**Chapter 2: Data Collection**

**2.1 Introduction**

The analyses in chapters 3, 4… use morphological and ecological data sets which I compiled for xx specimens representing xx species of mammals. This chapter describes how the data were collected and checked for errors. First I outline how I compiled my morphological data set from museum specimens and secondly I describe the sources which contributed to my ecological dataset. Subsequent chapters will refer to both of these data sources but I will only describe the methods I used for their creation in detail here. Other methods I used which have specific relevance to subsequent chapters are described in those chapters.

**2.1.1 Study species**

I collected morphological measurements of xxx species from four mammalian orders; Afrosoricida, Erinaceomorpha, Soricomorpha and Notoryctemorphia comprising seven families of mammals; tenrecs (Tenrecidae), golden moles (Chrysochloridae), hedgehogs and gymnures (Erinaceidae), shrews (Soricidae), solenodons (Solenodontidae), moles and desmans (Talpidae) and marsupial moles (Notoryctidae).

My aim was to include measurements of all tenrecs and their sister taxa, golden moles. For my comparative species from other mammalian orders, I chose a random sample of xx taxa which have been previously identified as convergent with tenrecs (e.g. [Gould and Eisenberg, 1966](#_ENREF_13), [Symonds, 2005](#_ENREF_31), [Poux et al., 2008](#_ENREF_27), [Olson, 2013](#_ENREF_23)). I used Wilson and Reeder’s Mammal Species of the World (MSW05), ([2005](#_ENREF_34)) as it is the most complete and accepted reference source for mammalian systematics. I used phylogenies for each order to select, at random, species representing the main sub-branches of each order which also represented the morphological diversity of that order. For example, within the Soricomorpha, I included both species of *Solenodon* but only xx (out of a total of xx) species of *Crocidura* as the former genus represents a separate subgroup to the rest of the order (*come back and rephrase this*).

**2.1.2 Taxonomy**

Although MSW05 is currently the most complete mammalian taxonomic reference, it does not include some known species. For example, Wilson and Reader ([2005](#_ENREF_34)) record 30 species of tenrec but more recent studies indicate that there are now 34 recognised species of tenrec ([Olson, 2013](#_ENREF_23)). The additional species belong to the shrew tenrec (*Microgale*) genus and represent either recognition of cryptic species boundaries ([Olson et al., 2004](#_ENREF_24)) or discovery of new species ([Goodman et al., 2006](#_ENREF_12), [Olson et al., 2009](#_ENREF_25)). Only one of these four recent additions to the *Microgale* genus, *M. jobihely*, was present in museum collections and therefore I could not include the three other newly recognised species in my analyses.

As MSW05 does not include all known species, I used the International Union for the Conservation of Nature ([IUCN, 2012](#_ENREF_16)) as an additional reference for the taxonomy and species diversity of each group. Table x outlines the number of species I measured from each family and how this sample relates to the overall number of species in that group as recorded by both MSW05 and the IUCN.

**Table x:** The number of species I measured in each family compared to the total number of species in that family according to two sources; ([Wilson and Reeder, 2005](#_ENREF_34)) and ([IUCN, 2012](#_ENREF_16)).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Order | Family | Number of species measured | Total number of species MSW05 | Total number of species IUCN | Number measured as a percentage of the total species |
| Afrosoricida | Tenrecidae |  | 30 | 34 |  |
| Afrosoricida | Chrysochloridae |  | 21 | 21 |  |
| Erinaceomorpha | Erinaceidae |  | 24 |  |  |
| Soricomorpha | Soricidae |  | 376 |  |  |
| Soricomorpha | Solenodontidae |  | 4 |  |  |
| Soricomorpha | Talpidae |  | 39 |  |  |
| Notoryctemorphia | Notoryctidae |  | 2 |  |  |

**2.2 Morphological data set**

To construct my morphological data set, I used museum specimens of my study species housed at five different institutions. I compiled morphological data using two complementary approaches; linear measurements using calipers and 2D landmark-based geometric morphometrics.

**2.2.1 Museums visited**

I measured and photographed the skulls, limbs and skins of tenrecs and the species they convergently resemble using the collections of five different museums; the Natural History Museum London (NHML), the Smithsonian Institute Natural History Museum (SI), the American Museum of Natural History (AMNH), Harvard’s Museum of Comparative Zoology (MCZ) and the Field Museum of Natural History (FMNH), Chicago. Between January and September 2013, I spent 9 weeks working in these collections and collected data from xxx skulls, xxx limbs and xxx skins of xxx species.

**2.2.2. Museum Label Data**

I recorded all the data on the specimen labels including any handwritten or printed notes which had been added by other users of the collection. The label data included the museum specimen ID number, genus, species, sex, collector’s name, the date and location of where the specimen was collected. Some of the labels attached to skins had additional information such as the body, tail, hind foot and ear lengths as well as the body mass of the live individual.

The level of detail recorded on the labels varied considerably. For example recently collected specimens were more likely to have detailed information about the collection location, and some specimens did not have even basic information such as the sex recorded.

*Put in a picture of a label here to show the lack of information: NB; take a label picture in Chicago*

**2.2.3 Linear Measurements**

**2.2.3.1 Measurement descriptions**

Using a 15mm digital calipers (Mitutoyo Absolute digimatic calipers), I took 20 measurements of the skulls and mandibles (table xx) and 19 measurements of the limbs (table xx). My choice of which measurements to include was based on three main criteria; 1) their relevance to biological and ecological traits such as diet specialisation and locomotory adaptation, 2) their usefulness for assessing the overall shape and size of the specimen and 3) the ease with which they could be repeated both within and among specimens from different species*.*

Figures x-x depict the linear measurements of skulls and figures xx show the limb measurements.

*(I’ll most likely take out these tables and just put in pictures instead but I’ve left them in for now just as a reminder)*

Table xx: Skull and mandible measurements including two variables marked with \* which could not be measured in every species. Variables marked with † were measured five instead of three times (see explanation below)

|  |  |  |
| --- | --- | --- |
| **Abbreviation** | **Measurement** | **Description and notes** |
| 1\_CB | Condylobasal length | Total skull length from the front of the premaxillary bones to the rear surface of the occipital condyles, measured from the ventral side of the skull. In golden moles, the front of the premaxillary bones projects further out than the teeth. Does not include any part of the upper skull that projects further back than the occipital condyles (e.g. the rear-pointing skull crest in *Tenrec ecaudatus*) |
| 2\_PL | Palate length | From the front of the palate (in between the anterior teeth, front of the pre-maxilla) to the posterior of the hard palate (excluding the palate spine when it’s present) |
| 3\_TR | Tooth row length | From the front of the alveolus of the first incisor to the rear of the alveolus of the last molar on the same side. Measured between these points even if the relevant teeth were missing. |
| 4\_PWa†  **(Need to sort out this pair)** | Palate Width anterior | Width across the palate measured between the rear, outermost points of the alveoli of the first pair of teeth (i.e. include the width of the teeth in the measurement). However, when there are a row of anterior incisors which stretch across the front of the palate (e.g. moles, *Euroscaptor klossi* SI\_261090) then measure PWa as the width across this row of incisors (i.e. just in front of the canines) |
| 4a\_PWa\_2teeth\*†  **(Need to sort out this pair)** |  | Only included for some species when the PWa is measured behind the end of the row of the front incisors (see above), this measurement is the palate width behind the first two incisors only (i.e. same definition as the first line of above) |
| 5\_maxPW | Maximum palate width | Maximum width across the roof of the mouth including the alveoli of the cheek teeth on either side – measured at the widest point of the palate |
| 6­­\_IncisorH† | Incisor height | Maximum height of the first incisor on the right side |
| 7\_ZW\* | Zygomatic width | Maximum width between the zygomatic arches – measured within the arches from below the skull. Entered with a value of 0mm when zygomatic arches were broken or in species that don’t have zygomatic arches. |
| 8\_MX  **(Probably not a good definition)** | Maxilla width | Width between the maxillary bones on either side, measured from above the skull. Where zygomatic arches are present; width from the innermost connection between the anterior of the arch and the skull. When arches are absent; width between the anterior skull constrictions. |
| 9\_SQ  **(Probably not a good definition)** | Squamosal width | Width between the squamosal bones on either side, measured from above the skull. Where zygomatic arches are present; width from the innermost connection between the posterior of the arch and the skull. When arches are absent; width between the posterior skull constrictions. Does not include the two round “bulbs” (*proper word??)* on either side of the golden mole skulls. |
| 10\_OL  **(Probably not a good definition)** | Orbit length *(probably not the right word?)*  Asher; orbit continuous with the temporal fossa | Longitudinal length of the orbit opening on the right side measured along the edge of the skull from the maxilla to the squamosal. |
| 11\_IFD† | Interorbital foramen diameter | The maximum (vertical) diameter of the Interorbital foramen measured on the right side |
| 12\_IFW | Interorbital foramen width | The width across the skull between the two Interorbital foramina |
| 13\_IFcanal†  **(May have measured this wrong in some hedgehogs)** | Interorbital foramen canal | The length of the IF canal measured from above (almost across the maxilla) between the two openings |
| 14\_BW | Braincase width | Width across the brain case at the widest point of the skull. Excludes any part of the skull that isn’t part of the braincase e.g. “wings” (*proper word??)* that stick out on either side of the golden mole skulls and the extra nodules *(word??)* on the *Desmana moschata* skulls. |
| 15\_ML | Mandible length | Maximum jaw length measured from the symphysis to the end of the jaw in a straight line – the end of the angular process. Does not include the length of any forwardly projecting lower incisors i.e. measured from the front of the jaw bone rather than the anterior tip of the lower incisors. |
| 16\_MTR | Mandible tooth row length | From the anterior edged of the alveolus of the first tooth to the posterior edge of the alveolus of the last tooth on the right side. Measure to the end of the last alveolus even if the tooth is missing |
| 17\_CorP  **(I didn’t record which species included the angular process)** | Coronoid process height | Perpendicular height from the top of the coronoid process to the base of the jaw bone. In some species this includes measuring to the base of the angular process (lowest projection of the mandible). In other species, the perpendicular height to the tip of the coronoid does not include the angular process e.g. Golden moles’ jaws. |
| 18\_ConY | Condyloid height | Perpendicular height from the top of the mandibular condyle to the base of the jaw. |
| 19\_CorCon | Coronoid –condyloid length | Diagonal distance from the coronoid tip to the condyloid crest/posterior notch which is in the middle of the curve between the coronoid and condyloid ([Carraway et al., 1996](#_ENREF_10)). |
| 20\_SkH | Skull Height | Height of the back of the skull – perpendicular height from the highest point on the braincase (includes the top of any skull crest e.g. *Tenrec ecaudatus*) to the base of the skull. |

*Put pictures of the skull measurements in here. Include pictures of the measurements in a tenrec, golden mole, hedgehog and mole to see all the variety?*

Table xx: Limb measurements; hind limb 1-9 and forelimb 10-19. Variables marked with † were measured five rather than three times (see below) and variables marked with \* were only measured in some species

|  |  |  |
| --- | --- | --- |
| **Abbreviation** | **Measurement** | **Description** |
| 1\_Inn  **(May have measured some of the early specimens differently)** | Innominate length | Maximum longitudinal length of the pelvic bone measured in a straight line from the anterior tip to the curved end (i.e. don’t measure diagonally across open space and the end point will usually be in the middle of the curve) |
| 2\_Obt  **(Problem with golden moles)** | Obturator foramen | Maximum diameter of the opening in the hip bone. Golden moles seemed to have either no opening or else a very slight “depression” in the bone |
| 3\_FemL | Femur length | Length of the bone excluding the hip joint but including the knee(i.e. does not include the femoral head even when the bones are disarticulated). When the bones are attached, measure to the end of the bone excluding the patella. |
| 4\_FemD† | Femur diameter | Minimum width across the shaft of the bone (*circumference would be better but difficult to measure in the small species*) |
| 5\_TibL | Tibia length | Maximum longitudinal length of the tibia (from between the knee and ankle joints or else from top to bottom of the bone) |
| 6\_TibU | Tibia unfused length | Length of the tibia which is not fused with the fibula (from the knee joint excluding the patella to the point where the tibia and fibula fuse) |
| 7\_TibD† | Tibia diameter | Minimum diameter across the shaft of the tibia bone |
| 8\_Foot | Foot length | Maximum length of the entire foot (heel to longest toe) |
| 9\_Toe | Toe length | Length of the longest toe bone (just the phalange bone up to the metatarsal joint) |
| 10\_ScapL  **(needs a better definition)** | Scapula length | Perpendicular length of the scapula from the curved end *to* the inner edge of the groove at the other end(doesn’t include the final bony tip on the non-curved end) |
| 11\_ScapW  **(problems with Sorex scapulae)** | Scapula width | Maximum perpendicular width across the bone. In the *Sorex* scapulae, it can be difficult to see which direction is the ScapW and which is the crest which runs along the length of the scapula – sometimes the crest is wider than the ScapW (?measured it like this for *Sorex arcticus*, *bendirii* and *cinereus* on 10/04) |
| 12\_HumL | Humerus length | Maximum length of the bone (from tip to tip – need names). In golden moles (L-shaped humerus) – diagonal distance between the two ends of the bone – see notebook 27­/03/2013 |
| 12a\_HumLvert\* | Humerus length vertical | Only measured in golden moles since they have an L-shaped humerus – length of the vertical (longer) side of the bone |
| 12b\_HumLhori\* | Humerus length horizontal | Only measured in golden moles since they have an L-shaped humerus – length of the horizontal (shorter) side of the bone |
| 13\_HumD† | Humerus diameter | Minimum diameter across the shaft of the humerus |
| 14\_UlnL  **(needs a better definition)** | Ulna length | Length of the bone from the distal tip (end sticks out behind the elbow) to the wrist joint. Measured in a straight line from the wrist distal tip (did not include the part of the elbow end which bends down in golden moles) |
| 15\_RadL  **(needs a better definition)** | Radius length | Length of the radius from the end to the wrist |
| 16\_UlnD† | Ulna diameter | Minimum diameter across the ulna |
| 17\_RadD† | Radius diameter | Minimum diameter across the radius |
| 18\_Hand | Hand length | Maximum length of the entire hand (wrist to longest finger) |
| 19\_Finger | Finger length | Length of the longest finger bone (to the metatarsal joint) |

*Put pictures of the limb measurements in here. Include pictures of the measurements in a tenrec, golden mole, hedgehog and mole – may need to include shrews too?*

*Separate panels for each of the elements? E.g. ~4 hip pictures, 4 femur pictures etc. – may be difficult since I probably don’t have separate pictures of each limb element for all of the species. See what happens; deal with the skulls first*

I took each linear measurement three times, cycling through all 20 skull or 19 limb measurements then repeating the cycle to avoid measuring the same variable twice in a row. Small measurements (<2mm) are particularly prone to high error rates ([Cardini and Elton, 2008](#_ENREF_9)). Therefore, I took five separate replicates of some of the variables which were most prone to errors (marked with † in tables xx and xx). These included four of the skull measurements (PWa, IncisorH, IFD and IFcanal) and five of the limb measurements (FemD, TibD, HumD, UlnD, RadD).

Five replicates should give a more reliable median value because even if there are one or two outlying measurements there should be at least three replicates which are in close agreement.

**2.2.4 Landmark morphometrics**

**2.2.4.1 Photographic setup**

In order to get 2D landmarks for my specimens, I first had to photograph them. I used photographic copy stands consisting of a camera attachment with an adjustable height bar, a flat stage on which to place the specimen and an adjustable light source to either side of the stage. I used the copy stands that were available at each museum which differed in how the camera height was adjusted and in the light sources available.

To take the light variability into account, on each day I took a picture of a white sheet of paper and used the custom white balance function on the camera to set the image as the baseline “white” measurement for those particular light conditions.

**2.2.4.2 Photographing specimens**

I photographed the specimens with a Canon EOS 650D camera fitted with either an EF 100mm f/2.8 Macro USM lens (skulls and limbs) or EFS 18-55mm lens (skins). I used a remote control (hähnel Combi TF) to take the photos to avoid shaking the camera and distorting the images. I photographed the specimens on a black material background. I placed the light source from the top left-hand corner of the picture and positioned a piece of white card on the bottom right side of the specimen which reflected the light back onto the specimen and minimised any shadows (figure xx below).



**Figure xx:** Photographic set up for taking images of my skulls. The camera is fitted to a copy stand, the light source is directed from the top-left corner of the image and the white card reflects the light back onto the skull. *(NB: I need a better picture to go here).*

I made small bean bags (12 x 5cm) from the same black material as the background and filled them with plastic beads. I used these bags as necessary to hold the specimens in position while being photographed. For example, when taking pictures of the lateral view of skulls (see below), I placed one bean bag under the nose of the skull and another bag lying along the top (cranial) side of the skull to ensure that the side I was photographing lay in a flat plane relative to the camera and did not tilt in any direction.

I used the grid-line function on the live-view display screen of the camera to position the specimens in the centre of each image.

**2.2.4.2.1 Skulls**

I photographed the skulls in three views; dorsal (top of the cranium), ventral (underside of the skull with the palate roof facing uppermost) and lateral (right side of the skull). I also photographed the outer (buccal) side of the right mandible. When the right side of either the skull or mandibles were damaged or incomplete, I photographed the left sides and later reflected the images so that they could be compared to pictures of the right sides ([e.g. Barrow and Macleod, 2008](#_ENREF_7)). (*Probably need a disclaimer somewhere about not being interested/ worried about bilateral asymmetry and also a quick test.)*

**2.2.4.2.2 Limbs**

Initially, I tried to take pictures of the limbs in similar orientations to the skulls (dorsal, ventral and lateral). However, there was considerable variation in how the limbs were preserved. For example, some limbs were still articulated while others had fragmented bones. It therefore proved impossible to place the limb bones in consistent orientations that would be comparable across species. Similarly, the small size of some limbs, combined with the frequently incomplete nature of postcranial museum collections, made landmark-based morphometric analyses of any limb pictures impractical. Therefore, I photographed the fore- and hind-limb bones in outer (the side facing away from the rest of the body) and inner (the side facing in towards the centre of the body) views for reference purposes only.

**2.2.4.2.3 Skins**

As I was limited by the maximum camera height available on the copy stands, most skins were too large to be photographed with the 100mm macro lens. Therefore, I used an EFS 18-55mm lens to take pictures of the skins. I photographed skins in the same three orientations as the skulls; dorsal (the upper surface of the animal), ventral (the belly side of the skin) and lateral (right flank of the animal with the skin held in position using bean bags). The dorsal and ventral views give very approximate estimates of the overall body shape of the animal. The lateral views are less biologically relevant since the taxidermic process is unlikely to produce specimens which represent the true body height of the animal.

**2.2.4.3 Saving and processing images**

Photographs were captured and saved in a raw file format. Before using the pictures for morphometric analyses, I converted the raw files to binary (grey scale) images and re-saved them as TIFF files. The black and white pictures were more useful for later analyses since I was not interested in including any colour comparisons and it is easier to see some biological features in binary images. TIFF files were the most appropriate to use for my morphometric analyses as they are uncompressed (in comparison to JPEG) images and therefore there is less chance of any picture distortions which may affect later analyses ([HERC, 2013](#_ENREF_15)).

**2.2.4.4 Landmark placement on images**

*I need a general “this is morphometrics” overview at some stage; it might fit in here?*

I conducted geometric morphometric analyses of my skull, mandible and skin (??) photographs. I used a combination of landmark and outline/curve analysis approaches to assess the shape variability of the specimens. In contrast to detailed morphometric studies of single taxa (e.g. Refs), the interspecific and comparative nature of my work limited the number of points which could be reliably identified as landmarks in all species….

I used the TPS software suite *(how to ref this??)* to digitise landmarks and curves on my pictures. I set the scale on each pictures individually to standardise for the different camera heights I used when photographing my specimens. All subsequent morphometric and statistical analyses were carried out in R v2.15.1 ([R Core Team, 2013](#_ENREF_28)).

Here I summarise the landmarks and curves which I used on each of my different sets of pictures.

(*I’ll need to put in pictures showing the landmarks for each set of photos- do another photo shop job on pictures and then add points to that.)*

**2.2.4.4.1 Skulls: ventral view**

Most of the landmarks in this view are concentrated around the dentition and palate of the animals. The high variability of my species’ basi-cranial region and difficulties associated with identifying developmentally or functionally homologous points precluded designation of additional landmarks towards the back of the skulls. For the landmarks placed around the molars (*I don’t know which numbers yet because I’m changing some landmarks after talking to Francois*) I used species’ dental formulae ([Nowak, 1983](#_ENREF_22), [MacPhee, 1987](#_ENREF_18), [Knox Jones and Manning, 1992](#_ENREF_17), [Marshall and Eisenberg, 1996](#_ENREF_19), [Nagorsen, 2002](#_ENREF_21), [Goodman et al., 2006](#_ENREF_12), [Asher and Lehmann, 2008](#_ENREF_6), [ADW, 2013](#_ENREF_4)) where available to identify the number of premolars and molars.

**2.2.4.4.2 Skulls: lateral view**

I reflected photographs of the left lateral side of the skull so that all pictures would be in the same orientation. I placed x landmarks on the lateral pictures (see figure x below) and also drew a curve of semilandmarks between points x and x.

**2.2.4.4.3 Skulls: dorsal view**

There were even fewer identifiable landmarks in this view because none of the dental characteristics are visible. Therefore, I chose to use outline analysis (refs?) to compare the overall skull shape among my species.

**2.2.4.4.4 Mandibles**

I placed seven landmarks and drew four curves on each mandible picture (again, reflecting any pictures of the left mandible so they could be compared to pictures of the right side). I chose to draw separate curves around each of the three processes of the ascending ramus; coronoid, condyloid and angular and along the base of the horizontal ramus of the jaw. While obviously part of an integrated jaw unit, the development of the mandibular processes are also, in some aspects, independent since they attach different muscles which exert different masticatory forces on the jaw ([Barrow and Macleod, 2008](#_ENREF_7)) (Why do we care about independence?). Therefore, by drawing separate curves around each of these elements, my ensuing analyses could assess the relative shape changes of different components of the jaw with relevance to variation in feeding strategies and capabilities.

*I don’t like how I’ve phrased the above paragraph but I just thought it was a nice point in the Barrow paper so I want to include it somewhere.*

**2.2.4.4.5 Skins**

*Outline analysis of skins for overall body shape?*

**2.2.5 Error Checking**

My data are prone to a number of different error sources. These include 1) taxonomic identification which has not been updated to currently accepted terms, 2) specimen ID errors, 3) possible variation associated with sex and age class of individuals, 4) the accuracy and repeatability with which species traits are measured, 5) morphometric errors associated with photographing specimens and the placement of landmarks. I address each of these possible sources of error below.

* + - 1. **Taxonomic**

I recorded species names as they were written on museum specimen labels and then corrected them to match Wilson and Reader’s Mammal Species of the World ([2005](#_ENREF_34)) as this is the most complete and accepted reference source for mammalian systematics. For recently identified species, such as *Microgale jobihely* ([Goodman et al., 2006](#_ENREF_12)), which are not included in Wilson and Reader (2005) , I used the taxonomy recorded on the labels.

* + - 1. **Specimen ID** *(add in Natalie’s centroid means?)*

There were four specimens from the Smithsonian Institute which had species labels which did not match between skulls and skins with the same specimen ID numbers. The four skulls were labelled as *Hemicentetes semispinosus*. The corresponding skins were originally labelled as *H. semispinosus* but this was crossed out and changed to *H. nigriceps*. The re-labelled skins looked clearly different to the undisputed *H. semispinosus* skins and also look more similar to other pictures of *H. nigriceps*. Therefore, I made the assumption that the re-labelling of the skins as *H. nigriceps* represents the true taxonomy and I treated the corresponding skulls as *H. nigriceps*.

* + - 1. **Specimen sex and age**

I included both male and female specimens in my data as significant sexual dimorphism in skull or body size has not been identified in any of my species (*need more references* e.g. ([Olson et al., 2004](#_ENREF_24))). I also tested this …. (*need to do a test of male vs. female??)*

Information about the sex and/or age of an individual is often missing from museum records. Mammalian species can often be identified as juveniles by looking for incomplete fusion of the crania and non-fully erupted dentition (*references??)* However, age classification in tenrecs is difficult using these criteria; in some species, the last molar does not erupt fully until the first molar has been shed so the full dentition is never present at any one time ([Nowak, 1983](#_ENREF_22)). It is also difficult to distinguish deciduous from permanent teeth in *Microgale* tenrecs ([Asher and Lehmann, 2008](#_ENREF_6)) which has led to confusion and misidentification of juvenile forms as separate species ([Olson et al., 2004](#_ENREF_24)). I excluded any obvious juvenile specimens from my data set. Where specimens could not be obviously identified as juveniles I treated them all as equivalent adult forms. *(Check the Potamogale against the other Potamogales?? Keep the \_sp for family or genus level analyses but exclude from species-level things)*

* + - 1. **Linear measurements**

As mentioned above, I took three replicate measurements of most of my variables and five replicates of other variables. Some morphometric studies take replicate measurements of a trait and use the average value for further analyses. (*is this true? check papers*)

Rather than taking the mean of each of three (or five) measures, I used the median as it is less likely to be skewed by others and gives a more accurate representation of the true value of the trait.

Before extracting the median values I followed the protocol for assessing measurement error outlined by Cooper and Purvis ([2009](#_ENREF_11)). This method assesses whether there is a reasonable correlation among the replicate measurements of the same variable. The error checking criteria are based on two calculations; the coefficient of variation and the percentage spread.

I calculated the coefficient of variation (standard deviation/mean\*100) for each measurement. This value estimates the extent to which replicate measurements deviate from the mean. When the coefficient of variation was less than 5%, I accepted the median value as an accurate measurement of the size of the structure.

If the coefficient of variation was greater than 5%, indicative of a low agreement between replicate measurements, I measured the percentage spread of the data. For variables measured three times, I calculated percentage spread as [(minimum difference between neighbouring measurements)/ (range of measured values)\*100].

For variables that were measured five times, the differences between neighbouring values were calculated and labelled from smallest to largest as a, b, c, and d with the range of the measured values designated as e ([Cooper and Purvis, 2009](#_ENREF_11)). For these variables, I calculated percentage spread as [(a/e + b/e + c/e)\*100].

Small percentage spread values indicate close agreement between repeated measurements. When percentage spread approaches 50% the data are evenly spread out and therefore there is no way of knowing whether the median value is an accurate measurement of the trait ([Cooper and Purvis, 2009](#_ENREF_11)). I chose to use to use 25% as a cut off point for accepting the accuracy of measured traits.

I used these error checking criteria to assess the accuracy of my repeated measurements of both skulls and limbs. *(I need to present all of this as more of a narrative but keep the detail in for the moment.)*

**2.2.5.4.1 Skulls**

Of the 20 measurements for xxx skulls, there were xx variables belonging to xx skulls which had coefficient of variation > 5% and percentage spread >25 % (Table x).

Table x: Summary of measurement error checking for my skulls data set. (*NB: Natalie said to quote coef.var and per.spread as mean + standard deviation but I don’t see how that makes sense with the way I have laid out the tables at the moment i.e. just the number of variables with coef.var>5%)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Total data set | | After removing xx specimens | |
| Number of; | Variables | Skulls to which the variables belong | Variables | Skulls to which the variables belong |
| Total |  |  |  |  |
| Coefficient of variation > 5% |  |  |  |  |
| Percentage spread >25% |  |  |  |  |

My final skull data set included xx replicates of xx variables from xx specimens comprising xx species.

* + - * 1. **Limbs**

Of the 19 measurements for xxx limbs, there were xx variables belonging to xx specimens which had coefficient of variation > 5% and percentage spread >25 % (Table x).

Table x: Summary of measurement error checking for my limbs data set.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Total data set | | After removing xx specimens | |
| Number of; | Variables | Specimens to which the variables belong | Variables | Specimens to which the variables belong |
| Total |  |  |  |  |
| Coefficient of variation > 5% |  |  |  |  |
| Percentage spread >25% |  |  |  |  |

My final limb data set included xx replicates of xx variables from xx specimens comprising xx species.

* + - 1. **Landmark morphometrics**

I used 2D morphometrics to compare the morphologies of the skulls. The small size of my specimens, combined with the number of specimens involved in my study made 3D imaging impractical. It takes roughly 1.5 hours for a good quality scan of each specimen so it would take xx hours to compile a useful data set. While 2D methods are an accepted means of comparing morphological shape (e.g. [Adams et al., 2004](#_ENREF_2), [Mitteroecker and Gunz, 2009](#_ENREF_20)), particularly for comparing skull morphologies of small mammals (e.g. [Cardini, 2003](#_ENREF_8), [Panchetti et al., 2008](#_ENREF_26), [White and Searle, 2008](#_ENREF_33), [Barrow and Macleod, 2008](#_ENREF_7), [Scalici and Panchetti, 2011](#_ENREF_30)), the inherent discrepancies associated with comparing three dimensional objects using two dimensional pictures do introduce some difficulties of possible distortion of the image ([Arnqvist and Mårtensson, 1998](#_ENREF_5)). Similarly, human error with how landmarks are positioned on specimens could also introduce noise into further analyses. In contrast to detailed intraspecific work (e.g. paper??) photographic or landmark placement errors are unlikely to be significant in interspecific studies since one would expect that the morphological variation among species is large enough to be detected as a signal above any background noise associated with methodological error (*reference?).* Nevertheless, it is still important to assess measurement error in a morphometric data set to increase confidence in the outcome of final analyses.

I identify two main sources of morphometric measurement error; specimen orientation and placement of landmarks.

* + - * 1. **Specimen orientation**

Variation in the orientation of specimens for photography is one of the main sources of error in 2D morphometric studies ([Adriaens, 2007](#_ENREF_3)). If specimens are not placed on a flat plane or in a consistent position relative to the camera, areas of the object which are tilted towards the camera will appear to be larger than reality, distorting any subsequent morphometric analyses of the shape.

I used a random subset of skulls comprised of one representative from each of my 89 species (probably don’t need to use this many for error checking but how should I choose which ones to include?) to estimate the overall specimen orientation error in my photographic dataset. This subset included representatives from each tenrec and golden mole species along with samples from my comparative species (total of xx moles, xx shrews, xx hedgehogs…) I took three sets of pictures of each view of the skulls and mandibles, cycling through the pictures so that the specimen was removed and re-positioned before every shot ([Viscosi and Cortini, 2011](#_ENREF_32)).

* + - * 1. **Landmark placement**

Another possible source of measurement error is associated with the placement of landmarks ([Arnqvist and Mårtensson, 1998](#_ENREF_5)). I placed the landmarks on each set of pictures so inter-observer variation is not an issue for my study. However, repeatability and reliability of my choice of landmarks could affect the final results of my analyses (see section xx below for a description of the landmarks used in for each image view).

I used a combined, nested approach to test for both orientation and landmark placement error ([Arnqvist and Mårtensson, 1998](#_ENREF_5), [Barrow and Macleod, 2008](#_ENREF_7)). For each of the 89 specimens in my random subset of species, I photographed their skulls (dorsal, ventral and lateral views) and mandibles three times. I then copied these images and placed landmarks on 3 copies of each image. I used a nested mixed mode ANOVA to assess the measurement error of the Procrustes-superimposed coordinates. There were three factors in my ANOVA; specimen, photo (3 pictures of each specimen) and landmark trial (placed landmarks on 3 copies of each of my photos).

*Effect size for the variance explained by each factor, based on inter landmark linear distances……..*

*(I could also use PC scores or a Mahalanobis distance matrix…)*

Summary of morphometric error checking steps

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Specimen view | Skull dorsal | Skull ventral | Skull lateral | Mandibles |
| Number of specimens |  |  |  |  |
| Number of landmarks |  |  |  |  |
| Number of curves? |  |  |  |  |
| Metric of difference |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

* + 1. **Summary of the morphological data set**

**2.3 Ecological data**

*This is the end of the morphological data set collection*

*Probably move the analyses to a different chapter*

**Analysis of linear measurements**

I don’t know what’s going here yet!

**Geometric Morphometric Analyses**

After placing landmarks and drawing outline curves in TPSDig ([Rohlf, 2013](#_ENREF_29)) I used TPSUtil to create a sliders file ([Zelditch et al., 2012](#_ENREF_35)) which depicts which points to treat as semilandmarks ([Gunz and Mitteroecker, 2013](#_ENREF_14)). I used the geomorph package *(*[*Adams et al., 2013*](#_ENREF_1)*)* to read my morphometric data into R and conduct my analyses. I scaled all coordinates using the scale factor recorded for each image (scaled coordinates=raw coordinates \* scale factor) and used partial Procrustes superimposition (*ref*) to superimpose the landmark configurations.

*PCA plots, RW analysis, maybe CVA and ellipses around each group….*

*I also need to mention how I incorporated the measurement error from above*

**References**

*Still need to fix the Nagorsen reference*

Adams, D. C., Otárola-Castillo, E. & Paradis, E. 2013. geomorph: an r package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution,* 4**,** 393-399.

Adams, D. C., Rohlf, F. J. & Slice, D. E. 2004. Geometric morphometrics: Ten years of progress following the "revolution". *Italian Journal of Zoology,* 71**,** 5-16.

Adriaens, D. 2007. *Protocol for error testing in landmark based geometric morphometrics*. Available: http://www.fun-morph.ugent.be/Miscel/Methodology/Morphometrics.pdf (Accessed 20/05/2013).

ADW. 2013. *Animal Diversity Web*. Available: http://animaldiversity.ummz.umich.edu/ (Accessed 22/06/2013).

Arnqvist, G. & Mårtensson, T. 1998. Measurement error in geometric morphometrics; empirical strategies to assess and reduce its impact on measures of shape. *Acta Zoologica Academiae Scientiarum Hungaricae,* 44**,** 73-96.

Asher, R. J. & Lehmann, T. 2008. Dental eruption in Afrotherian mammals. *BMC Biology,* 6.

Barrow, E. & Macleod, N. 2008. Shape variation in the mole dentary (Talpidae: Mammalia). *Zoological Journal of the Linnean Society,* 153**,** 187-211.

Cardini, A. 2003. The geometry of the marmot (Rodentia: Sciuridae) mandible: phylogeny and patterns of morphological evolution. *Systematic Biology,* 52**,** 186-205.

Cardini, A. & Elton, S. 2008. Does the skull carry a phylogenetic signal? Evolution and modularity in the guenons. *Biological Journal of the Linnean Society,* 93**,** 813-834.

Carraway, L. N., Verts, B. J., Jones, M. L. & Whitaker, J. J. J. 1996. A search for age-related changes in bite force and diet in shrews. *American Midland Naturalist,* 135**,** 231-240.

Cooper, N. & Purvis, A. 2009. What factors shape rates of phenotypic evolution? A comparative study of cranial morphology of four mammalian clades. *Journal of Evolutionary Biology,* 22**,** 1024-1035.

Goodman, S. M., Raxworthy, C. J., Maminirina, C. P. & Olson, L. E. 2006. A new species of shrew tenrec (*Microgale jobihely*) from northern Madagascar. *Journal of Zoology,* 270**,** 384-398.

Gould, E. & Eisenberg, J. F. 1966. Notes on the biology of the Tenrecidae. *Journal of Mammalogy,* 47**,** 660-686.

Gunz, P. & Mitteroecker, P. 2013. Semilandmarks: a method for quantifying curves and surfaces. *Hystrix*.

HERC, U. B. 2013. *RHOI Fossil Photography Protocol*. http://rhoi.berkeley.edu/RHOI\_photo/RHOI\_Photography\_Protocol.html. (Accessed 20/05/2013).

IUCN. 2012. *International Union for Conservation of Nature*. Available: http://www.iucnredlist.org/initiatives/mammals/description (Accessed 26/10/2012).

Knox Jones, J. & Manning, R. W. 1992. Insectivores. *Illustrated key to skulls of genera of North American land mammals.* Texas Tech University Press.

MacPhee, R. D. E. 1987. The shrew tenrecs of Madagascar: Systematic revision and holocene distribution of *Microgale* (Tenrecidae, Insectivora). *American Museum Novitates,* Number 2889**,** 1-45.

Marshall, C. D. & Eisenberg, J. F. 1996. *Hemicentetes semispinosus*. *Mammalian Species*. Available: http://www.science.smith.edu/msi/pdf/i0076-3519-541-01-0001.pdf [Accessed 20/06/2013].

Mitteroecker, P. & Gunz, P. 2009. Advances in geometric morphometrics. *Evolutionary Biology,* 36**,** 235-247.

Nagorsen, D. W. 2002. An identification manual to the small mammals of British Columbia.

Nowak, R. M. 1983. *Walker's Mammals of the World, 4th edition,* Baltimore, Johns Hopkins University Press.

Olson, L. E. 2013. Tenrecs. *Current Biology,* 23**,** R5-R8.

Olson, L. E., Goodman, S. M. & Yoder, A. D. 2004. Illumination of cryptic species boundaries in long-tailed shrew tenrecs (Mammalia: Tenrecidae; *Microgale*), with new insights into geographic variation and distributional constraints. *Biological Journal of the Linnean Society,* 83.

Olson, L. E., Rakotomalala, Z., Hildebrandt, K. B. P., Lanier, H. C., Raxworthy, C. J. & Goodman, S. M. 2009. Phylogeography of *Microgale brevicaudata* (Tenrecidae) and description of a new species from western Madagascar. *Journal of Mammalogy,* 90**,** 1095-1110.

Panchetti, F., Scalici, M., Carpaneto, G. M. & Gibertini, G. 2008. Shape and size variations in the cranium of elephant-shrews: a morphometric contribution to a phylogenetic debate. *Zoomorphology,* 127**,** 69-82.

Poux, C., Madsen, O., Glos, J., de Jong, W. W. & Vences, M. 2008. Molecular phylogeny and divergence times of Malagasy tenrecs: Influence of data partitioning and taxon sampling on dating analyses. *BMC Evolutionary Biology,* 8.

R Core Team. 2013. *R: A language and environment for statistical computing*. Vienna, Austria. Available: http://www.R-project.org/).

Rohlf, F. 2013. TpsDig2 ver 2.17. Department of Ecology and Evoluion, State University of New York, Stony Brook, NY.

Scalici, M. & Panchetti, F. 2011. Morphological cranial diversity contributes to phylogeny in soft-furred sengis (Afrotheria, Macroscelidea). *Zoology,* 114**,** 85-94.

Symonds, M. R. E. 2005. Phylogeny and life histories of the ‘Insectivora’: controversies and consequences. *Biological Reviews,* 80**,** 93-128.

Viscosi, V. & Cortini, A. 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLOS One,* 6**,** e25630.

White, T. A. & Searle, J. B. 2008. Mandible asymmetry and genetic diversity in island populations of the common shrew, *Sorex araneus*. *Journal of Evolutionary Biology,* 21**,** 636-641.

Wilson, D. E. & Reeder, D. M. (eds.) 2005. *Mammal species of the world. A taxonomic and geographic reference (3rd ed)*: Johns Hopkins University Press.

Zelditch, M. L., Swiderski, D. L. & Sheets, D. H. 2012. *Geometric Morphometrics for Biologists, second edition,* United States of America, Academic Press, Elsevier.