RESEARCH ARTICLE SUMMARY

AVIAN GENOMICS

Evidence for a single loss of mineralized teeth in the common avian ancestor

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INTRODUCTION: The absence of teeth or edentulism has evolved on multiple occasions within vertebrates, including birds, turtles, and a few groups of mammals (anteaters, baleen whales, and pangolins). There are also mammals with enamelless teeth (aardvarks, sloths, and armadillos). All toothless/enamelless vertebrates are descended from ancestors with enamelcapped teeth. In the case of birds, it is theropod dinosaurs. Instead of teeth, modern birds use a horny beak (rhamphotheca) and part of their digestive tract (muscular gizzard) to grind up and process food. The fossil record of early birds is fragmentary, and it is unclear whether tooth loss evolved in the common ancestor of all modern birds or convergently in two or more independent lineages.

RATIONALE: Tooth formation in vertebrates is a complicated process that involves many different genes. Of these genes, six are essential for the proper formation of dentin (*DSPP*) and enamel (*AMTN*, *AMBN*, *ENAM*,

AMELX, and MMP20). We examined these six genes in the genomes of 48 bird species, which represent nearly all living bird orders, as well as the American alligator. a representative of Crocodylia (the closest living relatives of birds), for the presence of inactivating mutations that are shared by all 48 birds. The presence of such shared mutations in dentin and enamel-related genes would suggest a single loss of mineralized teeth in the common ancestor of all living birds. We also queried the genomes of additional toothless/enamelless vertebrates, including three turtles and four mammals, for inactivating mutations in these genes. For comparison, we looked at the genomes of mammalian taxa with enamel-capped teeth.

RESULTS: All edentulous vertebrate genomes that were examined are characterized by inactivating mutations in *DSPP*, *AMBN*, *AMELX*, *AMTN*, *ENAM*, and *MMP20*, rendering these genes nonfunctional. The dentin-related gene *DSPP* is

functional in vertebrates with enamelless teeth (sloth, aardvark, and armadillo). All six genes are functional in the American alligator and mammalian taxa with enamelcapped teeth. More important, 48 bird species share inactivating mutations in both dentin-related (*DSPP*) and enamel-related

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Read the full article at http://dx.doi .org/10.1126/ science.1254390 genes (ENAM, AMELX, AMTN, and MMP20), indicating that the genetic machinery necessary for tooth formation was lost in the common ancestor of all modern

birds. Furthermore, the frameshift mutation rate in birds suggests that the outer enamel covering of teeth was lost about 116 million years ago.

CONCLUSIONS: We postulate, on the basis of fossil and molecular evidence, a two-step scenario whereby tooth loss and beak development evolved together in the common ancestor of all modern birds. In the first stage, tooth loss and partial beak development commenced on the anterior portion of both the upper and lower jaws. The second stage involved concurrent progression of tooth loss and beak development from the anterior portion of both jaws to the back of the rostrum. We propose that this progression ultimately resulted in a complete horny beak that effectively replaced the teeth and may have contributed to the diversification of living birds.

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Observed shared inactivating mutations in tooth formation. Related genes were mapped onto a time tree depicting evolutionary relationships and times of divergence between modern birds, the closely related extinct taxon *lchthyornis*, and the American alligator. The hypothesized loss of mineralized teeth on the modern bird branch at 116 million years ago (Ma) is based on frameshift mutation rates.

Evidence for a single loss of mineralized teeth in the common avian ancestor

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Edentulism, the absence of teeth, has evolved convergently among vertebrates, including birds, turtles, and several lineages of mammals. Instead of teeth, modern birds (Neornithes) use a horny beak (rhamphotheca) and a muscular gizzard to acquire and process food. We performed comparative genomic analyses representing lineages of nearly all extant bird orders and recovered shared, inactivating mutations within genes expressed in both the enamel and dentin of teeth of other vertebrate species, indicating that the common ancestor of modern birds lacked mineralized teeth. We estimate that tooth loss, or at least the loss of enamel caps that provide the outer layer of mineralized teeth, occurred about 116 million years ago.

ith ~10,000 species, birds are the most species-rich clade of edentulous (toothless) jawed vertebrates (1). All edentulous or enamelless amniote lineages are derived from ancestors that possessed enamel-capped teeth (Fig. 1). Edentulism also occurs in turtles and mammalian lineages (anteaters, baleen whales, and pangolins) (2, 3), with some mammals lacking tooth enamel (aardvarks and sloths) (4), but these clades are less diverse than birds and together comprise only ~385 species. Modern birds share a common ancestry with toothed, maniraptoran theropod dinosaurs (5). Instead of teeth, modern birds use two highly specialized structures, a rhamphotheca (horny beak) for food acquisition and, as part of the digestive tract, a muscular gizzard for food processing (5). These structures are proposed to have played a role in enabling birds to adapt to a multitude of previously unavailable ecospaces and diversify relative to other edentulous vertebrates (5).

Throughout their evolutionary history [~170 million years ago (Ma)], independent tooth reduction and loss has occurred in multiple lineages of Avialae (living birds and all fossil theropods that are more closely related to Neornithes than to the dinosaurs *Dromaeosaurus albertensis* or *Troodon formosus*) (6). Minimally, there are six lineages of Avialae that show evidence of tooth

*Corresponding author. E-mail: meredithr@mail.montclair.edu (R.W.M.); mark.springer@ucr.edu (M.S.S.) reduction, with four lineages exhibiting complete tooth loss. Neornithes are hypothesized to have become edentulous somewhere between 125 and 65.5 Ma (5, 7). Among avialaen lineages exhibiting tooth reduction or loss, a rhamphotheca has also independently evolved (5, 7). The multiple independent iterations of tooth reduction and loss in Avialae suggest that ancestral odontogenetic pathways may have been predisposed to tooth reduction and loss in this clade (5).

Most phylogenetic analyses suggest that teeth were lost in the common ancestor of modern birds (8, 9), although dentate taxa from the Mesozoic (*Hesperornis* and *Ichthyornis*) have been recovered inside of crown Neornithes in some cladistic studies, implying that tooth loss could have instead occurred convergently among different members of this clade (10). The oldest fossils of putative neornithines are from the Cretaceous, but these fossils are fragmentary, lack complete jaw elements, and fail to resolve whether edentulism evolved in the common ancestor of all modern birds or convergently in at least two subclades (5).

Parallel tooth and enamel loss

The inactivation of enamel-related genes parallels the loss of enamel in the mammalian fossil record (4, 11-13). The presence of shared, inactivating mutations in dentin- and enamel-related genes could therefore be used as a molecular proxy to assess whether teeth were lost once or on multiple occasions in the common ancestor of living birds, as well as to estimate the timing of tooth loss. We queried the genomes of 48 living bird species that span the phylogeny of modern Aves (14), including representatives of the two major clades of birds (Palaeognathae and Neognathae) and nearly all extant bird orders, as well as a representative of Crocodylia [Alligator mississippiensis (American alligator)], which is the extant sister group of birds, by performing a comparative analysis of six genes that have dentin (DSPP) or enamel-related (AMTN, AMBN, ENAM, AMELX, and MMP20) patterns of gene expression. Of the hundreds of genes associated with tooth development, most are pleiotropic and perform essential functions outside of tooth development (15). By contrast, the aforementioned six genes have been hypothesized to be tooth-specific based on mutagenesis studies in mice, inactivation of these genes in one or more edentulous/enamelless vertebrate species, and natural genetic variation in humans that causes nonsyndromic amelogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasia (11-13, 16-20) (table S1). At the same time, other evidence indicates that these six genes are pleiotropic and are expressed outside of tooth development (21-23) (table S1). In addition to querying bird genomes, we also queried the genomes of additional edentulous/enamelless vertebrates, including three cryptodiran turtles [Chrysemys picta bellii (Western painted turtle), Chelonia mydas (green sea turtle), and Pelodiscus sinensis (Chinese soft-shelled turtle)] and four mammals [Dasypus novemcinctus (nine-banded armadillo), Choloepus hoffmanni (Hoffmann's two-toed sloth), Orycteropus afer (aardvark), and Manis pentadactyla (Chinese pangolin)] for signatures of molecular decay in these genes. As a control, we queried the genomes of additional mammalian taxa with enamel-capped teeth [Canis lupus familiaris (domestic dog), Loxodonta africana (African bush elephant), and Physeter macrocephalus (giant sperm whale)].

AMTN, AMBN, ENAM, AMELX, MMP20, and DSPP were recovered from the genomes of living birds, albeit with numerous inactivating mutations that render these genes nonfunctional (figs. S1 to S8 and tables S2 and S5 to S10). However, the synteny of these six tooth-related genes is strongly conserved across species. The only exceptions occur in Meleagris gallopavo (turkey), where ENAM exon 9 is found on both chromosome 1 and Z (13), and in the Pterocles guturalis (sandgrouse), where there appear to be multiple copies of exon 5 (four copies), exon 6 (three copies), and exon 10 (two copies) of MMP20 scattered across five contigs (fig. S7). Similar to other vertebrate genomes, including human and crocodile, we found that AMBN is located between AMTN and ENAM in the secretory calciumbinding phosphoprotein (SCPP) gene cluster (24) (Fig. 2), AMELX is found within the ARHGAP6 gene (Fig. 2), DSPP is located within the SIBLING gene cluster between SPARCL1 and DMP1 (24) (Fig. 2), and MMP20 is clustered with other matrix metalloproteinase proteins between MMP27 and MMP7 (25) (Fig. 2 and fig. S8). These synteny results provide unequivocal evidence that the avian pseudogenes for AMBN, AMELX, AMTN, DSPP, ENAM, and MMP20 are strictly orthologous with functional copies of these genes in vertebrates with enamel-capped teeth.

Loss of dentin and enamel genes

The remnants of *DSPP*, *AMBN*, *AMELX*, *AMTN*, *ENAM*, and *MMP20* genes in all bird genomes are characterized by exonic deletions and/or other inactivating mutations, including exon deletions, frameshift mutations, stop codons, and

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and *Aetiocetus*) and extant dentate taxa are also shown. Estimates for the timing of enamel loss in mammals are from fossils, phylogenetics, molecular clocks, frameshift mutations, and/or ratio of nonsynonymous to synonymous substitutions (dN/dS) (*4, 12*). Convergent enamel loss within Xenarthra is inferred for Pilosa (sloths and anteaters) and multiple armadillo lineages (*4*). Information on the enam- and dentin-related genes for pygmy/dwarf sperm whales, anteaters, and baleen whales are from previous studies (*4, 11, 12, 16, 44*). Hypothesized loss of mineralized teeth on the stem neornithine branch is assumed on the basis of indel substitution rates (*27, 43*). Numbers in brackets indicate the number of bird genomes analyzed for that particular group. Ghost branches are arbitrarily shown as representing ~5 million years, except for *lchthyornis*, where the ghost branch was extended to accommodate the depiction of inactivating mutations on the stem Neornithes branch.

splice-site mutations (figs. S1 to S8; tables S2 and S5 to S10; and nexus files 21 to 26). Importantly, all 48 neornithine species share inactivating mutations in both dentin-related (*DSPP*) and enamel-related genes (*ENAM*, *AMELX*, *AMTN*, and

MMP20) (Fig. 1, Table 1, and tables S5 and S7 to S10). This pattern of shared inactivating mutations indicates that the genetic machinery for tooth formation, including both dentin and enamel, was lost on the branch leading to all

extant birds. Additional frameshift mutations in these genes have continued to accumulate in crown lineages and are diagnostic of Palaeognathae, Neognathae, and more nested lineages within these clades (Table 1). The remnants of







Fig. 2. Schematic representation of enam- and dentin-related genes (blue) and flanking genes (red) from their positions in *Homo* (Ensembl 67), one of the most accurately annotated vertebrate genomes. Exons are indicated by arrows. Details of likely inactivating mutations identified in all enamelless and edentulous species are provided in tables S4 to S21.

Table 1. Total number of shared inactivating mutations in enamel- and dentin-related genes within birds.

| | ENAM | AMBN | AMTN | AMELX | MMP20 | DSPP |
|---------------|------|------|------|-------|--------------|------|
| Neornithes | 4 | 0 | 1 | 1 | 1 | 4 |
| Palaeognathae | 13 | 3 | 1 | 0 | 2 | 30 |
| Neognathae | 2 | 0 | 2 | 1 | 2 | 0 |
| Galloanserae | 1 | 1 | 1 | 2 | 0 | 1 |
| Neoaves | 4 | 0 | 2 | 0 | 3 | 4 |

these genes in extant birds are in advanced states of molecular decay, and we cataloged more than 1300 independent exon deletions and frameshift mutations in these six genes in crown Aves (tables S2 and S5 to S10).

Most of the *DSPP* gene has been lost from the genomes of neognath taxa, whereas relicts of this gene can be found in the two palaeognaths (ostrich and tinamou). Of these, *Struthio camelus* (ostrich) *DSPP* is more complete and retains portions of exon 1 (noncoding) and all five protein-coding exons (2 to 6) (figs. S5 and S6). *Tinamus guttatus* (tinamous), in turn, retains fragments of exons 5 and 6, as well as introns 1, 2, 4, and 5. Sire *et al.* (*26*) reported the presence of *DSPP* exon 2 in *Gallus gallus* (chicken), but we did not find any remnants of exonic regions of *DSPP* in chicken or other members of Galloanserae. Neoaves species retain ~1.5 kb of intron 4, fragments of exon 5 that preserve frameshift mutations that are shared with paleognaths, and portions of the noncoding region of exon 6. The region separating *SPARCL1* and *DMP1* is truncated in birds (5 to 37 kb) relative to American alligator (45 kb). The diminutive size of this region was noted in *Gallus* (26), and this pattern is now documented in all newly sequenced bird genomes.

Remnants of *AMBN*, *AMELX*, *AMTN*, *ENAM*, and *MMP20* are more complete than *DSPP* (figs. S1 to S8 and tables S5 to S10), but in some cases whole exons or multiple-exon blocks have been excised from one or more genomes. For example, a block of *ENAM* containing exons 2 to 8 has been deleted in *Calypte anna* (Anna's hummingbird) (fig. S3 and table S2), and exon 1 of *MMP20* is absent from the genomes of living birds (figs. S7 and S8 and tables S2 and S10). *MMP20* is closely syntenic with other matrix metalloproteinase (*MMP*) genes in gnathostomes with sequenced genomes (25). In American alligator, *MMP20* is located between *MMP27* and *MMP7*, and the region separating *MMP27* and *MMP20* is ~41 kb (fig. S8). By contrast, this region is reduced by almost 30 kb across a range of diverse bird genomes, including both Palaeognathae and Neognathae, which suggests that exon 1 was excised from the genome in the common ancestor of extant birds (fig. S8). In addition to the loss of exon 1, exon 3 is present only in *Anas platyrhynchos domestica* (domestic duck) (figs. S7 and S8 and table S2), which suggests multiple independent losses of this exon in birds.

Inactivating mutations are also present in these dentin- and enamel-related genes in the three turtle species with genome sequences, including inactivating mutations in AMELX shared by the three turtle species we examined (figs. S11 and S12 and tables S17 to S22). However, the genomic record of the six tooth-related genes in turtles is largely incomplete, and additional shared inactivating mutations may have been lost by subsequent, more inclusive deletions. For example, exons 5 to 7 of ENAM are missing from all three turtle species (fig. S11), but one or more of these exons may have been deleted in the common ancestor of these taxa followed by subsequent overprinting (e.g., additional deletions) in Chrysemys and Pelodiscus (fig. S11 and table S3). These difficulties in reconstructing frameshift mutations in tooth-related genes in





the early evolutionary history of turtles are not unexpected given that tooth loss in Testudines is at least as old as the Upper Jurassic (~145.5 to 161.2 Ma) (Fig. 1) (*13*). The antiquity of tooth loss in turtles may therefore have provided an extended window for tooth gene degradation in this clade (*13*).

Among enamelless mammals with genome sequences (sloth, armadillo, and aardvark), the *DSPP* gene remains intact, whereas *MMP20*, *ENAM*, *AMTN*, *AMELX*, and *AMBN* genes have been inactivated, with the exception of *AMBN* and *MMP20* in armadillo (Fig. 1, figs. S9 and S10, and tables S11 to S16) (13). There are no inacti-

vating mutations shared by the two xenarthrans (sloth and armadillo), although sloths share frameshift mutations in the ENAM gene with anteaters (4). The enamel-related MMP20, ENAM, AMTN, AMELX, and AMBN genes have also been inactivated in the edentulous Chinese pangolin, and all of the protein-coding exons have been excised from the dentin-related DSPP gene in this species (Fig. 1 and tables S11 to S14 and S16) (13). The functionality of DSPP (pygmy/dwarf sperm whales and baleen whales), MMP20 (anteaters), AMTN (anteaters, pygmy/dwarf sperm whales, and baleen whales), and AMBN (anteaters) are currently unknown. We also did not find evidence for molecular decay of tooth genes in the genomes of any of the nonavian toothed sauropsids [*Alligator*, and *Anolis carolinensis* (Carolina anole)] or mammals (elephant, dog, and sperm whale) (Fig. 1) that were included in our study. These results confirm that inactivation of tooth genes has occurred convergently in birds, turtles, and multiple mammalian lineages.

Dating of mutations in genes associated with tooth loss in birds

The occurrence of shared inactivating mutations in both dentin- and enamel- related genes suggests that teeth were lost on the stem neornithine

branch, which extends from 244.2 to 101.6 Ma (14). On the basis of the rate at which frameshift mutations accumulate (27) in neutrally evolving, tooth-related pseudogenes in crown Neornithes and the avian time tree of the 48 species in (14), we estimate that the outer enamel covering of teeth was lost around 116 Ma (95% confidence interval = 105.7 to 128.3 Ma) (see the supplementary materials) in the common ancestor of all modern birds. The remnants of DSPP in modern birds are too incomplete to date dentin loss, but if the loss of DSPP is a molecular proxy for edentulism then tooth loss in birds occurred no latter than 101.6 Ma on the basis of patterns of shared mutations in DSPP in modern birds. The closest relatives of crown Neornithes belong to Ichthyorniformes (e.g., Guildavis, Iaceornis, and Ichthyornis) and Hesperorniformes (e.g., Baptornis and Hesperornis) (7, 28) and range from the late Cenomanian (Ichthyornis, ~93.5 Ma) to within 300,000 years of the Cretaceous-Paleogene (KPg) boundary (29) at 66 Ma when modern birds are thought to have undergone a rapid radiation of species (14). The presence or absence of teeth cannot be determined for most ichthyorniform and hesperorniform taxa owing to incomplete skeletal remains, but Ichthyornis and Hesperornis fossils possess both dentary and maxillary teeth (28). Our genomic results support the exclusion of these taxa, as well as all dentate bird fossils, from crown Neornithes. Instead, they suggest that members of Ichthyorniformes and Hesperorniformes were dentate contemporaries of edentulous crown birds for most of the Late Cretaceous. Other avian contemporaries of crown Aves in the Late Cretaceous included both toothed and edentulous birds belonging to the now extinct clade Entantiornithes ("opposite birds") (Fig. 2).

Discussion

Previous studies have documented that DSPP. AMBN, AMELX, AMTN, ENAM, and MMP20 are expressed outside of tooth development (21-23)(table S1). Our findings of widespread and convergent pseudogenization of these six genes across diverse edentulous and enamelless vertebrates provides evidence that these genes are toothspecific with respect to their essential functions that are maintained by natural selection. Viewed from another perspective, these six genes are pleiotropic with the caveat that their pleiotropic roles outside of tooth development are nonobligatory. DSPP, which is involved in dentin formation, has been independently inactivated in three lineages of edentulous amniotes (birds, turtles, and Chinese pangolin). Similarly, AMELX, AMBN, AMTN, ENAM, and MMP20 are critical for enamel formation (table S1) and have been independently inactivated in birds, turtles, pangolins, sloths, armadillos (except MMP20 and AMBN), and aardvarks. Anteaters, baleen whales, pygmy/dwarf sperm whales, and narwhals also show evidence of inactivation of enamel-related genes, although sequence coverage for these genes is limited to one or a few partial exons of AMBN, AMELX, ENAM, and MMP20 (4, 11, 12). If these genes played critical, essential roles out-

side of enamel or dentin development, we would instead expect them to be maintained by natural selection in edentulous and enamelless lineages. It is possible that redundancy in the transcriptome may have compensated for their non-toothspecific functions in edentulous and enamelless lineages. For example, MMP20's primary role is in enamel formation, but it is also expressed in developing dentin and together with MMP2 cleaves DSPP in dentin formation (30). The MMP2-MMP20 redundancy in DSPP cleavage (30) may have facilitated inactivation of MMP20 once it was released from performing its function in enamel formation in enamelless taxa that nevertheless retain dentin-based teeth such as aardvark and sloth.

It is also possible that tooth-related genes are incidentally expressed outside of tooth development via gene expression neighborhoods (31, 32). Genes such as AMBN, AMTN, and ENAM occur in the SCPP gene cluster (24) that also includes bone-related genes and salivary proteins, and incidental coexpression may be expected to occur in some of these tissues. Expression studies provide support for the expression of these toothrelated genes in other biomineralized tissues (22, 33, 34) (table S1). Similarly, MMP20 is located in one of the MMP gene clusters with several other matrix metalloproteinase genes, and its coexpression in nondental tissues (e.g., lung and large intestine) (21, 35, 36) with other MMPs may be attributed, in part, to gene expression neighborhoods.

The possibility of functional splice site variants also merits attention. Given that multiple splice site variants are associated with Amelogenin, the gene AMELX (37) may remain intact if inactivating mutations are confined to a subset of AMELX exons. However, our data identified widely distributed inactivating mutations in AMELX across all of the protein-coding exons of this gene, which suggests that no bird has viable splice site variants (table S9) (13).

Edentulism and the development of a complete rhamphotheca are integral features of modern birds. Beak development, in turn, is associated with the expression of an assemblage of beta-keratin genes that differs from the sets of beta-keratin genes associated with other epidermal appendages, including scales, claws, feathers, and the egg tooth (38). Expression studies suggest that inactivation of BMP4 in the oral epithelium of chick embryos leads to early-stage odontogenic arrest (39). Moreover, BMP4 expression levels in beak mesenchyme are correlated with beak morphology (40). The inactivation of tooth genes does not provide direct evidence for a complete rhamphotheca, but fossil-based evidence (e.g., Hesperornis and Ichthyornis) for the replacement of premaxilary teeth by a partial rhamphotheca in the first stage of rostral remodeling suggests that tooth loss and rhamphotheca development are tightly linked, as may be expected if keratinization of epithelial cells impeded tooth formation (41).

We postulate, on the basis of fossil and molecular evidence, a two-step scenario whereby tooth loss and rhamphotheca development evolved in concert with each other on the stem neornithine branch. We propose that the first stage included tooth loss on the premaxilla and the development of a partial rhamphotheca on the premaxilla and anterior mandible (5, 42). Direct evidence for this incipient stage in the remaking of the avian rostrum derives from the fossil record (5, 42). Importantly, the evolution of a partial rhamphotheca may have been a prerequisite for complete edentulism so that the latter was adaptively viable (5). The second stage involved concurrent rostrocaudal progression of tooth loss and rhamphotheca development from the already modified anterior rostrum (5) (Fig. 2). We propose that this progression ultimately resulted in a complete rhamphotheca that effectively replaced the teeth and contributed to the diversification of living birds. (Fig. 3)

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6215/1254390/suppl/DC1 Supplementary Text Figs. S1 to S12 Tables S1 to S22 References (46–111)

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Supplementary Materials for

Evidence for a single loss of mineralized teeth in the common avian ancestor

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This PDF file includes:

Materials and Methods Supplementary Text Figs. S1 to S12 Tables S1 to S22 References

Other Supplementary Materials for this manuscript include the following: (available at www.sciencemag.org/cgi/content/full/346/6215/1254390/DC1)

Nexus File Databases S1 to S38 as zipped archives

Nexus File 1: Bird and alligator AMTN scaffold alignments
Nexus File 2: Bird and alligator AMBN scaffold alignments
Nexus File 3: Bird and alligator ENAM scaffold alignments
Nexus File 4: Bird and alligator AMELX scaffold alignments
Nexus File 5: Bird DSPP scaffold alignments
Nexus File 6: Bird and alligator DSPP scaffold alignments with scaffolds spanning from SPARCL1-DMP1
Nexus File 7: Bird and alligator MMP20 scaffold alignments
Nexus File 8: Bird MMP27-MMP20 scaffold alignments
Nexus File 9: Mammal AMTN scaffold alignments
Nexus File 10: Mammal AMBN scaffold alignments
Nexus File 11: Mammal ENAM scaffold alignments
Nexus File 12: Mammal AMELX scaffold alignments
Nexus File 13: Mammal DSPP scaffold alignments
Nexus File 14: Mammal MMP20 scaffold alignments

- Nexus File 15: Turtle AMTN scaffold alignments
- Nexus File 16: Turtle AMBN scaffold alignments
- Nexus File 17: Turtle ENAM scaffold alignments
- Nexus File 18: Turtle AMELX scaffold alignments
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- Nexus File 20: Turtle MMP20 scaffold alignments
- Nexus File 21: Bird and alligator AMTN exon alignments
- Nexus File 22: Bird and alligator AMBN exon alignments
- Nexus File 23: Bird and alligator ENAM exon alignments
- Nexus File 24: Bird and alligator AMELX exon alignments
- Nexus File 25: Bird and alligator DSPP exon alignments with scaffolds spanning MMP27-MMP20
- Nexus File 26: Bird and alligator MMP20 exon alignments for birds
- Nexus File 27: Mammal AMTN exon alignments
- Nexus File 28: Mammal AMBN exon alignments
- Nexus File 29: Mammal ENAM exon alignments
- Nexus File 30: Mammal AMELX exon alignments
- Nexus File 31: Mammal DSPP exon alignments
- Nexus File 32: Mammal MMP20 exon alignments
- Nexus File 33: Turtle AMTN exon alignments
- Nexus File 34: Turtle AMBN exon alignments
- Nexus File 35: Turtle ENAM exon alignments
- Nexus File 36: Turtle AMELX exon alignments
- Nexus File 37: Turtle DSPP exon alignments
- Nexus File 38: Turtle MMP20 exon alignments

Materials and Methods

Identification and Annotation of Tooth Genes.

DSPP, ENAM, AMBN, AMTN, AMEL, MMP20 gene sequences from mammalian taxa with enamel-capped teeth (e.g., Homo sapiens, Loxodonta africana, Canis lupus familiaris, 13) were used to query the genomes of enamelless or toothless mammalian taxa (Dasypus novemcinctus, Choloepus hoffmanni, Orycteropus afer, Manis pentadactyla) on NCBI and identify homologous sequences. Sequences were aligned in Geneious (46).

DSPP, ENAM, AMBN, AMTN, AMEL, MMP20 genes in the bird and turtle genomes were identified by blasting (blastn; 47) with crocodylian mRNA sequences taken from Genbank: Paleosuchus palpebrosus (Cuvier's dwarf caiman); AMEL (AF095568), Caiman crocodylus (spectacled caiman); AMBN (AY043290), Crocodylus niloticus (Nile crocodile); ENAM (GU344683). Complete DSPP, MMP20, and AMTN mRNA sequences for crocodylians are unknown. As a result, the *Alligator* genome was blasted (blastn; 47) with Ensembl predicted Anolis carolinensis (Carolina anole) sequences (AMNT LOC100554538; MMP20, ENSACAG00000021026; DSPP, ENSACAG00000012488). The annotated Ensembl Anolis DSPP sequence is not complete. Mus musculus (house mouse; NM 010080) and human (NM 014208) DSPP sequences were first used to blast the Anolis genome prior to blasting the Alligator genome to identify all of the unannotated Anolis DSPP exons. DSPP exons were further verified with mRNA sequence data isolated from *Alligator mississipiensis* teeth (provided by the Crocodilian Genome Working Group). We were unable to assemble any of the enamel-related genes used in this study with any confidence given the low number of reads associated with the library $(\sim 2 \text{ million reads}).$

Genes flanking *MMP20* (*MMP27* and *MMP7*) and *DSPP* (*SPARCL1* and *DMP1*) were identified in the bird, turtle, and *Alligator mississipiensis* genomes by blasting (blastn; 47) crocodyliform and bird GenBank (experimentally obtained)/Ensembl (predicted) mRNA sequences: *Paleosuchus palpebrosus* (DMP1 [AB185286]), *Gallus gallus* (MMP27 [NM_205000]; MMP7 [NM_001006278]; SPARCL1 [ENSGALT00000017778]).

All scaffolds with significant blastn hits (E<10) for the 10-targeted genes were subsequently visually inspected to verify the hit and then manually annotated using Geneious (46). The annotated *Alligator* scaffolds were then aligned to all corresponding bird scaffolds containing the same genes one at a time using the Mauve genome aligner (mauveAligner algorithm; 48) in Geneious (46). Mauve was set to automatically calculate the seed weight and minimum Local Collinear Blocks. Furthermore, we assumed synteny of overlapping genes between genomes, which were subsequently verified by the scaffold alignments. Fine-tuning of the Mauve aligned annotated regions was then executed using Muscle and/or MAFT (46; default settings). In many cases the annotated scaffold Mauve alignments identified exons that blast searches failed to find, particularly for the small exons. Subsequently, each of the homologous annotated bird scaffolds were aligned to one another using the Mauve genome aligner followed by Muscle/MAFT refinements of subregions (same settings as stated above). Each of the aligned exonic regions was then visually inspected and all newly identified exons were annotated. Identified exonic regions for each of the tooth-specific genes were then incorporated in a multi-species alignment in Geneious (46) using Muscle/MAFT. Inactivating mutations were visually identified.

Dating of Enamel and Tooth Loss.

Inactivating mutations (frameshift indels, whole exon[s] deletions, deletions of start or stop codon) in five enamel-related genes (AMBN, AMEL, AMTN, ENAM, MMP20) were used to date enamel loss in the common ancestor of crown Aves following previously described methods (27). Inactivating mutations in these five genes were mapped onto Jarvis et al.'s (14) phylogeny with parsimony optimization. The total number of inactivating mutations in crown Aves (1344) was then divided by the total amount of time represented by branches in crown Aves (2753 million years; timetree from 14) to yield a rate for the accumulation of inactivating mutations (0.488 mutations per million years). Loss of enamel was then calculated as the sum of the age of crown Aves (101.64 Ma [14]) plus the ratio of the total number of inactivating mutations on the stem Aves branch (7) divided by the mutation rate of 0.488 inactivating mutations per million years based on the compilation of five enamel-specific genes. Ninety-five percent confidence intervals on this estimate were derived with bootstrap resampling and 1000 replicates. Each bootstrap replicate was resampled with replacement from the total pool of 1351 inactivating mutations (7 on stem branch, 1344 in crown clade). DSPP is the only dentin-specific gene and is too incomplete to infer the age of dentin loss using this method. However, dentin is inferred to have been lost no later than the common ancestor of crown Aves (101.64 Ma) based on the occurrence of inactivating mutations in DSPP that are shared across Aves.

Supplementary Text

Description and Sources of Fossil Species in Figure 1.

Divergence dates and fossil ages are based on the following references: crown Aves (14 Jarvis); mammals (49, 50); Chrysemys to Chelonia (51); Pelodiscus to Chrysemys (51); Amniota (52); Odontochelys (53); Eomaia (54); Ichthyornis (55); Aetiocetus (56).

Description and Sources of Fossil Species in Figure 3.

Archaeorhynchus (57); Patagopteryx (58); Jianchangornis (59); Schizooura (60); Vorona (61); Zhongjianornis (62); Jiuquanornis (45); Yixianornis (63); Yumenornis (45);
Yanornis (63); Songlingornis (see Chaoyangia); Gansus (64); Apsaravis (65); Ambiortus (66); Hollanda (67); Changmaornis (45); Ichthyornis (55); Limenavis (68); Enaliornis (69); Baptornis varneri (70); Parahesperornis (71); Hesperornis (61, 72); Vegavis (73); Gobipteryx (74); Protopterus (75); Longirostravis (76).

Chaoyangia (77): The type specimen IVPP V9934 consists of only poorly preserved postcranial material. Two additional specimens were later referred to *Chaoyangia* one of which contains a nearly complete skull (IVPP V10913) despite the lack of the overlapping preserved elements. Subsequently, one of the referred specimens (IVPP V10913) was placed into the new genus *Songlingornis* (78) that left the validity of *Chaoyangia* in question. O'Connor and Zhou (7) confirmed the distinction of

Songlingornis from *Chaoyangia*. As a result, the presence/absence of teeth in *Chaoyangia* was coded as unknown.

Longicrusavis: Dentaries could be edentulous but this is not known with certainty (79).

Hongshanornis: Originally described as edentulous (*80*) but O'Connor et al. (*79*) suggest the presence of alveoli in the premaxilla and maxilla in the holotype (IVPP V14533). Mandible could be edentulous but this is not known with certainty (*79*)

Baptornis advenus (81): We are unaware of any skull material with teeth preserved in jaw bearing elements. As a result we coded this taxon as unknown.



AMTN

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| Nestor notabilis scaffold5464 | |
| Nipponia nippon scaffold1734 | |
| Ophisthocomus hoazin scaffold400 | |
| Pelecanus crispus scaffold11738 | |
| Phaethon lepturus scaffold8778 | |
| Phalacrocorax carbo scaffold29799 | |
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Fig. S1.

Bird and alligator AMTN scaffold alignments.



AMBN

Exon

Alligator mississipiensis scaffold107

Acanthisitta chloris scaffold1839 Anas platyrhynchos domestica Ensembl66 Antrostomus carolinensis scaffold53789 Apaloderma vittatum scaffold14372 Aptendodytes fosteri scaffold412 Balearica regulorum scaffold210 Buceros rhinoceros scaffold45198 Buceros rhonoceros scaffold22210 Calypte anna scaffold207 Cariama cristata scaffold16682 Cathartes aura scaffold18569 Chaetura pelagica scaffold145 Charadrius vociferus scaffold586 Chlamydotis macqueenii scaffold7713 Corvus brachyrhynchos scaffold547 Cuculus canorus scaffold286 Egretta garzetta scaffold693 Eurypyga helias scaffold713 Falco peregrinus scaffold630 Fulmarus glacialis scaffold11512 Gallus gallus Ensembl66 Gavia stellata scaffoldC13393595 Gavia stellata scaffold52443 Geospiza fortis scaffold167 Haliaeetus albicilla scaffold43224 Haliaeetus albicilla scaffold6753 Haliaeetus leucocephalus scaffold1083 Leptosomus discolor scaffold10880 Manacus vitellinus scaffold365 Melopsittacus undulatus Ensembl66 Mesitornis unicolor scaffold29450 Nestor notabilis scaffold5464 Nipponia nippon scaffold1734 Ophisthocomus hoazin scaffold47 Pelecanus crispus scaffold11738 Phaethon lepturus scaffold8778 Phalacrocorax carbo scaffold C8218617 Phalacrocorax carbo scaffold61064 Phoenicopterus ruber scaffold9196 Picoides pubescens scaffold528 Podiceps cristatus scaffold7374 Podiceps cristatus scaffold74317 Pterocles guturalis scaffold12949 Pygoscelis adeliae scaffold311 Struthio camelus scaffold149 Taeniopygia gutatta Ensembl66 Tauraco erythrolophus scaffold46833 Tinamus guttatus scaffold7365 Tyto alba scaffold1725



Fig. S2.

Bird and alligator AMBN scaffold alignments.



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| Exon | 1: | 2 | 3 | 4 | 5 | 67 | |
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| Alligator mississipiensis scaffold 107 | |)= + | 5,000 | | 10,000 | 15,000 | 20,0 |
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| Antrostomus carolinensis scaffold1454 | | |) | • |) } |))) | |
| Antrostomus carolinensis scaffold54750 | | | н | | | * | |
| Apaloderma vittatum scaffold17664 | | | | | • | | |
| Apaloderma vittatum scaffold23232 | | • | • | - | | | |
| Apaloderma vittatum scaffold7390 | | | | • | | | |
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| Aptenooyles loisten scanoo412 | , |) |) | • |) | * | |
| Buearea rhipagarea giluantria coaffeld10511 | |) | | • | | * | |
| Calvote anna scaffold207 | |) |) | • | | • | |
| Cariama cristata scaffold16682 | | | | 100 | | | |
| Cathartes aura scaffold18569 | |) , IDH HAN |)⊧ ⊢aoana |) Haan- | | | |
| Chaetura pelagica scaffold145 | |) ===== |) |) H | - | | |
| Charadrius vociferus scaffold586 | | P | P | , | P | • | |
| Chlamydotis undulata scaffold7713 | | | -joo | | - | | |
| Colius striatus scaffold23606 | | | ÷ | | - | 17 | |
| Corvus brachyrhynchos scaffold547 | | | ήa. | , | | 1 01 | |
| Cuculus canorus scattold286 | | , | , | | | | |
| Foretta garzetta scaffold693 | | | - 1930 | -0-0 | - | * | |
| Europoa belias scaffold713 | | • | • | • | |))) H an i | |
| Falco peregrinus scaffold630 | | | | - 100 | - | | |
| Fulmarus glacialis scaffold11512 | |) ्वनसम्बन्ध |) ⊢(anaa |) Hijumu | - jait i | | |
| Gallus gallus PreEnsembl66 | | ף ,=∎ | Ļ | ÷ | ÷. | | |
| Gallus gallus PreEnsembl66 | | , | ťœ | ÷ | n i II- | | |
| Gavia stellata scaffold5705 | | P | , | , | | | |
| Geospiza fortis scaffold167 | | | - | | - | | |
| Haliaeetus albicilla scaffold43224 | | , 1011311 } | - 1010 | • | | • | |
| Haliaeetus leucocephalus scaffold1083 | | , 100111111 } | – ∎) | ÷ | | • | |
| Leptosomus discolor scaffold10880 | | | H | • | | | |
| Manacus vitellinus scaffold365 | |) |) | | | - | |
| Meleagris gallopavo Ensembl66 chr1 | | • | | | | | |
| Meleagris gallopavo Ensembl66 Z | | | | | |) | |
| Melopsittacus undulatus scf900160277086 | |)) |) | | | | |
| Mesitornis unicolor scaffold27563 | | • |) | • | - | | |
| Mesitornis unicolor scaffold/29450 | | | | | _ | | |
| Nestor notabilis scaffold31865 | | K ∎ol−alii |) - 0100 | | | * | |
| Nipponia nippon scaffold1734 | |) , mittitt |) — (1000) | ų. | - | • | |
| Ophisthocomus hoazin scaffold47 | | ⊳ ,=HaH | -jeene | ł |) | - 1 <u>1,</u> 11 | |
| Pelecanus crispus scaffold11738 | | P | | - | | | |
| Phaethon lepturus scaffold8778 | | ■ Hitti | -00 | - | | | |
| Phalacrocorax scaffoldC7938125 | | , 100-8444 } | <i>,</i> | ŕ | , | | |
| Phalacrocorax scaffoldC7941533 | | | | | |)) } | |
| Phalacrocorax carbo scaffold25746 | | | | | | | |
| Phoenicopterus ruber scaffold9196 | | œ⊢4# } |)) |) |) | ••••••• | |
| Picoides pubescens scaffold528 | | | | | | | |
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| Pterocles guturalis scaffold12949 | |) | • | • | • | · · | |
| r teroues guturans scattol052424 | | | | | | | |
| Pygoscelis adeliae scaffold311 | | 00 1131 | ⊢œœ) | • | • | | |
| Struthio camelus scaffold149 | | H |) |) | • | • | |
| Taeniopygia guttata Ensembl66 | |)) | • | • | | | |
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| Tauraco erythrolophus scaffold40693 | | | | _ | | | |
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| Tyto alba scattola 1725 | | |) | • | | | |

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Fig. S3. Bird and alligator ENAM scaffold alignments.

| Exon | | | AMELX | | | |
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| Start Codon | Exon | 1 : | 2 | 3 4 | | 5 |
| Stop Codon | Alligator mississipiensis scaffold34 | 1 2,000 4,000 | 6,000 8,000 | 10,000 12,000 | 14,000 | 16,000 17,692 |
| | | | • |) | • | |
| | Acanthisitta chloris scaffold3798 | | n n n n n nganan (nganga (ngan ())) | | 18 <mark>1 - 11 - 12</mark> * | |
| | Anas platyrhynchos domestica scaffold415 | , | • ••• •••••••••••••••••••••••••••••••• | | 100 10 1 = = = = = 000 1 | |
| | Antrostomus carolinensis scaffold4002 | , | , } | | H0 100000000000000000000000000000000000 | |
| | Apaloderma vittatum scaffold9186 | , | - | , | | |
| | Apaloderma vittatum scaffold9264 | , | | | H 9 | |
| | Aptenodytes forsteri scaffold200 | | ■ ■■ ■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■ | | H80 H80 B = H #Q HD H ⊳ | |
| | Balearica regulorum gibbericeps scaffold791 | · · · · · · · | , 0 000 | | H00 =0 8====0 H0 H ⊳ | |
| | Buceros rhinoceros silvestris scaffold1906 | , | • • • • • • • • • • • • • • • • • • • | | Ka ⊢ | |
| | Calypte anna scaffold134 | | • •• •• •• •• •• •• •• •• •• •• •• •• • | | 18 • - 1 •0 •••• 100 01 1 - | |
| | Cariama cristata scaffold19502 | • •••••••••••••••••••••••••••••••••••• | , ■ ■■ ■■■■■■■ ■■■■■■■■■■■■■■■■■■■■■■■■■ | | HO O H⊂ HO H = HINO HO H ⊳ | |
| | Cathartes aura scaffold7180 | | , } | | H80 I==in C HD I ⊳ | |
| | Chaetura pelagica scaffold178 | ····· | , | | 80 - #0 8==#0 10 1 - ⊳ | |
| | Charadrius vociferus scaffold105 | | | | H00 #0 #==#00 HD H ⊳ | |
| | Chlamydotis macqueenii scaffold6849 | | | | 100 | |
| | Colius striatus scaffold9014 | " | , | | K e = 0 8=4 40 - ₩ 1 | |
| | Columba livia scaffold617 | | • • • • • • • • • • • • • • • • • • • | | -00 | |
| | Corvus brachyrhynchos scaffold83 | | / | | 10 1 10 10 10 1 0 1 | |
| | Cuculus canorus scaffold262 | | | | 100 10 1 a alag al 1 | |
| | Egretta garzetta scaffold49 | NC AL ACT A AD ADDA | | | () | |
| | Eurypyga helias scaffold3464 | | / | | HO O HO I = D=O HO I | |
| | Falco peregrinus scaffold88 | #0 <mark>4 #0++ #0-####</mark> | | | | |
| | Fulmarus glacialis scaffold2185 | | | | 180 - 19 - 19 - 19 - 10 - 1 | |
| | Gallus gallus Ensembl66 | | | | | |
| | Gavia stellata scaffold22700 | | | | 180 140 1 = 440 10 1 | |
| | Geospiza fortis scaffold50 | | | | 180 - 19 - 19 - 10 - 10 - 1 0 - 10 - 1 0 - 10 - 1 0 - 10 - 1 0 | |
| | Haliaeetus albicilla scaffold15182 | | | | 18 0 - 19 - 19 - 19 - 19 - 1 9 - 19 - 19 - 1 | |
| | Haliaeetus leucocephalus scaffold371 | | | | 180 10 1 - 10 1 | |
| | Leptosomus discolor scaffold2806 | | / } = = = = ⊡4400 00 44 0 | | 190 - 194 - 194 - 19 | |
| | Manacus vitellinus scaffold197 | v • <u>•</u> •⊡••• • ₩₩₩ | | | | |
| | Meleagris gallopavo Ensembl66 | | | | 10 10 10 10 1 | |
| | Melopsittacus undulatus scf900160277062 | | | | 180 140 1 4 440 10 1 | |
| | Merops nubicus scaffold6501 | | | | | |
| | Mesitornis unicolor scaffold45569 | ÷ • • • • • • • • • • • • • • • • • • • | / _ == ================================= | | +0 #0 8=#0 10 1 | |
| | Nestor notabilis scaffold24904 | | / -0 | | =================================== | |
| | Nipponia nippon scaffold30 | | | | 180 - 10 - 1 - 400 - 10 - 1 | |
| | Ophisthocomus hoazin scaffold29 | | | | H I 10 10 10 1 | |
| | Pelecanus crispus scaffold20894 | | | | 180 | |
| | Phaethon lepturus scaffold2690 | | | | 180 140 1440 110 1 | |
| | Phalacrocorax carbo scaffold29682 | | | | | |
| | Phoenicopterus ruber scaffold4456 | (| | | 18 | |
| | Picoides pubescens scaffold132 | | | | 1810 - 140 - 119 - 040 - 110 - 1 | |
| | Podiceps cristatus scaffold58594 | / HD | | | 190 - 190 1940 10 1 - | |
| | Pterocles guturalis scaffold9222 | | / | | 180 - 18 - 18 - 18 - 18 | |
| | Pygoscelis adeliae scaffold69 | / | | | 100 100 1 10 1 | |
| | Struthio camelus scaffold57 | | / | | | |
| | Taeniopygia gutatta Ensembl66 | · · · · · · · · · · · · | / | | 180 - 19 - 19 - 19 - 19 - 19 - 19 - 19 | |
| | Tauraco erythrolophus scaffold15954 | | / | | 180 - 10 - 1 - 10 - 10 - 1 0 - 10 - 1 0 - 10 | |
| | Tinamus guttatus scaffold1092 | / #0#1#0+#0-# 8### 0 | / | | | |
| | Tyto alba C9707461 | <i>r</i> ■ | ٢ | ٣ | | • |
| | Tyto alba scaffold17183 | ▶ ■ • • • • • • • • • • • • • • • • • • • | ■ ■■ ■■ ■■ ■■ ■■ ■■ ■■ ■ } | | 180 1 0 1 0 | |

Fig. S4. Bird and alligator *AMELX* scaffold alignments.

| Exon Start Codon | SPA | RCL1 | DSPP |
|---------------------|--|--|--|
| | Exon | 1 | 1 24 5 6 |
| | Acanthisitta chloria scaffold2164 | 1 2,590 5,090 7,590 10,090 12,500 15,090 17,500 20,090 22,500 25,500 27,500 | 10,000 12,500 35,000 37,500 40,000 42,500 45,000 47,500 50,000 52,500 55,000 57,500 59,436 → → → → → → → → → → → → → → → → → → → |
| | Anas platyrhynchos domestica scattold229 Antrostrowa caminansis acattold13900 | manamananananan ang ang ang ang ang ang ang a | |
| | Antrostomus carolinensis scallold/5670 | (1001-1001-10-0-0-0-0-0-0-0-0-0-0-0-0-0- | |
| | Apaloderma vittatum acafloid19352 Antenveistes insteri scolloid1977 | | |
| | Balearica regulorum gibbericeps scalfold2998 | | |
| | Buceros rhinoceros silvestris scallold40089 Buceros drinoceros silvestris scallold40089 | (| ###################################### |
| | Calypte anna scaffold157 | | inden (slade) (det in to a late and an inter a set i i i i a sint state and a late s as i |
| | Cariama cristata acaffold1234 | 4 maintaine (al (anti-antine (anna)) (al (al (anna)) (al (anna)) (al (anna)) (al (anna)) (anna)) (anna)) (anna) | anitelijia (jan 1- 0) (10) - a nija 0-0) (4) (4) 0 (5 - 1) (1) (1 - 1 - 1 - 0 + a nija aniteliane) (2) a 0 (a) 0 (a) 0 (a) |
| | Cathartes aura scaffold19472 | 1 mm(+m)=()(((+m)) = ()(+m)=()(+m)(+m)=()(+m)(+m)(+m)(+m)(+m)(+m)(+m)(+m)(+m)(+ | |
| | Cathartes aura scattoid83458 Chaetura pelagica scattoid210 | | (=====)(#(============================= |
| | Charachius vocilierus scaffold178 | ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ ₩ | M(Hele of G()=++H)(m(-+))-()-=====++()+(+(++++++++++++++++++ |
| | Chiamydotis macqueenii acaffoid1744 | | b@0(ba)mi-skimi-ski iD_ol@@_%K(abm_0.b=o{(si+jasm)@bmaassaabkamCasa_ib; \$idbiam_aastaaim)+ sast |
| | Colius striatus scaffold17732 | and alam (in Canton and a canton a canton and (in Canton and (in Canton a canton and a canton | =\$ |
| | Columba livia scallold28 | | |
| | Conus brachymynchos scallold229 Cuculus canorus scallold4 | | |
| | Egrette garzette scaffold615 | (| ;=;==-==;=;=;=;=; =;=;=;=; |
| | Egnette garzette sceffold856 | | |
| | Eurypyga helias acaffold12919 | | = = - = = : = : = : = : = : = : =: = : =: =: =: =: =: =: =: =: =: =: =: =: =: =: =: =: =: |
| | Falco paregrinus scattold79 | perinter beiden i sense forsen opper 20> ; selle mere beiden an 40. – 2017. mene (m. e. e. etablishe etablishe { | |
| | Galus galus PreEnsembl68 | | |
| | Gavie stellate scalloid25040 | | |
| | Liecepiza forte scalloid?? Haliaeetus albicilla scalloid??488 | | |
| | Haliaeetus leucocephalus acaffold1739 | (| Mairingia ajaan kanimaa - ajin ku - a aaja di di dahaa - C. 10- a Histi ku - ki ku a sa aa aakkamadi ajaja - 130 - 31 digid aan - a aa kanima sa aa ku |
| | Leptosomus discolor scalloid14034 | | halistik dani hana + ne jalani - n 🖸 - n |
| | Leptosomus discolor scaffold9439 Manacus vitellinus scaffold229 | 1 midm= x {================================== | terre = terre ===300 terre = terre == 0 = terre = terre == 1 = 0 = materia ere = 0 = materia ere = 0 = materia ere = 1 = 0 = materia |
| | Meleagris gallopavo Ensembil66 | (| (a) (a a i i a a a a a a a a a a a a a a a |
| | Melopsittacus undulatus act900160277028 | Υ mi(nhm)(n)((-m) = nhm)+ (+3)(nhm+ 2) → (= nh)(n = m++(nh)(m)n = (= (3))(0)(0 = mm + m + nh)(2m = 0) | Madali ma (jum (- si); a); - m (a); - (; a); - a - ((s = a)); and (s = a); - (); - |
| | Merops nubicus scaffold8208 | (minimiz)(()(minimiz)() (minimiz)() () () () () () () () () () () () () | |
| | Mesitomis unicolor scaffold14953 | imi(nima)(m 6 mm) - nima = (been nime) - 0 - mi(b - namin(milities = n + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + | handada (hard hand - naile) - n faciliti - faciliti - fi - di hard na anda (filma) - handa - fini - handa anda - handa - na anda - na an |
| | Nestor notabilis scalfold14849 | | |
| | Ophisthocomus hoazin scalfold1213 | | |
| | Pelecanus crispus acafloid13020 | | |
| | Phalacrocorax carbo scaffold36739 | (| |
| | Phoenicoptensi ruber scallold929 | (| |
| | Pterocles guturalis scattoid27825 Pterocles guturalis scattoid2458 | 1 ==================================== | □ +: □ + + □ + |
| | Pygoscelis adeliae acafloid157 | | الا من |
| | Acanthisitta chloris scalloid37315 | 1 m==================================== | |
| | Struthio camelus scalloid25 | 7 1000000000000000000000000000000000000 | |
| | Taericpygia guttata Ensembi86 | ani dana juli 2 ani - ani | |
| | Tauraco erythrolophus scallold15785 | | |
| | A MARINE STRATES A CANON AND A CANON | | |

Fig. S5. Bird DSPP scaffold alignments.

| Exon Start Codon Stop Codon | PARCL1 | E | SPP | DMP1 |
|---|---|-------------|-------------------|--|
| Exon | 21 | 1 234 | 5 | 6 1 |
| Allinator mississiniansis scatfold5316 | 2 1 1 5,000 10,000 15,000 20,000 25,000 30,000 35,000 40,000 45,000 55,000 55,000 60,000 65,000 75,000 10,000 85,000 | 90,000 95,0 | 00 100,000 | 105,000 110,000 114,981 |
| ningator mississiplentilis searendoore | | | | |
| Anas platyrhynchos domestica scaffold22 | | | 3-) D=) | |
| Apaloderma vittatum scaffold19352 | | | | |
| Aptenodytes forsteri scaffold22 | (| | | 4 4)=0(4=====)(0;4=) |
| Calypte anna scaffold157 | ((= <u>1831 ==================================</u> | | ••• =• | • |
| Chaetura pelagica scaffold210 | { { | | +00+) •=) | • |
| Charadrius vociferus scaffold178 | ((#2017;m=m20;f;m=m20;f;m=m2;m+(=max;m+(=j;m);m+(=j;m);m(=(=m=1;m)) + ((=max) + (0)(= (= max;m);m=m+(=)(=max;m)) + ((=max;m);m(=max;m)) + ((=max;m)) + (| | | 4 =}=================================== |
| Chlamydotis macqueenii scaffold1744 | { { = | | | • |
| Colius striatus scaffold17732 | { { | | | |
| Columba livia scaffold28 | ((== 100 =============================== | | | 4 ==================================== |
| Corvus brachyrhynchos scaffold229 | ((| | | 4 #################################### |
| Cuculus canorus scaffold4 | | | | • |
| Gallus gallus PreEnsembl66 | | | | ► |
| Geosoiza fortis scattold11 | | | | t |
| Helieeska lauseeskelus eeskeld 720 | | | | • |
| Malaeetus leucocepitalus scalolu 1739 | ((| | | • |
| weieagris galiopavo Ensemblee | | | | 10000000000000000000000000000000000000 |
| Melopsittacus undulatus scf900160277028 | #_{#};####};#;#;######## | | | ++++++++++++++++++++++++++++++++++++++ |
| Mesitornis unicolor scaffold14953 | w 1011 (maxee of 5 m) - of 1(m) + (maxee maxee) + 1 (maxe) (m + 4 m) (m + 4 m) (m + 4 m) (m + 4 m) (m + 6 | | •••• | • # # # # # # # # # # # # # # # # # # # |
| Nestor notabilis scaffold14649 | | | | nindeland milder i |
| Nipponia nippon scaffold461 | | | | |
| Pygoscelis adeliae scaffold157 | , , , , , , , , , , , , , , , , , , , | | | |
| Struthio camelus scaffold25 | { | | | |
| Taeniopygia guttata Ensembl66 | ((₩ <u>₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩</u> | → | | |
| Tauraco erythrolophus scatfold15765 | ({ - 103 300 000 } } | | |) |

Fig. S6.

Bird and alligator *DSPP* scaffold alignments with scaffolds spanning from *SPARCL1-DMP1*.

| Exon |
|-------------|
| Start Codon |
| Stop Codon |

MMP20

| n | Exon | 1 | 2 | 3 | 4 5 | 6 | | 7 | 8 | 9 | | | 10 |
|------|--|---|--|----------|---|---------------------------------------|--|----------|--|--|-----------------------|---|--------------|
| Alli | gator mississipiensis scaffold8965 | 1 5, | 000 10 | 0,000 | 15,000 20,00 C In C In C In C | 10 25,000 | 30,000 35,000 | | 40.000 | \$5,000 | 50,000 55, •••01 0 | 000 60,000 113 -113 113 1130 | () 62,636 |
| Aca | nthista chloris scaffold9461 | ÷ ـــــــــــــــــــــــــــــــــــ |) | <u> </u> |)) | · • • | |) |) | (| | • 0 • 0 | |
| Ana | as platyrhynchos domestica scaffold78 | | | - |) | | | | - - a_ | • •• | = =} | \$ == = | |
| Ant | rostomus carolinensis scaffold2851 | | | ×′ | , | , | | , | , | , | | | |
| Ant | rostomus carolinensis scaffold3995 | | | • • | | | | • | ********* | | •• | | |
| Apa | loderma vittatum scaffold6998 | +++ | | • • | • • • • • • • • | | | • | |) (m)mees | • * | +316-1 00 10 | |
| Bal | earica regulorum gibbericeps scaffold9930 | | • | | >```` | • | | ۶. | - | | | | |
| Cal | ypte anna scaffold117 | €-1040-00 | | | | | | _ |) |) = =0100 | •• | · ··· | |
| Car | iama cristata scaffold2096 | | | • • |) | | | | - |) | | | -() |
| Cat | hartes aura scaffold14423 | · | , | • • | | | | ÷ | |) () | | (1)(a am a a am | <u>ب</u> |
| Cha | aetura pelagica scaffold85 | C−+0+00+0+−−-0− | | • • | | | | ý- | | • ••••• •• | •) === •E + • • • | | |
| Cha | aradrius vociferus scaffold87 | (| - | • + | ····· | | | ÷ | ************************************** |)-0=0==== } | ****** | 10) 00 0 0 - 00) | 8 |
| Chi | amydotis macqueenii scaffold8929 | · | | • • | | • • • • • • • • • • • • • • • • • • • | |) | +=)-(0)-=+ | =8441 =8 -0 (| ei 0 | | |
| Col | us stratus scattoid21822 | | • | | > | • • • • • • • • • • • • • • • • • • • | |) | **** |)) | | 100(() 10010 0 1001 | • |
| Col | umba livia scattold59 vus brachvrhvnchos scattold86 | |) | | ······································ | | |) | |) -(=)==(=)-(| | | Þ |
| Cu | culus canorus scaffold380 | |) | |) | | | | |) | | | • |
| Egr | etta garzetta scaffold547 | | → ===++++ | • • | | ·) · | | ÷. | ****** | | | ++:)+- +++ +++++ ++++++++++++++++++++++++++++ | •••• |
| Eur | ypyga helias scaffold8224 | · | | | | | | ÷ | | ۲ | | | ÷ |
| Fal | co peregrinus scaffold72 | | | | | | | ÷ | ÷- | - 0= 0==== 0 | | 000 0 + + + + + + + + + + + + + + + + + | 0101- |
| Ful | marus glacialis scaffold6760 | | · · | • • | + | • • • • • • • • • • • • • • • • • • • | | É. | ************************************** |) (1899 | | nia (n. 1910) - 1910 - 1910 - 1910 - 1910 | ••••• |
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Fig. S7.

Bird and alligator MMP20 scaffold alignments.

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| Charadrius vociferus scaffold87 | / |
| Chlamydotis macqueenii scaffold8929 | |
| Colius striatus scaffold21822 | ////////////////////////////////////// |
| Columba livia scaffold59 | |
| Corvus brachyrhynchos scaffold86 | / |
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Fig. S8.

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Bird and alligator MMP27-MMP20 scaffold alignments.

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Fig. **S9**.

Mammal AMTN, AMBN, and ENAM scaffold alignments.

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Fig. S10.

Mammal DSPP, AMELX, and MMP20 scaffold alignments.



Fig. S11.

Turtle AMTN, AMBN, and ENAM scaffold alignments.



Fig. S12. Turtle *AMELX*, *DSPP*, and *MMP20* scaffold alignments.

Table S1.

Summary of key studies debating specificity of expression for MMP20, AMBN, AMEL, ENAM, AMTN, and DSPP.

| Gene | Tooth- specific expression | Pseudogene evidence in edentulous/ enamelless taxa | Expression outside teeth (pleiotropy) | Mouse mutational study | Natural human variation (e.g., <i>amelogenesis</i> <i>imperfecta</i>) | Organism(s) | Review Article | Reference |
|-------|----------------------------------|---|---|------------------------------|---|---|-------------------|---|
| MMP20 | Yes | No | No | No | No | mouse | No | Begue-Kim et al. (82) |
| | Yes | N/A | N/A | N/A | N/A | N/A | Yes | Bartlett et al. (18) |
| | Yes | Yes | No | No | No | bird (chicken, zebra-finch) | No | Quesada et al. (83) |
| | Yes | No | No | No | Yes | humans | No | Chan et al. (84) |
| | Yes | No | No | No | No | baleen whales, pygmy and dwarf sperm whales | No | Meredith et al. (12) |
| | No | No | lung | No | No | human, mouse | No | Ryu et al. (35) |
| | No | No | large intestine | No | No | mouse | No | Turk et al. (36) |
| | No | No | lung | No | No | human, mouse | Yes | Greenlee et al. (21) (references 35) |
| | No | No | SNPs associated with kidney aging | No | No | human | No | Wheeler et al. (85) |
| AMBN | Yes | No | No | No | No | rat, mouse | No | Krebsbach et al. (86) |
| | Yes | No | No | No | No | mouse | No | Begue-Kim et al. (82) |
| | Yes | No | No | knockout | No | mouse | No | Fukumoto et al. (87) |
| | Yes | N/A | N/A | N/A | N/A | N/A | Yes | Bartlett et al. (18) |

| | Yes | Yes | No | No | No | baleen whales | No | Deméré et al. (11) |
|------|-----|-----|------------------------|----------|-----|--|-----|----------------------------|
| | Yes | No | No | knockout | No | mouse | No | Hatakeyama et al. (19) |
| | Yes | No | No | knockout | No | mouse | No | Smith et al. (88) |
| | Yes | Yes | No | No | No | bird (chicken, zebra-finch) | No | Quesada et al. (83) |
| | Yes | Yes | No | No | No | birds, armadillo, sloth, turtles, birds | No | Meredith et al. (13) |
| | No | No | mineralized tissues | No | No | mouse | No | Zeichner-David et al. (89) |
| | No | No | mineralized tissues | No | No | Bioinformatics modeling of human DNA sequence | No | Vymětal et al. (23) |
| | No | No | mineralized tissues | N/A | N/A | mouse | Yes | Caton and Tucker (90) |
| | No | No | mineralized tissues | No | No | mouse | No | Atsawasuwan et al. (34) |
| AMEL | Yes | Yes | No | No | No | bird (chicken, zebra-finch) | No | Quesada et al. (83) |
| | Yes | No | No | knockout | No | mouse | No | Gibson et al. (91) |
| | Yes | No | No | No | No | rat | No | Papagerakis et al. (92) |
| | Yes | Yes | No | No | No | baleen whales | No | Deméré et al. (11) |
| | Yes | Yes | No | No | No | chicken | No | Sire et al. (26) |
| | Yes | No | No | knockout | No | mouse | No | Smith et al. (88) |
| | Yes | Yes | No | No | No | birds, armadillo, sloth, turtles, birds | No | Meredith et al. (13) |
| | Yes | No | No | No | Yes | human | No | Chan et al. (84) |

| | No | No | brain; hematopoietic system cells | No | No | mouse | No | Deutsch et al. (22) |
|------|------|-----|---|----------------|-----|--|----|-------------------------|
| | No | No | bone, cartilage | No | No | rat | No | Haze et al. (33) |
| | No | No | mineralized tissues | No | No | mouse | No | Atsawasuwan et al. (34) |
| ENAM | Yes | No | No | No | No | mouse, human | No | Hu et al. (93) |
| | Yes | No | No | No | Yes | human | No | Rajpar et al. (94) |
| | Yes | No | No | knockout study | No | mouse | No | Hu et al. (20) |
| | Yes | No | No | No | Yes | human | No | Hart et al. (95) |
| | Yes | No | No | No | No | mouse | No | Masuya et al. (96) |
| | Yes | Yes | No | No | No | baleen whales | No | Deméré et al. (11) |
| | Yes | Yes | No | No | No | mammals | No | Al-Hashimi et al. (97) |
| | Yes | No | No | knockout | No | mouse | No | Smith et al. (88) |
| | Yes. | No | No | No | No | enamel-specific expression is dependent on ENAM promoter | No | Papagerakis et al. (98) |
| | Yes | Yes | No | No | No | aardvark, baleen whales, pygmy and dwarf sperm whales, pangolins, sloths, anteaters, armadillos | No | Meredith et al. (4) |
| | Yes | Yes | No | No | No | birds, armadillo, sloth, turtles, birds | No | Meredith et al. (13) |
| | Yes | No | No | No | Yes | human | No | Chan et al. (84) |
| | No | No | bone and energy | mutant mouse | No | mouse | No | Fuchs et al. (99) |

| | | | metabolism | lines | | | | |
|------|-----|-----|--|-------|--|--------------------------------|-----|---------------------------|
| | No | No | mineralized tissues | No | No | mouse | No | Atsawasuwan et al. (34) |
| AMTN | Yes | Yes | No | No | No | mammals | No | Gasse et al. (17) |
| | No | No | mineralized tissues | No | No | mouse | No | Atsawasuwan et al. (34) |
| DSPP | Yes | No | No | No | No | mouse | No | Begue-Kim et al. (82) |
| | Yes | Yes | No | No | No | mammals | | McKnight and Fisher (16) |
| | Yes | Yes | No | No | No | bird (chicken, zebra-finch) | No | Quesada et al. (83) |
| | No | No | bone | No | No | rat | No | Qin et al. (100) |
| | No | No | bone and osteoblasts | No | No | rat | No | Qin et al. (101) |
| | No | No | salivary glands | No | No | human, monkey, mouse | No | Ogbureke and Fisher (102) |
| | No | No | Kidney | No | No | monkey | No | Ogbureke and Fisher (103) |
| | No | No | sweat glands | No | No | human | No | Ogbureke and Fisher (104) |
| | No | No | kidneys, lungs | No | No | mouse | No | Alvares et al. (105) |
| | No | No | bone | No | No | mouse | No | Verdelis et al. (106) |
| | No | N/A | N/A | N/A | N/A | N/A | Yes | Suzuki et al. (107) |
| | No | N/A | N/A | N/A | N/A | N/A | Yes | Wang and Lee (108) |
| | No | No | SNPs in DSPP affect bone density | No | SNPs in DSPP affect bone density | human | No | Alam et al. (109) |
| | No | No | mandibular condylar cartilage | No | No | mouse | No | Liu et al. (110) |

| No | No | periodontal ligament stem cells | No | No | mouse | No | Ozer et al. (111) |
|----|----|---------------------------------------|----|----|-------|----|-------------------|

Table S2.

Scaffolds and exons recovered from the 48 bird genomes and American alligator for the six tooth related genes.

| | | | ENAM | | | | | | | | |
|------------------------------------|--|---------|----------|--------------|--------------|----------|----------|----------|----------|---------|--|
| Taxon | Scaffold | Exon 1* | Exon 2 | Exon3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 | |
| Alligator mississippiensis | scaffold-107 | x | x | x | x | x | x | x | x | x | |
| Acanthisitta chloris | scaffold1839 | ? | Excised | Excised | Excised | Excised | Excised | Excised | Excised | x | |
| Anas platyrhynchos | Stationa1053 | ? | N | | W | N | N. | | E. i. i | v | |
| domestica Antrostomus | scaffold247 scaffold1454: | ? | X | X | X | X | X | X | Excised | X | |
| carolinensis | scaffold54750 | | Excised? | Excised? | Х | Х | Х | Х | Х | Х | |
| Apaloderma vittatum | scaffold23232; scaffold17664; scaffold7390 | ? | х | Х | Х | Excised | Excised | Excised | Excised | X | |
| Aptenodytes forsteri | scaffold412 | ? | х | х | х | х | х | х | Excised | х | |
| Balearica | | ? | | | | | | | | | |
| gibbericeps | scaffold21214 | | х | Excised | х | Excised | Х | Х | Excised | х | |
| Buceros rhinoceros | scaffold10511 | ? | x | x | x | Excised | Excised | Excised | Excised | x | |
| Calvote anna | scaffold207 | ? | Excised | Excised | Excised | Excised | Excised | Excised | Excised | x | |
| Cariama orietata | seaffeld16692 | ? | v | v | v | Evolged | Exclored | Evolved | Evolved | v | |
| | scarloid10082 | ? | <u>л</u> | A | A | Excised | Excised | Excised | Excised | A | |
| Cathartes aura | scaffold18569 | ? | X | X | X | Excised | Excised | Excised | Excised | X | |
| Chaetura pelagica | scaffold145 | | Х | Х | Х | Х | Excised | Х | Excised | Х | |
| vociferus | scaffold586 | · | Excised? | Excised? | Excised? | Excised? | Excised? | Excised? | Excised? | Х | |
| undulata | scaffold7713 | ? | Х | х | Excised | х | х | Х | Х | х | |
| Colius striatus | scaffold23606 | ? | Х | х | х | Excised | Excised | Excised | Excised | Х | |
| Columba livia | EXCISED COMPLETELY | ? | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | |
| Corvus brachyrhynchos | scaffold547 | ? | х | х | Excised | Excised | Excised | Excised | Excised | х | |
| Cuculus canorus | scaffold286 | ? | Excised | Excised | Excised | Excised | Х | Х | х | х | |
| Egretta garzetta | scaffold693 | ? | х | Х | х | Excised | х | Х | х | х | |
| Eurvpvga helias | scaffold713 | ? | Excised | Excised | Excised | Excised | Excised | Excised | Excised | x | |
| Falco peregrinus | scaffold630 | ? | х | x | X | Excised | Excised | Excised | Excised | х | |
| Fulmarus glacialis | scaffold11512 | ? | х | х | x | x | X | х | х | х | |
| Gallus gallus | ProEnsembl66 | ? | v | v | v | v | v | v | Excised | v | |
| Gania stellata | saaffald5705 | ? | 2 | 2 | 2 | v | v | Evoised | Excised | v | |
| Gaogniza fortia | scalfold167 | ? | v | ? Evoiced | ? Excised | A | A | Excised | Excised | A V | |
| | scariold107 | ? | N | Excised V | Excised N | Excised | Excised | Excised | Excised | A V | |
| Haliaeetus albicilla Haliaeetus | scarrold43224 | ? | X | X | Х | Excised | Excised | Excised | Excised | X | |
| leucocephalus | scaffold1083 | 2 | Х | Х | Х | Excised | Excised | Excised | Excised | Х | |
| discolor | scaffold10880 | : | Excised | Excised | х | Excised | Excised | Excised | Excised | Х | |
| Manacus vitellinus | scaffold365 | ? | Х | Х | Excised | Excised | Excised | Excised | Excised | Х | |
| Meleagris gallopavo | Ensembl66 | ? | ? | ? | ? | ? | ? | х | ? | х | |
| Melopsittacus undulatus | PreEnsembl66 | ? | x | x | Excised | Excised | Excised | Excised | Excised | x | |
| Marons nubious | scaffold12602 | ? | v | v | v | Evoiced | Excised | Excised | Excised | v | |
| Mesitornis unicolor | scaffold29450; scaffold27563 | ? | x | x | Excised | Excised | X | X | X | X | |

| | | 2 | | | | | | | | |
|---------------------|----------------|---|---------|---------|---------|---------|---------|---------|---------|---|
| Nestor notabilis | scaffold31865 | - | х | Х | Excised | Excised | Excised | Excised | Excised | Х |
| | | ? | | | | | | | | |
| Nipponia nippon | scaffold1734 | | Х | Х | Х | Х | Х | Х | Х | Х |
| Ophisthocomus | | ? | | | | | | | | |
| hoazin | scaffold47 | | Х | Х | Х | Excised | Х | Х | Х | Х |
| | | ? | | | | | | | | |
| Pelecanus crispus | scaffold11738 | | Excised | Excised | Excised | Х | Х | Х | Excised | Х |
| | | ? | | | | | | | | |
| Phaethon lepturus | scaffold8778 | | Х | Х | Х | Х | Х | Х | Х | Х |
| | scaffold25746; | ? | | | | | | | | |
| Phalacrocorax | C7938125; | | | | | | | | | |
| carbo | C7941533 | | Х | ? | ? | ? | Х | Х | Х | Х |
| Phoenicopterus | | ? | | | | | | | | |
| ruber | scaffold9196 | | Х | Х | Х | Х | Х | Х | Х | Х |
| | | ? | | | | | | | | |
| Picoides pubescens | scaffold528 | | Excised | Х |
| | | ? | | | | | | | | |
| Podiceps cristatus | scaffold69430 | | ? | ? | ? | ? | ? | ? | ? | Х |
| | scaffold12949; | ? | | | | | | | | |
| Pterocles guturalis | scaffold52424 | | Х | Х | Х | Х | Х | Excised | Excised | Х |
| | | ? | | | | | | | | |
| Pygoscelis adeliae | scaffold311 | | Х | Х | Х | Х | Х | Х | Excised | Х |
| | | ? | | | | | | | | |
| Struthio camelus | scaffold149 | | Excised | Х | Х | Х | Х | Х | Х | Х |
| | | ? | | | | | | | | |
| Taeniopygia guttata | Ensembl66 | | Х | Х | Excised | Excised | Excised | Excised | Excised | Х |
| Tauraco | scaffold30607; | ? | | | | | | | | |
| erythrolophus | scaffold40693 | | Х | Х | Х | Х | ? | ? | ? | Х |
| | | ? | | | | | | | | |
| Tinamus guttatus | scaffold7365 | | Х | Excised | Excised | Х | Х | Х | Х | Х |
| | | ? | | | | | | | | |
| Tyto alba | scaffold1725 | 1 | Х | Х | Х | Excised | Excised | Excised | Excised | Х |

* = Exon 1 is non-coding and was not found in any bird genome. However, this exon could be present but is too divergent to detect.

| | | AMELX | | | | | |
|---------------------------------|----------------------------|---------|--------|---------|--------|--------|--|
| Taxon | Scaffold | Exon 2 | Exon 3 | Exon 5 | Exon 6 | Exon 7 | |
| Alligator mississippiensis | scaffold-34 | Х | Х | х | x | х | |
| Acanthisitta chloris | scaffold3798 | Х | х | х | х | х | |
| Anas platyrhynchos domestica | scaffold415 | Х | Х | х | х | х | |
| Antrostomus carolinensis | scaffold4002 | Х | Х | x | x | х | |
| Apaloderma vittatum | scaffold9186; scaffold9264 | Х | Х | х | х | х | |
| Aptenodytes forsteri | scaffold200 | Х | Х | х | х | х | |
| Balearica regulorum gibbericeps | scaffold791 | Х | Х | х | x | х | |
| Buceros rhinoceros silvestris | scaffold1906 | Х | х | х | x | х | |
| Calypte anna | scaffold134 | Х | х | х | х | х | |
| Cariama cristata | scaffold19502 | Х | х | х | х | х | |
| Cathartes aura | scaffold7180 | Х | х | х | х | х | |
| Chaetura pelagica | scaffold178 | Х | х | х | х | х | |
| Charadrius vociferus | scaffold105 | Х | х | х | х | х | |
| Chlamydotis undulata | scaffold6849 | Х | х | х | х | х | |
| Colius striatus | scaffold9014 | Х | х | х | х | х | |
| Columba livia | scaffold617 | Х | Х | х | х | Х | |
| Corvus brachyrhynchos | scaffold83 | х | х | Excised | х | х | |
| Cuculus canorus | scaffold262 | Х | х | х | х | Х | |
| Egretta garzetta | scaffold49 | Х | х | х | х | х | |
| Eurypyga helias | scaffold3464 | Excised | х | x | х | х | |
| Falco peregrinus | scaffold88 | х | х | x | х | х | |

| | | | 1 | r | | |
|--------------------------|-------------------------|---------|---|---|---------|---|
| Fulmarus glacialis | scaffold2185 | х | х | х | х | х |
| Gallus gallus | ENSEMBL66 | Х | Х | х | х | х |
| Gavia stellata | scaffold22700 | Х | Х | х | Х | х |
| Geospiza fortis | scaffold50 | Х | Х | х | Х | х |
| Haliaeetus albicilla | scaffold15182 | Х | Х | х | Х | х |
| Haliaeetus leucocephalus | scaffold371 | Х | Х | х | х | х |
| Leptosomus discolor | scaffold2806 | х | Х | х | х | х |
| Manacus vitellinus | scaffold197 | Excised | Х | х | Excised | х |
| Meleagris gallopavo | ENSEMBL66 | х | х | х | х | х |
| Melopsittacus undulatus | scf900160277062 | Х | Х | х | Х | х |
| Merops nubicus | scaffold6501 | х | Х | х | Excised | х |
| Mesitornis unicolor | scaffold45569 | х | Х | х | х | х |
| Nestor notabilis | scaffold24904 | Х | Х | х | Х | х |
| Nipponia nippon | scaffold30 | Х | Х | х | Х | х |
| Ophisthocomus hoazin | scaffold29 | Х | Х | х | Х | х |
| Pelecanus crispus | scaffold20894 | Х | Х | х | Х | х |
| Phaethon lepturus | scaffold2690 | х | Х | х | х | х |
| Phalacrocorax carbo | scaffold29682 | Х | Х | х | Excised | х |
| Phoenicopterus ruber | scaffold4456 | Excised | Х | х | х | х |
| Picoides pubescens | scaffold132 | х | Х | х | х | х |
| Podiceps cristatus | scaffold58594 | Excised | х | х | х | х |
| Pterocles guturalis | scaffold9222 | х | Х | х | х | х |
| Pygoscelis adeliae | scaffold69 | х | Х | х | х | х |
| Struthio camelus | scaffold57 | х | х | х | х | х |
| Taeniopygia guttata | ENSEMBL66 | Х | Х | х | Х | х |
| Tauraco erythrolophus | scaffold15954 | х | х | х | х | х |
| Tinamus guttatus | scaffold1092 | х | х | х | Excised | х |
| Tyto alba | C9707461; scaffold17183 | Х | Х | х | Х | Х |

| | | | | | | MM | P20 | | | | |
|---------------------------------------|-------------------------------|---------|---------|---------|---------|---------|---------|---------|--------|---------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 | Exon 10 |
| Alligator mississippiensis | scaffold8965 | x | x | х | х | х | х | х | х | х | х |
| Acanthisitta chloris | scaffold9461 | Excised | x | Excised | х | Excised | Excised | х | х | х | х |
| Anas platyrhynchos domestica | scaffold78 | Excised | x | x | х | Excised | x | х | x | х | х |
| Antrostomus carolinensis | scaffold2851; scaffold3995 | Excised | x | Excised | х | х | х | х | х | х | ? |
| Apaloderma vittatum | scaffold6998 | Excised | Excised | Excised | Excised | Х | х | Excised | х | Excised | ? |
| Aptenodytes forsteri | scaffold352 | Excised | х | Excised | Х | Х | х | х | х | х | х |
| Balearica regulorum gibbericeps | scaffold9930 | Excised | X | Excised | х | х | х | х | ? | ? | ? |
| Buceros rhinoceros silvestris | scaffold14690 | Excised | x | Excised | х | Excised | Excised | Excised | х | х | Excised |

| Calypte anna | scaffold117 | Excised | Excised | Excised | х | Excised | Excised | Excised | Excised | х | х |
|-----------------------------|---------------------------------|-----------|----------|-----------|----------|---------------|---------------|-----------|--------------|---------|---------------|
| Cariama cristata | scaffold2096 | Excised | х | Excised | х | х | х | х | х | х | х |
| Cathartes aura | scaffold14423 | Excised | х | Excised | х | х | х | х | х | Excised | х |
| Chaetura pelagica | scaffold85 | Excised | Excised | Excised | x | х | x | х | Excised | х | x |
| Charadrius | scaffold87 | Excised | Excised | Excised | x | x | x | x | x | x | x |
| Chlamydotis | scaffeld@020 | Excised | v | Excised | Engined | v | v | v | v | v | v |
| Coline attriatue | scaliold 323 | Excised | N V | Excised | v | N V | N V | x v | A Evaluat | x v | N V |
| Collus striatus | scarroid21822 | Excised | A | Excised | A V | | | A | Excised | A | A |
| Columba livia Corvus | scattold59 | Excised | X | Excised | X | Excised | Excised | X | X | X | X |
| brachyrhynchos | scaffold86 | Excised | Х | Excised | Х | Excised | Excised | Excised | Excised | Х | Х |
| Cuculus canorus | scaffold380 | Excised | Х | Excised | Х | Х | Х | Х | Х | Excised | Excised |
| Egretta garzetta | scaffold547 | Excised | Х | Excised | Х | Х | Х | Х | Х | Х | Х |
| Eurypyga helias | scaffold8224 | Excised | Х | Excised | Х | Х | Х | Х | Х | Excised | Х |
| Falco peregrinus | scaffold72 | Excised | Х | Excised | Excised | х | Х | х | Excised | х | Excised |
| glacialis | scaffold6760 | Excised | х | Excised | х | х | х | Excised | х | Excised | х |
| Gallus gallus | PreEnsembl66 | Excised | Excised | Excised | Excised | х | х | х | Excised | х | Excised |
| Gavia stellata | scaffold2583; scaffold10828 | Excised | х | Excised | Х | х | х | х | х | х | х |
| Geospiza fortis | scaffold16 | Excised | х | Excised | Х | Excised | Excised | Excised | Excised | х | х |
| Haliaeetus albicilla | scaffold15162 | Excised | х | Excised | х | x | x | х | x | х | х |
| Haliaeetus leucocephalus | scaffold191 | Excised | x | Excised | x | x | x | x | x | x | x |
| Leptosomus | scarfor1/20474 | Excised | v | Excised | v | v | v | v | v | v | v |
| Manacus | scarroid204/4 | Excised | <u>л</u> | Excised | <u>л</u> | | A | A | A | A | <u>л</u> |
| vitellinus Meleagris | scaffold54 | Excised | X | Excised | X | Excised | Excised | X | Excised | X | X |
| gallopavo Melopsittacus | Ensembl66 | Excised | Excised | Excised | Excised | Excised | X | Х | Excised | Х | Excised |
| undulatus | PreEnsembl66 | Excised | Х | Excised | Excised | Excised | Excised | Excised | Excised | Х | Excised |
| Merops nubicus | scaffold20609 | Excised | Х | Excised | Х | Excised | Excised | Х | Х | Х | Х |
| unicolor | scaffold5006 | Excised | х | Excised | х | Excised | Excised | Excised | х | х | х |
| Nestor notabilis | scaffold17558 | Excised | х | Excised | Excised | Excised | Excised | Excised | Excised | х | х |
| Nipponia nippon | scaffold60 | Excised | х | Excised | Х | х | Х | х | Х | х | х |
| Ophisthocomus hoazin | scaffold105 | Excised | х | Excised | Х | x | х | Excised | х | Excised | х |
| Pelecanus crispus | scaffold9361; scaffold48452 | Excised | Excised | Excised | x | ? | ? | х | x | х | x |
| Phaethon lanturus | scaffold6627; | Excised | v | Excised | v | v | Excised | v | 2 | 2 | v |
| Phalacrocorax | scaffold7716; | Excised | X | Excised | X | X | N | X | V | V | |
| Carbo Phoenicopterus | scaffold6679; | Excised | х | Excised | X | X | X | Х | X | Х | Excised |
| ruber Picoides | scaffold12578 | Excised | Х | Excised | Excised | Х | X | Х | X | Х | X |
| pubescens | scaffold2012 | Excised | Excised | Excised | Х | Excised | Excised | Excised | Excised | Excised | Excised |
| Podiceps cristatus | scaffold43225 | Excised | Х | Excised | Excised | Х | Х | Х | Х | Х | Х |
| | scaffold6311; | | | | | | | | | | |
| Pterocles guturalis | scaffold8459; scaffold17735; | | | | | х | х | | | | х |
| | scaffold17864; scaffold33671 | Excised | ? | ? | ? | (3 copies) | (3 copies) | Х | х | ? | (2 copies) |
| Pygoscelis adeliae | scaffold383 | Excised | х | Excised | х | X | X | Х | х | Х | х |
| Struthio camelus | scaffold229 | Excised | x | X | x | x | x | x | x | x | x |
| Taeniopygia | Encombl64 | Evolution | v | Evolution | v | Evolution | Evolution | Evolution | Evolution | v | v |
| zunana | LIISCHIDIOO | EAUISEU | Λ | Excised | Λ | EACISEU | Excised | Excised | Excised | Λ | Λ |

| Tauraco erythrolophus | scaffold10875 | Excised | Excised | Excised | х | х | х | Х | х | Х | х |
|--------------------------|---------------|---------|---------|---------|---|---|---|---|---------|---|---------|
| Tinamus guttatus | scaffold3445 | Excised | Excised | х | х | х | х | х | х | х | Х |
| Tyto alba | scaffold9771 | Excised | х | Excised | Х | Х | Х | Х | Excised | Х | Excised |

| | | AMBN | | | | | | | |
|----------------------------------|---------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 |
| Alligator mississippiensis | scaffold-107 | х | х | х | х | х | х | х | х |
| Acanthisitta chloris | scaffold1839 | x | x | x | x | Excised | x | x | x |
| Anas platyrhynchos | | | | - | | Excloca | | | |
| domestica Antrostomus | Ensembl66 | ? | ? | ? | X | X | X | X | X |
| carolinensis | scaffold53789 | Х | Х | Х | Х | Х | Х | Х | Х |
| Apaloderma vittatum | scaffold14372 | ? | ? | ? | ? | ? | ? | х | х |
| Aptenodytes forsteri | scaffold412 | х | х | x | х | х | х | х | х |
| Balearica | | | | | | | | | |
| regulorum gibbericeps | scaffold210 | х | х | х | х | х | х | х | х |
| | scaffold22210; | | | | | | | | |
| Buceros rhinoceros silvestris | scaffold46037; scaffold10511 | х | х | х | х | ? | ? | ? | х |
| Calvpte anna | scaffold207 | ? | ? | ? | х | ? | ? | ? | ? |
| Cariama cristata | scaffold16682 | х | х | х | х | х | х | х | х |
| Cathartes aura | scaffold18569 | x | x | x | x | x | x | x | х |
| Chaetura pelagica | scaffold145 | x | Excised | Excised | x | Excised | x | x | Excised |
| Charadrius | scallold145 | v | v | V | v | V | v | v | V |
| Chlamydotis | scarioid586 | А | А | А | А | А | А | А | А |
| undulata | scaffold7713 | Х | Х | Х | Х | Х | Excised | Х | Х |
| Colius striatus | scaffold23606 | Excised | Х | Х | Х | Х | Excised | Х | Х |
| Columba livia | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised |
| Corvus brachyrhynchos | scaffold547 | х | Excised | Х | Excised | х | х | х | х |
| Cuculus canorus | scaffold286 | х | х | Х | х | х | х | х | х |
| Egretta garzetta | scaffold693 | Х | Х | Х | Х | Х | Х | Х | х |
| Eurypyga helias | scaffold713 | х | х | Х | х | х | х | х | х |
| Falco peregrinus | scaffold630 | х | х | х | х | х | х | х | х |
| Fulmarus glacialis | scaffold11512 | х | х | х | х | х | х | х | х |
| Gallus gallus | Ensembl66 | х | х | Х | х | х | Excised | Excised | Excised |
| Gavia stellata | C13393595; scaffold52443 | х | х | Х | х | х | х | х | х |
| Geospiza fortis | scaffold167 | Excised | Excised | Excised | Excised | х | Excised | х | х |
| Haliaeetus albicilla | scaffold6753; scaffold43224 | х | х | х | х | х | х | х | х |
| Haliaeetus | CC 1 11002 | v | v | v | v | v | v | v | v |
| leucocephalus Leptosomus | scattold1083 | X | X | X | X | X | X | X | X |
| discolor | scaffold10880 | Х | Х | Excised | Excised | Excised | Excised | Excised | Excised |
| Manacus vitellinus | scaffold365 | Х | Х | Х | Х | Excised | Х | Х | Х |
| Meleagris gallopavo | NOT | ? | ? | ? | ? | ? | ? | ? | ? |
| Melopsittacus undulatus | Ensembl66 | х | х | x | х | Excised | Excised | x | x |
| Merons nubicus | scaffold13603 | Excised | x | x | x | X | Excised | x | x |
| | scaffold29450; | LAUISCU | | | | | LAUISCU | | |
| Mesitornis unicolor | scaffold430 | Х | ? | ? | Х | Х | Х | Excised | Excised |

| [| | | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
|--------------------------|--------------------------------|---------|---------|---------|---------|---------|---------|---|---------|
| Nestor notabilis | scaffold5464 | Х | х | Х | х | Excised | Excised | х | х |
| Nipponia nippon | scaffold1734 | х | х | х | х | х | х | х | х |
| Ophisthocomus hoazin | scaffold47 | Х | Х | Х | х | Excised | Excised | х | х |
| Pelecanus crispus | scaffold11738 | х | х | х | х | х | х | х | х |
| Phaethon lepturus | scaffold8778 | х | х | х | х | х | х | х | Excised |
| Phalacrocorax carbo | C8218617; scaffold5758 | х | х | х | х | х | х | х | х |
| Phoenicopterus ruber | scaffold9196 | х | х | х | Х | х | х | Х | Х |
| Picoides pubescens | scaffold528 | Х | х | Х | х | Excised | Excised | х | х |
| Podiceps cristatus | scaffold7374; scaffold74317 | Х | Х | Х | Х | Х | Х | Х | Х |
| Pterocles guturalis | scaffold12949 | Х | х | х | Excised | Excised | Excised | х | х |
| Pygoscelis adeliae | scaffold311 | Х | х | х | х | х | х | х | х |
| Struthio camelus | scaffold149 | Х | х | Excised | х | х | х | х | х |
| Taeniopygia guttata | Ensembl66 | Excised | Excised | Excised | х | Excised | х | х | х |
| Tauraco erythrolophus | scaffold46833 | ? | ? | х | Х | х | х | Х | Х |
| Tinamus guttatus | scaffold7365 | X | х | Х | X | Excised | Х | X | х |
| Tyto alba | scaffold1725 | х | Х | х | Х | Excised | х | Х | Х |

| | | AMBN (cont.) | | | | |
|---------------------------------------|---|--------------|---------|---------|--|--|
| Taxon | Scaffold | Exon 9 | Exon 10 | Exon 11 | | |
| Alligator mississippiensis | scaffold-107 | х | х | х | | |
| Acanthisitta chloris | scaffold1839 | х | х | х | | |
| Anas platyrhynchos domestica | Ensembl66 | х | Excised | Excised | | |
| Antrostomus carolinensis | scaffold53789 | х | х | Х | | |
| Apaloderma vittatum | scaffold14372 | Х | Х | Х | | |
| Aptenodytes forsteri | scaffold412 | х | х | х | | |
| Balearica regulorum gibbericeps | scaffold210 | X | х | х | | |
| Buceros rhinoceros silvestris | scaffold22210; scaffold46037; scaffold10511 | х | х | Х | | |
| Calypte anna | scaffold207 | ? | ? | ? | | |
| Cariama cristata | scaffold16682 | х | х | х | | |
| Cathartes aura | scaffold18569 | х | х | х | | |
| Chaetura pelagica | scaffold145 | Excised | Excised | Excised | | |
| Charadrius vociferus | scaffold586 | х | х | Х | | |
| Chlamydotis undulata | scaffold7713 | Excised | Excised | х | | |
| Colius striatus | scaffold23606 | Excised | Excised | х | | |
| Columba livia | Excised | Excised | Excised | Excised | | |
| Corvus brachyrhynchos | scaffold547 | Х | х | х | | |
| Cuculus canorus | scaffold286 | х | х | Х | | |
| Egretta garzetta | scaffold693 | Х | Х | Х | | |
| Eurypyga helias | scaffold713 | Х | Х | Х | | |
| Falco peregrinus | scaffold630 | Х | х | х | | |
| Fulmarus glacialis | scaffold11512 | Х | Х | х |
|-------------------------|--------------------------------|---------|---------|---------|
| Gallus gallus | Ensembl66 | Excised | Excised | Excised |
| Gavia stellata | C13393595; scaffold52443 | х | ? | ? |
| Geospiza fortis | scaffold167 | х | х | х |
| Haliaeetus alhicilla | scaffold6753; scaffold43224 | x | x | x |
| Haliaeetus | Seanora (S22) | | | |
| leucocephalus | scaffold1083 | Х | Х | Х |
| Leptosomus | | | | |
| discolor | scaffold10880 | Excised | Х | Х |
| Manacus vitellinus | scaffold365 | x | x | Х |
| Meleagris | NOT | | | |
| gallopavo | SEQUENCED | ? | ? | ? |
| Melopsittacus | | | | |
| undulatus | Ensembl66 | X | X | Х |
| Merops nubicus | scaffold13603 | Х | Excised | Х |
| Mesitornis unicolor | scaffold29450; scaffold430 | Excised | Excised | Excised |
| Nestor notabilis | scaffold5464 | x | x | Х |
| Nipponia nippon | scaffold1734 | х | х | Excised |
| Ophisthocomus hoazin | scaffold47 | х | х | х |
| Pelecanus crispus | scaffold11738 | х | х | Х |
| Phaethon lepturus | scaffold8778 | Excised | Excised | х |
| Phalacrocorax | C8218617; | | | |
| carbo | scatfold5758 | X | X | X |
| Phoenicopterus ruber | scaffold9196 | х | х | х |
| Picoides pubescens | scaffold528 | Excised | Excised | Excised |
| Podiceps cristatus | scaffold7374; scaffold74317 | х | х | Х |
| Pterocles guturalis | scaffold12949 | х | х | х |
| Pygoscelis adeliae | scaffold311 | x | x | х |
| Struthio camelus | scaffold149 | х | х | Excised |
| Taeniopygia guttata | Ensembl66 | х | х | х |
| Tauraco | | | | |
| erythrolophus | scaffold46833 | Х | Х | Х |
| Tinamus guttatus | scaffold7365 | Х | Х | ? |
| Tyto alba | scaffold1725 | x | x | x |

| | | | AMTN | | | | | | | |
|---------------------------------------|---------------|--------|--------|--------|-------|---------|--------|---------|--------|--|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | |
| Alligator mississippiensis | scaffold-107 | х | Х | Х | Х | Х | N/A | Х | Х | |
| Acanthisitta chloris | scaffold3710 | х | х | х | х | Excised | N/A | Excised | х | |
| Anas platyrhynchos domestica | scaffold247 | х | Х | Х | х | Х | N/A | Х | х | |
| Antrostomus carolinensis | scaffold11362 | х | Х | Х | х | Х | N/A | Х | Х | |
| Apaloderma vittatum | scaffold14372 | Х | Х | Х | Х | Excised | N/A | Х | Х | |
| Aptenodytes forsteri | scaffold412 | х | х | х | х | Excised | N/A | х | х | |
| Balearica regulorum gibbericeps | scaffold13680 | x | x | x | x | X | N/A | x | x | |
| Buceros rhinoceros silvestris | scaffold12548 | х | х | Х | х | Х | N/A | Х | Х | |

| Calypte anna | scaffold207 | ? | ? | ? | ? | ? | N/A | х | ? |
|----------------------------------|---------------------------------|---------|---------|---------|--------|----------|------|------------|---------|
| Cariama cristata | scaffold16682; scaffold34970 | х | х | х | х | х | N/A | х | Х |
| Cathartes aura | scaffold2023; scaffold42292 | х | х | х | х | ? | N/A | х | х |
| Chaetura pelagica | scaffold145 | х | х | Excised | х | х | N/A | х | х |
| Charadrius vociferus | scaffold586 | х | Excised | х | х | х | N/A | х | Х |
| Chlamydotis undulata | scaffold6206 | x | x | x | x | x | N/A | x | x |
| Colius striatus | scaffold23606 | 2 | 2 | 2 | 2 | 2 | N/A | 2 | 9 |
| Columba livia | scaffold33 | x | Excised | x | x | Excised | N/A | Excised | Excised |
| Corvus brachurhunchog | sooffold547 | v | v | v | v | Excised | N/A | Excised | v |
| Cuculus caporus | scaffold286 | x | x | x | x | v | N/A | v | x |
| Equation Canorias | scattold200 | N V | N V | N V | N V | A V | N/A | A V | x v |
| Egretta garzetta | scaffold21777; | Λ | Λ | Λ | Λ | Λ | IN/A | Λ | А |
| Eurypyga helias | scaffold24649 | Excised | Excised | X | X | X | N/A | X | X |
| Falco peregrinus | scaffold630 | Х | Х | Х | Х | Х | N/A | Excised | Excised |
| Fulmarus glacialis | scaffold51529 | Х | Х | Х | Х | Х | N/A | Х | х |
| Gallus gallus | PreEnsemb66 | Excised | Excised | Х | Х | х | N/A | х | х |
| Gavia stellata | scaffold18014 | х | х | Х | х | Х | N/A | Х | х |
| Geospiza fortis | scaffold167 | Х | Х | Х | Х | Excised | N/A | Excised | х |
| Haliaeetus albicilla | scaffold6753 | х | х | Х | х | Х | N/A | Х | х |
| Haliaeetus leucocephalus | scaffold1083 | х | х | Х | х | х | N/A | х | х |
| Leptosomus discolor | scaffold4770 | х | х | х | х | х | N/A | х | х |
| Manacus vitellinus | scaffold365 | х | х | х | х | Excised | N/A | Excised | х |
| Meleagris gallopavo | Ensembl66 | ? | ? | ? | ? | ? | N/A | x | X |
| Melopsittacus undulatus | scf9001602770 86 | x | x | x | x | Excised | N/A | Excised | x |
| | scaffold13603; | N | N | N | N | Lixeised | 27/1 | - Exclosed | |
| Merops nubicus | scatfold26897 | X | X | X | X | X | N/A | ? | ? |
| Mesitornis unicolor | scaffold430 | Excised | Excised | Excised | X | X | N/A | X | Excised |
| Nestor notabilis | scaffold5464 | X | X | X | X | Excised | N/A | Excised | X |
| Nipponia nippon Ophisthocomus | scaffold1734 | X | X | X | X | X | N/A | X | X |
| hoazin | scaffold400 | Х | Х | Х | Х | Х | N/A | Х | Х |
| Pelecanus crispus | scaffold11738 | Х | Х | Х | Х | Х | N/A | Х | Х |
| Phaethon lepturus | scaffold8778 | Х | Х | Х | Х | Excised | N/A | Excised | Excised |
| Phalacrocorax carbo | scaffold29799 | х | х | Х | х | х | N/A | Х | х |
| Phoenicopterus ruber | scaffold4648 | х | х | х | х | Х | N/A | х | х |
| Picoides pubescens | scaffold528 | х | х | Excised | х | Х | N/A | Х | х |
| Podiceps cristatus | scaffold1805; scaffold4120 | x | x | x | x | x | N/A | x | x |
| Pterocles guturalis | scaffold12949 | x | x | x | x | Excised | N/A | x | x |
| Pvgoscelis adeliae | scaffold311 | x | x | x | x | x | N/A | x | x |
| Struthio camelus | scaffold149 | x | x | x | x | x | N/A | x | X |
| Taeniopygia guttata | Ensembl66 | x | x | x | x | Excised | N/A | Excised | x |
| Tauraco | scaffold7020 | x | x | x | x | x | N/A | x | x |
| T. | Scarroid/020 | | | | A V | A V | | | |
| 1 inamus guttatus | scattoid/365 | Excised | Excised | Excised | А | А | N/A | Х | Excised |

| | | | | , | | | | | |
|----------------------------------|---------------------------------|---------|----------------|---------|---|---------|-----|---------|---------|
| Tyto alba | scaffold1725 | Х | Х | Х | Х | Excised | N/A | Excised | Excised |
| | | | 4147117 | -4.) | | | | | |
| | | | AMTN (con | it.) | | | | | |
| Taxon | Scaffold | Exon 9 | Exon 10 | Exon 11 | | | | | |
| Alligator mississippiensis | scaffold-107 | х | x | х | | | | | |
| Acanthisitta chloris | scaffold3710 | х | Excised | х | | | | | |
| Anas platyrhynchos domestica | scaffold247 | х | Excised | х | | | | | |
| Antrostomus carolinensis | scaffold11362 | х | Excised | х | | | | | |
| Apaloderma vittatum | scaffold14372 | х | Excised | х | | | | | |
| Aptenodytes forsteri | scaffold412 | х | Excised | х | | | | | |
| Balearica | | | | | | | | | |
| regulorum | sooffold12680 | v | Evoiced | v | | | | | |
| Buceros rhinoceros | scallolu15080 | Λ | Excised | Λ | | | | | |
| silvestris | scaffold12548 | Х | Excised | Х | | | | | |
| Calypte anna | scaffold207 | ? | ? | ? | | | | | |
| Cariama cristata | scaffold16682; scaffold34970 | х | Excised | Х | | | | | |
| Cathartes aura | scaffold2023; scaffold42292 | х | Excised | х | | | | | |
| Chaetura pelagica | scaffold145 | x | Excised | x | | | | | |
| Charadrius | | | | | | | | | |
| vociferus Chlamvdotis | scattold586 | X | Excised | X | | | | | |
| undulata | scaffold6206 | Х | Excised | Х | | | | | |
| Colius striatus | scaffold23606 | Excised | Excised | X | | | | | |
| Columba livia | scaffold33 | Excised | Excised | Excised | | | | | |
| brachyrhynchos | scaffold547 | х | Excised | Х | | | | | |
| Cuculus canorus | scaffold286 | Excised | Excised | х | | | | | |
| Egretta garzetta | scaffold693 | Х | Excised | Х | | | | | |
| Eurypyga helias | scaffold21777; scaffold24649 | х | Excised | х | | | | | |
| Falco peregrinus | scaffold630 | Excised | Excised | Excised | | | | | |
| Fulmarus glacialis | scaffold11512; scaffold51529 | х | Excised | х | | | | | |
| Gallus gallus | PreEnsemb66 | Excised | Excised | Х | | | | | |
| Gavia stellata | Scaffold18014 | Х | Excised | Х | | | | | |
| Geospiza fortis | scaffold167 | Х | Excised | Х | | | | | |
| Haliaeetus albicilla | scaffold6753 | Х | Excised | Х | | | | | |
| Haliaeetus leucocephalus | scaffold1083 | Х | Excised | Х | | | | | |
| Leptosomus discolor | scaffold4770 | Х | Excised | ? | | | | | |
| Manacus vitellinus | scaffold365 | Х | Excised | Х | | | | | |
| Meleagris gallopavo | Ensembl66 | ? | ? | ? | | | | | |
| Melopsittacus | scf9001602770 | v | F · · · | v | | | | | |
| undulatus | scaffold13603; | X | Excised | | _ | | | | |
| Merops nubicus | scattold26897 | X | ? | ? | _ | | | | |
| Mesitornis unicolor | scarroid430 | X | Excised | X | _ | | | | |
| Nestor notabilis | scattold5464 | X | Excised | X | _ | | | | |
| Nipponia nippon Ophisthocomus | scaffold1734 | X | Excised | X | _ | | | | |
| hoazin | scaffold400 | Х | Excised | Х | | | | | |

| Pelecanus crispus | scaffold11738 | Х | Excised | Х |
|---------------------|---------------|---------|---------|---|
| Phaethon lepturus | scaffold8778 | Excised | Excised | х |
| Phalacrocorax | | | | |
| carbo | scaffold29799 | Х | Excised | Х |
| Phoenicopterus | | | | |
| ruber | scaffold4648 | Х | Excised | Х |
| | 22.1.1.6.0.0 | | | |
| Picoides pubescens | scattold528 | Х | Excised | Х |
| | scaffold1805; | | | |
| Podiceps cristatus | scaffold4120 | Х | Excised | Х |
| Pterocles guturalis | scaffold12949 | х | Excised | Х |
| Pygoscelis adeliae | scaffold311 | х | Excised | Х |
| Struthio camelus | scaffold149 | Excised | Excised | x |
| | | | | |
| Taeniopygia guttata | Ensembl66 | Х | Excised | Х |
| Tauraco | | | | |
| erythrolophus | scaffold7020 | X | Excised | Х |
| Tinamus guttatus | scaffold7365 | Excised | Excised | х |
| Tyto alba | scaffold1725 | х | Excised | х |

| | | | | D | SPP | | |
|---------------------------------|------------------------------|---------|---------|---------|---------|---------|---------|
| Taxon | Scaffold | Exon1 | Exon2 | Exon3 | Exon4 | Exon5* | Exon6* |
| Alligator mississippiensis | scaffold-5316 | X | x | X | Х | x | х |
| Acanthisitta chloris | scaffold2164; scaffold37315 | Excised | Excised | Excised | Excised | X? | X? |
| Anas platyrhynchos domestica | scaffold229 | Excised | Excised | Excised | Excised | Excised | X? |
| Antrostomus carolinensis | scaffold5670; scaffold13399 | Excised | Excised | Excised | Excised | X? | Excised |
| Apaloderma vittatum | scaffold19352 | Excised | Excised | Excised | Excised | X? | X? |
| Aptenodytes forsteri | scaffold227 | Excised | Excised | Excised | Excised | X? | X? |
| Balearica regulorum gibbericeps | scaffold29984; scaffold30196 | Excised | Excised | Excised | Excised | X? | X? |
| Buceros rhinoceros silvestris | scaffold7806; scaffold40069 | Excised | Excised | Excised | Excised | X? | X? |
| Calypte anna | scaffold157 | Excised | Excised | Excised | Excised | X? | X? |
| Cariama cristata | scaffold1234; scaffold25080 | Excised | Excised | Excised | Excised | X? | X? |
| Cathartes aura | scaffold19472; scaffold63458 | Excised | Excised | Excised | Excised | X? | X? |
| Chaetura pelagica | scaffold210 | Excised | Excised | Excised | Excised | X? | X? |
| Charadrius vociferus | scaffold178 | Excised | Excised | Excised | Excised | X? | Excised |
| Chlamydotis undulata | scaffold1744 | Excised | Excised | Excised | Excised | X? | X? |
| Colius striatus | scaffold17732 | Excised | Excised | Excised | Excised | Excised | X? |
| Columba livia | scaffold28 | Excised | Excised | Excised | Excised | X? | X? |
| Corvus brachyrhynchos | scaffold229 | Excised | Excised | Excised | Excised | X? | X? |
| Cuculus canorus | scaffold4 | Excised | Excised | Excised | Excised | X? | X? |
| Egretta garzetta | scaffold615; scaffold856 | ? | ? | ? | ? | ? | ? |
| Eurypyga helias | scaffold11047; scaffold12919 | ? | ? | ? | ? | ? | ? |
| Falco peregrinus | scaffold79 | Excised | Excised | Excised | Excised | X? | X? |
| Fulmarus glacialis | scaffold42243; scaffold19072 | Excised | Excised | Excised | Excised | X? | X? |
| Gallus gallus | PreEnsembl66 | Excised | Excised | Excised | Excised | Excised | Excised |

| - | | | | | 1 | | |
|--------------------------|------------------------------|---------|---------|---------|---------|---------|---------|
| Gavia stellata | scaffold25940 | Excised | Excised | Excised | Excised | ? | ? |
| Geospiza fortis | scaffold11 | Excised | Excised | Excised | Excised | X? | X? |
| Haliaeetus albicilla | scaffold23488 | ? | ? | ? | ? | ? | ? |
| Haliaeetus leucocephalus | scaffold1739 | Excised | Excised | Excised | Excised | X? | X? |
| Leptosomus discolor | scaffold9439; scaffold14034 | ? | ? | ? | ? | ? | ? |
| Manacus vitellinus | scaffold229 | Excised | Excised | Excised | Excised | X? | X? |
| Meleagris gallopavo | Ensembl66 | Excised | Excised | Excised | Excised | Excised | Excised |
| Melopsittacus undulatus | scf900160277028 | Excised | Excised | Excised | Excised | X? | X? |
| Merops nubicus | scaffold8208 | Excised | Excised | Excised | Excised | X? | X? |
| Mesitornis unicolor | scaffold14953 | Excised | Excised | Excised | Excised | X? | X? |
| Nestor notabilis | scaffold14649 | Excised | Excised | Excised | Excised | X? | X? |
| Nipponia nippon | scaffold461 | Excised | Excised | Excised | Excised | X? | X? |
| Ophisthocomus hoazin | scaffold1213 | Excised | Excised | Excised | Excised | X? | X? |
| Pelecanus crispus | scaffold28177; scaffold13020 | Excised | Excised | Excised | Excised | X? | X? |
| Phaethon lepturus | scaffold11667 | Excised | Excised | Excised | Excised | X? | X? |
| Phalacrocorax carbo | scaffold36739 | Excised | Excised | Excised | Excised | X? | X? |
| Phoenicopterus ruber | scaffold929; scaffold36951 | ? | ? | ? | ? | ? | ? |
| Picoides pubescens | | ? | ? | ? | ? | ? | ? |
| Podiceps cristatus | scaffold2096 | Excised | Excised | Excised | Excised | Excised | Excised |
| Pterocles guturalis | scaffold9458; scaffold27625 | ? | ? | ? | ? | ? | ? |
| Pygoscelis adeliae | scaffold157 | Excised | Excised | Excised | Excised | X? | X? |
| Struthio camelus | scaffold25 | х | х | х | х | x | х |
| Taeniopygia guttata | Ensembl66 | Excised | Excised | Excised | Excised | X? | X? |
| Tauraco erythrolophus | scaffold15765 | Excised | Excised | Excised | Excised | X? | X? |
| Tinamus guttatus | scaffold1085 | Excised | Excised | Excised | Excised | X? | X? |
| Tyto alba | scaffold13941 | ? | ? | ? | ? | ? | ? |

X? = Possibly present. See text.

Table S3.

Scaffolds and exons recovered from the three turtle genomes for the six tooth related genes.

| | | | ENAM | | | | | | | |
|---------------------------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 |
| Chelonia mydas | JH205207 | х | х | х | х | Excised | Excised | Excised | Excised | х |
| Pelodiscus sinensis | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised |
| Chrysemys picta bellii | JH584398 | Excised | Х |

| | | | | AMELX | | |
|------------------------|------------|--------|--------|---------|---------|--------|
| Taxon | Scaffold | Exon 2 | Exon 3 | Exon 5 | Exon 6 | Exon 7 |
| Chelonia mydas | scaffold27 | х | х | Excised | Excised | х |
| Pelodiscus sinensis | JH208023 | х | х | х | х | х |
| Chrysemys picta bellii | JH584884 | Х | Х | Excised | Excised | х |

| | | | ММР20 | | | | | | | | |
|-----------------|-----------|---------|--------|--------|---------|---------|---------|--------|---------|---------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 | Exon 10 |
| Chelonia mydas | scaffold2 | Excised | х | х | Excised | х | х | х | х | х | х |
| | JH209032; | | | | | | | | | | |
| Pelodiscus | JH219023; | | | | | | | | | | |
| sinensis | JH221105 | Excised | Х | Х | Excised | Excised | Excised | Х | Excised | Excised | Excised |
| Chrysemys picta | | | | | | | | | | | |
| bellii | JH584710 | Excised | Х | Х | Excised | Х | Excised | Х | Х | Х | Х |

| | | | AMBN | | | | | | | |
|---------------------------|-------------|---------|---------|---------|---------|---------|---------|---------|---------|--|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | |
| Chelonia mydas | scaffold209 | х | х | Excised | Excised | х | х | х | Х | |
| Pelodiscus sinensis | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | Excised | |
| Chrysemys picta bellii | JH584398 | Х | Х | Х | Excised | Excised | Х | Х | Х | |

| | | 4 | AMBN (cont.) | | | | | |
|---------------------------|-------------|---------|--------------|---------|--|--|--|--|
| Taxon | Scaffold | Exon 9 | Exon 10 | Exon 11 | | | | |
| Chelonia mydas | scaffold209 | х | Х | х | | | | |
| Pelodiscus sinensis | Excised | Excised | Excised | Excised | | | | |
| Chrysemys picta bellii | JH584398 | Х | х | х | | | | |

| | | | | | AM | ITN | | | |
|---------------------------|-------------|---------|---------|---------|---------|---------|--------|---------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 |
| Chelonia mydas | scaffold209 | х | х | х | х | Excised | N/A | Excised | х |
| Pelodiscus sinensis | JH205207 | Excised | Excised | Excised | Excised | Excised | N/A | Excised | Excised |
| Chrysemys picta bellii | JH584398 | х | Х | х | Excised | Excised | N/A | Excised | Х |

| | | _ | AMTN (cont | .) |
|---------------------------|-------------|--------|------------|---------|
| Taxon | Scaffold | Exon 9 | Exon 10 | Exon 11 |
| Chelonia mydas | scaffold209 | х | Excised | Excised |
| Pelodiscus sinensis | JH205207 | Х | Excised | Excised |
| Chrysemys picta bellii | JH584398 | х | Excised | Excised |

| | | | | D | SPP | | |
|------------------------|------------|----------|---------|---------|---------|---------|---------|
| Taxon | Scaffold | Exon1 | Exon2 | Exon3 | Exon4 | Exon5 | Exon6 |
| Cholonia mudaa | anoffeld52 | Engineed | v | v | v | 9 | v |
| Chelonia myaas | scanolu32 | Excised | Excised | Excised | Excised | Excised | Excised |
| Pelodiscus sinensis | Excised | Excised | | | | | |
| | | | | | | | |
| Chrysemys picta bellii | JH584595 | Excised | Х | Х | Х | ? | Х |

Table S4.

Scaffolds/assemblies/amplicons and exons recovered from the eight mammalian genomes for the six tooth related genes.

| | | | | | | ENAM | | | | |
|---------------------------|------------------------------------|--------|--------|-------|--------|--------|---------|---------|---------|--------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 |
| Homo sapiens | Ensembl66 | х | Х | Х | х | х | х | х | х | Х |
| Orycteropus afer | ALYB01124787; ALYB01124786 | х | х | х | х | х | х | х | х | х |
| Canis lupus familiaris | Ensembl66 | х | х | х | х | х | х | х | х | х |
| Dasypus novemcinctus | JH575992 | х | х | х | Х | х | Х | Х | Х | Х |
| Loodonta africana | GL010057 | х | х | х | х | х | х | х | х | х |
| Physeter macrocephalus | KI405375 | х | х | х | х | х | х | х | х | х |
| Choloepus hoffmani | scaffold 46750; scaffold 275290 | х | х | х | х | х | Excised | Excised | Excised | х |
| Manis pentadactyla | JPTV01017537 | ? | ? | ? | ? | ? | ? | Х | Х | Х |

| | | | | | AMELX | | | |
|------------------------|--|--------|--------|--------|---------|--------|--------|--------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4* | Exon 5 | Exon 6 | Exon 7 |
| Homo sapiens | Ensembl66 | Х | х | х | х | х | х | Х |
| Orycteropus afer | ALYB01242161; ALYB01071525; ALYB01242162 | х | x | х | х | х | х | х |
| Canis lupus familiaris | Ensembl66 | Х | х | х | х | х | х | Х |
| Dasypus novemcinctus | JH568171 | Х | х | х | х | х | х | Х |
| Loodonta africana | GL010066 | х | х | х | х | х | х | х |
| Physeter macrocephalus | KI411331 | х | х | х | х | х | х | х |
| Choloepus hoffmani | scaffold 55074 | Х | Х | Х | х | Х | х | х |
| Manis pentadactyla | JPTV01051765 | Х | Х | Х | Х | Х | Х | Х |

* Only functional in Homo and Physeter

| | | | | | | MN | MP20 | | | | |
|---------------------------|---|--------|--------|---------|--------|--------|--------|--------|--------|--------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 | Exon 10 |
| Homo sapiens | Ensembl66 | х | х | х | х | х | х | х | х | х | х |
| Orycteropus afer | ALYB01162291; ALYB01162292; ALYB01162293 | х | х | х | х | х | х | х | х | х | х |
| Canis lupus familiaris | Ensembl66 | х | х | х | х | х | х | х | х | х | х |
| Dasypus novemcinctus | JH564871 | Х | Х | х | Х | Х | х | х | Х | Х | х |
| Loodonta africana | GL010062 | Х | Х | х | Х | Х | Х | Х | Х | Х | х |
| Physeter macrocephalus | KI404428 | Х | Х | х | Х | Х | х | х | х | Х | х |
| Choloepus hoffmani | scaffold 3741 | Х | Х | Excised | Х | Х | х | х | х | Х | х |
| Manis pentadactyla | JPTV01075382; JPTV01140158; JPTV01140157; JPTV01104240 | ? | ? | Х | Х | Х | Х | Х | Х | X | X |

| | | | | | AM | 1BN | | | |
|---------------------------|--|--------|--------|--------|--------|--------|---------|--------|---------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8* |
| Homo sapiens | Ensembl66 | х | х | x | х | х | х | х | х |
| Orycteropus afer | ALYB01124783 | х | х | Х | X | Х | Excised | Х | NA |
| Čanis lupus familiaris | Ensembl66 | х | Х | Х | Х | Х | Х | Х | NA |
| Dasypus novemcinctus | JH575992 | х | Х | х | X | х | х | х | NA |
| Loodonta africana | GL010057 | х | Х | х | Х | х | х | х | NA |
| Physeter macrocephalus | KI405375 | х | х | Х | X | Х | Х | Х | NA |
| Choloepus hoffmani | scaffold 6767; 1336370099; 1338793682; 1368206053 | Х | Х | Х | Х | Х | Х | Х | NA |
| Manis pentadactyla | JPTV01085286; JPTV01085287 | Х | Х | Х | Х | Х | Х | Х | NA |

| | | | | AMBN (con | t.) | |
|---------------------------|---|---------|---------|-----------|---------|---------|
| Taxon | Scaffold | Exon 9* | Exon 10 | Exon 11 | Exon 12 | Exon 13 |
| Homo sapiens | Ensembl66 | x | Х | х | x | x |
| Orycteropus afer | ALYB01124783 | NA | х | х | x | х |
| Canis lupus familiaris | Ensembl66 | NA | х | х | x | х |
| Dasypus novemcinctus | JH575992 | NA | х | х | x | х |
| Loodonta africana | GL010057 | NA | х | х | x | х |
| Physeter macrocephalus | KI405375 | NA | х | х | x | х |
| Choloemus | scaffold 6767; 1336370099; 1338793682 | NA | х | х | Х | Х |
| hoffmani | 1368206053 | | | | | |
| Manis pentadactyla | JPTV01085286; JPTV01085287 | NA | X | X | X | X |

* Present in Homo only

| | | | | | | AMTN | | | | |
|------------------------|---------------|--------|--------|---------|-------|---------|--------|--------|--------|--------|
| Taxon | Scaffold | Exon 1 | Exon 2 | Exon 3 | Exon4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 |
| Homo sapiens | Ensembl66 | х | x | Х | Х | х | х | Х | х | x |
| Orycteropus afer | ALYB01124779 | ? | ? | ? | ? | ? | х | х | х | х |
| Canis lupus familiaris | Ensembl66 | х | x | Х | Х | X | х | Х | х | х |
| Dasypus novemcinctus | JH575992 | ? | ? | х | X | x | х | х | x | x |
| Loodonta africana | GL010057 | x | x | x | x | x | х | x | x | x |
| Physeter macrocephalus | K1405375 | x | x | x | x | x | x | x | x | x |
| Choloenus hoffmani | scaffold 7283 | 2 | 2 | Excised | x | Excised | x | x | x | x |
| Manis pentadactyla | JPTV01085286 | ? | ? | ? | ? | ? | ? | ? | X | X |

| | | | | DSPP | | |
|------------------|---------------|-------|-------|-------|-------|-------|
| Taxon | Scaffold | Exon1 | Exon2 | Exon3 | Exon4 | Exon5 |
| Homo sapiens | Ensembl66 | х | х | х | х | х |
| Orycteropus afer | ALYB01395527; | | | | | |

| | ALYB01244079 | Х | ? | Х | Х | Х |
|------------------------|------------------------------------|---|---------|---------|---------|---|
| | | | | | | |
| Canis lupus familiaris | Ensembl66 | Х | Х | Х | Х | Х |
| Dasypus novemcinctus | JH569075 | х | х | х | х | х |
| Loodonta africana | GL010057 | х | х | х | х | х |
| Physeter macrocephalus | KI403123 | x | x | х | х | х |
| Choloepus hoffmani | scaffold 31678; scaffold 189150 | х | х | Excised | х | х |
| Manis pentadactyla | JPTV01058887 | Х | Excised | Excised | Excised | Х |

 Table S5.

 Bird AMTN inactivating mutations. I=Insertion; D=deletion

Neornithes: 1205D

Paleognathae: 151-152I

Struthio: 699D, 717D, 721-722D, 844D, 930-6632D, 6692-6699D (7 bp)

Tinamus: 1-99D (Exons 2+3D), 792-994D, 1225-6947D

Neognathae: 6012-6013D (with subsequent insertions in several lineages), 6042D (with subsequent frameshifts in a few taxa)

Galloanserae: 6650-6653D

Anas: 765D, 840-841I, 927-934D, 966-981I, 4604I

Gallus+Meleagris: 747D

Gallus: 1-57D (Exon 2+5' of exon 3), 65-69D, 73-74D, 86-95D, 153-159D, 696-697D, 848-852D, 933-1224D, 1409-1413D, 1460D, 4534I, 6042-6051D

Meleagris: 1-725D (Exons 2-5), 933-EndD

Neoaves: 732D, 4567-4568I

Phoenicopterus: 4659-5038I, 6013I, 6069-6606I, 6616-6617I, 6654-6685I

Podiceps: 822D, 1356-1366D, 1435-1436I

Columba: 1-54D (Exon 1), 100-107D, 170-EndD

Mesitornis: 1-99D, 792-938D, 1339-1360D

Pterocles: 42D, 170-725D (Exon 5), 822-933D, 1304-1307I, 1461-1468I, 6013I, 6046D, 6686-6697D (8 bp)

Cuculus: 1276D, 1342D, 4590I

Chlamydotis: 33D, 876I, 1402D, 4577D

Tauraco: 1206-1207D, 1261-1376D, 6618D, 6628-6632D

Antrostomus: 1225-6298D

Calypte: 1-725D, 739-743D, 759-762D, 792-EndD,

Chaetura: 55-99D (Exon 3), 146-150D, 6627D

Balaerica: 164D, 649-652D, 657-658D, 6039-6049D, 6058D

Charadrius: 1-54D (Exon 2), 941-942I, 958D, 1492-4510I, 6633-6640D, 6633-6640D

Opisthocomus: 58D, 867D, 1373D, 4582-4583D

Eurypyga: 1-54D, 158D, 763-764D, 960-994D, 1478-1486D (8 bp), 4539-4567D (11 bp), 6654-6655I

Phaethon: 726-1394D

Aptendodytes: 170-725D, 1224D

Fulmarus: 55-58D, 843-849D, 1209-1212I, 1456-1460D, 6705-6713D (7 bp)

Gavia: 4534I, 4604I

Pygoscelis: 6616-6617I

Egretta: 109D, 953D, 1327I, 4524-4533D, 5069-6013I

Nipponia: 759D, 783D, 6013I

Pelecanus: 714D, 6618D

Phalacrocorax: 939-956D, 995-1224D, 6047D

Cariama: 9-10D, 30D, 993-1224D, 6013I

Falco: 14D, 128I, 726-EndD,

Melopsittacus+Nestor: 146-150D, 1348-1358D, 4612-4658D

Melopsittacus: 6051D, 6644D

Nestor: 30D

Melopsittacus+*Nestor*+*Acanthista*+*Manacus*+*Corvus*+*Taeniopygia*+*Geospiza*: 170-791D (exons 5 and 6)

Acanthista+Manacus+Corvus+Taeniopygia+Geospiza: 12-54D

Acanthista: 1346-1392D, 1483I, 4605D, 4638-5066D

Manacus: 129-132I, 955-956D, 1226I, 4520-6938D

Corvus+Geospiza+Taeniopygia: 5058-5061D, 6708-6709I

Corvus: 63-75D, 6026-6030D

Geospiza: 109-113D, 797D, 1277D, 1485-1486D

Taeniopygia: 1286-1311D (5 bp), 1425D, 4659I

Cathartes: 157-725D, 1407-1408I

Haliaeetus: 767D, 877D

Tyto: 170-938D (Exons 5-7), 1243-1253D, 1483I, 4511-4514D, 4534I, 4590I, 4604I, 5998-6013I, 6616-6617I, 6623-6626I, 6692-6695I

Colius: 1-994D, 5039-5040D, 6043-6047D, 6607-6614D

Leptosomus: 792-801D, 846-849D, 1209-1212I, 1225-EndD

Apaloderma: 170-725D, 885-886D, 999-1197I, 6616-6617I, 6653D

Buceros: 46-50D, 75D, 172-173I, 806-816I, 876I, 1323D, 1336-1342D, 4533D

Picoides: 55-99D, 183-646I, 665D, 987I, 995-1224D, 1254-1260I, 1304-1307I, 1407-1408I, 1435-1436I, 1460D, 4654D, 6014-6634D, 6637D

Merops: 9-10D, 726-938D, 987-988I, 1225-EndD

Table S6.

Bird AMBN inactivating mutations. I=Insertion; D=deletion

Palaeognathae: 195-196D, 964-971D, 1377-EndD

Struthio: 56-175D, 1027-1045D, 1125-1172D, 1372-1373D, 1376D

Tinamus: 183D, 222-898D (Exon 5), 915-931D, 947D, 1018-1082D, 1099-1144D, 1347-1363D

Galloanserae: 201D

Anas: 1-175D, 1033-1045D, 1077I, 1257-1261D, 1331-1376D (Exon 10), 1377-EndD

Gallus: 1-55D, 899-EndD,

Phoenicopterus: 1001-1020D

Neoaves: 829-841D

Podiceps: 201-204D, 1938-1941D

Pterocles: 176-1174D (Exons 4-6), 1265D, 1310-1316D, 1376D

Mesitornis: 1-175D, 255-814I, 903-1152D, 1175-EndD

Cuculus: 901-902D, 1047-1165D, 1198D, 1213I, 1430-1497I, 1624I, 1883-1886D, 1921-1924D

Tauraco: 1-55D, 1420D, 2001I

Chlamydotis: 899-1174D, 1249-1395D, 1513-1514I, 1711-1729D, 1990D

Antrostomus: 60-175D, 249D, 900-910D, 1253-1256D, 1604-1605D, 1702-1706D, 1927-EndD

Chaetura: 1-102D, 106-115D, 222-898D (Exon 4), 1159-1165D, 1215-EndD

Calypte: 1-175D, 222-EndD

Ophisthocomus+Charadrius+Balearica: 1969D (with subsequent overprinting deletion in *Balearica*)

Ophisthocomus: 222-1174D (Exons 5, 6), 1217D, 1240D

Charadrius: 9-15D, 1377-1547D

Balearica: 919-934D, 1148-1154D, 1580I, 1647-EndD

Phaethon: 1045D, 1215-1376D, 1530D, 1671-1678D, 1867-1871I

Eurypyga: 21D, 85-88I, 244-251D, 1148-1154D, 1215-1216D, 1299-1300D, 1336I, 1926-1953D

Gavia: 1289I, 1331-EndD (possibly two deletions given that intron 10 aligns poorly with other birds), 1731-1732

Fulmarus: 222-225D, 856-862D, 1521D, 1986D

Pygoscelis: 1942D

Aptendodytes: 972I

Phalacrocorax: 195-196D, 1048D, 1975-1976I, 2100-2107D

Nipponia: 919-922D, 1377-EndD

Egretta: 1166I, 1528I, 1580-1583I, 1723-1839D, 1852D

Pelecanus: 1525D, 1679-1700D

Cariama: 1026-1027D, 1129D

Falco: 28-29D, 119-171I, 952-955D, 1076D, 1175-1196D, 1528I, 1753D

Melopsittacus+Nestor+Acanthisitta+Manacus+Corvus+Geospiza+Taeniopygia: 222-898D (Exon 4)

Melopsittacus+Nestor: 898-1174D, 1723-EndD

Nestor: 13-24D (11 bp), 1672-1687D (10 bp)

Acanthisitta+Manacus+Corvus+Geospiza+Taeniopygia: 1962-1977D (w/ subsequent deletion in *Corvus+Geospiza+Taeniopygia*)

Corvus+Geospiza+Taeniopygia: 1978-1985D

Melopsittacus: 1377-1381D, 1654-1680D (26 bp)

Acanthisitta: 211-214I, 1218-1227D, 1524D, 1657I, 1676I

Manacus: 2-9D, 56-59D, 176-201D, 964D, 1026-1040D, 1069D, 1646I, 1681-1685I, 1729D, 1884D, 2003D

Corvus: 1-55D, 900-903D, 1026-1040D, 1377-1754D (probably shared deletion with *Geospiza+Taeniopygia* with subsequent 1 bp deletion in *Corvus*)

Geospiza+Taenopygia: 1-175D, 1377-1753D

Geospiza: 898-1174D (Exon 6), 1331D

Taeniopygia: 1028-1040D, 1119-1120D

Cathartes: 236D, 995I, 2001I

Haliaeetus: 850D, 1026D, 1926-1929D

Tyto: 202D, 222-898D (Exon 5), 1298I, 1786D, 2110D

Colius: 865D, 899-1174D, 1284-1376D, 1936-1937I

Leptosomus: 20I, 56-1330D, 1346-1352D, 1578D, 1882I, 1968D, 2119I

Apaloderma: 1-1174D, 1797-1845D

Buceros: 70-73D, 222-1214D (Exons 5-7), 1548I, 1864D, 2112D

Picoides: 1-55D, 85-88I, 205-206D, 222-1174D, 1184D, 1215-EndD,

Merops: 56-71D, 899-1174D, 1949-1950D

Table S7.

Bird ENAM inactivating mutations. I=Insertion; D=deletion

Aves: 608D (requires subsequent single base insertion in *Anas*, but there are also alignment issues; probably not real given location of splice site in *Anas* and *Gallus*), 2386-2393D (requires subsequent 1 bp insertion in *Phoenicopterus*, alignment much better than previous frameshift candidate), 3824-3831D (with subsequent 2 bp insertion in *Cuculus*), 3844-3856D, 5753D

Neognathae: 4494-4497D (w/ subsequent 1bp insertion in Buceros), 5856-5862D

Galliformes+Anseriformes: 4514I

Struthio: Exon 2 deletion, 145D, 242-498D, 563D, 1381-1396D, 1546-1550D, 1580I, 2423-2427D (4 bp), 2478-2497D, 2586D, 4592-4593D, 5720-5721D, 5827D, 5970I

Tinamus: Exons 3-4 deletion, 253-498D, 513-519D, 617-623D, 664-665D, 1484I, 1492-1509D, 1522-1538D, 1583-1646D (38 bp), 1653-1885D, 1966-2278D, 2416-2422I, 2426I, 2475-2514D (38 bp), 2586-2587D, 2775I, 3723D, 3980-3992D, 4239-4250D, 4594I, 5754-5755D, 5780-5789D, 5831-5834D, 5886-5892I, 6126I, 11390-11522D, 11730-12358D

Palaeognathae: 668-684D, 1339-1348D, 1466I, 1580I, 2360-2361I, 3072D, 3723D, 4364D, 4514I, 4700-4707I, 4815-5716D, 5863-5867I, 11348D

Gallus: 132-139D, 191-195D, 253-254D, 313-498 (most of exon 7), Exon 8 deletion 1387-1388D, 1497-1667D, 1892D, 1976-1979D, 2011D, 2321-2343D, 2596D, 3751-3754D, 5817-5818D, 5831D, 5938I, 6039I

Meleagris: 577-592D, 605-612D, 1344-2394D, 2403-2477D, 2591-2596D (5 bp), 2694-2751D (44 bp), 3108D, 3751-3762D (11 bp), 4302-4305I, 11422D

Gallus+Meleagris: 735D, 2791-2794D, 3908D, 4142-4146D, 4594-4637I, 4765D, 4794-4798D, 5706-5707D, 5985-5989D, 6146I, 11377-11392D

Columba: Exons 1-9 deletion

Anas: 194-194D, exon 8 deletion, 248-498D, 1569D, 2287-2297, 2785-2791D, 3101D, 3773-3782I, 3913D, 4145-4146D, 4698-5949D, 5970I, 6148-11392D, 11644-11645, 11661D

Neoaves: 6D, 494-495D, 4512-4513D, 11644-11645D

Melopsittacus+*Nestor*: 1-6D (no start codon), 1496I, 1980I, 2236-2237D, 2498-2499I, 3722-3723D, 4252-4253I, 4262-4268D, 5974-6019D, 11683-11690I, 11711-11732D

Ophisthocomus+Balearica: Exon 5 deletion

Manacus: 1-19D (no start codon), 735D, 1490-4465D, 4757D, 4788D, 5850-5853D, 5895D, 6059D, 6116D

Corvus: 1-15D (no start codon), 18-19D, 668-5769D, 6115-6122D, 12415D

Corvus+Geospiza+Taeniopygia: 6067D, 12380I

Geospiza+Taeniopygia: 6116D

Geospiza: 1-22D (no start codon), 43D, exon 3 deletion, 668-5706D, 11675D, 12371I, 12416D

Acanthisitta+Manacus+Corvus+Geospiza+Taeniopygia+Melopsittacus+Nestor: exon 4 deletion

Cariama+Falco+Acanthisitta+Manacus+Corvus+Geospiza+Taeniopygia+Melopsittacus +*Nestor*: exons 5-8 deletion

Taeniopygia: 1-20D (no start codon), 668-5925D, 12415-12419D

Colius: 1-16D (no start codon), 681-690D, 1415D, 1422D, 2495-2504D (8bp), 2796D, 2884-2885D, 3755I, 3816-3819D, 3983-3984, 4179-4180D, 4353D, 4378-4384D, 4440D, 4672D, 4737D, 4798D, 5936D, 5970I, 11399D, 11643-11646

Leptosomus: Exons 2-3 deletion, 672-1349D, 1387-1388D, 2701-2704D, 2755I, 2786-2787D, 3754-end (deletion)

Cuculus: Exons 2-5 deletion, 564I, 1512-1519I, 1972-1975D, 2500-2501D, 3043-3053D, 3800D, 3910-3920D, 5685D, 5791-5794D, 11437-11455D

Tauraco: 202-205D, 2264-2265D, 2341D, 2416-2419I, 4402D, 4440-4443D, 4751I, 5789D, 6051D, 6126I, 11381-11382I, 11433D, 12409-12410D

Chaetura: 215-234D, exon 6 deletion, exon 8 deletion, 439I, 539-545D, 1633I, 2280-2281D, 2500D, 2592-2600D (8 bp), 3722-3723D, 3906-3910D, 3939D, 4366-4443D, 4592D, 4819-4826I, 6044D, 11453-11465D, 11643-11646D, 12418D

Calypte: Exons 2-8 deletion, 1390D, 1400D, 1494-1498D, 1532D, 2282-2286D, 2394-2403D, 2414-2415D, 2471I, 2683D, 2810-2817D, 2843I, 2865-2873D, 2896-2899I, 3055-3062D, 3755I, 3764D, 3796-3797D, 3809D, 3816-3819D, 3834-3861D (14 bp), 3883D, 3895-3898I, 3906D, 3917-3930D, 4109I, 4120-4152D, 4156D, 4163D, 4188I,

4199-4202D, 4205-4212D, 4220-4221I, 4248-4249I, 4252-4253I, 4274-4277D, 4292D, 4299-4338D, 4344D, 4353-4356D, 4363-4367D, 4374-4375D, 4377I, 4440-4456D, 4470-4471D, 4478D, 4488-4501D, 4514I, 4528-4529D, 4573-4574D, 4580-4645D, 4687-4691D, 4707I, 4750D, 4819-4820I, 5685-5686D, 5718-5745D, 5885D, 6120D, 115151-11514D, 11518D, 11614-11621D, 11659-11662D, 12370D

Chaetura+*Calypte*: 2280-2281D, 2906-3033I, 5969D, 5974-5975D

Eurypyga: Exons 2-8 deletion, 735D, 1351-1547D, 1556-1569D, 1581-2275D, 2283-4366D, 4467-4468D, 5706D, 5833-5839D, 5969D, 5990-5993D, 11346D, 11588D, 11646-11652D

Pelecanus: Exons 2-4 deletion, Exon 8 deletion, 243D, 414-421D, 564I, 598-607D, 1569-1639D, 1898D, 2500D, 2872D, 3055-3056D, 3722-3723D, 3794-3797D

Acanthisitta: Exons 2-3 deletion, 1483D, 1534I, 1864-1885I, 2465D, 2570-4515D, 5817-5818D, 5830I, 6118D, 11614-11621D, 11708-11717D, 12369-12373D

Balearica: Exon 3 deletion, Exon 8+most of exon 9 deletion (beginning of exon 8 through 11346),

Apaloderma: 87-90I, 127I, 681D, 1347-1375D, 1534I, 1539-1543I, 2270-2273I, 2437-2444D, 2524-2555D (28 bp), 2720D, 2843I, 3794-3797D, 3818-3819D, 3892-3898I, 3991-4105D (11 bp), 4334-4338D, 4404-4408D, 5878I, 5970I, 5976-5977I, 6152-6168I, 11438-11448D, 11500D

Gavia: Exon 7-8 deletion, 445D, 2283-2310D, 2489D, 2564-2565D, 3047-3048D, 3102-3115D, 3794-3797D, 3816-3819D, 4590D, 5817-5818D, 11707-end (deletion)

Mesitornis: Exons 4-5 deletion, 736-1331I, 1387-1388D, 2456-2478D (19 bp), 2595I, 2671-2672I, 3043-3053D, 3760-3764D, 3794-3797D, 3995-4088I, 4326-4333I, 4467-4468D, 4750D, 4798D, 5703I, 5970I, 6071I, 11646-11647, 11724-end

Phaethon: 98-101D, 594-606D, 1480-1491 (11 bp deletion), 1559I, 2678I, 2746I, 2852-2883D (29 bp), 4235-4245D, 4383-4386D, 4746-4757D (11 bp), 5817-5818D, 5903-5907I, 6143-11340D (11 bp), 11714-11715D

Fulmarus: 124-140D (16 bp), 2527-2531D, 5817-5818D, 11602I

Antrostomus: 98-101D, 1378-1384D, 1473D, 2410-2411D, 2456-2478D (19 bp), 2544-2550D, 3746-3760D (14 bp), 3902-3903D, 4213-4216D, 4301D, 4485-4486D, 4767D, 4809D, 5817-5818D, 6119I, 11507-11510I, 11643-11646D

Nipponia: 151-152I, 235D, 4101I, 4365I, 4467D, 5817-5818D, 6064I, 11517-11532D

Picoides: Exons 2-4 deletion, 1674-1885I, 1905-1965I, 2090-2219I, 2396-2400D, 2436D, 3049I, 3071-3072D, 3729-5852D, 11516I, 11597-11598D, 12366-12370

Apaloderma+Buceros+Cathartes+Haliaeetus+Leptosomus+Tyto+Merops+Picoides+Coli us: Exons 5-8 deletion

Egretta: Exon 5 deletion, 736-827I, 2560-2566D, 2630D, 2661-2670D, 3821-3837D (5 bp), 4179-4195D, 4366D, 4392D, 4472D, 4594I, 5703I, 5970I

Sphenisciformes (penguins): Exon 8 deletion

Pterocles: Exons 7-8 deletion, 406-428D, 2541I, 2867-2883D, 4147-4148I, 4278-4281I, 4377I, 4433-6148D, 11586D

Chlamydotis: Exon 4 deletion, 541-554D (8 bp deletion), 735D, 1632D, 1904-2385D, 266I, 4227-4234D, 4352-4355D, 4493-4504D, 4687-4693D

Podiceps: 668-1532 (beginning of exon 9), 1886-1887D, 2249D, 2263D, 2344-2347I, 4741I, 4799I, 5873-5883D, 5980I, 11643-11646D

Charadrius: 668-5723 (5' end of exon 9), 5874D

Merops: 6-7D, 1369I, 2382D, 2471-2474I, 2598-2602D, 2858-2868D, 4120D, 4533-4572D, 4683D, 4712D, 4898D, 5725-5741I, 5753I, 11643-11650D

Tyto: 1336-2533D, 2586D, 3794-3794D, 3820I, 5817-5818D, 11744-12357I

Buceros: 735D, 1510-1525 (8 bp deletion), 2239D, 2280-2281D, 2489D, 2560-2563D, 2579D, 2596-2624D (17 bp), 2696I, 2712-2713I, 3722-3723D, 3798I, 4119I, 4188I, 4333I, 4793-4804D (11 bp), 5720-5792D, 6100-6101D

Phalacrocorax: 735D, 1555I, 3800-3803D, 4213-4818D, 5970I, 11643-11646D, 11733I

Phoenicopterus: 1498D, 2386I, 2406I, 2755I, 3794-3797D, 3939D, 4770-5840D

Aptenodytes+Pygoscelis: 1484I, 3719-3723D

Pygoscelis: 4551-4552I

Haliaeetus (both species): 1539-1543I, 2593-2594D, 2882-2883D, 3113D, 3835I, 11496D

Nestor: 1997-2003I, 4593D, 11622I

Melopsittacus: 2000-2003I, 2433-2434D, 3093-3096D, 3116-3713I, 4101I, 4139D, 4353D, 5831-5834D, 5902-5913D, 11633-11643D, 12426-12430I

Cariama: 677-678D, 2316-end (deletion)

Falco: 2500D, 2696I, 3053-3075D, 3718-3724D, 4306-4313D, 4573-4581, 4819-5684I, 5817-5818D, 5871I, 5936-5946D, 6053D, 11642-11643D, 12402-12403I

Cathartes: 2585I, 3798-3799I, 4322-4323I, 4594I

Ophisthocomus: 2404D, 3713I, 3794-3797D, 3818-3819D, 4646-4671I, 6152-11337I, 11642-11643D, 12409-12410D

Podiceps+Phoenicopterus: 3722-3723D, 3816-3817D

Table S8.

Bird AMELX inactivating mutations. I=Insertion; D=deletion

Acanthisitta: 32-36D, 87D, 236-258D, 358-359D, 441-444D, 1183D

Anas: 1-20D, 102I, 140I, 1043I, 1149-1170D

Antrostomus: 245-248D

Antrostomus+Calypte+Chaetura: 308-309D

Apaloderma: 5I, 85-86I, 1128-1132D

Aptenodytes: 85-86I

Aptenodytes+Pygoscelis: 4D

Aves: 69D (w/ subsequent insertions within Aves)

Balaerica: 308-309D, 368-380D

Buceros: 26I, 265D

Calypte: 53-63D, 167-173D, 304-305D, 351D, 375-379I, 1131-1132D, 1164-1167D

Cariama: 12I, 278-296D, 278-296D (14 bp), 389-398D, 1259I

Cathartes: 349-350D

Chaetura: 32-36D, 45I, 151-167D, 281-284I, 375-379I, 1127D, 1217-1259I, 1275-1278D

Chlamydotis: 371-380D

Colius: 5I, 51D, 114I, 238-241D, 436-439D, 558-1033D, 1144-1151D, 1273-1274D

Columba: 255D, 259-1261D

Corvus: 87D, Exon 5 deletion, 358D

Corvus+Geospiza+Taeniopygia: 238-260D, 344D

Cuculus: 190-195D (5 bp), 366I, 1057-1087I, 1146D

Egretta: 86I, 304-307D, 1166D

Eurypyga: Exon 2 deletion, 1259I

Falco: 30-31D, 87D, 112-113D, 1057-1058I, 1200I

Fulmaris: 1097I

Galloanserae: 132-133D, 246-256D (8 bp)

Gallus: 257-258D, 432-439D, 1056D, 1275-1276D

Gallus+Meleagris: 15-18I, 193I

Gavia: 185D, 1094-1121D

Gavia+Fulmarus+Pygoscelis+Aptenodytes: 308-309D

Geospiza: 68D, 313D, 368-383D

Geospiza+Taeniopygia: 357-358D, 1182-1185D

Haliaeetus (both species): 50-63D

Leptosomus: 64-88D

Manacus: Exon 2 deletion, 87D, 172-187D, Exon 6 deletion

Meleagris: 328D, 425-542D, 1150-1166D

Melopsittacus: 355D, 1117-1118D, 1148-1154D

Melopsittacus+Nestor: 37I, 114I

Merops: 5I, Exon 6 deletion

Mesitornis: 272-276D, 307-311D, 1035D, 1275-1308 (5' end of exon 7 including stop codon)

Neognathae: 219D

Nestor: 277-278D, 351D, 1101-1116I

Nipponia: 5I, 398D

Opisthocomus: 349D

Phaethon: 292I, 450-541I

Phalacrocorax: 70-86I, Exon 6 deletion

Phoenicopterus: 193D

Phoenicopterus+Podiceps: Exon 2 deletion

Picoides: 71-86I, 1195-1208D

Podiceps: 86I

Pterocles: 87D, 233D, 368-380D, 1192-1202D

Pygoscelis: 87D, 554-561D

Struthio: 546-549D, 1130D, 1160-1161D

Tauraco: 13-63D, 318I, 1033D

Taeniopygia: 87D, 449D

Tinamus: 87D, 180-181D, Exon 6 deletion

Tyto: 20-63D, 319-326D, 339D

Table S9.Bird DSPP inactivating mutations. I=Insertion; D=deletion

Aves: 1332-1350D, 1588-1748D, 1814-1911D, 2206-2210D

*Shared mutations within Aves is based off of scaffolds that spanned from *SPARCL1* to *DMP1* (23 birds). Only Palaeognathae is annotated as sequence similarity is not highly compelling within Neognathae.

Table S10.

Bird MMP20 inactivating mutations. I=Insertion; D=deletion

Aves: Exon 1 (deletion)

Palaeognathae: 911-993D, 1646D

Struthio: 127-280D, 444-447D, 455-591D, 722D, 725-750D, 897D, 1075-1144D, 1394-1406D, 1572D

Tinamus: 127-640D, 709-710D, 725-748D, 820D, 873-880I, 1075-1135D, 1172-1190I, 1413I, 1534D

Neognathae: 200-206D, 911-1006D

Anas: 214-220I, 251-443D, 549-550D, 685-692D, Exon 5D, 911-1014D, 1306-1357D, 1551-1552D

Galliformes: Exon 3D, Exon 4D, 1244I, Exon 8D, Exon 10D

Gallus: Exon 2D, 733I, 1045-1052I

Meleagris: Exon 5D

Neoaves: Exon 3D, 660D, 1075-1134D

Phalacrocorax: 169-184I, 854-858D, 1333-1334I, Exon 10D

Columba: 135-150D, Exon 5+6D

Picoides: Exon 2D, Exon 7-10D

Pelecanus: Exon 2D, Exon 5+6D

Calypte+Chaetura: Exon 2D,

Calypte: 599-659D, 715-722D, Exon 5+6D, Exon 7+8D, 1471-1472I, 1583D

Tauraco: Exon 2D, 1348-1355D

Pterocles: Exon 2D, Exon 4D, 733I, 755-764I, 898I, 1159I, 1245-1248D, 1417-1549D

Apaloderma: Exon 2D, Exon 4D, 1027D, Exon 7D, 1250-1287D, 1291-1292D, 1328I, Exon 9D, Exon 10D

Mesitornis: 294-443D, Exon 5+6D, Exon 7D

Charadrius: Exon 2D, 1030-1034I

Taeniopygia: 169I, 1562I

Tyto: 237-238D, Exon 8D, 1547-1550D, Exon 10D

Manacus: 242-243D, 326-330D, 395-402D

Cariama: 390-393D, 851-852D, 1291-1292I

Corvus: 331-358I, Exon 7+8D

Phaethon: 282I, 298-299D, 848-909D, Exon 9D, 1563-1564D

Chlamydotis: 269I, Exon 4D, 826-837D

Podiceps: 283D, 1654I, 1711-1716D (includes last 5 bp of coding sequence with stop codon)

Falco: 264-272D (8 bp), 292-309D, Exon 4D, Exon 8D, 1451-1455D, 1471-1472I, 1487-1503I, 1538-1549D, Exon 10D

Manacus+Corvus+Geospiza+Taeniopygia+Acanthista: 300-303I, Exon 5+6D

Merops: 326-330D, 1445-1457D, 1473-1483D, 1576-1577I

Colius: 328D, 625-631D, Exon 8D

Melopsittacus+Nestor: 427-436I, Exon 4D, Exon 5D, Exon 6D, Exon 7+8D

Nestor: 1552-1886D

Melopsittacus: Exon 10D

Cuculus: 408I, 425-443D, 725-749D, 817D, 832-854D, 1415D, Exon 9+10D

Phoenicopterus+Podiceps: Exon 4D

Phoenicopterus: 1468-1550D

Gavia: 625-724D, 830-8311

Chaetura: 635D, 638-644D, 723D, 732D, 824D, 1171-1416D, 1434D, 1482-1483D, 1671-endD

Antrostomus: 1370-1377D, Exon 10D

Opisthocomus: 681D, 724D, 1060-1064D, Exon 7D, Exon 9D, 1576I

Balaerica: 687-691D, 1200-1206D, 1245D, Exon8-10D

Eurypyga: 633-640D, 691-701D, 730I, 734D, 1291D, Exon 9D, 1551-1578D, 1641-1650D, 1710-1719D (includes stop codon)

Leptosomus: 311D, 661-662D

Buceros+Picoides+Merops: Exon 5D

Egretta: 897-902D (5 bp), 1284-1288D, 1589-1590D

Fulmarus: Exon 7D, 1276-1332D, Exon 9D

Geospiza+Taeniopygia: Exon 7D, Exon 8D, 1429-1432I, 1596D

Buceros: Exon 7D, 1433-1442D, Exon 10D

Nipponia: 1290I, 1329D, 1448-1462D (13 bp)

Acanthista: 1320D, 1451-1455D, 1471-1472I, 1550D, 1591-1592D

Cathartes: 1388-1392D, Exon 9D

Haliaeetus: 1433-1437D

Table S11.Mammal AMTN inactivating mutations. I=Insertion; D=deletion

Dasypus: 637D

Orycteropus: 504D

Choloepus: Exon 2D

Physeter: None

Manis: Exon 1-7D, 498D, 508I

Table S12.

Mammal AMBN inactivating mutations. I=Insertion; D=deletion

Dasypus: None

Orycteropus: Exon 6D, 1239-1251D, 387-389 stop codon

Choloepus: Splice site mutation: AG to AT at intron 2 acceptor site

Physeter: None

Manis: 1053-1066D, 1217D, 1362-1365I

Table S13.Mammal *ENAM* inactivating mutations. I=Insertion; D=deletion

Dasypus: 3863I, 3916D, 3938D

Orycteropus: 805-981I, 1111D, 3144I, 3547-3550D

Choloepus: 1684D, 1708-1709D, 1760D, 2022D, 2486D, 2548-2549I, 2637-2638

Physeter: 1904-1905D

Manis: 1499I; 1657D; 1657D; 1712-1718D; 1744-1745D; 1852D; 2595D; 3183-3192I; 3328D; 3511-3512I; 3840D

Table S14.

Mammal *AMELX* inactivating mutations. I=Insertion; D=deletion

Exon 4 Only functional in *Homo*.

Dasypus: 887-903D

Orycteropus: 602-604 Stop codon

Choloepus: 106-1116D, 822D

Physeter: None

Manis: 810-813D

Table S15.

Mammal DSPP inactivating mutations. I=Insertion; D=deletion

Dasypus: 2161-2163 possible premature stop codons (Truncates full length CDS) but trace archives are of poor quality

Orycteropus: None

Choloepus: None; Exon 3 is near the end of the read and is most likely sequencing error

Physeter: None

Table S16.Mammal *MMP20* inactivating mutations. I=Insertion; D=deletion

Dasypus: None

Orycteropus: 9111

Choloepus: 14-28 (Start codon deletion), 302-836I, 1078D, 1169D, 1295-1301I, 1418D, 1830-1831D

Physeter: Functional

Manis: 1189D, 1270-1274D, 1475I, 1505-1508I, 1634-1641D, 1661D, 1723D,

Table S17.Turtle AMTN inactivating mutations. I=Insertion; D=deletion

Chelonia: 96-133D, 376-377D

Chrysemys: Exon 4D, 346-356D, 464I

Chelonia+Chrysemys: 41-90D (includes start codon), Exons5-6, Exon 9-10D

Pelodiscus: 460D

Table S18.

Turtle AMBN inactivating mutations. I=Insertion; D=deletion

Chelonia: Exon 3D, 3141-3147D

Chrysemys: Exon 5D, 462-466D, 469-475D, 593-606D, 648-669I, 825-835D, 852-855D, 1048-1067I, 1111-1112I, 2816-2922I, 2972-2984I, 2999I, 3018-3064I, 3084-3096I, 3148-3222I, 3257-3264I

Chelonia+Chrysemys: Exon 4D, 522-528D, 542D, 552-553D, 621D

Pelodiscus: All exons excised.
Table S19.

Turtle *ENAM* inactivating mutations. I=Insertion; D=deletion

Chelonia: 42-54D (Deleted start codon), 698-701D, 2310-2311D, 2554-2674I, 4021D, 5641-5680I, 6910D, 7073-7098I, 7591-7592I

Chrysemys: 1859-1865D, 1963-1964D, 2046-2059D, 2196-2206D, 2284D, 2340-2350D, 2415-2427D, 2554-2714I, 3819-3831D, 4104D, 7083-7098I, 7189D, 7197D, 7312-7633D

Chelonia+*Chrysemys*: Exons5-8D, 2159D, 5464-5467I, 5534-5543I, 5717-5808I, 7239-7257D

Pelodiscus: All exons excised.

Table S20.

Turtle *AMELX* inactivating mutations. I=Insertion; D=deletion

Chelonia: 142D

Chrysemys: 96D

Chelonia+Chrysemys: Mutated start codon (ATC), Exons 3-4D

Pelodiscus: 113-116D, 189D, 262-265I, 283D, 367-420D, 452-559D, 682D

Chelonia+Chrysemys+Pelodiscus: 157-158D, 166I

Table S21.Turtle DSPP inactivating mutations. I=Insertion; D=deletion

Chelonia: Exon1D, 242-252I, 418-422D, 2282-2444D

Chrysemys: Exon1D, 2054-2933D (includes stop codon)

Chelonia+*Chrysemys*: 405-408D, 439-455D, 489-645D, 655-748D, 781-997D, 1008-1406D, 1731-1735D, 1881-2000D

Pelodiscus: All exons excised.

Table S22.Turtle *MMP20* inactivating mutations. I=Insertion; D=deletion

Chelonia: 271-275I, 1202-1205D

Chrysemys: Exon6D, 1105-1106D

Chelonia+*Chrysemys*: Exon1D, 197I, Exon4D, 737D, 759D, 1217D, 1227-1242D, 1289D

Pelodiscus: 309-310I, 330I, 523-530D

Chelonia+Chrysemys+Pelodiscus (possible): 291-304I, 357-358D, 389D

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