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Barbara S. Carlsward

Eastern Illinois University, bscarlsward@eiu.edu

W. Mark Whitten

University of Florida

Morris H. Williams

Florida Museum of Natural History

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MOLECULAR PHYLOGENETICS OF NEOTROPICAL LEAFLESS ANGRAECINAE (ORCHIDACEAE): REEVALUATION OF GENERIC CONCEPTS

Barbara S. Carlsward,^{1,*} W. Mark Whitten,[†] and Norris H. Williamst

^{*}Department of Botany, University of Florida, Gainesville, Florida 32611-8526, U.S.A.; and [†]Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611-7800, U.S.A.

Phylogenetic relationships of leafless Neotropical Angraecinae (*Dendrophylax* Rchb.f., *Harrisella* Fawc. & Rendle, *Polyradicion* Garay, and *Campylocentrum* Benth.) were estimated using combined nuclear (ITS nrDNA) and plastid (*matK* and *trnL-F*) data sets with African angraecoid taxa as outgroups. Results indicate that *Harrisella*, *Polyradicion*, and *Campylocentrum filiforme* (Sw.) Cogn. are embedded within *Dendrophylax* and should be included in *Dendrophylax*. This contrasts with earlier generic concepts, which have been based mainly on gross differences in floral size and various morphological autapomorphies. Based on our current sampling, *Campylocentrum* includes both leafy and leafless species and is sister to a broadly defined *Dendrophylax*.

Keywords: Orchidaceae, *Dendrophylax*, *Campylocentrum*, phylogeny, ITS, *trnL-F*, *matK*.

Introduction

The majority of species in the orchid tribe Vandeeae (158 genera; ca. 1250 species; Dressler 1993) are found throughout the Old World tropics, while leafless genera of subtribe Angraecinae are restricted to the Neotropics. As defined by Dressler (1993), these New World endemics include *Campylocentrum* Benth. (ca. 55 species; Brazil to Mexico and Greater Antilles), *Dendrophylax* Rchb.f. (six to eight species; Greater Antilles), *Polyradicion* Garay (two to four species; Florida and Cuba), and *Harrisella* Fawc. & Rendle (one to three species; Mexico, Greater Antilles, Florida, El Salvador). No phylogenetic analysis of Angraecinae has been published, but most workers have assumed these Neotropical species form a clade, possibly the result of long-distance dispersal and subsequent radiation (McCartney 2000). This group is also remarkable for the occurrence of a leafless growth habit found only in the tribe Vandeeae. In several genera of Vandeeae (e.g., *Taeniophyllum* Blume from Asia, *Microcoelia* Lindl. from Africa, and the above-mentioned genera from the New World), the adult plants possess a very reduced stem and lack leaves; photosynthesis occurs in the chlorophyllous aerial roots and inflorescence axes.

All species of *Dendrophylax*, *Harrisella*, and *Polyradicion* are leafless, but *Campylocentrum* includes both leafy and leafless species. Flower size varies dramatically among and within genera. *Dendrophylax funalis* (Sw.) Benth. ex Rolfe, *Dendrophylax fawcettii* Rolfe, *Dendrophylax sallei* (Rchb.f.) Benth. ex Rolfe, and *Polyradicion lindenii* (Lindl.) Garay produce large white, nocturnally fragrant flowers with long, nectariferous spurs (ca. 15 cm in *D. fawcettii*), whereas other species of *Dendrophylax* produce much smaller, greenish flowers. *Harrisella porrecta* (Rchb.f.) Fawc. & Rendle has tiny, greenish

tan flowers with a short (ca. 1 mm), bilobed spur; the inflorescence is few flowered and relatively lax. In contrast, most species of *Campylocentrum* produce short, congested inflorescence axes bearing 10–20 small, white flowers with relatively short spurs. Many of the Old World Angraecinae have large white, spurred flowers that exhibit a hawkmoth pollination syndrome.

Because vegetative characters are reduced or greatly altered in these leafless orchids, the generic concepts of Neotropical Angraecinae have been based largely on gross floral and pollinarium morphology. Most species were originally placed in the genus *Aeranthus* Lindl. by early workers and were later separated from the Palearctic taxa into segregate genera. The most recent comprehensive taxonomic treatment of Neotropical Angraecinae is that of Nir (2000) in his examination of Antillean Orchidaceae. In this work, Nir (2000) transferred *Polyradicion* and *Campylocentrum constanzense* Garay into *Dendrophylax* and transferred *Harrisella* into *Campylocentrum*, leaving two Neotropical genera distinguished by flower resupination and fruit size.

To date, four species of *Harrisella* have been described: *H. porrecta*, *Harrisella filiformis* (Sw.) Cogn., *Harrisella monte-verdi* (Rchb.f.) Cogn., and *Harrisella uniflora* Dietrich. Ackerman (1995) examined the types of these taxa and concluded that *H. filiformis* and *H. monte-verdi* were synonymous with the leafless *Campylocentrum filiforme* (Sw.) Cogn. ex Kuntze. Citing unpublished studies of Cuban *Harrisella* by Jorge Ferro Díaz, Ackerman (1995) also regarded *H. uniflora* as a synonym of *H. porrecta*. Conversely, Nir (2000) regarded *H. uniflora* as a synonym of *C. filiforme*. In both cases, *Harrisella* was reduced to a single species, *H. porrecta*.

Combined molecular analyses of several orchid clades have shown that floral morphology is evolutionarily plastic (e.g., Oncidiinae, Chase and Palmer 1997; Catasetinae, Pridgeon and Chase 1998; Stanhopeinae, Whitten et al. 2000; Oncidiinae, Williams et al. 2001). Given the extreme morphological

¹ Author for correspondence; e-mail barbarac@botany.ufl.edu.

reduction in these leafless taxa and the probability of floral convergence based on pollination syndromes, DNA sequence data should provide a useful and independent data set for evaluating relationships. In this study, we use DNA sequence data to examine the monophyly and generic relationships within Neotropical Vandaeae as well as homoplasy of the leafless condition.

Material and Methods

Specimens were obtained from cultivated material, herbarium specimens, or wild-collected plants (table 1). Samples of *Polyradicion lindenii*, *Campylocentrum pachyrrhizum* (Rchb.f.) Rolfe, and *Harrisella porrecta* from Fakahatchee Strand State Preserve, Florida, and *H. porrecta* from Grand Cayman are unvouchered; we were only allowed to collect root tips because of the rarity of these species at these localities. Protocols for extraction, amplification, and DNA sequencing from fresh and silica gel-dried material are given in Whitten et al. (2000). We did not sample the following Antillean species of *Dendrophylax* and *Campylocentrum*: *Campylocentrum macrocarpum* Dod, *Dendrophylax constanzense* (Garay) Nir, *Dendrophylax gracilis* (Cogn.) Garay, *Dendrophylax helorhiza* Dod, and *Dendrophylax serpentilingua* (Dod) Nir.

Many of these taxa are rare in cultivation and in the field, so we attempted to amplify DNA from herbarium specimens. Extractions of 1–2 cm of root from herbarium material usually yielded degraded DNA. Attempts to amplify the entire ITS region in one piece were unsuccessful; however, we were able to amplify ITS 1 and ITS 2 separately by using the primers of Blattner (1999). Before amplification, total DNAs from herbarium material were cleaned using Qiagen QIAquick columns to remove inhibitory secondary compounds. The amplification mix consisted of 33 μ L water, 5 μ L Sigma buffer, 6 μ L $MgCl_2$ (25 mM), 1 μ L dNTPs (10 mM each), 1 μ L of each primer (10 pmol/ μ L), 5 μ L of template, and 0.2 μ L of Sigma *Taq* polymerase. The thermocycler protocol consisted of an initial denaturation at 94°C for 2 min followed by 35 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 3 min. Using these two primer sets (A/C and B/D), we were able to amplify and cleanly sequence the ITS region from herbarium specimens up to 63 yr old.

We attempted to amplify both nuclear and chloroplast regions for all Neotropical taxa, but efforts to amplify chloroplast regions (*matK* and *trnL-F*) proved unsuccessful for most taxa available from herbarium specimens. Therefore, parsimony analyses were conducted with several data sets with an uneven sampling of taxa: (1) an ITS data set containing 33 individuals of 20 ingroup species; (2) a *trnL-F* data set containing 24 individuals of 14 ingroup species; (3) a *matK* data set containing 25 individuals of 15 ingroup species; (4) a chloroplast data set with *matK* and *trnL-F* combined for 27 individuals of 14 ingroup species; and (5) a combined data set of ITS, *matK*, and *trnL-F* regions containing 37 individuals of 20 ingroup species. Sequences were aligned manually; data matrices are available from us. Cladistic analyses based on parsimony were performed using PAUP* 4.0b10 (Swofford 1999). Levels of support were estimated using the bootstrap method (BS; 1000 replicates). Outgroup taxa in all analyses (*Angraecum chevalieri* Summerh., *Angraecum cultriforme*

Summerh., and *Angraecum eichlerianum* Kraenzl.) were chosen on the basis of more extensive combined analyses using 130 taxa within Vandaeae (B. S. Carlswald, unpublished data) in which these three species of *Angraecum* were sister to the New World clade. Heuristic searches were performed with 1000 random-addition replicates, saving 10 trees per replicate, with the tree bisection/reconnection (TBR) algorithm. All characters were weighted equally, and no regions were excluded from the alignment. Trees resulting from this initial search were swapped to exhaustion. Bootstrap analyses utilized 1000 replicates, with 10 random-addition replicates (SPR swapping) per bootstrap replicate.

Results

In all analyses, species of *Campylocentrum* (excluding *Campylocentrum filiforme*) were monophyletic and sister to the clade including *Dendrophylax* spp., *Harrisella*, *C. filiforme*, and *Polyradicion*. These two clades are henceforth referred to as *Campylocentrum* and *Dendrophylax*, respectively. Authorities for taxa discussed in the results are listed in table 1.

ITS Matrix

The ITS matrix included 33 Neotropical individuals representing 20 species plus three outgroup taxa. Of the 711 aligned positions, 193 (27%) were variable and 77 (11%) were parsimony informative. Heuristic analyses produced 216 trees with length (L) = 307, consistency index (CI₁) = 0.75, consistency index excluding uninformative characters (CI₂) = 0.66, and retention index (RI) = 0.85 (fig. 1).

Within the moderately supported *Campylocentrum* clade (74% BS), five subclades were well supported (>90% BS): *Campylocentrum micranthum* (Panama) + *Campylocentrum schiedei* (94% BS), *C. micranthum* (Mexico)/*Campylocentrum robustum* (100% BS), *Campylocentrum fasciola*/*Campylocentrum ulei* (92% BS), *Campylocentrum lansbergii*/*Campylocentrum jamaicense* (99% BS), and *C. lansbergii*/*Campylocentrum neglectum* (99% BS). The large *C. fasciola*/*C. ulei* clade, which includes leafless and leafy species, is sister to the smaller leafy *C. lansbergii*/*C. neglectum* clade.

Species of *Dendrophylax* were also only moderately supported as a clade (79% BS), but there were many well-supported subclades: *C. filiforme* + *Dendrophylax barrettiae* (95% BS), *Dendrophylax varius*/*Dendrophylax sallei* (100% BS), *Dendrophylax fawcettii* + *Dendrophylax funalis* (99% BS), and *Harrisella porrecta* (97% BS). The *C. filiforme* + *D. barrettiae* clade is sister to the remaining members of *Dendrophylax* (79% BS).

trnL-F Matrix

The *trnL-F* matrix included 24 Neotropical individuals representing 14 species plus three outgroup taxa. Of the 1489 aligned positions, 267 (18%) were variable and 144 (10%) were parsimony informative. Heuristic analyses produced 72 trees with L = 337, CI₁ = 0.86, CI₂ = 0.77, and RI = 0.89 (fig. 2).

Within the well-supported *Campylocentrum* clade (96% BS), two subclades were also strongly supported: *C. lansbergii*/*C. jamaicense* (100% BS) and *C. fasciola* + *Campylocentrum*

Table 1
Voucher Specimens

Taxon	Voucher	Locality	GenBank accession number		
			ITS	<i>trnL-F</i>	<i>matK</i>
<i>Angraecum eichlerianum</i> Kraenzl. ^a	Carlsward 284 (FLAS)	Unknown garden origin	AF506322	AF506341	AF506365
<i>Angraecum chevalieri</i> Summerh. ^a	Carlsward 208 (FLAS)	Selby Botanical Gardens	AF506320	AF506339	AF506363
<i>Angraecum cultriforme</i> Summerh. ^a	Carlsward 298 (FLAS)	Countryside Orchids	AF506321	AF506340	AF506364
<i>Campylocentrum fasciola</i> (Lindl.) Cogn.	Carlsward 185 (FLAS)	Claude Hamilton, Jamaica	AF506294	AY147226	AF506342
<i>C. fasciola</i> (Lindl.) Cogn	Whitten 1933 (QCNE)	Ecuador	AF506295	na	AF506343
<i>Campylocentrum filiforme</i> (Sw.) Cogn.	Whitten 1842 (FLAS)	Puerto Rico	AF506296	AF506323	AF506344
<i>Campylocentrum jamaicense</i> (Rchb.f. & Wullschl.) Benth. ex Rolfe ^a	Whitten 1934 (FLAS)	Jamaica	AF506299	AF506326	AF506348
<i>Campylocentrum lansbergii</i> (Rchb.f.) Schltr. ^a	Carlsward 272 (FLAS)	Brazil	AF506297	AF506324	AF506345
<i>Campylocentrum micranthum</i> (Lindl.) Rolfe ^a	Ackerman 3341 (UPRRP)	Puerto Rico	AY147219	AF506325	AF506346
<i>C. micranthum</i> (Lindl.) Rolfe ^a	Carlsward 180 (FLAS)	CalOrchid, Mexico	AF506298	AY147227	AF506347
<i>C. micranthum</i> (Lindl.) Rolfe ^a	Carlsward 315 (FLAS)	Tropical Orchids, Panama	AY147220	AY147228	AY147235
<i>Campylocentrum neglectum</i> (Rchb.f. & Warm.) Cogn. ^a	Zardini 14995 (SEL)	Paraguay	AF506300	na	na
<i>Campylocentrum pachyrrhizum</i> (Rchb.f.) Rolfe	No voucher	Fakahatchee State Preserve, Florida	na	AF506327	AF506349
<i>C. pachyrrhizum</i> (Rchb.f.) Rolfe	Ackerman s.n. (UPRRP)	Puerto Rico	AF506301	AF506328	AF506350
<i>Campylocentrum poeppigii</i> (Rchb.f.) Rolfe	Carnevali 4507 (CICY)	Mexico	AF506302	AF506329	AF506351
<i>Campylocentrum robustum</i> Cogn. ^a	Höijer & Dalström 839 (SEL)	Ecuador	AF506303	na	na
<i>Campylocentrum schiedeii</i> (Rchb.f.) Benth. ex Hemsl. ^a	Whitten 1822 (FLAS)	Costa Rica	AF506304	na	AF506352
<i>Campylocentrum tyrridion</i> Garay & Dunst.	Carnevali 5145 (FLAS, CICY)	Mexico	AF506305	na	na
<i>Campylocentrum ulei</i> Cogn. ^a	Chagas & Silva 1333 (SEL)	Brazil	AF506306	na	na
<i>Dendrophyllax alcoa</i> Dod	Ackerman 2773 (UPRRP)	Dominican Republic	AF506307	na	na
<i>Dendrophyllax barrettiae</i> Fawc. & Rendle	Carlsward 199 (FLAS)	Claude Hamilton, Jamaica	AF506308	AF506330	AF506353
<i>Dendrophyllax faucettii</i> Rolfe	Whitten 1939 (FLAS)	Grand Cayman	AF506309	AF506331	AF506354
<i>Dendrophyllax funalis</i> (Sw.) Benth. ex Rolfe—specimen 1	Carlsward 302 (FLAS)	Jamaica	AY147221	AY147229	AF506355
<i>D. funalis</i> (Sw.) Benth. ex Rolfe—specimen 2	Whitten 1935 (FLAS)	Jamaica	AF506310	AF506332	na
<i>Dendrophyllax sallei</i> (Rchb.f.) Benth. ex Rolfe	Whitten 1945 (JBSD)	Dominican Republic	AY147225	AY147234	AY147239
<i>Dendrophyllax varius</i> (Gmel.) Urb.—specimen 1	Ackerman 3118 (UPRRP)	Dominican Republic	AF506311	AF506333	na
<i>D. varius</i> (Gmel.) Urb.—specimen 2	Thompson 10683 (SEL)	Dominican Republic	AF506312	na	na
<i>D. varius</i> (Gmel.) Urb.—specimen 3	Whitten 1960 (JBSD)	Dominican Republic	AY147222	AY147230	AY147236
<i>Harrisella porrecta</i> (Rchb.f.) Fawc. & Rendle	No voucher	Grand Cayman	AF506317	na	AF506361
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Whitten 1950 (JBSD)	Dominican Republic	AY147224	AY147233	AY147238
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Carlsward 329 (FLAS)	Florida	AY147223	AY147232	AY147237
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Carlsward 184 (FLAS)	Jamaica	AF506315	AY147231	AF506358
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Ackerman 3340 (UPRRP) B11	Puerto Rico	AF506313	AF506334	AF506356
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Carnevali 6312 (FLAS, CICY)	Campeche	AF506316	AF506337	AF506360
<i>H. porrecta</i> (Rchb.f.) Fawc. & Rendle	Carnevali et al. 5907 (FLAS, CICY)	Yucatán	AF506314	AF506335	AF506357
<i>Polyradicion lindenii</i> (Lindl.) Garay	Photo voucher (FLAS)	Claude Hamilton, Cuba	AF506318	AF506338	AF506362
<i>P. lindenii</i> (Lindl.) Garay	No voucher	Fakahatchee State Preserve, Florida	AF506319	na	na

Note. na = not sequenced.

^a Taxa with leaves.

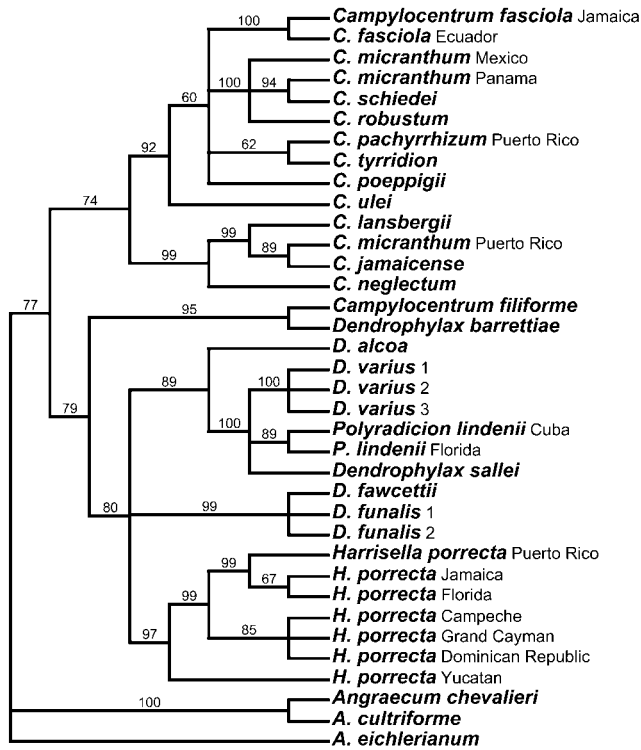


Fig. 1 Bootstrap consensus for ITS data set. Bootstrap percentages, shown above branches, are based on 1000 replicates.

poeppigii (93% BS). Species of *Dendrophyllax* were also well supported with 95% BS, and there were three well-supported subclades: *D. fawcettii* + *D. funalis* (99% BS), *Polyradicion lindenii* + *D. sallei* (96% BS), and *H. porrecta* (99% BS).

matK Matrix

The *matK* matrix included 25 Neotropical individuals representing 15 species plus three outgroup taxa. Of the 1354 aligned positions, 120 (9%) were variable and 68 (5%) were parsimony informative. Heuristic analyses produced 72 trees with $L = 147$, $CI_1 = 0.86$, $CI_2 = 0.79$, and $RI = 0.90$ (fig. 3).

As in the ITS matrix, *Campylocentrum* and *Dendrophyllax* were only moderately supported clades. Within *Campylocentrum*, *C. micranthum* (Mexico) + *C. schiedei* (100% BS) and *C. lansbergii*/*C. jamaicense* (98% BS) formed the only subclades with greater than 90% bootstrap support. Within *Dendrophyllax*, *D. fawcettii* + *D. funalis* (100% BS) and *H. porrecta* (98% BS) were the only two subclades with greater than 90% bootstrap support; these clades are also well supported in the ITS and *trnL-F* matrices. As in the *trnL-F* analysis, most relationships within *Dendrophyllax* were unresolved.

Combined Analysis of Chloroplast Data (matK and *trnL-F*)

The chloroplast matrix included 27 Neotropical individuals representing 15 species plus three outgroup taxa. Of the 2841 aligned positions, 392 (14%) were variable and 218 (8%) were

parsimony informative. Heuristic analyses produced 360 trees with $L = 500$, $CI_1 = 0.84$, $CI_2 = 0.76$, and $RI = 0.89$ (fig. 4).

Within the well-supported *Campylocentrum* clade (98% BS), several subclades were also strongly supported: *C. micranthum* (Mexico)/*C. schiedei* (100% BS); *C. fasciola*/*Campylocentrum pachyrrhizum* (99% BS), and *C. lansbergii*/*C. jamaicense* (100% BS). Species of *Dendrophyllax* also formed a well-supported clade with 97% BS, and two subclades were similarly supported: *D. fawcettii* + *D. funalis* (100% BS), *D. varius*/*D. sallei* (91% BS), and *H. porrecta* (100% BS). The topology of both *Dendrophyllax* and *Campylocentrum* within the chloroplast bootstrap consensus is very similar to that of the ITS data (figs. 1, 4).

Combined Analysis of Chloroplast and Nuclear Data

There are no conflicting, well-supported clades between the nuclear and chloroplast topologies. We therefore performed a combined analysis with all three data sets (ITS, *matK*, and *trnL-F*). The combined matrix included 37 Neotropical individuals representing 20 species plus three outgroup taxa. Of the 3555 aligned positions, 587 (16%) were variable and 335 (9%) were parsimony informative. Heuristic analyses produced 162 trees with $L = 814$, $CI_1 = 0.80$, $CI_2 = 0.71$, $RI = 0.87$ (fig. 5).

Campylocentrum and *Dendrophyllax* formed two well-supported clades (99% BS), sister to one another. Within *Campylocentrum*, the following subclades were supported with high bootstrap percentages: *C. fasciola*/*C. ulei* (98% BS), *C.*

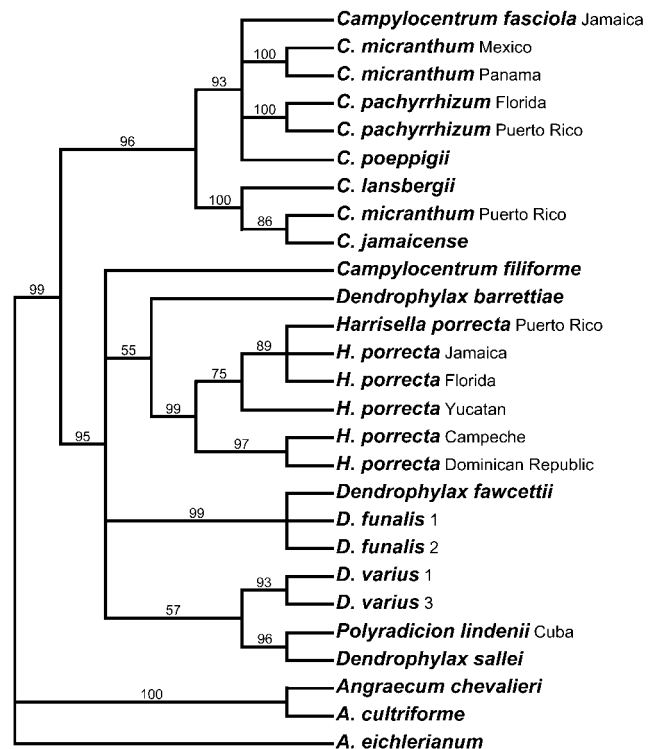


Fig. 2 Bootstrap consensus for *trnL-F* data set. Bootstrap percentages, shown above branches, are based on 1000 replicates.

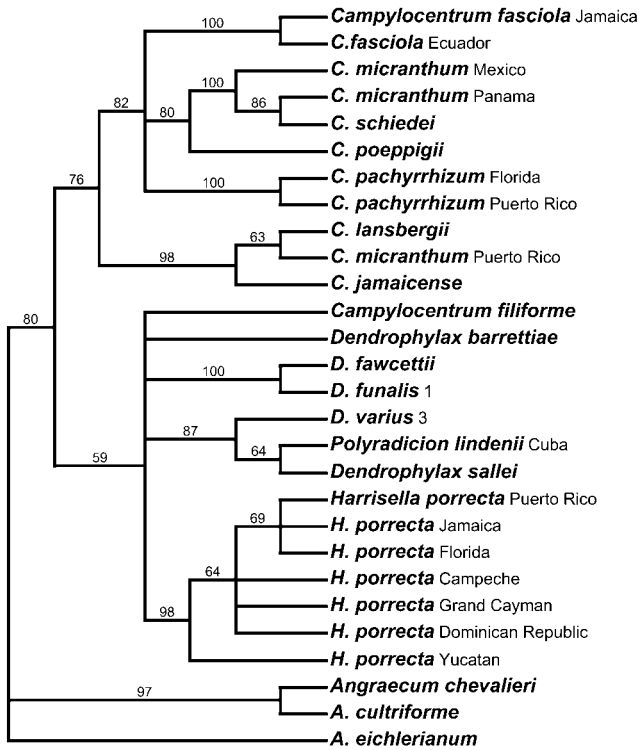


Fig. 3 Bootstrap consensus for *matK* data set. Bootstrap percentages, shown above branches, are based on 1000 replicates.

micranthum (Mexico)/*C. robustum* (100% BS), *C. lansbergii*/ *C. jamaicense* (99% BS), *C. micranthum* (Puerto Rico)/*C. jamaicense* (91% BS), and *C. lansbergii*/ *C. neglectum* (99% BS). There were also several strongly supported subclades within *Dendrophylax*: *C. filiforme* + *D. barrettiae* (95% BS), *Dendrophylax alcoal*/ *D. sallei* (91% BS), *D. varius*/ *D. sallei* (100% BS), *Polyradicion lindenii*/ *D. sallei* (94% BS), *D. fawcettii* + *D. funalis* (100% BS), and *H. porrecta* (100% BS).

Discussion

From more extensive analyses incorporating ITS, *matK*, and *trnL-F* data of Angraecinae and Aerangidinae, Palearctic Angraecinae form a basal grade within which the Neotropical Angraecinae constitute a derived, well-supported clade (99% BS; B. S. Carlswald, unpublished data). The most significant result of our current phylogenetic analyses of Neotropical Angraecinae (figs. 1–5) is the strongly supported polyphyly of *Dendrophylax*; intercalated among species of *Dendrophylax* are *Polyradicion*, *Harrisella*, and *Campylocentrum filiforme*.

The nomenclatural history of the large-flowered species of *Dendrophylax* is plagued with confusion. Originally, Pfitzer (1889) segregated the genus *Polyrrhiza* Pfitz. from *Dendrophylax* based on stipe morphology and flower size. He created *Polyrrhiza* based on several West Indian species originally described as *Dendrophylax*, but he only made an official transfer of one species, *Dendrophylax funalis*. Cogniaux (1910) later transferred the remaining species of *Dendrophylax* (*Dendro-*

phylax fawcettii, *Dendrophylax lindenii*, and *Dendrophylax sallei*) to the genus *Polyrrhiza* and named a new species (*Polyrrhiza gracilis* Cogn.), making a total of five species. Fawcett and Rendle (1910) were the first to begin disassembling *Polyrrhiza* by making *Polyrrhiza funalis* (Rchb.f.) Pfitzer synonymous with *D. funalis*. Garay (1969) later eliminated *Polyrrhiza* altogether by transferring two species (*Polyrrhiza lindenii* [Lindl.] Cogn. and *Polyrrhiza sallei* [Rchb.f.] Cogn.) into the genus *Polyradicion* and *P. gracilis* back to *Dendrophylax*. Finally, Nir (2000) made the transfer of the one remaining species of *Polyrrhiza* (*Polyrrhiza fawcettii* [Rolfe] Cogn.) and all species of *Polyradicion* into *Dendrophylax*.

Examination of the Lindley orchid herbarium at the Royal Botanic Gardens, Kew (K) revealed several *Harrisella* specimens annotated in Lindley's handwriting as the genus *Scroptella*, accompanied by a loose sheet of paper with a handwritten description of the new genus. This name, probably drawn from the saccate, bilobed spur of the lip, was never published. Several taxonomists have suggested that *Harrisella porrecta* be transferred into *Campylocentrum* (Williams 1951; McVaugh 1985; Dressler 1993), and Nir (2000) eventually made this transfer in his study of Antillean Orchidaceae. Based on our phylogenetic analyses, the small-flowered *Harrisella* is most closely related to large-flowered taxa of *Dendrophylax* and does not belong in *Campylocentrum*.

The relatively high levels of sequence divergence among the different accessions of *H. porrecta* (2% of the three-region matrix) indicate the possible existence of cryptic species. Al-

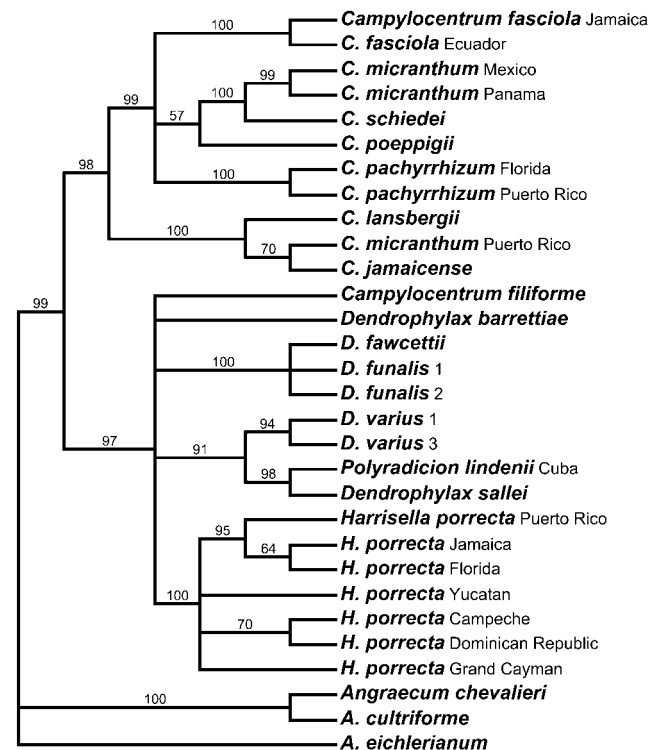


Fig. 4 Bootstrap consensus for the combined chloroplast data sets (*matK* and *trnL-F*). Bootstrap percentages, shown above branches, are based on 1000 replicates.

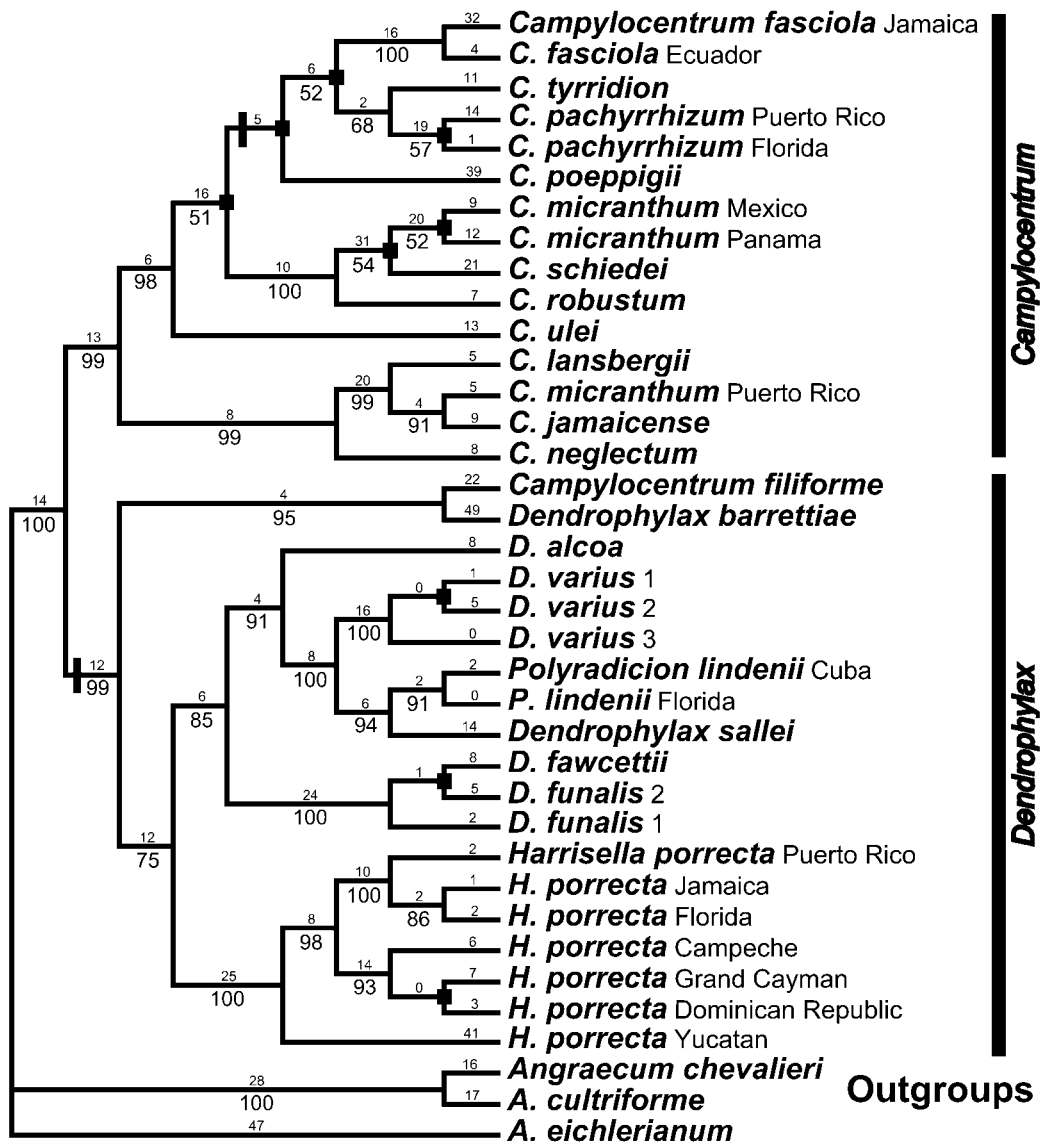


Fig. 5 One of 162 trees resulting from a heuristic search of the combined matrix for all data sets (ITS, *trnL-F*, and *matK*). Fitch lengths are shown above branches; bootstrap percentages (1000 replicates) are shown below branches. Branches that collapse in the strict consensus of all trees are indicated with a square. Bars along a branch indicate the character state change from leafy to leafless.

though floral morphology varies little among *H. porrecta* populations, there are dramatic differences in root thickness, plant size, and inflorescence size. Most plants produce thin roots (0.5–2.0-mm diameter), but some plants from the Yucatán peninsula (e.g., Carnevali 5907) are larger, possess thicker roots, and produce larger inflorescences. Germán Carnevali (personal communication) reports that both thin- and thick-rooted forms are sympatric in certain areas of the Yucatán. More extensive sampling of *Harrisella* throughout its range (perhaps coupled with chromosome counts and additional molecular markers) is needed to clarify the number of species present in this morphologically reduced clade.

Campylocentrum filiforme is strongly supported as a member of *Dendrophyllax* in all of our analyses. Its short, few-flowered inflorescence is unlike the many-flowered, distichous

inflorescence of other *Campylocentrum* species, and it is clearly misplaced within *Campylocentrum*.

In our analysis, *Campylocentrum micranthum* (Lindl.) Rolfe from Puerto Rico is more closely related to *Campylocentrum jamaicense* (Rchb.f. & Wullschl.) Benth. ex Rolfe than to mainland *C. micranthum* (Mexico and Panama). *Campylocentrum jamaicense* has previously been synonymized with *C. micranthum* (Ackerman 1995). Within the species complex of *C. micranthum*–*C. jamaicense*, there seems to be a distinction between the West Indian material and the mainland material based on the habit of the inflorescence; the West Indian taxa are distichous while the mainland taxa are secund (R. L. Dressler, personal communication; Ackerman 1995). While further sampling is required, our preliminary sampling supports this distinction.

It is clear from these analyses that floral size and gross morphology are not predictive of phylogenetic relationships within this Neotropical clade; the large-flowered (presumably, hawkmoth-pollinated) species do not form a monophyletic group. The relatively low levels of sequence divergence among *Harrisella*, *Dendrophylax*, and *Polyradicion* indicate that these taxa simply represent dramatic divergences in floral size and shape among closely related species. Our results are similar to those found in other orchid groups in which molecular analyses reveal extreme plasticity and convergence in floral morphology (Oncidiinae, Chase and Palmer 1997; Catasetinae, Pridgeon and Chase 1998; Stanhopeinae, Whitten et al. 2000; Oncidiinae, Williams et al. 2001). Although *Harrisella* and *Polyradicion* differ greatly in flower size, they simply represent extremes of a morphological continuum connected by species of *Dendrophylax* with small to medium-sized flowers. Other than flower size, we find no consistent differences to delimit genera within this clade. Capsule morphology and dehiscence vary among species, but these traits are correlated with plant and flower size. The large-flowered taxa (*D. funalis*, *D. fawcettii*, *D. sallei*, *Polyradicion lindenii*) produce long, cylindrical capsules, whereas the small-flowered *Dendrophylax* and *Harrisella* produce ovoid to globose capsules. *Harrisella* is unique in possessing capsules with valves that separate at the apex and reflex at maturity in addition to flowers with a bilobed spur, but these traits represent autapomorphies of a single species and do not necessarily merit generic status.

To reconcile our phylogenetic analyses with a generic classification, two options exist. The first is to maintain *Harrisella* and *Polyradicion* and to create several new genera from the monophyletic groups within the polyphyletic *Dendrophylax*. Although *Harrisella* and *Polyradicion* both possess several autapomorphies that make them very distinctive, the other clades within *Dendrophylax* are morphologically intermediate. If this first option for classification were taken, the result would be several new genera lacking morphological synapomorphies. The second option is to transfer *Harrisella* and *Polyradicion* into a broader, monophyletic *Dendrophylax* sister to *Campylocentrum*. Based on the relatively low sequence divergence among these taxa, the lack of distinguishing morphological synapomorphies, and the polyphyly of *Dendrophylax* (*sensu* Dressler 1993) in the separate and combined cladograms, we choose to recognize a broad *Dendrophylax*, thereby avoiding the creation of many monotypic genera. Our circumscription of *Dendrophylax* is similar to that of Nir (2000) but differs by our inclusion of several species that he places in *Campylocentrum* (*H. porrecta*, *C. filiforme*, *Dendrophylax helorrhiza* Dod, and *Dendrophylax barrettiae*). We were unable to obtain specimens for DNA analysis of *C. macrocarpum*, *Dendrophylax constanzense*, *Dendrophylax gracilis*, *D. helorrhiza*, and *Dendrophylax serpentilingua*. Therefore, our decision to include these taxa within *Dendrophylax* was based on morphological descriptions and illustrations of each species. Necessary nomenclatural combinations are made in appendix A and appendix B.

Unfortunately, our revised classification eliminates flower size as an easy field character for generic recognition, and it necessitates nomenclatural changes for several well-known taxa. Nevertheless, this classification more closely reflects the evolutionary relationships among Neotropical species than the

current system based on convergent pollination syndromes, and it should prove more predictive of other phenotypic traits. The most obvious synapomorphy for distinguishing these two genera is inflorescence condition. The flowers of *Campylocentrum* are arranged distichously on an unbranched raceme and are usually numerous (>10), whereas *Dendrophylax* has a loosely single-flowered to several-flowered raceme or panicle, with few flowers open at any given time. From his extensive observations of Antillean Angraecinae, John Beckner (personal communication) noted the presence of a small, swollen extension below the abscission layer (between the peduncle and fused ovary/pedicle) in *Dendrophylax s.s.* and in *Harrisella*; this structure is absent in *Polyradicion* and *Campylocentrum*. This swelling could also potentially be used as a synapomorphy, albeit a homoplasious one. Live root tip color may be another synapomorphy separating *Dendrophylax* and *Campylocentrum*. *Dendrophylax* root tips are always green, whereas *Campylocentrum* root tips are usually tinted orange-brown in living taxa we have examined (although this color may weaken in cultivated material). The vegetative anatomy of this clade is currently under study (B. S. Carlsward, unpublished data) but has yielded no synapomorphies useful in distinguishing these two genera. A complete key to the species of *Dendrophylax* is in preparation by James Ackerman for his treatment of the Orchidaceae of the Greater Antilles.

Based on our limited sampling, *Campylocentrum* appears monophyletic and includes both leafy and leafless species, whereas all *Dendrophylax* are leafless. This distinctive leafless habit found only in members of Vandeeae has probably arisen at least three times worldwide: (1) in Asia, (2) in Africa and Madagascar, and (3) in the Neotropics. Within the New World, leaflessness appears to have arisen at least twice: once in the ancestor of *Dendrophylax* and at least once within *Campylocentrum* (the *Campylocentrum fasciola*/*Campylocentrum poeppigii* clade). Several critical nodes within *Campylocentrum* (fig. 5) are poorly supported and collapse in the strict consensus of all trees, making the evolution of leaflessness within the genus uncertain. However, *Campylocentrum* is a large genus of ca. 55 species with moderate levels of sequence divergence, and increased taxon sampling should improve the phylogeny and clarify the evolution of the leafless habit.

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Appendix A

Key to Genera of Neotropical Angraecinae

1. Flowers few (one to six), with one or few flowers open at once; inflorescence lax, sometimes branching; plants always leafless; root tips green. *Dendrophylax*
 1. Flowers many (more than 10), distichously arranged

on unbranched raceme; usually many flowers open at once; plants leafy or leafless; root tips usually orange-brown. *Campylocentrum*

Appendix B

Summary of the Genus *Dendrophylax*

Dendrophylax Rchb.f., *Annales Botanices Systematicae* 6:903, 1864

Synonyms. *Polyrrhiza* Pfitzer, Die Natürlichen Pflanzenfamilien 2(6):208, 1888; *Harrisella* Fawc. & Rendle, Journal of Botany 47(559):265–266, 1909; *Polyradicion* Garay, Journal of the Arnold Arboretum 50:466, 1969.

Distribution. El Salvador, Florida, Greater Antilles, Mexico.

Dendrophylax alcoa Dod, *Moscoso* 2:5, 1983

Distribution. Hispaniola.

Dendrophylax barrettiae Fawc. & Rendle, *Journal of Botany* 47:266, 1909

Synonyms. *Campylocentrum arizae-juliae* Ames, Botanical Museum Leaflets 6(2):23, 1938.

Distribution. Hispaniola, Jamaica.

Dendrophylax constanzense (Garay) Nir, *Orchidaceae Antillanae* 83, 2000

Basionym. *Campylocentrum constanzense* Garay, Journal of the Arnold Arboretum 50:468, 1969.

Distribution. Hispaniola.

Dendrophylax fawcettii Rolfe, *Gardeners' Chronicle*, ser. 3, 4:533, 1888

Synonym. *Polyrrhiza fawcettii* (Rolfe) Cogn., *Symbolae Antillanae* 6:679, 1910.

Distribution. Grand Cayman.

Dendrophylax filiformis (Sw.) Carlswald & Whitten, comb. nov.

Basionym. *Epidendrum filiforme* Sw., *Nova Genera et Species Plantarum Seu Prodrumus* 126, 1788.

Synonyms. *Aeranthus monteaverdi* Rchb.f., *Flora* 48:279,

1865; *Campylocentrum filiforme* (Sw.) Cogn. ex Kuntze, *Revisio Generum Plantarum* 3(2):298, 1898; *Campylocentrum monteaverdi* (Rchb.f.) Rolfe, *The Orchid Review* 11:247, 1903; *Harrisella filiformis* (Sw.) Cogn., *Symbolae Antillanae* 6:687, 1910; *Harrisella monteaverdi* (Rchb.f.) Cogn., *Symbolae Antillanae* 6:687, 1910.

Distribution. Cuba, Hispaniola, Puerto Rico.

Dendrophylax funalis (Sw.) Benth. ex Rolfe, *Gardeners' Chronicle*, ser. 3, 4:533, 1888

Basionym. *Epidendrum funale* Sw., *Nova Genera et Species Plantarum Seu Prodrumus* 126, 1788.

Synonyms. *Limodorum funale* (Sw.) Sw., *Nova Acta Regiae Societatis Scientiarum Upsaliensis* 6:79, 1799; *Oeceoclades funalis* (Sw.) Lindl., *The Genera and Species of Orchidaceous Plants* 237, 1833; *Angraecum funale* (Sw.) Lindl., *Gardeners' Chronicle & Agricultural Gazette* 135, 1846; *Aeranthus funalis* (Sw.) Rchb.f., *Annales Botanices Systematicae* 6:902, 1864; *Polyrrhiza funalis* (Rchb.f.) Pfitzer, *Die Natürlichen Pflanzenfamilien* 2, pt. 6, 215, 1889.

Distribution. Jamaica.

Dendrophylax gracilis (Cogn.) Garay, *Journal of the Arnold Arboretum* 50:467, 1969

Basionym. *Polyrrhiza gracilis* Cogn., *Symbolae Antillanae* 6:679, 1910.

Synonym. *Polyradicion gracilis* (Cogn.) H. Dietr., *Wissenschaftliche Zeitschrift der Friedrich-Schiller-Universität Jena/Thüringen, Mathematisch-Naturwissenschaftliche Reihe* 32:61, 1983.

Distribution. Cuba.

Dendrophylax helorrhiza Dod, *Moscoso* 2:7, 1983

Synonym. *Campylocentrum helorrhizum* (Dod) Nir, *Orchidaceae Antillanae* 59, 2000.

Distribution. Hispaniola.

***Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe, Gardeners' Chronicle, ser. 3, 4:533, 1888**

Basionym. *Angraecum lindenii* Lindl., Gardeners' Chronicle 1846:135, 1846.

Synonyms. *Aeranthus lindenii* (Lindl.) Rchb.f., Annales Botanicæ Systematicæ 6:902, 1864; *Polyrrhiza lindenii* (Lindl.) Cogn., Symbolae Antillanae 6:680, 1910; *Polyradicion lindenii* (Lindl.) Garay, Journal of the Arnold Arboretum 50:467, 1969.

Distribution. Cuba, Florida.

***Dendrophylax macrocarpa* (Dod) Carlsward & Whitten, comb. nov.**

Basionym. *Campylocentrum macrocarpum* Dod, Moscosoa 1(2):39, 1977.

Distribution. Hispaniola.

***Dendrophylax porrectus* (Rchb.f.) Carlsward & Whitten, comb. nov.**

Basionym. *Aeranthus porrectus* Rchb.f., Flora 48:279, 1865.

Synonyms. *Campylocentrum porrectum* (Rchb.f.) Rolfe, The Orchid Review 11:247, 1903; *Harrisella porrecta* (Rchb.f.) Fawc. & Rendle, Journal of Botany 47:266, 1909; *Harrisella amesiana* Cogn., Symbolae Antillanae 6:687, 1910; *Harrisella uniflora* H. Dietr., Die Orchidee 33:18–19, 1982.

Distribution. El Salvador, Florida, Greater Antilles, Mexico.

***Dendrophylax sallei* (Rchb.f.) Benth. ex Rolfe, Gardeners' Chronicle, ser. 3, 4:533, 1888**

Basionym. *Aeranthus sallei* Rchb.f., Annales Botanicæ Systematicæ 6:902, 1864.

Synonyms. *Polyrrhiza sallei* (Rchb.f.) Cogn., Symbolae Antillanae 6:680, 1910; *Polyradicion sallei* (Rchb.f.) Garay, Journal of the Arnold Arboretum 50:467, 1969.

Distribution. Hispaniola.

***Dendrophylax serpentilingua* (Dod) Nir, Orchidaceae Antillanae 86, 2000**

Basionym. *Campylocentrum serpentilingua* Dod, Moscosoa 1(3):51, 1978.

Distribution. Hispaniola.

***Dendrophylax varius* (Gmel.) Urb., Repertorium Specierum Novarum Regni Vegetabilis 15:306, 1918**

Basionym. *Orchis varia* J. F. Gmel., Systema Vegetabilium, ed. 16:53, 1791.

Synonyms. *Limodorum flexuosum* Willd., Species Plantarum 4:128, 1805; *Dendrophylax hymenanthus* Rchb.f., Annales Botanicæ Systematicæ 6:903, 1864; *Aeranthus hymenanthus* (Rchb.f.) Griseb., Catalogus Plantarum Cubensium 264, 1866; *Dendrophylax flexuosus* (Willd.) Urb., Repertorium Specierum Novarum Regni Vegetabilis 15:108, 1917.

Distribution. Cuba, Hispaniola.

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