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SOLANUM PHYLOGENY INFERRED FROM CHLOROPLAST DNA SEQUENCE DATA

LYNN BOHS¹ & RICHARD G. OLMSTEAD²

¹Department of Biology, 201 Biology Building, University of Utah, Salt Lake City,
Utah 84112, U.S.A.

²Department of Biology, Box 355325, University of Washington, Seattle,
WA 98195-5325, U.S.A.

Abstract

A data set derived from gene sequences of *ndhF* is used to deduce phylogenetic relationships among the subgenera of *Solanum*, among related genera of the tribe Solanaceae, and within selected *Solanum* clades. Complete *ndhF* sequences were obtained for 12 species of *Solanum*, representing five of the seven subgenera. *ndhF* sequences also were obtained from species of *Capsicum*, *Cyphomandra*, *Datura*, *Jaltomata*, *Lycopersicon*, *Nicotiana*, and *Physalis*. Results of a parsimony analysis of these data indicate that the *Solanum* species form a monophyletic clade with *Capsicum* rather weakly supported as the sister group. *Lycopersicon* and *Cyphomandra* are included within the *Solanum* clade. Three to four clades can be identified within *Solanum*. The subgenera *Minon* and *Potatoe* are probably not monophyletic as currently defined. *Solanum wendlandii*, a species with recurved prickles but no stellate hairs, does not belong with the other spiny solanums in subgenus *Leptostemonum*. These results cast doubt on the utility of traditional characters such as anther shape and hair morphology in defining high-level infrageneric groups in *Solanum*.

Introduction

Solanum, with approximately 1000 to 1400 species (D'Arcy, 1991), is one of the largest genera of flowering plants. Its size and complexity have put it beyond the reach of a single monographer, and the last complete treatment of the entire genus dates to Dunal (1852). Adding to the taxonomic difficulties of *Solanum* is its largely tropical distribution. *Solanum* attains its highest diversity in the neotropics, with over 500 species endemic to the New World (D'Arcy, 1991).

Solanum also is an extremely important genus economically, including taxa with edible fruits (e.g., tomato, eggplant, pepino, naranjilla), edible tubers (potato), and medicinally valuable or poisonous alkaloids (e.g., nightshades [section *Solanum*]). Despite its economic importance, the current level of understanding of infrageneric groups in *Solanum* is very uneven. A few groups such as the potatoes, tomatoes, and nightshades have been examined extensively. Otherwise, large gaps remain in our knowledge of taxonomic groups and their phylogenetic affinities. Revisions or partial revisions are available for less than half of the approximately 60 sections currently recognized within *Solanum*. Sectional limits are well-defined in some cases and poorly defined in others. Above the rank of section, seven subgenera are recognized (D'Arcy, 1972, 1991). However, subgeneric circumscriptions are generally vague. Few phylogenetic hypotheses exist for relationships among infrageneric groups in *Solanum* at either the sectional or subgeneric level (see Spooner et al., 1993 and Whalen, 1984 for examples). Cladistic analysis of *Solanum*

subgroups has rarely been attempted, largely because of uncertainties about group circumscription and appropriate outgroups for comparison.

Molecular approaches have revolutionized the field of evolutionary systematics by providing data on character sets with relatively unambiguous homologies. Molecular approaches thus offer the opportunity to examine higher-order relationships without requiring exhaustive information on their component taxa. Molecular techniques most commonly used in inferring phylogenetic relationships in plants include restriction site mapping and DNA sequencing. Both methods have been successfully applied to problems in Solanaceae systematics (e.g., Olmstead & Palmer, 1991, 1992; Olmstead & Sweere, 1994; Palmer & Zamir, 1982; Spooner et al., 1993).

The present study focuses on the use of gene sequence data to infer phylogenetic relationships among subgeneric groups in *Solanum*. The gene chosen was *ndhF*, a chloroplast region approximately 2200 base pairs in length. Previous work (Olmstead & Sweere, 1994) has shown that *ndhF* has a higher base substitution rate than that of the chloroplast gene *rbcL*, which has been used to infer phylogenetic relationships among angiosperm subclasses and families (e.g., Chase et al., 1993; Olmstead et al., 1992). *ndhF*, then, is a promising way to investigate phylogenetic relationships at the generic and subgeneric level. The objectives of this study were to investigate the utility of *ndhF* sequence data in phylogenetic analyses of *Solanum* subgenera, sections, and related genera of the tribe Solaneae and, if successful, to identify monophyletic groups within *Solanum*. The resultant phylogenetic hypotheses can also be used to examine patterns of character evolution in the genus and its relatives.

Materials and Methods

Complete *ndhF* sequences were obtained from twelve *Solanum* species representing five of the seven subgenera of D'Arcy (1972) and representatives of the closely related genera *Jaltomata* and *Cyphomandra*. *Capsicum baccatum*, *Datura stramonium*, *Lycopersicon esculentum*, *Nicotiana tabacum*, and *Physalis alkekengi* were included from the study of Olmstead & Sweere (1994). Voucher information is given in Table 1. DNA was extracted from fresh or dried leaves by the modified CTAB technique of Doyle & Doyle (1987) and further purified by cesium chloride/ethidium bromide density gradient centrifugation. Sequencing was accomplished by standard dideoxy methods from single stranded DNA generated by using biotinylated PCR primers. The sequencing strategy for *ndhF*, PCR, and sequencing primers are described in Olmstead & Sweere (1994).

The data were analyzed by parsimony methods using PAUP (Swofford, 1993), with all character changes weighted equally. The Branch and Bound search algorithm was used with the MULPARS option. A bootstrap analysis (Felsenstein, 1985) was conducted with 100 replicates to determine relative support for clades found in the parsimony analysis. To determine the presence of phylogenetic signal in the data, the frequency distribution of 1000 trees sampled randomly from the set of all possible trees was examined, and Hillis' g_1 statistic (Hillis, 1991; Huelsenbeck, 1991) was generated.

Results

The data set consisted of 19 taxa, each with 2121 nucleotides of DNA sequence. The only length variation found was a 33 base pair insertion found at position 1473 in *Solanum wendlandii*. 81 nucleotide positions provide phylogenetically informative characters.

The parsimony analysis resulted in four equally parsimonious trees of 329 steps (Fig. 1) with a consistency index (CI; Kluge & Farris, 1969) of 0.854 and retention index (RI; Farris, 1989) of 0.751. Hillis' g_1 statistic was -0.741 , indicating strong

TABLE 1. Sources of DNA accessions sequenced for *ndhF*.

Taxon	Source ^a	Voucher ^b
<i>Capsicum baccatum</i> L.	2	Eshbaugh 1584 ^c
var. <i>pendulum</i> (Willd.) Eshbaugh	1	Bohs 2468 ^c
<i>Cyphomandra betacea</i> (Cav.) Sendtn.	2	RGO S-16 ^c
<i>Datura stramonium</i> L.	2	Davis 1189A
<i>Jaltomata procumbens</i> (Cav.) J.L. Gentry	3	none ^c
<i>Lycopersicon esculentum</i> Mill.	2	none ^{c,d}
<i>Nicotiana tabacum</i> L.	2	none ^c
<i>Physalis alkekengi</i> L.	2	D'Arcy 17707 ^c
<i>Solanum abutiloides</i> (Griseb.) Bitter & Lillo	2	BIRM S.0655
<i>Solanum allophyllum</i> (Miers) Standl.	1	Bohs 2339
<i>Solanum aviculare</i> Forst. f.	2	BIRM S.0809
<i>Solanum dulcamara</i> L.	2	none
<i>Solanum pseudocapsicum</i> L.	2	BIRM S.0870
<i>Solanum ptychanthum</i> Dunal	2	RGO.S-94 ^{c,e}
<i>Solanum rostratum</i> Dunal	1	none
<i>Solanum seaforthianum</i> Andr.	2	BIRM S.0051
<i>Solanum torvum</i> Swartz	2	BIRM S.0839
<i>Solanum tuberosum</i> L. ssp. <i>tuberosum</i>	2	WRF 1610 ^f
<i>Solanum wallacei</i> (Gray) Parish	1	Bohs 2438
<i>Solanum wendlandii</i> Hook.	2	BIRM S.0488

^aDNA extracts provided by: 1 – L. Bohs, University of Utah, Salt Lake City, UT. 2 – R.G. Olmstead, University of Colorado, Boulder, CO. 3 – T. Mione, Central Connecticut State University, New Britain, CT.

^bCollector and number of herbarium vouchers. Bohs vouchers are at UT, RGO vouchers at COLO. BIRM samples bear the seed accession number of the University of Birmingham Solanaceae collection.

^cSame DNA accession used in Olmstead & Palmer (1992).

^dCorrected sequence from Olmstead et al. (1993).

^eAs "*S. americanum*" in Olmstead and Palmer (1992).

^fCollection number from Sturgeon Bay USDA station. Sample also bears the annotation "PI (245793 x 245796)."

phylogenetic signal in the data. The four trees differ only in the relative placement of *S. seaforthianum*, *S. wallacei*, and *S. dulcamara*, and the strict consensus tree of the four most parsimonious trees is well-resolved (Fig. 2).

Among the genera of the Solaneae sampled, *Capsicum* emerges as the sister group to *Solanum*, albeit with weak support (64% of bootstrap replicates; Fig. 2). Relationships among the other genera of Solaneae are not well-resolved. There is strong support for the monophyly of *Solanum*, with *Lycopersicon* and *Cyphomandra* included within the *Solanum* clade. Three or four clades can be identified within *Solanum*: 1) the "spiny plus" clade, consisting of *S. abutiloides*, *S. wendlandii*, *S. allophyllum*, *S. pseudocapsicum*, *S. rostratum*, *S. torvum*, and *Cyphomandra betacea*, 2) subgenus *Archaeosolanum*, represented by *S. aviculare*, 3) the "Solanum proper" clade, with *S. ptychanthum*, *S. seaforthianum*, *S. wallacei*, and *S. dulcamara*, and 4) tomato, *Lycopersicon esculentum*, plus potato, *S.*

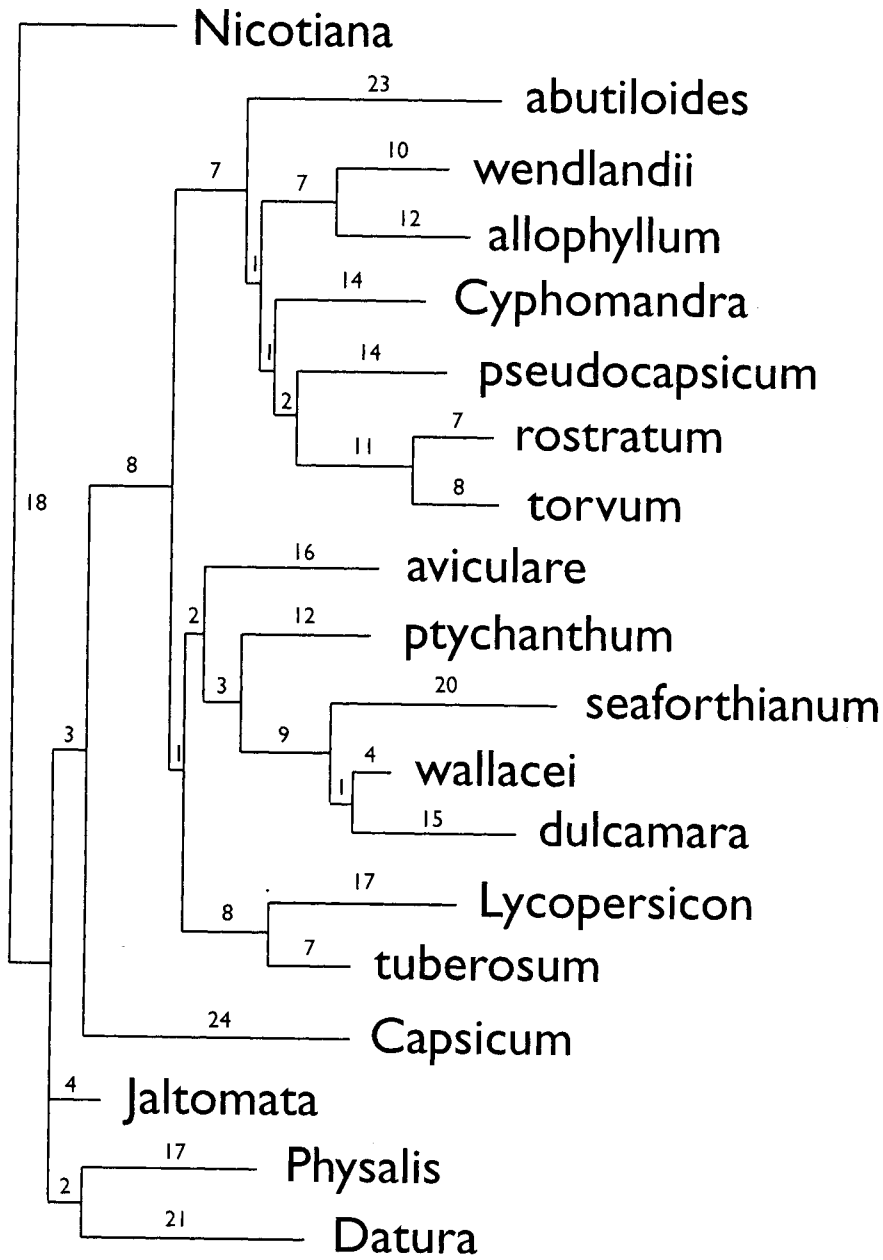


FIG. 1. One of four most parsimonious trees of 329 steps (CI = 0.854, RI = 0.751) with branch lengths indicated.

tuberosum. *Solanum aviculare* is included in the *Solanum* proper clade on the most parsimonious trees and the strict consensus tree derived from them, but with little character support for its inclusion. Other well-supported groups include the association of *S. wendlandii* with *S. allophyllum*, of *S. rostratum* with *S. torvum*, and of *S. seforthianum*, *S. wallacei*, and *S. dulcamara*.

Discussion

Certain caveats apply to the phylogenetic hypotheses presented here due to the fact that the trees derived from *ndhF* sequences are gene trees rather than species trees, i.e., that evolutionary events such as hybridization can confound phylogenies based on chloroplast characters, which are maternally inherited in most flowering plants, and that intraspecific variation in DNA can exist and is only detectable when multiple samples of the same taxon are studied (Doyle, 1992; Rieseberg & Soltis, 1991; Soltis et al., 1992). The small number of *Solanum* species sampled (about 1.5% of the genus) must also be considered when interpreting the phylogenetic hypotheses presented here. Given these considerations, however, the *ndhF* tree can be used as a starting point to examine systematic relationships and character evolution in *Solanum* and its allies.

Monophyly of *Solanum*

The present study finds strong support for the monophyly of *Solanum* if the genera *Lycopersicon* and *Cyphomandra* are included within it. The same result has been obtained by Olmstead & Palmer (1992) and Spooner et al. (1993) using data from chloroplast DNA restriction site analysis. Two taxonomic solutions are possible: the species of *Lycopersicon* and *Cyphomandra* can be transferred to *Solanum*, or *Solanum* can be broken up into smaller monophyletic genera. The former alternative was pursued by Spooner et al. (1993), who transferred all *Lycopersicon* epithets to *Solanum*. *Cyphomandra* was recently monographed by Bohs (1994), who left its status as a separate genus intact. If it proves desirable to consider *Lycopersicon* and *Cyphomandra* as synonyms of *Solanum*, the species of *Cyphomandra* will need to be transferred (see Addendum). On the other hand, *Solanum* is currently unwieldy and nearly unmanageable because of its large size, and an appealing case may be made for dismantling it into smaller monophyletic units. Although this study and those of Olmstead & Palmer (1992), Spooner et al. (1993), make contributions in this direction, much more work on infrageneric phylogeny of *Solanum* needs to be done before this course is followed.

Relationships of Tribe Solaneae

Phylogenetic relationships among *Solanum* and the other genera sampled from the tribe Solaneae (*Capsicum*, *Datura*, *Jaltomata*, and *Physalis*) are poorly resolved in the present analysis. The *ndhF* data point to *Capsicum* as the sister group to *Solanum*, but this grouping is only weakly supported. *Jaltomata* emerged as the sister group to *Solanum* in Olmstead & Palmer's (1992) restriction site analysis of the Solanaceae. Relationships at the base of the Solaneae were poorly resolved in their study. *Capsicum* was found by Olmstead & Sweere (1994) to be sister to *Solanum* in analyses based on combined data from *ndhF*, *rbcl*, and restriction sites, but *Jaltomata* was not included in their study. More extensive sampling among the genera of this tribe for *ndhF* is now in progress and may help to clarify relationships in this group.

Relationships of *Solanum* subgroups

Figure 2 shows the currently accepted subgenera of *Solanum* mapped onto the strict consensus tree. The subgenera *Minon* and *Potaloe* as currently defined are probably not monophyletic. Only one representative was sequenced from the subgenus

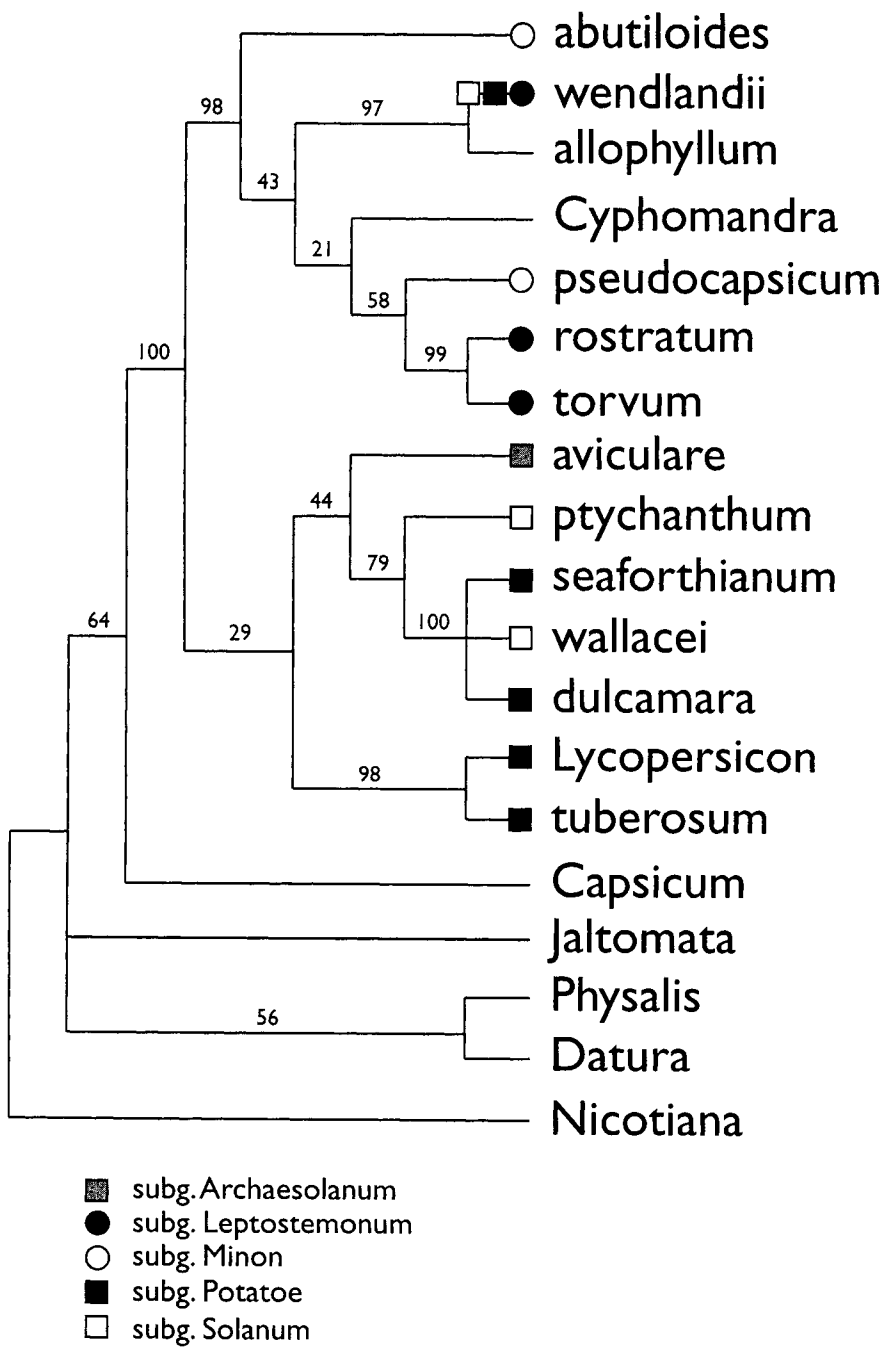


FIG. 2. Strict consensus of four most parsimonious trees. Numbers indicate percentage of bootstrap replicates obtained for each clade. The subgenus of *Solanum* (sensu D'Arcy, 1972, 1991) to which each species belongs is mapped onto the branches.

Archaeosolanum. Traditional members of the subgenus *Leptostemonum*, *S. rostratum* and *S. torvum*, come out together in a strongly supported branch on the *ndhF* tree. The taxonomic position of *S. wendlandii* has been controversial. It belongs to section *Aculeigerum*, which was placed by D'Arcy (1972) and Whalen (1984) in subgenus *Leptostemonum* because of its tapered anthers and recurved spines. However, members of section *Aculeigerum* lack stellate hairs and thus diverge from typical leptostemonoid solanums. D'Arcy (1972) notes that this section may warrant subgeneric status. Child (1990) included *Aculeigerum* as a section of subgenus *Potatoe*. Seithe (1962), who did not recognize subgenus *Potatoe*, considered *Aculeigerum* to belong to subgenus *Solanum*. According to the *ndhF* data, *S. wendlandii* is well removed from the subgenera *Potatoe* or *Solanum*, but also does not belong with the traditional spiny solanums in subgenus *Leptostemonum*. The data further indicate that spines may have evolved more than once in *Solanum*. With *S. wendlandii* removed, subgenus *Leptostemonum* probably forms a monophyletic group.

Solanum allophyllum has not yet been placed in a subgenus. This species belongs to section *Allophyllum* (Bohs, 1990), whose relationships have been obscure until now. *Solanum allophyllum* comes out strongly supported as the sister group to *S. wendlandii*, although no previous classification scheme has united the two taxa. Morphological features that the two sections share are pinnately lobed leaves and tapered anthers with small terminal pores.

All representatives from subgenus *Leptostemonum* and *Minon* sequenced for *ndhF* fall out with *S. wendlandii*, *S. allophyllum*, and *Cyphomandra* on a well-supported clade, here called the "spiny plus" group. No infrageneric group of *Solanum* currently exists to accommodate this clade. Further taxonomic sampling of this clade for *ndhF* is underway and may aid in its circumscription and naming.

A second *Solanum* clade includes *S. ptychanthum*, *S. seaforthianum*, *S. wallacei*, and *S. dulcamara*. *Solanum aviculare*, the sole representative of subgenus *Archaeosolanum* included in the study, is contained within this clade in the most parsimonious trees (Fig. 1), but the clade is weakly supported with only two synapomorphies and a bootstrap value of less than 50 per cent (Fig. 2). Without *S. aviculare*, this clade consists of members of subgenus *Solanum* (*S. ptychanthum*, *S. wallacei*) plus sections *Jasminosolanum* (*S. seaforthianum*) and *Dulcamara* (*S. dulcamara*), which form part of subgenus *Potatoe* in D'Arcy's (1972) scheme. *Solanum wallacei* was included in subgenus *Solanum* by Seithe (1962), but it appears to be more closely related to *S. seaforthianum* and *S. dulcamara* than to *S. ptychanthum*. Interesting biogeographical problems remain to be examined in this group, with section *Jasminosolanum* centered in tropical America, section *Dulcamara* distributed in temperate Asia and Europe, and *S. wallacei* representative of the California solanums.

Another well-supported *Solanum* subclade includes the tomato, *Lycopersicon esculentum*, plus the potato, *S. tuberosum*. A close relationship between the potatoes and tomatoes has been postulated by many previous workers (reviewed in part in Lester, 1991), and has been established on the basis of chloroplast DNA restriction site data (Spooner et al., 1993). The genus *Solanopsis* Börner exists to accommodate the members of this clade, and may be used in the future if it seems prudent to subdivide *Solanum* in this way (Spooner et al., 1993).

Despite good resolution within *Solanum* subgroups, the present *ndhF* data do not provide good resolution at the base of the *Solanum* clade. This could be an artifact of taxonomic sampling or due to morphological evolution without concomitant molecular evolution in *ndhF*. If the latter explanation turns out to be more likely, one might postulate that the evolution of poricidal anther dehiscence was a key innovation that promoted rapid diversification in *Solanum*, similar to the postulated evolutionary innovations that may have led to increased rates of diversification in the angiosperms as compared to other seed plants (Sanderson & Donoghue, 1994).

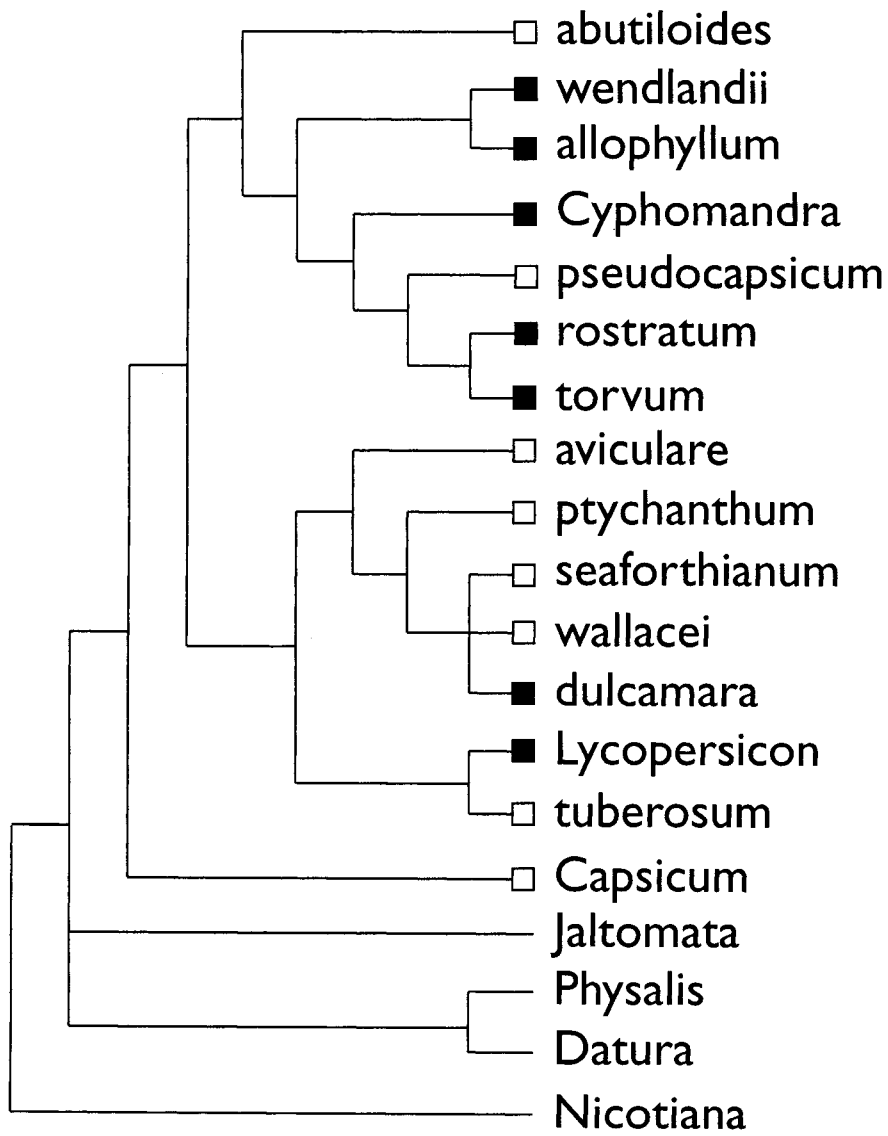


FIG. 3. Strict consensus tree with anther characters mapped onto the branches. = taxa with short, oblong anthers, = tapered anthers.

Character evolution

Anther shape has been used traditionally to delimit major infrageneric categories in *Solanum*. Dunal (1852) divided the genus into two parts based on anther shape. Section *Pachystemonum* included taxa with relatively short, oblong anthers, usually with large terminal pores. Species of section *Leptostemonum* had tapered anthers with small terminal pores. Dunal's system does not adequately reflect the true distribution of these anther characters because he segregated the traditional spiny solanums in *Leptostemonum* and left the rest of *Solanum* diversity in *Pachystemonum* without detailed consideration of anther shape. Furthermore, *Lycopersicon* and *Cyphomandra* were considered as separate genera, and thus were not included in this scheme.

Anther characters are mapped onto the *ndhF* tree in Figure 3. Allowing some latitude for character interpretation, it can be seen from outgroup comparison with *Capsicum* that tapered anthers are derived relative to short, oblong anthers. Furthermore, tapered anthers have probably evolved more than once in various *Solanum* clades.

Similarly, hair morphology has been used to divide *Solanum* into major groups (Seithe, 1962). Three broad categories of hairs can be recognized: simple (unbranched), dendritically branched, and stellate. Figure 4 shows these hair types mapped onto the *ndhF* tree. Simple hairs are apparently plesiomorphic relative to branched hairs. One major clade of *Solanum* has primarily simple hairs (the group composed of *S. aviculare*, the "solanum proper" clade, and tomato plus potato), whereas the "spiny plus" clade has representatives with all three hair types. However, the evolutionary pattern of these hair types is complex, with probable convergence in hair morphology between different lineages.

Two other conspicuous morphological characters, vining or scrambling habit and the presence of compound or deeply lobed leaves, are mapped onto the *ndhF* tree in Figures 5 and 6, respectively. Both characters show homoplastic distributions, indicating several evolutionary origins or reversals.

From the results above, it can be seen that character evolution in *Solanum* has been complex. It is not surprising that traditional characters such as anther shape and hair morphology have not been successful in defining satisfactory infrageneric groups. Elucidation of the genetic mechanisms underlying development of morphological characters such as hair or leaf shape may show that they are under relatively simple genetic control and have been switched on or off in disparate lineages. Alternatively, such characters may be under strong selection, but at present we know little or nothing about their effects on organismal fitness.

Prospects and Future Research

ndhF sequencing is continuing, emphasizing increased sampling within *Solanum* and among genera of the tribe Solaneae. The results from the present study show that *ndhF* sequence data generate well-resolved and well-supported trees for groups within *Solanum*. However, a revised phylogenetic classification for *Solanum* is premature. It is hoped that additional sampling will continue to refine our hypotheses of *Solanum* phylogeny to the point that a modern taxonomic scheme can be generated for the genus.

Although we have not yet reached the limit of resolution for *ndhF* sequence data, it is probable that insufficient variation will be found in *ndhF* to resolve relationships among closely related species, such as those within a section. Work in progress includes a sequencing study of the Internal Transcribed Spacer (ITS) regions of the nuclear ribosomal DNA array. ITS has a high substitution rate and has been helpful in resolving relationships among closely related species (Baldwin, 1992). In addition, ITS is a nuclear DNA sequence. Comparison between phylogenies generated from chloroplast and nuclear sequences may reveal episodes of hybridization, introgression, and lineage sorting.

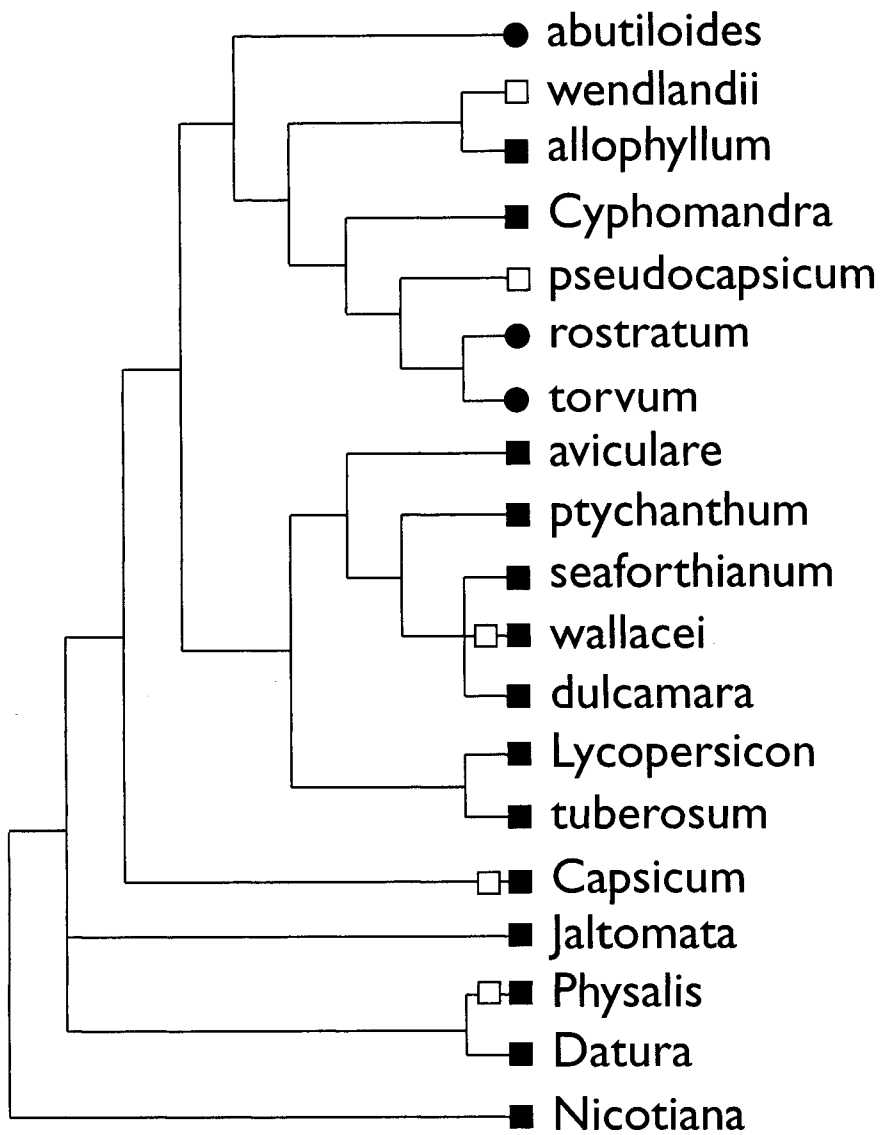


FIG. 4. Strict consensus tree with hair characters mapped onto the branches. Hair types indicated are not necessarily found in the species sequenced, but are known to occur in the section or species group to which it belongs. ■ = unbranched hairs, □ = dendritically branched hairs, ● = stellate hairs.

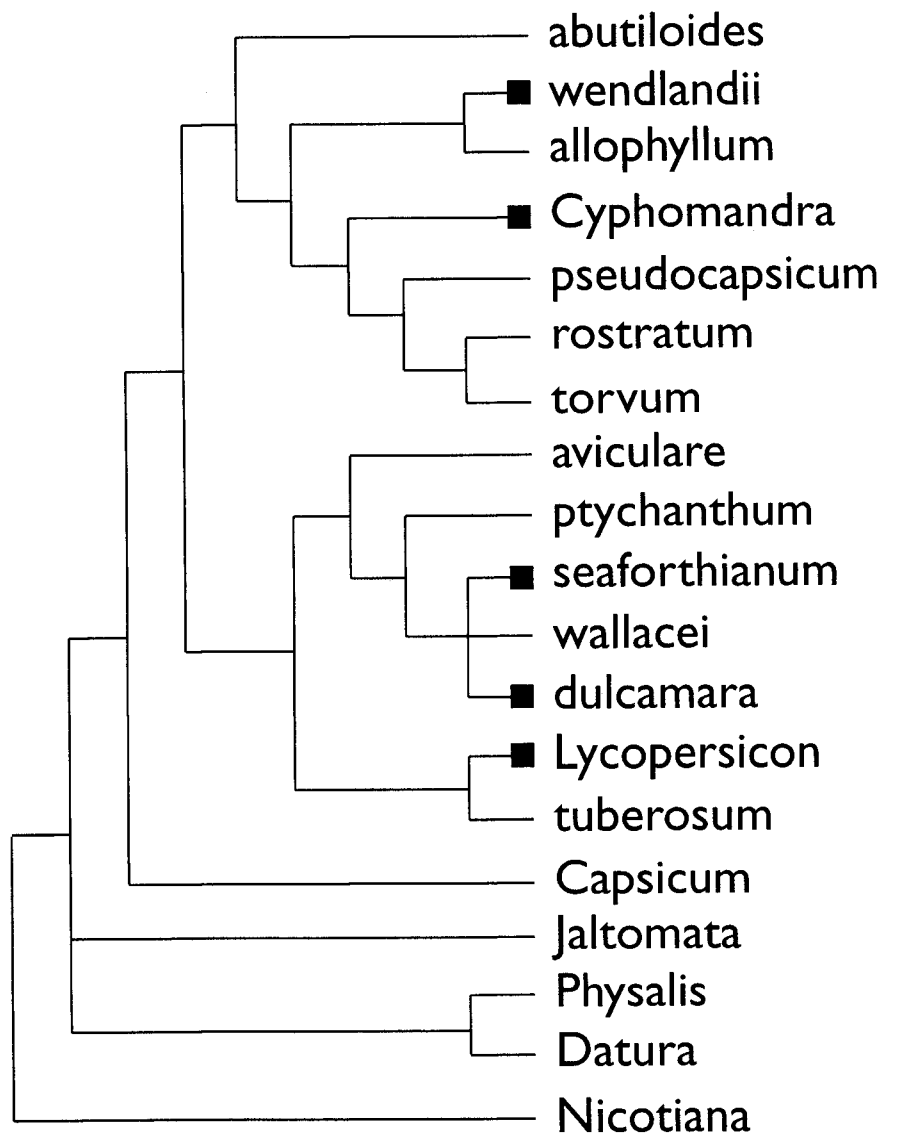


FIG. 5. Strict consensus tree showing distribution of vining or scrambling habit (■) in *Solanum* species sampled. Vining or scrambling habit is not necessarily found in the species sequenced, but is known to occur in other members of the section or species group.

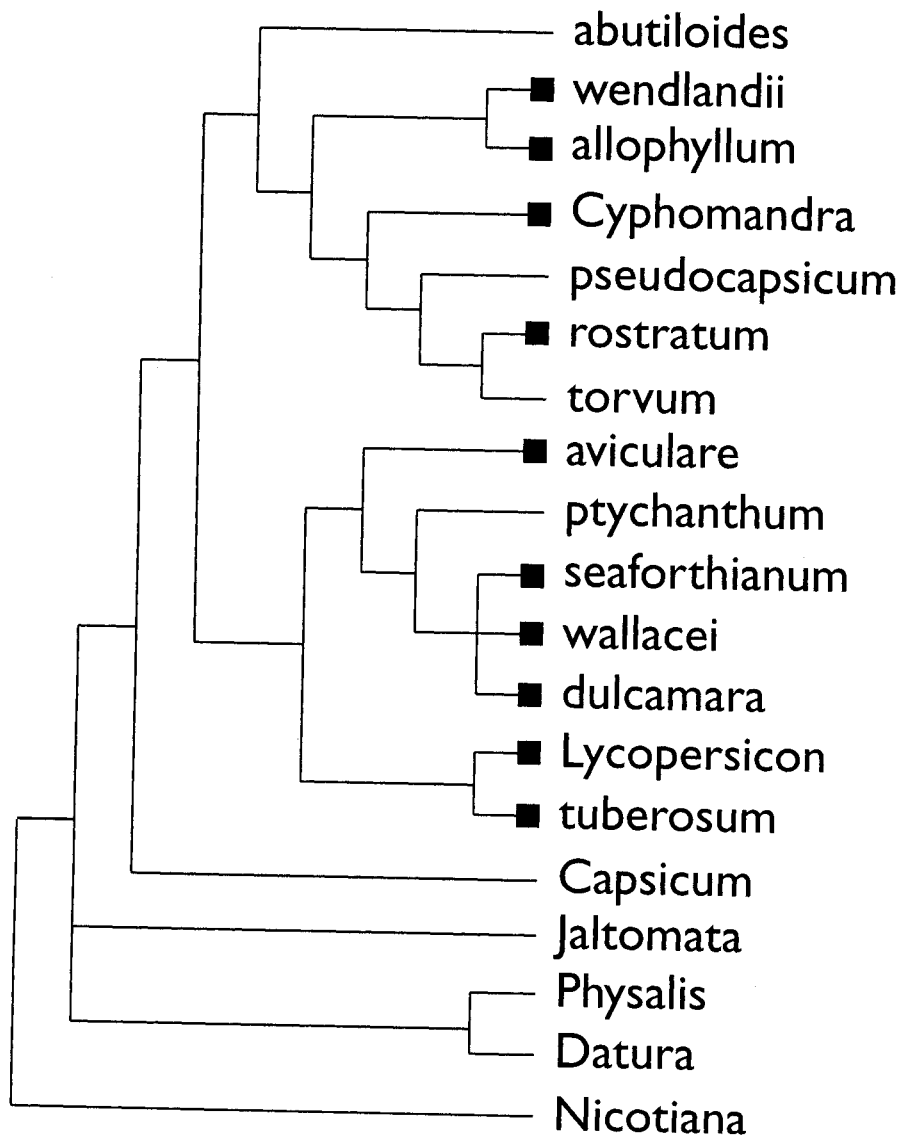


FIG. 6. Strict consensus tree showing distribution of compound or deeply lobed leaves (■) in sections or species groups to which the sequenced species belong.

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Addendum

All species of *Cyphomandra* have now been transferred to *Solanum* section *Pachyphylla* Dunal (Bohs, L. 1995 Transfer of *Cyphomandra* (Solanaceae) and its species to *Solanum*. *Taxon* 44: 583–587).