**Overview**

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 (SI), the American Museum for Natural History (AMNH), Harvard’s Museum for Comparative Zoology (MCZ) and the Field Museum for Natural History (FMNH). 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.

**Species and Taxonomy**

I collected morphological measurements of xxx species from four orders; Afrosoricida, Erinaceomorpha, Soricomorpha and Notoryctemorphia comprising 7 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 tenrec and their sister taxa, golden moles. Collecting morphological measurements of golden moles would allow me to assess morphological disparity within tenrecs compared to their nearest sister taxa (e.g. Harmon et al. 2003? Need to check if this is the right paper). For the comparison species, I chose a random sample of taxa which have been previously identified as convergent with tenrecs (e.g. [Gould and Eisenberg, 1966](#_ENREF_12), [Symonds, 2005](#_ENREF_28), [Poux et al., 2008](#_ENREF_26), [Olson, 2013](#_ENREF_22)). 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.

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; Mammal Species of the World ([MSW05, Wilson and Reeder, 2005](#_ENREF_31)) and the International Union for the Conservation of Nature ([IUCN, 2012](#_ENREF_15)).

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

Wilson and Reader ([2005](#_ENREF_31)) record 30 species of tenrec. However, more recent studies indicate that there are now 34 accepted species of tenrec ([Olson, 2013](#_ENREF_22)). The additional species belong to the shrew tenrec (*Microgale*) genus and represent either recognition of cryptic species boundaries ([Olson et al., 2004](#_ENREF_23)) or discovery of new species ([Goodman et al., 2006](#_ENREF_11), [Olson et al., 2009](#_ENREF_24)). 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.

**(Sexual dimorphism and adult/juvenile issues)**

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_23))).

I checked for obvious signs that specimens were juveniles by looking for incomplete fusion of the crania and non-fully erupted dentition (*references??)* However, basing age classification in tenrecs on dental studies has many associated difficulties. For example, in some species, the last molar does not erupt fully until the first molar has been shed so the full dentition is not present at any one time ([Nowak, 1983](#_ENREF_21)). In particular, it is difficult to distinguish deciduous from permanent teeth in *Microgale* tenrecs ([Asher and Lehmann, 2008](#_ENREF_5)) which has led to confusion and misidentification of juvenile forms as separate species ([Olson et al., 2004](#_ENREF_23)).

I identified one specimen of *Potamogale velox* in my data setas a possible juvenile since the lower and upper molars are not fully erupted. *(Remove this from the analysis??)* None of the other specimens I measured could be obviously identified as juveniles so, in the absence of any evidence to the contrary, I treated them all as equivalent adult forms.

**Linear Measurements**

**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.

**Measurement descriptions**

Using a 15mm digital calipers (Mitutoya 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 or ecological traits, 2) their usefulness for assessing overall shape and size of the specimen and 3) their ease of repeatability both within and among specimens from different species (*not phrased very well).*

*(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_9)). |
| 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.*

In my initial pilot study at the NHML, I took three separate replicates of each measurement, cycling through them in order to avoid measuring the same variable twice in a row. When I checked these data for measurement errors (see section x below), there was a high error rate associated with some of the measurements. Variables which were frequently (but not always) measured to be less than 2mm were particularly prone to high error rates, most likely due to the difficulties associated with placing the calipers in exactly the same position for each measurement of such small structures and due to the tendency for measurement error to be relatively larger in small structures ([Cardini and Elton, 2008](#_ENREF_8)). Therefore, during my time in subsequent museum collections, I chose to take 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 is one or two outlying measurements there should be at least 3 replicates which are in close agreement. *(I don’t like this explanation but it probably needs something here?).*

**Photography**

**Photographic setup**

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.

**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.

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.

**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_6)). (*Probably need a disclaimer somewhere about not being interested/ worried about lateral asymmetry.)*

**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 and it 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.

**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.

**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_14)).

**Error Checking**

Museum-based and morphometric studies are prone to a number of different error sources. These include 1) taxonomic identification which has not been updated to currently accepted terms, 2) curatorial misidentification of species, 3) the accuracy and repeatability with which species traits are measured, 4) morphometric errors associated with photographing specimens and the placement of landmarks. I address each of these possible sources of error below.

1. **Taxonomic errors**

I recorded species names as they were written on museum specimen labels and checked them for taxonomic accuracy using Wilson and Reader’s Mammal Species of the World ([2005](#_ENREF_31)) 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_11)), which are not included in Wilson and Reader’s text, I just used the taxonomy as it was recorded on the labels.

I corrected the species names from the museum labels as necessary. There were 25 species labelled with incorrect taxonomy due to their placement in incorrect genera, labelling with a no-longer accepted species’ synonym or misidentification of their sub or full species status.

1. **Curatorial errors** *(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. **Measurement errors**

I took three replicate measurements of most of my variables and five replicates of some variables which were often (but not always) less than 2mm in size (see section xx above). Some morphometric studies take replicate measurements of a trait and use the average value for further analyses. (*is this true? check papers*)

However, the average measured value can be skewed by any outlying measurements and may not give a good estimate of central tendency. Therefore, I chose to use the median value for each measurement because it is likely to be 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_10)). 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_10)). 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_10)). 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 a nicer way to present all of this)*

**3.1. 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 %. I repeated my measurement process for xx of these variables from xx skulls and re-measured the variables either 3 or 5 times as appropriate. I re-calculated the measurement error for these repeated measurements which reduced to xx variables from xx skulls which still had a coefficient of variation >5% and percentage spread >25% (Table x)..

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Total data set | | After re-measuring xx variables | |
| Number of; | Variables | Skulls to which the variables belong | Variables | Skulls to which the variables belong |
| Total |  |  |  |  |
| Coefficient of variation > 5% |  |  |  |  |
| Percentage spread >25% |  |  |  |  |
| Re-measured values |  |  |  |  |

Therefore, the final error calculations for all of my skull variables gave a list of xx variables belonging to xx skulls from xx species which had measurement error values higher than my accepted thresholds. These were discarded from further analyses (Table x.)

Table x: Skulls measurements which were discarded from my analysis due to measurement errors calculated as greater than threshold values (coefficient of variation >5%, percentage spread >25%).

|  |  |  |
| --- | --- | --- |
| Skull measurement | Number of skull specimens with measurement error greater than threshold values | Number of species to which the skulls belong |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

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 %. I repeated my measurement process for xx of these variables from xx specimens and re-measured the variables either 3 or 5 times as appropriate. I re-calculated the measurement error for these repeated measurements which reduced to xx variables from xx specimens which still had a 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 re-measuring xx variables | |
| Number of; | Variables | Specimens to which the variables belong | Variables | Specimens to which the variables belong |
| Total |  |  |  |  |
| Coefficient of variation > 5% |  |  |  |  |
| Percentage spread >25% |  |  |  |  |
| Re-measured values |  |  |  |  |

Therefore, the final error calculations for all of my limb measurements gave a list of xx variables belonging to xx specimens from xx species which had measurement error values higher than my accepted thresholds. These were discarded from further analyses (Table x.)

Table x: Limb measurements which were discarded from my analysis due to measurement errors calculated as greater than threshold values (coefficient of variation >5%, percentage spread >25%).

|  |  |  |
| --- | --- | --- |
| Limb measurement | Number of specimens with measurement error greater than threshold values | Number of species to which the limbs belong |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

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

1. **Morphometric errors**

I chose to use 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. While 2D methods are an accepted means of comparing morphological shape (e.g. [Adams et al., 2004](#_ENREF_1), [Mitteroecker and Gunz, 2009](#_ENREF_19)), particularly for comparing skull morphologies of small mammals (e.g. [Cardini, 2003](#_ENREF_7), [Panchetti et al., 2008](#_ENREF_25), [White and Searle, 2008](#_ENREF_30), [Barrow and Macleod, 2008](#_ENREF_6), [Scalici and Panchetti, 2011](#_ENREF_27)), 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_4)). Similarly, personal error with how landmarks are positioned on specimens could also introduce noise into further analyses. In contrast to detailed intraspecific work (e.g. paper??) morphometric measurement error is 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 measurement 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_2)). 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 of 89 species 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_29)).

* 1. **Landmark placement**

Another possible source of measurement error is associated with the placement of landmarks ([Arnqvist and Mårtensson, 1998](#_ENREF_4)). 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_4), [Barrow and Macleod, 2008](#_ENREF_6)). 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 |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**Analysis of linear measurements**

I don’t know what’s going here yet!

**Analysis of photographs; Geometric morphometrics**

*Do I need a general; “this is morphometrics” overview/introduction 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 *(I haven’t figured out how to put R into endnote as a reference)*

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. I have a tps screen shot below but the points come out too small so I’ll probably do another photo shop job on a picture and then add points to that.)*

**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_21), [MacPhee, 1987](#_ENREF_17), [Knox Jones and Manning, 1992](#_ENREF_16), [Marshall and Eisenberg, 1996](#_ENREF_18), [Nagorsen, 2002](#_ENREF_20), [Goodman et al., 2006](#_ENREF_11), [Asher and Lehmann, 2008](#_ENREF_5), [ADW, 2013](#_ENREF_3)) where available to identify the number of premolars and molars.



**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.

**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.

**Mandibles**

I placed x 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_6)). 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.*

**Skins**

Outline analysis of skins for overall body shape?

**Geometric Morphometric Analyses**

After placing landmarks and drawing outline curves in TPSDig *(how to reference this?)*, I used TPSUtil to create a sliders file ([Zelditch et al., 2012](#_ENREF_32)) which depicts which points to treat as semilandmarks ([Gunz and Mitteroecker, 2013](#_ENREF_13)). I used the geomorph package (*how to cite?)* 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**

*Some of the references aren’t formatted properly e.g. I need to fix Nagorsen 2002*

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