

1 Original article

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

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17 Running title: Fruiting calyx evolution in Physalideae

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19 **PREMISE OF THE STUDY:** The evolution of novel fruit morphologies has been integral
 20 to the success of angiosperms. The inflated fruiting calyx, in which the balloon-like calyx
 21 swells to completely surround the fruit, has evolved repeatedly across angiosperms and is
 22 postulated to aid in protection and dispersal. Here we investigate the evolution of this trait in
 23 the tomatillos and their allies (Physalideae, Solanaceae), using a newly estimated phylogeny
 24 and a suite of comparative methods to infer evolutionary gains and losses.

25
 26 **METHODS:** The Physalideae phylogeny was estimated using DNA sequences from four
 27 regions (ITS, LEAFY, *trnL-F*, *waxy*) using maximum likelihood and Bayesian Inference.
 28 Maximum likelihood model selection was used to determine the best fitting model of trait
 29 evolution. Using this model, we estimated ancestral states along with the numbers of gains
 30 and losses of fruiting calyx accrescence and inflation with Bayesian stochastic mapping.
 31 Also, phylogenetic signal in calyx morphology was examined with two metrics (parsimony
 32 score and Fritz and Purvis' D).

33
 34 **KEY RESULTS:** The well resolved phylogeny points to multiple taxa in need of revision,
 35 including the eight genera that are non-monophyletic as presently circumscribed. Model
 36 fitting indicated that calyx evolution has proceeded in stepwise fashion, from non-acrescent,
 37 to acrescent, to inflated. Moreover, these transitions appear to be largely irreversible.
 38 Among the 215 sampled Physalideae, we inferred 24 gains of fruiting calyx accrescence, 24
 39 subsequent transitions to a fully inflated calyx and only two reversals. A median of 50 shifts
 40 were estimated in total across the clade from the ancestral non-acrescent calyx. Nonetheless,
 41 fruiting calyx accrescence and inflation show strong phylogenetic signal.

42

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

49

50 **KEY WORDS**

51 Convergence; evolution; inflated calyx; Physalideae; Solanaceae; stochastic mapping

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54 Fruit evolution has long been considered a key contributor to the success of angiosperms,
 55 with bursts of morphological innovation closely tied to climatological events as well as the
 56 rise of frugivorous lineages of vertebrates (Tiffney, 1984; Eriksson et al., 2000; Knapp,
 57 2002). Variation in fruit traits across taxa is often correlated with differences in dispersal
 58 mode (e.g., Gautier-Hion et al., 1985; Lomáscolo et al., 2010), which in turn, can lead to
 59 shifts in diversification rates (e.g., Beaulieu and Donoghue, 2013; Lagomarsino et al., 2016;
 60 Larson & Johnson, 2016). Beyond their role in facilitating seed dispersal, fruits also serve to
 61 protect seeds from pathogens and predators (Tewksbury and Nabhan, 2001; Beckman and
 62 Muller-Landau, 2011) and promote successful germination (Traveset, 1998; Vander Wall,
 63 2001).

64 From an evolutionary perspective, fruit morphology is known not only for its tremendous
 65 diversity but also the high degree of convergence. For instance, fleshy fruits have evolved
 66 repeatedly in a wide variety of angiosperm clades (e.g. Malphigiaceae, Davis et al., 2001;
 67 Rubiaceae, Bremer et al., 1995; Solanaceae, Knapp, 2002), often in relation to shifts in
 68 ecological niche (Bolmgren and Eriksson, 2005; Givnish et al., 2005). Even seemingly
 69 complex fruit traits, such as heteroarthrocarpy, have been gained and lost multiple times at
 70 recent phylogenetic scales (Hall et al., 2011; Marcussen and Meseguer, 2017). However,
 71 unlike with floral traits, such as symmetry and coloration (Preston and Hileman, 2009; Sobel
 72 and Streisfeld, 2013), the extent to which convergent transitions in fruit traits occur through
 73 similar genetic and developmental mechanisms remains little explored (Pabón-Mora et al.,
 74 2014; Ortiz-Ramírez et al., 2018; but see Avino et al., 2012).

75 Here we focus on a charismatic but understudied fruit trait, the inflated fruiting calyx, which
 76 has evolved repeatedly across angiosperms. Inflated calyces develop by accrescence after
 77 anthesis such that the fruit becomes completely enclosed upon maturation (He et al., 2004).
 78 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 dramatic 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 which 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 fruiting calyx inflation in Physalideae with a greatly expanded phylogeny and statistical comparative analyses of character transitions. This tribe contains the highest generic-level diversity in Solanaceae, with 29 genera and ca. 300 species arranged in three subtribes (Iochrominae, Physalidinae

and Withaninae; Olmstead et al., 2008; Särkinen et al., 2013). Moreover, 13 of 19 Solanaceae genera with fruiting calyx inflation are placed in Physalideae. The wide variation in fruiting calyx form, from non-acrescent to greatly inflated (Fig. 1), has often been used for intergeneric delimitation (Hunziker, 2001; Sawyer, 2001; Li et al., 2013; Zamberlan et al., 2015), although phylogenetic studies suggest that these characters are homoplastic (Hu and Saedler, 2007). With a new phylogeny including 73 % of Physalideae species, we traced the evolution of the fruiting calyx accrescence and inflation 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-acrescent and inflated states or do they tend to transition through intermediate stages of accrescence? The answers to these questions will provide insight into evolutionary accessibility of the lantern-like fruit form and lay the foundation for future studies at the genetic and development 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 4 varieties (Appendix S1 and S2; see Supplemental Data with this article). The monotypic *Mellissia* Hook. f. and *Capsicophysalis* Averett & M. Martínez 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 already 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 regions internal transcriber spacer (ITS), 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 they only comprised from exon 8 to 10, whereas we are using from exon 2 to 9 for most taxa. Taxa 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 work (Smith and Baum, 2006; Deanna et al., 2018).

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. Trees were 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 partitioned 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. We also excluded the voucher R. Deanna 143 (which morphologically matches to the original description of *Cuatresia harlingiana* Hunz.) given its phylogenetic position outside of Physalideae. However, we included sequences of a voucher previously identified as *C. harlingiana* (Smith and Baum, 2006; Deanna et al., 2017, 2018), which does fall within *Cuatresia* and appears to belong to an undescribed taxon (appearing here as *Cuatresia* sp.).

The final combined matrix included 7988 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 fifty 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*. 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 TreeAnnotator v2.4.7. The trees were visualized in FigTree v.1.4.3 (Rambaut, 2016).

Codification 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 stramonifolius*), 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-acrescent 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-acrescent 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). Second, we computed Fritz and Purvis' D (FPD, Fritz and Purvis, 2010), a metric which 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 (Fritz and Purvis, 2010). For both measures, we tested whether the observed values differed 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 are predicted to 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-acrescent to inflated through the intermediate state of acrescent-appressed. Model 3 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* function from the {diversitree} package in R (FitzJohn, 2012).

RESULTS

Phylogeny of Physalideae—Our final combined matrix had a taxon coverage of 0.84 % (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 divided amongst 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 Brownian motion of evolution along the phylogeny (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-acrescent 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% HDR = 44.56–56.04). Among these changes, shifts from non-acrescent to accrescent-appressed calyces and accrescent-appressed to inflated calyces were inferred to occur at roughly equal frequencies (median = 24 (19.94–29.09) vs. 24 (19.96–27.71), Appendix S10). Loss of inflation to an accrescent-appressed calyx was infrequent (median = 2, 95% HDR = 0–3.93; Appendix S10). The ancestral state of the tribe was estimated by SM as non-acrescent 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).

278

279 **DISCUSSION**

280 ***Phylogenetic relationships and taxonomy of Physalideae***– We present the first well-resolved
281 and densely sampled phylogeny of the Physalideae tribe. This data set is a significant
282 expansion compared with previous studies (e.g. 33 species of *Physalis* in Zamora-Tavares et
283 al., 2016, vs 53 taxa here), and the sampling covers most of the taxonomic, morphological,
284 and geographic variation within this group. Although some parts of the tree (e.g. within
285 *Physalis*) will require additional data for better resolution, our results have recovered many
286 previously proposed relationships as well as several new ones, which we briefly review here.

287

288 Starting with the monophyletic and well-studied Iochrominae, only three out of six genera are
289 monophyletic, even after recent nomenclatural changes (Shaw, 2018a; b). The crossability
290 among genera, high convergence in traits used to delimitate generic taxonomy, and the
291 comparative lack of karyological variation (Smith and Baum, 2006; Smith et al., 2008; Shaw,
292 2018b) suggest that combining the genera into a single monophyletic *Iochroma* may be the
293 most stable solution. During the last 20 years, 19 new species of *Iochroma* and one *Saracha*
294 have been described (e.g. Leiva Gonzalez et al., 2003; Leiva González and Lezama, 2005;
295 Lezama Escobedo et al., 2007; Fernandez-Hilario and Smith, 2017) but no key for the entire
296 group has been proposed, increasing the necessity of a full taxonomic revision.

297

298 The subtribe Withaninae also presents taxonomic challenges, both at the subtribal and generic
299 levels. This subtribe was originally circumscribed by Olmstead et al. (2008) to contain seven
300 small genera, which were all Old World except for the South American *Aureliana*. Our
301 analysis provides strong support for the non-monophyly of the type genus, *Withania*, with
302 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 which have been placed in the subtribe are monophyletic (or nearly so) given extensive taxonomic work in recent years (Zamberlan et al., 2015; Deanna et al., 2018).

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*, *Schaderanthus*), 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 (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 2 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*) 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 which 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., accepted).

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 possibility 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 indicated that evolution of the inflated calyx proceeds in directional fashion, starting from the non-acrescent 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 which 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 the calyx and fruit development at early stages, wherein removal of sepals prior to fertilization completely abolish 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 which 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 Physalidae with non-acrescent, acrescent-appressed, and inflated calyxes 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 calyxes (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 (6) 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. 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 closely 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 tab for this article.

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696 TABLES

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

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TABLE 2. Phylogenetic signal metrics calculated on Physalideae species with accrescent and/or inflated calyx. Bolded values indicate the statistics that were significantly lower than the random distribution of traits or significantly greater than Brownian motion evolution ($p < 0.05$). *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

APPENDIX 1. Summary of taxon sampling, provenance, voucher (collector and number or barcode, in italics), herbarium 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, Minas Gerais, Caraça, *Barboza 3638b* (CORD), na, MH822153*, na, KX690166. *Aureliana brasiliensis* (Hunz.) Barboza & Hunz., BRAZIL, Rio de Janeiro, Itatiaia National Park, *Rodrigues et al. 106* (BHCB), KC832783, na, KC549634-KC549615, na. BRAZIL, Rio 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,

737 Paraty, *Stehmann et al.* 4856 (BHCB), KC832785, na, KC549636-KC549617, na. ***Aureliana***
738 ***fasciculata*** (Vell.) Sendtn (= *A. fasciculata* var. *fasciculata*), BRAZIL, São Paulo, Jundiá,
739 Serra do Japi, *Stehmann et al.* 4790 (BHCB), KC832786, na, KC549637-KC549618, na.
740 BRAZIL, Paraná, Morretes, La Graciosa, *Barboza et al.* 1630 (CORD), na, na, na,
741 EF537144. ***Aureliana martiana*** (Sendtn.) I. M. C. Rodrigues & Stehmann, BRAZIL, Minas
742 Gerais, Juiz de Fora, *Giacomin et al.* (BHCB), KC832787, na, KC549638-KC549619, na.
743 ***Aureliana micrantha*** Sendtn., BRAZIL, Bahia, Road São José, *Stehmann* 5064 (BHCB),
744 KC832780, na, KC549631-KC549612, na. ***Aureliana picta*** (Mart.) I.M.C. Rodrigues &
745 Stehmann, BRAZIL, São Paulo, Bananal, *Giacomin* 887 (BHCB), KC832789, na,
746 KC549640-KC549621, na. ***Aureliana pogogena*** (Moric.) I.M.C. Rodrigues & Stehmann,
747 BRAZIL, Bahia, Conservation Area Serra Bonita, Camacan, *Stehmann* 5084 (BHCB),
748 KC832790, na, KC549641-KC549622, na. BRAZIL, *Stehmann et al.* 5098 (BHCB), na,
749 MH822155*, na, MH796559*. ***Aureliana sellowiana*** (Sendtn.) Barboza & Stehmann,
750 BRAZIL, São Paulo, Parelheiros, *Rodrigues* 69 (BHCB), KC832781, na, KC549632-
751 KC549613, na. BRAZIL, São Paulo, desde Parelheiros rumbo a Eng. Marsilac, *Barboza et al.*
752 2024 (CORD), na, MH822156*, na, MH796560*. ***Aureliana sp. nov.*** (= *A. fasciculata* var.
753 *longifolia*), BRAZIL, São Paulo, Moji das Cruzes, *Stehmann* 4800 (BHCB), KC832798, na,
754 KC549649-KC549630, na. ***Aureliana tomentosa*** Sendtn. (= *A. fasciculata* var. *tomentella*),
755 BRAZIL, Espírito Santo, Santa Teresa, *Stehmann et al.* 4857 (BHCB), KC832791, na,
756 KC549642-KC549623, na. ***Aureliana velutina*** Sendtn. BRAZIL, Minas Gerais, Nova Lima,
757 *Stehmann et al.* 4543 (BHCB), KC832792, na, KC549643-KC549624, na. ***Aureliana***
758 ***wettsteiniana*** (Witasek) Hunz. & Barboza, BRAZIL, Santa Catarina, Porto União, *Thode* 300
759 (BHCB), KC832793, na, KC549644-KC549625, na. BRAZIL, Paraná, Morretes, *Barboza*
760 2020 (CORD), na, MH822157*, na, MH796561*.

- 761 ***Brachistus nelsonii*** (Fernald) D'Arcy, J.L. Gentry & Averett, MEXICO, Campeche,
762 Calakmul, Rancho El Sacrificio, *Martínez et al.* 28097 (MEXU), MH763701*, MH822158*,
763 MH752595*, MH796562*. ***Brachistus stramonifolius*** (Kunth) Miers, GUATEMALA,
764 Solola and Chimaltenango, *Williams* 41524 (DUKE), AY665845, na, na, na. MEXICO,
765 Veracruz, Xalapa, Sierra Madre Oriental, *Sousa-Peña* 738a (MEXU), na, MH822159*,
766 EU580963, na.
- 767 ***Calliphysalis carpenteri*** (Riddell) Whitson, UNITED STATES, Florida, *Whitson* 1133
768 (DUKE), AY665851, MH822160*, EU581042, MH796563*.
- 769 ***Capsicum lycianthoides*** Bitter, ECUADOR, Pichincha, Bellavista Cloud Forest Reserve,
770 *Smith* 203 (WIS), DQ314158, DQ309518, MH281754*, DQ309468.
- 771 ***Chamaesaracha arida*** Henrickson, UNITED STATES, New Mexico, Grant, San Vicente
772 Creek drainage, *Deanna et al.* 221 (COLO), MH763702*, MH822161*, MH752596*,
773 MH796564*. ***Chamaesaracha coniodes*** (Moric. ex Dunal) Benth. & Hook. f. ex B.D. Jacks.
774 & al., UNITED STATES, New Mexico, Harding, Ute Creek Valley, *Deanna et al.* 234
775 (COLO), MH763703*, MH822162*, MH752597*, MH796565*. ***Chamaesaracha***
776 ***coronopus*** (Dunal) A. Gray, UNITED STATES, Colorado, Pueblo, Lake Pueblo, *Deanna* &
777 *Carrasco* 237 (COLO, CORD), MH763704*, MH822163*, MH752598*, MH796566*.
- 778 ***Chamaesaracha crenata*** Rydb., MEXICO, Coahuila, Cepeda, Estación Marte, Talud norte,
779 *Villarreal et al.* 6646 (MEXU), MH763705*, MH822164*, MH752599*, MH796567*.
- 780 ***Chamaesaracha pallida*** Averett, MEXICO, Zacatecas, Concepción del Oro, Sierra Astillero,
781 *Villarreal & Ramírez* 9391 (MEXU), MH763706*, MH822165*, MH752600*, MH796568*.
- 782 ***Chamaesaracha rzedowskiana*** Hunz, MEXICO, Queretaro, Jalpan, Los Sarros, *López Ch.*
783 *546* (MEXU), MH763707*, MH822166*, MH752601*, MH796569*. ***Chamaesaracha***
784 ***sordida*** (Dunal) A. Gray, MEXICO, Sonora, Naco, Chihuahuan desert, *van Devender et al.*

- 785 2003-352 (MEXU), MH763708*, MH822167*, MH752602*, na. *Chamaesaracha villosa*
- 786 Rydb., UNITED STATES, Texas, Pecos, Picnic Area East of Iraan, *Deanna et al.* 211
- 787 (COLO), MH763709*, MH822168*, MH752603*, MH796570*.
- 788 *Cuatresia colombiana* Hunz., COLOMBIA, Cauca, El Tampo, PNN Munchique, *Orozco et*
- 789 *al.* 3816 (COL, CORD), MH763710*, MH822169*, MH752604*, MH796571*. *Cuatresia*
- 790 *cuneata* (Standl.) Bohs, NA, *Bohs* 2394 (UT), MH763711*, MH822170*, MH752605*,
- 791 MH796572*. *Cuatresia cuspidata* (Dunal) Hunz., COLOMBIA, Cundinamarca, Soacha,
- 792 *Deanna* 161 (CORD), MH763712*, MH822171*, MH752606*, MH796573*. *Cuatresia*
- 793 *exiguiflora* (D'Arcy) Hunz., NA., Bohs 2454 (UT), MH763713*, MH822172*, EU580981,
- 794 MH796574*. *Cuatresia foreroi* Hunz., ECUADOR, Sucumbios, from Lumbaqui to La
- 795 Bonita, *Croat & Ferry* 93692 (MO), MH763714*, na, na, na. *Cuatresia fosteriana* Hunz.,
- 796 NA, *Bohs* 2753 (UT), MH763715*, MH822173*, MH752607*, MH796575*. *Cuatresia*
- 797 *garciae* Hunz., COLOMBIA, Antioquia, Frontino, road to Murri, *Brant & Martínez* 1410
- 798 (MO), na, MH822174*, na, na. *Cuatresia plowmanii* Hunz., COLOMBIA, Bocayá, Santa
- 799 María, Calichana, La Almenara, *Orejuela et al.* 120 (COL), MH763716*, MH822175*,
- 800 MH752608*, MH796576*. *Cuatresia riparia* (Kunth) Hunz., NA, *Bohs* 2551 (UT), na,
- 801 MH822176*, EU580982, MH796577*. *Cuatresia sp.*, ECUADOR, Pichincha, Bellavista
- 802 Cloud Forest Reserve, *Smith* 204 (WIS), DQ314165, DQ301518, KM200029, DQ309475.
- 803 *Cuatresia trianae* Hunz. COLOMBIA, Caquetá, Florencia, corregimiento el Caraño,
- 804 *Trujillo & Sánchez* 3587 (HUAZ), MH763717*, MH822177*, MH752609*,
- 805 MH796578*.
- 806 *Darcyanthus spruceanus* (Hunz.) Hunz., PERU, Madre de Dios, Tambopata, Puerto
- 807 Maldonado, *Valenzuela & Huamantupa* 1011 (MO), na, na, MH752610*, na.

808 ***Deprea abra-patriciae*** (S. Leiva & Barboza) S. Leiva & Deanna, PERU, Amazonas,
809 Bongará, Área de Conservación Privada Abra-Patricia, *Deanna & Leiva González 41*
810 (CORD, HAO), KX557300, na, MH281755*, KX690167. ***Deprea altomayoensis*** (S. Leiva &
811 Quip.) Barboza & Deanna, PERU, San Martín, Rioja, Bosque de Protección Alto Mayo,
812 *Deanna & Leiva González 84* (CORD), KX557302, MH822178*, MH281756*, KX690168.
813 ***Deprea andersonii*** (N.W. Sawyer) Deanna & S. Leiva, ECUADOR, Napo, carretera Hollín-
814 Loreto, km 26.5 (Ruta E45A, Troncal amazónica), *Deanna & Leiva González 116* (CORD,
815 HAO), KX557301, MH822179*, MH281757*, KX690169. ***Deprea auccana*** S. Leiva,
816 Barboza & Deanna, PERÚ, Amazonas, Bongará, Nueva Cajamarca – Pomacochas, *Deanna*
817 *& Leiva González 44* (CORD), KX557303, MH822180*, MH281758*, KX690170. ***Deprea***
818 ***bitteriana*** (Werderm.) N.W. Sawyer & Benítez, COLOMBIA, Cundinamarca, Subachoque,
819 El Tablazo, *Orozco et al. 3871* (COL, CORD), KP267794, MH822181*, MH281760*,
820 KP267808. ***Deprea bongaraensis*** (S. Leiva) Deanna & Barboza, PERU, Amazonas, Bongará,
821 carretera Bongará-Nuevo Cajamarca, *Deanna & Leiva González 36* (CORD), KX557304,
822 MH822182*, MH281761*, KX690171. ***Deprea chotanae*** (S. Leiva, Pereyra & Barboza) S.
823 Leiva, PERU, Cajamarca, Chota, bosque El Pargo, La Loma, *Deanna & Leiva González 59*
824 (CORD), KX557305, MH822183*, MH281762*, KX690172. ***Deprea cuyacensis*** (N.W.
825 Sawyer & S. Leiva) S. Leiva & Lezama, PERU, Piura, Ayabaca, bosque de Cuyas, *Barboza*
826 *et al. 3367* (CORD), KP267793, MH822184*, MH281763*, KP267807. ***Deprea cyanocarpa***
827 Garzón & C.I. Orozco, COLOMBIA, Muñoz 2 (COL), KP267797, MH822185*,
828 MH281764*, KP267811. ***Deprea darcyana*** (N.W. Sawyer) Barboza & S. Leiva,
829 COLOMBIA, Cauca, El Tambo, Parque Nacional Munchique, *Orozco et al. 3860* (COL,
830 CORD), KX557306, na, MH281765*, KX690173. ***Deprea ecuatoriana*** Hunz. & Barboza,
831 ECUADOR, Zamora Chinchipe, Yanganá, rumbo al Cerro Toledo, *Orozco et al. 3952*
832 (CORD), KP267795, MH822186*, MH281767*, KP267809. ***Deprea glabra*** (Standl.) Hunz.,

833 COLOMBIA, Cauca, El Tambo, Parque Nacional Munchique, *Orozco et al.* 3812 (COL,
834 CORD, QCA), KP267799, MH822187*, MH281768*, KP267813. ***Deprea harlingiana***
835 (Hunz. & Barboza) Deanna & S. Leiva, ECUADOR, Zamora Chinchipe, Parque Nacional
836 Podocarpus, *Deanna & Leiva González* 12 (CORD, HAO), KX557307, MH822188*,
837 MH281769*, KX690174. ***Deprea hawkesii*** (Hunz.) Deanna, COLOMBIA, Cauca, El Tambo,
838 Parque Nacional Munchique, *Orozco et al.* 3824 (COL, CORD), KP267821, na,
839 MH281770*, KP267820. COLOMBIA, Huila, La Plata, Agua Bonita, Finca Meremberg,
840 *Orejuela & Deanna* 2568 (CORD, JBB), na, MH822189*, na, na. ***Deprea longipedunculata***
841 (S. Leiva, E. Rodr. & J. Campos) Barboza, PERU, Cajamarca, San Ignacio, Tabaconas,
842 caserío La Bermeja, *Deanna & Leiva González* 18 (CORD, HAO), KX557309, MH822190*,
843 MH281775*, KX690177. ***Deprea lutea*** (S. Leiva) Deanna, PERU, Cajamarca, Chota, km 46
844 desde desvío Llama-Huambos hacia La Granja, *Deanna & Leiva González* 68 (CORD,
845 HAO), KX557310, MH822191*, MH281779*, KX690178. ***Deprea macasiana*** (Deanna, S.
846 Leiva & Barboza) Barboza, ECUADOR, Pastaza, Macas, cerro San José del Quílamo,
847 *Deanna & Leiva González* 111 (CORD, HAO, QUSF), KX557311, MH822192*,
848 MH281780*, KX690180. ***Deprea maculatifolia*** (E. Rodr. & S. Leiva) S. Leiva, PERU,
849 Amazonas, Bagua, Imaza, Comunidad Aguaruna de Yamayakat, *Deanna & Leiva González*
850 82 (CORD, HAO), KX557313, na, MH281781*, KX690181. ***Deprea micrantha*** S. Leiva &
851 Barboza, ECUADOR, Zamora Chinchipe, Reserva Biológica San Francisco, Leiva González
852 & Barboza 6530 (CORD, HAO, LOJA), MH281823*, na, MH281776*, MH281832*.
853 ***Deprea nieva*** (S. Leiva & N.W. Sawyer) Barboza & Deanna, PERU, Amazonas, Bongará,
854 km 384, bordes de carretera Nueva Cajamarca-Pomacochas (Florida), *Deanna & Leiva*
855 *González* 46 (CORD, HAO), KP267769, MH304887*, MH281782*, KP267763. ***Deprea***
856 ***nubicola*** N.W. Sawyer, COLOMBIA, Magdalena, Ciénaga, Sierra Nevada de Santa Marta,
857 *Orejuela & Vélez* 215 (COL), KP267796, MH822193*, MH281783*, KP267810. ***Deprea***

858 *orinocensis* (Kunth) Raf., VENEZUELA, Benítez & Mancilla 7460 (MY), KP267767,
859 MH822194*, MH281784*, KP267762. ***Deprea paneroi*** Benítez & M. Martínez,
860 VENEZUELA, Benítez et al. 7454 (MY), KP267768, na, MH281785*, KP267761. ***Deprea***
861 ***parviflora*** (N.W. Sawyer & S. Leiva) S. Leiva, PERÚ, Cajamarca, Cutervo, km 1543-1544,
862 carretera Cutervo-La Capilla, Deanna & Leiva González 73 (CORD, HAO), KX557314,
863 MH822195*, MH281786*, KX690183. ***Deprea pauciflora*** Deanna, Barboza & S. Leiva,
864 ECUADOR, Zamora Chinchipe, límite del Parque Nacional Podocarpus, Deanna & Leiva
865 González 13 (CORD), KX557332, MH822196*, MH281787*, KX690182. ***Deprea pecaensis***
866 S. Leiva, Deanna & Barboza, PERU, Amazonas, Bagua, La Peca, puente El Arenal, Deanna
867 & Leiva González 49 (CORD, HAO), KX557315, MH822197*, MH281789*, KX690184.
868 ***Deprea pedrazae*** (S. Leiva & Barboza) Deanna & S. Leiva, PERU, Amazonas, Bagua, La
869 Peca, puente El Arenal, Deanna & Leiva González 48 (CORD, HAO), KX557316,
870 MH822198*, MH281788*, KX690185. ***Deprea physalidicalyx*** S. Leiva, Barboza & Deanna,
871 PERU, San Martín, San Martín, carretera Tarapoto hacia Bella Vista, Leiva González &
872 Barboza 5645 (CORD, HAO), KX557341, MH822199*, MH281790*, KX690186. ***Deprea***
873 ***pilosa*** (S. Leiva, E. Rodr. & J. Campos) Deanna, PERU, Cajamarca, San Ignacio, San José de
874 Lourdes, Estrella del Oriente, Deanna & Leiva González 32 (CORD, HAO), KX557317,
875 MH822200*, MH281791*, KX690187. ***Deprea pomacochaensis*** (S. Leiva) Barboza, PERU,
876 Amazonas, Bongará, carretera Bongará-Nueva Cajamarca, Deanna & Leiva González 33
877 (CORD, HAO), KX557318, MH822201*, MH281792*, KX690188. ***Deprea psilophyta***
878 (N.W. Sawyer) S. Leiva & Deanna, ECUADOR, Loja, Nudo de Sabanilla, sendero a
879 Ayupallas, Orozco et al. 3947 (COL, CORD), na, na, MH281793*, na. ECUADOR, Zamora
880 Chinchipe, carretera desde Yanganá hacia Valladolid, Sawyer 770 (CONN, LOJA),
881 KP267772, na, na, KP267766. ***Deprea pumila*** (S. Leiva, Barboza & Deanna) S. Leiva,
882 ECUADOR, Pastaza, Mera, camino al río Anzú, Orozco et al. 3890 (COL, CORD, QCA),

883 KX557320, MH304886*, MH281794*, KX690189. *Deprea purpurea* (S. Leiva) Barboza &
884 S. Leiva, PERU, Cajamarca, San Ignacio, San José de Lourdes, Estrella del Oriente, *Deanna*
885 & *Leiva González* 27 (CORD, HAO), KX557319, MH822202*, MH281795*, KX690192.
886 *Deprea purpureocarpa* (S. Leiva, Deanna & Barboza) Deanna, ECUADOR, Napo, carretera
887 Cosanga-Baeza, 5.4 km al sur de Baeza, Deanna & Leiva González 125 (CORD, HAO,
888 QCNE), KX557321, MH822203*, MH281800*, KX690193. *Deprea sachapapa* (Hunz.) S.
889 Leiva & Deanna, ECUADOR, Cotopaxi, San Francisco de las Pampas, Otonga, *Orozco et al.*
890 3985 (COL, CORD, QCA), KX557328, na, MH281796*, KX690197. ECUADOR,
891 Pichincha, *Smith* 205 (WIS), na, DQ301519, na, na. *Deprea sagasteguii* (S. Leiva, Quip. &
892 N.W. Sawyer) Barboza, PERU, Piura, Ayabaca, cerro Aypate, *Deanna & Leiva González* 97
893 (CORD, HAO), KX557330, MH822204*, MH281797*, KX690200. *Deprea sapalachensis*
894 S. Leiva & Barboza, PERU, Piura, Huancabamba, Carmen de la Frontera, *Barboza & Leiva*
895 *González* 4833 (CORD, HAO), na, na, MH752611*, MH796579*. *Deprea sawyeriana* (S.
896 Leiva, E. Rodr. & J. Campos) S. Leiva, PERU, Cajamarca, San Ignacio, Tabaconas, caserío
897 La Bermeja, *Deanna & Leiva González* 14 (CORD, HAO), KX557331, MH822205*,
898 MH281798*, KX690202. *Deprea sp.*, ECUADOR, Pastaza, Mera, desde la Plaza Mayor de
899 Mera hacia Cavernas del Río Anzú, *Deanna et al.* 114 (CORD), MH763718*, na,
900 MH752612*, na. *Deprea steyermarkii* (Hunz.) S. Leiva & Barboza, ECUADOR, Azuay,
901 carretera Gualaceo-Indanza, km 23, *Deanna & Leiva González* 108 (CORD, HAO),
902 KX557335, MH822206*, MH281803*, KX690203. *Deprea subtriflora* (Ruiz & Pav.)
903 D'Arcy, BOLIVIA, La Paz, Nor-Yungas, carretera desde Chuspipata a Coroico, *Barboza &*
904 *Leiva González* 3663 (CORD), KP267770, MH822207*, MH281805*, KP267764. *Deprea*
905 *sylvarum* (Standl. & C.V. Morton) Hunz., COSTA RICA, *Bohs* 2504 (UT), KP267800, na,
906 MH281806*, KP267814. *Deprea teresitae* Deanna & Orejuela, COLOMBIA, Valle del
907 Cauca, Reserva 'El Refugio', *Deanna & Calderón* 169 (PSO, CORD), MH281825*, na,

- 908 MH281801*, MH281833*. *Deprea toledoana* (Barboza & S. Leiva) Barboza, ECUADOR,
909 Zamora Chinchipe, a Valladolid desde Yanganá, *Orozco et al.* 3936 (COL, CORD, QCA),
910 KX557337, MH822208*, MH281807*, KX690205. *Deprea vasquezii* (S. Leiva, E. Rodr. &
911 J. Campos) Deanna, PERU, Cajamarca, San Ignacio, San José de Lourdes, Estrella del
912 Oriente, *Deanna & Leiva González* 28 (CORD, HAO), KX557339, MH822209*,
913 MH281808*, KX690207. *Deprea zakii* Barboza, S. Leiva & Deanna, ECUADOR, Napo,
914 Quijos, carretera Papallacta-Cuyuja, *Deanna et al.* 138 (CORD, QCNE), KX557340,
915 MH822210*, MH281802*, KX690208. *Deprea zamorae* Barboza & S. Leiva, ECUADOR,
916 Zamora Chinchipe, Parque Nacional Podocarpus, *Orozco et al.* 3926 (COL, CORD, QCA),
917 KP267792, MH822211*, MH281809*, KP267806.
- 918 *Discopodium penninervium* Horchst., TANZANIA, *Tanner* 3288, KC832794, MH822212*,
919 na, na. UGANDA, Kabarole, Burahya, *Knapp* 9808 (BM), na, na, EU580986, na.
- 920 *Dunalia brachyacantha* Miers, ARGENTINA, Jujuy, Valle Grande, *Nee & Bohs* 50811
921 (NY), DQ314172, DQ301527, MH281810*, DQ309482. *Dunalia obovata* (Ruiz & Pav.)
922 Dammer, PERU. Junin, *Smith et al.* 458 (HAO, F, MO, NY, USM, WIS), DQ314192,
923 DQ301547, MH281811*, MDQ309499. *Dunalia spathulata* Ruiz & Pav.) Braun & Bouché,
924 PERU, Huanuco, *Smith et al.* 452 (HAO, F, MO, NY, USM, WIS), DQ314198, DQ301554,
925 MH752613*, DQ309506. *Dunalia spinosa* (Meyen) Dammer, BOLIVIA, Potosí, Tomas
926 Frias, *Smith et al.* 379 (MO, WIS) DQ314188, DQ301543, MH281812*, DQ309495.
- 927 *Eriolarynx fasciculata* (Miers) Hunz., BOLIVIA, Cochabamba, *Smith et al.* 432 (HAO, F,
928 MO, NY, WIS), DQ314196, DQ301552, MH752614*, DQ309504. *Eriolarynx iochromoides*
929 (Hunz.) Hunz., ARGENTINA, Catamarca, Andalgalá, Río Potrero, *Barboza et al.* 1966
930 (CORD), KP267802, MH304888*, MH281813*, KP267816. *Eriolarynx australis* (Griseb.)
931 J.M.H Shaw, BOLIVIA, Chuquisaca, *Smith et al.* 390 (WIS), DQ314189, DQ301544,

- 932 KP756712, DQ309496. *Eriolarynx lorentzii* (Dammer) Hunz., ARGENTINA, Tucumán,
 933 *Hawkes et al.* 3452 (BIRM), DQ314171, DQ301525, KP756713, DQ309481.
- 934 *Iochroma amicum* M. Cueva, S.D. Sm. & S. Leiva, PERU, Oxapampa, Huancabamba, PN
 935 Yanachaga-Chemillen, *Smith* 542 (HAO, HOXA, MO, USM), KM514683, KM514684,
 936 MH752615*, KM521199. *Iochroma arborescens* (L.) J.M.H. Shaw, COSTA RICA,
 937 Puntarenas, Las Cruces, *Bohs* 2428 (UT), DQ314173, DQ301528, KP756700, DQ309483.
- 938 *Iochroma ayabacense* S. Leiva, PERU, Piura, Ayabaca, *Smith & Leiva González* 337A
 939 (HAO, F, MO, WIS), DQ314194, DQ301549, MH752616*, DQ309501. *Iochroma barbozae*
 940 S. Leiva & Deanna, PERU, Piura, Ayabaca, *Deanna et al.* 91 (CORD), MH763719*,
 941 MH822213*, MH752617*, MH796581*. *Iochroma baumii* S.D. Sm. & S. Leiva,
 942 ECUADOR, Napo, Papallacta, *Smith & López* 476 (QCNE, F, WIS), DQ314202, DQ301558,
 943 MH752618*, DQ309513. *Iochroma calycinum* Benth., ECUADOR, Pichincha, *Smith* 471
 944 (F, QCNE, WIS), DQ314201, DQ301557, MH281815*, DQ309512. *Iochroma*
 945 *confertiflorum* (Miers) Hunz., ECUADOR, Loja, *Smith et al.* 237 (QCNE, MO, WIS),
 946 DQ314176, DQ301531, MH752619*, DQ309486. *Iochroma cornifolium* (Kunth) Miers,
 947 ECUADOR, Loja, *Smith et al.* 242 (QCNE, MO, WIS), DQ314177, DQ301532,
 948 MH752620*, DQ309487. *Iochroma cyaneum* (Lindl.) G.H.M. Lawr. & J.M. Tucker,
 949 ECUADOR, Loja, Catamayo-El Cisne road, *Smith* 223 (QCNE, MO, WIS), DQ314180,
 950 DQ301535, MH281814*, DQ309490. *Iochroma edule* S.Leiva, PERU, La Libertad, *Smith et*
 951 *al.* 300 (HAO, F, MO, NY, USM, WIS), DQ314193, DQ301548, KP756703, DQ309500.
- 952 *Iochroma ellipticum* (Hook. f.) Hunz., ECUADOR, Galápagos, *Jager* 622 (CDS),
 953 DQ314199, DQ301555, MH752622*, DQ309507. *Iochroma fuchsoides* (Bonpl.) Miers,
 954 ECUADOR, Azuay, *Smith & López* 488 (QCNE, F, MO, WIS), DQ314203, DQ301559,
 955 KP756711, DQ309514. *Iochroma gesnerioides* (Kunth) Miers, ECUADOR, Pichincha,
 956 Reserva Geobotanica Pululahua, *Smith* 200 (QCNE, MO, WIS), DQ314179, DQ301534,

957 MH281816*, DQ309489. *Iochroma lehmannii* Dammer ex Bitter, ECUADOR, Cañar, *Smith*
958 & López 484 (QCNE, F, MO, WIS), DQ314200, DQ301556, MH752623*, DQ309511.
959 *Iochroma loxense* (Kunth) Miers, ECUADOR, Loja, *Smith* 220 (QCNE, MO, WIS),
960 DQ314175, DQ301530, MH752624*, DQ309485. *Iochroma nitidum* S. Leiva & Quip.,
961 PERU, Amazonas, *Smith et al.* 371 (HAO, F, MO, NY, USM, WIS), DQ314168, DQ301521,
962 MH752625*, DQ309478. *Iochroma parvifolium* (Roem. & Schult.) D'Arcy, PERU, La
963 Libertad, *Smith et al.* 303 (HAO, F, MO, NY, USM, WIS), DQ314195, DQ301551,
964 MH752626*, DQ309503. *Iochroma peruvianum* (Dunal) J.F. Macbr., PERU, Cajamarca,
965 *Smith & Hall* 353 (HAO, F, MO, NY, USM, WIS), DQ314197, DQ301553, KP756706,
966 DQ309505. *Iochroma piuranum* S. Leiva, PERU, Piura, Ayabaca, *Deanna et al.* 93 (CORD),
967 MH763721*, MH822215*, MH752627*, MH796582*. *Iochroma salpoanum* S. Leiva &
968 Lezama, PERU, La Libertad, *Smith* 364 (WIS), DQ314187, DQ301542, MH752628*,
969 DQ309509. *Iochroma squamosum* S. Leiva & Quip., PERU, Piura, Ayabaca, *Smith et al.*
970 330 (HAO, F, MO, NY), DQ314186, DQ301541, MH281817*, DQ309494. *Iochroma*
971 *stenanthum* S. Leiva, Quip. & N.W. Sawyer, PERU, Cajamarca, *Smith et al.* 313 (HAO, F,
972 MO, NY, USM, WIS), DQ314184, DQ301539, MH752629*, DQ309508. *Iochroma*
973 *tingoanum* S. Leiva, PERU, Amazonas, *Smith et al.* 370 (HAO, F, MO, NY, USM, WIS),
974 DQ314167, DQ301520, MH752630*, DQ309477. *Iochroma tupayachianum* S. Leiva,
975 PERU, La Libertad, *Smith et al.* 526 (F, MO, USM, WIS), KC290442, KC290441, na,
976 KC243428.
977 *Leucophysalis grandiflora* (Hook.) Rydb., UNITED STATES, *Olmstead* S-30 (WTU),
978 DQ314162, DQ301515, EU581013, DQ309472. *Leucophysalis nana* (A. Gray) Averett,
979 UNITED STATES, *Bartholomew* 5994 (MO), MH763722*, na, EU581014, na.
980 *Lycianthes inaequilatera* Bitter, ECUADOR, Pichincha, Alluriquin, *Smith* 210 (WIS),
981 DQ314159, DQ309519, na, DQ309469. BOLIVIA, *Bohs* 3089 (UT), na, na, EU581018, na.

- 982 *Nothocestrum breviflorum* A. Gray, HAWAII, Hamakua, Kailikaula Cliffs and Stream,
 983 *Wood et al.* 4862 (MO), MH763723*, MH822216*, MH752631*, MH796583*.
- 984 *Nothocestrum latifolium* A. Gray, HAWAII, Polynesia Hawaiian Islands, *H. St. John* 24469,
 985 KC832796, na, na, na. HAWAII, *Herbst et al.* 725 (COLO), na, na, EU581037, na. HAWAII,
 986 *Lorentz* 9063, na, MH822217*, na, MH796584*. *Nothocestrum longifolium* A. Gray,
 987 HAWAII, Main Hawaiian Islands, North Hilo, *Cuddihy* 743 (BISH), KC832795, na, na, na.
 988 HAWAII, *Oppenheimer s.n.* (BISH), na, MH822218*, EU581038, MH796585*.
- 989 *Nothocestrum peltatum* Skotts., HAWAII, Honopu, NW of Kainamanu, *Acacia koa*, *Wood*
 990 *& Query* 15166 (MO), na, MH822219*, MH752632*, MH796586*.
- 991 *Oryctes nevadensis* S. Watson, UNITED STATES, Nevada, Churchill, *Tiehm* 11982 (COLO,
 992 TEX), AY665864, na, EU581039, na.
- 993 *Physaliastrum echinatum* (Yatabe) Makino, CHINA, Yunyougu, Xinchengzi Town, Miyun
 994 District, Beijing, *Liu & Shi* 5186 (PE), MH763724*, MH822220*, MH752633*,
 995 MH796587*. *Physaliastrum japonicum* (Franch. & Sav.) Honda, NA, *YYZWF* 20387,
 996 KP894015, na, na, na. *Physaliastrum heterophyllum* (Hemsl.) Migo, CHINA, Zhejiang West
 997 Tianmu Mountain, *Li et al.* 435 (HSNU), KC768878, na, KC768880, na. *Physaliastrum*
 998 *sinense* (Hemsl.) D'Arcy & Z.Y. Zhang, CHINA, Sichuan, *Hungui* 1177 (MO), na,
 999 MH822221*, na, na.
- 1000 *Physalis acutifolia* (Miers) Sandwith, UNITED STATES, Arizona, *Cpcjose*, *Makings* 3742
 1001 (MO), na, MH822222*, MH752634*, MH796588*. UNITED STATES, cultivated, *NIJ*
 1002 *974750059*, AY665876, na, na, na. *Physalis angulata* L., ARGENTINA, Córdoba, Río Seco,
 1003 Ruta Nac. N° 9, pasando Va. de María, *Morero* 365 (CORD), MH763725*, MH822223*,
 1004 MH752635*, MH796589*. *Physalis angustifolia* Nutt., UNITED STATES, Florida,
 1005 Okalossa, *Miller et al.* 9107 (MO), na, MH822224*, MH752636*, na. UNITED STATES,

1006 Florida, *Whitson, no voucher*, AY665878, na, na, na. *Physalis angustiphysa* Nutt., MEXICO,
1007 Chiapas, *Ton 9286* (TEX), AY665879, na, na, na. *Physalis arenicola* Kearney, UNITED
1008 STATES, Florida, Putnam, Ordway-Swisher Biological Station, *Majure et al. 5075* (FLAS),
1009 na, MH822225*, MH752637*, MH796590*. UNITED STATES, Florida, *Whitson, no*
1010 *voucher*, AY665880, na, na, na. *Physalis campanula* Standl. & Steyerl., MEXICO,
1011 Veracruz, *Ventura 4882* (MO), AY665882, na, na, na. *Physalis campechiana* L., MEXICO,
1012 Tamaulipas, *Jimenez 454* (TEX), AY665867, MH822226*, MH752638*, MH796591*.
1013 *Physalis caudella* Standl., MEXICO, Chihuahua, *Quintana 3075* (TEX), AY665891, na, na,
1014 na. *Physalis chenopodifolia* Lam., MEXICO, México, Pirámides de Teotihuacan, *Chiarini et*
1015 *al. 1277* (CORD), na, MH304893*, MH752639*, MH304879*. UNITED STATES,
1016 cultivated, *Whitson 1287* (DUKE), AY665883, na, na, na. *Physalis cinerascens* (Dunal)
1017 Hitchc. **var. cinerascens**, UNITED STATES, Texas, Comal, Schmucks and Doeppens,
1018 roadsides, *Deanna et al. 206* (COLO, CORD), MH763726*, MH822227*, MH752640*,
1019 MH796592*. *Physalis cinerascens var. spathulifolia* (Torr.) J.R. Sullivan, UNITED
1020 STATES, Texas, Colorado, East to the Attwater Prairie Chicken National Wildlife Refuge,
1021 *Deanna et al. 203* (COLO), MH763727*, MH822228*, MH752641*, MH796593*. *Physalis*
1022 *cordata* Mill., PERU, Cajamarca, Contumazá, *Knapp et al. 10557* (CORD), MH763728*,
1023 MH822229*, MH752642*, MH796594*. *Physalis coztomatl* Dunal, MEXICO, *Garcia 264*
1024 (MO), AY665887, na, na, na. *Physalis crassifolia* Benth., UNITED STATES, California,
1025 *Sharples 744* (COLO), MH763729*, MH822230*, MH752643*, MH796595*. *Physalis x*
1026 *elliottii* Kunze, UNITED STATES, Florida, Sanibel Island, Bailey Tract, *Wheeler 14144* (SI),
1027 na, MH822231*, MH752644*, MH796596*. *Physalis fendleri* A. Gray, UNITED STATES,
1028 New Mexico, Grant, outside of Silver City, *Deanna et al. 219* (COLO), MH763730*,
1029 MH822232*, MH752645*, MH796597*. *Physalis glabra* Benth., MEXICO, Baja California
1030 Sur, La Paz, *Provance et al. 8003* (MO), MH763731*, na, MH752646*, na. *Physalis*

- 1031 *glutinosa* Schlecht., MEXICO, Durango, Sikes 375 (TEX), AY665892, na, na, na. *Physalis*
- 1032 *greenmanii* Waterf., MEXICO, Veracruz, Nee 22432 (MO), AY665893, na, na, na.
- 1033 MEXICO, Veracruz, Villa Aldama, Nee 32880 (CORD), na, MH822233*, na, na. *Physalis*
- 1034 *grisea* (Waterf.) M. Martínez, UNITED STATES, cultivated, NIJ 894750256, AY665915, na,
- 1035 na, na. *Physalis hederifolia* A. Gray, UNITED STATES, Texas, Uvalde, dry Frio River,
- 1036 *Deanna et al.* 209 (COLO), MH763732*, MH822234*, MH752647*, MH796598*. *Physalis*
- 1037 *heterophylla* Nees, UNITED STATES, Colorado, Larimer, Lory State Park, *Deanna et al.*
- 1038 199 (COLO), na, MH822235*, na, MH796599*. UNITED STATES, North Carolina,
- 1039 Caswell, Whitson, no voucher, AY665907, na, na, na. UNITED STATES, Olmstead S-64
- 1040 (WTU), na, na, EU581043, na. *Physalis hintonii* Waterf., MEXICO, Nuevo Leon, Villarreal
- 1041 4909 (MO), AY665895, na, na, na. *Physalis ignota* Britton, MEXICO, Chiapas, Breedlove
- 1042 52891 (MO), AY665897, na, na, na. *Physalis ixocarpa* Brot. ex Hornem., UNITED
- 1043 STATES, cultivated, *Deanna* 251 (CORD), MH763733*, MH822236*, MH752648*,
- 1044 MH796600*. *Physalis lagascae* Roem. & Schult., PERU, Cajamarca, Cutervo, Särkinen
- 1045 4548 (BM), na, MH304892*, MH752649*, MH304880*. *Physalis lanceolata* Michx.,
- 1046 UNITED STATES, North Carolina, Scotland, *Horn* 1133 (DUKE), AY665899, na, na, na.
- 1047 *Physalis lassa* Stand. & Steyerl., MEXICO, Comala, Sanders 11807 (MO), AY665900, na,
- 1048 na, na. *Physalis longifolia* Nutt., UNITED STATES, New Mexico, Bernalillo, Albuquerque,
- 1049 *Deanna et al.* 227 (COLO), na, MH822237*, MH752650*, MH796601*. UNITED STATES,
- 1050 Kansas, Riley, Whitson s.n. (DUKE 358627), AY665901, na, na, na. *Physalis macrosperma*
- 1051 ined. UNITED STATES, Arkansas, Miller, Gentry & Reid 3188, MH763734*, MH822238*,
- 1052 MH752651*, na. *Physalis melanocystis* (B.L. Rob.) Bitter, MEXICO, Tamaulipas, Martinez
- 1053 1940 (MO), AY665865, MH822239*, MH752652*, MH796602*. *Physalis microcarpa* Urb.,
- 1054 MEXICO, Chihuahua, Laferriere 1661 (MO), AY665903, na, na, na. *Physalis microphysa*
- 1055 A. Gray, MEXICO, Coahuila, Henrickson 11850 (TEX), AY665859, MH822240*,

1056 MH752653*, MH796603*. *Physalis minima* L., AUSTRALIA, South Australia, cultivated,
1057 *Symon 14813* (CORD), na, MH822241*, na, na. THAILAND, cultivated, *NIJ 974750167*,
1058 AY665904, na, na, na. *Physalis minimaculata* Waterf., MEXICO, Oaxaca, *Mayfield 986*
1059 (TEX), AY665906, na, na, na. *Physalis mollis* Nutt., UNITED STATES, Texas, Bastrop,
1060 *Deanna et al. 201* (COLO), na, MH822242*, MH752654*, MH796604*. *Physalis*
1061 *neomexicana* Rydb., UNITED STATES, New Mexico, Santa Fe, *Deanna et al. 228* (COLO),
1062 MH763735*, MH822243*, MH752655*, MH796605*. *Physalis nicandroides* Schltdl.,
1063 MEXICO, Veracruz, Acultzingo, *Nee 33132* (CORD), na, MH822244*, na, MH796606*.
1064 MEXICO, Morelos, *Hernandez 2488* (MO), AY665912, na, na, na. *Physalis orizabae* Dunal,
1065 MEXICO, Morelos, Lagunas de Zempoala, *Chiarini et al. 1280* (CORD), MH763736*,
1066 MH822245*, MH752656*, MH796607*. *Physalis patula* Mill., MEXICO, Ciudad de
1067 México, *Chiarini et al. 1273* (CORD), na, MH822246*, MH752657*, MH796608*.
1068 MEXICO, Veracruz, *Nee 32810* (MO), AY665913, na, na, na. *Physalis peruviana* L.,
1069 ECUADOR, Pichincha, cultivated, *Smith 217* (WIS), DQ314161, DQ301514, na, DQ309471.
1070 PERU, *Olmstead S-69* (WTU), na, na, EU581044, na. *Physalis philadelphica* Lam.,
1071 UNITED STATES, cultivated, *Bohs 2433* (UT), na, MH822247*, EU581045, MH796609*.
1072 UNITED STATES, cultivated, *Whitson s.n.* (DUKE), AY665871, na, na, na. *Physalis*
1073 *pruinosa* var. *argentina* J. M. Toledo & Barboza, ARGENTINA, Jujuy, Valle Grande, Ruta
1074 P.N. Calilegua-San Francisco-Valle Grande, *Smith & Chiarini 630* (COLO), na,
1075 MH822248*, MH752658*, MH796610*. *Physalis pubescens* L. var. *pubescens*, MEXICO,
1076 Morelos, Lagunas de Zempoala, *Chiarini et al. 1281* (CORD), na, MH304895*,
1077 MH752659*, MH304881*. COSTA RICA, La Selva Biological Station, *Whitson 3* (DUKE),
1078 AY665916, na, na, na. *Physalis pubescens* var. *higrophyla* (Mart.) Dunal, ARGENTINA,
1079 Jujuy, Ledesma, Libertador Gral. San Martín, *Toledo 1652* (CORD), MH763737*,
1080 MH822249*, MH752660*, MH796611*. *Physalis pumila* Nutt. var. *pumila*, UNITED

- 1081 STATES, New Mexico, San Miguel, Sangre de Cristo Mountains, *Deanna et al.* 230
- 1082 (COLO), MH763738*, MH822250*, MH752661*, MH796612*. ***Physalis pumila* var.**
- 1083 ***hispida*** (Waterf.) W.F. Hinton, UNITED STATES, Colorado, Larimer, next to Poudre River,
- 1084 *Deanna et al.* 200 (COLO), MH763739*, MH822251*, MH752662*, MH796613*. ***Physalis***
- 1085 ***purpurea*** Wiggins, BOLIVIA, La Paz, Sud-Yungas, *Barboza* 3657 (CORD), MH763740*,
- 1086 MH822252*, MH752663*, MH796614*. ***Physalis solanacea*** (Schltdl.) Axelius, MEXICO,
- 1087 Tamaulipas, Llera de Canales, *Nee & Calzada* 33199 (CORD), na, MH822253*, na,
- 1088 MH796615*. MEXICO, cultivated, *Olmstead S-37* (WTU), AY665877, na, EU581025, na.
- 1089 ***Physalis sordida*** Fernald, MEXICO, Nuevo Leon, *Hinton* 18464 (TEX), AY665869, na, na,
- 1090 na. ***Physalis subilsiana*** J.M. Toledo ARGENTINA, Salta, General José de San Martín,
- 1091 *Toledo & Domínguez* 226 (CORD), na, MH822254*, na, na. ***Physalis sulphurea*** (Fernald)
- 1092 Waterf., MEXICO, 1 km al N de San Juan Citlaltepec, *Rodríguez García* 116 (CORD),
- 1093 MH763741*, na, na, na. ***Physalis victoriana*** J.M. Toledo, ARGENTINA, Jujuy, Ruta
- 1094 Provincial N° 1, de Caimancito a Palma Sola, *Carrizo García* 5 (CORD), MH763742*, na,
- 1095 MH752664*, MH796616*. ***Physalis virginiana*** Mill., UNITED STATES, Colorado,
- 1096 Boulder, *Deanna & Smith* 238 (COLO), MH763743*, MH822255*, MH752665*,
- 1097 MH796617*. ***Physalis viscosa*** L., ARGENTINA, Córdoba, Calamuchita, *Deanna &*
- 1098 *Tamborini* 179 (CORD), na, MH304894*, MH752666*, MH304882*. UNITED STATES,
- 1099 cultivated, *Whitson* 1282 (DUKE), AY665870, na, na, na. ***Physalis walteri*** Nutt., UNITED
- 1100 STATES, Florida, Levy, Havens Island, *Majure* 3051 (FLAS), na, MH822256*,
- 1101 MH752667*, MH796618*. UNITED STATES, Florida, *Whitson*, no voucher, AY665918,
- 1102 na, na, na.
- 1103 ***Quincula lobata*** (Torr.) Raf., UNITED STATES, New Mexico, Harding, *Deanna et al.* 235
- 1104 (COLO), MH763744*, MH822257*, MH752668*, MH796619*.

- 1105 *Salpichroa tristis* Miers, BOLIVIA, Potosí, Tomas Frias, *Smith et al.* 382 (HAO, F, MO, NY,
1106 WIS), DQ314160, DQ309520, MH281774*, DQ309470.
- 1107 *Saracha andina* Rob. Fernandez, I. Revilla & E. Pariente, PERU, Ayacucho, Lucanas, *Smith*
1108 & *Fernandez* 594 (COLO, F, MO, USM), KY172041, KY172040, na, KY172039. *Saracha*
1109 *nigribaccata* J.M.H. Shaw, ECUADOR, Pichincha, *Smith* 211A (QCNE, MO, WIS),
1110 DQ314174, DQ301529, EU580988, DQ309484. *Saracha punctata* Ruiz & Pav., BOLIVIA,
1111 La Paz, Nor Yungas, Rio Unduavi, *Nee* 51804 (MO, NY), DQ314182, DQ301537,
1112 KP756709, DQ309492. *Saracha quitensis* (Hook.) Miers, ECUADOR, Napo, Laguna de
1113 Papallacta, *Smith* 257 (QCNE, MO, WIS), DQ314178, DQ301533, MH281777*, DQ309488.
- 1114 *Schraderanthus viscosus* (Schrad.) Averett, MEXICO, Oaxaca, *Torres* 7932 (MO),
1115 AY665848, na, na, na.
- 1116 *Trozelia grandiflora* (Benth.) J.M.H. Shaw, PERU, Cajamarca, *Smith et al.* 320A (HAO, F,
1117 MO, NY, USM, WIS), DQ314170, DQ301523, MH752669*, DQ309480. *Trozellia*
1118 *umbellata* (Ruiz & Pav.) Raf., PERÚ, La Libertad, *Smith et al.* 301 (HAO, F, NY, USM,
1119 WIS), DQ314169, DQ301522, MH281818*, DQ309479.
- 1120 *Tubocapsicum anomalum*, CHINA, *Chen* 231 (MO), DQ314163, DQ301516, EU581066,
1121 DQ309473.
- 1122 *Tzeltalia amphitricha* (Bitter) E. Estrada & M. Martínez, MEXICO, Chiapas, *Martínez*
1123 20523 (TEX), AY665853, na, na, na. *Tzeltalia calidaria* (Standl. & Steyerm.) E. Estrada &
1124 M. Martínez, GUATEMALA, *Lundell* 19625 (TEX), na, na, MH752670*, na. *Tzeltalia*
1125 *esenbeckii* M. Martínez & O. Vargas, MEXICO, Chiapas, La Independencia, from Las
1126 Margaritas to Campo Alegre, *Breedlove* 51325 (MEXU), MH763745*, na, MH752671*, na.

- 1127 ***Vassobia breviflora*** (Sendtn.) Hunz., BOLIVIA, Chuquisaca, *Smith 412* (WIS), DQ314190,
1128 DQ301545, MH281819*, DQ309497. ***Vassobia dichotoma*** (Rusby) Bitter, BOLIVIA, *Nee*
1129 *et al. 51797* (UT), na, na, EU581067, na. BOLIVIA, La Paz, *Smith 440* (WIS), DQ314191,
1130 DQ301546, na, DQ309498.
- 1131 ***Withania adpressa*** Cors., MORROCCO, *Lewalle 13205* (MO), na, na, MH752672*,
1132 MH796620*. ***Withania aristata*** Pauq., SPAIN, Canary Islands, *del Arco s.n.* (CORD),
1133 MH763746*, MH822258*, MH752673*, MH796621*. ***Withania coagulans*** (Stocks) Dunal,
1134 CENTRAL ASIA, *Olmstead S-109* (WTU), na, MH822259*, EU581068, MH796622*.
- 1135 ***Withania frutescens*** (L.) Pauquy, MOROCCO, Beldevere de Chicht, 15 km N of Essaouira,
1136 *Miller et al. 335* (MO), na, MH822260*, na, na. ***Withania riebeckii*** Balf. f., NA., *D'Arcy*
1137 *17750* (MO), na, MH822261*, KC549645-KC549626, MH796623*. ***Withania somnifera***
1138 (L.) Dunal, NA, *Whitson 1262* (KNK), na, MH304890*, na, MH304884*. NA, *Lester S.*
1139 *0960*, KC832797, na, na, na. SPAIN, Canary Is., Mediterranean to Central Asia, *Whitson s.n.*
1140 (KNK), na, na, EU581069, na.
- 1141 ***Witheringia asterotricha*** (Standl.) Hunz., COSTA RICA, *Bohs 3007* (UT), MH763747*,
1142 MH822262*, MH752674*, MH796624*. ***Witheringia coccoloboides*** (Dammer) Hunz.
1143 COSTA RICA, *Bohs 2568* (UT), MH281826*, MH304889*, MH752675*, MH304885*.
- 1144 ***Witheringia correana*** D'Arcy, PANAMA, Bocas del Toro, Fortuna, *D'Arcy 16415* (MO),
1145 MH763748*, MH822263*, MH752676*, MH796625*. ***Witheringia killipiana*** Hunz.,
1146 COLOMBIA, Cauca, El Tambo, *Orozco et al. 3858* (COL, CORD), MH763749*,
1147 MH822264*, MH752677*, MH796626*. ***Witheringia macrantha*** (Standl. & C.V. Morton)
1148 Hunz., COSTA RICA, Monteverde, *Bohs 2512* (UT), AY665857, MH822265*, EU581071,
1149 MH796627*. ***Witheringia meiantha*** (Donn. Sm.) Hunz., COSTA RICA, *Bohs 3015* (UT),
1150 AY665856, MH822266*, EU581072, MH796628*. ***Witheringia mexicana*** (B.L. Rob.)
1151 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.

APPENDICES 2-10: uploaded in separate files.

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-acrescent 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.

1177

1178 **FIGURE 2.** Phylogenetic relationships of Physalideae based on a maximum likelihood
 1179 analysis of the combined dataset of four markers (ITS, LFY, *trnL-F*, and *waxy*). Bootstrap
 1180 support (BS) values > 60 % are given above each branch, and bold numbers indicate BS > 80
 1181 %. Differentially coloured branches correspond to the subtribes proposed by Olmstead et al.
 1182 (2008) and followed by Särkinen et al. (2013).

1183

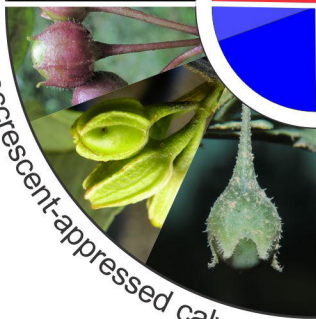
1184 **FIGURE 3.** Reconstruction of fruiting calyx evolution in the Physalideae tribe. Topology is
 1185 provided from four-gene BEAST analyses of 219 taxa. Circles at nodes indicate the posterior
 1186 probabilities from stochastic mapping and tip label colors represent tip states, with red, blue
 1187 and black representing inflated, accrescent-appressed, and non-acrescent fruiting calyces,
 1188 respectively. On the bottom, transitions between states are represented with arrows
 1189 proportional to number of estimated changes (see Appendix S10).

1190

inflated calyx



accrescent-appressed calyx



non-accrescent calyx



