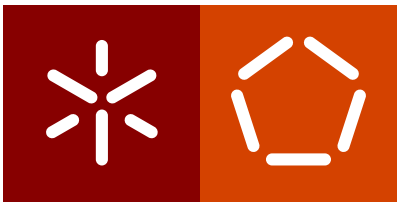

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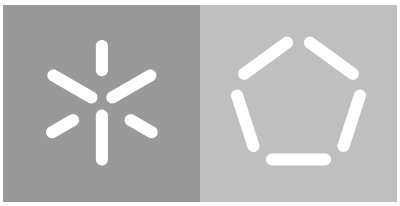

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Avian genomics: Insight into bitter taste receptors

Master dissertation
Master Degree in Bioinformatics

Dissertation supervised by
Prof. Dr. Agostinho Antunes
Prof. Dr. Miguel Rocha

January 2021

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

ABSTRACT

The detection of bitter taste is of major importance for animal survival since it provides an earlier evaluation of which food resources are safer, avoiding the ingestion of toxic compounds and regulating the feeding behavior. The taste receptor protein type 2 (T2R) family of G protein-coupled receptors (GPCRs) is responsible for bitter taste perception and its study is relevant to better understand the evolution of the sense of taste. Additionally, birds are a group of animals which are considered good models to evolutionary studies due to their abundance, high diversity of species and global widespread across varied ecological conditions.

Phylogenetic reconstructions and selection analysis present a great approach to understand the evolutionary history and diversification of avian T2Rs. Additionally, comparative methodologies can assess the selective pressures acting on these genes.

This work aims to assess the evolutionary genomics of the animal taste receptor gene type 2 (Tas2r) gene family in 245 bird species, distributed across 14 orders and, through a set of bioinformatics and genomic tools, to clarify their genomic representation, selective pressures and phylogenetic relationships. The results herein obtained reveal an acceleration of Tas2rs in the order Passeriformes. In addition, it was previously reported that diet has an influence on the Tas2r repertoire. Therefore, we studied the effect of additional ecological traits such as habitat and migration. Our results indicate that Tas2r show conservation on water birds and a stronger evolutionary pressure on non-migratory birds.

Keywords: T2R, Tas2r, Avians, Positive selection

RESUMO

A deteção de sabor amargo é muito importante para a sobrevivência animal uma vez que permite avaliar que fontes de alimento são seguras consumir, prevenindo assim a ingestão de xenobióticos. Para além disso, estes receptores também regulam o comportamento alimentar dos animais. Os recetores de sabor tipo 2 (T2R), uma família de receptores acoplados às proteínas G (GPCRs), são responsáveis pela deteção de sabor amargo e o seu estudo é relevante para clarificar a evolução do sentido do paladar. Adicionalmente, as aves são um grupo de animais considerados como sendo bons modelos de evolução devido à sua abundância, grande diversidade de espécies e distribuição global em diferentes condições ecológicas.

As reconstruções filogenéticas e análises de seleção, apresentam uma abordagem interessante para entender a história evolutiva e a diversificação de T2Rs em aves. Adicionalmente, metodologias comparativas podem avaliar as pressões seletivas que atuam nestes genes.

Este estudo tem o objetivo de analisar a genómica evolutiva da família de genes dos receptores de sabor tipo 2 de animais (Tas2r) em 245 espécies de aves em 14 ordens. Atráves de um conjuto de ferramentas bioinformáticas e genómicas, pretende-se também esclarecer a sua representação genómica, pressões seletivas e relações filogenéticas. Os resultados obtidos revelam uma aceleração da pressão seletiva na ordem Passeriformes. Para além disso, foi anteriormente reportado que a dieta influencia o repertório de T2R. Assim, analisou-se o efeito de traços ecológicos adicionais como migração e habitat. Os nossos resultados indicam que Tas2r apresenta conservação em aves aquáticas e uma maior pressão evolutiva em aves não migratórias.

Palavras-Chave: T2R, Tas2r, Aves, Seleção positiva

CONTENTS

ABBREVIATIONS

ω nonsynonymous/synonymous rate ratio. 26

aBSREL adaptive Branch-Site Random Effects Likelihood. 21
AIC Akaike information criteria. 20, 25, 26, 29

BEB Bayes empirical Bayes. 27
BI Bayesian inference. 21, 26, 30
BIC Bayesian information criteria. 20
BLAST Basic Local Alignment Search Tool. 2, 18, 24, 29
BUSTED Branch-site Unrestricted Statistical Test for Episodic Diversification. 22

cAMP cyclic adenosine monophosphate. 7
cGMP cyclic guanosine monophosphate. 7
cNMP cyclic nucleotide. 7
CR1 chicken repeat 1. 14

DAG diacylglycerol. 7
dLRT dynamical likelihood ratio tests. 20
DT decision theory method. 20
DXM dextromethorphan. 11

FA fatty acids. 15, 16
FEL Fixed Effects Likelihood. 21, 22, 27, 44, 46–49, 51, 52, 106, 109, 112, 118, 124, 128
FUBAR Fast Unconstrained Bayesian AppRoximation. 21, 22, 27, 44, 46–49, 51, 52, 106, 109, 112, 118, 124, 128

GARD Genetic Algorithm for Recombination Detection. 22
GPCR G protein-coupled receptor. v, vi, 1–6, 56
GRAFS Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, Secretin. 3
GTR General Time Reversible Model. 25

HKY Hasegawa, Kishino, and Yano Model. 25
hLRT hierarchical likelihood ratio tests. 20
HoT heads-or-tails. 19

IP$_3$ inositol 1,4,5-triphosphate. 7
ISS index score. 25, 29
ISS.C index critical score. 25, 29

LINE long interspersed elements. 14
LRT likelihood ratio tests. 20, 27, 28

MCMC Markov chain Monte Carlo. 21, 26
MEGA Molecular Evolutionary Genetics Analysis. 2, 18, 19, 25, 32
MEME Mixed Effects Model of Evolution. 21, 22, 27, 44, 46–49, 51, 52, 106, 109, 112, 118, 124, 128
ML maximum likelihood. 20–22, 25, 26, 30, 32–36, 38–43
MOE main olfactory epithelium. 4
MSA multiple sequence analysis. 18–22, 25, 29, 30

NCBI National Center for Biotechnology Information. 2, 18, 24, 29
NSS negative selected sites. 27, 44

OR olfactory receptors. 4, 6, 12
OTUs operational taxonomic units. 19, 25

PAML Phylogenetic Analysis by Maximum Likelihood. 2, 22, 27, 44, 46–49, 51, 52, 103, 108, 111, 114, 122, 126
PDE phosphodiesterase. 7
PIP2 phosphatidylinositol 4,5-bisphosphate. 7
PLCβ2 phospholipase C β2. 7
PROP 6-n-propyl-2-thiouracil. 8, 9
PSS positive selected sites. 27, 44, 46–49, 51, 52, 54–57
PTC phenylthiocarbamide. 9

RT-PCR reverse transcription-polymerase chain reaction. 10

SCCs solitary chemosensory cells. 10
SINEs short interspersed elements. 14
SLAC Single-Likelihood Ancestor Counting. 21, 22, 27, 44, 46–49, 51, 52, 106, 109, 112, 118, 124, 128

T1R taste receptor protein type 1. 3, 5, 6
T2R taste receptor protein type 2. v, vi, 3, 5–8, 10–12, 44, 56
TAS2R human taste receptor gene type 2. 6, 8, 10, 11
Tas2r animal taste receptor gene type 2. v, vi, viii, xii–xiv, 1, 2, 6–14, 16, 17, 23, 24, 28–31, 37, 44, 46–49, 51–54, 56, 103, 106, 108, 109, 111, 112, 114, 118, 122, 124, 126, 128
TEs transposable elements. 14
TM transmembrane. 3
TRC taste receptor cells. 4–7
TrC two-ratio constrained model. 28
TRPM5 transient receptor potential cation channel subfamily M member 5. 7, 14
TrU two-ratio unconstrained model. 28

V1R vomeronasal receptors type-1. 4–6
V2R vomeronasal receptors type-2. 4, 5
VNO vomeronasal organ. 4
VRs vomeronasal receptors. 4

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1

INTRODUCTION

1.1 Motivation

Animal genomic studies are increasing as the number of sequenced genomes grows. Comparative genomic studies are a good tool to analyse genomic data because they allow inference on differential gene variation and evolution. Without comparative genomics, it would be very hard to find consistent patterns in a large quantity of data from different species[1]. Birds are good models for evolutionary and ecological investigation since they are very diverse, widely distributed and the most species-rich class of tetrapods[2]. To detect and collect the diversity of environmental signals, birds use several senses such as sight, sound, smell, touch and taste[3]. Elucidation of the taste sense of avians is important, not only to help clarify the evolution of taste sense in birds, but also in animals in general[4]. Additionally, bitter taste may signal the presences of toxins, which suggests that bitter signalling is a key defense mechanism against the ingestion of toxic xenobiotics[5]. During the last years, the field of taste research has experienced rapid progress, especially regarding GPCR-mediated taste qualities umami, sweet and bitter[6–8]. Moreover, a large quantity of hypotheses inside GPCR chemosensory perception are still to be tested and we believe that bitter taste is a good candidate to clarify how environmental changes shape the chemosensory receptor gene repertoire.

1.2 Goals

The aim of this work is to clarify the Tas2r repertoire in 245 bird species from 14 avian orders. The proposed species genomes we explored in this project belong to a private consortium, many of them sequenced for the first time. For this reason, all the resulting findings and conclusions will be a novelty and reported by the first time.

In our study, we propose to analyze the relationship between bird migration and habitat patterns with the Tas2r repertoire. We examined species with different migratory patterns, from migrators, non-migratory birds to birds with partial migration. We also studied birds with diverse habitats: water birds, land birds and birds from intermediate regions. Additionally, we analyzed the patterns of Tas2r regarding different bird orders. The full species list, regarding their migration habits and habitats, can be consulted in Table S1. By doing a screening and analysing full-length Tas2rs from the aforementioned 245 bird species, we aim to find patterns that clarify the evolution of Tas2r. These analyses include phylogenetic reconstructions, codon level and branch level selection analysis.

All the obtained results were compared with available literature and databases in order to enforce the value of this work inside avian Tas2r comparative studies. The final goals are to write a thesis and publish a paper with the results obtained from the mentioned analysis. We hope this work provides novel insight into the evolutionary history of Tas2r in avian species.

1.3 Structure

The document is organized into five chapters: "Introduction", "State of the Art", "Computational Methods and Tools", "Results and Discussion" and "Conclusions and Further Work". There is an additional chapter named "Supplemental Material".

The initial chapter Introduction (1) is composed of both the motivation and the goals driving the study. In the subsequent chapter State of the Art (2), we briefly revise the state of the art. This comprises GPCRs in general and bitter taste receptors in particular. We also analyze previous work about the relation between bitter sense and diet, as also broad patterns of genome evolution. It is also constituted by the environmental aspects that we intend to study in relation to the Tas2r gene family.

The chapter Computational Methods and Tools (3) addresses the bioinformatics databases and tools used during this work (National Center for Biotechnology Information (NCBI), Basic Local Alignment Search Tool (BLAST), SeaView, Molecular Evolutionary Genetics Analysis (MEGA), DAMBE, GUIDANCE2, jModelTest 2, IQ-TREE, MrBayes, Datamonkey, PAML, Python, FigTree, Image Generator and Editor Tools).

In the "Results and Discussion" chapter (4) we describe the results of the phylogenetic and selective pressure analysis, not only regarding the different bird orders, but also considering habitat and migratory preferences. In this chapter, we also provide hypothetical explanations and present related studies which help to confirm the interpretation of our results.

The chapter "Conclusions and Further Work" (5) provides a summary of the main conclusions achieved through this work and suggests further steps that could be taken in the future to have a better understanding of the results herein obtained.

Lastly, in "Supplemental Material" we present additional information that enhance or support this thesis.

2

STATE OF THE ART

2.1 GPCRs and genome evolution

2.1.1 GPCRs

To survive in the external environment, animals depend on their senses. Vertebrates have five traditional senses including sight, sound, smell, touch and taste. These senses alert animals to adapt to external stimuli or even trigger responses accordingly[3].

Membrane receptors play an important physiological role by mediating communication between the cell and its environment. The largest group of membrane receptors are constituted by receptors of the GPCRs family. Structurally, GPCRs are characterized by an extracellular N-terminus, 7 transmembrane (TM) α-helices connected by three intracellular (IL-1, IL-2 and IL-3) and three extracellular loops (EL-1, EL-2 and EL-3), and an intracellular C-terminus. GPCRs respond to diversified extracellular stimuli such as neurotransmitters, ions, photons, hormones and tastants by signalling through heterotrimeric G-proteins[9].

Several classification systems have been proposed for the GPCRs super family. One of the classifications groups GPCRs into six classes (clans): A, B, C, D, E and F. In this classification, taste receptor protein type 1 (T1R) belong to class C (metabotropic glutamate/pheromone) and T2Rs are either a separate family or related to class A (rhodopsinlike)[10;11]. A more recent classification, called Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, Secretin (GRAFS), places T1Rs and T2Rs in distinct families: T1Rs in the glutamate family and T2Rs in a cluster within frizzled/taste2 family[12]. The frizzled receptors that form the second cluster are responsible for development and cell proliferation[10].

GPCRs comprise various physiological roles. One of these roles includes the visual sense, in which photoreceptors respond to visual stimuli through rods and cones[13]. Opsins are photoreceptive compounds which change their conformation from a resting state to a signalling state in response to light. This initiates a signalling cascade that culminates in physiological changes within the cell[14]. Further studies in rhodopsins revealed that opsins are constituted by a chromophore (a vitamin A-based retinaldehyde) and the opsin protein (a seven-transmembrane helical structure)[14;15].

In addition, GPCRs are involved in the sense of smell. Olfactory sensory perception is one of the most studied chemosensory systems[13] and is constituted by the main olfactory system and the vomeronasal system[16]. The sensory perception of these systems is mediated by two anatomically

and functionally different organs: the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), respectively[16]. In mammals, the odorants are detected by olfactory receptor neurons of the MOE[13;16]. The olfactory receptors (OR) family has a high number of pseudogenes and lacks introns in coding regions[16;17]. Studies in mammals found that each olfactory neuron expresses only 1 of up 1000 different olfactory receptors and a glomerulli has approximately 3000 projections from a set of neurons that express the same receptor[18]. Also, a single olfactory receptor detects multiple odorants and a single odorant is detected by multiple receptors[19]. Thus, the variety of odorants and odor concentrations in the environment elicit a coding combination of glomerulli[20]. In turn, vomeronasal receptors (VRs) have pheromone detection as their main role[16]. VRs are mainly expressed in the VNO and are divided in two superfamilies, type-1 vomeronasal receptors type-1 (V1R) and type-2 vomeronasal receptors type-2 (V2R), with different gene structure and expression location. V1Rs have a short N-terminal domain, lack introns in the coding region and the protein is encoded in one exon. V2Rs however, have a long N-terminal domain and the protein is encoded in six exons[16;21]. Until this date, a vomeronasal system was not found in birds[22].

GPCRs also has a role in mediating taste perception through taste receptors, which will be discussed in more detail further ahead.

2.1.2 Taste Perception

Animals need to ingest nutrients in order to survive, therefore the sense of taste is a powerful system that allows them to evaluate food resources. Taste can help animals decide if food is either beneficial and can be consumed or harmful and should not be ingested[10]. This way, animals ensure the ingestion of nutrients rather than poisonous substances based on the response that taste provokes. Besides evaluating food resources, taste also regulates the feeding behavior[3].

Biologically, taste is defined by the sensations mediated by the chemosensory gustatory system. The gustatory system includes taste receptor cellss (TRCs), which are sensory cells that enable taste perception. TRCs are distributed throughout the oral cavity. On the tongue, the main taste organ, TRCs are organized in taste buds. Taste buds are located within gustatory papillae, that belong to three types - foliate, fungiform and vallate - and are non-uniformly distributed on the tongue surface. There are also non linguae taste papillae in the epiglottis, oropharynx, larynx, upper esophagus and palate. Apical ends of TRCs interact with tastants present in the oral cavity. This interaction initiates an afferent signal transmitted to the brain by cranial nerves, resulting in taste perception[10;23].

Vertebrates usually have five taste modalities: bitter, sweet, salty, sour and umami[24]. Salty taste is mainly stimulated by sodium salts thus suggesting that salty taste signals the presence of sodium. Sodium is important for the maintenance of the osmotic balance of the body. From low to moderate concentration there is an appetitive stimulus whereas high concentrations become aversive[25]. Sour has an innate aversive response and signals the presence of acid[25]. The most common sweet stimulus is sugar, thereby indicating that there are carbohydrates in the food. L-glutamate is the most common umami taste stimulus, which can signal the presence of protein. Sour and bitter tastes can indicate that the food is spoiled[10;26;27]. Also, bitter taste may signal the presences of toxins in food

and animals have an aversive response to this tastant. This suggests that bitter signalling is a key defense mechanism against the ingestion of toxic xenobiotics. Many food compounds are perceived as bitter, such as esters and lactones, amino acids and peptides, flavonoids and terpenes, phenols and polyphenols, sulfimides (saccharin), methylxanthines (caffeine), and organic and inorganic salts[5]. These compounds have very distinct chemical structures that require molecular recognition to initiate taste perception[28].

Taste receptors are believed to be a part of the TRC membranes because most taste ligands are organic proteins that do not permeate cell membranes easily[29]. Bitter, sweet, umami are normally organic proteins and are thought to be detected trough GPCRs encoded by either the type 1 or 2 family of taste receptor genes, which are expressed on the surface of receptor cells[28;29]. However, some ligands such as sour and salty compounds, are typically ionic and smaller. Thus, they are able to penetrate cell membranes through ion channels and interact with intracellular targets to activate TRC. Thereby, it is unclear what is considered a taste receptor for such ligands[29].

According to Bachmanov et al.[10], a molecule functions as a taste receptor when:

- Has expression in TRCs;
- Possesses appropriate ligands;
- Demonstrates changes in taste function in result of changes in the taste receptor;
- Has an established molecular identity.

The T1R family

The T1R family is constituted by 3 genes. In 1999, T1R1 and T1R2 were identified for the first time[30]. These 2 receptors are part of the family of class C GPCRs and are distantly related to V2R pheromone receptors[6]. The third receptor of the T1R family, T1R3, was identified in the human genome in 2001[7]. Adler et. al[11] showed that T1Rs do not coexpress with T2Rs and Nelson et. al[7] showed that both the T1Rs subunits heteromers T1R1 and T1R2 coexpress with T1R3 but not with each other. Through *in situ* hybridization experiments, it was shown that T1Rs are expressed in ~30% of taste receptor cells[30]. These receptors that belong to the GPCRs family have a large N-terminal domain and ~850 amino acids[30].

Functional expression and mouse gene knockout studies confirmed that the T1R family of taste receptors mediate sweet and umami taste, combining activate different heterodimers of the T1R family: T1R2/T1R3 for sweet and T1R1/T1R3 for umami taste. In addition, taste receptors for sweet and umami qualities are closely related, suggesting that they have the same evolutionary origin[6].

The T2R family

The T2Rs are a group of chemoreceptors that belong to the GPCR superfamily and mediate signal transduction when stimulated by bitter agonists[26]. These receptors are distantly related to opsins and V1R vomeronasal receptors[11]. The genes and pseudogenes of this family were numbered according to their order of discovery[11]. Chandrashekar et al.[26] was the first to identify T2Rs as bitter taste

receptors, two decades ago. Unlike T1Rs, which normally do not colocalize with α-gustducin[30], taste receptor cells expressing T2Rs are a subset of α-gustducin positive cells, which suggests they function as gustducin-linked receptors[11]. Like umami and sweet, the other GPCR-mediated taste qualities, bitter taste receptors use the G-protein α-subunit α-gustducin for signal transduction[31]. When T2Rs interact with their agonists in the oral cavity, these receptors are activated[23].

T2Rs are encoded by individual genes. Like many other GPCRs genes, these genes are monoexonic thus do not have introns in its genomic organization[11;32]. Besides, the T2R family has a high number of pseudogenes, reaching nearly 17% of the mouse Tas2r sequences and approximately 40% of human taste receptor gene type 2 (TAS2R) sequences in the human[33;34]. Opposed to T1Rs, T2Rs have a short N-terminal extracellular domain and full-length of almost ~300-330 amino acids[10;30].

Adler et. al[11] estimates that the number of T2Rs in humans is between 80 and 120. If to this number we deduct the pseudogenes, we have a final number of functional human receptors between 40 and 80. Interestingly, human ORs and V1Rs receptors also present a high number of pseudogenes[16]. Even though there is a high number of pseudogenes on TAS2R sequences, it is still unknown if they play a role in the taste gene repertoire. However, they are likely implicated in evolutionary mechanisms and represent a source of variability in the chemosensory receptor repertoire, which is related with different preferences[28].

In the genome, TAS2R genes and pseudogenes are organized in clusters, both in humans and other animals[11;32–34]. Genome information and cell-based assays suggest that chicken has three putative bitter taste receptors, which the nomenclature is not consensual[4;35]. Additional behavioral studies show that only two of these avian paralogs in chicken are functional[4;36]. In humans, all TAS2R sequences are located on chromosomes 5, 7 and 12, but they mainly form 2 clusters: 10 TAS2Rs sequences on chromosome 7 and 20 on chromosome 12[33]. In mouse, all Tas2r genes and pseudogenes locate on chromosome 6 with the exception of Tas2r19 andTas2r34 that locate on chromosomes 15 and 2, respectively. They also form two clusters, 1 of 10 Tas2r sequences and another of 29 Tas2r sequences[34]. These clusters present a conserved synteny, sustaining the hypothesis that the genetic organization of TAS2Rs was originated prior to the divergence between rodents and primates lineages[34].

Based on the expression pattern of T2Rs, we can infer how the nervous system codes taste information. Previous studies propose ways of T2Rs expression: on one hand, the co-expression of T2Rs on the same TRC suggests identical perception of different bitter tastants; on the other hand, different T2Rs are expressed on different TRCs, which might indicate discrimination of different bitter stimuli. It is also possible that these differences in taste perception are related with T2R levels of expression in different TRC[10]. Given the high number of natural and synthetic bitter compounds and the need to detect them without having an enormous number of taste receptors, it is only natural that the receptor proteins are very distinct among themselves and that they respond to more than one bitter compound[8;23].

Oral bitter taste perception

α-gustducin is a key element in bitter taste signal transduction but its function is complemented by the other G-protein α-subunits of taste receptor cells[23]. The functional heterotrimeric G-protein complex also needs β- and γ-subunits, which were identified in taste cells[37]. This was supported by observations that α-gustducin knockout mice have an extremely reduce response to bitter taste stimulation but not null[38]. Therefore, to transduce a bitter taste signal, normally is necessary to form a heterotrimeric G-protein complex constituted by α-gustducin, Gγ13, Gβ3 and possibly a minor fraction of complexes with Gβ1[23] (Figure 1). When T2R is stimulated, the G-protein heterodimer is activated. Then, phospholipase C β2 (PLCβ2) is induced, leading to an increase of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) through the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP2). In its turn, IP_3 releases calcium from the intracellular reticulum, activating the transient receptor potential cation channel subfamily M member 5 (TRPM5). TRPM5 was the last component of bitter taste transduction to be identified. The induction of TRPM5 results in a depolarization across the TRC membrane. When a certain action potential is achieved, the neurotransmitters are released and act on sensory nerves that innervate the taste buds, thereby communicating with the brain centres linked with taste perception. Moreover, phosphodiesterase (PDE) is activated by the α-gustducin subunit. Consequently, cellular concentrations of cyclic nucleotides (cNMPs), like cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), decrease. It is still unknown why cNMPs levels vary[23;29].

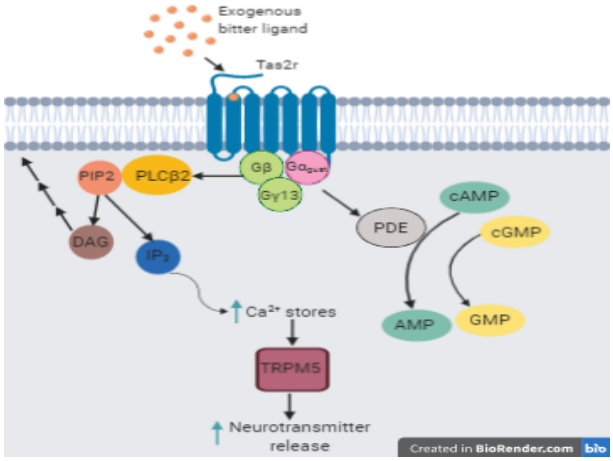


Figure 1: **Tas2r signalling in the oral cavity.** Detection of bitter ligands results in the activation of the G-protein complex and dissociation in Gα-gustducin, Gγ13 and Gβ3. G$\beta\gamma$ induces the cleavage of PIP2 and the activation of PLCβ2, which increases levels of DAG and IP_3. IP_3 induces the release of Ca^{2+} from internal stores followed by a release of neurotransmitters. The activation of Gα-gustducin induces a decrease in levels. Adapted from Martin et al.[8]. This image was created with BioRender[39].

Ligands

To elucidate the function of Tas2r, is important to identify the ligands that activate them. Despite the efforts, it is still unknown how such a small number of receptors is able to identify such a plethora

of bitter taste compounds[23]. However, studies have shown that different Tas2rs show different specificity and sensibility.

This difference in ligand specificity was explored in humans by Meyerhof et al.[40], in which some receptors are agonist specific or recognize few agonists, while others show extremely wide range of molecular receptors. For example, 3 TAS2Rs (TAS2R10, TAS2R14, and TAS2R46) were able to detect ~50% of the substances used. On the contrary, TAS2R3 is specific to chloroquine (Table 1)[40]. It is also important to note that quinine was the substance detected by the highest number of TAS2Rs, in a total of 9 out of 25 TAS2Rs[40].

Rodent bitter taste receptors appear to be selective, since only cycloheximide elicits a response in Tas2r5 and Tas2r8 is exclusively activated by denatonium and high concentrations of 6-n-propyl-2-thiouracil (PROP) (Table 1)[26].

Regarding avians, a study was performed using functional analysis of 3 Tas2rs paralogs of chicken (*Gallus gallus*), 2 Tas2rs paralogs of turkey (*Meleagris gallopavo*) and 3 Tas2rs paralogs of seven zebra finch (*Taeniopygia guttata*) Tas2rs[41]. Whereas chicken and turkey taste receptors recognized numerous substances, the three zebra finch Tas2rs, had reduced responses to agonists (Table 1)[41]. In addition, different species display different recognition of bitter compounds. Similar to what was observed in chicken, ginkgolide A, a substance that was not recognized by any of the human TAS2Rs[40], was found to be an agonist for one turkey bitter taste receptor (turkey Tas2r3) (Table 1)[41]. It is also interesting to note that some Tas2rs show ambiguity since both turkey receptors recognized 4 compounds (quinine sulfate, diphenidol, chloramphenicol and parthenolide).

Not only Tas2rs show differences in specificity, but they also have a wide range of activation threshold, thereby confirming different ligand sensitivity. For instance, TAS2R43 is activated by aristocholic acid at just 1.3 nM, yet sodium cyclamate needs to be at a concentration of 30 mM to activate TAS2R1[40].

Moreover, even animals from the same species can experience different ligand sensibility. When stimulating mice with cycloheximide, α-gustducin was activated by the cycloheximide receptor Tas2r5 in some mice but not in others. This can be explained by the fact that Tas2r5 in mice has different alleles for taster and non-taster strains. Thus, the sensibility of the mouse Tas2r5 receptor varies according to these amino acid changes[26].

Additionally, it was detected orthology of T2R. When assaying 11 human TAS2Rs, TAS2R4 was found to be ~70% identical in sequence to mouse Tas2r8[26]. This was also verified in avians: the turkey Tas2r3 and Tas2r4 are orthologues of the chicken Tas2r7 and Tas2r2, respectively[41]. However, the turkey receptor that corresponds to chicken Tas2r1 is pseudogenized, which further indicates that chicken Tas2r1 might have a more species-specific function. Perhaps due to the phylogenetic proximity between chicken and turkey, the two pairs of orthologues receptors display a similar set of agonists considering that turkey Tas2r4 and chicken Tas2r2 share 7 of agonists, while turkey Tas2r3 and chicken Tas2r7 share 13. Moreover, the chicken receptor Tas2r1 shares a common ancestral node with human TAS2R39 and TAS2R40 and these receptors detect similar agonists. Chicken Tas2r2 belongs to a different ancestral node but, of its eight identified agonists, shares 3 with TAS2R39 and 2 with TAS2R40. These results suggest that phylogenetically close and even more distantly related

receptors possess an overlapping agonist spectra, thereby suggesting functional conservation regarding to tuning range and agonist recognition [41].

Nowadays, over 80% of human TAS2Rs have been deorphaned, i.e., each human TAS2R has, at least, a corresponding bitter ligand [40;42]. However, only 6% of the mouse Tas2rs have been deorphaned [26] and the numbers are even lower in regard to avians [41]. It is necessary to make continuous efforts to deorphanize avian Tas2r in order to clarify bitter taste receptors selectivity, specificity and ambiguity. It is specially interesting to explore how avians, in comparison to other animals, generally possess only few Tas2r, while being able to detect a wide variety of bitter compounds. Additionally, the phylogenetic relationship between avian Tas2rs and the overlapping agonist recognition is still only beginning to be explored.

Table 1: Identified bitter compound/Tas2r combinations

Receptor	Identified agonists	References
TAS2R1	Amarogentin, arborescin, cascarillin, chloramphenicol, humulone isomeres, parthenolide, picrotoxinin, thiamine and yohimbine	[40]
TAS2R3	Chloroquine	[40]
TAS2R10	Amarogentin, arborescin, cascarillin, chloramphenicol, humulone isomeres, parthenolide, picrotoxinin, thiamine and yohimbine	[40]
TAS2R14	Absinthin, arborescin, arglabin, aristolochic acid, artemorin, campher, caffeine, cascarillin, coumarin, cucurbitacin B, falcarindiol, humulone isomeres, noscapine, papaverine, parthenolide, picrotoxinin, quassin, quinine, and (-)-α-thujone	[40]
TAS2R43	Aloin, arborescin, arglabin, aristolochic acid, caffeine, chloramphenicol, falcarindiol, grossheimin, helicin and quinine	[40]
TAS2R46	Absinthin, amarogentin, andrographolide, arborescin, arglabin, artemorin, brucine, caffeine, cascarillin, chloramphenicol, cnicin, colchicine, crispolide, grossheimin, parthenolide, picrotoxinin, quassin, quinine, strychnine, tatridin B and yohimbine	[40]
Mouse Tas2r5	Cycloheximide	[26]
Mouse Tas2r8	Denatonium and PROP	[26]
Chicken Tas2r1	Alkaloid nicotine, azathioprine, chloroquine, chlorpheniramine, coumarin, diphedrynamine, diphenidol, picrotoxinin and quinine sulphate	[41]
Chicken Tas2r2	Caffeine, chlorampenicol, coumarin, diphenidol, parthenolide, quinine sulphate and yohimbine	[41]
Chicken Tas2r7	Absinthin, amarogentin, andrographolide, carisoprodol, chlorampenicol, chlorpheniramine, colchicine, cycloheximide, diphenidol, diterpene ginkgolide A, erythromycin, parthenolide, picrotoxinin, quassin, quinine sulphate, (-)-α-thujone and yohimbine	[41]
Turkey Tas2r3	Amarogentin, andrographolide, carisprodol, chlorampenicol, colchicine, diphenidol, diterpene ginkgolide A, erythromycin, limonin, parthenolide, picrotoxinin, quinine sulphate, saccharine, (-)-α-thujone and yohimbine	[41]

Turkey Tas2r4	Azathioprine, caffeine, chlorampenicol, chlorpheniramine, coumarin, diphenidol, parthenolide and quinine sulphate	(41)
Zebra finch Tas2r5	Chlorampenicol, chloroquine, denatonium benzoate, diphenidol and phenylthiocarbamide (PTC)	(41)
Zebra finch Tas2r6	Andrographolide, camphor and diphenidol	(41)
Zebra finch Tas2r7	Andrographolide, camphor, chlorpheniramine, cycloheximide and denatonium benzoate	(41)

T2Rs Beyond the Oral Cavity

Functional bitter taste receptors have been found on several extra-oral tissues such as the gut[43], the pancreas[44], the airways[45], the adipose tissue[46], the nasal respiratory epithelium[13], and even more surprisingly, the testis[47]. T2Rs have also been found in the brain, the vasculature, the heart, the kidney, the thymus, the thyroid, the immune system, bone marrow stromal cells, skin keratinocytes and breast epithelium[29].

Since T2Rs have a protective role on the tongue, as toxicity detectors, it has been hypothesized that they have a similar function on organs that interact with the external environment. In external such as airways and urinary tract, T2Rs appear to, not only inhibit the uptake of toxic compounds, but also remove them out of the body[29]. In the nasal epithelium, two mouse bitter taste receptors, Tas2r8 and Tas2r19 were found to be expressed in putative solitary chemosensory cells (SCCs). These cells work as sentinels and when stimulated by bitter tastant compounds like denatonium, they trigger the terminal nerve. This results in protective reflexes such as sneezing, apnea, slowed breathing rate or coughing. It was reported that TAS2R47 expressed in nasal solitary SCCs, upon stimulation by denatonium benzoate, triggered a "calcium wave" resulting in antimicrobial peptides release, which prevents increased bacterial invasion[45]. The response obtained in mice and humans might indicate that, in the nasal cavity, are not perceived as tastes but rather as irritants[13]. In addition, microarrays and reverse transcription-polymerase chain reaction (RT-PCR) revealed expression of members of the T2R family in the motile cilia of human airways epithelial cells. When these receptors detected bitter compounds, the ciliary beat frequency increased. This further suggests that taste receptors potentially provides a defensive response to eliminate inhaled harmful compounds[48].

Besides the role of T2Rs in innate airway immunity, they are also involved in airway smooth muscle contraction. It was observed that bitter taste receptors on airway smooth muscle are functional and able to induce bronchial relaxation. Thus, T2Rs seem to regulate the immune activation, posing as therapeutic targets for the treatment of allergy in general and allergic asthma in specific, given their effects on bronchorelaxation, that surpassed the therapeutic efficiency of β_2-agonists, the general asthma treatment[49]. Perhaps in the future, a combination therapy of bitter taste receptor and β_2-agonists could be developed[8;49].

Tas2rs have also been found in internal organs such as the thyroid. Even though these receptors influence the production of thyroid hormone, it is still unclear which function it may serve[29]. In addition, mouse Tas2rs were found to be expressed in cholinergic cells of the thymic medulla, thereby

proposing a regulatory role in the maturation of T-cells[50]. Bitter taste receptors have also been found in gut endocrine cells and present agonist-mediated contractility[29].

In a study by Avau et al.[51], the influx of extracellular calcium and bitter tastant denatonium benzoate led to the release of intracellular calcium, resulting in contractions in human gastric smooth muscle, which was similar to what was previously observed in mice. In addition, the intra-gastric administration of denatonium induced gastric emptying delay. Bitter tastant compounds also had effects on healthy human volunteers, since the intra-gastric denatonium administration resulted in a lower tolerance of nutrient volume and increased hunger satiation. This further suggests that TAS2Rs participate in a protective negative feedback loop in the gut, in which ingestion of bitter and potentially harmful compounds results in a decrease in food intake[51]. In regard to avians, it was reported that chicken bitter taste receptors mRNAs were observed in the gastrointestinal tract, suggesting the involvement of taste pathways for sensing bitter compounds in these extra-gustatory tissues[52].

Maybe surprisingly, Tas2rs are strongly expressed in testis, which were one of the first organs where these receptors were found[32]. Tas2rs are also expressed in sperm since an immunocytochemistry and immunogold electron microscopy study detected high levels of α-gustducin in both differentiating spermatids and mature spermatozoa of different mammals. Even though the function of α-gustducin was not identified, it was suggested that Tas2r may have a role in chemotaxis and sperm motility[47].

TAS2Rs were also found to be expressed in two types of cancer tissues, pancreatic cancer[53] and breast cancer[54] with differential expression. Functional studies after the application of natural and synthetic bitter agonists such as quinine, dextromethorphan (DXM) and phenylthiocarbamide showed an increase in intracellular calcium mobilization, thereby implying that the endogenous TAS2Rs are functional in breast cancer cells. It should be noted that only 5 well studied TAS2Rs were used in this study (TAS2R1, TAS2R10, TAS2R4 and TAS2R38)[54]. In another study, TAS2R38 was found on the surface of lipid droplets of pancreatic cancer.

Moreover, bitter tastants like isoflavones, flavonoids, phenols and glucosinates display anti-carcinogenic and antioxidant effects and tumour-blocking properties[55;56]. In addition, vegetables and fruits rich diets, which normally richer include a bigger portion of bitter tastants, are believed to give protection against cancer[56;57]. These findings suggest that, in the future, T2Rs may be used as targets for new cancer therapies[8]. However, until this date, is still not possible to link TAS2Rs with either in inhibition or promotion of breast cancer growth or resistance to metastasis[54].

In conclusion, given the role of T2R in mediating the communication between the cell and the environment and their recently discovered functions, taste receptors are emerging as potential drug targets[54]. Therefore, a better further understanding of the physiological roles of these, possibly pluri-functional receptors, presents itself as an interesting study case.

Variation of T2R

Tas2rs display various types of variation. As seen before, different bitter tastants show different sensitivity and specificity to ligands and even within a species, some individuals can have different taste perceptions because of genetic variation of Tas2rs.

Structurally, Tas2rs also present variation: while the intracytoplasmatic loops and the transmembrane domains have highly conserved sequence motifs, the extracellular segments are the most divergent regions. The intracytoplasmic loops are the predicted sites of G-protein interaction and the extracellular regions are the predicted regions of ligand binding, which relates to the necessity of T2Rs recognize structurally different ligands. It is also interesting to note that each T2R family member has 30-70% amino acid identity between them[11;58].

Additionally, when comparing species it is possible to observe that Tas2rs vary in number of functional proteins and pseudogenes[23]. For example, a study by Li et al.[59] surveying various species revealed that the total number of Tas2r genes varies from 3 in chicken to 69 in the guinea pig. The proportion of Tas2r pseudogenes vary from 0% in the chicken to 100% in the dolphin. Another study[60] notes that except for chicken and platypus, which only encode three and four intact genes, respectively, all the other studied tetrapod genomes include a minimum of 15 intact Tas2r genes. Interspecies variance is likely linked to different species' feeding habits, which have different exposure to toxins[60]. Thus, the small number of platypus' Tas2r genes might be due to its semiaquatic diet but chicken has no apparent dietary explanation[61]. Like most birds, chickens only have a few taste buds and taste receptor genes, therefore they were believed to possess a less complex taste system[62]. However, they have a high sensitivity to different tastes and a well-developed taste system[63]. Thereby it was hypothesized that other genes on chicken's genome have obtained the ability to work as Tas2r[64]. This hypothesis is contested by Davis et al.[60], who believes that a low number of genes in the chicken genome is not related to acquired bitter taste perception by other genes, but instead linked with evolutionary expansion and contraction of this gene family. Combined, the lack of pseudogenes and the low number of Tas2r may indicate that a genome size reduction in avian lineage might be the cause. This would result in a lack of expansion of this gene family because of narrow gene expansion capacity[35]. It was noted that the avian Tas2r repertoire had an ancestral genome size reduction and the size may have been maintained until the Galliformes lineage, but experienced an expansion in the Passerine lineage[60].

In mammalian species, the evolutionary history is somewhat different: some species are almost duplication free whereas others possess more T2Rs because of species-specific duplication[65]. Some studies suggest that the primate and rodent lineages divergences were prior to the local gene duplication events that originated some of the species-specific groups of genes[34].

It is speculated that a "birth-and-death" model shapes the T2R gene family[66]. A similar model is observed in the OR family which is also comparable in sequence diversity and evolutionary history[67]. During the generation of new genes by mutation and duplication, an intermediary state may be reached, thereby resulting in pseudogene formation. Chemosensory pseudogenes are thought to contribute to the variability of the chemosensory receptor repertoire[11;32–34].

In conclusion, bitter taste receptors are a very complex family of chemosensory receptors with variations at structural and functional levels. Further investigation is still needed to understand the evolutionary history that led to these variations and how they influence taste perception.

2.1.3 Bitter sense and diet

Bitter taste perception has evolved as a mechanism to prevent the ingestion of toxic compounds, since almost all natural poisons taste bitter to humans. The bitter rejection response consists of negative affective responses and aversive reflexes to remove the toxic chemicals, such as rejecting food, vomiting, gaping, nausea, tongue retraction, lowered heart rate and increased latency to swallow and excretion[27;68–73]. This response is activated through neuronal and hormonal signalling cascades[68;73]. However, the response elicited by a bitter compound is not directly correlated with its toxicity. Therefore, bitter response can be stimulated by highly toxic or nontoxic compounds. Many compounds found in food and beverages such as beer, coffee and broccoli are not toxic when ingested at normal concentrations and can even be beneficial to health, providing, for example, chemoprotection[8].

Having a very low or very high bitter threshold is not ideal for animals because they would reject nutrients that are important for their diet or they would ingest toxic compounds without detecting them. Given these observations, it was hypothesised that an animal's bitter threshold was influenced by the occurrence of bitter and possibly harmful compounds in its diet[27]. Therefore, studying the relationship between bitter taste and diet posed as an interesting perspective.

Animals with a diet richer in bitter and potentially harmful compounds were predicted to have a higher bitter tolerance and threshold, as opposed to animals with a diet with a lower occurrence of bitter compounds[27]. Generally, plant tissues have more toxic compounds and are more bitter than animal tissues[27] thus herbivores consume many more bitter molecules than carnivores or omnivorous. For this reason, it is likely that they encode and express the largest bitter taste receptors repertoire[8]. In addition, herbivorous may have adapted to reduce their bitter sensitivity by repeated exposure to these compounds[27]. Also, herbivorous have undergone a stronger selective pressure to maintain Tas2r genes[74] and previous studies by Li et al.[59] show that a herbivorous diet is positively correlated with the number of Tas2r genes. Further comparative studies between carnivores and herbivorous birds are congruent with these results[75].

Animals with high bitter sensitivity (i.e., low bitter threshold) may reject nutritious and nontoxic food. Not only a reduction of bitter sensitivity in herbivores might help them avoid starvation, but they also may have acquired detoxification mechanisms. An example of this is the fermentation in ruminants[27;76]. Behavioral studies using quinine hydrochloride, a natural bitter compound, suggest that carnivores are more sensitive than omnivores to bitter compounds. In its turn, omnivores are more sensitive than herbivores. Thus, bitter sensitivity is inversely correlated with the widespread of bitter compounds in the diet.

Herbivores recognize a larger number of bitter compounds in comparison with carnivores but they also appear to have an increased tolerance to putative ingested poisons[59]. This tolerance evolved as a way to reduce the risk of poisoning when herbivorous unintentionally ingest food with poisonous substances. In contrast, carnivores have a low threshold that enables them to reduce the risk of ingesting toxic compounds by rejecting almost all possibly toxic food they find. However, they also have a lower tolerance to dietary poisons[27].

A study by Davis et al.[60] hypothesizes that, even though the differences in functional Tas2r repertoires between species may just be the result of contractions and expansions of this gene cluster, these differences are possibly linked with distinct taste perceptions and dietary adaptations. There is a precedent to associate morphological differences with dietary adaptation, being the variation in size and shape of beaks in Darwin's finches a classic example[77]. However, the relation between avian bitter taste perception and the evolution of Tas2rs in this lineage is still very much unknown.

It is interesting to note that, both in baleen and toothed whales, all except one Tas2r were pseudogenes[78;79]. This might be due to feeding behavior of swallowing the food whole, the high concentration of sodium in the ocean and dietary switch in ancient whales from plants to meat[78]. These explanations can be extended to our case of study, even though the feeding behavior of swallowing the food whole does not take into account the Tas2r evolution in bird species since all modern birds lack teeth and swallow food without mastication[80]. In addition, the ancestor of penguins has two pseudogenized Tas2rs, which are intact in their outgroup species. The fact that penguins experience extreme cold experienced in Antarctica and that TRPM5 is sensitive to low temperatures may have rendered the taste receptors that depend on this channel unusable[81]. Therefore, it was hypothesised that not only diet, but also other factors must be involved in shaping Tas2rs diversity[62].

2.1.4 Broad patterns of avian genome evolution

Birds originated from a tetrapod lineage during the Jurassic period and are presently, the only descendant from dinosaurs[82;83]. Due to the widespread of birds and the fact that birds are the most species-rich class of tetrapod vertebrates, they are used as models for ecological and evolutionary studies[2].

Amphibians and many fishes have a smaller genome than birds. However, birds have the smallest genomes among amniotes. Whereas genomes of reptiles and mammals range between 1.0 to 8.2 Gb, avian genomes range from 0.91 to just 1.3 Gb[84]. The smaller avian genome size may be due to some reasons. One of them is the proliferation and loss of transposable elements (TEs). These events appear to drive the evolution of the vertebrate genome size[85–87].

According to Zhang et al.[2], most avian genomes analyzed in their works contain lower levels of repeat elements ($\sim$ 4 to 10%) than tetrapod vertebrates (mammals have 34 to 52%[88]). The exception was the downy woodpecker (*Picoides pubescens*), which had higher levels of repeat elements ($\sim$22%), mainly because of species-specific expansion of long interspersed elements (LINE) type chicken repeat 1 (CR1) transposons. Additionally, the average length of short interspersed elements (SINEs) in birds is 10 to 27 times less than other reptiles, which suggests the reduction of SINEs occurred in the ancestral of birds. After comparing the average size of genomic elements of birds with three nonavian reptiles and 24 mammals genomes, Zhang et al.[2] found that avian protein-coding genes are on average 50% shorter than mammalians and 27% shorter than reptiles. This reduction may be caused by an increased gene density due to reduced intergenic distances and shortening of introns. The reason behind condensed genomes might be a rapid gene regulation required by powered

flight[89;90]. Also, after their divergence from other extant reptiles, the ancestral avian lineage had a large genomic sequence deletion resulting in the loss of a large number of genes. This large segmental loss suggests ancestral fission of macrochromosomes into a large number of microchromosomes[2].

2.2 Environmental Aspects

2.2.1 Migration

Migration is one of the most spectacular phenomenons observed in animal nature. It is characterized by the movement of a population twice a year between a breeding and a non-breeding area[91]. A set of biological requirements motivate birds to migrate, such as finding a favourable area for feeding, breeding and raising their young[92]. Other birds are non-migratory or residents and they do not migrate, thereby occupying the same habitat every year. Although their habitat remains the same, they might adjust their behavior as seasons change, e.g., changing their diet accordingly to which food is available[93]. Additionally, part of a population can migrate while the rest remains resident. This type of migrant is considered to be partial migration[91]. Competition for better territories can cause partial migration, in which birds in better condition are expected to remain residents and the remaining ones migrate[91;94]. Partial migration also varies with sex, since a higher proportion of adult females migrate in comparison with adult males. Age is also a crucial factor because juvenile birds migrate more than adults. The reason for this is that resident birds have bigger competition for breeding grounds and food. Additionally, females tend to subordinate to males and juveniles are less prone to succeed in competition compared with experienced adults[91].

Bird migratory journeys vary in a set of parameters. Their ability to flight predisposed them to move globally and take long migratory journeys that can go up to tens of thousand kilometres on long-distance migrants[95]. For example, Alaskan bar-tailed godwits (*Limosa lapponica barueri*) make the longest non-stop flight of 12,000 km across the Pacific ocean. The annual journey of these birds, joining the three main flights, adds up to an impressive 30,000 km[96]. However, some birds make short flights which might be just a few hundred kilometres in short-distance migrants[91;95]. Also, avians can fly at high or low altitudes, during the day or night, alone or in flocks, have different cycles of molting, breeding and migration, as also have different responses to winds and weather[97]. Closely related bird species and even birds from the same species but different subspecies can have a wide spectrum of short-distance, long-distance and resident birds[91].

Migration in birds seems to be driven by both endogenous mechanisms and exogenous factors. The annual migratory cycle generally exhibited is thought to be endogenous because circannual events such as molt timing are also observed in birds kept in captivity[98]. Endogenous mechanism include orientation, fattening, the existence of stopovers and the food selected[99]. Almost all birds experience fasting to different extents, according to their types of migration[100]. Fasting while migrating is very interesting since birds keep a very high metabolic rate and also do not drink[100;101]. Not only do birds fast while migrating, but they also perform other energetically demanding tasks such as molting and breeding. Moreover, they do so without functional or structural damage[100].

Bird's preparation for migration also includes storage of fuel[100], upgrade of their oxidative capacity and transport of fatty acids (FA) to the flight muscles before takeoff[102–104]. The oxidation of FA from molecular adipose tissue is responsible for almost all the energy metabolism (85-95%)[101;105;106] and it consists mainly of fat (95%) and a small portion of proteins (5%)[100;107]. Compared to mammals, the avian fuel metabolism reaches 20-fold higher rates of exogenous FA oxidation, thus having an enhanced capacity for FA uptake[104]. However, during this intense physical activity, long-distance migrating individuals might experience oxidative stress (i.e. the accumulation of oxidative damage)[108].

Birds also experience other physiological changes while performing long nonstop flights such as reducing the gut and increasing the pectoral muscles in the last days before takeoff. This is done to reduce the weight of organs not needed to flight and increase the necessary ones[109–111]. While the exhaustion of lipids is the main factor of the fast duration, lipids might not be the limiting factor. The fasting period, hence flight duration, can also be determined by the exhaustion of body water or proteins, which, at the end of the fasting period, are the only fuel left[112;113].

Moreover, stopovers are also very important to achieve a successful migration. Since birds spend most of the migration time on stopovers, the overall time of migration depends on the duration of these stops[114]. It is crucial for migrants to choose the right habitat because they find themselves at unfamiliar surroundings where competition, predation and food demands are likely to be high. Therefore, selecting the right stopover location will determine if migrant birds achieve the necessary fuel for the migration journey, which is imperative for their survival[97;115–119]. Migrants select their habitats given their morphology, preferences[120;121], food distribution, foraging strategies[116;122–124] and habitat carrying capacity[117]. While food is available and bird's fuel stores have been replenished, they suppress their motivation to depart to the next flight[114].

Besides food availability, current fuel reserves are accepted to be an important factor on making birds stay. While previous studies state that the departure fuel load limits the flight range, Eikenaar et al.[114] hypothesizes that the best predictor of stopover duration might be the fuel lost during the migration. Due to differences in the flight apparatus and fat metabolism, individuals of the same species can have use different amounts of fuel to travel the same distance. Additionally, environmental factors such as wind direction and wind speed change during the migration season. As result, birds that migrate at different stages of the season might experience different wind influence on flight performance, which might result in distinct fuel use during migration.

The migratory tendency can also be associated with exogenous factors that comprise photoperiod and climate variables like wind, precipitation and temperature. Not only do climatic conditions alter migration dynamics, but they also have an impact on the availability of food[125]. Therefore, climate changes can result in a mismatch between food availability and the arrival of avians, which may in turn cause higher mortality rates[125–127].

Extended stopovers and later arrivals to breeding grounds can lead to the obtaining of worse quality territories, produce fewer offspring, breed later, find a worse mating partner and produce worse offspring, thus diminishing their chances to having their offspring recruited to the breeding population[94;128]. Male birds have an additional pressure to arrive earlier (protandry) because male fitness generally depends more on the number of matings in comparison with female fitness[97].

To our knowledge, migratory behavior has not been studied alongside Tas2r in birds. Since avians show such diverse migration strategies, we found interesting to find the connection between the migration pattern and the Tas2rs. We propose that migratory, partially migratory and resident birds have different Tas2r repertoires and we intend to evaluate if migratory and resident birds present distinct evolutionary patterns Tas2rs.

2.2.2 Habitat

We used the term water birds to refer to birds that spend a significant part of their life in or around any source of water, such as rivers, oceans, streams, swamps, bays, estuaries, lakes, and marshes. On the other hand, we attributed the term land birds to those which live in scrub, forests and open country. The ones which do not exhibit a demarcated preference for aquatic or land habitats, were considered to be birds from intermediate regions.

Water birds seem to be specially interesting because they are good bio-indicators because, sensing environmental variations at short and long scales[129;130]. Also, many water bird species are top predators thus they accumulate contaminants from lower trophic levels, serving as indicators for the trophic chain they are in[131]. The effect of water pollution on the number and species diversity of water birds have been studied and reviewed in several publications[132;133]. It was reported that 6.5% of bird species are functionally extinct and 20% of bird species are prone to extinction[134]. Studies show that at least $1/3$ of the piscivorous, exotic and herbivorous birds and approximately $1/4$ of the omnivorous and frugivorous species are at risk of extinction because of aquatic pollution[135;136].

The relationship between habitat and Tas2r has not been studied until this point. As a result, we purpose to understand if Tas2r are evolving under distinct evolutionary pressures when considering distinct habitats (land birds, birds from intermediate regions or water birds).

3

COMPUTATIONAL METHODS AND TOOLS

3.1 Bioinformatics Databases and Tools

Natural selection plays a major role in evolutionary change. Therefore, it was important to understand the functional effects of neutral drift and positive selection [137]. Comparative genomics include a wide variety of methods, such as databases searches and bioinformatic tools that allow the evaluation of pressures acting on genes, which can subsequently lead to the understanding of gene evolution [138].

3.1.1 NCBI BLAST

The NCBI webserver provides numerous databases and resources on biological information [139]. One of the available databases included in NCBI is Pubmed, which comprises numerous scientific publications and online books. Additionally, NCBI's BLAST [140] is a search program that provides several ways to compare nucleotide or protein queries with a database of sequences that can be either nucleotide or protein databases. It is one of the most widely used bioinformatics research tools and it can be used a stand-alone tool or as a web interface. Using BLAST does not only performs alignments, but also provides statistical information about them [141]. For nucleotide searches and alignment, the options are discontiguous megablast, megablast and blastn. One of the best options of inter-species comparison is blastn. The blastn program enables the alignment of rRNA or tRNA sequences. It can also align mRNA or genomic DNA sequences which contain both coding and noncoding regions. For protein-protein searches there are several options, such as PHIBLAST and PSI-BLAST but blastp is the standard option [142]. NCBI's blastp approach was used in the execution of our work in order to verify the identity of each extracted sequence. These resources can be accessed at the NCBI website, www.ncbi.nlm.nih.gov.

3.1.2 SeaView and MEGA

In this project, we used the SeaView and MEGA software to visualize and edit sequences. SeaView enables molecular evolution analysis by performing multiple sequence analysis (MSA) and phylogenetic tree building through a graphical user interface. This program can read both nucleotide and protein

sequences of a genetic code given by the user or retrieved from a database. In addition, nucleotide sequences can be translated to protein[143].

To perform preliminary MSAs, SeaView uses two external programs: Muscle[144] and ClustalW version 2[145]. These programs are set to their default parameter values but can be altered through SeaView. It is possible to align all sequences, selected sequences or part of sequences. In addition, SeaView also permits the editing of the MSA by adding or removing gaps of one or more sequences at a time[143].

On the other hand, the MEGA software[146] enables the analysis of big datasets by generating sequence alignments and estimating sequence divergence. MEGA also allows the reconstruction and display of phylogenetic trees, and the testing of evolutionary hypotheses. Comparing SeaView and MEGA, they both provide a graphical user interface which easily allows MSA and parsimony tree reconstruction and visualization.

MEGA has some advantages, as it easily allows to edit sequences header, add/remove parts of the nucleotide or protein sequences and is freely made available in two interfaces: command line and graphical, at www.megasoftware.net. While SeaView is less versatile for pairwise distance computations and does not have neutrality or molecular tests, it enables maximum-likelihood tree reconstruction resorting to PhyML version 3[147] and is available free of charge for the major computer platforms (Mac OS X, Microsoft Windows, Linux and SPARC/Solaris) at http://pbil.univ-lyon1.fr/software/seaview[143].

3.1.3 DAMBE

DAMBE[148] is a user-friendly graphic interface that allows for genomic and phylogenetic comparative analysis. Some of the several methods available in this tool are computation of protein isoelectric points, identification of tRNA anticodon and position weight matrix to characterize and predict sequence motifs. In this study, we used the DAMBE's Xia et al. test[149], which is an index to measure saturation of a nucleotide sequence alignment. The critical values of the index are obtained from computer simulations with different operational taxonomic units (OTUs), sequence lengths and topologies. Through the critical values, the user is able to determine if the alignment is useful or not, since the phylogenetic information contained in the sequences is impaired by the substitution saturation.

3.1.4 GUIDANCE2

An important step into comparative analysis is the creation of a MSA. Therefore, it is crucial to guarantee that the MSA has the least amount of errors and uncertainties that would have a negative effect on downstream analyses. GUIDANCE2 is a user-friendly web-server that accepts a set of unaligned DNA, RNA or protein sequences in FASTA format. It was created to improve the accuracy of the resulting MSA by upgrading the identification of unreliable alignment regions. The server offers three different algorithm options to evaluate MSA uncertainties: the heads-or-tails (HoT)

method, the GUIDANCE score and the GUIDANCE2 score[150]. Afterwards, it is necessary to chose the progressive MSA algorithm: PRANK, ClustalW or MAFFT, which is the default. The number of bootstrap repeats is set for 100 but it can be changed by the user. As this number increases, so does the running time. The server generates a base and an alternative MSA, which are compared to estimate the confidence level. If unreliable columns or sequences are found, they can be automatically removed from the base MSA. This methodology considers the uncertainty in the assumed guide tree, equally optimal solutions in the pairwise alignments and the formation of indels (gaps). Additionally, it is possible to pick the cutoffs values for sequences and columns to be filtered out. GUIDANCE2 can be accessed for free at http://guidance.tau.ac.il[150–152].

3.1.5 jModelTest 2

jModelTest 2[153] is a program to statistically select the best-fit models of nucleotide substitution based on Phyml[147]. It incorporates likelihood ratio tests (LRT), which can be hierarchical likelihood ratio tests (hLRT) or dynamical likelihood ratio tests (dLRT). jModelTest 2 can also estimate the model selection uncertainty, model-averaged parameters and parameter importances. It is also possible to define equal or unequal base frequencies (+F), rate variation among sites (+G) and proportion of invariable sites. Additionally, the information criteria can be selected as Akaike information criteria (AIC), Bayesian information criteria (BIC) or decision theory method (DT).

jModelTest 2 is written in Java and can be used in a desktop version for multicore processors, in a cluster version that distributes the computational load among nodes or as a hybrid version that utilizes a cluster of multicore nodes at its advantage. The program runs under Mac OSX, Windows XP, and Linux with a Java Runtime Environment[154].

3.1.6 IQ-TREE

When dealing with large phylogenomic datasets, it is necessary to use fast tree inference methods. This is especially true for maximum likelihood (ML) phylogenies. The best-fitting phylogenetic tree is defined by best tree topology, branch lengths and substitution model parameters, even though tree topology is the most important factor. IQ-TREE[155] is an effective and fast stochastic algorithm to reconstruct ML trees. This program explores the tree space and enables:

- Model selection by partition;
- Selection of which phylogenomic data to analyse;
- Bootstrap approximation;
- Tests of several branches;
- Tree topology tests.

This stochastic algorithm does not have the local optima problem faced by hill-climbing algorithms. By maintaining candidate trees or allowing "downhill" moves, it escapes local optima and reduces

computation time. One of the most frequently used features is Tree Inference, which enables phylogenetic analysis on MSA. To to this, it is necessary to feed an MSA to IQ-TREE, choose the options and start the analysis. The biggest advantage of IQ-TREE is the ultra-fast bootstrap reconstruction that allows for a quick, but accurate phylogenetic reconstruction when using large MSA data[155;156]. IQ-TREE can be freely accessed at http://www.cibiv.at/software/iqtree.

3.1.7 MrBayes

MrBayes[157;158] is a command-driven program that performs Bayesian phylogenetic inference by joining heterogeneous datasets evolving under different stochastic evolutionary models. Normally, it is not possible to calculate the posterior probability distribution analytically. Therefore, MrBayes uses Markov chain Monte Carlo (MCMC) techniques to estimate posterior probabilities of phylogenetic trees. These techniques include not only the standard MCMC algorithm, but also a variant called Metropolis-coupled Markov chain Monte Carlo. MrBayes has a variety of stochastic models for nucleotide, protein, restriction site and morphological data, which is the default. For nucleotide data, it is also possible to chose single, doublet or codon models. There is also a range of fixed or variable rate matrices to analyze protein data. Additionally, the restriction site and standard models can be corrected for coding biases. Finally, the program allows for inference of ancestral states by integrating out uncertainty regarding tree topology and model parameters. The Bayesian phylogenetic analysis can be summarised in the following points:

- Reading of an aligned matrix of amino acid or DNA sequences from a Nexus data file;
- Choice of the evolutionary model;
- Execution of the analysis;
- Summary and diagnose of the results of the analysis.

We performed Bayesian inference (BI) in our dataset and compared the result with the ML reconstructions from IQ-TREE. MrBayes is written in ANSI C and is available for free at http://morphbank.ebc.uu.se/mrbayes/.

3.1.8 Datamonkey

Datamonkey[159;160] is a user-friendly web interface of phylogenetic analysis tools executed by the molecular evolution analysis package, HyPhy[161]. Through ML and Bayesian-based tools, Datamonkey enables the identification of sites under positive or negative selection, even in recombinant sequences, by determining dN and dS substitution rates. Datamonkey has selection pressure methods, which contain individual site models, individual branch models and gene-wide models. The individual site models include FEL[162], FUBAR[163], SLAC[162] and MEME[164]. The individual branch model is adaptive Branch-Site Random Effects Likelihood (aBSREL)[165], while the gene-wide models are constituted by Branch-site Unrestricted Statistical Test for Episodic Diversification (BUSTED)[166]

and RELAX[167] methods. Additionally, Datamonkey allows for recombination detection with Genetic Algorithm for Recombination Detection (GARD)[168] and has an additional method, HIV-TRACE[169]. In our work, we submitted our MSA to four selection methods of individual sites, which were the MEME, FEL, FUBAR and SLAC approaches[159;160]. The Datamonkey methods and documentation are freely available at http://www.datamonkey.org.

3.1.9 PAML

PAML[170] is a package of programs to carry out ML phylogenetic analyses of DNA and protein sequences. The PAML package includes the programs CODEML, PAMP, BASEML, BASEMLG, YN00, CHI2, EVOLVER and MCMCTREE. These programs can conduct several analysis, including:

- Comparison and tests of phylogenetic trees;
- Estimation of synonymous and nonsynonymous rates between two DNA sequences;
- Inference of positive selection through phylogenetic genes comparison;
- Reconstruction of ancestral sequences using codon amino acid and nucleotide models;
- Estimation of species divergence times incorporating uncertainties in fossil calibrations;
- Combined analysis of heterogeneous datasets from multiple gene loci;
- Construction of ancestral genes and proteins for molecular restoration studies of extinct life forms;
- Detection of adaptive molecular evolution under models of codon substitution;
- Simulation of molecular evolution.

PAML package is written in ANSI C and is compiled for UNIX, Windows and Mac OSX. The package can be freely accessed for academic use at http://abacus.gene.ucl.ac.uk/software/paml.html.

3.1.10 Programming language: Python

Python[171] is a high-level programming language which prioritizes easy code readability. It has a wide variety of applications such as scripting, rapid development of programs or acting as a "glue code" to existing components. This programming language includes several modules and packages, such as NumPy[172], Pandas[173] and xlwt, which are applicable in different areas like web development and data science.

NumPy[172] is the most popular Python package when dealing with arrays. Additionally, it can be used to work in fourier transform, matrices, and linear algebra. Pandas[173] is a library used for statistical data analysis and manipulation. It provides both tools and data structures that aim to make working with datasets easier. This package is well suited to work with ordered and unordered data,

tabular data, arbitrary matrix data or any statistical datasets[173]. Finally, xlwt is a Python package that allows for the development of spreadsheet files compatible with Microsoft Excel. Using this library it is possible to read Excel files and perform numerous modifications on them, such as creating elements within a Workbook, writing to different types of cells or formatting rows and columns.

The Python interpreter and its library are freely available on Python website, https://www.python.org/.

3.1.11 Image Generator and Editor Tools

Protter (http://wlab.ethz.ch/protter/)[174] is an interactive web-based tool that integrates annotated sequence features with experimental protein topology. In addition, Protter is useful to highlight features of membrane proteins by varying the color, shape frame or background color of the amino acid letter. Protter uses protein topology information from Phobious[175] or Uniprot[176], protein feature annotation from Uniprot and experimental data from the user or other database. Therefore, Protter is an open source application to customize and visualize protein sequences either with previously annotated protein topology or experimental protein data.

TimeTree is a public database of the evolutionary timescale of life that uses data from of several studies. It enables the building of a timetree of a custom list or group of species, the finding of the divergence time of two taxas or the understanding of the evolutionary branches that led to a specific species. TimeTree provides timepanels to compare astronomical history or geological time events with timetrees and timelines. The results can be exported in different formats and the Timetree knowledge-base[177–180] is freely accessible on the website, https://www.timetree.org.

FigTree[181] is a program for graphical visualization and generation of phylogenetic trees. FigTree allows for the edition of phylogenetic trees generated from a variety of programs at structure level (e.g. linear, circular or cladogram mode). It is designed to modify tree components such as node labels, rooting positions, scale axes and tip labels but also allows to emphasize branches or regions of the tree by changing branch colors or highlight tree sections. The resultant trees can be exported to other graphics programs or as PDF.

Additionally, to create scientific figures with pre-made templates and icons, the web-based tool BioRender[39] was used.

3.2 Methods

3.2.1 Genome data/sequences and Annotation

The genomes used in this project (Table S1) were retrieved from a private consortium that sequenced some avian species for the first time. The genomes were already annotated, therefore we started working from a library of protein coding regions. The Tas2r genes were obtained using a script previously developed by a member of our team. The script uses a keyword search, based on full gene name (e.g. Tas2r) or related keywords (e. g. taste receptor type 2) and retrieves the target genes through a BLAST approach. Other biological and ecological information about the birds considered

in this study, such as family, order, habitat preference and the type of migration was provided by the consortium as well (Table S1).

3.2.2 Gene identification

In order to confirm the identity of putative Tas2r genes obtained through the method described in the former section, we conducted blastp searches, using the Tas2r genes as queries. The command used to perform these searches was the following:

blastp -query query1p.fas -db /home/labpc10c/Documents/BLAST/nr/db/V5/09012020/ nr/v5 -evalue 0.001 -outfmt 6 -max/target/seqs 3 -num/threads 20 quer1p.out

The term 'query' corresponds to the file with the amino acid sequences of extracted Tas2r genes; 'db' refers to the database previously downloaded from the NCBIs server; 'evalue' is the sensibility of excepted value; 'outfm' defines the output format. Finally, 'max/target/seqs' refers to number of best matches and 'threads' sets the number of threads to be used in the computer during the process. Considering the best matches from blastp, two main datasets were created (Tas2r40 and Tas2r9).

3.2.3 Alignments and Saturation

The Tas2r40 and Tas2r9 datasets were manually inspected and pseudogenes, which are sequences with interleaved stop codons (Tables S2 and S3, respectively), and partial sequences (with less than 768 amino acids) were removed from the datasets. Each main Tas2r40 and Tas2r9 dataset were then subdivided accordingly with the main taxonomic divisions, as also migratory and habitat preferences, originating the subsets of sequences shown in Table 2 below:

Table 2: Tas2r40 and Tas2r9 datasets used in this study

Full sequences	**Phylogenetic/taxonomic division**	**Migratory preference**	**Habitat preference**
All-40	Basal-40	Migratory-40	Water-birds-40
	Strisores-Aequorlitornithes-40	Partially-migratory-40	Intermediate-regions-40
	Acanthisittidae-Tyranni-Passeri-40	Non-migratory-40	Land-birds-40
	Passeri-1-40		
	Passeri-2-40		
	Passeri-3-40		
All-9	Basal-9	Migratory-9	Water-birds-9
	Strisores-Aequorlitornithes-9	Partially-migratory-9	Intermediate-regions-9
	Piciformes-9	Non-migratory-9	Land-birds-9
	Acanthisittidae-Tyranni-Passeri-1-9		
	Acanthisittidae-Tyranni-Passeri-2-9		
	Tyranni-Passeri-1-9		
	Tyranni-Passeri-2-9		
	Tyranni-Passeri-3-9		
	Tyranni-Passeri-4-9		

The resulting coding sequences were used to perform a protein-based coding sequence alignment using the GUIDANCE2 web-server[150–152]. Only the columns with a score over 0.93 were retrieved from the GUIDANCE's MSA file. When the number of sequences per file exceeded the 500, we generated MSA using the MUSCLE methods implemented in MEGA7. Afterwards, all the MSA were manually verified. Each MSA was tested for nucleotide (base) substitution saturation using the Xia et al. test implanted in DAMBE[148] by comparing the index score (ISS) with the index critical score (ISS.C), using the adequate proportion of invariant sites and, whenever possible, testing for 100 replicates. When the datasets have more than 32 sequences, DAMBE randomly samples subsets of 4, 8, 16 and 32 OTUs numerous times and performs a saturation test for each subset.

3.2.4 Determination of substitution models

After the alignment, it was necessary to select the evolutionary model that best translated biological mutations into patterns using a small set of parameters. These models are based in some assumptions: all models assume that all nucleotide sites change independently, the substitution rate is constant over time and in different lineages, the base composition within the dataset is the same (at equilibrium) and all sites have the same constant probability to undergo substitution. However, different models assume different nucleotide substitution rates and different base frequencies[182].

To test for the best fitting nucleotide substitution model, we used jModelTest (version 2.1.10). The command used to perform this operation is as follows:

```
java -jar jModelTest.jar
```

This command opens a dialogue box that allows the specification of several likelihood settings, including the number of substitution schemes to be tested (3 in our study). The other settings specify whether unequal base frequencies are to be tested (+F), and whether a proportion of invariable sites (+I) and rate variation among sites with a number of rate categories in a discretized gamma distribution (+G) should be included. It is also possible to pick one of four options to infer the base tree used for likelihood calculations: Fixed BIONJ-JC, Fixed user topology, BIONJ, and ML optimized - we chose ML optimized. For base tree search, 'Best' was our preference. We tested for the most adequate evolutionary model by comparing the AIC scores. In the analyzed MSA, we obtained two models (Table S4): Hasegawa, Kishino, and Yano Model (HKY) and General Time Reversible Model (GTR). The HKY model assumes that base frequencies are not equal and that transversions and transitions occur at different rates[183]. On other hand, the GTR model assumes different base frequencies and different rates of nucleotide substitutions for each pair (6), therefore each possible substitution has its own rate[184].

3.2.5 Phylogenetic Tree Estimates

We used models of evolution and their respective parameters to construct phylogenetic trees of the former datasets, considering two distinct algorithms: ML reconstruction using IQ-TREE v1.6[155;156] and BI using MCMC via MrBayes v3.2.6[157;158].

To estimate the ML tree using IQ-TREE v1.6, the following command-line program was used:

iqtree -s `Basal-40.phy` -m GTR+I+G -i 0.000 -a 6.1090 -nt 3 -bb 1000

In this command-line program, 's' corresponds to the input file, which was previously converted into the Phylip format by the Seaview software[185] and 'm' to the model of evolution that was previously selected using jModelTest 2's AIC. When models have a variation across sites using a gamma distribution with none the sites being invariant, 'i' is defined, while a value is attributed to 'a' when models have variation across sites, using a gamma distribution with a proportion of the sites being invariant. Finally, 'nt' corresponds to the number of cores used in this computation and 'bb' to the number of ultrafast bootstraps.

On other hand, bayesian trees were constructed using MrBayes[157;158]. The software was opened by typing 'mb' on the terminal. Then, we used the command 'exe file.nxs'. The fasta files were previously converted into NEXUS format through the SeaView software[185]. The maximum likelihood models employed either two or six substitution types ('nst = 2' for HKY or 'nst = 6' for GTR), depending on the dataset. Some models have a variation across sites using a gamma distribution with none the sites being invariant ('rates=gamma') while others have a variation across sites using a gamma distribution with a proportion of the sites being invariant ('rates = invgamma'). The MCMC searches were run twice with four chains for 5,000,000 generations, with trees being sampled every 500 generations and diagnosed at each 100 generations. Convergence upon a specific topology was confirmed by ensuring that the standard deviation of split frequencies was lower than 0.05 (Table S6). Following this assessment, the first 2,500 trees (corresponding to 1,250,000 generations), were discarded as 'burn-in' in each of the analyses ('sump relburnin=yes burninfrac=0.25' and 'sumt relburnin=yes burninfrac=0.25') and the rest was used to generate the consensus tree.

Additionally, the divergence times of the tree with all the orders used in this study were inferred from previously available studies using the TimeTree database[177–180] (http://www.timetree.org/, last accessed February 2020).

3.2.6 Site Selection

Proteins experience mutations which can be considered synonymous or non-synonymous. Synonymous mutations do not modify the amino acid sequence while non-synonymous mutations change the amino acid sequence which can alter both protein's structure and function. Positive selection is the process by which advantageous mutations are fixed in a population. The evolutionary pressure can be analyzed through these mutations, by comparing the nonsynonymous/synonymous rate ratio (ω),

thus obtaining $\omega = \mathrm{d}N/\mathrm{d}S$. If there are positive, neutral or negative pressures acting on proteins, it is expected that the ω is greater, equal or less than 1, respectively[186–188].

We employed codon models implemented on Datamonkey[159;160] and PAML[170]. The Datamonkey webserver (http://www.datamonkey.org/), provides several methods to detect positive and negative selected sites. In this study, the following methods were selected: FUBAR[163], FEL[162], MEME[164] and SLAC[162]. FUBAR guarantees robustness when the models are misspecified. FEL calculates the $\mathrm{d}N$ and $\mathrm{d}S$ substitution rates on a site-by-site basis. MEME is able to detect sites evolving under episodic and pervasive positive selection, being most suitable to detect episodic selection. Finally, SLAC uses ancestral reconstruction and then counts the total number of synonymous and nonsynonymous changes at each site. We did 3 runs for each dataset using all the previously mentioned methods (Tables S9 to S20).

We used an integrative approach to collect both the positive and the negative selected sites in Datamonkey considering as final results the positive selected sites (PSS) selected by at least 3 of the 4 methods (FUBAR, FEL, MEME e SLAC) and the negative selected sites (NSS) detected by at least 2 of the 3 approaches (FUBAR, FEL and SLAC). This integrative approach was employed because sites detected by multiple methods are understood as having more support of positive selection[189–191]. To optimize the process of extraction of results, we firstly created individual scripts to collect the PSS and NSS of each method (FUBAR, FEL, SLAC and MEME). Then another two scripts were created to optimize the integrative approach by collecting the common number of PSS and NSS. The computing scripts are available at https://github.com/raquelsvcardoso/avian-tas2r. Considering the FEL, SLAC and MEME outputs, the ω value of each dataset was extracted and used to calculate the average ω value.

In addition, we employed codon models implemented on LMAP, an interface that allows for an easier use of the PAML software. The program CODEML, as implemented in PAML v.4.7, generates the maximum LRT. The LRT were used to compare site-specific models by calculating the difference of log likelihood between the two models multiplied by two, following the Chi square distribution. The degrees of freedom used match the difference in the number of parameters of the two models. The site-specific models were compared using the LRT: M7 (beta) vs M8 (beta + ω) and M8a (beta + ω = 1) vs M8. Both M7 and M8 assume a beta-distribution for $0 \leq \omega \leq 1$. However, M8 additionally assumes an extra class of ($\omega > 1$), allowing the occurrence of positively selected sites. M8a, on the other hand, tests the neutral evolution including a class of neutral evolving sites. This test was replicated with three different k values (0.2, 2 and 5). When two or more replicates had a p-value $<$ 0.05, the null models were rejected, and the LRT is significant. In those cases, the Bayes empirical Bayes (BEB) was tested to determine the posterior probabilities of positive selected sites. The sites which had PP $>$ 0.95% where noted as PSS. The M0 of each replicate calculated the global ω value of each dataset, which were later used to calculate the mean ω value.

In the last step, ω values retrieved by the Datamonkey and PAML methods were used to calculate a final average ω value. Additionally, considering a double applied methodology of codon level approach, we considered as PSS the sites validates by either the Datamonkey or the PAML approach.

3.2.7 Protein modelling and site mapping

Using Protter[174], we predicted the secondary structure and expected topology of seven transmembrane domains of a representative sequence of each dataset. Hence, we mapped all the significant positive selected sites on the respective predicted protein structure to show the location of selected amino acid residues. It was attempted to predict the 3D structure of Tas2r through the SwissModel webserver, but the obtained structures do not present parameters with enough quality to enable conclusions about residue interaction or 3D location.

3.2.8 Branch Selection

The branch selection analysis required the labelling of the Tas2r40 and Tas2r9 datasets according to a) their habitat preference and b) their migratory habits. This analysis tests for divergence among lineages by verifying if there are significant ω ratio variations between branches of each label. In this study, we observed the performance of 2 LRT comparisons among 3 models (M0 vs TrU and TrC vs TrU)[192–194]. While in the first comparison a null (M0) model is tested against a two-ratio unconstrained model (TrU), in the second one TrU is tested against a two-ratio constrained model (TrC) ($\omega = 1$)[195]. This test was replicated with three different k values (0.5, 1 and 1.5). If the null model were to be rejected, the ω obtained by the M0 would be accepted. Otherwise, there would be signals of divergence and the ω obtained by the TrU model would be assumed.

4

RESULTS AND DISCUSSION

4.1 Identification and Alignment of Tas2r genes

The first step of this work was a deep screening and extraction of 976 sequences of Tas2r genes present in 245 avian species (Table S1) distributed across 14 avian orders, as shown in Figure 2. The cladogram shows the relationships between the bird orders used in this study, as proposed by TimeTree[178]. The avian nomenclature of Tas2rs is ambiguous and not consistent, since some authors classified the three available chicken Tas2rs as Tas2r1-3[35], whereas other authors classified them as Tas2r1, Tas2r2 and Tas2r7[4]. In the literature, we found references to the existence of Tas2r3 and Tas2r4 in turkey, while in zebra finch the attributed nomenclature was Tas2r5-7[41]. Based on that, we applied a protocol of BLAST search through NCBI dataset to all extracted sequences, in order to clarify the identification of Tas2r genes. Our results suggest the division of taste receptors in two main groups: Tas2r40 and Tas2r9. The majority of the avian orders present elements of both groups. However, the distribution of Tas2r across species is not homogenous: while the presence of Tas2r9 is widespread across species, the distribution of Tas2r40 is more limited.

Afterwards, we removed partial sequences and also sequences that present intra-sequential stop codons. We verified that some Struthioniformes and Passeriformes species, as also all Pelecaniformes and all Suliformes orders present pseudogenization of Tas2r40. Moreover, some species of Sphenisciformes and Passeriformes present pseudogenized Tas2r9 genes. Additionally, one could observe that, even though the number of sequences of the Tas2r40 dataset is lower, the rate of pseudogenization in this group of genes is higher, and extended to a bigger number of species, than those observed in the Tas2r9 dataset. The presence of pseudogenes might mean that a Tas2r gene was duplicated, bringing no advantage to the species because of its redundancy. Therefore, after the duplication has occurred, if the gene is functionally redundant, it can become a pseudogene by mutations and/or deletions[205;206]. Afterwards, pseudogenes end up being deleted or diverge so much that they are no longer recognized[206]. In other words, pseudogenes can represent an intermediary state in evolution, during the generation of new genes by duplication and mutation[28].

In addition, the duplication rate in Tas2r9 is more frequent (up to 10 copies) than in Tas2r40. Not only the duplication phenomenon is less common in Tas2r40, but also some orders (Eurypygiformes, Gaviiformes, Pelecaniformes and Piciformes) apparently lost this group of receptors. In contrast, the order Sphenisciformes seems to have lost Tas2r9.

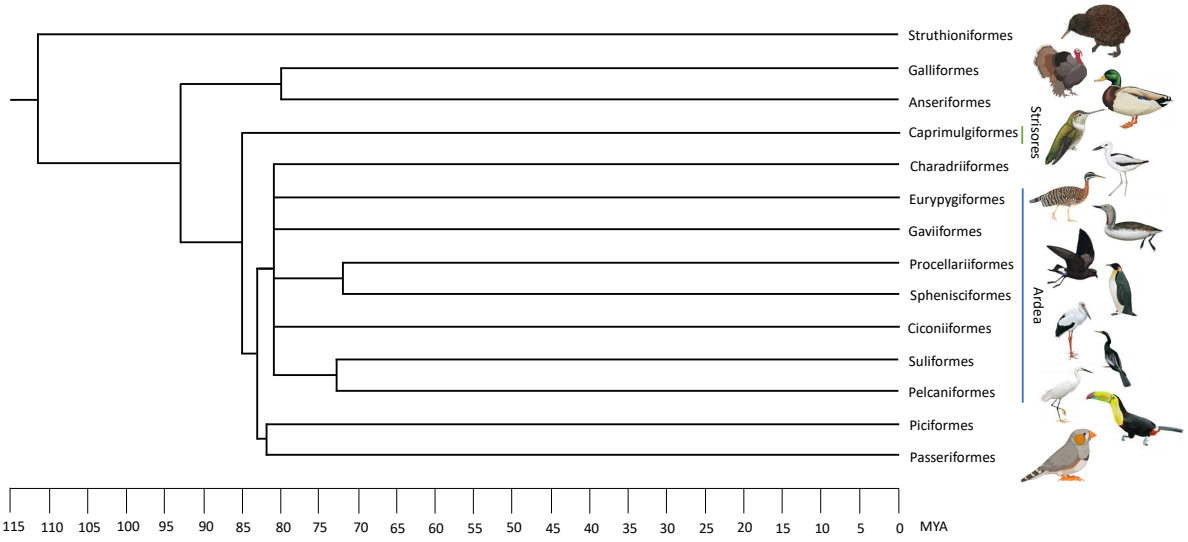


Figure 2: Phylogenetic tree with the orders used in this study. The divergence times were inferred using http://www.timetree.org/. Branch lengths were drawn to the scale.

All MSAs were submitted to the Xia et al. test to evaluate the presence of saturation. In all datasets, it was obtained a symmetric and asymmetric ISS.C higher than the ISS, which supports the absence of saturation (Table S5). We used jModelTest (version 2.1.10) with AIC to estimate the most appropriate model of evolution. Regarding the Tas2r40 dataset (Table S4), GTR+G was the most adequate model for most MSA. However, for Basal-40 the best-fit model was HKY+G and for Passeri-3-40 and All-40, the most appropriate model was GTR+G. In the case of the Tas2r9 dataset (Table S4), GTR+I+G was the most adequate model for all MSA, except Tyranni-Passeri-2-9, for which GTR+G was the best-fitting model.

4.2 Phylogenetic Tree Estimates/Reconstructions

In the next step, we used the formerly collected bird sequences to construct ML and Bayesian phylogenetic trees (by IQ-TREE v. 1.6 and MrBayes, respectively) and evaluated their duplication patterns. The average standard deviation of split frequencies obtained using MrBayes was lower than 0.05 in all datasets (Table S6). After rooting and swapping the position of certain species, the trees inferred under ML and BI approaches had very similar topology. However, since Bayesian trees have more polytomies, ML trees were chosen for all datasets, with the exception of Acanthisittidae-Tyranni-Passeri-40. We compared our phylogenetic inferences with phylogenetic trees reported in recent articles to infer comparative conclusions[196–200].

4.2.1 Tas2r40 Trees analysis

We reconstructed the phylogeny of all Tas2r40 sequences herein analyzed. The general tree (Figure 3a) presents the sub-dataset named as Basal-40 as the root, followed by Strisores-Aequorlitornithes-40 and then the 4 predefined groups of Passeriformes birds (Figure 3b), which is in accordance with previous studies[199;201].

Firstly, the group we defined as Basal-40 (Figure 4) is constituted by the orders Struthioniformes, Galliformes and Anseriformes, and has a single copy of Tas2r40, showing no signals of duplication. The dataset named as Strisores-Aequorlitornithes-40 (Figure 5) possesses species of the orders Caprimulgiformes, Charadriiformes, Ciconiiformes, Procellariiformes and Sphenisciformes. Each species only has one copy of Tas2r40. Some of the orders initially included in this dataset, such as Pelecaniformes and Suliformes, were later removed for being pseudogenized (Table S2). Additionally, were unable to find any sequence of Tas2r40 in the orders Eurypygiformes, Gaviiformes, and Piciformes.

Passeriformes order can be subdivided into three suborders: Acanthisittidae, Tyranni (Suboscines) and Passeri (Oscines). The distribution and duplication pattern of Tas2r40 genes, associated with high bootstrap values and elevated posterior probabilities of ancestral nodes of our phylogeny, lead us to divide the dataset into the Acanthisittidae-Tyranni-Passeri-40 group, composed by species of all the suborders of Passeriformes; and three additional datasets (Passeri-1-40, Passeri-2-40 and Passeri-3-40), formed exclusively by species of suborder Passeri. In Acanthisittidae-Tyranni-Passeri-40 (Figure 6) and Passeri-1-40 (Figure 7) groups, we verified several punctual events of duplication (Table S7) that do not appear to be family specific. In Passeri-2-40 (Figure 8) it is possible to observe 69 different species with only one copy of Tas2r40 for each one, therefore showing no signal of duplication. Finally, in Passeri-3-40 (Figure 9) we detected several duplication events that seems to be related with the Emberizidae, Fringillidae and Timaliidae families.

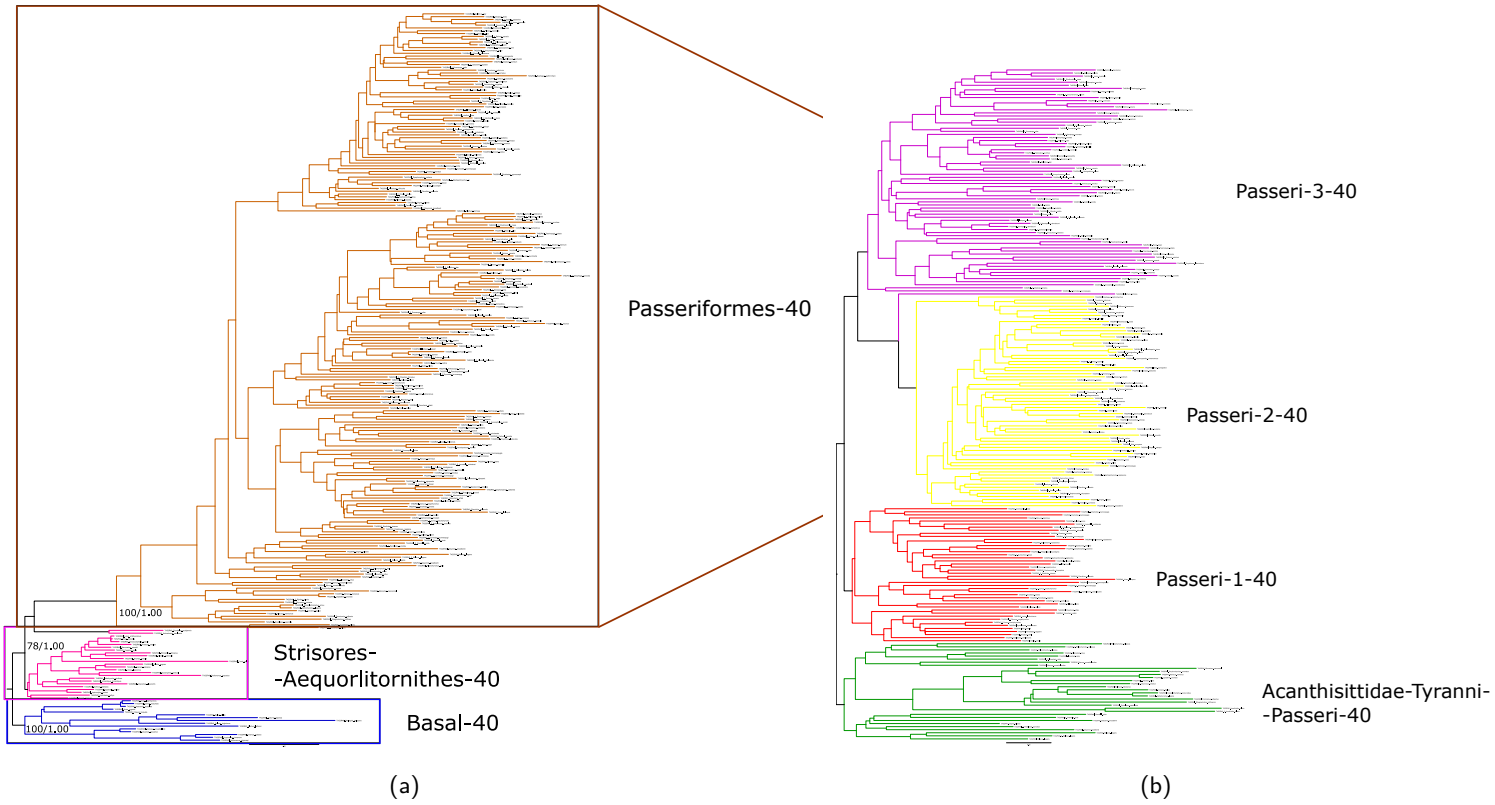


Figure 3: (a) Maximum Likelihood tree topology of All-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different orders: blue for Basal-40, pink for Strisores-Aequorlitornithes-40 and brown for Passeriformes-40. (b) Neighbour joining tree of Passeriformes-40 generated in MEGA. Different colors represent different subsets: green for Acanthisittidae-Tyranni-Passeri-40, red for Passeri-1-40, yellow for Passeri-2-40 and purple for Passeri-3-40.

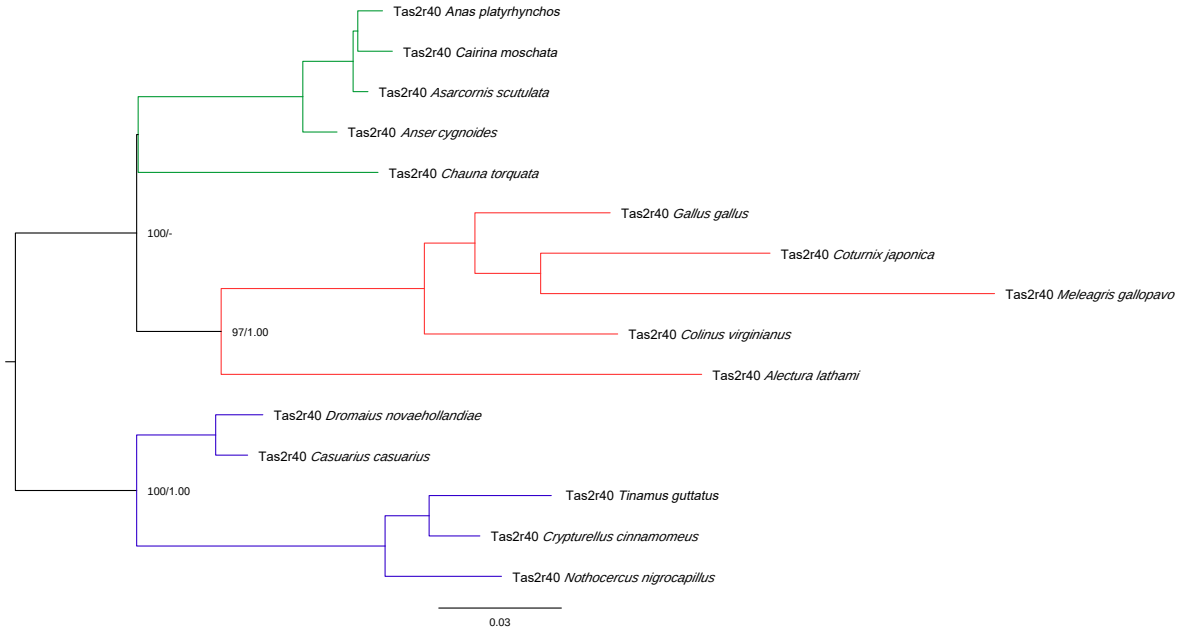


Figure 4: Maximum Likelihood tree topology of Basal-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different orders: blue for Struthioniformes, red for Galliformes and green for Anseriformes.

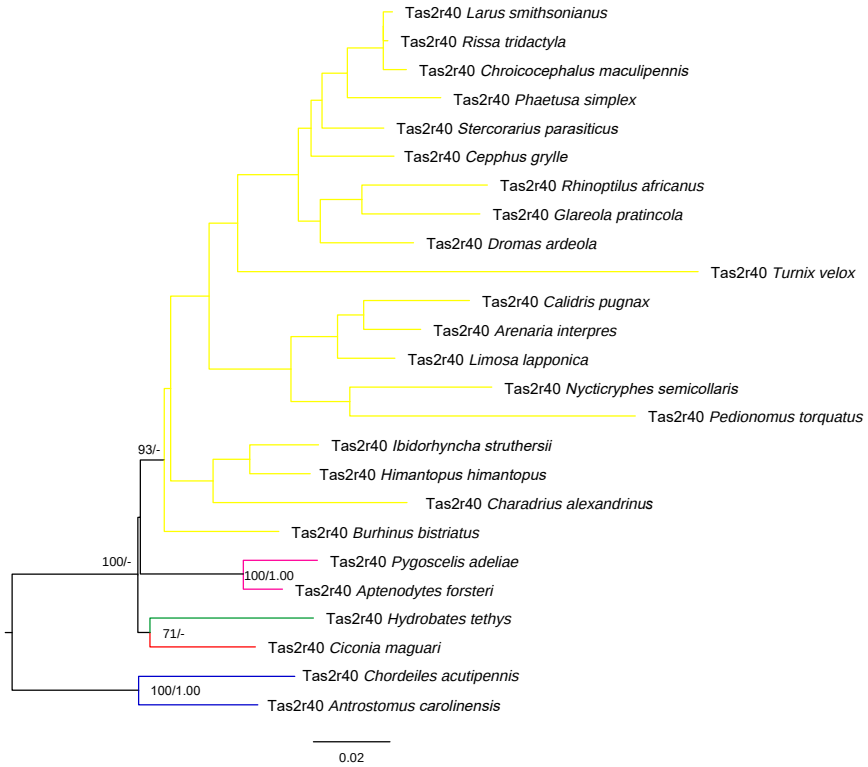


Figure 5: Maximum Likelihood tree topology of Strisores-Aequorlitornithes-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different subsets: blue for Struthioniformes, red for Ciconiiformes, green for Procellariiformes, purple for Sphenisciformes and yellow for Charadriiformes.

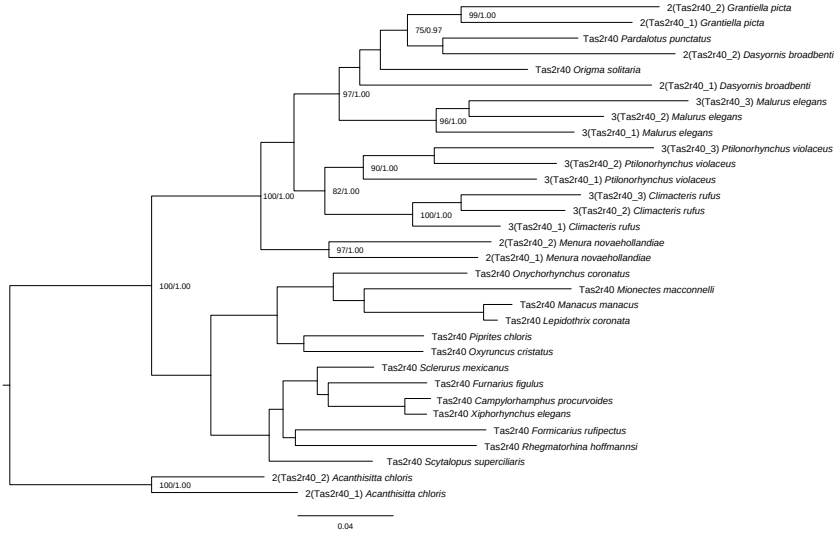


Figure 6: Bayesian tree topology of Acanthisittidae-Tyranni-Passeri-40 generated in MrBayes. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

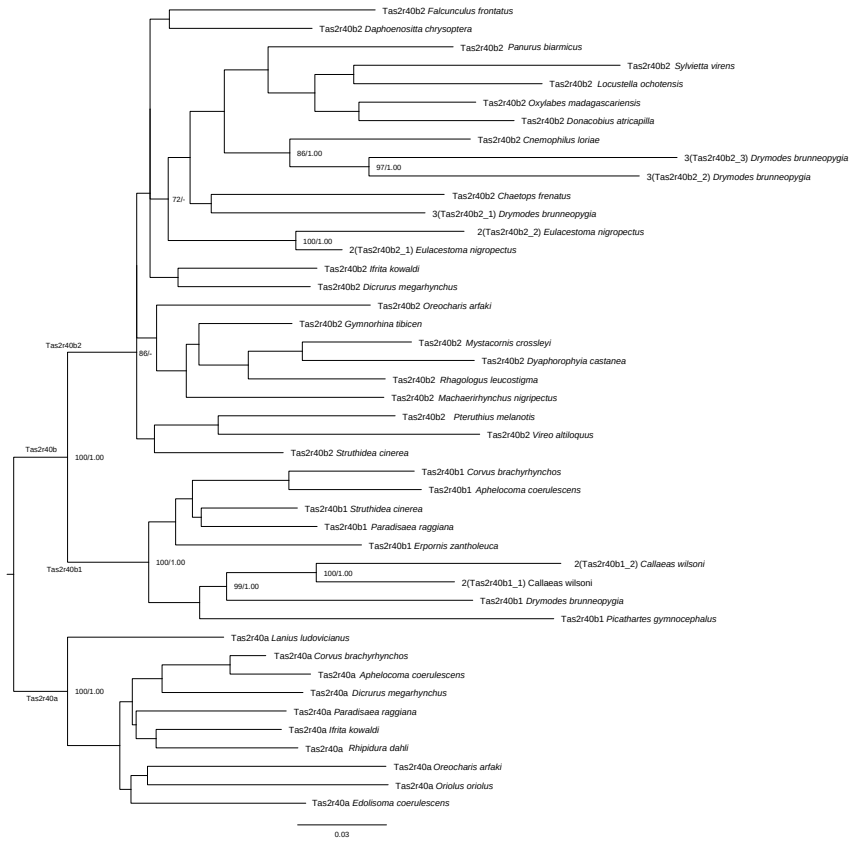


Figure 7: Maximum Likelihood tree topology of Passeri-1-40 generated in in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

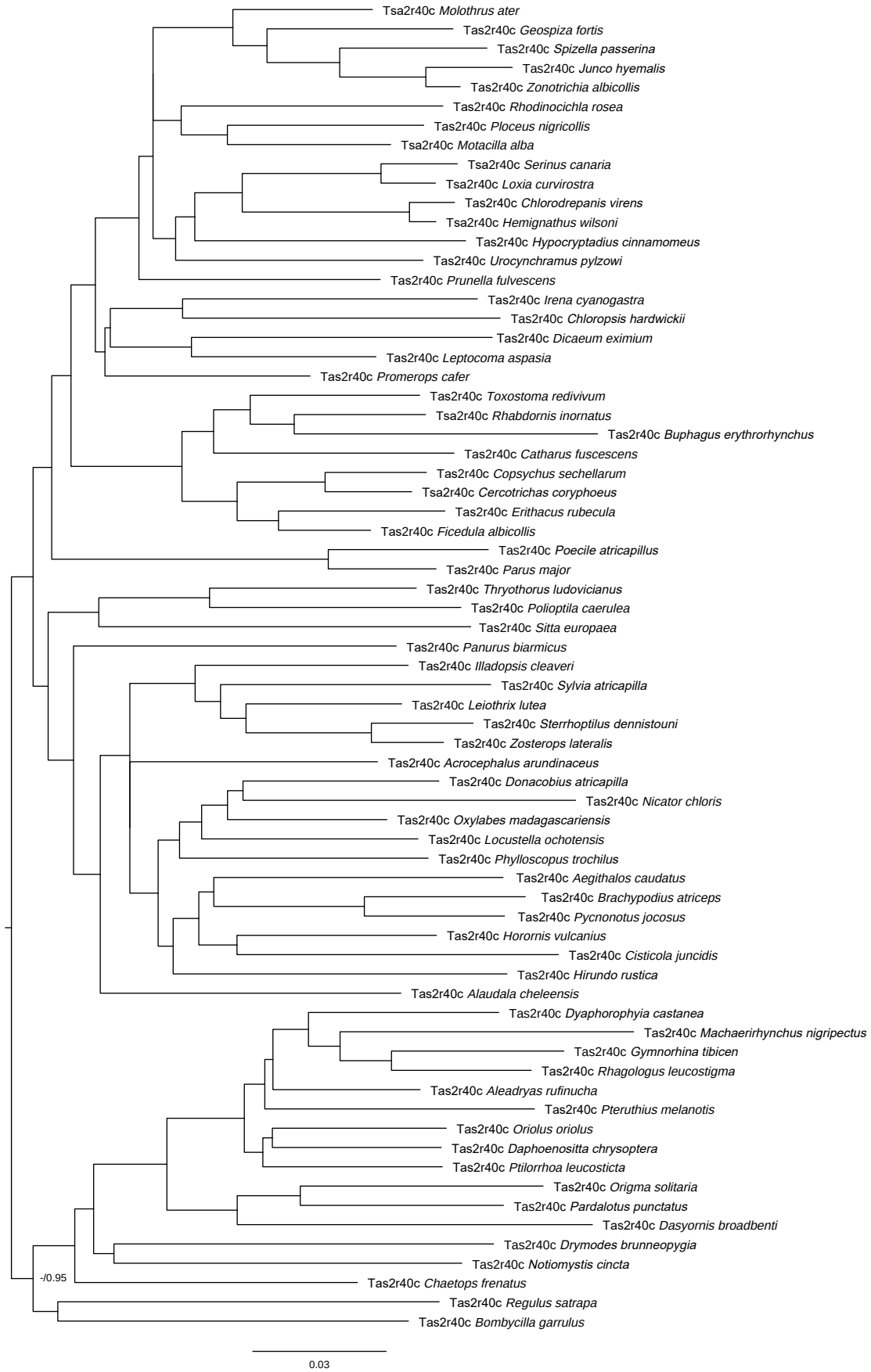


Figure 8: Maximum Likelihood tree topology of Passeri-2-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

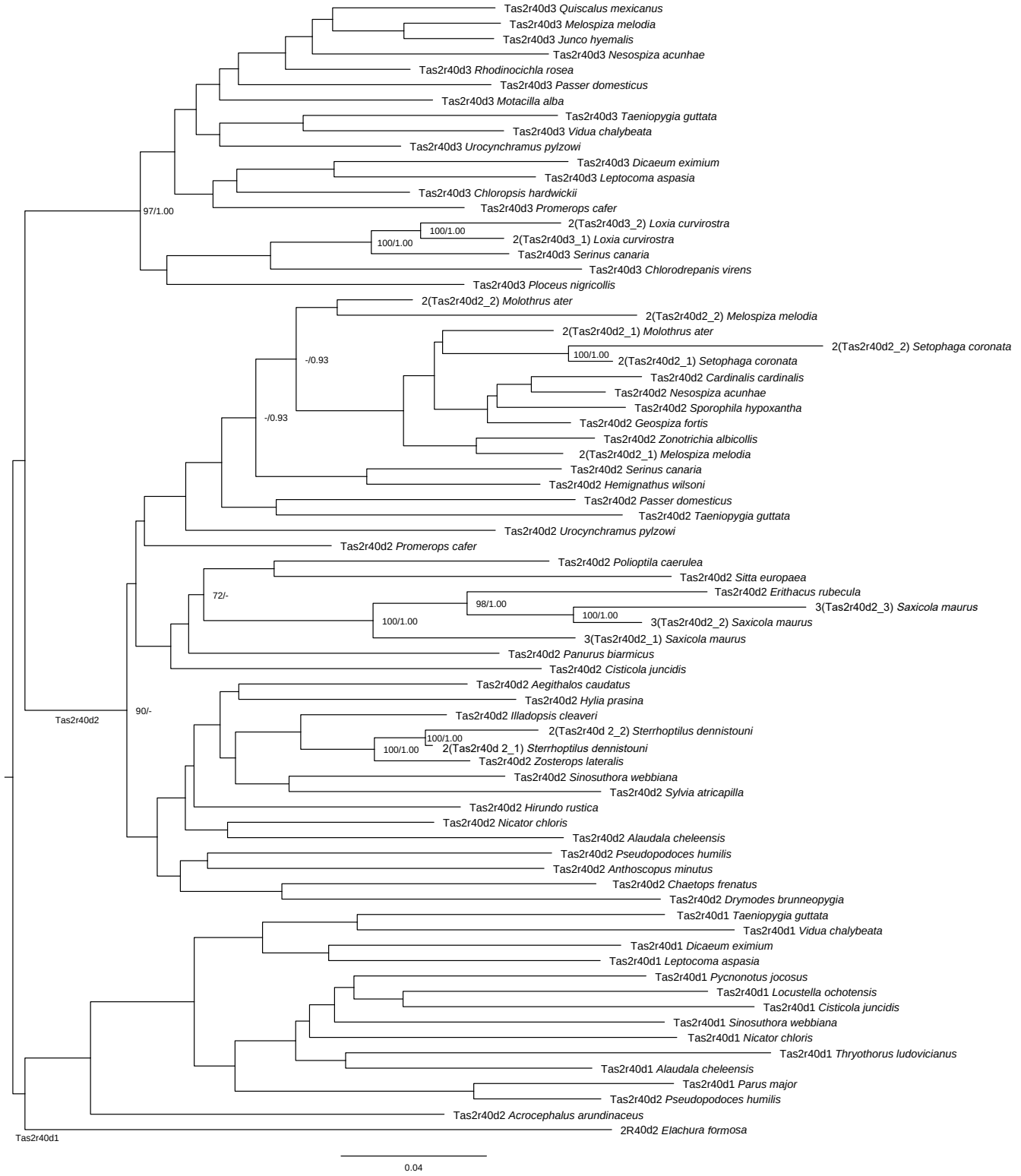


Figure 9: Maximum Likelihood tree topology of Passeri-3-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

4.2.2 Tas2r9 Trees analysis

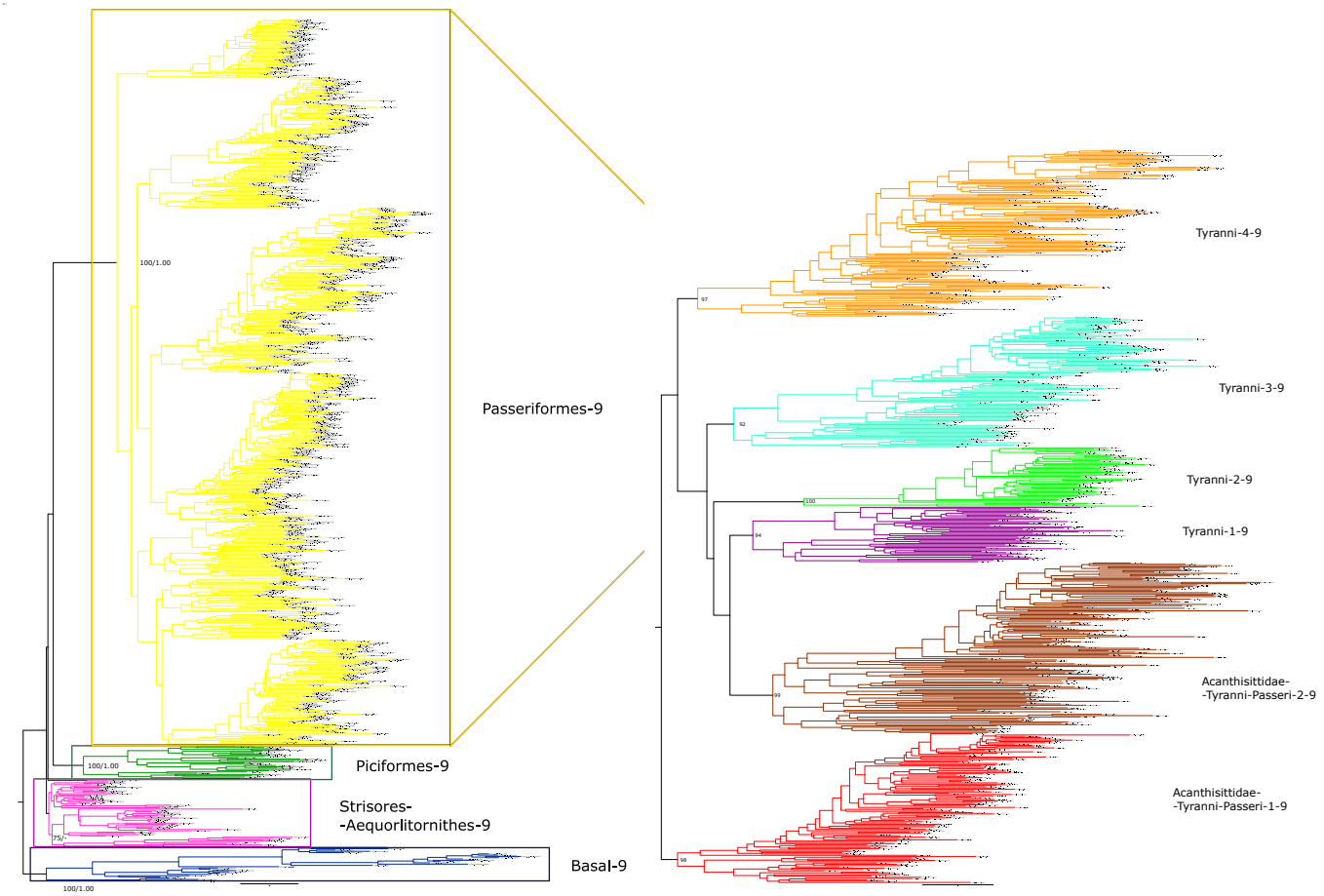
The general tree of Tas2r9 (Figure 10a) has Basal-9 group as the root of the tree, then Strisores-Aequorlitornithes-9, Piciformes-9 and, finally, the datasets formed by the sequences of the order Passeriformes (Figure 10b).

In the Basal-9 dataset (Figure 11) it is possible to verify the presence of multiple copies in Anseriformes and Galliformes orders (Table S8). These duplicates can be a result of intra-specific duplication events, like the ones observable for *Callipepla squamata*, but they can also be a result of inter-specific duplications, such as the ones seen in ansTas2r9a and ansTas2r9b for *Anas platyrynchus* and *Cairina moschata*.

The Strisores-Aequorlitornithes-9 dataset (Figure 12) shows several sets of Tas2r9 duplicates (Table S8). Some duplications took place inside some elements of Caprimulgiformes and Charadriiformes orders. In addition, we can observe that the phylogenetic position of some orders of this dataset is fragmented (Pelecaniformes and Suliformes). This may be due to lack of resolution of the clade Aequorlitornithes. Furthermore, this dataset had initially one more order, Sphenisciformes, that due to pseudogenized sequences had to be removed (Table S3).

Additionally, in Piciformes-9 (Figure 13) the most basal species (*Bucco capensis* and *Galbula dea*) present intra-specific duplications. The remaining species of this group, which belong to the Ramphastidae family, present numerous inter-specific duplications, which suggest that a duplication event occurred in ancestor of this family lineage.

In addition to these the aforementioned datasets, the Tas2r9 of the Passeriformes order were grouped into six distinct datasets. As was mentioned before, the Passeriformes order is constituted by the suborders Acanthisittidae, Tyranni (Suboscines) and Passeri (Oscines). Therefore, we divided the Tas2r9 genes into 2 groups with all the suborders of Passeriformes (Acanthisittidae-Tyranni-Passeri-1-9 and Acanthisittidae-Tyranni-Passeri-2-9) and four groups with the suborders Tyranni and Passeri (Tyranni-Passeri-1-9, Tyranni-Passeri-2-9, Tyranni-Passeri-3-9 and Tyranni-Passeri-4-9). While Acanthisittidae-Tyranni-Passeri-1-9 (Figure 14a) has 116 sequences and does not present events of duplication, inside the remaining subsets of Passeri suborders we verify the existence of multiple inter and intra-specific duplication events, with up to 10 copies in some species. Inside the Acanthisittidae-Tyranni-2-9 group (Figure 14b), the majority of species present 2 or 3 copies of Tas2r9, but *Pitta sordida* and *Xiphorhynchus elegans* present 6 copies and *Formicarius rufipectus* presents 5 copies. In the Tyranni-Passeri-1-9 dataset (Figure 15), the majority of species present a single Tas2r9 sequence, with exception of elements of Eurylaimidae, Pittidae, Thamnophilidae and Tyrannidae families, which have an extra Tas2r9 copy. In a similar way, all elements of Tyranni-Passeri-2-9 (Figure 16) present a single Tas2r9 copy with the exception of *Sakesphorus luctuosus*, that presents an additional copy. Moreover, it is possible to observe several cases of duplication in the Tyranni-Passeri-3-9 (Figure 17a) dataset (Table S8). However, all the copies found are distributed across distinct species or families, therefore suggesting the existence of independent duplication events. Finally, in Tyranni-Passeri-4-9 (Figure 17b) we detected some duplication events, with highlight in Zosterops lateralis (7 copies), Melospiza melodia (5 copies), Sterrhoptilus dennistouni (4 copies) and Serinus canaria (4 copies).



(a) (b)

Figure 10: (a) Maximum Likelihood tree topology of All-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different orders: blue for Basal-9, pink for Strisores-Aequorlitornithes-9 and yellow for Passeriformes-9. (b) Maximum Likelihood tree topology of Passeriformes-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6. Different colors represent different subsets: red for Acanthisittidae-Tyranni-Passeri-1-9, brown for Acanthisittidae-Tyranni-Passeri-2-9, purple for Tyranni-Passeri-1-9, light-green for Tyranni-Passeri-2-9, light-blue for Tyranni-Passeri-3-9 and orange for Tyranni-Passeri-4-9.

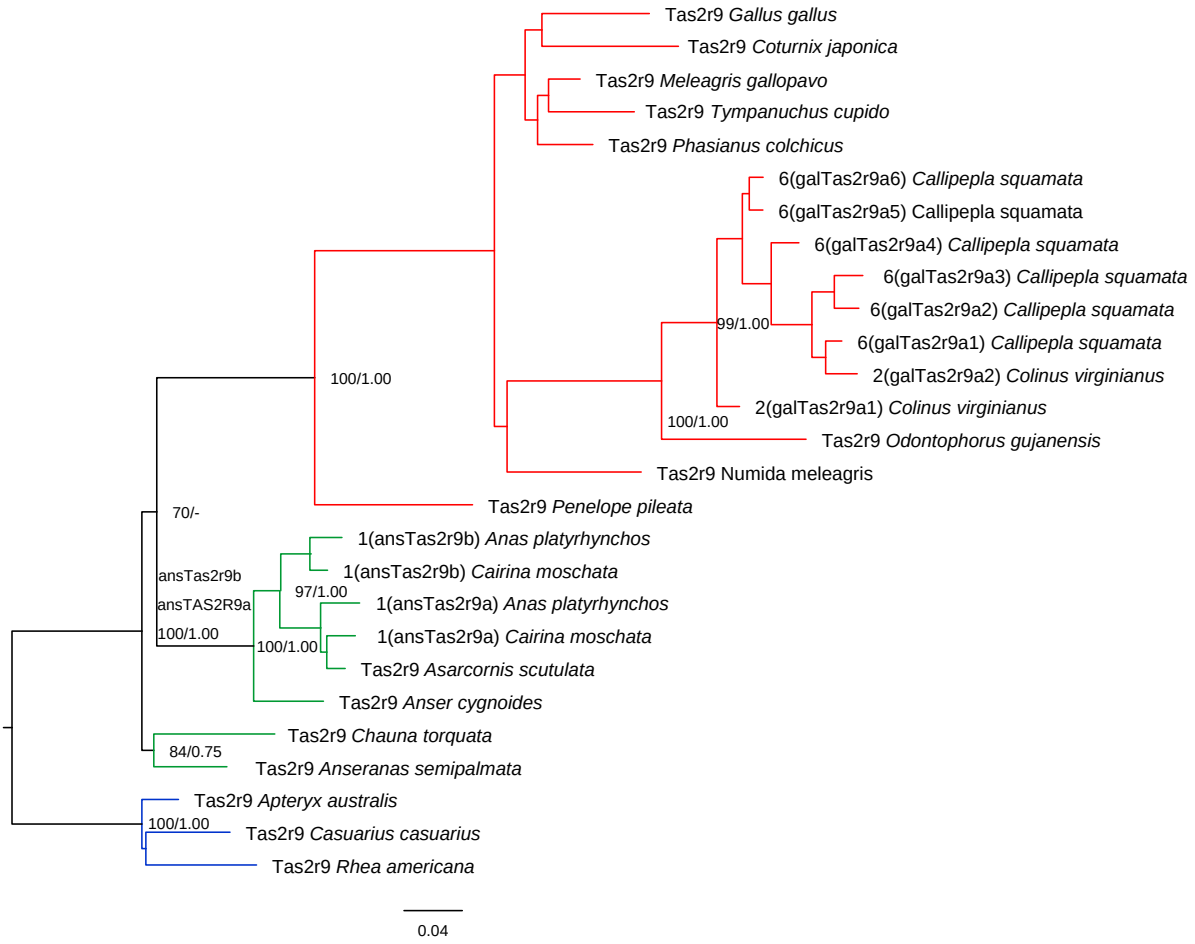


Figure 11: Maximum Likelihood tree topology of Basal-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different orders: blue for Struthioniformes, green for Anseriformes and red for Galliformes.

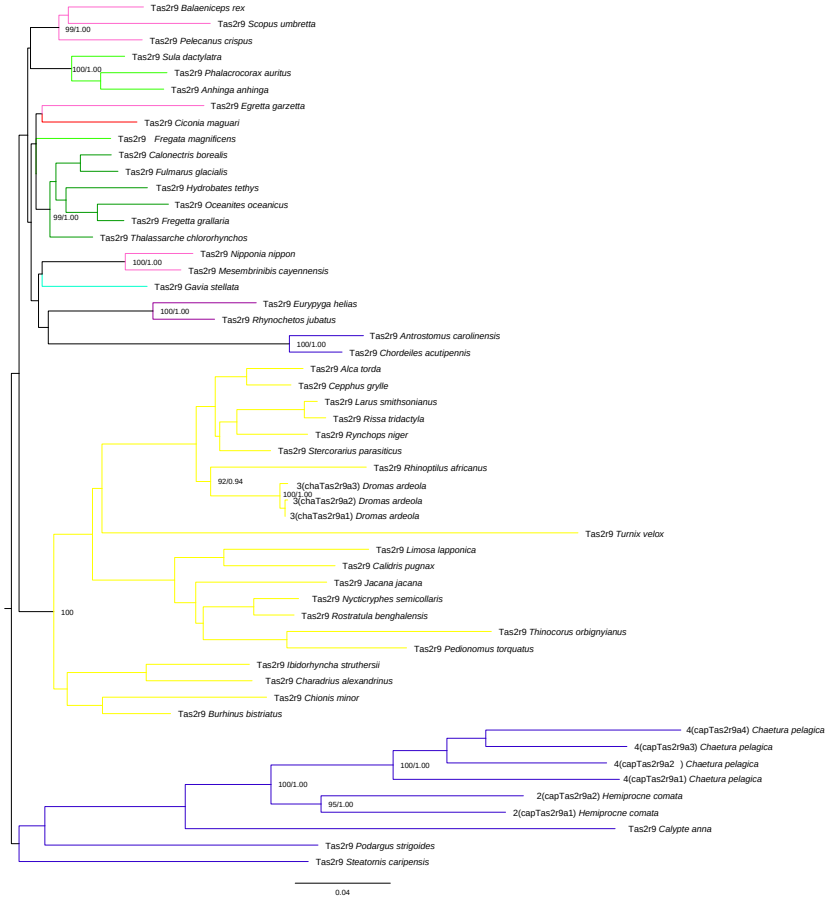


Figure 12: Maximum Likelihood tree topology of Strisores-Aequorlitornithes-40 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. Different colors represent different orders: blue for Struthioniformes, red for Ciconiiformes, green for Procellariiformes, purple for Sphenisciformes and yellow for Charadriiformes.

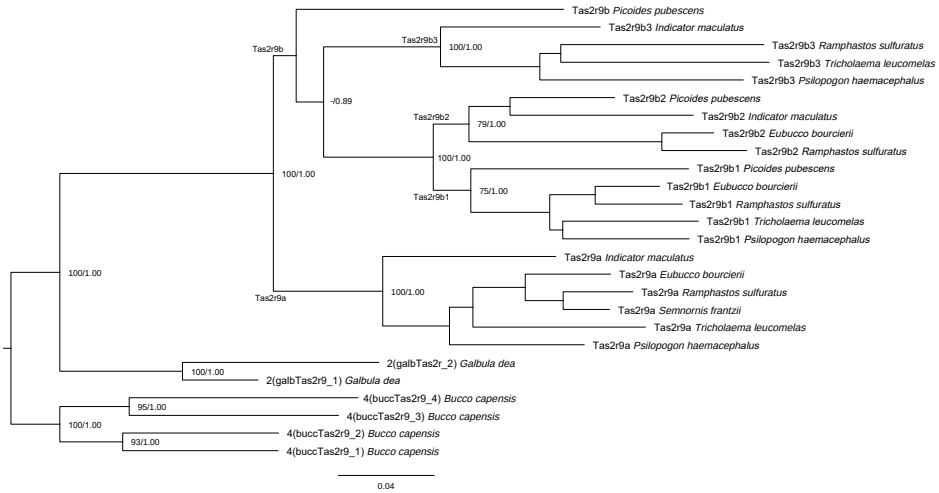


Figure 13: Maximum Likelihood tree topology of Piciformes-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

(a) (b)

Figure 14: (a) Maximum Likelihood tree topology of Acanthisittidae-Tyranni-Passeri-1-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. (b) Maximum Likelihood tree topology of Acanthisittidae-Tyranni-Passeri-2-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

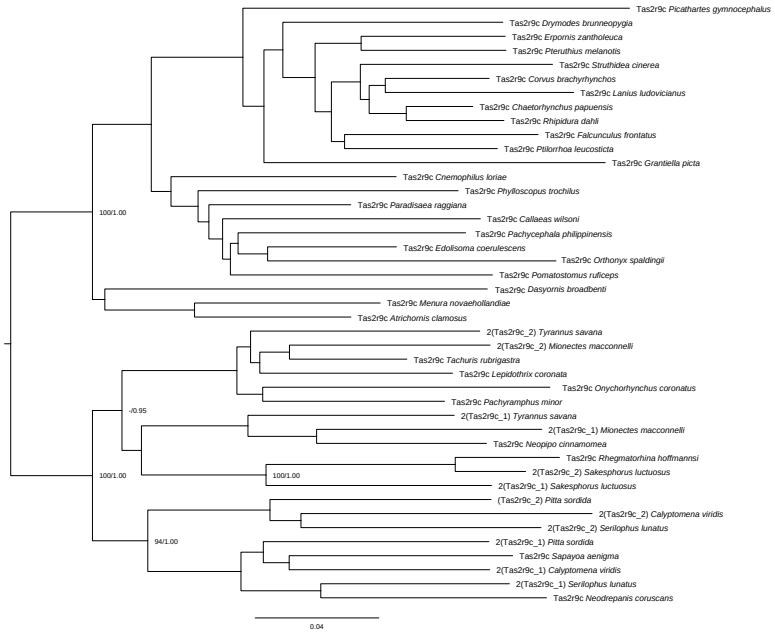


Figure 15: Maximum Likelihood tree topology of Tyranni-Passeri-1-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

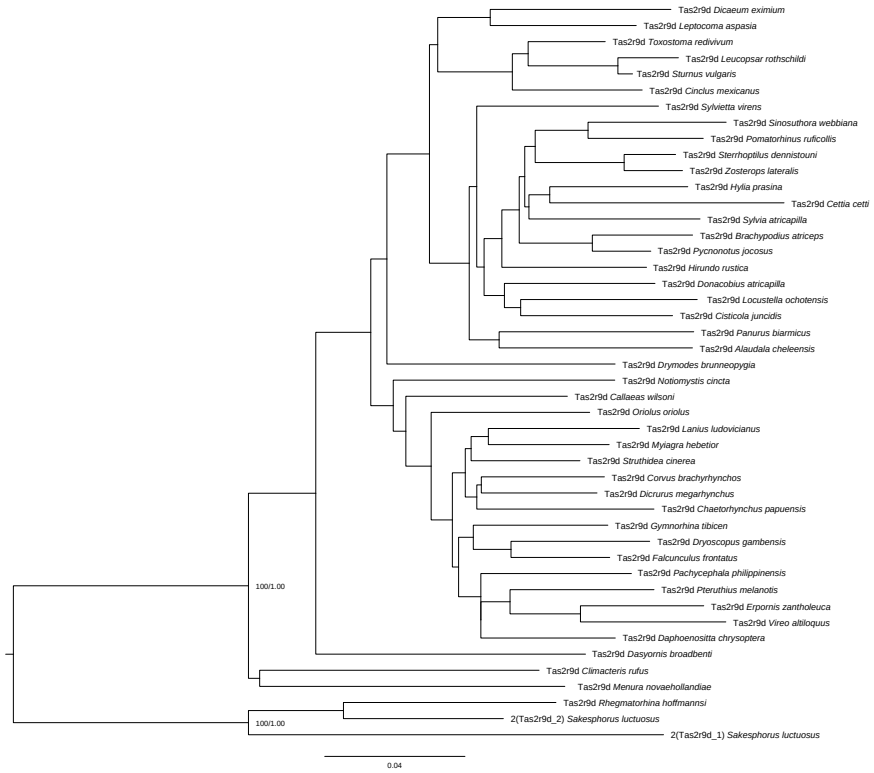
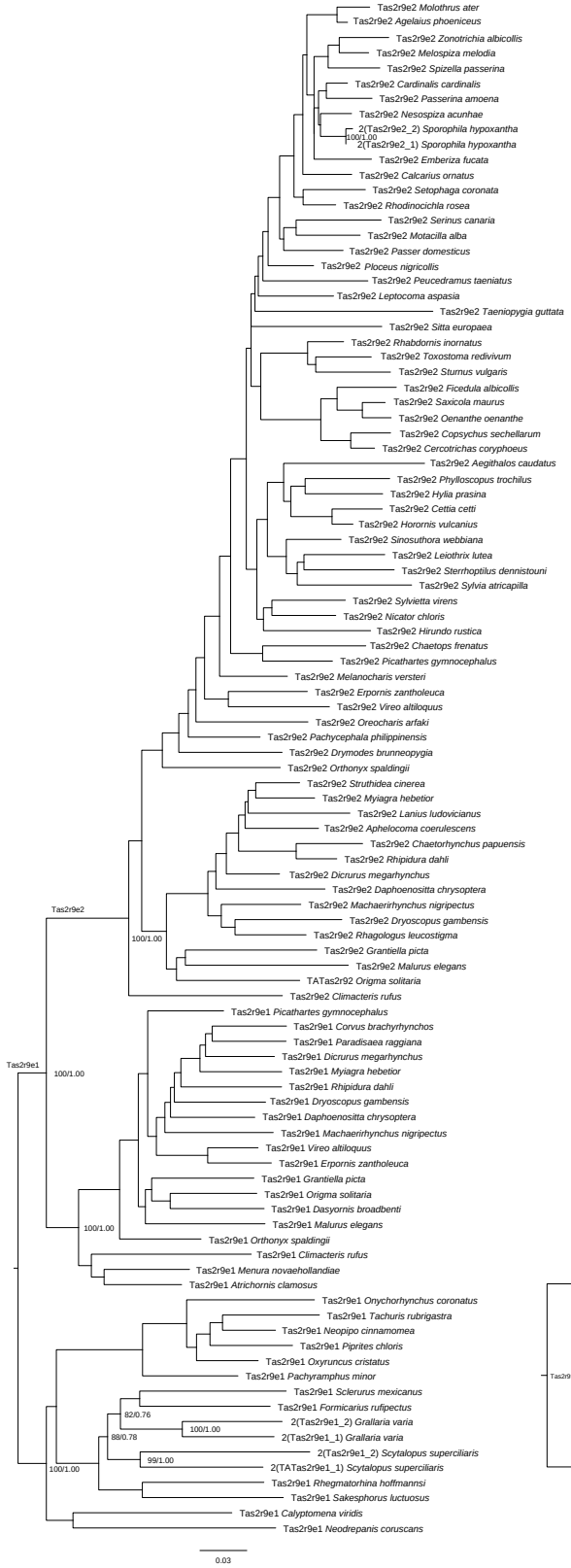


Figure 16: Maximum Likelihood tree topology of Tyranni-Passeri-2-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.



(a) (b)

Figure 17: (a) Maximum Likelihood tree topology of Tyranni-Passeri-3-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes. (b) Maximum Likelihood tree topology of Tyranni-Passeri-4-9 generated in IQ-TREE 1.6. Node support numbers represent ML bootstraps generated in IQ-TREE 1.6 and Bayesian posterior probabilities from MrBayes.

4.3 Codon level selection analysis

Considering the codon level selection analysis M8 model implemented in PAML, and also the SLAC, MEME, FEL and FUBAR approaches implemented in Datamonkey, signals of positive selection were detected in Tas2r40 and Tas2r9 subsets. As expected, the SLAC, FEL and FUBAR approaches also revealed the presence of NSS in all datasets (Figures 18b and 24b).

Despite the fact that the majority of Tas2r40 datasets presented PSS, this was not verified for the Basal-40 group (Figure 18a). It is also interesting to note that the majority of PSS were detected in Passeriformes subsets (Figure 18a). The highest ω (Figure 18c) tend to be found in datasets constituted exclusively by Passeriformes species, which suggests that Tas2r40 of this order present an acceleration of selective pressure when compared with other analyzed avian orders. Finally, the fact that there were no PSS found in Basal-40, together with the lowest ω value ($\omega = 0.632$), suggests that genes pertaining to this group present high degree of conservation.

Regarding the Tas2r9 dataset, we were able to find PSS for all subsets (Figure 24a). The Acanthisittidae-Tyranni-Passeri-2-9 and Tyranni-Passeri-4-9 subsets with 55 and 51 PSS, respectively, had the highest number of PSS. However, it was not detected high variation of ω values among all subsets (Figure 24c).

Additionally, our datasets were analyzed considering different habitats. Species that inhabit in aquatic environments (water birds) present reduced number of PSS and lower ω values when compared with species that exclusively habit in land (land birds) (Figures 20a, 20c, 26a and 26c).

Henceforth, our findings of a lower evolutionary pressure in water birds are consistent with the small number of Tas2rs found in other aquatic vertebrates such as the penguin[81] and the toothed and baleen whales[78;79], as reported in previous studies. Firstly, the bitter tastants might be diluted in the water and masked by the high concentration of sodium in the ocean[202;203]. Secondly, most of the toxic compounds are present on plants[27] and since the prevalent diet of water birds is carnivore, bitter taste perception might have been rendered useless. Our results further suggest habitat as a key factor in Tas2r evolution.

In regard to the migratory preference, when comparing migratory species with the non-migratory ones, the first show a lower number of PSS and ω value in both analyzed gene families (Figures 22a, 22c, 28a and 28c). Opposed to migratory birds, non-migratory birds need to adapt to low food abundance and worse climate conditions during their nomadic lifestyle[93]. This adaptation may justify the acceleration of selective pressure.

Protter was used to map the PSS identified by Datamonkey and PAML on the two-dimensional protein structures (Figures 19, 21, 23, 25, 27 and 29). In all the former analyzed datasets, the majority of detected PSS are located in the transmembrane region. This is an interesting result since the transmembrane domain (as also intracytoplasmatic loops) have the highest degree of sequence conservation while the most variable region of proteins tend to be the extracellular loops and amino- and carboxy- terminal regions. Even though the functions of intracellular and extracellular regions of T2R proteins are still broadly undiscovered, it is predicted that the intracytoplasmic loops are the sites of G-protein interaction, the extracellular regions are the regions of ligand binding and the transmembrane region have the role to anchor and stabilize the helical conformation of T2R[11;58].

It has been hypothesized that a conserved LXXSL motif, where L = leucine, X = any amino acid and S = serine, has an important structural role of stabilizing the fifth transmembrane helix at the cytoplasmatic end[204]. However, in the majority of analyzed datasets we detected positively selected sites in this putative highly conserved motif.

In a former study it was reported the presence of conserved cysteine residues within the extracellular domain[30]. However, the conserved cysteines present in our sequences are essentially located in transmembrane or intracelullar domains.

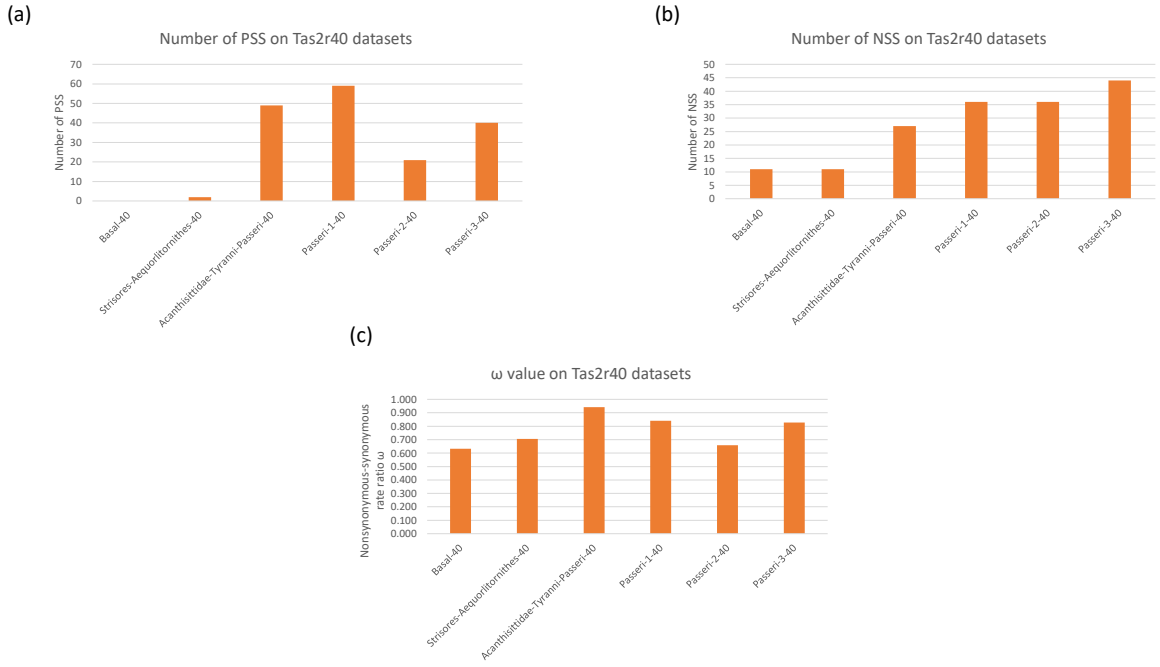


Figure 18: Codon level selection analysis on Tas2r40 datasets. (a) Number of PSS identified on Tas2r40 datasets by various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number of negative selected sites identified on Tas2r40 datasets by an integrative approach of various methods (SLAC, FEL, MEME and FUBAR). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r40 datasets by a consensus methodology of various methods (SLAC, FEL, FUBAR, MEME and PAML).

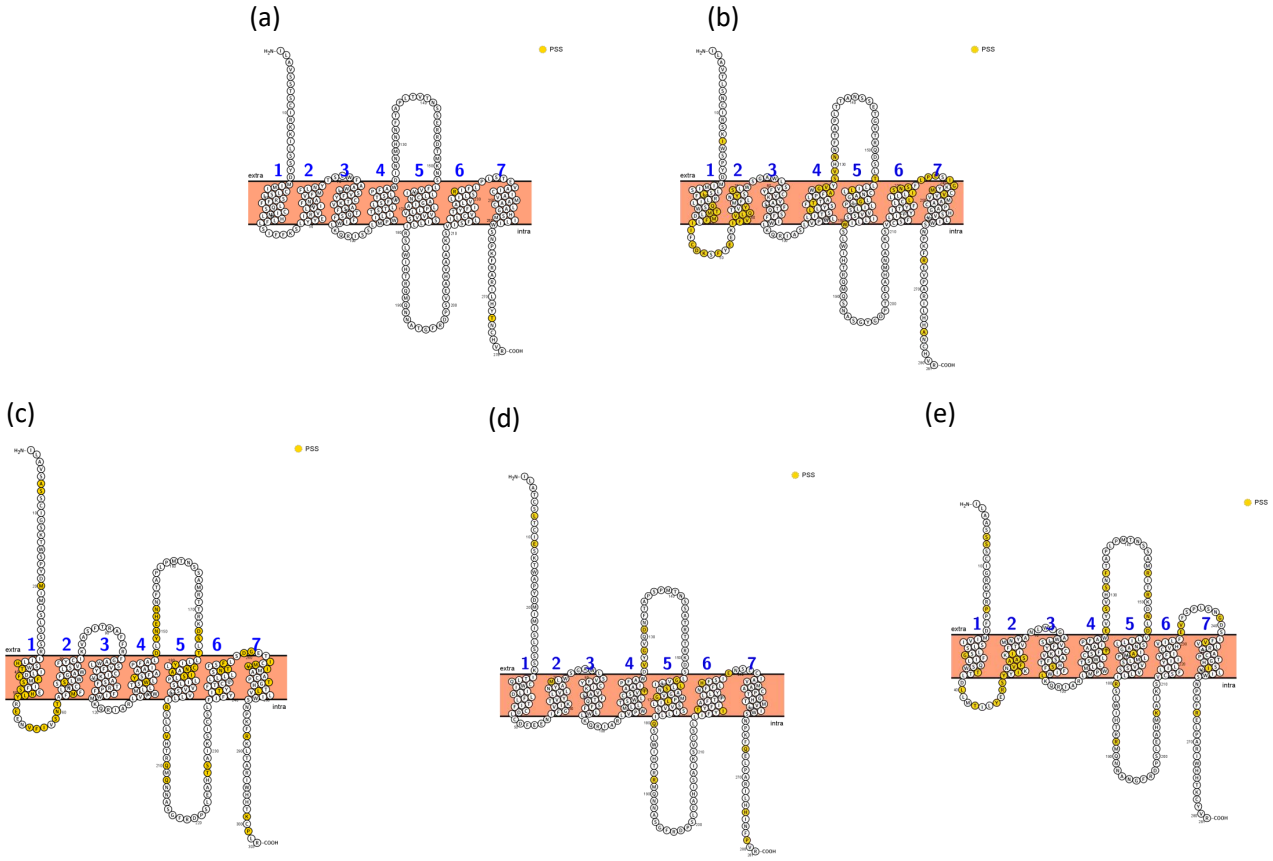


Figure 19: Taste receptor structural prediction with sites under diversifying selection (yellow) on Tas2r40 datasets. (a) Representation of Strisores-Aequorlitornithes-40. (b) Representation of Acanthisittidae-Tyranni-Passeri-40. (c) Representation of Passeri-1-40. (d) Representation of Passeri-2-40. (e) Representation of Passeri-3-40. There is supposed to be a positive selected site between the residues 158 and 159 which was detected by both methods; however, we were not able to identify it with Protter.

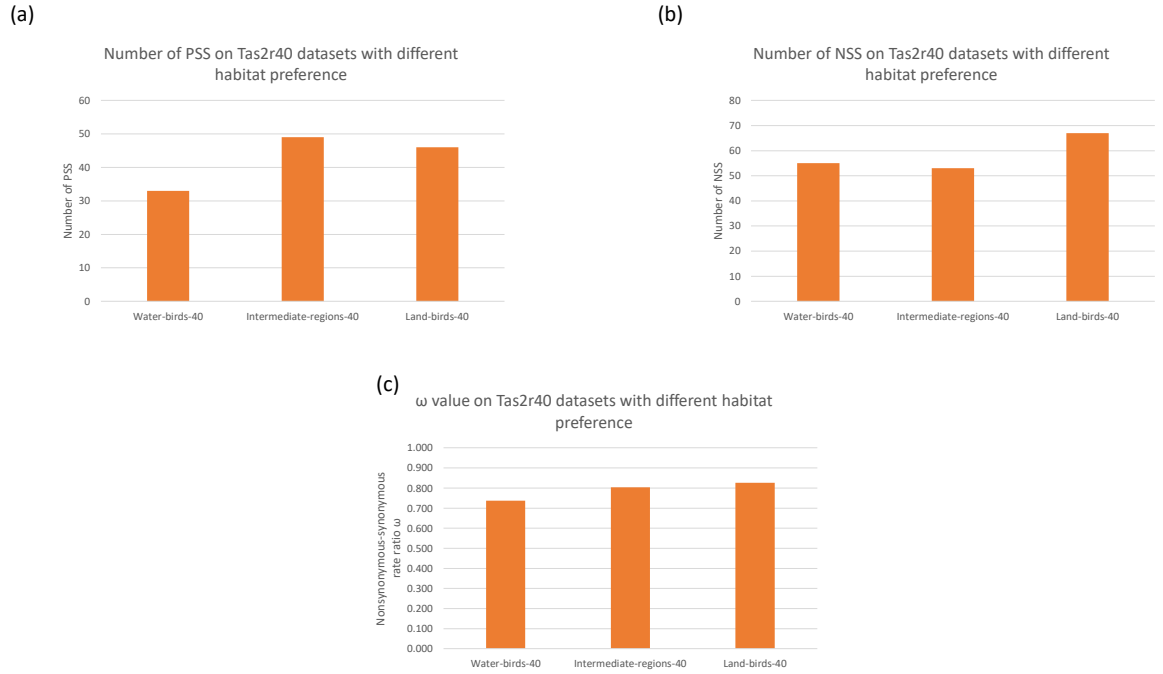


Figure 20: Codon level selection analysis on Tas2r40 datasets with different habitat preference. (a) Number PSS identified on Tas2r40 datasets with different habitat preference by an integrative response with various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number negative selected sites identified on Tas2r40 datasets with different habitat preference by an integrative response with various methods (SLAC, FEL, FUBAR and PAML). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r40 datasets with different habitat preference by an integrative approach of various methods (SLAC, FEL, MEME, and PAML).

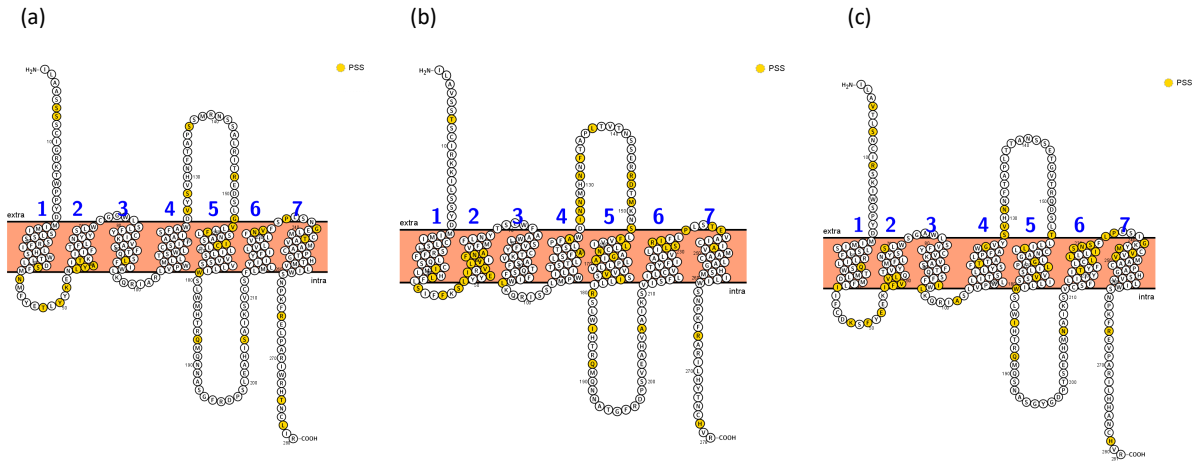


Figure 21: Taste receptor structural prediction with sites under diversifying selection (yellow) on Tas2r40 datasets with different habits. (a) Representation of Water-birds-40. There is supposed to be a positive selected site between the residues 130 and 131 which was detected by both methods; however, we were not able to identify it with Protter. (b) Representation of Intermediate-regions-40. (c) Representation of Land-birds-40.

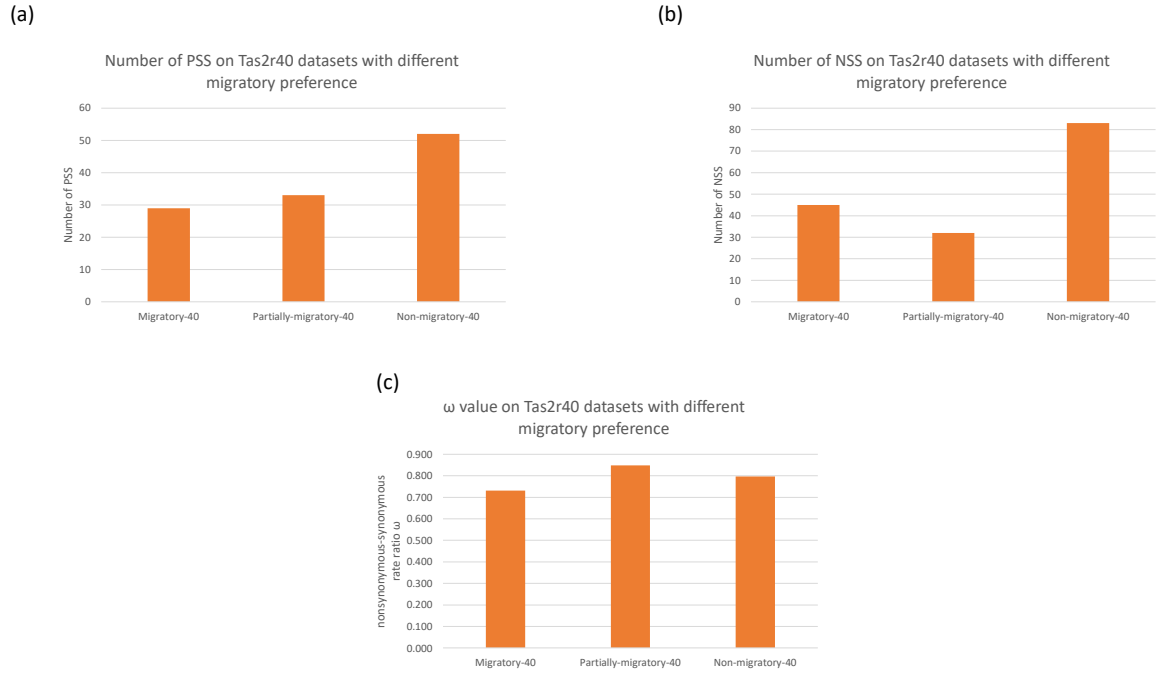


Figure 22: Codon level selection analysis on Tas2r40 datasets with different migratory preference. (a) Number PSS identified on Tas2r40 datasets with different migration preference by an integrative response with various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number negative selected sites identified on Tas2r40 datasets with different migration preference by an integrative response with various methods (SLAC, FEL, FUBAR and PAML). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r40 datasets with different migration preference by an integrative approach of various methods (SLAC, FEL, MEME and PAML).

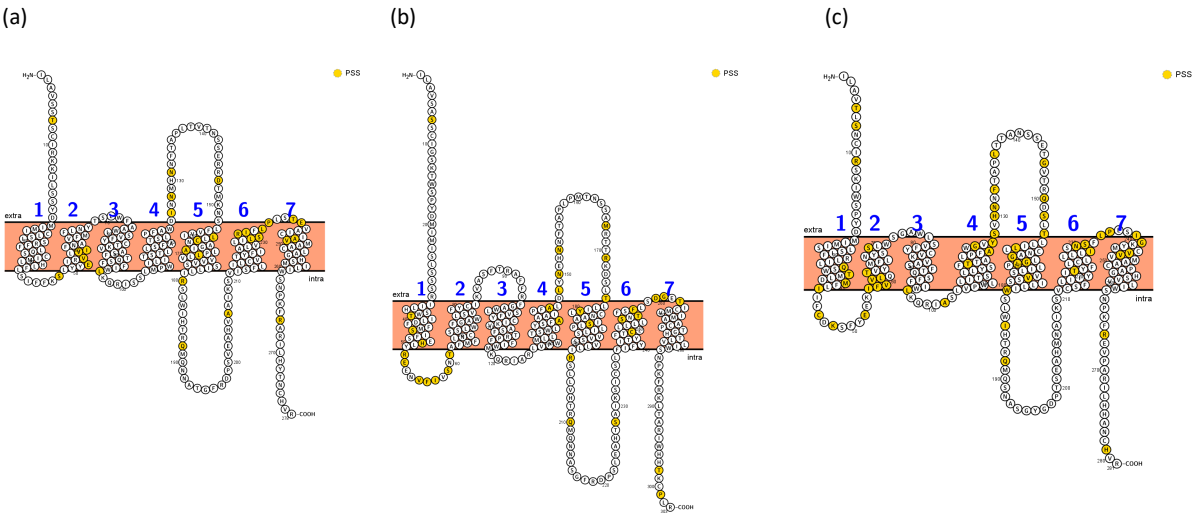


Figure 23: Taste receptor structural prediction with sites under diversifying selection (yellow) on Tas2r40 datasets with different migratory preference. (a) Representation of Migratory-40. (b) Representation of Partially-migratory-40. (c) Representation of Non-migratory-40.

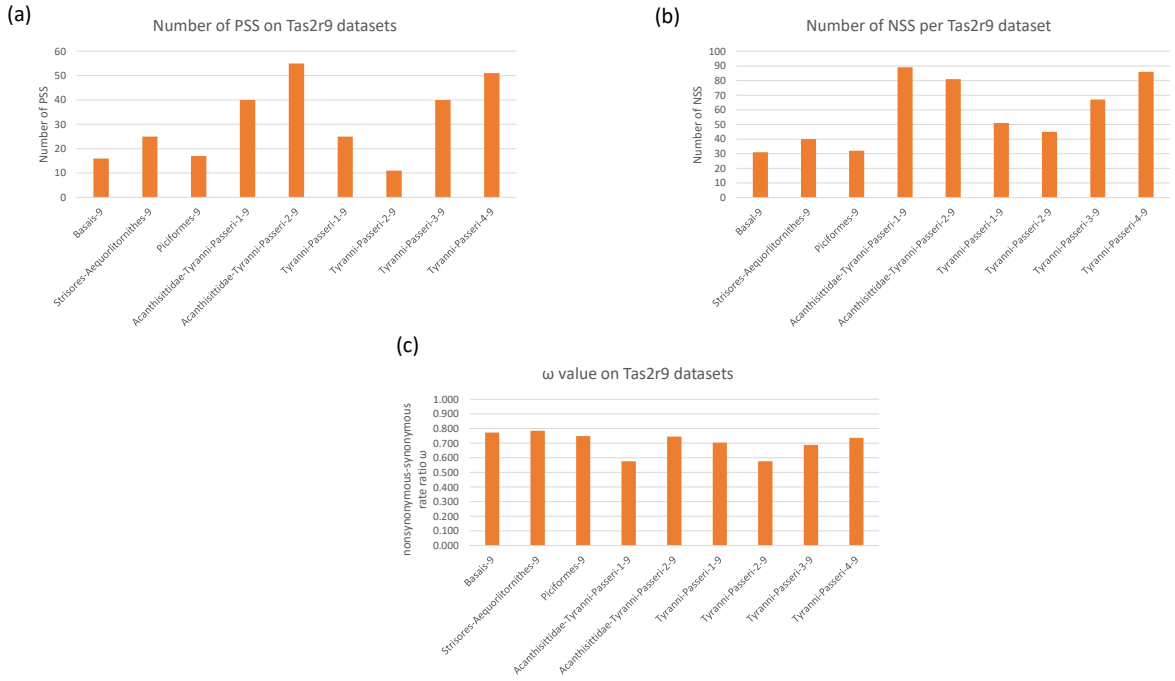


Figure 24: Codon level selection analysis on Tas2r9 datasets. (a) Number of PSS identified on Tas2r9 datasets by various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number of negative selected sites identified on Tas2r9 datasets by an integrative approach of various methods (SLAC, FEL, MEME and FUBAR). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r40 datasets by an integrative approach of various methods (SLAC, FEL, FUBAR, MEME and PAML).

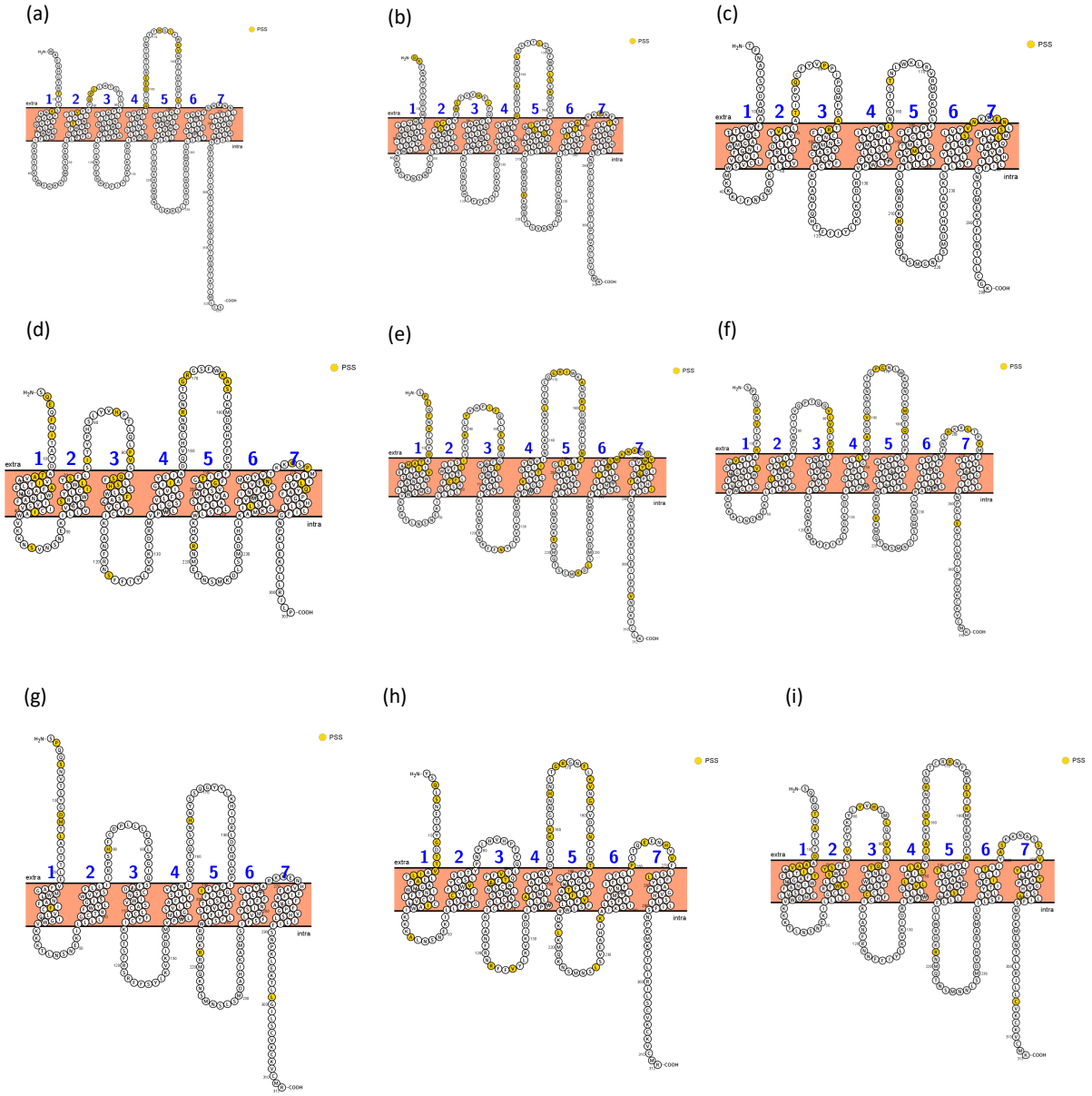


Figure 25: Taste receptor structural prediction with sites under diversifying selection (yellow) on Tas2r9 datasets. (a) Representation of Basal-9. (b) Representation of Strisores-Aequorlitornithes-9. (c) Representation of Piciformes-9. (d) Representation of Acanthisittidae-Tyranni-Passeri-1-9. (e) Representation of Acanthisittidae-Tyranni-Passeri-2-9. (f) Representation of Tyranni-Passeri-1-9. (g) Representation of Tyranni-Passeri-2-9. (h) Representation of Tyranni-Passeri-3-9. (i) Representation of Tyranni-Passeri-4-9.

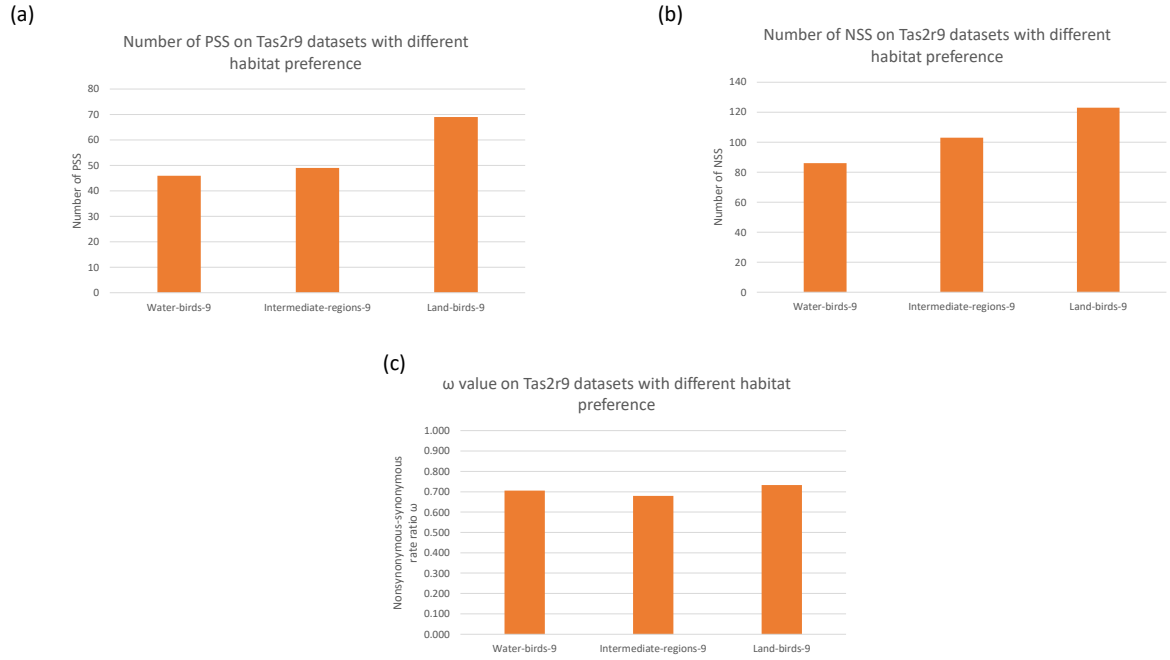


Figure 26: Codon level selection analysis on Tas2r9 datasets with different habitat preference. (a) Number of PSS identified on Tas2r9 datasets with different habitat preference by an integrative response with various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number of negative selected sites identified on Tas2r9 datasets with different habitat preference by an integrative response with various methods (SLAC, FEL, FUBAR and PAML). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r9 datasets with different habitat preference by an integrative approach of various methods (SLAC, FEL, MEME, and PAML).

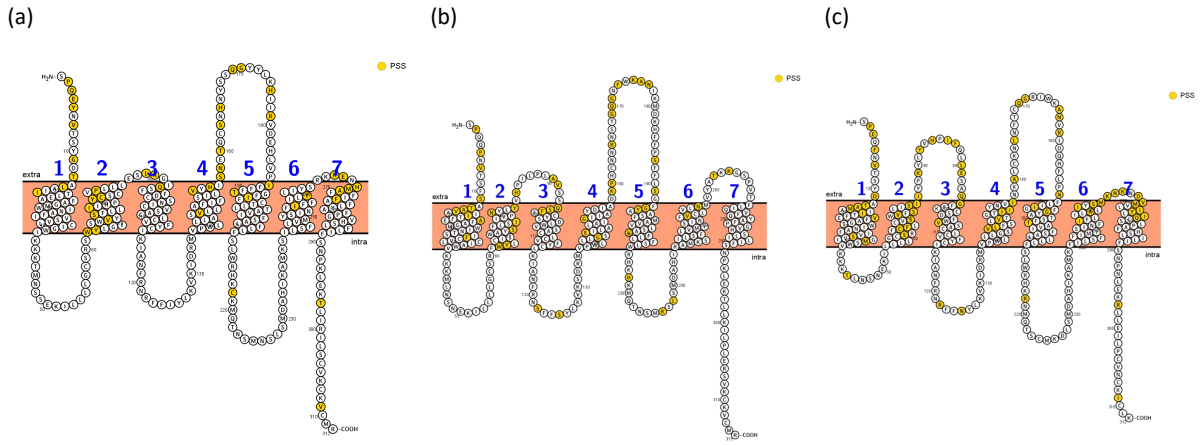


Figure 27: Taste receptor structural prediction with sites under diversifying selection (yellow) on dataset Tas2r9 with different habits. (a) Representation of Water-birds-9. (b) Representation of Intermediate-regions-9. (c) Representation of Land-birds-9.

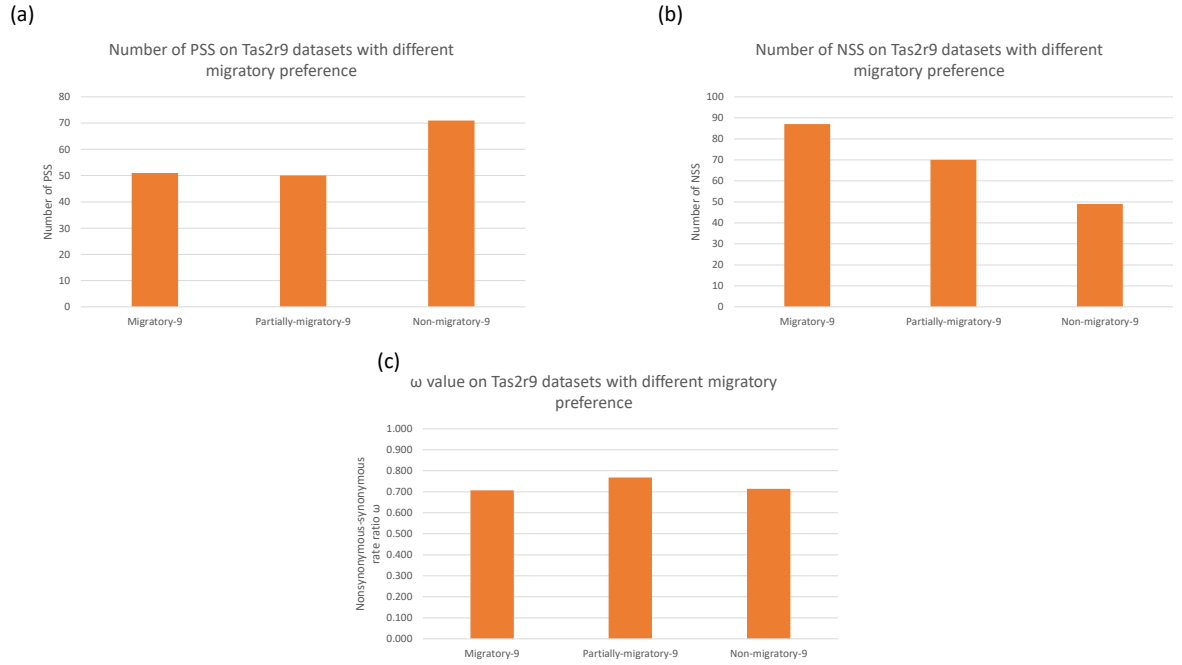


Figure 28: Codon I on Tas2r9 datasets with different migratory preference. (a) Number of PSS identified on Tas2r9 datasets with different migration preference by an integrative response with various methods (SLAC, FEL, FUBAR, MEME and PAML). (b) Number of negative selected sites identified on Tas2r9 datasets with different migration preference by an integrative response with various methods (SLAC, FEL, FUBAR and PAML). (c) Nonsynonymous-synonymous rate ratio ω on Tas2r9 datasets with different migration preference by an integrative approach of various methods (SLAC, FEL, MEME and PAML).

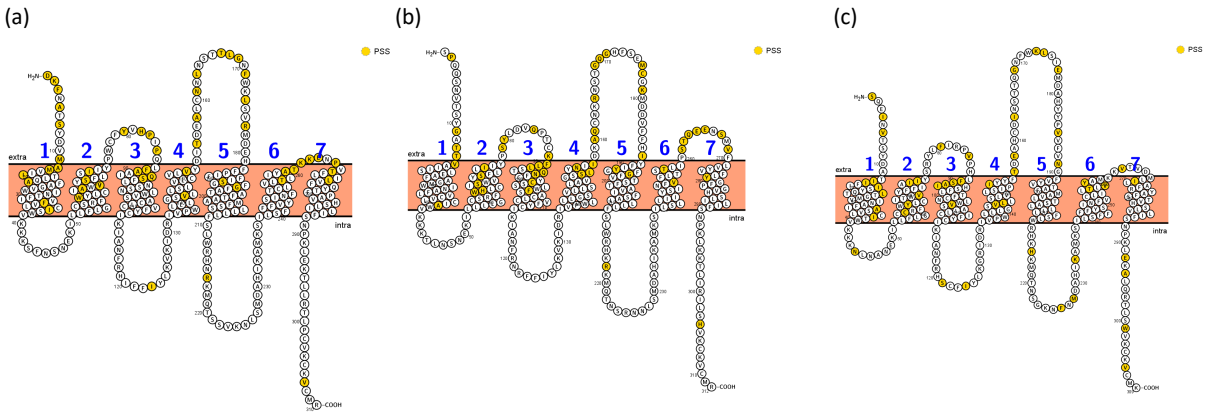


Figure 29: Taste receptor structural prediction with sites under diversifying selection (yellow) on dataset Tas2r9 with different migratory preference. (a) Representation of Migratory-9. (b) Representation of Partially-migratory-9. (c) Representation of Non/Migratory-9.

4.4 Branch selection analysis of Tas2r40 and Tas2r9 dataset

To assess the impact of migratory and habitat preference on Tas2r40 and Tas2r9, we performed branch-specific selection analysis, marking with distinct labels the ecological preference of the bird species used in this study.

Considering the habitat preference, in Tas2r40 and Tas2r9 datasets, we could verify that, despite very similar ω values, water birds tend to have a lower ω value than the species which live on land or intermediate regions (Figures 30a and 31a). This difference is more accentuated in Tas2r40 than in Tas2r9. These results seem to be in agreement with the codon level selection study.

Regarding the migratory preference, Tas2r40 and Tas2r9 (Figures 30b and 31b, respectively) do not show a big disparity among the different labels, but the non-migratory birds have the lowest ω value when compared with the others. These results are in accordance with the ones obtained by the codon level selection study.

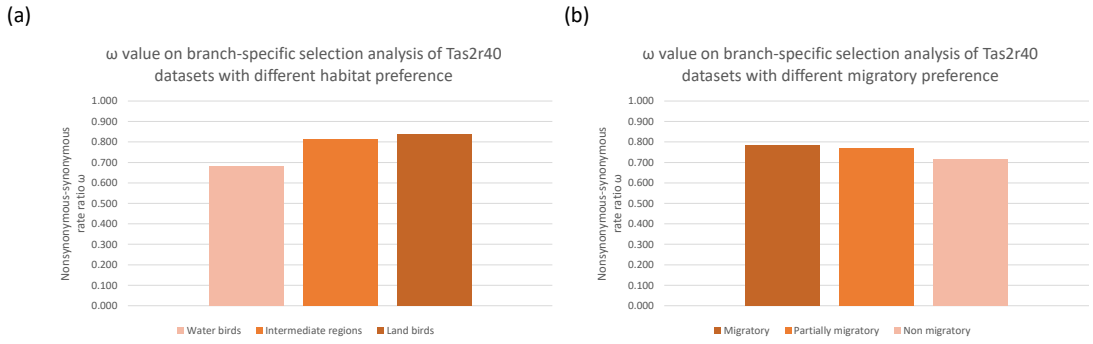


Figure 30: Branch-specific selection analysis of Tas2r40 datasets with different habiatat or migratory preference by branch-specific selection analysis. (a) Nonsynonymous-synonymous rate ratio ω of Tas2r40 datasets with different habitat preference by branch-specific selection analysis. (b) Nonsynonymous-synonymous rate ratio ω of Tas2r40 datasets with different migratory preference by branch-specific selection analysis.

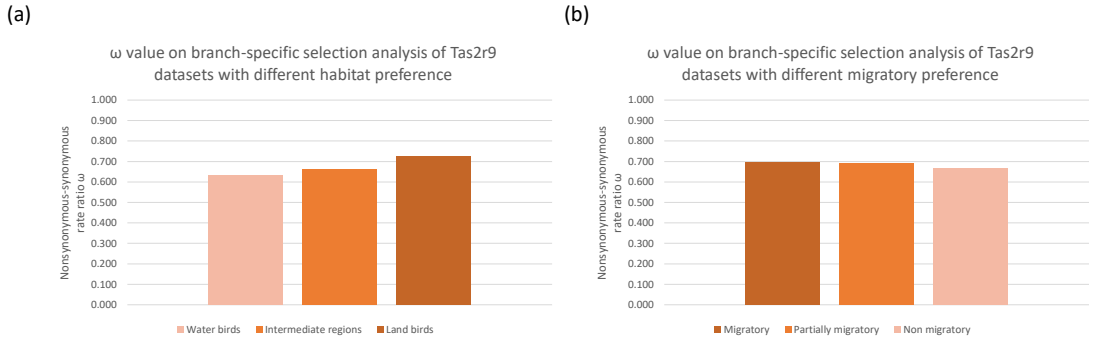


Figure 31: Branch-specific selection analysis of Tas2r9 datasets with different habiatat or migratory preference by branch-specific selection analysis. (a) Nonsynonymous-synonymous rate ratio ω of Tas2r9 datasets with different habitat preference by branch-specific selection analysis. (b) Nonsynonymous-synonymous rate ratio ω of Tas2r9 datasets with different migratory preference by branch-specific selection analysis.

5

CONCLUSIONS AND FURTHER WORK

Birds are established as important models of evolutionary studies given their global distribution and abundance when compared to other vertebrates[2].

Moreover, avians guide their behaviour by the stimuli detected by their senses, making them very important tools for survival[3]. Of these senses, taste is the one dedicated to regulate the feeding behavior. Bitter taste is specially important since it not only identifies food sources, but it also detects toxic compounds in very small concentrations to avoid potential lethal consequences[3]. The transduction of bitter tastants involves the activation of T2R, a member of the GPCR family[26].

To study the repertoire of Tas2r on birds, we used an integrative approach of comparative evolutionary-genomics and phylogeny-based methodologies in 245 avian species that allows the evaluation of selective pressures acting on bitter receptors[137].

This study revealed order-specific differences in the distribution of Tas2r40 and Tas2r9 among avians. Tas2r9 is more widespread than Tas2r40 and also possesses more duplicates.

Despite the fact that most birds present a single copy of Tas2r40, exceptions were detected in order Passeriformes (Acanthisittidae-Tyranni-Passeri-40, Passeri-1-40 and Passeri-3-40). Additionally, the datasets of this avian order present the highest number of PSS and ω value. It is interesting to note that the datasets with the highest ω values are the same datasets that present duplication events.

There are multiple copies of Tas2r9 in several orders, such as Anseriformes, Galliformes, Caprimulgiformes, Charadriiformes, Piciformes and Passeriformes. Considering this sub-family of taste receptors, the highest numbers of PSS were found for datasets with sequences of the Passeriformes order (Acanthisittidae-Tyranni-Passeri-2-9 and Tyranni-Passeri-4-9). Despite the fact that Tas2r9 seems to be under a greater evolutionary pressure in the order Passeriformes, this pressure is apparently felt in other orders as well.

The order Passeriformes has the highest number of sequences, as also has the largest quantity of duplicates and pseudogenized species. It had been previously reported that chemosensory pseudogenes probably embody a variability source that is related with each species preferences[28]. The large quantity of duplicates and existence of pseudogenes have a substantial contribution in evolutionary diversification by providing new genetic material through different mechanisms, which may result in new gene functions[206].

Our results of selection analysis indicate that, generally, an acceleration of selective pressure is detected in Passeriformes birds, especially in Tas2r40. This evolutionary pressure might explain why Passeriformes is the most diverse group of birds[2]. Also of note, Tas2r40 on basal species is the

only dataset without PSS and has the lowest ω value, indicating that these genes are under a more conserved evolutionary pressure.

To investigate how habitat preference influences the evolution of Tas2r, we conducted selection analysis at codon level and branch level considering distinct bird habitats. All approaches revealed that Tas2r of water birds have lowest ω values and reduced number of PSS when compared with birds inhabiting land regions. We hypothesized that the conservation of Tas2r in aquatic birds may be related with the dilution of tastants in water. Therefore, habitat poses as a significant factor of Tas2r evolution.

We additionally investigated the implications of different types of migration behaviours on Tas2r evolution. Our results indicate that Tas2r show a stronger evolutionary pressure on non-migratory birds, which could be related with the necessity of these nomadic birds to adapt to ecological alterations in their habitats.

Regarding the results of our 2D analysis, the PSS found do not seem to be located in regions of ligand recognition. However, further strategies to obtain a 3D structure by remote modelling homology will clarify the role of selected residues and unreveal the interactions between residues.

In a final stage of this work, the genomes assemblies of the species herein used was concluded, enabling further future work on the evaluation of the synteny of the genes analyzed, which might clarify the complex duplication patterns reported.

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SUPPLEMENTAL MATERIAL

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Table S1: List of species used in this work and its corresponding number of Tas2r40 and Tas2r9 genes and pseudogenes, habitat and migratory preference

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Acanthisitta chloris	Acanthisittidae	Passeriformes	2	4	Land birds	Non-migratory
Acrocephalus arundinaceus	Sylviidae	Passeriformes	2	3	Water birds	Migratory
Aegithalos caudatus	Aegithalidae	Passeriformes	2	2	Intermediate regions	Non-migratory
Agelaius phoeniceus	Icteridae	Passeriformes		3	Water birds	Migratory
Alaudala cheleensis	Alaudidae	Passeriformes	3	1	Water birds	Partially Migratory
Alca torda	Alcidae	Charadriiformes	(1)	1	Water birds	Migratory
Aleadryas rufinucha	Pachycephalidae	Passeriformes	1	2	Land birds	Non-migratory
Alectura lathami	Megapodiidae	Galliformes	1		Land birds	Non-migratory
Anas platyrhynchos	Anatidae	Anseriformes	1	2	Water birds	Partially Migratory
Anhinga anhinga	Anhingidae	Suliformes		1	Water birds	Non-migratory
Anser cygnoides	Anatidae	Anseriformes	1	1	Water birds	Migratory
Anseranas semipalmata	Anseranatidae	Anseriformes		1	Water birds	Partially Migratory
Anthoscopus minutus	Remizidae	Passeriformes	1	2	Water birds	Non-migratory
Antrostomus carolinensis	Caprimulgidae	Caprimulgiformes	1	1	Intermediate regions	Migratory
Aphelocoma coerulescens	Corvidae	Passeriformes	2	3	Intermediate regions	Non-migratory
Aptenodytes forsteri	Spheniscidae	Sphenisciformes	1		Water birds	Partially Migratory
Apteryx australis	Apterygidae	Struthioniformes	(1)	1	Land birds	Non-migratory
Arenaria interpres	Scolopacidae	Charadriiformes	1		Water birds	Migratory
Asarcornis scutulata	Anatidae	Anseriformes	1	1	Water birds	Non-migratory
Atrichornis clamosus	Atrichornithidae	Passeriformes		3	Intermediate regions	Non-migratory
Balaeniceps rex	Balaenicipitidae	Pelecaniformes		1	Water birds	Non-migratory
Bombycilla garrulus	Bombycillidae	Passeriformes	1	2	Land birds	Migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Brachypodius atriceps	Pycnonotidae	Passeriformes	1	4	Land birds	Non-migratory
Bucco capensis	Bucconidae	Piciformes		4	Land birds	Non-migratory
Buphagus erythrorhynchus	Sturnidae	Passeriformes	1		Water birds	Non-migratory
Burhinus bistriatus	Burhinidae	Charadriiformes	1	1	Water birds	Non-migratory
Cairina moschata	Anatidae	Anseriformes	1	2	Water birds	Non-migratory
Calcarius ornatus	Emberizidae	Passeriformes		4	Water birds	Migratory
Calidris pugnax	Scolopacidae	Charadriiformes	1	1	Water birds	Migratory
Callaeas wilsoni	Callaeatidae	Passeriformes	2	5	Land birds	Non-migratory
Callipepla squamata	Odontophoridae	Galliformes		6	Water birds	Non-migratory
Calonectris borealis	Procellariidae	Procellariiformes		1	Water birds	Migratory
Calypte anna	Trochilidae	Caprimulgiformes		1	Intermediate regions	Partially Migratory
Calyptomena viridis	Eurylaimidae	Passeriformes		6	Land birds	Non-migratory
Campylorhamphus procurvoides	Dendrocolaptidae	Passeriformes	1	1	Land birds	Non-migratory
Cardinalis cardinalis	Cardinalidae	Passeriformes	1	4	Intermediate regions	Non-migratory
Casuarius casuarius	Casuariidae	Struthioniformes	1	1	Land birds	Non-migratory
Catharus fuscescens	Turdidae	Passeriformes	1	1	Land birds	Migratory
Cepphus grylle	Alcidae	Charadriiformes	1	1	Water birds	Migratory
Cercotrichas coryphoeus	Muscicapidae	Passeriformes	1	3	Intermediate regions	Non-migratory
Certhia brachydactyla	Certhiidae	Passeriformes		2	Land birds	Non-migratory
Cettia cetti	Sylviidae	Passeriformes		4	Intermediate regions	Non-migratory
Chaetops frenatus	Turdidae	Passeriformes	3	3	Water birds	Non-migratory
Chaetorhynchus papuensis	Dicruridae	Passeriformes		4	Land birds	Non-migratory
Chaetura pelagica	Apodidae	Caprimulgiformes		4	Land birds	Migratory
Charadrius alexandrinus	Charadriidae	Charadriiformes	1	1	Water birds	Partially Migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Chauna torquata	Anhimidae	Anseriformes	1	1	Water birds	Non-migratory
Chionis minor	Chionidae	Charadriiformes		1	Water birds	Partially Migratory
Chlorodrepanis virens	Fringillidae	Passeriformes	2	1	Land birds	Non-migratory
Chloropsis hardwickii	Chloropseidae	Passeriformes	2	1	Land birds	Migratory
Chordeiles acutipennis	Caprimulgidae	Caprimulgiformes	1	1	Intermediate regions	Partially Migratory
Chroicocephalus maculipennis	Laridae	Charadriiformes	1		Water birds	Non-migratory
Ciconia maguari	Ciconiidae	Ciconiiformes	1	1	Water birds	Partially Migratory
Cinclus mexicanus	Cinclidae	Passeriformes		3	Water birds	Non-migratory
Cisticola juncidis	Cisticolidae	Passeriformes	3	2	Water birds	Migratory
Climacteris rufus	Climacteridae	Passeriformes	3	5	Intermediate regions	Non-migratory
Cnemophilus loriae	Cnemophilidae	Passeriformes	1	3(1)	Land birds	Non-migratory
Colinus virginianus	Odontophoridae	Galliformes	1	2	Intermediate regions	Non-migratory
Copsychus sechellarum	Muscicapidae	Passeriformes	1	3	Land birds	Non-migratory
Corvus brachyrhynchos	Corvidae	Passeriformes	2	5	Intermediate regions	Partially Migratory
Coturnix japonica	Phasianidae	Galliformes	1	1	Water birds	Migratory
Crypturellas cinnamomeus	Tinamidae	Struthioniformes	1		Land birds	Non-migratory
Daphoenositta chrysoptera	Neosittidae	Passeriformes	2	3	Intermediate regions	Non-migratory
Dasyornis broadbenti	Dasyornithidae	Passeriformes	3	6	Intermediate regions	Non-migratory
Dicaeum eximium	Dicaeidae	Passeriformes	3	1	Land birds	Non-migratory
Dicrurus megarhynchus	Dicruridae	Passeriformes	2	6	Land birds	Non-migratory
Donacobius atricapilla	Mimidae	Passeriformes	2	2	Water birds	Non-migratory
Dromaius novaehollandiae	Dromaiidae	Struthioniformes	1		Water birds	Non-migratory
Dromas ardeola	Dromadidae	Charadriiformes	1	3	Water birds	Non-migratory
Drymodes brunneopygia	Petroicidae	Passeriformes	6	6	Intermediate regions	Non-migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Dryoscopus gambensis	Malaconotidae	Passeriformes		4	Intermediate regions	Non-migratory
Dyaphorophyia castanea	Platysteiridae	Passeriformes	2		Land birds	Non-migratory
Edolisoma coerulescens	Campephagidae	Passeriformes	1	4	Land birds	Non-migratory
Egretta garzetta	Ardeidae	Pelecaniformes	(1)	1	Water birds	Partially Migratory
Elachura formosa	Timaliidae	Passeriformes	1	1	Land birds	Non-migratory
Emberiza fucata	Emberizidae	Passeriformes		3	Intermediate regions	Migratory
Erithacus rubecula	Muscicapidae	Passeriformes	2	2	Land birds	Migratory
Erpornis zantholeuca	Timaliidae	Passeriformes	1	6	Land birds	Non-migratory
Erythrocercus mccallii	Monarchidae	Passeriformes		1	Land birds	Non-migratory
Eubucco bourcierii	Ramphastidae	Piciformes		3	Land birds	Non-migratory
Eulacestoma nigropectus	Falcunculidae	Passeriformes	2	1	Land birds	Non-migratory
Eurypyga helias	Eurypygidae	Eurypygiformes		1	Intermediate regions	Non-migratory
Falcunculus frontatus	Falcunculidae	Passeriformes	1	5	Land birds	Non-migratory
Ficedula albicollis	Muscicapidae	Passeriformes	1	5	Land birds	Migratory
Formicarius rufipectus	Formicariidae	Passeriformes	1	7	Land birds	Non-migratory
Fregata magnificens	Fregatidae	Suliformes		1	Water birds	Migratory
Fregetta grallaria	Hydrobatidae	Procellariiformes		1	Water birds	Migratory
Fulmarus glacialis	Procellariidae	Procellariiformes		1	Water birds	Migratory
Furnarius figulus	Furnariidae	Passeriformes	1	3	Land birds	Non-migratory
Galbula dea	Galbulidae	Piciformes		2	Land birds	Non-migratory
Gallus gallus	Phasianidae	Galliformes	1	1	Land birds	Non-migratory
Gavia stellata	Gaviidae	Gaviiformes		1	Water birds	Migratory
Geospiza fortis	Emberizidae	Passeriformes	2	4	Intermediate regions	Non-migratory
Glareola pratincola	Glareolidae	Charadriiformes	1		Water birds	Migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Grallaria varia	Formicariidae	Passeriformes	(1)	6	Land birds	Non-migratory
Grantiella picta	Meliphagidae	Passeriformes	2	8	Intermediate regions	Migratory
Gymnorhina tibicen	Cracticidae	Passeriformes	2	2(2)	Intermediate regions	Non-migratory
Hemignathus wilsoni	Fringillidae	Passeriformes	2(1)	1	Land birds	Non-migratory
Hemiprocne comata	Hemiprocnidae	Caprimulgiformes		2	Land birds	Non-migratory
Himantopus himantopus	Recurvirostridae	Charadriiformes	1		Water birds	Partially Migratory
Hippolais icterina	Sylviidae	Passeriformes		2	Land birds	Migratory
Hirundo rustica	Hirundinidae	Passeriformes	2	3	Water birds	Migratory
Horornis vulcanius	Sylviidae	Passeriformes	1	2	Land birds	Non-migratory
Hydrobates tethys	Hydrobatidae	Procellariiformes	1	1	Water birds	Migratory
Hylia prasina	Sylviidae	Passeriformes	1	4	Land birds	Non-migratory
Hypocryptadius cinnamomeus	Zosteropidae	Passeriformes	1	1	Land birds	Non-migratory
Ibidorhyncha struthersii	Ibidorhynchidae	Charadriiformes	1	1	Water birds	Partially Migratory
Ifrita kowaldi	Orthonychidae	Passeriformes	2	2	Land birds	Non-migratory
Illadopsis cleaveri	Timaliidae	Passeriformes	2	2(1)	Land birds	Non-migratory
Indicator maculatus	Indicatoridae	Piciformes		3	Land birds	Non-migratory
Irena cyanogastra	Irenidae	Passeriformes	1(2)	1	Land birds	Non-migratory
Jacana jacana	Jacanidae	Charadriiformes		1	Water birds	Non-migratory
Junco hyemalis	Emberizidae	Passeriformes	2	3	Intermediate regions	Migratory
Lanius ludovicianus	Laniidae	Passeriformes	1	6	Water birds	Migratory
Larus smithsonianus	Laridae	Charadriiformes	1	1	Water birds	Partially Migratory
Leiothrix lutea	Timaliidae	Passeriformes	1	4	Land birds	Non-migratory
Lepidothrix coronata	Pipridae	Passeriformes	1	3	Land birds	Non-migratory
Leptocoma aspasia	Nectariniidae	Passeriformes	3	3	Intermediate regions	Non-migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Leucopsar rothschildi	Sturnidae	Passeriformes		3	Intermediate regions	Non-migratory
Limosa lapponica	Scolopacidae	Charadriiformes	1	1	Water birds	Migratory
Locustella ochotensis	Sylviidae	Passeriformes	3	2	Water birds	Migratory
Lonchura striata	Estrildidae	Passeriformes		3	Water birds	Non-migratory
Loxia curvirostra	Fringillidae	Passeriformes	3	4	Land birds	Partially Migratory
Machaerirhynchus nigripectus	Machaerirhynchidae	Passeriformes	2	5	Land birds	Non-migratory
Malurus elegans	Maluridae	Passeriformes	3(1)	5	Land birds	Non-migratory
Manacus manacus	Pipridae	Passeriformes	1	1	Land birds	Non-migratory
Melanocharis versteri	Melanocharitidae	Passeriformes		3	Land birds	Non-migratory
Meleagris gallopavo	Phasianidae	Galliformes	1	1	Intermediate regions	Non-migratory
Melospiza melodia	Emberizidae	Passeriformes	3	7	Intermediate regions	Partially Migratory
Menura novaehollandiae	Menuridae	Passeriformes	2	5	Land birds	Non-migratory
Mesembrinibis cayennensis	Threskiornithidae	Pelecaniformes		1	Intermediate regions	Non-migratory
Mionectes macconnelli	Tyrannidae	Passeriformes	1	6	Land birds	Non-migratory
Molothrus ater	Icteridae	Passeriformes	3	5	Intermediate regions	Migratory
Motacilla alba	Motacillidae	Passeriformes	2	2	Water birds	Migratory
Myiagra hebetior	Monarchidae	Passeriformes	(1)	6	Land birds	Non-migratory
Mystacornis crossleyi	Vangidae	Passeriformes	1		Land birds	Non-migratory
Neodrepanis coruscans	Philepittidae	Passeriformes		7	Land birds	Non-migratory
Neopipo cinnamomea	Tyrannidae	Passeriformes		4	Land birds	Non-migratory
Nesospiza acunhae	Emberizidae	Passeriformes	2	4	Intermediate regions	Non-migratory
Nicator chloris	Pycnonotidae	Passeriformes	3	4	Land birds	Non-migratory
Nipponia nippon	Threskiornithidae	Pelecaniformes		1	Intermediate regions	Non-migratory
Nothocercus nigrocapillus	Tinamidae	Struthioniformes	1		Land birds	Non-migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Notiomystis cincta	Meliphagidae	Passeriformes	1	2	Land birds	Non-migratory
Numida meleagris	Numididae	Galliformes	(1)	1	Intermediate regions	Non-migratory
Nycticryphes semicollaris	Rostratulidae	Charadriiformes	1	1	Land birds	Partially Migratory
Oceanites oceanicus	Hydrobatidae	Procellariiformes		1	Water birds	Migratory
Odontophorus gujanensis	Odontophoridae	Galliformes	(1)	1	Land birds	Non-migratory
Oenanthe oenanthe	Muscicapidae	Passeriformes		4	Water birds	Migratory
Onychorhynchus coronatus	Tyrannidae	Passeriformes	1	4	Land birds	Partially Migratory
Oreocharis arfaki	Melanocharitidae	Passeriformes	2	4	Land birds	Non-migratory
Origma solitaria	Acanthizidae	Passeriformes	2	6	Water birds	Non-migratory
Oriolus oriolus	Oriolidae	Passeriformes	2	3	Land birds	Migratory
Orthonyx spaldingii	Orthonychidae	Passeriformes	(1)	6	Land birds	Non-migratory
Oxylabes madagascariensis	Sylviidae	Passeriformes	2		Land birds	Non-migratory
Oxyruncus cristatus	Cotingidae	Passeriformes	1	5	Land birds	Non-migratory
Pachycephala philippinensis	Pachycephalidae	Passeriformes	(1)	6	Land birds	Non-migratory
Pachyramphus minor	Cotingidae	Passeriformes		5	Land birds	Partially Migratory
Panurus biarmicus	Timaliidae	Passeriformes	3	4	Water birds	Partially Migratory
Paradisaea raggiana	Paradisaeidae	Passeriformes	2	5	Land birds	Non-migratory
Pardalotus punctatus	Pardalotidae	Passeriformes	2	3(1)	Intermediate regions	Non-migratory
Parus major	Paridae	Passeriformes	2		Intermediate regions	Non-migratory
Passer domesticus	Passeridae	Passeriformes	2	1(1)	Water birds	Non-migratory
Passerina amoena	Emberizidae	Passeriformes		4	Intermediate regions	Migratory
Pedionomus torquatus	Pedionomidae	Charadriiformes	1	1	Water birds	Non-migratory
Pelecanus crispus	Pelecanidae	Pelecaniformes		1	Water birds	Partially Migratory
Penelope pileata	Cracidae	Galliformes	(1)	1	Land birds	Non-migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Peucedramus taeniatus	Peucedramidae	Passeriformes	(1)	3	Land birds	Partially Migratory
Phaetusa simplex	Laridae	Charadriiformes	1		Water birds	Partially Migratory
Phalacrocorax auritus	Phalacrocoracidae	Suliformes	(1)	1	Water birds	Partially Migratory
Phasianus colchicus	Phasianidae	Galliformes	(1)	1	Intermediate regions	Non-migratory
Phylloscopus trochilus	Sylviidae	Passeriformes	1	4(1)	Land birds	Migratory
Picathartes gymnocephalus	Picathartidae	Passeriformes	1(1)	5	Land birds	Non-migratory
Picoides pubescens	Picidae	Piciformes		3	Intermediate regions	Non-migratory
Piprites chloris	Pipridae	Passeriformes	1	4	Land birds	Non-migratory
Pitta sordida	Pittidae	Passeriformes		9	Land birds	Migratory
Ploceus nigricollis	Ploceidae	Passeriformes	2	2	Intermediate regions	Non-migratory
Podargus strigoides	Podargidae	Caprimulgiformes		1	Land birds	Non-migratory
Poecile atricapillus	Paridae	Passeriformes	1	1	Land birds	Non-migratory
Polioptila caerulea	Polioptilidae	Passeriformes	2	1	Intermediate regions	Migratory
Pomatorhinus ruficollis	Timaliidae	Passeriformes		2	Intermediate regions	Non-migratory
Pomatostomus ruficeps	Pomatostomidae	Passeriformes		3	Intermediate regions	Partially Migratory
Promerops cafer	Promeropidae	Passeriformes	3	3	Intermediate regions	Partially Migratory
Prunella fulvescens	Prunellidae	Passeriformes	1	2	Water birds	Partially Migratory
Pseudopodoces humilis	Paridae	Passeriformes	2	1	Water birds	Partially Migratory
Psilopogon haemacephalus	Ramphastidae	Piciformes		3	Intermediate regions	Non-migratory
Pteruthius melanotis	Timaliidae	Passeriformes	2	4	Land birds	Non-migratory
Ptilonorhynchus violaceus	Ptilonorhynchidae	Passeriformes	3	1	Land birds	Non-migratory
Ptilorrhoa leucosticta	Eupetidae	Passeriformes	1	2	Land birds	Non-migratory
Pycnonotus jocosus	Pycnonotidae	Passeriformes	2	3	Intermediate regions	Non-migratory
Pygoscelis adeliae	Spheniscidae	Sphenisciformes	1(1)		Water birds	Partially Migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Quiscalus mexicanus	Icteridae	Passeriformes	1	1	Water birds	Non-migratory
Ramphastos sulfuratus	Ramphastidae	Piciformes		4	Land birds	Non-migratory
Regulus satrapa	Regulidae	Passeriformes	1		Land birds	Migratory
Rhabdornis inornatus	Rhabdornithidae	Passeriformes	1	4	Land birds	Non-migratory
Rhagologus leucostigma	Pachycephalidae	Passeriformes	2	3	Land birds	Non-migratory
Rhea americana	Rheidae	Struthioniformes		1	Water birds	Non-migratory
Rhegmatorhina hoffmannsi	Thamnophilidae	Passeriformes	1	8	Land birds	Non-migratory
Rhinoptilus africanus	Glareolidae	Charadriiformes	1	1	Water birds	Non-migratory
Rhipidura dahli	Rhipiduridae	Passeriformes	1	6	Land birds	Non-migratory
Rhodinocichla rosea	Thraupidae	Passeriformes	2	3	Land birds	Non-migratory
Rhynochetos jubatus	Rhynochetidae	Eurypygiformes		1	Land birds	Non-migratory
Rissa tridactyla	Laridae	Charadriiformes	1	1	Water birds	Partially Migratory
Rostratula benghalensis	Rostratulidae	Charadriiformes		1	Land birds	Partially Migratory
Rynchops niger	Laridae	Charadriiformes	(1)	1	Water birds	Partially Migratory
Sakesphorus luctuosus	Thamnophilidae	Passeriformes		10(1)	Land birds	Non-migratory
Sapayoa aenigma	Sapayoaidae	Passeriformes	(1)	5	Land birds	Non-migratory
Saxicola maurus	Muscicapidae	Passeriformes	3	5	Intermediate regions	Migratory
Sclerurus mexicanus	Furnariidae	Passeriformes	1	5	Land birds	Non-migratory
Scopus umbretta	Scopidae	Pelecaniformes		1	Water birds	Partially Migratory
Scytalopus superciliaris	Rhinocryptidae	Passeriformes	1	7	Water birds	Non-migratory
Semnornis frantzii	Ramphastidae	Piciformes		1	Land birds	Non-migratory
Serilophus lunatus	Eurylaimidae	Passeriformes		7	Land birds	Non-migratory
Serinus canaria	Fringillidae	Passeriformes	3	6	Intermediate regions	Non-migratory
Setophaga coronata	Parulidae	Passeriformes	2	4	Land birds	Migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Sinosuthora webbiana	Timaliidae	Passeriformes	2	5	Intermediate regions	Non-migratory
Sitta europaea	Sittidae	Passeriformes	2	3	Land birds	Non-migratory
Smithornis capensis	Eurylaimidae	Passeriformes		3(2)	Intermediate regions	Non-migratory
Spizella passerina	Emberizidae	Passeriformes	1	2	Intermediate regions	Migratory
Sporophila hypoxantha	Emberizidae	Passeriformes	1	3(1)	Water birds	Partially Migratory
Steatornis caripensis	Steatornithidae	Caprimulgiformes		1	Land birds	Non-migratory
Stercorarius parasiticus	Stercorariidae	Charadriiformes	1	1	Water birds	Migratory
Sterrhoptilus dennistouni	Timaliidae	Passeriformes	3	8	Land birds	Non-migratory
Struthidea cinerea	Corcoracidae	Passeriformes	2	6	Intermediate regions	Non-migratory
Sturnus vulgaris	Sturnidae	Passeriformes		5	Water birds	Partially Migratory
Sula dactylatra	Sulidae	Suliformes	(1)	1	Water birds	Partially Migratory
Sylvia atricapilla	Sylviidae	Passeriformes	2	5	Land birds	Migratory
Sylvietta virens	Sylviidae	Passeriformes	1	4	Intermediate regions	Non-migratory
Tachuris rubrigastra	Tyrannidae	Passeriformes		3	Water birds	Non-migratory
Taeniopygia guttata	Estrildidae	Passeriformes	3	4	Intermediate regions	Partially Migratory
Thalassarche chlororhynchos	Diomedeidae	Procellariiformes		1	Water birds	Partially Migratory
Thinocorus orbignyianus	Thinocoridae	Charadriiformes	(1)	1	Water birds	Non-migratory
Thryothorus ludovicianus	Troglodytidae	Passeriformes	2	1	Intermediate regions	Non-migratory
Tichodroma muraria	Sittidae	Passeriformes		1	Land birds	Partially Migratory
Tinamus guttatus	Tinamidae	Struthioniformes	1		Land birds	Non-migratory
Toxostoma redivivum	Mimidae	Passeriformes	1	3	Intermediate regions	Non-migratory
Tricholaema leucomelas	Ramphastidae	Piciformes		3	Intermediate regions	Non-migratory
Turnix velox	Turnicidae	Charadriiformes	1	1	Water birds	Non-migratory
Tympanuchus cupido	Phasianidae	Galliformes		1	Water birds	Non-migratory

Species	Family	Order	Tas2r40 (Tas2r40p)	Tasr9 (Tas2r9p)	Habitat	Migration
Tyrannus savana	Tyrannidae	Passeriformes		3	Water birds	Migratory
Urocynchramus pylzowi	Urocynchramidae	Passeriformes	3	2(1)	Intermediate regions	Non-migratory
Vidua chalybeata	Viduidae	Passeriformes	2	3	Water birds	Non-migratory
Vireo altiloquus	Vireonidae	Passeriformes	1	6	Intermediate regions	Non-migratory
Xiphorhynchus elegans	Dendrocolaptidae	Passeriformes	1	8	Land birds	Non-migratory
Zonotrichia albicollis	Emberizidae	Passeriformes	2	5	Intermediate regions	Migratory
Zosterops lateralis	Zosteropidae	Passeriformes	2(1)	10	Intermediate regions	Non-migratory

Table S2: Removed pseudogenized Tas2r40

Order	Family	Species
Galliformes	Numididae	Tas2r40 *Numida meleagris*
	Odontophoridae	Tas2r40 *Odontophorus gujanensis*
	Cracidae	Tas2r40 *Penelope pileata*
	Phasianidae	Tas2r40 *Phasianus colchius*
Charadriiformes	Alcidae	Tas2r40 *Alca torda*
	Laridae	Tas2r40 *Rynchops niger*
	Thinocoridae	Tas2r40 *Thinocorus orbignyianus*
Struthioniformes	Apterygidae	Tas2r40 *Apteryx australis*
Pelecaniformes	Ardeidae	Tas2r40 *Egretta garzetta*
Suliformes	Phalacrocoracidae	Tas2r40 *Phalacrocorax auritus*
	Sulidae	Tas2r40 *Sula dactylatra*
Passeriformes	Formicariidae	Tas2r40 *Grallaria varia*
	Fringillidae	Tas2r40 *Hemignathus wilsoni*
	Irenidae	Tas2r40 *Irena cyanogastra*
	Irenidae	Tas2r40 *Irena cyanogastra*
	Maluridae	Tas2r40 *Malurus elegans*
	Monarchidae	Tas2r40 *Myiagra hebitor*
	Orthonychidae	Tas2r40 *Orthonyx spaldingii*
	Pachycephalidae	Tas2r40 *Pachycephala phillipensis*
	Peucedramidae	Tas2r40 *Peucedramus taeniatus*
	Picathartidae	Tas2r40 *Picathartes gymnocephalus*
	Sapayoaidae	Tas2r40 *Sapayoa aenigma*
	Zosteropidae	Tas2r40 *Zosterops lateralis*

Table S3: Removed pseudogenized Tas2r9

Order	Family	Species
Sphenisciformes	Spheniscidae	Tas2r9 *Pygoscelis adeliae*
Passeriformes	Cnemophilidae	Tas2r9 *Cnemophilus loriae*
	Cracticidae	Tas2r9 *Gymnorhina tibicen*
	Cracticidae	Tas2r9 *Gymnorhina tibicen*
	Timaliidae	Tas2r9 *Illadospsis cleaveri*
	Pardalotidae	Tas2r9 *Pardalotus punctatus*
	Passeridae	Tas2r9 *Passer domesticus*
	Sylviidae	Tas2r9 *Phylloscopus trochilus*
	Thamnophilidae	Tas2r9 *Sakesphorus luctuosus*
	Eurylaimidae	Tas2r9 *Smithornis capensis*
	Eurylaimidae	Tas2r9 *Smithornis capensis*
	Emberizidae	Tas2r9 *Sporophila hypoxantha*
	Urocynchramidae	Tas2r9 *Urocynchramus pylzowi*

Table S4: jModelTest2 results for Tas2r40 and Tas2r9 MSA

Dataset	Model	p-inv	gamma
Basal-40	HKY+G		1.933
Strisores-Aequoritornithes-40	GTR+G		1.038
Acanthisittidae-Tyranni-Passeri-40	GTR+G		0.846
Passeri-1-40	GTR+G		0.771
Passeri-2-40	GTR+I+G	0.016	0.971
Passeri-3-40	GTR+G		0.794
All-40	GTR+I+G	0.034	1.200
Basal-9	GTR+I+G	0.136	1.867
Strisores-Aequoritornithes-9	GTR+I+G	0.014	1.197
Piciformes-9	GTR+I+G	0.110	1.692
Acanthisittidae-Tyranni-Passeri-1-9	GTR+I+G	0.112	1.014
Acanthisittidae-Tyranni-Passeri-2-9	GTR+I+G	0.047	1.320
Tyranni-Passeri-1-9	GTR+I+G	0.077	1.410
Tyranni-Passeri-2-9	GTR+G		0.977
Tyranni-Passeri-3-9	GTR+I+G	0.048	1.367
Tyranni-Passeri-4-9	GTR+I+G	0.077	1.238
All-9	GTR+I+G	0.020	1.356

Table S5: Sequences of the Passeriformes order found in Tas2r40 datasets

Dataset	Iss	SymIssC	SymProb	AsymIssC	AsymProb	NumOTU
Basal-40	0.225	0.753	0.00	0.560	0.00	
Strisores-Aequorlitornithes-40	0.184	0.758	0.00	0.495	0.00	
Acanthisittidae-Tyranni-Passeri-40	0.278	0.735	0.00	0.420	0.00	
Passeri-1-40	0.197	0.814	0.00	0.783	0.00	4
	0.198	0.779	0.00	0.671	0.00	8
	0.207	0.761	0.00	0.557	0.00	16
	0.222	0.736	0.00	0.420	0.00	32
Passeri-2-40	0.154	0.814	0.00	0.783	0.00	4
	0.157	0.779	0.00	0.671	0.00	8
	0.163	0.761	0.00	0.557	0.00	16
	0.173	0.736	0.00	0.420	0.00	32
Passeri-3-40	0.211	0.814	0.00	0.783	0.00	4
	0.211	0.779	0.00	0.671	0.00	8
	0.229	0.761	0.00	0.557	0.00	16
	0.246	0.735	0.00	0.420	0.00	32
All-40	0.255	0.814	0	0.783	0	4
	0.251	0.779	0.00	0.671	0.00	8
	0.270	0.761	0.00	0.557	0.00	16
	0.282	0.736	0.00	0.420	0.00	32
Basal-9	0.412	0.758	0.00	0.486	0.000	
Strisores-Aequorlitornithes-9	0.220	0.818	0.00	0.786	0.00	4
	0.216	0.785	0.00	0.678	0.00	8
	0.230	0.767	0.00	0.567	0.00	12
	0.240	0.743	0.00	0.434	0	16
Piciformes-9	0.306	0.761	0.00	0.496	0.00	
Acanthisittidae-Tyranni-Passeri-1-9	0.190	0.817	0.00	0.785	0.00	4
	0.191	0.783	0.00	0.676	0.00	8
	0.201	0.765	0.00	0.564	0.00	12
	0.215	0.741	0.00	0.429	0.00	16
Acanthisittidae-Tyranni-Passeri-2-9	0.269	0.818	0.00	0.786	0.00	4
	0.277	0.784	0.00	0.678	0.00	8
	0.289	0.767	0.00	0.567	0.00	12
	0.309	0.743	0.00	0.433	0.00	16
Tyranni-Passeri-1-9	0.250	0.818	0	0.786	0.00	4

	0.258	0.784	0.00	0.678	0.00	8
	0.267	0.767	0.00	0.566	0.00	12
	0.286	0.742	0.00	0.432	0.00	16
Tyranni-Passeri-2-9	0.178	0.818	0.00	0.786	0.00	4
	0.180	0.785	0.00	0.678	0.00	8
	0.195	0.767	0.00	0.567	0.00	12
	0.206	0.743	0.00	0.434	0.00	16
Tyranni-Passeri-3-9	0.244	0.818	0.00	0.786	0.00	4
	0.243	0.784	0.00	0.678	0.00	8
	0.261	0.767	0.00	0.566	0.00	12
	0.285	0.742	0.00	0.432	0.00	16
Tyranni-Passeri-4-9	0.237	0.817	0.00	0.785	0.00	4
	0.242	0.784	0.00	0.677	0.00	8
	0.262	0.766	0.00	0.565	0.00	12
	0.273	0.742	0.00	0.431	0.00	16
All-9	0.330	0.818	0.00	0.786	0.00	4
	0.333	0.784	0.00	0.678	0.00	8
	0.351	0.767	0.00	0.567	0.00	12
	0.374	0.743	0.00	0.433	0.002	16

Table S6: MrBayes average standard deviation analysis

NSX files	**Average standard deviation of split frequencies**
Basal-40.nxs	0.0019
Strisores-Aequoritornithes-40.nxs	0.0010
Acanthisittidae-Tyranni-Passeri-40.nxs	0.0012
Passeri-1-40.nxs	0.0106
Passeri-2-40.nxs	0.0042
Passeri-3-40.nxs	0.0083
All-40.nxs	0.0454
Basal-9.nxs	0.0020
Strisores-Aequoritornithes-9.nxs	0.0036
Piciformes-9.nxs	0.0004
Acanthisittidae-Tyranni-Passeri-1-9.nxs	0.0103
Acanthisittidae-Tyranni-Passeri-1-9.nxs	0.0071
Tyranni-Passeri-1-9.nxs	0.0034
Tyranni-Passeri-2-9.nxs	0.0045
Tyranni-Passeri-3-9.nxs	0.0064
Tyranni-Passeri-4-9.nxs	0.0064
All-9.nxs	0.0222

Table S7: Sequences of the Passeriformes order found in Tas2r40 datasets

Family	Species	Acanthisittidae--Tyranni-Passeri-40	Passeri-1-40	Passeri-2-40	Passeri-3-40
Acanthisittidae	*Acanthisitta chloris*	2(Tas2r40/1-2)			
Acanthizidae	*Origma solitaria*	Tas2r40		Tas2r40c	
Aegithalidae	*Aegithalos caudatus*			Tas2r40c	Tas2r40d2
Alaudidae	*Alaudala cheleensis*			Tas2r40c	Tas2r40d1
					Tas2r40d2
Bombycillidae	*Bombycilla garrulus*			Tas2r40c	
Callaeatidae	*Callaeas wilsoni*		2(Tas2r40b1/1-2)		
Campephagidae	*Edolisoma coerulescens*		Tas2r40a		
Cardinalidae	*Cardinalis cardinalis*				Tas2r40d2
Chloropseidae	*Chloropsis hardwickii*			Tas2r40c	Tas2r40d3
Cisticolidae	*Cisticola juncidis*			Tas2r40c	Tas2r40d1
					Tas2r40d2
Climacteridae	*Climacteris rufus*	3(Tas2r40/1-3)			
Cnemophilidae	*Cnemophilus loriae*		Tas2r40b2		
Corcoracidae	*Struthidea cinerea*		Tas2r40b1		
			Tas2r40b2		
Corvidae	*Aphelocoma coerulescens*		Tas2r40a		
			Tas2r40b1		
Corvidae	*Corvus brachyrhynchos*		Tas2r40a		
			Tas2r40b1		
Cotingidae	*Oxyruncus cristatus*	Tas2r40			
Cracticidae	*Gymnorhina tibicen*		Tas2r40b2	Tas2r40c	
Dasyornithidae	*Dasyornis broadbenti*	2(Tas2r40/1-2)		Tas2r40c	

Dendrocolaptidae	*Campylorhamphus procurvoides*	Tas2r40			
Dendrocolaptidae	*Xiphorhynchus elegans*	Tas2r40			
Dicaeidae	*Dicaeum eximium*			Tas2r40c	Tas2r40d1
					Tas2r40d3
Dicruridae	*Dicrurus megarhynchus*		Tas2r40a		
			Tas2r40b2		
Emberizidae	*Geospiza fortis*			Tas2r40c	Tas2r40d2
Emberizidae	*Junco hyemalis*			Tas2r40c	Tas2r40d3
Emberizidae	*Melospiza melodia*				2(Tas2r40d2/1-2)
					Tas2r40d3
Emberizidae	*Nesospiza acunhae*				Tas2r40d2
					Tas2r40d3
Emberizidae	*Spizella passerina*			Tas2r40c	
Emberizidae	*Sporophila hypoxantha*				Tas2r40d2
Emberizidae	*Zonotrichia albicollis*			Tas2r40c	Tas2r40d2
Estrildidae	*Taeniopygia guttata*				Tas2r40d1
					Tas2r40d2
					Tas2r40d3
Eupetidae	*Ptilorrhoa leucosticta*			Tas2r40c	
Falcunculidae	*Eulacestoma nigropectus*		2(Tas2r40b2/1-2)		
Falcunculidae	*Falcunculus frontatus*		Tas2r40b2		
Formicariidae	*Formicarius rufipectus*	Tas2r40			
Fringillidae	*Chlorodrepanis virens*			Tas2r40c	Tas2r40d3
Fringillidae	*Hemignathus wilsoni*			Tas2r40c	Tas2r40d2
Fringillidae	*Loxia curvirostra*			Tas2r40c	2(Tas2r40d3/1-2)
Fringillidae	*Serinus canaria*			Tas2r40c	Tas2r40d2
					Tas2r40d3

Furnariidae	*Furnarius figulus*	Tas2r40			
Furnariidae	*Sclerurus mexicanus*	Tas2r40			
Hirundinidae	*Hirundo rustica*			Tas2r40c	Tas2r40d2
Icteridae	*Molothrus ater*			Tas2r40c	2(Tas2r40d2/1-2)
Icteridae	*Quiscalus mexicanus*				Tas2r40d3
Irenidae	*Irena cyanogastra*			Tas2r40c	
Laniidae	*Lanius ludovicianus*		Tas2r40a		
Machaerirhynchidae	*Machaerirhynchus nigripectus*		Tas2r40b2	Tas2r40c	
Maluridae	*Malurus elegans*	3(Tas2r40/1-3)			
Melanocharitidae	*Oreocharis arfaki*		Tas2r40a		
			Tas2r40b2		
Meliphagidae	*Grantiella picta*	2(Tas2r40/1-2)			
Meliphagidae	*Notiomystis cincta*			Tas2r40c	
Menuridae	*Menura novaehollandiae*	2(Tas2r40/1-2)			
Mimidae	*Donacobius atricapilla*		Tas2r40b2	Tas2r40c	
Mimidae	*Toxostoma redivivum*			Tas2r40c	
Motacillidae	*Motacilla alba*			Tas2r40c	Tas2r40d3
Muscicapidae	*Cercotrichas coryphoeus*			Tas2r40c	
Muscicapidae	*Copsychus sechellarum*			Tas2r40c	
Muscicapidae	*Erithacus rubecula*			Tas2r40c	Tas2r40d2
Muscicapidae	*Ficedula albicollis*			Tas2r40c	
Muscicapidae	*Saxicola maurus*				3(Tas2r40d2/1-3)
Nectariniidae	*Leptocoma aspasia*			Tas2r40c	Tas2r40d1
					Tas2r40d3
Neosittidae	*Daphoenositta chrysoptera*		Tas2r40b2	Tas2r40c	
Oriolidae	*Oriolus oriolus*		Tas2r40a	Tas2r40c	

Orthonychidae	*Ifrita kowaldi*		Tas2r40a Tas2r40b2		
Pachycephalidae	*Aleadryas rufinucha*			Tas2r40c	
Pachycephalidae	*Rhagologus leucostigma*		Tas2r40b2	Tas2r40c	
Paradisaeidae	*Paradisaea raggiana*		Tas2r40a Tas2r40b1		
Pardalotidae	*Pardalotus punctatus*	Tas2r40		Tas2r40c	
Paridae	*Parus major*			Tas2r40c	Tas2r40d1
Paridae	*Poecile atricapillus*			Tas2r40c	
Paridae	*Pseudopodoces humilis*				Tas2r40d1 Tas2r40d2
Parulidae	*Setophaga coronata*				2(Tas2r40d2/1-2)
Passeridae	*Passer domesticus*				Tas2r40d2 Tas2r40d3
Petroicidae	*Drymodes brunneopygia*		Tas2r40b1 3(Tas2r40b2/1-3)	Tas2r40c	Tas2r40d2
Picathartidae	*Picathartes gymnocephalus*		Tas2r40b1		
Pipridae	*Lepidothrix coronata*	Tas2r40			
Pipridae	*Manacus manacus*	Tas2r40			
Pipridae	*Piprites chloris*	Tas2r40			
Platysteiridae	*Dyaphorophyia castanea*		Tas2r40b2	Tas2r40c	
Ploceidae	*Ploceus nigricollis*			Tas2r40c	Tas2r40d3
Polioptilidae	*Polioptila caerulea*			Tas2r40c	Tas2r40d2
Promeropidae	*Promerops cafer*			Tas2r40c	Tas2r40d2 Tas2r40d3
Prunellidae	*Prunella fulvescens*			Tas2r40c	
Ptilonorhynchidae	*Ptilonorhynchus violaceus*	3(Tas2r40/1-3)			

Pycnonotidae	*Brachypodius atriceps*			Tas2r40c	
Pycnonotidae	*Nicator chloris*			Tas2r40c	Tas2r40d1
					Tas2r40d2
Pycnonotidae	*Pycnonotus jocosus*			Tas2r40c	Tas2r40d1
Regulidae	*Regulus satrapa*			Tas2r40c	
Remizidae	*Anthoscopus minutus*				Tas2r40d2
Rhabdornithidae	*Rhabdornis inornatus*			Tas2r40c	
Rhinocryptidae	*Scytalopus superciliaris*	Tas2r40			
Rhipiduridae	*Rhipidura dahli*		Tas2r40a		
Sittidae	*Sitta europaea*			Tas2r40c	Tas2r40d2
Sturnidae	*Buphagus erythrorhynchus*			Tas2r40c	
Sylviidae	*Acrocephalus arundinaceus*			Tas2r40c	Tas2r40d1
Sylviidae	*Horornis vulcanius*			Tas2r40c	
Sylviidae	*Hylia prasina*				Tas2r40d2
Sylviidae	*Locustella ochotensis*		Tas2r40b2	Tas2r40c	Tas2r40d1
Sylviidae	*Oxylabes madagascariensis*		Tas2r40b2	Tas2r40c	
Sylviidae	*Phylloscopus trochilus*			Tas2r40c	
Sylviidae	*Sylvia atricapilla*			Tas2r40c	Tas2r40d2
Sylviidae	*Sylvietta virens*		Tas2r40b2		
Thamnophilidae	*Rhegmatorhina hoffmannsi*	Tas2r40			
Thraupidae	*Rhodinocichla rosea*			Tas2r40c	Tas2r40d3
Timaliidae	*Elachura formosa*				Tas2r40d1
Timaliidae	*Erpornis zantholeuca*		Tas2r40b1		
Timaliidae	*Illadopsis cleaveri*			Tas2r40c	Tas2r40d2
Timaliidae	*Leiothrix lutea*			Tas2r40c	
Timaliidae	*Panurus biarmicus*		Tas2r40b2	Tas2r40c	Tas2r40d3
Timaliidae	*Pteruthius melanotis*		Tas2r40b2	Tas2r40c	

Timaliidae	*Sinosuthora webbiana*				Tas2r40d1
					Tas2r40d2
Timaliidae	*Sterrhoptilus dennistouni*			Tas2r40c	2(Tas2r40d2/1-2)
Troglodytidae	*Thryothorus ludovicianus*			Tas2r40c	Tas2r40d1
Turdidae	*Catharus fuscescens*			Tas2r40c	
Turdidae	*Chaetops frenatus*		Tas2r40b2	Tas2r40c	Tas2r40d2
Tyrannidae	*Mionectes macconnelli*	Tas2r40			
Tyrannidae	*Onychorhynchus coronatus*	Tas2r40			
Urocynchramidae	*Urocynchramus pylzowi*			Tas2r40c	Tas2r40d2
					Tas2r40d3
Vangidae	*Mystacornis crossleyi*		Tas2r40b2		
Viduidae	*Vidua chalybeata*				Tas2r40d1
					Tas2r40d3
Vireonidae	*Vireo altiloquus*		Tas2r40b2		
Zosteropidae	*Hypocryptadius cinnamomeus*			Tas2r40c	
Zosteropidae	*Zosterops lateralis*			Tas2r40c	Tas2r40d2

Table S8: Sequences of the Passeriformes order found in Tas2r9 datasets

Family	Species	Acanthisittidae--Tyranni-Passeri-1-9	Acanthisittidae-Tyranni--Passeri-2-9	Tyranni-Passeri--1-9	Tyranni-Passeri--2-9	Tyranni-Passeri--3-9	Tyranni-Passeri-4-9
Acanthisittidae	*Acanthisitta chloris*	Tas2r9a	3(Tas2r9b/1-3)				
Acanthizidae	*Origma solitaria*	Tas2r9a	2(Tas2r9b/1-2)			Tas2r9e1	Tas2r9f1
						Tas2r9e2	
Aegithalidae	*Aegithalos caudatus*	Tas2r9a				Tas2r9e2	
Alaudidae	*Alaudala cheleensis*				Tas2r9d		
Atrichornithidae	*Atrichornis clamosus*	Tas2r9a		Tas2r9c		Tas2r9e1	
Bombycillidae	*Bombycilla garrulus*	Tas2r9a					Tas2r9f1
Callaeatidae	*Callaeas wilsoni*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d		Tas2r9f1
Campephagidae	*Edolisoma coerulescens*	Tas2r9a	Tas2r9b	Tas2r9c			Tas2r9f1
Cardinalidae	*Cardinalis cardinalis*	Tas2r9a				Tas2r9e2	Tas2r9f1
							Tas2r9f3
Certhiidae	*Certhia brachydactyla*	Tas2r9a	Tas2r9b				
Chloropseidae	*Chloropsis hardwickii*	Tas2r9a					
Cinclidae	*Cinclus mexicanus*		Tas2r9b		Tas2r9d		Tas2r9f1
Cisticolidae	*Cisticola juncidis*				Tas2r9d		Tas2r9f1
Climacteridae	*Climacteris rufus*	Tas2r9a			Tas2r9d	Tas2r9e1	Tas2r9f1
						Tas2r9e2	
Cnemophilidae	*Cnemophilus loriae*	Tas2r9a		Tas2r9c			Tas2r9f1
Corcoracidae	*Struthidea cinerea*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e2	Tas2r9f1
Corvidae	*Aphelocoma coerulescens*	Tas2r9a	Tas2r9b			Tas2r9e2	
Corvidae	*Corvus brachyrhynchos*	Tas2r9a		Tas2r9c	Tas2r9d	Tas2r9e1	Tas2r9f1
Cotingidae	*Oxyruncus cristatus*	Tas2r9a	2(Tas2r9b/1-2)			Tas2r9e1	Tas2r9f1
Cotingidae	*Pachyramphus minor*	Tas2r9a	2(Tas2r9b/1-2)	Tas2r9c		Tas2r9e1	
Cracticidae	*Gymnorhina tibicen*		Tas2r9b		Tas2r9d		
Dasyornithidae	*Dasyornis broadbenti*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e1	Tas2r9f1

Dendrocolaptidae	*Campylorhamphus procurvoides*	Tas2r9a					
Dendrocolaptidae	*Xiphorhynchus elegans*	Tas2r9a	6(Tas2r9b/1-6)				Tas2r9f1
Dicaeidae	*Dicaeum eximium*				Tas2r9d		
Dicruridae	*Chaetorhynchus papuensis*	Tas2r9a		Tas2r9c	Tas2r9d	Tas2r9e2	
Dicruridae	*Dicrurus megarhynchus*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e1	Tas2r9f1
						Tas2r9e2	
Emberizidae	*Calcarius ornatus*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f1
Emberizidae	*Emberiza fucata*	Tas2r9a	Tas2r9b			Tas2r9e2	
Emberizidae	*Geospiza fortis*	Tas2r9a					2(Tas2r9f1/1-2)
							Tas2r9f2
Emberizidae	*Junco hyemalis*	Tas2r9a	Tas2r9b				Tas2r9f1
Emberizidae	*Melospiza melodia*	Tas2r9a				Tas2r9e2	Tas2r9f1
							2(Tas2r9f2/1-2)
							2(Tas2r9f3/1-2)
Emberizidae	*Nesospiza acunhae*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f3
Emberizidae	*Passerina amoena*	Tas2r9a				Tas2r9e2	Tas2r9f1
							Tas2r9f3
Emberizidae	*Spizella passerina*	Tas2r9a				Tas2r9e2	
Emberizidae	*Sporophila hypoxantha*	Tas2r9a				2(Tas2r9e2/1-2)	
Emberizidae	*Zonotrichia albicollis*	Tas2r9a				Tas2r9e2	Tas2r9f1
							Tas2r9f2
							Tas2r9f3
Estrildidae	*Lonchura striata*	Tas2r9a	Tas2r9b				Tas2r9f1
Estrildidae	*Taeniopygia guttata*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f1
Eupetidae	*Ptilorrhoa leucosticta*	Tas2r9a		Tas2r9c			
Eurylaimidae	*Calyptomena viridis*	Tas2r9a	Tas2r9b	2(Tas2r9c/1-2)		Tas2r9e1	Tas2r9f1
Eurylaimidae	*Serilophus lunatus*	Tas2r9a	3(Tas2r9b/1-3)	2(Tas2r9c/1-2)			Tas2r9f1
Eurylaimidae	*Smithornis capensis*	Tas2r9a	Tas2r9b				Tas2r9f1
Falcunculidae	*Eulacestoma nigropectus*						Tas2r9f1

Falcunculidae	*Falcunculus frontatus*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d		Tas2r9f1
Formicariidae	*Formicarius rufipectus*	Tas2r9a	5(Tas2r9b/1-5)			Tas2r9e1	
Formicariidae	*Grallaria varia*	Tas2r9a	2(Tas2r9b/1-2)			2(Tas2r9e1/1-2)	Tas2r9f1
Fringillidae	*Chlorodrepanis virens*						Tas2r9f3
Fringillidae	*Hemignathus wilsoni*						Tas2r9f3
Fringillidae	*Loxia curvirostra*	Tas2r9a	Tas2r9b				Tas2r9f1
							Tas2r9f3
Fringillidae	*Serinus canaria*		Tas2r9b			Tas2r9e2	Tas2r9f1
							2(Tas2r9f2/1-2)
							Tas2r9f3
Fumariidae	*Furnarius figulus*	Tas2r9a	Tas2r9b				Tas2r9f1
Fumariidae	*Sclerurus mexicanus*	Tas2r9a	2(Tas2r9b/1-2)			Tas2r9e1	Tas2r9f1
Hirundinidae	*Hirundo rustica*	Tas2r9a			Tas2r9d	Tas2r9e2	
Icteridae	*Agelaius phoeniceus*					Tas2r9e2	Tas2r9f2
							Tas2r9f3
Icteridae	*Molothrus ater*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f2
							Tas2r9f3
Icteridae	*Quiscalus mexicanus*						Tas2r9f3
Irenidae	*Irena cyanogastra*	Tas2r9a					
Laniidae	*Lanius ludovicianus*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e2	Tas2r9f1
Machaerirhynchidae	*Machaerirhynchus nigripectus*	Tas2r9a	Tas2r9b			Tas2r9e1	Tas2r9f1
						Tas2r9e2	
Malaconotidae	*Dryoscopus gambensis*		Tas2r9b		Tas2r9d	Tas2r9e1	
						Tas2r9e2	
Maluridae	*Malurus elegans*		2(Tas2r9b/1-2)			Tas2r9e1	Tas2r9f1
						Tas2r9e2	
Melanocharitidae	*Melanocharis versteri*	Tas2r9a	Tas2r9b			Tas2r9e2	
Melanocharitidae	*Oreocharis arfaki*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f1
Meliphagidae	*Grantiella picta*	Tas2r9a	2(Tas2r9b/1-2)	Tas2r9c		Tas2r9e1	2(Tas2r9f1/1-2)
						Tas2r9e2	

Meliphagidae	*Notiomystis cincta*		Tas2r9b		Tas2r9d			
Menuridae	*Menura novaehollandiae*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e1		
Mimidae	*Donacobius atricapilla*		Tas2r9b		Tas2r9d			
Mimidae	*Toxostoma redivivum*				Tas2r9d	Tas2r9e2	Tas2r9f1	
Monarchidae	*Erythrocercus mccallii*	Tas2r9a						
Monarchidae	*Myiagra hebetior*		2(Tas2r9b/1-2)		Tas2r9d	Tas2r9e1	Tas2r9f1	
						Tas2r9e2		
Motacillidae	*Motacilla alba*					Tas2r9e2	Tas2r9f2	
Muscicapidae	*Cercotrichas coryphoeus*	Tas2r9a				Tas2r9e2	Tas2r9f1	
Muscicapidae	*Copsychus sechellarum*	Tas2r9a	Tas2r9b			Tas2r9e2		
Muscicapidae	*Erithacus rubecula*						2(Tas2r9f1/1-2)	
Muscicapidae	*Ficedula albicollis*	Tas2r9a	Tas2r9b			Tas2r9e2	2(Tas2r9f1/1-2)	
Muscicapidae	*Oenanthe oenanthe*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f1	
Muscicapidae	*Saxicola maurus*	Tas2r9a	Tas2r9b			Tas2r9e2	2(Tas2r9f1/1-2)	
Nectariniidae	*Leptocoma aspasia*		Tas2r9b		Tas2r9d	Tas2r9e2		
Neosittidae	*Daphoenositta chrysoptera*				Tas2r9d	Tas2r9e1		
						Tas2r9e2		
Oriolidae	*Oriolus oriolus*	Tas2r9a			Tas2r9d		Tas2r9f1	
Orthonychidae	*Ifrita kowaldi*	Tas2r9a					Tas2r9f1	
Orthonychidae	*Orthonyx spaldingii*	Tas2r9a	Tas2r9b	Tas2r9c		Tas2r9e1	Tas2r9f1	
						Tas2r9e2		
Pachycephalidae	*Aleadryas rufinucha*	Tas2r9a					Tas2r9f1	
Pachycephalidae	*Pachycephala philippinensis*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e2	Tas2r9f1	
Pachycephalidae	*Rhagologus leucostigma*		Tas2r9b			Tas2r9e2	Tas2r9f1	
Paradisaeidae	*Paradisaea raggiana*	Tas2r9a	Tas2r9b	Tas2r9c		Tas2r9e1	Tas2r9f1	
Pardalotidae	*Pardalotus punctatus*	Tas2r9a	Tas2r9b				Tas2r9f1	
Paridae	*Poecile atricapillus*	Tas2r9a						
Paridae	*Pseudopodoces humilis*	Tas2r9a						

Parulidae	*Setophaga coronata*	Tas2r9a				Tas2r9e2	Tas2r9f1 Tas2r9f2
Passeridae	*Passer domesticus*					Tas2r9e2	
Petroicidae	*Drymodes brunneopygia*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e2	Tas2r9f1
Peucedramidae	*Peucedramus taeniatus*	Tas2r9a	Tas2r9b			Tas2r9e2	
Philepittidae	*Neodrepanis coruscans*	Tas2r9a	3(Tas2r9b/1-3)	Tas2r9c		Tas2r9e1	Tas2r9f1
Picathartidae	*Picathartes gymnocephalus*	Tas2r9a	Tas2r9b	Tas2r9c		Tas2r9e1 Tas2r9e2	
Pipridae	*Lepidothrix coronata*		Tas2r9b	Tas2r9c			Tas2r9f1
Pipridae	*Manacus manacus*		Tas2r9b				
Pipridae	*Piprites chloris*	Tas2r9a	2(Tas2r9b/1-2)			Tas2r9e1	
Pittidae	*Pitta sordida*		6(Tas2r9b/1-6)	2(Tas2r9c/1-2)			Tas2r9f1
Ploceidae	*Ploceus nigricollis*		Tas2r9b			Tas2r9e2	
Polioptilidae	*Polioptila caerulea*	Tas2r9a					
Pomatostomidae	*Pomatostomus ruficeps*	Tas2r9a		Tas2r9c			Tas2r9f1
Promeropidae	*Promerops cafer*	Tas2r9a	Tas2r9b				Tas2r9f1
Prunellidae	*Prunella fulvescens*	Tas2r9a	Tas2r9b				
Ptilonorhynchidae	*Ptilonorhynchus violaceus*	Tas2r9a					
Pycnonotidae	*Brachypodius atriceps*	Tas2r9a			Tas2r9d		2(Tas2r9f1/1-2)
Pycnonotidae	*Nicator chloris*	Tas2r9a	Tas2r9b			Tas2r9e2	Tas2r9f1
Pycnonotidae	*Pycnonotus jocosus*	Tas2r9a			Tas2r9d		Tas2r9f1
Remizidae	*Anthoscopus minutus*		Tas2r9b				Tas2r9f1
Rhabdornithidae	*Rhabdornis inornatus*	Tas2r9a				Tas2r9e2	2(Tas2r9f1/1-2)
Rhinocryptidae	*Scytalopus superciliaris*	Tas2r9a	3(Tas2r9b/1-3)			2(Tas2r9e1/1-2)	Tas2r9f1
Rhipiduridae	*Rhipidura dahli*	Tas2r9a	Tas2r9b	Tas2r9c		Tas2r9e1 Tas2r9e2	Tas2r9f1
Sapayoaidae	*Sapayoa aenigma*	Tas2r9a	3(Tas2r9b/1-3)	Tas2r9c			
Sittidae	*Sitta europaea*	Tas2r9a	Tas2r9b			Tas2r9e2	
Sittidae	*Tichodroma muraria*						Tas2r9f1

Sturnidae	*Leucopsar rothschildi*		Tas2r9b		Tas2r9d		Tas2r9f1
Sturnidae	*Sturnus vulgaris*	Tas2r9a			Tas2r9d	Tas2r9e2	2(Tas2r9f1/1-2)
Sylviidae	*Acrocephalus arundinaceus*	Tas2r9a	Tas2r9b				Tas2r9f1
Sylviidae	*Cettia cetti*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e2	
Sylviidae	*Hippolais icterina*	Tas2r9a	Tas2r9b				
Sylviidae	*Horornis vulcanius*	Tas2r9a				Tas2r9e2	
Sylviidae	*Hylia prasina*		Tas2r9b		Tas2r9d	Tas2r9e2	Tas2r9f1
Sylviidae	*Locustella ochotensis*				Tas2r9d		Tas2r9f1
Sylviidae	*Phylloscopus trochilus*	Tas2r9a		Tas2r9c		Tas2r9e2	Tas2r9f1
Sylviidae	*Sylvia atricapilla*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e2	Tas2r9f1
Sylviidae	*Sylvietta virens*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e2	
Thamnophilidae	*Rhegmatorhina hoffmannsi*	Tas2r9a	3(Tas2r9b/1-3)	Tas2r9c	Tas2r9d	Tas2r9e1	Tas2r9f1
Thamnophilidae	*Sakesphorus luctuosus*	Tas2r9a	3(Tas2r9b/1-3)	2(Tas2r9c/1-2)	2(Tas2r9d/1-2)	Tas2r9e1	Tas2r9f1
Thraupidae	*Rhodinocichla rosea*	Tas2r9a	Tas2r9b			Tas2r9e2	
Timaliidae	*Elachura formosa*	Tas2r9a					
Timaliidae	*Erpornis zantholeuca*	Tas2r9a	Tas2r9b	Tas2r9c	Tas2r9d	Tas2r9e1	
						Tas2r9e2	
Timaliidae	*Illadopsis cleaveri*	Tas2r9a	Tas2r9b				
Timaliidae	*Leiothrix lutea*	Tas2r9a				Tas2r9e2	2(Tas2r9f1/1-2)
Timaliidae	*Panurus biarmicus*	Tas2r9a	Tas2r9b		Tas2r9d		Tas2r9f1
Timaliidae	*Pomatorhinus ruficollis*				Tas2r9d		Tas2r9f1
Timaliidae	*Pteruthius melanotis*		Tas2r9b	Tas2r9c	Tas2r9d		Tas2r9f1
Timaliidae	*Sinosuthora webbiana*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e2	Tas2r9f1
Timaliidae	*Sterrhoptilus dennistouni*	Tas2r9a	Tas2r9b		Tas2r9d	Tas2r9e2	4(Tas2r9f1/1-4)
Troglodytidae	*Thryothorus ludovicianus*	Tas2r9a					
Turdidae	*Catharus fuscescens*	Tas2r9a					
Turdidae	*Chaetops frenatus*		Tas2r9b			Tas2r9e2	Tas2r9f1
Tyrannidae	*Mionectes macconnelli*	Tas2r9a	2(Tas2r9b/1-2)	2(Tas2r9c/1-2)			Tas2r9f1
Tyrannidae	*Neopipo cinnamomea*	Tas2r9a		Tas2r9c		Tas2r9e1	Tas2r9f1

Tyrannidae	*Onychorhynchus coronatus*	Tas2r9a	Tas2r9b	Tas2r9c		Tas2r9e1	
Tyrannidae	*Tachuris rubrigastra*	Tas2r9a		Tas2r9c		Tas2r9e1	
Tyrannidae	*Tyrannus savana*		Tas2r9b	2(Tas2r9c/1-2)			
Urocynchramidae	*Urocynchramus pylzowi*	Tas2r9a	Tas2r9b				
Viduidae	*Vidua chalybeata*	Tas2r9a	Tas2r9b				Tas2r9f1
Vireonidae	*Vireo altiloquus*	Tas2r9a	2(Tas2r9b/1-2)		Tas2r9d	Tas2r9e1	
						Tas2r9e2	
Zosteropidae	*Hypocryptadius cinnamomeus*	Tas2r9a					
Zosteropidae	*Zosterops lateralis*	Tas2r9a	Tas2r9b		Tas2r9d		7(Tas2r9f1/1-7)

Table S9: PAML results for site model comparisons for test of positive selection of the Tas2r40 datasets

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Basal-40	0.2	M7	p = 0.616, q = 0.374	-4234.922	-4.121	1.000	—
		M8	p0 = 0.994, p = 0.634 q = 0.421 (p1 = 0.006), w = 2.742	-4236.983			
		M8a	p0 = 0.516, p = 32.811, q = 99.000 (p1 = 0.484), w = 1.000	-4232.605	-8.755	1.000	
		M8	p0 = 0.994, p = 0.634, q = 0.421 (p1 = 0.006), w = 2.742	-4236.983			
	2	M7	p = 0.642, q = 0.412	-4235.016	6.681	0.035	—
		M8	p0 = 0.748, p = 1.586, q = 2.192 (p1 = 0.252), w = 1.475	-4231.675			
		M8a	p0 = 0.516, p = 32.811, q = 99.000 (p1 = 0.4838), w = 1.000	-4232.605	1.860	0.173	
		M8	p0 = 0.748, p = 1.586, q = 2.192 (p1 = 0.252), w = 1.475	-4231.675			
	5	M7	p = 0.548 q = 0.314	-4235.293	5.375	0.068	—
		M8	p0 = 0.859, p = 0.918, q = 0.804 (p1 = 0.141), w = 1.701	-4232.419			
		M8a	p0 = 0.516, p = 32.811, q = 99.000 (p1 = 0.484), w = 1.000	-4232.605	0.372	0.542	
		M8	p0 = 0.859, p = 0.918, q = 0.804 (p1 = 0.141) w = 1.701	-4232.419			
Strisores-Aequorlitornithes-40	0.2	M7	p = 0.651, q = 0.345	-5150.191	43.958	0.000	231, 276
		M8	p0 = 0.755, p = 77.686, q = 99.000 (p1 = 0.245), w = 1.968	-5128.212			
		M8a	p0 = 0.528, p = 37.626, q = 99.000 (p1 = 0.472), w = 1.000	-5141.065	25.706	0.000	
		M8	p0 = 0.755, p = 77.686, q = 99.000 (p1 = 0.245), w = 1.968	-5128.212			
	2	M7	p = 0.651, q = 0.341	-5147.952	34.978	0.000	48, 231, 276
		M8	p0 = 0.851, p = 1.259, q = 0.934 (p1 = 0.149), w = 2.375	-5130.463			
		M8a	p0 = 0.535, p = 39.756, q = 99.000 (p1 = 0.465), w = 1.000	-5141.103	21.279	0.000	
		M8	p0 = 0.851, p = 1.259, q = 0.934 (p1 = 0.149), w = 2.375	-5130.463			
	5	M7	p = 0.722, q = 0.415	-5147.937	13.745	0.001	231, 276
		M8	p0 = 0.831, p = 1.645, q = 1.435 (p1 = 0.169) w = 2.193	-5129.820			
		M8a	p0 = 0.528, p = 37.626, q = 99.000 (p1 = 0.472) w = 1.000	-5141.065	22.491	0.000	

		M8	p0 = 0.831, p = 1.645, q = 1.435 (p1 = 0.169), w = 2.193	-5129.820			
Acanthisittidae-Tyranni-Passeri-40	0.2	M7	p = 0.474, q = 0.321	-9711.558	234.997	0.000	33 , 36 , 37 , 40, 41, 43, 44, 46, 47, 48, 49, 51, 54, 55, 56, 58, 59, 67, 69, 111, 116, 122, 124, 125, 127, 128, 130, 153, 157, 164, 179, 228, 229, 230, 231, 233, 234, 237, 238, 241, 243, 265, 275
		M8	p0 = 0.799, p = 0.561, q = 0.446 (p1 = 0.201), w = 2.790	-9594.059			
		M8a	p0 = 0.507, p = 2.450, q = 11.117 (p1 = 0.494), w = 1.000	-9688.670	189.221	0.000	
		M8	p0 = 0.799, p = 0.561, q = 0.446 (p1 = 0.201), w = 2.790	-9594.059			
	2	M7	p = 0.480 q = 0.319	-9711.442	234.203	0.000	14, 33 , 36 , 37 , 40, 41, 43, 44, 46, 47, 48, 49, 51, 54, 55, 56, 58, 59, 67, 69, 111, 116, 122, 124, 125, 127, 128, 130, 153, 157, 164, 179, 228, 229, 230, 231, 233, 234, 237, 238, 241, 243, 265, 275
		M8	p0 = 0.800, p = 0.550, q = 0.435 (p1 = 0.200) w = 2.762	-9594.340			
		M8a	p0 = 0.507, p = 2.450, q = 11.120 (p1 = 0.494) w = 1.000	-9688.670	188.659	0.000	
		M8	p0 = 0.800, p = 0.550, q = 0.435 (p1 = 0.200) w = 2.762	-9594.340			
	5	M7	p = 0.187, q = 0.041	-9713.369	237.519	0.000	14, 33 , 36 , 37 , 40, 41, 43, 44, 46, 47, 48, 49, 51, 54, 55, 56, 58, 59, 67, 69, 111, 113, 116, 122, 124, 125, 127, 128, 130, 153, 157, 164, 179, 228, 229, 230, 231, 233, 234, 237, 238, 241, 243, 265, 275
		M8	p0 = 0.811, p = 0.540, q = 0.444 (p1 = 0.189), w = 2.768	-9594.610			
		M8a	p0 = 0.507, p = 2.450, q = 11.117 (p1 = 0.494), w = 1.000	-9688.670	188.120	0.000	
		M8	p0 = 0.811, p = 0.540, q = 0.444 (p1 = 0.189), w = 2.768	-9594.610			
Passeri-1-40	0.2	M7	p = 0.421 q = 0.386	-11405.799	256.626	0.000	6, 7, 20, 33, 36, 40, 41, 43, 46, 48, 49, 50, 52, 55, 56, 57, 59, 60, 61, 64, 70, 112, 114, 125, 127, 128, 129, 130, 131, 151, 152, 158, 160, 161, 163, 164, 165, 180, 184, 188, 190, 205, 206, 220, 231, 232, 235, 238, 239, 242, 244, 245, 246, 252, 256, 266, 277, 279
		M8	p0 = 0.745 p = 0.872 q = 1.130 (p1 = 0.255) w = 2.364	-11277.486			
		M8a	p0 = 0.598 p = 2.219 q = 8.395 (p1 = 0.402) w = 1.000	-11373.833	192.695	0.000	
		M8	p0 = 0.745 p = 0.872 q = 1.130 (p1 = 0.255) w = 2.364	-11277.486			
	2	M7	p = 0.469 q = 0.411	-11405.085	253.680	0.000	6, 7, 20, 33, 36, 40, 41, 43, 46, 48, 49, 50, 52, 55, 56, 57, 59, 60, 61, 64, 70, 112, 114, 125, 127, 128, 129, 130, 131, 151, 152, 158, 160, 161, 163, 164, 165, 180, 184, 188, 190, 205, 206, 220, 231, 232, 235, 238, 239, 242, 244, 245, 246, 252, 256, 266, 277, 279
		M8	p0 = 0.767 p = 0.860 q = 1.137 (p1 = 0.233) w = 2.325	-11278.245			
		M8a	p0 = 0.598 p = 2.219 q = 8.395 (p1 = 0.402) w = 1.000	-11373.833	191.177	0.000	
		M8	p0 = 0.767 p = 0.860 q = 1.137 (p1 = 0.233) w = 2.325	-11278.245			
	5	M7	p = 0.437 q = 0.387	-11405.189	62.711	0.000	6, 7, 20, 33, 36, 40, 41, 43, 46, 48, 49, 50, 52, 55, 56, 57, 59, 60, 61, 64, 70, 112, 114, 125, 127, 128, 129, 130, 131, 151, 152, 158, 160, 161, 163, 164, 165, 180, 184, 188, 190, 205, 206, 220, 231, 232, 235, 238, 239, 242, 244, 245, 246, 252, 256, 266, 277, 279
		M8	p0 = 0.777 p = 0.839 q = 1.090 (p1 = 0.223) w = 2.389	-11278.856			
		M8a	p0 = 0.598 p = 2.219 q = 8.395 (p1 = 0.402) w = 1.000	-11373.833	189.954	0.000	

		M8	p0 = 0.777 p = 0.839 q = 1.090 (p1 = 0.223) w = 2.389	-11278.856			
Passeri-2-40	0.2	M7	p = 0.581 q = 0.493	-15508.558	192.533	0.000	7, 11, 70, 117, 126, 128, 131, 154, 160, 165, 180, 188, 220, 235, 239, 266, 275, 279
		M8	p0 = 0.920 p = 0.669 q = 0.610 (p1 = 0.080) w = 2.609	-15412.292			
		M8a	p0 = 0.667 p = 1.297 q = 3.397 (p1 = 0.333) w = 1.000	-15489.545	154.507	0.000	
		M8	p0 = 0.920 p = 0.669 q = 0.610 (p1 = 0.080) w = 2.609	-15412.292			
	2	M7	p = 0.545 q = 0.489	-15509.467	194.352	0.000	7, 11, 70, 117, 126, 128, 131, 154, 160, 165, 180, 188, 220, 235, 239, 266, 275, 279
		M8	p0 = 0.920 p = 0.669 q = 0.610 (p1 = 0.080) w = 2.609	-15412.292			
		M8a	p0 = 0.667 p = 1.297 q = 3.397 (p1 = 0.333) w = 1.000	-15489.545	154.507	0.000	
		M8	p0 = 0.920 p = 0.669 q = 0.610 (p1 = 0.080) w = 2.609	-15412.292			
	5	M7	p = 0.551 q = 0.465	-15509.257	39.424	0.000	7, 11, 70, 117, 126, 128, 131, 154, 160, 165, 166, 180, 188, 220, 235, 239, 266, 275, 279
		M8	p0 = 0.928 p = 0.698 q = 0.676 (p1 = 0.072) w = 2.372	-15415.125			
		M8a	p0 = 0.667 p = 1.297 q = 3.396 (p1 = 0.333) w = 1.000	-15489.545	148.840	0.000	
		M8	p0 = 0.928 p = 0.698 q = 0.676 (p1 = 0.072) w = 2.372	-15415.125			
Passeri-3-40	0.2	M7	p = 0.106 q = 0.223	-19091.743	610.258	0.000	7, 36, 40, 43, 46, 48, 49, 52, 55, 56, 57, 59, 61, 117, 125, 128, 131, 149, 152, 154, 158, 164, 179, 187, 230, 231, 238, 243, 255, 265
		M8	p0 = 0.855 p = 0.679 q = 0.569 (p1 = 0.146) w = 2.593	-18786.613			
		M8a	p0 = 0.604 p = 1.530 q = 4.571 (p1 = 0.396) w = 1.000	-18950.591	327.956	0.000	
		M8	p0 = 0.855 p = 0.679 q = 0.569 (p1 = 0.146) w = 2.593	-18786.613			
	2	M7	p = 0.576 q = 0.480	-18987.839	402.451	0.000	7, 36, 40, 43, 46, 48, 49, 52, 55, 56, 57, 59, 61, 117, 125, 128, 131, 149, 152, 154, 158, 164, 179, 187, 230, 231, 238, 243, 255, 265
		M8	p0 = 0.855 p = 0.679 q = 0.569 (p1 = 0.146) w = 2.593	-18786.613			
		M8a	p0 = 0.604 p = 1.530 q = 4.571 (p1 = 0.396) w = 1.000	-18950.591	327.956	0.000	
		M8	p0 = 0.855 p = 0.679 q = 0.569 (p1 = 0.146) w = 2.593	-18786.613			
	5	M7	p = 0.094 q = 0.068	-19012.260	123.336	0.000	7, 36, 40, 43, 46, 48, 49, 52, 55, 56, 57, 59, 61, 117, 125, 128, 131, 149, 152, 154, 158, 164, 179, 187, 230, 231, 238, 243, 255, 265
		M8	p0 = 0.881 p = 0.663 q = 0.539 (p1 = 0.119) w = 2.727	-18788.235			
		M8a	p0 = 0.604 p = 1.530 q = 4.571 (p1 = 0.396) w = 1.000	-18950.591	324.714	0.000	

M8	p0 = 0.881 p = 0.663 q = 0.539 (p1 = 0.119) w = 2.727	-18788.235

Table S10: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r40 datasets

Dataset		SLAC	MEME	FEL	FUBAR
Basal-40	number of PSS	0	8	2	0
	sites		1, 6, 13, 85, 99, 149, 154, 200	154, 218	
Strisores-Aequorlitornithes-40	number of PSS	1	6	8	0
	sites	276	153, 154, 186, 238, 269, 276	49, 61, 123, 225, 238, 266, 268, 276	
Acanthisittidae-Tyranni-Passeri-40	number of PSS	11	23	18	13
	sites	27, 43, 57, 58, 153, 157, 224, 231, 233, 234, 235	14, 27, 43, 44, 51, 56, 57, 58, 62, 67, 122, 130, 146, 153, 157, 224, 229, 231, 233, 235, 247, 265, 279	27, 43, 57, 58, 62, 67, 100, 130, 147, 153, 154, 156, 157, 224, 231, 233, 235, 265	27, 57, 58, 62, 122, 130, 153, 157, 179, 224, 231, 233, 235

Passeri-1-40	number of PSS	10	34	20	13
	sites	20, 114, 154, 163, 180, 206, 232, 244, 245, 246	9, 20, 37, 52, 53, 57, 61, 73, 100, 114, 127, 128, 129, 131, 135, 146, 154, 158, 161, 163, 165, 173, 177, 180, 190, 206, 223, 229, 232, 235, 238, 244, 245, 269	20, 37, 41, 52, 61, 114, 129, 131, 140, 146, 154, 163, 180, 206, 223, 232, 244, 245, 246, 269	20, 41, 61, 114, 129, 154, 163, 180, 206, 229, 235, 244, 245
Passeri-2-40	number of PSS	15	30	19	10
	sites	10, 11, 86, 131, 147, 154, 158, 160, 165, 168, 173, 180, 219, 232, 235	10, 11, 30, 52, 57, 86, 113, 123, 131, 133, 135, 139, 147, 154, 158, 160, 168, 171, 173, 180, 206, 216, 219, 223, 232, 235, 255, 278, 280, 281	10, 11, 71, 79, 86, 131, 139, 147, 154, 158, 160, 168, 171, 173, 180, 216, 219, 232, 235	11, 131, 139, 154, 158, 160, 168, 180, 219, 235
Passeri-3-40	number of PSS	21	37	26	22
	sites	6, 16, 29, 36, 43, 49, 55, 57, 58, 61, 91, 96, 133, 146, 154, 158, 164, 179, 205, 243, 255	4, 6, 16, 22, 29, 36, 39, 43, 48, 49, 50, 55, 56, 57, 58, 61, 68, 91, 96, 101, 123, 127, 133, 137, 141, 144, 146, 148, 154, 158, 164, 179, 185, 189, 205, 243, 255	6, 10, 16, 29, 36, 43, 49, 50, 55, 57, 58, 61, 68, 91, 96, 133, 146, 154, 158, 164, 179, 205, 233, 243, 255, 265	6, 16, 36, 43, 46, 49, 50, 55, 57, 58, 61, 91, 96, 133, 149, 154, 158, 164, 179, 243, 255, 265

Table S11: PAML results for site model comparisons for test of positive selection of the Tas2r40 datasets regarding habitat preference

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Water-birds-40	0.2	M7	p = 0.344 q = 0.391	-17257.137	244.172	0.000	7, 43, 48, 52, 55, 56, 57, 59, 128, 131, 137, 149, 154, 158, 165, 180, 188, 206, 231, 232, 235, 239, 244, 266, 276, 279
		M8	p0 = 0.842 p = 0.772 q = 0.648 (p1 = 0.158) w = 2.153	-17135.051			
		M8a	p0 = 0.588 p = 1.561 q = 4.398 (p1 = 0.412) w = 1.000	-17213.533	156.963	0.000	
		M8	p0 = 0.842 p = 0.772 q = 0.648 (p1 = 0.158) w = 2.153	-17135.051			
	2	M7	p = 0.653 q = 0.438	-17239.291	202.789	0.000	7, 43, 48, 52, 55, 56, 57, 59, 128, 131, 137, 149, 152, 154, 158, 165, 180, 188, 206, 231, 232, 235, 239, 244, 266, 276, 279
		M8	p0 = 0.850 p = 0.751 q = 0.731 (p1 = 0.150) w = 2.060	-17137.897			
		M8a	p0 = 0.588 p = 1.561 q = 4.398 (p1 = 0.412) w = 1.000	-17213.533	151.272	0.000	
		M8	p0 = 0.850 p = 0.751 q = 0.731 (p1 = 0.150) w = 2.060	-17137.897			
	5	M7	p = 0.638 q = 0.409	-17239.564	52.063	0.000	7, 43, 48, 52, 55, 56, 57, 59, 128, 131, 137, 149, 154, 158, 165, 180, 188, 206, 231, 232, 235, 239, 244, 266, 276, 279
		M8	p0 = 0.861 p = 0.734 q = 0.636 (p1 = 0.139) w = 2.078	-17137.150			
		M8a	p0 = 0.588 p = 1.561 q = 4.398 (p1 = 0.412) w = 1.000	-17213.533	152.766	0.000	
		M8	p0 = 0.861 p = 0.734 q = 0.636 (p1 = 0.139) w = 2.078	-17137.150			
Intermediate-regions-40	0.2	M7	p = 0.544 q = 0.443	-22863.407	401.597	0.000	7, 36, 40, 43, 46, 48, 49, 52, 53, 55, 56, 57, 59, 60, 61, 117, 126, 127, 128, 131, 137, 149, 152, 154, 158, 163, 165, 180, 188, 206, 230, 231, 232, 235, 238, 239, 266, 279
		M8	p0 = 0.794 p = 0.706 q = 0.648 (p1 = 0.206) w = 2.189	-22662.608			
		M8a	p0 = 0.633 p = 1.256 q = 3.378 (p1 = 0.367) w = 1.000	-22828.530	331.843	0.000	
		M8	p0 = 0.794 p = 0.706 q = 0.648 (p1 = 0.206) w = 2.189	-22662.608			
	2	M7	p = 0.542 q = 0.432	-22863.231	398.574	0.000	7, 36, 40, 43, 46, 48, 49, 52, 53, 55, 56, 57, 59, 60, 61, 117, 126, 127, 128, 131, 137, 149, 152, 154, 158, 163, 165, 176, 180, 184, 188, 206, 230, 231, 232, 235, 238, 239, 266, 279
		M8	p0 = 0.808 p = 0.719 q = 0.682 (p1 = 0.192) w = 2.168	-22663.944			
		M8a	p0 = 0.633 p = 1.256 q = 3.380 (p1 = 0.367) w = 1.000	-22828.530	329.171	0.000	
		M8	p0 = 0.808 p = 0.719 q = 0.682 (p1 = 0.192) w = 2.168	-22663.944			
	5	M7	p = 0.532 q = 0.426	-22863.838	70.617	0.000	7, 36, 40, 43, 46, 48, 49, 52, 53, 55, 56, 57, 59, 60, 61, 117, 126, 127, 128, 131, 137, 149, 152, 154, 158, 163, 165, 176, 180, 184, 188, 206, 230, 231, 232, 235, 238, 239, 266, 279
		M8	p0 = 0.795 p = 0.708 q = 0.653 (p1 = 0.205) w = 2.164	-22663.459			

	M8a	p0 = 0.633 p = 1.256 q = 3.380 (p1 = 0.367) w = 1.000	-22828.530	330.141	0.000	
	M8	p0 = 0.795 p = 0.708 q = 0.653 (p1 = 0.205) w = 2.164	-22663.459			
Land-birds-40	0.2	M7	p = 0.314 q = 0.381	-28560.458	506.512	0.000	7, 11, 33, 43, 48, 50, 52, 55, 56, 57, 59, 70, 117, 125, 128, 129, 131, 154, 158, 165, 180, 184, 188, 206, 220, 230, 231, 232, 234, 235, 239, 242, 244, 246, 266, 279
		M8	p0 = 0.842 p = 0.738 q = 0.548 (p1 = 0.158) w = 2.255	-28307.202			
		M8a	p0 = 0.627 p = 1.215 q = 2.787 (p1 = 0.373) w = 1.000	-28499.250	384.096	0.000	
		M8	p0 = 0.842 p = 0.738 q = 0.548 (p1 = 0.158) w = 2.255	-28307.202			
	2	M7	p = 0.641 q = 0.449	-28533.944	447.964	0.000	7, 11, 33, 43, 48, 50, 52, 55, 56, 57, 59, 70, 117, 125, 128, 129, 131, 154, 158, 165, 180, 184, 188, 206, 220, 230, 231, 232, 234, 235, 239, 242, 244, 246, 266, 279
		M8	p0 = 0.870 p = 0.724 q = 0.553 (p1 = 0.130) w = 2.250	-28309.962			
		M8a	p0 = 0.627 p = 1.215 q = 2.787 (p1 = 0.373) w = 1.000	-28499.250	378.575	0.000	
		M8	p0 = 0.870 p = 0.724 q = 0.553 (p1 = 0.130) w = 2.250	-28309.962			
	5	M7	p = 0.652 q = 0.447	-28533.114	67.728	0.000	7, 11, 33, 36, 43, 48, 50, 52, 55, 56, 57, 59, 70, 117, 125, 128, 129, 131, 154, 158, 163, 164, 165, 180, 184, 188, 206, 220, 230, 231, 232, 234, 235, 239, 242, 244, 246, 266, 279
		M8	p0 = 0.890 p = 0.054 q = 0.104 (p1 = 0.110) w = 2.336	-28434.827			
		M8a	p0 = 0.627 p = 1.215 q = 2.787 (p1 = 0.373) w = 1.000	-28499.250	128.846	0.000	
		M8	p0 = 0.890 p = 0.054 q = 0.104 (p1 = 0.110) w = 2.336	-28434.827			

Table S12: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r40 datasets

Dataset		**SLAC**	**MEME**	**FEL**	**FUBAR**
Water-birds-40	number of PSS	12	26	15	15
	sites	6, 40, 50, 59, 126, 131, 154, 155, 158, 164, 206, 245	1, 6, 9, 40, 45, 50, 57, 59, 92, 100, 123, 126, 131, 137, 146, 154, 155, 158, 164, 186, 190, 200, 206, 216, 229, 281	6, 40, 50, 57, 59, 92, 126, 131, 146, 154, 155, 158, 164, 184, 216	6, 40, 50, 57, 58, 59, 92, 126, 131, 158, 164, 180, 206, 244, 266

Intermediate-regions-40	number of PSS	1	6	8	0
	sites	36, 55, 57, 59, 96, 123, 131, 146, 147, 154, 155, 158, 161, 165, 173, 180, 206, 229, 231, 232, 235, 245, 266	6, 14, 36, 38, 48, 53, 55, 57, 59, 61, 85, 86, 91, 96, 101, 112, 120, 123, 133, 146, 147, 148, 150, 153, 154, 158, 161, 165, 167, 172, 173, 180, 190, 206, 208, 224, 229, 231, 232, 245, 246, 256, 266, 275, 278, 280	6, 36, 55, 57, 59, 61, 90, 96, 101, 120, 123, 133, 146, 147, 149, 150, 154, 155, 158, 161, 165, 173, 180, 206, 229, 231, 232, 245, 246, 266	36, 57, 59, 60, 61, 96, 117, 123, 133, 147, 154, 158, 161, 165, 173, 180, 206, 229, 232, 245, 266
Land-birds-40	number of PSS	29	51	30	31
	sites	4, 11, 43, 50, 57, 58, 94, 96, 101, 128, 131, 135, 154, 158, 163, 165, 168, 172, 173, 206, 225, 226, 229, 231, 232, 234, 235, 245, 266	3, 4, 5, 12, 31, 42, 43, 48, 50, 52, 56, 57, 58, 59, 92, 94, 96, 101, 123, 128, 131, 133, 135, 139, 143, 144, 148, 154, 158, 160, 161, 165, 168, 173, 180, 186, 205, 206, 220, 224, 225, 226, 229, 232, 234, 235, 248, 266, 279, 280, 281	4, 11, 12, 43, 50, 57, 58, 92, 94, 96, 101, 131, 133, 139, 154, 158, 165, 168, 173, 180, 206, 220, 225, 226, 229, 232, 234, 235, 244, 266	4, 36, 43, 50, 52, 57, 58, 94, 96, 101, 125, 131, 154, 158, 164, 165, 168, 173, 180, 184, 206, 220, 225, 226, 229, 232, 234, 235, 244, 246, 266

Table S13: PAML results for nested site model comparisons for test of positive selection for Tas2r40 datasets with different migratory preference

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Migratory-40	0.2	M7	p = 0.560 q = 0.449	-14808.234	164.948	0.000	7, 48, 51, 54, 56, 57, 127, 130, 159, 164, 179, 187, 205, 230, 231, 233, 237, 238, 265
		M8	p0 = 0.800 p = 0.737 q = 0.745 (p1 = 0.200) w = 1.973	-14725.760			
		M8a	p0 = 0.578 p = 1.435 q = 4.156 (p1 = 0.422) w = 1.000	-14786.203	120.885	0.000	
		M8	p0 = 0.800 p = 0.737 q = 0.745 (p1 = 0.200) w = 1.973	-14725.760			
	2	M7	p = 0.584 q = 0.477	-14808.366	161.155	0.000	7, 40, 48, 51, 54, 56, 57, 125, 127, 130, 159, 164, 179, 187, 205, 229, 230, 231, 233, 237, 238, 265
		M8	p0 = 0.827 p = 0.715 q = 0.766 (p1 = 0.173) w = 1.999	-14727.789			
		M8a	p0 = 0.578 p = 1.435 q = 4.156 (p1 = 0.422) w = 1.000	-14786.203	116.828	0.000	
		M8	p0 = 0.827 p = 0.715 q = 0.766 (p1 = 0.173) w = 1.999	-14727.789			
	5	M7	p = 0.574 q = 0.417	-14808.338	44.271	0.000	7, 48, 51, 54, 56, 57, 125, 127, 130, 159, 164, 179, 187, 205, 229, 230, 231, 233, 237, 238, 265
		M8	p0 = 0.799 p = 0.750 q = 0.750 (p1 = 0.201) w = 1.949	-14726.345			
		M8a	p0 = 0.578 p = 1.435 q = 4.156 (p1 = 0.422) w = 1.000	-14786.203	119.716	0.000	
		M8	p0 = 0.799 p = 0.750 q = 0.750 (p1 = 0.201) w = 1.949	-14726.345			
Partially-migratory-40	0.2	M7	p = 0.071 q = 0.048	-10679.926	208.070	0.000	7, 36, 43, 48, 51, 52, 55, 56, 57, 59, 61, 117, 123, 126, 128, 131, 145, 149, 154, 158, 165, 180, 188, 206, 224, 230, 232, 235, 238, 239, 241, 279
		M8	p0 = 0.776 p = 0.704 q = 0.664 (p1 = 0.224) w = 2.387	-10575.891			
		M8a	p0 = 0.521 p = 1.919 q = 7.489 (p1 = 0.479) w = 1.000	-10651.281	150.781	0.000	
		M8	p0 = 0.776 p = 0.704 q = 0.664 (p1 = 0.224) w = 2.387	-10575.891			
	2	M7	p = 0.515 q = 0.330	-10671.699	190.865	0.000	7, 36, 43, 48, 51, 52, 55, 56, 57, 59, 61, 117, 123, 126, 128, 131, 145, 149, 154, 158, 165, 180, 188, 206, 224, 230, 232, 235, 238, 239, 241, 276, 279
		M8	p0 = 0.781 p = 0.686 q = 0.664 (p1 = 0.219) w = 2.362	-10576.267			
		M8a	p0 = 0.521 p = 1.919 q = 7.489 (p1 = 0.479) w = 1.000	-10651.281	150.029	0.000	
		M8	p0 = 0.781 p = 0.686 q = 0.664 (p1 = 0.219) w = 2.362	-10576.267			
	5	M7	p = 0.521 q = 0.330	-10671.366	40.169	0.000	7, 36, 40, 43, 48, 51, 52, 55, 56, 57, 59, 61, 117, 123, 126, 128, 131, 145, 149, 154, 158, 165, 180, 188, 206, 224, 230, 232, 235, 238, 239, 240, 241, 268, 276, 279
		M8	p0 = 0.802 p = 0.703 q = 0.660 (p1 = 0.198) w = 2.396	-10576.625			

	M8a	p0 = 0.521 p = 1.919 q = 7.489 (p1 = 0.479) w = 1.000	-10651.281	149.313	0.000	
	M8	p0 = 0.802 p = 0.703 q = 0.660 (p1 = 0.198) w = 2.396	-10576.625			
Non-migratory-40	0.2	M7	p = 0.345 q = 0.408	-42593.036	669.479	0.000	7, 11, 33, 36, 40, 43, 46, 48, 52, 55, 56, 57, 59, 61, 70, 117, 125, 127, 128, 130, 131, 137, 146, 152, 154, 158, 163, 165, 180, 184, 188, 220, 231, 232, 234, 235, 238, 239, 244, 266, 279
		M8	p0 = 0.806 p = 0.752 q = 0.702 (p1 = 0.194) w = 2.032	-42258.297			
		M8a	p0 = 0.645 p = 1.196 q = 2.791 (p1 = 0.355) w = 1.000	-42509.837	503.081	0.000	
		M8	p0 = 0.806 p = 0.752 q = 0.702 (p1 = 0.194) w = 2.032	-42258.297			
	2	M7	p = 0.336 q = 0.395	-42594.684	672.774	0.000	7, 11, 33, 36, 40, 43, 46, 48, 52, 55, 56, 57, 59, 61, 70, 117, 125, 127, 128, 130, 131, 137, 146, 152, 154, 158, 163, 165, 180, 184, 188, 220, 231, 232, 234, 235, 238, 239, 244, 266, 279
		M8	p0 = 0.806 p = 0.752 q = 0.702 (p1 = 0.194) w = 2.032	-42258.297			
		M8a	p0 = 0.645 p = 1.196 q = 2.791 (p1 = 0.355) w = 1.000	-42509.837	503.081	0.000	
		M8	p0 = 0.806 p = 0.752 q = 0.702 (p1 = 0.194) w = 2.032	-42258.297			
	5	M7	p = 0.340 q = 0.395	-42593.066	166.458	0.000	7, 11, 33, 36, 40, 43, 46, 48, 52, 55, 56, 57, 59, 61, 70, 117, 125, 127, 128, 130, 131, 137, 146, 152, 154, 158, 163, 165, 180, 184, 188, 220, 231, 232, 234, 235, 238, 239, 244, 266, 279
		M8	p0 = 0.872 p = 0.533 q = 0.669 (p1 = 0.128) w = 1.990	-42275.652			
		M8a	p0 = 0.645 p = 1.196 q = 2.791 (p1 = 0.355) w = 1.000	-42509.837	468.371	0.000	
		M8	p0 = 0.872 p = 0.533 q = 0.669 (p1 = 0.128) w = 1.990	-42275.652			

Table S14: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r40 datasets regarding migratory preference

Dataset		**SLAC**	**MEME**	**FEL**	**FUBAR**
Migratory-40	number of PSS	12	26	19	7
	sites	56, 57, 95, 125, 146, 157, 167, 205, 228, 234, 244, 245	48, 55, 56, 57, 58, 84, 95, 99, 122, 125, 132, 136, 145, 146, 147, 152, 157, 167, 172, 179, 205, 228, 234, 240, 244, 245	6, 56, 57, 58, 86, 95, 122, 125, 132, 146, 157, 167, 179, 187, 205, 228, 234, 244, 245	56, 57, 95, 125, 146, 228, 245

Partially-migratory-40	number of PSS	9	35	18	9
	sites	43, 57, 59, 61, 126, 154, 158, 165, 232	3, 4, 5, 6, 8, 29, 43, 44, 48, 49, 52, 57, 59, 61, 91, 125, 126, 127, 133, 147, 148, 154, 158, 165, 200, 206, 224, 229, 232, 241, 246, 255, 256, 278, 279	6, 29, 43, 48, 52, 57, 59, 61, 126, 133, 147, 149, 154, 158, 164, 165, 206, 232	43, 52, 57, 61, 126, 154, 158, 165, 232
Non-migratory-40	number of PSS	30	50	27	27
	sites	5, 57, 58, 61, 96, 101, 117, 123, 125, 128, 131, 135, 146, 150, 154, 158, 163, 165, 166, 173, 180, 184, 206, 229, 231, 232, 234, 235, 245, 266	1, 5, 20, 42, 46, 50, 52, 57, 58, 59, 61, 85, 86, 96, 101, 123, 128, 131, 133, 135, 139, 142, 143, 144, 146, 150, 154, 158, 161, 165, 166, 173, 180, 184, 186, 190, 201, 206, 224, 229, 232, 234, 235, 247, 248, 256, 266, 275, 280, 281	5, 57, 58, 59, 61, 96, 101, 123, 131, 133, 139, 146, 150, 154, 158, 165, 166, 173, 180, 184, 190, 229, 232, 234, 235, 245, 266	5, 36, 46, 49, 57, 59, 96, 101, 123, 131, 133, 146, 150, 154, 158, 165, 173, 180, 184, 220, 229, 232, 234, 235, 244, 245, 266

Table S15: PAML results for site model comparisons for test of positive selection of the Tas2r9 datasets

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Basal-9	0.2	M7	p = 0.430 q = 0.296	-8437.439	88.246	0.000	7, 11, 66, 75, 79, 80, 81, 155, 158, 159, 160, 169, 171, 174
		M8	p0 = 0.708 p = 0.640 q = 0.850 (p1 = 0.292) w = 1.945	-8393.316			
		M8a	p0 = 0.467 p = 2.811 q = 15.937 (p1 = 0.533) w = 1.000	-8422.239	57.845	0.000	
		M8	p0 = 0.708 p = 0.640 q = 0.850 (p1 = 0.292) w = 1.945	-8393.316			
	2	M7	p = 0.435 q = 0.285	-8437.217	86.954	0.000	7, 11, 66, 75, 79, 80, 81, 155, 158, 159, 160, 169, 171, 174
		M8	p0 = 0.718 p = 0.601 q = 0.811 (p1 = 0.282) w = 1.937	-8393.740			
		M8a	p0 = 0.467 p = 2.811q = 15.939 (p1 = 0.533) w = 1.000	-8422.239	56.999	0.000	
		M8	p0 = 0.718 p = 0.601 q = 0.811 (p1 = 0.282) w = 1.937	-8393.740			
	5	M7	p = 0.432 q = 0.282	-8437.164	86.438	0.000	7, 11, 66, 75, 79, 80, 81, 155, 158, 159, 160, 169, 171, 174
		M8	p0 = 0.751 p = 0.581 q = 0.683 (p1 = 0.249) w = 2.021	-8393.945			
		M8a	p0 = 0.516, p = 32.811, q = 99.000 (p1 = 0.484), w = 1.000	-8422.239	56.587	0.000	
		M8	p0 = 0.751 p = 0.581 q = 0.683 (p1 = 0.249) w = 2.021	-8393.945			
Strisores-Aequorlitornithes-9	0.2	M7	p = 0.525 q = 0.396	-14328.661	221.390	0.000	3, 4, 71, 74, 75, 79, 80, 84, 86, 155, 160, 170, 176, 177, 179, 187, 191, 194, 218, 252, 257, 261, 265, 269
		M8	p0 = 0.887 p = 0.586 q = 0.395 (p1 = 0.113) w = 2.842	-14217.967			
		M8a	p0 = 0.583 p = 1.214 q = 3.020 (p1 = 0.417) w = 1.000	-14314.862	193.791	0.000	
		M8	p0 = 0.887 p = 0.586 q = 0.395 (p1 = 0.113) w = 2.842	-14217.967			
	2	M7	p = 0.528 q = 0.390	-14328.211	217.500	0.000	3, 4, 71, 74, 75, 79, 80, 84, 86, 155, 160, 170, 176, 177, 179, 187, 191, 194, 218, 252, 257, 261, 265, 269
		M8	p0 = 0.901 p = 0.623 q = 0.449 (p1 = 0.099) w = 2.802	-14219.461			
		M8a	p0 = 0.583 p = 1.214 q = 3.020 (p1 = 0.417) w = 1.000	-14314.862	190.801	0.000	
		M8	p0 = 0.901 p = 0.623 q = 0.449 (p1 = 0.099) w = 2.802	-14219.461			
	5	M7	p = 0.535 q = 0.388	-14328.265	26.806	0.000	3, 4, 71, 74, 75, 79, 80, 84, 86, 155, 160, 170, 176, 177, 179, 187, 191, 194, 218, 252, 257, 261, 265, 269
		M8	p0 = 0.920 p = 0.584 q = 0.397 (p1 = 0.080) w = 2.862	-14221.330			
		M8a	p0 = 0.583 p = 1.214 q = 3.020 (p1 = 0.417) w = 1.000	-14314.862	187.065	0.000	

		M8	p0 = 0.920 p = 0.584 q = 0.397 (p1 = 0.080) w = 2.862	-14221.330			
Piciformes-9	0.2	M7	p = 0.468 q = 0.242	-10075.529	87.147	0.000	71, 75, 79, 84, 94, 162, 168, 197, 215, 254, 258, 259, 262, 265
		M8	p0 = 0.879 p = 0.513 q = 0.317 (p1 = 0.121) w = 2.477	-10031.955			
		M8a	p0 = 0.428 p = 1.377 q = 5.899 (p1 = 0.572) w = 1.000	-10066.854	69.797	0.000	
		M8	p0 = 0.879 p = 0.513 q = 0.317 (p1 = 0.121) w = 2.477	-10031.955			
	2	M7	p = 0.472 q = 0.251	-10074.610	85.068	0.000	71, 75, 79, 84, 94, 162, 167, 168, 197, 215, 254, 258, 259, 262, 265
		M8	p0 = 0.872 p = 0.521 q = 0.327 (p1 = 0.128) w = 2.464	-10032.076			
		M8a	p0 = 0.428 p = 1.377 q = 5.899 (p1 = 0.572) w = 1.000	-10066.854	69.556	0.000	
		M8	p0 = 0.872 p = 0.521 q = 0.327 (p1 = 0.128) w = 2.464	-10032.076			
	5	M7	p = 0.469 q = 0.249	-10074.558	15.409	0.000	71, 75, 79, 84, 94, 162, 168, 197, 215, 254, 258, 259, 262, 265
		M8	p0 = 0.872 p = 0.513 q = 0.323 (p1 = 0.128) w = 2.417	-10032.036			
		M8a	p0 = 0.428 p = 1.377 q = 5.899 (p1 = 0.572) w = 1.000	-10066.854	69.636	0.000	
		M8	p0 = 0.872 p = 0.513 q = 0.323 (p1 = 0.128) w = 2.417	-10032.036			
Acanthisittidae-Tyranni-Passeri-1-9	0.2	M7	p = 0.338 q = 0.414	-25076.969	326.902	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.857 p = 0.497 q = 0.706 (p1 = 0.143) w = 2.110	-24913.518			
		M8a	p0 = 0.712 p = 0.706 q = 2.190 (p1 = 0.288) w = 1.000	-25043.525	260.013	0.000	
		M8	p0 = 0.857 p = 0.497 q = 0.706 (p1 = 0.143) w = 2.110	-24913.518			
	2	M7	p = 0.058 q = 0.117	-25196.933	543.209	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.917 p = 0.434 q = 0.728 (p1 = 0.083) w = 2.152	-24925.328			
		M8a	p0 = 0.712 p = 0.706 q = 2.190 (p1 = 0.288) w = 1.000	-25043.525	236.392	0.000	
		M8	p0 = 0.917 p = 0.434 q = 0.728 (p1 = 0.083) w = 2.152	-24925.328			
	5	M7	p = 0.327 q = 0.414	-25077.864	68.679	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.876 p = 0.543 q = 0.817 (p1 = 0.124) w = 2.072	-24916.956			
		M8a	p0 = 0.712 p = 0.706 q = 2.190 (p1 = 0.288) w = 1.000	-25043.525	253.138	0.000	

		M8	p0 = 0.876 p = 0.543 q = 0.817 (p1 = 0.124) w = 2.072	-24916.956			
Acanthisittidae-Tyranni-Passeri-2-9	0.2	M7	p = 0.597 q = 0.500	-39965.669	496.688	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 70, 74, 78, 81, 90, 98, 142, 148, 170, 171, 175, 178, 179, 186, 187, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 267, 272, 276, 304
		M8	p0 = 0.834 p = 0.726 q = 0.655 (p1 = 0.166) w = 2.029	-39717.325			
		M8a	p0 = 0.648 p = 1.090 q = 2.309 (p1 = 0.352) w = 1.000	-39914.326	394.002	0.000	
		M8	p0 = 0.834 p = 0.726 q = 0.655 (p1 = 0.166) w = 2.029	-39717.325			
	2	M7	p = 0.567 q = 0.492	-39967.694	480.877	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 70, 74, 78, 81, 90, 98, 142, 148, 170, 171, 175, 178, 179, 186, 187, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 267, 272, 276, 304
		M8	p0 = 0.886 p = 0.723 q = 0.793 (p1 = 0.114) w = 1.905	-39727.255			
		M8a	p0 = 0.648 p = 1.090 q = 2.309 (p1 = 0.352) w = 1.000	-39914.326	374.140	0.000	
		M8	p0 = 0.886 p = 0.723 q = 0.793 (p1 = 0.114) w = 1.905	-39727.255			
	5	M7	p = 0.573 q = 0.504	-39967.883	107.115	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 70, 74, 78, 81, 90, 93, 98, 142, 148, 155, 170, 171, 175, 178, 179, 186, 187, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 267, 272, 276, 304
		M8	p0 = 0.900 p = 0.721 q = 0.682 (p1 = 0.100) w = 2.060	-39732.760			
		M8a	p0 = 0.619 p = 1.527 q = 4.904 (p1 = 0.381) w = 1.000	-39914.326	363.132	0.000	
		M8	p0 = 0.900 p = 0.721 q = 0.682 (p1 = 0.100) w = 2.060	-39732.760			
Tyranni-Passeri-1-9	0.2	M7	p = 0.519 q = 0.425	-14566.227	148.604	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.847 p = 0.648 q = 0.587 (p1 = 0.153) w = 2.146	-14491.925			
		M8a	p0 = 0.595 p = 1.168 q = 3.161 (p1 = 0.405) w = 1.000	-14551.329	118.809	0.000	
		M8	p0 = 0.847 p = 0.648 q = 0.587 (p1 = 0.153) w = 2.146	-14491.925			
	2	M7	p = 0.541 q = 0.436	-14565.848	147.846	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.847 p = 0.648 q = 0.587 (p1 = 0.153) w = 2.146	-14491.925			
		M8a	p0 = 0.595 p = 1.168 q = 3.161 (p1 = 0.405) w = 1.000	-14551.329	118.809	0.000	
		M8	p0 = 0.847 p = 0.648 q = 0.587 (p1 = 0.153) w = 2.146	-14491.925			
	5	M7	p = 0.278 q = 0.068	-14597.202	91.746	0.000	4, 10, 21, 61, 70, 88, 90, 93, 94, 152, 156, 159, 167, 177, 180, 215, 259, 262, 265, 292
		M8	p0 = 0.850 p = 0.657 q = 0.617 (p1 = 0.150) w = 2.097	-14492.528			
		M8a	p0 = 0.595 p = 1.168 q = 3.161 (p1 = 0.405) w = 1.000	-14551.329	117.604	0.000	

		M8	p0 = 0.850 p = 0.657 q = 0.617 (p1 = 0.150) w = 2.097	-14492.528			
Tyranni-Passeri-2-9	0.2	M7	p = 0.500 q = 0.489	-11433.215	65.114	0.000	5, 12, 13, 15, 80, 165, 191, 218, 299
		M8	p0 = 0.891 p = 0.701 q = 0.808 (p1 = 0.109) w = 1.881	-11400.658			
		M8a	p0 = 0.619 p = 1.527 q = 4.904 (p1 = 0.381) w = 1.000	-25043.525	260.013	0.000	
		M8	p0 = 0.891 p = 0.701 q = 0.808 (p1 = 0.109) w = 1.881	-11400.658			
	2	M7	p = 0.558 q = 0.523	-11430.923	57.178	0.000	2, 5, 12, 13, 15, 80, 165, 191, 218, 299
		M8	p0 = 0.920 p = 0.652 q = 0.773 (p1 = 0.080) w = 1.969	-11402.334			
		M8a	p0 = 0.619 p = 1.527 q = 4.904 (p1 = 0.381) w = 1.000	-11417.105	29.542	0.000	
		M8	p0 = 0.920 p = 0.652 q = 0.773 (p1 = 0.080) w = 1.969	-11402.334			
	5	M7	p = 0.483 q = 0.438	-11432.156	30.102	0.000	2, 5, 12, 13, 15, 80, 165, 191, 218, 299
		M8	p0 = 0.867 p = 0.770 q = 1.067 (p1 = 0.133) w = 1.709	-11402.595			
		M8a	p0 = 0.619 p = 1.527 q = 4.904 (p1 = 0.381) w = 1.000	-11417.105	253.138	0.000	
		M8	p0 = 0.867 p = 0.770 q = 1.067 (p1 = 0.133) w = 1.709	-11402.595			
Tyranni-Passeri-3-9	0.2	M7	p = 0.052 q = 0.098	-25973.793	655.373	0.000	5, 12, 13, 15, 80, 165, 191, 218, 299
		M8	p0 = 0.862 p = 0.822 q = 0.736 (p1 = 0.138) w = 2.123	-25646.106			
		M8a	p0 = 0.676 p = 1.249 q = 2.452 (p1 = 0.324) w = 1.000	-25759.963	227.713	0.000	
		M8	p0 = 0.862 p = 0.822 q = 0.736 (p1 = 0.138) w = 2.123	-25646.106			
	2	M7	p = 0.678 q = 0.571	-25789.175	286.138	0.000	1, 3, 9, 11, 12, 20, 60, 67, 93, 120, 139, 158, 163, 166, 167, 173, 175, 180, 184, 197, 215, 249, 257, 261, 264, 271
		M8	p0 = 0.862 p = 0.822 q = 0.736 (p1 = 0.138) w = 2.123	-25646.106			
		M8a	p0 = 0.676 p = 1.249 q = 2.452 (p1 = 0.381) w = 1.000	-25759.963	227.713	0.000	
		M8	p0 = 0.862 p = 0.822 q = 0.736 (p1 = 0.138) w = 2.123	-25646.106			
	5	M7	p = 0.690 q = 0.570	-25788.052	56.177	0.000	1, 3, 9, 11, 12, 20, 60, 67, 93, 120, 139, 158, 163, 166, 167, 173, 175, 180, 184, 197, 215, 232, 249, 257, 261, 264, 266, 271
		M8	p0 = 0.904 p = 0.736 q = 0.700 (p1 = 0.0960) w = 2.157	-25651.044			
		M8a	p0 = 0.676 p = 1.249 q = 2.452 (p1 = 0.324) w = 1.000	-25759.963	217.838	0.000	

		M8	p0 = 0.904 p = 0.736 q = 0.700 (p1 = 0.0960) w = 2.157	-25651.044			
Tyranni-Passeri-4-9	0.2	M7	p = 0.297 q = 0.418	-33611.442	609.683	0.000	4, 10, 12, 13, 14, 21, 61, 62, 66, 69, 70, 74, 81, 83, 86, 90, 93, 94, 109, 142, 149, 152, 155, 156, 159, 160, 170, 175, 176, 178, 185, 190, 217, 251, 256, 260, 261, 265, 267, 271, 275
		M8	p0 = 0.817 p = 0.615 q = 0.731 (p1 = 0.183) w = 2.180	-33306.601			
		M8a	p0 = 0.694 p = 0.971 q = 2.570 (p1 = 0.306) w = 1.000	-33539.676	227.713	0.000	
		M8	p0 = 0.817 p = 0.615 q = 0.731 (p1 = 0.183) w = 2.180	-33306.601			
	2	M7	p = 0.297 q = 0.419	-33611.670	588.638	0.000	4, 10, 12, 13, 14, 21, 61, 62, 66, 68, 69, 70, 74, 81, 83, 86, 90, 93, 94, 109, 142, 149, 152, 155, 156, 159, 160, 170, 175, 176, 178, 185, 190, 217, 251, 256, 260, 261, 265, 267, 271, 275
		M8	p0 = 0.880 p = 0.576 q = 0.795 (p1 = 0.120) w = 2.149	-33317.351			
		M8a	p0 = 0.694 p = 0.971 q = 2.570 (p1 = 0.306) w = 1.000	-33539.676	444.649	0.000	
		M8	p0 = 0.880 p = 0.576 q = 0.795 (p1 = 0.120) w = 2.149	-33317.351			
	5	M7	p = 0.298 q = 0.417	-33611.667	143.982	0.000	4, 10, 12, 13, 14, 21, 61, 62, 66, 69, 70, 74, 81, 83, 86, 90, 93, 94, 109, 142, 149, 152, 155, 156, 159, 160, 170, 175, 176, 178, 185, 190, 217, 251, 256, 260, 261, 265, 267, 271, 275
		M8	p0 = 0.890 p = 0.582 q = 0.757 (p1 = 0.110) w = 2.164	-33316.167			
		M8a	p0 = 0.694 p = 0.971 q = 2.570 (p1 = 0.306) w = 1.000	-33539.676	447.017	0.000	
		M8	p0 = 0.890 p = 0.582 q = 0.757 (p1 = 0.110) w = 2.164	-33316.167			

Table S16: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r9 datasets

Dataset		**SLAC**	**MEME**	**FEL**	**FUBAR**
Basal-9	number of PSS	2	15	11	2
	sites	175, 184	7, 15, 71, 75, 81, 144, 164, 169, 175, 179, 181, 182, 184, 258, 288	71, 81, 88, 143, 144, 164, 175, 181, 182, 184, 258	81, 184
Strisores-Aequorlitornithes-9	number of PSS	5	19	14	3
	sites	71, 165, 176, 194, 261	18, 30, 59, 70, 71, 75, 76, 80, 86, 90, 95, 104, 165, 176, 194, 204, 222, 261, 272	18, 70, 71, 76, 86, 90, 165, 176, 194, 204, 218, 254, 261, 272	71, 86, 261

Piciformes-9	number of PSS	5	24	15	4
	sites	92, 94, 168, 254, 270	14, 23, 50, 55, 62, 67, 71, 73, 74, 82, 92, 94, 98, 120, 143, 168, 173, 236, 254, 263, 270, 294, 295, 302	23, 55, 59, 73, 92, 94, 143, 168, 188, 254, 257, 263, 270, 294, 302	94, 168, 254, 263
Acanthisittidae-Tyranni-Passeri-1-9	number of PSS	27	35	27	24
	sites	4, 6, 13, 17, 37, 58, 68, 89, 94, 97, 98, 103, 147, 151, 167, 168, 173, 174, 189, 192, 203, 216, 239, 255, 263, 265, 280	4, 6, 13, 17, 23, 37, 44, 53, 58, 62, 68, 70, 74, 81, 86, 89, 90, 94, 98, 103, 140, 165, 168, 173, 174, 184, 189, 192, 216, 225, 239, 255, 263, 273, 300	4, 6, 13, 14, 17, 37, 44, 58, 68, 81, 89, 90, 94, 97, 98, 103, 151, 168, 173, 174, 189, 192, 216, 239, 255, 263, 273	4, 6, 13, 17, 44, 62, 68, 85, 89, 90, 94, 97, 98, 103, 121, 151, 163, 168, 173, 192, 216, 255, 263, 265
Acanthisittidae-Tyranni-Passeri-2-9	number of PSS	28	57	34	27
	sites	4, 6, 13, 14, 17, 81, 85, 86, 89, 92, 124, 142, 152, 164, 170, 172, 179, 186, 187, 190, 193, 217, 225, 227, 264, 271, 281, 285	4, 6, 10, 13, 14, 17, 23, 58, 66, 73, 75, 81, 85, 86, 91, 92, 98, 118, 124, 136, 142, 149, 152, 161, 164, 166, 170, 172, 179, 181, 186, 187, 190, 191, 193, 217, 223, 225, 226, 227, 229, 231, 240, 244, 251, 264, 268, 271, 274, 281, 285, 286, 298, 306, 309, 310	4, 6, 13, 14, 17, 75, 81, 85, 86, 89, 92, 124, 142, 152, 164, 172, 179, 181, 186, 187, 190, 193, 217, 225, 227, 229, 251, 252, 264, 267, 268, 271, 281, 285	4, 6, 10, 13, 14, 17, 62, 70, 81, 85, 86, 124, 142, 172, 179, 186, 187, 190, 193, 217, 225, 227, 251, 264, 267, 268, 281

Tyranni-Passeri-1-9	number of PSS	6	30	18	9
	sites	6, 14, 91, 156, 168, 215	4, 6, 14, 17, 22, 23, 33, 54, 58, 65, 70, 78, 85, 86, 88, 89, 90, 91, 101, 156, 168, 175, 179, 191, 197, 215, 254, 299, 301, 302	4, 6, 14, 17, 22, 23, 33, 58, 89, 91, 156, 168, 188, 198, 215, 254, 260, 302	4, 6, 14, 89, 91, 156, 170, 215, 269
Tyranni-Passeri-2-9	number of PSS	4	12	11	4
	sites	5, 15, 33, 184	5, 11, 15, 33, 104, 125, 163, 200, 204, 215, 269, 299	5, 15, 33, 104, 125, 139, 152, 163, 177, 184, 299	5, 15, 33, 299
Tyranni-Passeri-3-9	number of PSS	21	46	26	10
	sites	3, 12, 13, 15, 16, 31, 36, 43, 89, 95, 120, 123, 157, 170, 172, 201, 204, 215, 225, 232, 257	3, 9, 12, 13, 15, 16, 36, 43, 47, 52, 84, 85, 89, 93, 95, 116, 120, 123, 131, 151, 157, 170, 172, 173, 175, 180, 188, 189, 196, 197, 201, 204, 215, 219, 225, 232, 237, 257, 259, 269, 270, 283, 294, 298, 301, 306	3, 12, 13, 15, 16, 31, 36, 43, 47, 89, 95, 116, 120, 123, 157, 170, 172, 188, 201, 204, 215, 219, 225, 232, 237, 257	3, 12, 13, 15, 36, 120, 123, 157, 201, 215

	number of PSS	32	46	36	27
Tyranni-Passeri-4-9	sites	4, 6, 13, 14, 17, 62, 66, 70, 81, 82, 86, 89, 90, 93, 143, 144, 149, 151, 152, 156, 164, 170, 176, 178, 185, 190, 204, 217, 260, 275, 284, 302	4, 5, 6, 10, 13, 14, 17, 56, 57, 62, 66, 70, 74, 81, 86, 89, 90, 93, 139, 142, 143, 144, 149, 151, 152, 155, 156, 157, 164, 170, 172, 176, 178, 190, 196, 204, 217, 260, 262, 266, 267, 271, 275, 284, 297, 302	4, 6, 13, 14, 17, 37, 62, 66, 68, 74, 81, 86, 89, 90, 93, 143, 144, 149, 151, 152, 155, 156, 157, 164, 170, 176, 178, 185, 190, 204, 217, 260, 267, 275, 284, 302	4, 6, 13, 14, 17, 62, 66, 81, 86, 89, 90, 93, 143, 144, 152, 155, 156, 170, 176, 185, 190, 204, 217, 267, 271, 275, 284

Table S17: PAML results for site model comparisons for test of positive selection of the Tas2r9 datasets regarding habitat preferences

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Water-birds-9	0.2	M7	p = 0.419 q = 0.472	-38927.871	427.435	0.000	2, 3, 4, 5, 11, 13, 62, 69, 71, 75, 79, 80, 84, 91, 94, 143, 155, 157, 160, 164, 169, 170, 175, 178, 190, 193, 217, 251, 256, 261, 262, 264, 265, 266, 270
		M8	p0 = 0.821 p = 0.634 q = 0.674 (p1 = 0.179) w = 1.902	-38714.154			
		M8a	p0 = 0.665 p = 0.995 q = 2.293 (p1 = 0.335) w = 1.000	-38871.014	313.720	0.000	
		M8	p0 = 0.821 p = 0.634 q = 0.674 (p1 = 0.179) w = 1.902	-38714.154			
	2	M7	p = 0.514 q = 0.492	-38922.120	397.733	0.000	2, 3, 4, 5, 11, 13, 62, 69, 71, 75, 79, 80, 84, 91, 94, 143, 155, 157, 160, 164, 169, 170, 175, 178, 190, 193, 217, 251, 256, 261, 262, 264, 265, 266, 270
		M8	p0 = 0.869 p = 0.603 q = 0.763 (p1 = 0.131) w = 1.884	-38722.472			
		M8a	p0 = 0.665 p = 0.995 q = 2.293 (p1 = 0.335) w = 1.000	-38871.014	295.520	0.000	
		M8	p0 = 0.869 p = 0.603 q = 0.763 (p1 = 0.131) w = 1.884	-38722.472			
	5	M7	p = 0.493 q = 0.504	-38922.875	103.723	0.000	2, 3, 4, 5, 11, 13, 62, 63, 69, 71, 75, 79, 80, 84, 91, 94, 143, 155, 157, 158, 160, 164, 169, 170, 175, 178, 190, 193, 217, 251, 256, 261, 262, 264, 265, 266, 270, 294
		M8	p0 = 0.875 p = 0.642 q = 0.767 (p1 = 0.125) w = 1.886	-38723.253			
		M8a	p0 = 0.665 p = 0.995 q = 2.293 (p1 = 0.335) w = 1.000	-38871.014	313.720	0.000	
		M8	p0 = 0.875 p = 0.642 q = 0.767 (p1 = 0.125) w = 1.886	-38723.253			
Intermediate-regions-9	0.2	M7	p = 0.294 q = 0.464	-55269.263	602.707	0.000	2, 5, 11, 13, 14, 22, 62, 71, 75, 79, 84, 91, 94, 95, 122, 141, 150, 160, 165, 169, 170, 171, 176, 177, 191, 194, 218, 252, 257, 262, 264
		M8	p0 = 0.808 p = 0.717 q = 0.803 (p1 = 0.192) w = 1.727	-54967.909			
		M8a	p0 = 0.675 p = 1.011 q = 2.195 (p1 = 0.325) w = 1.000	-55130.743	325.668	0.000	
		M8	p0 = 0.808 p = 0.717 q = 0.803 (p1 = 0.192) w = 1.727	-54967.909			
	2	M7	p = 0.289 q = 0.454	-55275.532	615.247	0.000	2, 5, 11, 13, 14, 22, 62, 71, 75, 79, 84, 91, 94, 95, 122, 141, 150, 160, 165, 169, 170, 171, 176, 177, 191, 194, 218, 252, 257, 262, 264
		M8	p0 = 0.808 p = 0.717 q = 0.803 (p1 = 0.192) w = 1.727	-54967.909			
		M8a	p0 = 0.675 p = 1.011 q = 2.195 (p1 = 0.325) w = 1.000	-55130.743	325.668	0.000	
		M8	p0 = 0.808 p = 0.717 q = 0.803 (p1 = 0.192) w = 1.727	-54967.909			
	5	M7	p = 0.284 q = 0.449	-55276.762	292.037	0.000	2, 5, 11, 13, 14, 22, 62, 71, 75, 79, 84, 91, 94, 95, 122, 141, 150, 160, 165, 169, 170, 171, 176, 177, 191, 194, 218, 252, 257, 262, 264
		M8	p0 = 0.888 p = 0.686 q = 0.894 (p1 = 0.112) w = 1.680	-54981.669			

		M8a	p0 = 0.675 p = 1.011 q = 2.195 (p1 = 0.325) w = 1.000	-55130.743	298.148	0.000	
		M8	p0 = 0.888 p = 0.686 q = 0.894 (p1 = 0.112) w = 1.680	-54981.669			
Land-birds-9	0.2	M7	p = 0.558 q = 0.494	-102708.396	497.578	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 66, 68, 70, 73, 74, 78, 83, 85, 90, 93, 94, 142, 159, 164, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 261, 262, 263, 265, 266, 267, 271, 274
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			
		M8a	p0 = 0.703 p = 0.831 q = 1.784 (p1 = 0.297) w = 1.000	-102541.906	371.163	0.000	
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			
	2	M7	p = 0.555 q = 0.500	-102709.547	493.040	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 66, 68, 70, 73, 74, 78, 83, 85, 90, 93, 94, 142, 159, 164, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 261, 262, 263, 265, 266, 267, 271, 274
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			
		M8a	p0 = 0.703 p = 0.831 q = 1.784 (p1 = 0.297) w = 1.000	-102541.906	371.095	0.000	
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			
	5	M7	p = 0.536 q = 0.505	-102713.553	126.350	0.000	1, 2, 4, 10, 12, 13, 21, 61, 62, 66, 68, 70, 73, 74, 78, 83, 85, 90, 93, 94, 142, 159, 164, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 261, 262, 263, 265, 266, 267, 271, 274
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			
		M8a	p0 = 0.703 p = 0.831 q = 1.784 (p1 = 0.297) w = 1.000	-102541.906	375.632	0.000	
		M8	p0 = 0.791 p = 0.600 q = 0.769 (p1 = 0.209) w = 1.793	-102120.125			

Table S18: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r9 datasets regarding habitat preference

Dataset		SLAC	MEME	FEL	FUBAR
Water-birds-9	number of PSS	23	47	29	12
	sites	5, 7, 14, 15, 18, 71, 80, 90, 91, 143, 144, 153, 158, 163, 164, 175, 178, 186, 190, 217, 262, 294, 306	1, 5, 7, 11, 15, 17, 18, 54, 57, 63, 71, 74, 75, 76, 80, 82, 86, 87, 90, 91, 92, 94, 95, 99, 131, 143, 153, 158, 163, 164, 175, 177, 178, 186, 190, 193, 217, 226, 227, 252, 256, 262, 268, 269, 275, 294, 306	5, 7, 15, 18, 38, 63, 71, 80, 86, 90, 91, 125, 130, 143, 144, 153, 158, 163, 164, 175, 178, 186, 190, 217, 227, 262, 294, 298, 306	5, 14, 18, 63, 80, 143, 164, 175, 186, 187, 190, 217
Intermediate-regions-9	number of PSS	26	60	29	21
	sites	5, 7, 12, 14, 15, 18, 33, 63, 90, 91, 92, 125, 143, 157, 159, 165, 171, 173, 175, 187, 191, 195, 207, 218, 226, 257	1, 5, 7, 11, 13, 14, 15, 18, 24, 33, 45, 57, 63, 67, 71, 74, 75, 80, 82, 83, 84, 87, 90, 91, 92, 94, 96, 125, 133, 141, 143, 153, 157, 158, 159, 165, 166, 171, 173, 175, 178, 187, 188, 191, 194, 195, 200, 201, 207, 218, 225, 226, 227, 228, 253, 257, 259, 260, 265, 301	5, 7, 14, 15, 18, 33, 45, 63, 82, 87, 90, 91, 96, 125, 143, 159, 165, 171, 173, 175, 187, 191, 195, 207, 218, 226, 228, 257, 260	5, 14, 15, 18, 63, 75, 82, 90, 91, 96, 125, 159, 165, 173, 175, 177, 187, 191, 207, 218, 228

	number of PSS	49	80	51	40
Land-birds-9	sites	1, 4, 6, 13, 14, 17, 32, 33, 37, 44, 58, 62, 70, 73, 75, 81, 85, 86, 89, 90, 91, 103, 121, 124, 142, 143, 147, 151, 152, 156, 164, 169, 174, 175, 177, 185, 189, 192, 199, 216, 252, 255, 257, 259, 263, 270, 295, 302, 307	1, 3, 4, 5, 6, 10, 13, 14, 17, 33, 37, 44, 54, 56, 58, 62, 65, 66, 70, 73, 74, 75, 81, 85, 86, 87, 89, 90, 91, 93, 94, 97, 103, 121, 124, 132, 134, 136, 142, 143, 147, 151, 152, 156, 158, 164, 169, 171, 174, 175, 176, 177, 178, 181, 185, 186, 189, 192, 198, 216, 223, 228, 230, 239, 251, 255, 257, 259, 263, 270, 271, 273, 285, 295, 297, 302, 305, 307, 308, 309	1, 4, 6, 13, 14, 17, 22, 33, 37, 44, 58, 62, 70, 73, 74, 75, 81, 85, 86, 89, 90, 92, 103, 121, 124, 142, 143, 147, 151, 152, 156, 164, 169, 171, 174, 175, 177, 185, 189, 199, 216, 228, 255, 257, 259, 263, 270, 273, 284, 295, 307	1, 4, 6, 13, 14, 17, 22, 33, 37, 44, 58, 62, 70, 73, 74, 75, 78, 81, 89, 90, 121, 124, 142, 143, 152, 164, 169, 185, 189, 216, 255, 257, 259, 266, 267, 271, 273, 284, 295, 307

Table S19: PAML results for site model comparisons for test of positive selection of the Tas2r9 datasets with different migratory preferences

Dataset	κ	Model	Parameters	Likelihood (lnL)	$2\Delta \ln L$ (LRT)	Significance (p-value)	PSS
Migratory-9	0.2	M7	p = 0.332 q = 0.439	-37801.143	436.550	0.000	3, 4, 8, 12, 13, 21, 61, 68, 70, 74, 81, 83, 84, 90, 93, 94, 159, 168, 169, 170, 175, 178, 190, 193, 217, 256, 260, 261, 262, 263, 264, 266, 268, 272
		M8	p0 = 0.787 p = 0.689 q = 0.826 (p1 = 0.213) w = 1.809	-37582.868			
		M8a	p0 = 0.654 p = 1.047 q = 2.427 (p1 = 0.346) w = 1.000	-37724.828	283.921	0.000	
		M8	p0 = 0.787 p = 0.689 q = 0.826 (p1 = 0.213) w = 1.809	-37582.868			
	2	M7	p = 0.622 q = 0.504	-37775.498	366.037	0.000	2, 3, 4, 8, 12, 13, 21, 61, 66, 68, 70, 74, 81, 83, 84, 90, 93, 94, 156, 159, 168, 169, 170, 175, 178, 190, 193, 217, 256, 260, 261, 262, 263, 264, 266, 268, 272
		M8	p0 = 0.859 p = 0.527 q = 0.649 (p1 = 0.141) w = 1.857	-37592.479			
		M8a	p0 = 0.654 p = 1.047 q = 2.427 (p1 = 0.346) w = 1.000	-37724.828	264.698	0.000	
		M8	p0 = 0.859 p = 0.527 q = 0.649 (p1 = 0.141) w = 1.857	-37592.479			
	5	M7	p = 0.624 q = 0.491	-37774.425	99.194	0.000	2, 3, 4, 8, 12, 13, 21, 61, 66, 68, 70, 74, 81, 83, 84, 90, 93, 94, 156, 159, 168, 169, 170, 175, 178, 190, 193, 217, 256, 260, 261, 262, 263, 264, 266, 268, 272
		M8	p0 = 0.849 p = 0.621 q = 0.803 (p1 = 0.151) w = 1.835	-37590.397			
		M8a	p0 = 0.654 p = 1.047 q = 2.427 (p1 = 0.346) w = 1.000	-37724.828	268.863	0.000	
		M8	p0 = 0.849 p = 0.621 q = 0.803 (p1 = 0.151) w = 1.835	-37590.397			
Partially-migratory-9	0.2	M7	p = 0.072 q = 0.136	-24029.300	575.627	0.000	2, 11, 13, 14, 39, 62, 63, 67, 71, 75, 79, 80, 84, 88, 89, 91, 94, 95, 153, 155, 157, 160, 161, 164, 168, 169, 170, 175, 176, 178, 186, 190, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 268, 276
		M8	p0 = 0.764 p = 0.614 q = 0.712 (p1 = 0.236) w = 2.056	-23741.487			
		M8a	p0 = 0.641 p = 1.005 q = 2.808 (p1 = 0.359) w = 1.000	-23885.426	287.879	0.000	
		M8	p0 = 0.764 p = 0.614 q = 0.712 (p1 = 0.236) w = 2.056	-23741.487			
	2	M7	p = 0.129 q = 0.248	-23993.842	501.453	0.000	2, 11, 13, 14, 39, 62, 63, 67, 71, 75, 79, 80, 84, 88, 89, 91, 94, 95, 153, 155, 157, 160, 161, 164, 168, 169, 170, 175, 176, 178, 186, 190, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 268, 276
		M8	p0 = 0.782 p = 0.615 q = 0.752 (p1 = 0.218) w = 2.043	-23743.116			
		M8a	p0 = 0.641 p = 1.005 q = 2.808 (p1 = 0.359) w = 1.000	-23885.426	284.620	0.000	
		M8	p0 = 0.782 p = 0.615 q = 0.752 (p1 = 0.218) w = 2.043	-23743.116			
	5	M7	p = 0.134 q = 0.258	-23992.283	213.715	0.000	2, 11, 13, 14, 39, 62, 63, 67, 71, 75, 79, 80, 84, 88, 89, 91, 94, 95, 153, 155, 157, 160, 161, 164, 168, 169, 170, 175, 176, 178, 186, 190, 193, 217, 251, 256, 260, 261, 262, 263, 264, 266, 268, 276
		M8	p0 = 0.782 p = 0.622 q = 0.765 (p1 = 0.218) w = 2.051	-23742.915			

		M8a	p0 = 0.641 p = 1.005 q = 2.808 (p1 = 0.359) w = 1.000	-23885.426	285.022	0.000	
		M8	p0 = 0.782 p = 0.622 q = 0.765 (p1 = 0.218) w = 2.051	-23742.915			
Non-migratory-9	0.2	M7	p = 0.255 q = 0.415	-130860.647	1689.279	0.000	1, 2, 4, 10, 12, 13, 21, 61, 68, 70, 74, 78, 83, 85, 90, 93, 94, 121, 142, 154, 159, 163, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 262, 263, 265, 266, 270
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			
		M8a	p0 = 0.704 p = 0.886 q = 1.799 (p1 = 0.296) w = 1.000	-130470.196	908.378	0.000	
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			
	2	M7	p = 0.261 q = 0.426	-130857.832	1683.651	0.000	1, 2, 4, 10, 12, 13, 21, 61, 68, 70, 74, 78, 83, 85, 90, 93, 94, 121, 142, 154, 159, 163, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 262, 263, 265, 266, 270
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			
		M8a	p0 = 0.704 p = 0.886 q = 1.799 (p1 = 0.296) w = 1.000	-130470.196	908.378	0.000	
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			
	5	M7	p = 0.262 q = 0.426	-130859.766	779.140	0.000	1, 2, 4, 10, 12, 13, 21, 61, 68, 70, 74, 78, 83, 85, 90, 93, 94, 121, 142, 154, 159, 163, 168, 169, 174, 185, 189, 192, 198, 216, 250, 255, 259, 260, 262, 263, 265, 266, 270
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			
		M8a	p0 = 0.704 p = 0.886 q = 1.799 (p1 = 0.296) w = 1.000	-130470.196	908.378	0.000	
		M8	p0 = 0.838 p = 0.627 q = 0.736 (p1 = 0.162) w = 1.821	-130016.007			

Table S20: Datamonkey (SLAC, MEME, FEL, FUBAR) results for site model comparisons for test of positive selection of the Tas2r9 datasets regarding migratory preference

Dataset		SLAC	MEME	FEL	FUBAR
Migratory-9	number of PSS	22	46	24	15
	sites	4, 6, 13, 14, 17, 33, 37, 86, 89, 90, 142, 152, 156, 162, 164, 170, 172, 178, 190, 193, 217, 308	4, 5, 6, 8, 12, 13, 14, 17, 23, 33, 37, 56, 66, 70, 81, 85, 86, 89, 90, 91, 93, 124, 130, 140, 142, 152, 162, 164, 170, 172, 173, 175, 177, 178, 190, 193, 217, 226, 256, 259, 260, 264, 272, 274, 275, 308	4, 6, 13, 14, 142, 17, 152, 33, 162, 164, 37, 172, 178, 308, 190, 193, 81, 85, 86, 89, 90, 217, 124, 126	4, 6, 13, 17, 86, 89, 90, 124, 164, 175, 186, 190, 217, 260, 267
Partially-migratory-40	number of PSS	16	36	23	10
	sites	15, 63, 71, 90, 91, 99, 104, 152, 153, 157, 176, 190, 217, 262, 264, 303	5, 11, 15, 23, 30, 57, 63, 66, 71, 82, 88, 90, 91, 92, 96, 99, 104, 140, 152, 153, 158, 161, 163, 177, 181, 190, 193, 199, 226, 252, 259, 262, 264, 271, 272, 303	15, 18, 23, 38, 63, 71, 82, 90, 91, 93, 96, 99, 104, 152, 153, 163, 164, 176, 181, 190, 262, 264, 303	15, 63, 71, 80, 91, 93, 152, 153, 190, 264

	number of PSS	53	83	52	44
Non-migratory-40	sites	1, 4, 6, 13, 14, 17, 32, 33, 37, 44, 58, 62, 68, 75, 78, 81, 85, 89, 90, 91, 92, 103, 121, 124, 142, 143, 147, 151, 152, 156, 158, 163, 169, 173, 174, 175, 177, 185, 189, 199, 205, 216, 228, 233, 255, 257, 259, 263, 269, 292, 294, 301, 306	1, 5, 20, 42, 46, 50, 52, 57, 58, 59, 61, 85, 86, 96, 101, 123, 128, 131, 133, 135, 139, 142, 143, 144, 146, 150, 154, 158, 161, 165, 166, 173, 180, 184, 186, 190, 201, 206, 224, 229, 232, 234, 235, 247, 248, 256, 266, 275, 280, 281	1, 4, 6, 13, 14, 17, 22, 32, 37, 44, 58, 62, 68, 70, 73, 74, 75, 81, 85, 89, 90, 91, 92, 103, 121, 124, 142, 143, 152, 156, 158, 163, 169, 171, 173, 174, 177, 185, 189, 199, 216, 226, 233, 255, 257, 258, 263, 269, 292, 294, 301, 306	4, 6, 13, 14, 17, 22, 33, 37, 44, 58, 62, 65, 74, 75, 78, 85, 89, 90, 93, 94, 124, 142, 143, 158, 163, 169, 177, 185, 189, 216, 226, 228, 233, 250, 255, 257, 258, 259, 262, 265, 292, 294, 301, 306