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

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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.

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

104 *Jaltomata* (Solanaceae) includes approximately 60-80 species distributed from the
105 Southwestern United States to the Andean region of South America, with extensive and recently
106 evolved floral diversity [28-30]. Although the pollination biology of this genus has yet to be
107 formally evaluated, field observations (T. Mione, pers. comm.) reveal that different species are
108 visited by bees and hummingbirds. In addition, *Jaltomata* produce varying amounts of floral
109 nectar as a pollinator reward, unlike close relatives like *Solanum* [31-32]. Molecular phylogenies
110 based on one to several loci [28-30], as well as phylogenomic reconstruction using whole
111 transcriptomes (Wu, Kostyun, Moyle, unpubl.), indicate that the ancestor of *Jaltomata* had
112 flattened 'rotate' flowers, while both bell-shaped 'campanulate' and elongated 'tubular' flowers are
113 derived specifically within the genus (Fig. 1). Further, species with derived campanulate or
114 tubular forms generally produce more nectar per flower than those with ancestral rotate forms
115 [30]. Ancestral state reconstruction [30] also suggests that some of the derived campanulate and

tubular floral forms found in different *Jaltomata* species evolved independently, allowing comparison of developmental mechanisms underlying putatively convergent forms.

Because both derived campanulate and tubular forms might represent elaborated versions of the ancestral rotate form, we hypothesized that heterochronic changes (specifically peramorphic changes such as longer duration of development and/or growth acceleration, which often produce larger or more elaborated structures) underlie these transitions, particularly during later stages of ontology [1-2, 4]. In addition to evaluating the role of heterochrony in the evolution of derived floral forms, we also expected that comparing floral development between species with similar mature morphs--but unresolved phylogenetic relatedness--would provide insight into their evolutionary origin [30] (Wu, Kostyun, Moyle, unpub.). For instance, if similar mature forms result from similar developmental processes, this could suggest re-use of similar pathways or a shared single origin. In contrast, if they result from distinct developmental processes, this could indicate that these forms are independently derived, or that these forms share a common evolutionary origin (i.e. are homologous) but underwent developmental systems drift during their divergence. Follow-up analyses at the molecular level, identifying causative mutations contributing to trait divergence, could then be used to discriminate further between hypotheses, with shared mutations being more likely attributed to common descent than to parallel evolution.

Given these considerations, our main goals in this study were to: 1) assess evidence for heterochronic changes during floral development in species with derived campanulate and tubular forms; 2) determine which type(s) of heterochronic changes are associated with specific floral trait changes; and 3) assess whether putatively convergent forms might result from similar or different developmental processes (i.e. are associated with the same or different types of

139 heterochronic shifts). To address these goals, we examined floral ontogeny of five florally
140 diverse *Jaltomata* species (including one species with an ancestrally representative rotate corolla
141 form), as well as an outgroup species that also has an ancestrally representative rotate corolla
142 form. Our findings support the inference that a combination of parallel and convergent allometric
143 changes (i.e. shifts in the size of particular floral organs in relation to the entire flower) have
144 given rise to floral variation in this group, and show that heterochronic shifts explain these
145 changes. In particular, two types of peramorphic changes (extended duration of floral
146 development and accelerated growth) predominately explain changes in corolla traits. Thus,
147 peramorphism emerges as an important developmental mechanism controlling diversity of
148 *Jaltomata* corolla forms. Finally, species with similar mature campanulate corollas follow
149 similar but clearly not identical growth trajectories, consistent with phylogenetic inferences that
150 these campanulate floral morphs have independent origins (Wu, Kostyun, Moyle, unpubl.) (Fig.
151 1). In contrast, we found that species with similar mature rotate corollas follow nearly identical
152 developmental trajectories until the very last stages of ontology, suggesting a single evolutionary
153 origin in this case.
154

155 **Methods**

156 **Plant materials and growth conditions**

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

162 [32], floral development in this species has previously been characterized [21, 33], and--as a wild
163 species--its floral development has not been influenced by domestication (i.e. compared to
164 domestic tomato, *S. lycopersicum*). Our focal *Jaltomata* species included the Central American
165 species *Jaltomata darcyana* that has an ancestrally representative rotate corolla, and four species
166 found as natives exclusively in South America: *Jaltomata calliantha* and *Jaltomata dendroidea*
167 with putatively independently derived campanulate corollas, *Jaltomata umbellata* with a tubular
168 corolla, and *Jaltomata sinuosa* with a rotate corolla that is ambiguously ancestral or secondarily
169 derived (Fig. 1). Plants were cultivated in the Indiana University Research Greenhouse, under
170 standardized temperature (15-20°C) and light (16-hour days) conditions. All plants were watered
171 to field capacity on automatic driplines, and received weekly fertilizer treatment.
172

173 **Floral development**

174 To estimate the duration of floral development, young inflorescences were first identified when
175 the largest bud was approximately 1 mm, and then evaluated and measured every second day
176 until first anthesis. To assess relative organ growth during development, buds up to 4 mm in
177 diameter were collected into 70% ethanol or formalin-acetic acid-alcohol (FAA), dissected as
178 required, dehydrated through an ethanol series at room temperature, and incubated in a 4°C
179 fridge over-night. Samples were critical point dried, mounted on aluminum stubs, and sputter-
180 coated with gold-palladium. They were then imaged using a JEOL 6060 or a JEOL 5800LV
181 scanning electron microscope (SEM) at the University of Vermont or Indiana University,
182 respectively. Morphological traits were measured on the resulting bud micrographs using ImageJ
183 [34]. For buds greater than 4 mm in diameter, morphological traits were measured by hand on
184 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

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

241 Developmental timing of trichome initiation on sepals and petals is similar among all
242 species. However, qualitative differences become apparent by the 0.3-0.4 mm bud stage (Figs. 2-
243 7). Trichomes are first initiated on sepals in all species around the 0.2 mm stage, but both simple
244 (i.e. uniseriate) and non-viscous (i.e. non-secreting) glandular trichomes are apparent on *S.*
245 *pimpinellifolium* sepals, compared to just simple ones on *Jaltomata* species (Figs. 2a-b, 3a-b, 4a,
246 5d, 6a-b, 7a). By the 0.3 mm and 0.5 mm stages, dendritic (i.e. branched) trichomes become
247 apparent on *J. sinuosa*, *J. dendroidea*, and *J. umbellata* sepals, and viscous glandular trichomes
248 develop on *J. sinuosa* sepals, respectively (Figs. 3c-e, 5e, 6f). Trichomes on petals are initiated
249 by ~0.5 mm in all species (Figs. 2e, 3d, 4e, 5g, 6f, 7e) but, compared to only simple trichomes in
250 *Jaltomata* species, both simple and non-viscous glandular trichomes are present on *S.*
251 *pimpinellifolium* petals by this stage. By the 1.0 mm diameter bud stage (Figs. 2e-f, 5g), dendritic
252 trichomes in *J. dendroidea* and viscous glandular trichomes in *J. sinuosa*, are present on petals.
253

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

322 next highest growth rates for corolla fusion (and are not statistically different from one another; p
323 $= 0.45$) (Table 2).
324
325 **Mature floral traits differ markedly across species**
326 Mature, post-anthesis flowers differ substantially in overall size, as well as in relative sizes of
327 individual organs (Table 3). *Jaltomata calliantha* and *J. dendroidea* have the largest flowers,
328 while *S. pimpinellifolium* has the smallest; *Jaltomata* flowers also differ from *S. pimpinellifolium*
329 in having free stamens (compared to a fused anther cone, considered a derived trait in the wild
330 tomatoes [32]), and in producing nectar. Calyx diameter significantly differs across all species,
331 while both corolla and ovary diameter significantly differ between all species pairs, except *J.*
332 *umbellata* versus *S. pimpinellifolium* (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$) which
333 both have comparatively small flowers. Although floral organ sizes vary substantially amongst
334 *Jaltomata* species, ancestral rotate flowers of *J. darcyana* have the shortest petals (ANOVA $p <$
335 0.00001; Tukey HSD, $p < 0.0001$), and the smallest amount of corolla fusion (ANOVA $p <$
336 0.00001; Tukey HSD, $p < 0.00001$) (Table 3).
337 In addition to organ size and shape, our focal species also differ in the amount of nectar
338 produced per flower (ANOVA $p < 0.00001$; Tukey HSD, $p < 0.0001$) (Table 3), and the timing
339 of nectar secretion. The ancestral rotate species *J. darcyana* produces significantly less nectar
340 than the four other examined *Jaltomata* species. *Jaltomata darcyana* also produces nectar at the
341 earliest point during its developmental timeline; minute amounts of nectar are apparent in this
342 species in mid-day -1 day buds (i.e. the afternoon of the day before anthesis, or flower opening).
343 In contrast, for *J. calliantha*, *J. dendroidea*, and *J. sinuosa,* nectar is first apparent during the
344 morning in 0 day buds (i.e. the morning of the day the flower opens, typically a few hours prior

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

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

359

360 **Discussion**

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

368 forms and other floral traits. We found that early floral development (<1 mm stage) is very
369 similar among all examined species, that differences between *Jaltomata* and *Solanum* are
370 apparent by the 2 mm stage, and that corolla trait differences associated with divergent corolla
371 shapes among *Jaltomata* species first arise during mid-developmental stages (3-5 mm stages).
372 Elevated growth rates of corolla traits, combined with additional heterochronic and allometric
373 changes during late development (>5 mm stage), lead to observed differences in mature floral
374 traits among species (Table 3). Our data are therefore consistent with Darwin's insight that
375 diversity of form results from changes in later developmental stages [1-2]. In particular, these
376 developmental changes are heterochronic, with predominately accelerated growth rates and
377 extended development duration (that is, peramorphic changes) associated with variation in adult
378 corolla form.
379

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

382 One goal of this study was to assess whether derived tubular and campanulate corolla forms in
383 *Jaltomata* arise due to heterochronic developmental shifts. In particular, we hypothesized that
384 derived campanulate and tubular forms are elaborated versions of the ancestral rotate form, and
385 therefore might result from peramorphic changes (i.e. longer duration of floral development or
386 accelerated growth rates). Indeed, compared to ancestrally rotate flowers of *J. darcyana*, all
387 derived *Jaltomata* species show peramorphic changes during floral development. First, all
388 species except *J. umbellata* have significantly longer development duration (Table 1). Because
389 floral organs initiated at the same developmental stage across species, differences in the duration
390 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

414 mm bud stage when anthesis occurs in *J. darcyana*. Indeed, both average overall growth rate and
415 growth rates for corolla traits do not differ between these species until this stage (Table 1). After
416 the 5 mm stage, *J. sinuosa* continues to develop for significantly longer (Table 1), and
417 experiences an elevated growth rate for corolla fusion (Table 2). These data indicate that the
418 shared rotate floral morphology in these two species arises through a very similar developmental
419 process, but peramorphic changes (both an extended development period and growth
420 acceleration) during late floral development lead to an overall increased size of *J. sinuosa*
421 flowers, and a proportionately greater extent of corolla fusion (Table 3). These observations
422 again suggest that the rotate flowers in these species share a single evolutionary origin.
423 In contrast, in the two species with campanulate flowers (*J. calliantha* and *J.*
424 *dendroidea*), both exhibit extended development periods and accelerated growth rates in corolla
425 traits compared to *J. darcyana*; however, they differ from each other in the magnitude of change
426 (Tables 2-3). While both show significantly elevated overall rates for corolla depth, corolla
427 fusion, and petal length, rates for corolla depth and petal length are significantly elevated in *J.*
428 *dendroidea* compared to *J. calliantha*. Interestingly, *J. calliantha* actually shows a significantly
429 decreased growth rate (i.e. neoteny) for lobe length compared to *J. darcyana* (Table 2), whereas
430 the growth rate of lobe length does not significantly differ between *J. dendroidea* and *J.*
431 *darcyana*. Therefore, both campanulate species have similar corolla trait development until the 5
432 mm bud stage, including common differences compared to *J. darcyana* during the 3-5 mm
433 stages, but undergo different heterochronic changes during late development. These changes
434 include significantly greater corolla fusion in *J. dendroidea* during the 5-7 mm bud stages
435 (although *overall* rates of corolla fusion are similar between species (Table 2)), as well as
436 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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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552 **Authors' contributions**

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

556

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562

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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

689 during stamen thecae emergence. (g-h) Fused carpel development. (i) Petals remain only slightly
690 congenitally fused above the stamen filament in 3.5 mm wide flowers (corolla was opened prior
691 to fixation, so superficial fusion via trichomes is not visible). Scale bars = 100 μm.
692
693 **Fig. 5.** *Jaltomata dendroidea* campanulate flower development. (a-b) Sepals develop
694 asymmetrically from the abaxial side. (c) Once equal in size, sepals extend through growth of the
695 underlying ring meristem, giving rise to a fused tube. (d-e) Partial removal of sepals reveals
696 simultaneous development of petals and stamens. (f-h) Petals are largely free from early
697 development to the stamen thecae differentiation stage in 1.6 mm wide flowers, however, there is
698 a small zone of fusion above the stamen attachment point. (i) Carpels are partially fused above
699 the developing ovary. Scale bars = 100 μm.
700
701 **Fig. 6.** *Jaltomata umbellata* tubular flower development. (a) Sepal development occurs
702 asymmetrically, with the first sepal initiating on the abaxial side. (b) Once all sepal primordia
703 have expanded, organs become fused at the base to form a tube. (c-d) Removal of partially fused
704 sepals reveals the simultaneous initiation of petal and stamen primordia in an asymmetric
705 progression. (e-g) Separate petal primordia elongate without fusion until differentiation of
706 stamen thecae. (h-k) As stamen thecae differentiate, petals of ~1-3 mm diameter flowers become
707 increasingly fused above the stamen attachment point to form a corolla tube. (l-m) Carpel
708 development, including formation of the disk nectary by ~2 mm diameter buds. Scale bars = 100
709 μm.
710

711 **Fig. 7.** *Solanum pimpinellifolium* rotate flower development. (a) Sepals develop asymmetrically
712 and fuse at their base to form a fused ring. (b-c) Removal of sepals reveals simultaneous
713 development of petals and stamens, followed by carpel development (c). (d-h) Petals are fused at
714 the base above the stamen attachment point (visible in g) from early development (d), however,
715 most of the petal margin is free. (i) Late carpel development. (j) At the 2 mm flower diameter
716 stage, when anther thecae are well established, petals are mostly free, but remain partially fused
717 at the base. Scale bars = 100 μm.
718
719 **Fig. 8.** Growth trajectories for the focal floral organs and features. For all panels, the x-axis is
720 bud diameter (mm) and the y-axis is the focal organ (mm). Each species is color coded.
721
722 **Additional files**
723 **Additional file 1: Table S1.** Accessions of the species used in the present study and their
724 collection locations. All *Jaltomata* seed material provided by Dr. Thomas Mione at Central
725 Connecticut State University (CCSU); *S. pimpinellifolium* seed provided by the Tomato Genetics
726 Resource Center at UC Davis. Additional file 1 is in .docx format.
727
728 **Additional file 2: Figure S1.** Measured morphological traits on mature flowers. Floral organs
729 were measured on three flowers per individual, for at least three individuals per species, using
730 hand-held digital calipers to the nearest 0.01 mm. Additional file 2 is in .pdf format.

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

732 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]

733 Bud diameter was measured every other day, from 1 mm buds to anthesis. Significant differences among species are indicated by different letters (following

734 ANOVA and Tukey's HSD).

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743 **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***

744 Measurements were log-transformed prior to analysis. Significant differences against *J. darcyana* (ancestral rotate) indicated by * (* $p < 0.05$; ** $p < 0.001$; ***

745 $p < 0.0001$); significant differences between *J. calliantha* and *J. dendroidea* (campanulate) indicated by ^ (^ $p < 0.05$; ^^ $p < 0.001$; ^^^ $p < 0.0001$).

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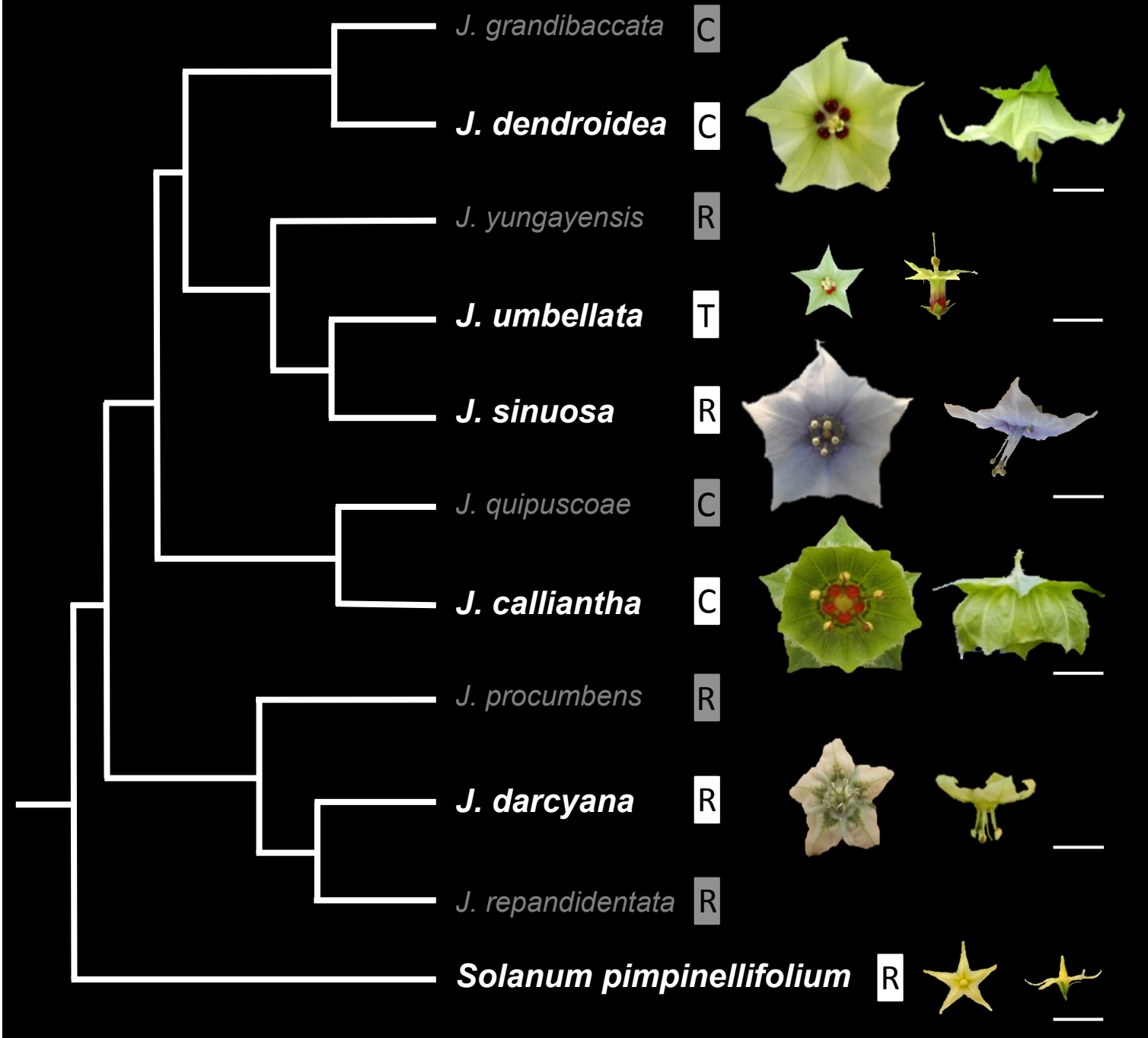
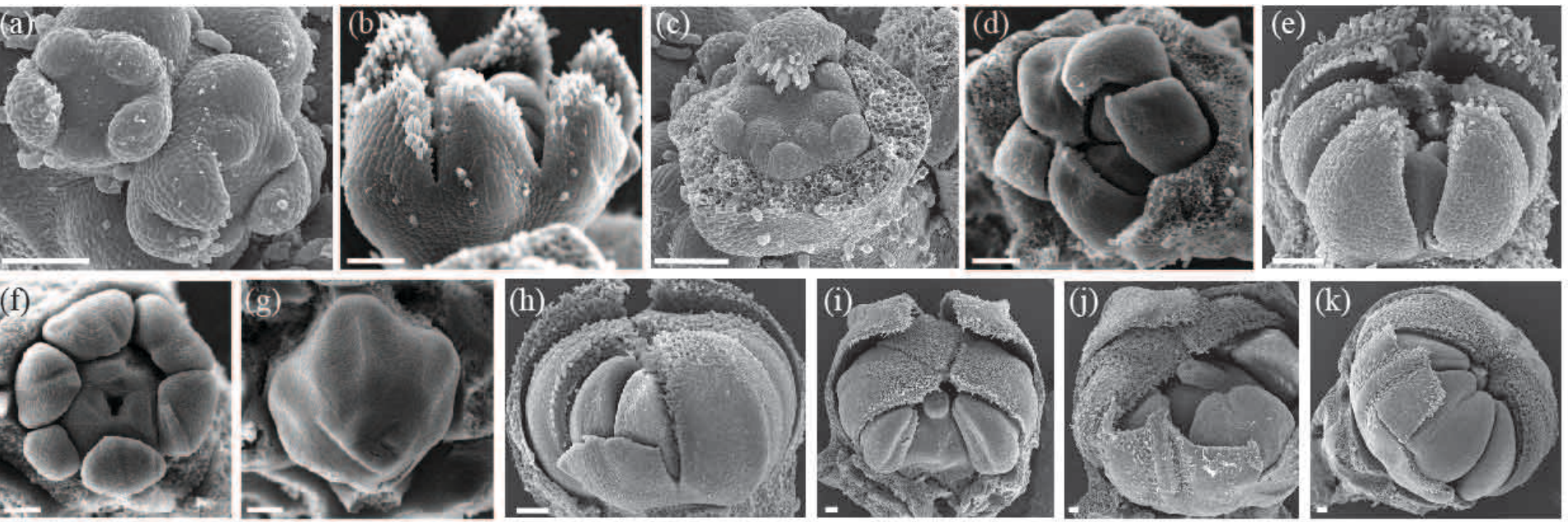
759 **Table 3.** Mature floral trait means for the five included *Jaltomata* species and *Solanum pimpinellifolium*.

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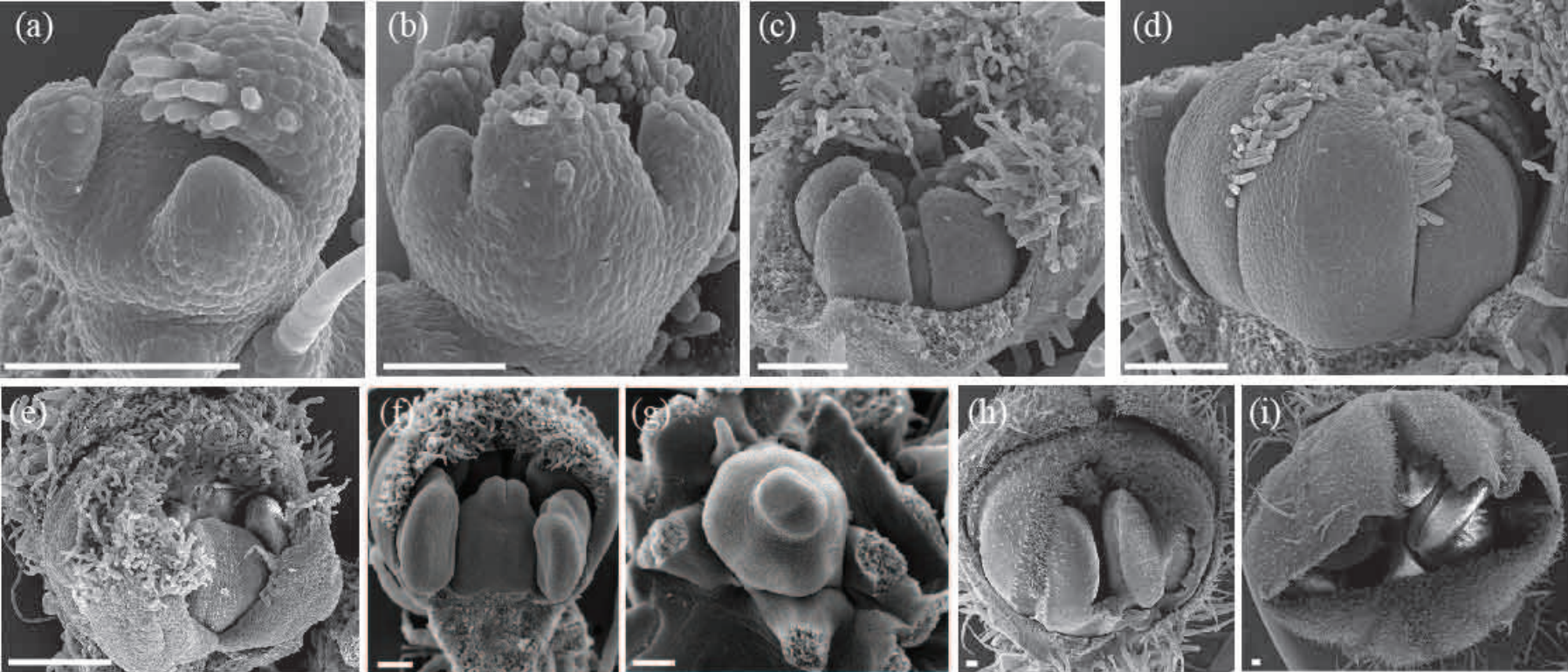
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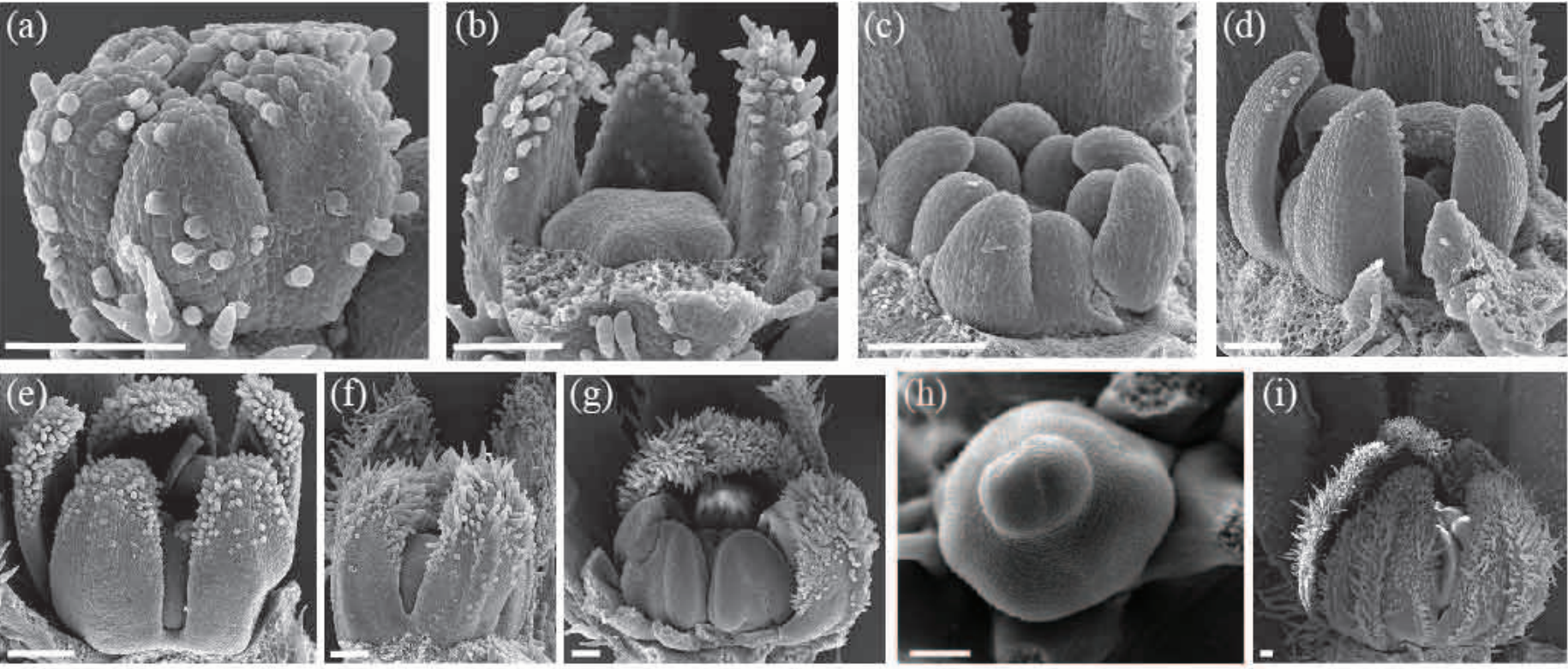
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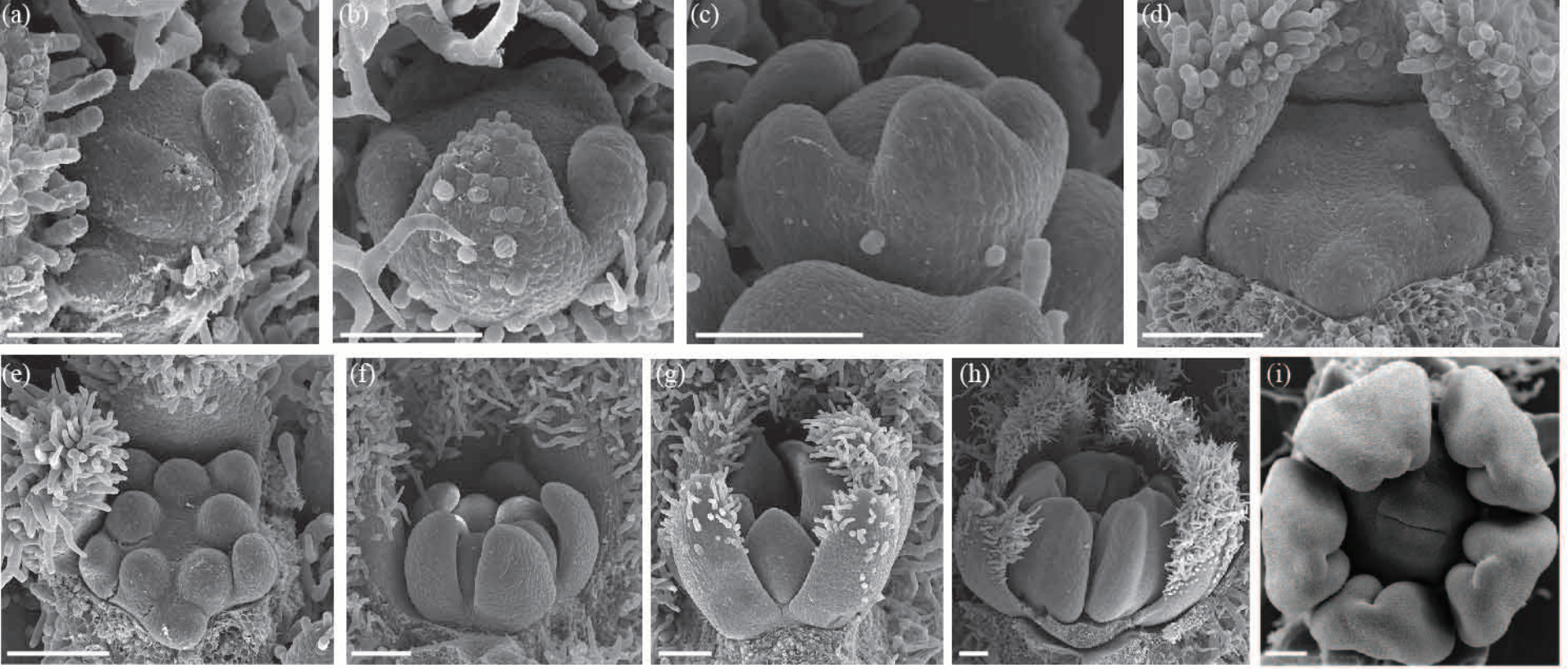
(a)
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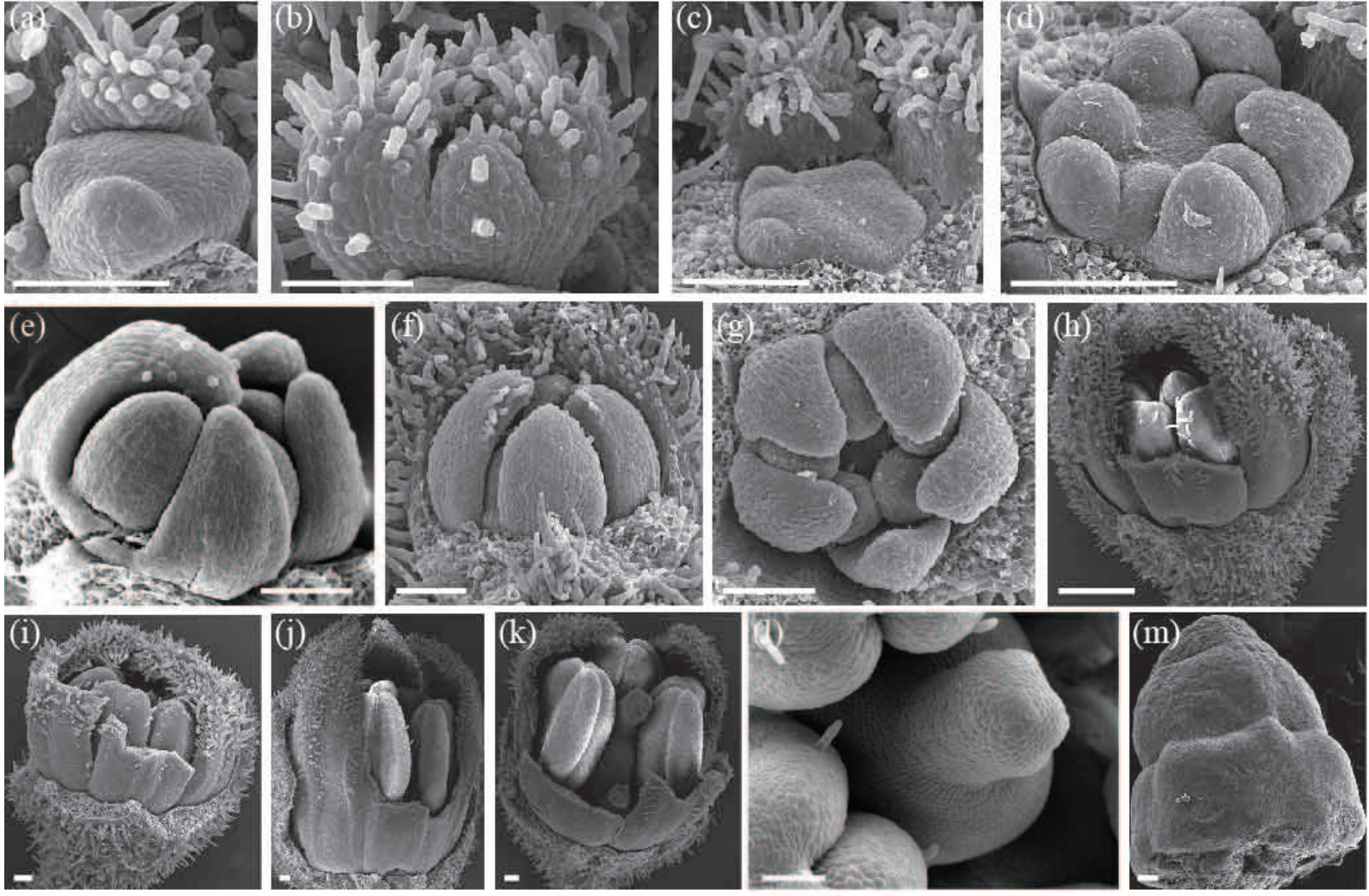
(a)
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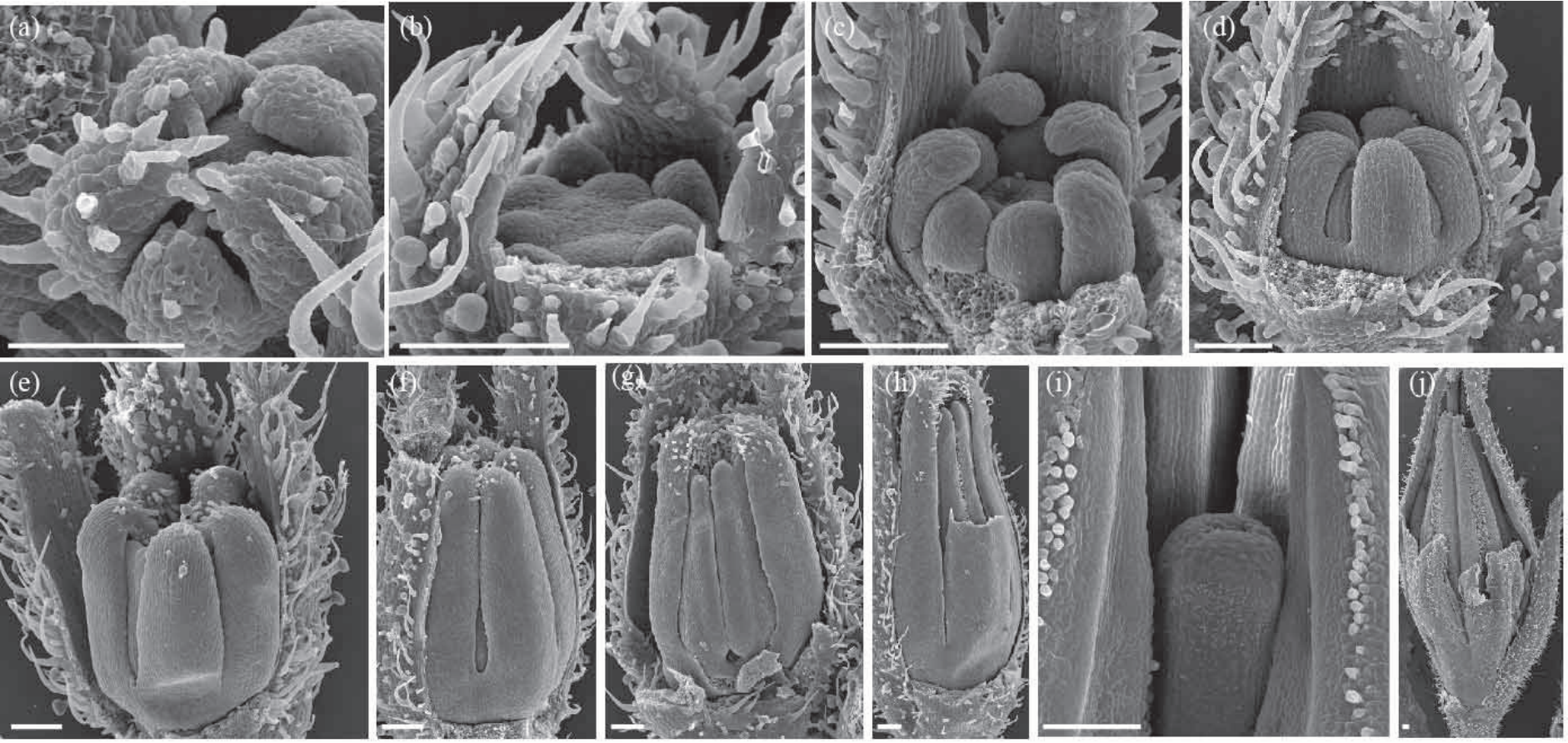
(a)
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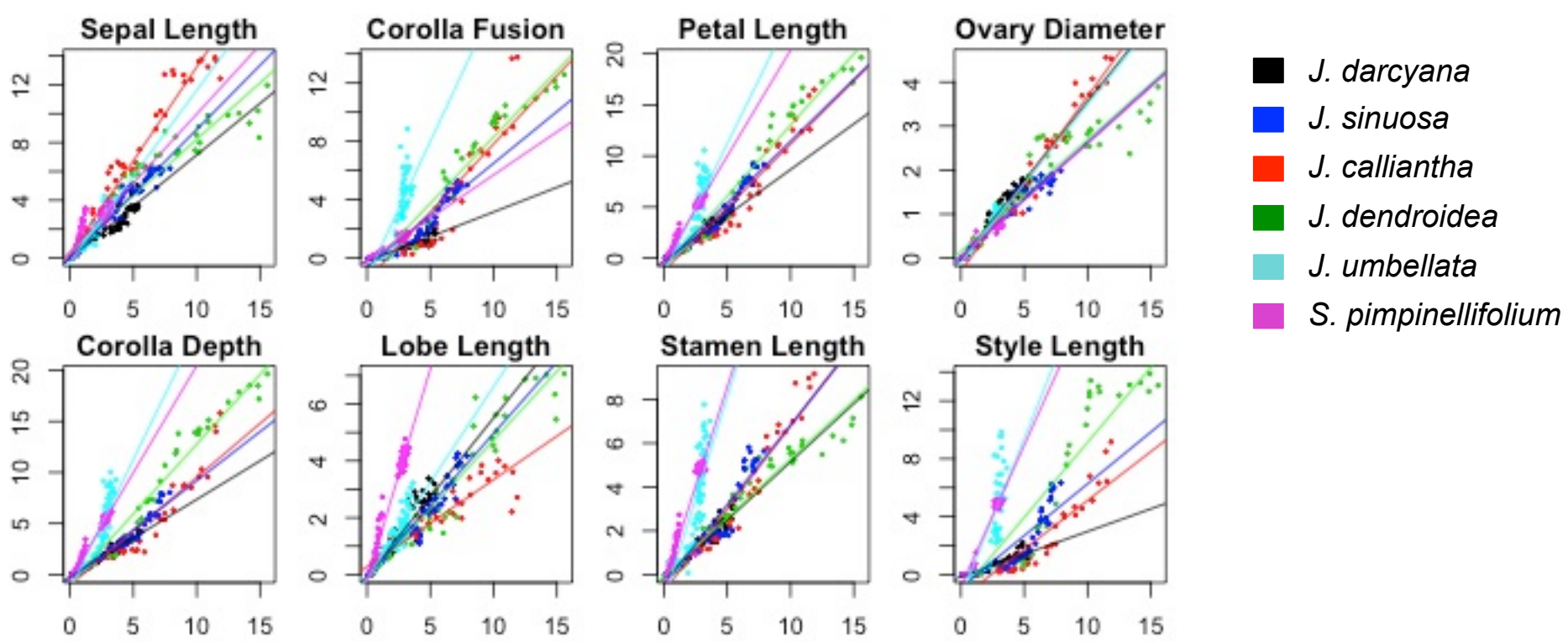
Sepal Length
Corolla Fusion
Petal Length
Ovary Diameter
Corolla Depth
Lobe Length
Stamen Length
Style Length
J. darcyana
J. sinuosa
J. calliantha
J. dendroidea
J. umbellata
S. pimpinellifolium