Maximising the frog biodiversity surveys in looking at tadpoles

Amphibians are organisms very sensitive to environmental changes such as habitat lost, appearance of new pathogens, pollution and climatic changes among others. Facing these threats efficient conservation plans must be worked out to protect this fauna (as well as other plant and animal species) especially in areas where biodiversity is maximum, that is in the tropics. Setting up such conservation planning requires accurate information about species composition of the sites, as well as knowledge of distribution areas and ecological requirements of species. Tadpoles are usually overlooked from biodiversity surveys while they are usually easy to detect and collect, present in great number, and sometime the only evidence of the occurrence of a species at a given time. The main reason is that identification of tadpoles is extremely difficult.

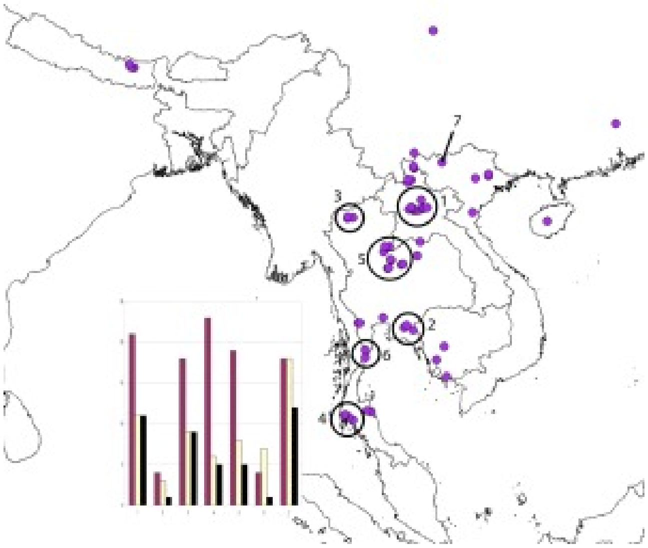

Fig. 1. Distribution of collecting localities for the samples analysed in this study. Numbers 1 to 7 refer to areas where an equivalent effort has been undertaken to collect both adults and tadpoles. The inserted histogram shows for each of the seven chosen localities the number of species collected in their adult stage (first column), the number of species collected in their tadpole stage (second column), and the number of species for which both adults and tadpoles were collected (third column).

Nevertheless their consideration in biodiversity surveys will optimize species count whereas the knowledge of their ecological and developmental requirements (dramatically different from that of adults) is crucial for the choice of habitats to protect. Since a decade a molecular taxonomic approach named barcoding propose to obtain a small part of the genome by sequencing and to compare it to the homologous region of a wide range of well-identified organisms from databases.

This very simple method allows to estimate the genetic divergence of the organism under scope with the species included in the database and then to identify it or to flag it as an unknown species (either because the species is not yet in the reference database or because it is a new species for science). In the case of tadpoles barcoding allows to accurately identify them.

In this study we compared the performance of two genes widely used in barcoding to identify tadpoles: the subunit 1 of the *cytochrome c oxidase* (or *COI*, a mitochondrial gene used for respiration) which has been proposed to be the universal barcoding marker and the *16S* rRNA gene (another mitochondrial gene coding for a cellular organelle, the ribosome), which has been advocated to be the standard barcoding marker for amphibians. We also estimated the rate of misidentification of tadpoles in the field and we estimated how tadpoles can improve the assessment of biodiversity.

For this we built our own barcoding database with the adults collected for ten years of fieldworks in Southeast Asia, a region of great biodiversity, and matched all the barcoding sequences of tadpoles available to us from the same sites.

From our data none of the two barcoding genes is “perfect” and each has its advantages and its drawbacks but the *16S* gene is advised for “local” barcoding (that is for barcoding all organisms of a site) because it ensures to obtain sequences for all the sample whereas the *COI* gene is recommended for “global” barcoding because it allow the building of huge databases gathering all organims.

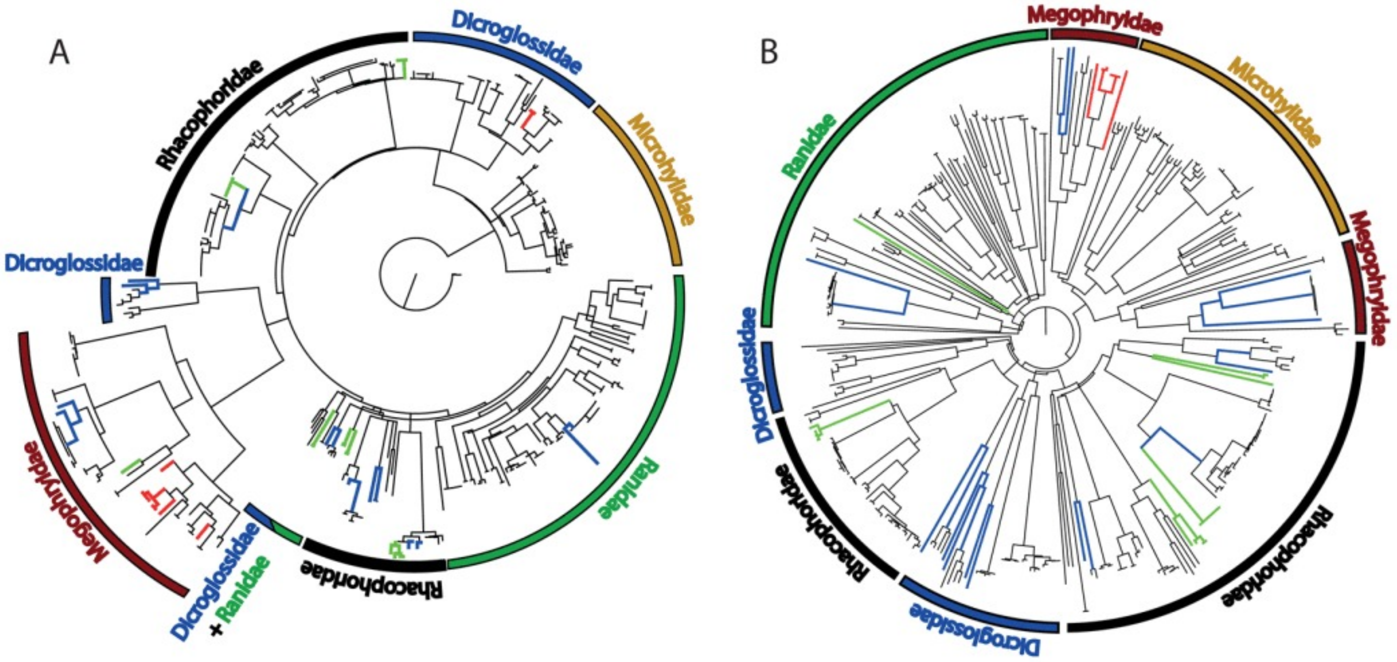

Fig. 2. Neighbour-Joining phylograms for the 16S barcode sequences (A) and the COI barcode

sequences (B). Blue branches indicate taxa with deep intraspecific genetic divergences that potentially represent several distinct species. Green branches indicate species represented only by their larval forms. Red branches indicate probable new species.

All tadpoles have been correctly assigned to their respective species except for one case of possible hybridization (a phenomenon which causes false barcoding results). About half of the collected tadpoles were badly identified or incompletely named in the field. The use of barcoding is then strongly recommended to any biologist for naming tadpoles. In most of the sites a variable but important proportion of species were represented only by their tadpoles (Fig. 1). As a consequence several tadpole sequences without corresponding adults were revealed stressing the importance to collect and take into account tadpoles in biodiversity survey (Fig. 2). At last this study detected 17 lineages (an increase of 20.5% of the initial number of species) which could be new species among which a new confirmed species detected by its tadpole only.

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