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Untangling *Physalis* (Solanaceae) from the Physaloids: A Two-Gene Phylogeny of the Physalinae

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ABSTRACT. *Physalis* (75+ species, Solanaceae) is most diverse in Mexico, with only the type, *P. alkekengi*, native to the Old World. Interspecific relationships are poorly known, and despite the distinctive inflated fruiting calyces, generic limits remain uncertain. Sequence data from part of the nuclear gene *waxy* (622 bp) and the internal transcribed spacer of the nrDNA (652 bp) were used to generate a phylogeny of subtribe Physalinae. Thirty-five species of *Physalis* and eight physaloid genera were sequenced. Data analysis included Bayesian and maximum parsimony methods. The Physalinae was monophyletic, but while the morphologically typical *Physalis* species formed a strongly supported clade, the morphologically atypical species made the genus paraphyletic. A grade of physaloid genera (*Quincula*, *Oryctes*, and *Chamaesaracha*) and *Physalis* subgenus *Physalodendron* separate *P. alkekengi*, *P. carpenteri*, and *P. microphysa* from other *Physalis* species. The *Physalis* clade consists of *Margaranthus* and species with solitary yellow flowers and highly inflated calyces. Most sections of *Physalis* do not appear to be monophyletic. *Leucophysalis viscosa* and the Central American physaloid genera *Brachistus*, *Tzeltalia*, and *Witheringia* formed a clade at the base of the Physalinae.

Like many genera in the Solanaceae, *Physalis* has a variety of economically important species, from edibles to ornamentals, as well as a variety of taxonomic problems. *Physalis* is one of the largest genera in the Solanaceae, with 75–90 species, most of which occur in Mexico. With one notable exception, all species are native to the New World, although cultivated species and weedy annuals have been introduced to warm areas worldwide. The most arresting feature of the genus is the calyx, which becomes greatly expanded in fruit, inflating until it completely envelops the berry. Of the species grown for their edible fruits, one of the best known is *P. philadelphica* Lam., the tomatillo, a key component of salsa verde. The Chinese lantern plant, *P. alkekengi* L., is also cultivated for its fruits, though their use is more decorative than culinary. Several species have been used medicinally, and recent research has focused on potential antibacterial and antitumor properties of their secondary compounds (Chiang et al. 1992; Kennelly et al. 1997; Pietro et al. 2000).

Historically, *Physalis* has been divided into species groups on the basis of characters such as habit, hair type, and number of calyx angles (Rydberg 1896; Martínez 1998, 1999). The most recent infrageneric revision used gross-morphological characters as well as micromorphological ones, such as trichome surface sculpturing, to define subgenera and sections (Martínez 1999; Table 1). However, the relationships both within and among the sections remain unclear.

The typical *Physalis* plant is an herb with solitary, bee-pollinated, axillary yellow flowers (Sullivan 1984). The nodding, bell-shaped corollas are unlobed, with darkly-spotted throats. Once pollination has occurred, the corolla falls off and the calyx expands until the developing berry is entirely hidden, often touching the fruit only at the base. In some species the mature calyces turn yellow or orange. The many-seeded berries range from greenish to yellow to tangerine and are sometimes flushed red or purple.

Accressant calyces appear throughout the Solanaceae, but the highly inflated calyx found in *Physalis* is unusual, and this feature makes *Physalis* one of the easiest solanaceous genera to recognize. However, the circumscription of the genus has been confounded by several small genera called "physaloids," a general term referring to those genera morphologically reminiscent of *Physalis*, due usually to the presence of some amount of calyx expansion (but not necessarily inflation). Historically, taxonomic treatments have differed with respect to which genera are physaloids, as well as to which ones should be subsumed within *Physalis*. Although the genera *Archiphysalis* Kuang, *Athenaea* Sendt., *Deprea* Raf., *Exodeconus* Raf., *Jaltomata* Schlecht., *Larnax* Miers, *Physalisatrum* Makino, *Nicandra* Adans., and *Saracha* Ruiz & Pav. have sometimes been considered physaloid, cpDNA data now suggest that these taxa are not closely related to *Physalis* (D'Arcy and Averett 1996; Olmstead et. al 1999).

Current debate centers on five North American genera that are close relatives of *Physalis*: *Chamaesaracha*, *Leucophysalis*, *Margaranthus*, *Oryctes*, and *Quincula*. Arguments over which of the five are worthy of genus-level recognition and which should be included within *Physalis* are longstanding (Rydberg 1896; Waterfall 1958, 1967; Averett 1970; Barboza 2000). *Margaranthus solanaceous* and *Quincula lobata* were treated as *Physalis solanaceous* and *P. lobata* in the latest revision of *Physalis* (Martínez 1999), but whether these plants are recog-

TABLE 1. Infrageneric classification of *Physalis* sensu Martínez (1999). The genus is currently divided into four subgenera and 12 sections.

Subgenera and sections of *Physalis*	Species	Native to
Physalis subgenus *Physalis*	1	China (and possibly Europe)
P. alkekengi (type)		
Physalis subgenus *Physalodendron* (G. Don) M. Martínez	2	S Mexico and Central America
P. arborescens, *P. melanocystis*		
Physalis subgenus *Quincula* (Raf.) M. Martínez	1	SW U.S. and N Mexico
P. lobata (syn. *Quincula lobata*)		
Physalis subgenus *Rydbergis* Hendrych	60+	New World, mostly Mexico
section *Angulatae* (Rydb.) M. Y. Menzel (includes *Margaranthus*)	10	U.S. to Central America
section *Campanulae* M. Martínez	2	Mexico
section *Carpenterianae* (Rydb.) M. Y. Menzel	1	SE U.S.
section *Coztomatae* M. Martínez	11	Mexico
section *Epeteiorhiza* G. Don	14	U.S. to Central America
section *Lanceolatae* (Rydb.) M. Y. Menzel	14+	U.S. and Mexico
section *Rydbergae* M. Martínez	2+	Mexico
section *Viscosae* (Rydb.) M. Y. Menzel	6	U.S. to South America
section *Tehuacanae* M. Martínez	1	Mexico

nized as unique genera or merely species of *Physalis* remains arbitrary.

Four subtribes of the tribe Physaleae D'Arcy have been proposed on the basis of cpDNA phylogenies, including the Physalinae (Table 2), which contains *Physalis*, the five North American physaloid genera, *Witheringia*, and its segregate *Brachistus* (D'Arcy et al. 1981). Because most species of *Witheringia* lack calyx expansion in fruit, the genus has not traditionally been considered physaloid. However, cpDNA data placed it firmly at the base of the Physalinae clade, and *Witheringia* does share the nodal inflorescences and longitudinally dehiscent anthers characteristic of other physaloids. Finally, the recent segregation of *Tzeltalia* from *Physalis* has given rise to a new physaloid genus that should be considered a member of subtribe Physalinae (Estrada and Martínez 1998).

For the purposes of this study, the term "physaloid" refers to genera provisionally placed in subtribe Physalinae based on phylogenetic analyses of cpDNA data (e.g., Olmstead et al. 1999; see Table 2). This is a broader view of the subtribe than that recognized by Hunziker (2000), who included only *Physalis*, *Quincula*, *Leucophysalis*, and *Chamaesaracha*—our definition of Physalinae follows that of Olmstead et al. (1999) rather than Hunziker. To emphasize that *P. lobata* and *P. solanaceous* are the subject of continuing nomenclatural debate, they will be referred to by their generic names (*Quincula* and *Margaranthus*, respectively).

By clarifying which taxa are closely related to *Physalis*, chloroplast DNA data have helped to end arguments over which genera are physaloid, but because the study of Olmstead et al. (1999) was focused on generic and higher level relationships, sampling within *Physalis* was limited. Within *Physalis*, taxonomic questions remain. There are several morphologically unusual species of *Physalis* whose affinities to other species are uncertain, and which may not belong within the genus. The type species, *P. alkekengi*, is one of these morphologically atypical species, and it is the only native Eurasian species in the Physalinae. This species has long been grown as an ornamental throughout China, Japan, and Europe. It is unclear where *P. alkekengi* originated, but China has been suggested (Hendrych 1989; Olmstead et al. 1999). After establishing *Leucophysalis*, Rydberg (1896) stated, "If . . . *P. alkekengi*

TABLE 2. List of genera in subtribe Physalinae, including species diversity and geographic range. **Tzeltalia* was segregated from *Physalis* after Olmstead et al. (1999) provided a provisional classification for the Physalinae. However, it can be placed in this subtribe on morphological grounds.

Genus	Species	Native to:
Brachistus	3	Central America
Chamaesaracha	10	SW U.S., Mexico, Central America
Leucophysalis	3 or more	U.S. and Central America
Margaranthus	1	SW U.S. to Central America
Oryctes	1	Nevada and California
Quincula	1	SW U.S. and N Mexico
Physalis	75+	the Americas, China, and naturalized worldwide
*Tzeltalia**	2	S Mexico and Guatemala
Witheringia	20	Central and South America

could be also removed, the genus would be a very natural one." Although the morphological differences seem slight, cpDNA phylogenies indicate that *P. alkekengi* is more distantly related to other species of *Physalis* than are the genera *Chamaesaracha* and *Margaranthus* (Mione et al. 1994; Olmstead et al. 1999).

Since the 1950s, cytological, biochemical, and morphological data have been collected for various sets of *Physalis* species and physaloid genera, but few studies have included a broad sampling of species both from within *Physalis* and from related genera. The purpose of this study was to examine species relationships within *Physalis* and relationships among the genera of subtribe Physalinae. Because chloroplast DNA data lack the variability required to resolve species relationships within *Physalis* (Martínez, personal communication; Whitson, unpublished data), two regions of more variable nuclear DNA were chosen for use in this study. Phylogenetic analysis of DNA sequences from the internal transcribed spacer (ITS) region of ribosomal DNA and from the gene *waxy* (also known as the granule bound starch synthase or GBBSI gene) was used to address the following questions: Is *Physalis* monophyletic? Are the sections of the genus *Physalis*, established primarily on the basis of gross morphology, generally congruent with DNA data? What are the relationships between the physaloid genera within subtribe Physalinae?

Materials and Methods

Taxon Sampling. ITS and *waxy* sequences were obtained from representatives of each genus in the Physalinae (Table 2), and one species of *Tzeltalia*. Thirty-five species of *Physalis* were also sequenced, representing all four subgenera (Table 1). Seven of the nine sections of subgenus *Rydbergis* were sampled. Material of the monotypic section *Tehuacanae* was not available, and *P. minimaculata*, one of the two species in section *Rydbergae*, was represented by only an ITS sequence.

DNA Extraction. Samples were extracted from fresh material as well as herbarium specimens (Table 3). Fresh leaves were extracted using a miniprep modification of Doyle and Doyle's CTAB procedure (1987), or DNeasy Plant Mini kits (QIAGEN Inc., Valencia, California, USA). Herbarium material was extracted via the CTAB procedure, then cleaned using the Elu-quik DNA purification kit (Schleicher and Schuell, Keene, New Hampshire, USA).

General PCR Protocols. PCR protocols were similar for both ITS and *waxy*. PCR reactions were carried out in 25 μL volumes, using Perkin Elmer (Norwalk, Connecticut, USA) AmpliTaq, Mg+ buffer, and dNTPs. The cocktail included: 0.75 μL H_2O, 1.25 μL DMSO (ITS only; Buckler et al. 1997), 2.5 μL dNTPs, 4.15 μL Mg+ buffer, 1.25 μL forward primer, 1.25 μL reverse primer, 1.25 μL glycerol and 0.1 μL *Taq*. This was added to 12.5 μL of diluted DNA sample (1/50–1/100 for high quality DNAs, 2/25 for herbarium DNAs and some *waxy* reactions). The thermocycler program used was: 96°C, 2 min; 30 cycles × 96°C, 1 min, 50°C, 1 min, 72°C, 45 sec; 72°C, 7 min; 4°C hold. For cleanup of PCR products, QIAquick PCR purification kits or QIAquick gel cleanup kits were used (QIAGEN Inc., Valencia, California, USA).

Herbarium PCR Protocols. DNA from herbarium material was often fragmented and very limited in quantity. To improve amplification, the general PCR protocol was modified to: 96°C, 2 min; 10 cycles × 96°C, 1 min, 50°C, 1.5 min, 72°C, 1 min; 25 cycles × 96°C, 1 min, 50°C, 1 min, 72°C, 45 sec; 72°C, 7 min; 4°C, hold. When amplification was weak or undetectable, 1 μL of the 'failed' PCR product was added to new cocktail and run again, generally using a set of primers internal to (or nested within) the set used for the initial PCR attempt. As most 'failed' PCR reactions did produce some copies of the desired gene to serve as templates for re-amplification, re-PCR of 'failed' initial reactions was generally successful. Product from multiple reactions was often pooled for sequencing. Sequences from different individuals of the same species, with one or both obtained from re-amplification of herbarium extractions, clustered together in the analysis, suggesting that any errors resulting from the re-amplification process are not enough to affect significantly the phylogenetic signal.

ITS Primers. Initially, ITS was amplified and sequenced using ITS-2, ITS-3, ITS-4 and ITS-5 (White et al. 1990). Samples from fresh material were also amplified using Leu1 (L. E. Urbatsch), ITS-5A (K. Wurdak) or ITS-5 and ITS-4 or 4A (external to ITS-4: 5′ GGAATCCTTGTAAGTTTC 3′). For herbarium DNAs, ITS was amplified in two halves using Leu1 × ITS-2 or 2C (5′ TGCGTTCAAAGACTCGAT 3′) and ITS-3 × ITS-4 or 4A. When re-amplification of a 'failed' PCR product was necessary, primers internal to the set used for the initial amplification attempt were generally used (ITS-5 or 5A × ITS-2, and ITS-3 × ITS-4). Most samples were sequenced using internal primers, including those mentioned above and ITS-3i (internal to ITS-3: 5′ AATGCGATACTTGGTGTGAA 3′). Two to four sequencing reactions were done per sample, such that most of the resulting sequence was double stranded. Some taxa could not be directly sequenced, and were cloned using either the Invitrogen TA cloning kit, or the Topo TA kit (Invitrogen, San Diego, California, USA).

Waxy *Primers.* In samples from fresh material, approximately 620 bp of *waxy*, between exons 8 and 10, was amplified using *waxy* 3F and 2R, primers originally designed for the Convolvulaceae (Miller et al. 1999). Amplification of *waxy* was limited to this region to avoid length variation within the introns, and direct sequencing of PCR products was successful for the majority of taxa. For herbarium samples *waxy* was amplified in two halves, using 3F and SR (5′ AAAGGTTCAGAYATTCTTGT 3′) and 2R and SF (5′ AGACTTGARGAGCAGAAAGG 3′). Primers SR and SF were designed for this study so that after sequencing, there would still be some overlap between the two amplified segments of the gene.

DNA Sequencing and Alignment. dRhodamine dyes (Applied Biosystems Inc., Foster City, California, USA) were used for cycle sequencing reactions, following the manufacturer's protocols. The resulting products were sequenced on an ABI 377 or 3700 automated sequencer. Sequences were initially corrected and aligned using Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). Further alignment was done manually.

Missing and Composite Taxa. The ITS region was successfully amplified in 75 taxa, but *waxy* proved to be more difficult, and was amplified for only 50 of these. About 45 species of *Physalis* were included in the ITS data set, whereas the *waxy* data set had approximately 35.

Leucophysalis nana was the only taxon represented by different individuals in the two-gene data set. Neither of the two accessions used would produce both an entire ITS and an entire *waxy* sequence. In a preliminary parsimony analysis of ITS data, the partial sequences from both *L. nana* accessions formed a clade, which justified using these individuals to represent the *L. nana* clade in the combined analysis. *Leucophysalis viscosa* was the only taxon included in the two gene data set that had a significant amount of missing data. While represented by an entire ITS sequence, 42% of *waxy* (3F to SR) would not amplify.

Overall, the amount of missing data was low. For the 80-taxon ITS data set, 0.4% of the characters were scored as missing. The 55-taxon *waxy* data set had 1.7% of its characters missing, most of which was due to 231 bp missing from the *L. viscosa* sequence and 91 bp missing from the 5′ end of the *P. acutifolia* sequence. For all data sets, the majority of missing base pairs fell at the 5′ or 3′ ends of sequences.

Outgroup Selection. Outgroups were initially selected on the basis of the earlier cpDNA study (Olmstead et al. 1999), or because their calyx morphology suggested physaloid affinities (D'Arcy and

Averett 1996). Gene trees were rooted with a set of five outgroup taxa, three of which Olmstead et al. (1999) planned to include in tribe Physaleae, including *Iochroma fuchsioides*, *Vassobia lorentzii*, and *Larnax sylvarum*. The two other outgroup taxa were *Capsicum eximium* and *Lycianthes amatitlanensis*, which were tentatively placed in tribe Capsiceae by Olmstead et al. (1999).

Phylogenetic Analyses. Modeltest 3.06 (Posada and Crandall 1998) was used in combination with PAUP* (Swofford 2001) to determine which models of evolution were most appropriate for use with each data set. PAUP* was used for initial parsimony and bootstrapping analyses, and MrBayes 3.0B (Huelsenbeck and Ronquist 2001) was used for the final Bayesian analyses of all data sets. PAUP* was also used to sort and draw the trees produced by MrBayes.

ITS and *waxy* data sets were analyzed separately and in combination. A total of four data sets were analyzed: an 80-taxon ITS data set, a 55-taxon subset of ITS sequences only from taxa for which *waxy* sequences were also available, the matching 55-taxon *waxy* data set, and the combined 55-taxon ITS/*waxy* data set. All data sets are available via TreeBASE (study accession number S1168, matrix accession numbers M2014-M2018).

An incongruence-length difference test (ILD or partition homogeneity test; Farris et al. 1995) was used to gauge the congruence of the two data sets prior to combined analysis. This test is known to be conservative (Yoder et al. 2001; Barker and Lutzoni 2002; Darlu and Lecointre 2002; Hipp et al. 2004), so comparison of changes in bootstrap support in trees from separate and combined analyses, as well as comparison of tree topologies from analyses of separate data sets was also used to pinpoint sources of incongruence.

For the parsimony analyses, heuristic searches were performed with 100 replicates of random addition, TBR branch swapping, and no max trees limit, with gaps treated as missing data. For the 80-taxon ITS data set and the 55-taxon *waxy* data set, each replicate was limited to saving and swapping on no more than 1000 trees because the relatively low numbers of variable characters and large numbers of taxa made full heuristic searches computationally infeasible.

Bootstrap analyses were conducted using a heuristic search of 100 replicates with 10 random addition cycles per replicate and TBR branch swapping. Again, the 80-taxon ITS data set and the 55-taxon *waxy* data set were subjected to a limit of 1000 trees per random addition cycle. Neither the combined data set nor the 55-taxon ITS data set were subjected to tree number limits.

Each data set was also analyzed twice in MrBayes, using 1,000,000 generations per run and sampling trees every 100 generations. Burn-in values were set to 50,000 generations. To verify that -lnL values stabilized before that point, generations (x) were graphed against the likelihood scores (y) of trees sampled after each analysis (Buckley et al. 2002; Miller et al. 2002). Curves from paired runs were compared to verify that they stabilized on similar likelihood values. Trees from each pair of runs were then pooled and used to estimate the posterior probabilities of clades.

Both bootstrap proportions (BS) and posterior probabilities (Pr) were calculated to provide measures of clade support (Felsenstein 1985). Clades with posterior probabilities of 0.95 or better and bootstrap proportions of 70% or more were considered to have strong support.

Results

80-Taxon ITS Data Set. The aligned ITS data set included 18 bp of the 18S gene. After alignment, ITS-1 was 267 bp long, followed by the 164-bp 5.8S gene, and ITS-2, which was 238 bp long. Twenty-two bp of the 26S gene were also included, for a total of 709 base pairs. There were two regions of ambiguous alignment: a 36-bp indel region in ITS-1 (bp positions 76 to 111), and a 21-bp region in ITS-2 (bp positions 474 to 494). In preliminary parsimony and Bayesian analyses, neither alternative alignments nor removing these regions from the analyses had a great effect on resulting tree topologies (not shown), and all rearrangements occurred in areas of the trees that had low clade support (Pr < 0.95, BS < 70%). These indel regions were excluded from the final analyses. After their exclusion, the remaining 652 bp of the ITS region included 427 invariant characters, 57 that were variable but not parsimony informative, and 168 variable and parsimony informative. Smaller indel regions (from 1–5 nucleotides) that were less difficult to align were left in the data matrix, but gaps were treated as missing data, comprising 2.4% of the characters used for analysis. After excluding uninformative characters, parsimony analysis found 56 islands of shortest trees, which produced 52,002 trees of 773 steps (CI = 0.3635, RI = 0.7497, RC = 0.2725). Of the 53 clades appearing in the strict consensus, 38 had Pr ≥ 0.95 in the Bayesian analysis (Fig. 1).

A hierarchical likelihood ratio test (hLRT) determined that the GTR+I+G model (Yang 1994) was the best model of DNA substitution for the ITS data, and this was the model used for the Bayesian analyses. After burn-in, the lnL values of the remaining trees ranged from −5547.205 to −5651.761. A consensus of the 19,002 trees pooled from both analyses resulted in 41 clades with Pr ≥ 0.95, 27 of which had Pr = 1.0.

55-Taxon ITS Data Set. The 55-taxon data set included only those taxa that also had *waxy* sequences (Fig. 1, underlined taxa). Of 652 characters, 443 were constant, 63 were variable but parsimony uninformative, and 146 were parsimony informative. After excluding uninformative characters, parsimony analysis resulted in 2,638 trees of 672 steps (CI = 0.3705, RI = 0.6761, RC = 0.2505) from 14 islands of shortest trees. Twenty-two of 30 clades appearing in the strict consensus (not shown) had Pr ≥ 0.95 in the Bayesian analysis, as did all clades with bootstrap support greater than 70%. There were no significant topological conflicts between the strict consensus of the most parsimonious trees and the 95% consensus of the Bayesian trees.

A hLRT determined that the GTR+I+G model was the best model of DNA substitution for the 55-taxon ITS data. This model was used for both Bayesian analyses. After burn-in, the lnL values of the remaining trees ranged from −4856.567 to −4970.742. A consensus of the 19,002 trees pooled from both analyses resulted in 24 clades with Pr ≥ 0.95, 19 of which had Pr = 1.0. All but one of these clades also had 50% or greater bootstrap support, although high posterior probabilities were not necessarily congruent with high bootstrap support.

ITS Trees. The ITS data strongly support a monophyletic Physalinae (Pr = 1.0, BS = 92%; Fig. 1A, clade A). The Central American physaloids (*Tzeltalia*, *Leu-*

TABLE 3. Voucher information for taxa from which DNA was extracted. The GenBank number for the ITS sequence is listed first, followed by the number for the *waxy* sequence, if applicable ("–" if none). Herbaria: BIRM – the Solanaceae collection at University of Birmingham, UK; DUKE – Duke University, USA; FLAS - University of Florida, USA; LL-TEX, TEX – University of Texas at Austin, USA; MO – Missouri Botanical Garden, USA; NCU –University of North Carolina at Chapel Hill, USA; NIJ – Radboud University Botanical and Experimental Garden, the Netherlands; UT - University of Utah, USA; WTU – University of Washington, USA.

Brachistus stramonifolius Miers, *L. Williams 41524* (DUKE), Solola and Chimaltenango, Guatemala, AY665845, AY665924; *Brachistus stramonifolius* Miers, *Cochrane 2018* (DUKE), Jalisco, Mexico, AY665846, –

Capsicum eximium Hunz., *Bohs 2463* (UT), Cultivated. Seeds from BIRM S038/83, AY665841, AY665923; *Chamaesaracha coronopus* A. Gray, *B. L. Turner 15854* (TEX), Texas, USA, AY665860, AY665937; *Chamaesaracha sordida* A. Gray, *R. G. Olmstead s. n.* (WTU), Cultivated at the Missouri Botanical Garden, AY665861, AY665938; *Chamaesaracha sordida* A. Gray, *Turner 97-0413* (LL-TEX), Crockett Co., TX, USA, AY665862, –

Iochroma fuchsioides Miers, *R. G. Olmstead S-29* (WTU), Cultivated. Seeds from Bogota Jardin Botanical, AY665840, AY665921

Larnax sylvarum (Standl. & C. V. Morton) N. W. Sawyer, *Almeda 2226* (DUKE), Heredia, Costa Rica, AY665839, AY665919; *Leucophysalis grandiflora* (Hook.) Rydb., *R. G. Olmstead S-30* (WTU), Michigan, USA, AY665846, AY665929; *Leucophysalis nana* (A. Gray) Averett, *Bartholomew 5994* (MO), Modoc Co., CA, USA, AY665847, –; *Leucophysalis nana* (Gray) Averett, *M. Williams, 82-108-1* (MO), Douglas Co., NV, USA, AY665847, AY665928; *Leucophysalis viscosa* (Schrader) Hunz., *Torres 7932* (MO), Oaxaca, Mexico, AY665848, AY665927; *Lycianthes amatitlanensis* Bitter, *Bohs 2552* (UT), Puntarenas, Costa Rica, AY665842, AY665922

Margaranthus solanaceous Schldl., also in *Physalis* subgenus *Rydbergis* sect. *Angulatae, R. G. Olmstead S-37* (WTU), Cultivated. Seeds from BIRM S.0610, AY665877, AY665939

Oryctes nevadensis S. Watson, *Tiehm 11982* (LL-TEX), Churchill Co., NV, USA, AY665864, AY665934

Physalis acutifolia (Miers) Sandwith subgenus *Rydbergis* sect. *Angulatae*, NIJ 974750059, Cultivated. Seeds from southwestern USA, AY665876, AY665941; *Physalis alkekengi* L. subgenus *Physalis, M. K. Whitson 1280* (DUKE), Cultivated, AY665850, –; *Physalis alkekengi* L., *M. K. Whitson 1283* (DUKE), also NIJ 914750013, Cultivated, AY665849, AY665931; *Physalis angulata* L. subgenus *Rydbergis* sect. *Angulatae, J. Horn 1284* (DUKE), Worth Co., GA, USA, AY665875, AY665950; *Physalis angustifolia* Nutt. subgenus *Rydbergis* sect. *Viscosae,* M. K. Whitson, no voucher, Florida, USA, AY665878, AY665972; *Physalis angustiphysa* Waterf. subgenus *Rydbergis* sect. *Epeteiorhiza, Ton 9286* (LL-TEX), Chiapas, Mexico, AY665879, –; *Physalis arborescens* L. subgenus *Physalodendron, Jimenez 454* (LL-TEX), Tamaulipas, Mexico, AY665867, AY665936; *Physalis arborescens* L., *Nee 28700* (MO), Veracruz, Mexico, AY665866; *Physalis arenicola* Kearney subgenus *Rydbergis, M. K. Whitson 987* (DUKE), Polk Co., FL, USA, AY665881, AY665964; *Physalis arenicola* Kearney, M. K. Whitson, no voucher, Florida, USA, AY665880, –

Physalis campanula Standl. & Steyerm. subgenus *Rydbergis* sect. *Campanulae, Ventura 4882* (MO), Veracruz, Mexico, AY665882, –; *Physalis carpenteri* Riddell subgenus *Rydbergis* sect. *Carpenterianae, M. K. Whitson 1133* (DUKE), Florida, USA, AY665851, AY665932; *Physalis carpenteri* Riddell, *W. J. Dunn 201*(FLAS 181229), Alachua Co., Florida, USA, AY665852, --; *Physalis caudella* Standl. subgenus *Rydbergis* sect. *Lanceolatae, Quintana 3075* (TEX), Chihuahua, Mexico, AY665891, AY665946; *Physalis chenopodifolia* Lam. subgenus *Rydbergis* sect. *Coztomatae, M. K. Whitson 1287* (DUKE), also NIJ 934750010, Cultivated, AY665883, AY665960; *Physalis cinerascens* A. S. Hitchcock subgenus *Rydbergis* sect. *Viscosae,* M. K. Whitson, no voucher, Kaufman Co., TX, USA, AY665884, AY665971; *Physalis cinerascens* A. S. Hitchcock, M. K. Whitson, no voucher, Kaufman Co., TX, USA, AY665885, --; *Physalis cordata* Mill. subgenus *Rydbergis* sect. *Epeteiorhiza, M. K. Whitson s.n.* (DUKE), Gadsden Co., FL, USA, AY665886, AY665952; *Physalis coztomatl* Dunal subgenus *Rydbergis* sect. *Coztomatae, Ventura 1006* (MO), D. F., Mexico, AY665888, –; *Physalis coztomatl* Dunal, *Garcia 264* (MO), Mexico, Mexico, AY665887, AY665961; *Physalis crassifolia* Benth. subgenus *Rydbergis* sect. *Angulatae,* Richmond, no voucher, California, USA, AY665889, AY665940; *Physalis crassifolia* Benth., *Panero 2824* (MO), Baja California Norte, Mexico, AY665890, –

Physalis glutinosa Schlecht. subgenus *Rydbergis* sect. *Campanulae, Sikes 375* (TEX), Durango, Mexico, AY665892, AY665943; *Physalis greenmanii* Waterf. subgenus *Rydbergis* sect. *Coztomatae, Nee 22432* (MO), Veracruz, Mexico, AY665893, AY665942; *Physalis grisea* (Waterf.) M. Martínez subgenus *Rydbergis* sect. *Epeteiorhiza,* NIJ 894750256, Cultivated , AY665915, AY665949

Physalis hederaefolia A. Gray subgenus *Rydbergis* sect. *Lanceolatae, Van Devender 85-36* (LL-TEX), Brewster Co., TX, USA, AY665894, AY665968; *Physalis hederaefolia* var. *puberula* A. Gray, *Henrickson 5869* (TEX), Chihuahua, Mexico, AY665874, AY665969; *Physalis heterophylla* Nees subgenus *Rydbergis* sect. *Lanceolatae,* M. K. Whitson, no voucher, Caswell Co., NC, USA, AY665907, AY665965; *Physalis* aff. *heterophylla, M. K. Whitson s.n.* (DUKE), Liberty Co., FL, USA, AY665872, --; *Physalis* aff. *heterophylla, M. K. Whitsons.n.* (DUKE), Liberty Co., FL, USA, AY665873, AY665963; *Physalis hintonii* Waterf. subgenus *Rydbergis* sect. *Coztomatae, Villarreal 4909* (MO), Nuevo Leon, Mexico, AY665895, –; *Physalis hintonii* Waterf., *Luckow 3050* (NCU), Veracruz, Mexico, AY665896, –

Physalis ignota Britton subgenus *Rydbergis* sect. *Epeteiorhiza, Breedlove 52891* (MO), Chiapas, Mexico, AY665897, AY665944

Physalis lagascae Roem. & Schult. subgenus *Rydbergis* sect. *Angulatae, Flores 1810* (MO), Nayarit, Mexico, AY665898, AY665954; *Physalis lanceolata* Michx. subgenus *Rydbergis* sect. *Lanceolatae, J. Horn 1133* (DUKE), Scotland Co., NC, USA, AY665899, AY665962; *Physalis lassa* Stand. & Steyerm. subgenus *Rydbergis, Sanders 11807* (MO), Comala, Mexico, AY665900, –; *Physalis longifolia* Nutt. subgenus *Rydbergis* sect. *Lanceolatae, Mona Whitson s.n.*, (DUKE 358627), Riley Co., KS, USA, AY665901, AY665958; *Physalis longifolia* Nutt., *M. K. Whitson 1281* (DUKE), also NIJ 964750022, Cultivated. Seeds from Colorado, USA, AY665902, –

Physalis melanocystis Bitter subgenus *Physalodendron, M. Martínez 1940* (MO), Tamaulipas, Mexico, AY665865, –; *Physalis microcarpa* Urb. & Eckman subgenus *Rydbergis* sect. *Angulatae, Laferriere 1661* (MO), Chihuahua, Mexico, AY665903, AY665947; *Physalis microphysa* A. Gray, *Henrickson 11850* (TEX), Coahuila, Mexico, AY665859, AY665933; *Physalis minima* L. subgenus *Rydbergis,* NIJ 974750167, Cultivated. Seeds from Thailand, AY665904, AY665953; *Physalis minimaculata* Waterf. subgenus *Rydbergis* sect. *Rydbergae, Torres 1595* (TEX), Michoacan, Mexico, AY665905, –; *Physalis minimaculata* Waterf., *Mayfield 986* (TEX), Oaxaca, Mexico, AY665906, --; *Physalis mollis* Nutt. subgenus *Rydbergis* sect. *Viscosae, M. K. Whitson s.n.* (DUKE), Van Zandt Co., TX, USA, AY665908, AY665970

Physalis nicandroides Schlecht. subgenus *Rydbergis* sect. *Epeteiorhiza, L. G. Hernandez 2488* (MO), Morelos, Mexico., AY665912, AY665945

TABLE 3. Continued.

Physalis patula Mill. subgenus *Rydbergis* sect. *Epeteiorhiza, Nee 32810* (MO), Veracruz, Mexico, AY665913, –; *Physalis peruviana* L. subgenus *Rydbergis* sect. *Lanceolatae,* N. Pitman, no voucher, Cultivated. Seeds from Ecuador, AY665914, AY665959; *Physalis philadelphica* Lam. subgenus *Rydbergis* sect. *Angulatae, M. K. Whitson s.n.* (DUKE), Cultivated, AY665871, AY665955; *Physalis* aff. *philadelphica,* NIJ 894750257, Cultivated, AY665868, AY665956; *Physalis pubescens* L. subgenus *Rydbergis* sect. *Epeteiorhiza, M. K. Whitson 3* (DUKE), Seedling 1: seeds from La Selva Biological Station, Costa Rica, AY665916, AY665951; *Physalis pubescens* L., *M. K. Whitson 3* (DUKE), Seedling 2: seeds from La Selva Biological Station, Costa Rica, AY665917; *Physalis pumila* Nutt. subgenus *Rydbergis* sect. *Lanceolatae, M. K. Whitson s.n.* (DUKE), Van Zandt Co., TX, USA, AY665909, AY665967

Physalis sordida Fernald subgenus *Rydbergis* sect. *Lanceolatae, Hinton 18464* (TEX), Nuevo Leon, Mexico, AY665869, AY665948

Physalis virginiana Mill. subgenus *Rydbergis* sect. *Lanceolatae,* M. K. Whitson, no voucher, North Carolina, USA, AY665911, AY665966; *Physalis virginiana* Mill., M. K. Whitson, no voucher, North Carolina, USA, AY665910; *Physalis viscosa* L. subgenus *Rydbergis* sect. *Viscosae, M. K. Whitson 1282* (DUKE), also NIJ 904750326, Cultivated, AY665870, AY665957

Physalis walteri Nutt. subgenus *Rydbergis* sect. *Viscosae,* M. K. Whitson, no voucher, N Florida, USA, AY665918, AY665973

Quincula lobata Raf. = *Physalis* subgenus *Quincula, R. G. Olmstead 93-74* (WTU), Boulder Co., CO, USA, AY665863, AY665935 *Tzeltalia amphitricha* (Bitter) Estrada & M. Martínez, *E. Martínez 20523* (LL-TEX), Chiapas, Mexico, AY665853

Tzeltalia calidaria (Standl. & Steyerm.) Estrada & Martínez, *Lundell 19625* (LL-TEX), Baja Verapaz, Guatemala, AY665855, AY665930; *Tzeltalia calidaria* (Standley & Steyermark) Estrada & M. Martínez, *Matuda 5199* (LL-TEX), Chiapas, Mexico, AY665854, –

Vassobia lorentzii (Dammer) Hunz, *R. G. Olmstead S-18* (WTU), Birmingham seed collection, S.0376, AY665843, AY665920

Witheringia macrantha (Stadl. & Morton) Hunz., *Bohs 2512* (UT), Monteverde, Costa Rica, AY665857, AY665925; *Witheringia meiantha* (Donn. Sm.) Hunz., *Bohs s.n.* (UT), No collection data, AY665856, –; *Witheringia solanacea* L'Her., *Bohs 2427* (UT), Alajuela, Costa Rica, AY665858, AY665926

cophysalis viscosa, Brachistus, and *Witheringia*) hold basal positions within the subtribe (Fig. 1A, clade B and surrounding taxa), followed by a grade of morphologically atypical *Physalis* species intermixed with physaloid genera. The morphologically typical, New World species of *Physalis* form a clade (Pr =1.0, BS = 99%; Fig. 1A, clade C), with the notable exclusion of the type species *P. alkekengi* (China), which is sister to *P. carpenteri* (southeastern U.S.).

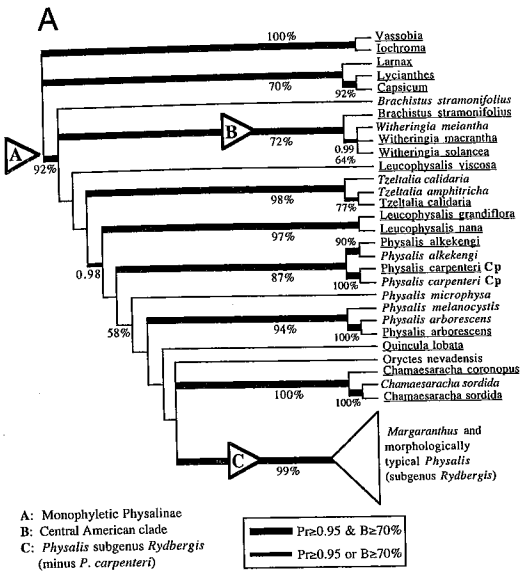
The morphologically typical *Physalis* clade (Fig. 1B) is generally congruent with subgenus *Rydbergis*. The other three subgenera of *Physalis* (*Physalis, Physalodendron,* and *Quincula*) do not group with the *Rydbergis* clade, but appear among the grade of physaloid taxa near its base. Both species in *Physalis* subgenus *Physalodendron* (*P. arborescens* and *P. melanocystis*) form a clade. Support for species groups within the *Rydbergis* clade is weak, but it appears that most sections of subgenus *Rydbergis* are not monophyletic.

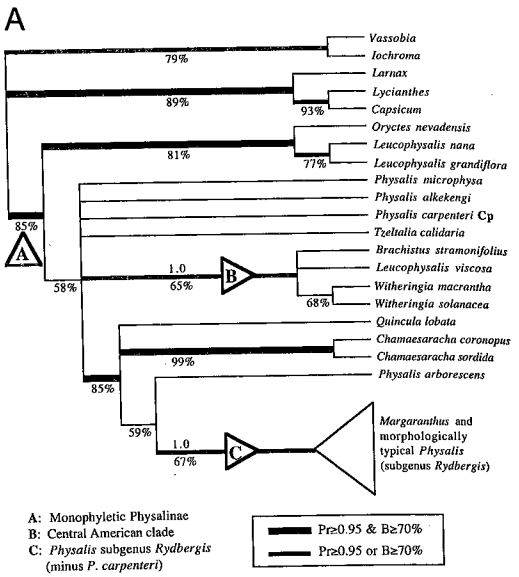
With the exception of *Margaranthus,* all physaloid genera are distinct both from the main *Physalis* clade and from each other (Fig. 1A). Only *Margaranthus* nests within the *Rydbergis* clade. The two species of *Chamaesaracha* form a clade, as do both species of *Tzeltalia*. The Central American species *L. viscosa* makes the otherwise North American genus *Leucophysalis* paraphyletic. The results of the 55-taxon ITS analysis (Fig. 1, underlined taxa) were congruent with those from the 80-taxon analysis.

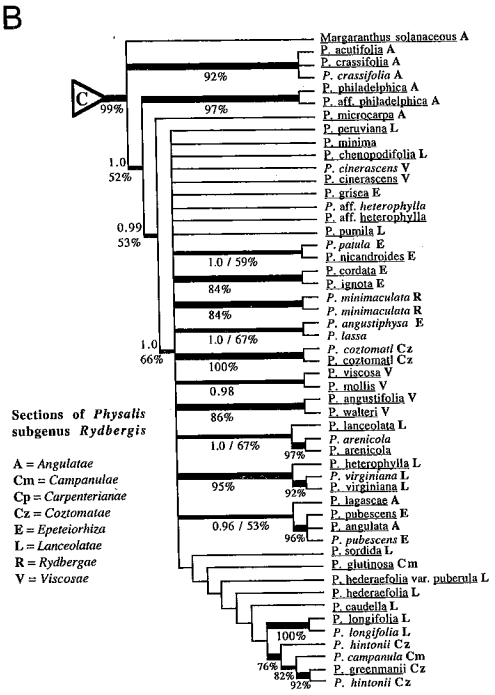
Waxy *Data Set.* The *waxy* data set had 622 characters, of which 433 were constant, 105 were variable but parsimony uninformative, and 84 were variable and parsimony informative. Gaps made up 0.2% of the parsimony informative characters used for analysis and were treated as missing data. Parsimony analysis found 76,000 shortest trees, each 195 steps long (CI = 0.5641, RI = 0.8055, RC = 0.4544), from 77 islands. Thirteen of 25 clades appearing in the strict consensus had Pr ≥ 0.95 in the Bayesian analysis (Fig. 2). There were no significant topological conflicts between the strict consensus of the most parsimonious trees and the 95% consensus of the Bayesian trees.

A hLRT determined that the HKY85+G model (Hasegawa et al. 1985) was the best model of DNA substitution for the *waxy* data. This model was used for both Bayesian analyses. After burn-in, the lnL values of the remaining trees ranged from −3082.137 to −3154.935. A consensus of the 19,002 trees pooled from both analyses resulted in 13 clades with Pr ≥ 0.95, 10 of which had Pr = 1.0. All but one of these clades also had 50% or greater bootstrap support in the parsimony trees (Fig. 2).

Waxy *Gene Trees.* Like the ITS data sets, the *waxy* data supported both a monophyletic subtribe Physalinae (Pr =1.0, BS = 85%) and a large clade of morphologically typical, New World *Physalis* species (Pr = 1.0, BS = 67%; Fig. 2A). Within the main *Physalis* clade (Fig. 2B), there was generally little support for smaller species groups. However, two clades did have strong support: a clade of seven perennial taxa with branched to stelliform hairs (Pr = 1.0, BS = 97%), most of which belong to *Physalis* section *Viscosae,* and a pair of annual species, *P. angulata* and *P. pubescens* (Pr = 1.0, BS = 99%). As with the ITS data, *Chamaesaracha* was supported as monophyletic (Pr = 1.0, BS = 99%) and *Leucophysalis* was paraphyletic. Unlike the ITS data, *Oryctes* is supported as sister to the two North American species of *Leucophysalis* (Pr = 1.0, BS = 81%), and a sister-species relationship between *P. carpenteri* and *P. alkekengi* was not supported. Though the *L. viscosa*







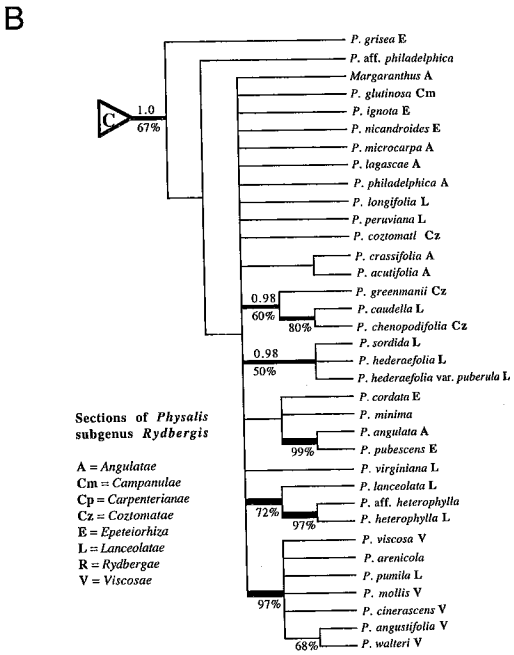
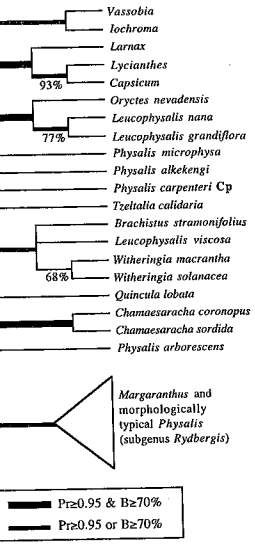


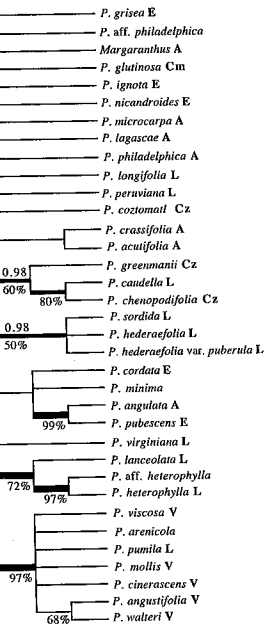
FIG. 1A. Basal clades of the strict consensus of 52,005 most parsimonious 80-taxon ITS gene trees. Bootstrap values (BS) are provided as percentages for all clades with support of 50% or more. Posterior probabilities (Pr) between 0.95 and 1.0 are shown for branches with bootstrap support less than 70%, and are otherwise indicated by branch width. Underlined taxa were used in the 55-taxon ITS and combined analyses. B. Fig. 1 continued. Detail of *Physalis* subgenus *Rydbergis* clade. Species not marked with sectional abbreviations were not included in Martínez's (1999) infrageneric classification.

FIG. 2A. Basal clades of the strict consensus of 76,000 most parsimonious *waxy* gene trees. Bootstrap values (BS) are provided as percentages for all clades with support of 50% or more. Posterior probabilities (Pr) between 0.95 and 1.0 are shown for branches with bootstrap support less than 70%, and are otherwise indicated by branch width. B. Fig. 2 continued. Detail of *Physalis* subgenus *Rydbergis* clade.

waxy sequence was incomplete (missing 42 of 84 parsimony informative characters), the *waxy* data agreed with the ITS data, placing *L. viscosa* with the Central American physaloids rather than with the other species of *Leucophysalis* (Fig. 2A, clade B).

Assessing Incongruence. An ILD test produced a





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2A, clade B).

ce. An ILD test produced a

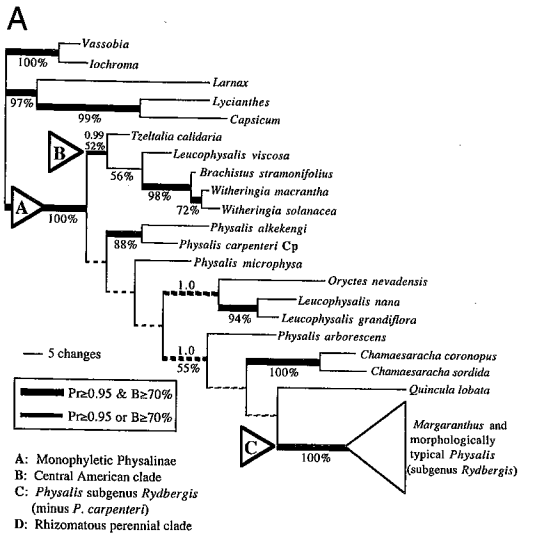
set of trees all longer than the sum of tree lengths from the original partition, indicating that the two data sets were significantly incongruent ($p = 0.01$). Performing the analysis without outgroups and other divergent taxa (e.g., *Oryctes*) did not affect this result. However, an increasing number of studies indicate that the ILD test may detect significant incongruence between data sets even when they produce trees with similar topologies, especially when character numbers are limited or there is rate heterogeneity (Barker and Lutzoni 2002; Darlu and Lecointre 2002; Hipp et al. 2004). Due to the conservative nature of the ILD test, a combined analysis of the two data sets was preformed, but only after separate analysis of each data set. Areas of well-supported disagreement between gene trees from separate analyses were noted prior to analysis of the combined data. Comparison of the strongly supported clades in trees from analyses of separate data sets revealed two points of incongruence: the placement of the monotypic genus *Oryctes*, and species relationships among the closely related North American members of *Physalis* section *Lanceolatae*. Relationships among taxa whose positions conflicted between separate data sets were considered tentative in the combined analysis, even in cases where statistical support was high.

Combined Data Set. The combined data set consisted of 230 parsimony informative characters for 55 taxa. Parsimony analysis found 40 shortest trees, each 909 steps long (CI = 0.3949, RI = 0.6845, RC = 0.2703), distributed in 20 islands.

Bayesian analysis of the combined data was conducted using a different model of evolution for data from each gene: HKY+G for the *waxy* data and GTR+I+G for the ITS data. After burn-in, the lnL values of the remaining trees ranged from −8135.648 to −8329.043. A consensus of the 19,002 trees pooled from both analyses resulted in 31 clades with Pr ≥ 0.95, and 23 with Pr = 1.0.

Two-gene Trees. The combined analyses produced 28 clades with either Pr ≥ 0.95 or bootstrap support of 70% or more (Fig. 3). For the areas of conflict between the separate data sets, the *waxy* data decided the position of *Oryctes* in the combined analyses and there was a loss of resolution among the members of the North American *Lanceolatae* complex. Most clades with strong support also appeared in one or both separate analyses, but one well-supported clade of perennial species (Fig. 3B, clade D) was unique to the combined analyses.

As with the analyses of separate data sets, the combined analyses strongly supported a monophyletic Physalinae (Pr = 1.0, BS = 100%). There was also support (Pr = 0.99, BS = 52%) for a basal clade of shrubby, Central American physaloid taxa, including *Tzeltalia*, *Leucophysalis viscosa*, *Brachistus*, and *Witheringia* (Fig. 3A, clade B). Among the herbaceous physaloids, *Oryctes* was supported as being sister to the North American members of *Leucophysalis*, and *P. alkekengi* and *P. carpenteri* were supported as sister taxa. One of the most strongly supported (Pr = 1.0, BS = 100%) branches in the tree separated the physaloids from the morphologically typical species of *Physalis*. There was little resolution among the majority of *Physalis* species,



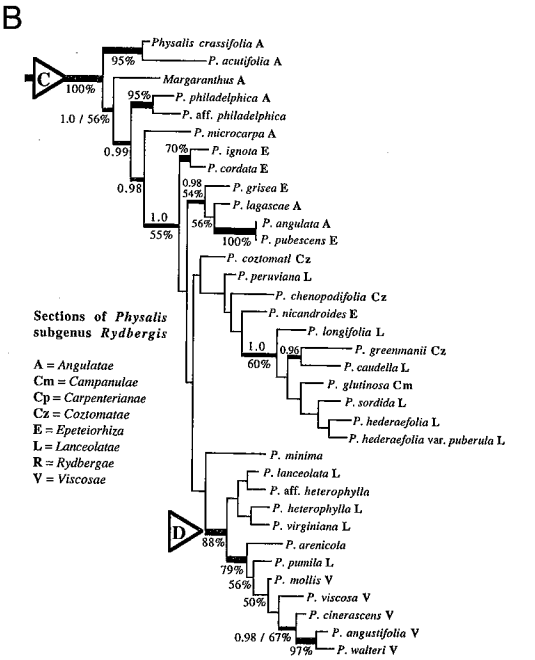


FIG. 3A. Basal clades of one of 40 most parsimonious trees from the two-gene phylogeny of the Physalinae. Bootstrap values (BS) are provided as percentages for all clades with support of 50% or more. Posterior probabilities (Pr) between 0.95 and 1.0 are shown for branches with bootstrap support less than 70%, and are otherwise indicated by branch width. B. Fig. 3 continued. Detail of *Physalis* subgenus *Rydbergis* clade.

except for two well-supported clades: five U.S. perennial species in section *Lanceolatae* and five species of section *Viscosae* formed a clade (Pr = 1.0, BS = 88%), and within that clade, all members of section *Viscosae* and two species with occasional branched hairs also formed a monophyletic group (Pr = 1.0, BS = 79%).

Discussion

Overview. Both ITS and *waxy* gene trees show that the genus *Physalis* is paraphyletic, as previous cpDNA studies suggested (Mione et al. 1994; Olmstead et al. 1999). The more extensive taxon sampling used here clarifies the extent of the problem and suggests that the highly-inflated fruiting calyx considered so definitive of *Physalis* has arisen multiple times throughout the Physalinae and in other genera outside this subtribe. However, the morphologically typical species of *Physalis* do form a clade that also includes the monotypic physaloid genus *Margaranthus* (Fig. 3B). The morphologically atypical *Physalis* species, including the type species *P. alkekengi*, are not included within the clade of morphologically typical species (Fig. 3A). The genera *Chamaesaracha*, *Leucophysalis*, *Quincula*, and *Tzeltalia* were all well separated from the clade of morphologically typical *Physalis* species, supporting their earlier exclusion from *Physalis* on morphological grounds (Fig. 3A).

Thirty of the 35 *Physalis* species sampled, representing the New World members of the genus, form the most derived clade within the Physalinae (Fig. 3B). This clade generally corresponds to *Physalis* subgenus *Rydbergis*. The group is morphologically homogeneous, with most species having a herbaceous habit, solitary flowers, unlobed yellow corollas and highly inflated fruiting calyces. *Margaranthus solanaceous* also falls within this group, supporting its current inclusion in *Physalis* (Martínez 1999; Axelius 1995).

The *Physalis* species not falling within the *Rydbergis* clade are all morphologically atypical, either having multiple flowers per node, corollas which are lobed or odd colors (e.g., purple or white), or unusual fruiting calyx morphology. These atypical species include the three remaining subgenera of *Physalis* recognized by Martínez (1999): *Physalis* (the type species *P. alkekengi*), *Physalodendron* (*P. arborescens* and *P. melanocystis*), and *Quincula* (*P. lobata*). Relationships among the atypical *Physalis* species and the North American physaloid genera (e.g., *Oryctes*, *Leucophysalis*, *Quincula*, and *Chamaesaracha*) are poorly resolved, but there is strong support for separating these taxa from the four Central American taxa that form the basal-most clades of the Physalinae (*Brachistus*, *Leucophysalis viscosa*, *Tzeltalia*, and *Witheringia*).

Nomenclatural Implications. To correct the paraphyly of *Physalis*, nomenclatural changes are required. Options include restricting the name *Physalis* to *P. alkekengi*, the type, and renaming the 75+ species of New World *Physalis*, or broadening the circumscription of *Physalis* by uniting the majority of the Physalinae into a single genus. However, the least taxonomically disruptive approach for dealing with this problem is to re-typify *Physalis* using a Linnaean species that is a member of the morphologically typical *Rydbergis* clade, such as *P. pubescens*. The atypical species could then be recognized as four small genera (for *P. carpenteri*, *P. alkekengi*, *P. microphysa*, and subgenus *Physalodendron*), which would produce a morphologically homogeneous *Physalis*. A proposal to re-typify *Physalis* is currently in progress.

***Species Relationships Within Subgenus* Rydbergis.** Although morphological characters seem to be reliable in delimiting monophyletic physaloid genera, they are not particularly useful for delimiting monophyletic species groups within *Physalis*. Most of the sections of *Physalis* appear to be paraphyletic, but species relationships within the *Rydbergis* clade were for the most part poorly supported. However, the monophyly of section *Viscosae* was well supported, and it proved to be nested within a clade of species from section *Lanceolatae*. This *Lanceolatae/Viscosae* group is made up of mostly U.S. species, and may represent a northward radiation from Mexico, which is the center of diversity for *Physalis*, and where the basally-branching members of the *Rydbergis* clade originate (Fig. 3B).

Morphological and geographical characters have been the primary criteria for establishing sections within *Physalis*, though Menzel (1951) used cytological data as well (Rydberg 1896; Martínez 1999). Four of the nine sections of subgenus *Rydbergis* are small, with only 1–2 species (Table 1). With the exception of *P. carpenteri*, the remaining species of subgenus *Rydbergis* form a clade, but relationships within this group are poorly resolved (Fig. 3B).

ITS data place *P. minimaculata*, one of two species in section *Rydbergae*, within the main clade of *Physalis* species. Neither the placement nor the branch length of this species justifies separation from the larger sections of the genus. Martínez (1999) noted that there are several unnamed species from Mexico that should belong to this section, and additional molecular data from these species may help to determine whether this section should be recognized.

The two species of section *Campanulae*, *P. campanula* and *P. glutinosa*, share unusually large flowers. Apart from that, they are morphologically distinct, and the ITS data provide no support for a sister-taxon relationship, suggesting that this section may be an artificial grouping. These species might better be recognized as distinctive members of one (or two) of the larger sections.

The remaining five sections of subgenus *Rydbergis* contain from 6–14 species, and are based primarily on

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kekengi, the type, and renaming the 75+ species of New World *Physalis*, or broadening the circumscription of *Physalis* by uniting the majority of the Physalinae into a single genus. However, the least taxonomically disruptive approach for dealing with this problem is to re-typify *Physalis* using a Linnaean species that is a member of the morphologically typical *Rydbergis* clade, such as *P. pubescens*. The atypical species could then be recognized as four small genera (for *P. carpenteri*, *P. alkekengi*, *P. microphysa*, and subgenus *Physalodendron*), which would produce a morphologically homogeneous *Physalis*. A proposal to re-typify *Physalis* is currently in progress.

***Species Relationships Within Subgenus* Rydbergis.** Although morphological characters seem to be reliable in delimiting monophyletic physaloid genera, they are not particularly useful for delimiting monophyletic species groups within *Physalis*. Most of the sections of *Physalis* appear to be paraphyletic, but species relationships within the *Rydbergis* clade were for the most part poorly supported. However, the monophyly of section *Viscosae* was well supported, and it proved to be nested within a clade of species from section *Lanceolatae*. This *Lanceolatae/Viscosae* group is made up of mostly U.S. species, and may represent a northward radiation from Mexico, which is the center of diversity for *Physalis*, and where the basally-branching members of the *Rydbergis* clade originate (Fig. 3B).

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The remaining five sections of subgenus *Rydbergis* contain from 6–14 species, and are based primarily on the gross morphological characters traditionally used in *Physalis* taxonomy. Sections *Angulatae* and *Epeteiorhiza* contain mostly annual species, and are distinguished from one another on the basis of calyx angles. In fruit, members of section *Angulatae* have rounded or 10-angled calyces, whereas most members of section *Epeteiorhiza* have five. The type species of the two sections, *P. angulata* and *P. pubescens* (respectively), formed one of the most strongly supported pairs of sister taxa in the *Rydbergis* clade. Both species are weedy annuals, but are easily distinguished by several morphological characters, and there has been no suggestion that they hybridize. Their identical *waxy* and ITS sequences (even after sampling multiple individuals of both species) are unusual examples of the lack of sequence differentiation at certain genic regions despite morphological divergence.

Several species of section *Angulatae* form the most basal clades of the monophyletic subgenus *Rydbergis* (Fig. 3B), including *Margaranthus*, a monotypic physaloid which Martínez places in this section as *P. solanaceous*. This result is congruent with a morphological cladistic analysis conducted by Axelius (1996), who also found that *Margaranthus* nested within a clade of morphologically typical *Physalis* species. The urceolate flowers of *Margaranthus*, although unique within *Physalis*, resemble partially open *Physalis* flowers, with their yellow color obscured by dark, basal spots extending most of the length of the corollas. The corolla vasculature is also quite similar to that of *Physalis* (Averett 1979). In fruit, *Margaranthus* resembles an annual *Physalis*, and its chromosome morphology and number ($x = 12$) are similar to those of the annual species surveyed by Menzel (1950).

Section *Coztomatae* contains 11 distinctive Mexican species characterized by dark corolla maculations formed from conglomerations of smaller spots. There is no support for a monophyletic *Coztomatae*, although this group is represented by only three taxa in the two gene data set. Resolution is poor, however, and neither species is strongly supported as being sister to taxa from another section. Sampling is slightly better in the 80-taxon ITS tree, but the results are similar. Compound corolla maculations throughout the Physalinae, and are either symplesiomorphic for the group or have evolved independently several times. It is unlikely that section *Coztomatae* is monophyletic, but further sampling may reveal monophyletic subsets of species.

Two sections of *Physalis* are devoted to rhizomatous, perennial species: *Viscosae*, including only taxa with branched, stelliform or dendroid-stelliform hairs (Seithe and Sullivan 1990), and *Lanceolatae*, including species with mostly unbranched trichomes. Section *Viscosae* is monophyletic and nested within the *Lanceolatae/Viscosae* clade (hereafter L/V; Fig. 3B, clade D). Section *Lanceolatae*, however, is polyphyletic, with the recently described Mexican species (Martínez 1999) and two species from the southwestern U.S. (*P. longifolia*, *P. hederifolia*) scattered throughout the rest of the *Rydbergis* clade. A rhizomatous habit and unbranched hairs, two of the major morphological characters defining the section *Lanceolatae*, are widespread within *Physalis*. The monophyletic or paraphyletic complex of *Lanceolatae* species, including *P. lanceolata*, (hereafter *Lanceolatae* s.s.) are endemic to the U.S., with at least part of their ranges in either the Southeast or Midwest.

Although *P. longifolia* has long been considered part of a complex of three wide-ranging taxa that also includes *P. heterophylla* and *P. virginiana*, the DNA data did not support a close relationship between *P. longifolia* and the other two species. These three species range from the eastern U.S. and southeastern Canada, across the Midwest and down to the southwestern U.S., but *Physalis longifolia* is the only species ranging south into Mexico. Two morphological features do differentiate *P. longifolia* from the *Lanceolatae* s.s., nearly glabrous shoots and corolla spotting that is dense and smudgy (as opposed to the distinct feathered spots seen in the *Lanceolatae* s.s.). *Physalis longifolia* appears to be more closely related to Mexican and southwestern-U.S. *Physalis* species than to southeastern-U.S. species. Unlike other southeastern-U.S. members of section *Lanceolatae*, *P. longifolia* does occur in Mexico, suggesting that its widespread presence in the eastern U.S. may exemplify a successful range expansion by a species originally of southwestern U.S. or Mexican origin.

Menzel (1951) reported successful crossing of *P. longifolia* and *P. virginiana*, with apparently fertile F_1 offspring resulting. At that time, however, species boundaries between these two taxa were confused, and two of the varieties of *P. virginiana* (var. *subglabrata* and var. *sonorae*) are now considered forms of *P. longifolia*. Hinton (1976) reported low seed set in crosses between *P. pumila* and *P. virginiana*, which are both in the L/V clade (Fig. 3B, clade D), but no seed set in crosses between *P. pumila* and *P. longifolia*, as would be expected if *P. longifolia* is not closely related to the species of the L/V clade.

While hybridization between *Physalis* species has often been suggested (Menzel 1951, 1960; Waterfall 1967), documented cases in the field are rare (Hinton 1975; Sullivan 1985). Most evidence for successful hybridization within *Physalis* has been found from artificial crosses among the closely related species of the L/V clade (Fig. 3B, clade D). Even then, most crosses between the more distantly related members of the clade either fail or result in low seed set and stunted F_1s (Hinton 1976; Sullivan 1985). Menzel (1951) found that crosses made between species in different sections of the genus were generally unsuccessful.

Two species pairs within the L/V clade are known to hybridize naturally. In the combined analysis, *Phys-*

alis angustifolia and *P. walteri* were strongly supported as sister species, and hybridize freely where their ranges overlap (Sullivan 1985). If fact, Waterfall (1967) treated both taxa as varieties of *P. viscosa* L. Natural hybrids between *Physalis heterophylla* and *P. virginiana* are occasionally reported (Hinton 1975), but only the ITS data strongly supported a sister-species relationship. Overall, natural hybridization within *Physalis* seems to be uncommon and limited to species which are very closely related. Hybridization between closely related species could obscure phylogenetic relationships and may account for poor resolution between such taxa, but it is difficult to distinguish this from a lack of resolution due to recent divergence of taxa (and thus few available differentiating characters). As hybridization between distantly related species has not been demonstrated, phylogenetic relationships between species groups within *Physalis* should not be affected.

The monophyly of section *Viscosae* is strongly supported by both molecular and morphological characters. Widespread within this section are dichotomously branched to stelliform hairs, often so short and dense that the plants appear velvety. Also common are flowers with distinct black maculations, and tangerine orange fruit with yellow to orange fruiting calyces. A notable aspect of the *Viscosae* is the predominance of coastal species. *Physalis walteri* is a common dune species from the Carolinas south to Florida, while *P. angustifolia* ranges along the coasts of Florida and west to Louisiana. *Physalis cinerascens* var. *spathulaefolia* inhabits coastal Louisiana and Texas, *P. vestita* is found along Mexican coasts, and *P. viscosa* occurs along coastlines from Mexico to northern South America. This is a closely related complex of species, most of which form hybrids in artificial crosses (Sullivan 1985), and includes one of the rare pairs of species (*P. walteri* and *P. angustifolia*) between which natural hybridization is common. *Physalis vestita* and *P. viscosa* are the only species in the complex with no native U.S. populations, although some populations of *P. viscosa* have been introduced.

The molecular analysis placed *P. pumila* in the *Viscosae* clade. Although Martínez (1999) classified *P. pumila* as a member of section *Lanceolatae*, definitive placement of this species has long been confounded by the fact that the plants have unbranched hairs typical of section *Lanceolatae* intermixed with dichotomously branched hairs more typical of species in section *Viscosae* (Menzel 1951; Seithe and Sullivan 1990). Hinton (1976) found that *P. pumila* generally produced some seed when crossed with members of section *Lanceolatae*. On the other hand, Sullivan (1985) found that *P. pumila* also produced seed when crossed with *P. mollis* in section *Viscosae*. These results are consistent with the fact that all members of the L/V clade appear to be closely related.

The placement of *P. arenicola* at the base of the *Viscosae* clade was unexpected, as Menzel (1951) considered it a member of *Lanceolatae*, and the majority of plants have simple hairs. This predominantly Floridian species was not included in Martínez's treatment of the genus (1999), but fits well morphologically with her section *Lanceolatae*. However, unlike most species of *Lanceolatae* s.s., which have the occasional branched hair intermixed with many unbranched hairs, rare individuals of *P. arenicola* may have mostly branched hairs. *Physalis arenicola* has a restricted range, being very common throughout Florida and quickly becoming scarce in neighboring states. Along with two other members of *Viscosae* (*P. angustifolia* and *P. walteri*), it is one of the most common perennial species in Florida, the only southeastern state mostly uninhabited by members of *Lanceolatae* s.s. (only *P. aff. heterophylla* and *P. virginiana* are occasionally reported from the northernmost counties). The L/V clade represents about half of the species native to the U.S., and is a striking example of a northern radiation into temperate habitats. Both morphology and molecular data support the recognition of section *Lanceolatae* s.s., and the Mexican and southwestern U.S. species that do not group with this clade should be reassigned to other sections. This will be done when there are additional molecular data to suggest where these non-*Lanceolatae* species should be placed.

The Physaloid Grade. Four physaloid genera and four morphologically atypical species of *Physalis* form a grade at the base of the *Rydbergis* clade (Fig. 1A), but support for the node separating these taxa from the basal Central American taxa is lost in the combined analysis. The taxa in the physaloid grade encompass most of the morphological variation within the Physalinae. They share some amount of calyx expansion in fruit, and most of them have multiple flowers per node (Appendix I). Relationships between these taxa were generally not well resolved by the molecular data, but morphology provides additional information.

Physalis alkekengi and *P. carpenteri* form one of the most strongly supported pairs of sister taxa in the physaloid grade. This relationship, however, is only supported by the ITS data, as the *waxy* gene tree is unresolved for these taxa. A sister-taxon relationship between the individuals sampled is also supported by cpDNA sequences from the *ndhF* gene (Bohs, unpublished). *Physalis carpenteri* and *P. alkekengi* share an odd morphological trait, corky bodies intermixed with their seeds (Estrada and Martínez 1999). Otherwise, these taxa are morphologically dissimilar. *Physalis alkekengi* is a rhizomatous, perennial herb with 5-lobed, white corollas and red-orange fruit and fruiting calyces, whereas *P. carpenteri* is a woody, taprooted perennial with unlobed, yellow corollas and brown fruiting calyces around yellow berries. However, the most strik-

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The placement of the North American species of *Leucophysalis* in this analysis of nuclear DNA is congruent with the placement of this taxon in earlier cpDNA studies (Mione et al. 1994; Olmstead et al. 1999). *Leucophysalis nana* and *L. grandiflora* are sister taxa, which is supported by the very similar morphology and flavonoid chemistry of the two species (Averett 1979). Like *P. alkekengi* and *P. carpenteri*, but certainly less extreme, these taxa have a broad geographical disjunction. *Leucophysalis nana* is a plant of the Sierra Nevada Range (Averett 1979), while *L. grandiflora* is one of the few north temperate members of the Physalinae, occurring in the Great Lakes region of the U.S. and Canada. *Leucophysalis viscosa*, an unusual Central American species recently transferred to the genus (Hunziker 1991), groups not with the North American *Leucophysalis*, but with other Central American taxa such as *Witheringia*.

Oryctes nevadensis has been recognized as a physaloid genus since Rydberg (1896) treated it in his monograph of *Physalis* and related genera. The molecular data strongly support the placement of this taxon within the Physalinae, a result suggested by cpDNA data (Olmstead et al. 1999), but the exact affinities of *Oryctes* remain unclear. The derived physaloids, including *P. arborescens*, *Chamaesaracha*, *Quincula*, and members of the *Rydbergis* clade, all share a 19 bp deletion in ITS1, which is also found in *Oryctes*, arguing for its placement among these taxa. *Oryctes* is rare and poorly known, and our current knowledge of its morphology offers little information about its affinities. The tubular, purple corollas are unique within the subtribe, but the flavonoids of *Oryctes* are typical of much of the Solaneae, as well as *Leucophysalis* and *Chamaesaracha* (Averett and D'Arcy 1983). *Oryctes* also has an unusual seed testa pattern, and while it is unlike those seen in *Physalis* or *Chamaesaracha*, seed testa patterns have not been exhaustively studied in physaloids, so whether *Oryctes* will share this trait with other taxa remains to be seen (Axelius 1992).

Physalis microphysa is unusual even for an 'anomalous' species of *Physalis*. While most morphologically atypical species are notable for multiple flowers per node and/or lobed corollas, *P. microphysa* has typical, *Physalis*-like, solitary yellow flowers, but unique fruiting calyces. The calyces only enlarge to about 1/2" long, are deeply lobed, and never close at the apices. Rydberg (1896) suggested removal of this species from *Physalis*. Martínez (1999) agreed, declining to treat this species in her revision of the genus and suggesting that *P. microphysa* and its putative sister species, *P. parvianthera*, be placed in a new genus, *Cascada*. In both separate and combined analyses, *Physalis microphysa* was separated from the clade of morphologically typical *Physalis* species which seems to support its removal from *Physalis* s.s. However, further sampling of physaloid taxa and of *P. parvianthera* would help determine whether *P. microphysa* merits a new genus.

The most strongly supported node along the backbone of the physaloid grade separates the basal members of the grade from *P. arborescens*, *Quincula*, and *Chamaesaracha* (Figs. 2A, 3A). Though ITS data support *P. arborescens* and *P. melanocystis* as sister species, and thus support the monophyly of *Physalis* subgenus *Physalodendron*, too little is known about the morphology of these species to discuss their affinities within the physaloid grade. The placement of this Central American subgenus among otherwise Chihuahuan/Sonoran taxa (*Chamaesaracha*, *Quincula*, and several basal species of *Physalis*) is interesting because most of the other Central American physaloids fall at the base of the Physalinae clade (Fig. 1A).

There is no support for a sister-taxon relationship between *Quincula* and *Chamaesaracha*, but in all analyses both genera fall together at the base of the *Physalis* subgenus *Rydbergis* clade, though this relationship does not always have strong support. Several morphological characters affirm the close relationship between *Quincula* and *Chamaesaracha*. Pinnatifid leaves occur in both *Chamaesaracha* and *Quincula*, a trait unique within the Physalinae. The two taxa also share fruit with basal placentation and similar corolla vasculature (Averett 1979). A trait unique to *Quincula*, and one often used to argue for the recognition of this genus, is its unique base chromosome number, $x = 11$. The other members of the Physalinae and the majority of the Solanoideae have $x = 12$. Barboza (2000) argued for recognizing *Quincula* as distinct from *Physalis* on the basis of several novel morphological traits, including calyx venation. That *Quincula* doesn't group with the main *Physalis* clade also supports its recognition as a distinct genus. While both *Quincula* and *Chamaesaracha* have similarities to *Physalis*, the molecular data are not decisive and it is unclear which, if either, genus is sister to the *Rydbergis* clade. The inflated fruiting calyces of *Quincula* look much like those of *Physalis*, while the pale, spotted flowers of *Chamaesaracha* are more *Physalis*-like than those of *Quincula*.

Relationships among the Basal Taxa of Physalinae. *Brachistus* is a small genus of three species, and has been considered a section of *Witheringia* (D'Arcy et al. 1981). Morphologically, *B. stramonifolius* is very similar to *Witheringia*, the main difference being the slight expansion of the fruiting calyces, which causes them to gently clasp the sides of the maturing berries. The two-gene phylogeny (Fig. 3A) supports *Brachistus* and *Witheringia* as sister taxa, though the 80-taxon ITS phylogeny (Fig. 1A) places the two accessions of *B. stramonifolius* in different positions. However, the genus *Brachistus* is poorly known, and it is quite possible that there are more than the three currently recognized species. *Brachistus* was segregated from *Witheringia* partly on the basis of its comparatively primitive calyx morphology (D'Arcy 1986), and its recognition as a distinct genus warrants further study.

Tzeltalia is a recent segregate of *Physalis*, and consists of the two shrubby species from Guatemala and southern Mexico, which were formerly placed in *Physalis* section *Calidariae* (*P. calidaria* and *P. amphitricha*) (Estrada and Martínez 1998). Both the monophyly of this small genus and its recognition as distinct from *Physalis* are supported by ITS data. Citing shared calyx, corolla, and inflorescence characters, as well as similar habits, Estrada and Martínez (1998) hypothesized a close relationship between *Tzeltalia* and the largely Andean genus *Deprea*. However, the molecular data groups *Tzeltalia* with other Central American physaloids, and places *Larnax sylvarum* (formerly *Deprea*) among the distant outgroups. This result demonstrates that characters such as floral lobing and calyx expansion are relatively plastic among the physaloid taxa and in the tribe Solaneae, and have likely been lost, gained, or modified numerous times.

The taxon currently called *Leucophysalis viscosa* has been placed in no less than six genera: *Athenaea*, *Chamaesaracha*, *Jaltomata*, *Physalis*, *Saracha*, and *Witheringia*. Its placement in the Central American clade (Fig. 3A, clade B) at the base of the Physalinae, as opposed to sister to the two North American species of *Leucophysalis*, indicates that this species should be removed from *Leucophysalis*. Morphology supports the placement of *L. viscosa* with *Brachistus* and *Witheringia*, as it is vegetatively similar to both genera, the major differences being its larger fruit size (1+ cm dia) and highly expanded fruiting calyx (Appendix 1). Further sampling of *Witheringia* and *Brachistus* would be helpful in clarifying whether recognition of *L. viscosa* as a distinct genus is justified.

Morphological Trends Within Physalinae. There are several morphological trends within the Physalinae (Fig. 4; Appendix 1). In general, there is a shift from a woody habit in the basal physaloids to a herbaceous habit in more derived taxa. Flower shape moves from a symplesiomorphic stellate form common throughout

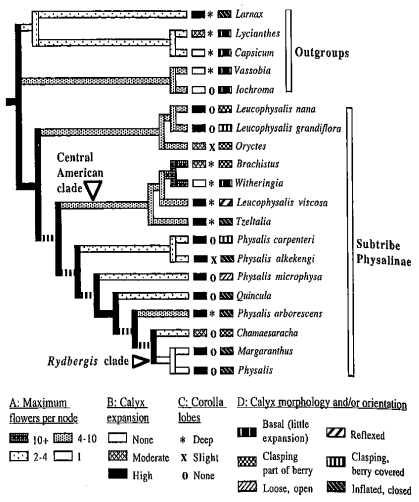

FIG. 4. Flower and fruiting calyx morphology (Appendix 1) mapped onto a simplified combined analysis tree. Dashed branches lack clade support. Due to low support along the backbone of the tree, no attempt was made to infer which traits are ancestral.

the Solanaceae to an unlobed form more typical of *Physalis* and its close relatives. Calyx shape and enlargement is more variable than flower form, but shape stabilizes to enveloping the fruit and amount of expansion generally increases as one moves up from the basal physaloids.

The Central American clade of the Physalinae (*Witheringia*, *Brachistus*, *L. viscosa* and *Tzeltalia*) are mostly woody perennials, with shrubby or sprawling habits (Fig. 3A, clade B; Appendix 1). They have densely clustered flowers with deeply lobed corollas. Corolla color varies from white to greenish or yellowish. The amount of calyx expansion ranges from none in many species of *Witheringia*, to inflated and *Physalis*-like in *Tzeltalia*.

In comparison, the taxa in the physaloid grade are more morphologically variable. Though many of them are woody, herbaceous taxa form the bulk of the diversity. Most of these taxa have clustered flowers, but the clusters are often small, with only 2–4 flowers. Flower color varies from white to purple to yellow. The feature that all of these taxa share is calyx expansion in fruit, though some, like *Oryctes*, have deeply lobed, tightly clasping calyces which only partially cover the berry, while others, such as *Quincula*, have inflated, *Physalis*-like calyces.

The *Rydbergis* clade (Fig. 3B) is morphologically homogeneous, characterized by solitary, yellow flowers, with unlobed corollas, and inflated calyces in fruit. Most of the major variation occurs near the base of this clade. *Physalis crassifolia* is one of the few truly woody species, while its sister *P. acutifolia* is the one species

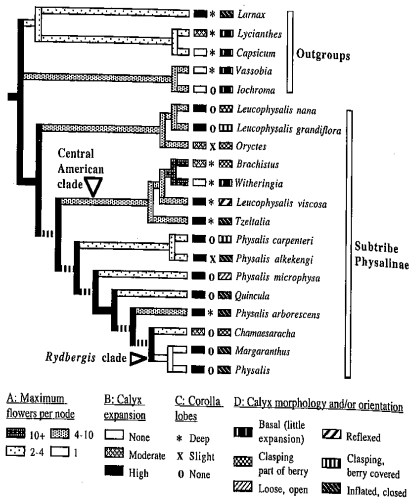

FIG. 4. Flower and fruiting calyx morphology (Appendix 1) mapped onto a simplified combined analysis tree. Dashed branches lack clade support. Due to low support along the backbone of the tree, no attempt was made to infer which traits are ancestral.

the Solanaceae to an unlobed form more typical of *Physalis* and its close relatives. Calyx shape and enlargement is more variable than flower form, but shape stabilizes to enveloping the fruit and amount of expansion generally increases as one moves up from the basal physaloids.

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with white flowers. *Physalis solanaceous* (Schldl.) Axelius, also one of the basal-most branches in the clade, is the only species with urceolate flowers, and has sometimes been recognized as the genus *Margaranthus*, though the DNA data does not seem to support this.

ACKNOWLEDGEMENTS. Richard Olmstead kindly provided DNAs of *Capsicum*, *Iochroma*, *Lycianthes*, and *Vassobia*, and also DNAs for *Margaranthus*, *Quincula*, *Chamaesaracha*, and the North American *Leucophysalis*. DNA for two species of *Witheringia* was kindly provided by Lynn Bohs. Several *Physalis* species were grown from seed supplied by the Solanaceae germplasm collection at Radboud University's Botanical and Experimental Garden, in Nijmegen, the Netherlands. The following herbaria graciously allowed sampling of leaf material from their specimens: DUKE, FLAS, MO, TEX, and NCU. James and Mona Whitson helped collect many of the U.S. *Physalis* species used in this study. Patrick Herendeen, Gregory Plunkett, Janet Sullivan, and an anonymous reviewer provided helpful comments for the improvement of this paper. This work represents a part of Maggie Whitson's doctoral thesis, submitted to the Department of Botany at Duke University, and would not have been possible without financial support from the Andrew Mellon Foundation.

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APPENDIX 1. Major morphological features of outgroups and physaloid taxa used in this study. Missing data is scored as ?. *Leucophysalis* s.s. refers to the North American species. Character information for sampled taxa includes: **A: Habit:** (1) perennial shrub; (2) perennial, woody only at base; (3) perennial herb; (4) annual herb. **B: Maximum flower number per node:** Flowers are axillary in all taxa. (1) numerous (10+); (2) several (4-10); (3) few (2-4); (4) solitary (1). **C: Flower color:** Spotting inside the corolla and flushes of color along the outer primary veins are common. Flower color refers to the predominant shade of the inner corolla. (1) pale (greenish to yellowish to creamy); (2) white; (3) purple; (4) bright yellow; (5) red-orange. **D: Corolla shape:** Physaloid flowers are generally campanulate to rotate (1), but occasionally tubular (2) or urceolate (3). **E: Corolla lobing:** (1) stellate = corolla distinctly star-shaped, lobes more than ⅓ the length of the corolla. (2) slightly lobed = lobes less than ⅓ corolla length; (3) unlobed = corolla pentagonal to round when flattened and viewed from the front. **F: Expansion of fruiting calyx:** All physaloid taxa have calyces which enlarge somewhat after flowering and persist in fruit. Three degrees of fruiting calyx expansion are used here: (1) not expanded = similar in size to flowering calyx; (2) somewhat expanded = larger than the flowering calyx, but smaller than the mature fruit; (3) highly expanded is as large as or larger than the mature fruit. **G: Arrangement of fruiting calyx:** The enlarged calyx may also have varying positions and shapes, including: (1) basal to fruit with little or no expansion; (2) reflexed; (3) tightly clasping, but not entirely covering the berry; (4) tightly covering entire berry; (5) loosely surrounding berry, but open at end; (6) inflated around fruit, closed at end. **H: Fruit color:** The predominant fruit color among the physaloid taxa is greenish yellow, ranging from pale green to mustard yellow. Four color categories are defined here: (1) red-orange; (2) greenish to yellow; (3) white; (4) orange.

	Characters							
Taxon	A	B	C	D	E	F	G	H
Witheringia	1	1	1	1	1	1	1	1
Brachistus	1	1	1	1	1	2	3	1
Leucophysalis viscosa	1	2	1	1	1	3	2	1
Tzeltalia	1	2	1	1	1	3	6	2
P. alkekengi	3	3	2	1	2	3	6	1
P. carpenteri	2	3	4	1	3	3	4	2
P. microphysa	2	3	4	1	3	3	5	2
Oryctes	4	2	3	2	2	2	3	2
Leucophysalis s.s.	3,4	2	2	1	3	3	3,4	3
P. arborescens	1	2	1	1	1	3	6	2
Quincula	3	3	3	1	3	3	6	2
Chamaesaracha	3-4	3	1	1	3	2	3	2
Margaranthus	4	4	3	3	3	3	6	2
Physalis s.s.	2,3,4	4	4	1	3	3	6	2,4
Outgroups								
Larnax	1	3	3	1	1	3	6	4
Capsicum	2	3	2	1	1	1	1	1
Lycianthes	1	3	1	1	1	2	1	1
Iochroma	1	2	5	2	3	1	1	?
Vassobia	1	2	3	1	1	1	1	?

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(2) greenish to yellow; (3) white; (4) orange.

Characters						
B	C	D	E	F	G	H
1	1	1	1	1	1	1
1	1	1	1	2	3	1
2	1	1	1	3	2	1
2	1	1	1	3	6	2
3	2	1	2	3	6	1
3	4	1	3	3	4	2
3	4	1	3	3	5	2
2	3	2	2	2	3	2
2	2	1	3	3	3,4	3
2	1	1	1	3	6	2
3	3	1	3	3	6	2
3	1	1	3	2	3	2
4	3	3	3	3	6	2
4	4	1	3	3	6	2,4
3	3	1	1	3	6	4
3	2	1	1	1	1	1
3	1	1	1	2	1	1
2	5	2	3	1	1	?
2	3	1	1	1	1	?

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Book Reviews

Flowering Plants of the Neotropics by N. Smith, S. A. Mori, A. Henderson, D. W. Stevenson, and S. V. Heald (eds.). 2004. 594 pp. ISBN 0-691-11694-6. $75.00 (hbk). Princeton University Press, 41 William Street, Princeton, NJ 08540.

This book attempts to be the "definitive guide" to the plant families of the Neotropics. And perhaps it is. To produce it, one hundred fifty botanists joined forces to cover 277 families of plants known to occur in the tropics of the Western Hemisphere, which is home to 30% of the Earth's plant diversity.

The families are arranged in the following manner: dicots before monocots, and alphabetically within each group. (The authors rejected phylogenetic listings due to anticipated changes in family relationships.) Nomenclature is Cronquistian for dicots and Dahlgrenian for monocots, with some lumping and splitting based on recent molecular studies.

Each family is depicted in a consistent, concise, and definitive manner. A "bullet list" of characteristics useful for a family's identification is followed by the number of genera and species contained therein, its distribution and habitat requirements, classification (and any related taxonomic controversies), basic family features, natural history (especially pollinators and dispersers), economic uses, and major references. Sixty-four plates (containing 308 color photos) and 258 line drawings complete the family portraits.

The family treatments are followed by an extensive glossary, and four appendices outline various classification schemes (and where these neotropical families are placed). A large key to the treated families precedes the index.

This book is not, by any means, a field guide to tropical families. (For such, you might try Maas & Westra's *Neotropical Plant Families*.) Its large format and detailed depictions lend it best to the office and herbarium, where it should serve as an important source of information on such plants.

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