PHYLOGENY OF THE TRIBE HYMENOCALLIDEAE (AMARYLLIDACEAE) BASED ON MORPHOLOGY AND MOLECULAR CHARACTERS[1]

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ABSTRACT

The generic limits of *Hymenocallis* have been variously proposed by different taxonomic workers, often without discussion or data. The genera *Leptochiton*, *Ismene*, *Elisena*, and *Pseudostenomesson* have been included with *Hymenocallis*, lumped together as the genus *Ismene*, or maintained as distinct genera. Recent cladistic analysis of plastid and nrDNA for Amaryllidaceae support a distinct tribe Hymenocallideae. Cladistic analyses of morphology, and plastid (*trn*L-F region) and nuclear ribosomal DNA (ITS) are presented alone and in combination for the tribe. *Leptochiton* is sister to the rest of the genera in the tribe in all analyses. While *Hymenocallis* is always resolved as monophyletic, *Ismene* is variably paraphyletic or monophyletic. The combined sequence data produce the most resolved and best-supported phylogeny, wherein *Hymenocallis* and *Ismene* are monophyletic sister genera. These data support an origin for the tribe in the Andes, with vicariant distribution of the largely Mesoamerican *Hymenocallis*. Formal recognition of *Ismene* subg. *Elisena* and *Pseudostenomesson* is established.

Key words: Amaryllidaceae, cladistics, molecular systematics, phylogeny.

Systematics of the genus *Hymenocallis* Salisb. (Amaryllidaceae) and its allies have defied precise systematic understanding at both the specific and generic levels (Flory, 1976; Meerow & Dehgan, 1985). The genera *Hymenocallis* and *Ismene* Salisb. were established by Salisbury (1812) for the Neotropical species with fleshy seeds originally assigned to the Old World genus *Pancratium* L. The zygomorphic-flowered *Elisena* was described by Herbert (1837), who recognized *Hymenocallis* and *Ismene* as distinct genera. Baker (1888) subsumed *Ismene* within *Hymenocallis* but retained *Elisena* as distinct, as did Pax (1890). While Stapf (1933) treated *H. quitoensis* Herb. as a species of *Pamianthe* Stapf, Sealy (1937) considered the species to exhibit sufficient morphological divergence to be recognized as a monotypic genus, *Leptochiton* Sealy. Hutchinson (1934, 1959) retained both *Elisena* and *Ismene* (presumably including *Leptochiton*) as distinct. Velarde (1949) established the Peruvian genus *Pseudostenomesson* for a fleshy-seeded species originally described as *Stenomesson morissonii* Vargas as well as one new species. Traub (1962) recognized all four erstwhile genera as subgenera of *Hymenocallis* in his synoptic treatment: subg. *Hymenocallis*, subg. *Ismene* (Salisb.) Baker ex Traub (including *Leptochiton*), subg. *Elisena* (Herb.) Traub, and subg. *Pseudostenomesson* (Velarde) Traub. Traub (1980) later reduced these subgenera to the rank of section without explanation. Ravenna (1980) in his description of *H. heliantha* (= *Leptochiton heliantha* (Ravenna) Gereau & Meerow) suggested that subgenera *Ismene* (including *Lepidochiton*), *Elisena*, and *Pseudostenomesson* should probably be all recognized as the genus *Ismene*, distinct from *Hymenocallis*. Meerow and Dehgan (1985) suggested that *Pseudostenomesson* might warrant recognition at the rank of genus due to its extreme phenetic divergence (funnelform-tubular perianth) versus the "pancratioid" flower of *Leptochiton*, *Ismene* subg. *Ismene*, and *Hymenocallis*. "Pancratoid" floral morphology refers to a large, white, fragrant, crateriform flower with a conspicuous staminal cup (cf. *Pancratium* L.). This type of flower appears to be adapted for sphingid moth pollination (Bauml, 1979; Grant, 1983; Morton, 1965). Meerow (1990) treated *Leptochiton* as a distinct genus and recognized *Hymenocallis* and *Ismene* (including *Elisena* and *Pseudostenomesson*) as distinct, a treatment followed by Gereau et al. (1993) and

[1] This work was supported in part by NSF Grant DEB 9628787 to AWM and GLG.

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Meerow and Snijman (1998). No cladistic analysis has focused exclusively on testing the validity of this treatment, although at least one representative of each subgenus was included in overall molecular studies of Amaryllidaceae (Meerow et al., 1999, 2000a).

Hymenocallis and its allied segregate genera are entirely Neotropical in distribution [a single West African taxon, *H. senegambica*, was treated by Sealy (1954) as an early adventive introduction of *H. caribaea*]. *Hymenocallis* sensu stricto, with 50 to 60 species, is chiefly Mesoamerican and extends into the West Indies and the southeastern United States. It is sparingly represented in northern South America. *Leptochiton* Sealy (2 spp.), *Ismene* (ca. 7 spp.), *Elisena* Herb. (2 to 4 spp.), and *Pseudostenomesson* Velarde (2 spp.) are all endemic to the Central Andean region of South America. *Hymenocallis*, *Ismene*, and *Leptochiton* are contrasted in Table 1.

Hymenocallis and allies have usually been allied with *Eucharis* Planch. in the tribe Eucharideae (Hutchinson, 1934, 1959; Traub, 1963; Dahlgren et al., 1985; Müller-Doblies & Müller-Doblies, 1996). Meerow (1989, 1995) argued that the linkage of these genera, largely through the perception that both lineages shared a fleshy seed, was misconstrued, and proposed that either subtribal or tribal recognition of *Hymenocallis* and allies was warranted. Müller-Doblies and Müller-Doblies (1996) placed them in Eucharideae subtribe Hymenocallidinae, while Meerow and Snijman (1998) recognized a distinct tribe, Hymenocallideae. Family-wide analysis of plastid sequences (Meerow et al., 1999) and nrDNA analyses of the monophyletic American clade of the family (Meerow et al., 2000a) support a distinct Hymenocallideae as sister to the newly recognized tribe Clinantheae (a segregate of the former Stenomesseae), but complete resolution of the intratribal relationships is not apparent in these large analyses. Both tribes are subclades of a well-supported, Andean, tetraploid clade of genera.

In this paper, we present phylogenetic analyses of morphological and molecular data for the tribe Hymenocallideae, and seek to clarify the relationships within the tribe.

Materials and Methods

Sampling

Sequences for the plastid *trn*L-F region were newly obtained for *H. eucharidifolia*, which, along with *H. latifolia*, was used as an exemplar taxon of *Hymenocallis* (Table 2). Previously cited sequences were used for one species each of the three subgenera of *Ismene*, one species of *Leptochiton*, and the outgroup *Pamianthe peruviana* (Table 2, Meerow et al., 1999). For ITS and the morphological data matrix, we increased our sampling with an additional four species of *Hymenocallis* and two additional species of *Ismene* subg. *Ismene* (Table 2). The aligned sequence matrices are available from the first author (miaam@ars-grin.gov).

Morphological Data

Morphological and cytological character state data were derived from the following sources: Traub (1962, 1980), Sealy (1954), Flory (1976), Velarde (1949), Bauml (1979), Meerow and Dehgan (1985); from examination of living material in research collections at the USDA, Miami, Florida; field observations of *Hymenocallis*, *Ismene*, and *Leptochiton* species; and examination of herbarium material. The morphological matrix consists of 12 species representing 4 genera and 23 characters (Tables 3, 4).

Sequence Data

The *trn*L-F (*trn*L intron and spacer region between *trn*L and *trn*F) matrix consisted of 6 taxa and 906 base positions. The nrDNA ITS sequence matrix (ITS1, 5.8s intron, ITS2) consisted of 12 taxa and 636 bp.

DNA Extraction, Amplification, and Sequencing Protocols

Genomic DNA was extracted from silica gel dried leaf tissue as described by Meerow et al. (2000a). The *trn*L-*trn*F region was amplified using the primers of Taberlet et al. (1991) as described by Meerow et al. (1999). Amplification of the ribosomal DNA ITS1/5.8S/ITS2 region was accomplished using flanking primers (18S, 26S) AB101 and AB102 (Douzery et al., 1999), and the original White et al. (1990) internal primers ITS2 and 3 to amplify the spacers along with the intervening 5.8S sequence, as described by Meerow et al. (2000a). Amplified products were purified using QIAquick (Qiagen, Valencia, California) columns, following manufacturer's protocols. All polymerase chain reactions (PCR) were performed on an ABI 9700 (Applied Biosystems, Foster City, California).

Cycle sequencing reactions were performed directly on purified PCR products on the ABI 9700, using standard dideoxy cycle protocols for sequencing with dye terminators on either an ABI 377 or ABI 310 automated sequencer (according to the manufacturer's protocols; Applied Biosystems, Foster City, California).

Table 1. Comparison of the genera and subgenera of Amaryllidaceae tribe Hymenocallideae.

Genus or subgenus	Number of species	Distribution	Elongate pseudostem	Floral morphology	Ovules per locule	Phytomelan on seed coat	Chromosome number
Leptochiton	2	SW Ecuador and NW Peru, at low elevations	Absent	Pancratioid, actinomorphic, large, white or yellow, fragrant, sessile, (sub)erect; tube long; staminal cup large and striped green within; free filament short and incurved.	16–20	Present	$2n = 34$
Ismene subg. *Ismene*	5–7	Central Andes at low to high elevations	Present	Pancratioid, actinomorphic large, white or yellow, fragrant, 2–10, subsessile to pedicellate, horizontal or declinate, tube ± long, staminal cup large, striped green within; free filament short and incurved.	2–4	Absent	$2n = 23$–86, 46, 104–110
Ismene subg. *Elisena*	2–4	Peru and Ecuador at mid to high elevations	Present	Zygomorphic, large, white, not fragrant, 2–10, subsessile, declinate; tube short; staminal cup ± large, deflexed from the tube, free filaments long and declinate.	2	Absent	$2n = 46$
Ismene subg. *Pseudostenomesson*	2	Peru, above 3000 m	Present	Funnelform-tubular, actinomorphic, ± small, green, not fragrant, numerous, pedicellate, pendulous; tube long; staminal cup subcylindrical, free filament straight.	2	Absent	$2n = 46$
Hymenocallis	ca. 50	SE U.S., West Indies, Mesoamerica	Absent	Pancratioid, actinomorphic, large, white, fragrant, 1 to many, mostly sessile, erect; tube long; staminal cup large or small and variable in shape, not striped within; free filament long and straight.	2–10	Absent	$2n = 46$, 40 most common but variable

Table 2. Vouchers and new GenBank accession numbers for DNA sequences of Hymenocallideae. All vouchers are deposited at FTG unless otherwise indicated.

Taxon	Voucher	Origin	GenBank accession no. or previous citation		
			*trn*L gene	*trn*L-F spacer	ITS
Hymenocallis acutifolia (Herb.) Sweet	*Meerow 2424*	Mexico	—	—	Meerow et al. (2000a)
H. glauca M. Roem.	*Meerow 2433*	Mexico	—	—	Meerow et al. (2000a)
H. latifolia (Mill.) Roem.	*Meerow 2438*	Florida, USA	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)
H. eucharidifolia Bak.	*Meerow 2439*	Mexico	AF411078	AF411079	Meerow et al. (2000a)
H. tubiflora Salisb.	*Meerow 2440*	Trinidad	—	—	Meerow et al. (2000a)
Ismene subg. *Ismene*					
Ismene amancaes (Ruiz & Pav.) Herb.	*Meerow 2452*	Peru	—	—	AF411080
I. hawkesii (Vargas) Gereau & Meerow	*Meerow 2441*	Peru	—	—	Meerow et al. (2000a)
I. narcissiflora Jacq.	*Meerow 2306*	Peru	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)
Ismene subg. *Elisena* (Herb.) Meerow					
I. longipetala (Lindl.) Meerow	*Sagastegui 15454*	Peru	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)
Ismene subg. *Pseudostenomesson* (Velarde) Meerow					
I. vargasii (Velarde) Gereau & Meerow	*Meerow 2308*	Peru	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)
Leptochiton quitoensis (Herb.) Sealy	*Meerow 1116*	Ecuador	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)
Pamianthe peruviana Stapf	*Meerow 2304*	Peru	Meerow et al. (1999)	Meerow et al. (1999)	Meerow et al. (2000a)

Table 3. Characters and character states used in the cladistic analyses of Hymenocallideae based on morphology.

Character	States and coding
1. Elongate pseudostem	absent (0); present (1)
2. Flower number	2–10 (0); solitary (1); >10 (2)
3. Flowers sessile/pedicellate	sessile (0); pedicellate (1)
4. Flower habit	erect (0); declinate/horizontal (1); pendent (2)
5. Tube length	shorter than tepals (0); longer than or equal to tepals (1)
6. Tube habit	straight (0); curved (1)
7. Perianth morphology	pancratioid (0); funnelform-tubular (1); ± funnelform (2)
8. Perianth symmetry	actinomorphic (0); zygomorphic (1)
9. Flower color	white (0); yellow (1); green (2)
10. Fragrance	present (0); absent (1)
11. Staminal cup shape	rotate or funnelform (0); cylindrical (1)
12. Staminal cup striping	present (0); absent (1)
13. Free filament	incurved (0); straight (1); declinate (2)
14. Free filament	longer than cup (0); shorter than cup (1)
15. Pollen grain size	very large (0); large (1); medium (2)
16. Pollen grain	auriculate (0); not (1)
17. Exine reticulum	coarse (0); medium (1)
18. Ovules per locule	>20 (0); 16–20 (1); 2–10 (2); 2–4 (3)
19. Seed per locule	numerous (0); 2–5 (1); 1 (2)
20. Phytomelan on testa	present (0); absent (1)
21. Seed coat	not fleshy (0); fleshy (1)
22. Seed shape	flat, winged (0); globose (1)
23. Most common diploid chromosome number	46 (0); 34 (1); 46, 40 (2)

SEQUENCE ALIGNMENTS

Both sequence matrices were readily aligned manually using the program Sequencher (Gene-Codes, Inc., Ann Arbor, Michigan) as few gaps needed to be inserted.

CLADISTIC ANALYSES

Pamianthe (tribe Clinantheae) was used as outgroup for all analyses. In larger sequence analyses (Meerow et al., 1999, 2000a), this genus resolves as most closely related to the Hymenocallideae. *Pamianthe* and *Leptochiton* (the latter putatively the least derived genus in the Hymenocallideae; see discussion below) share two four-base sequence elements in the *trn*L-F region (bp325–328, 821–824) that are absent from the rest of the Hymenocallideae. Phylogenetic analyses were run using PAUP* version 4.0b8 beta (Swofford, 1998). An exhaustive search of all possible tree topologies was conducted

Table 4. Character state matrix for cladistic analysis of 23 morphological characters in Hymenocallideae. Polymorphisms: + = (0,1); * = (0,1,2).

Taxon	Matrix
	1 2
Character	12345678901234567890123
Hymenocallis acutifolia	00001000000110000221112
Hymenocallis eucharidifolia	0*00100000130000221112
Hymenocallis glauca	0*00100000130000221112
Hymenocallis latifolia	0*00100000130000221112
Hymenocallis tubiflora	0*00100000130000221112
Ismene amancaes	10111+00100001000321110
Ismene hawkesii	10111+00000001000321110
Ismene longipetala	10110021011120211321110
Ismene narcissiflora	10111+00000001000321110
Ismene vargasii	10120110211110211321110
Leptochiton quitoensis	01001100+00001000110111
Pamianthe peruviana	10111000000001110000000

for *trn*L-F. For ITS, the morphological, and all combined analyses, branch and bound searches were conducted. Support for internal nodes of the trees was determined with 5000 replicates of branch and bound bootstrapping (Felsenstein, 1985) and by calculation of Bremer (1988) decay indices (DI) using the program TreeRot (Sorenson, 1999). A branch and bound search was implemented for each constraint statement postulated by TreeRot. A bootstrap value of 50–64% was considered weak, 65–74% moderate, and 75–100% strong support.

Combining independent character matrices, whether both molecular or molecular and morphological, very often increases the resolution of the ingroup and the bootstrap support of the internal nodes of the phylogenetic trees (Olmstead & Sweere, 1994; Chase et al., 1995; Yukawa et al., 1996; Rudall et al., 1998; Soltis et al., 1998; Meerow et al., 1999). Nonetheless, there is controversy about whether different data sets should be analyzed separately or together (De Queiroz et al., 1995; Huelsenbeck et al., 1996). Congruence of the independent matrices has generally been demonstrated before they are combined, but it has also been argued that incongruence should not be a predetermined factor against doing so (Dubuisson et al., 1998; Seelanan et al., 1997). Miyamoto and Fitch (1995) argued that data sets should always be analyzed independently, as underlying assumptions, constraints, or weighting strategies will vary from data set to data set. Kluge (1989) and Nixon and Carpenter (1996) argued that simultaneous analysis of multiple data sets better maximizes parsimony and allows secondary signals to appear from the combined data. Bull et al. (1993), Rodrigo et al. (1993), and De Queiroz (1993) advocated combining data only after a statistical test of congruence, what Huelsenbeck et al. (1996) called "conditional combination." Before combining the data sets, we performed a partition homogeneity test (Farris et al., 1994, 1995) on the variously combined matrices, using a branch and bound search.

Results

Morphological matrix

With all characters unordered, two most parsimonious trees (Fig. 1A, one shown) were found of length = 37, consistency index (CI) = 0.86, and retention index (RI) = 0.88. Sixteen of the 23 characters used were parsimony informative. In both trees, *Hymenocallis* is monophyletic (bootstrap = 89%, DI = 1), while *Ismene* is paraphyletic. *Ismene longipetala* (subg. *Elisena*) and *I. vargasii* (subg. *Pseudostenomesson*) are sisters in both trees. *Leptochiton* is sister to both *Hymenocallis* and *Ismene* in one tree (Fig. 1A). The 6 apomorphies at the ancestral node are an increase in pollen grain size, auriculate pollen grains, reduction in ovule number from more than 20 to 16 to 20; reduction in number of seeds per locule; and evolution of globose, fleshy seeds. Apomorphies for *Hymenocallis* (Fig. 1A) are the absence of an elongate pseudostem, predominantly sessile and erect flowers, and $2n = 46$, 40 chromosomes. Other than *Hymenocallis*, the only clade with strong bootstrap support is the sister relationship of *Ismene* subg. *Elisena* and subgenus *Pseudostenomesson* (100%, DI = 6), based on 7 apomorphies: perigone tube length reduction, nonpancratioid floral morphology, loss of floral fragrance, cylindrical staminal cup, and smaller nonauriculate pollen grains with less coarse exine reticulum. If all of the characters are ordered as irreversible, a single tree is found of length = 48, with CI = 0.67 and RI = 0.88 (Fig. 1B). There is moderate bootstrap support for a monophyletic *Ismene* (65%; DI = 2; apomorphies: elongate pseudostem, pedicellate and declinate/horizontal flowers, and 2–4 ovules per locule). There is weak support for the sister relationship of *Hymenocallis* and *Ismene* (56%, DI = 1; apomorphies: reduction in ovule and seed number, respectively; and the loss of phytomelan from the testa). *Leptochiton* is moderately supported as sister to both (65%, DI = 1; apomorphies: reduction in ovule and seed number and the evolution of a fleshy seed). *Ismene* subg. *Ismene* has a 91% bootstrap and DI = 2. *Ismene* subg. *Elisena* (*I. longipetala*) and subgenus *Pseudostenomesson* (*I. vargasii*) are again sister groups with 100% bootstrap and a DI = 9. A monophyletic *Hymenocallis* receives 87% bootstrap support with a DI = 4. *Hymenocallis latifolia*, *H. glauca*, and *H. eucharidifolia* form a monophyletic group with bootstrap support of 60 and DI = 1. This same tree topology (Fig. 1B) is 40 steps long with CI = 0.80 and RI = 0.80 if a branch and bound search is run with the topology as a constraint with all characters unordered.

Plastid *trn*L-F sequences

Using *trn*L-F sequences, which provide 7 parsimony-informative base substitutions, three equally most parsimonious trees are found of length = 82, CI = 0.99, and RI = 0.88 (Fig. 2, one tree shown). All three trees resolve a monophyletic *Ismene* with 81% bootstrap support (DI = 2), and *Leptochiton* as sister to the rest of the tribe but without support. A monophyletic *Hymenocallis* is resolved as sister to *Ismene* in one tree (Fig. 2), but *Hymenocallis* and

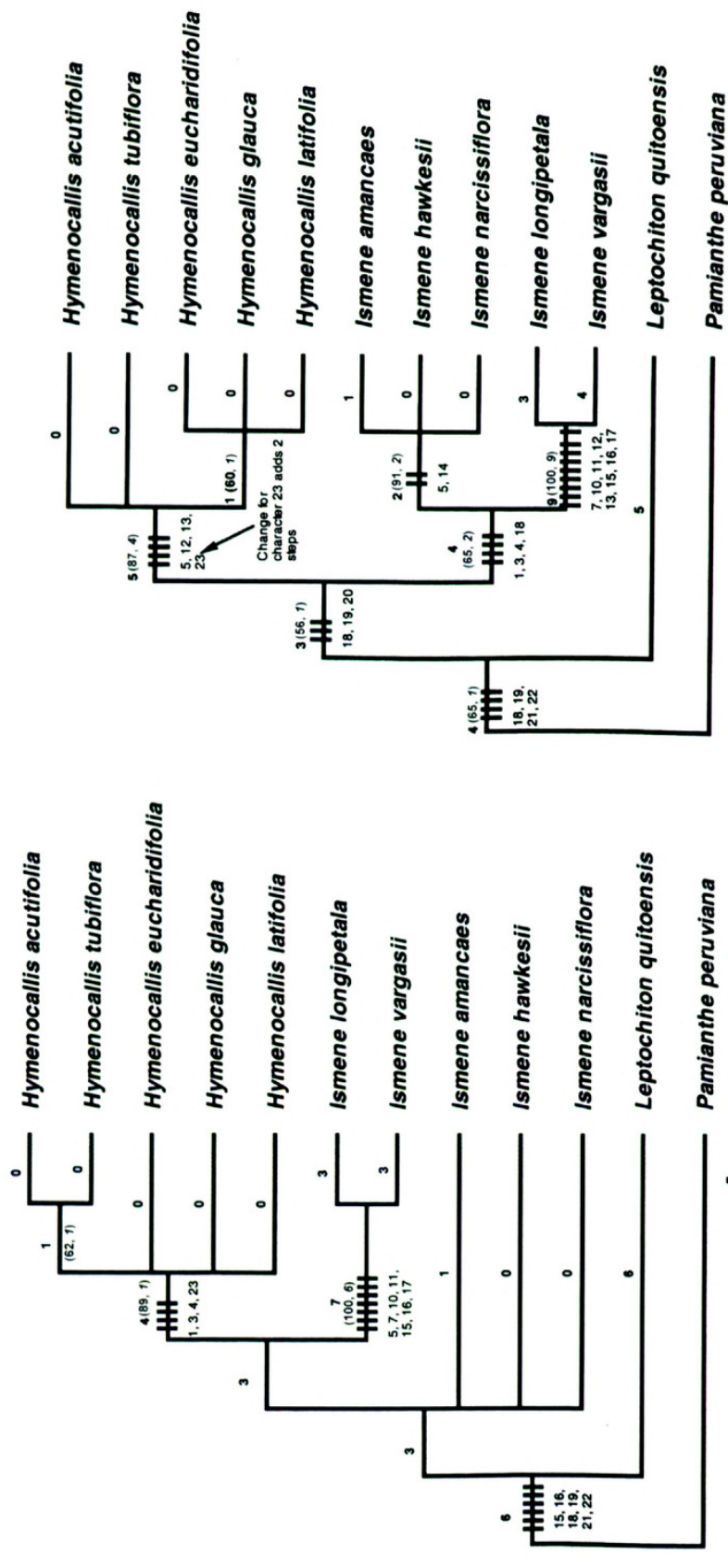

Figure 1. Cladograms for Hymenocallideae based on morphological characters. —A. One of two most parsimonious trees found with all characters unordered. —B. Single most parsimonious tree found if all characters are ordered as irreversible. Numbers above branches are branch lengths. Bootstrap percentages and decay indices (italic) are in parentheses. Vertical lines and numbers below branches are apomorphies along that branch (see Table 3).

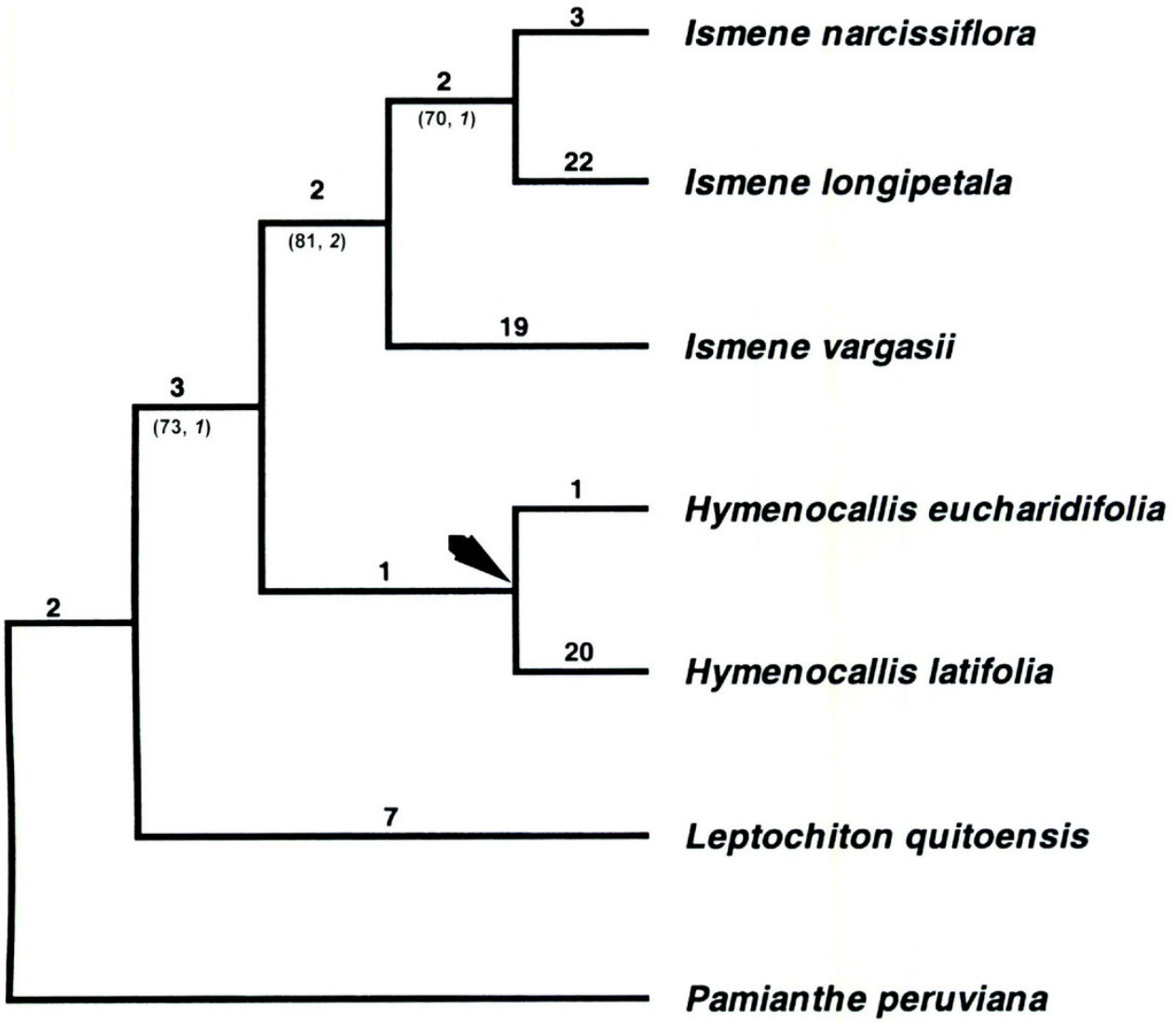

Figure 2. One of the three most parsimonious trees found by cladistic analysis of plastid *trn*L-F DNA sequences for the Hymenocallideae. Numbers above branches are branch lengths; numbers below branches are bootstrap percentages, followed by decay indices (italic). The large arrow indicates a node that collapses in the strict consensus of all three trees.

Ismene form a clade in all three (73% bootstrap, DI = 1). *Ismene* subg. *Ismene* (*I. narcissiflora*) and *Elisena* (*I. longipetala*) are resolved as sister groups in all three trees with a bootstrap of 70% (DI = 1).

ITS SEQUENCES

ITS provides 50 parsimony-informative characters, and 9 trees of length = 209, CI = 0.73, and RI = 0.77 were found (Fig. 3). In all of the trees, *Leptochiton* is resolved as sister to both *Hymenocallis* and *Ismene* (Fig. 3A), but without significant support. *Hymenocallis* is monophyletic (bootstrap = 97%, DI = 5), but *Ismene* is monophyletic in only 2 of the 9 trees (Fig. 3B, one shown). However, *Ismene* subg. *Ismene* (*I. amancaes*, *I. hawkesii*, *I. narcissiflora*) is monophyletic with weak bootstrap support (59%) and DI = 1 (Fig. 3B).

COMBINED *trn*L-F AND ITS SEQUENCES

The P value from the partition homogeneity test = 0.93, indicating that the *trn*L-F and ITS sequence matrices were highly congruent. Six most parsimonious trees were found of length = 292, CI = 0.92, and RI = 0.77 (Fig. 4). In all trees, *Hymenocallis* and *Ismene* are monophyletic sister genera with bootstrap support of 94% and a DI = 3. *Leptochiton* is sister to both, but without significant support. Bootstrap support for a monophyletic *Hymenocallis* is 98% (DI = 5), but only 68% (DI = 1) for a monophyletic *Ismene*. The only other internal resolution within *Ismene* that receives bootstrap support is a sister relationship between *I. narcissiflora* and *I. hawkesii* (both within subg. *Ismene*) at 84% with DI = 2.

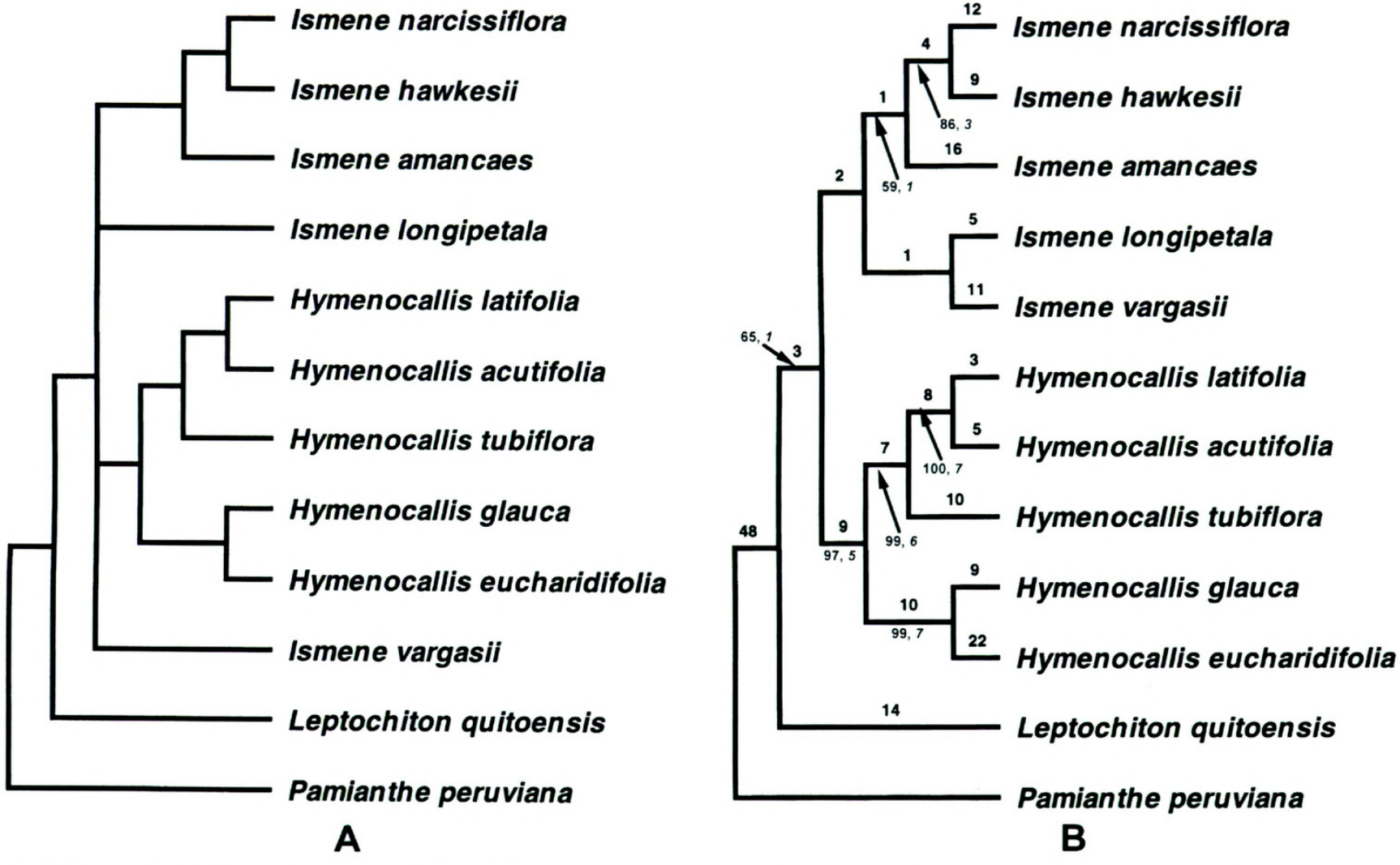

Figure 3. Cladograms for Hymenocallideae based on nrDNA ITS sequences. —A. Strict consensus of nine equally parsimonious trees. —B. One of two trees in which *Ismene* is a monophyletic sister group to *Hymenocallis*. Numbers above branches are branch lengths; numbers below branches are bootstrap percentages followed by decay indices (italic).

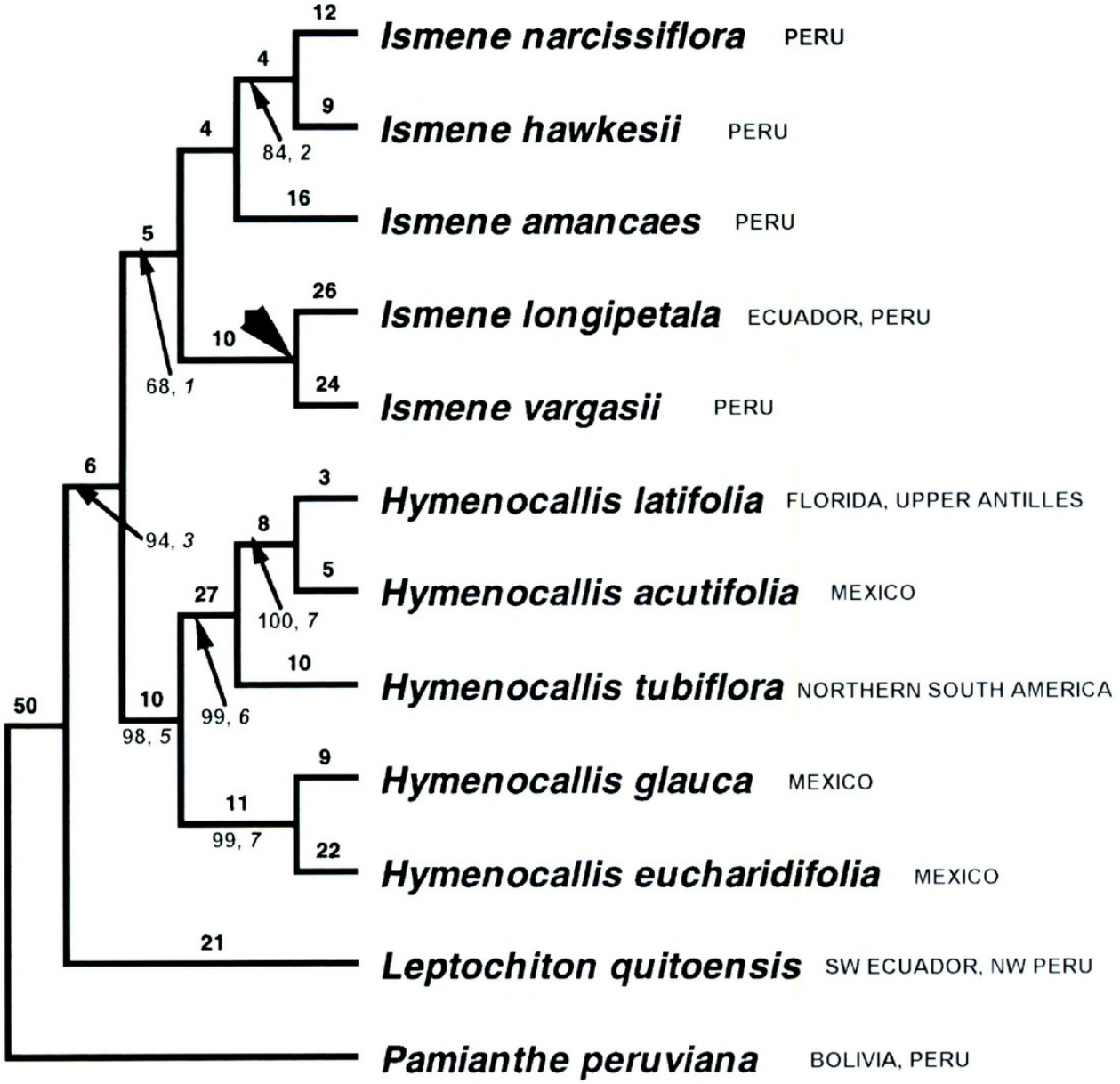

Figure 4. One of six most parsimonious trees found by cladistic analysis of combined plastid *trn*L-F and nrDNA ITS sequences. Numbers above branches are branch lengths; numbers below branches are bootstrap percentages followed by decay indices (italic). The larger arrow indicates a node that collapses in the strict consensus of all six trees.

COMBINED SEQUENCE AND MORPHOLOGICAL MATRICES

The P value of the partition homogeneity test was 0.0003, indicating significant incongruence between the morphological and DNA sequence data matrices. Much of the apparent incongruence can be attributed to the weak resolution of the morphologically based topologies, and we felt that it would still be informative to combine the two matrices in a single analysis. Of the 1565 characters included, 76 were parsimony informative. A single tree was found of length = 332, CI = 0.92, and RI = 0.79 (Fig. 5A). *Hymenocallis* is monophyletic with 100% bootstrap support (DI = 8), but *Ismene* is paraphyletic. Bootstrap support for the monophyly of *Ismene* subg. *Ismene* rises to 81% (DI = 2), but *Ismene* subg. *Elisena* (*I. longipetala*) and *Pseudostenomesson* (*I. vargasii*) are sister groups (bootstrap = 97%, DI = 4) weakly supported (bootstrap = 57%, DI = 1) as sister to *Hymenocallis*. *Leptochiton* is again sister to the other members of Hymenocallideae but without support. If trees one step longer were also retained in the search, in addition to the single shortest tree (Fig. 5A), a single, fully resolved tree of length = 333, CI = 0.90, and RI = 0.77 was found (Fig. 5B). In this tree (Fig. 5B), both *Hymenocallis* and *Ismene* are monophyletic sister genera, as are *Ismene* subg. *Elisena* and *Pseudostenomesson*.

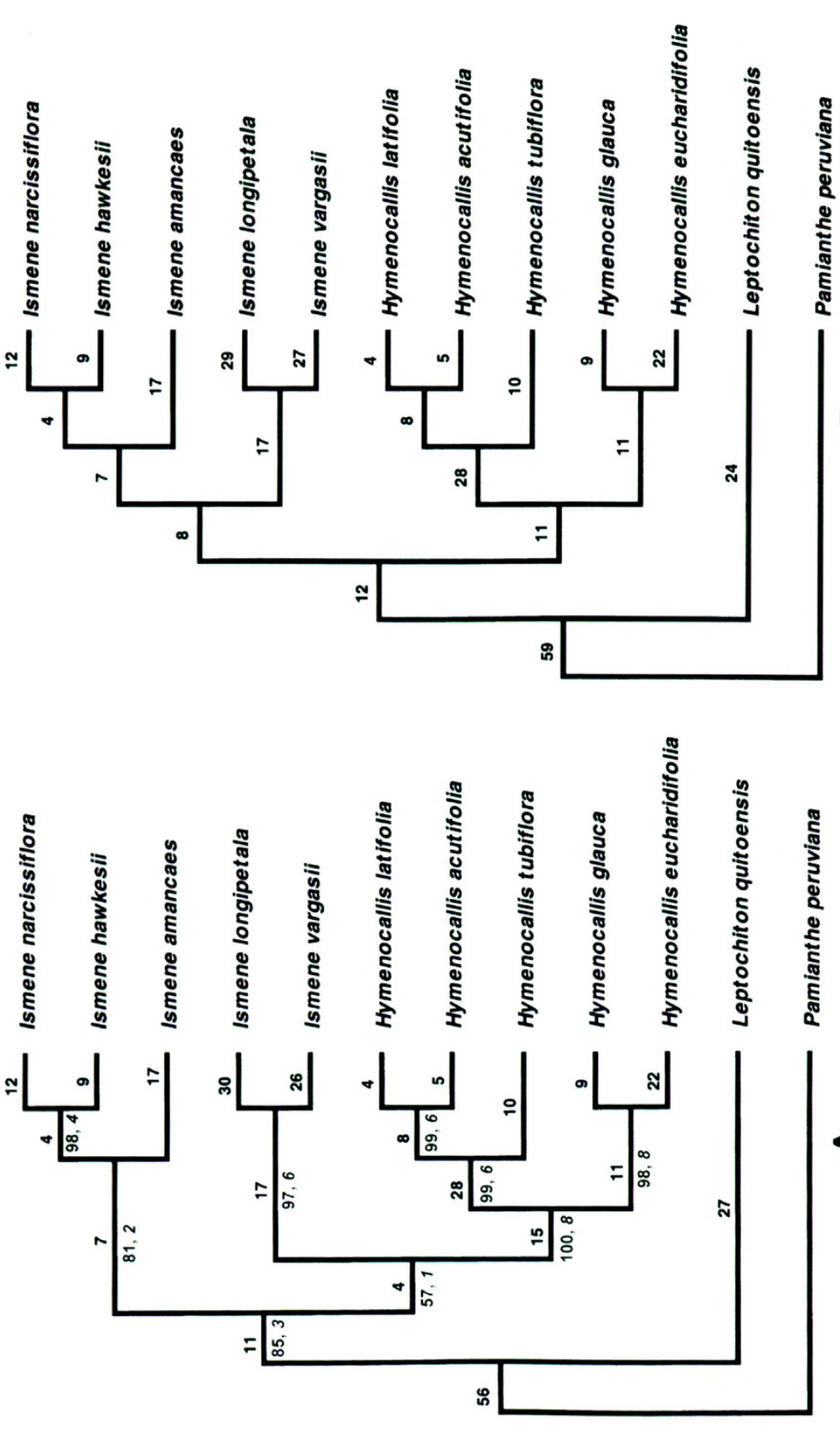

Figure 5. Cladograms for Hymenocallideae based on combined morphological and DNA sequence matrices. —A. Single most parsimonious tree. Numbers above branches are branch lengths; numbers below branches are bootstrap percentages followed by decay indices (italic). —B. Single additional tree found if trees one step longer than tree pictured in 5A are retained. Numbers above branches are branch lengths.

Discussion

Both plastid (Meerow et al., 1999) and ITS (Meerow et al., 2000a) sequences strongly support the position of the tribe Hymenocallideae as a monophyletic group within the Andean tetraploid clade of the endemic American Amaryllidaceae that is sister to the newly recognized tribe Clinantheae Meerow (Meerow et al., 2000a). The seeds of the Clinantheae are uniformly dry, flat, winged, and with phytomelanous testas. There are links between *Leptochiton* and *Pamianthe* that Stapf implicitly recognized, most notably the plesiomorphic presence of phytomelan in the testa of *Leptochiton*'s seed [of which Meerow & Dehgan (1985) were unaware], but also the numerous ovules of this genus (plesiomorphic as well). In the ITS phylogeny presented by Meerow et al. (2000a), support for *Pamianthe* as sister to the rest of Clinantheae (vs. a sister group relationship to Hymenocallideae or an unresolved position) was considerably weaker when the aligned matrix was not successively weighted. This is not surprising given that both genera occupy a basal phylogenetic position in their respective clades herein.

The difficulty of relying on morphological characters alone to generate phylogenies in Amaryllidaceae has been discussed (Meerow, 1995; Meerow et al., 2000b), given a high degree of homoplasy for many morphological characters in the family. Our analysis (Fig. 1) generates trees with relatively high CI and RI, but parsimony is still not able to resolve *Ismene* nor consistently place *Leptochiton* in the basal position within the tribe with unordered morphological characters alone, in contrast to sequence data (Figs. 2–4), which also provide (in the combined *trn*L-F and ITS matrix), over three times the number of phylogenetically informative characters of morphology alone. The combined plastid and nuclear sequence matrix produces the most fully resolved shortest trees. To "force" this topology upon any of the other conflicting data matrices requires either ordering characters or accepting longer trees (albeit only one step longer in the combined sequence and morphological analysis).

When biogeographic information is optimized upon the combined plastid and nrDNA tree (Fig. 4), the gene phylogeny supports an origin for the tribe in the central Andes, inarguably a locus of diversity for the Andean tetraploid clade of the Amaryllidaceae (Meerow et al., 2000a), with a vicariance event that gave rise to the largely North American *Hymenocallis*. *Leptochiton*, with 16 to 20 ovules per locule and a phytomelanous testa, occupies a relict position in the tribe with links to the non-fleshy seeded Andean endemic Clinantheae. However, it is the genus *Ismene* that reflects the patterns of floral morphological diversity that occur in the Eustephieae, Clinantheae, and Stenomesseae (sensu Meerow et al., 2000a). *Ismene* subg. *Ismene* retains the plesiomorphic pancratioid floral morphology of *Leptochiton*, *Pamianthe*, and *Hymenocallis*, while the smaller *Ismene* subg. *Elisena* and subg. *Pseudostenomesson* express floral novelties. *Ismene* subg. *Pseudostenomesson*, occurring at the highest elevations of any member of the tribe, might be the youngest element of the polymorphic *Ismene*, since the Andes likely did not extend above 1000 m elevation before the Pliocene (10 MYBP; Van der Hammen, 1974, 1979). Analogous patterns of floral diversity are found throughout the tetraploid Andean clade of the American Amaryllidaceae. In the Clinantheae, the low- to mid-elevation genera *Pamianthe* and *Paramongaia* Velarde have pancratioid floral morphology, while the mostly high-elevation *Clinanthus* Herb. has colorful, putatively ornithophilous flowers. In the more distantly related petiolate-leafed Stenomesseae, *Eucharis* has the pancratioid flower; *Plagiolirion* resembles a miniature *Ismene* subg. *Elisena*; and *Stenomesson* and *Urceolina* exhibit colorful, putatively ornithophilous flowers. Finally, in the Eustephieae, which is sister to rest of the Andean clade (Meerow et al., 2000a), the full range of variation is evident in a single genus, *Hieronymiella* Pax (Hunziker, 1969). This recurrent pattern suggests a scenario of rapid mosaic evolution (sensu Stebbins, 1984) within this monophyletic, tetraploid group (Meerow, 1987). The relatively low number of phylogenetically informative base substitutions in our sequence analyses of non-coding regions (7 for *trn*L-F; 50 for ITS) supports a hypothesis of a relatively recent radiation within the Hymenocallideae tied to the rise of the Andes. This seems most significant relative to *Ismene*, the most polymorphic of the three hymenocallid genera, and the only one that has adapted to high elevation.

Hymenocallis is most speciose in Mexico (Bauml, 1979), with a secondary area of diversity in the southeastern United States (Smith & Flory, 1990, 2001; Smith et al., 2001). Only three described species have been reported from South America: the broadly and coastally distributed *H. littoralis*, *H. pedalis*, and *H. tubiflora*. The genus does not occur at all in the Andes, and *H. tubiflora* is the only species of the three that is restricted to northern South America (including Trinidad-Tobago). The known distribution of the Hymenocallideae suggests two possible hypotheses, either a long-distance dispersal event from the Andean center of

origin, or extinction of intervening populations of a proto-*Hymenocallis* ancestor. The fleshy seed of *Hymenocallis* is the largest of all the endemic American Amaryllidaceae, exhibits no dormancy, and germinates within 3–4 weeks after release, whether or not in substrate (Whitehead & Brown, 1940; pers. obs.). The relatively heavy seed does not immediately seem amenable to long-distance dispersal, and no dispersal agent other than water has even been suggested for the genus. Thus ancestral extinction is a more convincing hypothesis, but without a better understanding of the historical biogeography of *Hymenocallis* and a well-resolved phylogeny of the genus a likely explanation for its distribution cannot be determined.

In summary, combined *trn*L-F and ITS sequences support the Meerow and Snijman (1998) treatment of Hymenocallideae with three genera: *Hymenocallis*, *Ismene*, and *Leptochiton*. *Leptochiton* is sister to the *Hymenocallis*/*Ismene* clade and retains two plesiomorphic characters of the Andean tetraploid clade: 16 to 20 ovules per locule and a phytomelanous seed coat. The central Andean endemism of *Ismene* and *Leptochiton* and the absence of *Hymenocallis* from this region further suggest a vicariance event at some point subsequent to the origin of the tribe. It is thus appropriate to formalize the recognition of the two new subgeneric combinations within *Ismene*.

Ismene subg. **Elisena** (Herbert) Meerow, comb. nov. Basionym: *Elisena* Herb., Amaryllidaceae, 75, 201. 1837. TYPE: *Ismene ringens* (Ruiz & Pav.) Gereau & Meerow, Novon 3: 29. 1993.

Ismene subg. **Pseudostenomesson** (Velarde) Meerow, comb nov. Basionym: *Pseudostenomesson* Velarde. Rev. Cienc. (Lima) 51: 47–51. 1949. TYPE: *Ismene vargasii* (Velarde) Gereau & Meerow, in L. Brako & J. Zarucchi, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1253. 1993.

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