PHYLOGENY AND EVOLUTION OF *SYMBOLANTHUS* AND *WURDACKANTHUS* (GENTIANACEAE–HELIEAE) IN THE GUAYANA HIGHLANDS AND ANDES, BASED ON RIBOSOMAL 5S-NTS SEQUENCES[1]

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ABSTRACT

Symbolanthus (Gentianaceae, Helieae) is a genus of small trees, shrubs, and herbs distributed in South America (the central and northern Andes and the Guayana Highlands) and southern Central America. To discover the pattern of *Symbolanthus* evolution and to assess the relationships between *Symbolanthus* and the closely related *Wurdackanthus* of the Guayana Highlands (Venezuela, Brazil) and the Lesser Antilles (St. Vincent), we performed cladistic analyses on members of both taxa. Molecular data (5S-NTS rDNA sequences) were gathered from individual plant samples, mainly from herbarium specimens. 5S-NTS sequence data did not resolve internal relationships well in *Symbolanthus* and show that the genus may be a recently and rapidly diverging clade. However, these results indicate that *Wurdackanthus* is either paraphyletic to *Symbolanthus* or that *W. argyreus* is nested within a Symbolanthus clade. Furthermore, *W. frigidus* may be the basalmost species of the Wurdackanthus–Symbolanthus clade. This species is noticeably divergent and distinct, molecularly and morphologically, from the rest of the clade. Testing an alternate phylogenetic hypothesis in which *Wurdackanthus* is monophyletic results in trees 11 steps longer than the shortest trees found, and still with *Wurdackanthus* nested within *Symbolanthus*. Ancestral morphological character evolution in the Wurdackanthus–Symbolanthus clade is discussed in the context of a 5S-NTS consensus tree.

Key words: 5S-NTS, biogeography, Gentianaceae, Helieae, molecular systematics, morphology, Neotropics, phylogeny, *Symbolanthus, Wurdackanthus.*

RESUMEN

El género *Symbolanthus* (Gentianaceae, Helieae) incluye árboles pequeños, arbustos y hierbas, distribuidos en Sur América (al centro y norte de Andes y en las tierras altas de la Guayana) y al sur de Centro América. Con el objeto de descubrir el patron de la evolución y biogeografía de *Symbolanthus,* realizamos un análisis cladístico con algunas especies de *Symbolanthus* y del cercanamente relacionado *Wurdackanthus,* distribuido en las tierras altas de la Guayana (Venezuela, Brazil) y en las Antillas Menores (St. Vincent). Los datos moleculares (secuencias de 5S-NTS rADN) se obtuvieron de muestras individuales de plantas, principalmente de especímenes de herbario. Las secuencias de 5S-NTS no resolvieron bien las relaciones al interior de *Symbolanthus,* y mostraron que el género puede haber divergido rápida y recientemente. Estos resultados indican ya sea que *Wurdackanthus* es parafilético respecto a *Symbolanthus,* o que *W. argyreus* se encuentra dentro del clado de Symbolanthus. Mas aún, *W. frigidus* podria ser la especie más basal del clado Wurdackanthus–Symbolanthus. Esta especie es notoriamente diferente, tanto molecular como morfológicamente del resto del clado. Al probar una hipótesis alterna, en la cuál *Wurdackanthus* es monofilético, se encuentran árboles 11 pasos más largos que los mas cortos obtenidos, aún con *Wurdackanthus* incluído dentro de *Symbolanthus.* Con base en el consenso obtenido usando 5S-NTS, se discute la evolución de caracteres morfológicos ancestrales en el clado Wurdackanthus–Symbolanthus.

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Symbolanthus G. Don (Gentianaceae) is a genus of about 30 species of small trees, shrubs, and (rarely) herbs distributed mainly in montane rain forests in the northern and central Andes (Bolivia, Colombia, Ecuador, Peru, Venezuela; ca. 21 spp.) and the Guayana Shield highlands in South America (Brazil, Guyana, Venezuela; 7 spp.), as well as southern Central America (Costa Rica, Panama; ca. 2–3 spp.). The Guayana Highland is one of the oldest land regions in South America, part of the original Gondwanan continent, which has not been inundated since the mid-Cretaceous (100 mya; Huber, 1995). The highland region comprises table mountains, known as tepuis, up to 3014 m altitude. Most tepuis consist of sandstones of the Roraima Formation overlying the Precambrian crystalline basement of the Guayana Shield (Ghosh, 1985). The age of the uplift of the sandstone plateau is unknown, but cycles of upheaval and erosion may go back to the Mesozoic (Kubitzki, 1989). Erosion of the sandstone resulted in the formation of isolated, vertical-cliffed mountains, the tepuis.

Symbolanthus is a member of the well-defined Helieae clade, which primarily consists of *Adenolisianthus* (Spruce ex Prog.) Gilg, *Aripuana* Struwe, Maas & V. A. Albert, *Calolisianthus* Gilg, *Chelonanthus* (Griseb.) Gilg, *Helia* Mart., *Irlbachia* Mart., *Macrocarpaea* (Griseb.) Gilg, *Rogersonanthus* Maguire & B. M. Boom, *Symbolanthus, Tachia* Aubl., *Tetrapollinia* Maguire & B. M. Boom, and *Wurdackanthus* Maguire (Struwe et al., 1997, 2002). Helieae are exclusively Neotropical with the highest concentration of genera on the Guayana Shield. They include the majority of non-montane Neotropical gentians and contain among the highest morphological and anatomical diversity within the family. Many Helieae, as well as *Symbolanthus,* species are endemic to single mountain tops, ridges or massifs, river drainages, or islands. *Symbolanthus* infrageneric taxonomy is fraught with difficult species delimitation problems, particularly among Andean entities, and many undescribed species (Struwe, 2003a, b).

Symbolanthus and the morphologically similar *Wurdackanthus* together form a well-supported clade within Helieae (Struwe et al., 2002; Fig. 1). They can be distinguished from other Helieae and gentians by pollen morphology and the presence of a corona or corona-like structure within the corolla. *Wurdackanthus* differs from *Symbolanthus* in having a much shorter calyx that is divided ¾ of its length, as opposed to being divided ⅞ or more of its length (Pringle, 1995; Struwe et al., 1999). *Wurdackanthus* consists of two species, *W. argyreus,*

which is endemic to the Guayana Highlands, and *W. frigidus,* found in the Lesser Antilles.

Our objectives in this study are to determine the phylogenetic relationship between *Symbolanthus* and *Wurdackanthus,* which share morphological characteristics that are unique within Helieae, and to investigate these putative synapomorphies in a phylogenetic context with a known outgroup, *Chelonanthus* (Struwe et al., 2002). Previous cladistic analyses of Helieae with nrDNA sequences of the internal transcribed spacer (ITS) region showed that this gene region, though highly variable in other Helieae lineages, including *Macrocarpaea* (Grant & Struwe, 2000), does not vary enough within *Symbolanthus* to resolve interspecific relationships (Struwe et al., 2002; Fig. 1). In order to assess relationships within *Symbolanthus,* it was necessary to use a more quickly evolving DNA fragment. DNA of the ribosomal 5S non-transcribed spacer (5S-NTS) region has been shown to be highly variable in closely related species of plants (e.g., *Alibertia* A. Rich., Persson, 2000; *Saintpaulia* Wendl., Lindqvist & Albert, 1999; see also Cox et al., 1992; Kellogg & Appels, 1995; Cronn et al., 1996). Like ITS sequences, 5S-NTS sequences are arranged in arrays of several hundred to several thousand tandemly repeated copies that occur at one or several chromosome loci and that normally become homogenized via concerted evolution processes (Hillis & Dixon, 1991; Playford et al., 1992).

MATERIALS AND METHODS

MOLECULAR METHODS

Due to the remote habitats of many *Symbolanthus* and *Wurdackanthus* species, we relied on DNA extraction from leaf material from herbarium sheets (Table 1). Total DNA was extracted from 0.5–1 cm² dried leaf tissue. Leaves were pulverized by means of a Bio 101/Savant FastPrep tissue disruptor, followed by extraction with a CTAB (cetyldimethylammonium bromide) buffer with the addition of 1% PEG 4000 (polyethylene glycol), and glassmilk (GeneClean, Bio 101) purification (modified from Struwe et al., 1998). For difficult samples, a chloroform:isoamyl alcohol (24:1) purification step was added following CTAB extraction. Amplification of the 5S-NTS region by polymerase chain reaction (PCR) was accomplished in standard 25 μl reactions on a Perkin Elmer Gene Amp 9600 in the presence of bovine serum albumin (BSA, 0.04%) and tetramethyl ammonium chloride (TMACl, 0.1 mM). PCR primers were "5S forward" and "5S reverse" designed by Cox et al. (1992). The cycle program included an initial incubation at 95°C for
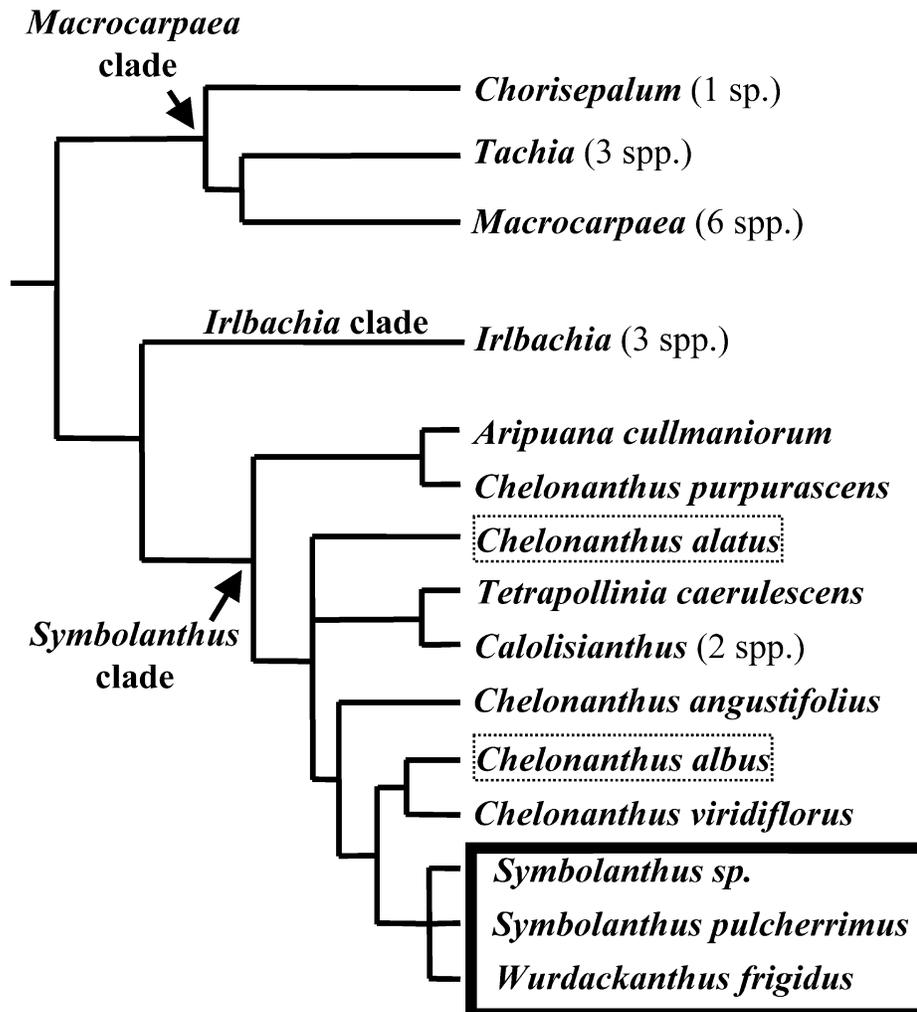
Figure 1. Preliminary phylogenetic relationships of the Helieae based on ITS sequences (adapted after Struwe et al. (2002) and L. Struwe, K. Gould, J. R. Grant & V. A. Albert, unpublished). Within Helieae, three major well-supported clades are identified, the Macrocarpaea clade, the Irlbachia clade, and the Symbolanthus clade (Struwe et al., 2002). The genus *Chelonanthus* is paraphyletic within the Symbolanthus clade; the two species used in this study are indicated with dotted boxes. The Symbolanthus–Wurdackanthus clade is indicated with a solid box.

2 min., followed by 27 cycles of 94°C for 1 min., 60°C for 1 min., and 72°C for 1 min.

Most PCR products were run on a low-melting agarose (1.2%) gel, from which the fragments were plugged, resuspended in 300 μl water, and reamplified as before to increase product yield. PCR products were then prepared for sequencing by column purification using QIAquick spin-columns (Qiagen). This was followed by PCR-based cycle sequencing reactions using a d-Rhodamine Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer Applied Biosystems) with AmpliTaq DNA polymerase, FS, following kit protocols, and using

the same PCR primers. Sequencing reactions were cleaned by means of Sephadex (Pharmacia Biotech AB) column purification.

Sequencing gels were run on an ABI 377 XL automated sequencer. Sequences obtained were edited and aligned using the software program Sequencher 3.0 (GeneCodes Corp.). Only clearly readable sequences were used. The aligned sequences were cut at an arbitrarily chosen position at both ends of the spacer where all sequences were readable. The complete alignment is available at <http://www.rci.rutgers.edu/~struwe/dnadata.htm>. All molecular-based phylogenetic work was done at the Lewis B.

and Dorothy Cullman Program for Molecular Systematics Studies of the New York Botanical Garden. Table 1 lists the species, voucher specimens, and Genbank accession numbers of sequences used in the study.

Eleven individuals and nine species of *Symbolanthus* and both species of *Wurdackanthus* were sampled. Attempts to amplify 5S-NTS for additional species of *Symbolanthus* from herbarium material (in particular from tepui species) failed, presumably due to poor specimen preservation. Two species of *Chelonanthus* were used as outgroups following sister-group relationships derived from ITS data (Struwe et al., 2002; Fig. 1). Thus a total of 15 individuals representing 13 species were included in the 5S-NTS data set. Species of *Calolisianthus* and *Tetrapollina caerulescens* (Aubl.) Maguire & B. M. Boom were also sequenced for 5S-NTS, but were very divergent from and therefore difficult to align with *Symbolanthus* and *Chelonanthus* and were not included in the analysis. During the course of this study, four undescribed species of *Symbolanthus* were identified among our herbarium samples. They are here indicated as "sp. ined. 1–4" (Table 1) and will be described in a later publication on the taxonomy of *Symbolanthus.*

Phylogenetic analyses were done using maximum parsimony in PAUP* version 4.0b4a (Swofford, 2000). Minimal length trees were searched for using the heuristic option, with TBR branch-swapping, 100 replicates of random addition sequence order, and branches collapsed if the maximum branch length is zero. Gaps in the aligned 5S-NTS sequences were treated as missing data. Branch lengths were calculated using the ACCTRAN option in PAUP*.

Internal clade support was evaluated using the parsimony jackknifing procedure, which was performed in Xac (Farris et al., 1996), using 1000 replicates with 5 random addition replicates each. Decay analyses (Donoghue et al., 1992) were done in PAUP* by means of the AutoDecay program (Eriksson, 1998). In addition, analyses were run with topological constraints imposed, first forcing *Wurdackanthus* and then *Symbolanthus* to be monophyletic, to determine their effects on tree lengths and to test alternate hypotheses of relationships. Heuristic searches were done with these constraints imposed and all other run options as above.

MORPHOLOGICAL METHODS

Morphological data were collected by examining the same specimens that were sequenced, as well as duplicates of the same collections and pickled flower and fruit material from the same collection when available. Output trees from PAUP* were imported into MacClade version 3.08a (Maddison & Maddison, 2000) in order to explore changes among morphological character states. Thirty vegetative and reproductive characters were examined, including five quantitative characters. Measurements for quantitative characters were divided into discrete ranges by means of two different methods described in Chappill (1989) (gap-coding and segment-coding) and coded as discrete character states.

RESULTS

5S-NTS

The aligned data set of the 5S-NTS region contains 322 characters, including 139 (43%) variable characters and 55 (17%) parsimony-informative characters. However, most of the variation found represents differences between the outgroup, *Chelonanthus,* and *Wurdackanthus–Symbolanthus,* as well as between *W. frigidus* and the remaining ingroup taxa (see below). Individual sequence lengths for the 5S-NTS region range from 292 bp (*Chelonanthus alatus*) to 311 bp (*Symbolanthus nerioides*), with most taxa having a length of 308 bp. Mean GC content for the data set calculated in PAUP* is 57%.

PARSIMONY ANALYSES

Results of the parsimony analyses are shown in Figure 2. The parsimony analysis of the 5S-NTS data set alone yielded 42 shortest trees of length 186 (consistency index excluding uninformative characters (CI) = 0.86, retention index (RI) = 0.85; Fig. 2A, B). In the strict consensus tree (Fig. 2A), with *Chelonanthus* spp. as outgroup, *Wurdackanthus frigidus* (St. Vincent) is sister to the remaining taxa, with 100% jackknife support and a decay value of 30. *Wurdackanthus argyreus* (Guayana Highlands: Sierra de la Neblina and Cerro Aracamuni) and all *Symbolanthus* species form a clade (jackknife = 100%, decay = 8) within which there is little resolution. However, two clades with good measures of support are found, one containing the two *S. macranthus* samples (Ecuadorean cloud forest species; jackknife = 73%), and a second containing two Central American species, *S. pulcherrimus* (Cartago region of Costa Rica) and *S.* sp. ined. 1 (Veraguas, Panama) (jackknife = 98%, decay = 4). Overall, there is relatively little molecular divergence within the W. argyreus + Symbolanthus clade, with the exception of a long terminal branch

Table 1. Material used in this study.

| Taxon	Country, State	Locality	Collector #	Herbarium voucher at:	GenBank number
Chelonanthus alatus (Aubl.) Pulle	French Guiana	Saül (3°37′N, 53°12′W) and vicinity, on road to airport	*S. A. Mori et al. 24076*	NY	AY143381
Chelonanthus albus (Spruce ex Prog.) Badillo	Brazil, Amazonas	Rio Negro between Manaus and São Gabriel, ca. 80 km N of São Gabriel, ca. 100 m alt., terra firme, 00°20′N, 66°45′W	*J. M. Poole 2049*	NY	AY143384
Wurdackanthus argyreus Maguire	Venezuela, Amazonas	Cerro de la Neblina, 6.5 km SSE of base camp, southern extension of range, elev. 1600 m, 0°47′N, 66°11′W, ridge top with bromeliads, *Heliamphora* and low shrubs	*B. Stein et al. 1617*	NY	AY143371
Wurdackanthus frigidus (Sw.) Maguire & B. M. Boom	St. Vincent	Charlotte Parish, on the W slope of Soufriere Mountain among scrub vegetation	*G. R. Cooley 8211*	NY, S	AY143370
Symbolanthus sp. ined. 1	Panama, Veraguas	Along road between Escuela Agricola and Alto Piedra (above Santa Fe) and Río Dos Bocas ca. 5–8 km from Escuela, 730–770 m elev.	*T. B. Croat 25922*	AAU, MO, NY	AY143382
Symbolanthus sp. ined. 2	Peru, Pasco	Border Prov. Oxampampa and Pasco, in dwarf forest, elev. 2700 m. Rich in Ericaceae, bamboo and *Blechnum*, with *Sphagnum* layer below, San Cotardo	*H. van der Werff et al. 8511*	F, MO, US	AY143378
Symbolanthus sp. ined. 3	Peru, Loreto	Provincia Requena, Distrito Sapuena, Poblado Jenaro Herrera, CIJH-IIAP; bosque de altura area W of herbarium, elev. ca. 200 m	*P. Aevedo & D. Daly 1592*	NY	AY143372
Symbolanthus sp. ined. 4	Bolivia, La Paz	Prov. Sud Yungas, 9 km from Huancané (16°22′S, 67°32′W) on the road to San Isidro, 2540 m, "Bosque de alta montaña con *Podocarpus*"	*L. J. Dorr et al. 6691*	NY	AY143383
Symbolanthus macranthus 1 (Benth.) Moldenke	Ecuador, Loja	Road to Cerro Toledo, 8–11 km from turnoff above town of Yangana, ca. 4°20′S, 79°10′W, 2440–2700 m, scrub vegetation and cloud forest	*J. L. Luteyn et al. 15099*	NY	AY143373
Symbolanthus macranthus 2 (Benth.) Moldenke	Ecuador, Zamora–Chinchipe	Road between Loja & Zamora ca. km 16–18, ecotone beween cloud forest and páramo, elev. 2500–2650 m	*J. L. Luteyn et al. 6620*	NY	AY143375
Symbolanthus brittonianus Gilg	Bolivia, La Paz	Prov. Larecaja, 19.0 km al SO de Guanay por el camino a Tipuani, bosque húmedo, 15°34′S, 67°59′W, 1200 m	*J. C. Solomon 17684*	MO, NY	AY143376
Symbolanthus pulcherrimus Gilg	Costa Rica, Cartago	Cantón del Guarco, Distrito de San Isidro, bosque secundario y robledales alterados en las cercanías de El Empalme, 9°43′04″N, 84°03′01″W, 2300 m	*J. F. Morales et al. 1600*	F, MO, NY (×2)	AY143379
Symbolanthus nerioides 1 (Griseb.) Ewan	Venezuela, Mérida	Cloud forest in sheltered valley, Estanques–Paramis de los Colorados Road, alt. ca. 2300 m	*G. L. Sobel & J. Strudwick 2164*	NY	AY143377
Symbolanthus nerioides 2 (Griseb.) Ewan	Venezuela, Mérida	Distrito Sucre, along carretera Estanque a El Molino, just E of Páramo Molino, elev. ca. 2500 m	*G. R. Proctor et al. 49150*	US	AY143380
Symbolanthus vasculosus Gilg	Venezuela, Barinas	Distrito Bolívar, Municipio Altamira, "La Gallineta," Caserío "El Celosos" near feldspar mine, ca. 1700 m elev. [ca. 8°50′N, 70°35′W]	*L. J. Dorr et al. 5474*	NY, US	AY143374

A.

B.
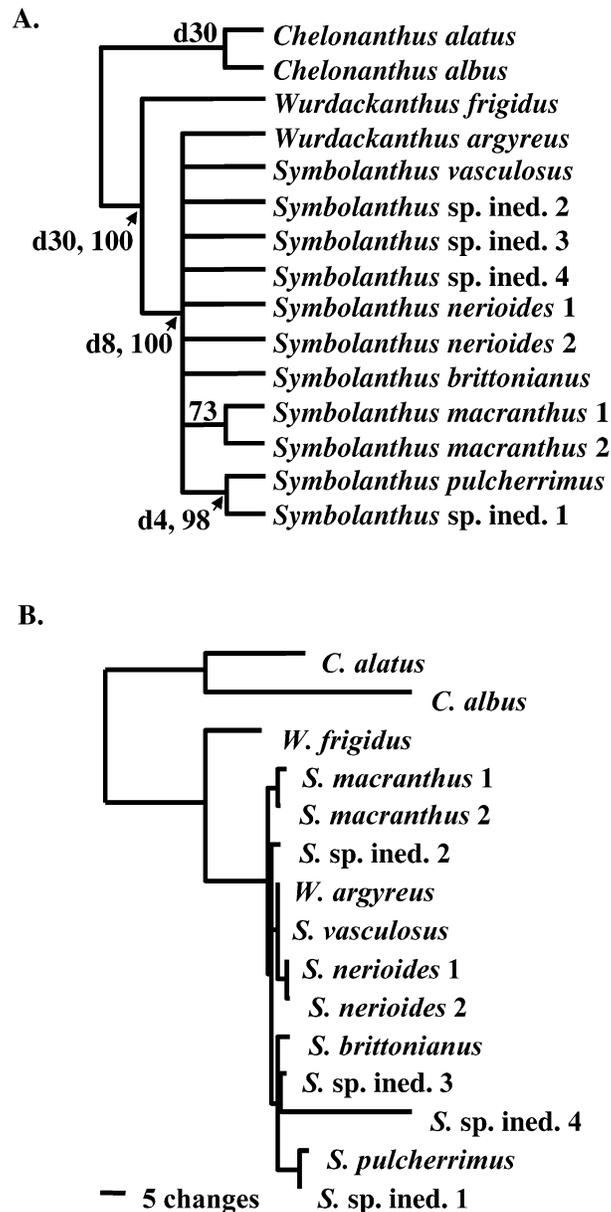
Figure 2. Results of analyses of *Symbolanthus–Wurdackanthus* based on 5S-NTS sequence data. —A. Strict consensus tree showing support indices for nodes including decay values greater than 1 (preceded by "d") and jackknife values greater than or equaling 50%. —B. Tree showing branch lengths for one of 42 shortest trees from the 5S-NTS analysis.

leading to *Symbolanthus* sp. ined. 4 (Bolivian Andes), containing 28 changes (Fig. 2B). Notably, there is a relatively large amount of divergence between the two species of *Wurdackanthus,* with a long branch containing 13 changes between *W. frigidus* and *W. argyreus* + *Symbolanthus.*

CONSTRAINT ANALYSES

The first constraint analysis kept only trees compatible with a pre-defined *Wurdackanthus* monophyly constraint tree. PAUP* found 491 trees of length 197, 11 steps longer than the most parsimonious trees (CI = 0.76, RI = 0.71). In all of

these trees, a Wurdackanthus clade is nested within a Symbolanthus clade, making *Symbolanthus* paraphyletic.

The second constraint analysis kept only trees compatible with a pre-defined *Symbolanthus* monophyly constraint tree. PAUP* found 30 trees of length 187, one step longer than the most parsimonious trees (CI = 0.85, RI = 0.84). In all of these trees, *Wurdackanthus* is paraphyletic toward *Symbolanthus,* with *W. frigidus* most basal followed by *W. argyreus.*

In summary, results indicate that *Wurdackanthus* is paraphyletic, with *W. frigidus* sister to a W. argyreus + Symbolanthus clade. *Wurdackanthus argyreus* is either sister to the *Symbolanthus* species or nested among them. The constraint analysis forcing *Wurdackanthus* to be monophyletic finds trees much longer than the most parsimonious trees and results in a non-monophyletic *Symbolanthus.*

MORPHOLOGICAL ANALYSES

Eight characters out of 30 that were examined were found to be potential synapomorphies for clades. These characters were corolla length, calyx length, depth of calyx division, number of flowers per inflorescence, capsule length, pollen type, presence of corona or staminal pockets inside the corolla, and flower bud shape (see Fig. 3). The different means of coding quantitative character states did not affect the outcome of the analysis of synapomorphies.

DISCUSSION

PHYLOGENY

Sequencing of the 5S-NTS region has now been done within three genera of Helieae, including the two largest, *Symbolanthus* and *Macrocarpaea.* We expected 5S-NTS data to be useful for resolving both higher- and lower-level relationships. For example, the 5S-NTS data do indicate the paraphyly of *Wurdackanthus* toward *Symbolanthus.* Because a monophyletic *Wurdackanthus* is not supported by these data, and because of its morphological and anatomical similarities to *Symbolanthus,* its circumscription should be reconsidered (Struwe & Gould, 2004).

However, we found that in Helieae, 5S-NTS data also highlighted the accumulation of different amounts of molecular change in lineages of presumably different ages. While there is little variation within *Symbolanthus,* considerably more variation occurs within *Macrocarpaea* (J. R. Grant & L. Struwe, unpublished), which based on relative

amounts of ITS variation (Grant & Struwe, 2000) is estimated to be a relatively much older lineage.

We did find a relatively large amount of molecular change between *Chelonanthus* and *Wurdackanthus–Symbolanthus* (branch length = 39), as well as between the two species of *Chelonanthus* we sampled (branch length = 27; see Fig. 2B), indicating that the 5S-NTS region can be useful in phylogenetic studies at higher levels, e.g., between closely related genera. Many changes also occurred along the branch between *Wurdackanthus frigidus* and *W. argyreus* + *Symbolanthus* (13), signifying a greater divergence between the two species of *Wurdackanthus* than between *W. argyreus* and *Symbolanthus* spp.

MORPHOLOGY

Compared to the outgroup, *Chelonanthus,* potential synapomorphies for the Wurdackanthus–Symbolanthus clade include having long corollas (greater than ca. 3 cm) and relatively large capsules. Other synapomorphies that distinguish *Wurdackanthus–Symbolanthus* from all other Helieae are common presence of a corona or staminal pockets inside the corolla and pollen of *Symbolanthus*-type (i.e., pollen grains united in tetrahedral tetrads with strongly reticulate exine; Nilsson, 1970) (Fig. 3).

Potential synapomorphies for the Wurdackanthus argyreus + Symbolanthus clade include even longer corollas (5.7 cm long or greater), pointed flower bud apices, and 1 to 3 flowers per inflorescence (vs. 5 or more) (Fig. 3). *Chelonanthus* bears many flowers per inflorescence on 1 to 4 long monochasial branches within a dichasium, and *Wurdackanthus frigidus* produces up to 7 flowers on monochasial branches, while *Wurdackanthus–Symbolanthus* have reduced 1- to 3-flowered mono- or dichasia. Deeply divided calyces is another possible synapomorphy for the W. argyreus + Symbolanthus clade (calyces divided 2/3 to nearly all the way to the base vs. 1/3 divided in *W. frigidus*; Fig. 3), but *Chelonanthus* is polymorphic for this character.

These morphological changes, as well as the relatively large number of nucleotide base changes along the branch to this clade, indicate that *Wurdackanthus argyreus* and *W. frigidus* are relatively divergent from one another. The character of calyx length has been used in the literature to separate *Wurdackanthus* from *Symbolanthus, Symbolanthus* having a longer calyx (e.g., Pringle, 1995; Struwe et al., 1999). However, according to our results, the relatively short calyx of *Wurdackanthus* is a plesio-
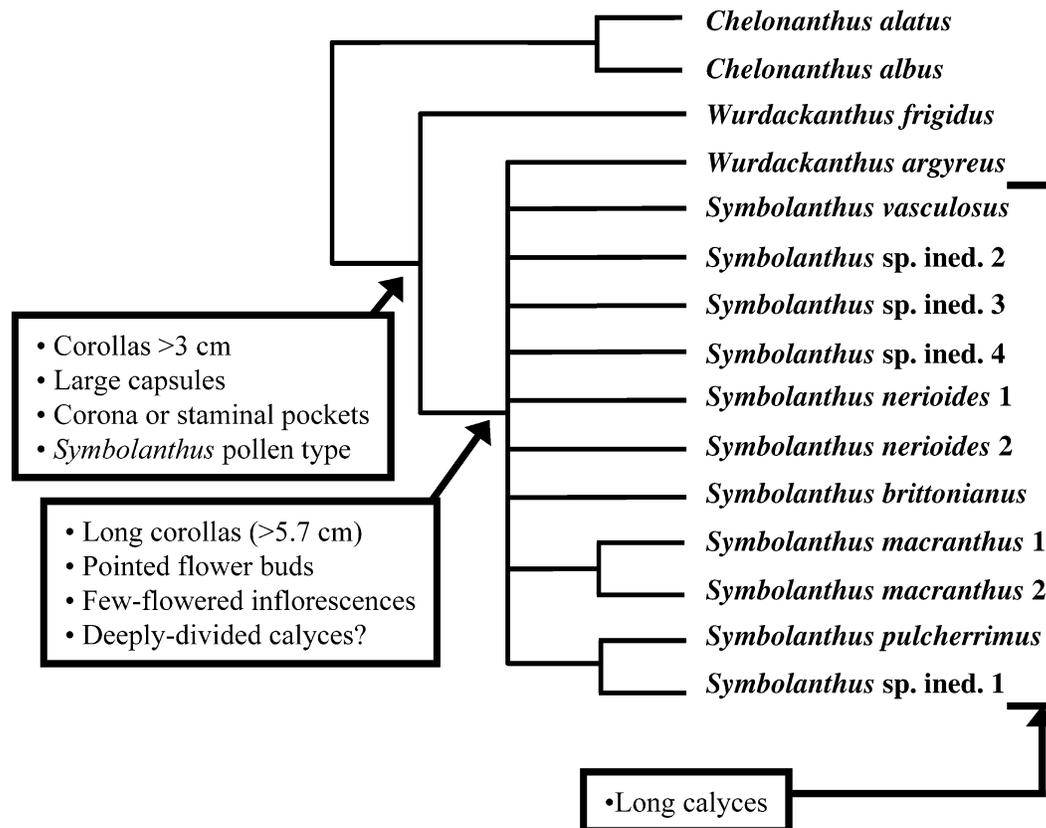
Figure 3. Potential morphological synapomorphies for *Wurdackanthus* + *Symbolanthus*, *W. argyreus* + *Symbolanthus* and *Symbolanthus* based on the 5S-NTS strict consensus tree.

morphic character state that is also found in *Chelonanthus*, not a synapomorphy for *Wurdackanthus* (Fig. 3). Although the two species of *Wurdackanthus* are relatively divergent from one another morphologically and in 5S-NTS sequences, other molecular data sets support a close relationship between *W. frigidus* and *Symbolanthus* spp. (nrITS sequences and cpDNA *trn*L intron sequences, Struwe et al., 2002).

BIOGEOGRAPHY

The *Symbolanthus* distribution is an excellent opportunity to test the influence of migration between the Guayana Highlands, the Andes, and Central America, and to test biogeographic hypotheses about the origin of the Guayana Highland flora. This study indicates the possibility that tepui species could have had Andean origins, since *Wurdackanthus argyreus* (Sierra de la Neblina and Cerro Aracamuni) is not consistently basal but nested among non-tepui *Symbolanthus* species. In the 50% majority rule consensus tree, it is nested

among two Venezuelan Andean species (*S. nerioides* and *S. vasculosus*). Future research directions on this group would be to obtain better material of tepui species for DNA sampling, or use morphological data to make a phylogeny of all species once a revision of *Symbolanthus* is complete (Struwe, in prep.). In addition, a higher-resolution data set, such as AFLPs, would probably be helpful.

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