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Skull and lower jaw development and asymmetry of Pond bats (*Myotis dasycneme*)

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Summary

This study used 2D landmark based geometric morphometrics to study developmental patterns of the cranium and lower jaw of the Pond bat (*Myotis dasycneme*), of which not much was known before. Furthermore, this study found that there are left-right asymmetries in the shape of the cranium and lower jaw of pond bats that may be linked to echolocation. Lastly this study found that there are differences in the shape of the cranium and mandible between males and females of the Pond bat that may be linked to dietary differences between the sexes.

Key words: Pond bat, *Myotis dasycneme*, development, asymmetry, geometric morphometrics, cranium, mandible

Introduction

Bats

Bats, with around 1400 species, make up around 1/5 of the mammal species on this world, only outnumbered by rodents (Burgin et al. 2018). All bats belong to the order of the Chiroptera, which is monophyletic (Gunnell and Simmons 2005). The name of the order, Chiroptera, is derived from the Greek words for 'hand' and 'wing'. The Chiroptera are classically divided into two suborders, the Megachiroptera and the Microchiroptera, as the name already suggests, the 'big bats' and the 'small bats'. Recent studies, however, have shown that those suborders are not monophyletic and proposed two new suborders, the Yinpterochiroptera and the Yangochiroptera (Sadier et al. 2020). These two monophyletic suborders are, however, not widely accepted. The phylogeny of the Chiroptera with its suborders can be seen below (Fig 1). The oldest fossil evidence of bats dates back to the Eocene of North America, approximately 51 million years ago and belongs to the newly described species *Icaronycteris gunelli* (Gunnell and Simmons 2005; Rietbergen et al. 2023).

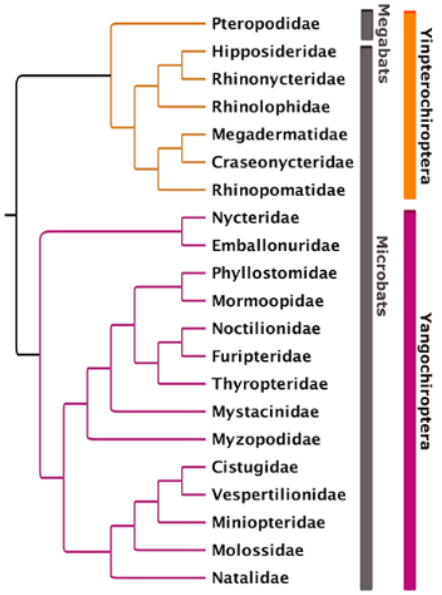

Figure 1. Phylogeny Chiroptera (Sadier et al. 2020)

Within the order of the Chiroptera, there is a huge diversity, for example in the size, head and body size vary from 25 to 406 mm (Walker and Nowak 1999). However, there are various other variations and interesting characteristics that make the Chiroptera an interesting research subject. For example, bats roost upside down and even give birth upside down. Also, many bat species hibernate. Furthermore, the diet varies enormously among those 1400 species, from insects or fruits to even meat, fish and blood (Freeman

1998; Walker and Nowak 1999). The way some bat species search for their food is also an interesting subject, namely by using echolocation.

Bats are not only rich in species number and ecomorphological diversity between those species, they are also present in most parts of the world. They even have one of the largest habitat ranges known to mammals, only humans and rodents have a bigger distribution (Thomas and Suthers 1972; Walker and Nowak 1999). Bats have been found all across the globe, except for the polar region and on some isolated oceanic islands (Wund and Myers 2005).

The diversity within bats is very high, so is also the research done on them. The research ranges from their habitat use (Krusic et al. 1996; Agosta 2002), to their role in the dispersal of seeds (Villalobos-Chaves and Rodríguez-Herrera 2021) or their role in the spread of diseases (Allocati et al. 2016; Letko et al. 2020).

Mandible and cranium characteristics and development in bats

Frequently researched body parts in bats, are the cranium and mandible (Arbour et al. 2019; Shi et al. 2020). Research has shown that the type of diet is reflected in the morphology of the cranium and mandible (Ghazali and Dzeverin 2013). Between different genera or families of bats with different diets morphological differences have been observed (Freeman 1998, 2000; Van Cakenberghe et al. 2002). This trend in cranium and lower jaw adaptation to diet is not unique to bats but is in line with what is known for other mammalian orders (eg. rodents (West and King 2018; Karamani et al. 2022) and humans (Holmes and Ruff 2011; Hoover and Williams 2016)).

However, the lower jaw is highly plastic and can change during a lifetime. In mice it has been shown that hardness of foods not only changes the morphology of the mandible within a lifetime (Anderson et al. 2014). An interesting example of this is a study by Renaud and Auffray (2010). This study showed that house mice, *Mus musculus domesticus*, fed with a different hardness of food, showed differences in the mandible form and shape after a few months. One group of mice was given solely very soft food, the other group was given solely hard foods, and this difference in food hardness influenced the mandible's form and shape (Renaud and Auffray 2010). Plasticity of the mandible has also been shown in other animals, such as the dwarf fat-tailed jerboa, *Pygeretmus piumilio* (Kryštufek et al. 2019) and Japanese macaques, *Macaca fuscata* (Kamaluddin et al. 2019).

Another example of plasticity or adaptation of the mandible to different or changing hardness of food, is the red squirrel, *Sciurus vulgaris*, in England. Dr Phil Cox and PhD candidate Kim Chandler are investigating the mechanisms behind the changing mandible of the red squirrel as a result of the increasing number of peanuts this species has been fed over the last years, this is ongoing research (Cox et al. 2020; Ashworth 2022).

Not only the mandible reflects the diet, the skull does the same. The cranium responds to selective pressures related to the diet (Freeman 2000; Van Cakenberghe et al. 2002). Bats have a 'diverse cranial morphology that appears to be related to changes in bite force' (Nogueira et al. 2009; Ramírez-Fráncel et al. 2021). However, the cranium is less plastic than the mandible, and will respond less to dietary changes. This is because the cranium and especially the upper jaw is more strongly related with echolocation, in contrast to the lower jaw which is more strongly related to dietary changes (Arbour et al. 2019).

Echolocation

Echolocation is the technique bats use to determine the location of objects, such as prey, using the reflection of sound. It is used by bats, dolphins and other animals. They use it to 'forage, avoid obstacles and orient in the absence of light' (Thomas et al. 2004). The sounds bat use for echolocation are for the most part not hearable for the human ear, which can hear from about 20 Hz to 20 kHz (Purves et al. 2001), bat calls are about 12 to 200 kHz. The echolocation calls of bats last from 0.3 to 300 ms (Whitlow 1997) Within the echolocation of bats there is quite some diversity in the types of calls they make. The Pond bat, used in this research, uses 'Short, broad-band, dominated by fundamental harmonic echolocation' (Jones and Teeling 2006).

Asymmetries

Not only is the cranium more strongly related with echolocation than the lower jaw, there also have been found asymmetries in the cranium that have been linked to echolocation. Parés-Casanova and Otin (2020) found directional asymmetry in skulls of the species *Carollia perspicillata*, a fruit eating bat. They found asymmetry in the neurocranial part on the dorsal aspect of the skull (Parés-Casanova and Otin 2020).

There are, however, different forms of asymmetry, namely directional asymmetry, fluctuating asymmetry, and antisymmetry (Cole et al. 2020; Manthey and Ousley 2020). Directional asymmetry is 'a systematic differentiation between the left and right sides for structures with bilateral symmetry or a systematic differentiation among repeated parts for complex symmetry' (Budečević et al. 2022). Fluctuating asymmetry, on the other hand is defined as 'non-directional variation between left and right sides of a bilateral character and it may arise as a result of an inability to control development under genetic or environmental stress' (Russell and Bauer 2005). Lastly, antisymmetry, which is defined as 'the condition where right-sided and left-sided—or dextral and sinistral—forms are equally common within a species, as seen in the major claws of lobsters and male fiddler crabs, the side to which the upper mandible crosses in most crossbill finches, or the spiral orientation of palm-tree trunks' (Palmer 2005).

The found asymmetry in the cranium by Parés-Casanova and Otin (2020) can therefore be classified as a directional asymmetry. This asymmetry linked to echolocation is not exclusive to bats: in Cetaceans cranial asymmetries have been widely found and described and are also linked to echolocation (Yurick and Gaskin 1987; Coombs et al. 2020; Lanzetti et al. 2022). Furthermore, a similar phenomenon is seen in owls, specifically in Tengmalm's Owl, *Aegolius funereus*, where there is a cranial asymmetry that is linked to directional hearing (Norberg 1978).

Pond bat

Within the Yangochiroptera, the family of the Vespertilionidae, commonly known as evening or vesper bats, is placed. As the name already suggests these bats are active at night. The species *Myotis dasycneme*, Boie 1825 is member of this family. *Myotis dasycneme* is commonly known as the pond bat, named after the habitat it lives in. This bat occurs in lowland areas with a cool and humid climate. It prefers open landscapes with large water surfaces, that are preferably calm (Van de Sijpe and Holsbeek 2007). This is because the pond bat is an insectivorous bat that uses water trawling with echolocation as its main hunting technique (van de Sijpe and Holsbeek 2007; Haarsma and Tuitert 2009; Ciechanowski and Zapart 2012). The pond bat also occurs in Europe, but is one of the rarer species and is more threatened than other species (Haarsma and Tuitert 2009). In 2009, the Netherlands had a total of 114 pond bat roosts, of which 59 were maternity roosts and 65 were male roosts (Haarsma and Tuitert 2009).

The pond bat hibernates, like many other bats, for a large part of the year, approximately from November up to and including February. After that the females migrate to the maternity roosts where they give birth and care for their young and then migrate back again to their hibernacula. Males follow a similar pattern (Fig 2), (Haarsma and Tuitert 2009). They, however, live in male and mating roosts, apart from the females.

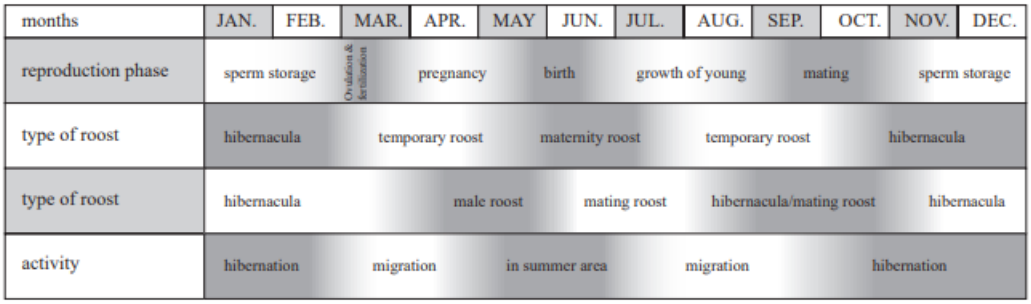

Figure 2. Pond bat behaviour over the year (Haarsma and Tuitert 2009)

Pond bat diet

A recent study investigated the diet of the pond bat. This study, (Haarsma et al. 2023), looked at the diet over different seasons, reproductive stadia and sexes in Dutch pond bats.

The diet of all pond bats consists mainly of insects from the families of the Chironomidae (nonbiting midges/lake flies) and Noctuidae (owlet moths). Furthermore, the research by Haarsma et al (2023) discovered that there are significant differences in the proportion of Chironomidae pupae in the diet of female pond bats of different reproductive stadia. Pregnant females ate the highest proportion of pupae, followed by the lactating females. The females that were not reproductively active ate the lowest proportion of pupae. The proportion of pupae in the diet of males was not included. The research also discovered that pregnant females had a less diverse diet and ate smaller and lighter prey.

Aims of this research

The main aims were to (I) to examine a large number of skull and lower jaw samples of *M. dasycneme* to learn more about the development from baby to adult in the pond bat (II) to test whether sexes differ in their development in relation to their differences in diet and (III) to test for asymmetries in either the shape of the mandible or cranium in relation to echolocation.

Material

For this research the cranium and lower jaw material of over 400 pond bats was available from the mammal collection of Naturalis Biodiversity Center in Leiden, the Netherlands. Out of these 400 specimens, 339 were selected based on completeness, see appendix I. Here, the sex and developmental stage of each specimen are mentioned, as well as which part(s) of the specimen were studied.

Of most, about 2/3, of the specimens the sex is known, indicated with F for females, M for males and X for the specimens of which the sex is unknown.
Furthermore, all specimens are categorised for their developmental stage. 1 for babies, 2 for juveniles and 3 for adults, developmental stage will also be abbreviated as “dev. stage” in this research.

The babies, developmental stage 1, are characterised by the presence of deciduous teeth and the coronoid process, mandibular condyle and angular process are not very pronounced yet in the lower jaw. Because the cranium is not completely fused yet, no skulls were available from this category to use in research. The Juveniles, developmental stage 2, are characterised by the presence of adult teeth, but in the lower jaw the canine is still erupting. The adults, developmental stage 3, are characterised by permanent teeth and fully erupted canines. Cases for which the developmental stage could not be determined, were classified as stage 0.

Only specimens that were intact or only had minor damage were considered suitable for this research. Therefore, for some specimens only the mandible, or one side of it, or the cranium was used. A complete overview, including which parts of which specimens where photographed and landmarked can be found in Appendix I.

All material is currently stored in the collection of Naturalis Biodiversity Center (Leiden).

Methods

In order to accomplish the three main aims of this research and to be able to look at the development and morphology of the pond bat cranium and mandible, 2D landmark-based geometric morphometric techniques were used. This was done on the labial side of the mandible, the ventral side of the cranium and the dorsal side of the cranium.

Digitisation

The first step in this research was to photograph all material. This was done using the Zeiss Stereo Discovery Microscope V20 with attached camera and AxioVision software in either the laboratories, Lab 03.01, or the collection lab, CoLa 1, of Naturalis biodiversity Center. Each image included a 2000 µm scalebar and the images were stored in JPG format on a shared google drive folder.

In order to make sure all pictures were taken as equally as possible, specimens were placed in sterilized black sand in a 9 cm plastic Petri dish under the microscope and the following guidelines have been maintained. The black sand was used for stability and to provide a contrasting background to the bone material.

For the mandibles, the specimens were placed with the labial side up on the sand, laying the body of the mandible as horizontal as possible and the ramus pointing slightly upwards. In the case the mandible was still fully intact, the side that was not being photographed was buried in the black sand to be able to photograph the other side in the right position. For the skulls, the specimens were placed as horizontal as possible on top of, or slightly buried in the black sand, with the ventral or dorsal side of the skull pointing upwards to the camera.

Geometric morphometrics

When all pictures were taken, the next step was to landmark them. In order to do so and be able to compare all of them, they were put in the same orientation. This was done by mirroring about half of the pictures. Then all pictures were, per category, combined in one TPS file using TPSutil32, version 1.82. When the TPS files were made, landmarks were placed using TPSdig264, version 2.32 64 bits.

The used landmarks can be seen below (Fig 3, 4 and 5). The complete list of exact definitions of each landmark can be found in Appendix II. The landmarks for the mandible were adapted from Jansky et al., (2016) and Sztencel-Jablonka et al., (2009), the landmarks for the ventral side of the cranium were adapted from Sztencel-Jablonka et al., (2009) and the landmarks for the dorsal side of the cranium were adapted from Parés-Casanova and Otin, (2020) and Sztencel-Jablonka et al., (2009). For the analysis of the mandibles of specimens that were marked as babies, a subset of the landmarks was used. This was done since the babies didn't have adult teeth yet. The subset of the landmarks used consisted of landmarks 1 – 9, 19 and 20. The landmarks 10-18 were placed randomly and later removed in MorphoJ in order to be able to compare between babies and the juveniles and adults.

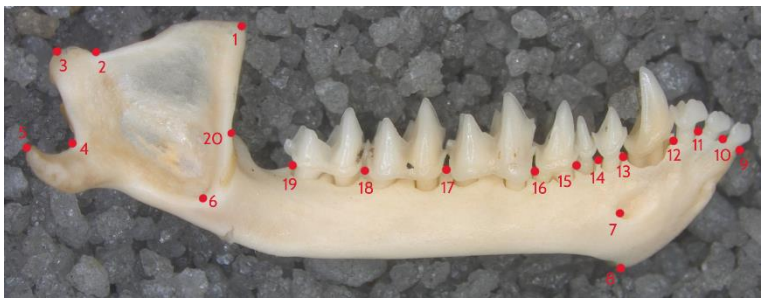

Figure 3. Landmarks placed on the mandible Pond bats. Photograph by Daniëlle Snijders

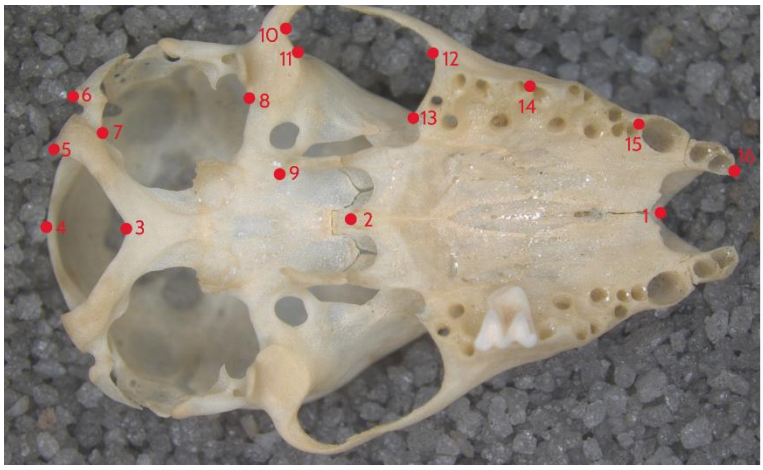

Figure 4. Landmarks placed on the ventral side of the cranium of an adult Pond Bat. Photograph by Daniëlle Snijders

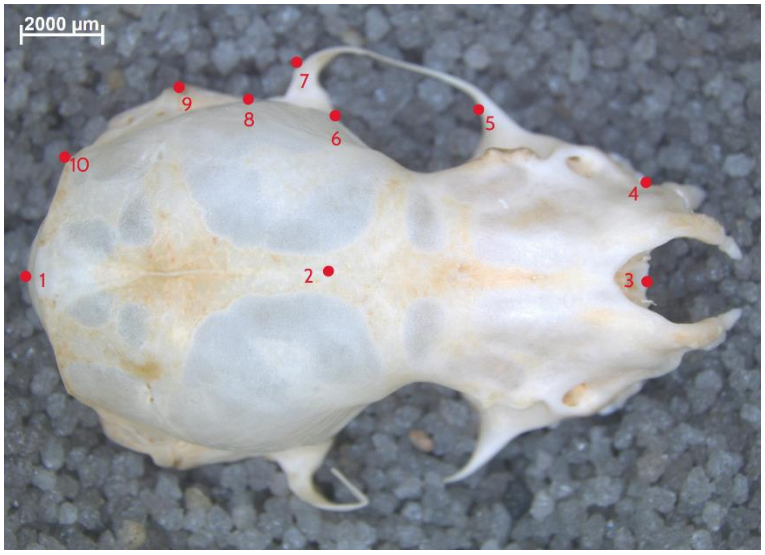

Figure 5. Landmarks placed on the dorsal aide of the cranium of an adult pond bat. Photograph by Daniëlle Snijders

When all landmarks were placed, analyses were done using MorphoJ version 1.08.01 (Klingenberg 2011).

In MorphoJ the material was classified in categories per sex, developmental stage and side. A Procrustes fit aligned by the principal axis was made, a covariance matrix was generated and a wireframe was created. After that a Principal Component Analysis, PCA, and Procrustes ANOVA were performed. The Procrustes ANOVAs were performed on the data per developmental category and only included specimens of which the sex was known. All Procrustes coordinates can be found in appendix III.

Results

Mandible

When comparing all mandible specimens from all three developmental categories, baby, juvenile and adult, the following results is seen. The biggest variation between the different developmental stages is along principal component 2, PC2, explaining around 25% of the variance (Fig. 6). Along PC2 the babies have lower values, seen as light blue (Fig. 7) and the adults have higher values, seen as dark blue.

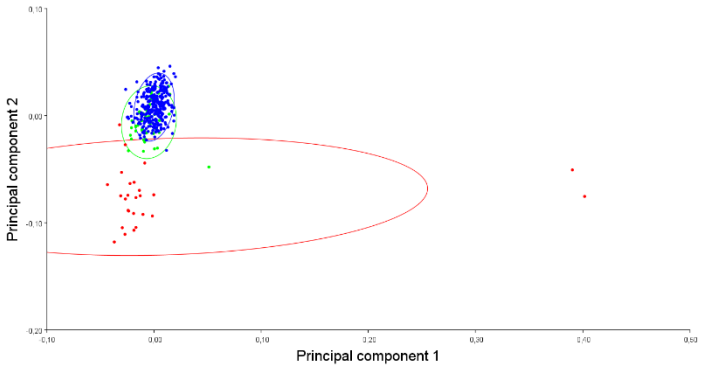

Figure 6. PCA 1 vs 2 of the mandible of pond bats. N= 516 Red = babies, green = juveniles and blue = adults

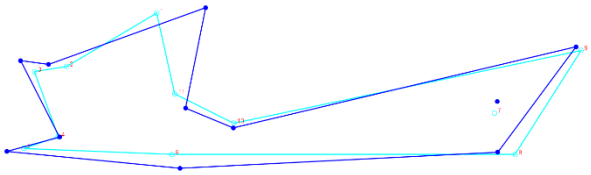

Figure 7. Shape changes in the mandible of pond bats along PC2

When only looking at the mandible of the specimens classified as babies, a significant difference (P=0.0065) in the shape between the left and the right side is seen.

When looking at the mandibles of juvenile specimens, a significant difference (P=0.0003) in the shape between left and right side is seen. The seen difference is along PC1, explaining around 37% of the variance. The left side has a greater variation in the shape change of PC1, where the right side shows less variation (Fig 8). The shape change of PC1 can be seen below (Fig 9).

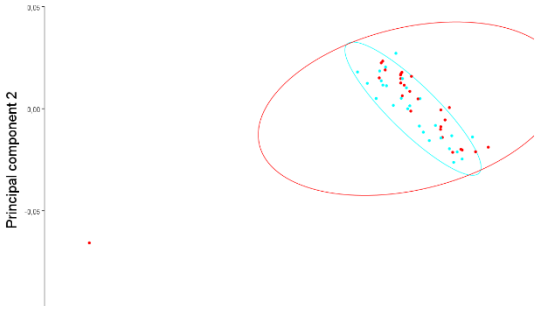

Figure 8. PCA 1 vs 2 of the juvenile mandible of pond bats. N= 104 Red = left side, blue = right side

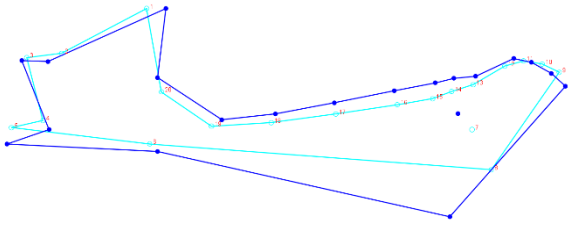

Figure 9. Shape changes in the juvenile mandible of pond bats along PC1

When looking at the mandibles of adult specimens, a significant difference (P<0.0001) in the shape between the sexes is seen, as well as significant differences (P<0.0001) in both shape and size between the left and right sides. The difference between the sexes is seen along PC2, which explains around 10% of the variance (Fig 10). The males are towards the higher values, seen as dark blue (Fig 12), and the females are towards the lower values, seen as light blue. The differences between left and right are seen along PC1, explaining around 24% of variance (Fig 11). The left side has lower values along PC1, seen as light blue (Fig 13), and the right side has higher values along PC1, seen as dark blue.

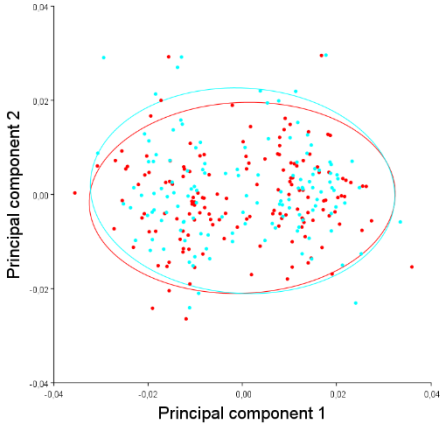

Figure 10. PCA 1 vs 2 of the adult mandible of Pond bats
N= 326 Red = female, blue = male

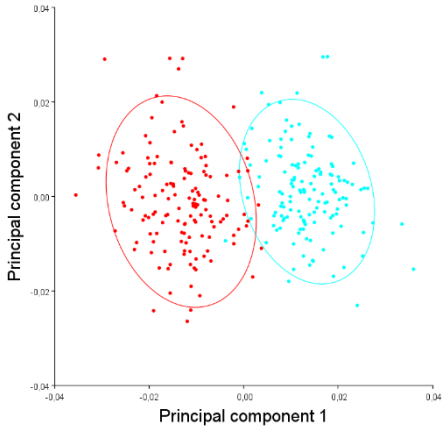

Figure 11. PCA 1 vs 2 of the adult mandible of Pond bats.
N= 326 Red = left side, blue = right side

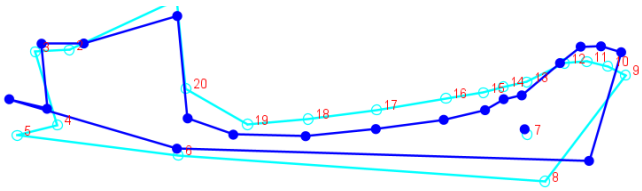

Figure 12. Shape changes in the adult mandible of Pond bats along PC2

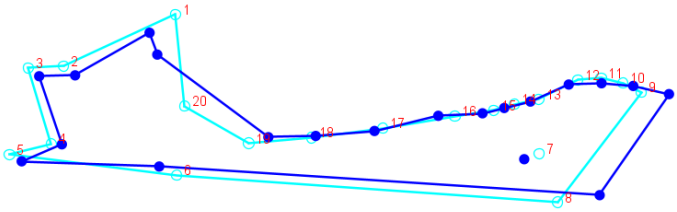

Figure 13. Shape changes in the adult mandible of Pond bats along PC1

Ventral side of the cranium

When looking at the ventral side of the cranium of juvenile specimens, a significant difference (P=0.0162) in the shape between left and right side is seen.

When looking at the ventral side of the cranium of adult specimens, a significant difference in the size (P=0.0258) and shape (P=0.0048) between the sexes is seen, as well as significant differences (P=0.0014) in shape between the left and right sides.

The differences between the sides are mainly along PC2, explaining around 18% of variation (Fig. 14). The left side has higher values, seen as dark blue (Fig 15), and the right side has lower values, seen as light blue.

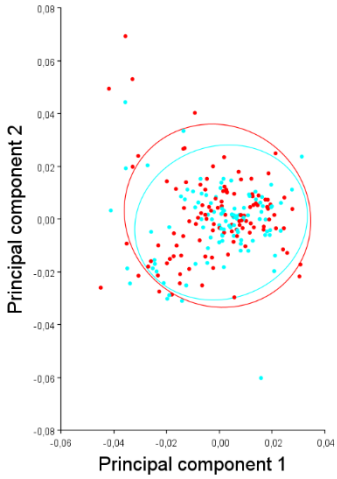

Figure 14. PC 1 vs 2 in the ventral view of the cranium of adult pond bats. N= 157 Red = left side, blue = right side

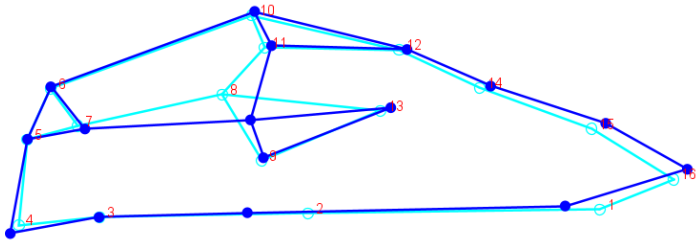

Figure 15. Shape changes in the ventral side of adult craniums of Pond bats along PC2

Dorsal side of the cranium

When looking at the dorsal side of the cranium of juvenile specimens, a significant difference (P=0.0294) in the shape between the left and right side is seen. However, only 18 skulls were available of juvenile pond bats.

When looking at the dorsal side of the cranium of adult specimens, a significant difference (P=0.0002) in the shape between the left and right side is seen. No significant differences in size of between sexes was found. The differences between the sides are mainly along PC2, which explains around 15% of the variation (Fig 16). The left side has higher values, seen as dark blue (Fig 17), and the right side has lower values, seen as dark blue.

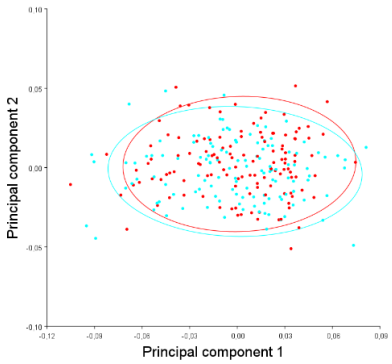

Figure 16. PC 1 vs 2 in the dorsal view of the cranium of Pond bats.
N= 157 Red = left side, blue = right side

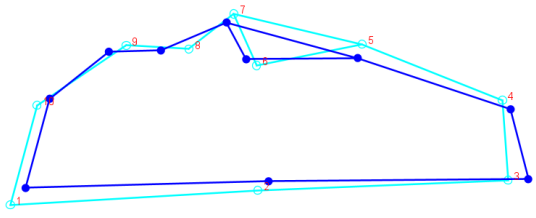

Figure 17. Shape changes in the dorsal side of adult craniums of Pond bats along PC2

Discussion

Within the pond bat *Myotis dasycneme*, there is a relatively big difference in body size between males and females, where the females are around 20% heavier than males (Stevens and Platt 2015; Haarsma et al. 2023). This also explains the found significant size differences between male and female cranium and lower jaw specimens investigated.

The lower jaw develops a more pronounced coronoid process from baby to juvenile

When looking at the development of the lower jaw, it can be seen that most development happens in the phase from baby to juvenile, most variation is between those developmental stages. Between the developmental stages of juvenile and adult there isn't as much difference and variation.

The biggest developmental difference that is seen is in the lower jaw is in the coronoid process, mandibular condyle and the angular process. Those three structures become more pronounced in the development. In babies these three structures are still very small and can barely be differentiated from the ramus base of the mandible. When fully developed, the coronoid process, mandibular condyle and the angular process are pronounced and are three clear structures. Of the three structures, the coronoid process is the one that changes the most in the development (Fig 7).

All pond bats have asymmetric mandibles

One of the results that stands out is the difference in shape and size between the left and right side of the mandible as reflected in the PCA (Fig 8 and 10). There is a significant ($P < 0.0001$) difference in both shape and size for adults and a significant difference in shape for juveniles ($P=0.0003$) and babies ($P=0.0065$). Although there was a significant difference in shape between left and right was seen in the mandibles of baby specimens, it has to be noted that only 49 specimens, left and right together, were available.

This raised the question if this could be a human error in the positioning of the mandibles for photography or the placing of the landmarks. However, further investigation showed that the shape difference found by the PCA and Procrustes ANOVA are already visible with the naked eye. This, combined with the facts that the protocols for placing the mandibles for photography and placing the landmarks have been followed meticulously and that similar asymmetries have been found in the cranium, make human error very unlikely in this case.

There are a few other possibilities to explain these deviations from perfect left-right symmetry. The first one is that bats are born with this asymmetry. Since the asymmetry was already present in the babies it is likely that this is the case. The second explanation has to do with echolocation and directional hearing. In other bats, but also in other species cranial asymmetries have been found and linked to directional hearing and echolocation (Yurick and Gaskin 1987; Coombs et al. 2020; Parés-Casanova and Otin 2020; Lanzetti et al. 2022). It is likely that the asymmetries in the cranium are reflected in the mandible as well. However, the shape of the mandible is more related to the diet than to echolocation (Arbour et al. 2019). Another possibility is that bats have a side preference for chewing which has an effect on mandibular symmetry. Similar observations have been made in humans (Martinez-Gomis et al. 2009; Tiwari et al. 2017) and there is anecdotal evidence, (Personal communication A-J Haarsma 2023), that bats also have a side preference. Although this is an alternative explanation and this falls outside the scope of this current research, it would be interesting future research to look into the mandibular asymmetries and potential side preferences in relation to chewing in bats.

Mature male and female pond bats have differently shaped mandibles

When looking at the other differences in the mandible, the significant ($P<0.0001$) difference in shape between the sexes in adult specimens stands out. This shape difference could not be detected in the juvenile specimens. A logical explanation for this shape differences between the sexes that arises in adulthood is the difference in diet. A recent study, (Haarsma et al. 2023), found a significant difference in diet composition between males and females that increased when females where pregnant or lactating, which is a large part of their adult life. The found differences between the diets were mainly in the higher percentage of pupae the females ate. That differences in diet, especially in the hardness of diet can have a quick and significant effect on the shape of the mandible has already been shown in mice (Renaud and Auffray 2010) so therefore it is very likely that the effect is present in bats as well.

All pond bats have asymmetric ventral craniums due to differently positioned auditory bulla

The cranium was, analysed from two different viewpoints (ventral and dorsal). The ventral side shows the most details and structures, including structures that are related to hearing. Therefore, it was expected that if there were cranial asymmetries that were linked to directional hearing or echolocation, they should be found here.

This expectation turned about to be correct. When looking at the asymmetry in the skull of the adult pond bats, the biggest difference in shape between left and right is around landmark 8, defined as the maximum curvature of the posterior margin of the zygomatic arch. Specifically, the biggest difference is the distance between landmarks 7 and 8 and, if you were to draw a line between them, the angle of that line. This difference is at the location of the auditory bulla. The auditory bulla is a hollow bony structure on the ventral, posterior end of the skull and holds parts of the middle and inner ear. It is a structure that sits loose in the skull and was not present in a large percentage of available skulls. For this reason, landmarks have been chosen that could be placed on images of both specimens that still had the bulla and the specimens were the bulla was not present anymore. The main shape difference between left and right was found between landmarks 7 and 8, which represents the size and angle of the auditory bulla or the place these were located in respectively.

All female and male pond bats have differently sized and shaped ventral craniums

Furthermore, at the ventral side of the cranium, a significant difference in shape between the sexes is seen in both juvenile and adult pond bats, as well as a significant size difference in the adults. The size difference of the adults is again easily explained by the size difference between males and females (Stevens and Platt 2015; Haarsma et al. 2023). The shape difference between the sexes is a little harder to explain. A potential explanation could be the same as in the mandible, namely the difference in diet, however, the cranium is more strongly related to echolocation than to diet (Arbour et al. 2019). Although there also was a significant difference in the shape of the ventral side of the cranium of the juvenile Pond bat between the sexes, it has to be noted that there were only 15 specimens of juveniles available. This is a small sample size, especially in contrast with the adults where over 150 specimens were available.

All pond bats have differently shaped dorsal craniums

Finally, at the dorsal side of the cranium, significant differences in shape between the left and right sides are seen in both juveniles ($P=0.0294$) and adults ($P=0.0002$). The observed differences in shape are located mainly around landmarks 8 and 9, the neurocranium. When comparing our results with those of Parés-Casanova and Otin (2020) we found the following; The authors state that ‘differences were located mainly on the neurocranium length, and orbital length and width’ (Parés-Casanova and Otin 2020). This is a very similar result to what is found in this here.

However, like with the ventral side of the cranium, it again has to be noted that although significant differences in the shape of the left and right side of the dorsal cranium have been found in juveniles, only around 15 specimens of juvenile skulls were available for this research.

Extreme specimens are extreme in both analyses of the cranium but not in the analyses of the mandible

Finally, when looking at the different specimens in the three different investigated parts of the skull and lower jaw, two things stood out. First of al when comparing the analyses of the ventral and dorsal side of the cranium, it is seen that the specimens that have extreme values in the PCA of the ventral side also have extreme values in the PCA of the dorsal side. This was also expected since the two datapoints in the two different PCA's represent the same specimen.

The second thing that stood out was that when comparing the specimens with extreme values in the PCA's of the cranium in only a few cases they had extreme values in the analyses of the mandible. So, the 'extreme' specimens of the mandible were not necessarily 'extreme' in their cranium.

Conclusions

This research applied 2D landmark based geometric morphometrics, applied to the mandible, ventral view of the cranium and the dorsal view of the cranium of juvenile and adult pond bats (*Myotis dasycneme*), with the aim to answer the following questions: (I) How do the skull and lower jaw develop from baby to adult? (II) Are there sex-related differences in the skull and lower jaw development and (III) are there asymmetries in either the shape of the cranium or lower jaw? Based on these results, the following conclusions can be drawn.

First of all, the development could be best seen in the mandible since there were specimens from all developmental categories available in relatively big sample sizes. Within the development of the mandible, most developmental changes happened at the ramus base of the mandible and especially in the coronoid process.

Secondly, there are shape asymmetries in the left and right mandible of both juveniles and adults, as well as size differences between the left and right sides of the adult mandible. This difference between the left and right sides requires more in-depth investigation.

Thirdly, we found a shape difference of the lower jaw between adult male and female pond bats. The shape differences between the males and females may be linked to the differences in diet, as shown by (Haarsma et al. 2023).

Furthermore, there is a shape asymmetry between the right and left side of the cranium from the ventral view, this asymmetry is only found in adult specimens and is not present in the juveniles. There is a shape difference between male and female juveniles as well as a size and shape difference between the adult males and females. Because of the location of the asymmetry in the adult specimens, the auditory bulla, this asymmetry might be linked to echolocation and directional hearing. The differences between the sexes could again be, explained by a difference in the diet.

Lastly, there are shape asymmetries between the right and left side of the cranium from the dorsal view, this asymmetry is found in both juvenile and adult pond bats. This asymmetry might also be linked to echolocation and directional hearing in bats, confirming earlier research with similar results (Parés-Casanova and Otin. 2020).

In conclusion, after previous research by Parés-Casanova and Otin (2020) where bat cranial asymmetries were found on the dorsal side that are hypothesised to be related to echolocation and directional hearing, this research provides more evidence for this hypothesis. This research includes more specimens and also the ventral side of the skull, where landmarks were placed and a shape difference could be pinpointed to the auditory bulla, which makes for more compelling evidence that this asymmetry is indeed linked with echolocation and directional hearing. This study also concludes that there is a shape difference between the sexes that is likely causes by a difference in diet as discovered by Haarsma et al. (2023).

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Appendix I – Pond bat (*M. dasycneme*) skull and lower jaw specimens studied in the mammal collection of Naturalis Biodiversity Center

All skull and lower jaw material used in this research from the mammal collection of Naturalis biodiversity center, Leiden, the Netherlands. Of most specimens the sex is known, indicated with a f for females, m for males and and x for the specimens of which the sex is unknown. All specimens are categorized for their developmental stage, abbreviated as 'Dev. Stage', 1 for babies, 2 for juveniles and 3 for adults. Cases for which the developmental stage could not be determined were classified as stage 0.

Only specimens that were intact or had minor damage were considered suitable for this research, therefore for some specimens only the mandible or one side of it or the cranium was used. Which parts were used can be seen below, indicated with an x in the column of the part that was used. For the mandible there is made a distinction between left, L, and right, R. For the cranium there is made a distinction between the ventral side, V, and the dorsal side, D.

Collectionnumber	Information		Mandible		Cranium	
	Sex	Dev. stage	L	R	V	D
RMNH.MAM.11067.a	m	3	x	x	x	x
RMNH.MAM.11096.a	m	3	x	x	x	x
RMNH.MAM.11108.a	f	3	x	x	x	x
RMNH.MAM.11109.a	m	3		x	x	x
RMNH.MAM.11149.a	f	3	x	x	x	x
RMNH.MAM.11154	f	3	x	x	x	x
RMNH.MAM.11713.a	f	3	x	x	x	x
RMNH.MAM.12246.a	f	3	x	x		
RMNH.MAM.12247.a	f	3	x	x	x	x
RMNH.MAM.12248.a	f	3	x	x	x	x
RMNH.MAM.12250.a	f	3	x			
RMNH.MAM.12251.a	f	3	x	x	x	x
RMNH.MAM.12252.a	f	3	x	x	x	x
RMNH.MAM.12253.a	m	3	x	x	x	x
RMNH.MAM.12254.a	f	3	x	x	x	x
RMNH.MAM.12255.a	m	3	x	x	x	x
RMNH.MAM.12256.a	f	2	x	x		
RMNH.MAM.12257.a	f	3	x	x	x	x
RMNH.MAM.12258.a	m	3	x	x	x	x
RMNH.MAM.12259.a	f	3	x	x	x	x
RMNH.MAM.12260.a	f	0	x	x		
RMNH.MAM.12261.a	f	3	x	x	x	x
RMNH.MAM.12262.a	f	2	x	x	x	x
RMNH.MAM.12263.a	m	0	x	x	x	x
RMNH.MAM.12264.a	m	3	x	x	x	x
RMNH.MAM.12265.a	m	3	x		x	x
RMNH.MAM.12266.a	m	3	x	x	x	x
RMNH.MAM.12267.a	f	3	x	x	x	x
RMNH.MAM.12268.a	f	3	x	x	x	x
RMNH.MAM.12269.a	f	3	x	x	x	x
RMNH.MAM.12270.a	f	3	x	x	x	x
RMNH.MAM.12271.a	f	3	x	x	x	x
RMNH.MAM.12272.a	m	3	x	x	x	x
RMNH.MAM.12273.a	m	3	x	x	x	x
RMNH.MAM.12274.a	f	3	x	x	x	x
RMNH.MAM.12275.a	m	3	x	x	x	x
RMNH.MAM.12276.a	f	3	x	x	x	x
RMNH.MAM.12277.a	m	3	x	x	x	x
RMNH.MAM.12278.a	m	3	x	x	x	x
RMNH.MAM.12279.a	f	3	x	x	x	x
RMNH.MAM.12280.a	f	3	x	x	x	x
RMNH.MAM.12281.a	f	3	x	x	x	x
RMNH.MAM.12282.a	m	0			x	x
RMNH.MAM.12283.a	f	3	x	x	x	x
RMNH.MAM.12284.a	f	3	x	x	x	x
RMNH.MAM.12285.a	m	3	x	x	x	x
RMNH.MAM.12286.a	f	3	x	x	x	x
RMNH.MAM.12287.a	f	3	x	x	x	x
RMNH.MAM.12288.a	f	3	x	x	x	x
RMNH.MAM.12289.a	f	3	x	x	x	x

RMNH.MAM.12291.a	f	3	x	x	x	x
RMNH.MAM.12292.a	f	3	x	x	x	x
RMNH.MAM.12293.a	f	3	x	x	x	x
RMNH.MAM.12294.a	m	3	x	x	x	x
RMNH.MAM.12295.a	m	3	x	x	x	x
RMNH.MAM.12296.a	f	3	x	x	x	x
RMNH.MAM.12297.a	f	3	x	x	x	x
RMNH.MAM.12298.a	f	3	x	x	x	x
RMNH.MAM.12299.a	f	3	x			
RMNH.MAM.12300.a	f	3	x	x	x	x
RMNH.MAM.12301.a	f	3	x	x	x	x
RMNH.MAM.12302.a	f	3	x	x	x	x
RMNH.MAM.12303.a	f	3	x	x	x	x
RMNH.MAM.12304.a	f	3	x	x	x	x
RMNH.MAM.12305	f	3	x	x	x	x
RMNH.MAM.12306.a	m	3	x	x	x	x
RMNH.MAM.12308.a	f	3	x	x	x	x
RMNH.MAM.12310.a	f	3	x	x	x	x
RMNH.MAM.12311.a	m	3	x	x	x	x
RMNH.MAM.12312.a	m	3	x	x	x	x
RMNH.MAM.12313.a	m	3	x	x	x	x
RMNH.MAM.12392.a	f	2	x		x	x
RMNH.MAM.13291.a	m	3	x	x	x	x
RMNH.MAM.1545.a	m	3	x	x	x	x
RMNH.MAM.16035.a	m	2	x	x	x	x
RMNH.MAM.16036.a	f	1	x	x	x	x
RMNH.MAM.17364	x	3	x	x	x	x
RMNH.MAM.1800.a	m	3	x	x	x	x
RMNH.MAM.2610.a	m	3	x	x	x	x
RMNH.MAM.27357	x	0			x	x
RMNH.MAM.27358	x	2	x	x		
RMNH.MAM.28506	f	3	x	x	x	x
RMNH.MAM.28757.a	m	3	x	x	x	x
RMNH.MAM.29073.a	f	3	x	x	x	x
RMNH.MAM.29074.a	f	3	x	x	x	x
RMNH.MAM.29075.a	m	3	x	x	x	x
RMNH.MAM.29076.a	m	3	x	x	x	x
RMNH.MAM.29077.a	m	3	x	x	x	x
RMNH.MAM.30755	x	3		x	x	x
RMNH.MAM.30780	x	3	x	x	x	x
RMNH.MAM.30781	f	3	x	x	x	x
RMNH.MAM.30790	x	3	x	x	x	x
RMNH.MAM.30820.a	f	3	x	x	x	x
RMNH.MAM.31347.a	m	3	x	x	x	x
RMNH.MAM.32267.a	m	2	x	x		
RMNH.MAM.32268.a	m	2	x	x		
RMNH.MAM.32288.a	m	3	x	x	x	x
RMNH.MAM.34408.a	m	3	x	x		
RMNH.MAM.3671.a	m	2	x	x	x	x
RMNH.MAM.37172.a	m	3	x	x	x	x
RMNH.MAM.37196.a	f	3	x	x	x	x
RMNH.MAM.37432	f	3	x	x	x	x
RMNH.MAM.38827.a	f	0			x	x
RMNH.MAM.4019.a	m	3	x	x	x	x
RMNH.MAM.40639.a	m	2	x	x	x	x
RMNH.MAM.40729.a	m	3	x	x		
RMNH.MAM.41303.a	m	3	x	x	x	x
RMNH.MAM.5223.a	m	3	x	x	x	x
RMNH.MAM.52807	x	2	x			
RMNH.MAM.52808	m	1	x			
RMNH.MAM.52809	x	2		x		
RMNH.MAM.52810	x	2	x	x		
RMNH.MAM.52811	x	2	x	x		
RMNH.MAM.52812	x	2	x	x		
RMNH.MAM.52813	x	0	x	x		
RMNH.MAM.52814	x	2	x	x		
RMNH.MAM.52815	x	2	x	x		
RMNH.MAM.52816	x	2	x	x		
RMNH.MAM.52817	x	2	x	x		
RMNH.MAM.52819	x	0	x	x		
RMNH.MAM.55047.c	f	3	x	x	x	x
RMNH.MAM.60367.a	f	2	x	x		
RMNH.MAM.60368.a	f	2	x	x		
RMNH.MAM.60369.a	m	2	x	x	x	x

RMNH.MAM.60370.a	m	1	x	x		
RMNH.MAM.60371.a	f	2	x	x		
RMNH.MAM.60373.a	m	3	x	x	x	x
RMNH.MAM.60392.a	m	2	x	x	x	x
RMNH.MAM.63941	f	3	x	x	x	x
RMNH.MAM.63942	f	3	x	x	x	x
RMNH.MAM.63943	m	3	x	x		
RMNH.MAM.63944	x	2	x	x	x	x
RMNH.MAM.63945	m	3	x	x	x	x
RMNH.MAM.63946	m	3	x	x	x	x
RMNH.MAM.63947	m	3	x	x	x	x
RMNH.MAM.63948	m	2	x	x	x	x
RMNH.MAM.63949	m	3	x	x	x	x
RMNH.MAM.63950	f	3	x	x	x	x
RMNH.MAM.63951	m	3	x	x	x	x
RMNH.MAM.63952	m	3	x	x	x	x
RMNH.MAM.63953	f	2	x	x		
RMNH.MAM.63954	f	2	x	x		
RMNH.MAM.63955	m	2	x	x		
RMNH.MAM.63956	x	3	x	x	x	x
RMNH.MAM.63957	m	2	x	x		
RMNH.MAM.63958	m	3	x	x	x	x
RMNH.MAM.63960	m	3	x	x	x	x
RMNH.MAM.63961	m	3	x	x	x	x
RMNH.MAM.63962	x	3	x	x	x	x
RMNH.MAM.63963	m	3	x	x	x	x
RMNH.MAM.63964	f	3	x	x	x	x
RMNH.MAM.63965	f	3	x	x	x	x
RMNH.MAM.63966	x	3	x	x	x	x
RMNH.MAM.63967	f	2	x	x		
RMNH.MAM.63968	x	3	x	x	x	x
RMNH.MAM.63969	m	0	x	x		
RMNH.MAM.63970	f	3	x	x	x	x
RMNH.MAM.63971	m	3	x	x	x	x
RMNH.MAM.63972	x	0	x	x		
RMNH.MAM.63973	m	0	x	x		
RMNH.MAM.63974	f	3	x	x	x	x

RMNH.MAM.63975	m	0	x	x		
RMNH.MAM.63976	m	0	x	x		
RMNH.MAM.63977	x	3	x	x	x	x
RMNH.MAM.63978	m	0	x	x	x	x
RMNH.MAM.63979	m	3	x	x	x	x
RMNH.MAM.63980	f	0	x	x	x	x
RMNH.MAM.63981	m	2	x	x		
RMNH.MAM.63982	m	3	x	x	x	x
RMNH.MAM.63983	m	3	x	x	x	x
RMNH.MAM.63984	m	3	x	x	x	x
RMNH.MAM.63985	m	3	x	x	x	x
RMNH.MAM.63986	f	0		x		
RMNH.MAM.63987	m	2	x	x		
RMNH.MAM.63988	m	3	x	x	x	x
RMNH.MAM.63989	m	0	x	x		
RMNH.MAM.63990	f	2	x	x	x	x
RMNH.MAM.63991	f	3	x	x	x	x
RMNH.MAM.63992	f	3	x	x	x	x
RMNH.MAM.63993	f	3	x	x	x	x
RMNH.MAM.63994	f	3	x	x	x	x
RMNH.MAM.63995	x	0	x	x	x	x
RMNH.MAM.63996	f	3	x	x	x	x
RMNH.MAM.63997	m	3	x	x	x	x
RMNH.MAM.63998	m	3	x	x	x	x
RMNH.MAM.64000	f	1	x	x		
RMNH.MAM.64001	m	1	x	x		
RMNH.MAM.64002	m	1	x	x		
RMNH.MAM.64003	m	1	x	x		
RMNH.MAM.64004	m	0	x	x		
RMNH.MAM.64005	f	1	x	x		
RMNH.MAM.64006	m	0	x	x		
RMNH.MAM.64007	m	1	x	x		
RMNH.MAM.64008	f	2	x	x		
RMNH.MAM.64009	f	1	x	x		
RMNH.MAM.64011	f	1	x	x		
RMNH.MAM.64012	f	1	x	x		
RMNH.MAM.64013	f	1		x		

RMNH.MAM.64014	f	3	x		x	x
RMNH.MAM.64015	f	3	x	x	x	x
RMNH.MAM.64016	f	3	x	x	x	x
RMNH.MAM.64017	f	3	x	x	x	x
RMNH.MAM.64018	f	3	x	x	x	x
RMNH.MAM.64019	x	3	x	x	x	x
RMNH.MAM.64020	x	3	x	x	x	x
RMNH.MAM.64021	m	0	x	x	x	x
RMNH.MAM.64025	x	3	x	x	x	x
RMNH.MAM.64026	x	0	x	x		
RMNH.MAM.64027	x	1	x	x		
RMNH.MAM.64028	m	3	x	x		
RMNH.MAM.64029	m	2	x	x		
RMNH.MAM.64030	m	1	x	x		
RMNH.MAM.64031	m	2	x	x		
RMNH.MAM.64032	m	0	x	x		
RMNH.MAM.64035	m	1	x	x		
RMNH.MAM.64038	m	1	x	x		
RMNH.MAM.64039	x	1	x	x		
RMNH.MAM.64040	x	0	x	x	x	x
RMNH.MAM.64041	x	0	x	x	x	x
RMNH.MAM.64042	f	0	x	x		
RMNH.MAM.64043	f	2	x	x		
RMNH.MAM.64044	x	0	x	x	x	x
RMNH.MAM.64045	m	0	x	x		
RMNH.MAM.64046	f	3	x	x	x	x
RMNH.MAM.64047	f	3	x	x	x	x
RMNH.MAM.64048	x	0	x	x		
RMNH.MAM.64049	m	0	x	x		
RMNH.MAM.64050	x	3	x	x		
RMNH.MAM.64051	m	3	x	x	x	x
RMNH.MAM.64052	m	0	x	x		
RMNH.MAM.64053	x	0	x	x		
RMNH.MAM.64054	m	3	x	x	x	x
RMNH.MAM.64055	x	3	x	x	x	x
RMNH.MAM.64056	x	0	x	x		
RMNH.MAM.64057	x	0	x	x		
RMNH.MAM.64058	x	3	x	x	x	x
RMNH.MAM.64059	x	0	x	x	x	x
RMNH.MAM.64060	x	3	x	x	x	x
RMNH.MAM.64061	x	0	x	x	x	x
RMNH.MAM.64062	m	0	x	x		
RMNH.MAM.64063	x	3	x	x	x	x
RMNH.MAM.64064	x	0	x	x		
RMNH.MAM.64065	x	0	x	x	x	x
RMNH.MAM.64066	x	3	x	x	x	x
RMNH.MAM.64067	m	3	x	x	x	x
RMNH.MAM.64068	x	2	x	x	x	x
RMNH.MAM.64069	x	2	x	x		
RMNH.MAM.64070	m	0	x	x		
RMNH.MAM.64071	x	0	x	x	x	x
RMNH.MAM.64072	x	2	x	x		
RMNH.MAM.64073	m	0	x	x	x	x
RMNH.MAM.64074	x	2	x	x		
RMNH.MAM.64075	x	0	x	x		
RMNH.MAM.64076	x	0	x	x		
RMNH.MAM.64077	m	2	x	x		
RMNH.MAM.64078	x	0	x	x		
RMNH.MAM.64079	x	1	x	x		
RMNH.MAM.64080	x	1	x	x		
RMNH.MAM.64081	x	3	x	x	x	x
RMNH.MAM.64082	x	3	x	x	x	x
RMNH.MAM.64083	f	0	x	x	x	x
RMNH.MAM.64084	x	0	x	x		
RMNH.MAM.64085	x	0	x	x	x	x
RMNH.MAM.64086	x	0	x	x	x	x
RMNH.MAM.64087	x	0	x	x		
RMNH.MAM.64088	m	0	x	x	x	x
RMNH.MAM.64089	m	0	x	x		
RMNH.MAM.64090	m	0	x	x	x	x
RMNH.MAM.64091	x	3	x	x	x	x
RMNH.MAM.64092	x	2	x	x	x	x
RMNH.MAM.64093	x	3	x	x	x	x
RMNH.MAM.64094	m	3	x	x	x	x

RMNH.MAM.64095	x	3	x	x	x	x
RMNH.MAM.64096	m	0	x	x	x	x
RMNH.MAM.64097	x	3	x	x		
RMNH.MAM.64098	x	0	x	x	x	x
RMNH.MAM.64099	x	2	x	x		
RMNH.MAM.64100	x	0	x	x	x	x
RMNH.MAM.64101	x	0	x	x	x	x
RMNH.MAM.64102	x	3	x	x	x	x
RMNH.MAM.64103	x	0	x	x		
RMNH.MAM.64104	m	3	x	x		
RMNH.MAM.64105	x	0	x	x	x	x
RMNH.MAM.64106	x	0		x		
RMNH.MAM.64107	f	3	x	x	x	x
RMNH.MAM.64108	x	0	x	x		
RMNH.MAM.64109	f	0	x	x		
RMNH.MAM.64110	m	0	x	x		
RMNH.MAM.64111	m	0	x	x		
RMNH.MAM.64112	m	0	x	x		
RMNH.MAM.64113	m	0	x	x		
RMNH.MAM.64114	m	3	x	x	x	x
RMNH.MAM.64115	m	0	x	x	x	x
RMNH.MAM.64116	m	0	x	x		
RMNH.MAM.64117	m	0	x	x		
RMNH.MAM.64118	m	2	x	x	x	x
RMNH.MAM.64119	m	3	x	x	x	x
RMNH.MAM.64120	x	2	x	x		
RMNH.MAM.6608.a	f	3	x	x	x	x
RMNH.MAM.6609.a	m	3	x	x	x	x
RMNH.MAM.6610.a	m	2	x	x	x	x
RMNH.MAM.6611.a	m	3	x	x	x	x
RMNH.MAM.9766.a	f	3	x	x	x	x
RMNH.MAM.9769.a	f	3	x	x	x	x
ZMA.MAM.23181.a	f	2			x	x
ZMA.MAM.23244.a	f	3	x	x	x	x
ZMA.MAM.24635.a	m	3	x		x	x

ZMA.MAM.25479.a	f	2			x	x
ZMA.MAM.30185	x	1		x		
ZMA.MAM.30187	x	1	x	x		
ZMA.MAM.30195	x	2	x	x		
ZMA.MAM.30197	x	2	x			
ZMA.MAM.30198	x	2		x		
ZMA.MAM.30203	x	2		x		
ZMA.MAM.30204	x	2		x		
ZMA.MAM.30205	x	2	x	x		
ZMA.MAM.30206	x	2		x		
ZMA.MAM.30207	x	2	x	x	x	x
ZMA.MAM.30209	x	2	x	x		
ZMA.MAM.30210	x	2	x	x	x	x
ZMA.MAM.30211	x	2	x			
ZMA.MAM.30212	x	2	x	x	x	x
ZMA.MAM.30261	x	2	x	x	x	x
ZMA.MAM.30263	x	3	x	x		
ZMA.MAM.30264	x	0			x	x
ZMA.MAM.30266	m	3	x		x	x
ZMA.MAM.30267	x	3	x	x	x	x
ZMA.MAM.30268	x	3	x	x	x	x
ZMA.MAM.30269	x	2	x	x	x	x
ZMA.MAM.30273	x	0			x	x
ZMA.MAM.30274	x	0			x	x
ZMA.MAM.30275	x	2	x			
ZMA.MAM.30293	x	1	x	x		
ZMA.MAM.30477	x	1	x	x		
ZMA.MAM.30481	x	2	x	x		
ZMA.MAM.30853	x	1	x	x		
ZMA.MAM.30857	x	1	x	x		
ZMA.MAM.31452	x	2	x	x		
ZMA.MAM.31492	x	2	x	x		

Appendix II – List of landmarks applied in this study

Mandible

1. Tip of the coronoid process: point of maximum curvature
2. Angle of mandibular condyle and coronoid process: point of maximum curvature
3. Tip of the mandibular condyle: point of maximum curvature
4. Angle of mandibular condyle and angular process: point of maximum curvature
5. Tip of angular process: point of maximum curvature
6. Ventral angle of masseteric fossa: point of maximum curvature
7. Midpoint of the mandibular corpus
8. Ventral most point of the mandibular symphysis: point of maximum curvature
9. Anterior most point of the mandible corpus
10. Posterior of the base of the first incisor
11. Posterior of the base of the second incisor
12. Posterior of the base of the third incisor
13. Posterior of the base of the canine
14. Posterior of the base of the first premolar
15. Posterior of the base of the second premolar
16. Posterior of the base of the third premolar
17. Posterior of the base of the first molar
18. Posterior of the base of the second molar
19. Posterior of the base of third molar
20. Point on the mandibular corpus at the ramus base

Ventral view of the skull

1. Midpoint of the posterior curvature of the internasal opening
2. Posterior end of the palatum
3. Posterior limit of the foramen magnum
4. Posterior limit of the foramen magnum
5. Lateral limit of the foramen magnum
6. Tip of the mastoid process
7. Posterior end of the stylomastoid foramen?
8. Maximum curvature of the posterior margin of the zygomatic arch
9. Tip of the hamular process of the pterygoid
10. Posterior most internal point of the zygomatic arch
11. Maximum curvature of the anterior margin of the zygomatic process
12. Anterior most internal point of the zygomatic arch
13. Metastyle of the third molar
14. Principal cusp of the first incisor
15. Top of the second premolar
16. Parastyle of second molar

Dorsal view of the skull

1. Posterior end of the cranium
2. Sagittal crest at the end of the frontal cranial bone
3. Anterior end of the cranium at the sagittal crest
4. Anterior end of the maxillary cranial bone
5. Point of maximum curvature on the inside of the anterior end of the zygomatic arch
6. Posterior most point on the inside of the zygomatic arch
7. Point of maximum curvature on the outside of the posterior end of the zygomatic arch
8. Asterion, posterior at occipital-parietal-temporal suture
9. Point at the intersection of the articular process and squamosal body
10. Point at the caudolateral end of the occipital bone

Appendix III – Procrustes coordinates

All Procrustes coordinates can be found via the following link.
https://drive.google.com/file/d/1Ed_UCZmjWLt5lj_bKgqWOksIjkaR_Nye/view?usp=sharing

a. Mandible babies

Procrustes coordinates of the mandible of the baby specimens, only known males and female are included.

b. Mandible juveniles

Procrustes coordinates of the mandible of the juvenile specimens, only known males and female are included.

c. Mandible adults

Procrustes coordinates of the mandible of the adult specimens, only known males and female are included.

d. Mandible all ages, subset of the landmarks

Procrustes coordinates of the mandible of all specimens, only known males and females are included, the subset of the landmarks was used.

e. Ventral side of the cranium of juveniles

Procrustes coordinates of the ventral side of the cranium of juvenile specimens, only known males and females are included.

f. Ventral side of the cranium of adults

Procrustes coordinates of the ventral side of the cranium of adult specimens, only known males and females are included.

g. Dorsal side of the cranium of juveniles

Procrustes coordinates of the dorsal side of the cranium of juvenile specimens, only known males and females are included.

h. Dorsal side of the cranium of adults

Procrustes coordinates of the dorsal side of the cranium of adult specimens, only known males and females are included.