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Phylogenetic Systematics of *Ipomoea* (Convolvulaceae) Based on ITS and *Waxy* Sequences

RICHARD E. MILLER and MARK D. RAUSHER

Department of Zoology, Duke University, Durham, North Carolina 27708-0325

PAUL S. MANOS

Department of Botany, Duke University, Durham, North Carolina 27708-0338

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ABSTRACT. *Ipomoea* is a large and complex genus containing over 600 species of vines and shrubs widely distributed throughout the tropics and subtropics. The phylogeny of 40 species representing the three currently recognized subgenera and nine sections within the genus was analyzed using sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and sequences for three exons and two introns of the 3' end of the nuclear gene *waxy*. Nucleotide data from each gene or region were analyzed singly and in combination using parsimony. Exon and intron sequences from the relatively unexplored *waxy* gene provided appreciable levels of site mutations, and intron sequences revealed several phylogenetically informative deletions. ITS provided greater resolution and was largely congruent with *waxy*. Combined analyses using *Merremia* and *Opeculina* as outgroups showed strong support for two major clades, including a novel assemblage of four Old World species and a larger clade composed of the remaining sample. Within the larger clade were numerous well-supported subclades, several of which corresponded to previously recognized taxonomic groups. Higher level hierarchical relationships within the two clades and the among the subclades did not support the most recent classification scheme, which divides *Ipomoea* into three subgenera, *Ipomoea*, *Quamoclit*, and *Eriospermum*. A striking result from this study was identifying a close relationship between species of section *Pharbitis* (subgenus *Ipomoea*) and species of subgenus *Quamoclit*. This clade is comprised of taxa with a broad range of morphological diversity, implying both floral and vegetative morphology may have been evolutionarily labile within the genus. The composition of three clades consisting largely of species of subg. *Eriospermum* suggests a novel set of relationships between New World and Australian species. Several clades identified in this study are prime candidates for future studies of character evolution, including several putative cases of independent pigment transformations of red and white flowers from purple flowers.

Central to understanding character evolution within an historical context is obtaining an accurate phylogeny for the group of organisms of interest (Donoghue 1989; Brooks and McLennan 1991; Harvey and Pagel 1991). This study is a first step towards developing a phylogenetic hypothesis for the species of the genus *Ipomoea* L. This genus is exceptionally diverse, containing over 600 species of vines and shrubs widely distributed throughout the tropics and subtropics (Van Ooststroom 1953; Standley and Williams 1970; Austin 1975b). *Ipomoea* species vary widely in habit, and vegetative and reproductive characters (e.g., House 1908; MacBride 1959; Standley and Williams 1970; Austin 1975b; McDonald 1991) making the genus a prime candidate for studies of character evolution. Particular species of *Ipomoea* have been the focus of a broad range of evolutionary studies including maintenance of floral polymorphisms (e.g., Brown and Clegg 1984; Epperson and Clegg 1987; Rausher et al. 1993; Fry and Rausher 1997), resistance and tol-

erance to herbivory and pathogens (e.g., Rausher and Simms 1989; Findeblum and Rausher 1995; Iwao and Rausher 1997), mating system evolution (e.g., Chang and Rausher 1998), evolution of seed size (e.g., Mojonner 1998), and complex inheritance of quantitative traits (e.g., Simms and Triplett 1996). In addition, there has been a growing understanding of the anthocyanin pathway (Donner et al. 1991; Martin et al. 1991; Quattrocchio et al. 1993; Koes et al. 1994), and species of *Ipomoea* have been a focus of some of this work (e.g., Durbin et al. 1995; Glover et al. 1996; Inagaki et al. 1996; Fukada-Tanak et al. 1997; Tiffin et al. 1998; Rausher et al. 1999). These evolutionary and molecular genetic studies could be used to generate externally based hypotheses regarding character evolution that could be tested within a phylogenetic context (e.g., Kohn et al. 1996).

Ipomoea is a member of the Convolvulaceae, one of two large families of the Solanales (Verdcourt 1963; Austin 1975b; Cronquist 1981). Within Con-

volvulaceae, *Ipomoea* is the sole genus of the tribe *Ipomoeae* which is often characterized by spinulose pollen (Austin 1975b). Closely related genera are the "Merremioids", which include *Merremia* Dennst. ex Endl., *Operculina* A. Silva Manso, and *Aniseia* Choisy, all of which have smooth pollen. Genera related to or potentially nested within *Ipomoea* are notoriously difficult to delimit (Meeuse 1957; Wilson 1960; Austin 1975b). For example, the genera *Batatas* Choisy, *Calonyction* Choisy, *Exogonium* Choisy, *Merremia*, *Mina* Llave & Lex., *Operculina*, *Pharbitis* Choisy, *Quamoclit* Moench., and *Turbina* Raf. have been included within *Ipomoea* (O'Donnell 1959; Meeuse 1957; Verdcourt 1963). For this study, the most current classification was used in which *Batatas*, *Calonyction*, *Exogonium*, *Mina*, *Pharbitis*, and *Quamoclit* are treated within *Ipomoea*, while *Merremia*, *Operculina*, and *Turbina* are recognized as separate genera (Austin 1975a, 1979, 1980, 1997; Austin and Huáman 1996).

Choisy (1845), Hallier (1893), and House (1908) provided early treatments recognizing subgenera and further infrageneric subdivisions within *Ipomoea*. A modern treatment of primarily African *Ipomoea* species was provided by Verdcourt (1957, 1963) recognizing eight subgenera and this was essentially parallel to the seven sections of van Ooststroom (1953) in his treatment of Asian *Ipomoea* species. The most comprehensive infrageneric treatment of *Ipomoea* is that proposed by Austin (1975a, 1979, 1980). This system has since been further refined to include a complete infrageneric treatment of American *Ipomoea* (Austin and Huáman 1996; Austin 1997). The classification developed by Austin (1975a, 1979, 1980, 1997; Austin and Huáman 1996) divided *Ipomoea* into three subgenera: *Eriospermum* (Hallier f.) Verdcourt ex Austin, *Ipomoea*, and *Quamoclit* (Moench) Clarke. This taxonomic treatment is similar to those of Verdcourt (1957) and van Ooststroom (1953); however, differences in rank are given for particular taxa due to differential character weighting (McDonald 1982). For example, Verdcourt (1957) recognized *Orthipomoea* Choisy, a group of small, erect herbs, at the level of subgenus retaining the traditional use of habit (e.g., Choisy 1845; Hallier 1893; House 1908). In contrast, Austin (1979, 1980) considered *Orthipomoea* to be a section with subgenus *Quamoclit*, presumably emphasizing seed shape and vestiture.

Austin's (1975a, 1979, 1980, 1997; Austin and Huáman 1996) division of *Ipomoea* into three subgenera suggests three major lineages within the genus (Table 2). Although particular synapomorphies

have not been explicitly presented, each subgenus can be generally characterized as follows: (a) subgenus *Eriospermum*—perennial woody species of varying habit, glabrous coriaceous sepals, hairy seeds, and two-locular gynoecia; (b) subgenus *Ipomoea*—pubescent vines, herbaceous pubescent sepals, puberulent seeds, and two or three-locular gynoecia; (c) subgenus *Quamoclit*—glabrous vines, glabrous sepals, puberulent to glabrescent seeds, and two or four-locular gynoecia (Wilson 1960; Austin 1979, 1997).

Within the broad concept of *Ipomoea* certain taxa that can be recognized on the basis of suites of traits (e.g., habit, sepal shape, sepal pubescence, corolla shape, seed vestiture, carpel number) are distinguished in most treatments (e.g., Hooker 1883; van Ooststroom 1953; Verdcourt 1957; McPherson 1979; Shinnars 1979; Gonçalves 1987). These include series *Batatas* (Choisy) D. F. Austin, sect. *Pharbitis* (Choisy) Griseb., and sect. *Mina* (Cerv.) Griseb. In contrast, other taxa within *Ipomoea* are generally recognized on the basis of one or two traits. For example, species of subgenus *Eriospermum* (except ser. *Batatas*) are defined by habit and seed vestiture. The most extreme case of a diffuse infrageneric group are the species that have been placed within section *Erpipomoea* Choisy, recognized loosely on the basis of being procumbent herbs, but in general the characteristics of the species included in this section are highly variable.

There is growing understanding of the diversification of *Ipomoea* from both floristics (e.g., van Ooststroom 1953; MacBride 1959; Matuda 1963; Verdcourt 1963; Standley and Williams 1970; Austin 1975b, 1982), as well as a variety of comparative studies (e.g., Keeler and Kaul 1984; McDonald 1992; Sinha and Sharma 1992; Armor-Prats and Harborne 1993). In addition, recent molecular phylogenetic studies have shown great promise towards refining the systematics of *Ipomoea*. McDonald and Mabry (1992) successfully used chloroplast DNA restriction fragment length polymorphisms (RFLPs) to investigate the relationships among 31 New World *Ipomoea* species. Their study supported the monophyly of several traditionally recognized taxa and led to the reclassification of series *Batatas*, from subgenus *Quamoclit* (Austin 1979, 1980) to subgenus *Eriospermum* (McDonald and Austin 1990; Austin and Huáman 1996; Austin 1997). Austin et al. (1993) examined the cladistic relationships among Australian *Ipomoea* species using both morphology and nuclear RFLPs. Two clades were identified among the twelve sampled species, with strong support for

I. batatas and *I. littoralis* Blume (series *Batatas*) as sister to the remaining taxa. Although these studies were limited in terms of taxonomic breadth, McDonald and Mabry (1992) in particular, suggested considerable homoplasy in several traditionally important morphological characters, such as glabrous or short-haired seeds, annual growth, foliose-pubescent sepals, and erect habit.

Despite these efforts, several outstanding questions concerning the systematics of *Ipomoea* remain unanswered. Competing hypotheses have been forwarded regarding which taxa within *Ipomoea* are ancestral. Austin (1979) suggested that section *Pharbitis* is basal and linked to Polemoniaceae on the basis of sharing three-locular gynoecea. In contrast, McPherson (1979) suggested *I. pedicellaris* Benth. is ancestral, which is supported, in part, by McDonald and Mabry (1992), who placed *I. pedicellaris* and *I. crinalyx* S. Moore as sister to other New World species. While most treatments of *Ipomoea* have been directed towards developing particular floras, some have been developed to reflect phylogenetic pattern. Along these lines, Austin (1979) presented an infrageneric classification that, while tentative, was proposed to represent major evolutionary branches of *Ipomoea*. Using independent data, this recent evolutionary scheme deserves testing. Another major subdivision within *Ipomoea* is one developed along biogeographic lines. Old World and New World *Ipomoea* have traditionally received separate taxonomic treatment, perhaps in part because of the notion that these two elements are distinct (Austin 1997). Worldwide sampling can allow these large-scale biogeographical patterns within *Ipomoea* to be considered.

The main focus of this study is to develop a phylogenetic hypothesis for a broad sample of *Ipomoea* using nucleotide data from two independent regions of nuclear DNA. We present data from the ribosomal 5.8S gene and associated transcribed spacers (ITS region), a well-established source of species-level phylogenetic data (e.g., Baldwin 1992; Wojciechowski et al. 1993; Baum et al. 1994, 1998; Baldwin et al. 1995; Sang et al. 1995; Soltis et al. 1996; Manos 1997), and the *waxy* gene, a relatively unexplored source of nucleotide variation. Specific objectives include (1) the use of broad taxonomic sampling to identify monophyletic groups; (2) re-examination of previously recognized taxa in light of results from new molecular data; and (3) reconsideration of characters traditionally used to delimit taxa. Finally, we hope to set the stage for directed

studies of character evolution by providing an explicit phylogenetic hypothesis for species of *Ipomoea*.

MATERIALS AND METHODS

Plant Material. Forty species of *Ipomoea* were included in this study representing the three currently recognized subgenera (*Ipomoea*, *Quamoclit*, *Eriospermum*) and most of the larger sections within the genus (e.g., Verdcourt 1957, 1963; Austin 1979, 1980, 1997; Austin and Huáman 1996; see Table 2). Species from all continents except Europe were included. The genera *Merremia* and *Operculina* were selected as outgroups based on preliminary generic level analyses within Convolvulaceae (Manos and Miller; unpublished data).

Live plant material was taken from field collections, and seeds obtained from the USDA Southern Regional Plant Introduction Station (courtesy of Dr. Robert Jarret), commercial suppliers, and from herbarium specimens. Seeds were scarified and the plants grown in the greenhouse using standard growing conditions. Species identifications were verified by growing all plants in the Duke University Greenhouse followed by comparative study with herbarium specimens and regional floristic taxonomic treatments. Voucher specimens were deposited in the Duke University Herbarium (DUKE).

DNA Extraction. Total genomic DNA was obtained from fresh material using a small-scale modification of the high-salt buffer procedure described by Bookjans et al. (1984) followed by the 2X CTAB method (Doyle and Doyle 1987). The DNeasy Plant Mini Kit (Qiagen; Valencia, CA) was also found to recover high-quality genomic DNA.

PCR and Sequencing Procedures. The ITS region (ITS 1-5.8S-ITS 2) was amplified for 42 taxa using slight modifications of the procedures described by Baldwin (1992). PCR was used to amplify double-stranded products spanning the entire ITS region in a Perkin Elmer 480 Thermal Cycler. The primers "ITS 5'" and "ITS 4'" (sequences given in Baldwin 1992) were used to generate products approximately 700 base pairs in length. These products were immediately cloned using the TA Cloning Kit (Invitrogen; Carlsbad, CA). A small sample of cells from a single colony was screened for the presence of the ITS region by PCR using the ITS 5 and ITS 4 primers under the same conditions used to

TABLE 1. Seed sources of *Ipomoea* species and outgroup accessions examined for ITS and *waxy* sequence variation. Vouchers deposited in the Duke University Herbarium. SRPIS indicates the USDA-Southern Regional Plant Introduction Station. SBE indicates the Southern Business Exchange.

Taxon	Source	Voucher	GenBank accession numbers
<i>I. alba</i> L.	Burpee Moonflower 'Giant White'	REM 58	ITS: AF110946, <i>waxy</i> : AF111154
<i>I. amnicola</i> Morong.	SRPIS-553010 (Texas)	REM 36	ITS: AF110928, <i>waxy</i> : AF111136
<i>I. aquatica</i> Forssk.	B & T World Seeds-22364	REM 3	ITS: AF110919, <i>waxy</i> : AF111127
<i>I. arborescens</i> G. Don	SBE Universal Seed Bank	REM 84	ITS: AF110924, <i>waxy</i> : AF111132
<i>I. argillicola</i> R. W. Johnson	SRPIS-538265 (Queensland, Australia)	REM 38	ITS: AF110934, <i>waxy</i> : AF111142
<i>I. asarifolia</i> (Desr.) Roem. & Schult.	Duke University Herbarium (Jamaica)	REM 97	ITS: AF110931, <i>waxy</i> : AF111139
<i>I. batatas</i> (L.) Lam.	SRPIS-561558 (Mexico)	REM 39	ITS: AF110938, <i>waxy</i> : AF111146
<i>I. carnea</i> Jacq.	B & T World Seeds-1472	REM 6	ITS: AF110920, <i>waxy</i> : AF111128
<i>I. coccinea</i> L.	Orange Co., North Carolina	REM 47	ITS: AF110941, <i>waxy</i> : AF111149
<i>I. conzattii</i> Greenm.	B & T World Seeds-32404	REM 71	ITS: AF110927, <i>waxy</i> : AF111135
<i>I. cordatotriloba</i> Dennst.	B & T World Seeds-74931	REM 73	ITS: AF110939, <i>waxy</i> : AF111147
<i>I. costata</i> F. Muell. ex Benth.	B & T World Seeds-31051	REM 62	ITS: AF110923, <i>waxy</i> : AF111131
<i>I. diamantinensis</i> J. W. Black	SRPIS-549256 (Queensland, Australia)	REM 37	ITS: AF110918, <i>waxy</i> : AF111126
<i>I. gracilis</i> R. Br.	SRPIS-538270 (Queensland, Australia)	REM 34	ITS: AF110933, <i>waxy</i> : AF111141
<i>I. graminea</i> R. Br.	SRPIS-53935 (Queensland, Australia)	REM 35	ITS: AF110915, <i>waxy</i> : AF111123
<i>I. hederacea</i> Jacq.	Orange Co., North Carolina	REM 46	ITS: AF110949, <i>waxy</i> : AF111157
<i>I. imperati</i> (Vahl) Griseb.	Brunswick Co., North Carolina	REM 99	ITS: AF110917, <i>waxy</i> : AF111125
<i>I. lacunosa</i> L.	Durham Co., North Carolina	REM 57	ITS: AF110937, <i>waxy</i> : AF111145
<i>I. leptophylla</i> Torr.	SRPIS-303327 (Nebraska)	REM 30	ITS: AF110929, <i>waxy</i> : AF111137
<i>I. lindheimeri</i> A. Gray	SRPIS-553011 (Texas)	REM 31	ITS: AF110944, <i>waxy</i> : AF111152
<i>I. lobata</i> (Cerv.) Thell.	B & T World Seeds-34004	REM 7	ITS: AF110940, <i>waxy</i> : AF111148
<i>I. muelleri</i> Benth.	SRPIS-538273 (North Territory, Australia)	REM 29	ITS: AF110921, <i>waxy</i> : AF111129
<i>I. nil</i> (L.) Roth	Kew Botanical Gardens-583 (India)	REM 50	ITS: AF110948, <i>waxy</i> : AF111156
<i>I. obscura</i> (L.) Ker Gawl.	SRPIS-530993 (Dominican Republic)	REM 28	ITS: AF110914, <i>waxy</i> : AF111122
<i>I. ochracea</i> (Lindl.) G. Don	SRPIS-549257 (Queensland, Australia)	REM 27	ITS: AF110913, <i>waxy</i> : AF111121
<i>I. pandurata</i> (L.) G. Meyer	Durham Co., North Carolina	REM 98	ITS: AF110930, <i>waxy</i> : AF111138
<i>I. parasitica</i> (Kunth) G. Don	SRPIS-279698 (Mexico)	REM 13	ITS: AF110950, <i>waxy</i> : AF111158
<i>I. pes-caprae</i> (L.) R. Br.	Kew Botanical Gardens-73215 (Mali)	REM 51	ITS: AF110932, <i>waxy</i> : AF111140
<i>I. pes-tigridis</i> L.	SRPIS-549258 (Queensland, Australia)	REM 15	ITS: AF110912, <i>waxy</i> : AF111120
<i>I. platensis</i> Ker Gawl.	B & T World Seeds-35975	REM 91	ITS: AF110925, <i>waxy</i> : AF111133
<i>I. plebeia</i> R. Br.	SRPIS-549259 (Queensland, Australia)	REM 16	ITS: AF110911, <i>waxy</i> : AF111119
<i>I. polpha</i> R. W. Johnson	B & T World Seeds-74933	REM 74	ITS: AF110922, <i>waxy</i> : AF111130
<i>I. purpurea</i> (L.) Roth	Durham Co., North Carolina	REM 100	ITS: AF110947, <i>waxy</i> : AF111155

TABLE 1. Continued.

Taxon	Source	Voucher	GenBank accession numbers
<i>I. quamoclit</i> L.	SRPIS-561555 (Australia)	REM 12	ITS: AF110943, <i>waxy</i> : AF111151
<i>I. saintronianensis</i> R. W. Johnson	SRPIS-538278 (Queensland, Australia)	REM 1	ITS: AF110926, <i>waxy</i> : AF111134
<i>I. setosa</i> Ker Gawl.	SRPIS-Grif 6151 (Texas)	REM 18	ITS: AF110935, <i>waxy</i> : AF111143
<i>I. ternifolia</i> Cav.	B & T World Seeds-64552	REM 78	ITS: AF110942, <i>waxy</i> : AF111150
<i>I. tricolor</i> Cav.	Shepherd's Garden Seeds	REM 53	ITS: AF110945, <i>waxy</i> : AF111153
<i>I. umbraticola</i> House	SRPIS-561557 (Mexico)	REM 24	ITS: AF110936, <i>waxy</i> : AF111144
<i>I. wrightii</i> A. Gray	SRPIS-518496 (Tabasco, Mexico)	REM 25	ITS: AF110916, <i>waxy</i> : AF111124
<i>Merremia tuberosa</i> (L.) Rendle	B & T World Seeds-1694	REM 18	ITS: AF110909, <i>waxy</i> : AF111117
<i>Operculina brownii</i> Ooststr.	B & T World Seeds-16342	REM 101	ITS: AF110910, <i>waxy</i> : AF111118

amplify ITS from total genomic DNA. For several samples, multiple ITS clones were examined to check for intragenomic heterogeneity. Sequences for ITS were obtained using both the external primers ITS 4 and 5, as well as using the internal primers ITS 2 and 3 (see Baldwin 1992 for sequences). Several of these putative ITS sequences were subjected to BLAST (Altschul et al. 1997) in GenBank.

The nuclear gene *waxy* (*Wx*) of plants encodes the 59 kDa granule-bound starch synthase (GBSS I) (Dry et al. 1992; Wang et al. 1995) and current evidence strongly indicates that this is a single-copy gene (Klosgen et al. 1986; Visser et al. 1989; Wang et al. 1990; van der Leij et al. 1991; Salehuzzaman et al. 1993). The 3' region was amplified for all taxa using PCR, where in all cases a single PCR product was obtained. The primers used in this study were developed using sequences of related taxa obtained from GenBank. In particular, *Ipomoea batatas* GBSS sequences obtained from mRNA, in conjunction with genomic sequences for GBSS from *Solanum tuberosum* L. were very informative. The results we report here are based on double-stranded PCR products that span the part of the gene that extends from exon 9 to exon 11 including two introns. These products were formed using a 5' primer ('GATACCCAAGAGTGGAAACCC') and a 3' primer ('GTTCCATACGCATAGCATG') under the following reaction conditions: one cycle of 1 min at 97°C and 40 cycles of 57°C for 1 min, 72°C for 45 sec with a 4 sec increase after each cycle. Sequences for the *waxy* gene were obtained for both the forward and reverse primers.

Sequence reactions were prepared using the ABI Prism Dye Terminator Cycle Sequencing Reaction Kit. Sequences were analyzed using the program Sequencher (GenCodes). Sequences were deposited in GenBank (accession numbers given in Table 1).

Phylogenetic Analysis. Sequences were aligned by direct visual determination. ITS sequences among *Ipomoea* species were relatively free of extensive length variation. Variable positions (61) of the ITS region that could not be aligned unambiguously were removed from further consideration. Sequences from *waxy* contained considerable length variation within intron regions, but only one variable position was excluded on the basis of alignment ambiguity. Complete data matrices (available from the authors) from each genic region were analyzed

separately and in combination using unweighted parsimony as implemented on Macintosh versions of PAUP 3.1.1 (Swofford 1993) and PAUP* (Swofford 1998). Heuristic search algorithms based on 100 rounds of random taxon-entry sequences were used in conjunction with the MULPARS option and TBR branch swapping. Branches with a minimum length of zero were collapsed (Nixon and Carpenter 1996). Consistency index (CI; Kluge and Farris 1969) and retention index (RI; Farris 1989) also were calculated. Consensus trees were constructed to evaluate branches common to each set of most-parsimonious trees. Bootstrap analysis (Felsenstein 1985) was used to determine the relative support for individual clades, and where possible, all minimum length trees were saved for each pseudoreplicate.

Incongruence between data sets was quantified using the Mickevich and Farris incongruence index (I_{MF} ; Mickevich and Farris 1981) and tested using the random partitions test of Farris et al. (1994), as implemented in PAUP*. This tests for character congruence by calculating the length difference among resampled partitions from the pool of characters in the combined original matrices. Incongruence between data sets is identified if the additive tree lengths taken from resampled matrices are greater than the sum of the tree lengths from the original data (e.g., Allard and Carpenter 1996). Relative bootstrap support also was used to evaluate the strength of the contrasting topologies (Mason-Gamer and Kellogg 1996).

Alternative topologies were assessed by implementing the TOPOLOGICAL CONSTRAINTS option in PAUP. Constraint trees were used to test how our results compared with previous treatments of *Ipomoea*. Specifically, we used the classification of *Ipomoea* species presented in Austin and Huáman (1996) as a general framework. We also recognized section *Tricolores* (Austin 1997; following McDonald 1982) and the placement of section *Orthipomoea* within subgenus *Quamoclit* (Austin 1979, 1980). Finally, we included *Ipomoea plebeia* within section *Orthipomoea* following Verdcourt (1963). We present this as the most current treatment of the taxa included in our study by Austin and coworkers (D. Austin, pers. comm.; see Table 2). To fairly represent the evolutionary component of Austin's classification scheme, a tree was constrained to form an unresolved trichotomy representing the three

subgeneric groups (*Eriospermum*, *Ipomoea*, and *Quamoclit*). Where sampling permitted, additional infrageneric groups (sections *Eriospermum*, *Eripipomoea*, *Pharbitis*, *Mina*, *Tricolores* J. A. McDonald; series *Batatas*, *Jalapae* (House) D. F. Austin, *Heterophyllae* (House) D. F. Austin) were constrained, but relationships within these groups were treated as polytomies. The minimum number of steps required to produce the resulting topologies was recorded. Because several Australian taxa included here have never been placed within a subgeneric treatment and the placement of *I. wrightii* is tentative (Austin and Huáman 1996), the constraint trees included 33 taxa.

RESULTS

ITS Sequence Data. The highest value of raw sequence divergence (18.8%), excluding 61 sites for which alignment was ambiguous, was obtained in pairwise comparisons of the outgroup *Operculina* and *Ipomoea quamoclit*. Divergence values between most combinations of *Ipomoea* species were less than 10%. Sequences from eight ITS clones from each of three taxa were found to differ in length by one to three base pairs in highly repetitive spans of both spacers. When these sets of clones were included in preliminary rounds of phylogenetic analysis they always formed species-specific clades, and thus had minor effects on the resolution of major clades.

From the final alignment of 573 nucleotide sites across 42 taxa, 163 phylogenetically informative sites formed the basis of subsequent parsimony analyses; there were no missing data. Parsimony-informative sites were distributed in the following way: ITS 1 = 79, 5.8S = 7, and ITS 2 = 77. When sets of *Ipomoea* ITS sequences were subjected to BLAST in GenBank, the highest values of sequence similarity were found with angiosperms classified within subclass Asteridae. Comparisons among the complete set of sequences showed that sequences from the 5.8S gene were highly conserved and therefore most likely representative of functional ITS (Buckler et al. 1997).

Unweighted parsimony analysis recovered six most-parsimonious trees of 644 steps (excluding autapomorphies). One of the most-parsimonious trees is shown to indicate branch lengths and nodes which collapse in the strict consensus

TABLE 2. *Ipomoea* species used in our study placed according to Austin and coworkers, and Verdcourt (Austin 1979; Austin & Huáman 1996; Austin 1997; Verdcourt 1957, 1963). Asterisk indicates type species.

Ipomoea L.

subg. *Eriospermum* (Hallier f.) Verdcourt ex Austin

sect. *Eriospermum* Hallier f.

Ipomoea platensis

ser. *Eriospermum* (Hallier f.) D. F. Austin

Ipomoea konzattii

ser. *Aborescentes* (Choisy)

*Ipomoea arborescens**

ser. *Setosae* (House) D. F. Austin

*Ipomoea setosa**

ser. *Batatas* (Choisy) D. F. Austin

*Ipomoea batatas**

Ipomoea lacunosa

Ipomoea cordatotriloba

Ipomoea umbraticola

ser. *Jalapae* (House) D. F. Austin

Ipomoea amnicola

Ipomoea leptophylla

Ipomoea pandurata

Ipomoea carnea

sect. *Erpipomoea* Choisy

*Ipomoea pes-caprae**

Ipomoea asarifolia

Ipomoea aquatica

Ipomoea imperati

Ipomoea obscura

Ipomoea ochracea

subg. *Ipomoea*

sect. *Ipomoea*

ser. *Ipomoea*

*Ipomoea pes-tigridis**

sect. *Pharbitis* (Choisy) Griseb.

ser. *Pharbitis* (Choisy) D. F. Austin

*Ipomoea purpurea**

ser. *Heterophyllae* (House) D. F. Austin

Ipomoea lindheimeri

Ipomoea hederacea

Ipomoea nil

subg. *Quamoclit* (Moench) Clarke

Ipomoea wrightii

sect. *Mina* (Cerv.) Griseb.

*Ipomoea coccinea**

Ipomoea lobata

Ipomoea quamoclit

TABLE 2. Continued.

sect. *Leptocallis* (G. Don) J. A. McDonald

Ipomoea terminifolia

sect. *Calonyction* (Choisy) Griseb.

*Ipomoea alba**

sect. *Tricolores* J. A. McDonald

Ipomoea parasitica

Ipomoea tricolor

sect. *Orthipomoea* Choisy

Ipomoea plebeia

(Fig. 1). In all trees, a clade consisting of *Ipomoea plebeia* + *I. pes-tigridis* and *I. ochracea* + *I. obscura* was sister to the remaining species of *Ipomoea*. Within the larger group, six subclades were moderately to strongly supported; two clades of two species each, *I. wrightii* + *I. imperati* and *I. aquatica* + *I. diamantinensis*; a third clade of seven species including *I. carnea*, *I. konzattii*, and *I. platensis*; a fourth clade of four species that includes *I. umbraticola* and *I. batatas*; a fifth clade of eight species that includes *I. muelleri*, *I. argillicola*, and *I. pes-caprae*, and a sixth clade of eleven species including *I. lobata*, *I. purpurea*, and *I. tricolor*. The ITS data also show strong support for subdivisions within these large clades, including some well-supported species pairs. In contrast, structure at intermediate hierarchical levels is generally not well supported as indicated by bootstrap values below 50% (not shown).

Waxy Sequence Data. Raw sequence divergence values generally were not as great as those of ITS, with most values between *Ipomoea* species less than 5%. The high for an outgroup species (*Operculina*) and an *Ipomoea* species (*I. purpurea*) was 8.8%, but *I. plebeia* and *I. hederacea* had the highest value (10.4%). Generally, intragenomic heterogeneity was not detected. However, the poorly resolved sequences of a few polyploid taxa (e.g., *I. batatas*) suggested potential length heterogeneity at the 3' end of the fragment. Several cloned fragments were sequenced and length variation was detected in the most labile portion of the intron between exon 11 and 12. The additional variation detected was not phylogenetically informative.

From the final alignment of 651 nucleotide sites sequenced from the 3' end of the *waxy* gene, 14 potentially variable sites were excluded from further analysis because of poor sequence reso-

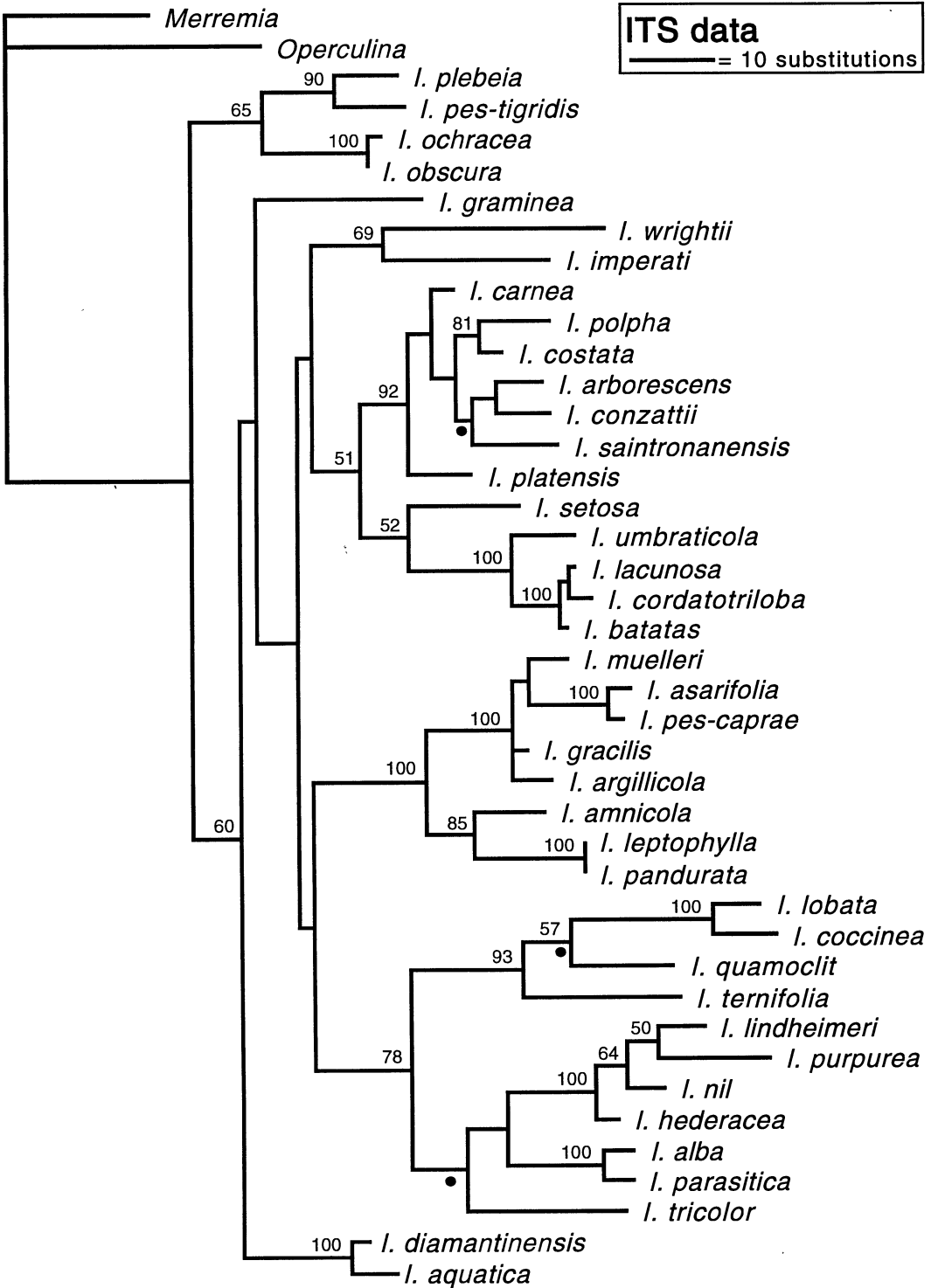


FIG. 1. One of 6 most-parsimonious trees for *Ipomoea* based on ITS sequences. Length = 644 steps, CI = 0.46, RI = 0.65; branch lengths drawn to scale. Percentage of 100 bootstrap replications is given for nodes with bootstrap values > 50%. Nodes not supported in the strict consensus tree are indicated with a solid circle.

lution close to the primer sites. Parsimony analysis was conducted on 86 phylogenetically informative sites, only four of which included missing data (0.4% of the matrix). These 86 sites were distributed in the following regions: exon 10 (partial) = 5, intron 10 = 17, exon 11 = 21, intron 11 = 29, and exon 12 = 14. Within the second intron, two well delimited deletions of 10 and 15 base pairs were inferred from the final alignment.

Unweighted parsimony analysis of the 86 sites resulted in 373 most-parsimonious trees of 189 steps (autapomorphies excluded). One of the most-parsimonious trees derived from *waxy* is shown to indicate branch lengths and nodes which collapse in the strict consensus (Fig. 2). With fewer phylogenetically informative sites per taxon, it was impossible to save all trees for many bootstrap pseudoreplicates, thus bootstrap values were derived by saving only 1000 trees for each pseudoreplicate. In general, the resolving power of *waxy* was much less than that of ITS as illustrated in part by the number of nodes which collapse in the strict consensus tree. All trees strongly supported a clade composed of *I. plebeia*, *I. pes-tigridis*, *I. ochracea*, and *I. obscura* as sister group to the remaining species. In spite of the numerous unresolved branches, six subclades corresponded to groups that were also resolved by the ITS data (see Fig. 2).

The most-parsimonious distribution of the two deletions detected within the second intron also is shown in Fig. 2. For the topologies based on sequence data alone, a 10 base deletion unambiguously supported the clade that includes *I. batatas*, *I. cordatotriloba*, *I. lacunosa*, and *I. umbraticola*, whereas the distribution of a 15 base pair deletion was mapped twice on the tree.

ITS and Waxy Combined Data. The amount of incongruence between the two data sets, as measured by the Mickevich-Farris incongruence index, was low ($I_{MF} = 0.024$). The results of the random partitions test could not reject the null hypothesis of congruence among data partitions; however, *P* values ranged from 0.05 to 0.03 indicating some degree of incongruence between data sets.

In comparing the topologies across both trees, there were few instances of strongly supported incongruence. For example, the placement of the well-supported species pair *I. aquatica* and *I. diamantinensis* in the ITS trees suggested a sister group relationship to a larger clade of *Ipomoea*

species included in this study, but with little confidence (Fig. 1). In contrast, *waxy* sequences and a 10 bp deletion placed these species within one of the large clades identified in this study (Fig. 2; discussed in more detail below). Another incongruent relationship detected was the placement of *I. konzattii* and *I. platensis*. The two data sets suggested different positions for these taxa with moderate to high bootstrap support (Figs. 1, 2). However, when the random partitions test was performed on data sets pruned to exclude one or both pairs of these taxa, levels of incongruence remained the same. In this case, different gene histories and homoplasy are equally likely sources of incongruence.

Combined parsimony analysis resulted in 56 most-parsimonious trees of 854 steps. The sum of the tree lengths of the original matrices was 833 steps, indicating that 21 extra steps were needed to explain character incongruence between data sets. One of the most-parsimonious trees is shown to illustrate relative branch lengths (Fig. 3) and the consensus is presented to serve as a reference guide for clade-specific discussion (Fig. 4). The two deletions detected within a *waxy* intron were parsimoniously mapped on the tree shown in Fig. 3. Based on the topology shown in Fig. 3, the distribution of the 15 bp deletion found in *waxy* was explained by three losses, whereas the 10 bp deletion unambiguously supported the clade including *I. batatas*.

Basal structure within these trees identified two strongly supported clades. The smaller of the two clades (clade 1) included two well-delimited species pairs of *I. ochracea* + *I. obscura* and *I. plebeia* + *I. pes-tigridis*. In the larger clade, four well-supported multi-species clades and a two species clade composed of *I. wrightii* + *I. imperati* were resolved (Figs. 3, 4); however, the interrelationships among the clades were weakly supported.

Within clade 2 (Fig. 4) was the basal species pair of *I. diamantinensis* + *I. aquatica*. Subclade 2A, which also was well-supported in separate analyses, consisted of a monophyletic group with two main subclades, 2A-1 and 2A-2. Subclade 2A-1 was well-defined, containing the strongly supported species pair *I. lobata* + *I. coccinea*. Within subclade 2A-2 was the species pair *I. alba* + *I. parasitica* and another well-supported subclade, 2A-3, which is sister to *I. tricolor* (though placement of *I. tricolor* varied in the ITS

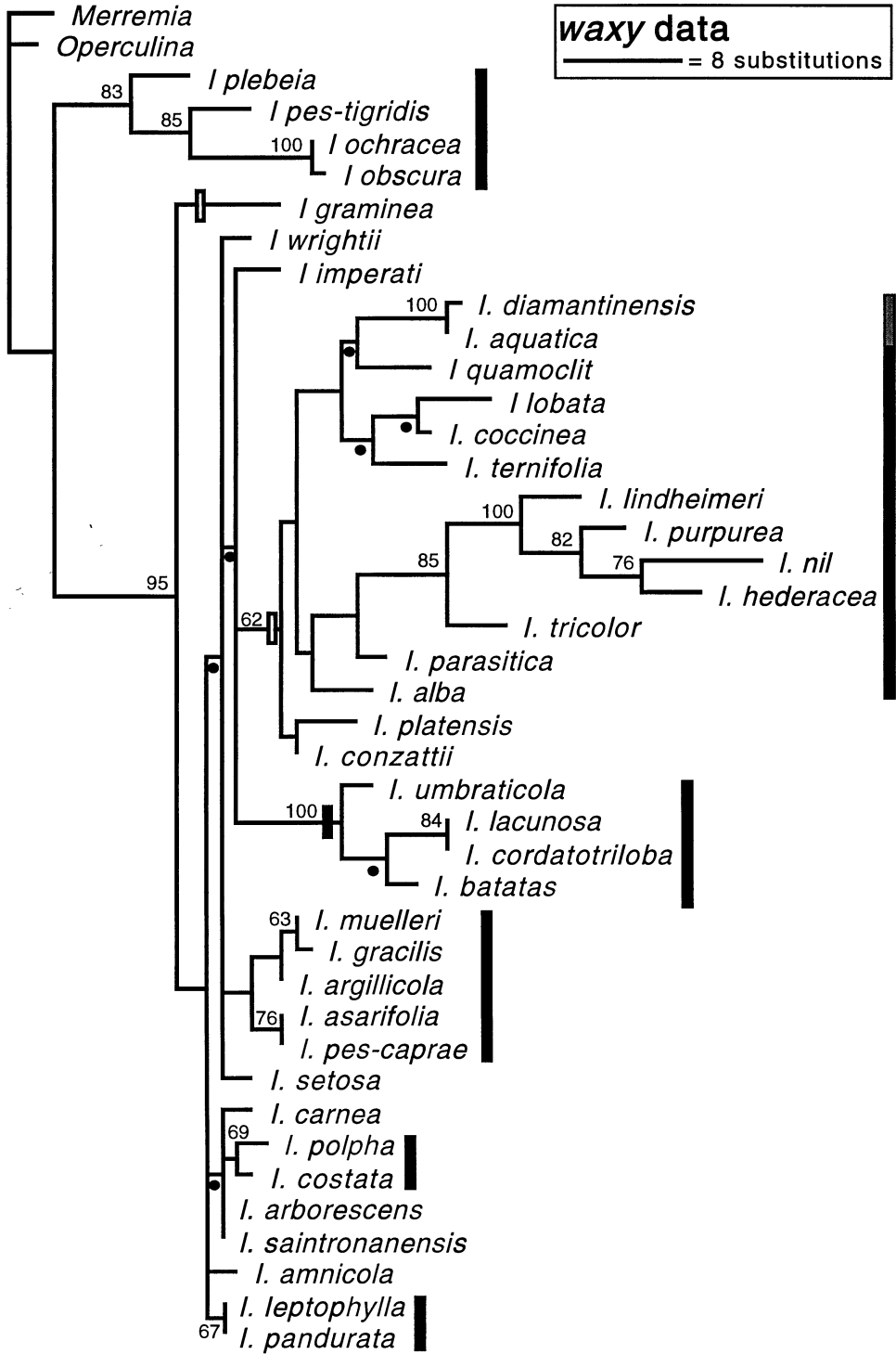


FIG. 2. One of 373 most-parsimonious trees for *Ipomoea* based on *waxy* sequences. Length = 189 steps, CI = 0.60, RI = 0.77, branch lengths drawn to scale. Percentage of 100 bootstrap replicates (see text for details) is given for node with bootstrap values > 50%. Nodes not supported in the strict consensus tree are indicated with a solid circle. Most-

and *waxy* trees). Subclade 2A-3 represents a monophyletic group that included *I. purpurea* and was strongly supported in separate analyses (Figs. 1, 2).

Clade 3 and clade 4 + *I. setosa* were shown to be weakly supported sister taxa, in accord with the ITS tree. Clade 3 was well-supported by ITS data and several of these species pairs also were resolved in the *waxy* trees (Figs. 1, 2). However, according to the *waxy* data, including a 15 base pair deletion (see Fig. 2), the species pair *I. conzattii* + *I. platensis* grouped with species resolved in the combined analysis as clade 2. The ITS data appeared to provide the strength of inference in placing both species within clade 3 in the combined analysis. A notable subclade within clade 3 is composed of two Australian endemics, *I. polpha* and *I. costata*.

Clade 4 was found to be sister to *I. setosa*, though this relationship does not have strong support in any of the trees presented (Figs. 1-4). Clade 4 was well-supported in separate and combined analyses, and a 10 base pair deletion in one of the *waxy* introns provided additional support. Within clade 4, there was strong support for the subclade 4A which united the species pair *I. lacunosa* and *I. cordatotriloba* with *I. batatas* and placed *I. umbraticola* as sister to these taxa.

The resolution of clade 5 was based mostly on the ITS sequence data. Within clade 5, two well-supported subclades were resolved. Subclade 5A included five species plus the well-supported species pair of *I. pes-caprae* and *I. asarifolia*. Subclade 5B included three species, with strong support for the sister taxa, *I. leptophylla* and *I. pandurata*.

One specific alternative hypothesis derived from the classification of *Ipomoea* of Austin and coworkers was tested. Thirty-one taxa and two outgroups, representing those species treated in this classification (Table 2), were reanalyzed using unweighted parsimony and then constrained to match the proposed classification. Parsimony analysis on this subset of taxa based on the combined molecular data produced two equally parsimonious trees of 696 steps (autapomorphies

excluded). The main structure within the treatment of Austin is the three subgenera within *Ipomoea*, which was forwarded as a viable evolutionary hypothesis of relationships. However, analysis of this alternative hypothesis resulted in trees at least 104 steps or 14.9% longer than the most-parsimonious trees based on the unconstrained molecular data for the same 33 taxa.

DISCUSSION

Simultaneous phylogenetic analysis of an exemplar sample of 40 *Ipomoea* species using molecular data from two nuclear DNA regions, ITS and the *waxy* gene, has generated a well-resolved cladistic hypothesis to consider the systematics of the genus (Figs. 3, 4). These results do not support the three subgenera of Austin's classification (Austin and Huáman 1996; Austin 1997), nor any other previous subgeneric arrangements of *Ipomoea* (e.g., van Ooststroom 1953; Verdcourt 1957, 1963). This disparity results from a novel arrangement of species composed of taxa previously considered to be members of each of the three subgenera (Figs. 1-3; Fig. 4, clade 1). Furthermore, we find that section *Erpipomoea* of subgenus *Eriospermum* is clearly not monophyletic with species from this group being scattered within several well-supported clades (Fig. 4, clades 1, 2, and 4a). In contrast, our analysis suggests a novel arrangement of two major clades within the sample. Within the larger of the two clades, some groups correspond to previously recognized taxa within *Ipomoea* (van Ooststroom 1953; Verdcourt 1957, 1963; Austin 1979, 1980, 1997; Austin and Huáman 1996), whereas others suggest relationships that are inconsistent with parts of the most recent infrageneric classification of the genus (Austin and Huáman 1996; Austin 1997).

An important cladistic result supported by separate and combined data is the resolution of a four species clade of *Ipomoea* from the remaining sample (Figs. 1-3; Fig. 4, clade 1). This sister group arrangement suggests a previously unrecognized taxon of Old World species. The four species clade includes species (*I. obscura*, *I. ochra-*

←

parsimonious distributions of 10 and 15 basepair deletions indicated with solid and open bars, respectively. Vertical lines indicate clades also resolved by ITS sequences, except for the position of the *I. diamantinensis* + *I. aquatica* clade which is shaded.

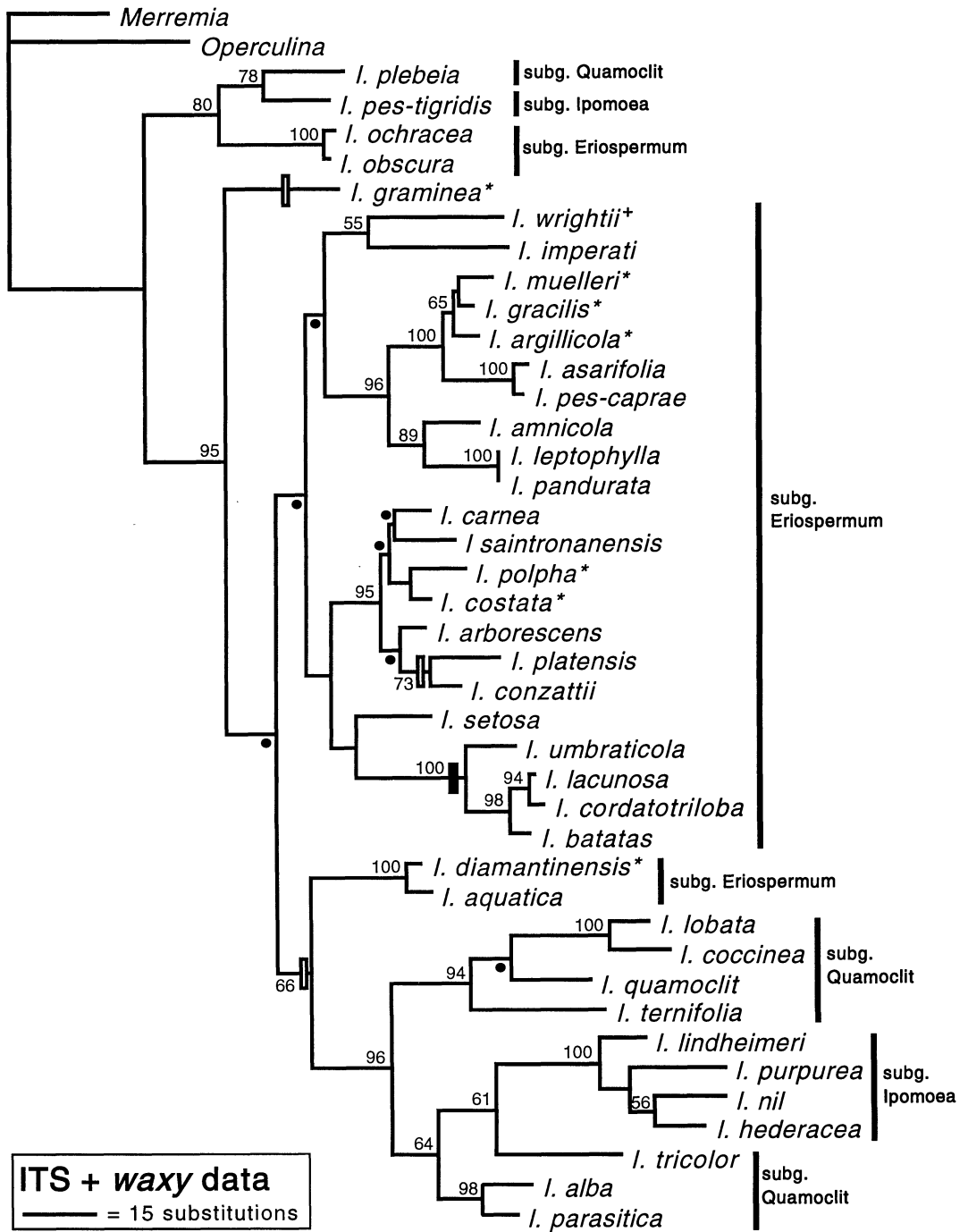


FIG. 3. One of 56 most-parsimonious trees for *Ipomoea* based on simultaneous analysis of ITS and *waxy* sequences. Length 854 steps, CI = 0.48, RI = 0.66, branch lengths drawn to scale. Percentage of 100 bootstrap replications is given for nodes with bootstrap values > 50%. Nodes not supported in the strict consensus tree are indicated with a solid circle. Most-parsimonious distributions of 10 and 15 basepair deletions indicated with solid and open bars, respectively. Placement of species within subgenera following the classification of Austin and coworkers are indicated by vertical lines (see Table 2). Species names followed by an asterisk indicate taxa that have not been placed within a subgeneric treatment. The plus following *I. wrightii* indicates its placement is tentative.

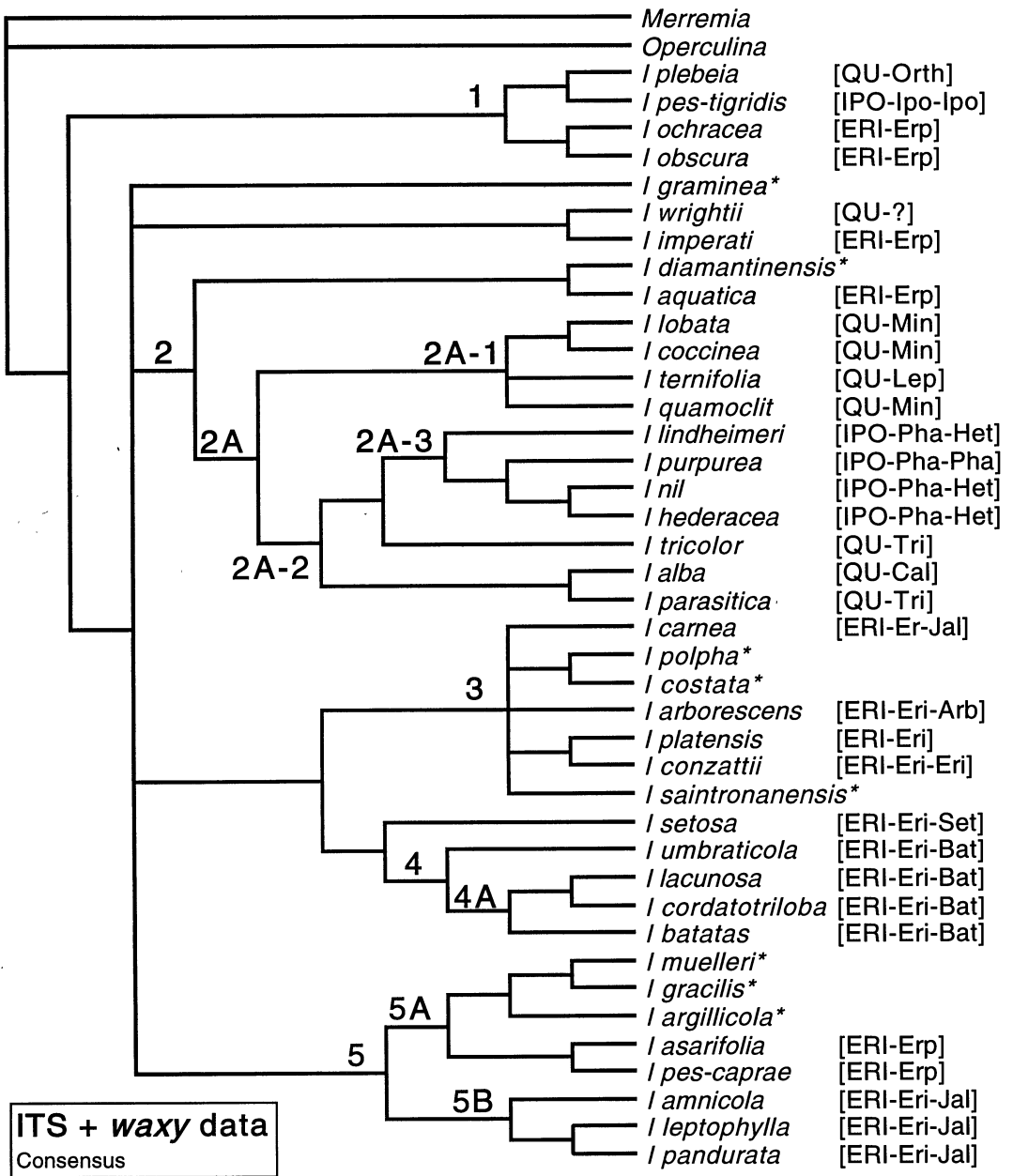


FIG. 4. Strict consensus tree for *Ipomoea* based on simultaneous analysis of ITS and *waxy* sequences. Clade numbers are given for reference. Species are followed by abbreviations referring to the classification of Austin and coworkers (see Table 2); Subgenera: ERI = *Eriospermum*, IPO = *Ipomoea*, QU = *Quamoclit*; Sections: Orth = *Orthipomoea*, Ipo = *Ipomoea*, Erp = *Erpipomoea*, Min = *Mina*, Lep = *Leptocallis*, Pha = *Pharbitis*, Tri = *Tricolores*, Cal = *Calonyction*, Eri = *Eriospermum*; Series: Ipo = *Ipomoea*, Het = *Heterophyllae*, Pha = *Pharbitis*, Jal = *Jalapae*, Arb = *Arborescentes*, Eri = *Eriospermum*, Set = *Setosae*; Bat = *Batatas*; ? = not classified to section or series. Species names followed by an asterisk indicate taxa that have not been placed within a subgeneric treatment.

cea, *I. pes-tigridis*, *I. plebeia*) from the three subgenera recognized by Austin (Austin and Huáman 1996; Austin 1997) therefore contributing to the nonmonophyly of the three subgeneric concept within *Ipomoea*. *Ipomoea obscura* and *I. ochracea* are morphologically similar species that have been previously thought to belong to subgenus *Eriospermum* section *Erpipomoea* (Austin and Huáman 1996). *Ipomoea pes-tigridis* is the type species of subgenus *Ipomoea* section *Ipomoea* (Austin 1979), whereas *I. plebeia* is regarded as a member of subgenus *Quamoclit* section *Orthipomoea* (Austin 1979, 1980; Verdcourt 1963). Although *Ipomoea plebeia* is the only representative of subgenus *Quamoclit* sect. *Orthipomoea* in our study, this position clearly indicates that it is not related to the more typical species treated within subgenus *Quamoclit* (Table 2). Additional sampling from section *Orthipomoea*; (e.g., *I. eriocarpa* R. Br., *I. polymorpha* Roem. & Schultes, *I. sinensis* (Desr.) Choisy) and section *Ipomoea* (e.g., *I. involucrata* Beauv., *I. pileata* Roxb., *I. heterotricha* F. Didr.) (van Ooststroom 1953; Verdcourt 1963) will further establish the taxonomic breadth of this novel assemblage identified here as clade 1 (Fig. 4).

Our results do not support the monophyly of section *Erpipomoea* Choisy (= *Leiocalyx* Hallier f. sensu stricto) of subgenus *Eriospermum*. This is due to the placement of *I. obscura* and *I. ochracea* within clade 1, the species pair *I. pes-caprae* and *I. asarifolia* within clade 5A, *Ipomoea aquatica* within clade 2, and *I. imperati* forming a species pair with *I. wrightii*. Previous treatments also point towards the artificiality of section *Erpipomoea*, as it is largely defined on the basis of habit (e.g., House 1908; van Ooststroom 1953).

In addition to the two major clades within *Ipomoea*, the resolution of several subclades provides a useful platform for reconciling the existing classification through some taxonomic restructuring (Fig. 4). According to the combined data, subgenus *Ipomoea* appears to be nonmonophyletic, suggesting that sections *Ipomoea* and *Pharbitis* are distinct and unrelated. Subgenus *Eriospermum* may not be monophyletic given the placement of taxa recognized within section *Erpipomoea*. Finally, inclusion of section *Orthipomoea* within subgenus *Quamoclit* is not upheld by our data. After these modifications, clade 2 would correspond to a broadened concept of subgenus *Quamoclit*, now including section *Pharbitis* (clade 2-A2). Clades 3, 4, and 5 correspond

to subgenus *Eriospermum*, though our data cannot be used to support these three clades as members of a larger a monophyletic group.

Subgenus *Quamoclit*—Clade 2. A major result of this study is the close relationship of the species of section *Pharbitis* (clade 2A-1) with other groups of species traditionally placed in sections *Calonyction*, *Tricolores*, *Mina*, and *Leptocallis* (G. Don) J. A. McDonald of subgenus *Quamoclit* (Austin and Huáman 1996; Austin 1997). Because section *Pharbitis* has always been treated within subgenus *Ipomoea*, our results therefore suggest a new concept of relationships among these species and a broadened concept of subgenus *Quamoclit*. The phylogenetic position of section *Pharbitis* is clearly derived within the genus, thus corroborating the results of cpDNA data (McDonald and Mabry 1992), while contradicting the notion of *Pharbitis* as ancestral within *Ipomoea* (Austin 1979).

The broadened concept of *Quamoclit* also encompasses a wider range of morphological diversity. This includes two morphologically distinct and commonly recognized taxonomic groups, section *Mina* and section *Pharbitis* (Hooker 1883; House 1908; van Ooststroom 1953; Meeuse 1957; Verdcourt 1963; Austin 1975, 1997; McPherson 1979; Austin and Huáman 1996). Species of section *Mina* have red or yellow salverform corollas, exserted stamens, and 4-locular gynoecea (Austin 1997). Morphologically, the monophyly of section *Pharbitis* appears to be supported by foliose-pubescent sepals, 3-locular gynoecea (Austin 1997), and the lack of extrafloral nectaries (Keeler and Kaul 1984). Together with the other species included in this novel assemblage, clade 2 is one of the most interesting in terms of phylogeny and morphological diversity.

An unexpected result is the position of the closely related species pair *I. aquatica* + *I. diamantinensis* as the sister group to the *Quamoclit* species. *Ipomoea aquatica* has consistently been placed in section *Erpipomoea* and is native to the Old World tropics. *Ipomoea diamantinensis*, however, is endemic to Australia and has a hollow stem, a characteristic shared by *I. aquatica* (R. Miller, pers. obs.). Morphologically, these species appear to represent a single widespread taxon in the Old World, but additional evidence and further sampling are needed to clarify the relationships between these species and the tradi-

tionally recognized members of subgenus *Quamoclit*.

Subgenus *Eriospermum*—Clades 3, 4, and 5. The most challenging and largest set of species within the genus *Ipomoea* are those belonging to subgenus *Eriospermum*. Clades 3, 4, and 5 from our analysis are broadly consistent with Austin's notion of subgenus *Eriospermum*, with clade 4 corresponding to series *Batatas*. The membership of species forming clades 3 and 5 is a surprising result indicating close relationships between two sets of New World species belonging to various subdivisions of section *Eriospermum* with two distinct elements of unclassified Australian species.

Clade 4 identifies another group of widely recognized taxa, the *Batatas* species (van Oostroom 1953; Austin 1975, 1978; McPherson 1979; McDonald and Austin 1990), most of which are distinct among other species traditionally placed in subgenus *Eriospermum* on the basis of glabrous seeds (Austin 1978; McDonald and Austin 1990). Within this clade, *I. umbraticola* is sister group to several highly typical *Batatas* species. Morphologically *I. umbraticola* shares many similarities with most *Batatas* species (e.g., annual habit, herbaceous stems), but differs in having trichomes along the seed margins. This sister group relationship is consistent with McDonald and Austin's (1990) interpretation of the relationships among these species based largely on seed vestiture. The putatively ancestral condition of hairy seeds was used by them to link the *Batatas* species, via *I. umbraticola*, to other taxa in subgenus *Eriospermum*. Our results support the cpDNA data (McDonald and Mabry 1992) in suggesting a close relationship of series *Batatas* to other *Eriospermum* species, *I. setosa* in particular.

The distribution of the constituent species that form clade 3 suggests close phylogenetic relationships between taxa from Australia (*I. costata*, *I. polpha*, and *I. saintronanensis*) and the Americas (*I. arborescens*, *I. carnea*, *I. conzattii*, and *I. platenis*). These American species have been placed in various sections within subgenus *Eriospermum*, while the Australian species remain unclassified (see Figs. 3 and 4). The phylogenetic affinities among the Australian species also was supported by Austin et al. (1993) on the basis of nuclear RFLP data. However, the monophyly of these biogeographically distinct species is a new result

from this study, obtained, in part, because of a wide biogeographic sampling.

While the woody and hairy-seeded species *I. carnea* and *I. arborescens* have been classified in separate series (ser. *Jalapae* and *Arborescentes* Choisy respectively), our results suggest they are related (although it is worth noting these species have distinct forms of woody growth, McDonald 1992). An association between *I. carnea* and *I. pracecana*, a species closely related to *I. arborescens* (McPherson 1981), also was supported by cpDNA data (McDonald and Mabry 1992). In contrast, the relationship between *I. carnea* and other species considered members of series *Jalapae* (*I. amnicola*, *I. leptophylla*, *I. pandurata*) is not supported by our data, suggesting series *Jalapae* is not monophyletic (see Fig. 4, clades 3 and 5B).

Combined analysis supports clade 5 and its two distinct subclades (5A and 5B). The species composition of clade 5A, which includes the Australian endemics, *I. muelleri*, *I. gracilis*, and *I. argillicola* and the pantropical species *I. pes-caprae*, is essentially equivalent to a clade reported by Austin et al. (1993) based on molecular data. Previous taxonomic treatments placed *I. pes-caprae* within subgenus *Eriospermum* section *Erpipomoea*, a taxonomic section not supported by our data. The strongly supported relationship between *I. pes-caprae* and *I. asarifolia* is completely consistent with their overall morphological similarity (Austin 1975b). While the close relationships among the Australian endemics is supported by morphological analysis (Austin et al. 1992), no clear morphological characters unite these taxa with *I. pes-caprae* + *I. asarifolia*. Clade 5B includes American species placed within section *Eriospermum* series *Jalapae*. There are no obvious morphological characters to support the well-defined groups within clade 5, however it does represent another independent example of a close phylogenetic relationship between taxa from Australia and the Americas.

Future Directions. Many of the clades identified in this study suggest novel phylogenetic associations to consider in future cladistic studies. Unfortunately, without complementary morphological data it is difficult to support the assertion that traditional morphological characters are prone to homoplasy (McDonald and Mabry 1992). However, using our molecular hypothesis of phylogeny as a preliminary guide, new combinations of characters are certain to emerge to

independently support and combine with the data presented here (P. Wilkin, unpubl. data). For example, it would be particularly informative to determine whether there are morphological characters that also delimit the two major clades resolved within our study, or the various well-supported subclades within the larger clade that do not correspond to previously recognized taxa. In addition, several groups of *Ipomoea* identified in this study bring together geographically disparate taxa, emphasizing the need for broader taxonomic comparisons to identify widespread monophyletic groups. Alternatively, increased sampling will identify sets of endemic taxa, thus providing a detailed framework to consider the historical biogeography of the genus.

Using the phylogenetic hypothesis presented here as a general starting point, studies of character evolution are also possible. For example, considering the wealth of floral variation within the genus, *Ipomoea* is ideally suited to a comparative study of pollination syndromes. Notable floral innovations include zygomorphy, exerted stamens, salverform corolla tubes, and changes to putatively derived corolla pigments (e.g., red, yellow, white). Many of these innovations represent transformations from more generalized character combinations (Grant and Grant 1965; Stebbins 1974) that are best evaluated within phylogenetic context (Armbruster 1993; Barrett et al. 1996). Specific hypotheses regarding the origin(s) of pollination transitions can be tested and evaluated to fully understand their role in promoting the diversification of the genus.

The floral diversity within clade 2 may provide some key examples to examine floral pigment transitions within a monophyletic group. An expanded sampling emphasizing species of section *Tricolores* and *I. alba* of section *Calonyction* is likely to indicate a transition from bee-pollinated *Tricolores* species to the derived floral morphology of *I. alba*, a clear example of the moth-pollination syndrome. Wilkin (1995) has proposed an interesting example of the same pollinator shift among two species of section *Pharbitis*. While most species in section *Pharbitis* have the floral morphology consistent with bee-pollinated flowers there are some species with white corollas (*I. ampullacea* Fernald, *I. igualensis* Weath., *I. petrophila* House, *I. sescossiana* Baill.), as well as polymorphic species with pigmented and white corollas (*I. indica* (Burm. f.) Merr., *I.*

purpurea). Determining whether this variation in floral morphology represents multiple or single transitions from bee to moth-pollination will require broader sampling, additional pollination studies, and a resolved species-level phylogeny.

Another evolutionarily interesting pollination transition within *Ipomoea* involves multiple shifts from bees to birds. Bird pollination and red corollas are most common among species of section *Mina*, but also occur in species of other lineages, including sections *Exogonium* and *Eriosperrum* (Austin 1997). Considering the well-supported broader relationships of section *Mina* species, these species provide a promising case study. Specific association of cause and consequence regarding the evolution of red flowers will require greater resolution between additional species of sections *Leptocallis* and *Mina*, as well as elucidation of the underlying genetic basis of pigment transition via the anthocyanin pathway.

Additional examples suggesting independent transitions from pigmented (purple, lavender, blue) flowers to white flowers occur within each of the clades 3, 4, 5A, and 5B. These putative transitions further support the notion of evolutionary lability of floral pigments within *Ipomoea*, and represent promising case studies for examining the molecular genetics of loss of function (loss of pigmentation) in the anthocyanin pathway (e.g., Tiffin et al. 1998). Specifically, clade 3 contains the white-flowered *I. arborescens*, which is recognized as belonging to series *Arborescentes*, a group of trees and shrubs with large white flowers (McPherson 1981; Austin 1997). Another species in clade 3, the Australian *Ipomoea saintro-nanensis* has large white salverform flowers (Johnson 1986; Austin et al. 1993). Even though the *Batatas* species (clade 4) have mostly lavender corollas, white-flowered species are also present within this group as represented by *I. lacunosa*. In addition, clades 5A and 5B both contain white-flowered representatives, *I. argillicola* and *I. pandurata*, respectively. Future analyses of flower color transitions will require broader sampling among this complex group and careful attention to the assumptions made in reconstructing ancestral character states (see Schultz et al. 1996; Cunningham et al. 1998).

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