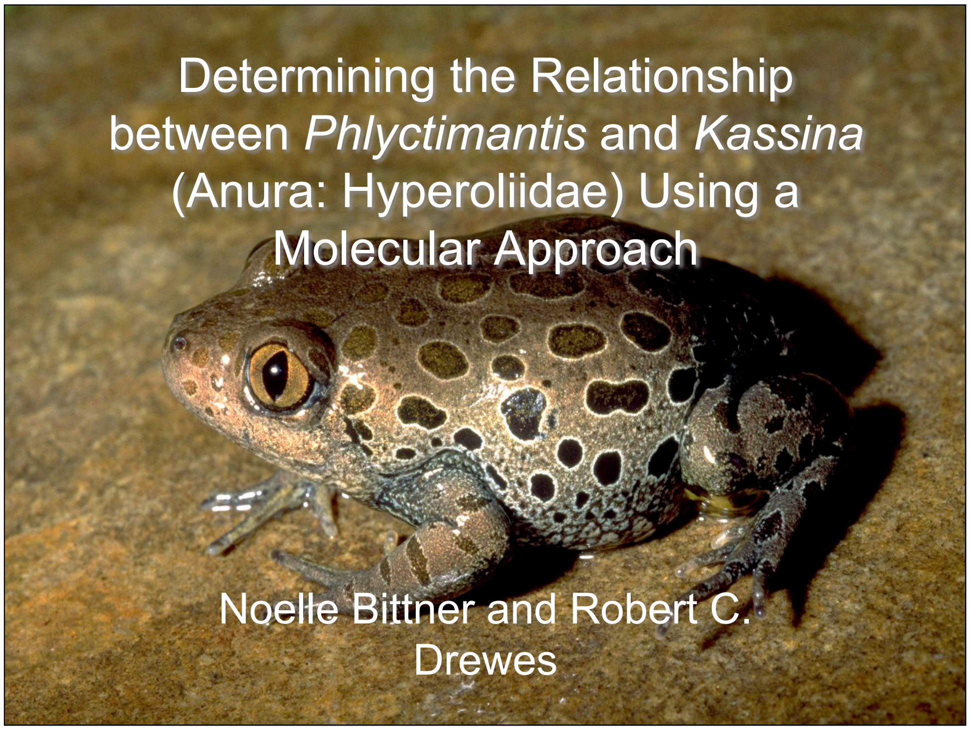
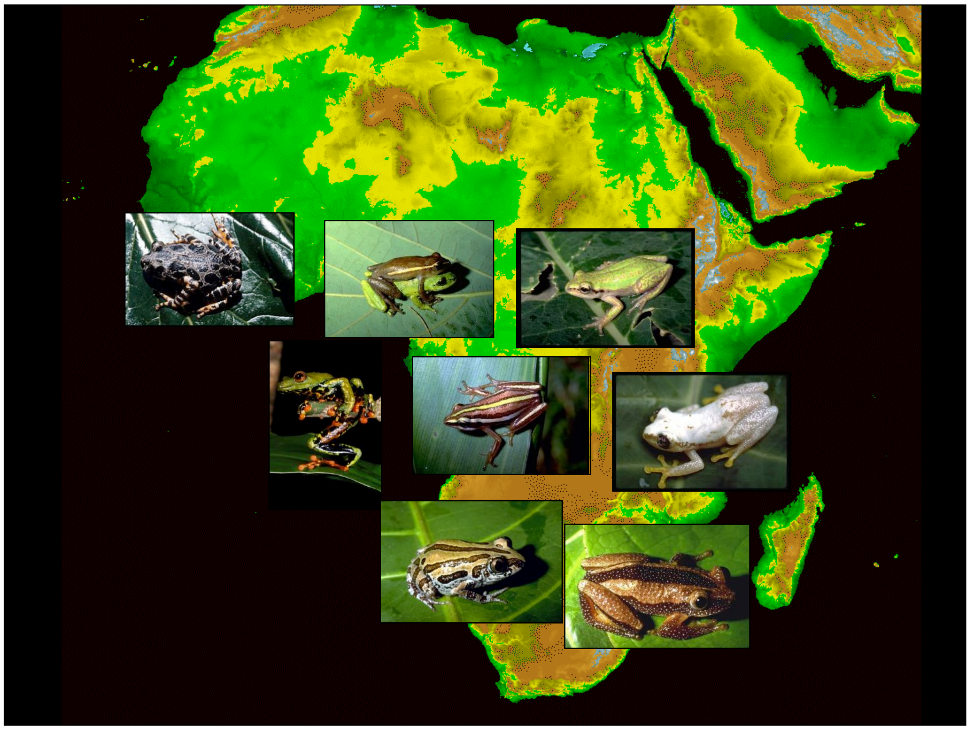


Slide 1 – Intro

Good morning. My name is Noelle Bittner and I worked in the herpetology department this summer with Dr. Bob Drewes. My project is an attempt to determine the relationship between *Phlyctimantis* and *Kassina*, both frogs of the family Hyperoliidae, using molecular data.

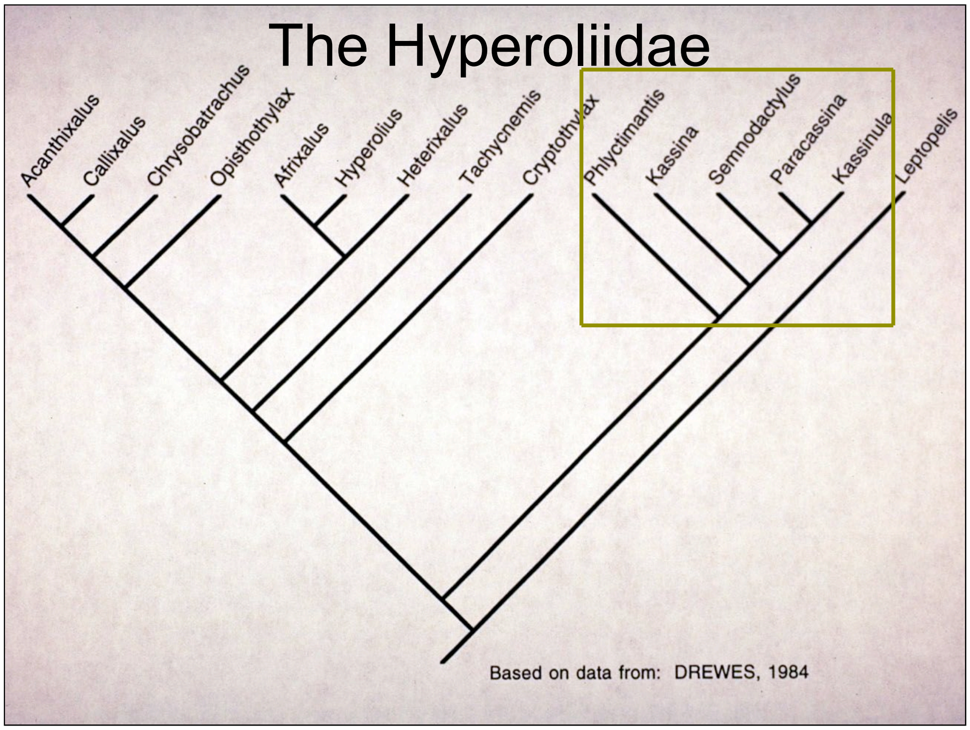


Slide 2 – Map

Almost all of the tree frogs in Africa fall into the family Hyperoliidae- cleverly known as the African Tree frogs. They are found throughout Africa south of the Sahara, on islands such as São Tome, on Madagascar and the Seychelles

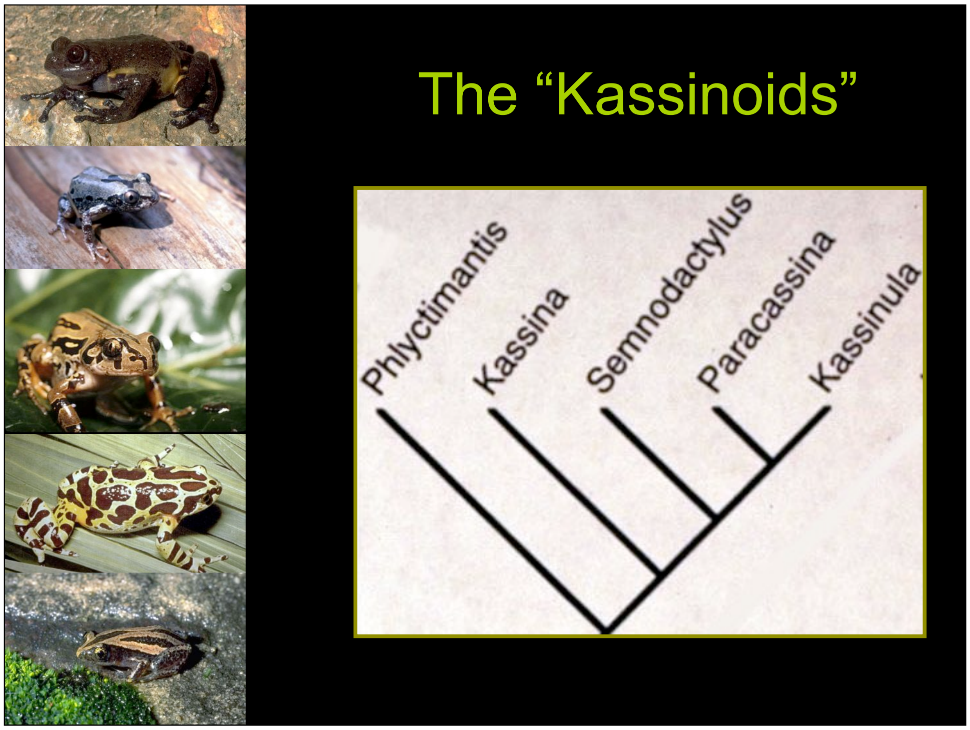
Slide 3 – General hyperoliid information

Most of the Hyperoliids are arboreal - hence their name - with the important exception of the genus *Kassina*, which I will later discuss. Almost all of the hyperoliids breed in or near water. There are currently 17 recognized genera.



Slide 4 – Phylogeny

With less than 17 taxa here, this is a slightly outdated phylogeny. It was based on my advisor's original morphological study from 1984. Frost et. al. from 2006 found the basal genus, *Leptopelis*, to be a member of a different family, which leaves the frogs that I studied in the most basal clade – the "kassinoids."



Slide 5 – The "kassinoids"

Very little work has been done on this clade since Drewes 1984 and while a few species have been analyzed and even sequenced – they have all been for larger projects focused on resolving larger clades – the lissamphibia or the African ranoids. This clade contains 5 genera pictured here in descending order- *Phlyctimantis, Kassina, Semnodactylus, Paracassina* and *Kassinula*. The latter four are well defined with synapomorphies to unite each as a genus. *Phlyctimantis*, on the other hand, has no unifying characters. Little recent work has been done on the subject since Drewes 1984, where it appears as an uncorroborated node. *Phlyctimantis* is a separate genus, more so because it does not share the synapomorphies that define each of the other genera.

It is possible that *Kassina* is the sister taxa to *Phlyctimantis* - an idea that has been tossed around for a while but has had no confirmation. It has also been suggested that they are all members of the same genus.

To try to get some resolution about the position of *Phlyctimantis* within the "kassinoids," my work this summer started with just the first step- trying to understand its relationship to *Kassina*.

There are a few reasons why I looked at *Kassina*- there was evidence that there is more to the relationship than what is already published.

Slide 6 – *Kassina* (I)

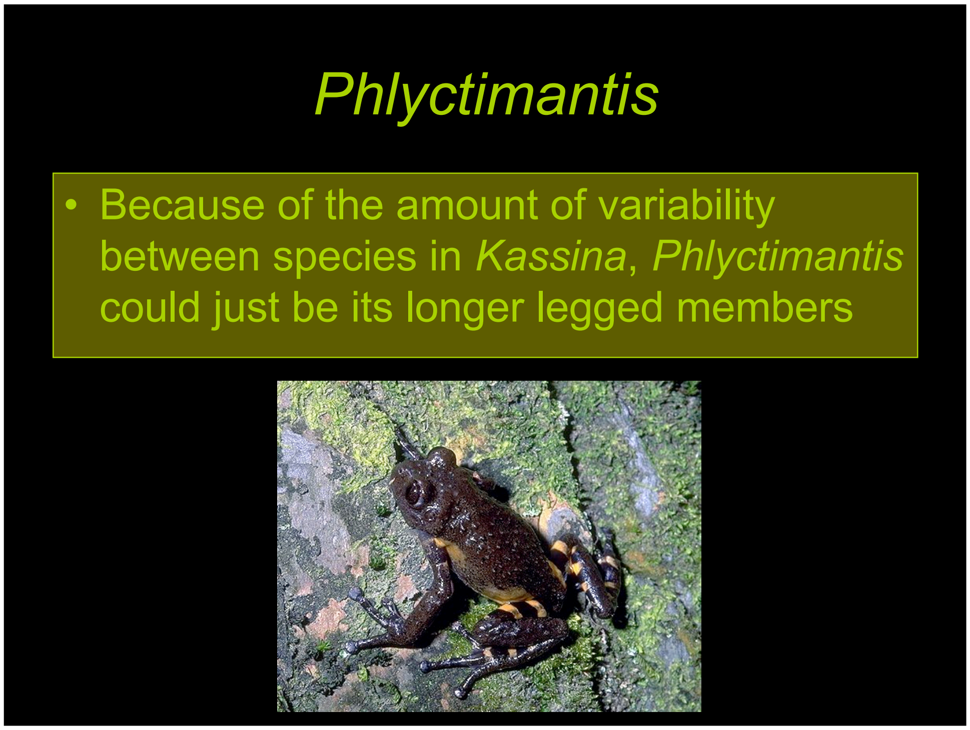
Kassina, as I have mentioned, is a well supported monophyletic genus. It is, however, the most variable genus within the Hyperoliidae with each of its 13 species highly variable in lifestyle and appearance.

Take *Kassina fusca* for example: a small, semi-fossorial frog it spends most of its life underground and comes out to breed. You can see their robust body which appropriate for their lifestyle. They also have light markings.

Compare this with *Kassina cochranae*, a much larger frog it has well defined dark dorsal markings. They are scansorial animals, which means they spend most of their life climbing on rocks and on trees.

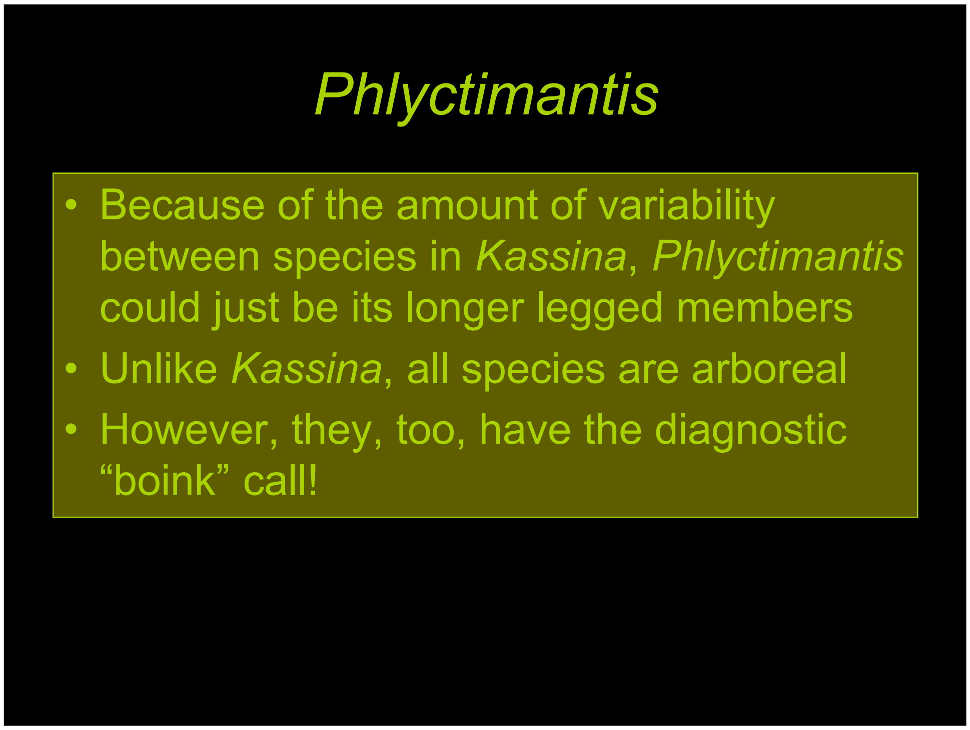
Slide 7 – *Kassina* (II)

On character that unites this genus is the male mating call- all *Kassina*s make some variation of a "boink" call. [Recording of frog calls.]



Slide 8 – *Phlyctimantis* (I)

Because there is so much variability between species within *Kassina*, it is possible that *Phlyctimantis* is just a longer legged *Kassina*.



Slide 9 – *Phlyctimantis* (II)

All of the species are arboreal, however, unlike *Kassina*.

And, most interestingly *Phlyctimantis* also shares the "boink" mating call.

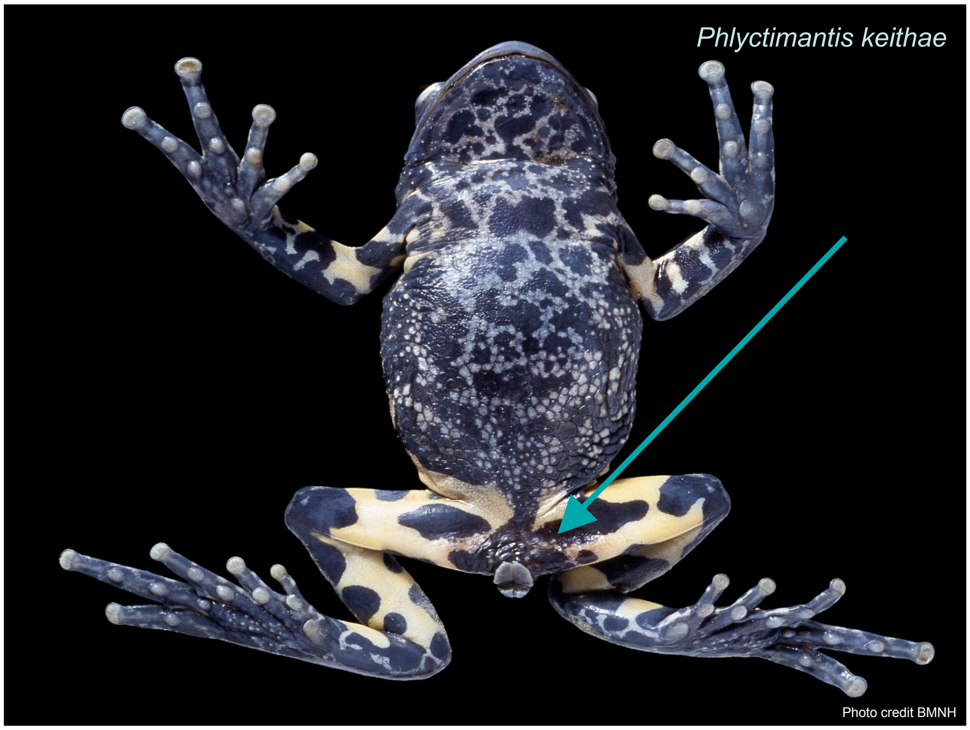
Now let me digress briefly on the significance of this- aside from frog calls just being wonderful things to listen to. Male frogs call to attract females during breeding seasons. The female follows the voice until hopefully she finds her mate. Because of this, mating calls are species-specific. Frogs, therefore, want to have the most dissimilar call as possible to avoid any competition – especially in the tropics where tons of frogs are calling at once. It doesn't make sense, therefore, that there would be convergent evolution in frog calls. The most likely reason two frogs would have similar calls would be because they share a common ancestor.

Slide 10 – The First Question

All of this information leads us to the first question, namely what is the relationship between *Kassina* and *Phlyctimantis*

Slide 11 – The Morphological Approach

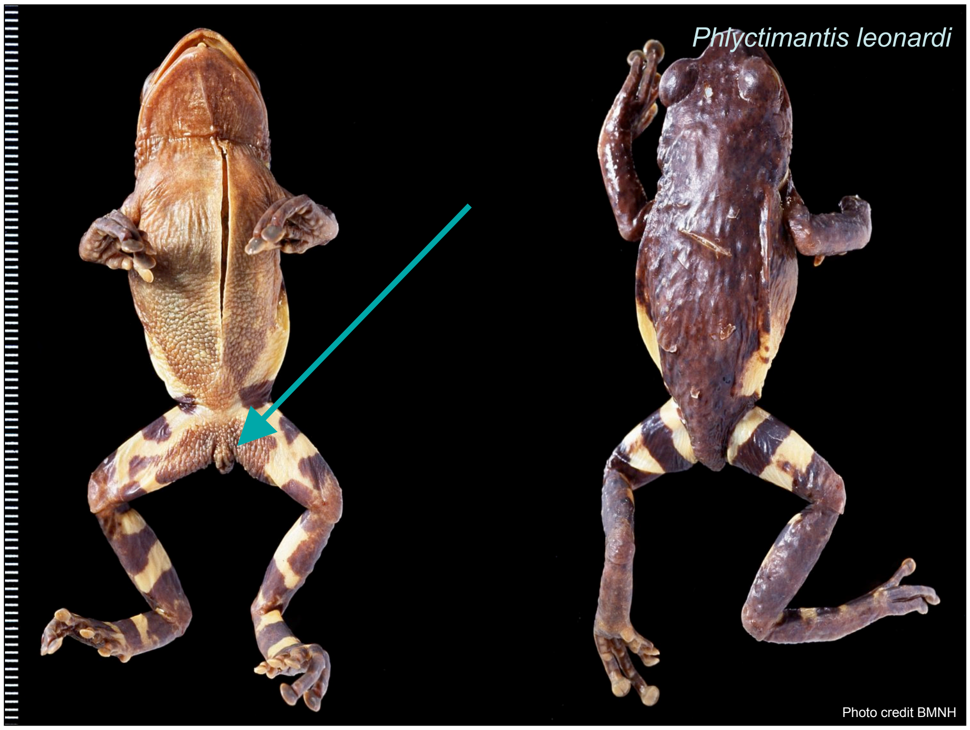
To answer this question I first took a morphological approach



Slide 12 – *P. keithae*

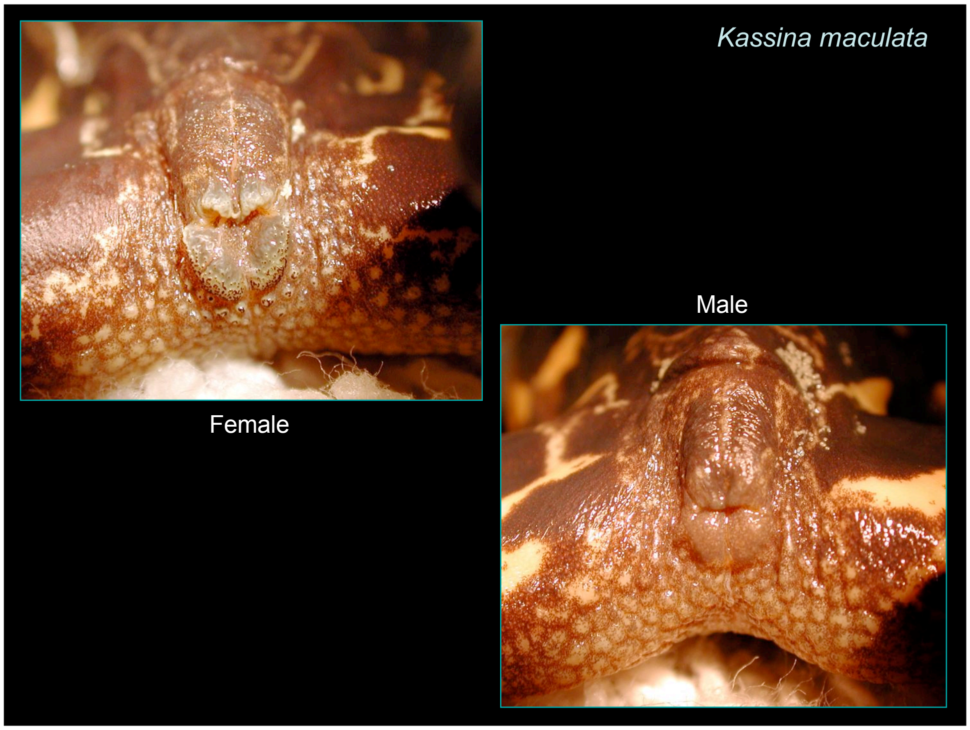
When I first arrived here at the beginning of the summer Bob told me that he had found a novel morphological character found in all four species of *Phlyctimantis* and in one oddball species of *Kassina*- *Kassina maculata* and that this could be the synapomorphy that would define *Phlyctimantis.* That was not the case.

First, let me talk a bit about the character. To give you some context this is the ventral view of *Phlyctimantis keithae* and right here is the character. Here it is in *Phlyctimantis leonardi*. It is also recorded in *P. boulengeri* and *P. verrucosus*.



Slide 13 - *P. leonardi*

Here it is in *Phlyctimantis leonardi*. It is also recorded in *P. boulengeri* and *P. verrucosus*.

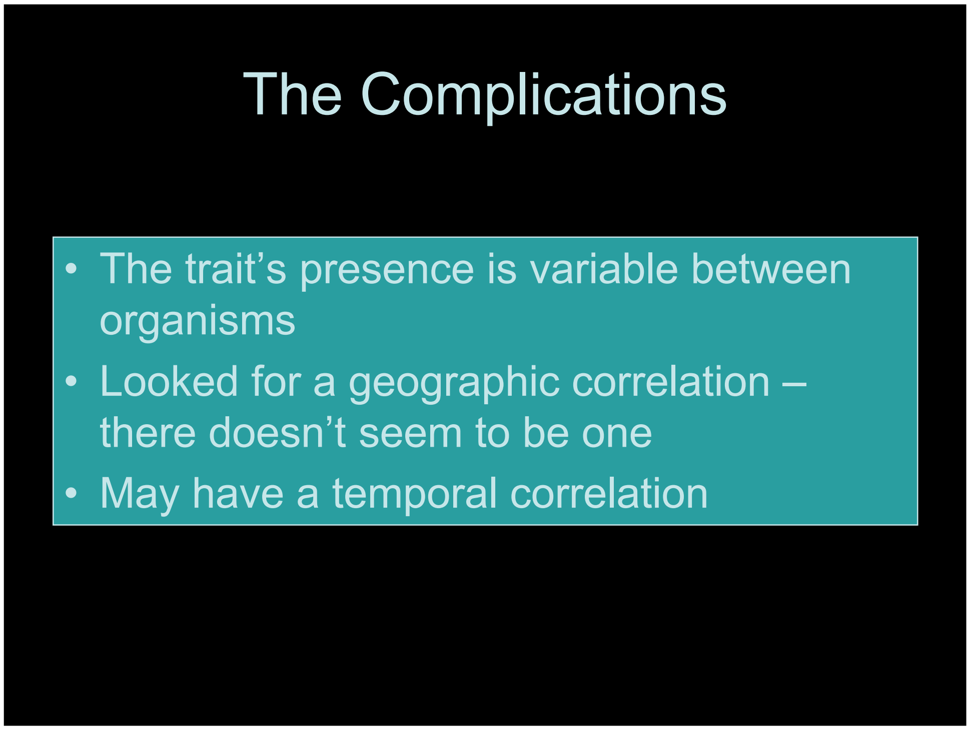


Slide 14 – *K. maculate*

And here is the *Kassina* and a close-up view of the structure – the elaborate cloacal papillae. There are many components to the structure which remain unnamed at the point but briefly there is a pronounced tub leading towards the cloacal aperture which is surrounded by these elaborate papillae. The ventral side has what looks almost like a shelf- two well developed lobes under the vent. You can see it here in the female (on the left) and in the male (on the right) to a lesser degree.

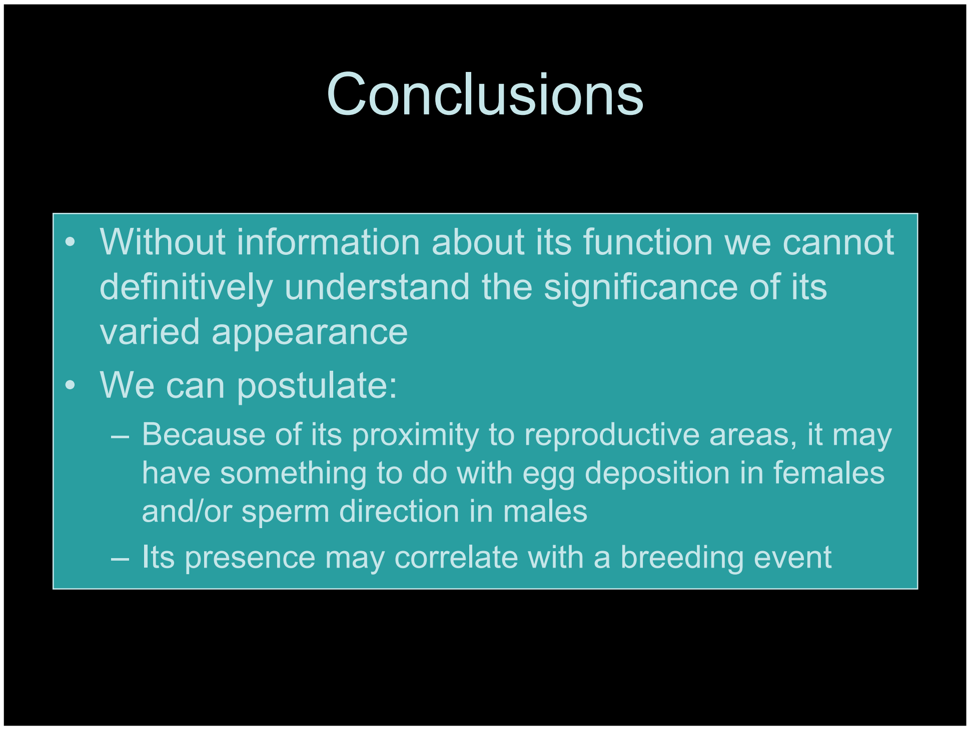
Slide 15 – Other *Kassinas*

Upon further inspection of this region in other *Kassina*s The structure was found in *K. senegalensis K. kuvangensis* and, although not pictured here, *K. fusca*, *K. decorate* and possibly more. As you can see here the females – again on the left – have much more defined structures than the males but the males do have the structure to a lesser degree. What is really interesting about this picture is the variation between species. You can see *K. senegalensis* has large, well defined papillae whereas *K. kuvangensis* has better defined lobes. There are many mysteries still surrounding this trait.



Slide 16 – The Complications

The reason the structure was likely not noted in the first place in more species is because its presence is variable between individuals even of the same species. To shed some light on this matter, I went through some of the collection housed here as well as some specimens on loan to determine why this was so. The specimens are kept here in jars containing animals from the same collection sites and same time frame. I first looked at a variety of jars from different locations of the same animals and found that there was little correlation between the presence of the structure and the location of the animal. I realized that within a jar, all the specimens either presented the structure or did not have one at all. This led me to hypothesize there may be some sort of temporal or season effect on the presence of this structure.



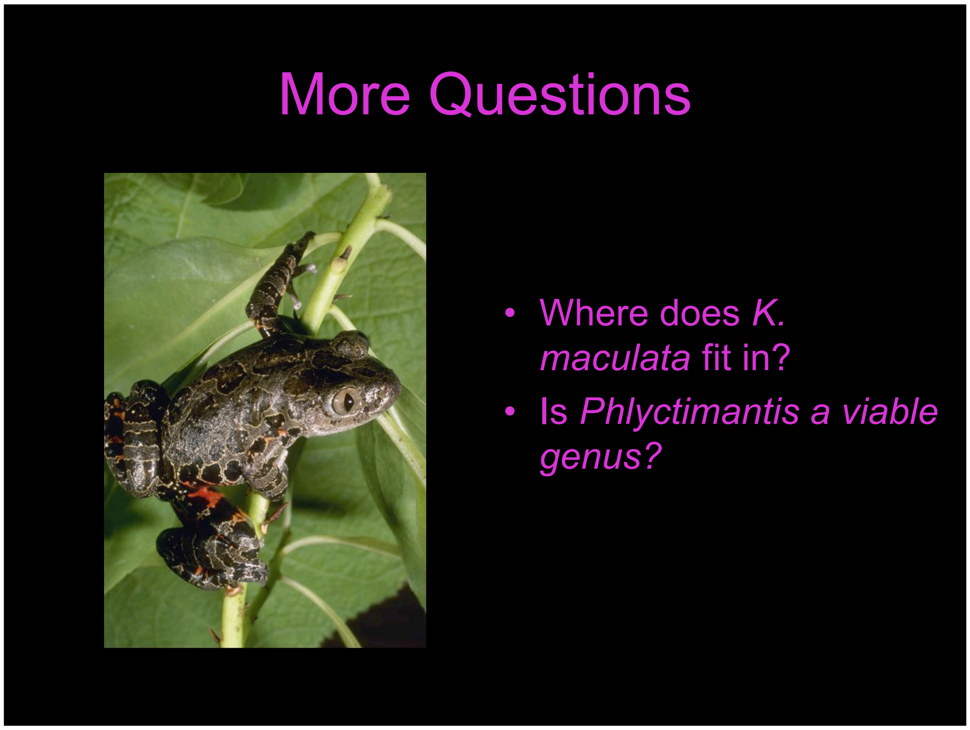
Slide 17 – Conclusion

Because we do not have any information on the true function of this structure, it is hard to understand its significance or the significance of its variability. We can however make some educated guesses on the subject.

The papillae are surrounding the cloaca. So, because of its proximity to the reproductive region, the structure may have to do with oviposition in females. The lower lobes could act to "soften" the landing of the egg or something similar. However, as far as we know, these frogs all lay their eggs in water and would therefore have no need for protection in this way.

Maybe, then, the male structure is for directing sperm – this, too is questionable because plenty of frogs with similar breeding habits are able to fertilize eggs just fine without a specialized structure.

Which leads us to wonder about the presence of the structure. It is possible that this structure appears near a breeding event. There are, too, some problems with this hypothesis.

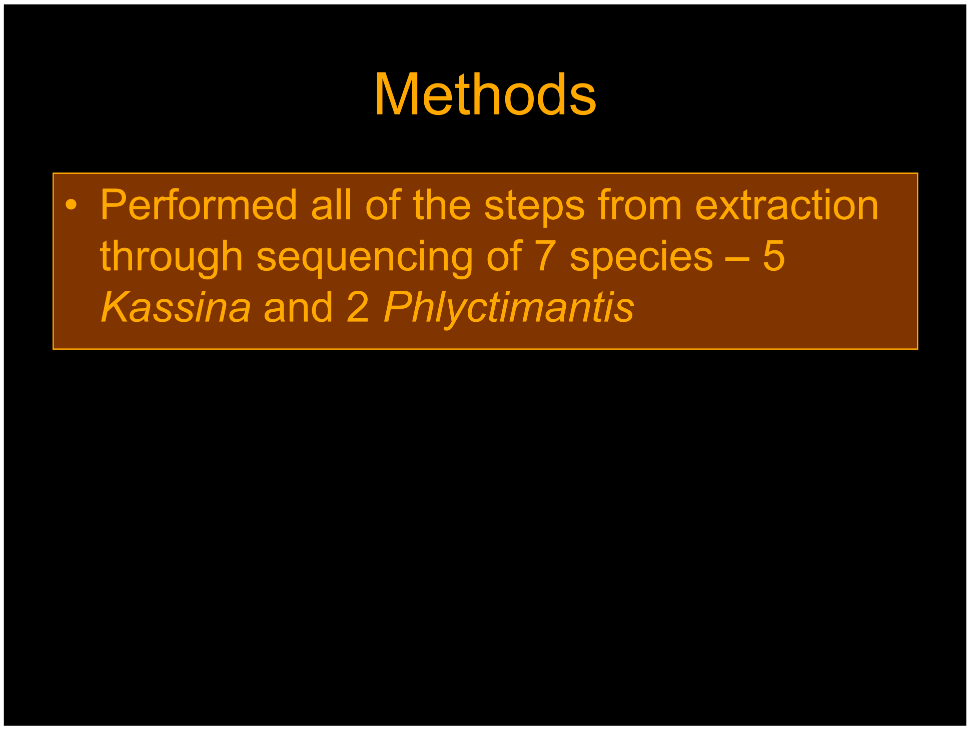


Slide 18 – More questions

Although the structure did not give us any more clarity on the relationship, it did lead us to new questions such as "Where does *K. maculata* fit in with the *Kassina* and *Phlyctimantis*? As you can see in this image, they are much more arboreal than the other *Kassinas* and are also the largest – similar in size to the *Phlyctimantis*. This also forces us to ask is *Phlyctimantis* really a viable genus?

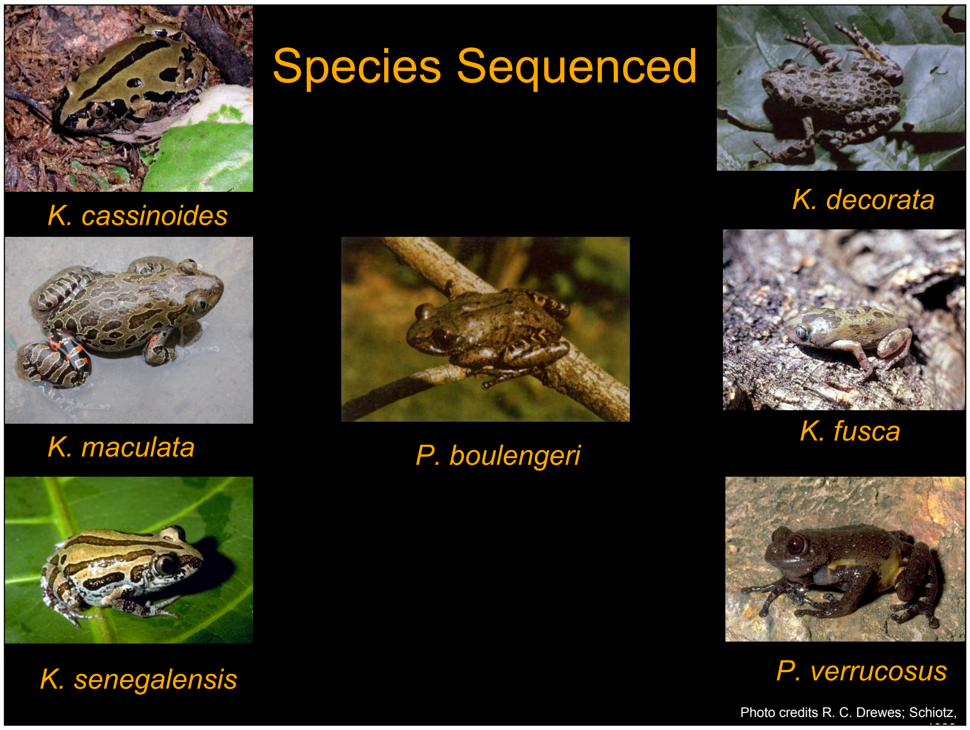
Slide 19 – A Molecular Approach

To answer these questions we started collecting molecular data.



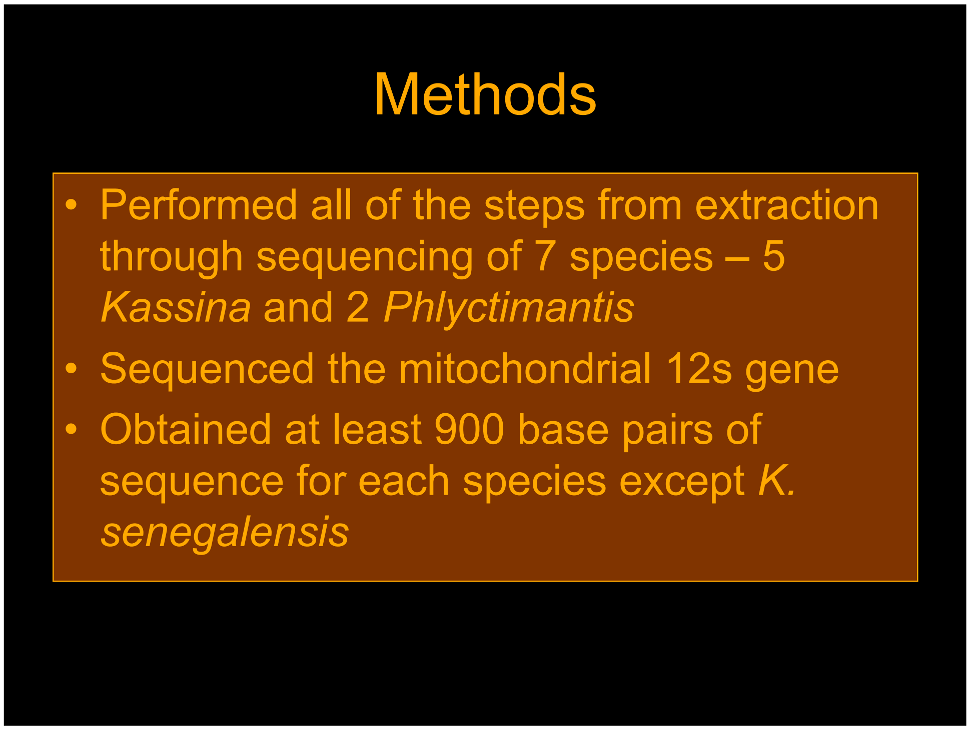
Slide 20 - Methods (I)

I performed all the steps from extraction to sequencing of DNA from seven species – 5 *Kassina* and 2 *Phlyctimantis*.



Slide 21 - Methods (II)

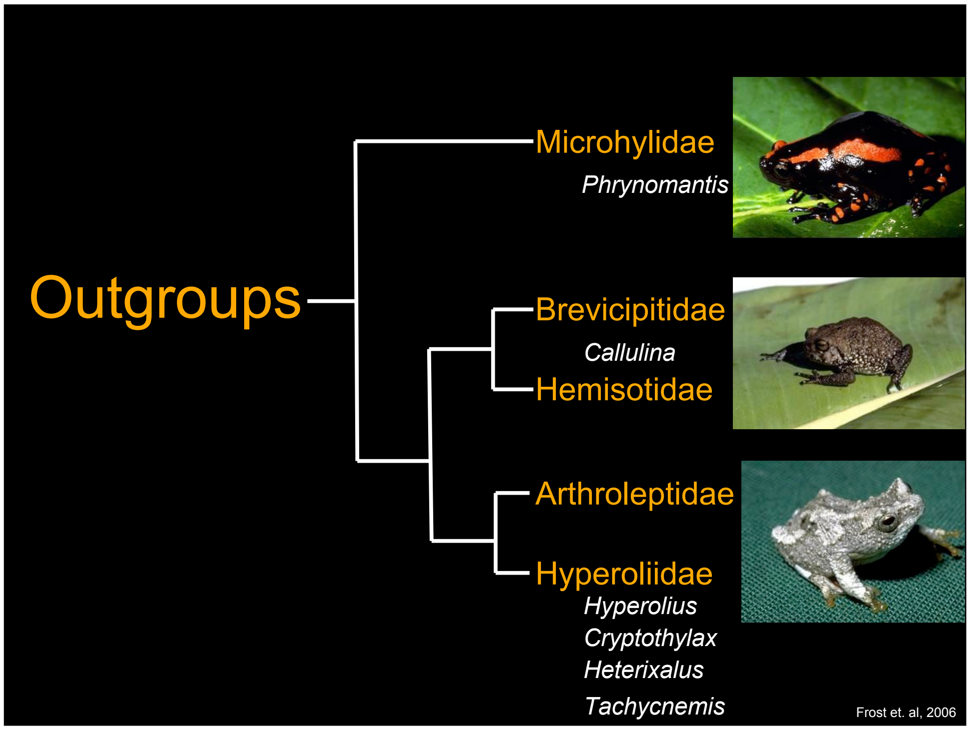
The seven species were *K. cassinoides, K. maculate, K. senegalensis, K. decorate, K. fusca, P. boulengeri* and *P.verrucosus.*



Slide 22 - Methods (III)

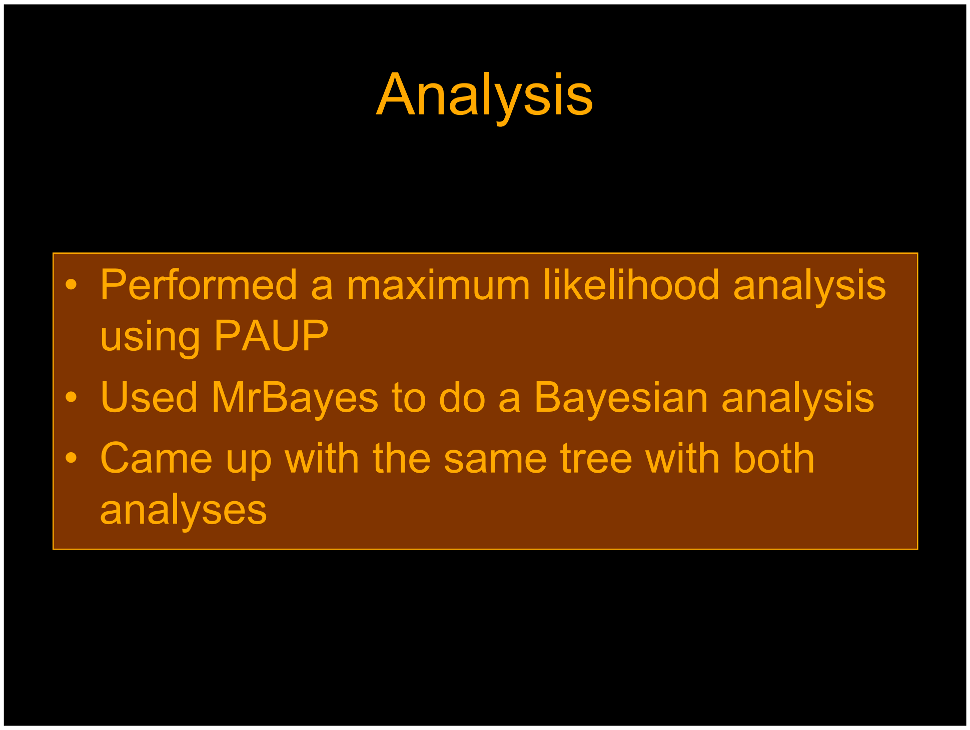
I sequenced the mitochondrial 12S gene, which has precedents in the literature for this family and other similar ones.

I obtained at least 900 base pairs from the gene of each species with the exception of *K. senegalensis* which I could only sequence half of the gene despite my best efforts.



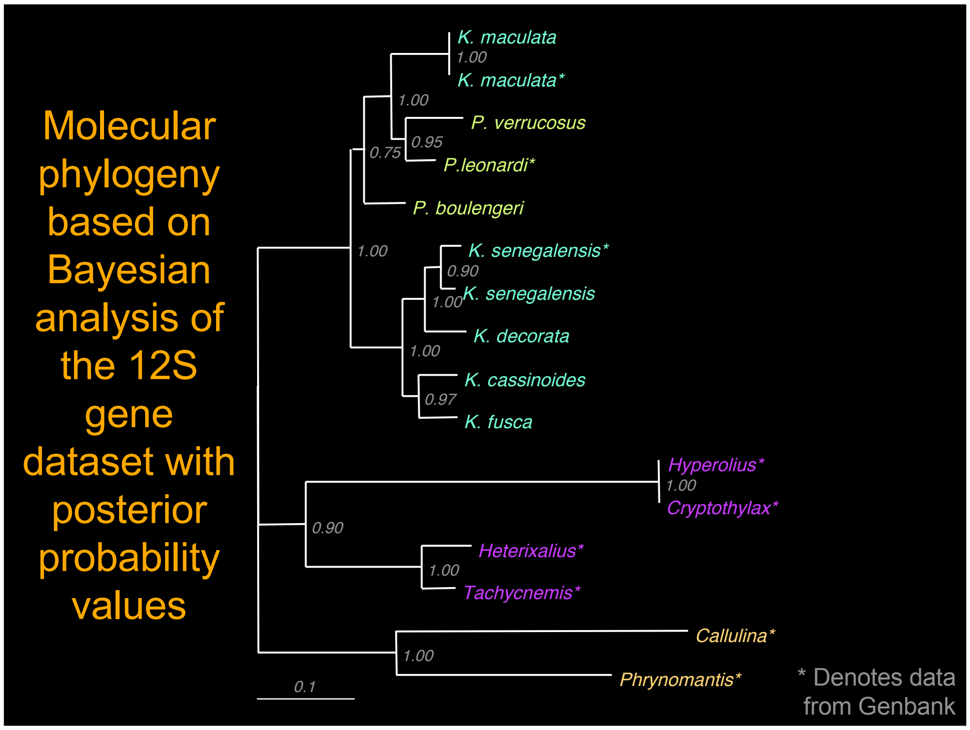
Slide 23 – Outgroups

In addition to my own data, I added outgroups from GenBank. Within the family that both *Phlyctimantis* and *Kassina* are in, the Hyperoliidae, I used four outgroups *Hyperolius, Cryptothylax, Heterixalus,* and *Tachycnemis.* Furthur out I used *Callulina* one of the Brevicipitidae and then a genus from the sister family of the two *Phrynomantis* from the Microhylidae.



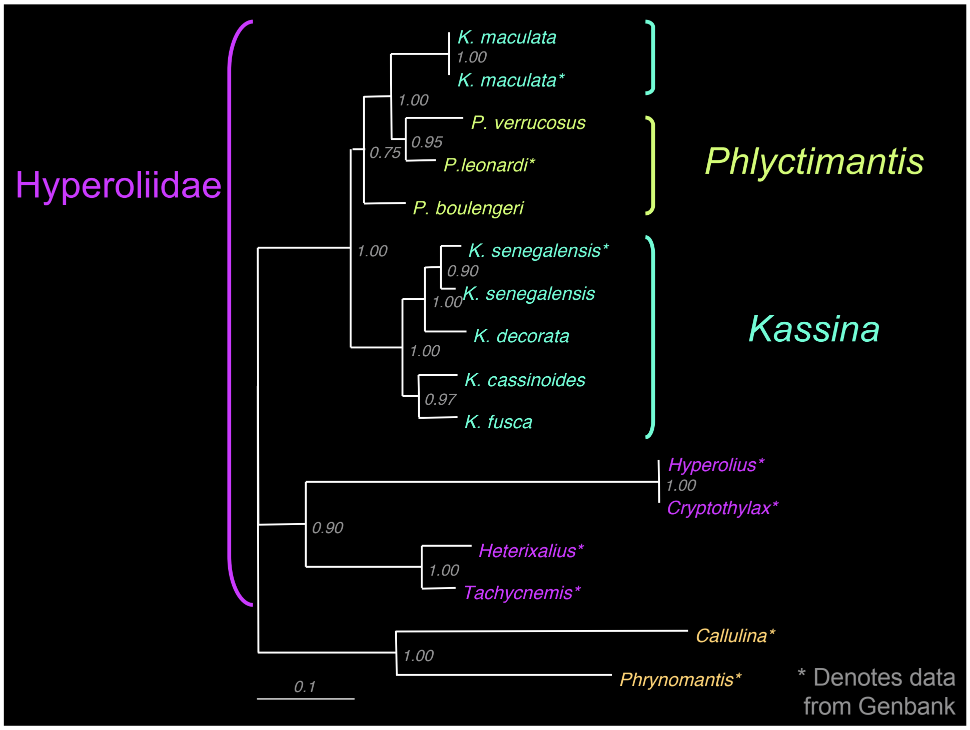
Slide 24 – Analysis

To analyze this data, I performed a likelihood analysis using PAUP and a Bayesian analysis from MrBayes. Both of these methods came up with the same tree.



Slide 25 – The Tree

Here is a phylogeny of my molecular 12s data based on a Bayesian analysis. In gray are the posterior probability values.



Slide 26 – Hyperoliidae tree

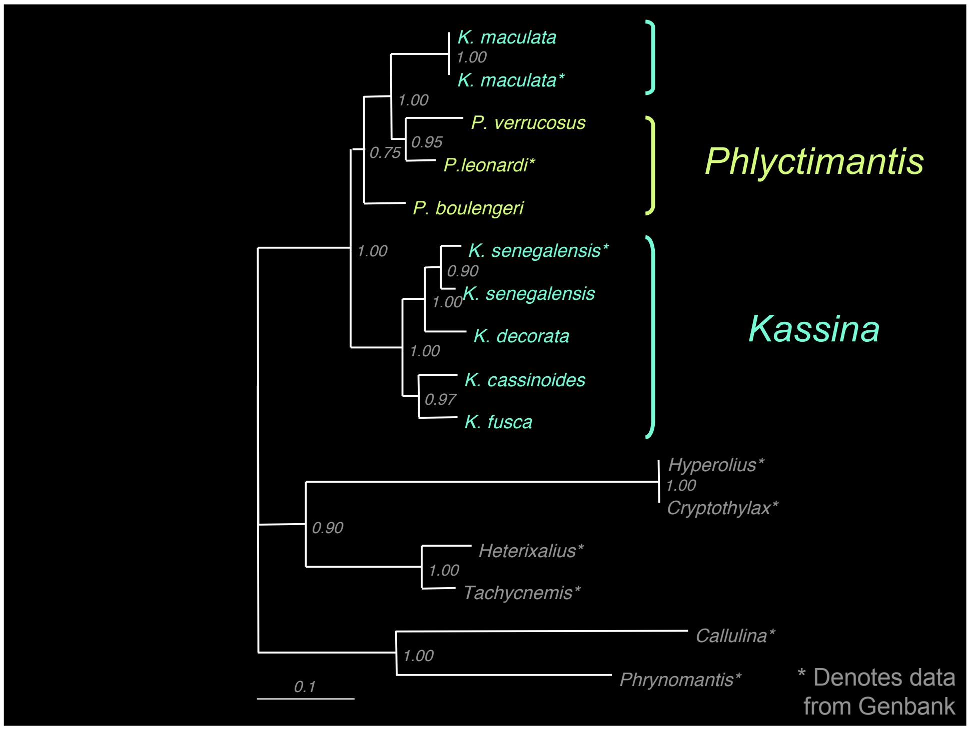
You can see two of my most distantly related outgroups here. All of these animals are within the African ranoids. Here are the hyperoliidae. *Kassina* and *Phlyctimantis.* For the deeper nodes, this phylogeny reflects the literature and the trees I showed you earlier.

Now let's focus in on the genera I looked at right here. *Kassina* and *Phlyctimantis* clade together, with the exception of *K. maculata,* which we will talk about in a moment.

Results

- *Kassina* and *Phlyctimantis* clade together, as expected

Slide 27 - Results



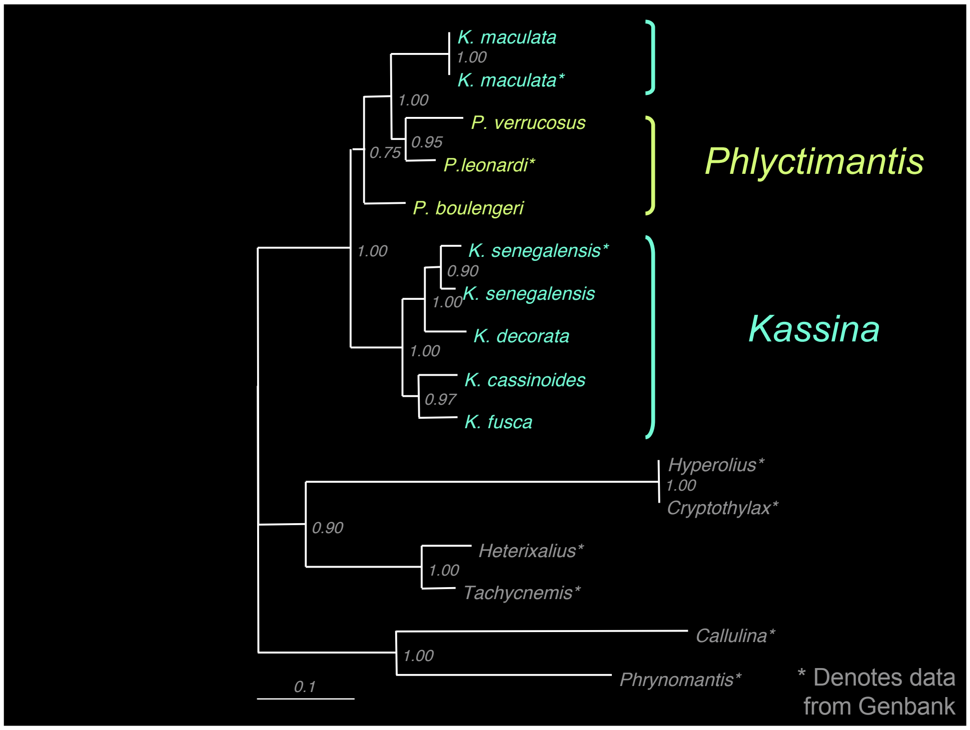
Slide 28 – Relationship between *Phlyctimantis* and *Kassina*

With a posterior probability value of 1.0 the *Phlyctimantis* and *Kassina* group is well supported by being each other's closest relatives in relation to the outgroup. Because we do not have data for any of the other *Kassinoids* yet, we cannot make any statements about how closely these two genera are related to each other. But we can also tell that there is a definite split between *Kassina* and *Phlyctimantis*.

Results

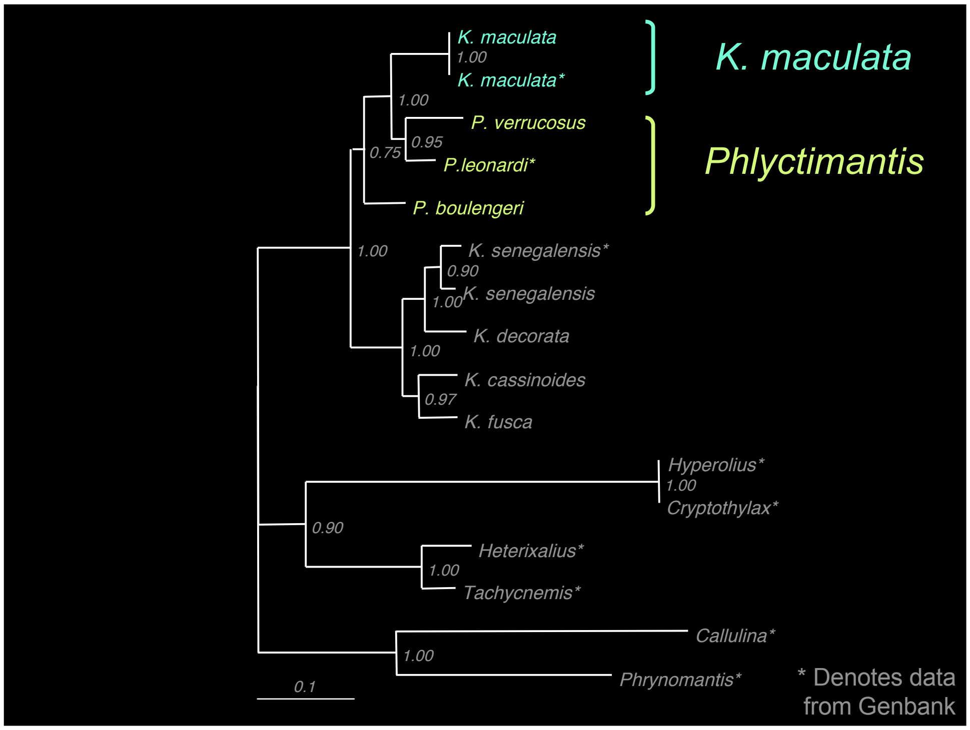
- *Kassina* and *Phlyctimantis* clade together, as expected
- There is a defined split between the two genera- with the exception of *K. maculata*

Slide 29 – Results



Slide 30 – *Kassina*, *Phlyctimantis* and *K. maculata*

Here the *Kassina* lump together (again with the exception of *maculata*) with a high posterior probability. *Phlyctimantis* is more problematic. Here at the base of the *Phlyctimantis* branch - there is not much support for it as a clade. However, the data I obtained for *Phlyctimantis boulengeri* was not as strong as my data for many of the others. I would definitely try to reproduce the data before putting any weight on this discrepancy. What you can see here is that there is strong support for a clade containing the two *Phlyctimantis* species, for which I have strong data.



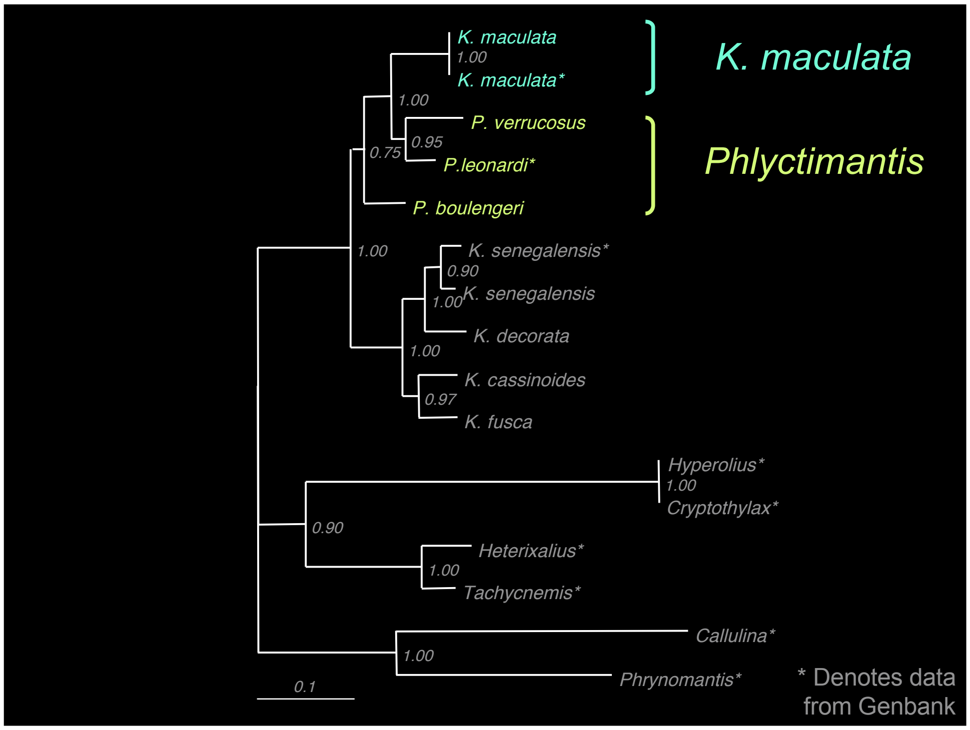
Slide 31 – *K. maculata*

Within what could be tentatively considered the *Phlyctimantis* clade, as I'm sure you've noticed, is the *Kassina* in question- *Kassina maculata*.

Results

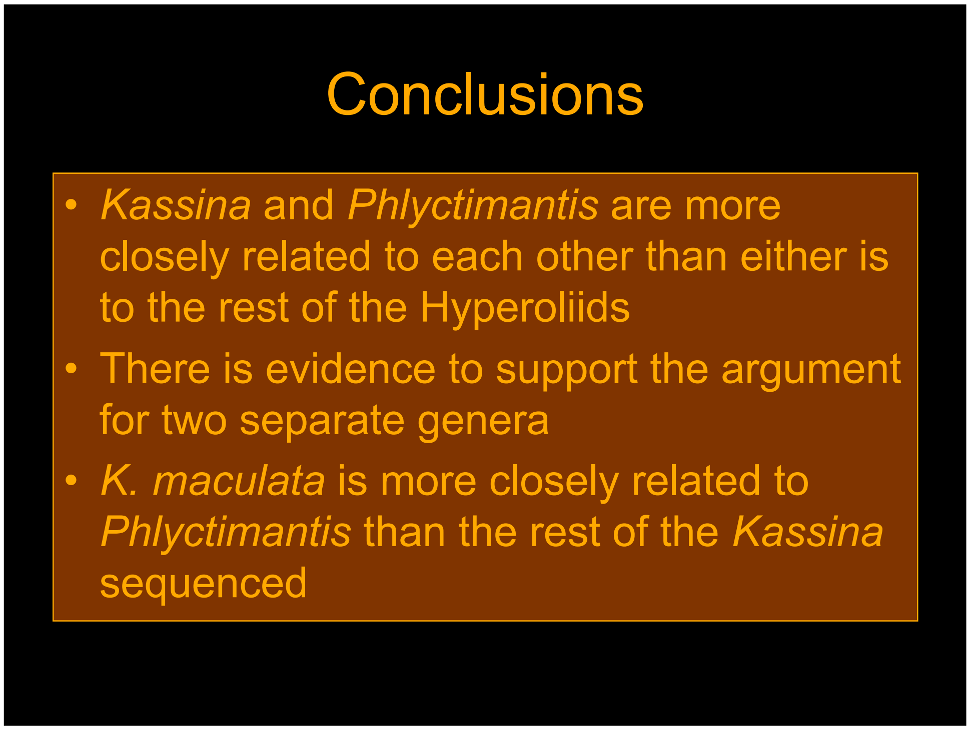
- *Kassina* and *Phlyctimantis* clade together, as expected
- There is a defined split between the two genera- with the exception of *K. maculata*
- *K. maculata* groups with *Phlyctimantis*

Slide 32 - Results



Slide 36 – *K. maculata*

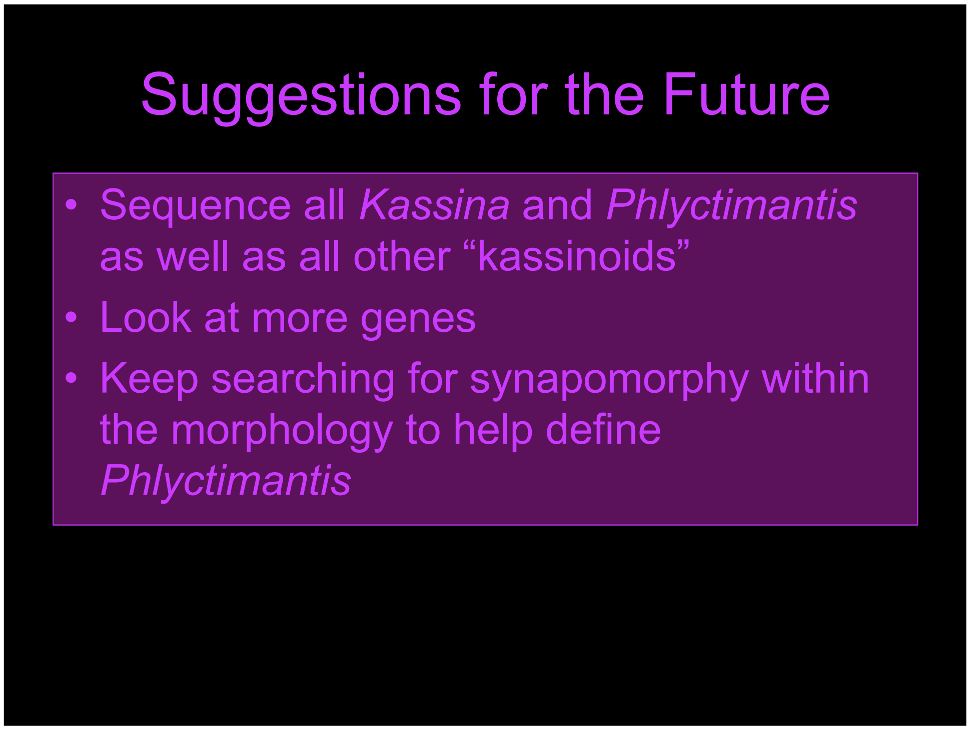
With such a high posterior probability value, this data shows that *K. maculata* falls in with *Phlyctimantis*.



Slide 34 - Conclusions

To sum what has already been discussed, I have concluded, from my very preliminary work this summer, first that *Kassina* and *Phlyctimantis* are closely related with respect to the rest of the Hyperoliidae, and that the two genera seem to have some definite division – they are not all lumped together or interspersed in one large clade – which suggests that they are separate entities, likely at the generic level but again more data is needed to be conclusive.

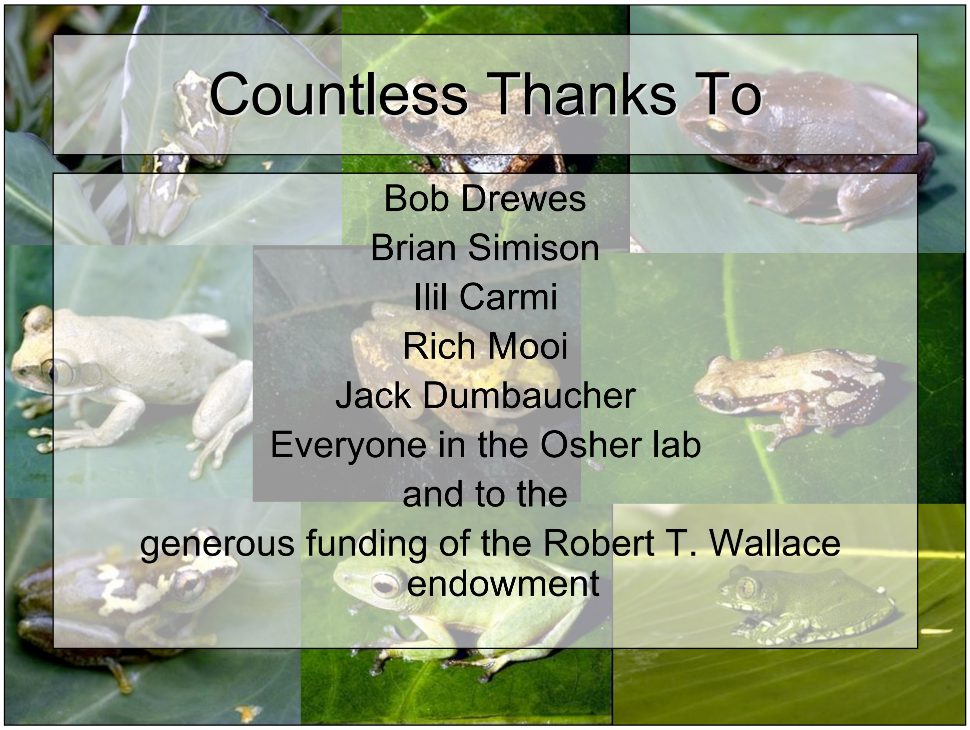
And finally that *Kassina maculata* is more closely related to *Phlyctimantis* than *Kassina* – or at least than the species we sequenced. There are still 8 other species in *Kassina* and one in *Phlyctimantis* that I do not have data for. Before rushing to conclude anything about the relationships of these animals, we must have more information. Therefore,



Slide 40 – Suggestions For The Future

I suggest for the future of this study that more sequences be obtained for all of the "kassinoids." Also, before making any conclusions about these animals, other genes should be examined, so I would add more genes to the data set. To reference the morphological section again, I would hope in the future to be able to find a definite morphological character to unite molecularly-defined clades.

One definite conclusion from my work this summer is that there is *something* there and that these relationships – not previously looked into on a molecular level to this degree – do need to be questioned.



Slide 36 - Thanks