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THE IMPROBABLE JOURNEYS OF EPIPHYTIC PLANTS ACROSS THE ANDES: HISTORICAL BIOGEOGRAPHY OF *CYCNOCHES* (CATASETINAE, ORCHIDACEAE)

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Abstract [200 words]:

The Andean uplift is one of the major orographic events in the New World and has impacted considerably the diversification of numerous Neotropical organisms. Despite its importance for biogeography, the specific role of mountain ranges as a dispersal barrier between South and Central American lowland plant lineages is still poorly understood. The swan orchids (*Cycnoches*) comprise *ca* 34 epiphytic species distributed in lowland and pre-montane forests of Central and South America. Here, we study the historical biogeography of *Cycnoches* to better understand the impact of the Andean uplift on the diversification of Neotropical lowland plant lineages. Using novel molecular sequences (five nuclear and plastid regions) and twelve biogeographic models with and without founder-event speciation, we infer that the most recent common ancestor of *Cycnoches* may have originated in Amazonia *ca* 5 Mya. The first colonization of Central America occurred from a direct migration event from Amazonia, and multiple bidirectional trans-Andean migrations between Amazonia and Central America took place subsequently. Notably, such biological exchange occurred well after major mountain building periods. The Andes have not acted as an impassable barrier for epiphytic lowland lineages such as orchids having a great potential for effortless dispersal because of the very light, anemochorous seeds.

Key words: ancestral area, Andean uplift, barrier, dispersal, historial biogeography, molecular clock.

Introduction

Neotropical landscape and biodiversity have long drawn the attention of naturalists [1,2]. The tropical Andes are of particular interest as the world's premier biodiversity hotspot, with both an extraordinary species richness and a remarkable level of endemism[3–5]. The combination of molecular phylogenies with species distributions and the fossil record has uncovered different biotic and abiotic factors that fostered diversification in the Neotropics[6–10]. However, biogeographical studies applying modern phylogenetic methods are only available for few Neotropical plant clades (e.g., *Begonia*[11], *Cyathostegia*[12], *Heliotropium*[13], *Lupinus*[14,15], palms[16], Rubiaceae[6]). These studies have generally demonstrated the importance of geological processes such as mountain building and establishing the Isthmus of Panama for the diversification of Neotropical plants[17].

One of the most biologically relevant abiotic processes in the diverse geological history of the Americas is the rise of the Andes [7,13,18]. Andean mountain building was driven by plate tectonic re-adjustments that started during the Palaeogene and continued until the Pliocene [7]. The fossil record (e.g., palynological[3,19] and geological data: isotope measurements[20], sediment loads, apatite fission-track data[7]) collectively indicate that the Andean uplift was a partially constant process punctuated by periods of intensified mountain building. Two of the most intense uplift periods occurred around 12 (mid-Miocene) and 4.5 million years ago (Mya; early Pliocene[7]). During these periods, the Northern Andes reached elevations as high as 4500 m in the Pliocene, whereas the Central Andes already peaked an altitude of 4500 m during the mid-Miocene [7,21].

Newly formed mountain ranges may had a strong impact on the adjacent Amazonian landscapes and the inhabiting organisms due to the transformation of its

drainage systems[22]. The Andes also influenced local and regional climates by forming the only barrier to atmospheric circulation in the Neotropics[23,24]. The rise of the Andes led to the formation of island-like habitats and of local microclimates and soil conditions that eventually fostered species diversification[14,25,26]. At the same time, the Andes provided physical and/or ecological barriers to species dispersal and migration. For instance, the efficiency of the Northern Andes as migration barrier is shown for Andean centred woody species of *Crematosperma*, *Klarobelia*, *Malmea*, and *Mosannona* (Annonaceae[27]). Propagules are dispersed by animals in these lineages[28], and none of their constituent species occur in both east and west of the Andes mountain range[27].

Recent phylogenetic studies provide solid evidence for the important role of Andean uplift in diversification of highland-dwelling plant groups (e.g., *Lupinus*[14]; *Bartsia*[29]; centropogonid Campanulaceae[9]). However, the impact of such orographic processes for the lowland flora is still poorly understood[4,6,30]. Thus, the question remains whether Andean uplift has indeed been an abiotic barrier to migration for epiphytic lineages such as lowland orchids and bromeliads, both being important components of Neotropical forests.

Epiphytic diversity is dramatically greater in the Neotropics than in any other tropical region around the world [31,32], being twice as high than, for instance, in Australasia [33–36]. Several traits shared by Neotropical epiphytic taxa, and related to their reproductive biology, may explain such overwhelming differences in diversity. One of the most prominent shared traits is the lightness and the very small size of the propagules, occasionally with highly elaborated surfaces (e.g., bromeliads, ferns, orchids, *Pinguicula*[33]). The potential of dust-like seeds for longer distance dispersals by wind might be much greater compared to other plants with propagules locally dispersed

100 by animals (e.g., Araceae[37]). Nonetheless, it remains largely unknown whether lowland
101 epiphyte lineages have been able to disperse across geological barriers such as the
102 Andes. Well-sampled phylogenies for epiphyte clades have been lacking to address this
103 issue.

104 Several wind-dispersed plant lineages (e.g., *Begonia*[11]; bromeliads[38]) span across
105 the Neotropical region, many of which are restricted to lowland elevations. One
106 example is the orchid tribe Cymbidieae comprising *ca* 3900 species that are mostly
107 distributed in the Neotropics (but with few representatives in the Old World Tropics[39]).
108 To Neotropical Cymbidieae belong the swan orchids (*Cycnoches*) that are known for the
109 striking sexual dimorphisms[40] and pollination syndrome[41]. Molecular phylogenetics and
110 morphological studies conducted to date confirm the inclusion of *Cycnoches* within
111 Catasetinae[42–44] and its sister group relationship to *Mormodes*[40,45,46].

112 *Cycnoches* encompasses 34 species of epiphytes[47] that are distributed from
113 Southern Mexico to Central Brazil and Bolivia. Its species are best represented in the
114 Amazonian forests of Brazil, Ecuador, and Peru, but also in the Llanos and Caribbean
115 regions of Colombia and Venezuela[47,48]. They commonly inhabit lowland tropical wet
116 forests, ranging from 0 to 800 m. *Cycnoches* species are pollinated by male euglossine
117 bees, which collect volatile compounds produced by flowers but also from other sources
118 (e.g., rotten wood, faeces[41]). They have rather a restricted geographical range, and most
119 of them are distributed in single biogeographical area. Nevertheless, one species (*C.*
120 *chlorochilon*) is distributed in both sides of the Northern Andes[47].

121 Because of the striking sexual system they have evolved[40], swan orchids have
122 long attracted the attention of several prominent botanists including[49]. Despite this long
123 interest, previous phylogenetic studies have only been included up to three species of
124 *Cycnoches* probably because of their scarceness both in the wild and in herbarium

125 collections[42,43,45,46,50]. Moreover, the lack of a solid and well-sampled phylogeny of
126 *Cycnoches* has precluded addressing specific questions concerning the role of Andean
127 uplift in their biogeographical history. The narrow geographical distribution of almost
128 all extant *Cycnoches* species and their restricted habitat preference given (i.e., lowland
129 wet forests, see above), we expect the swan orchids diversification to be strongly
130 influenced by the Andean uplift. In particular, we hypothesize that Andean uplift was an
131 impassable, isolative, barrier for *Cycnoches*, as already reported for other plant lineages.
132 To test this hypothesis, we generated a strongly supported 5-loci phylogeny, sampling
133 23 out of 34 accepted *Cycnoches* species and comprising its known diversity and
134 distribution, and performed models of biogeographical analyses.
135
136 **Results**
137 *Phylogeny of* Cycnoches
138 Our phylogeny comprised 23 out of 34 accepted species of *Cycnoches*. Table S5
139 provides detailed alignment descriptions. The concatenated 'nuc' alignment was 2395
140 bp in length and included 310 parsimony informative sites, and the concatenated 'cp'
141 alignment was 2419 bp and comprised 171 parsimony informative positions.
142 Independently derived 'nuc' and 'cp' phylogenies revealed topologies with conflicting
143 and highly supported phylogenetic placements. PACo analysis revealed 22 potential
144 outlier OTUs (see below; Figs S1–S2) belonging to *Cycnoches* (majority of 19),
145 *Dressleria*, and *Mormodes*. After inspection of the potentially confliting OTUs
146 placement in 'nuc' and 'cp' phylogenies (Fig. S2), 20 outliers were confirmed as
147 conflicting terminals. Thus, only two terminals (i.e., *Dressleria severiniana* and one
148 representative of *C. lehmannii*) were misclassified by PACo as conflicting (see
149 Appemdix S1 for a detailed explanation on outlier handling).

Within *Cycnoches*, the 'nuc' phylogeny recovered three maximally supported clades (A, B, C; Fig. 1). Clade A included all sequenced accessions of *Cycnoches hagii* and was recovered as sister group of the remaining species of *Cycnoches* comprising clades B and C. By contrast, the 'cp' phylogeny showed two main maximally supported clades (namely I and II), each including a set of intermingled species from clades A, B, and C. The best ML tree inferred from non-conflicting, concatenated 'nuc' and 'cp' datasets showing the internal phylogenetic relationships of *Cycnoches* is presented in Figure 2. Virtually, all internal nodes of the backbone phylogeny were highly, if not maximally supported by MLBS and PP values. *Cycnoches* segregated into three main lineages (clades A, B and C). Clade A (i.e., all specimens of *C. haagii*) was sister group of the remaining species of *Cycnoches* clustering in clades B and C.

Molecular dating of Cycnoches

Comparison of marginal likelihood estimates (MLE) of tree priors and clock models revealed that an uncorrelated molecular clock combined to a birth-death tree prior with incomplete sampling best fitted our data (MLE of –14244.47; Table 1). Nevertheless, Bayes factors (BF) clearly rejected the strict clock models (BF = 72, 45, and 40 for all tree priors), but did not provide strong evidence to support an incomplete sampling birth-death model *vs* a standard birth-death model (BF=1.08; Table 1). Analysis of the log file produced by dating analyses under the relaxed clock and tree models are also shown in Table 1. Overall, they yielded CV values between 0.35 and 0.42 (indicating there was among branch rate heterogeneity, which argued for the use of a relaxed molecular clock). Therefore, results obtained from a dated phylogeny with relaxed molecular clock and birth-death standard speciation model (Table 1) are presented only (Figs 3, S5).

175 A chronogram showing absolute ages estimated under a relaxed clock is
176 presented in Figure 3 (see also Fig. S5 for the 95% confidence intervals) and shows that
177 *Cycnoches* and *Mormodes* shared a common ancestor during the beginning of the late
178 Miocene (9.1 Mya ±3). Diversification of *Cycnoches* took place around 5 Mya ±2 at end
179 of the late Miocene. The split between *Cycnoches* clades B and C occurred during late
180 Pliocene (3 Mya ±2), whereas both clades were estimated to be of Pleistocene ages (1.2
181 and 1.6 Mya ± 1, respectively).
182
183 *Ancestral area estimations*
184 Table 2 provides ML statistics for the biogeographical models as inferred in
185 BioGeoBEARS. The best fitting parametric model was the BAYAREA star, including
186 the founder-event speciation. This model revealed a geographical origin in Amazonia as
187 the most likely ancestral area of *Cycnoches* (Figs 3, S6). The MRCA of clades B and C
188 was reconstructed to have inhabited in Amazonia region. The MRCA of clade C
189 occurred in Central America, whereas the MRCA of clade B inhabited in Amazonia
190 region.
191 Three independent trans-Andean migration events between Amazonia and
192 Central America could be identified. The first migration from Amazonia to Central
193 America took place towards the late Pliocene (±1 Mya), after the divergence of MRCAs
194 of clades B and C. A second migration from Central America to Amazonia took place
195 around mid-Pleistocene (±1 Mya) by the MRCA of *C. maculatum*, *C. manoelae*, *C.*
196 *peruvianum*, *C. quatuorcristis*, and *C. suarezii*. Last biotical exchange from Amazonia
197 to Central America was dated to late Pleistocene (±0.5 Mya) with the MRCA of *C.*
198 *chlorchilon*, *C. ventricosum*, and *C. warzsewiczii*.
199

Discussion

Influence of Andean orogeny on the biogeography of a Neotropical epiphyte group

Our study provides a solid phylogenetic framework, divergence times, and ancestral areas estimation for *Cycnoches*. Central America has been considered the most likely region of *Cycnoches* origin, probably because of its locally elevated species richness as compared to other areas in the Neotropics (Romero & Gerlach, *in press*). However, this evolutionary scenario is rejected by our analyses, which instead support a South American origin of *Cycnoches* in the late Miocene (ca. 5 Mya ±2; Fig. 3, Fig. S6). The ancestral area estimated at *Cycnoches* MRCA largely reflects the current distribution of early diverging lineages such as *C. haagii*, a species today distributed in Amazonia and the Guiana Shield. Thus, the early diversification of *Cycnoches* has taken place well after the most intense mountain building events of the Northern and Central Andes ca. 12 and 10 Mya, respectively[7,52–54].

Our results indicate a less important role of the Andes as a biogeographic barrier for the diversification of *Cycnoches*. Two migrations from Amazonia to Central America and one reverse colonisation event back imply vigorous ancient dispersals across the Andes during last 5 million years (Figs 3). During the early Pliocene (i.e., the period when *Cycnoches* has started to diversify), the Northern Andes of Colombia and Venezuela have already reached elevations up to at least 3000 m (Hoorn *et al.*, 2010). Moreover, three migrations from Amazonia to Central America and back, respectively, took place ~2 Mya, when the Northern and Central Andes already peaked at a mean elevation of 4000 m (see mean Andean elevation displayed in the inset of Fig. 3).

Similar trans-Andean migrations are reported for bromeliads and ferns. In Bromeliaceae (subfamily Bromelioideae), direct migrations from the Brazilian Shield towards Central America took place around 7 Mya[38], a period where Northern Andes

225 reached a palaeoelevation of ~2000 m[7]. Furthermore, biotical exchanges between South
226 and Central America have also been reported in the fern complex *Jamesonia-Eriosorus*
227 (Pteridaceae)[30]. Here, migrants from the Brazilian coast colonised and further
228 established in Central Andes, and from there subsequently migrated towards Central
229 America during the late Pleistocene[55].

230 Impermeability of the Andes as a barrier for reproductive isolation does not
231 appear as important for Neotropical epiphyte lineages such as *Cycnoches*. Our results
232 also point to a rapid colonization of the mainland Neotropics (i.e., colonization of
233 Central America, Amazonia, and Choco in ~5 million yr.). Such biotic invasion may be
234 related to the biology of the group having anemochorous seed dispersal and an epiphytic
235 habit. Most orchid seeds are characterized by their minute size and reduced weight, as
236 well as by their elaborated seed coats[56,57]. These traits allow them to easily remain
237 airborne for extended periods of time and to travel over long distances[57]. *Cycnoches*
238 species might have good dispersal potential as well because of their seeds, which are
239 between 100–300 µm long, 50–60 µm wide, and about 3.6 µg weight[56,58].

240 Empirical evidence strongly supports short mean dispersal distances (e.g., 4–5
241 m) of seeds from terrestrial orchids such as *Orchis purpurea*[59], yet there are no
242 experimental studies supporting putatively longer dispersal of epiphytic orchid seeds.
243 Nevertheless, disjunctive distributions of several orchid taxa occurring, for instance, in
244 remote islands (e.g., *Anacamptis pyramidalis* growing in Aldabra island and
245 Madagascar[60]) require dispersals over longer ranges as explanation. Authors have
246 already reported disjunctive distributions of several orchid species resulting from
247 putative long-distance dispersals[61,62]. However, to the best of our knowledge, no study
248 has yet reported ancient orchid long-distance dispersal across a geographical barrier
249 such as the Andes.

250

251 *Phylogenetic conflict between nuclear and plastid phylogenies in* Cycnoches

252 Our study brings important insights for the species relationships within
253 *Cycnoches*. Previous phylogenetic studies about Catasetinae have included not more
254 than three species of *Cycnoches*[42,43,45,46,50], hence keeping internal relationships (and
255 corresponding conflicts, see bellow) of the lineages unresolved. Serious phylogenetic
256 incongruence between 'nuc' and 'cp' tree topologies of Catasetinae has been firstly
257 identified by[63], but a discussion about the respective plausibility of the trees has been
258 remained undone.

259 All major clades in our 'nuc' phylogeny are consistent with morphological
260 concepts of *Cycnoches* (Fig. 1a). *Cycnoches hagii* (Clade A) differs from other
261 *Cycnoches* species by the fleshy oblong, curved pair set of calli located towards the base
262 of the labellum. Clade B includes species with conspicuously large male flowers and an
263 entire to 4-lobed labellum blade. Clade C comprises species with proportionately small
264 male flowers and labella with 8–10 marginal projections[48]. The consistence between
265 'nuc' molecules and morphology is challenged by an apparent correlation between the
266 'cp' molecular tree and the distributions of particular lineages (Fig. 1b): Clade I
267 comprises species occurring mostly in Amazonia, Caribbean-Llanos, and Guyana shield
268 regions (except *C. warzsewiczii* present in Panama and Costa Rica: Fig. S4). Clade II, in
269 turn, includes species exclusively distributed in Central America and Choco regions.

270 Topological incongruence between phylogenies derived from different DNA
271 data partitions is a widespread phenomenon in phylogenetic inferences[64,65]. Examples
272 from angiosperms (e.g., Araceae[37], Asteraceae[66], Saxifragaceae[67]) have long revealed
273 similar conflicting patterns, in which the 'nuc' phylogeny is in accordance with
274 morphology, whereas the 'cp' relationships correlate to geographical distributions. For

the particular case of *Cycnoches*, hybridization might have explanatory power for the nuclear-plastid conflict observed in the clade. Euglossine-bee-pollinated orchids such as *Cycnoches* produce a blend of volatile compounds, which attracts male Euglossine bees, and pollination takes place while bees collect such compounds produced by specialized tissues in the flower[68]. Species-specific production of floral blends and therefore attraction of a unique set of pollinator(s) has been accounted as an isolative reproductive barrier in Euglossine bee pollinated orchids[41,69]. Nevertheless, intra-specific variation of fragrances produced by the flower has been reported in several orchid lineages such as *Stanhopea*[70] and even *Cycnoches*[71]. Fragrance variation may result on attraction of a set of pollinators that are shared by species co-occurring in the same biogeographical region (e.g. *C. dianae, C. guttulatum*, and *C. pachydactylon*[71]; see also Fig. S4) with similar composition of the fragrance profile, providing an opportunity to hybridization to occur. Little is known about pollinators of *Cycnoches*, but our own observations of pollinator sharing between species and presence of polymorphic species may indicate past hybridization processes.

Conclusion

Based on a solid, comprehensively sampled phylogeny we provide macroevolutionary evidence for a South American origin of *Cycnoches.* Our biogeographical analysis indicates colonization of Central America via a direct migration from the Amazonian basin. More importantly, the analyses support three recent trans-Andean, bidirectional migration processes between Central America and Amazonia, which is indicative for a minor effect of the Andean barrier on swan orchids migration. Consequently, our study enlightens the limited role of Andean mountain

299 building on the range evolution and diversification of lowland Neotropical epiphytic
300 lineages.
301
302 **Material and methods**
303 *Taxon sampling, DNA sequencing and phylogenetic analyses*
304 Species names, geographical origins, voucher specimens, and GenBank
305 accession numbers of sequences included in phylogenetic analyses are provided in
306 Table S1. Our study builds-up upon the DNA data matrices generated by[40,46,63].
307 Genomic DNA was extracted from herbarium and fresh leaf material with the
308 NucleoSpin® plant kit (Macherey-Nagel; Düren, Germany). We amplified and
309 sequenced nuclear (consistently referred as 'nuc' henceforth) ribosomal external and
310 internal transcribed spacers (ETS and ITS, respectively), and a fragment of the *Xdh*
311 gene. We also sequenced a ~1500 bp fragment of the plastid (referred to as 'cp') gene
312 *ycf*1, as well as the *trn*S–*trn*G intergenic spacer. Amplification and sequencing settings,
313 as well as sequencing primers used for ITS, ETS, *Xdh*, *trn*S–*trn*G, and *ycf*1 are the same
314 as in[63,72] (Table S2). In this study, 84 sequences were newly generated (Table S1).
315 Loci were aligned separately using MAFFT 7.1[73]. For 'nuc' ribosomal RNA loci
316 and 'cp' *trn*S–*trn*G spacer, secondary structure of molecules was taken into account
317 (i.e., the -qINSi option). Congruence between 'nuc' and 'cp' datasets was assessed
318 following Pérez-Escobar et al.[63] and using PACo[74]. The procedure is available as a
319 pipeline (http://www.uv.es/cophylpaco/) and was also employed to identify operational
320 terminal units (OTUs) from the 'cp' dataset that conflicting with the 'nuc' dataset
321 (potential outliers detected by PACo are shown in Figs S1–S2). A detailed explanation
322 on PACo and a rationale on outlier handling is provided Extended Materials and
323 Methods of Appendix S1.

Phylogenetic analyses of separate and concatenated loci were carried out under maximum likelihood (ML) and Bayesian inference (BI). The best-fitting evolutionary models for ML and Bayesian analyses (for each data partition) were selected using jModelTest v.2.1.6[75], relying on a Likelihood Ratio Test (LRT) and the Akaike information criterion (AIC) (Table S3). Phylogenetic inference relied on the ML approach implemented in RAXML-HPC v.8.2.4[76] and BI as implemented in MRBAYES v.3.2.2[77] and were carried out on the CIPRES Science Gateway computing facility[78]. Bayesian inferences were performed with two independent runs, each with four Markov chains Monte Carlo (MCMC) running for 30 million generations each, and sampled every 1000th generation (all other prior settings by default). Log files derived from MRBAYES were examined, and the convergence of MCMC was assessed using TRACER (available at: http://tree.bio.ed.ac.uk/software/tracer/). Node support values were assessed for both the ML tree using Maximum Likelihood Bootstrap Support (MLBS) and the consensus Bayesian tree using posterior probabilities (PP).

Molecular clock dating

A few orchid macrofossils are available for Orchidaceae[79,80], but these are assigned to lineages very distantly related to our groups of interest. Using distant outgroups to calibrate our *Cycnoches* phylogeny would have created extensive sampling heterogeneities, which can result in spurious results[81]. Thus, we had to rely on secondary calibrations. In order to obtain the best secondary calibration points possible, we first generated an Orchidaceae-wide fossil-calibrated phylogenies, sampling 316 orchid species sampled as evenly as possible along the tree. Loci, number of sequences and settings for absolute age estimation of the Orchidaceae-wide fossil calibrate phylogeny are provided in the Extened Materials and Methods of Appendix S1. The

ages obtained were very similar to recent orchid dating studies[82,83] and the dated phylogeny is shown in Figure S3.

We selected two secondary calibrations for the dating of *Cycnoches*: (*i*) the crown group of Catasetinae was set to 19.8 Mya with a standard deviation of 4 to reflect the 95% CI, and (*ii*) and the root of the *Cycnoches* tree (i.e., MRCA of *Cyrtopodium* + Catasetinae) was set to 27.1 Mya with a standard deviation of 6. To explore the clock-likeness of the data, we used both strict clock and uncorrelated lognormal clock models, and compared different tree priors (pure-birth, standard birth-death, and incomplete sampling birth-death). For strict molecular clock calibration, we placed a single constraint only at the tree root (27.1 Mya with a standard deviation of 6) using a normal distribution. The best-fitting tree speciation model was selected using Bayes factors calculated from marginal likelihoods computed for every model using the stepping-stone sampling[84] (Table 1). For each clock model, we ran two MCMC analyses with 20 million generations each, sampled every 1000th generation. For the relaxed molecular clock analyses, we estimated the coefficient of variation (CV) to inform us on the rate heterogeneity among branches (CV approaching 0 indicates that a strict clock model cannot be rejected). Parameter convergence was confirmed using TRACER (http://tree.bio.ed.ac.uk/software/tracer/). All dating analyses were performed at the CIPRES Science Gateway computing facility[78].

Ancestral area estimations

Species ranges of *Cycnoches* of were coded from the collection site and type locality of the material sequenced, which reflect the distribution ranges for every taxa included in our phylogeny (except by *C. chlorochilon* and *C. pentadactylon,* which also occur in Central America and Southeastern South America, respectively; see bellow).

374 Distribution data of *Cycnoches* and outgroup taxa was obtained from own field
375 observations, literature[85,86] and from herbarium specimens (Herbaria AMES, COL, F,
376 M, MO, SEL, US); this information was employed to code outgroup distribution ranges.
377 Biogeographical areas were derived from distribution maps of the orchids under
378 investigation (Fig. S4) as well as species distributions observed in other plant lineages
379 (e.g., Rubiaceae[6]; Bromeliaceae[38]). We coded for eight biogeographical areas using the
380 R-package 'SpeciesGeocodeR'[87]: (1) Central America comprising southern Mexico
381 through Panama; (2) Caribbean-Llanos comprising the coastal northernmost areas and
382 plains of Colombia and Venezuela[88]; (3) Guiana Shield encompasses areas above 800 m
383 in Colombia, Venezuela, Brazil, Guyana, Suriname, and French Guiana; (4) Amazonia
384 encompassing lowlands and pre-montane forest below 800 m in Colombia, Ecuador,
385 Peru, Brazil, Venezuela, Guyana, Suriname, and French Guiana[6]; (5) Chocó comprising
386 lowlands below 500 m of the western Andes in Colombia and Ecuador; (6) Northern
387 Andes including elevated areas above 800 m from Southernmost Peru to Northern
388 Colombia and Northeast Venezuela; (7) Central Andes comprising areas above 800 m
389 in Northern Peru to Northern Chile and Northeast Argentina; (8) South-eastern South
390 America encompassing part of the Brazilian shield, the Atlantic forest, South-eastern
391 Bolivia, Paraguay, Uruguay, and Northern Argentina. A map with the eight
392 biogeographical areas, number of species for each area, and the corresponding
393 distribution records of *Cycnoches* plus outgroups are provided in Figure S4.

394 To infer ancestral areas in *Cycnoches*, we used the R-package 'BioGeoBEARS'
395 (Biogeography with Bayesian and Likelihood Evolutionary Analysis in R script[89]).
396 Using BioGeoBEARS, we tested the fit of six biogeographic models with and without
397 founder-event speciation (or jump speciation), altogether testing the role and
398 contribution of evolutionary processes that were taken into account to explain today's

399 observed distributions (i.e., range expansions, local extinctions, founder-event
400 speciation, vicariance, and speciation despite sympatry) in a joint statistical framework.
401 It is therefore capable of model testing and of determining, which process fits better the
402 geographical and phylogenetic data for any particular clade.

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Author contributions

O.A.P.E., M.G. and F.L.C. designed research; G.G. and O.A.P.E. collected samples; O.A.P.E. and E.P. performed all the lab work; O.A.P.E., F.L.C., and G.C. performed all analyses; O.A.P.E., M.G., F.C., G.C., G.G., B.K. and E.P. wrote the manuscript under the lead of O.A.P.E., M.G, and F.C.

Competing financial interest

The authors declare no competing financial interest.

Tables

Table 1. Marginal likelihoods of tree speciation models for relaxed and strict molecular clocks.

Table 2. Likelihood of biogeographic models as implemented in DEC, DIVA-like and BayArea-like.

Figures

Figure 1. Best scoring, ML tree of *Cycnoches* obtained from concatenated **A)** nuclear ETS, ITS, *Xdh* and **B)** plastid *trn*S-G, *ycf*1 loci. Node charts indicate Bootstrap Support (BS > 75), in where fully red diagrams indicate BS of 100. Numbers at nodes indicate Bayesian Posterior Probability (PP > 0.95). Representatives of each clade are shown in pictures: **1)** *C. haagii*; **2)** *C. ventricosum* (left) and *C. pentadactylon* (right); **3)** *C. herrenhusanum* (left) and *C. peruvianum* (right). The red arrows indicate autapomorphies of every clade: in 1) fleshy oblong, curved pair set of calli located towards the base of the labellum, 2) labellum blade entire to 4-lobed, and 3) labellum with 8-10 marginal projections. Geopolitical boundaries map generated by ArcMAP software (http://www.esri.com), using layers freely available at http://www.diva-gis.org/Data. Photos: O. Pérez & G.Gerlach.

Figure 2. Best scoring, ML tree of *Cycnoches* obtained from concatenated, non-conflicting 'nuc' ETS, ITS, and *Xdh* and 'cp' *trn*S–*trn*G and *ycf*1 loci. Node charts indicate Bootstrap Support (BS > 75), in where fully red diagrams indicate BS of 100. Numbers at nodes indicate Bayesian Posterior Probability (PP > 0.95).

Figure 3. Chronogram for *Cycnoches* obtained under a relaxed clock model, applied to a non-conflicting, concatenated 'nuc' (ITS, ETS, *Xdh*) and 'cp' loci (*trn*S–*trn*G, *ycf1*). Age

estimates, including maximum and minimum intervals for all nodes, are provided in Figure S5. Time scale is provided in million years ago (Mya). Node charts correspond to ancestral areas estimated under the BayArea-Like* model, including founder event process (*J*). Pink, orange and blue lines indicate mean elevations (m) on Colombian and Venezuelan Northern Andes, respectively (adapted from Hoorn et al., 2010). (Inset) Coded areas used for biogeographical analysis. Geopolitical boundaries map generated by ArcMAP software (http://www.esri.com). Political divisions and elevation data from DIVA-GIS (http://www.diva-gis.org/gdata).

Supplementary material

Appendix S1. Extended Materials and Methods, supplementary tables and figures.

Table 1

	Tree prior	MLE (SS)	CV	Yule	BD	BD sampling	Yule	BD	BD sampling
Strict clock	**Yule**	-14280,93	-	-					
	BD	-14267,36	-	27,14	-				
	BD sampling	-14264,73	-	32,4	5,26	-			
Relaxed clock	**Yule**	-14259,53	0,42	42,8	15,66	10,4	-		
	BD	-14245,01	0,35	71,84	44,7	39,44	29,04	-	
	BD sampling	-14244,47	0,35	72,92	45,78	40,52	30,12	1,08	-

Table 2

Model	LnL	AIC	AICc	AIC weighted
DEC	-119,3054256	242,6108513	242,9744877	1,06E-08
DEC+J	-110,4860209	226,9720418	227,7220418	2,63E-05
DEC*	-106,2412542	216,4825085	216,8461448	0,004984487
DEC+J*	-103,9600773	213,9201546	214,6701546	0,017948515
DIVA	-125,2827663	254,5655327	254,929169	2,68E-11
DIVA+J	-123,1895028	252,3790055	253,1290055	7,99E-11
DIVA*	-104,1616852	212,3233704	212,6870068	0,039880977
DIVA+J*	-104,9727468	215,9454937	216,6954937	0,006519762
BAYAREA	-131,2239925	266,4479849	266,8116213	7,04E-14
BAYAREA+J	-113,2503658	232,5007317	233,2507317	1,66E-06
BAYAREA*	-119,7393191	243,4786382	243,8422745	6,85E-09
BAYAREA+J*	**-100,011714**	**206,0234279**	**206,7734279**	**0,930638293**

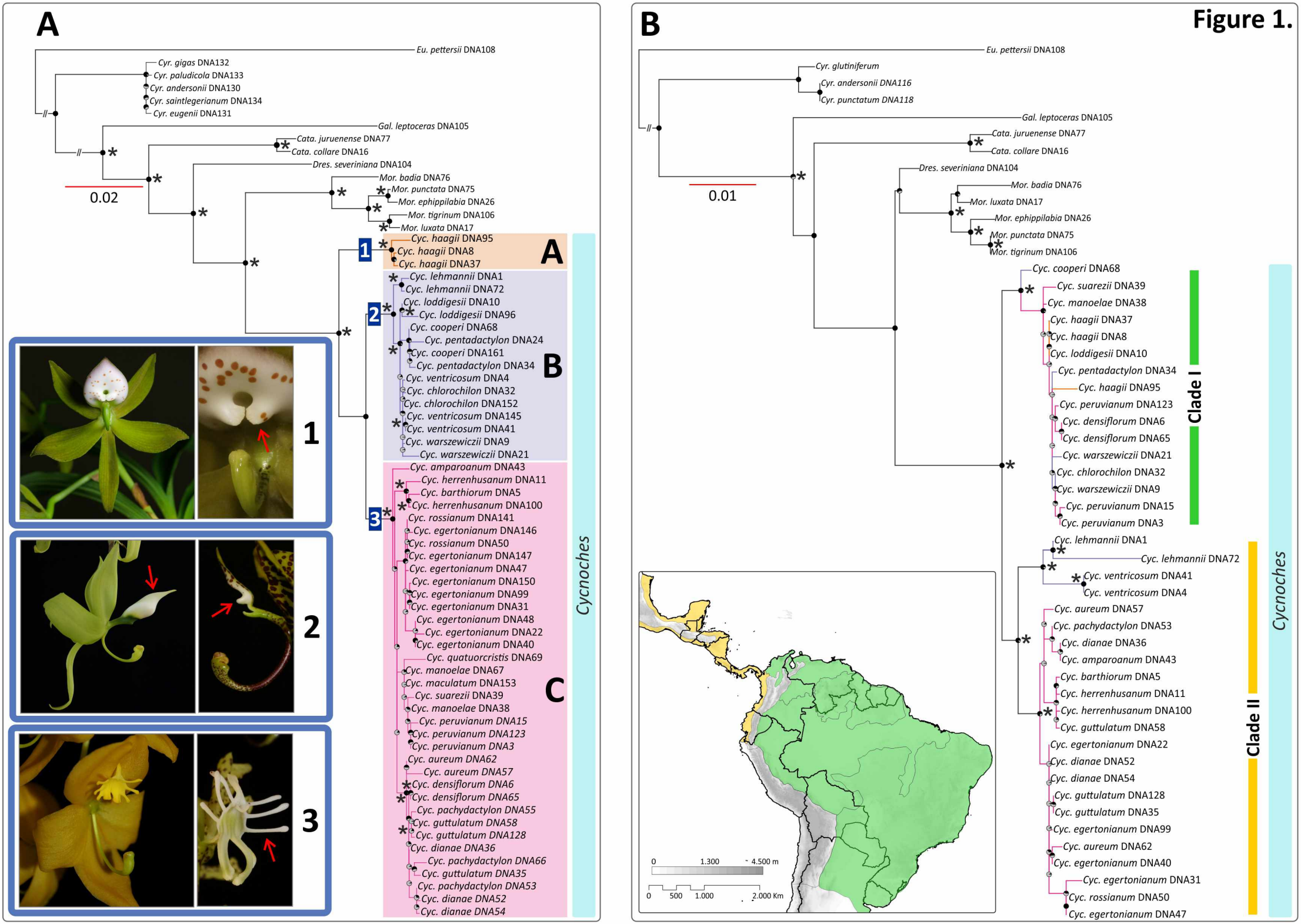
A
Eu. pettersii DNA108
Cyr. gigas DNA132
Cyr. paludicola DNA133
Cyr. andersonii DNA130
Cyr. saintlegerianum DNA134
Cyr. eugenii DNA131
Gal. leptoceras DNA105
Cata. juruenense DNA77
Cata. collare DNA16
Dres. severiniana DNA104
Mor. badia DNA76
Mor. punctata DNA75
Mor. ephippilabia DNA26
Mor. tigrinum DNA106
Mor. luxata DNA17
0.02
1
2
3
A
B
C
Cyc. haagii DNA95
Cyc. haagii DNA8
Cyc. haagii DNA37
Cyc. lehmannii DNA1
Cyc. lehmannii DNA72
Cyc. loddigesii DNA10
Cyc. loddigesii DNA96
Cyc. cooperi DNA68
Cyc. pentadactylon DNA24
Cyc. cooperi DNA161
Cyc. pentadactylon DNA34
Cyc. ventricosum DNA4
Cyc. chlorochilon DNA32
Cyc. chlorochilon DNA152
Cyc. ventricosum DNA145
Cyc. ventricosum DNA41
Cyc. warszewiczii DNA9
Cyc. warszewiczii DNA21
Cyc. amparoanum DNA43
Cyc. herrenhusanum DNA11
Cyc. barthiorum DNA5
Cyc. herrenhusanum DNA100
Cyc. rossianum DNA141
Cyc. egertonianum DNA146
Cyc. rossianum DNA50
Cyc. egertonianum DNA147
Cyc. egertonianum DNA47
Cyc. egertonianum DNA150
Cyc. egertonianum DNA99
Cyc. egertonianum DNA31
Cyc. egertonianum DNA48
Cyc. egertonianum DNA22
Cyc. egertonianum DNA40
Cyc. quatuorcristis DNA69
Cyc. manoelae DNA67
Cyc. maculatum DNA153
Cyc. suarezii DNA39
Cyc. manoelae DNA38
Cyc. peruvianum DNA15
Cyc. peruvianum DNA123
Cyc. peruvianum DNA3
Cyc. aureum DNA62
Cyc. aureum DNA57
Cyc. densiflorum DNA6
Cyc. densiflorum DNA65
Cyc. pachydactylon DNA55
Cyc. guttulatum DNA58
Cyc. guttulatum DNA128
Cyc. dianae DNA36
Cyc. pachydactylon DNA66
Cyc. guttulatum DNA35
Cyc. pachydactylon DNA53
Cyc. dianae DNA52
Cyc. dianae DNA54
Cycnoches
B
Eu. pettersii DNA108
Cyr. glutiniferum
Cyr. andersonii DNA116
Cyr. punctatum DNA118
Gal. leptoceras DNA105
Cata. juruenense DNA77
Cata. collare DNA16
Dres. severiniana DNA104
Mor. badia DNA76
Mor. luxata DNA17
Mor. ephippilabia DNA26
Mor. punctata DNA75
Mor. tigrinum DNA106
0.01
Cyc. cooperi DNA68
Cyc. suarezii DNA39
Cyc. manoelae DNA38
Cyc. haagii DNA37
Cyc. haagii DNA8
Cyc. loddigesii DNA10
Cyc. pentadactylon DNA34
Cyc. haagii DNA95
Cyc. peruvianum DNA123
Cyc. densiflorum DNA6
Cyc. densiflorum DNA65
Cyc. warszewiczii DNA21
Cyc. chlorochilon DNA32
Cyc. warszewiczii DNA9
Cyc. peruvianum DNA15
Cyc. peruvianum DNA3
Clade I
Cyc. lehmannii DNA1
Cyc. lehmannii DNA72
Cyc. ventricosum DNA41
Cyc. ventricosum DNA4
Cyc. aureum DNA57
Cyc. pachydactylon DNA53
Cyc. dianae DNA36
Cyc. amparoanum DNA43
Cyc. barthiorum DNA5
Cyc. herrenhusanum DNA11
Cyc. herrenhusanum DNA100
Cyc. guttulatum DNA58
Cyc. egertonianum DNA22
Cyc. dianae DNA52
Cyc. dianae DNA54
Cyc. guttulatum DNA128
Cyc. guttulatum DNA35
Cyc. egertonianum DNA99
Cyc. aureum DNA62
Cyc. egertonianum DNA40
Cyc. egertonianum DNA31
Cyc. rossianum DNA50
Cyc. egertonianum DNA47
Clade II
Cycnoches
0
1.300
4.500 m
0
500
1.000
2.000 Km
Figure 1.

Figure 2.

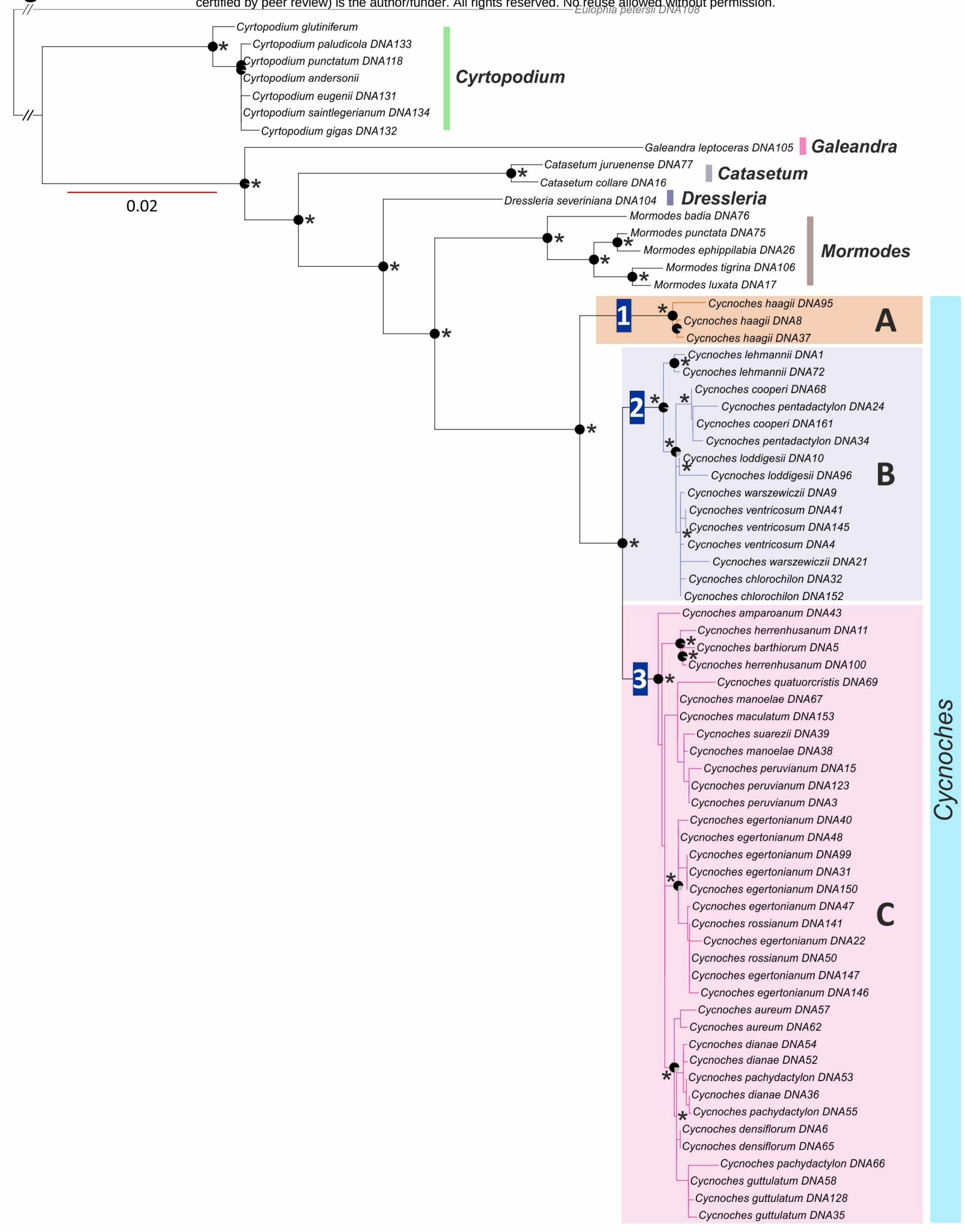

Figure 3.