What is proper vouchering in phylogenetic studies of birds?—A reply to Peterson et al. (2007)

1. Introduction

Peterson et al. (2007) present an argument for the importance of museum voucher specimens in molecular systematic studies, illustrating their points with reference to a paper recently published by us (Olsson et al., 2006). Their argument concerning specimen vouchers is a sound argument about scientific practice, with which we largely agree, but unfortunately, they also attempt to boost their case by incorporating what we feel are a number of irrelevant and erroneous allegations. We comment on these and also suggest alternative means of documentation in cases where voucher specimens cannot be collected.

1.1. There is a continued need for collection of specimens

We totally agree with Peterson et al. about the “enormous positive contributions that specimen material makes to a broader scientific understanding of biodiversity”, and that phylogenetic studies should be backed up by voucher specimens whenever possible. We also agree that there is a need for continued collecting efforts. However, there are circumstances when collecting of vouchers is not possible, while sampling of feathers and/or blood may be feasible. Although feathers and blood are evidently not as good as voucher specimens, we do not agree that such material is useless. In our opinion, photographs and/or sound recordings are a reasonable substitute for voucher specimens, when vouchers cannot be collected.

There is no doubt that study skins in museum collections have been, and continue to be, an invaluable source of information, especially in our studies of Old World warblers over the past three decades. These birds are appropriately characterised by Peterson et al. as difficult-to-identify. However, species regarded as impossible to identify a couple of decades ago, unless examined in the hand or compared to reference collections, are now routinely correctly identified in the field, even by beginners, aided by excellent field guides such as Grimmett et al. (1999), Robson (2000) and Rasmussen and Anderton (2005). These sources build on reciprocal illumination from field observations and studies in museum collections, which have taken field identification competence to a much higher level for birds than for other animal groups. Field records are now widely accepted in ecological research, revisions of distributional ranges, regional check-lists, notes on behaviour, etc. Contrary to the statement of Peterson et al. that “Field situations are not always the best conditions under which to make difficult identifications”, we instead argue that identification of live individuals in the field is superior to identifying museum material when it comes to Old World warblers. In the field, one can often study vocalisations, and other behaviours, which are generally more divergent than morphology in these birds. Certainly, not all birds are possible to identify without comparison to reference collections, but neither are all morphological characters clearly visible on study skins, especially if these have been badly prepared—which, in our experience, is often the case. In particular, face patterns, which are important for identification of Old World warblers, are much easier to judge on a live bird than on a museum specimen. In our experience, a small proportion of museum specimens remain misidentified, even after scrutiny by skilled ornithologists.

1.2. What kind of data are required to formulate phylogenetic hypotheses?

Peterson et al. state that “conclusions regarding phylogeny, relationships, and especially species limits require multiple character sets”, and suggest that our paper does not meet these standards. We fully agree that phylogenetic conclusions benefit from multiple independent data, and ours are based on two unlinked DNA loci in an effort to increase the basis for the conclusions. Both our genes converge on the same topology, and we therefore consider our hypothesis well founded. Furthermore, our conclusions rest on many years of studying these species, both in the field and in most of the main and many smaller museum collections in the world, and we have ample collections of photographs, sound recordings, video cuts, notes and DNA samples of these and related species. In retrospect, this should have been stated more clearly in the paper. In our experience, morphological characters are good for assigning Old World warblers specimens to least-inclusive taxa, but are generally too invariable and too sensitive to selection to be of much use in inferring relationships—as indicated for these Cettia species by the previous, much disparate, classifications based exclusively on studies of museum specimens. Peterson et al. imply that we draw conclusion based on insufficient data, but our omission of morphological and vocal characters is the result of a deliberate decision not to elaborate on phylogenetically uninformative data, not due to a lack of voucher specimens. The main scope of Olsson et al. (2006) was to present a phylogenetic and phylogeographic analysis, not to evaluate species limits. Nevertheless, it revealed a pattern worthy of comment. Our conclusions on species limits build on previous taxonomic work, with the addition of the more unbiased evidence from two independent molecular data sets. We believe that our comments on species limits do not go beyond what the data allows. Previous taxonomic arrangements were largely based on typological arguments. We present three taxonomic alternatives that are compatible with the phylogeny, and recommend the one we think is most appropriate without claiming that it being the final word in this matter.

Although specimen vouchers are to be preferred whenever possible, there are other means of verifying identifications. For all except one of the Cettia species in our study we have photographs and/or sound recordings of the actual individuals; the only individ-
ual lacking documentation (except for tail feathers) is Cettia cetti, a common European species with no confusion species. We regret that this was not clearly stated in the paper. These photos and sound recordings are deposited at the Swedish Museum of Natural History, linked to the museum numbers in our Appendix.

1.3. How to define science

Peterson et al. state that “information from more diverse character suites: plumage variation, morphometrics and vocalizations are...not available for analysis”. Although we agree that such data are useful, this is somewhat irrelevant to their main argument about specimen vouchers. Plumage variation would not have been available even if our samples had been vouchered, since we only used one individual per locality, and information about vocalizations cannot be extracted from voucher specimens anyway.

Furthermore, Peterson et al. propose two alternative interpretations of our data which they appear to consider equally probable as the one we present. In our opinion, their suggestions are without strong support, as both their alternative interpretations require the existence of two sympatric lineages of Cettia flavolivacea-like birds living in China and Vietnam, a situation of which no researchers, past or present, have found any evidence, neither in the field nor in collections.

From these imagined populations, we would have consistently studied and sampled only the one unknown to others. We thus consider both of Peterson et al.’s ad hoc explanations less parsimonious than that we actually sampled from the only population of C. flavolivacea s. l. that is known from these areas. This is also supported by comparison of our photos and field notes to specimens—done long before this study was published, but unfortunately not mentioned in the paper.

Peterson et al. further state that our study “cannot be replicated” and “therefore violates the basic tenets of scientific method”. This is incorrect. Our study can be replicated by resampling from the same localities where we collected our samples. Comparison with the sequences deposited in GenBank by us will provide evidence as to whether the skeptics have encountered the same populations as the ones we sampled. This approach would furthermore be an independent test of our hypothesis, in contrast to a second study based on the same specimens, and hence more “scientific”. Peterson et al. further claim that “because phenotypic information is unavailable, we do not believe that such unvouched studies fit the definition of ‘science’”. In our opinion, this is a real misunderstanding of the nature of science. What defines science in the context discussed here is whether there are possible critical tests to reject our hypothesis and not whether data for this is served on a silver platter or not. We thus disagree strongly with the statement by Peterson et al. that “Molecular phylogenies with such limitations [i.e. lack of voucher specimens; our clarification] are problematic and cannot be verified, leaving open questions of accurate reconstruction of relationships of these organisms”. In fact, studies that do not leave questions open are exceedingly rare. For example, most molecular phylogenetic studies rely on a small number of genes, often only of mitochondrial origin. Vouched or not, such hypotheses are of course less than conclusive. However, it is not a general requirement of scientific hypotheses that they present the complete and ultimate truth. All phylogenetic conclusions in Olsson et al. (2006) are open for test and falsification by independent data, and thus per definition scientific in a Popperian sense. Furthermore, our main conclusions concerning the phylogeny—e.g., that two independent gene-trees, one mitochondrial and one nuclear, suggest that two mainland taxa are more closely related to three Sundaic taxa than to other mainland taxa—are valid regardless of whether the taxa are backed up by vouchers, were represented by multiple samples or had been referred to as A, B, C, etc.

2. Alternatives when voucher specimens cannot be collected

There are many instances when collection of voucher specimens is not possible, leaving us with other sources of DNA like blood or feathers. Such samples can also be collected in connection with other activities, such as bird banding. Contrary to Peterson et al., we think that such samples may carry a high scientific value, overshadowing the drawback that they are not backed up by a museum specimen. We welcome the suggestions by Peterson et al. regarding minimum standards in presentation of sources of molecular data. We add the points below as useful in cases where voucher specimens cannot be obtained, and suggest that they are useful also when voucher specimens are collected.

2.1. Photographs

Colour pictures of the bird in the hand are in many cases sufficient to support both identification to taxon, sex and age of an individual. For taxa where subtle colour shades are of importance, we suggest that a relevant colour standard is held next to the individual in the photograph. Care should be taken to photographically document all characters of interest, such as colour patterns, wing formula and colours of soft parts. Multiple photographs from different angles are often required to achieve this. It is important to “preen” the birds, as especially wing patterns can be difficult to judge otherwise.

2.2. Biometrics

Proportions are often crucial for identification of birds. Standard measurements should therefore be taken when relevant. These typically include length of wing, tail, bill and tarsus. For certain taxa, wing formula or other measurements may be important, and should be documented as appropriate.

2.3. Sound recordings

For many taxa, such as Old World warblers, vocalisations often differ between morphologically similar taxa. Whenever possible, the vocalisations of individuals sampled should be tape recorded.

2.4. Locality description

For many taxa, characterisation of the locality and habitat where the sample originated may provide crucial evidence regarding the credibility of the identification. It is also important to provide the date when the sample was collected, as this provides evidence as to whether the individual may have been on the breeding grounds or elsewhere. Any indications of breeding, such as defending a territory, should be noted.

We do not suggest that all of this information must be provided in each case. Different pieces of evidence carry different weights, and the important thing is that enough evidence is provided to convincingly show which taxa were used in the study. It is further important that this evidence is made permanently available for inspection. For this purpose museums and comparable institutions should house the original photos, sound recordings and documents. Akin with sequence data, which is available in GenBank, photos deposited in Morphbank would be readily available for inspection. Recordings could be submitted to e.g., the British Library Sound Archive or the Macaulay Library, Cornell Lab of Orni-
ology. It should also be possible to supply both photographs and sound files in a digital format as additional data with electronically published journals.

In conclusion, we definitely do not advocate a specimen-free approach. To the contrary, we fully endorse the collection of voucher specimens, and we argue that these should be accompanied by photographs and sound recordings of the live specimens whenever possible. Indeed, we have ourselves directed expeditions both in Sweden and abroad to collect bird specimens to the benefit of the international scientific community. However, it is a reality that specimens may not always be possible to collect. In these cases our suggestions would provide an adequate and most often satisfactory substitute.

3. There is a need for improved annotation of GenBank sequences

We also urge authors of phylogenetic studies, including ourselves, to improve their annotation of sequence data. Most sequences available in GenBank are sadly deficient in information of importance in phylogenetic or biogeographic contexts, such as data on the geographic origin of the sample, date of collection and subspecific identity. Although this information is usually available in the original papers, having it in direct connection with the sequences would save a lot of time and effort for anyone using GenBank data. Furthermore, it is of crucial importance that deposited sequences are conclusively linked to the individual sample or voucher in a study.

References


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