Size Relationship of the Tympanic Bullae and Pinnae in Bandicoots and Bilbies (Marsupialia: Peramelemorphia)

by

Melissa Taylor

BSc

This thesis is presented for the degree of Bachelor of Science Honours, School of Veterinary and Life Sciences, of Murdoch University

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Author’s Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

________________________________________

Melissa Taylor
Abstract

Hearing is an important factor allowing species to obtain information about their environment. Variation in tympanic bullae and external pinnae morphology has been linked with hearing sensitivity and sound localisation in different mammals. Bandicoots and bilbies (Order Peramelemorphia) typically occupy omnivorous niches across a range of habitats from open, arid deserts to dense, tropical forests in Australia and New Guinea. The morphology of tympanic bullae and pinnae varies between peramelemorphian taxa. Little is known about the relationship between these structures, or the extent to which they vary with respect to aspects of ecology, environment or behaviour.

This thesis investigated the relationship between tympanic bulla and pinna size in 29 species of bandicoot and bilby. Measurements were taken from museum specimens to investigate this relationship using direct measuring methods and linear dimensions. It was hypothesised that an inverse relationship between bullae and pinnae may exist and that species residing in arid regions would have more extreme differences. Environmental variables were examined to determine the level of influence they had on bullae and pinnae.

This study found that there was a phylogenetic correlation between the structures and that they were significantly influenced by temperature (max/average) and precipitation (average). Species which inhabited more complex, temperate habitats had relatively smaller bullae and pinnae than those in less complex, more arid habitats. Species tended to have either a relatively larger bulla or pinna, with the relationship being more pronounced in species in more arid habitats. No inverse relationship was found between relative bulla and pinna size. Previous studies have found a relationship between relative bulla and pinna and predator evasion tactics; the findings of this study appeared to support this.

These findings suggest that ecological traits and habitat types may be linked with relative bullae and pinnae sizes.
Acknowledgements

I would like to thank the following people for contribution and support throughout this project. Without them this would not have been possible.

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Chapter 1  Introduction

1.1  General Introduction

Hearing plays an important role in how many groups of animals interact with their environment. It is used in several aspects of day to day life from locating possible hazards, both from the environment or from potential predators, to finding food and communicating with others (Vaughan et al., 2015). Sound is captured by the external ear (pinna) and travels through the external auditory meatus to the air-filled middle ear. This middle ear cavity may be enlarged to become a visible protrusion at the base of the skull known as the tympanic bulla. This bulla is often particularly large in small insectivorous species (Hildebrand and Goslow, 1998; Kardong, 2012; Keen and Grobbelaar, 1940). There are a number of external influences that impact the size and shape of these structures including temperature (Maloiy et al., 1982; Webster, 1966) and the frequencies of sound within the environment (Mason, 2015; Schleich and Busch, 2004). These structures have also been noted to be linked with behavioural traits including predator evasion tactics (Pavlinov and Rogovin, 2000).

Understanding how the tympanic bullae and pinnae are impacted by various factors may aid in understanding the ecology of species and how they interact with their environment. Through this it may also be possible to make informed assumptions on the ecological traits and habitat use of species which are hard to study or extinct.

This thesis focuses on determining the relative size relationship between the tympanic bulla and pinna in a number of bandicoot and bilby species (Marsupialia: Peramelemorphia) and the extent to which environmental factors impact this. This introduction provides a background on the mechanisms of hearing, the functional adaptations of tympanic bulla and pinna, how hearing may be linked with extinction risk, and what is known of bandicoots and bilbies.
1.2 Hearing

1.2.1 Mechanisms Behind Hearing

The ear is made up of three different sections: the outer, middle, and inner ear (Kardong, 2012).

Together these components capture sound and transfer them through the inner parts of the ear towards
the auditory nerves after which they are interpreted by the brain (Fig. 1).

The outer ear consists of an auditory meatus which is a channel that leads from the external
environment towards the middle ear. An external pinna is an enlargement surrounding the auditory
meatus that is composed of skin and often supported by cartilage. Pinnae are common amongst many
groups of mammals (Hildebrand and Goslow, 1998; Kardong, 2012). Where pinnae are present, they
function to capture and funnel sound through the auditory meatus towards the tympanic membrane
which separates the outer and middle ear (Kardong, 2012; Webster, 1966).

Three small bones, known as ossicles, are present in the air-filled middle ear cavity. These
ossicles (stapes, incus, and malleus) form a chain connecting the tympanic membrane and the oval
window, a membrane that separates the middle and inner ear (Hildebrand and Goslow, 1998;
Kardong, 2012; Mason, 2016). Ossicles help facilitate the transfer of sound into the inner ear through
impedance matching, a process through which the sound energy is matched to the resistance of the
fluid-filled inner ear while maintaining sound quality. The energy is matched as the ossicles are
arranged in such a way that sound is amplified by as much as ninety times; without this only about
0.1% of sound energy would reach the inner ear (Hickman et al., 2014; Kardong, 2012; Tucker, 2017).
The size of the middle ear cavity has been linked to the hearing threshold of the animal, with smaller
middle ears being linked with a higher frequency hearing (Lay, 1972; Novacek, 1977). Therefore,
larger mammals, who have larger middle ears due to scaling, are able to hear lower frequency sounds
than smaller mammals. To work around this, smaller mammals develop an enlarged middle ear cavity
which protrudes from the bottom of the skull, known as a tympanic bulla (Ravicz and Rosowski,
1997).
The inner ear consists of the vestibular apparatus and the surrounding fluid. The apparatus includes three semicircular canals and two fluid-filled sacks (ultriculus and sacculus) which aid in determining the orientation of the head, and the coiled cochlea (Hildebrand and Goslow, 1998; Kardong, 2012). The cochlea is made up of three fluid-filled coils: the vestibular canal, the base of which is enclosed by the oval window, the tympanic canal, which connects to the vestibular canal at the tip of the cochlea and closed at the base by the round window, and the cochlear duct, which contains the organ of Corti. The organ of Corti consists of hair cells that are thought to be tuned to different frequencies, with higher frequency sounds being detected at the base of the cochlea, and lower frequency sounds towards the tip. The hair cells rest on a basilar membrane which separates the cochlear duct and the tympanic canal, and are covered by a tectorial membrane (Hickman et al., 2014; Hildebrand and Goslow, 1998). When sound travels through the ossicles, the stapes pushes on the oval window which causes the fluid inside the tympanic and vestibular canals to move (Hickman et al., 2014; Kardong, 2012). The movement on this fluid causes shifts in the basilar membrane, which, in turn, causes movement in the hair cells of the organ of Corti. The auditory information is then passed...
into the nerves attached to each of the hair cells before moving towards the brain where it is then utilised to gather information about the animal’s surroundings (Hickman et al., 2014).

1.2.2 Adaptation of the Tympanic Bulla and Pinna

The pinnae are used to improve sound capture by funnelling sound into the auditory meatus. This is demonstrated to great effect in mammals with large, mobile pinnae, such as deer. This allows for greater accuracy in sound localisation by being able to turn in the direction of the source of sound. Depending on the size of the head, this can create an acoustic shadow which helps in creating different auditory cues for each ear that can be used for source localization (Heffner et al., 1996; Jones et al., 2011; Mason, 2015; Webster, 1966). In smaller mammals, it has been suggested that larger pinnae may also facilitate the detection of lower frequency sounds (Jones et al., 2011).

Smaller pinnae reduce the ability to detect sound directionality and therefore animals with small pinnae rely more on high-frequency pinna cues (Heffner et al., 1996; Mason, 2015) than individuals with larger pinnae. Experiments have demonstrated that localisation ability is reduced when higher frequency sounds are filtered out (Heffner et al., 1996).

Environmental factors affect pinnae size in mammals, such as the habitat in which species live. Mammalian species that live in subterranean or aquatic habitats, such as subterranean moles and many marine mammals, completely lack pinnae (Webster, 1966). Large pinnae would increase drag in marine environments and likely also be a hazard underground. Reduced pinnae size may be countered by the fact that lower frequency sounds travel greater distances through these environments than higher frequency sounds, making pinnae less useful and possibly even covering the auditory meatus, muffling and confusing the auditory cues (Paula Basso et al., 2017).

In addition to their auditory role, pinnae have also been linked to roles including thermoregulation. For mammals in arid environments, such as the fennec fox, enlarged pinnae are
frequently used as an avenue for heat loss due to their high vascularisation and surface area (Maloiy et al., 1982). It is possible that bilbies also use their ears to this end as they, like the fennec fox, live in arid areas and have enlarged pinnae. Though there have been no studies to test if this is the case. Larger mammals such as elephants, might also use large pinnae for heat loss and have been noted to flap their ears to cool down. In cold environments conversely, animals tend to have smaller pinnae to reduce heat lost. Many mammals have lost the ability to move their pinnae, and thus it could be hypothesised that they have become a predominantly thermoregulatory rather than a localisation adaptation in these mammals (Webster, 1966).

Like the pinnae, the middle ear region has also been noted to influence the hearing of animals. It has been observed that larger middle ears are correlated with a lower frequency hearing in mammals (Lay, 1972; Ravicz and Rosowski, 1997). An enlarged middle ear may therefore be an adaptation to lower the hearing threshold in smaller mammals that would otherwise have their hearing restricted to higher frequencies. This region, known as the tympanic bulla, is common in smaller mammals and is particularly large in insectivores (Keen and Grobbelaar, 1940).

Auditory cues are used by prey species to detect the presence of predators (Deecke et al., 2002; Schleich and Vassallo, 2003) and adaptations that allow for this would be selected for. Avian predators and snakes appear to generate low-frequency sounds (Huang et al., 2002; Schleich and Vassallo, 2003), early detection of which can trigger avoidance behaviour in prey species. In rodents, larger tympanic bullae are correlated with improved predator avoidance, lending further credibility to the notion that larger bullae allow for lower frequency hearing (Mason, 2015). Further, it has been noted in kangaroo rats (Dipodomys merriama) which had their tympanic bullae surgically reduced, that their ability to detect two desert predators: screech owl (Otus asio) and sidewinder rattlesnake (Crotalus cerastes), was greatly reduced. It is suggested the larger tympanic bullae reduce the dampening on the tympanic membrane, increasing auditory sensitivity (Webster, 1962).

Environmental factors may also influence the size of the tympanic bulla. It has been observed that species in arid areas have larger tympanic bullae than those in more temperate areas (Hunt, 1974;
Keen and Grobbelaar, 1940; Mason, 2015). This may be because the absorption of high frequency increases in arid conditions (Knudsen, 1931; Lay, 1972), making it more difficult to perceive higher-frequency sounds over a distance. Through the reduction of sound transmission in these environments, flying predators are likely to be favoured as their hunting methods tend to keep them at a distance from their prey without hindering their ability to locate it. This can cause issues in predator detection in arid regions where there are already limited sound sources and refuges for prey species, making early detection of threats important (Hunt, 1974; Lay, 1972). Avian predators and snakes have been documented to emit low-frequency sounds during attack (Huang et al., 2002). By having larger tympanic bullae it would increase the hearing sensitivity of prey species, adapting them to the high sound absorption of their environments and low sound producing predators. Other environmental factors, such as altitude have also been linked with bullae size, such as in daurian pika (Ochotona daurica) where individuals in higher altitudes had smaller bullae than those occupying lower altitudes (Liao et al., 2007)

A link between the size of the tympanic bullae and pinnae has been previously investigated in desert specialised rodents and related this relationship to predator avoidance tactics (Pavlinov and Rogovin, 2000). Two main trends were found regarding this relationship: a larger pinna where the tympanic bulla remained small, or a larger tympanic bulla where the pinna remained small. It was also found that this relationship influenced the escape tactics of the species. Individuals that had more passive tactics, such as hiding in nearby undergrowth or in burrows had larger bulla whereas individuals that made a speedy escape to outrun predators had larger pinna. It was theorised that this was due to the type of sound information most useful for each escape tactic. A passive approach benefited from early detection and therefore these species had larger bullae to increase hearing sensitivity. A speedy escape however benefited more from increased localisation and therefore favoured larger pinnae. Other studies have mentioned, anecdotally, patterns regarding bulla and pinna sizes. For instance, it has been observed that in species (eg. Dipodomys sp.) that inhabit arid areas
there is an inverse relationship between pinna size and bullae size, i.e. that as the pinna gets larger, the
tympanic bulla generally becomes smaller (Mason, 2015).

1.2.3 Hearing and the Critical Weight Range

Extinction risk in mammals tends to increase with body size due to a variety of factors including
generation length, low population density, and low fecundity (Johnson and Isaac, 2009). In most
countries, the main causes of modern extinction are human development, hunting, and habitat loss,
which predominantly target larger species that are most affected by these factors due to their home
range requirements and their desirability as hunting trophies (Cardillo et al., 2005; Woinarski et al.,
2015). However, the most influential factors contributing to extinction risk in Australian mammals are
predation from introduced predators and changing fire regimes (Woinarski et al., 2015). The red fox
*(Vulpes vulpes)* and domestic cat (*Felis catus*) were both introduced by European settlers and have
since been implicated as a major threat to native Australian mammals (Hunter et al., 2018; Johnson
and Isaac, 2009; Woinarski et al., 2015).

Australia has a very high extinction rate, with over 10% of the endemic mammal fauna having
gone extinct since European settlement just over 200 years ago, and a further 27% currently under
threat. Australian mammal extinctions make up about 35% of modern mammal extinctions (Woinarski
et al., 2015). Australian mammal species are believed to be particularly vulnerable to introduced
predators due to their evolutionary isolation for about 40 million years, making them naïve to
predation tactics of placental predators and the cues that indicate their presence (Russell and Banks,
2005). Mid-sized, ground dwelling native mammals ranging from 35g to 5.5kg have been shown to be
at the greatest risk of extinction (Burbidge and McKenzie, 1989; Johnson and Isaac, 2009).

The critical weight range (CWR) hypothesis attempts to explain why there is such a high
extinction rate within this weight range (Burbidge and McKenzie, 1989; Cardillo and Bromham, 2001;
Johnson and Isaac, 2009). It suggests that species that fall within the CWR have increased
vulnerability due to their limited mobility and relatively high metabolic rates, which due to the increased changes in the landscape for agricultural use means that there is less plant productivity and habitat available to them (Burbidge and McKenzie, 1989). This may predispose these species to being unable to recover efficiently from disturbances. The theory also proposes that this vulnerability is exploited by introduced predator species, and to a lesser extent introduced herbivorous species (Burbidge and McKenzie, 1989; Johnson and Isaac, 2009; Russell and Banks, 2005).

CWR species fall into the preferred prey size range for introduced predators such as the red fox and domestic cat, which predominantly prey on ground-dwelling species (Hunter et al., 2018; Martin and Palumbi, 1993; Russell and Banks, 2005; Woinarski et al., 2015). The effect of these species has been observed in areas where predators have been removed or excluded. The removal of foxes had a strong positive effect on the population size of CWR species (Hunter et al., 2018; Woinarski et al., 2015). Programs such as Western Australia’s Western Shield baiting scheme targets introduced predators and have been shown to decrease predator numbers, resulting in an increase in native fauna (Doherty and Algar, 2015; Woinarski et al., 2015).

Prey naivety is often cited as the reason for Australian mammal vulnerability to introduced predators (Frank et al., 2016). Studies have been conducted to test how native species react to predator cues such as scent, and have found that they do not respond to the scent cues of foxes (Mella et al., 2011; Russell and Banks, 2005). It has been noted that while native prey species do not appear to react to predator cues from foxes and feral cats, they will however avoid domestic dogs (Canis familiaris). This is thought to be due to the similarity between domestic dogs and the dingo (Canis familiaris dingo) which has been present on the Australian mainland for approximately 4000-5000 years (Frank et al., 2016; Hunter et al., 2018). Corroborating this are observations that bandicoot populations in Tasmania, with no previous exposure to dingoes, do not show any avoidance to dogs, in contrast to those on the mainland (Frank et al., 2016). It has been suggested however that Australian marsupials are more likely to use audio or visual cues to detect predators which provide a greater amount of information concerning predation risk (Mella et al., 2011). It is possible that the reaction of native
species that have been attributed to scent cues may be due to other cues present, or that scent cues play a less important role in predator detection than previously thought.

1.3 Bandicoots and Bilbies

The marsupial Order Peramelemorphia includes a number of bandicoot species in a single extant family and two extinct (Families Peramelidae, Chaeropodidae† and Yaralidae†) and a single extant bilby species (Thylacomyidae) which are native to Australia and New Guinea (Fig. 4) (Hall et al., 2016; Warburton and Travouillon, 2016). They inhabit a variety of habitats including rainforests, woodlands, heath, and arid areas throughout both countries, including Tasmania and other islands surrounding Australia (Driessen and Rose, 2015; Hall et al., 2016).

There are currently 31 recognised modern species within the order (Table 1), and a number of fossil species (Travouillon and Phillips, 2018; Warburton and Travouillon, 2016). Of the modern species, eight have gone extinct within the last century: the Desert Bandicoot (Perameles eremiana), the Marl (Perameles myosuros), the Nullabor Barred Bandicoot (Perameles papillon), the Liverpool Plains Striped Bandicoot (Perameles fasciata), the South-eastern Striped Bandicoot (Perameles notina), the Lesser Bilby (Macrotis leucura), the Southern Pig-footed Bandicoot (Chaeropus ecaudatus), and the Northern Pig-footed Bandicoot (Chaeropus yirratji) (Travouillon and Phillips, 2018; Travouillon et al., 2019; Warburton and Travouillon, 2016; Woinarski et al., 2015).

Bandicoots are predominantly nocturnal, opportunistic omnivore species that dig to forage for food using forelimbs adapted for this function (Hall et al., 2016; Warburton and Travouillon, 2016). They eat a variety of invertebrates, fungi, and plant material. They opportunistically will prey upon eggs, juvenile rodents (Driessen and Rose, 2015; FitzGibbon et al., 2007; Hall et al., 2016), with the long nosed bandicoots having been observed to excavate a pardalote (Pardalotus punctatus) nest and eat the juvenile birds (Guppy and Guppy, 2018). In urban areas they have been known to eat lawn pests and will visit camping and picnic areas to scavenge (Hall et al., 2016). Bandicoots and bilbies...
have the second shortest gestation of any mammal, averaging between 12 and 14 days (Dickman and Ganf, 2008; Flores et al., 2013; Hall et al., 2016; Warburton and Travouillon, 2016). Breeding seasons are long, lasting several months, with some species capable of breeding all year round and producing multiple litters in a year if conditions are favourable (Hall et al., 2016). This coupled with their generalist nature would normally indicate that these species should be more resistant to disturbance (Dickman and Ganf, 2008; Johnson and Isaac, 2009). Despite this, eight species have gone extinct in recent times (Woinarski et al., 2015) and currently five of the extant species in Australia are considered threatened to some degree (Table 1).

Introduced predators, habitat destruction, altered fire regimes, and competition from introduced grazers are common threatening factors within Australia (Hall et al., 2016). All Peramelemorphia species fall into the critical weight range which makes them particularly vulnerable to predation by introduced predators (Frank et al., 2016; Hall et al., 2016). While the threatening processes within Australia are fairly well understood and recognised, those that affect species in New Guinea are not as well studied. Three species in New Guinea have been classed as data deficient (DD in Table 1), lending itself to the claim by Warburton and Travouillon (2016) that these species are under studied. This could mean, that despite not knowing the threatening processes in New Guinea, these species may be more vulnerable than is currently believed.
Table 1 - Table summarising the 26 modern peramelemorphian species’ country of origin and status. Country abbreviations: AU, Australia; NG, New Guinea. IUCN Red List Categories: EX, extinct; EW, extinct in the wild; CR, critically endangered; EN, endangered; VU, vulnerable; NT, near threatened; LC, least concern; DD, data deficient; NE, not evaluated. Species list, status, and habitat sourced from (Hall et al., 2016; IUCN, 2019; Travouillon and Phillips, 2018; Travouillon et al., 2019; Warburton and Travouillon, 2016; Westerman et al., 2012)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Common Name</th>
<th>Country</th>
<th>Status</th>
<th>Habitat Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaeropodidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chaeropus ecaudatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. e. ecaudatus (NSW/VIC/SA)</td>
<td></td>
<td>AU</td>
<td>EX</td>
<td>Open, arid and semiarid grassland</td>
</tr>
<tr>
<td>C. e. occidentalis (WA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chaeropus yirratji</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroryctidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microperoryctes alpini</td>
<td>Arfak Pygmy Bandicoot</td>
<td>NG</td>
<td>DD</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Microperoryctes longicauda</td>
<td></td>
<td>NG</td>
<td>LC</td>
<td>Open/Closed, tropical forest, grassland</td>
</tr>
<tr>
<td>Microperoryctes murina</td>
<td>Mouse Bandicoot</td>
<td>NG</td>
<td>DD</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Microperoryctes ornata</td>
<td>Eastern Striped Bandicoot</td>
<td>NG</td>
<td>LC</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Microperoryctes papuensis</td>
<td>Papuan Bandicoot</td>
<td>NG</td>
<td>LC</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Peroryctes broadbenti</td>
<td>Giant Bandicoot</td>
<td>NG</td>
<td>EN</td>
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<tr>
<td>Peroryctes raffrayana</td>
<td>Raffray’s Bandicoot</td>
<td>NG</td>
<td>LC</td>
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<td>Rhynchomeles prattorum</td>
<td>Seram Bandicoot</td>
<td>NG</td>
<td>EN</td>
<td>Closed, forest</td>
</tr>
<tr>
<td>Peramelidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echymipera clara</td>
<td>Clara’s Spiny Bandicoot</td>
<td>NG</td>
<td>LC</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Echymipera davidi</td>
<td>David’s Spiny Bandicoot</td>
<td>NG</td>
<td>EN</td>
<td>Closed, tropical forest</td>
</tr>
<tr>
<td>Echymipera kalubu</td>
<td>Common Spiny Bandicoot</td>
<td>NG</td>
<td>LC</td>
<td>Closed, forest</td>
</tr>
<tr>
<td>Echymipera echinista</td>
<td>Menzie’s Spiny Bandicoot</td>
<td>NG</td>
<td>DD</td>
<td>Open/Closed, forest, savanna</td>
</tr>
<tr>
<td>Echymipera rufescens</td>
<td>Long-Nosed Echymipera</td>
<td>AU/NG</td>
<td>LC</td>
<td>Closed, tropical/vine forest (NG/AU), coastal heath (AU)</td>
</tr>
</tbody>
</table>
### Isoodon

**Isoodon auratus**  
*I. a. auratus* (mainland)  
*I. a. barrowensis* (Barrow Isl.)  
- **Golden Bandicoot**  
  - AU  
  - VU  
  - Open, grasslands, heath, coastal scrub

**Isoodon fusciventer**  
- **Quenda**  
  - AU  
  - LC  
  - Open/Closed, forest, shrub, grassland, wetlands

**Isoodon macrourus**  
*I. m. macrourus* (WA/NT)  
*I. m. moreshyensis* (NG)  
*I. m. toross* (QLD)  
- **Northern Brown Bandicoot**  
  - AU  
  - LC  
  - Open, forest, grassland, shrub

**Isoodon obesulus**  
*I. o. affinis* (TAS)  
*I. o. nauticus* (SA)  
*I. o. obesulus* (NSW/VIC)  
- **Southern Brown Bandicoot**  
  - AU  
  - EN  
  - Closed, forest, woodland, shrub, heath

**Isoodon peninsulae**  
- **Cape York Brown Bandicoot**  
  - AU  
  - LC  
  - Open, grassland, savanna

**Perameles bougainville**  
- **Shark Bay Bandicoot**  
  - AU  
  - EN  
  - Open, dense scrub, grassland

**Perameles eremiana**  
- **Desert Bandicoot**  
  - AU  
  - EX  
  - Open, arid/semi-arid, grassland

**Perameles fasciata**  
- **Liverpool Plains Striped Bandicoot**  
  - AU  
  - EX  
  - Open, grassland

**Perameles gunnii**  
*Pe. g. gunnii* (TAS)  
- **Eastern Barred Bandicoot**  
  - AU  
  - VU/EN  
  - Open grassland, woodland

**Perameles myosuros**  
- **Marl**  
  - AU  
  - EX  
  - Open

**Perameles nasuta**  
- **Long-Nosed Bandicoot**  
  - AU  
  - LC  
  - Open, forest, heath

**Perameles notina**  
- **South-eastern Striped Bandicoot**  
  - AU  
  - EX  
  - Open

**Perameles pallescens**  
- **Northern Long-Nosed Bandicoot**  
  - AU  
  - LC  
  - Open/Closed forest, heath

**Perameles papillon**  
- **Nullabor Barred Bandicoot**  
  - AU  
  - EX  
  - Open, arid/semi-arid

### Thylogomidae

**Macrotis lagotis**  
- **Bilby**  
  - AU  
  - VU  
  - Open, arid/semi-arid, grassland, woodland

**Macrotis leucura**  
*M. l. minor*  
- **Lesser Bilby**  
  - AU  
  - EX  
  - Open, arid, grassland

Since European settlement many Australian mammals, including bandicoots and bilbies, have seen large range reductions due to human activity (Claridge and Barry, 2000; Warburton and
Travouillon, 2016). Increasing urbanization has the potential to benefit generalist species, with species such as the southern brown bandicoot and the long-nosed bandicoot persisting in urban areas in Sydney (Chambers and Dickman, 2002; Dowle and Deane, 2009), northern brown bandicoots inhabiting areas within Brisbane (FitzGibbon et al., 2007), and quenda have been observed in urban areas within the greater Perth region, and in urban and suburban regions throughout the south west of WA (Bryant et al., 2017; Howard et al., 2014). While these species are capable of inhabiting urban environments, most peramelemorphian species are unable to exploit these areas and are forced out due to human impact leaving it unsuitable for them (Warburton and Travouillon, 2016).

Bandicoots and bilbies have varying pinnae sizes across different species (Hall et al., 2016) (Fig. 3). It has also been noted that there are varying sizes in tympanic bullae between species (Travouillon and Phillips, 2018), the bilby having an enlarged bullae (Flores et al., 2013), which fits the trend of arid species having larger, more developed bullae when compared to other species (Hunt, 1974; Keen and Grobbelaar, 1940; Lay, 1972).

1.4 Study objective

Hearing is an important factor which influences the ecological traits of individuals by providing information on their surroundings. Understanding how this impacts species can aid in informing management practices as these relationships can be complex (Travouillon and Phillips, 2018) and introducing a species or subspecies into a new range may result in them not detecting the right acoustic cues to avoid local predators. This relationship and the underlying factors that impact this can be used to extrapolate ecological information on hard to study or extinct species for which little is known.
This study aims to determine the relationship between the tympanic bullae and pinnae in bandicoots and bilbies and which environmental factors are the most influential in determining the relative size of these structures. It is predicted that species which have relatively large bullae will have smaller pinnae and vice versa as it was thought that species would rely more on either low-frequency sound detection or sound localisation. It is also predicted that this relationship will be at its most extreme in arid environments.

The specific aims of this thesis are as follows:

1) To determine the size relationship between tympanic bulla and pinna;

2) To determine the relationship between species and genera using comparisons of transformed bulla volume and pinna surface area and phylogenetic analysis;

3) To determine which environmental factors are influencing tympanic bulla and pinna size.
Chapter 2  Materials and Methods

2.1 Specimens

Skulls and skins preserved in alcohol of representative peramelemorphian species were sourced from the Western Australian Museum (WAM), Australian Museum (AM), Museum Victoria (NMV), Queen Victoria Museum & Art Gallery (QVMAG), South Australian Museum (SAM), Natural History Museum UK (NHMUK), American Museum of Natural History (AMNH), and Queensland Museum (QM). Only adult specimens were used: skulls were aged by dentition, being deemed adults if all of the teeth had fully erupted; wet skins were deemed adults by their body size and if the pouch or testes appeared fully developed. A total of 800 specimens were measured, with roughly equal numbers of skulls (n = 412) and skins (n = 388) (Table 2).

Only skulls that were deemed to be in a reasonable condition to obtain their dimensions were included. Wet skins were only used if at least one ear was in good condition with minimal damage and able to be flattened out in order to take reliable measures. One dry skin was used as the only accessible wet skin was for a juvenile (Pe. myosuros, n=1), though due to the shrinkage of dry tissues the results for this specimen should be considered an underestimate of actual size. Ten skulls were removed from the data set for the analysis due to some ambiguity in their taxonomy (Isoodon sp n =1 and I. obesulus n = 1), their tympanic bulla volume (TBV) being markedly smaller than the other individuals of that species (Mi. ornata, n = 4), or due to being outliers when comparing all species (I. fusciventer, n = 3; Ma. lagotis, n = 1).
Table 2 - Summary of specimens from which measures were collected. Does not include specimens which were removed for analysis. * denotes the use of a dry skin

<table>
<thead>
<tr>
<th>Species</th>
<th># of Skulls</th>
<th># of Skins</th>
<th>Total</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. yirratji</em></td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>SAM, NMV</td>
</tr>
<tr>
<td><em>E. clara</em></td>
<td>13</td>
<td>3</td>
<td>16</td>
<td>AM</td>
</tr>
<tr>
<td><em>E. davidii</em></td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>AM</td>
</tr>
<tr>
<td><em>E. kalubu</em></td>
<td>69</td>
<td>27</td>
<td>96</td>
<td>AM, WAM</td>
</tr>
<tr>
<td><em>E. rufescens</em></td>
<td>14</td>
<td>5</td>
<td>19</td>
<td>AM, WAM, QM</td>
</tr>
<tr>
<td><em>E. r. australis</em></td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>AM, QM</td>
</tr>
<tr>
<td><em>I. a. auratus</em></td>
<td>8</td>
<td>14</td>
<td>22</td>
<td>WAM</td>
</tr>
<tr>
<td><em>I. a. barrowensis</em></td>
<td>9</td>
<td>30</td>
<td>39</td>
<td>WAM</td>
</tr>
<tr>
<td><em>I. fasciventer</em></td>
<td>82</td>
<td>125</td>
<td>207</td>
<td>AM, WAM</td>
</tr>
<tr>
<td><em>I. m. macrourus</em></td>
<td>13</td>
<td>46</td>
<td>59</td>
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</tr>
<tr>
<td><em>I. m. moresbyensis</em></td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>AM</td>
</tr>
<tr>
<td><em>I. m. torosus</em></td>
<td>11</td>
<td>16</td>
<td>27</td>
<td>AM, WAM</td>
</tr>
<tr>
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<td>3</td>
<td>6</td>
<td>AM, WAM, QVMAG</td>
</tr>
<tr>
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<td>5</td>
<td>2</td>
<td>7</td>
<td>WAM, SAM</td>
</tr>
<tr>
<td><em>I. o. obesuslus</em></td>
<td>4</td>
<td>10</td>
<td>14</td>
<td>AM, WAM</td>
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<tr>
<td><em>Ma. lagotis</em></td>
<td>37</td>
<td>19</td>
<td>56</td>
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</tr>
<tr>
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<td>5</td>
<td>1</td>
<td>6</td>
<td>NMV, AM, NHMUK</td>
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<td><em>Mi. longicauda</em></td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>AM</td>
</tr>
<tr>
<td><em>Mi. murina</em></td>
<td>1</td>
<td>1</td>
<td>2</td>
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</tr>
<tr>
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<td>13</td>
<td>36</td>
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<tr>
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<td>10</td>
<td>AM, WAM</td>
</tr>
<tr>
<td><em>Pe. bougainville</em></td>
<td>13</td>
<td>15</td>
<td>28</td>
<td>WAM</td>
</tr>
<tr>
<td><em>Pe. eremiana</em></td>
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<td>1</td>
<td>3</td>
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</tr>
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<td>8</td>
<td>11</td>
<td>AM, WAM, NMV, QVMAG</td>
</tr>
<tr>
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<td>-</td>
<td>5</td>
<td>AM, WAM</td>
</tr>
<tr>
<td><em>Pe. myosuros</em></td>
<td>3</td>
<td>1*</td>
<td>4</td>
<td>AM</td>
</tr>
<tr>
<td><em>Pe. nasuta</em></td>
<td>33</td>
<td>27</td>
<td>60</td>
<td>AM, WAM</td>
</tr>
<tr>
<td><em>Pe. papillon</em></td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>WAM</td>
</tr>
<tr>
<td><em>Per. raffrayana</em></td>
<td>17</td>
<td>4</td>
<td>21</td>
<td>AM, WAM</td>
</tr>
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<td><strong>Total</strong></td>
<td><strong>402</strong></td>
<td><strong>388</strong></td>
<td><strong>790</strong></td>
<td></td>
</tr>
</tbody>
</table>

2.2 Data Collection

2.2.1 Skulls

Measurements were made of maximum skull length in millimetres (mm) using digital callipers (Absolute Cooland Proof IP67, Mitutoyo) (Cooper, 2000; Onar, 1999) (Fig. 2b). Maximum length, width and height of the right tympanic bulla (Paula Basso et al., 2017) were measured if it was intact or had suffered only superficial damage that would not affect these measurements (Fig. 2cde). In cases where the right tympanic bulla was deemed too damaged to be used, the left one was used in its stead.
The height of the bulla was measured from the glenoid fossa, where the mandible connects with the zygomatic arch (Figure 2de). The volume of the tympanic bulla was measured using a fine sand (EC Rainbow Sand, Chocolate Brown, Melbourne, Australia), the density of which was calculated \( D = \frac{M}{V} \); where \( D \) = Density, \( M \) = Mass, and \( V \) = Volume.

To measure tympanic bulla volume (TBV) a paper funnel was used to direct the sand into the tympanic bulla until full. The sand was then poured out of the bulla and into a vial of known weight and weighed using a small set of jewellery scales (MH-8028-series, Professional Digital Jewellery Scale). The mass of sand (minus the mass of the vial itself) was then used to determine the volume \( V = \frac{M}{D} \) of sand. For the New Guinea species, the bullae are incomplete, but rather are shaped as an overhang with one open side. In order to measure the volume of these specimens the gap was closed using a finger while the bulla was filled with sand. For some specimens the TBV measurements were unable to be collected using this method and so measurements were obtained from a pre-existing database (Travouillon, unpubl. data). To check for the reliability of the direct, non-destructive method of measuring the TBV the dimensions were used to calculate an estimated volume using half the volume of a sphere:

\[
\frac{1}{2} \left( \frac{4}{3} \pi \left( \frac{\text{TBL}}{2} + \frac{\text{TBW}}{2} + \frac{\text{TBH}}{2} \right)^{3} \right) \]; \quad \text{eq. 1}
\]

where TBL = Tympanic Bulla Length, TBW = Tympanic Bulla Width, and TBH = Tympanic Bulla Height.
Figure 2- Skull measurements for an Australian (*I. fusciventer*; a, b & d) and New Guinea (*E. kalubu*; c & e) species. a) & c) Length and width of tympanic bulla; b) Skull Length for both species groups; d) & e) Skull height and tympanic bulla height

2.2.2 Skins

Pinnae measurements were taken from wet skins preserved in formalin and ethanol as they are less likely to have shrunk than dry skins. The right pinna was used when intact. For those that were damaged or too stiffly bent in such a way that may influence the measurements, the left pinna was used instead. The height and width of the pinnae (Fig. 3cd) was measured (mm) using digital callipers (Absolute Cooland Proof IP67, Mitutoyo). Height was measured from the base of the tragus. Head height, width, and length were measured for the wet skins (Fig. 3ab). Most of these specimens still contained their skulls.
To directly measure pinna surface area (PSA), impression moulds of the inner surface of the pinnæ were made using a silicon-based mould (Pinkysil® putty, Barnes, Sydney, Australia). The Pinkysil mould was mixed according to the instructions on the packaging, left to set for 2-8 mins (depending on ambient temperature) until the mould felt firm, and then gently removed from the ear. Surface area calculation from these moulds was challenging due to the concave shape of the pinnæ, particularly in bilbies, and some shape irregularities. In order to more accurately measure surface area, the moulds were then coated in a layer of liquid latex (RubberLatex, Jacksons Drawing Supplies, Perth) to create a secondary mould, which was more malleable and could be flattened out to calculate surface area. The liquid latex was applied using a paint brush and allowed to dry before being removed from the Pinkysil mould (Fig. 3be). To calculate surface area the latex mould had its shape traced onto 5x5mm grid paper. This drawing was then scanned, made into a black and white image using the open source painting program Krita (Stitching Krita Foundation, 2018), and then the surface area was calculated using the open source program ImageJ (Rasband, 1997-2018) (Fig. 3f).

Some of the specimens from the Australian Museum were measured by museum staff after the initial collection period and therefore did not have a pinkysil mould taken of their ears (S. Ingleby, pers. comm.). The single dry skin was a mounted specimen; therefore, a piece of tissue paper was folded around the back of the ear and then the shape of the ear was traced onto it. From this the width and height of the pinna were able to be measured using digital callipers. As with the TBV, the dimensions were used to calculate an estimate of the pinnæ surface area. The area of a circle was used to calculate this:

\[
\pi(((PW/2)+(PH/2))/2)^2 \]

where \( PW = \) Pinna Width and \( PH = \) Pinna Height.
2.2.3 Predictor Variables

Habitat type was obtained from Warburton & Travoullion (2016) (Table 1) and categorised as open, closed or a mix of both (Table 3). A distribution map was made in QGIS (QGIS Development Team, 2019) for the species used in the analysis using data obtained from the IUCN (2017) terrestrial mammal database, Warburton & Travoullion (2016), and from personal communications with K. Travoullion (Fig. 4). The map was used to obtain temperature and precipitation maximum and average values for each of the species ranges using the worldclim database (Fick and Hijmans, 2017).

Habitat, temperature and precipitation data for each species were used as environmental predictor traits for further analysis (Table 3)
Table 3 – Environmental predictor traits used in phylogenetic analysis for each species. Tmax = Temperature max; Tavg = Temperature average; Pmax = Precipitation maximum; Pavg = Precipitation average.

<table>
<thead>
<tr>
<th>Sp</th>
<th>Habitat</th>
<th>Tmax (°C)</th>
<th>Tavg (°C)</th>
<th>Pmax (mm)</th>
<th>Pavg (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. yirratji</td>
<td>Open</td>
<td>42.4</td>
<td>22.7</td>
<td>513</td>
<td>249.2</td>
</tr>
<tr>
<td>E. clara</td>
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<td>33.2</td>
<td>25.85</td>
<td>4244</td>
<td>2797.9</td>
</tr>
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<td>Closed</td>
<td>31.29</td>
<td>26.5</td>
<td>3751</td>
<td>3517.5</td>
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<tr>
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<td>24</td>
<td>7322</td>
<td>3044</td>
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<tr>
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<td>26</td>
<td>2150</td>
<td>1558.9</td>
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<td>24</td>
<td>7322</td>
<td>3023.6</td>
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<td>1123</td>
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<td>25.7</td>
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<td>302.8</td>
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<tr>
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<td>26.2</td>
<td>3453</td>
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</tr>
<tr>
<td>l. m. torosus</td>
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<td>21.7</td>
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</tr>
<tr>
<td>l. o. affinis</td>
<td>Closed</td>
<td>22.6</td>
<td>10</td>
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<td>l. o. obesulus</td>
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<td>30.1</td>
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<td>248.6</td>
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<td>Mi. longicauda</td>
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<td>2695</td>
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<td>224</td>
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<tr>
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<td>34.1</td>
<td>20.1</td>
<td>7322</td>
<td>3045.8</td>
</tr>
</tbody>
</table>
2.3 Statistical Analysis

The calculated and measured TBVs values were transformed using a cube root and plotted on a scatterplot using Rstudio (R Core Team, 2013) with an added regression line and $R^2$ value (Fig. 6a).

The calculated and measured values for the pinnae surface areas were transformed using a square root and plotted on a scatterplot with an added regression line and an $R^2$ value (Fig. 6b). The slopes of the regression line was compared to a slope of linearity ($\beta = 1$) to test if there was any significant difference between the two using a linear regression comparison in StatistiXL (Roberts and Withers, 2019). Specimens for which only the calculated values were available for were not used in the comparison.
Four species (*E. kalubu, I. fusciventer, Ma. lagotis* and *Pe. nasuta*) were chosen due to their large sample size (Table 2) for intraspecific comparison between skull length and TBV, and head length and PSA (Fig. 7). The values calculated from the linear dimensions for both TBV and PSA were cube-root and square root transformed respectively, these values were used for all subsequent analyses to allow for direct comparison. Pearson correlation tests were run for each of the intraspecific and species pairwise comparisons to test for the significant differences in the slopes using StatistiXL (Roberts & Withers 2019). A slope comparison ANOVA was run for each species comparing the slopes for TBV and PSA, and for each species pair for TBV and PSA using StatistiXL (Roberts and Withers, 2019). The average values for skull length, head length, cube-root transformed TBV and square root transformed PSA were then calculated for each species. These were plotted against each other for interspecific comparison (Fig. 8).

Species averages were used to calculate residuals for TBV against skull length and PSA against head using Statistica (TIBCO Software Inc., 2017). The residuals were expressed as a proportion of the predicted values to obtain standardised residuals for the TBV or PSA to control for larger specimens having larger tympanic bullae or pinnae by virtue of their size.

The genera represented three bulla shapes: an open bulla (*Echymipera* spp., *Microperoryctes* spp. and *Per. raffrayana*) (Fig. 2), a closed spherical bulla (*C. yirratji* and *Perameles* spp.) (Travouillon and Phillips, 2018; Travouillon et al., 2019) and a closed conical bulla (*Isoodon* spp. and *Macrotis* spp.) (Fig. 2). The species residuals for each of the bullae types were used to conduct a single factor ANOVA test to compare the relative sizes of the genera of to see if there were significant size differences within bulla type.

A phylogenetic tree was made by combining multiple trees (Travouillon and Phillips, 2018; Travouillon et al., 2019; Westerman et al., 2012), creating a tree with arbitrary branch lengths and then built on Rstudio (Fig 5). Four species were not found in any of the existing trees (*Pe. myosuros, I. m.*
moralesyensis, *I. o. nauticus*, and *Mi. murina*) and were placed in polytomies with its expected closest relative (K. Travouillon pers. comm.). Phylogenetic tests were conducted to test for the influence that the relatedness of the species had upon pinnae surface area and tympanic bullae volume using the standardised species residuals. Due to the lack of specimens from which PSA was measured *Mi. longicauda* had a species residual calculated from the average of all the species residuals of *Microperoryctes* species and *Pe. g. gunnii* was allocated the same standardised residual as *Pe. gunnii* as it is a sub-species of it. Habitat type (open/closed/mixed), temperature (max/average) and precipitation (max/average) were used as predictor variables. The habitat type was used as a proxy to the type of ground and overhead cover available to each species. Dummy values for habitat were used in the analysis (Open = 0, Mixed = 0.5, Closed = 1). Temperature was used to assess the level of thermal pressure each species is under, with the maximum temperature used to represent the extremes of this pressure and how that may influence the pinnae in particular, as this structure has been known to facilitate thermoregulation through heat dissipation (Maloiy et al., 1982; Webster, 1966). Precipitation max and average were used as a proxy to habitat productivity and through this the extent of cover available to each of the species for foraging, predator avoidance, and resting.

A Blomberg’s K test (Blomberg et al., 2003) was conducted using an Excel macro (Withers, n.d) on standardised species residuals for both the tympanic bullae volume and pinnae surface area to test for the phylogenetic signal and whether the species are more or less similar than expected under Brownian evolution. The standardised species residuals for both the TBV and PSA, and the environmental predictor variables were run through the macro to obtain phylogenetic independent contrast (PIC) values for each of the traits.

The PIC values were used to conduct a linear/non-linear test in Statistica (TIBCO Software Inc., 2017) to generate models of trait subsets that would best describe the variation in either TBV or PSA and the corresponding Akaike Information Criterion (AIC). From this corrected AIC (AICc) values were calculated using the methods in Burnham, Anderson & Huyvaert (2011) for each of the
models and listed from smallest to largest AICc. Delta (Δ) values were calculated from the AICc values and models with a Δ value of two or less were considered to have substantial support (Burnham and Anderson, 2004) and therefore used in further analysis. A relative likelihood value for each model was calculated (exp(-0.5*Δ)), which was then used to calculate a model-weight by dividing the likelihood value of each model by the sum of the likelihood values and multiplying by a hundred to obtain a percentage.

Multiple regressions were run for each model using Statistica (TIBCO Software Inc., 2017) to test which of the traits had a significant influence. The standardised beta values obtained from the multiple regressions were multiplied by the model-weight to obtain weighted beta values. The sum of these for each trait can be divided by a hundred to remove the model weights and give an overall estimate of influence of each trait on either the TBV or the PSA (Table 6).

The test for which environmental trait had the greatest influence on TBV and PSA was conducted a second time without the use of PIC values to correct for the phylogenetic relatedness of the species. The test was conducted in an identical manner as stated above (Table 7).
Figure 5 – Phylogenetic tree showing relative relatedness of all species and subspecies in the analysis. * denotes species not present on existing trees.
Chapter 3  Results

3.1  Methods for Measurements

The two methods for calculating TBV (sand versus linear dimensions) had highly correlated results (Fig. 6a) and a relationship that was not significantly different from slope of linearity ($\beta = 1$) (slope comparison ANOVA test: $F_{1,756} = 0.91, p = 0.341$). The dimension method of calculating TBV (eq. 1) appeared to slightly underestimate the volume for *Echymipera* spp., *Macrotis* spp., *Microperoryctes* spp., and *Per. raffrayana*. when compared to the values obtained from the sand method, whereas it appeared to overestimate the TBV of *Isoodon* spp. (Fig. 6a).

The two methods for calculating PSA (moulds versus linear dimensions) also had highly correlated results (Fig. 6b), although the slope of this relationship was significantly different from $\beta = 1$ ($F_{1,388} = 8.01, p = 0.005$). The calculation of PSA (eq. 2) using linear dimensions appeared to overestimate the surface area for *Macrotis* spp. and *Perameles* spp. compared to the values calculated from the moulds, whereas it appeared to underestimate the surface area of *Microperoryctes* spp.. The other genera (*C. yirratji*, *Echymipera* spp., *Isoodon* spp., *Per. raffrayana*) appeared to have a more equal spread of values (Fig. 6b)
Figure 6 – Scatterplot comparing a) the cube-root transformed values for tympanic bulla volume (mm³) and b) log10 transformed values for pinna surface area (mm²). Line of best fit added (tympanic bulla volume: $y = 1.008x + 8.33$, $R^2 = 0.9601$; pinna surface area: $y = 1.061x - 0.078$, $R^2 = 0.8792$).
3.2 Intraspecific Variation in Tympanic Bulla Volume and Pinna Surface Area

For the four species where there were sufficient samples to investigate intraspecific correlations between TBV and skull length or PSA and head length, all four showed significant evidence of allometry in TBV and PSA (i.e. the slopes of the relationship were > 0) (Fig. 7). The Pearson correlation test found that each relationship was statistically significant (Table 4).

The allometry of TBV to skull length and PSA to head length could be seen in the slopes for each relationship (Fig. 7). Species that had larger differences in $\beta$ values had a more pronounced difference in allometry (Table 4). All of the species showed a stronger positive allometry in TBV than PSA (Table 4, Fig. 7). *Echymipera kalubu* had the lowest difference in allometry for TBV and PSA, having $\beta$ values that were the most similar to each other with *Pe. nasuta* having a slightly larger difference (Table 4). *Isoodon fusciventer* and *Ma. lagotis* showed the greatest allometric difference, their $\beta$ values for TBV being markedly larger than their PSA and that of the other two species (Table 4). TBV had a stronger correlation to skull length than PSA had to head length in all species except *E. kalubu* (Table 4).

*Echymipera kalubu* and *Pe. nasuta* had no significant difference in the slopes of TBV and PSA, while *I. fusciventer* and *Ma. lagotis* had a significant difference (Table 4). Each of the species ranked the same in TBV and PSA increase: *E. kalubu* had the lowest, *Pe. nasuta* had the second lowest, *I. fusciventer* had the second highest, and *Ma. lagotis* had the highest (Table 4).
Table 4 – Summary of slopes comparing cube-root transformed TBV to skull length and square root transformed PSA to head length for each species with Pearson Correlation test significance shown. * denotes significant value

<table>
<thead>
<tr>
<th></th>
<th>Echymipera kalubu</th>
<th>Isoodon fusciventer</th>
<th>Macrotis lagotis</th>
<th>Perameles nasuta</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TBV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.11*</td>
<td>0.46*</td>
<td>0.63*</td>
<td>0.47*</td>
</tr>
<tr>
<td><strong>PSA</strong></td>
<td>0.18</td>
<td>0.09</td>
<td>5.708</td>
<td>0.149</td>
</tr>
<tr>
<td><strong>β</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Slope Comparison ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>0.245</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*</td>
<td>0.231</td>
</tr>
</tbody>
</table>

Out of the six pairwise species comparisons for TBV allometry two had non-significant differences in slope (E. kalubu against Pe. nasuta and I. fusciventer against Ma. lagotis) while the other four comparisons had significant differences in slopes (Table 5). None of the species comparisons had a significant difference in PSA allometry slopes (Table 5).

Table 5 – P - value for slope comparison ANOVA test comparing slopes between pairs of species for cube-root transformed TBV (above the diagonal) and square root transformed PSA (below the diagonal). * denotes significance; p < 0.05

<table>
<thead>
<tr>
<th></th>
<th>Echymipera kalubu</th>
<th>Isoodon fusciventer</th>
<th>Macrotis lagotis</th>
<th>Perameles nasuta</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echymipera kalubu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*</td>
<td>0.299</td>
<td></td>
</tr>
<tr>
<td><strong>Isoodon fusciventer</strong></td>
<td>0.061</td>
<td>0.164</td>
<td>p &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td><strong>Macrotis lagotis</strong></td>
<td>0.108</td>
<td>0.135</td>
<td>p &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td><strong>Perameles nasuta</strong></td>
<td>0.482</td>
<td>0.562</td>
<td>0.376</td>
<td></td>
</tr>
</tbody>
</table>
Figure 7 – Scatterplots comparing cube-root transformed tympanic bulla volume (as calculated from linear dimensions) to skull length, and square-root transformed pinna surface area (as calculated from linear dimensions) to head length for *E. kalubu* (a & b), *I. fusciventer* (c & d), *Ma. lagotis* (e & f), and *Pe. nasuta* (g & h).
3.3 Interspecific Differences in Tympanic Bulla Volume and Pinna Surface Area

Bulla volume had no correlation with skull size ($R^2 = 0.044$) and appeared to be linked to phylogeny. *Macrotis lagotis* had the relatively largest tympanic bulla, *Isoodon* spp. and *Ma. le. minor* had intermediate sized bullae, *Perameles* spp., *C. yirratji* and *I. m. moresbyensis* had smaller bullae still distinguishable from *Per. raffrayana*, *Echymipera* spp., and *Microperoryctes* spp. which had relatively the smallest bullae (Fig. 8a).

The New Guinea genera (*Echymipera* spp., *Microperoryctes* spp. and *Per. raffrayana*) had similar sized bullae, though they had a large range of skull sizes from the smallest species (*Mi. murina*) to the second largest species (*E. clara*) (Fig. 8a). *Perameles* spp., and *C. yirratji* had larger bullae with skull sizes that split into two distinct groups whereby most of the species had skulls which averaged at approximately 60mm in length, and two species (*Pe. g. gunnii* and *Pe. nasuta*) which had skulls approximately 80mm in length (Fig. 8a). *Isoodon* spp. had the largest range of TBV, the smallest of which (*I. m. moresbyensis*) had a similar size to the larger of the *Perameles* species and *C. yirratji*. *Macrotis leucura minor* had a TBV larger than that of the *Isoodon* species, with a skull of intermediate size (Fig. 8a). *Macrotis lagotis* had the bullae which were relatively 1.5 times larger than the next largest species (*Ma. le. minor*); this species also had the largest skull size which was 1.08 times larger than the next largest species (*E. clara*) (Fig. 8a).

As with the tympanic bullae, PSA appeared to be linked to phylogeny, though within this it appeared to be linked with body size (Fig. 8b). Across all the species a weak correlation between pinnae size and head length was found ($R^2 = 0.193$). *Macrotis lagotis* had the largest pinnae, *Ma. le. minor* and *C. yirratji* had intermediate pinnae, *Perameles* spp. had smaller pinnae, though separated from *Echymipera* spp., *Isoodon* spp., *Microperoryctes* spp., and *Per. raffrayana* which had relatively the smallest pinnae (Fig. 8b). *Microperoryctes murina* had the smallest pinnae and head length with the remainder of *Microperoryctes*, *Echymipera* spp., *Isoodon* spp., and *Per. raffrayana* had larger,
though similar sized pinnae that increased with head size (Fig. 8b). *Perameles* spp. had larger pinnae with similar size, with the exception of *Pe. myosuros* which had pinnae of similar size to *Isoodon* spp. (Fig. 8b). *Chaeropus yirratji* and *Ma. le. minor* had intermediate head length which were similar to each other, however, *Ma. le. minor* had pinnae which were 1.27 times larger than that of *C. yirratji* (Fig. 8b). *Ma. lagotis* had the largest pinnae of all the species, which were 1.28 times larger than the next largest species (*Ma. le. minor*) and this species also had the largest head size which was 1.1 times larger than the next largest species (*Pe. nasuta*) (Fig. 8b).

There was no significant difference found in the slopes of TBV and PSA (slope comparison ANOVA: $F_{1,52} = 1.87, p = 0.187$).

ANOVA tests were conducted to test for significant differences in relative TBV size using standardised species residuals for each of the bulla shape types. The open bullae had a significant difference between the genera (ANOVA: single factor; $F_{2,7} = 6.641, p = 0.024$). Both the spherical and conical closed bullae did not have a significant difference in relative size between the genera (ANOVA: single factor, $F_{1,6} = 0.483, p = 0.513$ & $F_{1,9} = 3.286, p = 0.103$ respectively).
Figure 8 – Scatterplots comparing species averages for cube-root transformed tympanic bulla volume (as calculated from linear dimensions) to skull length (a), and square-root transformed pinna surface area (as calculated from linear dimensions) to head length (b).
3.4 Comparison of Tympanic Bulla Volume and Pinna Surface Area

The standardised species residuals for TBV and PSA were calculated from the species comparisons shown in Figure 9 and plotted against each other (Fig 9). These standardised residuals gave an estimate for how the relative size of the tympanic bullae and pinnae compared to the expected sizes given their skull or head length as calculated from the linear line of best fit in Fig. 8. The species reflected phylogenetic associations, and those species which only had TBV values clustered with the rest of their genera (Mi. longicauda and Pe. g. gunnii) (Fig. 9).

Species in the lower left quadrant of Fig. 9 have relatively small bullae and pinnae than was expected for their size. This group included all but one of the New Guinea species (Mi. papuensis). Echymipera spp., Per. raffrayana and Mi. ornata were clustered relatively close together in both relative tympanic bulla and pinna size. Microperoryctes murina and Mi. papuensis had TBV that clustered with the other species, though had larger pinnae, closer to what would be expected for their size. Microperoryctes longicauda had relatively the smallest bullae but did not have any specimens from which pinnae size was measured (Fig. 9).

Within genera, differences were found between the species. The Echymipera species clustered relatively close together, with E. r. australis separated slightly from the rest of the genus (Fig. 9). Echymipera rufescens australis had relatively the largest bullae and the smallest pinnae, E. davidi had relatively the largest pinnae and E. rufescens had relatively the smallest tympanic bullae. The Microperoryctes species clustered relatively close together in terms relative bullae size. They form two groups in terms of relative pinnae size with Mi. ornata having pinnae relatively smaller than the other species. Microperoryctes papuensis was the only species to have pinnae which were relatively larger than expected for their head size (Fig. 9).

Species in the lower right quadrant had tympanic bullae that were relatively larger than expected for their skull size, and pinnae that were relatively smaller than expected for their head size.
(fig. 9). This group comprises of almost all *Isoodon* spp. except for *I. m. moresbyensis* which had relatively smaller bullae falling into the lower left quadrant, clustered with the *Perameles* species. *I. a. auratus* had relatively the largest tympanic bulla while the other species in *Isoodon* had similar relative tympanic bulla size. The *Isoodon* species appeared to have similar relative pinna size except for *I. a. barrowensis* which had pinna that were closest to the expected size for their body size (Fig. 9).

Most *Perameles* spp. are placed in the upper left quadrant reflecting that they had tympanic bullae that were relatively smaller than expected for their skull size, and pinnae that were relatively larger than expected for their head size. The exceptions were *Pe. papillon* which had relatively larger tympanic bulla, *Pe. myosuros* and *Pe. nasuta* which had relatively smaller pinnae. *Pe. nasuta* had relatively the smallest tympanic bulla, *Pe. myoruros* had relatively the smallest pinna, *Pe. papillon* had relatively the largest tympanic bulla and *Pe. eremiana* had relatively the largest pinna. *Perameles* spp. were loosely clustered together by relative TBV and PSA, except for *Pe. eremiana* which had standardised residual value for PSA that was 6.33 times larger than the next largest species (*Pe. bougainville*). *Perameles gunnii gunnii* had a relative tympanic bulla size that clustered with the smaller *Perameles* species but did not have any specimens from which pinnae were measured (Fig. 9).

*Chaeropus yirratji* had tympanic bulla which were relatively smaller than expected for its skull size which had a similar relative size as *Pe. eremiana*. It had the third largest relative pinna size of all the species, with only *Macrotis* spp. having larger relative sizes (Fig. 9).

In the upper right quadrant, the two *Macrotis* spp. are extreme in their combination of tympanic bullae and pinnae that were relatively much larger than expected for their skull and head size. *Macrotis leucura minor* had the second largest relative bulla size overall, after *I. a. auratus* had the largest and it had the largest relative pinna size. *Macrotis lagotis* had the second largest relative pinna size which was roughly 1.6 times smaller than *Ma. le. minor’s* relative pinna size (Fig. 9).
### 3.5 Predictors of Tympanic Bulla Volume and Pinna Surface Area

Seven top models ($\Delta$AIC$_c$ <2) explained some of the variation in TBV while controlling for phylogenetic relatedness ($R^2$ and pa values given in Table 6a). Each of the models accounted for less than 30% of the variation found in TBV (Table 6a, $R^2$ range 0.207 – 0.288). Precipitation average ($P_{\text{avg}}$) was included in six of the seven models and was the most influential variable in five of these. Precipitation maximum ($P_{\text{max}}$) was included in the only model where $P_{\text{avg}}$ was not present. Temperature maximum ($T_{\text{max}}$) was present in four of the top models and strongest variable in two of these but was the second strongest variable in the other two models. Temperature average ($T_{\text{avg}}$) and PSA were present in two models. Habitat and $P_{\text{max}}$ were present in only of the models (Table 6a).
Ten top models (ΔAICc < 2) explained some of the variation in PSA while controlling for phylogenetic relatedness (Table 6b). Each of the models accounted for less than 17% of the variation found in PSA (Table 6b, $R^2$ range 0.093 – 0.161). None of the models included variables that had significant correlation with PSA. Habitat was included in six of these models and was the most influential in one of these. $P_{\text{max}}$ was included in five of the models and was the most influential in four of these. TBV was included in four of the models and was the most influential variable in one of these. $P_{\text{avg}}$ was included in two where it was the most influential variable. $T_{\text{avg}}$ was included in one model and $T_{\text{max}}$ was not present in any of the models (Table 6b).

Both TBV and PSA were found to have a strong phylogenetic signal. TBV were more similar than expected (Blomberg’s $K$: $K^* = 1.39$, $p < 0.001$) under Brownian motion whereas PSA was less similar than expected (Blomberg’s $K$: $K^* = 0.91$, $p < 0.001$).

Without controlling for phylogenetic relatedness four top models (ΔAICc < 2) explained some of the variation in TBV (Table 7a). Each of the models accounted for 40% or less of the variation found (Table 7a, $R^2$ range 0.378 – 0.401). $P_{\text{avg}}$ was included in all of the models and was the most influential variable in each of these. Habitat, $T_{\text{max}}$ and $T_{\text{avg}}$ were each included in only model. $P_{\text{avg}}$ was found to be of significant influence in each of the models. Across all of the models $P_{\text{avg}}$ had the highest influence on TBV, $T_{\text{avg}}$ the second highest, $T_{\text{max}}$ the second lowest, and habitat the lowest (Table 7a).

Without controlling for phylogenetic relatedness only one model (ΔAICc < 2) explained some of the variation in PSA (Table 7b). $T_{\text{max}}$ was the most influential variable, $P_{\text{max}}$ the second most influential and $T_{\text{avg}}$ the least influential. All of the variables included had a significant influence (Table 7b). The model accounted for approximately 53.6% of the variation found in PSA (Table 7b, $R^2 = 0.536$).
Table 6 - Models of phylogenetically independent contrast (PIC) variable subsets that best explain variation in a) tympanic bulla volume (TBV) and b) pinna surface area (PSA) with associated relative likelihood expressed as AIC model weight ($w_i$).

<table>
<thead>
<tr>
<th></th>
<th>$\Delta$AIC&lt;sub&gt;c&lt;/sub&gt;</th>
<th>$w_i$</th>
<th>$R^2$</th>
<th>$p$</th>
<th>Model-weighted standardised&lt;sup&gt;†&lt;/sup&gt; beta values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. TBV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PSA  Hab  $T_{max}$  $T_{avg}$  $P_{max}$  $P_{avg}$</td>
</tr>
<tr>
<td>Model 1</td>
<td>0</td>
<td>21.78</td>
<td>0.255</td>
<td>0.005*</td>
<td>5.99  -8.86*</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.09</td>
<td>20.76</td>
<td>0.288</td>
<td>0.005*</td>
<td>9.83*  -6.4  -8.29*</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.34</td>
<td>18.25</td>
<td>0.213</td>
<td>0.005*</td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td>1.19</td>
<td>11.99</td>
<td>0.259</td>
<td>0.008*</td>
<td>8.25*  -5.49  -4.31*</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.7</td>
<td>9.28</td>
<td>0.208</td>
<td>0.013*</td>
<td>-1.62  -3.9*</td>
</tr>
<tr>
<td>Model 6</td>
<td>1.76</td>
<td>9.03</td>
<td>0.207</td>
<td>0.013*</td>
<td>1.49  -3.9*</td>
</tr>
<tr>
<td>Model 7</td>
<td>1.81</td>
<td>8.79</td>
<td>0.243</td>
<td>0.011*</td>
<td>1.22  2.3  -3.18</td>
</tr>
<tr>
<td>Overall Beta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.027  0.016  0.263  -0.119  -0.043  -0.372</td>
</tr>
</tbody>
</table>

|                |                          |       |       |     | $T_{max}$  $T_{avg}$  $P_{max}$  $P_{avg}$ |
| **b. PSA**     |                          |       |       |     | TBV  Hab  $T_{max}$  $T_{avg}$  $P_{max}$  $P_{avg}$ |
| Model 1        | 0                        | 18.5  | 0.161 | 0.029* | -5.79  -5.88 |
| Model 2        | 0.96                     | 11.45 | 0.093 | 0.051  | -4.08  |
| Model 3        | 0.96                     | 11.41 | 0.093 | 0.051  | -4.07  |
| Model 4        | 1.06                     | 10.88 | 0.09  | 0.054  | -3.83  |
| Model 5        | 1.45                     | 8.93  | 0.116 | 0.059  | 2.17  -2.61 |
| Model 6        | 1.58                     | 8.38  | 0.112 | 0.064  | -2.07  -2.14 |
| Model 7        | 1.67                     | 8.02  | 0.151 | 0.048  | -2.87  -1.29  -2.8 |
| Model 8        | 1.75                     | 7.71  | 0.067 | 0.082  | 2.46  |
| Model 9        | 1.77                     | 7.61  | 0.148 | 0.0501 | 1.17  -2.01  -2.15 |
| Model 10       | 1.91                     | 7.09  | 0.102 | 0.075  | 1.59  -1.95 |
| Overall Beta   |                          |       |       |     | 0.074  -0.185  0  -0.013  -0.175  -0.062 |

Abbreviations: tympanic bulla volume (TBV), pinna surface area (PSA), Habitat = Hab, $T_{max}$ = Temperature maximum, $T_{avg}$ = Temperature average, $P_{max}$ = Precipitation maximum, and $P_{avg}$ = Precipitation average

†Standardised beta values are calculated after all variables are standardised to a mean = 0 and SD = 1, and are therefore directly comparable between variables.

* denotes models and variables which had a significant influence ($p < 0.05$).
Table 7 - Models of variable subsets that best explain variation in tympanic bulla volume (TBV) and pinna surface area (PSA) with associated relative likelihood expressed as a model weight.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta$AIC&lt;sub&gt;c&lt;/sub&gt;</th>
<th>$w_i$</th>
<th>R²</th>
<th>p</th>
<th>Weighted standardised beta values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PSA</td>
</tr>
<tr>
<td><strong>a. TBV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0</td>
<td>36.57</td>
<td>0.389</td>
<td>p &lt; 0.001*</td>
<td>-</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.68</td>
<td>26.04</td>
<td>0.401</td>
<td>p &lt; 0.001*</td>
<td>4.69</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.01</td>
<td>22.07</td>
<td>0.394</td>
<td>p &lt; 0.001*</td>
<td>3.68</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.74</td>
<td>15.31</td>
<td>0.378</td>
<td>p &lt; 0.001*</td>
<td>2.28</td>
</tr>
<tr>
<td>Overall Beta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

|                  |                        |      |     |                 |       |      |                |                |                |                   |
| **b. PSA**       |                        |      |     |                 |       |      |                |                |                |                   |
| Model 1          | 0                      | 100  | 0.536| p < 0.001*      | 77.91*|      |                | 46.3*           | 47.23*          |
| Overall Beta     |                        |      |     |                 | 0     | 0    | 0.779          | -0.472          | 0               |

Abbreviations: tympanic bulla volume (TBV), pinna surface area (PSA), Habitat = Hab, $T_{\text{max}}$ = Temperature maximum, $T_{\text{avg}}$ = Temperature average, $P_{\text{max}}$ = Precipitation maximum, and $P_{\text{avg}}$ = Precipitation average

†Standardised beta values are calculated after all variables are standardised to a mean = 0 and SD = 1, and are therefore directly comparable between variables.

* denotes models and variables which had a significant influence (p < 0.05).
Chapter 4  Discussion

4.1  Overall Summary of Key Results

Overall, no inverse relationship was found between relative bullae and pinnae sizes as was predicted. While species tended to have either relatively larger TBV or PSA, one did not get larger as the other got smaller. However, the relationship between the two was found to be at its most extreme in arid environments, if not in the way expected. Species in these environments tended towards having relatively the largest bullae and pinnae within their genera. This study also found that there were significant environmental factors influencing bulla size both when phylogeny was controlled for and not. Pinna size did not have any significant influencing factors when phylogeny was controlled for, but some were found when it was not controlled.

A significant correlation was found between the size of species and TBV and PSA in representatives of four of the seven genera (Table 4). This is not surprising as it is expected that as specimens get larger, they would have larger tympanic bullae and pinnae. It was found that was no significant difference at the rate of TBV and PSA growth in *E. kalubu* and *Pe. nasuta* whereas *I. fusciventer* and *Ma. lagotis* did show a significant difference (Table 4). Across all the species used in the intraspecific analysis a significant difference was found in the growth rates of TBV between the species with the steepest slopes (*I. fusciventer* and *Ma. lagotis*) and species with the flattest slopes (*E. kalubu* and *Pe. nasuta*). No significant difference was found in the growth rates of PSA across all of these species (Table 5).

Between all the species included in the analysis it was found that TBV and PSA clustered phylogenetically rather than by the average skull length for each species. There was a greater split between the genera regarding TBV and PSA (Fig. 8).

All the species showed a pattern of having either relatively larger TBV or PSA for their size (Fig. 9). This trend was smallest in *Perameles papillon* which had pinnae and bullae that were closest to having the same standardised residual. The species were shown to cluster according to phylogenetic relationships (Fig. 9). The New Guinea genera were clustered closely
together and had relatively smaller TBV and PSA than the other genera. They had similar relative sizes for tympanic bullae and pinnae except for *Microperoryctes* spp. which had relatively larger pinnae than the other New Guinea genera (Fig. 9).

The Australian species (*C. yirratji, Isoodon* spp., *Macrotis* spp., *Perameles* spp.) clustered together by genera, though the genera had more varied relative TBV and PSA. They had either tympanic bullae or pinnae being relatively larger than the species average, and the other trait being smaller. The exception to this was *Macrotis* spp., which had relatively larger TBV and PSA (Fig. 9).

A strong phylogenetic correlation was found for TBV and PSA, and when controlling for this the other traits (TBV/PSA and environmental predictor variables) were found to account for less than 30% of the variation (Table 6). These PIC values also produced more than five models for each trait to account for the variation. Some traits had a significant influence on TBV whereas no traits had a significant influence on PSA (Table 6). When phylogeny was not controlled for however, the tested traits accounted for a larger percentage of the variation and produced fewer models (Table 7). Fewer traits were included in these models. Only one trait was significant in the TBV models, and the single PSA model found that all three of the included traits (*T*\textsubscript{max}, *T*\textsubscript{avg} and *P*\textsubscript{max}) had a significant influence (Table 7). *P*\textsubscript{avg} was significant in both sets of tests (Table 6 & 8).

### 4.2 Tympanic Bulla and Perception of Sound

Tympanic bullae have been linked with low frequency hearing, the frequencies for which travel further in arid, low humidity environments (Knudsen, 1931; Lay, 1972). *T*\textsubscript{max} and *P*\textsubscript{avg} were significant traits impacting TBV (Table 6a & 8a), though *T*\textsubscript{max} was only significant when PIC values were used. These two traits can be used to estimate environmental aridity and humidity (Quan et al., 2013; Thornton et al., 2000). Species in more arid environments generally had larger TBV than their temperate counterparts (Table 3, Fig. 10).
The New Guinean species, which live in more humid, tropical environments (Table 1 & 3) had the smallest TBV, both in actual size and relative to their skull length (Fig. 7, 9 & 10). The lower frequency sounds that larger bullae are able to discern (Lay, 1972; Webster, 1962) may not be as important in these environments as higher-frequency sounds which would be able to travel further in these environments (Knudsen, 1931). These dense environments are also likely to have complex acoustic environments with a large variety of sound sources, both biotic and abiotic, that do not contain information relevant to individual survival. Denser habitats would also degrade acoustic signals by providing obstacles for sound to hit and be refracting from, possibly altering the frequency of the signals (Blumenrath and Dabelsteen, 2004; Boncoraglio and Saino, 2007). The dense environment would also result in predators which would be more likely to create higher-frequency sounds by stepping on the undergrowth and moving through the dense vegetation. By decreasing TBV it would increase the hearing threshold of the species to higher frequency sounds (Novacek, 1977), possibly to target sounds emitted by their prey and predators. Native predators for the New Guinea species are understudied however they are hunted by humans for their meat (Cuthbert and Denny, 2014).

The New Guinean species have a different bullae structure, with an open bulla shaped like an overhang, rather than the enclosed bulla present in the Australian species. This type of bulla appears to be ancestral trait for bandicoots (Gurovich et al., 2014; Travouillon et al., 2010) suggesting that these species have not required the development of a more complex bulla. The acoustic environments and sounds that are important to these species has therefore probably not changed. The effects this open bulla may have on the hearing sensitivity of these species is currently unknown. It is possible that as the tympanic bulla is not closed in these species that the middle ear cavity is larger than could be measured from the skulls.

The effect that the dense, tropical habitat and its associated environmental factors had on bullae size can be seen in *I. m. moresbyensis* which, while having a relative pinnae size similar to that of the rest of *Isoodon* spp. and *Microperoryctes* spp., had a markedly smaller bullae than the rest of its genus, closer to that of the other New Guinea species (Fig. 9).
The Australian species occupy habitats with a range of densities, temperatures and precipitation levels (Table 3). *Perameles* spp. had the smallest relative TBV of the Australian genera and occupy open habitats ranging from arid to more temperate climates (Table 1, Fig. 9). Within the genus, species occupying more arid environments tended to have larger relative TBV (Table 3, Fig. 9) as was predicted. *Perameles bougainville*, which inhabits one of the most arid regions however had a smaller relative TBV than the other arid species (Table 3, Fig. 9).

*Chaeropus yirratji* had relatively small TBV for its size, clustering with the larger of *Perameles* spp. (Fig. 9). These species had relatively spherical tympanic bullae which were relatively smaller than majority of the other Australian species (Fig. 8 & 10). *Chaeropus yirratji* and *Perameles* spp. had relatively larger TBV than the New Guinea species and reside in more arid habitats where avian and reptile predators have been documented for these species (Richards and Short, 2003) which produce low-frequency sounds (Huang et al., 2002). The larger TBV would therefore be useful in predator detection within these habitats.

Australian species that occupied a mix of open and closed habitats in more temperate regions had relatively large TBV and included all but *I. m. moresbyensis* of the genus *Isoodon* (Table 3, Fig. 9). *Isoodon auratus auratus* had the largest relative TBV of all the species (Fig. 9) despite occupying a less arid region than the other subspecies *I. a. barrowensis* (Table 3), going against the expected trend. This noticeably larger TBV is due to the addition of *I. a. auratus* specimens from the Canning Stock Route which have been noted to have much larger bullae (K. Travouillon, pers. comm.). This was noticed at too late a date to separate these specimens.

*Isoodon macrourus macrourus* had relatively larger TBV than the other subspecies *I. m. torosus* (Fig. 9) and inhabits an area with higher $P_{av}$ and $T_{av}$ (Table 3) increasing the relative humidity in the environment (Quan et al., 2013). It is likely that there are other factors that impact TBV which were not accounted for in this analysis that contribute to *I. a. auratus* and *I. m. macrourus* having larger bullae than predicted. The *I. obesulus* subspecies followed the expected pattern of
species in more arid environments having larger relative TBV (Table 3, Fig. 9). The predicted higher relative humidity in these environments would allow for higher-frequency sounds to travel further (Knudsen, 1931; Lay, 1972), reducing the overall environmental importance of lower-frequency sounds. Native predators for Isoodon spp. include avian and reptile species (Driessen and Rose, 2015) which emit lower-frequency sounds (Huang et al., 2002; Webster, 1962). The larger TBV increasing the sensitivity of their hearing (Lay, 1972; Pavlinov and Rogovin, 2000) may therefore be for early predator detection (Hunt, 1974; Novacek, 1977) rather than to collect general environmental acoustic information.

The species which resided in the most arid regions (Macrotis spp.) had relatively the largest TBV of all the species except I. a. auratus (Table 3, Fig. 9). The two Macrotis species live in open, arid habitats (Table 1 & 3) where low-frequency hearing is more important (Knudsen, 1931). Macrotis leucura minor had relatively larger TBV than Ma. lagotis and inhabited a more arid region (Table 3, Fig. 9). These species are predated upon by avian and reptile species (Rafferty, 2018)

Three types of tympanic bullae shape were observed within the species: an open bulla (Echymipera spp., Microperoryctes spp. and Peroryctes spp.) (Fig. 2), a closed spherical bulla (C. yirratji and Perameles spp.) (Travouillon and Phillips, 2018; Travouillon et al., 2019) and a closed conical bulla (Isoodon spp. and Macrotis spp.) (Fig. 2). The open bullae had significant differences in the relative size between the genera of this type. The two closed bulla types however did not find a significant difference within the genera of the spherical and conical bullae (Section 3.3). The denser, more complex tropical habitats of the New Guinean species may expose these species to a variety of acoustic environments that require significantly different bulla sizes not required by the other bulla types. However, the two closed bulla types may have significant differences that were not detected due to low species numbers in one of the two genera compared for each type (A single Chaeropus species and the two Macrotris species).
The advantages of spherical versus the more conical bulla shape that exists within these species is unclear as both types of bullae exist in a variety of habitats (Table 3). Significant differences in the rates in which TBV increased per millimetre of skull length were found between representatives of four of the genera included (Table 5). Interestingly, *E. kalubu* was not significantly different from *Pe. nasuta* despite their differently shaped bullae and *Pe. nasuta* residing in an open, more temperate environment. Likewise, no significant difference was found between *Ma. lagotis* and *I. fusciventer*. This difference in TBV growth could suggest that lower-frequency and high sensitivity hearing is more important in *Isoodon* spp. and *Macrotis* spp. than in *Echymipera* spp. (and by extrapolation the other New Guinean genera) and *Perameles* spp..

*Chaeropus yirratji*, which shares the spherical bulla shape and was not significantly different in relative bulla size from *Perameles* spp., may also not rely on low-frequency hearing to the same extent as *Isoodon* spp. and *Macrotis* spp..

Insects tend to produce higher frequency sounds, though some small species are known to produce lower-frequency sounds due to their size restricting the amount of energy available for producing sound (Bennet-Clark, 1998). It is possible that *Isoodon* spp. and *Macrotis* spp. have a larger proportion of insects in their diet compared to the other genera, or target species that emit lower-frequency sounds, explaining some of the variation in bulla size. However, it has been noted that *Ma. lagotis* relies more on smell than hearing while foraging (Johnson and Johnson, 1983). *Perameles* spp., which may target species with higher-frequency calls and *C. yirratji*, which was herbivorous (Travouillon et al., 2019) would therefore rely on their bullae more for predator detection than foraging. The New Guinean species may have a large proportion of fruit in their diet, relying less on invertebrates and thereby sound to forage (K. Travouillon, pers. comm.). This statement however would require further study to substantiate.
4.3 Thermoregulation and Pinna Size

Species that inhabited more arid areas tended to have relatively larger pinnae (Table 3, Fig. 9). This was seen to be the most extreme in *C. yirratji* and *Macrotis* spp. which had relatively the largest pinnae when compared to the other species (Fig. 8 & 10) and inhabited some of the more arid environments (Table 3). While some of this may be a result of needing larger, mobile pinnae for sound localisation (Jones et al., 2011; Pavlinov and Rogovin, 2000), it may at least partially be due to their large surface area being conducive to heat loss via thermoregulation (Maloiy et al., 1982; Webster, 1966). Amongst species of *Perameles*, those inhabiting more arid environments (*Pe. bougainville*, *Pe. eremiana* and *Pe. papillon*) had pinnae that were relatively large for their size (Fig. 9). *Isoodon* spp. and the New Guinean genera have relatively smaller pinnae overall suggesting that they do not rely on their pinnae for thermoregulation in their more temperate and tropical habitats.

This hypothesis was not supported in the predictor variable analysis when using PIC values (Table 6b), however $T_{avg}$ was only present in two of the ten models and was not considered a significant factor in either of these. PIC values may be less reliable in this instance as arbitrary branch lengths were used in creating the phylogenetic tree used (Fig. 5), reducing the accuracy of the tests conducted (Cruz-Neto et al., 2001). It is possible that as the larger pinnae are closely correlated with phylogeny that the effects of the predictor variables were masked by the strength of the relationship (section 3.5). However, when non-PIC values were used $T_{max}$, $T_{avg}$ and $P_{max}$ were included in the single model (Table 7b). These factors can be used to estimate the general aridity and humidity of an environment (Quan et al., 2013; Thornton et al., 2000). $T_{max}$ was found the most influential variable in PSA with $P_{max}$ being the second most. This would suggest that pinnae could be used for thermoregulation and would help explain why the more arid species have larger pinnae.

However, bandicoots and bilbies are nocturnal animals which reside in shelter throughout the day (Abbott, 2001; Warburton and Travouillon, 2016). They would therefore not be exposed to the higher temperatures in the arid environments that diurnal species such as the
fennec fox and elephant (Maloiy et al., 1982; Webster, 1966). It is therefore possible that
despite following existing patterns of arid species having larger pinnae that are used for
thermoregulation, that this is not the function that they serve in these species. Bilbies reside in
extensive burrows throughout the day and becomes active at night (Abbott, 2001; Johnson and
Johnson, 1983) as they have a low tolerance for heat stress (Gibson and Hume, 2000). Bilbies
do however have a higher field metabolic rate during the summer than they do in winter
(Gibson and Hume, 2000), suggesting they may be producing more excess heat when the
ambient temperature is higher. It is currently unknown if this excess heat would be enough to
cause heat stress if not dissipated. It was suggested by Gibson & Hume (2000) that this
difference in metabolic rate could be due to individuals spending more time in their burrows
during winter as they provide a stable thermal environment. The use of pinna as a
thermoregulatory structure could therefore be of a low priority within these species unlike more
diurnal species (Maloiy et al., 1982; Webster, 1966). Instead the larger pinnae in these species is
likely to be a result of the requirement for accurate sound localisation. In the open environments
that these species reside in it may not be adequate to only have early predator detection supplied
by larger bullae, but to be able to tell which direction they are coming from.

4.4 Habitat Complexity and Pinna Size

$P_{avg}$ can be used as an indicator of habitat complexity (Huxman et al., 2004; Sala et al.,
1988) and was a significant impact variable when non-PIC values were used in the predictor
variable analysis (Table 7b). While not significant $P_{max}$ and $P_{avg}$ were included in seven of the
ten top models found when PIC values were used (Table 6b).

Species that inhabited more complex environments (Table 3) tended towards relatively
smaller pinnae (Fig. 9). The New Guinea species have relatively some of the smallest PSA. It is
possible that this is due to the dense, tropical habitat that these species inhabit. In these kinds of
environments larger pinnae may be a detriment as they are more likely to get caught on the
surrounding vegetation and therefore either get damaged as a result. These dense environments
are also likely to result in some confusion in sound localization, as their complex structure can cause distortions in the sound (Michelsen and Larsen, 1983). Sound localisation is facilitated by larger pinnae (Heffner et al., 1996; Webster, 1966), which may be of lower priority in these environments.

The Australian species tended towards having relatively larger pinnae than the New Guinea species and inhabited less dense, more temperate habitats (Table 1, Table 3). In these environments pinnae are less likely to get caught on the surrounding vegetation, allowing for them to get larger without becoming a detriment. However, *Isoodon* spp. and some *Perameles* spp. are known to be territorial and aggressive towards conspecifics (Hall et al., 2016; Johnson and Johnson, 1983; Travouillon and Phillips, 2018). This kind of behaviour would likely select against larger pinnae as these could be more easily damaged in an encounter, partially explaining why *Isoodon* spp. have relatively some of the smallest pinnae of the Australian species. More arid species inhabit less complex habitats due to the lack of precipitation to allow for more productivity (Huxman et al., 2004; Sala et al., 1988) allowing for larger pinnae without the restriction of environmental obstructions. These less complex habitats would also have fewer objects within them to cause sound distortion (Blumenrath and Dabelsteen, 2004; Michelsen and Larsen, 1983) allowing sounds to travel greater distances in these environments. The larger pinnae here may therefore be a result of their larger surface area allowing for a greater amount of acoustic information to be received by the ear in an environment with fewer sound sources.

### 4.5 Interactions with Escape Behaviour

It is possible that the relationship between TBV and PSA influence the predator avoidance tactics of these species. Pavlinov & Rogovin (2002) found that in specialised desert rodents, species with larger bullae and smaller pinnae tended towards passive evasion tactics such as hiding in undergrowth or burrows; whereas species with smaller bullae and larger pinnae tended towards more active evasion tactics such as running to evade predation. Using this it is possible
to extrapolate possible predator escape behaviours within these species. The relatively larger TBV would allow for greater hearing sensitivity and early detection of low-frequency sounds that some predators emit while hunting (Huang et al., 2002; Webster, 1962). This early predator detection would allow individuals to seek out shelter either in the undergrowth or nearby burrows. Relatively larger PSA allow for more accurate sound localisation (Chen et al., 1995; Fuzessery, 1986; Mason, 2015) which would allow for individuals to be able to pinpoint the location of the predator and run away.

The New Guinea genera and *I. m. moresbyensis* had relatively smaller TBV than PSA (Fig. 9), which would suggest that they take more active tactics such as running away from predators. However, due to habitat complexity it would conserve more energy to use the undergrowth to hide from predators. As little is known of the predators and the overall ecology of the New Guinea species, the predation methods and subsequent predator evasion tactics cannot be commented on with any accuracy.

Within the Australian species *Perameles* spp. and *C. yirratji* had relatively smaller TBV than PSA (Fig. 9), suggesting that they employ more active evasion tactics. This is possible as these species occupy open, less complex habitats which would have fewer places which can be used as hiding spots from predators, particularly in the more arid habitats. These species are exposed to predators which produce low-frequency sounds (Richards and Short, 2003) and they may therefore use their larger pinna to accurately locate their predators and evade capture before running away (Webster, 1962).

Conversely *Isoodon* spp. had relatively larger TBV than PSA (Fig. 9) and occupy more complex habitats that would suggest that they use a more passive evasion tactic. It is possible that these species, when a predator is detected, quickly dash into nearby undergrowth where predators are less likely to be able to reach them.

*Macrotis* spp. had relatively larger TBV than PSA (Fig. 9) and occupy open environments which would have few refuges to be used to hide from predators. However,
bilbies are known to dig burrows (Johnson and Johnson, 1983; Warburton and Travouillon, 2016) within their territory which have been used to evade predators (Steindler et al., 2018). It would therefore be possible that the larger TBV aids in predator detection, allowing them to escape to their burrows. These species did have relatively larger pinnae than the other species, it is possible that these are used in conjunction with the larger pinnae to locate predators and select an appropriate refuge.

4.6 Methodology

Measuring the volume of the tympanic bullae using the non-destructive method of filling the bullae with sand was found to be viable for species with enclosed bullae (C. yirratji, Isoodon spp., Macrotis spp. and Perameles spp.), though it resulted in unreliable values in species with open bullae (Echymipera spp., Microperoryctes spp. and Per. raffrayana). Many of the values obtained from the sand method in the New Guinea species were identical to each other despite varying tympanic bullae dimensions, possibly due to the impractical method of covering part of the opening with a finger to allow the bullae to be filled. An alternative non-destructive method of measuring these kinds of bullae would need to be used if a direct volume measurement was required. Sand, while overall reliable for those with closed bullae, did encounter some issues when the tympanic membrane was partially intact, making the bullae more difficult to fill and empty. Some skulls also had minor obstructions within the bullae, such as ossicles, which may have impacted the overall volume of sand that could be poured into the bullae.

The equation used to calculate the volume of the bullae was found to generate values which were not significantly different from those obtained from the sand method (Fig. 6a). This equation could be used as a non-invasive method, particularly for those with bullae that are more difficult to measure. Though these values would not be as accurate as they could be as they do not take into account the thickness of the bone or any shape irregularities. Despite this, the lack of non-destructive method which is viable for different types of bullae structure leaves the equation as a useful alternative to other methods that could potentially damage the skull.
The linear dimensions method of measuring pinnae surface area was found to be less reliable when compared to the values obtained from the moulds (Fig. 6b). This unreliability could be due to the irregular shape of the pinnae for which the equations do not account for. Another possibility could be that the dimensions are slightly inaccurate due to some pinnae being folded which may not have flattened out properly while being measured. There could also be inaccuracies from the latex moulds from them being slightly stretched when they were flattened out to trace.

Due to the fact that the moulds preserve the shape of the pinnae this method of obtaining the surface would more accurately represent the actual surface area. However, due to the lack of moulds that could be taken from certain specimens, the values obtained from this method was unable to be used in the analysis. Preferably future studies would use moulds rather than dimensions for all specimens for increased accuracy and reliability.

The use of average dimension values for each species were not as accurate as they could be as many of the species had low sample size (Table 2) which would make these values non-representative of the population. Unfortunately, it is difficult to increase the sample size of rare or extinct species, limiting interpretations that can be made. Therefore, the average values used to calculate the species residuals used in this analysis should be regarded as possibly over or underrepresenting the size of both TBV and PSA.

The PIC values used to determine predictor variable influence should be considered as possibly reducing the accuracy of the tests (Cruz-Neto et al., 2001). The method of obtaining PIC values for each of the traits assumes Brownian motion, which may not be in effect and may therefore result in PIC values which are not phylogenetically independent (Freckleton, 2000). The use of both PIC and non-corrected values in this instance therefore take this into account and give alternative explanations for the observed relationships.
4.7 Conclusions and Future Studies

Significant relationships were found with both TBV and PSA for all species and were strongly linked with phylogeny. TBV was found to be significantly linked with $T_{\text{max}}$, $P_{\text{max}}$ and $P_{\text{avg}}$ when controlling for phylogeny, whereas only $P_{\text{avg}}$ was significant when non-phylogenetically controlled. PSA was not found to be significantly linked with any of the included environmental traits when controlling for phylogeny, a surprising result given that larger pinnae have been known to be linked with temperature in arid environments (Maloiy et al., 1982). When phylogeny was not controlled for the expected result of $T_{\text{max}}$, $T_{\text{avg}}$ and $P_{\text{max}}$ having a significant influence was found. However, as discussed above, the larger pinnae may be a result of open arid environments requiring a greater ability to detect sound origin for effective predator evasion. Species in more complex habitats with higher precipitation and lower temperatures were tended to have relatively smaller TBV and PSA than those in more open habitats with lower precipitation and higher temperatures within genera. The New Guinean species were found to have relatively the smallest TBV and PSA of all the species and inhabited dense, tropical regions. TBV appeared to be linked with temperature and precipitation, supporting the idea that bullae are linked with low-frequency hearing, which is useful in arid habitats which have higher temperatures and lower precipitation rates (Quan et al., 2013). It is unclear why Isoodon spp. had relatively larger TBV than Perameles spp. and C. yirratji despite residing in more temperate habitats. This may possibly be due to prey selection within these species as some invertebrate species have been known to generate lower-frequency sounds.

While this study did not investigate the predator evasion implications of this relationship, the findings of the relative sizes of TBV and PSA from this study with the habitat and known ecological traits of some species appeared to support the findings of Pavlinov and Rogovin (2002).
Further analysis of this relationship with associated behavioural observations for bandicoot and bilby species would aid in improving the knowledge of how these structures impact predator evasion tactics. This information could be used to inform future management practices as it would give an insight into how these species utilise their habitat using acoustic information. Future studies focusing on the New Guinea species would aid in understanding whether the bullae are in fact larger than what is represented by the skull and what the overall ecology of these species is to allow for better comparisons with the Australian species. Studies into how the different bullae perceive sounds could further understanding as to how each of the bulla shapes function and the relative advantages and disadvantages that these have.


IUCN, 2019. The IUCN Red List of Threatened Species.


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