

Commentary

## A need for continued collecting of avian voucher specimens in Africa: why blood is not enough

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Pioneers of African ornithology such as R Liversidge and PA Clancey spent most of their career documenting and describing avian diversity in Africa. In contributing this paper in honour of Richard Liversidge, we address issues related to the need for future collections to continue the progress that Liversidge, Clancey and others have made to our understanding of avian diversity in Africa. Specifically, we are concerned with problems related to gathering data for systematic and taxonomic purposes without collection of voucher specimens. In this paper, we will present a number of examples of the types of problems that can arise when there is no voucher to back up other observations (also see Ruedas *et al.* 1999). For those interested in papers on the value of scientific collecting in general, there are a number of excellent references (e.g. Goodman and Lanyon 1994, Remsen 1995, Payne and Sorenson 2003 and other examples in this volume). We would encourage anyone interested in this even broader debate to review these papers.

For African birds, the discovery and description of the Bulo-Burti Bush-Shrike (*Laniarius liberatus*, Smith *et al.* 1991) has proved a flashpoint for issues related to collecting. The description is based on a single individual held in captivity for over a year and later released far from the original capture point. Blood samples taken from the bird showed it to be genetically distinct from probable sister taxa (Smith *et al.* 1991). Morphological data were gathered from the bird prior to its release (Smith *et al.* 1991). There were obviously valid concerns about the collection of this individual, but some have questioned the approach of describing it as a new species without a traditional voucher (Banks *et al.* 1993). Our concern is not so much with this unique case, but rather that it may represent a growing trend in avian research, in which researchers increasingly see blood or feathers as an equivalent to a study skin, fluid specimen or skeleton.

The practice of gathering blood or feathers for genetic studies without also preserving complete voucher specimens is increasing in frequency. There are many types of studies where this is obviously the most appropriate procedure, e.g. long-term studies of marked populations or studies of endangered species with small populations. However,

there is another class of studies in which the lack of voucher specimens has led, or could have led, to misunderstandings. In presenting this paper, we urge the scientific community and government agencies to think broadly about the value of collecting so that scientists can continue to accurately document avian diversity today and to provide the material on which taxonomic and evolutionary studies can be conducted far into the future.

The advent of molecular studies, particularly those that generate DNA sequences, has revolutionised the study of systematics. The DNA can come from the tissues of a collected bird or from small samples of blood or a feather. Non-vouchered sampling for genetic studies has begun to be employed more extensively in general field studies. Bird ringers may sample blood from the birds they capture, or a scientific expedition to a remote locality may net birds and draw blood or save feathers for future taxonomic studies. As a consequence, some governmental agencies have become convinced that there is no longer any reason to ever collect a voucher. We believe that not collecting a voucher can be a poor scientific decision that hampers our understanding of systematics and evolution, and it is essential that permitting agencies are made aware of these situations.

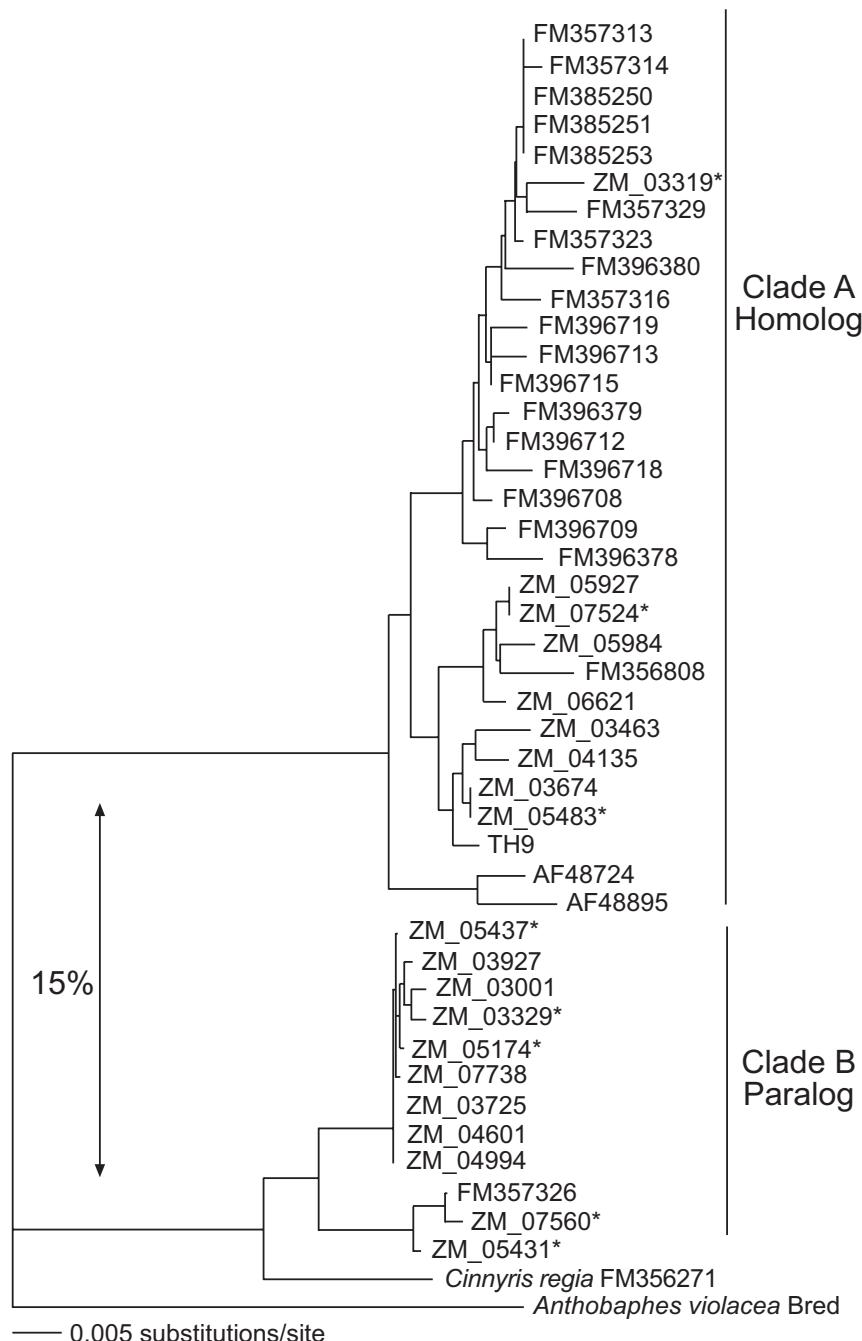
Two issues are often overlooked by researchers who collect blood samples without vouchers. First, for the most common types of DNA data gathered today, blood samples are often inferior sources because they can contain multiple copies of the genes being sequenced. Second, with the lack of voucher material accompanying a genetic sample, there is usually no recourse to assess problems that may arise with the sample after its collection.

The great majority of sequencing of DNA for avian studies at or below the species level is done with mitochondrial DNA. Mitochondrial DNA is so widely used because it is common in cells and it evolves rapidly; it can uncover differentiation between much more recently evolved taxa than we currently can with nuclear DNA (with the possible exception of short tandem repeats called microsatellites). The problem is that copies of mitochondrial genes can become incorporated into the nuclear genome (Sorenson and Quinn 1998, Williams and Knowlton 2001, Thalmann *et al.* 2004). When

this happens, they no longer evolve at the same rate as the genes in the mitochondria do. The nuclear copies are no longer homologous with the mitochondrial copies, but paralogous. They also are indistinguishable from the desired mitochondrial sequences in the most commonly used sequencing techniques. Including such sequences with mitochondrial sequences may lead to incorrect interpreta-

tions of the relationships of the samples from which the DNA were taken.

An illustration of this comes from a study of the phylogeography of Olive Sunbirds (*Cyanomitra olivacea/C. obscura*). A piece of the mitochondrial control region (363bp) was sequenced from 43 individuals and two outgroups (*Cinnyris regia* and *Anthobaphes violacea*, RCKB unpubl.



**Figure 1:** Phylogram of relationships among control region sequences from 43 Olive Sunbirds (*Cyanomitra olivacea/C. obscura*). Clade A represents the true relationships (homologous) among individuals sampled from different geographic localities, whereas Clade B presents a nuclear copy of the control region (the paralog). These two clades differ by 15% (uncorrected P) sequence divergence. Individuals labelled with an asterisk (\*) highlight localities where haplotypes from these disparate clades co-occur. FM — Field Museum of Natural History, ZM — Zoological Museum of the University of Copenhagen. See text for further discussion

data). Two distinct clades of DNA sequences (haplotypes) were recovered in a phylogenetic reconstruction (Figure 1). These two large groups are approximately 15% divergent from one another (uncorrected percent sequence divergence, P), a most unexpected finding, given that these clades do not correspond to geographically disjunct populations. Samples from three sites, Mafi Hill and the Ufipa Escarpment in Tanzania, and Kibale in Uganda (individuals marked with \* in Figure 1) fall into both clades. It is extremely unlikely that this result reflects real population history, because it implies extensive secondary contact following a long period of isolation. A more plausible explanation is that one of the two clades represents a nuclear paralog or pseudogene (as supported by analyses of the NADH3 gene, Bowie *et al.* 2004a). Close inspection of the actual DNA sequences suggests that clade B (where data were primarily collected from blood samples) is the paralog. If we had not realised this source of error in this dataset, then we would have developed an erroneous bio-geographical explanation.

Why are pseudogenes or paralogs of mitochondrial DNA more commonly associated with blood samples? Blood corpuscles contain relatively few mitochondria compared to nuclear DNA. In muscle or organ tissue samples, the opposite is true: copies of mitochondrial DNA far outnumber copies of the nuclear genome. Thus, when nuclear copies of a mitochondrial gene have evolved, they are much more likely to be encountered and sequenced in avian blood samples. As a result, obtaining useful samples for sequencing of mitochondrial DNA is far more difficult from blood samples, and one has to be much more cautious with respect to inspecting the data for the existence of nuclear copies.

The collection of feathers as an alternative to blood (Smith *et al.* 2003) may somewhat alleviate the homolog/paralog problem discussed above, although problems may still arise. A problem with non-vouchered material may only come to light long after the sample is collected, or it may not come to light at all, and erroneous conclusions could be drawn if that sample is used in a molecular study. For example, although some workers take photos of individuals from which they sample blood, many do not. Thus, there is complete reliance on the identification abilities of the person who took the sample. As good as many people are in the field, and as good as field guides have become, this approach has many pitfalls with respect to general sampling of any avifauna. Anyone who has ever worked on their own field collections in a museum setting can attest to the fact that material can be and is misidentified in the field, even by experienced field researchers (e.g. examples below, and Ruedas *et al.* 1999). The rate at which this happens will depend on the expertise of the worker, but even the most experienced misidentify birds in the field. Also, it is the comparison with material housed in collections that provides the comparative data necessary to assess geographic variation.

In our opinion, a pervasive rationale for collecting blood without vouchers is the erroneous idea that most or all morphological variation has been documented for birds, thereby making additional specimens superfluous. For Africa, despite the Herculean efforts of researchers such as Chapin, Prigogine, Liversidge and Clancey, among others, many countries or areas have received little attention in gen-

eral, but even fairly well-represented countries and areas (in terms of overall collections) have not received focused, evenly distributed sampling treatments. Although most individual species treatments describe variation across the entire species range, in many cases little evidence supports the geographic validity/uniformity of described subspecies (for North American birds see Zink and Remsen 1986, Zink 2004). Our preliminary genetic evidence for certain species shows strong differentiation between populations from Malawi and Uganda, and those from South Africa. Although it remains to be seen whether these genetic differences match already described subspecies, the existing collections from these countries and their neighbours are often sparse and not representative of even major intra-country regions. At the level of plumage variation within populations and species (such as age-related differences), lack of specimen data has been noted in recent field guides that have relied heavily on museum specimens (e.g. Zimmerman *et al.* 1996). Thus, it is critical that we continue to collect vouchers, because current subspecies boundaries certainly require revision, species limits are often poorly understood and cryptic new species likely await detection. The implications of this for conservation are obvious.

We use several examples from our own experience in the New and Old World tropics to illustrate how easily some of the issues raised above can occur. These include an example from South America, where general collection of a fairly common and widespread species was later shown to include an overlooked cryptic species. A second example from Madagascar characterises how voucher specimens can help unravel sampling errors, which can easily occur during general sampling, and a third from tropical Africa illustrates a problem with not having corroborating evidence for field identification of some West African sunbird species.

During an inventory of birds in Bolivia's Noel Kempff Mercado National Park in 1989, the late Theodore A Parker III and JMB collected several Suiriri Flycatchers (*Suiriri suiriri*), a species endemic to the Cerrado savannas south of the Amazon Basin. At the time, Parker was acknowledged as one of the most broadly experienced field ornithologists ever to study Neotropical birds (Bates and Schulenberg 1996). However, it was not until 10 years later that Zimmerman *et al.* (2001) realised that a cryptic species actually occurred with Suiriri Flycatcher in this part of South America. Re-examination of the specimens collected by Bates and Parker revealed that they had collected both species on the same day without realising it. Only through re-examination of the original voucher specimens was this possible (and it provided the only documented record for the new species, Chapada Flycatcher *Suiriri islerorum* for Bolivia). This example has another twist with respect to the genetic material collected from both specimens (Bates *et al.* in prep.). DNA sequences from these morphologically similar species demonstrate that they are very divergent genetically and that they actually belong to different genera in the family Tyrannidae. If we had examined only blood samples for analysis of genetic structure across the Suiriri Flycatcher's range, we might easily have concluded that the samples of the new species were simply contaminants from some other flycatcher or a misidentified sample.

Another recent example involves work on the genetic relationships in the Malagasy Ground-rollers (Brachypteraciidae). While gathering data for this project, we found that DNA sequences of several of the samples did not appear to represent the taxa to which they had been assigned in the field (Kirchman *et al.* 2001). We were able to verify from the voucher specimens that these samples were supposed to have come from ground-rollers. Because misidentification of the actual specimen could be ruled out, there had to be another source of the error. From the genetic material we were able to determine that the sequences we obtained from these samples were actually contaminants from taxa collected at the same time. In most instances it would not be possible to address such errors with non-vouchered blood samples. Voucher specimens can even provide a second independent source of genetic material (such as toe-pads, feathers or a piece of skin) if one is uncertain about the DNA sequences from a given tissue sample.

A third example of problems with the absence of vouchers comes from four dull, olive-green sunbirds of West Africa, placed in four different genera by Irwin (1993, 1999), primarily as a consequence of variation in bill shape and behaviour: *Anthreptes seimundi* (Little Green Sunbird), *Deleornis fraseri* (Scarlet-tufted Sunbird), *Cinnyris batesi* (Bates's Sunbird) and *Cyanomitra olivacea/C. obscura* (Olive Sunbird). Phylogenetic analyses (Bowie *et al.* in review) based on mtDNA sequence data show high statistical support for the close relationship of *Deleornis fraseri*, *Anthreptes seimundi* and *Cinnyris batesii* parallelling their shared plumage characters. Further, in three mitochondrial gene sequences *Anthreptes seimundi* and *Cinnyris batesi* were identical. Where the two species overlap in West Africa, it can be difficult to separate them in the field. A voucher specimen was collected for the *Anthreptes seimundi* sample (AMNH 831872), but not for the *Cinnyris batesi* individual, which was sequenced from a blood sample. Thus, we were able to confirm that the *Anthreptes seimundi* was correctly identified. However, unanswered is whether *Anthreptes seimundi* and *Cinnyris batesi* are genetically identical but morphologically plastic in bill dimensions (perhaps not unexpected for a nectar-feeder) or was the *Cinnyris batesi* sample simply a misidentified *Anthreptes seimundi*? The validity of this interesting result will remain unanswered until a vouchered tissue specimen of *Cinnyris batesi* is collected from which DNA data can be obtained.

Ongoing research on genetic structure in African warblers (CK and RCKB unpubl. data) constitutes yet another case illustrating the vital role that voucher specimens can play. For some especially difficult identification problems, our current understanding of a species' distribution may be affected by dubious unvouchered records. Specific cases include the Little Rush warbler (*Bradypterus baboecala*) versus the Grauer's Rush Warbler (*B. graueri*) in highland swamps, the Cinnamon Bracken Warbler (*B. cinnamomeus*) versus the Evergreen-forest Warbler (*B. lopesi*), and the Papyrus Yellow Warbler (*Chloropeta gracilirostris*) versus the African Yellow Warbler (*C. natalensis*) in papyrus. Presence of voucher specimens coupled with genetic data have helped us confirm the actual status of a number of taxa in those groups, thus adding value to field records by allow-

ing subsequent examination and verification.

Collar (1997) points out that it may not be possible to conserve all populations of a taxon that occur on isolated islands or mountain tops. What we do not necessarily know is just what these populations represent taxonomically and evolutionarily. If a blood sample from a population on an isolated mountain top proves genetically distinct from other populations, without voucher material, it may not be possible to determine what taxonomic status that population might deserve (e.g. Collar 2000). The importance of this argument is emphasised by recent work in the Eastern Arc Mountains of Tanzania, where Bowie *et al.* (2004b) argue that the Eastern Double-collared Sunbird (*Cinnyris mediocris*) complex does not comprise just two species, but five, with three of them very narrowly distributed. Further, these sorts of results are not restricted to sunbirds, but have also been found in thrushes (*Turdus* spp., Bowie 2003) and greenbuls (*Andropadus* spp., Roy *et al.* 1998) from the same geographic region. Without specimens from which morphological data could be gleaned to support the genetic data, the taxonomic status and therefore the conservation status of these montane populations would still be questioned.

A final issue is the widely-held perception that blood is somehow different from other collected material in that no permits are required for collection and export of samples. This is not the case. Blood is considered to be part of the organism under international treaties and by the laws of most countries. Blood samples cannot legally be collected or exported without permits from the country where the material is obtained, and without CITES permits for taxa covered by that treaty. It seems that many researchers are unaware of the strong concerns in many countries with respect to 'bio-prospecting.' Although this material has no commercial value and is not collected for such purposes, governmental officials are often sceptical about these assertions. As a result, the collecting and exporting of blood samples by well-meaning researchers without appropriate permits can have extremely detrimental consequences for all legitimately permitted research programmes within a country.

The above examples are specific to the value of specimens with respect to genetic sampling. Thus, our paper does not begin to address their value with respect to all other scientific purposes for which specimens are used (Goodman and Lanyon 1994, Payne and Sorenson 2003, and other examples in this volume). We hope that our examples will encourage those researchers who collect only blood to consider what information they may be losing or overlooking as well as the errors they may be propagating. However, our primary goal is to present examples that can be used to illustrate to government officials that blood samples alone often do not provide enough data or potentially erroneous data, about the individual birds studied. Scientific collecting provides important verifiable information that can be essential for both science and conservation.

**Acknowledgements** — We thank Mark Anderson for the invitation to contribute to this issue in honour of Richard Liversidge. We thank reviewers J van Remsen and M Brooke for their constructive comments on this manuscript.

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