A Conservation Strategy for the Amphibians of Madagascar

Monografie del Museo Regionale di Scienze Naturali di Torino, XLV (2008): pp. 233-252

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Which frogs are out there? A preliminary evaluation of survey techniques and identification reliability of Malagasy amphibians

ABSTRACT

We provide an estimate of identification reliability of Malagasy frog species based on different methods. According to our estimate, for 168 out of 358 species, a reliable identification based on morphology alone is not possible for reasonably trained researchers. By also considering colouration in life, this number went down to 116 species. Of 252 species for which calls are known, a reliable identification based exclusively on bioacoustics is not possible for 59 species. DNA barcoding performs distinctly better; problems with molecular identification are only known for 61 out of 347 species for which genetic data are available.

In a second approach we also present preliminary data on a comparative study of performance of various inventory techniques applied to three frog communities along eastern rainforest streams. At these streams tadpole collection and their subsequent identification via DNA barcoding allowed for an average detection success of 45% of all species per site, while standardized call surveys detected 28% and visual encounter surveys 29% of the species. However, these results varied widely among rough ecological guilds of frogs, with forest frogs that breed independently from open water, obviously, being undetectable in the tadpole surveys, arboreal frogs being poorly detectable in visual encounter surveys, and stream edge frogs being very poorly detectable in bioacoustic surveys. We suggest that a combination of methods is necessary to obtain a maximum of positively and reliably identified species records in a limited amount of time, and we emphasize the extreme importance of increasing data verifiability by

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listing voucher specimens, and as much as possible, including DNA barcoding, call recording, and photographs in life. For a public and easy access to such supplementary data to any amphibian survey in Madagascar, creation of a joint website is recommended.

Key words: Rapid surveys, Amphibians, DNA barcoding, Identification.

INTRODUCTION

Inventories of Madagascar's amphibian fauna are a major prerequisite for any efficient conservation strategy focused on these organisms (Vallan, 2000; Andreone et al., 2005). Furthermore, inventories are the only means to obtain more complete information on the distribution and biogeography of Madagascar's amphibian species, and even are the main driver of the ongoing discovery of new species. Traditionally, amphibian surveys are carried out in a combination with surveys of the reptile fauna (Andreone, 2004), and the results of both are presented in the form of species lists per site (e.g., Andreone et al., 2000, 2001, 2003; Andreone & Randriamahazo, 1997; Andreone & Randrianirina, 2000; Nussbaum et al., 1999; Rakotomalala, 2002; Ramanamanjato & Rabibisoa, 2002; Raselimanana et al., 2000; Raxworthy & Nussbaum, 1996a,b; Raxworthy et al., 1998; Vences et al., 2002a). Many of these surveys are based on major expeditions with a permanence of sometimes several weeks per mountain massif or forest, with various campsites. In other cases fast inventories of a few days only, so called Rapid Assessments (RAPs) have been carried out even at remote sites. For instance, the MacArthur foundation has funded a rapid assessment program of Malagasy researchers, known as the "RAP Gasy" (A. Raselimanana, pers. comm. in 2006) which over the past years has allowed herpetological surveys of numerous understudied sites in Madagascar, although most of these results are not yet published. There is no major methodological difference between long-term surveys and RAPs except for the study time at a particular site, but short-term studies are often logistically easier in remote areas and an efficient and precise inventorying methodology is particular important in such cases.

However, the existence of multiple sibling species of difficult morphological identification (e.g., Glaw et al., 2001; Köhler et al., 2005; Vences et al., 2002b) and the fast taxonomic progress in understanding the species diversity of this fauna (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 2000, 2003, 2006) casts doubts on the efficiency of the common amphibian survey practice in Madagascar and claims for comparisons of various detection and identification techniques, and for the development of precise recommendations to carry out surveys in the most cost- and time-effective way, and simultaneously to fully ensure data verifiability.

We here present first data from an ongoing project to develop specific recommendations for the most suitable methods for surveys of Madagascar's

amphibian fauna. We estimated the proportions of Malagasy frog species that can unambiguously be identified based on external morphology, morphology plus colour, bioacoustics, and DNA barcoding, and we present data on the survey efficiency of visual encounter, bioacoustic and tadpole-based methods along three short transects in Madagascar's eastern rainforests.

MATERIALS AND METHODS

To obtain estimates on the reliability of identification of Malagasy frogs, we compiled a list of all described species (ca. 232, based on the list of Glaw & Vences, 2003, plus subsequent descriptions) and of a large number of undescribed species which are well enough defined to include them in this analysis (i.e., in most cases by either a highly divergent advertisement call, or by a highly divergent DNA sequence accompanied with at least subtle differences in morphology, colouration, or call). The total number of species included was of 358. For each species we evaluated its similarity to its closest relatives and its morphological variability within and between populations, and estimated whether a moderately trained observer would be able to unambiguously assign a single and well-preserved adult male specimen (males being the specimens with the maximum amount of diagnostic characters such as vocal sacs, femoral glands, etc), without information on the precise locality of provenance, to this species, based on external morphology, or on morphology plus colouration in life. Based on call recordings published by Vences et al. (2006) we also compared for each species where data are available whether the calls can be distinguished from the most similar calls of other species. Large databases of the mitochondrial 16S rRNA gene for almost all species of Malagasy frogs (Vences et al., 2005a,b) were furthermore analyzed to assess whether particular species can be easily identified via DNA barcoding or if potential problems may occur due to known instances of haplotype sharing or of paraphyletic species (see Funk & Omland, 2003).

To obtain comparative data on the efficiency of various survey methods, fieldwork was carried out in February 2006 at various sites in eastern Madagascar and is scheduled to continue in the forthcoming years; in this paper, we present results from three of these sites from where reasonably complete and representative data sets are already available: (1) Imaloka forest in Ranomafana National Park, a stream in largely undisturbed mid-altitude rainforest, surveyed on 23 February 2006 (21°14.527' S, 47°27.909' E, 1020 m above sea level); (2) a stream in highly degraded low-altitude forest along the road from Ifanadiana towards Tolongoina, about 6 km from Ifanadiana, surveyed on 22 February 2006 (21°21.215' S, 47°36.467' E, 468 m a.s.l.); (3) a stream in the largely undisturbed mid-altitude rainforest of An'Ala near Andasibe, surveyed on 8 February 2006 (18.91926°S, 48.48796° E, 889 m a.s.l.). The amphibian community of this latter site has also been studied by Vallan et al. (2004).

At these sites, three different survey types were performed along stream transects of 50 or 100 m. Firstly, 1-2 researchers experienced in tadpole collection and identification collected tadpoles along these transects during daytime for 30-60 minutes, anesthetized them using chlorobutanol solution, and sorted them into series of morphospecies, creating duplicate series especially of the most commonly encountered tadpoles to increase the detection probability of species with similar tadpole morphology. Of each series, a tissue sample was taken from one individual, the whole series preserved in 4-6% formalin, and the DNA voucher specimen identified via DNA barcoding (for detailed descriptions of the methodology employed, see Thomas et al., 2005 and Vences et al., 2005a,b). Secondly, in the evening (roughly within the period of 19-21 h), one researcher experienced with bioacoustic recordings followed the same stream transect and recorded all sounds heard, pointing the microphone both randomly and in the directions of calling frogs, for 10-20 minutes. The recordings were analyzed using the software Cooledit (Syntrillium corp.) and a list of all frogs heard calling was compiled. Thirdly, a group of 3-6 experienced researchers followed the same stream transect for ca. 30 minutes and collected all encountered frogs, by randomly searching on leaves, in the water, on the banks of the stream, and in the adjacent forest to a distance of ca. 3 m from the stream. In this, calls were used as a guidance, but no extreme effort was directed towards collecting frogs calling from difficult positions, e.g., high in the canopy.

At all three sites, additional surveys were carried out on the days before and after the standardized inventories, and we considered all frog species encountered at one site in 2006 by all methods as the frog community present at the time of inventory. Species likely to occur at the site as well, or species encountered at the sites in previous years, were not considered to avoid too heavy biases in our data sets (well-known vs. less well-known sites). Inventory success was measured as the percentage of all species in the community that were detected using one of the three methods described above. We are aware that in such an approach, the data analysed are not independent from the test dataset. More thorough approaches in which the fauna occurring at a site will first be determined by a comprehensive survey, and subsequently the different methods tested against this dataset, are in progress. Considering this caveat, we refrained from performing any statistical analysis of our data.

To understand the dependence of detectability of particular frog species from their general habits, we divided the encountered frog species into four simplified ecological guilds: (1) treefrogs, that is, species that predominantly or exclusively are arboreal, living in bushes or trees and calling from the vegetation along lentic or lotic water bodies; (2) stream edge frogs: species that are terrestrial to semi-aquatic and are mainly found along streams, some species in the water or directly at the edge, some species at some meters from the streams in the leaf litter, all reproducing in the stream; (3) pond edge frogs: species that reproduce in ponds and outside of the reproductive season

occur either close to these water bodies or sometimes dispersed in the forest. A last, rather heterogeneous category is (4) forest frogs: these are species that do not reproduce in open lentic or lotic waterbodies and therefore usually occur relatively evenly spaced in the forest, although sometimes they are more common along streams. This category includes tree-hole breeders as well as species with putative direct development. We wish to emphasize that this categorization (as applied in Tab. I-III) is not based on any explicit analysis and is merely used as a convention to be able to refer to groups of frogs with roughly similar habits. A proper definition of ecomorphological guilds of adult frogs is highly needed but lies beyond the scope of the present paper.

RESULTS

A summary of our estimates of identification reliability is given in Fig. 1. As expected, the data show that a large number of frogs cannot be reliably identified using morphology alone. In fact this applied to 168 species, almost half of the total of species included in our analysis. If morphology was combined with colour in life, we still estimate that 116 species cannot be reliably identified if only single male specimens without locality and call data are studied. Also bioacoustic characters are not estimated to provide alone a clear diagnosis: of 252 species for which call data are available, 59 cannot be reliably identified to species level based on calls alone. DNA barcoding performs distinctly better; problems with molecular identification are only known for 61 out of 347 species for which genetic data are available.

The performance of surveys based on tadpole capture, bioacoustics and visual encounters at the three study sites are summarized in Fig. 2. Original data are given in Tables 1-3. At the three study sites Imaloka, Ifanadiana and An'Ala the total number of inventoried frog species was 30, 20 and 52. At all three sites, arboreal frogs were the majority, with 57%, 40% and 44% of all species recorded, followed by stream edge species which made up 33%, 35% and 30%, and forest species which made up 10%, 20% and 21%.

The different survey techniques performed with different success in these three ecological guilds (Fig. 3). Standardized bioacoustic surveys provided records of about one-third of the arboreal and forest species but no single record of any stream edge species. Visual encounter surveys (data only for Imaloka and Ifanadiana) were successful for stream-edge species, with an average of almost one-half of all species recorded, but performed poorly for arboreal and forest species (about one-sixth). Tadpole surveys were most successful, with over one-half of all species detected in arboreal and stream edge species, but with an extremely low but existing success for forest frogs. The latter result was highly surprising, since by definition forest frogs were not supposed to have free-living tadpoles in the streams. Nevertheless, at An'Ala

Species	Guild	Tadpoles	Calls	Visual Encounter
Boophis boehmei	arboreal	12	+	-
Boophis bottae	arboreal	-	+?	-
Boophis sp. aff. *goudoti*	arboreal	1	-	-
Boophis elenae	arboreal	8	-	-
Boophis luteus	arboreal	2	-	-
Boophis madagascariensis	arboreal	2	-	-
Boophis majori	arboreal	3	-	-
Boophis marojezensis	arboreal	1	+	4
Boophis picturatus	arboreal	-	+	-
Boophis pyrrhus	arboreal	3	-	-
Boophis reticulatus	arboreal	-	+	10
Boophis sibilans	arboreal	2	-	2
Boophis sp. aff. *sibilans*	arboreal	2	+	-
Boophis tasymena	arboreal	-	-	1
Gephyromantis sculpturatus	forest	-	-	-
Guibemantis tornieri	arboreal	7	-	-
Heterixalus alboguttatus	arboreal	-	-	-
Mantidactylus aerumnalis	stream edge	-	-	-
Mantidactylus sp. aff. *betsileanus*	stream edge	4	-	-
Mantidactylus sp. aff. *biporus*	stream edge	-	-	-
Mantidactylus femoralis	stream edge	-	-	1
Mantidactylus grandidieri	stream edge	-	-	1
Mantidactylus lugubris	stream edge	5	-	-
Mantidactylus majori	stream edge	27	-	15
Mantidactylus melanopleura	stream edge	1	-	-
Mantidactylus sp. aff. *mocquardi* 1	stream edge	1	-	-
Mantidactylus sp. aff. *mocquardi* 2	stream edge	2	-	-
Plethodontohyla sp. aff. *brevipes* 1	forest	-	-	-
Plethodontohyla sp. aff. *brevipes* 2	forest	-	-	-
Spinomantis aglavei	arboreal	-	+	-

Tab. 1. Frog species recorded from Imaloka study site (Ranomafana National Park) during our 2006 survey, their simplified ecological guild, and the efficiency of three different standardized survey methods in their detection along a stream transect of 50 m are indicated: (a) DNA-based identification of tadpole series collected during the day during ca. 30 minutes along the transect; (b) nocturnal bioacoustic recording of 10 minutes along the transect; (c) nocturnal visual encounter survey during 30 minutes along the transect. The table shows the number of tadpole series assigned to a particular species by DNA barcoding, and the number of metamorphosed frog specimens of each species collected during the visual encounter surveys. Bioacoustic data were not analyzed quantitatively. A "+" in the "Calls" column indicates existence of at least one positively identified call record for that species. Surveys were carried out on 23 February 2006.

Species	Guild	Tadpoles	Calls	Visual Encounter
Anodonthyla boulengeri	forest	-	+	1
Blommersia grandisonae	arboreal	-	-	-
Boophis albilabris	arboreal	1	-	-
Boophis madagascariensis	arboreal	3	+	2
Boophis opisthodon	arboreal	-	-	-
Boophis sp. aff. *rappiodes*	arboreal	-	-	-
Boophis pyrrhus	arboreal	1	+	4
Gephyromantis boulengeri	forest	-	+	-
Gephyromantis sculpturatus	forest	-	+	-
Guibemantis timidus	arboreal	-	-	-
Heterixalus alboguttatus	arboreal	-	-	-
Mantidactylus aerumnalis	stream edge	2	-	
Mantidactylus betsileanus	stream edge	-	-	1
Mantidactylus sp. aff. *betsileanus*	stream edge	1	-	
Mantidactylus femoralis	stream edge	-	-	2
Mantidactylus grandidieri	stream edge	-	-	-
Mantidactylus majori	stream edge	-	-	3
Mantidactylus melanopleura	stream edge	-	-	1
Ptychadena mascareniensis	pond edge	-	-	-
Stumpffia sp.	forest	-	+	-

Tab. 2. Frog species recorded from Ifanadiana study site (near Ranomafana) during our 2006 survey, their simplified ecological guild, and the efficiency of three different standardized survey methods in their detection along a stream transect of 100 m are indicated: (a) DNA-based identification of tadpole series collected during the day during ca. 30 minutes along the transect; (b) nocturnal bioacoustic recording of 10 minutes along the transect; (c) nocturnal visual encounter survey during 30 minutes along the transect. The table shows the number of tadpole series assigned to a particular species by DNA barcoding, and the number of metamorphosed frog specimens of each species collected during the visual encounter surveys. Bioacoustic data were not analyzed quantitatively. A "+" in the "Calls" column indicates existence of at least one positively identified call record for that species. Surveys were carried out on 22 February 2006.

we identified several series of tadpoles of *Gephyromantis asper,* previously supposed to have endotrophic development (Blommers-Schlösser, 1979a). This result, which demonstrates the power of tadpole DNA barcoding to understand the life-history of anurans, will be presented and discussed more in detail elsewhere. Averaged over all localities and guilds, tadpole surveys recorded an average of 45% of the species, standardized call surveys recorded 28% of the species, and visual encounter surveys recorded 29%.

Species	Guild	Tadpoles	Calls
Aglyptodactylus madagascariensis	pond edge	-	-
Blommersia blommersae	arboreal	-	-
Blommersia grandisonae	arboreal	-	-
Boophis albilabris	arboreal	2	-
Boophis boehmei	arboreal	9	-
Boophis bottae	arboreal	-	-
Boophis burgeri	arboreal	1	-
Boophis elenae	arboreal	1	-
Boophis liami	arboreal	1	-
Boophis lichenoides	arboreal	1	-
Boophis luteus	arboreal	4	-
Boophis madagascariensis	arboreal	5	-
Boophis marojezensis	arboreal	7	+
Boophis picturatus	arboreal	2	+
Boophis pyrrhus	arboreal	8	+
Boophis reticulatus	arboreal	4	+
Boophis rufioculis	arboreal	17	+
Boophis sibilans	arboreal	2	+
Boophis sp. aff. *sibilans*	arboreal	-	-
Boophis tasymena	arboreal	6	+
Gephyromantis asper	forest	2	-
Gephyromantis boulengeri	forest	-	-
Gephyromantis redimitus	forest	-	+
Gephyromantis sculpturatus	forest	-	-
Guibemantis depressiceps	arboreal	-	-
Guibemantis liber	arboreal	-	-
Guibemantis tornieri	arboreal	1	-
Guibemantis sp. aff. *albolineatus*	forest	-	-
Guibemantis pulcher	forest	-	-
Mantella baroni	stream edge	-	-
Mantella pulchra	stream edge	-	-
Mantidactylus aerumnalis	stream edge	13	-
Mantidactylus albofrenatus	stream edge	1	-
Mantidactylus argenteus	stream edge	6	-
Mantidactylus betsileanus	stream edge	1	-
Mantidactylus sp. aff. *betsileanus*	stream edge	-	-
Mantidactylus sp. aff. *biporus*	stream edge	-	-
Mantidactylus femoralis	stream edge	8	-
Mantidactylus grandidieri	stream edge	-	-
Mantidactylus lugubris	stream edge	-	-

Mantidactylus melanopleura	stream edge	13	-
Mantidactylus sp. aff. *mocquardi*	stream edge	5	-
Mantidactylus opiparis	stream edge	16	-
Mantidactylus sp. aff. *tricinctus*	stream edge	1	-
Mantidactylus zolitschka	stream edge	3	-
Platypelis barbouri	forest	-	-
Platypelis cf. *pollicaris*	forest	-	-
Platypelis tuberifera	forest	-	-
Plethodontohyla notosticta	forest	-	-
Plethodontohyla inguinalis	forest	-	-
Ptychadena mascareniensis	pond edge	-	-
Spinomantis aglavei	arboreal	1	+

Tab. III. Frog species recorded from An'Ala during our 2006 survey, their simplified ecological guild, and the efficiency of three different standardized survey methods in their detection along one stream transect of 50 m are indicated: (a) DNA-based identification of tadpole series collected during the day during ca. 30 minutes along the transect; (b) nocturnal bioacoustic recording of 10 minutes along the transect; no data from nocturnal visual encounter surveys are available from this site. The table shows the number of tadpole series assigned to a particular species by DNA barcoding. Bioacoustic data were not analyzed quantitatively. A "+" in the "Calls" column indicates existence of at least one positively identified call record for that species. Surveys were carried out on 8 February 2006.

DISCUSSION

Identification verifiability - a main theme for surveys

In species inventories and rapid assessments, amphibians and reptiles are usually inventoried together and included as a joint list in the corresponding report or publication. Although the search for calling males is certainly employed by most researchers in the field for frogs, the calls themselves are rarely used for species identification. However, as compared with reptiles, a main problem is the rareness of well-defined morphological characters in amphibians (e.g., Duellman, 1970; Glaw et al., 2001).

The poor performance of bioacoustics in species identification as reported here requires some additional comments, as bioacoustic characters have proven to be an excellent tool in diagnosing new frog species from Madagascar since the pioneering works of Blommers-Schlösser (1979a,b). In fact, the presence of constant bioacoustic differences between two frog species is a reliable indicator for specific distinctness. In general, sympatric species always differ distinctly in their calls. However, and this is reflected by the analysis here, instances of (almost exclusively) allopatric species exist where high genetic and morphological divergences clearly support a status as different species although their calls are still similar. In these cases, a species

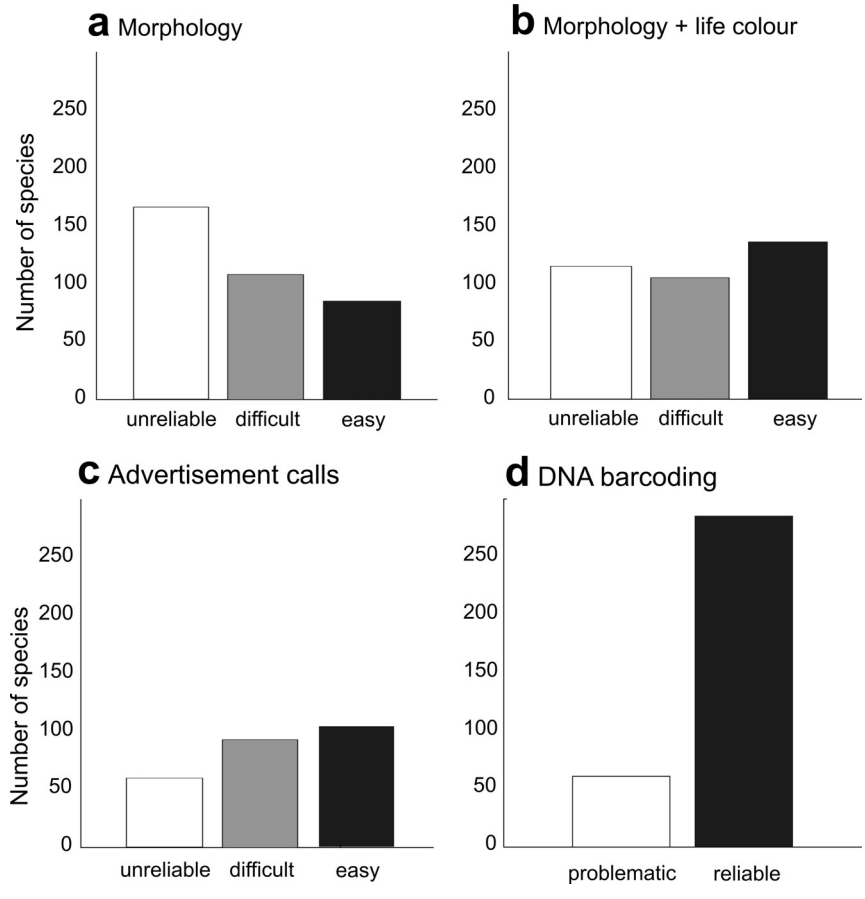

Fig. 1. Estimates of identification reliability for a total of 358 described and undescribed Malagasy frog species (not all species included in each separate estimate, depending on data availability). (a) numbers of species that can be identified, or not, from all other species using morphology of preserved specimens as only character set; (b) numbers of species that can be identified using morphology plus information on colour in life; (c) numbers of species that can be identified based only on call recordings; (d) numbers of species that can be identified using DNA barcoding. In (a) and (b), identification is considered easy if well-preserved adult male specimens can be determined at first glance, looking at only a few distinct characters or colour patterns, difficult if examination of hidden or small characters, or body proportions are necessary, and unreliable if overlap of character values compared to other species occurs or no diagnostic morphological characters are known. In (c), identification is considered easy if calls can immediately recognized by the human observer, difficult if analysis of temporal or spectral patterns in sonograms is necessary, and unreliable if calls are overlapping in all values with those of other species. In (d), identification is considered problematic if instances of haplotype sharing with other species are known, genetic divergences to other species are very low and haplotype sharing is to be expected, or if species are paraphyletic based on their mitochondrial phylogeny or nested within other paraphyletic species.

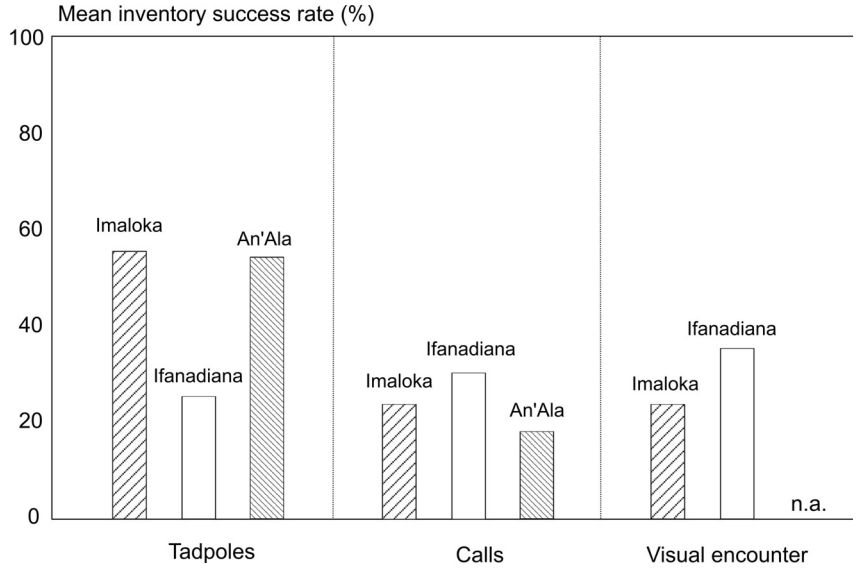

Fig. 2. Mean rate of success in standardized inventories along rainforest streams as described in the text at three sites (Imaloka, Ifanadiana and An'Ala), in percent of the total number of species known from each site. Values are given for three different inventory methods: tadpole surveys based on DNA barcoding identification, bioacoustic surveys, and visual encounter surveys. No visual encounter survey results are available for An'Ala.

identification based solely on advertisement calls must remain unreliable, although in concert with morphological data and/or locality information a better performance can be attained. In general, it remains true that a careful analysis of morphology, colouration in life and advertisement calls would allow to clearly diagnose almost all Malagasy frog species.

It also needs to be remarked that our estimates of identification reliability are based on own experience, and that in some groups, after very intensive study, it would probably become possible to elaborate morphological keys that lead to species identification of high reliability. However, we doubt that such keys would be applicable by less specialized researchers, and certainly such identification would require a very time-consuming study of various morphological characters and possibly morphometric ratios.

Our data on the difficulties in morphological identification of Malagasy frog species, plus the rapid taxonomic changes to which this fauna is currently subjected, have strong implications on the common practice on reporting the results of amphibian surveys in Madagascar in the form of mere species list. Except for a few easily recognizable species, we here make the

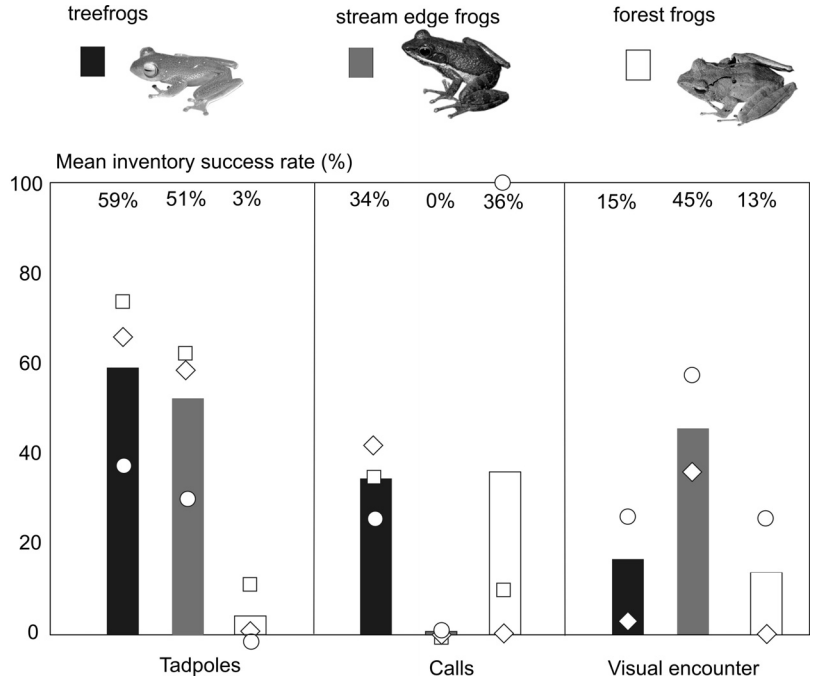

Fig. 3. Mean rate of success in standardized inventories along rainforest streams as described in the text, averaged over three sites (see Fig. 2), and given separately for three tentative ecological guilds of frogs: arboreal frogs (treefrogs), frogs living mainly on the edge of streams, and frogs living mainly dispersed in the forest. Values are given for three different inventory methods: tadpole surveys based on DNA barcoding identification, bioacoustic surveys, and visual encounter surveys. Symbols represent single data points from Imaloka (rhomboid), Ifanadiana (circle) and An'Ala (square).

drastic statement that these lists are almost worthless for amphibians, although such problems occur only to a much lower degree for reptiles. To allow verifiability of such survey data in amphibians, we encourage a practice in which the species lists are accompanied by a list of voucher specimens deposited in an accessible public collection. Collection of tissue samples clearly assignable to individual specimens, and sequencing of a standardized gene fragment from these tissue samples, would be a further ideal complement, and we envisage a future in which standardized DNA isolation and PCR for this purpose can be done in Madagascar, with a commercial or institutional outsourcing of the sequencing. Species lists in publications could then be accompanied by the Genbank accession numbers of the obtained sequences. Furthermore, it would be an enormous improvement if the

accompanying data, such as DNA sequences, specimen photographs, and call recordings, would be made available via a centralized website.

The advantages of a DNA-based identification system have often been emphasized (e.g., Savolainen et al., 2005). DNA sequences deposited in Genbank can be easily and quickly retrieved from any part of the world via internet, and directly and unambiguously compared to homologous sequences obtained by other research groups. Identifications even of juveniles or of not collected specimens can therefore be verified, also by researchers in Madagascar despite the less developed local laboratory infrastructure. Morphology-based identification, on the contrary, in a group as diverse and complex as Malagasy frogs is only possible by specialized researchers after intensive morphological training.

Capacities for DNA based identification in Malagasy laboratories

If a DNA based identification system is to be implemented for amphibian surveys in Madagascar, the financial costs are to be considered as well. At the time of writing the current article, no DNA sequencing facility exists at Madagascar, and DNA sequencing is commercially accessible for 3 EUR in some countries. Costs of DNA isolation, PCR and PCR purification can be estimated at a maximum of 2 Euro, although distinctly lower costs can be achieved in high-throughput systems. Altogether, a standardized marker sequence can therefore be obtained for 5-6 Euro in a relatively easy setup that at least partly could function under local conditions in Madagascar. However, automated DNA sequencers are not only extremely expensive machines but also require regular maintenance that is not available in Madagascar. Even without maintenance costs, it would be difficult to achieve sequencing costs as low as those of commercial companies if such a machine would be installed in Madagascar. Under current conditions and technical possibilities, we suggest a system in which DNA isolation and PCR would be carried out in Madagascar and the sequencing itself would be outsourced to commercial companies.

Perspectives and suggested methods for amphibian surveys in Madagascar

Besides the general suggestions for data presentation and listing of voucher specimens outlined above, there are also some obvious recommendations for field techniques in surveys following out of our results. The very good performance of tadpole surveys is encouraging and indicates that standardized tadpole collection should be included in any future species inventory study of Malagasy amphibians, also considering the importance of these larval amphibians for stream ecosystems (e.g., Whiles et al., 2006). At present we lack information on the comparative performance of the various techniques in the dry season, but we believe that the advantages of tadpole surveys are their relative independence from climatic and weather conditions: it should be possible to perform successful tadpole inventories both in dry intervals during the rainy season, and indeed during the dry season, when

calling activity, and reproductive activity in general, of most species is strongly reduced and bioacoustic and visual encounter methods will fail to produce sufficient data for the arboreal species. The drawback of tadpole inventories, i.e., the need for routine application of molecular techniques, is a challenge that should be overcome by a major institutional effort rather than by isolated efforts of each single research group.

Furthermore, although bioacoustic methods were not highly successful in our study, they still provide an easy means to reliably identify a large proportion of the arboreal species, and especially of the forest species that mostly do not have free-swimming tadpoles.

Besides tadpoles and bioacoustics, the need for collecting the visually encountered adult frog specimens is obvious: on one hand, these are important as voucher specimens for possible future morphological comparisons or taxonomic studies. On the other hand, for stream edge frogs, visual encounter collecting proved to be an efficient survey technique according to our results.

If surveys are carried out over longer periods, i.e., a week or more, it is likely that a large number of the frog community will be detected and voucher specimens collected even without tadpole surveys or bioacoustic recordings. Hence, it would seem that in such cases, the classical methodologies are sufficient. However, due to the low identification reliability if diagnosing the collected frogs based on their morphology alone, also in such cases the resulting list of species would be likely to be partly unreliable, incomplete and unverifiable. Therefore, also in such longer surveys, recording of calls and routine collection of DNA samples of every collected frog individual should be implemented.

As emphasized in the title and introduction, the data presented here are merely the first results of a more comprehensive comparison of survey techniques that will be carried out within the next years. Furthermore, in the present study we focused on survey techniques along streams, largely ignoring pond frogs and not exploring specific techniques for forest frogs living in leaf axils and tree holes, or in the leaf litter (for the latter group, for example, pitfall trapping is an important survey method). While developing a precise protocol for amphibian surveys is beyond the scope of this paper and would be premature at the present stage, we can advance here that a combination of call recordings, collection of voucher specimens of adult and larval stages, and DNA barcoding will provide a cost-effective means to obtain quick and verifiable inventories of Madagascar's amphibians.

Lessons for frog monitoring in Madagascar

The data presented here were directed towards the development of a more efficient methodology for surveys of the amphibian species diversity at particular sites in Madagascar. Such surveys are and will remain extremely important to understand the status of particular sites and to prioritize conservation efforts. For remote sites, rapid surveys will remain the only

feasible option if many localities are to be surveyed in a limited time. However, a second important need is the establishment of a regular monitoring of a number of representative sites in Madagascar: on one hand, to understand population dynamics and status of threatened or commercialized species, on the other hand to understand community dynamics and possible declines, especially in the light of a possible spread of emerging diseases such as chytridiomycosis (e.g., Lips et al., 2006). The protocols used herein are not or only in a limited way directly applicable for such monitoring of communities, but still there are a number of particularities that need to be considered when specific monitoring protocols are to be developed. (1) The high number of arboreal species along streams in Madagascar's rainforests makes it difficult to apply any pure visual encounter technique, since these frogs often call from high in trees, and are not usually encountered when not reproducing. (2) The low-intensity calls of most stream-edge frogs will not be captured sufficiently by approaches relying solely on automated bioacoustic recording (see Peterson & Dorcas, 1994). (3) Although tadpole identification turned out to be the most efficient survey method in our study, this technique relies on routine regular application of DNA sequencing and killing of large number of specimens, and such methods will usually not be applicable to long-term monitoring approaches. (4) Although bioacoustics performed poorly in our results, it remains an excellent and straightforward method to detect arboreal species during the breeding season, and it is a very reliable identification technique if applied at particular sites where the amphibian fauna is known. (5) The problems in identification reliability make an initial intensive inventory of any monitoring site necessary, of which DNA barcoding would be a crucial component to allow subsequent allocation of the monitoring results to changing taxonomies. As a conclusion, approaches to monitor amphibian communities in Madagascar's rainforests need to apply an initial inventory carried out by experts, and subsequently should apply a combination of bioacoustic and visual encounter techniques to detect all major ecological guilds of frogs.

ACKNOWLEDGEMENTS

We are grateful to the Malagasy authorities for issuing research permits. Emile Rajeriarison and Theo Rajaofiarison are to be acknowledged for their help in the field. Special thanks are due to Franco Andreone for taking the initiative for ACSAM workshop and to publish this proceedings volume, and to Conservation International for funding and organizing the workshop. Fieldwork was supported by the Volkswagen Foundation. Field work of YC was supported by an Ambassadorial Fellowship of the Rotary Foundation. DRV is currently funded by the NSF ATOL Grant EF-0334939.

RÉSUMÉ

Quelle grenouille y a-t-il là-bas? Une évaluation préliminaire des techniques d'inventaire et de la fiabilité d'identification des amphibiens malgaches.

Cette étude présente une estimation de la fiabilité des différentes méthodes d'inventaire et de détermination des espèces de grenouilles malgaches sur le terrain. Ainsi nous présentons les premières données d'une étude comparative de différentes techniques d'inventaire des communautés de grenouilles vivant aux alentours des ruisseaux de la forêt tropicale humide de Madagascar. D'après notre évaluation, une identification crédible basée seulement sur la morphologie n'est pas possible même pour les spécialistes en herpétologie pour 168 sur 358 espèces. En incluant la coloration en vie, ce nombre régresse jusqu'à 116 espèces. Parmi les 252 espèces dont leurs vocalisations sont connus, une identification fiable exclusivement basée sur la bioacoustique n'est pas possible pour 59 espèces. La séquence d'ADN leur permet une meilleure détermination, car seulement 61 sur 347 espèces dont les données génétiques sont disponibles, ont des problèmes avec l'identification moléculaire. Dans trois différents ruisseaux de la forêt tropicale humide de l'Est de Madagascar, la collection des têtards suivie par leur identification par le biais de leur séquence d'ADN a permis de détecter les 45% des espèces inventoriées par site, tandis que l'écoute des cris et l'observation aléatoire des adultes ont permis de découvrir respectivement 28% et 29%. Cependant, ces résultats sont largement variés suivant le type écologique des grenouilles: les grenouilles forestières qui vivent indépendamment des plans d'eau n'ont pas été décelable dans les études de têtards, les grenouilles arboricoles ont été pauvrement dépistable par l'observation aléatoire, et les grenouilles vivant au bord du ruisseau ont été très pauvrement détectable par la bioacoustique. Nous suggérons donc qu'une combinaison de ces trois méthodes soit nécessaire pour obtenir un nombre maximum d'espèces qui sont bien déterminées dans un intervalle de temps limité et nous signalons également l'importance majeure de la vérifiabilité des données par l'existence des spécimens de référence, en incluant autant que possible la séquence d'ADN, l'enregistrement de cris et les photos de l'animal en vie. La création d'un site web commun est recommandée afin de permettre un accès facile et public à de telles données supplémentaires concernant toutes les études des amphibiens de Madagascar.

Mots clés: Inventaire rapide, Amphibiens, séquence d'ADN, Identification.

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