

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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6 Rocío Deanna^{1,2,3,4}, Maximilien D. Larter², Gloria E. Barboza^{1,2,3}, Stacey D. Smith²

7 ¹Instituto Multidisciplinario de Biología Vegetal, IMBIV (CONICET-UNC). CC 495,

8 Córdoba 5000, Argentina.

9 ²Department of Ecology and Evolutionary Biology, University of Colorado, Boulder,

10 Colorado 80305, United States of America.

11 ³Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas (FCQ, UNC).

12 Medina Allende s.n., Córdoba 5000, Argentina.

13 ⁴Corresponding author. E-mail: rociodeanna@gmail.com

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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,

79 1990; Padmaja et al., 2014), although it is best known from the tomato family, Solanaceae,
80 where it is referred to as a ‘chinese-lantern’ fruit or, more formally, the ‘inflated calyx
81 syndrome’ (ICS; He et al., 2004; He and Saedler, 2005; Wang et al., 2015). This enlarged
82 fruiting calyx has been proposed to aid in dispersal by acting as a tumbleweed (Knapp, 2002)
83 or by providing flotation in flooded environments (Wilf et al., 2017). Pre-dispersal, the
84 inflated calyx may also serve to protect the developing fruit from predators as well as from
85 desiccation (Cedeño and Montenegro, 2004; Riss, 2009).

86 The evolution and development of inflated calyces has been studied in detail in only one
87 clade, the tomatillos and their allies (tribe Physalideae, Solanaceae). Using comparative gene
88 expression studies and transformation experiments, He and Saedler (2005) demonstrated that
89 expression of a MADS-box transcription factor (*MPF2*) is required for the development of
90 the dramatic inflated calyx in *Physalis*, and that overexpression of this gene in tomato can
91 induce some degree of fruiting calyx accrescence. Subsequent studies across Physalideae
92 revealed that many taxa which lack inflated calyces express *MPF2*, indicating that additional
93 factors are required for development of the trait (Hu and Saedler, 2007). These and
94 subsequent authors suggested that, given the shared expression of *MPF2* across Physalideae,
95 ICS could be the ancestral state with multiple subsequent losses (Hu and Saedler, 2007;
96 Zhang et al., 2012). Nonetheless, progress in reconstructing the history of gains and losses of
97 this morphological innovation has been hampered by the sparse taxon sampling of
98 Physalideae in existing phylogenies, which include only 37 % of the extant taxa (Särkinen et
99 al., 2013).

100 In the present study, we aim to elucidate the evolutionary history of fruiting calyx inflation in
101 Physalideae with a greatly expanded phylogeny and statistical comparative analyses of
102 character transitions. This tribe contains the highest generic-level diversity in Solanaceae,
103 with 29 genera and ca. 300 species arranged in three subtribes (Iochrominae, Physalidinae

104 and Withaninae; Olmstead et al., 2008; Särkinen et al., 2013). Moreover, 13 of 19 Solanaceae
105 genera with fruiting calyx inflation are placed in Physalideae, The wide variation in fruiting
106 calyx form, from non-acrescent to greatly inflated (Fig. 1), has often been used for
107 intergeneric delimitation (Hunziker, 2001; Sawyer, 2001; Li et al., 2013; Zamberlan et al.,
108 2015), although phylogenetic studies suggest that these characters are homoplastic (Hu and
109 Saedler, 2007). With a new phylogeny including 73 % of Physalideae species, we traced the
110 evolution of the fruiting calyx accrescence and inflation to address the following questions:
111 (i) is fruiting calyx inflation a convergent trait in Physalideae?; (ii) if so, how many times has
112 this trait been gained or lost? (iii) can lineages move directly between non-acrescent and
113 inflated states or do they tend to transition through intermediate stages of accrescence? The
114 answers to these questions will provide insight into evolutionary accessibility of the lantern-
115 like fruit form and lay the foundation for future studies at the genetic and development levels.

116

117 **MATERIALS AND METHODS**

118 ***Taxon sampling***– The ingroup sampling spanned 27 of the 29 genera of Physalideae and
119 included 215 species of the 294 species plus 4 varieties (Appendix S1 and S2; see
120 Supplemental Data with this article). The monotypic *Mellissia* Hook. f. and *Capsicophysalis*
121 Averett & M. Martínez were the only genera not sampled. *Capsicum lycianthoides*,
122 *Lycianthes inaequilatera*, and *Salpichroa tristis* (Appendix S1) were used as outgroups.
123 Newly sampled plant material was either gathered from herbaria (CORD, CSU, MO, SI) or
124 collected during several field trips to Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru,
125 and United States in the last ten years. Leaves were dried in silica and vouchers were
126 prepared and housed at local herbaria of each country (Argentina: CORD; Bolivia: LPB;
127 Brazil: BHCB; Colombia: COL, JBB, PSO; Ecuador: LOJA, QCA, QCNE, QUSF; Peru:

128 HAO, HUT; United States: COLO, CSU, MO). We also obtained already extracted DNA
129 from L. Bohs, R. Olmstead, and L. Freitas.

130 ***Phylogenetic reconstruction of Physalideae***—We used de novo (407, ca. 55 %) and published
131 (339, ca. 45 %) sequences from four regions to estimate relationships within Physalideae
132 (Appendix S1, including GenBank accession numbers): the nuclear regions internal
133 transcriber spacer (ITS), granule-bound starch synthase (GBSSI or *waxy*) gene, the second
134 intron of LEAFY (LFY), and the chloroplast spacer *trnL-F*. GBSSI regions previously
135 sequenced by Whitson and Manos (2005) were not included in the analyses because they only
136 comprised from exon 8 to 10, whereas we are using from exon 2 to 9 for most taxa. Taxa
137 coverage was 92.8 % for ITS, 77.9 % for LFY, 78.4 % for *waxy*, and 87 % for the chloroplast
138 fragment (Appendix S3). DNA extractions were done following a modified 2 x CTAB
139 procedure (Doyle and Doyle, 1987); primers and PCR conditions followed previous work
140 (Smith and Baum, 2006; Deanna et al., 2018).

141 Sequence quality was inspected using GENEIOUS v4.6.1 (Drummond, et al., 2006), and
142 sequence alignments were performed in MEGA 6 (Tamura et al., 2013) using the MUSCLE
143 algorithm (Edgar, 2004) followed by manual adjustments. For *trnL-F*, a variable repeat
144 region towards the 5' end of the intergenic spacer was removed because this is where putative
145 pseudogenic copies of *trnF* have been found in *Solanum* (Poczai and Hyvönen, 2011). Gene
146 trees were estimated individually for each region with maximum likelihood (ML) in RAxML
147 v.8 (Stamatakis, 2014) on the CIPRES server (Miller et al., 2010). We implemented the GTR
148 + GAMMA model and used the rapid bootstrap (BS) algorithm with 1000 replicates to assess
149 nodal support. Trees were compared across genes to identify areas of hard incongruence (BS
150 > 70%; Mason-Gamer and Kellogg, 1996).

151 Given the absence of hard incongruence, we conducted ML and Bayesian analyses on the
152 combined dataset. Matrices were concatenated with SequenceMatrix 1.8 (Vaidya et al., 2011)

153 and partitioned by gene before analysis. We also identified unstable tips based on the ML
154 bootstrap analyses using the software RogueNaRok (Aberer et al., 2013). Two iterations of
155 RogueNaRok were run with settings according to Särkinen et al. (2013), and rogue taxa were
156 removed after each iteration, resulting in the pruning of 10 tips in total. We also excluded the
157 voucher R. Deanna 143 (which morphologically matches to the original description of
158 *Cuatresia harlingiana* Hunz.) given its phylogenetic position outside of Physalidaeae.
159 However, we included sequences of a voucher previously identified as *C. harlingiana* (Smith
160 and Baum, 2006; Deanna et al., 2017, 2018), which does fall within *Cuatresia* and appears to
161 belong to an undescribed taxon (appearing here as *Cuatresia* sp.).

162 The final combined matrix included 7988 bp of aligned sequences of 222 taxa, including
163 outgroups. We performed ML phylogenetic inference partitioned by gene using RAxML
164 according to the parameters used for individual region analyses (see above) on the CIPRES
165 server (Miller et al., 2010). Bayesian analyses were conducted for the combined dataset with
166 four partitions in BEAST 2 (Bouckaert et al., 2014), also on the CIPRES server. Best models
167 of substitution were incorporated for each partition according to a previous selection with the
168 Akaike Information Criterion (AIC) using jModelTest 2.1.3 (Appendix S3; Posada and
169 Crandall, 1998; Darriba et al., 2012). Two independent BEAST analyses were run for fifty
170 million generations each with tree sampling every 1000 generations, using an uncorrelated
171 lognormal relaxed clock model to describe the branch-specific substitution rates (Drummond,
172 et al., 2006). We used a Birth-Death tree prior, which accounts for both speciation and
173 extinction (Gernhard, 2008), and a constraint of monophyly for all species excluding
174 *Salpichroa tristis*. Convergence and stationarity of the parameters were inspected using
175 Tracer v1.7 (Rambaut et al., 2018), targeting minimum effective sample sizes (ESS) of at
176 least 200. The initial 20 % of trees were discarded as burn-in, and the results were combined
177 using LogCombiner as implemented in the BEAST package. The phylogenetic relationships

178 were summarized in a maximum clade credibility (MCC) tree, and their posterior
179 probabilities (PP) for all nodes were derived using TreeAnnotator v2.4.7. The trees were
180 visualized in FigTree v.1.4.3 (Rambaut, 2016).

181 **Codification of fruiting calyces**—All fruiting calyces from taxa included in the phylogeny
182 were scored using specimens housed at herbaria (COL, COLO, CORD, CSU, MO, SI), the
183 JSTOR Plants database, and the literature (Appendix S2). Following Hu and Saedler (2007),
184 we scored a fruiting calyx as accrescent-appressed when there is an increase in calyx length
185 of 50 % or more from flower to fruit stage (e.g. *Brachistus stramoniiifolius*), or the berry is
186 entirely covered but there is not a space between calyx and berry (e.g. *Cuatresia exiguiflora*).
187 Fruiting calyx was coded as non-acrescent when it grows less than 50 % from flower to fruit
188 stage (e.g. *Witheringia solanacea*), and as inflated when the fruit is entirely enclosed by the
189 calyx and there is also a space between calyx and berry (e.g. *Physalis peruviana*; see matrix
190 in Appendix S4). Note that following this definition, species of *Iochroma* are coded as non-
191 accrescent despite being described as often having accrescent calyces (Hunziker, 2001; Smith
192 and Baum, 2006; Lezama Escobedo et al., 2007; Cueva Manchego et al., 2015). In *Iochroma*,
193 accrescence is usually less than the 50 % of the length present at the flowering stage. In a
194 handful of species (e.g. *I. calycinum*, *I. barbozae*; Khan et al., 2012a; Leiva González et al.,
195 2013), the fruiting calyx covers the berry (or nearly so), but this is due to the large size of the
196 flowering calyx.

197

198 **Testing for phylogenetic signal**—We implemented two metrics to examine the level for
199 phylogenetic signal in fruiting calyx morphology. First, we calculated the parsimony score
200 using the *parsimony* function in the {phangorn} R package (Schliep, 2011). Second, we
201 computed Fritz and Purvis' D (FPD, Fritz and Purvis, 2010), a metric which captures the sum
202 of sister clade differences, also available in {phangorn}. The FPD statistic takes a value of 1

203 if the trait has a phylogenetically random distribution and 0 if the trait has evolved under
204 Brownian motion (Fritz and Purvis, 2010). For both measures, we tested whether the
205 observed values differed those expected by chance (no phylogenetic signal) as well as those
206 expected under Brownian motion. In the former case, the null distribution was created by
207 randomly reshuffling the tip states 1000 times, and in the latter case, by evolving these traits
208 on the phylogeny under a Brownian motion model 1000 times. These null distributions were
209 created with the *treestat* function in the {phylogenetics} package (Hua and Bromham, 2016).
210 Traits with phylogenetic signal are predicted to differ significantly from the random
211 distribution ($p < 0.05$) but not the distribution expected under Brownian motion. As the FPD
212 statistic can only be applied to binary traits, we considered fruit accrescence and inflation
213 separately (Appendices S5 and S6), while for parsimony, we were able to examine them
214 jointly as three-state character (Fig. 1). These analyses were conducted using the MCC tree.

215

216 ***Reconstructing the evolutionary transitions to fruiting inflated calyces***—We estimated the
217 history of fruit calyx evolution across Physalideae using maximum likelihood and Bayesian
218 approaches. We first compared the fit of alternative models of trait evolution using the {ape}
219 package in R (Paradis et al., 2004) and the MCC tree from the BEAST analyses. We
220 considered six models with the first having transition rates between all states free to vary (the
221 all rates different model) and the second with all rates equal. We then fit four stepwise
222 models, where lineages move from non-acrescent to inflated through the intermediate state
223 of accrescent-appressed. Model 3 has all steps being reversible while the last three models
224 have one or more of these steps constrained to be irreversible (Table 1). Model selection was
225 conducted with the Akaike Information Criterion (AIC) score, with the best model having a
226 score at least two AIC units lower than the model with the next lowest AIC score (Burnham
227 and Anderson, 2002).

228 Using the best fitting model, we next estimated ancestral states and the number of transitions
229 between states with Bayesian stochastic mapping (SM). Through rounds of simulation
230 ('realizations'), SM generates a sample of histories of discrete character evolution on a
231 phylogeny that should approximate the posterior distribution of histories (Huelsenbeck et al.,
232 2003). In order to incorporate phylogenetic uncertainty, we performed 500 simulations of
233 character history on a sample of 100 trees from the BEAST analysis with the combined
234 dataset. The simulations, carried out with the *make.simmap* function in {phytools} package
235 (Revell, 2012), were summarized on the MCC tree to provide the posterior probability of
236 each state at each node. We also estimated the median number of changes for each transition
237 type from the histories and computed 95% credibility intervals using the *hdr* function from
238 the {diversitree} package in R (FitzJohn, 2012).

239

240 **RESULTS**

241 ***Phylogeny of Physalideae***—Our final combined matrix had a taxon coverage of 0.84 %
242 (Appendix S3) and comprised 215 species of Physalideae. This represents 73.1 % of the total
243 species within the tribe and 55 % of the species within *Physalis*. The plastid *trnL-F* and the
244 nuclear region ITS were the most densely sampled, whereas ITS contributed most parsimony-
245 informative characters (Appendix S3). Hard incongruence was not found among gene trees
246 (Appendix S7). The maximum likelihood and Bayesian topologies were largely congruent
247 (Fig. 2 and Appendix S8, respectively) and showed strong to moderate support for
248 Physalidinae (BS = 63 %, PP = 1) and Iochrominae (BS = 100 %, PP = 1), which is resolved
249 as sister to the remaining Physalideae taxa (BS = 89 %, PP = 1). The previously proposed
250 subtribe Withaninae (Olmstead et al., 2008; Särkinen et al., 2013) does not appear to be
251 monophyletic but instead divided amongst two clades with the Hawaiian *Nothocestrum* and
252 allied Old World genera more closely related to Physalidinae than other members of

253 Withaninae. Moreover, eight of the 27 sampled genera are non-monophyletic as presently
254 circumscribed (e.g. *Iochroma*, *Cuatresia*, *Physalis*).

255

256 ***Testing for phylogenetic signal of accrescent and inflated fruiting calyces***—We found strong
257 phylogenetic signal for fruiting calyx accrescence and inflation with both implemented
258 approaches. These traits have a significantly lower parsimony score and lower FPD compared
259 to the random null distribution, suggesting that species with accrescent and inflated calyces
260 are more closely related than expected by chance (Table 2). Consistent with this result,
261 neither of the traits significantly differed from expectations under Brownian motion of
262 evolution along the phylogeny (Table 2).

263

264 ***Evolutionary transitions to fruiting inflated calyces***—The best fitting maximum likelihood
265 model for fruiting calyx evolution was the stepwise model with transitions between
266 accrescent and non-acrescent fruiting calyces being irreversible (reverse transition rate not
267 different from zero). This model had the lowest AIC score and was greater than two AIC
268 units lower than any competing model (Table 1; Appendix S9). Our stochastic mapping
269 simulations with this model estimated a median of 50 changes across the clade (95% HDR =
270 44.56–56.04). Among these changes, shifts from non-acrescent to accrescent-appressed
271 calyces and accrescent-appressed to inflated calyces were inferred to occur at roughly equal
272 frequencies (median = 24 (19.94–29.09) vs. 24 (19.96–27.71), Appendix S10). Loss of
273 inflation to an accrescent-appressed calyx was infrequent (median = 2, 95% HDR = 0–3.93;
274 Appendix S10). The ancestral state of the tribe was estimated by SM as non-acrescent in all
275 stochastic maps (100% posterior probability, Fig. 3). Similarly, high support was inferred for
276 this ancestral state at many nodes throughout the phylogeny, revealing multiple independent
277 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

303 Withaninae sensu Olmstead et al. (2008) and the other two species (*W. aristata* and the type
304 species *W. frutescens*) closely related to *Aureliana*. In a prescient review, Hepper (1991)
305 pointed out that these two western African species, *W. aristata* and *W. frutescens*, are
306 morphologically unlike others in *Withania* and suggested that their closest relatives may
307 instead be across the Atlantic. Beyond the rearrangement of Withaninae necessitated by this
308 apparent split within *Withania*, most of the genera which have been placed in the subtribe are
309 monophyletic (or nearly so) given extensive taxonomic work in recent years (Zamberlan et
310 al., 2015; Deanna et al., 2018).

311

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

325

326 ***Repeated evolution of fruiting calyx accrescence and inflation***—Our analyses demonstrate
327 that the highly-inflated fruiting calyx considered so characteristic of *Physalis* has evolved

328 repeatedly in Physalideae. While previous studies had suggested homoplasious patterns in
329 fruiting calyx variation in the tribe (Whitson and Manos, 2005; Hu and Saedler, 2007), we
330 provide the first estimates of the numbers of gains and losses, with ca. 24 gains of
331 accrescence, 24 subsequent gains of inflation and 2 reversals from inflation to the accrescent-
332 appressed state (Fig. 3). Despite these many gains of calyx accrescence and inflation, we
333 recovered significant phylogenetic signal in these traits overall. Indeed, the character states
334 appear clustered on the phylogeny, with some large clades (e.g. Iochrominae, *Physalis*) being
335 invariant in the degree of fruiting calyx accrescence.

336

337 The many independent origins of calyx inflation may have occurred through recurring
338 modifications of the shared underlying pathway, which is well studied in several Physalideae.
339 The development of ICS requires the expression of *MPF2*-like MADS-box transcription
340 factors in flowering calyces (He and Saedler, 2005). Nonetheless, *MPF2* expression in the
341 calyx is widespread across taxa with and without ICS in Physalideae and even in Capsiceae
342 (Hu and Saedler, 2007), suggesting the development of ICS is determined by other factors.
343 Indeed, the effect of *MPF2* on calyx morphology appears to hinge on interactions with
344 cytokinin and gibberellin, which are released upon fertilization (He and Saedler, 2007; Khan
345 et al., 2012b). Thus, genetic changes which modify these hormonal signals, *MPF2*
346 expression, or *MPF2* function could all contribute to variation in calyx inflation (Riss, 2009).
347 Comparative molecular and developmental studies to-date implicate both regulatory and
348 structural mutations in *MPF2*-like genes (Hu and Saedler, 2007; Riss, 2009; Khan et al.,
349 2009), coupled with shifts in copy number due to the many ploidy changes in the tribe (Iqbal
350 and Datta, 2007; Deanna et al., accepted).

351

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

365

366 ***Loss vs. gain of inflation through a stepwise model of evolution***– Our comparative analyses
367 indicated that evolution of the inflated calyx proceeds in directional fashion, starting from the
368 non-acrescent state, moving first to an accrescent but appressed state before finally
369 becoming inflated. This pattern contradicts the hypothesis that, given the complex
370 developmental pathway required to produce ICS, inflation should be easier to lose than to
371 gain (Hu and Saedler, 2007). This frequent and directional transitions toward inflation
372 suggest not only that the trait is genetically accessible (perhaps given the background of
373 *MPF2* expression in Physalideae calyces) but also that inflation is generally retained by
374 lineages in which it evolves. Still, the adaptive advantages which could favor the fixation of
375 this trait (e.g. protection from desiccation, deterrence of predators, enhanced dispersal) have
376 been largely untested (but see Wilf et al., 2017). In fact, the only evidence for adaptive

377 evolution of ICS comes indirectly from molecular studies, which have estimated positive
378 selection acting on *MPF2*-like genes in *Withania* and *Physalis* (Khan et al., 2009; Zhang et
379 al., 2012).

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

391 Despite the strong directionality inferred from our analyses, it is important to note that such
392 patterns may be confounded by state-dependent differential diversification. For example, the
393 abundance of inflated calyxes (as in Physalideae) can occur through biased transitions toward
394 this state or by increased diversification of lineages with the state (Ng and Smith, 2014). A
395 thorough analysis of the effects of calyx evolution on speciation and extinction rates will
396 require a larger phylogeny (Beaulieu and O'Meara, 2016), ideally at the family level and
397 including all of the remaining genera (6) and species (76) with ICS. Diversification analyses
398 would also benefit from new divergence time estimates in light of the recent discovery of
399 Eocene lantern fruit fossils. These fossils, placed in crown group *Physalis*, are dated to 52.2
400 mya, which is roughly the age inferred for the entire crown group Solanaceae in previous
401 work (Särkinen et al., 2013; De-Silva et al., 2017). This contrast highlights the need for a

402 complete reassessment of Solanaceae fossils (Wilf et al., 2017; Särkinen et al., 2013, 2018),
403 together with a new family-wide dating analysis including all reliable fossil taxa.

404

405 **CONCLUSIONS**

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

415

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430

431 **AUTHOR CONTRIBUTIONS**

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

436

437 **DATA ACCESSIBILITY**

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

441

442 **SUPPORTING INFORMATION**

443 Additional Supporting Information may be found online in the supporting information tab for
444 this article.

445

446 **LITERATURE CITED**

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694

695

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

702

703

704 **TABLE 2.** Phylogenetic signal metrics calculated on Physalideae species with accrescent
 705 and/or inflated calyx. Bolded values indicate the statistics that were significantly lower than
 706 the random distribution of traits or significantly greater than Brownian motion evolution ($p <$
 707 0.05). *FPD can adopt negative values up to -0.5 when the phylogenetic signal is high (Fritz
 708 and Purvis, 2010).
 709

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

710

711

712 **APPENDIX 1.** Summary of taxon sampling, provenance, voucher (collector and number or
713 barcode, in italics), herbarium where vouchers were housed between parenthesis (acronyms
714 follow Index Herbariorum; Thiers, 2017), and GenBank accession numbers provided in the
715 following order: ITS, LEAFY, *trnL-F*, *waxy*. ‘NA’ indicates either voucher or provenance
716 information was not found, and ‘na’ that the region was not sampled for this accession.
717 Newly generated sequences are indicated with an asterisk following the accession number.

718

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

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

726 *Aureliana angustifolia* Alm.-Lafetá, BRAZIL, Minas Gerais, Juiz de Fora, *Giacomin et al.*
727 965 (BHCB), KC832782, na, KC549633-KC549614, na. *Aureliana anonacea* (Sendtn.)
728 I.M.C. Rodrigues & Stehmann (= *A. pereirae*), BRAZIL, Minas Gerais, Caraça Sanctuary,
729 *Oliveira et al.* 388 (BHCB), KC832788, na, KC549639-KC549620, na. BRAZIL, Mina
730 Gerais, Caraça, *Barboza 3638b* (CORD), na, MH822153*, na, KX690166. *Aureliana*
731 *brasiliiana* (Hunz.) Barboza & Hunz., BRAZIL, Rio de Janeiro, Itatiaia National Park,
732 *Rodrigues et al.* 106 (BHCB), KC832783, na, KC549634-KC549615, na. BRAZIL, Río de
733 Janeiro, Petrópolis, *Barboza et al.* 2055 (CORD), na, MH822154*, na, MH796558*.

734 *Aureliana cuspidata* (Witasek) I.M.C. Rodrigues & Stehmann, BRAZIL, Sao Paulo,
735 Conservation Area Boracéia, *Stehmann et al.* 4812 (BHCB), KC832784, na, KC549635-
736 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, Espiritu 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 *Nothoctrum breviflorum* A. Gray, HAWAII, Hamakua, Kailikaula Cliffs and Stream,
983 *Wood et al. 4862* (MO), MH763723*, MH822216*, MH752631*, MH796583*.
- 984 *Nothoctrum 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*. *Nothoctrum 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 *Nothoctrum 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, *YYZWF20387*,
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. higrrophylla* (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 ***hispid*** (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***

1152 *mortonii* Hunz., COSTA RICA, *Bohs 2594* (UT), MH763751*, MH822268*, MH752678*,
1153 MH796630*. *Witheringia solanacea* L'Hér., COSTA RICA, *Bohs 2416* (UT), na, na,
1154 EU581074, na. PANAMA, *D'Arcy 16399* (MO), DQ314164, DQ301517, na, DQ309474.
1155 *Witheringia stellata* (Greenm.) Hunz., MEXICO, *Stone 1522* (UT), MH763752*,
1156 MH822269*, MH752679*, MH796631*. *Witheringia wurdackiana* Benítez, VENEZUELA,
1157 Táchira, Fernández Feo, *Benítez de Rojas & Rojas 5433* (MO), MH763753*, na, na, na.

1158

1159 **APPENDICES 2-10:** uploaded in separate files.

1160

1161

1162 **FIGURE LEGENDS**

1163

1164 **FIGURE 1.** Distribution of fruiting calyx states across the tribe Physalideae. The size of the
1165 pie slices represents the proportion of taxa in each state, i.e. species with inflated calyces
1166 (red, 49.7%), non-acrescent calyces (black, 24.8%) and accrescent but still appressed
1167 calyces (blue, 25.5%). The darker shade in each pie slice corresponds to the percentage of
1168 taxa with that state sampled in the present study (64.4%, 86.3%, and 77.3%, respectively).
1169 Images from red to blue (moving clockwise) are *Physalis hederifolia* (Deanna *et al.* 209,
1170 photo by S. Carrasco), *Deprea pumila* (Orozco *et al.* 3890, photo by S. Leiva González),
1171 *Aureliana cuspidata* (Stehmann *et al.* 6457, photo by R. Deanna), *Witheringia solanacea*
1172 (Deanna 160, photo by R. Deanna), *Aureliana wettsteiniana* (Stehmann *et al.* 6448, photo by
1173 R. Deanna), *Iochroma arborescens* (Orejuela & Castillo 2697, photo by A. Orejuela),
1174 *Chamaesaracha coronopus* (Deanna *et al.* 237, photo by S. Carrasco), *Cuatresia exiguiflora*
1175 (Orozco *et al.* 3853, photo by G. E. Barboza), *Deprea sawyeriana* (Deanna & Leiva
1176 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

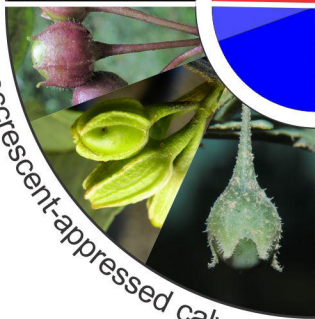
1184 **FIGURE 3.** Reconstruction of fruiting calyx evolution in the Physalideae tribe. Topology is
1185 provided from four-gene BEAST analyses of 219 taxa. Circles at nodes indicate the posterior
1186 probabilities from stochastic mapping and tip label colors represent tip states, with red, blue
1187 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



