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Molecular Systematics and *Columnea*: Tracing Evolutionary History

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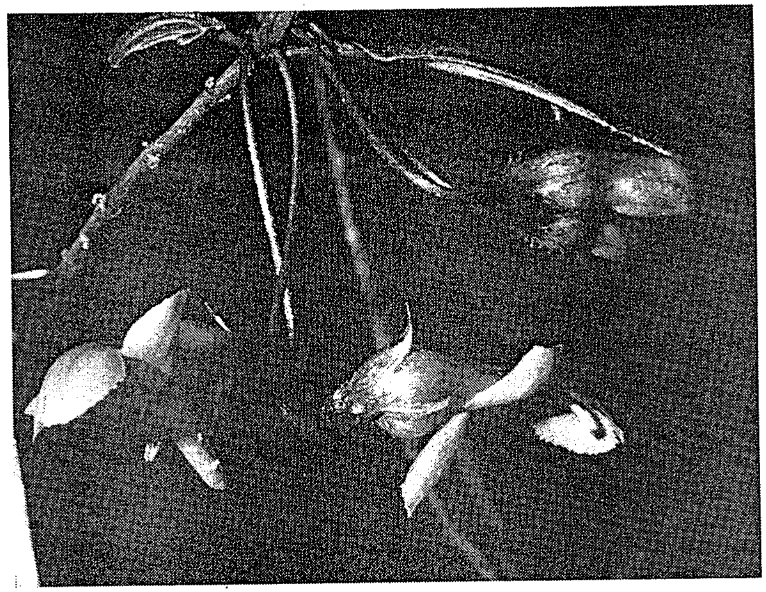
It can be almost a certainty that anyone reading this article will be familiar with the extensive and fascinating range of variation in the Gesneriaceae. The different leaf forms and colors are worthy of note without even mentioning the different forms and colors of the flowers. Entirely within the genus *Columnea* one can find almost any leaf shape that can be found within the family from small, round leaves to large, long leaves that are represented by the appropriately named *Columnea microphylla* and *C. gigantifolia* respectively. This morphological variation is an intriguing feature of the Gesneriaceae and if I am at all representative of gesneriad enthusiasts, it is this variability that makes the family attractive. As a scientist, I wanted to investigate the evolutionary history of the variation within the Gesneriaceae and so I set out on a career to study the classification and evolutionary history of the Gesneriaceae and started it all off with the largest New World genus *Columnea.*

Columnea angustata (Section *Stygnanthe*) from Colombia

To begin, we need a brief introduction to the taxonomic history of *Columnea.* The genus was first described in 1753 by the Swedish botanist who is considered the father of taxonomy, Linnaeus. The name is the Latin version of Colonna, named for the Italian botanist Fabio Colonna. Between 1753 and 1973 nearly 200 more species of *Columnea* were described by numerous botanists in North America and Europe. Throughout this period some of the species were removed from *Columnea* and placed in another genus, *Trichantha*, and later placed back into *Columnea.* Hans Wiehler in 1973 and 1983 was the first person to treat the genus as a whole and divided *Columnea* into four separate genera: *Columnea, Trichantha, Dalbergaria,* and *Pentadenia.* In addition, another genus *Bucinellina* was described that was closely related to the other four *Columnea* genera. Lars Peter Kvist and Laurence Skog (1993) examined the *Columnea* group for the Flora of Ecuador and determined that the species belonged in a single genus with Wiehler's genera as sections. (*Pentadenia* was split into two sections, *Pentadenia* and *Stygnanthe.*) Now if you are not yet thoroughly confused, here is where I make my entrance.

I knew that it was not possible to investigate all 200+ species of *Columnea* at one time and because I was interested in the evolution of the genus, I couldn't confine myself to a geographic region such as Ecuador. Therefore, I chose to work on a smaller group within the genus and chose to examine the species in sections *Pentadenia* and *Stygnanthe* (Wiehler's genus *Pentadenia*).



Columnea strigosa (Section *Pentadenia*) from Peru

My goal was to construct what is known as a phylogeny. A phylogeny is very similar to a family tree, except rather than placing aunts and cousins at the end of the branches, we place the species we are studying. A phylogeny differs from a family tree in one other important way. In a family tree, we trace all the ancestors, grandparents, etc. and put their names down. In a phylogeny this is never done. The ancestors are a part of the distant past and cannot be known by examining the plants of the present. We can only represent them as a place on the tree where branches occur that are called nodes.

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The fact that we don't name any ancestors and assume that we cannot identify ancestors makes the construction of family trees substantially different from the construction of phylogenies. A family tree is constructed by knowing the ancestors, and we can't do that with plant species. So instead we have to use other methods that infer the relationships. We have to look at features of the plants and assume that species that share particular features are more closely related to each other than they are to other species. What we are actually assuming in doing this is that the ancestor to these species is the one that acquired the feature and that it was passed on to all of its offspring.

Confused? Here's an example that doesn't involve plants and might make more sense. You get the announcement for this year's AGGS convention and decide to make some photocopies for some friends that aren't members yet. In between getting the announcement and copying it, you spill coffee on the form and it makes a stain that appears on all of the copies. Meanwhile other AGGS members are out there making copies of the same form but don't spill coffee on it before copying. Now we could put all the photocopies together and compare them and we would know that all of the ones with the copied coffee stain were all made from the same form and are, therefore, more closely related than any of the other copies. Essentially when we construct a phylogeny we're looking for copied coffee stains in the features of the plant. Unfortunately, evolution isn't as kind as a photocopier, and there are many features in plants that look like they show relationships but have actually arisen independently in different lines. There is no way to detect these features and to overcome what may be errors, so we try to use as many different features as possible. Here is where we go molecular, so prepare yourself, get some coffee, and be careful not to spill any.

Morphology, or the study of plant forms and features, is extremely useful for studies of phylogeny and has been the main source of information for decades. However, scientists recently started to look for differences in the DNA of organisms to use in phylogenic analyses. DNA is a very complicated and large molecule found in every living cell. It is the main component of chromosomes and carries all of the genetic information for all living organisms. Genes are made of DNA. Despite the complexity of the DNA molecule, we can simplify it into smaller units called nucleotides. DNA is made of only four nucleotides and we abbreviate their chemical names as A, G, C, and T. These nucleotides are attached to each other in a line, and the sequence in which they occur is unique to each individual. This is the basis for DNA fingerprinting that is being used more widely in court cases and will also be the basis for its utility in phylogenetic studies. As phylogeneticists, we are not interested in finding variation within individuals, but instead want to find variation between and among species. Fortunately the changes that occur in DNA do not occur at the same rate in every part of the molecule. Therefore we can use regions that are less variable than those used in court cases and find regions that vary between species but not much within a species.

This is all done by first extracting DNA from the plant tissue. Preferably fresh green leaves are used although frozen leaves also work well. Currently the technology is developing such that dried leaves and even fossils yield DNA! Once the DNA is extracted and cleaned, it is subjected to a restriction enzyme. This is another chemical that causes a change in the DNA, but is not used up in the reaction itself. Restriction enzymes work by recognizing a certain sequence of nucleotides in the DNA molecule and then cutting the DNA only at that sequence and every time it comes across that sequence. See Figure 1 for an example of this.

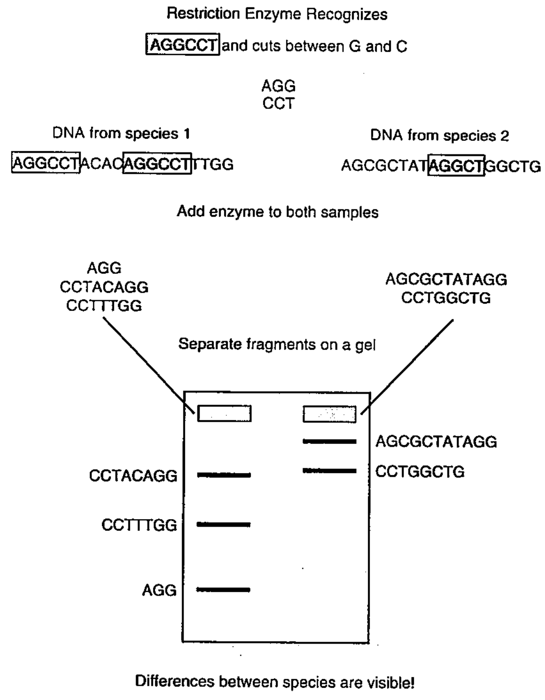
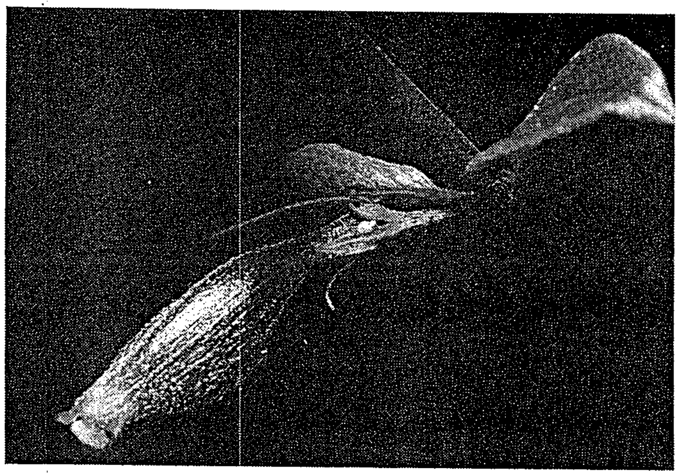


Figure 1: Restriction Enzyme Action on DNA.

We now have a tube of DNA that is all chopped up. This will be repeated for every species and all of the tubes kept separately. What we hope for is that there will be differences among the species where the restriction enzyme cut the DNA, and that these differences will serve as our coffee stains. Unfortunately, the mixture tells us nothing. We have to separate all of those fragments to see if there are any differences between the species. This is done by placing the contents of the tube into a well that has been made in a gel (Figure 1). A gel is essentially a block of clear gelatin. All DNA has a negative electric charge; therefore, if we apply a negative electrode near the well that contains the DNA and the positive electrode at the other end and turn on the current, the DNA will be pulled through the gel. Smaller fragments can move around and through the spaces in the gel faster than the bigger fragments. After a period of several hours, the smaller fragments will have travelled to the bottom of the gel, and the larger fragments will be near the wells. We stain the DNA in a way that makes it visible and look for differences in sizes. If we've been successful, we have some (Figure 1).

This is done repeatedly with many different enzymes for each species, and all of the information is recorded. The information is then analyzed using a computer program that will produce a phylogeny. By using molecular techniques it may be possible to augment the amount of information available by ten times. Using my own example from *Columnea*, I was able to find about 30 variable features from morphology and over 200 from DNA. It is important to understand that DNA does not replace morphology as a tool for phylogeny, it is simply an extra one to work with. Speaking of *Columnea*, wasn't I going to talk about that as well?

As I mentioned above, I gathered about 30 morphological features and over 200 DNA differences to use in a phylogenetic analysis. I used the computer program and produced a tree for the species I was studying. The result? Well, it's Figure 2 if you haven't already looked ahead to it. My taxonomic conclusions based on these data are that *Columnea* should be retained as a single genus with sections within the genus. Further studies will help to resolve some of the other questions regarding exactly which species will belong in each of the sections and whether the sections as marked here will be retained in the future. As to the evolution of all those intriguing features that I mentioned earlier, I'm really only beginning that part of the study. You'll have to wait for the next installment. Before I finish, I want to make one very important caveat. Although the phylogeny represented in Figure 2 is the best estimate we have at this time for these species, it is only an estimate. As more species are added to the analysis, and more data is added, the relationships could change. Just like the names, our estimates of phylogeny can change as well. I know this is frustrating, but it's how science works. So my advice is to keep using pencils and keep an eraser on hand. ☐



Columnea moesta (Section *Stygnanthe*) from Bolivia

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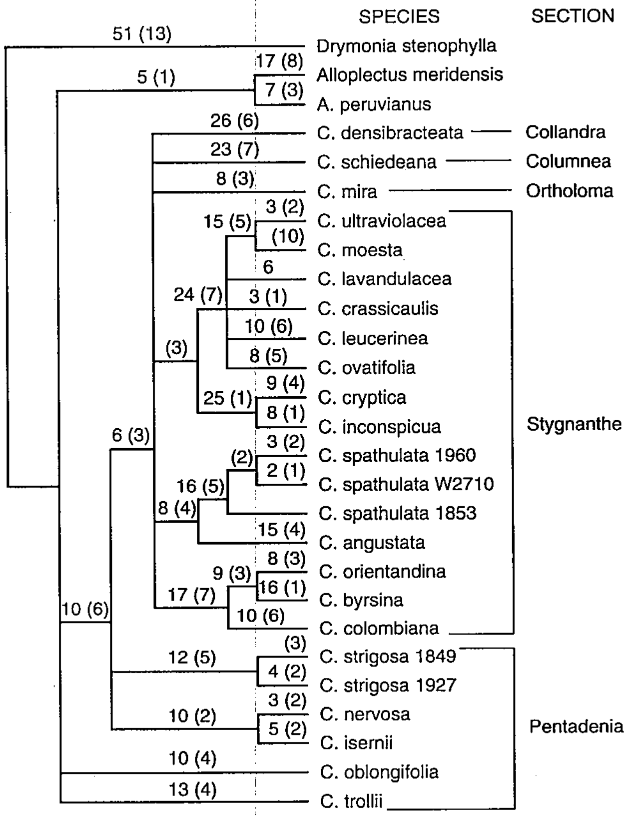

Figure 2

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J.F.S.

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Eucodonia verticillata 'Frances'