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Phylogeny of the American Amaryllidaceae based on nrDNA ITS sequences

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ABSTRACT. Analysis of three plastid DNA sequences for a broad sampling of Amaryllidaceae resolve the American genera of the Amaryllidaceae as a clade that is sister to the Eurasian genera of the family, but base substitution rates for these genes are too low to resolve much of the intergeneric relationships within the American clade. We obtained ITS rDNA sequences for 76 species of American Amaryllidaceae and analyzed the aligned matrix cladistically, both with and without gaps included, using two species of *Pancratium* as outgroup taxa. ITS resolves two moderately to strongly supported groups, an Andean tetraploid clade, and a primarily extra-Andean ''hippeastroid'' clade. Within the hippeastroid clade, the tribe Griffineae is resolved as sister to the rest of Hippeastreae. The genera *Rhodophiala* and *Zephyranthes* are resolved as polyphyletic, but the possibility of reticulation within this clade argues against any re-arrangement of these genera without further investigation. Within the Andean subclade, Eustephieae resolves as sister to all other tribes; a distinct petiolate-leafed group is resolved, combining the tribe Eucharideae and the petiolate Stenomesseae; and a distinct Hymenocallideae is supported. These Andean clades are all at least partially supported by plastid sequence data as well. We infer from our data that a great deal of the diversity of the family in the Americas is recent, and that the American Amaryllidaceae may have been reduced to peripheral isolates some time after its initial entry and spread through the Americas. While the sister relationship of the American and Eurasian clades might argue for a Boreotropical origin for the family in America, the cladistic relationships within the American clade based on ITS do not provide any further support for this or any other hypothesis of the family's entry into America. The new tribe **Clinantheae** is described (four genera: *Clinanthus, Pamianthe, Paramongaia,* and *Pucara*), and the lorate-leafed species of *Stenomesson* are transferred to *Clinanthus.*

Amaryllidaceae J. St.-Hil., a cosmopolitan (predominantly pantropical) family of petaloid monocots, represent one of the elements of the Linnaean *Hexandria monogynia* (Linnaeus 1753), the 51 genera of which have been variously classified since as liliaceous or amaryllidaceous. Meerow et al.(1999) reviewed the taxonomic history of the family. Despite a lack of consensus on generic limits and tribal delimitations within Amaryllidaceae, cladistic analysis has only rarely been applied to problems in the family, such as by Nordal and Duncan (1984) for *Haemanthus* and *Scadoxus,* two closely related, baccate-fruited African genera; Meerow (1987, 1989) for *Eucrosia* and *Eucharis* and *Caliphruria* respectively; Snijman (1994) and Snijman and Linder (1996) for various taxa of tribe Amaryllideae; and Meerow et al. (1999) for the entire family using three plastid DNA sequences. Phylogenetic studies for the entire family using morphological characters are made difficult by homoplasy for many conspicuous characters within this highly canalized group (Meerow et al., 2000).

The four most recent infrafamilial classifications of Amaryllidaceae are those of Traub (1963), Dahlgren et al. (1985), Müller-Doblies and Müller-Doblies (1996) and Meerow and Snijman (1998). Traub's scheme included Alliaceae, Hemerocallidaceae, and Ixioliriaceae as subfamilies, following Hutchinson (1934, 1959) in part. Within his subfamily Amarylloideae, he erected two informal taxa, ''infrafamilies'' Amarylloidinae and Pancratioidinae, both of which were polyphyletic (Meerow 1995). Dahlgren et al. (1985) dispensed with any subfamilial classification above the level of tribe, recognizing eight, and treated as Amaryllidaceae only those genera in Traub's Amarylloideae. Stenomesseae and Eustephieae were combined. Meerow (1995) resurrected Eustephieae from Stenomesseae and suggested that two new tribes needed to be recognized, Calostemmateae and Hymenocallideae. Müller-Doblies and Müller-Doblies (1996) recognized ten tribes (among them Calostemmateae) and nineteen subtribes, many of them monogeneric; Meerow and Snijman (1998) recognized 14 tribes, with two subtribes only in one of them. These classifications are compared in detail in Meerow et al. (1999).

Meerow et al. (1999) presented cladistic analyses of combined plastid DNA sequences *rbcL, trnL* gene and the *trnL-F* intergenic spacer for 48 genera of Amaryllidaceae and 29 genera of related Asparagales. Good support was provided for the monophyly of Amaryllidaceae. The infra-familial relationships of Amaryllidaceae were resolved along biogeographic lines. Ito et al. (1999) resolved a very similar topology for a more limited sampling of Amaryllidaceae and related asparagoids using plastid *matK* sequences. The most surprising conclusion from the plastid sequence phylogeny was that the Eurasian and American elements of the family are each monophyletic sister clades. However, there was insufficient base substitution among the American genera to resolve their relationships sufficiently with plastid sequences.

The 18S and 26S subunits of nuclear ribosomal DNA (nrDNA) are separated by two internal transcribed spacer regions, ITS1 and ITS2, and a 5.8S gene located between the two ITS regions. ITS1 varies in angiosperms from 187–298 bp, and ITS2 from 187–252 bp (Baldwin et al. 1995). The two ITS regions evolve more rapidly than the coding regions they separate. For closely related taxa, rapidly evolving, non-coding regions of nuclear genes such as ITS can potentially yield a greater degree of informative sequence variation than the more highly conserved coding regions. From a practical standpoint, the small size of the ITS region, and its location between highly conserved sequences, make the spacers easy to amplify by PCR (Baldwin et al. 1995). Potential problems of paralogous sequences due to the multiple copy nature of nrITS can be at least partially overcome by sequencing more than one clone of each taxon or pooling PCR extracts of several clones for the sequence reactions (Baldwin et al. 1995).

ITS shows greatest utility for generating gene phylogenies at the rank of family and below (Baldwin et al. 1995). To date, ITS sequences have been sparingly successful in resolving family-wide phylogenies (Fouquieriaceae, Schultheis and Baldwin 1999; Nothofagaceae, Manos 1993; Winteraceae, Suh et al. 1992), but have been most valuable when applied to single genera or at the subfamilial rank; e.g., *Aquilegia* (Ro et al. 1997), *Fraxinus* (Jeandroz et al. 1997), *Lupinus* (Kass and Wink 1997), *Saintpaulia* (Moeller and Cronk 1997), Adoxaceae: Adoxoideae (Eriksson and Donoghue 1997), Rosaceae subfamily Maloideae (Campbell et al. 1995), Orchidaceae subtribe Orchidinae (Bateman et al. 1997; Pridgeon et al. 1997), Poaceae subfamily Arundinoideae (Hsiao et al. 1998).

In this paper we present cladistic analyses of the internal transcribed spacer region (ITS) of nuclear ribosomal DNA for 76 species of American Amaryllidaceae (representing all but five of the American endemic genera) and discuss our results in the context of previous plastid sequence phylogenies (Ito et al. 1999; Meerow et al. 1999). We also discuss the biogeographic implications of these data.

MATERIALS AND METHODS

Plant Materials. Species used in the sequence analyses, voucher specimens and GenBank accession numbers for the sequences are listed in Table 1. All genera of the American Amaryllidaceae were sampled except *Mathieua* (presumed extinct), *Plagiolirion* (repeated attempts to amplify DNA from a recent herbarium specimen failed) and *Placea, Pucara,* and *Traubia* (material not available). The presence of the pantropical *Crinum* (tribe Amaryllideae) in the Americas is understood to represent a dispersal event unrelated to the origins of the endemic American tribes of Amaryllidaceae (Arroyo and Cutler 1984; Meerow et al. 1999) and no American species of this genus are considered here.

DNA Extraction. Genomic DNA was extracted from silica gel dried leaf tissue using a modified CTAB procedure of Doyle and Doyle (1987).

PCR and Sequencing Protocol. 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) primers ITS5, 4, 2 and 3 to amplify the spacers along with the intervening 5.8S sequence. Amplified products were purified

TABLE 1. Taxa used in the cladistic analyses nrDNA (ITS) sequence. Tribal and generic assignments in Amaryllidaceae follow Meerow and Snijman (1998). All vouchers are deposited at FTG unless otherwise noted.

Taxon	Voucher	GenBank Accession No.
Euchariddeae		
Caliphruria korsakoffii (Traub) Meerow	*Meerow 1096* (FLAS)	AF223529
Caliphruria subedentata Bak.	*Meerow 1109* (FLAS)	AF223549
Eucharis amazonica Lind. ex Planch.	*Schunke 14057* (FLAS)	AF223538
Eucharis castelnaeana (Baill) Macbr.	*Schunke 14156* (FLAS)	AF223525
Eucharis formosa Meerow	*Whitten et al. 95020* (FLAS)	AF223539
Urceolina microcrater Kränzl.	*Plowman & Kennedy 5721* (GH)	AF223531
Eustephieae		
Chlidanthus boliviensis Traub & I. S. Nelson	*Traub 529a* (MO)	AF223545
Chlidanthus fragrans Herb.	*Meerow 2312*	AF223524
Eustephia darwinii Vargas	*Meerow 2436*	AF223543
Hieronymiella argentina (Pax) A. T. Hunz. & S. Arroyo-L.	*M. W. Chase 1901* (K)	AF223541
Hippeastreae		
Griffinia hyacinthina Ker Gawler	*Meerow 2106*	AF223473
Griffinia nocturna Rav.	*Pereira & Paula 2351* (UB)	AF223491
Griffinia rochae Morel	*Meerow 1154*	AF223486
Habranthus brachyandrus (Bak.) Sealy	*Meerow 2400*	AF223504
Habranthus immaculatus Traub & Clint	*Meerow 2401*	AF223500
Habranthus martinezii Rav.	*Meerow 2437*	AF223497
Habranthus sp.	*Meerow 2402*	AF223499
Habranthus tubispathus (L'Hérit.) Traub	*Meerow 2403*	AF223498
Haylockia americana (Hoffgg.) Herter	*M. W. Chase 3585* (K)	AF223506
Hippeastrum blumenavium (K. Koch & Bouche ex Carr) Sealy	*Meerow 2404*	AF223501
Hippeastrum brasilianum (Traub & Doran) Dutilh	*Meerow 2405*	AF223479
Hippeastrum macbridei (Vargas) Gereau & Brako	*Meerow 2435*	AF223509
Hippeastrum molleviquensis (Cárd.) Van Scheepen	*Doran 1538* (MO)	AF223489
Hippeastrum papilio (Rav.) Van Scheepen	*Meerow 2406*	AF223496
Hippeastrum parodii A. T. Hunz. & Cocucci	*Meerow 2434*	AF223508
Hippeastrum reticulatum Herb.	*Meerow 2407*	AF223484
Phycella ignea Lindl.	*Meerow 2408*	AF223505
Pyrolirion sp.	*M. W. Chase 3639* (K)	AF223493
Rhodophiala bagnoldii (Herb.) Traub	*Meerow 2425*	AF223476
Rhodophiala bifida (Herb.) Traub	*Meerow 2410*	AF223477
Rhodophiala chilensis (L'Hérit.) Traub	*Meerow 2426*	AF223480
Rhodophiala moelleri (R. Phil.) Traub	*M. W. Chase 1908* (K)	AF223481
Sprekelia formosissima (L.) Herb.	*Meerow 1151*	AF223483
Worsleya rayneri (Hook.) Traub & Moldenke	*Meerow 2411*	AF223475
Zephyranthes atamasco (L.) Herb.	*Meerow 2412*	AF223474
Zephyranthes simpsonii	*Meerow 2413*	AF223472
Zephyranthes candida Herb.	*Meerow 2414*	AF223503
Zephyranthes cearensis Bak.	*Meerow 2415*	AF223507
Zephyranthes citrina Bak	*Meerow 2416*	AF223495
Zephyranthes drummondii D. Don.	*Meerow 2417*	AF223488
Zephyranthes filifolia Herb. ex Baker	*M. W. Chase 1836* (K)	AF223490
Zephyranthes flavissima Rav.	*Meerow 2418*	AF223510
Zephyranthes grandiflora Lindl.	*Meerow 2419*	AF223485
Zephyranthes mesochloa Herb.	*Meerow 2420*	AF223502
Zephyranthes morrisclintii Traub & T. M. Howard	*Meerow 2421*	AF223492
Zephyranthes pulchella F. D. Smith	*Meerow 2422*	AF223494
Zephyranthes rosea Lindl.	*Meerow 2429*	AF223487
Zephyranthes smallii (Alexander) Traub	*Meerow 2423*	AF223482

TABLE 1. Continued.

Taxon	Voucher	GenBank Accession No.
Hymenocallideae		
Hymenocallis acutifolia (Herb.) Sweet	*Meerow 2424*	AF223514
Hymenocallis glauca M. Roem.	*Meerow 2433*	AF223515
Hymenocallis latifolia (Mill.) Roem.	*Meerow 2438*	AF223516
Hymenocallis speciosa Salisb.	*Meerow 2439*	AF223512
Hymenocallis tubiflora Salisb	*Meerow 2440*	AF223513
Ismene hawkesii (Vargas) Gereau & Meerow	*Meerow 2441*	AF223519
Ismene longipetala (Lindl.) Meerow	*Sagastegui 15454*	AF223520
Ismene narcissiflora Jacq.	*Meerow 2306*	AF223518
Ismene vargasii (Velarde) Gereau & Meerow	*Meerow 2308*	AF223517
Leptochiton quitoensis (Herb.) Sealy	*Meerow 1116*	AF223521
Pancratieae		
Pancratium canariensis L.	*Meerow 1142*	AF223531
Pancratium tenuifolium Hochst.	*Meerow 2427*	AF223537
Stenomesseae		
Eucrosia bicolor Ker Gawl.	*Meerow 1113*	AF223511
Eucrosia dodsonii Meerow & Dehgan	*Meerow 1115*	AF223544
Eucrosia eucrosioides (Pax) Traub	*Meerow 1117*	AF223548
Eucrosia stricklandii (Bak.) Meerow	*Meerow 2428*	AF223540
Pamianthe peruviana Stapf	*Meerow 2304*	AF223546
Paramongaia weberbaueri Velarde	*Meerow 2303*	AF223536
Phaedranassa cinerea Rav.	*Meerow & Meerow 1045*	AF223527
Phaedranassa tunguraguae Rav.	*Meerow & Meerow 1130*	AF223526
Phaedranassa ventricosa Bak.	*Meerow 1129*	AF223528
Rauhia decora Ravenna	*Meerow 1160*	AF223523
Rauhia multiflora (Kunth) Rav.	*Meerow 2441*	AF223522
Stenomesson aurantiacum Herb.	*Meerow 1061* (FLAS)	AF223542
Stenomesson flavum (R. & P.) Herb.	*Meerow 2430*	AF223532
Stenomesson humilis Bak.	*Meerow 2442*	AF223547
Stenomesson incarnatum Bak.	*Meerow 1120*	AF223533
Stenomesson mirabile Rav.	*S. Leiva et al. 2000* (HAO)	AF223534
Stenomesson variegatum (R. & P.) Bak.	*Meerow 1159*	AF223535

using QIAquick (Qiagen, Valencia, CA) columns, following manufacturers protocols. PCR amplifications were performed on an ABI 9700 (Perkin-Elmer Applied Biosystems, Inc., Foster City, CA), running 28 cycles of the following program: 4 min at 94° C, 1 min at 52°, and 3 min at 72°.

Cycle sequencing reactions were performed directly on purified PCR products in the ABI 9700, using standard dideoxy cycle protocols for sequencing with dye terminators on an ABI 377 automated sequencer (according to the manufacturer's protocols; Applied Biosystems, Inc.). It was determined via cloning prior to beginning our ITS work on the neotropical Amaryllidaceae that paralogous variation among the tandem repeats of the ITS region (Baldwin et al. 1995) is not an issue in the group.

Sequence Alignment. ITS sequences were aligned in two ways. First, we used the sequence editing and alignment program Sequencher (Gene Codes, Inc., Ann Arbor, MI) to align sequences of closely related taxa with subsequent builds of these smaller alignments performed manually. We also used the program ClustalX (Higgins and Sharp 1988, Thompson et al. 1997) to align the sequences with a gap opening penalties of both 15 and 30 and gap extension penalties of 0.5 to 6.66. We also loaded our Sequencher alignment into ClustalX, and allowed the program to re-align the sequences but without removing gaps we had previously inserted. Low-scoring areas in all of the alignments were carefully examined. By comparing alignments among the three methods, as well as running heuristic searches with PAUP (see below) on each reiteration, we were able to refine our ultimate alignment considerably before cladistic analysis. Our manually edited alignment consistently produced the shortest trees when analyzed cladistically. The matrix and resulting trees are available from TreeBASE (http://www.herbaria.harvard.edu/treebase).

Analyses. Aligned matrices were analyzed using the parsimony algorithm of the software package PAUP* for Macintosh (v4.02 beta; Swofford 1998), with the MULPARS option and ACCTRAN optimization invoked. Zero length branches were collapsed only if the maximum length = 0. A successive weighting (SW) strategy (Farris 1969) was implemented. SW is a useful tool employed to globally reduce the effect of highly homoplasious base positions on the resulting topologies (Lledó et al. 1998; Meerow et al. 1999; Wenzel 1997). Whole category weights (codon or transversion) exhibit broad and overlapping ranges of consistency (Olmstead 1997), whereas successive weighting independently assesses each base position of the multiple alignment based on their consistency in the initial analysis.

The initial tree search was conducted under the Fitch (equal) weights (Fitch 1971) criterion with 1000 random sequence additions and SPR (subtree pruning-regrafting) branch swapping, permitting only ten trees to be held at each step to reduce the time spent searching trees at suboptimal levels. All trees collected in the 1000 replicates were swapped to completion or an upper limit of 5000 trees. The characters were then reweighted by the rescaled consistency index, and a further 50 replications of random sequence additions conducted with the weighted matrix saving 15 trees per replication. These trees were then swapped to completion or an upper limit of 5000 trees. The resulting trees were then used to reweight the matrix a second time by the rescaled consistency index, and another 50 replicates of random sequence addition conducted, saving 15 trees per replication, with subsequent swapping on those trees. This cycle was repeated until two successive rounds found trees of the same length.

Internal support was determined by bootstrapping (5000 replicates) with the final reweighted character matrix, and with 5000 replications of the jackknife procedure (Farris et al. 1996), the later with equal weight applied. In this way, support for both the weighted and unweighted matrices could be presented. The cut-off bootstrap percentage is 50; that for the jackknife is 63%. The ITS matrix consisted of 76 taxa, 74 American species and two species of *Pancratium* as outgroup taxa. *Pancratium* is part of the Eurasian clade that is the sister group to the American Amaryllidaceae (Ito et al. 1999; Meerow et al. 1999). Other members of the Eurasian clade were also used as outgroup taxa, alone or together, without any topological changes in the American clades, but the *Pancratium* sequences presented the least alignment ambiguities. Bremer (1988) decay indices (DI) were calculated using the program Tree Rot (Sorenson 1996). One hundred replicate heuristic searches were implemented for each constraint statement postulated by Tree Rot, saving 10 trees per replicate.

The matrix was analyzed with gaps coded as missing data but a gap matrix was also constructed from the alignment using the program PAUPGAP (Anthony Cox, RBG Kew), which applies a strict interpretation of gaps (i.e., no partial homology). This binary matrix was added to the alignment and the combined data set analyzed cladistically as previously detailed.

RESULTS

Gaps Coded as Missing Data. Of 697 included base positions in the analyses, 347 were parsimony informative. Our initial 1000 replications with equal weights applied found 9600 equally most parsimonious trees of length = 1458 with a consistency index (CI) of 0.54 and a retention index (RI) of 0.82. SW produced at least 5000 equally parsimonious trees with a length of 553270 (Fitch length = 1459), a CI = 0.73 (Fitch = 0.54), and RI = 0.91 (Fitch = 0.82). The additional step of the SW trees is essentially the ''cost'' of optimizing consistent characters over highly homoplastic base positions (Lledó et al. 1998; Meerow et al. 1999). Both the SW and Fitch topologies were very similar; however, as the consensus of the SW trees was slightly more resolved than that of the Fitch trees, they will be the focus of the discussion.

Two large subclades are resolved in the monophyletic American Amaryllidaceae (Figs. 1, 2). The first, or ''hippeastroid'' clade (Fig. 1) is referred to as the diploid (n = 11, 12 or less primarily, though polyploid species do occur), primarily extra-Andean element of the family (though several of the genera have Andean representatives), comprising the genera treated as the tribe Hippeastreae in most recent classifications (Dahlgren et al. 1985; Müller-Doblies and Müller-Doblies 1996; Meerow and Snijman 1998). The second subclade constitutes the tetraploid-derived (n = 23), Andean-centered tribes. In both subclades, one tribe is sister to all of the other genera in their respective groups. The Eustephieae resolve as sister to the rest of the Andean tribes (Fig. 2), but with weak support (DI = 2, 56%

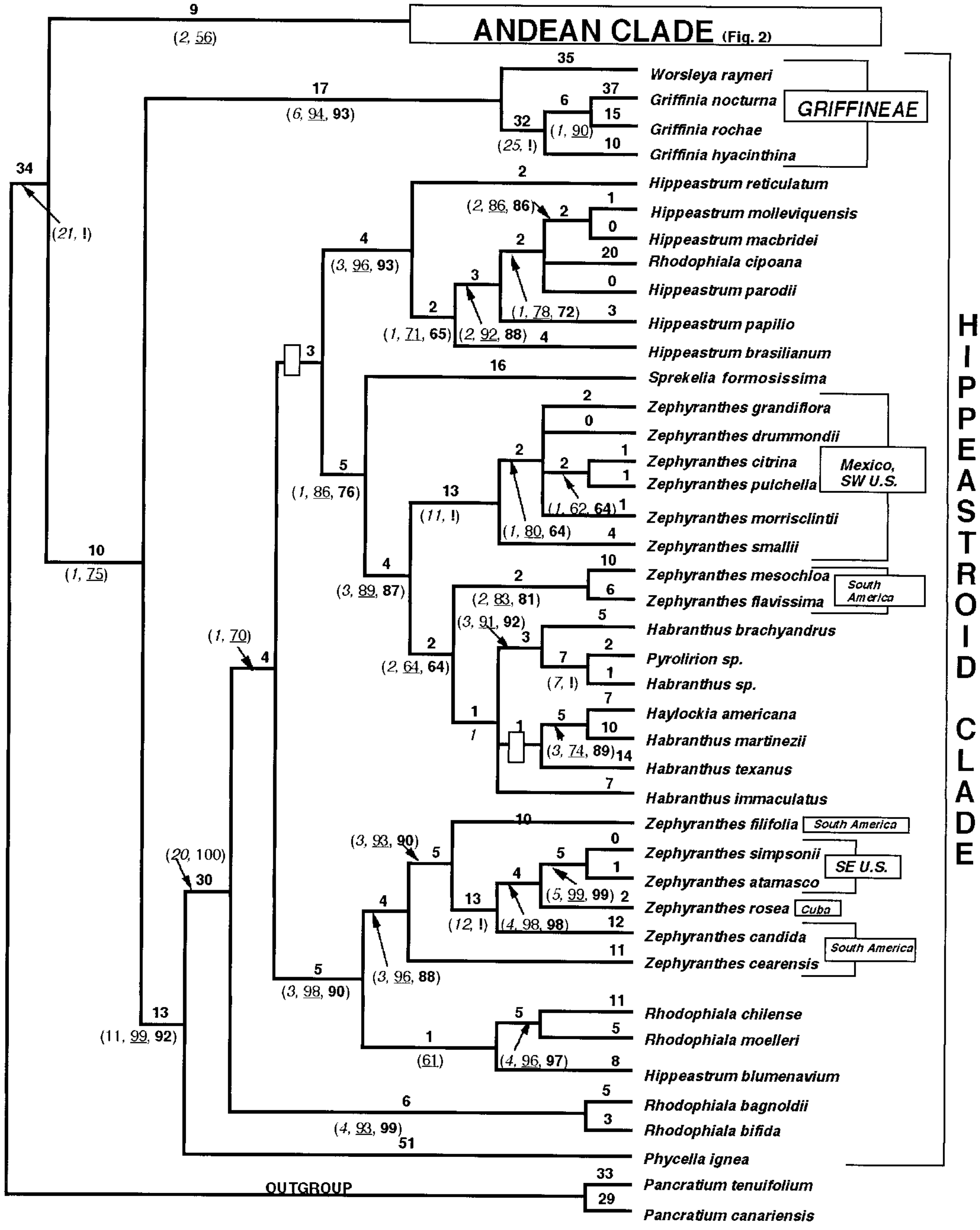

FIG. 1. One of 5000 equally most parsimonious trees found by SW heuristic search of ITS sequence matrix for the American Amaryllidaceae. Only the outgroup and "hippeastroid" clade is shown in detail; the tree is continued in Fig. 2. Numbers above branches are branch lengths; numbers below branches are DI (italic), bootstrap support (SW, underline), and jackknife support (equal weights, boldface). A white bar across a branch signifies a collapsed node in the strict consensus of all trees. An exclamation point (!) in the support values indicates that both bootstrap and jackknife = 100%.

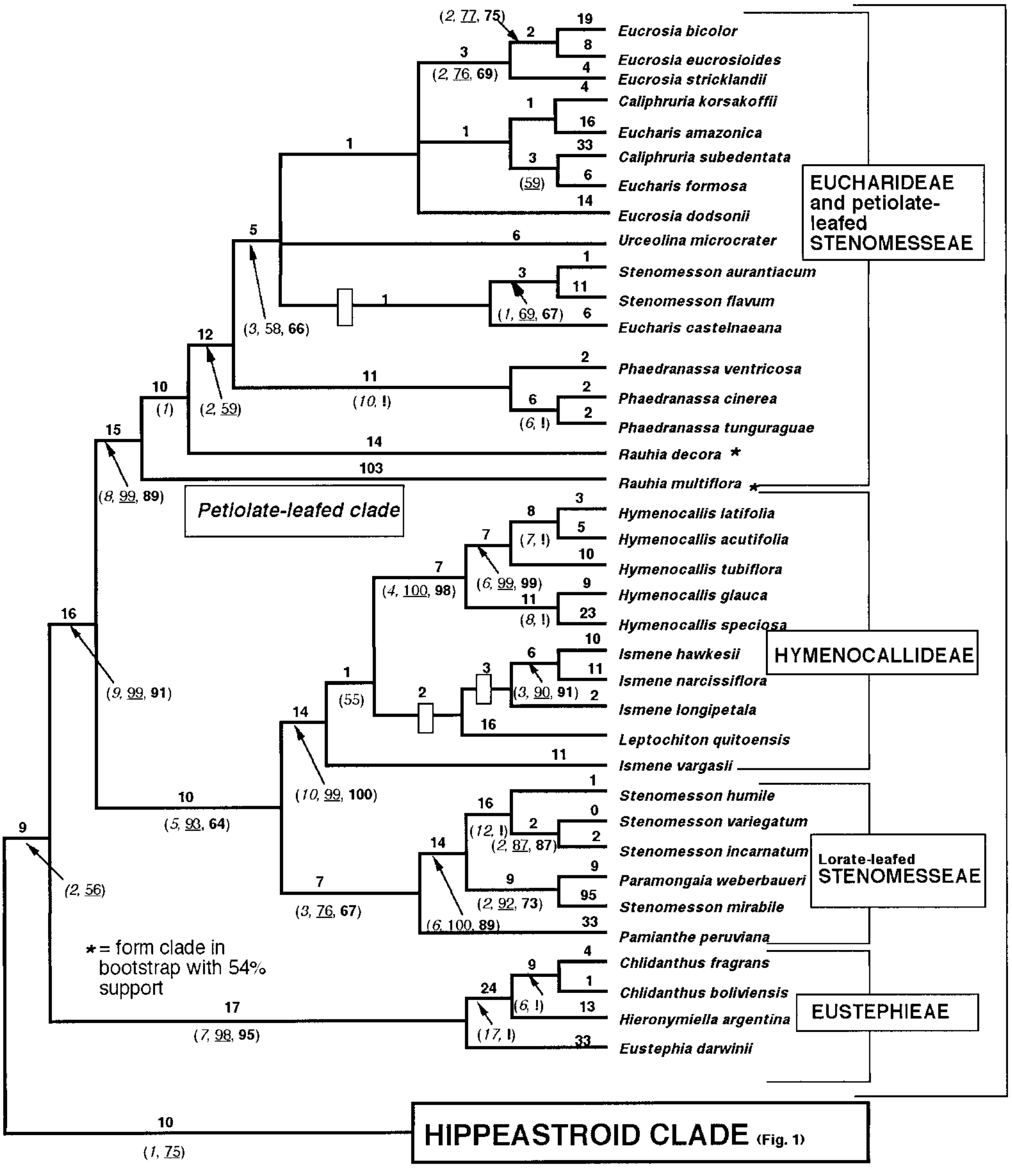

FIG. 2. One of 5000 equally most parsimonious trees found by SW heuristic search of ITS sequence matrix for the American Amaryllidaceae. Only the "Andean" clade is shown in detail; the tree is continued in Fig. 1. Numbers above branches are branch lengths; numbers below branches are DI (italic), bootstrap support (SW, underline), and jackknife support (equal weights, boldface). A white bar across a branch signifies a collapsed node in the strict consensus of all trees. An exclamation point (!) in the support values indicates that both bootstrap and jackknife = 100%.

bootstrap, no jackknife). In the hippeastroid clade, the Griffineae (*Worsleya* and *Griffinia*) are sister to the rest of the genera, with DI = 1, 75% bootstrap, and no jackknife support.

HIPPEASTROID CLADE. The Griffineae are a well supported clade (DI = 6, 94% bootstrap and 93% jackknife). *Phycella* is sister to the remaining hippeastroids (DI = 11, 99% bootstrap, 92% jackknife), followed by two species of *Rhodophiala* (DI = 4, 93% bootstrap, 99% jackknife), with strong support only in the SW trees (100% bootstrap, DI = 20). Two larger clades are then resolved, but with only DI = 1, 70% bootstrap and no jackknife support. The first unites two additional species of *Rhodophiala* and *Hippeastrum blumenavium* in a weakly supported subclade (DI = 0, 61% bootstrap and no jackknife support) that is in turn sister to a well-supported subclade of *Zephyranthes* species from North America, South America and the West Indies (DI = 3, 97% bootstrap and 88% jackknife). The overall support for this clade is strong (DI = 3, 98% bootstrap, 90% jackknife).

The largest clade of hippeastroid genera is split into two smaller monophyletic groups in the SW topology (Fig. 1). [In the consensus of our original 9600 trees with equal weights imposed, these two subclades form a trichotomy with the heterogeneous clade described previously (not shown)]. The smaller of the two represents the genus *Hippeastrum,* with the exception of *H. blumenavium,* and includes a species described as a *Rhodophiala* from Brazil (all other species in this genus are from Chile and Argentina), *R. cipoana.* Support for this clade is strong (DI = 3, 96% bootstrap, 93% jackknife). *Hippeastrum reticulatum* is resolved as sister to all other species in this clade. The second subclade essentially represents what has been called tribe Zephyrantheae (Traub 1963) or subtribe Zephyranthinae (Müller-Doblies and Müller-Doblies 1996), excluding the *Zephyranthes* species that resolve elsewhere. This clade, in which *Sprekelia* is resolved as sister to the rest of the group, has only modest support (DI = 1, 86% bootstrap, 76% jackknife). After *Sprekelia* branches, a more strongly supported clade (DI = 3, 89% bootstrap, 87% jackknife) is resolved consisting of two smaller subclades. The first is a very well supported group of entirely Mexican *Zephyranthes* (including *Cooperia*) species (DI = 11, 100% bootstrap and jackknife). The second is a weakly supported (DI = 2, 64% bootstrap, 64% jackknife) subclade that unites two additional South American *Zephyranthes* species as sister group to *Habranthus,* the latter within which is embedded the genera *Haylockia* (= *Zephyranthes pusila*) and *Pyrolirion.* The *Habranthus* subclade has no support and a DI = 1.

ANDEAN CLADE. The Eustephieae are well resolved (DI = 7, 98% bootstrap, 95% jackknife, Fig. 2) as a clade distinct from Stenomesseae, though the sister group relationship of this clade to the rest of the Andean clade is weak (DI = 2, 56% bootstrap, no jackknife). Hymenocallideae is also well-supported as a clade (DI = 10, 99% bootstrap, 100% jackknife), though only *Hymenocallis* is resolved as monophyletic (DI = 4, 100% bootstrap, 98% jackknife). The Hymenocallideae is sister to a clade comprising some elements of Stenomesseae (sensu Meerow and Snijman 1998) with DI = 5, strong bootstrap (93%) but weak jackknife (64%) support.

The petiolate-leafed Andean genera form a well-supported clade (DI = 8, 99% bootstrap, 89% jackknife) comprising elements of Stenomesseae and all of Eucharideae (sensu Meerow and Snijman 1998). However, only some of the component genera are resolved. *Phaedranassa* is well supported (DI = 10, 100% bootstrap and jackknife) and *Eucrosia,* less the mesophyte *E. dodsonii,* is weakly supported (DI = 2, 74% bootstrap, 70% jackknife). *Stenomesson* (petiolate-leafed) also is resolved with weak support (DI =1, 69% bootstrap and jackknife). The two species of *Rauhia* form a weak clade in only the bootstrap consensus (54%). By contrast, relationships among the genera of Eucharideae sensu Meerow and Snijman (1998) are poorly resolved.

Gap Matrix Added to Alignment. Of 797 total characters, 396 were parsimony informative. Our initial 1000 replicate heuristic search with equal weights applied found at least 7950 trees of length = 1644, with CI = 0.54 and RI = 0.82. SW found 198 trees of length = 634628 (Fitch length = 1644), with CI = 0.75 (Fitch = 0.54) and RI = 0.91 (Fitch = 0.82).

As with the analysis of base substitutions alone (Figs. 1, 2), the hippeastroid and Andean subclades are resolved, with Griffineae and Eustephieae as sister to each, respectively (Figs. 3, 4). Bootstrap support for both subclades is higher than with the base substitution matrix alone (84% for the hippeastroid group, 90% for the Andean group), but only the Andean subclade receives jackknife support (92%). DI = 5 for the Andean group, but only 1 for the hippeastroid subclade.

There are several consistent and significant gaps in our alignment that represent synapomorphies for the Andean clade (Fig. 5), and these strengthened the support values for this group: 1) base position (bp) 43–52, bp 90–99 in ITS1 and 22–28 in ITS2 (except Eustephieae).

HIPPEASTROID CLADE. When the gap matrix is included in the analysis (Fig. 3), resolution of these taxa is very similar to that of the base substitution matrix alone (Fig. 1). Polyphyletic *Zephyranthes* and *Rhodophiala* are once again indicated, and *Phycella* is sister to the rest of the Hippeastreae.

ANDEAN CLADE. The SW trees from the

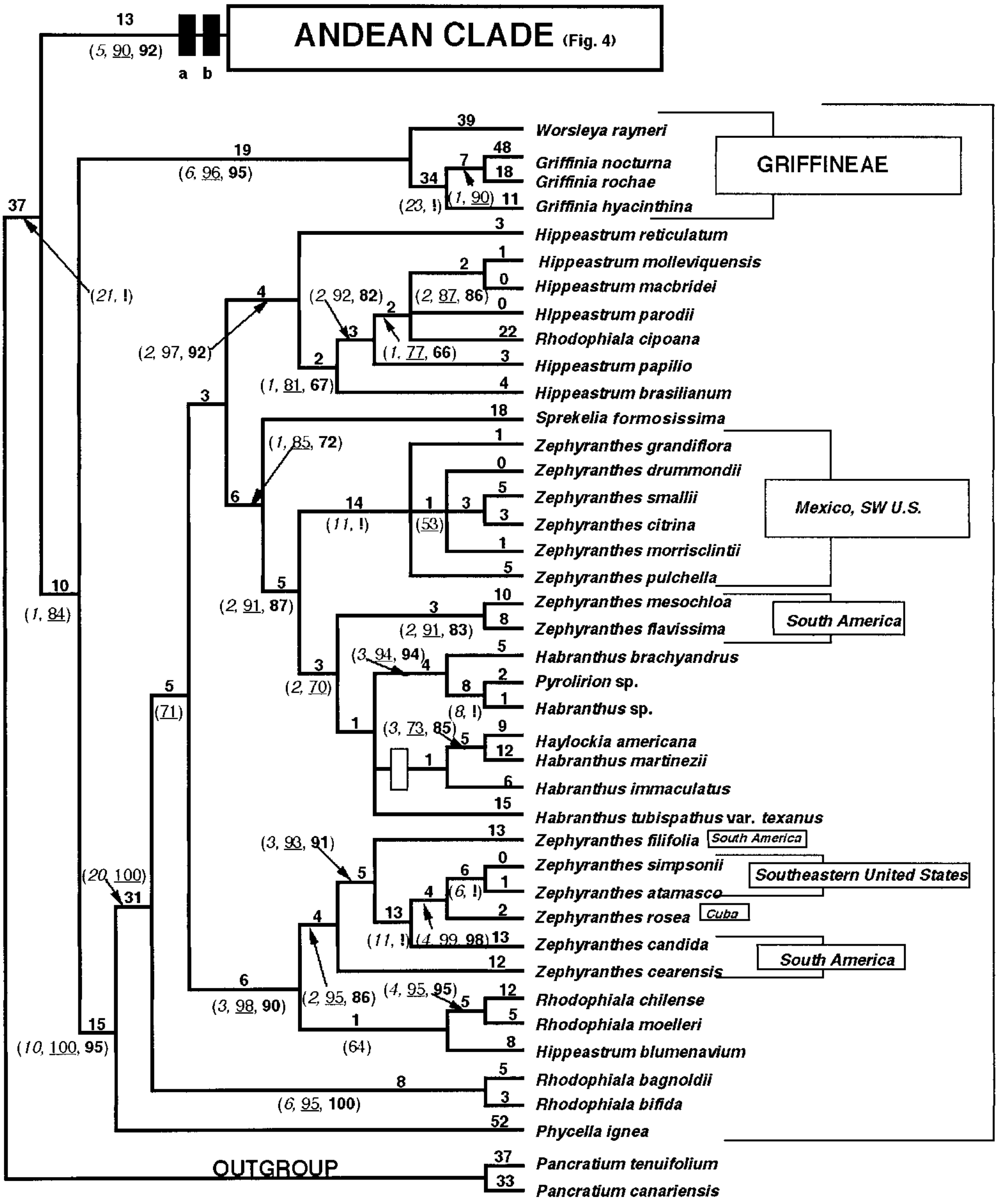

FIG. 3. One of 196 equally most parsimonious trees found by SW heuristic search of ITS sequences plus gap matrix for the American Amaryllidaceae. Only the "Andean" clade is shown in detail; the tree is continued in Fig. 4. Numbers above branches are branch lengths; numbers below branches are DI (italic), bootstrap support (SW, underline), and jackknife support (equal weights, boldface). A white bar across a branch signifies a collapsed node in the strict consensus of all trees. Black bars and lower case letters refer to synapomorphic indels illustrated in Fig. 5. An exclamation point (!) in the support values indicates that both bootstrap and jackknife = 100%.

combined base substitution and gap matrix did add further resolution to the Andean clade, though the overall topology is not changed. Within the Hymenocallideae, *Ismene* and *Leptochiton* are resolved as sister to *Hymenocallis,* though *Ismene* is still not monophyletic unless *Leptochiton* is included. Support is weak for this group (DI = 0, 54% bootstrap). In the petiolate-leafed clade, *Rauhia* is resolved as a

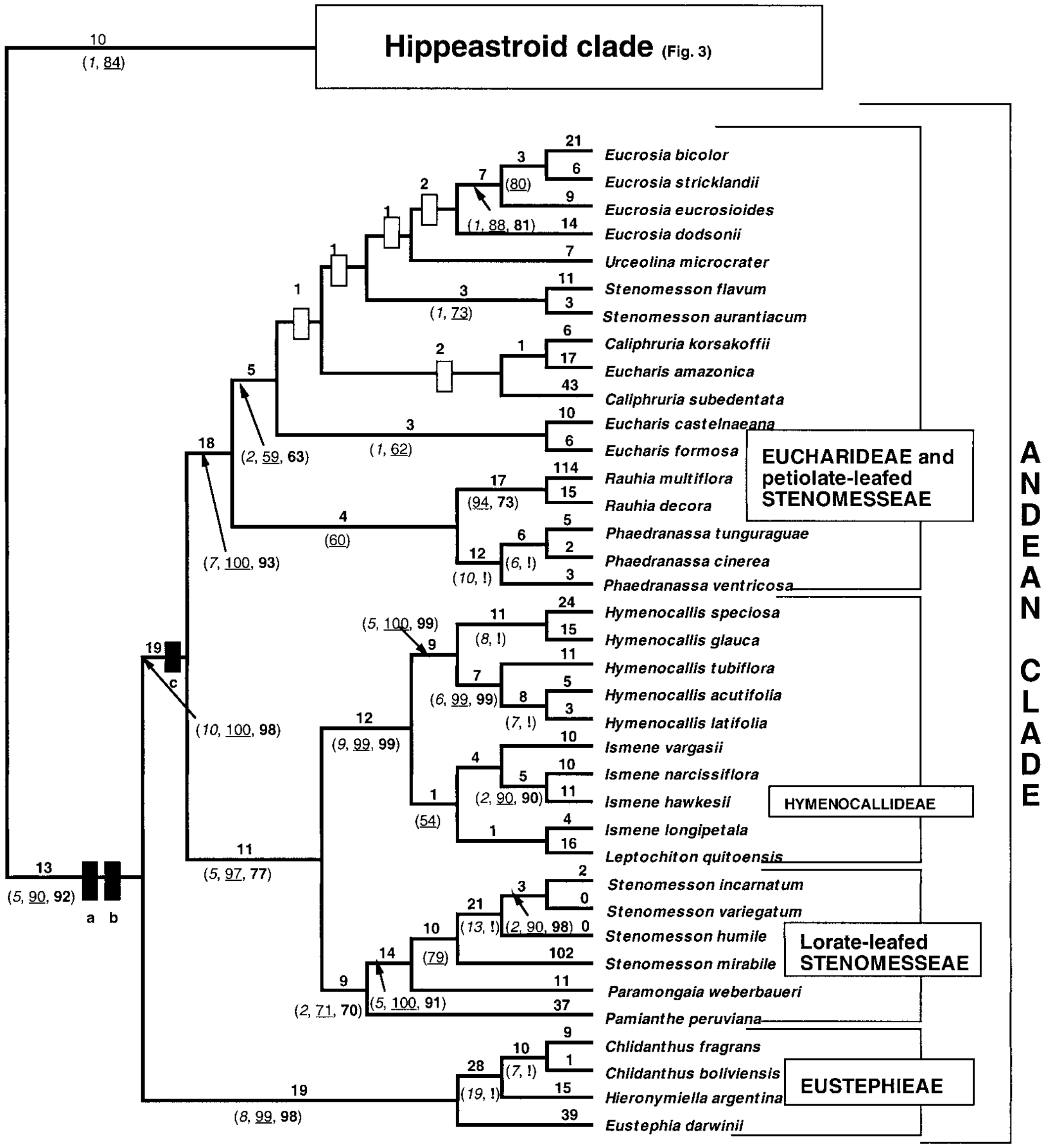

FIG. 4. One of 196 equally most parsimonious trees found by SW heuristic search of ITS sequence matrix alone for the American Amaryllidaceae. Only the "Andean" clade is shown in detail; the tree is continued in Fig. 3. Numbers above branches are branch lengths; numbers below branches are DI (italic), bootstrap support (SW, underline), and jackknife support (equal weights, boldface). A white bar across a branch signifies a collapsed node in the strict consensus of all trees. Black bars and lower case letters refer to synapomorphic indels illustrated in Fig. 5. An exclamation point (!) in the support values indicates that both bootstrap and jackknife = 100%.

monophyletic sister group to *Phaedranassa* but with weak support (bootstrap = 60%). *Rauhia* itself is monophyletic, however (DI = 0, 94% bootstrap, 73% jackknife). The Eucharideae sensu Meerow and Snijman (1998) is again dispersed throughout the petiolate-leafed clade.

DISCUSSION

Hippeastroid Clade and the Position of Griffineae. All but two of the genera treated by Meerow and Snijman (1998) as part of Hippeastreae are resolved as a well supported monophy-

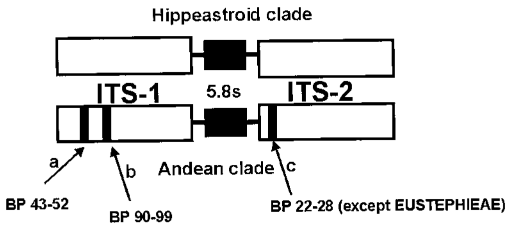

FIG. 5. Significant insertion/deletions in the ITS region that mark the "hippeastroid" and Andean clades, respectively, of the American Amaryllidaceae.

letic group in all the analyses (Figs. 1, 3). The two genera that lie outside of this clade are *Worsleya* and *Griffinia,* both Brazilian endemics, exhibiting the rare character of blue pigmentation in the flowers. In our previous combined plastid analysis (Meerow et al. 1999), both are positioned within the American clade, but unresolved with either Hippeastreae s.s. or a weakly supported tetraploid Andean clade. ITS strongly resolves *Worsleya* and *Griffinia* as sister genera, both resolved on long branches. Ravenna (1974) hinted at a perceived relationship between these genera, though he did not include *Worsleya* in his concept of Griffineae, and further allied this tribe with Amaryllideae (as Crineae). Clearly, Müller-Doblies and Müller-Doblies's (1996) placement of *Worsleya* in *Hippeastrum* is not supported by this study. Within the hippeastroid clade, recognition of a distinct tribe Griffineae appears well justified (Figs. 1, 3) with strong bootstrap and jackknife support (95%). Together these Brazilian endemics would appear to represent an isolated Brazilian shield element of the family's diversification in the neotropics. Bootstrap support for the sister group status of Griffineae to Hippeastreae is only moderate, however (75 or 84%, Figs. 1, 3), and the lack of jackknife support indicates that this resolution is only supported when the matix is successively weighted.

In a survey of internal morphology of American and African Amaryllidaceae, Arroyo and Cutler (1984) noted that all American species surveyed have scapes with obvolute bracts. All African tribes have equitant bracts (in *Agapanthus,* sister to Amaryllidaceae, the bracts are fused along one side). Meerow et al. (1999) reported that Calostemmateae (*Calostemma* and *Proiphys*), *Lycoris,* and *Pancratium* species with free bracts show the equitant condition. We only recently confirmed that *Worsleya* has a solid scape (like *Griffinia*), but equitant bracts like the rest of the family (in *Griffina* the bracts are fused on one side). In both analyses, the sister relationship of Griffineae and Hippeastreae has a DI of only 1; i.e., in trees only one step longer than those shown in Figs. 1 and 3, the position of Griffineae is unresolved within the American group. Thus there is a lingering possibility that Griffineae is the most ancient lineage in the Americas, and possibly sister to both Hippeastreae and the Andean clade.

The Chilean genus *Phycella* is next resolved as sister to the rest of tribe Hippeastreae (Fig. 3). The next clade resolved is also composed of species with a southern displacement from the equator, two species of *Rhodophiala,* one Chilean and one from Argentina. *Rhodophiala* is the first of several American genera that ITS indicates are polyphyletic. Another two Chilean species of *Rhodophiala* resolve in a grade as part of a weakly supported clade including *Hippeastrum blumenavium. Rhodophiala cipoana,* from Minas Gerais, Brazil, a considerable disjunct from the rest of genus, is firmly nested with *Hippeastrum,* strongly suggesting a misdiagnosis of this small-flowered, dwarf species' affiliation by Ravenna (1970). In fact, the species has 2n = 22 chromosomes (J. Dutilh, pers. comm.), a number unknown in *Rhodophiala,* (Flory 1977) but characteristic of *Hippeastrum* (Naranjo and Andrada 1975). *Rhodophiala* has at times been treated as part of *Hippeastrum* (e.g., Baker 1888; Bentham and Hooker f. 1883; Traub and Moldenke 1949), but few specialists in the family question Traub's later (Traub 1952, 1953) segregation of the genus. The limits of the genus have continued to be controversial, however (e.g., Hunziker 1985). Two chromosome groups have been reported in the genus, 2n = 16 and 2n = 18 (Ficker 1951; Flory 1977; Naranjo 1969; Satô 1942). *Rhodophiala bifida* has $2n = 16$ chromosomes (Naranjo 1969); no published reports exist for *R. bagnoldii.* While our data leaves little doubt that *Rhodophiala* is distinct from *Hippeastrum,* its apparent polyphyly is puzzling and requires wider sampling of the genus (but see discussion below). It should be noted that the resolution of the *Rhodophiala bifida/R. bagnoldii* clade as sister to the remaining Hippeastreae has no jackknife support, indicating that this resolution is only well-supported when highly homoplasious base positions are down-weighted.

Hippeastrum is inarguably monophyletic if *Rhodophiala cipoana* is included (Figs. 1, 3) with the exception of a single species, *H. blumenavium. Hippeastrum blumenavium* is an unusual species morphologically (petiolate leaves; few, wedge-shaped seeds with an elaisome). It was first described as

Griffinia blumenavia Koch and Bouche ex Carr. ITS sequences indicate that it is much more closely related to *Rhodophiala* than to *Hippeastrum.* Satô (1938) reported a chromosome number of 2*n* = 77 for this species, while Arroyo (1981) found 2*n* = 20. Independent unpublished studies (G. Smith pers. comm.; J. Dutilh pers. comm) report 2*n* = 18, the most common chromosome number in *Rhodophiala* Ficker 1952; Flory 1977; Naranjo 1969; Satô 1942). The sister relationship to two *Rhodophiala* species is only weakly supported (bootstrap = 64%, DI = 0), and ultimately this species may be best treated as a monotypic genus.

Hippeastrum reticulatum resolves as sister to the rest of the genus. Endemic to the understory of Atlantic rain forest of Brazil, *H. reticulatum* exhibits novel fruit and seed morphology. The seeds are round and turgid, and the dehisced capsule is bright red on the inner surface, perhaps functioning mimetically. As the next two terminal species of *Hippeastrum* are also Brazilian, a Brazilian origin for the genus seems likely. Further species relationships in this genus require additional sequence data; we originally sampled nearly 3 dozen species, but ITS base substitution rates are too low for adequate resolution beyond what is presented in Figs. 2 and 4. *Hippeastrum parodii* is endemic to Argentina, and *H. molleviquensis* (Bolivia) and *H. macbridei* (Peru) represent the Andean species. Though *Hippeastrum* is monophyletic, excluding *H. blumenavium,* the sister relationship of the genus to one clade of Zephyranthinae lacks any support in our trees (Fig. 2), and is unresolved by the sequence only matrix (Fig. 1).

Tribe Zephyrantheae (Traub 1963; Traub also included *Rhodophiala* in this tribe) or Hippeastreae subtribe Zephyranthinae (Müller-Doblies and Müller-Doblies 1996) are clearly polyphyletic. At present this is largely due to the apparent polyphyly of *Zephyranthes* itself, a genus which Meerow (1995) suggested may represent convergence of two distinct (albeit related) lineages in North (Meso) America and South America. Our wider sampling of this genus now indicates a putative triple origin for this genus. A most unexpected result is the position of the two species of this genus (*Z. atamasco* and *Z. simpsonii*) from the southeastern United States. Rather than appearing as sister to the well supported Mexican clade, *Z. atamasco* and *Z. simpsonii* are nested within the clade of South American/West Indian species (very well supported) that is sister to the *Rhodophiala/Hippeastrum blumenavium* clade. *Sprekelia,* a Mexican endemic (anecdotal reports of an Andean species have never been documented) with a highly zygomorphic perianth adapted for hummingbird pollination (two *Hippeastrum* species, *H. cybister* and *H. angustifolium,* have evolved similar perianth) resolves with moderate support as sister to the rest of Zephyranthineae (Fig. 1, 3). The latter clade consists of a paraphyletic *Habranthus* (with no support and a DI = 1) as a weakly supported sister to two South American *Zephyranthes* (*Z. flavissima* and *Z. mesochloa*), and a well-supported Mexican *Zephyranthes* clade. This heterogeneous subclade of Zephyranthinae has weak internal support, however. The segregate genus *Cooperia* (crepuscular, long-tubed Zephyranthes; represented in this study by *Z. smallii* and *Z. drummondii*) is nested within the Mexican *Zephyranthes* clade, the internal resolution of which is poor. Both *Pyrolirion* and *Haylockia* (= *Zephyranthes pusilla*) are embedded in *Habranthus.* These two genera have actinomorphic flowers, while those of *Habranthus* are zygomorphic. If zygomorphy is indeed plesiomorphic in the family (Meerow and Snijman 1998), our data suggest that the change to actinomorphy is relatively facile, and probably under simple genetic control.

The Zephyranthineae have been broadly categorized as having a basic chomosome number of x = 6 (Flory 1968, 1977), but considerable variation is exhibited among the species of *Zephyranthes* and *Habranthus*, the two largest genera, including euploid and aneuploid series. There is little consistency within the species of each of the three *Zephyranthes* clades resolved by ITS; at least some of the species within each has 2n = 24 chromosomes. If the results of this analysis are accurate, then chromosome number as well as morphology has evolved in parallel among the three subclades. An alternative hypothesis might be that reticulation early in the evolution of *Zephyranthes* and its allies completely obscures the phylogenetic relationships (Doyle 1992; McDade 1990, 1992, 1995). Intergeneric hybrids among genera of the Zephyranthinae are not unknown (Traub 1952, 1963; Flory 1968). The considerable amount of homoplasious base substitutions within the Hippeastreae (evidenced by the lack of internal branch support in trees resolved by the unweighted matrices) may be an indication that reticulation did occur in this clade. We plan to investigate this further by looking for corroboration of this pattern with plastid sequence data, as well as with tests for recombination within sequence data.

Andean Clade. Eustephieae are well resolved with very strong support as distinct from Stenomesseae (Figs. 2, 4) as argued by Meerow (1995). Müller-Doblies and Müller-Doblies (1996) followed Dahlgren et al. (1985) in placing this tribe within Stenomesseae. Support for this tribe as sister to the rest of the Andean clade rises from weak in the sequence topology (Fig. 2) to very strong in the sequences plus gaps trees (Fig. 4). This is a striking parallel to the situation in the hippeastroid clade, where a small clade is sister to all of the rest of the genera in their respective groups, though the positioning of Eustephieae is much stronger than the analogous relationship of Griffineae to the Hippeastreae (Figs. 1, 3). The Eustephieae represents the southern limits of the tetraploid Andean clade, occurring in the southern Andes of Peru, and the northern Andes of Argentina, Bolivia and Chile. They possess the putatively plesiomorphic condition of leaf mesophyll palisade (Meerow and Snijman 1998), unlike any other genus in the Andean clade. Support for the internal resolution of Eustephieae is also strong.

The fleshy seeded Hymenocallideae are also well-supported as a tribe distinct from Eucharideae (bootstrap = 99%). In fact, the affinities of Hymenocallideae are more with the lorate-leafed Stenomesseae (dry, flat, obliquely winged seed) than Eucharideae (turgid, oily seed). The infratribal relationships of Hymenocallideae are ambiguous, however, except for the strong monophyly of *Hymenocallis.* The ambiguity surrounds the relationships of the Andean genera *Ismene* and *Leptochiton.* The combined bp and gaps matrix very weakly resolves monophyly for these Andean taxa, but renders *Ismene* polyphyletic. *Hymenocallis* has only 2–3 species in South America, and represents the North and Meso-American vicariant of the tribe. *Leptochiton* and the three subgenera of *Ismene* (*Ismene, Elisena, Pseudostenomesson*) are endemic to the central Andes. *Leptochiton* is the only member of the tribe with phytomelanous seeds and also has the lowest chromosome number [$2n = 24$ according to Snoad (1952); $2n = 34$ in our accessions (Meerow, unpublished data)]. Plastid sequences resolve *Ismene* as monophyletic (Meerow et al. 1999), but with weak support.

ITS was most helpful in elucidating the relationships of the tribes Stenomesseae and Eucharideae, two tribes which showed insufficient plastid DNA divergence to resolve their phylogenetic relationships (Meerow et al. 1999). Most surprising is the resolution of a petiolate-leafed clade containing elements of both Eucharideae and Stenomesseae [(100% bootstrap, 93% jackknife, DI =7 with both bp and gaps (Fig. 4)]. Despite the fact that petiolate leaves have evolved independently several times elsewhere in Amaryllidaceae (Amaryllideae, Calostemmateae, Griffineae, Haemantheae, Hymenocallideae, Hippeastreae), the molecular data indicate that it is a synapomorphy for this clade. Whereas Meerow (1987) pointed out the probable paraphyly of *Stenomesson,* suggesting that the petiolate-leafed species of that genus might be ancestral to *Rauhia, Phaedranassa,* and *Eucrosia* (and the extinct *Mathieua galanthoides*), ITS sequences clearly indicate that *Stenomesson* is polyphyletic, and that petiolate leaves evolved only once in the Andean Amaryllidaceae. All of the petiolate-leafed Stenomesseae are more closely related to Eucharideae sensu Meerow and Snijman (1998) than to the lorate-leafed Stenomesseae. The sampling of this clade is still incomplete, however, with only *Stenomesson* s.s., *Phaedranassa,* and *Rauhia* resolving as monophyletic (*Rauhia* only when the gap matrix is included) with good bootstrap support for the latter two when the gaps matrix is included in the analysis (Fig. 4). The sister relationship of *Rauhia* and *Phaedranassa* resolves only in the combined bp and gap matrix. While three species of *Eucrosia* form a well-supported clade, the relationships of the only mesophyte in the genus, *E. dodsonii,* are unresolved. Although seed morphology for this species is unknown, ovule morphology is more like that of *Caliphruria, Eucharis,* and *Urceolina* (Eucharideae), all of which have globose ovules, than the winged, flattened ovules of Stenomesseae sensu Meerow and Snijman (1998). A further link of *E. dodsonii* to these genera is the rain forest understory habitat that all of them share. Meerow and Dehgan's (1985) placement of this species in *Eucrosia* may therefore require re-analysis once we obtain a broader sampling of the petiolate clade. Not only does Eucharideae sensu Meerow and Snijman (1998) fail to resolve as a monophyletic group, neither *Eucharis* nor *Caliphruria* appear monophyletic. The lack of resolution in this group may in part be attributable to a relatively recent evolutionary history tied to the uplift of the Andes in the Pliocene (van der Hammen 1974, 1979; Taylor 1995).

The lorate-leafed remnants of the Stenomesseae form a weakly supported clade with the epiphytic *Pamianthe* at the base. In trees 2 steps longer, *Pamianthe* is unresolved relative to Hymenocallideae or the lorate-leafed Stenomesseae. *Paramongaia weberbaueri* and *Stenomesson mirabile* (the latter on an extremely long branch) are well supported sister taxa (Fig. 2) in the SW (bootstrap = 92%), less so in the unweighted trees (jackknife = 73%) from the sequence matrix alone. With the gap matrix added (Fig. 4), *S. mirabile* is sister to the rest of *Stenomesson* but with only 70% bootstrap support in the SW trees. As in *Paramongaia,* the free portion of the staminal filament in *S. mirabile* is inserted below the rim of the staminal cup.

Biogeographic and Evolutionary Implications. Raven and Axelrod (1972), Arroyo and Cutler (1984), and Meerow (1995) have interpreted the endemic American Amaryllidaceae as a classical Gondwanaland disjunct from an African ancestor. However, combined plastid sequence phylogenies of the family (Meerow et al. 1999) indicate that the sister group relationships of the American genera are with the Eurasian tribes of the family, rather than any African clade. Meerow et al. (1999) were reluctant to suggest when or by what migration path the family entered the Americas, but invoked the Madrean-Tethyan hypothesis (Axelrod 1973, 1975) as a possibility. To note, a single species of *Pancratium, P. canariensis,* part of the Eurasian sister clade to the American Amaryllidaceae, is endemic to the Canary Islands and could conceivably represent an isolated remnant of migrations across the islands of the mid-Atlantic ridge in the late Cretaceous or early Paleocene times. The complete absence of a fossil record for Amaryllidaceae [except for some recent pollen deposits from Brazil (Behling 1995)] makes it very difficult to assess any of several hypotheses of the family's history in America.

Within each of the two major clades of American amaryllids, we have a parallel topology in the lower branches. In both cases, a small clade resolves as sister to a larger, more diverse clade. In the hippeastroid clade, the ''little'' sister is the eastern Brazil endemic Griffineae; in the Andean clade, it is the Eustephieae, which occurs in its present distribution considerably disjunct from the central Andean locus of diversity for the tetraploid Andean amaryllids.

This pattern, and the low base substitution rates apparent in the more terminal subclades of both larger groups, might indicate that that the family was subjected to a bottleneck in its evolution some time after its initial colonization of the Americas. Thus, one hypothesis of the history of the American Amaryllidaceae could be that the family was reduced to two peripheral isolates from its former range, a southern Andean group (Eustephieae) and a Brazilian Shield element (Griffineae), from which all modern diversity of the family in America later evolved. Glaciation would be the most reasonable factor to have been involved in reducing the former range of the family in America. Both groups exhibit a morphological plesiomorphy: leaf mesophyll palisade in the Eustephieae, and equitant bracts in Griffineae (*Worsleya*). They also exhibit a considerable amount of unique sequence in the ITS regions. Alternatively, the Eustephieae and Griffineae could merely represent early isolates from the rest of their respective clades, with no particular claim of greater antiquity.

Within the larger hippeastroid subclade, the next terminal clades are primarily displaced towards the southern region of South America, with *Phycella* (Chile) followed by two *Rhodophiala* species (Chile and Argentina). The next clade to resolve is the most geographically heterogeneous group in the entire topology, including species from Brazil (*Hippeastrum blumenavium, Zephyranthes cearensis*), Argentina (*Z. candida, Z. filifolia*), the West Indies (*Z. rosea* from Cuba), and North America (*Z. atamasco* and *Z. simpsonii*). The broadly dispersed *Zephyranthes* subclade is particularly intriguing. If the ITS phylogeny is accurate, the sister relationship of *Z. rosea* to the North American species would suggest that the genus entered the southeastern U.S. via Cuba after migrating from South America.

The boreotropical hypothesis postulates that, during the early Tertiary, biotic interchange occurred between paleotropical floras of Eurasia and North America over a direct land bridge or narrow water gaps (Wolfe 1975; Tiffany 1985a, 1985b; Taylor 1988; Lavin and Luckow 1993; Lavin 1995). During this time, little or no migration was possible between North and South America (Raven and Axelrod 1974). Fossil evidence has accumulated that supports the existence of a diverse tropical flora in North America up until the late Eocene or early Oligocene when the North Atlantic widened and temperate climates began to prevail in North America (Taylor 1988; Lavin 1995). That the American and Eurasian Amaryllidaceae are sister groups (Meerow et al. 1999) provides one congruent piece of evidence to suggest that this model might fit the Amaryllidaceae. However, a further expectation from phylogenetic analysis would be that the South American Amaryllidaceae should resolve as derived from North American progenitors, as Lavin (1995) has demonstrated for the legume tribe Robinieae. This is not explicit in our trees (Figs. 1–4). However, within the hippeastroid clade (Figs. 1, 3), we have a well-supported clade of *Zephyranthes* species that includes species endemic to North America, the West Indies, and South America, whereas the other clades of *Zephyranthes* species show geographic congruence. If this resolution represents the true phylogeny (versus the consequence of recombination early in the evolution of the hippeastroid clade), then North American species of *Zephyranthes* may represent the remnants of an early North American lineage in the family. It should be noted, however, that the two North American species are the terminal-most taxa in their clade, their sister species is a West Indian taxon (*Z. rosea*), and all three appear to be derived from South American progenitors. As no Tertiary fossil record exists for the Amaryllidaceae on any continent, we conclude at this point that the evidence for a Boreotropical origin for the American Amaryllidaceae is at best ambiguous. Our data do not suggest that extant South American Amaryllidaceae were derived from North American progenitors, despite the sister group relationship of the entire American clade to the Eurasian (Meerow et al. 1999), since South American taxa are in all of the basal positions in our trees. Based on our sequence topologies, South America seems the most likely center of origin for the family's extant diversity in the Americas, with later migration to North America, probably across the Panamanian Isthmus (Raven and Axelrod 1974; Gentry 1982; Taylor 1990; Graham 1995), or, in the case of *Zephyranthes* in the southeastern U.S., via island hopping through the Caribbean. Given the weak to moderate support for the Griffineae as sister to the Hippeastreae (versus an unresolved position in the American clade) only in our SW topologies, and the symplesiomorphic scape bract morphology of *Worsleya,* a reasonable hypothesis for a migration pattern would have the progenitor entering northern South America from North or tropical Africa. A land bridge did exist between the Brazilian Bulge and Nigeria until the end of the Cretaceous (Rand and Mabesoone 1982). This same ancestor may have been shared with the Eurasian sister clade of the American group. The American species of *Crinum,* the only pantropical amaryllid genus, are nested within a clade of North and tropical African species of the genus by ITS sequences (unpub. data), suggesting a similar pattern for the independent entry of this group into America. In any event, direct evidence for this or any other hypothesized pathway for Amaryllidaceae is lacking.

In conclusion, ITS sequences provide a well-resolved phylogeny of the monophyletic American Amaryllidaceae, but the early origins of the family in America remain ambiguous. The genera resolve into two subclades, an Andean-centered, tetraploid-derived group, and a clade conforming to the Hippeastreae sensu Meerow and Snijman (1998). In both cases, a small, geographically constrained South American tribe is sister to the more diverse and dispersed elements of the group. At present, the resolution of the Griffineae as part of the hippeastroid subclade receives moderate support only with SW weighting of the data matrices. The resolution of Eustephieae as sister to rest of the Andean genera is strongly supported when sequence gaps are included as part of the data matrix analyzed. The Griffineae and Eustephieae represent either ancient or isolated lineages within the American clade of the family. Within the hippeastroid clade, the apparent polyphyly of two genera, *Rhodophiala* and *Zephyranthes,* for which any other support is lacking, opens the possibility that reticulation might have occurred in the early evolution of the lineage. This issue requires further investigation.

Taxonomic Implications. At present, it is premature to suggest any taxonomic changes within the hippeastroid clade of our ITS phylogeny, other than recognition of the tribe Griffineae as distinct. However, within the Andean clade ITS indicates that the tribe Stenomesseae is polyphyletic, a resolution that was also suggested by plastid sequences (Meerow et al., 1999). A distinct petiolate-leafed clade, comprising the Euchariderae and the petiolate genera of the Stenomesseae, is strongly supported. As the type species of *Stenomesson* (the oldest name in the clade) is a petiolate-leafed species [*S. flavum* (R. & P.) Herb.], this clade must bear the name Stenomesseae. ITS also strongly supports recognition of Hymenocallideae as a distinct tribe that is sister to the lorate-leafed remnants of the Stenomesseae. The earliest prior name for the lorate-leafed species of *Stenomesson* is *Clinanthus* Herbert (1821) and the name Clinantheae is proposed for the new tribe. It should be noted that the ITS sequence topology without gaps resolves *Stenomesson mirabile* (and *S. viridiflorum* by inference) as sister to *Paramongaia* rather than the rest of the lorate-leafed *Stenomesson* species (Fig. 2). The latter resolution occurs when the gap matrix is included (Fig. 4), but is not as well supported as the former. We feel it is best to treat these two erstwhile *Stenomesson* species as part of *Clinanthus* and retain *Paramongaia* as a monotypic genus until the clade is more fully sampled.

Clinantheae Meerow, tribus novus.

Tribus novus herbarum perennium bulbosarum andinarum, foliis loratis vel linearibus, floribus cupula staminea, et seminibus phytomelanis complanatis alatis.

Bulbous perennial herbs, terrestrial, rarely epiphytic. Bulbs tunicate, often forming a long neck. Leaves linear or lorate, hysteranthous or synanthous, rarely persistent, often glaucous, lacking palisade in the mesophyll. Inflorescence scapose, pseudoumbellate (reduced helicoid cymes), the scape solid, terminated by two obvolute spathe bracts that enclose the flowers in bud, bracts rarely fused into a tube. Perianth crateriform, funnelform tubular, tubular, or campanulate, often brightly colored, pedicellate, each subtended by a bracteole, consisting of six tepals in two series fused below into a tube of varying length. Stamens 6, the filaments fused below into a staminal cup, free filament inserted at or below the rim of the cup. Stigma capitate; ovary inferior, trilocular, ovules numerous, axile in placentation, compressed. Fruit a papery or woody loculicidal capsule; seeds dry, flattened, obliquely winged with a black or brown phytomelanous testa. $2n = 46$.

TYPE: *Clinanthus luteus* Herbert. App. Edwards's Bot. Reg. 40 (1821).

Genera: *Clinanthus* (ca. 20), *Pamianthe* (2) *Paramongaia* (1), *Pucara* (1).

The following transfers of currently recognized linear or lorate-leafed *Stenomesson* species to *Clinanthus* are necessary. Only the basionym is provided, pending full synonomy in a monograph of the genus.

Clinanthus callacallensis (Ravenna) Meerow, comb. nov.
Stenomesson callacallense Ravenna. Pl. Life 30: 76 (1974).

Clinanthus campodensis (Ravenna) Meerow, comb. nov.
Stenomesson campodense Ravenna. Pl. Life 27: 75 (1971).

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