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The new DNA-DNA avian classification

What's it all about?

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In 1988, a new avian classification system was published (Sibley *et al.* 1988) that arranged and classified birds in a new hierarchy and sequence, significantly different from that currently used in ornithology, based entirely upon a laboratory technique known as DNA-DNA hybridisation (shortened hereafter in this article to just DNA-DNA). Although a large portion of the data supporting parts of this classification had been published during the decade of the 1980s through the many papers by Sibley and Ahlquist, it was not until December 1990 that the full documentation was available (Sibley & Ahlquist 1990). That monumental work was accompanied by a companion volume (Sibley & Monroe 1990) that placed all the bird species of the world in the DNA-DNA-based classification and updated their distribution, ecology, and taxonomic information pertinent to that listing.

The first reaction most birders experience when seeing these two massive books and confronted with this system for the first time ranges from complete frustration to stark terror, as exemplified by these comments: 'Having just spent the better part of my life learning the classification of birds so that I can use all the books, am I going to have to trash all that and learn a new system all over again?', or 'Why can't professional ornithologists leave well enough alone and keep the system as it is?', or perhaps even 'Why do we need a classification system—can't we just list birds in alphabetical order?'. The answer to all these questions is simple: it is the goal of biological scientists everywhere, whether studying plants, butterflies, fish, birds, or any other of the myriad groups of living things, to express the natural world in terms of the relationships of organisms one to another and, thus, to classify animals and plants in a natural system, not an artificial one. That is why whales are always listed with other mammals, and not with fish, which they resemble superficially much more closely than they do their nearer relatives. The big question in taxonomy is how to determine who is related to whom. This new classification of birds is intended to do this better than any system heretofore.

So now the ornithological world is faced with the problem of what to do with a system that purports to be the best at showing true relationships. The decision will have to be faced either to accept this system or to discard it in favour of one already in place; in other words, is the new system real and of value in our understanding of ornithology, or is it just another system based on data that may or may not improve upon our present classification? In order for one to make judgment in such a case, it is essential to know just what the system is supposed to show, and whether there are sufficient data from other sources to indicate that this classification warrants supplanting any other in existence. I am going to give you an insight into the background of this system, expressed from the viewpoint of one who is not a biochemist or geneticist and who has not been involved directly in the detailed laboratory experiments that developed the database for the system.

Up to now, all classification systems have been based primarily upon morphology, the hierarchy in taxonomy being developed by comparing similarities and differences between birds and deciding through a set of procedures which of these factors are important in showing the true relationship between kinds of birds. Two problems exist with any system of this sort: (1) there has to be a degree of subjectivity in any decision as to the weight (importance) of similarities, leading often to incorrect groupings of non-related birds, and (2), perhaps more importantly, evolutionary convergence (such as we see with whales and sharks) often obscures the true relationships, sometimes leading to major errors in taxonomy (refer to the songbird case when you get to it later in this article). The DNA-DNA system is designed to remove all subjectivity by showing which birds are more closely related *genetically* to which others, the idea being that the more alike genetically two birds are, the more recently in geological time they shared a common ancestor and thus are more closely related to one another. The DNA-DNA hybridisation technique is not all that new in science, but Sibley and Ahlquist were the first to apply the technique seriously to bird systematics, examining literally thousands of species to determine genetic relationships. And here's how the system basically works.

DNA (deoxyribonucleic acid) is a double-stranded molecule that is found in every living organism; it contains the coding system that makes specific animals what they are. Think of the DNA double strand as a ladder, with two parallel sides connected by the cross pieces (rungs); grab each end of the ladder and give the whole thing a twist, and the resultant twisted, spiral piece can be likened to the DNA molecule. At each side where a rung joins the side pieces, there is a nucleotide in the DNA molecule; in a single copy (which would be one side of the ladder) of the whole genome of one bird, there are about 1.7 *billion* nucleotides. Despite this staggering number, there are only four different kinds of nucleotides in DNA, each with a different nitrogenous 'base' in its chemical structure (adenine, cytosine, guanine, and thymine, abbreviated A, C, G, and T, respectively). The sides of the ladder represent the two strands, which are complements of each other; that means that where there is an 'A' on one strand there is a 'T' on the opposite end of the rung on the other side, and where there is a 'C' it pairs with a 'G'. The rungs of the ladder correspond to the hydrogen bonds that hold A-T and G-C together; if any other combination is there on the opposite sides of the rung (e.g., an 'A' on one side and a 'C', 'G', or another 'A' on the other side), the hydrogen bond cannot form and the rung is 'missing'. These hydrogen bonds are what hold the two strands together. The more matches there are (i.e. the more rungs there are), the stronger the strands are bound together. This principle is the basis of the DNA-DNA hybridisation technique, for it is known that the more closely related two organisms are, the more alike is their DNA (i.e., more matches and 'rungs' would be present if complementary strands of their respective DNAs were matched); conversely, distantly related things are more different, and there would be fewer matches in their respective DNA strands.

The laboratory technique is generally as follows. The DNA from a bird is extracted from red blood cells or tissue and purified of RNA, protein, and other materials. The long DNA strands are 'sheared' into shorter pieces (about 500 bases long) so that pairing is made easier. Through a special technique, multiple copies of DNA are removed so that but a single copy of each gene remains.

The technique takes advantage of the fact that, when DNA is heated to boiling (thus breaking the hydrogen bonds between the two strands) and then cooled, the hydrogen bonds are again established between complementary bases, and the double-stranded structure is re-formed. The trick is to label the DNA of one species with a radioactive tracer and make hybrids, first with itself (as a control) and then with the DNAs of other species.

For the control, both strands of the hybrids consist of DNA of the same species, so the amount of base-pair matching is the greatest. When these 'hybrids' are subjected to a controlled elevation of temperature, in which they are heated incrementally up to boiling, a melting curve is produced beginning with 0% single-stranded DNA (= 100% double-stranded) at 60°C and ending with 100% single-stranded DNA at about 95°C after all the hydrogen bonds are broken. The temperature at which the curve intercepts the 50% line is called the '$T_{50}H$', or the temperature at which half the strands are double and half are single. For birds and, indeed, most vertebrates, that is somewhere around 85°C.

That gives the base $T_{50}H$ figure for that species. Next, a small number of these radio-labelled single strands are mixed with a large number of single strands from a *second* species (not radioactively tagged). Now, when the complementary strands find each other, most of them form 'hybrid' molecules consisting of a radioactive half from the first species paired with a non-radioactive half from the second species. These hybrid DNA molecules do not pair up exactly, the degree of non-match reflecting the amount of genetic difference between the two species. When these hybrids are subjected to an elevation in temperature, they produce a curve with a $T_{50}H$ at a lower temperature (say, 75° in this case); that is because, with the fewer base matches and fewer hydrogen bonds, the strands break apart more easily (i.e., at a lower temperature). By comparing the two $T_{50}H$ temperatures, one gets a difference of 10°; this figure is called the $\Delta T_{50}H$ ('delta $T_{50}H$'), and it is that figure that dictates the hierarchy of classification. For example, if the two species that were tested above gave a $\Delta T_{50}H$ of 10, they would be classified in separate families of birds; to be sufficiently closely related to be placed in the same family, the delta $T_{50}H$ would have to be less than 9. If above 11, the two species would not only be in separate families, but also in separate superfamilies; the number would have to be 20 or greater for the birds to be classified in different orders. And so on, through the entire classification system. The correlation between $\Delta T_{50}H$ figures and the taxonomic categories is given in both Sibley *et al.* (1988, p. 412) and in Sibley & Ahlquist (1990, p. 254).

The big advantage to such a system is that the classification is totally objective: there is no subjective guess as to weight or importance of any characters, and the classification is based entirely on a single value showing genetic relationship. The biggest disadvantage is that it is totally cladistic: it does not pay any attention to morphological divergence (different from genetic divergence, because of the adaptive nature of some characters and the resultant rapid way external characters may evolve in nature). Thus, morphologically divergent groups such as falcons and penguins have a $\Delta T_{50}H$ of only 16.4 and end up being sufficiently closely related to be placed in the same order instead of the traditional separate orders. For the most part, however, the DNA-DNA classification is closely similar to the morphological systems that are currently used, and the higher categories are the same.

Perhaps the most surprising thing to be discovered as a result of the technique, and which appeared unexpectedly, was the fact that most of the Australian songbirds (oscines) are related to one another, thus having gone through an adaptive radiation in Australia similar to that which we find with the marsupial mammals. This fact remained hidden to science until the DNA-DNA data revealed it because the birds do not have a shared, external morphological character similar to the marsupial pouch by which we long ago realised the true relationships between the mammals. There have been a number of other surprises, including the close relationship of starlings and mockingbirds, and of New World vultures and storks; most of these had been suggested before, but the evidence for them had been considered insufficient to cause a change in classification.

The second book (Sibley & Monroe 1990), which is perhaps of more direct interest to birders, is an entirely different sort of publication. It is a world list that follows the DNA-DNA classification for purposes of higher taxonomic categories (tribe on up), but species are usually so closely related (with resultant low $\Delta T_{50}H$ figures) that the DNA-DNA technique becomes less reliable at showing relationships; the $\Delta T_{50}H$ figures approach the level of experimental error, and, thus, exact relationships at this level cannot be easily ascertained. Limits in species (and to a lesser degree genera) were determined primarily on the basis of the most recent studies made on the group. A modified biological species approach was taken for the species level, and those taxa that displayed differences, particularly in vocalisations, ecology or behaviour, indicating an isolating mechanism to prevent free interbreeding, were treated as different species rather than subspecies. And it was my intention in preparation of the manuscript to provide a reference for all situations in which there were controversial or conflicting classifications, so there is extensive documentation and many literature citations throughout the book. Other sections were designed to make the book more 'user friendly' (such as the world numbering system for computerising lists, the maps and accompanying gazetteer, and the extensive index).

Perhaps the most controversial feature (and one that is controversial in nearly all books and lists) is that of the English names that were used. Although I formed an international group to provide input on the best choice of names, there are some obvious problems in any attempt to have a list of standardised English names. It is hoped that a new international committee, established by the International Ornithological Congress for the purpose of developing a worldwide list of standardised English names, will be more successful and attain a better selection. Of the 11 members on this committee, two are from the United Kingdom (your editor, Tim Sharrock, and Tim Inskipp); in the next few years, if you have any input regarding names in the Sibley-Monroe book that you think need to be changed, please contact one of these two and supply them with reasons for using a different name. As chairman of this committee, I want to assure all readers that all input of this nature to the committee will be considered in the final selection of names.

I cannot tell you how all this classification business is going to end. I can only say that, for more than a decade, a myriad of people have taken aim at trying to discredit the DNA-DNA classification or technique, even to the point of accusing Sibley and Ahlquist of fraud. Not only have the system and the authors withstood all that criticism, but there have also been more and more data supporting the basic elements of the classification. For example, after the Australian radiation news hit the bird journals (Diamond 1983; Gould 1985), a morphological character (the number of fossae in the head of the humerus) was re-evaluated throughout the thousands of songbird species and showed a higher-than-90% correlation with the DNA-based classification. In the case of the condors (New World vultures) and storks, an extensive morphological study done previously by J. David Ligon (1967) indicated the same condor-stork relationship. Another surprising relationship revealed in the DNA study was the close relationship between pelicans and the Shoebill *Balaeniceps rex*, an African stork-like bird. After publication of the DNA data, however, it came to light that Patricia Cottam, a graduate student in England, had discovered the same relationship through a morphological study in her thesis (Cottam 1957), but nobody believed it at the time, thus burying the information for almost three decades.

If one looks at all the data—and literally thousands of DNA-DNA comparisons have been made—the amount of correlation with the generally known and accepted relationships among birds is impressive. There do not seem to be major discrepancies, such as finding a species of duck closely related to a dove, or a finch among the woodpeckers. It is beginning to look as if the DNA-DNA system may become the basis for avian classification in the future. So, take a deep breath and dig into the taxonomy. I know for a fact that at least a few field guides or lists already in press will follow the DNA-DNA classification, and I'm sure that more will come along in the future.

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Simplified summary of Sibley & Monroe (1990) bird classification

ORDERS and Families only; groups including at least one species on the West Palearctic list are shown in bold; figures in parentheses are total number of species in each Family, followed by number of species on the West Palearctic list, if any.

STRUTHIONIFORMES
- **Struthionidae** *Ostrich (1, 1)*
- Rheidae *rheas (2)*
- Casuariidae *cassowaries, Emu (4)*
- Apterygidae *kiwis (3)*

TINAMIFORMES
- Tinamidae *tinamous (47)*

CRACIFORMES
- Cracidae *guans, chachalacas, etc. (50)*
- Megapodiidae *megapodes (19)*

GALLIFORMES
- **Phasianidae** *grouse, turkeys, pheasants, partridges, etc. (177, 23)*
- **Numididae** *guineafowls (6, 1)*
- **Odontaphoridae** *New World quails (6, 2)*

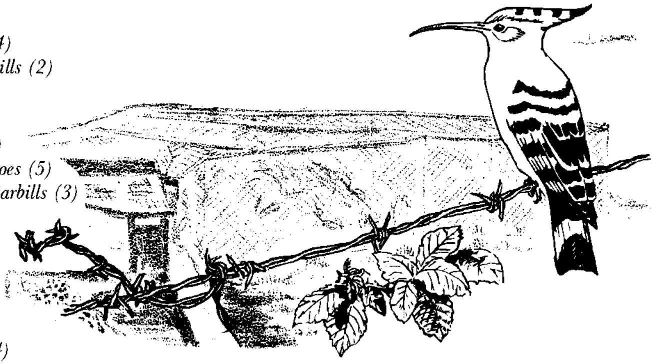
ANSERIFORMES
Anhimidae *screamers (3)*
Anseranatidae *Magpie Goose (1)*
Dendrocygnidae *whistling-ducks (9, 2)*
Anatidae *ducks, swans, geese (148, 65)*

TURNICIFORMES
Turnicidae *buttonquails (17, 1)*

PICIFORMES
Indicatoridae *honeyguides (17)*
Picidae *woodpeckers (215, 13)*
Megalaimidae *Asian barbets (26)*
Lybiidae *African barbets (42)*
Ramphastidae *New World barbets, toucans (55)*

GALBULIFORMES
Galbulidae *jacamars (18)*
Bucconidae *puffbirds (33)*

BUCEROTIFORMES
Bucerotidae *hornbills (54)*
Bucorvidae *ground-hornbills (2)*



UPUPIFORMES
Upupidae *hoopoes (2, 1)*
Phoeniculidae *woodhoopoes (5)*
Rhinopomastidae *scimitarbills (3)*

TROGONIFORMES
Trogonidae *trogons (39)*

CORACIIFORMES
Coraciidae *rollers (12, 4)*
Brachypteraciidae *ground-rollers (5)*
Leptosomidae *Courol (1)*
Momotidae *motmots (9)*
Todidae *todies (5)*
Alcedinidae *Alcedinid kingfishers (24, 1)*
Dacelonidae *Dacelonid kingfishers (61, 2)*
Cerylidae *Cerylid kingfishers (9, 2)*
Meropidae *bee-eaters (26, 3)*

COLIIFORMES
Coliidae *mousebirds (6)*

CUCULIFORMES
Cuculidae *Old World cuckoos (79, 5)*
Centropodidae *coucals (30, 1)*
Coccyzidae *American cuckoos (18, 2)*
Opisthocomidae *Hoatzin (1)*
Crotophagidae *anis, Guira Cuckoo (4)*
Neomorphidae *roadrunners, ground-cuckoos (11)*

PSITTACIFORMES
Psittacidae *parrots, etc. (358, 2)*

APODIFORMES
- **Apodidae** *swifts (99, 11)*
- Hemiprocnidae *crested-swifts (4)*

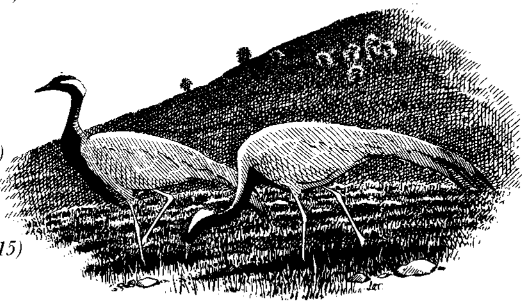
TROCHILIFORMES
- Trochilidae *hermits, hummingbirds (319)*

MUSOPHAGIFORMES
- Musophagidae *turacos, plantain-eaters (23)*

STRIGIFORMES
- **Tytonidae** *barn-owls, grass-owls (17, 1)*
- **Strigidae** *owls (161, 16)*
- Aegothelidae *owlet-nightjars (8)*
- Podargidae *Australian frogmouths (3)*
- Batrachostomidae *Asian frogmouths (11)*
- Steatornithidae *Oilbird (1)*
- Nyctibiidae *potoos (7)*
- Eurostopodidae *eared-nightjars (7)*
- **Caprimulgidae** *nighthawks, nightjars (76, 5)*

COLUMBIFORMES
- Raphidae *Dodo, solitaires (3: all extinct)*
- **Columbidae** *pigeons, doves (310, 13)*

GRUIFORMES
- Eurypygidae *Sunbittern (1)*
- **Otididae** *bustards (25, 6)*
- **Gruidae** *cranes (15, 4)*
- Heliornithidae *Limpkin, sungrebes (4)*
- Psophiidae *trumpeters (3)*
- Cariamidae *seriemas (2)*
- Rhynochetidae *Kagu (1)*
- **Rallidae** *rails, gallinules, coots (142, 15)*
- Mesitornithidae *mesites (3)*



CICONIIFORMES
- **Pteroclidae** *sandgrouse (16, 7)*
- Thinocoridae *seedsnipes (4)*
- Pedionomidae *Plains-wanderer (1)*
- **Scolopacidae** *woodcocks, snipes, sandpipers, curlews, phalaropes (88, 57)*
- **Rostratulidae** *paintedsnipes (2, 1)*
- Jacanidae *jacanas (8)*
- Chionididae *sheathbills (2)*
- **Burhinidae** *thick-knees (9, 2)*
- **Charadriidae** *oystercatchers, avocets, stilts, plovers, lapwings (89, 22)*
- **Glareolidae** *Crab-plover, pratincoles, coursers (18, 6)*
- **Laridae** *skuas, skimmers, gulls, terns, auks (129, 64)*
- **Accipitridae** *Osprey, hawks, eagles (240, 40)*
- Sagittariidae *Secretarybird (1)*
- **Falconidae** *caracaras, falcons (63, 13)*
- **Podicipedidae** *grebes (21, 6)*
- **Phaethontidae** *tropicbirds (3, 1)*
- **Sulidae** *boobies, gannets (9, 5)*
- **Anhingidae** *anhingas (4, 1)*
- **Phalacrocoracidae** *cormorants (38, 6)*
- **Ardeidae** *herons, bitterns, egrets (65, 25)*
- Scopidae *Hammerkop (1)*
- **Phoenicopteridae** *flamingos (5, 2)*
- **Threskiornithidae** *ibises, spoonbills (34, 5)*
- **Pelecanidae** *Shoebill, pelicans (9, 3)*

Ciconiidae *New World vultures, storks (26, 4)*
Fregatidae *frigatebirds (5, 1)*
Spheniscidae *penguins (17)*
Gaviidae *divers (5, 4)*
Procellariidae *petrels, shearwaters, diving-petrels, albatrosses, storm-petrels (115, 26)*

PASSERIFORMES
Acanthisittidae *New Zealand wrens (4)*
Pittidae *pittas (31)*
Eurylaimidae *broadbills (14)*
Philepittidae *asities (4)*
Tyrannidae *Mionectine flycatchers, tyrant-flycatchers, tityras, becards, cotingas, manakins, etc. (537, 2)*
Thamnophilidae *antbirds (188)*
Furnariidae *ovenbirds, woodcreepers (280)*
Formicariidae *ground-antbirds (56)*
Conopophagidae *gnateaters (8)*
Rhinocryptidae *tapaculos (28)*
Climacteridae *Australo-Papuan treecreepers (7)*
Menuridae *lyrebirds, scrub-birds (4)*
Ptilonorhynchidae *bowerbirds (20)*
Maluridae *fairywrens, emuwrens, grasswrens (26)*
Meliphagidae *honeyeaters, etc. (182)*
Pardalotidae *pardalotes, bristlebirds, scrubwrens, thornbills, etc. (68)*
Eopsaltriidae *Australo-Papuan robins (46)*
Irenidae *fairy-bluebirds, leafbirds (10)*
Orthonychidae *Logrunner, Chowchilla (2)*
Pomatostomidae *Australo-Papuan babblers (5)*
Laniidae *shrikes (30, 8)*
Vireonidae *vireos, peppershrikes, etc. (51, 3)*
Corvidae *quail-thrushes, whipbirds, Australian Chough, Apostlebird, sitellas, shrike-tits, whistlers, shrike-thrushes, crows, birds-of-paradise, currawongs, wood-swallows, orioles, cuckooshrikes, fantails, drongos, monarchs, magpie-larks, ioras, bush-shrikes, helmet-shrikes, etc. (647, 18)*
Callaeathidae *New Zealand wattlebirds (3)*
Picathartidae *rock-jumpers, rockfowls (4)*
Bombycillidae *Palmchat, silky-flycatchers, waxwings (8, 1)*
Cinclidae *dippers (5, 1)*
Muscicapidae *thrushes, Old World flycatchers, chats (449, 62)*
Sturnidae *starlings, mynas, mockingbirds, thrashers, catbirds (148, 9)*
Sittidae *nuthatches, Wallcreeper (25, 8)*
Certhiidae *treecreepers, Spotted Creeper, wrens, gnatcatchers, gnatwrens (97, 3)*
Paridae *tits, penduline-tits (65, 10)*
Aegithalidae *long-tailed-tits, bushtits (8, 1)*
Hirundinidae *river-martins, swallows (89, 11)*
Regulidae *kinglets (6, 4)*
Pycnonotidae *bulbuls (137, 3)*
Hypocoliidae *Grey Hypocolius (1, 1)*
Cisticolidae *African warblers (119, 3)*
Zosteropidae *white-eyes (96)*
Sylviidae *leaf-warblers, grass-warblers, laughingthrushes, babblers, Wrentit, scrub-warblers (552, 62)*
Alaudidae *larks (91, 23)*
Nectariniidae *sugarbirds, flower-peckers, sunbirds, spiderhunters (169, 3)*
Melanocharitidae *berrypeckers, longbills (10)*
Paramythiidae *Tit Berrypecker, Crested Berrypecker (2)*
Passeridae *sparrows, rock-sparrows, wagtails, pipits, accentors, weavers, Estrildine finches, whydahs (386, 41)*
Fringillidae *Olive Warbler, chaffinches, Cardueline finches, Hawaiian honeycreepers, buntings, longspurs, towhees, New World wood-warblers, tanagers, Neotropical honeycreepers, seedeaters, flower-piercers, cardinals, troupials, meadowlarks, New World blackbirds, etc. (993, 96)*