

Integrated Specimen-Based Studies of Philippine Birds and Avian Influenza: Applications in Conservation, Taxonomy, Wildlife Enforcement, and Disease

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Abstract

Conservation is practiced in many ways, but often overlooked is the role of specimens in conservation biological research. With increasingly powerful phylogenetic analyses necessary to describe dwindling genetic diversity, the preservation of biodiversity can only be as successful as the infrastructure of specimens available. Taxonomic units of biodiversity may not completely reflect true genetic diversity, so new phylogenetic tools useful for the recognition of biodiversity require specimen-based resources to be as complete as possible. Here, we discuss how specimen-based, integrated research has multiple goals, and how conservation biology remains a key focus and outcome of these studies. Our sample archiving methods are among the most comprehensive in the discipline, and critical for quality research in conservation, phylogenetics, taxonomy, wildlife enforcement, and avian influenza studies. Although we have not isolated any avian influenza from Philippine samples, we have learned much from these negative results about avian influenza in the Philippines, and our methodology is producing quality multidisciplinary science.

Keywords: avian influenza, bird specimens, conservation, taxonomy, wildlife enforcement.

Introduction

There are many ways in which conservation is practiced – habitat preservation, wildlife management, contaminant control and environmental cleanup, restoration, and breeding programs, just to name a few. All of these efforts are important and certainly make a contribution to conservation. Often overlooked is the role of specimen-based research and its application in conservation biology. Few recognize this important role, yet with increasingly powerful phylogenetic analyses that are routinely necessary to describe and quantify dwindling genetic diversity, preservation of biodiversity can only be as successful as the infrastructure of specimens available for such research. Further elucidating the very units of biodiversity that we need to protect is a dynamic field in ornithology today, with much cryptic diversity being discovered.

With limited resources for preservation, it is dangerous to rely on old models and taxonomic units of biodiversity that may not completely reflect true genetic diversity. For example, widely distributed “superspecies” may undergo range restrictions that can essentially be described as extinction events of cryptic species that have never been recognized. Many widely distributed Asian taxa may follow this pattern. In this way, it would be a grave mistake to claim that all the important archiving of specimens has already occurred, when new phylogenetic tools useful for recognition of biodiversity require specimen-based resources to be as complete as possible. This is not the only role that specimens play in conservation biology, but we can be assured that specimen-based research, combined with integrated phylogenetics, can maximize the benefits for science and conservation.

Here, we discuss how we use specimen-based, integrated research for multiple goals, and how conservation biology remains a key focus and outcome of these studies. These specimens are not only essential to current multidisciplinary studies, but also possess potentially unlimited opportunities for future studies.

Materials and Methods

Our sample archiving methods are among the most comprehensive in the discipline (see Winker, 2000). We routinely preserve skin, skeleton, tissue, and stomach samples from birds. In regards to our influenza screening, there are two compelling reasons for using a sample archiving approach. The first is that such an approach is the

only practical means of collecting the full suite of data needed to resolve detailed questions about the avian virus transport system. The second is that sample archives of wild animal tissues are an invaluable resource for tracing the origins, distribution, and transport of emergent zoonotic pathogens. A full understanding of these zoonoses requires retrospective analyses. There are now many powerful techniques for such studies, but retrospective research can only be as good as the archived samples from which to draw. Avian influenza (AI), replicated in the gut, is not likely to be present in traditionally archived animal tissues, however. As a consequence, we have added intestinal tissue samples to our sampling and archiving program.

Standardized morphological measurements are made, and sex, age class (hatching-year/after-hatching year), and the size and condition of the gonads are recorded. The cloaca is swabbed to collect any potential live influenza virus, and we also preserve intestine samples and ca. 6 g of muscle tissue from birds collected. Cloacal swabs are placed in cryovials containing viral medium. Tissues and cloacal swabs from all of the specimens we collect are stored on liquid nitrogen in the field the same day they are collected. Voucher specimens are prepared as study skins, skeletons, and often spread wings and deposited and catalogued at the University of Alaska Museum and the National Museum of the Philippines. Cloacal swabs are shipped frozen to the U.S. Department of Agriculture's Southeast Poultry Research Lab in Athens, Georgia, for real-time reverse transcriptase PCR (RT-PCR) influenza screening and genome sequencing (Spackman et al., 2002).

Our research is providing basic information about viral infection rates, host specificity, genomics of viral genetic reassortments (from regions where we have obtained positive samples), and the population genetics of the bird vectors. Outside of game species, we know more of non-breeding avian movements through comparative study of research skins than by any other methods, including bird banding. Additionally, data such as age, sex, condition, reproductive status, parasite loads, and diet are all useful means of determining individual susceptibility to infection and transmission. Dissection and specimen archiving enable full capture and use of the above data - in addition to making these materials available in perpetuity for a suite of other scientific studies. It is in this way that our approach to specimen-based research maximizes the scientific benefit of each specimen.

Results

We have not yet isolated any AI viruses from the Philippines, and this is good news from national and international perspectives. This project continues to document some of the baseline rates of AI infection in wild birds in the Philippines, and this should help us learn more of the potential origin and transport of this emerging health and economic crisis. An example of how study of these transport systems can illuminate risk levels was presented by Winker et al. (2007) for our avian influenza research in Alaska. Continuation of this screening in the Philippines is of paramount importance in monitoring the presence of viruses known to be in the region and thereby providing scientists and policy-makers the best possible information. Conversely, lack of knowledge in this emerging crisis could lead to costly and detrimental actions.

Twenty-eight new distributional and seasonal records for localities have been established based on our 2001-2003 work. Phenotypic and genotypic analyses have yielded new information about migratory bird routes. In one long-distance migrant species (*Limosa lapponica baueri*), we have genetically linked a Philippine bird to host populations in Siberia and not to breeding Alaska populations (unpublished data), demonstrating the effectiveness of such research and providing new evidence on migratory routes. Phenotypic analyses have provided multiple new migratory path developments. One discovery provides the possibility that Philippine birds are not only linked to the Asian/Alaskan migration but to the European/Asian migration route as well (unpublished data).

For example, in terms of phenotypic analyses, the following species have been identified to subspecies and the implications of migratory routes are described. We were able to identify a Philippine Kentish Plover (*Charadrius alexandrinus dealbatus*) to this subspecies. Thomas Braille was also able to collect several individuals of this species in Mongolia (2002), and this makes an interesting discovery. The Philippine specimen is the same subspecies as a specimen collected in Mongolia, but differs from other Mongolian specimens of Kentish Plovers of the nominate race which breeds substantially west of *C. a. dealbatus*. This discovery provides the possibility that Philippine birds are not only linked to the Asia/Alaska migration but also to European/Asian migration through contact of Philippine Kentish Plovers with the nominate race. Philippine-collected Ruddy Turnstones (*Arenaria interpres interpres*) have been identified to the

nominate race that differ from breeding Alaska Ruddy Turnstones, indicating that these birds have a Palearctic origin and may not be mixing with Alaska birds. Further analysis will be necessary to determine whether some of the nominate subspecies occur in the Aleutian Islands in migration. Phenotypic analysis of a Philippine-collected Bar-tailed Godwit (*Limosa lapponica baueri*) indicates that it is of the same subspecies as both Siberian and Western Alaskan breeding Bar-tailed Godwits. We hope that genetic analyses of these birds will provide finer resolution in the identification of host populations. Philippine Whimbrels (*Numenius phaeopus variegatus*) is the same subspecies that we have identified in the Aleutian Islands. While differing from breeding Alaska Whimbrels, Philippine Whimbrels may be a regular component of the Aleutian migration. A Philippine Little Egret (*Egretta garzetta nigripes*) specimen has been identified as a subspecies endemic to the Philippines, Sunda Archipelago, and New Guinea. A Philippine specimen of *Anthus gustavi gustavi* has been identified as belonging to the Russian and Kamchatka breeding populations.

Finally, in terms of traditional museum studies of biodiversity, we are finding that even common species in Southeast Asia are likely composed of multiple cryptic species (unpublished data). In preliminary phylogenetic analyses, we have already found at least one such case. We are continuing our search for more, and other cryptic species are undoubtedly present.

Discussion

Applications of our specimen-based approach can have enormous conservation impact. Human health crises have the potential to cause panic, and many times conservation is forgotten in efforts to protect human health. We understand that there was a serious proposal here in the Philippines to attempt to kill massive numbers of migratory birds in a misguided attempt to prevent the transport of bird flu. In this case we, together with others in the international community, worked with the Philippine Department of Environment and Natural Resources to help convince policy makers that this was the wrong thing to do for both conservation and the spread of disease. Averting this effort was a major boost for conservation of birds. In this way, our collection of a relatively small number of birds can better inform us about the potential risks from avian influenza and avoid a panic that could result in extreme conservation problems.

The discovery of cryptic species will also have a major conservation impact (Bickford et al., 2007), and the phenomenon may be widespread among Philippine birds and elsewhere in Asia. An example of this is the interest from conservation organizations, and the resulting support from them and the public in their recognition of the increased number of both endemic and total species found in Philippine birds. Newly discovered species and heightened attention placed on them is bound to have a profound impact for the protection of their habitat and raise awareness for their conservation needs. The discovery of new species will simply not occur without specimen-based phenotypic and genotypic research.

New migratory connectivities are being discovered in this project, and this not only helps in tracking potential influenza transport but also in the conservation of migratory birds. Critical habitat in the form of staging and stopover areas can be identified for specific breeding populations, enabling improvements in population monitoring and in analysis of factors influencing potential population declines. Knowing when and where populations are going on a fine scale is highly necessary in the protection of migratory birds across political boundaries. New discoveries in both phenotypic and genotypic analyses will certainly aid in this effort.

The gradual buildup of decent sample sizes of specimens from across species ranges will also enable wildlife trade to be monitored in ways previously not possible. Too often the source of illegally traded birds cannot be traced to the host locality or population. A base of specimens would allow for phylogenetic linking of traded birds to the host population, and wildlife enforcement could then concentrate on important areas for the prevention of illegal trapping and transport.

Some conservationists are very sensitive about the collection of birds, and we understand that. However, we must place the collection of birds in context. An understanding of population dynamics confirms that the relatively small numbers of birds that are collected for science are not having impacts to their conservation. Endangered species and other sensitive species are not being collected. Birds collected for science can be compared to birds being killed by human influence that does not contribute to conservation or science. If one considers as an example, the human support of domesticated cats, which kill millions of birds in North America alone, a rational mind will not single out the extremely small number of collected birds by the specimen-based researcher (Winker, 1996). There are many other ways in which humans are

killing birds, all of us only need to look in the mirror after we drive a car or fly a plane to the conference, or even the paper on which our conservation reports are printed on.

It is fine to choose to pursue conservation and science in other areas, but it is unproductive to single out the specimen-based researcher as being “anti-conservation.” Specimen-based bird researchers are also ardent conservation biologists. It is important that all biologists recognize the need of specimen-based research for science and conservation benefits. If you are really against the idea of collecting, I suggest you use that energy to promote the salvage of “found dead” birds. Modern collections routinely contain 50-70% salvage birds as opposed to collected birds. If all biologists worked harder to salvage specimens, then the need for collecting birds could be reduced. Let us all work together as biologists to promote good conservation biology.

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