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# Analysis of rhizome morphology of the Zingiberales in Payamino (Ecuador) reveals convergent evolution of two distinct architectural strategies

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# Analysis of rhizome morphology of the Zingiberales in Payamino (Ecuador) reveals convergent evolution of two distinct architectural strategies

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**Abstract:** Rhizome morphology of 18 Zingiberales species growing *in situ* in lowland Ecuadorian rainforest (Payamino) covering six of the eight families of the order is presented. Phenetic and morphological analyses reveal two strategies that vary starkly in their mode of construction and geometry. Furthermore, parsimony-based character evolution in a resolved phylogenetic framework identifies convergent evolution of these strategies. The two strategies uncovered by the phenetic and morphological analyses are correlated with branching localization and timing: species showing delayed, non-positional–preferential branching exhibit poorly predictable, non-geometric rhizomes whereas species exhibiting immediate branching occurring at specific internodes display predictable, highly geometric rhizome morphologies. Hence, the control of two simple developmental parameters defines two distinct modes of construction in basitonically branched, rhizomatous plants. Multiple switches in the state of these characters during the evolution of Zingiberales resulted in diversification and convergence of rhizome morphologies in the order.

**Keywords:** rhizomes; Zingiberales; convergent evolution; Tomlinson's model; phenetic analysis; branching timing; branching localization; starch storage

## Introduction

The term "rhizome" has recurrently been defined by many researchers of both plant morphology and physiology; so a consensual meaning of the term can be difficult to grasp. Rhizome generally refers to an underground shoot system (Bell 2008), a definition which excludes many rhizomatous plants, including epiphytes (Bell and Tomlinson 1980). Other definitions imply that rhizomes are thickened underground axes that play a storage and resting role (Warming 1918; Raunkiær 1934; Lorougnon 1969; Rogers, Dunn and Brown 1976; Paull, Jung Chen and Goo 1988; Granéli, Weisner and Sytsma 1992; Karunaratne, Asaeda, and Yutani 2004). Rhizomes often bear scale leaves, but in some species such as *Nypa fruticans*, the rhizome bears foliage leaves. Bell and Tomlinson (1980), in their extensive morphological study of rhizomatous plants, adopt a generous definition of rhizome as "vegetative extension over or within the substrate by means of axis elongation, and includes organs which may be distinguished more precisely as stolons, offsets or suckers and which may intergrade with tubers and corms". This topological definition, presenting fuzzy Arberian morphology features (*sensu* Rutishauser and Isler 2001), allows conceptualizing rhizome as an architectural habit, which has convergently evolved in

many plant groups. In the architectural system of Hallé and Oldeman (1970) and Hallé, Oldeman and Tomlinson (1978), updated in Hallé (2004), most rhizomatous plants conform to two models: Tomlinson's model if the rhizome is sympodial (the axis is edified by a succession of meristems and therefore each axis is constructed by a series of determinate modules) and Bell's model if monopodial (the axis is edified by a single meristem). A unique type of rhizome occurs in the mangrove palm *Nypa fruticans*, which is characterized by a plagiotropic stem branching by true dichotomy (Tomlinson 1971) (Schoute's model – [Hallé et al. 1978]). Tomlinson's model is characterized by a succession of orthotropic axes that branch basitonically. The succession of the proximal parts of the modules forms the sympodial rhizome while each distal portion of the modules forms the aerial part. Important differences in the architecture of the aerial part of the modules led to the recognition of several variants of Tomlinson's model, notably with regard to the position of the sexuality, which can be either terminal, as in Holttum's model, or lateral as in Corner's model (Hallé, Oldeman and Tomlinson 1978). Since in the initial definition of Tomlinson's model it was assumed that the aerial module part was generally unbranched, the model of McClure was established by Hallé, Oldeman and

Tomlinson (1978) for plants, most notably bamboos, in which the aerial part of the modules conforms to Roux's model. An extensive study of over 50 basitonically branched species has revealed a great diversity of aerial architectures with seven distinct types including architectures that conform to the models of Petit, Massart, Chamberlain and Leeuwenberg (Cremers and Edelin 1995). While the aerial part of modules has received much interest, little attention has been manifested for the proximal parts of the modules.

Many architectural traits have been regarded as strategies from an ecological viewpoint (Hallé, Oldeman and Tomlinson 1978; Bellingham and Sparrow 2000). Architectural strategy also refers to the variation of architecture in different environments such as closed or open canopies, and different soil types or wind regimens (Tomlinson 2009; Charles-Dominique, Edelin and Bouchard 2010; Charles-Dominique et al. 2012; Castellanos et al. 2011). Because of their growth orientation and structure, some axes may be involved in space or substrate exploration while others may be centred on exploitation by means of light capture or specialized in sexual reproduction (Barthélémy and Caraglio 2007). Therefore, the architectural specialization of rhizomatous plants can be considered as a strategy in that it is both a means of vegetative extension and of resource storage, thus impacting on population structure and resource allocation.

The evolution of plant architecture has received relatively little attention despite a well-resolved global angiosperm phylogeny (APGIII 2009). However, many studies have illustrated the relevance of architectural studies to track architectural variation and diversification within a group (Guillaumet 1973; Cremers 1975; Veillon 1978; Donogue 1981; Hallé et al. 2004 and references therein), some of which have envisaged evolutionary scenarios (Vester 1999; Enriquez et al. 2008). Likewise, the evolution of rhizome structure has been poorly studied, despite being an ancient type of organization which characterized many early land plants (some Protracheophytes, Zosterophylls and Early Lycopods, from the Early Devonian – Stewart and Rothwell 1993) and a growth strategy that convergently evolved in many plant groups from a wide range of habitats.

The Zingiberales is a major order of monocotyledons with pan-tropical geographic repartition comprising about 2300 species. The circumscription of the order was recognized by taxonomists and botanists alike as being "natural" before molecular phylogenetics (Tomlinson 1962; Cronquist 1981; Dahlgren, Clifford and Yeo 1985; Kress 1990). The order contains species of high economic importance such as banana, arrow-root and ginger as well as species of horticultural importance such as *Strelitzia reginae* and *Heliconia* species. Modular growth with presence of subterranean stems that most often correspond to rhizomes *sensu* Bell (2008) is one of the defining features of Zingiberales. The Zingiberales is divided into eight families namely Musaceae, Heliconiaceae, Strelitziaceae, Marantaceae, Lowiaceae, Cannaceae, Zingiberaceae and Costaceae. The Zingiberales phylogeny is well-resolved (Smith, Kress and Zimmer 1993; Kress et al. 2001) comprising a basal grade with the so-called "banana families" (Musaceae, Heliconiaceae, Strelitziaceae and Lowiaceae) and a clade containing the "ginger families" (Zingiberaceae, Costaceae, Marantaceae and Cannaceae) (Kirchoff et al. 2009).

Although the diversity of aerial architectures of basitonically branched plants has been stressed (e.g. Cremers and Edelin 1995), the structure of the proximal part of the axis has been essentially reduced to the distinction of "clustering" versus "stoloniferous" behaviours in Tomlinson's model with regard to the length of the subterranean axis (Hallé, Oldeman and Tomlinson 1978). This has been an incentive to focus on the proximal, underground part of modules in this study. The study has been centred on 18 phylogenetically related taxa from the order Zingiberales in order to gain insights into the evolution of rhizome morphology and structure in this group. A total of 16 morphological and anatomical characters were recorded for the 18 species; this allows the performance of a phenetic analysis to evaluate overall similarity of rhizomes between species. Maximum parsimony optimization of rhizome characters onto the Zingiberales phylogeny revealed trends in the evolution of Zingiberales rhizomes. Architectural analysis and cluster analysis of a morphological matrix identifies two groups of rhizomes that vary in the timing and location of branching and as a result exhibit strikingly different rhizome architectures. The first group is characterized by rhizomes with immediate branching at a precise location (i.e. from particular nodes) on the rhizome module, is referred to as the "geometric species", whereas the second group exhibits delayed branching on a non-restricted location (i.e. that can potentially occur from any node), resulting in poorly predictable rhizomes and are referred to as "non-geometric species". Maximum parsimony optimization onto a phylogeny reveals convergent evolution of these architectural strategies in the Zingiberales.

## Materials and methods

### Plant material and study site

The plant material used for this study has been collected in the surroundings of San José de Payamino, Ecuador, a small village of the Payamino community situated at approximately 2 hours from Francisco de Orellana by boat, position 00°28'55" S, 77°17'06" W. The study area is a typical lowland Ecuadorian rainforest with an altitude of 200 m above sea level.

Three mature specimens per species were collected for the analysis. All specimens were kept in water during the study and were replanted afterwards. *Zingiber officinale* Roscoe was cultivated and was kindly

provided by the Payamino. *Musa × paradisiaca* L. and *Dimerocostus strobilaceus* Kuntze were collected in a disturbed area at some distance of the Rio Payamino bank; *Hylaeanthe hexantha* (Poepp. and Endl.) A.M.E. Jonker and Jonker was collected on the bank of Rio Payamino. The four Marantaceae species (*Calathea ecuadoriana* H.A. Kenn., *Calathea inocephala* (Kuntze) T. Durand and B.D. Jacks., *Calathea roseopicta* (Linden) Regel and *Maranta arundinacea* L.) were growing in a low-light environment as understorey herbs. *Canna indica* L. was growing in disturbed areas, close to and within the cleared land where the camp was based, a zone characterized by high light level. *Heliconia rostrata* Ruiz and Pav., *Heliconia episcopalis* Vell., *Heliconia chartacea* Lane ex Barreiros and *Heliconia stricta* Huber were growing in a partially shaded zone, in the rainforest but relatively close to the Rio Payamino banks. *Heliconia hirsuta* L.f. and *Heliconia irrasa* R.R. Sm. were found in more disturbed environments at the boundary between the rainforest and recently partially cleared land. *Costus pulverulentus* C. Presl and *Costus scaber* Ruiz and Pav. were present close to the Rio Payamino banks as well as at boundaries between the rainforest and partially cleared land whereas *Costus erythrophyllus* Loes was exclusively found in lower light environments in the forest understorey. A summary of the light environments where each species was encountered is provided in Table 1.

### Character description and coding

A total of 16 rhizome characters have been used for the phenetic analysis (Table 2). The characters were a combination of architectural, morphological and anatomical features. The characters were coded using *Musa × paradisiaca* as reference since Musaceae is the basal-most family in the Zingiberales (Kress et al. 2001). Binary or two-state characters were coded (0) and (1) whereby (0) represents the state exhibited by *Musa × paradisiaca*. Discrete multistate characters were arbitrarily coded (0) for the state exhibited by *Musa* and (1), (2)…(n) for the states of apparent increased complexity. Continuous characters were coded directionally whereby the character state exhibited by *Musa* (0) was opposed to all other Zingiberales (Table 2). For each continuous character, $n = 10$ measurements were taken and the average was recorded for each species and used further in the analyses.

The first two characters refer to the aerial part of the mixed axis constituting the architecture of the Zingiberales: whether it is a true stem or a pseudostem (1) and the phyllotaxy of foliage leaves on the aerial axis (2). Phyllotaxy of cataphylls (scale leaves) on the rhizome was not used as it is often difficult to establish given that the cataphylls are often deciduous or degrade rapidly. Characters (3) refers to the origin of branching: whether new rhizome modules can arise at any node (of the subterranean stem), a condition that is referred to as

non-restricted branching, as opposed to the condition in which branching is restricted to certain specific node(s), it is referred to as restricted branching. Quantitative characters relating to the size of rhizomes included (5) rhizome module length (the underground part of each sympodial module (see Supplementary material, Figure S1); average of $n = 10$ units), (6) rhizome diameter at mid-module, (average of $n = 10$ units) (10) internode number per unit, (12) rhizome internode length (average of $n = 50$ internodes, measured on fully expanded modules). The rhizome branching level (7) was coded according to both its extent and variability as three evident patterns were present: high variable (coded 0) refers to a high (over three branches per rhizome module in average) but variable branching level; low variable (coded 1) refers to a low but variable number of branches per module (one or more rarely two); poorly variable (coded 2) refers to a level of branching which is highly conserved over modules (Table 2). Geometric and topological criteria were also used and included (8) the angle between succeeding rhizome modules and (9) the angle between sister rhizome units, that is between two rhizome modules having the same parent module and a more or less equivalent position on this parent module. The way the rhizome develops in relation to substrate was taken into consideration by recording the curvature per rhizome unit (13) and the height between succeeding rhizome modules (14) as Bell and Tomlinson (1980) showed that they can either be horizontal, ascending or descending. Finally, two anatomical characters used were the cortex/stele ratio (15) and the pattern of starch accumulation (16). For the determination of both the cortex/stele ratio and the starch accumulation pattern, rhizomes were cut at mid-module. Cortex/stele ratios were determined by image analysis of the photographed section in the software imagej (http://rsb.info.nih.gov/ij). Starch accumulation was determined by staining with Lugol's iodine. Schemes illustrating the measurements taken for characters 5, 8, 9, 13 and 14 are provided in Supplementary material, Figure S1.

### Phenetic and statistical analysis

A hierarchical clustering analysis was performed using the JMP Statistical Package (SAS Institute, Inc., Cary, NC, USA). The clustering was effectuated on the basis of the character matrix constructed (Table 3). The software evaluated the number of clusters based on the root-mean square (RMS) distance. In the unweighted analysis, each non-binary character of the matrix was multiplied by a scalar $\lambda_i$ so that the maximum value of all characters was 1. Since some characters showed less variability than others within a single species, a second analysis with weighting of these characters was performed. For the weighted analysis, the same normalized matrix was used but the state values of characters (1), (2), (3), (4), (7), (9), (13) and (16) (Table 2) were multiplied by 2; hence these characters

Table 1.    List of the Zingiberales species studied.

| Species | Family | Origin | Light environment |
|---|---|---|---|
| *Musa × paradisiaca* L. | Musaceae | Cultivated | Open canopy |
| *Heliconia rostrata* Ruiz and Pav. | Heliconiaceae | Native | Partially closed canopy |
| *Heliconia stricta* Huber | Heliconiaceae | Native | Partially closed canopy |
| *Heliconia epicospalis* Vell. | Heliconiaceae | Native | Partially closed canopy |
| *Heliconia chartacea* Lane ex Barreiros | Heliconiaceae | Native | Partially closed canopy |
| *Heliconia hirsuta* L.f. | Heliconiaceae | Native | Open canopy |
| *Heliconia irrasa* R. R. Sm. | Heliconiaceae | Native | Open canopy |
| *Costus scaber* Ruiz and Pav. | Costaceae | Native | Open canopy |
| *Costus pulverulentus* C. Presl. | Costaceae | Native | Open canopy |
| *Costus erythrophyllus* Loes. | Costaceae | Native | Closed canopy |
| *Dimerocostus strobilaceus* Kuntze | Costaceae | Native | Open canopy |
| *Zingiber officinale* Roscoe | Zingiberaceae | Cultivated | Open canopy |
| *Canna indica* L. | Cannaceae | Native | Open canopy |
| *Calathea ecuadoriana* H.A. Kenn. | Marantaceae | Native | Closed canopy |
| *Calathea inocephala* (Kuntze) T. Durand and B.D. Jacks. | Marantaceae | Native | Closed canopy |
| *Calathea roseopicta* (Linden) Regel | Marantaceae | Native | Closed canopy |
| *Hylaeanthe hexantha* (Poepp. and Endl.) A.M.E. Jonker and Jonker | Marantaceae | Native | Open canopy |
| *Maranta arundinacea* L. | Marantaceae | Native | Closed canopy |

Table 2.    List of characters studied and their coding for the phenetic and mapping analyses.

| No. | Character | Character coding |
|---|---|---|
| 1 | Aerial stem type | Pseudostem (0) – Stem (1) |
| 2 | Phyllotaxy on stem/pseudostem | Spiral (0) – Distichous (1) – Spiro-distichous (2) |
| 3 | Rhizome branching type | Axillary (0) – Subapical (1) |
| 4 | Branching timing | Proleptic (0) – Sylleptic (1) |
| 5 | Rhizome module length | 20–15 cm (0) – 15–10 cm (1) – 10–5 cm (2) – 5–0 cm (3) |
| 6 | Rhizome diameter at mid-rhizome module | Over 10 cm (0) – 10–5 cm (1) – 5–3.75 cm (2) –3.75–2.5 (3) – 2.5–1.25 (4) – 1.25–0 (5) |
| 7 | Rhizome module branching level | High but variable (0) – low but variable (1) – Fixed (2) |
| 8 | Variability of angles between (n) and (n+1) rhizome module | Highly variable (>50%) (0) – quite variable (<50%) (1) – Poorly variable (<25%) (2) |
| 9 | Variability of angles between sister rhizome module | Highly variable (>50%) (0) – quite variable (<50%) (1) – Poorly variable (<25%) (2) |
| 10 | Internode number per rhizome module | 15–10 (0) – 10–5 (1) – 5–1 (2) |
| 11 | Variability of internode number per module | Highly variable (>50%) (0) – quite variable (<50%) (1) – poorly variable (>25%) (2) |
| 12 | Internode length | 0–0.5 cm (0) – 0.5–0.75 cm (1) – 0.75–1 cm (2) – 1–1.25 cm (3) – 1.25–1.25 + (4) |
| 13 | Rhizome module curvature (a/b) | 1–0.75 (0) – 0.75–0.5 (1) – 0.5–0.25 (2) |
| 14 | Height between successive rhizome modules (z) | $\neq 0$ (0) – null (1) |
| 15 | Ratio cortex/stele | 0–0.35 (0) – 0.35–0.7 (1) – 0.7–1 (2) – 1–1.30 (3) – 1.30–1.65 (4) |
| 16 | Starch accumulation pattern in rhizome | Musa type (0) – Cortex + Stele (1) – Stele (2) – Cortex (3) – Vascular Bundles (4) |

had twice as much weight as the others in this analysis. Therefore, in the weighted analysis, the maximum value of a weighted character state was 2 whereas for every other character it was 1.

Student's two sample *t*-tests were performed in R (The R foundation for Statistical Computing). The null and alternative hypotheses were as follows ($H_0$): there is no difference in means between the two groups; ($H_1$): there is a difference in means between the two groups.

### Character mapping

Character mapping was effectuated on Mesquite version 2.75 (Maddison and Maddison 2011). The topology used

for the phylogeny was integrated from previously published molecular phylogenies in the Zingiberales (Andersson and Chase 2001; Kress et al. 2001; Specht et al. 2001; Kress, Prince and Williams 2002; Prince and Kress 2006a,b; Specht 2006; Specht and Stevenson 2006; Marouelli et al. 2010). When a species used in this study was absent from a published phylogeny, it was collapsed as a polytomy with its closest relatives. Character mapping was effectuated by using maximum parsimony ancestral state reconstruction as implemented in Mesquite v. 2.75. The matrix of characters coded was entered as categorical characters and unordered state assumption was applied.

Table 3.   Matrix of the 16 coded characters for the 18 Zingiberales species.

| Species studied | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Musa × paradisiaca* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Heliconia rostrata* | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 2 | 1 |
| *Heliconia stricta* | 0 | 1 | 1 | 1 | 1 | 4 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 4 | 0 |
| *Heliconia episcopalis* | 0 | 1 | 1 | 1 | 0 | 3 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 |
| *Heliconia chartacea* | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 1 |
| *Heliconia hirsuta* | 1 | 1 | 1 | 1 | 3 | 3 | 1 | 2 | 1 | 2 | 2 | 0 | 2 | 1 | 4 | 1 |
| *Heliconia irrasa* | 0 | 1 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 1 |
| *Costus scaber* | 1 | 2 | 1 | 1 | 3 | 4 | 1 | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 0 | 1 |
| *Costus pulverulentus* | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 3 | 2 | 1 | 0 | 1 |
| *Costus erythrophyllus* | 1 | 2 | 1 | 1 | 3 | 1 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 1 | 0 | 4 |
| *Dimerocostus strobilaceus* | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 0 | 2 | 0 | 0 | 1 |
| *Canna indica* | 0 | 1 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Zingiber officinale* | 1 | 1 | 1 | 0&1 | 2 | 5 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| *Calathea ecuadoriana* | 0 | 1 | 0 | 0 | 3 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| *Calathea inocephala* | 0 | 1 | 0 | 0 | 2 | 5 | 1 | 1 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 1 |
| *Calathea roseopicta* | 0 | 0 | 0 | 0 | 3 | 5 | 1 | 1 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 2 |
| *Hylaeanthe hexantha* | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 |
| *Maranta arundinacea* | 1 | 0 | 1 | 0 | 1 | 5 | 2 | 2 | 1 | 0 | 2 | 2 | 1 | 0 | 1 | 1 |

## Results

### Developmental morphology of the rhizomes

The term rhizome module is used here to refer to the proximal, underground part (i.e. the rhizome) of each module that constitutes the basic unit of organization of the Zingiberales.

### Heliconia chartacea Lane ex Barreiros (Figure 1A(a))

The development of the rhizome in this species is relatively similar to that of *H. rostrata* and *H. stricta* except that the succession between once and twice-branched rhizome module is a one-to-one alternation. However, in some instances, two two-branched rhizome modules are produced. Succession of two one-branched modules has not been observed. In two-branched modules, the two sister modules diverge by ∼100° from each other. The succession of modules occurs with a divergence angle of ∼60° from each other, resulting in a Z-shaped pattern. Branching is always immediate.

### Heliconia rostrata Ruiz and Pav. (Figure 1A(b))

*Heliconia rostrata* Ruiz and Pav. is also characterized by pseudostems, measuring 2–3 m. The plant is a succession of plagiotropic rhizome modules that shows a bilateral symmetry. The branching pattern is very consistent and characterized by once-branched modules, generally at the eighth node (Figure 1C(b)) of the rhizome module with a ∼45° angle divergence that alternates. Moreover, at the opposing region of the rhizome module, a short rhizome shoot is produced, which seems to remain dormant. However, some rhizome modules exhibit two sister branches diverging at about ∼70° from each other. When they develop, the second rhizome module arises from the exact same position as the short shoot that exists on the rhizome modules with a single branch, and short shoots are absent from twice branched rhizome modules. Branching is always immediate.

### Heliconia irrasa R. R. Sm. (Figure 1A(c))

*Heliconia irrasa* is also a relatively small species, about 1.20 m high. The mode of construction of this species differed starkly from the other *Heliconia* species observed. The development of the young plant consists of the succession of modules, which occurs without particular pattern; all modules are one-branched and branching occurs on the distal part of the rhizome unit, similarly to other *Heliconia* observed, although there is no conservation of branching angles of the successive modules. However, the adult plant differs in that the modules are highly curved and in that branching is highly variable, ranging from unbranched modules to four-branched modules. Moreover, branching – which is delayed – can occur at any internode of the rhizome module, including in the middle or the proximal part, contrasting with the other species of *Heliconia* in which branching occurs only on the latest internodes. Consequently to the high curvature of the modules, the geometry of the rhizome is tridimensional by contrast to the essentially two-dimensional geometry of other *Heliconia* species. No geometric pattern was observed.

### Heliconia stricta Huber (Figure 1A(d))

The shape of *H. stricta* rhizome is circular and radially symmetrical. Rhizome modules are either once or twice

branched; however, twice-branched modules occur more regularly in *H. stricta* with a ratio of one two-branched module for two one-branched modules. Nevertheless, in two-branched modules, although the formation of the two branches is simultaneous, outgrowth of the distal part of the module (i.e. the pseudostem) is not concurrent and in some instances, one of the two sister rhizome modules remains as a dormant shoot, similar to those of *H. rostrata*. The two sister modules diverged ∼80° from each other. Short dormant shoots were rare on one-branched rhizome modules.

### *Heliconia hirsuta L.f.* (Figure 1(e))

This is a small *Heliconia* species with a real stem of about 1 m high. The rhizome modules are short, cylindrical and orthotropic, similar to those of many

Costaceae. They show evidence of orthotropy. Modules are either one- or two-branched. The alternation was however less precise with examples of 1-to-1 alternation, of two one-branched or conversely of two two-branched modules. The divergence angle between succeeding modules was close to 70° and is highly conserved; however, in two-branched modules, the divergence angles between sister modules is variable by about 50%.

### *Heliconia epicospalis Vell.* (Figure 1A(f))

This species is characterized by one-branched rhizome modules which occur in a linear sequence; branching in the linear sequence is always immediate. However, each rhizome module produces a short rhizomatous shoot arising at ∼90° from the linear module succession. Most
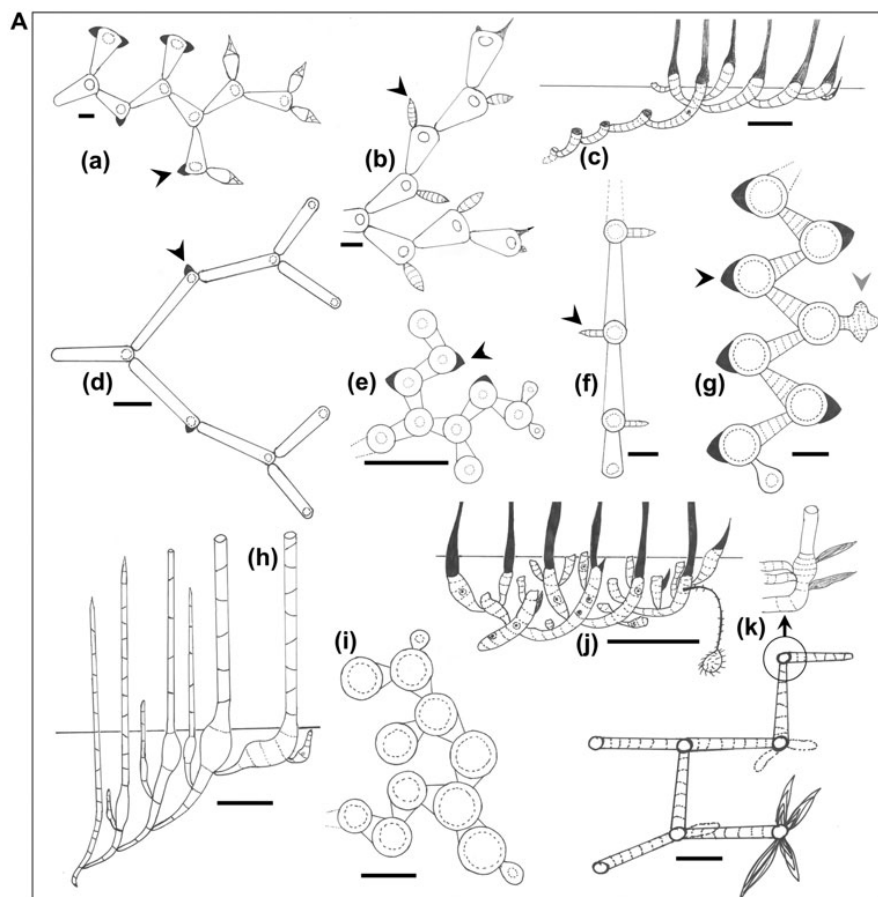


Figure 1. Zingiberales rhizome morphologies. (**A**) Morphological schemes (top or side view) for 11 species. (a) *Heliconia chartacea*. (b) *Heliconia rostrata*. (c) *Heliconia irrasa*. (d) *Heliconia stricta*. (e) *Heliconia hirsuta*. (f) *Heliconia epicospalis*. (g) *Dimerocostus strobilaceus*. (h) *Costus scaber*. (i) *Costus pulverulentus*. (j) *Calathea roseopicta*. (k) *Maranta arundinacea*. Black arrowheads point to dormant buds or short shoots. Grey arrowhead in (g) shows a young reiterate that developed from a dormant bud. Scale bar represent 5 cm on each scheme. (**B**) Phenetic analysis of rhizome morphologies reveal two strategies. (a) Unweighted analysis. (b) Weighted analysis. (**C**) Pattern of internode length with internode number along rhizome module in eight species. Red bars indicate branching restriction at the corresponding internodes, green bars denote absence of branching restriction.
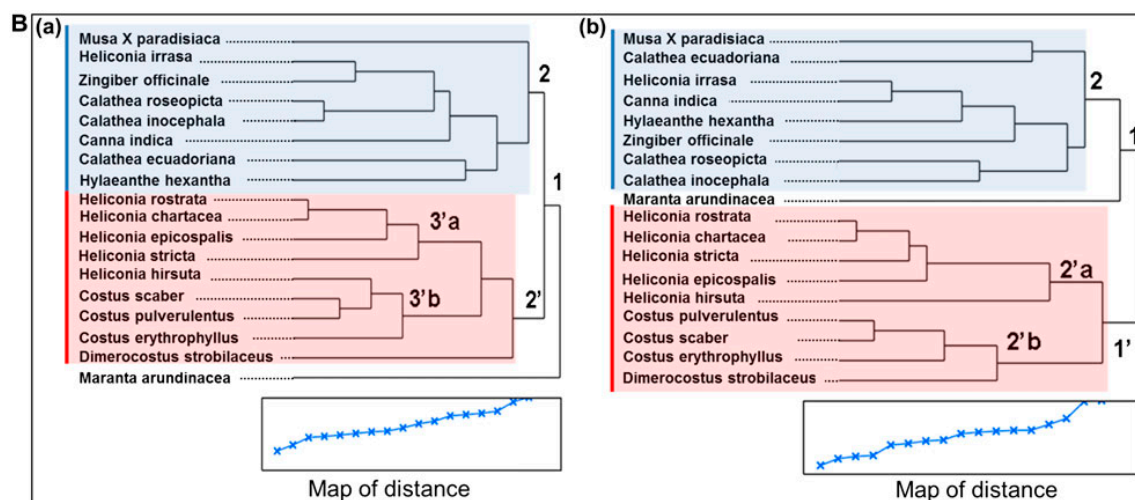
Figure 1. (*Continued*).

of these short shoots were found to be dormant; although some showed evidence of meristematic activity.

*Dimerocostus strobilaceus Kuntze* (Figure 1A(g))

This is a very tall species reaching 3–4 m; the aerial part of modules often branch distally but not in the first year of establishment. The rhizome of this species is highly geometric. Branching is always immediate. Rhizome modules are short and thick; they are one-branched, each module diverging by ∼75° from the preceding module, forming a Z-pattern. Each unit contains a short shoot that resembles a bud and has the potential to reproduce this architectural pattern (see Figure 1A(g) for the position and example of outgrowth). An equivalent model of rhizome development has been described in *Costus cylindricus* Jacq. (Bell and Tomlinson 1980).

*Costus scaber Ruiz and Pav.* (Figure 1A(h,i))

As for all Costaceae, in *Costus scaber*, the aerial part of modules is a stem. The mode of development of the rhizome is equivalent in *C. scaber* and *C. pulverulentus*. Observation of specimens at different stages, especially young but fully established specimens, allowed uncovering the establishment growth in this species, which differs from those recorded by Bell (2008, 205). The first modules are characterized by the absence of clear-cut distinction between the rhizome and the aerial part of the stem since there is no thickening of the rhizome, which in addition is poorly curved. Following these slender rhizome modules, there is a gradual shortening and thickening of the next rhizome modules. This considerable enlargement of the rhizome module occurs concurrently with a significant reduction of internode length. Importantly, the changes in the morphology of the rhizome are accompanied by a restriction of branching: in the first 8–12 modules, branching can occur from virtually any node of the rhizome unit and the level of branching of each module may vary from zero- to four-branched modules while in the adult plant, branching is restricted to the *n-2* internode of the rhizome unit (generally three to five) and modules are generally one- or two-branched, sometimes unbranched. As for all Costaceae, scale leaves (cataphylls) persist on the most proximal region of the aerial part of the module. Branching is delayed in the establishment phase but immediate in the adult phase.

*Calathea ecuadoriana H.A. Kenn., Calathea inocephala (Kuntze) T. Durand and B.D. Jacks. and Calathea roseopicta (Linden) Regel* (Figure 1A(j))

The rhizome of these understorey species is comparable and close to that of mature *Heliconia irrasa*. The rhizome modules are orthotropic, modules are generally highly branched and branching can occur at any internode, resulting in the absence of defined geometry. Tubers can develop at the apex of roots borne on the rhizome. Branching is always delayed.

*Maranta arundinacea L.* (Figure 1A(k))

This species is widely known and cultivated as arrow-root. The rhizome module comprises 13–14 internodes and branching occurs only in the distal part of the module, the last two to four internodes after the curvature. The angles between successive modules present some conservation, although the level of branching of modules is variable (from zero- to four-branched modules) and the angles between sister modules are not conserved. Thus the
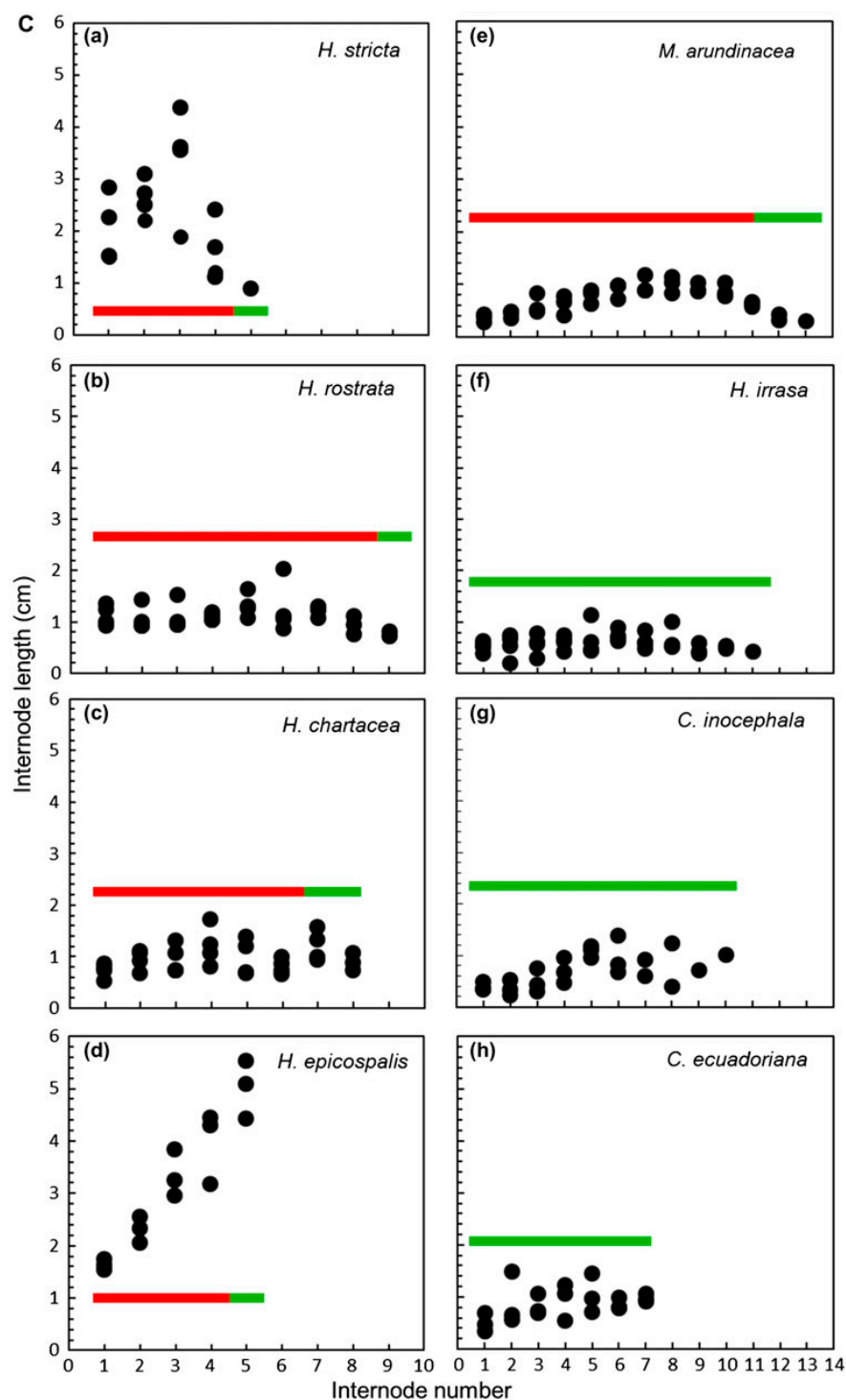
Figure 1.    (*Continued*).

geometry of *M. arundinacea* rhizome is somewhat intermediate between the "defined" and the non-geometric species. Importantly, branching is delayed.

*Costus erythrophyllus Loes.*

The rhizome of *C. erythrophyllus* is close to that of the *C. scaber* and *C. pulverulentus* except that the modules

touch each other. The great majority of rhizome modules are one-branched modules. As the angles are not alternating and the modules develop a single branch, the pattern is often a spiral in which the growing modules can become stuck. Branching is immediate.

### Zingiber officinale Roscoe

The rhizome of this species is poorly organized. Since the plane of distichy is vertical, modules develop linearly. However, buds are formed from any nodes, the position of which cannot be predicted, resulting in poorly organized rhizome morphology with no consistent geometry. Vegetative and reproductive functions are separated and occur on different modules. The linear

series that constitute the basic organization of the rhizome is characterized by immediate branching. By contrast, the buds that develop from any nodes constitute delayed branching. As this reiterative mode is prevalent in the development of this species, no clear geometric pattern can be recognized.

### Canna indica L.

In this species, highly curved modules terminate in a 50–80 cm pseudostem. Modules can develop from any internodes of the rhizome module, resulting in poorly predictable rhizome morphology. The level of branching is highly variable. Branching is always delayed.



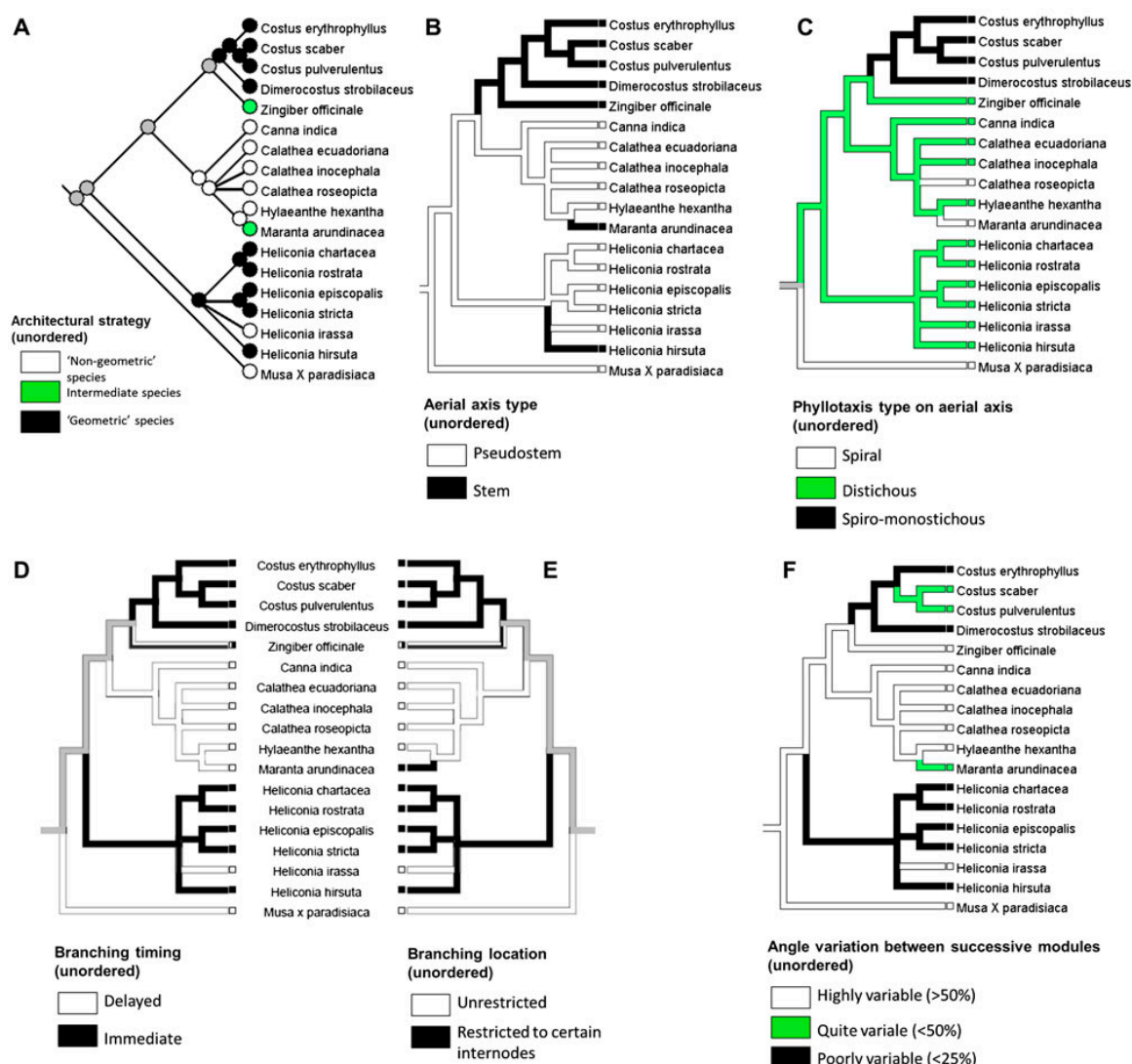Figure 2. Evolution of rhizome morphology in the Zingiberales as revealed by maximum parsimony character optimization on the phylogeny of the order. Grey depicts equivocal character state. (**A**) Architectural strategies. (**B**) Aerial axis type. (**C**) Phyllotaxy on aerial axis (foliage leaves phyllotaxy). (**D**) Branching timing. (**E**) Branching localization. (**F**) Angle variation between successive modules.

*Hylaeanthe hexantha (Poepp. and Endl.) A.M.E. Jonker and Jonker*

The rhizome of this small species (1 m) has the ability to branch from any internode of the module. However, modules are generally poorly branched (rarely over two-branched). Internodes are long and rhizome module varies drastically in length, a characteristic shared with the *Calathea* species, *Heliconia irrasa*, and *Canna indica*. In the fully developed module (i.e. when the pseudostem is established), a starch sheath separates the cortex and the stele, but there is no starch in the developing rhizome module before pseudostem establishment. Branching is always delayed.

*Musa × paradisiaca L.*

*Musa × paradisiaca L.*, the cultivated banana, exhibits pseudostems that can measure over 7 m high. The rhizomatous part of the module is short; branching is delayed and can virtually occur from any node of the rhizomatous part and the level of branching is highly variable between modules and individuals.

### Phenetic analysis revealed two groups matching branching type and timing

To perform a phenetic analysis, a hierarchical clustering method was applied to the character matrix established (Table 3, see also Materials and methods). In both the weighted and unweighted analyses, the taxa are separated in the same two groups with *Maranta arundinacea* having a particular position as either the outgroup of all taxa in the unweighted analysis (Figure 1B(a)) or sister of one of the groups in the weighted analysis (Figure 1B(b)). Importantly, the two groups revealed by the phenetic analysis match almost perfectly the branching localization and timing (characters 3 and 4, Table 3; Figure 2D, E), except for *Maranta arundinacea* in the unweighted analysis (Table 3, Figure 1A(k), Figure 2D). The first group comprises *Musa × paradisiaca*, *Heliconia irrasa*, *Zingiber officinale*, *Calathea roseopicta*, *Calathea inocephala*, *Calathea ecuadoriana*, *Canna indica* and *Hylaeanthe hexantha* and is characterized by poorly predictable rhizome geometry as a result of delayed branching occurring from any internodes and non-conserved geometry of the angles between successive or sister rhizome modules. The second group comprised *Heliconia rostrata*, *H. chartacea*, *H. epicospalis*, *H. hirsuta*, *H. stricta*, *Costus scaber*, *C. pulverulentus*, *C. erythrophyllus*, *Dimerocostus strobilaceus* and by opposition to the preceding group, exhibits highly geometric rhizomes with typically conserved angles between sister and succeeding modules, immediate branching occurring only in a restricted number of internodes. *Maranta arundinacea* is a transitional case between the two rhizome types as branching occurs on a restricted number of internodes, as for the "geometric"

species but delayed branching of modules is shared with the "non-geometric" species. This intermediate position is reflected in the phenetic tree: in both the unweighted and weighted analyses, *M. arundinacea* arises either as sister of both groups or sister to the "non-geometric" group (Figure 1B). An extra morphological feature of the "geometric" species is hypotonic branching, meaning that the next module arises on the lower side of the preceding module; by contrast, in non-geometric species and *M. arundinacea*, branching seemed to be less constrained: examples of basitonic, mesotonic and epitonic shoots were observed within the same specimen (in particular in *Calathea* species, Figure 1A(j)), although in *M. arundinacea*, such branching is limited to the distal-most internodes of the rhizome module (Figure 1C(e)).

### Internode length and number in "geometric" and "non-geometric rhizomes"

The pattern of internode length in relation to internode number is indicative of the extension growth of an axis (Moulia et al. 1999); thus, it has been determined for eight species encompassing the diversity of rhizome morphology observed. With the exception of the short dormant shoots observed in several species of *Heliconia*, only one type of rhizomatous axis can be distinguished. The rhizomatous versus aerial developmental phases of these shoots show drastic changes in ontogeny, developmental dynamics and organogenesis. In *Maranta arundinacea*, the range of internode length for the rhizome part of the shoot is 0.1–1.2 cm (Figure 1C(e)) whereas in the aerial part, it exceeds 30 cm (data not shown). *Heliconia stricta* exhibited five or six internodes that increased and subsequently decrease in length with their position ($\sim$2 cm $\rightarrow$ $\sim$3 cm $\rightarrow$ $\sim$1 cm) (Figure 1C (a)). *Heliconia rostrata* exhibited eight or nine internodes with slightly longer middle internodes (Figure 1C(b)). *Heliconia chartacea* had eight internodes that showed two bell-like increase–decreases in length along the module (Figure 1C(c)). *Heliconia epicospalis* was very distinctive in that its five internodes showed a steep, linear increase towards the distal end of the module (Figure 1C(d)). The non-geometric species *Heliconia irrasa* also presented a slight bell-like distribution of the 9–11 internodes, with somewhat more variability. Finally, *Calathea inocephala* and *Calathea ecuadoriana* were variable in terms of number and length of internodes (Figure 1C(g,h)). Overall, the number of internodes per module was more conservative in geometric species than in non-geometric species (Figure 1C; see also Tables 2 and 3). No clear pattern of internode differentiation separated rhizomes of both architectural strategies. The internodes at which branching can occur are outlined for each species (Figure 1C). Importantly, no correlation was found between internode length and restriction of branching in the geometric species (Figure 1C).

### Evolutionary trends in rhizome morphology in the Zingiberales

A phylogenetic framework was used to determine evolutionary trends in Zingiberales rhizomes. In order to evaluate how many times key rhizome characters evolved, maximum parsimony optimization was performed onto a Zingiberales phylogeny. Maximum parsimony mapping of rhizome strategies revealed that geometric rhizomes evolved at least twice, once in Heliconiaceae and once in Costaceae (Figure 2A). Non-geometric rhizomes have been lost at least once after the divergence of Musaceae from the rest of Zingiberales and further gained at least twice in Heliconiaceae and Marantaceae (Figure 2A). The presence of pseudostem is basal in the order (Figure 2B), and characterizes all members of Musaceae (Skutch 1932). The evolution of aerial stem from pseudostem occurred at least three times in Heliconiaceae, Marantaceae and [Zingiberaceae + Costaceae] (Figure 2B). No reversion of stem to pseudostem was identified in this data set (Figure 2B). However, as many Zingiberaceae species exhibit pseudostems, it seems likely that true stems evolved at least four times in the order. Several switches between distichous and spiral phyllotaxis occurred during the evolutionary history of Zingiberales (Figure 2C). By contrast, spiro-monostichous phyllotaxis – an unusual phyllotaxis defined by low angle divergence (30–50°) between succeeding leaf primordia at the shoot apex meristem (Kirchoff and Rutishauser 1990) – is deemed to have evolved only once after the divergence between Costaceae and Zingiberaceae (Figure 2C). Importantly, the evolution patterns of branching timing and location are correlated, except in *Maranta arundinacea*, which exhibits delayed branching restricted to certain internodes (Figure 2D,E). The evolution of angle variation between successive modules is also correlated to the two preceding characters, suggesting a direct link.

### Rhizome anatomy and starch storage

It was pivotal to investigate the extent of relative size of the cortex and the stele in relation to the pattern of starch accumulation in order to evaluate possible rhizome function. The relative size of the cortex and stele was evaluated by calculating the ratio cortex/stele (Table 2). The cortex/stele ratio varied from 0.15 to 1.65, indicative of very thin cortex versus very thick cortex, respectively (Figure 3B, see also Tables 2 and 3). In order to see whether the non-geometric species had a significantly different cortex/stele ratio from the non-geometric species, a two-sample Student's $t$-test was performed. There was no significant difference in the means of the cortex/stele ratio of the two strategies ($t = 1.23$, df = 11.5, $p = 0.24$). Since no difference was detected between the two strategies, the hypothesis that species from phylogenetically distinct groups may have a different cortex/stele ratio was tested by comparing Costaceae with Heliconiaceae, both of which exhibit geometric rhizome architectures in the species sampled (except *H. irrasa*). A strong phylogenetic signal was identified ($t = 7.64$, df = 4.68, $p = 0.0008$). Therefore, Heliconiaceae have significantly higher cortex/stele ratio than Costaceae (at least in the species sampled). Likewise, the pattern of starch accumulation varied across species, without presenting differences between strategies (Figure 3A, C). Five distinct patterns have been recognized. The *Musa*-type, and therefore likely the plesiomorphic type in the Zingiberales is characterized by starch accumulation in the inner part of the cortex and the stele and is also present in *Heliconia stricta* and *Canna indica* (Figure 3A, C). The second pattern corresponded to starch accumulation in the entire cortex and stele, which was the most common pattern found in 11 out of the 18 species investigated (Figure 3A, C). The third pattern was accumulation only in the stele, and was found in *Calathea roseopicta* only. The fourth pattern consisted of accumulation in the cortex only, and was recorded in two species: in *Hylaeanthe hexantha*, where only the inner cortex accumulates starch, while in *Heliconia epicospalis* the whole cortex strongly accumulated starch. Lastly, the fifth pattern identified was specific to *Dimerocostus strobilaceus* and consisted of small accumulations around each vascular bundle, especially in the cortex. The cortex + stele type is clearly derived from the related *Musa*-type. The other types of starch accumulation may be genus or species specific.

## Discussion

### Two strategies of rhizome architecture occur in Zingiberales

Although from a purely topological viewpoint, the species described here are assigned to Tomlinson's model, for their sympodial rhizomes (Hallé, Oldeman and Tomlinson 1978), two clearly distinct architectural strategies of rhizome are described here. The distinction between species with "geometric" rhizomes (*Costus scaber, Costus pulverulentus, Costus erythrophyllus, Dimerocostus strobilaceus, Heliconia stricta, H. rostrata, H. epicospalis, H. chartacea, H. hirsuta*) and with "non-geometric" rhizomes (*Musa × paradisiaca, Canna indica, Calathea ecuadoriana, Calathea inocephala, Calathea roseopicata, Hylaeanthe hexantha, Zingiber officinale* and *Heliconia irrasa*) is supported by both the architectural and phenetic analyses (Figure 1). These two types of rhizome architectures vary essentially in their geometric properties, which are dependent upon the timing and position of branching (Figures 1 and 2). An important point regarding immediate branching in the geometric species is that the next module is always formed simultaneously with its parent axis but it does not necessarily extend concurrently. The recognition of these two modes of construction in this study differs from the work of Bell and Tomlinson (1980), who distinguish linear versus hexagonal systems. However, this distinction does not account for the non-geometric
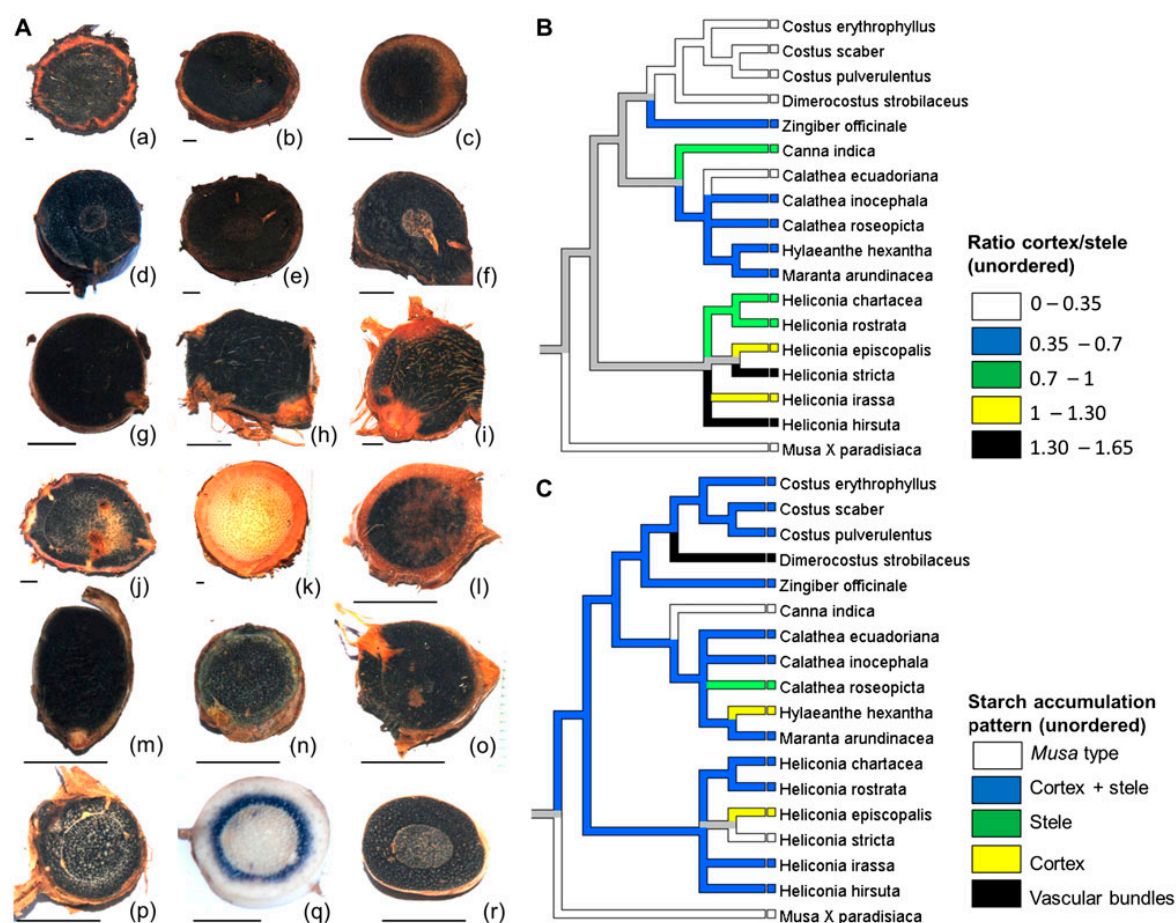
248    *G. Chomicki*



Figure 3.    Evolution of rhizome anatomy and starch accumulation pattern in Zingiberales rhizomes. (**A**) General anatomy and starch accummulation pattern in the rhizome of the species studied determined with Lugol's iodine. Scale bar is 1 cm for all sections. (a) *Musa × paradisiaca*. (b) *Heliconia rostrata*. (c) *Heliconia stricta*. (d) *Heliconia epicospalis*. (e) *Heliconia chartacea*. (f) *Heliconia hirsuta*. (g) *Heliconia irrasa*. (h) *Costus scaber*. (i) *Costus pulverulentus*. (j) *Costus erythrophyllus*. (k) *Dimerocostus strobilaceus*. (l) *Canna indica*. (m) *Zingiber officinale*. (n) *Calathea roseopicta*. (o) *Calathea ecuadoriana*. (p) *Calathea inocephala*. (q) *Hylaeanthe hexantha*. (r) *Maranta arundinacea*. (**B**) Reconstruction of the evolution of cortex/stele ratio in the Zingiberales species studied. (**C**) Reconstruction of the evolution of starch accumulation pattern in the Zingiberales species studied. Grey depicts equivocal character state.

rhizome species or for the diversity of geometries that cannot be reduced to linear and hexagonal. Moreover, linear topologies can be found in both geometric and non-geometric species. Thus this simple but morphologically tractable distinction between "geometric" and "non-geometric" rhizomes seems more appropriate that the topological distinction between "linear" and "hexagonal" rhizomes. However, a third class distinguished by these authors seems perfectly adequate; "mixed rhizomes" such as those of *Afromomum luteo-album* (K. Schum.) K. Schum. (Bell and Tomlinson 1980) are characterized by non-geometric types of rhizomes on which some modules give rise to geometric systems. Perhaps *Zingiber officinale* L. could be placed in such a category as in essence it is a geometric linear system in which delayed branching forms iteratively resulting in a non-geometric architecture.

Plagiotropy, the genetically or physiologically controlled horizontal growth of a shoot axis (Massart 1924), characterized the rhizome of many species such as *Heliconia stricta, H. rostrata* and *H. chartacea*; the last two exhibiting in turn bilateral symmetry of the rhizome modules, a diagnostic feature of plagiotropy (Caraglio and Barthélémy 2007). Rhizome plagiotropy was always associated with hypotonous branching; a correlation that is well-known in aerial shoots (plagiotropy by apposition or substitution; Caraglio and Barthélémy 2007), the latter type being to some extent applicable to plagiotropic rhizomes as all species had terminal inflorescences. The cylindrical modules of the Costaceae or of *H. hirsuta* were orthotropic but the high curvature of their modules (character 13) defined an upper and lower side and branching was hypotonous. By contrast, the species with non-geometric rhizomes generally exhibited orthotropic

rhizomes (e.g. the three *Calathea* species, *H. irrasa*) and absence of privileged arrangement of the rhizome modules with examples of hypotonic, epitonic or amphitonic branching (considering that these axes are curved). Hypotonous branching was therefore an important characteristic of the geometric species.

### Convergent evolution of "geometric" and "non-geometric" architectural strategies in the Zingiberales

The independent acquisition of geometric rhizome architectures in Costaceae and in Heliconiaceae (Figure 2A), but also in Zingiberaceae and probably in Marantaceae (Bell 1979; Bell and Tomlinson 1980) revealed convergent evolution of this architectural strategy. Likewise, non-geometric rhizome architecture is convergent in Musaceae, in at least one Heliconiaceae species and in Marantaceae. The fact that five of the six *Heliconia* species exhibit "geometric" rhizomes suggests a phylogenetic signal of this morphology in the genus. It also seems to be the case for Costaceae; although, interestingly, in certain *Costus* species there is a shift from non-geometric to geometric morphology during ontogeny (Figure 1A(h)). By contrast, the two genera of Marantaceae studied convey the idea that a non-geometric strategy is dominant in the family; other intermediate systems occur in this family (Bell and Tomlinson 1980) and so possibly species with geometric rhizomes. It is difficult to judge Zingiberaceae as only one species was studied. However, previous studies showed that a very geometric rhizome occurs, such as in the hexagonal grid of *Alpinia* (Bell 1979) or in the linear system of *Hedychium* (Bell and Tomlinson 1980), indicating that the geometric mode of construction also evolved independently in this family. In Musaceae, although only one species has been studied here, it is well known that this clustering habit is generalized in the genus *Musa* (Cheesman 1947); however, most species of *Ensete* have no capacity for basitonic branching and are hence monocarpic.

Correlated evolution of branching timing and location is significant (Figure 2D, E). Firstly, this suggests that the "geometric" versus "non-geometric" architectural strategies, that are precisely defined by the co-occurrence of these two combinations of character states (immediate and localized [geometric] versus delayed and non-localized [non-geometric]), might have biological significance. This correlated evolution might reflect developmental constraints or the two combinations may have higher adaptive values, perhaps in relation to exploration and exploitation of the substrate. In particular, the combination of immediate and non-selective branching was never observed, perhaps a developmental constraint in relation to the sub-apical origin the (n + 1) shoot.

Moreover, the correlation of the evolution of angle variation with the two preceding characters is strongly suggestive of a causal relationship between branching

timing and location and rhizome geometry. The spatial restriction of branching on the rhizome module clearly results in more geometric rhizomes. However, the angle between sister rhizome modules might be more restricted if the two sister modules are formed concurrently with the parent rhizome module due to potential developmental constraints. Interestingly, the angles of twice-branched species with immediate branching (e.g. the sister branches in *Heliconia rostrata, H. strica* or *H. chartacea*) were highly conserved. By contrast, those of the intermediate species *Maranta arundinacea*, which exhibits delayed but restricted branching, were less conserved and buds were observed to develop independently of each other at varying angles, supporting this hypothesis.

The genetic basis of rhizome development is poorly understood. Quantitative trait loci (QTL) mapping revealed a potential master regulator gene for rhizomatousness in rice and *Sorghum*, providing a genetic basis for the convergent evolution of rhizome in these two species that diverged some 50 million years ago (Hu et al. 2003). Candidate genes involved in rhizome bud development have been identified in the bamboo species *Phyllostachys violascens* Rivière & C. Rivière (=*Phyllostachys praecox*) (Wang et al. 2010). Although genetic studies on non-model organisms are often difficult, it would be interesting to investigate the genetic basis of branching timing and location in the Zingiberales and therefore provide a mechanistic basis for this architectural convergence.

### Latent buds, reiteration and Tomlinson's model

The presence of latent buds or short shoots on very specific positions in the rhizome of the geometric species (Figure 1A) with the potential to reproduce the geometric pattern of the species, has been previously described in other Zingiberales species with geometric rhizome architectures (Bell and Tomlinson 1980). Their role in the delayed, total reiteration of the rhizome organization has been well established, including Zingiberales species such as *Alpinia speciosa* L. or *Afromomum luteo-album* (K. Schum) K. Schum (Bell 1979; Bell and Tomlinson 1980). In this study, substantial evidence has been found for *Dimerocostus strobilaceus*, in which one of these buds started its Z-shaped development (Figure 1G); although the difficulty of finding and excavating very large rhizome has hampered an irrefutable demonstration of total delayed reiteration for all "geometric" species in which these buds or short shoots are described. However, support from previous studies further sustains the idea that these latent buds or short shoots have a reiterative function in the species described in this study.

Sequential total reiteration (Edelin 1977; 1984; Nicolini 1997) is another interesting yet difficult question which has been demonstrated to occur in the rhizome of *Carex arenaria* L. (Noble, Bell and Harper 1979). In the geometric species, which present a consistent alternation between differently branched modules, this sequential

branching can hardly be referred to as reiteration, although there is some subjectivity. By contrast, in species where the pattern is more defined, for instance in *Heliconia rostrata* where one-branched rhizomes develop in a Z-shaped pattern and a second module develops in the position of the short shoot contiguously with the other module, sequential ("automatic" sensu Edelin (1977)) reiteration may be hypothesized.

In the non-geometric species, the mode of development of the rhizome does not follow a predictable pattern; instead, modules develop in a more iterative mode. By contrast, in the geometric species, the rhizome, despite being sympodial, presents a developmental and structural integrity (i.e. the geometric pattern can be described as the architectural unity) while in the non-geometric species the rhizome is simply the underground continuity of the aerial module and therefore the architectural unity remains the module. Such high integration of a basitonic branching system has been well described in the orchid *Encyclia vespa* (Vell.) Dressler (Barthélémy 1988). Hence, in the geometric species, a developmental and architectural unity can be found in the development of the rhizome while in the non-geometric species the unity remains the module, not the rhizome. Given that in the non-geometric species, the architectural unity is represented by the module, they could be considered as architectural variants of the Holttum model (i.e. a single determinate orthotropic shoot which terminates in an inflorescence, Hallé et al. (1978)) with the ability to reiterate basally.

Such difference recalls the discussion of Cremers and Edelin (1995) on the nature of basitonically branched plants. These authors carried out a thorough study of basitonically branched plants and showed that the aerial architecture of such plants corresponds to Corner, Holttum, Chamberlain, Leeuwenberg, Massart or Petit's model and so can be considered as variants with basal reiteration. This led the authors to a questioning about the suitability of Tomlinson's model, which may just encapsulate a variation that can potentially occur in any architectural model. Moreover, basitonic branching can occur in a normally non-basitonically branched taxa, such as in many temperate woody trees, as well as being established variants of a species (e.g. *Lophophora williamsii* var. *caespitosa*), resulting in a physiological and evolutionary continuum for basitonic branching. Besides, the genetic basis of basitonic branching is relatively well understood from work on several model species (*Arabidopsis*, Petunia, Pea, Rice) and it is clear that it is regulated by the balance between auxin and strigolactone signalling, the latter being dependent upon the MAX pathway (see Domagalska and Leyser 2011 for review). Mutation in the gene *MAX2* (or its orthologues), a gene coding for a protein involved in strigolactone signalling, leads to basitonic branching in *Arabidopsis*, Petunia and Pea (ibid.). This gives further support to the idea of Cremers and Edelin (1995) that basitonic branching is a simple variation that can occur

in any architectures, which may apply for the non-geometric species.

However, the mode of development of the geometric species, like that of *Encyclia vespa* (Vell.) Dressler (Barthélémy 1988), presents a basitonically branched system that is integrated at the developmental, ontogenetic and architectural level and so cannot be satisfactorily described as variants of other architectural models. Immediate branching of the rhizome at defined internodes on the module resulting in a patterned geometry clearly illustrates an architectural unity. Therefore, any repetition of this unity – either immediate or delayed – can be interpreted as reiteration. Hence, the architectural unity of these shoot systems, in addition to the distinctiveness of this mode of construction, advocate to attribute these plants to an architectural model – perhaps a revised version of that of Tomlinson.

### The evolution of starch accumulation and rhizome function in Zingiberales rhizomes

The evolution of the pattern of starch accumulation is also of interest with regard to rhizome function. Although a majority of species accumulate starch in their rhizome, genus- or species-specific patterns exist, suggesting differential storage function in rhizomes of distinct species. Tuber production through adventitious rooting (e.g. in *Calathea roseopicta*, Figure 1A(j)) illustrates partial transfer of the storage function to specialized organs.

Thus, rhizomes are underground shoot systems that characterize certain basitonically branched plants. The present study revealed that rhizomes can be classified by the level of integration they exhibit, defined as either the proximal part of a module (non-geometric species) or a unified sympodium (geometric species). This characteristic may also hold for non-rhizomatic basitonically branched plants. A common feature that unifies both types of rhizome is their propensity to starch accumulation, although rhizomes differ in their storage function. Perhaps a further difference between rhizome and stolon – in addition to their position below (rhizome) or above (stolon) ground – lies in the fact that stolons can store starch only transiently (e.g. Bouchart et al. 1998) while rhizomes could be stable starch sinks. Likewise, the role of resting, already mentioned by Raunkiær (1934) is also more specific to rhizome than stolons, the latter exhibiting long internodes and being often short-living. Dormant buds and short shoots that have been described in many geometric species in this study on one hand and dormant axillary meristems or buds along the modules of non-geometric rhizomes on the other hand are all resting organs.

### Conclusion

This study presents the rhizome morphology of 18 Zingiberales species from Payamino, Ecuador. Morphological analyses, further supported by phenetic

analyses, reveal two fundamentally distinct modes of construction: one that develops an integrated sympodial rhizome by contrast to one that privileges the module as unity. Hence, the geometric and non-geometric rhizomes differ in their basic level of organization: for one it is an indeterminate sympodium (each module is determinate but the axis is indeterminate in that it is a continuous succession of determinate units; the rhizome) while for the other it is a determinate monopodium (the module). These geometric rhizomes evolved convergently in Heliconiaceae and in Costaceae, but also in Zingiberaceae where geometric species have been described (Bell 1979; Bell and Tomlinson 1980). The evolution of a distinct mode of construction can be tracked by simple and concrete morphological characters: the switch from delayed to immediate branching and reduction of the number of internodes at which branching can occur. Both characters co-evolved convergently preceding the evolution of geometric rhizome morphologies in Costaceae, Heliconiaceae, Zingiberaceae and perhaps Marantaceae.

## Acknowledgements

## Biography

Guillaume Chomicki is a Fellow of the Linnean Society of London, who is currently doing a PhD at the Ludwig Maximilan University, Munich.

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