Heterochronic Developmental Shifts Underlie Floral Diversity within *Jaltomata* (Solanaceae)

Jamie L. Kostyun[1*], Jill C. Preston[2,a], and Leonie C. Moyle[1,b]

[1] *Department of Biology, Indiana University, Bloomington, Indiana 47405, USA*

[2] *Department of Plant Biology, The University of Vermont, Burlington, Vermont 05405, USA*

[a] Jill.Preston@uvm.edu

[b] lmoyle@indiana.edu

*Corresponding author: jkostyun@indiana.edu

Abstract

Background: Haeckel and Darwin, respectively, proposed heterochronic shifts during mid to late stages of organismal development as key mechanisms generating phenotypic diversity. To determine whether late heterochronic shifts underlie derived floral morphologies within *Jaltomata* – a genus with extensive and recently evolved floral diversity – we compared floral development of four diverse species (including an ambiguously ancestral or secondarily derived rotate, two putatively independently evolved campanulate, and a tubular morph) to the ancestral rotate floral form, as well as to an outgroup that shares this ancestral floral morphology.

Results: We determined that early floral development (<1 mm bud stage) is very similar among all species, but that different mature floral forms are distinguishable by mid-development (3-4 mm stages) due to differential growth acceleration of corolla traits. Floral ontogeny among similar mature forms remains comparable until late stages (>5 mm), followed by species-specific heterochronic shifts in corolla traits.

Conclusions: Our data suggest shared floral patterning during early-stage development, continued shared patterning or re-use of similar floral developmental pathways at mid-stage development, and distinct heterochronic shifts during late-stage development. Heterochrony thus appears to have been important in the rapid and repeated diversification of *Jaltomata* flowers.

Keywords: Convergent Evolution, Flower development, Heterochrony, *Jaltomata*, Solanaceae.

Background

In The Origin of Species, Darwin stated "we can actually see in embryonic crustaceans and in many other animals, and in flowers, that organs, which when mature become extremely different, are at an early stage of growth exactly alike." [1-2]. This prescient statement both suggests the importance of understanding diversity in a developmental and phylogenetic framework, and raises the question about which mechanisms underlie these hypothesized mid to late stage shifts in ontogeny. Heterochrony—a change in the relative timing or rate of a developmental process between a derived lineage and its ancestor—is one potential mechanism contributing to such trait variation [3-4]. Since being formally defined by Haeckel [5], and expanded upon by Gould [6-7], the concept of heterochrony has been applied to understanding morphological changes, predominantly in animal taxa. For instance, the classic example of axolotls that retain more juvenilized features than their ancestors [8], the large variation in cranial morphology among different dog breeds [9], and even the evolution of extremely complex traits such as metamorphosis in insects [10], have largely be attributed to heterochronic shifts during development.

The general concept of heterochrony, as well as the distinct processes that comprise this mechanism of developmental change, can also be meaningfully applied to plant evolution [11-12]. However, while heterochronic shifts are typically determined in animals by comparing the relative timing of reproductive versus somatic maturation, in plants this can be challenging given their indeterminate development. Despite this, heterochrony can still be identified in plants by examining individual organs or functional units, such as flowers or individual leaves [4]. Broadly, heterochronic shifts include changes in the relative timing of initiation (i.e. onset) or termination (i.e. offset) of a developmental process – which together determine the duration of

this developmental process. Additionally, changes can occur in the rate at which a developmental process proceeds. Shorter developmental duration or decreased growth rate (termed pedomorphosis) often results in the reduction or juvenilization of a trait, while longer developmental duration or increased growth rate (termed peramorphosis) often results in the elaboration of a trait. Both types of shifts – duration or rate – can conceivably contribute to observed variation in plant traits [3-4, 13].

Unlike plant architecture in general, flowers undergo determinate development, and thus are particularly attractive subjects for studying the contribution of heterochrony to morphological divergence. While most flowers conform to a shared ground plan, i.e. egg and pollen-producing structures surrounded by a non-reproductive perianth (sepals and petals), many lineages exhibit abundant inter- and intra-specific variation in the organization (phyllotaxy), number (merosity), size and shape, degree of fusion, and specific identity of floral organs [14-15]. Numerous studies have examined the potential role of heterochronic changes in floral trait evolution, most often those associated with mating system transitions. The shift from outcrossing to predominately selfing is considered one of the most common evolutionary transitions among plants [16-17], and is typically associated with reduction of flower size, petal size, and/or anther-stigma separation (herkogamy) – collectively referred to as the 'selfing syndrome' [18-19]. Comparative work examining closely related outcrossing and selfing species has generally revealed that reductions in floral size result from pedomorphic changes, either from decreased growth rates (i.e. neoteny; e.g. [20]), or truncation of the growth period [21]. However, some studies have also found that smaller flowers or those with reduced herkogamy in selfing lineages result from an increased growth rate over either short [22-23] or long [24] developmental periods. Thus, several distinct heterochronic processes can result in convergent selfing syndromes.

Apart from developmental studies of mating system transitions, comparative ontogenetic studies have examined floral trait variation likely related to pollinator shifts [25-26]. A classic example is Guerrant's [27] examination of developmental differences between a pair of *Delphinium* species – one bee-pollinated, the other hummingbird-pollinated. In that instance, the derived hummingbird-pollinated flower results from an overall decreased growth rate (pedomorphosis via neoteny), but also accelerated growth for an extended period (peramorphosis via acceleration and hypermorphosis) specifically in the reward-providing nectariferous petals. Nonetheless, despite this increasing attention to the role of heterochrony in plant development, several critical questions remain, including how frequently heterochronic shifts underlie changes in floral form, and whether convergent phenotypes may be the result of similar or divergent developmental mechanisms.

Jaltomata (Solanaceae) includes approximately 60-80 species distributed from the Southwestern United States to the Andean region of South America, with extensive and recently evolved floral diversity [28-30]. Although the pollination biology of this genus has yet to be formally evaluated, field observations (T. Mione, pers. comm.) reveal that different species are visited by bees and hummingbirds. In addition, *Jaltomata* produce varying amounts of floral nectar as a pollinator reward, unlike close relatives like *Solanum* [31-32]. Molecular phylogenies based on one to several loci [28-30], as well as phylogenomic reconstruction using whole transcriptomes (Wu, Kostyun, Moyle, unpubl.), indicate that the ancestor of *Jaltomata* had flattened 'rotate' flowers, while both bell-shaped 'campanulate' and elongated 'tubular' flowers are derived specifically within the genus (Fig. 1). Further, species with derived campanulate or tubular forms generally produce more nectar per flower than those with ancestral rotate forms [30]. Ancestral state reconstruction [30] also suggests that some of the derived campanulate and

116 tubular floral forms found in different *Jaltomata* species evolved independently, allowing
117 comparison of developmental mechanisms underlying putatively convergent forms.
118 Because both derived campanulate and tubular forms might represent elaborated versions
119 of the ancestral rotate form, we hypothesized that heterochronic changes (specifically
120 peramorphic changes such as longer duration of development and/or growth acceleration, which
121 often produce larger or more elaborated structures) underlie these transitions, particularly during
122 later stages of ontology [1-2, 4]. In addition to evaluating the role of heterochrony in the
123 evolution of derived floral forms, we also expected that comparing floral development between
124 species with similar mature morphs--but unresolved phylogenetic relatedness--would provide
125 insight into their evolutionary origin [30] (Wu, Kostyun, Moyle, unpub.). For instance, if similar
126 mature forms result from similar developmental processes, this could suggest re-use of similar
127 pathways or a shared single origin. In contrast, if they result from distinct developmental
128 processes, this could indicate that these forms are independently derived, or that these forms
129 share a common evolutionary origin (i.e. are homologous) but underwent developmental systems
130 drift during their divergence. Follow-up analyses at the molecular level, identifying causative
131 mutations contributing to trait divergence, could then be used to discriminate further between
132 hypotheses, with shared mutations being more likely attributed to common descent than to
133 parallel evolution.
134 Given these considerations, our main goals in this study were to: 1) assess evidence for
135 heterochronic changes during floral development in species with derived campanulate and
136 tubular forms; 2) determine which type(s) of heterochronic changes are associated with specific
137 floral trait changes; and 3) assess whether putatively convergent forms might result from similar
138 or different developmental processes (i.e. are associated with the same or different types of

heterochronic shifts). To address these goals, we examined floral ontogeny of five florally diverse *Jaltomata* species (including one species with an ancestrally representative rotate corolla form), as well as an outgroup species that also has an ancestrally representative rotate corolla form. Our findings support the inference that a combination of parallel and convergent allometric changes (i.e. shifts in the size of particular floral organs in relation to the entire flower) have given rise to floral variation in this group, and show that heterochronic shifts explain these changes. In particular, two types of peramorphic changes (extended duration of floral development and accelerated growth) predominately explain changes in corolla traits. Thus, peramorphism emerges as an important developmental mechanism controlling diversity of *Jaltomata* corolla forms. Finally, species with similar mature campanulate corollas follow similar but clearly not identical growth trajectories, consistent with phylogenetic inferences that these campanulate floral morphs have independent origins (Wu, Kostyun, Moyle, unpubl.) (Fig. 1). In contrast, we found that species with similar mature rotate corollas follow nearly identical developmental trajectories until the very last stages of ontology, suggesting a single evolutionary origin in this case.

Methods

Plant materials and growth conditions

Field-collected seeds of our five target *Jaltomata* species were provided by Dr. Thomas Mione (Central Connecticut State University), and seed for one wild tomato outgroup (*Solanum pimpinellifolium*, accession LA1589) was obtained from the Tomato Genetics Resource Center at the University of California, Davis (Table S1). We selected this outgroup because *Solanum* is the sister genus to *Jaltomata* [28-29], this species has an ancestrally representative rotate corolla

[32], floral development in this species has previously been characterized [21, 33], and--as a wild species--its floral development has not been influenced by domestication (i.e. compared to domestic tomato, *S. lycopersicum*). Our focal *Jaltomata* species included the Central American species *Jaltomata darcyana* that has an ancestrally representative rotate corolla, and four species found as natives exclusively in South America: *Jaltomata calliantha* and *Jaltomata dendroidea* with putatively independently derived campanulate corollas, *Jaltomata umbellata* with a tubular corolla, and *Jaltomata sinuosa* with a rotate corolla that is ambiguously ancestral or secondarily derived (Fig. 1). Plants were cultivated in the Indiana University Research Greenhouse, under standardized temperature (15-20°C) and light (16-hour days) conditions. All plants were watered to field capacity on automatic driplines, and received weekly fertilizer treatment.

Floral development

To estimate the duration of floral development, young inflorescences were first identified when the largest bud was approximately 1 mm, and then evaluated and measured every second day until first anthesis. To assess relative organ growth during development, buds up to 4 mm in diameter were collected into 70% ethanol or formalin-acetic acid-alcohol (FAA), dissected as required, dehydrated through an ethanol series at room temperature, and incubated in a 4°C fridge over-night. Samples were critical point dried, mounted on aluminum stubs, and sputter-coated with gold-palladium. They were then imaged using a JEOL 6060 or a JEOL 5800LV scanning electron microscope (SEM) at the University of Vermont or Indiana University, respectively. Morphological traits were measured on the resulting bud micrographs using ImageJ [34]. For buds greater than 4 mm in diameter, morphological traits were measured by hand on freshly collected samples using digital calipers. Given the potential role of trichomes in

regulating corolla shape during bud development [35], we also determined the developmental timing of trichome initiation and maturation. Because our focal species also vary in nectar volume per flower, we determined the onset of nectar secretion using a 20x hand-lens.

Mature floral trait measurements

Mature floral traits were measured with hand-held digital calipers on three flowers each for at least three individuals per species, and included calyx diameter, sepal length, corolla diameter, corolla depth, corolla fusion, petal length, lobe length, stamen length, anther length, ovary diameter, style length, and herkogamy (stigma-anther separation) (Fig. S1). We also measured nectar volume per flower to the nearest 1 μL with a pipette. To reduce potential environmental effects on nectar production, nectar volume per flower was always measured during the early afternoon following watering. Trichome type(s) present on sepals and petals was also scored using a 20x hand lens.

Statistical analyses

We used analyses of variance (ANOVA), followed by Tukey's honest significant difference (HSD) post-hoc tests, to assess differences among species in three comparisons: floral traits on mature flowers; log-transformed floral organ size at discrete stages during development; and duration (i.e. number of days) of floral development. To compare relative floral organ growth rates during development, we used linear regression on log-transformed bud measurements. In particular, we regressed organ sizes on bud diameter (as a standard measure of developmental floral stage; e.g. [21]), and assessed whether there were significant interaction effects between bud diameter and species, as a measure of differential growth rates. We used *J. darcyana* as the

baseline in these analyses to assess differences between ancestral and derived floral forms, and *J. calliantha* as the baseline when comparing the two derived campanulate forms (i.e. *J. calliantha* versus *J. dendroidea*). All analyses were performed within the R statistical environment [36].

Results

The early sequence of floral development is similar across *Jaltomata* species

As expected based on their common origin, the five focal species of *Jaltomata* share a common floral ground plan with each other as well as with the *Solanum* outgroup *S. pimpinellifolium*. All six species have very similar development up until the 1 mm bud diameter stage. Sepals are the first floral organs to emerge from the ~0.15 mm diameter floral meristem, and do so in an asymmetric manner from the abaxial, to adaxial, to lateral sides (Figs. 2a, 3a, 5a-b, 6a, 7a). Once sepals are approximately the same size and have started to accumulate trichomes, they elongate from a ring primordium at the base to form a partially fused structure (Figs. 2b, 3b, 4a, 5c, 6b, 7a). At the ~0.25-0.3 mm floral diameter stage, petals and stamens emerge almost simultaneously, but slightly asymmetrically, followed by gynoecial development by the ~0.5 mm diameter stage (Figs. 2c-d, 3c, 4b-c, 5d-e, 6c-d, 7b-c). Similar to sepals, petals initially emerge as free strap-like primordia, but begin to elongate from the congenitally fused region at the base (just above the stamen attachment point) prior to the ~1 mm diameter bud stage in all species (Figs. 2e, 3d, 4e-f, 5g, 6g, 7d). Because floral organs initiate at the same developmental stage across species, observed differences in mature flowers are therefore not a result of differences in growth onset.

Despite these general ontogenetic similarities, specific differences in growth rate are apparent by the 1 mm stage (Fig. 8). This includes when style elongation is first apparent, which

ranges from ~0.8 mm in *S. pimpinellifolium* and *J. sinuosa*, to ~1.5 mm in *J. darcyana* and *J. calliantha*. Relative to all *Jaltomata* species, *S. pimpinellifolium* shows significantly longer sepals, petals, corolla depth, and stamens (ANOVA $p < 0.00001$; all Tukey HSD, $p < 0.00001$), and wider ovaries (Tukey HSD, $p < 0.04$) at the 1 to 1.5 mm stage (Figs. 7-8). Among *Jaltomata* species, differences in sepal and corolla traits are also apparent by the 1 mm bud stage, but are not associated with rotate vs. campanulate vs. tubular mature floral morphologies (Fig. 1): sepals are significantly longer in campanulate *J. calliantha* compared to campanulate *J. dendroidea* and rotate *J. darcyana* (ANOVA $p < 0.001$; Tukey HSD, $p < 0.001$ and $p < 0.02$), while petals are longer in *J. calliantha* and tubular *J. umbellata* than *J. dendroidea* (ANOVA $p < 0.0001$; Tukey HSD, $p < 0.05$) (Fig. 8).

Developmental timing of trichome initiation on sepals and petals is similar among all species. However, qualitative differences become apparent by the 0.3-0.4 mm bud stage (Figs. 2-7). Trichomes are first initiated on sepals in all species around the 0.2 mm stage, but both simple (i.e. uniseriate) and non-viscous (i.e. non-secreting) glandular trichomes are apparent on *S. pimpinellifolium* sepals, compared to just simple ones on *Jaltomata* species (Figs. 2a-b, 3a-b, 4a, 5d, 6a-b, 7a). By the 0.3 mm and 0.5 mm stages, dendritic (i.e. branched) trichomes become apparent on *J. sinuosa*, *J. dendroidea*, and *J. umbellata* sepals, and viscous glandular trichomes develop on *J. sinuosa* sepals, respectively (Figs. 3c-e, 5e, 6f). Trichomes on petals are initiated by ~0.5 mm in all species (Figs. 2e, 3d, 4e, 5g, 6f, 7e) but, compared to only simple trichomes in *Jaltomata* species, both simple and non-viscous glandular trichomes are present on *S. pimpinellifolium* petals by this stage. By the 1.0 mm diameter bud stage (Figs. 2e-f, 5g), dendritic trichomes in *J. dendroidea* and viscous glandular trichomes in *J. sinuosa*, are present on petals.

Mid stage corolla patterning distinguishes divergent adult *Jaltomata* corolla types

While *S. pimpinellifolium* is conspicuously dissimilar from *Jaltomata* species by the 2 mm stage, pronounced differences in corolla traits among *Jaltomata* species are not apparent until the 3-5 mm stages (Fig. 8). Furthermore, these corolla trait differences are associated with the divergent mature corolla forms. Specifically, by the 3 mm stage, petal length, corolla depth, and the extent of congenital corolla fusion are significantly longer in tubular *J. umbellata* than in all other *Jaltomata* species (ANOVA $p < 0.00001$; all Tukey HSD, $p < 0.00001$). Further, by this stage rotate and campanulate species have superficial post-genital corolla fusion via interlocking trichomes that extends from the region of congenital fusion to petal tips (Figs. 2j-k, 3h-i, 4i). This is in striking contrast to development of rotate *S. pimpinellifolium* petals that remain only slightly fused at the base at this stage (Figs. 7j, 8). By the 4 mm stage, the extent of the congenitally fused corolla region becomes significantly longer in both campanulate species compared to both rotate species. Finally, by the 5 mm stage, both campanulate-flowered species also have significantly longer petals and corolla depths relative to the ancestrally rotate *J. darcyana* (ANOVA $p < 0.0004$; Tukey HSD, $p < 0.001$) (Fig. 8).

In addition to differences in corolla traits between species with divergent mature corolla forms, several species-specific differences in other floral traits are apparent during mid-development stages. For instance, sepals are shortest in rotate *J. darcyana* and longest in campanulate *J. calliantha* (ANOVA $p < 0.0001$; Tukey HSD, $p < 0.001$ and $p < 0.03$), stamens and styles (in addition to petals and corollas) are longest in tubular *J. umbellata* (ANOVA $p < 0.00001$; all Tukey HSD, $p < 0.004$), and the ovary is widest in *J. darcyana* (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.05$) (Fig. 8).

Corolla trait differences between species with similar forms arise during late floral development

For species with similar corolla forms in mature flowers, major species-specific corolla trait differences only arise during late stages (i.e. after the 5 mm bud stage). The ancestrally rotate flowers of *J. darcyana* and the ambiguously ancestral or secondarily derived rotate flowers of *J. sinuosa* appear nearly identical up until this stage. Indeed, none of the petal or corolla traits significantly differ between them until flowers of *J. darcyana* open shortly after the 5 mm stage, whereas the buds of *J. sinuosa* continue to grow until they open following the 6 mm stage (Table 1). Likewise, corolla traits are very similar between species with campanulate corollas (*J. calliantha* and *J. dendroidea*) until the 5 mm stage (although petals are shorter in *J. dendroidea* at the 1 mm stage). However, after the 5 mm stage, petal length, corolla depth, and corolla fusion all become significantly longer in *J. dendroidea* (Tukey HSD, $p < 0.007$; $p < 0.0001$; $p < 0.007$). Both petal length and corolla depth remain significantly longer in *J. dendroidea* until anthesis; however, by the 7 mm stage, corolla fusion expands in *J. calliantha* to match that in *J. dendroidea*. Finally, lobe length remains similar in these two species until the 8 mm stage, when it becomes significantly longer in *J. dendroidea* until anthesis (Tukey HSD, $p < 0.021$).

Overall duration of floral development and relative growth rates differ among species

We measured the duration of floral development in days to assess evidence that an extended growth period underpinned species floral differences, and calculated an average overall growth rate (bud diameter in mm per day) to assess potential accelerated growth of whole flowers. In addition, given observed allometric differences in organ sizes among species (Table 3), we also calculated relative growth rates of individual floral organs. For instance, even if species do not

differ in their overall absolute growth rate (diameter per day), differences in the relative growth rates among floral organs (floral organ in mm per mm of bud diameter) could explain observed differences in mature flowers.

Floral development duration ranged from 14 days in outgroup *S. pimpinellifolium* to 32.4 days in *J. dendroidea* (Table 1). Among *Jaltomata* species, the ancestrally rotate flowers of *J. darcyana* (19.23 days) and derived tubular flowers of *J. umbellata* (18.62 days) had the shortest development times (ANOVA $p < 0.00001$; Tukey HSD against all other species, $p < 0.00001$; Tukey HSD against each other, $p = 0.525$). Similarly, overall average bud growth rate (bud diameter in mm per day) ranged from 0.172 mm/day in *S. pimpinellifolium* to 0.448 mm/day in campanulate *J. dendroidea* (Table 1). Among *Jaltomata* species, tubular *J. umbellata* had the slowest overall growth rate at 0.181 mm/day (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0004$).

In addition to species differing in duration of floral development and overall average growth rate, we also detected several differences in relative growth rates for particular floral organs. First, growth rates for all measured floral traits (except ovary diameter) were significantly higher in *S. pimpinellifolium* than in *J. darcyana* (both ancestrally rotate); growth rates for petals and stamens (which primarily determine the overall size and shape of *S. pimpinellifolium* flowers), and for the style, are significantly higher in *S. pimpinellifolium* than all *Jaltomata* species except *J. umbellata* (Table 2; Fig. 8). Compared to the ancestrally rotate flowers in *J. darcyana*, all other *Jaltomata* species have significantly elevated growth rates for all floral organs except lobe length and ovary diameter. The tubular flowers of *J. umbellata* have the highest growth rates for corolla depth, corolla fusion, petal length, stamen length, and style length, while both species with campanulate flowers (*J. calliantha and J. dendroidea*) have the

next highest growth rates for corolla fusion (and are not statistically different from one another; $p = 0.45$) (Table 2).

Mature floral traits differ markedly across species

Mature, post-anthesis flowers differ substantially in overall size, as well as in relative sizes of individual organs (Table 3). *Jaltomata calliantha* and *J. dendroidea* have the largest flowers, while *S. pimpinellifolium* has the smallest; *Jaltomata* flowers also differ from *S. pimpinellifolium* in having free stamens (compared to a fused anther cone, considered a derived trait in the wild tomatoes [32]), and in producing nectar. Calyx diameter significantly differs across all species, while both corolla and ovary diameter significantly differ between all species pairs, except *J. umbellata* versus *S. pimpinellifolium* (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$) which both have comparatively small flowers. Although floral organ sizes vary substantially amongst *Jaltomata* species, ancestral rotate flowers of *J. darcyana* have the shortest petals (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$), and the smallest amount of corolla fusion (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.00001$) (Table 3).

In addition to organ size and shape, our focal species also differ in the amount of nectar produced per flower (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$) (Table 3), and the timing of nectar secretion. The ancestral rotate species *J. darcyana* produces significantly less nectar than the four other examined *Jaltomata* species. *Jaltomata darcyana* also produces nectar at the earliest point during its developmental timeline; minute amounts of nectar are apparent in this species in mid-day -1 day buds (i.e. the afternoon of the day before anthesis, or flower opening). In contrast, for *J. calliantha*, *J. dendroidea*, and *J. sinuosa,* nectar is first apparent during the morning in 0 day buds (i.e. the morning of the day the flower opens, typically a few hours prior

to full anthesis); in *J. umbellata*, nectar secretion is not apparent until the evening of day 0 flowers (after anthesis has occurred), or until the morning of +1 day flowers (i.e. the day following anthesis, just prior to anther dehiscence in this species). Nectar secretion dynamics therefore appear to have undergone a combination of heterochronic shifts in more derived forms, including a delayed onset of the start of nectar secretion, as well as increased rates of nectar production.

Finally, mature flowers differ in the types and density of trichomes on mature floral tissues. From simple to complex: *J. darcyana* has sparse simple (uniseriate) trichomes on both sepals and petals, *J. calliantha* dense simple trichomes on both sepals and petals, *J. umbellata* dense simple and dendritic trichomes on sepals but only dense simple ones on petals, *J. dendroidea* very dense dendritic trichomes on both sepals and petals, *S. pimpinellifolium* dense simple and non-viscous glandular trichomes on both sepals and petals, and *J. sinuosa* very dense simple, dendritic and viscous glandular trichomes on sepals and dense simple and viscous glandular ones on petals.

Discussion

Heterochronic shifts during development are often considered a primary mechanism underlying phenotypic evolution [6]. Indeed, heterochrony appears to be a common mechanism underlying morphological diversification in numerous animal lineages [8], as well as in flowers, including shifts in both duration of floral development and growth rates leading to phenotypic shifts in descendent lineages [3-4, 13]. In this study, we examined floral ontogeny in five florally diverse species of *Jaltomata*, as well as a closely related outgroup that shares the ancestral rotate corolla form, to assess evidence for heterochrony contributing to observed variation in adult corolla

forms and other floral traits. We found that early floral development (<1 mm stage) is very similar among all examined species, that differences between *Jaltomata* and *Solanum* are apparent by the 2 mm stage, and that corolla trait differences associated with divergent corolla shapes among *Jaltomata* species first arise during mid-developmental stages (3-5 mm stages). Elevated growth rates of corolla traits, combined with additional heterochronic and allometric changes during late development (>5 mm stage), lead to observed differences in mature floral traits among species (Table 3). Our data are therefore consistent with Darwin's insight that diversity of form results from changes in later developmental stages [1-2]. In particular, these developmental changes are heterochronic, with predominately accelerated growth rates and extended development duration (that is, peramorphic changes) associated with variation in adult corolla form.

Extended duration of floral development and accelerated growth rates underlie derived tubular and campanulate adult corolla shapes in *Jaltomata*

One goal of this study was to assess whether derived tubular and campanulate corolla forms in *Jaltomata* arise due to heterochronic developmental shifts. In particular, we hypothesized that derived campanulate and tubular forms are elaborated versions of the ancestral rotate form, and therefore might result from peramorphic changes (i.e. longer duration of floral development or accelerated growth rates). Indeed, compared to ancestrally rotate flowers of *J. darcyana*, all derived *Jaltomata* species show peramorphic changes during floral development. First, all species except *J. umbellata* have significantly longer development duration (Table 1). Because floral organs initiated at the same developmental stage across species, differences in the duration of floral development in derived forms result from changes in the timing of offset (i.e. growth

period is extended). Second, growth rates for most of our measured floral traits are significantly elevated in *J. umbellata*, including petal length and extent of corolla fusion (Table 2, even though *J. darcyana* and *J. umbellata* have similar development periods (19.23 days vs. 18.62 days)). These differences appear to explain how flowers of *J. umbellata* are generally smaller than those of *J. darcyana*, but nonetheless have significantly longer petals, stamens, and styles, and greater corolla fusion at anthesis (Table 3). Similarly, growth rates of most floral organs are significantly elevated in both campanulate species – including rates of petal length and extent of corolla fusion – in comparison to *J. darcyana* (Table 2), even though both also have longer development periods and faster overall growth rates in terms of bud diameter (Table 1). Thus, derived campanulate flowers in *J. calliantha* and *J. dendroidea* result from both accelerated organ growth rates and an extended development period, while derived tubular flowers in *J. umbellata* appear to result from accelerated growth rates of particular floral organs/features.

Alike adult corolla forms result from similar heterochronic changes during mid-stages, but not necessarily during late stages

Another goal of this study was to assess whether similar mature corolla shapes arise from similar or divergent developmental trajectories. Overall, we found that floral development was very similar, including nearly identical corolla development, during early and mid-stages within both species with rotate flowers, suggesting that these forms may share a single evolutionary origin. In contrast, the two species with campanulate flowers showed similar heterochronic changes in direction but not in magnitude during mid-stages.

Floral ontology in general and corolla development in particular are very similar between rotate flowers of *J. sinuosa* and ancestrally rotate flowers of *J. darcyana*, until shortly after the 5

mm bud stage when anthesis occurs in *J. darcyana*. Indeed, both average overall growth rate and growth rates for corolla traits do not differ between these species until this stage (Table 1). After the 5 mm stage, *J. sinuosa* continues to develop for significantly longer (Table 1), and experiences an elevated growth rate for corolla fusion (Table 2). These data indicate that the shared rotate floral morphology in these two species arises through a very similar developmental process, but peramorphic changes (both an extended development period and growth acceleration) during late floral development lead to an overall increased size of *J. sinuosa* flowers, and a proportionately greater extent of corolla fusion (Table 3). These observations again suggest that the rotate flowers in these species share a single evolutionary origin.

In contrast, in the two species with campanulate flowers (*J. calliantha* and *J. dendroidea*), both exhibit extended development periods and accelerated growth rates in corolla traits compared to *J. darcyana*; however, they differ from each other in the magnitude of change (Tables 2-3). While both show significantly elevated overall rates for corolla depth, corolla fusion, and petal length, rates for corolla depth and petal length are significantly elevated in *J. dendroidea* compared to *J. calliantha*. Interestingly, *J. calliantha* actually shows a significantly decreased growth rate (i.e. neoteny) for lobe length compared to *J. darcyana* (Table 2), whereas the growth rate of lobe length does not significantly differ between *J. dendroidea* and *J. darcyana*. Therefore, both campanulate species have similar corolla trait development until the 5 mm bud stage, including common differences compared to *J. darcyana* during the 3-5 mm stages, but undergo different heterochronic changes during late development. These changes include significantly greater corolla fusion in *J. dendroidea* during the 5-7 mm bud stages (although *overall* rates of corolla fusion are similar between species (Table 2)), as well as pedomorphosis via neoteny in lobe length growth in *J. calliantha*. These differences, as well as

437 shorter petals in *J. dendroidea* at the 1 mm stage (see above), suggest that campanulate corollas
438 in these two species are independently derived. Alternatively, these forms could share a single
439 origin, but could have experienced subsequent developmental changes without large changes in
440 the mature phenotype (i.e. developmental systems drift) during lineage divergence. In the
441 absence of other data, we prefer the former hypothesis based on our phylogenetic inference (Wu,
442 Kostyun, Moyle, unpubl.) (Fig. 1). Together, these observations agree with prior studies
443 suggesting that different heterochronic changes contributing to similar mature floral phenotypes
444 may actually be quite common; for instance, multiple distinct developmental shifts have been
445 shown to produce flowers with the 'selfing syndrome' across lineages [3-4, 19] and among
446 closely related populations and species [37].
447
448 **Heterochronic shifts as a first step to identifying specific mechanisms underlying floral**
449 **divergence and convergence in *Jaltomata***
450 Our data suggest an important role for several different heterochronic developmental shifts in
451 generating floral trait diversity among *Jaltomata* species, especially peramorphism (via growth
452 acceleration and extended growth period), in shaping corolla diversity. If these heterochronic
453 shifts are caused by relatively simple genetic changes, this could have contributed to the apparent
454 rapid floral trait diversification within the genus (<5 my; [29]). Future work identifying the
455 specific genetic and developmental mechanisms underlying these heterochronic shifts will
456 provide a better understanding of the pace of phenotypic change, as well as likely candidate
457 genes contributing to these developmental shifts. For instance, changes in the regulation of cell
458 proliferation and/or cell expansion can lead to variation in overall floral size or size of particular
459 floral organs, and several key candidate genes have been identified in *Arabidopsis* that function

460 during these processes [19, 38]. Given our observed patterns of developmental heterochrony (i.e.
461 extended floral development duration and accelerated growth), we anticipate that a combination
462 of molecular heterochrony (e.g. growth promoting factors are expressed for longer) and
463 heterometry (e.g. a higher amount of growth promoting gene products are produced) might
464 underlie these shifts.

465 In addition to intrinsic (genetic) regulation of cell growth within floral organs, external
466 signals or structures could also influence mature floral morphology [39-40]. For instance, the
467 involvement of trichomes in regulating flower shape was recently reported in cotton (*Gossypium*
468 *hirsutum*), in which the petal trichome gene *GhMYB-MIXTA-Like10* (*GhMYBML10*) is strongly
469 expressed at the point of petal overlap, resulting in trichome cross-linking that physically hold
470 petals in place [35]. We identified similar superficial corolla fusion via interlocking trichomes in
471 both rotate and campanulate *Jaltomata* species examined here, although different growth
472 dynamics during mid- and late-stages was observed between these forms. In particular, in rotate
473 corollas of *J. darcyana* and *J. sinuosa*, the proportion of the congenitally to post-genitally fused
474 (via interlocking trichomes) region remains similar throughout mid-stages; in contrast, in
475 campanulate corollas of *J. calliantha* and *J. dendroidea* the congenitally fused region becomes
476 proportionately larger during this time. In both rotate and campanulate species however,
477 trichomes in post-genitally fused regions become unlocked during anthesis, releasing petals,
478 while congenitally fused regions of the corolla remained fused in mature flowers. Although
479 trichome cross-linking occurs in these species, it remains to be determined whether it is essential
480 for normal corolla development (as in [35]) and if this phenotype is regulated by a *MYBML10*
481 orthologue (including whether expression differs between rotate and campanulate vs. tubular
482 *Jaltomata* forms).

The identification of specific developmental mechanisms and candidate genes will also clarify whether similar mature floral morphs result from shared evolutionary history or from convergent or parallel changes at the molecular level. In the *Jaltomata* species that we examined here, floral development (especially for corolla traits) in both rotate species was extremely similar until *J. darcyana* matured shortly after the 5 mm stage, while in the campanulate species, we identified heterochronic shifts in the same direction but not of the same magnitude. Thus, it is likely that *J. darcyana* and *J. sinuosa* share their rotate form from common ancestry, while *J. calliantha* and *J. dendroidea* represent convergent or parallel evolution of the campanulate form. In this way, determining the developmental mechanisms underlying these heterochronic shifts can be used both to pinpoint how changes in existing floral development pathways lead to phenotypic variation, and to differentiate alternative evolutionary histories (common ancestry versus convergence) for similar mature floral forms. In particular, shared mutations would be more indicative of common descent rather than parallel evolution.

Pollinator-mediated selection may have shaped *Jaltomata* floral diversification

Understanding how developmental trajectories differ between species with different mature floral traits also broadly informs the ways in which these traits are most able to respond to selection (e.g. whether there is developmental constraint). Such inferences are especially relevant to floral trait evolution across the *Jaltomata* genus, which shows both high levels of floral trait divergence, as well as multiple putatively independent shifts to similar floral forms, within a relatively short timescale (<5mya, [29]). From an evolutionary perspective, such shifts are likely to have been shaped by pollinator behavior. Observations indicate that some species are predominantly visited by distinct pollinator functional groups (e.g. hymenopterans vs.

506 hummingbirds, T. Mione, pers. comm.; J.L. Kostyun, unpub.), and these species have floral trait
507 suites consistent with different pollinator syndromes [41]. In particular, hummingbirds have been
508 observed visiting campanulate *J. calliantha* and tubular *Jaltomata viridiflora* whose flowers have
509 copious amounts of dilute nectar, while hymenopterans have been observed visiting *J. sinuosa*
510 and *Jaltomata repandidentata* (a close relative to *J. darcyana*; [30]) that have rotate corollas
511 with relatively small amounts of concentrated nectar. Because differential pollinator behavior
512 often leads to reproductive isolation between lineages [42], understanding the underlying
513 developmental basis of floral trait evolution can also reveal factors that could accelerate
514 speciation in this rapidly-evolving and florally diverse system.
515
516 **Conclusions**
517 As initially proposed by Darwin, much diversity of form (including in flowers) likely results
518 from changes in late developmental stages. One such type of developmental change is
519 heterochrony, which is a shift in rate or timing in a descendant compared to its ancestor. By
520 comparing floral ontogeny among five *Jaltomata* species and an outgroup, we determined that
521 heterochronic shifts during mid- and late stages of floral development distinguish divergent
522 corolla forms. In particular, two types of peramorphosis (differential growth acceleration and
523 delayed offset leading to an extended development period) predominately explain these changes.
524 Our data therefore support Darwin's insight that even highly divergent mature floral traits result
525 from modifications to initially similar structures. These relatively simple heterochronic shifts
526 during later stages of development contribute to observed floral trait variation among *Jaltomata*
527 species and, as such, could act as a mechanism allowing rapid floral trait diversification in this
528 florally diverse system.

529

530 **Declarations**

531 **Ethics approval and consent to participate**

532 Not applicable.

533

534 **Consent for publication**

535 Not applicable.

536

537 **Availability of data and material**

538 The datasets used and/or analyzed during the current study are available from the corresponding

539 author on reasonable request.

540

541 **Competing interests**

542 The authors declare that they have no competing interests.

543

544 **Funding**

545 This work was supported by a National Science Foundation Graduate Research Fellowship (NSF

546 DEB 1342962) and Amherst College Graduate Fellowship to JLK, a National Science

547 Foundation Award (NSF IOS 1353056) to JCP, and a National Science Foundation Award (NSF

548 DEB 1136707) to LCM. Any opinions, findings, and conclusions or recommendations expressed

549 in this material are those of the author(s) and do not necessarily reflect the views of the National

550 Science Foundation.

551

Authors' contributions

All authors planned and designed the research, JLK performed the experiments, JLK and JCP analyzed the data, and all wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Armin Moczek for providing feedback on a previous draft of this manuscript, Barry Stein of the IU Electron Microscopy Center and Michele von Turkovich of the UVM Microscopy Imaging Center for SEM and other instrument training, the IU Greenhouse staff for plant care, and Thomas Mione for sharing seed material.

References

1. Darwin C. On the Origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. London: J. Murray; 1859.

2. Friedman WE, Diggle PK. Charles Darwin and the origins of plant evolutionary developmental biology. Plant Cell 2011; 23:1194-1207.

3. Li P, Johnston MO. Heterochrony in plant evolutionary studies through the twentieth century. Botanical Review 2000; 66:57-88.

4. Box MS, Glover BJ. A plant developmentalist's guide to paedomorphosis: reintroducing a classic concept to a new generation. Trends Plant Sci 2010; 15:241-246.

5. Haeckel E. Die Gastrula and die Eifurchung der Thiere. Jena Z. Naturwiss 1875; 9:402-508.

6. Gould SJ. Ontogeny and phylogeny. Cambridge, MA: Harvard University Press; 1977.

7. Gould SJ. Ontogeny and phylogeny: revisited and reunited. BioEssays 1992; 14:275-279.

575 8. McNamara KJ. Heterochrony: the evolution of development. Evo Edu Outreach 2012; 5:203-
576 218.
577 9. Wayne RK. Cranial morphology of domestic and wild canids: the influence of development on
578 morphological change. Evolution 1986; 40:243-261.
579 10. Truman JW, Riddiford LM. The origins of insect metamorphosis. Nature 1999; 401:447-452.
580 11. Cartolano M, Pieper B, Lempe J, Tattersall A, Huijser P, Tresch A, Darrah PR, Hay A,
581 Tsiantis M. Heterochrony underpins natural variation in *Cardamine hirsuta* leaf form.
582 Proc Natl Acad Sci USA 2015; 112:10539-10544.
583 12. Lemmon ZH, Park SJ, Jiang K, Van Eck J, Schatz MC, Lippman ZB. The evolution of
584 inflorescence diversity in the nightshades and heterochrony during meristem maturation.
585 Genome Res 2016; 26:1676-1686.
586 13. Geuten K, Coenen H. Heterochronic genes in plant evolution and development. Front Plant
587 Sci 2013; 4:381.
588 14. Endress PK. Evolutionary diversification of the flowers in angiosperms. Am J Bot 2011;
589 98:370-396.
590 15. Angiosperm Phylogeny Group, The. An update of the Angiosperm Phylogeny Group
591 classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc
592 2016; 181:1-20.
593 16. Stebbins GL. Self fertilization and population variability in the higher plants. Am Nat 1957;
594 91:337-354.
595 17. Stebbins GL. Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination
596 mechanisms. Annu Rev Ecol Evol Syst 1970; 1:307-326.

597 18. Barrett SCH, Harder LD. Floral variation in *Eichhornia paniculata* (Spreng) Solms
598 (Pontederiaceae). 2. Effects of development and environment on the formation of selfing
599 flowers. J Evol Biol 1992; 5:83-107.
600 19. Sicard A, Lenhard M. The selfing syndrome: a model for studying the genetic and
601 evolutionary basis of morphological adaptation in plants. Ann Bot 2011; 107:1433-1443.
602 20. Li P, Johnston MO. Comparative floral morphometrics of distyly and homostyly in three
603 evolutionary lineages of *Amsinckia* (Boraginaceae). Canadian Journal of Botany 2001;
604 79:1332-1348.
605 21. Georgiady MS, Lord EM. Evolution of the inbred flower form in the currant tomato,
606 *Lycopersicon pimpinellifolium*. Int J Plant Sci 2002; 163:531-541.
607 22. Minter TC, Lord EM. A comparison of cleistogamous and chasmogamous floral
608 development in *Collomia grandiflora* Dougl ex Lindl (Polemoniaceae). Am J Bot 1983;
609 70:1499-1508.
610 23. Runions CJ, Geber MA. Evolution of the self-pollinating flower in *Clarkia xantiana*
611 (Onagraceae). I. Size and development of floral organs. Am J Bot 2000; 87:1439-1451.
612 24. Li P, Johnston MO. Flower development and the evolution of self-fertilization in *Amsinckia*:
613 The role of heterochrony. Evol Biol 2010; 37:143-168.
614 25. Box MS, RM Bateman, BJ Glover, Rudall PJ. Floral ontogenetic evidence of repeated
615 speciation via paedomorphosis in subtribe Orchidinae (Orchidaceae). 2008; Bot J Linn
616 Soc 157:429-454.
617 26. Armbruster WS, J Lee, ME Edwards, Baldwin BG. Floral paedomorphy leads to secondary
618 specialization in pollination of Madagascar *Dalechampia* (Euphorbiaceae). Evolution
619 2013; 67:1196-1203.

620 27. Guerrant EO. Neotenic evolution of *Delphinium nudicaule* (Ranunculaceae) - a
621 hummingbird-pollinated larkspur. Evolution 1982; 36:699-712.
622 28. Olmstead RG, L Bohs, HA Migid, E Santiago-Valentin, VF Garcia, Collier SM. A molecular
623 phylogeny of the Solanaceae. Taxon 2008; 57:1159-1181.
624 29. Sarkinen T, L Bohs, RG Olmstead, Knapp S. A phylogenetic framework for evolutionary
625 study of the nightshades (Solanaceae): a dated 1000-tip tree. BMC Evol Biol 2013;
626 13:214.
627 30. Miller RJ, T Mione, HL Phan, Olmstead RG. Color by numbers: nuclear gene phylogeny of
628 *Jaltomata* (Solanaceae), sister genus to *Solanum*, supports three clades differing in fruit
629 color. Syst Botany 2011; 36:153-162.
630 31. Hansen DM, JM Olesen, T Mione, SD Johnson, Muller CB. Coloured nectar: distribution,
631 ecology, and evolution of an enigmatic floral trait. Biological Reviews 2007; 82:83-111.
632 32. Knapp S. On 'various contrivances': pollination, phylogeny and flower form in the
633 Solanaceae. Phil Trans R Soc B 2010; 365:449-460.
634 33. Xiao H, C Radovich, N Welty, J Hsu, DM Li, T Meulia, van der Knaap E. Integration of
635 tomato reproductive developmental landmarks and expression profiles, and the effect of
636 SUN on fruit shape. BMC Plant Biol 2009; 9:49.
637 34. Schneider CA, WS Rasband, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis.
638 Nature Methods 2012; 9:671-675.
639 35. Tan J, Walford S-A, Dennis ES, Llewellyn D. Trichomes control flower bud shape by linking
640 together young petals. Nature Plants 2016; 2:16093.
641 36. R Core Team. R: A language and environment for statistical computing. R Foundation for
642 Statistical Computing, Vienna, Austria. 2015. https://www.R-project.org/.

643 37. Summers HE, SM Hartwick, Raguso RA. Geographic variation in floral allometry suggests
644 repeated transitions between selfing and outcrossing in a mixed mating plant. Am J Bot
645 2015; 102:745-757.
646 38. Krizek BA, Anderson JT. Control of flower size. J Exp Bot 2013; 64:1427-1437.
647 39. Gilbert SF. Ecological developmental biology: environmental signals for normal animal
648 development. Evol Dev 2012; 14:20-28.
649 40. Sampathkumar A, Yan A, Krupinski P, Meyerowitz EM. Physical forces regulate plant
650 development and morphogenesis. Curr Biol 2014; 24:R475-R483.
651 41. Fenster CB, WS Armbruster, P Wilson, MR Dudash, Thomson JD. Pollination syndromes
652 and floral specialization. Annu Rev Ecol Evol Syst 2004; 35:375-403.
653 42. Kay KM, Sargent RD. The role of animal pollination in plant speciation: integrating ecology,
654 geography, and genetics. Annu Rev Ecol Evol Syst 2009; 40:637-656.
655 43. Kostyun JL, Moyle LC. Multiple strong postmating and intrinsic postzygotic reproductive
656 barriers isolate florally diverse species of *Jaltomata* (Solanaceae). Evolution 2017;
657 71:1556-1571.

658

659

660 **Figure legends**

661 **Fig. 1.** Simplified phylogenetic relationships in *Jaltomata* and the outgroup *Solanum*
662 *pimpinellifolium* based on [30, 43] and (Wu, Kostyun, Moyle, unpubl.). Representative mature
663 flowers are depicted for the species examined here (in bold font). White scale bars = 1 cm. R,
664 rotate; C, campanulate; T, tubular.

665

Fig. 2. *Jaltomata darcyana* rotate flower development. (a-b) Sepals develop asymmetrically from the abaxial side, and once of similar lengths, grow from a basal ring meristem to form a fused tube. (c) Removal of sepals reveals simultaneous development of petal and stamen primordia. (d-e) Petals grow freely as stamen thecae become differentiated. (f-g) Carpels become fused during gynoecial development. (h-k) Petals are congentially fused at the base just above the stamen insertion point (i), but then become superficially and postgenitally fused via interlocking trichomes along their lengths by the 3.5 mm flower diameter stage. Scale bars = 100 μm.

Fig. 3. *Jaltomata sinuosa* rotate flower development. (a-b) Sepals initiate in an asymmetric fashion from the abaxial side, and quickly fuse at the base. (c) Five free petals develop around initiating stamen primordia. (d-e) As anther thecae differentiate, petals remain mostly free, but are fused at the base. (f-g) Removal of mostly free petals reveals development of fused carpels. (h-i) Opening of the corolla in 2.5-4 mm wide flowers shows a fused petal base from just above the stamen attachment point to about mid-way up the length of the corolla (i), and superficial fusion of petals above the mid-point, post-genitally via interlocking trichomes. Scale bars = 100 μm.

Fig. 4. *Jaltomata calliantha* campanulate flower development. (a) Free sepals extend through the growth of a basal ring primordium to form a lower fused tube. (b) Opening of partially fused sepals reveals initiation of petal, followed by stamen, primordia. (c) Free petals and stamens grow simultaneously prior to initiation of the carpel primordia. (d-f) Free petals develop trichomes on the adaxial side, becoming slightly fused just above the stamen attachment point

during stamen thecae emergence. (g-h) Fused carpel development. (i) Petals remain only slightly congenitally fused above the stamen filament in 3.5 mm wide flowers (corolla was opened prior to fixation, so superficial fusion via trichomes is not visible). Scale bars = 100 μm.

Fig. 5. *Jaltomata dendroidea* campanulate flower development. (a-b) Sepals develop asymmetrically from the abaxial side. (c) Once equal in size, sepals extend through growth of the underlying ring meristem, giving rise to a fused tube. (d-e) Partial removal of sepals reveals simultaneous development of petals and stamens. (f-h) Petals are largely free from early development to the stamen thecae differentiation stage in 1.6 mm wide flowers, however, there is a small zone of fusion above the stamen attachment point. (i) Carpels are partially fused above the developing ovary. Scale bars = 100 μm.

Fig. 6. *Jaltomata umbellata* tubular flower development. (a) Sepal development occurs asymmetrically, with the first sepal initiating on the abaxial side. (b) Once all sepal primordia have expanded, organs become fused at the base to form a tube. (c-d) Removal of partially fused sepals reveals the simultaneous initiation of petal and stamen primordia in an asymmetric progression. (e-g) Separate petal primordia elongate without fusion until differentiation of stamen thecae. (h-k) As stamen thecae differentiate, petals of ~1-3 mm diameter flowers become increasingly fused above the stamen attachment point to form a corolla tube. (l-m) Carpel development, including formation of the disk nectary by ~2 mm diameter buds. Scale bars = 100 μm.

Fig. 7. *Solanum pimpinellifolium* rotate flower development. (a) Sepals develop asymmetrically and fuse at their base to form a fused ring. (b-c) Removal of sepals reveals simultaneous development of petals and stamens, followed by carpel development (c). (d-h) Petals are fused at the base above the stamen attachment point (visible in g) from early development (d), however, most of the petal margin is free. (i) Late carpel development. (j) At the 2 mm flower diameter stage, when anther thecae are well established, petals are mostly free, but remain partially fused at the base. Scale bars = 100 μm.

Fig. 8. Growth trajectories for the focal floral organs and features. For all panels, the x-axis is bud diameter (mm) and the y-axis is the focal organ (mm). Each species is color coded.

Additional files

Additional file 1: Table S1. Accessions of the species used in the present study and their collection locations. All *Jaltomata* seed material provided by Dr. Thomas Mione at Central Connecticut State University (CCSU); *S. pimpinellifolium* seed provided by the Tomato Genetics Resource Center at UC Davis. Additional file 1 is in .docx format.

Additional file 2: Figure S1. Measured morphological traits on mature flowers. Floral organs were measured on three flowers per individual, for at least three individuals per species, using hand-held digital calipers to the nearest 0.01 mm. Additional file 2 is in .pdf format.

Table 1. Average length of development, size of -1 day buds (day before anthesis), and overall bud growth rates for the 5 *Jaltomata* species and *Solanum pimpinellifolium*.

Species	Length (days)	-Day Bud Diameter (mm)	Growth rate (mm/day)
J. darcyana (n=13)	19.23 [a]	4.52 [a]	0.236 [a]
J. sinuosa (n=11)	28.55 [b]	6.36 [b]	0.223 [a]
J. calliantha (n=10)	28.80 [b]	11.35 [c]	0.395 [b]
J. dendroidea (n=5)	32.40 [c]	14.50 [d]	0.448 [c]
J. umbellata (n=13)	18.62 [a]	3.37 [e]	0.181 [d]
S. pimpinellifolium (n=8)	14.00 [d]	2.41 [f]	0.172 [d]

Bud diameter was measured every other day, from 1 mm buds to anthesis. Significant differences among species are indicated by different letters (following ANOVA and Tukey's HSD).

Table 2. Relative growth rates for focal floral organs and features for the examined species, measured against bud diameter.

	***J. darcyana* (100)**		***J. sinuosa* (88)**		***J. calliantha* (66)**		***J. dendroidea* (85)**		***J. umbellata* (96)**		***S. pimpinellifolium* (106)**	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Sepal Length	0.826	0.043	0.967***	-0.002*	1.159***	0.013^	0.948***^^^	0.013^	1.153***	-0.083***	0.960**	0.061
Corolla Depth	0.906	-0.024	1.012*	-0.063	1.032*	-0.173***^	1.225***^^^	-0.173***^	1.881***	-0.423***	1.651***	-0.253***
Corolla Fusion	0.522	0.085	0.887***	-0.071***	1.015***	-0.166***	1.067***	-0.166***	1.652***	-0.386***	0.767**	0.015
Lobe Length	0.734	0.049	0.683	0.069	0.537***	0.037^^^	0.721^^^	0.037^^^	0.795	0.029	1.354***	-0.143***
Petal Length	1.005	-0.060	1.149**	-0.122*	1.129*	-0.183***	1.244***^^	-0.183***	1.873***	-0.418***	1.633***	-0.239***
Stamen Length	0.722	0.035	0.863**	-0.027*	0.928***	-0.009^	0.815**^	-0.009^	1.577***	-0.320***	1.540***	-0.232***
Ovary Diameter	0.551	0.107	0.449***	0.152*	0.667***	0.116^^^	0.524^^^	0.116^^^	0.519	0.112	0.387***	0.177**
Style Length	0.481	0.116	0.802*	-0.031	0.896***	-0.160***	1.059***	-0.160***	1.747***	-0.408***	1.684***	-0.330***

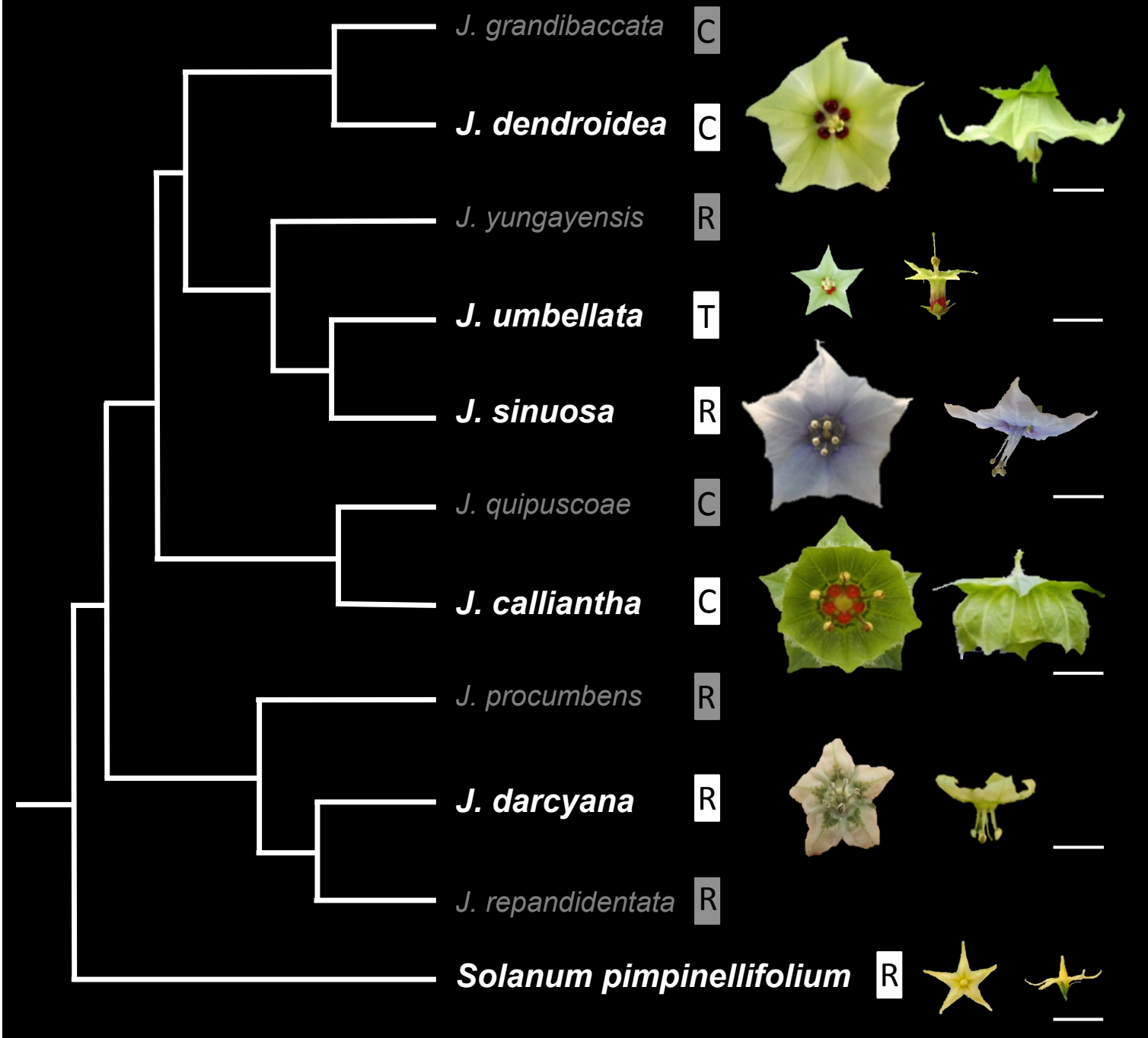
Measurements were log-transformed prior to analysis. Significant differences against *J. darcyana* (ancestral rotate) indicated by * (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$); significant differences between *J. calliantha* and *J. dendroidea* (campanulate) indicated by ^ (^ $p < 0.05$; ^^ $p < 0.001$; ^^^ $p < 0.0001$).

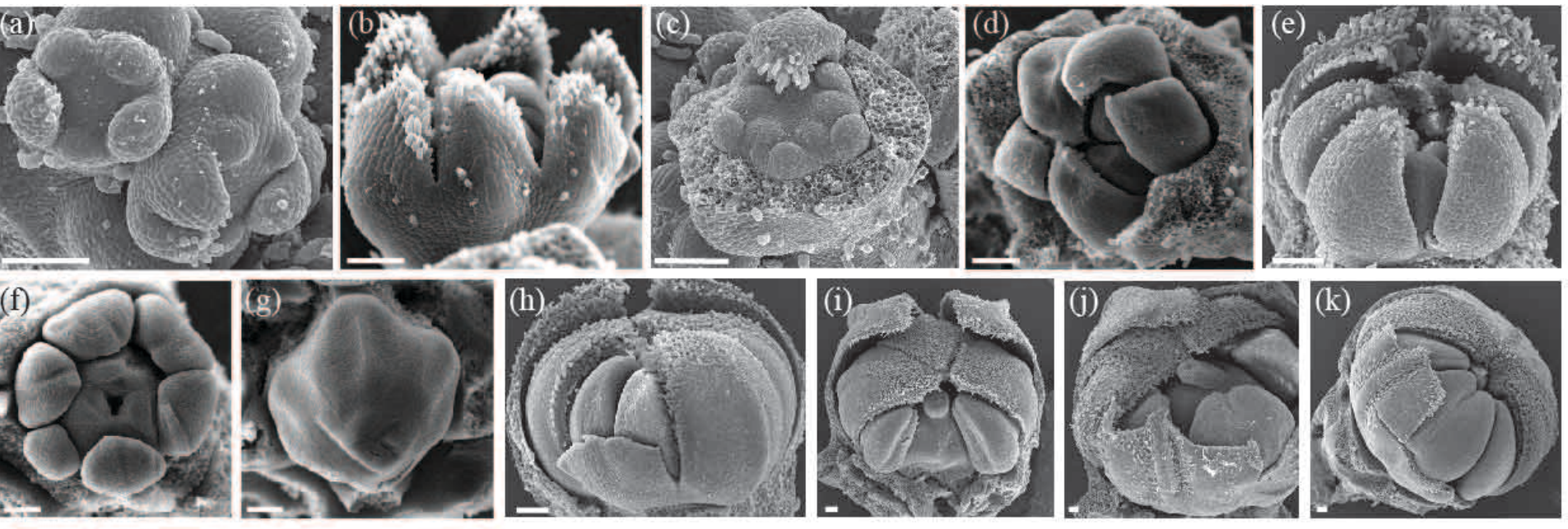
759 **Table 3.** Mature floral trait means for the five included *Jaltomata* species and *Solanum pimpinellifolium*.

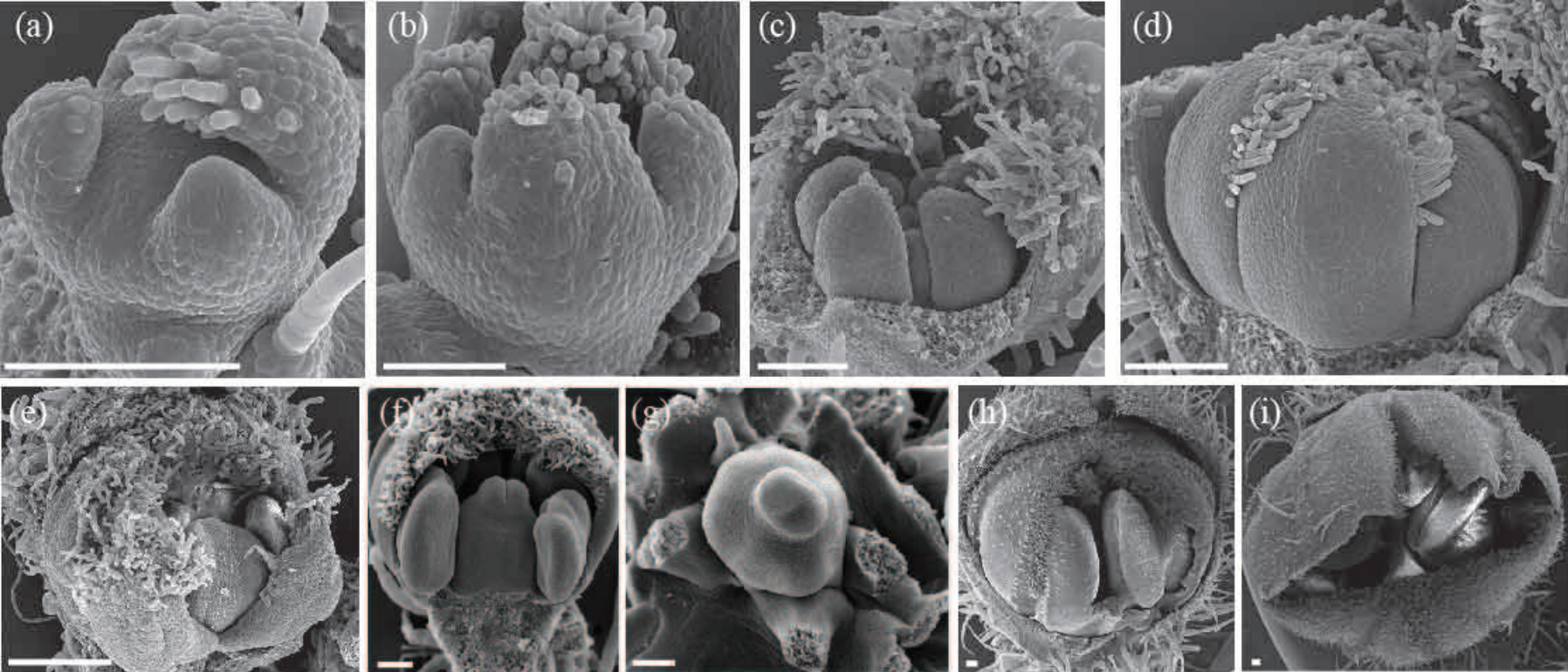
	J. darcyana (7)		*J. sinuosa* (7)		*J. calliantha* (7)		*J. dendroidea* (3)		*J. umbellata* (5)		*S. pimpinellifolium* (5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calyx Diameter (mm)	11.88 [a]	0.37	15.49 [b]	0.40	33.88 [c]	1.21	19.69 [d]	1.74	8.14 [e]	1.63	5.77 [f]	0.43
Sepal Length (mm)	6.32 [a]	0.16	7.48 [b]	0.25	18.01 [c]	1.10	9.67 [d]	0.80	4.41 [e]	0.73	3.45 [f]	0.23
Corolla Diameter (mm)	20.48 [a]	2.18	29.82 [b]	2.27	27.48 [c]	3.48	35.35 [d]	3.27	15.32 [e]	2.52	15.58 [e]	0.58
Corolla Depth (mm)	0.77 [a]	0.09	1.38 [a]	0.27	15.57 [b]	1.84	12.72 [c]	0.88	10.25 [d]	2.27	0.45 [a]	0.07
Corolla Fusion (mm)	3.96 [a]	0.28	9.45 [b]	0.46	15.64 [c]	0.44	14.91 [c]	3.78	10.33 [b]	1.22	1.60 [d]	0.25
Corolla Fusion Proportion	0.42 [a]	0.07	0.64 [b]	0.05	0.86 [c]	0.08	0.65 [b]	0.10	0.72 [b]	0.08	0.21 [d]	0.03
Lobe Length (mm)	5.48 [a]	1.42	5.40 [a]	0.70	2.77 [b]	1.83	7.65 [c]	0.66	4.08 [a]	0.96	5.97 [a]	0.11
Petal Length (mm)	9.53 [a]	1.39	14.85 [b]	1.04	18.41 [c]	1.92	22.56 [d]	2.98	14.40 [b]	0.81	7.57 [e]	0.22
Stamen Length (mm)	4.76 [a]	0.38	10.80 [b]	0.60	10.48 [b]	1.00	10.94 [b]	0.35	10.45 [b]	0.57	6.15 [c]	0.09
Anther Length (mm)	1.43 [a]	0.10	1.59 [b]	0.10	3.27 [c]	0.19	1.86 [d]	0.05	1.34 [a]	0.15	5.75 [e]	0.12
Ovary Diameter (mm)	1.78 [a]	0.14	2.28 [b]	0.19	5.30 [c]	0.41	3.15 [d]	0.18	1.37 [e]	0.26	1.12 [e]	0.06
Style Length (mm)	4.79 [a]	0.53	7.90 [b]	0.73	8.38 [b]	0.66	12.74 [c]	0.86	14.64 [d]	1.47	5.45 [a]	0.21
Herkogamy (mm)	1.47 [a]	0.14	-2.20 [b]	0.19	5.19 [c]	0.75	4.85 [c]	0.35	5.37 [c]	2.14	-0.08 [d]	0.12
Nectar Volume (µl)	2.19 [a]	0.48	6.86 [b]	1.31	50.62 [c]	3.76	32.89 [d]	7.03	17.80 [e]	3.84	0.00 [a]	0.00

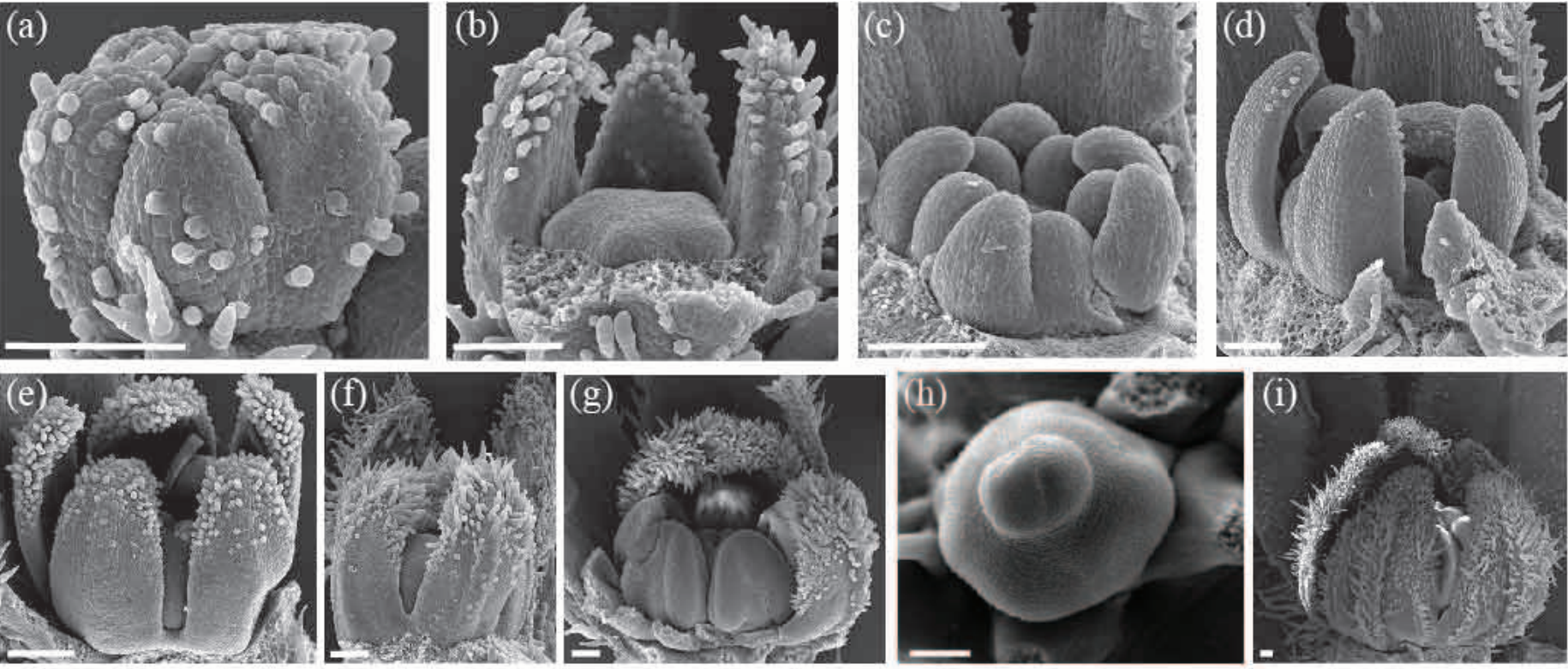
760 Sample sizes in parentheses refer to the number of individuals measured (at least three flowers per individual). Significant differences among species are

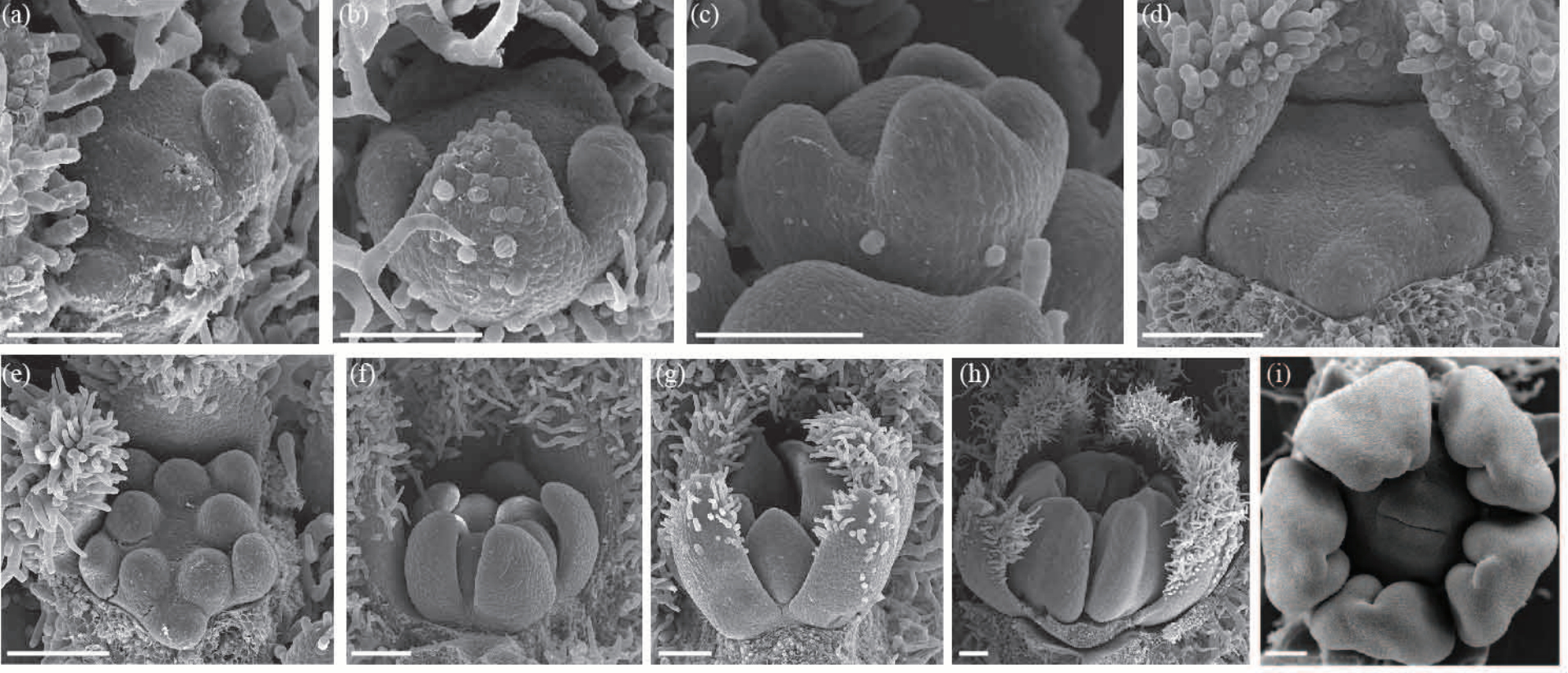
761 indicated by different letters ($p<0.001$, ANOVA and Tukey's HSD). *Jaltomata* data from [43].

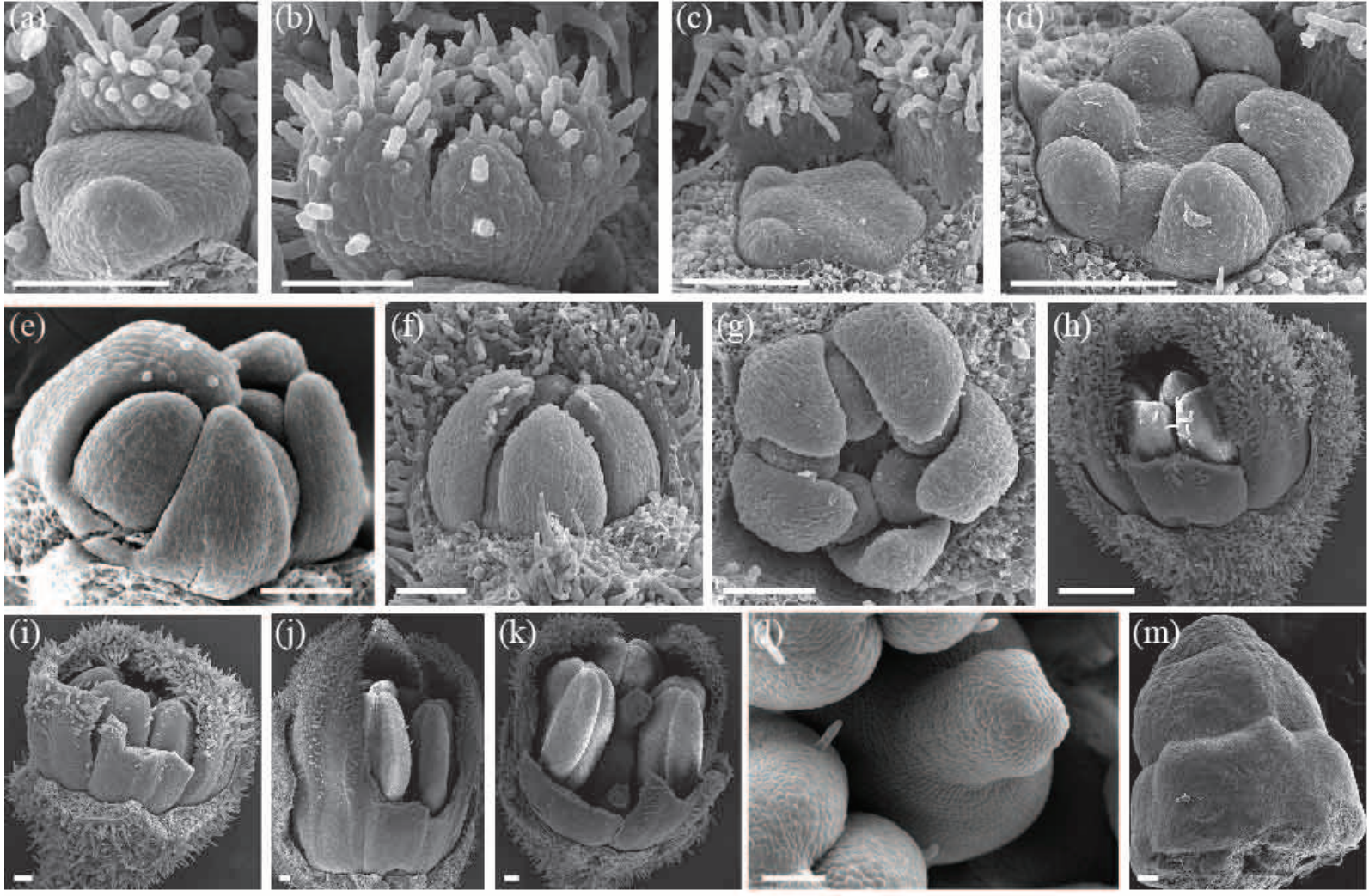

J. grandibaccata
C
J. dendroidea
C
J. yungayensis
R
J. umbellata
T
J. sinuosa
R
J. quipuscoae
C
J. calliantha
C
J. procumbens
R
J. darcyana
R
J. repandidentata
R
Solanum pimpinellifolium
R

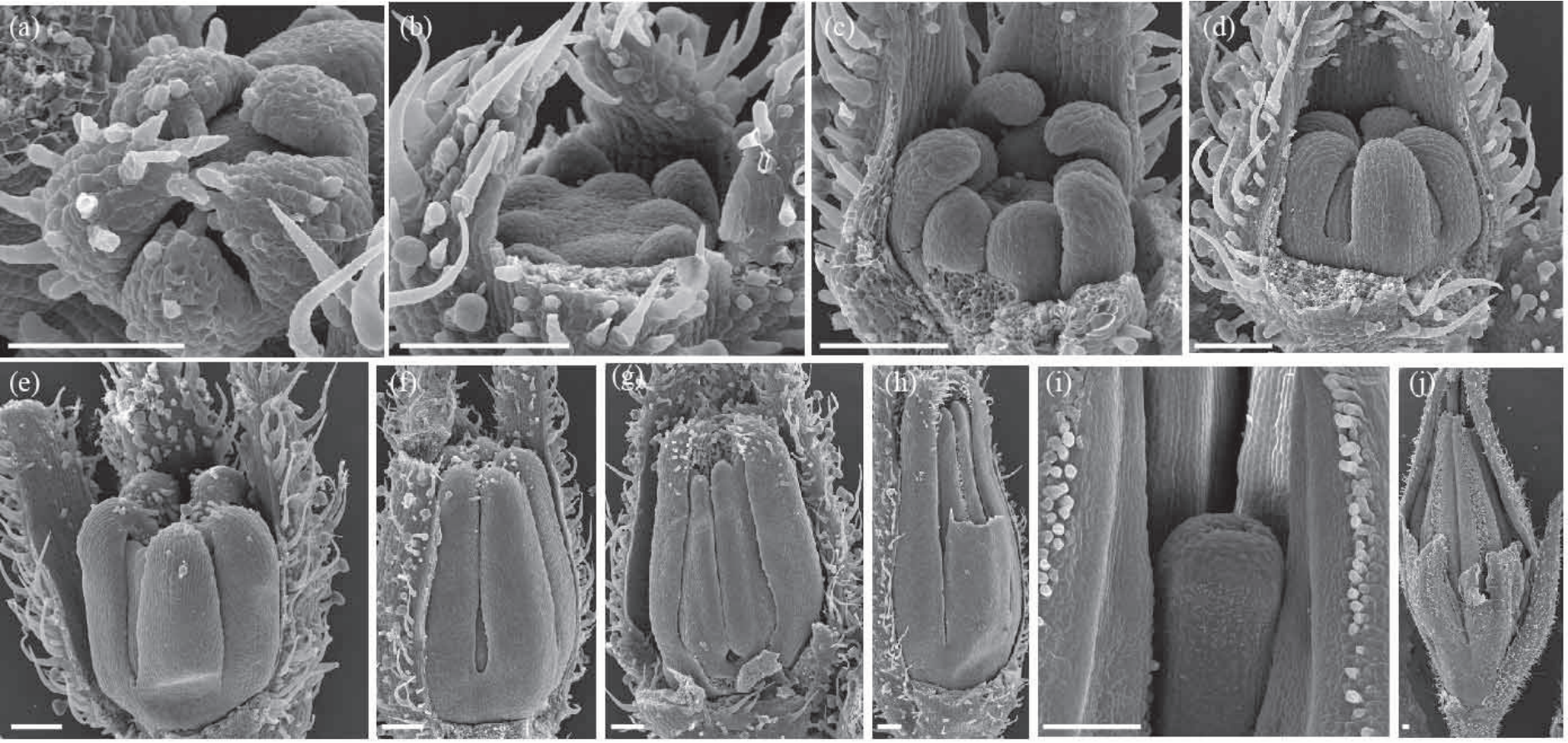

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)

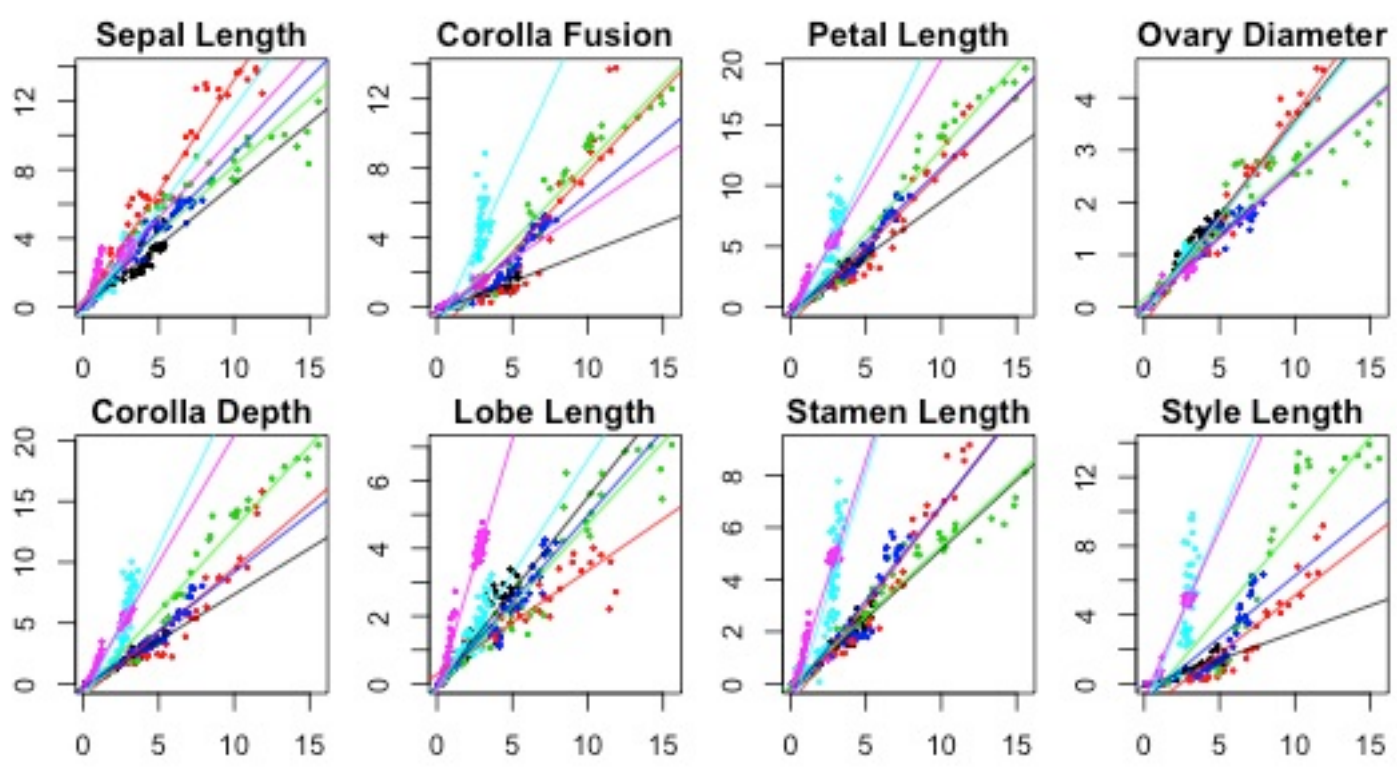

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)


Sepal Length
Corolla Fusion
Petal Length
Ovary Diameter
Corolla Depth
Lobe Length
Stamen Length
Style Length
J. darcyana
J. sinuosa
J. calliantha
J. dendroidea
J. umbellata
S. pimpinellifolium