Phylogeny of the Cyphomandra clade of the genus *Solanum* (Solanaceae) based on ITS sequence data

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About 13 major clades can be recognized within the genus *Solanum* (Solanaceae) based on chloroplast DNA sequence data. One of these is the Cyphomandra clade, which includes about 50 neotropical species. These have traditionally been placed into two or three sections: *S.* section *Pachyphylla* (formerly recognized as the genus *Cyphomandra*), *S.* section *Cyphomandropsis*, and *S.* section *Glaucophyllum* (monotypic and sometimes placed in *S.* section *Cyphomandropsis*). Phylogenetic relationships among 61 accessions of 35 species of the Cyphomandra clade are investigated using sequence data from the nuclear ITS region analyzed by parsimony and Bayesian inference. The Cyphomandra clade forms a monophyletic group, but the ITS data are equivocal as to the monophyly of sections *Pachyphylla* and *Cyphomandropsis*. Four well-supported groups of species can be recognized within the Cyphomandra clade; these conform in part to species groups proposed on the basis of morphology. The distribution of self-incompatible and self-compatible breeding systems is mapped onto the ITS cladogram, and patterns of evolution of enlarged anther connectives, osmophores, and volatile composition are discussed in light of hypothesized phylogenetic relationships.

KEYWORDS: *Cyphomandra*, ITS, osmophores, self-incompatibility, Solanaceae, *Solanum*

INTRODUCTION

Solanum is one of the largest angiosperm genera, with approximately 1,500 species distributed worldwide. The genus includes important economic plants such as the tomato, potato, and eggplant, as well as a number of lesser-known cultivated species such as the pepino (*S. muricatum* Aiton), naranjilla (*S. quitoense* Lam.), cocona (*S. sessiliflorum* Dunal), and tree tomato (*S. betaceum* Cav.). Although its large size, morphological complexity, and largely tropical distribution have hindered taxonomic understanding of the genus, molecular approaches are proving useful in elucidating its overall phylogenetic structure. For instance, sequence data from the chloroplast *ndhF* gene as well as the nuclear ITS and *waxy* regions have identified at least twelve major clades within *Solanum* with high bootstrap support (Bohs & Olmstead, 2001; Bohs, 2005; Weese & Bohs, 2007). Several of these clades conform to infrageneric groups recognized by previous systematists on the basis of morphological similarity. Others, however, represent novel groupings that have not been suggested previously.

The Cyphomandra clade (sensu Bohs, 2005 and Weese & Bohs, 2007) is one of these well-supported major groups. It encompasses about 50 neotropical species that have been placed into three sections: *Solanum* section *Pachyphylla* (Dunal) Dunal, *S.* section *Cyphomandropsis* Bitter, and *S.* section *Glaucophyllum* A. Child. All taxa of the clade are woody shrubs or small trees and most have relatively small anther pores that do not ultimately open into longitudinal slits. The most consistent morphological synapomorphy of the group is the presence of very large chromosomes and large amounts of nuclear DNA, which have been found in all species of the clade investigated to date (Roe, 1967; Pringle & Murray, 1991a; Moscone, 1992; Bohs, 1994, 2001).

Solanum section *Pachyphylla* was formerly recognized as the genus *Cyphomandra* Sendtn. and includes the cultivated tree tomato, *S. betaceum*. Species of this section are found in mesic forests from Mexico to northern Argentina and southeastern Brazil. The morphological synapomorphy that defines section *Pachyphylla* is the presence of enlarged anther connectives (Sendtner, 1845; Fig. 1A–D) that function in at least some species as floral osmophores to attract male euglossine bees (Gracie, 1993; Sazima & al., 1993). In addition, many species of section *Pachyphylla* have a distinctive branching pattern and architecture that conforms to Prévost's model in the architectural scheme of Hallé & al. (1978; see Bohs, 1989, 1994 for details). A taxonomic monograph of the genus *Cyphomandra* was published in 1994 (Bohs, 1994). The 32 recognized species were placed into five provisional species groups, with three species not placed in any group. Molecular data subsequently showed that the genus *Cyphomandra* is nested within *Solanum* (Olmstead & Palmer, 1992, 1997; Spooner & al., 1993; Bohs & Olmstead, 1997, 1999), and all species of *Cyphomandra* were transferred to *Solanum* (Bohs, 1995). A new species, *S. maternum* Bohs, closely related to the tree tomato, was described in 1997 (Bohs &

Nelson, 1997), bringing the total number of described species in this section to 33.

Solanum section *Cyphomandropsis* was revised in 2001 (Bohs, 2001). Thirteen species were recognized in this treatment. These were placed into four provisional species groups, with two species not put into any group. The members of section *Cyphomandropsis* also have very large chromosomes, but they lack the discrete and elaborated anther connectives found in section *Pachyphylla* (Fig. 1E). In general, plants of section *Cyphomandropsis* exhibit Leeuwenberg's to Chamberlain's architectural model (Hallé & al., 1978; see Bohs, 2001 for details) in contrast to Prévost's model that is typical of section *Pachyphylla*. The fruit mesocarp in species of section *Cyphomandropsis* is usually scant and gummy, and eight of the thirteen species have thick, angled seeds and few seeds per fruit (Bohs, 2001). Taxa of section *Cyphomandropsis* occupy cooler, drier, and higher areas than those of section *Pachyphylla*, and the group as a whole is restricted to western and southern South America (Bohs, 2001).

Child (1984) and Child & Lester (2001) maintained the distinction between the genera *Cyphomandra* and *Solanum* (Table 1). In their latest treatment of the *Cyphomandra* group, Child & Lester (2001) included in *Cyphomandra* the 33 species recognized in Bohs' 1994 *Cyphomandra* monograph as well as all the taxa of section *Cyphomandropsis*. They recognize five sections within the genus *Cyphomandra*: *Cyphomandra* (33 species), *Cyphomandropsis* (11–12 species), *Allophylla* (3–4 species), *Rhynchantherum* (1 species), and *Cornigera* (1 species). [The exact number of species placed in each of Child's (1984, 1986) and Child & Lester's (2001) groups is ambiguous because of uncertainties in species delimitation and synonymy and uncertain sectional placement of several species.] Bohs (1989, 1990, 1994, 2005) and Bohs & Olmstead (1997) excluded section *Allophylla* from the Cyphomandra clade on the basis of morphological and molecular data. Bohs (1994, 2005) used morphological characters to exclude the monotypic *Solanum* section *Rhynchantherum* from the Cyphomandra clade. The monotypic *Cyphomandra* section *Cornigera* includes

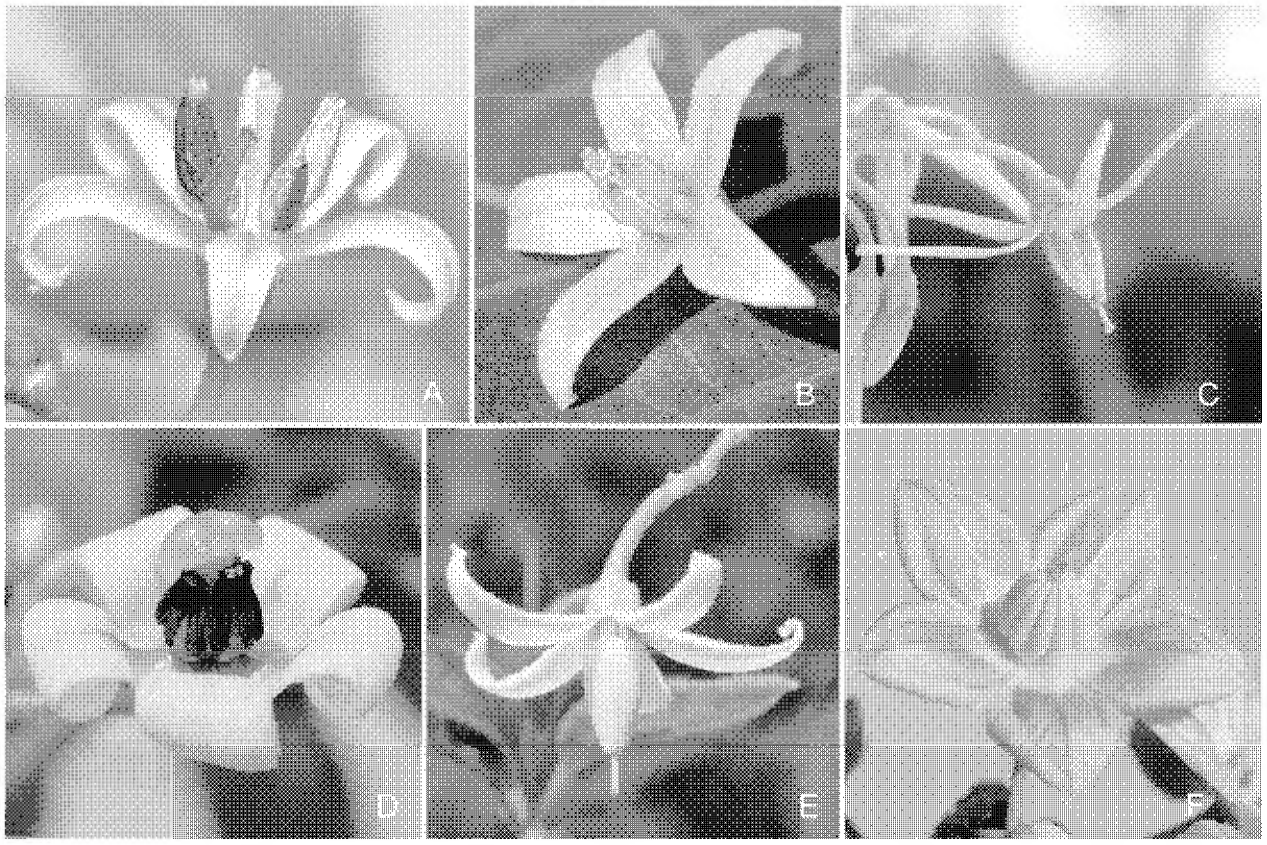


Fig. 1. Flowers of selected species of the Cyphomandra clade. A, *Solanum roseum* (sect. *Pachyphylla*); B, *Solanum maternum* (sect. *Pachyphylla*); C, *Solanum circinatum* (sect. *Pachyphylla*); D, *Solanum sciadostylis* (sect. *Pachyphylla*); E, *Solanum stuckertii* (sect. *Cyphomandropsis*); F, *Solanum glaucophyllum* (sect. *Cyphomandropsis* or *Glaucophyllum*). Enlarged anther connectives characteristic of sect. *Pachyphylla* are visible in A–D (A, connective brownish, anther thecae pink; B, connective orange, anther thecae white; C, connective light purple, anther thecae white; D, connective tan, anther thecae purple).

Table 1. Comparison of classification schemes for taxa of the Cyphomandra clade.

Child (1984, 1986)	
Cyphomandra Mart. ex Sendtn.	
C. section *Cyphomandra*	ca. 38 spp.
C. section *Ceratostemon* Miers	ca. 7 spp.
C. section *Cyphomandropsis* (Bitter) D'Arcy	ca. 15 spp.
C. section *Cornigera* A. Child	2 spp.
C. section *Allophylla* A. Child	3 spp.
C. section *Rhynchantherum* (Bitter) A. Child	1 sp.
Incertae sedis	5 spp.
Solanum section *Glaucophyllum* A. Child	1 sp.
Child & Lester (2001)	
Cyphomandra Mart. ex Sendtn.	
C. section *Cyphomandra*	33 spp.
C. section *Cyphomandropsis* (Bitter) D'Arcy	11–12 spp.
C. section *Cornigera* A. Child	1 sp.
C. section *Allophylla* A. Child	3–4 spp.
C. section *Rhynchantherum* (Bitter) A. Child	1 sp.
Solanum section *Glaucophyllum* A. Child	1 sp.
Bohs (1994)	
Cyphomandra Mart. ex Sendtn. included *C.* section *Cyphomandra* and *C.* section *Ceratostemon* (Miers) A. Child	32 spp.
Solanum section *Cyphomandropsis* Bitter included *C.* section *Cornigera* A. Child	12 spp.
Excluded from *Cyphomandra* and *Solanum* section *Cyphomandropsis*:	
C. section *Allophylla* A. Child = *S.* section *Allophyllum* (A. Child) Bohs	3 spp.
C. section *Rhynchantherum* (Bitter) A. Child = *S.* section *Rhynchantherum* Bitter	1 sp.
Not treated:	
S. glaucophyllum Desf.	
Bohs (2001)	
Solanum section *Pachyphylla* (Dunal) Dunal included all of genus *Cyphomandra* sensu Bohs (1994)	33 spp.
Solanum section *Cyphomandropsis* Bitter included *C.* section *Cornigera* A. Child and *S.* section *Glaucophyllum* A. Child	13 spp.

only the Brazilian species *C. cornigera* Dunal (Child, 1984). This species was included in section *Cyphomandropsis* by Bohs (2001) under the name *S. pelagicum* Bohs.

Child (1986) created the monotypic *Solanum* section *Glaucophyllum* to accommodate the single species *S. glaucophyllum* Desf. Its glaucous foliage, lavender rotate-stellate corollas, and purple-black fruits are distinctive with respect to other species in sections *Pachyphylla* and *Cyphomandropsis* (Fig. 1F). The majority of previous workers included *S. glaucophyllum* in section *Cyphomandropsis* and *S. glaucophyllum* has the large chromosomes diagnostic for the Cyphomandra clade (Moscone, 1992). However, Child (1986), Dottori (1995), Mansilla & al. (1999), Child & Lester (2001), and Hunziker (2001) excluded it from section *Cyphomandropsis* on morphological and anatomical grounds and considered it to be unrelated to members of the Cyphomandra clade.

Aside from these morphological studies, several species of sections *Pachyphylla*, *Cyphomandropsis*, and *Glaucophyllum* have been used in molecular phylogenetic analyses aimed at clarifying relationships and major clades in the genus *Solanum* and family Solanaceae. *Solanum betaceum* (sect. *Pachyphylla*) and *S. luteoalbum* (sect. *Cyphomandropsis*) were included in phylogenetic studies using chloroplast DNA restriction sites and cpDNA sequence data (Olmstead & Palmer, 1992; Bohs & Olmstead, 1997; Olmstead & al., 1999), and the two species form a clade with strong support. In addition to these two taxa, *S. glaucophyllum* (sect. *Glaucophyllum*) was included in an analysis of *Solanum* and related genera based on chloroplast *ndhF* and nuclear ITS sequence data (Bohs & Olmstead, 2001); the three taxa form a strongly supported clade in analyses of separate and combined datasets. In Bohs (2005), *S. diploconos* (sect. *Pachyphylla*) was included along with *S. betaceum*, *S. luteoalbum*, and *S. glaucophyllum* in a genus-wide analysis of chloroplast *ndhF* sequence data, and all four taxa form a well supported clade. *Solanum betaceum*, *S. luteoalbum*, and *S. glaucophyllum* form a polytomy with bootstrap support of 60%, and *S. diploconos* is sister to this clade. This Cyphomandra clade is part of a larger polytomy within *Solanum* such that the sister group to the Cyphomandra clade cannot be unequivocally identified. Olmstead & Palmer (1997) sampled five taxa from the Cyphomandra clade in their cpDNA restriction site analyses. These were *S. betaceum*, *S. circinatum*, *S. corymbiflorum*, and *S. diploconos* from section *Pachyphylla* and *S. luteoalbum* from section *Cyphomandropsis*. All five taxa form a clade with 88% bootstrap support. *Solanum luteoalbum* appears to be nested within section *Pachyphylla*, calling the monophyly of sect. *Pachyphylla* into question. Once again, the Cyphomandra clade appears in a relatively isolated position within the genus *Solanum* and its closest relatives are unclear.

The motivation for the current study was to increase sampling in the Cyphomandra clade in order to examine phylogenetic relationships among species of the group using molecular data. Monophyletic groups identified with these data are noted and these groups are compared with the sections and provisional species groups set up by Bohs (1994, 2001) in systematic treatments of the Cyphomandra clade based on morphology. Where possible, comparison is also made with the sectional classifications of Child (1984) and Child & Lester (2001). These data allow the relationships of *S. glaucophyllum* and the monophyly of *Solanum* sections *Pachyphylla* and *Cyphomandropsis* to be examined. Three species of section *Allophylla* are included to clarify whether they form a monophyletic group distinct from the Cyphomandra clade.

MATERIALS AND METHODS

Eighty-seven ITS sequences are analyzed here for 60 species of *Solanum*, including 61 accessions of 35 species from the Cyphomandra clade sensu Bohs (2005) and Weese & Bohs (2007). These represent 26 species from *Solanum* section *Pachyphylla* and 9 species of section *Cyphomandropsis*, including *S. glaucophyllum*. Outgroup taxa were chosen on the basis of previously published results of Bohs & Olmstead (2001) and Bohs (2005). These include representatives of the Leptostemonum, Brevantherum, Geminata, and Wendlandii/Allophyllum clades sensu Bohs (2005) that were found to be possible sister groups to the Cyphomandra clade in the analysis of Bohs (2005), as well as six examples of more distantly related outgroups occupying relatively basal positions within *Solanum*. Provenance and voucher information are given in the Appendix.

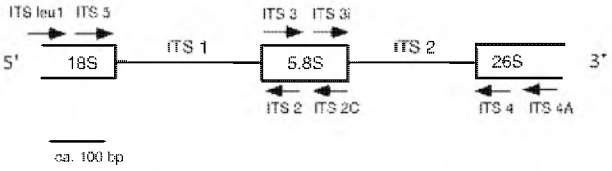
DNA was extracted from fresh or silica-dried leaves or from herbarium specimens using the modified CTAB technique of Doyle & Doyle (1987) or a microextraction protocol that substituted QiaQuick columns and buffer (Qiagen, Inc.) for the isopropanol precipitation step in the CTAB procedure. Samples extracted with the modified CTAB method were purified using cesium chloride density gradient centrifugation or a phenol-chloroform cleanup. Amplification of the ITS region, including the ITS 1 and ITS 2 transcribed spacers and the 5.8S coding region was achieved by one of two methods. For DNA samples from fresh or silica-dried leaves, both ITS regions were amplified in one step using primers ITS leu1 and ITS 4 (Fig. 2) and the PCR program given in Bohs & Olmstead (2001). DNA extracted from herbarium specimens generally did not amplify or sequence well with this procedure. To circumvent this problem, DNA from herbarium extractions were amplified in two halves using primers designed by M. Whitson (Duke University). Amplification primers were ITS leu1 and ITS 2C for the 5′ end of the region and ITS 4A and ITS 3 for the 3′ end (Fig. 2). Primers ITS 5, ITS 2, ITS 3i, and ITS 4 were used for sequencing (Fig. 2). Accessions amplified in two halves from herbarium material are noted in the Appendix. PCR products were cleaned with QiaQuick spin columns and sequenced on an ABI automated sequencer. Sequences were edited and contigs assembled using Sequencher (Gene Codes Corp.) and manual alignments were performed using Se-Al (Rambaut, 1996). All new sequences generated in this study were deposited in GenBank and the aligned dataset and representative phylogenetic trees were submitted to TreeBASE (study accession number S1714, matrix accession number M3102).

Initial parsimony analyses used the parsimony ratchet technique (Nixon, 1999) in concert with PAUP* 4.0b10 (Swofford, 2002) as implemented by PAUPRat (Sikes & Lewis, 2001). Five replicate searches of 200 iterations each were performed using the default PAUPRat parameters. The shortest trees from all iterations were saved and combined into a consensus tree. A subset of the shortest trees found by PAUPRat was then used as starting trees for a heuristic PAUP* search using the TBR and MulTrees options and equal weights for all characters and character state changes. 18,800 equally parsimonious trees were saved from this analysis before the memory capacity of the computer was exceeded, and a strict consensus was constructed from these 18,800 trees.

A parsimony bootstrap analysis was conducted using 500 pseudoreplicates and the heuristic search option with random addition, TBR, and MulTrees, Maxtrees set to 1,000, and rearrangements limited to 1,000,000 per replicate.

The best fit model of sequence evolution was determined using Modeltest 3.06 (Posada & Crandall, 1998). This model (TIM + I + G), which assumes unequal nucleotide frequencies, four substitution types, some invariant sites, and variable sites conforming to a gamma distribution, was then used to analyze the data by Bayesian inference using the program MrBayes 2.01 (Huelsenbeck & Ronquist, 2001) with the following settings: nst = 6, rates = gamma, ngen = 1,500,000, printfreq = 1,000, samplefreq = 100, nchains = 4, basefreq = estimate, ncat = 4.

Constraint trees were used to test the hypothesis of monophyly of *Solanum* sections *Pachyphylla* and *Cyphomandropsis*. A tree was constructed where species of each of the two sections (sensu Bohs, 1994, 2001) were constrained to monophyly. *Solanum fallax* was constrained to the Pachyphylla clade and *S. glaucophyllum* was constrained to the Cyphomandropsis clade. A parsimony analysis was performed using the parameters described above. A Shimodaira-Hasegawa (S-H) test (Shimodaira & Hasegawa, 1999) was performed in PAUP* which uses likelihood ratio tests to examine whether the constrained trees differed signifi-



ITS leu 1: GTC CAC TGA ACC TTA TCA TTT AG
ITS 5: GGA AGG AGA AGT CGT AAC AAG G
ITS 2: GCT GCG TTC TTC ATC GAT GC
ITS 2C: TGC GTT CAA AGA CTC GAT
ITS 3: GCA TCG ATG AAG AAC GCA GC
ITS 3i: AAT GCG ATA CTT GGT GTG AA
ITS 4: TCC TCC GCT TAT TGA TAT GC
ITS 4A: GGA ATC CTT GTA AGT TTC

Fig. 2. Location and sequences of primers used in amplification and sequencing ITS in *Solanum*. All primer sequences are written 5′ to 3′.

cantly from the unconstrained topology. One randomly chosen most parsimonious tree from the unconstrained analysis was compared to 20 randomly chosen trees from the constrained analysis. Default settings (RELL, 1,000 bootstrap replicates) were used.

RESULTS

In this study, new ITS sequences were generated for 65 accessions; the remainder were obtained from GenBank. Complete ITS sequences were obtained for all the taxa listed in the Appendix with the following exceptions. In nine cases readable sequence could not be obtained for a region in ITS 1 between positions 74 and 100 in the aligned sequence data matrix. The affected accessions are the following with the inferred number of missing nucleotides in parentheses: *S. circinatum* 2301 (23), *S. circinatum* 2442 (11), *S. circinatum* 2532 (17), *S. circinatum* 2542 (9), *S. circinatum* 4982 (25), *S. occultum* (13), *S. proteanthum* (22), *S. sibundoyense* (9), and *S. tegore* (19).

The total length of the aligned dataset including ITS 1, ITS 2 and the 5.8S coding region was 676 nucleotides, of which 85 represented indels. Of these characters, 280 were variable and 195 of these were parsimony-informative. Of the 1,000 iterations, PAUPRat found 217 shortest trees of 941 steps with a consistency index (excluding uninformative characters) of 0.380 and an retention index of 0.723. 18,800 trees of 941 steps were saved from the PAUP* heuristic search (Fig. 3). The strict consensus of the 217 PAUPRat trees and the 18,800 PAUP heuristic search trees were identical except that the PAUPRat consensus tree resolved two additional nodes within the *S. betaceum* clade (see Fig. 4).

A plot of the posterior probabilities of the 1,500 trees saved from the Bayesian analysis revealed that stationarity was reached after the first 10,000 generations, so the first 100 trees in the dataset were discarded. In general, the parsimony and Bayesian topologies were congruent, and branches with high bootstrap support also had high Bayesian posterior probabilities (Fig. 4). However, the Bayesian analysis resolved a few clades that received low bootstrap support (Fig. 4). The parsimony and Bayesian analysis mainly differed in the position of the three taxa of *Solanum* section *Allophyllum* (see below).

All species of the Cyphomandra clade (sensu Bohs 1994, 2001, 2005) form a monophyletic group (Fig. 3), with a posterior probability (PP) of 100% and bootstrap support of 59% (Fig. 4). Branch lengths were relatively short in the Cyphomandra clade compared to the outgroups (Fig. 3). *Solanum allophyllum* lies outside the Cyphomandra clade on a very long branch. However, *S. allophyllum* does not form a monophyletic group with other species of *Solanum* section *Allophyllum* (*S. mapiriense*, *S. morellifolium*), which emerge together as sister taxa in the parsimony analysis (Figs. 3, 4) and as a grade in the Bayesian analysis (Fig. 4). The Cyphomandra clade is nested within a larger monophyletic group with a number of spiny and non-spiny *Solanum* species, but no groups clearly emerge as sister to the Cyphomandra clade. *Solanum glaucophyllum* falls within the Cyphomandra clade.

Within the Cyphomandra clade, sections *Pachyphylla* and *Cyphomandropsis* do not emerge as monophyletic groups. Constraining each of the sections to monophyly results in trees six steps longer than the unconstrained trees. However, comparison of constrained vs. unconstrained trees using the S-H test showed no significant difference between the likelihoods of the constrained and unconstrained topologies (p values ranged from 0.354 to 0.562), indicating that the ITS data alone do not rule out the possibility of monophyly of the two sections.

Four well-supported groups (bootstrap values 63%–99%; PP 94%–100%) can be discerned within the Cyphomandra clade. The *S. betaceum* clade, composed of all accessions of *S. betaceum*, *S. maternum*, *S. roseum*, and *S. unilobum*, forms a monophyletic group with a posterior probability of 94% and 91% bootstrap support. The *S. circinatum* clade includes *S. circinatum*, *S. endopogon*, *S. occultum*, *S. proteanthum*, *S. sibundoyense*, *S. tegore*, and *S. tenuisetosum* (bootstrap 63%; PP 100%). The *S. luteoalbum* clade includes *S. luteoalbum*, *S. confusum*, *S. hibernum*, and *S. stuckertii* (bootstrap 99%; PP 100%). The *S. diploconos* clade includes the Brazilian taxa *S. corymbiflorum*, *S. diploconos*, *S. latiflorum*, *S. pinetorum*, and *S. sciadostylis* as well as the Andean species *S. cacosmum* and *S. exiguum* (bootstrap 66%; PP 100%). The relationships of other taxa within the Cyphomandra clade are not well resolved. Several of the well-supported clades from the ITS data correspond with species groups designated by Bohs (1994, 2001) on the basis of morphological similarity, but others do not. Comparisons with morphological species groups are made in more detail below.

DISCUSSION

Monophyly of the Cyphomandra clade. — The ITS data support the monophyly of a group that includes *S. glaucophyllum* plus the species sampled from *Solanum* sections *Pachyphylla* and *Cyphomandropsis* sensu Bohs (1994, 2001). This group corresponds to the Cyphomandra clade of Bohs (2005) and Weese & Bohs (2007). Thus, these data do not support Child's exclusion of *S. glaucophyllum* from the Cyphomandra clade and are consistent with the morphological and cytological evidence that ally *S. glaucophyllum* with sections *Pachyphylla* and *Cyphomandropsis* (Morton, 1976; Moscone, 1992; Bohs &

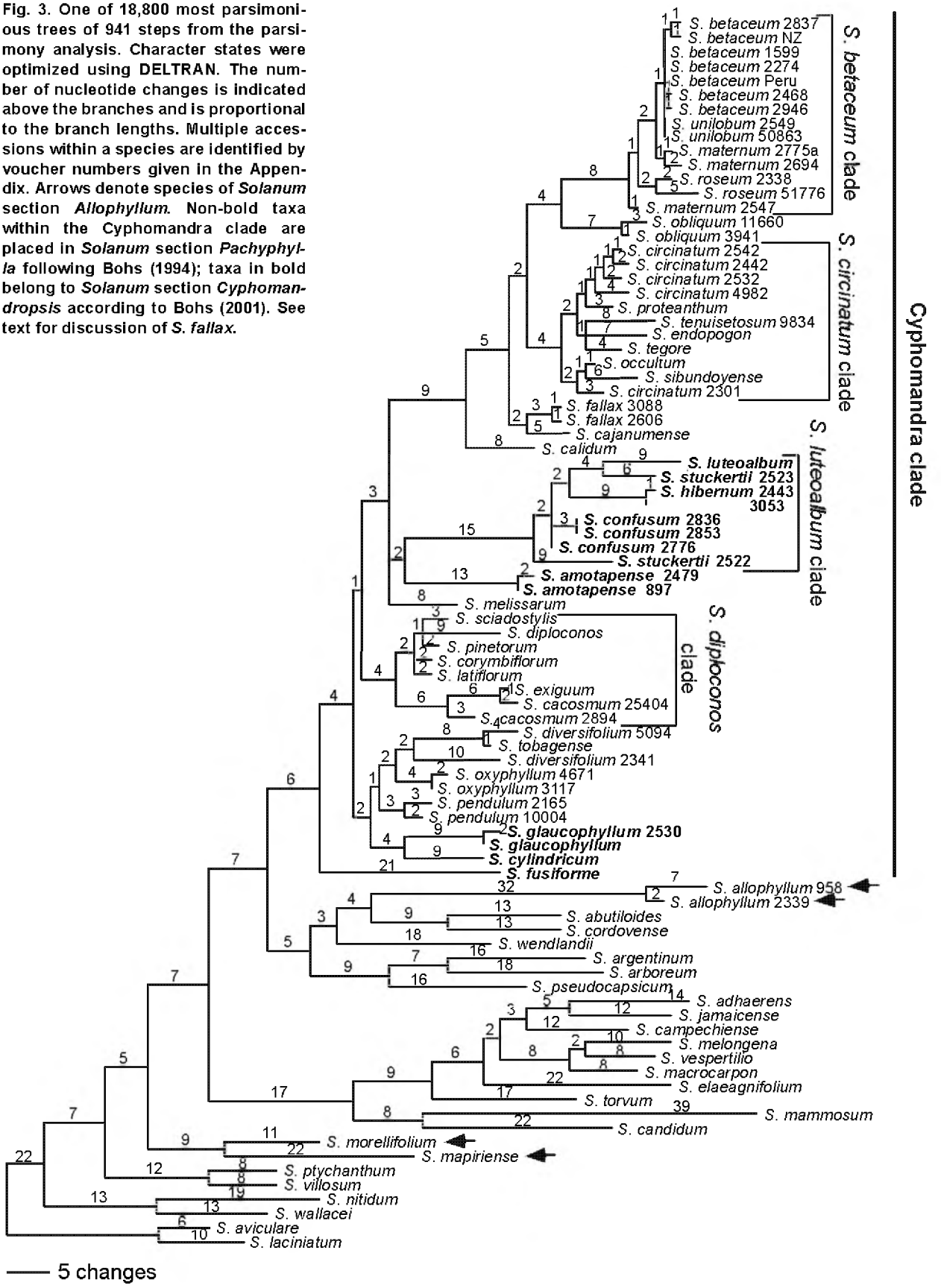


Fig. 3. One of 18,800 most parsimonious trees of 941 steps from the parsimony analysis. Character states were optimized using DELTRAN. The number of nucleotide changes is indicated above the branches and is proportional to the branch lengths. Multiple accessions within a species are identified by voucher numbers given in the Appendix. Arrows denote species of *Solanum* section *Allophyllum*. Non-bold taxa within the Cyphomandra clade are placed in *Solanum* section *Pachyphylla* following Bohs (1994); taxa in bold belong to *Solanum* section *Cyphomandropsis* according to Bohs (2001). See text for discussion of *S. fallax*.

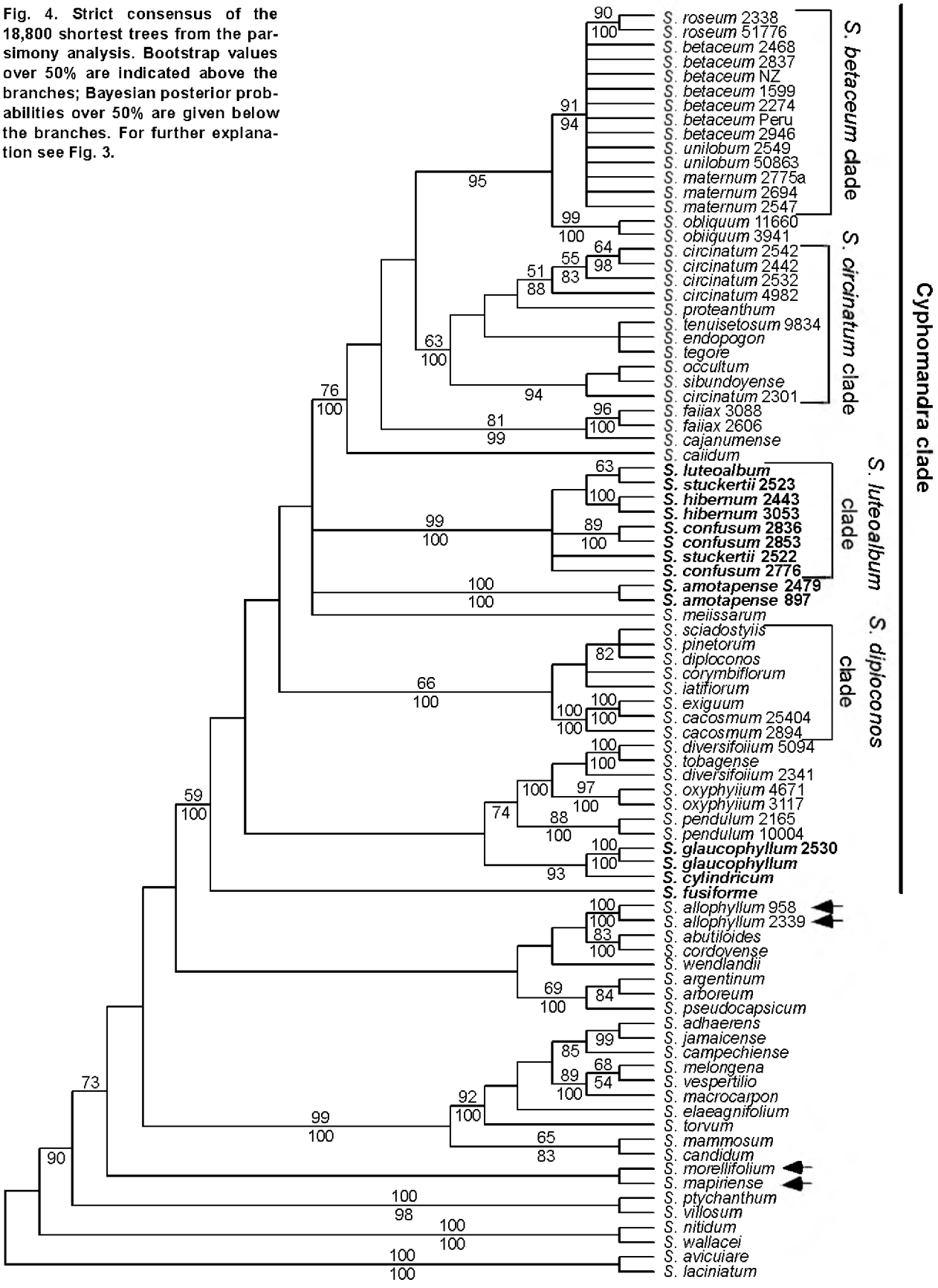


Fig. 4. Strict consensus of the 18,800 shortest trees from the parsimony analysis. Bootstrap values over 50% are indicated above the branches; Bayesian posterior probabilities over 50% are given below the branches. For further explanation see Fig. 3.

Olmstead, 2001; Bohs, 2005). Furthermore, the ITS data do not support the inclusion of the species of section *Allophyllum* (*S. allophyllum*, *S. mapiriense*, *S. morellifolium*) in the Cyphomandra clade, contrary to Child's scheme (Child, 1984). This finding is also consistent with morphological and cytological evidence (Bohs, 1989, 1990). The three species of section *Allophyllum* do not form a monophyletic group in the ITS trees, with *S. allophyllum* emerging in a clade with *S. abutiloides*, *S. cordovense*, *S. wendlandii*, *S. arboreum*, *S. pseudocapsicum*, and *S. argentinum*; *S. mapiriense* and *S. morellifolium* form a separate, distinct group. However, there is little support for the grouping of *S. allophyllum* with the six taxa mentioned above; likewise, the grouping of *S. mapiriense* and *S. morellifolium* has low support. Additional data should be sought to clarify the relationships of the members of section *Allophyllum*.

Sister group to Cyphomandra clade. — The ITS data do not definitively resolve the possible sister group(s) to the Cyphomandra clade. Although the Cyphomandra clade is sister to a group including *S. argentinum*, *S. pseudocapsicum*, *S. arboreum*, *S. wendlandii*, *S. allophyllum*, *S. abutiloides*, and *S. cordovense* in all of the most parsimonious ITS trees (Fig. 4), this association is not well supported. Thus, the ITS results are similar to those from *ndhF* sequence data (Bohs, 2005) in identifying the Cyphomandra clade as a distinct but isolated monophyletic group within *Solanum*.

Monophyly of sections *Pachyphylla* and *Cyphomandropsis*. — The ITS data reveal four discrete, fairly well-supported clades within the Cyphomandra clade. These largely conform to the informal species groups in sections *Pachyphylla* and *Cyphomandropsis* inferred from morphological characters (Bohs, 1994, 2001). Although the species of each section largely cluster together, the sections do not form monophyletic groups in the most parsimonious trees. Constraining the sections to monophyly results in trees that are six steps longer than the unconstrained trees. However, a S-H test showed no significant differences between the constrained and unconstrained trees, indicating that the ITS data are ambiguous in support of the monophyly of the two sections.

Comparisons to morphological species groups. — Three of the four well-supported clades in the ITS analyses conform to species groups proposed by Bohs (1994) for section *Pachyphylla*. The first is comprised of the species *S. betaceum*, *S. unilobum*, *S. maternum*, and *S. roseum*. *Solanum obliquum* is a part of this group in the strict consensus tree (Fig. 4), but with low bootstrap support. All five of these taxa form part of the larger *S. obliquum* species group of Bohs (1994). They are morphologically homogeneous in possessing simple, cordate, and often subcoriaceous leaf blades; stellate, coriaceous corollas; short and broad anthers with expanded abaxial anther connectives not extending below the anther thecae (Fig. 1A, B); relatively thick, often distally dilated styles; and glabrous, yellow to reddish fleshy fruits. These five species are native to western South America. *Solanum obliquum* is found in Colombia, Peru, and northwestern Brazil (Estados Acre and Amazonas). *Solanum maternum*, *S. roseum*, and *S. unilobum* are endemic to Bolivia, and *S. betaceum* is probably native to southern Bolivia and northwestern Argentina (Bohs, unpub.). Bohs (1994) also includes *S. rojasianum* (Standl. & Steyerm.) Bohs, *S. paralum* Bohs, and *S. sycocarpum* Mart. & Sendtn. in the *S. obliquum* species group, but ITS sequences of these species were not obtained in the present study. The Central American species *S. rojasianum* is morphologically similar to the five taxa of this group included in the molecular analyses and is expected to belong to this clade. Although Bohs (1994) placed *Solanum paralum* and *S. sycocarpum* in the *S. obliquum* species group due to their coriaceous leaves and corollas, short broad anthers, and distally expanded styles, these two species diverge from the other members of the *S. obliquum* group in their lobed or compound leaves, apically prolonged anther connectives, and seaside habitats in southeastern Brazil. Perhaps these two species do not belong with the other members of the *S. obliquum* group, but the lack of molecular data does not allow a firm conclusion to be drawn.

The close relationship among *S. betaceum*, *S. maternum*, *S. roseum*, and *S. unilobum* seen in the ITS trees agrees not only with their morphological similarity but also with crossing data. All four species formed fertile hybrids in at least some crossing combinations in greenhouse pollinations (Bohs, 1991; Bohs & Nelson, 1997). There is little to no ITS sequence divergence among these four taxa (Figs. 3, 4); pairwise sequence divergence calculated by the Kimura 2-parameter model ranged from 0% to 1.9%. However, these four species are ecologically distinct, their ranges are largely allopatric, and there is no evidence from field observations or herbarium specimens of natural hybrids among them. The ITS data convincingly show that *S. maternum*, *S. roseum*, and *S. unilobum* are likely the closest wild relatives of the cultivated tree tomato, *S. betaceum*.

The place of origin and wild status of *S. betaceum*, the tree tomato or tamarillo, has been unclear. Grown worldwide in suitable climates, this species has often been described as being known only from cultivation. Recent field investigations in southern Bolivia and northwestern Argentina have located putatively wild populations of *S. betaceum* (Bohs, unpub.), confirming reports of previous botanists (Brücher, 1968, 1977; J. Solomon, pers. comm.; E. Zardini, pers. comm.). Sequences of individuals from these wild populations (*S. betaceum* 2837 & 2946) were included along with cultivated *S. betaceum* accessions from Bolivia (2468), Colombia (1599), Ecuador

(2274), Peru (Peru), and New Zealand (NZ) in the ITS analyses (Figs. 3 & 4). The ITS sequences of all *S. betaceum* samples, both wild and cultivated, were nearly identical (Kimura 2-parameter distances range from 0% to 0.48%) and these, in turn, were nearly identical to accessions of the exclusively wild species *S. maternum* (0%–0.96%) and *S. unilobum* (0%–0.32%). The lack of sequence divergence in ITS among species of the *S. betaceum* clade and between wild and cultivated accessions of *S. betaceum* imply that the tree tomato may have diverged rather recently from its wild relatives.

Another clade inferred from the ITS data corresponds largely to the *S. circinatum* species group of Bohs (1994). Of the eleven species placed in this group by Bohs (1994), ten were sampled for ITS. Six of these taxa (*S. circinatum*, *S. proteanthum*, *S. endopogon*, *S. tegore*, *S. occultum*, *S. sibundoyense*) form a clade in the ITS analysis. *Solanum tenuisetosum*, hypothesized to belong to the *S. pendulum* group in Bohs (1994), also emerges here. Morphological characters shared by the seven taxa of this clade include simple to pinnately lobed leaves; often very elongated inflorescences; purplish or green, usually stellate corollas; long narrow anthers (Fig. 1C); long cylindrical styles with usually truncate to capitate stigmas; and fruits with large flattened seeds and prominent stone cell aggregates. The species of this clade are mainly distributed in western South America, with *S. circinatum* reaching Central America and southern Mexico and *S. endopogon*, *S. proteanthum*, and *S. tegore* extending into the Amazon basin and the Guianas. *Solanum cacosmum*, *S. oxyphyllum*, and *S. tobagense*, postulated by Bohs (1994) to belong to this species group on the basis of morphological characters, are more closely related to other species in the ITS analyses.

Solanum circinatum is the most widely distributed and one of the most morphologically variable species of *Solanum* section *Pachyphylla*, and five accessions of this species were included in the ITS analyses. Four of these accessions correspond to *S. circinatum* subsp. *circinatum* (*Cyphomandra hartwegii* (Miers) Walp. subsp. *hartwegii* in Bohs, 1994) and these form a monophyletic group. Accession *Bohs 2301* from Dept. Huila, Colombia was grown from seeds of the type collection of *Cyphomandra hartwegii* subsp. *ramosa* Bohs (transfer of infraspecific epithet to *Solanum* not yet made). Its ITS sequence does not cluster with the other *S. circinatum* accessions, suggesting that this taxon might best be considered a separate species rather than a subspecies of *S. circinatum*.

Associated with the *S. betaceum* and *S. circinatum* clades are three taxa, *S. cajanumense*, *S. fallax*, and *S. calidum*, whose affinities have been uncertain. *Solanum cajanumense* was doubtfully placed in the *S. circinatum* species group in Bohs (1994), but it emerges as sister to *S. fallax* in the ITS trees. *Solanum fallax* has been variously treated as a member of *Solanum* section *Pachyphylla* (Bohs, 1994) or of section *Cyphomandropsis* (Bohs, 2001); the ITS data suggest that it is more closely related to species of section *Pachyphylla* and that it is sister to *S. cajanumense*. *Solanum cajanumense* and *S. fallax* occupy similar ranges in western parts of Colombia and Ecuador. *Solanum cajanumense* is variable with respect to leaf shape, pubescence, and flower and fruit characters, but in general both taxa have large cordate leaves, stellate corollas, anther connectives not prolonged dorsally beyond the bases of the anther thecae, and cylindrical styles with truncate stigmas. *Solanum fallax* and many individuals of *S. cajanumense* have pubescent fruits. The close association of *S. fallax* and *S. cajanumense* is unexpected, however, and should be tested with data from other genes.

Solanum calidum appears as sister to the rest of the taxa in the large *S. betaceum*/*S. circinatum*/*S. fallax* clade. The affinities of *Solanum calidum* have been problematical and Bohs (1994) did not place it in a species group. It is native to western South America and has pubescent fruits similar to those of *S. fallax* and many *S. cajanumense* collections. In all, this more inclusive clade consists of species mainly distributed in Andean South America and all originally placed in *Solanum* section *Pachyphylla*. Except for *S. fallax*, these species have the expanded anther connectives typical of the section, but non-molecular synapomorphies of this clade are not immediately apparent.

Two other clades with bootstrap support over 50% emerge from the ITS analyses. The first consists of species previously included in *Solanum* section *Pachyphylla* and designated as the *S. diploconos* clade in Figs. 3 and 4. This clade includes taxa placed in four different species groups in Bohs (1994) and consists of two subclades. The first includes *S. diploconos*, *S. pinetorum*, *S. latiflorum*, *S. sciadostylis*, and *S. corymbiflorum*, species native to southeastern Brazil and adjacent parts of Argentina and Paraguay. These taxa were originally split into two species groups in Bohs (1994), but this division is apparently unwarranted. Sister to the Brazilian species are *S. cacosmum* and *S. exiguum*, two taxa from the eastern Andean slopes and Amazon basin. These two species are morphologically unlike the species from southeastern Brazil, but are similar to each other in their stellate corollas and pubescent fruits. However, *S. cacosmum* is morphologically most similar to *S. tegore* of the *S. circinatum* clade, whereas *S. exiguum* was allied by Bohs (1994) with *S. pendulum* because of its dense tomentum on the abaxial corolla surfaces. Neither of these relationships are supported by the ITS data.

The last well-supported clade to emerge from these analyses includes four species of *Solanum* section *Cyphomandropsis* from two different species groups (Bohs, 2001). *Solanum stuckertii*, *S. hibernum*, and *S. luteoalbum* are morphologically cohesive and were previously placed in the *S. luteoalbum* species group. According to the ITS

data, *S. confusum* also belongs here, although its stellate-campanulate corollas and relatively broad anthers with roughened dorsal papillae were thought to ally it with the *S. glaucophyllum* species group (Bohs, 2001). Members of this clade are found on the Andean slopes from Ecuador to Argentina.

Solanum amotapense is associated with the *S. luteoalbum* clade in some of the most parsimonious trees (Fig. 3), but this relationship does not appear in the strict consensus and has low bootstrap support (Fig. 4). *Solanum amotapense*, like members of the *S. luteoalbum* clade, is an Andean species traditionally placed in section *Cyphomandropsis*. Bohs (2001) proposed that the cordate leaves and swollen calyx tube of *S. amotapense* allied it with *S. fallax*, but the ITS data argue against this view. Members of the *S. luteoalbum* group and *S. amotapense* have very large angled seeds, but this character is also found in *S. glaucophyllum*, which is far removed from both groups in the ITS trees.

The ITS data do not resolve the affinities of *S. fusiforme* or *S. mellisarum*, both of which appear as isolated taxa in the strict consensus tree (Fig. 4). Both *S. fusiforme* and *S. melissarum* are enigmatic taxa whose affinities are not well understood on morphological grounds as well. *Solanum melissarum* is found in southeastern Brazil from Santa Catarina north to the states of Bahia and Paraiba, whereas *S. fusiforme* occurs further to the west in the drainages of the Rios Uruguay and Paraná in Argentina, Paraguay, and adjacent parts of Brazil. These two species are morphologically dissimilar and have been considered to belong to different sections, with *S. melissarum* treated as a member of section *Pachyphylla* and *S. fusiforme* included in section *Cyphomandropsis*. Within these respective sections, each is morphologically anomalous and neither was placed in a species group. ITS data suggest that *S. fusiforme* is sister to the other species of the Cyphomandra clade as defined here. However, additional data and taxon sampling should be sought to clarify this relationship.

Another group of taxa comprised of *S. diversifolium*, *S. tobagense*, *S. oxyphyllum*, *S. pendulum*, *S. glaucophyllum*, and *S. cylindricum* form a clade in the strict consensus tree, but with little bootstrap support. Of these taxa, the first four have been included in section *Pachyphylla*, whereas *S. glaucophyllum* and *S. cylindricum* have been treated in section *Cyphomandropsis*. *Solanum glaucophyllum* and *S. cylindricum* are taxa of southeastern Brazil and adjacent areas of Argentina and Uruguay to Bolivia. In Bohs (2001), *S. cylindricum* was allied with *S. pelagicum* from coastal Brazil, whereas *S. glaucophyllum* formed part of a group with the Andean *S. confusum* and with *S. luridifuscescens* Bitter and *S. matadori* L.B. Sm. & Downs from southeastern Brazil. The ITS data indicate that *S. confusum* and *S. glaucophyllum* are not closely allied, but the relationships among *S. glaucophyllum* and the Brazilian taxa are less clear due to poor resolution in the ITS trees and lack of molecular data from *S. pelagicum*, *S. luridifuscescens*, and *S. matadori*.

The other part of this clade comprises *S. diversifolium*, *S. tobagense*, *S. oxyphyllum*, and *S. pendulum*, all included in section *Pachyphylla* and all from northern or western South America. *Solanum diversifolium*, *S. oxyphyllum*, and *S. pendulum* have pinnately compound trunk leaves, and Bohs (1994) placed *S. diversifolium* and *S. pendulum* together in a species group along with two other taxa, *S. exiguum* and *S. tenuisetosum*. However, the sister relationship of *S. diversifolium* and *S. tobagense* is the only group within this clade to receive high bootstrap support. *Solanum tobagense* was thought to belong to the *S. circinatum* species group by Bohs (1994) by virtue of its simple leaves, stellate corollas, and elongated anthers with narrow connectives, but the ITS data do not place *S. tobagense* within the *S. circinatum* clade. *Solanum diversifolium* has urceolate corollas, a unique character state in section *Pachyphylla*. Similarities between *S. diversifolium* and *S. tobagense* include elongated fruits with acute apices in most collections and overlapping ranges in northeastern Venezuela. However, they differ in many other characters. There is poor support for branches throughout this clade, so definitive ideas of relationships among these four taxa must await additional data.

Character evolution in the Cyphomandra clade. Breeding systems. — The distribution of self-incompatibility (SI) and self-compatibility (SC) has been investigated in 13 species of *Solanum* section *Pachyphylla* (Bohs, 1991, 1994; Soares & al., 1989; Pringle & Murray, 1991b; Bohs & Nelson, 1997; Bohs, unpubl. data) and six species of section *Cyphomandropsis* (Passarelli, 1999; Bohs, 2001). These breeding systems are mapped onto a simplified version of the parsimony strict consensus tree in Fig. 5. Breeding systems of the *Solanum* species outside the Cyphomandra clade were taken from the literature (summarized in Whalen & Anderson, 1981) or from personal observations from greenhouse-grown plants (Bohs, unpubl. data). Of the 19 species investigated from the Cyphomandra clade, only five were SC. Given the ITS strict consensus tree topology, there was one unambiguous change from SI to SC and up to five independent instances of the evolution of SC in taxa of the Cyphomandra clade. In no case did SI arise from within SC clades. Although this type of analysis gives an overview of general patterns in breeding systems, the true picture may change when breeding systems of more species in the clade are investigated. Self-incompatibility may be plesiomorphic in the Cyphomandra clade; however, the breeding system of the basal taxon in the clade, *S. fusiforme*, is unknown. Some reports should be reinvestigated (e.g., *S. cajanumense*, *S. obliquum*), and

in nearly all cases a single accession was investigated per species, obscuring any infraspecific polymorphisms in breeding system. Also, a caveat should be mentioned about the pattern of SI/SC among the *Solanum* species outside the Cyphomandra clade in Fig. 5: although the breeding systems of these taxa, where known, are exclusively SC, this is a sampling artifact. Self-incompatible taxa and clades are well-known in the non-spiny solanums (Whalen & Anderson, 1981), but those species were by chance not included in this study.

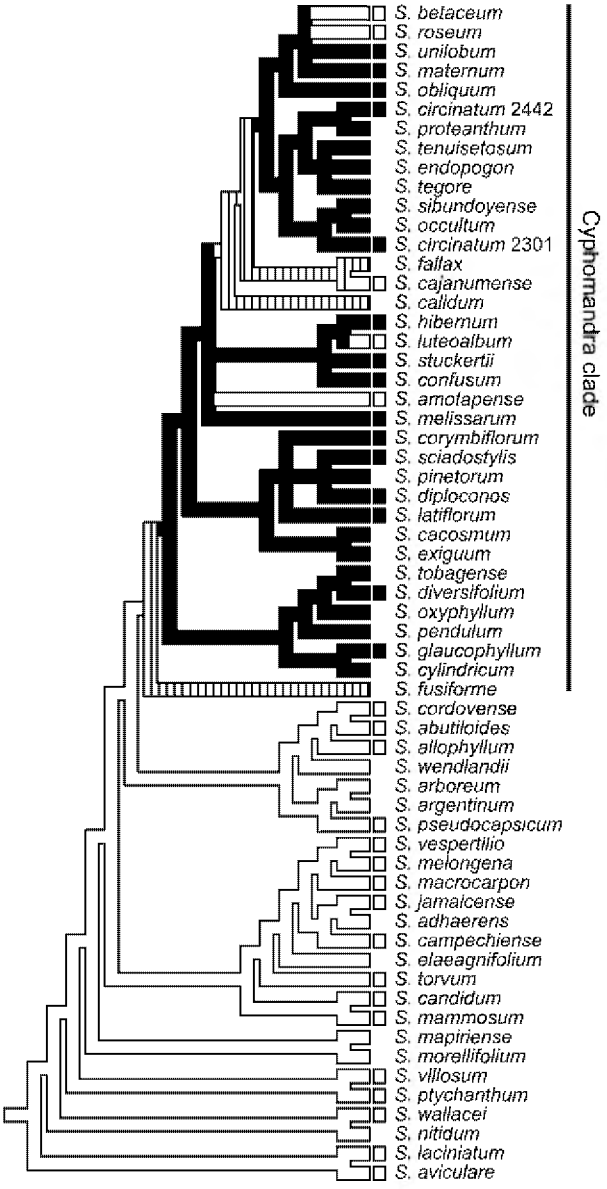


Fig. 5. Distribution of self-incompatible (SI, black branches) and self-compatible (SC, white branches) breeding systems mapped onto a simplified version of the strict consensus tree of Fig. 4. Squares at ends of branches denote taxa whose breeding systems have been investigated; striped branches: equivocal.

Anther connectives, osmophores, and volatiles. — The presence of a discrete, swollen anther connective is the morphological character that differentiates *Solanum* section *Pachyphylla* from all other sections of *Solanum* (Fig. 1A–D). In many, perhaps all, species that possess this structure, it functions as a floral osmophore that emits volatile compounds. In all species of section *Pachyphylla* that have been investigated thus far (summarized in Table 2), the volatiles attract male euglossine bees that gather the scents by brushing the surfaces of the anther connectives with their forelegs. These compounds are possibly used by the bees as precursors of sex pheromones, although their exact fate is unknown. In these taxa, the walls of the anther thecae are very thin and elastic and the thecae themselves are air-filled. When the bees come in contact with the thecae, even slight pressure causes pollen to be emitted from the anther pores in a process called the "bellows mechanism" by Sazima & al. (1993). In contrast, species of *Solanum* section *Cyphomandropsis* do not possess an enlarged anther connective (Fig. 1E–F) and apparently do not produce volatiles or attract scent-gathering bees (L. Passarelli, pers. comm.). Presumably these species are buzz pollinated by female bees, the common situation in most *Solanum* taxa. Thus far, section *Pachyphylla* is the only group in the Solanaceae known to exhibit the male euglossine syndrome. However, few species have been investigated from either section, either from the point of view of volatile production or insect visitors. It is hoped that the availability of a phylogeny for the Cyphomandra clade will stimulate further research in these areas. A summary of currently known information is given below, with suggestions on key taxa that may shed light on the evolution of the male euglossine syndrome in *Solanum*.

Volatiles and insect visitors have been most thoroughly studied in members of the *S. diploconos* and *S. circinatum* clades. In all cases where volatiles were detected, the species attracted male euglossine bees. Sazima & al. (1993) analyzed volatiles from *S. diploconos* and *S. sciadostylis* of the *S. diploconos* clade using gas chromatography (GC) of head space fragrance collections as well as hexane anther extracts. Soares & al. (1989) and Sazima & al. (1993) catalogued insect visitors to *S. diploconos*, *S. sciadostylis*, *S. latiflorum*, and *S. pinetorum* of the *S. diploconos* clade. The chemical compounds isolated from *S. diploconos* and *S. sciadostylis* were dominated by terpenes and aliphatic hydrocarbons, with benezenoids almost completely absent (Sazima & al., 1993). Floral visitors included male euglossines as well as pollen-gathering female non-euglossines, except in the case of *S. pinetorum*. Sazima & al. (1993) report no volatiles from this species and observed that it was visited exclusively by non-euglossine female bees. Though *S. pinetorum* has an expanded anther connective, it is not as prominent as in other members of the *S. diplo-*

conos clade. This species should be investigated more thoroughly to document whether it does not possess a connective osmophore. If so, this may indicate a reversal to buzz pollination by female bees within the *S. diploconos* clade, as suggested by Sazima & al. (1993).

Dressler (1979), Williams (1982), Sazima & al. (1993), Gracie (1993), and G. Gerlach (pers. comm.) investigated volatiles and insect visitors in *S. circinatum* and *S. endopogon* of the *S. circinatum* clade. These species emit volatiles and are visited by male euglossines. Benzenoids and monoterpenes dominated in their fragrances, whereas sesquiterpenes were absent. However, the analyses of Sazima & al. (1993) and Gerlach (pers. comm.) do not completely agree in their fragrance profiles (cf. the two profiles of *S. circinatum* in Table 2). Whether this reflects intraspecific variation among plants or is a result of differing methodologies for fragrance collection and analysis is unknown.

Solanum fallax is a curious case that deserves further attention from the perspective of pollination and anther morphology. The enlarged connective in this species is present but small, and *S. fallax* was treated as a member of *Solanum* section *Pachyphylla* by Bohs (1994; as *Cyphomandra hypomalaca* Bitter) and as *S.* section *Cyphomandropsis* by Bohs (2001). As it falls squarely with other members of section *Pachyphylla* on the ITS trees, perhaps this species has lost a functional osmophore as is speculated for *S. pinetorum*.

Two other areas of investigation are ripe for further study with relation to osmophores and pollination. First, nothing is known about volatile production or insect visitors in the *S. betaceum* clade, although this clade includes the economically important tree tomato (*S. betaceum*). Bumblebees and honeybees are frequent visitors to *S. betaceum* in New Zealand tree tomato orchards (Pringle

Table 2. Major fragrance compounds identified in species of *Solanum* section *Pachyphylla*.

Species	Major compounds	Reference
S. circinatum	benzyl alcohol 1,8-cineole benzyl acetate β-inene myrcene tricosane	Sazima & al., 1993
S. circinatum subsp. *circinatum*	1,8-cineole sabinene myrcene methyl salicylate β-pinene	R. Kaiser & G. Gerlach, pers. comm.
S. diploconos	myrcene β-pinene α-pinene limonene ipsdienol heneicosane tricosane	Sazima & al., 1993
S. diversifolium	trans-carvone epoxide terpinolene limonene carvone 1,8-cineole	R. Kaiser & G. Gerlach, pers. comm.
S. endopogon subsp. *guianense*	ocimene?	Gracie, 1993
S. melissarum	1,8-cineole (E)-ocimene scatole 2-methoxy-6-methyl-acetophenone[a]	R. Kaiser & G. Gerlach, pers. comm.
S. sciadostylis	trans-β-ocimene germacrene D 1,8-cineole heneicosane	Sazima & al., 1993

This is a selection of compounds identified in headspace trappings and/or hexane anther extracts. Consult original references for additional compounds and details of analyses. G. Gerlach's analyses were run by R. Kaiser, Givaudan Fragrance Research, Dübendorf, Switzerland.

[a]A new natural compound that will be published elsewere (R. Kaiser, pers. comm.).

& Murray, 1991b), but this is an artificial situation where the plants and the insects have been introduced. The native range of *S. betaceum* is thought to include Andean forests in southern Bolivia and northwestern Argentina (L. Bohs, unpub. data) where bumblebees are also native; however, there are no records of floral visitors to this species in South America. *Solanum betaceum* flowers have a strong odor and prominent anther connective (L. Bohs, pers. obs.), so presumably the male euglossine syndrome is operating in this species. Since members of the *S. betaceum* clade are phylogenetically distant from the *S. diploconos* and *S. circinatum* clades, it would be interesting to know if their volatiles are chemically distinct as well.

Finally, anther structure, odor production, and flower visitors should be more thoroughly documented in all members of *Solanum* section *Cyphomandropsis*. The conventional wisdom is that all members of this group lack osmophores and attract female pollen-gathering bees. Insect visitors have been reported from just two species of the section, *S. stuckertii* and *S. glaucophyllum*, and both were buzz-pollinated by female bees (L. Passarelli, pers. comm.). However, more detailed studies should be undertaken to confirm that these species do not emit volatiles and do not attract male euglossines. For instance, Sazima & al. (1993) report that *S. stuckertii* flowers have a papillose dorsal anther surface reminiscent of an osmophore and that the anther cone exudes a fruity, cinnamon-like, or cucumber-like odor. Because members of section *Cyphomandropsis* occur in at least two separate clades in the ITS strict consensus tree, they may represent independent instances of the pollen-reward buzz pollination syndrome: perhaps plesiomorphically without volatiles in the case of the relatively basal taxa *S. fusiforme*, *S. cylindricum*, and *S. glaucophyllum*, and reflecting a loss of osmophores in the *S. luteoalbum* clade and *S. amotapense*.

Because of their architectural and floral diversity as well as their unusual chromosomes and pollination syndromes, members of the Cyphomandra clade can serve as model organisms for the investigation of questions in plant development, genome evolution, and plant/animal interactions. The availability of a phylogenetic hypothesis of evolutionary relationships within the clade provides a starting point for the examination of these questions in a historical context and allows appropriate taxa to be chosen for comparative studies. Future phylogenetic studies can build on this groundwork by using other genes and more extensive taxonomic sampling. At the same time, additional observations of floral visitors and analyses of volatile emissions in taxa of the Cyphomandra clade are badly needed to understand the extent of the male euglossine perfume syndrome and its significance in maintaining species identity and promoting species diversification within the genus *Solanum*.

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Appendix. Taxa included in this study [sectional affiliation within *Solanum*].

Voucher (herbarium acronym), collection locality, ITS GenBank accession number.

S. abutiloides* (Griseb.) Bitter & Lillo** [*Brevantherum*], *Olmstead S-73* (WTU), BIRM S.0655, AF244716. ***S. adhaerens* Roem. & Schult.** [*Micracantha*], *Bohs 2473* (UT), Costa Rica, AF244723. ***S. allophyllum* (Miers) Standl.** [*Allophylla*], *Bohs 2339* (UT), Panama, AF244732; *Hammel & Grayum 19929* (MO), Costa Rica, AY523928[a]. ***S. amotapense* Svenson** [*Cyphomandropsis*], *Bohs 2479* (UT), BIRM S.0034, AY523869; *Whalen & Dillon 897* (BH), Peru, AY523870[a]. ***S. arboreum* Dunal** [*Geminata*], *Bohs 2521* (UT), Costa Rica, AF244719. ***S. argentinum* Bitter & Lillo** [*Holophylla*], *Bohs 2539* (UT), Argentina, AF244718. ***S. aviculare* G. Forst.** [*Archaesolanum*], no voucher, BIRM S.0809, AF244743. ***S. betaceum* Cav.** [*Pachyphylla*], no voucher, New Zealand (cultivated), AY523872; *Bohs 1599* (GH), Colombia (cultivated), AY523873; *Bohs 2274* (GH), Ecuador (cultivated), AY523874; no voucher, Peru (cultivated), AY523876; *Bohs 2837* (UT), Tarija, Bolivia (wild), AY523871; *Bohs 2468* (UT), Cochabamba, Bolivia (cultivated), AF244713; *Bohs 2946* (UT), Jujuy, Argentina (wild), AY523875. ***S. cacosmum* Bohs** [*Pachyphylla*], *Cid 2894* (GH), Brazil, AY523877; *Prance & al. P25404* (MO), Brazil, AY523878[a]. ***S. cajanumense* Kunth** [*Pachyphylla*], no voucher, New Zealand (cultivated), AY523879. ***S. calidum* Bohs** [*Pachyphylla*], *Cerón 2143* (UT), Ecuador, AY523880. ***S. campechiense* L.** [*Cryptocarpum*], *Bohs 2536* (UT), Costa Rica, AF244728. ***S. candidum* Lindl.** [*Lasiocarpa*], *Olmstead S-100* (WTU), BIRM S.0975, AF244722. ***S. circinatum* Bohs subsp. *circinatum [*Pachyphylla*], *Bohs 2442* (UT), Colombia, AY523881; *Bohs 2532* (UT), Panama, AY523882; *Bohs 2542* (UT), Colombia, AY523883; *Clark 4982* (US), Ecuador, AY523884. ***S. circinatum* Bohs** [*Pachyphylla*], *Bohs 2301* (GH), Colombia, AY523914. ***S. confusum* C.V. Morton** [*Cyphomandropsis*], *Bohs 2776* (UT), Bolivia, AY523885; *Bohs 2836* (UT), Bolivia, AY523886; *Bohs 2853* (UT), Argentina, AY523887. ***S. cordovense* Sessé & Moç.** [*Extensum*], *Bohs 2693* (UT), Costa Rica, AF244717. ***S. corymbiflorum* (Sendtn.) Bohs** [*Pachyphylla*], *Bohs 2343* (GH), Brazil, AY523888. ***S. cylindricum* Vell.** [*Cyphomandropsis*], *Severo & al. s.n.* (NY), Brazil, AY523889. ***S. diploconos* (Mart.) Bohs** [*Pachyphylla*], *Bohs 2335* (GH), Brazil, AY523890. ***S. diversifolium* Dunal** [*Pachyphylla*], *Bohs 2341* (GH), Venezuela, AY523891; *Benitez de Rojas & al. 5094* (MO), Venezuela, AY523892. ***S. elaeagnifolium* Cav.** [*Leprophora*], *Olmstead S-82* (WTU), U.S.A., AF244730. ***S. endopogon* (Bitter) Bohs** [*Pachyphylla*], *Bohs 2716* (UT), French Guiana, AY523925. ***S. exiguum* Bohs** [*Pachyphylla*], *Bohs 2758* (UT), Bolivia, AY523893. ***S. fallax* Bohs** [*Cyphomandropsis*], *Ramos & al. 3088* (UT), Colombia, AY523895; *Silverstone-Sopkin & al. 2606* (UT), Colombia, AY523894[a]. ***S. fusiforme* L.B. Sm. & Downs** [*Cyphomandropsis*], *Moscone & Daviña 217* (CORD), Argentina, AY523896. ***S. glaucophyllum* Desf.** [*Glaucophyllum*], no voucher, D'Arcy collection, AF244714; *Bohs 2530* (UT), Argentina, AY523897. ***S. hibernum* Bohs** [*Cyphomandropsis*], *Bohs 2443* (UT), Bolivia, AY523898; *Bohs 3053* (UT), Bolivia, AY523899. ***S. jamaicense* Mill.** [*Eriophylla*], *Olmstead S-85* (WTU), BIRM S.1209, AF244724. ***S. laciniatum* Aiton** [*Archaesolanum*], *Bohs 2528* (UT), New Zealand, AF244744. ***S. latiflorum* Bohs** [*Pachyphylla*], *Soares s.n.* (UT), Brazil, AY523900. ***S. luteoalbum* Pers.** [*Cyphomandropsis*], *Bohs 2336* (UT), BIRM S.1543, AF244715. ***S. macrocarpon* L.** [*Melongena*], *Olmstead S-88* (WTU), BIRM S.0133, AF244725. ***S. mammosum* L.** [*Acanthophora*], *Olmstead S-89* (WTU), BIRM S.0983, AF244721. ***S. mapiriense* Bitter** [*Allophylla*], *Nee & Solomon 30305* (UT), Bolivia, AY523901. ***S. maternum* Bohs** [*Pachyphylla*], *Bohs 2547* (UT), Bolivia, AY523902; *Bohs 2694* (UT), Bolivia, AY523904; *Bohs 2775a* (UT), Bolivia, AY523903. ***S. melissarum* Bohs** [*Pachyphylla*], *Bohs 2476* (UT), Brazil, AY523926. ***S. melongena* L.** [*Melongena*], *Olmstead S-91* (WTU), BIRM S.0657, AF244726. ***S. morellifolium* Bohs** [*Allophylla*], *Cerón & Cerón 4549* (MO), Ecuador, AY523929[a]. ***S. nitidum* Ruiz & Pav.** [*Holophylla*], *Nee 31944* (NY), Bolivia, AF244740. ***S. obliquum* Ruiz & Pav.** [*Pachyphylla*], *Lewis & al. 11660* (UT), Peru, AY523906[a]; *Stein & Kallunki 3941* (MO), Peru, AY523905[a]. ***S. occultum* Bohs** [*Pachyphylla*], *Cerón & Cerón 4632* (UT), Ecuador, AY523907. ***S. oxyphyllum* C.V. Morton** [*Pachyphylla*], *Cerón & Cerón 4671* (UT), Ecuador, AY523908; *Zak & Jaramillo 3117* (MO), Ecuador, AY523909[a]. ***S. pendulum* Ruiz & Pav.** [*Pachyphylla*], *Daly & al. 10004* (NY), Brazil, AY523910[a]; *Bohs & Schunke 2165* (MO), Peru, AY523911[a]. ***S. pinetorum* (L.B. Sm. & Downs) Bohs** [*Pachyphylla*], *Kummrow & Soares s.n.* (UT), Brazil, AY523912. ***S. proteanthum* Bohs** [*Pachyphylla*], *Nee 41288* (UT), Bolivia, AY523913. ***S. pseudocapsicum* L.** [*Geminata*], no voucher, BIRM S.0870, AF244720. ***S. ptychanthum* Dunal** [*Solanum*], *Olmstead S-94* (WTU), U.S.A., AF244735. ***S. roseum* Bohs** [*Pachyphylla*], *Bohs 2338* (GH), Bolivia, AY523915; *Nee & al. 51776* (NY), Bolivia, AY523916. ***S. sciadostylis* (Sendtn.) Bohs** [*Pachyphylla*], *Bohs 2453* (UT), Brazil, AY523917. ***S. sibundoyense* (Bohs) Bohs** [*Pachyphylla*], *Bohs & Juajibioy 2222* (GH), Colombia, AY523918. ***S. stuckertii* Bitter** [*Cyphomandropsis*], *Bohs 2522* (UT), Argentina, AY523927; *Bohs 2523* (UT), Bolivia, AY523919. ***S. tegore* Aubl.** [*Pachyphylla*], *Lindeman & al. 732* (MO), Suriname, AY523920[a]. ***S. tenuisetosum* (Bitter) Bohs** [*Pachyphylla*], *Sánchez 9834* (UT), Peru, AY523921[a]. ***S. tobagense* (Sandwith) Bohs** [*Pachyphylla*], *Aymard 4799* (MO), Venezuela, AY523922[a]. ***S. torvum* Sw.** [*Torva*], *Olmstead S-101* (WTU), BIRM S.0839, AF244729. ***S. unilobum* (Rusby) Bohs** [*Pachyphylla*], *Bohs 2549* (UT), Bolivia, AY523923; *Nee & Bohs 50863* (NY), Bolivia, AY523924. ***S. vespertilio* Aiton** [*Nycterium*], *Olmstead S-103* (WTU), BIRM S.2091, AF244727. ***S. villosum* Mill.** [*Solanum*], *Bohs 2553* (UT), Iran, AF244736. ***S. wallacei* (A. Gray) Parish** [*Californisolanum*], *Bohs 2438* (UT), U.S.A., AF244741. ***S. wendlandii* Hook. f.** [*Aculeigerum*], no voucher, BIRM S.0488, AF244731.

Sectional designations follow Bohs (1990) for sect. *Allophylla*, Bohs (1994) for sect. *Pachyphylla*, Bohs (2001) for sect. *Cyphomandropsis*, Child (1986) for sect. *Glaucophyllum*, Knapp (2002) for sect. *Geminata*, Symon (1981) for sect. *Archaesolanum*, and D'Arcy (1972, 1991) and Nee (1999) for all others. BIRM samples bear the seed accession number of the University of Birmingham Solanaceae collection, now held at Radboud University, Nijmegen, The Netherlands.

[a]Accessions amplified in two halves using the protocol and primers described in the text.