

## **Supplementary Material**

### **Resolving phylogenetic relationships within Passeriformes based on mitochondrial genes and inferring the evolution of their mitogenomes in terms of duplications**

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## **Phylogeny and evolution of Passeriformes**

### **1. Introduction**

It was initially assumed that the evolution of passerines was driven by the breakup of the southern megacontinent Gondwana as well as the subsequent split and drift of their fragments (Barker, et al. 2002; Barker, et al. 2004; Edwards and Boles 2002; Ericson, et al. 2002; Ericson, et al. 2003). These geological events were supposed to be reflected in the passerine diversification and the suborder classification (Ericson, et al. 2002; Ericson and Johansson 2003). However, more recent studies based on fossil records postponed the diversification of passerines to the beginning of Cenozoic (Claramunt and Cracraft 2015; Gibb, et al. 2015; Jarvis, et al. 2014; Mayr 2013; Oliveros, et al. 2019; Prum, et al. 2015;

Selvatti, et al. 2017; Selvatti, et al. 2015). Passerines originated most likely in the Australo-Pacific region and their dispersal and diversification were affected by a number of climatic and geologic events, e.g. Oligocene glaciation and inundation of New Zealand (Oliveros, et al. 2019). The first lineage branched off from all other passerines grouped into Eupasserres was Acanthisitti (or Acanthisittia), which is endemic to New Zealand. This separation was previously assumed to be associated with the rupture of Neozelandic plate and was dated to the late Cretaceous, ca. 80 Ma (Aggerbeck, et al. 2014; Barker, et al. 2004; Ericson, et al. 2002; Ericson, et al. 2014) or ca. 78-76 Ma (Pacheco, et al. 2011), but the recent multi-taxon and phylogenomic estimations shifted this divergence to later times, ca. 62 Ma, i.e. the early Palaeocene (Gibb, et al. 2015), ca. 57-50 Ma, i.e. the late Palaeocene/early Eocene (Claramunt and Cracraft 2015; Prum, et al. 2015; Selvatti, et al. 2015) or even ca. 47 Ma, i.e. the Middle Eocene (Oliveros, et al. 2019). Most likely, a gene flow could occur for some time between New Zealand and Gondwana avian populations until their complete isolation (Selvatti, et al. 2015). The split of Eupasserres into two suborders, the Suboscines (or Tyranni) and the Oscines (or Passeri) could be associated with the separation of Eastern Antarctica and Australia (Claramunt and Cracraft 2015; Selvatti, et al. 2015). The members of the former group left on Western Gondwana corresponding to current Antarctica and South America, while the latter drifted northwards on the Australian plate. According to the older estimations, this divergence occurred in the late Cretaceous, ca. 89-71 Ma (Ericson, et al. 2014; Pacheco, et al. 2011), but it is more probable that this diversification occurred in the middle Palaeocene, ca. 60 Ma (Gibb, et al. 2015), the early/middle Eocene, ca. 52-47 Ma (Prum, et al. 2015; Selvatti, et al. 2015) or the middle Eocene, ca. 44 Ma (Oliveros, et al. 2019). The latter biogeographic analysis suggested an Eurasian origin of crown suboscines but the dating is still compatible with the hypothesis of trans-Antarctic separation of oscines and suboscines (Oliveros, et al. 2019).

The further biogeographic history of Suboscines is well reflected in the current distribution of their two infraorders: Tyrannides, endemic to the New World, i.e. Central and South America (Ohlson, et al. 2013), and Eurylaimides, which is widespread in the Old World, i.e. Africa, Madagascar and Southeast Asia, but one monotypic family lives in South America (Moyle, et al. 2006). Fossils of Eurylaimides were also discovered in Europe (Mayr and Manegold 2006). This separation could occur ca. 50 Ma in the early Eocene (Gibb, et al. 2015) or ca. 46-39 Ma in the middle/late Eocene (Claramunt and Cracraft 2015; Oliveros, et al. 2019; Prum, et al. 2015; Selvatti, et al. 2015), i.e. much later than older estimations, ca. 79-61 Ma in the Cretaceous/Palaeocene boundary (Ericson, et al. 2014; Pacheco, et al. 2011). The split of these groups could be associated with the isolation of South America and Africa if we assume the New World origin of Eurylaimides (Selvatti, et al. 2017; Selvatti, et al. 2015) but other studies indicate that this group originated in Eurasia (Moyle, et al. 2006; Oliveros, et al. 2019).

Oscines achieved the greatest evolutionary success covering almost 80% of all Passeriformes diversity. Two parvorders, Corvida and Passerida, are traditionally recognized in Oscines (Barker, et al. 2002; Barker, et al. 2004; Ericson, et al. 2002; Sibley and Ahlquist 1990). The evolution of the former has been associated with the Southern hemisphere, while the latter with the Northern hemisphere (Selvatti, et al. 2015). Passerida proved to be a monophyletic lineage and nested within Corvida, which turned out paraphyletic in molecular analyses. In consequence, additional groups were elevated in this classification, such as: Basal Oscines, Corvoidea (or core Corvoidea) and Transitional Oscines (or Basal Passerida) (Gibb, et al. 2015; Jonsson, et al. 2011; Selvatti, et al. 2015). Basal Oscines are the earliest diverged lineages of all Oscines, while Transitional Oscines are sister to Passerida. Both groups also turned out paraphyletic and are restricted to the Australian-Oceanian realms. Corvoidea is the main monophyletic group of Corvida, which is currently distributed in Eurasia, Africa and

Americas. Its diversification and dispersion from Australian region, ca. 22 Ma (Moyle, et al. 2016), ca. 25.5-28 Ma (Gibb, et al. 2015; Oliveros, et al. 2019; Selvatti, et al. 2015), was enabled by a closer connection of Australasia with continental Asia in the Oligocene/Miocene and could be associated with the Oligocene glaciation (Oliveros, et al. 2019). Previous estimations dated the Corvoidea radiation to the Eocene/Oligocene, ca. 37-32 Ma (Aggerbeck, et al. 2014; Jonsson, et al. 2011) or the Eocene, ca. 46-37 Ma (Claramunt and Cracraft 2015; Ericson, et al. 2014).

The Transitional Oscines are considered closely related to Passerida (or Eupasserii) (Beresford, et al. 2005; Gibb, et al. 2015; Jonsson, et al. 2011; Jonsson, et al. 2007; Selvatti, et al. 2015). The origin of the latter group is still controversial and is explained by two hypotheses. One assumes that its Australasian ancestors migrated to continental Asia across the Sunda Shelf and next expanded into Africa and the New World (Barker, et al. 2004; Johansson, et al. 2008). The other view claims an African origin of this group, from an Australian ancestor dispersed directly to Africa across stepping-stones in the southern Indian Ocean (Beresford, et al. 2005; Jonsson and Fjeldsa 2006; Jonsson, et al. 2007). Next, Passerida dispersed from this continent to Eurasia and the rest of the world. According to the results of some researchers, Passerida diverged ca. 30-25 Ma in the late Oligocene, which is in agreement with the Asian-first hypothesis (Gibb, et al. 2015; Oliveros, et al. 2019; Selvatti, et al. 2015). Much older estimations, ca. 54-44 Ma in the early Eocene or ca. 39 Ma in the middle Eocene were obtained by Ericson, et al. (2014) and Claramunt and Cracraft (2015), respectively. On the other hand, (Moyle, et al. 2016) estimated the divergence to much later time, ca. 22 Ma, i.e. at the beginning of the Miocene. The parvorder Passerida is presently very widespread in Eurasia, Africa and two Americas.

## 2. Results and Discussion

The high-level groups of Passeriformes: Acanthisitti, Suboscines and Oscines, are significantly distinguished (Fig. 1, 2, S1 and S2). Acanthisitti is sister to the others (Eupasserres) as in other phylogenies. Subgroups of Suboscines, Eurylaimides and Tyrannides (divided into Furnariida and Tyrannida) are also highly supported. The basal position to other Oscines is occupied by Menurida followed by Meliphagida as in other analyses (Barker 2014; Barker, et al. 2004; Claramunt and Cracraft 2015; Ericson, et al. 2014; Gibb, et al. 2015; Moyle, et al. 2016; Oliveros, et al. 2019; Prum, et al. 2015; Selvatti, et al. 2015). The next diverged lineage is Corvides.

### 2.1. Relationships among Corvides

Compared with other authors (Aggerbeck, et al. 2014; Barker 2014; Barker, et al. 2004; Claramunt and Cracraft 2015; Jonsson, et al. 2016; Jonsson, et al. 2011; Moyle, et al. 2016; Oliveros, et al. 2019; Selvatti, et al. 2015), we also obtained compatible relationships for some families classified to Corvides: Rhipiduridae, Monarchidae, Laniidae and Corvidae, but we received much higher support by more methods (Fig. S1). However, relationships among earlier diverged lineages of Corvides, *sensu* Aggerbeck, et al. (2014) and Jonsson, et al. (2016), are different. All three methods applied by us showed significant ( $PP_{MrBayes}=1$ ,  $PP_{PhyloBayes}=0.99$ ,  $aLRT=99$ ,  $BP=81$ ) grouping of Campephagidae, Oriolidae and Vireonidae. Such relationships were not present in other phylogenies but the alternative topologies were weakly supported or not at all (Aggerbeck, et al. 2014; Barker, et al. 2004; Jonsson, et al. 2011; Selvatti, et al. 2015). Jonsson, et al. (2016) obtained better resolved relationships in BEAST based on 8 nuclear and 4 mitochondrial markers. In this case, Campephagidae was sister to the clade that included, among others, Rhipiduridae, Monarchidae, Laniidae and Corvidae, while Oriolidae and Vireonidae were clustered together. In a better supported

genome-scale phylogeny (Moyle, et al. 2016; Oliveros, et al. 2019), the Campephagidae diverged earlier than Mohouidae, after which the other above-mentioned families diverged. The grouping of Oriolidae and Vireonidae was also obtained in other phylogenies (Aggerbeck, et al. 2014; Ericson, et al. 2014; Jonsson, et al. 2011; Moyle, et al. 2016; Oliveros, et al. 2019; Selvatti, et al. 2015) but not always with high support. In our phylogenetic analyses, these families were, however, not clustered, similarly to Barker, et al. (2004) and Claramunt and Cracraft (2015). However, it was not highly supported either.

*Mohoua*, a representative of Mohouidae, has also an incompatible position among various authors. In all three trees obtained by us, it was sister (with  $PP_{MrBayes}=0.98$ ,  $PP_{PhyloBayes}=0.93$ ,  $aLRT=72$ ,  $BP=61$ ) to the clade including Rhipiduridae, Monarchidae, Laniidae and Corvidae, which was highly supported (Fig. S1). According to Moyle, et al. (2016) and Oliveros, et al. (2019), *Mohoua* evolved after Campephagidae and before the divergence of the mentioned families. In the phylogeny based on 1 mitochondrial and 4 nuclear markers (Jonsson, et al. 2011), Mohouidae is very poorly grouped with Campephagidae, while in a phylogeny based on 5 mitochondrial and 4 nuclear genes (Selvatti, et al. 2015), insignificantly with Oriolidae and Vireonidae. On the other hand, Aggerbeck, et al. (2014) obtained the basal position of *Mohoua* in relation to the other families mentioned above, which was highly supported in the MrBayes tree based on 22 nuclear loci. Such position was also received by Claramunt and Cracraft (2015), but they did not provide support values. A further study is necessary to solve this disagreement.

## **2.2. Relationships involving Transitional Oscines**

The sister lineage to Corvides is maximally supported and includes parvorder Passerida as well as several lineages diverged earlier and described under the common name Transitional Oscines (Aggerbeck, et al. 2014; Barker 2014; Jonsson, et al. 2011; Selvatti, et al. 2015). The phylogenetic position of the Transitional Oscines is not consistent across many

analyses. The earliest diverged lineage in IQ-TREE and MrBayes trees received by us includes members of Melanocharitidae, as well as the clade of Notiomystidae and Callaeidae (Fig. S1). In turn, the PhyloBayes tree has Callaeidae branched off first followed by the lineage containing Notiomystidae and Melanocharitidae. However, none of these relationships are highly supported. Nevertheless, all three phylogenetic trees demonstrated consistently, with high support  $PP \geq 0.98$  and  $aLRT \geq 97$  values, the subsequent divergence of Petroicidae and Picathartidae. Members of Melanocharitidae, Notiomystidae, Petroicidae and Picathartidae included in the mitogenomic phylogenies received by Barker (2014) also showed relationships described above in most approaches applied. The close relationship between Notiomystidae and Callaeidae is also present in the trees calculated by Selvatti, et al. (2015) and Oliveros, et al. (2019), but Melanocharitidae is clustered with the rest Transitional Oscines and Passerida. The arrangement of Petroicidae and Picathartidae is the same in Selvatti, et al. (2015) as in our trees, too. Unfortunately, these parts of the tree are not well supported. In phylogenies based on much smaller number of markers and much weaker supported, Picathartidae diverged earlier than Petroicidae (Barker, et al. 2004; Beresford, et al. 2005; Jonsson, et al. 2011) or had a common origin (Jonsson, et al. 2007). The clade grouping members of this families is better supported ( $BP > 70\%$ ) in phylogenomic study by Moyle, et al. (2016) and Oliveros, et al. (2019).

The closer relationship of Petroicidae or Picathartii to Passerida has interesting implications for determination of biogeographic origin of the latter. One hypothesis supported by the common grouping of Passerida with Australasian Petroicidae claims that continental Asia was firstly inhabited by emerging Passerida (Barker, et al. 2002; Barker, et al. 2004; Johansson, et al. 2008). On the other hand, the close connection of Passerida and Picathartii indicates that Africa was the place of origin (Beresford, et al. 2005; Jonsson and Fjeldsa 2006; Jonsson, et al. 2007). However, not all members of Picathartii live in Africa. *Eupetes*

*macrocerus* inhabits Southeast Asia and is placed in phylogenetic trees among African members of Picarthartii, which does not exclude the first hypothesis completely. *Eupetes* can represent a relic lineage that was previously much more widespread in Southeast Asia, while Africa was inhabited later independently. In agreement with that, the recent estimations of divergence time among Passerida indicate that the crossing from Australia to Africa by the potential ancestor of Passerida could not be easy because a putative dispersal corridor across the southern Indian Ocean did not already exist (Moyle, et al. 2016; Selvatti, et al. 2015). More probable is a direct dispersal from Australia to Asia via island chains, which occurred due to an uplift in the Wallacea region (Oliveros, et al. 2019). Additional analyses including more representatives of Transitional Oscines are required to verify these hypotheses from a phylogenetic point of view.

### **2.3. Relationships among Passerida**

Three main groups of Passerida are traditionally recognized: Passeroidea, Muscicapoidea and Sylvioidea (Ericson and Johansson 2003; Sibley and Ahlquist 1990). Nabholz, et al. (2010) separated also the fourth lineage belonging in the other classifications to Sylvioidea and represented by Paridae, which can correspond to Paroidea, proposed by Alstrom, et al. (2014) and including additionally Remizidae, as well as Hyliotidae and Stenostiridae in Boyd's classification (Boyd 2016). Alstrom, et al. (2014) and Boyd (2016) split also Muscicapoidea (*sensu lato*) into four superfamilies: (core) Muscicapoidea, Certhioidea, Bombycilloidea and Reguloidea. The phylogenetic relationships between these groups differ depending on the authors.

Johansson, et al. (2008) obtained the basal position of Passeroidea, next diverged Paroidea (including, however, Regulidae) and the clade of Muscicapoidea and Sylvioidea in a three-nuclear marker phylogeny. Bombycillidae had an unresolved position. In their phylogeny based on different set of markers (1 mitochondrial and 5 nuclear ones),



Passeroidea occupied the same position, but the next evolved lineage was Muscipoidea (now including Regulidae) and Sylvioidea grouped with Paroidea members. However, only the latter grouping was significantly supported with PP=0.98. The similar relationships, but better resolved, are presented by Alstrom, et al. (2014) based on six markers (2 mitochondrial and 4 nuclear). Sylvioidea and all families classified to Paroidea by Boyd (2016) are clustered with PP=1 and BP=85. However, the highly supported clade including Muscipoidea, Certhioidea, Bombycilloidea and *Spalaeornis* is weakly grouped with Sylvioidea+Passeroidea and does not contain Reguloidea whose sisterhood to all above-mentioned groups is also poorly supported.

The tree based on 12 mitochondrial protein-coding genes is characterized by the following order of branching: Muscipoidea, Paroidea, and Sylvioidea grouped with Passeroidea (Nabholz, et al. 2010). The first two groups were, however, represented only by one species. The highest support was obtained for Sylvioidea+Passeroidea (PP=1) as well as the clade including the latter group and Passeroidea (PP=0.99), although, by only one out of six approaches. A similar relationships were received by Wu, et al. (2015) based on 12S rRNA, but members of Paroidea did not form a monophyletic clade and were mixed within Sylvioidea or even Muscipoidea. The BP support of Sylvioidea+Passeroidea was at best only 71.

Our three trees are similar to that based on mitogenomes and obtained by Gibb, et al. (2015) and most approaches applied by Barker (2014), but are better resolved (Fig. 1, 2 and S1). Two significantly supported groups of Passerida can be recognized. One of them (PPs=1, aLRT=99, BP=79) includes Sylvioidea (*sensu stricto*) and Paroidea, which is represented by Hyliotidae, Paridae and Remizidae. Sylvioidea and Paroidea are also well defined groups with PPs≥0.99, aLRT≥97 and BP≥80. The second significant group (with PPs=1, aLRT=100, BP=95) comprises monophyletic Passeroidea with all four support values maximal and

monophyletic Muscicapoidea (*sensu lato*) with  $PP_{MrBayes}=1$ ,  $PP_{PhyloBayes}=0.89$ ,  $aLRT=97$  and  $BP=82$ . The latter clade contains members of Certhioidea (Sittidae, Troglodytidae), Bombycilloidea (Bombycillidae, Mohoidae), core Muscicapoidea (Sturnidae, Muscicapidae, Turdidae) and Reguloidea (Regulidae). Bombycilloidea and Reguloidea are significantly grouped together ( $PP_{MrBayes}=1$ ,  $PP_{PhyloBayes}=0.97$ ,  $aLRT=98$ ,  $BP=74$ ).

The relationships between these four groups of Passerida are congruent with those of Barker, et al. (2004) based on two RAG genes, in which Paroidea (Paridae and Remizidae) were very highly supported by three methods, and Paroidea+Sylvioidea by Bayesian approach ( $PP\geq 0.95$ ). The two main groups obtained by us, i.e. Paroidea+Sylvioidea and Passeroidea+Muscicapoidea (*sensu lato*) were also inferred in the 1156-nuclear gene phylogeny (Claramunt and Cracraft 2015), but support values were not presented. However, these groups received maximal support in phylogenomic analyses by Moyle, et al. (2016) and  $>70\%$  BP by (Oliveros, et al. 2019), but the clade of Hyliotidae (and Stenostiridae) was placed at the base to other members of Paroidea and Sylvioidea, and did not cluster with the clade of Paridae and Remizidae as in our trees ( $PP_{MrBayes}=1$ ,  $PP_{PhyloBayes}=0.99$ ,  $aLRT=97$ ,  $BP=88$ ). These phylogenomic studies provided the same relationships among families of Muscicapoidea (*sensu lato*) as our results except for Regulidae, which clustered with Certhioidea members with differentiated support ( $BP<70\%$ ,  $PP=1$ ). The two main groups of Passerida were also inferred in the 7-nuclear gene tree, but with poor support (Ericson, et al. 2014), as well as in the multi-taxa phylogeny based on 5 mitochondrial and 4 nuclear genes (Selvatti, et al. 2015), where Paroidea+Sylvioidea were supported with  $BP=83$ , while Passeroidea+Muscicapoidea (*sensu lato*) with  $BP=62$ . However, the position of Stenostiridae and Regulidae was not well resolved. The former was sister to Paroidea+Sylvioidea and the latter sister to Passeroidea+Muscicapoidea (*sensu lato*). The relationships between Passerida inferred in our study are also present in the global bird phylogeny using 259 nuclear genes

(Prum, et al. 2015). Although the groups in the question are not represented by many taxa, all nodes referring to Passerida received the highest posterior probability. In the light of our and other results, we can state that the partition of Passerida into Paroidea+Sylvioidea and Passeroidea+Muscicapoidea (*sensu lato*) is currently the most probable.

The group of New World Nine-Primaried Oscines (Fuchs, et al. 2012; Sibley and Ahlquist 1990) is nested within other Passeroidea (Fig. 1, 2 and S1). This assemblage is monophyletic, but only one tree, calculated in PhyloBayes, provided the highest support PP=1. The PP in MrBayes tree is 0.9 and bootstrap percentage in IQ-TREE, only 58. In the maximum likelihood tree calculated by Selvatti, et al. (2015), this group was much better supported (BP=96).

The relationships at lower taxonomic levels in phylogenetic trees received by us are well resolved and three approaches produced consistent topology (Fig. 1, 2 and S1). However, we also found some genera and families non-monophyletic, which suggests that their nomenclature and taxonomy should be revised. In agreement with others (Gibb, et al. 2015; Oliveros, et al. 2019; Selvatti, et al. 2015), our phylogenetic analyses showed that Callaeidae and Petroicidae classified to one superfamily Callaeoidea by Boyd (2016) are not significantly clustered together. Therefore, this superfamily should be revised. *Passer ammodendri* is grouped in the IQ-TREE phylogeny with other members of its genus, but in the Bayesian trees it is placed unexpectedly among *Emberiza*. This inconsistent placement may result from contamination or misassembly of its mitochondrial genome. We found that 22 subsequent genes in the region from 2719 bp to 9972 bp show greater similarity to the sequences from *Emberiza* than from *Passer*, while the control region and 14 genes in other regions (1-2718 bp and 9974-16782 bp) are more similar to *Passer*. Therefore, the sequence of this genome should be verified.

### 3. References

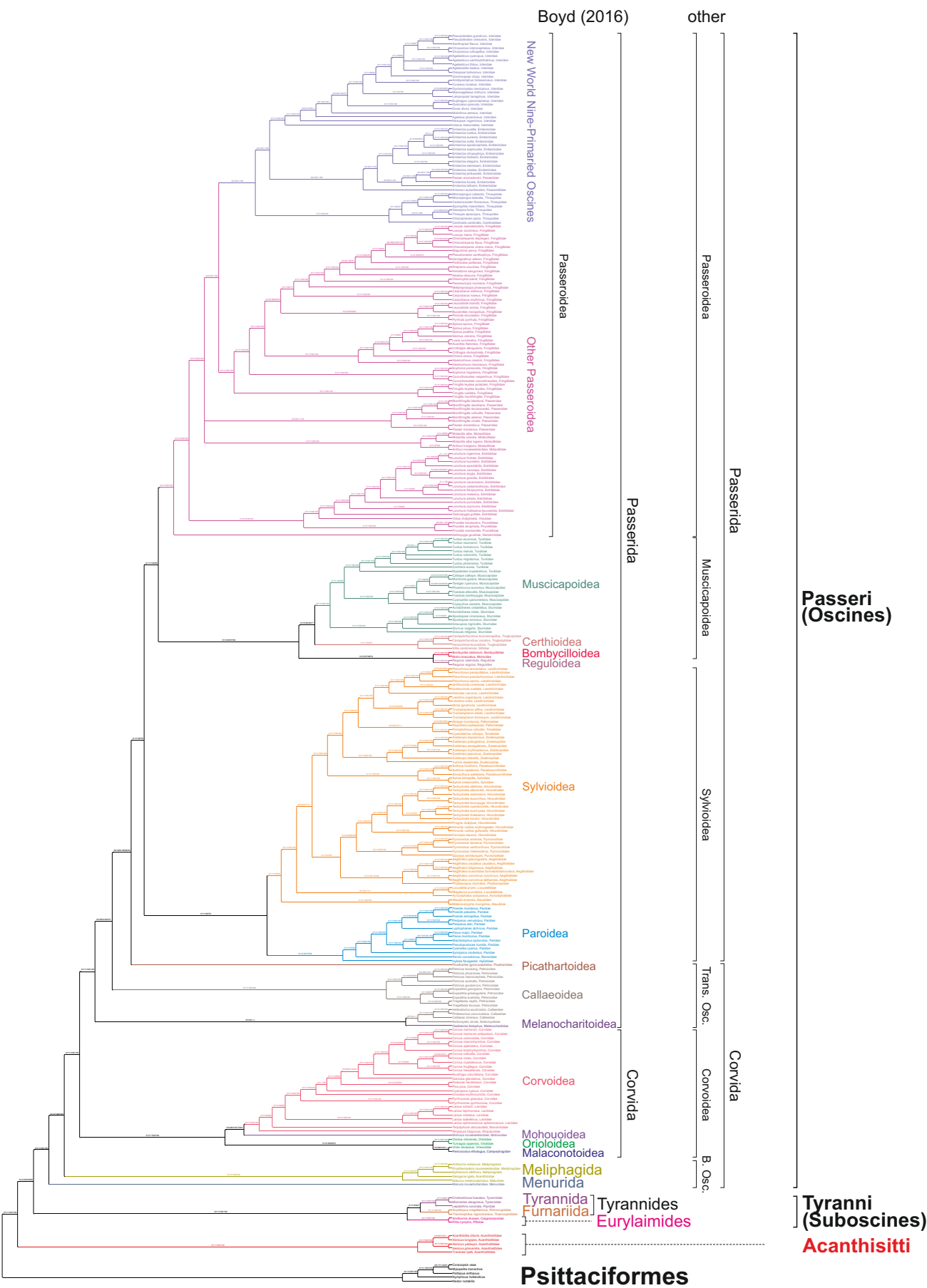
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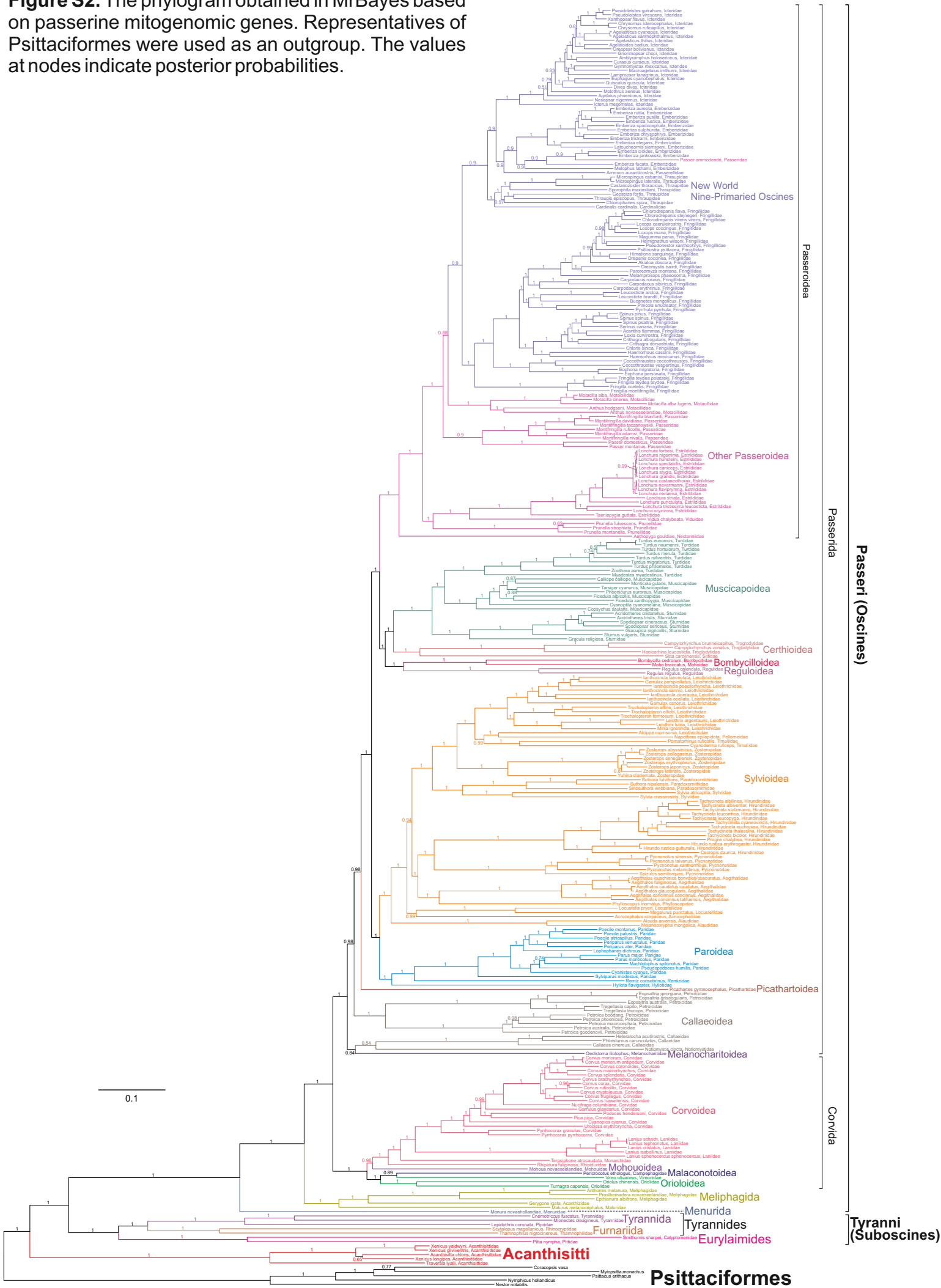
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**Figure S1.** The consensus cladogram of three trees obtained in MrBayes, PhyloBayes and IQ-TREE based on passerine mitogenomic genes. Representatives of Psittaciformes were used as an outgroup. The values at nodes, in the following order N/MB/PB/SH/BP, indicate: the number of trees containing a given node (N), posterior probabilities found in MrBayes (MB) and PhyloBayes (PB) as well as SH-aLRT (SH) and non-parametric bootstrap (BP) percentages calculated in IQ-TREE. The posterior probabilities < 0.5 and the percentages < 50% were omitted or indicated by a dash “-“. Two classification systems of Passeriformes at the medium taxonomic level were presented. B. Osc. – Basal Oscines; Trans. Osc. – Transitional Oscines.

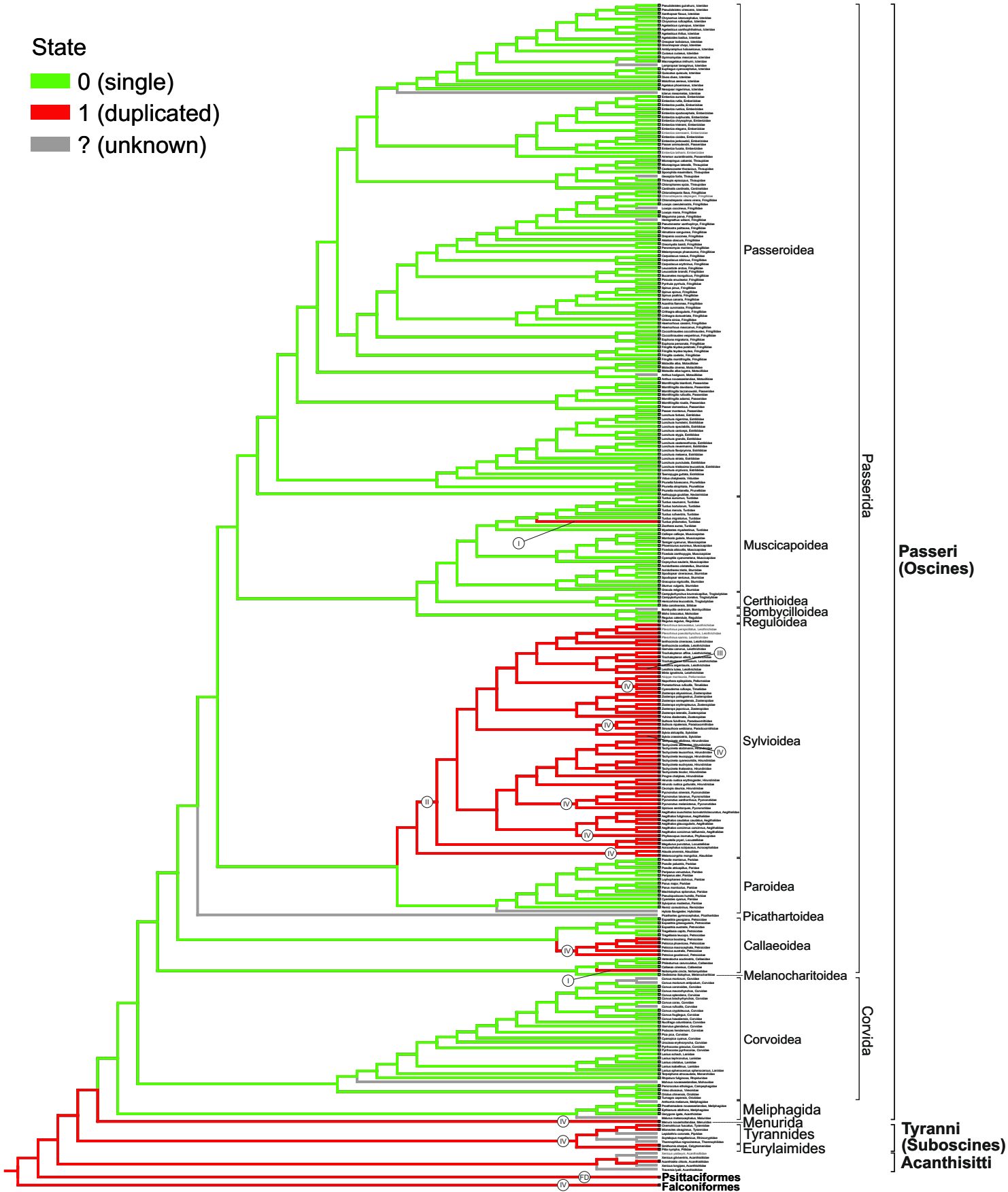


**Figure S2.** The phylogram obtained in MrBayes based on passerine mitogenomic genes. Representatives of Psittaciformes were used as an outgroup. The values at nodes indicate posterior probabilities.

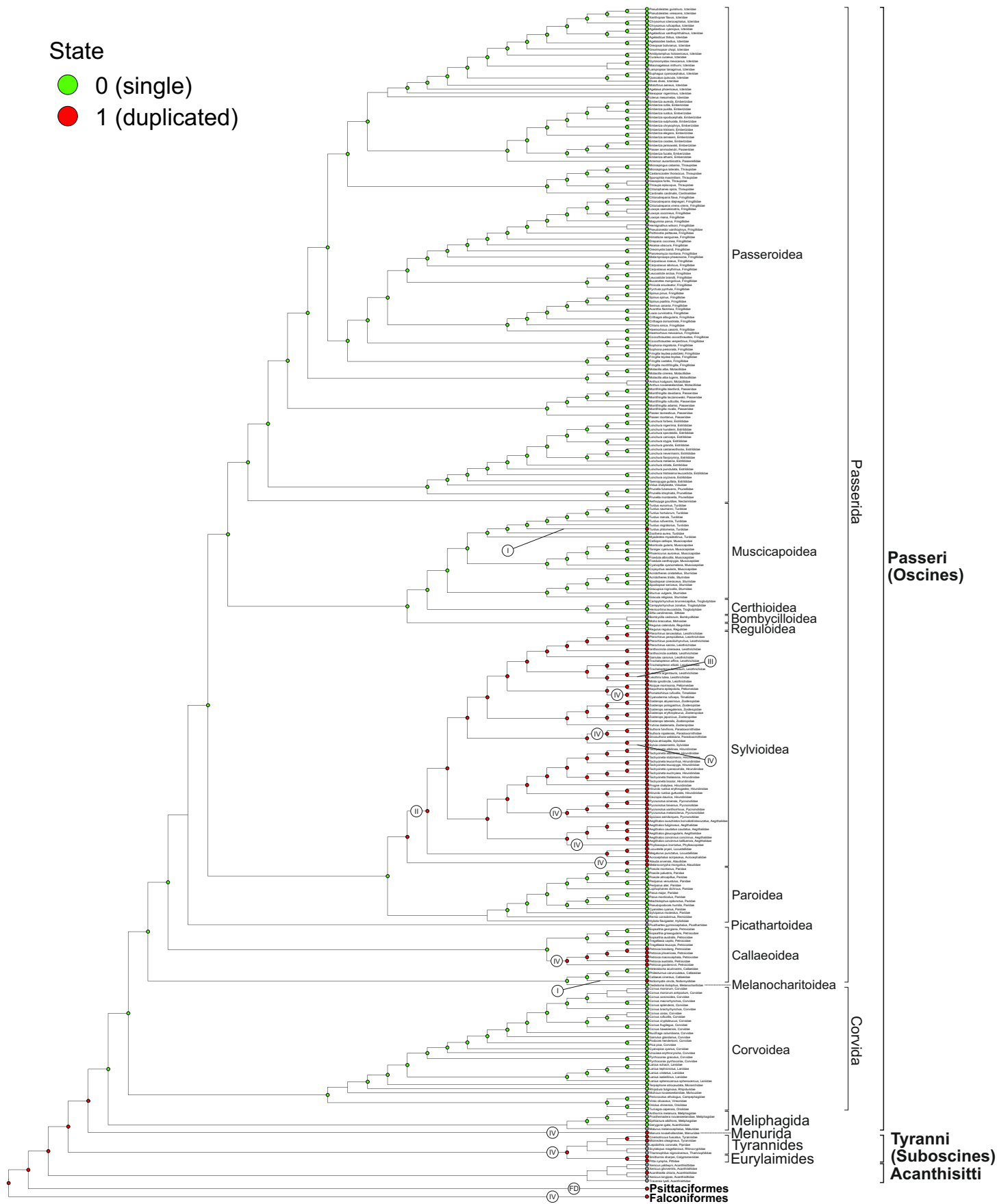




**Figure S3.** The maximum parsimony reconstruction of ancestral states and mapping of mitogenomic duplications onto the phylogenetic tree of passerines with verified presence or absence of the duplication. The parrot and falcon lineages are also included. Gene orders I, II, III and IV were marked at appropriate lineages.

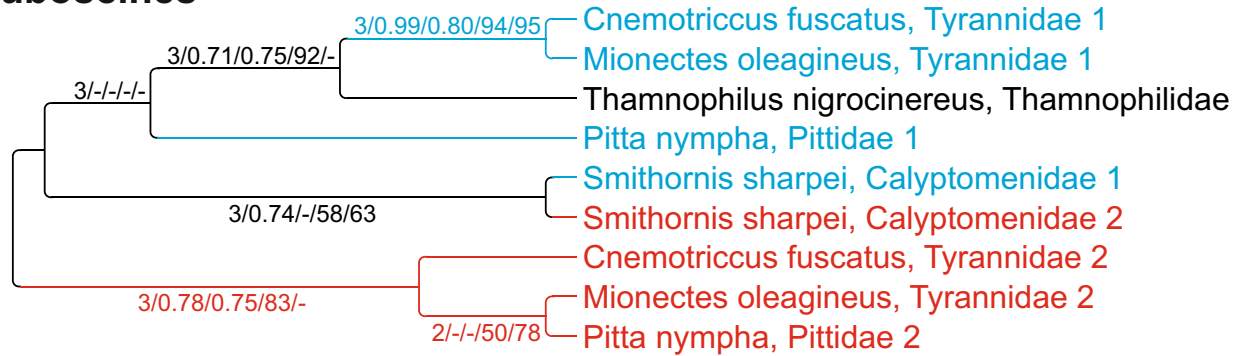


**Figure S4.** The maximum likelihood reconstruction of ancestral states and mapping of mitogenomic duplications onto the phylogenetic tree of passerines with verified presence or absence of the duplication. The area of colours at nodes corresponds to the probability of the given state, single or duplicated region. The parrot and falcon lineages are also included. Gene orders I, II, III and IV were marked at appropriate lineages.

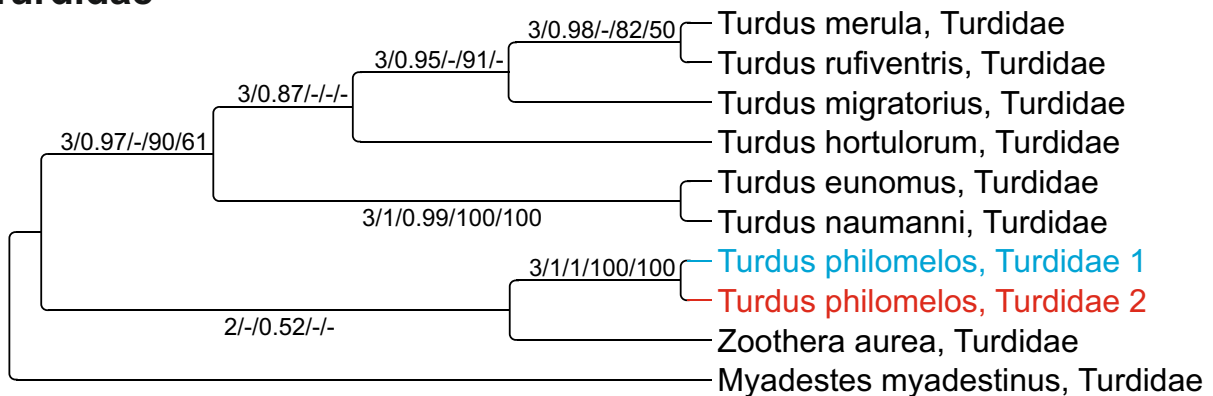


**Figure S5.** The consensus cladograms of three trees obtained in MrBayes, PhyloBayes and IQ-TREE based on control regions for Suboscines, Turdidae, Petroicidae, Sylvioidea and Falconiformes. The blue and red colours correspond to the first and the second copy of CR, respectively. The values at nodes, in the following order N/MB/PB/SH/BP, indicate: the number of trees containing a given node (N), posterior probabilities found in MrBayes (MB) and PhyloBayes (PB) as well as SH-aLRT (SH) and non-parametric bootstrap (BP) percentages calculated in IQ-TREE. The posterior probabilities < 0.5 and the percentages < 50% were omitted or indicated by a dash “-”.

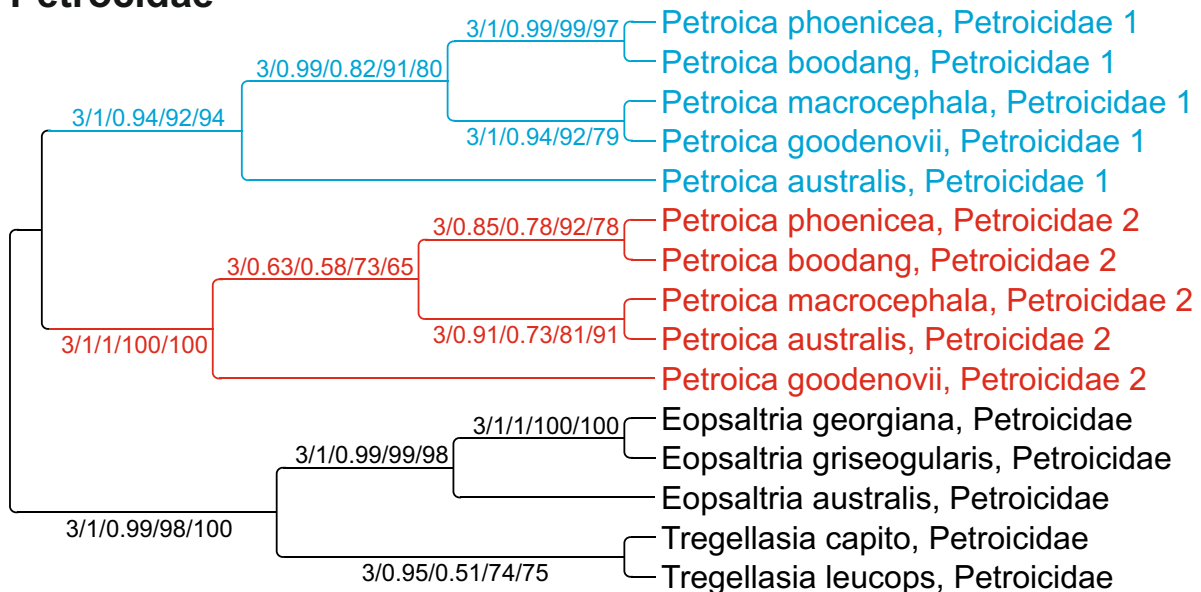
## Suboscines



## Turdidae



## Petroicidae



## Sylvioidea

Falconiformes

