Long eared bat hybridization in the UK

Abstract:
Project aim was to amplify several loci from the bat genome from brown long eared (BLE), grey long eared (GLE) and the GLE/BLE samples in the lab using PCR and sequencing DNA to establish whether they are affiliated with BLE or GLE. This evidence will be used to establish whether hybridisation has occurred.

Genes studied include Thy1, Thy2, RAG1, STAT5A and DMP1 which covers a range on interesting functions diverging from fur colour and bone formation to assisting DNA substrate binding. However, data could not be collected for DMP1. Results suggests that there are high possibilities of hybridisation occurring between the two species, as seen in Thy2 genes but further research would be required to confidently conclude and support this theory.

Introduction:

Plecotus austriacus, commonly known as grey long eared bats (GLEs), are one of our rarer bat species in the UK, closely related to *Plecotus auritus*, brown long eared bats (BLE), which are incredibly common. GLEs are particularly interesting because they are climatic indicators; they are currently restricted to southern areas of England, but have been noted in very recent years to be undergoing an expansion northward.

Dr Allaby's group was the first to genotype the presence of GLE in southern Wales in 2013. However, many sightings have been made of bats which are similar morphometrically to GLEs but have been genotyped as BLE. This raised the questions of whether BLE have a wider morphometric range that was previously appreciated or whether that BLE and GLE have hybridised in recent times. The later may also be implicated in the range expansion of GLE if proven to be true. This project will test several loci of BLEs and GLEs as well as samples that are genotyped BLE but appear to look like GLEs to provide insight to this important conservation question.

The most reliable distinguishing features between brown and grey long eared bats are dorsal hair colour (dark and light bands along the hairs in the BLE), thumb length, thumb to forearm ratio, tragus width, face colour and shape (pinkish-brown and shorter lighter muzzle in BLE)

Figure 1. Grey long eared bat (left) and brown long eared bat (right) with distinctively different tragus shapes and fur colour.

Methods:

Chemical and physical methods were used to extract the DNA from faecal samples collected via non-invasive sampling methods around UK. PCR is used to amplify the genes being studied and gel electrophoresis to isolate and identify the genes. Once a clean sequence has been identified, DNA sequencing is completed; data analysis uses software such as Clustal2 and Figtree, which includes bioinformatics, visual representation and phylogenetic trees. Phylogenetic techniques allow the study of the relationship, similarities and differences in gene sequences between the two species as well as comparison with other bat species.

Results:

Main findings of the project are that there is potential hybridisation occurring between the two species as demonstrated by the presence of SNPs present in the genes studied. Further research is needed to confidently conclude this. However, DMP1 could not be isolated and is critical for proper mineralisation of bone and dentin. Mutations in the gene are known to cause autosomal recessive hypophosphatemia, a disease that manifests as rickets and osteomalacia; therefore the locus is highly conserved due to significance for survival. This is seen in both species of bats studied.

The function of Thy1 has not yet been fully elucidated; it has speculated roles in cell-to-cell and cell-to-matrix interactions with implication in neurite outgrowth, nerve regeneration, apoptosis, metastasis, inflammation and fibrosis. Looking at the Fig.2, results of Thy1, we can see that there are not many SNPs which indicate that the gene is independent to each species. Looking at the Fig.3, we can see that the two long eared species are closely related in comparison to the control species, *Myotis brandtii.*

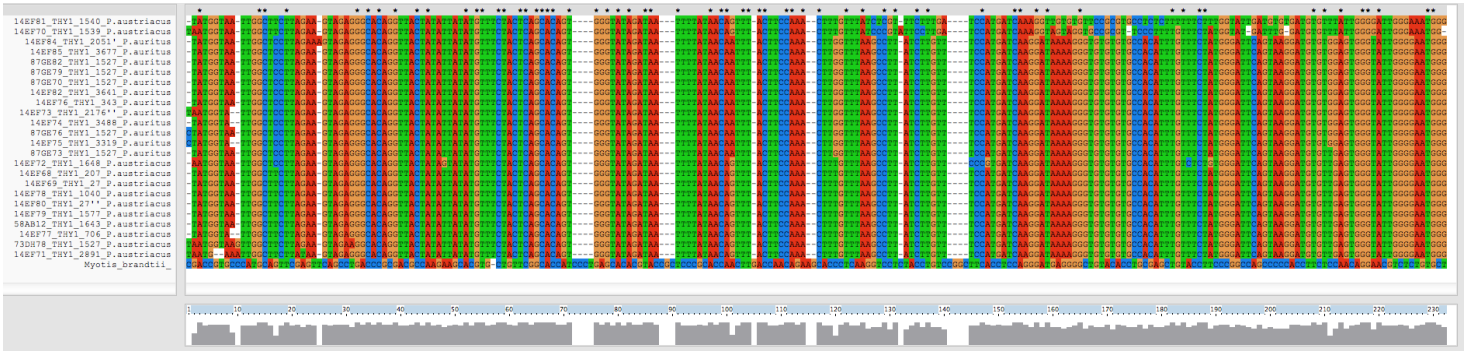


Figure 2: Clustal results of Thy1 gene from *P. auritus* and *P.austriacus* compared to *Myotis brandtii.*

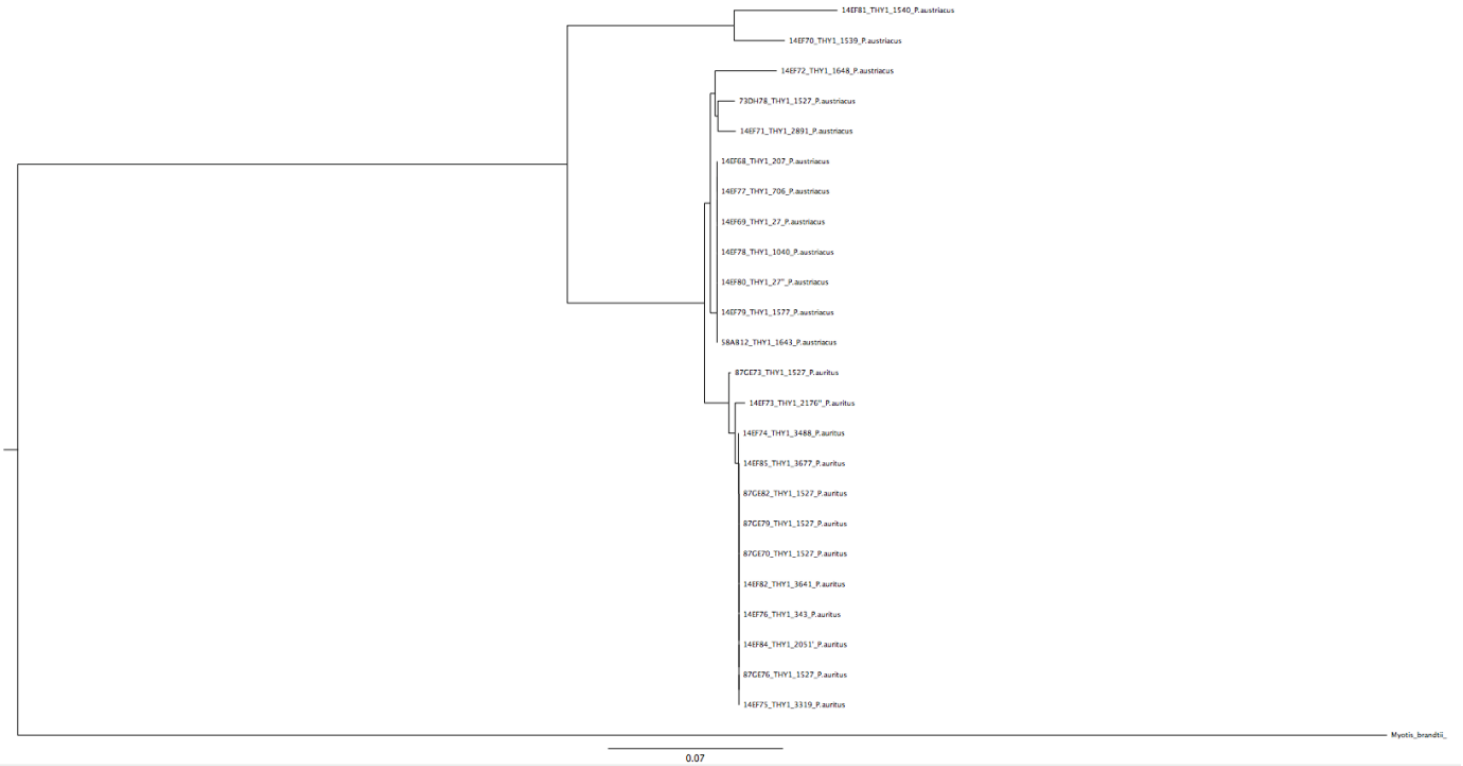


Figure 3: Figtree results of Thy1 gene from *P. auritus* and *P.austriacus* compared to *Myotis brandtii.*

Thy2 is a murine thymocyte-brain alloantigen. Both Thy1 and Thy2 are both thought to contribute to fur colour, which could be the most likely genes to show hybridisation, especially if the phenotypes of the two species are getting confused due to similarities which are rumoured to be appearing. Potential hybridisation is supported by the presence of a SNP at around 185 base pairs in Fig.4, of which the T and C are swapped between species. More sequencing samples are needed to come to a confident conclusion.

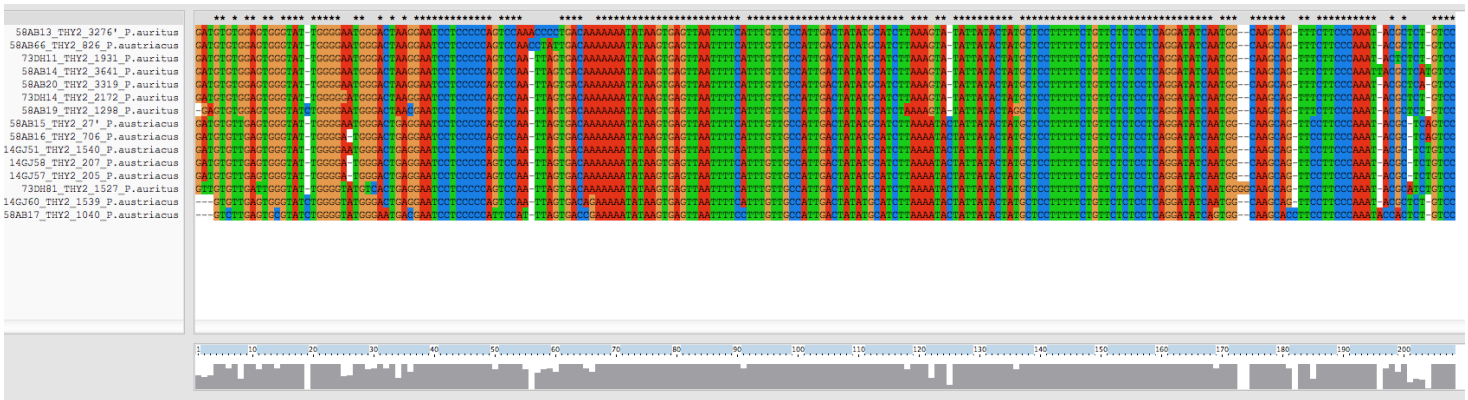


Figure 4: Clustal results of Thy2 gene from *P. auritus* and *P.austriacus* without comparison to other species.

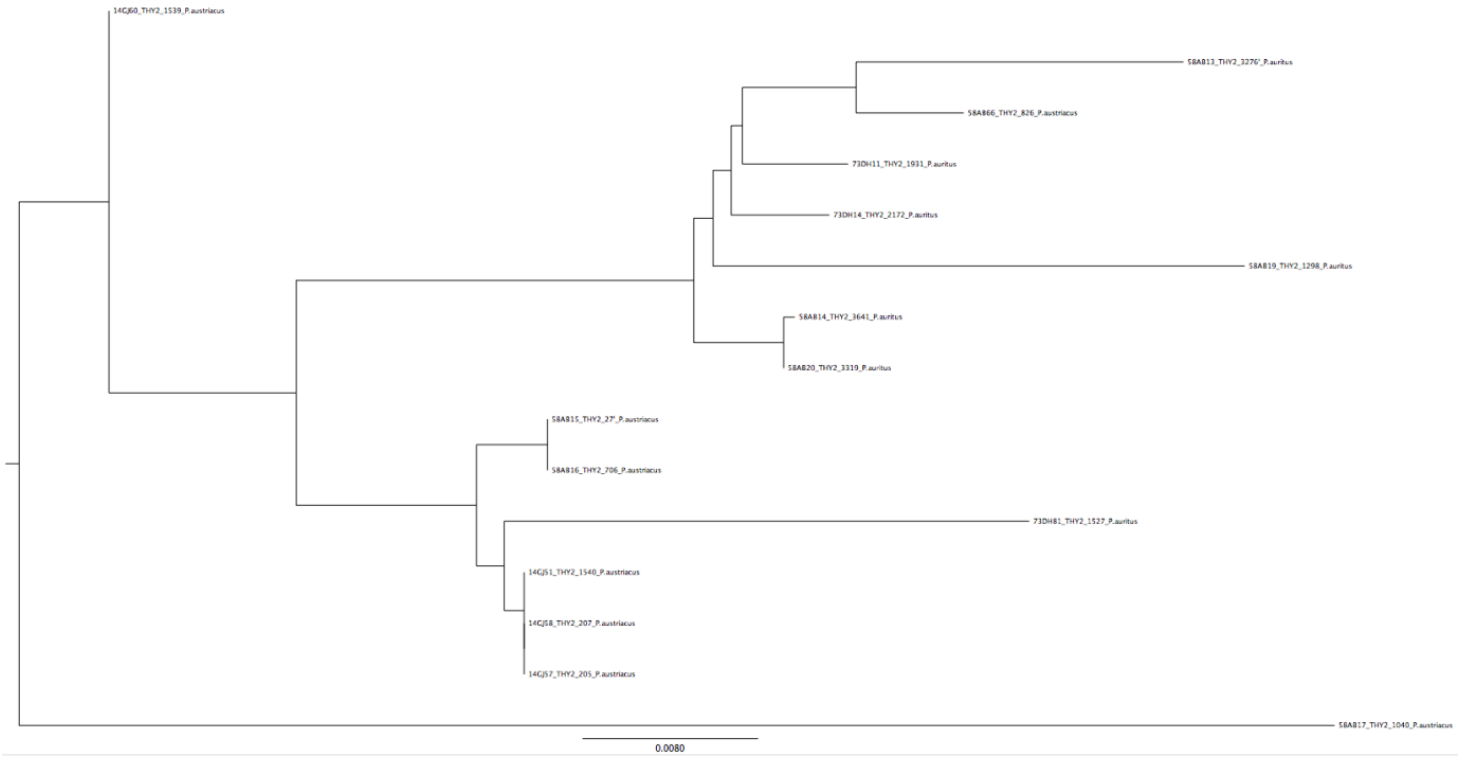


Figure 5: Figtree results of Thy2 gene from *P. auritus* and *P.austriacus* without comparison to other species.

RAG is involved in activation of immunoglobulin VDJ recombination by stabilising binding cleavage activity in the recognition of DNA substrate. Defects in this gene can be the cause of several diseases; therefore this gene is most likely to be conserved between the species due to its importance for survival. As predicted, the sequences are very similar, showing no SNPs between the two species (Fig.6). Interestingly, with comparison to *Pteropus*, the sequence varies dramatically, however this could be due to experimental error.

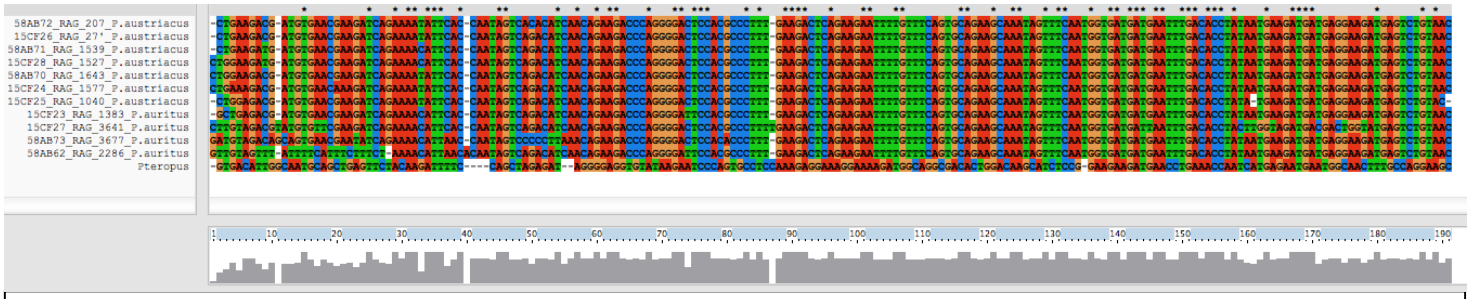


Figure 6: Clustal results of RAG gene from *P. auritus* and *P.austriacus* with comparison to *Pteropus.*

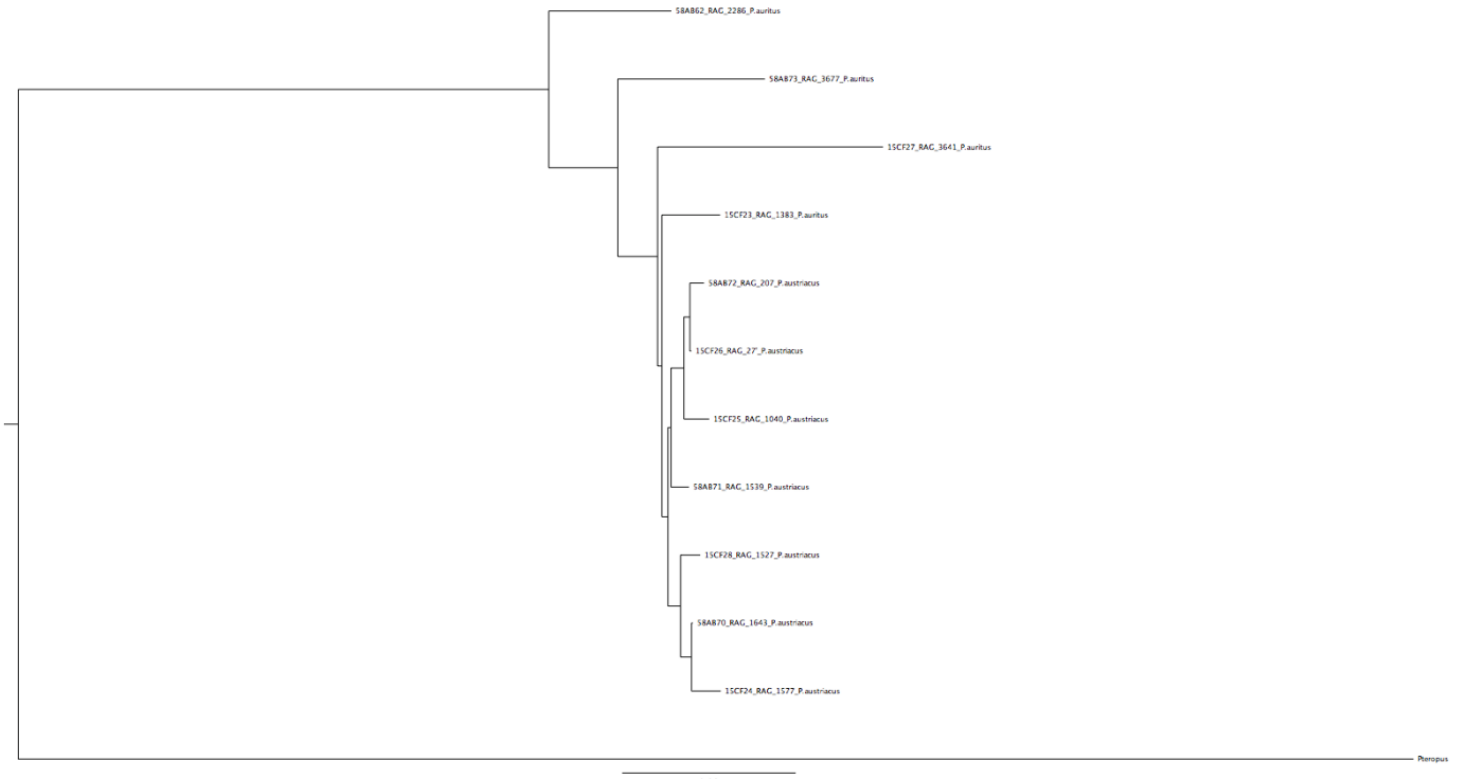


Figure 7: FigTree results of RAG gene from *P. auritus* and *P.austriacus* with comparison to *Pteropus*.

STAT5A is involved in the response to cytokines and growth factors; STAT family members are phosphorylated by the receptor associated kinases, and then form dimers that translocate to the cell nucleus where they act as transcription activators.

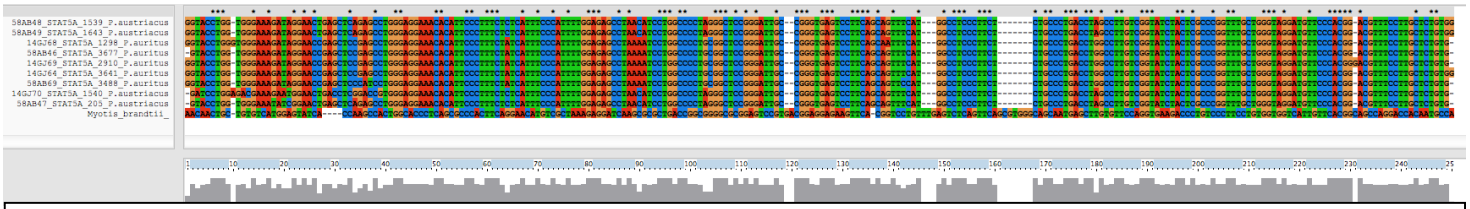


Figure 8: Clustal results of STAT5A gene from *P. auritus* and *P.austriacus* with comparison to *Myotis brandtii.*

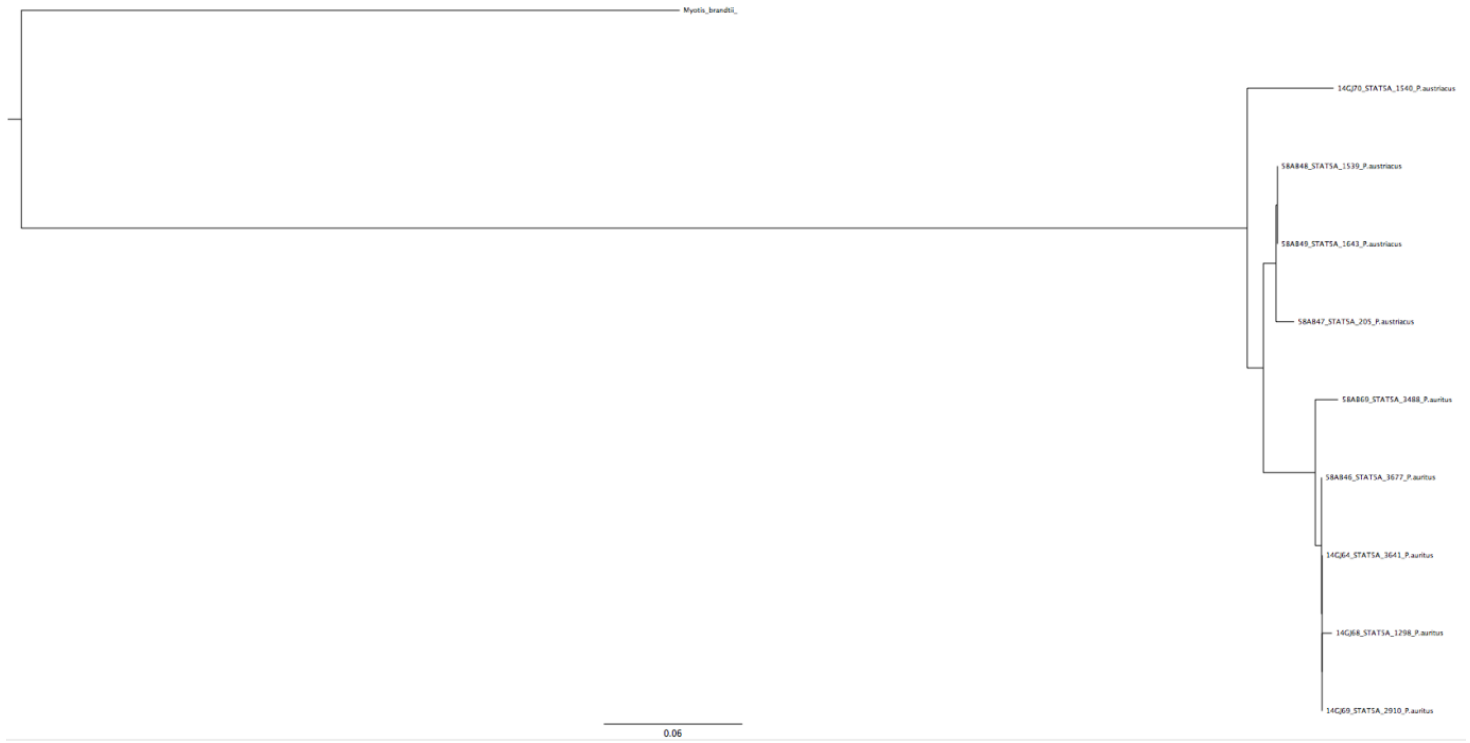


Figure 9: FigTree results ofSTAT5A gene from *P. auritus* and *P.austriacus* with comparison to *Pteropus*.

Looking at the results for each gene, there seems to be evidence of potential hybridisation, but there is low confidence in this conclusion due to limited sample size. However, if further research is carried out, the theory of hybridisation could be confirmed and further supported. It would appear to be natural hybridisation, however, whether the offspring is fertile, we do not know. Only invasive sampling could establish and to confirm that, but it would be unethical to do so, especially the fact GLEs is a protected species in the UK.

Discussion and Conclusions

Since the two long eared bat species are not widely studied, it was a challenge to find suitable genes to study given the limited nuclear database available. Studying less known genes such as Thy1 and Thy2 which are related to fur colour, I thought could discover new mechanisms, gene expressions and how they interact with relation to their phenotypic appearances could help in explaining why the two bat species are easily mistaken for one another. Choosing a range of genes will allow a better understanding of the extent of hybridisation and potentially provide evidence that hybridisation has occurred. The results shows likely chance of hybridisation, but with the limited data collected, the analysis is inconclusive.

A range of different genes were studied in this project and each of them have different variation rates, for example, some mutations may hinder the genes ability to function and if it plays critical role required for survival then the gene is much more likely to be conserved or the life of the animal would be at high risk and could not pass on its genes to the next generation.

If more time could be available in optimising primers and designing them, it would provide better and more detailed results to analyse. However, due to time constraints and costs, a compromise was taken. Ideally, primers designed contained overlapping regions of a gene as this would provide a clearer and more accurate sequencing data to be analysed.

The success rate for sequencing DNA in this project is rather low, as larger sequences are harder to find due to being nuclear DNA. However, mitochondrial DNA could not be used as it is maternal and we are looking at hybridisation which requires genetic information from both parents. The DNA extracted were from faecal samples, this limits the amount of DNA we can extract and also depends on the quality and conditions of the samples sent in. The use of invasive samples is likely to provide better data, however ethics and disruption to habitat especially to rare species is not ideal.

A possibility that the hybridisation is not very apparent when the project was taking place is that mutation rates will vary within different genes, some taking longer to evolve, so hybridisation may have already happened, but it will take some time for the mutations to be more frequently seen in the species and noticed through experiments. The use of bootstrap analytical method allows us to accept the data with confidence as the data is repeated multiple times and therefore limits the results to occur by chance.

As GLEs are such a rare species, hybridisation could be intentional to increase chances of survival and accidental due to the lack of same specie mates available; either way, it allows them to pass their genes onto future generation which is the ultimate goal of each organism.

Further research potential could be to look at whether if it is location specific hybridisation and if more densely populated bat areas have higher degree of hybridisation than those with fewer species present. Since there is a sign of hybridisation, it is wondered how long would it take for the GLEs and BLEs fully hybridise? And would that mean that the GLE species would disappear altogether? Do they have synchronised mating seasons and how does their roosting location, diet and human activity influences impact their chances of hybridisation? There are so many more questions to explore and speculate to contribute to a better understanding of the fascinating and mysterious world of bats.

References:

1. Harris S., Morris, P., Wray, S. & Yalden, D. (1995) *A review of British mammals: population estimates and conservation status of British mammals other than cetaceans*. JNCC, Peterborough.
2. Russ, J.M. 1999. *The Microchiroptera of Northern Ireland: community composition, habitat associations and ultrasound*. Unpublished PhD thesis. Queen's University, Belfast.
3. Dietz, C., von Helversen, O., & Nill, D. (2009) Bats of Britain, Europe & Northwest Africa. A & C Black Publishers Ltd., London.
4. Joint Nature Conservation Committee. 2007. *Second Report by the UK under Article 17 on the implementation of the Habitats Directive from January 2001 to December 2006.* JNCC, Peterborough. Available from: http://jncc.defra.gov.uk/page-4060.
5. Razgour O. (2012) From genes to landscapes: conservation biology of the grey long-eared bat, *Plecotus austriacus*, across spatio-temporal scales. PhD thesis, University of Bristol, UK.
6. Razgour, O., Hanmer, J. & Jones, G. (2011) Using multi–scale modelling to predict habitat suitability for species of conservation concern: the grey long–eared bat as a case study. *Biological Conservation* 144: 2922–2930.
7. http://www.bats.org.uk/pages/-brown_long-eared_bat-829.html
8. http://jncc.defra.gov.uk/page-6316
9. http://www.bats.org.uk/pages/grey_long-eared_bat.html
10. Figure 1 left: http://www.pbase.com/samwald/image/145809995
11. Figure 1 right: http://i.telegraph.co.uk/multimedia/archive/01514/bat_1514736i.jpg

Extra:

This additional data analysis section was done out of curiosity and not needed for the project itself. I wanted to compare the differences between the individual species of long eared bats with each other using a control of a not so related bat species to see how different the genes were. I then decided to also compare a gene from humans, *Homo sapiens,* with the sequenced sample data.

The following figures, Fig.10-12, show the differences between the individual species with comparison to a control species, for example *Myotis brandtii* for Thy1. From Fig.10 we can clearly see the differences between the sequences between the two species with very few base pairs being aligned. However, we can see that the species are related from the branching in the Figtree diagram in Fig.11, where sample 1530 *Plecotus austriacus* share the same branch with the *Myotis brandtii*; but they are not too closely related due to the distance from the majority of the rest of the samples.

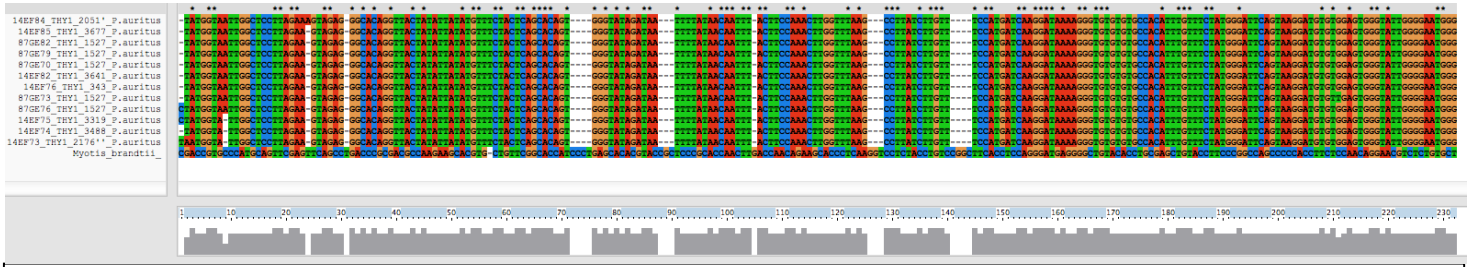


Figure 10: Clustal results of Thy1 gene from *P. auritus* compared to *Myotis brandtii.*

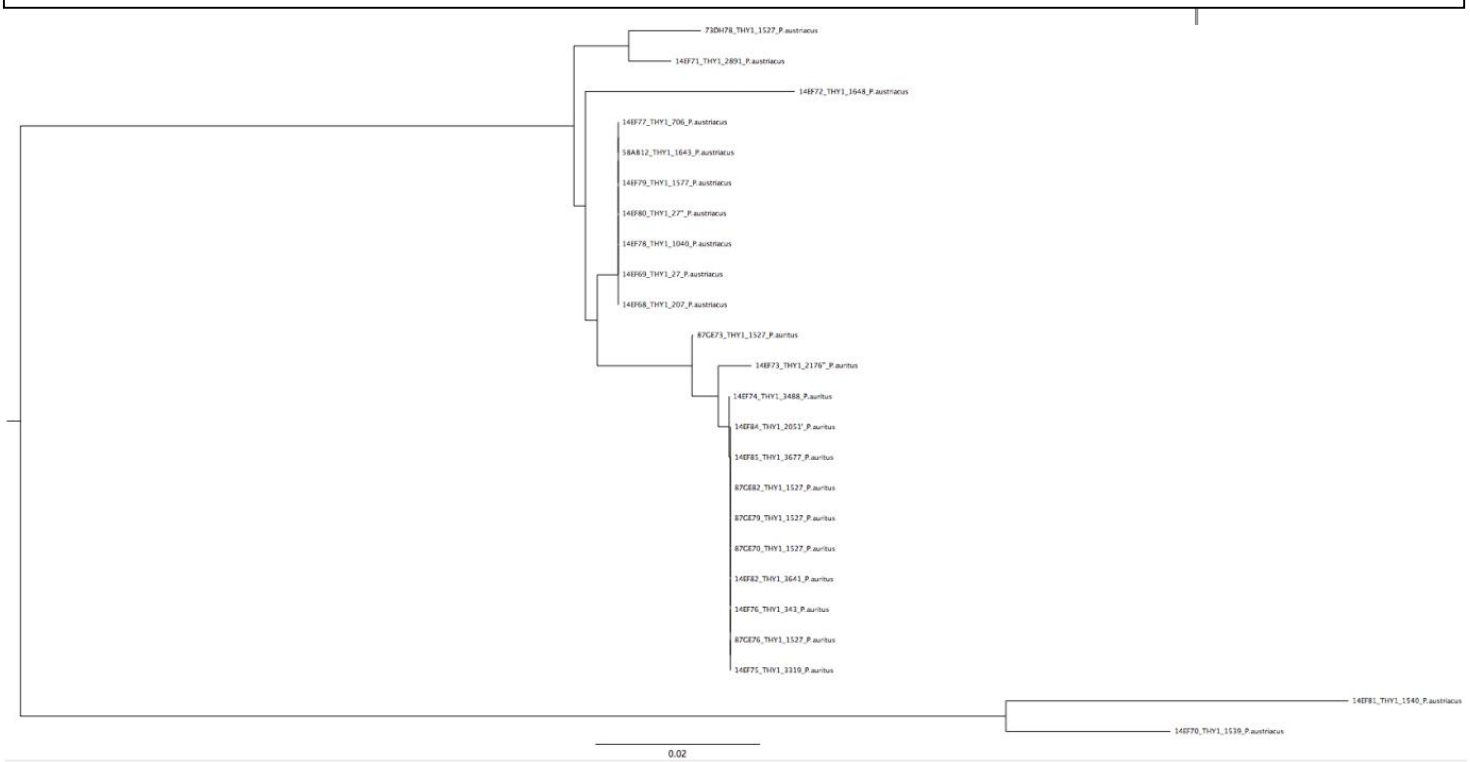


Figure 11: Figtree results of Thy1 gene from *P. auritus* compared to *Myotis brandtii.*

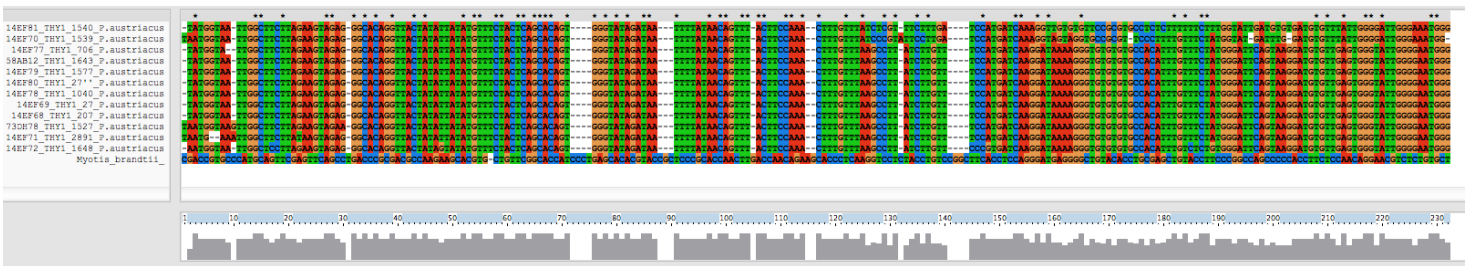


Figure 12: Clustal results of Thy1 gene from *P.austriacus* compared to *Myotis brandtii.*

Difference between *P. auritus* and *P. austriacus* with comparison to *Homo sapiens;* once again shows great variation in the base pair sequences between the two species, Fig.13, not surprisingly they align more loosely than the Thy1 gene between *Plecotus auritus* and *Myotis brandtii,* supporting that the two bat species are more closely related than we are to bats. This is further reflected in the Figtree diagram, Fig.14.

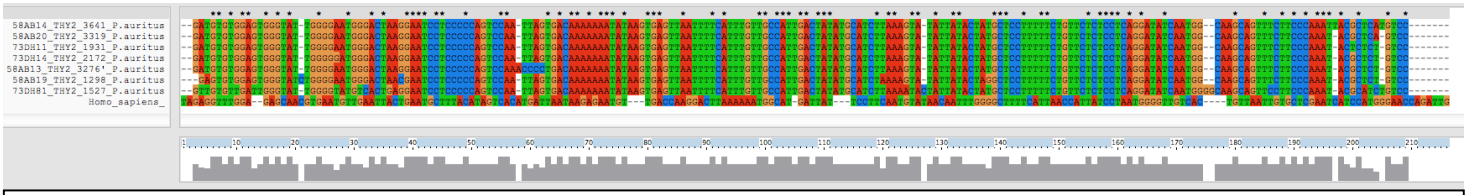
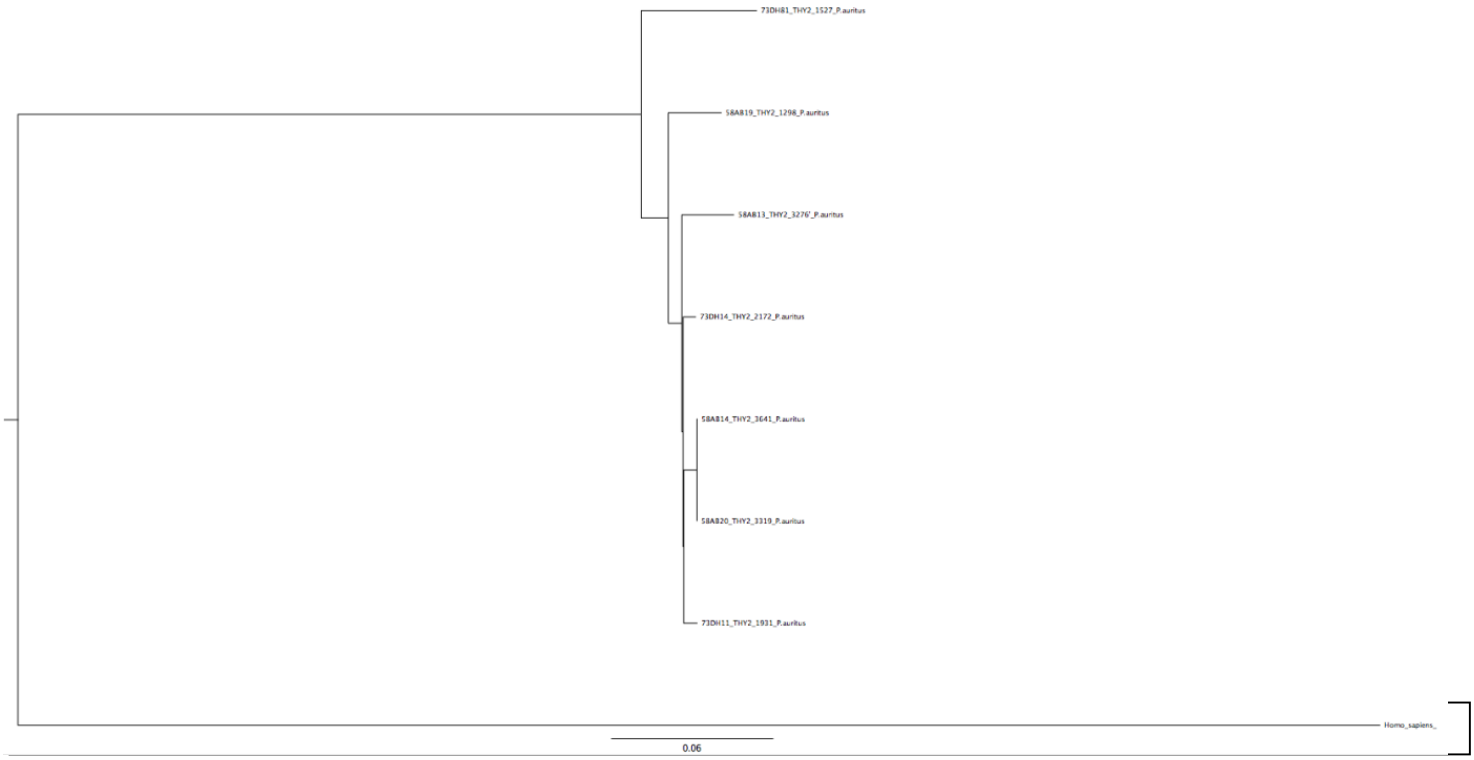


Figure 13: Clustal results of Thy2 gene from *P. auritus* with comparison to *Homo sapiens*.



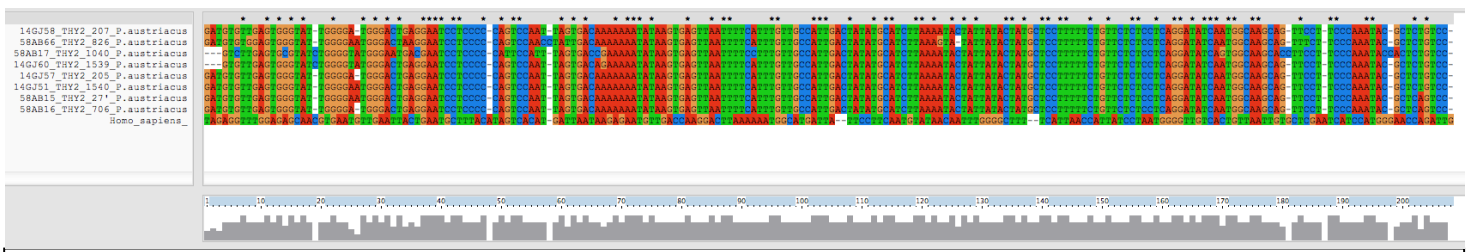


Figure 15: Clustal results of Thy2 gene from *P.austriacus* with comparison to *Homo sapiens*.

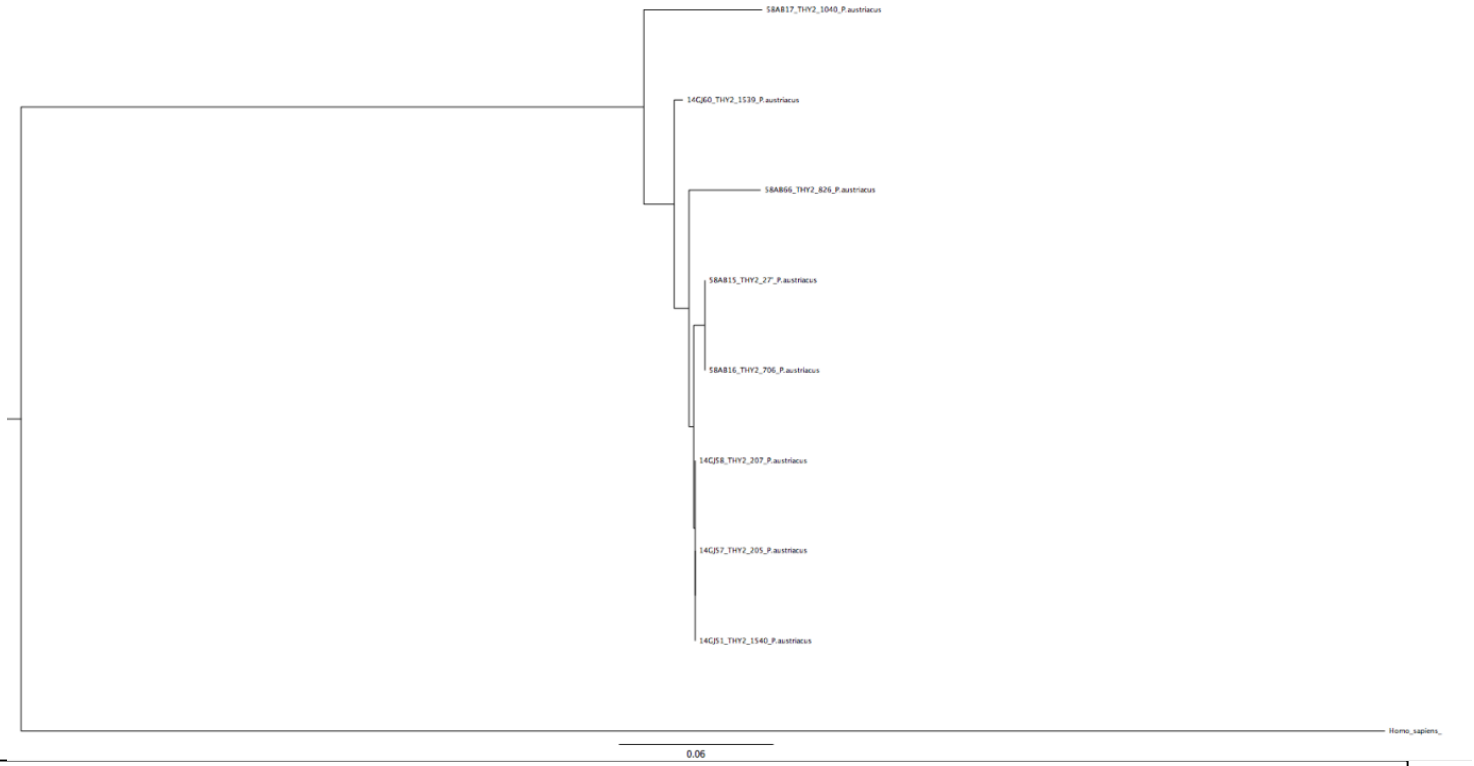


Figure 16: Figtree results of Thy2 gene from *P.austriacus* with comparison to *Homo sapiens*.

A different and visually interesting way of displaying analysed data is in the form of a phylogenetic tree, here are some created using the data of RAG genes from *Plecotus auritus* and *Plecotus austriacus,* Fig.17 and STAT5A genes from the same 2 species, Fig.18.

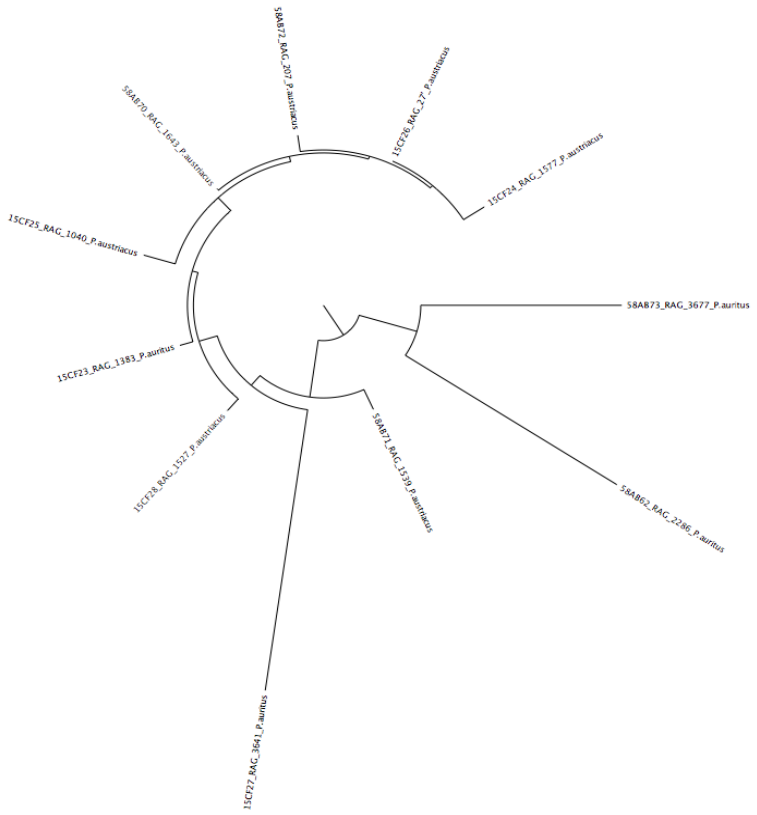


Figure 17: Phylogenetic tree of RAG gene from *P.austriacus* and *P. auritus.*

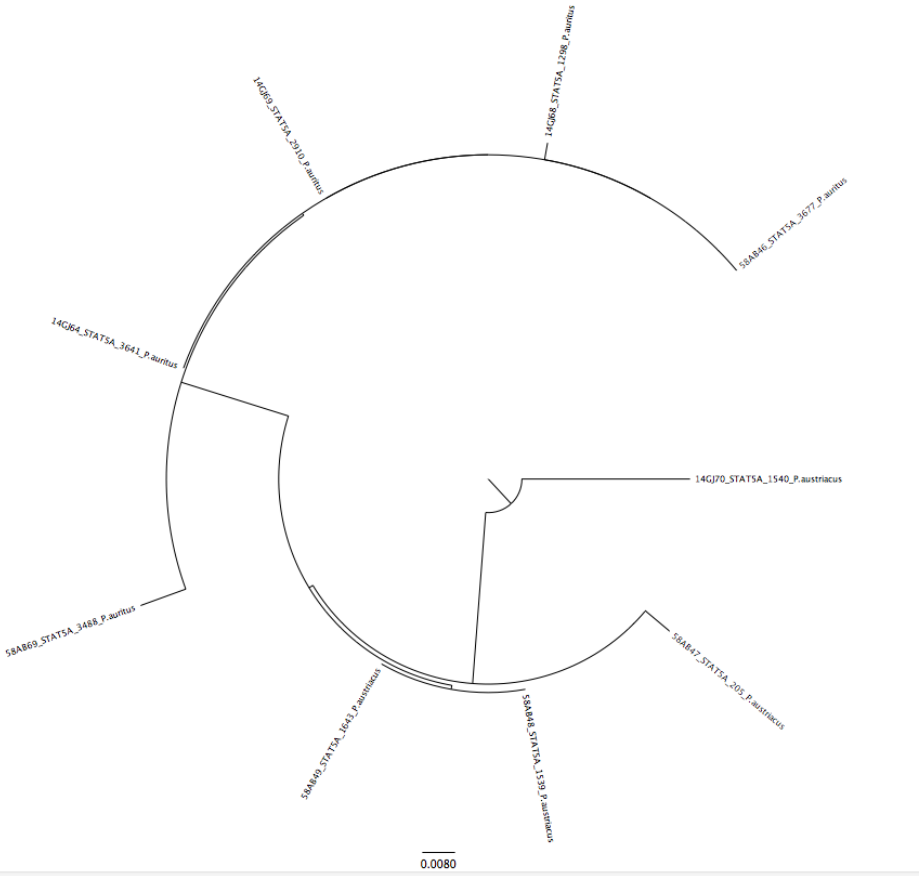


Figure 18: Phylogenetic tree of STAT5A gene from *P.austriacus* and *P. auritus.*