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# DNA Analyses Have Revolutionized Studies on the Taxonomy and Evolution in Birds

Michael Wink

## Abstract

Whereas Linné aimed to classify all species of our planet by a unique binomial Latin name, later generations of taxonomists and systematists intended to place the taxa in a natural system according to their phylogeny. This also happened in ornithology and still scientists are on the way to find the ultimate “Avian Tree of Life”. Formerly, systematic relationships were studied by comparing morphological characters. Since adaptive character evolution occurred frequently, convergences could lead to misleading conclusions. An alternative to morphological characters are biochemical markers, especially nucleotide sequences of marker genes or of complete genomes. They are less prone to convergent evolution. The use of DNA sequences of marker genes for bird systematics started around 1990. The introduction of Next Generation Sequencing (NGS) facilitated the sequence analysis of large parts of bird genomes and to reconstruct the Avian Tree of Life. The genetic analyses allowed the reconstruction of phylogenetic trees and the detection of monophyletic clades, which should be the base for a phylogenetic classification. In consequence, several orders, families and genera of birds had to be rearranged. In addition, a number of species was split into several new species because DNA data could point out hidden lineages in cryptic species or in species complexes.

**Keywords:** systematics, taxonomy, convergence, cladistics, monophyletic clades, phylogenomics, marker genes, sequence analysis, next generation sequencing

## 1. Introduction

Apparently, humans always tried to classify the animals, which they saw or hunted. They gave them local names. Only during the time of classical Greek scholars, a more systematic approach emerged. The first scholar was Aristotle (384–322 BC), the known father of Natural history and Science. He described the appearance, behavior, and occurrence of more than 140 bird species [1–3]. The next progress came with Plinius (23–79 AC), a known Roman writer. Plinius analyzed the form of feet and legs to classify birds in his *Historia naturalis*. Aristotle and Plinius were the main sources of information until the Renaissance (from 1400 onwards). The Renaissance brought progress in many fields of science, including ornithology. New knowledge was no longer transmitted in hand-written books but in printed books when Johannes Gutenberg in Mainz (Germany) invented book printing around 1450 [1–3]. In consequence, many illustrated books on

plants and animals were published. William Turner (1500–1568), Conrad Gessner (1516–1565), and Pierre Belon (1517–1564) were three known ornithologists in the 16th century. Gessner reported on 180 bird species in the illustrated *Historia animalium*. John Jonston (1603–1675) published the *Historiae naturalis de avibus libri VII* in 1650 illustrated by Matthaeus Merian (**Figure 1**). However, the classification was only based on morphology, leading to wrong relationships. As can be seen from **Figure 1**, bats were included in birds and the cuckoo and shrike were treated as raptors.

After 1600, the ornithological landscape quickly changed. New species were brought in from everywhere in the world by early explorers, and systematic collections of specimens were started facilitating the study of avian taxonomy. Known ornithologists of the 17th century were Walter Charleton (1619–1707), John Ray (1628–1704), and Francis Willughby (1635–1672). John Ray became famous since he produced with *Ornithologiae libri tres* a first modern ornithology handbook, based it on authentic observations [4].

Another breakthrough came in the 18th century: Carl Linnaeus (1707–1778), a naturalist and medical doctor from Uppsala (Sweden) revolutionized taxonomy by introducing a binary nomenclature, in which every animal and plant species obtained its own and unequivocal Latin name [1–3]: The Chaffinch was called *Fringilla coelebs* L.; the first name indicates the genus and the second the species. This name is exclusive for the Chaffinch. By comparing the outer morphology of animals and plants, Linné arranged species with a similar anatomy and morphology into genera, orders and classes. For birds, Linné used the morphology of feet and beaks to distinguish six orders of birds, which included 85 genera. As there are several



**Figure 1.** Illustrations from *Historiae naturalis de avibus libri VII*. As can be seen, shrikes and cuckoo were grouped with raptors because of their bill morphology. Cuckoo = *Cuculus*; shrike = *Lanius*; raptors = *Tinnunculus*, *Dendrofalco* (a) and even bats were classified as birds because of their wings. *Nycticorax* = Night Heron; *Caprimulgus* = Nightjar; Bats = *Fledermaus* (b). (photo M. Wink).



events of convergent evolution, some of his systematic assumptions were wrong and could not survive. In the 10th edition of *Systema naturae* (1758), six orders were distinguished: 1. **Accipitres**: Raptors, owls, parrots, waxwings, and shrikes; 2. **Picae**: Woodpeckers, hornbills, cuckoos, hoopoes, birds of paradise, crows, and creepers; 3. **Anseres**: all water birds, pelicans, cormorants, loons, grebes, gulls, and terns; 4. **Grallae**: Ratites, waders, flamingos, storks, herons, cranes, coots, and bustards; 5. **Gallinae**: wild fowl, guans, grouse, and quails and 6. **Passeres**: Pigeons, thrushes, larks, humming birds, nightjars, swifts, crossbills, wagtails, and tits [1].

During the 18th and 19th century, the knowledge on taxonomy and systematics of birds rapidly increased. Many explorers and travelers explored Europe, Africa, Asia, Australia and the Americas, and brought back many unknown species. Taxidermy improved [5] and specimens could be stored in skin collections, which were then created in Paris (1793), London (1881), Frankfurt, Halle, Munich, and Dresden [1, 2]. These curated collections enabled a better comparison and study of related and unrelated taxa. Already at that time, the status of species and subspecies was extensively debated.

The 19th century was strongly influenced by the new concept of evolution and phylogeny through natural selection formulated by Charles Darwin (1809–1882) and Alfred Russel Wallace (1823–1913). Species were no longer considered to be unchangeable (or created by God) but were seen in a phylogenetic context. This means, ancestral taxa had existed from which the extant taxa derived. Charles Darwin came up with the concept of a phylogenetic tree, which can illustrate the descent from common ancestors [1–3].

## 2. Towards a new avian classification

After Darwin, ornithologists overturned the typological species concept and tried to build up a “natural system”, based on shared ancestry and common descent. According to [6, 7], more than 40 classifications were proposed during the last two centuries. Since 1900, the order of bird families in handbooks and field guides was based on these classification systems [8–12].

Traditionally, morphology, such as plumage, beak and head shape, had been used to make inferences in systematics and taxonomy [1, 3]. Since 1900 new characters were included, coming from ecology, biogeography, and biochemistry. The main concept of classification remained overall similarity; the more similar two taxa, the more closely related they should be.

Whereas the inclusion of similar taxa into a common genus was mostly unambiguous, the circumscription of families and orders was however more difficult. In many taxa, a variation of plumage can be seen in relation to age, sex or season. Large skin collections were helpful to find out if the variable forms belonged to a single species. Several bird species (e.g. ducks and geese) can hybridize, which generate more confusion. We already noticed that adaptive characters can occur convergently. In consequence, similar adaptive features might have evolved in unrelated group of taxa. If such adaptive characters are used for taxonomy, artificial and polyphyletic groups (clades with members from unrelated lineages) may be created (**Figure 1**).

Over the last 200 years, different species concepts have also strongly influenced taxonomy and systematics [3, 4, 10]. Although ornithologists loved the typological species concept for a long time, it was substituted by Ernst Mayr by the Biological Species Concept (BSC). Presently, the “Phylogenetic Species Concept (PSC)” has been widely accepted, because it better fits the molecular data [1].

The German entomologist Willi Hennig (1913–1976) introduced the concept of cladistics. He distinguished plesiomorphic, apomorphic and synapomorphic traits

to define common ancestry in clades. Clades, which comprise all descendants of a common ancestor, are termed “monophyletic”. According to cladistics, a natural system of classification should be only based on monophyletic groups. If scientists obtain evidence for para- and polyphyletic clades, taxa in such groups need to be either lumped or split until all clades are monophyletic. The consequences for bird taxonomy are discussed in Part 5.

### 3. Impact of DNA analysis on avian systematics and phylogeny

When James Watson and Francis Crick discovered the structure of DNA in 1953 [1–3], a new era started in biology and with some delay, also in ornithology. In the decades following the discovery of DNA, new technologies emerged to study DNA and genetics: DNA sequencing was established in 1978, the polymerase chain reaction (PCR) was discovered in 1985 by Kary Mullis and Next Generation Sequencing (NGS) appeared after 2000. NGS or High-throughput Sequencing enable the parallel and concomitant sequencing of millions of DNA sequences. NGS is thus the method of choice for the analysis of complete genomes and transcriptomes [1–3, 13, 14].

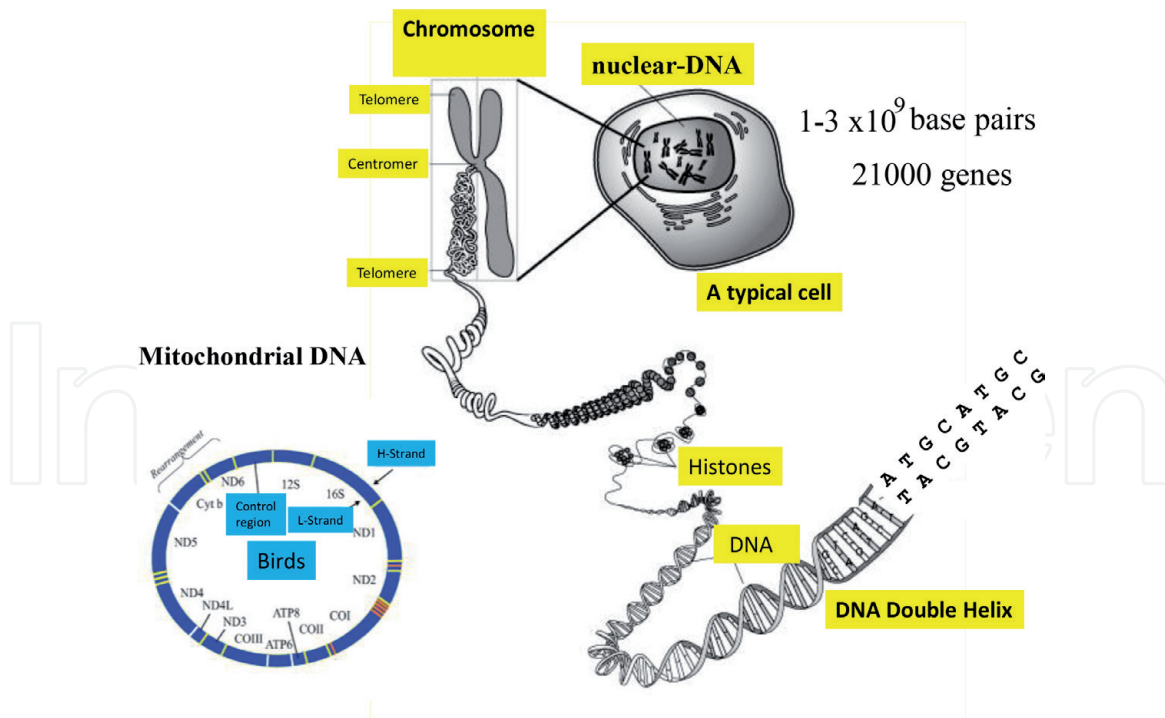
#### 3.1 DNA as a marker for phylogeny

Deoxyribonucleic acid (DNA) is a macromolecule composed of linearly coupled nucleotides. The pyrimidine bases cytosine (C) and thymine (T) have two N atoms, and the purine bases adenine (A) and guanine (G) each have four N atoms. In addition, deoxyribose (a sugar called pentose) and a phosphate group belong to a nucleotide building block. Unlike DNA, ribonucleic acid (RNA) contains uracil (U) instead of thymine and ribose (which lacks the hydroxyl group in the 2-position) instead of deoxyribose. DNA thus contains the bases A, T, G, and C, and RNA the bases A, U, G, and C. The DNA strands are complimentary and form a double helix, in which A pairs with T and G with C (**Figure 2**) [1, 3].

The DNA double helix is located in the nucleus of all eukaryotic cells as a linear, i.e. filamentous, macromolecule (**Figure 2**). Depending on the species, the nuclear genome (i.e., the DNA in the nucleus) is organized in specific number of chromosomes [1–3]. During the growth of an organism, cells have to multiply at a high rate. During cell division, the DNA of a mother cell is duplicated by a process, termed DNA replication. Consequently, daughter cells obtain an identical genome copy of the mother cell. All cells, which exist today, are never generated *de novo* but always derive from a mother cell. And this continuous flow of cell divisions must have existed since the first ancestral cell; thus all cells which exist today are connected and their DNA can be traced back to the origin of life.

Except for germ cells, all vertebrate cells have a double (diploid) set of chromosomes. All offspring receive each a haploid (single) set of chromosomes from the mother and father, respectively with the gametes (germ cells that unite at fertilization). These haploid genomes are similar, but not 100% identical. Genetic variability of individuals is generated during the generation of germ cells by a process called meiosis.

The vertebrate genome is thought to have 21,000 genes encoding proteins and another 9,000 genes encoding diverse RNAs. These genes correspond to the genotype of an individual. Since not all genes are active at the same time, but are regulated in a cell- and development-specific manner, the expression of the respective active genes is called phenotype. Epigenetic processes can influence the phenotype and phenotypic variability [3].



**Figure 2.**  
 Schematic view of nuclear and mitochondrial DNA in birds.

In addition to the nuclear genome (ncDNA), all animals have additional DNA in their mitochondria (mtDNA), cell organelles that originally arose from bacteria through symbiosis and whose main function is to provide ATP, the fuel for the cell [3]. Similar to bacteria, mtDNA exists as a ring-shaped chromosome and consists of approximately 16,000 to 19,000 base pairs in vertebrates. It contains 13 genes encoding enzymes or other proteins involved in electron transport, 22 genes for tRNAs (tRNA is the abbreviation for transfer RNA, which is required in protein biosynthesis), and two for rRNAs (rRNA is the abbreviation for ribosomal RNA, which is important for the structure and function of ribosomes) (**Figure 2**). Since each animal cell contains several 100 to 1000 mitochondria and each of the mitochondria contains five to ten mtDNA copies, the total number of identical mtDNA copies is several thousand per cell. The mtDNA makes up about 1% of the total DNA of a cell and is particularly suitable for research in molecular evolution and phylogenetics. In contrast to nuclear DNA, mtDNA is almost exclusively inherited maternally. Because mtDNA exhibits more sequence variation than protein coding ncDNA, the sequence analysis of mtDNA has widely used to study bird taxonomy and phylogenetics [13–16].

Most sequence differences in DNA, i.e. an exchange of one of the four DNA bases A, T, G and C, are due to point mutations. Point mutations are triggered by internal mechanisms that occur spontaneously and regularly. These include biochemical alterations of DNA bases (through depurination, deamination, dimerization, and oxidation) and the incorporation of tautomeric bases [3]. External factors for point mutations include high-energy radiation such as UV, X-ray, and high-energy ionizing radiation from radioactivity or cosmic rays, and mutagens (mutation-inducing substances). Most mutations are repaired by special enzymes before the duplication of chromosomes during cell division. This is one of the great advantages of the double helix: even if information on one DNA strand has been altered by mutation, it is still correctly present on the complementary strand and can be used by the repair enzymes as a back-up copy [3].

Most mutations are observed in somatic cells (body cells), which are not passed onto the offspring and perish with the death of the individual (somatic mutations).

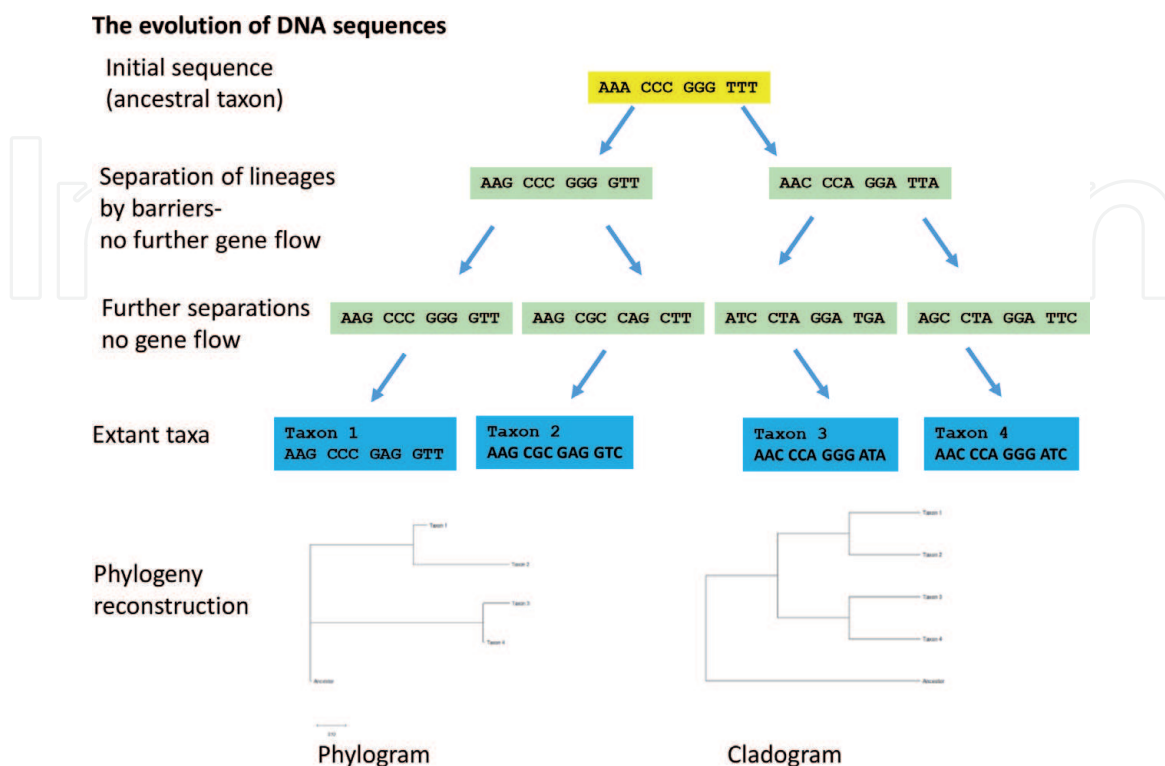


Only mutations in germline cells (gametes or sex cells) can be inherited. Most mutations have no or negative consequences. Only in rare cases does a mutated gene or allele provide a carrier with a selective advantage to better adapt its bearer to its environment and thereby increase the reproductive success of its offspring. When we analyze DNA sequences or genome structures of organisms living today, we essentially see only mutations that were either neutral or had a positive selection value. Carriers of mutations with negative consequences have logically not withstood the selection pressure - they often had no or little reproductive success and just disappeared.

Only germline mutations may end up in the next generation. If they are successful, they may survive in subsequent generations. If we look at the DNA of an individual, its DNA may differ by millions of nucleotide exchanges in its genome from conspecifics, which were inherited from the ancestors. These nucleotide exchanges can be discovered by DNA sequencing and can be used to reconstruct the Tree of life. A driver for the evolution of divergent DNA sequence lineages is their geographic or ecological separation. If a population gets isolated on an island and if there is no further exchange of individuals with the ancestral population, then an independent sequence evolution sets in, as outlined in **Figure 3**. This phenomenon and feature is the base for the Tree of life.

The rate of mutations is typical for individual genes and can be used to infer the date of ancient evolutionary divergence events. This is the concept of the “Biological Clock” which is widely used in phylogenetics [3, 14].

Darwin demanded variability of traits within populations as a prerequisite for Natural Selection. We now know that this variability exists and is due to diverse mutations in protein-coding genes and in genes for transcription factors. Mutations in regulatory genes sometimes lead to more pronounced morphological changes. This variability is used, for example, in artificial selection for animal and plant breeding. Darwin already recognized the high plasticity of our genomes, from which a breeder can generate new forms in just a few generations, such as the various cabbage vegetables bred from the wild cabbage plant or domestic dogs from wolves (see [3]).



**Figure 3.** Geographic or ecological separations of populations lead to sequence evolution and phylogeny.

### 3.2 DNA–DNA hybridization

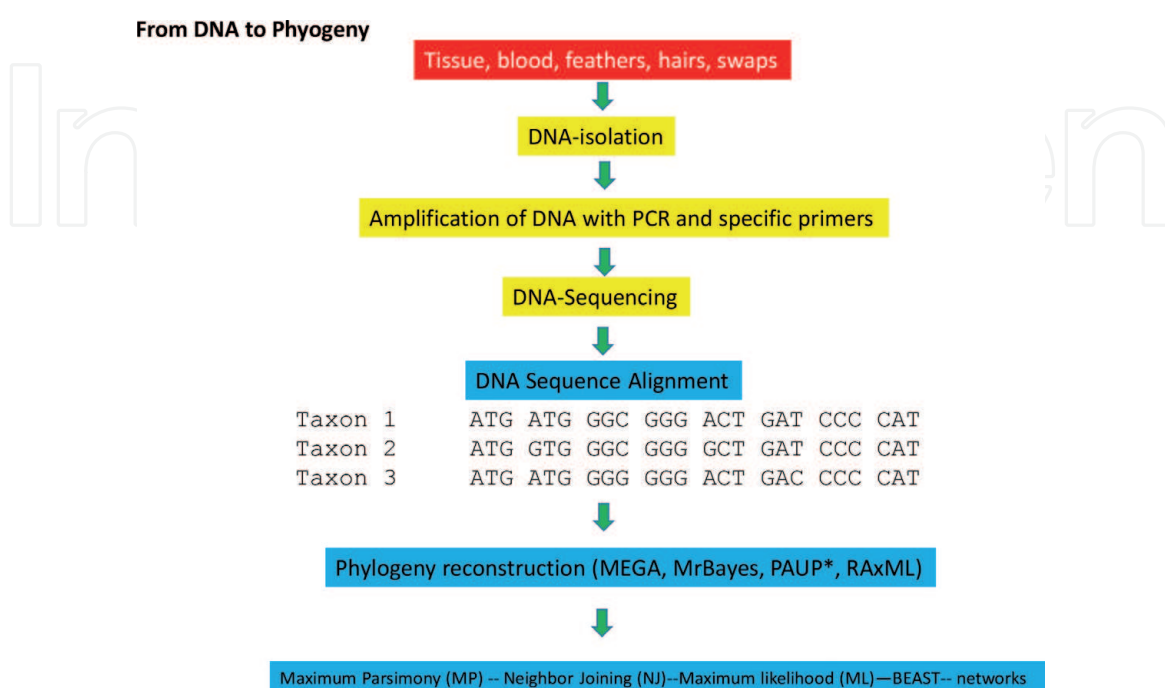
Charles Sibley was the first scientist to utilize DNA analysis to study avian systematics. When in 1975 Sibley embarked on his DNA work, DNA sequencing was not yet invented. Sibley employed DNA–DNA hybridization analysis instead, in which DNA melting temperatures are compared. Together with Jon Ahlquist Charles Sibley investigated the DNA melting profiles of more than 1700 bird taxa. In 1990, they published their results as “Phylogeny and Classification of Birds” [7]. Sibley employed the DNA–DNA hybridization data to postulate a novel avian taxonomy, published in 1990 as “Distribution and Taxonomy of Birds of the World” [12].

Sibley and Ahlquist [12] grouped many of orders and families of birds correctly, but as we know today, they were completely wrong with others [1]. For example, New World vultures are not storks, as Sibley had assumed, but cluster at the base of the Accipitriformes. DNA–DNA hybridization has severe shortcomings, because it does not provide sufficient resolution and suffers from laboratory artifacts. Sibley and Ahlquist [7] knew the limitations of the DNA–DNA hybridization, but had no choice, because at that time, it was the only DNA method around.

### 3.3 DNA sequence analysis

We can isolate DNA from any bird tissue, such as blood and muscle, but DNA also occurs in feathers or in buccal swaps. Using PCR with specific primers, single genes (so-called marker genes) can be amplified and sequenced using the Sanger chain termination method. A schematic view of the procedure, how to go from DNA to a phylogeny is illustrated in **Figure 4**.

Already the sequence analysis of marker genes from mitochondria (e.g. COI, cytochrome b, ND2) or the nuclear genome is often very informative and enables informative and reliable phylogeny reconstructions. The choice of marker genes differs between animals and plants and furthermore, depends on whether one wants to study evolutionarily young or old relationships.



**Figure 4.**  
*From a sample with DNA to a phylogeny reconstruction.*



After 2000, next generation sequencing (NGS) became available in which whole genomes are analyzed by parallel sequencing [13]. Hundreds of millions of short DNA sequences can be generated in a single NGS run. These sequences are then assembled into longer DNA segments by bioinformaticians and assigned to known genes (“annotation”). Homologous DNA sequences are aligned and, as with marker genes, evaluated using phylogeny programs. A larger and more comprehensive collection of genes or even complete genomes and transcriptomes can be sequenced by the new High-Throughput Sequencers [13, 14].

The pyrosequencer 454 from Roche represented the first generation of NGS sequencers. Several companies developed new NGS strategies and sequencers, such as Illumina, SOLiD, IonTorrent, and PacBio [1, 13, 14]. The Illumina technology is a market leader at present; these sequencers generate of up to 250 million short sequences (50 to 200 nucleotides) in a single lane. The short sequences introduce a number of problems for bioinformatics, thus new developer look sequencers that generate longer reads. 3rd generation sequencers from PacBio or Nanopore Sequencing are beginning to reach the laboratory. The longer sequences allow a localization of the sequence on a chromosome and to reconstruct complete gene assemblies including repetitive elements. Longer and high quality reads are important to reconstruct phylogenies [14].

Several thousand genome sequences are now available, mainly from prokaryotes. The number of genome sequences from animals is comparably small. But already many genome sequences are available to reconstruct the large-scale phylogenomics of animal groups, such as birds: It is foreseeable that the phylogeny of most evolutionary lineages can be reliably reconstructed via genome sequencing in a few years (see Chapter 4).

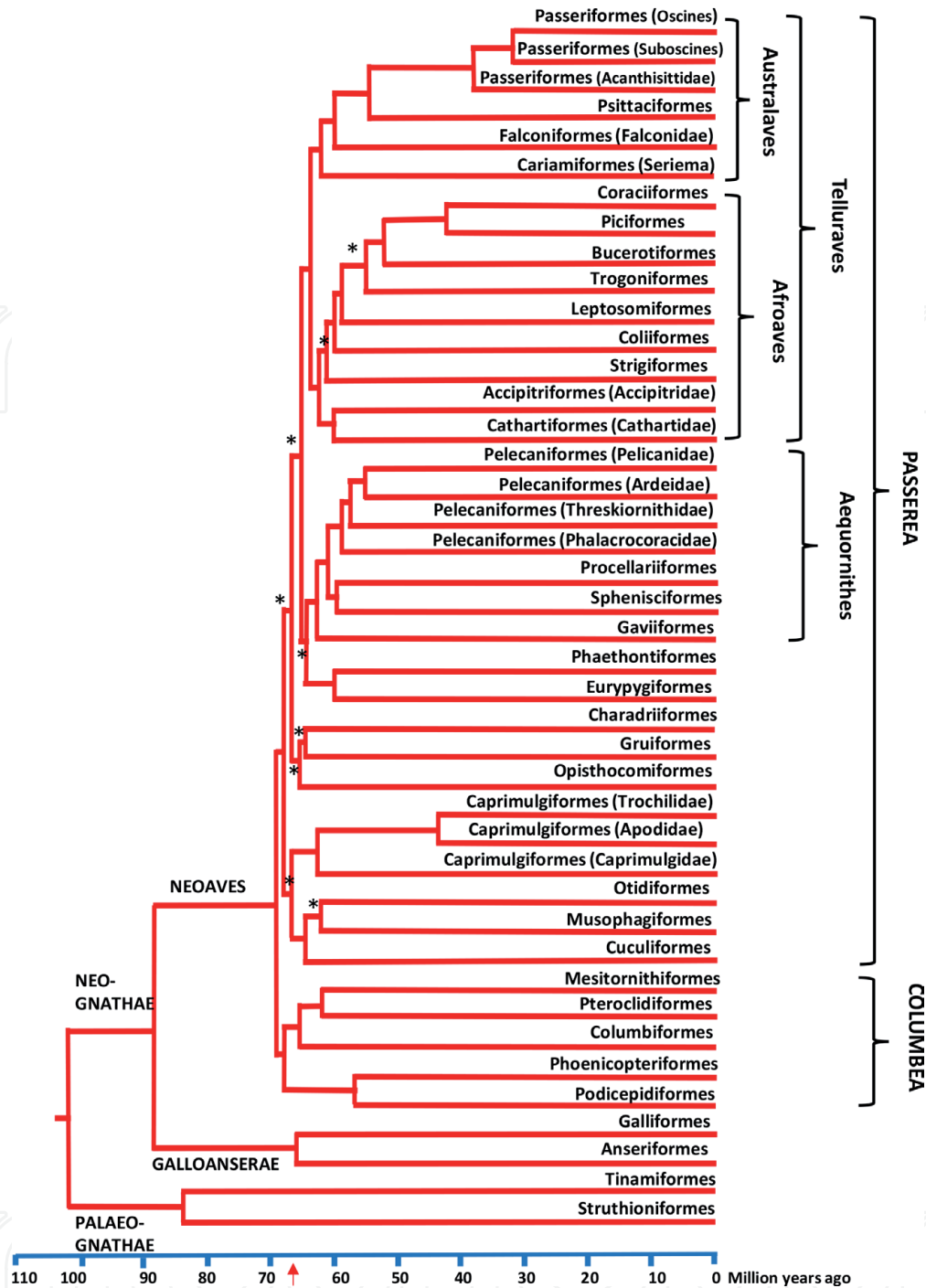
#### 4. Towards a new “Avian Tree of Life”

Genome studies of birds started later than in other animal groups [13, 14]. Following the genome of *Gallus gallus*, the next in line were *Taeniopygia guttata*, *Meleagris gallopavo*, *Ficedula hypoleuca*, *F. albicollis*, *Falco peregrinus*, *Falco cherrug*, and *Anas platyrhynchos* [13, 14, 17]. Today, several hundred genomes have been sequenced and the information is available in open databases, such as NCBI and GenBank. The initial genome data were instrumental for avian phylogenomics as the sequences could be used to assemble and align the millions of sequence snippets obtained via NGS.

The Avian Phylogenetic Consortium [18] published in 2014 a first phylogenomic Tree of life (**Figure 5**). 2015 saw a more detailed DNA analysis [19] based on target sequencing of 259 nuclear genes and a total of 394,000 nucleotides, covering 198 species in 122 families and 40 orders (**Figure 6**). The study of Prum et al. [19] can be discussed as a follow-up of Hackett et al. [14] who had sequenced 19 nuclear genes of each of the major bird families using traditional Sanger sequencing.

Simplified phylogenies [18, 19] are illustrated in **Figures 5** and **6**. Main findings include a common ancestry of swifts and nightjars, the sister-pair relationship of grebes and flamingos, the separation of falcons from diurnal raptors, inclusion of New World vultures in the raptor clade and a new clade combining falcons, parrots and passerine birds [1, 13, 14, 18, 19].

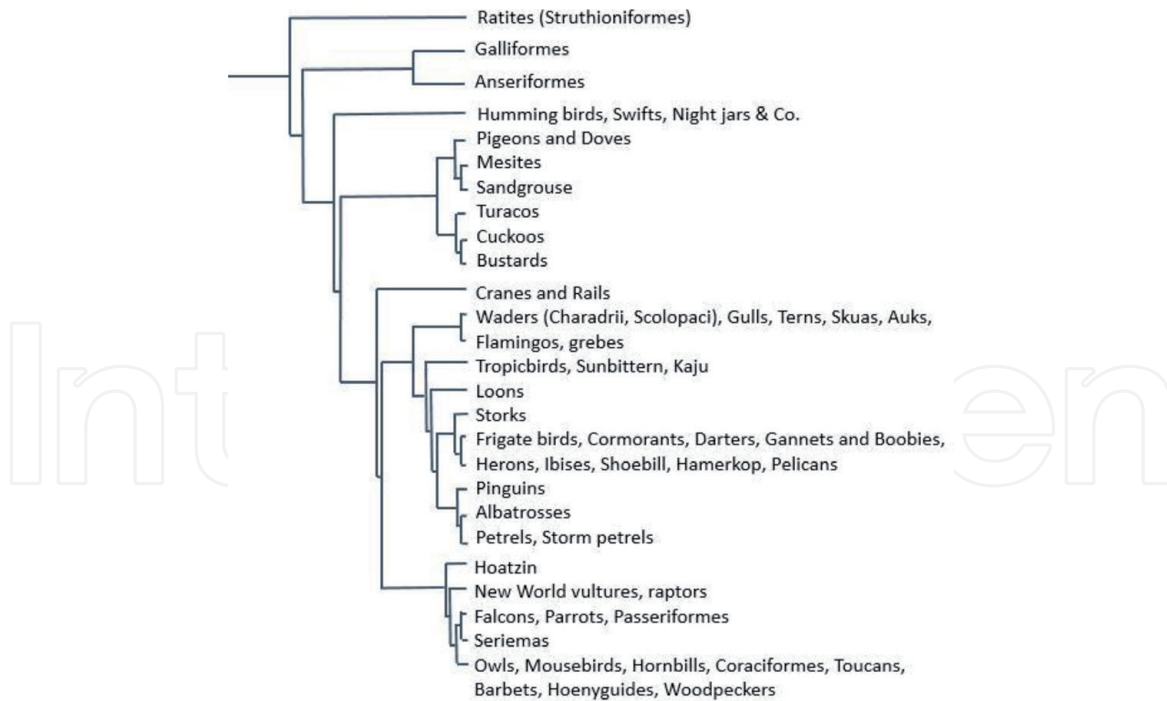
A new phylogenomic analysis covering 363 taxa from 92% of all bird families was published by Feng et al. [20]. This phylogeny contains for the first time information for many of the families within Passeriformes. The new data are combined with putative data from over 10100 bird taxa to generate a phylogeny hypothesis as



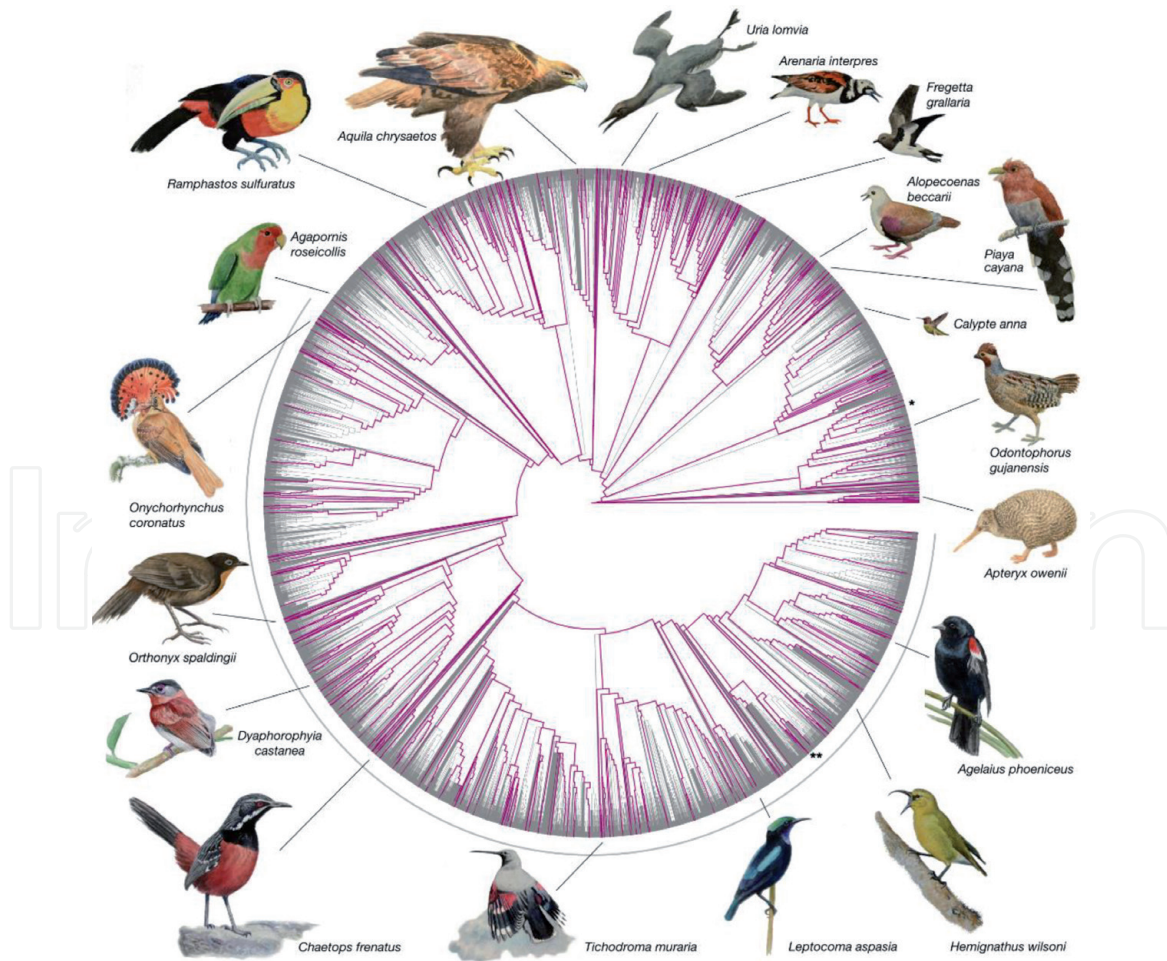
**Figure 5.**  
 The first phylogenomic avian phylogeny (modified from [18]).

shown in **Figure 7**. This analysis is preliminary and phylogenetic trees shown were reconstructed based on transposable elements. For non-passerine orders, the new phylogeny is very similar to the tree of Jarvis et al. [18] (**Figure 5**), maybe because the same taxa and genome sequences were used. For Passeriformes, the phylogeny is similar to that of Fjeldså et al. (**Figure 8**) [21].

More than 60% of all birds (6204 species) belong to the Order Passeriformes. Its systematics has seen great advantages recently. In “The Largest Avian Radiation” Jon Fjeldså, Les Christidis and Per Ericson [21] have put all evidence together to reconstruct its complex phylogeny. Passerines (also parrot and falcons) apparently evolved about 55 to 50 million years ago, just after the Cretaceous/Tertiary boundary in Australasia and then immigrated all over the world. The main radiation

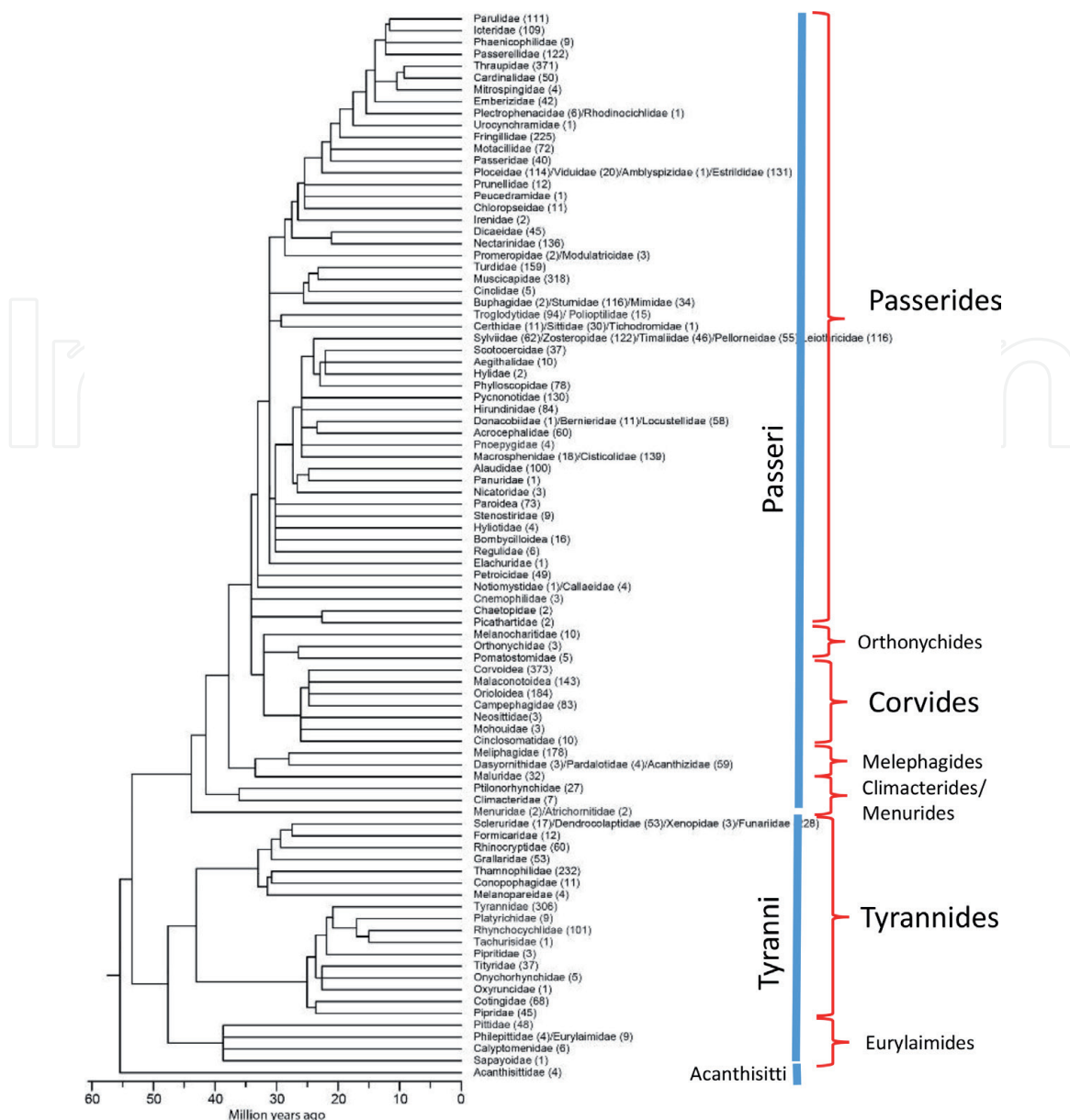


**Figure 6.** A simplified phylogeny of birds according to Prum et al. [19] based on nucleotide sequences of 259 nuclear genes.



**Figure 7.** A comprehensive avian tree of life [20]. (the article is licensed under a creative commons attribution 4.0 international license, which permits use, sharing, adaptation, distribution and reproduction in any medium or format).





**Figure 8.** A time-calibrated phylogeny of families within the Passeriformes (after [21]). Names of suborders (blue line) and infraorders (right of the red brackets).

of passerine families occurred later between 20 and 35 million years ago. The Passeriformes (**Figure 8**) are divided into three Suborders: Acanthisitti, Tyranni and Passeri. They are divided into several Infraorders and Parvorders. **Figure 8** shows a phylogeny reconstruction of the majority of families with an indication of Suborders and Infraorders. Species numbers are uneven in these groups: The Acanthisitti comprise 4 species, the Tyranni about 1290 taxa and the Passeri 4910 species. In Passeri, the largest Infraorder Corvides comprises 775 species, whereas the Passerides contain the majority of 3800 species. The book of Fjeldså et al. [21] provides phylogenies of most families of passerine birds, if available. The book is a milestone in the history of bird systematics and outlines many of the open questions.

High-throughput sequencing can also be used to study the transcriptome of birds. This information is important to understand the phenotype of an individual or adaptations to ecological or biological challenges (review in [22]). Examples are studies of the migratory phenotype of birds and the question which genes influence timing and spacing of migration events [23, 24].

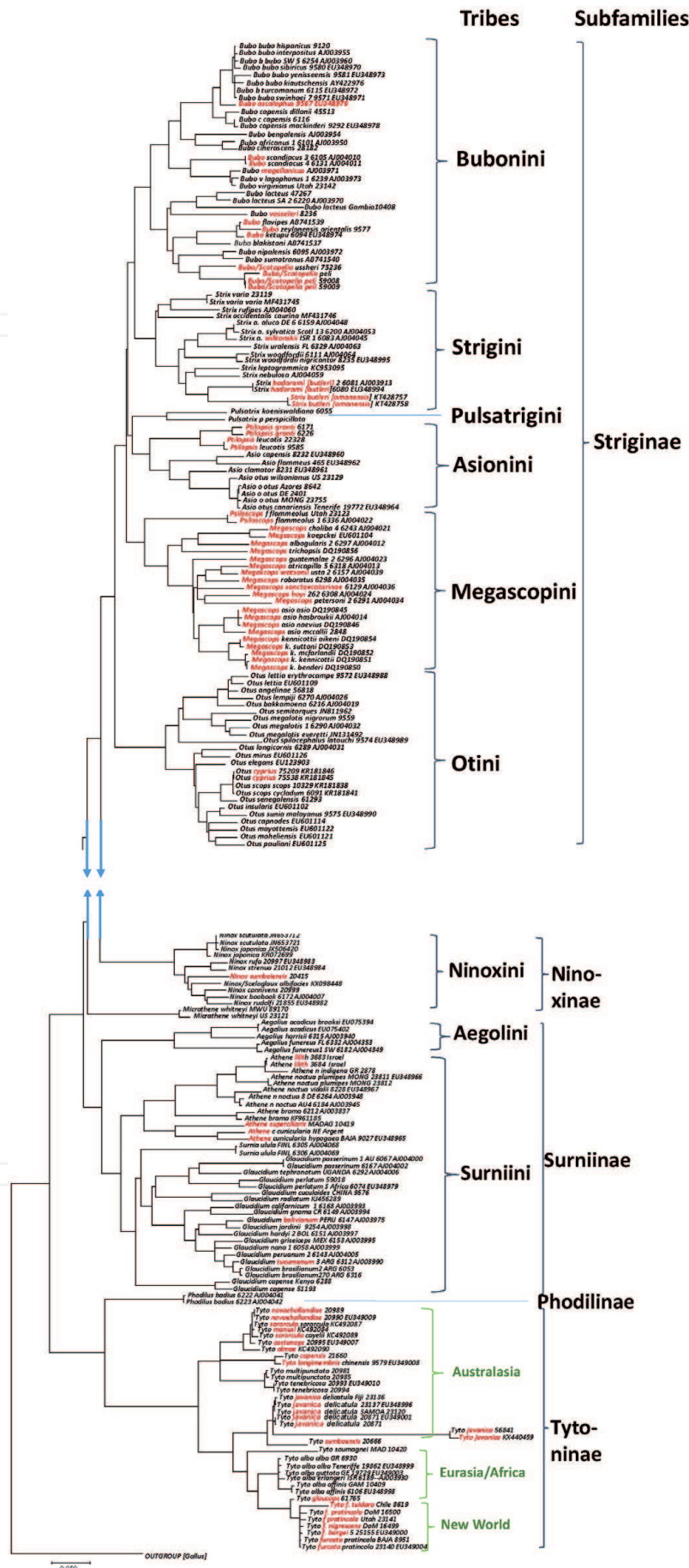
## 5. Consequences of cladistic evaluations

Progress was not only achieved at the level of orders, but also at the level of species, genera and families. With advent of DNA sequencing, more and more bird phylogenies were reconstructed from nucleotide sequences of one or more marker genes [in the beginning only mtDNA, later mtDNA and nuclear DNA (ncDNA) were used] from each species. These phylogenies provide a good resolution at the family and genus level, but often failed to infer divergences in the far past [13, 14].

As an example for the taxonomic changes within a bird family, I would like to document our own work on owl systematics [25, 26]. In **Figure 9**, a phylogram (reconstructed from cytochrome b sequences) indicates the major groupings within Tytonidae and Strigidae. In red, I have pointed out all the taxa, where DNA data either helped to define a species or a genus. In particular, the former genera *Nyctea*, *Ketupa* and *Scotopelia* were lumped into the genus *Bubo*, in order to avoid a polyphyletic genus *Bubo*. The former genus *Otus* was clearly polyphyletic and was split into new genera *Megascops*, *Psilosops* and *Ptilopsis*. *Ninox superciliaris* from Madagascar is not a member of *Ninox*, but apparently belongs to *Athene*. Linné only recognized a single species *Tyto alba* with worldwide distribution. DNA data clearly distinguish between *Tyto* from Europe/Africa (*Tyto alba* complex) and the New World (*Tyto furcata* complex). The Australasian Barn owls are quite diverse with four major lineages and many new species on isolated islands. Apparently, barn owls had evolved in Australia.

Similar splits and lumpings occurred in many bird families, just to name a few (see [27]) for a comprehensive list of accepted names).

- Gulls and terns
- Petrels and albatrosses
- Bustards
- Waders
- Woodpeckers
- Swifts
- Larks
- Shrikes
- Wagtails
- Pipits
- Warblers (*Sylvia*, *Acrocephalus*, *Cisticola*, *Hippolais*, *Phylloscopus*)
- Turdids (*Saxicola*, *Phoenicurus*, *Oenanthe*, *Turdus*)
- Tits
- Sparrows
- Finches and buntings



**Figure 9.** Phylogeny of owls (Tytonidae, Strigidae) (after [26]). Names in red are those, which had changed because of DNA data.



Thus a birder, who started his career 40 years ago will sometimes no longer recognize the Latin names of a species and their order of arrangement in modern field guides.

All these efforts have expanded the world checklist of birds. The IOC World Bird List 11.1 [27] actually (2021) comprises **10,806** extant species (and **158** extinct species) organized in **40** Orders, **252** Families and **2,353** Genera. **19,990** subspecies, their ranges and authors are also included. The number so of new bird species is increasing continuously. It has been speculated that we will end up with more than 18000 bird species, when all of them have been sequenced and re-classified [28].

## 6. Phylogeography

Another area of interest is the distribution and evolution of a species over time and space. This is the realm of phylogeography [15]. In order to use DNA for such analyses, we require highly informative DNA and methods with a high degree of resolution. Although variable mtDNA is useful in many instances, a better resolution can be obtained from the analysis of microsatellite markers. Increasingly, partial (RADSeq) and complete genome analyses from High-throughput sequencing are also used to study phylogeography because we can obtain information of millions of single nucleotide polymorphisms (SNPs). In case of human evolution, such data could trace human migrations over time and ancient hybridizations with Neanderthals and Denisovans in fascinating details [3]. It will take some time, until we will have similar data for any species of birds. But, as the costs for NGS come down, it is probably only a matter of time, until we will get there.

We have analyzed the phylogeography of several birds and reptile species on oceanic islands (Macaronesia), in the Amazon region and in Eurasia. The pattern, which we discovered, differed substantially between regions. Although the Macaronesian islands (including Canary Islands, and Madeira and Azores) are sometime not far from each other, the local bird populations are resident and do not exchange between islands [29, 30]. All these oceanic islands are of volcanic origin and between 20 to 1 million years old. They are known for their richness of endemic fauna and flora.

When we studied the variation of mitochondrial DNA sequences of birds from different Macaronesian islands, we discovered, that many of them had specific and unique island haplotypes, suggesting that gene flow between islands is very low or not existing [29, 30]. As a consequence, some of the islands species obtained species rank, such as *Phylloscopus canariensis*. In *Fringilla coelebs*, *Cyanistes caeruleus*, *Erythacus rubecula*, *Regulus regulus*, *Sylvia melanocephala*, and others we could define new island specific subspecies (see references in [29, 30]). A similar diversification can be seen on the Island archipelago of the Wallace zone in Australasia [31]. However, if we look at bird population on the Aegean Islands in Greece or Turkey (except for Cyprus), little or no differentiation can be seen [32]. The Aegean islands have been connected with each other during the last few million years, which allowed gene flow among island taxa.

We also studied some bird taxa in the Amazon region and to our surprise found a strong degree of phylogeographic patterning, which correlated with the large river systems in the area. As a result, a number of morphologically similar species could be split into new taxa mostly on account of DNA data, sometimes also because of differences in vocalization [33–37].

To our surprise, we found some genetic variation in Eurasian bird species, but could often not discover a robust phylogeographic pattern. Examples are: *Lanius collurio*, *Merops apiaster*, *Upupa epops*, *Dendrocopos major*, *Tyto alba*, *Athene noctua*, *Falco peregrinus* or *Acrocephalus palustris* [38–43]. The apparent reason for this

phenomenon concerns the climate in the last two million years, which saw a continuous cycle of warm and cold periods. During cold periods (ice ages) large parts of the northern hemisphere was covered by ice and bird populations, which settled these areas during warm periods, had to escape further south to climatically more favorable refugia, which existed on the Iberian peninsula, in North Africa and the Near East. In refugia, bird lineages met, mixed and then spread north again when the next warm period came. This has happened more than 10–20 times during the last 2 million years when most species of extant birds evolved. This has led to a complex mixing of genetic lineages in most Eurasian bird species (review in [44]).

The last ice age ended about 12000 years ago and gradually, woodland and wetland habitats in Central, North and Eastern Europe developed, which were then colonized from birds out of their southern refugia. When humans cleared forest and created agricultural landscapes, species of open land also settled in Europe. As a consequence, even if local bird populations are philopatric by now, the time period was too short to develop new haplotypes in different parts of Eurasia. Thus, Eurasian birds offers a great challenge for the phylogeographic analysis. However, if we would use similar markers for birds (SNPs) as used for humans, we might solve these problems.

The analysis of bird migration is still a challenge. The use of bird ringing and tracking system (geolocators, GPS sensors, satellite transmitters) have brought substantial progress. Since each individual bird carries a unique DNA profile, it should also be possible to connect a bird on migration or in the wintering grounds to its place of birth [44]. As discussed before, we need DNA markers of extremely resolution to solve this problem. MtDNA and microsatellite analyses are not informative enough in most cases [38, 45]. Genome-wide SNP analyses should help, as they did with human migrations.

## 7. Outlook

As a consequence of new DNA analyses and the use of cladistics, the number of extent bird species is growing from year to year. We presently recognize well over 10,806 bird species; some estimates assume even more than 18,000 bird taxa if subspecies will attain species level [28]. Even if we see very good progress over recent years, it will certainly take some time until the final “Avian Tree of Life” will be published, in which the phylogenetic position and history for each of the avian species is reconstructed. A Tree of Life, will enable a better understanding of avian evolution in general, of systematics but also of the evolution of traits and adaptations.

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## Conflict of interest

The author declares no conflict of interest.

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