for the Zingiberales, are reduced in the Zingiberaceae. In the outer whorl one organ is entirely absent and the other two are only visible as two small remnant lobes which are referred to as staminodes. In the inner whorl there is only a single fertile stamen and the other two organs are fused to form the conspicuous label-

lum (Kress 1990, Bartlett & Specht 2010). This ‘labellum’ is shared with the sister family Costaceae and often used in taxonomic determinations in that family due to its interspecies variability (Specht 2001). The labellum has an important function in floral display for pollination (Specht 2001). Great variation within and between genera in Costaceae and Zingiberaceae is based on the different proportion of size and shape of floral organs rather than different numbers and relative positions of the different floral parts. The family of Zingiberaceae is pantropically distributed with the diversity centre in the Indomalayan region and many studies have been conducted on floral diversity and pollination systems in Asia (Troll 1929, Sun et al. 2007), however, very little has been reported from Africa with the exception of a discussion on vegetative and floral characters for a taxonomic grouping by

Lock & Hall (1975) and pollinator observations in *Aframomum melegueta* by Lock et al. (1977).

Within the family Zingiberaceae in Africa, *Aframomum* (61 species) is the most diverse genus distributed from Senegal to Madagascar (Larsen et al. 1998, Auvray et al. 2010). It comprises perennial rhizomatous herbs most often growing in the rainforest understorey. In *A. melegueta* from Ghana, Lock et al. (1977) observed that flowers were generally short lived, opening in the morning and wilting by mid-day. A taxonomic revision has recently been prepared by Harris & Wortley (submitted) including twelve newly described species. A phylogeny based on molecular data of rather low resolution is available including 30% of all species suggesting a recent diversification of the genus (Auvray et al. 2010).

In taxonomic groups of great flower morphological diversity, pollination has long been hypothesized as an important factor of mechanical isolation reinforcing the diversification of flowering plants (Darwin 1859, Dodd et al. 1999, Ollerton 1999, Lunau 2004, van der Niet & Johnson 2012). Through co-adaptation leading to a close morphological fit between flowers and pollinators (Faegri & van der Pijl 1966, Pauw et al. 2008) two mechanisms of mechanical isolation can establish: (a) isolation by pollen transport via different pollinator species, and (b) isolation by pollen deposition on different positions on the same pollinator (Grant 1994). These two modes of mechanical isolation arise from two slightly different mechanisms; the close matching with specific pollinators and the exclusion of others; and evolutionary changes in the relative position of pollinators and flower reproductive organs (Armbruster et al. 1994, Johnson & Steiner 1995, Goldblatt & Manning 2005).

In this study we wanted to explore the potential of floral types and their hypothesized associated pollinators in mediating mechanical isolation a potentially important force for speciation and species maintenance in *Aframomum*. We further consider allopatry and habitat differentiation. We did this by (1) investigating the flower morphological diversity across the genus, identifying floral types and the key morphological changes differentiating these types; (2) evaluating intra-type variability; (3) proposing for each type potential pollinators by matching morphological traits in flowers and pollinators; (4) mapping floral types onto the phylogeny of the genus to trace shifts in floral types which might be associated with changes in pollinators and (5) analyzing species distribution pattern and habitat specificities to detect cases of related sympatric species with the same floral types where, we would expect factors, other than pollinator differences and allopatry, to contribute to the maintenance of species boundaries.

MATERIAL AND METHODS

Study material

In total, 55 of the currently known 61 species (~86% of all species) of *Aframomum* (Zingiberaceae) from tropical Africa were included in this study (electronic appendices 1 & 2). Morphological measurements and photographs (Nikon Coolpix 995) of flowers were taken under the binocular microscope on fresh and alcohol preserved (70% ethanol)

material of seven and eleven species, respectively. Voucher specimens of these are deposited at the Royal Botanic Garden Edinburgh (E). For a further 38 species morphological and distributional information was taken from the literature ((Dhetchuvi 1993, Dhetchuvi 1995, Dhetchuvi & Fischer 2006, Dhetchuvi et al. 2011, Hallé 1967, Harris et al. 2000, Harris & Wortley submitted, Hepper 1967, Koechlin 1964, Koechlin 1965, Lock & Hall 1973, Lock & Hall 1975, Lock 1976, Lock et al. 1977, Lock 1978a, Lock 1978b, Lock 1978c, Lock 1979, Lock 1980, Lock 1984, Lock 1985, Poulsen & Lock 1997). Additional digital colour photographs for eleven of these 38 species were available. Five species did not yield any flower morphological data relevant for this study.

Morphological data

For the eighteen species with fresh and alcohol preserved material available (electronic appendix 1) a dataset of 29 quantitative and five qualitative flower morphological characteristics was established (see table 1, fig. 1, data in electronic appendix 3). For the taxa with only a monographic description and no fresh or preserved material we assembled from these descriptions four quantitative floral characters important for pollination (minimum and maximum length of dorsal petal and labellum) (electronic appendix 2).

Statistical analysis

The datamatrix of 29 quantitative and five qualitative flower morphological characteristics (electronic appendix 3) for eighteen species was submitted to a multidimensional scaling analysis in CANOCO version 4.5 (ter Braak & Šmilauer 2002) searching for groups of similar floral morphologies to describe flower types. The quantitative characteristics were summarized by the retrieved groupings (= floral types: average \pm standard deviation) to estimate variability within groupings and to compare between types. As floral types were distinct from each other and therefore able to be distinguished and sorted at first sight, we proceeded further by assigning 38 species with data only available from the literature (morphological descriptions and photos) manually to the already defined floral types. Values of the length of dorsal petal and labellum taken from Harris & Wortley (submitted) were summarized per floral type to estimate variability within the types. Five species could not be assigned to floral types due to insufficient knowledge on their flowers.

Experiments

Experiments concerning pollen deposition and the flexibility of the anther and the potential for it to be pushed away by a pollinator in the Trumpet type were undertaken on fresh material of *A. aulacocarpos* and *A. touchoui* by introducing a pen into the floral tube between anther and labellum. The movement and pollen deposition was filmed with a Nikon Coolpix 995 and is available in the online material.

Table 1 – Coding of 29 quantitative and five qualitative flower morphological characteristics.

ID	Character	Character state
Quantitative floral characteristics		
Floral measurements [mm]		
1	Length of calyx	
2	Length of lateral petal	
3	Width of lateral petal	
4	Length of dorsal petal	
5	Width of dorsal petal	
6	Length of labellum	
7	Width of labellum	
8	Length of anther	
9	Length of the anther's appendix	
10	Length of appendage at base of anther	
11	Length of anther above floral tube	
12	Width of arch formed by lateral appendage of anther	
13	Distance between thecae and labellum surface	
14	Length of thecae	
15	Length of epigynal glands	
16	Width of epigynal glands	
17	Length of style	
18	Width of stigmatic cavity	
19	Height of stigmatic cavity	
20	Length of floral tube	
21	Distance below floral tube narrowing	
22	Distance between style and nectar	
23	Difference in length between labellum and anther	
24	Length of labellum above floral tube	
25	Length of anther above floral tube	
26	Difference in length between dorsal petal and anther	
27	Difference in length between labellum and dorsal petal	
Floral elements		
28	Number of appendages at the base of the anther	
29	Number of epigynal glands	
Qualitative floral characteristics		
30	Colour of dorsal petal	1, yellow; 2, purple; 3, red; 4, white
31	Comparison of labellum and petal colour	0, different; 1, identical
32	Colour of labellum	1, yellow; 2, purple; 3, red; 4, white
33	Nectar guides	0, absent; 1, present
34	Opening of the thecae	1, half; 2, three-quarters; 3, entirely

Phylogenetic character mapping and biogeography

The five floral types identified in this study were mapped onto the existing phylogenetic tree of *Aframomum* (from Auvray et al. 2010) and ancestral states were reconstructed using Maximum Parsimony Analysis assuming unordered states (Fitch 1971) in Mesquite (Maddison & Maddison 2006).

Centers of diversity per floral type were established on a 1° grid in Q-GIS Version 1.6 (“*Quantum GIS (2010) Système d’Information Géographique, Projet de l’Open Source Geospatial Foundation*”; <http://qgis.osgeo.org>”) using collection data from Harris & Wortley (submitted) assuming for each species a continuous distribution area within the limits of its most distant/peripheral collection points. Habitat descriptions were taken from Harris & Wortley (submitted). Both

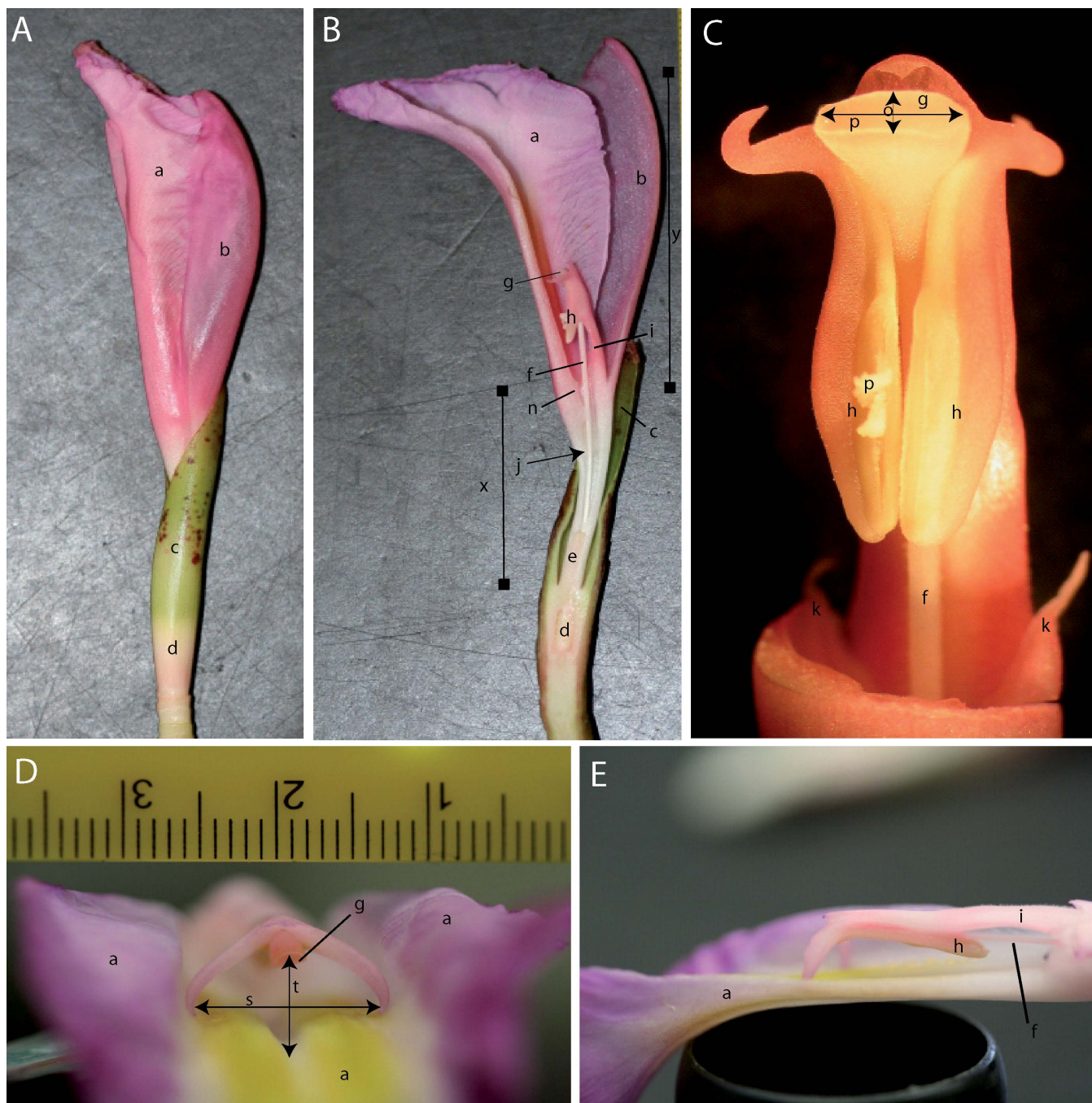


Figure 1 – Flower morphological measurements exemplified on *Aframomum tchoutoui*. A, lateral view of entire flower; B, longitudinal section of flower; C, anther (illustrating thecae opening 3/4); frontal (D) and lateral (E) view of the complex of anther, style and labellum. Abbreviations: a, labellum; b, dorsal petal; c, calyx; d, ovary; e, epigynal glands; f, style; g, style head; h, thecae; i, filament; n, narrowing of the floral tube; p, pollen; q, width of stigmatic cavity; o, height of stigmatic cavity; s, arch formed by lateral anther appendages; t, distance between thecae and labellum surface; x, floral tube; y, floral tube elongation.

datasets were then interpreted in the context of the species phylogeny (Auvray et al. 2010), focusing on the comparison of sister species.

RESULTS

Floral types in *Aframomum*

The multidimensional scaling analysis of the morphological datasets suggests the establishment of five floral types (fig. 2) which can be described as follows:

1. Trumpet type – This floral type occurs in 45 species (eleven identified from fresh or alcohol material; 34 from monographic descriptions; electronic appendices 2 & 3; fig. 3A & B) and is the most frequent type (60% of all species) found in *Aframomum*. The flowers of this type are characterized by the delicate tissue and the mostly purple colour of petals and the labellum (occasionally rather dark in e.g. *A. sceptrum*, or very pale to almost white in, for example, *A. alboviolaceum* and *A. mildbraedii*), and an elongation (y) of the floral tube (x, 3.86 ± 0.65 cm; fig. 1). This elongation of the floral tube is achieved by a lateral overlapping of the broad dorsal petal (dp) and the labellum (l) to a total flower length of 8.74 ± 1.48 cm ($n = 11$). The dorsal petal and labellum are much broader than in any of the other types (dp: 2.87 ± 0.62 cm; l: 4.35 ± 0.37 cm; $n = 11$; table 2). Apically, the dorsal petal and labellum are slightly bent, so that the floral entrance is rather horizontally arranged. The

labellum is usually a bit longer than the dorsal petal and occasionally quite elaborate at its tip (e.g. *A. limbatum*) forming a broad conspicuous ring around the floral entrance and a kind of landing platform for pollinators (fig. 3A & B). In its centre and inside the floral tube, but sometimes visible from the outside, the labellum often exhibits yellow nectar guides – the intensity of the yellow colour varies between species (compare fig. 1A & B). Additionally, exactly opposite to the thecae there is a channel in the labellum which achieves its maximum depth opposite to the thecae (fig. 1D). Due to the significant elongation of the floral tube the anther is about 3 cm below the end of the tube and is thus invisible from the outside. The filament is arranged parallel to the labellum and the thecae are thus positioned at a distance of 0.45 ± 0.07 cm from the labellum surface (fig. 1D). The thecae split open only along a proportion of their length (see fig. 1C). In most species this is only half the length of the thecae from the base but in some species such as *A. limbatum* the split reaches $\frac{3}{4}$ of the length from the base. In the species where fresh material was available nectar was observed filling the floral tube up to the narrowing of the floral tube (n) (fig. 1).

In the multidimensional scaling analysis all species of this type cluster together, although there is considerable variability in size. The largest species are *A. aulacocarpos* and *A. citratum* (labellum length incl. floral tube: 11.95 ± 0.78 cm, $n = 2$) and the smallest is *A. tchoutoui* (labellum length incl. floral tube: 7.8 cm). These species thereby represent the two

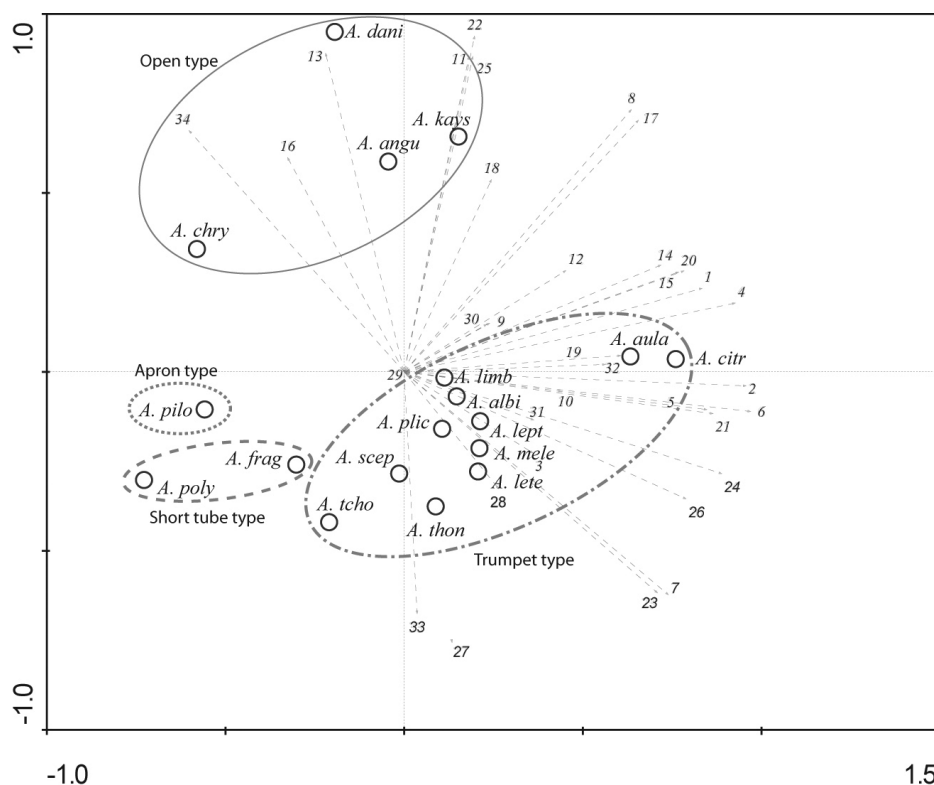


Figure 2 – Multi-dimensional scaling analysis of 29 quantitative and five qualitative flower morphological characteristics in eighteen *Aframomum* species and indications of floral types. Dotted-hatched line, Trumpet type; solid line, Open type; dotted line, Apron type; hatched line, Short tube type. Numbers indicate morphological character traits in table 1. Species names are abbreviated to the first four letters of the species name. For entire names see electronic appendices 1 & 2.

Table 2 – Summary statistics of 29 quantitative and five qualitative flower morphological characteristics in four floral types (average +/- standard deviation) collected on eighteen species of *Aframomum*.
a., anther; dp, dorsal petal; l., labellum; nb., number.

Characteristics	Trumpet Type (PTT) (n = 11)	PTT all except aulacocarpos /citrat	PTT only aulacocarpos /citrat	Open Type (n = 4)	Short Tube Type (n = 2)	Apron type (n = 1; <i>A.pilosum</i>)
floral measurements [mm]						
1 Length of calyx	4.45 +/- 1.13	4.1 +/- 0.9	6 +/- 0.71	3.93 +/- 0.96	2.25 +/- 0.35	3.2
2 Length of lateral petal	8.25 +/- 1.4	7.73 +/- 0.87	10.6 +/- 0.14	6.35 +/- 1.48	4.75 +/- 1.34	6
3 Width of lateral petal	0.91 +/- 0.26	0.87 +/- 0.19	1.1 +/- 0.57	0.65 +/- 0.13	1.05 +/- 0.21	0.4
4 Length of dorsal petal	8.75 +/- 1.49	8.2 +/- 0.95	11.2 +/- 0.42	7.9 +/- 1.42	4.5 +/- 1.27	6
5 Width of dorsal petal	2.87 +/- 0.62	2.7 +/- 0.53	3.65 +/- 0.21	2.03 +/- 0.5	1.6 +/- 0.57	1.2
6 Length of labellum	9.61 +/- 1.48	9.09 +/- 0.99	11.95 +/- 0.78	7.2 +/- 1.35	5.65 +/- 1.48	5.6
7 Width of labellum	4.35 +/- 0.57	4.26 +/- 0.58	4.75 +/- 0.35	1.88 +/- 0.44	2.85 +/- 1.2	1.9
8 Length of anther	5.53 +/- 0.89	5.22 +/- 0.65	6.9 +/- 0	6.5 +/- 1.11	3.7 +/- 0.71	4.9
9 Length of the anther's appendix	0.31 +/- 0.22	0.31 +/- 0.25	0.33 +/- 0.11	0.2 +/- 0.08	0.05 +/- 0.07	0.1
10 Length of appendage at base of a.	0.33 +/- 0.28	0.31 +/- 0.26	0.45 +/- 0.49	0.33 +/- 0.1	0.25 +/- 0.07	0.1
11 Length of anther above floral tube	1.69 +/- 0.38	1.6 +/- 0.36	2.1 +/- 0.14	2.8 +/- 0.68	1.3 +/- 0.14	0.9
12 Width of arche formed by lateral appendage of a.	0.99 +/- 0.19	0.96 +/- 0.19	1.15 +/- 0.07	1.08 +/- 0.19	1 +/- 0.28	0.7
13 Distance between thecae and l surface	0.45 +/- 0.1	0.44 +/- 0.11	0.45 +/- 0.07	1.85 +/- 0.37	0.5 +/- 0.14	0.4
14 Length of thecae	1.2 +/- 0.3	1.1 +/- 0.22	1.65 +/- 0.07	1.2 +/- 0.12	1 +/- 0	0.7
15 Length of epigynal glands	1.07 +/- 0.24	1.02 +/- 0.22	1.3 +/- 0.28	0.98 +/- 0.39	0.5 +/- 0.28	0.25
16 Width of epigynal glands	0.14 +/- 0.03	0.14 +/- 0.04	0.15 +/- 0	0.21 +/- 0.06	0.18 +/- 0.04	0.2
17 Length of style	5.45 +/- 0.9	5.14 +/- 0.64	6.85 +/- 0.07	6.33 +/- 1.14	3.7 +/- 0.71	4.9
18 Width of stigmatic cavity	0.3 +/- 0.08	0.28 +/- 0.08	0.35 +/- 0.07	0.36 +/- 0.07	0.25 +/- 0.07	0.3
19 Height of stigmatic cavity	0.11 +/- 0.03	0.1 +/- 0	0.18 +/- 0.04	0.1 +/- 0.04	0.13 +/- 0.04	0.05
20 Length of floral tube	3.86 +/- 0.66	3.66 +/- 0.52	4.8 +/- 0.14	3.7 +/- 0.88	2.4 +/- 0.57	4
21 Distance below floral tube narrowing	2.42 +/- 0.59	2.19 +/- 0.31	3.45 +/- 0.35	1.65 +/- 0.56	1.65 +/- 0.21	2.1
22 Distance between style and nectar	3.11 +/- 0.39	3.03 +/- 0.37	3.45 +/- 0.35	4.85 +/- 0.7	2.05 +/- 0.49	2.8
23 Difference in length between l. and a.	4.08 +/- 0.89	3.87 +/- 0.79	5.05 +/- 0.78	0.7 +/- 0.77	1.95 +/- 0.78	0.7
24 Length of l above floral tube	5.75 +/- 1	5.43 +/- 0.78	7.15 +/- 0.64	3.5 +/- 0.5	3.25 +/- 0.92	1.6
25 Length of anther above floral tube	1.66 +/- 0.43	1.57 +/- 0.41	2.1 +/- 0.14	2.8 +/- 0.68	1.3 +/- 0.14	0.9
26 Difference in length between dp and a.	3.22 +/- 1.06	2.98 +/- 1.01	4.3 +/- 0.42	1.4 +/- 0.65	0.8 +/- 0.57	1.1
27 Difference in length between l and dp	0.86 +/- 0.81	0.89 +/- 0.9	0.75 +/- 0.35	-0.7 +/- 0.22	1.15 +/- 0.21	-0.4

Table 2 (continued) – Summary statistics of 29 quantitative and five qualitative flower morphological characteristics in four floral types (average \pm standard deviation) collected on eighteen species of *Aframomum*.
a., anther; dp, dorsal petal; l., labellum; nb., number.

Characteristics	Trumpet Type (PTT) (n = 11)	PTT all except aulacocarpos /citrat	PTT only aulacocarpos /citrat	Open Type (n = 4)	Short Tube Type (n = 2)	Apron type (n = 1; <i>A. pilosum</i>)
Floral elements						
28 Nb of appendages at the base of the a	1.36 \pm 0.5	1.33 \pm 0.5	1.5 \pm 0.71	1 \pm 0	1 \pm 0	1
29 Number of epigynal glands	2 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0	2
Qualitative floral characteristics						
30 Colour of dorsal petal	Purple			white, yellow, red	yellow, red	yellow
31 Comparison of l and petal colour	Purple			white, yellow, red	yellow, red	yellow
32 Colour of labellum	Purple			white, yellow, red	yellow, red	yellow
33 Nectar guides	yes			no	yes	no
34 Opening of the thecae	1/2 to 3/4			entirely	half to entirely	entirely

extremes in floral size found in this type within the genus (see electronic appendix 2).

The pollination experiments have shown that the anther, although it seems to be rather stout, is actually slightly flexible in this floral type (see electronic appendices 5 & 6 in supplementary material). It can be pushed backwards to broaden the space between thecae and labellum surface.

2. Open type – This type is found in five species (four identified from fresh or alcohol material; one from monographic descriptions; electronic appendices 2 & 3, fig. 3). As the name of this type suggests these flowers are rather open, thus above the floral tube (3.7 ± 0.88 cm) dorsal petal and labellum do not overlap to elongate the tube. While the dorsal petal stands straight upright (total floral length: 7.9 ± 1.42 cm; $n = 4$), the labellum is bent backwards and much narrower (1.87 ± 0.44 cm; $n = 4$) than in the Trumpet type. Labellum and petals are made up of a much stronger tissue than in the Trumpet type. Due to the backwards bending of the labellum the anther is entirely visible. The filament is arranged parallel to the dorsal petal and only bends a very little towards the labellum at its apical end so that the thecae are positioned at a greater distance to the labellum (1.85 ± 0.37 cm; $n = 4$). The thecae open entirely in all the investigated species. The floral colour of these species is either yellow, pink, red or white (in *A. kayserianum*). There are no nectar guides present; however, there is a groove on the adaxial side of the labellum all along the mid-vein.

In the multidimensional scaling analysis all species of this type are well separated from species of all other types. The distant position of *A. chrysanthemum* in this analysis from all other species of this Open type is due to its much smaller size.

3. Apron type – Three species have been attributed to this type based on alcohol material and the literature descriptions (*A. luteoalbum*, *A. pilosum*, *A. wuerthii*) (electronic appendices 2 & 3). These species present again a more open flower where the dorsal petal and labellum are not overlapping, and like in the Open type, the dorsal petal is upright and the labellum bent backwards. In contrast to the Open type, the labellum is generally very broad and elaborate forming a landing platform and due to a slight forward bent of the anther the distance between anther and labellum surface is rather small (*A. pilosum*: 0.4 cm). The colour of these flowers is white or yellow. *A. wuerthii* additionally possesses bright red bracts. The species vary greatly in size the dorsal petal being only 0.3 cm long above the floral tube in *A. wuerthii*, 2 cm in *A. pilosum* and 4 to 5 cm in *A. luteoalbum*.

For the multidimensional scaling analysis we possessed only data for *A. pilosum* and it clustered far from all other floral types, stressing its distinct combination of characters

4. Short tube type – This type includes two species, both identified from fresh or alcohol material (*A. polyanthum*, *A. fragrans*) (electronic appendices 2 & 3; fig. 3). The characteristic of this type is the rather short elongation of the floral tube (2.4 ± 0.57 cm; $n = 2$) to a total floral length of 4.5 ± 1.27 cm by a lateral overlap of dorsal petal and labellum. The anther reaches up to 1.4 cm below the rim of this elongated tube. The distance between the labellum and the parallel-arranged anther is comparable to the Trumpet type (0.5

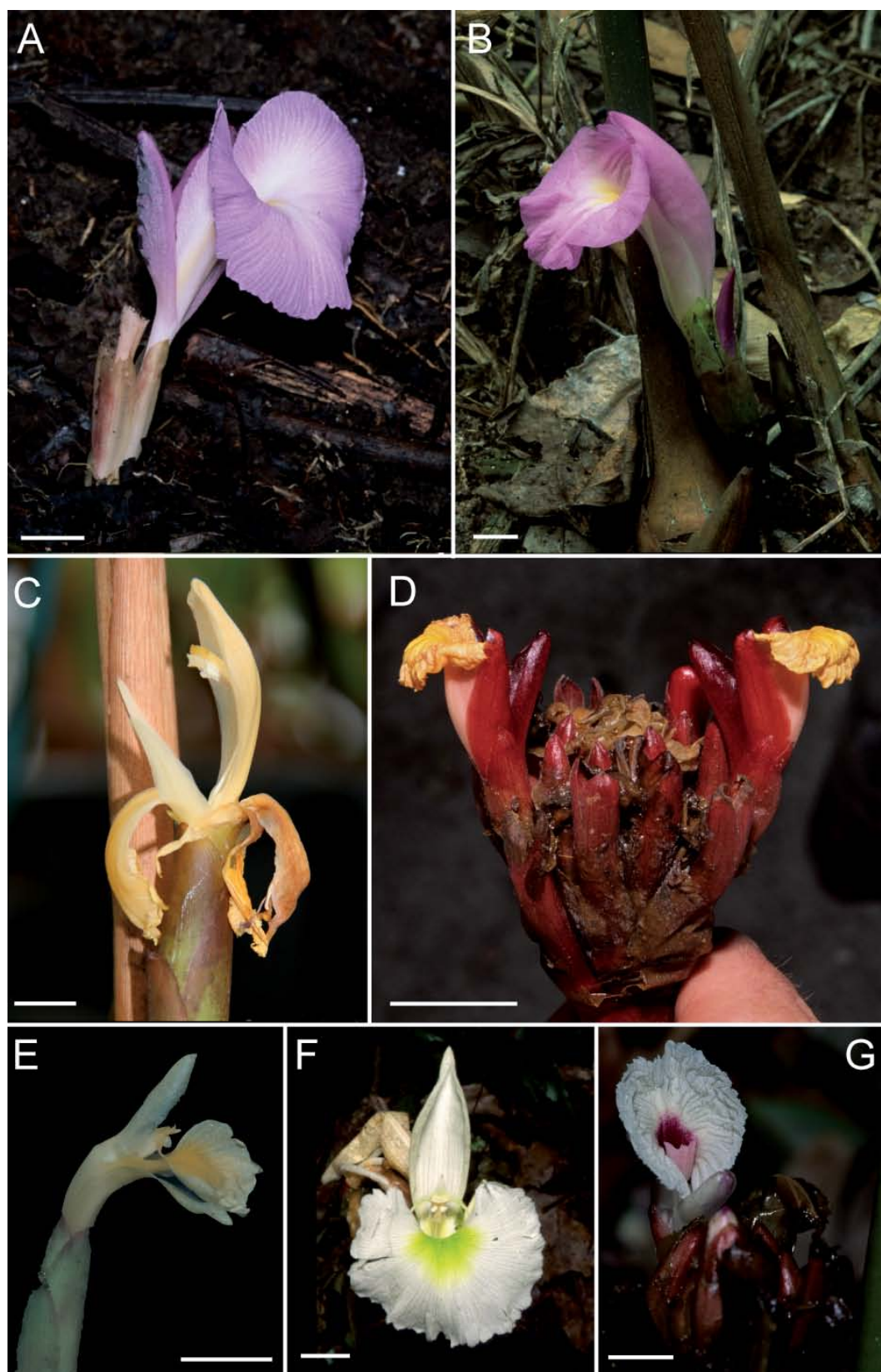


Figure 3 – Five floral types in *Aframomum*. Trumpet type exemplified by *A. limbatum* (Harris 6741) (A) and *A. thonneri* (Harris 5749) (B); the open type represented by *A. daniellii* (Harris 6753) (C); the Short tube type exemplified by *A. polyanthum* (Harris s.n.) (D); the Apron type depicted by *A. pilosum* (Harris 5570) (E) and *A. luteoalbum* (Poulsen 670) (F, photo by A.D. Poulsen); the collar type found in *A. zambesiaceum* (Harris 6761) (G).

+/- 0.14 cm), however, the entrance of the tube is directed vertically and not horizontally as in the Trumpet type. Still, a narrow landing platform for pollinators is formed through the backwards bending of the labellum tip. The colour of these flowers is generally yellow or red. In *A. fragrans* corolla and labellum are yellow and in *A. polyanthum* the flower is reddish with a yellow tip to the labellum and bright red bracts. There are red nectar guides on yellow background in *A. polyanthum*. In *A. fragrans* nectar guides are absent.

In the multidimensional scaling analysis *A. fragrans* is located half way between *A. polyanthum* and species of the Trumpet type and could be termed an intermediate between those two types. Its tube is a bit longer than in *A. polyanthum*, the tissue of petals and labellum rather delicate and its thecae opens only half as in the Trumpet type. In *A. polyanthum* the thecae opens entirely. However, floral colour and the direction of the floral entrance in *A. fragrans* are more similar to *A. polyanthum* than to the Trumpet type.

5. Collar type – So far only one species, *A. zambesiaceum*, has been identified in this type (identified from the literature descriptions only) (electronic appendices 2 & 3; fig. 3). As in the Trumpet and Apron type the labellum is the most conspicuous organ, however, this time it stands upright and looks like an elaborate white collar or ruff, underlined by its lengthwise plications. The white labellum overlaps laterally with the dorsal petal; in its centre there is a conspicuous pink anther. This anther appears particular thick on the photo leaving only a tiny space towards the labellum. The dorsal petal is transparent and inconspicuous.

As no flower material was present for direct inspection this species is absent from the multidimensional scaling analysis.

Evolutionary reconstruction of floral types

The mapping of floral types onto the phylogeny of 21 *Aframomum* species suggested a repeated independent evolution of each of the described floral types (see fig. 4). Six shifts in floral types were inferred (~30% of all speciation events). The most common floral type, the Trumpet type, was recovered as the ancestral state.

Biogeography of *Aframomum* in tropical Africa

The genus *Aframomum* is widely distributed in tropical Africa from Senegal to Ethiopia and Mozambique, with one species, *A. angustifolium* extending as far as Madagascar. Almost all species occur in tropical rainforest with only one species, *A. alboviolaceum*, widely distributed in the savanna. The species diversity is at its highest in Southern Cameroon. The distribution of some species is widespread (5 spp. / 7% of species span across Upper and Lower Guinea and Congolia (sub-centres of endemism defined by White 1979); 1 spp. / 1% span across Upper and Lower Guinea; 14 spp. / 25% of species occur across Lower Guinea and Congolia) but most species are locally restricted within one centre of endemism only (41 spp. / 67%) (electronic appendix 2).

The distribution of the Trumpet type (45 species) ranges from Senegal in West Africa to Ethiopia and Mozambique in East Africa (fig. 5A). The centre of diversity is situated in

Southern Cameroon and the Republic of Congo with a maximum of thirteen sympatric species in central South Cameroon and the border triangle of Cameroon, the Republic of Congo and Central Africa. Looking at individual species, their known distribution ranges are generally rather small being restricted to one of the centres of endemism (after White 1979: Upper Guinea: *A. cordifolium*, *A. longiscapum*, *A. sulcatum*; Lower Guinea: *A. arundinaceum*, *A. citratum*, *A. pseudostipulare*, *A. aulacocarpos*, *A. sericeum*; Congolia: *A. singulariflorum*; Lower Guinea + Congolia: *A. letestuanum*, *A. subsericeum*, *A. thonneri*, *A. verrucosum*) or even smaller units, such as the Cameroonian Mountain range (e.g. *A. kayserianum*, *A. kodmin*), the mountains of East Africa (e.g. *A. korarima*, *A. luteoalbum*, *A. mildbraedii*), the Eastern Arc Mountains of Tanzania (*A. laxiflorum* and *A. alpinum*) the Tanzanian coast (*A. orientale*), or a part of Eastern central Africa from South East DR Congo across to Tanza-

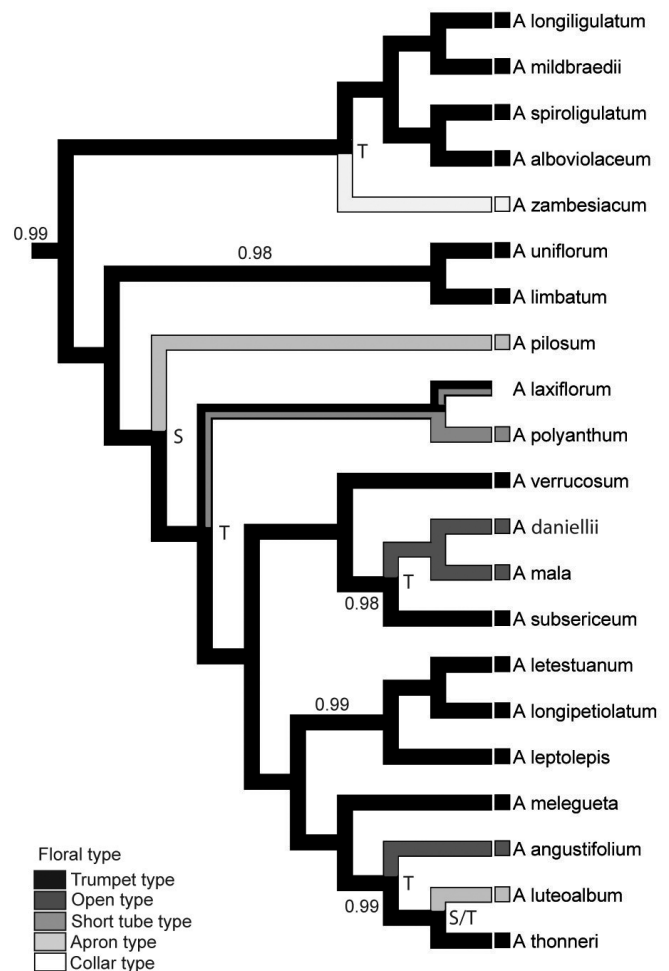


Figure 4 – Maximum parsimony mapping of floral types (A) onto a simplified Bayesian phylogeny of a combined dataset of ITS and *trnL-F* region after Auvray et al. (2010) using Mesquite (Maddison & Maddison 2006). Hypothesized main pollinator indicated behind each taxon. Values along branches indicate posterior probabilities (only values above 0.98 are given). T, potential pollinator shift from bee to bird. S, potential shift between different bee species.

nia, Zambia, Malawi and Mozambique (*A. albiflorum*). Only three species show a continuous wide distribution from West to East Africa (*A. alboviolaceum*, *A. limbatum* and *A. sceptrum*).

The distribution areas of the species of the Open type (5 species, fig. 5B) are mostly allopatric being restricted either in upper Guinea (*A. chrysanthemum*) or central Africa (*A. daniellii*), in the Cameroonian mountains (*A. kayserianum*)

or Eastern arc mountains (*A. mala*). Only *A. angustifolium* is widespread throughout the area from Senegal to Madagascar overlapping with the respective distribution range of all other species. In this group, however, we can often observe a habitat differentiation. In the Dzanga-Sangha area in Central African Republic (Harris 2002), for example, where *A. angustifolium* and *A. daniellii* both occur, *A. angustifolium* occurs only in wet places and *A. daniellii* on *terra firma*.

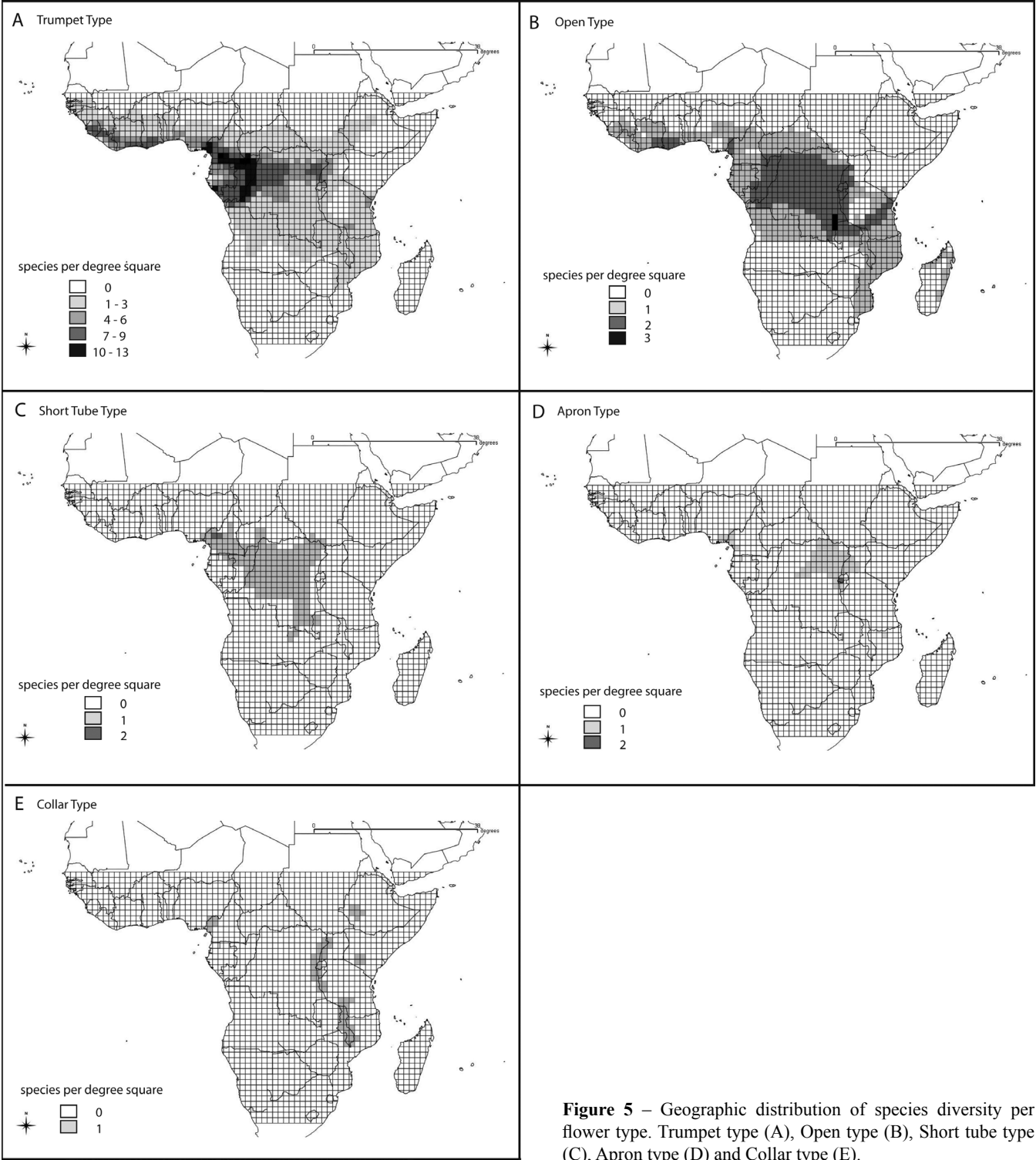


Figure 5 – Geographic distribution of species diversity per flower type. Trumpet type (A), Open type (B), Short tube type (C), Apron type (D) and Collar type (E).

Table 3 – Key flower morphological traits and sample species of five floral types in *Aframomum*.

Length measurements: average +/- standard deviation; * data based on a combined dataset with data from this paper and Harris & Wortley (submitted).

Floral type		Number of species	Description	Dorsal petal (cm)*	Labellum (cm)*	Example species
1	Trumpet type	45	Purple to white colour; long tube elongation formed by lateral overlapping dorsal petal and labellum; broad elaborate labellum	3.94 +/- 3.43	5.61 +/- 2.51	<i>A. giganteum</i>
2	Open type	5	Yellow, white or red colour; no floral tube elongation; anther aligned with dorsal petal; large distance (>2 cm) between thecae and labellum	3.8 +/- 0.82	3.2 +/- 0.79	<i>A. daniellii</i>
3	Apron type	3	Yellow, white and red colour; open tube; elaborate (“apron”) labellum; anther aligned with labellum	1.82 +/- 5.51	2.12 +/- 6.24	<i>A. pilosum</i>
4	Short tube type	2	Yellow and red colour; short tube elongation by lateral overlapping of dorsal petal and labellum	1.35 +/- 0.04	1.75 +/- 0	<i>A. polyanthum</i>
5	Collar type	1	Labellum upright standing with longitudinal folds (“collar”); tube elongation formed by labellum and anther	0.6	2	<i>A. zambesiaccum</i>
Unassigned		5		na	na	
Total		61				

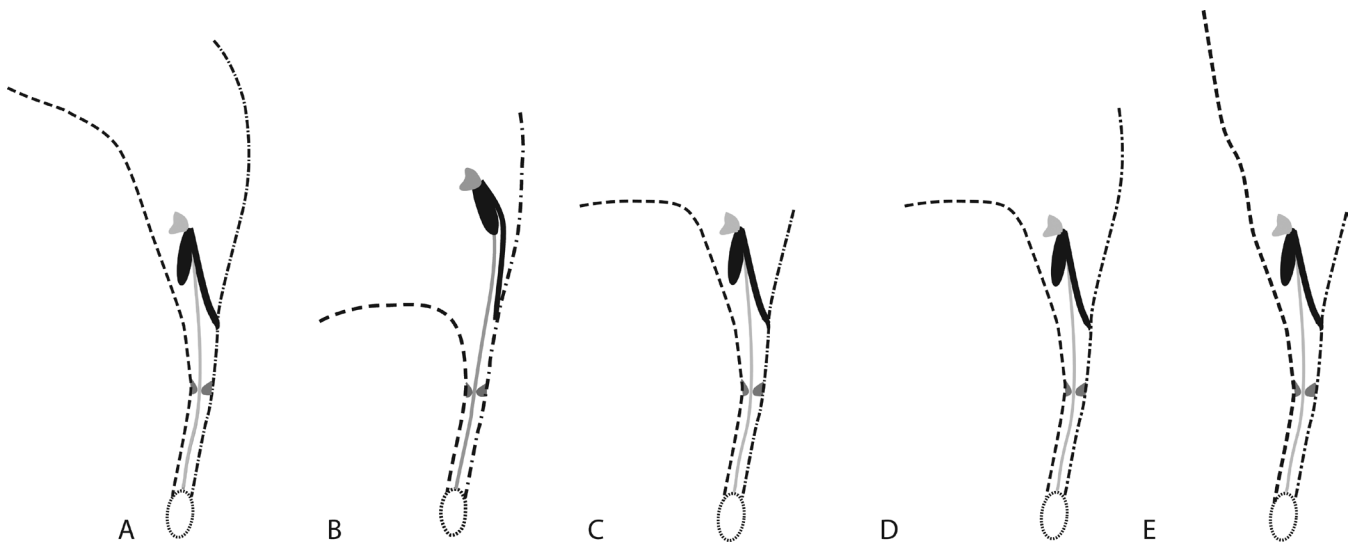


Figure 6 – Model of *Aframomum* flower and the derivation of five distinct floral types based on differences in the relative length and position of the dorsal petal, the labellum and the complex of style and anther. (A) Trumpet type: lateral overlap of dorsal petal and labellum resulting in an elongation of the floral tube which is much longer than the complex of style and anther (csa), horizontally arranged floral entrance; (B) Open type: dorsal petal longer than csa, labellum short and backwards bent basal to the csa, with csa parallel to the dorsal petal resulting in large distance between thecae and labellum surface; (C) Short tube type: dorsal petal as long as the csa, labellum reflexed at the height of the apical end of the csa, floral entrance vertically arranged; (D) Apron type: dorsal petal longer than csa, labellum backwards bent basal to the csa, with csa parallel to the labellum, labellum and dorsal petal non-overlapping; (E) Collar type: dorsal petal as long as the csa, labellum upright standing and much longer than the csa. Thick black line, anther basally fused with floral tube; light grey line, style; dark grey, thickening of the wall of the floral tube resulting in a narrowing of the tube; dotted line, ovary; hatched line, labellum; dotted-hatched line, dorsal petal.

Of the two species of the Short tube type (fig. 5C) *A. polyanthum* is widely distributed in Lower Guinea occurring in swamps and along streams usually in full sunlight. It overlaps in the west with *A. fragrans* which is restricted to the Western Cameroonian mountain range and found there mainly in the shady forest understorey.

The species of the Apron type (three species, fig. 5D) show a disjunct distribution with *A. luteoalbum* in east D.R.Congo and Uganda, *A. wuerthii* restricted to Rwanda and *A. pilosum* only found in the Atlantic forests of Cameroon and Nigeria.

The only species of the Collar type (fig. 5E), *A. zambeziacum*, presents a disjunct area (above 1000 m) in between the more restricted highlands of Cameroon and the much wider highlands of East Africa from Ethiopia to Malawi.

DISCUSSION

General 'Bauplan' and floral type variation in the *Aframomum* flower

Comparing all investigated *Aframomum* flowers reveals a common 'Bauplan'. The flower consists of an inferior ovary, three free inconspicuous sepals, a floral tube due to basal fusion of all organs of corolla and androecium with an apical interior thickening resulting in a narrowing of the tube, two generally inconspicuous narrow lateral petals and one larger dorsal petal, a labellum of varying conspicuousness depending on floral type, a single fertile anther and a style that forms a complex with the anther by being positioned between the thecae and ending in a globular head with the opening of the stigmatic cavity directed slightly towards the front of the flower instead of straight up in the direction of the apex.

The universal interior narrowing of the floral tube in all floral types could serve to hold the nectar within the flower as already proposed in many other taxa (Wester & Claßen-Bockhoff 2007, Ley 2008) however since the flowers are held upright it is more probable in our opinion that the narrowing acts to prevent non-pollinator from stealing nectar. Lateral hairs in the floral tube might contribute to capillary upward transport of nectar in the corolla tube) and have been reported in a similar position in the related genus *Etlingera* by Poulsen (2006) but apparently without the same narrowing of the tube that occurs in *Aframomum*.

Distinct floral types differ only in the relative length and position of the dorsal petal, the labellum and the complex of style and anther (fig. 6, table 3). In four of the five types the dorsal petal and labellum are broad and overlap laterally so that they form an elongation (y) of the floral tube (= floral part where all inner organs except the sepals are fused). This results in the anther not being visible from the outside of the flower (fig. 6A, C, D & E). Only in the Open type (fig. 6B) is the labellum comparatively narrow, and it bends outwards directly above the floral tube so that there is no elongation of the floral tube and the thecae are entirely visible. Another peculiarity of the Open type is the arrangement of the filament parallel to the dorsal petal and not to the labellum as in all other types resulting in a large distance between thecae and labellum. The floral tube elongation, made by the lateral overlap of labellum and dorsal petal in most floral types, is

extremely long in the Trumpet type (fig. 6A) and rather short in the Short tube type (fig. 6C). In both types the dorsal petal and the reflexed labellum form a common rim at the floral entrance. In contrast, in the Collar type (fig. 6E) the labellum is not reflexed but stands straight and is thus significantly longer than the dorsal petal. Finally, in the Apron type (fig. 6D) the flowers also appear rather open due to a basally reflexed labellum, comparable to the Open type. However, the filament is arranged in parallel to the labellum and not to the dorsal petal and additionally the labellum tip is much broader and more elaborate than in the Open type.

Floral types are further distinct in their colouration. Most species with the Trumpet type are purple with a few species either very pale purple or white. The colour purple is basically absent from all other types in which the dominant colours are white, yellow and red.

These simple differences in position and colour might explain the recurrent independent evolutionary origin of the same floral type from the most frequent Trumpet type which has been inferred as the ancestral state in the phylogeny of this genus (fig. 4).

The diversity of floral types – preliminary hypotheses on pollinators

The great variation in size, colour, position of thecae and the direction of the floral entrance in different floral types suggests adaptations to a variety of different pollinators (Faegri & van der Pijl 1966, Johnson et al. 1998). As only very few direct observations on pollinators have so far been made, we want here to only briefly focus on the morphological differences between floral types. Each of these types has specific cues which are often summarized as floral syndromes (Hess 1983, Ollerton & Watts 2000). These suggest the reception of different pollinators (see also Miyake & Yahara 1998). We propose preliminary hypotheses about pollinators for each floral type based on knowledge from other plant groups and the available pollinators. These hypotheses need to be tested by direct observations in the field.

To our knowledge, so far the only published observations refer to flowers of the Trumpet type from Ghana, where regular visits of a long-tongued bee (*Anthophora vivida* Smith) have been reported (Lock et al. 1977). The narrow and long elongation of the floral tube of the Trumpet type flowers made up of a very soft tissue suggests that medium-sized bees are probably the only pollinators. They present the adequate size to touch the thecae which are at a distance of about 0.5 cm from the labellum on which bees have been observed to land and walk down to the nectar (Lock et al. 1977). The yellow nectar guides and the longitudinal depression in the labellum are recognized guiding devices for the bees on their way towards the nectar at the bottom of the flowers (Osche 1983, Lunau 1993, 1995, 1996, 2000). Birds are excluded by the very long and curved tube elongation which inhibits the penetration of the relatively short beak and large head to access the nectar. The hypothesis that large carpenter bees (*Xylocopa* spp.) act as pollinators for Trumpet type flowers is less clear. The carpenter bees prefer the canopy and forest gaps rather than the understorey (Davis 1987, Ley & Claßen-Bockhoff 2009) and concentrate on mass flowering species

to satisfy their energetic requirements (Louw & Nicolson 1983, Gerling et al. 1989). Some species of *Aframomum* of the Trumpet type are common in forest gaps and along forest edges but others are more restricted to the understory. Their flowers are generally very close to the ground and only few of them open synchronously per inflorescence (Harris & Wortley submitted) but each flower provides a large quantity of nectar. Nevertheless, different sized bees might act as specific pollinators in different *Aframomum* species of this floral type considering the large range of floral sizes detected.

Another floral type for which the main pollinators are probably bees is the Apron type due to the rather delicate nature of its petals and labellum, the presence of pronounced nectar guides and the rather horizontally arranged floral entrance which might render the access for birds rather difficult, however, not impossible.

In contrast, the flowers of the Open type are probably only pollinated by birds as the distance between thecae and reflexed labellum (> 1.5 cm), which might serve bees as a landing platform (1.85 ± 0.37 cm; $n = 4$), exceeds the size of any known bee (Eardley & Urban 2010). The pollen could be deposited in large quantities by the entirely opened thecae on the bird's head when the latter enters the beak into the floral tube to access the nectar (Wester & Claßen-Bockhoff 2007). A bird might be able to perch on either the inflorescence stem or the base of an adjacent leafy shoot. The bilateral floral symmetry might force birds into the same position each time to ensure pollen transfer between conspecific flowers. The yellow, red and white coloration has been reported repeatedly in bird pollinated flowers (Vogel 1954, Wester & Claßen-Bockhoff 2007, Ley & Claßen-Bockhoff 2009).

The species of the Short tube type and the Collar type might be predominantly bird pollinated based on their coloration (yellow, red, white) and the rather restricted or even absent landing platform, respectively, which might partially or entirely exclude bees. The stout inflorescence dominated by red bracts could serve as attraction and landing device especially for birds. Similar red bracts attract birds in *Marantochloa conferta* (treated as *Ataenidia conferta* in Ley & Claßen-Bockhoff 2009). The rather narrow floral entrance of the Collar type might prevent bees from reaching the nectar. Here alternatively, butterflies might play a role in pollination transmitting pollen deposited onto their proboscis.

Mixed pollination systems including birds and bees as pollinators have been observed in other tropical understorey herbs (Holsinger 1991, Kato et al. 1993, Sakai et al. 1999, Sakai & Nagamasu 2003, see Marantaceae in Ley & Claßen-Bockhoff 2009) and this may turn out to be the situation in the Apron, Short tube and Collar type.

The inclusion of further species into the multidimensional scaling analysis in the future depending on data availability might reveal even greater variability within types and indicate potential developmental pathways between floral types.

The function of different floral types, habitat adaptations and geographic distribution in the maintenance of species boundaries in *Aframomum*

The adaptation to different habitats, the occurrence in different geographic areas (allopatry) and the pollination by different pollinators or the differential deposition of pollen onto the same pollinator, i.e. mechanical isolation (Grant 1994) within groups of closely related taxa are often regarded as potential mechanisms for the maintenance of species boundaries (van der Niet & Johnson 2012).

In the currently available phylogenetic tree of *Aframomum* there are indications for at least four splits that show a change in floral types which might be associated with a shift in pollinators: here especially from bees to birds (see fig. 4: T) but also between different sized bee species (see fig. 4: S), both of which might have contributed to the formation and maintenance of species boundaries. We further predict several incidences of genetic isolation between species from different floral types which presumably use the same pollinator, e.g. the Open type (bird pollinated) versus the floral types of potentially mixed (bird and bee) pollination systems such as Apron, Short tube and Collar type. This is because there are pronounced differences in thecae-labellum distance between these types suggesting pollen deposition onto the bird's head in the Open type and onto the bird's beak in Apron, Short tube and Collar type. Within the Open type species boundaries might be maintained through incompatibility of sympatric species due to larger phylogenetic distances (e.g. *A. daniellii* and *A. angustifolium*, fig. 4), differential pollen deposition onto the same bird head due to distinct thecae positions (*A. daniellii* against all other species within this floral type see electronic appendix 3: character 11) and allopatry (e.g. *A. chrysanthum* against *A. angustifolium* and *A. kayserianum*, fig. 5B).

Comparing the Trumpet and Apron type we assume pollen transport by different bee pollinators due to different stimulating cues (e.g. different colours or open flower versus long tube elongation) (see indicated splits in phylogenetic tree; fig. 4: S). Whereas in these two types pollen would be deposited onto the back of bees, pollen might be deposited onto the bees' proboscis in the Collar type due to the small space between thecae and labellum which might prevent bees from entirely entering the floral tube. We do not yet see any isolation of pollen transport between species of the Trumpet and the Short tube type, as the thecae is always positioned within the elongation of the floral tube at the same distance from the labellum so that pollen can always be deposited onto the bees' back.

A further enigma is the preservation of species boundaries between species of the Trumpet type. Whereas we might expect very small bee species as pollinators in the very small flowers of *A. tchoutoui* with a distance between thecae and labellum of only 0.2 cm which might prevent larger bees from entering these flowers, floral morphology in all other species seems to be rather uniform with a distance of thecae-labellum of about 0.5 cm (including the largest flowers *A. aulacocarpos* and *A. citratum*) and adapted to efficient pollen placement onto the back of medium-sized bees. Thus this high similarity within all these species of the Trumpet

type which forms the largest group of flowers in *Aframomum* (~45 species) with many closely related sympatric species (e.g. *A. mildbraedii*, *A. spirologulatum*, compare electronic appendix 2 and fig. 5B) and little difference in pollinator size (e.g. distance thecae-labellum) or colour variation, all of which suggest isolation mechanisms other than mechanical isolation in the pollination system. In the Sangha Trinational Area, for example, open flowers of the Trumpet type have been recorded at the same time for more than one species within a few metres of each other (for example *A. limbatum*, *A. sericeum*, *A. subsericeum* and *A. thonneri*) (Harris, pers. com.). Here phylogenetic isolation might prevent crossing between species (compare fig. 4). Instead species might take advantage of pollinator sharing (Tachiki et al. 2010). Further, a peculiarity of the species from the Trumpet type is the large number of species with a very restricted range size, suggesting that at present, persisting allopatry might play a major role in species maintenance.

CONCLUSION

The flowers of *Aframomum* prove to be quite plastic in establishing very distinct floral types based on a few changes in relative size and shape of organs but following a consistent general 'Bauplan' with no variation in number or relative position of floral organs. This suggests close adaptations to highly different pollinators. The combined analyses of floral morphology, phylogeny, distribution area and habitat within a large proportion of species in *Aframomum* indicate the occasional correlation of shifts in floral type and speciation events. These might be attributed to pollinator shifts in the evolutionary history of the genus in Africa. However, we predict that several other factors such as persisting allopatry, habitat differentiation and genetic incompatibility due to large phylogenetic distances have been and are still involved in species diversification and maintenance of species boundaries.

The analyses conducted here are intended to guide further research on speciation in *Aframomum*. First steps will be to improve the sampling and resolution of the phylogeny and confirm pollination scenarios in the field.

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>), and consist of: (1) list of fresh and/or in ethanol fixed material of plant species investigated in detail morphologically and the respective voucher specimen (pdf format); (2) geographic distribution and morphological characteristics (length of dorsal petal and labellum in centimeters) of 61 *Aframomum* species (pdf format); (3) morphological data matrix of 29 quantitative and five qualitative flower morphological characteristics measured in eighteen species of *Aframomum* arranged by floral type (pdf format); (4) experiments on anther flexibility in *A. tchoutoui* (Trumpet type) (film, MOV format); and (5) experiments on anther flexibility in *A. aulacocarpos* (Trumpet type) (film, MOV format).

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