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Molecules and morphology: congruence of data in *Columnea* (*Gesneriaceae*)

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Abstract: The cladistic analysis and comparison of molecular and morphological data has been the source of much recent debate. In this study, independent analyses of molecular and morphological data from *Columnea* L. sects. *Pentadenia* and *Stygnanthe* (*Gesneriaceae*) are compared. Comparative methods include consensus, visually comparing trees from independent analyses and combined data analysis. Consensus methods provided little resolution. Comparison of trees obtained from the independent analyses revealed some differences although the trees are highly similar. However, a combined analysis found that the level of incongruence between the two data sets was low. The tree resulting from the combined data has aspects of both the morphological and molecular trees despite the larger number of molecular characters. In addition, the combined data tree has greater resolution than either of the two data sets singly, indicating that the two types of data are congruent, and complementary to each other.

Recent advances in molecular systematics have reduced interest in morphological data as a phylogenetic tool. Frequently, morphological data are either ignored (Palmer & al. 1983, Fritzche & al. 1987, Gottlieb 1988, Duvall & Doebley 1990, Debener & al. 1990, Song & al. 1990) or explained in light of a cladogram derived from molecular characters (Sytsma & Gottlieb 1986 a, b; Doebley & al. 1987; Jansen & Palmer 1987; Sytsma & Smith 1988; Ranker & al. 1990; Bruneau & al. 1990; Soltis & al. 1990 a, b; Sytsma & al. 1990, 1991; Albert & al. 1992; Baldwin 1993). The justification used for omitting morphological data in a cladistic analysis is the high level of homoplasy traditionally associated with morphological data (Givnish & Sytsma 1992). In addition, homology is frequently difficult to interpret with morphology when careful developmental analyses are lacking. In contrast, chloroplast DNA (cpDNA) restriction site data has gained a reputation for low levels of homoplasy (Givnish & Sytsma 1992, however, see Sanderson & Donoghue 1989 for another comparison of morphological and molecular data) and characters that can be unambiguously scored as simple binary characters. Therefore the large numbers of characters that generally result from cpDNA restriction site analysis may not only provide greater resolution of phy-

logenetic relationships, but are also viewed with greater reliability (PALMER & al. 1988, CRAWFORD 1990).

However, the relative facility in scoring molecular characters does not reduce the usefulness of morphological data in a cladistic analysis. Several arguments address the problems of molecular data (BREMER & al. 1987, PATTERSON 1987, SYVANEN & al. 1989) and discuss the general utility of morphological data (HILLIS 1987, DONOGHUE & SANDERSON 1992). Molecular data is generally obtained from only a small portion of the entire genome. The analysis of this data may result in cladograms that reflect the evolutionary history of the genes, but not the organisms (ATCHLEY & FITCH 1991, DOYLE 1992). Morphological data are assumed to be controlled from many loci distributed throughout the genome and therefore problems of gene trees are avoided. Another advantage of morphological data is that it allows the inclusion of fossil data (DONOGHUE & al. 1989). With the exception of a few particular types (GOLENBERG & al. 1990, SOLTIS & al. 1991), fossils can not be included in molecular analyses.

The debate of morphology versus molecules has been reviewed in several articles and books (PATTERSON 1987, HILLIS 1987, CRACRAFT & MINDELL 1989, SYTSMA 1990, DONOGHUE & SANDERSON 1992). One of the main arguments regarding molecules vs. morphology is the relative utility of the different data sets. The utility of molecular (cpDNA restriction site data) characters in phylogenetic studies has been reviewed elsewhere (PALMER & al. 1988, CRAWFORD 1990). In general the large number of characters, relative unambiguity in scoring, and low levels of homoplasy have been promoted as reasons for preferring molecules over morphology. While the arguments for morphology have not been as concisely recorded, nearly 40 years of cladistic analysis, and hundreds of years of classification, all based entirely on morphology, have provided substantial knowledge toward our understanding of evolution. DONOGHUE & SANDERSON (1992) provide several arguments for morphology in a cladistic analysis, and emphasize the inclusion of morphological data in phylogenetic studies of molecular data.

There are obviously clear advantages to both molecular and morphological data. Both types of data have different strengths, and rather than argue for or against morphology, it should be used in conjunction with molecular data. The problem arises in how to compare the different resulting cladograms if they are not completely congruent with one another, and how to weigh one data set against the other. Several methods have been proposed to compare cladograms from different types of data (HILLIS 1987, SYTSMA 1990, DONOGHUE & SANDERSON 1992, SYTSMA & SMITH 1992). These include: 1) a consensus of all trees produced from the two data sets independently (ADAMS 1972, NELSON 1979, BREMER 1990), 2) independent data analyses using the results of one data set (either morphology or molecules) to examine the other (OLMSTEAD 1989, RANKER 1990, SMITH & SYTSMA 1990, SYTSMA 1990, BULL & al. 1993), and 3) combining the data before analysis and performing global parsimony (MIYAMOTO 1983, HILLIS 1987, CRACRAFT & MINDELL 1989, DE SA & HILLIS 1990, RANKER 1990, DONOGHUE & SANDERSON 1992, WHEELER & al. 1993, KIM & JANSEN 1994). The initial comparison of trees is important should hybridization, introgression, or matriarchal lineage sorting (NEIGEL & AVISE 1986) be suspected as a potential problem either a priori or a

posteriori (SMITH & SYTSMA 1990). This paper examines these methods with data from *Columnea* L.

The *Gesneriaceae* is a family of plants well suited for comparing trees obtained from molecular and morphological data. Patterns of morphological variation are complex and there is a high level of homoplasy in morphological characters within the family. Throughout the taxon, suites of characters are used to define groups ranging from subfamilies to intrageneric sections (BURTT 1977, WIEHLER 1983, KVIST & SKOG 1993). However, almost invariably, some species of one group possess characters that are used to define an entirely different group (BURTT 1990, CHAUTEMS 1990, BOGGAN 1990). An example of the confusing morphological variation in the *Gesneriaceae* is leaf arrangement. Most members of the family possess opposite leaves. However, in some species of *Columnea*, leaf pairs develop such that one leaf enlarges while the other becomes arrested in development (SÁNCHEZ-BURGOS & DENGLER 1988). This trait, known as anisophylly, has been used to delimit sect. *Collandra* (LEM.) HANST. of *Columnea* and was even considered of sufficient taxonomic importance to recognize this section as the genus *Dalbergaria* by WIEHLER (1973, 1983). Despite the use of anisophylly as a diagnostic character, four of the six sections of *Columnea* contain species with at least some degree of anisophylly (sects. *Columnea, Stygnanthe* HANST., *Ortholoma* BENTH., and *Collandra*) (KVIST & SKOG 1993). In fact, anisophylly can be found in many other genera throughout the *Gesneriaceae* and other families (GIVNISH 1984). Because the morphological variation in *Columnea* is complex, a cladogram based entirely on morphological characters may be less likely to reflect the evolution of the species and may instead reflect selection on certain characters. Therefore it is desirable to have additional data that may be under less direct environmental selection. Molecular data is an appropriate source of information in this instance.

Cladograms for species of *Columnea* sects. *Pentadenia* (PLANCH.) BENTH. and *Stygnanthe* are not fully resolved regardless of data set (SMITH & SYTSMA 1994 a, b). Therefore the addition of data can enhance the information obtained from the cladistic analysis. In this case the molecular data may enhance the morphological and vice versa. Phylogenetic studies based on chloroplast DNA and morphology of *Columnea* sects. *Pentadenia* and *Stygnanthe* provide an opportunity to compare empirically cladograms obtained from morphological and molecular data.

Material and methods

Data from the molecular phylogenetic studies of *Columnea* sects. *Pentadenia* and *Stygnanthe* (*Gesneriaceae*) were obtained by examining cpDNA with 42 different restriction enzymes (SMITH & SYTSMA 1994 a). This resulted in 119 restriction site mutations, scored as gains and losses that are not autapomorphic for the taxa used in these analyses. Concurrently, morphological data were obtained from 33 vegetative and reproductive characters. Twenty of the characters were scored as binary and 13 as unordered multi-state characters resulting in 73 total character-states (SMITH & SYTSMA 1994 b). *Drymonia stenophylla* (J. D. SMITH) H. E. MOORE was used as an outgroup in all analyses. See SMITH & SYTSMA (1994 a, b) for further details and descriptions of characters.

The morphological and molecular data sets were reanalyzed to include only the 27 species common to both data sets (SMITH & SYTSMA 1994 a, b). Data were analyzed with the computer package PAUP (version 3.1.1) (SWOFFORD 1991 a) using Wagner parsimony and the general heuristic option, saving minimal trees only and with the collapse zero-

length branches, tree bissection-reconnection, and ignore uninformative characters options in effect. All autapomorphies were excluded from all tree length calculations in this study. To minimize the possibility that these searches were finding only a subset of the potentially numerous islands of trees (MADDISON 1991), a random search of 1000 replicates was performed. The two data sets were then combined and a third analysis performed using the same options. Bootstrap values were determined using 100 replicates and decay indices (BREMER 1988) were determined for each of the trees as a means of demonstrating the support for individual clades.

Incongruence between the morphological and molecular data sets was determined by calculating I_{MF} (MICKEVICH & FARRIS 1981) and I_M (SWOFFORD 1991 b). Incongruence was also statistically compared using the computer program developed by FARRIS & al. (1994). This program compares the congruence of the data to the congruence in randomized distributions of the character states. The data for *Columnea* was compared with 10,000 random distributions of the data.

In addition, congruency of data was also examined by comparing the topology of the most-parsimonious trees from one analysis with the data from the two remaining sets. This was conducted by opening the tree file for each of two data sets using the data file for the third and examining the lengths of each of the trees. This was repeated for each pairwise data set combination.

Results

Chloroplast DNA topology. The molecular data yielded 332 most-parsimonious trees of 149 steps each (consistency index 0.80, retention index 0.89). A strict consensus of the 332 trees was compiled (Fig. 1) and is topologically identical to the molecular tree obtained using all data (SMITH & SYTSMA 1994 a). Thirty homoplastic steps are necessary to construct the most-parsimonious trees with the molecular data (Table 1). When morphological data and combined data are used to construct the cpDNA trees, 157 to 168, and 307 to 318 steps were required, respectively (Table 2).

Morphological topology. The morphological data resulted in 145 most-parsimonious trees of 137 steps each (consistency index of 0.42, retention index 0.62). A strict consensus of the 145 trees was compiled and is shown in Fig. 2. Sixty-four homoplastic steps are necessary to construct the most-parsimonious trees based on morphological data (Table 1). When cpDNA data and combined data are used to construct the morphological trees, 237 to 278, and 374 to 417 steps were required, respectively (Table 2).

Combined data topology. The combined data resulted in 16 most-parsimonious trees of 304 steps each (consistency index of 0.60, retention index 0.74). A strict consensus of these 16 trees is presented in Fig. 3. When the morphological and cpDNA data were used to construct the combined data trees, 147 to 152, and 151 to 156 steps were required, respectively (Table 2). The topology of this consensus tree is distinctly different from either the tree based on cpDNA restriction site mutations, or the tree derived from morphology. The consensus tree based on the combined data has aspects of both the morphological and molecular tree as well as characteristics not seen in either. Sect. *Pentadenia* is distinct from most of the species of sect. *Stygnanthe*, an aspect of the morphological tree. In contrast, *Columnea moesta* POEPP. appears as the sister species of *C. ultraviolacea* in the combined tree as it does in the molecular tree. Clade A (Figs. 1–3) is the sister group of clade B (Figs. 1–3) in the combined data set consensus tree. The position of this clade

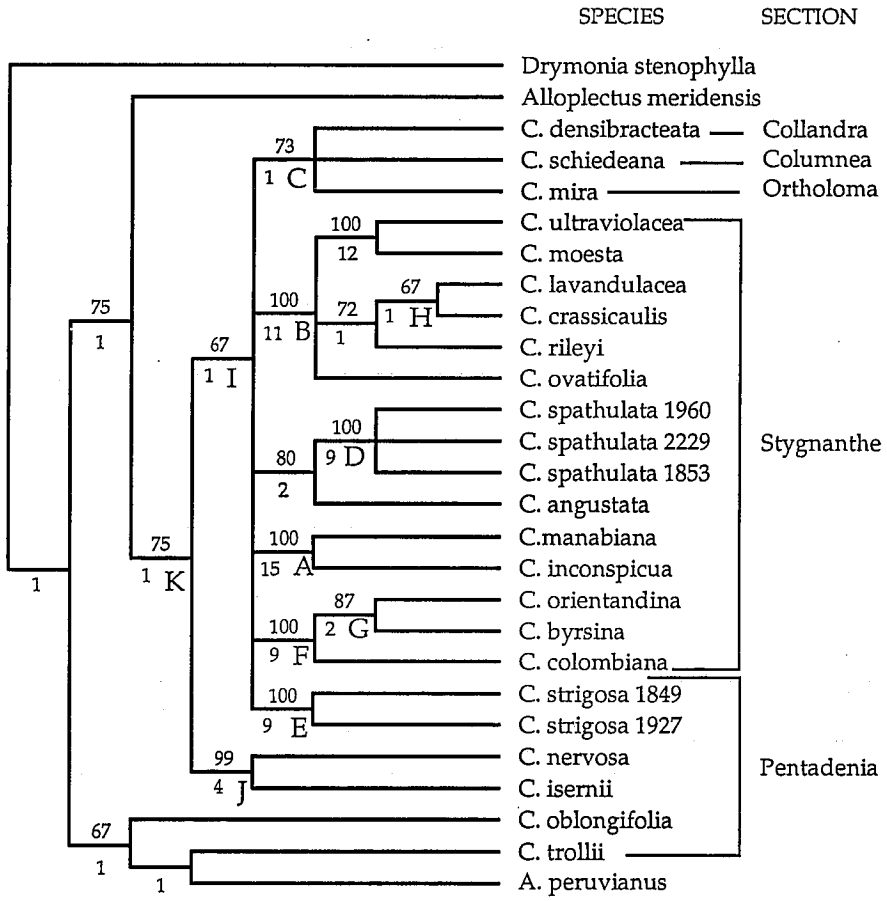

Fig. 1. Strict consensus tree of 332 most-parsimonious trees of 149 steps each (C.I. 0.80) derived from cpDNA restriction site data for *Columnea* sects. *Stygnanthe* and *Pentadenia* (*Gesneriaceae*). Numbers above branches indicate bootstrap values. Numbers below branches are the decay index values. Lettered nodes are discussed in text

Table 1. Data derived from most-parsimonious trees based on cpDNA, morphology, and a combined data set of cpDNA and morphology

Data set	Number of trees	Number of steps	Minimum steps	Extra steps
Morphology	145	137	73	64
cpDNA	332	149	119	30
Combined	16	304	192	112

in the combined data tree is not apparent in either the molecular or morphological tree.

Consensus. Strict (NELSON 1979), ADAMS (1972), and combinable (BREMER 1990) consensus trees were constructed from the 477 total trees obtained from the morphological and molecular analyses independently. None of the three consensus trees

Table 2. Comparison of tree topologies derived from one type of data to data from the remaining data sets. The full range of steps is presented although only minimal values have been used in the calculations in the text. Steps greater than the steps obtained for each parsimonious tree follow in parentheses

	Topology		
	Morphology	cpDNA	Combined
Morphology	137	157–168 (20–31)	147–152 (10–15)
cpDNA	237–278 (88–129)	149	151–156 (2–7)
Combined	374–417 (70–113)	307–318 (3–14)	304

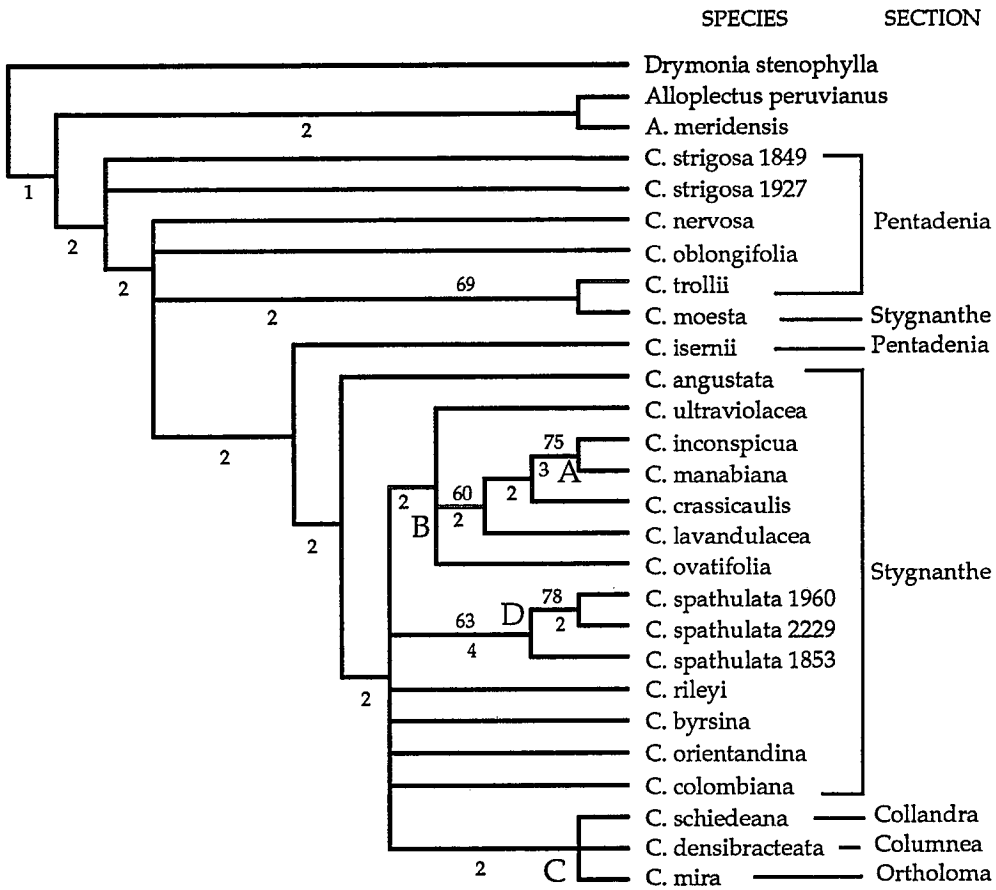

Fig. 2. Strict consensus tree of 145 most-parsimonious trees of 137 steps each (C.I. 0.42) based on morphology for *Columnea* sects. *Stygnanthe* and *Pentadenia* (*Gesneriaceae*). Numbers above branches indicate bootstrap values. Numbers below branches are the decay index values. Lettered nodes are dicussed in text

provided adequate resolution for the species involved. The strict consensus tree (NELSON 1979) and combinable consensus tree (BREMER 1990) were identical and

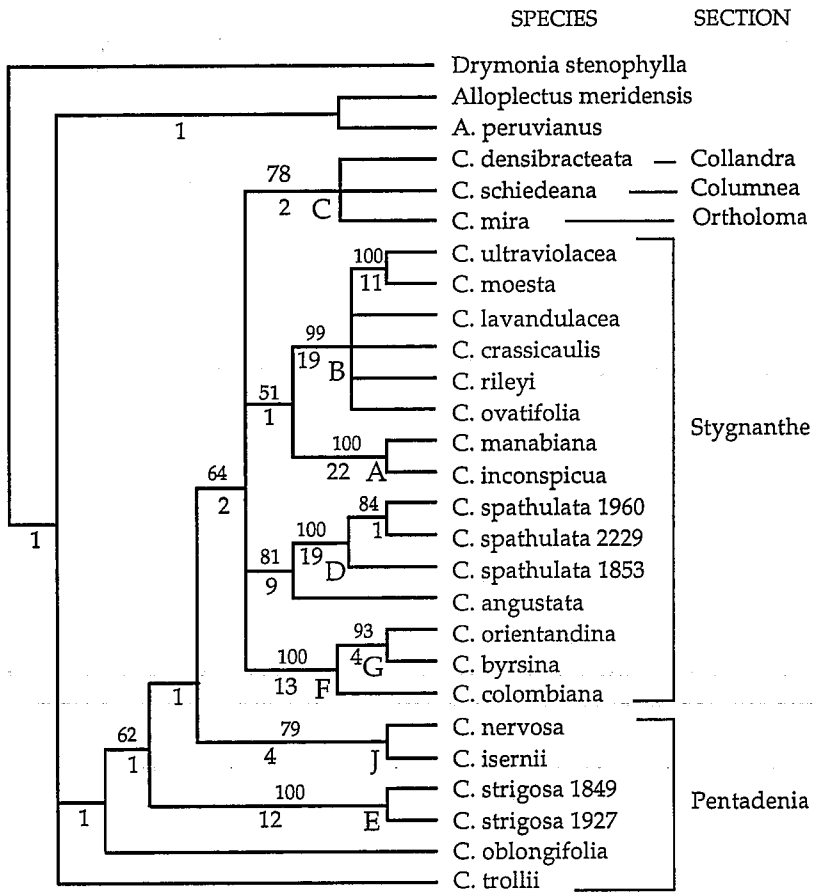

Fig. 3. Strict consensus tree of 16 most-parsimonious trees of 304 steps each (C.I. 0.60) derived from the combined data set of cpDNA and morphology for *Columnea* sects. *Stygnanthe* and *Pentadenia* (*Gesneriaceae*). Numbers above branches indicate bootstrap values. Numbers below branches are the decay index values. Lettered nodes are discussed in text

resulted in the resolution of only four clades (C, D, and A; Figs. 1, 2). The ADAMS (1972) consensus tree was slightly more resolved with 12 clades (A–K of Fig. 1, with the exception that *Columnea moesta* is not present in clade B and clades E and I are unresolved sister groups to clade J). This consensus method most closely approximates the results of the combined analysis (Fig. 3). However, as suggested by BARRETT & al. (1991), the placement of some taxa (most notably *C. moesta*) in the ADAMS (1972) consensus is not consistent with any of the original trees.

Congruency. Discrepancy between the data sets was determined by comparing the data sets according to MICKEVICH & FARRIS (1981) and SWOFFORD (1991 b). Incongruency (I_{MF}) according to MICKEVICH & FARRIS (1981) is calculated by subtracting the total homoplastic steps produced from the data sets independently (64 + 30) from the homoplastic steps produced in the combined analysis (112 − 94 = 18) and dividing by the number of homoplastic steps produced in the combined analysis (18/112 = 16.1%). Because the I_{MF} value may underestimate

incongruence among data sets, especially when the number of characters in the data sets are unequal, an alternative measure of incongruence was calculated. This measure is the I_M index of SWOFFORD (1991 b). To calculate I_M, the minimum number of extra steps generated by mapping each data set onto the trees computed for the other data sets was used (Table 2). The homoplastic steps produced from each of the data sets independently (94) was subtracted from the total number of additional steps (20 + 88 = 108) for morphological and cpDNA data based on cpDNA and morphological trees, respectively (108 − 94 = 14) and divided by the total number of additional steps based on mapping data sets onto other trees (14/108 = 13.0%).

In addition, comparisons of congruency were made by comparing the *Columnea* data to 10,000 random distributions of the data set (FARRIS & al. 1994). This resulted in an α value of 1. This high value indicates that the two data sets are not significantly incongruent.

Discussion

Consensus. Theoretical and practical problems exist for all methods of comparing cladograms. A consensus of all trees derived from different types of data seems the most logical and conservative. However, it is possible that such a consensus tree will not only be poorly resolved but potentially may yield relationships not found in any of the fundamental trees (CRACRAFT & MINDELL 1989, BARRETT & al. 1991). Strict (NELSON 1979), ADAMS (1972), and combinable (BREMER 1990) consensus trees of the 477 trees from the separate analyses of morphology and cpDNA provided very little resolution (see results). The lack of resolution in the consensus trees for these data is largely due to a few taxa whose positions differ strongly between the two sets of trees, and several taxa whose positions differ only slightly. As a result, no consensus between the two data sets adequately resolves relationships among these species.

Another type of consensus tree is a composite consensus where different parts of trees are chosen to construct yet another tree. Composite trees (HILLIS 1987, OLMSTEAD 1989) do not consider the potential effects the characters have on each other. The composite tree may be well resolved, but is neither a reflection of a topology derived from global parsimony of all data, nor a rigorous consensus. HILLIS (1987) provides an example in which different data sets produce trees that are not in conflict, but lack resolution in different branches. The composite of all three data sets results in a tree that is completely resolved. HILLIS (1987) suggests that not all data sets will work as well as this example, especially when the resulting trees from different types of data are in conflict. A composite tree of the two strict consensus trees obtained from morphology and cpDNA (Figs. 1, 2) would be impossible to construct without high subjectivity. There are only a few instances where resolution not common to both trees already is not in conflict with resolution of the other (F, and G of Fig. 1).

Comparison. Comparison of trees derived from different data sets independently is a more conservative approach for comparing data sets. Comparison of trees is necessary if the data are incongruent because of specific biological problems such as introgression (SMITH & SYTSMA 1990, BULL & al. 1993). However, comparisons may fail to produce a single evolutionary history if the resulting trees are at all

incongruent. Comparison of the strict consensus trees obtained from the separate analysis of morphology and cpDNA reveals some clades common to both cladograms but fails to resolve the difference between the two trees when clades are in conflict.

The topology of the morphological and molecular trees generally are congruent with the exception of minor differences in the position of some of the species in sect. *Stygnanthe*. One major difference is the position of the Bolivian species *Columnea moesta*. In the molecular tree, *C. moesta* is the sister species to another Bolivian species, *Columnea ultraviolacea* J. F. SMITH & L. SKOG, and is deeply embedded in the clade leading to *C. ultraviolacea*. Its position in the morphological tree is strikingly different. It is not only distinct from *C. ultraviolacea*, but it is also removed from the clade containing *C. ultraviolacea* and the clade marking the majority of species in sect. *Stygnanthe*. The possibility that this species is a hybrid between *C. ultraviolacea* and *C. trollii* MANSF., another Bolivian species, is eliminated by examination of the nuclear ribosomal DNA (rDNA) (SMITH 1991, SMITH & SYTSMA 1994 a). Although restriction site mutations in the rDNA were few for this study, there are sufficient mutations to group *C. moesta* with the taxa that it is allied with in the cpDNA tree. Likewise, there is no sign of morphological intermediacy between these two species. Therefore, the possibility that the accession of *C. moesta* used in this study is a hybrid (at least recent), is extremely unlikely.

Another apparent discrepancy between the morphological and molecular based trees is the position of the two *Alloplectus* species used in this analysis (Figs. 1, 2). Although these taxa undoubtedly have different placements in the two trees and contribute to measures of incongruency, the differences are more likely a reflection of inadequate sampling within the genus rather than a reflection of any phylogenetic relationships within *Alloplectus*, or between *Alloplectus* and *Columnea*.

Combined. Data that are in conflict can also pose problems for combining data. Conflicting data can potentially yield an unresolved tree if the conflicts are serious enough (BULL & al. 1993). Therefore it is essential to compare trees from the different analyses separately first and then decide to what degree the data sets are congruent before combining.

Another potential problem with combining data sets is that the generally larger number of molecular characters may outweigh the generally fewer characters in the morphological data (KLUGE 1983, MIYAMOTO 1985). Weighting schemes (MIYAMOTO 1985), and transformation series (DOYLE 1992) have been proposed to eliminate this potential effect, but have no objective defense (HILLIS 1987). A more important factor is the distribution of character-state changes throughout the cladogram (DONOGHUE & SANDERSON 1992). The distribution patterns of molecular and morphological characters differ between the two data sets in *Columnea*. The majority of molecular mutations are in the terminal clades, whereas the morphological character-state changes are more evenly distributed (SMITH & SYTSMA 1994 a, b). Therefore, the larger number of molecular characters do not override the morphological characters when combined.

Lastly, combining data may be impractical depending on the ultimate use of the cladogram. If the purpose of the cladogram is to examine the origin of morphological traits, it is best to remove the specific characters to be examined from the analysis, or defer combining data.

This study illustrates that molecular and morphological data sets can be complementary (WHEELER & al. 1993). The most probable reason the combined data work well for *Columnea* is the different distribution of characters on each tree. The majority of molecular characters are clustered at the terminal clades where they strongly support these clades (SMITH & SYTSMA 1994 a). In these areas the molecular characters undoubtedly outweigh the effects of morphological characters as repositioning these taxa would require anywhere from 4 to 23 extra steps. However, there is very weak support in the molecular tree for clades below this level (SMITH & SYTSMA 1994 a). The morphological data, on the other hand, has a more even distribution of character-state changes (SMITH & SYTSMA 1994 b). Therefore, as some of the morphological characters were forced to be homoplastic in the clades strongly supported by the cpDNA data, parsimony would minimize further homoplasies. Thus, morphological data outweighs the molecular characters in those parts of the tree where few (1–5) molecular characters support clades. The molecular data strengthens weakly supported clades based on morphological data (the terminal clades in *Columnea* cladograms) and the morphological data strengthens weak clades based on molecular data (the basal branches in *Columnea* cladograms).

Congruency of data in *Columnea*. Before the data from the molecular and morphological data sets are combined, the degree of incongruency between the two data sets must be determined (BULL & al. 1993). The discrepancy between the two data sets for *Columnea* appears high (I_{MF} = 16.1%, I_M = 13.0%), but is comparable to previous analyses where incongruency was determined to be low. Performing a similar test, MICKEVICH & FARRIS (1981) found 5.0% (I_{MF}) incongruence between allozymes and morphology in *Menidia*. With the comparison performed as in this analysis, KLUGE (1989) found 11.4% (I_{MF}) incongruence in *Epicrates*, and KIM & JANSEN (1994) found 13.3% (I_{MF}) and 22.5% (I_M).

FARRIS & al. (1994) have developed a computer program that compares the congruency found in the data to congruency found in trees based on a random distribution of the character states. With adequate replication this can be calculated as a statistical test. The data for *Columnea* showed no incongruence (α = 1) with 10 000 replicates. This does not mean that incongruence is lacking, but that it is not detectable within the limits of the test, and that the two data sets are not significantly different.

In addition to congruency, the three data sets were compared by calculating the number of steps required to construct the topology of the most-parsimonious trees for each analysis using the other two data sets. The results show that a morphological or molecular tree will require a minimum increase of 20 and 88 steps, respectively, when the other data set is used to construct the same topology (Table 2). However, the topology resulting from the combined data requires only three and two additional steps when the morphological and molecular characters are used, respectively. This indicates that the combined data tree does not deviate tremendously from either the morphology or cpDNA tree and that the data are not incongruent to a large degree.

Source of incongruence. The cpDNA and morphology data sets for *Columnea* are somewhat in conflict (I_{MF} = 16.1%, I_M = 13.0%), and the discrepancy between the data sets is real, as is demonstrated by the difference in position for some of

the taxa (e.g., *C. moesta*). However, the tree resulting from the combined data retains aspects of both the molecular and morphological data (Fig. 3).

Despite the debate over molecules versus morphology, most reviews conclude that there is seldom a difference between the two (PATTERSON 1987, SYTSMA 1990, DONOGHUE & SANDERSON 1992). Discrepancies that do exist between data sets have been attributed to either procedural or biological problems (SYTSMA 1990, BULL & al. 1993).

Procedural errors result when cladograms based on phenetic analyses are compared with those based on cladistic analyses, or when inappropriate data is used for the level of analysis. For *Columnea*, the analysis used for the morphological data was identical to that used for the molecular data, suggesting that this can be eliminated as a potential problem in this study.

The potential for a procedural error resulting from poor character choice is also unlikely. The characters used for both morphological and molecular analyses (SMITH & SYTSMA 1994 a, b) are generally regarded as suitable for phylogenetic analysis. Similar characters have been used with success in numerous other studies employing either molecular or morphological characters at the species level. For example, restriction site mutations have proven highly successful for phylogenetic analysis at the species level (e.g., SYTSMA & SCHAAL 1985, SMITH 1988, BALDWIN & al. 1990, DOYLE & al. 1990, MORGAN & SIMPSON 1992, BRUNEAU & DOYLE 1993, MORGAN 1993). Although the exact morphological characters useful for a particular group depend on the degree of morphological variation, the characters should be homologous, minimally susceptible to environmental variation, unambiguously scorable, and consistent within a taxon. All of these criteria were met for the morphological characters chosen for this analysis and have proven successful in previous morphological phylogenetic analyses in plants (e.g., DONOGHUE 1983, JUDD 1989, CRISCI & BERRY 1990, BOUFFORD & al. 1990, KRON & JUDD 1990, WEN & STUESSY 1993). Therefore, it is unlikely that the discrepancy between the two data sets is a result of poor character choice.

The discrepency may also be a biological factor. Potential factors are hybridization, polyploidy, convergence, or unequal rates of evolution (SYTSMA 1990, BULL & al. 1993). Hybridization, although a potential problem in the *Gesneriaceae* due to its wide outcrossing capabilities (MORLEY 1971, 1975, 1976; WIEHLER 1976), is unlikely to be a problem in these species of *Columnea* (SMITH 1991). The lack of sympatry and morphological intermediates between potential parent species argues against the possibility of natural hybrids in *Columnea* sects. *Pentadenia* and *Stygnanthe*. In addition, the evidence from rDNA is in accordance with the data from cpDNA and not indicative of hybridization (DOYLE & al. 1985, RIESEBERG & al. 1988, SMITH & SYTSMA 1990, SPOONER & al. 1991, RIESEBERG & BRUNSFELD 1992). If hybridization had been present, the data sets would have been truly incongruent and combining the data would not have been recommended unless the discrepant taxa could be easily removed from the analysis. The type of data analyzed is not particularly sensitive to polyploidy, as hybridization would again have to be invoked to create a discrepancy.

Either convergence, unequal rates of evolution, or both are potentially sources of incongruence. There is clearly convergence in both data sets as indicated by homoplasy. Convergence in either data set potentially results in a cladogram that

deviates from the evolutionary history of the species in some manner. Unless a similar pattern of convergence occurred in both data sets, they would appear incongruent at some level. Unequal rates of evolution, either between data sets, or among clades within each data set, may also explain the discrepancy between the morphological and molecular tree. Differing rates among lineages may result in long branch effects (FELSENSTEIN 1978) that will cause two taxa that are not evolutionarily related to occur in a single clade because they are more different from the other taxa in the data set.

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