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The evolution of unique cranial traits in leporid lagomorphs

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Background

The leporid lagomorphs (rabbits and hares) are adapted to running and leaping (some more than others) and consequently have unique anatomical features that distinguish them from ochotonid lagomorphs (pikas) and from their rodent relatives. One such feature is an intracranial joint that circumscribes the back of the skull, thought to facilitate skull mobility. This joint separates the anterior portion of the cranium (including the dentition, rostrum and orbital apparatus) from the posterior portion of the cranium (which encompasses the occipital and the auditory complex). Aside from the observation that the intracranial joint is absent in pikas (generalist locomotors) and appears more elaborate in the genera with cursorial and saltatorial locomotory habits, the evolutionary history, biomechanical function and comparative anatomy of this feature in leporids lacks a comprehensive evaluation.

Methodology

The present work analysed the intracranial joint (as well as facial tilting and lateral fenestration of the maxilla) in the context of leporid evolutionary history using a Bayesian inference of phylogeny (18 genera, 23 species) and ancestral state reconstruction. These methods were used to gather information about the likelihood of the presence of these three traits in ancestral groups.

Results

Our phylogenetic analysis found it highly likely that the last common ancestor between all living leporids had an intracranial joint (92.9% likelihood) and that the last common ancestor of all living lagomorphs did not (70.1% likelihood). These findings provide a broader context to further studies of evolutionary history and will help inform the formulation and testing of functional hypotheses.

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Introduction

The order Lagomorpha is a highly successful, geographically widespread mammalian group, with a rich taxonomic history ~~which~~ has waned and dates almost to the Cretaceous-Paleogene (K-Pg) extinction event (Lopez-Martinez 2008). As herbivores, generally adapted to a cursorial locomotory form, lagomorphs have a set of anatomical features that distinguish them from their rodent relatives, but these features did not grant them ordinal status until relatively recently (Gidley 1912). A general understanding that lagomorphs are morphologically conservative with an "evolutionary picture [that is] one of the simplest of any group of mammals" (Wood 1957), has somewhat exacerbated the lack of research focusing on the group, relative to rodents. However, the extensive use of the European rabbit (*Oryctolagus cuniculus*) as a model organism in medical research, particularly that relating to disease and disorders of the musculoskeletal system (Esteves et al., 2018; Li et al., 2015), warrants further understanding of the general gross anatomy and evolutionary history of lagomorphs as a whole.

The literature on functional anatomy in lagomorphs has primarily focused on the limbs in relation to locomotion (Camp & Borell 1937; Fostowicz-Frelik 2007; Gambaryan 1974; Young et al., 2014). Comparatively little research has been undertaken on the cranium (Bramble 1989; Kraatz et al., 2015; Stott, Jennings & Harris 2010; Watson et al., 2014; Watson et al., 2021). Many cranial features appear to correlate with posture and gait (DuBrul 1950) and there are a number of unique traits that are poorly understood in terms of how they relate functionally to ecological factors such as diet, locomotion and burrowing (Bramble 1989; Feijó et al., 2020; Gambaryan 1974; Kraatz et al., 2015; Moss & Feliciano 1977). One interesting cranial feature in leporid lagomorphs (rabbits and hares) is ~~the presence of~~ an intracranial joint that may facilitate cranial kinesis (Bramble 1989). The intracranial joint is located between the parietal and occipital bones dorsally, the basioccipital-basisphenoid ventrally and between the squamosal and otic complex at the sides of the cranium. This feature ~~therefore~~ divides the cranium into anterior and posterior units and is thought to provide ~~a degree of~~ movement that ~~sets it apart~~ from the cranial sutures (Bramble 1989). It is most elaborate in the extant genus *Lepus* (hares and jackrabbits), although the ~~exact nature of this elaboration~~ is not yet well understood. Intracranial joints are common in vertebrates such as reptiles and birds but ~~its~~ presence in leporids is unique for mammals. In other animals, intracranial joints span a wide range of joint types and functions primarily in feeding; e.g., Holliday & Witmer (2008) but in leporids, the function is currently hypothetical and lacks comparative evolutionary, histological and biomechanical data (Bramble 1989). Furthermore, the influence of other ecological factors, such as diet, have not been sufficiently explored.

Other unique features of the leporid cranium that have had more recent attention include fenestration of the lateral maxilla and the presence of marked facial tilting. Maxillary fenestration appears in all leporid genera, albeit to varying degrees (Moss & Feliciano 1977). Ochotonids, the sister-family to leporids, also share this trait; however, in *Ochotona,* it presents as a single vacuity (Moss & Feliciano 1977). There are two primary hypotheses regarding the function of this trait: the first postulates that it serves to lighten the crania, reducing torque forces during high-speed locomotion (DuBrul 1950) and the second, is that it relates to the lack of masticatory forces transmitted through the area (Moss & Feliciano 1977). However, a recent biomechanical investigation of the strains generated during mastication show that fenestrations do not diminish the transmission of masticatory forces, and therefore likely supports the first hypothesis: minimising bone while maintaining a mechanically resistant morphology (Watson et al., 2021). Facial tilting in leporids was identified by Kraatz et al., (2015) who noted that, across leporids, there is variation in the angle between the upper diastema and occipital plane. They hypothesised that this functions to increase frontation of the orbits in order to aid vision in taxa that have specialised, high-speed locomotion. The presence and complexity of these cranial specialisations have been found to vary with locomotory form; for example, the fastest running species have the greatest degree of fenestration in their crania (and subsequently, markedly lighter skulls) (Bramble 1989; DuBrul 1950), higher degrees of tilting in the facial region (Kraatz et al., 2015) and more elaborate intracranial joints (Bramble 1989). Given that these features tend to appear together, it is ~~somewhat~~ likely that they form a functional complex that allows the cranium to withstand the mechanical forces present during high-speed locomotion.

Lagomorphs are notable in exhibiting higher diversity in the fossil record than the extant biosphere with 12 extant genera (11 leporid, one ochotonid) and ~94 extant species (61-63 leporid, 30 ochotonid) compared to approximately 78 genera and 234 species from the fossil record (Lopez-Martinez 2008). ~~Due to this, and the conservative lagomorph body plan, lagomorph evolutionary history remains a contentious issue (Matthee et al., 2004).~~ Although large-scale molecular phylogenetic studies have aided the general systematics, the use of morphological data in character-based phylogenetic methods remains important for time calibration, ancestral state reconstruction and trait evolution rates (Donoghue & Yang 2016). The identification of new, derived morphological characters for discrete character-based phylogenetics would therefore be welcomed (Ruf 2014). Furthermore, the use of comparative phylogenetic methods in the field of functional anatomy allows for the study of functional traits (or groups of functional traits) in the context of the evolutionary history of a group (Blanke et al., 2017; McElroy, Hickey & Reilly 2008)

Due to the difficulties preserving the anterior and posterior portions of the crania together *in situ*, as the two parts separate easily, the posterior portion of the crania is often poorly preserved or entirely absent in many fossil remains (Quintana, Köhler & Moyà-Solà 2011). This complicates any attempt to directly classify an intracranial joint in extinct species. Furthermore, the identification of facial tilt angle and fenestration of the lateral maxilla also requires high levels of cranial preservation in the fossil record. By applying various methods to a morphological discrete character matrix, which includes data from extant and extinct species such as *Palaeolagus*, it is possible to predict the most likely character state at internal nodes on the resultant phylogenetic tree (Reyes et al., 2018).

Hence, there are two specific aims of this work. First, to develop a lagomorph phylogeny using morphological characters and generated via Bayesian inference, that is mostly concordant with major clades and divergence estimates reconstructed by large-scale molecular lagomorph phylogenies (such as Matthee et al., 2004 and Ge et al., 2013). Secondly, to utilise this tree to undertake an ancestral state reconstruction to better understand where these unique cranial traits likely arose in the lagomorph lineage. Results from this work will be used to ascertain whether any of these unique cranial traits would be useful as morphological characters for leporid systematics in general.

Methodology

Reconstructing phylogeny

Phylogenetic analyses were run using Mr Bayes v. 3.2.7a (Ronquist et al., 2012) via CIPRES Science Gateway (Miller, Pfeiffer & Schwartz 2010). The matrix used was primarily based on that published by Asher et al., (2005) (which is based on a matrix developed by Meng, Hu & Li (2003) supplemented with additional characters. Character definitions for original characters are identical to Asher et al., (2005). In addition to adding characters, the number of taxa was expanded to better reflect extant species diversity. The resulting morphological data matrix contains 23 taxa and 228 characters. The ~~resulting~~ taxa are extant and extinct lagomorphs with a tree-shrew genus *Tupaia* serving as the outgroup.

Additional taxa

The matrix developed by Asher et al., (2005) includes a broad range of both extant and extinct genera belonging to (or close to) the supraorder Glires. The taxa used by Asher et al., (2005) were chosen in order to place new *Gomphos* material in the context of Glires systematics. Since our study is concerned with a morphology that is only found in (extant) leporids, we have adjusted our data accordingly. For extant lagomorphs, the original inclusion of *Lepus, Sylvilagus, Oryctolagus* and *Ochotona* was expanded to include all extant genera and multiple species for genera that are polyspecific (Table 1). For fossil data, only genera that are part of, or close to, the lagomorph lineage were included (*Mimolagus, Gomphos, Mimotona, Paleolagus, Prolagus*).

Additional characters

Three new characters were added to the matrix. These characters represent cranial traits that are likely linked to locomotory habit; perhaps forming a suite of functional traits that perform somewhat together (Figure 1).

Character 95: angle between the upper diastema and the occipital plane (facial tilt) (Kraatz et al., 2015) – -- (0) more obtuse: >39.9° (e.g., Ochotona) (1) more acute: <39.9° (e.g., Pronolagus).

The non-leporids included in the taxon list are defined as having more obtuse facial tilt angles. For leporids, a species is defined by having a more obtuse degree of facial tilt if the median angle between the upper diastema and the occipital plane is greater than 39.9°. They are defined as having a more acute degree of facial tilt if the median angle between the upper diastema and occipital plane of a species is less than 39.9°. This character is based on data collected by Kraatz et al., (2015). It was found by Kraatz et al., (2015) that leporid species that locomote in a specialised, cursorial or saltatorial, way generally exhibit a more acute degree of facial tilting than those who locomote in a more generalised way. Therefore, leporids can be grouped into one or the other of these categories.

Character 113 lateral fenestration of maxilla (if present)--- (0) large single opening (e.g., Ochotona), (1) a latticework of small openings (reduced) (e.g., Oryctolagus), (2) a latticework of small openings (advanced) (e.g., Lepus). Ordered (see discussion).

These states are based on observations by (Wible 2007). A multi-state character was necessary to expand on the original character for lateral fenestration of the maxilla (character M121, MW66, A111) as the differences appear to correlate with locomotory form. This character is coded as inapplicable for those without lateral fenestration of the maxilla.

Character 136 (new) intracranial joint --- (0) absent (e.g., Ochotona), (1) present (e.g., Lepus).

The intracranial joint is located along the occipito-parietal union dorsally and continues down either side of the braincase between the squamosal and the otic complex. In *Oryctolagus*, which has an unfused interparietal bone, the joint is diverted around the posterior edge of the interparietal. Mid-ventrally, it is completed by a union at the basioccipital-basisphenoid articulation (Bramble 1989). Due to the lack of published data pertaining to the variation (in complexity) of this feature between genera and species, there is no justification for a multi-state character (as states are currently only observational). Therefore, it is coded as absent or present.

Phylogenetic approach

A relaxed clock analysis was implemented using a fossilised birth-death model in the program Mr Bayes v. 3.2.7a (Ronquist et al., 2012) via CIPRES Science Gateway (Miller et al., 2010). Some groups were constrained (using prset tologypr=constraints) in order to better fit the topology of published trees which used molecular data (Ge et al., 2013; Matthee et al., 2004). In this instance, constraining was justified as the difficulty of producing accurate topologies from morphological data for lagomorphs is well reported (Kraatz et al., 2021). The fossil taxa were calibrated by age of fossil occurrence (via fossilworks.org) and a soft upper bound constraint was placed on the age of the tree (prset treeagepr=offsetexp) based on the molecular estimate of the age of *Mimotona* (the oldest taxon) (dos Reis, Donoghue & Yang 2014). A calibration was also placed on the age of the genera *Lepus, Ochotona* and *Sylvilagus* based on the posterior distribution of the divergence estimates from Matthee (2004) (Table 2). The strategy under which the species were sampled was set to represent all major lineages (diversity sampling) (prset samplestrat=diversity). The base of the clock rate was set using an informative prior derived from a non-clock analysis of the dataset (prset clockratepr=lognorm). The clock model for rate variation among lineages was set to a relaxed uncorrelated clock with values sampled from a gamma distribution (IGR). Six MCMC chains were run twice for 7000000 generations and sampled every 1000 generations. The first 25% of each run were discarded at the burnin phase.

Reconstructing ancestral state

Due to poor preservation of the lagomorph posterior cranium during the taphonomic process, it is difficult to ascertain the presence (or lack thereof) of the intracranial joint in fossil individuals. Ancestral state reconstruction allows for the combination of observed state data at the tips of a tree and information regarding the phylogenetic relationships between taxa – resulting in the ability to predict states of heritable traits (characters) at internal nodes (Holland et al., 2020). Ancestral state reconstructions were undertaken in the R-language toolkit MBASR (MrBayes Ancestral States with R) (Heritage 2021; R Studio Team 2020). This toolkit performs ancestral state reconstruction using the continuous-time Markov model via MrBayes and automates many of the steps included in packages with similar functions (Heritage 2021).

The consensus tree from the relaxed clock analysis was loaded into MBASR with a file including the specific trait data examined. The number of samples generated was set at 10000 (having been compared to other, lower, values). MBASR applies a likelihood filter (the threshold for this filter is 25% of the likelihood range) and so this value allows enough generations to reach optimum proposals in terms of likelihoods. Each run reconstructed a single character.

The characters reconstructed were as follows: ch. 95 (facial tilt), ch. 113 (fenestration) and ch. 136 (intracranial joint). Character 113, relating to the state of lateral fenestration of the maxilla, is eligible for ordering (as there is good evidence for a progression of states). This was tested and ordering the states was found to make very little difference to results.

Results and Discussion

Phylogeny

The relaxed-clock phylogenetic reconstruction was derived from morphological data and includes members of every extant genus of lagomorph (Figure 2). The clade groupings by previously published phylogenies are not always consistent with one another and our phylogeny is no exception. Matthee et al., (2004), Ge et al., (2013) and our phylogeny agree that *Nesolagus* is closely related to *Pronolagus,* although *Poelagus* is also included in this group for Matthee et al., (2004) and here. Matthee et al., (2004) and our reconstruction agree that *Bunolagus, Oryctolagus, Caprolagus* and *Pentalagus* form a clade. Ge et al., (2013) recovered this clade as well, but also included *Romerolagus* (which branches off earlier in Matthee et al., 2004) and *Sylvilagus* (which is grouped with *Brachylagus* in Matthee et al., 2004 and *Brachylagus* and *Romerolagus* in our data) (Figure 3).

The divergence time estimates in our phylogenetic reconstruction roughly match those in molecular studies, with a key difference being the divergence estimate for the leporid/ochotonid split. For this, Ge et al., (2013) give a median value of divergence time as 50.3 million years and Matthee et al., (2004) give 31.7 million years. Our phylogeny gives a median estimate of 27.0 million years. The estimates for the divergence of leporids are consistent within all three phylogenies (21.0 Mya here, 15.2 Mya for Matthee et al., 2004 and 18.1 Mya for Ge et al., 2013).

Clade groupings for extant lagomorphs are notoriously difficult to resolve (using molecular or morphological data) due to morphological conservatism, the absence of chromosomal synapomorphies and the saturation of mitochondrial DNA sequences (Matthee et al., 2004). Given that we used morphological data alone, it was necessary to give the model information derived from molecular phylogenies. The relevant divergence time estimates in our phylogenetic reconstruction generally fall within the published ranges, with the exception of the leporid/ochotonid split in Ge et al., (2013). This was largely aided by the calibration of the age of the genera *Lepus, Ochotona* and *Sylvilagus.* Without these calibrations, the divergence time estimates are far younger than expected; for example, it places the divergence between leporids and ochotonids at around 10.9 million years. This reflects the young estimates for clade divergence that morphological data alone, with a poor sampling of fossil specimens, tends to produce (Barba-Montoya, Tao & Kumar 2021). By placing a few key calibrations on large extant genera, we compute a tree with estimations that are concordant with previous studies.

Ancestral state reconstruction

The results from the first reconstructed trait, the angle between the upper diastema and occipital plane (facial tilting) are inconclusive (Figure 4). That is, that MBASR could not confidently assert one way or another to the state at key internal nodes such as the last common ancestor of extant leporids, or the last common ancestor of extant lagomorphs as a whole. Previous work on leporid facial tilting found that it was strongly homoplastic across leporid evolutionary history and that there was weak phylogenetic signal in the facial tilt angle (Kraatz & Sherratt 2016; Kraatz et al., 2015). Furthermore, disaggregating the raw data for these angles reveals a large amount of intraspecific variation (in some species, up to 20.2° - as in *Pronolagus crassicaudatus*)

(Kraatz et al., 2015), suggesting that it is likely a trait driven more by environmental than evolutionary factors. Specifically, Kraatz et al., (2015) found that in generalised locomotors, such as *Brachylagus idahoensis*, there is reduced facial tilt angle in comparison to cursorial and (to a lesser extent) saltatorial locomotors such as *Lepus californicus* and *Sylvilagus audubonii.* This suggests that perhaps locomotion might be a driver for facial tilt angle, rather than phylogeny. Due to the lack of significant phylogenetic signal, high homoplasy and the influence of ecological factors (primarily locomotion), the ancestral state reconstruction did not bear informative results for facial tilt angle. This trait is therefore not a promising morphological character for use in phylogenetics.

The second reconstructed trait, fenestration of the rostrum, indicates that the intermediate fenestration seen in rabbits such as *Oryctolagus, Pronolagus* and *Bunolagus* is the likely ancestral state of leporids and all lagomorphs (Figure 5). Therefore, the advanced and singular opening states in *Lepus* and *Ochotona* are likely derived traits. Whilst fossil lagomorph taxa are often only represented by teeth or mandibular sections, well preserved members of *Palaeolagus* (33.9 – 20.43 Mya) and *Alilepus* (13.6 – 1.8 Mya) appear to also feature the intermediate, rabbit-like, state (Wolniewicz & Fostowicz-Frelik 2021; Wu & Flynn 2017), supporting our results. Our ancestral state reconstruction also suggests that the development of the advanced form of fenestration seen in *Lepus* and some other taxa (such as *Sylvilagus* and *Brachylagus*) has evolved on two separate occasions in the lineages of extant leporids, whereas the single vacuity state seen in ochotonids likely evolved once (in the common ancestor between *Ochotona* and *Prolagus*). However, in phylogenies reconstructed by Matthee et al., (2004) the genus *Lepus* is in a clade with *Sylvilagus, Brachylagus* and other taxa. If we are to accept their reconstructions as correct, then the advanced fenestration in these taxa would have likely evolved just once (in the common ancestor of *Lepus, Brachylagus* and *Sylvilagus*).

Fenestration of the maxilla is considered a diagnostic feature of the leporid cranium and its state varies between taxa. There are multiple hypotheses as to the function of these fenestrations, including lightening the rostrum for running speed vs lack of masticatory force transmission (DuBrul 1950; Moss & Feliciano 1977; Watson et al., 2021). More recently, a study utilising both multibody dynamics analysis and finite element analysis suggests that the fenestration is optimised to reduce mass in the rostrum whilst maintaining structural stability during mastication (Watson et al., 2021). In this scenario, both primary functional hypotheses (lightening the skull for locomotion and masticatory force response) could be correct. The ancestral state reconstruction presented here does not bolster any functional hypothesis, however the presence of the advanced fenestrations in taxa that run at slower speeds (such as *Brachylagus,* which locomotes at a top speed of around 23 km per hour, as opposed to *Lepus europaeus,* which reaches speeds of 75 km per hour) suggests that the function is not entirely related to running speed. This trait also needs more study in order to identify the amount of intraspecific variation and measure the extent and complexity of the maxillary fenestrations. This trait could be utilised as a morphological character in further phylogenetic analyses; whilst the original character set included a character for the presence of fenestration in the maxilla, information regarding the degree of the fenestration could help to separate extant taxa further.

The third trait reconstructed at internal nodes, the leporid intracranial joint, is shown mapped on the consensus tree of the relaxed clock analysis (Figure 6). This suggests that the last common ancestor of all extant leporids likely possessed the joint, whereas the last common ancestor of all extant lagomorphs likely did not. This outcome was expected, as we predicted that this trait arose as leporids became more specialised in morphology, – possibly relating to the transition from more generalised to more specialised high-speed locomotion (Gambaryan 1974). The ancestral state reconstruction also suggests that it is likely (although with less certainty) that the last common ancestor of all extant lagomorphs did not have this trait, an outcome that was also expected given a close relative of this ancestor, *Palaeolagus*, was likely a generalist locomotor and appears to have a posterior cranium that resembles ochotonid morphology (Wolniewicz & Fostowicz-Frelik 2021). For large-scale phylogenies, where distinguishing between leporids and ochotonids is necessary, the presence/absence of an intracranial joint could be a useful morphological character. However, it is rarely preserved in fossil taxa and in the character's current state (just presence/absence), it does not provide any means of differentiating between extant leporid taxa. Future work on the variation of this trait among leporids may allow us to categorise different degrees of complexity, aiding our ability to use this feature as a useful character in leporid systematics, and potentially identifying links to locomotion.

Conclusion

This study found that the last common ancestor of extant leporids likely had an intracranial joint, but the last common ancestor of extant lagomorphs likely did not – indicating that this trait was potentially driven by changes to locomotory form in the leporids (in particular, as they became more specialist runners and jumpers). It was also found that the ancestral state of maxillary fenestration was likely the intermediate rabbit-like form, with the extreme advanced and singular forms in *Lepus* and *Ochotona* representing derived features. As expected, the ancestral state reconstruction could not resolve states for facial tilting at internal nodes; this is probably due to the homoplastic nature of this trait (Kraatz et al., 2015). In future work, broader sampling of fossils (particularly those closer to the leporid/ochotonid split) is necessary to avoid the need to calibrate clade divergence times. Furthermore, the study of these traits would benefit from a total evidence approach, combining molecular and morphological characters to ensure the accuracy of resolved phylogenetic relationships.

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Figure 1

Morphological characters added to the matrix developed by Meng et al. (2003), Asher et al. (2005) and Rose et al. (2008).

A. the intracranial joint can be seen here between the squamosal and the otic complex in *Lepus* (right), it is not visible in *Ochotona* (left). The angle of the upper diastema to line of the occipital plane is illustrated in black. B. the morphological differences in maxillary fenestration are shown in *Ochotona* (left), *Oryctolagus* (middle) and *Lepus* (right). *Ochotona* features a singular vacuity. *Oryctolagus* has extensive fenestration, but noticeably less advanced than that seen in *Lepus.*

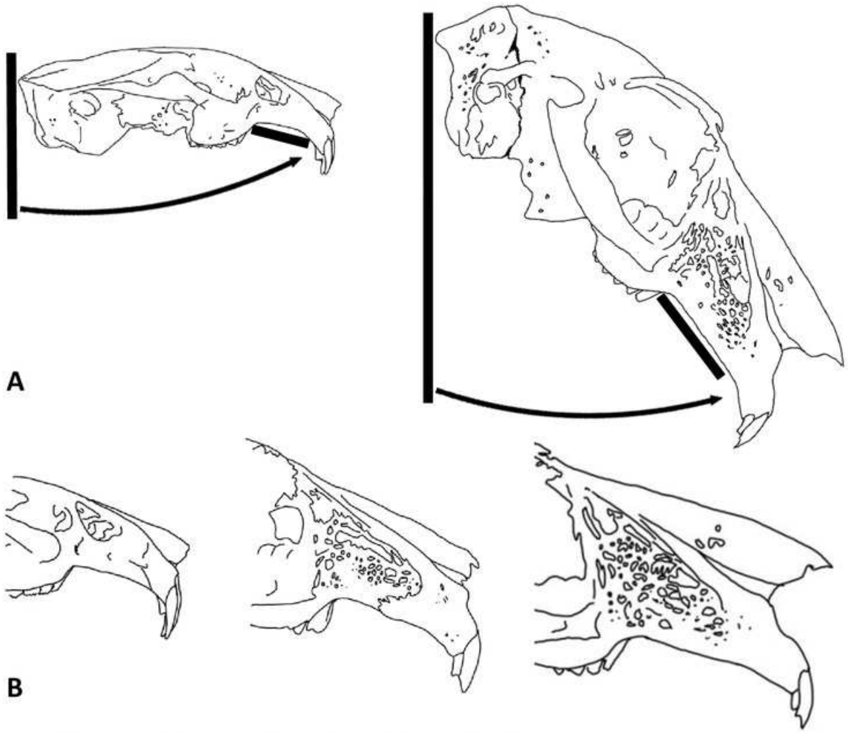

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Figure 2(on next page)

Our relaxed-clock phylogenetic reconstruction derived from morphological data.

The tree includes at least one member of every extant genus of lagomorph. Fossil taxa are denoted with “zz”.

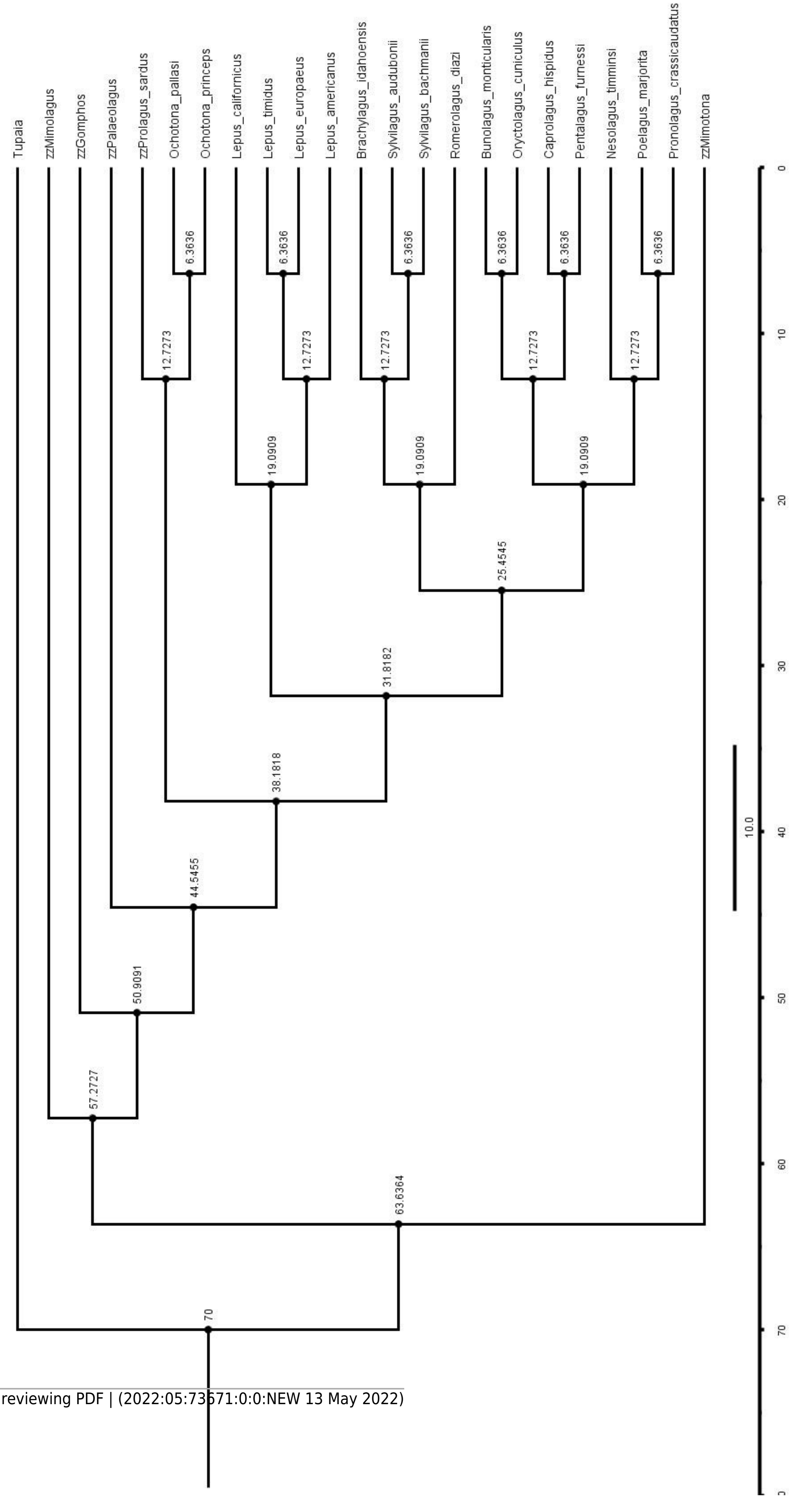
Tupaia
zzMimolagus
zzGomphos
zzPalaeolagus
zzProlagus_sardus
Ochotona_pallasi
Ochotona_princeps
Lepus_californicus
Lepus_timidus
Lepus_europaeus
Lepus_americanus
Brachylagus_idahoensis
Sylvilagus_audubonii
Sylvilagus_bachmanii
Romerolagus_diazi
Bunolagus_monticularis
Oryctolagus_cuniculus
Caprolagus_hispidus
Pentalagus_furnessi
Nesolagus_timminsi
Poelagus_marjorita
Pronolagus_crassicaudatus
zzMimotona
6.3636
6.3636
6.3636
6.3636
6.3636
6.3636
12.7273
12.7273
12.7273
12.7273
12.7273
19.0909
19.0909
19.0909
25.4545
31.8182
38.1818
44.5455
50.9091
57.2727
63.6364
70
10.0
0
10
20
30
40
50
60
70

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Figure 3

The phylogenetic relationships offered by Matthee et al. (2004) (left), Ge et al. (2015) (middle) and our phylogeny (right).

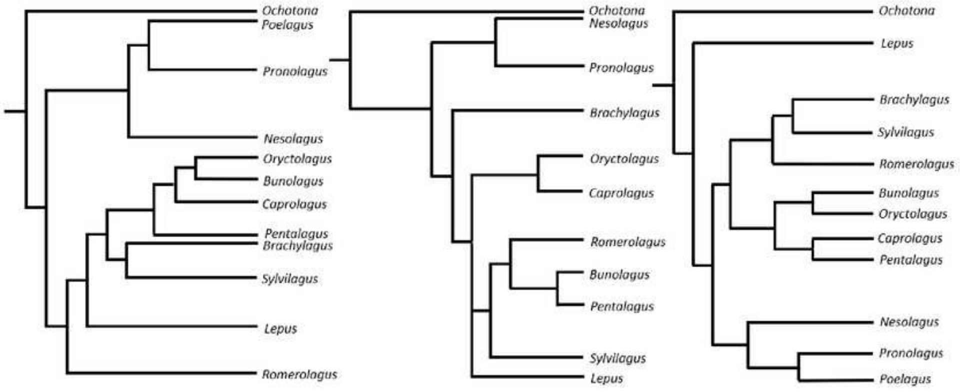

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Figure 4

Ancestral state reconstruction of leporid facial tilt.

Results from this reconstruction are inconclusive due to the homoplastic nature of this trait. The orange bracket encompasses all lagomorphs, the blue surrounds leporid lagomorphs and in green, the ochotonids. Red in the nodal markers refers to an obtuse facial tilt (as in extant Ochotonids), orange refers to a present intracranial joint (as in extant Leporids).

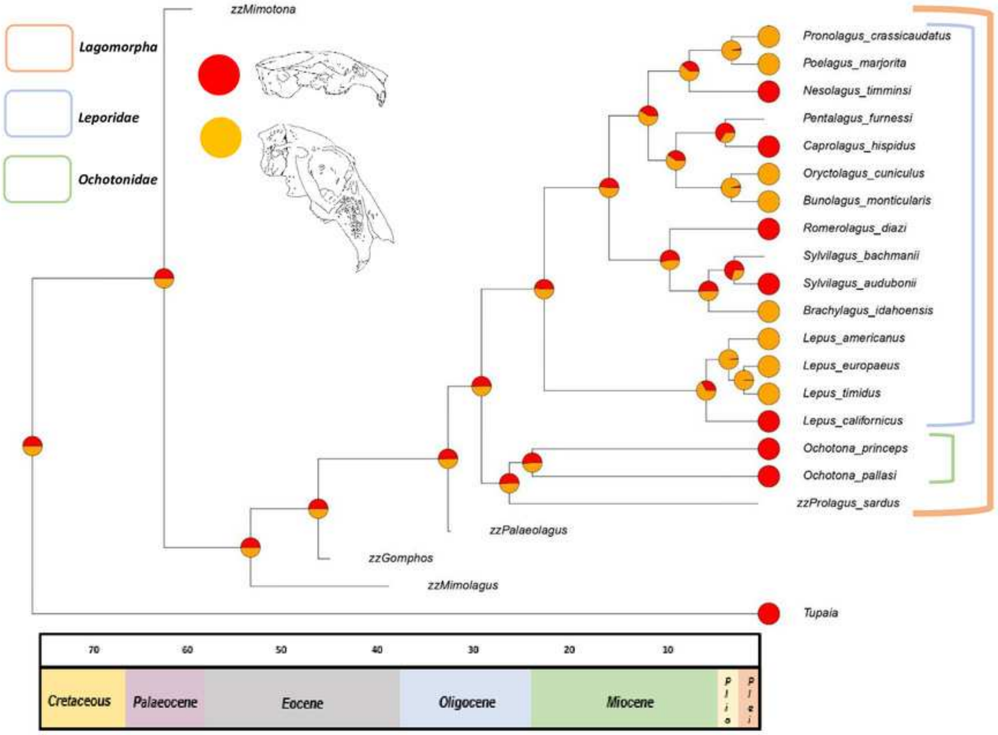

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Figure 5

Ancestral state reconstruction of the maxillary fenestration

Rabbit-like, intermediate fenestration is likely ancestral. More extreme morphologies, the *Ochotona* single paucity and the *Lepus* advanced fenestration are likely derived traits. The orange bracket encompasses all lagomorphs, the blue surrounds leporid lagomorphs and in green, the ochotonids. Red in the nodal markers refers to the single paucity fenestration type (as in Ochotonids), orange refers to a reduced type (as in *Oryctolagus*) and yellow refers to the advanced type (as in *Lepus*).

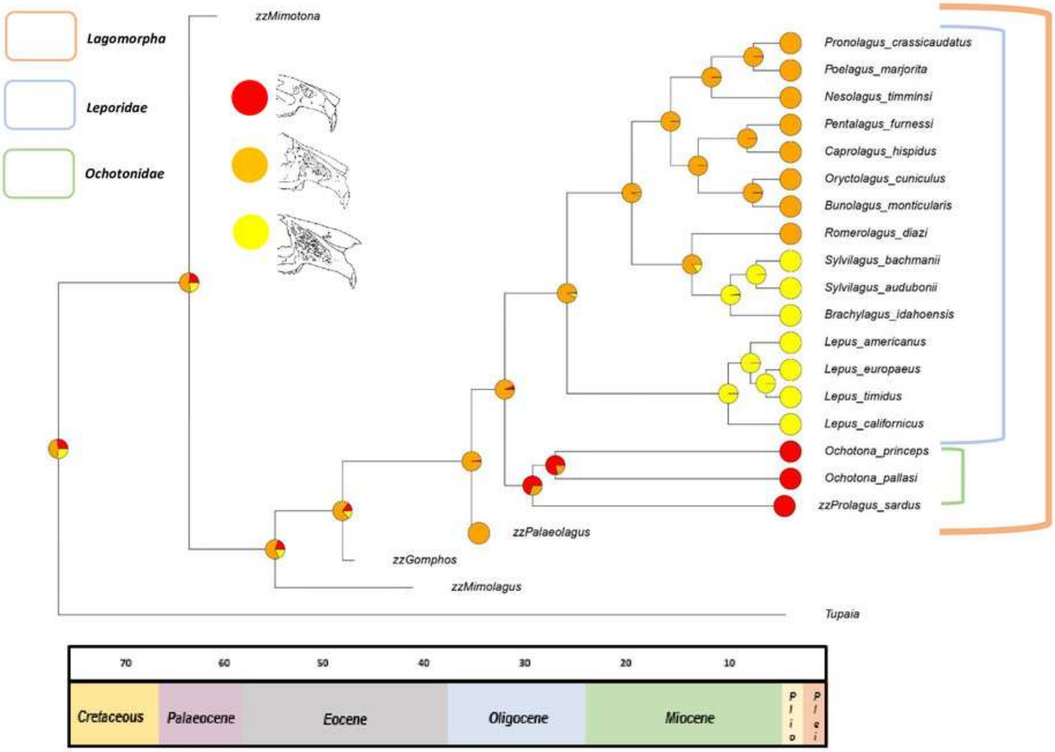

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Figure 6

Ancestral state reconstruction of the leporid intracranial joint

The last common ancestor of extant leporids likely did have the intracranial joint, whereas the last common ancestor of extant lagomorphs likely did not. The orange bracket encompasses all lagomorphs, the blue surrounds leporid lagomorphs and in green, the ochotonids. Red in the nodal markers refers to a lack of intracranial joint (as in extant Ochotonids), orange refers to a present intracranial joint (as in extant Leporids).

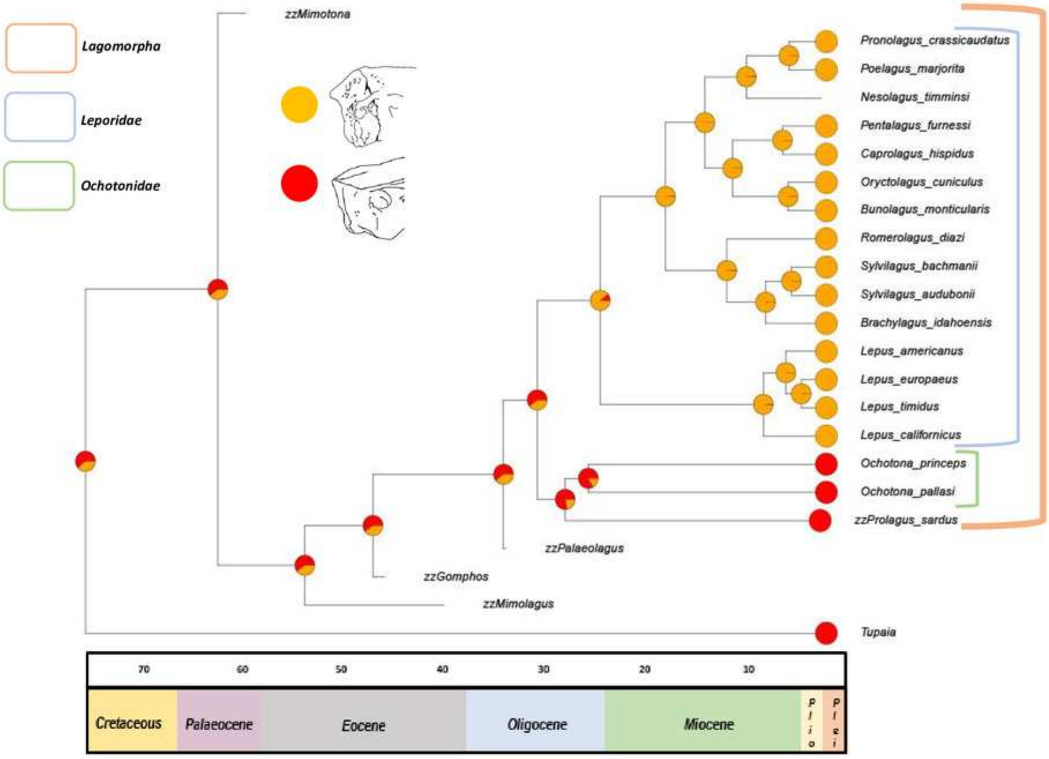

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Table 1(on next page)

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Table 1: The genera included in previous datasets (Meng et al. 2004, Asher et al. (2005) and Rose et al. (2008) versus the genera and species included in the present study. The new dataset adds a member of every extant lagomorph genus (and multiple species for genera that are polyspecific).

Genera incl. in previous datasets	Genera and species incl. in present study
Lepus	*Lepus californicus* *Lepus timidus* *Lepus europaeus* *Lepus americanus*
Oryctolagus	*Oryctolagus cuniculus*
Sylvilagus	*Sylvilagus bachmanii* *Sylvilagus audubonii*
	Brachylagus idahoensis
	Bunolagus monticularis
	Caprolagus hispidus
	Pentalagus furnessi
	Poelagus marjorita
	Romerolagus diazi
	Pronolagus crassicaudatus
Ochotona	*Ochotona pallasi* *Ochotona princeps*
zzMimolagus	*zzMimolagus*
zzGomphos	*zzGomphos*
zzMimotona	*zzMimotona*
zzPalaeolagus	*zzPalaeolagus*
zzProlagus	*zzProlagus sardus*
Tupaia (outgroup for Glires)	*Tupaia (outgroup for Glires)*

Table 2(on next page)

The constraints and calibrations placed on clades

This ensures that the reconstructed topology and divergence time estimates of our tree are more concordant with published molecular phylogenies.

Table 2: The constraints and calibrations placed on clades so that the reconstructed topology and divergence time estimates of our tree is more concordant with published molecular phylogenies.

Constraint	Taxa	Divergence calibration
ingroup	All taxa bar *Tupaia*	N/A
Lepus	*L. californicus, L. timidus, L. europaeus, L. americanus*	4.03 - 5.90 (Matthee et al. 2004)
Ochotona	*O. pallasi, O. princeps*	23.31 - 39.26 (Matthee et al. 2004)
Sylvilagus	*S. bachmanii, S. audubonii*	2.43 - 6.65 (Matthee et al. 2004)
Leporids	All *Lepus* sp., *B. idahoensis, B. monticularis, C. hispidus, N. timminsi, P. furnessi, P. marjorita, R. diazi, P. crassicaudatus, O. cuniculus, S. audubonii, S. bachmanii*	N/A
Clade_one	*O. pallasi, O. princeps, P. sardus*	N/A
Clade_two	*N. timminsi, P. marjorita, P. crassicaudatus*	N/A
Clade_three	*C. hispidus, O. cuniculus, B. monticularis, P. furnessi*	N/A
Clade_four	*R. diazi, B. idahoensis, S. audubonii, S. bachmanii*	N/A