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## Vouchering, integrative taxonomy and natural history collections: a case study with the amphibians of Madagascar

### SUMMARY

Natural history collections have played an important role in the origin of natural history museums. Over the time these institutions have become places where “voucher specimens” and other kinds of natural history objects were preferably deposited. In this paper we further highlight the importance to consider collecting biological specimens as a useful / needed action for the expansion and diffusion of scientific collections, as well as for advancing sciences and, indirectly, conservation. We also report on a recent trend of underestimating the need of voucher specimens and provide evidences that the collection of a selected number of individuals as voucher specimens is necessary to contribute to such a goal and duty of cataloguing the world biodiversity and promote its conservation. Voucher specimens play the crucial function – among the others - to confirm the presence of a species at a locality at a given time, document the original ecological and biological diversity before change, can enable long-term studies and comparisons (i.e., using for example morphological or molecular tools) and can serve as a basis for ecological studies. Specifically, we used the amphibians of Madagascar as a case-study to highlight the need to continuing the collection activities, showing that their collection has been crucial to the cataloguing of Malagasy biodiversity and thus moving towards their conservation. The use of ancillary data alone (including photographs, videos, acoustic recordings, and genetic analysis carried out on tissue samples), frequently considered in alternative to vouchering, although useful, remains insufficient.

*Keywords:* biodiversity, collections, natural history museums, voucher specimens.

### INTRODUCTION

With an estimate of about 8.7 million of eukaryotic species present on the Earth, of which barely two millions are currently described (Mora *et al.*, 2011; but see Wiens, 2023), field surveys and taxonomic assessments are still necessary to fill

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the knowledge of our planet's biodiversity and the exploration and discovery of nature and its biota (Dijkstra, 2016).

As a common and widespread practice in studies aiming to assess the biodiversity in specific areas (particularly in little known territories), a small number of individuals found during the field surveys are usually collected, sacrificed, and preserved as “vouchers” or “voucher specimens” (Funk *et al.*, 2005). Vouchers are crucial not only for the description of new taxa (i.e., becoming part of the typical series and providing the biological standard that determines the application of a name; i.e. name-bearing types), but also for assuring a wide set of information on collected organisms and/or biological communities (i.e., longevity, anatomy, pathogenic presence, alimentation, and ecology), and when associated with other metadata they may provide the opportunity of witnessing species presence in precise places and times (Clemann, 2013; Clemann *et al.*, 2014). By using preserved specimens it is also possible to investigate, among the other things, the trend of the distribution range through times with comparison analysis with new up dated information.

The collections of “mirabilia”, “naturalia”, and “exotica” as natural history objects were often called in the 16<sup>th</sup> century, were originally assembled to show the “wonders” of the natural world (Beck, 2018). This became more explicit in coincidence with the travels around the world of naturalists in the 19<sup>th</sup> and 20<sup>th</sup> centuries (Valdecasas & Correas, 2010). In parallel, specimens increasingly became the basic reference for species descriptions and taxonomic revisions (West, 1990), as well as to understand and witness their variability, ecology and habitat preferences. More recently, collections also acquired a more prominent role in understanding the changes occurring on our planet, being part of the scientific process of data accumulation. For example, old voucher series are also useful to unveil the effects of global warming, anthropogenic impacts, or determining origins of infectious diseases on species or ecosystems (i.e., Fischer, 2015; Byrne *et al.*, 2019). When associated and compared to newly collected specimens they allow to compare time series and provide a vast array of information.

Natural history museums (NHMs, from here onwards) are the preferred places where natural history collections and specimens are housed and valorized (Salick *et al.*, 2014; Schilthuizen *et al.*, 2015; Funk *et al.*, 2018), although they may also be deposited in biorepositories in universities, agencies, and associations. It is therefore clear that NHMs, which are the direct descendants of the 17<sup>th</sup> century's “Wunderkammern”, progressively became engaged in scientific documentation, research, and awareness (Butler *et al.*, 1998; McCarter *et al.*, 2001; Andreone *et al.*, 2022) and over time became important dissemination hubs, where outreach, education, and research meet. Today, many large (mostly state or national) museums further evolved into study centers to support research and science dissemination, and document the biodiversity of the natural world: personnel charged with managing and preserving collections are usually specialists carrying out systematic and taxonomic research that contributes to valorize the collections under their care (Fischer, 2015).

Biodiversity assessments and voucher collection are often tightly linked and both contribute to document the occurrence of species in a certain place and at a certain time in history, providing the basis to document, for example, the effects of global warming (Lacey *et al.*, 2017) and other biotic alterations (Sanders *et al.*, 2023). Without a proper valorization of voucher collecting and legitimating their gathering, museums risk transforming themselves into something different, and possibly less efficient in preserving biodiversity. So far, although voucher collecting finds its full justification in scientific purposes and conservation fall-outs, its use and implementation are increasingly questioned, sometimes suggesting that voucher collection could/should be replaced by other methods including photographs, bioacoustics, and/or the collection of tissue samples (Pape, 2016). In some cases, collection of voucher specimens is also questionably proposed as a direct cause or con-cause for local extinction, contributing providing a negative image of this practice (Minteer *et al.*, 2014; Pape, 2016; Russo *et al.*, 2017). Contrary to this idea, and in agreement with several other authors (i.e., Gippoliti, 2018; Ceriaco *et al.*, 2016), we are certain that museums still play a pivotal role in documenting life on earth and voucher collecting remain a fundamental part of this global endeavor.

#### *Field surveys and integrative taxonomic activity on the amphibians of Madagascar*

In their paper, based on the study of mammals and scorpions, Waeber *et al.* (2017) admitted that specimen availability in mammals and birds was critical (and necessary) for advancing the knowledge on Madagascar's biodiversity at the onset of zoological work on the island, but also assumed that this activity is apparently currently less necessary, sometimes almost useless and even possibly affecting their conservation. However, we believe that the situation cannot be generalized, and is different within other groups, such as amphibians and invertebrates, where patterns of species richness and diversity strongly correspond to collection effort, suggesting that their cataloguing is far from being complete (Antonelli *et al.*, 2022).

As an example, the recent catalogue of Malagasy amphibians enumerates more than 400 species (AmphibiaWeb, 2023), and another ca 200 candidate species are waiting formal description (Vieites *et al.*, 2009; Perl *et al.*, 2014; Rakotoarison *et al.* 2017, Scherz *et al.*, 2022; and Vences *et al.*, 2022). Here we provide a few examples on how collection of voucher specimens of amphibians at different sites in Madagascar (i.e., the Réserve Naturelle Intégrale (RNI) de Betampona; the Parc National (PN) de l'Isalo; the Sahamalaza-Iles Radama Biosphere Reserve) has been useful to advance the amphibian knowledge of Madagascar.

1. The *Réserve Naturelle Intégrale de Betampona* includes a parcel of low altitude rainforest (around 29.18 square kilometers of extension) located about 40 km North from the seaport town of Toamasina, on Madagascar's East coast. Isolated from the rest of lowland rainforests and surrounded by degraded and over-exploited areas, this forest fragment hosts more than 80 frog species (Rosa *et al.*, 2012), of which 36 taxa were candidate species at the time of the first

survey release (Rosa *et al.*, 2012). Of these species, eleven were subsequently described / resurrected based on specimens collected during the survey at Betampona: *Boophis fayi* (Köhler *et al.*, 2011), *Blommersia angolafa* (Andreone *et al.*, 2010), *Platypelis karenae* (Rosa *et al.*, 2014), *P. ranjomena* (Glaw *et al.*, 2020), *Plethodontohyla laevis* (Bellati *et al.*, 2018), *Stumpffia analanjirofo*, *S. betampona*, *S. garaffoi*, *S. jeannoeli*, *Stumpffia davidattenboroughi* and *S. pardus* (Rakotoarison *et al.*, 2017). In addition, surveys at this site enable to confirm the extension of the distribution range of multiple other species (Glaw *et al.*, 2020).

2. The *Parc National de l'Isalo*. In this area of south-western Madagascar our survey enabled the identification of 24 species, of which nine were considered candidate species at the time of the first surveys (Mercurio *et al.*, 2008; Cocca *et al.*, 2018). The possibility to collect specimens over several repeated expeditions gave Cocca *et al.* (2018) the chance to identify two new species with respect to Mercurio *et al.* (2008), *Mantidactylus riparius* and *Scaphiophryne obscura*, and characterise *Mantella* sp. aff. *expectata* "South". Six species were described or resurrected or placed in synonymy thanks to the analysis of specimens collected in the surveys carried out in the area since 2004: *Gephyromantis kintana* (Cocca *et al.*, 2020), *Mantidactylus noralottae* (Mercurio & Andreone, 2007), *Scaphiophryne obscura* (Scherz *et al.*, 2021), *Blommersia bara* (Vences *et al.*, 2023), *Mantidactylus riparius*, and *Mantidactylus mahery* (Scherz *et al.*, 2022).

3. The *Sahamalaza-Iles Radama Biosphere Reserve*. At this site, including a transitional forest (Penny, 2017) 20 species of amphibians were identified in the course of two surveys, of which seven were considered candidate species at the time of the first visit (Andreone *et al.*, 2001) and four were described since then thanks to the analysis of specimens collected in these surveys (Raselimanana, 2008; Penny *et al.*, 2017): *Boophis ankarafensis* (Penny *et al.*, 2014), *Boophis tsilomaro* (Vences *et al.*, 2010), *Cophyla berara* (Vences *et al.*, 2000), and *Stumpffia froeschaueri* (Crottini *et al.*, 2020). Of these four recently described species, two have been newly identified only after the first three surveys of the area: *Boophis ankarafensis* and *Stumpffia froeschaueri*, suggesting that multiple surveys covering different seasons are often needed to compile the biological diversity of one area.

### *Collecting Malagasy amphibians: an historical overview*

Data on the number of amphibian specimens of Malagasy origin in museum collections was obtained through the GBIF database (GBIF, 2018). These data show that Malagasy amphibians entering museum collections were very limited up to 1965, with most specimens obtained through opportunistic field activities (Fig. 1).

The description of the amphibian diversity of Madagascar organically began in the 19<sup>th</sup> Century, when a few specimens were sent to museums in Europe and elsewhere by missionaries or local workers (i.e., Tschudi, 1838). After this initial

phase, we observed a peak in voucher specimen collecting in coincidence with the fieldwork and taxonomic activity by scientists from the Muséum national d'Histoire naturelle in Paris and the Natural History Museum of London (i.e., Grandidier, 1872; Boulenger, 1881), and with the exploration of the humid forests of the high mountains of Madagascar (i.e., Andohahela, Andringitra, Marojejy, and Tsaratanàna), which have greatly contributed to the discovery of several species. Their work was continued by other colleagues such as C. Domergue, J. Guibé, R.M.A Blommers-Schlösser and C.P. Blanc (Blommers-Schlösser & Blanc, 1991a, b, 1993), among others.

A sharp increase starting from the 80s of the 20<sup>th</sup> century (Fig. 1) corresponds to the intensification of survey investigations carried out by a third vague of field researchers, including an Anglo-American (C.J. Raxworthy, and R.A. Nussbaum), a German-American (F. Glaw, M. Vences, J. Koehler, J. Glos, C. Hutter and M. Scherz), and an Italian-Portuguese (F. Andreone, A. Crottini, and G. M. Rosa) teams, all of which have closely collaborated with several Malagasy herpetologists (A.P. Raselimanana, N. Rabibisoa, O. Ramilijaona, F.C.E. Rabemananjara, J.B. Rabemananjato, J.E. Randrianirina, F.M. Ratsoavina, and A. Rakotoarison, among many others). This period coincided with a sharp increase in species description, in an increasing productive collaborative teamwork (Andreone, 2008; Vences *et al.*, 2008; Antonelli *et al.*, 2022) and the publication of ecological (e.g. Dubos *et al.* 2019) and conservation contributions, including also the two editions of the ACSAM (A Conservation Strategy for the Amphibians of Madagascar): Andreone, 2008; Andreone *et al.*, 2006.

This period also saw the desire of local researchers to create a reference collection within the Faculty of Sciences at the University of Antananarivo (Ramilijaona *et al.*, 2008). Thanks to a fruitful collaboration with multiple foreigner colleagues and the reinforcement of systematic skills within Madagascar, this newly created collection room began to be known at the international level with the acronym UADBA (University of Antananarivo Department of Biological Animal), now Mention Zoologie et Biodiversité Animale. The increase in the species description activity was also backed by a significant change in the applied methodological techniques, which mostly moved from a morphological, ecological and kariological work (i.e., Blommers-Schlösser & Blanc, 1991, 1993) to an integrative taxonomic approach (i.e., Rakotoarison *et al.*, 2017; Scherz *et al.*, 2022).

We interpret the decrease in number of deposited specimens as visible in the histogram after the decade of 2000 (Fig. 1) as the result of a generalized and/or diffused decreasing in the field collecting activity, mostly due to increased alleged ethical issues and the introduction of limits to the number of authorized captures, and a shift to other kinds of field activities (not necessarily limited to taxonomic surveys), and to the use of new techniques (such as molecular taxonomic identification) which favors the collection of a limited number of voucher specimens in front of the collection of a larger number of tissue samples, which when analyzed, if they may confirm the existence of previously undetected candidate species, can guide the priority of future surveys in the same area.

*Field surveys; gaps and opportunities*

A general consideration for Madagascar, but likely valid for many other areas in the World, is that only a few study sites are regularly visited by researchers. This is mostly due, among other reasons, to the difficult access to many key areas, including protected areas, hampering a regular monitoring of the local wildlife.

These are, for example, Isalo, Montagne d'Ambre, Ranomafana, and Andasibe (i.e., Raxworthy & Nussbaum, 1994; Andreone *et al.*, 2001; Penny *et al.*, 2017; Mercurio *et al.*, 2008; Cocca *et al.*, 2018), all of which are also well-known touristic destinations (Gardner *et al.*, 2018; Goodman *et al.*, 2018).

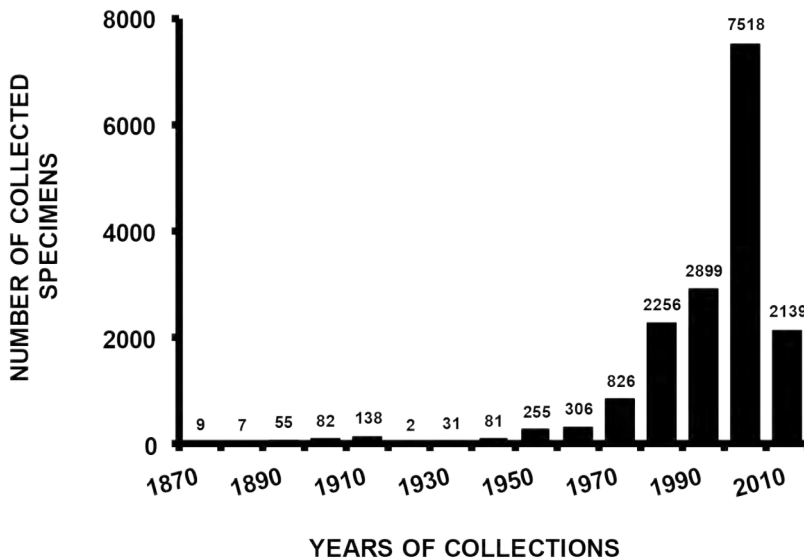


Fig. 1. Number of collected vouchers of amphibians in Madagascar from 1870 to 2010. Data extracted from the GBIF database.

Other sites, like the ones visited by Andreone *et al.*, (2000) and Gehring *et al.* (2010) are instead very seldom visited and never surveyed repeatedly. These sites often benefit of some sort of natural protection, given their remoteness, although, they also usually suffer of severe habitat alteration and deforestation (Harper *et al.*, 2007), since there is virtually no control and formal protection is very limited or even inexistent formal protection. At these sites, field surveys represent a “once in a lifetime” opportunity to discover, witness and catalogue the diversity of these sites for specific taxonomic groups.

Collection of voucher specimens when visiting these remote areas and/or other sites excluded from the network of protected areas is badly needed as this approach enables to witnessing biodiversity while allowing at the same time to continue the cataloguing of the unique biodiversity of Madagascar.

Current deforestation rates in Madagascar make some collecting sites almost impossible to re-investigate simply because they may disappear within a short time lapse (Harper *et al.*, 2007; Vieilledent *et al.*, 2018). An emblematic example of this unfortunate events is the survey that took place at the fragment of low-altitude rainforest of Nahampoana (around 200 m elevation) close to Tolagnaro (SE Madagascar; 24.58°S, 46.58°E). This forest parcel was frequently visited at the beginning of the '90s, and several species have been reported and subsequently described from there (i.e., Andreone, 1991, 1993). This site was massively logged around 1995 (C.J. Raxworthy, pers. comm.), and today the original forest habitat is not longer existing. The specimens collected before 1995 are the only documentation of the herpetofauna from that area. Multiple forest fragments of Ambohitantely Special Reserve (the network of relic humid forest fragments in the high central plateau of the Island) share a similar fate, with the area being heavily fragmented and degraded due to the almost annually bush fire which are affecting this protected area. Interestingly, new species are still being discovered there (i.e., Mullin *et al.*, 2022). Taken into consideration the micro-endemicity of many Malagasy frogs, it is likely that deforestations could lead to extinction several undescribed species.

A final consideration should be done on the conservation implications of voucher collection. A regulated collection of a standardised number of voucher individuals likely does not play a significant role in species extinctions (Remsen, 1995; Collar, 2000) and we think that it remains an essential tool to continue the understanding the world biodiversity, a fundamental step for its conservation (Dijkstra, 2016). In their paper Waeber *et al.* (2017) suggested that alternative, bloodless and not lethal methods should be used, at least for well-known taxonomic groups, and that efforts need to be invested in capitalizing on the specimens that are already available in NHMs, while always avoiding to collect large series of voucher specimens. Of course, we agree with the need to regulate the collection of voucher specimens, favoring the collecting of poorly taxonomically investigated groups, where the cataloguing of their diversity is still far from being completed (i.e., Olson & Soarimalala, 2018; Rakotoarison *et al.*, 2017; Scherz *et al.* 2022; Vences *et al.* 2022b).

Around 17,000 specimens of Malagasy amphibian are currently recorded on GBIF and are housed in registered world NHMs. Despite this remains an underestimation (i.e., some collections do not deposit their records in GBIF), we assume that this number is a good proxy of the amphibians' specimens that were collected for scientific purposes over a period of 150 years. With a richness of more than 400 amphibian species in Madagascar, this means that an average of around 40 specimens per species were collected over several decades. This number did not contribute (or contributed only marginally) to the decline of collected species, which

instead have been severely threatened by the destruction or fragmentation of their original habitat (i.e., the Nahampoana Forest: Andreone, 2023; Gehring *et al.*, 2010). In Madagascar, over the last couple of decades, the number of allowed amphibian voucher specimen collection per survey is small - generally two per candidate species per campsite. We consider this to be a sustainable approach to amphibian voucher collecting in Madagascar, since obviously does not significantly impact population viability. Under these strict limitations, vouchering, next to different conservation activities (e.g. cryopreservation) should also be considered compulsory for named species, so that long-term series can be built and opportunities for reintroductions in the future, be created.

### *Museums and voucher collecting*

Museums own irreplaceable items that attract scientists and amateurs and educate new generations. However, natural history museum differ significantly from other institutions, like universities and science centers. Our long-lasting taxonomic work on Malagasy amphibians was always done in collaboration with museological institutions and with a particular attention to the implementation of natural history collections. We use our direct experience to highlight the value of these collections, voucher collecting, and survey work as necessary activities to contribute to the global goal of cataloging world biodiversity and to warrant the life and subsistence of museums.

The presence of scientific collections usually associated to exhibitions and dissemination activities constitutes a distinctive mark for NHMs, where these collections are seen as a scientific heritage that need to be valorized and properly safeguarded (Beck, 2018; Andreone *et al.* 2021, 2022). However, the lack of economic support for collections in some countries and the decrease of their research activities often determined their depletion, and in some instances this led to their dismantling (Andreone *et al.*, 2014). In Madagascar, the maintenance of the reference specimen collection has proved to be challenging. Thanks to the support of Institutions and foreign scientists working in Madagascar and foreign scientists working in the Island, the specimens housed at the collection room of the Mention Zoologie et Biodiversité Animal continue to receive some care. However, the lack of a full time collection manager and the unavailability of appropriate tools (e.g. alcohol, jars) and storage space is preventing to maintain and manage adequately the specimens hosted at UADBA, which instead represent one of the most representative collection of Malagasy amphibians worldwide.

Fischer (2015) emphasized how collections need to be valorized not only as historical heritages (as they are often seen in a country like Italy that feature an historico-centric approach to its museums), but also as irreplaceable bio-repositories and research centers. Indeed, if collections are not used as research tools their use remain limited to exhibits, contributing even further to shift the role of NHMs from research centers to exposition centers only. In some cases, museums switched from a

“collection-based” conception towards a more generic perception of “science centers”, where the scientific activity of biodiversity assessment and species cataloguing progressively loose importance and where didactic and educational components become more relevant (either economically or in terms of priority targets).

Although exhibits are obviously an important parts of museum activities, there is a realistic risk that the collections’ central role will dramatically be reduced (Card, 2023). If this will happen, natural history museums would fail in conveying one of their most important messages to society: the importance of knowing, describing, and hence preserve, biodiversity. Finally, we think that there is a chance that the alleged threat posed by vouchering (i.e., Minter *et al.*, 2014; Pape *et al.*, 2016) is now paving the way for a decrease of collection activities in NHMs, with some people and institutions now more prone to perform other types of activities. If this is confirmed to be true, it will be yet another difficult trend to reverse, further weakening the role of collections.

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#### RIASSUNTO

*Vouchering, tassonomia integrata e collezioni di storia naturale: un caso di studio con gli anfibi del Madagascar.*

Le collezioni naturalistiche hanno generalmente svolto un ruolo importante nell’origine dei musei di storia naturale. Queste istituzioni sono diventate nel corso del tempo luoghi in cui venivano preferibilmente depositati esemplari voucher e altri tipi di campioni. In questo articolo sottolineiamo l’importanza di considerare la raccolta di campioni biologici un’azione utile/necessaria per l’espansione delle collezioni scientifiche, nonché per il progresso delle scienze e, indirettamente, della conservazione. In questa sede, inoltre, riportiamo una recente tendenza a declassare la necessità di esemplari voucher e forniamo prove del fatto che la raccolta di un numero limitato e autorizzato di individui come esemplari voucher è necessaria per contribuire all’obiettivo globale e al dovere di catalogare la biodiversità mondiale e promuoverne la conservazione. Gli esemplari voucher svolgono la funzione cruciale - tra le altre - di confermare la presenza di una specie in una località in un determinato momento, di documentare la diversità ecologica e biologica originaria prima del cambiamento, di consentire studi e confronti a lungo termine (utilizzando, ad esempio, strumenti morfologici o molecolari) e di servire come base per le analisi ecologiche. In particolare, abbiamo utilizzato gli anfibi del Madagascar come caso-studio per sottolineare la necessità di continuare le attività di raccolta su questo gruppo, dimostrando che la loro raccolta è stata molto importante per descrivere le specie e quindi procedere verso la loro

conservazione. L'uso dei soli dati accessori (tra cui fotografie, video, registrazioni acustiche e analisi genetiche effettuate su campioni di tessuto), più spesso considerati in alternativa alla voucherizzazione, sebbene utile, è considerato insufficiente.

*Parole chiave:* biodiversità, collezioni, musei di storia naturale, vouchering.

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