The species has been treated as a fundamental unit in biology (Hull, 1977) and, more recently, in biodiversity conservation (Sites and Crandall, 1997). Almost all studies in biology, whether at the level of molecules, cells, individuals or populations, are typically referenced to the level of the species. In the field of conservation biology, assessments of biodiversity are made at the level of the species: typical criteria include species richness, numbers of endemic species, and the number or presence of endangered species in given areas (Myers et al. 2000). The accurate identification of species is crucial both to research in all areas of biology and to biodiversity conservation. It is therefore surprising that, in the field of systematics, species are currently used mostly as terminal taxa in the reconstruction of phylogenetic trees, whereas the methods by which they are delimited and identified receive scant attention (Wiens and Penkrot, 2002).

Species Identification: Practical Problems

The taxonomic classification and identification of species is an endeavour with a long and complicated history (Gilmour, 1951; Ridley, 1986). The Linnaean system provided a successfully established and universally accepted code of classification and nomenclature that has served us well over centuries. Whereas the development of these formal rules, together with the existence of voucher specimens (holotypes) in museum collections for verification of species identity is of undeniable value, species identification still remains a difficult problem for practising taxonomists, particularly in tropical countries. The reasons for this are manifold and are briefly outlined below.

The first reason is the inadequacy of currently available taxonomic keys: on the Indian subcontinent, for example, identification keys for most groups of invertebrates have not been updated since the publication of the ‘Fauna of British India’ series of volumes in the mid-1900s. Whereas many of the monographs in the above series are monumental works, the taxonomic keys are often not good enough to unambiguously identify specimens to the species level. The reasons for this difficulty include imprecise character definition and a paucity of illustrations to effectively convey the exact nature of the defined characters. These, combined with completely hierarchical, dichotomous key structures, do not allow unambiguous identification to the species level. Further, the species descriptions are not exhaustive and often suffer from an inconsistent inclusion of characters, even among descriptions of very similar species.

The second reason is the lack of an objective criterion for defining an individual specimen as the standard reference holotype for a species: holotypes have traditionally been designated by taxonomists without an objective argument and sometimes without examination of a sufficient number of specimens to take into account interindividual variability in different characters. For example, the discrimination between some species of crickets (Chopard, 1969) is made on the basis of differences in the number of tibial spines. There were several cases that I examined, however, where the difference in spine number between the left and right tibiae of the same specimen exceeded or equaled the variation in spine numbers between the designated species. In the absence of other distinguishing characters in the keys, it was essentially impossible to assign such specimens to a given species.

These problems are further compounded for taxonomists in tropical countries by the inaccessibility of both the taxonomic literature on previous descriptions and the reference specimens or holotypes of their local fauna and flora. These are largely available, for historical reasons, only in museums in Europe and North America. Added to this is the worldwide decline in the number of professional taxonomists, particularly for invertebrate animal groups (Gaston and May, 1992). The impact of this is becoming severe, with the increase in numbers of comparative studies in biology that require accurate species identification. All of the above problems are well-recognized and have been the subject of much discussion in recent years (Gaston and May, 1992; Godfray, 2002; Wheeler et al. 2004).

Species Identification: Conceptual and Methodological Problems

The process of allocating individuals to a given species obviously depends on the criteria by which species are defined and delimited, which are in turn determined by the concept of what a species is. The species concept continues to be the subject of much debate (Cracraft, 2000) and, until there evolves a consensus, there can be no well-defined universal criteria by which species may be delimited or identified. In the following paragraphs, I therefore examine some of the major species
concepts and methods of species delimitation implied by them.

Classical taxonomists classified individuals as members of a species based on a suite of shared morphological characters that were diagnostic and differentiated them from other such morphologically defined groups. In recent times, behavioral and ecological characters have also been increasingly used. Numerical taxonomy shares the concept of a morphological (or rather, phenetic) species and differs from classical taxonomy mainly in its methodology: the use of a large number of phenotypic characters, rather than only diagnostic ones, with equal a priori weighting, and in its emphasis on species as clusters derived from measures of overall similarity (Sneth and Sokal, 1973). The phenetic species concept is practical and applicable to all taxa and therefore of high operational value.

The biological species concept of Dobzhansky (1937) and Mayr (1942) defines species based on their ability to interbreed: species are considered as “natural” entities distinguishable from other species by the criterion of reproductive isolation and not overall phenotypic similarity. The biological species concept has been criticized for several reasons, including its lack of universality (for example, inapplicability to asexual taxa: Mishler and Theriot, 2000; Wheeler and Platnick, 2000), lack of a temporal dimension (Willmann and Meier, 2000), logical fallacy, and the difficulty of applying it to allopatric populations (Mallet, 1995). Although the biological species concept provides an unambiguous criterion for differentiating and delimiting species, one of the major problems is the practical impossibility of ascertaining reproductive isolation between large numbers of populations in the wild. As a result, most taxonomists, even those that accept the biological species concept, continue to use morphology and other phenotypic characters in order to delimit species boundaries.

Phylogenetic species concepts are increasingly being proposed as alternatives to the biological species concept. These concepts also have both philosophical and practical problems: one of the frequently raised philosophical problems is the validity of a phylogenetic approach to units at and below the species level (Mallet, 1995; Willmann and Meier, 2000). Even assuming this validity, there are several problems with species delimitation and identification using a phylogenetic approach. The various phylogenetic species concepts (de Quieroz, 1998) may be classified (following Baum and Donoghue, 1995) into two major types: character-based and lineage-based.

The character-based species concepts define species as “the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)” (Nixon and Wheeler, 1990). This “pattern cladistic” species concept has both philosophical and practical flaws that have been convincingly argued by Baum and Donoghue (1995). The major practical difficulties include determining whether shared traits have attained fixation in the population (Wiens and Servedio, 2000), determining how many diagnostic traits to consider and the inapplicability of this approach to most continuously varying quantitative traits (Willmann and Meier, 2000).

Lineage-based species concepts include the Hennigian species concept (itself rooted strongly in the idea of reproductive isolation) which views species as “a temporal series of populations connecting two speciation events” (quoted from Willmann and Meier, 2000). Operationally, the Hennigian species concept is essentially a more restricted form of the biological species concept that can only be tested by reproductive isolation together with a phylogenetic reconstruction. The “genealogical species concept” of Baum and Donoghue (1995) defines a species as a “basal group of organisms all of whose genes coalesce more recently with each other than with those of any organism outside the group.” Operationally, this species concept would involve tracing the genealogy of several different alleles and would require extensive molecular analysis of a large number of genetic loci, including nuclear and mitochondrial genes. Although philosophically and methodologically the most attractive of the phylogenetic concepts, it would be highly impractical, time-consuming, and prohibitively expensive if all species of all taxonomic groups were required to be delimited in this fashion, particularly in the species-rich and financial resource-limited environment of the tropics. In this context, it is worth noting that seven of the nine methods of delimiting species boundaries that were recently reviewed by Sites and Marshall (2003) require molecular data, which would be impractical for species identification in the assessment of tropical biodiversity.

In recent years, there have been several attempts to delimit species boundaries based on phylogenetic reconstructions, mostly using mitochondrial DNA haplotypes. Although some of these studies have revealed concordance with species defined by classical taxonomy, others have not (Wiens and Penkrot, 2002). More seriously, there have been observations of discord between phylogenetic trees or phylogenetic species boundaries based on morphological versus molecular characters, character versus lineage based reconstructions (Wiens and Penkrot, 2002) or mitochondrial versus nuclear DNA (Shaw, 2002). The ability of mitochondrial DNA sequences to accurately reflect species boundaries and species histories is being increasingly questioned (Sites and Crandall, 1997; Puorto et al., 2001; Shaw, 2002).

Avise and Wollenberg (1997) have provided a thoughtful exposition of the connections between population genetics, gene genealogies, and reproductive isolation. They have pointed out that reproductive isolation is an important component of all lineage-based phylogenetic species concepts: it provides the tokogenetic-phylogenetic border of Hennigian cladism, the restriction or elimination of gene flow between populations that results in the “independently evolving lineages” of the evolutionary species concept (Wiley and Mayden, 2000), and is the basis of the “deep” and concordant genealogical splits that are the basis of the
genotypic exclusivity criterion of the “genealogical species concept.”

One may then argue that, for sexually reproducing taxa, reproductive isolation does provide a sound criterion for delimiting species. Of course, several species maintain their identities in the face of a high level of gene flow between them (Mallet, 1995). Reproductive isolation may thus be looked upon as a sufficient but not necessary condition for delimiting species boundaries: where it does exist, it is likely to unambiguously delimit species.

If one accepts reproductive isolation as a sufficient criterion for delimiting species, but is constrained to delineate species boundaries using morphology, it becomes imperative to examine the concordance between morphological and “biological” species boundaries. This is relatively easy in certain groups such as crickets (Order Orthoptera, Family Gryllidae) because the calling songs of cricket species are often reliable indicators of reproductively isolated populations (Shaw, 1999). Concordance in the species boundaries provided by song and morphological characters would imply that phenetic clusters based on morphology correctly reflect the species boundaries defined by reproductive isolation. This then validates the use of phenetic clustering and ordination techniques (Sneath and Sokal, 1973) on morphological and/or song data to unambiguously classify and identify cricket species. For taxa that do not possess such behavioural “signatures” of reproductive isolation, the species boundaries implied by phenetic clustering or ordination will have to be compared with those implied by genetic distances (Sites and Marshall, 2003; Hebert et al., 2003).

The Future: A Pluralistic Approach?

I have illustrated how both conceptual and practical problems have resulted in the lack of accurate and accessible species identification keys. The philosophical debate on the validity of different species concepts is unlikely to be resolved in the near future. One may even dispute the wisdom of imposing a universal species concept on philosophical or conceptual grounds alone, given that systematics has two related, yet different, aims: to reconstruct the historical paths of evolution and to serve as a frame of reference for all studies in biology. At the level of the species, at least, the two may not be easily reconciled. A universal species concept should not be imposed without careful consideration of both objectives: a concept that is philosophically sound may well make species identification practically impossible.

Testing the concordance between species boundaries implied by different species concepts and using different data sets should be a prioritized research theme in systematics. Concordance between boundaries obtained using different species concepts or different data sets within a particular taxon, say an order or family, would, in the context of that taxon, relegate the debate on species concepts to the realm of theory and philosophy and enable the construction of identification keys. Currently, there are too few studies on the problem of species delimitation to know whether there are taxon-specific differences in levels of concordance or discordance of species boundaries implied by different data sets and different species concepts. The majority of studies examining this question have been carried out on reptiles, where discordant species boundaries appear to be common (Sites and Crandall, 1997; Puorto et al., 2001; Wiens and Penkrot, 2002). It is quite possible that this will not be the case for insect taxa, where distinct and stereotyped genitalic and behavioral characters are often good indicators of reproductive isolation (Otte, 1994; Shaw, 1999 for crickets).

The recent demonstration (Hebert et al., 2003, 2004) 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. 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 characters. 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 to create databases that allow identification using independent data sets. For example, parallel identification keys based on molecular, morphological and/or behavioral data may be developed. Even if DNA barcoding is highly accurate for species identification, it may be too expensive to be used in biodiversity surveys, where the number of specimens to be identified can be very large. Further, field identification and noninvasive sampling, two increasingly important requirements in biodiversity surveys, are not always possible using molecular methods. Field identification methods such as acoustic sampling (Riede, 1993) for taxa such as birds, frogs, and crickets, and visual sampling based on diagnostic morphological characters are rapid and inexpensive and can be developed to be accurate. Identification keys using nonmolecular data will therefore remain crucial to the process of species identification, particularly in the tropics.

The problem of accessibility to taxonomic information is beginning to be addressed via the development of Internet-accessible taxonomic databases (Godfray, 2002; Mallet and Wilmott, 2003). For example, in the case of orthopteran insects, the Orthoptera Species File Online (Otte and Naskrecki, 1997) is an invaluable first step in this direction. Nonetheless, it is not adequate for the purpose of species identification. What are urgently needed, in addition, are Web-based identification keys for different taxonomic groups based on an extensive and detailed examination of several characters of a sufficient number of individuals of each species. These should be verified against the established holotypes for historical continuity of nomenclature but one would like to envisage the consequent freedom from conventional holotype referencing for users of such databases, whether based on molecular or morphological characters. The ‘holotype’
in the new system would be a virtual one: the centroid of the cluster used to define the species in the database. Such a population-based approach has been previously advocated (Mayr, 1976). The current insistence on species verification using inaccessible type specimens is a major impediment to rapid and accurate species identification, particularly for those working in the tropics.

The development of accurate Web-based keys for several thousands of taxonomic groups using different data sets is a formidable task and requires both an increase in the number of professional taxonomists specializing in different taxa in tropical and temperate countries, as well as research into the bioinformatic methods required to construct optimal key structures, in terms of efficiency and accuracy. A conspicuous feature of the majority of keys available today, whether on- or off-line, is the absence of any measure of validation: all keys should ideally be validated by probing them with specimens of known identity to arrive at the probability of correct identification and assignment (Sneath and Sokal, 1973). Unfortunately, the construction and validation of identification keys is not currently a popular research area in systematics, as a glance at the publication titles of most journals in the area will reveal: the majority of articles deal with intricacies of phylogenetic reconstruction (mostly above the species level) or, in more specialised journals, with species descriptions. The difficulty in publishing studies that explore the problem of identification in journals that specialize in systematics can only worsen the current situation with regard to species identification.

The problem of species identification should be placed in the context of the current global biodiversity crisis (Wheeler et al., 2004). In order to decide conservation priorities, biologists in tropical countries are increasingly called upon by governmental and legal bodies to carry out rapid assessments of species diversity in different areas. Accurate assessments require the availability of rapid, inexpensive, accessible, and accurate methods of species identification, that are unavailable given the current state of the art in systematics.

Debates and decisions on species concepts and optimal methods for species delimitation cannot and should not be divorced from the practical issue of species identification upon which they impact so heavily. There is need as never before for a dialogue between systematists, evolutionary biologists, and conservation biologists on the one hand, and between systematists in tropical and temperate countries on the other. The problems faced by biologists in the tropics with regard to accessing information on their native flora and fauna should also be a cause for concern among the global community of systematists and inspire action to remedy the situation, given that these problems impact directly on biodiversity conservation.

REFERENCES


Since Pauling and Zuckerkandl first suggested it more than 40 years ago, the idea of reconstructing ancestral proteins and DNA sequences from the information contained in sequences of present day species has held considerable fascination (Pauling and Zuckerkandl, 1963). Such reconstructions can provide a unifying framework for understanding the molecular origins and evolution of key components in living organisms. However, only recently has it become relatively straightforward to perform such reconstructions and then test the reconstructed molecules functionally in the lab. Now there is a surge of activity in this area. Ancestral protein sequences for rhodopsin (Chang et al., 2002), ultraviolet vision gene SWS1 (Shi and Yokoyama, 2003), ribonucleases (Jermann et al., 1995; Zhang and Nei, 1997) and steroid receptors (Thornton et al., 2003), have been reconstructed and tested (see the reviews by Chang and Donoghue [2000] and Thornton [2004]). In addition, DNA from the common ancestor of placental mammals has been reconstructed for a megabase-sized region containing the cystic fibrosis gene (Blanchette et al., 2004) for several families of transposons (Adey et al., 1994; Ivics et al., 1997; Smit and Riggs, 1996; Jurka, 2002), and for some complete small genomes like HIV (Hillis et al., 1994). In the latter case the predicted ancestral sequences were compared to the known ones, so the accuracy of the reconstruction could be measured directly. However, in other cases theoretical results (Yang et al., 1995; Schultz et al., 1996; Schultz and Churchill, 1999) and computer simulations (Zhang and Nei, 1997; Blanchette et al., 2004; Cai et al., 2004) are required to assess the accuracy of the reconstructed sequence.

In these investigations it has been observed that the topology of the phylogenetic tree relating the present day species to the target ancestral species can affect the accuracy obtainable in reconstruction of the target ancestral character states. Simulations show that a starlike phylogeny, i.e., a rapid radiation of many different lineages from a common target ancestor, such as occurred in the radiation of placental mammals, allows the ancestral character states of that target ancestor to be more accurately reconstructed than those of more recent ancestors in parts of the tree where speciation events are more regularly spaced (Blanchette et al., 2004). More generally, it has been claimed that the star phylogeny always “represents the best case for ancestral character state reconstruction, because each observation is conditionally independent and yields maximum information about the ancestor” (Schultz et al., 1996), see also Schultz and Churchill (1999). Here we show that the actual situation is more complex and depends on the branch lengths. This complexity occurs even for the simplest evolutionary model with only one parameter: the Poisson model (known also as the Neyman r-state model, generalized Jukes-Cantor model, and the Potts model), where the parameter determines the rate of substitution and all substitutions are equally likely.

Consider the tree topology shown in Figure 1, where $A$ represents the common ancestor of three present day species, designated by $C_1$, $C_2$, and $C_3$. $B_2$ represents the common ancestor of $C_2$ and $C_3$ whereas $B_1$ represents the ancestor of $C_1$ at the same moment in evolutionary time as $B_2$. This tree contains a subtree with the simplest star topology, the two-leaf subtree shown in bold in Figure 1b, and it also contains a subtree with the simplest

**Counterexample to a Claim About the Reconstruction of Ancestral Character States**

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