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Abstract:	The evolution of novel fruit morphologies has been integral to the success of angiosperms. The inflated fruiting calyx, in which the balloon-like calyx swells to completely surround the fruit, has evolved repeatedly across angiosperms and is postulated to aid in protection and dispersal. Here we investigate the evolution of this trait in the tomatillos and their allies (Physalideae, Solanaceae). The Physalideae phylogeny was estimated using four regions (ITS, LEAFY, trnL-F, waxy) with maximum likelihood (ML) and Bayesian Inference. Under the best fitting ML model of trait evolution, we estimated ancestral states along with the numbers of gains and losses of fruiting calyx accrescence and inflation with Bayesian stochastic mapping. Also, phylogenetic signal in calyx morphology was examined with two metrics (parsimony score and Fritz and Purvis' D). Based on our well-resolved and densely sampled phylogeny, we infer that calyx evolution has proceeded in a stepwise and directional fashion, from non-accrescent, to accrescent, to inflated. In total, we inferred 24 gains of accrescence, 24 subsequent transitions to a fully inflated calyx and only two reversals. Despite this lability, fruiting calyx accrescence and inflation showed strong phylogenetic signal. Our phylogeny greatly improves the resolution of Physalideae and highlights the need for taxonomic work. The comparative analyses reveal that the inflated fruiting calyx has evolved many times and that the trajectory towards this phenotype is generally stepwise and irreversible. These results provide a strong foundation for studying the genetic and developmental mechanisms responsible for the repeated origins of this charismatic fruit trait.
Keywords:	convergence; irreversibility; physaloids; stochastic mapping; trait evolution; transition rates
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Original article

Repeated evolution of a morphological novelty: a phylogenetic analysis of the inflated fruiting calyx in the Physalideae tribe (Solanaceae)

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PREMISE OF THE STUDY: The evolution of novel fruit morphologies has been integral to the success of angiosperms. The inflated fruiting calyx, in which the balloon-like calyx swells to completely surround the fruit, has evolved repeatedly across angiosperms and is postulated to aid in protection and dispersal. Here we investigate the evolution of this trait in the tomatillos and their allies (Physalideae, Solanaceae).

METHODS: The Physalideae phylogeny was estimated using four regions (ITS, LEAFY, *trnL-F*, *waxy*) with maximum likelihood (ML) and Bayesian Inference. Under the best fitting ML model of trait evolution, we estimated ancestral states along with the numbers of gains and losses of fruiting calyx accrescence and inflation with Bayesian stochastic mapping. Also, phylogenetic signal in calyx morphology was examined with two metrics (parsimony score and Fritz and Purvis' D).

KEY RESULTS: Based on our well-resolved and densely sampled phylogeny, we infer that calyx evolution has proceeded in a stepwise and directional fashion, from non-accrescent, to accrescent, to inflated. In total, we inferred 24 gains of accrescence, 24 subsequent transitions to a fully inflated calyx and only two reversals. Despite this lability, fruiting calyx accrescence and inflation showed strong phylogenetic signal.

CONCLUSIONS: Our phylogeny greatly improves the resolution of Physalideae and highlights the need for taxonomic work. The comparative analyses reveal that the inflated fruiting calyx has evolved many times and that the trajectory towards this phenotype is generally stepwise and irreversible. These results provide a strong foundation for studying the genetic and developmental mechanisms responsible for the repeated origins of this charismatic fruit trait.

KEY WORDS

Convergence; irreversibility; physaloids; stochastic mapping; trait evolution; transition rates

Fruit evolution has long been considered a key contributor to the success of angiosperms, with bursts of morphological innovation closely tied to climatological events as well as the rise of frugivorous lineages of vertebrates (Tiffney, 1984; Eriksson et al., 2000; Knapp, 2002). Variation in fruit traits across taxa is often correlated with differences in dispersal mode (e.g., Gautier-Hion et al., 1985; Lomáscolo et al., 2010), which in turn, can lead to shifts in diversification rates (e.g., Beaulieu and Donoghue, 2013; Lagomarsino et al., 2016; Larson- Johnson, 2016). Beyond their role in facilitating seed dispersal, fruits also serve to protect seeds from pathogens and predators (Tewksbury and Nabhan, 2001; Beckman and Muller-Landau, 2011) and promote successful germination (Traveset, 1998; Vander Wall, 2001).

From an evolutionary perspective, fruit morphology is known not only for its tremendous diversity but also the high degree of convergence. For instance, fleshy fruits have evolved repeatedly in a wide variety of angiosperm clades (e.g., Malpighiaceae, Davis et al., 2001; Rubiaceae, Bremer et al., 1995; Solanaceae, Knapp, 2002), often in relation to shifts in ecological niche (Bolmgren and Eriksson, 2005; Givnish et al., 2005). Even seemingly complex fruit traits, such as segmentation and seed ejection, have been gained and lost multiple times at recent phylogenetic scales (Hall et al., 2011; Marcussen and Meseguer, 2017). Despite this evolutionary lability, relatively little is known about the extent to which convergent transitions in fruit traits occur through similar genetic and developmental mechanisms (Pabón-Mora et al., 2014; Ortiz-Ramírez et al., 2018; but see Avino et al., 2012).

Here we focus on a charismatic but understudied fruit trait, the inflated fruiting calyx, which has evolved repeatedly across angiosperms. Inflated calyces develop by accrescence after anthesis such that the fruit becomes completely enclosed upon maturation (He et al., 2004). This feature is found in at least 11 plant families, such as Malvaceae and Lamiaceae (Paton, 1990; Padmaja et al., 2014), although it is best known from the tomato family, Solanaceae, where it is referred to as a ‘chinese-lantern’ fruit or, more formally, the ‘inflated calyx syndrome’ (ICS; He et al., 2004; He and Saedler, 2005; Wang et al., 2015). This enlarged fruiting calyx has been proposed to aid in dispersal by acting as a tumbleweed (Knapp, 2002) or by providing flotation in flooded environments (Wilf et al., 2017). Pre-dispersal, the inflated calyx may also serve to protect the developing fruit from predators as well as from desiccation (Cedeño and Montenegro, 2004; Riss, 2009).

The evolution and development of inflated calyces has been studied in detail in only one clade, the tomatillos and their allies (tribe Physalideae, Solanaceae). Using comparative gene expression studies and transformation experiments, He and Saedler (2005) demonstrated that expression of a MADS-box transcription factor (*MPF2*) is required for the development of the dramatically inflated calyx in *Physalis*, and that overexpression of this gene in tomato can induce some degree of fruiting calyx accrescence. Subsequent studies across Physalideae revealed that many taxa that lack inflated calyces express *MPF2*, indicating that additional factors are required for development of the trait (Hu and Saedler, 2007). These and subsequent authors suggested that, given the shared expression of *MPF2* across Physalideae, ICS could be the ancestral state with multiple subsequent losses (Hu and Saedler, 2007; Zhang et al., 2012). Nonetheless, progress in reconstructing the history of gains and losses of this morphological innovation has been hampered by the sparse taxon sampling of Physalideae in existing phylogenies, which include only 37% of the extant taxa (Särkinen et al., 2013). In the present study, we aim to elucidate the evolutionary history of Physalideae, which contains the vast majority of Solanaceae species with inflated calyces (146 of ca. 222 in total). Previous phylogenetic studies at the family-level have provided strong support for the monophyly of Physalideae and its close relationship to Capsiceae (*Capsicum* + *Lycianthes*) and Salpichroina (*Salpichroa* + *Nectouxia*) (Olmstead et al., 2008; Särkinen et al., 2013). These authors recognized three subtribes, Iochrominae, Physalidinae and

Withaninae, the last of which was recently proposed to include additional genera (*Deprea*, *Cuatresia*) (Deanna et al., 2017). Nonetheless, many areas of the Physalideae remain unresolved, in particular relationships within and among the genera of Physalidinae (Whitson and Manos, 2005; Zamora-Tavares et al., 2016). We seek to improve the resolution within Physalidinae and across Physalideae broadly by expanding taxon sampling (to 73% of extant species) and including additional fast-evolving markers.

In the context of this new phylogeny, we trace the evolution of fruiting calyx inflation, leveraging the wide variation in form across the clade. Within Physalideae, calyces range from non-accrescent (not increasing in size during fruit development) to highly inflated (growing to completely envelop the fruit) (Fig. 1). This variation in fruiting calyx morphology has often been used for generic delimitation (Hunziker, 2001; Sawyer, 2001; Li et al., 2013; Zamberlan et al., 2015), although phylogenetic studies suggest that these characters are homoplastic (Whitson and Manos, 2005; Hu and Saedler, 2007), resulting in several non-monophyletic genera (e.g., *Physalis* and *Chamaesaracha*, Zamora-Tavares et al., 2016). Here we score fruiting calyx morphology across Physalideae and use statistical comparative methods to address the following questions: (i) is fruiting calyx inflation a convergent trait in Physalideae?; (ii) if so, how many times has this trait been gained or lost? (iii) can lineages move directly between non-accrescent and inflated states or do they tend to transition through intermediate stages of accrescence? The answers to these questions will provide insight into evolution of the lantern-like fruit form and lay the foundation for future studies at the genetic and developmental levels.

MATERIALS AND METHODS

Taxon sampling**–** The ingroup sampling spanned 27 of the 29 genera of Physalideae and included 215 species of the 294 species plus four varieties (Appendix S1 and S2; see

Supplemental Data with this article). The monotypic *Mellissia* and *Capsicophysalis* were the only genera not sampled. *Capsicum lycianthoides*, *Lycianthes inaequilatera*, and *Salpichroa tristis* (Appendix S1) were used as outgroups. Newly sampled plant material was either gathered from herbaria (CORD, CSU, MO, SI) or collected during several field trips to Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru, and United States in the last ten years. Leaves were dried in silica and vouchers were prepared and housed at local herbaria of each country (Argentina: CORD; Bolivia: LPB; Brazil: BHCB; Colombia: COL, JBB, PSO; Ecuador: LOJA, QCA, QCNE, QUSF; Peru: HAO, HUT; United States: COLO, CSU, MO). We also obtained previously extracted DNA from L. Bohs, R. Olmstead, and L. Freitas.

Phylogenetic reconstruction of Physalideae–We used de novo (407, ca. 55%) and published (339, ca. 45%) sequences from four regions to estimate relationships within Physalideae (Appendix S1, including GenBank accession numbers): the nuclear internal transcribed spacer region (ITS), the granule-bound starch synthase (GBSSI or *waxy*) gene, the second intron of LEAFY (LFY), and the chloroplast spacer *trnL-F*. GBSSI regions previously sequenced by Whitson and Manos (2005) were not included in the analyses because these included exons eight through ten while we used exons two through nine for most taxa. Taxon coverage was 92.8% for ITS, 77.9% for LFY, 78.4% for *waxy*, and 87% for the chloroplast fragment (Appendix S3). DNA extractions were done following a modified 2 x CTAB procedure (Doyle and Doyle, 1987); primers and PCR conditions followed previous works (Smith and Baum, 2006; Deanna et al., 2018a).

Sequence quality was inspected using GENEIOUS v4.6.1 (Drummond et al., 2006), and sequence alignments were performed in MEGA 6 (Tamura et al., 2013) using the MUSCLE algorithm (Edgar, 2004) followed by manual adjustments. For *trnL-F*, a variable repeat region towards the 5’ end of the intergenic spacer was removed because this is where putative pseudogenic copies of *trnF* have been found in *Solanum* (Poczai and Hyvönen, 2011). Gene trees were estimated individually for each region with maximum likelihood (ML) in RAxML v.8 (Stamatakis, 2014) on the CIPRES server (Miller et al., 2010). We implemented the GTR + GAMMA model and used the rapid bootstrap (BS) algorithm with 1000 replicates to assess nodal support. Sequences leading to long branches were double-checked by searching for sequences highly similar to the introns and exons with BLAST in order to exclude contamination problems. Trees were then compared across genes to identify areas of hard incongruence (BS > 70%; Mason-Gamer and Kellogg, 1996).

Given the absence of hard incongruence, we conducted ML and Bayesian analyses on the combined dataset. Matrices were concatenated with SequenceMatrix 1.8 (Vaidya et al., 2011) and partioned by gene before analysis. We also identified unstable tips based on the ML bootstrap analyses using the software RogueNaRok (Aberer et al., 2013). Two iterations of RogueNaRok were run with settings according to Särkinen et al. (2013), and rogue taxa were removed after each iteration, resulting in the pruning of 10 tips in total.

The final combined matrix included 7,988 bp of aligned sequences of 222 taxa, including outgroups. We performed ML phylogenetic inference partitioned by gene using RAxML according to the parameters used for individual region analyses (see above) on the CIPRES server (Miller et al., 2010). Bayesian analyses were conducted for the combined dataset with four partitions in BEAST 2 (Bouckaert et al., 2014), also on the CIPRES server. Best models of substitution were incorporated for each partition according to a previous selection with the Akaike Information Criterion (AIC) using jModelTest 2.1.3 (Appendix S3; Posada and Crandall, 1998; Darriba et al., 2012). Two independent BEAST analyses were run for 50 million generations each with tree sampling every 1000 generations, using an uncorrelated lognormal relaxed clock model to describe the branch-specific substitution rates (Drummond, et al., 2006). We used a Birth-Death tree prior, which accounts for both speciation and extinction (Gernhard, 2008), and a constraint of monophyly for all species excluding

Salpichroa tristis (the outgroup most distantly related to Physalideae). Convergence and stationarity of the parameters were inspected using Tracer v1.7 (Rambaut et al., 2018), targeting minimum effective sample sizes (ESS) of at least 200. The initial 20% of trees were discarded as burn-in, and the results were combined using LogCombiner as implemented in the BEAST package. The phylogenetic relationships were summarized in a maximum clade credibility (MCC) tree, and their posterior probabilities (PP) for all nodes were derived using TreeAnotator v2.4.7. The trees were visualized in FigTree v.1.4.3 (Rambaut, 2016).

Character coding of fruiting calyces–All fruiting calyces from taxa included in the phylogeny were scored using specimens housed at herbaria (COL, COLO, CORD, CSU, MO, SI), the JSTOR Plants database, and the literature (Appendix S2). Following Hu and Saedler (2007), we scored a fruiting calyx as accrescent-appressed when there is an increase in calyx length of 50% or more from flower to fruit stage (e.g., *Brachistus stramoniifolius*), or the berry is entirely covered but there is not a space between calyx and berry (e.g., *Cuatresia exiguiflora*). Fruiting calyx was coded as non-accrescent when it grows less than 50% from flower to fruit stage (e.g., *Witheringia solanacea*), and as inflated when the fruit is entirely enclosed by the calyx and there is also a space between calyx and berry (e.g., *Physalis peruviana*; see matrix in Appendix S4). Note that following this definition, species of *Iochroma* are coded as non-accrescent despite being described as often having accrescent calyces (Hunziker, 2001; Smith and Baum, 2006; Lezama Escobedo et al., 2007; Cueva Manchego et al., 2015). In *Iochroma*, accrescence is usually less than the 50% of the length present at the flowering stage. In a handful of species (e.g., *I. calycinum*, *I. barbozae*; Khan et al., 2012a; Leiva González et al., 2013), the fruiting calyx covers the berry (or nearly so), but this is due to the large size of the flowering calyx.

***Testing for phylogenetic signal*–**We implemented two metrics to examine the level for phylogenetic signal in fruiting calyx morphology. First, we calculated the parsimony score using the *parsimony* function in the {phangorn} R package (Schliep, 2011). This metric represents the minimum number of changes necessary to describe the trait distribution on the tree. Second, we computed Fritz and Purvis' D (FPD; Fritz and Purvis, 2010), a metric that captures the sum of sister clade differences, also available in {phangorn}. The FPD statistic takes a value of 1 if the trait has a phylogenetically random distribution and 0 if the trait has evolved under Brownian motion, where shared history will lead closely related taxa to have similar trait values. For both measures, we tested whether the observed values differed from those expected by chance (no phylogenetic signal) as well as those expected under Brownian motion. In the former case, the null distribution was created by randomly reshuffling the tip states 1000 times, and in the latter case, by evolving these traits on the phylogeny under a Brownian motion model 1000 times. These null distributions were created with the *treestat* function in the {phylometrics} package (Hua and Bromham, 2016). Traits with phylogenetic signal differ significantly from the random distribution ($p<0.05$) but not the distribution expected under Brownian motion. As the FPD statistic can only be applied to binary traits, we considered fruit accrescence and inflation separately (Appendices S5 and S6), while for parsimony, we were able to examine them jointly as three-state character (Fig. 1). These analyses were conducted using the MCC tree.

***Reconstructing the evolutionary transitions to fruiting inflated calyces*–**We estimated the history of fruit calyx evolution across Physalideae using maximum likelihood and Bayesian approaches. We first compared the fit of alternative models of trait evolution using the {ape} package in R (Paradis et al., 2004) and the MCC tree from the BEAST analyses. We considered six models with the first having transition rates between all states free to vary (the all rates different model) and the second with all rates equal. We then fit four stepwise models, where lineages move from non-accrescent to inflated through the intermediate state of accrescent-appressed. Model three has all steps being reversible while the last three models have one or more of these steps constrained to be irreversible (Table 1). Model selection was conducted with the Akaike Information Criterion (AIC) score, with the best model having a score at least two AIC units lower than the model with the next lowest AIC score (Burnham and Anderson, 2002).

Using the best fitting model, we next estimated ancestral states and the number of transitions between states with Bayesian stochastic mapping (SM). Through rounds of simulation (‘realizations’), SM generates a sample of histories of discrete character evolution on a phylogeny that should approximate the posterior distribution of histories (Huelsenbeck et al., 2003). In order to incorporate phylogenetic uncertainty, we performed 500 simulations of character history on a sample of 100 trees from the BEAST analysis with the combined dataset. The simulations, carried out with the *make.simmap* function in {phytools} package (Revell, 2012), were summarized on the MCC tree to provide the posterior probability of each state at each node. We also estimated the median number of changes for each transition type from the histories and computed 95% credibility intervals using the *hdr* (highest density region) function from the {diversitree} package in R (FitzJohn, 2012).

RESULTS

Phylogeny of Physalideae–Our final combined matrix had a taxon coverage of 84% (Appendix S3) and comprised 215 species of Physalideae. This represents 73.1% of the total species within the tribe and 55% of the species within *Physalis*. The plastid *trnL-F* and the nuclear region ITS were the most densely sampled, whereas ITS contributed most parsimony-informative characters (Appendix S3). Hard incongruence was not found among gene trees

(Appendix S7). The maximum likelihood and Bayesian topologies were largely congruent (Fig. 2 and Appendix S8, respectively) and showed strong to moderate support for Physalidinae (BS = 63%, PP = 1) and Iochrominae (BS = 100%, PP = 1), which is resolved as sister to the remaining Physalideae taxa (BS = 89%, PP = 1). The previously proposed subtribe Withaninae (Olmstead et al., 2008; Särkinen et al., 2013) does not appear to be monophyletic but instead was divided between two clades, with the Hawaiian *Nothocestrum* and allied Old World genera more closely related to Physalidinae than other members of Withaninae. Moreover, eight of the 27 sampled genera are non-monophyletic as presently circumscribed (e.g., *Iochroma*, *Cuatresia*, *Physalis*).

Testing for phylogenetic signal of accrescent and inflated fruiting calyces**–**We found strong phylogenetic signal for fruiting calyx accrescence and inflation with both implemented approaches. These traits have a significantly lower parsimony score and lower FPD compared to the random null distribution, suggesting that species with accrescent and inflated calyces are more closely related than expected by chance (Table 2). Consistent with this result, neither of the traits significantly differed from expectations under a Brownian motion model of evolution, which makes closely related taxa more likely to exhibit the same state due to their shared history (Table 2).

Evolutionary transitions to fruiting inflated calyces–The best-fitting maximum likelihood model for fruiting calyx evolution was the stepwise model with transitions between accrescent and non-accrescent fruiting calyces being irreversible (reverse transition rate not different from zero). This model had the lowest AIC score and was greater than two AIC units lower than any competing model (Table 1; Appendix S9). Our stochastic mapping simulations with this model estimated a median of 50 changes across the clade (95%

credibility interval (CI) = 44.56–56.04). Among these changes, shifts from non-accrescent to accrescent-appressed calyces and accrescent-appressed to inflated calyces were inferred to occur at roughly equal frequencies (median = 24 (95% CI = 19.94–29.09) vs. 24 (95% CI = 19.96–27.71), Appendix S10). Loss of inflation to an accrescent-appressed calyx was infrequent (median = 2, 95% CI = 0–3.93; Appendix S10). The ancestral state of the tribe was estimated by SM as non-accrescent in all stochastic maps (100% posterior probability, Fig. 3). Similarly, high support was inferred for this ancestral state at many nodes throughout the phylogeny, revealing multiple independent gains of accrescence and inflation (Fig. 3).

DISCUSSION

***Phylogenetic relationships and taxonomy of Physalideae*–** We present the first well-resolved and densely sampled phylogeny of the Physalideae tribe. This data set is a significant expansion compared with previous studies (e.g., 33 species of *Physalis* in Zamora-Tavares et al., 2016, vs 53 taxa here), and the sampling covers most of the taxonomic, morphological, and geographic variation within this group. Our results point to multiple taxa in need of re-circumscription, underscoring the importance of phylogenetic work to inform taxonomic revision (e.g., Olmstead et al., 2008). Although some parts of the tree (e.g., within *Physalis*) will require additional data for better resolution, our results have recovered many previously proposed relationships as well as several new ones, which we briefly review below. Taxonomic changes based on these results and an extensive revision of herbarium collections will be forthcoming in future work (Deanna et al., in. prep.).

Starting with the monophyletic and well-studied Iochrominae, only three out of six genera are monophyletic, even after recent nomenclatural changes (Shaw, 2018a; b). The crossability among genera, high convergence in traits used to delimitate generic taxonomy, and the comparative lack of karyological variation (Smith and Baum, 2006; Smith et al., 2008; Deanna et al., 2018b; Shaw, 2018b) suggest that combining the genera into a single monophyletic *Iochroma* may be the most stable solution. During the last 20 years, 19 new species of *Iochroma* and one *Saracha* have been described (e.g., Leiva Gonzalez et al., 2003; Leiva González and Lezama, 2005; Lezama Escobedo et al., 2007; Fernandez-Hilario and Smith, 2017) but no key for the entire group has been proposed, increasing the necessity of a full taxonomic revision.

The subtribe Withaninae also presents taxonomic challenges, both at the subtribal and generic levels. This subtribe was originally circumscribed by Olmstead et al. (2008) to contain seven small genera, which were all Old World except for the South American *Aureliana*. Deanna et al. (2017) placed the re-circumscribed *Deprea* within Withaninae and suggested that *Cuatresia* could also be included based on the phylogeny. Our analysis provides strong support for the close relationship of *Aureliana* to *Cuatresia* and *Deprea*, but also for the non-monophyly of the type genus, *Withania*, with three species (*W. coagulans*, *W. riebeckii*, and *W. somnifera*) closely related to other taxa in Withaninae sensu Olmstead et al. (2008) and the other two species (*W. aristata* and the type species *W. frutescens*) closely related to *Aureliana*. In a prescient review, Hepper (1991) pointed out that these two western African species, *W. aristata* and *W. frutescens*, are morphologically unlike others in *Withania* and suggested that their closest relatives may instead be across the Atlantic. Beyond the rearrangement of Withaninae necessitated by this apparent split within *Withania*, most of the genera that have been placed in the subtribe are monophyletic (or nearly so) based on extensive taxonomic work in recent years (Zamberlan et al., 2015; Deanna et al., 2018a).

The largest subtribe Physalidinae, with 12 genera, was recovered as a monophyletic group although relationships among and within the genera are complex and, in some cases, unresolved. One complicating factor is the large number of monotypic genera (*Alkekengi*, *Calliphysalis*, *Oryctes*, *Quincula*, *Schraderanthus*), some of which are nested within other genera. Nonetheless, several of the affinities that we uncovered have been proposed by previous authors using morphological data (e.g., between *Brachistus* and *Witheringia*, Hunziker, 1969; between xerophytic *Chamaesaracha* but excluding *C. rzedowskiana*, Averett, 1973; Turner, 2015), suggesting viable avenues for future taxonomic rearrangements. Perhaps the greatest challenge will be estimating relationships within *Physalis*, which remain largely unclear in this study as they have in previous analyses (Whitson and Manos, 2005; Zamora-Tavares et al., 2016). The lack of resolution within this clade may reflect a history of rapid diversification and hybridization, which will likely be elucidated only with phylogenomic approaches (e.g., Stenz et al., 2015).

Repeated evolution of fruiting calyx accrescence and inflation–Our analyses demonstrate that the highly-inflated fruiting calyx considered so characteristic of *Physalis* has evolved repeatedly in Physalideae. While previous studies had suggested homoplasious patterns in fruiting calyx variation in the tribe (Whitson and Manos, 2005; Hu and Saedler, 2007), we provide the first estimates of the numbers of gains and losses, with ca. 24 gains of accrescence, 24 subsequent gains of inflation and two reversals from inflation to the accrescent-appressed state (Fig. 3). Despite these many gains of calyx accrescence and inflation, we recovered significant phylogenetic signal in these traits overall. Indeed, the character states appear clustered on the phylogeny, with some large clades (e.g., Iochrominae, *Physalis* subg. *Rydbergis*) being invariant in the degree of fruiting calyx accrescence.

The many independent origins of calyx inflation may have occurred through recurring modifications of the shared underlying pathway, which is well studied in several Physalideae. The development of ICS requires the expression of *MPF2*-like MADS-box transcription factors in flowering calyces (He and Saedler, 2005). Nonetheless, *MPF2* expression in the calyx is widespread across taxa with and without ICS in Physalideae and even in Capsiceae (Hu and Saedler, 2007), suggesting the development of ICS is determined by other factors. Indeed, the effect of *MPF2* on calyx morphology appears to hinge on interactions with cytokinin and gibberellin, which are released upon fertilization (He and Saedler, 2007; Khan et al., 2012b). Thus, genetic changes that modify these hormonal signals, *MPF2* expression, or *MPF2* function could all contribute to variation in calyx inflation (Riss, 2009). Comparative molecular and developmental studies to-date implicate both regulatory and structural mutations in *MPF2*-like genes (Hu and Saedler, 2007; Riss, 2009; Khan et al., 2009), coupled with shifts in copy number due to the many ploidy changes in the tribe (Iqbal and Datta, 2007; Deanna et al., 2018b).

Inflated calyces have convergently evolved in many taxa outside of Solanaceae, although the possibility that these rely on the same genetic pathway has not been explored. The 11 families with highly accrescent calyces, in which the fruits may be berries, drupes or capsules, are spread across eudicots, from rosids (e.g., Caryophyllaceae, Malvaceae, Aptandraceae) to asterids (e.g., Lamiaceae, Boraginaceae, Campanulaceae) (Paton, 1990; Francis, 2000; Gottschling and Miller, 2006; Wilf et al., 2017). Solanaceae is the only family in which the developmental genetics of the trait has been studied in detail (Wang et al., 2015). Intriguingly however, overexpression of *MPF2*-like genes in *Arabidopsis* results in enlarged and persistent calyces (Khan et al., 2013) and the *MPF2*-like promoters from Physalideae are able to drive sepal-specific gene expression, also in *Arabidopsis* (Khan et al., 2012b). These patterns suggest that many elements of networks regulating sepal growth are widely conserved, raising the possibilty that the evolution of inflated calyces in other clades has involved similar mechanisms.

Loss vs. gain of inflation through a stepwise model of evolution– Our comparative analyses indicate that evolution of the inflated calyx proceeds in directional fashion, starting from the non-accrescent state, moving first to an accrescent but appressed state before finally becoming inflated. This pattern contradicts the hypothesis that, given the complex developmental pathway required to produce ICS, inflation should be easier to lose than to gain (Hu and Saedler, 2007). This frequent and directional transitions toward inflation suggest not only that the trait is genetically accessible (perhaps given the background of *MPF2* expression in Physalideae calyces) but also that inflation is generally retained by lineages in which it evolves. Still, the adaptive advantages that could favor the fixation of this trait (e.g., protection from desiccation, deterrence of predators, enhanced dispersal) have been largely untested (but see Wilf et al., 2017). In fact, the only evidence for adaptive evolution of ICS comes indirectly from molecular studies, which have estimated positive selection acting on *MPF2*-like genes in *Withania* and *Physalis* (Khan et al., 2009; Zhang et al., 2012).

The retention of ICS following its evolution may reflect not only selective advantages, but also developmental constraints acting on reversals. Ablation experiments in two ICS taxa (*Physalis* and *Withania*) reveal a complex crosstalk between calyx and fruit development at early stages, wherein removal of sepals prior to fertilization completely abolishes fruit setting (He and Saedler, 2007; Khan, et al. 2012b); even ablations at later stages result in the development of smaller berries. These results suggest that genetic changes that reduce sepal size in ICS taxa might also reduce fruit size, which would presumably carry negative consequences for plant fitness. In the future, it would be valuable to conduct similar ablation experiments across Physalideae with non-accrescent, accrescent-appressed, and inflated calyces to determine whether the negative effect of calyx damage on fruit development scales with the degree of accrescence of the fruiting calyx.

Despite the strong directionality inferred from our analyses, it is important to note that such patterns may be confounded by state-dependent differential diversification. For example, the abundance of inflated calyces (as in Physalideae) can occur through biased transitions toward this state or by increased diversification of lineages with the state (Ng and Smith, 2014). A thorough analysis of the effects of calyx evolution on speciation and extinction rates will require a larger phylogeny (Beaulieu and O'Meara, 2016), ideally at the family level and including all of the remaining genera (six) and species (76) with ICS. Diversification analyses would also benefit from new divergence time estimates in light of the recent discovery of Eocene lantern fruit fossils (Wilf et al., 2017). These fossils, placed in crown group *Physalis*, are dated to 52.2 mya, which is roughly the age inferred for the entire crown group Solanaceae in previous work (Särkinen et al., 2013; De-Silva et al., 2017). This contrast highlights the need for a complete reassessment of Solanaceae fossils (Wilf et al., 2017; Särkinen et al., 2013, 2018), together with a new family-wide dating analysis including all reliable fossil taxa.

CONCLUSIONS

Our phylogeny provides a starting point for re-circumscription of taxa and lays the foundation for ongoing research into morphological diversification of Physalideae and its spread around the globe. The charismatic lantern fruits, characteristic of the genus *Physalis*, have evolved repeatedly among its close relatives in Physalideae. In each case, lineages have moved stepwise towards the inflated calyx, with many extant lineages exhibiting intermediate states of accrescence. This well-resolved evolutionary history for Physalideae, together with the growing knowledge of fruit developmental pathways, will facilitate future work to trace the genetic changes that lead to ICS and may also explain the apparent directionality of transitions toward this morphological novelty.

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AUTHOR CONTRIBUTIONS

R.D. and S.D.S designed the study; R.D. and S.D.S extracted DNA and performed PCR; R.D. analyzed sequences, performed alignments and phylogenetic analyses; R.D. and M.D.L. applied phylogenetic comparative methods; R.D. and S.D.S. wrote the paper, with contributions from M.D.L. and G.E.B.

DATA ACCESSIBILITY

All sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) with accessions numbers and voucher information detailed in Appendix S1. Gene trees are presented in Appendix S7.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

Appendix S1. Summary of taxon sampling, provenance, voucher, and GenBank accession numbers.

Appendix S2. Total number of species per genus, including the distribution for each fruiting calyx character state (inflated, accrescent-appressed, and non-accrescent) and proportion of species sampled.

Appendix S3. Matrix information. List of the four regions used for building the Physalideae phylogeny, showing level of sampling, aligned length of matrix, variable sites per region, and proportion of parsimony informative (PI) characters.

Appendix S4. Matrix with the scoring of all species sampled as non-accrescent, accrescent-appressed, or inflated fruiting calyx.

Appendix S5. Matrix with the scoring of all species sampled as non-accrescent or accrescent fruiting calyx.

Appendix S6. Matrix with the scoring of all species sampled as non-inflated or inflated fruiting calyx.

Appendix S7. Phylogenetic relationships of Physalideae based on a maximum likelihood analysis of each marker (ITS, LFY, *trnL-F*, and *waxy*).

Appendix S8. Maximum clade credibility (MCC) tree of Physalideae obtained from a Bayesian analysis of the combined dataset of four markers (ITS, LFY, *trnL-F*, and *waxy*).

Appendix S9. Transition rates estimated for different models tested for fruiting calyx accrescence and inflation evolution in Physalideae, using a Maximum Likelihood analysis with the ace function from the {ape} package.

Appendix S10. Summary of the stochastic character mapping for fruiting calyx accrescence and inflation, using the best estimated model (stepwise with irreversible 0-1 transition).

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ZHANG, J., M.R. KHAN, Y. TIAN, Z. LI, S. RISS, and C. HE. 2012. Divergences of *MPF2*-like MADS-domain proteins have an association with the evolution of the inflated calyx syndrome within Solanaceae. *Planta* 236: 1247–1260.

TABLES

TABLE 1. Comparison of likelihood models tested for fruiting calyx accrescence and inflation, including log-likelihood (lnLik) and Akaike Information Criterion (AIC) scores. The lowest AIC score is bolded. The character states are: 0 = non-accrescent, 1 = accrescent-appressed, 2 = inflated fruiting calyx, and thus q_{01}, for example, denotes the transition rates from non-accrescent to accrescent-appressed.

Model tested	Constraints	Free parameters	lnLik	AIC
1. All rates different	--	6: q_{01}, q_{10}, q_{02}, q_{20}, q_{12}, q_{21}	-109.364	230.727
2. Equal rates	$q_{01} = q_{10} = q_{02} = q_{20} = q_{12} = q_{21}$	1: q	-123.646	249.291
3. Stepwise reversible	$q_{02}=0$, $q_{20}=0$	4: q_{01}, q_{10}, q_{12}, q_{21}	-109.374	226.747
4. Stepwise 0-1 irreversible	$q_{02}=0$, $q_{20}=0$, $q_{10}=0$	3: q_{01}, q_{12}, q_{21}	-109.374	**224.747**
5. Stepwise 1-2 irreversible	$q_{02}=0$, $q_{20}=0$, $q_{21}=0$	3: q_{01}, q_{12}, q_{10}	-111.630	229.259
6. Stepwise irreversible	$q_{02}=0$, $q_{20}=0$, $q_{21}=0$, $q_{10}=0$	2: q_{01}, q_{12}	-111.630	227.259

TABLE 2. Phylogenetic signal for fruiting calyx accrescence. All characters show significant signal, i.e., have parsimony scores and FBD values that are significantly lower than observed under the random distribution (bolded values, $p < 0.05$) but are not significantly different from the distribution under Brownian motion ($p > 0.05$). *FPD can adopt negative values up to -0.5 when the phylogenetic signal is high (Fritz and Purvis, 2010).

Trait	Parsimony score (PS)	P-value of observed vs. random distribution	P-value of observed vs. Brownian motion evolution	Fritz and Purvis' D (FPD)	P-value of observed vs. random distribution	P-value of observed vs. Brownian motion evolution
Fruiting calyx accrescence and inflation	**33**	**0.000**	0.910	NA	NA	NA
Fruiting calyx accrescence	**14**	**0.000**	0.960	**-0.483***	**0.002**	0.961
Fruiting calyx inflation	**20**	**0.003**	0.934	**-0.356***	**0.000**	0.949

FIGURE LEGENDS

FIGURE 1. Distribution of fruiting calyx states across the tribe Physalideae. The size of the pie slices represents the proportion of taxa in each state, i.e., species with inflated calyces (red, 49.7%), non-accrescent calyces (black, 24.8%) and accrescent but still appressed calyces (blue, 25.5%). The darker shade in each pie slice corresponds to the percentage of taxa with that state sampled in the present study (64.4%, 86.3%, and 77.3%, respectively). Images from red to blue (moving clockwise) are *Physalis hederifolia* (Deanna *et al.* 209, photo by S. Carrasco), *Deprea pumila* (Orozco *et al.* 3890, photo by S. Leiva González), *Aureliana cuspidata* (Stehmann *et al.* 6457, photo by R. Deanna), *Witheringia solanacea* (Deanna 160, photo by R. Deanna), *Aureliana wettsteiniana* (Stehmann *et al.* 6448, photo by R. Deanna), *Iochroma arborescens* (Orejuela & Castillo 2697, photo by A. Orejuela), *Chamaesaracha coronopus* (Deanna *et al.* 237, photo by S. Carrasco), *Cuatresia exiguiflora* (Orozco *et al.* 3853, photo by G. E. Barboza), *Deprea sawyeriana* (Deanna & Leiva González 14, photo by S. Leiva González). Photos not to scale.

FIGURE 2. Phylogenetic relationships of Physalideae based on a maximum likelihood analysis of the combined dataset of four markers (ITS, LFY, *trnL-F*, and *waxy*). Bootstrap support (BS) values > 60% are given above each branch, and bold numbers indicate BS > 80%. Differentially coloured branches correspond to the subtribes proposed by Olmstead et al. (2008), followed by Särkinen et al. (2013) and modified by Deanna et al. (2017). The asterisk indicates *Physalis* subg. *Rydbergis*.

FIGURE 3. Reconstruction of fruiting calyx evolution in the Physalideae tribe. Topology is provided from four-gene BEAST analyses of 219 taxa. Circles at nodes indicate the posterior

probabilities from stochastic mapping and tip label colors represent tip states, with red, blue and black representing inflated, accrescent-appressed, and non-accrescent fruiting calyces, respectively. On the bottom, transitions between states are represented with arrows proportional to number of estimated changes (see Appendix S10).

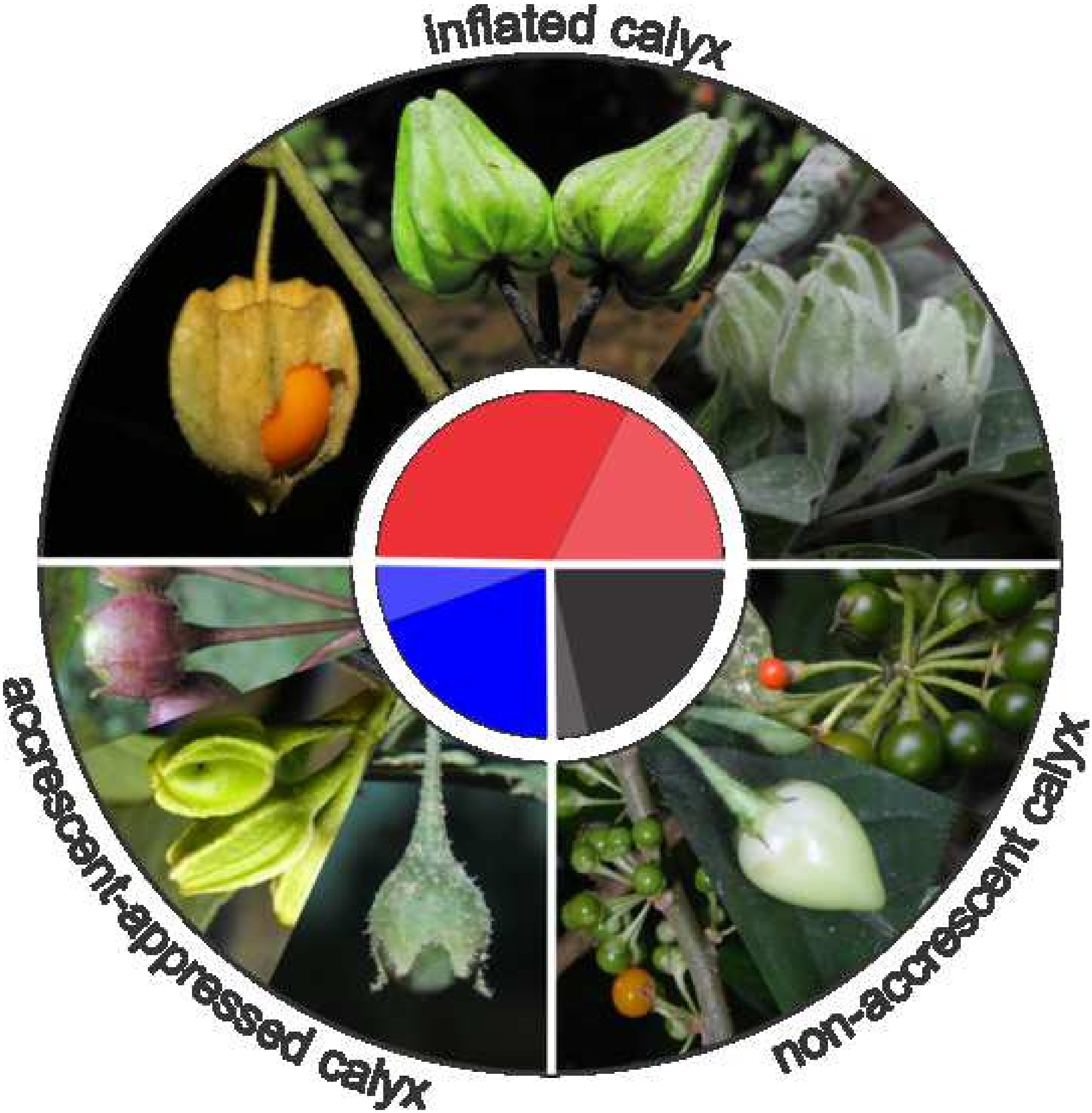
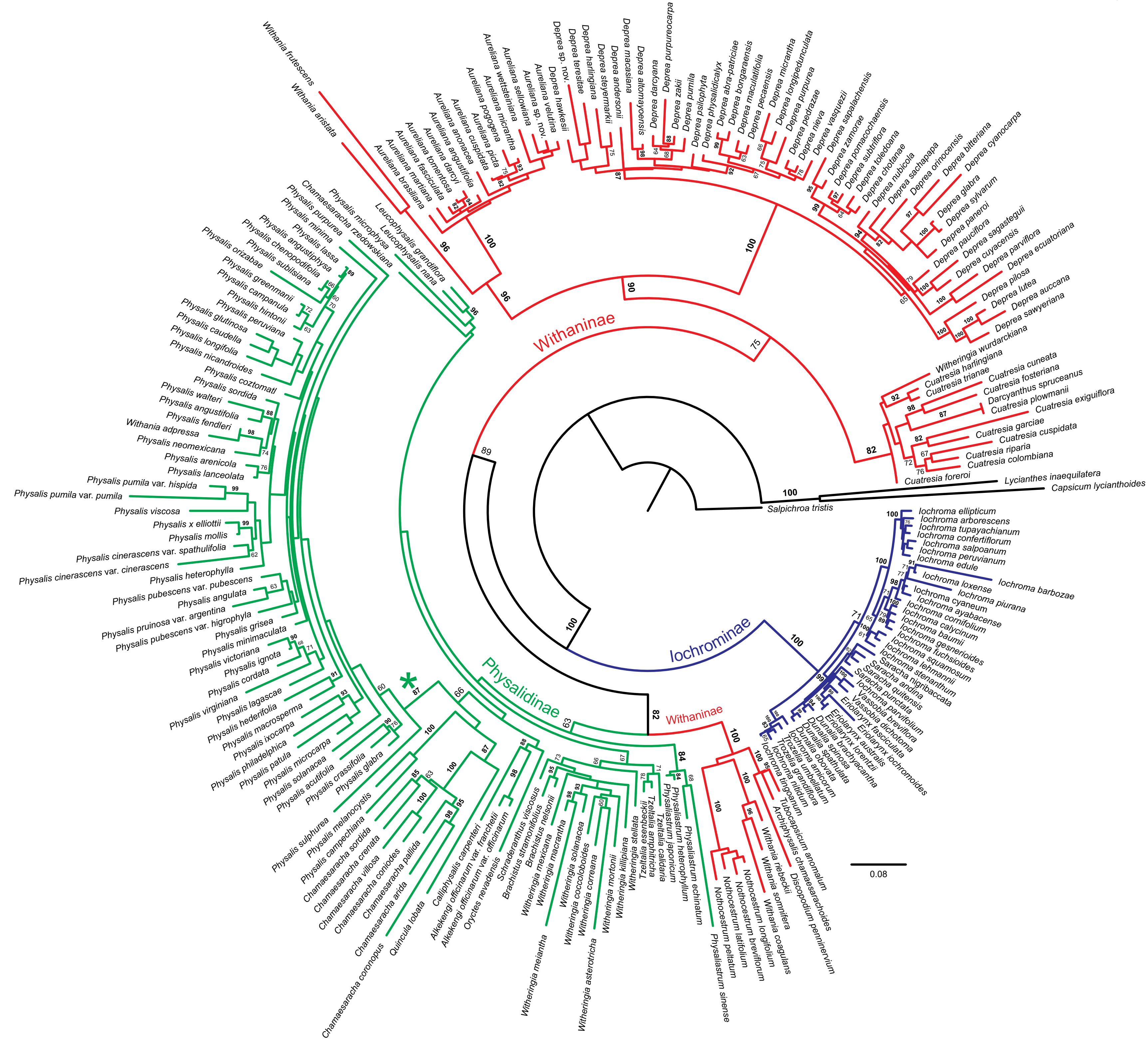

inflated calyx
accrescent-appressed calyx
non-accrescent calyx

Figure 2


Withaninae
Iochrominae
Physalidinae
Withaninae
*
0.08
Withania frutescens
Withania aristata
Aureliana brasiliana
Aureliana martiana
Aureliana fasciculata
Aureliana tomentosa
Aureliana darcyi
Aureliana anonacea
Aureliana angustifolia
Aureliana cuspidata
Aureliana pogogena
Aureliana picta
Aureliana wettsteiniana
Aureliana micrantha
Aureliana sellowiana
Aureliana sp. nov.
Aureliana velutina
Deprea hawkesii
Deprea sp. nov.
Deprea teresitae
Deprea harlingiana
Deprea steyermarkii
Deprea andersonii
Deprea macasiana
Deprea altomayoensis
Deprea darcyana
Deprea purpureocarpa
Deprea zakii
Deprea pumila
Deprea psilophyta
Deprea physalidicalyx
Deprea abra-patriciae
Deprea bongaraensis
Deprea maculatifolia
Deprea pecaensis
Deprea micrantha
Deprea longipedunculata
Deprea purpurea
Deprea pedrazae
Deprea nieva
Deprea vasquezii
Deprea sapalachensis
Deprea zamorae
Deprea pomacochaensis
Deprea subtriflora
Deprea toledoana
Deprea chotanae
Deprea nubicola
Deprea sachapapa
Deprea orinocensis
Deprea bitteriana
Deprea cyanocarpa
Deprea glabra
Deprea sylvarum
Deprea paneroi
Deprea pauciflora
Deprea sagasteguii
Deprea cuyacensis
Deprea parviflora
Deprea ecuatoriana
Deprea pilosa
Deprea lutea
Deprea auccana
Deprea sawyeriana
Witheringia wurdackiana
Cuatresia harlingiana
Cuatresia trianae
Cuatresia cuneata
Cuatresia fosteriana
Darcyanthus spruceanus
Cuatresia plowmanii
Cuatresia exiguiflora
Cuatresia garciae
Cuatresia cuspidata
Cuatresia riparia
Cuatresia colombiana
Cuatresia foreroi
Lycianthes inaequilatera
Capsicum lycianthoides
Salpichroa tristis
Iochroma ellipticum
Iochroma arborescens
Iochroma tupayachianum
Iochroma confertiflorum
Iochroma salpoanum
Iochroma peruvianum
Iochroma edule
Iochroma barbozae
Iochroma loxense
Iochroma piurana
Iochroma cyaneum
Iochroma ayabacense
Iochroma cornifolium
Iochroma calycinum
Iochroma baumii
Iochroma gesnerioides
Iochroma fuchsioides
Iochroma squamosum
Iochroma lehmannii
Iochroma stenanthum
Saracha nigribaccata
Saracha andina
Saracha quitensis
Saracha punctata
Iochroma parvifolium
Vassobia breviflora
Vassobia dichotoma
Eriolarynx fasciculata
Eriolarynx iochromoides
Eriolarynx australis
Eriolarynx lorentzii
Dunalia brachyacantha
Dunalia spinosa
Dunalia spathulata
Dunalia obovata
Iochroma amicorum
Iochroma umbellatum
Trozelia grandiflora
Trozelia nitidum
Iochroma tingoanum
Tubocapsicum anomalum
Archiphysalis chamaesarachoides
Discopodium penninervium
Withania riebeckii
Withania somnifera
Withania coagulans
Nothocestrum longifolium
Nothocestrum breviflorum
Nothocestrum latifolium
Nothocestrum peltatum
Physaliastrum sinense
Physaliastrum echinatum
Physaliastrum heterophyllum
Physaliastrum japonicum
Tzeltalia calidaria
Tzeltalia amphitricha
Tzeltalia esenbeckii
Witheringia stellata
Witheringia killipiana
Witheringia mortonii
Witheringia asterotricha
Witheringia correana
Witheringia coccoloboides
Witheringia solanacea
Witheringia meiantha
Witheringia macrantha
Witheringia mexicana
Brachistus nelsonii
Brachistus stramoniifolius
Schraderanthus viscosus
Oryctes nevadensis
Alkekengi officinarum var. officinarum
Alkekengi officinarum var. franchetii
Calliphysalis carpenteri
Quincula lobata
Chamaesaracha coronopus
Chamaesaracha arida
Chamaesaracha pallida
Chamaesaracha coniodes
Chamaesaracha villosa
Chamaesaracha crenata
Chamaesaracha sordida
Physalis campechiana
Physalis melanocystis
Physalis sulphurea
Physalis glabra
Physalis crassifolia
Physalis acutifolia
Physalis solanacea
Physalis microcarpa
Physalis patula
Physalis philadelphica
Physalis ixocarpa
Physalis macrosperma
Physalis hederifolia
Physalis lagascae
Physalis virginiana
Physalis cordata
Physalis ignota
Physalis victoriana
Physalis minimaculata
Physalis grisea
Physalis pubescens var. higrophyla
Physalis pruinosa var. argentina
Physalis angulata
Physalis pubescens var. pubescens
Physalis heterophylla
Physalis cinerascens var. cinerascens
Physalis cinerascens var. spathulifolia
Physalis mollis
Physalis x elliottii
Physalis viscosa
Physalis pumila var. pumila
Physalis pumila var. hispida
Physalis lanceolata
Physalis arenicola
Physalis neomexicana
Withania adpressa
Physalis fendleri
Physalis angustifolia
Physalis walteri
Physalis sordida
Physalis coztomatl
Physalis nicandroides
Physalis longifolia
Physalis caudella
Physalis glutinosa
Physalis peruviana
Physalis hintonii
Physalis campanula
Physalis greenmanii
Physalis orizabae
Physalis subilsiana
Physalis chenopodifolia
Physalis angustiphysa
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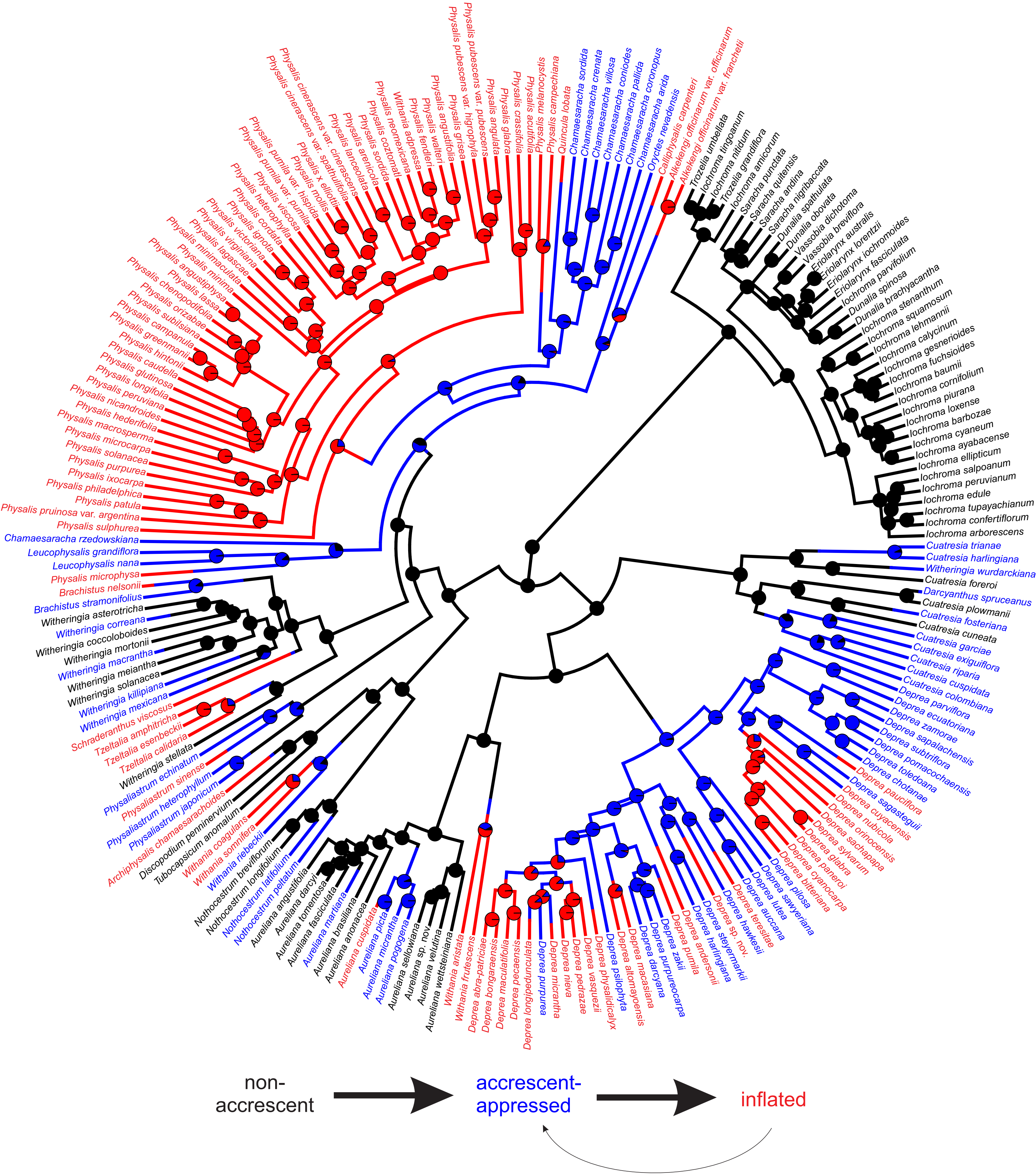
Physalis purpurea
Chamaesaracha rzedowskiana
Physalis microphysa
Leucophysalis nana
Leucophysalis grandiflora

Figure 3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Physalis peruviana
Physalis longifolia
Physalis glutinosa
Physalis caudella
Physalis hintonii
Physalis greenmanii
Physalis campanula
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Physalis lassa
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Physalis ignota
Physalis cordata
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Physalis viscosa
Physalis pumila var. pumila
Physalis pumila var. hispida
Physalis mollis
Physalis x elliottii
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Physalis walteri
Physalis angustifolia
Physalis grisea
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Physalis pubescens var. pubescens
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Physalis campechiana
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Chamaesaracha crenata
Chamaesaracha villosa
Chamaesaracha coniodes
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Iochroma confertiflorum
Iochroma arborescens
Cuatresia trianae
Cuatresia harlingiana
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Cuatresia foreroi
Darcyanthus spruceanus
Cuatresia plowmanii
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Cuatresia exiguiflora
Cuatresia riparia
Cuatresia cuspidata
Cuatresia colombiana
Deprea parviflora
Deprea ecuatoriana
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Aureliana fasciculata
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Witheringia correana
Witheringia asterotricha
Brachistus stramonifolius
Brachistus nelsonii
Physalis microphysa
Leucophysalis nana
Leucophysalis grandiflora
Chamaesaracha rzedowskiana
non-accrescent
accrescent-appressed
inflated

Dear Drs. Diggle and Potter,

We acknowledge these changes to improve the manuscript. We made the corrections suggested (accepting all the track-changes), as described below.

For this version of the manuscript, please accept all the track-changes in the text and do not make additional changes using this feature for this final submission. **Done. We also fixed minor things, such as the required comma after e.g.**

Key words can greatly enhance the discovery of your article. Please review your key words and consider whether they will help the reader find your work, i.e., by using words and phrases that are not already in the title or the abstract and/or including words that will widen the search-window of topics in your manuscript. If applicable, include the name of the plant family here (instead of in the title of the article). For more details see “c. Key Words” at https://onlinelibrary.wiley.com/page/journal/15372197/homepage/ForAuthors.html#ps
We replaced the previous key words: ‘Convergence; evolution; inflated calyx; Physalideae; Solanaceae; stochastic mapping’ by ‘Convergence; irreversibility; physaloids; stochastic mapping; trait evolution; transition rates’

In the “Supporting Information” statement, after “Additional Supporting Information may be found online in the supporting information section at the end of the article”, please list each Appendix S# and its title. **Done.**

Please change Appendix 1 to be Appendix S1 and upload it separately (not with the ms text file) as you did for each of the other online supplements/appendices, with a header and its title. **We changed the name to Appendix S1 and uploaded it separately.**

Appendix S2 – please add a header and a title for this appendix to the file itself. Be sure to explain the different sheets (which can be labeled Appendix S2a and S2b) and what they contain so that this appendix is fully understandable to the reader.
Be sure to provide headers and titles on the files for Appendices S4, S5, and S6 as well.
We provided the header and titles for all the Appendices now, and to keep the formatting, we changed the .csv files to .xlsx files. We also provided titles for each sheet of Appendix S2 inside the file.

APPENDIX S1. Summary of taxon sampling, provenance, voucher (collector and number or barcode, in italics), herbaria where vouchers were housed between parenthesis (acronyms follow Index Herbariorum; Thiers, 2017), and GenBank accession numbers provided in the following order: ITS, LEAFY, *trnL-F*, *waxy*. 'NA' indicates either voucher or provenance information was not found, and 'na' that the region was not sampled for this accession. Newly generated sequences are indicated with an asterisk following the accession number.

Alkekengi officinarum Mill. **var. *officinarum***, HUNGARY, cultivated, *ISZ 10-02*, na, na, HM006825, na. UNITED STATES, cultivated, *Whitson 1280* (DUKE), AY665850, na, na, na; NA, *D'Arcy 17707* (MO), na, MH822152*, na, DQ169012. ***Alkekengi officinarum* var. *franchetii*** (Mast.) R.J.Wang, NA, *Lester S. XYZ* (BIRM), na, MH822151*, MH752594*, MH796557*.

Archiphysalis chamaesarachoides (Makino) Kuang, CHINA, Zhejiang, Gutian Mountain, *Li et al. 393* (HSNU), KC768877, na, KC768879, na.

Aureliana angustifolia Alm.-Lafetá, BRAZIL, Minas Gerais, Juiz de Fora, *Giacomin et al. 965* (BHCB), KC832782, na, KC549633-KC549614, na. ***Aureliana anonacea*** (Sendtn.) I.M.C. Rodrigues & Stehmann (= *A. pereirae*), BRAZIL, Minas Gerais, Caraça Sanctuary, *Oliveira et al. 388* (BHCB), KC832788, na, KC549639-KC549620, na. BRAZIL, Mina Gerais, Caraça, *Barboza 3638b* (CORD), na, MH822153*, na, KX690166. ***Aureliana brasiliana*** (Hunz.) Barboza & Hunz., BRAZIL, Rio de Janeiro, Itatiaia National Park, *Rodrigues et al. 106* (BHCB), KC832783, na, KC549634-KC549615, na. BRAZIL, Río de Janeiro, Petrópolis, *Barboza et al. 2055* (CORD), na, MH822154*, na, MH796558*. ***Aureliana cuspidata*** (Witasek) I.M.C. Rodrigues & Stehmann, BRAZIL, Sao Paulo, Conservation Area Boracéia, *Stehmann et al. 4812* (BHCB), KC832784, na, KC549635-KC549616, na. ***Aureliana darcyi*** Carvalho & Bovini, BRAZIL, Rio de Janeiro, Trindade, Paraty, *Stehmann et al. 4856* (BHCB), KC832785, na, KC549636-KC549617, na. ***Aureliana fasciculata*** (Vell.) Sendtn (= *A. fasciculata* var. *fasciculata*), BRAZIL, São Paulo, Jundiaí, Serra do Japi, *Stehmann et al. 4790* (BHCB), KC832786, na, KC549637-KC549618, na. BRAZIL, Paraná, Morretes, La Graciosa, *Barboza et al. 1630* (CORD), na, na, na, EF537144. ***Aureliana martiana*** (Sendtn.) I. M. C. Rodrigues & Stehmann, BRAZIL, Minas Gerais, Juiz de Fora, *Giacomin et al.* (BHCB), KC832787, na, KC549638-KC549619, na. ***Aureliana micrantha*** Sendtn., BRAZIL, Bahia, Road São José, *Stehmann 5064* (BHCB), KC832780, na, KC549631-KC549612, na. ***Aureliana picta*** (Mart.) I.M.C. Rodrigues & Stehmann, BRAZIL, São Paulo, Bananal, *Giacomin 887* (BHCB), KC832789, na, KC549640-KC549621, na. ***Aureliana pogogena*** (Moric.) I.M.C. Rodrigues & Stehmann, BRAZIL, Bahia, Conservation Area Serra Bonita, Camacan, *Stehmann 5084* (BHCB), KC832790, na, KC549641-KC549622, na. BRAZIL, *Stehmann et al. 5098* (BHBC), na, MH822155*, na, MH796559*. ***Aureliana sellowiana*** (Sendtn.) Barboza & Stehmann, BRAZIL, São Paulo, Parelheiros, *Rodrigues 69* (BHCB), KC832781, na, KC549632-KC549613, na. BRAZIL, São Paulo, desde Parelheiros rumbo a Eng. Marsilac, *Barboza et al. 2024* (CORD), na, MH822156*, na, MH796560*. ***Aureliana* sp. nov.** (= *A. fasciculata* var. *longifolia*), BRAZIL, São Paulo, Moji das Cruzes, *Stehmann 4800* (BHCB), KC832798, na, KC549649-KC549630, na. ***Aureliana tomentosa*** Sendtn. (= *A. fasciculata* var. *tomentella*), BRAZIL, Espiritu Santo, Santa Teresa, *Stehmann et al. 4857* (BHCB), KC832791, na, KC549642-KC549623, na. ***Aureliana velutina*** Sendtn. BRAZIL, Minas Gerais, Nova Lima, *Stehmann et al. 4543* (BHCB), KC832792, na, KC549643-KC549624, na. ***Aureliana wettsteiniana*** (Witasek) Hunz. & Barboza, BRAZIL, Santa Catarina, Porto União, *Thode 300* (BHCB), KC832793, na, KC549644-KC549625, na. BRAZIL, Paraná, Morretes, *Barboza 2020* (CORD), na, MH822157*, na, MH796561*.

Brachistus nelsonii (Fernald) D'Arcy, J.L. Gentry & Averett, MEXICO, Campeche, Calakmul, Rancho El Sacrificio, *Martínez et al. 28097* (MEXU), MH763701*, MH822158*, MH752595*, MH796562*. **Brachistus stramonifolius** (Kunth) Miers, GUATEMALA, Solola and Chimaltenango, *Williams 41524* (DUKE), AY665845, na, na, na. MEXICO, Veracruz, Xalapa, Sierra Madre Oriental, *Sousa-Peña 738a* (MEXU), na, MH822159*, EU580963, na.

Calliphysalis carpenteri (Riddell) Whitson, UNITED STATES, Florida, *Whitson 1133* (DUKE), AY665851, MH822160*, EU581042, MH796563*.

Capsicum lycianthoides Bitter, ECUADOR, Pichincha, Bellavista Cloud Forest Reserve, *Smith 203* (WIS), DQ314158, DQ309518, MH281754*, DQ309468.

Chamaesaracha arida Henrickson, UNITED STATES, New Mexico, Grant, San Vicente Creek drainage, *Deanna et al. 221* (COLO), MH763702*, MH822161*, MH752596*, MH796564*. ***Chamaesaracha coniodes*** (Moric. ex Dunal) Benth. & Hook. f. ex B.D. Jacks. & al., UNITED STATES, New Mexico, Harding, Ute Creek Valley, *Deanna et al. 234* (COLO), MH763703*, MH822162*, MH752597*, MH796565*. ***Chamaesaracha coronopus*** (Dunal) A. Gray, UNITED STATES, Colorado, Pueblo, Lake Pueblo, *Deanna & Carrasco 237* (COLO, CORD), MH763704*, MH822163*, MH752598*, MH796566*. ***Chamaesaracha crenata*** Rydb., MEXICO, Coahuila, Cepeda, Estación Marte, Talud norte, *Villarreal et al. 6646* (MEXU), MH763705*, MH822164*, MH752599*, MH796567*. ***Chamaesaracha pallida*** Averett, MEXICO, Zacatecas, Concepción del Oro, Sierra Astillero, *Villarreal & Ramírez 9391* (MEXU), MH763706*, MH822165*, MH752600*, MH796568*. ***Chamaesaracha rzedowskiana*** Hunz, MEXICO, Queretaro, Jalpan, Los Sarros, *López Ch. 546* (MEXU), MH763707*, MH822166*, MH752601*, MH796569*. ***Chamaesaracha sordida*** (Dunal) A. Gray, MEXICO, Sonora, Naco, Chihuahuan desert, *van Devender et al. 2003-352* (MEXU), MH763708*, MH822167*, MH752602*, na. ***Chamaesaracha villosa*** Rydb., UNITED STATES, Texas, Pecos, Picnic Area East of Iraan, *Deanna et al. 211* (COLO), MH763709*, MH822168*, MH752603*, MH796570*.

Cuatresia colombiana Hunz., COLOMBIA, Cauca, El Tampo, PNN Munchique, *Orozco et al. 3816* (COL, CORD), MH763710*, MH822169*, MH752604*, MH796571*. ***Cuatresia cuneata*** (Standl.) Bohs, NA, *Bohs 2394* (UT), MH763711*, MH822170*, MH752605*, MH796572*. ***Cuatresia cuspidata*** (Dunal) Hunz., COLOMBIA, Cundinamarca, Soacha, *Deanna 161* (CORD), MH763712*, MH822171*, MH752606*, MH796573*. ***Cuatresia exiguiflora*** (D'Arcy) Hunz., NA., Bohs 2454 (UT), MH763713*, MH822172*, EU580981, MH796574*. ***Cuatresia forereoi*** Hunz., ECUADOR, Sucumbios, from Lumbaqui to La Bonita, *Croat & Ferry 93692* (MO), MH763714*, na, na, na. ***Cuatresia fosteriana*** Hunz., NA, *Bohs 2753* (UT), MH763715*, MH822173*, MH752607*, MH796575*. ***Cuatresia garciae*** Hunz., COLOMBIA, Antioquia, Frontino, road to Murri, *Brant & Martínez 1410* (MO), na, MH822174*, na, na. ***Cuatresia harlingiana***, ECUADOR, Pichincha, Bellavista Cloud Forest Reserve, *Smith 204* (WIS), DQ314165, DQ301518, KM200029, DQ309475**.** ***Cuatresia plowmanii*** Hunz., COLOMBIA, Bocayá, Santa María, Calichana, La Almenara, *Orejuela et al. 120* (COL), MH763716*, MH822175*, MH752608*, MH796576*. ***Cuatresia riparia*** (Kunth) Hunz., NA, *Bohs 2551* (UT), na, MH822176*, EU580982, MH796577*. ***Cuatresia trianae*** Hunz. COLOMBIA, Caquetá, Florencia, corregimiento el Caraño, *Trujillo & Sánchez 3587* (HUAZ), MH763717*, MH822177*, MH752609*, MH796578*.

Darcyanthus spruceanus (Hunz.) Hunz., PERU, Madre de Dios, Tambopata, Puerto Maldonado, *Valenzuela & Huamantupa 1011* (MO), na, na, MH752610*, na.

Deprea abra-patriciae (S. Leiva & Barboza) S. Leiva & Deanna, PERU, Amazonas, Bongará, Área de Conservación Privada Abra-Patricia, *Deanna & Leiva González 41* (CORD, HAO), KX557300, na, MH281755*, KX690167. ***Deprea altomayoensis*** (S. Leiva & Quip.) Barboza & Deanna, PERU, San Martín, Rioja, Bosque de Protección Alto Mayo, *Deanna & Leiva González 84* (CORD), KX557302, MH822178*, MH281756*, KX690168. ***Deprea andersonii*** (N.W. Sawyer) Deanna & S. Leiva, ECUADOR, Napo, carretera Hollín-Loreto, km 26.5 (Ruta E45A, Troncal amazónica), *Deanna & Leiva González 116* (CORD, HAO), KX557301, MH822179*, MH281757*, KX690169. ***Deprea auccana*** S. Leiva, Barboza & Deanna, PERÚ, Amazonas, Bongará, Nueva Cajamarca – Pomacochas, *Deanna & Leiva González 44* (CORD), KX557303, MH822180*, MH281758*, KX690170. ***Deprea bitteriana*** (Werderm.) N.W. Sawyer & Benítez, COLOMBIA, Cundinamarca, Subachoque, El Tablazo, *Orozco et al. 3871* (COL, CORD), KP267794, MH822181*, MH281760*, KP267808. ***Deprea bongaraensis*** (S. Leiva) Deanna & Barboza, PERU, Amazonas, Bongará, carretera Bongará-Nuevo Cajamarca, *Deanna & Leiva González 36* (CORD), KX557304, MH822182*, MH281761*, KX690171. ***Deprea chotanae*** (S. Leiva, Pereyra & Barboza) S. Leiva, PERU, Cajamarca, Chota, bosque El Pargo, La Loma, *Deanna & Leiva González 59* (CORD), KX557305, MH822183*, MH281762*, KX690172. ***Deprea cuyacensis*** (N.W. Sawyer & S. Leiva) S. Leiva & Lezama, PERU, Piura, Ayabaca, bosque de Cuyas, *Barboza et al. 3367* (CORD), KP267793, MH822184*, MH281763*, KP267807. ***Deprea cyanocarpa*** Garzón & C.I. Orozco, COLOMBIA, *Muñoz 2* (COL), KP267797, MH822185*, MH281764*, KP267811. ***Deprea darcyana*** (N.W. Sawyer) Barboza & S. Leiva, COLOMBIA, Cauca, El Tambo, Parque Nacional Munchique, *Orozco et al. 3860* (COL, CORD), KX557306, na, MH281765*, KX690173. ***Deprea ecuatoriana*** Hunz. & Barboza, ECUADOR, Zamora Chinchipe, Yanganá, rumbo al Cerro Toledo, *Orozco et al. 3952* (CORD), KP267795, MH822186*, MH281767*, KP267809. ***Deprea glabra*** (Standl.) Hunz., COLOMBIA, Cauca, El Tambo, Parque Nacional Munchique, *Orozco et al. 3812* (COL, CORD, QCA), KP267799, MH822187*, MH281768*, KP267813. ***Deprea harlingiana*** (Hunz. & Barboza) Deanna & S. Leiva, ECUADOR, Zamora Chinchipe, Parque Nacional Podocarpus, *Deanna & Leiva González 12* (CORD, HAO), KX557307, MH822188*, MH281769*, KX690174. ***Deprea hawkesii*** (Hunz.) Deanna, COLOMBIA, Cauca, El Tambo, Parque Nacional Munchique, *Orozco et al. 3824* (COL, CORD), KP267821, na, MH281770*, KP267820. COLOMBIA, Huila, La Plata, Agua Bonita, Finca Meremberg, *Orejuela & Deanna 2568* (CORD, JBB), na, MH822189*, na, na. ***Deprea longipedunculata*** (S. Leiva, E. Rodr. & J. Campos) Barboza, PERU, Cajamarca, San Ignacio, Tabaconas, caserío La Bermeja, *Deanna & Leiva González 18* (CORD, HAO), KX557309, MH822190*, MH281775*, KX690177. ***Deprea lutea*** (S. Leiva) Deanna, PERU, Cajamarca, Chota, km 46 desde desvío Llama-Huambos hacia La Granja, *Deanna & Leiva González 68* (CORD, HAO), KX557310, MH822191*, MH281779*, KX690178. ***Deprea macasiana*** (Deanna, S. Leiva & Barboza) Barboza, ECUADOR, Pastaza, Macas, cerro San José del Quílamo, *Deanna & Leiva González 111* (CORD, HAO, QUSF), KX557311, MH822192*, MH281780*, KX690180. ***Deprea maculatifolia*** (E. Rodr. & S. Leiva) S. Leiva, PERU, Amazonas, Bagua, Imaza, Comunidad Aguaruna de Yamayakat, *Deanna & Leiva González 82* (CORD, HAO), KX557313, na, MH281781*, KX690181. ***Deprea micrantha*** S. Leiva & Barboza, ECUADOR, Zamora Chinchipe, Reserva Biológica San Francisco, Leiva González & Barboza 6530 (CORD, HAO, LOJA), MH281823*, na, MH281776*, MH281832*. ***Deprea nieva*** (S. Leiva & N.W. Sawyer) Barboza & Deanna, PERU, Amazonas, Bongará, km 384, bordes de carretera Nueva Cajamarca-Pomacochas (Florida), *Deanna & Leiva González 46* (CORD, HAO), KP267769, MH304887*, MH281782*, KP267763. ***Deprea nubicola*** N.W. Sawyer, COLOMBIA, Magdalena, Ciénaga, Sierra Nevada de Santa Marta, *Orejuela & Vélez 215* (COL), KP267796, MH822193*, MH281783*, KP267810. ***Deprea***

orinocensis (Kunth) Raf., VENEZUELA, *Benítez & Mancilla 7460* (MY), KP267767, MH822194*, MH281784*, KP267762. ***Deprea paneroi*** Benítez & M. Martínez, VENEZUELA, *Benítez et al. 7454* (MY), KP267768, na, MH281785*, KP267761. ***Deprea parviflora*** (N.W. Sawyer & S. Leiva) S. Leiva, PERÚ, Cajamarca, Cutervo, km 1543-1544, carretera Cutervo-La Capilla, *Deanna & Leiva González 73* (CORD, HAO), KX557314, MH822195*, MH281786*, KX690183. ***Deprea pauciflora*** Deanna, Barboza & S. Leiva, ECUADOR, Zamora Chinchipe, límite del Parque Nacional Podocarpus, *Deanna & Leiva González 13* (CORD), KX557332, MH822196*, MH281787*, KX690182. ***Deprea pecaensis*** S. Leiva, Deanna & Barboza, PERU, Amazonas, Bagua, La Peca, puente El Arenal, *Deanna & Leiva González 49* (CORD, HAO), KX557315, MH822197*, MH281789*, KX690184. ***Deprea pedrazae*** (S. Leiva & Barboza) Deanna & S. Leiva, PERU, Amazonas, Bagua, La Peca, puente El Arenal, *Deanna & Leiva González 48* (CORD, HAO), KX557316, MH822198*, MH281788*, KX690185. ***Deprea physalidicalyx*** S. Leiva, Barboza & Deanna, PERU, San Martín, San Martín, carretera Tarapoto hacia Bella Vista, *Leiva González & Barboza 5645* (CORD, HAO), KX557341, MH822199*, MH281790*, KX690186. ***Deprea pilosa*** (S. Leiva, E. Rodr. & J. Campos) Deanna, PERU, Cajamarca, San Ignacio, San José de Lourdes, Estrella del Oriente, *Deanna & Leiva González 32* (CORD, HAO), KX557317, MH822200*, MH281791*, KX690187. ***Deprea pomacochaensis*** (S. Leiva) Barboza, PERU, Amazonas, Bongará, carretera Bongará-Nueva Cajamarca, *Deanna & Leiva González 33* (CORD, HAO), KX557318, MH822201*, MH281792*, KX690188. ***Deprea psilophyta*** (N.W. Sawyer) S. Leiva & Deanna, ECUADOR, Loja, Nudo de Sabanilla, sendero a Ayupallas, *Orozco et al. 3947* (COL, CORD), na, na, MH281793*, na. ECUADOR, Zamora Chinchipe, carretera desde Yanganá hacia Valladolid, *Sawyer 770* (CONN, LOJA), KP267772, na, na, KP267766. ***Deprea pumila*** (S. Leiva, Barboza & Deanna) S. Leiva, ECUADOR, Pastaza, Mera, camino al río Anzú, *Orozco et al. 3890* (COL, CORD, QCA), KX557320, MH304886*, MH281794*, KX690189. ***Deprea purpurea*** (S. Leiva) Barboza & S. Leiva, PERU, Cajamarca, San Ignacio, San José de Lourdes, Estrella del Oriente, *Deanna & Leiva González 27* (CORD, HAO), KX557319, MH822202*, MH281795*, KX690192. ***Deprea purpureocarpa*** (S. Leiva, Deanna & Barboza) Deanna, ECUADOR, Napo, carretera Cosanga-Baeza, 5.4 km al sur de Baeza, Deanna & Leiva González 125 (CORD, HAO, QCNE), KX557321, MH822203*, MH281800*, KX690193. ***Deprea sachapapa*** (Hunz.) S. Leiva & Deanna, ECUADOR, Cotopaxi, San Francisco de las Pampas, Otonga, *Orozco et al. 3985* (COL, CORD, QCA), KX557328, na, MH281796*, KX690197. ECUADOR, Pichincha, *Smith 205* (WIS), na, DQ301519, na, na. ***Deprea sagasteguii*** (S. Leiva, Quip. & N.W. Sawyer) Barboza, PERU, Piura, Ayabaca, cerro Aypate, *Deanna & Leiva González 97* (CORD, HAO), KX557330, MH822204*, MH281797*, KX690200. ***Deprea sapalachensis*** S. Leiva & Barboza, PERU, Piura, Huancabamba, Carmen de la Frontera, *Barboza & Leiva González 4833* (CORD, HAO), na, na, MH752611*, MH796579*. ***Deprea sawyeriana*** (S. Leiva, E. Rodr. & J. Campos) S. Leiva, PERU, Cajamarca, San Ignacio, Tabaconas, caserío La Bermeja, *Deanna & Leiva González 14* (CORD, HAO), KX557331, MH822205*, MH281798*, KX690202. ***Deprea* sp.,** ECUADOR, Pastaza, Mera, desde la Plaza Mayor de Mera hacia Cavernas del Río Anzú, *Deanna et al. 114* (CORD), MH763718*, na, MH752612*, na. ***Deprea steyermarkii*** (Hunz.) S. Leiva & Barboza, ECUADOR, Azuay, carretera Gualaceo-Indanza, km 23, *Deanna & Leiva González 108* (CORD, HAO), KX557335, MH822206*, MH281803*, KX690203. ***Deprea subtriflora*** (Ruiz & Pav.) D'Arcy, BOLIVIA, La Paz, Nor-Yungas, carretera desde Chuspipata a Coroico, *Barboza & Leiva González 3663* (CORD), KP267770, MH822207*, MH281805*, KP267764. ***Deprea sylvarum*** (Standl. & C.V. Morton) Hunz., COSTA RICA, *Bohs 2504* (UT), KP267800, na, MH281806*, KP267814. ***Deprea teresitae*** Deanna & Orejuela, COLOMBIA, Valle del Cauca, Reserva ‘El Refugio’, *Deanna & Calderón 169* (PSO, CORD), MH281825*, na, MH281801*, MH281833*. ***Deprea toledoana*** (Barboza & S. Leiva) Barboza, ECUADOR, Zamora Chinchipe, a Valladolid desde Yanganá, *Orozco et al. 3936* (COL, CORD, QCA), KX557337, MH822208*, MH281807*, KX690205. ***Deprea vasquezii*** (S. Leiva, E. Rodr. & J. Campos) Deanna, PERU, Cajamarca, San Ignacio, San José de Lourdes, Estrella del Oriente, *Deanna & Leiva González 28* (CORD, HAO), KX557339, MH822209*, MH281808*, KX690207. ***Deprea zakii*** Barboza, S. Leiva & Deanna, ECUADOR, Napo, Quijos, carretera Papallacta-Cuyuja, *Deanna et al. 138* (CORD, QCNE), KX557340, MH822210*, MH281802*, KX690208. ***Deprea zamorae*** Barboza & S. Leiva, ECUADOR, Zamora Chinchipe, Parque Nacional Podocarpus, *Orozco et al. 3926* (COL, CORD, QCA), KP267792, MH822211*, MH281809*, KP267806.

Discopodium penninervium Horchst., TANZANIA, *Tanner 3288*, KC832794, MH822212*, na, na. UGANDA, Kabarole, Burahya, *Knapp 9808* (BM), na, na, EU580986, na.

Dunalia brachyacantha Miers, ARGENTINA, Jujuy, Valle Grande, *Nee & Bohs 50811* (NY), DQ314172, DQ301527, MH281810*, DQ309482. ***Dunalia obovata*** (Ruiz & Pav.) Dammer, PERU. Junin, *Smith et al. 458* (HAO, F, MO, NY, USM, WIS), DQ314192, DQ301547, MH281811*, MDQ309499. ***Dunalia spathulata*** Ruiz & Pav.) Braun & Bouché, PERU, Huanuco, *Smith et al. 452* (HAO, F, MO, NY, USM, WIS), DQ314198, DQ301554, MH752613*, DQ309506. ***Dunalia spinosa*** (Meyen) Dammer, BOLIVIA, Potosí, Tomas Frias, *Smith et al. 379* (MO, WIS) DQ314188, DQ301543, MH281812*, DQ309495.

Eriolarynx fasciculata (Miers) Hunz., BOLIVIA, Cochabamba, *Smith et al. 432* (HAO, F, MO, NY, WIS), DQ314196, DQ301552, MH752614*, DQ309504. ***Eriolarynx iochromoides*** (Hunz.) Hunz., ARGENTINA, Catamarca, Andalgalá, Río Potrero, *Barboza et al. 1966* (CORD), KP267802, MH304888*, MH281813*, KP267816. ***Eriolarynx australis*** (Griseb.) J.M.H Shaw, BOLIVIA, Chuquisaca, *Smith et al. 390* (WIS), DQ314189, DQ301544, KP756712, DQ309496. ***Eriolarynx lorentzii*** (Dammer) Hunz., ARGENTINA, Tucumán, *Hawkes et al. 3452* (BIRM), DQ314171, DQ301525, KP756713, DQ309481.

Iochroma amicorum M. Cueva, S.D. Sm. & S. Leiva, PERU, Oxapampa, Huancabamba, PN Yanachaga-Chemillen, *Smith 542* (HAO, HOXA, MO, USM), KM514683, KM514684, MH752615*, KM521199. ***Iochroma arborescens*** (L.) J.M.H. Shaw, COSTA RICA, Puntarenas, Las Cruces, *Bohs 2428* (UT), DQ314173, DQ301528, KP756700, DQ309483. ***Iochroma ayabacense*** S. Leiva, PERU, Piura, Ayabaca, *Smith & Leiva González 337A* (HAO, F, MO, WIS), DQ314194, DQ301549, MH752616*, DQ309501. ***Iochroma barbozae*** S. Leiva & Deanna, PERU, Piura, Ayabaca, *Deanna et al. 91* (CORD), MH763719*, MH822213*, MH752617*, MH796581*. ***Iochroma baumii*** S.D. Sm. & S. Leiva, ECUADOR, Napo, Papallacta, *Smith & López 476* (QCNE, F, WIS), DQ314202, DQ301558, MH752618*, DQ309513. ***Iochroma calycinum*** Benth., ECUADOR, Pichincha, *Smith 471* (F, QCNE, WIS), DQ314201, DQ301557, MH281815*, DQ309512. ***Iochroma confertiflorum*** (Miers) Hunz., ECUADOR, Loja, *Smith et al. 237* (QCNE, MO, WIS), DQ314176, DQ301531, MH752619*, DQ309486. ***Iochroma cornifolium*** (Kunth) Miers, ECUADOR, Loja, *Smith et al. 242* (QCNE, MO, WIS), DQ314177, DQ301532, MH752620*, DQ309487. ***Iochroma cyaneum*** (Lindl.) G.H.M. Lawr. & J.M. Tucker, ECUADOR, Loja, Catamayo-El Cisne road, *Smith 223* (QCNE, MO, WIS), DQ314180, DQ301535, MH281814*, DQ309490. ***Iochroma edule*** S.Leiva, PERU, La Libertad, *Smith et al. 300* (HAO, F, MO, NY, USM, WIS), DQ314193, DQ301548, KP756703, DQ309500. ***Iochroma ellipticum*** (Hook. f.) Hunz., ECUADOR, Galápagos, *Jager 622* (CDS), DQ314199, DQ301555, MH752622*, DQ309507. ***Iochroma fuchsioides*** (Bonpl.) Miers, ECUADOR, Azuay, *Smith & López 488* (QCNE, F, MO, WIS), DQ314203, DQ301559, KP756711, DQ309514. ***Iochroma gesnerioides*** (Kunth) Miers, ECUADOR, Pichincha, Reserva Geobotanica Pululahua, *Smith 200* (QCNE, MO, WIS), DQ314179, DQ301534, MH281816*, DQ309489. ***Iochroma lehmannii*** Dammer ex Bitter, ECUADOR, Cañar, *Smith & López 484* (QCNE, F, MO, WIS), DQ314200, DQ301556, MH752623*, DQ309511. ***Iochroma loxense*** (Kunth) Miers, ECUADOR, Loja, *Smith 220* (QCNE, MO, WIS), DQ314175, DQ301530, MH752624*, DQ309485. ***Iochroma nitidum*** S. Leiva & Quip., PERU, Amazonas, *Smith et al. 371* (HAO, F, MO, NY, USM, WIS), DQ314168, DQ301521, MH752625*, DQ309478. ***Iochroma parvifolium*** (Roem. & Schult.) D'Arcy, PERU, La Libertad, *Smith et al. 303* (HAO, F, MO, NY, USM, WIS), DQ314195, DQ301551, MH752626*, DQ309503. ***Iochroma peruvianum*** (Dunal) J.F. Macbr., PERU, Cajamarca, *Smith & Hall 353* (HAO, F, MO, NY, USM, WIS), DQ314197, DQ301553, KP756706, DQ309505. ***Iochroma piuranum*** S.Leiva, PERU, Piura, Ayabaca, *Deanna et al. 93* (CORD), MH763721*, MH822215*, MH752627*, MH796582*. ***Iochroma salpoanum*** S. Leiva & Lezama, PERU, La Libertad, *Smith 364* (WIS), DQ314187, DQ301542, MH752628*, DQ309509. ***Iochroma squamosum*** S. Leiva & Quip., PERU, Piura, Ayabaca, *Smith et al. 330* (HAO, F, MO, NY), DQ314186, DQ301541, MH281817*, DQ309494. ***Iochroma stenanthum*** S. Leiva, Quip. & N.W. Sawyer, PERU, Cajamarca, *Smith et al. 313* (HAO, F, MO, NY, USM, WIS), DQ314184, DQ301539, MH752629*, DQ309508. ***Iochroma tingoanum*** S. Leiva, PERU, Amazonas, *Smith et al. 370* (HAO, F, MO, NY, USM, WIS), DQ314167, DQ301520, MH752630*, DQ309477. ***Iochroma tupayachianum*** S. Leiva, PERU, La Libertad, *Smith et al. 526* (F, MO, USM, WIS), KC290442, KC290441, na, KC243428.

Leucophysalis grandiflora (Hook.) Rydb., UNITED STATES, *Olmstead S-30* (WTU), DQ314162, DQ301515, EU581013, DQ309472. ***Leucophysalis nana*** (A. Gray) Averett, UNITED STATES, *Bartholomew 5994* (MO), MH763722*, na, EU581014, na.

Lycianthes inaequilatera Bitter, ECUADOR, Pichincha, Alluriquin, *Smith 210* (WIS), DQ314159, DQ309519, na, DQ309469. BOLIVIA, *Bohs 3089* (UT), na, na, EU581018, na.

Nothocestrum breviflorum A. Gray, HAWAII, Hamakua, Kailikaula Cliffs and Stream, *Wood et al. 4862* (MO), MH763723*, MH822216*, MH752631*, MH796583*. ***Nothocestrum latifolium*** A. Gray, HAWAII, Polynesia Hawaiian Islands, *H. St. John 24469*, KC832796, na, na, na. HAWAII, *Herbst et al. 725* (COLO), na, na, EU581037, na. HAWAII, *Lorentz 9063*, na, MH822217*, na, MH796584*. ***Nothocestrum longifolium*** A. Gray, HAWAII, Main Hawaiian Islands, North Hilo, *Cuddihy 743* (BISH), KC832795, na, na, na. HAWAII, *Oppenheimer s.n.* (BISH), na, MH822218*, EU581038, MH796585*. ***Nothocestrum peltatum*** Skottsb., HAWAII, Honopu, NW of Kainamanu, Acacia koa, *Wood & Query 15166* (MO), na, MH822219*, MH752632*, MH796586*.

Oryctes nevadensis S. Watson, UNITED STATES, Nevada, Churchill, *Tiehm 11982* (COLO, TEX), AY665864, na, EU581039, na.

Physaliastrum echinatum (Yatabe) Makino, CHINA, Yunyougu, Xinchengzi Town, Miyun District, Beijing, *Liu & Shi 5186* (PE), MH763724*, MH822220*, MH752633*, MH796587*. ***Physaliastrum japonicum*** (Franch. & Sav.) Honda, NA, *YYZWF20387*, KP894015, na, na, na. ***Physaliastrum heterophyllum*** (Hemsl.) Migo, CHINA, Zhejiang West Tianmu Mountain, *Li et al. 435* (HSNU), KC768878, na, KC768880, na. ***Physaliastrum sinense*** (Hemsl.) D'Arcy & Z.Y. Zhang, CHINA, Sichuan, *Hunggui 1177* (MO), na, MH822221*, na, na.

Physalis acutifolia (Miers) Sandwith, UNITED STATES, Arizona, Cpcjose, *Makings 3742* (MO), na, MH822222*, MH752634*, MH796588*. UNITED STATES, cultivated, *NIJ 974750059*, AY665876, na, na, na. ***Physalis angulata*** L., ARGENTINA, Córdoba, Río Seco, Ruta Nac. Nº 9, pasando Va. de María, *Morero 365* (CORD), MH763725*, MH822223*, MH752635*, MH796589*. ***Physalis angustifolia*** Nutt., UNITED STATES, Florida, Okalossa, *Miller et al. 9107* (MO), na, MH822224*, MH752636*, na. UNITED STATES, Florida, *Whitson, no voucher*, AY665878, na, na, na. ***Physalis angustiphysa*** Nutt., MEXICO, Chiapas, *Ton 9286* (TEX), AY665879, na, na, na. ***Physalis arenicola*** Kearney, UNITED STATES, Florida, Putnam, Ordway-Swisher Biological Station, *Majure et al. 5075* (FLAS), na, MH822225*, MH752637*, MH796590*. UNITED STATES, Florida, *Whitson, no voucher*, AY665880, na, na, na. ***Physalis campanula*** Standl. & Steyerm., MEXICO, Veracruz, *Ventura 4882* (MO), AY665882, na, na, na. ***Physalis campechiana*** L., MEXICO, Tamaulipas, *Jimenez 454* (TEX), AY665867, MH822226*, MH752638*, MH796591*. ***Physalis caudella*** Standl., MEXICO, Chihuahua, *Quintana 3075* (TEX), AY665891, na, na, na. ***Physalis chenopodifolia*** Lam., MEXICO, México, Pirámides de Teotihuacan, *Chiarini et al. 1277* (CORD), na, MH304893*, MH752639*, MH304879*. UNITED STATES, cultivated, *Whitson 1287* (DUKE), AY665883, na, na, na. ***Physalis cinerascens*** (Dunal) Hitchc. **var. *cinerascens***, UNITED STATES, Texas, Comal, Schmucks and Doeppens, roadsides, *Deanna et al. 206* (COLO, CORD), MH763726*, MH822227*, MH752640*, MH796592*. ***Physalis cinerascens* var. *spathulifolia*** (Torr.) J.R. Sullivan, UNITED STATES, Texas, Colorado, East to the Attwater Prairie Chicken National Wildlife Refuge, *Deanna et al. 203* (COLO), MH763727*, MH822228*, MH752641*, MH796593*. ***Physalis cordata*** Mill., PERU, Cajamarca, Contumazá, *Knapp et al. 10557* (CORD), MH763728*, MH822229*, MH752642*, MH796594*. ***Physalis coztomatl*** Dunal, MEXICO, *Garcia 264* (MO), AY665887, na, na, na. ***Physalis crassifolia*** Benth., UNITED STATES, California, *Sharples 744* (COLO), MH763729*, MH822230*, MH752643*, MH796595*. ***Physalis* x *elliottii*** Kunze, UNITED STATES, Florida, Sanibel Island, Bailey Tract, *Wheeler 14144* (SI), na, MH822231*, MH752644*, MH796596*. ***Physalis fendleri*** A. Gray, UNITED STATES, New Mexico, Grant, outside of Silver City, *Deanna et al. 219* (COLO), MH763730*, MH822232*, MH752645*, MH796597*. ***Physalis glabra*** Benth., MEXICO, Baja California Sur, La Paz, *Provance et al. 8003* (MO), MH763731*, na, MH752646*, na. ***Physalis glutinosa*** Schltdl., MEXICO, Durango, *Sikes 375* (TEX), AY665892, na, na, na. ***Physalis greenmanii*** Waterf., MEXICO, Veracruz, *Nee 22432* (MO), AY665893, na, na, na. MEXICO, Veracruz, Villa Aldama, *Nee 32880* (CORD), na, MH822233*, na, na. ***Physalis grisea*** (Waterf.) M. Martínez, UNITED STATES, cultivated, *NIJ 894750256*, AY665915, na, na, na. ***Physalis hederifolia*** A. Gray, UNITED STATES, Texas, Uvalde, dry Frio River, *Deanna et al. 209* (COLO), MH763732*, MH822234*, MH752647*, MH796598*. ***Physalis heterophylla*** Nees, UNITED STATES, Colorado, Larimer, Lory State Park, *Deanna et al. 199* (COLO), na, MH822235*, na, MH796599*. UNITED STATES, North Carolina, Caswell, *Whitson, no voucher*, AY665907, na, na, na. UNITED STATES, *Olmstead S-64* (WTU), na, na, EU581043, na. ***Physalis hintonii*** Waterf., MEXICO, Nuevo Leon, *Villarreal 4909* (MO), AY665895, na, na, na. ***Physalis ignota*** Britton, MEXICO, Chiapas, *Breedlove 52891* (MO), AY665897, na, na, na. ***Physalis ixocarpa*** Brot. ex Hornem., UNITED STATES, cultivated, *Deanna 251* (CORD), MH763733*, MH822236*, MH752648*, MH796600*. ***Physalis lagascae*** Roem. & Schult., PERU, Cajamarca, Cutervo, *Särkinen 4548* (BM), na, MH304892*, MH752649*, MH304880*. ***Physalis lanceolata*** Michx., UNITED STATES, North Carolina, Scotland, *Horn 1133* (DUKE), AY665899, na, na, na. ***Physalis lassa*** Stand. & Steyerm., MEXICO, Comala, *Sanders 11807* (MO), AY665900, na, na, na. ***Physalis longifolia*** Nutt., UNITED STATES, New Mexico, Bernalillo, Albuquerque, *Deanna et al. 227* (COLO), na, MH822237*, MH752650*, MH796601*. UNITED STATES, Kansas, Riley, *Whitson s.n.* (DUKE 358627), AY665901, na, na, na. ***Physalis macrosperma*** ined. UNITED STATES, Arkansas, Miller, *Gentry & Reid 3188*, MH763734*, MH822238*, MH752651*, na. ***Physalis melanocystis*** (B.L. Rob.) Bitter, MEXICO, Tamaulipas, *Martinez 1940* (MO), AY665865, MH822239*, MH752652*, MH796602*. ***Physalis microcarpa*** Urb., MEXICO, Chihuahua, *Laferriere 1661* (MO), AY665903, na, na, na. ***Physalis microphysa*** A. Gray, MEXICO, Coahuila, *Henrickson 11850* (TEX), AY665859, MH822240*, MH752653*, MH796603*. ***Physalis minima*** L., AUSTRALIA, South Australia, cultivated, *Symon 14813* (CORD), na, MH822241*, na, na. THAILAND, cultivated, *NIJ 974750167*, AY665904, na, na, na. ***Physalis minimaculata*** Waterf., MEXICO, Oaxaca, *Mayfield 986* (TEX), AY665906, na, na, na. ***Physalis mollis*** Nutt., UNITED STATES, Texas, Bastrop, *Deanna et al. 201* (COLO), na, MH822242*, MH752654*, MH796604*. ***Physalis neomexicana*** Rydb., UNITED STATES, New Mexico, Santa Fe, *Deanna et al. 228* (COLO), MH763735*, MH822243*, MH752655*, MH796605*. ***Physalis nicandroides*** Schltdl., MEXICO, Veracruz, Acultzingo, *Nee 33132* (CORD), na, MH822244*, na, MH796606*. MEXICO, Morelos, *Hernandez 2488* (MO), AY665912, na, na, na. ***Physalis orizabae*** Dunal, MEXICO, Morelos, Lagunas de Zempoala, *Chiarini et al. 1280* (CORD), MH763736*, MH822245*, MH752656*, MH796607*. ***Physalis patula*** Mill., MEXICO, Ciudad de México, *Chiarini et al. 1273* (CORD), na, MH822246*, MH752657*, MH796608*. MEXICO, Veracruz, *Nee 32810* (MO), AY665913, na, na, na. ***Physalis peruviana*** L., ECUADOR, Pichincha, cultivated, *Smith 217* (WIS), DQ314161, DQ301514, na, DQ309471. PERU, *Olmstead S-69* (WTU), na, na, EU581044, na. ***Physalis philadelphica*** Lam., UNITED STATES, cultivated, *Bohs 2433* (UT), na, MH822247*, EU581045, MH796609*. UNITED STATES, cultivated, *Whitson s.n.* (DUKE), AY665871, na, na, na. ***Physalis pruinosa* var. *argentina*** J. M. Toledo & Barboza, ARGENTINA, Jujuy, Valle Grande, Ruta P.N. Calilegua-San Francisco-Valle Grande, *Smith & Chiarini 630* (COLO), na, MH822248*, MH752658*, MH796610*. ***Physalis pubescens* L. var. *pubescens***, MEXICO, Morelos, Lagunas de Zempoala, *Chiarini et al. 1281* (CORD), na, MH304895*, MH752659*, MH304881*. COSTA RICA, La Selva Biological Station, *Whitson 3* (DUKE), AY665916, na, na, na. ***Physalis pubescens* var. *higrophyla*** (Mart.) Dunal, ARGENTINA, Jujuy, Ledesma, Libertador Gral. San Martín, *Toledo 1652* (CORD), MH763737*, MH822249*, MH752660*, MH796611*. ***Physalis pumila*** Nutt. **var. *pumila***, UNITED STATES, New Mexico, San Miguel, Sangre de Cristo Mountains, *Deanna et al. 230* (COLO), MH763738*, MH822250*, MH752661*, MH796612*. ***Physalis pumila* var. *hispida*** (Waterf.) W.F. Hinton, UNITED STATES, Colorado, Larimer, next to Poudre River, *Deanna et al. 200* (COLO), MH763739*, MH822251*, MH752662*, MH796613*. ***Physalis purpurea*** Wiggins, BOLIVIA, La Paz, Sud-Yungas, *Barboza 3657* (CORD), MH763740*, MH822252*, MH752663*, MH796614*. ***Physalis solanacea*** (Schltdl.) Axelius, MEXICO, Tamaulipas, Llera de Canales, *Nee & Calzada 33199* (CORD), na, MH822253*, na, MH796615*. MEXICO, cultivated, *Olmstead S-37* (WTU), AY665877, na, EU581025, na. ***Physalis sordida*** Fernald, MEXICO, Nuevo Leon, *Hinton 18464* (TEX), AY665869, na, na, na. ***Physalis subilsiana*** J.M. Toledo ARGENTINA, Salta, General José de San Martín, *Toledo & Domínguez 226* (CORD), na, MH822254*, na, na. ***Physalis sulphurea*** (Fernald) Waterf., MEXICO, 1 km al N de San Juan Citlaltepec, *Rodríguez García 116* (CORD), MH763741*, na, na, na. ***Physalis victoriana*** J.M. Toledo, ARGENTINA, Jujuy, Ruta Provincial N° 1, de Caimancito a Palma Sola, *Carrizo García 5* (CORD), MH763742*, na, MH752664*, MH796616*. ***Physalis virginiana*** Mill., UNITED STATES, Colorado, Boulder, *Deanna & Smith 238* (COLO), MH763743*, MH822255*, MH752665*, MH796617*. ***Physalis viscosa*** L., ARGENTINA, Córdoba, Calamuchita, *Deanna & Tamborini 179* (CORD), na, MH304894*, MH752666*, MH304882*. UNITED STATES, cultivated, *Whitson 1282* (DUKE), AY665870, na, na, na. ***Physalis walteri*** Nutt., UNITED

STATES, Florida, Levy, Havens Island, *Majure 3051* (FLAS), na, MH822256*, MH752667*, MH796618*. UNITED STATES, Florida, *Whitson, no voucher*, AY665918, na, na, na.

Quincula lobata (Torr.) Raf., UNITED STATES, New Mexico, Harding, *Deanna et al. 235* (COLO), MH763744*, MH822257*, MH752668*, MH796619*.

Salpichroa tristis Miers, BOLIVIA, Potosí, Tomas Frias, *Smith et al. 382* (HAO, F, MO, NY, WIS), DQ314160, DQ309520, MH281774*, DQ309470.

Saracha andina Rob. Fernandez, I. Revilla & E. Pariente, PERU, Ayacucho, Lucanas, *Smith & Fernandez 594* (COLO, F, MO, USM), KY172041, KY172040, na, KY172039. ***Saracha nigribaccata*** J.M.H. Shaw, ECUADOR, Pichincha, *Smith 211A* (QCNE, MO, WIS), DQ314174, DQ301529, EU580988, DQ309484. ***Saracha punctata*** Ruiz & Pav., BOLIVIA, La Paz, Nor Yungas, Rio Unduavi, *Nee 51804* (MO, NY), DQ314182, DQ301537, KP756709, DQ309492. ***Saracha quitensis*** (Hook.) Miers, ECUADOR, Napo, Laguna de Papallacta, *Smith 257* (QCNE, MO, WIS), DQ314178, DQ301533, MH281777*, DQ309488.

Schraderanthus viscosus (Schrad.) Averett, MEXICO, Oaxaca, *Torres 7932* (MO), AY665848, na, na, na.

Trozelia grandiflora (Benth.) J.M.H. Shaw, PERU, Cajamarca, *Smith et al. 320A* (HAO, F, MO, NY, USM, WIS), DQ314170, DQ301523, MH752669*, DQ309480. ***Trozelia umbellata*** (Ruiz & Pav.) Raf., PERÚ, La Libertad, *Smith et al. 301* (HAO, F, NY, USM, WIS), DQ314169, DQ301522, MH281818*, DQ309479.

Tubocapsicum anomalum (Franch. & Sav.) Makino, CHINA, *Chen 231* (MO), DQ314163, DQ301516, EU581066, DQ309473.

Tzeltalia amphitricha (Bitter) E. Estrada & M. Martínez, MEXICO, Chiapas, *Martínez 20523* (TEX), AY665853, na, na, na. ***Tzeltalia calidaria*** (Standl. & Steyerm.) E. Estrada & M. Martínez, GUATEMALA, *Lundell 19625* (TEX), na, na, MH752670*, na. ***Tzeltalia esenbeckii*** M. Martínez & O. Vargas, MEXICO, Chiapas, La Independencia, from Las Margaritas to Campo Alegre, *Breedlove 51325* (MEXU), MH763745*, na, MH752671*, na.

Vassobia breviflora (Sendtn.) Hunz., BOLIVIA, Chuquisaca, *Smith 412* (WIS), DQ314190, DQ301545, MH281819*, DQ309497. ***Vassobia dichotoma*** (Rusby) Bitter, BOLIVIA, *Nee et al. 51797* (UT), na, na, EU581067, na. BOLIVIA, La Paz, *Smith 440* (WIS), DQ314191, DQ301546, na, DQ309498.

Withania adpressa Cors., MORROCCO, *Lewalle 13205* (MO), na, na, MH752672*, MH796620*. ***Withania aristata*** Pauq., SPAIN, Canary Islands, *del Arco s.n.* (CORD), MH763746*, MH822258*, MH752673*, MH796621*. ***Withania coagulans*** (Stocks) Dunal, CENTRAL ASIA, *Olmstead S-109* (WTU), na, MH822259*, EU581068, MH796622*. ***Withania frutescens*** (L.) Pauquy, MOROCCO, Beldevere de Chicht, 15 km N of Essaouira, *Miller et al. 335* (MO), na, MH822260*, na, na. ***Withania riebeckii*** Balf. f., NA., *D'Arcy 17750* (MO), na, MH822261*, KC549645-KC549626, MH796623*. ***Withania somnifera*** (L.) Dunal, NA, *Whitson 1262* (KNK), na, MH304890*, na, MH304884*. NA, *Lester S. 0960*, KC832797, na, na, na. SPAIN, Canary Is., Mediterranean to Central Asia, *Whitson s.n.* (KNK), na, na, EU581069, na.

Witheringia asterotricha (Standl.) Hunz., COSTA RICA, *Bohs 3007* (UT), MH763747*, MH822262*, MH752674*, MH796624*. ***Witheringia coccoloboides*** (Dammer) Hunz.

COSTA RICA, Bohs 2568 (UT), MH281826*, MH304889*, MH752675*, MH304885*. ***Witheringia correana*** D'Arcy, PANAMA, Bocas del Toro, Fortuna, *D'Arcy 16415* (MO), MH763748*, MH822263*, MH752676*, MH796625*. ***Witheringia killipiana*** Hunz., COLOMBIA, Cauca, El Tambo, *Orozco et al. 3858* (COL, CORD), MH763749*, MH822264*, MH752677*, MH796626*. ***Witheringia macrantha*** (Standl. & C.V. Morton) Hunz., COSTA RICA, Monteverde, *Bohs 2512* (UT), AY665857, MH822265*, EU581071, MH796627*. ***Witheringia meiantha*** (Donn. Sm.) Hunz., COSTA RICA, *Bohs 3015* (UT), AY665856, MH822266*, EU581072, MH796628*. ***Witheringia mexicana*** (B.L. Rob.) Hunz., NA, *Bohs 3294* (UT), MH763750*, MH822267*, na, MH796629*. ***Witheringia mortonii*** Hunz., COSTA RICA, *Bohs 2594* (UT), MH763751*, MH822268*, MH752678*, MH796630*. ***Witheringia solanacea*** L’Hér., COSTA RICA, *Bohs 2416* (UT), na, na, EU581074, na. PANAMA, *D’Arcy 16399* (MO), DQ314164, DQ301517, na, DQ309474. ***Witheringia stellata*** (Greenm.) Hunz., MEXICO, *Stone 1522* (UT), MH763752*, MH822269*, MH752679*, MH796631*. ***Witheringia wurdackiana*** Benítez, VENEZUELA, Táchira, Fernández Feo, *Benítez de Rojas & Rojas 5433* (MO), MH763753*, na, na, na.

Appendix S2

Appendix S3. Matrix information. List of the four regions used for building the Physalideae phylogeny, showing level of sampling, aligned length of matrix, variable sites per region, and proportion of parsimony informative (PI) characters.

DNA region	ITS	LEAFY	*trnL-F*	*waxy*	Total
Genera	26	22	25	21	27
Taxa	206	173	193	174	222
Taxon coverage density	0.928	0.779	0.87	0.784	0.84
Missing data (%)	7	22	13	22	16
Aligned length (including gaps)	758	4504	1213	1513	7988
Variable characters	609	2722	340	877	4548
Variable characters (%)	80.3	60.4	28	58	56.94
PI characters	432	1595	152	528	2707
PI characters (%)	57	35.4	12.5	34.9	33.9
Best fitting nucleotide model	TPM1uf+I+G	TPM1uf+I+G	F81+I+G	HKY+I+G	--

Appendix S4

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Appendix S4.xlsx

Appendix S5

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Appendix S6

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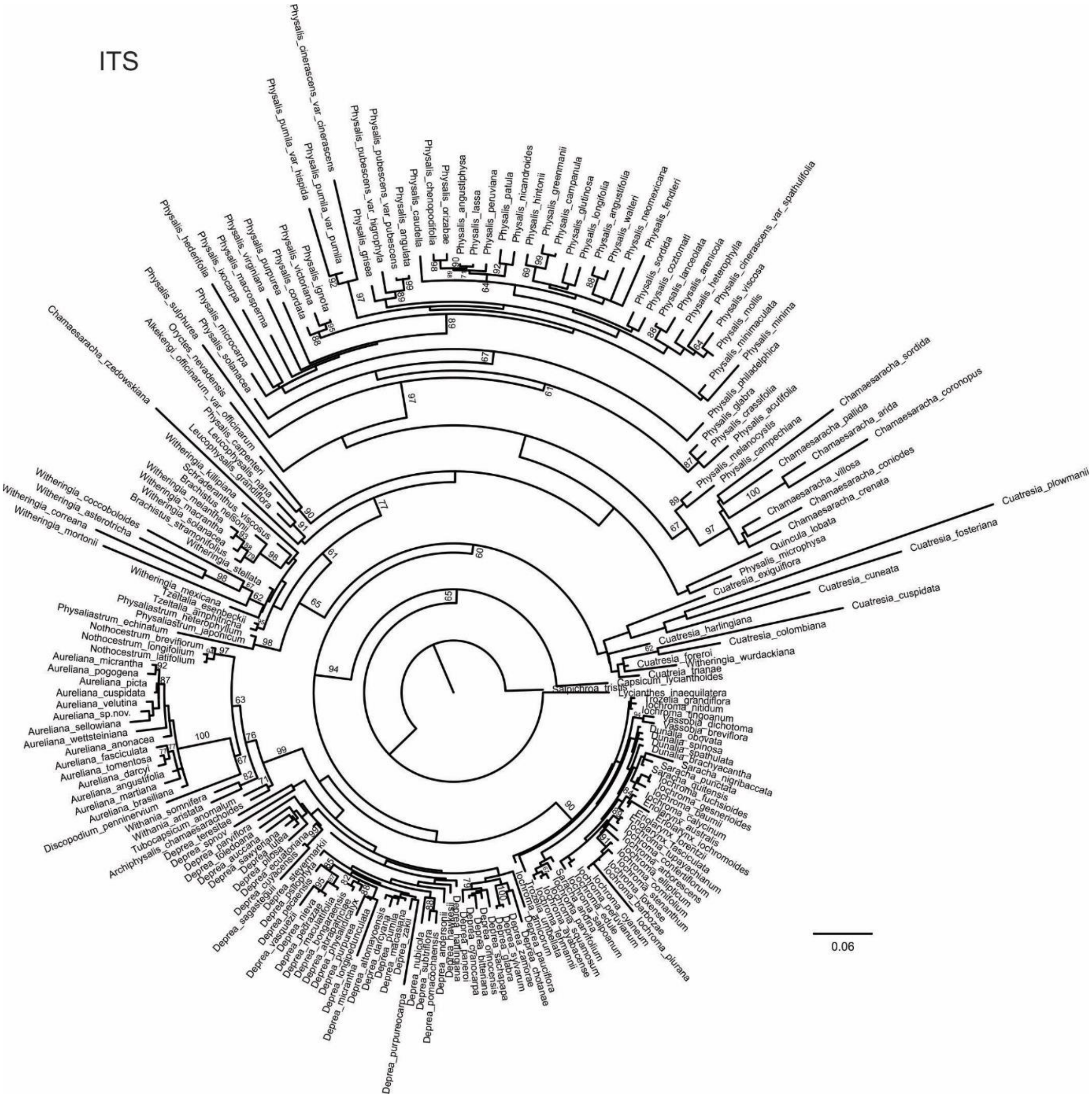
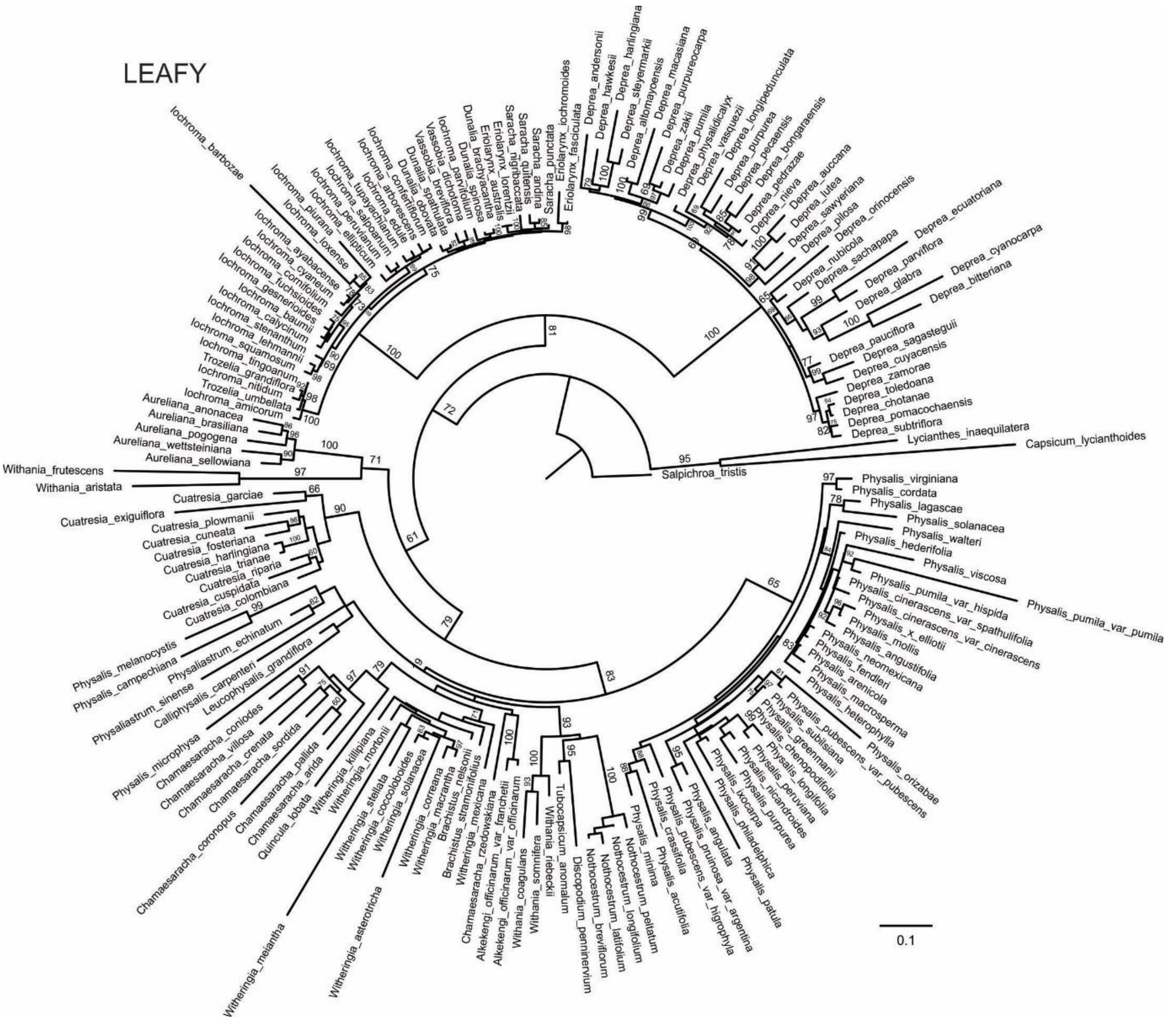
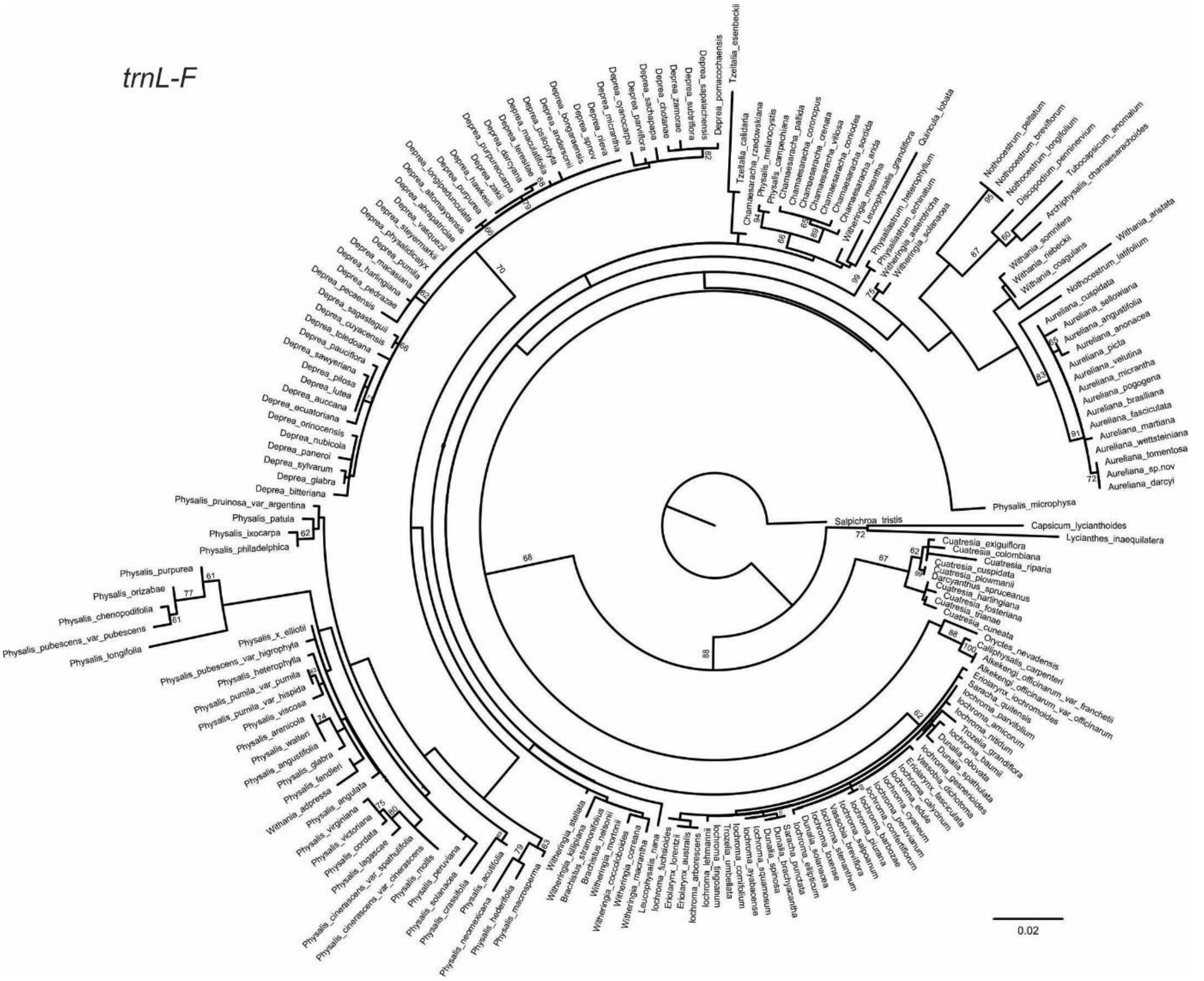
APPENDIX S7. Phylogenetic relationships of Physalideae based on a maximum likelihood analysis of each marker (ITS, LFY, *trnL-F*, and *waxy*). Bootstrap support (BS) values > 60 % are given above each branch.




LEAFY
Withania_frutescens
Withania_aristata
Aureliana_sellowiana
Aureliana_wettsteiniana
Aureliana_pogogena
Aureliana_brasiliana
Aureliana_anonacea
Iochroma_amicorum
Trozelia_umbellata
Iochroma_nitidum
Trozelia_grandiflora
Iochroma_tingoanum
Iochroma_squamosum
Iochroma_lehmannii
Iochroma_stenanthum
Iochroma_calycinum
Iochroma_baumii
Iochroma_gesnerioides
Iochroma_fuchsioides
Iochroma_cornifolium
Iochroma_cyaneum
Iochroma_ayabacense
Iochroma_barbozae
Iochroma_loxense
Iochroma_piurana
Iochroma_ellipticum
Iochroma_peruvianum
Iochroma_salpoanum
Iochroma_tupayachianum
Iochroma_edule
Iochroma_arborescens
Iochroma_confertiflorum
Dunalia_obovata
Vassobia_spathulata
Vassobia_breviflora
Vassobia_dichotoma
Iochroma_parvifolium
Dunalia_spinosa
Dunalia_brachyacantha
Eriolarynx_australis
Eriolarynx_lorentzii
Saracha_nigribaccata
Saracha_quitensis
Saracha_andina
Saracha_punctata
Eriolarynx_iochromoides
Eriolarynx_fasciculata
Deprea_andersonii
Deprea_hawkesii
Deprea_harlingiana
Deprea_steyermarkii
Deprea_altomayoensis
Deprea_macasiana
Deprea_purpureocarpa
Deprea_zakii
Deprea_pumila
Deprea_physalidicalyx
Deprea_vasquezii
Deprea_longipedunculata
Deprea_purpurea
Deprea_pecaensis
Deprea_bongaraensis
Deprea_pedrazae
Deprea_nieva
Deprea_auccana
Deprea_lutea
Deprea_sawyeriana
Deprea_pilosa
Deprea_orinocensis
Deprea_nubicola
Deprea_sachapapa
Deprea_ecuatoriana
Deprea_parviflora
Deprea_glabra
Deprea_cyanocarpa
Deprea_bitteriana
Deprea_pauciflora
Deprea_sagasteguii
Deprea_cuyacensis
Deprea_zamorae
Deprea_toledoana
Deprea_chotanae
Deprea_pomacochaensis
Deprea_subtriflora
Lycianthes_inaequilatera
Capsicum_lycianthoides
Salpichroa_tristis
Physalis_virginiana
Physalis_cordata
Physalis_lagascae
Physalis_solanacea
Physalis_walteri
Physalis_hederifolia
Physalis_viscosa
Physalis_pumila_var_pumila
Physalis_pumila_var_hispida
Physalis_cinerascens_var_spathulifolia
Physalis_cinerascens_var_cinerascens
Physalis_x_elliotii
Physalis_mollis
Physalis_angustifolia
Physalis_neomexicana
Physalis_fendleri
Physalis_arenicola
Physalis_macrosperma
Physalis_heterophylla
Physalis_orizabae
Physalis_pubescens_var_pubescens
Physalis_subilsiana
Physalis_greenmanii
Physalis_chenopodifolia
Physalis_longifolia
Physalis_peruviana
Physalis_nicandroides
Physalis_purpurea
Physalis_ixocarpa
Physalis_philadelphica
Physalis_patula
Physalis_angulata
Physalis_pruinosa_var_argentina
Physalis_pubescens_var_higrophyla
Physalis_crassifolia
Physalis_acutifolia
Physalis_minima
Nothocestrum_peltatum
Nothocestrum_longifolium
Nothocestrum_latifolium
Nothocestrum_breviflorum
Discopodium_penninervium
Tubocapsicum_anomalum
Withania_riebeckii
Withania_somnifera
Withania_coagulans
Alkekengi_officinarum_var_officinarum
Alkekengi_officinarum_var_franchetii
Chamaesaracha_rzedowskiana
Witheringia_mexicana
Brachistus_stramonifolius
Brachistus_nelsonii
Witheringia_macrantha
Witheringia_correana
Witheringia_asterotricha
Witheringia_solanacea
Witheringia_coccoloboides
Witheringia_stellata
Witheringia_meiantha
Witheringia_mortonii
Witheringia_killipiana
Quincula_lobata
Chamaesaracha_arida
Chamaesaracha_pallida
Chamaesaracha_coronopus
Chamaesaracha_sordida
Chamaesaracha_crenata
Chamaesaracha_villosa
Chamaesaracha_coniodes
Physalis_microphysa
Leucophysalis_grandiflora
Calliphysalis_carpenteri
Physaliastrum_sinense
Physaliastrum_echinatum
Physalis_campechiana
Physalis_melanocystis
Cuatresia_colombiana
Cuatresia_cuspidata
Cuatresia_riparia
Cuatresia_trianae
Cuatresia_harlingiana
Cuatresia_fosteriana
Cuatresia_cuneata
Cuatresia_plowmanii
Cuatresia_exiguiflora
Cuatresia_garciae
0.1


trnL-F
Tzeltalia_esenbeckii
Deprea_pomacochaensis
Deprea_sapaiachensis
Deprea_subtriflora
Deprea_zamorae
Deprea_chotanae
Deprea_sachapapa
Deprea_parviflora
Deprea_micrantha
Deprea_cyanocarpa
Deprea_nieva
Deprea_sprov
Deprea_bongaraensis
Deprea_andersonii
Deprea_maculatifolia
Deprea_pilophyta
Deprea_ferrarsiae
Deprea_darcyana
Deprea_purpurocarpa
Deprea_zakii
Deprea_hawkesii
Deprea_purpurea
Deprea_longipedunculata
Deprea_altomayoensis
Deprea_abrajaticiae
Deprea_vasquezii
Deprea_steyermarkii
Deprea_physalidicalyx
Deprea_pumila
Deprea_macasiana
Deprea_harlingiana
Deprea_pedrazae
Deprea_pecaensis
Deprea_sagasteguii
Deprea_cuyacensis
Deprea_toledoana
Deprea_pauciflora
Deprea_sawyeriana
Deprea_pilosa
Deprea_lutea
Deprea_auccana
Deprea_ecuatoriana
Deprea_orinocensis
Deprea_nubicola
Deprea_paneroi
Deprea_sylvarum
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Deprea_bitteriana
Physalis_pruinosa_var_argentina
Physalis_patula
Physalis_ixocarpa
Physalis_philadelphica
Physalis_purpurea
Physalis_orizabae
Physalis_chenopodifolia
Physalis_pubescens_var_pubescens
Physalis_longifolia
Physalis_x_elliotii
Physalis_pubescens_var_higrophyla
Physalis_heterophylla
Physalis_pumila_var_pumila
Physalis_pumila_var_hispida
Physalis_viscosa
Physalis_arenicola
Physalis_walteri
Physalis_angustifolia
Physalis_glabra
Physalis_fendleri
Withania_adpressa
Physalis_angulata
Physalis_virginiana
Physalis_victoriana
Physalis_cordata
Physalis_lagascae
Physalis_cinerascens_var_spathulifolia
Physalis_cinerascens_var_cinerascens
Physalis_mollis
Physalis_peruviana
Physalis_solanacea
Physalis_crassifolia
Physalis_neomexicana
Physalis_acutifolia
Physalis_hederifolia
Physalis_macrosperma
Witheringia_stellata
Witheringia_solanacea
Brachistus_stramoniifolius
Brachistus_nelsonii
Witheringia_mortonii
Witheringia_coccoloboides
Witheringia_correana
Witheringia_macrantha
Leucophysalis_nana
Iochroma_fuchsioides
Eriolarynx_australis
Eriolarynx_lorentzii
Iochroma_arborescens
Iochroma_lehmannii
Iochroma_tingoanum
Iochroma_umbellatum
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Iochroma_cornifolium
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Iochroma_ayabacense
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Vassobia_breviflora
Iochroma_stenanthum
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Iochroma_peruvianum
Iochroma_cyaneum
Iochroma_edule
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Dunalia_obovata
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Saracha_quitensis
Eriolarynx_iochromoides
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Calliphysalis_carpenteri
Oryctes_nevadensis
Cuatresia_cuneata
Cuatresia_trianae
Cuatresia_fosteriana
Cuatresia_harlingiana
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Darcyanthus_spruceanus
Cuatresia_plowmanii
Cuatresia_cuspidata
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Aureliana_sp.nov
Aureliana_tomentosa
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Aureliana_martiana
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Aureliana_cuspidata
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Withania_somnifera
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Witheringia_asterotricha
Physaliastrum_echinatum
Physaliastrum_heterophyllum
Leucophysalis_grandiflora
Chamaesaracha_arida
Witheringia_meiantha
Chamaesaracha_sordida
Chamaesaracha_coniodes
Chamaesaracha_villosa
Chamaesaracha_crenata
Chamaesaracha_coronopus
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Physalis_campechiana
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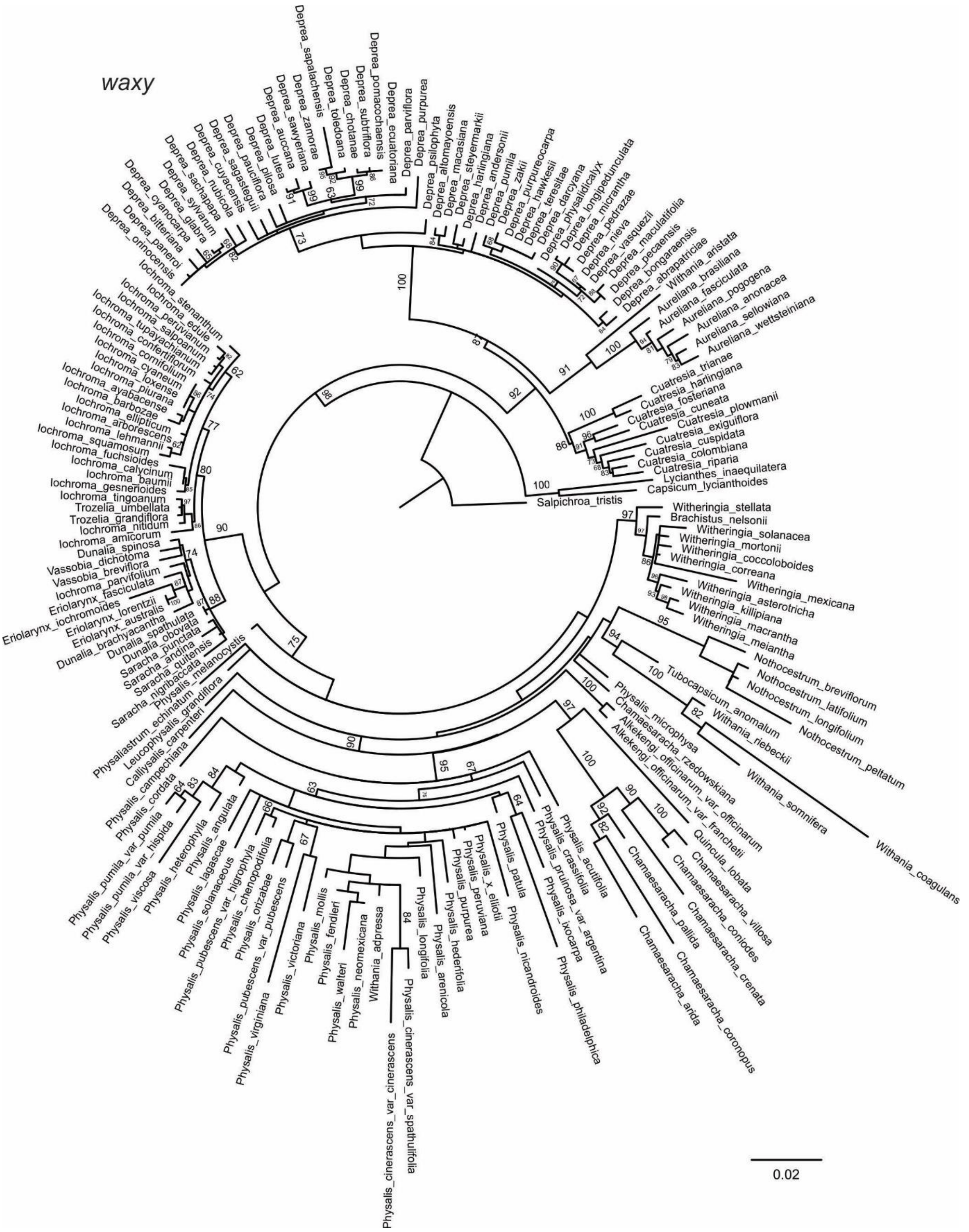
waxy
0.02
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Deprea_harlingiana
Deprea_andersonii
Deprea_pumila
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Deprea_purpureocarpa
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Deprea_teresitae
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Deprea_micrantha
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Deprea_nieva
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Deprea_vasquezii
Deprea_pecaensis
Deprea_bongaraensis
Deprea_abrapatriciae
Withania_aristata
Aureliana_brasiliana
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Witheringia_meiantha
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Nothocestrum_peltatum
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Withania_riebeckii
Withania_somnifera
Withania_coagulans
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Chamaesaracha_rzedowskiana
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Alkekengi_officinarum_var_franchetii
Quincula_lobata
Chamaesaracha_villosa
Chamaesaracha_coniodes
Chamaesaracha_crenata
Chamaesaracha_pallida
Chamaesaracha_coronopus
Chamaesaracha_arida
Physalis_acutifolia
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Physalis_pruinosa_var_argentina
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Physalis_neomexicana
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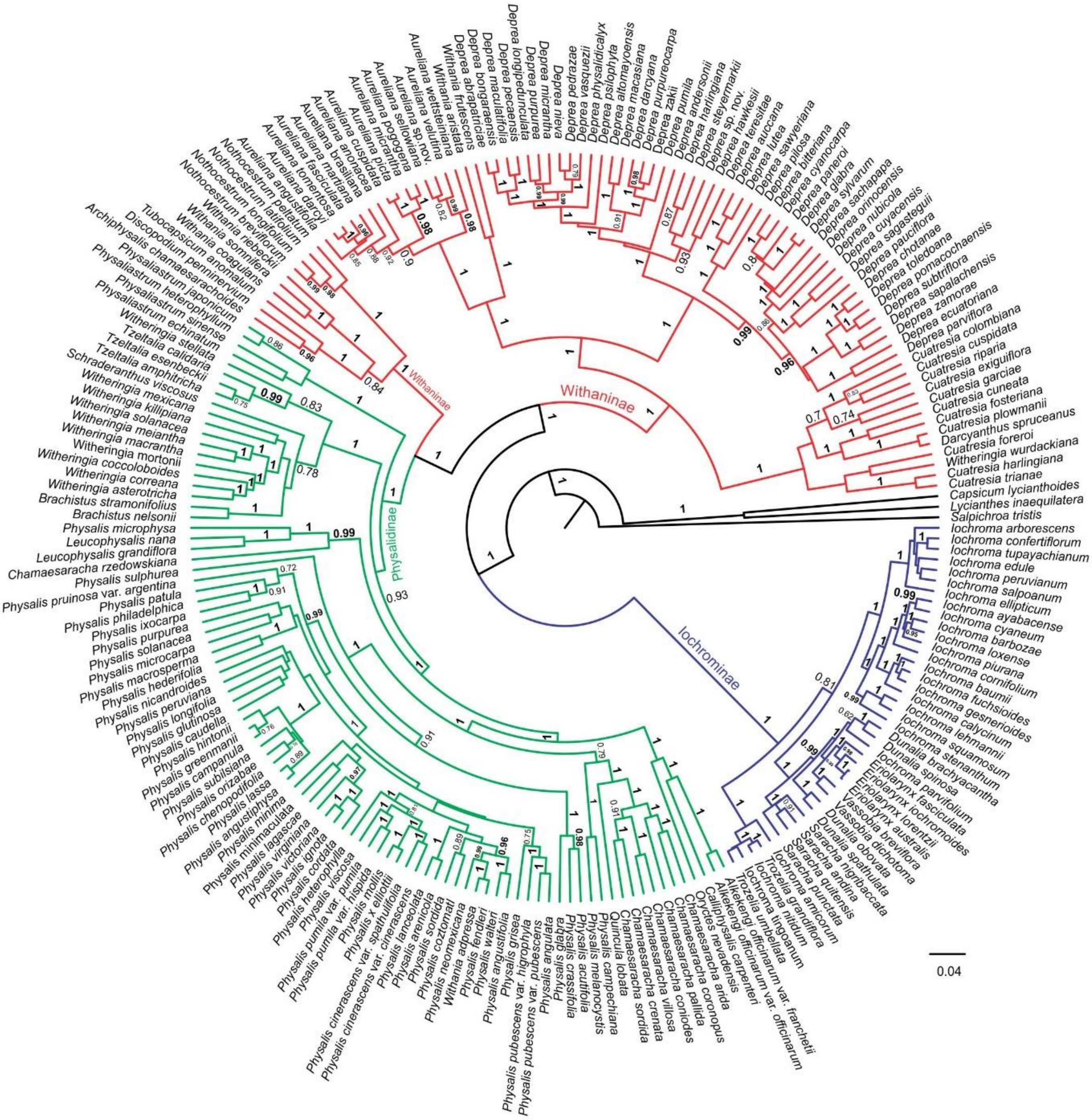
Physalis_cordata
Physalis_campechiana
Calliphysalis_carpenteri
Leucophysalis_grandiflora
Physaliastrum_echinatum
Physalis_melanocystis
Saracha_nigribaccata
Saracha_quitensis
Saracha_andina
Saracha_punctata
Dunalia_obovata
Dunalia_spathulata
Dunalia_brachyacantha
Eriolarynx_australis
Eriolarynx_lorentzii
Eriolarynx_iochromoides
Eriolarynx_fasciculata
Iochroma_parvifolium
Vassobia_breviflora
Vassobia_dichotoma
Dunalia_spinosa
Iochroma_amicorum
Iochroma_nitidum
Trozelia_grandiflora
Trozelia_umbellata
Iochroma_tingoanum
Iochroma_gesnerioides
Iochroma_baumii
Iochroma_calycinum
Iochroma_fuchsioides
Iochroma_squamosum
Iochroma_lehmannii
Iochroma_arborescens
Iochroma_ellipticum
Iochroma_barbozae
Iochroma_ayabacense
Iochroma_piurana
Iochroma_loxense
Iochroma_cyaneum
Iochroma_cornifolium
Iochroma_confertiflorum
Iochroma_tupayachianum
Iochroma_salpoanum
Iochroma_peruvianum
Iochroma_edule
Iochroma_stenanthum

Appendix S8. Maximum clade credibility (MCC) tree of Physalideae obtained from a Bayesian analysis of the combined dataset of four markers (ITS, LFY, *trnL-F*, and *waxy*). Posterior probabilities (PP) > 0.7 are given above each branch, and bold numbers indicate PP > 0.95. Differentially coloured branches correspond to the subtribes proposed by Olmstead et al. (2008), followed by Särkinen et al. (2013), and modified by Deanna et al. (2017).



Appendix S9. Transition rates estimated for different models tested for fruiting calyx accrescence and inflation evolution in Physalideae, using a Maximum Likelihood analysis with the ace function from the {ape} package. 0 = non-accrescent, 1 = accrescent-appressed, 2 = inflated fruiting calyx. Best model is highlighted in bold.

Model tested	Free parameters	Parameter estimates	Standard deviation
All rates different	q_{01}	5.3134	0.9025
	q_{10}	0.0000	0.5142
	q_{02}	0.0840	0.4271
	q_{20}	0.0000	0.6861
	q_{12}	5.152	0.4271
	q_{21}	0.3010	0.2266
Equal rates	q	1.5099	0.1971
Stepwise reversible	q_{01}	5.4018	0.7355
	q_{10}	0.0000	NaN
	q_{12}	5.2389	0.8051
	q_{21}	0.3001	0.2208
Stepwise 0-1 irreversible	$\mathbf{q_{01}}$	**5.4018**	**0.8154**
	$\mathbf{q_{12}}$	**5.2389**	**0.8087**
	$\mathbf{q_{21}}$	**0.3001**	**0.2204**
Stepwise 1-2 irreversible	q_{01}	5.4276	0.7308
	q_{10}	0.0000	NaN
	q_{12}	5.9227	0.8282
Stepwise irreversible	q_{01}	5.4276	0.8180
	q_{12}	5.9227	0.8319

Appendix S10. Summary of the stochastic character mapping for fruiting calyx accrescence and inflation, using the best estimated model (stepwise with irreversible 0-1 transition). MT= percentage mean total time spent in each state, TC = median number of total changes, C = median number of changes per transition, 95% CI = 95% credibility interval of number of changes. Most frequent transitions are in bold.

Fruiting calyx states	MT	TC	TC (95% CI)	Transition	C	C (95% CI)	State at the Physalideae root
0 = non-accrescent	31.69	50	44.56–56.04	**0-> 1**	24	19.94–29.09	0
1 = accrescent-appressed	32.17			**1-> 2**	24	19.96–27.71	
2 = inflated	36.14			2-> 1	2	0–3.93	