



Modern Tools of Species Identification Can Save Millions of Animals Killed in Identification and Animal Taxonomy

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Summary

A large number of animals are taken from the wild every year into practical classrooms for demonstrations of anatomy, physiology, biochemistry, and biosystematics studies. Besides the use of animals in vivisection/ dissection, the next large-scale exploitation is in making an animal museum for the study of identification, classification, taxonomy, and phylogeny. A large number of animals are collected regularly from nature for making museum specimens, irrespective of their IUCN status. Over-exploitation of animals in such studies has resulted in almost complete removal of many species from nature. Although many digital alternatives have come into existence in the recent past for the replacement of animals in vivisection, dissections, physiological, and biochemical exercises, very few efforts have been made in search of alternatives biosystematics studies. High-resolution photographs of animals with close-ups of some of their key features, with a biometric database and digitization associated with bioacoustics and molecular characteristic can convincingly replace animal killing in biosystematics studies as well.

Keywords: biosystematics, biometric database, bioacoustics, sonotaxonomy

1 Use of animals in teaching and research

A large number of animals are used in teaching and research all over the world. This has created serious problems for the existence of many species. Laboratory dissection or vivisection has been a common practice in Zoology since the 1920's as a way of demonstrating the internal organization of an animal. In physiological and biochemical tests as well, the complete animal often is used to study a few parameters. Such practices in Zoology laboratories have resulted in severe loss of biodiversity because greedy animal-catchers often totally sweep large numbers of animals from their natural habitats. Generally, most animals used in the practical exercises are wild-caught species such as *Pila globosa*, *Hirudinaria* sp., *Echinus* sp., *Scoliodon laticaudus*, *Hoplobatrachus tigerinus*, *H. crassus*, etc. Some species are even listed under Schedule I (*Scoliodon laticaudus*) and Schedule IV (*Hoplobatrachus tigerinus*) of the Wildlife (Protection) Act of 1972 in India. Over-exploitation of these and other species in vivisection and dissection, combined with other reasons, has resulted in almost complete removal of several species from nature. The best example of such a loss is *Sara hardwickii* (*Uromastix hardwickii*), which we report from Rajasthan. This lizard used to be a common animal for the dissection tray in many institutions until it was realized

in the 1980's that it had disappeared almost completely from most parts of India.

After animal dissection, the next most important aspect in Zoology in which large numbers of animals are collected by the animal suppliers is to make museum specimens for the study of classification, taxonomy, and phylogeny (biosystematics) (Anderson, 1965; Knudsen, 1966; Longair et al., 1991; Miller and Nagorsen, 1992). This process generally involves exhibiting formalin-fixed or resin-embedded animals (Henry et al., 1997). A large number of animals are collected from nature regularly to make museum specimens, irrespective of their IUCN status. A large number of Zoology museums are maintained in institutions with millions of animals in formalin-filled glass jars. In conventional biosystematics studies, for identification of a species, hundreds of individuals are killed and preserved. Although many digital alternatives have been developed for the replacement of animals in vivisection, dissections, physiological and biochemical exercises, little effort has been made to search for alternatives in biosystematics (Sharma, 2009; Sharma et al., 2011a).

We have found that high resolution photographs of an animal with close-ups of some key features, combined with a biometric database, and their digitization and bioacoustics and molecular characteristics can convincingly replace animal killing in biosystematics studies (Sharma et al., 2011b).



2 Species identification using diagnostic features of morphology with high resolution imaging (HRI)

The morphological and morphometric observations of individuals using images of certain diagnostic morphological features can help in identification up to species. For example, four species of genus *Hemidactylus* of family Geckonidae, namely *H. flaviviridis*, *H. brooki*, *H. leachaunalti*, and *H. triedrus* can be identified with the HRI of their lamellae, skin, and toes. Similarly, frogs of order Anura, *Duttaphrynus stomaticus* and *D. melanostictus* can be identified with the help of HRI of their warts of the head region. Among birds the long tailed shrike (*Lanius schach*), southern grey shrike (*Lanius meridionalis*) and brown shrike (*Lanius cristatus*) are good examples. High-resolution imaging can be done conveniently using optical and digital zooming facilities available in almost all still- and video digital cameras.

3 Molecular tools

The recent demonstration of the efficacy of “DNA barcoding” using the cytochrome c oxidase I (COI) gene in correctly identifying specimens of a large number of species of birds and moths provides hope for an unambiguous and standard method of species identification at the molecular level (Hebert et al., 2003). In addition, the studies by Hebert et al. (2003, 2004) also demonstrate for North American birds and for three moth families the concordance in species boundaries implied by molecular phylogenetic versus classical taxonomic methods based on morphological and behavioral characteristics. If this turns out to be the case for a large number of taxa and for groups of closely related species, it opens up the possibility of creating databases that allow identification using independent data sets. For example, parallel identification keys based on molecular, morphological, and/or behavioral data may be developed.

Recent developments in genomics have identified many marker genes. About 26 marker genes have been identified and may be used in biosystematics studies. The technique for identification of these marker genes is based on isolation and characterization of DNA from a few cells – without killing the animals (Sharma, 2010). The cells obtained from the surface of the body or from a drop of blood can be used to produce DNA copies by PCR for partial or complete sequencing using an auto-sequencer. Such information can be used for identification of species.

4 Bioacoustic tools: Sonotaxonomy

Field identification methods involving acoustic sampling for taxa such as birds, frogs, and crickets, and visual sampling based on diagnostic morphological characters are rapid and inexpensive, and they can be developed to be accurate (Riede, 1993).

Morphological characteristics often are found insufficient for the identification of cryptic species. Several cryptic species of anurans display a high level of morphological similarities that often make them virtually impossible to distinguish on the basis of morphological parameters. The taxonomic status of some very poorly known groups of frogs of the family Dicroglossidae from the central Aravalli ranges of Western India and the family Microhylidae from the southern parts of India, is assessed by means of acoustic and statistical analyses of differences in temporal parameters of advertisement calls, such as the number of pulses and the call duration, as well as a spectral parameter, dominant frequency, harmonics, peak frequency, amplitude, and power, etc. As these species usually are misidentified or ignored because of their taxonomic complexity in both ecologically diversified regions, we have found bioacoustical diagnosis for each species in order to facilitate identification in the field. Differences in acoustic parameters support the specific status of *Sphaerotheca breviceps*, *S. rolandae*, *Microhyla ornata*, and *M. rubra*. Populations from these distinct biodiversity regions can be recognized by distinctive advertisement calls, usually corresponding to a recognized species.

The individuals of family Dicroglossidae (*S. breviceps* and *S. rolandae*) and Microhylidae (*M. ornata* and *M. rubra*), being sympatric species, show great similarities in their morphological characteristics as well as eco-biological needs, but their advertisement call characteristics analyzed using sound analysis softwares, viz., Raven, Avisoft, and Sound Ruler, are very different and species-specific, and they are very useful, particularly in field identification and monitoring (Sharma, 2005). Furthermore, identification and monitoring of species using bioacoustics tools is a humane approach that avoids unnecessary killing of animals.

4.1 Sound analysis system

The call analysis system includes the following steps: Transmitter > Medium (air) > Receiver. The transmitter, e.g., a male frog, emits the sound, which is transmitted through the medium, usually the air, as longitudinal pressure waves. The receiver processes the sound and presents the waves as visual spectrograms that are used for the identification of species.

The system of call analysis includes:

1. Recording
 2. Storage and conversion into a proper format
 3. Generation of a spectrogram
 4. Analysis of spectral pattern and development of classifiers
- Investigation of animal sounds includes signal recording with electronic recording equipment. Due to a wide range of signal properties and media they propagate, specialized equipment is used instead of the usual microphones. Video cameras are used for the confirmation of the call of a particular species. A sound bank is prepared to store calls in a format applicable to the software. Specific computer programming is designed for the

storage and analysis of recorded data, and specialized sound analyses are used for describing and storing signals according to their intensity, frequency, duration, and other parameters. Before the analysis of an unknown call the software is calibrated with the help of audio-frequency generators. All the sound signals are analyzed on the same frequency and time-scale to ensure that the recorded sound belongs to a different species or the same species.

Data collection involves two main components: sampling followed by processing. Sampling depends on rate and resolution. Rate should be greater than twice the highest frequency to be sampled. Resolution depends on processor intake, i.e., 8-bit or 16-bit. The resolution of 8-bit code is 256 combination steps and that of 16-bit code is 65,536 combination steps.

Most graphical display devices present sound as a time domain feature. The time domain display of sound waves has limitations in analysis. Frequency domain representatives of spectrum are an improved display system achieved by Fourier transform that provides better opportunities for sound analysis. The Fourier transform is a mathematical function that converts the time domain forms of a signal (produced by most measuring and graphical display devices) to a frequency domain representation or spectrum. The input to the DFT is a sequence of digitized amplitude values ($x_0, x_1, x_2, \dots, x_{N-1}$) at N discrete points in time. The output is a sequence of amplitude values ($(A_0, A_1, A_2, \dots, A_{N/2-1})$) at $N/2$ discrete frequencies. The highest frequency, $(N/2)-1$, is equal to half the sampling rate ($=1/(2T)$), where T is the sampling period. The output could be plotted as a magnitude spectrum. Frequency composition of a signal changes over time and can be plotted as a sound spectrogram using spectrum generation and analysis software (Avisoft SAS PRO, Raven 1.4, Sound Ruler). The spectrograms produced by sound plot have frequency on the vertical axis versus time on the horizontal; the amplitude of a given frequency component at a given time is represented by color combinations as per the parameters of sub-menus of the software.

4.2 Micro-scale analysis of calls

Furthermore, microanalysis of a spectrogram could be achieved by slicing the spectrogram (Raven 1.4). A spectrogram slice view is a plot of relative intensity versus frequency at a particular point in time within a signal. A spectrogram slice represents a vertical cross section through a spectrogram at a single time, but rotated 90° so that the frequency axis is horizontal. In fact, a spectrogram is built of a series of spectrogram slices stacked side by side (with their frequency axis running vertically). Whereas a spectrogram view shows a series of slices at successive points in time and represents power at each frequency by a color (by default, grayscale) value, a spectrogram slice view shows only one slice and represents power at each frequency on a line graph. Using a sound ruler, pulse rate, call rate, dominant frequency,

fundamental frequency, etc., are recorded. Classifiers and filters are used to sort particular elements or symbols of a call. Generally, sound spectra are discrete in most species, and such diagnostic characteristics of the spectral pattern can be used for the identification of a species. Sound-based identification, classification of call, and spectral parameters can be developed as a strong tool in taxonomy as sono-taxonomy. Sono-taxonomy may be used independently for the identification and categorization of various taxa or as a supporting tool to the conventional taxonomy.

5 Conclusion

High-resolution imaging associated with molecular and sound characteristics can be used not only for the identification of a species but also to resolve confusion among the cryptic species that look morphologically quite similar – without killing the animal.

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