DNA barcoding of reptiles: practical aspects

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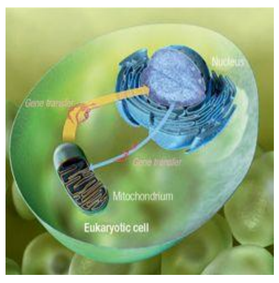
Context

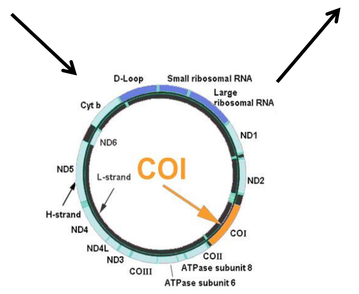
The number of DNA barcoding campaigns focusing on reptiles is very limited compared to other vertebrate taxa like fishes and birds, although reptiles also represent a major group of tetrapods with more than 9300 described species. First barcoding studies have proven to be useful to identify reptile species like marine turtles, alligators and crocodiles. Here we give a brief overview of available primers and present our results on a first DNA barcoding project on Malagasy squamates.

Methods

The DNA barcoding approach enables a rapid species identification based on a single, standardized molecular genetic marker. First, well-identified voucher specimens are needed in order to establish a reference database.

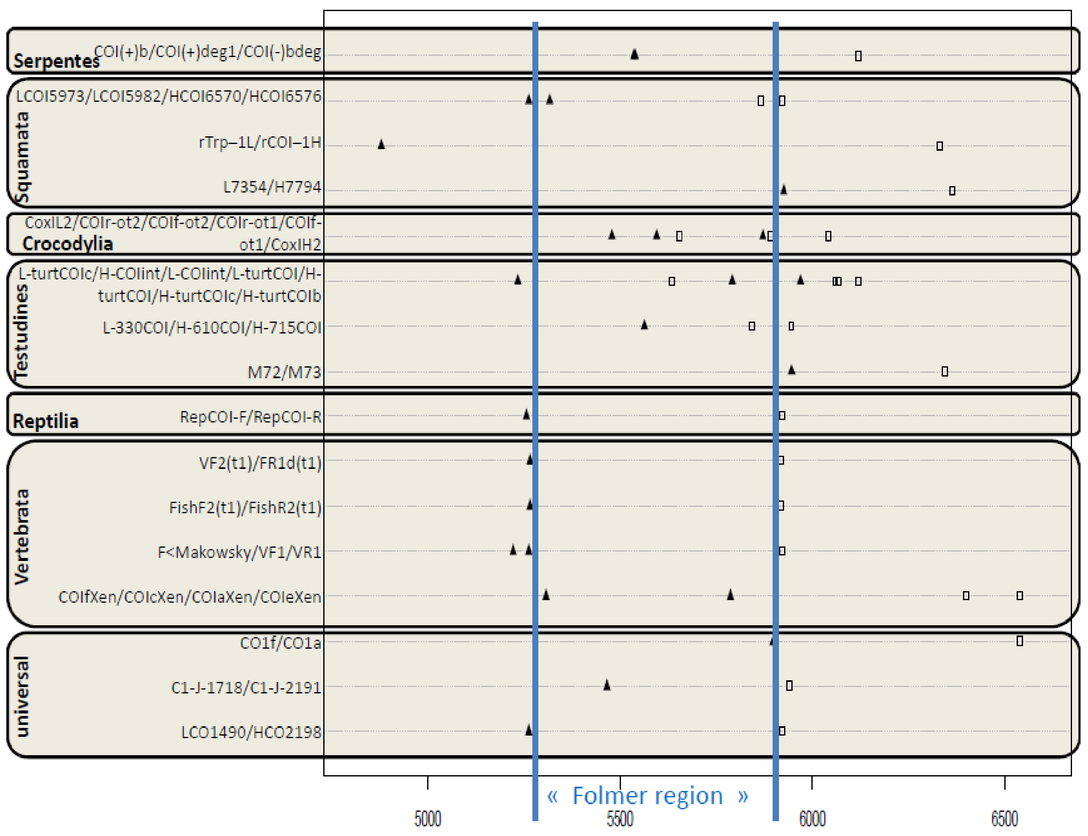
The DNA barcoding of animals is based on a fragment of the mitochondrial cytochrome oxidase I (COI) gene.





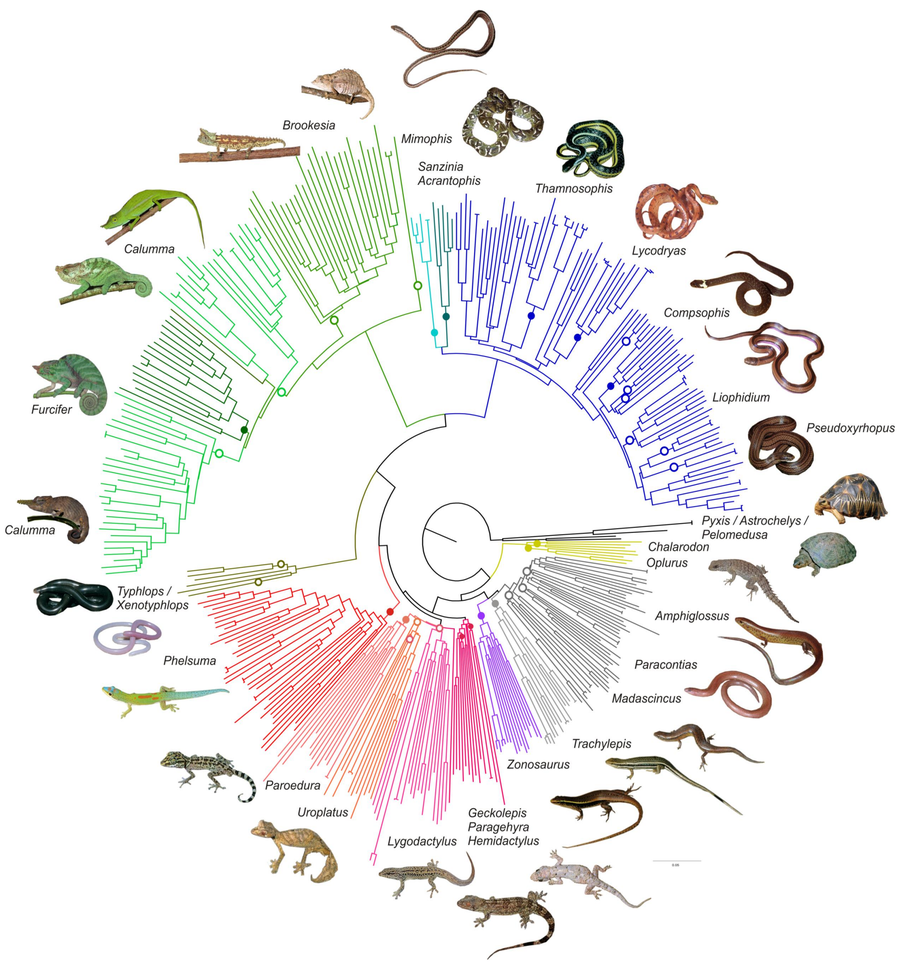
Results and discussion

Reptile species are typically old, strongly divergent, and contain deep conspecific lineages which might lead to problems in species assignment with incomplete reference databases. Furthermore, it can accentuate the problem of primer failure in single samples even within species or species complexes. Single primer pairs are likely to have a failure rate between 5-50% if taxa of a wide taxonomic range are targeted. In such cases the use of primer cocktails or subsequent hierarchical usage of different primer pairs is necessary.



If the target group is taxonomically limited, many studies have followed a strategy of designing specific primers which then allow an easy and reliable amplification of all samples. A compilation of available primers used for reptiles is given above.

We developed an easy-to-use PCR protocol including a newly developed primer pair enabling a rapid assessment of the samples. The success rate of the PCR amplification and DNA sequencing was constantly high (around 85%) even if having only degraded DNA available or minimal amount of tissue samples.



The use of these relatively short sequences succeeded in assigning samples correctly to the taxonomic groups of any ranks in case of Malagasy reptiles. Moreover, most families were well supported (consistently receiving high bootstrap values and posterior probabilities in neighbour-joining, maximum likelihood and Bayesian analyses, respectively).