

# Deciphering the complex architecture of an herb using micro-computed X-ray tomography, with an illustrated discussion on architectural diversity of herbs

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Differing from most animals, plants have an indeterminate body plan, allowing them to add new body parts throughout their lifetime. The realized modular construction of a plant is the result of endogenous processes and exogenous constraints. Plant architectural analysis provides a holistic approach to whole-plant development by disentangling endogenous processes from exogenous constraints. Such analyses have focused more on trees than herbs. We here report a rare growth form discovered in the tropical herb *Centella asiatica* (Apiaceae) using micro-computed X-ray tomography to understand organogenesis in the meristem. Seeds of *C. asiatica* germinate to give rise to an orthotropic monochasium (sympodium) with terminal inflorescences. A stolon subsequently emerges from the oldest leaf axils of the sympodium. The primary growth of stolons is indeterminate (monopodial), and they only bear scale leaves. The stolon not only produces new leafy sympodial shoots at each node but also an axillary inflorescence. To place this growth form in context, we illustrate all architectural models so far reported from herbs. Our study highlights the complexity of herb architectures and illustrates the use of a novel methodology to decipher plant branching modes.

ADDITIONAL KEYWORDS: Apiaceae – *Centella* – electron microscopy – plant architecture.

## INTRODUCTION

The architecture of a plant is the result of the fate of its meristems in time and space. Plant growth forms have long attracted naturalists and botanists, and a series of classification schemes, dating back as far as Theophrastus (c. 350 BC; Du Rietz, 1931) who first separated herbs, trees and shrubs, have been developed (von Humboldt, 1806; Raunkiær, 1904; Warming, 1909; De Candolle, 1818, reviewed by Du Rietz, 1931 and updated by Ellenberg &

Mueller-Dombois, 1967). These systems considered either natural groups (von Humboldt, 1806) or ecological attributes (e.g. Raunkiær, 1904) to classify growth forms. Morphologists, either from the school of classical plant morphology or from the school of the more recent ‘fuzzy’ Arberian morphology, focused more on organs and their clear-cut (classical) or blurred (fuzzy Arberian) boundaries (see Rutishauser & Isler, 2001, for a review) than on comparing realized plant growth forms.

In the 1970s, a developmental approach to whole-plant morphology emerged, plant architectural analysis, which aimed to distinguish the endogenous processes

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moulding the architecture of a plant from the constraints exerted by the environment (Hallé & Oldeman, 1970; Hallé, Oldeman & Tomlinson, 1978; Barthélémy & Caraglio, 2007). A few morphological characters, such as the position of sexual structures or the orientation of axis growth, have permitted distinguishing some 23 architectural models that capture the main tree growth patterns (Hallé & Oldeman, 1970; Hallé *et al.*, 1978; Hallé, 2004). This approach allows an integrative understanding of whole-plant development (Barthélémy & Caraglio, 2007; Chomicki, Coiro & Renner, 2017). The initial focus on trees, however, has meant that the architectural diversity of herbs is still poorly documented and understood.

Plant architecture is typically analysed by macro-morphological observations. For herbs, with their numerous and diverse types of meristems, macro-morphological observation of growth form, however, can be problematic. We therefore studied the architecture of the worldwide tropical aquatic herb *Centella asiatica* (L.) Urb. (Apiaceae) with micro-computed X-ray tomography (CT scanning), in addition to traditional microscopic approaches. The results reveal a novel growth form and illustrate the potential of CT scanning to decipher complex architectures. We also place the architecture of *C. asiatica* in the context of growth forms so far known from herbs.

## MATERIAL AND METHODS

### ARCHITECTURAL ANALYSIS

The architectural analysis of *C. asiatica* was performed according to the concepts and methods developed by Hallé & Oldeman (1970) and Hallé *et al.* (1978), and modified by Edelin (1977, 1984) and Barthélémy *et al.* (1989, 1991), and more recently revisited by Hallé (2004) and Barthélémy & Caraglio (2007). We selected individuals at all developmental stages and qualitatively described their types of axes; quantitative measurements are not relevant to our research question. Plants came from a single environment (greenhouse grown, full light, water and nutrient replete), but the first author has also observed the growth pattern of the species under full sun (water and nutrient replete), in Montpellier and, in the wild, in Fiji (Taveuni Island, close to Matei) in different periods (wet season, March 2015; dry season, July and August 2016, September 2014). The branching pattern and shoot tropisms of *C. asiatica* do not change with environment, in contrast to some other species, for example species of *Cornus* L. and *Rhamnus* L. (Charles-Dominique, Edelin & Bouchard, 2009; Charles-Dominique *et al.*, 2012) and *Zea mays* L. (Mouliat *et al.*, 1999).

### SCANNING ELECTRON MICROSCOPY

Stolon buds of *C. asiatica* were collected in the aquatic plant house of the Munich Botanical Garden, voucher S.S. Renner & Chomicki 2863 (deposited in the herbarium M), fixed in formalin–acetic acid–alcohol (FAA) and dehydrated in an ethanol–acetone series and dried with a Bio-Rad E3000 critical-point dryer (Watford, UK). Buds dried at the critical point were mounted on aluminium stubs with colloidal graphite and coated with platinum using a Bal-Tec SCD 050 sputter-coater (Witten, Germany). They were observed with a Supra 55VP scanning electron microscope (LEO 438 VP; Cambridge, UK).

### LIGHT MICROSCOPY

Light microscopy was carried out on a Nikon microscope eclipse E600 fitted to a Nikon camera Ds-Fi 1. A Nikon bright field filter was used. Lignin was stained with phloroglucinol and hydrochloric acid, with pink/reddish stains marking lignin-impregnated vascular tissues.

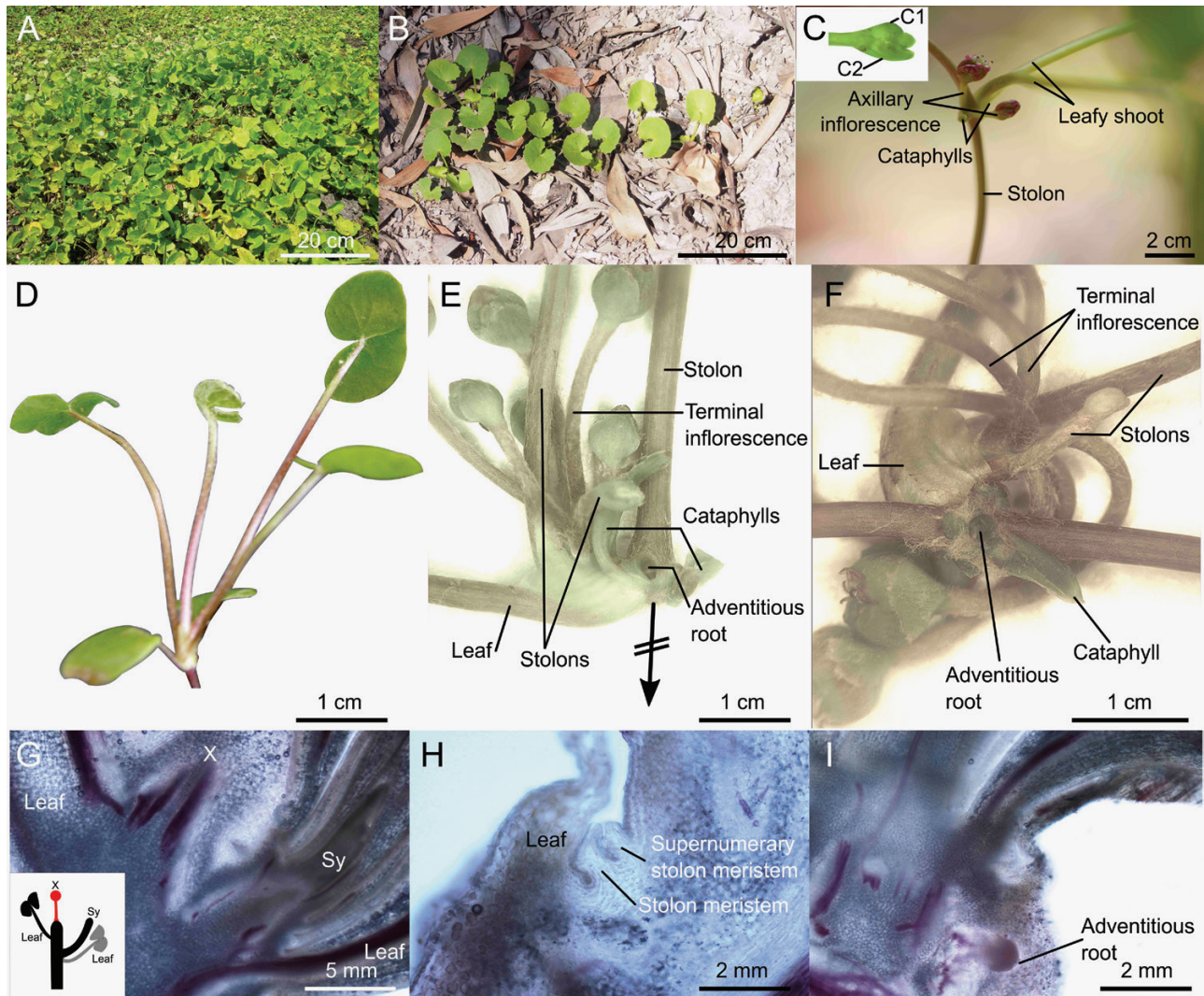
### X-RAY MICRO-COMPUTED TOMOGRAPHY (CT SCANNING)

Stolon buds were fixed in FAA. For X-ray micro-CT, all samples were treated with a solution of 1% (w/v) phosphotungstic acid in FAA for several months, following the protocol of Staedler *et al.* (2013). The buds were imaged at 2.0–33.7 µm voxel size with a microXCT-200 X-ray tomography system from Zeiss Microscopy (Jena, Germany). This system uses a 90-kV microfocus X-ray source (L9421-02 from Hamamatsu, Hamamatsu City, Japan), a cooled 2k CCD camera and switchable scintillator objective lens units. The software Amira (version 6.0.1, TGS Europe, S.A., Merignac Cedex, France; Mercury Computer Systems Inc., Chelmsford, MA, USA) was used to perform the 3D reconstruction from the CT scanning data. For samples that were scanned in several steps, the same software was used to stitch together the resulting scan data and to acquire images of the samples.

## RESULTS

### ARCHITECTURE OF *CENTELLA ASIATICA*

*Centella asiatica* is a prostrate, perennial herb that grows in dense colonies in humid areas in the tropics. The architectural unit of *C. asiatica* consists of two types of axes (Fig. 1A–C). Germination leads to the establishment of a leafy shoot, the first type of axis (Fig. 1D). The leafy shoot has a determinate primary growth, and the terminal meristem gives rise to an inflorescence after elongating



**Figure 1.** Habit and vegetative morphology of *Centella asiatica*. A, B, *Centella asiatica* forms large, clonal colonies via continuously growing stolons. C, stolons produce pairs of cataphylls (scale leaves), inflorescences and leafy shoots. D, seeds germinate to form a sympodial leafy shoot. E, F, leafy shoots produce further stolons in the leaf axils. All newly developed leafy shoots grow adventitious roots. G, following the inflorescence that terminates the development of the leafy shoot, a relay meristem develops from the uppermost leaf axil, leading to sympodial branching. X denotes meristem death (via termination in inflorescence); Sy, sympodial shoot meristem. H, some leaf axils produce a supernumerary stolon meristem (produced in a vertical series), leading to two stolons that emerge from a single leaf axil (see Fig. 1E, F). I, section through the base of a leafy shoot showing the emergence of an adventitious root.

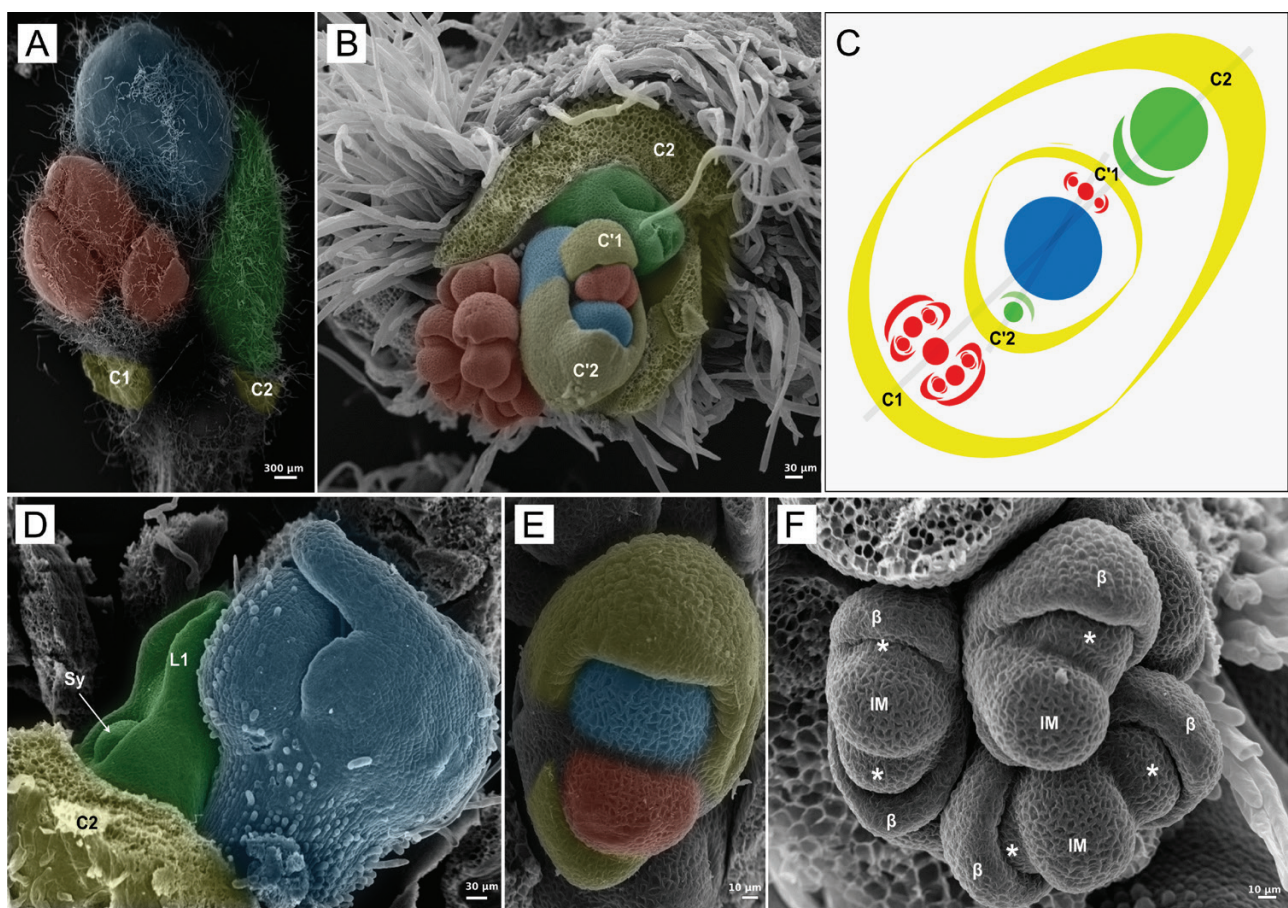
one to five phytomers; the inflorescence consists of a contracted umbel with one to five flowers (Fig. 1E, F). A relay meristem takes over from the uppermost leaf axil (Fig. 1G), resulting in a sympodial, modular growth of the leafy shoot, with a single relay meristem being produced at each node, leading to a monochasium. The leafy shoot thus at first conforms to Chamberlain's model (Hallé *et al.*, 1978). The relay meristem develops by immediate branching (concurrently with the development of the axis, not from a dormant bud). The leafy sympodium has a spiral phyllotaxis. There are no morphological markers of

growth rhythmicity in *C. asiatica*. Observations of growth of *C. asiatica* in a greenhouse between 2010 and 2017 and in the field at different times of the year (Material and Methods) always revealed meristematic activity, indicating that growth is continuous (without endogenous growth cessation), probably as in most herbs. A second type of branching occurs in the sympodium, this time delayed and occurring in the basal-most leaf axils, resulting in the formation of a stolon (Fig. 1E, F). Frequently, two stolons develop in the same axil (Fig. 1E, F) as the result of supernumerary buds produced in a vertical



succession (Fig. 1H). The stolon bears no leaves, but instead photosynthetic cataphylls (scale leaves) arranged in an opposite phyllotaxis but never fully decussate nor perfectly aligned (with secondary internode torsion) Fig. 2B, C. In addition to leafy shoots, the stolon produces inflorescences, but from macroscopic observations, it is unclear if they are terminal or axillary. Each leafy shoot produces adventitious roots (Fig. 1E, F I), leading to the formation of dense, clonal colonies (Fig. 1A). Each pair of cataphylls is asymmetric, with a minor cataphyll (C1) next to the inflorescence and a major cataphyll (C2) subtending the leafy shoot (Fig. 1C, inset). The structure of the stolon is unclear from our macroscopic

observations. In 1971, Hallé, who developed plant architectural analysis, observed *C. asiatica* and concluded that there were two competing hypotheses for the structure and development of the stolon and that it was impossible to distinguish by macroscopic observations (F. Hallé, pers. comm. to G.C. in 2009). The stolons either had a monopodial structure, where inflorescences were axillary on the stolon and terminal in the sympodium, or had a sympodial structure, where inflorescences are terminal and the major cataphyll has two buds (one supernumerary), the closest to the leaf axil which gives rise to the leafy sympodium and the other which gives rise to the stolons.



**Figure 2.** Scanning electron micrographs of the stolon apex. A, stolon bud (cataphylls removed, yellow) showing the next node of the stolon bud (blue), the sympodial leafy shoot (green) and the inflorescence (red). B, dissected secondary stolon bud [shown in blue in (A)], showing the same structure, with a new stolon bud in the centre (blue, with cataphylls yellow), and a sympodial leafy shoot (green), and the inflorescence (red) on each side. The stolon bud apparently has the same structure, with an inflorescence and the next stolon bud in the centre, but the cataphyll C'2 obscures the structure. C, hypothetical organ arrangements in the stolon bud shown in (B), assuming the stolon is monopodial. Note the difference of angle of the latest phytomer and the next to last. D, side view of a dissected apical meristem showing the stolon bud (blue) and sympodial shoot (green), with its shoot apex meristem (Sy) and first leaf (L1). E, close-up of a young (third series) stolon bud, showing the stolon meristem and an inflorescence meristem. F, close-up of a second-series stolon inflorescence [similar to the one shown in (B)], showing the inflorescence meristems (IMs), flower meristems (\*) and bracts (β).

DEVELOPMENT OF STOLONS IN *CENTELLA ASIATICA*

To determine whether the stolon of *C. asiatica* has a monopodial or sympodial architecture (and thus decide between Hallé's two hypotheses), we first used scanning electron microscopy (SEM) on dissected buds (Fig. 2). Removing the cataphylls from the stolon bud revealed a well-developed inflorescence and a leafy shoot, each apparently in the axil of an opposed cataphyll (C1 and C2, respectively), and a young, secondary stolon bud in the centre (Fig. 2A). Dissecting this second-series stolon bud revealed the same basic structure, with an inflorescence (Fig. 2B, C, F) and leafy shoot on the sides and a third-series stolon bud in the centre (Fig. 2B, C). An attempt to dissect a third-series stolon bud (Fig. 2E), with only the inflorescence and stolon meristem visible, failed. Since we could not determine the structure of the stolon from SEMs alone, we decided to study the youngest meristematic stages with micro-CT. A series of virtual sections revealed that inflorescences are in axillary positions and are not always produced at each node; the same is true for sympodial shoots (Fig. 3; Supporting Information, Movies S1, S2). Three-dimensional reconstructions of the whole bud showed the same pattern (Fig. 4A, B). They sometimes alternate with leafy shoots (Fig. 4A, B; Supporting Information, Movies S3, S4). Zooming into the second-series stolon meristem revealed that the next stolon module with the next pair of cataphylls is produced before inflorescence or leafy shoot meristems, demonstrating the indeterminacy of the stolon meristem and thus its monopodial growth (Fig. 4C, D). The development of the inflorescence and leafy shoot well after the cataphylls and axils is produced, and the general topology of the structures clearly rules out sympodial branching of the stolon.

## DISCUSSION

THE ARCHITECTURE OF *CENTELLA ASIATICA*

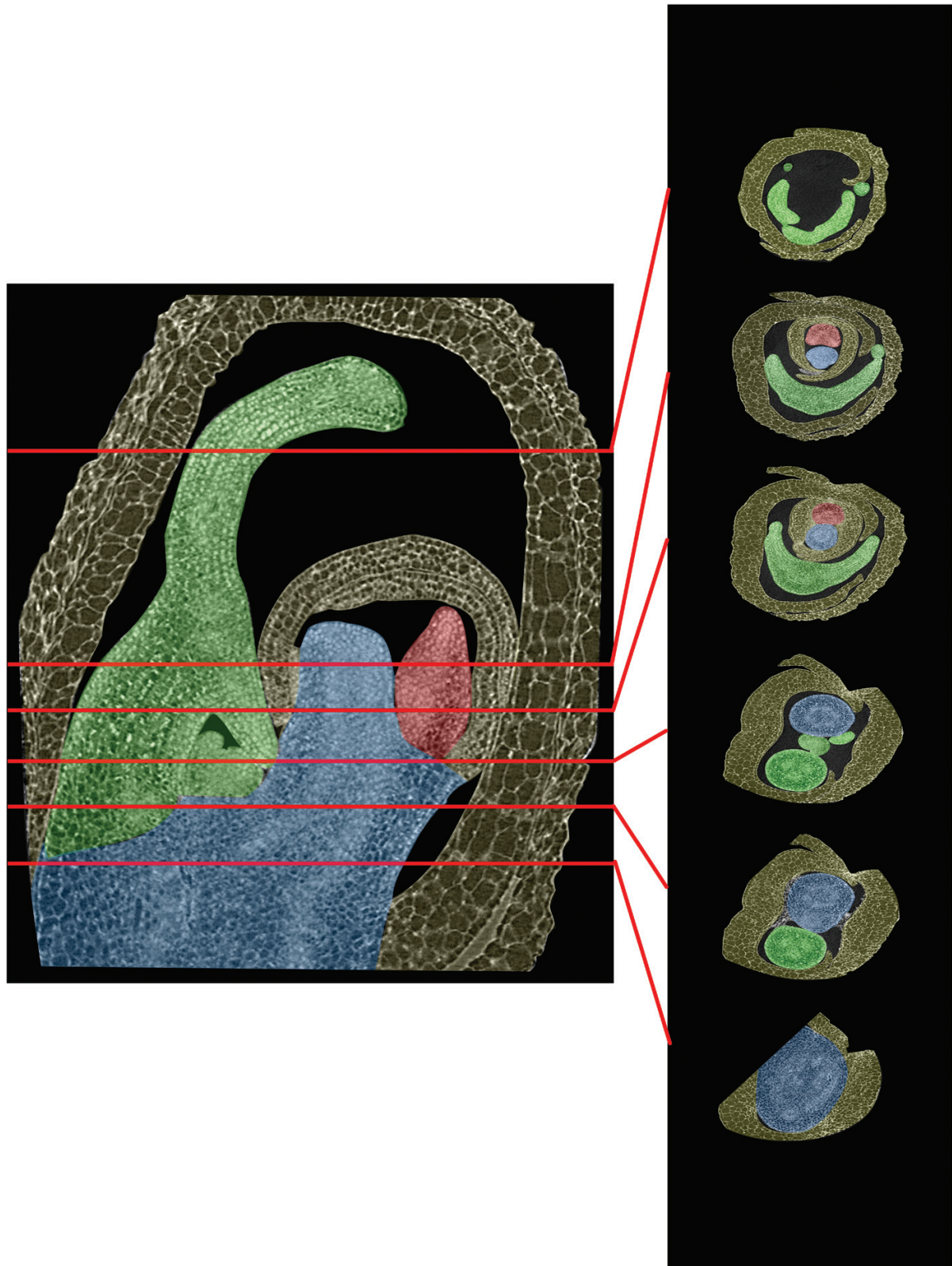
The developmental stages of *C. asiatica* are summarized in Figure 5. Seeds of *C. asiatica* germinate to produce a leafy shoot, which develops as a sympodial shoot system, in which typically a single relay meristem grows. This structure initially conforms to Chamberlain's model (Hallé *et al.*, 1978), but the production of stolons from older leaf axils gradually leads to a multi-branched system. The monopodial stolons are central to *C. asiatica* development, as they lead to vegetative multiplication of sympodial shoots. The architectural unit of *C. asiatica* consists of two types of axes, and their description is summarized in Table 1. This architecture could be considered a variant of Bell's model (Hallé, 2004; Fig. 6L), which lumps all plants with an architecture that involves a monopodial stolon. However, stolons are typically

sterile axes, whereas the stolons of *C. asiatica* have a reproductive function. It is unusual that both orthotropic and plagiotropic axes produce inflorescences. Another unusual aspect is the position of terminal inflorescences on the leafy sympodium and axillary ones on the monopodial stolon. Typically, axis differentiation leads to decreased morphogenetic potential of the sister axes, as in many plants with plagiotropic branches, such as *Araucaria heterophylla* (Salisb.) Franco (Massart, 1924). In *C. asiatica*, the leafy sympodium produces stolons, and the stolons produce leafy sympodia. The combination of strong axis differentiation and maintained morphogenetic potential to develop the other axis type leads to the formation of large, highly reiterated axes colonies. Each axis type only rarely reproduces itself: the leafy sympodium rarely produces two relay meristems and, likewise, the stolon rarely produces two stolons. Occasionally, however, new stolons can arise from an older stolon, for example in case of ablation or damage of the stolon meristem or when the plant is growing under abundant light and nutrients; we interpret these occurrences as traumatic (delayed) and adaptive reiteration (immediate), respectively. The architecture of *C. asiatica* does not fit well into any of the known architectural models, and we therefore advocate that Bell's model be subdivided to account for the diversity of aerial axes in stoloniferous plants, analogous to the subdivision of Tomlinson's model for basally branched herbs (Cremers, 1992; Cremers & Edelin, 1995; Chomicki, 2013). A morphological study of *Hydrocotyle vulgaris* L. (Araliaceae; Apiales), not closely related to *C. asiatica*, identified a similar architecture (Froebe, 1967), implying two independent origins of this growth form in the order. Strawberry (*Fragaria* L.) shows a similar architecture, but the stolons are not reproductive, and each sympodium branches, whereas in *C. asiatica*, the stolons are reproductive and the sympodium branches rarely. Further work is needed to determine whether other *Centella* spp. and related genera share a similar growth form and how many times it has evolved.

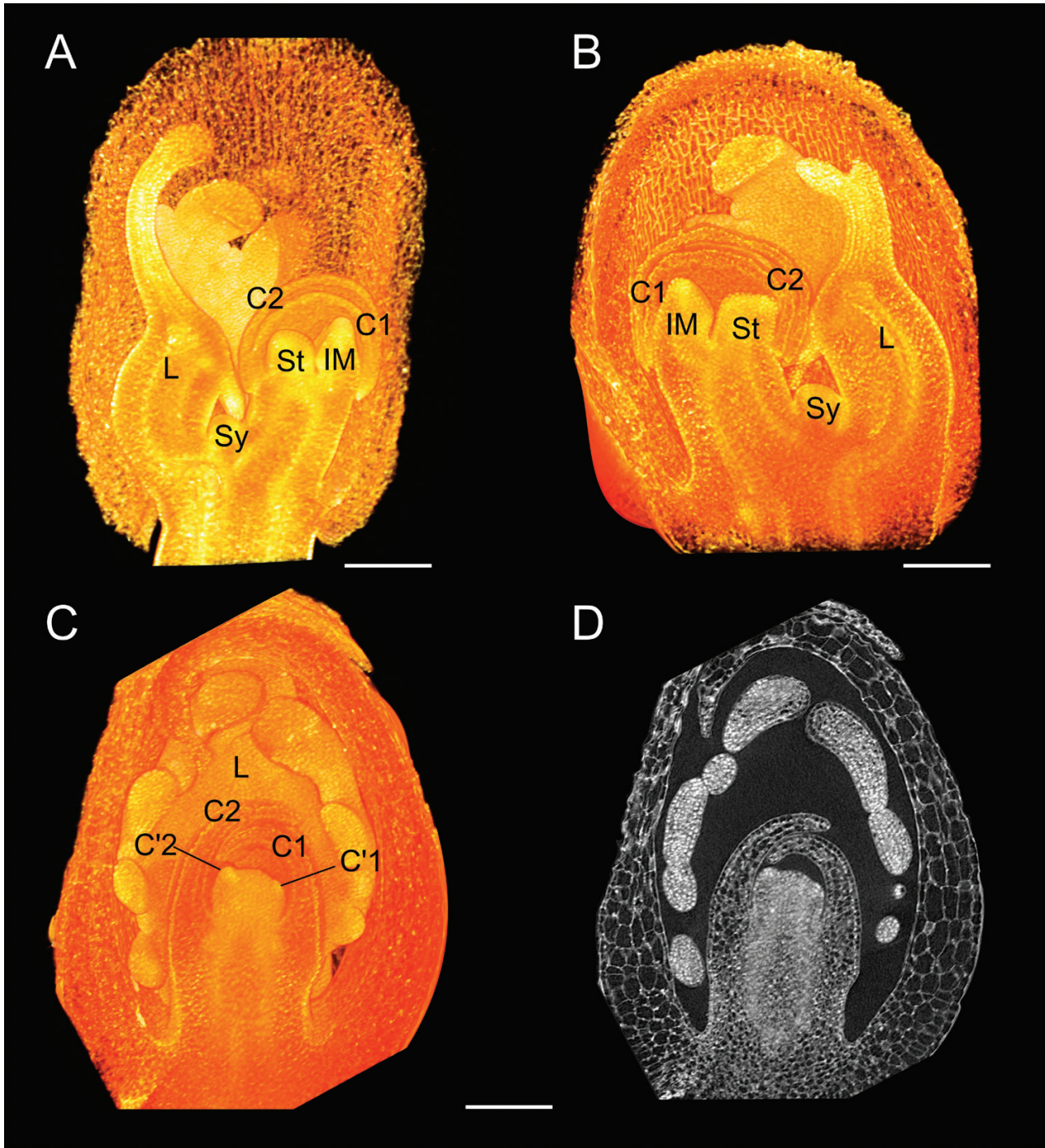
## USING MICRO-COMPUTED X-RAY TOMOGRAPHY TO STUDY ARCHITECTURE OF HERBS

The use of CT scanning has contributed to our understanding of extant and extinct plant morphology (Stuppy *et al.*, 2003; Dhondt *et al.*, 2010), but as far as we know, has not been used to analyse plant architecture. Our CT-scanning methodology allowed deciphering the architecture of the tropical aquatic herb *C. asiatica*. Compact growth, high numbers of primordia or meristems, continuous growth and immediate branching all render architectural analysis difficult if performed solely based on macro-morphological observations. SEM

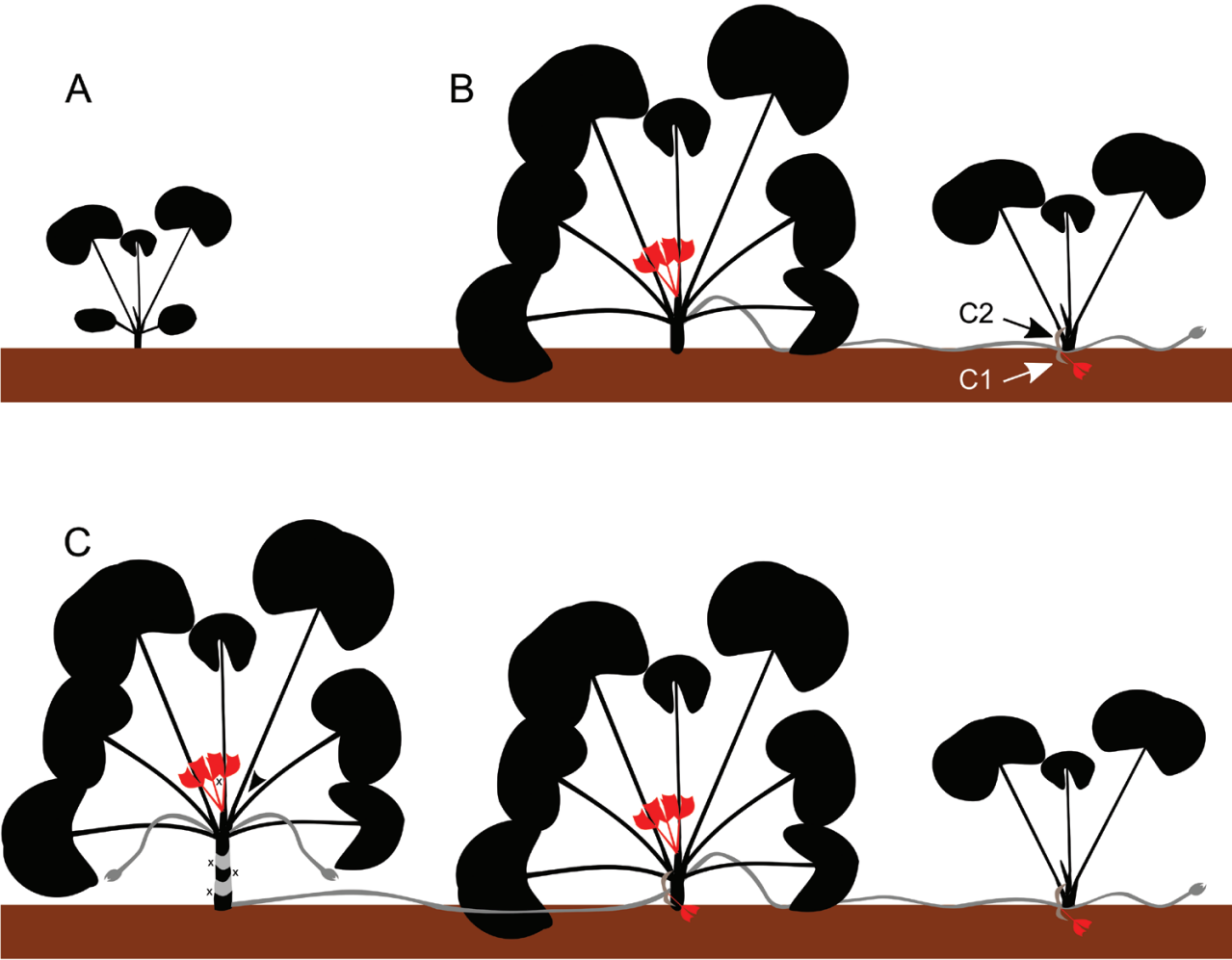




**Figure 3.** Micro-computed tomography scan showing virtual sections through a stolon bud. Colour coding: cataphylls in yellow; stolon and stolon meristem in blue; leafy shoot in green and inflorescence in red.



**Figure 4.** Micro-computed tomography scan showing 3D sections through stolon buds. A, B, longitudinal (virtual) section through stolon (first series) buds. C, longitudinal (virtual) section through stolon showing second- and third-series buds, third being the highest level of preformation in the bud. D, same section as in (C), shown in 2D. Abbreviations: L, leaf; Sy, sympodial leafy shoot meristem; C1 and 2, Cataphylls 1 and 2 (second series); St, stolon meristem; IM, Inflorescence meristem. C'1 and 2, third series cataphylls 1 and 2.

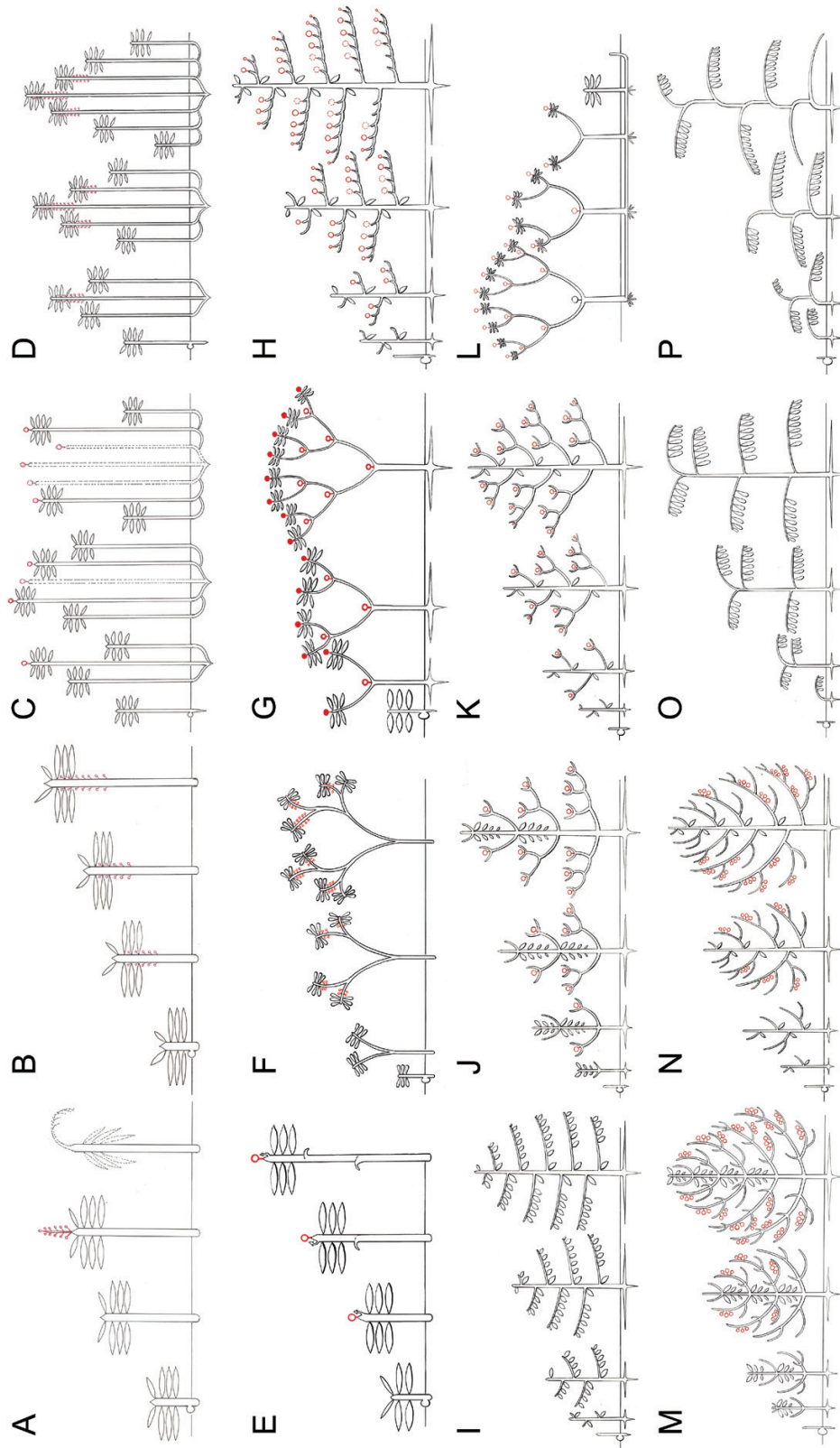


**Figure 5.** Schematic representation of the main developmental stages of *Centella asiatica*. A, the germinating seed gives rise to a leafy shoot. B, the initial leafy shoot apical meristem terminates in an inflorescence (in red), and monopodial stolons are produced, with paired cataphylls; from the axil of one cataphyll (C1, smaller one), an axillary inflorescence emerges; the other one (C2, larger) produces a leafy shoot in its axil, which then produces adventitious roots (not shown). C, older leafy shoots branch sympodially, but a single module replaces the preceding one (as in Chamberlain’s model), a dense network of monopodial stolons is produced, leading to a large clonal colony, and both the leafy sympodia and the cataphyll-bearing monopodial stolons produce inflorescences (terminal and axillary, respectively). X denotes meristem death, with the alternation of black and grey stem portions showing each module. The arrowhead points to the relay shoot.

**Table 1.** The architectural unit of *Centella asiatica*

	Leafy shoot (axis 1)	Stolon (axis 2)
Growth direction	Orthotropic	Plagiotropic
Growth	Continuous, determinate	Continuous, indeterminate
Leaves	Fully developed	Scale leaves (cataphylls)
Phyllotaxis	Spiral	Opposite-decussate, but secondary internode torsion
Branching	Immediate (relay meristem)/ delayed (stolon)	Immediate (leafy shoot)
Flowering	Terminal	Lateral





**Figure 6.** The diversity of herb architectures so far known. A, Holttum's model. B, corner's model. C, Tomlinson's model, version with terminal inflorescences. D, Tomlinson's model, version with axillary inflorescences. E, Chamberlain's model. F, Schoute's model. G, Leeuwenberg's model. H, Petit's model. I, Roux's model. J, Scarrone's model. K, Stone's model. L, Bell's model. M, Rauh's model. N, Attim's model. O, Troll's model, monopodial version. P, Troll's model, sympodial version. All drawings were prepared by Yasumin Sophia Lerner, University of Munich (LMU) and Munich Botanical Garden, and re-drawn following [Hallé \*et al.\* \(1978\)](#) and [Hallé \*et al.\* \(2004\)](#).

**Table 2.** Examples of herbs conforming to the architectural models shown in [Figure 6](#)

Model	Examples	References
Holttum ( <a href="#">Fig. 6A</a> )	<i>Ensete</i> (all eight species), e.g. <i>E. ventricosum</i> (Musaceae); <i>Aeonium nobile</i> , <i>A. tabuliforme</i> , <i>A. urbicum</i> ; <i>Kalanchoe gastonis-bonnieri</i> (Crassulaceae); <i>Geranium yeoi</i> ; <i>G. maderense</i> (Geraniaceae); <i>Boea havilandii</i> (Gesneriaceae)	<a href="#">Hallé <i>et al.</i> (1978)</a>
Corner ( <a href="#">Fig. 6B</a> )	<i>Hypolytrum heteromorphum</i> , <i>Mapania baldwinii</i> (Cyperaceae); <i>Byblis liniflora</i> (Byblidaceae); <i>Geranium canariense</i> (Geraniaceae); <i>Paraboea lanata</i> (Gesneriaceae); <i>Dorstenia contrajerva</i> (Moraceae); <i>Biophytum</i> spp. (several species, Oxalidaceae);	<a href="#">Hallé <i>et al.</i> (1978)</a>
Tomlinson, version with terminal inflorescences ( <a href="#">Fig. 6C</a> )	<i>Musa</i> (all species), <i>Musella lasiocarpa</i> (Musaceae); <i>Aechmea lingulata</i> , <i>Ananas comosus</i> , <i>Araeococcus micranthus</i> , <i>Guzmania lingulata</i> (Bromeliaceae); <i>Costus dinklagei</i> and many other species (Costaceae); <i>Cyperus esculentus</i> and many other <i>Cyperus</i> species, <i>Kyllinga erecta</i> (Cyperaceae); <i>Cynodon dactylon</i> , <i>Hierochloa odorata</i> , <i>Zea mays</i> (Poaceae) and most species in that large family. <i>Heliconia</i> spp. (most species, Heliconiaceae); <i>Alpinia boia</i> (Zingiberaceae) and most species in that family; <i>Carlina acanthifolia</i> (Asteraceae) and many other species in this huge family; <i>Bryophyllum fedtschenkoi</i> (Crassulaceae); <i>Euphorbia characias</i> (Euphorbiaceae) and many other <i>Euphorbia</i> spp.; <i>Lysimachia punctata</i> (Primulaceae); <i>Helleborus foetidus</i> (Ranunculaceae)	<a href="#">Hallé <i>et al.</i> (1978)</a> ; <a href="#">Cremers (1992)</a> ; <a href="#">Cremers &amp; Edelin (1995)</a> ; <a href="#">Moulia <i>et al.</i> (1999)</a> ; <a href="#">Chomicki (2013)</a>
Tomlinson, version with axillary inflorescences ( <a href="#">Fig. 6D</a> )	<i>Elytraria marginata</i> (Acanthaceae); <i>Geranium palmatum</i> (Geraniaceae); <i>Piper peltatum</i> (Piperaceae)	<a href="#">Hallé <i>et al.</i> (1978)</a>
Chamberlain ( <a href="#">Fig. 6E</a> )	<i>Alocasia lauterbachiana</i> , <i>Arum maculatum</i> , <i>Dieffenbachia pica</i> (Araceae); <i>Afrotrilepis pilosa</i> (Cyperaceae); <i>Kalanchoe rhombopilosa</i> (Crassulaceae);	<a href="#">Hallé <i>et al.</i> (1978)</a>
Schoute (dichotomous branching) ( <a href="#">Fig. 6F</a> )	<i>Strelitzia regina</i> (Strelitziaceae); <i>Mammillaria parkinsonii</i> , <i>M. perbella</i> , <i>M. karwinskiana</i> , <i>M. rosensis</i> , <i>M. auriareoli</i> (Cactaceae); <i>Asclepias syriaca</i> (Apocynaceae)	<a href="#">Craig (1945)</a> ; <a href="#">Nolan (1969)</a> ; <a href="#">Boke (1976)</a> ; <a href="#">Fisher (1976)</a>

also failed to reveal the structure of the stolon because the youngest, most fragile buds could not be successfully dissected. CT scanning of whole bud complexes proved to be an efficient method to decipher architecture because it simultaneously reveals micro-morphology and anatomy and allows 3D visualizations of structure and sections without destruction of the sample.

#### THE ARCHITECTURAL DIVERSITY OF HERBS

All architectural models so far known to be represented in herbs are illustrated in [Figure 6](#), and [Table 2](#) provides examples of herbs conforming to these models. The great diversity of herb architecture probably reflects their deeply convergent evolution ([Zanne \*et al.\*, 2014](#)) and repeated radiation into temperate regions from initially tropical woody plants, as inferred in Apiaceae, Rubiaceae, Malvaceae,

Dipsacaceae and other families ([Smith & Donoghue, 2008](#); [Beaulieu, O'Meara & Donoghue, 2013](#)). Investigating the evolution of plant architecture in clades that transited from trees to herbs (or *vice versa*) is pivotal to illuminating the morphogenetic changes that have shaped much of the current dissimilarities between temperate and tropical floras. Studying plant architecture in these transitions requires (1) plant architectural analysis in multiple species of a clade in which such transitions occurred and (2) a phylogenetic framework for the relevant clade, allowing for ancestral state reconstructions. Shifts from trees to herbs could either involve (1) *miniaturization*, if the herb retains the identical architecture of the tree; (2) *neoteny*, in which all axes will quickly enter the reproductive phase, leading to small plants, with potentially distinct architecture; (3) *fragmentation*, when a fraction of the architecture of a tree is present



Table 2. Continued

Model	Examples	References
Leeuwenberg (Fig. 6G)	<i>Achyranthes aspera</i> (Amaranthaceae); <i>Datura stramonium</i> , <i>Capsicum annuum</i> (Solanaceae); <i>Sphenoclea zeylanica</i> (Sphenocleaceae); <i>Acanthospermum hispidum</i> , <i>Pallenis hierochuntica</i> , <i>Carlina racemosa</i> , <i>Asteriscus aquaticus</i> , <i>Pectis ciliaris</i> , <i>Psiadia altissima</i> , <i>Spilanthes acmella</i> , <i>Synedrella nodiflora</i> (Asteraceae); <i>Kalanchoe streptantha</i> (Crassulaceae); <i>Scabiosa prolifera</i> (Dipsacaceae); <i>Erica tetralix</i> (Ericaceae); <i>Croton hirtus</i> (Euphorbiaceae); <i>Ocimum campechianum</i> (Lamiaceae); <i>Phytolacca americana</i> (Phytolaccaceae); <i>Polygala pepelis</i> (Polygalaceae)	Hallé <i>et al.</i> (1978); G.C. (pers. observ.)
Petit (Fig. 6H)	<i>Waltheria indica</i> (Malvaceae)	Hallé <i>et al.</i> (1978)
Roux (Fig. 6I)	<i>Phyllanthus urinaria</i> , <i>P. niruri</i> (Phyllanthaceae)	Hallé <i>et al.</i> (1978)
Scarrone (Fig. 6J)	<i>Bidens</i> sp. (Asteraceae); <i>Aeonium decorum</i> (Crassulaceae); <i>Erysimum</i> × <i>cheiri</i> (Brassicaceae); <i>Scabiosa cretica</i> (Dipsacaceae); <i>Polygala myrtifolia</i> (Polygalaceae)	Hallé <i>et al.</i> (1978)
Stone (Fig. 6K)	<i>Sphenoclea zeylanica</i> (Sphenocleaceae); <i>Ageratum conyzoides</i> (Asteraceae)	Hallé <i>et al.</i> (1978)
Bell (Fig. 6L)	<i>Equisetum arvense</i> (and other species), <i>Calamites</i> spp. [fossil], (Equisetaceae); <i>Centella asiatica</i> (Apiaceae), <i>Cymodocea serrulata</i> , <i>Thalassodendron ciliatum</i> (Cymodoceaceae); <i>Remirea maritima</i> (Cyperaceae); <i>Halophila ovalis</i> , <i>Thalassia testudinum</i> (Hydrocharitaceae); <i>Arundinaria amabilis</i> , <i>Phyllostachys aurea</i> , <i>P. edulis</i> , <i>P. nigra</i> , <i>Semiarundinaria fastuosa</i> , <i>Sasa veitchii</i> , <i>Spinifex littoreus</i> , <i>Thuarea involuta</i> (Poaceae), <i>Rungia paxiana</i> (Acanthaceae), <i>Rhus aromatica</i> (Anacardiaceae); <i>Matelea rivularis</i> (Apocynaceae); <i>Enhydra sessilis</i> , <i>Wedelia trilobata</i> (Asteraceae); <i>Brasenia schreberi</i> (Cabombaceae); <i>Linnaea borealis</i> (Caprifoliaceae); <i>Honckenya peploides</i> (Caryophyllaceae); <i>Vitex trifolia</i> (Lamiaceae); <i>Piper sarmentosum</i> (Piperaceae); <i>Ardisia japonica</i> , <i>A. pusilla</i> (Primulaceae); <i>Geophylla obvallata</i> , <i>G. renaris</i> , <i>G. repens</i> , <i>Bertiera adamsii</i> , <i>Diodia maritima</i> , <i>D. vaginalis</i> , <i>Hydrophyllax madagascariensis</i> , <i>Lasianthus repens</i> (Rubiaceae); <i>Utricularia</i> spp. (Utriculariaceae); <i>Phyla nodiflora</i> (Verbenaceae)	Hallé (2004); note that in <i>Equisetum</i> , the branching mechanism is not axillary, and thus, the fit to Bell's model is only topological.
Rauh (Fig. 6M)	<i>Oleandra pistillaris</i> (Oleandraceae/Polypodiaceae); <i>Erica herbacea</i> , <i>E. multiflora</i> , <i>E. vagans</i> , <i>Calluna vulgaris</i> (Ericaceae); <i>Paraboea suffruticosa</i> (Gesneriaceae)	Hallé <i>et al.</i> (1978)
Attims (Fig. 6N)	<i>Phyllanthus lacunarius</i> , <i>P. polygonoides</i> (Phyllanthaceae); <i>Ludwigia abyssinica</i> (Onagraceae); <i>Turnera ulmifolia</i> (Passifloraceae)	Hallé <i>et al.</i> (1978)
Troll, monopodial version (Fig. 6O)	<i>Phyllanthus alpestris</i> , <i>P. caroliniensis</i> (Phyllanthaceae); <i>Columnea</i> sp., <i>Drymonia</i> sp. (Gesneriaceae); <i>Chamaecrista mimosoides</i> (Fabaceae); <i>Cuphea carthagenensis</i> (Lythraceae)	Hallé <i>et al.</i> (1978)
Troll, sympodial version (Fig. 6P)	<i>Klugia notoniana</i> (Gesneriaceae); <i>Sida spinosa</i> (Malvaceae); <i>Argostemma parvifolium</i> var. <i>involutum</i> (Rubiaceae); <i>Pilea microphylla</i> (Urticaceae)	Hallé <i>et al.</i> (1978)

Two architectural models of herbs described in Hallé *et al.* (1978) have since been reframed in Hallé (2004): Cook's model, which is now considered to be a variation of Roux's model, in which the branches are phyllomorphic, and McClure's model, which is now considered to be a variation of Tomlinson's model.

in the herb, notably in the case of rhythmically growing tree ancestors; or (4) *loss of tropism*, when a shift from orthotropic to plagiotropic habit drives the shift to the herbaceous habit (Hallé *et al.*, 1978). Architectural analyses reveal that some herbs have an architecture comparable to trees, whereas others have distinct architectures (Jeannoda-Robinson, 1977). Therefore, only a phylogenetic comparative framework allows one to infer whether an herb conforming to Corner's model (see Fig. 6B), an architecture also found in trees, results from neoteny or not.

Basal plagiotropic or mixed axes able to form colonies by vegetative reproduction are a frequent feature of herb architecture, especially in perennial species. These axes can be lumped into the definition of a rhizome as a 'vegetative extension over or within the substrate by means of axis elongation, and [including] organs which may be distinguished more precisely as stolons, offsets or suckers and which may intergrade with tubers and corms' (Bell & Tomlinson, 1980: 126). A study of Zingiberales rhizomes from Ecuador revealed two modes of basal branching: (1) geometric rhizomes, in which basal branching is integrated into the development of the plant and occurs at specific internodes, often concurrently with the parent shoot development (immediate branching), leading to highly patterned, 'geometric' rhizomes, and (2) non-geometric rhizomes that opportunistically branch from any internodes (also delayed branching) (Chomicki, 2013). In *C. asiatica*, branching of the sympodium (stolons) is delayed, but occurs regularly, regardless of light conditions (cf. Bidel *et al.*, 2015) or field *vs.* greenhouse conditions (G.C., pers. observ. on the Fiji island of Taveuni: wet season, March 2015; dry season, July and August 2016, September, 2014).

## CONCLUSIONS

Our 3D imaging approach unveiled the rare architecture of the tropical aquatic herbaceous *C. asiatica* (Apiaceae); it has sympodial orthotropic leafy shoots that are multiplied by a plagiotropic and monopodial stolon. Unique features include the reproductive function of the leafless stolon and terminal *vs.* axillary inflorescences on orthotropic and plagiotropic axes, resulting in sympodial and monopodial branching in two types of axes. The finding of such a complex architecture in a common and much-cultivated plant, such as *C. asiatica*, suggests that further work is needed on the diversity of herb architectures, and our 3D imaging technique using micro-computed X-ray tomography illustrates the utility of this approach to understand the structure of tiny, condensed meristems. Architecture impacts the extent and timing of sexual and vegetative reproduction,

and herb architectural analysis might prove a useful tool in trait analyses in the context of community ecology of savannas and grasslands.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

- Movie S1.** Transversal sections through a bud of *Centella asiatica* scanned using micro-computed tomography.
- Movie S2.** Transversal sections through a bud of *Centella asiatica* reconstructed in 3D and coloured artificially, scanned using micro-computed tomography.
- Movie S3.** Longitudinal sections through a bud of *Centella asiatica* scanned using micro-computed tomography.
- Movie S4.** Longitudinal sections through a bud of *Centella asiatica* reconstructed in 3D and coloured artificially, scanned using micro-computed tomography.