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Squamate tongues: Disentangling Natural and Sexual Selection

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This thesis is presented for the Degree of

Master of Philosophy (Environment and Agriculture)

of

Curtin University

August 2023

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Statement of contribution by others

All experimental work was undertaken by Christabel Khoo. Interpretation of results and discussion were done by all authors. Professor Bill Bateman initiated the project and aided with the editing the manuscript. Professional editing was done by L. N. Gilson and J. Barr.

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Figure 3.2 was created by Christabel Khoo with assistance from L.N. Gilson. Line images 3.2 a-d and g were generated using graphic sketching software on actual photographs of tongue specimens; images 3.2 e-f hand-drawn by Christabel Khoo in Photoshop, with guidance from K. M. Trinajstic. SEM photographs in Figures 3.3, 3.4, 3.5, 3.5, 3.7, 3.8, and 3.9 produced by Christabel Khoo.

Chapters 3 and 4 have been prepared as co-authored manuscripts for peer-reviewed publication in the scientific literature.

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Acknowledgements

I would like to give a huge thanks to my supervisors, Professor **Kate Trinajstic** and A/Prof. **Bill Bateman** for all the wonderful support, guidance and encouragement in the creation of this thesis over the last three years. Thanks to A/Prof. **Bill Bateman** for initiating the project. A huge thanks to Professor Kate Trinajstic for being a wonderful guide for me for manuscript and graphic formatting. Special thanks to Dr **Catherine Boisvert** and **Lauren Gilson** and Dr **Michael Just** for their support, particularly in developing my writing and technical skills.

A shoutout to **Rebecca Bray** and Dr **Paul Doughty** at the **Western Australian Museum** for allowing the usage of dissection equipment and access to preserved snake specimens. Finally, a big thank you to my parents, **Anne** and **Clarence Khoo**, for supporting me through this experience.

Animal Ethics

The research study did not require animal ethics approval from the **Curtin University Animal Ethics Committee**.

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General Abstract

Squamates (lizards and snakes) use their tongues for multiple purposes. As a result of natural selection, different tongue uses such as feeding and mate selection give rise to variation in tongue structure between squamate species. This work aims to determine if naturally and sexually selected traits, such as tongue morphology, are measurable at microscale. Measurements were taken from squamate specimens and scanning electron microscopy was performed to look for differences in tongue macro- and micromorphology between squamates. We aim to investigate if convergence in lizard tongue structure reflect convergence in diets and behaviour and to determine whether sexual selection is acting on macro- and micromorphological traits of snake tongues.

To address the knowledge gap of the relation between lizard species' tongue morphology and diet and behaviour and determine if habitat and diet have an effect on tongue structure, tongue macro- and micromorphology of seven WA lizard species were investigated. Analyses determined diet and behaviour as opposed to phylogeny are stronger influences on lizard tongue morphology as tongue microstructure of lizard species with similar diet and hunting strategies exhibited similar tongue microstructure rather than lizard species which are phylogenetically related. Further research will allow exploration of lizard feeding habits and behavioural niches in order to understand the roles of different squamate species within various ecosystems.

Sexual selection can co-opt organs, such as snake tongues, for roles in mate finding, choosing and gaining mates. Previous studies have identified evidence that the process of sexual selection occurs in snake tongues, but only at macroscale. Investigation of tongue tines of male and female snakes from two species of WA elapid snakes was done to fill this knowledge gap by addressing sexual selection at both macro- and microscale. Quantification of gross morphology and microfacet density between male and female snake tongues was examined with regard to sexual selection in two WA elapid snakes. Results showed that sex based divergence in tongue tine lengths and microfacet densities is driven by differences in roles between the sexes. Further investigation for differences in tongue tines between the sexes in other snake species as well as other squamates with deeply bifurcated tongues, such as varanids, will need to be addressed in future studies.

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List of abbreviations

BW: body width

e: eye

fr.sc: frontal scale

HWer: Head with at ears

HWey: Head width at eyes

HWno: Head width at nostrils

HL: Head length

NW: Neck Width

sn: snout

SEM: Scanning electron microscopy

SSD: Sexual size dimorphism

SVL: Snout-vent length

TL: Total body length

TaL: Tail length

ToL: Total tongue length

TiL: Tongue tine length

ToW: Tongue tine width

TiW: Tongue tine width

VNO: Vomeronasal organ

Chapter 1. The effect of natural and sexual selection on squamate tongue structure

1.1 Tongue structure in squamates

The tongue is a muscular organ, lined with epithelial cells and located in the ventral part of the mouth. The tongue is attached to the base of the mouth by the frenulum (Kent, 1973), allowing flexibility and motility (Sokoloff & Burkholder, 2013). Protractor muscles, which protrude the tongue, and retractor muscles, which allow the tongue to be retracted back into the mouth, run along the ventral surface of the tongue (Herrel *et al.,* 2005). In vertebrates, the tongue is used for the uptake and consumption of food and water by assisting during swallowing, known as intraoral transport (Kardong, 2006a), as well as for making sounds; in humans, it is particularly important for the process of speech (Iwasaki *et al.,* 2002; Weichert, 1970). German scientist David P. Hänig first described tongue structures and areas in his thesis in 1901, dividing the tongue into different sections based on what it can taste (Hänig, 1901 in Munger, 2015). More recent studies indicate that the tongue is functionally divided into four sections from anterior to posterior based on the number and type of papillae present: the tongue tip, fore-tongue, mid-tongue and hind-tongue (Delheusy *et al*., 1994; Iwasaki *et al.*, 2019).

The dorsal surface of the tongue is covered in papillae: tiny microscopic structures made of epithelial cells that enable the tongue's various sensory purposes. Papillae vary in shape, size and density, which facilitates variable function across the surface of the tongue. Squamates – lizards and snakes - have a range of papillae which function similarly to filiform papillae in mammals, e.g. movement of food and handling (Kardong, 2006a), that vary in being conical (Abbate *et al*., 2009), dome shaped (Iwasaki, 1990; Jamniczky *et al*., 2009; Bayoumi *et al.,* 2011), overlapping scale shaped (Schwenk, 1988; Yang & Wang, 2016), diamond shaped (Al-Ahmady *et al*., 2017), mosaic (Salem *et al.*, 2019), and plumerose (Rabinowitz & Tandler, 1986; Zghikh *et al*., 2014). In squamates, conical filiform papillae are the most common, distributed across the tongue tip and the anterior part of the fore-tongue. Fungiform papillae, which are round and knob-like, are scattered around the fore-tongue and mid-tongue in mammals and squamates. In most tetrapod species, the fungiform papillae contain tastebuds - small rounded structures which pick chemical signals, providing an organism's sense of taste (Kardong, 2006a; Iwasaki *et al*., 2002). Foliate papillae, named for their leaf-like scale shape, are located near the lateral sides of the tongue. These spikey papillae aid with holding food inside the oral cavity in order to prevent it from falling out of the mouth (Al-Fartwsy *et al.,* 2015; Parves & Alam, 2015; Jamniczky *et al*., 2009). Circumvallate papillae, which aid in the process of pushing food towards the oesophagus and swallowing, are located near the hind-tongue and tongue base (Weichert, 1965). Other structures on the tetrapod tongue's dorsal surface include secretory cells, which are located all over the tongue and excrete mucus to aid in the process of breaking down food prior to the process of swallowing (Andrew & Hickman, 1974) and taste buds, located on the tongue surface, which contribute to the uptake of chemical signals from food (Kardong, 2006b).

In squamates, the tongue is important in the process of smell and is crucial for the process of locating prey or food or mates via chemical signals detected by the use of tongue flicks (Cooper, 1997). Squamate tongues are also used to detect predators (Bealor & Krekorian, 2002; Kabes & Clark, 2016; Van Damme *et al.,* 2001) and during food discrimination (Cooper, 2000b). The tongues of some squamates (mainly the snakes) have microfacets, small bauble-like structures which increase the surface area of the tongue, allowing more chemical signals to be picked up from the atmosphere for prey seeking (Mao *et al*., 1991). The tips of squamate tongues are covered in secretory fluid, which enables the chemical signals which they pick up to be diffused into the tongue tip's epithelial cells, then transferred to the vomeronasal organ (VNO; Dial & Schwenk, 1996).

Within the highly diverse Squamata, there is much variation in tongue macrostructure (overall shape of the organ structure) among species. The shape of the tongue tip of squamates ranges from rounded to deeply bifurcated (illustrated in Schwenk, 1995). Most squamate tongue tips are at least slightly bifurcated, which is an adaptation that allows them to pick up chemical signals at a wider range than if un-bifurcated (Schwenk, 1989). Some skinks (e.g. *Tiliqua scincoides*) and the night lizards (Xantusiidae) have rounded tongue tips with no bifurcation (Badiane *et al.,* 2018; Pianka & Vitt, 2003; Schwenk *et al.,* 1995), while some other skinks such as *Scincella tsinlingensis* (Yang & Wang, 2016) have rounded tongues with only slightly bifurcated ends (Schwenk *et al.,* 1995). Deeply bifurcated tongues, typical of monitor lizards (Varanidae) and snakes, are, unlike those of other lizard species, retractable into sheaths made of epithelial cells, located at the ventral part of the mouth's posterior (Andrew & Hickman, 1979; Baer, 1964). The extreme bifurcation of these tongue tips allow signals to be picked up at wider gradients, to facilitate foraging for widely dispersed prey over long distances (Pough *et al.,* 2009).

For most squamates, with the exception of snakes and varanids, the tongue is used in the uptake of food (Schwenk & Throckmorton, 1989). Scale-shaped papillae on the fore-tongue and mid-tongue point towards the back of the mouth, and aid in the swallowing process. Secretory cells, which cover the tongues of several squamate species, encase their tongues in mucus, which allows them to trap their prey, such as insects and spiders (Iwasaki *et al.,* 2002). In some species, the tongue is used for the process of signalling (Badiane *et al*., 2018). The mid-tongues of *Tiliqua scincoides* (bluetongue skinks) are vividly pigmented and reflect large amounts of UV light, giving them a striking blue colour, allowing these lizards to use their tongues as a warning signal (Abbate *et al.,* 2009; Badiane *et al*., 2018). Similarly, *Tiliqua rugosa* (bobtail) tongues have a darker blue pigment, which also reflects UV light for signalling against predators (Nagloo *et al.,* 2022). All squamate reptiles rely on their tongues for prey and mate seeking via a process known as chemoreception.

1.1.1 The use of the tongue in chemoreception

In squamates, chemoreception is a two-step process that allows for prey and mate seeking using chemical cues from the environment. The first step involves flicking their tongues to pick up chemical signals. The tongue tip is then withdrawn into the mouth and thrust into a duct between the upper mandible of the mouth and nasal cavity (Noble & Kumpf, 1936). Chemicals in the environment which are picked up by the tongue tip are then converted into a liquid form, allowing them to be transmitted to the olfactory nerve, then transported into the vomeronasal organ (VNO) in the brain (Filoramo & Schwenk, 2009). Both senses of taste and smell in vertebrates allow the uptake of chemical signals to the VNO in the mandible attached to the brain, in a process known as chemoreception (Døving *et al*., 1998).

The VNO is located in the upper mandible of the mouth in tetrapods. Also known as the Jacobson's organ after Danish physician Ludvig Lewin Jacobson, it was first described in animals in 1811 by Dutch anatomist Frederik Ruysch (Coelho *et al*., 2005; Lekakis, 2003). This organ is present in many mammals (though absent in bats and aquatic mammals), amphibians, and reptiles (except for crocodiles and most turtles) (Eisthen, 1992; Mackay-Sims *et al.,* 1985). In mammals, the VNO is located in an isolated area of the nasal cavity. The nasopalatine duct connects this area to the mouth (Kardong, 2006b). In reptiles, the VNO is connected to the oral membranes by a narrow vomeronasal duct (Halpern, 1992). Squamates that rely heavily on using their tongues to seek out prey using chemical signals have evolved to retract their tongues into a tongue sheath in the oral cavity. The process of chemoreception in squamates is achieved by picking up chemical signals via the use of tongue flicks (Mao *et al.,* 1991; Schwenk, 1994). The sheath positions the tongue tips to directly deliver chemical cues to the VNO (Schwenk, 1995).

The VNO is made of three types of epithelia. The sensory epithelium, which makes up a large area of the VNO, consists of many neurons and lines the organ's dorsal area. This epithelium is lined with goblet cells, which secrete fluids which aid in taking in chemical signals taken in by the nasal system (in mammals) or tongue tines (in reptiles) during the process of chemoreception. The second type, known as mushroom body epithelium, composes the round ventral structure of the VNO known as the mushroom body. The mushroom body is covered in ciliated, column-like epithelium and is responsible for processing those signals that allow the organism to remember prey or mates (Kratzing, 1975; Rehorek *et al*., 2000). The third type, intermediate epithelium, is located on the tip and base of the VNO, which allows the organ's sensory epithelium to be attached to the mushroom body epithelium (Rehorek *et al.,* 2000).

Mammals, especially canines and rodents, have a very highly developed VNO as they rely on their sense of smell for communication (Graziadei, 1977; Kostov, 2007; Wysocki & Lepri, 1991). In amphibians, the VNO of male *Plethodon cinereus* (red-backed salamander; Plethodontidae) is important for processing chemical signals taken in by chemoreception, allowing them to seek out mates (Dawley & Crowder, 1995). Compared to mammals and amphibians, there have been fewer studies on the function of the VNO in reptiles. However, studies by Allison (1953) and Burghardt (1970) have shown that in squamates the VNO is highly developed and that, in contrast to other tetrapods, squamates rely on the use of their tongues rather than the noses to pick up chemical signals for transfer to the VNO (Gillingham & Clarke, 1981). Removal or sealing of the VNO squamate reptiles hinders their ability to mate-search and communicate, as well as impairing their ability to forage (Graves & Halpern, 1990).

Squamate VNO size is affected by an organism's ecological niche; a species' role and function with the elements of its environment, living and nonliving (Pough *et al.*, 2013). Many squamates, including some skink species, geckos, and iguanids, have only moderately developed VNOs as their dietary niche of invertebrate prey does not require them to wander long distances in search of prey (Schwenk, 1993a). Lacertid lizards tend to have a moderately developed VNO but also moderate amounts of sensory epithelium. This allows them to process information when foraging for long distances for small invertebrate prey (Saito *et al.*, 2010). Night lizards (*Xantusia spp.*), in contrast to all the aforementioned lizard species, have very small VNOs with only small amounts of sensory epithelium, and non-bifurcated tongues. One reason suggested for this is that they do not rely on chemoreception to seek out their preferred diet of small invertebrates (Halpern, 1992) and plant matter (Fellers & Drost, 1991) because they forage over small areas where prey and vegetation are readily available (Fellers & Drost, 1991; Miller, 1951). Chameleons lack a VNO altogether as their tongues are not used for chemoreception. Rather, they rely on sight for prey seeking (Haas, 1937) and have highly specialized tongues with modified musculature and secretory cells for prey capture (de Groot, 2004; Fouda *et al.*, 2015; Iwasaki *et al.*, 2002).

Snakes and larger varanid species, such as *Varanus panoptes* (yellow-spotted monitor) and *Varanus gouldii* (Gould's monitor), have highly developed VNOs which aid them in mate-seeking as well as foraging for prey that consists of vertebrates and, in the case of the large varanids, smaller varanid species and mammals. The VNO of snakes and varanids have large amounts of sensory epithelium with globular cells, which allow them to take in considerable amounts of chemical signals while mate seeking (Takami & Hirosawa, 1990; Wang & Halpern, 1980).

1.2 Effect of diet and behaviour on tongue structure

Evolutionary effects are known for driving changes in squamate tongue macro- and micromorphology. The macro- and micromorphology of a species evolves through the process of natural selection to reflect its role in its ecological niche. Two contributing factors that affect natural selection for niche occupancy are diet and habitat and these may drive both macro- and micromorphological differentiation, as seen in the variety of papillae shapes found on squamate tongues.

1.2.1 Dietary niche and its effect on tongue structure and function

There is great diversity in tongue macromorphology (overall shape of the organ) and micromorphology (fine features of the tongue surface) among squamate species (Schwenk, 1995) that is due to diet. Geckos and many skink species, such as *Scincella tsinlingensis,* forage for insects and other invertebrates and rely on bifurcated tongue tips for prey seeking (Schwenk, 1994) and prey discrimination for geckos (Cooper, 1994; Schwenk, 1993b; Cooper, 1998). The tongue of *S. tsinlingensis* is covered with overlapping scale-shaped papillae on the fore-tongue and mid-tongue. This allows ease of transport of prey towards the oesophagus (Yang & Wang, 2016). The leopard gecko's (*Eublepharis macularius*) fore-tongue and mid-tongue have densely packed leaf-like papillae as well as conical papillae, which allow the prey to adhere to its tongue while it gets transported to the oesophagus. It also aids in ingestion and intraoral transport of prey and water. Its (Jamniczky *et al*., 2009).

Similar to the geckos, *Pogona vitticeps* (bearded dragon; Agamidae) is omnivorous agamid that forages for various invertebrate prey and plant matter as well as occasional small vertebrate prey, such as smaller lizards (Cooper, 2000a). *Pogona vitticeps* has a bifurcated tongue tip, which allows it to distinguish plant or animal matter (Schwenk, 1995; Zghikh *et al.,* 2014). Its tongue microstructure consists of multiple plumerose papillae and mucosal cells on its fore-tongue, which allows it to capture and take in various invertebrate prey (Cooper, 2000a; Zghikh *et al*., 2014). In behavioural studies in which the number of tongue flicks made in response to food scents presented on swabs indicated preferences for specific food odours (Cooper, 2000a), *Pogona vitticeps* were responsive to live food odours (e.g. cricket, but not carrot or alfalfa odour), a result that supported their foraging preference for active prey (Schaerlaken *et al.,* 2007). Its head is wide (Cogger, 2018) but its tongue macrostructure is narrow at the tongue tip which gradually increases in width from tip to hind-tongue (Zghikh *et al.,* 2014). The multiple plumerose papillae on its tongue surface allows for capture of active prey, correlating to its dietary niche (Zghikh *et al*., 2014).

Skinks of the genus *Tiliqua* are omnivorous and mostly forage for plant and animal material. The tongue of *Tiliqua scincoides* is broad, flexible, and extensible from the oral cavity (macro features) and has conical papillae on the fore-tongue that promote intraoral transport of food. The conical papillae are useful for handling and ingesting snails and smaller lizards (Abbate *et al*., 2009; Cogger, 2018).

Lacertid lizards such as *Podarcis sicula* have bifurcated tongue tips but, compared to geckos and many skink species, their tongues have a deeper bifurcation, presumably because they forage for prey and mates in a manner more similar to that of varanids and snakes (Abbate *et al.,* 2010; Baeckens *et al*., 2017; Schwenk, 1994). Chameleons are specialized insectivorous ambush predators that, in contrast to foraging species, have highly specialised non-bifurcated tongues adapted for prey capture from a motionless posture (Fouda *et al.*, 2015). Their tongues are anchored at the mouth's posterior by the intralingual accelerator muscle, which is attached to the ventro-posterior of the floor of the mouth and allows the tongue to extend approximately 1.5 body lengths (Baer, 1964; de Groot, 2004). Chameleon tongues are covered in secretory cells, which coat them in mucus, enhancing prey capture success (Fouda *et al*., 2015; Iwasaki *et al*., 2002). The tongues of most Xantusiidae (night lizards) are evolved for detecting nearby predators and small invertebrate prey, as well as intraoral transport of food into the mouth (Kabes & Clark, 2016; Schwenk, 1988). Their tongues have non-bifurcated tongue tips (Schwenk, 1995), and widely distributed tastebuds on the dorsal fore-tongue (Schwenk, 1985). No studies have been done on Xantusiidae tongue microstructure in relation to dietary niche.

Varanids and snakes have highly specialised, deeply bifurcated tongues which function in prey seeking (Schwenk *et al.,* 1994; Smith *et al.,* 2008) and play little to no role in food ingestion or handling; snakes and varanids depend more on teeth and jaw musculature for ingesting prey items. Varanids are more likely to tear up prey (King & Green, 1999; Schaerlaeken, 2011) in contrast to snakes which have unhinged jaws that allow them to swallow large prey whole (Lee *et al.,* 1999; Zaher, 1994). *Pseudonaja affinis* (dugite: Elapidae), one of Australia's most common elapid snake species, forages from desert dunes to urban areas in search of vertebrate prey which includes reptiles, mice and birds (Maryan & Gaikhorst, 2005; Wolfe *et al*., 2018). *Notechis scutatus* (tiger snake: Elapidae) is a top predator in Australia's wetlands which uses chemoreception to seek out its preferred prey of frogs (Shine, 1977a). *Varanus gouldii*, one of Australia's largest varanid species, is known to forage for snakes, birds, frogs, mammals, and smaller lizards including other varanids (Godwin *et al.,* 2020; Wilson & Swan, 2020). Both *P. affinis* and *V. gouldii* have been shown to resort to cannibalism occasionally (Maryan & Gaikhorst, 2005; Wolfe *et al*., 2018).

Moloch horridus (thorny devil: Agamidae), a diurnal agamid native to Western Australia, is a sit-and-wait ambush predator which has a specific diet of ants and some small insect eggs. It uses its specialized tongue which, like a chameleon's tongue, is covered in secretory cells (Withers & Dickman, 1995) to catch its prey (Pianka & Pianka, 1970). Though Pianka and Pianka (1970) have shown that the secretory cells' mucus production enables *M. horridus* to take up to 45 ants per minute, no further information on its tongue microstructure is known.

The main diet for many arboreal lizard species in Australia, including *Cryptoblepharus australis* (inland snake-eyed skink: Scincidae) has been analysed through faecal samples (Nordberg *et al.,* 2017). Faecal samples from *C. australis* and several other arboreal lizard species consisted largely of insects. From the results of Nordberg *et al.* (2017)'s study, one would predict that other arboreal lizard species such as *Christinus marmoratus* (marbled gecko: Gekkonidae) would have a similar tongue structure, although no information is available on the tongue structure of these geckos. Based on published evidence of relationships between tongue macrostructure and diet, we hypothesised that tongue microstructure also is affected by dietary niche and behaviour, as opposed to phylogeny. Researching the tongue microstructures and diets and habitats of a range of Australian squamate species would allow us to evaluate this hypothesis. With regard to Australian lizards, details of the fore-tongue papillae are only available for two species: *Tiliqua scincoides* (bluetongue skink) and *Pogona vitticeps* (bearded dragon) (Table 1.1). In order to find the relation between tongue structure and diet/habitat and behavioural studies, we further explored the fore-tongue papillae of these two species and other lizard taxa, both through microstructure measurements of opportunistically obtained specimens and through comparison to species for whom descriptions of tongue microstructure and diet were available in the published literature.

1.2.2 Habitat size, behaviour, and their effect on tongue structure and functions

A species' habitat size and its foraging behaviour are other factors which may contribute to squamate tongue morphology. Smaller squamates in general have a smaller habitat range in comparison to large squamates (Cechin *et al.,* 2011; Pianka, 1994; Smith & Griffiths, 2009). For example, small varanid species such as *Varanus* (*caudolineatus* (stripe-tailed goanna; mean SVL= 101.2 mm) and *V. tristis* (black-headed goanna; mean SVL= 213.0 mm) prefer arboreal lifestyles and have smaller home ranges of up to 40.1 ha (Pianka, 1994; Thompson *et al*., 1999). Insects and invertebrate prey make up most of their diet, though both species will occasionally prey on smaller lizards and birds' eggs (Pianka, 1969; Pianka, 1994; Thompson, 1993; Thompson *et al*., 1999).

By contrast, larger varanid species such as *Varanus gouldii* (mean snout-vent length (SVL)= 310.8 mm) and *Varanus giganteus* (perentie monitor) (mean SVL= 689.0mm) have a wide diet of mammals, birds, and smaller reptiles (Pianka, 1994; Wilson & Swan, 2020) and have home ranges of up to 63.5 ha (Thompson, 1994) and 325.0 ha (Horn & King, 2004) respectively. *Varanus giganteus* forages over long ranges of up to 21.5 ha in search of vertebrate prey (King *et al.*, 1989), while large male *V. gouldii* have foraging ranges of up to 48.2 ha (Thompson, 1994).

There have been several studies on varanid home ranges and dietary differences (Thompson *et al*., 1999; Pianka, 1969; King *et al*., 1989; Thompson, 1993), but rarely any studies relating tongue morphology of varanids to their diet and habitat. Tongue micromorphology of *Moloch horridus* in relation to its very specific diet, and morphological and behavioural studies on the family Xantusiidae (night lizards) in relation to tongue structure have not been covered in detail. For this research, we explored a variety of lizard species for further assessment of the relationship between tongue morphology and lifestyle.

1.3 Natural selection in tongue structures

1.3.1 Natural selection in squamate tongue structures

Natural selection, according to Darwin (1859), is the selective evolutionary process in which individuals retain traits which enable them to survive in their habitat niches, which results in the rise of new species. Perhaps the most famous example is that of the species of finches on the Galapagos Islands as described by Darwin (cited in Podos, 2001). Differential survival rates can be driven by such pressures such as predation, resulting in mechanisms such as cryptic colouration that allows prey animals to evade predators (Endler, 1983; Orton *et al*., 2018; Stuart-Fox *et al*., 2004), e.g. the evolution of the peppered moth (Kettlewell, 1958). Other forms of natural selection include the differentiation of limbs in relation to habitat and behaviour of squamate reptiles (Richardson *et al*., 2009; Skinner, 2012; Stanley, 2014).

Studies on squamates have shown the rise of several species phenotypes due to natural selection driven by environmental conditions. Gamble *et al.* (2012) showcased the diversity of gecko species, some having evolved to lose their toe pads to fit their role in a specific environmental niche. Night lizards such as the *Xantusia henshawi* and *X. vigilis* live in crevices such as cracks between rocks and prefer to hunt their prey in enclosed spaces, and therefore have evolved round, non-bifurcated tongues with taste buds on the dorsal parts of the fore-tongue (Brattstrom, 1952; Schwenk, 1985). By contrast, large varanid species such as *V. gouldii* are diurnal, have evolved deeply bifurcated tongues to seek out active vertebrate prey, and foraging over areas as large as 1000m^2 daily in search of vertebrate prey (Thompson, 1992; Wilson & Swan, 2020). While there have been many studies of natural selection at macroscale in reptiles regarding their colouration (Stewart-Fox *et al*., 2007; Krohn & Rosenblum, 2016) and obvious physical features like their leg shape in relation to habitat (Aerts *et al.*, 2000; Richardson *et al.*, 2009), there have been fewer studies regarding the relation between tongue macro- or microstructure and environmental niches.

Information on microscale features that could be naturally selected, such as the shapes and distributions of tongue papillae, is only available for two Australian lizard species, the skink *T. scincoides* and the agamid *P. vitticeps*. Although *T. scincoides* and *P. vitticeps* are both ground-dwelling, diurnal, omnivorous lizards, they have notably dissimilarly shaped papillae on the fore-tongue. *Pogona vitticeps* has multiple plumerose papillae on its fore-tongue (Zghikh *et al.,* 2014); *T. scincoides,* however, has more conical shaped papillae on its fore-tongue (Abbate *et al.,* 2009). Both tongues, however, have globular cells and are used for the uptake of food (Abbate *et al*., 2009; Zghikh *et al*., 2014). Details of the tongue microstructure of other Australian agamid or gecko species, varanids, or other Australian skink species, especially common ones like *Egernia striolata* (tree crevice skink) are unknown. Most comparisons of squamate tongue structure have been made between varanids and snakes, but mainly focused on macrostructure as opposed to microstructure (Schwenk, 1994; Young, 1997; Cooper, 1994). More comparisons between omnivorous lacertids and skinks need to be made, regarding comparing tongue microstructure, as there are little to no comparisons of *Xantusia* lizard tongue morphology with other insectivorous species of other lizard families. To determine if convergent evolution in tongue microstructure is affected by phylogenetic relationships or habitat and diet, we examined a wide range of lizard taxa to answer this question.

1.3.2 Convergent evolution in squamate tongue structures

Convergent evolution is the process in which widely separated evolutionary lineages evolve morphologically similar structures due to the requirement of undertaking a similar function within a similar ecological niche e.g. specialized feeding or burrowing. Squamates provide an insight to examples of convergent evolution via comparison of the tongue macrostructures of phylogenetically distant species. *Pogona vitticeps,* a diurnal agamid lizard, has a semi-bifurcated tongue which allows it to seek out prey as well as distinguish plant and animal matter (Cooper, 2000a; Schaerlaeken *et al*., 2007). This type of tongue structure enables it to forage over vegetation and rocky outcrops in search of insect prey or plant matter (Cogger, 2018). *Eublepharis macularius*, a crepuscular gecko which also prefers rocky outcrops, also has a bifurcated tongue similar to *P. vitticeps* (Hunziker, 1994; Rawat *et al.,* 2019) and also relies on its bifurcated tongue tip for foraging for small invertebrate prey over rocky outcrops (Jamniczky *et al.,* 2009). *Tiliqua scincoides* and gekkonid geckos from the genus *Rhacodactylus* both have rounded tongues with semi-bifurcated tips due to their similar diet and use of chemoreception to distinguish between plant and prey matter, although they are phylogenetically distant (Abbate *et al*., 2009; Cooper & Habegger, 2001). *Takydromus tachydromoides* (Japanese grass lizard) and *Gekko japonicus* (Japanese gecko) share similar tongue morphology despite being from different families (Lacertidae and Gekkonidae respectively), due to their prey seeking behaviour and insectivorous diet (El-Bakry & Hamdi, 2016; Iwasaki, 1990). From these comparisons, it is apparent that shared diet and prey seeking behaviour between the species can result in tongue macromorphological similarities, regardless of phylogenetic relatedness.

At a micromorphological scale, however, there is a knowledge gap on fine structures which may also be naturally selected, considering their roles in food handling. In order to observe the full effect of natural selection in squamate tongue morphology, I made comparative studies at a morphological microscale of seven Western Australian lizard species and compared them to SEM images of tongue microstructures of squamate species from other lizard families from previous literature.

Table 1.1: Comparison of niche, diet, and foraging behaviour with tongue structures of six Australian lizard species from previously published literature.

Species (Family)	**Niche**	**Diet**	**Foraging Habits**	**Hunting Strategy**	**Tongue Macrostructure**	**Tongue Microstructure**	**References**
Christinus marmoratus (Gekkonidae)	Terrestrial	Insectivorous	Nocturnal	Foraging	Unknown	Papillae not known	Cogger, 2018; Nordberg *et al*., 2017
Tiliqua scincoides (Scincidae)	Terrestrial	Omnivorous	Diurnal	Foraging	Bifurcated tip	Conical papillae on fore-tongue	Cogger, 2018; Abbate *et al.,* 2009
Tiliqua rugosa (Scincidae)	Terrestrial	Omnivorous,	Diurnal	Foraging	Bifurcated tip	Previously Undescribed*	Cogger, 2018; This study
Egernia kingii (Scincidae)	Terrestrial	Omnivorous	Diurnal	Foraging	Bifurcated tip	Previously undescribed	Cogger, 2018; This study
Cryptoblepharus buchananii (Scincidae)	Arboreal	Insectivorous	Diurnal	Foraging	Bifurcated tip	Papillae not known[ǂ]	Cogger, 2018
Varanus panoptes (Varanidae)	Terrestrial	Carnivorous	Diurnal	Foraging	Deeply Bifurcated	Papillae not known	Cogger, 2018; Wilson & Swan, 2020
Varanus tristis (Varanidae)	Arboreal	Insectivorous	Diurnal	Ambush/ Foraging	Deeply Bifurcated	Papillae not known	Cogger, 2018; Thompson *et al.,* 1999
Moloch horridus (Agamidae)	Terrestrial	Ants (exclusively)	Diurnal	Ambush	Bifurcated tip	Papillae not known	Cogger, 2018; Pianka & Pianka, 1970
Pogona vitticeps (Agamidae)	Terrestrial	Omnivorous	Diurnal	Foraging	Bifurcated tip	Multiple plumerose papillae on fore-tongue	Cogger, 2018; Zghikh *et al.,* 2014

Explaination of table 1. *Previously undescribed refers to taxa examined in this study. [ǂ]Papillae undescribed refers to taxa which are yet to be studied.

1.4 Sexual selection in snake tongues and mate seeking in snakes

A form of natural selection, sexual selection refers to the differential success of individuals solely with respect to gaining access to mates. Sexual selection can result in morphological differences between the sexes other than in reproductive organs, as well as influencing the ability for an organism to attract and copulate with a mate (Darwin, 1872). Sexual selection occurs at an obvious morphological macroscale, as notable extravagant plumage in male birds (Petrie *et al*., 1991; Pruett-Jones *et al*., 1990), bright colouration in male squamates (Johnson & Wade 2010; Marshall *et al*., 2005; Husak *et al*., 2005; Chen *et al*., 2012), or the presence of weapons in male organisms in general (Clutton-Brock *et al*., 1979; Emlen, 2008; Berglund, 2013). These mechanisms allow males to compete for dominance and access to fertilize females with their sperm, passing their genes to the next generation. As such it seems at odds with natural selection, in that it may make individuals susceptible to be preyed on through their behaviour or appearance (Marshall *et al*., 2015; Husak *et al*., 2005).

Sexual selection can also result sexual size dimorphism (SSD), where one sex is significantly different in size and/or shape to the other. Male-biased sexual sized dimorphism tends to occur in species where male-male combat occurs, as larger males are better able to win battles and gain access to many females, as seen in many snake species (Mattos *et al*., 2017; Valencia, 2020; Shine, 1978; Shine, 1994). Female-biased SSD, also known as reversed sexual dimorphism (RSD) also occurs, but often in invertebrates or herpetofauna where size is associated with higher fecundity; for example, *Eunectes murinus* (green anaconda: Boidae), where a larger size in females result in more eggs to be produced (Rivas & Burghardt, 2001). Reversed sexual dimorphism is also the norm in raptors like *Pandion haliaetus* (Osprey) (Muriel *et al*., 2010) and *Bubo scandiacus* (snowy owl) (Wiklund & Stigh, 1983). Smaller body sizes allow males birds to be more successful in hunting in flight, while females with larger body sizes have an advantage in incubation during colder months (Muriel *et al*., 2010; Widen, 1984; Wiklund & Sitgh, 1983).

Sexual selection can also have more subtle effects on anatomy; for example, because males of some primate species such as those of the *Macaca* and *Presbytis* genera, are known to partake in defending their territory and mates, they have larger canine sizes compared to females as larger teeth are a better defence mechanism (Harvey *et al*., 1978). Another example of a subtle forms of sexual selection is the size of the VNO. In *Microtus mandarinus* (Mandarin vole), males have a larger VNO size in comparison to females (Tai *et al*., 2004), while in male *Plethodon cinereus*, the VNO increases in size during the breeding season (Dawley *et al*., 1992). Snakes have evolved deeply bifurcated tongues not for the process of food handling, but for prey and mate seeking (Schwenk, 1994).

More subtle forms of sexual selection in snake tongue morphology may also be occurring at a microsurface level. Microfacets located on the tongue tines augment the detection of chemical signals from the environment, allowing the snake to seek out more distant mates (Mao *et al*., 1991) and therefore may also be sexually selected. Microfacets are not apparent to the naked eye, but require microscopy for evaluation. Scanning electron microscopy (SEM) has been used previously for analysis of snake tongue microstructure (Morgans & Heidt, 1978; Mao *et al*., 1991), but not in relation to assessing the presence of subtle forms of sexual dimorphism. To address this knowledge gap, I investigated differences between tongue microstructure in males and females in two Western Australian snake species using SEM images.

1.5 Thesis aim: significance of studying natural selection in Western Australian squamates- closing knowledge gaps

There have been several studies on tongue microstructure in reptiles (Abbate *et al*., 2009; Fouda *et al.,* 2015; Mao *et al.*, 1991; Iwasaki *et al*., 2002; Rabinowitz & Tandler, 1986; Zghikh *et al.,* 2014) as well as reptile ecological and behavioural studies on their lifestyles and/or diet (Cooper & Vitt, 1986; Mason & Parker; 2010) in isolation. However, there has not been any form of study which combines the two topics together. The aim of this study was to determine whether squamate tongue macro- and microstructure are affected by phylogeny or by their ecological niche and diets. Currently, little is known about the relationship between tongue microstructure, diet and habitat.

While many studies on tongue papillae shape in squamates have been undertaken (Abbate *et al.,* 2009; Jamniczky et al., 2009; Salem *et al*., 2019; Yang & Wang, 2016; Zghikh *et al*., 2014), information on the relation between tongue microstructure and natural selection is still unknown, and little is known of whether tongue microstructure is affected by phylogenetic relationships or by diet and behaviour. For example, evidence from the behavioural study of *Egernia kingii* indicates that it is an omnivorous squamate that prefers to forage for insects and birds' eggs. One would therefore predict that *E. kingii* would have conical papillae, which enhances the movement of prey towards the back of the throat (Abbate *et al.,* 2009; Cogger, 2018; Hewes & Schwenk, 2020), on its fore-tongue, like *T. scincoides,* as both skink species are diurnal omnivorous foragers (Cogger*,* 2018).

To determine if tongue microstructure is under the influence natural selection, comparisons of tongue microstructures from a wide range of squamate species must be made. To achieve this, a comparative study at a morphological microscale of seven Western Australian lizard species was undertaken and results were compared with published data on tongue microstructures of key squamate species representing major families encompassing a global distribution. If tongues are under natural selection, squamate species from related families should share similar papillae structure. Using known information on papillae shape and function and assessing it in relation to the diet and behaviour of squamates will allow us to investigate if diet and habitat have an impact on squamate tongue microstructure.

Regarding studies on sexual selection in squamates, many studies on mate selection cover sexually dimorphic traits at obvious levels (Johnson & Wade 2010; Marshall *et al*., 2005; Husak *et al*., 2005; Chen *et al*., 2012). There has been only one study which combines the study of squamate tongue structure and sexual selection (Smith *et al*., 2008) with focus on the differences in tongue tine lengths between the sexes in copperheads. Also, while research involving studies of tongue microstructure in snakes have been done previously (Mao *et al*., 1991; Morgans & Heidt, 1978), studies that combine investigation of squamate tongue microstructure and sexual selection have yet to be covered.

In snakes, because microfacet density augments chemoreception, I hypothesised that an increase in microfacet density in tongue tines enhances the ability to seek out mates. Because chemoreception is involved in mate-finding, microfacet density in snake tongues should therefore be under sexual selection. I investigated the macro- and microstructure of the snake tongue tines for morphological differences between the sexes. Combining Smith *et al.* (2008)'s methodology with the use of scanning electron microscopy allowed me to address the differences in both tongue tine length and microfacet density between the sexes in snakes.

1.5.1 The importance of studying natural selection in Western Australian lizards

The first of my two research studies involve investigation of the presence of natural selection on lizard tongue macro- and microstructure. Squamate reptiles play a very important role in Australia's ecosystems with a wide range of roles in their ecological niches. These roles may range from small insectivores such as the *Menetia greyii* (Cogger, 2018; Wilson & Swan, 2020) to medium-sized larger omnivorous lizards like *Tiliqua scincoides* (bluetongue skink), which feeds on insects, smaller reptiles and vegetation (Abbate *et al*., 2009; Cogger, 2018; Wilson & Swan, 2020), to large apex predators like *Varanus panoptes* (yellow-spotted monitor) which forages for prey ranging from shell fish (Shannon & Mendyk, 2009), fish, birds, smaller reptiles and small mammals (Shine, 1986). I focused on seven Western Australian lizard species from the families Carphodactylidae, Scincidae, and Agamidae. All species of lizards chosen for this study are common and accessible as three of the species, two skinks and one agamid were found roadkill and four were donated non-data specimens, making them suitable for dissection, macroscale measurement, and microscopy. They also exhibit strong diversity, as they are from three different families with different lifestyles and habitats, making them excellent choices for considering natural selection as a driver of their diversity.

In the family Carphodactylidae, geckos lack specialized toe pads which enable geckos from other families to scale walls. They are mostly nocturnal, reflected by their large eyes, have clawed toes instead of toe pads, and fat tails. One of the genera of carphodactylid geckos, *Underwoodisaurus*, consists of only two species— *U. seorsus* (Pilbara barking gecko) and the focus species for this study, *U. milii* (thick-tailed gecko or barking gecko) (Cogger, 2018).

Underwoodisaurus milii has a widespread distribution ranging from South Western Australia to South Australia, New South Wales, southern parts of Queensland, and northern parts of Victoria. It is noted by its fat tail, which is a dark purplish-red, has five white stripes and a thin tip. Its body is deep purplish-red in colour with specks of cream-white spots and a pale purplish underbelly, with large eyes, a set of smooth scales across its dorsal and ventral area, and five clawed toes on its feet. This species has a preference for rocky outcrops, coastal wetlands, leaf litter, and crevices. Its foraging habits are nocturnal and it prefers invertebrate prey, but occasionally eats smaller lizard species (Cogger, 2018).

The family Scincidae, the largest of Australia's lizard families, exhibits an excellent example of divergent evolution, as there are a 1741 species within this family (Uetz *et al.,* 2023). Scincidae can be identified via their snake-like heads and overlapping shiny scales (Hutchinson, 1993). The *Tiliqua* genus consists of medium to large (SVL 90-300mm), stout-bodied, mostly short-tailed skinks that are diurnal and omnivorous. All species in this genus are viviparous. *Tiliqua rugosa* (bobtail) is one of the most well-known species of skinks in Australia. Endemic throughout the southwest to South Australia and inland East Australia, *T. rugosa* can be identified by its short limbs, fat, short, round ended tail and its rugose thick scales, which run across on its dorsal area all the way to its tail, giving it a pine-cone like texture. It prefers a variety of open shrublands and desert dunes and is opportunistic in its diet, preying on snails, insects, eggs and even carrion (Cogger, 2018). The subspecies of *T. rugosa* focused on in this study is *T. r. rugosa* (Western bobtail). This subspecies can be distinguished from others by its reddish-brown hue and pale underbelly (Cogger, 2018).

Skinks of the genus *Egernia* are also medium to large (SVL 100-200 mm) and are omnivorous and viviparous, but have longer tails in comparison to *Tiliqua*. In contrast to the genus *Tiliqua*, some species of *Egernia* are arboreal. Some species of *Egernia* have notable small tail spines, which members of *Tiliqua* lack. *Egernia* skinks often prefer rocky outcrops, wet to dry sclerophyll forests, and woodlands. *Egernia kingii* (King's skink) is the focal *Egernia* species of this study. Found in southwestern Australia, this notable skink is identified by its dark olive brown to black scales, with its offspring having tiny flecks of white or cream on its dorsal and lateral areas. Like *T. rugosa, E. kingii* can be found in rocks and crevices as well as woodlands and scrublands as opposed to open dunes. It is omnivorous and eats vegetation and may also occasionally feed on insects and birds' eggs (Cogger, 2018).

The *Eremiascincus* genus consists of seven species of small (SVL 70-100mm) sandswimmer skinks which have visible ears and very smooth, glossy scales. *Eremiascincus richardsonii* (broad-banded sandswimmer), the species analysed in this study, is widespread throughout Western Australia, South Australia, the Northern Territory, inland New South Wales, and Queensland. It has notable dark bands on its dorsal surface giving its namesake and is yellow-gold with a pink underbelly. *Eremiascincus richardsonii* burrows for invertebrate prey, but will occasionally eat fruit (Cogger, 2018; James & Losos, 1991).

Skinks of the genus *Hemiergis are* small, fossorial, and mostly insectivorous. They have three to five toes on their feet and are found across mainland Australia (Cogger, 2018). The sampled member of this genus in this study, *Hemiergis initialis initialis* (Southwestern earless skink), can be told apart from other members of its genus via a blackish stripe on its lateral areas and its medium to dark brown dorsal area. It has five toes on each foot (Reeder & Reichert, 2011; Shapiro *et al*., 2003) and has small eyes which is a reflection of its burrowing habits. It is also insectivorous (Cogger, 2018).

In contrast to *Hemiergis*, the similarly sized (SVL 25-30 mm), also insectivorous skinks from the genus *Menetia* have larger eyes and prefer to seek prey by sight on the surface as opposed to living a fossorial lifestyle. *Menetia* skinks have four toes on their front limbs and five toes on their back limbs. *Menetia greyii* (the common dwarf skink; included in this study), is widespread across Australia and is adaptable to a variety of forest environments and can be found under leaf litter (Storr *et al.*, 1999; Cogger, 2018).

The Agamidae or dragon lizard family is one of the largest lizard families worldwide. Agamids can be identified via the presence of notable spikes (or loose skin in some genera) on their bodies, dull or rough scales and longer limbs compared to the skink or gecko families (Cogger, 2018). They also have acrodont teeth and at five digits on each foot, in contrast to geckos and skinks, which have varying numbers of digits on their front and hind limbs (Witten, 1993). All species of agamids are oviparous and exhibit a range of body sizes. Fifty species of agamids reside in Australia. The sole agamid species in this study is from the genus *Pogona*. Lizards of the *Pogona* genus have triangular heads, tiny spinose scales across their lateral sides and notable spiny scales which form a ‘beard’ under their throats. Habitat-wise, *Pogona* lizards can range from terrestrial to arboreal. The second smallest of the *Pogona* agamids, *Pogona minor* (pygmy bearded dragon) is brown-grey with small spinose scales across the back of its head and along its lateral area and dorsal area (Cogger, 2018). The focal subspecies for this study is *P. minor minima* (Southwestern pygmy bearded dragon), which is brownish-grey with tiny spine-like scales and a triangular head.

I conducted a comparative study of the macro- and micromorphology of lizard tongues from three families of Western Australian lizards by generating scanning electron microscopy (SEM) images of their tongues, and comparing their tongue micromorphology with SEM images of tongues from species from 15 other lizard families from previous literature. The aim of the experiment was to assess whether diet has a stronger impact on tongue morphology than does phylogeny. Schwenk (1995) addressed the phylogenetic relationship by showing that related reptiles share similarly shaped tongues. By researching the tongue morphologies of lizard species from related families along, with their diet, behaviour and roles in their ecological niches, I expected to find variation in tongue macro- and micromorphologies within related lizards. Because my specimens were limited to three families, I evaluated published accounts of tongue microstructure from other families for which there were also SEM images, for a broader phylogenetic scope. Considering the large variety of lizard species with different types of diets and roles in ecological niches (Schalk & Cove, 2018), I hypothesised that natural selection occurs at a subtle, microscopic level, evident in the microstructural diversity of their tongues.

The importance of comparative study of a wide range of squamate tongue structures from different lizard species is that it allows us to investigate natural selection across a wider phylogenetic sample. Previous work on squamate sensory organs by Filoramo and Schwenk (2009)'s concentrated of VNOs and Bayoumi *et al.* (2011)'s who did do a comparative study of the tongue microstructure utilised only two unrelated lizard species, the agamid *Uromastyx aegyptia* (Egyptian uromastyx) and the gecko *Tarentola annularis* (white spotted wall gecko). Further comparative studies, especially the overlooked microanatomy of tongues, using a wider range of squamates will allow us to investigate the processes, if any, of natural selection occurring at a morphological microscale. This will indicate if sexual dimorphism is more prevalent in the natural world, although unrecognised as it is at the microscale and not readily determined.

Studying squamate tongue macro- and microstructure will allow us to better understand the anatomy and relate differences in tongue anatomy to previous works about diverse diet (Bauer & Sadlier, 1994; Hendersen & Pauers, 2012; Kolodiuk *et al*., 2010), prey and mate seeking strategies (Barbosa *et al*., 2021; Rivas & Burghardt, 2005; Schwenk, 1994). Since squamates are extremely reliant on chemoreception, urban pollution poses a threat as pollutants in the air may interfere with their prey seeking.

1.5.2 The importance of studying sexual selection in Western Australian snakes

The second study presented in this thesis investigates if sexual dimorphism and/or selection present in two species of elapids *Pseudonaja* (brown snakes) and *Notechis* (tiger snakes) at the micro-scale. Sexual selection at a morphological microscale within snake has only been documented in one species *Agkistrodon contortrix* (American easter copperhead). Smith *et* *al.,* (2008) determined male *Agkistrodon contortrix* (Eastern copperhead: Viperidae) have longer tongue tines compared to females and suggested this was because they seek females out during the breeding season using chemoreception. He determined the differences in tongue tine between sexes was the result of sexual selection on tongue tine lengths (Smith *et al.,* 2008. My study extends this work as it not only examines tongue tie length but also looks the microfacet density between the tines of males and females in two Western Australian elapid snakes.

The family Elapidae make up a majority of Australia's venomous snake species, which have a range of habitats from terrestrial to marine. This study will focus on single representatives of two genera: *Pseudonaja* (brown snakes) and *Notechis* (tiger snakes) in order to determine the presence or absence of sexual selection. The *Pseudonaja* genus is widespread throughout Australia and mainly consists of many species of brown snakes. These range from a variety of patterns, but can be noted by their smooth mid-body scales, which run in rows of 17 to 21 and 7 to 12 teeth in the maxilla which follow the fang. *Pseudonaja affinis* (dugite) is an active diurnal foraging predator similar to *P. textilis* (Eastern brown snake). *Pseudonaja affinis* can grow up to 1.5m in length, is oviparous, and prefers open sand dunes and arid scrubs (Cogger, 2018; Wolfe *et al*., 2018). It is also capable of adapting to urban environments to seek out prey, such as smaller squamates, birds, and reptiles (Maryan & Gaikhorst, 2005; Wolfe *et al*., 2018). *Pseudonaja affinis'* colouration can range from being plain light to dark brown with a pale underbelly to brown and speckled with dark flecks and an olive underbelly. Its frontal scale between the eyes is also narrow and it has large eyes which point towards its narrow snout (Cogger, 2018). The species chosen for this study is *Pseudonaja affinis affinis* (mainland dugite) which can be easily identified via its brown colour and paler head, along with its olive underbelly (Cogger, 2018). Significantly, male and female snakes of both *Pseudonaja* species appear to rely on visual cues and chance meetings for reproductive pairing (Whitaker & Shine, 2003; Wolfe *et al*., 2018).

In contrast to the more widespread *Pseudonaja* genus, snakes of the *Notechis* genus are only found in southwest and southeast Australia. Like the *Pseudonaja* genus, *Notechis* snakes have smooth mid-body scales that run in rows of 17 to 21. In contrast to the *Pseudonaja* snakes, *Notechis* snakes exhibit a broader neck, and rows of four to five teeth in the maxilla following the fang (Cogger, 2018). There is one species of snake in this genus— *Notechis scutatus* (tiger snake). In contrast to *P. affinis*, *N. scutatus* is smaller (1.2m), and is viviparous and crepuscular. This darkish brown elapid can be identified through the presence of its yellow stripes and yellow underbelly. Its eyes are forward facing near its narrow snout and its frontal scale is much broader and wider compared to P. *affinis* (Cogger, 2018)*. Notechis scutatus* prefers being around damp environments, such as the wetlands where it forages for its preferred prey of frogs (Shine, 1977a), but may occasionally eat smaller birds (Cogger, 2018). It also exhibits male-biased sexual size dimorphism due to male-male combat (Shine, 1977b; Shine, 1978 and Shine, 1979). *Notechis scutatus* has five subspecies, but for this study, the focal subspecies is *Notechis scutatus occidentalis* (Western tiger snake).

P. affinis and *N. scutatus* are from different genera and have different life histories, which may result in behavioural differences e.g. hunting and mate seeking. Studying tongue microstructure in Western Australia's elapids will allow us to look for specific forms of sexual dimorphism between the sexes in each species and relate these to published data on mate seeking behaviour and diet (Lettoof *et al.,* 2020a).

The objective of this research is to investigate if squamate tongue structure is affected by natural selection. We aimed to investigate if a) lizard tongue microstructure is affected by diet or phylogeny and b) snake tongue microstructure is under sexual selection.

The current lack of data on the microanatomy of squamate tongues indicates that morphological dimorphism in squamates is being underreported resulting gaps in our understanding of the evolutionary drivers in these animals.

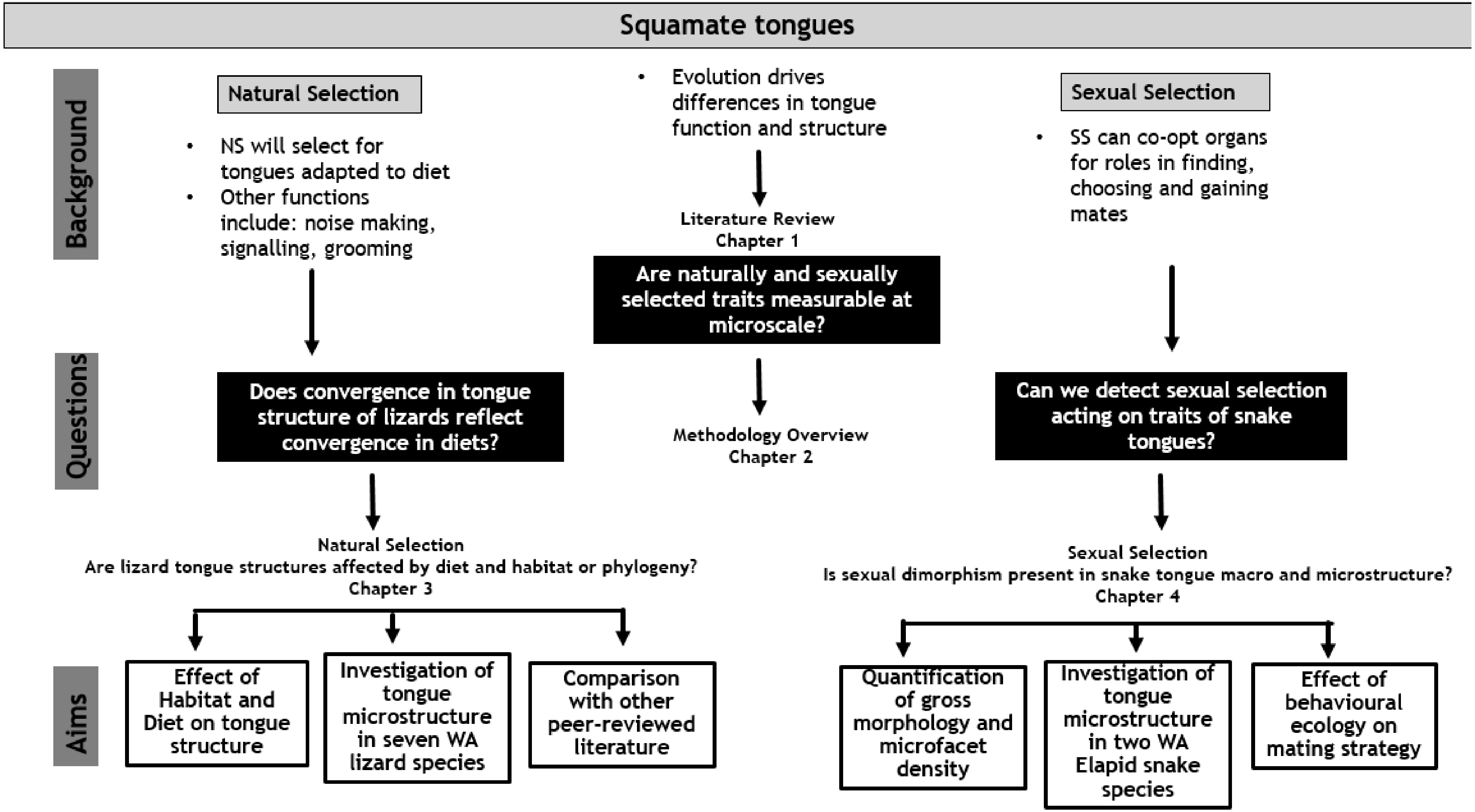

Figure 1.1: Schematic organisation of the research questions and aims addressed in this thesis

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Chapter 2. Materials and methods

Methodology relevant to each experimental undertaking will be repeated in chapters three and four.

2.1 Sources of research materials and general methods

To assess the roles of natural and sexual selection in shaping the diversity of squamate tongues, the principal methodology of these research experiments involved taking morphological measurements from squamate specimens at both macroscale (whole body and body parts) and microscale (fine features only evident with the aid of microscopy). A total of 82 specimens were measured, 47 of which were road-kill, five were ethanol-preserved non-data snake specimens loaned by the Western Australian Museum, and 30 were specimens collected as part of a research projects of Lettoof *et al*. (2020). Body measurements of lizards were taken in order to assess differences in sizes in relation to tongue shape within families. Body measurement of the snakes were taken in order to investigate if head and snout vent length, which are known to be sexually selected, affect tongue tine length. Microscale features included investigating the differences in papillae shape on the tongues in relation to the species' diets, and microfacet counts on the tongue tines to quantify differences in chemoreceptive capacity of both sexes.

2.2 Preparation of materials and specimen measurements

Three road killed and four donated non-data samples of seven Western Australian lizard species were used in this study. The species examined were one adult *Underwoodisaurus milii* (barking gecko), one adult *Tiliqua rugosa rugosa* (western bobtail), one adult *Egernia kingii* (King's skink), one adult *Eremiascincus richardsonii* (broad banded sandswimmer), one adult *Hemiergis initialis initialis* (Southwestern earless skink*)*, one adult *Menetia greyii* (common dwarf skink) and one adult *Pogona minor minima* (southwestern pygmy bearded dragon). The seven Western Australian lizard species are from three different families and have divergent dietary niches and lifestyles. These differences, reflected in head shapes (Figure 2.1, with content adapted from Cogger, 2018 and Storr *et al*., 1999), allowed the investigation of whether tongue macrostructure is also under natural selection— with tongue structures diversifying a result of specific dietary niches.

A set of standard measurements (following Smith, 2007 and Cogger, 2018) were taken of the total body length and body width, snout vent length (SVL) (tip of the snout to the cloaca), head length (tip of snout to start of neck), head width at eyes, head width at nostrils, neck width, and tail length (cloaca to the tip of the tail) using digital calipers or, where the preserved specimen was twisted, a flexible measuring tape. Measurements were taken to the nearest mm. Tongue dissection was then undertaken and imaged using scanning electron microscopy (SEM; see Section 2.3 below). The images of the tongue microstructures were then compared with the published literature of a variety of lizards (details of species provided in Chapter 3).

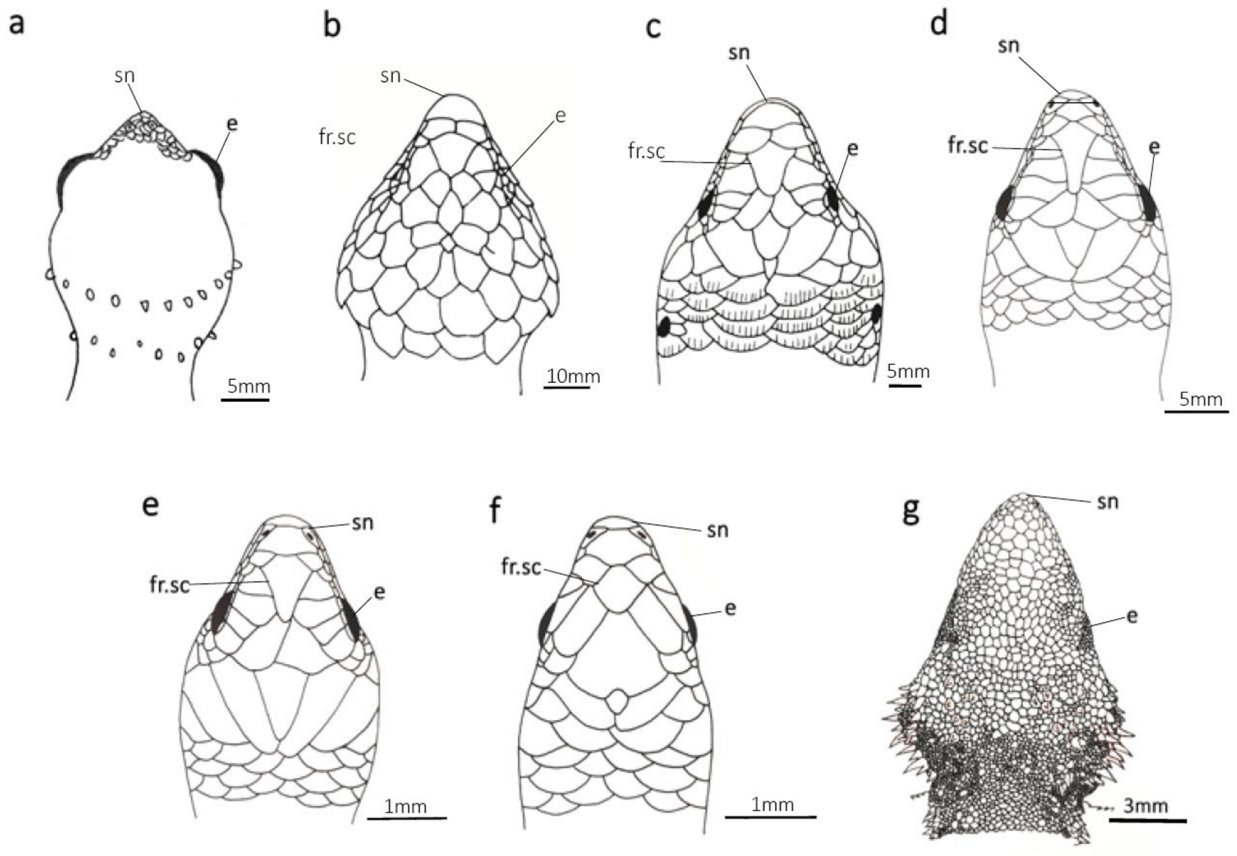

Figure 2.1 Schematic comparison of dorsal view of heads of seven Western Australian lizard species.
(a) *Underwoodisaurus milii* (barking gecko, Family: Carphodactylidae. Image: C.M Khoo). Note the large eyes (e), small pointed snout (sn), very small,fine, scales and no notable frontal scale (fr.sc) compared to that of the skinks (Figure 1.1b, c, d, e & f) and *Pogona minor minima* (Figure 1.1g). (b) *Tiliqua rugosa rugosa* (western bobtail, Family: Scincidae. Image: Storr *et al.*, 1999). Dorsal view of head displays a pointed triangular snout (sn) similar to that of *Egernia kingii* (Figure 1.1c) but has larger, rugose scales, a broader frontal scale (fr.sc). It also has the most broad head width compared to the other four skink species (Figures 1.1c, d, e & f) and deep set eyes (e). (c) *Egernia kingii* (King's skink, Family: Scincidae. Image: Storr *et al.*, 1999). Dorsal view of head displays a pointed triangular snout (sn) and deep set eyes (e) similar to that of *T. r. rugosa*, but has less rugose scales, and a less broad, longer frontal scale (fr.sc).
(d) *Eremiascincus richardsonii* (sandswimmer skink, Family: Scincidae. Image: Storr *et al.*, 1999). Dorsal view displays deep set eyes, pointed snout (sn) and the most elongated frontal scale (fr.sc) of five selected skink species (Figure 1.1b, c, d, e & f). Head is streamlined in shape. (e) *Hemiergis initialis initialis* (southwestern earless skink, Family: Scincidae. Image: Storr *et al.*, 1999). Dorsal view displays deep set eyes similar to that of *E. richardsonii* (Figure 1.1d)and a more eleongated frontal scale (fr.sc) and thicker width behind the eyes compared to that of the simiarly sized *Menetia greyii* (f). (f) *Menetia greyii* (common dwarf skink, Family: Scincidae. Image: Storr *et al.*, 1999). Dorsal view displays large eyes similar to that of *U. milii* (Figure 1.1a), but has larger, less fine scales similar to that of *E. kingii* (Figure 1.1c), *E. richardsonii* (Figure 1.1d) and *H. i. initialis* (Figure 1.1e). *M. greyii* also has the most streamlined head of all skink species(Figures 1.1b, c, d, e, & f) and a broader frontal scale (fr.sc) compared to the similarly sized *H. i. initialis* (Figure 1.1e).
(g) *Pogona minor minima* (West Australian pygmy bearded dragon. Family: Agamidae. Image: Cogger, 2018). Dorsal view displays small, spiny scales which become prominent toward the throat area and no notable frontal scale (fr.sc) compared to that of *U. milii* (Figure 1.1a) and the skinks (Figure 1.1b, c, d, e & f). Scales are more spiny compared to that of *U. milii* (Figure 1.1a) and the skinks (Figures c, 1.1d, e & f). Eyes (e) are deep set similar to *T. r. rugosa* (Figure 1.1b) and pointed head is streamlined similar to that of *M.greyii* (Figure 1.1f). Scale bars = 5mm (a, c & d), 10mm (b), 1mm (e & f) and 3mm(g).

For the study to determine if sexual selection is present in Western Australian snakes, two focal species were chosen- *Pseudonaja affinis affinis* (western dugite) and *Notechis scutatus occidentalis* (western tiger snake). These two elapid snake species are common, allowing for large sample sizes. To determine if sexual selection was present on both macro- and micromorphological features, the specimens of both snake species were sexed. A total of five ethanol-preserved and 15 male road-killed adult dugites, 25 female road-killed adult dugites, and 15 male and 15 female adult tiger snakes sourced from another study (Lettoof *et al*., 2020) were used. The preserved dugite specimens were loaned by the Western Australian Museum; roadkill specimens were collected by Professor Bateman, Curtin University and the euthanized specimens were provided by Damian Lettoof, Curtin University.

Body measurements were taken to determine if sexual size dimorphism was present between the sexes of the snakes. Measurements of the total length, body width between the shoulder girdle and the hip girdle, snout vent length and tail length were taken using a flexible measure tape and ruler. Digital calipers were used to take the measurements of head length, head width at eyes, head width at nostrils and neck width (Figure 2.2). All measurements were recorded to the nearest mm. Comparisons of total body length and head length and between males and females of each snake species were undertaken.

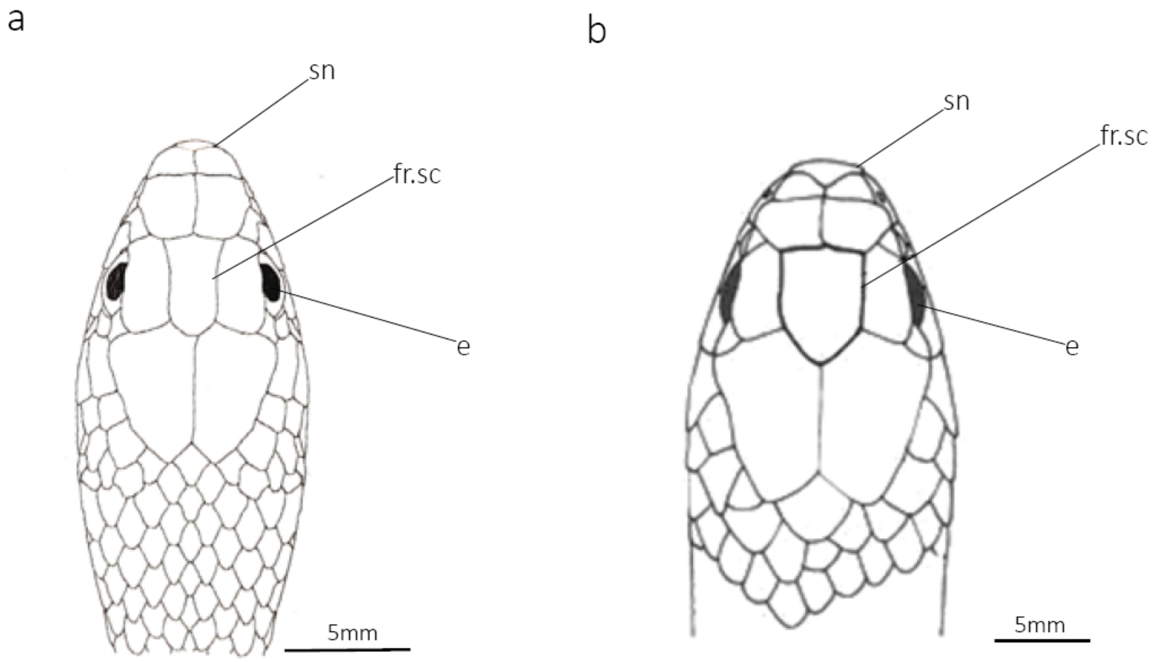

Figure 2.2: Schematic comparison of dorsal view of two Western Australian elapid snake specie**s**.
(a) ***Pseudonaja affinis affinis*** (mainland dugite. Image: Cogger, 2018) head displaying forward facing eyes (e) and wider head compared to its neck. Note the pointed snount (sn) and the narrow frontal scale (fr.sc).
(b) Dorsal view of head of ***Notechis scutatus occidentalis*** (Western tiger snake. Image: Department of Natural Resources and Environment Tasmania, 2022) also displaying forward facing eyes (e) and a pointed snout (sn). In contrast to ***P. affinis affinis***, ***N. scutatus occidentalis*** has a broader frontal scale (fr.sc) and a wider neck. All scale bars= 5mm.

2.3 Dissection and tongue measurements

For each lizard specimen (n= 7), road-killed dugite specimen (n= 35), and tiger snake specimen (n= 30), the tongues were dissected out of the oral cavity. The lower mandible and movable maxillary of the jaw were held open. For lizard samples, the tongue was held out from the oral cavity and cut near the frenulum. To ensure total tongue length was obtained for snake samples, snake tongues were held out from its sheath below the glottis and before being cut near the frenulum (Figure 2.3). For both lizard and snake samples, total tongue length and width were measured to the nearest mm. For snake samples, total tine length was also measured and recorded to the nearest mm.

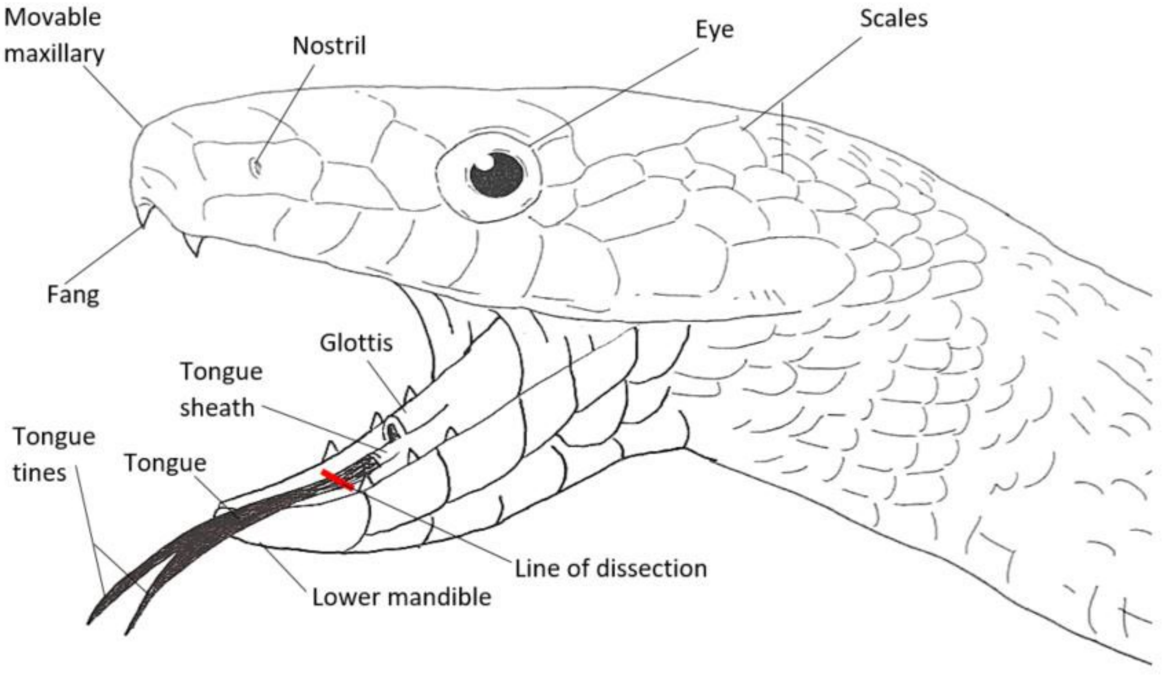

Figure 2.3: An elapid snake displaying scales, forward facing eyes, pointed snout, oral cavity, glottis, and bifurcated tongue in sheath. Dissections were performed by locating the sheath below the glottis and holding the tongue out with forceps prior to dissection with scissors. The red line indicates the point of dissection at the frenulum.

For museum-preserved snake specimens, the lower mandible and the movable maxillary were held open using a cut plastic pipette in order for the tongue to be measured without dissection (Figure 2.4). The tongue was kept in the mouth and held out from the tongue sheath using a pair of forceps and measured. The measurement of total tongue length and tongue tine length was done to the nearest mm (Figure 2.5).

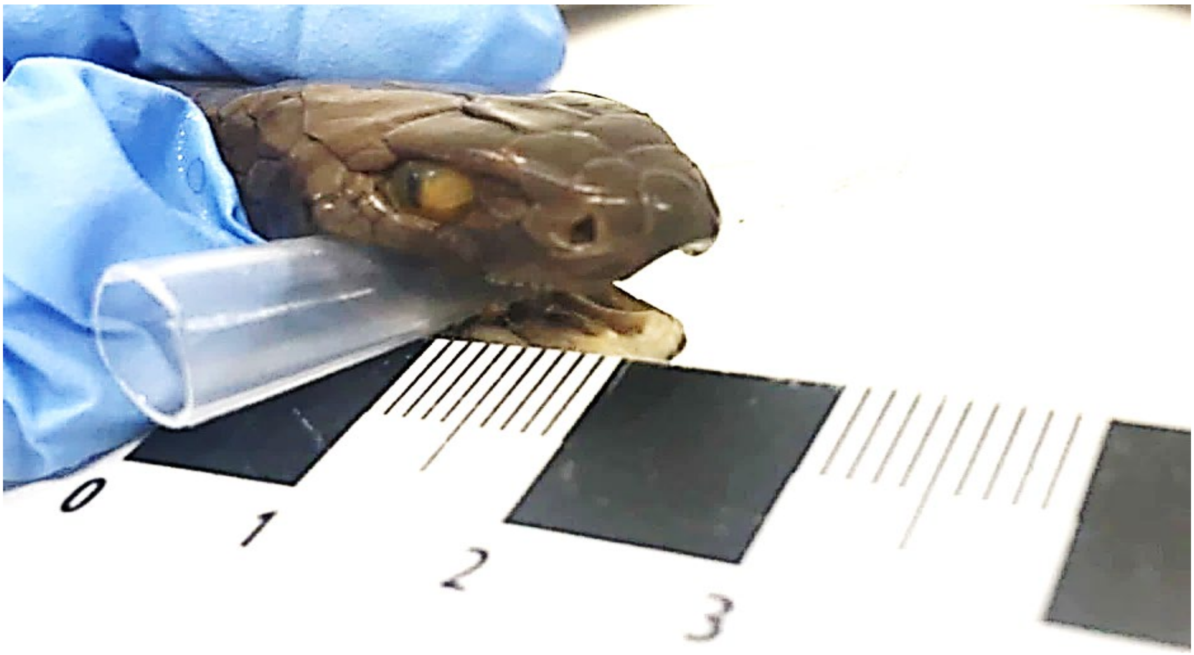

Figure 2.4: A museum-preserved *Pseudonaja affinis affinis* specimen with its mouth held open to ensure accurate tongue measurement. Measurement of the tongue tine was done by holding out the tongue from the sheath below the glottis using a pair of forceps. Tongues were not dissected from museum specimens, as they were for the road-killed specimens (see Figure 2.5).

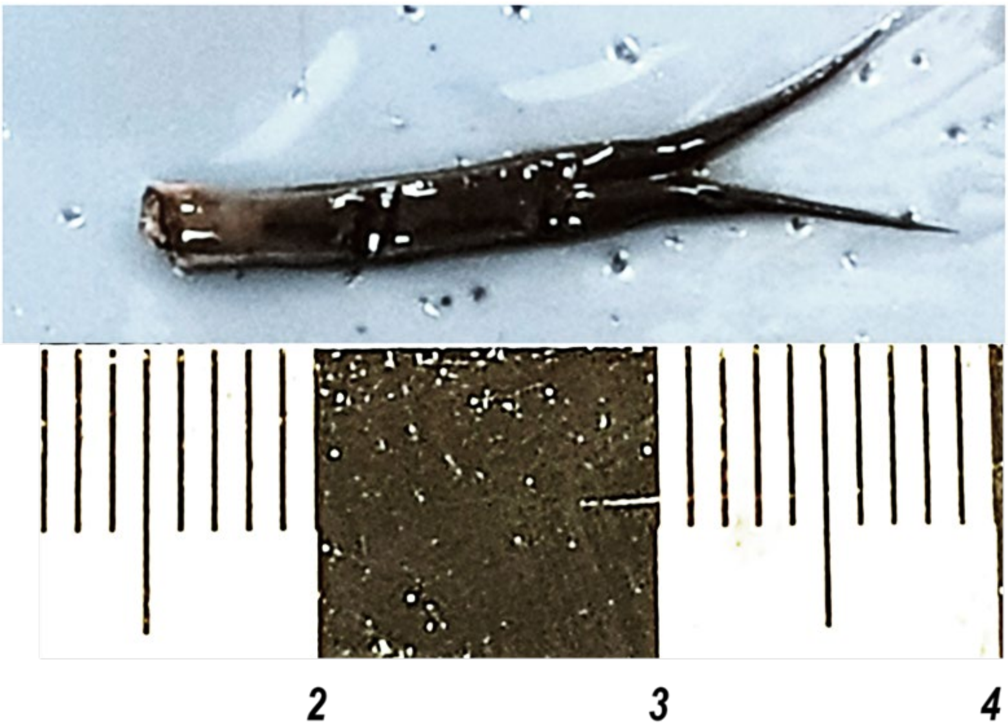

Figure 2.5: Example of a snake tongue tine length dissected from a road-killed specimen being measured on a 1x1 cm scale. This procedure was performed and recorded for both sexes in *Pseudonaja affinis affinis* and *Notechis scutatus occidentalis.*

2.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to investigate the microstructure of the squamate tongues. SEMs were used for this study as it is the only way to view the details of tongue morphology at this scale. To prepare the SEM samples, seven lizard tongues and 65 snake tongues were fixed in 5 ml of glutaraldehyde for 24 hours at 4˚C. They were then removed from the glutaraldehyde. Glutaraldehyde has a covalent property that allows adherence to proteins, stabilizing cellular structures for the binding of proteins, while also doing little to no damage to the specimen (Hayat, 1986). The most common technique of tongue specimen fixing involves 2.5-3.0% glutaraldehyde (Morgans & Heidt, 1978). Fixation of biological tissues in glutaraldehyde is necessary in order to prevent further biological sample decomposition, but glutaraldehyde causes some shrinkage (Hayat, 1986). This is solved by washing in 0.1M phosphate buffer to rehydrate the tissues. The tongues were immediately washed in 5ml of 0.1M phosphate buffer following removal from glutaraldehyde. Phosphate buffer, which shares the same pH level as blood (7.4), prevents autolysis of tissues (Choudhary, 2021).

Dehydration of the tongues was achieved by placing them in a series of ethanol concentrations (75%, 86% and then 96%) for 18 minutes at each concentration. The tongues were then air dried for 24 hours, allowing samples to be ready for mounting on stubs. Biological specimens need to be dry before being placed in the SEM to prevent moisture damage to both the sample and the electron microscope chamber (Stadtländer, 2007). A series of increasing ethanol concentrations allows for a gradual decrease in water content in the specimens without damaging the specimen (Murtey & Ramasamy, 2016). It is also more suitable than critical point drying for the biological specimen preparation, as the process allows the specimen to undergo less shrinkage compared to critical point drying (Gusnard & Kirschner, 1977).

All tongue samples were affixed to SEM stubs using double-sided carbon tape. For snake tongue samples, one tine was removed and positioned with its ventral side up so that microstructural variation could be evaluated for both surfaces (Figure 2.6). Mounted lizard and snake tongue samples were then sputter coated with gold ions (DII-29030SCTR Smart Coater). Sputter coating with gold ions enables the conduction of electron beams (Murtey & Ramasamy, 2016) while preventing the tissues from being destroyed by high voltages

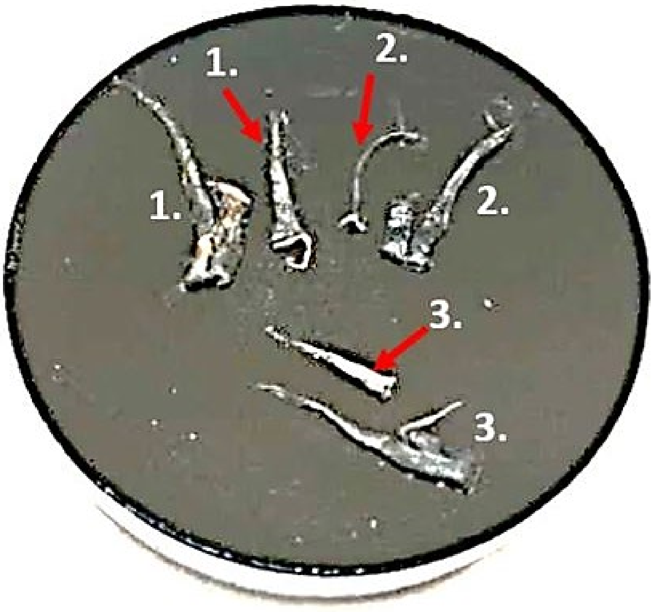

Figure 2.6: Example of a mounted tongue stub showcasing mounted tines in preparation for gold sputtering. Note: for each tongue, one tine is turned over to ensure the dorsal side is viewed under the SEM. This stub displays tongue tines from different three dugite specimens. Numbered red arrows indicate inverted tines of respectively numbered tongue samples.

caused by the build-up of electrons attracted by the sample's glutaraldehyde and phosphate coating (Stadtländer, 2007). The negative ions of glutaraldehyde and phosphate adhere firmly to the positive charged gold ions allowing for tight coating and a clearer resolution on an SEM camera (Horisberger & Rosset, 1977; Li *et al*., 2010). Images of lizard and snake tongue tines were then taken using an SEM camera (JEOL JCM-6000Plus). From previous literature (Jamniczky *et al*., 2009; Yang & Wang, 2016; Zghikh *et al.,* 2014), tongue papillae were known to range in size and shape from 10 µm to 1mm. I chose magnifications 10 µm, 20 µm, 50 µm, 100 µm, 200 µm, 500 µm and 1 mm to as they captured the known range of sizes. All specimens were viewed at 15 kV.

For research in natural selection in squamates, SEM images of the tongue microstructure of seven Western Australian lizard species were produced. The shape and types of papillae were examined and described for four areas of the tongue: tongue tip, fore-tongue, mid-tongue and hind-tongue. These SEM images were then compared with SEM images of tongue macro- and microstructure of other lizard species from previous peer-reviewed literature (See Chapter 3), representing 34 genera from 16 families. This allowed us to comparatively evaluate if tongue microstructure (papillae type and distribution) is affected by variation in dietary niche and lifestyles between lizard species as a result of natural selection.

For research into sexual selection in snakes, SEM images of the dorsal surfaces of tongue tines of male and female *P. a. affinis* and female *N. s. occidentalis* were taken to count the number of microfacets on the tongue tines between the sexes. This use of SEM imagery to evaluate sexual selection at a microscopic scale in snakes addresses a knowledge gap which had yet to be covered.

2.5 Statistical analysis for snake specimens

To investigate the scaling relationship between body sizes and sexual dimorphism in male and female snakes for both focal species, two-tailed t-tests were conducted using StatistiXL (version 2). A two-tailed t-test was used as it is one of the most effective statistical choices for investigating differences between two large sized samples (Pillemer, 1991). Body measurements compared between the sexes were SVL, head length, and tongue tine lengths. Head length and SVL were predicted to be factors affecting tongue tine length and therefore were chosen to be the independent variables against which tongue tine length was measured in a linear regression. To assess difference in tongue tine lengths between the sexes while accounting for potential differences in head length, the residuals of head length were calculated from the regression of head length against tine length for each sex. The residuals come from the difference between the individual observed values on the regression line and their respective predicted values obtained using the regression equation (Jemna *et al*., 2020). Head length was chosen as the independent variable because head size has a positive linear effect on tongue tine length (Smith *et al*., 2008; this study); tine length was chosen the dependent variable (See Chapter 4).

Following Smith *et al.* (2008), we plotted the measurements of tongue tine length against SVL and head length in two line graphs for identifying correlation between tongue tine length and body measurements to investigate for potential sexual size dimorphism. The line graphs were plotted in Excel.

To identify differences in tongue microstructure between the sexes in both snake species, microfacet counts were done on 5µm^2 segments randomly distributed across the dorsal surface of the tine tips and mid-tines. Mid-tine regions were determined from anterior to posterior of each tine by the decreasing density of microfacets. A total of four 5µm^2 segments were counted for each *P. a. affinis* tine tip image and a total of seven 5µm^2 segments were counted for each *N. s. occidentalis* tine tip image. Number of microfacets were analysed using StatistiXL. The mean number of microfacets in male and female *P. a. affinis* and male and female *N. s. occidentalis* were calculated and additional two tailed t-tests were also performed to assess significant differences between the number of microfacets in male and female dugites and male and female tiger snakes (See Chapter 4).

2.6 Significance of experimental design

Previous literature that has focused on squamate tongue morphology using SEM data in the experimental designs have used the SEM imagery to investigate tongue structures in a single species (Delheusy *et al*. 1994; Rabinowitz & Tandler, 1986; Yang & Wang, 2016), a pair (Bayoumi *et al*., 2011; Morgans & Heidt, 1978), or a trio (Salem *et al*., 2019) of squamate species. Only one study focused on the effect of behaviour on tongue macro- and microstructure (Bayoumi *et al*., 2011), another on the effect of lifestyle (Morgans & Heidt, 1978), and a third on the effect of diet (Salem *et al*., 2019). However, there have been no studies which use SEM imagery in order to investigate natural selection in tongue microstructure, especially at a phylogenetic scale. In addition, very few studies have combined the use of SEMs and morphological measurements to investigate natural selection of tongue tines in squamate reptiles at a broad level. My experiments on natural selection in squamate reptiles focuses on using SEM and morphological measurements of lizards to investigate the effect of dietary niche and lifestyle on tongue macro- and microstructure in Western Australian lizard species. Comparing the SEM images with those from previous peer-reviewed literature will allow us to investigate the presence of natural selection in squamate tongue structures at macro- and microscopic level, covering previous knowledge gaps which have yet to be addressed.

Regarding sexual selection in snakes, there has only been one study which focuses on investigating sexual selection in tongue macrostructure, whose statistical analyses that can be easily replicated (Smith *et al*., 2008) with additional species. However, there have been no studies exploring sexual selection in snake tongue microstructure. Therefore, in addition to using statistical analysis of macrostructures, we are also using SEMs in order to assess measurable differences in tongue microstructure in Western Australian elapid snakes. This experimental method will allow us to cover a knowledge gap on the topic of sexual selection in squamates.

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Chapter 3. Natural selection in lizard tongues

This chapter has been prepared for submission to the *Journal of Zoology, London*. As a result, relevant parts of the methodology have been repeated.

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3.1 Abstract

Within squamate families, structures of papillae are diverse and have a wide range of shapes. To investigate if diet, as opposed to phylogeny, influences squamate tongue structure, scanning electron microscopy was used to examine the tongue macro- and microstructure for seven lizard species from three different squamate families (Carphodactylidae, Scincidae, Agamidae). Comparison of tongue SEM images were made to that of tongue micromorphologies of other squamates from other families studied in previous literature. *Tiliqua rugosa rugosa* exhibits elevated papillae, which appears to reflect its opportunistic omnivorous diet of plant matter, snails, and small lizards. By contrast, *Hemiergis initialis initialis* and *Menetia greyii*, both insectivorous skinks, have non-elevated papillae. *Underwoodisaurus milii* exhibits overlapping papillae similar to *M. greyii*, but its papillae are scale shaped, reflecting its diet of both invertebrates and occasionally small vertebrates. Agamid *Pogona minor minima* and skink *Egernia kingii* both displayed round, non-elevated papillae on their tongue tips, indicating a primary diet of vegetation and occasionally insects. *Eremiascincus richardsonii* has round non-elevated papillae similar *to P. m. minima* and *E. kingii* but its tongue macrostructure lacks bifurcation that the other six lizard species have, indicating a different hunting strategy. The results overall indicate that diet and behaviour as opposed to phylogeny has an effect on squamate tongue microstructure.

3.2 Introduction

The tongue is a muscular organ located on the floor of the mouth and mainly used in the process of the intake and manipulation of food. In vertebrates, the tongue's primary function is intraoral transport: the uptake and consumption of food and water by assisting during swallowing (Kardong, 2006), as well as contributing to making sounds. Across the tetrapods, including mammals (Emura *et al.,* 2001), birds (Emura *et al.,* 2010) and reptiles (Iwasaki, 2002), tongue morphology is highly variable and gross morphological structure of the tongues appears to be related broadly to different kinds of feeding in a terrestrial and aquatic environments (Iwasaki, 2002; Iwasaki *et al*., 1996; Park & Lee, 2009). Squamate reptiles – lizards and snakes— are an extremely diverse order of tetrapods that occur in a wide variety of habitats, resulting in varied overall morphology, lifestyles, and diets. Squamates heavily rely on their tongues to capture and handle prey (Herrel *et al.,* 1995; Smith *et al.,* 1999) and/or seek out prey with the use of chemical cues (chemoreception) (Schwenk, 1995). Squamate reptiles use chemoreception in a way that is unique to them as they rely on picking up chemical signals using their tongues (Schwenk, 1995).

The vertebrate tongue is covered in epithelial cells and tiny structures called papillae, which come in a variety of shapes that are involved in a variety of functions (Iwasaki, 2002). It has four specific areas based on the papillae: the tongue tip, the fore-tongue, the mid-tongue, and hind-tongue (Delheusy *et al*., 1994; Iwasaki *et al.*, 2019). Filiform papillae are conical-shaped and are the most common form of papillae, distributed across the tongue tip and the anterior part of the fore-tongue. Tetrapod tongues rely on filiform papillae for the movement of food towards the back of the mouth (Kardong, 2006). Papillae which are found in squamates that function similarly to filiform papillae may be conical in shape (Abbate *et al.,* 2009), but can also range from being dome-shaped (Iwasaki, 1990; Jamniczky *et al*., 2009; Bayoumi *et al.,* 2011), to overlapping scale-shaped (Schwenk, 1988; Yang & Wang, 2016), diamond-shaped (Al-Ahmady *et al*., 2017; Elsheikh *et al.,* 2013), mosaic-patterned (Salem *et al*., 2019) and even plumerose with secretory cells (Rabinowitz *et al*., 1989; Zghikh *et al*., 2014), depending on the squamate species.

Fungiform papillae, which are round and knob-like, are scattered around the fore-tongue and mid-tongue in most tetrapods. In most tetrapods, apart from snakes, fungiform papillae contain taste buds, small rounded structures which help at picking chemical signals via an organism's sense of taste (Kardong, 2006). Foliate papillae, named for their leaf-like scale shape, are located along the lateral sides of the tongue. These spiky papillae aid with keeping food within the mouth, and in preventing live prey from escaping (Jamniczky *et al.,* 2009). The circumvallate papillae, which aid in the process of swallowing and pushing food towards the oesophagus, are located on the hind-tongue and near the tongue base (Weichert, 1965). Other structures on the tongue's dorsal surface include secretory cells, which are located all over the tongue and excrete mucus (Andrew & Hickman, 1974) and microfacets, small bauble-like structures that increase the surface area of the tongue. In squamate reptiles, microfacets aid in detecting chemical signals via chemoreception (Mao *et al.,* 1991).

The variation in hunting strategies and diet between lizard taxa has resulted in the evolution of diverse tongue morphology (Schwenk, 1995). For example, chameleons have an extremely specialised tongue with sticky mucosal glands on the tongue tip that enable them to catch prey via extension of tongues. Chameleon tongues also have an intralingual accelerator muscle and specialized collagenous tissue, which allows them to extend their tongues to capture their insect prey (Fouda *et al*., 2015; de Groot, 2004; Moulton *et al*., 2016; Van Leeuwen, 1997). Agamids are closely related to chameleons (Matthee *et al.,* 2004), but their tongues lack the specialized collagenous tissue and spiral-shaped muscle fibres of chameleon tongues which allow a specialized extension mechanism (Moulton *et al.,* 2016; Van Leeuwen, 1997). The tongue of the agamid *Moloch horridus* (thorny devil), however, is like that of the chameleon: covered in sticky mucosal glands which enable them to catch insect prey (Meyers & Herrel, 2005). Many other agamid species such as *Pogona vitticeps* (bearded dragon) use their tongues for swallowing (food intake), and chemoreception which enables them to forage for prey and seek mates. *Pogona vitticeps* will eat insects, but it is also likely to forage for plant matter and the occasional smaller lizard rather than just sit and wait for insect prey; therefore, it lacks the sticky mucosal glands on the tongues of chameleons and *M. horridus* (Abbate *et al.,* 2009; Cogger, 2018). Other distinctive features such as the deeply bifurcated tongue tips of many varanid species such as *Varanus gouldii* (Gould's monitor) as well as snakes, enable them to search for vertebrate prey via chemoreception while travelling long distances, as their highly specialized tongues allow them to pick up chemical signals at wide ranges (Schwenk, 1995; Cooper, 1989). Snakes and varanids both seek out active vertebrate prey via foraging and roam while mate seeking, and therefore appear to have deeply bifurcated tongues as a result of convergent evolution (Cooper, 1995; Schwenk, 1994; Young, 1997).

While several studies on squamate tongue structure and morphology have been carried out, these studies mainly focus on one species (Čížek *et al*., 2019; Iwasaki *et al.,* 1994; Yang & Wang, 2016). Only two studies have focused on the effect of lifestyle (Morgans & Heidt, 1978) or diet (Salem *et al*., 2019) on tongue structure, without placing their findings within a phylogenetic context. Direct comparisons have only been made between a pair (Morgans & Heidt, 1978) or trio (Salem *et al*., 2019) of squamate species.

For our study, we hypothesize that diet and niche are more important at influencing tongue structure than is relatedness. We predict that, regardless of position on the phylogenetic tree, species that are more similar in lifestyle will share greater similarities in tongue structure as a result of natural selection. To test the relationship between tongue morphology and natural selection in lizards, we will focus on seven Western Australian squamates – one carphodactylid gecko, *Underwoodisaurus milii* (barking gecko); five skinks, *Tiliqua rugosa rugosa* (western bobtail), *Egernia kingii* (King's skink), *Eremiascincus richardsonii* (broad-banded sandswimmer), *Hemiergis initialis initialis* (southwestern earless skink) and *Menetia greyii* (common dwarf skink); and one agamid, *Pogona minor minima* (southwestern bearded dragon). Most of the lizard species in this study are from the family Scincidae, but all fill different niches. Selective pressures caused by diet and lifestyles may result in convergent evolution and similar tongue morphologies between unrelated lizard species from different families. For example, a skink and an agamid with similar diets may share similarly shaped papillae.

The aim of this study is to compare the tongue macro- and microstructure of these seven native Australian lizard species with each other and then with other lizard species for whom SEM images are available in published literature (Figure 3.2). Comparison with a wide range of other lizard families will allow us to test the hypothesis that diet and niche exert stronger influence on squamate tongue morphology as opposed to phylogeny.

3.3 Materials and methods

3.3.1 Morphological measurements of Western Australian squamate species

Donated non-data and road killed specimens of one adult *Underwoodisaurus milii*, one adult *Tiliqua rugosa rugosa*, one adult *Egernia kingii*, one adult *Eremiascincus richardsonii*, one adult *Hemiergis initialis initialis*, one adult *Menetia greyii*, and one adult *Pogona minor minima* were measured to assess overall body size dimorphism. Standard measurements of the total body length and body width, snout vent length (SVL) (tip of the snout to the cloaca), and tail length (cloaca to the tip of the tail) were taken using a flexible measure tape and a plastic ruler. Digital calipers were used to take the measurements of the head length (tip of snout to front of neck), head width at nostrils, head width at eyes and neck width. All measurements were recorded to the nearest mm.

For each specimen, the lower mandible and movable maxillary of the jaw were held open and the tongue was dissected from the oral cavity near the frenulum. Total tongue length and width were recorded in mm. Tongue macrostructure variation among the seven lizard species was compared (Figure 3.1) using morphometric measurements.

3.3.2. Preparation of squamate tongues for scanning electron microscopy (SEM)

The dissected tongue samples were fixed in 2.5% glutaraldehyde solution for 24 hours at 4˚C. The tongues were then rinsed in 0.1M phosphate buffer before undergoing a dehydration process in 75%, 86% and then 96% ethanol for 18 minutes at each concentration, then air dried for 24 hours. Excised tongue and tine samples were then mounted, ventral side down, onto metal mounting stubs. Mounted samples were then sputter coated with gold ions (DII-29030SCTR Smart Coater) before being placed in an SEM for viewing. Images of tongue microstructures at the tip, fore-tongue, mid-tongue, and hind-tongue were taken with an SEM camera at 15 kV (JEOL JCM-6000Plus). Images were viewed at magnifications of 20 µm, 50 µm, 100 µm and 1mm in order for tongue structure morphology to be analysed between squamate species of differing body sizes (SVL ranges from 34.00mm to 287.00mm).

3.3.3. Comparison of tongue macrostructure between seven Western Australian squamate species

Following Herrel *et al.* (1998) and Rabinowitz and Tandler (1986), the tongues of all seven lizard specimens were partitioned into three sections- the fore-tongue which also features the tongue tip, the mid-tongue, and the hind-tongue. Comparisons of tongue macrostructure were made with regard to variation in total tongue length, tongue width, tip shape and presence and absence/degree of bifurcation.

3.3.4. Comparison of SEM imagery with previously published material

Papillae type and spatial distribution were described for each of the seven specimens. These descriptive summaries and their associated macro- and microstructure details were then tabulated with descriptions derived from SEMS of 33 other lizard species available in the published literature (Table 3.2). The sole carphodactylid species, *U. milii*, was compared to five other members of the Gekkota superfamily: *Eublepharis macularius* (leopard gecko: Eublepharidae) (Jamniczky *et al.,* 2009), *Coleonyx variegatus* (Western banded gecko: Eublepharidae) (Filoramo, 2007), *Ptyodactylus guttatus* (Sinai fan-fingered gecko: Phyllodactylidae) (Gewily *et al.,* 2021), *Gekko japonicus* (Japanese gecko: Gekkonidae) (Iwasaki, 1990), and *Hemidactylus flaviviridis* (yellow-bellied gecko: Gekkonidae) (Al-Fartwsy *et al*., 2016). The five native skink species were compared with five skink species from published literature: *Tiliqua scincoides* (bluetongue skink) (Abbate *et al*., 2009), *Scincella tsinlingensis* (Tsingling dwarf skink) (Yang & Wang, 2016), *Chalcides ocellatus* (ocellated skink) (Elsheikh *et al.,* 2013), *Scincus scincus* (sandfish skink) (Salem *et al*., 2019), and a single species from the genus *Lygosoma* (*Scincella*) (Schwenk, 1988). *Pogona minor minima* was compared to five other agamid species: *Uromastyx aegyptia* (Egyptian uromastyx) (Al-Ahmady *et al.,* 2017), *Pogona vitticeps* (Central bearded dragon) (Zghikh *et al.,* 2014), *Uromastyx ornata* (ornate uromastyx), *Uromastyx acanthinura* (spiny-tailed uromastyx) (Herrel *et al.,* 1998), and *Stellagama stellio* (starred agama) (Herrel *et al.,* 1995). A total of 16 lizard families were used for this broad comparison of tongue morphology within squamates (Figure 3.1).

Fifteen species from the families Cordylidae, Teiidae, Lacertidae, Helodermatidae, Chamaeleonidae, Anguidae, Iguanidae, Phrynosomatidae, Opluridae and Dactyloidae were used to compare published SEM-based data with the images generated in this study in order to make a comparison at a wider phylogenetic scale. Comparison of the two Cordylidae species *Ouroborus cataphractus* (armadillo girdled lizard) and *Karusasaurus polyzonus* (southern karusa lizard) (Broeckhoven & Mouton, 2013) were made with Agamidae *P. vitticeps*, *U. ornata* and *S. stellio* (Zghikh *et al*., 2014; Salem *et al*., 2019; Herrel *et al*., 1998), Dactyloidae *Anolis carolinensis* (green anole) and Opluridae *Oplurus cuvieri* (Madagascan collared iguana) (Rabinowitz *et al*., 1986; Delheusy *et al.*, 1994).

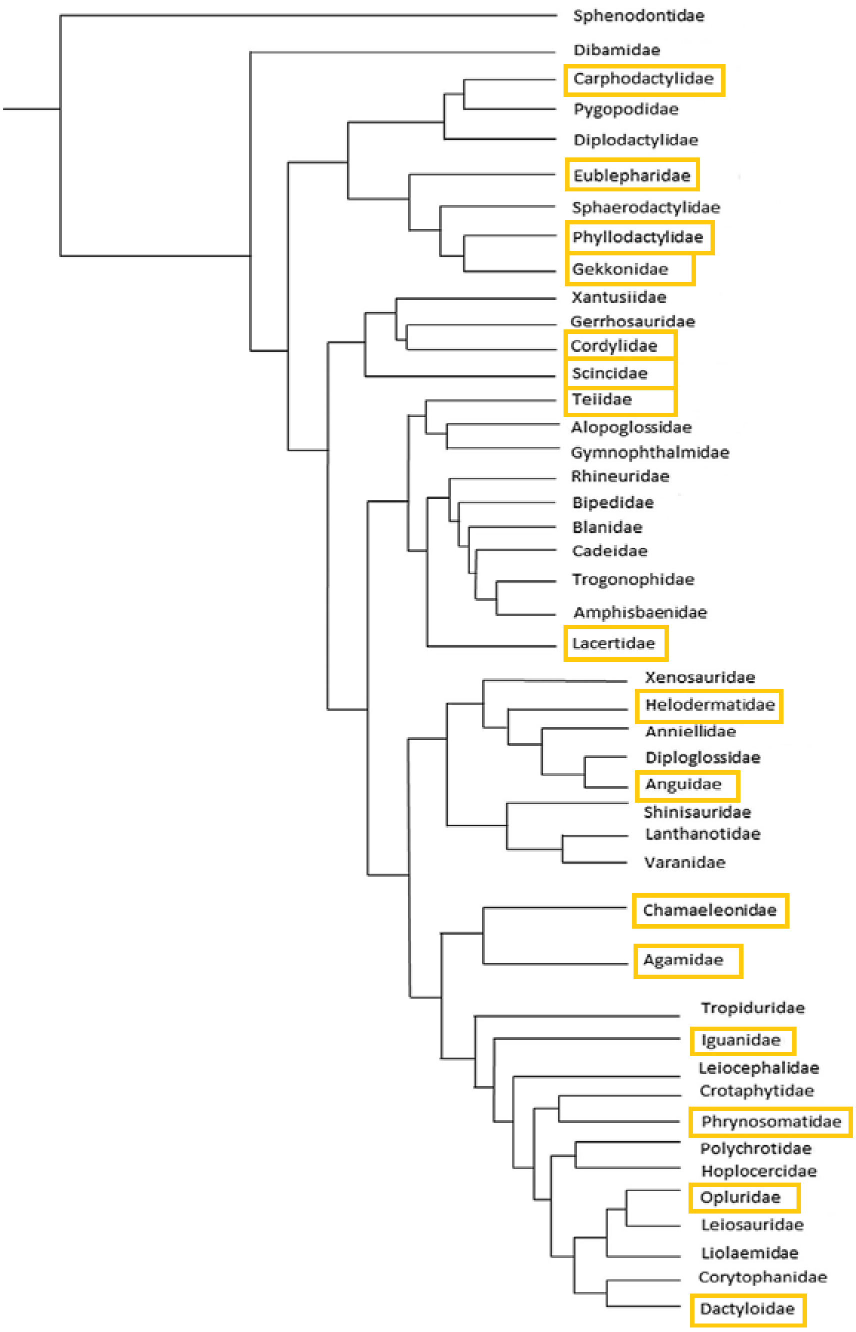

Figure 3.1 Cladogram of the lizard families comparatively analysed for tongue microstructure. SEM imagery of lizard tongues were available for the following families (highlighted in yellow boxes), with the number of species for which SEM data are available indicated per family: Carphodactylidae- 1, Eublepharidae- 2, Phyllodactylidae- 2, Gekkonidae- 2, Cordylidae- 3, Scincidae- 10, Teiidae- 1, Lacertidae- 3, Helodermatidae- 1, Anguidae- 1, Chamaeleonidae- 3, Agamidae- 6, Iguanidae- 1, Phrynosomatidae- 2, Opluridae- 1, and Dactyloidae- 1. Cladogram adapted from Barr *et al.,* (2020). The ecology, diet and tongue macro- and micromorphology of all 40 species, are collated in Table 3.2 in phylogenetic order.

3.4 Results

All seven study species are described with 33 other squamate species from 16 families in Table 3.2 in phylogenetic order. Tongue macro- and micromorphologies as well as diet, behaviour and niche of the seven West Australian species were compared to that of closely related squamate species from similar families listed in Table 3.2. Tongue, head, and full body measurements in Table 3.1 provide a comparative scale of morphometric diversity among the Western Australian lizard specimens sampled. Proportion of the tongue length to head length indicates that head length has a stronger influence than snout vent length (SVL) on tongue length (Table 3.1).

3.4.1. Examination of size differences and tongue macro- and microstructure between Western Australian squamate specimens

Tiliqua rugosa rugosa and *Egernia kingii* are both large species of skinks with large tongue length to head length proportions (*T. r. rugosa*= 54.2%, *E. kingii*= 59.5%) (Table 3.1), reflecting their foraging and food handling behaviours that allow them to capture active prey. *Tiliqua rugosa rugosa* relies on its large body size (378.00mm) and head length (72mm) to defend its mate and territory during the breeding season (Bull & Pamula, 1996). The similarly large *E. kingii* (410.00mm) also relies on its large body size, but as defence against predators like snakes via biting and writhing (Masters & Shine, 2003). Differences in tongue macro- and microstructure of these large skinks result from their differences in dietary preferences and food handling behaviour.

The tongue tip of *T. r. rugosa* is bifurcated and has a dark pigment (Figure 3.2b). Its tongue width gradually increases from tongue tip to fore-tongue to mid-tongue (Figure 3.2b). The dorsal area of the fore-tongue of *T. r. rugosa* shows elevated diamond-shaped papillae arranged in a mosaic like pattern (Figure 3.4a-d). The presence of diamond-shaped elevated papillae extends to the mid-tongue, where the elevation is more pronounced (Figure 3.4c, d). The base of the tongue shows the presence of triangular, flattened papillae (Figure 3.4e). *Egernia kingii* also features a dark pigment on its tongue (Figure 3.2c) but in contrast to *T. r. rugosa*'s broad tongue, it has a narrow tongue bifurcated tongue tip which is less visible compared to that of *T. r. rugosa*. The dorsal tongue tip of *E. kingii* has rounded non-elevated papillae arranged in a mosaic-like pattern as opposed to T. r. rugosa's diamond shaped papillae (Figure 3.5a, b). The presence of these rounded non-elevated papillae decreases from fore-tongue to mid-tongue. Fleshy fungiform papillae are present from the mid-tongue (Figure 3.5c) to hind-tongue (Figure 3.5d). These differences in papillae are a reflection on the two large skinks' foraging dietary preferences. *Tiliqua rugosa rugosa* is an opportunist that prefers open dunes. Its elevated diamond shaped papillae and broad tongue allow easy intake of carrion and invertebrates like snails. It only occasionally eats plant matter (Cogger, 2018). By contrast, *E. kingii* prefers woodlands and shrublands and its non-elevated round papillae is a reflection of its primary diet of plants. The fleshy fungiform papillae allow the movement of its occasional food of birds' eggs to the back of the throat (Cogger, 2018; this study).

Eremiascincus richardsonii's large tongue length to head length ratio (75.0%) and thin head width at the eyes (6.27mm) (Table 3.1), reflect its fossorial habits for thermal regulation in a desert environment via burrowing headfirst (Pianka, 2011). Its small body width (11.13mm) (Table 3.1) allows it to easily forage for both insect and vertebrate prey via 'swimming' in the sand (James & Losos, 1991). *Eremiascincus richardsonii*'s narrow tongue (Table 3.1) shows no bifurcation of the tongue tip (Figure 3.2d & Figure 3.6a) and is broad in shape compared to *Egernia kingii* (Figure 3.2d). The dorsal surface of the tongue from the fore-tongue shows round, non-elevated papillae similar to *E. kingii* (Figure 3.6a & b). These papillae become more elevated as they reach the mid-tongue and hind-tongue (Figure 3.6b). Back of the hind-tongue has a smooth surface with no papillae (Figure 3.6b).

Both the large *Egernia kingii* and the smaller *Eremiascincus. richardsonii* have narrow tongues with round non-elevated papillae on the tongue tips, reflecting their omnivorous dietary preferences of insects and plant matter (Cogger, 2018). However, while *E. kingii* 's tongue displays a bifurcated tip and fleshy fungiform papillae on its hind-tongue, the tongue tip of *E. richardsonii* by contrast has no bifurcation and the papillae on its tongue tip get more elevated towards the hind-tongue (this study) , which aid in the uptake of occasional smaller lizard prey (Cogger, 2018; James & Losos, 1991), in contrast to *E. kingii's* diet of vegetation and birds' eggs (Cogger, 2018) (Table 3.2). The lack of bifurcation on *E. richardsonii*'s tongue tip indicates that it prefers to seek out prey by sensing vibrations in the sand (Cogger, 2018; James and Losos, 1991) as opposed to using chemoreception like *E. kingii* (Cogger, 2018).

The two small crepuscular, insectivorous skinks *Hemiergis initialis initialis* and *Menetia greyii* have thin body widths (*H. i. initialis*= 5mm, *M. greyii*= 6mm) reflecting their fossorial behaviour, allowing them to move through soil with ease for small invertebrate prey. Their small ratio of tongue length to head length proportions (*H. i. initialis*= 14.3%, *M. greyii*= 16.7%) and broad, slightly bifurcated tongues (Figure 3.2f, Figure 3.2g) indicating their foraging habits. Both skinks also have shared habitat and dietary preferences (Cogger, 2018): their main difference lies in tongue microstructure (this study). The dorsal area of the tongue tip of *Hemiergis initialis initialis* is bifurcated and elevated (Figure 3.2e, Figure 3.7a) with round, irregular flattened mosaic papillae cover the dorsal fore-tongue (Figure 3.7b). Overlapping scale shaped papillae cover the mid-tongue and hind-tongue (Figure 3.7c, d). They are flat and are inconsistent in shape. The overlapping papillae on the hind-tongue are more diamond shaped compared to that of the mid-tongue (Figure 3.7d). By contrast, *Menetia greyii* also has a broad, bifurcated tongue, but its dorsal tongue tip folds are not as elevated compared to *H. i. initialis* (Figure 3.2f, Figure 3.8a). *Menetia greyii* also consistently exhibits overlapping scale shaped papillae on its dorsal surface throughout its fore-tongue, mid-tongue and hind-tongue. These papillae have prominent scalloped edges (Figure 3.8a, b & c). These subtle tongue structure and papillae variations indicate differences in prey capture and food handling between the two small skink species despite having similar diet and habitat preferences.

The snout vent lengths of *Underwoodisaurus milii*, the sole carphodactylid gecko (88.00mm), and *Pogona minor minima*, an agamid (96.00mm), along with their tongue length to head length ratios (*U. milii*= 37%, *P. m. minima*= 30.6%) reflect their preferences for foraging for insects and occasional lizard prey (Table 3.1). *Underwoodisaurus milii* is nocturnal while *P. m. minima* is diurnal and in addition to insects, also eats plant matter (Cogger, 2018) (Table 3.2). *Underwoodisaurus milii* has a larger tongue length to head length ratio (37%) compared to that of *P. m. minima* (30.6%) because *U. milii* uses its tongue for cleaning purposes in addition to prey seeking (Griffiths, 1987). Further differences between the two species also can be identified via their tongue microstructure. The tongue tip of *U. milii* is predominantly rounded with the exception of a slight bifurcation (Figure 3.3a), that measured only 344 µm deep. The tongue microstructure of *U. milii* has a multitude of overlapping scale-shaped papillae on the dorsal area of its tongue tip (Figure 3.3a). In addition, conical-shaped papillae are also located on the edge of the tongue tip (Figure 3.3b). The papillae on its mid-tongue are rounded yet irregular in shape and less elevated. Arrangement of rounded papillae on the fore-tongue and mid-tongue is mosaic-like (Figure 3.3c & d). In contrast to *U. milii*, *Pogona minor minima* lacks a bifurcated tongue tip, but is wider in comparison to *E. richardsonii* (Figure 3.2g). The fore-tongue of *Pogona minor minima* exhibits rounded, non-elevated papillae on the dorsal surface (Figure 3.9a, b & c). In contrast to *E. kingii* and *E. richardsonii*, these rounded non-elevated papillae are notably clumped and almost shapeless. They are smooth and almost resemble bumpy microfacets (Figure 3.9a, b & c). Examination of the mid-tongue shows overlapping foliate-shaped papillae (Figure 3.9d). The conical shaped papillae on *U. milii*'s tongue tip aid not only its food handling habits of occasionally taking in smaller lizard prey, but also aid process of grooming (Griffiths, 1987). By contrast, *Pogona minor minima*'s rounded, non-elevated papillae on its fore-tongue are similar to that of *E. kingii* (this study) as both lizards forage for vegetation and insects. Its foliate papillae on its mid tongue aid with moving occasional smaller lizard prey towards the back of its throat (Cogger, 2018).

Underwoodisaurus milii and *Eremiascincus richardsonii* have narrow tongues (Table 3.1), and both are foragers with similar dietary preferences of insects and occasional reptile prey (Cogger, 2018) (Table 3.2). However, *E. richardsonii* is crepuscular (Cogger, 2018) and has a non-bifurcated tongue with rounded non-elevated papillae on the fore-tongue, which become slightly elevated towards the mid- and hind-tongue. By contrast, *U. milii* is nocturnal (Cogger, 2018), has slightly bifurcated tongue with elevated conical shaped papillae on the tongue tip and fore-tongue, mosaic papillae on the mid-tongue and a smooth lingual surface on the hind tongue (this study). The differences in tongue macro- and microstructure reflect these similarly sized lizards' divergent hunting habits as *E. richardsonii* is a fossorial lizard which burrows through the sand for insects and occasional small lizards and is more likely to rely on sensing its prey using vibrations as opposed to chemoreception. By contrast, *U. milii* prefers to forage for prey using sight and chemoreception (Cogger, 2018). Both carphodactylid gecko *U. milii* and agamid *P. m. minima* both exhibit variation on the mid-tongue (Figure 3.3c & 3.3d, Figure 3.9d).

Both *Pogona minor minima* and *Eremiascincus richardsonii* are similar in snout vent length (*P. m. minima*= 96mm, *E. richardsonii*= 70.10mm) (Table 3.1) and display round non-elevated papillae on their tongue tips and fore-tongue (Figure 3.6a, Figure 3.9), two morphological similarities attributable to these two squamates having insects and vegetation as part of their dietary preferences. The overlapping round papillae on *E. richardsonii*'s hind tongue (Figure 3.6b) and foliate papillae on *P. m. minima*'s mid-tongue (Figure 3.9d) aid in a similar function with moving their occasional lizard prey towards the back of their throats.

Analysis of macro- and micromorphology of these seven lizard samples and their related kin confirms that that diet, behaviour and habitat niches have more impact on tongue macro- and microstructure than does phylogenetic affiliation. Unrelated species with similar diets and lifestyles are more likely to share similar tongue macro and microstructures. Further comparison of macro- and microstructure in relation to diet, habitat and behaviour of the seven WA lizard species will be done with that of the other 33 lizard species from previous literature.

Table 3.1: Body measurements of seven Western Australian lizard species obtained opportunistically as roadkill and non-data specimens. Species are presented in phylogenetic order. Head length has a stronger influence on tongue length compared to snout vent length.

Species	**Total body length (mm)**	**Body width (mm)**	**Snout-vent length (mm)**	**Tail length (mm)**	**Head length (mm)**	**Head width at eyes (mm)**	**Head width at nostrils (mm)**	**Neck width (mm)**	**Total tongue length (mm)**	**Total tongue width (mm)**
Underwoodisaurus milii	113.00	18.00	88.00	25.00	27.00	11.00	5.00	11.00	10.00	3.00
Proportion of TL to SVL									*11.4%*	
Proportion of TL to Head L									*37.0%*	*11.1%*
Tiliqua rugosa rugosa	378.00	48.45	287.00	91.00	72.00	20.96	9.54	34.86	39.00	19.68
Proportion of TL to SVL									*13.6%*	
Proportion of Head L									*54.2%*	*27.3%*
Egernia kingii	410.00	44.00	223.00	187.00	42.00	30.00	8.00	28.00	25.00	8.00
Proportion of TL to SVL									11.2%	
Proportion of TL to Head L									59.5%	19.0%
Eremiascincus richardsonii	(70.10)[1]	11.13	70.10	NA[1]	14.00	6.27	2.88	9.24	10.50	3.49
Proportion of TL to SVL									15.0%	
Proportion of TL to Head L									75.0%	24.9%
Hemiergis initialis initialis	60.00	5.00	41.00	19.00	7.00	3.00	1.00	1.00	1.00	1.00
Proportion of TL to SVL									2.4%	
Proportion of TL to Head L									14.2%	14.2%
Menetia greyii	58.00	4.00	34.00	24.00	6.00	3.00	1.00	1.00	1.00	1.00
Proportion of TL to SVL									2.9%	
Proportion of TL to Head L									16.7%	16.7%
Pogona minor minima	289.00	11.93	96.00	113.00	34.00	6.83	4.35	6.07	10.40	5.00
Proportion of TL to SVL									10.8%	
Proportion of TL to Head L									30.6%	14.7%

[1] Specimen damaged, no tail available. Total body length is presented as equivalent to snout-vent length.

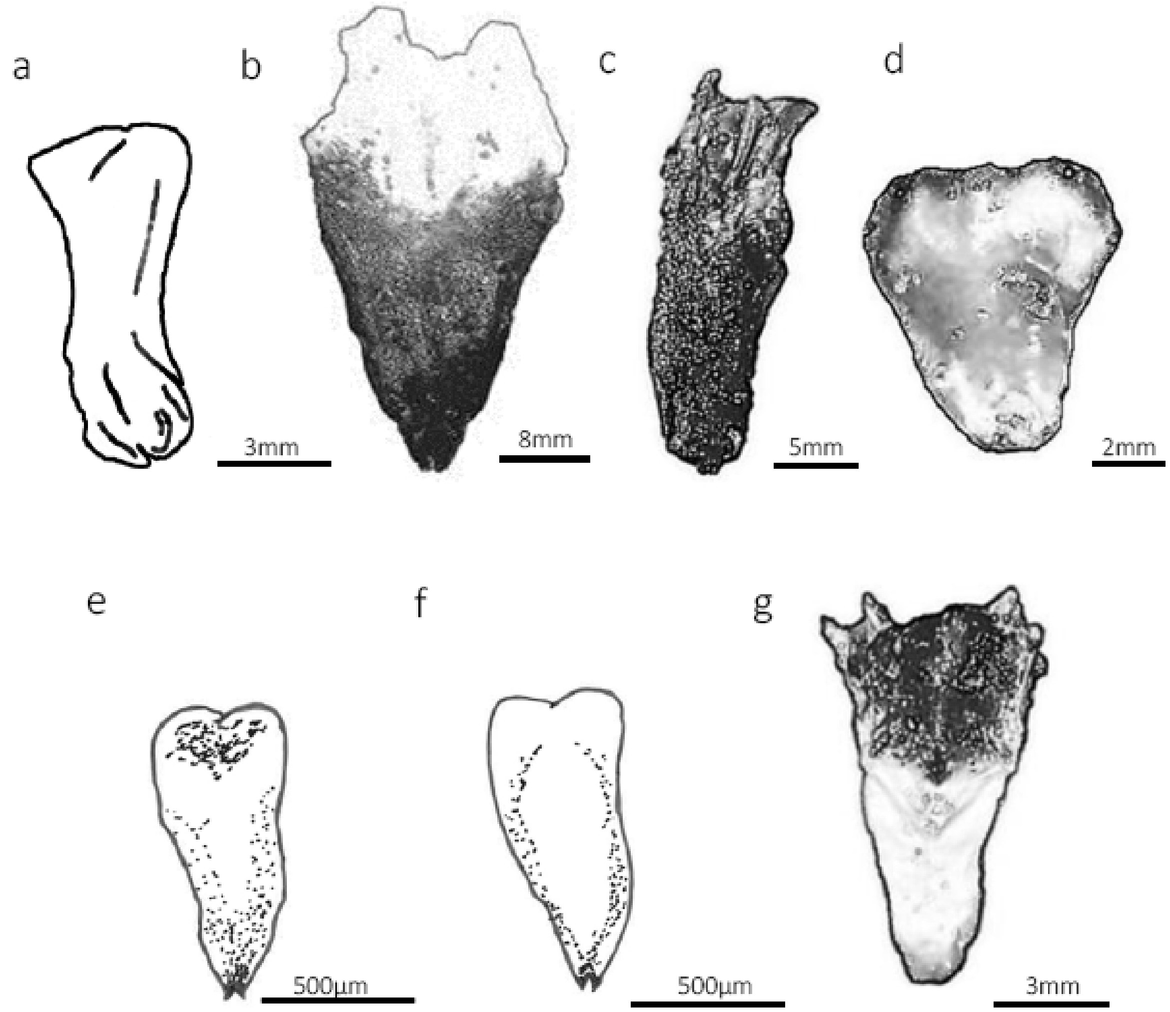

Figure 3.2: Schematic comparison of tongue macro-structure of the seven Western Australian squamate species. a) *Underwoodisaurus milii* (barking gecko. Family: Carphodactylidae). Tongue tip has slight bifurcation (Figure 3.3a) and remains constant in width from tongue tip to fore-tongue to mid-tongue. Folds on tongue indicated by lines. b) *Tiliqua rugosa rugosa* (Southwestern bobtail. Family: Scincidae). Tongue tip has slight bifurcation and tongue width gradually increases as it goes from tongue tip to fore-tongue c) *Egernia kingii* (King's skink. Family: Scincidae). Tongue has slight bifurcation at tip and has a sudden increase in width as it goes from tongue tip to fore-tongue. Tongue width size remains constant from fore-tongue to mid-tongue. d) *Eremiascincus richardsonii* (sandswimmer skink. Family: Scincidae). Tongue tip lacks bifurcation and gradually increases in size as it goes from tongue tip to fore-tongue to mid-tongue. e) *Hemiergis initialis initialis* (Southwestern earless skink. Family: Scincidae) Tongue tip is bifurcated and tongue gradually increases in width from tongue tip to fore-tongue to mid-tongue. f) *Menetia greyii* (Common dwarf skink. Family: Scincidae). Tongue tip is bifurcated and tongue gradually increases in width from tongue tip to fore-tongue to mid-tongue. g) *Pogona minor minima* (Pygmy bearded dragon.Family: Agamidae). Tongue tip lacks bifurcation and tongue width gradually increases in size as it goes from tongue tip to fore-tongue. Scale bars at 3mm (a and g), 8mm (b), 5mm (c), 2mm (d) and 500µm (e and f).

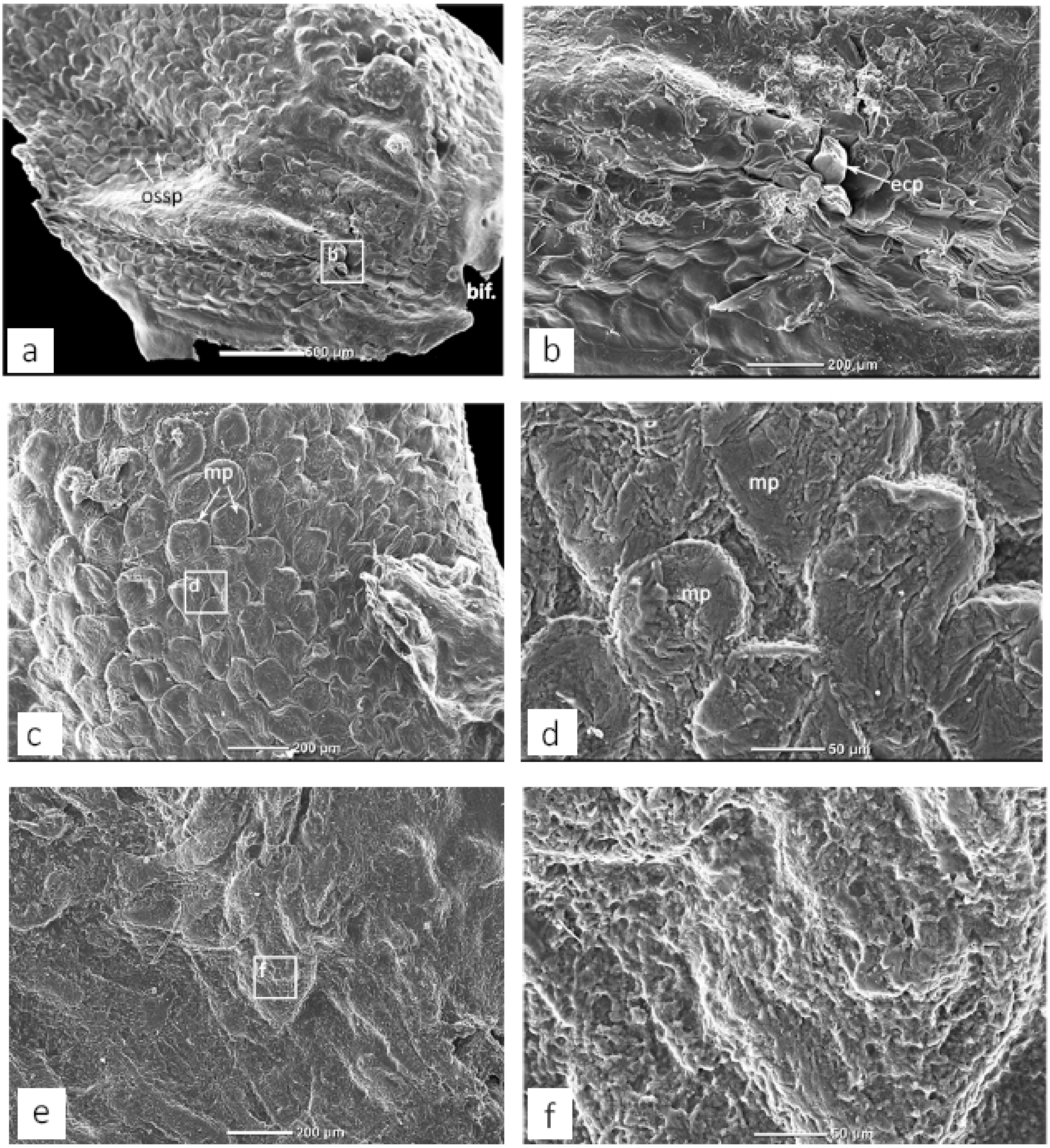

Figure 3.3: Scanning electron microscope image of the dorsal surface of *Underwoodisaurus milii* tongue. (a) *U. milii* dorsal fore-tongue showing the bifurcated tongue tip (bif.) and overlapping scale shaped papillae (ossp). Box indicates area in section (b). (b) Close up of *U. milii* tongue tip with elevated conical papillae (ecp). (c) Mid-tongue showing rounded, yet irregular mosaic papillae (mp). Box indicates area in section (d). (d) Close up of the mid-tongue section with a closer look at the mosiac papillae (mp). (e) Hind-tongue exhibiting a smooth lingual surface with raised areas and no papillae. Box indicates area in section (f). (f) Closeup of hind-tongue showing elevated ridges with no papillae. Scale bars = 500µm (a), 200µm (b, c & e) and 50µm (d & f).

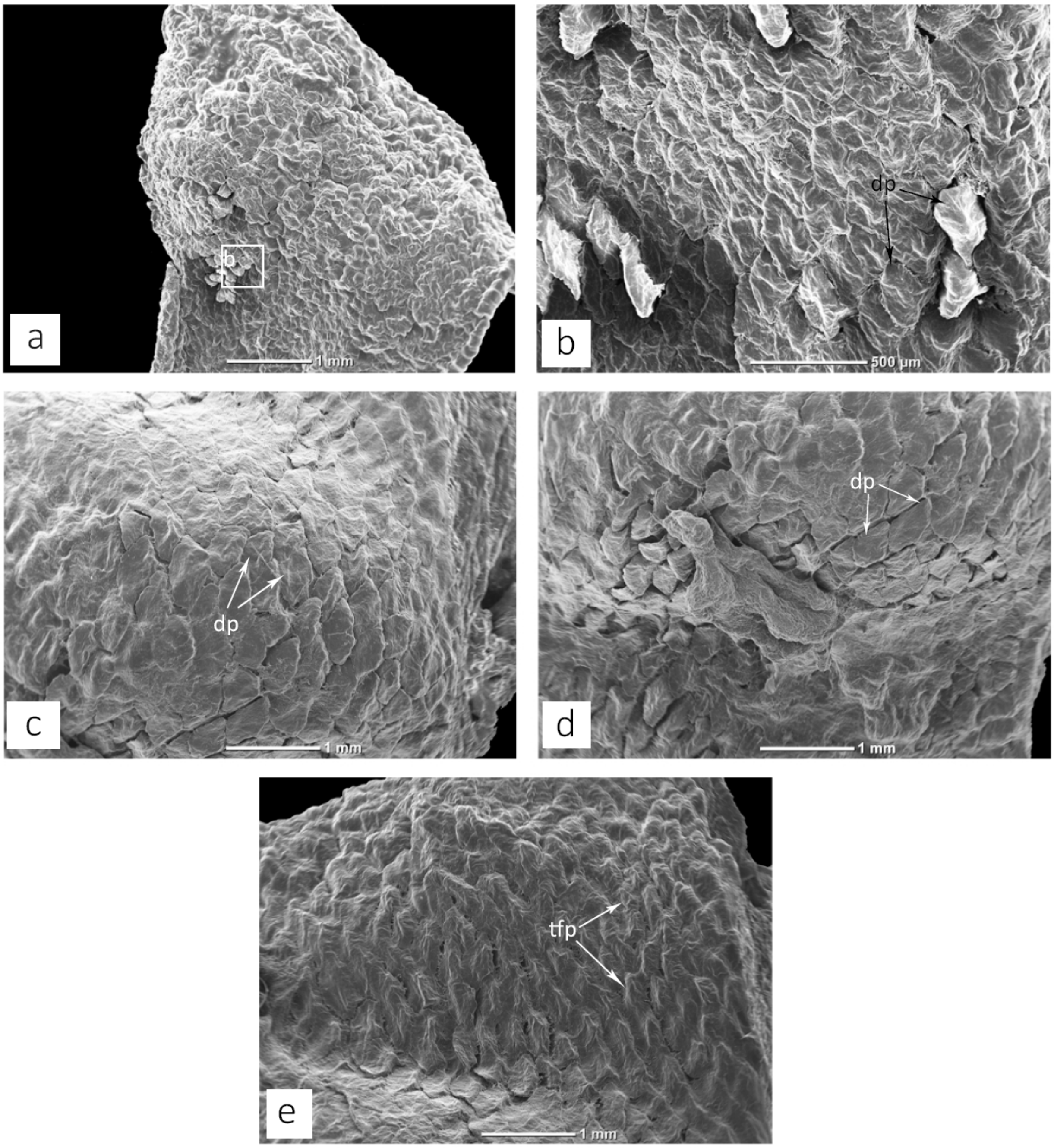

Figure 3.4: Scanning electron microscope image of dorsal view of *Tiliqua rugosa rugosa tongue*.
(a) *T. r. rugosa* tongue tip showing diamond shaped papillae (dp) arranged in a mosaic like fashion.
(b) Close up of *T. r. rugosa* tongue tip section exhibiting the diamond shaped papillae (dp).
These papillae are also elevated and are arranged in a mosiac pattern. (c) and (d) Mid-tongue close up of *T. r. rugosa* showing thick and more irregular diamond shaped papillae. (e) *T. r. rugosa* hind-tongue showing triangular flattened papilae (tfp). Scale bars = 1mm (a,c, d & e) and 500µm (b).

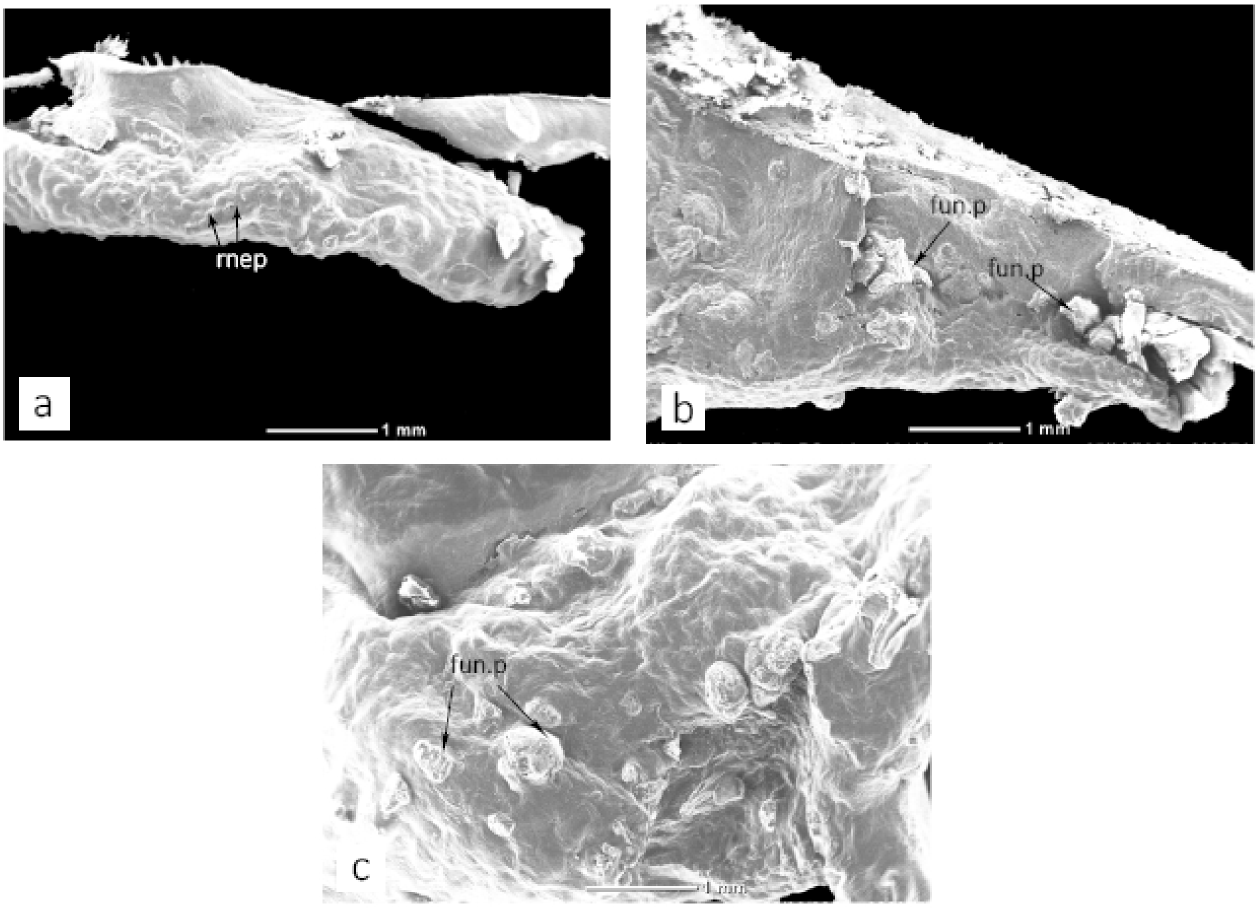

Figure 3.5: Scanning electron microscope image of *Egernia kingii* tongue at dorsal view. a) *E. kingii* tongue tip, fore-tongue and mid-tongue showing round, non-elevated papillae (rnep). b) and c) Fleshy fungiform papillae (fun.p) on hind-tongue. All scale bars = 1 mm.

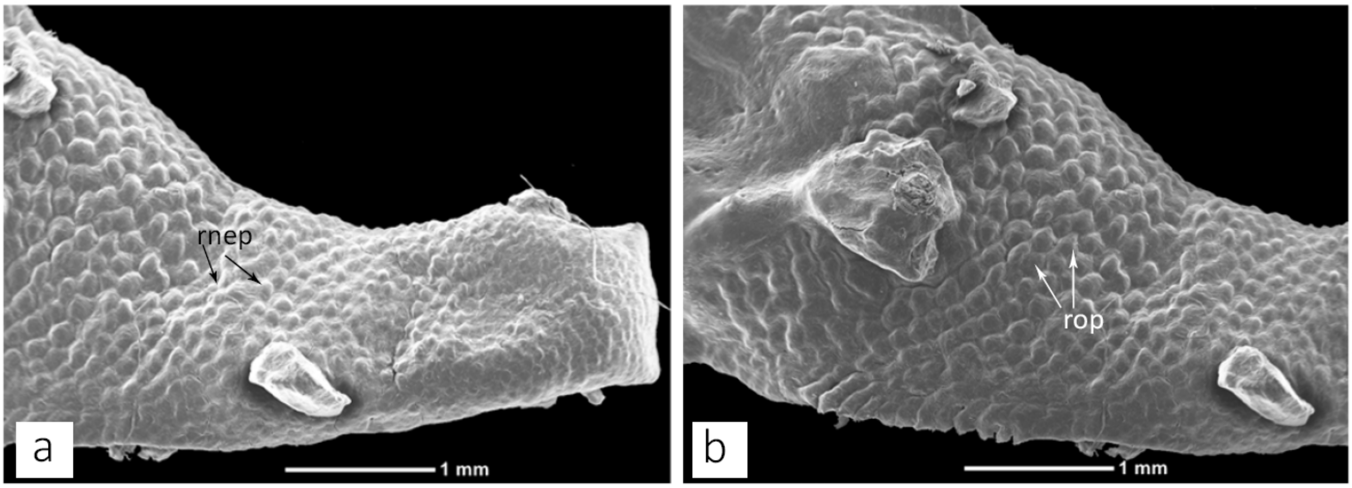

Figure 3.6: Scanning electron microscope image of *Eremiascincus richardsonii* tongue in dorsal view. (a) Tongue tip and fore-tongue of *E. richardsonii* exhibiting a non-bifurcated tongue tip and flattened round non-elevated papillae (rnep) in a mosaic like pattern. (b) Mid-tongue and hind-tongue exhibiting rounded overlapping papillae (rop). All scale bars = 1mm.

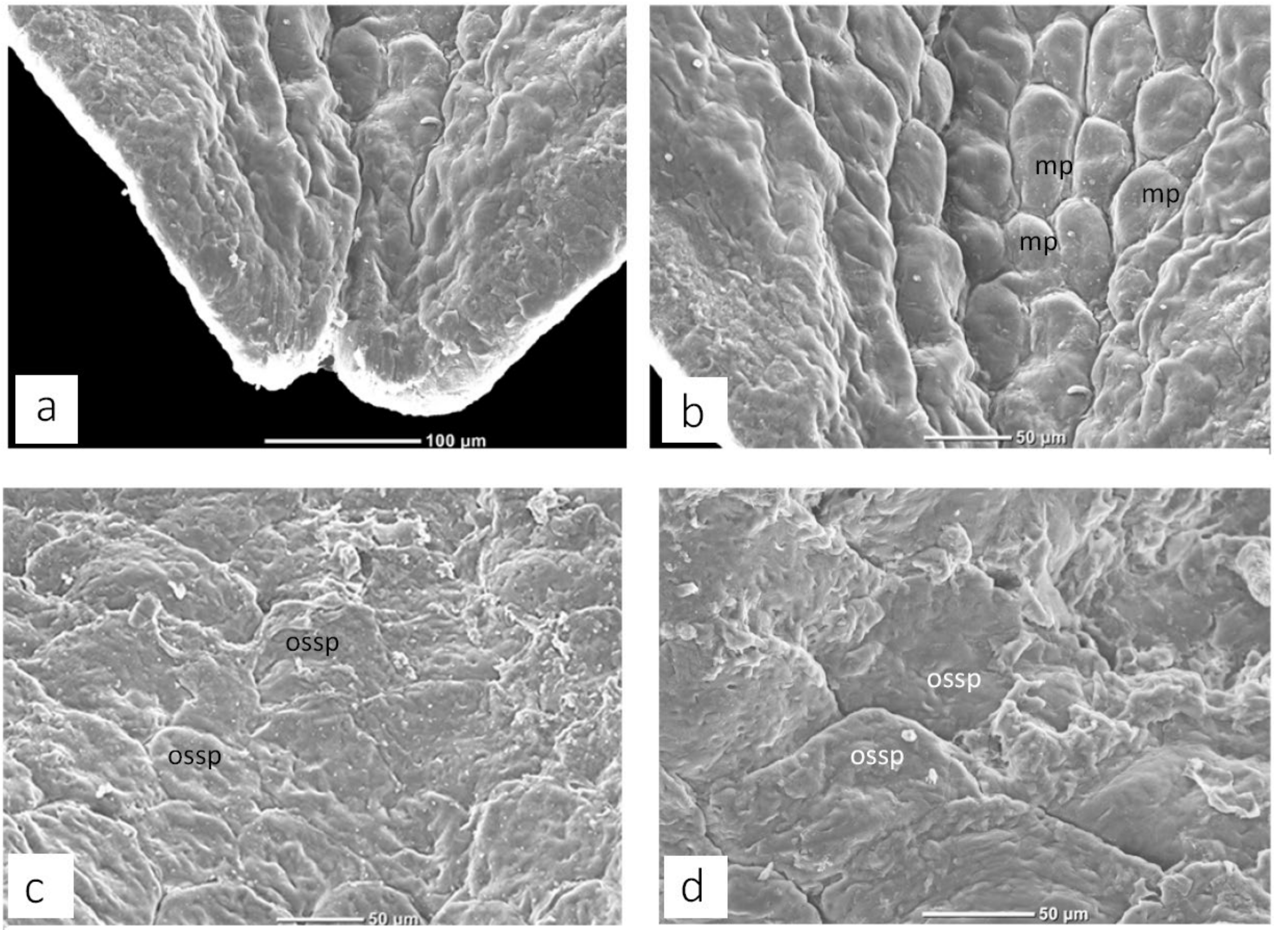

Figure 3.7: Scanning electron microscope image of *Hemiergis initialis initialis* tongue in dorsal view. (a) *H. i. initialis* tongue tip exhibiting a bifurcated tongue tip and raised lateral sides. (b) Close up of fore-tongue showing irregular mosiac papillae (mp). (c) Mid-tongue of *H. i. initialis* showing irregular shaped overlapping scale shaped papillae (ossp). (d) Close-up of hind-tongue showing overlapping scale shaped papillae (ossp), notably becoming more diamond shaped. Scale bars = 100µm (a) and 50µm (b, c & d).

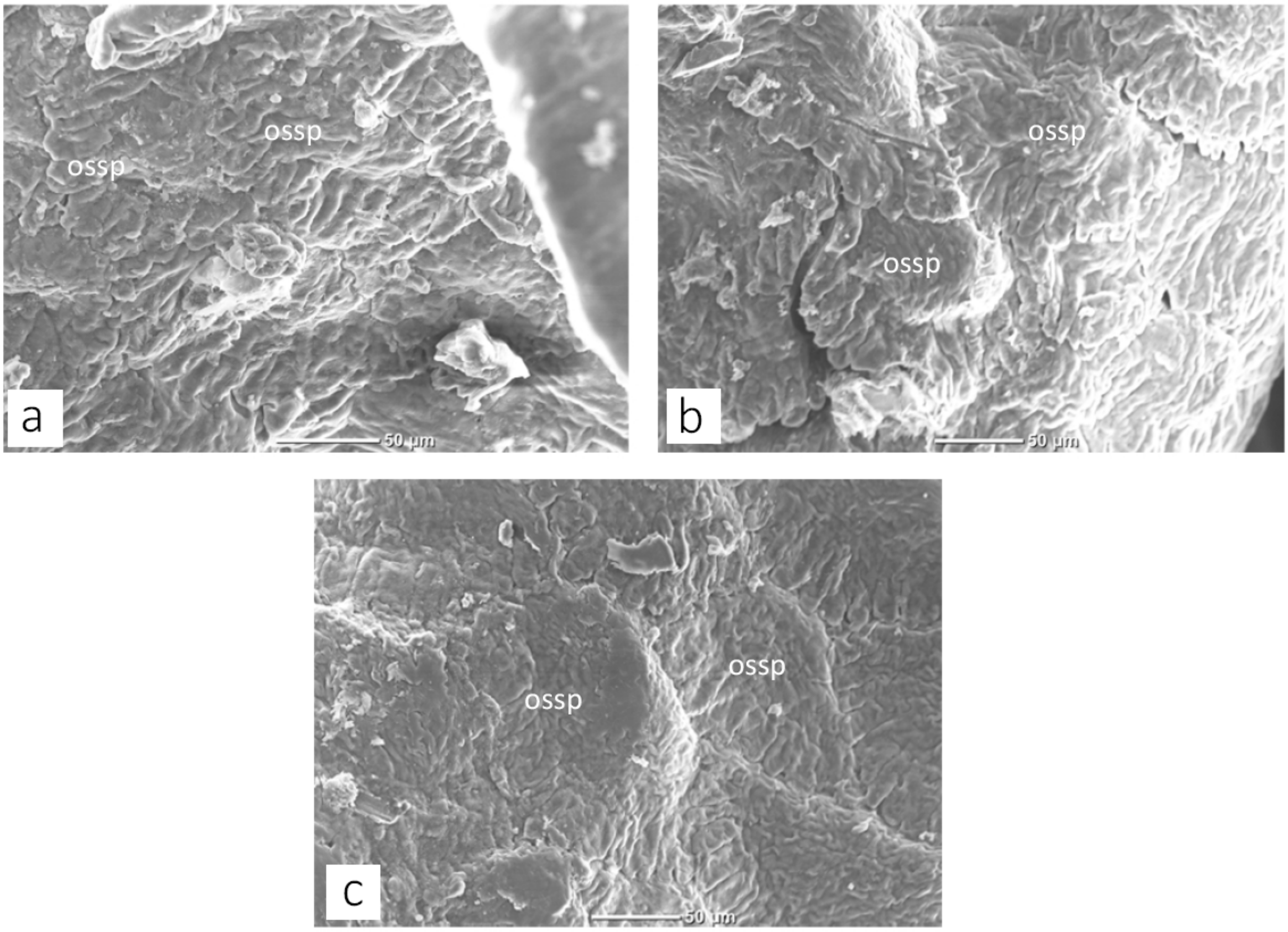

Figure 3.8: Scanning electron microscope image of *Menetia greyii* tongue at dorsal view. *(*a) Fore-tongue and (b) mid-tongue of *M. greyii* showing overlapping scale shaped papillae (ossp). Note the frilled edges on these papillae. (c) Hind-tongue showing overlapping scale shaped papillae (ossp). Hind-tongue papillae appear to be flattened, but frilled edges are still pronounced. All scale bars = 50µm.

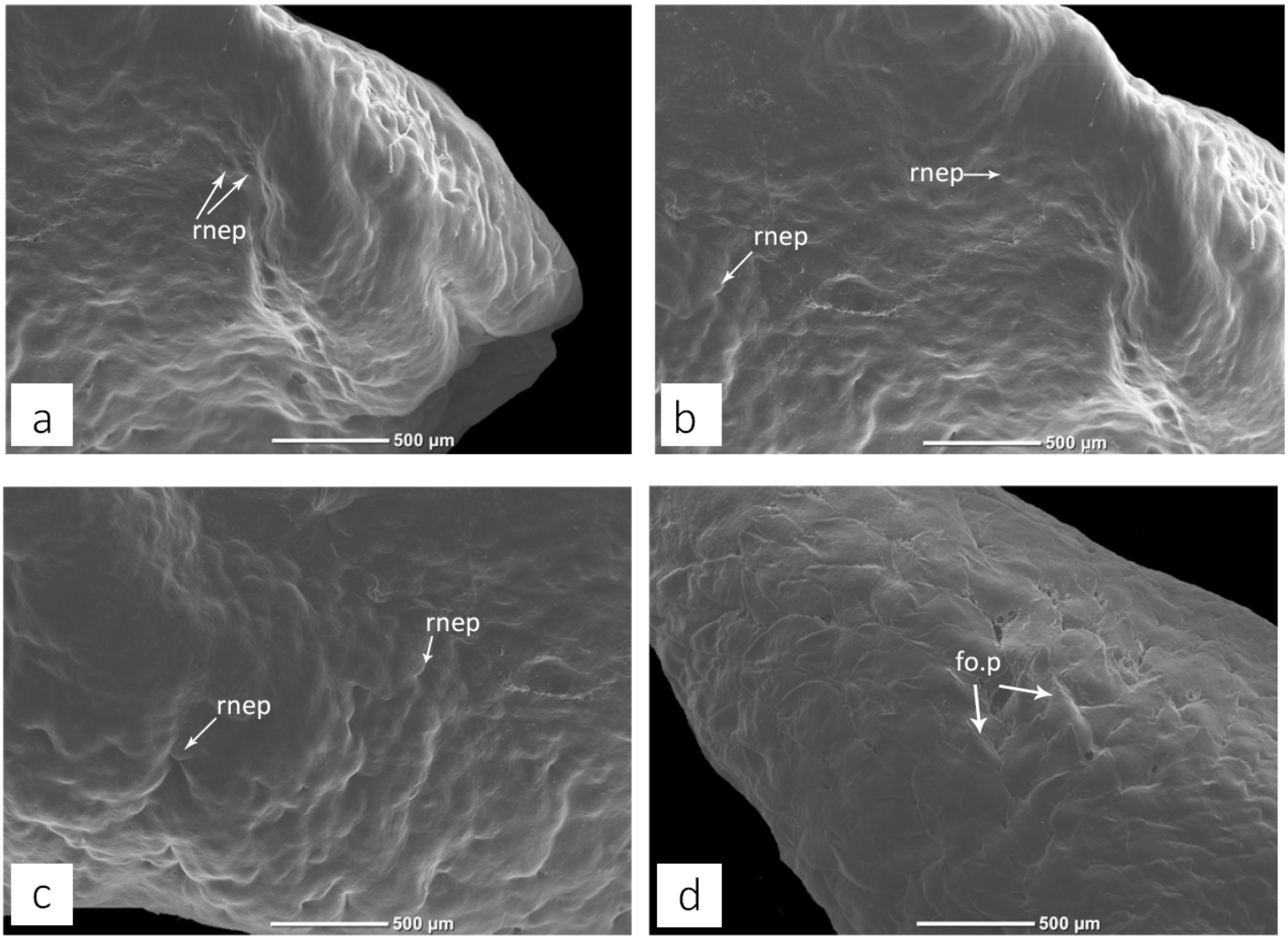

Figure 3.9: Scanning electron microscope image of the dorsal view of *Pogona minor minima* tongue. (a) *P. m. minima* tongue tip and (b), (c) fore-tongue showing nearly shapeless rounded, non-elevated papillae (rnep). (d) Mid-tongue exhibiting overlapping foliate papillae (fo.p). All scale bars = 500µm.

Table 3.2: Comparison of lizard species diet, habitat, and tongue structure from this study and previously published literature.

Species, (Family), Location	Habitat	Diet	Foraging Habits	Hunting Strategy	Tongue Macrostructure	Tongue Microstructure	References
Underwoodisaurus milii (Carphodactylidae) Western Australia	Rocky outcrops and coastal wetlands and crevices under litter and debris.	Insectivorous but will occasionally eat smaller lizards	Nocturnal	Forager	Narrow & bifurcated at the very tip	Complex mix of overlapping scale shaped papillae and elevated conical papillae tongue tip. Rounded mosaic papillae on the mid-tongue.	THIS STUDY; Cogger, 2018.
Eublepharis macularius (Eublepharidae) Pakistan and Nepal	Under rocky outcrops	Invertebrates but may feed on young mice	Nocturnal	Forager or Ambush predator	Broad	Dome shaped papillae on tongue tip, foliate papillae on lateral sides, and globular cells on foliate papillae on hind-tongue.	Jamniczky *et al.,* 2009; Henkel & Schmidt, 1995; Hunziker, 1994; Khan, 1999; Rawat *et al.*, 2019.
Coleonyx variegatus (Eublepharidae) Western United States	Open bushlands, desert and open juniper forests	Many invertebrate species	Nocturnal	Ambush predator	Broad	Foliate papillae on lateral side of tongue, dome shaped papillae on tongue tip	Filoramo, 2007; Cooper, 1998; Cooper *et al.*, 1985; Parker, 1972.
Ptyodactylus guttatus (Phyllodactylidae) Egypt and Middle East	Open desert	Small invertebrates, mainly scorpions	Crepuscular or nocturnal	Ambush predator	Narrow & bifurcated	Tongue tip has dome shaped non-overlapping papillae, mid-and hind-tongue has mosaic like papillae.	Gewily *et al.,* 2021; Werner, 2003.
Tarentola annularis (Phyllodactylidae) Egypt	Urban areas or rocky outcrops	Small invertebrates, but will eat smaller lizards	Crepuscular	Ambush predator	Narrow, bifurcated	Dome shaped papillae on tongue tip and mid-tongue. Foliate papillae on lateral side of tongue.	Bayoumi *et al.,* 2011; Ibrahim, 2004; El-Bakry *et al.,* 2016.

Hemidactylus flaviviridis (Gekkonidae) South East Asia	Prefers urban environments	Opportunistic (often feeds on insects but also smaller lizards and small snakes)	Nocturnal	Forager	Narrow	Mid-tongue/body has plenty of elevated conical papillae and fungiform papillae, while hind-tongue has foliate papillae with open goblet cells.	Al-Fartwsy *et al.,* 2015; Parves & Alam, 2015.
Gekko japonicus (Gekkonidae) Japan	Prefers urban environments	Insectivorous but will occasionally drink sap	Nocturnal	Forager	Broad	Dome and fan shaped papillae on fore-tongue. Dome shaped papillae covered in microvilli	Iwasaki, 1990; Murai *et al.,* 2013; Zhao & Adler, 1993; Ota & Tanaka, 1996; Lee *et al.,* 2004.
Hemicordylus capensis (Cordylidae) South Africa	Mountain dwelling	Invertebrates and small vertebrates	Diurnal	Sit-and-wait/ambush	Broad with bifurcated tip	Overlapping irregular papillae on ventral side of mid-tongue	Broeckhoven & Mouton, 2013; Cooper *et al.,* 1997; Mouton & Van Wyk, 1997; Brach, 1998.
Karusasaurus polyzonus (Cordylidae) South Africa	Lower mountain slopes and rocky outcrops	Large number of invertebrate species, occasionally plant matter. May resort to cannibalism.	Diurnal	Forager	Broad with Bifurcated tongue tip	Non-plumerose irregular papillae found at ventral side of tongue.	Broeckhoven & Mouton, 2013; van Blerk *et al.*, 2021.
Ouroborus cataphractus (Cordylidae) South Africa	Rocky outcrops and desert dunes	Termites, beetles, and occasionally plant matter	Diurnal	Forager	Broad	Plumerose papillae found at tip of ventral side of mid-tongue.	Bates *et al.*, 2018; Broeckhoven & Mouton, 2013;
Lygosoma (Scincella) sp. (Scincidae)	Shrublands, forest habitats and soil under fallen timber	Insectivorous	Not available	Forager	Not available	Irregular overlapping Scale	Schwenk, 1988; Sumonth

South-east Asia						Shaped papillae on mid-tongue with frilled edges	a & Cota, 2018.
Eremiascincus richardsonii (Scincidae) Western Australia	Desert environment	Small invertebrates but will occasionally eat small lizards or fruit	Crepuscular	Forager	Narrow	Round, non-elevated papillae on tongue tip, more elevated on hind-tongue.	THIS STUDY; Cogger, 2018; James & Losos (1991).
Egernia kingii (Scincidae) Western Australia	Woodlands and shrublands. Also rocks and crevices	Mostly herbivorous but will occasionally eat insects and bird eggs.	Diurnal	Forager	Narrow & bifurcated at the very tip	Round-non elevated papillae on tongue tip.	THIS STUDY; Cogger, (2018).
Hemiergis initialis initialis (Scincidae) Western Australia	Shrublands, forest habitats and soil under fallen timber	Insectivorous	Crepuscular	Forager	Broad with bifurcated tip	Irregular round mosaic papillae on tongue tip. Overlapping scale shaped papillae on mid- to hind-tongue.	THIS STUDY; Cogger, 2018; Wilson & Swan, (2020); Storr *et al.,* (1999).
Menetia greyii (Scincidae) Western Australia	Dry Shrublands, temperate, tropical and mallee forest habitats under fallen timber	Insectivorous	Crepuscular	Forager	Broad with bifurcated tip	Irregular scale shaped papillae on mid-tongue. Pronounced scalloped edges and overlapping	THIS STUDY; Cogger, 2018.
Scincella tsinlingensis (Scincidae) Szechwan, China	Shrublands, forest habitats and soil under fallen timber	Insectivorous	Diurnal	Forager	Broad with bifurcated tip	Irregular scale shaped papillae on mid-tongue. VERY pronounced scalloped edges and overlapping	Yang & Wang, 2016; Hu & Zhao, 1966.
Scincus scincus (Scincidae) Northwest African and Southwest Asia	Fossorial dessert environment	Insectivorous	Diurnal	Forager	Narrow with bifurcated tip	Mosaic like papillae with a couple of mucosal cells on fore-tongue.	Salem *et al.,* 2019.
Chalcides ocellatus (Scincidae)	Semi-fossorial, open forests, archaeological sites	Opportunistic, (insects, smaller	Diurnal	Forager	Broad	Diamond like papillae on fore-tongue,	Elsheikh *et al.,* 2013; Kalbouss

Greece, Southern Italy, Israel		lizards, plant matter, shell fragments and snails)				elevated and arranged in a mosaic like pattern.	i & Nouira, 2004.
Tiliqua scincoides (Scincidae) Australia	Open desert	Omnivorous (Insects, live reptiles and plant matter)	Diurnal	Forager	Broad	Elevated square shaped papillae on tongue tip, mosaic square papillae on mid-tongue and hind-tongue.	Abbate *et al.,* 2009.
Tiliqua rugosa rugosa (Scincidae) Western Australia	Shrub lands and desert dunes	Opportunist (Carrion, snails and insects. Occasionally plants.)	Diurnal	Forager	Broad with bifurcated tip	Diamond shaped elevated papillae on tongue tip and mid-tongue	THIS STUDY; Cogger, 2018; Cogger, 1975.
Cnemidophorus (Aspidoscelis) tigris (Teiidae) South West USA	Flat hills and open areas	invertebrates and smaller lizards	Diurnal	Forager	Not Available	Scale shaped, non-elevated, semi-overlapping papillae	Best & Gennaro, 1985; Burkholder and Walker; 1973 Schwenk, 1985; Schwenk, 1988.
Lacerta agilis (Lacertidae) France and Lake Baikal, Russia	Sand dunes	Insectivorous/ Will eat plant matter occasionally	Diurnal	Forager	Narrow with deep bifurcation	Dome and scale shaped papillae on fore-tongue and mid-tongue. Deep bifurcation of tongue tip	Čížek *et al.,* 2019; Crovetto & Salvidio, 2013.
Podarcis sicula (Lacertidae) Italy	Urban, shrubs, rocky shores, sandy shores (adaptive to many environments)	Omnivorous, eats insects, may forage for carrion, may resort to cannibalism	Diurnal	Forager	Narrow with bifurcated tongue tip	Dome shaped papillae on fore-tongue. Foliate papillae on the lateral areas of the tongue. Base of tongue has imbricated wicket-like papillae	Abbate *et al.,* 2010; Capula & Aloise, 2011.
Takydromus tachydromoides	Fossorial, yet adaptable to	Insectivorous	Diurnal	Ambush predator	Not available	Irregular mosaic-like	Iwasaki *et al.,*

(Lacertidae) Japan	paddy fields, riverbanks and urban areas					papillae on mid-tongue. Secretory cells help catch prey	2002; Jackson & Telford, 1975; Takeishi, 1987.
Heloderma suspectum (Helodermatidae) Northwest Mexico	Open desert	Carnivorous	Diurnal	Forager	Narrow & deeply bifurcated	Lack tastebuds, smooth, tiny microfacets present	Cooper & Arnett, 1995; Ernst, 1992; Filoramo, 2007; Garrett *et al.*, 1996.
Anguis fragilis (Anguidae) Eastern Europe	Semi fossorial, long grass	Slugs and worms, may result to cannibalism	Crepuscular	Forager	Narrow with widely bifurcated tongue tip	Dome shaped papillae located on fore-tongue under the smooth, widely bifurcated tip.	Toubeau *et al.*, 1994; Čeirāns, 2004; Çiçek *et al.*, 1981.
Chamaeleo africanus (Chamaeleonidae) Sahel, Nile Valley Africa	Semi-arboreal	Mainly insectivorous, but may eat smaller lizards and birds	Diurnal	Ambush predator	Narrow	Conical papillae on tongue. Further magnification shows conical papillae in a rosette shape	Fouda *et al.,* 2015; Dimaki *et al.*, 2005.
Chamaeleo chameleon (Chamaeleonidae) Southern Spain, Southern Italy, Portugal	Arboreal	Mainly insects but may eat smaller lizards. May resort to cannibalism.	Diurnal	Ambush predator	Narrow	Fungiform and foliate papillae with taste buds and secretory cells	Fouda *et al.*, 2015; Dimaki *et al.*, 2005; Keren-Rotem *et al.* 2006.
Chamaeleo vulgaris (Subsp. Chameleon) (Chamaeleonidae) North Africa	Arboreal	Mainly insects but may eat smaller lizards.	Diurnal	Ambush predator	Narrow	Longitudinal papillae on closer inspection show plumerose like papillae	Fouda *et al.*, 2015.
Pogona minor minima (Agamidae) Western Australia	Woodlands and shrublands	Plants, seeds and flowers, occasionally insects (grasshoppers). Smaller lizards	Diurnal	Forager	Broad	Overlapping foliate papillae on mid-tongue. Rounded lumpy papillae on tongue tip	THIS STUDY; Cogger (2018).

Pogona vitticeps (Agamidae) Australia	Woodlands and shrublands	Smaller invertebrates (mainly termites), plant matter, occasionally smaller lizards	Diurnal	Forager	Broad	Plumerose papillae and mucosal cells on the tongue tip to help with prey capture	Zghikh *et al.*, 2014; Oonincx *et al.*, 2015; Cogger, 2018.
Uromastyx acanthinura (Agamidae) North Africa	Rocky outcrops	Herbivorous, occasionally insectivorous (ants and beetles)	Diurnal	Forager	Broad with bifurcated tongue tip	Irregular non-overlapping scaled papillae on tongue tip. Tastebuds on mid-tongue, smooth irregular papillae.	Herrel *et al.,* 1998; Bartlett, 2003.
Uromastyx aegyptia (Agamidae) Egypt	Open desert dunes	Opportunist, (insects and plant matter) but also sheep droppings.	Diurnal	Forager	Broad with bifurcated tip	Diamond shaped elevated non-overlapping papillae on tongue tip. Conical papillae on mid-tongue	Al-Ahmady *et al.,* 2017; Castilla *et al.*, 2011.
Uromastyx ornata (Agamidae) Egypt, Israel and Saudi Arabia	Rocky outcrops and open desert	Herbivorous and occasionally insects	Diurnal	Forager	Narrow with bifurcated tongue tip	Overlapping plumerose papillae on tongue tip. Overlapping scaly papillae on mid-tongue	Salem *et al.,* 2019,
Stellagama stellio (Agamidae) Northeast Africa and Southwest Asia	Rocky outcrops	Invertebrates and occasionally small vertebrates	Diurnal	Forager	Broad with slightly bifurcated tongue tip	Plumerose papillae on tongue tip to allow prey capture.	Herrel *et al.,* 1998; Baker *et al.*, 2021.
Iguana iguana (Iguanidae) Central and South America	Arboreal	Herbivorous	Diurnal	Forager	Broad and fleshy	Mosaic, flattened dome shaped papillae on mid-tongue	Čížek *et al.,* 2011; Rand *et al.,* 1990.
Callisaurus draconoides (Phrynosomatidae) Southwest United States	Open desert	Insectivorous but will eat plant matter	Diurnal	Forager	Broad, narrowing to a non-bifurcated tip	Elevated mosaic like papillae with elevated tastebuds	Schwenk, 2021; Schwenk, 1985; Pianka & Parker, 1972.
Phrynosoma platyrhinos	Prairies, open forest, desert	Insectivorous (termites,	Diurnal	Sit-and-wait/Ambush	Broad, waisted, narrowing to a non-	Mosaic papillae with visible tastebuds	Schwenk, 2021; Schwenk, 1985;

(Phrynosomatidae) Western North America		ants and beetles)			bifurcated tip		Pianka & Parker, 1975.
Oplurus cuvieri (Opluridae) Madagascar	Semi-Arboreal, dry forests and savannahs	Insectivorous	Diurnal	Ambush predator	Broad with bifurcated tip	Irregular shaped, elevated non-overlapping papillae with tastebuds similar to Iguanids. Plumerose papillae also on the mid-tongue	Delheusy *et al.,* 1994, Itoh & Ito, 2015.
Anolis carolinensis (Dactyloidae) South-East United States	Arboreal	Insectivorous (beetles and flies) but will also eat plant matter	Diurnal	Ambush predator	Narrow	Elevated plumerose papillae which allow capture of prey.	Rabinowitz *et al*., 1986; Losos, 2009; Bartlett & Bartlett.

3.5 Discussion

Our hypothesis that difference in tongue structure between the species is a result of convergent evolution was supported. Diet rather than phylogeny is the greater influencing factor in tongue structure and morphology amongst the seven West Australian squamate species sampled. The same findings apply to the other 33 species from the literature, spanning 16 squamate families.

Variation in tongue macro- and micromorphology within lizard families occurs as a result of not just dietary niches, but also habitat and hunting strategies. Unrelated lizard species show convergence in tongue macro- and microstructure as a result of similar hunting strategies, habitat and dietary preferences. Movement of food towards the oesophagus (swallowing) is universal for all lizard species, which is why the hind tongue lacks variation in papillae types. Greater variation on the mid-tongue of all lizard species studied showed that prey handling features vary between lizard species' diets. The tongue tip and fore-tongue are the most important areas for prey capture and discrimination, and food uptake. This is why we see the most variation in the tongue tip and fore-tongue.

3.5.1 Comparison of tongue microstructures of Western Australian specimens in reflection to diet

Comparison of the seven WA lizard samples, especially the five skink species, indicate that dietary choices, lifestyles and habitats are the driving forces of variation in tongue microstructure, as opposed to relation and phylogeny. Comparison of tongue samples of *T. r. rugosa* and the similarly sized *E. kingii* (Table 3.1, Figure 3.2) show differences in tongue tip and mid-tongue that are due to *T. r. rugosa* being a forager which prefers open desert environments as it has preferred diet of carrion and snails. It also has a broader tongue with pigment, which it uses for signalling and display (Nagloo *et al.,* 2022) like *T. scincoides* (Cogger; 2018; Badiane *et al.,* 2018). In contrast to *T. rugosa*, *E. kingii* has a narrow tongue with a darker pigment (Figure 3.2c), and there have been no records of it using its tongue for signalling to ward off predators. Its fore-tongue round papillae are not as raised and its mid-tongue has a smooth dorsal surface with little to no papillae (Figure 3.5c). This reflects its preferred diet of plant matter, thought it will prey on insects and occasionally eat birds' eggs (Cogger, 2018).

Eremiascincus richardsonii has rounded non-elevated papillae, similar to *E. kingii* (Figure 3.6a) but unlike *E. kingii,* its papillae have become more elevated and pronounced towards its mid-tongue and its hind-tongue (Figure 3.6b). Its lack of bifurcation on its tongue tip, seen in the other four skink species, is due to the fact it senses its prey by vibrations in the sand as opposed to chemoreception (James & Losos, 1991). *Eremiascincus richardsonii* prefers sandy desert environments.

Hemiergis initialis initialis and *Menetia greyii* are of a similar body size, have bifurcated tongue tips (Figure 3.2e, f), and are both insectivorous (Cogger, 2018; Storr *et al.,* 1999; Wilson & Swan, 2020), but *M. greyii's* scale shaped papillae have pronounced scalloped edges, which *H. i. initialis* lacks. *Hemiergis initialis initialis* also has mosaic papillae on its fore-tongue (Figure 3.7a, b) and overlapping scale-shaped papillae on its hind-tongue (Figure 3.7c, d). Differences in papillae variation on the dorsal tongue surfaces and shape of overlapping scale shaped papillae between *H. i. initialis* and *M. greyii* indicate different methods of prey handling between the two species.

Comparison of the single carphodactylid gecko specimen, *Underwoodisaurus milii* with the single agamid specimen *Pogona minor minima*, shows variation between the tongue tip and mid-tongue. Both species have diets of insects and occasional lizard prey. However, U. milii has elevated conical papillae as well as overlapping scale shaped papillae on its tongue tip. By contrast, *Pogona minor minima*, exhibits rounded, non-elevated papillae on its tongue tip and fore-tongue (Figure 3.9a, b & c), similar to the rounded papillae of *E. kingii*'s fore-tongue and mid-tongue, which reflects *P. m. minima*'s preferred plant and insect diet. In contrast to *E. kingii*'s mid-tongue microstructure however, the microstructure of *P. m. minima*'s mid-tongue has spiky leaf-like papillae (Figure 3.9d), which reflects its occasional diet of vertebrate prey, which are smaller lizards.

3.5.2 Comparison with other squamate species from previous literature of tongue microstructure in relation to diet and hunting strategies

All members of the Gekkota superfamily have variation in their tongue morphology. Variation in tongue macrostructures as well as papillae shape in geckos (Table 3.2) are a sign of divergent evolution, which is caused by differences in their diet and hunting strategies.

Underwoodisaurus milii has a narrow tongue, conical papillae and overlapping scale-shaped papillae on its dorsal tongue tip, which presumably aids in the stronger grip and movement of its prey, insects and smaller lizards, towards the back of its throat (Figure 3.3). In contrast to *U. milii*, *Eublepharis macularius* has a broad tongue width, non-overlapping, elevated dome shaped papillae on its dorsal tongue tip as well as foliate papillae on the lateral sides of its tongue, which reflects the presence in its diet of small mammals such as nesting mice (Henkel & Schmidt, 1995; Jamniczky *et al*., 2009). Both species have bifurcated tongue tips, reflecting their foraging behaviour (Cogger; 2018, Jamniczky *et al*., 2009; Rawat *et al*., 2019) and use their tongues for grooming (Deperno & Cooper, 1996; Griffiths, 1987) but behaviour wise, unlike *U. milii*, *E. macularis* will resort to being an ambush predator (Rawat *et al.,* 2019). *Coleonyx variegatus* also belongs in the Eublepharidae family, but in contrast to *E. macularius,* it prefers small invertebrate prey as opposed to vertebrate prey (Cooper, 1998; Cooper et al., 1985; Parker, 1972). Both *Coleonyx variegatus* and *Eublepharis maulcarus* have broad tongues, but *C. variegatus* exhibits conical papillae on its tongue tip. It also lacks foliate papillae on the sides of its tongue. (Filoramo, 2007).

Comparisons of *U. milii* and gecko *Hemidactylus flaviviridis* (Gekkonidae; Fig. 3.1) show that both geckos exhibit narrow tongues with conical papillae present which aid with the intake of insects and smaller lizard prey (Al-Fartwsy *et al*., 2015, Cogger, 2018). However, unlike *U. milii* which has mosaic papillae on its mid tongue, *H. flaviviridis* has fungiform papillae on its mid-tongue and foliate papillae on its tongue base, which aid it with swallowing its vertebrate prey of smaller reptiles (Al-Fartwsy *et al.,* 2015; Parves & Alam, 2015). This variation in tongue microstructure between the species alludes to divergence in dietary preferences and associated differences in food handling requirements between the two species of geckos- *U. milii* prefers insects but will occasionally eat smaller lizards, while *H. flaviviridis* prefers smaller reptiles and occasionally eats insects (Cogger, 2018; Parves & Alam, 2015).

Underwoodisaurus milii and the Phyllodactylid gecko *Tarentola annularis* have narrow tongues with bifurcated tips. Both gecko species have a preferred habitat of rocky outcrops and similar dietary preferences of invertebrates and smaller lizards. However, comparisons of their tongue microstructure reveals that *T. annularis* has dome shaped papillae one its tongue tip as well as foliate papillae on the side tongue (Bayoumi *et al.,* 2011). This reflects different lifestyles as *U. milii* has a preference for hunting for invertebrates over smaller lizards, showing a lack of foliate papillae. By contrast, *T. annularis* prefers smaller vertebrate prey as indicated by the presence of dome shaped and foliate papillae, which allow the movement of active vertebrate prey towards its oesophagus.

Both *Gekko japonicas* and *H. flaviviridis* are nocturnal foragers (Al-Fartwsy *et al*., 2015; Lee *et al.,* 2004; Ota & Tanaka, 1996; Zhao & Adler, 1993) but have other dietary differences which affect the microstructure of their tongues. *Gekko japonicus* has dome-shaped papillae on its tongue tip like but also has fan shaped papillae as well as tiny microvilli covering its tongue tip. These tiny structures aid with the intake of insects and tree sap, which make up its diet (Iwasaki *et al*., 1990, Murai *et al*., 2013).

From all these comparisons of the tongue microstructure of the superfamily Gekkota, we conclude that lizard species that prefer larger, more active vertebrate prey are noted to have more elevated papillae on their tongue tip, fore-tongue and mid-tongue (Bayoumi *et al.,* 2011, Jamniczky *et al*., 2009; this study). Such elevated papillae enable the movement of larger prey towards the oesophagus; in the case of geckos *Underwoodisaurus milii* and *Eublepharis macularis*, grooming (Deperno & Cooper, 1996; Griffiths, 1987). Foliate papillae, located on the lateral sides of the tongue, are also important for preventing large or more active vertebrate prey from escaping the oral cavity as well as the movement of prey towards the oesophagus.

Tiliqua rugosa rugosa and *Chalcides ocellatus* are open forest dwelling, opportunistic foragers. Both species prey on plant matter, snails and smaller lizards, reflected by the presence of diamond shaped papillae on their fore-tongue and mid-tongue, which aid with the grip of vertebrate and mollusc prey (Cogger, 2018; Elsheikh *et al.*, 2013). *Tiliqua rugosa rugosa*'s fore-tongue and mid-tongue is similar in structure to the agamid *Uromastyx aegyptia* as both lizard species have minimally forked tongue tips and their dorsal tongue tips covered in elevated diamond-shaped papillae (Al-Ahmady *et al.,* 2017; Bayoumi *et al.,* 2011). Both lizard species are large diurnal foragers that prefer open environments, mainly desert dunes, and are opportunistic feeders (Castilla *et al.,* 2011; Cooper Jr, 2000), but *T. r.rugosa* prefers open scrublands and desert dunes, preys on snails and insects, and scavenges on carrion as its main food sources but will also eat plant matter (Cogger, 2018). Similarly, *U. aegyptia* will eat plant matter and insects, also scavenging on sheep droppings (Castilla *et al*., 2011). The papillae on *T. r. rugosa*'s and *U aegyptia's* fore-tongue and mid-tongues are also elevated and arranged in a mosaic pattern, as we see in *U. milii*. As with *U. milii*, it is likely that these papillae aid in taking vertebrate prey, as *U. milii* and *T. r. rugosa* occasionally eat smaller lizard species (Cogger, 2018).

Eremiascincus richardsonii shares its fossorial behaviour with that of the larger *Scincus scincus*, and both skink species use vibrations in the sand to detect their prey (Hetherington, 1989; James & Losos, 1991). Their tongue macro- and micromorphologies however, are different as *S. scincus* has a narrow tongue with mosaic papillae on its bifurcated tongue tip (Salem *et al.,* 2019). *Scincus scinus*' fore-tongue also displays a few mucosal cells (Salem *et al.,* 2019). While both *S. scincus* and *H. i. initialis* may be insectivorous foragers, their differences in tongue microstructure indicate variation in feeding habits at a microscopic level.

The insectivorous skinks *Hemiergis initialis initialis*, *Menetia greyii*, and *Scincella tsinglingensis* all forage for prey in enclosed environments under fallen timber (Cogger, 2018; Hu & Zhao, 1966; Wilson & Swan, 2020). All three skinks have scale shaped overlapping papillae on their mid-tongues (Yang & Wang; 2016; this study), which allow the ease of movement of insect prey towards the back of the throat. However, *H. i. initialis'* overlapping scale shaped papillae on its mid tongue have smooth edges. In addition, *H. i. initialis* also has irregular round mosaic papillae on its tongue tip and fore-tongue. By contrast, *Menetia greyii*'s scale shaped papillae are present all over its tongue tip, fore-tongue and mid-tongue. *Menetia greyii*'s scale shaped papillae also has scalloped edges similar to that of diurnal *Scincella tsinglingensis* (Yang & Wang, 2016) indicating possible similarities in food handling and hunting strategies between the two skink species. In addition, there is also a possibility that niche partition is occurring in order to reduce competition between species with similar diet and foraging habits. Further research would need to address why similarly sized lizards within the same family with similar dietary niches such as that of *Menetia greyii* and *Hemiergis initialis initialis* have subtle differences in tongue microstructure.

All agamid species analysed (Table 3.2) showed a great variation in tongue microscale morphology which again appears to reflect a wide range in diet. For example, *Pogona minor minima* and its larger relative *Pogona vitticeps* are diurnal foraging species (Cogger, 2018), but have different tongue macro- and microstructure. *Pogona vitticeps* has a tongue that is broad from tip to hind-tongue. Its tongue tip is covered in plumerose papillae and mucosal cells which help with the intake of invertebrate prey like termites. It only occasionally eats plant matter and smaller lizards (Cogger, 2018; Zghikh *et al*., 2014). By contrast, the tongue of *Pogona minor minima* is broad at the hind-tongue but gradually gets narrow at the tip. Its tongue microstructure displays round non-elevated papillae on its tongue tip like the skinks *E. kingii* and *E. richardsonii,* but also foliate papillae on its mid-tongue to hind-tongue (this study). These structures reflect its primary diet of seeds and vegetation. It occasionally eats insects like grasshoppers and also vertebrate prey in the form of smaller lizards (Cogger, 2018) (Table 3.2). Its lack of plumerose papillae on its tongue tip shows that it has a different strategy of prey capture to *Pogona vitticeps* and *Stellagama stellio*, while the foliate papillae on its mid-tongue to hind-tongue prevent more active vertebrate prey from escaping.

Pogona vitticeps and *S. stellio* have multiple plumerose papillae which allows it to capture insect and small vertebrate prey and will occasionally eat vegetation and fruit (Zghikh *et al*., 2014; Herrel *et al*., 1998). *Pogona minor minima* prefers vegetation and smaller insect prey but will occasionally eat smaller lizard prey (Cogger, 2018). This is reflected in rounded, non-elevated papillae on its fore-tongue and foliate papillae on its mid-tongue, the latter which aids in swallowing smaller vertebrate prey by keeping it in its mouth. By contrast to the two *Pogona* species, *U. aegyptia* is an opportunistic predator that prefers to forage for both plant and animal matter, which include sheep droppings, and therefore has elevated diamond shaped papillae similar to that of the skink *T. r. rugosa* (Al-Ahmady *et al.,* 2017).

3.5.3 Comparison of tongue microstructure among other lizard families from previous literature in relation to dietary niches

Comparison of the remaining 15 species from the families Cordylidae, Teiidae, Lacertidae, Helodermatidae, Chamaeleonidae, Anguidae, Iguanidae, Phrynosomatidae, Opluridae and

Dactyloidae for which there is data on tongue structure showed further evidence that diet has a much stronger impact on tongue microstructure compared to phylogeny. This is seen through comparing the diet and lifestyles of lizard species from different families. The comparison between *U. acanthinura* (an agamid) with *Iguana iguana* (an iguanid- Figure 3.1) shows that both species have large taste buds present on their mid-tongue. Both *U. acanthinura* and *I. iguana* dwell in rocky outcrops and have a primary diet of plant matter (Bartlett, 2003; Alberts, 2004), though *I. iguana* prefers to be arboreal (Alberts, 2004) and only eats plant matter as reflected by flattened mosaic dome-shaped papillae on its mid-tongue (Čížek *et al.,* 2011) as opposed to *U. acanthinura*, which has a preference rocky outcrops (Bartlett, 2003) and has irregular shaped papillae on its mid-tongue, which helps with its occasional intake of ants and beetles (Herrel *et al*., 1998). Comparison of the agamid *U. acanthinura* with the phrynosomatid lizard *Callisaurus draconoides* (zebra-tailed lizard), provides additional evidence that unrelated lizard species with plant matter in their diets have prominently displayed tastebuds on their tongues' dorsal surfaces (Herrel *et al*., 1998; Schwenk, 1985; Pianka & Parker, 1972).

Comparing the two Africa cordylid (Figure 3.1) species *Ouroborus cataphractus* and *Karusasaurus polyzonus* with that of the agamid *U. acanthinura* reveals that all three lizard species are diurnal forgers that prefer sand dunes and rocky outcrops. However, while *K. polyzonus* and *O. cataphractus* have a primarily insectivorous diet, with both species occasionally eating plants and *K. polyzonus* occasionally resorting to cannibalism (Mouton *et al*., 2013; van Blerk *et al*., 2021), *U. acanthinura* prefers vegetation and occasionally eats insects by contrast (Bartlett, 2003). This is reflected in their tongue structure in which *U. acanthinura* has large taste buds and non-overlapping scale shaped papillae covering its mid-tongue dorsal surface while the two cordylid species have irregular-shaped papillae or scattered plumerose on the lateral sides of the mid-tongue surface, which prevent active prey from escaping (Broeckhoven & Mouton, 2013; Herrel *et al*., 1998).

All lizard species with a diet of both insects and vegetation have varied forms of scattered plumerose papillae or rounded non-elevated papillae present on various areas of their tongues (Table 3.2). Scattered plumerose papillae and rounded, non-elevated papillae allow easier capture of insect prey and intake of vegetation, which make up a primary part of the diet of all listed lizard species.

Overlapping scale shaped papillae are present on the unidentified *Scincella* species (Schwenk, 1988), the teiid species *Cnemidophorus (Aspidoscelis) tigris* (Western whiptail) (Schwenk, 1988) and the cordylid *Hemicordylus capensis* (Broeckhoven & Mouton, 2013), in addition to the skinks *S. tsinlingensis* (Yang & Wang, 2016) and *M. greyii*, and the gecko *U. milii*. All of these lizard species have a foraging behaviour and a primarily insectivorous diet (Cogger, 2018; Best & Gennaro, 1985; Burkholder and Walker; 1973), but *U. milii* will occasionally eat smaller lizard species (Cogger, 2018), which result in a slightly more raised mosaic like papillae on its mid-tongue. Secretory cells are also present in primarily insectivorous lizards as we see by comparing the lacertid, *Takydromus tachydromoides* (Japanese grass lizard, Family Lacertidae- Figure 3.1) with *Chamaeleon chameleon* (common chameleon, Family Chameleonidae- Figure 3.1). Both unrelated lizard species have secretory cells present in their tongue microstructure (Iwasaki et al., 2002; Fouda *et al*., 2015) and are ambush predators as opposed to foragers. These microstructures allow strong capture of insect prey, which these two lizard species have as a primary source of their diet (Jackson & Telford, 1975; Dimaki *et al*., 2005).

Examining the tongue microstructure of *Podarcis sicula* (Lacertidae- Figure 3.1) and *Hemidactylus flaviviridis* (Gekkonidae- Figure 3.1) shows the presence of elevated dome or conical shaped papillae, which allow the occasionally large prey to be moved towards the back of the throat (Abbate *et al*., 2010; Al-Fartwsy *et al*., 2015). The Italian wall lizard *P. sicula*, has dome-shaped papillae on its tongue tip similar to *Eublepharis macularius,* a member of the family Eublepharidae. Both species prefer to forage for smaller vertebrate prey in sandy environments, with *P. sicula* preferring sandy shores as opposed to desert dunes (Abbate *et al*., 2010; Jamniczky *et al*., 2009). Dome shaped papillae can also be found in the tongue structures of two unrelated gecko species from different families, *Tarentola annularis (*Phyllodactylidae- Figure 3.1) and *E. macularius* (Eublepharidae- Figure 3.1) (Bayoumi *et al*., 2011, Jamniczky *et al.,* 2009). Both lizard species are ambush predators that have dome shaped papillae on their tongue tips and foliate papillae on their tongue sides and will eat smaller vertebrate prey, which include smaller lizards (Bayoumi *et al.,* 2011; Jamniczky *et al.,* 2009) and in the case of *E. macularius*, young mice (Henkel & Schmidt, 1995).

Finally, the sole member of Helodermatidae, *Heloderma suspectum* (gila monster) has a deeply bifurcated tongue with some microfacets on the dorsal surface not unlike that of the varanids and snakes (Filoramo, 2007; Garett *et al.*, 1996; Schwenk, 1995). This is a reflection of its carnivorous diet, and that it uses its tongue to seek out active vertebrate prey (Ernst, 1992; Cooper & Arnett, 1995).Both *Heloderma suspectum* and the Lacertid *Lacertis agilis* have deeply bifurcated tongues as they wander over long distances in search of food. However unlike *H. susepectum*, *L. agilis* does not forage for vertebrate prey, as it prefers insect prey and plant matter (Crovette & Salvidio, 2013). There is a possibility that *L. agilis* uses its bifurcated tongue for mate seeking as bifurcation enhances mate detection (Schwenk, 1995), a knowledge gap that needs to be addressed.

3.6 Conclusion

This study's comparison of tongue macro- and micromorphologies between members of 16 lizard families reveals the evidence that diet and behaviour cause greater convergence in squamate tongue morphology than does relatedness between species and phylogeny. Squamate species with similar dietary preferences but from diverse families have more similar tongue macro- and micromorphologies than those of other species from the same family. Behavioural differences in food handling between squamate species also results in tongue macro- and micromorphology variation, as similar species with the same dietary preferences display variation in tongue microstructure as a result of differences in hunting and feeding habits.

For future studies, we need to look at a wider range of squamates and SEM images of tongue structures in order to get a broader picture of the effect of natural selection at a macro- and microscale level. We would need to address if deep bifurcation of the tongue tip is crucial to mate seeking in species which are not varanids, snakes or Helodermatids. Connections between foraging strategies and mate finding behaviour would need to be addressed in further studies as there could be possible tradeoffs between investing in structural features for prey handling and chemical receptors. More research on other behaviours such as grooming and signalling will also need to be covered as they also affect differences in squamate tongue macro-and microstructure. For example, we would need to see the significance of pigmented tongues for both signalling and non-signalling lizard species and if more elevated papillae are present on their tongue tips and fore-tongues of lizard species that use their tongues for grooming. These discoveries will help us fill more knowledge gaps complementing animal biology and behavioural ecology in reptiles.

3.7 Acknowledgements

I would like to give a huge thanks to my supervisors. Special thanks to Dr Bill Bateman for founding the project and for letting me have access to roadkill and donated non-data specimens in building 311. Huge thanks to Professor Kate Trinajstic and Lauren Gilson for being wonderful guides for me for manuscript editing and graphic formatting. Special thanks to Dr Catherine Boisvert in helping organize the project and Michael Just for educating me in the use of scanning electron microscopy at building 304 at Curtin University.

Author Contributions:

PWB, KT, CB and CK designed the experiment. Catherine Boisvert assisted in giving instructions and helping out with scanning electron microscopy. CK undertook CT scanning with the help of PWB. CK wrote the manuscripts with KT, PWB and LNG helping with the subsequent drafts. PWB and CK contributed with data interpretation. PWB and LNG created Figure 3.1. CK, LNG, and KT created Figure 3.2. CK created Figures 3.3-3.9. LNG, PWB and CK collated Table 3.2. KT assisted in giving instructions and helping out with Photoshop.

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Chapter 4. Sexual selection in snakes

This chapter has been prepared for submission to the *Journal of Anatomy*. As a result, relevant parts of the methodology have been repeated.

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4.1 Abstract

Many snake species demonstrate little obvious sexual differences apart from overall body size, but may differ in more subtle ways. To investigate for the presence of male-biased sexual dimorphism of the tongue macro- and microstructure of two venomous elapid snake species — the mainland dugite (*Pseudonaja affinis affinis*) and the Western tiger snake (*Notechis scutatus occidentalis*) – morphological measurements and Scanning Electron Microscopy (SEM) were utilized. Comparisons of SEMs images of the tongue tines of male and female dugites and male and female tiger snakes were made. Both morphological measurements and SEM images showed the presence of male-biased sexual dimorphism in macro- and micromorphological tongue structures in *N. s. occidentalis,* but at neither level in *P. a. affinis*. Behavioural differences between the two snake species can explain the absence or presence of sexual differences of tongue micromorphology between the sexes. The foraging niche of *P. a. affinis* suggests both sexes are equally active and may find each other visually. By contrast, the behaviour of *N. s. occidentalis* is similar to other ambush predator snake species, in which males seek out females and partake in male-male combat, resulting in not only male-biased sexual size dimorphism, but also differences in tongue tine length and chemoreceptivity between the sexes. These behavioural differences between the species may explain why male *N. s. occidentalis* require additional sensory structures to seek out females, whereas male *P. a. affinis* do not.

4.2 Introduction

Differences between the sexes other than in genitalia are a result of sexual selection, a form of natural selection as described by Darwin (Darwin, 1872). Studies of sexual selection have tended to concentration on morphological sexual dimorphisms such as crests, horns, antlers, weapons, size or colour (Bonduriansky, 2007). However, many traits show that no obvious sexual dimorphism might nevertheless still be under sexual selection; for example, for modes of communication via chemical cues, males may have developed higher sensitivity than females if they have to search for females. This may be particularly prevalent in squamates that heavily rely on chemical cues both for foraging (Cooper & Alberts, 1990; Cooper, 1997; Roth *et al.,* 1999) and mate searching (Bryant *et al.,* 2011; Kubie *et al.,* 1978; Halpern & Kubie, 1980).

Patterns of sexual size dimorphism (SSD) in snakes appear to be broadly linked to reproductive strategies, of which snakes show a wide variety, from polygyny to polyandry (Gregory, 2004; Shine & Fitzgerald, 1995; Reed, 2003; Rivas & Burghardt, 2001). In species where males are larger than females, there is an increased role of male-male combat; where females are larger than males there is both a role for increased fecundity advantages for the female and reduced direct combat in the males, with greater reliance on sperm competition strategies (Shine, 1994). Before any snake can breed, however, it needs to locate a mate, and this is where chemical cues become important.

Snakes detect chemical cues through the tongue and the Jacobson's or vomeronasal organ (VNO) (Schwenk, 1995). The tongue of snakes (and some lizards) is deeply bifurcated, forming two tines, each of which has receptors involved in detecting scent cues and transferring them to the VNO (Schwenk, 1995). The dorsal surface of snake tongues has raised bump-like structures known as microfacets. These structures are embedded on the epithelial surface of each tongue tine, which increases surface area. Microfacets allow snakes to pick up an increased number of chemical signals (chemoreception) left by the opposite sex or prey (Mao *et al.,* 1991). During chemoreception, microfacets are responsible for transferring chemical signals to a snake's Jacobson's organ, which allow the snake to identify prey, a threat, or a potential mate (Morgans & Heidt, 1978).

Schwenk's (1994) review on snake tongues suggests that increased tongue tine length or forking, between and within the species, may reflect increased cue-detection ability through tropotaxis, or being able to compare a wider gradient on each side of the body (Frankel & Gunn, 1961). If increased tine length improves the tropotactic capacity of one sex (usually males) to follow pheromone trails left by potential mates, then we would predict that it would be under sexual selection and show signs of sexual dimorphism.

Supporting this hypothesis, Smith *et al.* (2007) found that male and female copperheads, *Agkistrodon contortrix*, a North American pit viper which also exhibits male-biased SSD (Vincent *et al.,* 2004), showed significant sexual dimorphism in tongue tine lengths between the sexes. The increased tine length of male *A. contortrix* was interpreted as being a result of sexual selection on the tropotactic ability of males to find females through chemical cues. If this hypothesis is generally valid, then even snakes without SSD would be expected to show sexual selection on tongue tine length in taxa in which males search for females.

To test this predicted relationship between SSD and tongue morphology, we looked at two Western Australian elapid snakes— *Pseudonaja affinis affinis* (mainland dugite) and *Notechis scutatus occidentalis* (Western tiger snake). The dugite is a large, primarily diurnal, active-hunting species of elapid in which gross morphological SSD has not been recognised (Shine, 1989; Wolfe *et al*., 2018). By contrast the Western tiger snake (*N. s. occidentalis*) is medium in length and, while diurnal, is also recorded to also have crepuscular and nocturnal hunting behaviour (Shine, 1979) and has some evidence of male-biased SSD (Shine, 1977).

Here, we investigate the size and structure of the tongue tines in male and female *P. a. affinis* and *N. s. occidentalis* to determine if subtle sexual dimorphism occurs in either species. In addition, we also investigate the density of the microfacets on the tongue which may be related to mate seeking behaviours (Mao *et al.,* 1991). The aim of this research is to discover if the tongues of dugites and tiger snakes are under sexual selection. If this hypothesis is supported, we predict that male snakes have (a) longer tongue tine lengths than females to increase tropotactic efficiency, and (b) that the tines of male tongues will have a higher density of microfacets than those of females.

4.3 Materials and methods

4.3.1. Morphological measurements of two elapid species

A total of 15 male and 25 female adult road-killed and five male ethanol-preserved non-data museum dugite specimens were measured, along with 15 male and 15 adult female tiger snakes sourced from Lettoof *et al.* (2020). The preserved specimens were provided by the West Australian Museum. All specimens were measured using a flexible measuring tape and ruler to access overall body size dimorphism. Standard measurements of the total length and body width (Figure 4.1a), snout vent length (SVL- tip of nose to cloaca), and tail length (cloaca to tail tip) (Figure 4.1b) were taken using a flexible measuring tape and ruler and recorded to the nearest mm.

To examine the scaling of macrostructures, measurements of the tongues, head length (tip of snout to front of the neck), head width at eyes, head width at nostrils and neck width were taken using digital calipers (Figure 4.1c). Head region of elapid snakes exhibit extendable jaws that consist of the movable maxillary and lower mandible (Figure 4.2a & 4.2b). For road-killed specimens, the movable maxillary and the lower mandible were held open in order for the tongue to be removed from the oral cavity. Total tongue length and total tongue tine length (Figure 4.1d) were measured. All measurements were recorded to the nearest mm.

4.3.2. Preparation of snake tongues for SEM

Immediately after dissection, tongue specimens were fixed in 5mls of 2.5% glutaraldehyde for 24 hours at 4˚C. Tongues were then rinsed in 5mls of 0.1M phosphate buffer before being given a dehydration process in 75%, 86% and then 96% ethanol for 18 minutes at each concentration, then left to air dry in labelled petri dishes for 24 hours. Tongue were mounted ventral side down onto metal mounting stubs, with one tine from each tongue dissected and turned onto its dorsal surface to assess whether microfacets are present on the ventral surface of the tongue (Morgan and Heidt, 1978; Bayoumi *et al.,* 2011). Mounted samples were then sputter coated with gold ions (DII-29030SCTR Smart Coater) before being viewed under an SEM. A total of four male and four female dugite samples and four male and four female tiger snake samples were viewed under the SEM camera (JEOL JCM-6000Plus). SEM images were viewed at 10 µm for *P. a. affinis* and 20 µm for *N. s. occidentalis* and scanned at 15 kV to produce images suitable for quantifying microfacet density variation.

From taken images, microfacet counts were done on 5µm^2 segments randomly distributed across the tine tips and mid-tines of the dorsal surface. Mid-tine regions were determined from anterior to posterior of each tine by the decreasing density of microfacets. A total of four 5µm^2 segments were counted for each *P. a. affinis* image and a total of seven 5µm^2 segments were counted for each *N. s. occidentalis* image. Number of microfacets were statistical analysed were performed using statisiXL (Figure 4.3).

4.3.3 Statistical analysis of tongue macro- and microstructural differences.

For looking for differences in morphological measurements in male and female snakes, two-tailed t-tests were conducted. Recorded body measurements for both snake species were subjected to two tailed t-tests comparing the snout-vent length (SVL), head length, and tongue tine lengths. Head length and SVL were chosen to be independent variables to be measured against tongue tine length. To detect differences in tongue tine length between the sexes while still taking into account potential allometric effects, the residuals of head length were calculated from the regression of head length against tine length, with the results presented as a box plot for each sex for both elapid species. Head length was chosen as an independent variable for calculating results because head size has a positive linear effect on tongue tine length (Smith *et al.*, 2008), but could also be sexually dimorphic. Additional two tailed t-tests were also performed to find significant differences between the number of microfacets in male and female dugites and male and female tiger snakes.

To find any form of difference in relationship between tongue tine length and head length between the sexes while still taking into account potential SSD, StatistiXL (v2) was used to calculate the residuals of head length for the regression of head length against tine length, in for both sexes of elapid species, and carried out by a t-test by sex.

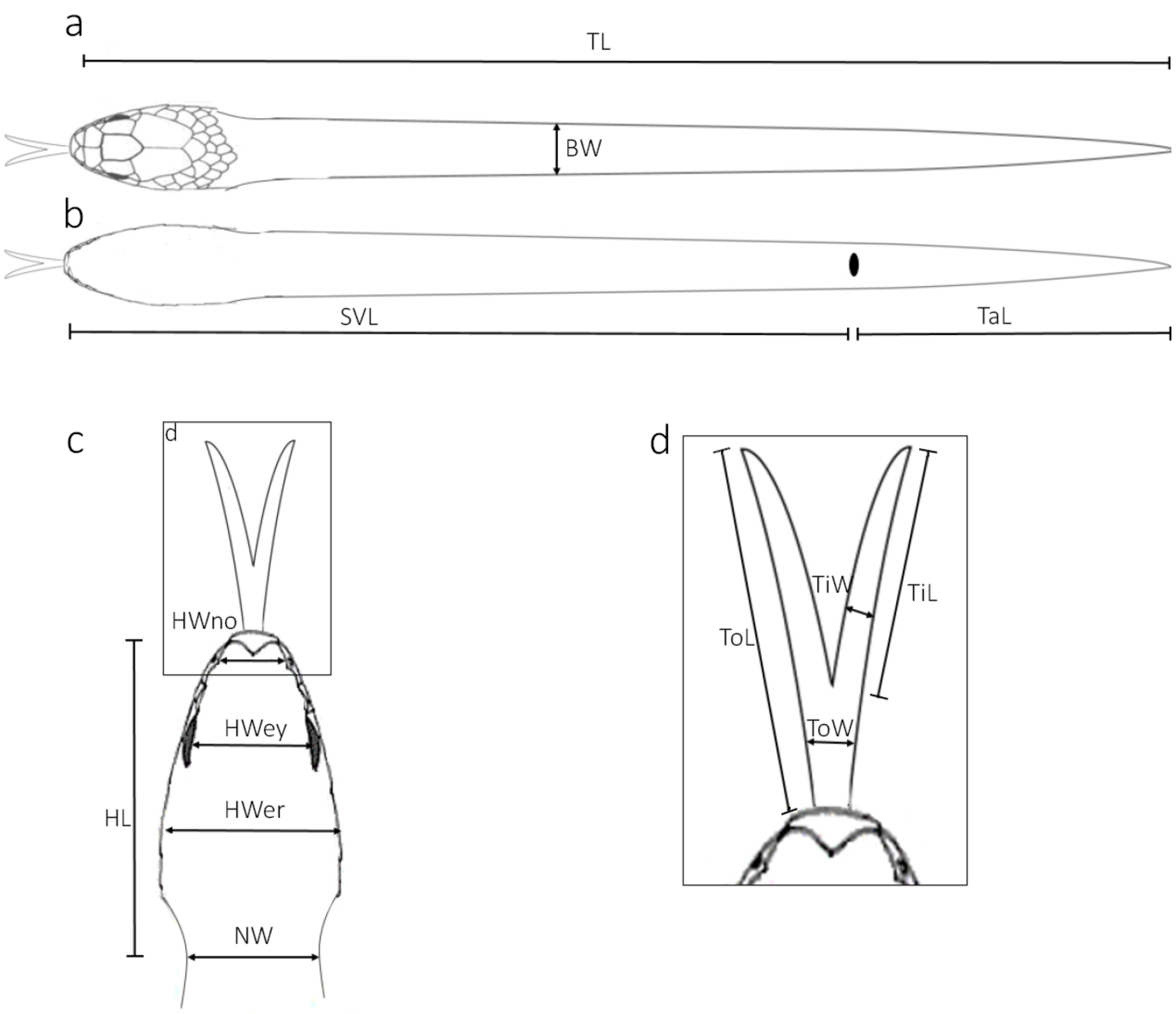

Figure 4.1: Body measurements taken for *Pseudonaja affinis affinis* and *Notechis scutatus occidentalis* samples. a) Dorsal view of the snake indicating position of measurement, exhibiting the total body length (TL) and body width (BW). b) Ventral view of the snake indicating measurements of snout vent length (SVL) and tail length (TaL). c) Dorsal view showing measurements of head width at nostrils (HWno), head width at eyes (HWey), head width at ears (HWer), neck width (NW) and head length (HL). d) Insert indication measurements of the tongue; total tongue length (ToL), total tongue width (ToW), tongue tine length (TiL) and tongue tine width (TiW). Head graphic taken from Department of Natural Resources (2022).

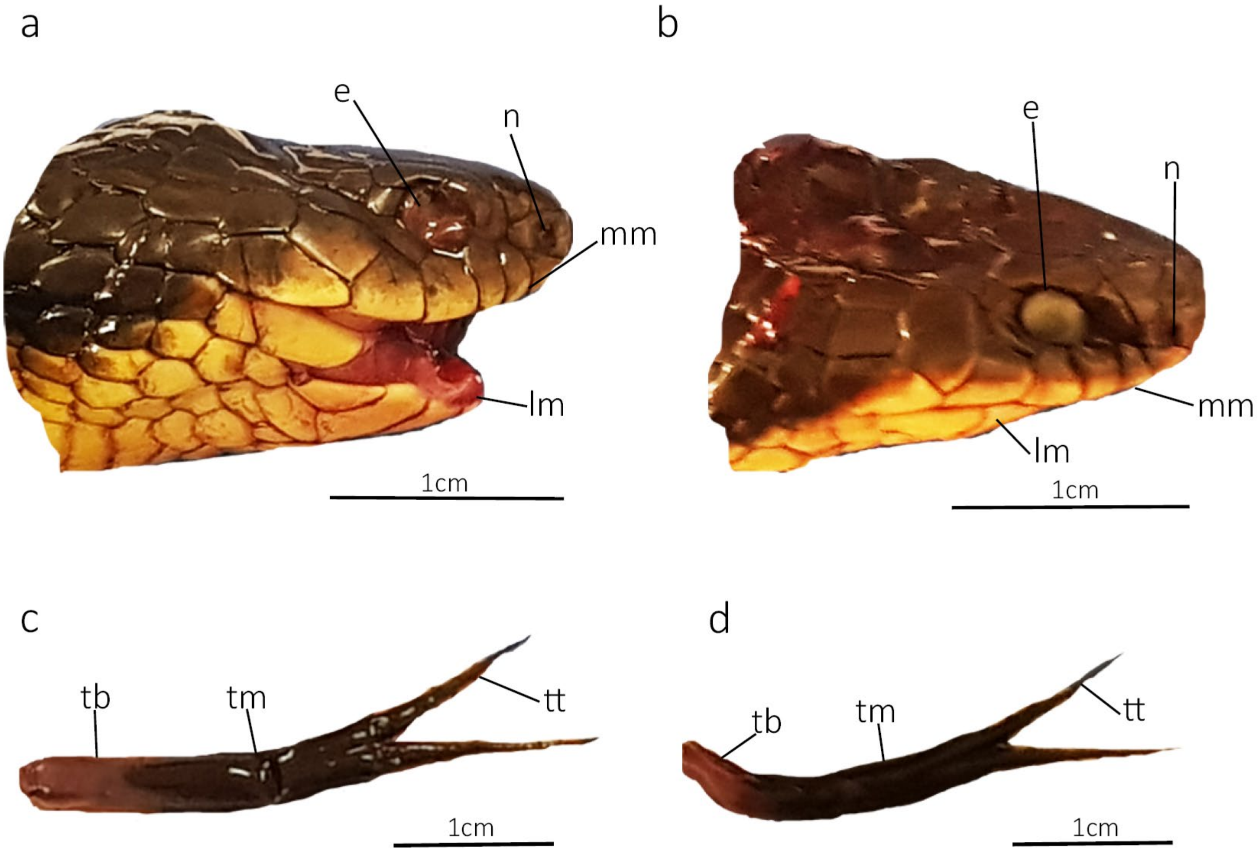

Figure 4.2: Comparison of the head and tongue anatomy between male ((a) and (c)) and female ((b) and (d)) tiger snakes (*Notechis scutatus occidentalis*). (a) Head of a male; and (b) female *N. s. occidentalis*. Both sexes exhibit forward set eyes (e), extendable jaws with a lower mandible (lm) and movable maxillary (mm), a smooth and anteriorly pointed snout and forward orientated nostril (n). (c) Tongue from a male; and (d) a female *N. s. occidentalis,* exhibiting the tongue base (tb), tongue mid (tm) and tongue tip (tt). Tongue tips of both sexes are deeply bifurcated. All scale bars = 1cm.

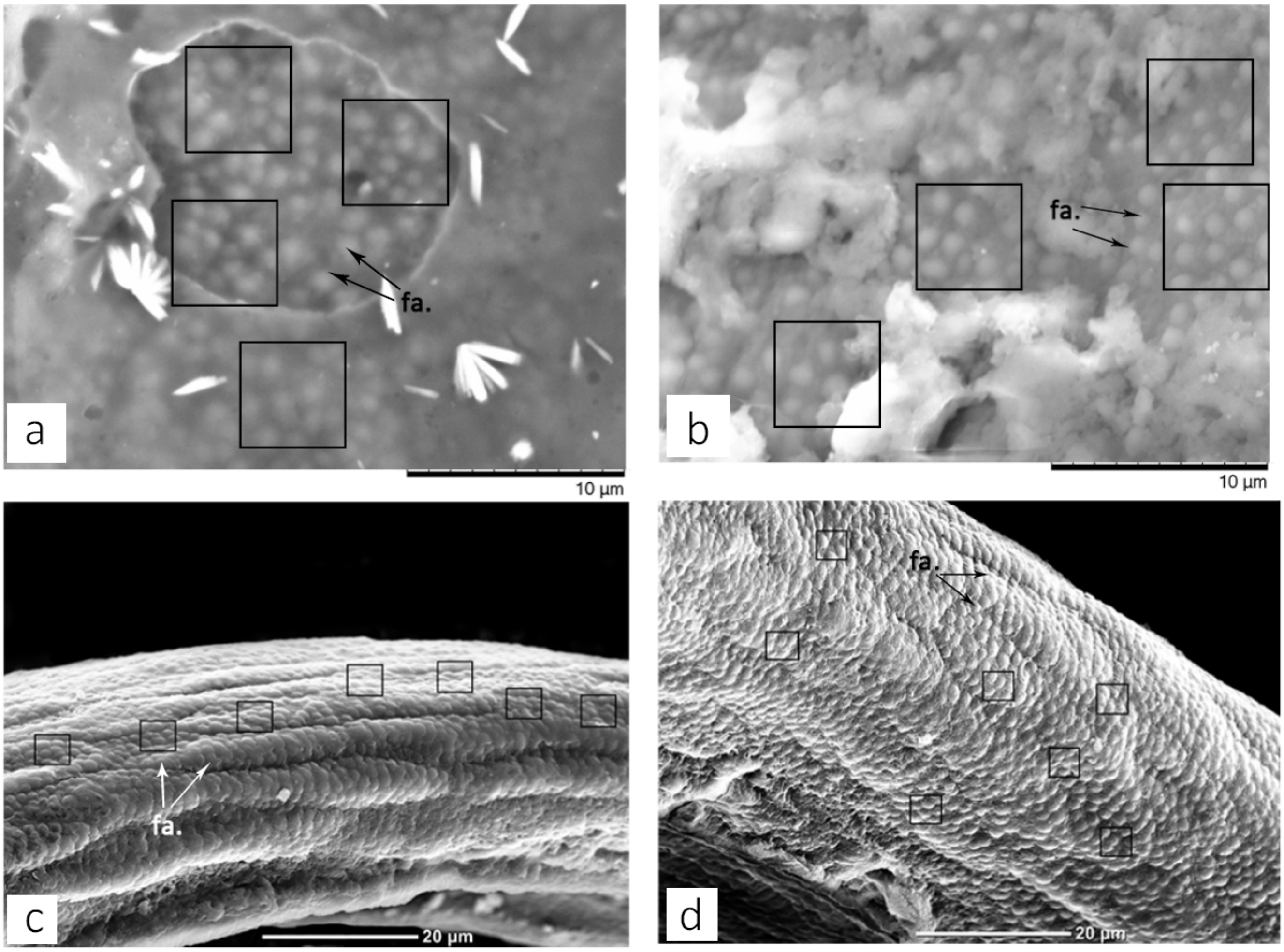

Figure 4.3: Scanning electron microscope images of dorsal surface of male (a) and female (b) ***Pseudonaja affinis affinis*** and male (c) and female (d) ***Notechis scutatus occidentalis*** showing tiny microfacets **(fa.)**. Squares indicate indicate 5μm^2 areas used for counting of microfacets. Scale bars = 10μm (a & b) and 20μm (c & d).

4.4 Results

4.4.1 Linear regression for body measurements of male and female snakes

There was no significant relationship in the linear regressions between SVL and tine length of both males and female dugites (R^2=0.761, P=0.116 and R^2=0.677, P=0.981, respectively; Figure 4.4a). Only females showed a significant relationship between head length and tine length (R^2=0.394, P=0.265 and R^2=0.392, P=0.002 respectively; Figure 4.4b).

For tiger snakes, a significantly positive relationship was found between SVL and head length of males and females (R^2= 0.373, P= 0.000 for males, R^2 = 0.101, P= 0.059 for females; Figure 4.5a) and between tine length and head length in males and females (R^2=0.666, P=0.000, R^2=0.496, P= 0.000 respectively; Figure 4.5b). The overall linear regression of the relationship between tongue tine length and head length for males and females combined for both species exhibited a positive relationship for both species, with tiger snakes exhibiting a stronger R value (dugites R^2= 0.387, P= 0.001; tiger snakes R^2=0.674, P= 0.000) (Figure 4.6).

4.4.2 Comparative assessment of macrostructural differences between males and female snakes

For dugites, there was a significant difference in head length (t= -2.608, df= 43, P =0.012), but not in SVL (t= -0.366, df= 43, P= 0.716) or in tine length (t= -1.120, df= 43, P= 0.269) between males and females (Table 4.1). For tiger snakes, all three body measurements differed significantly between the sexes (SVL t= 24.096, df= 28, P= 0.000; head length t= -2.938, df= 28, P= 0.007; tongue tine length t= -2.368, df= 28, P= 0.025) (Table 4.2). No significant difference was found in the residuals of head length against tongue tine length for male and female dugites (t= 0.000, df= 43, P = 1.000) (Figure 4.7a) or for male and female tiger snakes (t= 0.000, df= 28, P = 1.000) (Figure 4.7b).

4.4.3 Comparative assessment of microstructural differences between male and female snakes

There were no microfacets present on the ventral surface of the tongue tines for males or females in either snake species. Only the dorsal surfaces of the tongues had microfacets present. The series of microfacet counts on the dorsal areas of the tine tips and mid-tines indicated no significant difference in the microfacets per unit area on the tongues of male and female dugites (t=1.419, df= 3.25, P =0.251; Table 4.1). By contrast, there was a significant difference in the number of microfacets per 5µm^2 area on the tine tips and mid-tines between male and female tiger snakes (t= 4.415, df= 22.989, P=0.000; Table 4.2).

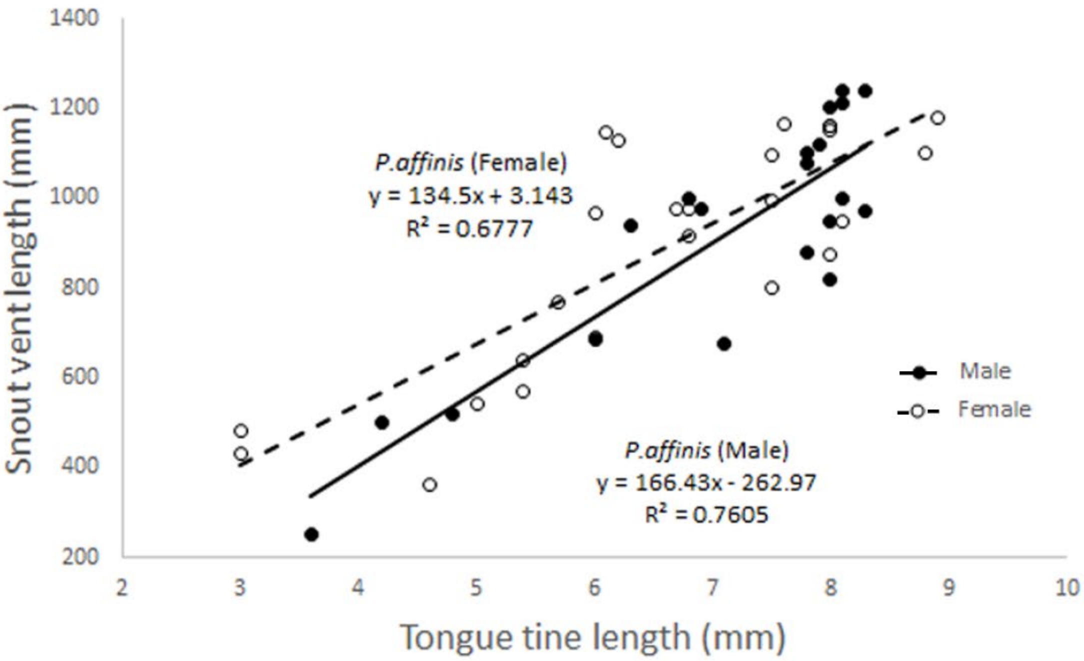

Figure 4.4a: Linear regression of Snout vent length against tongue tine length for male (R^2=0.761, P=0.116) and female (R^2=0.677, P=0.981) *Pseudonaja affinis affinis*. There is no significant relationship between the tongue tine length and snout vent length for both sexes.

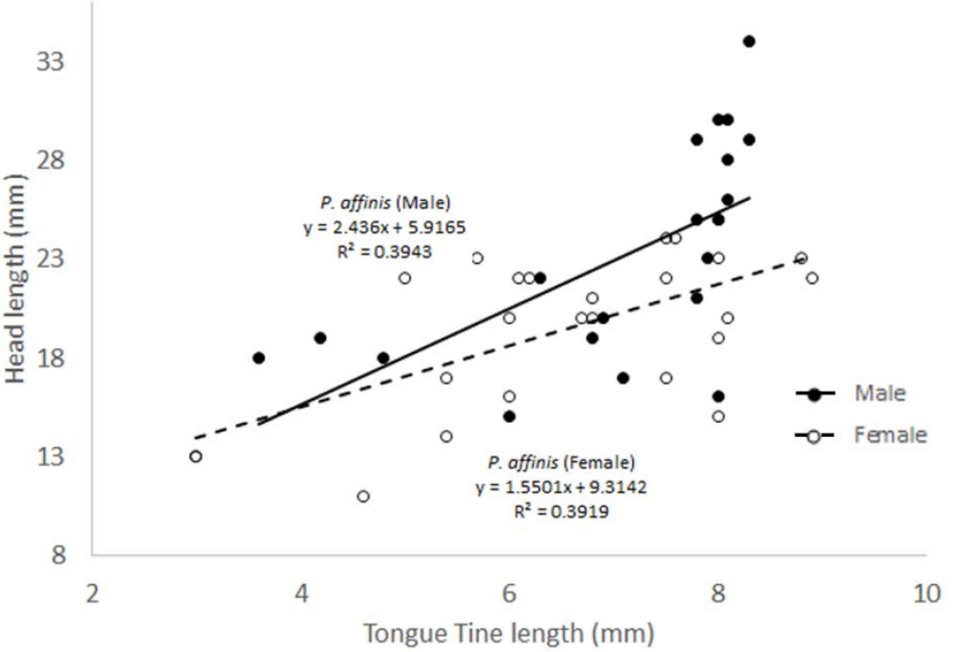

Figure 4.4b) Linear regression of head length against tongue tine length for male (R^2= 0.394, P=0.265) and female (R^2=0.392, P=0.002) *Pseudonaja affinis affinis*. There is a significant relationship between head length and tongue tine length in female *P.affinis*, but not males.

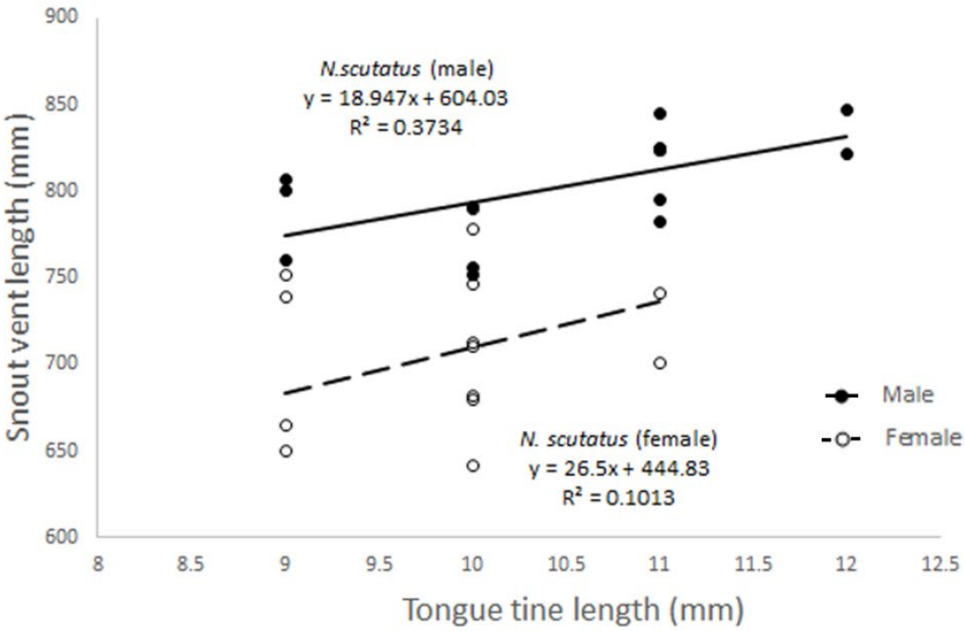

Figure 4.5a: Linear regression of snout vent length vs head length in male (R^2= 0.373, P=0.000) and female (R^2= 0.1013, P=0.059) *Notechis scutatus occidentalis*. Both sexes show a significantly positive relationship between snout vent length and head length.

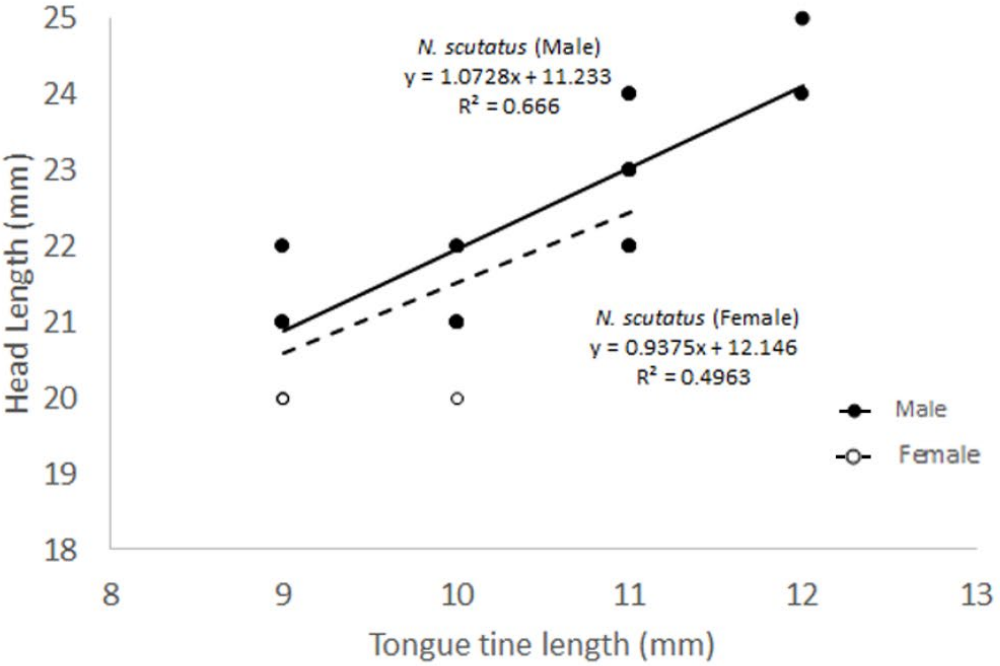

Figure 4.5b: Regression of tongue tine length vs head length in male (R^2= 0.666, P= 0.000) and female (R^2= 0.496, P=0.000) *Notechis scutatus occidentalis*. There is significant relationship between the tongue tine length and head length for both sexes.

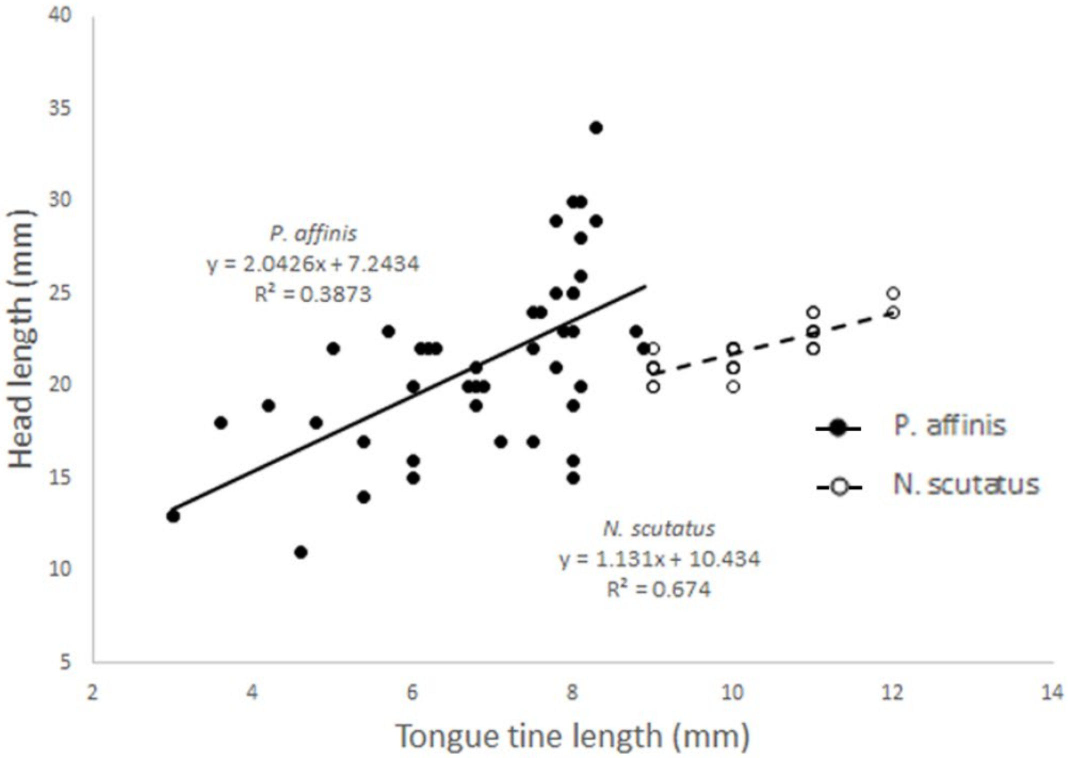

Figure 4.6: Linear regression of head length against tongue tine length for *Pseudonaja affinis affinis* (R^2 =0.387, P=0.001) and *Notechis scutatus occidentalis* (R^2= 0.674, P=0.000). Both species show a strong relationship between tongue tine length and head length, with *N.scutatus occidentalis* having a stronger overall relationship indicated by the R value.

Table 4.1: Sex differences in macro- and micromorphological measurements of *Pseudonaja affinis affinis.* Significant P-values are in bold.

	Male (n=20)	Female (n=25)	t	df	P-value
SVL (mm) ±SD; **(range)**	917.85 ± 271.14 (250.0 – 1240.0)	888.72 ± 260.55 (60.0 – 1178.0)	-0.366	43.0	0.716
Head Length (mm) ±SD; **(range)**	23.20 ± 5.51 (15.0 – 34.0)	19.52 ± 3.95 (11.0 – 24.0)	-2.608	43.0	**0.012**
Tongue tine length (mm) ±SD; **(range)**	7.095 ± 1.42 (3.60 - 8.30)	6.548 ± 1.595 (3.0 - 8.90)	-1.120	43.0	0.296
Density of tongue microfacets/5µm^2 $^\pm$SD; **(range)**	17.50 ± 0.5 (17.0 – 19.0)	14.00 ± 2.45 (7.0 – 18.0)	1.419	3.25	0.251

Table 4.2: Sex differences in macro- and micromorphological measurements of *Notechis scutatus occidentalis.*

	Male (n=15)	Female (n=15)	t	df	P-value
SVL (mm) ± SD; **(range)**	803.6 ± 30.708 (752.0 – 847.0)	704.533 ± 56.287 (580.00 – 790.0)	24.096	28.0	**0.000**
Head length (mm) ±SD; **(range)**	22.533 ± 1.302 (21.0 – 25.0)	21.333 ± 0.900 (20.0 – 23.0)	-2.937	28.0	**0.007**
Tongue tine length (mm) ±SD; **(range)**	10.533 ± 0.676 (9.0 – 12.0)	9.8± 0.990 (9.0 – 11.0)	-2.368	28.0	**0.025**
Density of tongue microfacets/5µm^2 ±SD; **(range)**	11.267 ± 2.153 (8.0 – 15.0)	8.4 ± 1.298 (6.0 – 11.0)	4.415	22.99	**0.000**

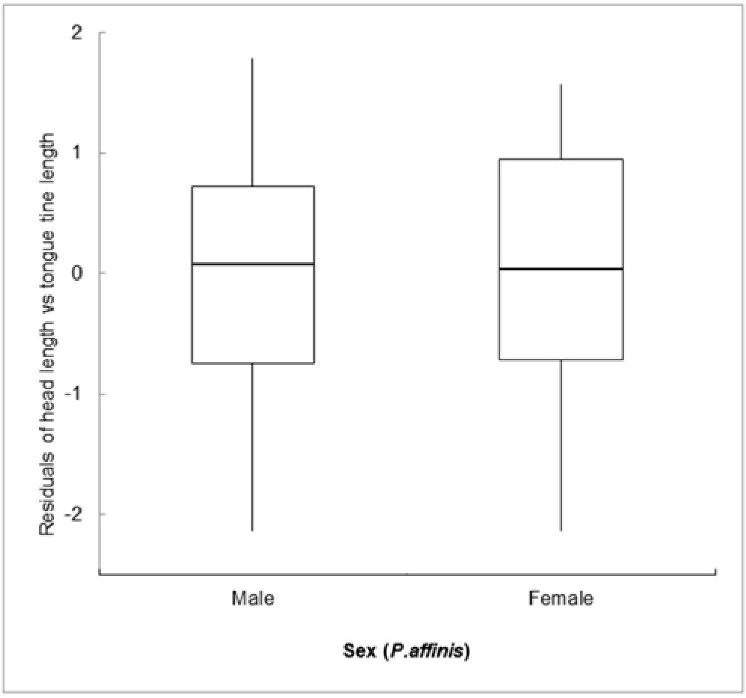

Figure 4.7a: Box and whisker plot showing differences in residual values of head length vs tongue tine length in male and female *Pseudonaja affinis affinis.* No significant differences demonstrated between male and female residuals (Table 4.3, P-value: 1.000)

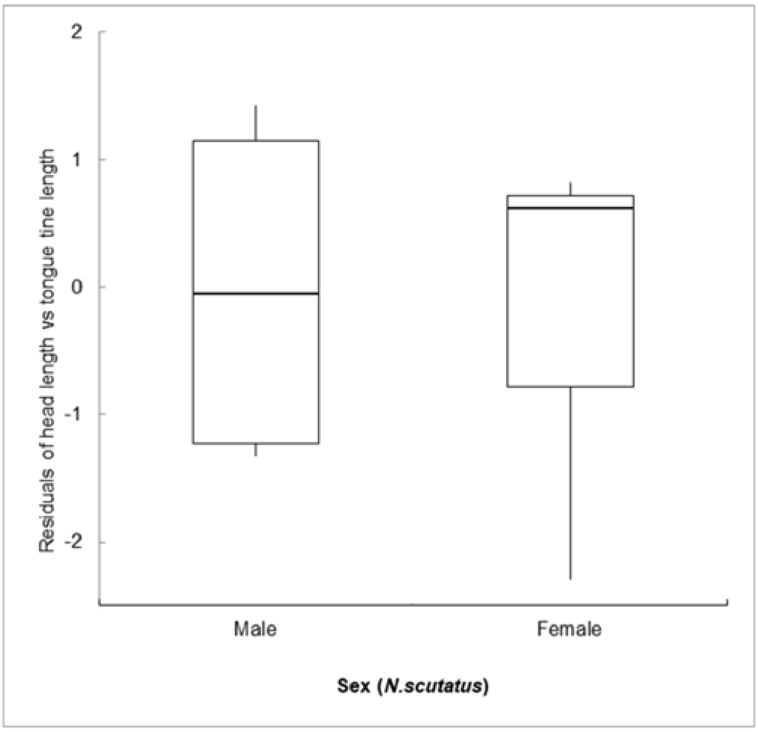

Figure 4.7b: Box and whisker plot showing a difference in residuals of head length vs tongue tine length in male and female *Notechis scutatus occidentalis*. No significant differences demonstrated between male and female residuals (Table 4.3, P = 1.000).

Table 4.3: Relationships between head length and tongue tine length in male and female *Pseudonaja affinis affinis* and *Notechis scutatus occidentalis.* The residuals quantify the differences between the individual observed values on the regression line and their respective predicted values obtained using the regression equation. Residuals were used in order to find any form of difference in tongue tine length while taking into account potential sexual size dimorphism (Smith *et al.*, 2008).

2-tailed t-test results for residuals for:	Mean Difference	SE Difference	P-value	df	t
Head length vs Tongue tine length (*P. a. affinis*)	0.000	0.293	1.000	43.000	0.000
Head Length vs Tongue tine length (*N. s. occidentalis*)	0.000	0.352	1.000	28.000	0.000

4.5 Discussion

Our hypothesis that there would be sexual dimorphism in the tongue tine length of *P. a. affinis* was not supported. Furthermore, we could find no significant differences in the number of microfacets on the tongues of male and female *P. a. affinis*, suggesting that there is no difference in chemo-receptivity between the sexes of this species. It appears, therefore, that there is no evidence that tongue size or surface microstructure is under sexual selection in *P. a. affinis*. By contrast, sexual dimorphism was present in the tongue tine lengths of *N. s. occidentalis* as male *N. s. occidentalis* had longer tongue tines and a higher density of microfacets than females. There were also significant differences in the number of microfacets on the tongues of male and female snakes. For both snake species, our results supports those of Bayoumi *et al.* (2011) and Morgans and Heidt (1978) that there are no microfacets on the ventral surface of the tongue.

4.5.1 Subtle sexual selection in tongue tines in relation to behavioural variability in snakes

As there have only been two studies on sexual selection on snake tongues – our present study on two elapids and that of Smith *et al.* (2008) on the viperid *Agkistrodon contortrix* – it is difficult to speculate on what this means for our hypothesis across snakes in general. It is possible that *A. contortrix* (the North American copperhead) is the only snake species with SSD in tongue tines, but it is more likely that other life history factors may explain the observed morphological differences between *A. contortrix, P. a. affinis*, and *N. s. occidentalis* tongues.

The dugite and tiger snake are both Australian elapid snakes, while the copperhead is a North American viperid. Most elapids are active predators with shorter fangs compared to viperids, and non-flexible maxilla (Kardong, 1982), that mostly use visual cues to seek out prey and mates. They may also successfully locate mates using both visual cues and chance meetings in their well-known and overlapping home ranges. By contrast, most viperids are sedentary ambush predators with longer fangs and very flexible maxillae (Kardong, 1982) that typically inhabit a limited area for most of the year and thus require a more developed sensory system to detect mates during the breeding season (de Cock Bunning, 1983; Bryant *et al.,* 2012). As an ambush predator, *A. contortrix* generally stays in one place for long periods of time waiting for prey to pass opportunistically (Smith, 2007), while active predators such as *P. a. affinis* and *N. s. occidentalis* rely on visual cues and chance meetings used in mate seeking and for seeking out prey.

The results of our study indicate that the presence of sexual dimorphism in body size, tongue tine length, tongue microstructure is determined by variation in mating strategies in snakes. The forked structure of the snake tongue has two defining characteristics which aid in prey and mate seeking: prominent tongue bifurcation which enables them to pick up chemical signals in a spatial radius of detection, and microfacets which increase the snake's sensitivity to chemical signals detected (Ridlon, 1985; Schwenk, 1994). Mate seeking is a selective pressure, driving sexual difference in tongue tine lengths and number of microfacets between male and female snake tongue tines.

While Smith *et al.* (2008) did not look at the number of microfacets on the tongues of the North American Copperhead, their experiment and findings indicate that male *A. contortrix* travel long distances using chemoreception to detect potential mates during the breeding season, resulting in males having longer tongue tines in comparison to females as chemoreception is necessary for tongue-mediated mate searching (Smith, 2007). These results for *N. s. occidentalis* and *A. contortrix* are a clear contrast to that of *P. a. affinis* in which dimorphism in tongue tine length and number of microfacets is absent. This is because dugites are more likely to encounter mates during casual contact while hunting for prey or travelling to seek shelter and sunnier spots (Wolfe *et al.,* 2018), which indicates the absence of sexual dimorphism between the sexes in the presence of more active, foraging behaviours.

4.5.2 Lifestyle comparisons between two Western Australian elapids and a North American viperid

Lifestyle differences, habitat ranges and prey preferences are also key drivers of sexual selection in size and tongue structure between male and female snakes. Elapid *N. s. occidentalis'* behavioural ecology shares closer characteristics with the viperid *A. contortrix*, with tongue-mediated searching being common during the breeding season as well as a preference for damp habitats such as grassy woodlands near creeks and marshes (Butler *et al.*, 2005; Smith *et al.*, 2009). Frogs are the preferred prey of both *N. s. occidentalis* and *A. contortrix*, which means they have food-based home ranges near the water (Lettoof *et al.*, 2020; Shine, 1977b). However, *A. contortrix* may also travel further distances in search of preferred, small mammalian prey such as mice and rats (Shine, 1977b; Greenbaum, 2004). In addition, Smith (2007) found that telemetered male *A. contortrix* had larger mean annual home ranges than did females (17.9 ha vs. 4.8 ha respectively), due entirely to males searching long distances for females in spring. This study also notes that the general mean home range sizes between the female copperheads and tiger snakes are similar (4.8 ha vs 4.94 ha) (Smith, 2007; Butler *et al*., 2005). Both copperheads and tiger snakes express male-biased sexual size dimorphism as a result of males of both species partaking in male-male combat during the breeding season (Shine, 1978). In contrast to *N. s. occidentalis* and *A. contortrix*, data for *P. a. affinis* indicates that both males and females travel long distances in search of their preferred prey, which include small terrestrial mammals, birds, and reptiles (Maryan & Gaikhorst, 2005; Shine, 1977b; Wolfe *et al.,* 2018) and may even resort to cannibalism (Maryan & Gaikhorst, 2005). In addition, they are more adaptable to urban environments, where not only will they forage for a large number of prey mammals and birds, but also have access to water supplies from bore water in urban gardens, where they also encounter reptile prey, especially *Tiliqua rugosa* (Wolfe *et al.*, 2018). The dugite is a foraging species related to *Pseudonaja textilis* (Eastern brown snake). Both snake species are foragers with have large home ranges, with *P. textilis* having a mean home range of 6 ha (Whitaker & Shine, 2003), which is larger compared to that of *N. S. occidentalis* and female *A. contortrix*.

4.6 Conclusion and further studies- snakes, varanids, and sexual selection

The presence of sexual dimorphism in body size and tongue tine length and structure in snake species is not a result of phylogenetic histories, but behavioural and life history differences between species. Although our hypothesis was supported by the presence of sexual selection in *N. s. occidentalis* tongue tines and the absence of sexual selection in *P. a. affinis*, in order to improve future results, we would need larger snake samples. Furthermore, we would need reptiles of confirmed aged to accurately assess sexually mature individuals.

The results of the presence of male-biased sexual size dimorphism in snake species supported by Shine & Fitzgerald (1995)'s study on sexual size dimorphism in Australian carpet pythons (*Morelia spilota*). *Morelia spilota* demonstrates variation in sexual size dimorphism and mating strategies depending on the geographic location (Shine & Fitzgerald, 1995). In northern New South Wales, the climate is warmer, allowing both sexes of carpet pythons to be active all year round. While both sexes of carpet pythons actively move around for much of the year, males are larger than females, which appears to be due to the presence of male-male combat over females. By contrast, female-biased sexual size dimorphism occurs in southern New South Wales, where the climate is much cooler. For carpet python species occurring in Southern New South Wales, mating aggregations occur, where male snakes use chemoreception to seek out females once a year, during the breeding season (Shine & Fitzgerald, 1995). Apart from climate, other factors affecting variation in sexual size dimorphism include prey availability during the seasons, where drought conditions decrease the levels of female SSD due to the scarcity of prey (Winne *et al*., 2010). Such a situation is ideal to explore evolutionary trade-offs between investment in mate searching (tine length), competitive combat (SSD), sperm competition (testes size) and female fecundity (clutch size).

Bryant *et al.* (2011), in their study on chemoreception in southwest carpet pythons (*M. spilota imbricata*) noted the similarities in ambush foraging behaviour between these pythons and *Agkistrodon contortrix* of Smith (2007) and suggested looking for differences in tine lengths between the sexes in carpet pythons for future studies. While no studies on tongue tine lengths have been recorded for either case of *M. spilota* in northern or southern New South Wales, we would predict from our studies that male for both northern and southern species of New South Wales carpet python have longer tongue tine lengths as well as a greater number of microfacets as they seek out females during the breeding season.

In conclusion, to further our studies, we need to look at more snake species, and other squamates – such as monitor lizards (Varanidae), which also have deeply bifurcated tongues like those of snakes – with different life histories, to be able to distinguish between different selective pressures.

4.7 Acknowledgements

I would like to give special thanks to Professor Kate Trinajstic, Dr Bill Bateman and Dr Catherine Boisvert helping organize the project and Michael Just for educating me in the use of scanning electron microscopy at building 304 at Curtin University. I thank Rebecca Bray and Dr Paul Doughty at the Western Australian Museum for allowing the usage of dissection equipment and access to pickled snake specimens, and Dr Damien Lettoof for use of tongues from his snake specimens.

Author Contributions:

PWB, KT and CK designed the experiment. CK undertook CT scanning with the help of PWB. CK wrote the manuscripts with KT, PWB and LNG helping with the subsequent drafts. CK calculated the data in Excel and StatistiXL. PWB, LNG, and KT contributed to data interpretation. CK created Figures 4.1 through 4.7. LNG helped with Figure 1. KT helped with Figure 4.2.

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Chapter 5. General discussion and conclusion

5.1 Summary of findings

For this thesis, I explored the macro- and microscale features of squamate tongue structures with regard to the relative roles of natural and sexual selection in shaping their diversity. For both analyses, I focused on not just the macroscale aspect of differences between the species or sexes, but attempted to fill the knowledge gap in this field by studying tongues at a microscale level.

In Chapter 3, I explored whether diet and habitat have a greater effect on tongue structure variation than does phylogenetic relationships. I used a combination of morphometric analysis for gross morphological features and scanning electron microscopy for microscopic features that could possibly be under natural selection. I made use of opportunistically road-killed specimens of seven lizard species native to Western Australia and compared the morphological variation that I captured by SEM with published SEM results from 29 peer-reviewed research papers.

My results for Chapter 3 indicate that niche and diet as opposed to phylogeny are the driving forces of natural selection of lizard tongue structure. An example in my results can be seen within the five skink species, which all have diverse tongue microstructures on the tongue tip, fore-tongue and mid-tongue. There was a lack of diversity in the hind-tongue as movement of food towards the oesophagus is universal for all lizard species. In addition, a comparison of my gecko specimen with other members of the superfamily Gekkota and my agamid specimen with other agamids from peer reviewed articles also showed a wide range of tongue microstructures within the families. I also found, through the broad comparison of lizard species with similar habits and diet both from my samples and from the peer-reviewed articles, that convergent evolution on the tongue tip and fore-tongue is present across squamate families. Further studies on the evolutionary convergence of tongue microstructures are warranted, as investigation of tongues from a wider range of other lizard families is necessary in order to understand how niche, diet, and tongue usage indicate differences in behaviour and lifestyles between species in lizard families.

For Chapter 4, I investigated for the presence subtle forms of sexual dimorphism between male and female tongue tine structure in two Western Australian elapid snake species to see if they are sexually selected. I used a morphometric analysis of males and female dugites and tiger snakes to assess if macroscale differences would indicate sexual selection. A gross morphometric approach had already been undertaken by Smith *et al.* (2008), but no exploration had been done to date at the microsurface level, so I also used Scanning electron microscopy for analysis of microfacet density between the sexes for microscopic analysis of sexual dimorphism. I found that for tiger snakes, males have greater chemoreceptivity compared to females. Apart from males having longer tongue tines than female snakes, a result also found by Smith *et al.* (2008), I found that they also have a higher microfacet density, which increases their ability to discriminate scents. This enables males to pick up more chemical signals in order to navigate toward high chemical signal concentrations. Male tiger snakes seek out females during the breeding season, such that sexually selected longer tines with more microfacets allow them to find mates more efficiently.

Sexual differences in tongue microstructure would need to be examined further in future studies as only two studies have been done on tongue length, but none on tongue structure regarding microfacet density. An examination of tongues from different snake families with different lifestyles is necessary in order to assess sexual selection at microscopic levels and support my conclusion with a broader range of evidence.

5.2 Future studies

Apart from food intake and mate- and prey-finding duties (or functions), tongues in squamates have a wide variety of uses which include cleaning (Pedersen, 1988) and signalling (Abramjan *et al.,* 2012; Badiane *et al.,* 2018). I would expect to see greater specialization in tongue structures made for cleaning in certain lizard species with cleaning functions such as barking geckos (Griffiths, 1987), leopard geckos, and members of the Pygopodidae family (Deperno & Cooper, 1996; Spinner *et al.,* 2013). Knowledge gaps which we could use an SEM approach to resolve include determining if there are tradeoffs in tongue microstructure between signalling capacity and tongue microstructure, as well as tradeoffs between chemoreception and cleaning capability. Further studies will allow us to examine squamate tongues at microscopic levels, which may include looking at more SEMs of tongue structure in squamate species which use their tongues for grooming and examining the fluorescence levels of bluetongue skink tongues in relation to microsurface structure to see if investment in colour for signalling has an effect on other microscopic features and their associated functions.

Natural selection of tongue structure and shape indicates the use for survival while sexual selection drives dimorphism that promotes sexual reproduction. Studying natural and sexual selection is vital especially since our findings indicate that these forms of selection occur cryptically and at a microscopic level, which allow us to fill many undiscovered knowledge gaps in the field of behavioural ecology. It is therefore important that we discover more knowledge gaps which allow us to understand animal behaviour. Such information will enable us to educate the general public about our ecosystem's wildlife.

As demonstrated in this thesis, tongue morphology in squamates is highly complex and variable, clearly reflects natural selection, and can even reflect sexual selection at the microscopic scale, where microfacet density enhances male chemoreception for mate-finding. Tongue macrostructure has been a well-studied area of research for over 100 years. Aristotle was the first to observe the snake's bifurcated tongue, which he described as being used as a 'twofold pleasure' for taste (Schwenk, 1994). Shakespeare claimed their tongues could sting, while 17th Century Italian astronomer Giovanni Hodierna believed their tongues were used to clean their noses, which would be far from what would be eventually understood of the relationship between the tongue tines and the vomeronasal organ. In the 9th Century, early evolutionist Jean-Baptiste Lamarck was the first to make an observation that the snake's tongue was used as an organ of touch to feel its way around, a belief that would be supported in the late 1800s (Hopley, 1882). This belief would be further supported with the discovery of the VNO by Danish physician Ludvig Levin Jacobson, an organ, while first detected by Frederik Ruysch in 1800s, was later discovered to process chemical signals received by smell, or in the case of squamates by the tongue via the process of chemoreception (Coelho *et al.,* 2005; Helmenstine, 2020; Lekakis, 2003). Even with these discoveries regarding tongue function and macrostructure, many significant knowledge gaps remain with regard to microstructure, which has only been "under the microscope" since 1933 (Haguenau *et al*., 2003). Below, I discuss several important areas which I believe will greatly improve our understanding of natural and sexual selection in squamate tongues and provide suggestions for future research.

5.3 Future directions

The scope of the study was limited as we only had seven lizard species from three families and two snake species from one family. Future studies on natural selection in lizard tongues at a macro- and micromorphological scale should be further addressed by looking at more tongue morphologies from a wider range of lizard families. Sexual selection in snake tongue macro- and micromorphologies will need to be addressed by comparing wider range of snake species from different families.

As demonstrated in Chapter 3, dietary conditions appear to drive fine-scale modifications as seen in the macro- and microstructure of many lizard species. Further studies on natural selection in lizard tongues would allow me to gain access to more specimens from other lizard families. So far, I have discovered that divergent evolution results in tongue macro and microstructure to be diverse within lizard families and superfamilies. Also, lizards from different families and superfamilies that share the same tongue microstructure are a result of species having similar dietary preferences due to the process of convergent evolution. In order to further investigate this topic, I highly recommend a look at more Australian geckos from various families within the superfamily Gekkota as well as a wide range of native agamid species (*Ctenophorus parviceps*, *Moloch horridus,* etc.) as it will allow further knowledge gaps on squamate tongue macro and microstructure to be filled. Further inspections on the macro and microstructure of various other lizard species native to Western Australia will be highly desirable, especially from unexplored families which I have not covered in my thesis. A set of SEM analysis of lizard species similar to the ones in this thesis and other peer reviewed papers would be highly recommended to get a broader view of natural selection in lizard tongue structures. As I explain within chapter three, we need to investigate a wider range of tongue macro- and microstructures from other lizard families in order to understand the evolution of lizard tongue structure and how behaviour and diet as well as tongue usage give a good indication of tongue macro- and microstructure differences in lizard behaviour and lifestyles between lizard families, but as far as I am aware, no study has investigated this.

In Chapter 4, I provided the first confirmatory evidence of sexual selection at the microsurface level of tongue morphology. Further comparative analyses would be necessary to provide broad support for this. Of particular interest would be to explore species with variable behavioural tactics, an example being the species of *Morelia spilota* that has variable behaviour. Shine and Fitzgerald (1995) have looked at the differences in courtship behaviour of *M. spilota* subspecies throughout Australia. These subspecies will be useful for future studies in order to broaden the scope of analysing the presence of sexual selection at macro and microscopic levels in tongue structures between the sexes within a snake species. Further analysis on male and female tongue tines within other snake species from different snake subfamilies (colubrids, viperids) would also be used to provide board support of sexual selection in tongue microstructure at a microscopic level as many snake families share different life histories and habitat ranges, resulting in different mate seeking behaviours within the species.

Further knowledge gaps such as investigation of natural and sexual selection of the size and structure of the squamate VNO will also be addressed. The VNO is especially crucial in the process of chemoreception which many tetrapods, especially squamates, rely on for the process of chemical signals for information. So far only one comparative study of VNO structure within a range of lizard families has been addressed by Filoramo and Schwenk (2009). Further investigations will look at a wider range of squamates to see the result of natural selection at a morphological microscale. Most studies regarding sexual selection in the size and structure of the VNO have been done in mammals (Tai *et al*., 2004; Segovia *et al*., 2006) and amphibians (Dawley, 1992). As chemoreception is an important process of mate seeking in squamates, the size and structure of the VNO between the sexes should be addressed as a form of behavioural studies for breeding and conservation programmes for Australia's squamates.

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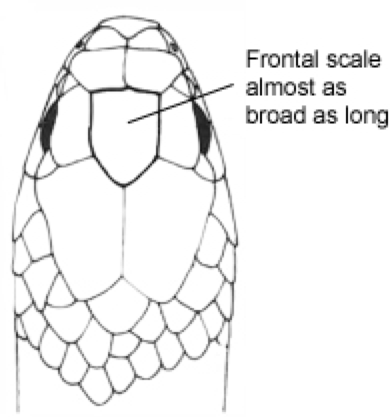

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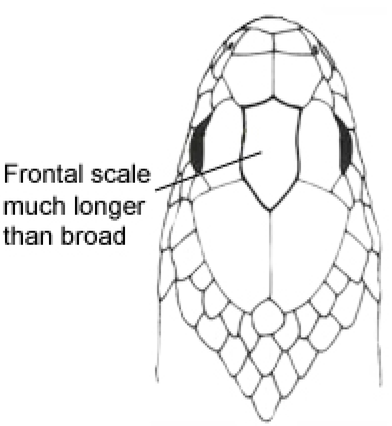

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