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A reassessment of *Normania* and *Triguera* (Solanaceae)

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Abstract. *Normania* and *Triguera* comprise two genera of the Solanaceae whose affinities have been uncertain. *Normania* encompasses two species endemic to Macaronesia; *Triguera* is monotypic and found in Spain and northwestern Africa. Both have slightly zygomorphic flowers and horned anthers that dehisce by both apical pores and longitudinal slits. Micromorphological similarities include trichotomously colporate pollen grains and seed surface cells with radially thickened extensions. Molecular data from the chloroplast *ndh*F gene and the nuclear ITS region establish that *Normania* and *Triguera* are nested within the large genus *Solanum*, where together they form a well supported clade. However, the relationship of this clade to other *Solanum* subgroups is not resolved. Transfer of the *Normania* and *Triguera* epithets to *Solanum* is made, necessitating one new name. The molecular data confirm that the species of *Solanum* endemic to Macaronesia belong to two distinct clades, each showing an independent evolution of heteromorphic anthers.

Key words: *Normania*, *Triguera*, *Solanum*, Macaronesia, *ndh*F, ITS.

Since the time of Darwin (1845), oceanic islands have served as living laboratories for the study of evolutionary questions. Researchers have targeted archipelagos such as the Galapagos, Macaronesia, and the Hawaiian and Juan Fernandez Islands as sites for investigation of speciation, adaptive radiation, morphological specialization, and long distance dispersal in plants. Island endemics often exhibit highly divergent morphologies compared to their mainland relatives and in many cases these disjunct but evolutionarily close relationships have been clarified only with the recent advent of molecular data. Such is the case with several endemic Macaronesian taxa of the Solanaceae. Olmstead and Palmer (1997) included the Macaronesian endemic *Solanum vespertilio* in their phylogenetic study based on chloroplast DNA restriction fragment variation, but the systematic placement of the other endemic solanaceous taxa has not been examined using molecular data and their nearest relatives have not been identified with certainty.

The purpose of this study is to elucidate the systematic position of two small and enigmatic genera of the Solanaceae, *Triguera* and *Normania*. *Triguera* is monotypic and native to the Iberian peninsula and northwestern Africa. *Normania* includes two species endemic to the Macaronesian islands of Madeira and the Canaries. Both have been placed in subfamily Solanoideae, tribe Solaneae, which includes genera with flattened seeds, curved embryos, generally valvate corolla aestivation, and basifixed anthers. Because of their unusual distribution and aberrant morphology, the relationships of these two genera have been obscure and misunderstood. Molecular data now offer the opportunity to examine the phylogenetic position of these taxa within the Solanaceae and clarify the close affinity between them, as has been suggested by previous authors (Lowe 1872, Francisco-Ortega et al. 1993).

Triguera osbeckii was first described by Linnaeus (1753) as a species of *Verbascum*, a genus later assigned to the Scrophulariaceae. In 1786, Cavanilles erected the genus *Triguera* and described the new species *T. ambrosiaca* Cav. and *T. inodora* Cav. The genus *Triguera* Cav. (1786; Solanaceae) is conserved over *Triguera* Cav. (1785; Bombacaceae; Farr et al. 1979). Gmelin in 1791 described another new species in the genus, *T. baccata* J. F. Gmel. Subsequent authors (e.g. Poiret 1808, Roemer and Schultes 1819, Sprengel 1825, Miers 1849a, Willkomm 1870, Hawkes 1972) recognized two to three species in *Triguera*, but Hansen and Hansen (1973) consider it likely that the genus is monotypic, with *T. osbeckii* (L.) Willk. as the only species. However, Hansen and Hansen (1973) were unable to reach a conclusion about the identity of *T. inodora*, whose type has not been located. In all probability, *T. inodora* is either a synonym of *T. osbeckii* or represents a taxon unrelated to *Triguera*.

Triguera osbeckii ranges from southern Spain to adjacent northern Africa in Morocco and northwestern Algeria. It is a small herb or weakly woody shrub, apparently annual, with alternate, sessile, obovate, coarsely dentate leaves. The flowers are solitary or paired in the leaf axils, and have rather large, foliaceous calyces densely covered with curled, unbranched, whitish hairs. The rotate-campanulate corolla is dark purple, shallowly five-lobed at the apex, and slightly zygomorphic. The five stamens are equal or subequal in size and shape, the filaments short (ca. 1 mm long), with short (4–5 mm) anthers that dehisce by terminal pores located beneath two small apical horns. As the anthers age, the pores apparently elongate into longitudinal slits. The style is straight, ca. 5–7 mm long and included, and the stigma is minute. The fruits are globose berries with a dry or membranous texture, ca. 10 mm in diameter, and are subtended by the somewhat accrescent foliaceous calyx. Each fruit contains 4–6 large, dark brown, deeply pitted seeds.

All previous Solanaceae taxonomists have recognized *Triguera* as a distinct genus. Miers (1849a) suggested that *Triguera* is closely allied with *Solanum* on the basis of its stamen structure and corolla aestivation. He reiterates this view in another paper (Miers 1849b), in which he lists *Triguera* in the tribe Solaneae along with the genera *Solanum*, *Lycopersicon*, and *Cyphomandra*. D'Arcy (1991) likewise associated *Triguera* with other poricidally dehiscent genera such as *Solanum*, *Lycopersicon*, *Cyphomandra*, and *Lycianthes*. Molecular phylogenetic studies have resulted in the placement of *Lycopersicon* and *Cyphomandra* within *Solanum*, and have established that *Lycianthes* is probably distinct from *Solanum* (Spooner et al. 1993; Bohs 1995; Bohs and Olmstead 1997, 1999; Olmstead and Palmer 1992, 1997; Olmstead et al. 1999). None of these molecular studies have examined the phylogenetic placement of *Triguera*.

The unusual distribution and floral morphology of *Normania* have attracted the attention of several previous workers. The genus consists of two species, *N. nava* and *N. triphylla*, both endemic to Macaronesia. *Normania nava* is one of the rarest species of Macaronesia and is restricted to the islands of Tenerife and Gran Canaria in the Canary Island archipelago. Only two living plants of this species have been found since the time of its original description in the first half of the nineteenth century (Francisco-Ortega et al. 1993). *Normania triphylla* is likewise rare in its natural range on the island of Madeira. However, seeds gathered from a single wild plant in 1994 were taken to the National Botanical Conservatory in Brest, France, where plants were successfully cultivated and have even became locally naturalized

(R. Lester, pers. comm.). Seeds have been distributed to various botanic gardens and Solanaceae specialists.

Like *Triguera osbeckii*, the species of *Normania* are herbs to weakly woody short-lived shrubs. The stems and leaves are covered with soft, unbranched, often glandular hairs. The leaves of both *Normania* species are membranaceous and elliptic-ovate in outline, with those of *N. triphylla* usually pinnately lobed or dissected. The inflorescences are pedunculate, unbranched, and relatively few- (less than 15-) flowered. Calyces are large, leafy, and soft-pubescent as in *Triguera osbeckii*. The corolla is purple, rotate-campanulate, shallowly five-lobed, and slightly zygomorphic (Fig. 1). Despite these similarities with *Triguera*, the flowers of both *Normania* species are distinct due to their remarkable stamens. The five anthers differ greatly in size and structure: two are long (6–11 mm) and curved, two are shorter (4.5–8.5 mm) and also curved, and one is quite short (3–4.5 mm). The four longer anthers have a projection or horn at the middle or near the base. Although a small pore is apparent near the tips of the longer anthers, they mainly dehisce by a longitudinal slit that develops from near the base of the pore and opens proximally. *Normania nava* and *N. triphylla* have minor differences in the color and form of their anthers (R. N. Lester, pers. comm.), but both are similar in the overall morphology of the androecium. Unlike *Triguera*, the style in *Normania* is long (ca. 11–12 mm) and curved and extends through the two longest anthers. The fruits are bright red to orange, globose, somewhat fleshy, and subtended by the accrescent calyx (Fig. 2). Plants cultivated at the University of Utah greenhouse were self-compatible and autogamous.

Normania triphylla was originally placed in the genus *Nycterium* Vent. by Lowe (1838). Webb and Berthelot (1845) originally described *Normania nava* as a species of *Solanum*. Dunal (1852) considered them both to belong to *Solanum*, and placed them in section *Pachystemonum* Dunal subsection *Tuberarium* Dunal because of their supposed basal pedicel articulation. Lowe (1872) later transferred these species to his new genus *Normania* and proposed that they were closely related to the genus *Triguera*. Later botanists did not recognize *Normania* and included *N. triphylla* and *N. nava* within *Solanum* (Bentham 1876, Bitter 1912, D'Arcy 1972, Child 1990). Bitter (1912) disagreed with placement of the two species near the potato group (section *Tuberarium* Bitter) and moved them into his new section *Normania*. D'Arcy (1972) and Child (1990) retained Bitter's section *Normania*, but considered it to be included in subgenus *Potatoe* (G. Don) D'Arcy. Most recently, Francisco-Ortega et al. (1993) have supported recognition of *Normania* as a separate genus within the tribe Solaneae due to its distinctness in macro- and micromorphological characters. They suggested a close relationship with *Triguera* on the basis of similarities in overall morphology and in pollen and seed structure. Whether recognized as *Normania* or considered as *Solanum*, the phylogenetic position of *N. triphylla* and *N. nava* has not been resolved.

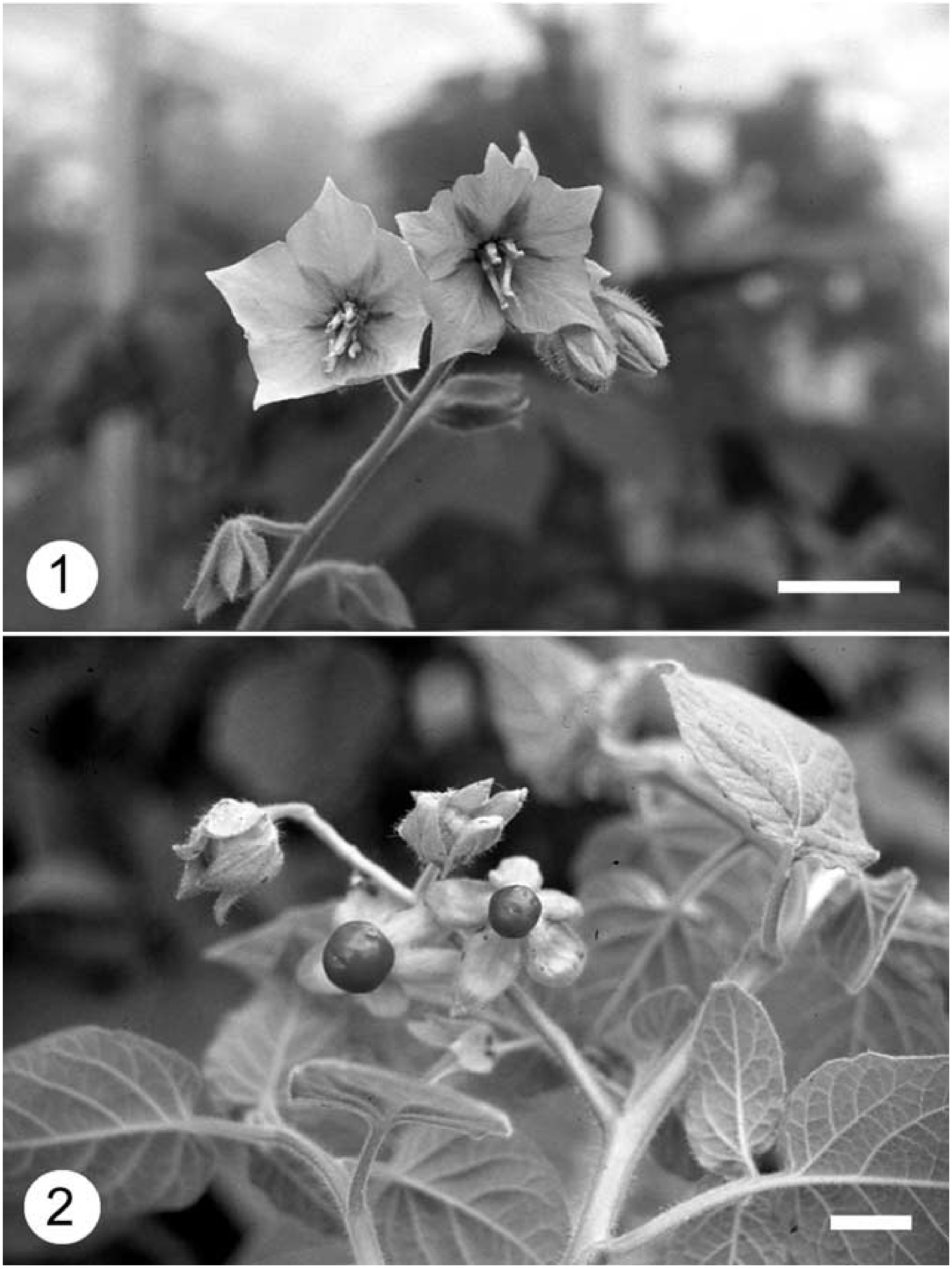
Four of the solanaceous species endemic to Macaronesia (*Normania nava*, *N. triphylla*, *Solanum vespertilio*, and *S. liddii* Sunding) have anthers that are markedly unequal in size. Two of these are included in the molecular analyses reported here. *Triguera* has equal or subequal anthers, but has been suggested as a possible relative of *Normania* by Lowe (1872) and Francisco-Ortega et al. (1993). This study thus affords the opportunity to ascertain whether heterandry (unequal anthers within a single flower) evolved convergently in separate lineages, as suggested by Lester et al. (1999).

Materials and methods

The data presented here are a subset of a larger analysis of over 100 species of *Solanum* and related genera. In this paper, we present data from 40 species of Solanaceae, including taxa representing broad sampling among subgroups of *Solanum* and selected outgroups from subfamily

Solanoideae. Outgroup taxa were chosen based on previously published phylogenetic analyses of Olmstead and Palmer (1992, 1997), Bohs and Olmstead (1997), and Olmstead et al. (1999). Provenance and voucher information is given in Table 1.

DNA was extracted from fresh or silica dried leaf samples using the modified CTAB method of



Figs. 1–2. Fig. 1. Flowers of *Solanum trisectum*. Scale bar = 1 cm. **Fig. 2.** Fruits of *Solanum trisectum*. Scale bar = 1 cm

Table 1. Sources of taxa sequenced for *ndh*F and ITS

Taxon	Source[a]	Voucher[b]	GenBank accession numbers	
			*ndh*F	ITS
Capsicum baccatum L. var. *pendulum* (Willd.) Eshbaugh	2	*Eshbaugh 1584*	U08916	AF244708
Jaltomata procumbens (Cav.) J. L. Gentry	3	*Davis 1189A*	U47429	AF244710
Lycianthes heteroclita (Sendtn.) Bitter	1	*Bohs 2376*	U72756	AF244709
Normania triphylla (Lowe) Lowe	1	*Bohs 2718*	AF224063	AF244733
Physalis alkekengi L.	2	*D'Arcy 17707*	U08927	AF244711
Solanum abutiloides (Griseb.) Bitter & Lillo	2	*RGO S-73*	U47415	AF244716
Solanum adhaerens Roem. & Schult.	1	*Bohs 2473*	AF224061	AF244723
Solanum allophyllum (Miers) Standl.	1	*Bohs 2339*	U47416	AF244732
Solanum appendiculatum Dunal	2	*Anderson 1401* (CONN)	AF224062	AF244746
Solanum arboreum Dunal	1	*Bohs 2521*	U47417	AF244719
Solanum argentinum Bitter & Lillo	1	*Bohs 2539*	U72752	AF244718
Solanum aviculare G. Forst.	2	BIRM S.0809	U47418	AF244743
Solanum betaceum Cav.	1	*Bohs 2468*	U47428	AF244713
Solanum campechiense L.	1	*Bohs 2536*	AF224071	AF244728
Solanum candidum Lindl.	2	*RGO S-100*	AF224072	AF244722
Solanum cordovense Sessé & Moç.	1	*Bohs 2693*	U72751	AF244717
Solanum dulcamara L.	2	none	U47419	AF244742
Solanum elaeagnifolium Cav.	2	*RGO S-82*	AF224067	AF244730
Solanum glaucophyllum Desf.	2	none	U72753	AF244714
Solanum jamaicense Mill.	2	*RGO S-85*	AF224073	AF244724
Solanum laciniatum Aiton	1	*Bohs 2528*	U47420	AF244744
Solanum luteoalbum Pers.	1	*Bohs 2337*	U72749	AF244715
Solanum lycopersicum L.	2	none	U08921	AF244747
Solanum macrocarpon L.	2	*RGO S-88*	AF224068	AF244725
Solanum mammosum L.	2	*RGO S-89*	AF224074	AF244721
Solanum melongena L.	2	*RGO S-91*	AF224069	AF244726
Solanum nitidum Ruiz & Pav.	1	*Nee 31944* (NY)	AF224075	AF244740
Solanum palitans C. V. Morton	1	*Bohs 2449*	AF224064	AF244739
Solanum physalifolium Rusby var. *nitidibaccatum* (Bitter) Edmonds	1	*Bohs 2467*	U47421	AF244737
Solanum pseudocapsicum L.	2	BIRM S.0870	U47422	AF244720
Solanum ptychanthum Dunal	2	*RGO S-94*	U47423	AF244735
Solanum torvum Sw.	2	BIRM S.0389	L76286	AF244729
Solanum tripartitum Dunal	1	*Bohs 2465*	U72750	AF244738
Solanum trizygum Bitter	1	*Bohs 2511*	U72754	AF244745
Solanum vespertilio Aiton	2	*RGO S-103*	AF224070	AF244727
Solanum villosum Mill.	1	*Bohs 2553*	AF224066	AF244736
Solanum wallacei (A.Gray) Parish	1	*Bohs 2438*	U47426	AF244741
Solanum wendlandii Hook. f.	2	BIRM S.0488	U47427	AF244731
Triguera osbeckii (L.) Willk.	2	*Jury 13742* (RNG)	AF224065	AF244734
Witheringia solanacea L'Her.	1	*Bohs 2416*	U72755	AF244712

[a] DNA extracts provided by: 1 – L. Bohs, University of Utah, Salt Lake City, UT. 2 – R. G. Olmstead, University of Washington, Seattle, WA. 3 – T. Mione, Central Connecticut State University, New Britain, CT

[b] Collector and number of herbarium vouchers. Bohs vouchers are at UT, RGO vouchers at WTU. BIRM samples bear the seed accession number of the University of Birmingham Solanaceae collection

Doyle and Doyle (1987). Where sampling coincided with the previous studies cited above, the same DNA extracts were used. PCR amplification of the *ndh*F region was accomplished using the methods described in Bohs and Olmstead (1997). Amplification of the ITS region used primers ITS 4 and ITS leu1 (5′-GTCCACTGAACCTTATCATTTAG-3′) in 25 μl reactions containing the following: 12.25 μl water, 1.25 μl each 10 μM primer, 4.15 μl Perkin Elmer 10X buffer containing 15 mM $MgCl_2$, 2.5 μl 2.5 mM dNTPs, 1.25 μl glycerol, 1.25 μl DMSO, 0.1 μl AmpliTaq. The PCR program used for ITS amplification was 97° C for 2 min followed by 30 cycles of 97° C for 1 min, 50° C for 1 min, 72° C for 45 sec, with a 3 sec extension per cycle, and a single cycle of 72° C for 7 min. Amplified products were cleaned using QiaQuick spin columns (Qiagen, Inc., Valencia, CA) and were sequenced on an ABI automated sequencer. Sequencing of *ndh*F used the eight to ten primers described in Bohs and Olmstead (1997). Sequencing of ITS used primers ITS 4 and ITS 5 of White et al. (1990); ITS 2 and ITS 3 were also used in some taxa.

Sequence data were edited and contigs constructed using the computer program Sequencher (Gene Codes Corp.). After a consensus sequence was obtained from all primer data, it was aligned by eye to a template sequence [*Nicotiana tabacum* L. for *ndh*F, *Solanum diploconos* (Mart.) Bohs for ITS]. Base changes relative to the template sequence were double-checked against the chromatograms, and alignments were adjusted by eye using the program Se-Al (Rambaut 1996). Due to ambiguities in alignment of the ITS data, 78 characters were excluded from subsequent analyses of the individual ITS and combined ITS and *ndh*F data sets. All new sequences obtained in this study were submitted to GenBank (Table 1), and the complete data set and trees depicted in Figs. 3–5 have been submitted to TreeBASE.

A partition homogeneity test with 1000 replicates was performed on the combined data set using PAUP* 4.0b2a (Swofford 1999) to determine if the data sets exhibited significant heterogeneity. Parsimony and maximum likelihood analyses were conducted on the individual and combined data sets using PAUP* 4.0b2a. The parsimony analyses used the heuristic search algorithm with the TBR and MulTrees options, equal weights for all nucleotide positions, gaps treated as missing data, and 100 random-order entry replicates. Additional heuristic searches using the same parameters, but with *Solanum* (excluding *Normania* and *Triguera*) constrained to monophyly were conducted to examine how much less parsimonious it would be to exclude those taxa. Bootstrap analyses were performed with 500 replicates using the heuristic search option, TBR, and MulTrees, with Maxtrees set to 1000. Initial runs of the maximum likelihood analyses used one of the most parsimonious trees from the parsimony analyses and varied the substitution model, base frequencies, and among-site rate variation using the options supplied in PAUP*. For each combination of parameters, a likelihood score was computed and scores were compared using likelihood ratio tests. For all data sets, the best likelihood score was obtained using a general-time-reversible model with rate heterogeneity and with the base frequencies, rate matrix parameters, proportion of invariable sites, and shape of the gamma distribution estimated from the data using maximum likelihood. These estimated values were then used to compute a likelihood tree and score for each data set using 1000 replicates of quartet puzzling.

In order to determine whether ITS sequence divergence could be used to calculate the approximate age of *Normania* in Macaronesia, likelihood scores were computed on one of the ITS trees from the parsimony analysis using the Enforce Molecular Clock option. The likelihood scores were compared by likelihood ratio tests to those obtained without invoking the clock option.

Results

The *ndh*F sequences obtained for all taxa except *S. wendlandii* and *L. heteroclita* were 2086 base pairs long, corresponding to positions 24 through 2109 in the tobacco *ndh*F sequence. *Solanum wendlandii* had a 33 bp insertion and *L. heteroclita* a 15 bp insertion between positions 1476 and 1477 in the *ndh*F sequence. All sequences were easily alignable by eye.

The *ndh*F data set contained 341 variable characters, of which 175 were parsimony informative. Pairwise sequence divergence calculated using the Kimura 2-parameter model ranged from 0.24% for the closely related taxon pairs *S. villosum* vs. *S. ptychanthum* and

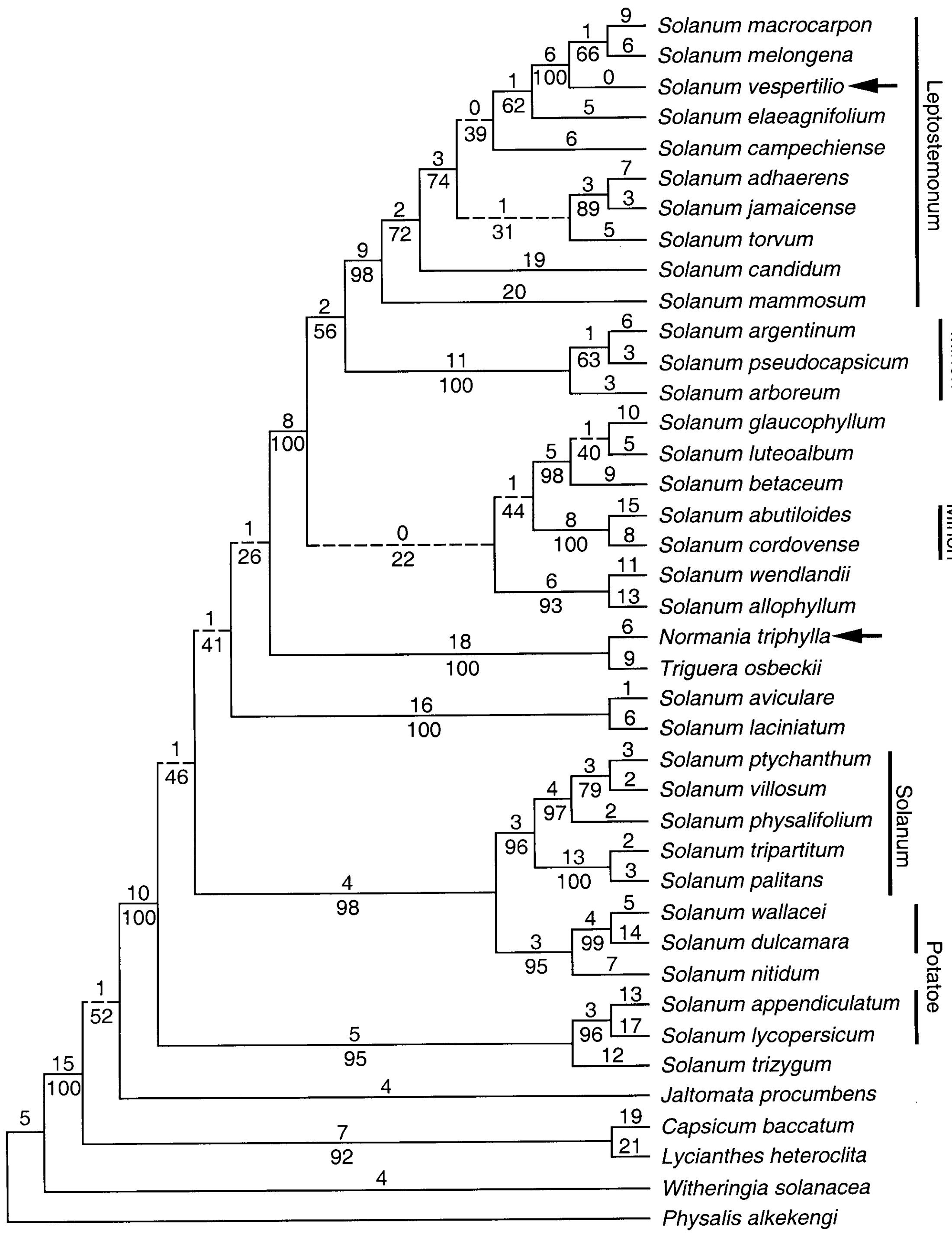


Fig. 3. One of 240 most parsimonious trees of 499 steps from the parsimony analysis of the *ndh*F data. Numbers above branches are branch lengths; numbers below branches are bootstrap values. Dashed lines indicate branches that collapse in the strict consensus tree. Taxa assigned to *Solanum* subgenera *Leptostemonum*, *Minon*, *Potatoe*, and *Solanum* as defined by D'Arcy (1972, 1991) are indicated. Arrows mark species endemic to Macaronesia

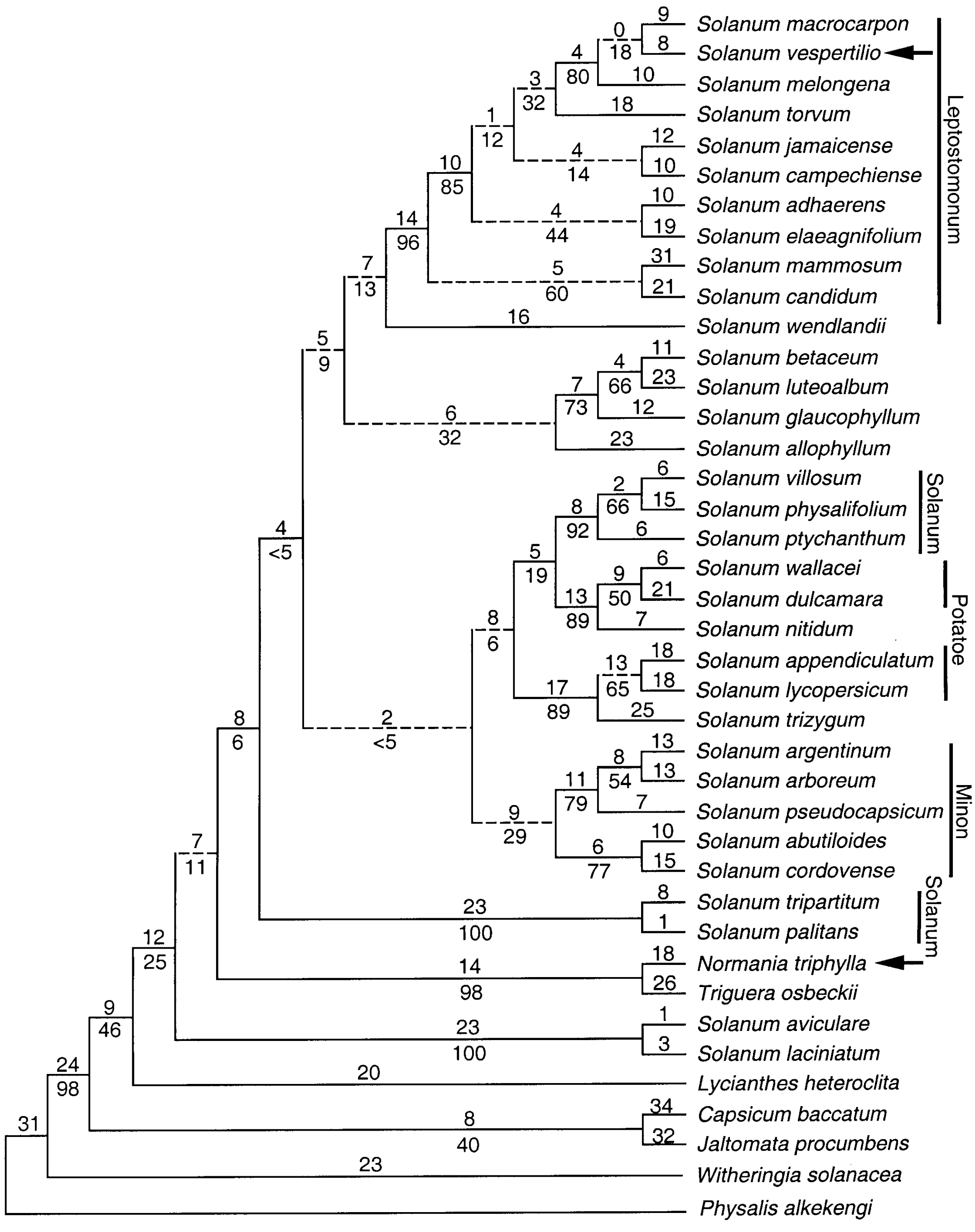


Fig. 4. One of 22 most parsimonious trees of 927 steps from the parsimony analysis of the ITS data matrix. Numbers above branches are branch lengths; numbers below branches are bootstrap values. Dashed lines indicate branches that collapse in the strict consensus tree. Taxa assigned to *Solanum* subgenera *Leptostemonum*, *Minon*, *Potatoe*, and *Solanum* as defined by D'Arcy (1972, 1991) are indicated. Arrows mark species endemic to Macaronesia

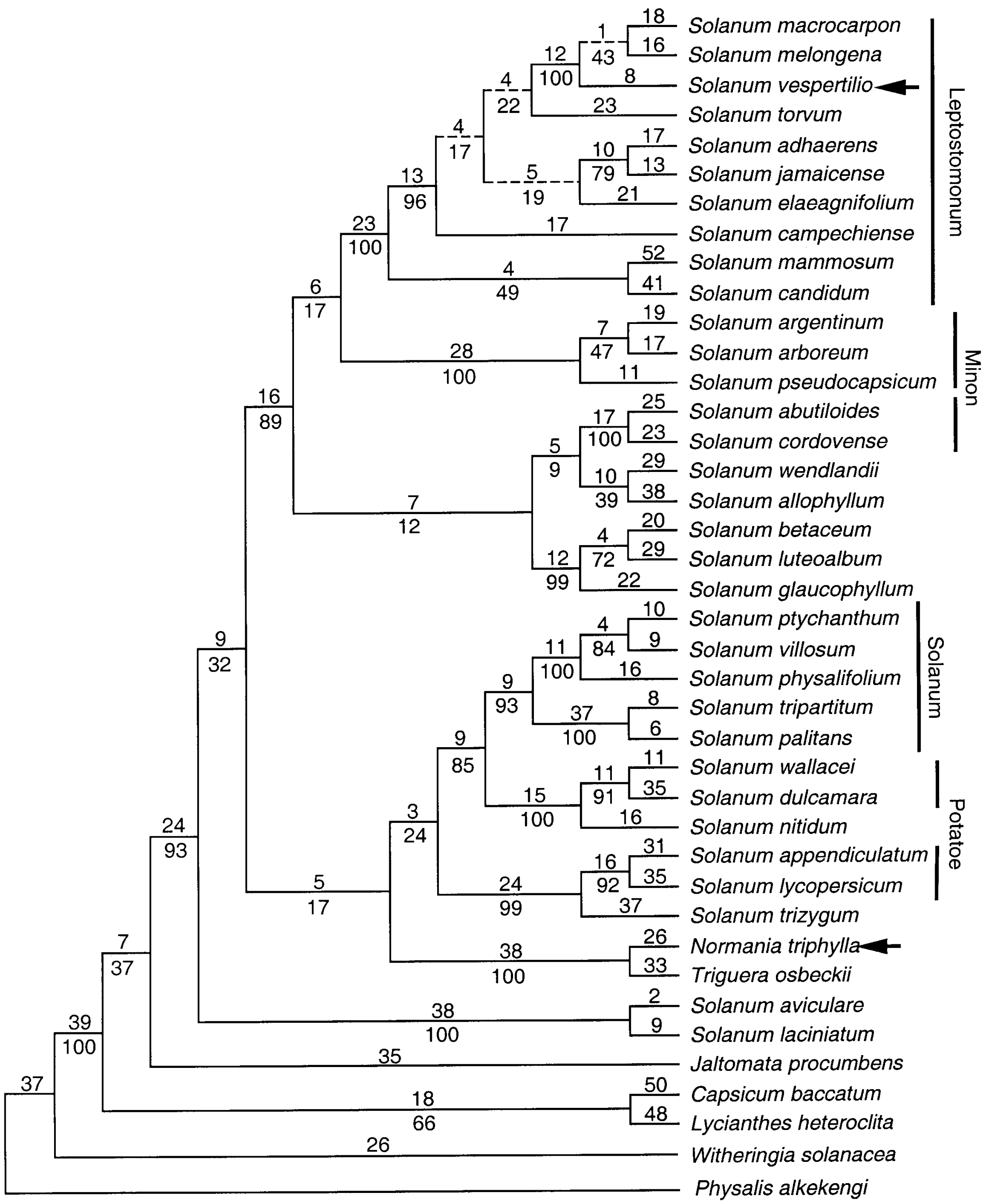


Fig. 5. One of 16 most parsimonious trees of 1444 steps from the parsimony analysis of the combined *ndh*F and ITS data matrix. Numbers above branches are branch lengths; numbers below branches are bootstrap values. Dashed lines indicate branches that collapse in the strict consensus tree. Taxa assigned to *Solanum* subgenera *Leptostemonum*, *Minon*, *Potatoe*, and *Solanum* as defined b D'Arcy (1972, 1991) are indicated. Arrows mark species endemic to Macaronesia

S. tripartitum vs. *S. palitans* to 3.4% between *S. candidum* and *Lycianthes heteroclita*. Pairwise sequence divergence between *Normania* and *Triguera* in *ndh*F was 0.72% using this model.

Parsimony analysis of the *ndh*F data resulted in 240 most parsimonious trees of 499 steps, with a CI (excluding uninformative characters) of 0.656 and RI of 0.822. *Normania triphylla* and *Triguera osbeckii* form a clade and in all most parsimonious trees. This clade is nested within a large, well-supported clade that includes the rest of *Solanum* (Fig. 3). The relationship of *Normania* and *Triguera* with respect to the sampled *Solanum* species is unresolved, however, because this clade forms part of a polytomy at the base of *Solanum*. The best trees obtained by the constrained search in which *Normania* and *Triguera* are excluded from *Solanum* are one step longer and place *Normania* and *Triguera* as the sister group to *Solanum*. A maximum likelihood analysis of the *ndh*F data resulted in a topology (not shown) congruent with that of the strict consensus tree from the parsimony analysis, in which *Normania* and *Triguera* form a well-supported clade nested within *Solanum*, but relationships of this clade to other *Solanum* subgroups are unresolved.

The length of the aligned ITS sequences is 710 nucleotides, including 9 bp of the 18S rDNA gene at the 5′ end of the sequence, 14 bp of the 26S gene at the 3′ end of the sequence, and 164 bp of the 5.8S rDNA gene intercalated between ITS-1 and ITS-2. The length of ITS-1 ranged from 210 bp in *S. palitans* to 257 bp in *S. mammosum*. The length of ITS-2 ranged from 206 bp in *S. candidum*, *S. physalifolium*, *S. ptychanthum*, *S. villosum*, and *L. heteroclita* to 226 bp in *Capsicum baccatum* and *Jaltomata procumbens*. Of the 710 nucleotides, 78 were excluded from analyses due to alignment ambiguities. Of the remaining 632 characters, 255 were variable and 170 were parsimony informative. Pairwise sequence divergence calculated using the Kimura 2-parameter model ranged from 0.65% in *S. aviculare* vs. *S. laciniatum* to 16.3% in *S. trizygum* vs. *Physalis alkekengi*. Pairwise sequence divergence was 7.62% between *Normania* and *Triguera*.

A parsimony analysis of the ITS data resulted in 22 most parsimonious trees of 927 steps, with a CI (excluding uninformative characters) of 0.368 and RI of 0.534. *Normania* and *Triguera* form a clade nested within *Solanum* in all most parsimonious trees (Fig. 4). The *Normania*/*Triguera* clade emerges as a basal lineage within *Solanum*, but, as in the *ndh*F trees, its relationships to other *Solanum* subgroups are unresolved. The constrained search required three additional steps to find a monophyletic *Solanum*, excluding *Normania* and *Triguera*. Analysis of the data using maximum likelihood resulted in a tree topology (not shown) much different from the parsimony trees. *Normania* and *Triguera* belong to the same clade, but they form a polytomy with *S. luteoalbum*. Support for this grouping is low (17% of the quartet puzzling replicates). Furthermore, there is no support for the relationship of this clade to other subgroups of *Solanum*. The incongruence in topologies resulting from the parsimony and maximum likelihood analyses indicates substantial differences in branch lengths throughout the tree, and this is confirmed by the likelihood ratio tests comparing scores with and without a molecular clock. The hypothesis of rate homogeneity was rejected in all comparisons using the HKY and GTR models with a variety of parameters. These results indicate that ITS sequence divergence values cannot be used to estimate the age of divergence of *Normania* and *Triguera*.

Resampling the data partitions with 1000 replicates of the partition homogeneity test gave a value of $p = 0.10$, indicating that the data sets were not significantly different from random partitions of the combined data set. The *ndh*F and ITS data were then analyzed together, giving a matrix of 2751 characters. Of these, 596 were variable and 345 were parsimony informative. A parsimony analysis found 16 shortest trees of 1444 steps, with a CI (excluding uninformative characters) of 0.441

and an RI of 0.625. *Normania* and *Triguera* form a well-supported clade, as in the separate analyses (Fig. 5). The *Normania/Triguera* clade is sister to a clade consisting mainly of members of the *Solanum* subgenera *Solanum* and *Potatoe*. Although this relationship appears in all most parsimonious trees, it has little support (17% of the bootstrap replicates). Likewise, this relationship was not supported in the maximum likelihood analysis (results not shown). Though the *Normania/Triguera* clade appeared in 94% of the quartet puzzling replicates, it emerges within *Solanum* near the base of the clade with little support for a close relationship with any other subgroup in the genus. The constrained parsimony search required three additional steps to find a monophyletic *Solanum* s.s.

Discussion

Three main conclusions can be drawn from this study: 1) *Normania* and *Triguera* are closely related, 2) *Normania* and *Triguera* clearly belong to a well-supported clade along with *Solanum* and are best considered as species of *Solanum*, and 3) heterandry (unequal anthers within a single flower) has evolved at least twice in the endemic Macaronesian Solanaceae. Each of these points is discussed in more detail below.

***Normania* and *Triguera* are closely related.** Sequence data from both genes support the close relationship between these two taxa, as suggested by previous authors (Lowe 1872, Francisco-Ortega et al. 1993). Macro- and micromorphological characters shared by both taxa include somewhat zygomorphic corollas, large leafy calyces, horned anthers, pollen colpi joined at the poles, and cells of the seed coat with radially extended walls.

The flora of Macaronesia has strong Mediterranean affinities and in part may represent a relict from a formerly widely distributed moist forest flora of Tertiary age (e.g. Bramwell 1976, Sunding 1979, Francisco-Ortega et al. 1997, Helfgott et al. 2000). The maximum age of Tenerife and Gran Canaria is estimated at 12–14 myr and that of Madeira at 5 myr (Francisco-Ortega et al. 1996). Thus, *Normania* could have been present in Madeira and the Canary Islands since the Pliocene. The sister relationship of *Normania* and *Triguera* is an example of a connection between endemic Macaronesian taxa and a mainland group from Iberia and northwestern Africa. Other examples of this distribution pattern that have been investigated using molecular data include *Argyranthemum* and the *Asteriscus* alliance (Asteraceae; Francisco-Ortega et al. 1995, 1997, 1999), *Echium* (Boraginaceae; Böhle et al. 1996), and *Ixanthus* (Gentianaceae; Thiv et al. 1999). The endemic status of the *Normania* species, their morphological distinctness, and their occurrence in moist laurel forest may indicate that they are Tertiary relicts, rather than relatively recent arrivals to the archipelago via long-distance dispersal. Unfortunately, the data at hand for *Normania* and *Triguera* are not sufficient to discriminate between these two hypotheses. Substantial molecular divergence has occurred between *Normania* and *Triguera* in both *ndh*F and ITS. However, no good calibrations exist for determining the rate of *ndh*F sequence evolution in Solanaceae, and rates of *ndh*F divergence for other taxa are unknown. Approximate rates of ITS sequence divergence have been estimated for other taxonomic groups (e.g. Suh et al. 1993; Sang et al. 1994, 1995; Böhle et al. 1996), but the rates vary considerably among taxa, and rejection of a molecular clock assumption for the Solanaceae ITS data set means that these values cannot be used reliably in formulating a hypothesis of the age of the *Normania/Triguera* split. Furthermore, *Normania* and *Triguera* form an isolated clade without obvious relationships to other *Solanum* subgroups, so it cannot be ascertained whether this clade has its closest relatives among other Old World taxa and when it may have diverged from its closest living relatives.

***Normania* and *Triguera* are included within *Solanum*.** All analyses of the nuclear and chloroplast data sets, singly and in combination, result in the *Normania*/*Triguera* clade being nested within *Solanum*. Currently *Solanum* is broadly defined, and several segregate genera which have recently been found to be included within the *Solanum* clade have been subsumed within it [e.g. *Lycopersicon* (Spooner et al. 1993), *Cyphomandra* (Bohs 1995)]. In accordance with this taxonomic concept, we recommend that the use of the generic names *Normania* and *Triguera* be abandoned and their species transferred to *Solanum*. Names are available in *Solanum* for both *Normania* species, but a new name is needed for the transfer of *Triguera osbeckii* to *Solanum*. Synonymy of the three species is given below:

Solanum nava Webb & Berthel., Hist. nat. Iles Canaries 2. 3(3): 123. 1845; tab. 174. 1849. Type: Canary Islands. Caidero de Coruña, montium Saucillo, supra vicum Tentenigua-dam, Herbarium Webbianum s.n., Solanaceae no. 9 (lectotype, FI). Designated by León et al., Vieraea 13: 21. 1984.

= *Solanum nava* var. *undulatidentatum* Bitter, Repert. Spec. Nov. Regni Veg. 11: 253. 1912. Type: In silva Teneriffe, Agua Garcia, Webb 44 (lectotype, W, #288851). Designated by Francisco-Ortega et al. (1993).

≡*Normania nava* (Webb & Berthel.) Franc.-Ort. & R. N. Lester, Pl. Syst. Evol. 185: 202. 1993.

Solanum trisectum Dunal in DC. Prodr. 13(1): 36. 1852. Non *S. triphyllum* Vell. Fl. Flum. 2: 120. 1827.

≡*Nycterium triphyllum* Lowe, Trans. Cambridge Philos. Soc. 6: 536. 1838. Syntypes: Madeira, S. Vicente below the Gingeiras, on the roadside to the Paul, ca. 1000 ft, July 1837, Lemann 1030 (BM, G-DC); Madeira, in the east near Portella, Lippold (W).

≡*Normania triphylla* (Lowe) Lowe, Man. fl. Madeira 2(1): 87. 1872.

Solanum herculeum Bohs, nom. nov. Non *S. osbeckii* Dunal in DC. Prodr. 13(1): 179. 1852.

≡*Verbascum osbeckii* L., Sp. Pl. 1: 179. 1753. Type: Ex Hispania, Osbeck (lectotype, S-LINN G-6305; Microfiche IDC 87.17). Designated by Hansen & Hansen (1973).

≡*Triguera osbeckii* (L.) Willk. in Willk. & Lange, Prodr. fl. hispan. 2: 524. 1870.

≡*Fontqueriella osbeckii* (L.) Rothm. in Font-Quer & Rothm., Brotéria 36: 151. 1940.

?= *Triguera ambrosiaca* Cav., Diss. 2, p. 2 and tab. A. 1786. Type: Southern Spain. In argillaceis Carmona, Hispalis, Córdoba, et per totam fere inferiorem Baeticam, D. de Trigueros s.n. (holotype, MA; isotypes, P).

? = *Triguera baccata* J. F. Gmel., Syst. nat. 2(1): 338. 1791. Type: unknown.

The new name, *Solanum herculeum*, is taken from Herculeum Fretum, a classical name for the Strait of Gibraltar. *Solanum herculeum* occurs both north and south of this strait. *Triguera ambrosiaca* and *T. baccata* are regarded as synonyms in accordance with Hansen and Hansen (1973). The specific status of *Triguera inodora* is in doubt (Hansen and Hansen 1973), and is not considered here. Likewise, Lowe (1872) and Francisco-Ortega et al. (1993) have suggested that *Normania nava* and *N. triphylla* are conspecific. A critical examination of species boundaries among the taxa of *Triguera* and *Normania* is beyond the scope of the current paper.

Relationships of the *Normania*/*Triguera* clade to other groups within *Solanum* are obscure. Though placed near the tuber-bearing *Solanum*s by previous authors [Dunal, 1852 (as subsection *Tuberarium*); D'Arcy, 1972 and Child, 1990 (as subgenus *Potatoe*)], molecular data indicate that this clade is probably not closely allied with the potatoes and their relatives (Figs. 3–5). Parsimony analysis of the combined molecular data set resulted in an association of the *Normania*/*Triguera* clade with a clade consisting mainly of members of *Solanum* subgenera *Potatoe* and *Solanum* (Fig. 5), but this relationship is not well supported. No obvious macromorphological synapomorphies define this larger clade, and Francisco-Ortega et al. (1993) concluded that micromorphological features of the pollen grains and seed coat cell wall thickenings were quite different between *Normania*/*Triguera* and members of *Solanum* subgenus *Potatoe*. To date, the molecular and morphological data suggest that the *Normania*/*Triguera* clade may be a relatively basal lineage of *Solanum* without extant close relatives.

Solanum section *Normania* (Lowe) Bitter was set up by Bitter (1912) to accommodate the two Macaronesian taxa *S. nava* and *S. trisectum*. It is useful to retain this section, but amend its definition to include the former genus *Triguera*. As such, it would be comprised of three species, *Solanum nava*, *S. trisectum*, and *S. herculeum*.

Heterandry has evolved at least twice in the endemic Macaronesian species of *Solanum*. Aside from *S. nava* and *S. trisectum*, two other *Solanum* species, *S. liddii* and *S. vespertilio*, are endemic to Macaronesia. Both belong to section *Nycterium* in the spiny subgenus *Leptostemonum* and are obviously closely related. Although *S. liddii* and *S. vespertilio* possess spines and stellate hairs typical of the other members of the subgenus, they are unusual in having highly zygomorphic flowers with unequal anthers. Heterandry (the presence of unequal anthers in a single flower) is found in several sections of subgenus *Leptostemonum* [e.g. sect. *Nycterium* (Vent.) Dunal, sect. *Androceras* (Nutt.) Marzell, sect. *Anisantherum* Bitter, sect. *Mondolichopus* Bitter, sect. *Aculeigerum* Seithe] as well as in non-spiny groups [e.g. sect. *Jasminosolanum* (Bitter) Seithe, sect. *Geminata* (G. Don) Walp., sect. *Lycopersicum* (Mill.) Wettst.] and has apparently evolved multiple times in the genus *Solanum*. The functional significance of heterandry in pollination was studied in *S. rostratum* Dun. of section *Androceras* by Bowers (1975), who concluded that outcrossing was promoted by heteromorphic anthers in combination with enantiostyly (asymmetrical style placement). It is not known if heterandry facilitates outcrossing in other *Solanum* species with unequal anthers.

Solanum vespertilio was included in the molecular analyses, and is indeed nested within the spiny *Solanum* clade (Figs. 3–5). The results do not support a close evolutionary relationship between *Normania*/*Triguera* and *S. vespertilio*, indicating that floral zygomorphy and heterandry have evolved more than once within the Macaronesian *Solanum* species. Among the sampled taxa, *S. vespertilio* appears to be most closely related to *S. melongena* and *S. macrocarpon* of the *S. incanum* group sensu Whalen [1984; sections *Melongena* (Mill.) Dunal or *Andromonoecum* Bitter], an Old World group with actinomorphic flowers and equal anthers. Other molecular results (Olmstead and Palmer 1997; Bohs, unpublished) further indicate that *S. vespertilio* is not closely related to the New World members of section *Nycterium*, despite their similar zygomorphic flowers with unequal anthers.

The inclusion of the genera *Normania* and *Triguera* within *Solanum* in no way diminishes the evolutionary and biogeographic importance of these taxa and their priority for conservation. Regardless of their taxonomic designation, they should be the focus of intensive efforts to locate new populations and conserve existing ones. We are in complete agreement with Francisco-Ortega et al. (1993), who advocate preservation of the Macaronesian laurel forest habitat as well as ex situ conservation measures for *S. trisectum* and *S. nava*. Although not endangered at present, efforts should be made to monitor and preserve populations of *S. herculeum* and to introduce this species into seed collections and/or botanical gardens where its biology and morphology can be studied in detail. Close comparison of living material of these taxa may reveal other morphological similarities and will help to resolve species boundaries in the group. Field studies of the pollination biology of the Macaronesian species of *Solanum* as well as other taxa of *Solanum* with heteromorphic anthers are needed to understand the possible adaptive significance of this striking convergence in floral morphology in disparate clades within the genus. In addition, it would be useful to know what significance the horned anthers of *S. nava*, *S. herculeum*, and *S. trisectum* might have in attracting or manipulating pollinators. Investigations of floral visitors to *S. nava* and *S. trisectum* in the Canary Islands are even more urgent given the rare status of these plants in their native habitats. Certainly an effective conservation plan should consider aspects of the reproductive biology of these plants such as their floral visitors or pollinators.

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