

S23-5 Developing guidelines to assist in defining species limits

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Abstract This review describes the reasoning behind the “Guidelines for assessing species rank” developed by the Taxonomic Subcommittee of the British Ornithologists’ Union, and recently published in the *Ibis*. It sets out criteria for delimiting species taxa based primarily on the Evolutionary Species Concept and General Lineage Concept of Species (Mayden, 1997; de Queiroz, 1999), and secondarily on the Biological Species Concept, Recognition Species Concept and Phylogenetic Species Concept. Procedures and scenarios are canvassed for defining diagnosability and for determining species limits in situations of sympatry, parapatry, allopatry and hybrid zones. The role and significance of DNA sequence data is also addressed.

Key words Species criteria, Species concepts, Diagnosability, Sympatry, Parapatry, Allopatry, Hybrid zones, DNA sequence data

1 Introduction

The period since the publication of the “Voous” list of Holarctic birds (Voous, 1977) has been one of comparative stability for Europe’s avian taxonomy. Changes in species limits have occurred, but these have been relatively modest. Recently, however, there have been rapid, even accelerating, developments in biology with profound impacts upon our ability to define species limits. Enhanced data sets and improved statistical methodology have allowed more refined analysis of morphology. The opening-up of hitherto little known parts of Asia has resulted in increased knowledge of the region’s zoogeography, and the relationships of taxa previously known from only a few specimens, such as the herring gull (*Larus argentatus*) complex. Research into bioacoustics has revealed the importance of song and call in promoting reproductive isolation (Slabbekoorn and Smith, 2002). But, especially, the application of molecular genetics has allowed more detailed investigations of taxon divergence (Helbig et al., 1995) and monophyly (Friesen et al., 1996).

These have combined with a more critical view of the nature of species (Mayden, 1997) to improve our appreciation of avian biodiversity. With a growing awareness of the need to quantify biodiversity, and the consequences of anthropogenic effects such as global warming, habitat destruction, population fragmentation and over-exploitation, conservationists have increasingly sought a standardized inventory of avian species and their inter-relationships.

The British Ornithologists’ Union (BOU) has been

mindful of its responsibility for this, especially within Great Britain and Northern Ireland, and the taxonomic list maintained by the BOU (the “British List”) has now become the “official” list adopted by statutory and conservation agencies in the United Kingdom. With this responsibility came a need for a consistent treatment of taxonomic problems, and three years ago the BOU formalized a Taxonomic Subcommittee (TSC) to advise on changes to the taxonomy of the British List for all end-users.

The TSC quickly recognized the need for a set of guidelines to improve consistency in taxonomic decision-making, and to assist non-specialists to understand the rationale behind any changes to the British List. It was well aware that such guidelines could prove a “hostage to fortune”, since any departure — however well intentioned — could open them to criticism. The guidelines were finally published in 2002 (Helbig et al., 2002), the result of extensive debate among members of the TSC and 24 significant revisions; their conclusions are summarized below.

2 Species concepts

The species concept to adopt presented immediate problems. Committee views ranged from the Biological Species Concept to the Phylogenetic Species Concept, but it was finally agreed to adopt criteria close to the General Lineage Concept of Species (GLCS) of de Queiroz (1999). Thus, the TSC regards a species as an extant segment of a lineage of populations, similar to the Evolutionary Species Concept (ESC; Mayden, 1997), but the TSC preferred the

name General Lineage Concept because essentially all species concepts are evolutionary.

There are many alternative species concepts (see Mayden, 1997), and the TSC concluded that those most relevant to avian systems are the Biological (BSC), Recognition (RSC) and Phylogenetic (PSC). The TSC agreed to apply the concepts that were most appropriate to the data under investigation. When population genetic data were available, such as fitness of individuals and their hybrids, the arguments of the BSC would be applied. If behavioral or acoustic data were presented, the RSC would be implemented. When phylogenetic reconstructions had been undertaken, with analyses of monophyly and diagnosability, the data would be interpreted according to the tenets of the PSC. Effectively then, the TSC followed Mayden (1997) and de Queiroz (1999) in recognizing the ESC/GLSC as the primary species *concept* to tell it what a species is, and the BSC, RSC, PSC as secondary species concepts or *species criteria* to tell it how to recognize their limits. This approach was adopted as a compromise. The TSC also agreed that, in general, only material published in peer-reviewed journals would be considered.

3 Species limits

When considering species limits, the TSC addressed two key questions:

Are the taxa diagnosable? Are they likely to maintain their genetic and phenotypic integrity in the future?

3.1 Diagnosability

The TSC deemed it necessary to define its view of diagnosability. In birds, there are generally two kinds of characters: qualitative and quantitative. Qualitative characters are presence-or-absence features, such as wing bars in *Phylloscopus* warblers, as opposed to discontinuities in a continuously varying trait. The TSC accepted diagnosability if members of at least one age/sex class could be distinguished from those of all other taxa in at least one qualitative difference.

Quantitative diagnosability is more problematic, since if large enough samples are available, it is theoretically possible for an individual of almost any size to emerge in a population. However, the TSC recognizes the practicalities of finite populations, and accepted quantitative diagnosability when at least one age/sex class is separated by a complete discontinuity in at least one continuously varying character from the same age/sex class in an otherwise similar taxon. Judgement and statistical interpretation are necessary here, but an example is to be found in the extent of yellow on the bill of the two forms, *columbianus* and *bewickii*, of the tundra swan, *Cygnus columbianus* (Evans and Sladen, 1980). The TSC agreed not to rely on characters that are evolutionarily labile, such as wing lengths of migrant and non-migrant populations. It also agreed to multivariate diagnosability, when overlap occurs in a single character. Thus, if a major component in a Principal Com-

ponents Analysis showed a complete discontinuity in the same age/sex class, then the taxa would be regarded as diagnosably distinct.

3.2 Genetic integrity

The TSC then turned to the more vexed problem of whether two diagnosable taxa would retain their differences into the future. This reduced to a question of the level of gene flow, actual or potential, between them. Four scenarios requiring different approaches were recognized here: sympatry, parapatry, hybrid zones and allopatry.

3.3 Sympatry

Taxa that coexist at pair formation will remain distinct if they are reproductively isolated. The TSC did not know of any examples where absolute reproductive isolation had broken down. Thus, it decided to rank as species diagnosable taxa that are broadly sympatric without hybridization, e.g. marsh and willow tits, *Parus montanus* and *P. palustris*. Most sympatric species fall into this category. However, diagnosable taxa were also ranked as species if they were broadly sympatric and hybridized only rarely, so that gene flow is so limited that gene pools are unlikely to merge, e.g. *Larus argentatus* and *L. fuscus*. Here can be inferred a strong barrier to gene flow that may be pre- or post-zygotic (or both), since hybrid young produced from cross-fostering experiments apparently survive less well than pure bred progeny (Harris et al., 1978).

3.4 Parapatry

Strictly parapatric taxa — those that exclude each other geographically — are considered species because their parapatry implies reproductive isolation. Icterine and melodious warblers (*Hippolais icterina*, *H. polyglotta*) are largely parapatric, and hybridize only rarely (Cramp, 1992). They are essentially reproductively isolated. Although hybridization in the contact zone is slightly more frequent, a similar situation holds for pied and collared flycatchers (*Ficedula hypoleuca*, *F. albicollis*), although here the reduced fitness of the hybrids has been demonstrated experimentally (e.g., Saetre et al., 1999). Secondary contact is involved here. Diagnosable taxa are ranked as species if their contact is relatively recent, through whatever cause, and if their overall divergence suggests that they will remain distinct. An example of recent contact is the spread of ruddy duck (*Oxyura jamaicensis*) into the Iberian range of the globally endangered white-headed duck (*O. leucocephala*). Although there is currently hybridization (Green and Hughes, 2001), increases in the abundance of the ruddy ducks may lead to a resumption of positive assortative mating.

3.5 Hybrid zones

The TSC distinguishes between hybrid zones and clines. The first occurs when populations within the zone contain one or both parental forms in addition to the hybrids. In a cline, the phenotype changes progressively through

space. In both scenarios, the population mean will change across the zone, but variance is higher in populations through a hybrid zone because of the wider range of phenotypes present. An example of a hybrid zone is the region of contact between carrion and hooded crows, *Corvus cornix* and *C. corone*, in various regions of the Palearctic (Parkin et al., 2003). A cline is typified by the progressive increase in wing-length with latitude in the Western Palearctic, or the transition from olive brown to grey in common chiffchaffs (*Phylloscopus collybita*) across Europe.

Hybrid zones probably always indicate some intrinsic barrier to gene flow between the two taxa, as has been demonstrated in the crows (Parkin et al., 2003). The TSC will rank as species diagnosable taxa that hybridize following secondary contact, if a stable and distinct hybrid zone connects them. It will regard the area of contact as a hybrid zone if local populations contain one or both parental phenotypes, plus F1 and backcross individuals. Stability implies that the zone has changed little in breadth through historical time, although it may have moved in position. Such taxa are regarded by some as semispecies (see below).

3.6 Allopatry

Here lie the real problems, for reproductive isolation cannot be tested in nature. Captive breeding is generally of little use (cf. Lanyon, 1979), since genetic incompatibility may be preceded by a variety of behavioral and ecological barriers to reproduction. Acoustic (playback) experiments may help, but are not conclusive. The taxonomic status of diagnosable taxa can only be assessed through the extent of their divergence, so comparison with species pairs that are taxonomically close, but occur in sympatry, is important. Even so, the assignment of species rank will always be hypothetical rather than proven. Here the TSC recognizes three more or less distinct scenarios that require different treatment with procedures slightly more rigorous than for sympatric or parapatric taxa.

First, allopatric taxa are regarded as species if they are fully diagnosable in each of several discrete or continuous characters that relate to different functional contexts, the sum of which corresponds with those distinguishing related sympatric species. Different functional contexts implies independently varying characters such as morphology, vocalizations and DNA sequences. Excluded are covarying characters such as leg and bill length in wading birds, or wing and tail length in small passerines. Bonelli's warbler complex (*Phylloscopus bonelli* / *P. orientalis*) is an example (Helbig et al., 1995). Its members are allopatric, with differences in vocalizations (marked), morphology (slight) and DNA sequences that are as great in magnitude as those between these taxa and the wood warbler (*P. sibilatrix*). If the latter is regarded as specifically distinct from *bonelli* / *orientalis*, then logically *bonelli* and *orientalis* must themselves be treated as separate species.

Secondly, allopatric taxa that do not fulfill the previous criteria are still regarded as species if at least one char-

acter is fully diagnostic and overall divergence in other characters is equivalent to that in closely related sympatric species. For example, male *Anas crecca* and *A. carolinensis* are fully diagnosable on the basis of the white line along the sides (vertical or horizontal), and are as divergent from one another at molecular levels as they are from the northern pintail, *Anas acuta* (Johnson and Sorenson, 1999).

Thirdly, allopatric taxa are regarded as species if they are statistically diagnosable by a combination of two or three functionally independent characters, and overall divergence in other characters is equivalent to that in closely related sympatric species. Covarying characters relating to body size or wing/tail length are not independent, although size and color are. DNA sequence data can be used as a character here, but the TSC considers it unwise to use a single base (or amino acid) difference as diagnostic, even though this may imply a substantial period of isolation. It seems unwise to invoke a character that might be reversed by a single mutational event. An example of statistically diagnosable taxa is the Armenian and herring gull complex (*Larus armenicus*, *L. argentatus*), the two members of which can be differentiated by Principal Components Analysis (Liebers and Helbig, 1999).

4 Superspecies

Taxa that meet in a hybrid zone are sometimes referred to as "semispecies" (Short, 1969), and those that are allopatric as "allospecies". While these are useful terms, a majority of the TSC did not wish to use them formally, preferring instead to apply them adjectivally in describing species that are very closely related and which come together to form a hybrid zone or are allopatric.

5 DNA sequences

There is no doubt that DNA sequence data are a powerful tool for elucidating evolutionary relationships, and their application continues to grow, especially in phylogenetics. However, although they can be used as an objective measure of genetic divergence in comparative studies, and applied in phylogenetic reconstructions to develop hypotheses of evolutionary relationships, molecular difference is not a taxonomic character. Hypotheses of relationship based upon a single sequence are greatly strengthened when supported by a second independent analysis, whether based upon comparative morphology or a functionally independent DNA sequence. A phylogeny based upon the mitochondrial gene cytochrome-*b* cannot be regarded as supported by a second mitochondrial sequence, however, since these are effectively inherited as a single unit or haplotype. On the other hand, a concordant phylogeny based upon one or more independent nuclear sequences will greatly strengthen confidence in the overall result.

The TSC also recognizes that paraphyly is to be avoided in classification, and that molecular phylogenetic evidence is a powerful technique for testing monophyly and revealing paraphyly. In the case of *Anas crecca* and *A.*

carolinensis (Sangster et al., 2001), a molecular phylogeny indicates that the sister group of *carolinensis* is not the Eurasian *crecca*, but the South American Speckled Teal, *A. flavirostris* (Johnson and Sorenson, 1999). This species shows no sexual dimorphism, its sexes share in parental care, and it differs in behavior and ecology from *carolinensis* (Madge and Burn, 1988). No one has suggested that *flavirostris* is conspecific with *carolinensis*. Using the criterion of monophyly, if *flavirostris* and *carolinensis* are sister taxa and distinct species, then logically *crecca* should be separated as well.

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