



The phylogeny of *Heliconia* (Heliconiaceae) and the evolution of floral presentation



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ABSTRACT

Heliconia (Heliconiaceae, order Zingiberales) is among the showiest plants of the Neotropical rainforest and represent a spectacular co-evolutionary radiation with hummingbirds. Despite the attractiveness and ecological importance of many *Heliconia*, the genus has been the subject of limited molecular phylogenetic studies. We sample seven markers from the plastid and nuclear genomes for 202 samples of *Heliconia*. This represents ca. 75% of accepted species and includes coverage of all taxonomic subgenera and sections. We date this phylogeny using fossils associated with other families in the Zingiberales; in particular we review and evaluate the Eocene fossil *Ensete oregonense*. We use this dated phylogenetic framework to evaluate the evolution of two components of flower orientation that are hypothesized to be important for modulating pollinator discrimination and pollen placement: resupination and erect versus pendant inflorescence habit. Our phylogenetic results suggest that the monophyletic Melanesian subgenus *Heliconiopsis* and a small clade of Ecuadorian species are together the sister group to the rest of *Heliconia*. Extant diversity of *Heliconia* originated in the Late Eocene (39 Ma) with rapid diversification through the Early Miocene, making it the oldest known clade of hummingbird-pollinated plants. Most described subgenera and sections are not monophyletic, though closely related groups of species, often defined by shared geography, mirror earlier morphological cladistic analyses. Evaluation of changes in resupination and inflorescence habit suggests that these characters are more homoplasious than expected, and this largely explains the non-monophyly of previously circumscribed subgenera, which were based on these characters. We also find strong evidence for the correlated evolution of resupination and inflorescence habit. The correlated model suggests that the most recent common ancestor of all extant *Heliconia* had resupinate flowers and erect inflorescences. Finally, we note our nearly complete species sampling and dated phylogeny allow for an assessment of taxonomic history in terms of phylogenetic diversity. We find approximately half of the currently recognized species, corresponding to half of the phylogenetic diversity, have been described since 1975, highlighting the continued importance of basic taxonomic research and conservation initiatives to preserve both described and undiscovered species of *Heliconia*.

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1. Introduction

Heliconia L. is a charismatic member of the lowland tropical rainforests of the Neotropics and Melanesia, easily recognized by banana-like leaves and inflorescences with large, showy bracts. *Heliconia* is the sole genus in Heliconiaceae (order Zingiberales) and comprises approximately 194 recognized species (Govaerts and Kress, 2016) and a number of undescribed species (ca. 25, C. D.S., unpublished data). Most species were described in the last quarter of the 20th Century, and many were the result of substantial taxonomic research carried out by the late Lennart Andersson and W. John Kress in the 1980s and 1990s (Andersson, 1992,

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1985a, 1985b, 1981; Kress, 1990, 1984), which left only subgenus *Griggsia* as incompletely treated taxonomically, described partially in regional floras (Andersson, 1985b; Kress, 1984). Andersson and Kress also published cladistic morphological analyses which informed their infrageneric taxonomic systems (see Table 1; Andersson, 1992; Kress, 1984; Kress et al., 2004). Based on cladistic analyses, the genus was divided into five subgenera (Table 2), with three of these subgenera corresponding to taxa with putative synapomorphies involving flower orientation; either as sharing (1) twisting of the flower pedicel (resupination) or (2) whole inflorescence habit (erect versus pendant) (Table 2). Despite taxonomic and morphological interest in this genus and its important ecological presence in tropical forests, studies of the molecular diversity of *Heliconia* have been infrequent (but see: Isaza et al., 2012; Marouelli et al., 2010) or focused on population level processes (Cortes et al., 2013; Stein et al., 2014; Suárez-Montes et al., 2011; Westerband and Horvitz, 2015). Bridging the gap between our knowledge of the morphological diversity in *Heliconia* and its molecular diversity is necessary for understanding the evolution of the genus and for characterizing co-evolution with other lineages.

As an ubiquitous member of Neotropical rainforests, *Heliconia* is involved in a number of co-evolutionary relationships: as prey items for herbivorous rolled-leaf beetles (McKenna and Farrell, 2006; Strong, 1982, 1977), as hosts for parasitic floral mites (Colwell and Naskrecki, 1999; Dobkin, 1990, 1985; Pessoa et al., 2015), and, perhaps most famously, with their co-adapted hummingbird pollinators in the Neotropics (Fig. 1) (Feinsinger, 1978; McDade, 1983; Stiles, 1979; Taylor and White, 2007; Temeles et al., 2016, 2000; Temeles and Kress, 2003). At local scales, hummingbirds and *Heliconia* partition their mutual resources (nectar and pollination services) through co-adapted changes involving the length and curvature of the hummingbird bill and the *Heliconia* flower. This may involve pollination by different species of hummingbirds (Taylor and White, 2007) or even differential morphology and food plant preferences between the sexes of a single hummingbird species (Temeles et al., 2016, 2000; Temeles and Kress, 2003). The *Heliconia* species involved are often closely related species pairs (e.g., *H. bihai* and *H. caribaea* [Temeles and Kress, 2003] or *H. beckneri* and *H. tortuosa* [Taylor and White, 2007]), and partitioning pollinators and changing the location of pollen placement on a pollinator via differences in flower size, shape, and orientation serves to limit inter-species pollen interference (Galen and Gregory, 1989). Thus, such floral and inflorescence characters act as a pre-pollination reproductive barrier before prezygotic incompatibility barriers are established (Kress, 1983), and are likely closely connected to speciation in this genus.

While flower length and curvature are the primary means by which *Heliconia* modulates pollinator visitation preferences and behavior, changes in flower orientation via resupination and inflorescence habit should also impact pollination through both pollen placement and pollinator behavior (e.g., favoring perching versus hovering by hummingbird visitors; Taylor and White, 2007). These aspects of *Heliconia* reproductive morphology have been used to define subgenera (Table 2). If the current classification of *Heliconia* reflects evolutionary relationships, changes in these characters are expected to be relatively rare and their character states phylogenetically consistent (non-homoplasious) compared with changes in flower length and curvature. However, morphological analyses suggested that resupination and inflorescence habit may be more homoplasious than indicated by current classification (Kress, 1984). As a result, changes in flower orientation as either a consequence or driving force of diversification in *Heliconia* may be more common than previously considered.

The goal of this study is to infer a species level phylogeny for the genus, sampling from all subgenera and sections of *Heliconia*. This

Table 1

Comparison of recent and informal infrageneric classifications for *Heliconia*. Species composition is generally similar but not necessarily identical between the sections of these two systems (this disparity is most pronounced in the sections of subgenus *Griggsia*).

Andersson, 1992	Kress et al., 2004 (with updates by C.D.S.)
subg. <i>Heliconia</i>	subg. <i>Heliconia</i>
sect. <i>Episcopales</i> (Griggs) L. Andersson	sect. <i>Episcopales</i> (Griggs) L. Andersson
sect. <i>Heliconia</i>	sect. <i>Heliconia</i>
sect. <i>Tenebria</i> L. Andersson	sect. <i>Tenebria</i> L. Andersson
sect. <i>Tortex</i>	sect. <i>Tortex</i>
	sect. <i>Complanatae</i> Kress, ined.
	sect. <i>Farinosae</i> Kress, ined.
subg. <i>Heliconiopsis</i> (Miq.) Kress	subg. <i>Heliconiopsis</i> (Miq.) Kress
subg. <i>Stenochlamys</i> Baker	subg. <i>Stenochlamys</i> Baker
sect. <i>Cannastrum</i> L. Andersson	sect. <i>Cannastrum</i> L. Andersson
sect. <i>Lanea</i> L. Andersson	sect. <i>Lanea</i> L. Andersson
sect. <i>Lasia</i> L. Andersson	sect. <i>Lasia</i> L. Andersson
sect. <i>Proximochlamys</i> L. Andersson	sect. <i>Proximochlamys</i> L. Andersson
sect. <i>Stenochlamys</i>	sect. <i>Stenochlamys</i>
sect. <i>Zingiberastrum</i> L. Andersson	sect. <i>Zingiberastrum</i> L. Andersson
subg. <i>Taeniostrobos</i> (Kuntze) Griggs	subg. <i>Taeniostrobos</i> (Kuntze) Griggs
subg. <i>Griggsia</i> L. Andersson	subg. <i>Griggsia</i> L. Andersson
<i>H. griggsiana</i> group	sect. <i>Griggsia</i> ined.
<i>H. pogonantha</i> group	sect. <i>Barbatae</i> Kress, ined.
	sect. <i>Arcuatae</i> Kress, ined.
<i>H. longa</i> group	sect. <i>Longae</i> Kress, ined.
<i>H. platystachys</i> group	sect. <i>Pendulae</i> Kress, ined.
<i>H. rostrata</i> group	sect. <i>Rostratae</i> Kress, ined.
<i>H. trichocarpa</i> group	sect. <i>Sigmoideae</i> Kress, ined.
<i>H. obscura</i> group	sect. <i>Obscurae</i> Kress, ined.
<i>H. nutans</i> group	sect. <i>Contortex</i> Kress, ined.
	sect. <i>Dromedarius</i> Kress, ined.
	sect. <i>Retiformes</i> Kress, ined.

Table 2

Reproductive characters, plant habit, and distribution for the subgenera of *Heliconia* (Andersson, 1992, 1985a; Kress, 1990). Plant habit in *Heliconia* is either banana-like (musoid) with large petiolate leaves comprising a prominent pseudostem, ginger-like (zingiberoid) with a pronounced aerial stem with relatively small leaves arising along it at regular intervals, or somewhat in-between these two extremes (cannoid).

Subgenus	Reproductive characters	Plant habit	Distribution
<i>Heliconia</i>	Erect inflorescence, flowers non-resupinate ^a	Musoid	Neotropics
<i>Heliconiopsis</i>	Erect or pendant inflorescence, non-resupinate	Musoid	Melanesia
<i>Stenochlamys</i>	Erect inflorescence, flowers resupinate	Musoid, cannoid, zingiberoid	Neotropics
<i>Taeniostrobos</i>	Erect inflorescence, flowers resupinate or non-resupinate, bracts overlapping	Musoid	Neotropics
<i>Griggsia</i>	Pendant inflorescence, non-resupinate	Musoid	Neotropics

^a Andersson (1992) considered *Heliconia clinophila*, *H. gracilis*, *H. ignescens*, and *H. librata* to be resupinate (which he nonetheless placed in subg. *Heliconia*); based on photographic evidence (e.g., Berry and Kress, 1991) and personal observations (C.D.S.) we here consider these species to be non-resupinate, in agreement with the rest of the subgenus.

allows us to evaluate infrageneric classifications and evolutionary history of characters described in previous morphological analyses (Andersson, 1992; Kress, 1984; Kress et al., 2004). The dense species sampling included in this analysis permits macroevolutionary hypothesis testing, particularly in regards to correlations between the evolution of resupination and inflorescence habit. Although our species level sampling is nearly complete, species boundaries in *Heliconia* are often unclear because of complex patterns of reproductive and vegetative traits, both within and between species (Andersson, 1992, 1985a, 1981; Kress, 1990, 1984). Comprehensive

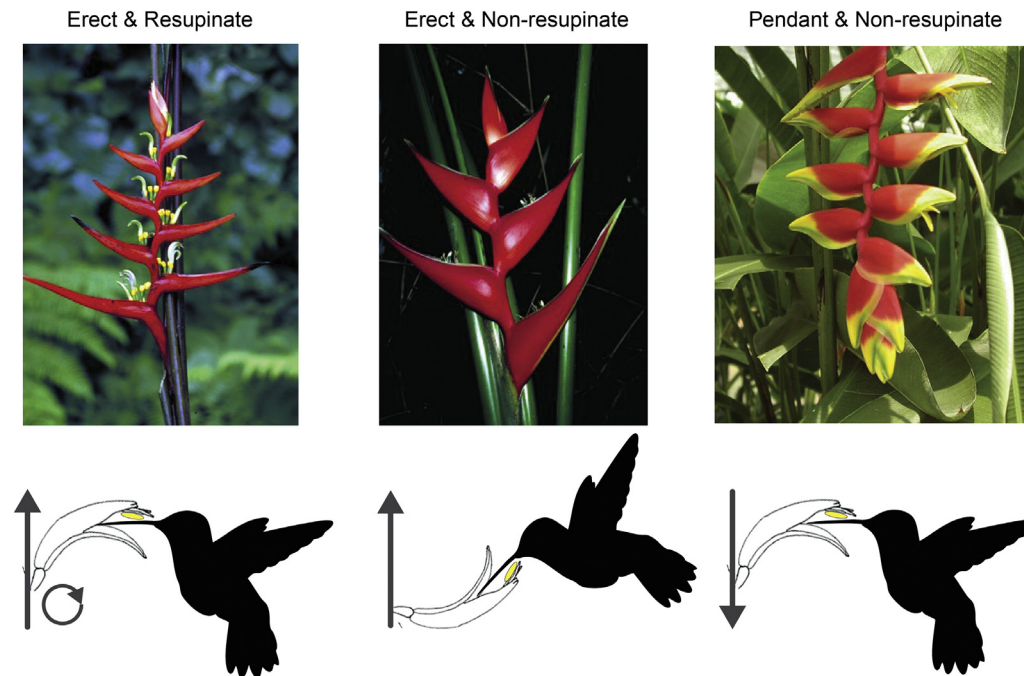


Fig. 1. Images of *Heliconia* species representing pendent and erect inflorescences and non-resupinate and resupinate flowers, demonstrating interactions with hummingbirds and the resulting position of placement of the pollen (yellow ovals) on the forehead/beak or chin of the pollinator. With pendent inflorescences (right), stamens are positioned dorsally with respect to an approaching pollinator resulting in pollen placement on the top of the beak or the forehead. This is also true with erect inflorescences bearing resupinate flowers (left, circular arrow). Erect inflorescences with non-resupinate flowers (center) present stamens at the ventral position, resulting in pollen deposition on the lower part of the bill or the chin of the visiting hummingbird. Species names from left to right: *H. acuminata*, *H. stricta*, *H. rostrata*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

population-level analysis of phylogenetic relationships in order to completely address species monophyly is beyond the scope of this project, and exemplars are used to represent most species. By focusing on macroevolutionary trends across a dense sampling of phylogenetic diversity, the problem with species boundaries should not affect our ability to understand the evolution of these reproductive traits.

2. Material and methods

2.1. Taxonomic sampling

Nomenclature generally follows Govaerts and Kress (2016) (for synonymies present in our sampling see Appendix A) and taxonomic circumscription primarily follows Andersson (1992, 1985a, 1985b, 1981) and Kress (1990, 1984). Names are not always consistently applied by the two authors; for example Andersson (1985a) considers Kress' (1984) *Heliconia curtispatha* and *H. stilesii* to be unwarranted segregates of *H. longa*, and considers Kress' *H. sessilis* to be a synonym of *H. curtispatha sensu* Andersson. Kress considers all these taxa to be distinct, with a close relationship between *H. curtispatha sensu* Kress and *H. stilesii*, but with *H. sessilis* distinct. Since the application of the name *H. curtispatha* is thus at odds, the species name in this analysis is followed by an 'A' for *sensu* Andersson or a 'K' for *sensu* Kress according to the original sample identification.

We obtained material from natural history collections (herbaria and botanical gardens) as well as targeted collecting in the wild (with vouchers deposited in natural history collections as indicated). Our strategy was to include samples from all 5 subgenera and all 23 sections identified by Kress et al. (2004) (see Table 1). In total, we include 202 samples that represent 152 of 194 accepted species *sensu* Govaerts and Kress (2016), one hybrid species, and five undetermined specimens. Seven of the samples

represent type collections. We sampled one to six species from each of the remaining seven families of Zingiberales (Cannaceae, Costaceae, Lowiaceae, Marantaceae, Musaceae, Strelitziaceae, and Zingiberaceae) as outgroups. Appendix B includes voucher data for all samples.

2.2. Genomic sampling

For genomic sampling we selected the plastid markers *trnL-F* and *trnL-rpl32*, the nuclear ribosomal internal transcribed spacers 1 and 2 (ITS) and the external transcribed spacer (ETS), and introns from the putatively single copy nuclear genes calmodulin (CaM), glyceraldehyde 3-phosphate dehydrogenase (G3PDH), and phosphoribulokinase (PRK). We used the following primers for amplification and sequencing: 'e' and 'f' from Taberlet et al. (1991) for *trnL-F*; 'trnL^{UAG}' and 'rpl32-F' from Shaw et al. (2007) for *trnL-rpl32*, 'ITS5A' and 'ITS4' from Stanford et al. (2000) and White et al. (1990) for ITS, '5'-primer' and '18S-IGS' from Baldwin and Markos (1998) and Kay et al. (2005) for ETS, newly developed primers 'CaMH-F' (5'-CGGAGATGGTTAGATCCCTTC-3') and 'CaMH-R' (5'-TTCTCGCCGAGGTTGGTCAT-3') for CaM, 'Fwd-Primer' from Sass and Specht (2010) and a newly developed primer 'G3A-R' (5'-TCCCTCBGATTCCTC CCG-3') for G3PDH, and newly developed primers 'PRK-F' (5'-CTTGACCCACCTGAGCTGATA-3') and 'PRK-R' (5'-TCTGTTGTGTACCTCTCCA-3') for PRK. G3PDH exists as two copies in *Heliconia* that are easy to distinguish based on sequence dissimilarity; here, we considered only a single copy, which we abbreviate G3A. DNA extraction followed modified CTAB or SDS protocols (Doyle and Doyle, 1987; Edwards et al., 1991; Konieczny and Ausubel, 1993) and PCR and thermocycler conditions follow standard and optimized protocols (details available upon request). Sanger sequencing was carried out using standard protocols on an Applied Biosystems 3100 in the Plant & Microbial Biology Department or a 3700 at the Evolutionary Genetics Laboratory

(UC Berkeley, Berkeley, CA USA). Sanger sequence base calling and contig assembly were done in Sequencher version 4.7 (Gene Codes Corporation, Ann Arbor, MI USA, www.genecodes.com).

For many samples taken from herbarium specimens, as well as for several silica dried ingroup and outgroup specimens, one or more of the seven gene regions proved recalcitrant to PCR and Sanger sequencing; these were obtained with an in-solution capture approach using PCR generated probes (SCPP, Peñalba et al., 2014). DNA was extracted from samples taken from herbarium sheets using a modified CTAB protocol (Doyle and Doyle, 1987). During extraction and library preparation, steps were taken to limit contamination from outside sources and increase total DNA yield: (i) extractions were performed in a laminar flow hood in a dedicated clean room free from PCR amplicons and using previously unopened reagents and filter tips; (ii) centrifugation and cold incubation steps were lengthened; (iii) library preparation steps (Meyer and Kircher, 2010) were performed separately for herbarium sampled material and those of fresh or silica dried leaf tissue.

For in-solution capture, PCR probes for the seven gene regions were generated using several individuals spanning the *Heliconia* phylogeny (based on a preliminary Sanger sequence only tree). All PCR products were gel extracted with a QIAquick gel extraction kit (Qiagen, Hilden, Germany, www.qiagen.com) to ensure probe purity. Genomic DNA was prepared for hybridization and sequencing as in [Meyer and Kircher \(2010\)](http://Meyer and Kircher (2010)). Probe generation, hybridization, and elution followed [Peñalba et al. \(2014\)](http://Peñalba et al. (2014)). We performed six captures corresponding to herbarium versus non-herbarium, plastid versus nuclear, and outgroup versus ingroup DNAs separately to limit capture competition between putatively difficult to capture and easy to capture DNA. In addition, all outgroups DNA was extracted from fresh or silica preserved (not herbarium) material. After capture, pools were subject to limited PCR enrichment, size verification with the Bioanalyzer DNA 1000 kit (Agilent Technologies, Santa Clara, CA USA, www.agilent.com), quantification by qPCR using KAPA Library Quantification kit (Kapa Biosystems, Wilmington, MA USA, www.kapabiosystems.com), and pooled based on qPCR, size, and proportional number of base pairs captured. The pool was sequenced on a MiSeq (Illumina, San Diego, CA USA, www.illumina.com) at the UC Davis Genome Center. After sequencing, reads were processed according to [Sass et al. \(2016\)](http://Sass et al. (2016)) with the addition that reads were evaluated for ancient DNA degradation with mapDamage2.0 (Jónsson et al., 2013). Additionally, some outgroups were selected from the dataset of [Sass et al. \(2016\)](http://Sass et al. (2016)). Sequence data from a putative hybrid taxon, *Heliconia* × *rauliniana*, was captured using the SCPP method from silica dried material and was readily phased into two distinct alleles for most of the non-plastid genes, which we label A and B indicating the two parental genomes. The final combined matrix is 78% complete, with gene specific completeness ranging from 56% to 90%.

2.3. Alignment and phylogenetic analysis

Each genic region was manually aligned in PhyDE 0.9971 (Müller et al., 2010) using published protocols (Graham et al., 2000; Graham and Olmstead, 2000). Two sections of ITS and of ETS were not alignable for the outgroup taxa and so these sections were removed for the outgroups only; for ITS, these correspond to positions 372–583 and 760–1026 and for ETS, to positions 1–113 and 202–600 in their respective alignments. Final alignment lengths are as follows: ITS, 1259 bp; ETS, 947 bp; *trnL-F*, 1078 bp; *trnL-rpl32*, 1856 bp; CaM, 1680 bp; G3A, 1161 bp, and PRK, 1580 bp.

We used PartitionFinder 1.1.1 (Lanfear et al., 2012) to find the optimal partitioning scheme and model of sequence evolution for the concatenated data set. The seven loci correspond to base subsets that are clustered. We used the greedy clustering algorithm

with all models considered (except those with a portion of invariant sites, see Yang, 2014) and branch lengths linked, and schemes were evaluated using the Bayesian information criterion (BIC). We repeated the procedure, but limited the models explored to only GTR+ Γ for downstream use in RAXML (Stamatakis, 2014), which does not allow for simpler models.

Maximum likelihood (ML) phylogenetic analysis was conducted using RAXML version 8.2.8 (Stamatakis, 2014) hosted on the CIPRES Science Gateway server (Miller et al., 2010). We used the optimal partitioned data set selected by PartitionFinder for RAXML which has four subsets corresponding to ITS, ETS, plastid regions combined, and single copy nuclear regions combined. We used the -f option to assess bootstrap support and full ML searches in a single run. For the full ML search, 12 replicates were evaluated under the GTR+ Γ_4 model of sequence evolution; for the bootstrap analysis, 1008 replicates were evaluated under the GTRCAT₂₅ model of sequence evolution (Stamatakis, 2006) using the rapid bootstrap algorithm (Stamatakis et al., 2008). We also conducted a Bayesian analysis in MrBayes version 3.2.6 (Ronquist et al., 2012), also hosted on CIPRES. Here, we used the optimal partitioned data set selected by PartitionFinder considering all models, which corresponds to all genes having their own subset except CaM and G3A, which were combined into a single subset. We implemented the partitioned analysis in MrBayes by allowing each partition its own mixed model (Ronquist and Huelsenbeck, 2003). We ran MrBayes with two independent analyses of four chains each for 5×10^7 generations sampling every 5×10^3 generations and used a burnin of 25% before downstream analysis. We ran all of these steps on both ingroup only and ingroup and outgroup data sets in order to explore the potentially stochastic influence of outgroups on ingroup relationships (Barrett et al., 2014; Graham et al., 2002; Graham and Iles, 2009).

Because of an unexpected placement for *Heliconia subulata* (see Phylogeny Results 3.2 and Fig. 2) we considered a single alternative constraint tree, in which members of subgenus *Griggsia* near *H. subulata* (subgenus *Stenochlamys*) are constrained to be monophyletic exclusive of *H. subulata*. We then re-evaluated the relationships of the small resulting polytomy using the -g option of RAXML. The original ML tree was then compared to this constraint tree using the approximately unbiased test (AU; Shimodaira, 2002) implemented in Consel version 0.1j (Shimodaira and Hasegawa, 2001). Individual linked loci (ITS&ETS, *trnL-F*&*trnL-rpl32*, CaM, G3A, and PRK) were also evaluated using RAXML. These individual loci trees were, in general, poorly resolved and topological relationships had limited support. Although there was some evidence of topological incongruence, we did not pursue coalescent methods or methods to detect hybridization due to the limited information content of these individual gene trees and large errors associated with their inference. Alignments and partitions are available from Figshare (doi: 10.6084/m9.figshare.4238951 and doi: 10.6084/m9.figshare.4238954).

2.4. Molecular dating

2.4.1. Fossil calibrations

There are no Heliconiaceae fossils for which character data would enable us to reliably calibrate the ingroup. Accordingly, we use two calibration points referable to Zingiberales and a root calibration referable to monocots. *Liliacidites* sp. 'A' represents the oldest reliable monocot fossil (Doyle and Hickey, 1976; reviewed in Iles et al., 2015); here we use its youngest age estimate (Aptian-Albian boundary) as a soft maximum age on the root of our phylogeny. This is a conservative estimate as crown Zingiberales (i.e., the limit of our taxon sampling) are unlikely to be as old as the oldest recorded monocot fossil. We use the fossil *Spirematospermum chandlerae* E.M.Friis as a calibration on stem

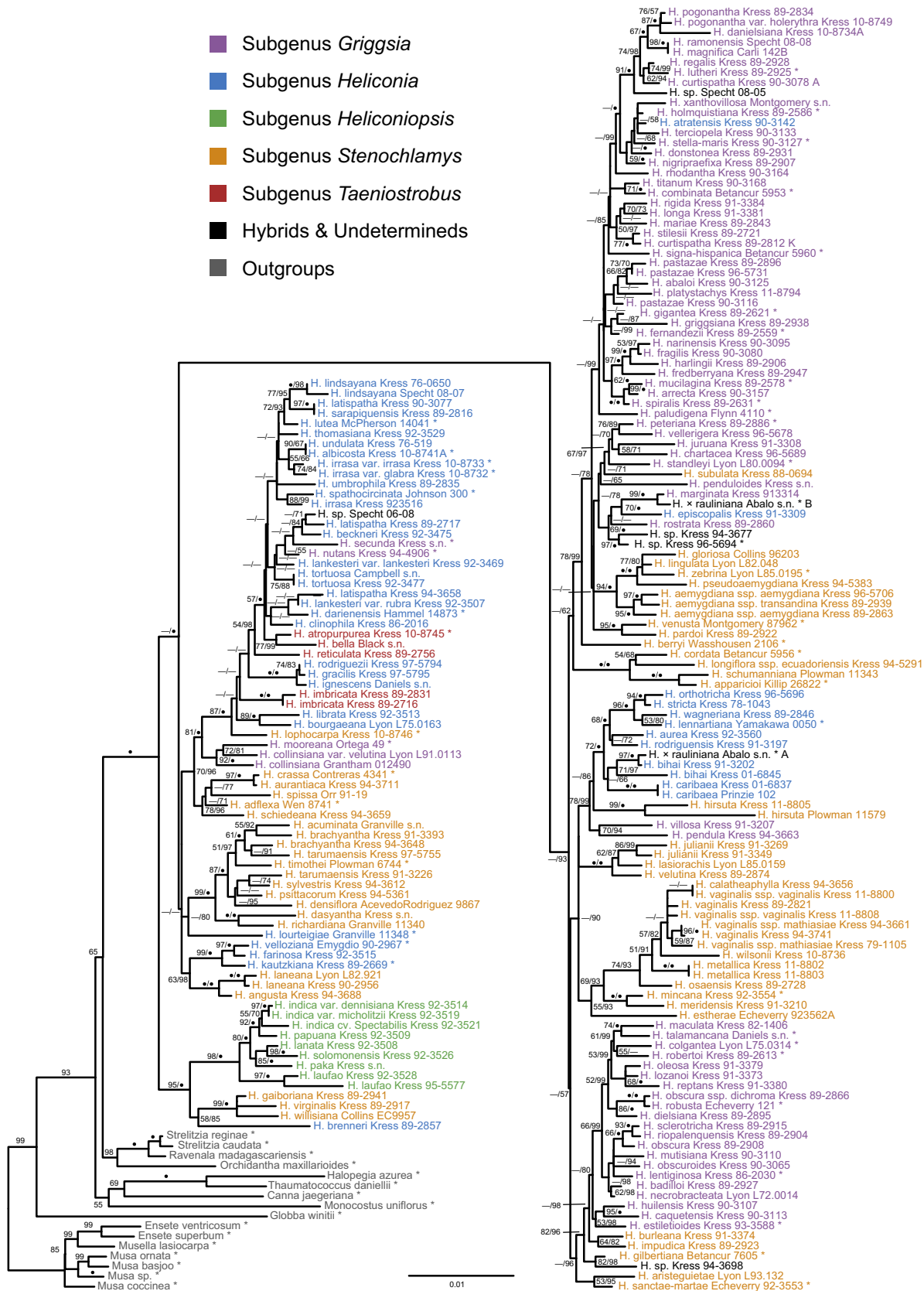


Fig. 2. Phylogeny of *Heliconia* estimated with a concatenated partitioned maximum likelihood (ML) analysis of intergenic plastid *trnL*-F and *trnL*-*rpl32*, nuclear ribosomal ITS and ETS, and nuclear markers *CaM*, *G3A*, and *PRK*. The analysis includes 203 *Heliconia* terminals that represent 202 *Heliconia* samples (*H. x rauliniana* was split into its parental components). An asterisk indicates those samples sequenced using the in-solution sequence capture method (see Text), except some outgroups were captured using the method of Sass et al. (2016). Support values are indicated adjacent to branches and are for ML bootstrap percentages and Bayesian posterior percentages, respectively. A dash indicates support <50%, while a filled circle indicates 100%. Note that the Bayesian analysis did not include outgroups (see Phylogeny Results 3.2) and so the Bayesian support for the root of Heliconiaceae is an artefact and only represents the unrooted bipartition support. Scale is in expected substitutions per site but outgroup branches are quarter length (including the stem branch of Heliconiaceae).

Zingiberaceae, following recent work suggesting that the fossil taxon belongs in Zingiberaceae (reviewed in: Iles et al., 2015 and Smith et al., 2013). Finally, we reviewed the taxonomic and geological context of *Ensete oregonense* Manchester & W.J.Kress (holotype: UF 6621, Florida Museum of Natural History), described from a suite of chalcedony and permineralized seeds from the middle Eocene Nut Beds flora, Clarno Formation, Oregon, USA (Manchester and Kress, 1993). The fossil possesses apomorphic and quantitative characters (a wide, deep hilum with a hilar rim and consistent seed size) that associate it with *Ensete* Bruce ex Horan. to the exclusion of other Musaceae (Manchester and Kress, 1993); we therefore consider it suitable for calibrating the stem node of *Ensete*. The Nut Beds flora and the larger Clarno Formation have been extensively radiometrically dated, with most dates falling between 44–43 Ma, with one older result at 48.3 Ma (reviewed in Hanson, 1996; Retallack et al., 2000). Some samples assignable to *E. oregonense* were also collected from near Wenatchee, Chelan Co., Washington, and initially attributed to the Swauk Formation (57–53 Ma) (reviewed in Manchester and Kress, 1993; fig. 8. Specimen OMSI-Pb 1061, however, their label is erroneous and the specimen should be UCMP 10689, pers. comm. Diane Erwin, U. California Museum of Paleontology, Berkeley, CA); however, more recent geologic maps suggest that this locality is more likely to be from the Roslyn/Chumstick Formation (46–43 Ma) (Cheney and Hayman, 2009), whose age is consistent with the age assigned to the Nut Beds flora. We thus discount the possible older dates for the Washington samples, and use the oldest date reported by B.D. Turrin in Manchester (1994) (i.e., 43.8 Ma) to calibrate *E. oregonense*.

2.4.2. Dating analysis

MCMCtree from PAML version 4.9a (Yang, 2007) was used to date the full ML tree with outgroups, following modification of the tree such that outgroups reflect hypothesized relationships based on more complete datasets (Sass et al., 2016). We used the approximate likelihood method where branch lengths are first estimated using ML, and the Markov chain Monte Carlo (MCMC) is performed on an approximation of the likelihood (Dos Reis and Yang, 2011). The HKY+ Γ_5 model of sequence evolution, which is the most complex model allowed using the approximate likelihood method, was used for tree building. While we initially tried using the optimal partitioning scheme, missing data resulted in problems during the ML branch length estimation stage; these were alleviated when we used a single combined subset. We used an autocorrelated model of rate evolution as a prior for the relaxed clock (Rannala and Yang, 2007). Several priors were initially estimated from exploratory analyses: the birth-death tree prior in MCMCtree and the gene substitution priors in the BASEML program from PAML (Yang, 2007). Calibration priors were implemented as uniform with soft bounds (Yang and Rannala, 2006). For calibration priors we considered the soft bounds to extend from the youngest possible age to 10 Myr past the oldest possible age of the fossil in question; for *Ensete oregonense* this is 53.8–43.8 Ma and for *Spirematospermum chandlerae*, which is less precisely dated, this is 93.6–72.1 Ma. *Liliacidites* sp. 'A' only has a soft maximum bound, which is 113 Ma (Fig. 3a). Because the calibration priors do not represent the actual time priors used during the MCMC run (Yang, 2014), we evaluated the actual time priors for suitability by running the analysis without sequence data (Fig. 3a). We ran the MCMC for 2.5×10^5 generations sampling every 5 generations and completed the analysis four times to ensure convergence.

2.5. Floral evolution

We gathered information on flower resupination and inflorescence habit (erect or pendant) from the literature (Andersson,

1992, 1985a, 1985b, 1981; Kress, 1990, 1984), with several exceptions enumerated here. *Heliconia brenneri* we consider to have an erect inflorescence as described in the original publication (Abalo, 1983) and based on personal observations (C.D.S., unpublished data) and not a pendant inflorescence as suggested by Andersson (1985a). We consider *H. clinophila*, *H. gracilis*, *H. ignescens*, and *H. librata* to be non-resupinate based on photographic evidence (Berry and Kress, 1991) and personal observations (C.D.S., unpublished data), not resupinate as suggested by Andersson (1992). All subsequent character evolution analyses were carried out on the MCMCtree dated phylogeny (Fig. 3) with outgroups and the putative hybrid, *Heliconia* \times *raulianiana*, trimmed prior to analysis. We tested for correlated evolution using a Bayesian extension of Pagel (1994) as implemented in the Discrete function in BayesTraits version 2.0 (Barker and Pagel, 2005). This method compares a dependent (correlated) model of evolution between two binary characters with an independent (un-correlated) model of evolution. We use the log-Bayes factor (BF) to compare the independent and dependent models of evolution. The model marginal likelihoods were estimated using the stepping stone method with 100 'stones' and 1.0×10^5 generations per stone. The MCMC analysis was run for 5×10^6 generations and sampled every 5×10^3 generations; burn-in was set at 5×10^4 generations. The Bayesian version allows us to enforce bounds via priors on poorly estimated rate parameters: for the *Heliconia* dataset, these are transitions in the dependent model involving the resupinate and pendant character state combination, which does not exist among living species (Table 2). We evaluated a number of priors: a uniform prior of 0–100 on rates, followed by exponential distribution priors with means of 10, 1, 0.1, and 0.01 ($\lambda = 0.1, 1, 10$, and 100, respectively). Transitions involving pendant and resupinate were variable among these prior options and did not converge, except for the exponential prior with 0.1 mean (Table 3). This process of prior evaluation was repeated for the independent model as well, in which case parameter estimates were stable and converged regardless of prior used (this is not surprising as all character states in the independent model are well represented in sampled species). In order to visualize ancestral states and obtain numbers of transitions between characters states, we used stochastic mapping (Huelsenbeck et al., 2003) using the estimated parameters of the Q matrix from the BayesTraits analysis as inputs. Stochastic mapping was performed in the R package Phytools version 0.5–38 (Revell, 2012) using a modified make.simmap function to allow for missing character states in the species data. We simulated 1000 mappings to approximate the posterior distribution.

Pagel's (1994) method of correlated evolution has recently been critiqued on the grounds that it produces spurious significant results under some conditions, such as when there is a single instance of two co-varying traits on the tree (Maddison and FitzJohn, 2015). Although Maddison and FitzJohn (2015) do not offer a definitive solution to this problem, they suggest use of the threshold model (Felsenstein, 2012, 2005; Wright, 1934) might side-step some of these issues. In this model, the discrete characters change when an unobserved quantitative trait (called a liability) crosses a threshold value. Felsenstein (2012, 2005) models the evolution of the liability with Brownian motion, for binary traits thresholds are set to 0 and variances to 1. The model can be extended to multiple traits where correlations can then be calculated between the liabilities. We use the Bayesian implementation of the threshold model implemented in the R package Phytools as it automatically provides an indicator of uncertainty through the posterior distribution (Revell, 2014). The threshBayes function was used with 1.0×10^8 generations, sampling every 1.0×10^4 .

All manual tree manipulations were performed in Mesquite version 3.03 (build 702) (Maddison and Maddison, 2009).

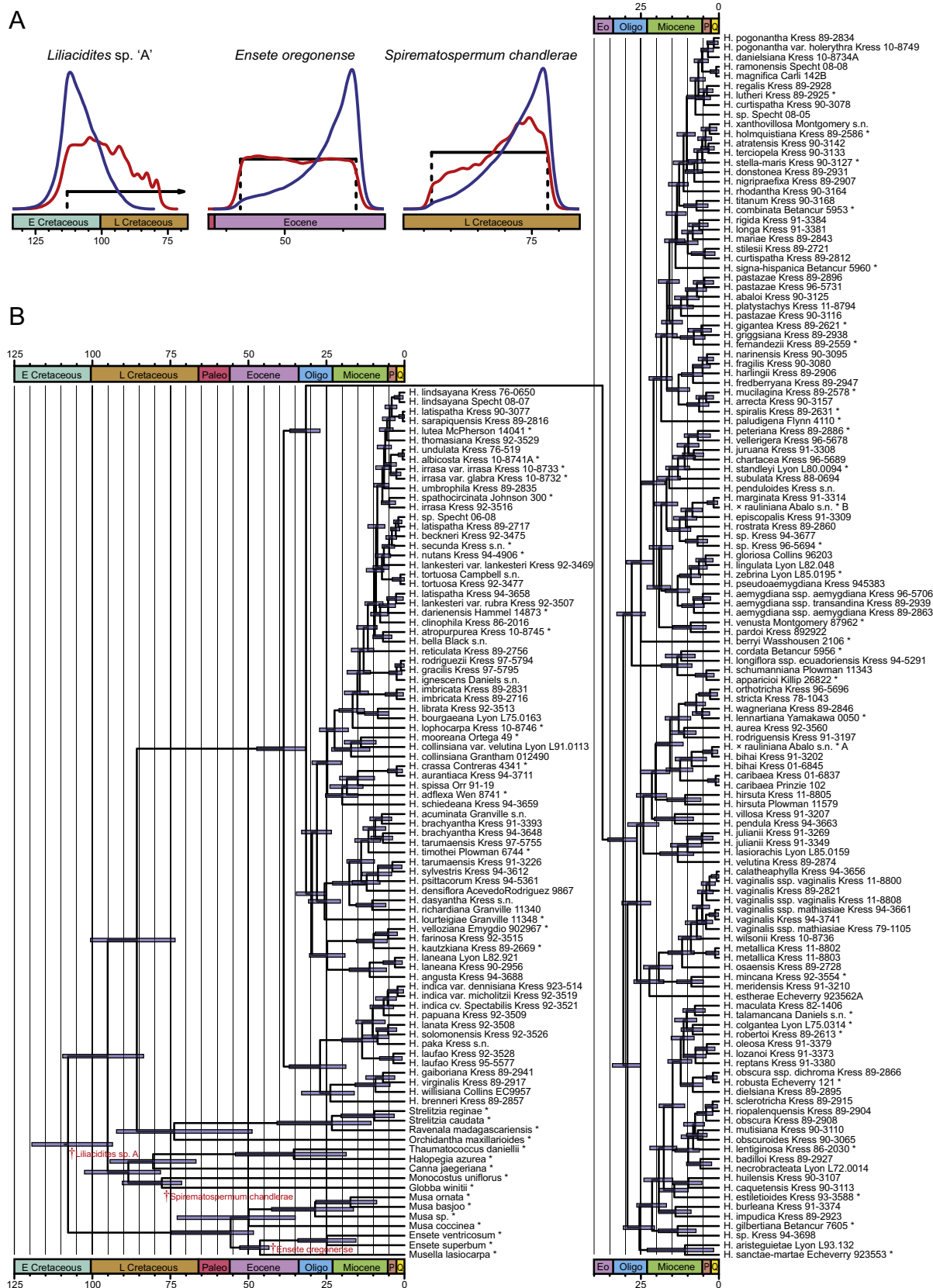


Fig. 3. *Heliconia* phylogeny through time. (a) Calibration prior, time prior, and posterior densities for the fossil constraints. The calibration prior (solid black line) with soft bounds (dashed black line) is the human-chosen prior. The time prior (red line) is the induced prior considering all the interactions with other calibration and model priors. The posterior (blue line) is the estimated time density for these calibrated nodes. Scale is relative density, areas under the curve integrate to 1, except for the calibration priors which integrate to 0.95 for *Ensete oregonense* and *Spirematospermum chandlerae* and 0.975 for *Liliacites* sp. 'A' (entire range to 0 Ma not shown but indicated by the arrow). (b) The dated *Heliconia* phylogeny (see Fig. 2). The bars associated with each node are the 95% HPD for that node's age. Fossil calibrations are indicated next to the associated node in red and with a dagger preceding them. Chronostratigraphic sequence follows Cohen et al. (2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

The effect of transition rate priors on parameter estimates and convergence in the analysis of correlated evolution between resupination and inflorescence habit for *Heliconia* species. The number of iterations (generations) remains constant between runs. The transition code, 'qij', is from the first state (i) to the second state (j). The states are as follows: 1, resupinate and erect; 2, resupinate and pendant; 3, non-resupinate and erect; 4, non-resupinate and pendant. 'Rt' is the inferred root state using the same codes as above. Parameter estimates are given as mean with 95% highest posterior density in parentheses.

	Uniform 0–100	Exponential 10	Exponential 1	Exponential 0.1	Exponential 0.01
–Log-likelihood	85.62 (89.71–82.46)	85.32 (88.90–81.65)	85.73 (90.20–82.46)	85.26 (85.26–88.88)	85.46 (85.46–89.57)
q12	0.008 (0–0.077) ^a	0.038 (0–0.13) ^a	0.020 (0–0.060) ^a	0.008 (0–0.017)	0.086 (0–0.26) ^a
q13	0.008 (0–0.015)	0.008 (0–0.014)	0.008 (0–0.015)	0.008 (0–0.015)	0.008 (0–0.015)
q21	0.14 (0.006–6.30) ^a	5.20 (0.009–11.88) ^a	1.35 (0.007–3.154) ^a	0.15 (0.002–0.36)	10.01 (3.92–13.92) ^a
q24	0.24 (0.032–3.54) ^a	1.79 (0.089–4.046) ^a	0.92 (0.092–2.11) ^a	0.21 (0.017–0.42)	1.20 (0–2.75) ^a
q31	0.011 (0–0.028)	0.012 (0–0.027)	0.012 (0–0.029)	0.011 (0–0.026)	0.012 (0–0.029)
q34	0.012 (0.002–0.026)	0.012 (0.002–0.024)	0.013 (0.003–0.026)	0.012 (0.002–0.023)	0.013 (0.002–0.025)
q42	0.007 (0–0.023) ^a	0.006 (0–0.015) ^a	0.007 (0–0.021) ^a	0.004 (0–0.010)	0.004 (0–0.010)
q43	0.005 (0–0.011)	0.005 (0–0.011)	0.005 (0.001–0.012)	0.005 (0–0.010)	0.005 (0–0.010)
Rt 1	0.52 (0–0.87) ^a	0.50 (0–0.76) ^a	0.54 (0–0.89) ^a	0.60 (0–0.93)	0.42 (0–0.61) ^a
Rt 2	0.23 (0–0.42) ^a	0.26 (0–0.45) ^a	0.20 (0–0.41) ^a	0.15 (0–0.39)	0.34 (0–0.47) ^a
Rt 3	0.24 (0–0.97)	0.23 (0–0.96)	0.26 (0–0.98)	0.25 (0–0.98)	0.24 (0–0.98)
Rt 4	0.005 (0–0.014)	0.004 (0–0.015)	0.004 (0–0.015)	0.002 (0–0.008)	0.004 (0–0.015)

^a Estimated sample size <200, no evidence of proper convergence.

2.6. Taxonomic and phylogenetic diversity

To explore the relationship between the taxonomic history of *Heliconia* and its phylogenetic diversity we used the species specific equal splits metric of evolutionary distinctiveness (Redding and Mooers, 2006) implemented in the R package Picante (Kembel et al., 2010). This was calculated on a per year basis whenever new species were described using the dated phylogeny (Fig. 3) trimmed to the species diversity known for that year. In cases where a species was represented by more than one sample in our phylogeny we chose a single exemplar.

3. Results

3.1. In-solution sequence capture

Sequence capture using PCR-generated probes worked effectively for all ingroup samples. All genes were captured for all individuals, although coverage varied. Samples with very low input DNA and all outgroup samples had the lowest average coverage, especially in low copy nuclear intronic DNA regions (Supplemental Fig. S1a–d). One herbarium specimen from 1923 had coverage comparable to silica dried specimens, although a second specimen from 1929 had very low input DNA and therefore coverage was very low, indicating that age of the herbarium specimen might not be as important as the pressing, drying, and storage conditions that might cause variability in DNA yield from extractions. Despite variable coverage in low copy nuclear intronic regions, input quantities of less than 1 ng of DNA resulted in coverage of plastid and nuclear ribosomal DNA greater than 20X. DNA damage as measured by 3' G to A and 5' C to T conversion in DNA extracted from herbarium specimens was not different from frequencies found for DNA extracted from silica-dried tissue.

3.2. Phylogeny

The ML phylogeny is characterized by short and poorly supported internal branches along the *Heliconia* backbone (Fig. 2, Supplemental nwk file 1). Subgenus *Heliconiopsis*, comprising the geographically disjunct Old World Melanesian species, is monophyletic and is the sister group to a small clade of Ecuadorian species; together these are sister to the rest of the family (Fig. 2). All previously defined subgenera for Neotropical species (*Stenochlamys*, *Heliconia*, *Grigisia*, and *Taeniostrobilus*) are not monophyletic, although groups of species that were placed in the same subgenus are often clustered together with high support (Fig. 2). Interest-

ingly, these smaller groups often represent species that had been placed in the same taxonomic section, largely based upon biogeographic affinities (data not shown). The placement of samples sequenced using SCPP (indicated by an asterisk in Fig. 2) accords with their taxonomic position. Likewise the phased parental components of the hybrid, *Heliconia* × *raulianiana*, were also correctly identified (i.e., *H. bihai* and *H. marginata*) using this method (Fig. 2). The ingroup-only ML phylogeny shows similar relationships and support values, but some backbone branches had higher support when outgroup taxa were excluded (Supplemental nwk file 2). The Bayesian analysis that included outgroups failed to converge; despite this, general relationships, including the root for Heliconiaceae, remain the same as found in the ML tree including outgroups (Supplemental nwk file 3). These also mirrored the Bayesian ingroup phylogeny, which showed appropriate estimated sample size statistics (i.e., >200) and similar log-likelihood scores between the two runs (Fig. 2, Supplemental nwk file 4). However, the average standard deviation of split frequencies remained between 0.03 and 0.04 for the last 2 × 10⁷ generations. The constrained tree (Supplemental nwk file 5) differed significantly from the unconstrained tree in the AU test, and is not considered further (p-value <0.05).

3.3. Dated phylogeny

The calibration priors impose similar time priors indicating that prior interactions of the whole model are not inducing an unreasonable set of time priors (Fig. 3a). Replicated MCMC runs indicated appropriate convergence. The dated phylogeny (Fig. 3b, Supplemental nwk file 6) suggests that Heliconiaceae split from other Zingiberales during the Late Cretaceous (86 Ma, 101–73 Ma; mean, 95% highest posterior density [HPD]) and that current biodiversity of *Heliconia* originated in the Eocene to early Oligocene (39 Ma, 47–32 Ma; mean, 95% HPD) followed by an initially rapid diversification that subsequently slowed down by the Miocene (Fig. 3b).

3.4. Floral evolution

We find very strong evidence of correlated evolution between resupination and inflorescence habit (BF = 27) using the correlation test of Pagel (1994). Ancestral state reconstruction using the correlated model is shown in Fig. 4b. Resupinate flowers on an erect inflorescence is the most likely ancestral state combination for *Heliconia*. Pendant inflorescences with non-resupinate flowers evolved convergently multiple times (ca. 13 times), from either

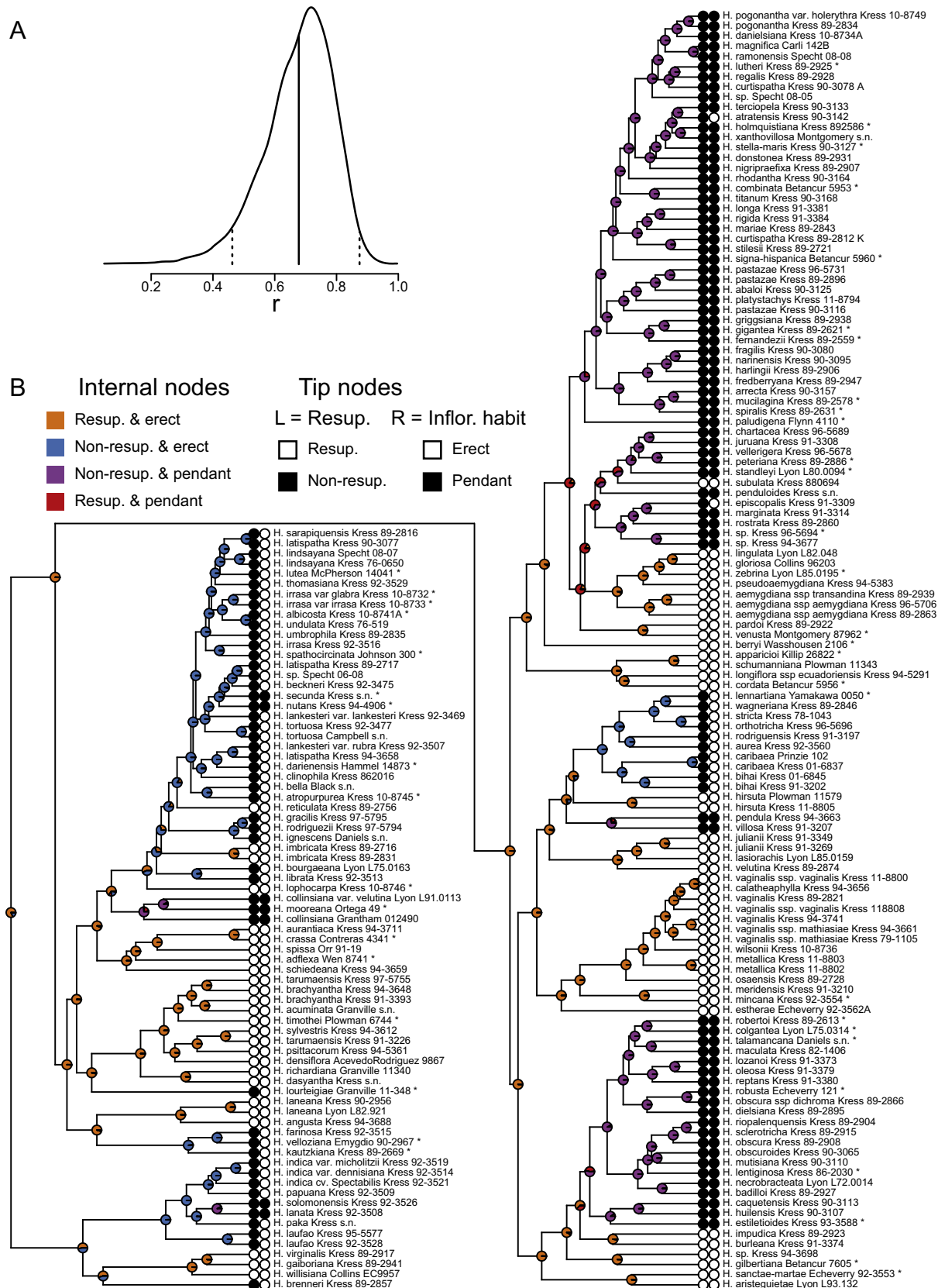


Fig. 4. Correlation and ancestral state reconstruction of resupination and inflorescence habit for *Heliconia* based on the dated tree. (a) The posterior distribution of the correlation coefficient (r) for the liabilities of resupination and inflorescence habit under the threshold model. Solid and dashed lines represent the mean and 95% HPD values for the correlation coefficient, respectively. Note that the posterior density does not cross $r = 0$. (b) Ancestral (joint) character states were estimated using the stochastic mapping method based on the BayesTraits analysis parameter values for correlated (dependent) evolution. Individual character states are shown for sampled species to show their co-distribution.

non-resupinate and erect (ca. 4 times) or resupinate and pendant (ca. 9 times) character state combinations (Fig. 4b, Supplemental File 1). The threshold model also finds strong evidence for correlation between the underlying liabilities (Fig. 4a, $r = 0.68$, 0.46–0.87; mean, 95% HPD). The root ancestral liability for inflorescence habit is 5.2 (0.5–9.5 95% HPD) standard deviations lower than that for resupination.

3.5. Taxonomic and phylogenetic diversity

Approximately half of the known species (Fig. 5) and half the cumulative phylogenetic diversity (Fig. 6) have been described since 1975, this includes many species with individually high evolutionary distinctiveness scores (Fig. 6).

4. Discussion

The phylogeny and character evolution of *Heliconia* suggest a rapid and morphologically complex radiation starting in the Eocene within the Neotropics (Fig. 3). Although the backbone of *Heliconia* remains largely unresolved, strongly supported subclades occur throughout the tree (Fig. 2), many of them with a mix of resupination and inflorescence habit character states (Fig. 4). As a result, any resolved and well-supported phylogeny will be marked by substantial plesiomorphy and homoplasy for these reproductive characters. The evolution of resupination and inflorescence habit are strongly correlated ($r = 0.64$, Fig. 4a), consistent with the hypothesis that these aspects of flower orientation are influenced by selection by hummingbirds. The correlated model also suggests that resupinate flowers on an erect inflorescence is the ancestral state combination, and that non-resupinate flowers on either erect or pendant inflorescences are derived states (Fig. 4). In addition, our in-solution sequence capture proved effective at recovering sequence data from otherwise recalcitrant specimens, including herbarium specimens (Fig. 2, Suppl. Fig. S1).

4.1. Species, classification, and phylogeny

The phylogeny of *Heliconia* is characterized by very short backbone branches that lack statistical support (Fig. 2). However, many shallow branches are well supported, allowing us to suggest sets of relationships that can inform species boundaries and infrageneric taxonomy.

Infrageneric taxa of *Heliconia* frequently cluster together within the phylogeny, but are only rarely completely monophyletic. Only the subgenus *Heliconiopsis*, which is geographically disjunct in the islands of Melanesia, is monophyletic; the other subgenera are all non-monophyletic. The non-monophyly of subgenus *Stenochlamys*, which is defined in part by resupinate flowers borne on erect inflorescences (Andersson, 1985a), can largely be explained by the plesiomorphic nature of this character state combination (see Fig. 4). Similarly, the polyphyly of the other Neotropical subgenera, especially the large subgenera *Heliconia* and *Griggsia*, can be explained by the convergence of other combinations of the traits we examined; this includes *Heliconia* with erect inflorescences and non-resupinate flowers, and *Griggsia* with pendant inflorescences and non-resupinate flowers. Early morphological analyses predicted this result by suggesting three independent origins of pendant habit among Central American species (Kress, 1984).

While the subgenera of *Heliconia* were not found to represent lineages, many of the sections were found to be monophyletic or nearly so. For example, subgenus *Griggsia* sections *Pendulae* and *Barbatae* are each largely monophyletic, consistent with a previous morphological analysis (Andersson, 1992); these subclades could be considered subgenera in an updated classification once the phy-

logeny is more completely resolved. Additional sections are found to be nearly-monophyletic including sections *Lasia*, *Lanae*, *Cannastrium*, and *Zingiberastrum* in subgenus *Stenochlamys*, and sections *Farinosae* and *Tortex* in subgenus *Heliconia*, among others. Interestingly, the two species placed in *Griggsia* section *Contortex* (*Heliconia nutans* and *H. secunda*) are resolved in a clade comprising members of subgenus *Heliconia* section *Tortex*, supporting observations by both Andersson (1985b) and Kress (1984) that these taxa appear affiliated to section *Tortex*, despite their pendant inflorescences that placed them taxonomically within subgenus *Griggsia* in the traditional classification scheme. The monophyly or near monophyly of many of these sections largely mirrors biogeography; for example, *Heliconia* sections *Heliconia*, *Zingiberastrum*, and *Tortex*; *Stenochlamys* sections *Lanae* and *Stenochlamys*; *Griggsia* section *Sigmoidea* among others. This indicates that there is a trend for regional diversification with little subsequent dispersal, and that biogeography should inform future classifications schemes, as is currently in this case with the monophyletic subgenus *Heliconiopsis*. Further, we provide strong evidence for the convergent nature of floral resupination and inflorescence habit, suggesting that while these traits cannot be used to define monophyletic taxa in classification, their evolution is incredibly dynamic, and likely driven by selection by hummingbird pollinators.

The short internal branches of the phylogeny of *Heliconia* suggest that methods that incorporate models of incomplete lineage sorting are appropriate for this group. Further supporting the use of these methods, we find that only 46% of 28 species (according to Govaerts and Kress, 2016; Fig. 2) with multiple samples are monophyletic and we detect some instances of inter-gene incongruence. These results are not necessarily surprising as incomplete lineage sorting is expected to produce conflicts between species and gene trees and among gene trees (Maddison, 1997; Rieseberg and Brouillet, 1994). Methods that account for this biological process, such as *BEAST or ASTRAL (Heled and Drummond, 2010; Mirarab et al., 2014) should be applied to this group in the future. However, the application of these methods was not possible in this study due to a limited number of loci and few samples per species as well as the lack of resolution recovered in individual gene trees. In addition to biological causes, species non-monophyly can also result from taxonomic or phylogenetic inference error. Given the complex set of patterns displayed by morphological characters in *Heliconia* (Andersson, 1992, 1985a, 1985b, 1981; Kress, 1990, 1984), the lack of recent revisions for newly described species (Fig. 5), and only partial coverage of subgenus *Griggsia* (Andersson, 1985b; Kress, 1984), taxonomic error may be the cause of some of the species non-monophyly seen in our phylogeny. Biological, taxonomic, and phylogenetic inference issues are amenable to resolution with greater sampling of individuals within putative species-complexes and increased sequence data for these individuals (e.g., Huelsenbeck et al., 2011; Yang and Rannala, 2014).

4.2. Flower orientation and character evolution

Results from Pagel's test (1994) and from Felsenstein's threshold model (2012, 2005) provide strong evidence for the correlated evolution of resupination and inflorescence habit in *Heliconia* (Fig. 4). In living species, pendant inflorescences always have non-resupinate flowers, while species with erect inflorescences may have either resupinate or non-resupinate flowers. A consequence of using the correlated evolution model (Fig. 4) is the ephemeral existence (<2% of total tree time, Supplemental File 1) of a resupinate and pendant character state combination, which is not observed in living species. Despite the short time that is spent in this combination of character states, twice as many origins of the non-resupinate and pendant combination originate through

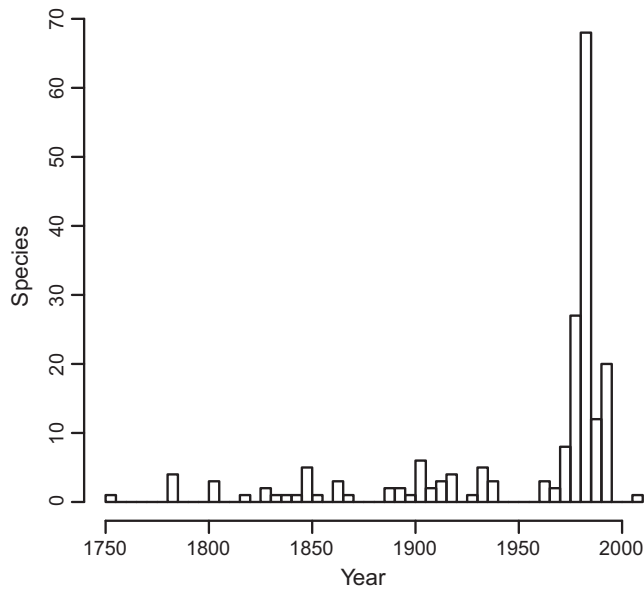


Fig. 5. Histogram of the publication date for currently accepted *Heliconia* species names. Accepted species names are according to Govaerts and Kress (2016) and publication date corresponds to the date of publication for the species name (or its basionym where applicable).

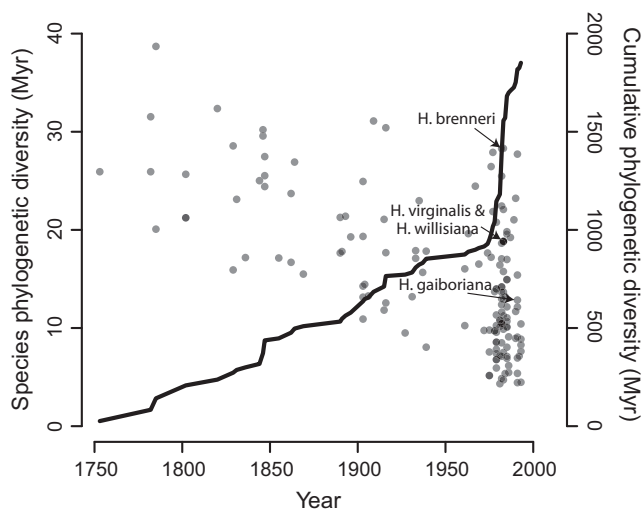


Fig. 6. Taxonomic history of *Heliconia* and its relation to phylogenetic diversity. Points (left-hand scale) correspond to species specific phylogenetic diversity calculated using the equal splits metric of evolutionary distinctiveness. The line (right-hand) corresponds to the cumulative phylogenetic diversity which is simply tree length trimmed to the species known for that year. Four species that belong to the Ecuadorian clade are highlighted (see Conclusion 5).

this hypothetical intermediary than through non-resupinate and erect (which accounts for 34% of total tree time, Supplemental File 1). This appears to be a function of the relatively high transition rates entering and exiting this character state combination (i.e., q12, q21, q24 in Table 3), despite efforts to constrain these rates through priors (note that the prior we use, exponential 0.1, gives lower rate estimates than the other priors, see Table 3). Given these high rates, it is tempting to interpret transitions of this type as representing a more-or-less simultaneous change from resupinate and erect to non-resupinate and pendant, although technically this transition is not allowed by the model. An alternative to trying to control the rates involved in unobserved character state combinations is to simply remove them from the model

completely (Beaulieu and Donoghue, 2013). We do not attempt that here because it makes interpreting the result of Pagel's test difficult, and, unlike in some scenarios, there is nothing intrinsically implausible about a resupinate and pendant inflorescence.

Given the spatial and temporal separation in the development of whole inflorescence morphology and individual pedicels, it seems that correlations that arise between these two characters are unlikely to result from developmental constraints (Specht and Howarth, 2015) and are more likely indicative of a response to pollinator-mediated selection pressure. In our framework, we consider changes in flower orientation through resupination and inflorescence habit to be the product of co-adaptation with hummingbirds as a result of resource partitioning by both parties (Stiles, 1979). However, co-evolutionary studies have focused on the effect of flower size and shape, not resupination or inflorescence habit (Taylor and White, 2007; Temeles et al., 2016, 2000; Temeles and Kress, 2003). It would be informative to study a system where closely related species of *Heliconia* differ in resupination or inflorescence habit, such as members of subgenus *Griggsia* section *Contortex* and subgenus *Heliconia* section *Tortex*, to see if similar levels of pollinator partitioning occur. Our focus has been on hummingbird pollinators, but we note that the Melanesian subgenus *Heliconiopsis*, that also contains a pendant lineage (Fig. 4), is bat pollinated (Kress, 1985). This appears to mimic the situation in the closely related Old World banana family (Musaceae, Zingiberales), which comprises species with erect and pendant inflorescences that are bat pollinated (Andersson, 1998).

In both *Heliconia* and Musaceae, species with pendant inflorescences comprise the largest species (overall above-ground body size) and species with the longest inflorescences (Andersson, 1998; C.D.S., W.J.D.I., unpublished data). For example, *Heliconia gigantea* has a 3 m long pendant inflorescence and a pseudostem (overlapping leaf petioles) 6.5 m tall (Kress et al., 1993). The evolution of pendant inflorescences may facilitate gigantism by lowering the plant's center of gravity, possibly creating a more biomechanically stable configuration. This is relevant in both *Heliconia* and Musaceae as all the mechanical support is born by the pseudostem and no secondary growth is possible in these overgrown monocotyledonous herbs. Alternatively, a pendant inflorescence likely has a lower construction cost due to a reduced need for structural elements, thereby reducing the marginal cost of each additional bract and facilitating the evolution of longer inflorescences in pendant species. A comparative study of both these closely related families together with the large erect inflorescences found in the woody (*sensu* Fitzjohn et al., 2014) Strelitziaceae (e.g., the Madagascan traveler's palm, *Ravenala* Adans.) would reveal the magnitude and extent of this possible association between pendant inflorescences and inflorescence length as well as overall plant size.

The period of rapid speciation in *Heliconia* during the Oligocene and early Miocene (Fig. 3) is co-incident with radiations in *Heliconia*'s predators, the hispine rolled-leaf beetles (McKenna and Farrell, 2006) and in *Heliconia*'s Neotropical pollinators, the hummingbirds (McGuire et al., 2014). This suggests that ancient co-diversification between *Heliconia* and hummingbirds may have driven morphological diversification in *Heliconia*, and has likely continued to do so since then, just as it does in current ecosystems (Feinsinger, 1978; Stiles, 1979; Taylor and White, 2007; Temeles et al., 2016, 2000; Temeles and Kress, 2003). Dating analyses of extant hummingbirds suggest a much younger age—22 Ma—of *Heliconia*'s obligate pollinators, the hummingbirds (McGuire et al., 2014). However, there is a substantial gap between Neotropical crown group hummingbirds and the only known stem fossil (*Eurotrochilus inexpectatus* Mayr, 2004), an Old World taxon from ca. 32 Ma in Frauenweiler, Germany (Mayr, 2007, 2004). The age of this fossil overlaps with the HPD age estimate of crown group

Heliconia. This suggests that *Heliconia* may have been one of the first clades to interact with the stem-group hummingbirds that dispersed to the New World and perhaps paved the way for a more expanded hummingbird-pollinated plant guild. The old age of *Heliconia* relative to other hummingbird-pollinated clades further supports the hypothesis that *Heliconia* underwent diffuse co-evolution with hummingbirds (*sensu* Tripp and McDade, 2013), bolstered by the correlated evolution of traits involved in precise pollen placement during this interaction. Hummingbird pollination in other taxa was likely facilitated by this pre-existing relationship, and explains the much younger crown ages inferred for other species-rich, largely hummingbird pollinated clades (e.g., Neotropical Campanulaceae–Lobelioideae at ca. 5 Ma: Lagomarsino et al., 2016; Acanthaceae–Ruellia at ca. 9 Ma: Tripp and McDade, 2013; Gesneriaceae–Columneinae at 22.4 Ma: Roalson and Roberts, 2016; *Passiflora* supersection *Tacsonia* at ca. 8.4 Ma: Abrahamczyk et al., 2014; Bromeliaceae at 22.7 Ma: Givnish et al., 2014).

4.3. Conclusion

The spectacular radiation of *Heliconia* reveals that characters related to flower orientation evolve in a correlated fashion and are much more homoplasious than previously indicated by taxonomic subdivisions. Flower orientation is clearly important for precise pollen presentation, especially in large-bodied pollinators such as hummingbirds; this has direct implications for the evolution of reproductive isolation, and thus may be important in the speciation process in *Heliconia*. About half the species diversity in *Heliconia* has been discovered since 1975 (Fig. 5), which mirrors the large number of Amazonian tree species described in the second half of the 20th Century (ter Steege et al., 2016). Crucially, many of these newly described *Heliconia* species are phylogenetically distinct, adding substantial amounts of evolutionary history to *Heliconia* phylogeny (see right-hand Fig. 6). Perhaps the most phylogenetically important species described during this time include those Ecuadorian species that, along with the Melanesian *Heliconiopsis*, comprise the sister group to all other *Heliconia* species (see left-hand Fig. 6). This may indicate that there is unsampled biodiversity and that continued taxonomic exploration is of ongoing importance for understanding extant diversity and setting conservation priorities for *Heliconia* (EDGE, 2016; Isaac et al., 2007).

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Appendix A

The nomenclature used herein for *Heliconia* generally follows the checklist of Govaerts and Kress (2016) with a few exceptions listed below.

Our phylogeny	Govaerts and Kress (2016)
<i>H. aristeguietae</i>	<i>H. scarlatina</i>
<i>H. aurea</i>	<i>H. bihai</i>
<i>H. gilbertiana</i>	<i>H. scarlatina</i>
<i>H. laneana</i>	<i>H. angusta</i>
<i>H. lennartiana</i>	<i>H. wagneriana</i>
<i>H. lindsayana</i>	<i>H. sarapiquensis</i>
<i>H. longa</i>	<i>H. curtispatha</i>
<i>H. mincana</i>	<i>H. meridensis</i>
<i>H. sylvestris</i>	<i>H. psittacorum</i>
<i>H. tarumaensis</i>	<i>H. acuminata</i>
<i>H. undulata</i>	<i>H. irrasa</i>
<i>H. vaginalis</i> ssp. <i>mathiasiae</i>	<i>H. mathiasiae</i>
<i>H. velloziana</i>	<i>H. farinosa</i>

Appendix B

Heliconia and outgroup voucher and GenBank accession information are in the following order: species, collection and source, ITS, ETS, *trnL*(UAA)-F, *trnL*(UAG)-rpl32, CaM, G3A, PRK. Herbarium acronyms follow (Thiers, 2012), other acronyms and abbreviations are for the following living collections: Lyon, Harold L. Lyon Arboretum at University of Hawai'i at Mānoa; US-GH, National Museum of Natural History Greenhouse, Smithsonian Institute; HeliGard, *Heliconia* Garden, Costa Rica (private collection of C. Black); UCBG, University of California, Berkeley, Botanical Garden; Huntington, Huntington Botanical Garden. Additional symbols are as follows: dash (–) indicates that the gene was not sequenced; asterisk (*) indicates the sample was sequenced using the in-solution sequence capture method (see Text); dagger (†) indicates a type specimen; double dagger (‡) indicates *affinis* determination.

Heliconia abalo G. Morales, W.J. Kress 90-3125 (US, US-GH 1990014), KY214954, –, –, KY214625, KY215330, KY215512, KY214782. *H. acuminata* A. Rich., J.-J. Granville s.n. (W.J. Kress 94-3689 [US, US-GH 1994742]), KY214955, KY215135, –, KY214626, KY215331, KY215513, KY214783. *H. adflexa* (Griggs) Standl. *, J. Wen 8741 (US), KY214956, KY215136, KY214495, KY214627, KY215332, KY215514, KY214784. *H. aemygdiana* Burle-Marx subsp. *aemygdiana*, W.J. Kress et al. 89-2863 (US), –, –, KY214496, KY214628, KY215333, KY215515, KY214785. *H. aemygdiana* subsp. *aemygdiana*, W.J. Kress et al. 96-5706 (US, US-GH 1996303), KY214957, KY215137, –, KY214629, KY215334, KY215516, KY214786. *H. aemygdiana* subsp. *transandina* L. Andersson, W.J. Kress et al. 89-2939 (US), KY214958, KY215138, KY214497, KY214630, KY215335, KY215517, KY214787. *H. albicosta* (G.S. Daniels & F.G. Stiles) L. Andersson *, W.J. Kress 10-8741A (US, INB), KY214962, KY215143, KY214499, KY214633, KY215339, KY215519, KY214790. *H. angusta* Vell., W.J. Kress 94-3688 (US), KY214963, KY215144, –, KY214634, KY215340, KY215520, KY214791. *H. apparicioi* Barreiros *, E.P. Killip & A.C. Smith 26822 (US), KY214964, KY215145, KY214500, KY214635, –, –, KY214792. *H. aristeguietae* Abalo & G. Morales, ex hort. (Lyon L93.132), KY215095, –, –, –, –, KY214895. *H. arrecta* W.J. Kress & Betancur †, W.J. Kress et al. 90-3157 (US), KY214965, KY215146, KY214501, KY214636, KY215341, KY215521, KY214793. *H. atratensis* Abalo & G. Morales, W.J. Kress et al. 90-3142 (US), KY214966, KY215147, KY214502, KY214637,

- KY215342, KY215522, –. *H. atropurpurea* G.S. Daniels & F.G. Stiles *, W.J. Kress 10-8745 (US, INB), KY214967, KY215148, KY214503, KY214638, KY215343, KY215523, KY214794. *H. aurantiaca* Ghiesbr. ex Lem., ex hort., W.J. Kress 94-3711 (US-GH-1994740), KY214968, KY215149, –, KY214639, KY215344, KY215524, KY214795. *H. aurea* G. Rodr., W.J. Kress et al. 92-3560 (US, US-GH 1992028), KY214969, KY215150, KY214504, KY214640, KY215345, KY215525, KY214796. *H. badilloi* Abalo & G. Morales, W.J. Kress 89-2927 (US), KY214970, KY215151, KY214505, KY214641, KY215346, KY215526, KY214797. *H. beckneri* R.R. Sm., W.J. Kress et al. 92-3475 (US), –, KY215152, KY214506, KY214642, KY215347, KY215527, KY214798. *H. bella* W.J. Kress, C. Black s.n. (HeliGard), KY214971, –, –, KY214643, KY215348, KY215528, –, *H. berryi* Abalo & G. Morales *, D.C. Wasshousen 2106 (US), KY214972, KY215153, KY214507, KY214644, KY215349, KY215529, KY214799. *H. bihai* (L.) L., W.J. Kress 91-3202 (US), KY214974, KY215155, –, KY214646, KY215350, KY215530, KY214800. *H. bihai*, W.J. Kress 01-6845 (US), KY214973, KY215154, –, KY214645, –, –, *H. bourgaeana* Petersen, Lyon L75.0163, KY214975, KY215156, –, –, KY215351, KY215531, KY214801. *H. brachyantha* L. Andersson, W.J. Kress et al. 91-3393 (US, US-GH 1991051), –, KY215157, –, KY214647, KY215352, KY215532, KY214802. *H. brachyantha* ‡, W.J. Kress 94-3648 (US, US-GH 1994733), KY214959, KY215139, KY214498, KY214631, KY215336, –, –, *H. brenneri* Abalo & G. Morales, W.J. Kress et al. 89-2857 (US), KY214976, KY215158, KY214508, KY214648, KY215353, KY215533, KY214803. *H. burleana* Abalo & G. Morales, W.J. Kress 91-3374 (US), KY214977, KY215159, KY214509, KY214649, KY215354, KY215534, KY214804. *H. calatheaphylla* G. S. Daniels & F.G. Stiles, W.J. Kress 94-3656 (US, US-GH 1994706), KY214978, –, –, –, –, *H. caquetensis* Abalo & G. Morales, W.J. Kress 90-3113 (US), KY214979, KY215160, KY214510, KY214650, KY215355, KY215535, KY214805. *H. caribaea* Lam., W.J. Kress 01-6837 (US), –, KY215161, –, –, KY215356, KY215536, –, *H. caribaea*, T. Prinzie & R.F. Baker 102 (Lyon L80.0034, W.J. Kress 92-3531), KY214980, KY215162, –, KY214651, KY215357, KY215537, KY214806. *H. chartacea* Lane ex Barreiros, W.J. Kress 96-5689 (US, US-GH 1996299), KY214981, KY215163, –, KY214652, KY215358, KY215538, –, *H. clinophila* R.R. Sm., W.J. Kress 86-2016 (US, US-GH 1994708), –, KY215164, –, KY214653, KY215359, KY215539, –, *H. colgantea* R.R. Sm. ex G.S. Daniels & F. G. Stiles *, Lyon L75.0314, KY214982, KY215165, KY214511, KY214654, KY215360, KY215540, KY214807. *H. collinsiana* Griggs, M. Grantham & J. Parsons 0124-90 (UCBG 90.1610), KY214983, KY215166, –, –, –, –, *H. collinsiana* var. *velutina* W.J. Kress, ex hort. (Lyon L91.0113), KY214984, KY215167, KY214512, KY214655, KY215361, –, KY214808. *H. combinata* Abalo & G. Morales *, J. Betancur 5953 (US), KY214985, KY215168, KY214513, KY214656, KY215362, KY215541, KY214809. *H. cordata* L. Andersson *, J. Betancur 5956 (US), KY214986, KY215169, KY214514, KY214657, KY215363, KY215542, KY214810. *H. crassa* Griggs *, E. Contreras 4341 (US), KY214987, KY215170, KY214515, KY214658, KY215364, KY215543, KY214811. *H. curtispatha* Petersen sensu Kress, W.J. Kress 89-2812 (US), KY214988, KY215171, KY214516, KY214659, KY215365, KY215544, KY214812. *H. curtispatha* sensu Andersson, W.J. Kress et al. 90-3078 (US), –, KY215172, –, KY214660, KY215366, –, KY214813. *H. danielsiana* W.J. Kress, W.J. Kress 10-8734A (US), KY214989, KY215173, –, –, KY215367, KY215545, –, *H. darienensis* L. Andersson *, B. Hammel 14873 (MO, W.J. Kress 89-2705 [US]), KY214990, KY215174, KY214517, KY214661, KY215368, KY215546, KY214814. *H. dasyantha* K. Koch & C.D. Bouché, W.J. Kress s.n. (US-GH 1998073), –, KY215175, –, KY214662, KY215369, KY215547, –, *H. densiflora* Verl., P. Acevedo-Rodriguez 9867 (US-GH 1997151), –, –, KY214663, KY215372, KY215550, KY214815. *H. dielsiana* Loes., W.J. Kress et al. 89-2895 (US), KY214993, KY215176, KY214518, KY214664, KY215373, –, KY214816. *H. donstonea* W.J. Kress & Betancur, W.J. Kress et al. 89-2931 (US), KY214994, KY215177, KY214519, KY214665, KY215374, KY215551, KY214817. *H. episcopalis* Vell., W.J. Kress et al. 91-3309 (US), KY214995, KY215178, KY214520, KY214666, KY215375, KY215552, KY214818. *H. estherae* Abalo & G. Morales, B. Echeverry et al. 92-3562A (US-GH 1992031, W.J. Kress 92-3562), –, KY215179, KY214521, KY214667, KY215376, –, KY214819. *H. estiletioides* Abalo & G. Morales *, W.J. Kress et al. 93-3588 (US), KY214996, KY215180, KY214522, KY214668, KY215377, KY215553, KY214820. *H. farinosa* Raddi, W.J. Kress 92-3515 (US), KY214997, KY215181, KY214523, KY214669, KY215378, –, KY214821. *H. fernandezii* Abalo & G. Morales *, W.J. Kress & B. Echeverry 89-2559 (US), KY214998, KY215182, KY214524, KY214670, KY215379, KY215554, KY214822. *H. fragilis* Abalo & G. Morales, W.J. Kress et al. 90-3080 (US), KY214999, KY215183, KY214525, KY214671, KY215380, KY215555, KY214823. *H. fred-berryana* W.J. Kress †, W.J. Kress et al. 89-2947 (US), KY215000, KY215184, KY214526, KY214672, KY215381, KY215556, KY214824. *H. gaiboriana* Abalo & G. Morales, W.J. Kress 89-2941 (US), KY215001, –, KY214527, KY214673, KY215382, KY215557, –, *H. gigantea* W.J. Kress & Betancur * †, W.J. Kress & B. Echeverry 89-2621 (US), KY215002, KY215185, KY214528, KY214674, KY215383, KY215558, KY214825. *H. gilbertiana* Abalo & G. Morales *, J. Betancur & H. Mendoza 7605 (US), KY215003, KY215186, KY214529, KY214675, KY215384, KY215559, KY214826. *H. gloriosa* Abalo & G. Morales, M. Collins 96.203 (Lyon L98.0166), KY215004, KY215187, KY214530, KY214676, KY215385, KY215560, KY214827. *H. gracilis* G.S. Daniels & F.G. Stiles, W.J. Kress & B. Hammel 97-5795 (US, US-GH 1997107), KY215006, KY215189, KY214531, KY214677, KY215387, KY215561, KY214828. *H. griggsiana* L.B. Sm., W.J. Kress et al. 89-2938 (US), KY215007, KY215190, –, –, KY215388, KY215562, KY214829. *H. harlingii* L. Andersson, W.J. Kress 89-2906 (US), KY215008, KY215191, KY214532, KY214678, KY215389, KY215563, KY214830. *H. hirsuta* L.f., W.J. Kress 11-8805 (HLDC), KY215010, –, –, –, KY215390, KY215565, –, *H. hirsuta*, T. Plowman 11579 (Lyon L82.0048), KY215009, KY215192, –, –, –, KY215564, –, *H. holmquistiana* Abalo & G. Morales *, W.J. Kress & B. Echeverry 89-2586 (US), KY215011, KY215193, KY214533, KY214679, KY215391, KY215566, KY214831. *H. huilensis* Abalo & G. Morales, W.J. Kress et al. 90-3107 (US), KY215012, KY215194, KY214534, KY214680, KY215392, KY215567, KY214832. *H. ignescens* G.S. Daniels & F.G. Stiles, G.S. Daniels s.n. (Lyon L80.0098), KY215013, KY215195, –, –, KY215393, –, –, *H. imbricata* (Kuntze) Baker, W.J. Kress 89-2716 (US), –, –, –, –, KY215395, –, –, *H. imbricata*, W.J. Kress 89-2831 (US), KY215014, KY215196, –, –, KY215394, KY215568, KY214833. *H. impudica* Abalo & G. Morales, W.J. Kress et al. 89-2923 (US), KY215015, KY215197, KY214535, KY214681, KY215396, KY215569, KY214834. *H. indica* Lam. cv. "Spectabilis", W.J. Kress 92-3521 (US), KY215016, KY215198, –, KY214682, KY215397, KY215570, KY214835. *H. indica* var. *dennisiana* W.J. Kress, W.J. Kress 92-3514 (US), KY215017, –, KY214536, KY214683, KY215398, KY215571, KY214836. *H. indica* var. *micholitzii* (Ridl.) W.J. Kress, W.J. Kress 92-3519 (US), KY215018, –, KY214537, KY214684, KY215399, –, KY214837. *H. irrada* R.R. Sm., W.J. Kress 92-3516 (US), KY215020, KY215199, KY214538, KY214685, KY215400, KY215572, –, *H. irrada* var. *glabra* G.S. Daniels & F.G. Stiles *, W.J. Kress 10-8732 (US, LC), KY215021, KY215200, KY214539, KY214686, KY215401, KY215573, KY214838. *H. irrada* var. *irrada* *, W.J. Kress 10-8733 (US), KY215022, KY215201, KY214540, KY214687, KY215402, KY215574, KY214839. *H. julianii* Barreiros, W.J. Kress et al. 91-3269 (US, US-GH 1991046), KY215023, KY215202, –, KY214688, KY215403, KY215575, –, *H. julianii*, W.J. Kress et al. 91-3349 (US), –, KY215203, –, –, KY215404, –, –, *H. juruana* Loes., W.J. Kress

- et al. 91-3308 (US), KY215024, KY215204, KY214541, KY214689, KY215405, –, KY214840. *H. kautzkiana* Emygdio & E. Santos *, W.J. Kress 89-2669 (US), KY215025, KY215205, –, KY214690, KY215406, KY215576, KY214841. *H. lanata* (P.S. Green) W.J. Kress, W.J. Kress 92-3508 (US), KY215026, KY215206, KY214542, KY214691, KY215407, KY215577, KY214842. *H. laneana* Barreiros ‡, W.J. Kress et al. 90-2956 (US, US-GH 1990022), KY214960, KY215140, –, KY214632, KY215337, –, KY214788. *H. laneana*, ex hort. (Lyon L82.921), KY215027, KY215207, KY214543, KY214692, KY215408, KY215578, –, *H. lankesteri* Standl. var. *lankesteri*, W.J. Kress et al. 92-3469 (US), KY215028, KY215208, –, KY214693, KY215409, KY215579, KY214843. *H. lankesteri* var. *rubra* G.S. Daniels & F.G. Stiles, W.J. Kress 92-3507 (US), KY215029, –, –, KY214694, KY215410, –, KY214844. *H. lasiorachis* L. Andersson, ex hort. (Lyon L85.0159), KY215030, KY215209, KY214544, KY214695, –, KY215580, KY214845. *H. latispatha* Benth., W.J. Kress 89-2717 (US), KY215031, KY215210, KY214545, KY214696, KY215411, KY215581, –, *H. latispatha*, W.J. Kress et al. 90-3077 (US), KY215032, KY215211, –, –, –, –, KY214846. *H. latispatha*, W.J. Kress 94-3658 (US, US-GH 1994720), KY215033, –, –, –, –, –, *H. laufao* W.J. Kress, W.J. Kress 92-3528 (US), KY215034, KY215212, KY214546, KY214697, KY215412, KY215582, KY214847. *H. laufao*, W.J. Kress 95-5577 (US, US-GH 1995239), KY215035, –, –, –, –, –, *H. lennartiana* W. J. Kress *, R. Yamakawa 0050 (Lyon L94.0113), KY215036, KY215213, KY214547, KY214698, KY215413, KY215583, KY214848. *H. lentiginosa* Abalo & G. ***Morales *, W.J. Kress et al. 86-2030 (US), KY215037, KY215214, KY214548, KY214699, KY215414, KY215584, KY214849. *H. librata* Griggs, W.J. Kress 92-3513 (US), KY215038, KY215215, –, –, KY215415, –, KY214850. *H. lindsayana* W.J. Kress †, W.J. Kress 76-0650 (US, US-GH 19760650), –, KY215216, KY214549, KY214700, KY215416, –, KY214851. *H. lindsayana* ‡, C.D. Specht 08-07 (NYBG), –, KY215141, –, –, KY215338, KY215518, KY214789. *H. lingulata* Ruiz & Pav., Lyon L82.048, KY215039, KY215217, –, –, KY215417, –, KY214852. *H. longa* (Griggs) H.J.P. Winkl., W.J. Kress et al. 91-3381 (US), KY215040, KY215218, KY214550, KY214701, KY215418, KY215585, KY214853. *H. longiflora* subsp. *ecuadoriensis* L. Andersson, Lyon L86.0480 (W.J. Kress 94-5291 [US]), KY215041, KY215219, KY214551, KY214702, KY215419, –, KY214854. *H. lophocarpa* G.S. Daniels & F.G. Stiles*, W.J. Kress 10-8746 (US, INB), KY215042, KY215220, KY214552, KY214703, KY215420, KY215586, KY214855. *H. lourteigiae* Emygdio & E. Santos*, J.-J. Granville 11348 (Lyon L91.0309, W.J. Kress 94-5250 [US]), KY215043, KY215221, KY214553, KY214704, KY215421, KY215587, KY214856. *H. lozanoii* Abalo & G. Morales, W.J. Kress et al. 91-3373 (US), KY215044, KY215222, KY214554, KY214705, KY215422, KY215588, KY214857. *H. lutea* W.J. Kress*, G. McPherson 14041 (US), KY215045, KY215223, KY214555, KY214706, KY215423, KY215589, KY214858. *H. lutheri* W.J. Kress* †, W.J. Kress et al. 89-2925 (US), KY215046, KY215224, KY214556, KY214707, KY215424, KY215590, KY214859. *H. maculata* W.J. Kress, W.J. Kress 82-1406 (US, Lyon L88.0430), KY215047, KY215225, –, KY214708, KY215425, –, –, *H. magnifica* W.J. Kress, D. Carli 142B, ex hort. (Lyon L90.0146), KY215048, KY215226, KY214557, KY214709, KY215426, KY215591, KY214860. *H. marginata* (Griggs) Pittier, W.J. Kress 91-3314 (US), KY215049, KY215227, KY214558, KY214710, KY215427, KY215592, KY214861. *H. mariae* Hook.f., W.J. Kress 89-2843 (US), KY215050, KY215228, –, KY214711, KY215428, KY215593, KY214862. *H. meridensis* Klotzsch, W.J. Kress 91-3210 (US), KY215052, KY215230, KY214559, KY214713, KY215430, KY215594, KY214864. *H. metallica* Planch. & Linden ex Hook., W.J. Kress 11-8802 (PMA), KY215053, –, –, –, KY215431, KY215595, –, *H. metallica*, W.J. Kress 11-8803 (HLDC), KY215054, –, –, –, KY215432, KY215596, –, *H. mincana* Abalo & G. Morales *, W.J. Kress et al. 92-3554 (US), KY215055, KY215231, KY214560, KY214714, KY215433, KY215597, KY214865. *H. mooreana* R.R. Sm. *, J.G. Ortega 49 (MO), KY215056, KY215232, KY214561, KY214715, KY215434, KY215598, KY214866. *H. mucilagina* Abalo & G. Morales *, W.J. Kress & B. Echeverry 89-2578 (US), KY215057, KY215233, KY214562, KY214716, KY215435, KY215599, KY214867. *H. mutisi-ana* Cuatrec., W.J. Kress 90-3110 (US), KY215058, KY215234, –, KY214717, KY215436, KY215600, KY214868. *H. narinensis* Abalo & G. Morales, W.J. Kress et al. 90-3095 (US), KY215059, KY215235, KY214563, KY214718, KY215437, KY215601, KY214869. *H. necrobracteata* W.J. Kress, ex hort. (Lyon L72.0014), KY215060, KY215236, KY214564, KY214719, KY215438, –, KY214870. *H. nigriprefixa* Dodson & A.H. Gentry, W.J. Kress et al. 89-2907 (US, Lyon L90.0110), KY215061, KY215237, KY214565, KY214720, KY215439, KY215602, KY214871. *H. nutans* Woodson *, W.J. Kress 94-4906 (US, US-GH 1994621), KY215062, KY215238, KY214566, KY214721, KY215440, KY215603, KY214872. *H. obscura* Dodson, W.J. Kress et al. 89-2908 (US), KY215063, KY215239, KY214567, KY214722, KY215441, KY215604, KY214873. *H. obscura* subsp. *dichroma* W.J. Kress†, W. J. Kress et al. 89-2866 (US, Lyon L90.0115), KY215064, KY215240, KY214568, KY214723, KY215442, KY215605, –, *H. obscuroides* L. Andersson, W.J. Kress et al. 90-3065 (US), KY215065, KY215241, KY214569, KY214724, KY215443, KY215606, –, *H. oleosa* Abalo & G. Morales, W.J. Kress et al. 91-3379 (US, US-GH 1991049), KY215066, KY215242, KY214570, KY214725, KY215444, KY215607, KY214874. *H. orthotricha* L. Andersson, W.J. Kress 96-5696 (US), –, KY215243, –, –, –, –, *H. osaensis* Cufod., W.J. Kress & C.S. Roesel 89-2728 (US), KY215067, KY215244, KY214571, KY214726, KY215445, KY215608, –, *H. paka* A.C. Sm., US-GH 1996352, KY215068, KY215245, –, KY214727, KY215446, KY215609, KY214875. *H. paludigena* Abalo & G. Morales*, T.W. Flynn et al. 4110 (US), KY215069, KY215246, KY214572, KY214728, KY215447, KY215610, KY214876. *H. papuana* W.J. Kress, W.J. Kress 92-3509 (US), KY215070, –, KY214573, KY214729, KY215448, KY215611, –, *H. pardoi* Abalo & G. Morales, W.J. Kress et al. 89-2922 (US, Lyon L90.0111), KY215071, KY215247, KY214574, KY214730, –, –, –, *H. pastazae* L. Andersson, W.J. Kress et al. 89-2896 (US), KY215081, KY215258, KY214578, KY214738, KY215457, –, KY214882. *H. pastazae*, W.J. Kress et al. 90-3116 (US), –, KY215249, –, KY214732, –, KY215613, –, *H. pas-tazae*, W.J. Kress et al. 96-5731 (US), KY215072, KY215248, KY214575, KY214731, KY215449, KY215612, KY214877. *H. pen-dula* Wawra, W.J. 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(Lyon L94.0034), KY215133, KY215312, –, –, –, KY215666, –, *H. zebirina* Plowman, W.J. Kress & H. Kenn. *, ex hort. (Lyon L85.0195), KY215134, KY215313, KY214608, KY214781, KY215511, KY215667, KY214925. *H. sp.*, ex hort. (US-GH 1993089, W.J. Kress 94-3677), KY215077, KY215254, –, KY214736, KY215453, KY215617, KY214879. *H. sp.*, Kress 94-3698, –, KY215280, KY214591, KY214757, KY215479, KY215639, –, *H. sp.* *, W.J. Kress et al. 96-5694 (US, US-GH 1996193), KY215129, KY215307, KY214607, KY214779, KY215506, KY215664, KY214922. *H. sp.*, C. D. Specht 06-08 (NYBG), KY215102, –, –, –, KY215477, KY215637, KY214901. *H. sp.*, C.D. Specht 08-05 (NYBG), KY215103, KY215279, –, –, KY215478, KY215638, –, *Canna jaegeriana* Urb., Sass et al. (2016), KY214934, KY214942, KY214481, KY214611, KY215316, –, –, *Ensete superbum* (Roxb.) Cheesman, Sass et al. (2016), KY214928, KY214943, KY214484, KY214614, KY215319, –, –, *Ensete ventricosum* (Welw.) Cheesman *, M. 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Appendix C. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jympev.2016.12.001>.

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