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Phylogenetics of the Florally Diverse Andean Clade Iochrominae (Solanaceae)

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Phylogenetics of the florally diverse Andean clade Iochrominae (Solanaceae)[1]

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Recent molecular phylogenetic studies of Solanaceae have identified many well-supported clades within the family and have permitted the creation of a phylogenetic system of classification. Here we estimate the phylogeny for Iochrominae, a clade of Physaleae sensu Olmstead et al. (1999), which contains 34 Andean species encompassing an immense diversity of floral forms and colors. Using three nuclear regions, ITS, the second intron of *LEAFY*, and exons 2 to 9 of the granule-bound starch synthase gene (*waxy*), we evaluated the monophyly of the traditional genera comprising Iochrominae and assessed the extent of interspecific hybridization within the clade. Only one of the six traditionally recognized genera of Iochrominae was supported as monophyletic. Further, comparison of the individual nuclear data sets revealed two interspecific hybrid taxa and a third possible case. These hybrid taxa occur in the Amotape–Huancabamba zone, a region between the northern and central Andes that has the greatest diversity of *Iochroma* species and offers frequent opportunities for hybridization in areas of sympatry. We postulate that periodic hybridization events in this area coupled with pollinator-mediated selection and the potential for microallopatry may have acted together to promote diversification in montane Andean taxa, such as Iochrominae.

Key words: floral evolution; granule-bound starch synthase; interspecific hybridization; *LEAFY*; phylogeny; pollination; reticulate evolution; speciation.

The tropical Andes comprise the pre-eminent hotspot of plant biodiversity, with approximately 15% of all plant species native to that region (Myers et al., 2000). Many plant families, though cosmopolitan, have centers of diversity in western South America, for example, Ericaceae, Orchidaceae, and Solanaceae (Dressler, 1981; D'Arcy, 1991; Luteyn, 2002). An important contributor to the origin of this diversity is the topological and environmental variation resulting from the uplift of the Andes (Gentry, 1982; Hooghiemstra et al., 2002). Phylogenetic studies support an association between the diversification of Andean plants (von Hagen and Kadereit, 2003; Kay et al., 2005) and animals (Patton and Smith, 1992; Bates and Zink, 1994; Brower, 1994) and the major episodes of Andean uplift, beginning in the early Miocene (ca. 20 mya) and ending in the Pliocene (ca. 3 mya) (Hoorn et al., 1995; Hooghiemstra and van der Hammen, 1998). Indeed, the parallel invasions of higher elevations by numerous plant groups and the coincident radiations of pollinating animals, e.g., hummingbirds (Bleiweiss, 1998), may explain the "explosive" speciation seen in some Andean groups (Gentry, 1982; Luteyn, 2002). Here we investigate the phylogenetic history of Iochrominae, a group of Andean Solanaceae, which have radiated in floral morphology and pollination system (Cocucci, 1999) and which may serve as a model system for other Andean radiations.

Recent phylogenetic analyses using plastid genes have greatly clarified relationships within Solanaceae and allowed for the creation of a phylogenetic system of classification (Olmstead and Sweere, 1994; Olmstead et al., 1999; Martins and Barkman, 2005; Olmstead et al., University of Washington, personal communication). Iochrominae sensu Olmstead et al. (1999) is a clade of Physaleae comprising around 34 mainly Andean species traditionally assigned to six genera: *Acnistus* Schott, *Dunalia* H.B.K, *Eriolarynx* (Hunz.) Hunz., *Iochroma* Benth., *Saracha* R. and P., and *Vassobia* Rusby (Table 1). In the Olmstead et al. (1999; R. G. Olmstead, University of Washington, unpublished manuscript) scheme, Iochrominae together with Physalinae and Withaninae form the large clade Physaleae, which is sister to Capsiceae. Although the phylogenetic classification was not accompanied by a morphological reassessment, Iochrominae can be distinguished from other subtribes in Physaleae by the fact that they are all woody shrubs or small trees and often have showy tubular flowers. In a recent morphological phylogenetic analysis (Sawyer, 2005), all Iochrominae genera except one, *Acnistus*, were found to be monophyletic, united most notably by the rounded-mucronate shape of the fruiting calyx margin and the presence of sclerosomes in the fruit wall.

Although it contains only one-third the number of species in its probable sister group Physalinae, Iochrominae boasts a greater floral diversity, spanning all major flower colors and forms found in the entire Solanaceae. Iochrominae flowers may be red, orange, yellow, green, blue, purple, or white, and the corolla varies from rotate to tubular, with over eight-fold variation in tube length across species (Shaw, 1998; Hunziker, 2001; Table 1). In contrast, a vast majority of taxa within

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TABLE 1. Summary information for genera of Iochrominae. Number of species is from recent treatments and descriptions (*Acnistus* [Hunziker, 1982], *Eriolarynx* [Hunziker, 2000, 2001], *Dunalia* [Hunziker, 1960, 2001], *Iochroma* [Leiva, 1995; Leiva et al., 1998, 2003; Shaw, 1998], *Saracha* [Alvarez, 1996], *Vassobia* [Hunziker, 1984, 2001]).

Genus	No. species	No. sampled	Distribution	Elevation (m a.s.l.)	Distinctive features
Acnistus	1	1	Southern Mexico, Central America, the Caribbean, northern South America and eastern Brazil	300–2000	Campanulate or funnel-shaped fragrant flowers; valvate bud aestivation; triangular corolla lobes; green markings inside corolla lobes; edible fruit
Dunalia	5	5	Colombia to Argentina	1600–3700	Often spiny; a few dioecious or gynodioecious; tubular flowers with wing-like appendages of stapet (filament base)
Eriolarynx	3	2	Bolivia and Argentina	1000–3000	Rotate or campanulate flowers with a dense ring of trichomes at base of corolla tube; stapet with small projections ("auricles")
Iochroma	21 + 3 undescribed taxa	21 + 3 undescribed taxa	Colombia to Peru, with one species in the Galapagos*	1100–3500	Tubular, often colorful flowers, with inflated calyces in some species
Saracha	2	2	Venezuela to Bolivia	2700–4500	Occasionally spiny; coriaceous to subcoriaceous leaves; campanulate or funnel-shaped flowers; pyrenes in fruit
Vassobia	2	2	Bolivia, Argentina, Paraguay, Uruguay and Brazil	300–2700	One spiny; campanulate flowers mostly glabrous; stapet with auricles

Note: *The range for *Iochroma* excludes the two southern Andean species shown in this study not to belong in *Iochroma*.

Physalinae, Withaninae, and Capsiceae have small, white or yellow, rotate flowers, and there are no instances of long, tubular, red or purple corollas in these three clades. Thus, the brightly colored tubular flowers likely represent a derived feature that arose within or at the base of Iochrominae.

The great floral diversity of Iochrominae sensu Olmstead et al. (1999) has misled classifications based on morphology. For example, Hunziker's (2001) morphologically delimited Iochrominae included *Oryctes* S. Watson, a monotypic tubular-flowered genus native to California and Nevada. *Oryctes* has since been shown to be nested within Physalinae, probably sister to *Leucophysalis* (Whitson and Manos, 2005; Olmstead et al., University of Washington, personal communication). Similarly, Sawyer's (2005) morphological cladistic analysis of Physaleae identified an Iochrominae clade that included all genera except *Acnistus*, which appeared with *Tubocapsicum* in a distant clade. Although *Acnistus* and the monotypic Japanese *Tubocapsicum* share small campanulate-infundibuliform flowers with valvate bud aestivation, molecular studies strongly suggest that *Tubocapsicum* is more closely related to other Physaleae (e.g., *Nothocestrum* and *Withania*) than to *Acnistus* and other Iochrominae (Olmstead et al., 1999; Olmstead et al., University of Washington, personal communication).

Another challenge in the systematics of Iochrominae is the potential for hybridization among species and across generic boundaries. Horticulturists have generated several hybrids (e.g., *I. australe* × *I. cyaneum*), and botanists have occasionally encountered hybrid populations in nature (Shaw, 1998; S. D. Smith, personal observation). The ease of crossing, the overlapping species ranges of many Iochrominae, and the observation of natural hybrids suggest that hybridization may have been important in the evolutionary history of Iochrominae. Combined with external sources of information such as morphology, biogeography, and cytology, phylogenetic estimation using multiple genetic markers can help identify instances of hybridization.

In this study, we used three nuclear regions, the internal transcribed spacer (ITS), exons 2 through 9 of the nuclear granule-bound starch synthase gene (GBSSI or *waxy*), and the second intron of LEAFY (*LFY*) to estimate the phylogeny of Iochrominae. Both ITS and *waxy* have been useful in clarifying specific and generic relationships in Solanaceae (e.g., Marshall et al., 2001; Peralta and Spooner, 2001; Whitson and Manos, 2005). *LFY* introns are increasingly utilized for resolving interspecific relationships and identifying hybrid taxa (e.g., Oh and Potter, 2003; Howarth and Baum, 2005), although this is the first study to use *LFY* in Solanaceae systematics. Our specific objectives were to evaluate the monophyly of the six traditional genera of Iochrominae and to assess the extent of interspecific hybridization. We close by considering our results in a biogeographical context.

MATERIALS AND METHODS

Taxon sampling—This study includes a nearly complete sampling of Iochrominae (Table 1) and a broad sampling of related lineages in the Solanoid radiation. Thirty-three of the 34 commonly recognized species of Iochrominae (all but *Eriolarnyx iochromoides*) were sampled in this study, as well as three as yet undescribed taxa (Appendix 1). The status of these unnamed taxa is under review by S. Leiva G., and for the purposes of this study, we will use temporary names, indicated by quote marks, based on their likely species epithets (S. Leiva G., Herbario Antenor Orrego, personal communication). For *Iochroma peruvianum*, a species known only from the type collection, our determination remains tentative because we were unable to find the species in its type locality and have here sampled individuals from another locality that closely resemble the type but have some small differences. For one ingroup species, *A. arborescens*, multiple individuals were included because the species is extremely widespread and variable.

Three ingroup taxa were suspected to have recent hybrid ancestry: *Iochroma* "sagasteguii," *I. ayabacense*, and *I. stenanthum*. These species are endemic to northern Peru and are often found in sympatry with other species of *Iochroma* and *Acnistus*. They share some characteristics of *Iochroma* (e.g., tubular flowers, purple coloration in the latter two) and some of *Acnistus* (e.g., yellow-green markings inside the corolla lobes), making their taxonomic affinity unclear. Preliminary chromosome counts for one of these three species, *I. ayabacense* suggest that it is $n = 12$ (S. D. Smith and V. Kolberg, University of Wisconsin, unpublished data) as are other species and genera of Iochrominae (Hunziker, 2001, and references therein). We, therefore, considered these taxa to be possible homoploid hybrids.

The 10 outgroup taxa were selected by reference to the plastid phylogeny of Solanaceae (Olmstead et al., 1999) and included Nicandreae (*Nicandra*), Solaneae (*Solanum*), Capsiceae (*Capsicum* and *Lycianthes*) and other members of Physaleae (*Leucophysalis*, *Physalis, Salpichroa, Tubocapsium*, and *Witheringia*) (Appendix 1). Also, included were the Andean genera *Cuatresia* and *Larnax*, which have not yet been incorporated into the phylogenetic classification scheme for the family, but appear to belong in Physaleae (R. G. Olmstead et al., University of Washington, unpublished manuscript).

Data collection—Total genomic DNA was extracted from silica-dried leaf material (Chase and Hills, 1991) using a modified 2× CTAB protocol (Doyle and Doyle, 1987). ITS was amplified as described in Baum et al. (1998) with primers ITS leu.1 (Andreasen et al., 1999) and ITS4 (White et al., 1990) and sequenced with these two primers plus ITS2 (White et al., 1990) and ITS3B (Baum et al., 1994).

The *waxy* region was amplified using primers 5′ and 3′ and sequenced using primers GBSSI-A, -B, -C_R, and -D_R designed by Peralta and Spooner (2001). For difficult taxa, four Iochrominae specific primers were designed: F41, F420, R991, and R1235 (Appendix 2). Each 25 μL *waxy* PCR reaction contained 2.5 μL 10× PCR Buffer (Qiagen, Valencia, California, USA), 2.5 μL of 25 mM $MgCl_2$, 1.0 μL of 10 mM dNTPs, 1.0 μL of each primer (10 μM solutions), 0.125 μL *Taq* polymerase (5 units/μL), and approximately 100 ng of template DNA. The PCR program was 95°C for 2 min, then 35 cycles of 95°C for 45 s, 56°C for 30 s, 72°C for 3 min, followed by a final extension of 72°C for 5 min.

The second intron of *LFY* was initially amplified and cloned from a subset of taxa using degenerate primers F2 and R1 (Howarth and Baum, 2005). These sequences were used to create Solanoid specific primers (LFYSOL-F7, -F68, -R700, -R1000 in Appendix 2). The PCR reactions for *LFY* differed from the *waxy* reactions in that they contained 2.0 μL $MgCl_2$ and 1.0 μL Q-solution (Qiagen). The PCR program for *LFY* amplification was 95°C for 4 min, then 35 cycles of 95°C for 1 min, 48°C for 1 min, 72°C for 3 min, followed by a final extension of 72°C for 5 min. All PCR products were purified with AMPure using manufacturer's protocols (Agencourt Bioscience, Beverly, Massachusetts, USA).

Copy number and allelic variants are of concern when using nuclear genes for phylogenetics. The ITS region, as part of the repeating units of rDNA in the nuclear genome, undergoes concerted evolution, potentially homogenizing the many copies (Hamby and Zimmer, 1992). This may explain why direct sequencing was possible for ITS for all taxa. With the single or low copy nuclear loci, *LFY* and *waxy*, direct sequencing often failed to yield a single sequence. In these cases, PCR products were gel-purified with the QIAquick gel extraction kit (Qiagen) and cloned using the pGEM-T easy vector system (Promega, Madison, Wisconsin, USA) following the manufacturer's protocol. Five to eight clones from each product were sequenced.

Sequencing used ABI sequencing reagents (Applied Biosystems, Foster City, California, USA). Each 10-μL cycle sequencing reaction contained 1.0 μL of purified PCR product, 2 pM of primer, 2 μL Big Dye, and 2 μL of sequencing buffer and was cycled through a program of 94°C for 2 min, then 30 cycles of 94°C for 20 s, 47°C for 20 s, and 60°C for 3 min. Reactions were cleaned using CleanSEQ (Agencourt) and run on ABI PRISM 3700 DNA analyzer at the University of Wisconsin Sequencing Facility. Sequences were edited in Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA) and aligned manually in MacClade 4.0 (Maddison and Maddison, 2000). Unique clones were maintained in the alignment until data collection was complete. Minor allelic variants (five or fewer substitutions per kilobase) were combined to create a consensus sequence for the species with differences coded as ambiguities. When alleles from a single species differed markedly in sequence or in length (due to indels) but still formed a clade in phylogenetic analyses, the allele giving the shortest branch in a parsimony tree was kept for analysis. In cases where the two alleles of a given species did not consistently form as a clade, both were kept for analysis. We use the term "divergent alleles" for such sequences for the remainder of the paper.

Sequences were examined for evidence of intragenic recombination by visual examination of the spatial distribution of different site patterns. Additionally, the sequences were analyzed for evidence of recombination using the MaxChi (Maynard Smith, 1992) and GENECONV (Padidam et al., 1999) methods (with default settings) in the program RDP (Martin and Rybicki, 2000). These methods have a limited ability to detect recombination, but are still potentially informative (Posada and Crandall, 2001; Posada, 2002). Final sequence alignments were deposited in TreeBASE (study accession number S1498, http://www.treebase.org).

Phylogenetic reconstruction—For parsimony analyses, all characters were equally weighted, and gaps were treated as missing characters. Heuristic searches were conducted in PAUP*, version 4.0b10 (Swofford, 2002), using 1000 random taxon addition sequences (holding two trees at each step) with tree-bisection-reconnection (TBR) branch swapping and keeping up to 100 most parsimonious trees (MPTs) per random addition replicate. Similar to Catalán et al. (1997), we next completed a heuristic search using the same settings but with 5000 random taxon additions and retaining only trees not compatible with the strict consensus of the first parsimony search (by enforcing the strict consensus as a reverse constraint). If the second search returned only trees longer than first search, then we considered the MPTs from the first search an adequate sample of parsimony tree space. If we found shorter trees, we repeated the process until no additional MPTs were recovered. To estimate clade support, heuristic searches were completed for 1000 bootstrap replicates with 10 random sequence additions (holding one tree at each step), TBR branch swapping, and maxtrees set to 100.

For likelihood analyses, the best fitting model was chosen by hierarchical likelihood ratio tests. Likelihood scores were calculated in PAUP* (Swofford, 2002) for the following models (in order of increasing complexity): JC, K2P, HKY, HKY+Γ, HKY+Γ+I, GTR+Γ, and GTR+Γ+I (Swofford et al., 1996, and references therein). The most-parsimonious tree (MPT) with the highest likelihood under the JC model was used for calculating likelihoods under more complex models. Likelihood searches were carried out in PAUP* (Swofford, 2002) using the best fitting model with all the MPTs used as starting trees, TBR branch swapping, and model parameters estimated during the hierarchical likelihood ratio tests.

Bayesian analyses were performed with MrBayes, version 3.1.1 (Ronquist and Huelsenbeck, 2003). The ITS and *LFY* intron data sets were each treated as a single data partition, whereas the *waxy* data set was divided into three partitions: first and second codon positions, third codon positions, and introns. Thus, the combined data set had five total data partitions. Each partition was assigned the best fitting model as suggested by likelihood ratio tests using MPTs from each partition as described previously. Transition/transversion ratio, substitution rates, state frequencies, gamma shape parameters, and proportion of invariant sites were unlinked across partitions and estimated during the Markov Chain Monte Carlo (MCMC) runs. For the individual and combined data sets, we conducted four independent MCMC runs, each with two internal runs (nruns = 2), to give eight tree files for each data set. Each run was initiated with a different starting seed and comprised four linked chains with temperature of 0.2. The chains were run for 5 000 000 generations, sampling every 100 generations, except for ITS, for which we used 15 000 000 generations, sampling every 150 generations. Adequate mixing (sampling of tree and parameter space) was judged by movement among chains and acceptance rates, which should be between 10 and 70%, and, most importantly, by convergence among independent runs with different starting points (Huelsenbeck et al., 2002). Inadequate mixing in some initial runs was corrected by adjusting the temperature and re-running the analysis. We considered that the runs had converged when the convergence diagnostics provided in sump output approached 1 and when clade credibilities (post burn-in), branch lengths, and topologies were similar across the four independent runs. We discarded 10% of trees as our burn-in period, which appeared to be very conservative given visual inspection of likelihood-by-generation plots. Posterior probabilities (PP) were averaged across runs.

Statistical tests—We estimated the *g*1 statistic, a measure of phylogenetic signal, for each data set in PAUP* using 10 000 random trees. Significance of the statistic was assessed following Hillis and Huelsenbeck (1992).

Incongruence between the three data sets was estimated with the incongruence length difference (ILD) test (Farris et al., 1994), implemented as the partition homogeneity test in PAUP*. The test was conducted with 1000 replicate partitions, each subjected to heuristic parsimony searches, comprising 10 random taxon addition replicates with TBR branch swapping and keeping no more than 100 trees per random addition replicate. The difference in phylogenetic signal from the three data sets as manifested in differing tree topologies was further examined using Wilcoxon signed-ranks (WSR) tests, also known as Templeton tests (Templeton, 1983), implemented in PAUP*. A detailed description of the use of WSR tests to compare phylogenetic hypotheses is given in Larson (1994). Constrained searches completed in conjunction with WSR tests were carried out with the same settings as unconstrained parsimony searches (described previously).

We also examined incongruence between data sets in a Bayesian framework as described in Buckley et al. (2002). We determined whether the combined topology existed within the 95% credible set of trees from each gene. If not, we assumed that the gene in question evolved under a different topology or that the model of evolution was inappropriate. In these cases, we attempted to localize areas of discordance by comparing individual clade credibilities between the individual and combined analyses.

TABLE 2. Summary statistics and analysis parameters for individual and combined data sets for phylogenetic analysis of Iochrominae. The number of most parsimonious trees includes only the unique trees after collapsing zero-length branches.

Region	No. characters	No. variable characters	No. parsimony informative characters	CI/RI (excluding uninformative characters)	$g1$	Tree length	No. most parsimonious trees	Best-fitting likelihood model	Temperature used in Bayesian analysis
ITS	803	239	138	0.42/0.54	−0.35*	670	16 353	GTR + Γ + I	0.07
waxy	1472 (exons, 750; introns, 722)	489	167	0.64/0.84	−0.67*	687	264	HKY + Γ	0.05
LFY	1806	764	322	0.63/0.78	−1.49*	1230	458	HKY + Γ	0.2
Combined ITS and *waxy*	2275	728	305	0.48/0.66	−0.47*	1393	180	GTR + Γ + I[a]	0.06
Combined all (excluding non-Physaleae outgroups)	4023	1204	511	0.57/0.73	−1.22*	1991	12	HKY + Γ + I[a]	0.1

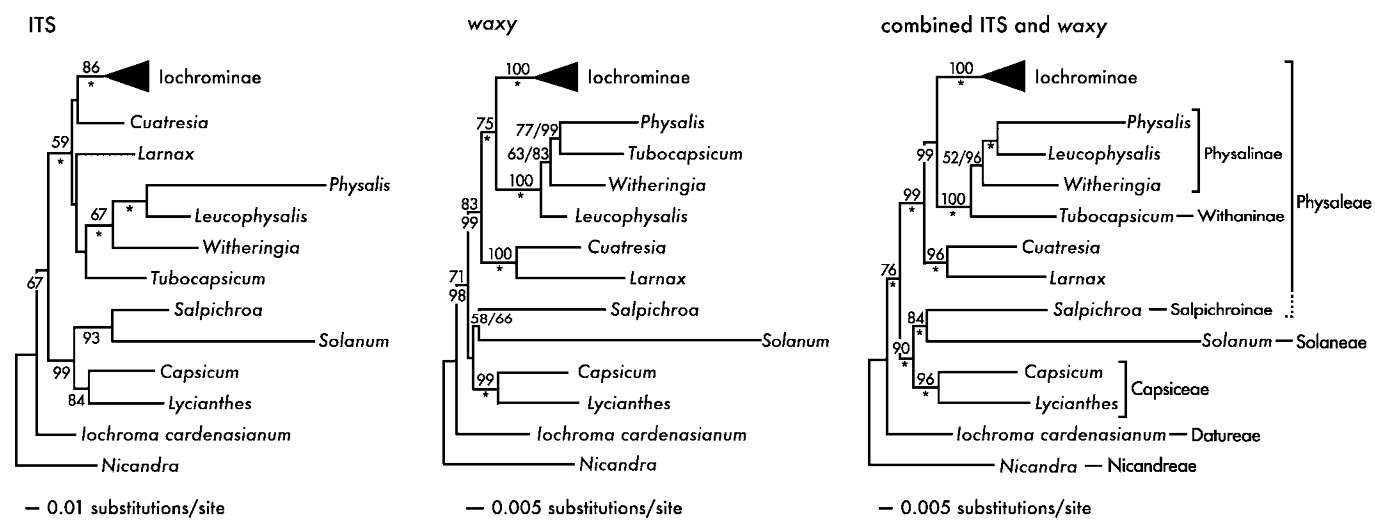
Notes: CI = consistency index; RI = retention index. *, significant phylogenetic signal ($P < 0.01$) according to the $g1$ statistic (Hillis and Huelsenbeck, 1992).

[a] For likelihood searches of combined data sets, we used the indicated model, but for Bayesian searches, we applied the best fitting model for each individual data set to its partition.

RESULTS

Phylogenetic analyses of individual data sets—*ITS*—Sequences were completed for all taxa (Appendix 1) and easily aligned to provide a matrix of 803 characters (described in Table 2). We found no evidence of intragenic recombination, either by visual inspection or by use of MaxChi and GENECONV methods in the program RDP ($P > 0.05$). Relative to *LFY* and *waxy*, ITS had low consistency and low phylogenetic signal (Table 2), resulting in many more MPTs. Our initial Bayesian analyses showed variation in clade credibilities across runs, but lengthening the runs to 15 000 000 generations (sampling every 150 generations) produced identical majority-rule consensus trees with less than 5% difference in PP across independent runs for clades with over 50% PP. Also, the convergence diagnostic was 1.0 for each run and acceptance rates were between 10 and 70%, indicating good mixing. Despite thorough exploration of tree space, ITS provided little resolution at any level and showed only a few strongly supported clades, which appeared consistently in the MP, ML, and Bayesian analyses. At the broader level, ITS data suggested that the mostly closely related taxa to Iochrominae are other Physaleae (bootstrap support [BS] 59%, PP = 1.0; Fig. 1), but it did not provide support for any specific Physaleae lineage being sister to Iochrominae. Iochrominae appeared monophyletic (BS = 86%, PP = 1.0; Fig. 1), excluding the spiny Bolivian endemic *I. cardenasianum*, which apparently is not a member of *Iochroma*. Within Iochrominae, ITS resolved only small clades, such as *Vassobia* (BS = 76%, PP = 1.0; Fig. 2) and the C clade (BS = 93%, PP = 1.0, see Fig. 2 legend for explanation of clade names). One interesting feature of the ITS phylogeny was the placement of the U group, which appeared as a clade (BS = 52%; not shown) sister to the rest of Iochrominae (BS = 33%; not shown) in parsimony analyses or as a basal grade in likelihood and Bayesian analyses (Fig. 2).

waxy—Most taxa were directly sequenced for *waxy* and only a few (*Lycianthes*, *Larnax*, *Iochroma parvifolium*, *I. fuchsioides*, and the three putative hybrids) required cloning. In



Revised Fig. 1. Maximum likelihood trees showing placement of Iochrominae within the Solanoideae. Regions analyzed are listed to the upper left of each tree. All trees are shown with branch lengths proportional to the estimated average number of substitutions per site under the models indicated in Table 2. Bootstrap support (BS) values > 50% are shown above branches or before the slash, and posterior probabilities (PP) > 0.75 (shown as percentages) are below branches or after the slash. Asterisks indicate a PP of 1.0. The rightmost tree is labeled with Olmstead et al. (1999) tribal and subtribal groupings. Solid vertical lines label monophyletic groups; dashed vertical lines indicate non-monophyly. **This figure differs from the print journal: the bootstrap value 90 with asterisk has been moved to the branch subtending the *Salpichroa* + *Solanum* + *Capsicum* + *Lycianthes* clade in the rightmost tree (combined ITS and waxy).**

A formal erratum appears in the September issue.

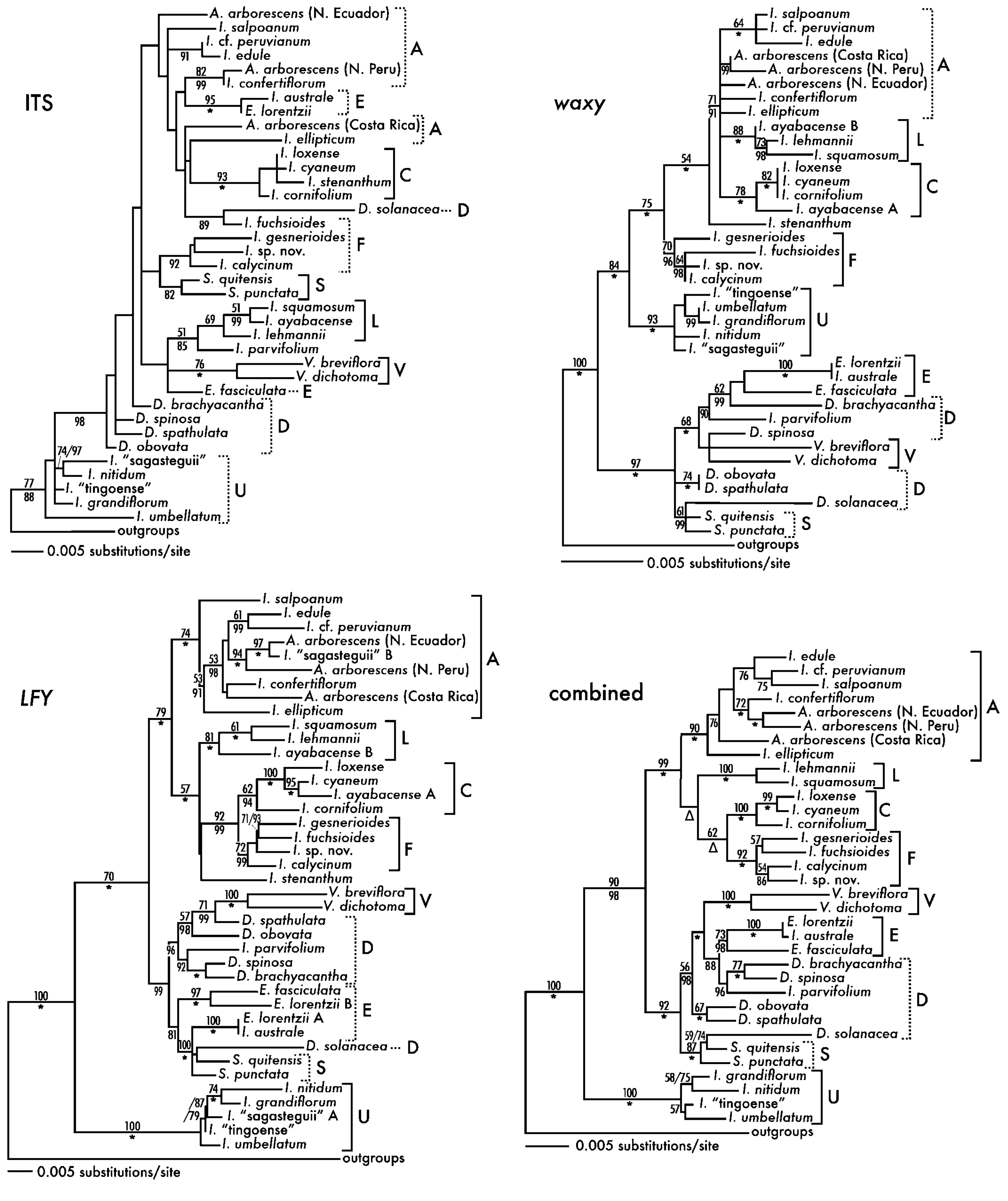


Fig. 2. Maximum likelihood trees of Iochrominae for individual and combined analyses. All trees are shown with branch lengths proportional to the estimated average number of substitutions per site under the models indicated in Table 2. Outgroups for ITS and *waxy* include all taxa shown in Fig. 1; outgroups for *LFY* and combined include only Physaleae (*Physalis*, *Leucophysalis*, *Witheringia*, *Tubocapsicum*, *Cuatresia* and *Larnax*; Fig 1.). Bootstrap support (BS) values > 50% are shown above branches or before the slash, and posterior probabilities (PP) > 0.75 (shown as percentages) are placed below branches or after the slash. Asterisks indicate a PP of 1.0. To facilitate comparison of relationships among trees, groups of interest are labeled with vertical

those cases, we conducted initial parsimony analyses to see if the separate clones formed a clade. When they did, we either created a consensus sequence (when clones differed by fewer than five bases per kilobase) or selected a single exemplar sequence. For *I. ayabacense* and *I.* "sagasteguii," two distinct sequence variants were found that did not form a clade. Both divergent alleles were retained in the final data set. We found no evidence of intragenic recombination among ingroup *waxy* sequences, either by visual inspection or by use of MaxChi and GENECONV methods in RDP ($P > 0.05$). Characteristics of the data set are given in Table 2.

Bayesian analyses of the *waxy* data set mixed well as indicated by the convergence diagnostics and the low variation among independent runs. The *waxy* analyses strongly supported the monophyly of Iochrominae (BS = 100, PP = 1.0; Fig. 1) and its inclusion in Physaleae (BS = 83, PP = 1.0; Fig. 1), perhaps as sister to Physalinae plus *Tubocapsicum* (BS = 75, PP = 1.0; Fig. 1). Like ITS, *waxy* showed *I. cardenasianum* to be distantly related from other iochromas. Further, all *waxy* analyses divided Iochrominae into a principally northern Andean clade containing *Acnistus* and *Iochroma* (A, C, L, F, and U clades; Fig. 2) and a mixed northern, central, and southern Andean clade containing members of *Dunalia*, *Eriolarynx*, *Saracha*, and *Vassobia* (D, E, S, and V; Fig. 2).

LFY—This region was more variable than *waxy* (Table 2), and could not be directly sequenced for many taxa. Nevertheless, most clones constituted minor sequence variants that were represented in the final matrix by consensus sequences. However, three of 49 taxa (*Eriolarynx lorentzii*, *I. ayabacense*, and *I.* "sagasteguii") contained two alleles that did not form a clade with others from the same accession. These divergent alleles were kept in the final matrix for phylogenetic analysis. We found no evidence of intragenic recombination in Iochrominae, either by visual inspection or with MaxChi and GENECONV methods implemented in RDP ($P > 0.05$).

Although *LFY* sequences were completed for all taxa, this intron could not be aligned outside Physaleae due to the enormous length variation (2.2 kb in Capsiceae vs. 1.4 kb in Iochrominae). Characteristics of the final data set of 43 taxa are given in Table 2. Similar to *waxy*, final Bayesian analyses mixed well as judged by acceptance rates and agreement among runs. Although *LFY* was too variable to be informative outside of Physaleae, it provided a good resolution within Iochrominae. Using other Physaleae as outgroup taxa, as indicated by *waxy* and ITS (Fig. 1), *LFY* produced an ingroup topology with many of the same well-supported clades that appear in ITS and *waxy*, but with some differences in relationships among the groups. Unlike *waxy* but similar to ITS, *LFY* placed the U clade sister to the rest of Iochrominae (BS 70%, PP 1.0; Fig. 2). As in *waxy* analyses, *LFY* supported a northern Andean clade with *Acnistus* and most of *Iochroma* (A, C, L, and F; Fig. 2) and a clade with *Dunalia*, *Eriolarynx*, *Saracha*, and *Vassobia* (D, E, S, and V; Fig. 2). *LFY* supported a monophyletic group of *Acnistus* and *Acnistus*-like iochromas (the A clade, Fig. 2) sister to a clade comprising other *Iochroma* subclades (C, L, and F; Fig. 2). This is in contrast with *waxy*, which placed the F clade sister to a clade comprising A, L, and C (but with A unresolved).

Divergent alleles in LFY and waxy—Three species, *Eriolarnyx lorentzii*, *Iochroma* "sagasteguii," and *I. ayabacense*, had divergent *LFY* alleles, and *I. ayabacense* also had divergent *waxy* alleles. In the case of *E. lorentzii*, one *LFY* allele formed a clade with *E. fasciculata* and the other with *I. australe* (Fig. 2). When the two alleles are constrained to be sister, the resulting trees are significantly longer than the optimal trees (WSR, $P = 0.0001$–0.0017), suggesting that *E. lorentzii* alleles are not exclusive and that there may be true genealogical discordance (e.g., due to lineage sorting or hybridization).

One *LFY* allele of *Iochroma* "sagasteguii" was sister to a sample of *Acnistus arborescens*, whereas the other fell in the distantly related U clade (Fig. 2). The *LFY* alleles of *I. ayabacense* were split between the C and L clades. When either *I.* "sagasteguii" or *I. ayabacense* alleles were forced to form a clade, the resulting trees were significantly longer than unconstrained trees (WSR, $P = 0.0001$–0.004 for *I. ayabacense* and $P < 0.0001$ for *I.* "sagasteguii"). *Iochroma ayabacense* also showed divergent *waxy* alleles, with one allele in the C clade and the other in the L clade, consistent with the *LFY* analysis (Fig. 2). Constraining the two *waxy* alleles from *I. ayabacense* to form a clade resulted in some significantly longer trees (WSR, $P = 0.025$–0.096). The distant placement of *I.* "sagasteguii" *LFY* alleles and *I. ayabacense LFY* and *waxy* alleles points to a hybrid origin for these taxa, a possibility that will be explored in more detail in the discussion.

Discordance among genes—The ILD (Farris et al., 1994) was used as an initial test of "global" congruence among and within data partitions. An ILD test indicated that the assignments of characters to the three *waxy* partitions (first and second codon positions, third codon positions, and introns) was not significantly different from random ($P = 0.70$), suggesting that *waxy* can be treated as a single data partition. In contrast, pairwise comparisons of the ITS, *LFY*, and *waxy* (excluding non-Physaleae outgroups and putative hybrids) all yielded significant ILD tests ($P < 0.01$), indicating that the three data sets are not drawn from the same population of characters (but see Darlu and Lecointre, 2002; Hipp et al., 2004). We attempted to localize the discordance by repeating the ILD test with successively pruned data sets (Table 3). We divided the data set into three parts, the ACLF group, the DESV group and the U group, and we found that only the DESV returned significant P-values (Table 3). However, simply deleting the DESV taxa from the larger clade did not result in insignificant ILD results (not shown), suggesting that it was not the sole source of incongruence.

Templeton tests—Although ILD tests suggested significant differences in signal among data sets, inspection of the

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brackets on each tree. The labels D, E, S, and V indicate members of the traditional genera *Dunalia*, *Eriolarynx*, *Saracha* and *Vassobia*, respectively. *Iochroma* has been divided into smaller clades: A, including *Acnistus* and *Acnistus*-like iochromas; C, containing the type *I. cyaneum*; L, after *I. lehmannii*; F, after *I. fuchsioides*; and U after *I. umbellatum*. Solid bracket lines indicate monophyly; dashed bracket lines indicate non-monophyly. The Δ's in the combined tree designate points of difference between the combined likelihood tree and combined Bayesian consensus; PP for clade ACL is 0.97 and PP for clade AL is 1.0 (see text, Results, *Combined analysis*).

Table 3. *P* values from incongruence length difference (ILD) tests of combined data sets. In each case, the putative hybrids, *Iochroma stenanthum*, *I. ayabacense*, and *I.* "sagasteguii," were already removed.

Data set	ITS vs. *waxy*	ITS vs. *LFY*	*LFY* vs. *waxy*
Iochrominae + Physaleae outgroups	0.001**	0.001**	0.001**
Iochrominae	0.001**	0.002**	0.007**
Clades A, C, L, F only	0.446	0.057*	0.479
Clades D, E, S, V only	0.206	0.01**	0.001**
Clade U only	1.0	1.0	1.0

Notes: Clade names (A, C, etc.) are explained in caption for Fig. 2. **, significant at $P < 0.01$; *, marginally significant values.

individual trees revealed only eight points of hard incongruence (conflicting clades with BS > 70; Mason-Gamer and Kellogg, 1996) among the three gene trees (Fig. 2). Three of these cases were due to differences in the placement of divergent *LFY* or *waxy* alleles. We used Templeton tests to compare the remaining five sources of hard incongruence (Table 4). In all cases, one or the other partition failed to reject the conflicting resolution at the $P < 0.05$ level. This suggests that the incongruence detected by ILD tests is "diffuse" rather than due to particular points of discordance.

Combined analysis—Before conducting combined analyses, we removed all of the putative hybrids, *Iochroma stenanthum*, *I. ayabacense*, and *I.* "sagasteguii," as they appeared to be a source of conflict among data sets. We chose not to remove *E. lorentzii* despite its divergent alleles because that would severely reduce our sampling of *Eriolarynx*. Instead, we removed the *E. lorentzii LFY* allele B, whose position conflicts with that supported by ITS and *waxy*. In addition, we reduced the outgroup sampling to include only other Physaleae (*Physalis*, *Leucophysalis*, *Witheringia*, *Tubocapsicum*, *Cuatresia*, and *Larnax*; Fig. 1).

Parsimony analysis of the combined data set of 40 taxa and 4023 characters yielded 12 MPTs (Table 2) and increased support for many of the clades observed in individual data sets (Fig. 2). Similar results were obtained for ML and Bayesian analyses. For example, among individual analyses, the A clade only appeared in the *LFY* tree (BS = 74%; PP = 1.0; Fig. 2), but it appeared in the combined analysis with a BS of 90% and PP of 1.0. Likewise, the placement of the U clade sister to the rest occurred with moderate support in *LFY* (BS = 70%, PP = 1.0; Fig. 2) and weak support in analyses of ITS (BS = 33%, PP = 0.12; not shown), but appeared strongly supported in the combined analysis (BS = 90%, PP = 0.99; Fig. 2). Nonetheless several areas on the combined tree remain unresolved, most notably within the DESV clade and within the A clade. Also, there were differences among modes of analysis. Clades C, L, and F together formed a clade in parsimony and ML searches of the combined data (Fig. 2), but Bayesian analyses showed clade F as sister to an A, C, and L clade and clade A sister to clade L with high posterior probability (PP = 0.96–1.0) at all relevant nodes (tree not shown, but see Fig. 2 caption). Exploration of pruned data sets (not shown) established that the resolution among the A, C, L, and F clades in a Bayesian framework is very sensitive to the inclusion or exclusion of L and to model choice (e.g., whether data partitions were allowed to evolve under different models or whether they were linked as in traditional likelihood searches).

Congruence in a Bayesian framework—As an additional assessment of congruence, we compared the results of the Bayesian analysis of the combined data set (described previously) with the results from Bayesian analyses of individual data sets that had been pruned to the same 40 taxa (Buckley et al., 2002). The results of these runs are provided in Appendix S1 (see Supplemental Data accompanying the online version of this article). We found that there were no trees that were shared between the posterior distributions (post burn-in) of the individual and combined data sets. This is perhaps not surprising given that there are 1.3×10^{55} possible unrooted trees for 40 taxa and thus a fairly small chance that different data sets would sample exactly equivalent topologies.

We next examined localized points of disagreement among data sets in the Bayesian framework. Within the ACLF group, we observed that *LFY* had a PP of 0.0 for F sister to ACL, whereas *waxy* had a PP of 0.0 for the F sister to C topology. This suggests that there may be true genealogical discordance between *LFY* and *waxy* within the ACLF clade (Table 4, conflict 5). A contrasting result was found with respect to the placement of the U clade (sister to the rest in *LFY* and sister to A, C, L, and F in *waxy*). We found that trees with U sister to the rest of Iochrominae (the "U-sister" topology), as suggested by *LFY*, appeared in *waxy* posterior distributions with a PP of 0.0078. Similarly U was sister to A, C, L, and F (the "U-nested" topology) in the *LFY* posterior with a PP of 0.001. While these values are lower than the traditional 0.05 threshold, the fact that both topologies were present in the posterior distributions for both data sets suggests that there may not be hard incongruence (consistent with the WSR tests, Table 4). On the other hand, the fact that the combined analysis supports the U-sister topology more strongly than does *LFY* alone, suggests that U-sister is a more plausible hypothesis at this time than U-nested.

DISCUSSION

Position of Iochrominae in Solanaceae—Our goal in outgroup sampling was to confirm the monophyly of Iochrominae and verify that it belongs in Physaleae as indicated by plastid data. Indeed, once *Iochroma cardenasianum* is excluded, Iochrominae appears to be monophyletic. Plastid data (Olmstead et al., University of Washington, personal communication) confirm the distant relationship of *I. cardenasianum* to Iochrominae and place it within the Datureae.

Our data support the inference that Iochrominae is part of Physaleae, but its relationship to other taxa remains unclear. Of the three markers, the *LFY* intron could not be readily aligned with the more distant outgroups, and ITS provided little resolution among Physaleae (Fig. 1). However, analysis of *waxy* alone and combined analysis of *waxy* and ITS strongly supported Iochrominae as sister to Physalinae sensu Olmstead et al. (1999) plus *Tubocapsicum*. This result disagrees with the most recent plastid phylogeny, which places *Deprea* plus *Larnax* sister to Iochrominae, albeit with weak support (Olmstead et al., University of Washington, personal communication). Resolving the lineages that comprise Physaleae and the relationships among them will require increased sampling and perhaps additional markers.

Taxonomic implications for genera of Iochrominae—Acnistus—In Hunziker's (1982) revision of *Acnistus*, he acknowledged that *Acnistus* has greatest affinity to the genus

TABLE 4. Wilcoxon signed-ranks tests of conflicting phylogenetic hypotheses. The instances of conflict described can be observed in the gene trees in Fig. 2. See Fig. 2 legend for explanation of clade names.

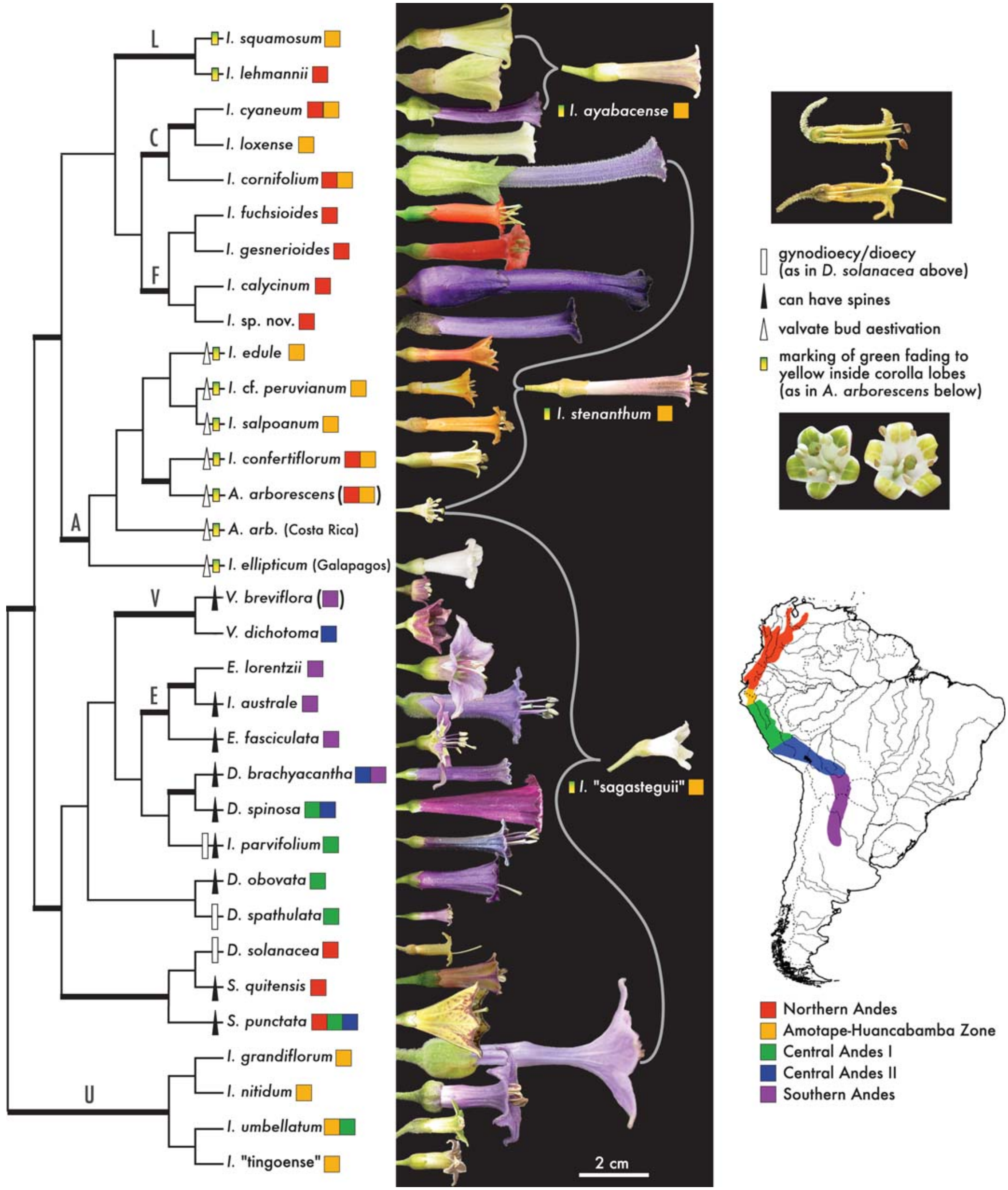
Conflicting topologies	Constraint	Result
1. *I. stenanthum* in clade C (ITS: BS 93%) vs. outside clade C (*LFY*: BS 92%, *waxy*: BS 71%)	Force *I. loxense*, *I. cyaneum* and *I. cornifolium* to form a clade in ITS	ITS: $P = 0.85–1.0$
	Force *I. loxense*, *I. cyaneum* and *I. cornifolium* and *I. stenanthum* form a clade in *LFY* and *waxy*	*LFY*: $P = 0.06–0.18$ *waxy*: $P = 0.01–0.03$
2. *A. arborescens* (Peru) sister to *I. confertiflorum* (ITS: BS 82%) vs. sister to *A. arborescens* (Ecuador) (*LFY*: BS 94%)	Force *A. arborescens* (Peru) sister to *A. arborescens* (Ecuador) in ITS	ITS: $P = 0.68–0.87$
	Force *I. confertiflorum* sister to *A. arborescens* (Peru) in *LFY*	*LFY*: $P = 0.16–0.51$
3. *D. spathulata* sister to *Vassobia* (*LFY*: BS 71%) vs. sister to *D. obovata* (*waxy*: BS 70%)	Force *D. spathulata* sister to *D. obovata* in *LFY*	ITS: $P = 0.76–0.83$
	Force *D. spathulata* sister to *Vassobia* in *waxy*	*LFY*: $P = 0.10$
4. Clade U sister to rest of Iochrominae (*LFY*: BS 75%) vs. sister to clade with A, C, L, and F (*waxy*: BS 70%)	Force U to form a clade with A, C, L, and F (including putative hybrids) in *LFY*	*LFY*: $P = 0.18–0.55$
	Force all members of A, C, L, and F (including *I. stenanthum* and *I. ayabacense*) and D, E, S, and V form a clade in *waxy*	*waxy*: $P = 0.08–0.18$
5. C forms a clade with F (*LFY*: BS 92%) vs. with A and L (*waxy*: BS 71%)	Force A, L, and C (including *I. ayabacense*) to form a clade in *LFY*	*LFY*: $P = 0.03–0.13$
	Force C (including *I. ayabacense* A) and clade F to form a clade in *waxy*	*waxy*: $P = 0.05–0.16$

Iochroma. The important differences he noted between them were the small flowers and anthers of *Acnistus*, the calyx (accresent in *Iochroma* but not in *Acnistus*), and the bud aestivation (induplicate in *Iochroma* and valvate in *Acnistus*). Confusing this demarcation are a few species currently placed in *Iochroma* that have the latter two characteristics of *Acnistus*. For example, *I. ellipticum* and *I. confertiflorum*, two large-flowered species that were transferred from *Acnistus* by Hunziker (1977, 1982), have valvate bud aestivation and lack a strongly accresent calyx. This combination of traits is also found in two recently named species, *I. edule* and *I. salpoanum* (Leiva, 1995; Leiva et al., 2003) and in *I. peruvianum*. Furthermore, field observations of these five iochromas (S. D. Smith, personal observation) indicate that they share with *Acnistus* a conspicuous green mark on the inner surface of the corolla lobe, which fades to yellow as the flower ages (Fig. 3). Thus, it is not surprising that *Acnistus* and these five other species form a well-supported clade in our analyses (clade A), but whether this group should be officially segregated from *Iochroma* deserves careful consideration and will be discussed further (see *Iochroma*).

The small-flowered form traditionally named *Acnistus arborescens* occurs from Argentina to Mexico and the Caribbean and is morphologically variable, with 28 synonyms in the taxonomic literature (Hunziker, 1982). The three accessions representative of this traditional species do not form a monophyletic group on any of the gene trees (though a clade is not contradicted by *waxy*). One possible explanation is that there has been incomplete lineage sorting within the A clade. This seems unlikely because our screen of clones revealed no allele sharing among other species in the A clade: all alleles from a given group A species formed a clade at all loci. Another interpretation is that *A. arborescens* refers to a lowland progenitor form that has given rise to multiple novel higher-elevation forms, similar to the case of *Lisianthus skinneri* (Sytsma and Schaal, 1985). Alternatively, because *A. arborescens* may occasionally hybridize in nature with related higher-elevation taxa such as *Iochroma confertiflorum* (S. D. Smith, personal observation), it is possible that different *A. arborescens* populations have acquired different introgressed alleles from other members of the A clade.

Dunalia—Hunziker's (1960) delimitation of *Dunalia* centered on a single character, the presence of enlarged and showy "stapets," which appear as winged or toothed lateral appendages emerging from the filament bases at the point of their insertion on the corolla tube. Our analyses suggest that *Dunalia* sensu Hunziker (1960) is not monophyletic. Notably, the type species, *D. solanacea* appears more closely related to *Saracha* than to other *Dunalia* species. Whereas other *Dunalia* species are xerophytes of the central and southern Andes, *D. solanacea* is a northern Andean cloud forest shrub with a dense indumentum of stellate hairs, anisogeminate leaves, and small, yellow-green, trumpet-shaped flowers. Although its placement within *Saracha* could be a phylogenetic artifact (note that *D. solanacea* has a long terminal branch for all genes), there is no evidence of an association between this species and the other "*Dunalia*" species.

The remaining four *Dunalia* species are similar to each other in morphology, distribution, and habit; however, they do not form a clade in any of the trees. Furthermore, one species traditionally placed in *Iochroma*, *I. parvifolium*, appears more closely related to some dunalias. However, the association of *I. parvifolium* with *D. brachyacantha* and *D. spinosa* is reasonable given its spiny xerophytic habit and tubular purple flowers. *Iochroma parvifolium* was placed in *Iochroma* as opposed to *Dunalia* because it lacks the showy stapets (Hunziker, 1977). Nevertheless, close examination of fresh flowers of *I. parvifolium* in the field revealed small, tooth-like expansions of the stapets, which are hard to detect in dried specimens (S. D. Smith, personal observation). Also, during the course of collection trips, one population of *I. parvifolium* was found to be gynodioecious, a condition found in some *Dunalia* species (S. D. Smith, personal observation). *Iochroma* species (members of A, C, L, F, and U) are invariably hermaphroditic and never spiny, making *I. parvifolium* an unlikely *Iochroma*. The epithet "parvifolia" does not exist in *Dunalia*, but transferring *I. parvifolium* to *Dunalia* is confounded by the fact that *D. solanacea*, the type species, is not associated with the other "*Dunalia*" species, making the taxonomic future of *Dunalia* uncertain.



Revised Fig. 3. Floral diversity, biogeography, and hybridization in Iochrominae. Cladogram showing relationships from combined analysis with the well-supported (BS > 70%, PP > 0.95) branches bolded. See Fig. 2 caption for explanation of clade names (L, C, F, etc.); members of *Dunalia* and *Saracha* are not labeled as the genera are non-monophyletic. Colored boxes indicate entire geographic distribution with the exception of *A. arborescens* (widespread, with samples from Ecuador, Peru, and Costa Rica included in this analysis) and *V. breviflora*, (widespread through southern South America).

Eriolarynx—The three species of *Eriolarynx*, recently segregated from *Vassobia*, can be distinguished from other Iochrominae by the dense ring of trichomes inside the corolla (Hunziker, 2000). Our analysis upholds the monophyly of *Eriolarynx*, with the addition of *I. australe*. This species was originally described in *Iochroma* (Grisebach, 1874), but later transferred to *Acnistus* (Grisebach, 1879) and then to *Dunalia* (Sleumer, 1950). *Iochroma australe* was not a good fit in *Iochroma* because its variable flowers can sometimes be short and funnel-shaped and because the corolla interior is densely pubescent near the base, whereas other Iochromas are typically glabrous. Further, it lacks the valvate aestivation of *Acnistus* and the characteristic filament appendages of *Dunalia*. The hairy flowers suggest a better fit with *Eriolarynx* despite the fact that the three described species typically have rotate or campanulate flowers, while *I. australe* has a funnel-shaped or tubular corolla. Geography also argues for this placement because both *I. australe* and *Eriolarnyx* are restricted to Bolivia and Argentina. There is no good argument against creating the new combination *E. australe*, except that this may prove to be only a temporary solution if it becomes necessary to combine the entire DESV clade into a single genus (with or without other elements of Iochrominae).

Iochroma—Species currently identified as *Iochroma* were not found to form a clade, even after the misplaced *I. australe* and *I. parvifolium* are ignored. One group of iochromas, the U clade appears as sister to remainder of Iochrominae. We consider this "U-sister" position to be strongly supported by our study for three reasons. First, two of the three loci sampled, ITS and *LFY*, support or are compatible with the "U-sister" topology. Second, heuristic searches using the *waxy* data constrained to be consistent with "U-sister" topology do not result in trees that are significantly longer than unconstrained trees (Table 4). Last, despite the differences in topology among loci, support for a "U-sister" relationship is highest in the combined analysis. Specifically the combined analysis of all three genes yielded a 90% bootstrap, as contrasted with a 78% bootstrap support for this relationship in a two-gene combined analysis of *LFY* and ITS (not shown). This pattern suggests that even though *waxy* does not return U as sister to the rest of Iochrominae, the *waxy* data do contain some support for this topology (Olmstead and Sweere, 1994).

The U group is distinguished from species in the ACLF clade by the form of the corolla and the androecium. Flowers of ACLF (excluding *Acnistus*) are funnel-shaped or tubular, whereas those of the U group are salverform. Also, the filaments are attached near the base in ACLF, while in the U group they are attached near the middle of the corolla tube (often with a visible bump at the point of attachment, e.g., *I. grandiflorum*, Fig 3.). The most extreme example of filament adnation in the U group is *I.* "tingoense" in which the anthers are more or less sessile on the corolla. Thus, even if one doubted the sister group relation between the U clade and other Iochrominae, there is reason to believe that the U clade is divergent from other traditional iochromas.

If the U clade (and *I. australe* and *I. parvifolium*) were excluded from *Iochroma* and if *Acnistus* were expanded to include the entire A clade (discussed previously), then one could imagine assigning only members of clades C, L, and F to *Iochroma*. However, this decision would be premature considering that it is not certain from these data that C, L, and F form a clade. Furthermore, there are no clear morphological differences between *Acnistus* and *Iochroma,* largely because *I. squamosum* and *I. lehmannii* (clade L) possess a mixture of traits from clade A on the one hand and clades C and F on the other; the bud aestivation is induplicate, resulting in wide corolla lobes and plaits in the corolla tube, like C and F, but the yellow flowers lack anthocyanins (Hunziker, 1982) and have the green markings on the corolla lobes, as in clade A. The other alternative, if we are to only recognize monophyletic groups, is to sink *Acnistus* into *Iochroma*. We also note that in a rank-independent system of nomenclature, *Acnistus* could be defined as a monophyletic group within a monophyletic *Iochroma*.

Saracha—This small genus of high-elevation treelets is morphologically well defined, including two species of páramo treelets with small coriaceous or subcoriaceous leaves and funnel-shaped or campanulate flowers that can be purple or yellow with purple spots (Alvarez, 1996). *Dunalia solanacea*, which often appears nested within *Saracha*, does not share any obvious features with *Saracha* except for its high-elevation distribution and occurrence in the northern Andes. As noted, *D. solanacea* has a long terminal branch for all tree genes, raising the possibility that its placement within *Saracha* is an artifact. Moreover, although *Saracha* only appears monophyletic in ITS trees and not in *LFY* or *waxy* trees, we note the sister relationship of *S. quitensis* and *S. punctata* does appears in Bayesian analyses of *LFY* with PP 0.08 and in those of *waxy* with PP 0.33 (Appendix S1, see Supplemental Data accompanying the online version of this article). Thus, we consider it premature to conclude that *Saracha* is nonmonophyletic.

Vassobia—Among the genera of Iochrominae, *Vassobia* is the only one that appeared monophyletic in all analyses. *Vassobia* includes two southern Andean species with small, purple, campanulate, glabrous flowers: *V. dichotoma* a cloud forest tree restricted to Bolivia, and *V. breviflora*, a widespread spiny shrub (Hunziker, 1984, 2001). The stapets of *Vassobia* are expanded to form small "auricles" similar to the appendages found in *Eriolarynx* (Hunziker, 2001). Considering that species of *Eriolarynx* formerly belonged to *Vassobia*, one might have expected a sister relationship between the genera. These data neither support nor strongly contradict this inference.

Hybridization in Iochrominae—Identifying hybrid taxa is a challenge for phylogenetics because reticulation erodes the strictly tree-like process of evolution assumed by most phylogenetic methods (McDade, 1990). Nonetheless, even when species trees are reticulate, gene trees will be strictly divergent structures so long as the rate of intragenic

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The samples of *A. arborescens* from Peru and Ecuador are condensed to a single line because they appear to be sister taxa in the combined analysis; the *A. arborescens* from Costa Rica is abbreviated "*A. arb.*" Gray, curved lines connect the putative hybrids, *I. ayabacense*, *I. stenanthum*, and *I.* "*sagasteguii*" to their putative parents. **This figure differs from the print journal: the branch and associated symbols for *V. breviflora* and for *V. dichotoma* have been interchanged to align with the correct flower images.**

A formal erratum appears in the September issue.

recombination is low relative to the rate at which lineage sorting occurs. Given that homoploid hybrid taxa potentially carry genetic contributions from one or both parents, we may observe divergent alleles on a single gene tree (with alleles associated with each parent) or disagreement among gene trees, with hybrid alleles appearing related to one parental lineage on one tree and to the other parent on a different gene tree. Thus, we can test hypotheses of hybrid ancestry by identifying divergent alleles or points of conflict among gene trees (Doyle, 1992; Maddison, 1997). In this study, our sampling included three taxa, *Iochroma ayabacense*, *I.* "sagasteguii," and *I. stenanthum*, which we had hypothesized to be of hybrid origin due to their distribution and morphology.

Iochroma ayabacense was hypothesized to be an interspecific hybrid between *I. cyaneum* and *I. squamosum*. *Iochroma ayabacense* occurs in at high elevations (2600–2700 m a.s.l.) around the city of Ayabaca in northern Peru, often in proximity to populations of its putative parents, *I. squamosum* and *I. cyaneum*. The infrequent *I. squamosum* favors mildly disturbed habitats like forest gaps or riparian areas, whereas the widespread *I. cyaneum* tolerates drier conditions and open habitats like roadsides and pastures. The two putative parents are, however, found occasionally in close proximity, for example, when a road passes through a patch of forest. Several morphological features pointed to the possibility that *I. ayabacense* was a hybrid between these two. It has peculiar yellowish-purple flowers intermediate between the yellow *I. squamosum* and the purple *I. cyaneum*, and it has yellow-green markings inside the corolla, which are signatures of clades A and L. Our phylogenetic analyses revealed divergent alleles of *I. ayabacense* in both *waxy* and *LFY* trees, and in each case, one *I. ayabacense* allele fell in clade C and one in clade L (Fig. 2). In ITS trees, *I. ayabacense* appeared to be sister to *I. squamosum* in the L clade (Fig. 2). Considering these gene trees together with its distribution and morphology, we conclude that *I. ayabacense* is a hybrid between *I. cyaneum* and *I. squamosum*. Further field research and genetic data would be needed to determine if *I. ayabacense* is best interpreted as a hybrid species or a transient hybrid form that lacks sufficient permanence to warrant species status.

Iochroma "sagasteguii" has small white flowers with greenish markings inside the corolla that resemble *Acnistus*. However, the pubescence on the calyx and corolla, the slightly induplicate bud aestivation, and the extended area of filament adnation are reminiscent of species in the U group. Although the distribution of *I.* "sagasteguii" is not well known, in some localities in northern Peru, it grows within a few kilometers of populations of *I. stenanthum*, *I. cornifolium*, *I. grandiflorum*, and *I.* cf. *peruvianum* and within 15 km of populations of *Acnistus arborescens*. Similar to *I. ayabacense*, genetic evidence supported the hypothesis of hybrid ancestry in *I.* "sagasteguii." We found divergent alleles in the *waxy* tree, with one allele in the U group and another in clade A. The genetic data, the morphology, and the geography point to *I. grandiflorum* and *A. arborescens* as the most likely parental species.

Iochroma stenanthum was the third suspected hybrid. It occurs in northern Peru and has long, tubular, pubescent flowers, most similar to *I. cornifolium* (Leiva et al., 1998), but with more triangular corolla lobes and yellow-green markings inside the corolla lobes as in clade A. The corolla color, which fades from cream at the base to purple at the apex, suggests that it is the result of crossing a white-flowered species (e.g., *Acnistus arborescens*) and a purple-flowered species. *Iochroma stenanthum* occurs in close proximity of populations of the putative parents, *I. cornifolium* and *A. arborescens*. However, our data were insufficient to resolve the relationship of *I. stenanthum* to other Iochrominae. Its position varied among gene trees and was generally poorly supported. This pattern might be ascribed to lineage sorting, but its morphology is so strongly indicative of a hybrid ancestry that we favor the hypothesis that *I. stenanthum* is the product of a more ancient hybridization event whose genetic signatures have been blurred by subsequent evolution.

Biogeographical context of the Iochrominae radiation—Simpson (1975) recognized that phytogeographical distributions in the Andes tend to coincide with the geologically defined structural units of the Cordilleras. Many subsequent authors have observed such a relationship (e.g., Berry, 1982; Luteyn, 2002), although the exact delimitation of the structural units and associated phytogeographic zones varies slightly among studies. For instance, Berry (1982) modified Simpson's (1975) structural units by recognizing the Amotape–Huancabamba zone (A–H zone), an area of low elevation between the northern and central Andes (4–8°S), as a separate unit. Weigend (2002; Weigend et al., 2004) supported Berry's distinction, noting the large number of A–H zone endemics, and additionally suggested distinguishing the Andes below 18°S as the southern Andes (as in Fig. 3). As a basis for discussing the biogeography of Iochrominae, it is useful to divide the tropical Andes into northern, central, and southern regions, to recognize the A–H zone as a distinct unit, and to divide the central Andes into a region north of the Pisco deflection (14°S; Berry's Cordillera Central and Occidental) and a region south of the deflection (Berry's Cordillera Oriental).

Similar to other plant groups that have radiated in the Andes (e.g., *Fuchsia* and *Nasa*), distribution patterns of Iochrominae species and clades strongly reflect the structural units of the Andes (Fig. 3). The diverse ACLF clade, excluding the weedy *Acnistus arborescens* and the Galapagos endemic *Iochroma ellipticum*, is restricted to the Andes from 5°N to 8°S, the southern boundary of the A–H zone. The DEV group contains taxa that only occur below 8°S, while its probable sister group, clade S, is widely distributed from 9°N to 16°S. Clade U straddles the ACLF and the DEV groups, with a distribution from 4°S (the northern limit of A–H zone) to 10°S.

Despite the clear patterns along the latitudinal gradient, we do not observe strong east–west separation of clades as has been the case in many Andean groups (Berry, 1982; Slade and Moritz, 1998; Brower, 1994). Although there is some tendency for greater species richness on the western cordilleras, several taxa, e.g., *I. calycinum* and *Dunalia solanacea*, are known to occur on both sides of the Andes. However, the distribution of many species remains poorly characterized. With increased collecting effort, it may eventually be possible to determine if Iochrominae distributions follow east–west structural units as closely as they do north–south units.

Iochrominae show a center of diversity in the A–H zone, where 16 of 33 (48%) species (excluding *I. cardenasianum*) occur, 11 of which are restricted to this zone. This enhanced diversity can be attributed to the overlap of the ACLF and U clades. The A–H zone is characterized by fragments of the Cordilleras, usually less than 3500 m a.s.l, separated by valleys that dip down to ca. 1000 m a.s.l. Iochrominae prefer cloud forest or Andean scrub forest between 2300 and 2800 m a.s.l and are abundant in the high elevation valleys of the A–H zone. In some areas, as many as five species may occur over the distance of a few kilometers. The proximity coupled with the ease of crossing has resulted in several hybrid taxa, as revealed by this study, all of which are confined to this A–H zone (Fig. 3).

Here we have examined the three putative hybrid taxa with three loci, but this represents only a first attempt at exploring hybridization in Iochrominae. Further investigation into the potential hybrid ancestry of all Iochrominae should include samples of multiple individuals and populations per taxon, additional chromosome counts, statistical morphometric studies, characterization of species distributions, and analysis of mitochondrial or plastid markers. Greater sampling of individuals, taxa, and genes will permit a more fine-tuned estimate of the frequency of hybridization and introgression in Iochrominae history.

As documented in this study, episodes of hybridization have clearly impacted the evolutionary history of Iochrominae. However, considering the amount of agreement among the three nuclear markers, it appears that these events have not entirely obscured the underlying divergent phylogenetic history, having only clouded the branching pattern in some parts of the tree. Furthermore, the presence of leaky species boundaries has not apparently precluded the diversification of Iochrominae. In addition to being the most florally diverse subtribe in Physaleae and perhaps Solanoideae, Iochrominae also boasts the greatest diversity of pollination systems (Cocucci, 1999). Perhaps the combination of pollinator-mediated selection, microallopatry in dissected Andean habitats and episodic hybridization have together permitted the explosion of floral diversity seen in Iochrominae.

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APPENDIX 1. Taxon sampling within Solanoideae, GenBank accession numbers (ITS, *LFY*, *waxy*), and voucher information. Tribes (ending -eae) and subtribes (ending -inae) are given when available (Olmstead et al., 1999; R. G. Olmstead et al., University of Washington, personal communication). Voucher specimens have been deposited in the following herbaria: BIRM = University of Birmingham; CDS = Charles Darwin Research Station, NY = New York Botanical Garden, UT = University of Utah, WIS = University of Wisconsin-Madison.

Tribe
Subtribe
Taxon—GenBank accession nos.: ITS, *LFY*, *waxy*; voucher information.

Nicandreae
Nicandra physaloides (L.) Gaertn.—DQ314155, DQ309515, DQ309465; Peru. Dept. Amazonas. Prov. Chachapoyas, 6.24291°S 77.87443°W, 2250 m, 11-II-04, *Smith 369*, WIS.

Datureae
Iochroma cardenasianum Hunz.—DQ314156, DQ309516, DQ309466; Bolivia. Dept. Potosí. Carretera Potosi-Orkhola-Tumusla. 20.39638°S 65.56287°W, 3099 m, 18-II-04, *Smith 385*, WIS.
Solaneae
Solanum lycopersicum L.—DQ314157, DQ309517, DQ309467; UW–Madison, Botany Living Collections s.n.
Capsiceae
Capsicum lycianthoides Bitter—DQ314158, DQ309518, DQ309468; Ecuador. Prov. Pichincha. 0.0157°S 78.680°W, 2250 m, 23-XII-02, *Smith 203*, WIS.
Lycianthes inaequilatera Bitter—DQ314159, DQ309519, DQ309469; Ecuador. Prov. Pichincha. 0.326°S 79.000°W 800 m, 25-XII-02, *Smith 210*, WIS.
Physaleae
Salpichroinae
Salpichroa tristis Walp.—DQ314160, DQ309520, DQ309470; Bolivia. Dept. Potosí. Ca. 19.5°S 65.45°W, 4020 m, 18-II-04, *Smith 382*, WIS.
Physalinae
Physalis peruviana L.—DQ314161, DQ301514, DQ309471; Ecuador. Prov. Pichincha. Gardens of Herbario Nacional (QCNE), 2800 m, 1-I-03, *Smith 217*, WIS.
Leucophysalis grandiflora (Hook.) Rydb.—DQ314162, DQ301515, DQ309472; *Olmstead S-30*, WTU.
Witheringia solanacea L'Herit.—DQ314164, DQ301517, DQ309474; *D'Arcy 16399*, MO.
Withaninae
Tubocapsicum anomalum (Franchet & Savat.) Makino—DQ314163, DQ301516, DQ309473; *Chen 231*, MO.
[Subtribe not known]
Cuatresia harlingiana Hunz.—DQ314165, DQ301518, DQ309475; Ecuador. Prov. Pichincha. 0.0157°S 78.680°W, 2250 m, 24-XII-02, *Smith 204*, WIS.
Larnax sachapapa Hunz.—DQ314166, DQ301519, DQ309476; Ecuador. Prov. Pichincha. 0.0157°S 78.680°W, 2250 m, 24-XII-02, *Smith 205*, WIS.
Iochrominae
Acnistus arborescens (L.) Schlecht.—DQ314173, DQ301528, DQ309483; Costa Rica. Prov. Puntarenas. Las Cruces B. S., 1992, *Bohs 2428*, UT. ***A. arborescens*** (L.) Schlecht.—DQ314181, DQ301536, DQ309491; Ecuador. Prov. Pichincha. 0.3260°S 79.000°W, 750 m, 25-XII-02, *Smith 209*, WIS. ***A. arborescens*** (L.) Schlecht.—DQ314183, DQ301538, DQ309493; Peru. Dept. Cajamarca. 7.42409°W 78.90111°S, 1976 m, 10-I-04, *Smith 312*, WIS.
Dunalia brachyacantha Miers—DQ314172, DQ301527, DQ309482; Argentina. Prov. Jujuy, 2100 m, 20-IX-02, *Nee and Bohs 50811*, NY. ***D. obovata*** Dammer—DQ314192, DQ301547, DQ309499; Peru. Dept. Junin. 11.34919°S 75.57408°W, 2679 m, 8-III-04, *Smith 458*, WIS. ***D. spathulata*** (Ruíz & Pav.) Braun & Aschers—DQ314198, DQ301554, DQ309506; Peru. Dept. Huanuco. 9.83831°S 76.11503°W, 1842 m, 6-III-04, *Smith 452*, WIS. ***D. solanacea*** H. B. & K.—DQ314174, DQ301529, DQ309484; Ecuador. Dept. Pichincha, ca. 0.23°S 78.75°W, ca. 2200 m, 31-XII-02, *Smith 211*, WIS. ***D. spinosa*** Dammer—DQ314188, DQ301543, DQ309495; Bolivia. Dept. Potosi, ca. 19.6°S 65.6°W, 4020 m, 18-II-04, *Smith 379*, WIS.
Eriolarynx lorentzii (Dammer) Hunz.—DQ314171, DQ301525/DQ301526 (allele A/allele B); DQ309481; Argentina. Prov. Tucuman, 26.633°S 65.467°W, 1700 m, 12-II-1966, *Hawkes et al. 3452*, BIRM. ***E. fasciculata*** (Miers) Hunz.—DQ314196, DQ301552, DQ309504; Bolivia. Dept. Cochabamba, 17.46477°S 65.75217°W, 3180 m, 26-II-04, *Smith 432*, WIS.
Iochroma australe Griseb.—DQ314189, DQ301544, DQ309496; Bolivia. Dept. Chuquisaca. 20.78477°S 65.04088°W, 3038 m, 18-II-04, *Smith 390*, WIS. ***I. ayabacense*** S. Leiva—DQ314194, DQ301549/DQ301550 (allele A/allele B), DQ309501/DQ309502 (allele A/allele B); Peru. Dept. Piura. 4.61462°S 79.71178°W, 2701 m, 15-I-04, *Smith 337*, WIS. ***I. calycinum*** Benth.—DQ314201, DQ301557, DQ309512; Ecuador. Prov. Pichincha. 0.24577 S 78.80903 W, 1834 m, 27-XII-04, *Smith 471*, WIS. ***I. confertiflorum*** (Miers) Hunz.—DQ314176, DQ301531, DQ309486; Ecuador. Prov. Loja, 4.1316°S 79.9218°W, 1582m, 15-I-03, *Smith 237*, WIS. ***I. cornifolium*** Miers—DQ314177, DQ301532, DQ309487; Ecuador. Prov. Loja, 4.0869°S 79.9356°W, 2570 m, 15-I-03, *Smith 242*, WIS. ***I. cyaneum*** (Lindl.) M. L. Green—DQ314180, DQ301535, DQ309490; Ecuador. Prov. Loja, 3.849°S 79.427°W, 2325 m, 4-I-03, *Smith 223*, WIS. ***I. edule*** Leiva—DQ314193, DQ301548, DQ309500; Peru. Dept. La Libertad, 7.928183°S 78.58368°W, 2550 m, 8-I-04, *Smith 300*, WIS. ***I. ellipticum*** (Hook. f.) Hunz.—DQ314199, DQ301555, DQ309507; Ecuador. Galapagos, 0.2203°S 90.7627°W, 700 m, 6-X-02, *Jager 622*, CDS. ***I. fuchsioides*** Miers—DQ314203, DQ301559, DQ309514; Ecuador. Prov. Azuay. 2.75117°S 78.88629°W, 2828 m, 9-I-05, *Smith 488*, WIS. ***I. gesnerioides*** Miers—DQ314179, DQ301534, DQ309489; Ecuador. Prov. Pichincha, 00.0394°N 78.500°W, 2500 m, 20-XII-02, *Smith 200*, WIS. ***I. grandiflorum*** Benth.—DQ314170, DQ301523, DQ309480; Peru. Prov. Cajamarca, 7.37908°W 78.89332°S, 2781 m, 11-I-04, *Smith 320*, WIS. ***I. lehmannii*** Bitter—DQ314200, DQ301556, DQ309511; Ecuador. Prov. Cañar, 2.31973 S 78.92679 W, 2475 m, 8-I-05, *Smith 484*, WIS. ***I. loxense*** Miers—DQ314175, DQ301530, DQ309485; Ecuador. Prov. Loja, 3.999°S 79.306°W, 2050 m, 3-I-03, *Smith 220*, WIS. ***I. nitidum*** S. Leiva & Quipuscoa—DQ314168, DQ301521, DQ309478; Peru. Dept. Amazons, 6.38960°S 77.98795°W, 2605 m, 11-I-04, *Smith 371*, WIS. ***I. parvifolium*** (Roem. & Schult.) D'Arcy—DQ314195, DQ301551, DQ309503; Peru. Dept. La Libertad, 7.9477167°W 78.56195°S, 2759 m, 8-I-04, *Smith 303*, WIS. ***I.* cf. *peruvianum*** (Dunal) J. F. Macbr.—DQ314197, DQ301553, DQ309505; Peru. Dept. Cajamarca, 7.38597°S 78.89774°W, 2602 m, 16-I-04, *Smith 353*, WIS. ***I. salpoanum*** S. Leiva & Lezama—DQ314187, DQ301542, DQ309509; Peru. Dept. La Libertad. 8.00878°S 78.63911°W, 2696 m, 10-II-04, *Smith 364*, WIS. ***I. squamosum*** S. Leiva & Quipuscoa—DQ314186, DQ301541, DQ309494; Peru. Dept. Piura. 4.659652°S 79.74038°W, 2730 m, 14-I-04, *Smith 330*, WIS. ***I. stenanthum*** S. Leiva, Quipuscoa & N. W. Sawyer—DQ314184, DQ301539, DQ309508; Peru. Dept. Cajamarca, 7.40116°S 78.89658°W, 1976 m, 10-I-04, *Smith 313*, WIS. ***I.* sp. nov.**—DQ314202, DQ301558, DQ309513; Ecuador. Prov. Napo, 0.36794°S 78.10471°W, 2811 m, 4-I-05, *Smith 476*, WIS. ***I.* sp. nov. "sagasteguii"**—DQ314185, DQ301524/DQ301540 (allele A/allele B), DQ309510; Peru. Dept. Cajamarca. 7.38489°S 78.89720°W, 2603 m, 10-I-04, *Smith 317*, WIS. ***I.* sp. nov. "tingoense"**—DQ314167, DQ301520, DQ309477; Peru. Dept. Amazonas, 6.37972°S 77.90962°W, 1800 m, 11-I-04, *Smith 370*, WIS. ***I. umbellatum*** (Ruíz & Pav.) D'Arcy — DQ314169, DQ301522, DQ309479; Peru. Dept. La Libertad, 7.9344167°S 78.592367°W, 2468 m, 8-I-04, *Smith 301*, WIS.
Saracha punctata Ruíz & Pav.— DQ314182, DQ301537, DQ309492; Bolivia. Dept. La Paz, 16.32694°S 67.89167°W, 2850 m, 12-V-01, *Nee 51804*, NY. ***S. quitensis*** (Hook.) Miers—DQ314178, DQ301533, DQ309488; Ecuador. Prov. Napo, 0.3824°S 78.1600°W, 3400 m, 21-I-03, *Smith 257*, WIS.
Vassobia breviflora (Sendt.) Hunz.—DQ314190, DQ301545, DQ309497; Bolivia. Dept. Chuquisaca, 18.89368°S 65.11555°W, 1922 m, 22-II-04, *Smith 412*, WIS. ***V. dichotoma*** (Rusby) Bitter—DQ314191, DQ301546, DQ309498; Bolivia. Dept. La Paz, 16.32033°S 67.82700°W, 2157 m, 28-II-04, *Smith 440*, WIS.

APPENDIX 2. PCR primers designed for this study. All primers listed 5′ to 3′.

Locus	Primer	Sequence
waxy	F41	GGT GAT GTT CTT GCT GGA CTA C
waxy	F420	CGT GGG GTT GAT CGT GTT TTT G
waxy	R991	CCT TCG CAT TCA CAT AGA TTC C
waxy	R1235	GCT TCT AAA CTT GGT GGT CTG A
LFY	LFYSOL-F7	AAY GGY YTR GAT TAY YTG TTC CAT
LFY	LFYSOL-F68	AGA MTA TTG CYA AGG AAC GRG GTG
LFY	LFYSOL-R700	GAC AMT RTW GAR AAG TRC GTA GCA
LFY	LFYSOL-R1000	CAA GGT TAC AGG TGG AGR TAC TYG