

**THE EVOLUTION OF *CAYAPONIA* (CUCURBITACEAE):
REPEATED SHIFTS FROM BAT TO BEE POLLINATION AND
LONG-DISTANCE DISPERSAL TO AFRICA 2–5 MILLION
YEARS AGO¹**

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- *Premise of the study:* The Cucurbitaceae genus *Cayaponia* comprises ~60 species that occur from Uruguay to the southern United States and the Caribbean; *C. africana* occurs in West Africa and on Madagascar. Pollination is by bees or bats, raising the question of the evolutionary direction and frequency of pollinator shifts. Studies that investigated such shifts in other clades have suggested that bat pollination might be an evolutionary end point.
- *Methods:* Plastid and nuclear DNA sequences were obtained for 50 accessions representing 30 species of *Cayaponia* and close relatives, and analyses were carried out to test monophyly, infer divergence times, and reconstruct ancestral states for habitat preferences and pollination modes.
- *Key results:* The phylogeny shows that *Cayaponia* is monophyletic as long as *Selysia* (a genus with four species from Central and South America) is included. The required nomenclatural transfers are made in this paper. African and Madagascan accessions of *C. africana* form a clade that is part of a polytomy with Caribbean and South American species, and the inferred divergence time of 2–5 Ma implies a transoceanic dispersal event from the New World to Africa. The ancestral state reconstructions suggest that *Cayaponia* originated in tropical forests from where open savannas were reached several times and that bee pollination arose from bat pollination, roughly concomitant with the shifts from forests to savanna habitats.
- *Conclusions:* *Cayaponia* provides the first example of evolutionary transitions from bat to bee pollination as well as another instance of transoceanic dispersal from the New World to Africa.

Key words: ancestral state reconstruction; bat pollination; bee pollination; *Calycophysum*; *Cionosicyos*; Cucurbitaceae; long-distance dispersal; molecular clock; *Selysia*.

The evolutionary direction of shifts between bee pollination and bat pollination has been studied in few clades, and in each case, the change was from bees to bats (Mori et al., 2007; Perret et al., 2007; Knox et al., 2008; Tripp and Manos, 2008). It has therefore been suggested that bat pollination may be an evolutionary dead end with respect to the potential for exploiting new pollinators, based on the idea that specialized states can be difficult to move away from (Tripp and Manos, 2008). Bat pollination is interpreted as specialized because it involves a distinct set of correlated adaptations such as nocturnal anthesis, abundant nectar, relatively large flowers exposed on sturdy peduncles or in suitably placed inflorescences, and a musty, fetid, and pungent smell that is unattractive to bees (Helversen, 1993; Pettersson et al., 2004; Fleming et al., 2009). It is unclear, however, why selection might not sometimes favor returns to less energy-costly pollination modes and thus shifts away from bat pollination.

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A medium-sized genus with well-documented bat and bee pollination is *Cayaponia* (Vogel, 1958, 1969; Heithaus, 1979; Steentoft, 1988; Sazima et al., 1996; Queiroz-Cámara et al., 2004; Ramirez, 2004). Most of its ~60 species are large perennial climbers growing in rainforest or along forest margins; fewer species occur in deciduous forest or scrubland. Appendix S1 (see Supplemental Data with the online version of this article) lists all names published in the genus and their current status. The geographic range of *Cayaponia* extends from 35°S in Uruguay to 35°N in Oklahoma. *Cayaponia* species are usually monoecious and have whitish or yellow-greenish flowers measuring 1–3 cm in diameter.

Besides its apparent bee/bat pollinator shifts, *Cayaponia* is interesting biogeographically because it has one or two species in Madagascar and West Africa (São Tomé, Senegal, Guinea-Bissau, Ivory Coast, Ghana, Cameroon, Gabon, Congo) as well as an endemic species on Fernando de Noronha Island, 357 km off the coast of Brazil (Fig. 1). A phylogeny of the genus therefore contributes to our understanding of trans-Atlantic range disjunctions. Such range disjunctions are known from 110 genus-level groups of flowering plants (Renner, 2004). Some 20 have now been analyzed with molecular-phylogenetic data, and in most cases, molecular clocks yield such young ages that long-distance dispersal is the only plausible explanation for the disjunctions. Each additional transatlantic clade that is analyzed contributes data to an eventual meta-analysis answering the question whether dispersal was predominantly in one direction, as suggested by wind patterns, but not sea currents (Renner, 2004).

Family-wide molecular-phylogenetic analyses have confirmed that *Cayaponia* belongs in the Cucurbitaceae, where it is



Fig. 1. Global distribution of the genus *Cayaponia*, obtained from collecting localities of 1908 specimens on loan from different herbaria.

most closely related to *Selysia*, *Abobra*, and *Cionosicys* (Kocyan et al., 2007; Schaefer et al., 2009). These family studies, however, only included three species of *Cayaponia* and one each of *Cionosicys*, *Abobra*, and *Selysia*. Therefore, they could not fully evaluate relationships among these genera. Morphologically, *Cionosicys*, with four or five species, differs from *Cayaponia* in its fleshy, melon-type fruit (with >14 seeds), compared with the typical berry of the latter (Jeffrey, 1971; Grayum, 2009). The monotypic *Abobra* has narrow, nonreflexed stigmas and linearly elongated seeds, while *Cayaponia* has broad, reflexed stigmas and ovate-oblong to compressed seeds (Cogniaux, 1881; P. Duchon, personal observation). The third genus, *Selysia*, with four species, is traditionally keyed out against *Cayaponia* by its triangular seeds and stamen morphology (Jeffrey, 1971).

Here we use molecular markers from the chloroplast and nuclear genomes, molecular clock dating, and ancestral trait reconstruction to test the monophyly and relationships of *Cayaponia*, *Cionosicys*, and *Selysia* (*Abobra* has only one species) and then to answer three questions: (1) What was the frequency and direction of shifts between bats and bees as pollinators, and what was the ecological context of the shifts? (2) What is the role of habitat shifts in the evolution of *Cayaponia*? and (3) what is the relationship of the African and Madagascan species (or forms) of *Cayaponia* to each other and to the South American ones? Because we found *Selysia* (including the type of the genus name) deeply nested inside *Cayaponia*, the necessary nomenclatural changes are made at the end of this paper.

MATERIALS AND METHODS

Taxon sampling—Appendix 1 provides a list of the species included in this study, with the geographic origin of material and GenBank accession numbers for all sequences. To detect intraspecific variation in *C. africana*, we sequenced seven accessions for this species that came from Senegal, Guinea-Bissau, Liberia, Gabon, Sao Tomé, and Madagascar. We generated 184 new sequences for this study. In addition, the first author studied 1908 collections from 19 European and American institutions (AAU, B, BM [only *Selysia*], BR, C, COI, F, G, GH, K, LISC, LPB, M, MO, SEL, U, USF, VEN and WAG, herbarium acronyms follow Index Herbariorum at <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). Of the four species of *Selysia* [*S. bidentata* R. J. Hampshire, *S.*

cordata Cogn., *S. prunifera* (Poepp. & Endl.) Cogn., and *S. smithii* (Standl.) C. Jeffrey], we sampled all except *S. bidentata*. *Cionosicys* was represented by two of its four or five species (Grayum, 2009), both with multiple accessions. Following the results of Kocyan et al. (2007) and Schaefer et al. (2009), trees were rooted on *Schizocarpon*.

DNA isolation, amplification, sequencing, and alignment—Total genomic DNA was isolated from silica-dried leaves and herbarium specimens using the NucleoSpin plant kit (Macherey-Nagel, Düren, Germany). DNA amplification by polymerase chain reaction, fragment purification, and cycle sequencing followed the protocols described in Kocyan et al. (2007). We amplified the following chloroplast markers: the *trnL* intron, the adjacent *trnL-F* intergenic spacer, the *rpl20-rps12* intergenic spacer between the ribosomal protein genes S12 and L20, and the *trnH-psbA* intergenic spacer. Primers for the first two regions were those of Taberlet et al. (1991) and Kocyan et al. (2007). The *trnH-psbA* spacer was amplified with the primers listed in Volz and Renner (2009). The ITS region, including 130 nucleotides of the 3' end of the 18S gene, the 5.8S gene, and 110 nucleotides of the 5' end of the 25S gene, was amplified using the primers of Balthazar et al. (2000). Sequencing primers were the same as used for DNA amplification. Both strands were sequenced and compared to generate consensus sequences in Sequencher (v. 4.1.2, Gene Codes Corp., Ann Arbor, Michigan, USA). Sequence alignment was done manually in the program MacClade v. 4.0.6 (Maddison and Maddison, 2003). Forty-four informative insertions or deletions (indels), most of them in the *trnL* intron, were manually coded as characters.

Phylogenetic analyses—Maximum likelihood (ML) searches and ML bootstrapping (using 100 replicates) relied on the program RAxML v. 7.0.4 (Stamatakis et al., 2008) and used the GTR + Γ model with 25 rate categories. Analyses of the separate plastid and nuclear data partitions produced congruent phylogenetic estimates, with all areas of discordance being restricted to branches with low support (i.e., bootstrap proportions <60%). Given this congruence, the data were concatenated, with separate model parameters for the plastid and nuclear data partitions estimated in RAxML. We also analyzed the data with and without the coded indel characters.

Bayesian inference also used the GTR + Γ model (with the default four rate categories) and relied on the program MrBayes v. 3 (Ronquist and Huelsenbeck, 2003). We again analyzed the combined data set with separate models for the plastid and nuclear data partitions, allowing partition models to vary by unlinking gamma shapes and transition matrices. Markov chain Monte Carlo (MCMC) runs started from independent random trees, were repeated twice, and extended for 1 million generations, with trees sampled every 100th generation. Convergence was assessed by checking that the standard deviations of split frequencies were <0.01; that the convergence diagnostic (the potential scale reduction factor given by MrBayes) approached one; and by examining the plot provided by MrBayes of the generation number vs. the log probability of the data. Trees saved prior to convergence were

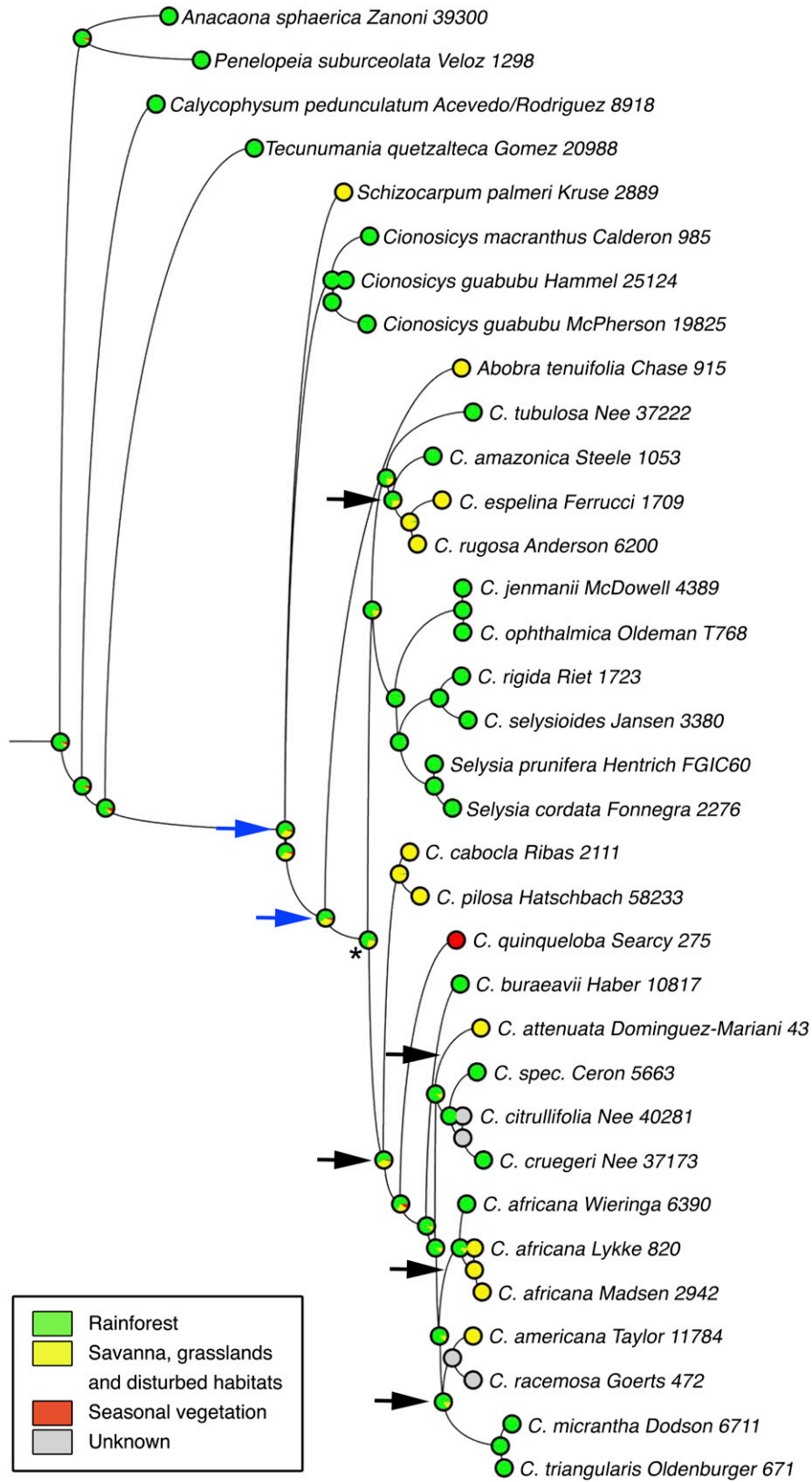


Fig. 3. Shifts in preferred habitat type during the evolution of *Cayaponia* inferred on a maximum likelihood tree under maximum likelihood optimization. Color codes for character states are explained in the legend, and pie diagrams at nodes represent likelihoods. Arrows indicate shifts from rainforest to savanna in the ingroup (black arrows) and the outgroup (blue arrows). The asterisk indicates the most recent common ancestor of *Cayaponia*. Voucher information is given after the species names.

(3) Central America, and (4) South America. *Cayaponia quinqueloba*, which occurs in North America, was grouped with Central America. A comparison of the parsimony-based DIVA approach with the maximum likelihood approach taken in the biogeographic program Lagrange (Clark et al., 2008), using an empirical study system, found that these methods yielded similar results, suggesting that it may be worthwhile to explore the implications of a simple DIVA analysis.

RESULTS

Paraphyly of *Cayaponia* without *Selysia*—The plastid DNA matrix comprised 1768 aligned nucleotides of *rpl20-rps12*, *trnH-psbA*, *trnL*, and *trnL-F* from 42 accessions representing 30 ingroup species and eight accessions representing four outgroup species. The nuclear ITS matrix consisted of 893 aligned nucleotides from 35 accessions representing 34 ingroup and one outgroup species. With the 44 coded indels included, the matrix comprised 2705 characters. Maximum likelihood analyses of the individual loci and of the matrix with or without the coded indels revealed no topological contradictions in any of the statistically supported nodes. The combined matrix comprised 27% empty cells. The topology of the resulting ML tree (Fig. 2 from the matrix that included the coded indels) is identical to the Bayesian consensus tree (not shown) in all statistically supported nodes. *Cayaponia* is monophyletic (with 99% ML bootstrap support and 100% posterior probability) with *Abobra* as sister, and *Cionosicyus* as the next closest clade. *Selysia cordata*, *S. prunifera*, and *S. smithii* form a clade that is deeply embedded in *Cayaponia*. The seven accessions of *C. africana* also cluster together, but some (e.g., *C. africana* var. *madagascariensis*) have rather long branches (Fig. 2).

Habitat shifts—The inferred habitat of the most recent common ancestor of *Cayaponia* is tropical rainforest (Fig. 3). Shifts to more open (savanna) vegetation occurred five times: (1) in the common ancestor of *C. pilosa* and *C. cabocla* (restricted mostly to southern Brazil), (2) within *C. africana*, which began diverging on the African continent 1–2 Ma (Fig. 4), (3) in the common ancestor of *C. espelina* and *C. rugosa* (Argentinean pampas and Brazilian Cerrado), which diverged from its sister clade about 6 ± 3 Ma (Fig. 4), (4) in *C. americana* or the common ancestor of *C. racemosa* and *C. americana*, and (5) in the Mexican *C. attenuata*. A shift from rainforest to subtropical deciduous vegetation occurred in the North American *C. quinqueloba*.

Biogeography—The DIVA analysis yielded South America as the ancestral region of the most recent common ancestor of *Cayaponia*. Central America (here including North America, which has only one species), and the Caribbean were occupied more recently. Some islands in the Greater Antilles and West Caribbean harbor both *C. americana* and *C. racemosa*, which differ in fruit size and whether fruits are borne solitary or in racemes (Jeffrey, 1971; P. Duchen, personal observation); *C. racemosa*, but not *C. americana*, also occurs in the Guianas. Dispersal of *Cayaponia* to Africa occurred between 5 and 2 Ma, as inferred from the stem and crown ages of *C. africana* (Fig. 4). Of the numerous species not included in the DIVA analysis, 18–19 are from the Amazon basin, 3–4 from Central America, and 14–15 from southern Brazil/northern Argentina. Running a DIVA analysis using the full species tree (with 30 ingroup species) was not possible because the program cannot handle polytomies.

Pollinator shifts—The most recent common ancestor of *Cayaponia* likely was bat-pollinated (Fig. 5). Shifts to bee pol-

lination occurred at least twice, once in the most recent common ancestor of the *C. quinqueloba/C. triangularis* clade around 8.5 Ma (Fig. 4), and a second time in the ancestor of *C. espelina* or the common ancestor of *C. espelina* and *C. rugosa* at around 6 Ma (Fig. 4). Of the outgroups, *Calycophysum pedunculatum* and *Cionosicyus guabubu* are bat-pollinated, while *C. macranthus* and *Abobra tenuifolia* apparently shifted to bee pollination (Fig. 5). Recoding *C. cabocla* as bee-pollinated instead of unknown (Materials and Methods) did not change these inferences, but reduced the likelihood of bat pollination being ancestral at the nodes immediately below *C. cabocla* and *C. pilosa*.

DISCUSSION

Inclusion of *Selysia* in *Cayaponia*—Results of this study show that *Selysia* belongs in *Cayaponia*. *Selysia* was thought to differ mainly in its triangular seeds, compared to oblong-ovate seeds in *Cayaponia*, and its free anther-thecae compared to the joined ones in *Cayaponia* (Jeffrey, 1971). However, free thecae are also found in some *Cayaponia* (e.g., *C. quinqueloba*), and *Selysia bidentata* has ovate rather than triangular seeds (Knapp and Hampshire, 1994), breaking down these distinctions. The four species of *Selysia* differ from each other by a combination of leaf shape (lobed vs. unlobed) and the presence/absence of hair tufts on the underside of the leaf base on either side of the midrib (Knapp and Hampshire, 1994). In habit, they all resemble *Cayaponia*. Both genera occur in similar habitats, and the geographic range of *Selysia* (Nicaragua to Peru) is enclosed in that of *Cayaponia* (North America to Uruguay plus Africa). There is thus no morphological or ecological trait arguing against the inclusion of *Selysia* in *Cayaponia*, and we therefore make the formal name transfers at the end of this paper.

Biogeography, dispersal, and habitat shifts—Dispersal to Africa took place during the Pliocene, 2–5 Ma and may have been mediated by the Atlantic current from northeastern Brazil to northwest Africa (as hypothesized for other angiosperms with trans-Atlantic disjunctions; Renner, 2004). That *Cayaponia* fruits or seeds may sometimes be dispersed considerable distances overseas is implied by *C. noronhae*, the seeds of which are eaten (and presumably occasionally dispersed) by the spotted dove *Zenaida maculata* (Vieill.), which is endemic on the island Fernando de Noronha, 357 km off the coast of northeast Brazil (Ridley, 1930). Also, tropical cyclones (hurricanes, typhoons) were much more frequent during the early Pliocene (3–5 Ma) than they are today (Fedorov et al., 2010). On land, *Cayaponia* is dispersed by a range of animals, including the maned wolf [*Chrysocyon brachyurus* (Illiger)] (Guimarães, 2002) and spider monkeys [*Ateles belzebuth* (É. Geoffroy Saint-Hilaire)] (van Roosmalen, 1985; Link and Fiore, 2006); freshwater herbivorous fishes also take the fruits (herbarium specimen label *N. M. Heyde 469*, Herbarium Utrecht). There is even evidence of *C. cruegeri* occurring on floating mats in the Suriname River, already close to the Atlantic ocean (herbarium specimen label *J. van Donselaar 3854*, Herbarium Utrecht). These observations fit with the inferred long-distance dispersal of the ancestor of *C. africana* from South America to Africa. Conceivably, there was a stepping-stone progression, with islands such as Fernando de Noronha perhaps providing one “foothold,” and the islands São Tomé, Príncipe, and Annobon, 400 km off the coast of West Africa (which harbor *C. africana*), another. Due to lack of suitable material, we were unable to assess the

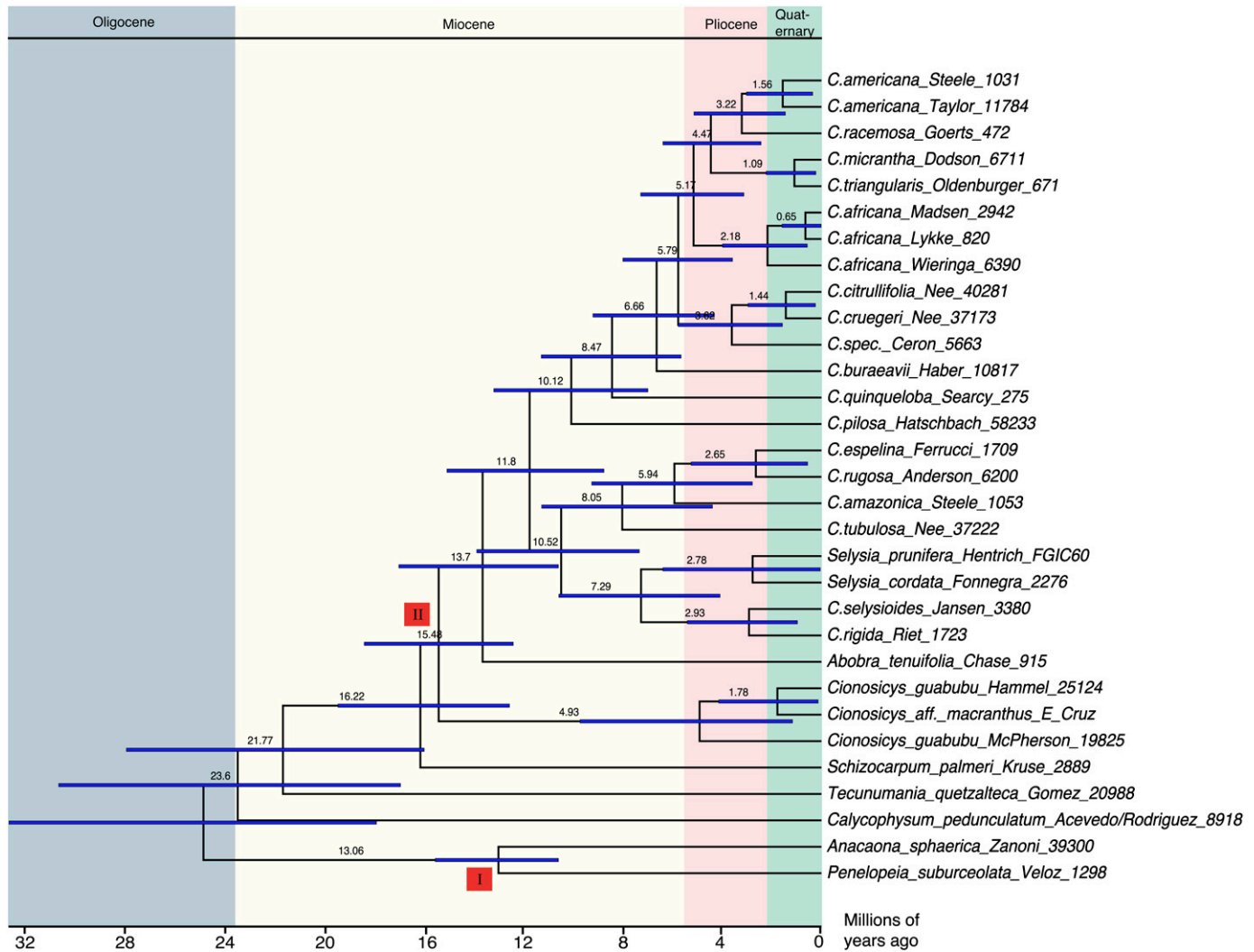


Fig. 4. Chronogram for 27 species of *Cayaponia* plus relevant outgroups obtained under a model of uncorrelated rate change using two calibration points, labeled I and II (see text for details). Node heights are mean ages, and bars at nodes indicate 95% confidence intervals around estimates.

genetic distance between *C. noronhae* and *C. africana*, but the presence of *Cayaponia* on these islands attests to its capacity to establish following transoceanic dispersal.

Regarding the African species *C. africana*, our results show intraspecific variation among the West African accessions from Senegal south to Gabon (see Fig. 2, orange clade), fitting with the Angolan material sometimes being assigned to a separate species, *C. multiglandulosa* (Fernandes, 1959). This species is supposed to differ from *C. africana* in the larger number of glands on the leaf base (7–9 glands at each side of the rib). Material from Angola will need to be sequenced to assess whether some of the haplotypes in our sampling actually represent *C. multiglandulosa*, not *C. africana*. The seven accessions we studied did not differ in the number of glands at their leaf bases, however. The single accession from Madagascar (Figs. 1 and 2) is insufficient to resolve whether the Madagascan populations of *C. africana* derive from anthropogenic introductions. Madagascan plants have been ranked as var. *madagascariensis* based on difference in fruit shape (Keraudren, 1968), and it has been conjectured that they are native (Keraudren, 1968). At least one *Cayaponia* species, however, has definitely been introduced to the Old World by man, namely

C. martiana, which occurs naturally in northern Argentina, Paraguay, and Uruguay, but was brought to the Bogor Botanical Garden (Buitenzorg, Java) during the second decade of the 20th century and then escaped from cultivation (Jeffrey, 1971). A collection made by van Steenis (*nr.* 12038, Gray Herbarium Harvard) in forest near Besuki in 1940 suggests that the species has become naturalized in the Javanese flora (Jeffrey, 1971).

Habitat reconstruction (Fig. 3) yielded rainforest as the most likely ancestral habitat of *Cayaponia* (including *Selysia*). The extant radiation of *Cayaponia* appears to date to the Miocene, some 12 Ma (Fig. 4), with many of today's species originating over the course of the Pliocene. At the beginning of the Miocene, rainforests in South America were well established but began being replaced by woodland and savannas during the relatively dry-cool climates that predominated in Miocene South America (Bredenkamp et al., 2002). The evolution of species adapted to open habitats (Fig. 3), as well as shifts from bat to bee pollination (Fig. 5), both correlate with the Miocene origin of savannas. Clearly, however, our habitat categories of open vegetation (grasslands, scrubland, savannas) vs. forest are insufficient descriptors of the habitat diversity occupied by *Cayaponia* (the state “seasonal

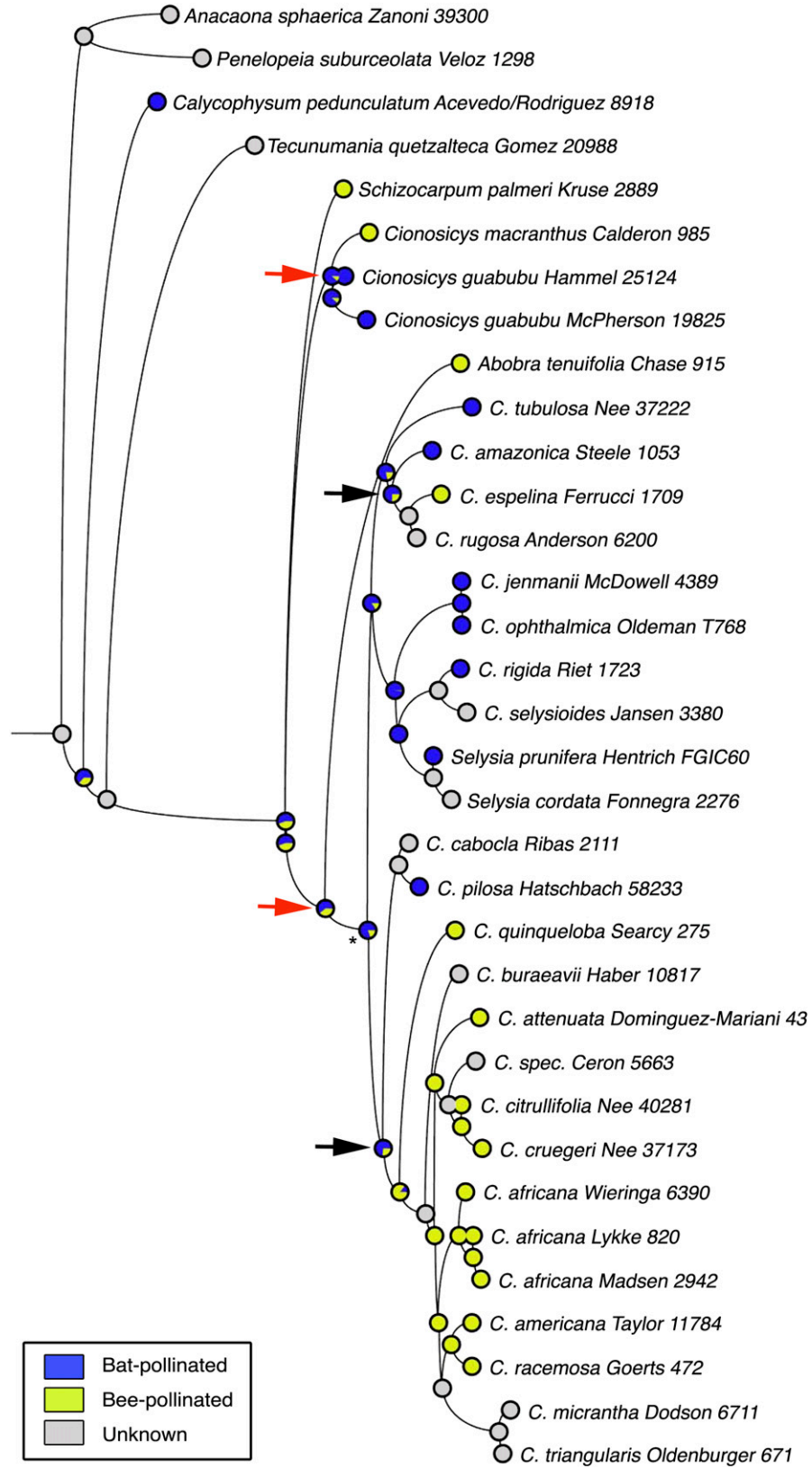


Fig. 5. Shifts between bat and bee pollination during the evolution of *Cayaponia* inferred on a preferred maximum likelihood tree under maximum likelihood optimization. Color codes for character states are explained in the legend, pie diagrams at nodes represent likelihoods, and Appendix 2 lists the sources for each scoring. Arrows indicate shifts from bat to bee pollination in the ingroup (black arrows) and the outgroup (red arrows). The asterisk indicates the most recent common ancestor of *Cayaponia*.

forest' occurred only once and therefore had no weight in the ancestral habitat reconstruction). Most species of *Cayaponia* (including *Selysia*) occur at <1000 m a.s.l., a few occur at 1000–2000 m, and only *C. boliviensis*, *C. buraevii*, *C. glandulosa*, and *C. simplicifolia* reach altitudes up to 3000 m a.s.l. The highest collection we have seen is a *C. simplicifolia* plant from 3750 m a.s.l. in the Andes of Colombia. In the United States, *C. quinqueloba* grows in areas with winter temperatures as low as -6°C (Oklahoma). Unfortunately, there is no pollinator information for any of the highest-ranging species, so that a possible correlation between pollinators and altitude cannot yet be tested.

Pollinator shifts—To our knowledge, *Cayaponia* is the first clade in which pollinator shifts from bats to bees have been inferred. Other studies of genera with bee- and bat-pollinated species all inferred shifts in the other direction (Mori et al., 2007; Perret et al., 2007; Knox et al., 2008; Tripp and Manos, 2008; N. Muchhala, University of Miami, personal communication), and bat pollination has even been interpreted as an evolutionary dead end (Tripp and Manos, 2008). Clearly, flowers pollinated by bees or bats differ in numerous traits. Bat-pollinated species produce large nocturnal flowers with copious nectar and usually a musty smell (Helvesen, 1993; Pettersson et al., 2004; Fleming et al., 2009). Bee-pollinated species have much smaller, diurnal, and sweet-smelling flowers. In *Cayaponia*, the transition between these syndromes appears to have happened not only once but twice, with some species, such as *C. cabocla*, apparently in transition between bat and bee pollination. We therefore ran analyses with *C. cabocla* coded as bee-pollinated or as unknown (Materials and Methods; Results). This dioecious Brazilian liana has nocturnal bell-shaped greenish flowers that have a slightly sweet smell (Sazima et al., 1996). Anthesis lasts from 0200 to 1800 hours, and the flowers are pollinated early in the morning by a large species of Colletidae (*Caupolicana*) (Sazima et al., 1996). Nectar production in both male and female flowers is abundant, reaching 214 μL , with a sugar concentration of around 23–29%. Other “transitional” species may be *Cayaponia macrocalyx* and *Cionosicyos macranthus*, both of which are bee-pollinated early in the morning (Appendix 2).

The ecological context of the shifts from bats to bees in *Cayaponia* appears to have been expansion into open habitats, such as the Brazilian savannas (Cerrado) and Argentinean pampas (Fig. 3 and 5). The North American *C. quinqueloba*, which occurs in swampy seasonal forest and on riverbanks, also is bee-pollinated, judging from its small flowers and diurnal anthesis. That pollinator shifts between bees and bats may correlate with habitat shift makes ecological sense since nectar-taking bats, mainly Glossophaginae, are reluctant to forage in open, disturbed habitats (Quesada et al., 2003). The four angiosperm clades in which the inferred direction of pollinator shifts went from bees to bats are the tree family Lecythidaceae (Mori et al., 2007; but only one of three bat-pollinated species is included in the relevant phylogeny), certain shrubby Gesneriaceae (Perret et al., 2007; bat pollination evolved at least twice), shrubby Lobeliaceae (Knox et al., 2008; bat pollination evolved from hummingbird pollination, and there is at least one reversal), and a shrub genus of Acanthaceae (Tripp and Manos, 2008). None of these four cases involves habitat shifts away from forest vegetation to savannas, which appear to have been the driving factor for the pollinator shifts from bats to bees in *Cayaponia*.

A caveat concerning our inferences about the habitat and pollinator shifts in *Cayaponia* is that they are based on only 22–23 of the 60 species of *Cayaponia*. Adding the numerous species

not included in our trait reconstructions might change the inferred ancestral pollination syndrome, although probably not the ancestral habitat, given that the majority of the non-included species occur in rainforests. The species sequenced for our study are those most commonly collected; of 1764 collections identified to species, 1340 (76%) belong to the 30 *Cayaponia* species sequenced here. To test the inferred correlation between transition into open habitat and transition from bat to bee pollination it will be necessary to include the rarely collected species, together with information on their pollinators. It would be surprising, however, if the addition of such information were to affect both inferred switches from bat to bee pollination since the likewise bat-pollinated species-poor outgroup genera are well represented in our study and partly determine the likely evolutionary direction from bats to bees.

FORMAL TRANSFERS OF *SELYSIA* INTO *CAYAPONIA*

Selysia Cogniaux in Alph. de Candolle et A. C. de Candolle, Monogr. Phan. 3: 735. June 1881. Lectotype: *S. prunifera* (Poeppig et Endlicher) Cogniaux (*Melothria prunifera* Poeppig et Endlicher).

Cayaponia prunifera (Poepp. & Endl.) Duchon and Renner, comb nov.—Basionym: *Melothria prunifera* Poepp. & Endl., Nova Genera ac Species Plantarum 2: 55, tab. 174. 1838. *Selysia prunifera* (Poepp. & Endl.) Cogn., Monographiae Phanerogamarum 3: 736. 1881. Peru: Maynas [Loreto]: Poeppig 2265 (holotype W, photo available online).

Note that two invalid names also belong here: *Selysia aspera* Killip ex C. Jeffrey, Kew Bulletin 25: 235. 1971, nom. inval., as syn., = *S. prunifera* fide Wunderlin (1978); and *Selysia perllobata* Killip ex C. Jeffrey, Kew Bulletin 25: 235. 1971, nom. inval., as syn., = *S. prunifera* fide Wunderlin (1978).

Cayaponia cordata (Cogn.) Duchon and Renner, comb nov. —Basionym: *Selysia cordata* Cogn., Monographiae Phanerogamarum 3: 736. 1881. Type: Goudot s.n., c. 1825–1827, Colombia: Boyacá: Muzo (holotype P, photo seen).

No transfer is needed for *Cayaponia smithii* Standl., Field Museum of Natural History, Botanical Series 13(6/2): 380. 1937.—*Selysia smithii* (Standl.) C. Jeffrey, Kew Bulletin 25(2): 235. 1971. Type: Peru: Loreto, Rio Marañon Valley, Rio Morona, 150 m, 20 Aug 1929 to 08 Sep 1929, Killip & Smith 29162 (holotype, US n.v. (on loan to V. Gomes-Klein as of Feb. 2010); isotype F!, leaf fragment and one fruit).

Cayaponia bidentata (R. J. Hampshire) Duchon and Renner, comb nov.—Basionym: *Selysia bidentata* R. J. Hampshire, Novon 4(1): 35, fig. 1. 1994. Type: Panama, Comarca de San Blas: El Llano-Carti Road 16–20 km from Interamerican Hwy; 9°19'N, 78°55'W, 350 m a.s.l., 6 Sep. 1984, G. de Nevers 3855 (holotype BM!; isotype MO!).

The differences among the four species of *Selysia* are discussed in Jeffrey (1971) and Knapp and Hampshire (1994).

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APPENDIX 1. Species included in this study, with the geographic origin of material and GenBank accessions for all sequences. The letters GT after a species name indicate that the species is the type of the respective genus. Herbarium acronyms follow the Index Herbariorum (<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Species	DNA source	Geographic origin	<i>trnH-psbA</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	ITS
Ingroup							
<i>Cayaponia africana</i> var. <i>madagascariensis</i> Keraudren	P. de la Bathie 6783 (P)	Madagascar: Sambirano mountains, along the river Bemarivo	—	—	—	HM015048	—
<i>Cayaponia africana</i> (Hook. f.) Exell	J. G. Adam 25122 (MO)	Liberia: Yekepa, Mt. Gangra	HM015007	—	HM015110	—	—
<i>Cayaponia africana</i> (Hook. f.) Exell	E. Figueiredo 249 (LISC)	São Tomé and Príncipe	HM015009	DQ536759	DQ536759	DQ536621	HM057414
<i>Cayaponia africana</i> (Hook. f.) Exell	R. Fernandes 3759 (MO)	Guinea Bissau: Gabu, between Sonaco and Nova Lamago	HM015008	—	HM015111	—	—
<i>Cayaponia africana</i> (Hook. f.) Exell	A. M. Lykke 820 (AAU)	Senegal: Region Sine Saloum, Delta of Saloum National Park (16°30'W, 13°40'N)	HM015010	HM015079	HM015112	HM015046	HM057415
<i>Cayaponia africana</i> (Hook. f.) Exell	J. E. Madsen 2942 (MO)	Senegal: Tambacounda, Niokolo Koba National Park (13°15'N, 13°01'W)	HM015011	HM015080	GU727864	HM015047	HM057396
<i>Cayaponia africana</i> (Hook. f.) Exell	J. J. Wieringa 6390 (WAG)	Gabon: Haut-Ogooue, 22 km on road from Okonkja to Akiéni (00°46.21'S, 13°46.79'E)	HM015012	HM015081	HM015113	HM015049	HM057416
<i>Cayaponia amazonica</i> (Poepp. & Endl.) Cogn.	R. Steele 1053 (TEX)	Peru: Loreto, Rio Momon (03°41'S, 73°16'W)	HM015013	—	HM015114	HM015050	HM057397
<i>Cayaponia americana</i> (Lam.) Cogn.	C. Taylor 11784 (MO)	Puerto Rico: Mun. Rio Grande, Punta Picua (18°25'N, 65°50'W)	HM015014	DQ536793	DQ536793	DQ648158	HM057399
<i>Cayaponia americana</i> (Lam.) Cogn.	R. Steele 1031 (TEX)	Puerto Rico: Prov. Fajardo, along Rt. 984, must N of Rt. 976 (18°19'N, 65°41'W)	HM015036	HM015082	HM015115	HM015051	HM057398
<i>Cayaponia attenuata</i> (Hook & Arn.) Cogn.	A. Dominguez-Mariani 43 (GH)	Mexico: Nayarit, Mpio. Compostela, road to Turtle Camp 300 m from the bay of Platanitos town	HM015015	HM015083	HM015116	HM015052	HM057400
<i>Cayaponia boliviensis</i> Cogn.	J. C. Solomon 8616 (U)	Bolivia: La Paz, Prov. Nor Yungas, 14.4 km NE (below) Chuspipata (15.8 km above Yolosa) (16°14'S, 67°47' W)	HM015017	HM015085	HM015118	HM015054	HM057402
<i>Cayaponia bonariensis</i> (Mill.) Mart. Crov.	S. G. Tressens 4677 (U)	Argentina: Misiones, Guarani, Predio Guarani, on Road 15 (26°54'-59' S, 54°12'-18'W)	HM015018	—	HM015119	HM015055	HM057403
<i>Cayaponia buraeavii</i> Cogn.	W. Haber 10817 (USF)	Costa Rica: Puntarenas, Section of Puntarenas Monteverde, Tract X (10°18'N, 84°48'W)	HM015019	HM015086	HM015120	HM015056	HM057404
<i>Cayaponia buraeavii</i> Cogn. (incl. <i>C. austin-smithii</i> Standl.)	P. Raven 21795 (F)	Costa Rica: Puntarenas, above Wilson's finca, 6 km S of San Vito de Java	HM015016	HM015084	HM015117	HM015053	HM057401
<i>Cayaponia cabocla</i> (Vell.) Mart.	O. Ribas 2111 (C)	Brazil: Parana, Mun. Tijucas do Sul, Rio do Fojo	HM015020	HM015087	HM015121	HM015057	HM057426
<i>Cayaponia citrullifolia</i> (Griseb.) Griseb.	M. Nee 40281 (LPB)	Bolivia: Santa Cruz, Prov. Ichilo, 11 km NE of Buena Vista, 1 im N of Buena Vista—Portachuelo highway on road to Palacios (17°23'S, 63°35' W)	HM015021	HM015088	HM015122	HM015058	HM057417 HM057427
<i>Cayaponia citrullifolia</i> (Griseb.) Cogn. in Griseb.	R. Steele 1041 (TEX)	Bolivia: Santa Cruz, right side of pathway S of Mistad bridge (17°19'31"S, 63°19'31"W)	HM015022	—	HM015123	HM015059	HM057418 HM057428
<i>Cayaponia</i> aff. <i>citrullifolia</i> (Griseb.) Griseb.	R. Vanni 2951 (GH)	Argentina: Misiones, Dep. Iguazu, Iguazu National Park, on road to Pto. de las Canoas	HM015023	—	HM015124	HM015060	HM057419 HM057429
<i>Cayaponia cruegeri</i> (Naud.) Cogn.	M. Nee 37173 (LPB)	Bolivia: Beni, Prov. Cercado, Casarabe, 51 km E of Trinidad on road to Ascencion de Guarayos (14°50'S, 63°40'W)	HM015024	HM015089	HM015125	HM015061	HM057420 HM057430
<i>Cayaponia espelina</i> (Manso) Cogn.	S. Ferrucci 1709 (F)	Paraguay: Amambay, 1 km S of Road 5, on the way to Colonia Aceite.	HM015025	HM015090	HM015126	HM015062	HM057431

APPENDIX I. Continued.

Species	DNA source	Geographic origin	<i>trnH-psbA</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	ITS
<i>Cayaponia glandulosa</i> (Poepp. & Endl.) Cogn.	I. A. Chacon 1006 (F)	Costa Rica: Prov. Heredia, Finca La Selva, the OTS Field Station on Rio Puerto Viejo just E of its junction with Rio Sarapiquí, Sendero El Atajo	HM015026	HM015091	HM015127	HM015063	—
<i>Cayaponia jenmanii</i> C. Jeffrey	T. McDowell 4389 (U)	Guyana: Region Barima-Waini, trail between Aranka Head and Barima Head, NW of Kariako River (07°30'N, 60°35'W)	HM015027	HM015092	HM015128	HM015064	HM057421
<i>Cayaponia micrantha</i> Cogn.	C. H. Dodson 6711 (SEL)	Ecuador: Los Rios, Rio Palenque Biological Station, km 56 Road Quevedo, Sto. Domingo	HM015028	HM015093	HM015129	HM015065	HM057405
<i>Cayaponia ophthalmica</i> R. E. Schult.	R. A. A. Oldeman T-768 (U)	French Guiana: Haut Oyapock, downstream the mouth of river Eureupoucigne	HM015029	HM015094	HM015130	HM015066	HM057406
<i>Cayaponia palmata</i> Cogn.	S. Ferrucci 471 (F)	Argentina: Misiones, Iguazu, Road 12, 20 km SE of Puerto Iguazu	HM015030	HM015095	—	—	—
<i>Cayaponia pilosa</i> (Vell.) Cogn., incl. synonym <i>C. diffusa</i> Silva Manso = GT	G. Hatschbach 58233 (C)	Brazil: Parana, Mun. Guaratuba, Rio Cubataozinho	HM015031	HM015096	HM015131	HM015067	HM057407
<i>Cayaponia podantha</i> Cogn.	Seeds from Hudson Seed Co., California, cult. B. Toskey	Argentina	HM015032	DQ536760	DQ536760	DQ648159	HM057408
<i>Cayaponia quinqueloba</i> (Raf.) Shinnery	D. Searcy 275 (USF)	United States of America: Florida, Escambia Co., small park/recreational area along E side of US 29 at the junction with FL 4, Century	HM015034	HM015098	HM015133	HM015069	HM057423
<i>Cayaponia quinqueloba</i> (Raf.) Shinnery	V. Bates 10234 (GH)	United States of America: Oklahoma, Little River, about 3 mi. S of Kulichito Church	HM015033	HM015097	HM015132	HM015068	HM057422
<i>Cayaponia racemosa</i> (Mill.) Cogn.	A.R.A. Goerts 472 (U)	Guyana: Santa Mission (03°30'N, 58°23'W)	HM015035	—	HM015134	HM015070	HM057409
<i>Cayaponia rigida</i> (Cogn.) Cogn.	Ek, R. C. Riet 1723 (U)	French Guiana: Tresor Reserve (4.35 N, 52.16 W)	HM015037	HM015109	HM015135	HM015071	HM057410
<i>Cayaponia rugosa</i> Gomes-Klein & Pirani	W. R. Anderson 6200 (C)	Brazil: Goias, ca. 20 km by road N of Alto Paraiso	HM015038	HM015099	HM015136	HM015072	HM057424
<i>Cayaponia selysioides</i> C. Jeffrey	M. J. Jansen-Jacobs 3380 (U)	Guyana: Rupununi, Kanuki Mts., Crabwood Cr., Camp 2 (03°07'N, 59°06'W)	HM015039	HM015100	HM015137	HM015073	HM057425
<i>Cayaponia</i> spec.	C. E. Cerón 5663 (AAU)	Ecuador: Pichincha, Pululahua Geobotanic Reserve, on the border of highway between Pululahua and Niebli (00°05'N, 78°30'W)	HM015040	HM015101	HM015138	HM015074	HM057411
<i>Cayaponia triangularis</i> (Cogn.) Cogn.	F. H. F. Oldenburger 651	Suriname: W border of Great Sipaliwini savanna, in wide valley in kawfoetoe zone	HM015041	HM015102	HM015139	HM015075	HM057412
<i>Cayaponia tubulosa</i> Cogn.	M. Nee 37222 (LPB)	Bolivia: Santa Cruz, Prov. Ichilo, 4 km WSW of Buena Vista on road to El Cairo (17°28' S, 63°41' W)	HM015042	HM015103	HM015140	HM015076	HM057413
<i>Selysia cordata</i> Cogn. = <i>Cayaponia cordata</i> (Cogn.) Duchén and Renner	R. Fonnegra & F. J. Roldan 2276 (MO)	Colombia: Antioquia, Anorí; Cañón del Río Porce, near desembocadura del Río Riachon	HM104674	HM104677	HM015143	—	HM104679
<i>Selysia prunifera</i> (Poepp. & Endl.) Cogn., GT = <i>Cayaponia prunifera</i> (Poepp. & Endl.) Duchén and Renner	H. Hentrich FGIC60 (ULM)	French Guiana: Inselberg Forest Reserve Les Nouragues (4°5'N, 52°41'W)	—	DQ536862	DQ536862	DQ536590	—
<i>Selysia prunifera</i> = <i>Cayaponia prunifera</i>	T. D. Croat 13846 (NY)	Panama, Colón, Santa Rita	HM104675	HM104678	—	—	—
<i>Selysia</i> aff. <i>prunifera</i> =	S. Smith 303 (NY)	Peru, Madre de Dios, Río Tambopata, Explorer's Inn.	HM104676	HM164539	—	—	—

APPENDIX 1. Continued.

Species	DNA source	Geographic origin	<i>trnH-psbA</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	ITS
<i>Selysia smithii</i> (Standl.) <i>C. Jeffrey</i> = <i>Cayaponia smithii</i> Standl.	T. D. Croat 70987 (MO)	Colombia: Valle del Cauca, Mun. Buenaventura, Region of Bajo Calima, along road between Buenaventura and Malaga (04°09' N, 77°12'W)	HM015045	HM015108	HM015144	—	—
Outgroups							
<i>Abobra tenuifolia</i> (Gillies ex Hook.) Cogn., incl. synonym <i>A. viridiflora</i> Naudin = GT	M. W. Chase 915 (K), Kew 1967-25606, aliquot	Brazil, Argentina, Uruguay	—	DQ536782	DQ536782	DQ536616	—
<i>Cionoscycs guabubu</i> Grayum & J. A. González	G. McPherson 19825 (MO)	Panama: Colon, Teck Cominco Petaquilla mining concession (08°49'23"N, 80°39'35"W)	—	HM015106	—	—	—
<i>Cionoscycs guabubu</i> Grayum & J. A. González	B. Hammel 24495 (MO)	Costa Rica: Limón: Pococí. Parque Nac. Braulio Carrillo, Cuenca del Sarapiquí. Along path to Botarama (10°09'20"N, 83°56'30"W)	HM015044	HM015105	HM015142	HM015078	—
<i>Cionoscycs guabubu</i> Grayum & J. A. González	B. Hammel 25124 (MO)	Costa Rica: Slope of Volcán Tenorio	HM015043	HM015104	HM015141	HM015077	HM057432
<i>Cionoscycs macranthus</i> (Pittier) C. Jeffrey	G. Martinez Calderón 985 (MO)	Mexico: Oaxaca, Puerto Eligio Mun. Comaltepec, road Tuxtepec to Oaxaca, Sierra Juarez km 149	—	HM015107	—	—	—
<i>Cionoscycs macranthus</i> (Pittier) C. Jeffrey	B. Hammel 25099 (MO)	Costa Rica: Heredia, Santo Domingo, cult. from seeds brought from Turrialba	—	—	HM104680	—	—
<i>Cionoscycs</i> aff. <i>macranthus</i> (Pittier) C. Jeffrey	E. Cruz, 6 May 2002, unvouchered	Costa Rica: Alajuela: Río Peñas Blancas	—	DQ536797	DQ536797	DQ536536	—
<i>Schizocarpum palmeri</i> Cogn. & Rose	H. Kruse 2889 (M)	Mexico: Guerrero	—	DQ536858	DQ536858	DQ536586	—

APPENDIX 2. *Cayaponia* habitat and pollinator codings used in this study. The information on species ranges is based on label information from 1908 herbarium specimens. Michael Nee checked species identifications during a visit to the Munich herbarium in February 2010. *Cayaponia macrocalyx* and *C. tayuya* were not included in the ancestral reconstructions because of missing sequences. Traits are coded as follows: h = 0, rainforest, h = 1, savannas, grasslands, and disturbed habitat, h = 2, seasonal forest, h = ?, unknown; p = 0, bats, p = 1, bees, p = ?, unknown. *Cayaponia* cf. *granatensis*, voucher Vogel 94 (WU), from Anchicayá, Dept. Valle del Cauca, Colombia, mentioned in Vogel (1969) as bat-pollinated, cannot be securely identified.

Species	Corolla color, time of anthesis (if known)	Pollinator	Species range and habitat	Reference
<i>Cayaponia</i> and <i>Selysia</i>				
<i>C. africana</i>	White, diurnal	Bee	West Africa; Swampy, open habitats (h = 1, p = 1)	Steenft (1988)
<i>C. amazonica</i>	White with pale green stripes	Bat	NW South America; Rainforest (h = 0, p = 0)	Inferred from color photograph by R. Steele of her collection 1053 (TEX)
<i>C. americana</i>	White with pale green stripes; diurnal	Bee	Caribbean; Disturbed sites (h = 1, p = 1)	Inferred from color photograph by R. Steele of her collection 1031 (TEX)
<i>C. attenuata</i>	Yellowish-greenish; diurnal	Bee	Central America; Scrubland (h = 1, p = 1)	Heithaus (1979)
<i>C. buraeavii</i>	Color unknown	Unknown (bees?)	Colombia to Costa Rica; Rainforest (h = 0, p = ?)	Hypanthium ca. 4–4.5 mm diam.; corolla ca. 6–7 mm (Grayum, 2009)
<i>C. cabocla</i>	Greenish white; anthesis 2–18 h	Bee	Brazil; Savanna (Cerrado) (h = 1, p = ? or 1)	Sazima et al. (1996); Pollinated by Colletidae early in the morning; coded once as bee-pollinated, once as unknown (see Materials and Methods; Results)
<i>C. citrullifolia</i>	White; diurnal	Bee	Argentina, Paraguay, Bolivia; Scrubland or forest (h = ? or 1, p = 1)	M. Nee, New York Botanical Garden, personal communication, Feb. 2010
<i>C. cruegeri</i>	Yellowish	Bee	Bolivia to Guianas; Rainforest (h = 0, p = 1)	Flower morphology as in <i>C. attenuata</i> (P. Duchon, personal observation)
<i>C. espelina</i>	White-greenish; diurnal	Bee	Brazil to Argentina; Savanna (Cerrado) (h = 1, p = 1)	Inferred from color photograph by V. L. Gomes-Klein, http://www.cucurbit.org
<i>C. jenmanii</i>	Greenish-white; nocturnal	Bat	Guianas, Brazil; Forest (h = 0, p = 0)	Label information on G. T. Prance 25469 (U)

APPENDIX 2. Continued.

Species	Corolla color, time of anthesis (if known)	Pollinator	Species range and habitat	Reference
<i>C. macrocalyx</i>	Yellowish-greenish; diurnal	Bee	Northern South America; Rainforest	D. Roubik, Smithsonian Institution, Panama, personal communication, 20 Feb. 2009: At Yasuní, Ecuador, pollinated by <i>Melipona</i> early in the morning
<i>C. micrantha</i>	Unknown	Unknown (bees?)	Northern South America; Forest (h = 0, p = ?)	Plants slender and fragile for the genus.
<i>C. ophthalmica</i>	White	Bat	Northern South America; Forest (h = 0, p = 0)	Hequet (2003)
<i>C. pilosa</i>	Green	Bat	Southern Brazil; Cerrado (h = 1, p = 0)	Flower morphology as in <i>C. cabocla</i> (P. Duchén, personal observation)
<i>C. quinqueloba</i>	White	Bee	Southern North America; Seasonal forest (h = 2, p = 1)	Extrapolated from the small size and color of the flowers and from the relatively northern distribution of the species
<i>C. racemosa</i>	Greenish; diurnal	Bee	Central America and Caribbean; Scrubland or forest (h = ? or 1, p = 1)	Ramírez (2004)
<i>C. rigida</i>	Green	Bat	French Guiana to N Brazil; Forest (h = 0, p = 0)	Hequet (2003)
<i>C. rugosa</i>	White to greenish	Unknown	Brazil; Cerrado (Savanna) (h = 1, p = ?)	Flowers only known in bud (on the type specimen)
<i>C. selysioides</i>	White-greenish	Unknown	Northern South America; Forest (h = 0, p = ?)	Hypanthium 5-8 mm long
<i>C. sp. Ceron 5663</i>	Unknown	Unknown	Ecuador; Rainforest (h = 0, p = ?)	–
<i>C. tayuya</i>	White-greenish; flowering diurnal	Bee	NW Brazil, Bolivia; Forest and Scrubland	Queiroz-Cámara et al. (2004)
<i>C. triangularis</i>	Unknown	Unknown (bee?)	Northern South America; Forest (h = 0, p = ?)	Flowers small: hypanthium 4 mm long
<i>C. tubulosa</i>	Green tubular flowers	Bat	Peru, Ecuador, Brazil; Forest (h = 0, p = 0)	Flower morphology as in <i>C. amazonica</i> (P. Duchén, personal observation)
<i>Selysia cordata</i>	Greenish-white	Unknown	Colombia; Forest (h = 0, p = ?)	–
<i>Selysia prunifera</i> (<i>C. prunifera</i>)	Greenish-white; nocturnal	Bat	Peru; Forest (h = 0, p = 0)	Extrapolated from the large greenish white flowers (B. Hammel, personal communication)
Outgroups				
<i>Abobra tenuifolia</i>	Greenish white; diurnal	Bee	S Brazil, Uruguay, Argentina; Savanna (h = 1, p = 1)	Flowers inconspicuous, sweet-scented, and petals spreading
<i>Calycophyllum pedunculatum</i>	Yellowish; nocturnal	Bat	Colombia to Peru; Forest (h = 0, p = 0)	Vogel (1958; 1969). Vogel first identified this as <i>Cayaponia</i> sp. (1958, p. 509, fig. 5), but C. Jeffrey (in Vogel 1969, p. 201) reidentified the voucher, Vogel 63 (WU), as <i>C. pedunculatum</i>
<i>Anacaona sphaerica</i>	Unknown	Unknown	Hispaniola (h = 0, p = ?)	Flowers >1 cm in diameter
<i>Cionosicyus guabubu</i>	Greenish cream; nocturnal	Bat	Central America; Rainforest (h = 0, p = 0)	Information on flowering time and color photos from B. Hammel, 19 Aug. 2009
<i>Cionosicyus macranthus</i>	Cream with green lines; diurnal	Bee	Central America; Rainforest (h = 0, p = 1)	B. Hammel, personal communication, 19 Aug. 2009
<i>Penelopeia suburceolata</i>	Unknown	Unknown	Hispaniola (h = 0, p = ?)	Extrapolated from small flower size
<i>Schizocarpum palmeri</i>	Yellow; diurnal	Bee	Mexico; Scrubland (h = 1, p = 1)	Inferred from color photograph at http://www.conabio.gob.mx . Identification confirmed by M. Nee, Feb. 2010
<i>Tecunumania quetzalteca</i>	White	Unknown	Costa Rica, Mexico, Guatemala (h = 0, p = ?)	Nadkarni and Wheelwright (2000)