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THE LEAVES GOT IT RIGHT AGAIN: DNA PHYLOGENETICS SUPPORTS A SISTER-GROUP RELATIONSHIP BETWEEN *EURYSTYLES* AND *LANKESTERELLA* (ORCHIDACEAE: SPIRANTHINAE)

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Abstract. In spite of noticeable non-floral similarities such as their unusual epiphytic habit and vegetative morphology, *Eurystyles* and *Lankesterella* have been regarded by taxonomists who rank floral characters above all other sources of information as only distantly related. Here we assess the phylogenetic relationships of these genera, analyzing over 4500 characters of nuclear (nrITS) and plastid (*matK-trnK, trnL-trnF*) DNA sequences from 29 species/22 genera of Spiranthinae (plus appropriate outgroups); three structurally distinctive species of *Eurystyles* and two of *Lankesterella* were included. Both our parsimony and Bayesian phylogenetic analyses recovered *Eurystyles* and *Lankesterella* as sister taxa with strong internal support. The *Eurystyles/Lankesterella* clade is in turn supported as sister to the "Spiranthes clade." Our results agree with previous interpretations of a close relationship between these two genera based on their shared epiphytic habit and similar vegetative morphology, indicating that floral morphology is evolutionarily labile in these groups and thus less reliable as an indicator of phylogenetic relationship than more conservative vegetative morphology.

Resumen. A pesar de notables similitudes en características no florales, tales como su desusado hábito epífito y su morfología vegetativa, *Eurystyles* y *Lankesterella* han sido considerados como sólo distantemente relacionados entre sí por los taxónomos que valoran los atributos florales por encima de cualquier otra fuente de información. En este trabajo evaluamos las relaciones filogenéticas de estos géneros analizando más de 4500 caracteres de secuencias de ADN nuclear (nrITS) y de plástidos (*matK-trnK, trnL-trnF*) de 29 especies/22 géneros de Spiranthinae (y grupos externos apropiados); tres especies de *Eurystyles* estructuralmente distintas entre sí y dos de *Lankesterella* fueron incluidas. Tanto nuestro análisis de parsimonia como el de inferencia bayesiana recobran a *Eurystyles* y *Lankesterella* como taxones hermanos con fuerte apoyo interno. El clado *Eurystyles/Lankesterella* a su vez está apoyado como hermano del "clado Spiranthes." Nuestros resultados concuerdan con interpretaciones previas de una relación cercana entre *Eurystyles* y *Lankesterella* basadas en el hábito epífito que comparten y su similar morfología vegetativa, indicando que la morfología floral es evolutivamente lábil en estos grupos y por lo tanto menos confiable como indicador de relaciones filogenéticas que la morfología vegetativa, más conservadora.

Key words: Orchidaceae, Spiranthinae, *Eurystyles, Lankesterella*, molecular phylogeny

> "It is ironic that within a family known for its spectacular flowers and specialized floral morphology, vegetative characters are quickly gaining recognition as a better indicator of phylogenetic relationships for particular groups."
>
> Kenneth M. Cameron, *American Journal of Botany* 92: 1025-1032 (2005).

The genera *Eurystyles* Wawra and *Lankesterella* Ames share several attributes that make them unique in subtribe Spiranthinae. The most obvious of these is their consistently epiphytic habit (Fig. 1A-B, 2A-B), in contrast with the ubiquitous terrestrial habit displayed by the nearly 40 other genera currently recognized in the subtribe (Schlechter, 1920; Dressler, 1981, 1993; Balogh, 1982; Garay, 1982; Burns-Balogh *et al.*, 1985; Szlachetko, 1992; Salazar, 2003, 2005; Salazar *et al.,* 2003). Furthermore, plants in both of these genera consist of small rosettes of lustrous, usually ciliate leaves that persist during several growth seasons, *i.e.* they are not deciduous as in other Spiranthinae, and their roots are fasciculate but comparatively slender instead of tuberous. Indeed, *Eurystyles* and *Lankesterella* are vegetatively so similar to one another that, in the absence of inflorescences, it is not always easy to identify to which genus a particular plant belongs (Johnson, 2001; Soto, 1993).

In contrast to their vegetative similarity, these two genera show striking differences in their reproductive structures. On the one hand, in *Eurystyles* the raceme is condensed (thus appearing capitate) and the numerous flowers are densely arranged in a spiral (Fig. 1B-D), whereas in *Lankesterella* the inflorescence consists of a lax, one-sided raceme bearing a few (usually 1-4) flowers (Fig. 2B, C). On the other hand, flowers of the two genera also differ in various structural details. In *Eurystyles* the bases of the sepals are inflated but do not form a distinct, retrorse spur. The base of the labellum usually is clawed and bears a retrorse, fleshy lobule at each side above the claw (except in *Eurystyles* subgenus *Pseudoëurystyles* (Hoehne) Szlach., in which the lip is sessile and lacks basal lobules). The gynostemium is free and elongated, and the rostellum, when present, usually forms a shallowly notched, membranaceous rostellum remnant upon removal of the pollinarium (Fig. 1E-H). In contrast, flowers of *Lankesterella* bear a retrorse spur, sessile labellum with marginal thickenings near the base, abbreviated gynostemium, and hard, pointed rostellum remnant (Fig. 2C-F).

The noticeable similarity in habit and vegetative structure between *Eurystyles* and *Lankesterella* led Dressler (1981), Soto (1993), and Salazar (2003, 2005) to advocate a close relationship between these two genera. Nevertheless, taxonomists who have preferred to classify orchids on the basis of floral characters to the exclusion of virtually all other biological evidence have disregarded such non-floral resemblance and instead considered these genera as distantly related, placing them in different generic alliances (Schlechter, 1920; Balogh, 1982; Burns-Balogh *et al.,* 1985) or even distinct subtribes (Szlachetko, 1995; Szlachetko and Rutkowski, 2000; Szlachetko *et al.,* 2005).

A recently published molecular phylogenetic analysis of Spiranthinae based on sequences of nuclear ribosomal ITS DNA (nrITS; Górniak *et al.,* 2006) sustained a sistergroup relationship between *Eurystyles* and *Lankesterella*. However, that analysis included only one species each of *Eurystyles* and *Lankesterella* and was based on a single DNA region, which raised the question of whether such an "unorthodox" result (by floral taxonomist's standards) would hold true if increased numbers of taxa and characters were included in the analysis. In this study, we assess the phylogenetic position of *Eurystyles* and *Lankesterella* by conducting cladistic analyses with expanded sampling of both taxa and characters and also by analyzing nuclear (nrITS) as well as plastid (*matK-trnK* and *trnLtrnF*) DNA sequence data. Our aims are to clarify the relationships of *Eurystyles* and *Lankesterella* with one another and other members of Spiranthinae, as well as evaluate the reliability of vegetative versus floral characters as predictors of phylogenetic relationships in these genera against the background of the DNA sequence trees.

Materials and methods

Taxonomic sampling—Exemplars of three species of *Eurystyles*, two of *Lankesterella*, and 24 additional species of Spiranthinae (comprising in total 22 genera) were analyzed in this study. Representative species of all other subtribes of Cranichideae *sensu* Salazar *et al.* (2003, 2009) were used as outgroups. A list of the species analyzed with voucher information and GenBank accessions for DNA sequences is given in Table 1.

Although we sampled only three of the about 20 species of *Eurystyles*, they represent much of the reproductive structural variation displayed by the genus, which is reflected in the fact that the three species have been assigned by taxonomists to different sections, subgenera, and even different genera in the case of "*Synanthes*" [=*Eurystyles*] *borealis* (Heller) Burns-Bal., Robinson & Foster (Burns-Balogh *et al.,* 1985; Szlachetko, 1992). As for *Lankesterella*, the genus encompasses six to eight species (Garay, 1982; Szlachetko *et al.,* 2005), all of which seem to be relatively uniform in most vegetative and reproductive morphological attributes.

Molecular methods—DNA extraction, gene amplification, and sequencing were carried out using standard protocols explained in Salazar *et al.* (2003) and Figueroa *et al.* (2008). Bidirectional sequence reads were obtained for all the DNA regions; chromatograms

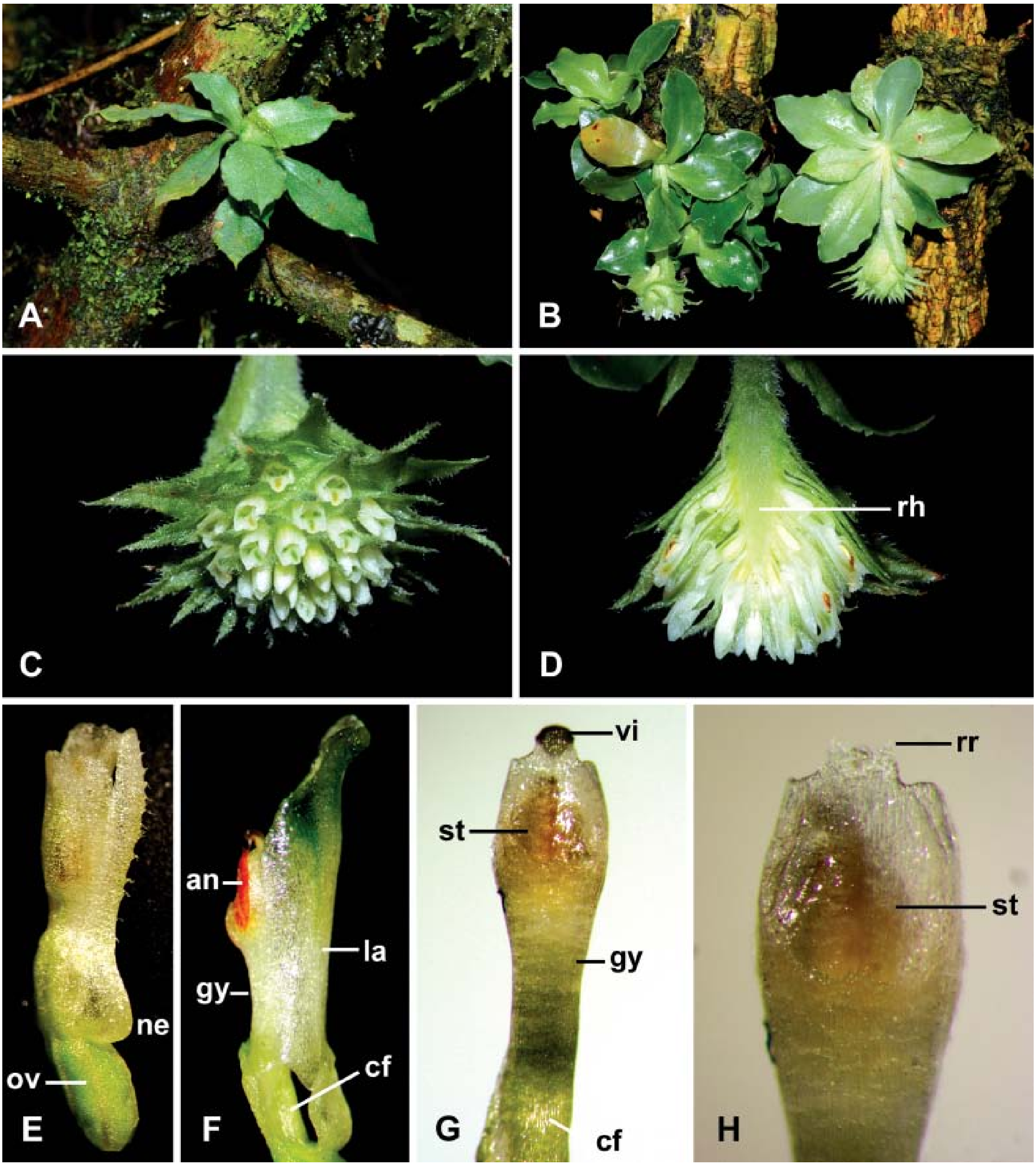

Figure 1. Morphology of *Eurystyles*. A. Plant of *Eurystyles cotyledon in situ* in a cloud forest of southern Ecuador. B. Flowering plants of *E. auriculata* (left) and *E. cotyledon* (right) in cultivation. C. Inflorescence of *E. cotyledon* from below. D. Longitudinal section of the same inflorescence. E. Individual flower of *E. cotyledon* viewed obliquely from above and the side. F. Labellum and gynostemium of previous flower after the sepals and petals were excised. G. Gynostemium of *E. cotyledon* from below. H. Apex of previous gynostemium after removal of the pollinarium, showing the broadly notched rostellum remnant. Abbreviations: an = anther; cf = column foot; gy = gynostemium; la = labellum; ne = nectary; ov = ovary; rh = rachis of the raceme; rr = rostellum remnant; st = stigma; vi = viscidium. (B left, from *Salazar 7646*; B right-H, from *Salazar 7642*). Photographs by G. A. Salazar.

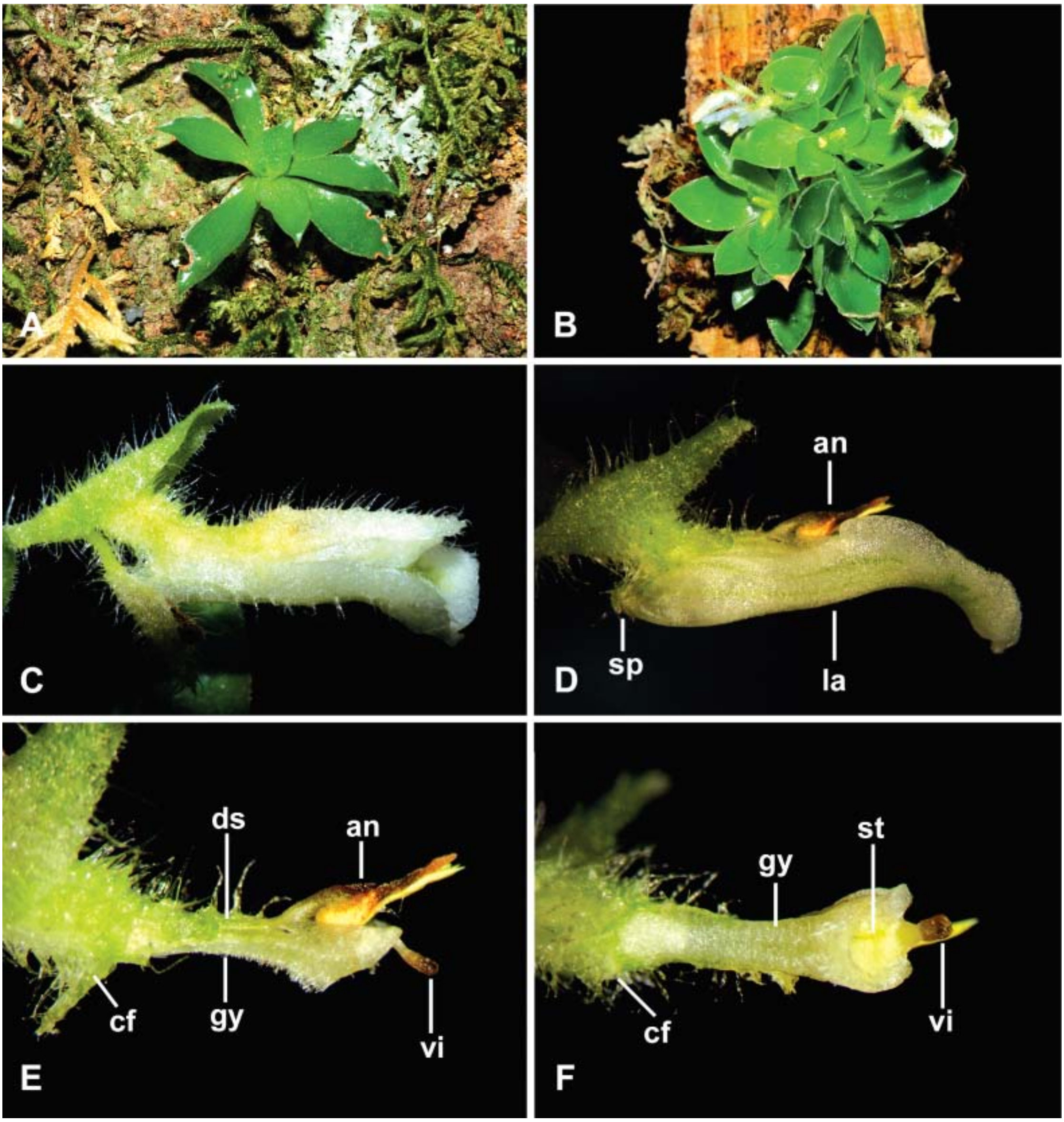

FIGURE 2. Morphology of *Lankesterella*. A. *Lankesterella gnoma in situ* in a mata atlántica of southeastern Brazil. B. Flowering plant of *Lankesterella ceracifolia* in cultivation. C. Close-up of a single-flowered inflorescence of the same plant. D. Labellum and column of previous flower after the sepals and petals were excised. E. Gynostemium from the side. F. Gynostemium from below. Abbreviations: an = anther; cf = column foot; ds = base of dorsal sepal (or ovary apex?) adnate to gynostemium; gy = gynostemium; la = labellum; sp = spur; st = stigma; vi = viscidium. (B-F from *Salazar 7535*). Photographs by G. A. Salazar.

were edited and assembled with Sequencher version 4.8 (GeneCodes Corp.). Alignment of the sequences was achieved by visual inspection in order to maximize sequence similarity (Simmons, 2004). No data were excluded from the analyses due to unambiguous alignment or for other reason, but individual gap positions were treated as missing data.

Cladistic analyses—From our previous molecular phylogenetic studies of Spiranthinae and other

TABLE 1. Taxa studied, voucher information, and GenBank accessions.

Taxon	Voucher	Gen Bank Accession		
		trnL-F	*matK-trnK*	ITS
Subtribe Achlydosinae M.A.Clem. & D.L.Jones				
Achlydosa glandulosa (Schltr.) M.A.Clem. & D.L.Jones	New Caledonia, *Clements D-285*, CANB	AJ544506	AJ543950	AJ539525
Subtribe Chloraeinae Rchb.f.				
Chloraea magellanica Hook.f.	Chile, *Ryan 1*, K (spirit)	AJ544504	AJ543948	AJ539523
Gavilea lutea (Pers.) M.N.Correa	Chile, *Ryan 3*, K (spirit)	AJ544505	AJ543949	AJ539524
Subtribe Cranichidinae Lindl.				
Aa colombiana Schltr.	Colombia, *Aldana 2*, ANDES	AM412731	AM900802	AM419766
Altensteinia fimbriata Kunth	Ecuador, *Salazar 6789*, MEXU (spirit)	AM412737	AM900801	AM419765
Cranichis engelii Rchb.f.	Ecuador, *Schott s.n.*, K (spirit)	AM412721	AM900814	AM419779
Galeoglossum thysanochilum (B.L.Rob. & Greenm.) Salazar	Mexico, *Tenorio 17900*, MEXU	AM412725	AM900810	AM419775
Gomphichis caucana Schltr.	Colombia, *Díaz 159*, ANDES	AM412736	AM900805	AM419770
Ponthieva racemosa (Walt.) C.Mohr	Mexico, *Salazar 6049*, MEXU	AJ544490	AJ543936	AJ539508
Porphyrostachys pilifera Rchb.f.	Peru, *Whalley s.n.*, K (photograph)	AJ544496	AJ543942	AJ539514
Prescottia plantaginea Lindl.	Brazil, *Salazar 6350*, K (spirit)	AJ544493	AJ543939	AJ539511
Pterichis habenarioides Schltr.	Colombia, *Aldana 12*, COL	AJ544491	AJ543937	AJ539509
Stenoptera ecuadorana Dodson & C.Vargas	Ecuador, *Salazar 6357*, K (spirit)	AJ544494	AJ543940	AJ539512
Subtribe Galeottiellinae Salazar & M.W.Chase				
Galeottiella sarcoglossa (A.Rich. & Galeotti) Schltr.	Mexico, *Jiménez 2334*, AMO	AJ544500	AJ543945	AJ539518
Subtribe Goodyerinae Klotzsch				
Ludisia discolor (Ker-Gawl.) A.Rich.	Tropical Asia (cultivated specimen), *Salazar 6354*, K (spirit)	AJ544466	AJ543911	AJ539483
Pachyplectron arifolium Schltr.	New Caledonia, *Chase 529*, K	AJ544503	AJ310051	AJ539522
Subtribe Manniellinae Schltr.				
Manniella cypripedioides Salazar, T.Franke, Zapfack & Benkeen	Cameroon, *Salazar et al. 6323*, YA	AJ544498	AJ543943	AJ539516

Taxon	Voucher	Gen Bank Accession		
		trnL-F	*matK-trnK*	ITS
Subtribe Pterostylidinae Pfitz.				
Pterostylis curta R.Br.	Australia, *Chase 572*, K	AJ544507	AJ543951	AJ539526
Subtribe Spiranthinae Lindl.				
Aulosepalum tenuiflorum (Greenm.) Garay	Mexico, *Salazar 6017*, MEXU	–	AJ543919	–
Aulosepalum tenuiflorum (Greenm.) Garay	Mexico, *Salazar et al. 6150*, MEXU	AJ544474	–	AJ539591
Beloglottis costaricensis (Rchb.f.) Schltr.	Mexico, *Soto 8129*, MEXU	AJ544475	AJ543920	AJ539492
Coccineorchis cernua (Lindl.) Garay	Panama, *Salazar et al. 6249*, MEXU (spirit)	AJ544485	AJ543930	AJ539502
Cyclopogon epiphyticum (Dodson) Dodson	Ecuador, *Salazar 6355*, K	AJ544482	AJ543927	AJ539499
Deiregyne diaphana (Lindl.) Garay	Mexico, *Salazar et al. 6172*, MEXU	AJ544467	AJ543912	AJ539484
Dichromanthus aurantiacus (La Llave & Lex.) Salazar & Soto Arenas	Mexico, *Salazar 6351*, K (spirit)	AJ544468	AJ543913	AJ539485
Dichromanthus cinnabarinus (La Llave & Lex.) Garay	Mexico, *Linares 4469*, MEXU	AJ544469	AJ543914	AJ539486
Eltroplectris calcarata (Sw.) Garay & H.R.Sweet	Brazil, *Soares s.n.*, K (photograph)	AJ519452	AJ519450	AJ519448
Eurystyles auriculata Schltr.	Costa Rica, *Dressler s.n.*, MEXU (spirit)	FN556166	FN556171	FN556161
Eurystyles borealis A.H.Heller	Mexico, *Soto 9149*, AMO	AJ544480	AJ543925	AJ539497
Eurystyles cornu-bovis Szlach.	Panama, *Maduro 230*, FLAS	FN556167	FN556172	FN556162
Funkiella hyemalis (A.Rich. & Galeotti) Schltr.	Mexico, *Salazar et al. 6128*, MEXU	AJ544478	AJ543923	AJ539495
Hapalorchis lineatus (Lindl.) Schltr.	Puerto Rico, *Ackerman 3648*, UPRRP	FN556170	FN556175	FN556165
Lankesterella ceracifolia (Barb.Rodr.) Ames	Argentina, *Salazar 7535*, MEXU (spirit)	FN556169	FN556174	FN556164
Lankesterella gnoma (Kraenzl.) Hoehne	Brazil, *Warren s.n.*, K (spirit)	FN556168	FN556173	FN556163
Mesadenella petenensis (Standl. & L.O.Williams) Garay	Mexico, *Salazar 6069*, MEXU	AJ544486	AJ543931	AJ539503
Mesadenus lucayanus (Britt.) Schltr.	Mexico, *Salazar 6043*, MEXU	AJ544471	AJ543916	AJ539488

Taxon	Voucher	Gen Bank Accession		
		trnL-F	*matK-trnK*	ITS
Subtribe Spiranthinae Lindl.				
Microthelys minutiflora (A.Rich. & Galeotti) Garay	Mexico, *Salazar et al. 6129*, MEXU	AJ544477	AJ543922	AJ539494
Odontorrhynchus variablis Garay	Chile, *Wallace 130/85*, CANB	AJ544481	AJ543926	AJ539498
Pelexia adnata (Sw.) Poit. ex Spreng.	Mexico, *Salazar 6012*, MEXU	AJ544484	AJ543929	AJ539501
Sacoila lanceolata (Aubl.) Garay	Brazil, *Da Silva 874*, MG	AJ544529	AJ543933	–
Sacoila lanceolata (Aubl.) Garay	Panama, *Förther 2545*, M	–	–	AJ539504
Sarcoglottis acaulis (J.E.Sm.) Schltr.	Trinidad, *Salazar 6356*, K (spirit)	AJ544483	AJ543928	AJ539500
Schiedeella faucisanguinea (Dod) Burns-Bal.	Mexico, *Jiménez s.n.*, AMO	AJ544479	AJ543924	AJ539496
Schiedeella llaveana (Lindl.) Schltr.	Mexico, *Salazar 6073*, MEXU	AJ544470	–	–
Schiedeella llaveana (Lindl.) Schltr.	Mexico, *Salazar 6105*, MEXU	–	AJ543915	AJ539487
Spiranthes cernua (L.) Rich.	USA, *Nickrent 4188*, MEXU	AJ544472	AJ543916	AJ539489
Spiranthes spiralis (L.) Cheval.	United Kingdom, *Bateman s.n.*, K (spirit)	AJ544473	AJ543918	AJ539490
Stenorrhynchos glicensteinii Christenson	Mexico, *Salazar 6090*, MEXU	AJ544487	AJ543532	AJ539505
Svenkoeltzia congestiflora (L.O.Williams) Burns-Bal.	Mexico, *Salazar 6143*, MEXU	AJ544476	AJ543921	AJ539493

Cranichideae (Salazar *et al.,* 2003, 2009; Figueroa *et al.,* 2008), it has become evident that combined analyses of the DNA sequence data that we are analyzing here increases resolution and internal clade support as compared with the separate analyses. Therefore, our approach here was to analyze all data sets in combination. The combined matrix was thus analyzed by two different phylogenetic methods: maximum parsimony and Bayesian inference. The parsimony analysis was conducted with the computer program PAUP* version 4.02b for Macintosh (Swofford, 2002), and consisted of a heuristic search with 1000 replicates of random taxon addition for the starting trees and tree rearrangements using tree bisection-reconnection ("TBR") branch-swapping; the option "MULTREES" was activated (to allow for storage in memory of multiple trees), and all most-parsimonious trees (MPTs) were saved. All characters were treated as unordered and had equal weights (Fitch, 1971). Internal support for clades was assessed by bootstrapping (Felsenstein, 1985), for which 300 bootstrap replicates were performed, each with 20 replicates with random taxon addition and TBR branch-swapping, keeping up to 20 most-parsimonious trees from each addition replicate. The Bayesian analysis was carried out using the program MrBayes version 3.1.2 for Macintosh (Ronquist *et al.,* 2005). The best-fitting models of nucleotide substitution for five character partitions (nrITS region, *matK* pseudogene, *trnK* intron, *trnL* intron, and *trnL-trnF* intergenic spacer) were selected using the Akaike Information Criterion (Akaike, 1974) with the program Modeltest 3.7 (Posada and Crandall, 1998). In all instances, a six-parameter model with among-site rate heterogeneity modeled according to a gamma distribution and a proportion of invariant characters was selected, except that for the *trnK* intron there were no invariant characters. Thus, two character partitions were declared in MrBayes, one including the *trnK* intron and another encompassing all the other sequence data, specifying the appropriate models. All model parameters were unlinked among the five character partitions, allowing each group of characters to have its own set of parameters (Ronquist *et al.,* 2005). Two simultaneous analyses were run for 1,000,000 generations, sampling from the trees every hundredth generation under the default conditions of MrBayes for the Markov chains. The first 250,000 generations (2500 trees) of each run were discarded as the burn-in. Inferences about relationships and posterior probabilities of clades (PP) were based on a majority-rule summary tree constructed by pooling the remaining 15,000 trees.

Results

Parsimony analysis — The concatenated data set consisted of 4549 characters, 1066 (23%) of which were potentially parsimony-informative. The heuristic search recovered six most parsimonious trees with a length of 4381 steps, consistency index (excluding uninformative characters) of 0.45, and retention index of 0.64. The six cladograms differed only in the resolution among species of *Eurystyles* and in whether or not the *Stenorrhynchos* and *Pelexia* clades (see below) are sisters with one another. However, none of the alternative resolutions received bootstrap support [BS] greater than 50%. One of the six trees is shown in Figure 3A. The same tree, which is topologically identical to the Bayesian consensus and on which bootstrap percentages and posterior probabilities (from the Bayesian analysis; see below) were included for the pertinent clades, is depicted in Figure 3B. Four major clades were recovered within strongly supported Spiranthinae (BP 100; Fig. 3A, B): 1) the *Stenorrhynchos* clade (*Stenorrhynchos glicensteinii* through *Sacoila lanceolata*; BP 98%); 2) the *Pelexia* clade (*Coccineorchis cernua* through *Pelexia adnata*; BP 80%); 3) a strongly supported clade consisting of *Eurystyles* and *Lankesterella* (BP 100%); and 4) a strongly supported *Spiranthes* clade (*Hapalorchis lineatus* through *Dichromanthus cinnabarinus*; BP 86%). *Eurystyles* and *Lankesterella* are in turn moderately supported as collective sisters to the *Spiranthes* clade.

Bayesian analysis — The majority-rule consensus calculated from 15,000 trees from the Bayesian analysis was fully resolved and topologically identical to the parsimony tree of Figure 3A. Posterior probabilities (PP) of clades are displayed in Figure 3B. Like parsimony, the Bayesian analysis recovered a strongly supported clade consisting of *Eurystyles* and *Lankesterella* (PP 1.00).

Discussion

Our parsimony and Bayesian analyses recovered the same phylogenetic patterns, both supporting *Eurystyles* and *Lankesterella* as forming a strongly supported clade. These congruent results are significant, given the disproportionately long branches subtending *Eurystyles* and *Lankesterella* (in the parsimony tree portrayed Figure 3A, the branch leading to *Eurystyles* is 124 steps long, whereas that of *Lankesterella* is 116 steps long). It has been proposed that parsimony may be inconsistent as a method of phylogeny reconstruction when different groups within a lineage evolve at dissimilar rates (Felsenstein, 1978, 2004; Hendy & Penny, 1989) because of an analytical artefact known as "long-branch attraction," which occurs when long-branched lineages that are not each other's closest relatives are grouped because of similarities due to independent substitutions to the same base from different ancestral bases (e.g. Lewis, 1998; Sanderson *et al.,* 2000; Anderson & Swofford, 2004; Bergsten, 2005). Methods based on stochastic models of nucleotide substitution such as maximum likelihood and Bayesian inference, however, have been shown to be less sensitive to such conditions (see Swofford *et al.,* 1996; Lewis, 1998; Felsenstein, 2004; Bergsten, 2005). In these analyses, both parsimony and Bayesian inference recovered the same tree topology, indicating that the strongly supported placement of *Eurystyles* and *Lankesterella* as sister groups is not an artefact of long branches misleading parsimony.

Our results are in full agreement with the remarkable similarities in habit and vegetative structure between *Eurystyles* and *Lankesterella*, in spite of their disparity in reproductive attributes. Such disparity might actually have been overstated, and

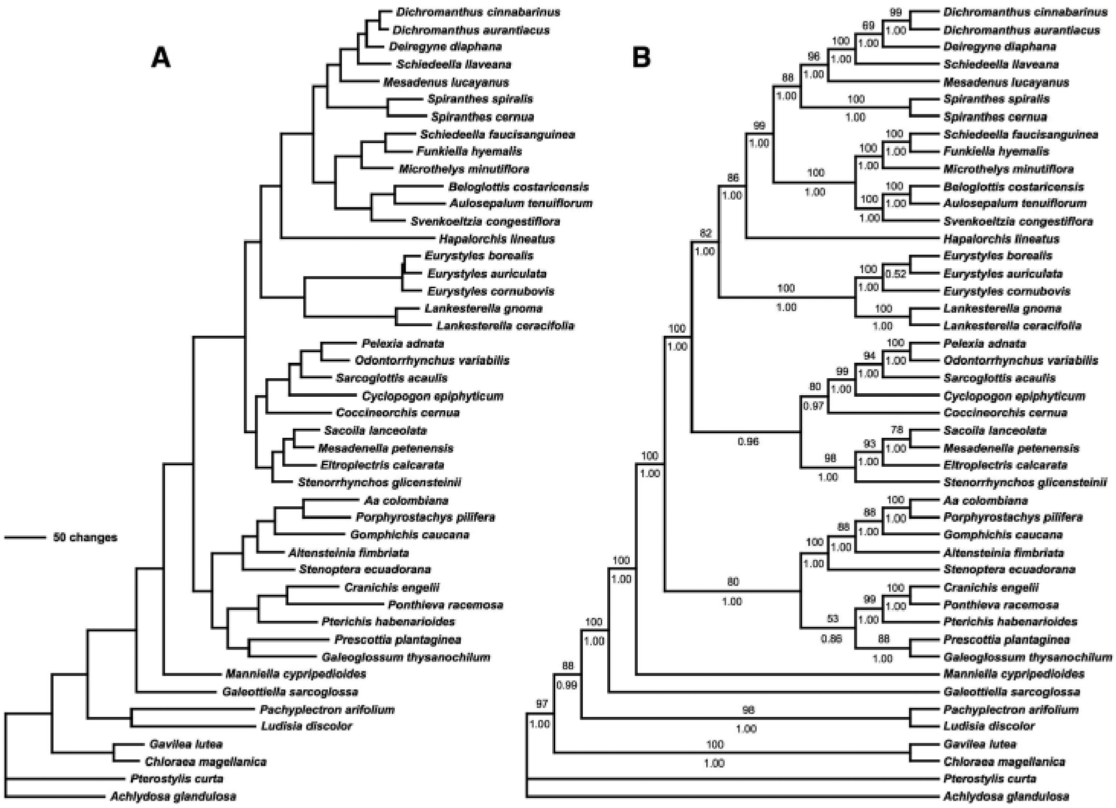

Figure 3. Phylogenetic relationships of *Eurystyles*, *Lankesterella*, and other Spiranthinae inferred from combined nuclear ITS and plastid *trnK-matK* and *trnLtrnF* DNA sequences. A. One of the six shortest trees recovered by the parsimony analysis, with branch lengths drawn proportional to the number of character changes supporting them; arrows point to clades that collapse in the strict consensus. B. Same parsimony tree as in A, which was topologically identical to the Bayesian summary tree, and on which bootstrap percentages (numbers above branches) and posterior probabilities from the Bayesian analysis (numbers below branches) are superimposed (see text).

upon closer scrutiny the morphological gap between these genera appears not as large as it would appear at first glance. For instance, and quoting Szlachetko (1992), labellum structure between *Eurystyles* subgen. *Pseudoëurystyles* and *Lankesterella* "is almost identical." Overall flower structure is much the same in both genera, and although species of *Eurystyles* lack the pointed retrorse spur, the base of their floral tube always is distinctly inflated (compare Fig. 1E and 2D).

Gynostemium morphology between these genera as described in the literature seems to be dissimilar, with the gynostemium of *Lankesterella* often being interpreted as relatively short, with an "obscure to short" column part (Szlachetko & Rutkowski, 2000; Szlachetko *et al.,* 2005) and a prominent column foot. However, in the fresh material of *Eurystyles* and *Lankesterella* that we have examined the gynostemia look similar, with the main difference being that, in *Lankesterella*, the basal portion of the dorsal sepal or the apex of the ovary (or both) is adnate to the back of the gynostemium (indicated in Figure 2E as "ds"). Were it not for this adnation, the proportions of column part to column foot of both genera would look even more similar.

The most distinctive feature separating the two genera is the rostellum, which after removal of the pollinarium in *Lankesterella* usually leaves a hard narrow point that is absent in *Eurystyles*. However, *Eurystyles* shows substantial structural variation (see Szlachetko, 1992; Szlachetko & Rutkowski, 2000), and both *Eurystyles* and *Lankesterella* are known for their frequent, seemingly autogamous forms, in which rostella and viscidia are reduced, non-functional or missing altogether (Szlachetko, 1992; Salazar, 2003). These phenomena indicate that rostellum morphology in these groups is evolutionarily labile.

The abovementioned differences in reproductive structures between these two genera are almost certainly related to different pollination mechanisms. Unfortunately, nothing is known about their natural pollination besides the aforementioned recurrence of autogamy. The inflorescences of *Eurystyles auriculata* and *E. cotyledon* produce conspicuous, pleasant, diurnal odors, which might play a role in pollinator attraction. Their pendulous, dense clusters of tubular, fragrant flowers with nectar at the bottom of the floral tube may be pollinated by small insects (possibly lepidopterans) that probe many flowers in search of food. However, we have had limited opportunities to examine fresh inflorescences of *Lankesterella* in the field, and we are unable at this time to confirm whether they are also fragrant or not.

Given the unusual (in subtribe Spiranthinae) epiphytic habit of *Eurystyles* and *Lankesterella* on the one hand and their long branches in the molecular tree on the other, one has to ask whether there may be a relationship between epiphytism and an accelerated rate of molecular evolution in these genera. However, the branch of *Hapalorchis lineata* (Fig. 3A), a terrestrial species, is even longer (194 steps). Therefore, drawing conclusions in this respect is not possible at this time, but it will be interesting to address this matter as more sequences of these and other genera of the subtribe become available.

In closing, it is worth mentioning that our results mirror those of Cameron's (2005) molecular phylogenetic study of Malaxideae (from which our epigraph was taken) in showing that at least in some particular orchid groups vegetative structure might be a better predictor of phylogenetic relationships than floral attributes. In both the epidendroid tribe Malaxideae and the orchidoid subtribe Spiranthinae, vegetative features prove to be more conservative than floral details. Indeed, speciation in these groups often appears to involve the evolution of different floral features in closely allied species, with the result that the species are pollinated by different agents, or in different ways, and the species coexist without interbreeding. There may also be parallelisms in floral features between quite different groups, as in *Trichocentrum* Poepp. & Endl. and other Oncidiinae, flowers of which mimic the flowers of Malpighiaceae, with or without oil glands (see Chase *et al.,* 2009 and references included there). Thus, one might expect that floral features are more variable and vegetative features more conservative, despite unwarranted assumptions to the contrary (e.g. Szlachetko & Rutkowski, 2000). This, of course, is a generalization. There are certainly parallelisms to be found among vegetative features just as there are among floral features. When in doubt, trust neither the leaves nor the flowers, but the DNA.

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