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So similar and yet so different: taxonomic status of Pallid Swift *Apus pallidus* and Common Swift *Apus apus*

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ABSTRACT

Capsule: Common Swift *Apus apus* and Pallid Swift *Apus Pallidus* are morphologically very similar but are genetically distinct and diverged 1.9–2.1 million years ago (mya).

Aims: To examine genetic differentiation and to estimate separation time between Common and Pallid Swifts.

Methods: Estimation of differences in three different mitochondrial DNA markers (*COI*, *ND2* and control region), and a fourth marker, the *cytb*, that did not differ between taxa. Fossils were used to calibrate the estimate of separation date between the two taxa.

Results: The genetic between-species distances were 0.010, 0.006 and 0.033 for the three markers, respectively. These values were from three to ten times higher than within-species distances. Results show that the separation dates back to 1.9–2.1 mya, at the Plio-Pleistocene transition, when global climate underwent a period of significant cooling and Northern latitudes were probably more favourable to the Common than to the Pallid Swift.

Conclusion: Differences in breeding biology, migration, moult and vocalizations between the two species are mirrored by a clear genetic separation.

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The taxonomy and phylogeny of swifts (Apodiformes: Apodidae) have long been debated (e.g. Lack 1956a, 1956b). This is because the family comprises more than 90 species that share homogeneous morphological characteristics related to their highly aerially foraging strategy (Chantler & Driessens 1995). One of the better known swift genera is the Old World distributed *Apus*. While the crown group Apodidae goes back to the late Oligocene-early Miocene (Mayr 2015), the earliest representatives of the genus *Apus* are *Apus gaillardi* from the middle Miocene (12.5–11 million years ago (mya)) of La Grive St. Alban, France (Brodkorb 1971), followed by *Apus wetmorei* from the latest Miocene (6 mya) of Gargano, Italy (Ballmann 1976) and *Apus baranensis* from the late Pliocene (around 3 mya) of Czech Republic (Jánossy 1977). These fossil representatives clearly show that the morphological evolution of the genus has been highly constrained due to life history, and so other methods (e.g. genetics) are required to develop robust hypotheses of *Apus* relationships. For example, Päckert et al. (2012) determined that *Apus* can be subdivided into three major clades:(1) an East Asian clade comprised of two species, (2) an African-Asian clade, comprised of five species and (3) an African-Palearctic clade, comprised of eight species.

In the African-Palearctic clade, only two species are widespread breeders in Europe. The first is the Common Swift *Apus apus*, which has a dense breeding population across its range (6 900 000–17 000 000 breeding pairs). The second is the Pallid Swift *Apus pallidus* which by comparison has far fewer breeding pairs (39 000–160 000) and a smaller distribution range. Swift populations are declining in some European areas, due to major threats such as loss of nesting cavities and pollution (BirdLife International 2004).

Common and Pallid Swifts are phenotypically very similar (Glutz von Blotzheim & Bauer 1980, Cramp 1985) and thus difficult to tell apart (Lack 1956b), particularly at the nestling stage. However, they differ considerably in biological traits, with differences in migration behaviour, arrival and departure dates on breeding grounds (Boano & Malacarne 1999, Boano & Perosino 2014), moult timing (Ahmed & Adriaens 2010, Boano *et al.* 2015) and breeding biology. Pallid Swifts usually raise two broods during each breeding season,

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while Common Swifts raise only one brood (Boano & Cucco 1989). A clear separation between these two *Apus* species was found in bioacoustic characteristics as well (Malacarne *et al.* 1989, Malacarne & Cucco 1990). The two species have not been found to form hybrid pairs (but see Oberli *et al.* 2013).

Genetic differences between Pallid and Common Swifts were first investigated by Randi and Boano (1993), via a mtDNA restriction fragment analysis. Their results indicated a divergence between these species dated to about 1 mya. Several subsequent molecular studies focused on phylogenetic relationships within Apodiformes have not explored the relationship between these two species (Lee *et al.* 1996, Thomassen *et al.* 2003, 2005). However more recently, a revision of the molecular phylogeny of Old World swifts suggested that these two species differed from one another by just 0.6% (cytochrome *b* divergence) and so proposed that they originated from a very recent dispersal and/or speciation event (Päckert *et al.* 2012). The conclusion of a very recent divergence is strikingly different from that reached by Randi & Boano (1993). Further, the widely accepted super-species classification of these species (Sibley & Monroe 1990, Chantler 1999) was not acknowledged by Päckert *et al.* (2012).

The aim of this study was to detect and quantify the genetic differences between Common and Pallid Swifts through the use of different mitochondrial DNA markers. Mitochondrial DNA sequences are particularly useful to clarify taxonomic uncertainties, especially in cases of cryptic species (Hebert *et al.* 2003, Bickford *et al.* 2007, Avise 2010). Moreover, we used Apodiformes fossil data to calibrate phylogeny and to estimate the divergence time between the two species.

Methods

We obtained 11 Pallid Swift and 6 Common Swift samples from tissue collections kept at the Natural History Museum of Carmagnola (Italy) and Texas A&M University (Texas, USA). Details on localities and collection dates are reported in online Appendix 1. Moreover, we sequenced two samples of Little Swift *Apus affinis* from Burkina Faso, and one Bradfield's Swift *Apus bradfieldi* from South Africa, to use as outgroups. Tissues were preserved at −20°C in 95% ethanol, and total genomic DNA was isolated by a NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany).

We amplified three different mitochondrial markers: Cytochrome c oxydase I (*COI*, primers CO1-ExtF and CO1-ExtR, Johnsen *et al.* 2010), the hypervariable domain I of the control region (newly designed primers ApusCrF AGGGCAGGCAAAAACACTCT and ApusCrR CGCAAACCGTCTCATTCAGC) and NADH dehydrogenase subunit 2 (ND2, primers L5215 (Hackett 1996) and H6313 (Johnson & Sorenson 1998)).

Polymerase chain reactions (PCR) were performed in a final volume of 25 µl containing 0.4 µl dNTPs (10 mM), 1 µl MgCl2 (25 mM), 0.3 µl of each primer (25 pmol/µl), 2.5 µl 10 × buffer, 0.4 µl Taq polymerase (5 unit/µl; QIAGEN), ddH2O and genomic DNA (about 50 ng/µl). PCR amplifications were carried out in the Bio-Rad thermal cycler (Bio-Rad Laboratories Inc., Hercules, California, USA). A fourth mitochondrial marker, cytochrome *b* (*cytb*), was amplified with the L14841-Cytb (Kocher *et al.* 1989) and H15917-Cytb (Päckert *et al.* 2012) primers.

The amplification protocol began at 94°C for 5 min, followed by 29–32 cycles at 94°C for 45 s, annealing temperature (60°C for COI and control region, 54°C for ND2) for 45 s, 72°C for 45 s, and a final extension at 72°C for 7 min. DNA concentration was determined after electrophoresis in 1.8% agarose gels (TBE 1%) using GelRedTM nucleic Acid gel stain (Biotium Inc., Hayward, California, USA) and quantified in a UV-trans illuminator Gel Doc XR (Bio-Rad Laboratories Inc., Hercules, California, USA) with Molecular Imager ChemiDoc XRS Systemand Quantity One software (Bio-Rad). PCR products were cleaned with the EXO-SAP procedure (Exonuclease I and Shrimp Alkaline Phosphatase Fermentas, Burlington, Canada).

Electropherograms were read using Finch TV 1.4.0 (http://www.geospiza.com), and sequences were aligned with ClustalW algorithm in MEGA 6 (Tamura *et al.* 2013). Unique haplotypes and their nucleotide composition, polymorphic and parsimony informative were analysed with DNASP v. 5.1 (Librado & Rozas 2009). Haplotype sequences were deposited in GenBank (accession numbers: MF579506–MF570526 and MF595051–MF595061).

Median-joining networks were calculated with Network 5 software (fluxus-engineering.com). Neighbour Joining trees (Saitou & Nei 1987) were calculated in MEGA 6.0 (Tamura *et al.* 2013), with clustering pairwise Tamura-Nei's genetic distances between haplotypes (TN93: Tamura & Nei 1993). Nodal support was assessed by bootstrap percentages (Felsenstein 1988) derived from 1000 resampling steps.

For the Bayesian analysis, the most appropriate model of sequence evolution for each gene was selected using Akaike's information criterion (Akaike 1974) in JMODELTEST, version 2.1.7 (Darriba *et al.* 2012). The Bayesian analysis was conducted using MRBAYES, version 3.1, with data partitioned according to the different mtDNA markers (Ronquist & Huelsenbeck 2003). The default settings (four Markov chains at four

different temperatures) were used. Markov chains were sampled every 100 generations and run for 5 million generations. Resulting trees were drawn using TREEVIEW, version 1.6.6 (Page 1996). In the main text we only show the tree originated from the concatenated sequences; individual gene trees are shown in the online Appendices.

In order to estimate a divergence time between Common and Pallid Swifts, we used our sequences in a Bayesian analysis conducted in BEAST (Drummond & Rambaut 2007). In this analysis, we included fossil calibrations and additional sequences obtained from GenBank: the Apodidae *Chaetura pelagica* (KT809406), the Trochilidae (also Apodiformes) *Amazilia versicolor* (NC024156) and *Archilochus colubris* (NC010094), and the Caprimulgiformes (sister order to Apodiformes) *Caprimulgus indicus* (KM272749). For fossil calibration, we used the stem Apodiformes *Eocypselus vincenti* from the early Eocene (51 mya) (Mayr 2009, 2010, Ksepka *et al.* 2013, Ksepka & Clarke 2015), the earliest stem Trochilidae *Parargornis messelensis* from the middle Eocene (47 mya), the oldest crown group Apodinae represented by *Collocalia buday* and *Collocalia* sp. swiftlets found in late Oligocene-early Miocene (25–20 and 19–16 mya, respectively) (Boles 2001, Worthy *et al.* 2007), and the earliest representative of *Apus*: *A. gaillardi* from the middle Miocene (12.5–11 mya). We estimated divergence times using both a strict and a relaxed molecular clock (Ho 2007). For *Collocalia* we chose the record described by Whorty *et al.* (2007), as it is better geologically and chronologically constrained.

To further evaluate differences between taxa, we used two additional approaches. First, we used the Automatic Barcode Gap Discovery (ABGD, with following parameters: Pmin: 0.001; Pmax: 0.1; Steps: 10; X: 1.5; Nb: 20 and JC69 distance) for species delimitation, as implemented in ABGD website (Puillandre *et al.* 2012). Second, we used Poisson tree processes (PTP) (Zhang *et al.* 2013), as a model for delimiting species in terms of number of substitutions, with the phylogenetic trees computed by MEGA and using the bPTP server (http://species.h-its.org) with the settings: MCMC generations: 100 000; thinning: 100; burn-in: 0.1; seed: 123.

Results

Common and Pallid Swifts had identical *cytb* sequences (856 bp), but did differ for the other three gene regions. We found five different haplotypes in *COI* (636 bp), with three specific to Pallid Swift and two to Common Swift. We found nine haplotypes (five for Pallid, four for Common Swift) in ND2 (955 bp). We found 11 control region (549 bp) haplotypes (five for Pallid, six for Common Swift). Overall, we found12 haplotypes in the concatenated sequences (2140 bp) (Table 1).

For *COI*, haplotype (Hap) 3 (*A. pallidus*) was the most frequent haplotype, being found in seven individuals, Hap 6 (*A. pallidus*) was the most frequent for *ND2* (four individuals), and Hap 6 (*A. pallidus*) for control region both (six individuals). The list of individual haplotypes is reported in online Appendix 1. Haplotype (Hd) and nucleotide (π) diversities are reported in Table 1. The highest nucleotide and haplotype diversity was found in the control region. We found nine variable sites in *COI* sequences, 12 polymorphic sites in *ND2*, and 34 polymorphic sites in the control region. The concatenated sequences showed 54 polymorphic sites, of which 36 were parsimony informative.

The mean number of mutations per haplotype (k) was higher in the control region than in *ND2* and *COI*. And, *COI* sequences showed the least variation in number of haplotypes and nucleotides (Table 1), as well as the number of mutations per haplotype. Overall, Common Swifts showed higher haplotype and nucleotide diversity in comparison to Pallid Swifts, as well as a larger number of mutations per haplotype.

The median-joining network obtained from concatenated sequences shows the presence of two groups, separated by 28 mutations, each group enclosing all the samples pertaining to Common or Pallid Swift, respectively (Figure 1). The three networks

Table 1. Molecular diversity indices (±*SD*): *n* – sample size, *S* – number of segregating sites, *H* – number of haplotypes, π – nucleotide diversity, Hd – haplotype diversity, *k* – average number of pairwise differences.

Species	*n*	*S*	*H*	$\pi \pm SD$	Hd ± SD	*k*
COI						
Apus pallidus	11	2	3	0.00048 ± 0.00027	0.295 ± 0.156	0.308
Apus apus	7	1	2	0.00052 ± 0.00034	0.333 ± 0.215	0.333
ND2						
A. pallidus	11	6	5	0.00136 ± 0.00045	0.628 ± 0.143	1.051
A. apus	7	4	4	0.00233 ± 000036	0.857 ± 0.102	1.809
CR						
A. pallidus	11	7	5	0.0024 ± 0.00084	0.667 ± 0.141	1.303
A. apus	7	22	6	0.0139 ± 0.00298	0.952 ± 0.096	7.524

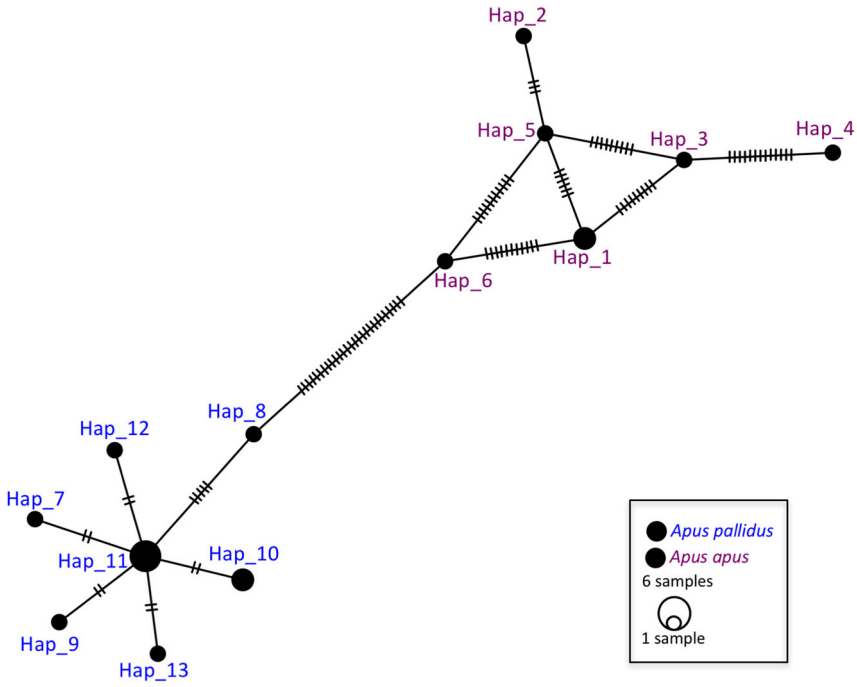

Figure 1. Median-joining haplotype network over the concatenated haplotypes. The sizes of the circles are proportional to the number of individuals sharing each haplotype. Small lines between haplotypes indicates mutation steps.

obtained from the discrete mtDNA markers (*COI*, *ND2* and control region) confirmed a clear distinction between the two species (online Appendix 2). For individual genes, the largest separation between species haplogroups was found in the control region (ten mutations), while *COI* (six mutations) and *ND2* (three mutations) showed lower values (online Appendix 2).

The neighbour joining tree computed from the concatenated sequences, with *A. affinis* and *A. bradfieldi* as outgroups, confirms a clear divergence between *A. apus* and *A. pallidus* (Figure 2). The neighbour joining trees computed from the *COI*, control region and ND2 markers separately were in accord with the concatenated tree, with a clear separation between Common Swift and Pallid Swift samples (Appendix 3).

The between- and within-taxa genetic distances are reported in Table 2. All within-taxa distances were lower than between-taxa genetic distances. In the comparison between Common Swift and Pallid Swift, the largest distance was observed in the control region (0.033 ± 0.007), and the lowest distance was found in *ND2* sequences (0.006 ± 0.002).

ABGD analyses recovered the same separation between *A. pallidus* and *A. apus* as found in the

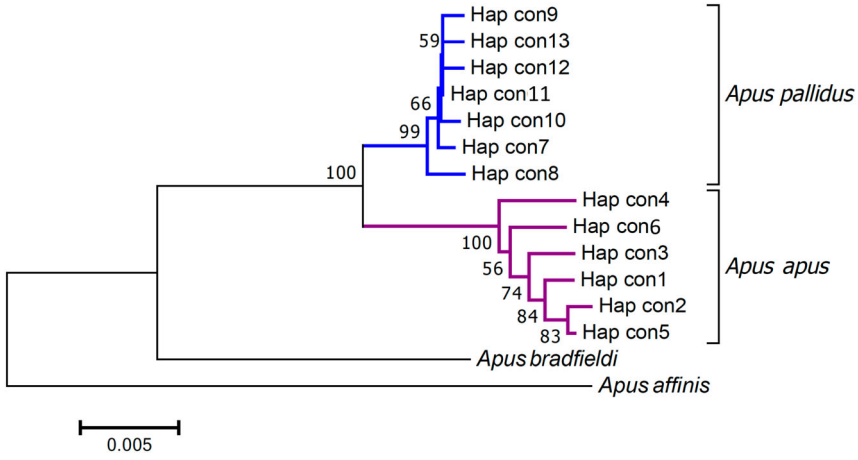

Figure 2. Neighbour joining tree with Tamura & Nei's (1993) genetic distances among concatenated haplotypes. The phylogenetic tree was rooted using *Apus affinis* and *Apus bradfieldi*. Numbers on the internodes indicate all the bootstrap percentage values (>50%).

Table 2. Kimura 2-parameterdistance calculated on single mitochondrial markers and on concatenated sequence within and among haplogroups.

Genetic distances within haplogroups		Genetic distance among haplogroups		
COI		*Apus apus*	*Apus pallidus*	*Apus affinis*
A. apus	0.002 ± 0.001	–		
A. pallidus	0.002 ± 0.001	0.012 ± 0.004	–	
A. affinis	–	0.049 ± 0.009	0.039 ± 0.008	–
A. bratfieldi	–	0.024 ± 0.006	0.014 ± 0.005	0.041 ± 0.009
ND2				
A. apus	0.003 ± 0.001	–		
A. pallidus	0.003 ± 0.001	0.008 ± 0.002	–	
A. affinis	–	0.040 ± 0.007	0.041 ± 0.007	–
A. bratfieldi	–	0.015 ± 0.004	0.016 ± 0.004	0.032 ± 0.006
CR				
A. apus	0.016 ± 0.003	–		
A. pallidus	0.005 ± 0.002	0.033 ± 0.006	–	
A. affinis	–	0.097 ± 0.013	0.089 ± 0.013	–
A. bratfieldi	–	0.088 ± 0.013	0.075 ± 0.012	0.099 ± 0.014
Concatenated sequence				
A. apus	0.006 ± 0.001	–		
A. pallidus	0.001 ± 0.000	0.015 ± 0.003	–	
A. affinis	–	0.058 ± 0.006	0.053 ± 0.005	–
A. bratfieldi	–	0.038 ± 0.005	0.031 ± 0.004	0.053 ± 0.005

phylogenetic analyses, with *P* values ranging from 0.001 to 0.05. The identified groups were recovered regardless of the chosen model (Jukes Cantor or Kimura). Results obtained from the PTP analysis were congruent with those produced by ABGD and support the splitting of the two species (Appendix 4).

The BEAST estimation of divergence time is reported in Figure 3. The separation between Common Swift and Pallid Swift was estimated at 1.898 mya (1.374–2.412, 95% highest posterior density) with the strict clock model, and 2.137 (1.225–3.063, 95% highest posterior density) with the relaxed clock model.

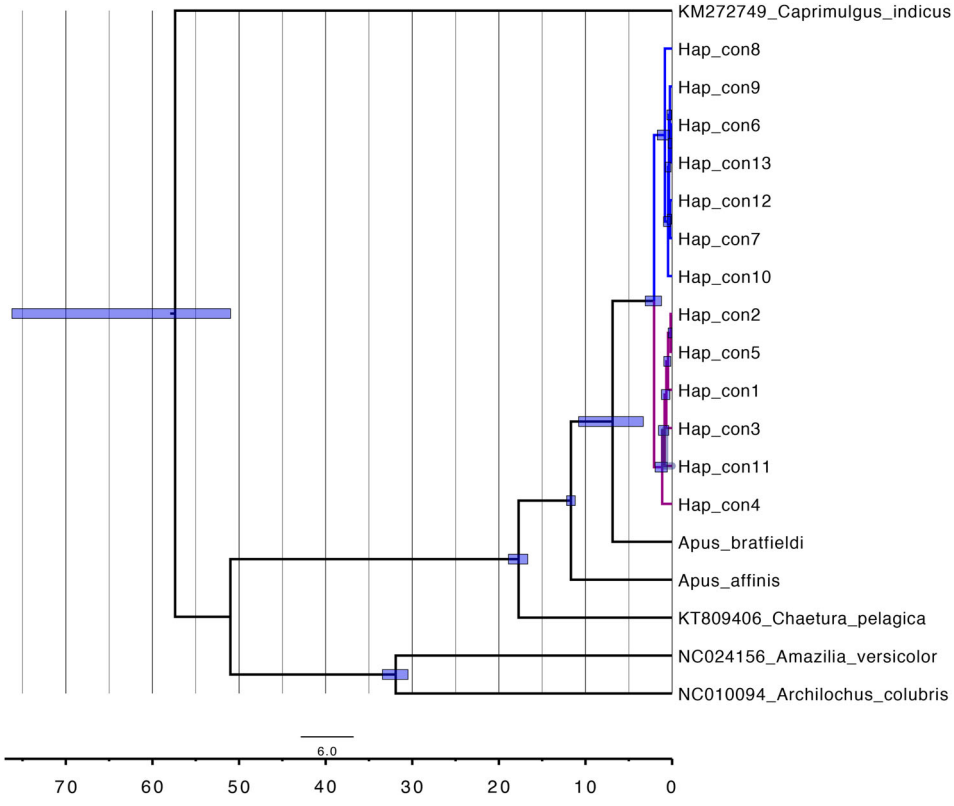

Figure 3. Estimates of the tree from the BEAST analyses. Nodal support is indicated with Bayesian posterior probabilities. Divergence time estimates using Bayesian relaxed clock. Bars show 95% Highest Posterior Density intervals of age nodes.

Discussion

In this study we found a clear separation between the samples pertaining to the Common versus those from the Pallid Swift. Each of the three mtDNA markers we analysed (*COI*, *ND2* and *CR*) reflected genetic variability (albeit at different levels), through the identification of unique haplotypes for each species.

Our results indicating Common Swift and Pallid Swift are genetically well differentiated from each other supports an earlier study involving an analysis of restriction fragment length polymorphisms (Randi & Boano 1993). Päckert *et al.* (2012) suggested that Common and Pallid Swifts are not diagnosable by DNA. Indeed, in our study the two species were indistinguishable by *cytb*, but we observed a high genetic distance when analysing the *CR* sequences, while genetic distances calculated from *ND2* sequences were lower. This could be associated with a different rate of evolution in the two markers. The traits of mtDNA that possess the lowest replacement rate are in fact the genes coding for rRNA, tRNA or enzymes such as NADH dehydrogenase, while the more variable are the non-coding sequences such as *CR*, whose divergence rates are from 1 to 4–5 times faster than those of genes that code for proteins (Garcia-Moreno 2004, Caramelli 2009, Ho & Duchene 2014).

The *COI* genetic distance between the two species was 0.010 ± 0.004, a value fully in the range of interspecific distances (Hebert *et al.* 2004). The mean intraspecific distances was considerably lower (Common Swift: 0.001, Pallid Swift: 0.001).

The neighbour joining trees and the haplotype networks showed a clear-cut separation of all samples pertaining to the Common Swift with respect to the samples from the Pallid Swifts. Both ABGD and PTP analyses confirmed the separation of these two swift species as well. Overall, haplotype and nucleotide diversity was higher in the Common Swift than in the Pallid Swift. This could be due to the differences related to the area from which the specimens originated (Frankham 1996, Rubidge *et al.* 2012): the Pallid Swifts were collected over a smaller geographic area than were the Common Swifts.

Our findings provided new data for a better understanding of the relationship between Common and Pallid Swifts. Some previous works hypothesized that the two species represent a super-species complex (Sibley & Monroe 1990, Chantler 1999). A recent revision of molecular phylogeny of Old World swifts suggested that this species-pair originated from very recent events (Päckert *et al.* 2012), due to very small difference in mtDNA (*cytb* and *12S* sequences) and nuclear intron genes. This finding is in line with our results, as in our study the *cytb* sequences were identical. For some time there has been a debate as to whether *cytb* or *COI* is the best gene for species identification (Linacre & Tobe 2011). In our case, *cytb* did not provide useful insights for a comparison of these two swifts species. Some studies have suggested that *ND2* is a more appropriate marker than *cytb* (Johnson & Sorenson 1998, Cicero & Johnson 2001). However, it is noteworthy that while in Apodidae this difference did affect some terminal taxa relationships, it did not affect the overall tree topology (Price *et al.* 2005).

Divergence time estimation

Our divergence time estimate, which used Apodiformes fossil calibrations, returned a date of 1.898 mya (1.37–2.41; 95% highest posterior density) for the split between Pallid and Common Swifts when enforcing a strict molecular clock, or a slightly earlier date of 2.137 mya (1.225–3.06) under a relaxed molecular clock. These values are in line with the age of the oldest Common Swift fossils from the Early Pleistocene of the Czech Republic (Mlíkovský 2002) and the other Early and Middle Pleistocene records of *Apus* (Tyrberg 1998). It is worth mentioning here that osteological differences between the two species have never been investigated, so the fossil records of Common and Pallid Swifts should be referred to *A. apus*/*pallidus*, especially those from Mediterranean area.

Our results show that the divergence dates coincide with important climatic and environmental transitions which occurred in the Early Pleistocene. Evidence from various fossil localities shows that most of the extant bird species appeared in the Western Palaearctic in the Early Pleistocene, between 2 and 1.5 mya (Bedetti & Pavia 2013). In fact, the European fossil record of birds up to the Pliocene is characterized by extinct species with Neogene origins (Mourer-Chauviré 1993). These extinct taxa include swifts, with the late Pliocene *A. baranensis* similar in size and morphology to the late Miocene *A. wetmorei*, both of which have been replaced by modern taxa during the Pleistocene. At the beginning of the Pleistocene, the global climate underwent a period of significant cooling (Kahlke *et al.* 2011) and Central-Northern Europe was probably more favourable to birds attempting a single brood per year, like the Common Swift, than to birds with a long breeding season and two broods each year, such as the Pallid Swift. Pollen records suggest that in Europe 3–5 mya the forests were dominated by subtropical taxa, but that 3.2–2.3 mya a series of climatic changes took place that caused retraction of the subtropical forests,

and increased the distribution of conifers. The differences in climatic conditions between the northern and the southern part of the distributional range of swifts could have been the driver for the separation and genetic differentiation between the Common Swift, with its adaptations to more northern climates, and the Pallid Swift, with its adaptations to more southern climates.

Conclusion

Currently, Common Swift and Pallid Swift are not included in the world list of threatened birds; however, their significant dependence on human construction (houses, churches, castles) for nesting raises some concern (BirdLife International 2004). The renovation of buildings often includes the closure of the cavities and under-hangs that swifts use for nesting. While in some cases this is directly related to excluding swifts, in many cases these closures are aimed at reducing nesting opportunities for feral pigeons, a species that widely plagues cities (Ferri *et al.* 2015). In this context, a correct species determination based on mtDNA would help with the distinction between individuals of these cryptic species, particularly of the chicks dropped out of the nest during unfavourable weather conditions or taken out during building restoration. These nestlings are often delivered to wildlife recovery centres or to associations for the protection of nature, in anticipation of their insertion in host nests of the correct species for adoption (Holzgang *et al.* 2013). As noted above, these difficult to identify sister species are even more difficult to identify to species in the nestling stage. Moreover, the possibility to attribute every single individual of *Apus* to the correct species with certainty could be very useful for specimens found outside the normal distribution range (Thorup 2001), especially in Africa where the higher number of similar *Apus* species often causes identification problems even of preserved museum specimens (Davies 2013).

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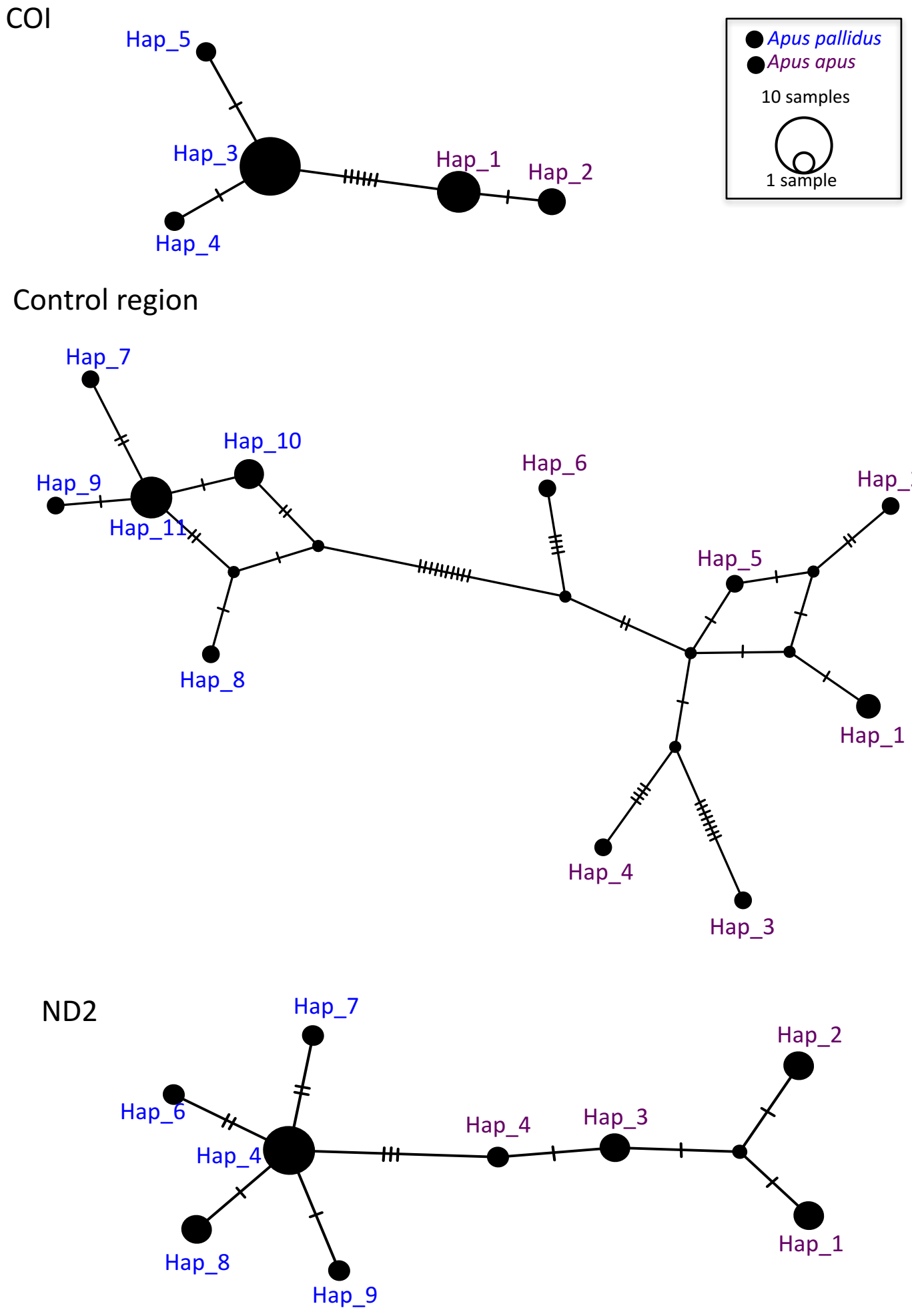
Appendix 1 - List of samples analysed in this study with locality, year of sampling, origin and haplotype designation for the mtDNA markers.

	COI haplotype	ND2 haplotype	Dloop haplotype	Concatenated haplotype	Date	Species	Country	Region	Locality	Source	Cod	Catalog N.
R1	Hap_COI_1	Hap_ND2_1	Hap_dloop_1	Hap_1	2000	*Apus apus*	Italy	Piemonte	Torino (TO), centro città	Carmagnola Natural History Museum	25734	MCCI 3527
R11	Hap_COI_3	Hap_ND2_5	Hap_dloop_11	Hap_11	29/07/2013	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO), Via Lomellini	Carmagnola Natural History Museum	26526	MCCI 3889
R12	Hap_COI_4	Hap_ND2_6	Hap_dloop_8	Hap_8	29/09/2013	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Carmagnola Natural History Museum	26551	MCCI 3913
R14	Hap_COI_5	Hap_ND2_5	Hap_dloop_9	Hap_9	08/01/2011	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Carmagnola Natural History Museum	25733	MCCI 3526
R16	Hap_COI_3	Hap_ND2_7	Hap_dloop_10	Hap_10	30/09/2014	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Carmagnola Natural History Museum		MCCI 4165
R17	Hap_COI_3	Hap_ND2_5	Hap_dloop_11	Hap_11	18/08/2010	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Carmagnola Natural History Museum	23065	MCCI 3134
R19	A. affinis_COI_1	A. affinis_ND2_1	A. affinis_dloop_1	A. affinis_1	18/02/2010	*Apus affinis*	Bukina Faso	Cascades	Banfora	Carmagnola Natural History Museum	23468	MCCI 3214
R2	Hap_COI_2	Hap_ND2_2	Hap_dloop_2	Hap_2	26/04/2003	*Apus apus*	Italy	Liguria	La Spezia (SP)	Carmagnola Natural History Museum	2259	MCCI 1380
R20	A. affinis_COI_1	A. affinis_ND2_1	A. affinis_dloop_2	A. affinis_2	18/02/2010	*Apus affinis*	Bukina Faso	Cascades	Banfora	Carmagnola Natural History Museum	23466	MCCI 3212
R22	Hap_COI_3	Hap_ND2_7	Hap_dloop_10	Hap_10	2013	*Apus pallidus*	Italy	Basilicata	Prov. Matera (MT)	Carmagnola Natural History Museum	GB15-03	MCCI 4211
R23	Hap_COI_1	Hap_ND2_3	Hap_dloop_6	Hap_6	2014	*Apus apus*	Italy	Puglia	Prov. Lecce (LE)	Carmagnola Natural History Museum	GB15-04	MCCI 4212
R25	Hap_COI_3	Hap_ND2_8	Hap_dloop_11	Hap_12	14/07/2015	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Gary Voelker, Texas A&M University		JAH091_AC01
R28	Hap_COI_3	Hap_ND2_9	Hap_dloop_11	Hap_13	13/07/2015	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Gary Voelker, Texas A&M University		JAH079_AC01
R29	Hap_COI_3	Hap_ND2_5	Hap_dloop_11	Hap_11	13/07/2015	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Gary Voelker, Texas A&M University		JAH076_AC01
R3	Hap_COI_1	Hap_ND2_3	Hap_dloop_3	Hap_3	05/05/2007	*Apus apus*	Italy	Piemonte	Torino (TO), Piazza Statuto	Carmagnola Natural History Museum	10907	MCCI 2635
R32	A. bradfieldi_1	A. bradfieldi_1	A. bradfieldi_1	A. bradfieldi_1	23/09/2009	*Apus bradfieldi*	South Africa	Northern Cape	Kimberley	Gary Voelker, Texas A&M University	V3363	TCWC 15572
R4	Hap_COI_1	Hap_ND2_1	Hap_dloop_1	Hap_1	20/06/2014	*Apus apus*	Italy	Piemonte	Carmagnola (TO), Via Gardezza	Carmagnola Natural History Museum	27109	MCCI 4134
R6	Hap_COI_1	Hap_ND2_4	Hap_dloop_4	Hap_4	Ante 2002	*Apus apus*	Italy	Piemonte	Undetermined	Carmagnola Natural History Museum	1137	MCCI 998
R7	Hap_COI_2	Hap_ND2_2	Hap_dloop_5	Hap_5	23/04/2004	*Apus apus*	Italy	Piemonte	Asti (AT) Torre Trojana	Carmagnola Natural History Museum	10859	MCCI 2586
R8	Hap_COI_3	Hap_ND2_5	Hap_dloop_11	Hap_11	16/06/2014	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO), Via Lomellini	Carmagnola Natural History Museum	27098	MCCI 4124
R9	Hap_COI_3	Hap_ND2_5	Hap_dloop_7	Hap_7	17/09/2013	*Apus pallidus*	Italy	Piemonte	Carmagnola (TO)	Carmagnola Natural History Museum	26548	MCCI 3910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Hap_3
Hap_1
Hap_2
Hap_4
Apus pallidus
Apus apus
10 samples
1 sample
Control region
Hap_7
Hap_10
Hap_9
Hap_11
Hap_6
Hap_2
Hap_5
Hap_8
Hap_1
Hap_4
Hap_3
ND2
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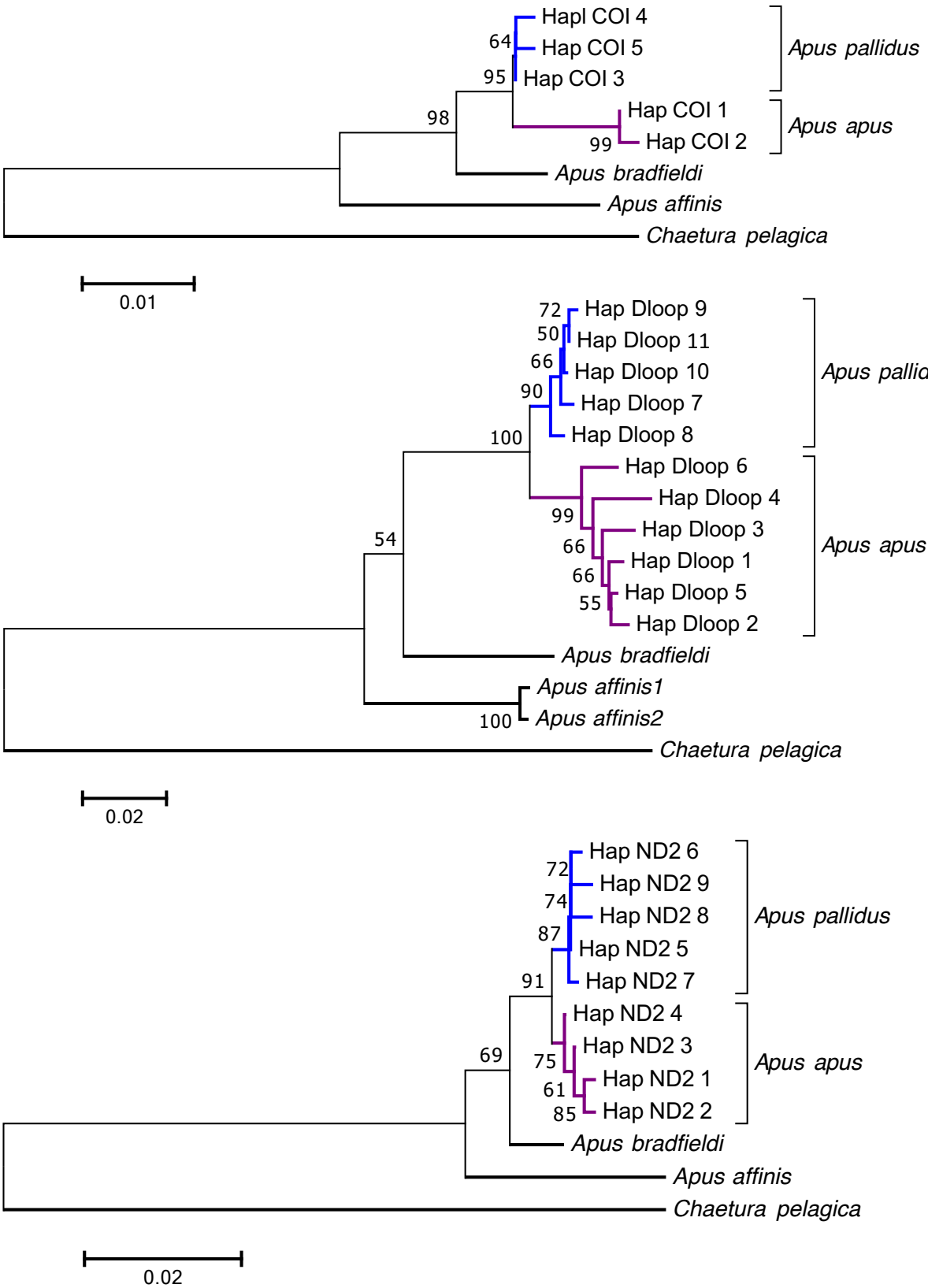
Hap_2
Hap_6
Hap_4
Hap_3
Hap_4
Hap_1
Hap_8
Hap_9


Hapl COI 4
64
Hap COI 5
95
Hap COI 3
Apus pallidus
98
Hap COI 1
99
Hap COI 2
Apus apus
Apus bradfieldi
Apus affinis
Chaetura pelagica
0.01
72
Hap Dloop 9
50
Hap Dloop 11
66
Hap Dloop 10
90
Hap Dloop 7
Apus pallidus
100
Hap Dloop 8
Hap Dloop 6
Hap Dloop 4
99
Hap Dloop 3
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66
Hap Dloop 1
66
Hap Dloop 5
55
Hap Dloop 2
Apus apus
Apus bradfieldi
Apus affinis1
100
Apus affinis2
Chaetura pelagica
0.02
Hap ND2 6
72
Hap ND2 9
74
Hap ND2 8
87
Hap ND2 5
Apus pallidus
91
Hap ND2 7
Hap ND2 4
69
Hap ND2 3
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Hap ND2 2
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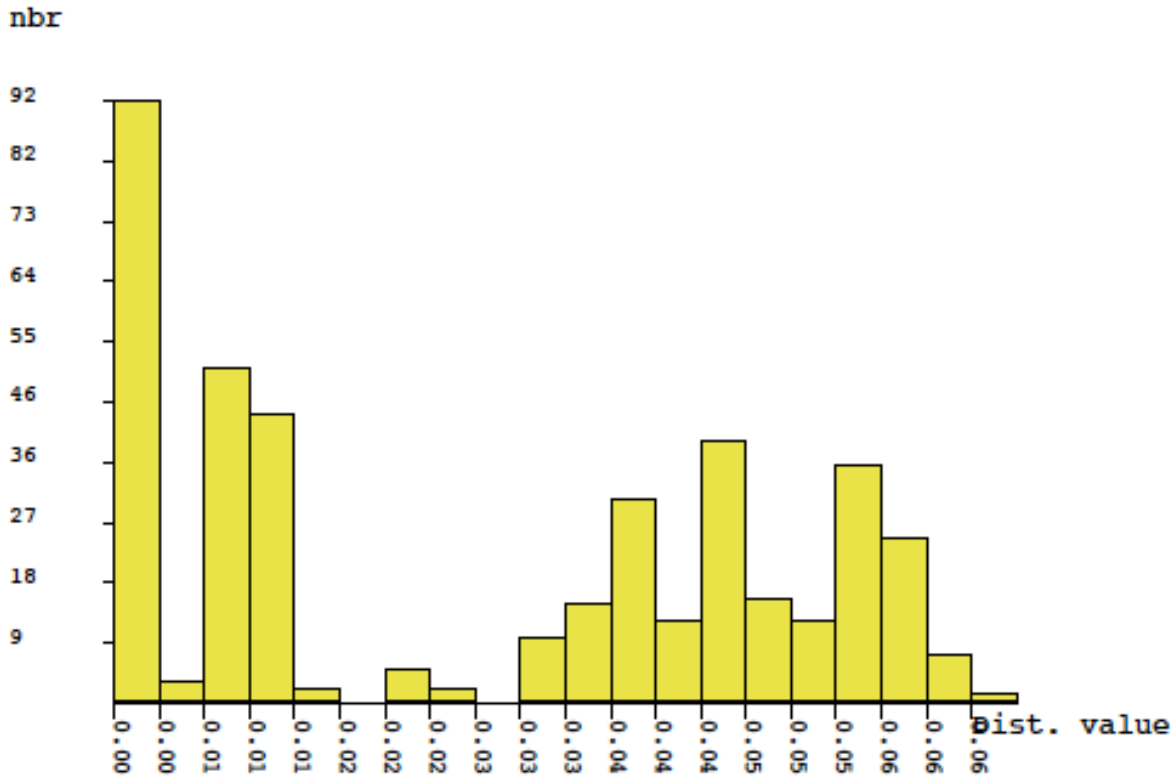
Apus bradfieldi
Apus affinis
Chaetura pelagica
0.02

(a)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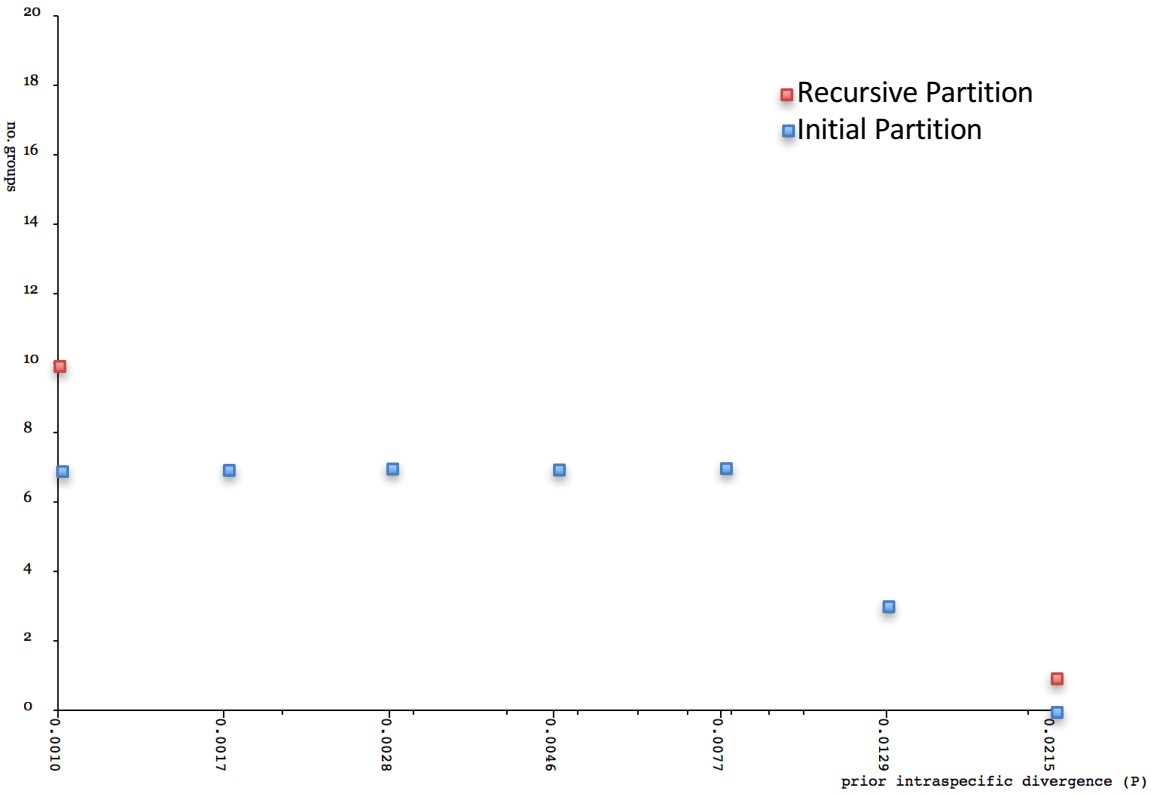
Dist. value

(b)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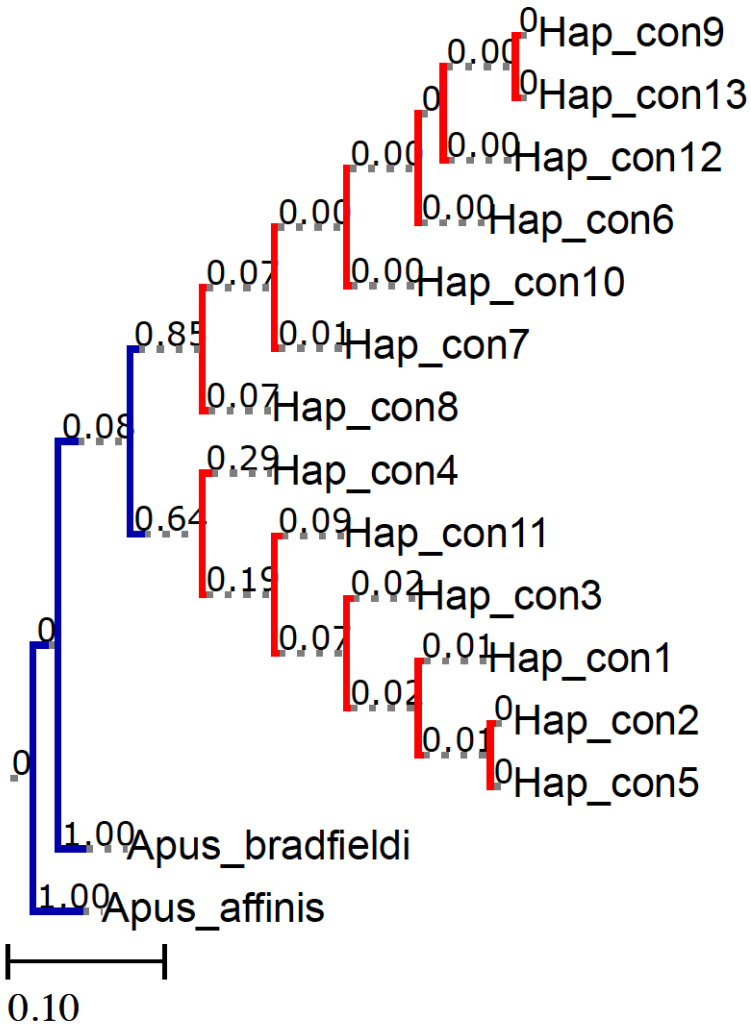
prior intraspecific divergence (P)

(c)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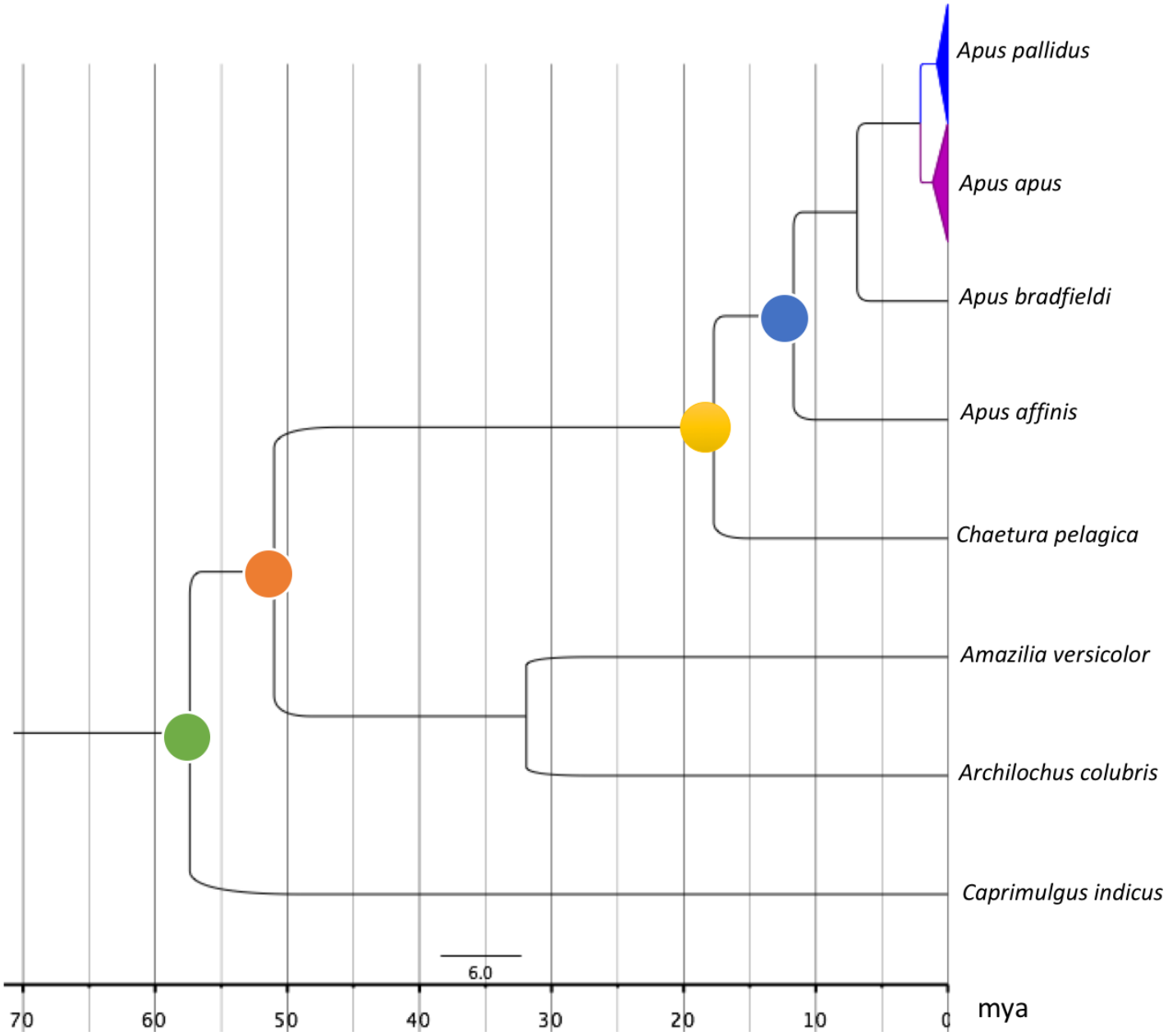

Apus gaillardi
mean=11.75; stdev=0.25

Collocalia sp.
mean=17.5 ; stdev=0.5

Parargornis messelensis
mean=32; stdev=0.75

Eocypselus vincenti
mean=51; stdev=0.1