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PHYLOGENETIC RELATIONSHIPS AND BIOGEOGRAPHY OF *FUCHSIA* (ONAGRACEAE) BASED ON NONCODING NUCLEAR AND CHLOROPLAST DNA DATA[1]

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To examine relationships and test previous sectional delimitations within *Fuchsia*, this study used parsimony and maximum likelihood analyses with nuclear ITS and chloroplast *trnL-F* and *rpl*16 sequence data for 37 taxa representing all sections of *Fuchsia* and four outgroup taxa. Results support previous sectional delimitations, except for *F. verrucosa*, which is related to a Central American clade rather than to section *Fuchsia* and is described here as a new section *Verrucosa*. The basal relationships within *Fuchsia* are poorly resolved, suggesting an initial rapid diversification of the genus. Among the species sampled, there is strong support for a single South Pacific lineage, a southern South American/southern Brazilian lineage, a tropical Andean lineage, and one or two Central American and Mexican lineages. There is no clear support for an austral origin of the genus, as previously proposed, which is more consistent with *Fuchsia*'s sister group relationship with the boreal *Circaea*. An ultrametric molecular clock analysis (all minimal dates) places the split between *Fuchsia* and *Circaea* at 41 million years ago (mya), with the diversification of the modern-day lineages of *Fuchsia* beginning at 31 mya. The South Pacific *Fuchsia* lineage branches off around 30 mya, consistent with fossil records from Australia and New Zealand. The large Andean section *Fuchsia* began to diversify around 22 mya, preceded by the divergence of the Caribbean *F. triphylla* at 25 mya. The Brazilian members of section *Quelusia* separated from the southern Andean *F. magellanica* around 13 mya, and the ancestor of the Tahitian *F. cyrtandroides* split off from the New Zealand species of section *Skinnera* approximately 8 mya.

Key words: biogeography; *Fuchsia*; ITS; molecular clock; Onagraceae; penalized likelihood; *rpl*16 intron; *trnL-F* spacer.

Fuchsia is a distinctive genus in the Onagraceae (order Myrtales), comprising nearly 110 species. It is the only genus in the family with fleshy berries and largely biporate pollen, and it typically has bird-pollinated flowers. Based on different patterns of morphological traits among the species of *Fuchsia*, taxonomists specializing in the genus have come to recognize 11 sections (Munz, 1943; Berry, 1982; Godley and Berry, 1995; Table 1). The genus consists mostly of mesic shrubs confined to cool, moist habitats, with close to three-quarters of the species concentrated in the tropical Andes. The remaining species occur in Mexico and Central America, Hispaniola, southeastern Brazil, and the southern Andes. In a notable disjunction, four species are native to the South Pacific, three on New Zealand, and one on the island of Tahiti.

In the first explicit biogeographic scenario proposed for *Fuchsia*, Raven (1972) suggested that the genus may have reached New Zealand from South America via long-distance dispersal across the Pacific, using Tahiti as a stepping stone. However, with the documentation of numerous fossil records of *Fuchsia* pollen from both Australia and New Zealand dating back to the Late Oligocene (Berry et al., 1990), it appeared more likely that *Fuchsia* formed part of the Antarcto-Tertiary Geoflora that could have spread between South America, Antarctica, and Australia by either direct overland connections or across narrow water gaps during the Eocene and possibly into the Oligocene. In terms of vicariance biogeography, a significant geographic separation between the South Pacific and the neotropical species of *Fuchsia* would have been established at least 35–40 million years ago (mya). For the Tahitian species, a long-distance dispersal event from a New Zealand ancestor was hypothesized by Sytsma et al. (1991), based on chloroplast DNA restriction site analysis and the isolation and recent volcanic origin of Tahiti (±2 mya; Dymond, 1975).

Among the American sections of *Fuchsia*, section *Quelusia* has a disjunct distribution between the southern temperate forests of the Andes and the cool montane forests of southeastern Brazil. This follows a pattern shared with other austral-Antarctic groups such as *Araucaria*, *Cordyline*, *Drimys*, and *Griselinia*, which inhabit the relicts of a once widespread Southern Hemisphere temperate forest (Zinmeister, 1987; Berry, 1989; Katinas et al., 1999). Based on the evidence for austral biogeographical connections in *Fuchsia* during the Tertiary from section *Quelusia* and from the South Pacific species, along with suggestions that the more species-rich tropical Andean sections were more recent radiations associated with the Neogene uplift of the northern Andes (Berry, 1982, 1985), Berry (1989) postulated that southern South America was the most likely area of origin for the genus.

The first molecular studies of *Fuchsia* used chloroplast restriction site mutations to explore the relationships of exemplars from each of the currently recognized 11 sections (Sytsma and Smith, 1988; Sytsma et al., 1991). The consensus cladogram of the combined studies (Sytsma and Smith, 1992) showed the South Pacific species to be sister to the rest of the

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TABLE 1. The 12 sections[a] of *Fuchsia* and their native geographical distribution.

Section	No. species	Distribution
Procumbentes	1	New Zealand (North Island)
Skinnera	3	New Zealand (both islands), Tahiti
Quelusia	9	SE Brazil, southern Chile & Argentina
Kierschlegeria	1	Central coastal Chile
Fuchsia	64	Tropical Andes, Hispaniola
Hemsleyella	15	Tropical Andes
Pachyrrhiza	1	W slopes of Peruvian Andes
Verrucosa[a]	1	Colombia and Venezuela
Ellobium	3	Mexico and Central America
Encliandra	6	Mexico and Central America
Schufia	2	Mexico and Central America
Jimenezia	1	Costa Rica and Panama

[a] Includes a new twelfth section added in this paper.

genus. The next branch was the monotypic section *Jimenezia* from Central America, followed by a trichotomy formed by section *Quelusia* along with the monotypic *F. lycioides* (section *Kierschlegeria*) from central Chile, the Andean section *Hemsleyella*, and a clade consisting of the remaining South and Central American sections. It was later realized (P. E. Berry, unpublished data) that the identity of the *F. jimenezii* accession in these studies was suspect and probably represented a cultivar of mixed parentage. In several family-wide molecular analyses of the Onagraceae, the circumboreal genus *Circaea* has consistently received strong support as the sister group of *Fuchsia* (Bult and Zimmer, 1993; Conti et al., 1993; Levin et al., 2003). This is paradoxical with the proposed austral origin of *Fuchsia* suggested earlier.

The current study uses a more complete sampling of species within *Fuchsia* than did previous studies, as well as sequence data from both the nuclear and chloroplast genomes, to (1) determine if the morphologically based sectional delimitations are supported by the molecular data and (2) resolve basal relationships in the genus to determine where and when the initial diversifications occurred.

MATERIALS AND METHODS

Taxon sampling—Outside of *Fuchsia*, two species of *Circaea* and two species of *Hauya* were included in the analysis; the latter were designated as the outgroup in all analyses. Based on previous phylogenetic studies of Onagraceae, *Circaea* is sister to *Fuchsia*, and *Hauya* is either sister to *Circaea* + *Fuchsia* or else it is sister to the rest of the family except *Ludwigia* (Bult and Zimmer, 1993; Conti et al., 1993; Levin et al., 2003). Within *Fuchsia*, 37 accessions were used, comprising 32 species, one natural hybrid, and one cultivar. At least one species from all 11 currently recognized sections in the genus are represented. Two different accessions were sampled for *F. fulgens*, *F. triphylla*, and *F. boliviana*. The 41 accessions with their corresponding voucher information and GenBank accessions are listed on the Botanical Society of America website (Appendix 1, in Supplemental Data accompanying the online version of this article). Most samples were taken from cultivated plants, which were generally progeny of well-documented, wild-collected plants. The exceptions include *F. hybrida*, a cultivar of mixed parentage mainly involving members of section *Quelusia* (Berry, 1989). Also, the different accessions of both *F. fulgens* and *F. triphylla* were taken from plants that have long been cultivated. Both species are known to have been used in crosses to develop novel cultivars (Manthey, 1987), so some of these accessions may have undergone crossing with other species or cultivars at some point in their history; morphologically, however, they are within the normal range of variation of wild-collected members of their species. Finally, the accession of *F. vargasiana* differs somewhat from wild-collected plants and may have been inadvertently crossed with another species or cultivar in its recent cultivation history.

Extractions, amplification, and sequencing—Total genomic DNA was extracted from fresh, frozen, silica-dried, or herbarium samples using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987; Smith et al., 1991) or DNeasy Plant Mini Kits (Qiagen Corporation, Valencia, California, USA). Standard polymerase chain reaction (PCR) and cycle sequencing techniques were used to amplify and sequence double-stranded DNA. The *trnL-trnF* spacer was amplified using primers "e" and "f" (Taberlet et al., 1991), and the *rpl*16 intron was amplified using primers F71 (Jordan et al., 1996) and R1516 (Kelchner and Clark, 1997). Amplification of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, composed of ITS1, the 5.8S gene, and ITS2 (Baldwin, 1992; Baldwin et al., 1995) was conducted using primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White et al., 1990) and ITS5HP (5′-GGAAGGAGAAGTCGTAACAAGG-3′; Hershkovitz and Zimmer, 1996). Additional sequencing primers used were ITS2 (5′-CGTAGCTACTTCTTGCATCG-3′; White et al., 1990), ITS3B (5′-GCATCGATGAAGAACGTAGC-3′; White et al., 1990), C5.8S (5′-TGCGTTCAAAGACTCGAT-3′; Suh et al., 1993), and N5.8S (5′-ATCGAGTCTTTGAACGCA-3′; Suh et al., 1993). The PCR products were cleaned using QiaQuick PCR purification kits (Qiagen) or the polyethylene glycol (PEG)/NaCl method of Kusakawa et al. (1990). Sequences were generated on an ABI Prism 373 or 377 DNA sequencer (Applied Biosystems, Foster City, California, USA).

Sequences were initially aligned manually using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Insertions and/or deletions (indels) that were parsimony-informative (e.g., shared by two or more taxa) were recorded and mapped onto the trees. In the *trnL-trnF* spacer sequence of *Fuchsia insignis*, there was a large deletion (194 bp) in a region where other taxa had smaller indels. These smaller indels were scored when present, except for *F. insignis*, where they were treated as unknown. Areas of ambiguous alignment or containing poly-N stretches were excluded from the analyses.

Phylogenetic analysis—We reconstructed the phylogeny of *Fuchsia* and close relatives using both maximum parsimony (MP) and maximum likelihood (ML) optimality criteria in PAUP* version 4.0b8 (Swofford, 2002). The heuristic MP searches employed 1000 random taxon addition sequences and tree bisection-reconnection (TBR) branch swapping. All characters were equally weighted and treated as unordered (Fitch, 1971). Besides standard tree parameters (tree length, branch length [BL], consistency index [CI], and retention index [RI]), support for individual branches was estimated using nonparametric bootstrapping (BS; Felsenstein, 1985) and decay analysis (index, DI; Bremer, 1988). Bootstrap analyses used 1000 replicates (simple addition, saving up to 1000 trees per replicate, TBR branch swapping, Multrees) on the individual and combined data sets. Decay indices for branches were obtained using reverse topological constraint with 1000 random taxon addition se-

TABLE 2. Date estimates of the *Fuchsia–Circaea* split (node B in Fig. 5) based on calibrated *rbcL* + *ndhF* sequences for Myrtales using three fossils (from Sytsma et al., in press) and resulting estimated rates of cpDNA + ITS for *Fuchsia* and outgroups. Both calibrations are based on maximum likelihood trees with branch lengths fitted to a clocklike pattern with the penalized likelihood method of Sanderson (2002).

Calibration point	Rate for *rbcL* + *ndhF* (substitutions/my)	Date of *Fuchsia–Circaea* split (mya)	Estimated rate for cpDNA + ITS (substitutions/my)
1. Myrtaceae	0.00140	B = 42.7	B_1 = 0.00097
2. Melastomataceae sensu stricto	0.00145	B = 41.2	B_2 = 0.00101
3. Combretaceae	0.00147	B = 40.7	B_3 = 0.00102

quences for each search, as suggested by Swofford (1993) and implemented by Baum et al. (1994). Decay analyses were only performed on the combined chloroplast and nuclear data set.

Because an excessive number of most parsimonious trees did not allow searches to be completed for the combined cpDNA data set under the described search parameters, an initial heuristic search of 100 random addition replicates was conducted, with 10 trees saved per replicate. The resulting consensus tree was then used as a backbone constraint to search for trees inconsistent with the initial trees. This strategy searches for possible shorter trees and tests if the strict consensus tree reflects all most parsimonious trees, even though all equal-length trees have not been found (Catalán et al., 1997).

The incongruence length difference (ILD) test (Farris et al., 1994, 1995) was employed to measure conflict between the three data sets, using the partition homogeneity test in PAUP*. We ran 1000 replicates on parsimony-informative characters using the TBR branch swapping algorithm (simple addition sequence, Multrees, steepest descent), with the number of trees retained for each replicate limited to 1000. This procedure may reduce the likelihood of finding most parsimonious topologies, but Farris et al. (1994) noted that exact tree lengths are not critical to the test. Alternative relationships suggested by individual analyses were also examined by enforcing topological constraints while running maximum parsimony tests using 100 random addition replicates and calculating the number of additional steps required.

Maximum likelihood analyses were conducted on the individual and combined data sets in PAUP*. We used hierarchical likelihood ratio tests (hLRTs) in Modeltest 3.06 (Posada and Crandall, 1998; alpha value of 0.01) to choose the preferred model of sequence evolution for the individual and combined data sets from among the 56 models tested by that program. An heuristic maximum likelihood search with TBR branch-swapping was then run using parameters determined by Modeltest for the selected model of sequence evolution.

Molecular clock analysis—A likelihood ratio test (Felsenstein, 1988) was performed on the single ML tree (cpDNA and nuclear ITS sequences), by comparing the scores of the ML tree with and without the clock. This rejected the clock assumption, so we used the penalized likelihood (PL) method (Sanderson, 2002) in the program r8s, version 1.60 (Sanderson, 2003). The PL averages local differences in the rate of DNA evolution on different branches, taking into account the topology of branching. Unlike nonparametric rate smoothing (NPRS; Sanderson, 1997), PL assigns a penalty for rapid rate changes among branches based on a smoothness parameter. We used the cross-verification approach in r8s to obtain the most likely smoothness parameter for this data. The ML tree was converted to an ultrametric tree (in which the lengths of all branches from the root are identical) in PL using the recommended algorithm = TN, collapsing zero length branches, and fixing the age of the *Fuchsia-Circaea* split. This split and thus calibration of the cpDNA and ITS sequence evolution for *Fuchsia* and relatives was based on results from a larger *rbcL* + *ndhF* analysis of Onagraceae and relatives in the Myrtales (Sytsma et al., in press). Three fossils (Myrtaceae, Melastomataceae, and Combretaceae) generated quite similar rates for *rbcL* + *ndhF* sequence evolution and provided three time estimates for the *Fuchsia–Circaea* split (Table 2; see Sytsma et al., in press, for details). These dates are consistent with the earliest known fossils of *Circaea* (fruits from the Oligocene in Europe; Boufford, 1982) and *Fuchsia* (pollen from the Oligocene in Australia, Berry et al.; 1990).

RESULTS

Fuchsia is supported as monophyletic in the cpDNA, ITS, and combined analyses (Figs. 1–4), with varying degrees of support (55% bootstrap in cpDNA, 96% in ITS, and 95% in the combined analysis). Although relationships at the base of the genus remain unresolved, four clades were recovered in all analyses: (1) the South Pacific clade of *F. excorticata*, *F.* × *colensoi*, *F. cytrandroides*, and *F. procumbens* (sections *Skinnera* and *Procumbentes*); (2) the southern South American clade of *F. brevilobis*, *F. coccinea*, *F. glazioviana*, *F. hatschbachii*, *F. regia*, *F. hybrida*, *F. magellanica*, and *F. lycioides* (sections *Quelusia* and *Kierschlegeria*); (3) the Mexican/Central American clade of *F. arborescens*, *F. paniculata*, and *F. jimenezii* (sections *Schufia* and *Jimenezia*); (4) and the tropical Andean clade of *F. inflata*, *F. insignis*, and *F. pilaloensis* (section *Hemsleyella*). The three species for which two accessions were sampled (*F. boliviana*, *F. fulgens*, and *F. triphylla*) are each supported as monophyletic.

Analysis of cpDNA—The aligned length of the 41 accessions for the *trnL-trnF* intergenic spacer is 634 bp, with individual lengths ranging from 611 bp (*F. inflata*) to 426 bp (*F. insignis*). Seven indels varying from 4 to 6 bp were introduced during alignment (Appendix 2, in Supplemental Data accompanying the online version of this article). Because of ambiguous alignment or poly-N stretches, 26 bp were excluded from the analyses. Of the 597 bp included, 37 (6.20%) were parsimony-informative. Aligned length of the *rpl*16 intron is 1122 bp, with 43 bp excluded from analyses due to uncertain alignment. Individual sequence lengths ranged from 1027 bp in *Hauya elegans* to 983 bp in *F. hatschbachii*, *F. magdalenae*, and *F. nigricans*. There are 67 (6.21%) parsimony-informative characters in the *rpl*16 data set, and five indels were introduced during alignment (Appendix 2, in Supplemental Data accompanying the online version of this article). With the low number of parsimony-informative characters in the *trnL-trnF* data set, few relationships are supported by the individual analysis. Under the assumption that both cpDNA data sets share a common evolutionary history, they were combined to give a total of 104 parsimony-informative characters.

Our initial analyses were unable to run to completion due to an excessive number of most parsimonious trees, but of 520 trees recovered in our constraint analysis, the consensus tree had a length of 290, CI = 0.814, and RI = 0.828. We then ran a reverse constraint analysis, where PAUP* searched for any shortest trees incongruent with the strict consensus of the partial pool of shortest trees. During the constrained search, only trees one step longer (length = 291) were recovered, indicating that the initial consensus tree is representative of the most parsimonious trees. One of the most parsimonious

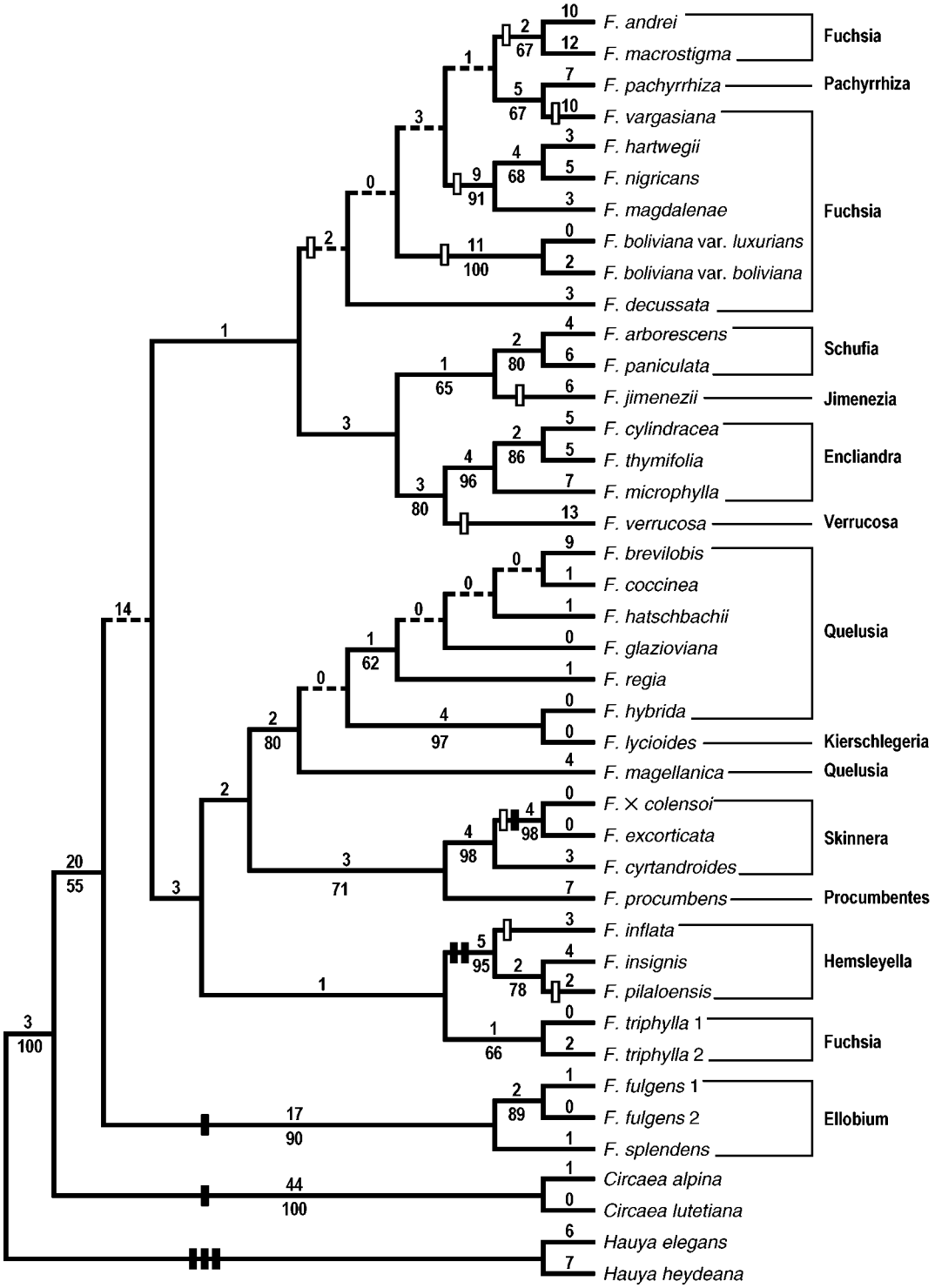

Fig. 1. One of the 520 most parsimonious trees (length 290) based on the combined cpDNA analyses (*rpl*16 and *trnL-trnF*). Branch lengths are above branches and bootstrap values are below. Branches that collapse in the strict consensus tree are indicated by dashed lines. The 12 indels are indicated by boxes; closed boxes indicate single-origin indels, and open boxes are indels requiring parallel or reversal events (see Appendix 2, in Supplemental Data accompanying the online version of this article).

trees is shown in Fig. 1, showing the branches that collapse in the consensus tree.

Modeltest 3.06 recognized the F82 + I + G model as the preferred one for the cpDNA data, with base frequencies A = 0.3776, C = 0.1710, G = 0.1670, T = 0.2843; proportion of invariable sites [I] = 0.5597; and gamma distribution shape [G] = 0.9157. An heuristic search resulted in one tree (ln L = −4316.72 555; tree not shown) that is nearly identical to the parsimony analyses—areas that collapse in the consensus tree are polytomies in the likelihood tree.

Analysis of ITS—The aligned length for the 41 accessions is 654 bp, with individual sequences ranging from 634 (*F. cylindracea* and *F. triphylla*) to 623 (*Fuchsia brevilobis*, *F. glazioviana*, and *F. hatschbachii*). A single indel groups all the Brazilian members sampled in section *Quelusia*. No nucleotide positions were excluded from analyses, and 123 (18.8%) characters were parsimony-informative. Two islands of trees were recovered (one island consisted of 18 trees and the other island of one) with length 312, CI = 0.776, and RI = 0.814. One of the most parsimonious trees is shown in Fig. 2, showing the branches that collapse in the consensus tree. With the increased number of parsimony-informative characters, there is higher support for some of the terminal relationships, but as with the cpDNA data, there is no clear support for basal relationships in the genus.

Modeltest 3.06 recognized the TrN + G model as the preferred one for the ITS data, with base frequencies A = 0.2322, C = 0.2986, G = 0.2621, T = 0.2071; substitution rates A-C = 1.0, A-G = 2.4703, A-T = 1.0, C-G = 1.0, C-T = 5.6018, G-T = 1.0; and gamma shape parameter = 0.6188. The single tree found under this model (ln L = −2834.6610; tree not shown) is topologically very similar to the MP analyses, with a large basal polytomy, except that *F. splendens* appears with a very short branch as sister to all remaining *Fuchsia*. All clades with a bootstrap greater than 50% in the parsimony analysis are recovered in the maximum likelihood analysis.

Combined analysis—Using a three-way comparison of the *trnL-trnF*, *rpl*16, and ITS data sets, the ILD test indicates significant incongruence ($P = 0.001$). Based on differences in the topologies of individual analyses, there were six taxa that had different positions in the cpDNA and ITS analyses (*Fuchsia hybrida*, *F. magdalenae*, *F. pachyrrhiza*, *F. verrucosa*, *F. triphylla*, and *F. vargasiana*). When an ILD test was conducted with these taxa removed, the data sets yielded insignificant *P* values, indicating congruence ($P = 0.111$). In most cases, the positions of the taxa that account for the nonhomogeneity between data sets vary within the same major clade in a way that does not contradict the overall results. In the ITS analyses, for example, *F. magdalenae* is sister to *F. macrostigma*, with these two taxa sister to *F. andrei*. In the cpDNA analyses, *F. magdalenae* is sister to *F. nigricans* and *F. hartwegii*. In both cases, *F. madgalenae* remains within the same moderately supported section *Fuchsia* clade. *Fuchsia hybrida*, which is a horticultural cultivar, groups with *F. magellanica* in the ITS analyses and with *F. lycioides* in the cpDNA analyses. In all analyses, *F. lycioides* is sister to *F. magellanica*, and this apparent conflict is consistent with *F. hybrida* being of mixed parentage with these two closely related species. *Fuchsia pachyrrhiza* is embedded within the section *Fuchsia* clade in the cpDNA analyses, while in the ITS analyses it is sister to the remaining members of the section *Fuchsia* clade; in both cases, there are no species from other sections embedded in the section *Fuchsia* + *F. pachyrrhiza* clade. In the ITS analyses, *F. verrucosa* is sister to section *Hemsleyella*, whereas in the cpDNA analyses it is sister to section *Encliandra*; these are conflicting results, but in both cases, *F. verrucosa* falls outside of section *Fuchsia*, where the species was previously placed. Lastly, the two accessions of *F. triphylla* are sister to section *Hemsleyella* in the cpDNA analyses but embedded within section *Fuchsia* in the ITS analyses. This could be due to past hybridization in cultivation within the *triphylla* cultivars. Because the alternative topologies of the taxa deemed incongruent by the ILD test largely do not involve shifts between major clades, we considered the incongruence to be minor and proceeded to combine the data sets.

Fitch parsimony analyses on the three combined data sets recovered one island of 156 trees (length = 626, CI = 0.764, RI = 0.600). Of the 2329 characters, 227 were parsimony-informative. One of the most parsimonious trees is shown in Fig. 3. As expected with increasing number of characters (Givnish and Sytsma, 1997), the trees were more resolved than in the individual analyses. However, this increased resolution and support occurs mainly at the terminal branches. There was still very little resolution at the base of the tree.

Modeltest 3.06 recognized the GTR + I + G model as the preferred one for the combined data. This model allows for independent rates of substitution for all nucleotide pairs, and among-site rate heterogeneity is modeled by allowing some sites to be invariant while the rest have rates drawn from a discrete approximation to a gamma distribution (base frequencies A = 0.3366, C = 0.2032, G = 0.1928, T = 0.2674; substitution rates A-C = 1.00, A-G = 1.1592, A-T = 0.4622, C-G = 0.4622, C-T = 2.2905, G-T = 1.00; I = 0.4727; G = 0.8474). An heuristic search resulted in one tree (ln L = −7442.9364; Fig. 4). The lack of basal resolution in the genus is also reflected in the topology of the maximum likelihood tree, although there is agreement with the parsimony trees in the terminal clades. Figure 4 is also used to show character evolution and is the basis of the molecular clock analysis that follows.

Molecular clock analysis—Figure 5 shows the result of the PL analysis with a smoothing rate of 31.62. Using the intermediate B_2 substitution rate for the *Fuchsia* data (Tables 2 and 3), the minimal age of the split between *Hauya* and *Circaea* occurs at about 52 mya (node A), and the *Fuchsia-Circaea* split occurred at least 41 mya (node B). According to the results of this analysis (all dates given are *minimal* ages), the initial diversification of what we now recognize as the main lineages of *Fuchsia* occurred about 31 mya (node C). The South Pacific *Fuchsia* lineage branches off around 30 mya, which is consistent with known fossil records of *Fuchsia* from Australia and New Zealand, which date back 25–30 mya (Berry et al., 1990). The split between the Caribbean clade represented by *F. triphylla* and the much more species-rich Andean section *Fuchsia* occurred at about 25 mya (node D). It appears that at least an initial diversification of the large Andean section *Fuchsia* occurred over 22 mya (node E). The New Zealand section *Procumbentes* splits off from the other South Pacific section *Skinnera* at 18 mya (node F). Two other significant dates suggested by the molecular clock analysis are at nodes G and H. The first represents the split between the southern Andean species (*F. lycioides* and *F. magellanica*) and the Brazilian members of section *Quelusia*, at about 13 mya. The second, at just over 8 mya, represents the split between the New Zealand species of section *Skinnera* and the ancestor of the Tahitian *F. cyrtandroides*.

DISCUSSION

Support for the genus and sectional delimitations—Our results confirm that *Fuchsia* is a monophyletic group, identifiable by its fleshy fruits. Two-porate pollen is also unique to *Fuchsia* and appears to be the basal condition in the genus,

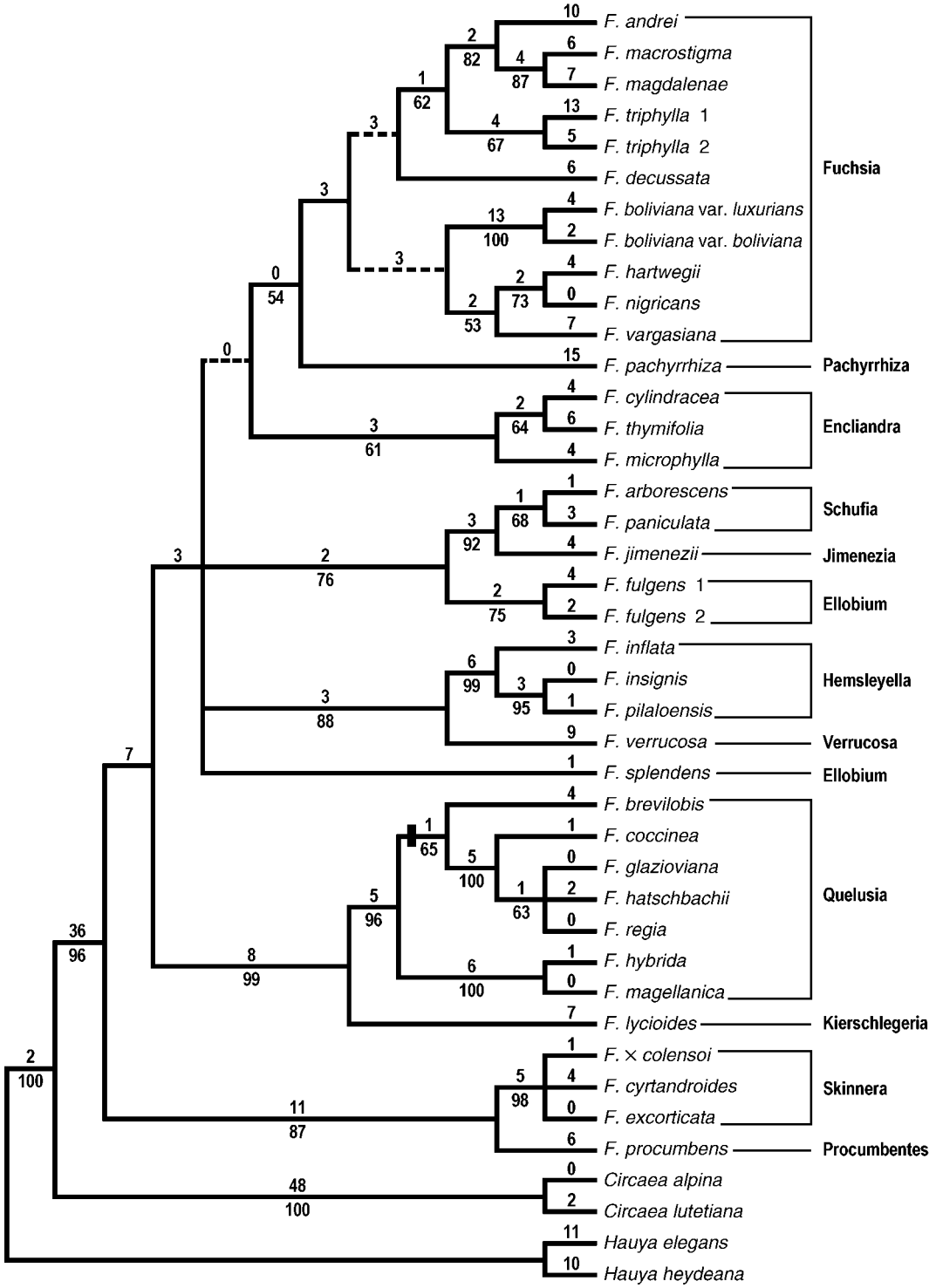

Fig. 2. One of the 19 most parsimonious trees of length 312 based on heuristic searches of ITS nDNA sequences. Branch lengths are above branches and bootstrap values greater than 50% are below. Branches that collapse in the strict consensus tree are indicated by dashed lines. The single indel is mapped on the tree.

with triporate pollen found mainly in the polyploid clade of sections *Kierschlegeria* and *Quelusia*.

Within *Fuchsia*, the monophyly of most currently recognized sections is supported by the molecular data. For example, the six species sampled from section *Quelusia* all form a well-supported clade (BL = 6, BS = 87, DI = 3; Fig. 3), which in turn forms a more strongly supported clade with the monotypic sister section *Kierschlegeria* (BL = 11, BS = 99, DI = 7). Within section *Quelusia*, the southern Andean *F. magellanica* is supported as sister to the remaining, disjunct

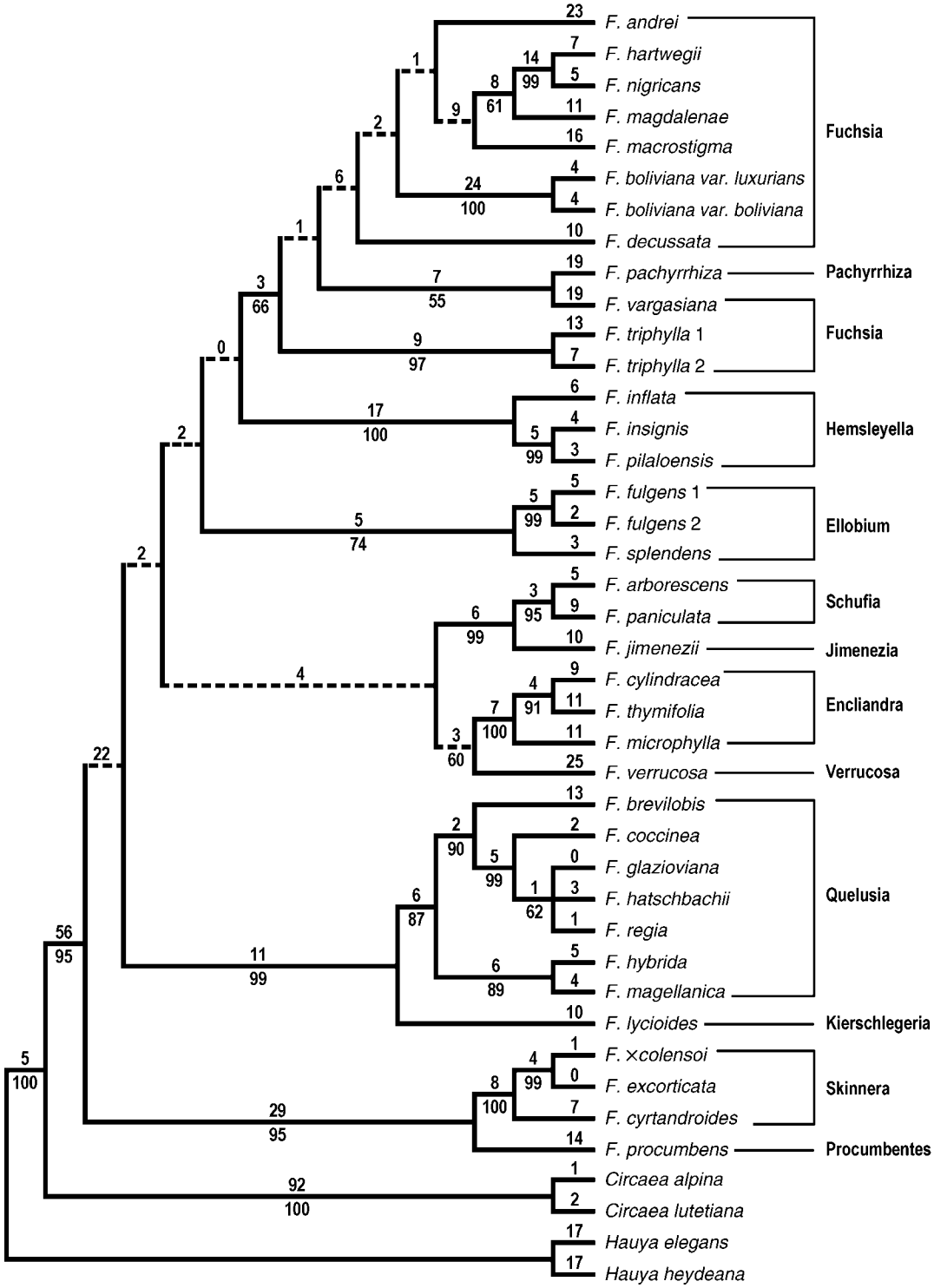

Fig. 3. One of the 156 most parsimonious trees of length 626 resulting from analyses of 41 sequences in the combined ITS, *trnL-trnF*, and *rpl*16 data sets. Branch lengths are provided above each branch, with bootstrap values above 50% and decay indices below. Branches that collapse in the consensus tree are indicated by dashed lines.

group of southern Brazilian species. The sole indel found in the ITS data set is a synapomorphy for the five Brazilian species sampled in section *Quelusia* (Fig. 2; Appendix 2, in Supplemental Data accompanying the online version of this article).

The South Pacific species are supported as a monophyletic clade, with the highly autapomorphic *F. procumbens* (section *Procumbentes*) sister to the members of section *Skinnera*, as previously found by Sytsma et al. (1991) using chloroplast restriction enzyme analyses. There is similar strong support

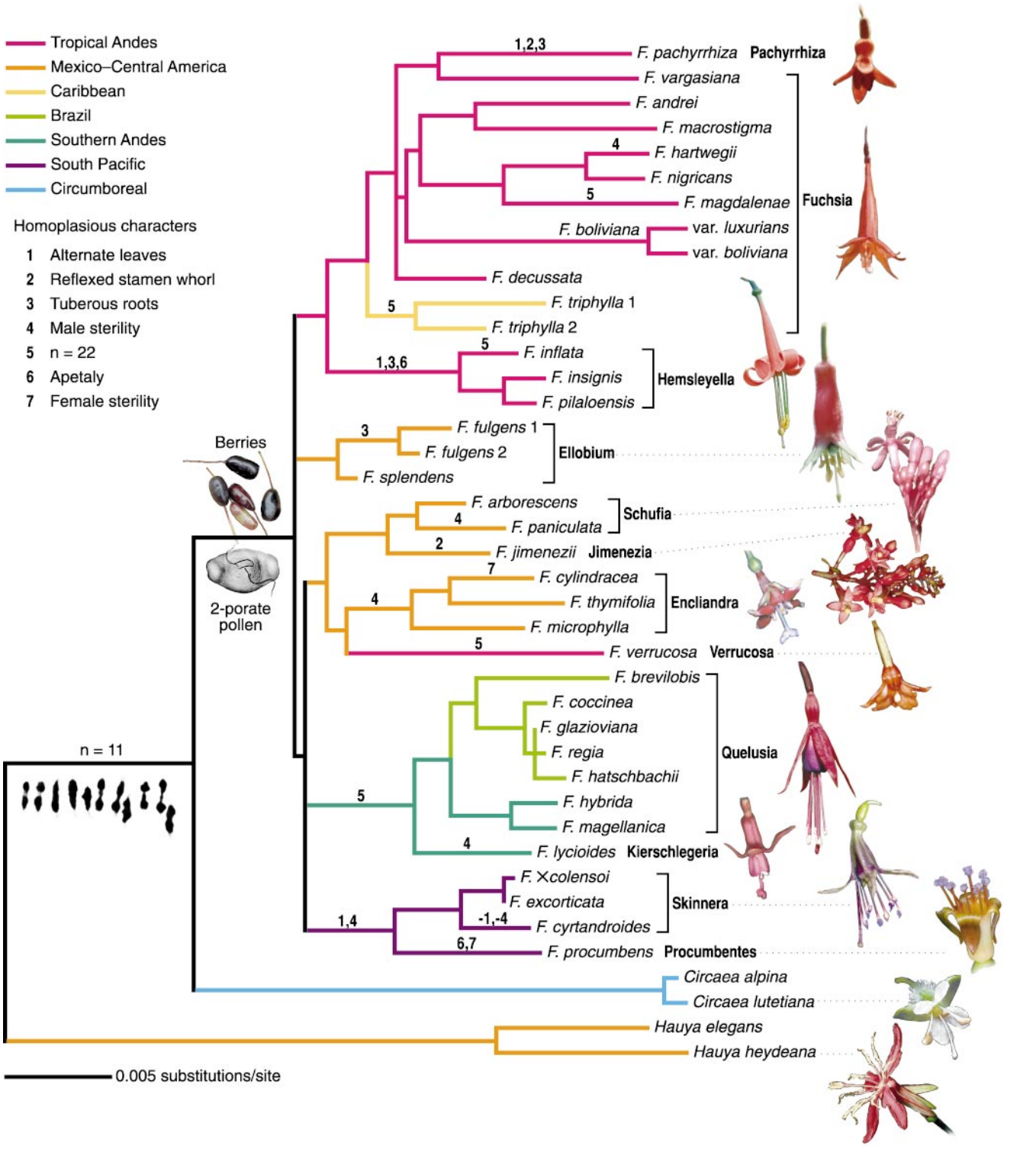

Fig. 4. Maximum likelihood tree of *Fuchsia* based on the combined ITS, *trnL-trnF*, and *rpl*16 data sets (ln L = −7442.9364 under the GTR + I + G model), with branch lengths proportional to the number of base changes along each branch. Superimposed upon the tree are geographical distributions of the taxa and enumeration of the most notable morphological or cytological homoplasies in the genus. The topology of this tree is very similar to the consensus tree of the most parsimonious trees (Fig. 3), except that the very short lowermost branch at the base of *Fuchsia* collapses to a larger polytomy in the maximum parismony (MP) consensus tree.

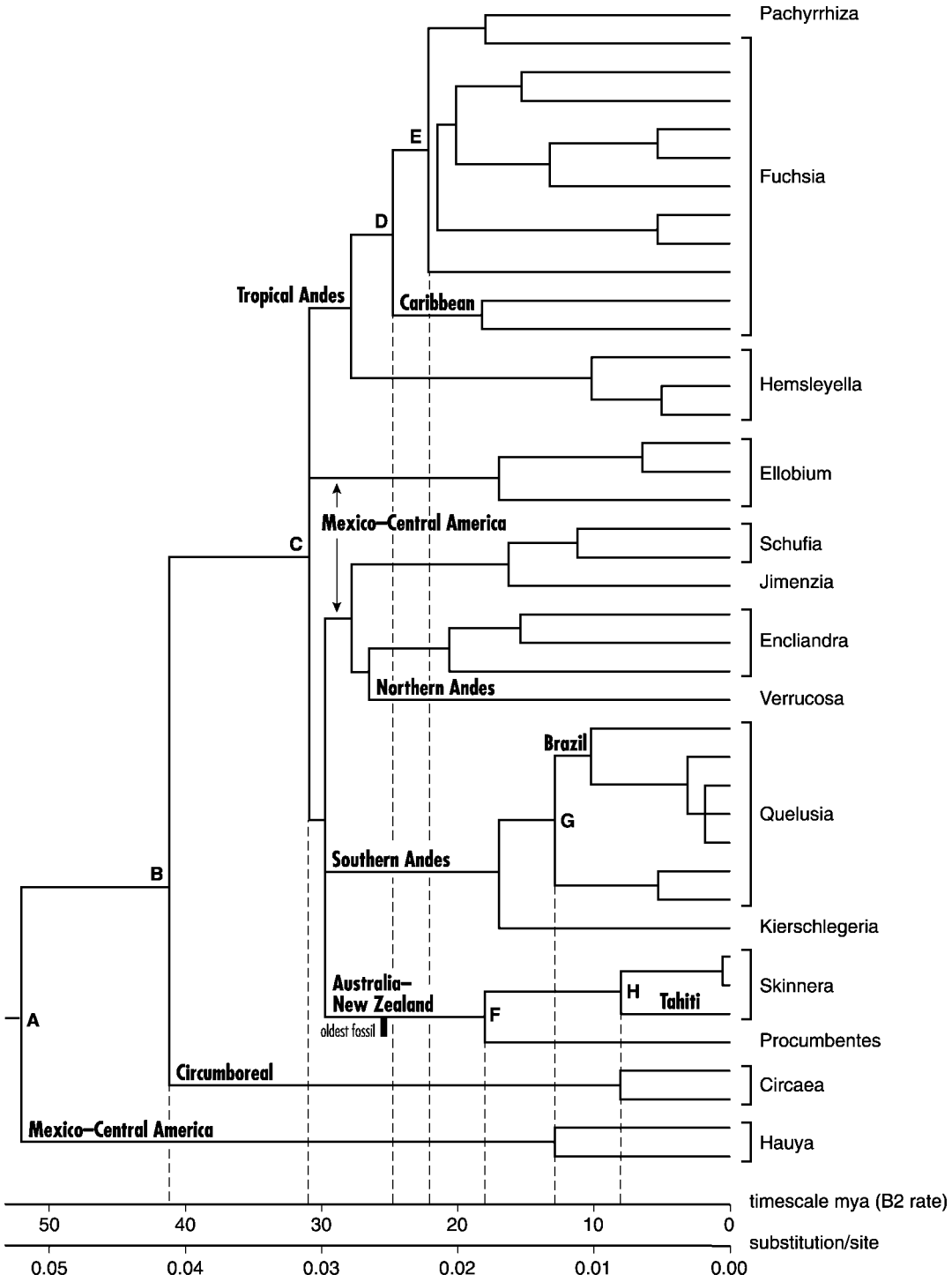

Fig. 5. Maximum likelihood tree of *Fuchsia* based on the combined ITS, *trnL-trnF*, and *rpl*16 data sets with branch lengths subjected to penalized likelihood rate smoothing. The analysis uses the intermediate B_2 substitution rate in Table 2 for the *Fuchsia* data. Nodes identified by letter are discussed in the text.

for section *Ellobium* (two of three species and three accessions sampled); section *Encliandra* (three of six species sampled); section *Hemsleyella* (three of 15 species sampled); and section *Schufia* (both species sampled); the latter is a close sister group to the monotypic section *Jimenezia*. For the large section *Fuchsia* (64 species), there is fairly weak support (BL = 3, BS = 66, DI = 1) grouping 10 of the 11 species sampled (excepting *F. verrucosa*). Because many of the informal species groups recognized by Berry (1982) are represented in our sample, as well as anomalous or geographically outlying species (e.g., *F. verrucosa*, *F. triphylla*, and *F. magdalenae*), we do not expect the addition of other species from section *Fuchsia* to fall outside this group.

The single species excluded from section *Fuchsia* in the

TABLE 3. Date estimates of splits within *Fuchsia* and with relatives based on calibrated cpDNA + ITS sequences (see Table 2).

	Nodal dates (mya)[a]				
Calibrated rate	A *Hauya–Fuchsia/ Circaea* split	B *Fuchsia–Circaea* split	C Basal radiation of *Fuchsia*	E Major Andean radiation of *Fuchsia*	F South Pacific differentiation of *Fuchsia*
B_1 = 0.00097	54.1	42.7	32.1	23.2	18.8
B_2 = 0.00101	52.2	41.2	31.0	22.5	18.1
B_3 = 0.00102	51.6	40.7	30.6	22.3	17.9

[a] See Fig. 5.

molecular analyses, *F. verrucosa*, is anomalous within that section, as pointed out by Berry (1982). Its large leaves and Andean distribution led to its original placement in section *Fuchsia*, but its small quadrangular flowers, very short floral tube, unique nectary type, large club-shaped style, long ovary, and pollen with smooth viscin threads are unlike any other member of the section (Fig. 6a). In the ITS analysis, *F. verrucosa* is sister to section *Hemsleyella*, but in the cpDNA and in the combined analysis, *F. verrucosa* is nested within a lineage of the Mexican and Central American sections *Encliandra*, *Schufia*, and *Jimenezia*. This is consistent with the small flower size, lobed nectaries, and smooth viscin pollen threads of *F. verrucosa* (Nowicke et al., 1984). However, it is the only tetraploid species in this group (Berry, 1982; Breedlove et al., 1982), and while the axillary flowers are shared with section *Encliandra*, the large leaves are like those of sections *Jimenezia* and *Schufia*. *Fuchsia verrucosa* is morphologically so unlike any extant member of section *Hemsleyella* that the ITS results placing it sister to this group are puzzling, and it seems very unlikely this would be the result of some past hybridization event with a member of that section. Although the position of *F. verrucosa* is not well resolved among the Central American and Mexican sections of the genus, we are recognizing it (see below, Taxonomic changes) as a new section in the genus because of its disjunct geographical distribution in northern South America, its clear separation from section *Fuchsia* in the molecular analyses, and its possession of numerous autapomorphies. Interestingly, *F. verrucosa* shares a unique indel with *F. jimenezii* in the *trnL-trnF* spacer sequence. The possibility that these are sister species needs to be explored with additional molecular markers.

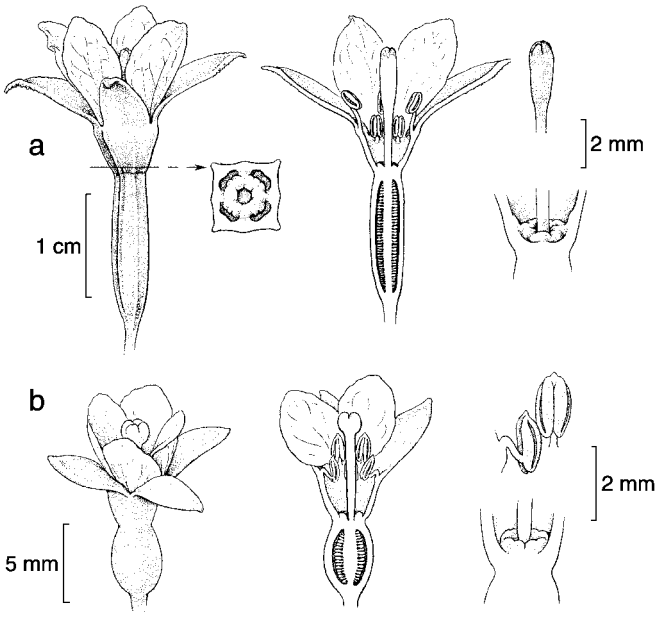

Fig. 6. (a) Floral details of *Fuchsia verrucosa*. Left, flower at anthesis with transverse section just above the nectary. Middle, longitudinal cross-section of the flower. Right, details of the stigma and the nectary. Note the very short tetragonal floral tube, short filaments, large clavate stigma, elongate ovary, and complex nectary. (b) Floral details of *Fuchsia jimenezii*. Left, flower at anthesis. Middle, longitudinal cross-section of the flower. Right, details of an antepetalous (above left) and an antesepalous anther (above right) and the nectary (below). This is the same kind of stamen reflexion that occurs in sections *Encliandra* and *Pachyrrhiza*.

The last section in the genus to be considered is the monotypic section *Pachyrrhiza*. In our combined analyses, it is nested within the large section *Fuchsia* clade, but with little internal support. In the ITS analyses, *F. pachyrrhiza* is sister to the other members of section *Fuchsia*. Given the large suite of characters in *F. pachyrrhiza* that differ fundamentally from all other members of section *Fuchsia* (Berry et al., 1988) and doubt about whether our accession of *F. vargasiana* (sister to *pachyrrhiza* in the combined analysis) had crossed with another species while in cultivation, it seems justifiable to maintain this as a distinct section from section *Fuchsia*. Also, *F. pachyrrhiza* lacks an indel in the *rpl*16 intron (Fig. 1; character 12 in Appendix 2, in Supplemental Data accompanying the online version of this article) that is present in all other Andean members sampled in section *Fuchsia* except for *F. decussata*. From the standpoint of our molecular results, *F. pachyrrhiza* is one of the best examples of the high degree of homoplasy in a number of key morphological characters that can occur in the genus. First, it has tubers as in the morphologically distinct sections *Ellobium* and *Hemsleyella*. It has alternate leaves, which occur elsewhere only in section *Hemsleyella*, the two South Pacific sections, and section *Kierschlegeria*. It has one of its whorls of stamens recurved into the floral tube, which only occurs elsewhere in sections *Encliandra* and *Jimenezia* (see Fig. 6b). Finally, its pollen has smooth viscin threads as in *F. lycioides*, *F. verrucosa*, and most of the Mesoamerican sections (Berry et al., 1988).

Molecular clock and lack of basal resolution among sections—In contrast to the overall support for the current sectional delimitation of *Fuchsia*, we found little basal resolution in the genus. Prior hypotheses pointed to the South Pacific sections as being sister to the New World sections, with the austral sections *Kierschlegeria* and *Quelusia* basal within the New World lineages (Sytsma and Smith, 1988; note that the basal position of section *Jimenezia* in that paper was likely due to a misidentification, particularly in light of the results obtained here). Although data from more genes would be desirable for our analysis, it is possible that the lack of basal resolution in *Fuchsia* is an indication of an ancient, rapid radiation, as was demonstrated by Fishbein et al. (2001) in the

Saxifragales. The PL analysis (Fig. 5) indicates that this initial radiation occurred about 31 mya, in the mid-Tertiary. It is unclear from our results where *Fuchsia* most likely originated, but given the strong support of the boreal genus *Circaea* as the closest outgroup of *Fuchsia* and of the Central American *Hauya* as the sister group to *Circaea* + *Fuchsia*, it seems just as likely that the genus arose in northern South America or even in southern North America or Mesoamerica, rather than in southern South America, as previously hypothesized (Berry, 1989).

Our results confirm that there is a single South Pacific lineage (two sections, four species) in *Fuchsia*, as well as a separate southern South America/southern Brazil lineage (two sections, 10 species). The maximum likelihood tree also suggests that there was a single major Andean radiation, comprising sections *Fuchsia*, *Hemsleyella*, and *Pachyrrhiza* (a total of 80 species, including two on the Caribbean island of Hispaniola), as well as a separate appearance in South America of the monotypic section *Verrucosa*. Lastly, there was either a single evolutionary lineage that developed in Mexico and Central America, comprising modern-day sections *Encliandra*, *Jimenezia*, *Schufia*, and *Ellobium*, or alternatively two separate lineages developed there, one comprising section *Ellobium* (three species) and the second the other three sections (nine species).

Fossil pollen evidence indicates that *Fuchsia* was present in Australasia by the early Oligocene, about 30 mya, which was taken as evidence to support an early Tertiary origin of the genus in southern temperate forests (Berry et al., 1990). The only extant sections in the genus that are found exclusively in the Southern Hemisphere are *Skinnera* and *Procumbentes* in the South Pacific and *Kierschlegeria* and *Quelusia* in South America. Given the long physical separation of Australia from Antarctica (ca. 50 mya) and the later opening of the Drake Passage between Antarctica and South America around 27 mya (Raven and Axelrod, 1974), we expected to see sections *Skinnera* and *Procumbentes* appear as a well-supported sister clade to the American species, and within the New World species, sections *Kierschlegeria* and *Quelusia* as sister to the tropical Andean, Central American, and Caribbean taxa. It is important to note that our data do not contradict these relationships, but they offer no significant support for them either. The PL analysis places the South Pacific–American split minimally at about 30 mya, which is consistent with the fossil pollen data from Australasia, but somewhat late for a direct exchange between South America, Antarctica, and Australia (Taylor, 1991, 1995). According to our current understanding of Caribbean plate tectonics (Taylor, 1995; Graham, 2003), there could have been an opportunity for a fairly continuous biotic exchange between North and South America via the proto-Antilles in the current Panama–Costa Rica Arc during the mid-Tertiary, between 25 and 35 mya. This would be consistent with the diversification of separate South American and Central American lineages of *Fuchsia* at this time, according to our PL analysis.

Hummingbirds are the primary pollinators for all of the South American species of *Fuchsia* (Berry, 1982, 1985, 1989). According to DNA hybridization distances among extant hummingbirds and a fossil divergence date corrected for incompleteness in the geological record, Bleiweiss et al. (1997) and Bleiweiss (1998a) determined that the major lineages of hummingbirds began to diverge in the Early Miocene (ca. 18 mya), even though the estimated divergence of hummingbirds from swifts dates back to the Paleocene (Sibley and Ahlquist, 1990). Some hummingbird lineages, however, notably high-elevation Andean groups and North American taxa, have diverged only within the last 2–8 my, including the emeralds, the mountain gems, and the bee hummingbirds (Bleiweiss, 1998a,b). These radiations were presumably correlated with the marked uplift of the northern Andes in the Pliocene and Quaternary and the severe climatic fluctuations associated with a long sequence of alternating glacial and interglacial periods (van der Hammen, 1995). For the Andean fuchsias, particularly the large section *Fuchsia*, we need a considerably denser molecular sampling of taxa to determine if certain groups, such as the high-elevation *Fuchsia petiolaris* species group (Berry, 1982), are similarly the result of recent (e.g., Pliocene or Quaternary) radiations. According to Gentry (1982) and Luteyn (2002), one reason the Neotropics are so much richer in plant species than the African or Asian tropics was the ''explosive speciation'' of many shrubby and herbaceous genera of plants centered in the northern Andes, including many hummingbird-pollinated groups like *Fuchsia*, *Heliconia*, Ericaceae, and Lobeliaceae.

There are only two Antillean species of *Fuchsia*, *F. triphylla* and *F. pringsheimii*, both native to Hispaniola. Our results indicate that *F. triphylla* was an early offshoot of section *Fuchsia*, possibly branching off from an Andean ancestor around 25 mya. Both *F. triphylla* and *F. pringsheimii* are tetraploid, whereas most members of section *Fuchsia* are diploid (Berry, 1982). Although the two species form natural hybrids in areas of geographical overlap in the Dominican Republic (Berry, 1982), they are morphologically very distinct. With wild-collected material of both species, we should be able to carry out molecular analyses and test if they are sister taxa and date their divergence.

The date obtained here for the split between the southern Andean and the southeastern Brazilian members of the *Kierschlegeria* + *Quelusia* clade, 13 mya, is significant because we could find no other molecular-based dates reported for this particular disjunction pattern. It will be particularly interesting to examine other clades with the same disjunction pattern, particularly more ancient lineages such as the southern Andean *Araucaria araucana* and the mainly Brazilian *A. angustifolia*, to determine if their divergence from a common ancestor in South America is concordant with the date obtained here for *Fuchsia*.

Finally, the date of 8 mya for the divergence of the Tahitian *F. cyrtandroides* from ancestors on New Zealand is close to the age of 10 mya that was estimated by Sytsma et al. (1991) based on cpDNA restriction site mutations. Because Tahiti is part of a volcanic arc that emerged from the Pacific only 2–4 mya (Dymond, 1975), this implies that *F. cyrtandroides* or its immediate ancestor either evolved for a considerable amount of time in New Zealand and then became extinct there after dispersing to Tahiti or else it dispersed earlier on to older, now submerged islands in the mid-Pacific.

Extensive morphological homoplasy—Our molecular results highlight a notable homoplasy in *Fuchsia*, namely the presence of male sterility, as manifested in species that are gynodioecious or dioecious (Fig. 4). In the subdioecious *F. procumbens* from New Zealand, the female is heterogametic, with male sterility controlled by a dominant gene (Godley, 1963). The members of the closely related section *Skinnera* are gynodioecious, with the exception of *F. cyrtandroides*, which is hermaphroditic and also disjunct on the mid-Pacific

island of Tahiti. The molecular results of Sytsma et al. (1991) led to the conclusion that gynodioecy is the ancestral condition in the South Pacific sections, with *F. cyrtandroides* becoming secondarily hermaphroditic through the loss of male sterility (presumably from a founder population that was double recessive for the male sterility gene). In contrast, the male sterility that is known in all six species of section *Encliandra* is determined differently than in the South Pacific species; in this section, the hermaphrodites are heterogametic, and there is probably more than one gene involved (Breedlove, 1969; Arroyo and Raven, 1975). The lability of the loss or gain of male sterility in *Fuchsia* is also shown by the presence of some hermaphroditic populations of *F. paniculata* (section *Schufia*) north of the Isthmus of Tehuantepec in Mexico, but gynodioecious populations present in all populations south of the Isthmus and into Central America (Breedlove et al., 1982). The closely related *F. arborescens* is entirely hermaphroditic. In South America, male sterility is known in *F. lycioides* (sole member of section *Kierschlegeria*; Atsatt and Rundel, 1982), which is tetraploid and sister to a lineage of hermaphroditic species (section *Quelusia*). Recently, P. E. Berry (unpublished data) found the first evidence of male sterility in the large section *Fuchsia*, with populations of *F. hartwegii* from Colombia that are gynodioecious.

Fruits with few, large seeds characterize both sections *Encliandra* and *Kierschlegeria*, but these sections occur in different clades, making it likely that this character evolved independently in the two groups. Opposite or whorled leaves is the most common phyllotaxy in the genus, but alternate leaves characterize sections *Kierschlegeria* and *Pachyrrhiza* (both monotypic). In addition, section *Hemsleyella* is mostly alternate-leaved, but at least four of the 15 species in the section have opposite leaves. The two South Pacific sections are alternate-leaved as well, except for the opposite-leaved Tahitian *F. cyrtandroides*. This distribution of alternate leaves shows that this character is widely homoplasious in *Fuchsia*, even though most sections—including the large sections *Fuchsia* and *Quelusia*—have entirely opposite or whorled leaves.

Root tubers are of limited occurrence in *Fuchsia*, but they are found in most species of section *Hemsleyella*, in *F. pachyrrhiza*, and in some or all members of section *Ellobium*. None of these three groups show any indication of close sister group relationships in the molecular data, again pointing to separate origins of root tubers in the genus. Another seemingly apomorphic character in *Fuchsia* is the reflexion of the antepetalous set of anthers down into the floral tube, instead of the typical exserted arrangement (Figs. 4, 6b). This character occurs in sections *Encliandra*, *Jimenezia*, and *Pachyrrhiza*. The first two form part of a weakly supported Central American clade, but each is sister in turn to another section that has exserted anthers (*Encliandra* with *Verrucosa* and *Jimenezia* with *Schufia*). Axillary flowers are the norm for most sections of *Fuchsia*, but paniculate inflorescences characterize the *Schufia* + *Jimenezia* clade. In the large Andean sections *Fuchsia* and *Hemsleyella*, however, both axillary flowers and different types of terminal or axillary inflorescences are found, showing that inflorescences have evolved independently in several different lineages.

Earlier knowledge about extensive homoplasy in morphological characters in *Fuchsia* and the paucity of additional reliable characters had dissuaded us in the past from attempting a morphologically based cladistic analysis of the genus. In fact, most sections have been defined by unique *combinations* of character states, rather than by true synapomorphies. The results outlined earlier and shown in Fig. 4 support our earlier doubts about using morphological characters that in many other groups might be considered appropriate for cladistic morphological analysis. An analogous situation occurs in the Brassicaceae, which show many instances of convergence in morphological characters (e.g., gamosepaly, fruit morphology, and cotyledon position). Many of these characters were used in earlier classifications but have since been shown by molecular evidence to be poor predictors of phylogenetic relationships (Koch et al., 2003).

Cytology and pollen morphology—*Fuchsia* and *Circaea* appear to share a basal chromosome number of $n = 11$ (Levin et al., 2003; Fig. 4). Except for some members of the two largest sections of *Fuchsia* (sections *Fuchsia* and *Hemsleyella*), chromosome counts have been made for all remaining species and sections (Breedlove, 1969; Berry, 1982, 1985, 1989; Breedlove et al., 1982; Berry et al., 1988; Hoshino and Berry, 1988, 1989). Most species and sections of *Fuchsia* are consistently diploid ($n = 11$), with two-porate pollen. The entire *Quelusia* + *Kierschlegeria* clade, however, is polyploid (all are tetraploid except for some octoploid populations of *F. regia*), and all have three-porate pollen. There are no known cases of three-porate pollen in any diploid species of *Fuchsia*, but there are several cases of tetraploid species with entirely two-porate pollen, such as *F. magdalenae*, *F. pringsheimii*, and *F. triphylla* in section *Fuchsia* (Berry, 1982) and *F. verrucosa* in its own new section (see Taxonomic changes, below). Two species in section *Hemsleyella* are tetraploid, and they have a mixture of two-porate and three-porate pollen (Berry, 1985). If our interpretation of *Fuchsia* phylogeny is correct, then polyploidy evolved independently on at least four (but probably more) occasions in the genus: in the ancestor of sections *Quelusia* + *Kierschlegeria* (in association with three-porate pollen), in section *Verrucosa*, at least once in section *Hemsleyella*, and several times in section *Fuchsia* (Fig. 4).

Segmented-beaded viscin pollen threads characterize most species and sections of *Fuchsia* (Nowicke et al., 1984), but the distribution of smooth viscin threads is mainly restricted to three Central American sections (*Encliandra*, *Jimenezia*, and *Schufia*) and the northern South American section *Verrucosa*, all of which form part of the same lineage in our maximum likelihood tree. Only two additional species, *F. lycioides* in the southern South American section *Kierschlegeria* and *F. pachyrrhiza* from Peru, also have smooth viscin threads (Nowicke et al., 1984; Berry et al., 1988).

Taxonomic changes—*Fuchsia* section *Verrucosa* P.E. Berry, sect. nov.—TYPE: *Fuchsia verrucosa* Hartweg (Fig. 6A). Frutex; folia opposita; flores axillares, bisexuales, ovario tetragono tubo florale duplo longiore, nectario cristis antepetalis; pollen biporatus filis rasilibus non segmentatis; bacca multiseminalis; chromosomatum numerus gameticus $n = 22$.

Shrubs; leaves opposite, elliptic, 5–16 × 2–8 cm; flowers solitary, axillary, bright orange; ovary elongate, tetragonous, 10–12 × 2.5–3 mm; floral tube four-angled, obconic, 3–6 mm long; petals 8–9 × 5–6 mm; nectary with four antesepalous ridged lobes 1.5 mm high; filaments erect, 2–3 and 1–1.5 mm long; style stout, 6–8 mm long, topped by a clavate stigma 2.5–4 mm long; pollen biporate, with smooth viscin threads; berry many-seeded; gametic chromosome number $n = 22$.

Conclusions—It is unclear from our molecular data how *Fuchsia* diverged early in its evolutionary history, but the data are consistent with an early, rapid divergence into several well-supported lineages that are now geographically and morphologically distinguishable, namely the South Pacific sections *Skinnera* and *Procumbentes*; the southern Andean/southern Brazilian sections *Quelusia* and *Kierschlegeria*; the Central American/Mexican sections *Encliandra*, *Jimenezia*, and *Schufia* together with the monotypic section *Verrucosa* of northern South America; the mainly tropical Andean sections *Fuchsia*, *Hemsleyella*, and *Pachyrrhiza*; and a possible second Central American lineage comprising section *Ellobium*. According to molecular clock analysis, *Fuchsia* diverged from a common ancestor with the boreal genus *Circaea* about 41 mya, and these two diverged from the Central American genus *Hauya* about 52 mya, which suggests that *Fuchsia* may not have had an austral origin as previously postulated. Similarly, the diversification of the tropical Andean fuchsias is considerably older than previously hypothesized (mid-Tertiary rather than late Tertiary or Quaternary), with the divergence of a small Antillean clade from South American ancestors occurring during the Miocene. The long evolutionary history of the genus may have contributed to the high levels of homoplasy evidenced in many key morphological characters, with multiple independent occurrences of polyploidy, male sterility, reflexed stamens, alternate leaves, and tuberous roots. Future molecular studies will address the origin of the major cultivar lines in *Fuchsia* to reconstruct which species and sections have been instrumental in the development of the myriad hybrids and cultivars. Also, carrying out a denser taxon sampling in the larger sections *Fuchsia*, *Hemsleyella*, *Quelusia*, and *Encliandra* will allow us to assess evolutionary and geographical relationships at considerably finer scales than in this paper.

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