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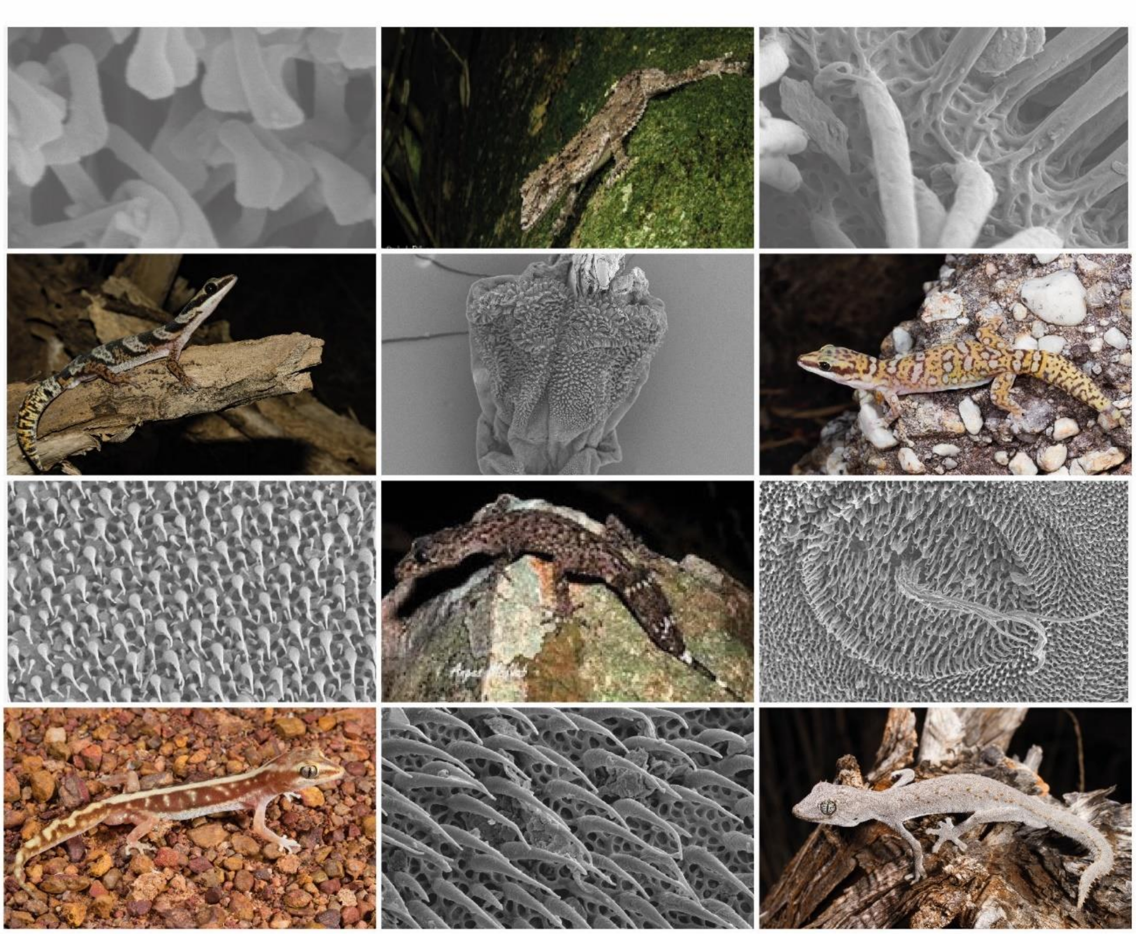
Evolution and ecological adaptations of microornamentation in Australian geckos (Gekkota, Squamata)



Thesis submitted by

Nils Jendrian Riedel

For the degree of Doctor of Philosophy in the College of Science and Engineering at
James Cook University

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Title page photographs

First row:

Left: Spatula at the tips of the setae from the Paluma-W lineage of *Heteronotia binoei* (credit: Jendrian Riedel)

Middle: *Saltuarius cornutus* (credit: Rishab Pillai)

Right: pits and struts between the setae stalks from the Paluma-W lineage of *Heteronotia binoei* (credit: Jendrian Riedel)

Second row:

Left: *Oedura castelnaui* (credit: Rishab Pillai)

Middle: setae covered terminal subdigital scales of the Paluma-W lineage of *Heteronotia binoei* (credit: Jendrian Riedel)

Right: *Oedura coggeri* (credit: Stephen Zozaya)

Third row:

Left: Spinules on a dorsal midbody scale of *Lucasium immaculatum* (credit: Jendrian Riedel)

Middle: *Phyllurus amnicola* (credit: Rishab Pillai)

Right: Cutaneous sensillum from *Oedura coggeri* (credit: Jendrian Riedel)

Fourth row:

Left: *Lucasium immaculatum* (credit: Stephen Zozaya)

Middle: Spinules on a lateral mid-distal subdigital scale from the Blencoe lineage of *Heteronotia binoei* (credit: Kevin Blake)

Right: *Strophurus krisalys* (credit: Stephen Zozaya)

Statement of Contributions and Ethics Declaration

AUTHORS CONTRIBUTIONS

A PhD thesis is never a solo project, and I was blessed with the opportunity to collaborate fruitfully with other researchers, both inside and outside my department. Lin Schwarzkopf, my primary supervisor, provided invaluable advice throughout my project and therefore is a co-author on all my data chapters and the appendix chapters in my thesis. We constantly discussed all steps of my research from experimental design and statistical analysis to interpretation of results, and she assisted me with editing of the manuscripts resulting in this thesis.

When I started working on my thesis, I was in the lucky situation to inherit a large data set from Matt Vucko, which he used for his master's thesis in 2008, but never published. So, I could immediately start to analyse his dataset, a process, which resulted in the chapters 2 and 3 of my thesis. Therefore, Matt Vucko is a co-author on Chapter 2 and 3, in which he collected the data, gave advice on study design, and edited the manuscript. Simone Blomberg also is a co-author on these two chapters, as she helped me with statistical analysis and troubleshooting in R for these two projects. Simon Robson is a co-author on Chapter 2, where he edited the manuscript and helped with the original data-collection by Matt Vucko.

Stephen Zozaya and Conrad Hoskin are both co-authors on Chapter 4, were I designed the study together with Stephen and Lin. Stephen also provided specimen for the project and we both collected specimen and data in the field. He conducted the phylogenetic part of the analysis (DNA extraction and phylogenetic tree reconstruction), took the macroscopic images of the digits of the specimen and measured toepad width, while I conducted the scanning electron microscopy work and analysed all the morphological data. Conrad Hoskin provided helpful advice on study design. I lead the writing of the manuscript while Stephen, Conrad and Lin edited the various drafts.

Eric Nordberg is a co-author on Chapter 5, where we designed the study and analysed the data together. Of the field observations used for this study, Eric collected the data from Wambiana Research Station, while I collected the data from all other sampled locations.

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I received an International Postgraduate Research Scholarship from the Australian government. My research was supported by funding I obtained through the College of Science and Engineering at James Cook University. My field work was partially funded by two research funds from the Skyrail Rainforest Foundation.

ETHICS APPROVAL AND PERMITS

All data was collected within the guidelines for ethical treatment of animals and approved by James Cook University (animal ethics permit No. 2409) and the Queensland Department of Environment and Heritage Protection (research permit No. WA0005590) and the Department of National Parks, Sports, and Racing (research permit No. WTIK18258517).

Preface

The following publication have resulted from this thesis.

Thesis Chapters

Chapter 2 – published:

Riedel, J., Vucko, M. J., Blomberg, S. P., Robson, S. K. A., & Schwarzkopf, L. (2019). Ecological associations among epidermal microstructure and scale characteristics of Australian geckos (Squamata: Carphodactylidae and Diplodactylidae). *Journal of Anatomy*, 234, 853–874.

Chapter 3 – published:

Riedel, J., Vucko, M. J., Blomberg, S. P. & Schwarzkopf, L. (2020). Skin hydrophobicity as an adaptation for self-cleaning in geckos. *Ecology and Evolution*, 00, 1-12.

Chapter 4 – under review:

Riedel, J., Zozaya, S. M., Hoskin, C. J., & Schwarzkopf, L. (under Review). Parallel evolution of toepads in rock dwelling lineages of a terrestrial gecko (*Heteronotia binoei*, Gekkota: Gekkonidae). *Zoological Journal of the Linnean Society*.

Chapter 5 – published:

Riedel, J., Nordberg, E. J., & Schwarzkopf, L. (2020). Ecological niche and microhabitat use of Australian geckos. *Israel Journal of Ecology & Evolution*, 1, 1–14.

Apendix Chapters

Appendix I – published:

Fushida, A., Riedel, J., Nordberg, E. J., Pillai, R., & Schwarzkopf, L. (2020). Can Geckos Increase Shedding Rate to Remove Fouling? *Herpetologica*, 76, 22–26.

Appendix II – published:

Pillai, R., Nordberg, E. J., Riedel, J., & Schwarzkopf, L. (2020). Nonlinear variation in clinging performance with surface roughness in geckos. *Ecology and Evolution*, 10, 2597–2607.

Acknowledgments

Moving to Australia from Germany to work on geckos for a PhD is like a dream for every reptile enthusiast. I am extremely grateful for this opportunity and to all the people who supported me traveling this path. It was also a big step to leave behind my family and many good friends to move to the other side of the world, but I did not need to take this step alone. My wife Linda Stanke joined me on this great adventure, pursuing her own career in Townsville. Linda has supported me in many ways throughout this process and encouraged me to follow this path. So, my deepest thanks go to you, Linda! Thank you for joining and supporting me these past 3.5 years!

I am extremely grateful to my primary supervisor Lin Schwarzkopf, who made it possible for me to join her lab in the first place. I could not have asked for of better supervisor and mentor for my project and my degree. Lin created an extremely positive, supportive and welcoming atmosphere in her lab full of scientists from a diversity of backgrounds and many different research areas. Lin constantly encouraged me to improve my skills and abilities as a researcher, from designing and planning my research, to analysing data, to writing and presenting results in a precise, correct way, but also in a style that is interesting to read. She always had time to discuss projects, problems, or just random interesting topics, and to read and correct manuscripts over and over again. She was also really supportive in connecting me with other researchers in Australia.

I am also thankful to my co-supervisors for support and helpful advice. Will Edwards, Deb Bower and Scott Keough where always there for me when I needed them. Will helped me to improve some of my manuscripts and grant proposals. Deb helped me planning the ecological part of my thesis, and also contributed greatly to the positive and welcoming atmosphere in My lab. Scott gave valuable advice on the morphological parts of my project, including data that did not make it into the thesis in the end… but will hopefully be published soon!

Research nowadays is highly collaborative, and I had the privilege to collaborate with quite a few great researchers for the different chapters of my thesis. Matt Vucko was so kind to share unpublished data from his master's thesis and gave me valuable support in analysing these datasets and writing the respective parts of my thesis. I'm also indebted to Simone Blomberg and her seemingly unlimited knowledge and expertise on statistics - I learned a lot about statistical analysis, while working with her. Simon Robson gave me valuable comments for one of the manuscripts for my thesis. Both Stephen Zozaya and Conrad Hoskin not only helped me with planning, conducting and writing one of my thesis chapters, but also provided helpful advice on where to go for my field trips to target the gecko species I was looking for. I am also very grateful to Eric Nordberg, who was the 'senior' PhD student in My lab, when I started. He not only contributed to one of my chapters and shared his data, but also generally supported me at different stages during my candidature form planning fieldwork to support and advice with R scripts.

Additionally, I would like to thank Don McKnight and Michalis Mihalitis, who also helped me with discussing statistical analysis and writing or sharing R scripts at one point or another during my work. I am particularly thankful to Rishab Pillai, who started as a master's student in My lab, shortly after I started my project, and which I had the honour to co-supervise. He continued to assist me during my candidature by joining most of my field trips and supporting me with lab work or gecko husbandry. I also want to thank Kevin Blake and Shane Askew from the Advanced Analytical Centre at JCU, who were always happy to help, when I got stuck with the scanning electron microscope, or just couldn't the get image as crisp as I wanted.

As I mentioned before, I really enjoyed the honOur to work in such a nice, open minded, and productive lab group. Lin's legendary "lizard lunch" lab meetings provided the platform for many group discussions on all aspects of research, as well as practice talks for conferences. Thanks to Heather Neilly, Eric Nordberg, Ben Muller, Don McKnight, Lexie Edwards, Deb Bower, Juan Mula, Rishab Pillai, Jodie Nordine, Cat Kelly, Sheryn Brodie, Ayano Fushida, Stewart Macdonald, Kyana Pike, Hiro Komine, Tom Bruce, Lily Leahy, Wytamma Wirth, Jamie Hopkins, Tash Ryan, Anna Pintor, Mat Vickers, Ross Alford, Elizah Nagombi, Slade Allen Ankins, Elliot Budd, Jari Cornelis and Ben Hirsh for comments and suggestions, as well as for having a good time during my candidature.

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Abstract

The integument, or skin, is the outermost layer of an organism, and its main area of contact with the environment. This multifunctional organ provides both protection against external damage, for example from predators, diseases, insolation, or though water loss, as well as a means of interaction with the environment, including sensory organs or specialised locomotor structures. Different taxa have evolved a huge variety of specialized skin morphologies as adaptations to different ecological demands. Squamate reptiles have evolved microscopic structures on their outermost skin surface, called microornamentation or microstructures. Geckos are a particularly diverse group of squamate reptiles, comprising around 1900 species world-wide. One hotspot of gecko biodiversity is Australia, where they occupy nearly all microhabitats and bioregions. Geckos are famous for their adhesive toepads, whose function is based on hair-like microornamentation called setae. These setae have probably evolved from shorter, hair-like microstructures called spinules, which cover the whole body and have hydrophobic, self-cleaning properties. Additionally, geckos feature small sense organs called cutaneous sensilla, which normally bear one or more hair-like extensions called bristles. For this thesis, I have studied different aspects of the evolution and ecological adaptation of these microstructures.

To examine the variation of microornamentation and scale dimensions, I described the diversity of microornamentation on the dorsal scale surface of 27 species from two families of geckos, the Carphodactylidae (8) and the Diplodactylidae (19), which are closely related and have evolved in Australia. To describe their microornamentation, epoxy-resin moulds from living geckos were used, based on negatives made with dental imprint material. These moulds were analysed with a scanning electron microscope (SEM) and then the microstructures were measured and described. For each species, I described the presence and quantified scale size, spinule length and density, and several other skin structures called cutaneous sensilla, lenticular sense organs, and knobs. Additionally, as a first step to examine factors influencing the evolution of these microstructures, I used a phylogenetic flexible discriminant analysis (pFDA) to describe the relationships of these traits with habitat selection (arboreal, saxicoline or terrestrial) and relative humidity of each species' habitat (xeric, mesic or humid), and then used a modelling approach to examine each trait individually. My analysis showed that terrestrial species tended to have long spinules and cutaneous sensilla with more bristles, saxicoline species had larger diameter cutaneous sensilla and arboreal species tended to have large granule scales and small intergranule scales. There was high overlap in microstructural morphology among species from xeric and mesic environments, whereas species from humid environments had different morphology, driven mainly by their large diameter cutaneous sensilla with few bristles. Significant associations between epidermal morphology and environmental humidity and habitat suggest that epidermal microstructures have evolved in response to environmental variables.

Hydrophobicity is common in plants and animals, functioning to keep exposed surfaces clean. Although the occurrence and physical causes of hydrophobicity are well understood, ecological factors promoting its evolution are unclear. The highly hydrophobic integument of

geckos makes them an ideal candidate with which to analyse the evolution of hydrophobicity. I predicted that, because the ground is dirty and filled with pathogens, high hydrophobicity should coevolve with terrestrial microhabitat use. Advancing contact angle (ACA) measurements of water droplets were used to quantify hydrophobicity in 24 species of Australian gecko. I reconstructed the evolution of ACA values, in relation to habitat use of geckos (arboreal, saxicoline or terrestrial). To determine the best set of structural characteristics associated with the evolution of hydrophobicity, I used linear models fitted using phylogenetic generalized least squares (PGLS), and then model averaging based on AIC_c values. All species were highly hydrophobic (ACA > 132.72°), but terrestrial species had significantly higher ACA values than arboreal ones. The evolution of longer spinules and smaller scales were correlated with high hydrophobicity. These results suggest that hydrophobicity has co-evolved with terrestrial microhabitat use in Australian geckos *via* selection for long spinules and small scales, likely to keep their skin clean and prevent fouling and disease.

The function of adhesive setae in geckos has been studied extensively, and it is well established that the multiple branched setae with their triangular tips (spatulae) enhance contact with the substrate on the nano- scale. This enhanced contact leads to adhesion though van der waals forced and enhanced friction (higher shear forces). With this baseline knowledge, the research on adhesive microstructures has recently shifted to examine their evolution. There are two ways adhesive toepads can evolve in geckos, either immediately at the end of the toe, *via* enlarged terminal scales (terminal toepads), or *via* broadening of the scale rows under the inflection point (knuckle) of the toe (basal toepads). The evolution of adhesive toepads is poorly understood because functionally intermediate morphological configurations between pad-less terrestrial and pad-bearing climbing forms are rare. One study has examined the evolution of basal pads, but no study has addressed the evolution of terminal pads. To shed light on terminal toepad evolution, I assessed the subdigital morphology of phylogenetically distinct lineages of the Bynoe's gecko species complex (*Heteronotia binoei*). Most populations of *H. binoei* are terrestrial, but two distantly related lineages use saxicoline habitats, and have enlarged terminal subdigital scale areas. I reconstructed the ancestral terminal subdigital scale size of nine lineages of *H. binoei* in north-east Australia, including the two saxicoline lineages. Additionally, I compared the subdigital microstructures of four lineages, the two saxicoline lineages and their respective terrestrial sister lineages. All four lineages had fully developed setae, but the setae of the two saxicoline lineages were significantly longer, branched more often, and had higher aspect ratios than those of the terrestrial sister clades. I concluded that the saxicoline lineages represented an example of parallel evolution of adhesive structures in response to vertical substrate use, and their morphology represented a candidate for an intermediate state in terminal toepad evolution.

To apply modelling techniques to answer broad-scale evolutionary questions, as I did in this thesis, a thorough knowledge of the ecology of the species analysed is paramount. But detailed information on species–habitat interactions are often only available for a few species, and Australian geckos are particularly data-deficient. For most species, information is available only as scattered, anecdotal, or descriptive entries in the taxonomic literature or in field guides, but not often from peer-reviewed, quantitative ecological studies. Therefore, I surveyed gecko communities from 10 sites, and 15 locations across central and northern Queensland, Australia, to quantify ecological niche and habitat use of geckos from these communities. My surveys

included deserts, woodlands, and rainforests, examining 34 gecko species. I assigned species to habitat niche categories: arboreal (9 species), saxicoline (4), or terrestrial (13), if at least 75% of the observations fell in one microhabitat; otherwise I classified geckos as generalists (8). I described perch height and perch diameter for arboreal species and assigned them to 'ecomorph' categories, originally developed for Anolis lizards. There was lower species richness in rainforests than in habitats with lower relative humidity; the highest species richness occurred in woodlands. Most arboreal and generalist species used perch heights and diameters consistent with those of the 'trunk-ground ecomorph, except those in the genus *Strophurus*, whose members preferred shrubs, twigs of small trees, or, in two cases, spinifex grass hummocks, thus occupying a perch space similar to that of 'grass-bush' anoles. I provide quantified basic ecological data and habitat use for a large group of previously poorly documented species.

Microornamentation has been studied for over a century, and adhesive toepads of geckos have gained a disproportionate share of this research interest. My thesis contributes to this research area on a broad scale, addressing some aspect of all microstructures found in geckos. The main foci of my thesis were the evolution and ecological adaptations of microstructures. For cutaneous sensilla and the spinules, my study provides, to my knowledge, the first detailed comparative study of ecological adaptations of these microstructures, applying a statistical modelling approach in a phylogenetically informed framework. In addition, I showed that hydrophobic, self-cleaning spinules have co- evolved with terrestrial microhabitats as an adaptation to higher exposure to dirt and debris on the ground. This part of my research also highlights the need to analyse function directly, in an evolutionary morphological framework. As a contribution to the study of adhesive toepads, I could demonstrate that two lineages within the *Heteronotia binoei* species complex have independently evolved enlarged subdigital scales and represent a candidate model to further study the evolution of adhesive toepads. Furthermore, my study highlights the need for more studies on the ecology of many reptile groups, including Australian geckos, as a reliable framework for modern biological research programs to answer broad-scale questions from evolution to adaptation to climate change. I contribute to this framework by presenting a large dataset on habitat use and ecological niche of Australian geckos, to be used for future studies.

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Figure 2-8 Dorsal scales of the genus *Lucasium* (SEM images). **(A)** Overview of *L. immaculatum*: Several granule scales with one cutaneous sensillum (CS) at the posterior end of each scale and surrounded by intergranules. Some scales have lenticular sense organs (LSO) at their anterior margins. **(B)** Evenly spaced long spinules amongst pits and struts (*L. immaculatum*). **(C)** Cutaneous sensillum of *L. immaculatum* with a slight depression but with no surrounding moat. The bristle (BR) is heavily barbed with setules and the surrounding spinules are sparser than those on the rest of the scale. (D) Lenticular sense organ of *L. steindachneri* near the anterior edge of the granule scale. **(E)** Cutaneous sensillum of *L. damaeum* with a distally tapered, smooth bristle (BR) surrounded by long spinules. **(F)** Lenticular sense organ (LSO) of *L. damaeum* which is recessed into the scale.

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Figure 2-10 Dorsal scales of the genus *Strophurus* (SEM images). **(A)** One hexagonal granule scale of *S. krisalys* with a bare area (BA) at the peak and one cutaneous sensillum (CS) at the posterior end. **(B)** One granule scale of *S. taeniatus* with two cutaneous sensilla at the posterior end of the scale. **(C)** Detail of a Cutaneous sensillum of *S. taeniatus* lacking a bristle with elongated spinules surrounded by a sparse spinulate area towards the edge of the scale. **(D)** Cutaneous sensillum of *S. williamsi* with one smooth bristle (BR) divided in two at the distal end. The outline of the cell boundaries can also be seen.

Figure 2-11 Plotted results of the (phylogenetic) flexible discriminant analysis of the habitat with lambda = 0. Group centroids (large symbols) are shown as well as the individual species (smaller symbols labelled with (abbreviated) species names), 95% (dotted lines) and 50% (solid lines) confidence intervals. The most important contributors are shown on the axes of the respective graphs, and the coefficients for these contributors are given here in brackets. Positive contributors for pFDA 1 are: Intergranule size (IGS): 67.27; spinule length (SL): 3.41; the number of bristles per cutaneous sensillum (Br/CS): 1.08. Negative contributors are: Granule size (GS): -11.55, the percentage of the scale area covered by knobs or hillocks (Knobbiness; KN): -3.54. For pFDA 2, positive contributors are: IGS: 85.50, diameter of the pits (PDM): 4.49, KN: 3.88, diameter of the cutaneous sensilla (CSD): 3.78. And negative contributors are: GS: -2.68, Br/CS: -1.55. All other measured traits had coefficients smaller than 0. Abbreviations for species names are: A. rho = *Amalosia rhombifer*; D. con. = *Diplodactylus conspicillatus*; D. plat = *D. platyurus*; D. tes = *D. tessellatus*; L. dam = *Lucasium damaeum*; L. imm = L. immaculatum; L .stein = *L. steindachneri*; L. steno = *L. stenodactylum*; N. laev = *Nephrurus leavissimus*; O. cas = *Oedura castelnaui*; O. cog = *O. coggeri*; O. mon = *O. monilis*; P. amni = *Phyllurus amnicola*; P. nep = *P. nepthys*; R. orm = *Rhynchoedura ormsbyi*; S. cor = *Saltuarius cornutus*; S. kri = *Strophurus krisalys*; S. tae = *S. taeniatus*; S. wil = *S. williamsi.*

Figure 2-12 Plotted results of the (phylogenetic) flexible discriminant analysis of the habitat with lambda = 0. Group centroids (large symbols) are shown as well as the individual species (smaller symbols labelled with (abbreviated) species names), 95% (dotted lines) and 50% (solid lines) confidence intervals. The most important contributors are shown on the axis of the respective graphs, and the coefficients for these contributors are given here in brackets. For pFDA1, the positive contributors are: IGS: 113.98; KN: 6.56; CSD: 2.16; and the negative are: GS: -13.90; Br/CS: -1.11. For pFDA these are: positive: IGS: 40.81; KN: 1.88; Br/CS: 1.30. Coefficients for all other traits were smaller than 0. For abbreviations used see Fig. 11.

Figure 3-1 Photographic sequence of a “growing” drop used to obtain advancing contact-angle (ACA) measurements on the dorsal scales of geckos. **(A)** Initial image of dorsal surface with the exact location where the scales crested (CR). **(B)** Initial, sessile drop placed on the dorsal surface with a contact angle (θ). **(C)** Droplet just previous to that which “popped out” **(D)**, where θ represents the ACA that was obtained by adding 0.25 µl of distilled water to the initial water droplet (b) numerous times. (d) One increment after the ACA measurement (c) in which the droplet had popped out (PD) and, in this case, projected toward the camera

Figure 3-2 Effects plot of the best model (Star model with λ = 0) of the ACA values grouped by microhabitat use. Terrestrial species have significantly higher ACA values than arboreal species, with the saxicoline ones falling in between both

Figure 3-3 Reconstructed ancestral states of hydrophobicity **(a)** and microhabitat use **(b)**. For hydrophobicity, the size of the dots correlates with the ACA measurements for the species of this study (yellow dots) and reconstructed for the nodes (blue dots). For microhabitat use, the dots correspond to the reconstructed probability of microhabitat use for nodes: A (green), arboreal; S (black), saxicoline; T (orange), terrestrial. Note the correspondence between brown nodes (terrestrial species) and large yellow circles (hydrophobic species).

Figure 4-1 Phylogeny and distribution of *Heteronotia binoei* lineages used in this study. **A)** Maximum-likelihood phylogeny of the relevant *H. binoei* lineages inferred from nd2 sequences using RAxML, with bootstrap values shown. See S2 for the full phylogeny. **B)** Distribution of the four relevant lineages in north-east Queensland, colour coded as in A. **C)** An individual of the MI lineage foraging on a granite boulder. **D)** Subdigital view on the right hindfoot (pes) of the terrestrial EA6 lineage. **E)** Subdigital view on the right pes of the saxicoline MI lineage.

Figure 4-2 Microhabitat use of the *Heteronotia binoei* lineages. Bars show the proportion of observations that each lineage was found on the ground (orange), on rock (grey), or on trees (green). The number of observations for each lineage are shown below the respective bars. While CC, MI, Paluma-E and Paluma-W are saxicoline (>75% on rocks), Blencoe is terrestrial (>75% on ground), and the remaining linages are generalist.

Figure 4-3 Terminal scale width plotted against snout-vent-length (SVL) for all 85 measured specimens (small symbols), and the mean values for each lineage (larger symbol), colour-coded by lineage and with symbol shape indicating habitat use. The black line indicates the regression line of the linear model of toe width against SVL. Note that saxicoline lineages (triangles) are mostly above the regression line, while the terrestrial (circles) and generalist (squares) lineages are on or below the regression line.

Figure 4-4 Ancestral state reconstruction of size-corrected terminal subdigital scale width. Trait values (depicted by the colour scale) represent the mean values of the residuals of terminal subdigital scale width regressed against SVL for each of the nine lineages (compare Fig. 3 and supplementary table S3). The habitat use is indicated by shape (EA6 is marked as generalist and terrestrial, see results). The reconstruction shows that the Paluma-W lineage and the clade containing CC, MI, and Paluma-E have independently evolved relatively large terminal subdigital scales. Note that the relatively low relative terminal scale width of MI (relative to the other saxicoline linages), is mainly driven by their relatively large SVL (comp. Fig. 3).

Figure 4-5 Boxplots illustrating variation in microornamentation among the four focal lineages of *Heteronotia binoei*. Terrestrial lineages appear as warm colours (EA6 in yellow, Blencoe in red) and saxicoline lineages appear as cool colours (CC in blue, Paluma-W in purple). **A)** Size-adjusted toepad area of the terminal (FD) and sub-inflection (IN) scales. **B)** Setae length across all scale regions in µm. **C)** Number of branching events of the setae on the FD and IN scales. **D)** Setae density approximated as inverse of setae spacing (measured in µm). The bottom of the figure shows a montage SEM image to illustrate the different toe regions.

Figure 4-6 Microornamentation on the terminal (FD) scales. Terrestrial lineages are displayed on the left (A, C), whereas saxicoline lineages are on the right (B, D). Magnification of each tile is shown in lower left corner with a scale bar in the lower right corner. **A)** Fully developed setae of terrestrial EA6 lineage with an average length of 14 µm (Table S4). **B)** Fully developed setae of saxicoline MI lineage (av. length: 28 µm, Table S4). **C)** Detail of the branched setae tips with spatulae of terrestrial EA6 lineage. White arrows indicate branching points of a single seta **D)** Detail of the branched setae tips with spatulae of saxicoline Paluma-W lineage. White arrows again indicate branching points of a single seta, illustration that setae of the saxicoline lineages branch more often. Note the different magnifications between C and D (and to a lesser extend between A and B).

Figure 5-1 Survey sites across Queensland, Australia. **(A)** Cape York Peninsula, showing the locations surveyed at the Iron Range site. **(B)** Townsville region with the sites Hervey Range, Hidden Valley, Paluma Range, Mt. Elliot and Wambiana. **(C)** Locations surveyed in the area around Winton. **(D)** Locations surveyed around Windorah. Each point refers to an area surveyed. Areas of similar habitat at each site are called 'locations' (Table 1).

Figure 5-2 Perch locations: log (1), tree trunk (2), primary branch (3), secondary branch (4), tertiary branch (5), grass (6), horizontal on rocks (7), on overhanging rock or crevice (8), vertically on rock (9), bush and shrub (10), ground (11).

Figure 5-3 Gecko community perch locations across Queensland, Australia. Arb. = arboreal species, Gen. = generalist species, Sax. = saxicoline (rock-dwelling) species, Ter. = terrestrial species.

Figure 5-4 Saxicolous species' perch orientation on rocks.

Figure 5-6 Perch space (height and diameter) used by Australian geckos, overlayed on polygons indicating the range in mean perch spaces occupied by anole ecomorphs (adapted from Hagey, Harte et al. 2017). Points for geckos are centroid means ± SE.

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Chapter 1 - General Introduction

Although morphology is an old field of biological research, dating back into pre-Darwinian times, it is still a very active research area (e.g. Gray *et al.*, 2019; Zhuang, Russell, & Higham, 2019; Ord *et al.*, 2020; Young & Chadwell, 2020). Modern morphological research combines traditional and modern analytical tools, such as histology, light microscopy, scanning electron microscopy, µCT (e.g. Nyakatura *et al.*, 2019; Rühr & Lambertz, 2019; Russell, Stark, & Higham, 2019), and functional experiments with phylogenetic and ecological research methods to link descriptive and comparative morphology with evolution (evolutionary morphology) and ecology (ecomorphology) (Bock, 1994; Richter & Wirkner, 2014). Evolutionary morphology analyses the evolution of morphological traits, commonly applying character mapping of traits onto a phylogeny (Finarelli & Flynn, 2006; Hwang & Weirauch, 2012; King & Lee, 2015) or identification of correlations between traits and ecological factors, in a phylogenetic context (Kohlsdorf, Garland Jr, & Navas, 2001; Rothier, Brandt, & Kohlsdorf, 2017; Mihalitsis & Bellwood, 2019). Modern statistical analytical approaches are often crucial to disentangle the evolutionary history of morphological traits across the tree of life (Melville, Harmon, & Losos, 2006; Wainwright *et al.*, 2012; Garcia-Porta & Ord, 2013). But over-simplified trait descriptions and overly broad categorisation can cause misleading or false interpretations (Gamble *et al.*, 2017). Therefore, detailed morphological descriptions are still a key element of morphological research, particularly for complex, multi-functional, morphological traits (Russell & Gamble, 2019). For complex morphological traits there are often multiple ways to evolve and be optimised for particular outcomes, which can be conceptualised with many-to-one mapping (Alfaro, Bolnick, & Wainwright, 2005; Wainwright, 2007). Thus, if the function of a complex trait is known, analysing the evolution of the function of a complex trait can be advantageous compared analyse the morphology directly (Garland Jr & Losos, 1994; Tiatragul, Murali, & Stroud, 2017). Conversely, if the function of a morphological structure is unknown, correlations between morphological traits and environmental factors can hint on potential functions (Hagey, Harmon, & Schwarzkopf, 2014a). In my PhD thesis, I will combine descriptive, comparative and functional morphological methods with a variety of phylogenetic statistical analytical approaches to examine the evolution and ecological adaptations of a complex multifunctional morphological structure, the integument, or skin, focussing on skin microstructures of Australian geckos as my model.

THE INTEGUMENT

The Integument is the outermost layer, separating an organism from its environment, and therefore functioning as a protective barrier against a diversity of potentially harmful environmental agencies. These include abiotic factors, such as desiccation, UV radiation, or extreme temperatures, and biotic factors, like diseases or parasites, as well as damage from predators, or competitors (Schliemann, 2015). But the integument also functions as a means for interaction with the environment. Many species use bright colours or exotic skin appendages to attract conspecifics, especially potential mating partners (e.g. Stuart–Fox & Ord, 2004; Prötzel *et al.*, 2018), or species with whom they interact in symbiosis, for example flowers attracting insects (Proctor, Yeo, & Lack, 1996; Marino, Raguso, & Goffinet, 2009; Farré-Armengol *et al.*, 2013). Skin colouration or ornaments may be used to warn potential predators of toxicity (aposematism) or in mimicry of toxic species (Servedio, 2000; Stevens & Ruxton, 2012), or to impress or overcome competitors (Storch, 2015). On the other hand, skin structures or colours can also enhance crypsis to increase survival probability (Spinner *et al.*, 2013b, 2014a; Resetarits & Raxworthy, 2016). The integument normally also features a diversity of sense organs, helping organisms to sense their surroundings and navigate in their environment. Skin sense organs vary from relatively simple mechanoreceptors that can detect touch, heat, or damage, to more complex organs, including lateral-line organs of fish and aquatic Lissamphibia, which detect water currents, or the ampullae of Lorenzini of some aquatic taxa, which detect electric fields (Schliemann, 2015). Additionally, the integument can also be modified to assist in locomotion. Examples include adhesive structures in spiders, insects, frogs and reptiles (Arzt, Gorb, & Spolenak, 2003; Spolenak *et al.*, 2005; Chan & Carlson, 2019), and skin folds for swimming, gliding, or flying (e.g. Ord *et al.*, 2020). The integument is renewed regularly and constantly to repair damage, and to cope with growth, in a process called shedding or ecdysis. Integumental renewal occurs either continuously in small pieces, for example in mammals, or regularly as a whole, as occurs in insects or squamate reptiles (Maderson, 1966; Landmann, 1986; Maderson *et al.*, 1998).

The integument of vertebrates (Vertebrata or Craniota) is highly differentiated and multi-layered, consisting of the outer cutis and the inner subcutis. The former is further divided into epidermis (outermost layer) and the corium (inner layer) (Schliemann, 2015). The detailed morphology of these layers, and of the integument in general, varies substantially among the major radiations of vertebrates, as different taxa had to adapt to different needs, but also evolved different solutions to similar problems. These disparate evolutionary trajectories lead to the development of a huge variety of different specialised structures, and often these were modified to serve additional or alternative functions (Landmann, 1986). For example, odontodes, or dermal teeth, have originally developed in early vertebrates, most likely to toughen the skin for protection, but were modified in the jaw apparatus to capture and manipulate prey items, and independently on the body of sharks and rays (Chondrichtyes), to reduce friction for aquatic locomotion (Oeffner & Lauder, 2012). In addition, both the feathers of birds and the hair of mammals most likely originally evolved for insulation, but has been modified repeatedly, for communication, to from mechanoreceptive sense organs or, for locomotion, that is flight in birds (Maderson & Alibardi, 2000; Clarke, 2013; Schliemann, 2015; Storch, 2015). Scales are another specialised structural component of the integument of vertebrates. Scales evolved independently in bony fish (Osteichthyes) (Schliemann, 2015), some mammals, like pangolins and armadillos (Storch, 2015), and in reptiles (Landmann, 1986; Böhme & Sander, 2015). They evolved as a means for protection from damage, but in terrestrial taxa they also assist in protection from water loss. The latter, among other adaptations, allowed early amniotic taxa to become totally independent from water (Sumida & Martin, 1997).

THE INTEGUMENT OF THE SQUAMATA

Reptiles are the most common terrestrial, scaled animals, but they are a paraphyletic group consisting of crocodiles, turtles, tuataras, and squamate reptiles (Squamata) (Böhme &

Sander, 2015). Of these taxa, squamates probably have the most complex, multi-layered skin. Squamata differ from all other scaled animals, in that they shed the entire outer epidermal layers in regular intervals, either as one piece (e.g. snakes) or in large fragments (e.g. many lizards). These intervals are the shedding phases, which are separated by a resting phase (Maderson, 1966; Landmann, 1979).

There are two types of keratin, which can form scales in reptiles, the relatively rigid and tough ß-keratin and the softer, more flexible, α-keratin. In squamate reptiles, both types of keratin contribute to the formation of the scales, forming distinct layers, which are superimposed on one another (Landmann, 1979). The layer bordering the outside environment is called the oberhäutchen, and is part of the outermost ß-layer, composed of ß-keratin and providing good protection against external damage as well as wear and tear (Maderson, 1966; Landmann, 1979). Below the ß-layer is the mesos-layer, which is composed of α-keratin with a high content of lipids. This layer is the most important for protection against water loss (Landmann, 1979, 1986). Next comes the α-layer, which is the innermost layer during the resting phase, and provides a soft and flexible underlayer for the scales. Shortly before shedding, two additional layers are formed below the α-layer. These are the lacunar and the clear layer, and both are part of the old epidermal generation and are, accordingly, removed during shedding. They help to facilitate the shedding process itself (Maderson, 1966; Landmann, 1979)

MICROORNAMENTATION

The integument of the Squamata is unique, in that the outermost layer is covered with a microscopic relief, formed from extensions and modification of the oberhäutchen (Ruibal, 1968). The structures forming this relief are most commonly referred to as microornamentation or microstructures, but they have also been referred to as ultrastructure (Bryant, Breathnach, & Bellairs, 1967), microdermatoglyphics (Stille, 1987) or other names in older publications (compare Irish, Williams, & Seling, 1988). As microornamentation and microstructures are the most common terms, I will use these two exclusively but interchangeably for this thesis.

The earliest records of microstructures date back to the end of the 19th century (Cartier, 1872; Leydig, 1873; Schmidt, 1912, 1920), but detailed analyses were not possible until scanning electron microscopy provided a tool for advanced analysis (Maderson, 1964; Ruibal & Ernst, 1965; Hiller, 1968, 1972). The earliest works were mostly descriptive (Ruibal, 1968; Stewart & Daniel, 1972, 1973), but soon functional studies followed (Maderson, 1964; Hiller, 1968). Early works suggested a potential role of microornamentation in the sloughing cycle (Maderson, 1966; Irish *et al.*, 1988), but this function could not really explain the high diversity of microstructures among taxa, and among body regions within taxa. This diversity across species lead to the use of microornamentation for taxonomic studies, for example in Iguanidae (Peterson, 1984a), Xenosauridae (Harvey, 1993), Cordylidae, Gerrhosauridae (Harvey & Gutberlet Jr, 1995), or Agamidae (Ananjeva & Matveyeva-Dujsebayeva, 1996). Therefore, functional studies focussed on different functions of distinct microstructures in different taxa. One of the earliest encountered microstructures, (Schmidt, 1912) were the long, hair-like setae on the subdigital scales of geckos, anoles, chameleons, and some skinks (Ernst & Ruibal, 1966; Ruibal, 1968; Williams & Peterson, 1982; Peterson, 1983). Their function is related to the astonishing climbing abilities of these animals (see below) and they have, therefore, gained a major share of the research interest of biologists (Russell, 2002; Russell *et al.*, 2019), but also of engineers for biomimetic research for human application (Bhushan, 2007; Abdel-Aal, 2013; Kim & Varenberg, 2019). Other microstructures fulfil different functions. For example, the ventral scales of snakes facilitate locomotion by providing frictional anisotropy, or low friction in a longitudinal direction (along the movement direction of the animals), but high friction in a perpendicular direction to prevent slipping (Hazel *et al.*, 1999; Berthé *et al.*, 2009; Baum *et al.*, 2014). Further functions of microstructures include dirt shedding, aided by strong hydrophobic abilities of some microstructures in snakes (Gans & Baic, 1977; Spinner *et al.*, 2014a) and geckos (Hiller, 2009; Watson *et al.*, 2015c), or increased crypsis through dispersal of light in snakes (Spinner *et al.*, 2013b) and lacertid lizards (Arnold, 2002). With more and more data on form and function of microornamentation available, recent research projects shifted to analyse the evolution (Arnold, 2002; Riedel *et al.*, 2015; Russell *et al.*, 2015; Higham, Gamble, & Russell, 2017) and ecological adaptation (Höfling & Renous, 2009; Russell & Johnson, 2014; Collins, Russell, & Higham, 2015) of microstructures, mostly focussing on those related to locomotion.

GECKOS AS A MODEL

As geckos are the most extensively studied squamate group, in terms of their microornamentation, they are an obvious choice for further evolutionary studies of microstructures. Geckos (Gekkota) are an ancient lineage of small- to medium-sized, primarily nocturnal lizards, which evolved around 150 myo ago (Gamble *et al.*, 2008a; Daza & Bauer, 2012; Garcia-Porta & Ord, 2013). Phylogenetic reconstructions place them as a sister taxon to the Dibamidae, at the base of the squamate tree (Wiens *et al.*, 2012; Reeder *et al.*, 2015). Geckos are the second most species-rich of the major clades of squamates, comprising more than 1900 species, distributed across seven families (Bauer, 2019; Uetz *et al.*, 2020). They split into two clades, the Diplodactyloidea and the Gekkomorpha. The Gekkomorpha is by far the more species-rich radiation, containing the Families Eublepharidae, Sphaerodactylidae, Phyllodactylidae, and Gekkonidae, with the latter contributing to more than half of the overall gecko species richness (Gamble *et al.*, 2017; Uetz, Freed, & Hošek, 2019). The Diplodactyloidea consist of three Families: Carphodactylidae, Diplodactylidae and Pygopodidae, and the Diplodactylidae are the sister taxon to the other two families (Brennan & Oliver, 2017; Skipwith, Bi, & Oliver, 2019).

Overall, geckos have a world-wide distribution and occur on all continents except Antarctica. They are most species-rich in tropical- and sub-tropical bioregions, but also occur in temperate areas (Meiri, 2020; Uetz *et al.*, 2020). The Gekkomorpha most likely evolved in Asia and their distribution spans from the Americas to south-east Asia, and the Gekkonidae also reach Oceania (Gamble *et al.*, 2008b,a, 2011; Daza, Bauer, & Snively, 2014). The Diplodactyloidea, in contrast, are restricted to Oceania, and the Carphodactylidae occur exclusively in Australia, whereas the Pygopodidae also occur in New Guinea, and the Diplodactylidae reach to New Caledonia and New Zealand (Brennan & Oliver, 2017; Skipwith *et al.*, 2019). With four of the seven families present, and in terms of species richness, Australia is one of the hotspots of gecko biodiversity (Meiri, 2020).

Ecologically, geckos occupy a wide variety of different niches and can be found in nearly all habitat types. Although primarily nocturnal, some gecko lineages have become secondarily diurnal (Gamble *et al.*, 2015; Stark, Schwarz, & Meiri, 2020). They are mostly carnivorous, but some eat fruits and nectar (Meiri, 2020). Geckos are most renowned for their astonishing climbing abilities, but while many species are skilful climbers in arboreal or saxicoline (rock-dwelling) microhabitats (Higham *et al.*, 2019), there are also many terrestrial gecko species, with some highly specialised forms (Russell & Bauer, 1990a; Bauer & Russell, 1991). They also use a wide range of habitats, occurring over diverse humidity and temperature gradients from deserts to rainforests, to cold temperate regions, e.g. in New Zealand or Patagonia (Aguilar & Cruz, 2010; Meiri, 2020). Within the food-web, geckos are normally small- to medium-sized predators of insects, and smaller vertebrates, and are preyed upon by medium-sized vertebrate and large invertebrate predators (Nordberg *et al.*, 2018b; Nordberg, Edwards, & Schwarzkopf, 2018a). Their high diversity and variation in niche use make them an ideal model system in which to study evolution and ecological adaptations. But, although detailed knowledge of their ecology is available for some species (e.g. Nordberg, 2019), for the majority of species, even broad-scale ecological information, for example if they are arboreal, or terrestrial, are often either unavailable or only available from anecdotal observations, rather than detailed ecological studies, which could limit the conclusions of studies of evolutionary adaptations (Meiri, 2018; Vidan *et al.*, 2019).

Morphologically, geckos share many features with other typical lizards, like their sprawling gait or autotomy, which is when tail breakage occurs as a anti predator response (Russell *et al.*, 2014). They are typically small- to medium-sized, including some of the smallest terrestrial vertebrates (Meiri, 2020), but the largest recorded species can reach nearly 40-cm snout-to-vent-length (Bauer and Russell 1986) Apart from the Eublepharidae, geckos have fused eyelids, leading to the typical, much photographed, eye-licking behaviour (Meiri, 2020). But their most well-known morphological characters are their adhesive toepads, facilitating their astonishing climbing abilities (Russell, 1972, 2002), which were noted by Aristotle and have led to a major branch of modern biomimetic research (Chan & Carlson, 2019). But not all geckos have adhesive toepads. While many are primarily pad-less, other lineages have reduced toe pads, for example when they became secondarily terrestrial (Gamble *et al.*, 2012; Higham *et al.*, 2015; Russell & Gamble, 2019). The Pygopodidae have even evolved a snake-like body, with reduced legs (Kluge, 1976; Jennings, Pianka, & Donnellan, 2003; Brennan, 2014). Also, within pad-bearing gecko species, there is high variation in the morphology of the adhesive toepads. This variation can generally be split into two or three distinct types of toepads in geckos (Russell & Bauer, 1990b; Gamble *et al.*, 2012; Russell & Gamble, 2019). There are basal toepads, which typically consist of many relatively small but broad lamellae (broadened subdigital scales) mostly along the central and basal part of the digits. In contrast, terminal (and fan-like) toepads primarily consist of two enlarged pad areas at the distal tip of the digit, flanking the claw. These can either consist of a single scansor each (terminal) or of an arrangement of multiple small lamellae (fan-like) (Russell & Bauer, 1989, 1990b; Russell & Gamble, 2019).

MICROORNAMENTATION IN GECKOS

Geckos are one of the main groups in which microornamentation was studied early on, mainly because of the general interest in the ability of many geckos to climb smooth, vertical, or overhanging surfaces. Geckos adhere to surfaces with their adhesive toepads, a complex organ consisting of laterally expanded scales (lamellae and scansors), which are controlled by internal muscles and soft tissue, and in, some species, specialized bones (paraphalanges) (Russell, 1975, 1986; Russell & Bauer, 1988; Russell *et al.*, 2019). But the most integral part of the adhesive toepads is their microornamentation (Russell, 2002; Pianka & Sweet, 2005). Lamellae and scansors are covered with microfibrillar stalks called setae, which are typically branched multiple times terminating in multiple triangular tips, called spatulae. This arrangement leads to enhanced contact area at the nano-scale between the toepad and the naturally irregular surface of the substrate, generating Van-der-Waals forces (adhesion) in addition to shear forces (friction) (Autumn & Peattie, 2002; Huber *et al.*, 2005b; Autumn, 2006, 2007). With the function of adhesive toepads now well understood, research interest recently shifted to the evolution and ecological adaptations of adhesive toepads (Collins *et al.*, 2015; Hagey *et al.*, 2017b; Bauer, 2019; Zhuang *et al.*, 2019).

Setae have most likely evolved from shorter, unbranched, hair-like microstructures called spinules or spines, which cover the remaining (non-toe pad) body regions of geckos (Ruibal & Ernst, 1965). Spinules have highly hydrophobic properties because they help ensure low contact area between the scale surface and surrounding liquids (Hiller, 2009; Spinner, Gorb, & Westhoff, 2013a), and hydrophobicity leads to self-cleaning abilities (Watson *et al.*, 2015c,a). Additionally, recent studies revealed that spinules are also bactericidal, piercing, stretching or squashing bacteria on the geckos' surface (Watson *et al.*, 2015b; Li *et al.*, 2016).

In terms of sensory organs, gecko skin is equipped with cutaneous sensilla. These are small, disc-like structures, which typically have one or more small hair-like extensions (bristles) and are mechanoreceptive (Hiller, 1971). On the feet of geckos, they may assist in the placement of the adhesive toepads (Hiller, 1968; Lauff, Russell, & Bauer, 1993), while on the tail they supposedly facilitate tail autotomy, as well as controlling the movement of the tail after breaking (Russell *et al.*, 2014). But the high variation in sensilla morphology among species and body regions have led other authors to propose additional functions, for example the detection of temperature, humidity, or wind movement (Ananjeva, Dilmuchamedov, & Matveyeva-Dujsebayeva, 1991; Matveyeva-Dujsebayeva & Ananjeva, 1995).

THESIS CHAPTER OUTLINE

My thesis aims to analyse the evolution and ecological adaptations of microornamentation in Australian geckos. Because gecko microornamentation covers a range of different structures, each with different knowledge gaps, different questions must be asked to address different microstructures, and aspects of their evolution and potential adaptations. My thesis is, therefore, structured as a series of stand-alone publications that target different microstructures and different aspects of their function, evolution and ecological adaptations.

In Chapter 2, I used scanning electron microscopy to describe the microornamentation on the dorsal midbody of 27 diplodactylid and carphodactylid gecko species (spinules and cutaneous sensilla). I use these descriptions, and measurements of scale size and different microstructural features to examine associations between microornamentation and scale dimensions, and different aspects of their ecology. I focussed particularly on habitat use, specifically whether they were arboreal, saxicoline or terrestrial, and habitat humidity, specifically whether it was xeric, mesic or hydric, representing desert, savannah and rainforest habitats respectively.

Based on these results I explored some of the proposed functional implications of these associations (Chapter 3). I used measurements of hydrophobicity (advancing contact angles) to analyse the correlations between hydrophobicity and the morphological measurements (spinule dimensions and scale size) obtained in the research conducted for Chapter 2. I also examined the relationships between hydrophobicity and the use of the three habitat categories, arboreal, saxicoline or terrestrial, and whether they occurred in xeric, mesic or hydric habitats. This analysis included an ancestral state reconstruction of hydrophobicity and the ecological categories, to determine if hydrophobicity co-evolved with habitat use or habitat humidity.

In chapter 4, I shifted my focus to adhesive toepads. I examined the subdigital scale morphology and microornamentation of genetically distinct lineages of the Bynoe's gecko (*Heteronotia binoei*) species complex. These lineages included two saxicoline populations with enlarged terminal subdigital scales, and their respective terrestrial sister populations, which did not have enlarged terminal subdigital scales. This analysis included a reconstruction of ancestral terminal subdigital scale sizes and an analysis of differences in subdigital microornamentation among lineages.

In chapter 5, I pooled my own ecological field observation with published data to examine the habitat use and ecological niche categories of a set of 34 Australian gecko species, because detailed published information about the ecology of most species is often primarily available from field guides, or descriptions in the taxonomic literature. If at least 75% of all observations fell into one microhabitat, I categorised species as arboreal (9), saxicoline (4), or terrestrial (13). Otherwise they were classified as generalists (8). Furthermore, I described average perch height and perch diameter for the arboreal species, and assigned them to ecomorph categories originally developed for *Anolis* lizards.

I concluded my thesis with a final chapter providing a general discussion and synthesis, including suggestions for future research.

STUDENT PROJECTS

During my PhD I also co-supervised undergraduate student projects. Two of the students I co-supervised have published their projects in scientific journals, and these two publications form the appendix of my thesis.

In appendix I, Ajano Fushida analysed shedding behaviour of four gecko species in response to fouling for her science research internship supervised by me, Eric Nordberg and Lin Schwarzkopf. We found that one species, *Hemidactylus frenatus*, could increase their shedding rate in response to external fouling, while the other three species, *L. steindachneri*, *S. williamsi* and *Oedura castelnaui* could not.

In appendix II, Rishab Pillai tested the variation in clinging performance of two gecko species (*Oedura coggeri* and *Pseudothecadactylus australis*) on substrates with different roughness (glass, fine-grained sandpaper and coarse-grained sandpaper) for his master's minor project, supervised by me, Eric Nordberg and Lin Schwarzkopf. We found that in both species, clinging performance did not decline gradually with increasing roughness, as was suggested by some authors, but instead was lowest on fine-grained sandpaper, with no significant difference in clinging performance to coarse-grained sandpaper or glass. We emphasised the need to examine the performance of gecko toepads, not only on artificially smooth substrates, but also on substrates with similar roughness to that they encounter in nature, to understand not only the physical principles but also ecological and evolutionary aspects of adhesive toepads of geckos.

Chapter 2 - Ecological associations among microstructure and scale characteristics of Australian geckos (Squamata: Carphodactylidae and Diplodactylidae)

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Abstract

A first step in examining factors influencing trait evolution is demonstrating associations between traits and environmental factors. Scale microstructure is a well-studied feature of squamate reptiles (Squamata), including geckos, but few studies examine ecology of microstructures, and those focus mainly on toe pads. In this study, the ecomorphology of cutaneous microstructures on the dorsum was described for 8 Australian species of carphodactylid (Squamata: Carphodactylidae) and 19 diplodactylid (Squamata: Diplodactylidae) geckos. I examined scale dimensions, spinule and cutaneous sensilla (CS) morphology, using scanning electron microscopy, and described associations of these traits with microhabitat selection (arboreal, saxicoline or terrestrial) and relative humidity of each species' habitat (xeric, mesic, or humid). I used a phylogenetic flexible discriminant analysis (pFDA) to describe relationships among all traits, and then a modelling approach to examine each trait individually. My analysis showed that terrestrial species tended to have long spinules, and CS with more bristles, saxicoline species larger diameter CS, and arboreal species tended to have large granule scales and small intergranule scales. There was high overlap in cutaneous microstructural morphology among species from xeric and mesic environments, whereas species from humid environments had large diameter CSs, and few bristles. Significant associations between epidermal morphology and environmental humidity and habitat suggest that epidermal microstructures have evolved in response to environmental variables. In summary, long spinules, which aid self-cleaning in terrestrial geckos, are consistent with greater exposure to dirt and debris in this habitat. Long spinules were not clearly correlated to environmental humidity. Finally, more complex CS (larger diameter with more bristles) may facilitate better perception of environmental variation in geckos living in drier habitats.

Key words: Oberhäutchen, scanning electron microscopy, microornamentation, ecomorphology, cutaneous sensilla

Introduction

Skin, comprising the epidermis (outer layer) and the dermis (inner layer), provides vertebrates with direct protection from desiccation, radiation, moisture, irritants, and infectious agents (Schliemann, 2015). The Oberhäutchen (the outermost layer of epidermis) of squamate reptiles expresses external relief in the form of microscopic structures collectively known as microornamentation or microstructures (Ruibal & Ernst, 1965; Ruibal, 1968; Gans & Baic, 1977; Jackson & Sharawy, 1980; Bauer & Russell, 1988; Arnold & Poinar, 2008). Microstructures have been studied for over a century (Cartier, 1872; Schmidt, 1912; Maderson, 1964), with most contributions focusing on either the taxonomic utility of these structures (Stille, 1987; Harvey, 1993; Ananjeva & Matveyeva-Dujsebayeva, 1996; Harvey & Gutberlet Jr, 2000; Bucklitsch, Böhme, & Koch, 2016), or their possible function (Hiller, 1968; Hazel *et al.*, 1999; Russell, 2002; Autumn, 2006; Berthé *et al.*, 2009; Russell *et al.*, 2014). Some studies have demonstrated that microstructures vary adaptively with specific life-histories, for example, subdigital (toepad) microstructures are associated with habitat use in some geckos and iguanids (Höfling & Renous, 2009; Collins *et al.*, 2015). There have, however, been few studies on the relationship between the ecology of geckos in relation to microstructures that are not associated with locomotion. Such studies are necessary because they can tentatively demonstrate the functions of cutaneous morphological traits (Hagey *et al.*, 2014a).

Geckos are an ancient (>100 myo) taxon of primarily nocturnal lizards, exhibiting great diversity and a worldwide distribution (Gamble *et al.*, 2008a; Garcia-Porta & Ord, 2013), occupying a wide variety of habitats and environmental conditions (Böhme & Sander, 2015; Wilson & Swan, 2017). Their wide habitat use makes them excellent candidates for studying epidermal microstructural features, and the associations of these features with habitats and climatic zones.

The external surface of the skin of geckos exhibits small, hair-like microstructures called spinules or spines (Ruibal, 1968), which vary in length (from 0.3 to 3.0 µm) among species (Stewart & Daniel, 1975; Rosenberg, Russell, & Cavey, 1992; Bauer, 1998; Peattie, 2009; Spinner *et al.*, 2013a). Recent studies have demonstrated that high spinule length and density are correlated with hydrophobicity (Vucko, 2008; Hiller, 2009), and produce self-cleaning and antibacterial epidermal characteristics (Watson *et al.*, 2015a,c, 2016). Bactericidal properties occur because large bacteria are pierced by the spinules, while small bacteria are damaged by compression, stretching or tearing between the spinules (Li *et al.*, 2016). Spinule length, or density, or both, may therefore increase if geckos live in an environment exposed to dirt and debris, or with a higher load of harmful microorganisms. For example, I would expect terrestrial species to have longer or more densely packed spinules (or both) than arboreal or saxicoline (rock-dwelling) species, because the accumulation of dirt and debris is more prevalent on the ground compared to higher strata (Ungar *et al.*, 1995), and terrestrial species are also more exposed to microorganisms than are arboreal or saxicoline species (Nunn, Gittleman, & Antonovics, 2000; McCabe, Reader, & Nunn, 2015). Conversely, species from humid areas, such as rainforests, may have long or dense spinules, or both, because bacterial and fungal growth rates may be greater in rainforests, leading to an increased prevalence of these microorganisms (Bouskill *et al.*, 2012). If microstructures are indeed adaptive and are linked to species' habitats, I would expect to see consistent variation in spinule morphology among habitat types.

Gecko skin also features mechanoreceptors, called cutaneous sensilla (Hiller, 1976; Bauer & Russell, 1988). For example, the distribution and form of cutaneous sensilla on the dorsal surface of the feet of the Tokay gecko (*Gekko gecko*) aid in the correct placement of the toes to maximize adhesive toe pad function (Lauff *et al.*, 1993). Also, the density and distribution of cutaneous sensilla on the tail of the leopard gecko (*Eublepharis macularius*) suggests that they mediate the location of tail breakage, and the movement of the tail after breaking (Russell *et al.*, 2014). Other authors have argued, however, that mechanoreception cannot be the sole functional explanation for the huge variation in the distribution and morphology of cutaneous sensilla in different taxa, and suggest additional functions, such as the perception of humidity or temperature (Ananjeva *et al.*, 1991; Matveyeva-Dujsebayeva & Ananjeva, 1995). Therefore, although the functional significance of sensilla is still unclear, it is likely that they fulfill different functions on different parts of the body. If cutaneous sensilla do play a role in perception of temperature or humidity, their morphology or distribution should vary among species exposed to different environmental conditions. These differences should be most obvious on body regions weakly influenced by other mechanoreceptive functions like toe placement, tail breakage or prey consumption. For example, terrestrial species in arid environments may depend upon detailed perception of disturbances on their body surface, as they are more likely to be susceptible to desiccation by wind (Tingley & Shine, 2011). If cutaneous sensilla do play a role in detection of humidity or temperature, terrestrial species from arid biomes may have more, or more complex (i.e., larger, with more bristles, or bristles with more complex structure), cutaneous sensilla, or both. On the other hand, microhabitat use (e.g., burrow or crevice use) may confound simple correlations between habitat temperature and especially humidity, reducing correlations among skin features and microhabitats (Aguilar & Cruz, 2010).

In this study, I examine a range of Australian carphodactylid (Squamata: Carphodactylidae) and diplodactylid geckos (Squamata: Diplodactylidae), using scanning electron microscopy to describe the microstructure of the Oberhäutchen. Then, accounting for phylogeny, I test the associations between scale characteristics and habitat and between scale characteristics and the humidity of the environment, to determine whether (1) terrestrial species have longer or more densely arranged spinules than arboreal or saxicoline species; (2) species from humid habitats have longer or more densely arranged spinules than species from more arid habitats; and (3) terrestrial species from drier habitats have more or more complex cutaneous sensilla than arboreal species, or species from more humid habitats.

Materials and methods

STUDY SPECIES

The two closely related families Carphodactylidae and Diplodactylidae originate from Australia and occupy nearly all habitats and ecosystems. Carphodactylines tend to be larger, and include more species occupying humid habitats. Geckos from Queensland, Australia were captured at night by hand, while those from South Australia were captured in 20 L pitfall traps. Only healthy, adult specimens were returned to the laboratory, and dental imprint moulds were taken from the skin four to eight days after each individual had shed, as described by Vucko et al. (2008). Since it was not possible to wait for shedding in *Diplodactylus wiru*, *Nephrurus* *laevissimus*, and *N. levis*, moulds were obtained at an unknown stage of the resting phase of their shedding cycle, but were still considered usable, as individual scales in good condition could be located and measured. In total, 27 species representing 10 genera of the Carphodactylidae and Diplodactylidae were examined (Table 2.1). Species were selected to include a broad range of habitats and humidity regimes.

DESCRIPTIVE TERMINOLOGY

The skin of Australian Carphodactylidae and Diplodactylidae is covered by up to three types of scales, termed tubercles (enlarged, conical scales), granules (medium-sized scales), and intergranules (smaller scales surrounding the medium-sized scales; Figures 2.1A & C) in accordance with Vanderduys (2016).

Descriptive terminology for microstructures follows that of Ruibal (1968), Peterson and Williams (1981), Peterson (1984a), Irish et al. (1988), Bauer and Russell (1988), Lang (1989), Harvey (1993), Harvey and Gutberlet (1995), and Arnold (2002), and includes some new terms. Hair-like microstructures (spinules) are surrounded by connecting radial lines (struts) with small indentations (pits) in between the spinules and the struts (Fig 2.1A). The long hair- or brush-like structures arising from the cutaneous sensilla are termed bristles (Fig. 2.1B). In some species, the bristles themselves are covered with microscopic projections called setules (Fig. 2.1C). Uprisings of the scale surface are generally termed knobs if they are devoid of microstructures, or hillocks if they are covered by spinules (Fig. 2.1D). As these two structures appear otherwise similar, and since knobs arise out of hillocks in some species, I have combined them for statistical analyses.

The categories for substrate use were terrestrial, saxicoline and arboreal, while categories for environmental relative humidity were xeric, mesic, and hydric. Lizards from xeric conditions were collected from deserts in central Australia, lizards from mesic conditions were collected from open savannah woodland in northern Australia, and lizards from hydric conditions were collected from the rainforests of the Australian wet tropics. Geckos were assigned to habitat-use and relative humidity groups using published data (Wilson & Swan, 2017; Cogger, 2018) and observations of habitat-use recorded during collection (Table 2.1).

EPIDERMAL MICROSTRUCTURES

Dorsal epidermal scale moulds were taken from the mid-dorsal region halfway between the front and the hind limbs, because microstructures (especially cutaneous sensilla) in this area may be less likely to perform specialized functions such as detecting body movement or posture, tail breakage, or prey capture (Lauff *et al.*, 1993; Russell *et al.*, 2014). Detailed epoxy-resin moulds of live specimens were made, according to methods described by Vucko et al. (2008). Images of microstructures of each species were taken from the epoxy-resin moulds with a JEOL JSM-5410LV (JEOL, Tokyo, Japan) scanning electron microscope (SEM) at magnifications between x15 and x10,000 and analysed using ImageJ (V.1.36b (Schneider, Rasband, & Eliceiri, 2012)). In all SEM images reproduced here, unless otherwise stated, the anterior of the animal is towards the top of the image. ImageJ was also used to measure all scale characteristics and microstructures observed. Measurements included the area of granules and intergranules (mm^2 [n = 50]; Table 2.2), the length of spinules (μm [n = 50]; Table 2.3), the density of spinules per 10 μm^2 (n = 2.3; Table 2.3), the diameter of pits (μm [n = 40]; Table 2.3), the density of pits per 5 μm^2 (n = 3; Table 2.3), the number of cutaneous sensilla per scale (n = 10) and per mm^2 (Table 4), the number of bristles per sensor (n = 10) and per mm^2 (Table 2.4), the diameter of cutaneous sensilla (µm [n = 10]; Table 2.4), and the percentage of each granule covered by knobs or hillocks, including partial and fully formed knobs and thick spinule-like structures (n = 10), with n representing the number of features measured per individual (Table 2.3). Cutaneous sensilla and number of bristles per mm^2 on each gecko were calculated from the same images used for counts per scale.

STATISTICAL ANALYSES

Supertree construction

A Matrix Representation with Parsimony (MRP) supertree was constructed using the following data and methods: Trees were derived from the literature (Donnellan, Hutchinson, & Saint, 1999; Hoskin, Couper, & Schneider, 2003; Han, Zhou, & Bauer, 2004; Melville *et al.*, 2004; Oliver, Hutchinson, & Cooper, 2007; Oliver *et al.*, 2012; Oliver & Bauer, 2011) and an unpublished tree by P. Oliver. Only maximum-likelihood trees from these sources were used, as these trees tended to be more resolved. Trees were encoded using both Baum-Ragan (Baum, 1992; Ragan, 1992) and Purvis (1995) coding, using the program SuperTree version 0.85b (Salamin, Hodkinson, & Savolainen, 2002). For each coding, tree characters were considered as both ordered and unordered, producing four tree matrices. The unordered character matrices were analysed using Wagner parsimony (Eck & Dayhoff, 1966; Kluge & Farris, 1969) and the ordered matrices were analysed using Camin-Sokal parsimony (Camin & Sokal, 1965). To build the trees, species were added to the tree in random order (10,000 times), and a heuristic search algorithm with local and global rearrangements was used to find the most parsimonious trees. The best 100 trees were saved in each analysis, and an extended majority-rule consensus tree was constructed from these trees for each matrix. The consensus trees were then pruned to include only taxa for which there were morphological data. The relationships of the *Diplodactylus conspicillatus* species complex were assigned using the species in (Oliver, Couper, & Pepper, 2014), and their relationships within the species complex were assumed to follow from their biogeographic ranges and locations (species with closer distributions were considered closer relatives). Of these four matrices I used the Camin-Sokal tree for subsequent analyses, as it was in accordance with the published literature (see results). Phylogenetic analyses were performed using the MIX and CONSENSE programs in the PHYLIP suite V. 3.696 (Felsenstein, 2018).

The supertree approach used here does not produce branch lengths, which are necessary for phylogenetic comparative analyses. Thus, three types of arbitrary branch lengths were used: those of Grafen (1989), Pagel (1992) and Nee (cited in Pagel, 1992). For each of the three branch length types, a tree with the same topology as above was constructed, and the performance of each branch length type was assessed by calculating phylogenetically independent contrasts (PICs) for each (log transformed) trait and set of branch lengths, and then calculating the Pearson correlation coefficient between the PICs and their standard error, following Garland et al. (1992). For each trait, the branch length type that produced the lowest (absolute value) correlation was chosen. Since the regression analysis of PICs is equivalent to a Phylogenetic Generalised Least Squares (PGLS) regression assuming a Brownian motion model of evolution (Blomberg *et al.*, 2012), the correct standardisation of PICs by an appropriate set of branch lengths means that set of branch lengths is appropriate for analyses using PGLS. Grafen's branch lengths were computed using the 'compute.brlen' function in the *ape* package for R (Paradis, Schliep, & Schwartz, 2018; R Core Team, 2018). Pagel's and Nee's branch lengths were calculated using the PDAP:PDTREE package for Mesquite (Midford, Garland Jr, & Maddison, 2005; Maddison & Maddison, 2018).

Multivariate analyses

A Discriminant Function Analysis (DFA) was done following Motani and Schmitz (2011). This method extends the Flexible Discriminant Analysis (FDA) methods of Hastie et al. (1994) to include a PGLS step with a Pagel's lambda model to flexibly allow for potential phylogenetic effects in both the trait variables and the classification variables (habitat use or relative humidity). The optimal value of lambda (minimizing the residual sum of squares, RSS) was quantified using the 'optim' function in R and the plot of lambda versus RSS was used to determine whether the relationship between these two variables was unimodal or monotonic. Confusion matrices were extracted from each analysis, and I computed the classification performance measures of Garczarek (2002) using the 'ucpm' function in the *klaR* package for R (Weihs *et al.*, 2005). Canonical function plots were generated, and group centroids with 50% and 95% confidence ellipses based on the standard error were calculated. The coefficients of the FDA function were used to determine the characteristics that loaded most heavily on the two discriminant functions (pFDA1 and p FDA2). The plots of the pFDA analysis where generated using the R package *ggplot2* (Wickham, 2009).

Univariate analyses of individual traits

All the trait variables were log-transformed, except for presence/absence of lenticular sense organs, which was treated as a binary variable. All continuous traits were analysed as response variables using PGLS, fitting the two explanatory variables (habitat use and relative humidity) in separate models as the dataset was too small to include both explanatory variables in the same model. Because of the tight correlation between SVL and log Body Mass (see Fig. S2.1), these two explanatory variables were included and removed together in the models, thus avoiding the need for variable selection between them (Harrell, 2015). I considered these two variables together to represent body size. Four models for each explanatory variable were fitted: (1) the "full" model with explanatory variables interacting with body size; (2) an "additive" model which included body size, but not interacting with the other explanatory variable; (3) a model with only the explanatory variable, and (4) a model with no explanatory variables (other than an intercept). In addition, I fitted evolutionary models assuming Brownian Motion, Independence, and Pagel's lambda model for each combination of explanatory variables, resulting in 12 models fitted per response variable. Branch lengths for each response variable were chosen based on the results of the analysis described above. Model adequacy was assessed by examining normal quantile-quantile plots of the (normalised) residuals, and by examining a plot of the residuals versus the fitted values for each model.

For each response variable an information-theoretic model averaging approach was used, based on the Akaike Information Criterion corrected for small samples (AICc)(Burnham & Anderson, 2004). All models were fitted using Maximum Likelihood using the 'gls' function in the *nlme* package for R (Pinheiro *et al.*, 2018; R Core Team, 2018), with the exception of the presence/absence of lenticular organs, which was analysed with a Bayesian logistic regression using the *MCMCglmm* package for R (Hadfield, 2010; Hadfield & Nakagawa, 2010). Regression parameter estimates were averaged according to their AICc weights and Wald tests were used to assess statistical significance of the fully averaged parameter estimates. Model averaging was performed using the *MuMIn* package for R (Bartoń, 2018). Because of the large number of response variables examined (13) and the large number of models for each variable (12), p-values for multiple testing were adjusted by controlling the False Discovery Rate using the method of Benjamini and Hochberg (1995).

Results

MORPHOLOGY

All species observed in this study had several characteristic skin features in common:

1. Dark lines were visible on all species, forming a web (see Fig. 2.4D). These represent either the cell borders of the Oberhäutchen cells, or the impression of the cell borders on the clear layer (the bottom-most layer of the old epidermal generation), or both (Irish *et al.*, 1988).
2. All species have granules and smaller intergranules surrounding these (Fig. 2.2A, 2.3A, 2.7A). All the Carphodactylidae and some species of the genus *Strophurus* also have larger tubercles (Fig. 2.2C, 2.5B). The tubercles are scattered across the scale surface in all tubercled species, if not stated otherwise. All granules, intergranules, and tubercles lack imbrication (i.e., overlap of scales) found in many other squamate species (Burstein, Larsen, & Smith, 1974; Peterson, 1984a; Lang, 1989; Alibardi & Toni, 2006).
3. The intergranules are always triangular but vary in size and exact shape.
4. Scales contain spinules, surrounded by radial struts and pits, which were uniform except at the very edges of the scale (Fig. 2.1A). Therefore, one average measurement was calculated for each species, taken from the centre of the scales (Table 2.3).
5. Tactile sense organs vary among species where (A) cutaneous sensilla occur in all species, but differ among species in distribution, number, and type; and (B) lenticular sense organs (LSO) are present in one carphodactylid and several diplodactylid species and are reported here for the first time in geckos. All tactile sense organs are present on scales and tubercles, but never on granules.
6. Detailed accounts of the microstructures are described for each genus separately, following the phylogenetic order given in Fig. S2.2. For measurements of microstructures see tables 2.3 & 2.4.

Carphodactylidae

Carphodactylidae generally have granules, intergranules, and tubercles. Knobs or hillocks, or both, appear on all granules and tubercles, and can form long keels on the tubercles. The area of all scales is evenly covered with spinules, except for the areas occupied by knobs. Cutaneous sensilla appear on all granules and tubercles.

Saltuarius cornutus features knobs, which are relatively long and slender and are often inclined towards the centre of the granule (Figs. 2.2A and B). There are few cutaneous sensilla per scale scattered across the granules (Fig. 2.2B), but over twenty on the tubercles (Fig. 2.2C). Each sensillum has one or two short and broad bristles (Fig. 2.2D).

In the genus *Phyllurus*, spinules are relatively short and dense (Fig. 2.3F), with the notable exception of *P. ossa* (Table 2.3). Knobs are scattered across the surface of the granules, and are broad and stout in *P. amnicola* (Fig. 2.3B) but resemble those in *S. cornutus* in the examined congeners (Fig. 2.3E). In the centre of the granules, there are short, stout, spinule-like structures (Figs. 2.3B and E), which gradually coalesce to form the knobs in *P. nepthys* and *P. ossa* (Fig. 2.3D).

Tubercles bear several cutaneous sensilla that are just below the peak, and multiple keels that begin below the peak and run down the sides of the tubercle in a radial fashion (Fig. 2.3A). Thicker spinule-like structures fill the areas between the keels near the peak of the tubercle and occur on the keels themselves towards the bottom of the tubercle. The base of each tubercle has several knobs resembling keels that break into smaller sections. In *P. amnicola*, the lower half of the tubercle bears lenticular sense organs (Fig. 2.3A), whereas hillocks are found at the same region in *P. nepthys and P. ossa* (see Fig. 2A in Vucko et al. (2008)).

In *P. amnicola*, multiple cutaneous sensilla are located around the edges of each granule (Fig. 2.3E), whereas *P. nepthys* and *P. ossa* have fewer sensilla scattered across the granules (Figs. 2.3B). Each sensillum has one bristle, which is thick, short and lightly covered by setules in *P. nepthys* (Fig. 2.3C), but notably longer and narrower in *P. amnicola* and *P. ossa* (Fig. 2.3F).

Carphodactylus laevis has long spinules, and the granules and intergranules are less distinct from each other than in other species in this study (Fig. 2.4A). Granules have hillocks, covered with spinules that are slightly longer and denser than on the remaining scale surface (Fig. 2.4B & D). Between the hillocks there are multiple cutaneous sensilla per scale, each bearing many lateral setules, giving them the appearance of a bottle brush (Fig. 2.4C). Tubercular scales occur in only two rows along the dorsal midline of this species. They have smooth, rounded peaks whereas their lower reaches are covered with hillocks and carry cutaneous sensilla similar to those on the granules.

All species of the genus *Nephrurus* examined have relatively short spinules varying in density among species. Broad and stout knobs are scattered across granules and tubercles, arising from surrounding hillocks and thus forming their tips (Figs. 2.1D and 2.5C). Tubercles also have a keel running along their anterior end, and bear several cutaneous sensilla at their peak, arranged in a V-shape around the keel, with the apex directed posteriorly (Fig. 2.5B). Cutaneous sensilla are positioned either centrally, or towards the posterior end of the granules of *N. asper* and *N. levis* (Fig. 2.5A), whereas they are always located centrally in *N. laevissimus* (Fig. 2.5C). While the number of bristles per sensillum is comparable in all three species (Table 2.3), their shape is distinctively different in *N. asper* compared to the other species. In *N. asper* the shafts of the bristles have short thick setules at their broadened end, and appear slightly barbed, resembling a mace (Fig. 2.5D). In contrast, the cutaneous sensilla of *N. laevissimus* and *N. levis* have bottle brush-shaped bristles, bearing long and thin setules from base to tip (Fig. 2.1C).

Diplodactylidae

The Diplodactylidae have granules and intergranules but lack tubercles (except for some *Strophurus*). Scales are evenly covered with spinules, unless otherwise noted, and always lack knobs or hillocks. Cutaneous sensilla are located at the posterior ends of the granules and are almost exclusively single-bristled. In contrast, lenticular sense organs, when present, are located on the anterior margins in all species except *Oedura*, where they are scattered all over the scales.

Oedura have relatively large granules with relatively long spinules (Tables 2.1 and 2.2). Cutaneous sensilla bear one smooth bristle, which is split at its distal end and is surrounded by longer spinules (Fig. 2.6B). While there are only a few sensilla per scale in *O. coggeri, O. bella* and *O. cincta; O. castelnaui* and *O. monilis* have distinctly more cutaneous sensilla per scale (Table 2.3).

Apart from *O. bella*, all species have lenticular sense organs (LSO) scattered across the scale surface (Figs. 2.6A and C), some of which appear distinctively crater-like (Fig. 2.6D). These crater-like LSOs are more numerous towards the centre of scales.

Amalosia rhombifer has granules with one cutaneous sensillum at their posterior end. The sensilla are without bristles but are covered entirely with elongated spinules (Fig. 2.7B). Lenticular sense organs are rare in this species (Fig 2.7A).

In *Lucasium*, all species examined have relatively long spinules (Fig. 2.8B). The number of cutaneous sensilla per scale is generally small in this genus (Table 2.3). The bristles taper and are smooth in *L. damaeum* and *L. stenodactylum* (Fig. 2.8E) but are heavily covered by setules at the distal end in *L. immaculatum* and *L. steindachneri* (Figs. 2.8C). In the first two species, the cutaneous sensilla are surrounded by longer spinules.

Lenticular sense organs are absent in *L. immaculatum*, but occur around the anterior edges, and occasionally towards the centre, of scales of *L. damaeum* and *L. stenodactylum* (Figs. 2.8A and D). In *L. steindachneri*, they occur around the edges of the scale, including posterior to the cutaneous sensilla (Fig. 2.8F).

In *Rhynchoedura ormsbyi* the granules along the dorsal midline are elongated and oval (Fig. 2.7D) but are hexagonal on the rest of the dorsal body surface (Fig. 2.7C). All scales have long spinules (Fig. 2.1A) and each granule has a cutaneous sensillum at its posterior end, with a smooth and tapered bristle. No lenticular sense organs occurred in this species.

All species of the genus *Diplodactylus* had up to seven rows of scales running along the dorsal midline that were larger than the scales on the remainder of the dorsal surface. The scales of all species were covered evenly with spinules, which is the only type of microstructure present in *D. wiru* (Fig. 2.9A). The granules of *D. tessellatus* exhibit a centrally located bare area (Fig. 2.9B), whereas the granules of the *D. conspicillatus* group (*D. ameyi, D. conspicillatus* and *D. platyurus*) have honeycomb structures covering the entire scale surface, with spinules within the walls of the honeycombs. These walls are consistently narrow in *D. conspicillatus* (Figs. 2.9D), whereas they are broadened at the junction points, but vanish between junctions in *D. ameyi* and *D. platyurus*. Thus, they form triangles (Fig. 2.9C).

Cutaneous sensilla occur at the posterior end of the granules. The spinules on the cutaneous sensilla are longer than the surrounding spinules (Fig. 2.9E & F). In *D. conspicillatus* and *D. platyurus*, the bristle of the cutaneous sensilla is covered with setules and has a tapered distal end (Fig. 2.9E). In contrast, the cutaneous sensilla of *D. wiru* and *D. tessellatus* have bristles that are quite smooth (without setules) but are still somewhat tapered at their distal end (Fig. 2.9F).

Lenticular sense organs occur around the edges of the granules (Fig. 2.9A). In *Diplodactylus conspicillatus* and *D. platyurus* these usually lie anterior to the cutaneous sensilla and are situated towards the anterior end of the scales, whereas in *D. wiru* they are numerous and found around the entire edge of the scale. In these species they have a concave, slightly inverted morphology (Fig. 2.9G). In *D. tessellatus*, they are uncommon and occur around the edge of the scales towards the anterior end, displaying a disc-like elevation (Fig. 2.9H).

The three species of the genus *Strophurus* have short spinules and no lenticular sense organs. *S. krisalys* and *S. williamsi* have bare areas without any microstructure at the centre of the granules (Fig. 2.10A), but there are no bare areas in *S. taeniatus* (Fig. 2.10B).The cutaneous sensilla of *S. krisalys* and *S. taeniatus* have no bristle, but only elongated spinules (Fig. 2.10C), while those of *S. williamsi* have one smooth bristle that is split at its distal end (Fig. 2.10D).

STATISTICAL ANALYSES

Supertree

Of the analyses of the four matrices, the two coding schemes produced identical results for the Camin-Sokal analyses, and known congeneric and familial relationships were preserved, but the Wagner trees did not always place known congeners together, and did not reproduce monophyly for the two families (Carphodactylidae and Diplodactylidae). Hence, I used the Camin-Sokal tree for subsequent analyses (Fig. S2.2).

Grafen's branch lengths provided the best standardizations for the PIC's for most traits, except for ecological traits (habitat use and humidity of the environment), size (mass and SVL) and cutaneous sensilla diameter, granule size and the number of sensilla per scale. For these traits Pagel's branch lengths provided a better result (Table 2.5).

Discriminant Function Analysis

For both habitat and relative humidity of the environment, the optimal value of lambda was close to zero, and the relationship between lambda and RSS was monotonically increasing (Fig. S2.3). Hence, I used a lambda value of zero, which reduces the Motani and Schmitz method to the ordinary FDA method of Hastie et al. (1994).

Habitat

All 27 species were classified correctly by the analysis to the habitat from which they were collected (Table 2.6A) and there was a perfect separation of the groups (Fig. 2.11), and all classification performance measures were higher than 0.9 (Table 2.7). The most important positive contributors to the first discriminant function (pFDA 1) were intergranule size, spinule length and the number of bristles per sensillum, whereas granule size and knobbiness index were the most important negative contributors. For pDFA 2, the most important positive contributors were granule size, pit diameter, knobbiness index and the diameter of the cutaneous sensilla (CS). The most important negative contributors were granule size and number of bristles per sensor (Fig. 2.11). (For the coefficient values for all traits in the analysis see supplementary material S2.1.) Terrestrial species were separated from both other groups mainly along pFDA 1, whereas pFDA 2 separated the saxicoline species from both other groups (Fig. 2.11). Overall, terrestrial species were more strongly separated from both arboreal and saxicoline species than were these two groups from one another.

Terrestrial species can, thus, be separated from both other groups by a combination of longer spinules, more bristles per CS, and, in the Carphodactylidae, smaller areas covered with knobs. Saxicoline species differ from both other groups by having pits and CS with larger diameters and, in the Carphodactylidae, larger areas covered with knobs. Arboreal species can be separated from both groups by larger granules and smaller intergranules, and have intermediate numbers of bristles per CS, and areas covered with knobs (Carphodactylidae).

Relative humidity

When examining the relative humidity of the environment of origin, 78% of the species were classified correctly, with most of the misclassifications between species from mesic and xeric conditions (Table 2.6B). Hydric species were classified more accurately, with only one species wrongly classified as mesic, and consistently had a higher performance classification coefficient (0.92 compared to 0.84 (mesic) and 0.80 (xeric)). Overall, these performance measurements were still reasonably high (Table 2.7). The plot also showed high overlap between mesic and xeric groups, whereas the hydric species were well separated (Fig. 2.12). The most important positive factors influencing pFDA 1 were intergranule size, knobbiness index, pit diameter and CS diameter, whereas granule size and bristles per CS were the strongest negative factors. For pFDA 2, the most important positive factors were intergranule size, knobbiness index and bristles per CS, while CS diameter and pit density were the most important negative factors. Spinule length had a weak positive influence on both axes (see S2.1). The hydric species are separated from both xeric and mesic species, mostly by pFDA1, whereas on pFDA 2 only xeric and hydric species are reasonably separated, with the mesic ones falling in between, and overlapping both other categories.

Thus, species from a hydric environment differ from species from drier relative humidity via a combination of higher pit diameter, CS with larger diameters but fewer bristles, smaller granules, and a higher density of pits. Species from mesic environments can be separated from both other groups by having smaller intergranules, slightly shorter spinules, and in the Carphodactylidae they have areas covered by knobs. Species from xeric environments have a lower density of pits compared to both other groups.

Univariate analysis of individual traits

The number of bristles and the number of cutaneous sensilla per mm^2 were significantly different in model averaging after controlling for the False Discovery Rate. There was a significant difference in the number of bristles between terrestrial and arboreal species, and terrestrial species differed significantly from arboreal and saxicoline species in terms of the number of cutaneous sensilla per mm2, with both trait values being higher in terrestrial species (compare Table 2.4). The model parameters for these two traits are reported in Table 2.8.

Discussion

GENERAL RESULTS

This study describes the dorsal microstructures of carphodactylid and diplodactylid geckos for the first time for most species (but see Bauer and Russell (1988) for *Nephrurus asper* and *N. levis*), as well as exploring associations between cutaneous microstructures and the habitat and humidity of the environment from which the geckos originated. Three hypotheses were tested: first, that terrestrial species would have longer, or more densely arranged spinules than arboreal species, which was confirmed (Fig. 2.12). Thus, there is an ecological association between long spinules and a terrestrial habitat in geckos. This is consistent with the idea that some functions of skin, such as self-cleaning and bactericidal properties, would be more beneficial for terrestrial species, compared to arboreal or saxicoline species. Second, I hypothesized that species from humid habitats would have longer or more densely arranged spinules than species from drier habitats, but this was only partially supported by My analysis, in that long spinules were weakly correlated with both xeric and hydric environments (compared to mesic environments). My final hypothesis was that terrestrial species from dry habitats would have more, or more complex, cutaneous sensilla, and this was supported in that both terrestrial species and those from dry habitats had more bristles per sensillum, while the sensilla tended to have smaller diameters, especially in species from dry habitats. Also, both the number of CS and bristles per mm^2 were significantly greater in terrestrial species after adjusting for phylogeny.

ECOLOGICAL ADAPTATION OF MICROSTRUCTURES

Although studies often discuss possible associations between microstructures and ecology (Gans & Baic, 1977; Hagey *et al.*, 2014a), they have seldom been examined analytically. Anoles (Dactyloidae) are the only reptile group for which ecological adaptations of microstructures have been studied in detail, by comparing morphology among closely related species occupying different ecological niches. These studies, however, focused exclusively on adhesive pad morphologies in relation to different ecological niches in rainforest habitats (Macrini, Irschick, & Losos, 2003; Renous, Höfling, & Da Rocha, 2010; Stuart *et al.*, 2014). Similarly, the few studies of geckos discussing connections between microstructures and ecology also addressed only the evolution of adhesive pads as adaptations to different habitats (Johnson & Russell, 2009; Collins *et al.*, 2015). In iguanid lizards, Peterson (1984a) could not detect an obvious relation between spinules (occurring only in some species of this clade) and habitat, but Peterson's (1984a) study was focused on the evolution of spinules, and did not analyse ecological associations in detail. Thus, my study is the first to analyse ecological associations of (non-toe pad) microstructures in detail, allowing us to address associations with habitat type (arboreal, terrestrial, saxicoline), and habitat relative humidity (xeric, mesic, hydric). My study was limited in that only the microstructures from the dorsal mid-body region were analysed, but this made it less likely that habitat associations would be masked by the evolutionary effects of other functional demands (i.e., feeding movements or tail-breakage) on cutaneous sensilla.

INTERACTION BETWEEN HABITAT AND RELATIVE HUMIDITY

There were associations between skin morphology (epidermal microstructures and scale size) and ecological factors (habitat type and relative humidity), although skin morphology was more strongly associated with habitat type than relative humidity (compare Figs. 2.11 and 2.12). Apparently, the occurrence of longer or more densely arranged spinules was more strongly associated with the habitat than with the humidity of the environment. Possibly, selection from habitat and humidity are not independent (Arnold, 2002). In My study, most of the species from xeric environmental conditions were terrestrial, and there was only one terrestrial hydric species (*Carphodactylus laevis*). Unfortunately for My hypothesis predicting the association between longer spinules and rainforest living (compared to species from drier habitats) any possible selection for longer spinules driven by the necessity for bacterial and fungicidal skin in rainforest environments may be overridden by even stronger selection for long spinules in dusty, and (possibly) disease-ridden terrestrial environments.

When I examined epidermal structure in relation to habitat relative humidity, I found overlap between species from mesic and xeric conditions (Fig. 2.12), which manifested as incorrect classifications in the analysis (Table 2.6B). Although open savannah woodland can range from quite mesic to quite dry, the environmental conditions (humidity, temperature) in open savannah woodland are much more similar to those of deserts than to tropical rainforests (Bureau of Meteorology, 2018). Consistent with this, some Diplodactylidae from xeric or mesic environmental conditions have changed from one relative humidity regime to the other repeatedly during their evolution (Oliver *et al.*, 2014). In addition, geckos may avoid environmental extremes by choosing appropriate habitats (e.g., using moist burrows in drier habitats (Aguilar & Cruz, 2010)), which may decrease the correlation between relative humidity and values of the traits in question. The ecology of and behaviour of these geckos is very poorly known, and more detailed information on habitat selection and preferred conditions of humidity used by each species are required to more clearly define associations between specific features and particular relative humidity regimes.

COMPARATIVE MORPHOLOGY OF MICROSTRUCTURES

Cutaneous microstructures of the Carphodactylidae and Diplodactylidae are mostly consistent with those of gecko species previously examined. Spinules have been described for all geckos examined thus far (Ruibal, 1968; Peattie, 2009), and are hypothesized to be the origin of the adhesive setae present in most climbing geckos (Ruibal & Ernst, 1965; Maderson *et al.*, 1998; Russell *et al.*, 2015). The spinule lengths described in this study (0.28 – 1.12 µm) lie within, or close to, the described ranges of gecko spinule length (0.3 – 3 µm), although situated towards the lower end (Ruibal, 1968; Stewart & Daniel, 1975; Peterson & Bezy, 1985; Rosenberg *et al.*, 1992; Spinner *et al.*, 2013a). Cutaneous sensilla have also been reported for all geckos examined so far, and the numbers I recorded lie within previously reported ranges (Matveyeva-Dujsebayeva & Ananjeva, 1995; Russell *et al.*, 2014).

The most remarkable observation from this study was the discovery of lenticular sense organs in the Diplodactylidae, and on the tubercular scales of *Phyllurus amnicola* (Carphodactylidae). Lenticular sense organs have never been reported for geckos, and are normally associated with non-gekkotan squamates. Tubercle-like sense organs, which resemble the LSO I report, occur on the heads of some snakes (Jackson, 1977; Jackson & Sharawy, 1980), and similar sense organs, termed 'sensory pits' by some authors, are described for the Cordylidae, Gerrhosauridae (Harvey & Gutberlet Jr, 1995), Phrynosomatidae (Sherbrooke & Nagle, 1996), Varanidae (Bucklitsch *et al.*, 2016), and Xantusidae (Peterson & Bezy, 1985; Harvey, 1993). Lenticular sense organs are also well known for the Agamidae, and some families of the Iguanidae (Ananjeva *et al.*, 1991; Ananjeva, Dujsebayeva, & Joger, 2001; Ananjeva & Matveyeva-Dujsebayeva, 1996). For both these clades, Matveyeva and Ananjeva (1995) report that species or genera have either cutaneous sensilla or lenticular sense organs, and suggest that when both types of sense organs are observed on the same area of a single species they are actually artefacts associated with observing skin structure in different stages in the shedding cycle of preserved specimens. The results presented here, which originate from moulds of freshly shed living specimens, demonstrate unequivocally that lenticular sense organs do co-occur with cutaneous sensilla, at least in geckos.

COMPARISON BETWEEN CARPHODACTYLIDAE AND DIPLODACTYLIDAE

Overall, microstructures varied greatly between Carphodactylidae and Diplodactylidae, strongly separating the two clades morphologically, consistent with a very early evolutionary divergence, between 66 and 102 mya (Gamble *et al.*, 2012). Cutaneous sensilla were generally single-bristled in the Diplodactylidae, whereas they could be multi-bristled in the Carphodactylidae, consistent with the pattern detected by Bauer and Russell (1988), who described single-bristled sensilla for the Diplodactylidae they examined from New Zealand and New Caledonia, and multi-bristled sensilla in the Carphodactylidae. Multiple bristles per sensillum also occur in the pygopodid genus *Lialis* (Spinner *et al.*, 2013a); and are widespread but sporadic within the Gekkomorpha (Schmidt, 1912; Hiller, 1976; Ananjeva *et al.*, 1991; Dujsebayeva, 1995; Nikitina & Ananjeva, 2003; Yonis, Bayomy, & Shalaby, 2009; Darwish, 2012; Russell *et al.*, 2014). Lauff et al. (1993) described bristleless sensilla and sensilla with branched bristles co-occurring, with simple, unbranched bristles on the feet of *Gekko gecko*. Thus, different sensilla morphologies occur even within a single species, although My findings suggest that sensilla morphology does not vary on the dorsal surface.

Additionally, cutaneous sensilla of the Carphodactylidae not only have more than one bristle, but they tend to have large diameters (21-27 µm compared to 14-20 µm in the Diplodactylidae). Single-bristled sensilla in other gekkotans are in the same size range as the sensilla of the Diplodactylidae for most species (Lauff *et al.*, 1993; Nikitina & Ananjeva, 2003), but some larger species like *Gekko gecko* (Gekkonidae) or *Tarentola chazaliae* (Phyllodactylidae) have sensilla diameters of 29–30 µm (Hiller, 1971). Also, the multi-bristled sensilla of gekkonid and sphaerodactylid geckos are similar in size to those of the single-bristled sensilla of the Diplodactylidae reported here (Hiller, 1971; Dujsebayeva, 1995).

The microstructures that most clearly distinguish the Diplodactylidae and the Carphodactylidae are the absence or presence of knobs and hillocks, which occur only in the Carphodactylidae. Bauer and Russell (1988) also found knobs in *Nephrurus*. As knobs or hillocks are absent in the Pygopodidae (the sister taxon of the Carphodactylidae (Gamble *et al.*, 2012)), these traits can be regarded as a synapomorphy of the Carphodactylidae. Most geckos whose microstructures have been examined so far do not have knobs or hillocks (Ruibal & Ernst, 1965; Stewart & Daniel, 1975; Spinner *et al.*, 2013a; Russell *et al.*, 2015). Only Peterson and Bezy (1985) report hillocks (there termed microtubercles) for the gekkonids *Pachydactylus bibronii* (now *Chondrodactylus bibronii*) and *Hemidactylus brookii*, and Yonis et al. (2009) describe hillocks on the dorsal head and trunk scales of *Tropiocolotes tripolitanus*. These traits have most likely evolved (or re-evolved) independently in the Carphodactylidae and in these three species, as the latter are clearly nested within the Gekkonidae (Gamble *et al.*, 2012).

Although sparse in geckos, knob-like structures are reported repeatedly in other squamate reptiles. Some of the ‘tooth-like structures’ described for leaf-chameleons (Riedel *et al.*, 2015) appear similar to the knobs in species of *Saltuarius* and *Phyllurus* examined in this study. Gans and Baic (1977) described knob-like structures on the scales of uropeltid snakes, terming them ‘cones’, and Peterson and Williams (1981) report them on the subdigital scales of Anoles (there termed ‘bosses’).

FUNCTION OF MICROSTRUCTURES

Apart from basic mechanosensitive abilities (Hiller, 1968; Düring & Miller, 1979), the details of the function of cutaneous sensilla are not fully understood, especially with regard to their morphological variation (Ananjeva *et al.*, 1991; Matveyeva-Dujsebayeva & Ananjeva, 1995). Results from this study suggest that at least some of the traits of sensilla (diameter and the number of bristles per sensillum) are associated with environmental humidity. This is consistent with the hypothesis suggested by Ananjeva et al. (1991) that the sensilla may detect humidity. However, more detailed analysis of the morphology of the sensilla in relation to these factors are necessary.

The adaptive significance of knobs and hillocks within the Carphodactylidae may be to increase camouflage. The leaf-tailed geckos (*Phyllurus* and *Saltuarius*), in particular, are very cryptic species relying heavily on camouflage. Arnold (2002) showed that Lacertids with smooth scales and few microstructures were shinier than species with highly structured scales and more microstructures, because their skin reflected light directly, whereas the skin scatters light in species with raised skin topography. Also, recent studies of snakes have revealed microstructures enhancing camouflage of the Gaboon viper (*Bitis rhinoceros*) (Spinner *et al.*, 2013b). Although the microstructures of this snake are far more hierarchically structured than those of the geckos I studied, knobs and hillocks found on gecko scales could still enhance their crypsis by scattering light more than uniformly flat, spinule-covered surfaces (Arnold, 2002). This contention is consistent with a similar pattern present in the Chamaeleonidae, in which the extremely cryptic leaf-chameleons (*Palleon, Brookesia, Rieppeleon* and *Rhampholeon*) have developed a bumpy skin surface, with knobs and hillocks (Riedel *et al.*, 2015). If this is correct, I predict that the equally cryptic Malagasy leaf-tailed geckos of the genus *Uroplatus* should also exhibit functionally similar microstructures. The tubercle scales, which were also more common in the Carphodactylidae compared to the Diplodactylidae, and also appear in many other gecko species, may have a similar effect.

Conclusion

This study described the epidermal microstructures of a range of Australian geckos, and established associations between epidermal microstructures and aspects of their ecology. These associations suggest that microstructures may be adaptations to these environmental factors, although further studies are required to directly test this hypothesis. As predicted, spinule length was associated with dry and dirty environments, consistent with the proposed functions of spinules in dirt-shedding and killing bacteria. Species from terrestrial habitats are likely exposed to more dirt and debris and potentially harmful microorganisms than are saxicoline or arboreal groups (Ungar *et al.*, 1995; Nunn *et al.*, 2000; McCabe *et al.*, 2015), thus self-cleaning and bactericidal properties associated with long spinules could be important drivers of their evolution (Watson *et al.*, 2015b). Although only weakly supported by My analysis, the anti-bacterial function proposed in recent studies (Watson *et al.*, 2015a, 2016; Li *et al.*, 2016) may also be a driver for the evolution of long spinules in rainforest species, which may be exposed to more fungi and bacteria than species from drier habitats (Bouskill *et al.*, 2012). Further studies should address non-independent selection for long spinules on geckos from dry habitats and those with high relative humidity (Arnold, 2002). In addition, I found that certain features of cutaneous sensilla were associated with both the habitat and the relative humidity (diameter and number of bristles per sensillum) and their density (bristles and sensilla per mm^2). Although difficult to interpret because the functions of the different morphologies of cutaneous sensilla are not known, I expect differences in skin sensory abilities required to negotiate different habitats. For example, small, nocturnal animals in xeric habitats may need to detect minute wind currents or moisture differentials, and this may select for an increase in sensilla number, or in number of bristles per sensillum. The many types of sensilla I detected, and the differences among species in their distributions and morphologies are consistent with the hypothesis that mechanoreception alone cannot explain the high variation in sensilla morphology among geckos (Ananjeva *et al.*, 1991; Matveyeva-Dujsebayeva & Ananjeva, 1995). Although it is important to remember that when examining many characters, some of which may have similar functions in different combinations, may produce similar evolutionary outcomes through convergent evolution from different evolutionary origins (Sherbrooke *et al.*, 2007), but examining direct correlations between morphological features and habitat provides useful information on possible evolutionary associations.

Figures

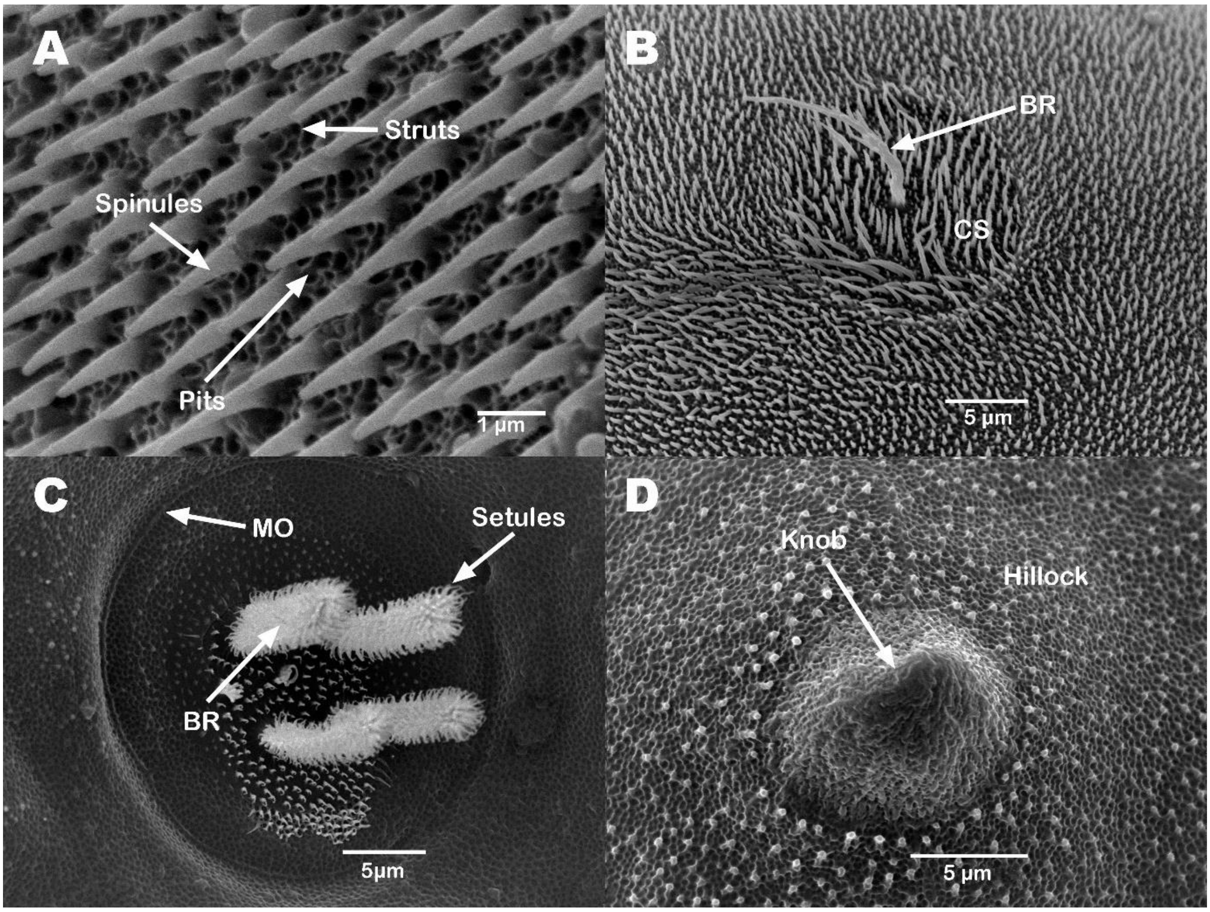


Figure 2-1 SEM images showing the microstructure terminology used for this chapter**.** **(A)** Long spinules, surrounded by pits and struts (*Rhynchoedura ormsbyi*). **(B)** A cutaneous sensillum (CS) of *Lucasium damaeum* with a single bristle (BR). **(C)** A cutaneous sensillum of *Nephrurus levis* has multiple bristles (BR), each covered by setules, and is surrounded by a moat (MO). **(D)** Detail of a knob of *Nephrurus asper*, arising from the top of a setae covered hillock.

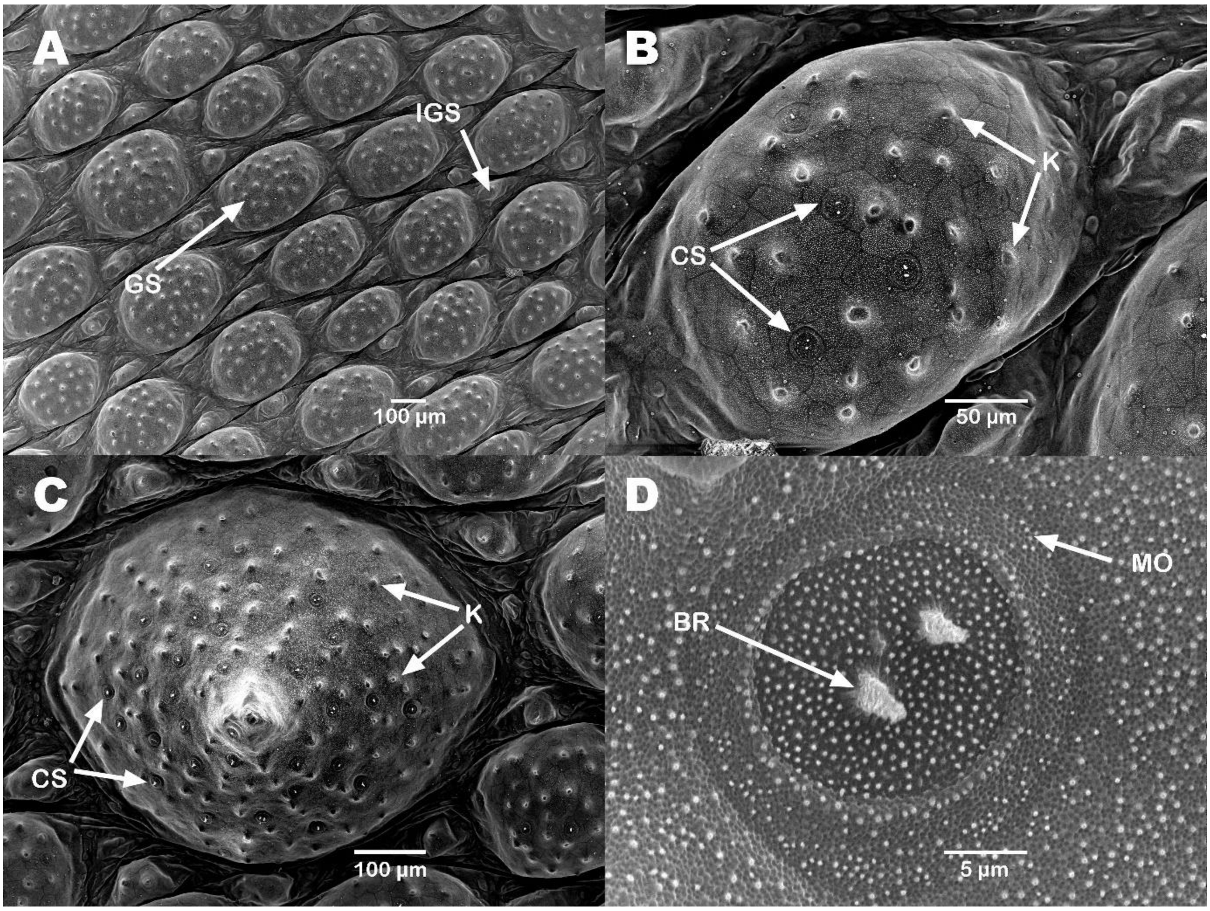


Figure 2-2 Dorsal scales of *Saltuarius cornutus* (SEM images). **(A)** Overview showing granule (GS) and intergranule (IGS) scales. **(B)** Detail of a granule scale partially covered by large knobs (K) and 4 cutaneous sensilla (CS). **(C)** Tubercle scale covered with knobs (K) and cutaneous sensilla (CS) from the peak to the base. **(D)** Cutaneous sensillum with two bristles (BR) and a very shallow moat (MO).

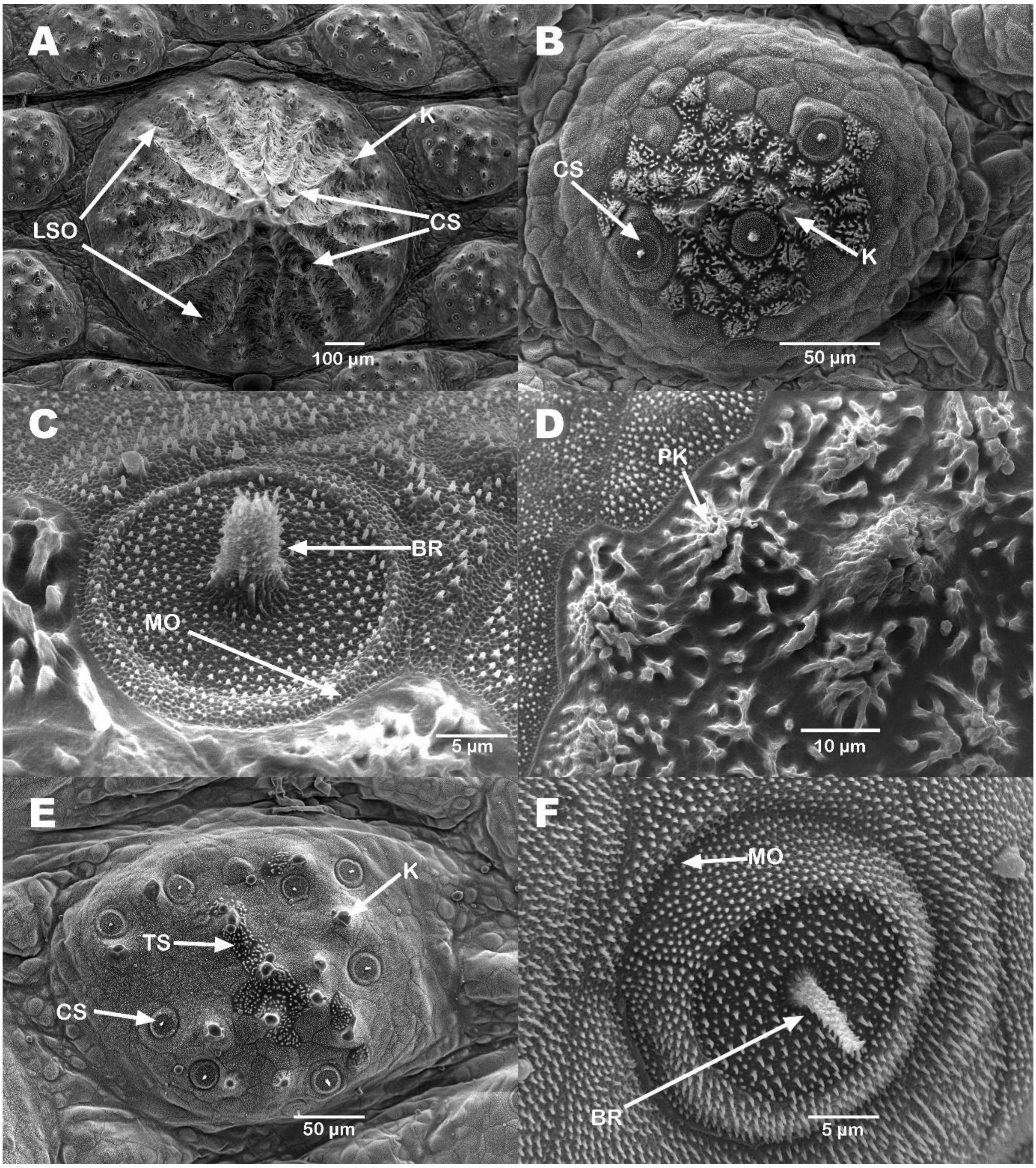


Figure 2-3 Dorsal scales of the genus *Phyllurus* (SEM images). *P. nepthys* is displayed in panels A - D, whereas panels E & F show *P. amnicola* **(A)** A tubercle scale of *P. amnicola* with keels (K) forming a radial pattern. Cutaneous sensilla (CS) are scattered between the keels on higher elevations, whereas lenticular sense organs (LSO) can be found on the lower parts of the same areas. **(B)** A single granule scale with three cutaneous sensilla (CS) and an area, where large, thick spinules gradually fuse to form knobs (K) in the centre. **(C)** Detail of a cutaneous sensillum surrounded by a shallow moat (MO) that is level with the rest of the scale, with a very short, thick bristle (BR), with short, rounded barbs. **(D)** Detail of the central area of (B), showing partially formed knobs (PK). (E) A granule scale with eight cutaneous sensilla (CS) around the edges. Knobs (K) and areas of thicker and larger spinule-like structures (TS) are more centrally located. **(F)** A slightly raised cutaneous sensillum surrounded by a wide moat (MO). Bristle (BR) is barbed and surrounded by longer spinules

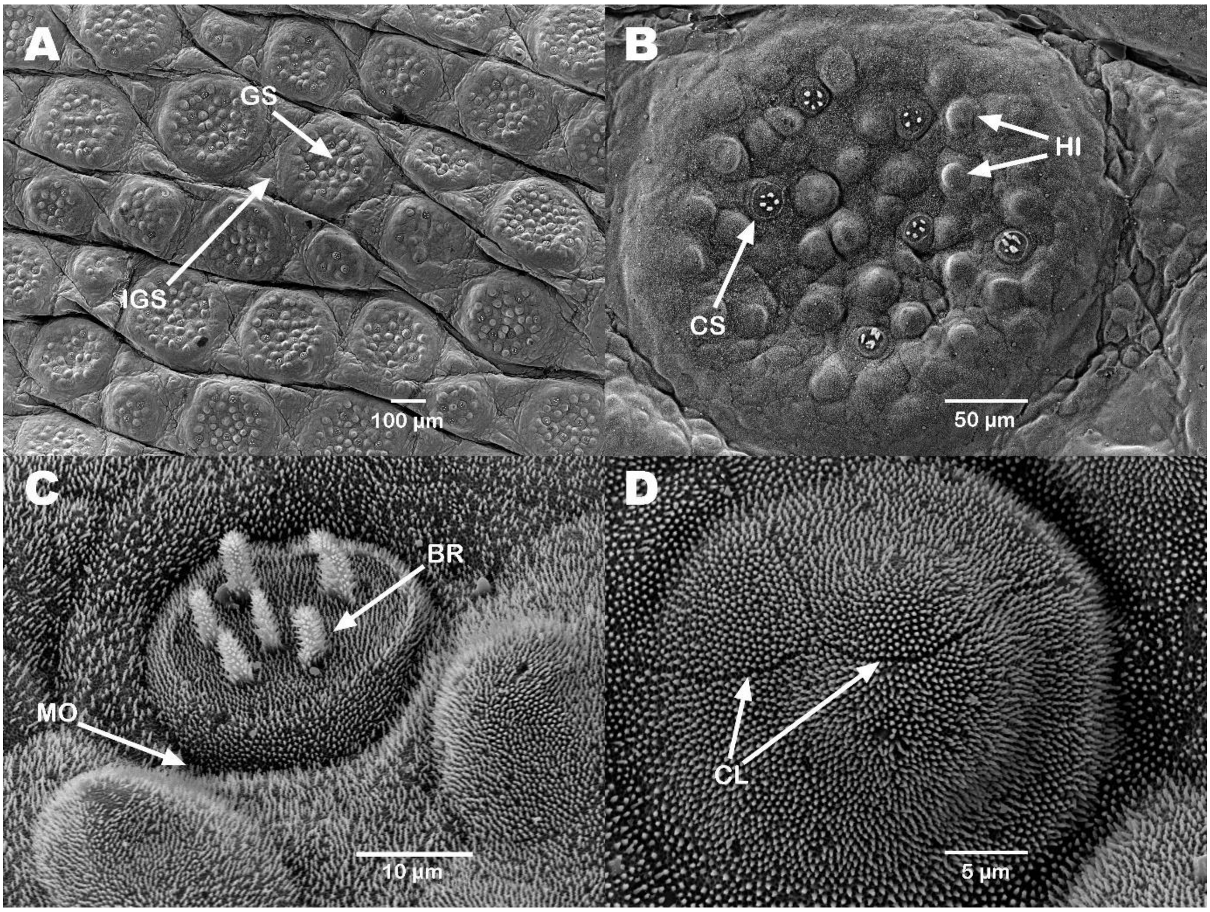


Figure 2-4 Dorsal scales of *Carphodactylus laevis* (SEM images). **(A)** Overview showing granules (GS) and intergranules (IGS). **(B)** Granule scale with hillocks (HI) scattered over the scale area and cutaneous sensilla (CS) in between the hillocks. **(C)** Cutaneous sensillum with five bottlebrush-shaped bristles (BR) surrounded by a deep moat (MO). **(D)** A single hillock covered with spinules, which are slightly longer and denser compared to those in the top left and right of the image. Three darker lines splitting the hillock are separations between cells indicating the outline of the clear layer (CL) from the previous skin generation.

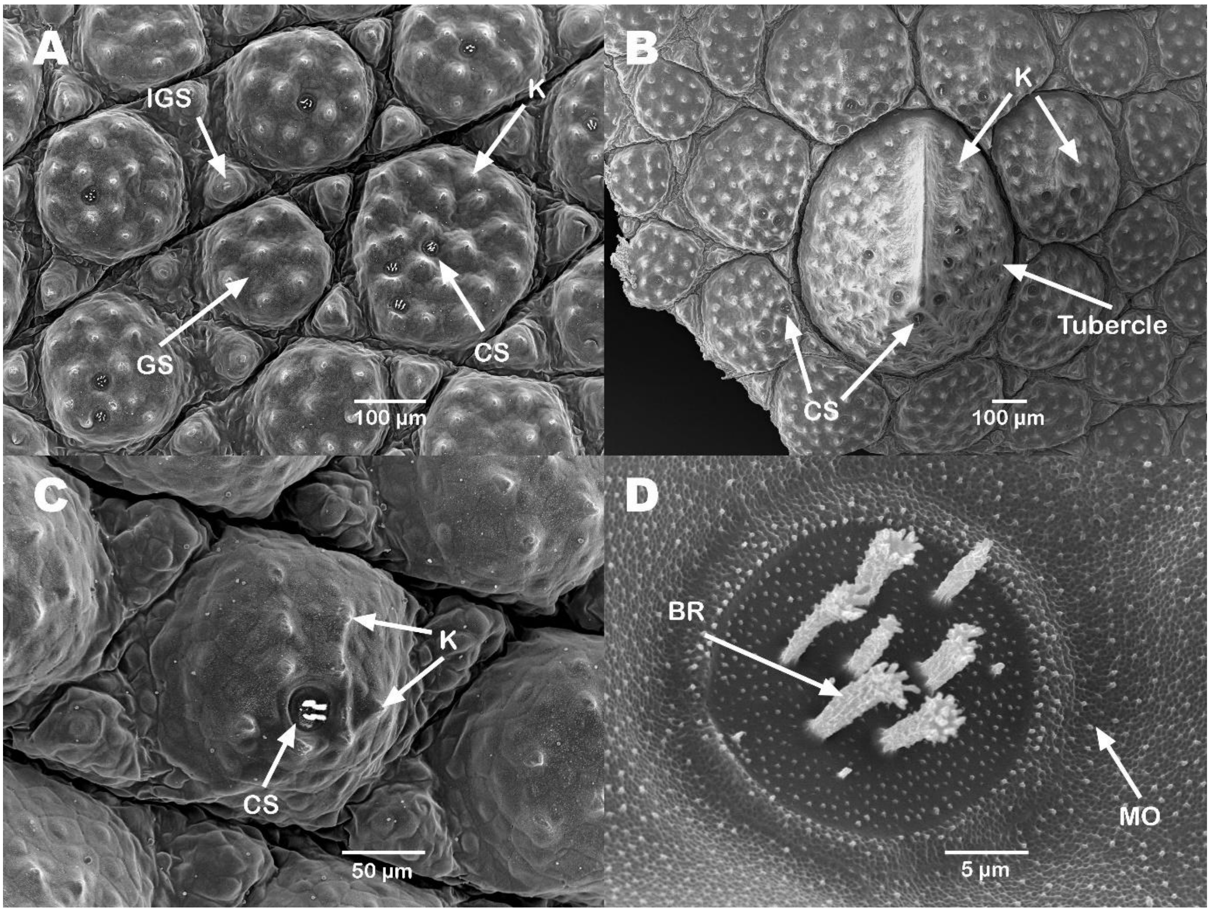


Figure 2-5 Dorsal scales of the genus *Nephrurus* (SEM images). **(A)** Overview of *N. asper* showing granules (GS) and intergranules (IGS). Knobs (K) are scattered over the granule scales, with cutaneous sensilla (CS) in between. **(B)** Tubercle scales of *N. asper* are keeled towards the anterior end of the scale and surrounded by larger granule scales. Six cutaneous sensilla (CS) occur at the peak forming a V-shape with the apex facing posteriorly while the rest of the tubercle is covered with knobs (K). **(C)** A single granule scale of *N. levis* with one centrally located cutaneous sensillum (CS), surrounded by knobs (K). **(D)** Detail of a cutaneous sensillum of *N. asper* with seven, slightly barbed bristles (BR) which are divided into a series of knobs at the end. A shallow moat (MO) surrounds the sensillum.

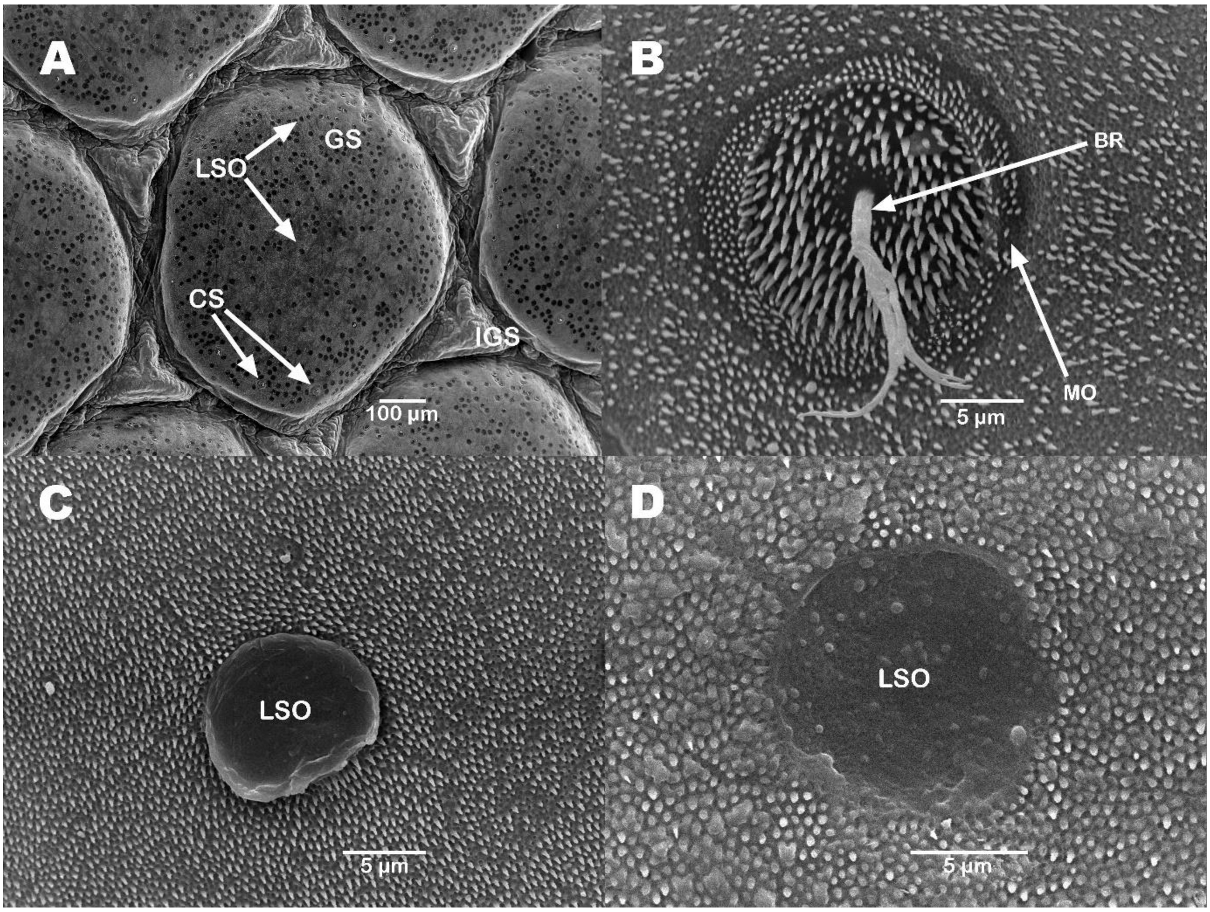


Figure 2-6 Dorsal scales of the genus *Oedura* (SEM images). **(A)** A single granule scale of *Oedura castelnaui* surrounded by small, wrinkled intergranules. and many lenticular sense organs (LSO) covering the entire scale surface. Cutaneous sensilla (CS) were also observed at the posterior end of the scale. **(B)** A Cutaneous sensillum on a granule scale of *O. cincta* in a recession surrounded by a moat (MO) filled with small, densely packed spinules. The bristle (BR) is split at the distal end and surrounded by long spinules. **(C)** A lenticular sense organ of *O.* castelnaui with a disc-like elevation in the centre. These LSO's are primarily found at the anterior margin of the scales. **(D)** A crate-like LSO of *O. coggeri*. These LSO's are more common towards the centre of the scale and at the distal margins

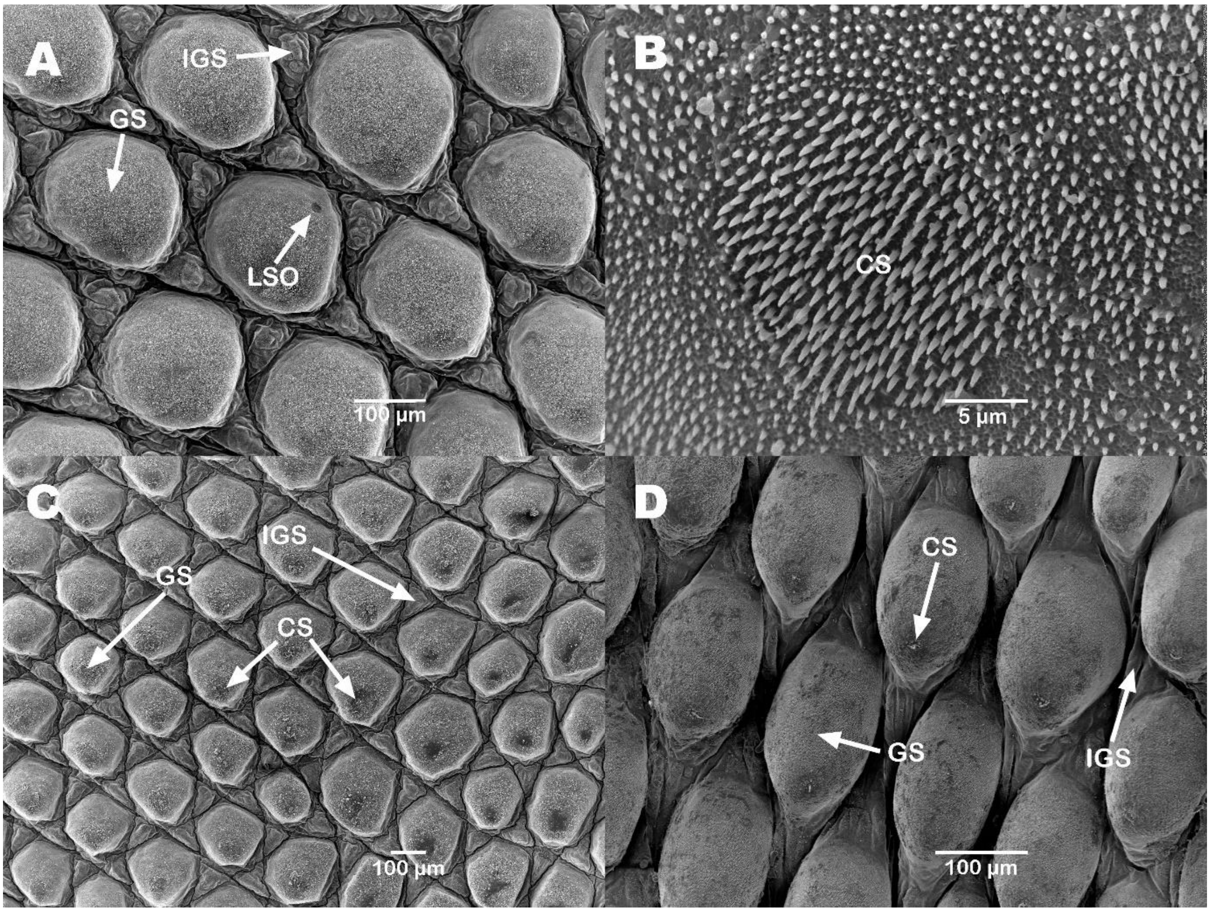


Figure 2-7 Dorsal scales of *Amalosia rhombifer* (A - B) and *Rhynchoedura ormsbyi* (D - F) (SEM images). **(A)** Domed-shaped granule scales surrounded by six intergranule scales, which are wrinkled and equilateral. One granule showing a lenticular sense organ (LSO) at the anterior scale margin. **(B)** Cutaneous sensilla of *A. rhombifer* are bristleless and covered in slightly denser and longer spinules. **(C)** Granule scales of *R. ormsbyi* located away from the dorsal midline are hexagonal but rounded at the posterior end and more hexagonally defined at the anterior end, and surrounded by six intergranules. Each of these granule scales has one cutaneous sensillum located at the posterior end. **(D)** Granule scales of *R. ormsbyi* running along the dorsal midline are elongated and oval while intergranules are very reduced and attached at the anterior and posterior ends. One cutaneous sensillum occur on each granule at the posterior end, which is the peak of the scale.

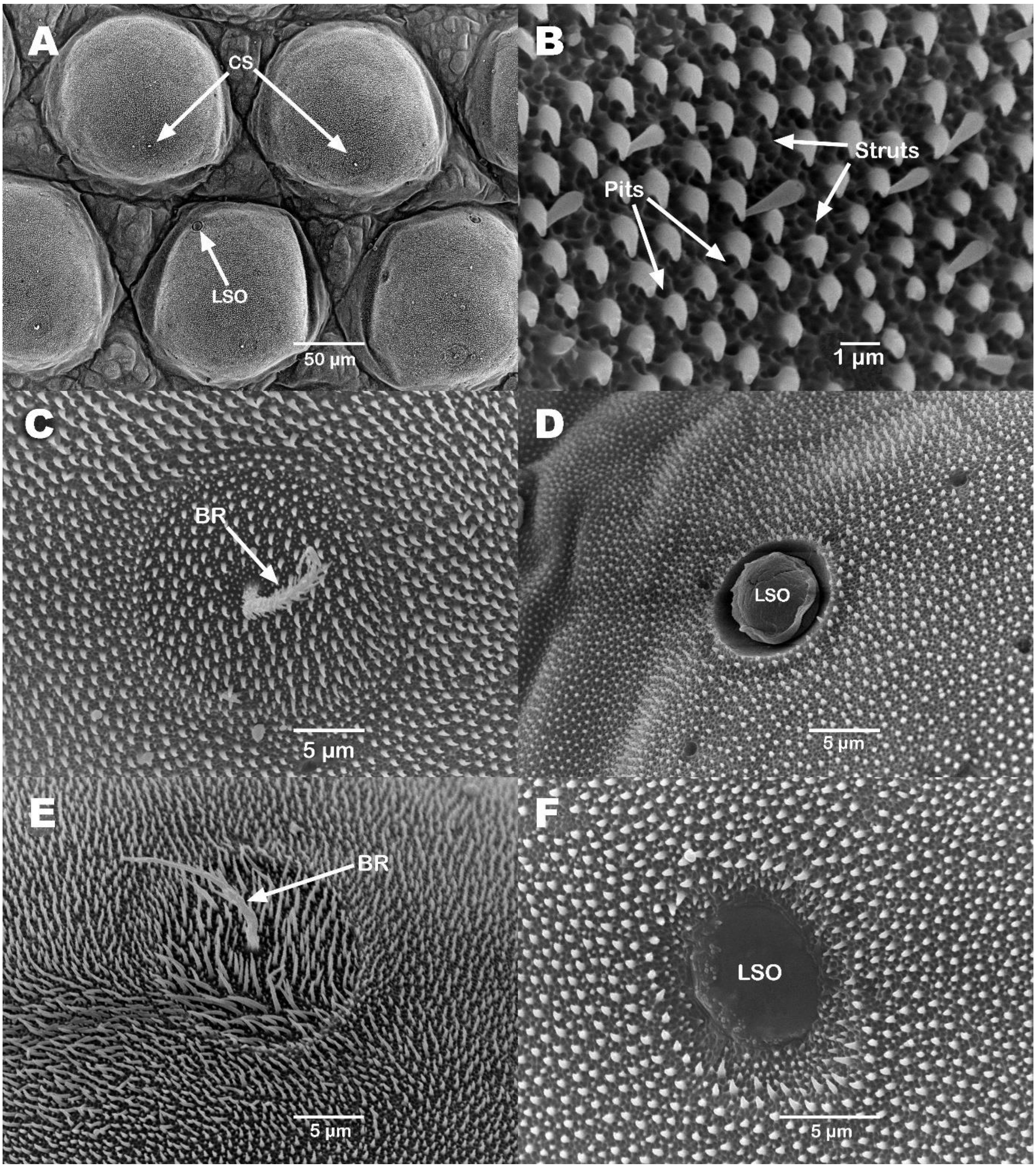
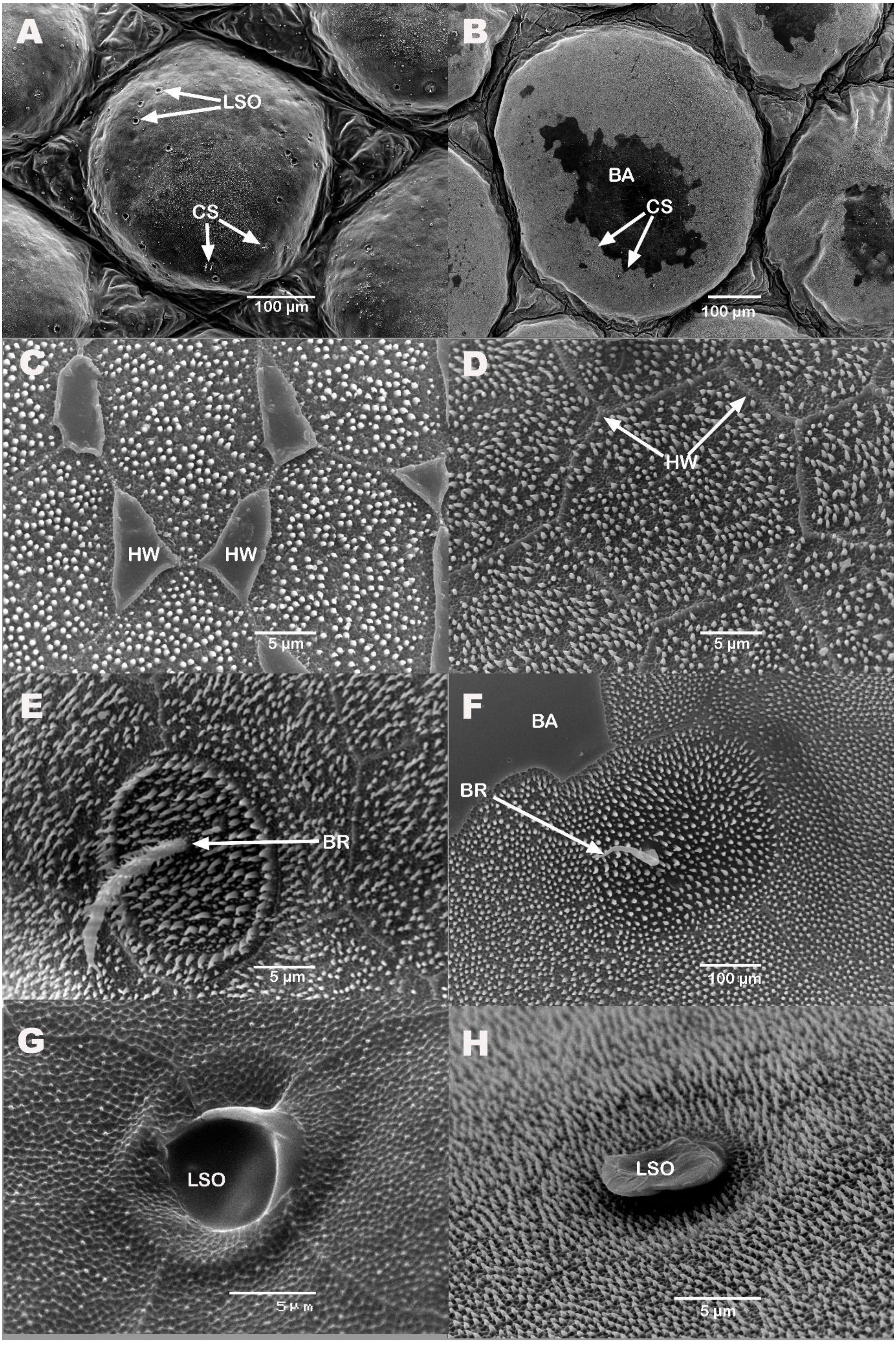


Figure 2-8 Dorsal scales of the genus *Lucasium* (SEM images). **(A)** Overview of *L. immaculatum*: Several granule scales with one cutaneous sensillum (CS) at the posterior end of each scale and surrounded by intergranules. Some scales have lenticular sense organs (LSO) at their anterior margins. **(B)** Evenly spaced long spinules amongst pits and struts (*L. immaculatum*). **(C)** Cutaneous sensillum of *L. immaculatum* with a slight depression but with no surrounding moat. The bristle (BR) is heavily barbed with setules and the surrounding spinules are sparser than those on the rest of the scale. (D) Lenticular sense organ of *L. steindachneri* near the anterior edge of the granule scale. **(E)** Cutaneous sensillum of *L. damaeum* with a distally tapered, smooth bristle (BR) surrounded by long spinules. **(F)** Lenticular sense organ (LSO) of *L. damaeum* which is recessed into the scale.


A
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LSO
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Figure 2-9 (previous page) Dorsal scales of the genus *Diplodactylus* (SEM images). **(A)** Granule scale of *D. wiru* with lenticular sense organs (LSO) surrounding the scale and two cutaneous sensilla (CS) at the posterior end. **(B)** A large granule scale from along the dorsal midline of *D. tessellatus* showing the centrally located bare area. Cutaneous sensilla (CS) are located at the posterior end of the scale. **(C)** Detail of a granule scale of *D. ameyi* showing modified honeycomb structures, where the walls broaden at the meeting point, but nearly dissolve in the middle between two meeting points. This way forming triangulate structures. **(D)** Detail of a granule scale of *D.* conspicillatus. The whole surface is covered with honeycomb structures and spinules. **(E)** Detail of a cutaneous sensillum of *D. platyurus.* The sensillum is slightly depressed with a barbed bristle (BR) that is tapered at the distal end. **(F)** Cutaneous sensillum of *D. tessellatus* with a smooth, tapered bristle (BR) located next to a bare area. **(G)** A lenticular sense organ (LSO) of *D. wiru* located at the edge of the scale where spinules were extremely short and sparse with clear layer outlines from the previous skin generation. **(H)** LSO of *D. tessellatus* at the anterior end of a granule.

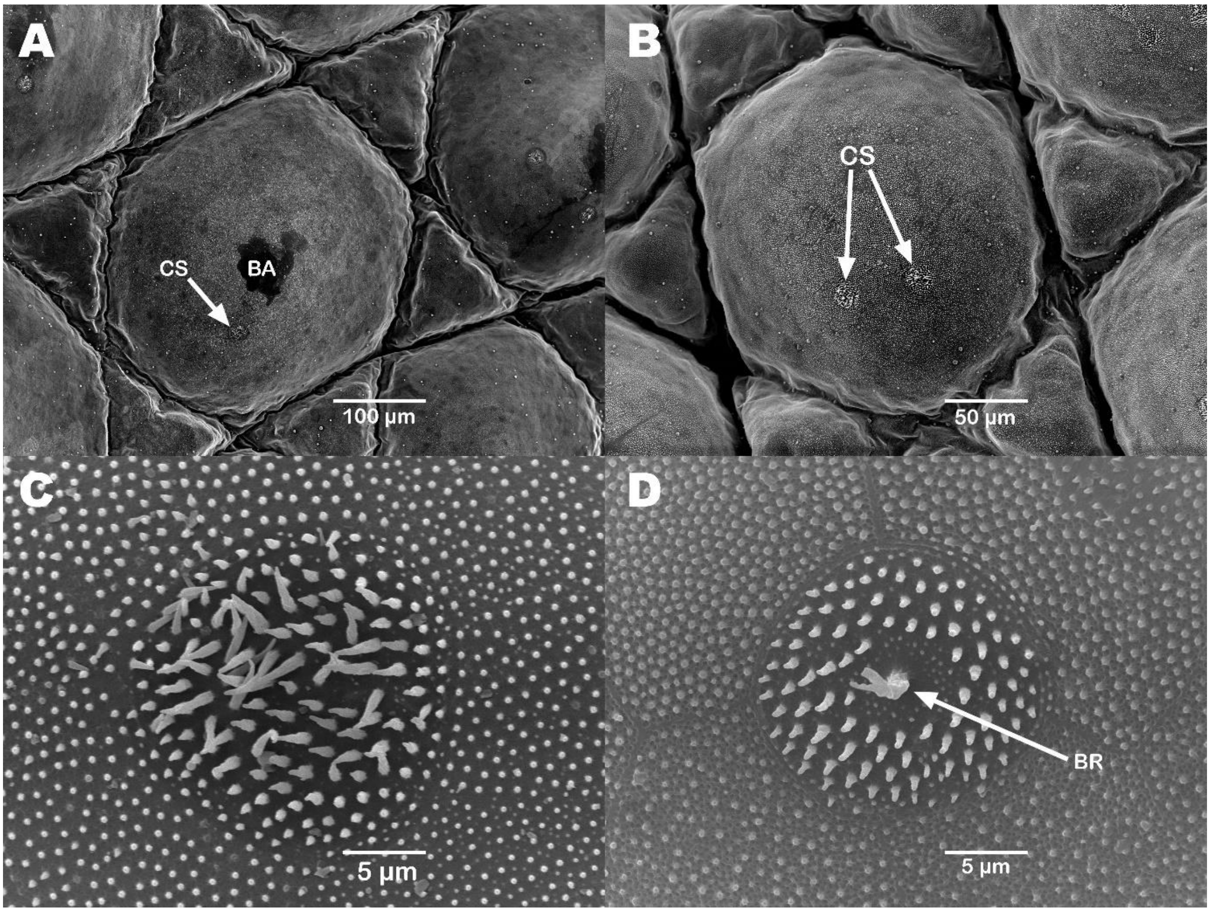


Figure 2-10 Dorsal scales of the genus *Strophurus* (SEM images). **(A)** One hexagonal granule scale of *S. krisalys* with a bare area (BA) at the peak and one cutaneous sensillum (CS) at the posterior end. **(B)** One granule scale of *S. taeniatus* with two cutaneous sensilla at the posterior end of the scale. **(C)** Detail of a Cutaneous sensillum of *S. taeniatus* lacking a bristle with elongated spinules surrounded by a sparse spinulate area towards the edge of the scale. **(D)** Cutaneous sensillum of *S. williamsi* with one smooth bristle (BR) divided in two at the distal end. The outline of the cell boundaries can also be seen.

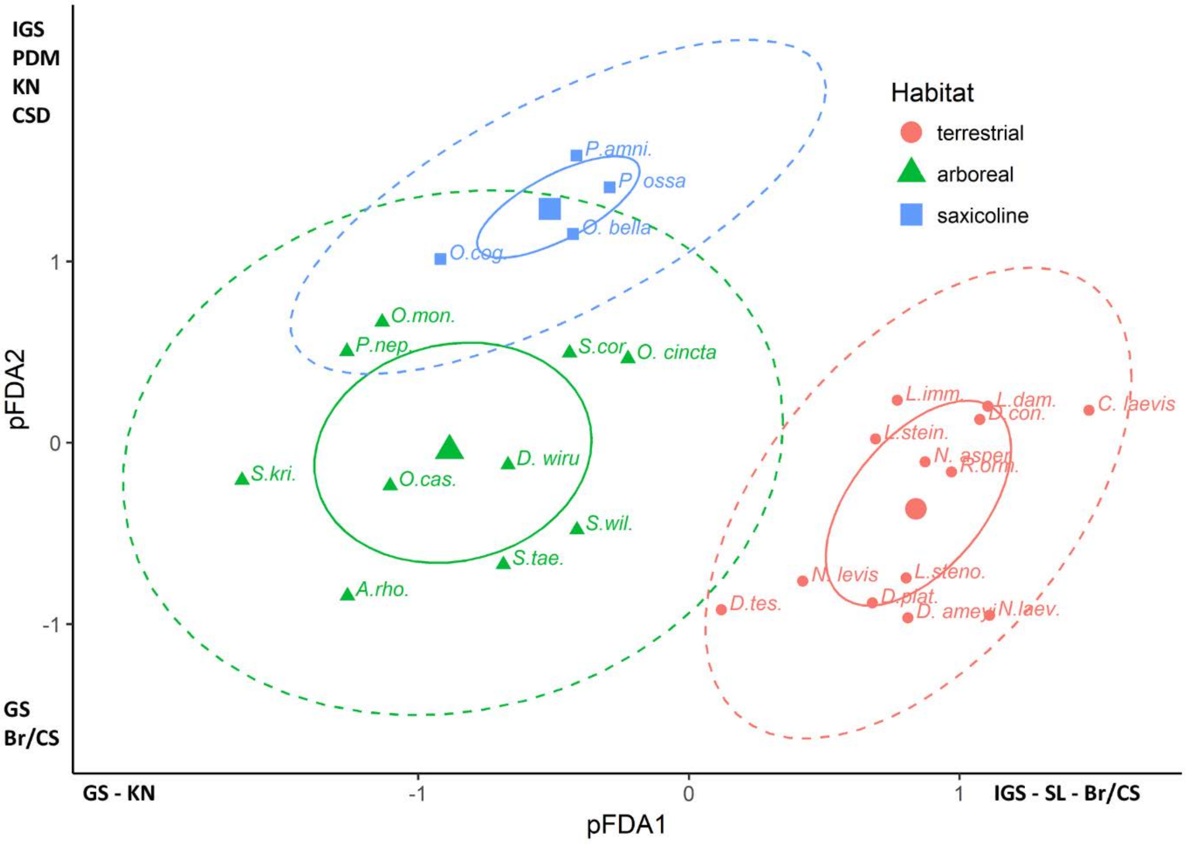


Figure 2-11 Plotted results of the (phylogenetic) flexible discriminant analysis of the habitat with lambda = 0. Group centroids (large symbols) are shown as well as the individual species (smaller symbols labelled with (abbreviated) species names), 95% (dotted lines) and 50% (solid lines) confidence intervals. The most important contributors are shown on the axes of the respective graphs, and the coefficients for these contributors are given here in brackets. Positive contributors for pFDA 1 are: Intergranule size (IGS): 67.27; spinule length (SL): 3.41; the number of bristles per cutaneous sensillum (Br/CS): 1.08. Negative contributors are: Granule size (GS): -11.55, the percentage of the scale area covered by knobs or hillocks (Knobbiness; KN): -3.54. For pFDA 2, positive contributors are: IGS: 85.50, diameter of the pits (PDM): 4.49, KN: 3.88, diameter of the cutaneous sensilla (CSD): 3.78. And negative contributors are: GS: -2.68, Br/CS: -1.55. All other measured traits had coefficients smaller than 0. Abbreviations for species names are: A. rho = *Amalosia rhombifer*; D. con. = *Diplodactylus conspicillatus*; D. plat = *D. platyurus*; D. tes = *D. tessellatus*; L. dam = *Lucasium damaeum*; L. imm = L. immaculatum; L. stein = *L. steindachneri*; L. steno = *L. stenodactylum*; N. laev = *Nephrurus leavissimus*; O. cas = *Oedura castelnaui*; O. cog = *O. coggeri*; O. mon = *O. monilis*; P. amni = *Phyllurus amnicola*; P. nep = *P. nepthys*; R. orm = *Rhynchoedura ormsbyi*; S. cor = *Saltuarius cornutus*; S. kri = *Strophurus krisalys*; S. tae = *S. taeniatus*; S. wil = *S. williamsi*.

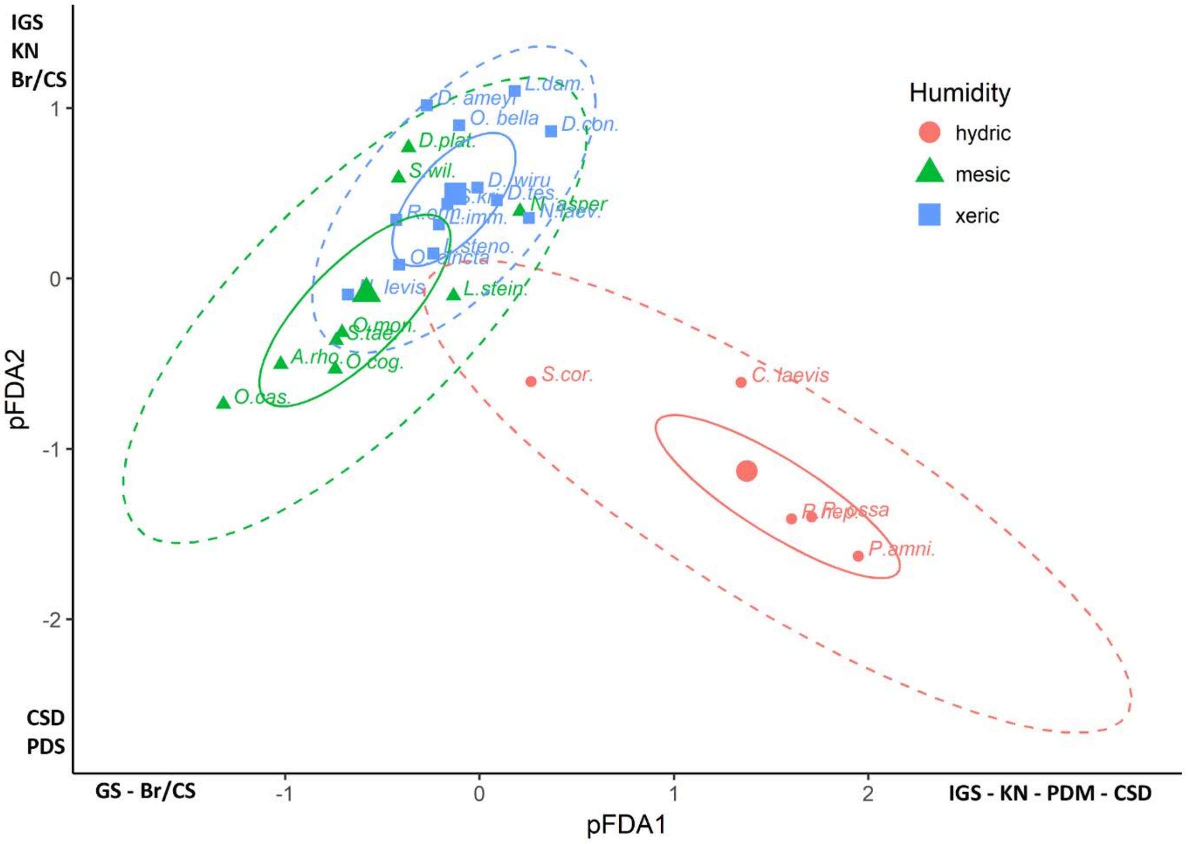


Figure 2-12 Plotted results of the (phylogenetic) flexible discriminant analysis of the habitat with lambda = 0. Group centroids (large symbols) are shown as well as the individual species (smaller symbols labelled with (abbreviated) species names), 95% (dotted lines) and 50% (solid lines) confidence intervals. The most important contributors are shown on the axis of the respective graphs, and the coefficients for these contributors are given here in brackets. For pFDA1, the positive contributors are: IGS: 113.98; KN: 6.56; CSD: 2.16; and the negative are: GS: -13.90; Br/CS: -1.11. For pFDA these are: positive: IGS: 40.81; KN: 1.88; Br/CS: 1.30. Coefficients for all other traits were smaller than 0. For abbreviations used see Fig. 11.

Tables

Table 2.1: Overview over all species examined in this study, showing collection site, humidity and habitat category assigned, morphometrics and scale sizes.

Species	Collection Site	Habitat	Humidity
Carphodactylidae			
Carphodactylus laevis Günther, 1897	Mount Hypipamee NP, QLD [S 17° 25’ 33”, E 145° 29’ 10”]	terrestrial	hydric
Nephrurus asper Günther, 1876	Mingela Range, QLD [S 20° 06’ 39”, E 146° 52’ 39”]	terrestrial	mesic
Nephrurus laevissimus Mertens, 1958	Great Victoria Desert, SA [S 29° 21’ 07”, E 132° 41’ 57”]	terrestrial	xeric
Nephrurus levis De Vis, 1886	Great Victoria Desert, SA [S 29° 21’ 07”, E 132° 41’ 57”]	terrestrial	xeric
Phyllurus amnicola Couper et al., 2000	Bowling Green Bay NP, QLD [S 19° 28' 50", E 146° 58' 59”]	saxicoline	hydric
Phyllurus nepthys Couper et al., 1993	Eungella NP, QLD [S 21° 08' 43", E 148° 29' 57"]	arboreal	hydric
Phyllurus ossa Couper et al., 1993	Airlie Beach, QLD [S 20° 20’ 08”, E 148° 40’ 19”]	saxicoline	hydric
Saltuarius cornutus (Ogilby, 1892)	Crater Lakes NP, Lake Eacham Section, QLD [S 17° 17' 12", E 145° 37' 17"]	arboreal	hydric
Diplodactylidae			
Amalosia rhombifer (Gray, 1845)	Airlie Beach, QLD [S 20° 20’ 08”, E 148° 40’ 19”]	arboreal	mesic
Diplodactylus ameyi Couper & Oliver, 2016	Winton, QLD [S 22° 27’ 26”, E 142° 57’ 39”]	terrestrial	xeric
Diplodactylus conspicillatus Lucas & Frost, 1897	Great Victoria Desert, SA [S 29° 21’ 07”, E 132° 41’ 57”]	terrestrial	xeric
Diplodactylus platyurus Parker, 1926	Mingela Range, QLD [S 20° 01’ 56”, E 146° 48’ 17”]	terrestrial	mesic
Diplodactylus tessellatus (Günther, 1875)	Boulia, QLD [S 22° 35’ 50”, E 139° 42’ 59”]	terrestrial	xeric
Diplodactylus wiru Hutchinson et al., 2009	Ifould Lake, SA [S 30° 52’ 60”, E 132° 09’ 00”]	terrestrial	xeric
Lucasium damaeum (Lucas & Frost, 1896)	Great Victoria Desert, SA [S 29° 24’ 13”, E 132° 50’ 19”]	terrestrial	xeric
Lucasium immaculatum (Storr, 1988)	Winton, QLD [S 22° 28’ 34”, E 142° 55’ 45”]	terrestrial	xeric
Lucasium steindachneri (Boulenger, 1885)	Mingela Range, QLD [S 20° 08’ 06”, E 146° 52’ 32”]	terrestrial	mesic
Lucasium stenodactylum (Boulenger, 1896)	Great Victoria Desert, SA [S 29° 25’ 37”, E 132° 56’ 42”]	terrestrial	xeric
Oedura bella Oliver & Doughty, 2016	Mt. Isa, QLD [S 20° 51’ 42”, E 139° 27’ 42”]	saxicoline	xeric
Oedura castelnaui (Thominot, 1889)	Mingela Range, QLD [S 20° 22’ 06”, E 146° 57’ 39”]	arboreal	mesic
Oedura cincta De Vis, 1888	Winton, QLD [S 22° 27’ 18”, E 142° 58’ 18”]	arboreal	xeric
Oedura coggeri Bustard, 1966	Hidden Valley, QLD [S 19° 00' 06", E 146° 04' 47"]	saxicoline	mesic

Oedura monilis De Vis, 1888	Eungella NP, QLD [S 21° 10' 07", E 148° 30' 09"]	arboreal	mesic
Rhynchoedura ormsbyi Wells & Wellington, 1985	Winton, QLD [S 22° 28’ 34”, E 142° 55’ 45”]	terrestrial	xeric
Strophurus krisalys Sadlier, 2005	Mt. Isa, QLD [S 20° 35' 51", E 139° 34' 10"]	arboreal	xeric
Strophurus taeniatus (Lönnberg & Anderson, 1913)	Mt. Isa, QLD [S 20° 49' 30", E 139° 27' 42"]	arboreal	mesic
Strophurus williamsi (Storr, 1983)	Mingela Range, QLD [S 20° 12' 56", E 146° 52' 20”]	arboreal	mesic

Table 2.2: Morphometric and scale measurements for all species in this study. Sample size indicates the number of specimens subject to morphometric measurements. When two scale sizes are given, the higher number is for the scales along the dorsal midline, while the smaller number is for the remaining dorsal body area.

Species	Sample size	SVL [mm]	Mass [g]	Granule size	Intergranule size
Carphodactylidae					
Carphodactylus laevis	*2*	110.24 ± 13.09	28.52 ± 2.65	0.06 ± 0.02	0.01 ± 0.003
Nephrurus asper	*11*	91.84 ± 7.87	18.42 ± 6.13	0.04 ± 0.01	0.01 ± 0.001
Nephrurus laevissimus	*2*	59.79 ± 1.58	4.06 ± 048	0.02 ± 0.004	0.001 ± 0.0003
Nephrurus levis	*3*	68.75 ± 13.41	8.76 ± 3.28	0.03 ± 0.01	0.004 ± 0.001
Phyllurus amnicola	*1*	92.00	13.42	0.06 ± 0.01	0.01 ± 0.003
Phyllurus nepthys	*3*	87.17 ± 10.06	11.56 ± 4.26	0.03 ± 0.01	0.003 ± 0.001
Phyllurus ossa	*5*	77.29 ± 7.05	8.36 ± 2.58	0.03 ± 0.01	0.003 ± 0.001
Saltuarius cornutus	*13*	134.27 ± 8.95	35.62 ± 9.02	0.07 ± 0.02	0.008 ± 0.002
Diplodactylidae					
Amalosia rhombifer	*6*	56.31 ± 3.21	3.01 ± 0.16	0.03 ± 0.01	0.004 ± 0.001
Diplodactylus ameyi	*3*	52.02 ± 4.33	3.20 ± 0.61	0.13 ± 0.02, 0.06 ± 0.01	0.01 ± 0.003, 0.01 ± 0.002
Diplodactylus conspicillatus	*2*	58.13 ± 0.33	4.02 ± 0.28	0.11 ± 0.01, 0.06 ± 0.01	0.01 ± 0.002, 0.01 ± 0.002
Diplodactylus platyurus	*8*	47.53 ± 2.96	1.98 ± 0.33	0.08 ± 0.01, 0.04 ± 0.01	0.003 ± 0.001, 0.004 ± 0.001
Diplodactylus tessellatus	*11*	47.31 ± 7.36	2.22 ± 0.61	0.13 ± 0.02, 0.08 ± 0.02	0.02 ± 0.003, 0.01 ± 0.002
Diplodactylus wiru	*2*	55.35 ± 5.37	4.93 ± 0.19	0.18 ± 0.03, 0.07 ± 0.01	0.01 ± 0.002, 0.01 ± 0.001
Lucasium damaeum	*2*	57.31 ± 3.76	2.45 ± 0.48	0.05 ± 0.01	0.01 ± 0.001
Lucasium immaculatum	*4*	51.74 ± 5.23	2.65 ± 0.76	0.03 ± 0.01	0.004 ± 0.001
Lucasium steindachneri	*26*	55.72 ± 3.58	2.72 ± 0.53	0.03 ± 0.01	0.004 ± 0.001

Lucasium stenodactylum	*2*	46.79 ± 13.11	2.3 ± 1.45	0.05 ± 0.01	0.01 ± 0.001
Oedura bella	*1*	77.41	10.97	0.3 ± 0.07	0.03 ± 0.007
Oedura castelnaui	*8*	92.09 ± 3.11	15.36 ± 2.82	0.26 ± 0.04	0.01 ± 0.003
Oedura cincta	*8*	90.48 ± 5.53	13.54 ± 1.78	0.24 ± 0.05	0.02 ± 0.004
Oedura coggeri	*7*	79.03 ± 3.57	7.34 ± 1.82	0.3 ± 0.05	0.02 ± 0.003
Oedura monilis	*15*	98.05 ± 5.75	16.04 ± 2.71	0.39 ± 0.07	0.03 ± 0.008
Rhynchoedura ormsbyi	*3*	49.38 ± 8.64	1.92 ± 0.49	0.02 ± 0.004, 0.02 ± 0.003	0.003 ± 0.001, 0.003 ± 0.001
Strophurus krisalys	*16*	70.29 ± 6.19	5.32 ± 1.47	0.1 ± 0.03	0.02 ± 0.003
Strophurus taeniatus	*1*	43.58	1.16	0.04 ± 0.01	0.01 ± 0.001
Strophurus williamsi	*19*	58.52 ± 4.98	3.14 ± 0.64	0.07 ± 0.02	0.01 ± 0.002

Table 2.3: Measurements of spinules, pits and percentage of area covered by knobs or hillocks (knobbiness index). Sample size indicates the number of specimens subject to morphometric measurements

Species	**sample size**	**Spinule length (µm) (n = 50)**	**Spinule density (per 10 µm²) (n = 3)**	**Pit diameter (µm) (n = 40)**	**Pit density (per 5 µm²) (n = 3)**	**Knobbiness index (%) (n = 10)**
Carphodactylidae						
Carphodactylus laevis	1	0.73 ± 0.07	477 ± 12.2	0.24 ± 0.03	232 ± 5.3	11.21
Nephrurus asper	2	0.28 ± 0.004	112 ± 3.8	0.25 ± 0.04	164 ± 6.5	6.85
Nephrurus laevissimus	1	0.42 ± 0.05	347 ± 10.1	0.28 ± 0.02	164 ± 12.5	11.32
Nephrurus levis	2	0.3 ± 0.07	62 ± 4.5	0.29 ± 0.03	151 ± 3.1	4.32
Phyllurus amnicola	1	0.47 ± 0.06	174 ± 18.9	0.29 ± 0.04	138 ± 5.6	10.09
Phyllurus nepthys	2	0.54 ± 0.1	170 ± 25.5	0.3 ± 0.03	186 ± 6.7	29.81
Phyllurus ossa	1	1.05 ± 0.16	75 ± 5.5	0.33 ± 0.04	144 ± 6.6	25.97
Saltuarius cornutus	1	0.46 ± 0.07	120 ± 1.7	0.28 ± 0.03	170 ± 4.7	5.53
Diplodactylidae						
Amalosia rhombifer	1	0.79 ± 0.1	112 ± 3.2	0.32 ± 0.03	163 ± 6.1	0
Diplodactylus ameyi	1	0.91 ± 0.07	170 ± 1.5	0.22 ± 0.03	130 ± 5.5	0
Diplodactylus conspicillatus	1	0.99 ± 0.14	169 ± 6.03	0.25 ± 0.03	160 ± 3.5	0
Diplodactylus platyurus	1	0.98 ± 0.09	144 ± 5.5	0.36 ± 0.04	134 ± 3.1	0

Diplodactylus tessellatus	1	0.48 ± 0.05	343 ± 26.8	0.31 ± 0.03	180 ± 3	0
Diplodactylus wiru	1	0.28 ± 0.05	159 ± 12.1	0.34 ± 0.04	135 ± 10.97	0
Lucasium damaeum	2	0.98 ± 0.14	213 ± 9.6	0.26 ± 0.02	191 ± 5.03	0
Lucasium immaculatum	1	1.03 ± 0.17	84 ± 6.3	0.31 ± 0.05	152 ± 4.6	0
Lucasium steindachneri	1	0.94 ± 0.09	130 ± 7.5	0.26 ± 0.02	182 ± 6.8	0
Lucasium stenodactylum	1	0.97 ± 0.1	115 ± 3.4	0.24 ± 0.02	181 ± 4.5	0
Oedura bella	1	0.77 ± 0.09	300 ± 10.1	0.29 ± 0.03	162 ± 5.5	0
Oedura castelnaui	2	0.97 ± 0.1	269 ± 14.4	0.39 ± 0.04	127 ± 5.03	0
Oedura cincta	1	0.97 ± 0.1	274 ± 10.6	0.3 ± 0.03	174 ± 2.1	0
Oedura coggeri	3	0.84 ± 0.08	287 ± 0.6	0.25 ± 0.03	267 ± 2.6	0
Oedura monilis	2	0.86 ± 0.07	232 ± 7.4	0.29 ± 0.03	158 ± 5.03	0
Rhynchoedura ormsbyi	3	1.12 ± 0.13	77 ± 2.4	0.26 ± 0.03	233 ± 10.4	0
Strophurus krisalys	2	0.43 ± 0.07	255 ± 11.5	0.26 ± 0.03	112 ± 3.1	0
Strophurus taeniatus	1	0.96 ± 0.09	95 ± 5.8	0.23 ± 0.03	233 ± 7.4	0
Strophurus williamsi	1	0.43 ± 0.05	117 ± 4.04	0.29 ± 0.03	126 ± 13.2	0

Table 2.4: Overview of the measurements taken of the cutaneous sensilla. This table also indicates the absence or presence of lenticular sense organs. Sample size indicates the number of specimens subject to morphometric measurements.

Species	Sample size	Cutaneous sensilla (CS) per scale (n = 10)	CS per mm²	Bristles per sensor (n = 10)	Bristles per mm²	CS diameter (μm) (n = 10)	Lenticular sense organs
Carphodactylidae							
Carphodactylus laevis	1	3 - 8	71	2 – 6	143	22.60 ± 1.60	-
Nephrurus asper	2	0 - 2	50	4 – 7	200	24.51 ± 1.68	-
Nephrurus laevissimus	1	1 - 2	60	5 – 9	300	22.36 ± 1.23	-
Nephrurus levis	2	1	20	4 – 6	80	24.25 ± 0.86	-
Phyllurus amnicola	1	5 - 10	75	1	75	22.57 ± 1.14	Y*
Phyllurus nepthys	2	1 - 3	7	1	7	23.39 ± 1.76	-
Phyllurus ossa	1	1 - 3	13	1	13	26.11 ± 0.91	-
Saltuarius cornutus	1	0 - 5	63	1 – 2	63	21.69 ± 1.45	-
Diplodactylidae							
Amalosia rhombifer	1	1	33	0	0	19.98 ± 1.07	Y
Diplodactylus ameyi	1	1 - 3	33	1	33	15.82 ± 1.78	Y

Diplodactylus conspicillatus	1	2 - 4	167	1	167	15.60 ± 0.75	Y
Diplodactylus platyurus	1	1 - 3	35	1	35	15.60 ± 0.86	Y
Diplodactylus tessellatus	1	3 - 6	50	1 - 2	50	14.56 ± 1.39	Y
Diplodactylus wiru	1	2 - 5	100	1	100	16.00 ± 1.23	Y
Lucasium damaeum	2	0 - 1	133	1	133	17.89 ± 1.64	Y
Lucasium immaculatum	1	0 - 1	75	1	75	20.07 ± 0.74	-
Lucasium steindachneri	1	0 - 1	67	1	67	20.17 ± 0.63	Y
Lucasium stenodactylum	1	1 - 3	13	1	13	17.49 ± 1.24	Y
Oedura bella	1	1 - 2	50	1	50	18.88 ± 0.45	-
Oedura castelnaui	2	5 - 9	20	1	20	19.18 ± 1.26	Y
Oedura cincta	1	2 - 3	33	1	33	18.72 ± 0.45	Y
Oedura coggeri	3	2 - 4	100	1	100	19.04 ± 1.93	Y
Oedura monilis	2	11 - 14	14	1	14	17.78 ± 0.92	Y
Rhynchoedura ormsbyi	3	1	86	1	86	17.26 ± 0.63	-
Strophurus krisalys	2	1 - 2	100	0	0	19.88 ± 1.02	-
Strophurus taeniatus	1	1 - 3	36	0	0	18.20 ± 1.02	-
Strophurus williamsi	1	1	33	1	33	17.84 ± 0.79	-

Table 2.5: The best branch length type (Best Tree) is shown for each trait, derived from the Pearson correlation between PIC and their standard error (Garland et al. 1992). For each trait and tree, the respective r and p values are shown as well.

Trait	Best Tree	r value	p value
bristles/mm²	grafen	-0.0342	0.8684
CS diameter	pagel	-0.0037	0.9855
CS/mm²	grafen	-0.1460	0.4768
Humidity	pagel	0.1220	0.5528
Habitat	pagel	-0.2814	0.1638
knobbiness index	grafen	0.1603	0.4340

LSO	grafen	0.0728	0.7239
Mass	pagel	-0.0832	0.6861
Pit density	grafen	0.1515	0.4599
Pit diameter	grafen	-0.2071	0.3100
Granule size	pagel	-0.1215	0.5543
Intergranule size	grafen	0.0705	0.7321
spinule density	grafen	0.1587	0.4387
spinule length	grafen	0.0943	0.6468
SVL	pagel	-0.1002	0.6261
bristles/CS	pagel	0.0939	0.6482
CS/scale	grafen	-0.1383	0.5005

Table 2.6: Classification of habitat use **(A)** and the humidity of the habitat **(B)** of the phylogenetic Flexible Discriminant (pFDA) analysis of skin characteristics compared to the actual habitat or humidity use of the species respectively. Lambda = 0 for both analyses.

A)		**Habitat**		
	terrestrial	arboreal	saxicoline	% correct
predicted as				
terrestrial	13	0	0	100%
arboreal	0	10	0	100%
saxicoline	0	0	4	100%
total	13	10	4	**100%**

B)		**Humidity**		
	hydric	mesic	xeric	% correct
predicted as				
hydric	4	0	0	80%
mesic	1	6	2	67%
xeric	0	3	11	85%
total	5	9	13	**78%**

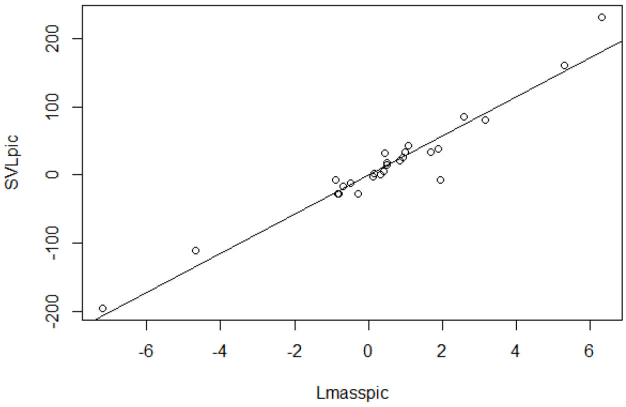
Table 2.7: Classification performance measures of Garczarek (2002) for the both analysis (habitat and humidity). CR = correctness rate; AC = accuracy; AS = ability to separate; CF = confidence; CFvec = confidence for each class.

	Habitat	**Humidity**
Lambda	**0**	**0**
CR	1.0000	0.7778
AC	0.9284	0.5307
AS	0.9284	0.7270
CF	0.9586	0.8416
CF vec	**terrestrial**	**hydric**
	0.9999	0.9213
	saxicoline	**mesic**
	0.9199	0.8454
	arboreal	**xeric**
	0.9214	0.8083

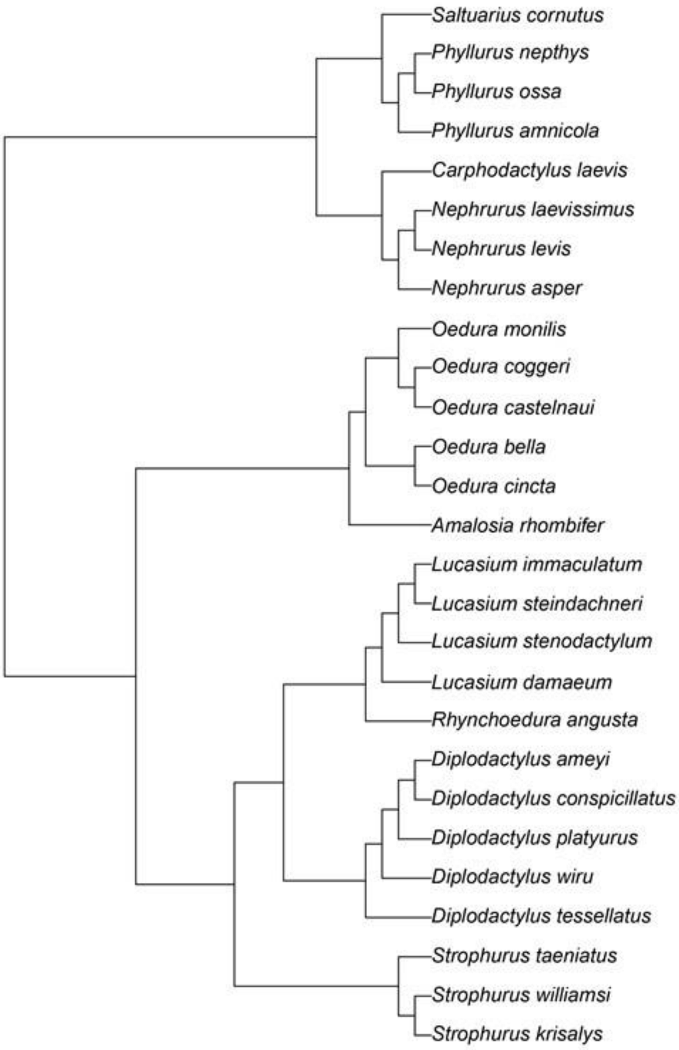
Table 2.8: Model parameter for the best fitting model for morphological traits which showed significant results ($p < 0.05$) after adjusting for False Discovery Rate.

response variable	**Model**	**mode of evolution**	**df**	**log likelihood**	**AICc**	**ΔAICc**	**weight**	**significant differences between**
Bristles / mm²	2 (habitat + SVL + Lmass)	independent	6	-39.3	94.8	0	0.68	terrestrial & arboreal
CS / mm²	3 (habitat)	Pagel’s lambda	5	-11.884	36.6	0	1	terrestrial & arboreal terrestrial & saxicoline

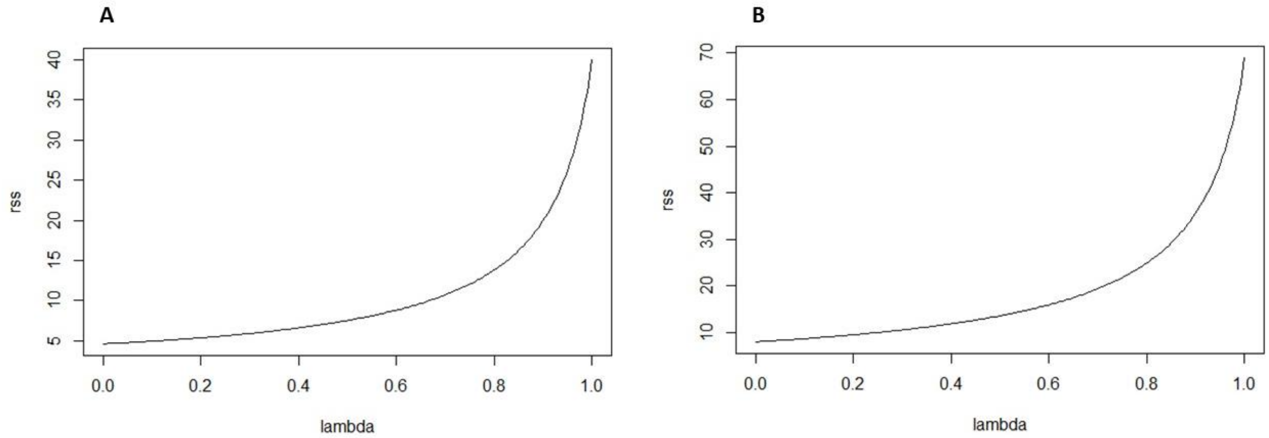
Supplementary material



Supplementary figure 2.1: Correlation between SVL and body mass displayed as phylogenetic independent contrast values of SVL (SVLpic) and of log body mass (Lmasspic).



Supplementary figure 2.2: Supertree of the species used in this analysis. Constructed with matrix representation with parsimony (MRP). Branch lengths are Grafen's arbitrary branch lengths.



Supplementary figure 2.3: The optimal lambda values are plotted against the residual sum squares (rss) for the pFDA analysis of **(A)** the habitat, and **(B)** the humidity of the environment.

Table S2.1: pFDA coefficients

	Habitat		**Humidity**	
Variable	**pFDA1**	**pFDA2**	**pFDA1**	**pFDA2**
Percentage	91.92%	100.00%	90.49%	100.00%
Trait				
bristles/mm²	0.2377	0.4958	0.4565	-0.1907
CS diameter	-0.2153	**3.7812**	**2.1635**	**-4.9336**
CS/mm²	-0.1441	0.0392	-0.1845	0.3149
knobbiness index	**-3.5371**	**3.8806**	**6.5594**	**1.8777**
Pit density	-0.1077	0.4049	0.0157	**-1.0029**
Pit diameter	-0.4255	**4.4925**	**3.3763**	-0.5957
Granule size	**-11.5532**	**-2.6780**	**-13.9033**	0.5382
Intergranule size	**67.2750**	**85.5005**	**113.9751**	**40.8107**
spinule density	0.1959	-0.2157	0.3800	-0.1255
spinule length	**3.4062**	0.4343	0.3940	0.8651
bristles/CS	**1.0754**	**-1.5472**	**-1.1059**	**1.2976**
CS/scale	0.0287	0.2945	0.5108	-0.8789

pFDA coefficients are given for each trait, for both axis of both analyses. Traits which have a strong influence on each respective axis are marked in bold.

Chapter 3 - Skin hydrophobicity as an adaptation for self-cleaning in geckos

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Abstract

Hydrophobicity is common in plants and animals, typically caused by high relief microtexture functioning to keep the surface clean. Although the occurrence and physical causes of hydrophobicity are well understood, ecological factors promoting its evolution are unclear. Geckos have highly hydrophobic integuments. I predicted that, because the ground is dirty and filled with pathogens, high hydrophobicity should coevolve with terrestrial microhabitat use. Advancing contact angle (ACA) measurements of water droplets were used to quantify hydrophobicity in 24 species of Australian gecko. I reconstructed the evolution of ACA values, in relation to microhabitat use of geckos. To determine the best set of structural characteristics associated with the evolution of hydrophobicity, I used linear models fitted using phylogenetic generalized least squares (PGLS), and then model averaging based on AICc values. All species were highly hydrophobic (ACA > 132.72°), but terrestrial species had significantly higher ACA values than arboreal ones. The evolution of longer spinules and smaller scales were correlated with high hydrophobicity. These results suggest that hydrophobicity has co-evolved with terrestrial microhabitat use in Australian geckos via selection for long spinules and small scales, likely to keep their skin clean and prevent fouling and disease.

Key words: Evolution, contact angle, ancestral state reconstruction, ecomorphology, Gekkota, hydrophobic surface properties, integument, PGLS

Introduction

The study of evolutionary morphology tackles questions examining how morphological traits may have evolved (Richter & Wirkner, 2014). Common study designs include mapping traits onto a phylogeny (Finarelli & Flynn, 2006; Hwang & Weirauch, 2012; King & Lee, 2015) or identifying correlations between traits and ecological factors (Kohlsdorf *et al.*, 2001; Rothier *et al.*, 2017; Mihalitsis & Bellwood, 2019; Riedel *et al.*, 2019 [Chapter 2]). Although these studies provide useful insights into the complex evolution of organisms and potential processes of adaptation, they only constitute the starting point of evolutionary research. Morphological traits often evolve to serve certain (and often multiple) functions, and therefore the ecomorphology paradigm predicts that natural selection does not act on the morphological traits directly, but rather on their function (Kluge, 1983; Garland Jr & Losos, 1994). Functional morphological units often consist of complex multi-trait structures, and there are multiple ways for a functionally complex organ to evolve and be optimised for particular outcomes, which can be conceptualised with many-to-one mapping (Alfaro *et al.*, 2005; Wainwright, 2007).

However, if complex traits are analysed as single traits, results can be misleading or difficult to interpret, so evolutionary morphology studies should address the evolution of functional outcomes directly (Garland Jr & Losos, 1994; Hagey *et al.*, 2017b; Tiatragul *et al.*, 2017; Russell & Gamble, 2019).

The integument, which is the outermost layer of an organism, is a good example of a complex organ that serves multiple functions, the most important of which is protection from the surrounding environment (e.g., water loss, ultra-violet radiation, pathogens, and predators). The integument can also be used for intra- or inter-specific communication (Schliemann, 2015), locomotion (Maderson & Alibardi, 2000; e.g. feathers in birds; Clarke, 2013; Homberger & de Silva, 2015) , or adhesion (e.g. in anoles or geckos; Maderson, 1964; Russell, 2002).

To ensure its functionality, the integument must be kept clean of dirt or debris, which may interfere with its functions. The ability to shed dirt is essential to prevent wear and tear (Irish *et al.*, 1988), to reduce the accumulation of excess weight, to avoid interfering with crypsis, signalling, or other specialised functions (Gans & Baic, 1977; Arnold, 2002; Hansen & Autumn, 2005), and to reduce exposure to pathogens (Neinhuis & Barthlott, 1997; Watson *et al.*, 2015b). Various functional and behavioural mechanisms have evolved to keep living surface structures clean and pathogen-free. A common behavioural mechanism is grooming (Sparks, 1967; i.e. Bauer, 1981), and shedding may assist in dirt removal (Böhme & Fischer, 2000; Fushida *et al.*, 2020 [Appendix I]). A common structural solution to fouling is increased surface hydrophobicity (Wagner, Neinhuis, & Barthlott, 1996; Wagner *et al.*, 2003; Neinhuis & Barthlott, 1997; Fusetani, 2004). This "self-cleaning" phenomenon, often termed the "lotus effect" (Barthlott & Neinhuis, 1997; Carbone & Mangialardi, 2005), helps to avoid fouling (Neinhuis & Barthlott, 1997; Watson *et al.*, 2015a) or enable floating (Gao & Jiang, 2004; Perez-Goodwyn, 2009) and occurs in plants, insects, and vertebrates (Neinhuis & Barthlott, 1997; Wagner *et al.*, 2003; Hiller, 2009; Spinner *et al.*, 2013a).

Hydrophobic surfaces repel water *via* surface texture or chemistry, such that the forces within the water droplet are stronger than those attracting the water to the surface, therefore, water beads on hydrophobic surfaces (Cassie & Baxter, 1944). A surface is defined as hydrophobic if the contact angle of a drop of water placed on that surface is greater than 90°, and superhydrophobic if the contact angle is greater than 150° (Li, Reinhoudt, & Crego-Calama, 2007). In structural hydrophobicity, hydrophobic properties are enhanced by increasing surface roughness, a product of complex hierarchical microstructures, which greatly reduce the contact angles of water droplets (Cassie & Baxter, 1944; Barthlott & Neinhuis, 1997; Shah & Sitti, 2004).

The occurrence and function of hydrophobicity has been well studied in plants (Neinhuis & Barthlott, 1997, 1998; Wagner *et al.*, 2003) and insects (Byun *et al.*, 2009; Watson *et al.*, 2010; Voigt, Boeve, & Gorb, 2012). For example, the wings of insects are covered with self-cleaning microstructures, thought to maintain flying ability (Wagner *et al.*, 1996; Watson *et al.*, 2010), and in plants, hydrophobic self-cleaning surfaces may protect against harmful microorganisms, which are growth-inhibited by the dry plant surface (Neinhuis & Barthlott, 1997). In some plants (e.g., water fern [Salvinia]), hydrophobic leaf surfaces ensure efficient gas exchange by keeping a thin film of air clinging to the surface when the leaves are submerged (Cerman, Striffler, & Barthlott, 2009). Underwater breathing has also evolved in insects (especially Heteroptera) and spiders, where some species associated with water form a thin layer of air (plastron) around the body (Perez-Goodwyn, 2009; Stratton & Suter, 2009), and potentially also in some *Anolis* lizards (Swierk, 2019). Another function of hydrophobic skin surfaces is to prevent submersion, allowing animals to live on or at the water surface (e.g., water striders (Gerrophora, Heteroptera) (Gao & Jiang, 2004) and spiders (Stratton & Suter, 2009). In contrast, the hydrophobic properties of the integument of vertebrates have not been well-documented (but see Hiller, 2009; Watson *et al.*, 2015b; Nirody *et al.*, 2018; Stark & Mitchell, 2019). More importantly, while the physical principles and function of hydrophobic surfaces is well understood (Li *et al.*, 2007), it is unclear which selective pressures promote the evolution of this functional adaptation.

My study aims to analyse the evolution of hydrophobicity, specifically hydrophobic surface characteristics, using geckos (Gekkota) as a model system. Geckos are an ideal model in that they are a successful, species-rich clade with a worldwide distribution (Uetz *et al.*, 2019; Meiri, 2020), and their skin surfaces are hydrophobic because of small microstructures (spinules) covering the outermost layer of the epidermis (Ruibal, 1968; Hiller, 2009). Recent studies have demonstrated that these microstructures are not only hydrophobic and lead to self-cleaning, but spinules are also bactericidal, making them a good example of multi-functional morphological traits (Watson *et al.*, 2015a,b). This bactericidal quality occurs because larger bacteria are pierced by the spinules and smaller bacteria get damaged by stretching and tearing between spinules (Watson *et al.*, 2015a; Li *et al.*, 2016).

If hydrophobicity has evolved to promote self-cleaning and bactericidal functions in geckos, I expect that terrestrial species would be better adapted for self-cleaning, and possess better bactericidal skin properties than species using other habitats, as dust, dirt, debris, and bacteria tend to accumulate on the ground (Ungar *et al.*, 1995; Nunn *et al.*, 2000; McCabe *et al.*, 2015). Conversely, as microbial growth rates are rapid in humid rainforest habitats, compared with more arid environments, and as high humidity enhances soiling because water facilitates the spread of dirt particles, hydrophobic and bactericidal skin properties may also be more prominent in species from habitats with a higher average humidity (Arnold, 2002; Bouskill *et al.*, 2012).

A recent study of Australian geckos found that long, dense spinules, in combination with small scales, were associated with terrestrial microhabitats, but not with a high habitat humidity (Riedel *et al.*, 2019 [Chapter 2]). According to the ecomorphology paradigm, trait selection should operate on the function of a trait, in this case, on its hydrophobic, self-cleaning, and bactericidal properties, instead of directly on the morphology of the traits, scale size and spinule length, density, or morphology. Therefore, if the association between small scales and long and dense spinules is truly an adaptation to terrestrial microhabitats, these morphological traits should affect the proposed function (hydrophobicity), which should in turn be the target of natural selection. Subsequently, this selection should be reflected in the evolutionary history of geckos (Kluge, 1983; Garland Jr & Losos, 1994). Thus, I would expect terrestrial gecko species to evolve hydrophobicity at greater rates than those of arboreal or saxicoline (rock-dwelling) gecko species, and that hydrophobicity has evolved in association with terrestrial microhabitat use. Using the same reasoning, species occupying habitats with high average humidity (e.g., rainforests) may also select for higher hydrophobicity compared with those from drier habitats (e.g., savannahs or deserts).

In this study, I examined the evolution of hydrophobicity of gecko skin, using the Diplodactyloid families Diplodactylidae and Carphodactylidae. The sessile drop technique (Kwok *et al.*, 1997; Drelich, 2013) was modified for use on living animals to quantify advancing contact angles (ACA) on 24 species, for which detailed measurements of skin microornamentation have been made previously (Riedel *et al.*, 2019 [Chapter 2]). I analysed the correlation of ACA values with both microhabitat use and habitat humidity in a phylogenetically informed context, using a modified version of a published phylogeny from Brennan and Oliver (2017). In addition, I reconstructed the ancestral states of the ACA values and microhabitat use and habitat humidity to test if these traits have evolved together. I predicted that 1) hydrophobicity has evolved together with terrestrial microhabitat use in geckos, and may be associated with habitats that have high humidity, and 2) hydrophobicity is primarily driven by relatively dense, long spinules and relatively small scale size. Both predictions are consistent with the hypothesis that the hydrophobic integument of Australian geckos has evolved as an adaptation to promote self-cleaning and bactericidal properties in a terrestrial microhabitat.

Material and Methods

STUDY SPECIES

Specimens of 24 species of Australian Carphodactylidae (6) and Diplodactylidae (18) were collected at night by hand, and habitat humidity (hydric, mesic, and xeric) (Bureau of Meteorology, 2018) and microhabitat use (terrestrial, arboreal, and saxicoline) were assigned to each species (Table 3.1) in the latter case using both data recorded at the time of collection and the literature (Cogger, 2018; Meiri, 2018; Nordberg & Schwarzkopf, 2019a). I classified white-striped geckos (*Strophurus taeniatus*) as 'terrestrial' for this analysis as this species is strongly associated with spinifex hummock or porcupine grass (*Triodia spp*.) (Nielsen *et al.*, 2016; Laver *et al.*, 2017), which is a very low growing plant, and in My study *S. taeniatus* was always found close to, or on, the ground (pers. obs.). After collection, healthy adult specimens were brought back to the laboratory to quantify hydrophobic properties. Microornamentation measurements were taken from Riedel et al. (2019 [Chapter II]), which were obtained from the same specimens used for the ACA measurements.

ADVANCING CONTACT ANGLE MEASUREMENTS

Increases in ACA of water droplets on surfaces are correlated with increasing hydrophobic properties of that surface (Li *et al.*, 2007). Therefore, the sessile drop technique was used to quantify contact angles of droplets of distilled water, incrementally increased in size (see below) and photographed at high resolution. The contact angles could then be used to predict the hydrophobicity of the dorsal skin surface (Li *et al.*, 2007). All contact-angle measurements were carried out under laboratory conditions at room temperature (23°C) between four and eight days after each individual shed its skin.

Measurements were only successful if the animals remained completely still and, to ensure this, their fore and hind limbs were outstretched and taped to the body using 3M Micropore™ tape, which was easily removable, leaving no residue. Limb immobilisation was accomplished without touching the dorsal scales to avoid affecting any dorsal scale microornamentation, or leaving chemical residues from fingers or tape. In addition to being restrained, it was necessary for each individual to present a flat surface for the accurate measurement of contact angles. To compensate for vertebral column unevenness and motion due to breathing, each lizard was rolled slightly to one side, to ensure that the area just lateral to the vertebral column was level. Movement due to breathing was mitigated by measuring contact angles towards the posterior of the torso on a flat area, and within the area assessed for skin microornamentation in Riedel et al. (2019 [Chapter 2]). If breathing movements affected the entire length of the body, tape was placed lengthwise along each side of the lizard for a short period (30 sec maximum), minimising breathing movements, without affecting the well-being of the lizard.

Once the lizard was immobilised and a suitable, level area on the dorsal surface was available (Figure 3.1A), ACA was quantified. Droplets were placed onto the lizard's body using an Eppendorf® pipette with a volume capacity of 0.1 - 2.5 µL and expanded slowly by adding water to the droplet in increments of 0.25 µL (Figure 3.1B). The initial, sessile drop was placed at the point at which the scales crested (Figure 3.1A), and the ACA values were attained by adding 0.25 µL to the sessile water droplet numerous times (Figure 3.1C). The expanding water droplet was photographed at each 0.25 µL increment, until the base of the drop appeared to "pop" out to the side or the droplet started to roll (Figure 3.1D). The ACA was measured on the photograph taken immediately before the drop "popped out" or rolled (Figure 3.1C). Although very difficult to see with the naked eye, on enlarged photographs ACA was clearly visible. Typically, less than 12 increments of 0.25 µL (3 µL) or 12 photographs were required to produce a drop suitable for an ACA measurement. Angles were measured using Image J (v. 1.36b, Schneider *et al.*, 2012) and median contact angles were calculated for each individual (individuals within each species were measured between 4-18 times on average, see Table 3.1) and used to calculate means for each species.

STATISTICAL ANALYSIS OF ECOLOGY AND HYDROPHOBICITY

Phylogenetic generalised least square analysis (PLGS) (Martins & Hansen, 1997; Revell & Collar, 2009) was used to examine the association between hydrophobicity (ACA measurements), and the microhabitat (terrestrial, arboreal, and saxicoline) and habitat humidity (hydric, mesic, and xeric) used by geckos, respectively. A modified version of the phylogenetic tree published by Brennan and Oliver (2017) was used, which included all the species from this study except for *Phyllurus nepthys*, for which no genetic sample was available. Therefore, I replaced *P. championae* on the tree with *P. nepthys*, as it is the closest relative for which data was available (Hoskin & Couper, 2013; C. Hoskin, pers. com.). The PGLS models were fitted comparing three different models of trait evolution, Brownian motion (λ = 1; 'BM') (Felsenstein, 1985), a relaxed maximum likelihood value of λ ('Pagel'), and without any phylogenetic signal in the trait evolution ('star model', λ set to 0) (Pagel, 1992). The ACA values were the response variable, and microhabitat and humidity were explanatory variables (both as fixed effects, combined in each model without an interaction term). Model averaging was then used (Bumham & Anderson, 2002), based on AIC_c, to combine the inferences from all valid models ($AIC_c < 2$), and type II ANOVAs (Langsrud, 2003), followed by a Tukey's *post hoc* test using the R package *emmeans* (R Core Team, 2018; Russell, 2018) to determine significant differences.

The ancestral states of ACA values were constructed using the ‘ace’ function from the *ape* package in R (Paradis, Claude, & Strimmer, 2004), and complemented with an ancestral state reconstruction of the explanatory variable(s). A "symmetrical" model was then fitted, which constrained forward transitions to be equivalent to backward transitions, as preliminary analysis demonstrated that there were not enough transitions to estimate a more complicated model (Schluter *et al.*, 1997; Pagel, 1999).

STATISTICAL ANALYSIS OF MORPHOLOGY AND PERFORMANCE

The morphological dataset from Riedel et al. (2019 [Chapter 2]) was used to analyse the influence of microornamentation and skin characteristics (both log-transformed) on ACA values, but excluded all cutaneous sensilla measurements, as cutaneous sensilla only covered a minute proportion of the skin surface and were unlikely to have a large effect on hydrophobicity. Therefore, seven measurements for microornamentation were used: spinule length (SL), spinule density (SD), diameter (PDM) and density (PDE) of the pits (small indentations between the spinules), the percentage of area covered by knobs (KI), and two measures of scale size (granule size [GS] and intergranule size [IGS]). Two sets of PGLS models were then constructed, one including all seven of these morphological traits (M1), and one containing only those traits that contributed strongly to separate the species by microhabitat (spinule length, pit diameter, knobbiness, granule size, and intergranule size) in Riedel et al. (2019 [Chapter 2]) (M2). To ensure the validity of these models, all traits were tested for multicollinearity (Mundry, 2014). As the scale size measurements for both types of scales (GS and IGS) are strongly correlated ($r(22) = 0.86$; $P < 0.001$), the above mentioned models were constructed twice, once containing only GS (M1G and M2G) and once containing only IGS (M1I and M2I). This produced four models with specific sets of predictor variables. Otherwise, the models were constructed using the same approach already described (see ‘Statistical analysis of ecology and hydrophobicity’). Notably, as the optimal λ value was inside a biologically meaningful range of 0 to 1 only for the M2I model, the optimal λ model was not fitted or used for all other models in the set of candidate models.

Results

ADVANCING CONTACT ANGLE MEASUREMENTS

All gecko species examined were hydrophobic, in that they had contact angles above 90°, but some species were more hydrophobic than others, including several that were superhydrophobic, with mean contact angles above 150° (Table 3.1). These included *L. steindachneri* (150.45 ± 0.39°), *A. rhombifer* (150.63 ± 0.70°), *S. taeniatus* (152.43 ± 1.14°), *D. conspicillatus* (152.60 ± 0.47°) and *L. damaeum* (155.58 ± 0.52°).

CORRELATION BETWEEN HYDROPHOBICITY AND MICROHABITAT

There was a significant effect of microhabitat on ACA in all models, such that terrestrial species had higher ACA values than arboreal ones, and there were no significant differences between the saxicoline species and the other two groups (Table 3.2B; Figure 3.2). Habitat humidity did not have a significant effect on ACA in any of the models (Table 3.2B). The optimal λ value of the Pagel model was 0.85 and both the Star model and the Pagel model had high support values (ΔAIC_c 0.00 and 0.36, respectively). However, the BM model also had reasonable support just outside the cut off (Delta $AIC_c = 2.01$; Table 2A), therefore an ANOVA (Type II) was used to determine significance of terms for all evolutionary models. The reconstructed ancestral states of the analysed traits are presented in Figures 3.3 and 3.4. The reconstructed evolution of the ACA values was reflected in the evolution of microhabitat use, and both traits had a similar evolutionary history (Fig. 3.3). Notable exceptions from this pattern are *Amalosia rhombifer*, which had a high ACA (150.63 ± 0.70°) despite being arboreal, and *Nephrurus asper*, which had a relatively low ACA (133.94 ± 0.33°) despite being terrestrial. The reconstructed evolution of habitat humidity use did not show any correlation with the evolution of ACA values (Fig. 3.4).

CORRELATION OF HYDROPHOBICITY AND MORPHOLOGY

Pagel's λ was only estimable ($\lambda = 0.13$) for the M2I model, which included the subset of traits and IGS. λ was negative for all other models, indicating a poor fit of the lambda model to the data. Thus, only the BM and Star models were used for all other trait combinations, producing nine estimate models (Table 3.3). The model comparison revealed two models with $\Delta AIC_C < 2$, namely the two variants of the M2 model, which included only the traits that were strongly correlated with microhabitat use in Riedel et al. (2019), and no phylogenetic signal in trait evolution assumed by the models (M2G.Star and M2I.Star; Table 3.3).

In both models, spinule length was significantly positively associated with ACA values, whereas the scale size trait (GS or IGS) included in each model was significantly negatively associated with ACA measurements. In the second-best model, which used IGS, pit diameter was also significantly negatively associated with the ACA measurements (Table 3.4).

Discussion

The outcomes of My study were consistent with My first prediction, and the hydrophobicity of geckos using terrestrial microhabitats was higher than those using arboreal habitats (Figure 3.2). In addition, I found that hydrophobicity and terrestrial microhabitat use have co-evolved (Figure 3.3). There was no support for the second part of My first prediction, that species from habitats with high humidity (rainforest) would have more hydrophobic skin (Figure 3.4). My second prediction, that hydrophobicity would be driven primarily by relatively long spinules and small scale size, was supported, such that longer spinules and smaller scale size were important predictors of higher hydrophobicity. Contrary to My prediction, spinule density had no effect on hydrophobicity, but pit diameter, which contributes to the spacing between spinules, was an important predictor in the model using inter-granule size, though not in the model using granule size. These results support My hypothesis that the hydrophobic integument of diplodactylid and carphodactylid geckos has probably evolved as an adaptation to keep their surfaces clean of dirt and debris and to inhibit the growth of potentially harmful bacteria prevalent in a terrestrial environment. Notably, the microhabitat was apparently a stronger selective force than was habitat humidity (Riedel *et al.*, 2019 [Chapter 2]). Hydrophobicity is related to highly irregular microscopic surface structures (Wagner *et al.*, 2003; Koch, Bhushan, & Barthlott, 2008) that, in the case of geckos, consist of small scales and long spinules (Ruibal, 1968; Hiller, 2009). My study confirms the importance of small scales and long spinules for the hydrophobic, self-cleaning, and bactericidal functions of gecko skin.

I developed a method to quantify the hydrophobic properties of lizard skin in living lizards for this study, and established that, while all geckos were hydrophobic, several species were superhydrophobic, and that most of the superhydrophobic species were terrestrial (Table 3.1). Although working with living lizards prevented us from strictly controlling humidity and vapour-level conditions, at the time of measurement as was suggested by Drelich (2013), all measurements were conducted under stable laboratory conditions (23°C and ~50% relative humidity). Combined with relatively high numbers of repeated measures, I received measurements with standard errors within the range of those (1 – 3°) reported by Drelich (2013).

Although the physical and functional basis of biological surfaces with hydrophobic properties has been well documented (Neinhuis & Barthlott, 1997; Carbone & Mangialardi, 2005; Autumn & Hansen, 2006; Zhai *et al.*, 2006; Stark *et al.*, 2015a,b), there has been little attention to the evolution of hydrophobic integumental properties. Associations of hydrophobic microstructures with aquatic or semi-aquatic microhabitats in Heteroptera (Perez-Goodwyn, 2009) are typically made in descriptive studies not analysed using evolutionary statistics or in a phylogenetic context. For plants, Tellechea-Robles et al. (2019) tested the hypothesis that plants from coastal wetlands would be more hydrophobic if they grew in areas that flooded regularly, compared with areas that stayed dry, but, the hypothesis was not supported by their study. Therefore, this is the first study to successfully test the predictions from a hypothesis about when and under which circumstances hydrophobicity may have evolved in nature, and provides phylogenetic statistical support for the evolution of hydrophobic surfaces as an apparent adaptation to the ecological pressures of living on the ground.

HYDROPHOBICITY IN GECKOS AND OTHER SQUAMATE REPTILES

The ACA measurements in this study were within the range measured by previous studies for geckos. The arboreal gecko *Phelsuma laticauda* has a relatively low ACA of 139° (Hiller, 2009), whereas the highly derived ground-dwelling legless gecko *Lialis jicari* (Pygopodidae) was superhydrophobic with an ACA of 160° on body regions not modified for their snake-like locomotion (Spinner *et al.*, 2013a). Both examples support My hypothesis that terrestrial gecko species should be more hydrophobic than arboreal species. Saxicoline species fall between arboreal and terrestrial species, overlapping with both. Saxicoline species live on rock walls and in crevices between boulders, which can range from a few centimetres to many meters above the ground, and thus species can be nearly terrestrial to almost never terrestrial, with their hydrophobicity likely varying in relation to their habitat requirements. Although variation in hydrophobicity was highest within saxicoline species, both arboreal and terrestrial species also varied considerably in their measured ACA. This variation could be correlated with differences in the rate of exposure to particle contamination within different microhabitats of the same category. For example, some tree species (e.g. paperbarks *Melaleuca spp.*) tend to be more granular and flakier than some others (e.g. iron barks *Eucalyptus* spp.), which may increase exposure to bark debris. Similarly, different soil types may lead to differences in exposure to dust particles. More detailed knowledge of microhabitat use, and particle exposure is necessary to elucidate this.

Long spinules as an adaptation to terrestrial microhabitats is particularly interesting in conjunction with the evolution of adhesive toepads. Adhesive toepads are adaptations to climbing, used in arboreal or saxicoline microhabitats (Russell, 1972, 2002). Setae, the microstructures generating adhesion in toepads, are proposed to have evolved by elongation of spinules (Ruibal & Ernst, 1965; Ernst & Ruibal, 1966; Russell *et al.*, 2015; Russell & Gamble, 2019). Therefore, hair-like microstructures in geckos appears to be an example in which a change in a morphological structure, which has evolved as an adaptation to one microhabitat, leads to a change in the trajectory of the adaptative potential of the changed morphological structure. For example, the elongation of spinules as an adaptation to terrestrial microhabitats, may contribute to the elongation of setae on the subdigital scales, which in turn may lead to an enhanced adaptive potential to occupy climbing (arboreal or saxicoline) microhabitats. The elongated setae on toepads are still highly hydrophobic, and the hydrophobic properties of the setae are important to maintain the adhesive properties of the toepads in geckos, especially for species from humid environments with high rainfall such as rainforests (Stark, Sullivan, & Niewiarowski, 2012; Stark *et al.*, 2015b; Stark & Mitchell, 2019). More detail on the relationship between spinule length and setae length of geckos from a range of habitats are required to examine evolution or co-evolution of these characters.

Geckos are not the only reptilian taxon featuring spinule-covered integuments. They share this trait with anoles and chameleons, as well as with some clades of skinks, agamids, and iguanids (Ruibal, 1968; Peterson, 1984a). Anoles are a prime model group for ecomorphological studies, but previous studies have focussed on morphometrics, such as relative limb dimensions, among species occupying different arboreal niches (Losos, 1990, 1992; Irschick *et al.*, 1997; Losos *et al.*, 1998), and have not examined the role of spinules. Chameleons are primarily arboreal, but also have exclusively terrestrial species. Interestingly, the mostly terrestrial leaf chameleons (*Brookesia* spp.), and the terrestrial Namib chameleon (*Chameleo namaquensis*) have evolved honeycomb microstructures instead of, or in addition to, spinules (Riedel *et al.*, 2015). Unfortunately, no studies on the hydrophobic properties of the integument of these two clades are available.

FUNCTION OF HYDROPHOBICITY IN GECKOS

The proposed function of hydrophobic surfaces in nature is to keep the surface of the integument free of dirt and debris, which can seriously obstruct other skin functions (Barthlott & Neinhuis, 1997; Hansen & Autumn, 2005; Watson *et al.*, 2015c). For this self-cleaning ability, the surface needs to be hydrophobic, and also must exhibit low adhesion forces for dirt particles. The integument of box-patterned geckos (*Lucasium steindachneri*) has extremely low adhesion of artificial fouling particles (Watson *et al.*, 2015a), consistent with the terrestrial microhabitat use of this species, and the high ACA measures found in the present study. This combination of high hydrophobicity and low adhesion of fouling particles results in efficient self-cleaning properties (Watson *et al.*, 2015c). Additional studies comparing self-cleaning and adhesion forces for dirt particles could further enhance My understanding of this functional link. The spinule-covered integument of geckos also has bactericidal properties (Watson *et al.*, 2015b; Li *et al.*, 2016). Because exposure to potentially harmful microorganisms is higher in a terrestrial microhabitat (Nunn *et al.*, 2000; McCabe *et al.*, 2015), and bacterial growth rates and thus prevalence of microorganisms may be higher in habitats featuring high humidity

(Bouskill *et al.*, 2012), bactericidal properties could be prevalent in both terrestrial microhabitats and habitats with high humidity (e.g., rainforests). I found support only for the former expectation.

Possibly, hydrophobicity in rainforest geckos prevents drowning. In some insects and spiders, hydrophobic integumental properties facilitate the prevention of submersion (Gao & Jiang, 2004; Stratton & Suter, 2009). Although geckos are normally not associated with aquatic ecosystems, some geckos have advanced swimming abilities due to their hydrophobic skin (Nirody *et al.*, 2018). Possibly, hydrophobicity may have evolved as an adaptation for species that are regularly threatened by flooding of their habitat. Under this hypothesis, I would expect terrestrial species to be more hydrophobic than arboreal species, but I would also expect stronger hydrophobic properties in rainforest habitats due to higher rainfall and more regular flooding. I would also expect saxicoline rainforest species (e.g., *Phyllurus amnicola* or *P. ossa*) to be strongly hydrophobic as they occur on boulders alongside rainforest streams. Therefore, drowning prevention is a plausible function promoting the evolution of hydrophobicity in some terrestrial geckos, but My results do not support drowning prevention as the dominant cause, because in My study, geckos from habitats most likely to flood were not the most hydrophobic. My results more clearly support self-cleaning as the main adaptive purpose for hydrophobicity, because in My study geckos from dusty environments were the most hydrophobic.

Another hypothetic function of hydrophobic surfaces could be to reduce evaporative chill caused by water accumulation on non-hydrophobic surfaces (Cowles, 1958). If this was the main function of hydrophobicity in geckos, I would expect species from habitats with higher average rainfall (like rainforests) to be more hydrophobic than species from drier habitats, and no difference among microhabitats (arboreal, saxicoline, terrestrial). As the opposite signal was found in My study, this hypothesis was not supported by My results.

Figures

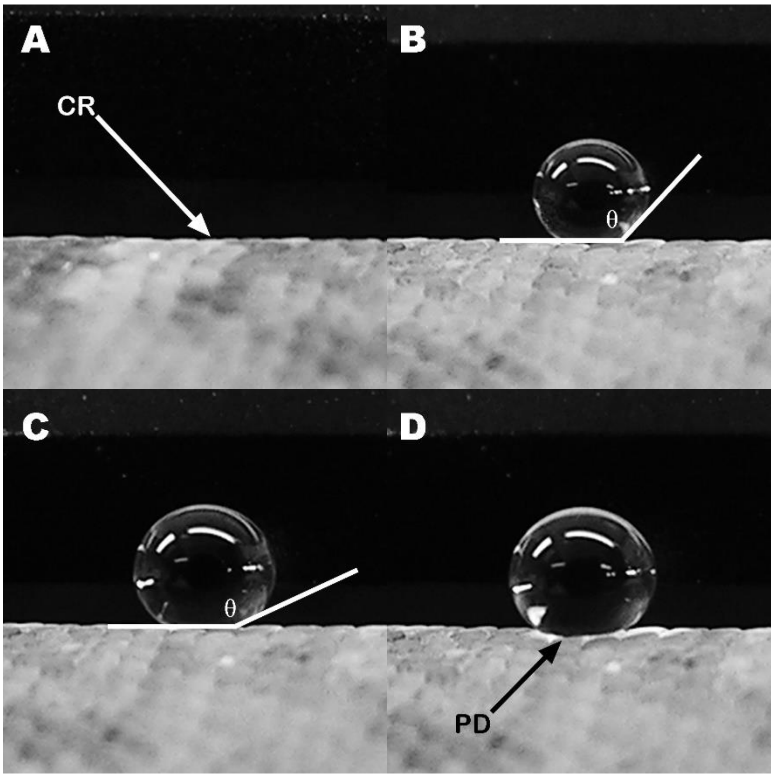

Figure 3-1 Photographic sequence of a “growing” drop used to obtain advancing contact-angle (ACA) measurements on the dorsal scales of geckos. **(A)** Initial image of dorsal surface with the exact location where the scales crested (CR). **(B)** Initial, sessile drop placed on the dorsal surface with a contact angle (θ). **(C)** Droplet just previous to that which “popped out” **(D)**, where θ represents the ACA that was obtained by adding 0.25 µl of distilled water to the initial water droplet (b) numerous times. (d) One increment after the ACA measurement (c) in which the droplet had popped out (PD) and, in this case, projected toward the camera

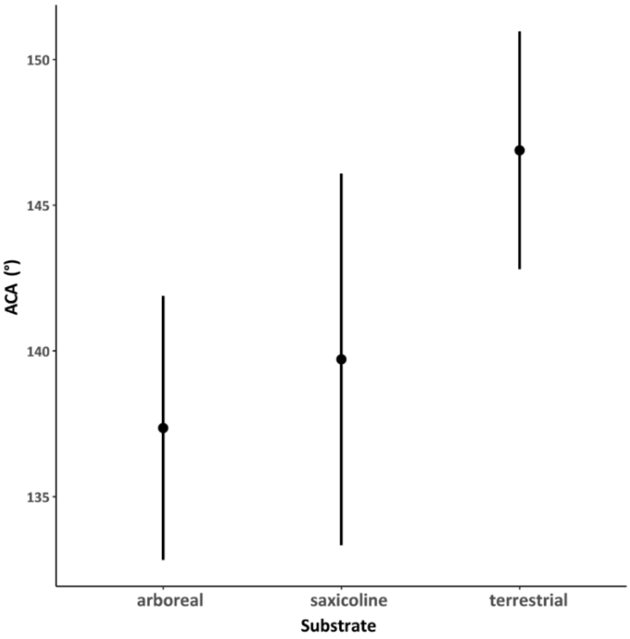

Figure 3-2 Effects plot of the best model (Star model with λ = 0) of the ACA values grouped by microhabitat use. Terrestrial species have significantly higher ACA values than arboreal species, with the saxicoline ones falling in between both

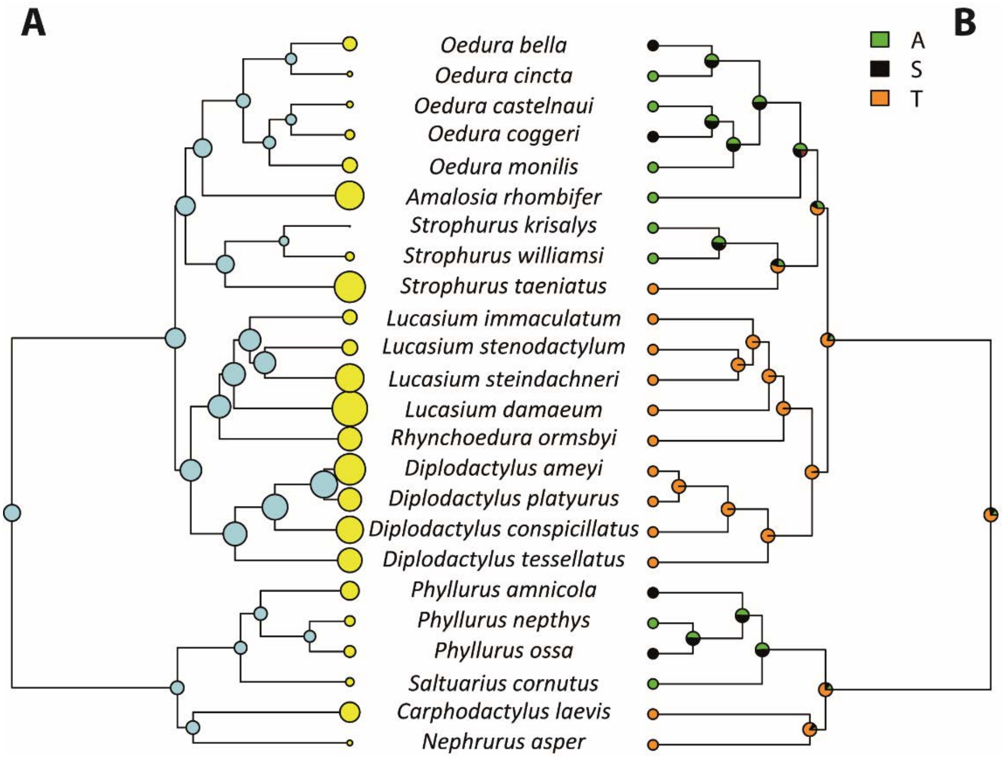

Figure 3-3 Reconstructed ancestral states of hydrophobicity **(A)** and microhabitat use **(B)**. For hydrophobicity, the size of the dots correlates with the ACA measurements for the species of this study (yellow dots) and reconstructed for the nodes (blue dots). For microhabitat use, the dots correspond to the reconstructed probability of microhabitat use for nodes: A (green), arboreal; S (black), saxicoline; T (orange), terrestrial. Note the correspondence between brown nodes (terrestrial species) and large yellow circles (hydrophobic species)

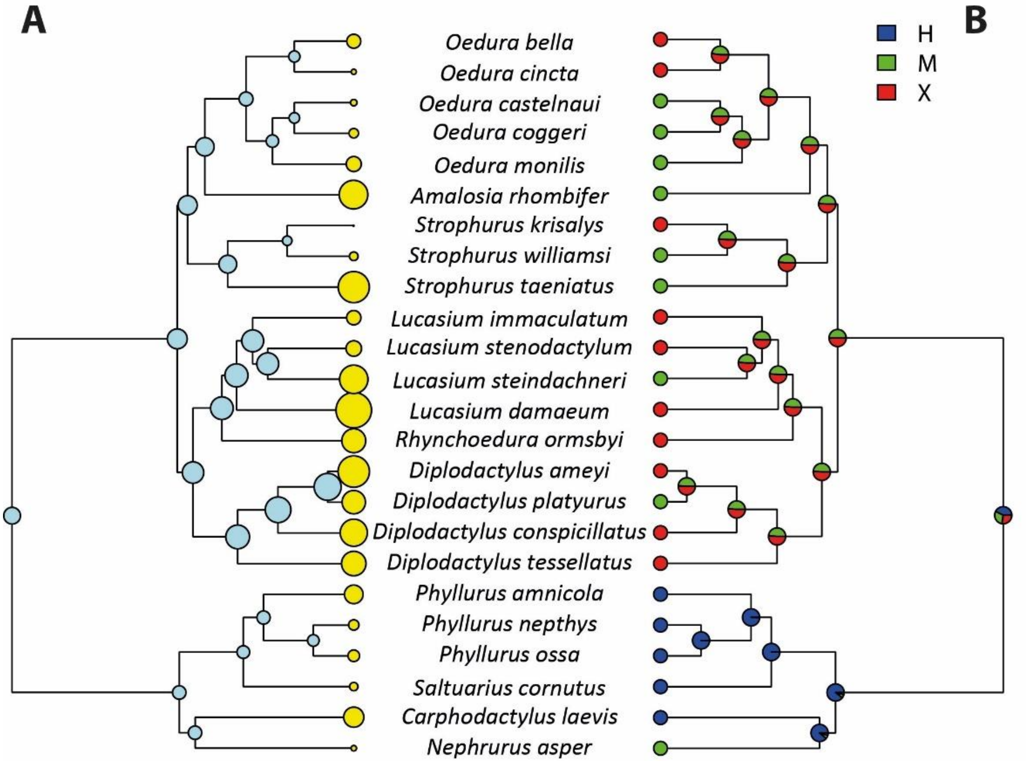

Figure 3-4 *Reconstructed ancestral states of hydrophobicity* ***(A)*** *and habitat humidity* ***(B)****. The hydrophobicity reconstruction is identical to Figure 3a. For habitat humidity, the dots correspond to the reconstructed probability of microhabitat use for nodes: H (blue), humid (rainforest); M (green), mesic (savanna); X (orange), xeric (desert) Neither high nor low hydrophobic species correspond with a particular habitat humidity regime*

Tables

Table 3.1 Microhabitat use, habitat humidity, and mean (± SE) advancing contact-angle (ACA) measurements for each species. The number of specimens measured for each species are given (NS), as well as the mean number of measurements for each individual per species (NI).

Species	Habitat	Humidity	ACA (°)	N_S	N_I
Carphodactylidae					
Carphodactylus laevis	Terrestrial	Hydric	144.49 ± 0.67	2	14
Nephrurus asper	Terrestrial	Mesic	133.94 ± 0.33	11	12
Phyllurus amnicola	Saxicoline	Hydric	143.40 ± 0.71	1	16
Phyllurus nepthys	Arboreal	Hydric	137.43 ± 0.86	3	12
Phyllurus ossa	Saxicoline	Hydric	138.32 ± 0.53	5	18
Saltuarius cornutus	Arboreal	Hydric	136.02 ± 0.32	13	10
Diplodactylidae					
Amalosia rhombifer	Arboreal	Mesic	150.63 ± 0.70	6	13
Diplodactylus ameyi	Terrestrial	Xeric	152.60 ± 0.47	3	8
Diplodactylus conspicillatus	Terrestrial	Xeric	149.57 ± 0.80	2	6
Diplodactylus platyurus	Terrestrial	Mesic	146.81 ± 0.40	12	11
Diplodactylus tessellatus	Terrestrial	Xeric	147.63 ± 0.57	12	9
Lucasium damaeum	Terrestrial	Xeric	155.58 ± 0.52	2	7
Lucasium immaculatum	Terrestrial	Xeric	140.32 ± 0.64	4	13
Lucasium steindachneri	Terrestrial	Mesic	150.45 ± 0.39	23	8
Lucasium stenodactylum	Terrestrial	Xeric	141.16 ± 0.61	2	14
Oedura bella	Saxicoline	Xeric	140.04 ± 0.79	1	16
Oedura castelnaui	Arboreal	Mesic	134.66 ± 0.41	9	12
Oedura cincta	Arboreal	Xeric	133.71 ± 0.41	9	14
Oedura coggeri	Saxicoline	Mesic	136.82 ± 0.38	7	14
Oedura monilis	Arboreal	Mesic	140.82 ± 0.49	15	10
Rhynchoedura ormsbyi	Terrestrial	Xeric	147.23 ± 1.07	7	8
Strophurus krisalys	Arboreal	Xeric	130.72 ± 0.45	16	9
Strophurus taeniatus	Terrestrial	Mesic	152.43 ± 1.14	1	4
Strophurus williamsi	Arboreal	Mesic	136.23 ± 0.37	19	10

Table 3.2 A) Results of the model selection for the different modes of trait evolution to test if hydrophobicity (ACA measurements) could be explained by either microhabitat use (substrate) or habitat humidity. **B)** P-values for each of the two explanatory variables for each model and results of a post-hoc pairwise comparisons for the significant variables.

A) model comparison

Mode of trait evolution	**df**	**log Likelihood**	**AIC$_C$**	**ΔAIC**	**AICc weight**	**Cumulated weight**
Star	6	-74.23	165.39	0	0.45	0.45
Pagel (λ = 0.85)	6	-74.41	165.76	0.36	0.38	0.83
BM	6	-75.23	167.41	2.01	0.17	1

B) ANOVA results

Explanatory variable	**df**	**χ^2**	**p**	Tukey's *Post hoc* pairwise comparison
Star model				
Substrate	2	11.473	**0.003**	**Terrestrial > Arboreal**
Habitat humidity	2	0.164	0.921	
Pagel model				
Substrate	2	8.016	**0.018**	**Terrestrial > Arboreal**
Habitat humidity	2	1.912	0.384	
BM model				
Substrate	2	6.369	**0.041**	**Terrestrial > Arboreal**
Habitat humidity	2	1.316	0.518	

Table 3.3. Model comparison for the morphological traits sorted by ΔAIC values. The two models with high support are highlighted in bold. Morphological traits (explanatory variables) are spinule length (SL), spinule density (SD), pit diameter (PDM), pit density (PDE), granule scale size (GS), intergranules scale size (IGS), and percentage of area covered by knobs (KI).

Model	**Explanatory variables**	**λ**	**df**	**Log likelihood**	**AIC$_C$**	**ΔAIC**	**AICc weight**	**Cum. weight**
M2G.Star	**SL, PDM, KI, GS**	0	6	-72.01	160.97	**0**	0.57	0.57
M2I.Star	**SL, PDM; KI, IGS**	0	6	-72.99	162.91	**1.95**	0.22	0.79
M2I.Pagel	SL, PDM, KI, IGS	0.13	6	-73.59	164.12	3.16	0.12	0.9
M1G.Star	SL, SD, PDM, PDE, GS, KI	0	8	-70.48	166.56	5.59	0.03	0.94
M2G.BM	SL, PDM, KI, GS	1	6	-74.89	166.71	5.75	0.03	0.97
M2I.BM	SL, PDM, KI, IGS	1	6	-75.39	167.72	6.76	0.02	0.99
M1I.Star	SL, SD, PDM, PDE, IGS, KI	0	8	-71.86	169.31	8.35	0.01	1
M1G.BM	SL, SD, PDM, PDE, GS, KI	1	8	-74.31	174.21	13.25	0	1

M1I.BM	SL, SD, PDM, PDE, IGS, KI	1	8	-75.1	175.8	14.81	0	1

Table 3.4: Predictors (morphological traits) in the two models with the highest support. Significant p-values are highlighted in bold and coefficients represent a positive or negative correlation with the ACA measurements.

Trait	**M2G.Star**				**M2I.Star**			
	coefficient	**P-value**	**χ^2**	**df**	**coefficient**	**P-value**	**χ^2**	**df**
Spinule length	49.24	**0.007**	7.09	1	49.24	**0.039**	4.23	1
Granule size	-84.44	**0.006**	7.52	1	NA	NA	NA	NA
intergranule size	NA	NA	NA	NA	-912.39	**0.019**	5.45	1
Pit diameter	-99.31	0.25	1.32	1	-99.31	**0.049**	3.89	1
Knobbiness	-56.67	0.15	2.07	1	-56.67	0.161	1.97	1

Chapter 4 - Parallel evolution of toepads in rock dwelling lineages of a terrestrial gecko (*Heteronotia binoei*, Gekkota: Gekkonidae)

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Abstract

Effective locomotion in complex habitats selects for specialized morphological adaptations. Adhesive toepads, which have arisen independently in different lizard clades, allow use of vertical and inverted substrates. Their evolution is poorly understood because functionally intermediate morphological configurations between pad-less terrestrial and pad-bearing climbing forms are rare. To shed light into toepad evolution, I assessed the subdigital morphology of phylogenetically distinct lineages of the Bynoe's gecko species complex (*Heteronotia binoei*). Most populations of *H. binoei* are terrestrial, but two distantly related lineages use saxicoline habitats and have enlarged terminal subdigital scales. I reconstructed the ancestral terminal subdigital scale size of nine lineages of *H. binoei* in north-east Australia, including the two saxicoline lineages. Additionally, I compared the subdigital microstructures of four lineages, the two saxicoline lineages and their respective terrestrial sister lineages. All four lineages had fully developed setae, but the setae of the two saxicoline lineages were significantly longer, branched more often, and had higher aspect ratios than those of the terrestrial sister clades. I conclude that the saxicoline lineages represent an example of parallel evolution of adhesive structures in response to vertical substrate use, and their morphology represents a candidate for an intermediate state in toepad evolution.

Key words: adaptation - adhesive system - setae – microornamentation – scanning electron microscopy (SEM) – species complex

Introduction

Effective locomotion in complex habitats is a challenging task for animals that can drive the evolution of specialised morphological structures. This can lead to similar morphological adaptations for locomotion arising independently in multiple taxa due to similar ecological and functional constraints, leading to parallel or convergent evolution (Cody, 1973; Ehleringer *et al.*, 1981; Losos *et al.*, 1998; Wiens *et al.*, 2006; Qi *et al.*, 2012; da Silva *et al.*, 2014). For example, many burrowing (fossorial) tetrapods feature an elongated snake-like body with reduced limbs and a compact, robust skull (Gans, 1975; Wiens & Slingluff, 2001). Similarly, species climbing on rocks (saxicoline) or vegetation (arboreal) often evolve modifications of the extremities for climbing, such as prehensile tails in some mammals and lizards (German, 1982; Meldrum, 1998; Zippel, Glor, & Bertram, 1999), zygodactyl feet in birds and chameleons for grasping small perches (Peterson, 1984b; Bock, 1999; Mayr, 2015; Molnar *et* *al.*, 2017), or subdigital adhesive structures in insects, frogs, and lizards, to adhere to vertical or inverted surfaces (Irschick *et al.*, 1996; Arzt *et al.*, 2003; Labonte *et al.*, 2016; Chan & Carlson, 2019). In some cases, morphological novelties can function as key innovations and facilitate adaptive radiations (Simpson, 1953; Schluter, 2000; Poe *et al.*, 2018).

Adhesive toepads have been proposed as an example of a key innovation influencing adaptive radiations in some lizards (Larson & Losos, 1996; Pianka & Sweet, 2005; Losos, 2010), but other studies have found no support for this hypothesis (Garcia-Porta & Ord, 2013; Kulyomina, Moen, & Irschick, 2019). Subdigital adhesive toepads have evolved independently in anoles, some skinks, and multiple times in geckos (Williams & Peterson, 1982; Russell, 2002; Irschick, Herrel, & Vanhooydonck, 2006; Gamble *et al.*, 2012, 2017; Russell & Gamble, 2019). Toepads provide these animals with astonishing climbing abilities that allow them to use previously inaccessible habitats, such as vertical or inverted rock-faces, tree-trunks, or leaf surfaces (Pianka & Sweet, 2005; Irschick *et al.*, 2006; Higham *et al.*, 2019). In anoles, distinct ecomorphs have evolved independently and repeatedly in different habitats, but their toepad morphology is rather uniform (Losos, 1990, 1994a, 2010; Glossip & Losos, 1997; Irschick *et al.*, 1997; Losos *et al.*, 1998). In contrast, geckos are an older clade with far more species and a worldwide distribution (Uetz *et al.*, 2019; Meiri, 2020), and show more variation in toepad morphologies, complicating the analysis of their evolution (Gamble *et al.*, 2012; Hagey *et al.*, 2017b; Russell & Gamble, 2019).

In the toepads of climbing lizards, adhesion is generated *via* microornamentation covering the subdigital scales: microfibrillar stalks (setae) that terminate in triangular tips (spatulae) (Maderson, 1964; Ruibal & Ernst, 1965; Hiller, 1968). These setae enhance the contact area with the substrate at the nano scale, generating Van-der-Waals forces (adhesion) in addition to shear forces (friction) (Autumn & Peattie, 2002; Autumn *et al.*, 2002; Arzt *et al.*, 2003; Gao *et al.*, 2005; Autumn, 2006; Tian *et al.*, 2006; Chan & Carlson, 2019). In geckos, setae often branch multiple times, further enhancing the contact area (Ruibal & Ernst, 1965; Peattie, 2001; Bhushan, 2007). Overall, the adhesive pads of geckos are especially complex, hierarchical structures normally consisting of multiple lamellae or scansors (laterally expanded scales) supported by specialised muscles, tendons, and sometimes bones (paraphalanges) that mediate the attachment and detachment of the lamellae (Russell, 1975, 1986; Russell & Bauer, 1988; Russell *et al.*, 2019).

While the function of the adhesive toepads is well understood, research interest has only recently turned to understanding evolution and ecological adaptations of toepads (Collins *et al.*, 2015; Hagey *et al.*, 2017b; Bauer, 2019; Kumar *et al.*, 2019; Naylor & Higham, 2019). Adhesive setae have evolved from shorter, more simple hair-like structures called spinules, covering the whole body surface of geckos, anoles, and many other lizards (including the subdigital scales of primarily terrestrial gecko species) (Ruibal & Ernst, 1965; Schleich & Kästle, 1986; Peattie, 2009). Within geckos, adhesive toepads have evolved multiple times (Russell, 1979; Peattie, 2001; Gamble *et al.*, 2012, 2017; Russell *et al.*, 2015), including multiple independent reductions (Lamb & Bauer, 2006; Higham *et al.*, 2015). This complex evolutionary history has led to two major toepad types: basal toepads, typically consisting of many small but broad lamellae in the middle and proximal region of the digits, and terminal toepads with two enlarged or fan-like lamellae at the distal part of the digit, which can be accompanied by additional lamellae along the more proximal digit regions (Russell & Bauer, 1989; Röll, 1995; Gamble *et al.*, 2012; Russell & Gamble, 2019) (see supporting information S4.1 for examples of terminal and basal toepads).

Comparative morphological studies are a useful method to reveal patterns in the evolution of complex structures, such as adhesive toepads (Russell & Gamble, 2019). Although there are many detailed studies of morphological variation in gecko toepads (Ruibal & Ernst, 1965; Schleich & Kästle, 1986; Peattie, 2001, 2009), evolutionary conclusions from these studies are often limited because extant forms are mostly either completely padless or have fully developed adhesive pads. A crucial step in understanding the evolution of complex structures, including adhesive toepads, is the examination of intermediate forms: populations or species that have evolved some, but not all, of the parts forming the complex structure in question (Russell, 1976; Peattie, 2009). In the case of toepad evolution in geckos, this would be a population or species with morphology somewhere between padless terrestrial and padded climbing forms that could, for example, either have evolved adhesive microstructures but no specialised internal support structures (specialised muscles or tendons), or have evolved microstructures that are structurally and functionally somewhere between unbranched spinules with round tips, and multiply branched, spatula-bearing (spatulated) setae (Peattie, 2009; Higham *et al.*, 2017; Russell & Gamble, 2019). Although there are a few candidate species macroscopically resembling an intermediate toepad morphology (Russell, 1976; Russell & Gamble, 2019), the only species studied in detail with an intermediate toepad morphology is the Trinidad Gecko *Gonatodes humeralis* (Guichenot, 1855), which has evolved relatively simple adhesive microstructures on slightly enlarged subdigital scales, with no modifications of the internal morphology typical of climbing forms (Russell *et al.*, 2015; Higham *et al.*, 2017), and retains the 'narrow-toed' digit morphology typical of terrestrial forms (Peattie, 2009). As these morphological changes have occurred primarily on the sub-inflexion scale in the middle of the digits, their intermediate toepad morphology supports an evolutionary series from a padless morphology to basal toepads, beginning with morphological changes at the proximal or central parts of the digits (Russell, 1976; Russell & Bauer, 1990b). But for terminal toepads a more distal origin of morphological changes as been suggested (Russell & Bauer, 1990b; Peattie, 2009) (S4.1).

Bynoe's geckos *Heteronotia binoei* (Gray, 1845) are gekkonid lizards distributed widely across Australia (Wilson & Swan, 2017; Cogger, 2018). Although *H. binoei* is currently recognised as a single taxon, phylogenetic analyses reveal a morphologically cryptic species complex comprised of a dozen or more independently evolving but currently undescribed species (Fujita *et al.*, 2010; Moritz *et al.*, 2016; Zozaya *et al.*, 2019). The species complex is normally regarded as either generalist or terrestrial (Pianka & Pianka, 1976; Henle, 1991; Meiri, 2018; Riedel, Nordberg, & Schwarzkopf, 2020 [Chapter 5]) and lacking the adhesive toepads typical of most climbing geckos (Gamble *et al.*, 2012; Cogger, 2018; Russell & Gamble, 2019). Recent fieldwork, however, has revealed that some populations living on granite outcrops in north-eastern Australia possess enlarged subdigital scales with the superficial appearance of terminal toepads (pers. obs.) (Fig. 4.1).

I hypothesised that these saxicoline populations possessed phenotypically intermediate terminal toepads, which, if so, provide a good opportunity for studying the evolution of adhesive toepads. I predicted that these saxicoline lineages could have evolved enlarged subdigital scales with longer, more complex adhesive microstructures (i.e., longer, more branched setae with flattened or triangular tips (Russell *et al.*, 2015; Higham *et al.*, 2017)). To test this, I first quantified microhabitat use and measured toepad size of individuals from both saxicoline and non-saxicoline populations of *H. binoei*, followed by phylogenetic analysis to assess relationships and determine the direction of toepad evolution among these populations (i.e., genetic lineages). Finally, I performed scanning electron microscopy (SEM) on specimens from four focal populations (two saxicoline groups and their respective generalist or terrestrial sister lineages) to test whether saxicoline populations possessed more complex adhesive microstructures.

Material and methods

APPROACH

For the first part of the study I sampled specimens from nine closely related lineages of *Heteronotia bionei* from north-eastern Australia (Fig. 4.1A), including all known granite outcrop occupying populations with enlarged subdigital terminal scales. For all these lineages, I quantified microhabitat use, performed a phylogenetic analysis, and measured and analysed the width of the subdigital terminal scales, including an ancestral state reconstruction of the size-corrected subdigital terminal scale width.

In the second part of the study, I selected four focal lineages for more detailed analysis. These were the two granite outcrop populations Paluma-W and CC (as a representative of the CC/MI/Paluma-E clade, see results), and Blencoe and EA6 as the ‘typical’ *H. binoei* lineages, which are the respective sister lineages to My granite outcrop lineages (in the case of EA6 to the whole granite outcrop clade) (Fig 4.1A, B). For these focal lineages, I measured and analysed the area of both the subdigital terminal and the sub-inflexion scales, and the microornamentation of the subdigital scales.

SAMPLING AND MICROHABITAT USE

I assessed microhabitat use for 208 individuals from nine deeply divergent genetic lineages of *H. binoei* from north-eastern Queensland, Australia. These included two distantly related saxicoline lineages: Paluma-W, and a monophyletic clade of three related sub-lineages (MI, CC, and Paluma-E) (Fig. 4.1; see Results). I captured 7–44 individuals per lineage and recorded if they occurred on rocks, on trees, or on the ground. I used these data to categorise each as terrestrial or saxicoline, if they spent more than 75% of all encounters on the ground or on rocks, respectively. Otherwise they were classified as generalist. Few specimens were encountered on trees; thus, none were classified as arboreal (see results).

To assess the size of the subdigital scales, I first sampled 85 specimens for My broader dataset of nine lineages, including the four lineages sampled for detailed analysis (see below), to confirm differences in scale size among saxicoline, terrestrial, and generalist lineages, and to determine the direction of the evolution of scale size (i.e., if the ancestors of My four focal lineages had larger or smaller terminal subdigital scales). Additionally, to test if scales were enlarged only on the terminal scales, or also on the inflexion point, I sampled 21 specimens of My four focal lineages for a more detailed measure of the area of both the terminal and the sub-inflexion scales. A total of 23 specimens of My four focal lineages (4–8 per lineage) were collected for scanning electron microscopy (SEM) analysis. All specimens sampled for morphometrics or SEM were euthanised using MS 222 injection (Conroy *et al.*, 2009), immediately set in 100% ethanol for 8–12 hours, and then stored in 75–80% ethanol.

DNA SEQUENCING AND ANALYSIS

I performed phylogenetic analysis to determine the relationships of the saxicoline populations of *H. binoei*. Three to eight individuals from each collection site were sequenced for *nd2* (NADH dehydrogenase subunit 2). DNA was extracted using the salting-out method of Sunnucks and Hales (1996) and amplified using the PCR primers tRNAI and tRNAA from Strasburg and Kearney (2005). I followed the PCR protocols of Fujita et al. (2010). The resulting sequences were edited and aligned with the sequence alignment from Zozaya et al. (2019) using the Geneious algorithm in Geneious version 6.1.8 (Drummond *et al.*, 2008). The alignment was visually checked and verified by translating the *nd2* coding region into amino acids. A phylogenetic tree was inferred using maximum-likelihood in RAxML version 8.2.11 (Stamatakis, 2006) with *Heteronotia planiceps* Storr, 1989 used as an outgroup. I applied the GTRCAT approximation of rate heterogeneity without codon partitions and performed a rapid bootstrap analysis with 100 bootstrap replicates for statistical support. Finally, I calculated uncorrected pairwise sequence divergence among lineages.

MORPHOMETRICS AND SUBDIGITAL SCALE AREA

I measured snout-to-vent length (SVL) of all specimens used in this study. For the broader sample of 85 specimens from nine lineages (3–19 per lineage), I measured the width of the terminal scales of the fourth toe of the left pes using a digital calliper. In addition, from 5–6 specimens of My four focal lineages, subdigital scale area was measured separately for the terminal and the sub-inflexion scale (21 in total, again from the fourth toe of the left pes). To do this I took photos of the subdigital side of the toe with an Apple iPhone 7 mounted with a Moment M-series macro lens (Donihue *et al.*, 2018). Photos were taken by placing a transparent petri dish on the lens with the respective gecko's left hindfoot (pes) placed on the petri dish so the terminal scale and the scale at the inflection point were flat on the surface. A small plastic mm ruler was taped flat against the petri dish to serve as a length reference for each photo. Area was measured with ImageJ V 1.52a (Schneider *et al.*, 2012) for both the terminal scale and the scale of the inflexion point of the fourth toe of the left pes. Each measured scale of each specimen was measured three times and then averaged to yield values for further statistical analysis.

SCANNING ELECTRON MICROSCOPY

The fourth toe of the left pes was dissected within 24 hours of each specimen's preservation and stored in 70% ethanol. The dissected toepad was mounted on an SEM stub with the pad facing up for top-down images. After these images were taken, some toes were dissected again sagittally and re-mounted on a stub, this time sideways for cross-section images. All mounted toes were sputter-coated with titanium using a JEOL JUC-5000 sputter-coater (JEOL, Tokyo, Japan) and then imaged with a Hitachi SU5000 FE-SEM (Hitachi, Tokyo, Japan) scanning electron microscope.

SEM images were used to assess the overall subdigital microornamentation of all subdigital scales of the toe. I classified subdigital hair-like microstructures as either setae (long structures with flattened or triangular tips) or spinules (shorter structures with round tips) in accordance with published literature (Ruibal & Ernst, 1965; Johnson & Russell, 2009; Russell *et al.*, 2015). Additionally, I assessed the branching patterns of the setae or spinules and classified the tips as 'round', 'flattened', or 'spatulated'. The dimensions of the microstructures were measured with the software ImageJ V 1.52a (Schneider *et al.*, 2012) as follows: I first measured length, diameter and spacing (as an approximation for density (Peattie, 2001)) of the setae or spinules (n = 10 per specimen) for each subdigital scale for a subset of one specimen per lineage. I used these measurements and the images from a preliminary assessment to classify the subdigital scales according to functional units and defined five functional scale regions for further analysis (Russell *et al.*, 2015): far-distal (FD), mid-distal (MD), sub-inflexion (friction point, IN), mid-proximal (PM), and far-proximal (FP) (See results, Fig. 4.2). I then took the same measurements for the setae or spinules of these scales of all 23 specimens for statistical analysis. For the mid-distal and mid-proximal regions, I measured the scale halfway between IN and FD or FP respectively. The aspect ratio of the setae or spinules was calculated from the average values (length and diameter) for each toe region. For the spatulated setae, I also measured the width of the spatulae (n = 10 per specimen) and counted the number of branching events of the stalks (n = 10 per specimen). Although the exact number of branching events could not be counted with certainty (because some branches were probably hidden behind other branches in the images) I assumed that this bias was approximately the same for all lineages, and therefore the comparison was still justified, even though the number of branching events I counted in all cases was probably an underestimate of the actual number. All counts were conducted halfway between the proximal and the distal end of the respective scale region (Webster, Johnson, & Russell, 2009).

MORPHOLOGICAL ANALYSIS

All analyses were performed in R version 3.3.1 (R Core Team, 2018). Prior to each analysis I first used the package *fitdistrplus* (Delignette-Muller & Dutang, 2015) to determine the distribution that best fit My data, which was then used for downstream analysis. As subdigital scale size is likely to be affected by the size of the gecko (Irschick *et al.*, 1996; Webster *et al.*, 2009; Pillai *et al.*, 2020 [Appendix II]), I performed a linear regression between SVL and all scale size measures, and used the residuals from these regressions for subsequent analysis to account for body size. I used standard generalised linear models because preliminary analysis showed that I did not have enough statistical power to reliably apply phylogenetically informed methods.

ANALYSIS OF SUBDIGITAL TERMINAL SCALE WIDTH

To test if size-corrected scale width in all 9 sampled lineages is associated with habitat, I performed a generalised linear mixed-effects model (GLMM) with habitat (terrestrial or generalist or saxicoline) as a fixed effect. I then used the R package *emmeans* (Russell, 2018) for *post hoc* comparisons. To infer if large terminal subdigital scales have evolved independently in each of My saxicoline lineages, or only once within *H. binoei*, or were ancestral for My lineages, I reconstructed the ancestral state of the residuals (mean values per lineage) from the linear regression I constructed from the width dataset using the 'fastanc' algorithm with the 'contMap' function form the R package *phytools* (Revell, 2012).

ANALYSIS OF SUBDIGITAL SCALE AREA AND MICROORNAMENTATION

For My four focal lineages, I analysed size-corrected subdigital scale area separately, once for the FD scale and once for the IN scale. To test for associations between body-size-corrected subdigital scale area and habitat (terrestrial or saxicoline), I grouped the two sister clades (eastern (EA6 + CC) and western (Blencoe + Paluma-W), Fig. 4.1) together as 'clades' in the analysis to partially control for phylogenetic relatedness. I used a GLMM with habitat (terrestrial or saxicoline) and clade as interacting fixed effects, and analysed the two different scale regions separately because sample size was too low to justify one model with scale region as a fixed effect.

I tested whether variation in microornamentation is associated with habitat using a GLMM for each microornamentation trait (setae length, diameter and density, and spatulae width) separately with habitat (terrestrial or saxicoline), 'clade' (EA6/CC or Paluma-W/Blencoe) and functional scale region (FD, MD, etc.) as fixed effects, including an interaction between habitat and clade. The specimen ID was included as a random effect (Bates *et al.*, 2015). Each model was performed as a type II ANOVA, followed by a type III ANOVA if the interaction was significant in the type II ANOVA (Langsrud, 2003; Fox & Weisberg, 2019).

Results

LINEAGE DELIMITATION

Nd2 sequences from 20 individuals of *H. binoei* were added to the alignment from Zozaya et al. (2019), yielding an alignment of 249 sequences. The resulting phylogeny is largely congruent with the phylogeny from Zozaya et al. (2019) and clearly shows that the populations of *H. binoei* with enlarged terminal scales fall into two lineages that are not each other's closest relatives: Paluma-W, and a lineage containing the MI, CC, and Paluma-E sub-lineages (Fig. 4.1; see Fig. S4.2 for the full phylogeny). Each of the two saxicoline lineages were recovered as sister to a terrestrial lineage without enlarged terminal scales (Figure 4.1A; S4.2). Support for the lineage containing Paluma-W and Blencoe was strong (and this pair is, in turn, sister to the lineage containing CYA6-N and CYA6-S; Fig. 4.1A), but support for the lineage containing EA6 and the MI, CC, and Paluma-E lineages was weak (bootstrap value 57; Fig. 4.1A). However, nuclear exome data strongly supports both these saxicoline and terrestrial sister lineage pairs (Zozaya et al., in prep.). Uncorrected pairwise sequence divergence ranges from 7.2% between Paluma-W and Blencoe, to 11.3% between EA6 and CC.

MICROHABITAT USE

The microhabitat use of all nine lineages is shown in Figure 4.2. The focal lineages with enlarged terminal subdigital scales were classified as saxicoline, with between 100% (MI, Paluma-E) and 88% (Paluma-W) of the respective encounters on rocks, whereas Blencoe was clearly terrestrial (96% on the ground). EA6 was a generalist, being found on the ground in 59% of observations (Fig. 2). At the two sites where I sampled EA6 specimens for subdigital scale area measurements and for SEM analysis, they were exclusively found on the ground, (at both locations they co-occurred with a saxicoline lineage) therefore I defined them as terrestrial for the respective analysis, but as generalist for the toe width analysis. The remaining lineages (CA6, CYA6-N, CYA-6S) were generalist lineages and did not co-occur with a saxicoline lineage.

GENERAL SUBDIGITAL SCALE MORPHOLOGY

The overall subdigital scale morphology was similar across all four lineages: on the distal end of the toe there is a pair of at least slightly enlarged scales (FD), followed by a series of mostly four to five (exceptionally up to 7) small, overlapping MD scales, and the most proximal is comparatively larger (Fig. 4.5, S4.3). These are followed by a considerably larger IN scale forming the friction plate for terrestrial locomotion (Padian & Olsen, 1984; Russell & Bauer, 1990b). Proximal to the friction plate is a row of six to eight (occasionally up to ten) MP scales that are smaller than the friction plate and become gradually smaller towards the FP scale (supporting information S4.3).

SUBDIGITAL TERMINAL SCALE WIDTH

Terminal scale width of all nine lineages was positively correlated with SVL ($F_{1,83} = 61.285$, $p < 0.001$, $R^2 = 0.4245$. The residuals of this model were normally distributed. There was a significant association between habitat and terminal scale width, with *post hoc* comparisons revealing that saxicoline lineages have significantly wider body-size-adjusted terminal scales than terrestrial lineages ($p < 0.001$) or generalist lineages ($p < 0.001$). But generalist and terrestrial lineages do not differ significantly ($p = 0.933$, Table 4.1).

The plot of SVL against terminal scale width shows that saxicoline lineages tend to be above the regression line, whereas terrestrial and generalist lineages tend to be below or on the regression line (Fig. 4.3, supporting information S4.3). Ancestral state reconstruction indicates that enlarged terminal scales have evolved independently in the Paluma-W and the CC/MI/Paluma-E clade (Fig 4.4), suggesting parallel evolution of toepads in these saxicoline lineages.

SUBDIGITAL SCALE AREA

Subdigital scale area of the four focal lineages was positively correlated with SVL for both the terminal scales (FD: $F_{1,19} = 30.75$, $p < 0.001$) and the inflection point scales (IN: $F_{1,19} = 9.03$, $p = 0.009$). However, the relationship was tighter for the terminal scales (FD: $R^2 = 0.618$) compared to those at the inflexion point (IN: $R^2 = 0.322$). The data was normally distributed for both scales. Saxicoline lineages had a significantly larger terminal scale area (FD, $p < 0.001$), but sub-inflexion scale area (IN) was not significantly different ($p = 0.727$, Table 4.1, Fig. 4.5A). ‘Clade’ had no significant influence on the area of either scale (Table 4.1). Therefore, both saxicoline lineages had larger terminal scales compared to their respective terrestrial or generalist sister clades (Fig. 4.5A).

SUBDIGITAL MORPHOLOGY

The terminal (FD) scales of all four lineages were covered with fully developed setae (branched multiple times and terminating in spatulae, Fig. 4.6). The MD scales were covered in spinules, except for the most proximal one, neighbouring the friction scale, which was covered by relatively short (9 µm (EA6) to 14 µm (CC)) setae with flattened and intermediately branched tips. The IN scale was covered with fully developed setae, comparable in structural complexity to those on the FD scales. On the MP scales, the microornamentation also consisted of setae that became gradually shorter and less branched from scale to scale in a proximal direction, thus gradually transforming into spinules again towards the FP scale. Along the same gradient, the tips, which are spatulated on the IN, gradually transform into flattened tips and then round tips (see supporting information S4.4 and S4.5).

All microornamentation traits had gamma distributions, except for branching, which was log-normally distributed. I therefore log-transformed the branching values and used a normal distribution for the respective model. The length of the subdigital microstructures (setae and spinules) differed among the four lineages, and was significantly longer in the saxicoline lineages (Table 4.1, Fig. 4.5B, 4.6A & B). The setae on the FD and the IN scale were about the same size in the terrestrial lineages, whereas in the saxicoline lineages the setae on the terminal scales were longer than on the friction scale (Table 4.1, supporting information S4.4). The differences in setae length were more pronounced in CC/EA6 than in Paluma-W/Blencoe, driving the significant interaction between habitat and 'clade' in the model for both the type II and type III ANOVAs (Table 4.1, Fig. 4.5B). The setae on the FD scale were also branched more often in the saxicoline lineages (Table 4.1, Fig. 4.5C, 4.6C–D). Setae spacing was significantly higher (and thus setae density lower) in the saxicoline lineages, compared to the terrestrial lineages. (Table 4.1, Fig. 4.5D). Setae diameter and spatulae width were not significantly different among lineages (neither habitat nor 'clade' was significant in the model, only scale region (FD, MD, IN, MP, FP)).

Discussion

MICROHABITAT USE AND TOE-PAD MORPHOLOGY

I predicted the *Heteronotia* lineages associated with granite outcrops (Paluma-W and the CC/MI/Paluma-E clade) would represent intermediate stages in an evolutionary series towards terminal adhesive toepads, and could have evolved enlarged subdigital scales covered with longer, more complex microstructures. Consistent with My predictions, subdigital scale area was significantly larger on the terminal scales of the saxicoline lineages compared to their terrestrial (or generalist) sister lineages (Fig. 4.2), and the ancestral state reconstruction revealed that enlarged terminal scales evolved independently in both saxicoline lineages (Fig. 4.3). Contrary to My predictions, all lineages—even terrestrial ones—had fully developed setae with spatulae on the FD and the IN scales. Spatula width was not significantly different between saxicoline and terrestrial lineages, but spatula width on the inflexion point was wider in the saxicoline CC lineage when compared only to its terrestrial sister lineage EA6. Consistent with My hypothesis, setae morphology was significantly different between saxicoline and terrestrial lineages—setae were longer, had a higher aspect ratio and where branched more often in the saxicoline lineages on all subdigital scales, and the differences were more prominent on the terminal (FD) scales (Fig. 4.2). Given the two saxicoline lineages have independently evolved enlarged terminal scales, and most likely independently shifted from a terrestrial or generalist to a saxicoline ecology, this is a case of parallel evolution, towards enlarged terminal adhesive pads (Serb & Eernisse, 2008).

My assessment of the habitat use of the lineages of this study confirmed that Paluma-W, CC, MI, and Paluma-E can be regarded as saxicoline, while Blencoe was near-exclusively encountered on the ground and can be regarded as terrestrial. EA6 occupied both terrestrial and saxicoline microhabitats, and can be regarded as generalist (Fig. 4.2). Interestingly, EA6 co-occurred with a saxicoline lineage at two of the sampled sites, and in those locations, they were found exclusively on the ground, whereas they also used saxicoline microhabitats at all other sites (Fig. 4.1B). The apparent competitive exclusion could suggest that these lineages compete for habitat, although more data is needed to test this. The remaining lineages were generalists and did not co-occur with strictly saxicoline lineages. Previous ecological studies found that *H. binoei* was either terrestrial (Pianka & Pianka, 1976; Henle, 1991), or generalist, using both terrestrial and arboreal microhabitats (Mesquita *et al.*, 2016a; Meiri, 2018; Riedel *et al.*, 2020 [Chapter 5]). These studies did not discriminate between the different lineages of the *H. binoei* species complex, and My results indicate that habitat use differs among these lineages, and even among populations within the lineages (i.e., EA6).

MICROSTRUCTURES AND FUNCTIONAL IMPLICATIONS

Adhesive toepads with fully developed setae work through a combination of adhesion *via* Van-der-Waals forces and friction *via* shear forces (Autumn *et al.*, 2002; Gao *et al.*, 2005; Autumn, 2006, 2007). Compared to setae, spinules alone can only enhance friction *via* shear forces. Having spinules only under the toes is characteristic of (mostly terrestrial) narrow-toed geckos (Johnson, Russell, & Delannoy, 2009; Peattie, 2009; Russell *et al.*, 2015), chameleons (Khannoon *et al.*, 2014; Spinner, Westhoff, & Gorb, 2014b), and potentially some skinks (Irschick *et al.*, 1996, 2006). In general, species with spinules are less efficient climbers (Irschick *et al.*, 1996), with the exception of chameleons, which use a completely different method of climbing with zygodactyl hands and feet (Khannoon *et al.*, 2014; Spinner *et al.*, 2014b). My results that all four lineages (terrestrial, generalist, and two saxicolous) had fully developed, spatulae-bearing setae, imply that they all should be capable of applying some adhesive force (Peattie, 2009). The differences in both size-adjusted subdigital scale area, however, as well as setal morphology, imply that the saxicoline lineages should be capable of applying greater forces, and thus should be better climbers. Greater subdigital scale areas are directly linked to improved clinging ability (Irschick *et al.*, 1996, 2006; Pillai *et al.*, 2020 [Appendix II]), and longer setae are better adapted for adhesion on naturally rough surfaces (Autumn, 2006; Johnson *et al.*, 2009). The lower setal density of the saxicoline lineages may help to enhance toepad function, because although long, thin (high aspect ratio) setae enhance adhesion on rough surfaces, they also have the tendency to clump, hampering adhesion. So, for species adapted to rough surfaces *via* long thin setae, there is likely a limit on how densely these can be arranged (Sitti & Fearing, 2003; Johnson & Russell, 2009).

The setae of the four lineages of *H. binoei* assessed in detail were overall considerably shorter than the setae of most arboreal or saxicoline gecko species with fully developed toepads (Peattie, 2001). However, while the setae of the terrestrial lineages were comparable in length to the mostly unbranched spinules of narrow-toed species (e.g., *Cyrtopodion* Fitzinger, 1843*, Nactus* or *Aeluroscalabotes* Günther, 1864) (Peattie, 2009), setae length of the saxicoline lineages were at the lower end of the range reported for some climbing, pad-bearing species (Schleich & Kästle, 1986). Together with the overall toe morphology of *H. binoei* (including the saxicoline lineages), which resembled the basic narrow-toed condition of terrestrial forms (Peattie, 2009; Gamble *et al.*, 2012; Russell *et al.*, 2015), these findings further support My hypothesis that the saxicoline lineages represent intermediate stages in terminal toepad evolution.

TOEPAD EVOLUTION

Toepads in geckos may arise from the sub-inflection scale, leading to basal pads (Higham *et al.*, 2015, 2017). Many climbing gecko species, however, have terminal pads (Russell & Bauer, 1989, 1990b). The evolution of terminal pads more likely follows a different trajectory from that of basal pads, starting instead with the development of enlarged, adhesive terminal scales (Russell & Bauer, 1989; Peattie, 2009), rather than enlarged sub-inflection scale pads (Russell *et al.*, 2015; Higham *et al.*, 2017)(see S4.1). I observed enlargement of terminal subdigital scales in the two saxicoline lineages of the *H. binoei* complex.

Alternative toepad morphologies may have evolved in response to different microhabitats. Terminal pads are often associated with saxicoline habitats (Russell & Bauer, 1989), and recent studies indicate that they are better suited to naturally dusty and friable rock surfaces (Cole, Jones, & Harris, 2005; Russell & Delaugerre, 2017) because their internal morphology allows independent control of claw and scansors (Russell, 1976, 1979). On the other hand, basal toepads may have evolved in association with arboreality (Russell, 1979; Higham *et al.*, 2019). Consistent with this hypothesis, the previously established intermediate form for basal toepads (*G. humeralis)* is arboreal (Russell *et al.*, 2015; Higham *et al.*, 2017), whereas My proposed intermediate forms for terminal toepads are associated with granite outcrops.

EVOLUTIONARY HISTORY OF THE GENUS *HETERONOTIA*

Contrary to My expectations, terrestrial *H. binoei* lineages had short but fully developed setae, including multiple branching of the tips, complete with spatulae. They were short, but for the saxicoline lineages not outside the range comparable to those previously reported for climbing geckos (Schleich & Kästle, 1986; Peattie, 2001). Thus, although most lineages of these geckos are generalists or terrestrial, they have microornamentation apparently adapted to climbing, which raises the question of the evolutionary history of toe morphology of this genus. While dwarf New World geckos (*Gonatodes*), the other group studied in relation to toepad evolution, are primarily terrestrial (Higham *et al.*, 2017), the evolutionary history of *Heteronotia* is more complex. The sister clade of *Heteronotia* is the South-east Asian genus *Dixonius* (Bauer, Good, & Branch, 1997), which has leaf-shaped, terminal, adhesive toepads (Bauer *et al.*, 1997; Gamble *et al.*, 2012), and is mostly saxicoline (although some members are terrestrial) (Das, 2004; Botov *et al.*, 2015; Ziegler *et al.*, 2016; Sumontha *et al.*, 2017). The sister taxon to this *Heteronotia–Dixonius* clade is *Nactus* Kluge, 1983, a terrestrial and padless genus (Jackman, Bauer, & Greenbaum, 2008). Therefore, the ancestral state of the *Nactus–(Dixonius–Heteronotia)* clade is likely to be pad-less (Gamble *et al.*, 2012; Russell & Gamble, 2019), but not necessarily. The subdigital microornamentation of *Nactus pelagicus* (Girard, 1858) consists of short (5µm), unbranched spinules (Russell, 1972; Peattie, 2009), in accordance with a primarily terrestrial lifestyle. But My results, not only in terms of microornamentation, but also in terms of ancestrally medium sized terminal toe width (Fig. 4.4), provide at least some support for an ancestrally terminally padded character-state in the *Heteronotia–Dixonius* clade, which would mean *Heteronotia* is secondarily terrestrial or generalist, and the terminal pads of My saxicoline lineages could constitute a re-acquisition of a previously reduced state. This hypothesis is contradicted by osteological findings that the phalangeal arrangement, and overall internal digit morphology, of *H. binoei* is identical to that of the terrestrial *N. pelagicus*, whereas that of *Dixonius* resembles arrangements typical for terminal padded, climbing species (Russell, 1972). All this remains speculative at this point but underlines the complexity of the evolution of adhesive toepads in geckos, and of complex hierarchical morphological systems in general (Russell & Gamble, 2019). Detailed examination of the internal foot anatomy of *Heteronotia* and their sister lineages could provide valuable information to assess if this genus is primarily or secondarily terrestrial (Russell, 1976, 1979; Gamble *et al.*, 2012; Russell & Gamble, 2019)

Figures

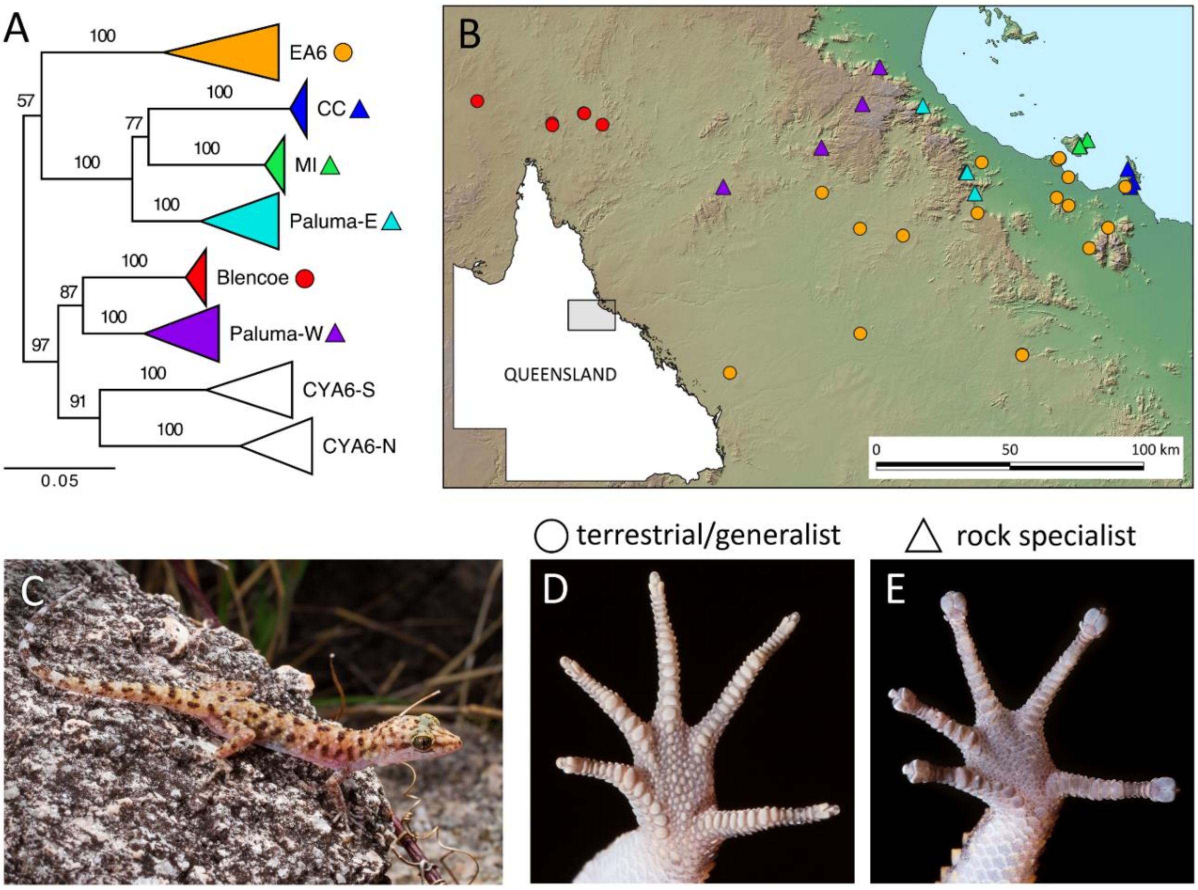


Figure 4-1 Phylogeny and distribution of *Heteronotia binoei* lineages used in this study. **A)** Maximum-likelihood phylogeny of the relevant *H. binoei* lineages inferred from nd2 sequences using RAxML, with bootstrap values shown. See S2 for the full phylogeny. **B)** Distribution of the four relevant lineages in north-east Queensland, colMy coded as in A. **C)** An individual of the MI lineage foraging on a granite boulder. **D)** Subdigital view on the right hindfoot (pes) of the terrestrial EA6 lineage. **E)** Subdigital view on the right pes of the saxicoline MI lineage.

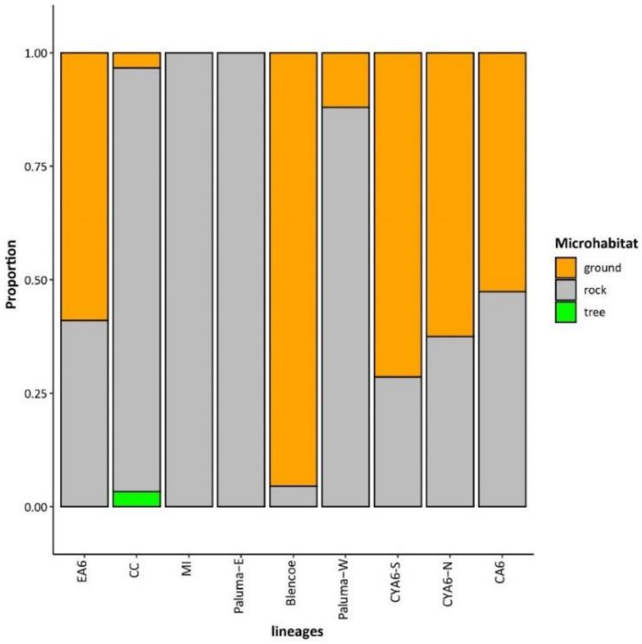


Figure 4-2 Microhabitat use of the *Heteronotia binoei* lineages. Bars show the proportion of observations that each lineage was found on the ground (orange), on rock (grey), or on trees (green). The number of observations for each lineage are shown below the respective bars. While CC, MI, Paluma-E and Paluma-W are saxicoline (>75% on rocks), Blencoe is terrestrial (>75% on ground), and the remaining linages are generalist.

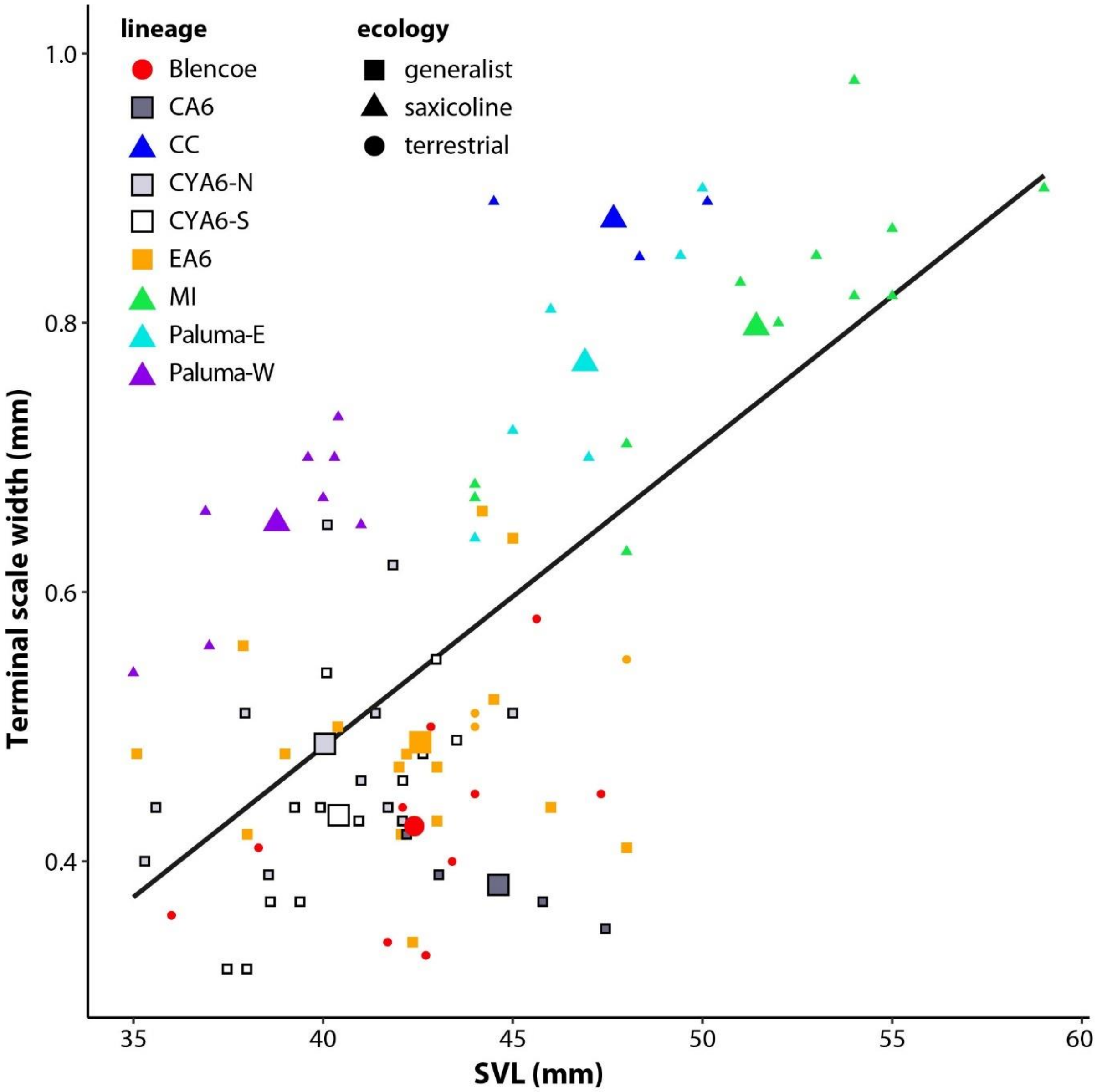


Figure 4-3 Terminal scale width plotted against snout-vent-length (SVL) for all 85 measured specimens (small symbols), and the mean values for each lineage (larger symbol), colour-coded by lineage and with symbol shape indicating habitat use. The black line indicates the regression line of the linear model of toe width against SVL. Note that saxicoline lineages (triangles) are mostly above the regression line, while the terrestrial (circles) and generalist (squares) lineages are on or below the regression line.

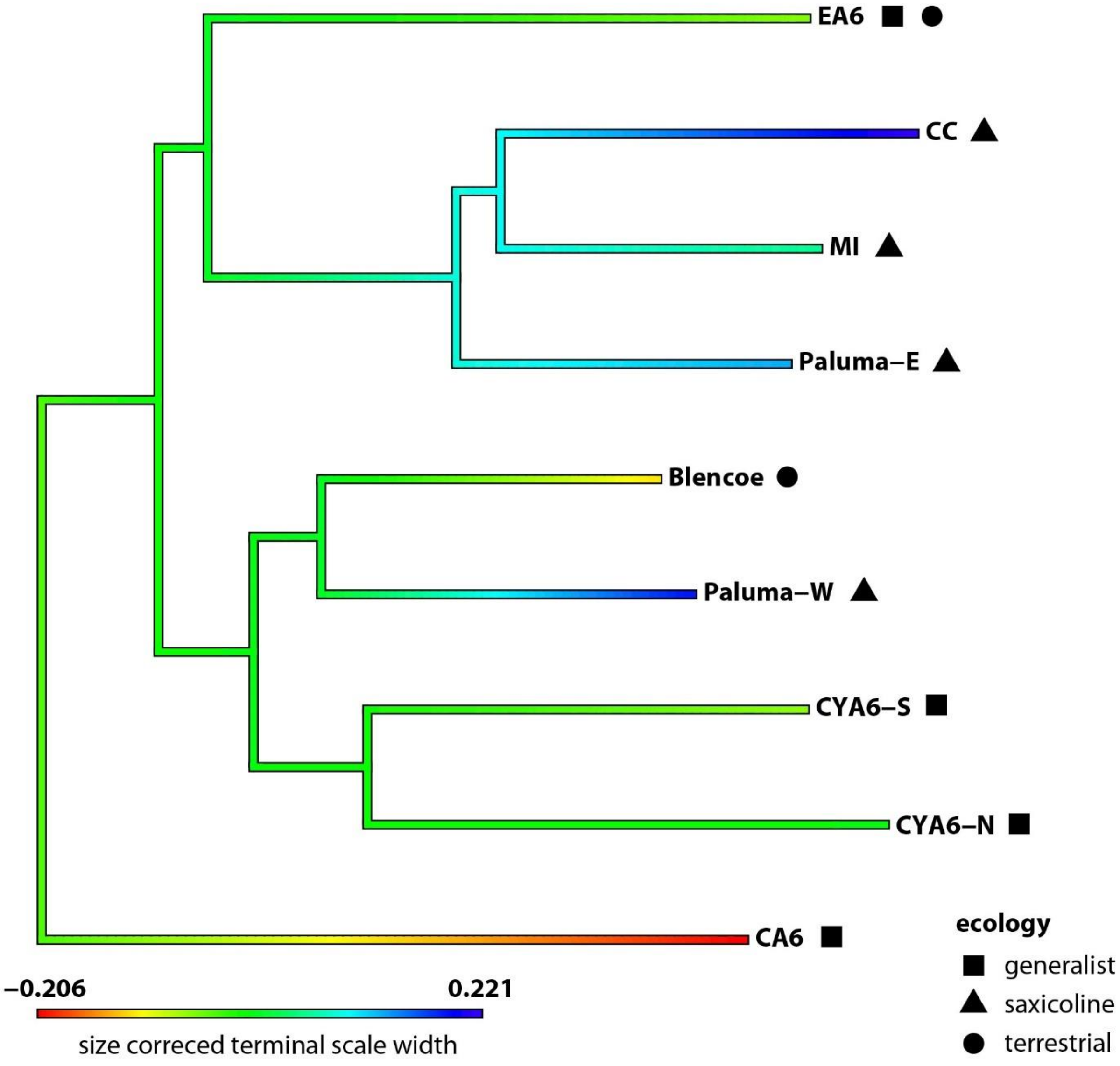


Figure 4-4 Ancestral state reconstruction of size-corrected terminal subdigital scale width. Trait values (depicted by the colMy scale) represent the mean values of the residuals of terminal subdigital scale width regressed against SVL for each of the nine lineages (compare Fig. 3 and supplementary table S3). The habitat use is indicated by shape (EA6 is marked as generalist and terrestrial, see results). The reconstruction shows that the Paluma-W lineage and the clade containing CC, MI, and Paluma-E have independently evolved relatively large terminal subdigital scales. Note that the relatively low relative terminal scale width of MI (relative to the other saxicoline linages), is mainly driven by their relatively large SVL (comp. Fig. 3).

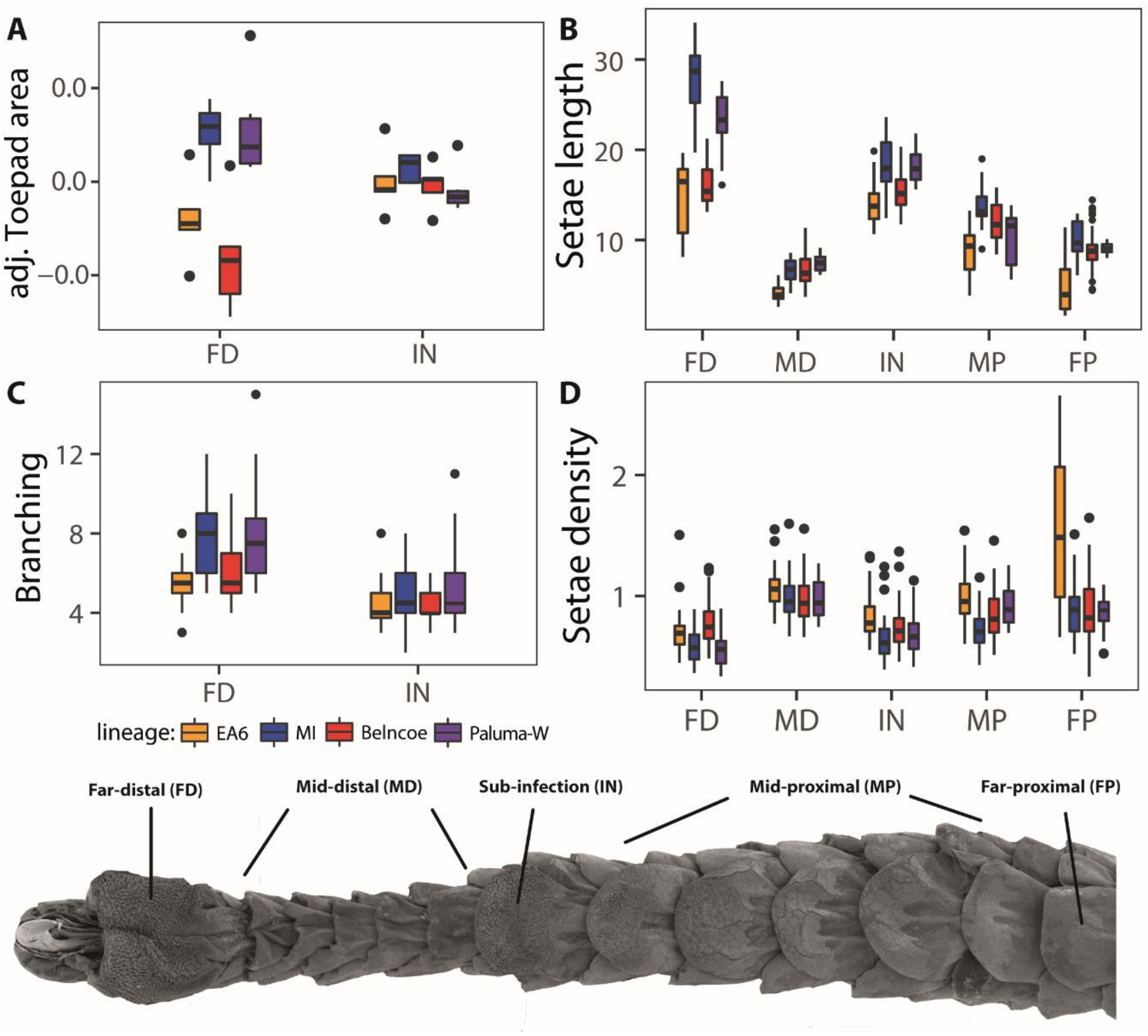


Figure 4-5 Boxplots illustrating variation in microornamentation among the four focal lineages of *Heteronotia binoei*. Terrestrial lineages appear as warm colours (EA6 in yellow, Blencoe in red) and saxicoline lineages appear as cool colours (CC in blue, Paluma-W in purple). **A)** Size-adjusted toepad area of the terminal (FD) and sub-inflection (IN) scales. **B)** Setae length across all scale regions in µm. **C)** Number of branching events of the setae on the FD and IN scales. **D)** Setae density approximated as inverse of setae spacing (measured in µm). The bottom of the figure shows a montage SEM image to illustrate the different toe regions.

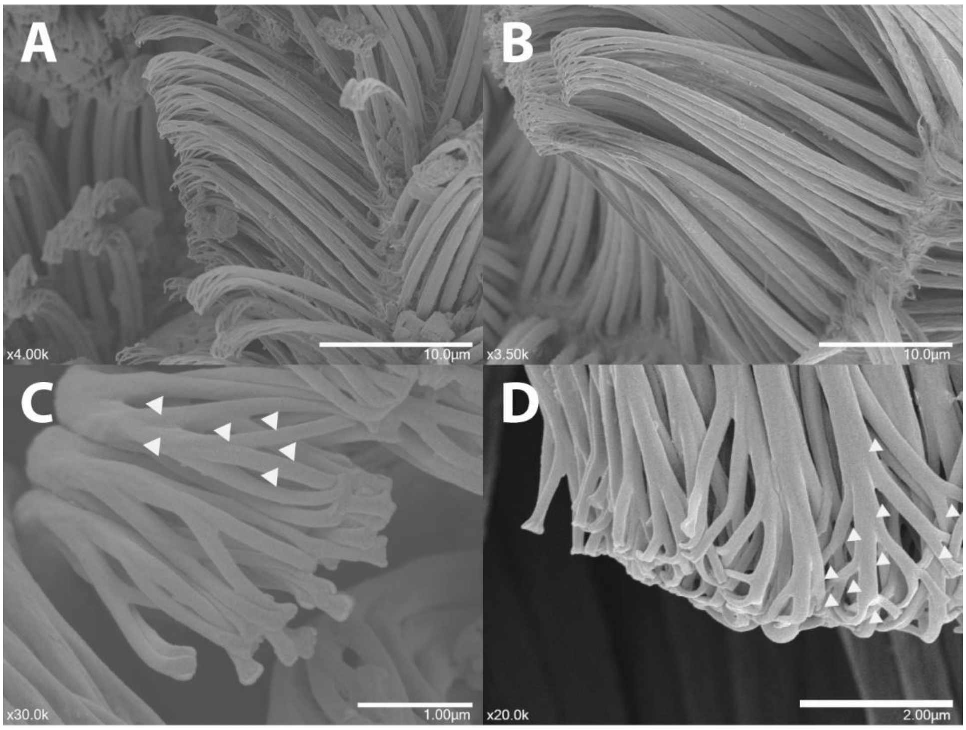


Figure 4-6 Microornamentation on the terminal (FD) scales. Terrestrial lineages are displayed on the left (A, C), whereas saxicoline lineages are on the right (B, D). Magnification of each tile is shown in lower left corner with a scale bar in the lower right corner. **A)** Fully developed setae of terrestrial EA6 lineage with an average length of 14 µm (Table S4). **B)** Fully developed setae of saxicoline MI lineage (av. length: 28 µm, Table S4). **C)** Detail of the branched setae tips with spatulae of terrestrial EA6 lineage. White arrows indicate branching points of a single seta **D)** Detail of the branched setae tips with spatulae of saxicoline Paluma-W lineage. White arrows again indicate branching points of a single seta, illustration that setae of the saxicoline lineages branch more often. Note the different magnifications between C and D (and to a lesser extend between A and B).

Tables

Table 4.1: Results of the statistical analysis of toepad size and microstructures. Toe width was analysed as a linear model with ecology as fixed effect. For the other traits, a linear model was constructed with ecology (microhabitat) and ‘clade’ (MI/EA6 & Blencoe/Paluma-W, see Fig. 1) as interacting fixed effects. Scale region was included as a fixed effect for the microstructural traits, but not for toepad area, which was analysed separately for the FD (terminal) and IN (sub inflexion) scales.

	ANOVA	**Ecology**		**Clade**		**Region**		**Interaction**		**Tukey's Post hoc comparison**
		p-value	**F (DF)**	**p-value**	**F (DF)**	**p-value**	**F (DF)**	**p-value**	**F (DF)**	
Terminal scale width	**type 2**	**<0.001**	35.236 (2)	NA	-	NA	-	NA	-	**saxicoline > terrestrial** **saxicoline > generalist**
Terminal scale area (FD)	type 2	**<0.001**	27.38 (1)	0.442	0.62 (1)	NA	-	0.326	1.02 (1)	
Sub-inflexion scale area (IN)	type 2	0.727	0.12 (1)	0.200	1.64 (1)	NA	-	0.426	0.67 (1)	
Setae length	type 2	**<0.001**	158.07 (1)	**0.030**	5.32 (1)	**<0.001**	465.15 (4)	**0.013**	24.31 (1)	
	type 3	**<0.001**	158.07 (1)	0.526	5.32 (1)	**<0.001**	465.15 (4)	**0.013**	24.31 (1)	
setae diameter	type 2	0.596	3.94 (1)	0.732	0.04(1)	**<0.001**	218.66 (4)	0.303	5.41 (1)	
setae spacing	type 2	**0.001**	29.50 (1)	0.228	0.75 (1)	**< 0.001**	89.99 (4)	0.08	8.49 (1)	
spatulae width	type 2	0.855	0.15 (1)	0.131	4.11 (1)	**< 0.001**	81.32 (1)	0.19	8.83 (1)	
Branching	type 2	**0.009**	5.10 (1)	0.74	< 0.01 (1)	**< 0.001**	138.887 (1)	0.515	0.424 (1)	

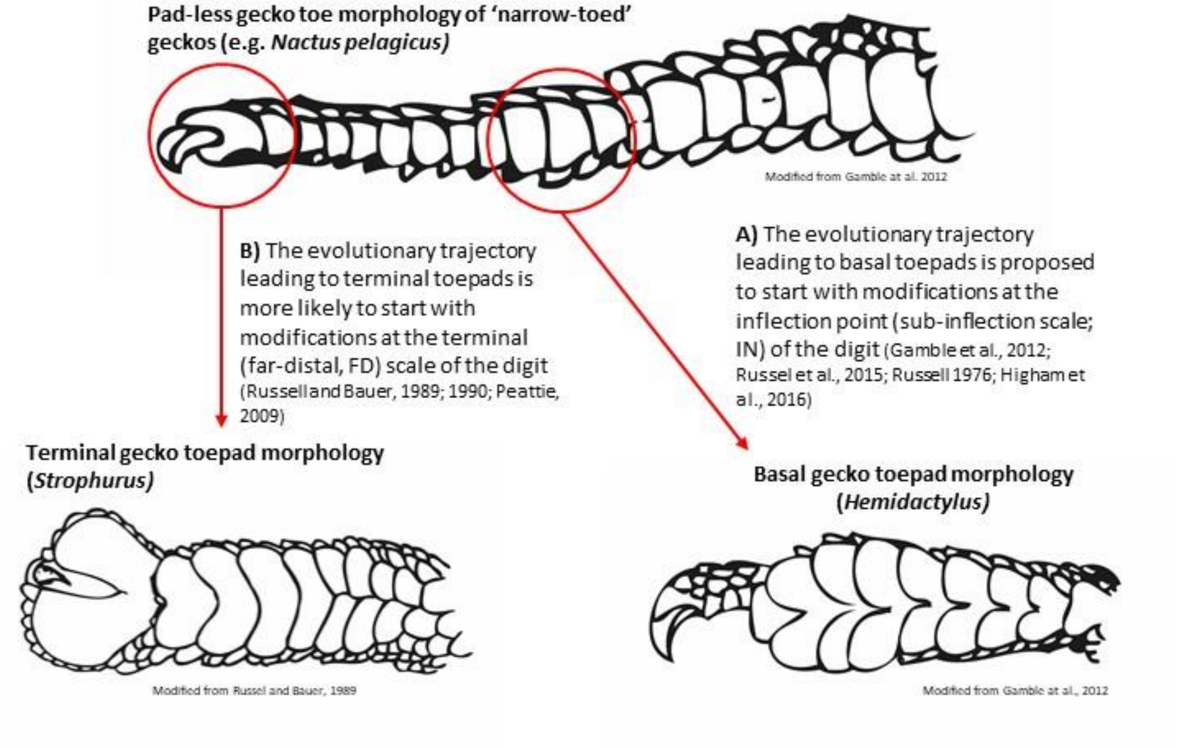
Supplementary material

Supplementary material S4.1:
Toepad types and their proposed evolutionary trajectories

1) Examples of the two main types of gecko toepads:

A) The foot of *Oedura murrumanu* (Diplodactylidae, Gekkota), a species with terminal toepads.
B) The foot of *Pseudothecadactylus australis* (Diplodactylidae, Gekkota) as a typical example for basal toepads.

2) Proposed evolutionary trajectories for the evolution of basal and terminal toepads



Pad-less gecko toe morphology of 'narrow-toed' geckos (e.g. *Nactus pelagicus*)

Modified from Gamble at al. 2012

B) The evolutionary trajectory leading to terminal toepads is more likely to start with modifications at the terminal (far-distal, FD) scale of the digit (Russell and Bauer, 1989; 1990; Peattie, 2009)

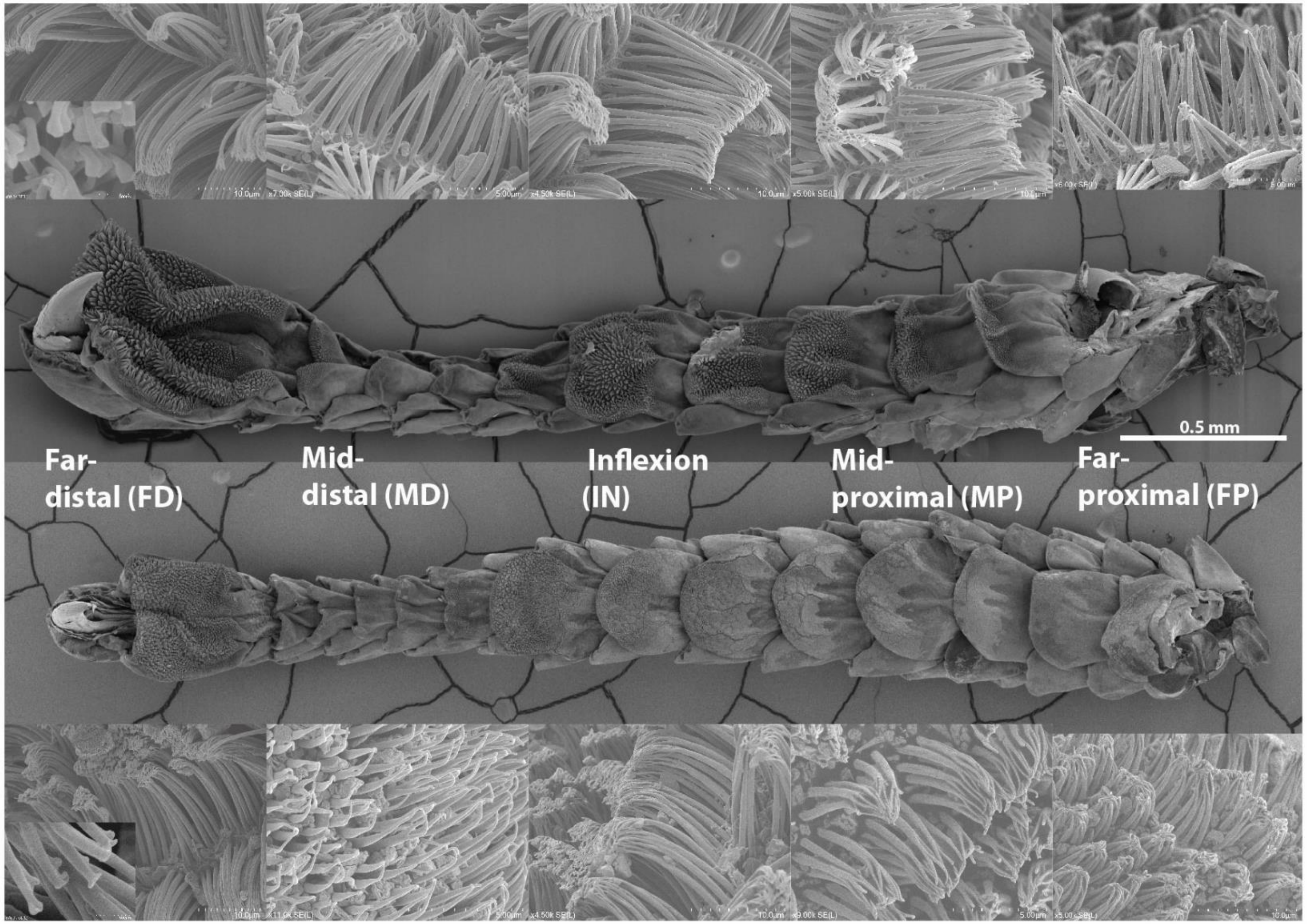
A) The evolutionary trajectory leading to basal toepads is proposed to start with modifications at the inflection point (sub-inflection scale; IN) of the digit (Gamble et al., 2012; Russel et al., 2015; Russell 1976; Higham et al., 2016)

Terminal gecko toepad morphology (*Strophurus*)

Modified from Russel and Bauer, 1989

Basal gecko toepad morphology (*Hemidactylus*)

Modified from Gamble at al., 2012



Supplementary material S4.2: example SEM images of a saxicoline (upper half) and a terrestrial (lower half) *H. binoei*. The detailed images (upper and lower rows) show the microornamentation at the different sampled digit areas (FD, MD, IN, MP, FP).

S4.4 Supplementary Tables

S4.1:

Raw measurements of setae dimensions used for analysis. All values in µm. Aspect ratio is calculated from measured average values.

Station along digit	Datum	EA6	CC	Blencoe	Paluma-W
Far distal	Length	13.57 ± 4.06	27.72 ± 3.81	15.96 ± 2.07	23.21 ± 3.05
	Diameter	0.72 ± 0.18	0.94 ± 0.16	0.75 ± 0.12	0.77 ± 0.16
	Aspect ratio	18.85	29.48	21.28	30.14
	Spacing	1.48 ± 0.30	1.80 ± 0.42	1.36 ± 0.33	1.90 ±0.43
Mid-distal	Length	4.07 ± 0.92	6.71 ± 1.26	6.80 ± 1.91	7.41 ± 0.86
	Diameter	0.44 ± 0.10	0.47 ± 0.05	0.48 ±0.08	0.46 ± 0.07
	Aspect ratio	9.25	14.28	14.17	16.11
	Spacing	0.96 ± 0.15	1.05 ±0.19	0.06 ± 0.18	1.05 ± 0.18
Inflection scale	Length	13.98 ± 2.11	18.41 ± 2.96	15.24 ± 1.81	18.28 ± 1.82
	Diameter	0.73 ± 0.16	0.75 ± 0.12	0.68 ± 0.11	0.69 ± 0.08
	Aspect ratio	19.15	24.55	22.41	26.49
	Spacing	1.28 ± 0.25	1.68 ±0.42	1.43 ±0.31	1.55 ± 0.38
Mid-proximal	Length	8.49 ± 2.63	13.74 ± 2.09	11.91 ± 2.10	10.38 ± 2.65
	Diameter	0.58 ± 0.10	0.64 ± 0.08	0.68 ± 0.13	0.52 ± 0.07
	Aspect ratio	14.64	21.47	17.52	19.96
	Spacing	1.06 ± 0.25	1.46 ± 0.33	1.25 ± 0.30	1.13 ± 0.19
Far proximal	Length	4.74 ± 2.82	10.02 ± 2.08	8.82 ± 2.26	9.09 ± 0.57
	Diameter	0.45 ± 0.21	0.59 ± 0.08	0.61 ± 0.13	0.53 ± 0.07
	Aspect ratio	10.53	16.98	14.46	17.15
	Spacing	0.74 ± 0.28	1.20 ± 0.31	1.25 ± 0.42	1.18 ±0.21

S4.2:

Mean terminal scale width residual values (TWR) for each lineage. Standard deviation (sd), standard error (se), and the 95% confidence interval (ci) are also given, as are the number of specimens sampled for each lineage (n).

lineages	N	TWR	sd	se	ci
CA6	4	-0.2057	0.0833	0.0416	0.1325
EA6	19	-0.0538	0.1008	0.0231	0.0486
Paluma-E	6	0.1309	0.0590	0.0241	0.0619
MI	12	0.0567	0.0595	0.0172	0.0378
CC	3	0.2207	0.0726	0.0419	0.1803
Blencoe	10	-0.1126	0.0733	0.0232	0.0524
Paluma-W	8	0.1936	0.0406	0.0144	0.0340
CYA6-S	12	-0.0599	0.0489	0.0141	0.0311
CYA6-N	11	0.0012	0.0863	0.0260	0.0580

Chapter 5 - Ecological niche and microhabitat use of Australian geckos

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Abstract

Modern biological research often uses global datasets to answer broad-scale questions using various modelling techniques. But detailed information on species–habitat interactions are often only available for a few species. Australian geckos, a species-rich group of small nocturnal predators, are particularly data-deficient. For most species, information is available only as scattered, anecdotal, or descriptive entries in the taxonomic literature or in field guides. I surveyed gecko communities from 10 sites, and 15 locations across central and northern Queensland, Australia, to quantify ecological niche and habitat use of these communities. My surveys included deserts, woodlands, and rainforests, examining 34 gecko species. I assigned species to habitat niche categories: arboreal (9 species), saxicoline (4), or terrestrial (13), if at least 75% of My observations fell in one microhabitat; otherwise I classified geckos as generalists (8). For arboreal species, I described perch height and perch diameter and assigned them to ecomorph categories, originally developed for *Anolis* lizards. There was lower species richness in rainforests than in habitats with lower relative humidity; the highest species richness occurred in woodlands. Most arboreal and generalist species fit the trunk-ground ecomorph, except those in the genus *Strophurus*, whose members preferred shrubs, twigs of small trees, or, in two cases, spinifex grass hummocks, thus occupying a perch space similar to that of grass-bush anoles. Habitat use by *Pseudothecadactylus australis*, *Saltuarius cornutus*, and *Gehyra dubia* fit the trunk-crown ecomorph. I provide quantified basic ecological data and habitat use for a large group of previously poorly documented species.

Key words: Gecko - ecological niche – ecomorph - habitat use – ecology - perch space

Introduction

In recent decades, scientific effort has shifted from more detailed, descriptive observations about species (e.g. Fitch, 1970; Wright & Vitt, 1993), to answering broad questions with global implications (e.g. Harfoot *et al.*, 2014). Robust studies in many fields of biology, ranging from evolution, biogeography, and conservation biology, to ecomorphology, frequently rely on large datasets of combined information (Melville *et al.*, 2006; Garcia-Porta & Ord, 2013; e.g. Davis & Betancur-R, 2017; Vidan *et al.*, 2019; Wölfer, Arnold, & Nyakatura, 2019). But these large datasets may have limited scope or include only coarse-scale information (e.g. presence – absence data), because detailed baseline knowledge, especially regarding natural history and ecology, are unavailable for individual species (Vidan *et al.*, 2019; Meiri, 2020). More and more detailed, autecological studies for many species in many parts of the world, including abundant and common species, may enhance future global studies and the conclusions that can be drawn from these.

Lizards are some of the most widespread and abundant vertebrates in the world. In particular, geckos (Gekkota) are the second most speciose lizard group (after snakes), comprising nearly 1900 species or 27.5% of all lizards (if snakes are excluded), with the highest rate of new species descriptions in squamate reptiles (Roll *et al.*, 2017; Uetz *et al.*, 2019; Meiri, 2020). Geckos have a worldwide distribution, mostly in tropical and subtropical regions. Australia is one of the global hotspots for gecko diversity, where they constitute a dominant part of the overall lizard biodiversity (Vidan *et al.*, 2019; Meiri, 2020). Geckos are small- to medium-sized predators of invertebrates (Nordberg *et al.*, 2018b) and small vertebrates (Nordberg, 2019) and are themselves depredated by birds, mammals, larger reptiles, frogs, and even large invertebrates (Nordberg *et al.*, 2018a). Thus, they form an important part of tropical and subtropical food webs. Geckos have colonized diverse habitats, occupying terrestrial habitats and vertical rocks and trees, including overhanging microhabitats (Gamble *et al.*, 2012; Collins *et al.*, 2015; Russell *et al.*, 2019).

Recent studies have described broad-scale evolutionary history, or revealed species complexes by examining biogeography, mapping species distributions, and conducting taxonomic analyses (e.g. Han *et al.*, 2004; Gamble *et al.*, 2008b, 2012; Skipwith *et al.*, 2016; Brennan & Oliver, 2017). But to understand the causes of diversification in particular bioregions, we require more detailed understanding of their ecology, for example which habitat niches, or which roles in the food-web are occupied (Meiri, 2018). Many gecko groups are severely data deficient, especially in terms of natural history and ecological data, including habitat use. Detailed studies on gecko field ecology are often only available for small areas or single species (e.g. Henle, 1990; Augros *et al.*, 2018; Neilly *et al.*, 2018; Nordberg *et al.*, 2018b; Nordberg & Schwarzkopf, 2019b,a). For most species, information on basic ecological traits are only available as anecdotal information in field guides or the taxonomic literature, and may be based on limited personal observations by the authors from restricted geographic areas (Kulyomina *et al.*, 2019; Vidan *et al.*, 2019; Zhuang *et al.*, 2019).

The lack of detailed natural history knowledge is problematic for conservation and management purposes, because we may underestimate threats due to data deficiency (Roll *et al.*, 2017; Meiri, 2020). For example, we cannot accurately assess the problems caused by invasive weeds if we do not understand preferred habitat structures and characteristics (Valentine *et al.*, 2008). Nor can we predict the influence of climate warming on lizard communities (Sinervo *et al.*, 2010), if we do not know their thermal preferences and thresholds, or which microhabitats are needed to access temperatures vital for digestion, gamete development, or optimal performance. Further, geckos are often used as a model system for evolutionary (Garcia-Porta & Ord, 2013; Nielsen *et al.*, 2016; Hagey *et al.*, 2017b) and ecomorphological studies (Zaaf & Van Damme, 2001; Hagey *et al.*, 2017a; Rothier *et al.*, 2017; Kulyomina *et al.*, 2019; Riedel *et al.*, 2019 (Chapter 2); Zhuang *et al.*, 2019). Yet, to fully understand the evolution of morphological structures, we need to understand gecko habitat use, and how they exploit various microhabitats, ideally within some kind of ecological classification system, which can be used to describe niches.

One group of lizards for which such a classification system is already established, and which has a substantial body of literature describing ecomorphology and natural history are the

Anolis lizards (Roughgarden, 1995; Losos, 2011). *Anolis* lizards are a well-studied model for ecomorphological analyses and, like geckos, some have adhesive toepads (Losos, 1992, 1994b, 2010; Irschick *et al.*, 1996; Russell, 2002; Hagey *et al.*, 2017b). Therefore, they provide an obvious starting point to use to classify gecko perch space use, and given the similarity in evolution and adaption to vertical habitats by geckos and *Anolis* lizards, niche classifications designed for anoles may be useful in this regard (c.f. Hagey *et al.*, 2017a; Kulyomina *et al.*, 2019).

The goal of My study was to quantify the microhabitat and niche space for a broad range of Australian gecko species, thereby providing baseline ecological information for use in future studies of gecko biology. Using the structural habitat categories perch height and perch diameter, I described the niche space of arboreal geckos (including padless Carphodactylidae), and compared their niche space use to those established for *Anolis* lizard ecomorphs (Losos, 1992; Irschick *et al.*, 1997; Langerhans, Knouft, & Losos, 2006; Poe & Anderson, 2019), to provide a basis for classification of gecko ecomorphs. This study contributes valuable ecological data to the literature for many species that lack such information, which can be used and applied in future ecomorphological, evolutionary or conservation studies.

Methods

FIELD WORK

Geckos were surveyed at 10 sites during multiple field trips to 15 locations (distinct habitat types within the different sites) spanning a wide array of habitats across northern and central Queensland, Australia, between 2014 and 2018 (Fig. 5.1). Locations included 1 - 10 replicates of similar habitat, close together (< 50 kms apart). Habitats were classified as rainforest (which could be further distinguished into lowland and upland rainforest), savannah woodland, woodland, desert, and heath (Table 5.1). In the Cape York Peninsula bioregion (in and near Iron Range National Park), I sampled lowland rainforest, woodland and heath (Fig. 5.1A). Upland rainforests were sampled at three sites across the Australian Wet Tropics (AWT) bioregion (Mt. Elliot, Paluma Range and the Tablelands) and at one site in the Central Queensland Coast bioregion (Eungella National Park). Woodlands were sampled in the Greater Townsville Region (Brigalow Belt (BB)), at Hidden Valley (Einsleigh Uplands [bordering the AWT]) and at the Wambiana Cattle Station (Desert Uplands [bordering BB]; Fig. 5.1B). In the area close to Winton (Mitchell Grass Downs) I sampled both woodland and savannah woodlands (Fig. 5.1C). Locations around Windorah (Channel Country), included savannah woodlands and desert sites (Fig. 5.1D).

Locations were visited for an average of 5 days (range 1 - 12) to assess gecko species during nightly spotlight surveys. At each surveyed location, I either repeatedly surveyed 3-6 replicates of the same habitat (approximately 1 km^2 each, often along a road, on average 5 km apart) or walked transects (e.g., on rainforest tracks) of 5 km on average. An exception to this was Wambiana Station, at which 24 1-ha locations were surveyed intensely for a 3-year research project (Nordberg, 2018).

I attempted to capture all observed geckos to collect morphometric data (mass, snout-vent-length, sex). Habitat and perch height and diameter were recorded for each gecko, regardless whether it was captured or not. I recorded perch location (e.g., tree trunk, grass, primary branch, on the ground; Fig. 5.2), perch height, perch diameter, body orientation (horizontal, vertical, inverted), and a general categorical classification for the type of microhabitat used (tree, shrub, sapling, vine, bamboo, rock, man-made structure) for each observation. Perch diameter was only recorded for arboreal habitats as it is irrelevant for wide or flat substrates such as building walls, boulders, or the ground.

HABITAT NICHE CLASSIFICATION AND PERCH LOCATION

I classified gecko species into one of four broad habitat niche categories: arboreal, terrestrial, saxicoline, or generalist. Species were classified as arboreal when geckos were captured above the ground on shrubs, bushes, trees, or grass; terrestrial if I captured them on the ground, sand, or leaf litter; or saxicoline if captured on rocks or boulders. Species that occurred on a variety of different substrata and did not have at least 75% of all captures in a single broad niche group were classified as generalists.

Additionally, I recorded gecko perch locations, which included subsets of arboreal habitats, for example on tree trunks, or primary, secondary, tertiary branches, or terrestrial habitats like logs, or on the ground (Fig. 5.2).

COMPARISON TO *ANOLIS* ECOMORPHS

I plotted mean perch height (cm) and perch diameter (cm) for 16 gecko species in My communities, which frequent used vegetation, although they may have occasionally used rocks or the ground. Furthermore, I compared gecko perch ecomorphs to those of *Anolis* lizards by overlaying existing perch data for the *Anolis* ecomorph system (Losos, 1992, 1994b; Irschick *et al.*, 1997; Hagey *et al.*, 2017a) on My gecko data. Based on current knowledge and data, I compare overlap of gecko and *Anolis* ecomorphs, to describe gecko habitats using terms established for perch-space niches.

Results

SAMPLING OF HABITAT ASSEMBLAGES

I sampled 2063 geckos across 35 species belonging to the families Gekkonidae, Diplodactylidae, Carphodactylidae and Pygopodidae (Table 5.2). The widespread Australian native house gecko, *Gehyra dubia*, of which 1544 individuals were sampled, mostly at Wambiana Cattle Station, dominated My data. For the remaining species, I sampled between 1 and 61 individuals. My dataset represents a broad sampling distribution across species, geographic regions, and habitat types, with varying sample size pending on location and species abundance. I present data on all species but caution that the results from species with low sample sizes be interpreted with care. In particular, species with low sample sizes include: *Carphodactylus laevis* (n=4), *Strophurus elderi* (n=1), *Strophurus taeniatus* (n=2), *Cyrtodactylus hoskini* (n=3), *Lialis burtonis* (n=3), *Delma tincta* (n=1), and *Pygopus schraderi* (n=1). There are few ecological observations available for these species, so while My sample sizes are low, they still provide valuable data.

SPECIES COMPOSITION AND COMMUNITY STRUCTURE

In terms of species composition and community structure in different habitats and bioregions, the rainforest habitats were generally less species-rich than habitats with lower average humidity, characterised by one or two species per location for rainforest compared to between 3 and 10 in other habitats (Table 5.1). Because of the high endemism of leaf tailed geckos (*Phyllurus* and *Saltuarius*), total species richness of all rainforest habitats (6) is only slightly lower than savannah woodland (9), but higher than heath (5) or desert (4) habitats. Woodland habitats had the highest total species richness (23, Table 5.2). The upland rainforest sites in the Australian Wet Tropics and adjacent regions typically hosted only a single species of leaf-tailed gecko (*Saltuarius* or *Phyllurus*), whereas the tablelands (upland rainforest) included the chameleon gecko (*Carphodactylus laevis*). The dominant gecko species in lowland rainforest in the Iron Range were the giant tree gecko (*Pseudothecadactylus australis*), with occasional *Gehyra dubia*. Woodland and desert habitats, in comparison, were often characterised by 3 to 6 species, and the area around Winton (10 species) and the woodland and heath habitats in Cape York (adjacent to the rainforest of the Iron Range National park; 9 species) were the most species-rich areas I sampled (Table 5.1). Woodland habitats often contained (at least) one species of velvet gecko (genus *Oedura*), a relatively large, and mostly climbing (arboreal, saxicoline, or generalist) species, at least one smaller, mostly climbing species in the genera *Gehyra, Amalosia*, or *Strophurus*, and at least one terrestrial species such as a *Lucasium*, or *Diplodactylus*. In more open habitats, species of mostly arboreal genera tended to be more generalist. Thus, in this study woodland habitats were characterised by *Oedura cincta* and *Strophurus ciliaris* or *S. krisalys*, which were found more often on the ground than their congeners from more eastern regions, which have higher tree density (Table 5.2, Fig. 5.3).

In terms of distribution across habitat types, species using rainforest tended to be restricted to that habitat, whereas species occupying drier habitats, like woodlands, savannah woodlands, heath, or deserts, often occupied more than one of these, but only occasionally used rainforest habitats (Table 5.2). The only exceptions were the giant tree gecko (*Pseudothecadactylus australis*), which occurred in rainforests, but also in adjacent woodlands and heath, and the native house gecko (*Gehyra dubia*), which occurred in rainforest at Iron Range. Notably, I found only two native house geckos in rainforest, and both on trees relatively close to a campground, where they could have been transported by human activity or vehicles.

MICROHABITAT

Throughout all sampled bioregions and habitat types, 9 species were arboreal, 4 saxicoline, 13 terrestrial and 8 generalists (Fig. 5.3, Table 5.2). Generalist species, by definition, used a combination of perch locations including natural substrates, like vegetation and rocks, and anthropogenic substrates, like wooden or concrete walls. Most species classified as terrestrial were nearly exclusively found on the ground, only *Nactus eboracensis* and *Diplodactylus tessellatus* were occasionally encountered on logs (20% and 7%, respectively). Similarly, three out of four saxicoline species were found exclusively on rocks, with only *Oedura monilis* also using vegetation (tree trunks in rocky habitats). Of the arboreal species, six were occasionally found on the ground, and the chameleon gecko (*Carphodactylus laevis*) occupied terrestrial microhabitats 25% of the time.

PERCH LOCATION AND ORIENTATION

For most species, especially leaf-tailed geckos (*Saltuarius* and *Phyllurus*), and species in the genera *Oedura*, and *Gehyra*, tree trunks were the most frequently used perch, followed by primary and secondary branches. In contrast, species in the genus Strophurus use predominantly small-diameter, low, complex, vegetation structures, such as shrubs, grass, or tertiary branches of trees and bushes. Carphodactylus leavis was exclusively found on small saplings (on which they perch head down), when not using the ground. *Amalosia rhombifer* occupied both tree trunks and shrubs quite frequently (Fig. 5.3). Perch orientation for most saxicoline species was predominantly vertical, but horizontal areas were also frequently used by all species. Only *Cyrtodactylus hoskini* and *Phyllurus amnicola* were found on overhanging surfaces (Fig. 5.4).

ECOMORPHS

Most gecko species fell within one or two of the perch-space niches originally described for *Anolis* ecomorphs, according to their habitat use (Fig. 5.5). *Strophurus* species, which were associated with shrubs, bushes, and small trees, typically clustered within the perch-space of the 'grass – bush' ecomorph, except *S. elderi*, which occupied a smaller perch diameter and lower perch height, below the mean perch-space occupied by *Anolis* ecomorphs. Similarly, trunk-using species, such as *Oedura tryoni*, *Oedura castelnaui*, clustered in or near the space occupied by 'trunk' and 'trunk – ground' ecomorphs. *Saltuarius cornutus, Pseudothecadactylus australis* and *Gehyra dubia* fit within the 'trunk – crown' perch-space, with the former also overlapping with the 'crown – giant' perch-space, which is appropriate as they are often found in the canopy of rainforest trees. *Amalosia rhombifer*, a generalist species, was situated in between 'ground – bush' and 'trunk – ground' perch-space, appropriate for its diverse habitat use. The perch heights of generalist species *O. cincta* and *G. versicolor* were below the mean range established for any anoles. In addition, the generalist Heteronotia binoei and the terrestrial *Nactus eboracensis* fell beneath the perch space occupied by *Anolis* ecomorphs, using large perch diameters at very low perch heights.

Discussion

GENERAL HABITAT NICHE AND HABITAT USE

Based on quantitative data, I classified the habitat niche categories, macro- and microhabitat use of 35 gecko species from four families across a wide range of available habitat types in central and north Queensland, Australia. Additionally, I classified perch-space niches for these gecko species using the perch-space niches established for *Anolis* ecomorphs, and found the *Anolis* ecomorphs broadly useful (Losos, 2010, 2011; Hagey *et al.*, 2017a), although My geckos seemed to use the ground more. Although the results for some species should be interpreted with care due to low sample sizes, this study provides an overview of species composition in tropical gecko communities, and a detailed account of habitat use for a variety of Australian gecko species. To My knowledge, this study represents the first detailed account of microhabitat use and especially perch-site behaviour for some species (Table 2). Quantified assessments of habitat use are available for some Australian species, e.g. for native house geckos (*Gehyra dubia*), eastern spiny tailed geckos (*Strophurus williamsi*) and northern velvet geckos (*Oedura castelnaui*) (e.g. Nordberg & Schwarzkopf, 2019a), and for some desert gecko communities in varying degrees of detail (Pianka, 1969; Pianka & Pianka, 1976; Pianka *et al.*, 2017). Perch location data was previously only reported by Hagey, Harte, et al. (2017a) from between three and nine observations for 13 species, 12 of which overlap with this study (Table 5.2).

The quantified habitat niche categories of my study are typically similar to the above-mentioned studies, and with commonly ascribed habitat niche categories from the published taxonomic descriptions and other literature (Wilson, 2015; Nielsen *et al.*, 2016; Hagey *et al.*, 2017a; Cogger, 2018), including the species for which I only have low sample sizes. The three species belonging to the Pygopodidae (*Delma tincta* [n=1], *Lialis burtonis* [n=3] and *Pygopus schraderi* [n = 1]) are unquestionably terrestrial, normally preferring leaf-litter or ground layer vegetation (Macdonald *et al.*, 2013; Wall & Shine, 2013; Cogger, 2018). Although I have limited records for the spiny tailed geckos *Strophurus taeniatus* (n=2) and *Strophurus elderi* (n=1), both were found in spinifex grass hummocks, consistent with previous descriptions of their habitat use as grass-dwelling (graminicolous) (Nielsen *et al.*, 2016; Laver *et al.*, 2017; Cogger, 2018).

In agreement with Hagey, Harte, et al. (2017a), I found that *Gehyra robusta* is a generalist species, using both the ground (n=4) and rocks (n=3). I found, however, that ocellated velvet geckos (*Oedura monilis*) were rock-dwelling (saxicolous), rather than generalist (Hagey *et al.*, 2017a) or arboreal (Henle, 1991; Mesquita *et al.*, 2016b; Nielsen *et al.*, 2016; Meiri, 2018), because I found individuals mostly on rocks (9 on rocks and 2 on trees). These habitat niche classifications could vary among populations and ecoregions, so possibly this species uses a wider variety of microhabitats than I detected. My results for *Cyrtodactylus hoskini* were consistent with the sparse descriptive information on their natural history (Shea, Couper, & Worthington Wilmer, 2011; Cogger, 2018). I describe *Carphodactylus laevis* as arboreal, as I often found C. laevis foraging close to the ground on slender branches and twigs, consistent with Wilson (2015). Other studies describe it more generally as scansorial, i.e., adapted for climbing (Nielsen *et al.*, 2016). *Heteronotia binoei* is typically described as terrestrial (Wilson, 2015; Cogger, 2018), although Mesquita et al. (2016a) classified them as arboreal. Henle (1991) reported *H. binoei* as mostly terrestrial but using bushes and trees up to 0.8 m as retreats, which was corroborated by My study. It must be noted, however, that *H. binoei* is a cryptic species complex (Fujita *et al.*, 2010; Moritz *et al.*, 2016), and different lineages use available microhabitats to different degrees, including rocks (Zozaya *et al.*, 2019; Chapter 4).

COMPARISON WITH ANOLE ECOMORPHS

My results show that the perch-space use of Australian geckos overlaps, at least partially, with *Anolis* ecomorphs. Consistent with (Hagey *et al.*, 2017a), I describe arboreal *Oedura* as using habitat similar to 'trunk' and 'trunk – ground' anoles. Species of the genus *Strophurus* use habitat structure similar to that of 'grass – bush' anoles (consistent with Hagey, Harte, et al. (2017a)), such as low-growing shrubs or small-diameter tertiary branches of trees at relatively low heights (Fig. 5.3, 5.5). Although most *Strophurus* species fall within the broad perch-space of the 'grass – bush' anoles, there is a clear separation between the spinifex-associated *Strophurus elderi* (which was not included in Hagey, Harte, et al. (2017a)), and *S. williamsi, S. krisalys*, and *S. ciliaris*. *Strophurus elderi* had perch heights lower even than means for 'ground – bush' anoles, whereas the latter three species used shrubs and twigs of small trees, and fall within the 'ground – bush' niche space (Fig. 5.3, 5.5). The differences in habitat use I note among *Strophurus spp.* were consistent with morphological and taxonomic distinction between the so-called 'graminicolous' and 'scanso-arboreal' groups (Greer, 1989; Storr, Smith, & Johnstone, 1990), and assessments of microhabitat use in these species (Nielsen *et al.*, 2016; Laver *et al.*, 2017). Although I did not record perch height and diameter for *S. taeniatus*, I found them in spinifex, suggesting they might occupy a perch space similar to *S. elderi.* Thus, although My suggestions are preliminary because I have only a small sample of *S. elderi,* I suggest that there are two distinct 'grass – bush' ecotypes in Australian geckos: a spinifex-hummock grass-associated 'grass' ecotype and a 'bush-twig' ecotype, using higher and thicker perches of shrubs and small trees (Nielsen *et al.*, 2016). Perch height for *Amalosia rhombifer* fell within the overlapping area between the 'grass' and the 'trunk – ground' perch-space area, consistent with its generalist habitat use (Fig. 5.3). As the generalist species *Gehyra versicolor* and *Oedura cincta* both fell outside of the perch spaces plotted for *Anolis*, but were close to *A. rhombifer*, I propose a new 'generalist' or 'ground – bush – twig' ecotype for Australian geckos, overlapping with the 'bush – twig' and the 'trunk – ground' perch-space area.

Hagey, Harte, et al. (2017a) suggested that *Pseudothecadactylus australis* was in the overlapping area between 'trunk – crown' and 'crown – giant' ecotype. My more extensive sampling revealed that it does use thick trunks of rainforest trees, but also thin branches of the same trees, vines, bamboo, and occurs outside rainforest in heath and woodland habitats, where it uses lower-growing trees with thin branches as well (Fig. 5.3, 5.5). Thus, I agree they are 'trunk – crown' ecotypes, but not in the 'crown – giant' group. My extensive sampling of *Gehyra dubia* reveals that it uses higher perches on average than previously recorded (Hagey *et al.*, 2017a). This suggests that extensive sampling of habitat use can be useful, even for common species (in Australia). The primarily padless Carphodactylidae were not included in Hagey, Harte, et al. (2017a), and detailed ecological data, including perch height and diameter, are reported here for the first time. While *Saltuarius cornutus* occupies a perch space similar to 'trunk – crown'/'crown – giant' ecotypes, *Phyllurus nepthys* fits within the 'trunk – ground' ecotype similar to *Oedura* or *Gehyra* species outside rainforest habitats. Notably, I found *Phyllurus nepthys* using its full range of microhabitats (trees, rocks, ground) only in the highest elevation areas of its habitat (Dalrymple Heights, nearly 1000 m), whereas they used boulders or man-made structures (concrete bridges) in or near rainforest streams in the lower elevations of their range (Finch Hatton Gorge, 300 - 400 m; Broken River, 600 - 700 m). Leaf-tailed geckos are dependent on habitats with high humidity, and these ancient rainforest lineages use rocky landscapes (lithorefugia) as habitats (Couper & Hoskin, 2008). *Carphodactylus laevis*, which exclusively used small saplings to perch head-down, low to the ground, potentially falls in My proposed 'grass' ecotype. Both, *Heteronotia binoei* and *Nactus eboracensis* occupied perch spaces far outside those plotted for anoles. And although *H. binoei* is a generalist, while *N. eboracensis* is terrestrial, both species used the ground in more than 50% of captures (Fig. 5.3). Therefore, these two species might be described as part of a 'ground-log' or 'ground-log-trunk' ecotype. I would need additional perch data from more Australian gecko species to validate the consistency of these proposed ecotypes.

IMPORTANCE OF NATURAL HISTORY STUDIES

Australia supports some of the world's most diverse gecko communities, yet most species are data deficient, even in terms of basic ecological or natural history data (Meiri, 2018). To better manage communities and understand the impacts of environmental changes on communities, I need to understand how species use their environment. Overall, My results are in accord with previous detailed studies, where they are available (Pianka, 1969; Pianka & Pianka, 1976; Henle, 1990; Nordberg *et al.*, 2018b,a; Nordberg & Schwarzkopf, 2019a), emphasizing that even anecdotal observations can provide useful insights into animal ecology. My study adds considerable new or updated information about the microhabitat use, perch-space, and ecological niche space of Australian geckos, and provides an ecomorph classification of geckos similar to that established for anoles. I encourage field biologists in all research areas to collect data on the ecology of the species they collect, and to publish them, or make them publicly available in other venues, such as public databases.

Figures

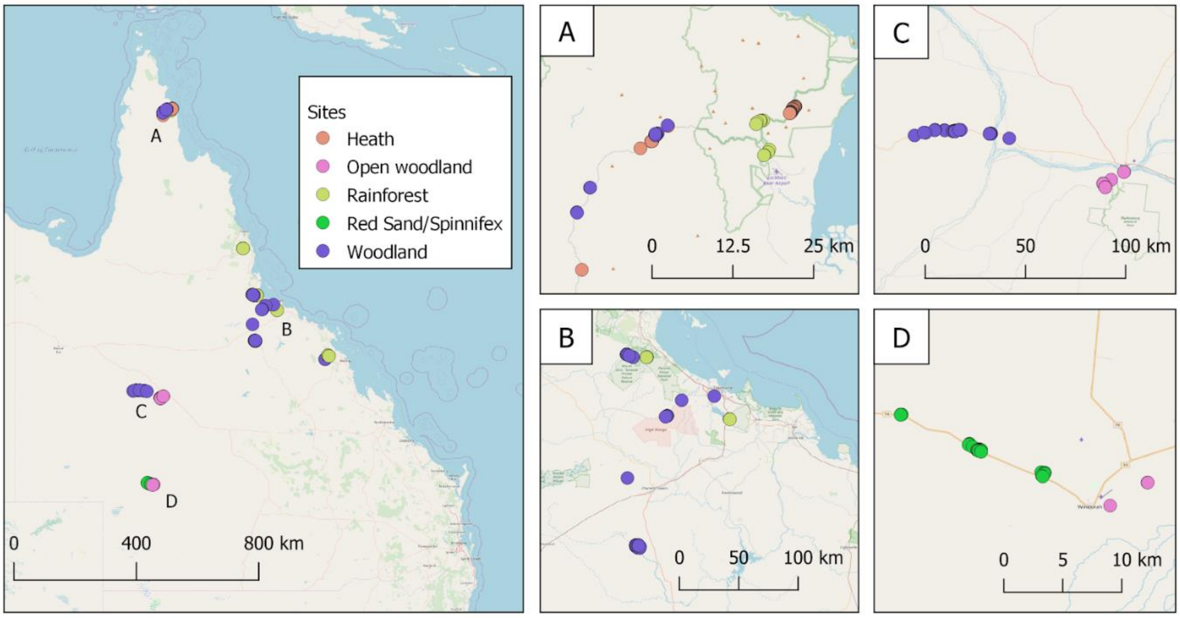

Figure 5-1 Survey sites across Queensland, Australia. **(A)** Cape York Peninsula, showing the locations surveyed at the Iron Range site. **(B)** Townsville region with the sites Hervey Range, Hidden Valley, Paluma Range, Mt. Elliot and Wambiana. **(C)** Locations surveyed in the area around Winton. **(D)** Locations surveyed around Windorah. Each point refers to an area surveyed. Areas of similar habitat at each site are called 'locations' (Table 1).

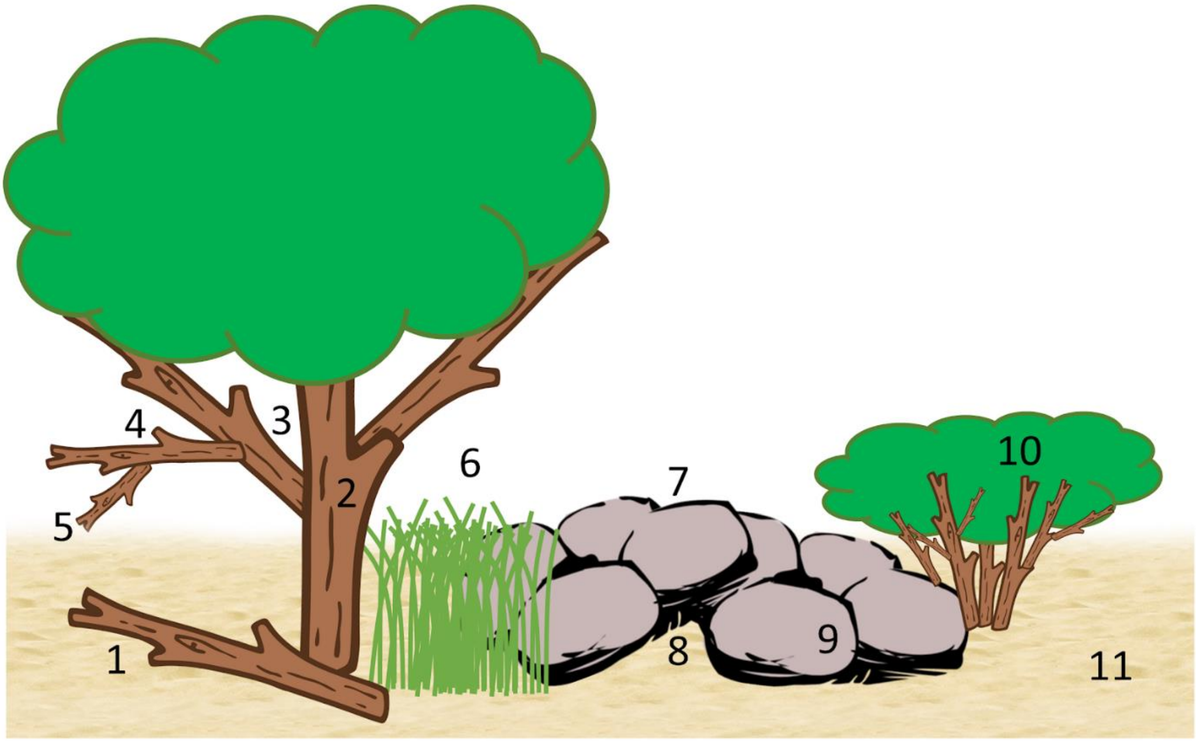

Figure 5-2 Perch locations: log (1), tree trunk (2), primary branch (3), secondary branch (4), tertiary branch (5), grass (6), horizontal on rocks (7), on overhanging rock or crevice (8), vertically on rock (9), bush and shrub (10), ground (11).

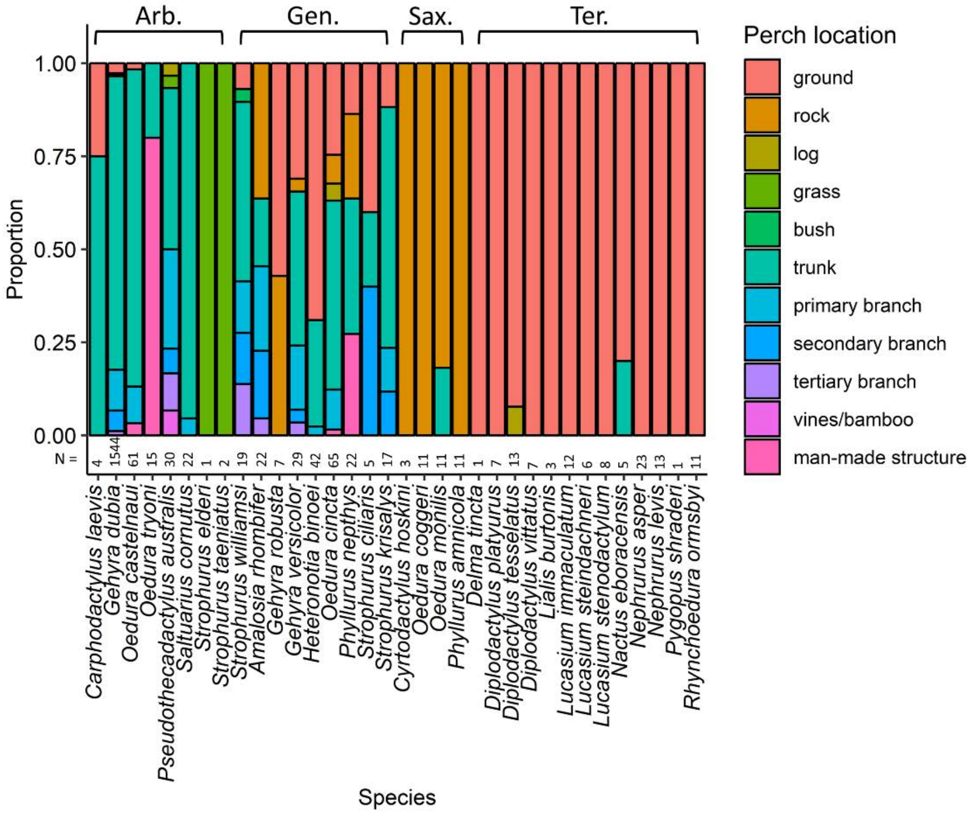

Figure 5-3 Gecko community perch locations across Queensland, Australia. Arb. = arboreal species, Gen. = generalist species, Sax. = saxicoline (rock-dwelling) species, Ter. = terrestrial species.

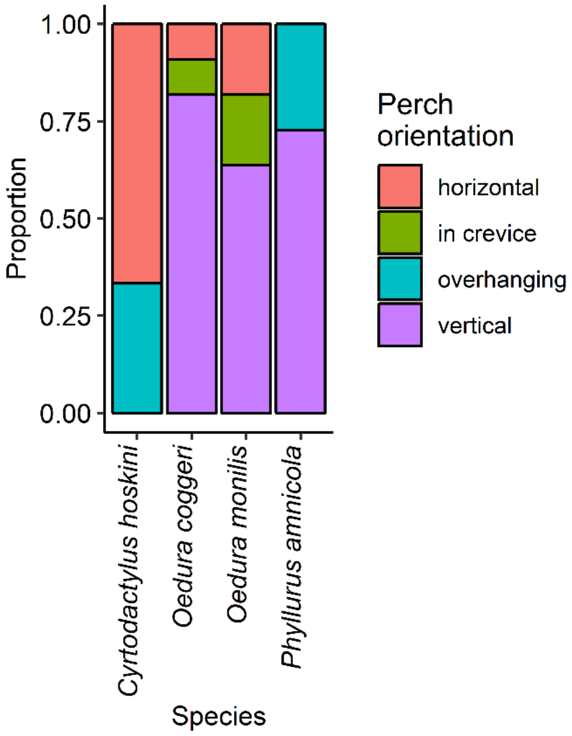

Figure 5-4 Saxicolous species' perch orientation on rocks.

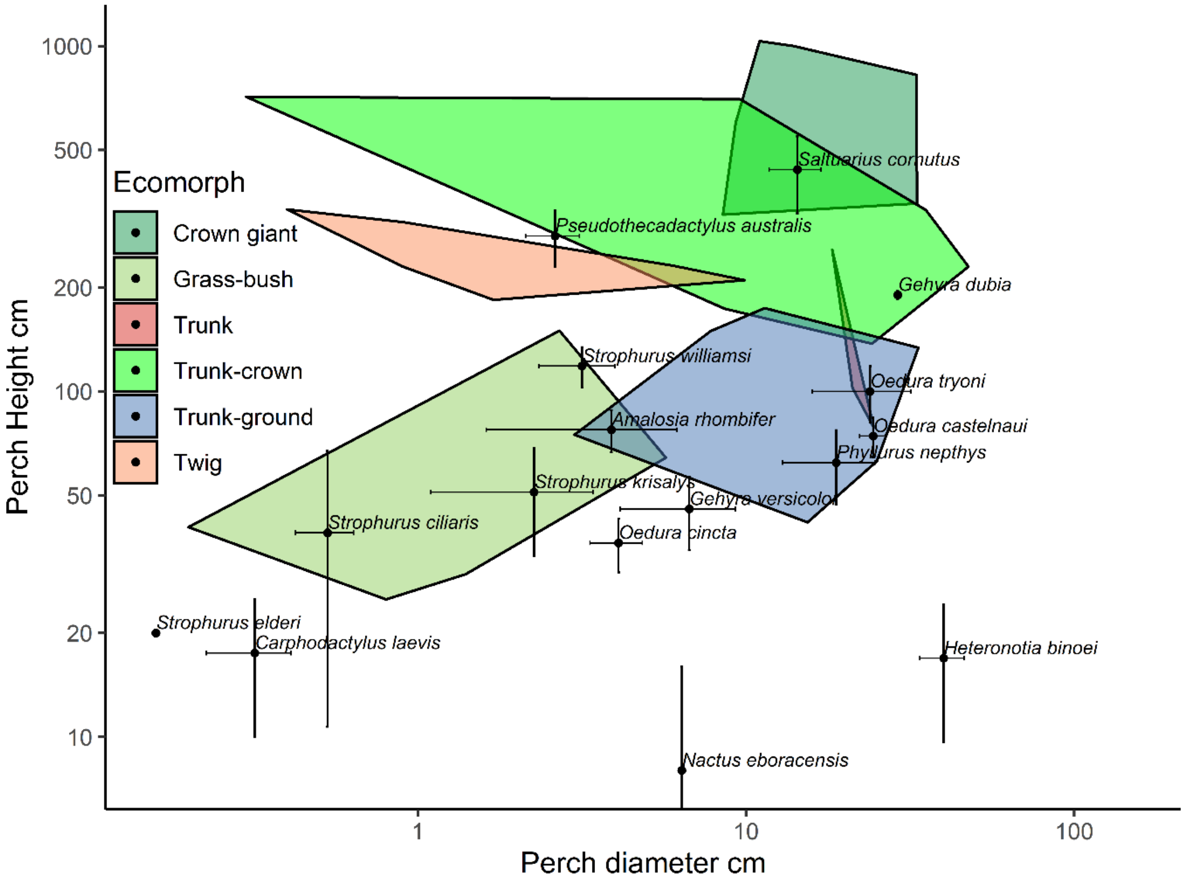

Figure 5-5 Perch space (height and diameter) used by Australian geckos, overlayed on polygons indicating the range in mean perch spaces occupied by anole ecomorphs (adapted from Hagey, Harte et al. 2017). Points for geckos are centroid means ± SE.

Tables

Table 5.1: Overview over the areas surveyed for this study and the bioregion to which these belong. The habitat categories, which we assigned, are shown as well as the standardized reginal ecosystem codes (Queensland Herbarium, 2019) for the areas in question. Species richness displayed the number of species detected in My surveys, with an ID matching that given for each species Table 2. Each habitat type in a geographic area (Site) is summarized as one location for this study.

Geographic Area	Bioregion	Habitat	Regional ecosystem codes: BVG1M (% covered)	Species richness	Species ID
Iron Range	Cape York Peninsular	Rainforest	3.11.1 / 3.11.3 / 3.11.11 (70/20/10)	2	8, 26
		Woodland	3.12.10 / 3.12.21 / 3.12.41 /3.12.28 / 3.12.11 (50/20/10/10/10)	8	1/2/8/11/12/16/19/26
		Heath	3.3.5a / 3.5.42 / 3.7.6x2 (40/40/20) 3.12.47 / 3.12.41 (80/20)	4	1/8/16/17/34
Tablelands	Wet tropics	Rainforest	7.8.2a	2	2/29
Paluma Range	Wet tropics	Rainforest	7.12.16	1	29
Mt. Elliot	Wet tropics	Rainforest	11.12.4	1	24
Eungella	Central Queensland Coast	Rainforest	8.12.2 / 8.12.3a / 8.12.19 (40/30/30)	1	25
		Woodland	8.12.4 / 8.12.7a (60/40)	3	8/23/25
Hervey's Range / Townsville Region	Bringalow Belt	Woodland	9.11.2a/9.11.5	6	5/7/12/14/19/27
Hidden Valley	Einasleigh Uplands	Woodland	9.12.19 7.12.65k	4	1/21/22/34
Wambiana Station	Desert Uplands	Woodland		6	8/11/12/14/19/34
Winton	Mitchell Grass Downs	Woodland	4.9.14x44 / 4.4.1xb (70/30) 4.7.1a / 4.7.2 / 4.7.2x1a / 4.7.4a (50/20/20/10)	10	1/6/9/10/11/13/17/20/32/33
		Savannah Woodland	5.7.1 / 4.5.6x4 / 4.7.2x2 (50/30/20)	6	6/13/14/20/28/32
Windorah	Channel Country	Desert	5.6.5a	4	10/15/18/30
		Savannah Woodland	5.5.2 / 5.3.16a (90/10)	3	10/30/31

Table 5.2: Comparison of habitat use and size data available from the literature and the data added in this study for the species surveyed in this study (ID's for species are given in brackets to match Table 1). In cases where conflicting information is available from the literature, different information from different sources are separated by a semicolon, and the sources are separated accordingly. SVL data from the literature are maximum values, unless marked with and *, in which case they are average values. PH and PD refer to average perch height and perch diameter respectively. References: 1) (Wilson, 2015). 2) (Cogger 2015). 3) (Michael *et al.*, 2015). 4) (Bustard, 1965). 5)(Nordberg *et al.*, 2018a). 6) (Pianka & Pianka, 1976). 7) (Neilly *et al.*, 2018). 8) (Pianka, 1969). 9) (Zozaya, Alford, & Schwarzkopf, 2015). 10) (Wilson & Knowles, 1988). 11) (Michael & Lindenmayer, 2010). 12) (Meiri 2018). 13) (Nielsen et al. 2016) 14) (Storr *et al.*, 1990) 15) (Shea *et al.*, 2011). 16) (Johansen, 2012). 17) (Henle 1991). 18) (Mesquita *et al.*, 2016a). 19) (Oliver *et al.*, 2017). 20) (Henkel, 2010). 21) (Oliver & Doughty, 2016). 22) (Pepper *et al.*, 2011). 23) (Couper, Covacevich, & Moritz, 1993). 24) (Vanderduys, 2017). 25) (Hagey, Harte et al. 2017)

	Existing Knowledge			**Updated Information - this study**		
Species			**Ref**			**n**
Amalosia rhombifer (1)	Macrohabitat	Widespread forests, woodlands	1,3,13	Macrohabitat	Heath and woodlands	22
	Microhabitat	Under bark	3	Microhabitat	Trees and rocks	22
	Lifestyle	Arboreal; Generalist	1,13; 12,14	Lifestyle	Generalist	22
	Perch location	PH: 81.4 cm and PD: 11.8 cm	25	Perch location	Branches and rocks	22
	SVL	70; 80	1,13; 12	SVL	48.94 ± 1.59	17
	Mass			Mass	2 ± 0.2	17
Carphodactylus laevis (2)	Macrohabitat	Wet tropics - rainforests	1	Macrohabitat	Rainforest	4
	Microhabitat	Leaf litter, slender twigs	1	Microhabitat	Trees, ground	4
	Lifestyle	Scansorial	13	Lifestyle	Arboreal	4
	Perch location	Slender twigs	1	Perch location	Tree trunks, ground	4
	SVL	130	1	SVL	93.5 ± 6.96	4
	Mass			Mass	14.71 ± 2.9	4
Cyrtodactylus hoskini (3)	Macrohabitat	Endemic - western edge of Iron Range	15,1	Macrohabitat	Woodland	3
	Microhabitat	Granite boulders, open forest	15,1	Microhabitat	Rocks	3
	Lifestyle	Saxicoline	12	Lifestyle	Saxicoline	3
	Perch location			Perch location	Rocks	3
	SVL	64;112	15,1	SVL	111 ± 2.08	3
	Mass			Mass	24.96 ± 0.9	3
Delma tincta (4)	Macrohabitat	Widespread, woodlands	1,3	Macrohabitat	Woodland	1
	Microhabitat	Under log, rocks	3	Microhabitat	Leaf litter	1
	Lifestyle	Fossorial, terrestrial	12	Lifestyle	Terrestrial	1
	Perch location			Perch location	Ground	1
	SVL	92	1	SVL		
	Mass			Mass		
Diplodactylus platyurus (5)	Macrohabitat	Widespread, arid woodlands, scrublands	1	Macrohabitat	Woodland	7
	Microhabitat			Microhabitat	Open ground	7

	Lifestyle	Terrestrial	1,12	Lifestyle	Terrestrial	7
	Perch location			Perch location	Ground	7
	SVL	60	1	SVL	39.1 ± 1.41	7
	Mass			Mass	2.18 ± 0.22	7
Diplodactylus tessellatus (6)	Macrohabitat	Clay soils; arid regions	1; 13	Macrohabitat	Savannah woodland, woodland	
	Microhabitat			Microhabitat	Open ground	13
	Lifestyle	Terrestrial	1,13,16,17	Lifestyle	Terrestrial	13
	Perch location			Perch location	Ground	13
	SVL	50; 58	1,13; 12	SVL	47.83 ± 2.11	11
	Mass			Mass	2.91 ± 0.29	11
Diplodactylus vittatus (7)	Macrohabitat	Woodlands	1,3	Macrohabitat	Woodland	7
	Microhabitat	Leaf litter, under log/rock; surface debris	3; 10	Microhabitat	Ground	7
	Lifestyle	Terrestrial	1, 12	Lifestyle	Terrestrial	7
	Perch location	Fallen twigs	1	Perch location	Ground, twigs	7
	SVL	50; 59.5	1; 12	SVL	46.81 ± 0.66	7
	Mass			Mass	3.06 ± 0.16	7
Gehyra dubia (8)	Macrohabitat	Widespread, woodlands	1, 5	Macrohabitat	Heath, rainforest, woodland	1544
	Microhabitat	Tree trunks; man-made structures	5,7;9	Microhabitat	Trees	1544
	Lifestyle	Arboreal	5,17,25	Lifestyle	Arboreal	1544
	Perch location	Trunk; PH: 85.5 cm, PD: 18.4 cm	5,7; 25	Perch location	Trunk, branches	1544
	SVL	65; 42.9*	1; 5	SVL	53.58 ± 0.37	636
	Mass	3.3	5	Mass	4.04 ± 0.07	636
Gehyra robusta (9)	Macrohabitat	Endemic - Northwest Highlands, Mitchell grass Downs	1	Macrohabitat	Woodland	7
	Microhabitat	Rocky ranges and outcrops	1	Microhabitat	Rocks, ground	7
	Lifestyle	Saxicoline	1,12	Lifestyle	Generalist	7
	Perch location	trunk; PH: 35.3 cm, PD: 10.8 cm	5,7; 25	Perch location	Rocks, ground	7
	SVL	75	1,12	SVL		
	Mass			Mass		
Gehyra versicolor (10)	Macrohabitat	Widespread, dry woodlands	1	Macrohabitat	Savannah woodland, desert	29
	Microhabitat			Microhabitat	Trees, ground, rocks	29
	Lifestyle	Arboreal and saxicoline	1,12,16,18	Lifestyle	Generalist	29
	Perch location			Perch location	Trunk, branches	29

	SVL	54	1, 12	SVL	45.17 ± 2.33	12
	Mass			Mass	2.47 ± 0.34	12
Heteronotia binoei (11)	Macrohabitat	Widespread, woodlands	3,4	Macrohabitat	Woodland	42
	Microhabitat	Under bark, log, and rocks; spinifex; shrubs, burrows	3,4,6,8	Microhabitat	Trees, ground	42
	Lifestyle	Terrestrial; arboreal and terrestrial	1,6,19,17; 12,18	Lifestyle	Generalist	42
	Perch location			Perch location	Trunk, ground	42
	SVL	54; 55	1,6	SVL		
	Mass			Mass		
Lialis burtonis (12)	Macrohabitat	Widespread, woodlands	1, 3	Macrohabitat	Woodland	3
	Microhabitat	Under rock; spinifex, ubiquitous	3,4,8	Microhabitat	Open ground	3
	Lifestyle	Terrestrial	1,12,13,17,18	Lifestyle	Terrestrial	3
	Perch location			Perch location	Ground	3
	SVL	85	1, 12	SVL	112.09 ± 92.59	2
	Mass			Mass	19.09 ± 0.65	2
Lucasium immaculatum (13)	Macrohabitat	Stony open woodlands; arid savannah	1; 13	Macrohabitat	Savannah woodland, Woodlands	12
	Microhabitat			Microhabitat	Open ground	12
	Lifestyle	Terrestrial	1,12,16	Lifestyle	Terrestrial	12
	Perch location			Perch location	Ground	12
	SVL	85	1, 12	SVL	46.7 ± 0.77	9
	Mass			Mass		
Lucasium steindachneri (14)	Macrohabitat	Woodlands, red soil plains	1,11	Macrohabitat	Savannah woodland, woodland	6
	Microhabitat	Spider burrows, dead vegetation, sparse ground cover	11	Microhabitat	Leaf litter, open ground	6
	Lifestyle	Terrestrial	1,12,18,16	Lifestyle	Terrestrial	6
	Perch location			Perch location	Ground	6
	SVL	55; 59	1; 12	SVL	47.67 ± 1.74	6
	Mass			Mass	3.04 ± 0.27	6
Lucasium stenodactylum (15)	Macrohabitat	Widespread, dry shrublands	1	Macrohabitat	desert	8
	Microhabitat			Microhabitat	Sandy soil	8
	Lifestyle	Terrestrial	1,12,18,16	Lifestyle	Terrestrial	8
	Perch location			Perch location	Ground	8

	SVL	57	1, 13	SVL	52.43 ± 1.53	7
	Mass			Mass	3.04 ± 0.19	7
Nactus eboracensis (16)	Macrohabitat	Tropical woodlands and outcrops	1	Macrohabitat	Heath and woodlands	5
	Microhabitat			Microhabitat	Trees and ground	5
	Lifestyle	Terrestrial	1, 12	Lifestyle	Terrestrial	5
	Perch location			Perch location	Trunk, ground	5
	SVL	57; 58	1, 12	SVL		
	Mass			Mass		
Nephrurus asper (17)	Macrohabitat	Dry woodlands, rocky outcrops	1	Macrohabitat	Heath and woodlands	23
	Microhabitat			Microhabitat	Open/rocky ground	23
	Lifestyle	Terrestrial; saxicoline and terrestrial	1; 20	Lifestyle	Terrestrial	23
	Perch location			Perch location	Ground	23
	SVL	115; 117	1; 12	SVL	86.6 ± 2.15	21
	Mass			Mass	16.18 ± 1.23	21
Nephrurus levis (18)	Macrohabitat	Sandy regions	1	Macrohabitat	Desert	13
	Microhabitat	Dunes with spinifex; open ground, litter	1,8; 6	Microhabitat	Sandy soil	13
	Lifestyle	Terrestrial	1,12,17,18	Lifestyle	Terrestrial	13
	Perch location			Perch location	Ground	13
	SVL	102; 105	1; 12	SVL	62.46 ± 5.21	13
	Mass			Mass	10.02 ± 2.28	13
Oedura castelnaui (19)	Macrohabitat	Widespread, woodlands, rocky outcrops, savannah	1 5,7; 13	Macrohabitat	Woodland	61
	Microhabitat	Dead trees, trunks	5,7	Microhabitat	Trees, logs	61
	Lifestyle	Arboreal; arboreal and terrestrial	1,5; 12	Lifestyle	Arboreal	61
	Perch location	Trunk; PH: 96.3 cm, PD: 16.0 cm	5,7; 25	Perch location	Dead trees	61
	SVL	90; 80.8*; 97	1; 5; 12	SVL	79.91 ± 2.57	31
	Mass	13.3	5	Mass	13.31 ± 0.95	31
Oedura cincta (20)	Macrohabitat	Dry open woodlands, rock outcrops	1	Macrohabitat	Savannah woodland and woodland	65
	Microhabitat			Microhabitat	Trees, rocks, ground	65
	Lifestyle	Arboreal and saxicoline; arboreal	1,12,21; 25	Lifestyle	Generalist	65
	Perch location	PH: 70.4 cm, PD: 18.2 cm	25	Perch location	Trunks, rocks, ground	65
	SVL	110; 108	1,12	SVL	82.36 ± 1.84	12
	Mass			Mass	10.4 ± 0.88	12
Oedura coggeri	Macrohabitat	Dry open woodlands, savannah	1,13	Macrohabitat	Woodland	11

(21)	Microhabitat	Rocks and boulders	1	Microhabitat	Boulders	11
	Lifestyle	Saxicoline; saxicoline and arboreal	1,13; 12	Lifestyle	Saxicoline	11
	Perch location			Perch location	Rocks	11
	SVL	70; 80.4	1; 12	SVL	70.27 ± 2.63	11
	Mass			Mass	7.66 ± 0.79	11
Oedura monilis (22)	Macrohabitat	Dry woodlands; sclerophyll	1,13	Macrohabitat	Woodland	11
	Microhabitat			Microhabitat	Trees and rocks	11
	Lifestyle	Arboreal; generalist	1,12,13,17; 25	Lifestyle	Saxicoline	11
	Perch location	PH: 13.3 cm, PD: 2.9 cm	25	Perch location	Trunks and boulders	11
	SVL	85; 98.1		SVL	82.64 ± 1.71	11
	Mass			Mass	11.35 ± 0.56	11
Oedura tryoni (23)	Macrohabitat	Woodlands, granite outcrops; sclerophyll	1, 3; 13	Macrohabitat	Woodland	15
	Microhabitat	Under bark, rocks	3	Microhabitat	Tree trunks, concrete drainage tunnels	15
	Lifestyle	Generalist; arboreal; saxicoline; terrestrial	12; 18; 13; 22	Lifestyle	Arboreal	15
	Perch location	Rocks and tree trunks	1	Perch location	Man-made structures, trunks	15
	SVL	87	1; 12,13	SVL	82.83 ± 5.11	12
	Mass			Mass	13.51 ± 1.92	12
Phyllurus amnicola (24)	Macrohabitat	Granit boulders in rainforest	1	Macrohabitat	Rainforest	11
	Microhabitat	Creek line boulders	1	Microhabitat	Boulder fields	11
	Lifestyle	Saxicoline; arboreal and saxicoline	1; 12	Lifestyle	Saxicoline	11
	Perch location			Perch location	Rocks	11
	SVL	113	1; 12	SVL	91.6 ± 3.67	10
	Mass			Mass	13.02 ± 2.23	10
Phyllurus nepthys (25)	Macrohabitat	Endemic - rainforest in Clark Range	1	Macrohabitat	Rainforest	22
	Microhabitat			Microhabitat	Trees and rocks	22
	Lifestyle	Arboreal	12	Lifestyle	Generalist	22
	Perch location			Perch location	Trunks and boulders	22
	SVL	103	1,12	SVL	86.24 ± 4.05	21
	Mass			Mass	12.73 ± 1.25	21
Pseudothecadactylus australis (26)	Macrohabitat	Endemic - northern Cape York, woodlands, mangrove forests	1	Macrohabitat	Heath and rainforest, occasionally Woodland	30
	Microhabitat			Microhabitat	Trees	30

	Lifestyle	Arboreal	1,12,13, 26	Lifestyle	Arboreal	30
	Perch location	PH: 380.0 cm, PH: 15.8 cm	25	Perch location	Bamboo/vines, trunks	30
	SVL	120	1,12	SVL	102.88 ± 2.07	16
	Mass			Mass	20.41 ± 1.23	16
Pygopus schraderi (27)	Macrohabitat	Widespread - dry woodlands and open habitats	1	Macrohabitat	Woodland	1
	Microhabitat			Microhabitat	Open ground	1
	Lifestyle	Terrestrial	1,12	Lifestyle	Terrestrial	1
	Perch location			Perch location	Ground	1
	SVL	198	1,12	SVL	112	1
	Mass			Mass	7.64	1
Rhynchoedura ormsbyi (28)	Macrohabitat	Widespread - dry arid regions	1	Macrohabitat	Savannah woodland	11
	Microhabitat			Microhabitat	Open ground	11
	Lifestyle	Terrestrial	1,12,22	Lifestyle	Terrestrial	11
	Perch location			Perch location	Ground	11
	SVL	50	1,12	SVL	38.18 ± 1.34	9
	Mass			Mass		
Saltuarius cornutus (29)	Macrohabitat	Wet tropical rainforests	1	Macrohabitat	Rainforest	22
	Microhabitat	Rainforest trees	1	Microhabitat	Trees	22
	Lifestyle	Arboreal and saxicoline	1,12,23	Lifestyle	Arboreal	22
	Perch location			Perch location	Trunks	22
	SVL	144; 160	1,12	SVL	116.75 ± 6.33	12
	Mass			Mass	30.31 ± 3.4	12
Strophurus ciliaris (30)	Macrohabitat	Widespread, arid shrublands	1,13	Macrohabitat	desert	5
	Microhabitat	Spinifex, shrubs, leaf litter	6	Microhabitat	Trees, shrubs, rocks, ground	5
	Lifestyle	Arboreal; arboreal and terrestrial; scansorial and arboreal;	25; 1,12,18,16; 13	Lifestyle	Generalist	5
	Perch location	PH: 21.8 cm, PD: 3.1 cm	26	Perch location	Trunk, shrubs	5
	SVL	77; 90; 86		SVL	69.8 ± 2.82	5
	Mass			Mass	6.31 ± 0.89	5
Strophurus elderi (31)	Macrohabitat	Arid regions, sandy deserts	1,4,13	Macrohabitat	Savannah woodland	1
	Microhabitat	Spinifex; leaf litter	1 4,8; 6	Microhabitat	Spinifex	1
	Lifestyle	Graminicolous; arboreal and terrestrial	1,13; 12,16	Lifestyle	Arboreal	1

	Perch location	Spinifex	4,6,8	Perch location	Spinifex	1
	SVL	48; 51	1,13	SVL	44	1
	Mass			Mass	2.47	1
Strophurus krisalys (32)	Macrohabitat	Shrublands, mulga woodlands; arid savannas	1; 13	Macrohabitat	Savannah woodland and woodland	17
	Microhabitat			Microhabitat	Trees, ground	17
	Lifestyle	Arboreal; scansorial	1,26; 12,13	Lifestyle	Generalist	17
	Perch location	PH: 62.9 cm, PD: 1.4 cm	25	Perch location	Tree branches	17
	SVL	70; 76	1; 13	SVL	60.8 ± 3.43	14
	Mass			Mass	4.96 ± 0.63	14
Strophurus taeniatus (33)	Macrohabitat	Northwest highlands, savanna	1; 13	Macrohabitat	Woodland	2
	Microhabitat	Spinifex	1	Microhabitat	Spinifex	2
	Lifestyle	Graminicolous; arboreal and terrestrial	1,13; 12,16	Lifestyle	Arboreal	2
	Perch location			Perch location	Spinifex	2
	SVL	44; 50	1; 13	SVL		
	Mass			Mass		
Strophurus williamsi (34)	Macrohabitat	Dry sclerophyll woodlands	1,5; 13	Macrohabitat	Heath and Woodland	29
	Microhabitat	Shrubs, bushes	5;7	Microhabitat	Trees and shrubs	29
	Lifestyle	Arboreal; arboreal and saxicoline; scansorial	1,5,17,25; 12,13,24	Lifestyle	Arboreal	29
	Perch location	Thin branches, twigs	5	Perch location	Trunks and branches	29
	SVL			SVL	56.89 ± 1.37	17
	Mass			Mass	4.05 ± 0.35	17

Chapter 6 - Concluding Remarks and Synthesis

The highly structured integument of squamate reptiles includes a diversity of different scale shapes and types (Baeckens *et al.*, 2019). The oberhäutchen, the outermost layer of the squamate integument, features a relief of microscopic structures or microornamentation (Ruibal, 1968). Microornamentation has been studied for over a century (Cartier, 1872; Leydig, 1873), and has influenced and generated a broad variety of research, ranging from descriptive or comparative morphology (e.g. Ruibal, 1968; Stewart & Daniel, 1972, 1973; Gower, 2003; Bucklitsch, Böhme, & Koch, 2012), to taxonomy (Harvey, 1993; Harvey & Gutberlet Jr, 1995, 2000; Bucklitsch *et al.*, 2016) functional and behavioural biology (e.g. Russell & Johnson, 2007; Baum *et al.*, 2014; Khannoon *et al.*, 2014; Spinner *et al.*, 2014b; Stark *et al.*, 2015a; Niewiarowski, Stark, & Dhinojwala, 2016), and has spawned new areas in biomimetic research (Sitti & Fearing, 2003; Shah & Sitti, 2004; Huijing *et al.*, 2005; Kumar *et al.*, 2018). Recently, interest in microornamentation has shifted to incorporate functional evolutionary and ecomorphological biology (Collins *et al.*, 2015; Russell *et al.*, 2015; Higham *et al.*, 2017; Zhuang *et al.*, 2019). Geckos have been a focus group for microstructure research since early on, because of their amazing climbing abilities generated by their adhesive toepads (Russell, 2002; Pianka & Sweet, 2005). Especially since the early 2000s, fuelled by improvements in microscopy, ground-breaking studies deeply enhanced My understanding of the physical principals associated with the function of the adhesive setae, including measurements of the adhesive forces generated by single spatulae (Autumn & Peattie, 2002; Huber *et al.*, 2005a,b; Autumn, 2007). To understand these physical and functional principles, most of these studies focussed on a single model organism, the tokay gecko (*Gekko gecko*). And although model organisms are useful for functional studies, they can limit My ability to generalize (Peattie, 2001). And although descriptions of microornamentation, especially of setae, are available for some non-model gecko species (Schleich & Kästle, 1986; Peattie, 2001, 2009; Hagey *et al.*, 2014b), broad scale knowledge of variation in microornamentation is still limited, and these limitations mean evolutionary and ecomorphological studies on gecko adhesion tend to focus either on performance and macroscopic toepad area (Kulyomina *et al.*, 2019; Zhuang *et al.*, 2019) or on a few species of a single genus (Johnson, Russell, & Bauer, 2005; Collins *et al.*, 2015; Russell *et al.*, 2015; Higham *et al.*, 2017). Furthermore, other skin microstructures, such as spinules and cutaneous sensilla, have largely been overlooked, and descriptions of non-setal microstructures are mostly limited to old publications, in which image quality is low compared to modern standards (Ruibal, 1968; Bauer & Russell, 1988). The function of the spinules has only recently gained some attention (Hiller, 2009; Spinner *et al.*, 2013a; Watson *et al.*, 2015a; Li *et al.*, 2016), and to my knowledge, no study so far has examined the evolution or ecological adaptation in either spinules or cutaneous sensilla.

This thesis contributes to a better understanding of the evolution and ecological adaptations of microornamentation on different levels for several of the different microstructures found in geckos. For spinules and cutaneous sensilla I provide broad comparative morphological data for a range of Australian geckos, and I analyse associations among these microstructures and aspects of the species' ecology in a phylogenetic framework (Chapter 2), something seldom done before for microstructures.

Then, to examine the function of spinules more deeply, I used functional measurements of hydrophobicity of the skin of those species to determine which features of the microornamentation is associated with hydrophobicity, and the association of hydrophobicity and aspects of the geckos' ecology (Chapter 3). Although hydrophobicity is widespread, not only in geckos, but also in invertebrates and plants (Barthlott & Neinhuis, 1997; Neinhuis & Barthlott, 1997; Wagner *et al.*, 2003; Watson *et al.*, 2010), it has never before been successfully associated with ecological factors, in a statistical framework (Tellechea-Robles *et al.*, 2019).

I also examined an aspect of the evolution of setae, describing the microornamentation and general subdigital scale morphology of various lineages of the *Heteronotia binoei* species complex, and I proposed that two, unrelated saxicoline lineages may be functional intermediates in the evolution of terminal adhesive toepads (Chapter 4). Functional intermediates are important to understand the evolution of adhesive toepads, but previously only one example has been established for basal toepads (Russell *et al.*, 2015; Higham *et al.*, 2017), and a few others suggested (Russell, 1976, 1977; Russell & Gamble, 2019).

Finally, as ecological data for most Australian gecko species is only available from scattered, anecdotal or descriptive entries in the taxonomic literature or in field guides, I also proved detailed ecological observations for a broad range of gecko species I sampled in Queensland, Australia, in some cases to obtain measurements for the other chapters (Chapter 5).

EVOLUTION AND ADAPTATION OF SPINULES AND CUTANEOUS SENSILLA

I provide a detailed description of the scale size and microornamentation on the dorsal midbody region of 27 species of Australian diplodactylid and carphodactylid geckos (Chapter 2). Using a phylogenetic flexible discrimination function analysis, I established associations among scale size and microstructure measurements, and aspects of the geckos' ecology, namely habitat humidity and habitat use. For the former I categorised the geckos as xeric, mesic, or humid, representing desert, savannah and rainforest habitats, and for the latter I categorized them as terrestrial, saxicoline or arboreal, using published literature (Wilson, 2015; Cogger, 2018). Species from humid environments had larger cutaneous sensilla diameter and a smaller number of bristles per sensillum compared to species from drier habitats. Terrestrial species had cutaneous sensilla with more bristles per sensillum. Both terrestrial species and those from xeric habitats had long spinules. Spinule length has been associated with hydrophobicity and self-cleaning (Hiller, 2009; Watson *et al.*, 2015c) and bactericidal properties (Watson *et al.*, 2015b; Li *et al.*, 2016) in functional studies of gecko skin, and species from terrestrial habitats are likely exposed to more dirt and debris and potentially harmful microorganisms than are saxicoline or arboreal groups (Ungar *et al.*, 1995; Nunn *et al.*, 2000; McCabe *et al.*, 2015). Therefore, self-cleaning and bactericidal properties, associated with long spinules, could be important drivers of their evolution (Watson *et al.*, 2015b). In contrast, I found no support for the hypothesis that the proposed bactericidal functions (Watson *et al.*, 2015a,b; Li *et al.*, 2016) could also have led to the evolution of long spinules in rainforest species, in spite of expected higher prevalence and growth rates of bacteria and fungi in rainforest habitats (Bouskill *et al.*, 2012).

EVOLUTION OF HYDROPHOBICITY

If hydrophobicity has evolved to promote self-cleaning and bactericidal functions in geckos, I expected that terrestrial species would be better adapted for self-cleaning, and possess better bactericidal skin properties than species using other habitats, as dust, dirt, debris, and bacteria tend to accumulate on the ground (Ungar *et al.*, 1995; Nunn *et al.*, 2000; McCabe *et al.*, 2015). To test this hypothesis, I used measurements of hydrophobicity to analyse: 1) if hydrophobicity is associated with long spinules, and 2) if hydrophobicity has evolved as an adaptation to terrestrial habitat use (Chapter 3). Using phylogenetic generalised least squares regressions and ancestral state reconstructions, I found that, although all gecko species were strongly hydrophobic, terrestrial species were more hydrophobic than arboreal or saxicoline species, with no significant difference between the latter two. In contrast, I found no significant difference among species from habitats with different humidity regimes, corroborating my previous results (Chapter 2). Accordingly, the ancestral state reconstruction indicates that high hydrophobicity has co-evolved with terrestrial habitat use, but not with any particular humidity regime. Equally in accordance with my results from chapter 2, the evolution of long spinules and small scales were correlated with high hydrophobicity.

EVOLUTION OF ADHESIVE TOEPADS

Shifting to the morphology of setae and adhesive toepads in Chapter 4, I analysed the evolution of adhesive toepads in the *Heteronotia binoei* species complex. Within the terrestrial and generalist species complex *Heteronotia binoei* (Fujita *et al.*, 2010; Moritz *et al.*, 2016; Zozaya *et al.*, 2019), two clades, one consisting of three lineages (Magnetic Island, Cape Cleveland, and Paluma East) and the other consisting of a single lineage (Paluma West) exploit saxicoline habitats on large granite outcrops on both sides of the Paluma Range in North Queensland. The two saxicoline clades are an example of parallel evolution, as they have independently developed larger terminal scales, along with setae that are longer and more complex compared to their terrestrial sister lineages (at least for CC and Paluma-W). They, therefore, represent a candidate for an intermediate state in a morphological series between a terrestrial pad-less state and a terminal 'leaf-toed' state, in gecko toe-pad evolution. The saxicoline microhabitat use of these morphologically intermediate lineages is in accord with the association of terminal pads with saxicoline habitats (Russell & Bauer, 1989; Russell & Delaugerre, 2017).

ECOLOGY OF AUSTRALIAN GECKOS

Finally, I classified the habitat use of 34 Australian gecko species using my own field work observations (Chapter 5), extended by data from a colleague from my lab (Nordberg, 2018). I classified 9 species as arboreal, 4 as saxicoline, and 13 as terrestrial, because least 75% of the observations for the respective species fell within one habitat category. The remaining 8 species were categorised as generalists. Furthermore, I collected perch height and perch diameter measurements for those species using arboreal microhabitats. I used this perch dataset to determine whether geckos fell within similar ecomorph categories as those established for anoles (Losos, 1992; Irschick *et al.*, 1997; Langerhans *et al.*, 2006; Poe & Anderson, 2019). Most gecko species fell within one of those categories, e.g. *Pseudothecadactylus australis, Saltuarius cornutus*, and *Gehyra dubia* fit the 'trunk-crown' ecomorph. The generalist *Amalosia rhombifer* fell in between 'grass-bush' and 'trunk-ground' ecomorphs. On the other hand, some species, e.g. *Carphodactylus laevis* and the spinifex-associated *Strophurus elderi*, potentially formed an additional gecko-specific ecomorphs outside of the anole ecomorph system (Losos, 1992, 1994a; Irschick *et al.*, 1997; Hagey *et al.*, 2017a).

CONCLUSION AND FURTHER RESEARCH

The function of cutaneous sensilla

Although microornamentation of geckos has been studied for over a century (Schmidt 1920), there are still knowledge gaps concerning these fascinating structures. Of the three microstructures found in geckos, spinules and cutaneous sensilla have often been neglected compared to the research interest in setae and their adhesive properties. After initial confirmation of the basic mechanoreceptive function of the cutaneous sensilla (Hiller, 1968; Düring & Miller, 1979), and early comparative descriptions of sensilla morphology (Hiller, 1971; Bauer & Russell, 1988), further functional implications have been deduced from sensilla distribution and morphology in single species (Lauff *et al.*, 1993; Russell *et al.*, 2014). Therefore, my thesis is, to my knowledge, the first to compare the morphology of cutaneous sensilla in a phylogenetically informed statistical analysis (Chapter 2). Although interpretations were hampered by the lack of knowledge of the function of different parts of the cutaneous sensilla, my study provided at least some support for the hypothesis that cutaneous sensilla may have further functions, in addition to the detection of touch (Ananjeva *et al.*, 1991; Ananjeva & Matveyeva-Dujsebayeva, 1996), but these must be tested further. This is particularly true, because many characters, some of which may have similar functions in different combinations, may produce similar evolutionary outcomes through convergent evolution from different evolutionary origins (Sherbrooke *et al.*, 2007). If cutaneous sensilla fulfil different functions among different body regions, I expect there will be variation of sensilla morphology and numbers among body regions, as described for some species (Bauer & Russell, 1988; Lauff *et al.*, 1993; Russell *et al.*, 2014). Furthermore, if cutaneous sensilla only detect touch, I expect more, or more complex, cutaneous sensilla, particularly around the mouth for feeding, on the extremities for locomotion (Lauff *et al.*, 1993), and on the tail to control autotomy and post-autotomic movement (Russell *et al.*, 2014). Examining the quantity and morphology of cutaneous sensilla on different body regions for a broader range of species, such as the Australian diplodactylids and carphodactylids examined here, should be instructive in this regard. Preliminary data collected in the course of this thesis suggests that, whereas cutaneous sensilla morphology is similar all over the body of particular species, and cutaneous sensilla morphology varies only among species, there are, indeed, more CS near the mouth, toes and on the tail (unpublished data), suggesting that cutaneous sensilla may primarily be for detecting touch, in spite of their morphological differences among species. But to more directly measure the function of cutaneous sensilla, functional experiments would be useful. To test if cutaneous sensilla can detect humidity or temperature, I would propose that researchers should determine the humidity and temperature preference of geckos under normal conditions, and also after temporarily numbing the cutaneous sensilla using a topical anaesthetic (see Hiller, 1968). Another promising avenue for future research would be detailed neurobiological and histological research on the structure and function of cutaneous sensilla.

Hydrophobicity

With the establishment of scanning electron microscopy for microstructure research, spinules have been described for a range of geckos, and other lizard species (Ruibal, 1968; Rosenberg *et al.*, 1992; Spinner *et al.*, 2014b), but detailed studies of their functions have started comparatively recently. The hydrophobic, self-cleaning properties of the spinule-covered gecko skin has now been confirmed by multiple studies (Hiller, 2009; Spinner *et al.*, 2013a; Watson *et al.*, 2015a,c). I found that, at least in Australian geckos, the hydrophobic, self-cleaning microstructures have apparently co-evolved with terrestrial microhabitat use (Chapters 2 and 3). I suggested that rainforest geckos may be exposed to fouling from microscopic plants, and fungi (Böhme & Fischer, 2000), and that arid zone geckos may be exposed to more dust, leading to selection for hydrophobicity in relation to habitat humidity. Contrary to my predictions, I found no association between habitat humidity and hydrophobicity. Adaptation to habitat use may supersede adaptation to habitat humidity (Arnold, 2002). Therefore, it would be interesting to test non-independent selection for long spinules and high hydrophobicity in, for example, a sample comprising only arboreal species, ranging from highly humid to dry habitats.

Spinules have evolved not only in geckos, but also in anoles, chameleons, and in some clades of skinks and iguanids (Ruibal, 1968; Peterson, 1984a; Irish *et al.*, 1988; Spinner, Westhoff, & Gorb, 2013c). Thus, I would be interested to repeat and expand my study to include an examination of the hydrophobicity of other lizard clades featuring spinules. Also, as other squamate clades have evolved completely different microstructures, such as honeycombs (Irish *et al.*, 1988; Harvey, 1993; Höfling & Renous, 2009; Riedel *et al.*, 2015), or longitudinal ridges (Stewart & Daniel, 1972, 1973; Arnold, 2002), it would be fascinating to understand the function of those structures. Different microstructures have different, and partially contrasting advantages and disadvantages, which may explain these different patterns. A problem for many lizards, particularly from desert habitats, is evaporative water loss (Thompson & Withers, 1997). Small scales enhance hydrophobicity and self-cleaning, but increase exposed skin surface area, which leads to increased evaporative water loss in geckos (Withers, Aplin, & Werner, 2000; Vucko, 2008). Geckos are ancestrally primarily nocturnal lizards (Gamble *et al.*, 2015; Meiri, 2020; Stark *et al.*, 2020), typically hiding in crevices or other shelters during the day (Croak *et al.*, 2010; Pike, Webb, & Shine, 2011; Nordberg, 2018). Diurnal shelter use could reduce the danger of desiccation for them, as they are less exposed to direct sunlight. On the other hand, some species, such as *Strophurus williamsi* tend to spend the day on small twigs exposed to sunlight and high temperature (Wilson, 2015, personal observation), and it may be worth noting that these species have larger scales than species using diurnal shelter (Chapter 2). In general, and examination of the trade-off between hydrophobicity and evaporative water loss would be very instructive for geckos, especially in relation to diurnal habitat selection.

The other two main clades with spinules are chameleons and anoles. Both clades are primarily rainforest species, living in environments with high humidity (Losos, 2011; Riedel *et al.*, 2015), where desiccation may not be a critical factor. Hydrophobic, self-cleaning surface properties have also convergently evolved in many arthropods (Wagner *et al.*, 2003; Watson *et al.*, 2010) and plants (Barthlott & Neinhuis, 1997). Studies on hydrophobic surfaces in plants mostly focus on their function (Neinhuis & Barthlott, 1997), but no correlation between habitat and humidity has yet been detected in plants (Tellechea-Robles *et al.*, 2019). In arthropods, associations between ecology and hydrophobicity has been described in comparative morphological studies (Byun *et al.*, 2009; Perez-Goodwyn, 2009), but never been tested statistically in a phylogenetic framework. I am, therefore, the first to successfully examine the evolution of hydrophobicity, statistically, and in a phylogenetic framework. Comparative analysis could be conducted for arthropods as well and could provide many more independent evolutions of hydrophobicity to work with than were available with Australian geckos.

Adhesive toepads

Although the function of setae and adhesive toepads is well studied (Russell, 2002; Russell *et al.*, 2019), their evolution is still not well understood. That is, it is unknown if adhesive toepads have evolved only once in the common ancestors of all geckos (Hagey *et al.*, 2017b; Harrington & Reeder, 2017), or multiple times within geckos (Gamble *et al.*, 2012, 2017), although recent morphological and phylogenetic studies favour the latter hypothesis (Russell *et al.*, 2015; Higham *et al.*, 2017; Russell & Gamble, 2019). Comparative morphological studies, and especially intermediate forms along morphological series, are important to disentangle the evolutionary history of complex morphological traits, such as adhesive toepads (Russell & Gamble, 2019). The only established case of a morphological intermediate in toepad evolution, in which the microornamentation is known, is *Gonatodes humeralis*, a proposed intermediate in the evolution of basal adhesive toepads (Russell *et al.*, 2015; Higham *et al.*, 2017). Based on internal morphology and subdigital scale shape, members of the genus *Cyrtodactylus* have also been proposed as intermediates for the evolution of basal toepads (Russell, 1976, 1977; Russell & Gamble, 2019), but their microornamentation is mostly unknown (Russell, 1976; but see Peattie, 2009). My thesis is unique, in that I studied and propose an intermediate form for the evolution of terminal adhesive toepads (Chapter 4). I would be interested to examine the subdigital microornamentation of the remaining lineages of the *Heteronotia binoei* species complex (particularly the remaining saxicoline lineages: Paluma-E and MI), to determine if they also show convergent morphology of toepads. Furthermore, I would like to quantify the adhesive performance of the different *H. binoei* lineages, comparing saxicoline to terrestrial and generalist lineages. I unexpectedly found short, but fully spatulated 'true' setae in the terrestrial lineages of *H. binoei,* highlighting the complexity of toe-pad evolution and raising questions about the ancestral state of the subdigital morphology within the genus *Heteronotia*. Therefore, I would be interested in examining the internal digit morphology and subdigital microornamentation in the other species of the genus *Heteronotia*, as well as in their climbing, terminal toepad-bearing sister genus *Dixonius,* and in the terrestrial and pad-less genus *Nactus,* which is the sister genus to both *Heteronotia* and *Dixonius* (Bauer *et al.*, 1997). These studies could help clarify if *Heteronotia* as a genus is primarily or secondarily pad-less and terrestrial, or generalist, and could be a promising avenue to further study the evolution of adhesive terminal toepads.

Gecko ecology

With my thesis I also contributed to overcoming data deficiency on the ecology of Australian geckos, and could provide a starting point for future ecomorphology research on geckos (Chapter 5). Although the quantified habitat niche categories were mostly in line with their assignment derived from the published literature (Chapters 2 & 3, Table 5.2), there were differences, for example, I found that *Amalosia rhombifer* is a generalist, using both rock and vegetation (Table 5.2), while it is often described as arboreal in the literature (Chapters 2, 3) (Wilson, 2015; Nielsen *et al.*, 2016). Conversely, I found that *Oedura monilis* is saxicoline, whereas the literature suggested it was arboreal (Chapter 2,3,) (Henle, 1991; Nielsen *et al.*, 2016; Meiri, 2018) or generalist (Hagey *et al.*, 2017a). These disparities illustrate the need for further, detailed, ecological studies of gecko species in Australia and around the world (Vidan *et al.*, 2019; Meiri, 2020). In addition, as they are mostly indistinguishable morphologically, *H. binoei* was treated as a single species in Chapter 5, albeit being a species complex (Fujita *et al.*, 2010; Moritz *et al.*, 2016; Zozaya *et al.*, 2019). As there are clearly both ecological and morphological differences among different lineages of *H. binoei* (Chapter 4), future research should examine and quantify these differences. in particular, my research indicates potential competitive exclusion between one generalist lineage (EA6) and a saxicoline lineage (CC) (Chapter 4). More detailed ecological sampling could properly test this hypothesis. Furthermore, this also underlines the need for more taxonomic studies to entangle cryptic and unknown gecko species in Australia (Meiri, 2020).

Student projects

In the time I worked on this thesis, I also supervised two undergraduate students. Ayano Fushida, who worked on shedding frequency in relation to mite load and fouling, and found that 1) shedding reduced mite load, and that 2) the gekkonid *Hemidactylus frenatus* could apparently increase their shedding rate in response to an artificial fouling agent, while three diplodactylid geckos did not increase their shedding rate (Appendix I). Previously, shedding was primarily seen as a fully obligate process (Maderson), while My study showed that at least some species can change their shedding frequency in response to fouling. Additional studies examining shedding frequency in relation to other external stressors, and in other species, could determine how widespread the ability to mobilize shedding might be, and if this distribution is mainly phylogenetic or maybe an adaptation to ecological factors.

Rishab Pillai analysed the performance of adhesive toepads (measured as shear force exerted) of two gecko species (*Oedura coggeri* and *Pseudothecadactylus australis*) on three substrate types of increasing roughness (Appendix II). While most studies have tested adhesive performance on glass or other artificially smooth substrates (Higham *et al.*, 2019), the substrates encountered by geckos in nature are rough and unregular (Russell, Johnson, & Delannoy, 2007; Russell & Johnson, 2014). We found that adhesive performance decreased on moderately rough surfaces compared to both smooth and very rough surfaces, contrary to previous suggestions that adhesive performance would decrease continuously with increasing roughness (Cole *et al.*, 2005). My study further highlights that we need to take natural, unregular rough surfaces into account, when studying ecological adaptations or the evolution of adhesive toepads in geckos (Higham *et al.*, 2019).

Conclusion

In my thesis, I examined different aspects of the evolution and ecological adaptations of microornamentation in Australian geckos, contributing to the accumulation of knowledge of these fascinating structures in a highly interesting model group. But as so often in science, my thesis also generates at least as many new questions as it answers. Geckos are a really diverse group of squamate reptiles, spanning a large range of variation in morphology, behaviour and ecology (Bauer, 2019; Higham *et al.*, 2019; Meiri, 2020). Similarly, microornamentation covers a large range of different structures across squamate reptiles, fulfilling a diversity of functions (Irish *et al.*, 1988; Arnold, 2002; Hiller, 2009; Spinner *et al.*, 2014a,b; Riedel *et al.*, 2015). I therefore could highlight only a few potentially instructive future studies here, stemming directly from my thesis, but many more are possible and worth to pursue.

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Appendix I - Can geckos increase shedding rate to remove fouling?

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Abstract

All vertebrates shed the outer layer of their epidermis, usually continuously, but squamate reptiles shed periodically, losing large pieces of this layer at once. While the cellular processes leading to loss of the outer epidermal layer, or shedding, in squamates have been studied in detail, few studies have examined the factors associated with shedding frequency. Shedding is an obligate event, linked to somatic growth and the regeneration of damaged or worn epidermal areas. Another proposed role for periodic shedding in squamates is the removal of ectoparasites and fouling substances stuck on the epidermis. It is unclear whether the removal of ectoparasites and fouling substances is completely passive, only mediated by a fully obligate shedding cycle, or if shedding can be mobilized directly in response to parasite attachment or fouling. To test these hypotheses, we first assessed whether shedding reduced the adherence of parasites to the skin of six different species of geckos by counting mites on the outer epidermis before and after shedding events. Next, we assessed whether shedding was triggered by fouling. Using four species of geckos, we applied artificial substances (marker pen [Sharpie^{TM}], and wood glue [polyvinyl acetate]) to the outer layer of the epidermis and recorded the time between shedding events (shedding interval) compared to unmanipulated controls. There was a clear decrease in parasite loads after shedding events, confirming that shedding reduces adherence of parasites. Our experiments with artificial substances applied to the outer epidermis showed that most gecko species did not change their shedding intervals, regardless of skin-fouling treatment. *Hemidactylus frenatus*, however, decreased their shedding interval in response to the application of wood glue. Thus, we found that parasites, if present, are removed by shedding, and external fouling can trigger shedding at least in one species of gecko.

Key words: Australia - Ectoparasites – External stressor - Reptile – Skin - Sloughing

Introduction

A common feature of all vertebrates is that the outermost layer of the epidermis is removed and replaced by a new layer in a process referred to as shedding (ecdysis). The process and frequency of shedding differs among taxa. Squamate reptiles have a particularly complex, multi-layered epidermis and a regular shedding cycle, shedding the entire outer epidermal layers (including the stratum corneum) either at once, or in large fragments (Landmann 1979; Maderson 1985; Irish et al. 1988; Harkewicz 2002). Before the old epidermal layer (exuviae) is sloughed, new keratinocytes fully differentiate underneath to form a new multi-layered epidermis, and shedding is triggered once these keratinocytes mature (Alibardi 1995, 1998, 2014; Lillywhite 2006). Shedding occurs for several reasons. First, it allows for growth and regeneration (Maderson and Licht 1967; Irish et al. 1988; Harkewicz 2002). As body size increases, the epidermal layers must expand, or if the epidermis is damaged, it must be replaced (Ling 1972; Irish et al. 1988). Shedding also restores epidermal functions that may be reduced by fouling from substances sticking to, or growing on, the epidermal surface (Böhme and Fisher 2000; Cramp et al. 2014). For example, geckos have water repellent skin, allowing self-cleaning by water droplets rolling over hydrophobic skin surfaces (Hiller 2009; Watson et al. 2015), and both fouling and damage can reduce this self-cleaning ability, so renewal by shedding is useful Finally, parasites may adhere to the epidermis, and shedding may remove them and reduce their impact, although demonstrations of this are rare (Zann et al. 1975; Chinnasamy and Bhupathy 2013; Lillywhite and Menon 2019), and some studies have found that shedding does not reduce ectoparasite numbers (Klukowski and Nelson 2001). Although the histology and ultrastructure of the outer epidermis and the cellular and biochemical processes associated with shedding in squamates are relatively well studied (Maderson 1966, 1967; Alibardi 1998; Maderson et al. 1998), few studies examine the factors affecting shedding rate. It is, therefore, unclear whether shedding occurs exclusively as an obligate event, at regular intervals, or if shedding frequency can be modified facultatively, for example by increasing shedding rate in response to external skin stressors such as parasites, damage, or fouling. A high shedding frequency in association with fouling has been reported in some reptiles; for example, some sea snakes, which are often fouled by ectoprocts, shed frequently (Kropach and Soule 1973). To our knowledge, however, no studies have examined if shedding frequency can be increased as a direct response to ectoparasites or fouling. Some lizards shed more frequently at higher ambient temperatures (Chiu and Maderson 1980), and humidity can influence shedding interval (Maderson et al. 1998), whereas hormones appear to have no effect on shedding interval at a given temperature (Chiu et al. 1986). It remains unclear, however, whether shedding can be triggered by external stressors on the epidermis itself, such as when the skin is damaged or fouled, or when parasitic attachment has taken place. We used geckos as a model system to determine (1) whether shedding frequency was influenced by fouling, and (2) if shedding was an important mechanism to remove parasites. We quantified if shedding rate was increased by the presence of (artificial) fouling substances on the skin. We also determined if shedding was associated with removal of ectoparasites in these lizard species, and if the shedding interval was related to parasite load. To accomplish these aims, we quantified the shedding interval of four species of geckos before and after experimental skin fouling was applied, and we counted ectoparasites before and after shedding in six species of geckos while recording shedding interval in relation to parasite load.

Material and methods

COLLECTION AND HOUSING

Individuals of six species of gecko were collected and used in this study: Common House Geckos (*Hemidactylus frenatus*; SVL = 49 6 0.75 mm, SE), Eastern Spiny-tailed Geckos (*Strophurus williamsi*; SVL = 52 6 1.58 mm, SE), Box-patterned Geckos (*Lucasium steindachneri*; SVL = 49 6 1.32 mm, SE), Northern Velvet Geckos (*Oedura castelnaui*; SVL = 74 6 3.80 mm, SE), Northern Spotted Velvet Geckos (Oedura coggeri; SVL = 71 6 2.63 mm, SE), and Ocellated Velvet Geckos (*Oedura monilis*; SVL = 83 6 1.70 mm, SE). A total of 73 *H. frenatus*, 10 *S. williamsi*, 5 *L. steindachneri*, 10 *O. castelnaui*, 11 *O. coggeri*, and 10 *O. monilis*, were captured. *Hemidactylus frenatus, S. williamsi, L. steindachneri*, and *O. castelnaui* were all hand-captured from the buildings and bushland surrounding the James

Cook University (JCU) campus, Townsville, Queensland, Australia (1981903400S, 14684502600E; in all cases datum = WGS84), while *O. coggeri* and *O. monilis* were collected near Hidden Valley, Queensland, Australia (1885803900S, 14680201400E), between March and September in 2017. All geckos used for experiments were sexually mature. Geckos were transported to a controlled-temperature room at JCU and were kept in a 12:12-h light: dark room at 288 6 28C during the day and 228 6 28C at night, at a constant relative humidity (67%). Geckos were held individually in translucent plastic containers (28 mm 3 19 mm 3 10 mm), with mesh lids to allow air exchange. Geckos were provided with a heat strip running under one side of the enclosure to allow for thermoregulation (maximum temperature = 338C) and supplied with a water dish, and several layers of paper towel and a tile, which served as shelter. Geckos were fed European Domestic Crickets (*Acheta domestica*) three times per week.

SHEDDING INTERVAL

We documented the shedding interval of *H. frenatus* over a period of 5 mo and of three other species of geckos (*S. williamsi, L. steindachneri,* and *O. castelnaui*) over a period of 3 mo. To establish that shedding had occurred, enclosures were visually examined daily for skin fragments in the enclosure (except for *H. frenatus*, see below). Treatment groups included (1) a control with no fouling agent, (2) a 1- cm line (0.25-cm width) applied with a nontoxic permanent marker (glyceride, pyrrolidone, and resin; Sharpie^{TM}) to the dorsal surface of the geckos, or (3) a 1-cm strip (0.25-cm width) of transparent-drying white wood glue (polyvinyl acetate; PARFIX^{TM}) mixed with nontoxic food colouring (Queen^{TM}) or fluorescent powder for visual detection, applied to the dorsal surface of the geckos. We chose two types of fouling agents (marker and glue) because they were inexpensive, easy to apply, and had different physical properties. By using both glue and a marker, we covered both viscous and fluid adherent types. Because we had a large number of *H. frenatus* available, we compared shedding interval among groups of this species. Ten *H. frenatus* represented controls (no external fouling applied), 30 were exposed to permanent marker, and 10 had wood glue applied to their epidermis. Some individuals were tested more than once by reapplying the treatment after shedding. Individuals of the other three species of geckos (*S. williamsi*, n = 10*; L. steindachneri*, n = 5; and *O. castelnaui*, n = 10), received each of the three treatments sequentially in a random order, such that all individuals (n = 25) were exposed to all treatments (control, permanent marker, and wood glue). When an individual shed, a new treatment was applied the same day. We were not able to monitor the shedding frequency for *O. coggeri* and *O. monilis* long enough to collect data as they were added later in the project.

DETECTION OF SHEDDING IN KERATOPHAGIC SPECIES

Hemidactylus frenatus was the only keratophagic species in Our experiment (they eat skin fragments as they shed; Mitchell et al. 2006), so shedding could not be easily or accurately detected for this species by looking for shed epidermis in their enclosures. For *H. frenatus*, we recorded the dates when fouling disappeared from the dorsum as our measure of shedding interval. As this was not possible for the control group, we measured shedding interval of control animals by checking for epidermal remnants in the feces. We estimated gut passage times to determine how long epidermal fragments would remain in the gut by feeding geckos small inert plastic beads and watching for their reappearance in feces. Two to three beads were injected into the body cavity of a cricket and fed to each gecko, after which all fecal samples were collected in the days following and searched for beads to calculate approximate gut passage time. We calculated gut passage time as the interval between consumption of the beads and the appearance of the first bead in the feces. Small fragments of epidermis could also be identified from fecal dissections under a microscope, and therefore, given estimated gut passage times, we were able to back-calculate the date of shedding. We applied both the disappearance of the fouling and the appearance of epidermal fragments in the feces to determine shedding rate in the geckos that had been fouled with marker pen. In this way, we could ensure that the shedding interval calculated with these different methods (looking for shed skin in feces and looking for the disappearance of marker pen) matched the actual shedding interval.

ECTOPARASITE LOAD

A total of 69 geckos (*H. frenatus*, n = 23; *S. williamsi*, n =10; *L. steindachneri*, n = 5; *O. castelnaui*, n = 10; *O. coggeri*, n = 11; *O. monilis*, n = 10) were used to test for differences in external parasite loads before and after shedding. Initially we did not include *H. frenatus* in Our ectoparasite experiment because they are keratophagic, and we could not identify when they shed their skin. However, after running the fouling experiment, we realized that marker pen could be used to determine shedding interval. All observed ectoparasites were pterygosomatid mites (Barnett et al.2018). The total number of mites on the skin surface of each gecko was counted using a hand lens, and all mites were counted before (upon capture) and 1 to 2 d after shedding for each gecko.

STATISTICAL ANALYSES

To detect differences in the abundance of mites before and after shedding for those species that had mites upon capture, we used a linear mixed-effect model (LME) in the *nlme* package (Pinheiro et al. 2017). Our model included the number of mites as the response variable, species and time (before or after shedding) as fixed-effects, and the gecko individual as a random factor. In addition, correlation between shedding interval and number of mites in *H. frenatus* was examined with Pearson's correlation coefficient. All analyses were conducted using the statistical software Rv3.4.1 (RStudio Inc., Boston, MA).

To determine the effects of the fouling treatments on shedding interval, we used an LME model with shedding interval as the response variable, species and treatments as fixed factors, and the gecko individual as a random factor. Using these models to identify significant effects, Wald's χ^2 test was used to find overall significance, and a Type II analysis of variance table was produced using the *car* package (Fox and Weisberg 2011). Where appropriate, multiple comparisons using Tukey's procedure were conducted with the package *lsmeans* (Lenth 2016) to compare among species, as well as among treatments.

Results

SHEDDING INTERVAL IN RESPONSE TO FOULING

There was a significant difference in natural shedding periods (determined by comparing control animals) among the four species of geckos ($F_{3,71} = 8.52$, $P < 0.01$; Fig. I-1). *Strophurus williamsi* had significantly longer shedding intervals than *H. frenatus* and *L.*

steindachneri ($P < 0.01$and $P = 0.021$, respectively; Fig. I-1), whereas there was no significant difference in shedding interval between *S. williamsi* and *O. castelnaui* ($P = 0.23$).

In *H. frenatus*, there was no significant difference in the shedding intervals determined by the presence of shed skin fragments in feces versus the disappearance of marker pen (mean shedding rate interval using the appearance of skin fragments in the feces: 21 ± 2.73 d, SE, n = 11; mean shedding rate interval using the disappearance of marker pen: 17.17 ± 1.20 d, SE, n = 42; $t = 1.92$, df = 1, $P > 0.05$). Thus, we used the data from both of these methods together in the rest of these analyses. There was a significant difference in shedding rate among fouling treatments in *H. frenatus*, such that geckos fouled with wood glue had a significantly shorter shedding interval than controls or those fouled with marker pen (wood glue versus the control, $P < 0.01$; wood glue versus marker pen, $P < 0.01$; Table I-1).There was no significant difference in the shedding intervals of control unmarked lizards and lizards marked with marker pen ($P = 0.79$; Table I-1). Moreover, there was no significant effect of the fouling treatments on shedding interval in the other three species (*S. williamsi*, *L. steindachneri*, and *O. castelnaui*; Fig. I-1).

THE INFLUENCE OF SHEDDING ON ECTOPARASITE LOADS

Of the six species we examined, 91.3% of *Hemidactylus frenatus* (21 of 23 individuals, an average of 62.0 ± 13.26 mites per individual), 72.7% of *O. coggeri* (8 of 11individuals, 19.2 ± 11.08 mites per individual), and 80% of *O. monilis* (8 of 10 individuals, 5.6 ± 1.86 mites per individual) had mites upon capture (i.e., pre shedding),whereas *L. steindachneri*, *O. castelnaui*, and *S. williamsi* had no mites present upon capture. After shedding, *H. frenatus* carried an average of 38.19 ± 9.36 mites per individual, *O. coggeri* carried 0 mites per individual, and *O. monilis* carried 0.13 ± 0.13 mites per individual. The LME showed significant differences among species ($\chi^2 = 12.97$, df = 2, $P < 0.01$; Fig. I-2), and before and after shedding in the number of mites ($\chi^2 = 21.58$, df = 1, $P < 0.01$; Fig. I-2). *Hemidactylus frenatus* had higher natural average parasite loads than did *O. monilis* and *O. coggeri* (Fig. I-2). Importantly, *H. frenatus* ($t_{20} = 4.25$, $P < 0.01$; Fig. I-2), *O. coggeri* ($t_7 = 3.41$, $P = 0.011$), and *O. monilis* ($t_7 = 2.57$, $P = 0.037$) all had significantly fewer mites after shedding. There was a weak, marginally significant correlation between parasite number and shedding interval in *H. frenatus* ($R^2 = -0.35$, $P = 0.10$, $n = 23$).

Discussion

Geckos benefit from shedding their epidermis by reducing the total abundance of ectoparasitic mites, as parasites negatively impact host fitness in lizards (Pence and Selcer 1988; Pare 2008; Caballero et al. 2015). Similarly, shedding reduced the number of cutaneous microbes on frogs (e.g., Cramp et al. 2014; Ohmer et al. 2015). *Hemidactylus frenatus*, an introduced species, had the highest parasite loads, and individuals lost about 50% of their parasites when they shed. In contrast, native species had much lower parasite loads, and lost all their mites upon shedding. We assessed mite numbers when geckos were captured, and then counted mites after shedding, after geckos had been in captivity for some time. It is possible that mite load was somehow reduced by the time in captivity, rather than by shedding. We think this unlikely, however, as mites are notoriously difficult to eliminate in captivity, and simply reinfest untreated reptiles (Harkewicz 2001). Instead, we think it most likely that shedding was the factor that eliminated the mites in this study. Another study also found high mite loads in *H. frenatus* compared to Australian native geckos (Torchin et al. 2003), and one study found two species of ectoparasitic mites, restricted to *H. frenatus*, that have apparently coevolved with them, suggesting that high host specificity of mites may explain high infestation (Coates et al. 2017). Also, there was a trend in Our study for higher mite loads in *H. frenatus* to be associated with slightly shorter shedding intervals, suggesting this species may mobilize shedding to eliminate parasites.

Consistent with a previous study (Weldon et al. 1993), we found *H. frenatus* was keratophagous. In squamates, keratophagy is widely documented, especially for geckos (Bustard and Maderson 1965). In our study, *S. williamsi*, *L. steindachneri*, and *O. castelnaui* did not consume their shed epidermis, which was particularly interesting as keratophagy is reported for some of their close relatives (e.g., *Diplodactylus elderi* [now *Strophurus elderi*], Bustard and Maderson1965; *O. marmorata*, and *O. tryoni*, Weldon et al. 1993).Shed epidermis may be consumed to reduce parasite loads (Mitchell et al. 2006). Possibly, *H. frenatus* is keratophagous in an attempt to reduce the relatively high mite loads they carry, compared to native geckos.

Another role suggested for keratophagy is to recover nutrients (Noble 1954; Bustard and Maderson 1965; Weldon et al. 1993; Mitchell et al. 2006). In our study, the occurrence of keratophagy was associated with the ability to mobilize shedding in response to fouling. Perhaps mobilization of shedding, or a decrease in shedding interval, was possible in *H. frenatus* because they can recover the lost nutrients in the shed epidermis more easily than geckos that do not consume their shed epidermis. Further study of the causes and functions of keratophagy may reveal why it occurs in some species but not others, and whether it is more generally coincident with mobilization of shedding. Comparison of a wider range of gekkonids and diplodactylids may help elucidate the variation in and causes of keratophagy.

We examined how shedding interval responded to epidermal fouling in a range of geckos. Fouling, while similar to ectoparasites in some ways, is a different epidermal integrity problem faced by animals. We found that *H. frenatus* individuals responded to the presence of glue on their epidermis by decreasing their shedding interval, perhaps indicating that they can increase their shedding frequency as a reaction to fouling. As fouling, or attachment of substances to the skin, is potentially harmful (Böhme and Fisher 2000; Cramp et al. 2014), this suggests that at least some squamate reptiles can increase their shedding rate to remove these kinds of external stressors, as suggested for some species of sea snakes (Zann et al. 1975; Lillywhite and Menon 2019). Contrary to *H. frenatus*, we found no evidence that fouling with polyvinyl acetate (glue) and ink altered shedding rate in the other species, suggesting that shedding interval is influenced by fouling only in some species. We therefore suggest that, rather than altering shedding cycles in response to fouling events, most species have evolved an optimal shedding frequency which balances the resources required to generate a new epidermis with the rate at which their epidermis becomes sufficiently fouled, dirty, or damaged, so its function is maintained.

There was a wide range in shedding intervals, from 18 d in *H. frenatus* and *L. steindachneri*, to 40 d in *S. williamsi*. In contrast, the shedding interval reported for Tokay Geckos (Gecko gecko) was approximately 25 d (Chui and Masterson 1980). Some studies have shown that the level of hydration in the epidermis plays an important role in shedding for squamates (Maderson et al. 1998; Lillywhite 2006), but factors causing variation in these periods still needs further exploration. Possibly, food availability and environmental conditions (e.g., humidity), as well as the amount of damage or fouling typically encountered may be factors causing differences in shedding intervals. In our study, it was not clear what was driving such differences. *Hemidactylus frenatus* had the fastest shedding cycle of the species we examined, which may be associated with high levels of fouling by ectoparasites. The effect of fouling on shedding frequency may differ among species that occupy different microhabitats; for example, ground-dwelling species may accumulate more epidermal adherents than do tree-dwelling species (Riedel et al. 2019 [Chapter 2]). However, *H. frenatus*, *O. castelnaui*, and *S. williamsi* are all arboreal, whereas *L. steindachneri* is terrestrial, so the differences in shedding interval we observed do not align well with microhabitat, and we have too small a group of species from which to draw conclusions.

In general, *H. frenatus* reacted differently from the other species. *Hemidactylus frenatus* had the highest mite load, lost the smallest proportion of that load when shedding, had the ability to activate shedding in response to fouling, shed in small pieces, and was keratophagous. All these differences could be related to phylogenetic affiliation, as *H. frenatus* belongs to the family Gekkonidae, whereas the other species we studied all belong to the Diplodactylidae. It is difficult, therefore, to generate adaptive explanations for differences between *H. frenatus* and the other species, as there may be phylogenetic influences on their physiology that produce differences that are characteristic of the group. Further studies comparing larger groups of gekkonids and diplodactylids are required to examine these questions.

Figures

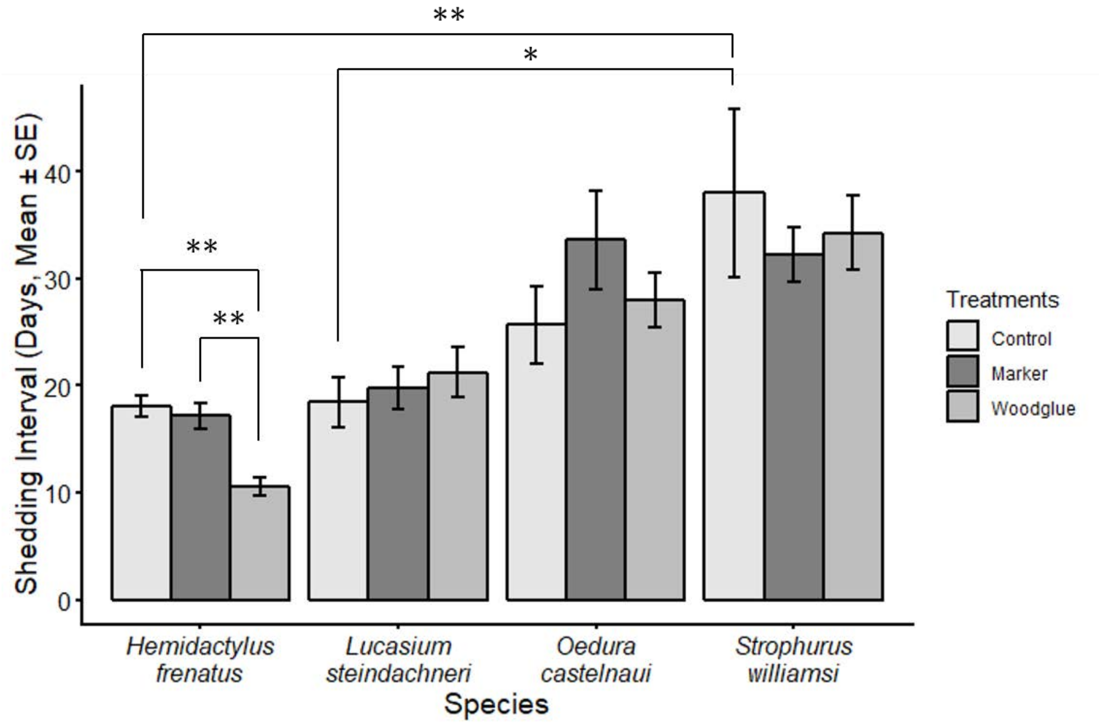

Figure I-1 The number of days between shedding events in four different species of geckos, Asian House Gecko (*Hemidactylus frenatus*, n = 73), Box-patterned Gecko (*Lucasium steindachneri*, n = 5), Northern Velvet Gecko (*Oedura castelnaui*, n = 10), and Eastern Spiny-tailed Geckos (*Strophurus williamsi, n = 10)*, unfouled or fouled with two different treatments (ink, Sharpie™ marker pen; glue, polyvinyl acetate adhesive). The bars show shedding intervals. Asterisks indicate a significant decrease in shedding interval among treatments from *H. frenatus*. * $P < 0.005$. ** $P < 0.01$.

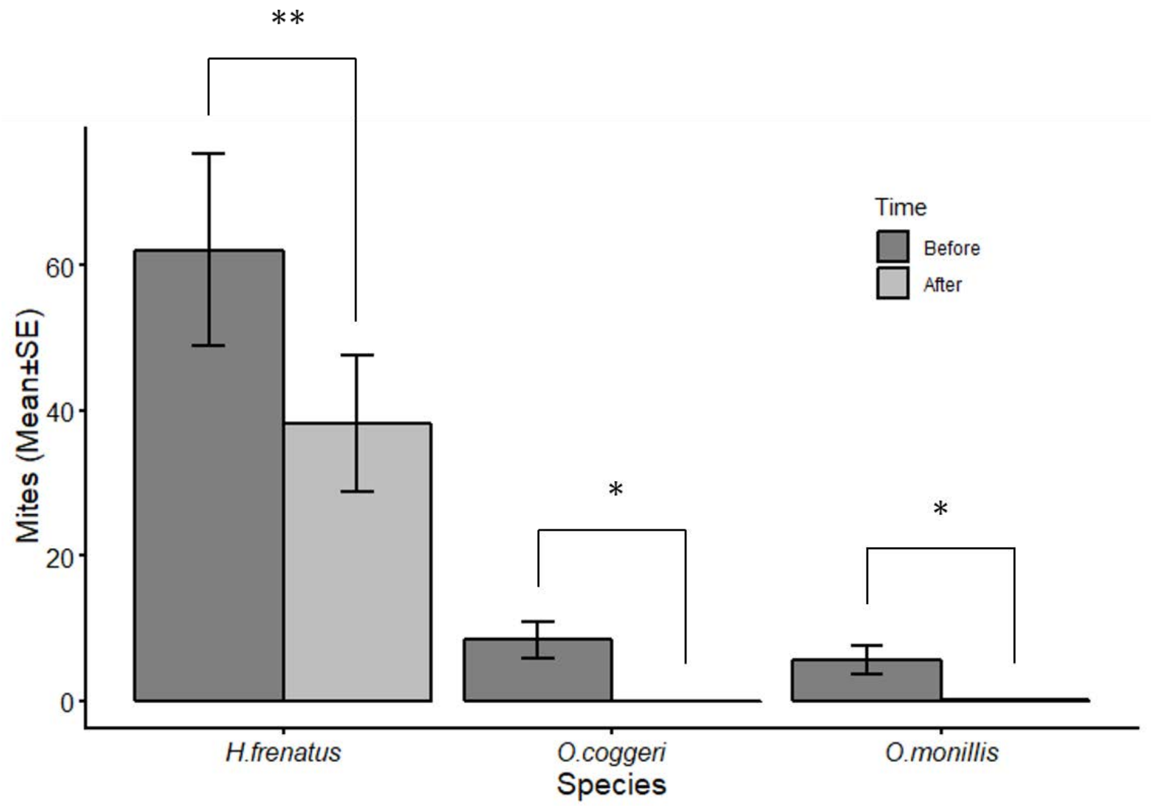

Figure I-2 Differences in the number of mites before and after shedding for *Hemidactylus frenatus* (n = 23), *Oedura coggeri* (n = 11)., and *O. monilis* (n = 10). The bars indicate the mean number of mites and the error bars show the standard error of the mean. Asterisks indicate a significant reduction in mite loads after shedding for each species. * $P < 0.005$. ** $P < 0.01$

Tables

Table I-1. Pairwise comparisons showing the significant differences in shedding interval among three different fouling treatments using Tukey's post hoc tests for *H. frenatus*.

Multiple Comparison	Mean difference	SE	df	*p*-value	
Control - Sharpie	0.94	1.43	95	0.788	
Control - Woodglue	7.50	1.47	95	< 0.0001	***
Sharpie - Woodglue	6.56	1.50	95	0.0001	***

Authors contribution

JR and LS and EN designed the study and planned the project. AF collected and analysed the data, with JR, EN, and RP assisting. AF, RP, EN, and JR conducted field work. AF lead the writing with all co-authors contributing and editing. All authors gave final approval.

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Appendix II - Nonlinear variation in clinging performance with surface roughness in geckos

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Abstract

Understanding the challenges faced by organisms moving within their environment is essential to comprehending the evolution of locomotor morphology and habitat use. Geckos have developed adhesive toe pads that enable exploitation of a wide range of microhabitats. These toe pads, and their adhesive mechanisms, have typically been studied using a range of artificial substrates, usually significantly smoother than those available in nature. Although these studies have been fundamental in understanding the mechanisms of attachment in geckos, it is unclear whether gecko attachment simply gradually declines with increased roughness as some researchers have suggested, or whether the interaction between the gekkotan adhesive system and surface roughness produces nonlinear relationships. To understand ecological challenges faced in their natural habitats, it is essential to use test surfaces that are more like surfaces used by geckos in nature. We tested gecko shear force (i.e., frictional force) generation as a measure of clinging performance on three artificial substrates. We selected substrates that exhibit microtopographies with peak-to-valley heights similar to those of substrates used in nature, to investigate performance on a range of smooth surfaces (glass), and fine-grained (fine sandpaper) to rough (coarse sandpaper). We found that shear force did not decline monotonically with roughness, but varied nonlinearly among substrates. Clinging performance was greater on glass and coarse sandpaper than on fine sandpaper, and clinging performance was not significantly different between glass and coarse sandpaper. Our results demonstrate that performance on different substrates varies, probably depending on the underlying mechanisms of the adhesive apparatus in geckos.

Key words: Adaptation – Adhesion biomechanics – Ecomechanics - Gekkota – Physiology - Zoology

Introduction

An animal's fitness is strongly influenced by its locomotor ability, which is fundamental for successful prey capture and predator avoidance (Alexander, 2003). Successful locomotion in particular habitats is dependent on morphology, physiology, and habitat structure and is constrained by evolutionary history (Schriefer & Hale, 2004; Zani, 2000). Natural selection favours traits that optimize locomotor performance in various habitats, and variation in physiological and morphological characters may, in turn, increase performance in certain habitats (Kohlsdorf et al., 2004). Therefore, studies of ecological morphology and evolution often link morphology, performance, and ecology to suggest adaptation (Hagey, Puthoff, Crandell, Autumn, & Harmon, 2016; Wainwright & Reilly, 1994).

The ability to climb is widespread in the animal kingdom (Labonte & Federle, 2015). Adhesive toe pads evolved in many taxa as an adaptation to enhance clinging ability. These structures have independently evolved in multiple lineages such as lizards (Irschick et al., 1996; Russell, 2002), tree frogs (Hanna, Jon, & Barnes, 1991; Langowski, Dodou, Kamperman, & Leeuwen, 2018), arachnids (Niederegger & Gorb, 2006; Wolff & Gorb, 2016), and many insect orders (Bullock & Federle, 2011). The mechanisms of adhesion vary among taxa, however. Tree frogs use a combination of wet and dry adhesion (Labonte & Federle, 2015; Langowski et al., 2018), whereas lizards, insects, and arachnids have evolved a hierarchical adhesive system using van der Waals forces, although they act at different scales in different taxa (Labonte et al., 2016).

Subdigital adhesive toe pads in geckos represent a classic example of the evolution of locomotory traits that have evolved independently, on multiple occasions (Gamble, Greenbaum, Jackman, Russell, & Bauer, 2012, 2017; Irschick et al., 1996; Russell & Gamble, 2019), and enabled the exploitation of several habitat types. In geckos, subdigital pads consist of laterally expanded scales (called lamellae) covered with modified scale derivatives in the form of stalks termed setae (Maderson, 1964; Russell, 2002). Fields of microfibrillar setae adhere to contacted surfaces through van der Waals forces (Autumn, Dittmore, Santos, Spenko, & Cutkosky, 2006; Autumn et al., 2000; Tian et al., 2006). The ability to cling to substrates by means of subdigital pads has long been a topic of research (Collette, 1962; Delannoy, 2006; Elstrott & Irschick, 2004; Ernst & Ruibal, 1966; Gamble et al., 2012; Hagey, Puthoff, Holbrook, Harmon, & Autumn, 2014; Ruibal & Ernst, 1965), and several studies have aimed to determine factors that allow geckos to adhere to and detach from the substrates they move across, examining the locomotory substrate characteristics (Gillies et al., 2014; Meine, Kloss, Schneider, & Spaltmann, 2004; Persson & Gorb, 2003; Pugno & Lepore, 2008; Spolenak, Gorb, Gao, & Arzt, 2005), the mechanisms of adhesion (Autumn et al., 2002; Autumn, Niewiarowski, & Puthoff, 2014; Gao, Wang, Yao, Gorb, & Arzt, 2005; Irschick, Herrel, & Vanhooydonck, 2006; Mahendra, 1941; Tian et al., 2006), and variation in adhesion among species (Bergmann & Irschick, 2005; Garner, Stark, Thomas, & Niewiarowski, 2017; Hagey et al., 2014, 2017; Irschick et al., 1996; Stark, Klittich, Sitti, Niewiarowski, & Dhinojwala, 2016; Stark et al., 2015).

The gekkotan adhesive system has evolved to enable the exploitation of inclined and inverted surfaces on rocks, or vegetation, with recent expansions onto man-made structures by some species (Glossip & Losos, 1997; Gamble et al., 2017; Hagey et al., 2017; Ruibal & Ernst, 1965). The mechanism and dynamics of adhesion, however, have almost exclusively been examined using a variety of smooth (Autumn et al., 2000; Gillies & Fearing, 2014; Irschick et al., 996; Peressadko & Gorb, 2004; Russell & Johnson, 2007; Stewart & Higham, 2014) and very fine-grained man-made surfaces (i.e., glass, Teflon, variations of polyethylene, polyvinyl chloride, aluminium bonding wire, acrylic, and acetate sheets; Campolo, Jones, & Fearing, 2003; Gillies & Fearing, 2014; Huber, Gorb, Hosoda, Spolenak, & Arzt, 2007; Meine et al., 2004; Persson, 2003; Persson & Gorb, 2003; Pugno & Lepore, 2008; Vanhooydonck, Andronescu, Herrel, & Irschick, 2005; Winchell, Reynolds, Prado-Irwin, Puente- Rolón, & Revell, 2016), most of them not encountered by geckos under natural conditions. Such research has revealed that geckos perform better on substrates that are smooth, clean, and have uniform surface chemistry (Stark et al., 2015), apparently because these substrates provide a greater surface area with which setae can make contact (Russell & Johnson, 2007; Vanhooydonck et al., 2005).

Natural substrates are usually structurally and chemically substantially different from those used in laboratories (Russell & Johnson, 2007, 2014; Stark et al., 2015). A few recent studies have examined the surface topography of natural substrates and how it affects adhesion in geckos, highlighting the unpredictability (i.e., nonuniform amplitude and wavelengths of asperities creating varying undulance) of natural substrates, especially in comparison with artificial substrates previously used in gecko adhesion studies (Cole, Jones, & Harris, 2005; Naylor & Higham, 2019; Russell & Johnson, 2014; Vanhooydonck et al., 2005). Other studies have also stressed the importance of using ecologically relevant substrates to better understand performance in insects (Bullock & Federle, 2011), tree frogs (Langowski et al., 2019), and geckos (Hagey et al., 2014; Higham, Russell, Niewiarowski, Wright, & Speck, 2019; Niewiarowski, Stark, & Dhinojwala, 2016; Peattie, 2007). Most recently, Higham et al. (2019) summarized the importance, methods, and reasons for including ecological parameters like surface characteristics in gecko adhesion studies.

When setal fields are first deployed, spatulae make direct contact with the surface microtopography, and they go through a proximal pull, undergoing a preloading phase. This enables the generation of shear forces and increases the overall strength of the bond (Autumn, 2007; Autumn et al., 2000; Russell & Johnson, 2007). Hence, substrate surface microtopography has a major influence on the area available for attachment from a single spatula to the whole setal field and significantly influences the magnitude of force generated by the adhesive apparatus (Russell & Johnson, 2007). The peak-to-valley heights of the surface topology are one way to estimate roughness and therefore are also one way to assess the area available for setal contact at different microtopographies. Investigating the performance of geckos on surfaces with specific kinds of micro- and nanotopography is an important element of understanding adhesion in nature (Gamble et al., 2012; Russell & Johnson, 2007, 2014). Although studies on smooth artificial surfaces have been important for unravelling the physical principles behind gecko adhesion, it is not clear if such studies can be used to estimate performance, or relative performance, of different species of geckos on rougher or nonuniform surfaces, such as those they encounter in their natural environment

Based on mechanisms predicted from observing gecko adhesion on artificial surfaces that are uniform and allow a very high proportion (nearing 100%, Russell & Johnson, 2007) of setae to make contact, we might expect a consistent decline in gecko attachment force with increasing roughness, presumably as setal fields find less purchase on uneven surfaces (Cole et al., 2005; Fuller & Tabor, 1975; Vanhooydonck et al., 2005; Figure 1a). Researchers have, however, found that setal fields can accommodate rougher surfaces, even though they are thought to have evolved for adhering to smooth substrates (e.g., *Rhoptropus* cf. *biporosus*; Russell & Johnson, 2014). In addition, recent studies have highlighted a multifunctional and synergistic relationship between claws and toe pads in geckos. Rough substrates that may provide limited surface area for setal attachment do allow mechanical purchase for claws. When substrates permit attachment of both claws and toe pads, that may increase clinging performance, even though there is limited surface area available for the setal fields by themselves. On the other hand, certain fine-grained substrates do not permit secure attachment of claws or setal fields, leading to diminished clinging performance (Naylor & Higham, 2019). These combined processes may lead to a trend in which smooth substrates (permitting maximal engagement of setal fields) allow generation of great clinging performance, whereas, on certain coarse substrates, an intermediate proportion of the setal field can engage in conjunction with mechanical interlocking of claws. Further, the lowest performance presumably occurs on substrates of intermediate roughness, which provide poor purchase for both claws and setal fields (Figure 1b). Thus, surfaces with intermediate roughness may permit only partial contact, producing a nonlinear performance curve, if performance is plotted against peak-to-valley height, or roughness (Huber et al., 2007). In addition, some studies at very small scales suggest that surfaces with very low and quite high levels of roughness will permit increased contact between spatulae and the surface compared to surfaces with intermediate roughness (Huber et al., 2007), which would also give rise to a nonlinear graph of shear forces in relation to roughness.

Thus, we suggest there are multiple ways in which the adhesive apparatus of geckos could interact with substrates, which may give rise to different relationships between substrate roughness and shear forces generated. We predicted one of two possible trends in gecko attachment when examined on substrates with varying roughness (glass, fine sandpaper, and coarse sandpaper). (a) Performance might decline monotonically with increasing roughness (Figure II-1a), or (b) performance might be lowest on surfaces with intermediate roughness forming a nonlinear trajectory (Figure II-1b). We quantified shear forces produced by two gecko species with different morphology, body size, and habitats, along a roughness gradient. We aimed to investigate the shape of the response, as shear force generated versus peak-to-valley height of each surface.

Methods

STUDY SPECIES

This study was conducted between August 2017 and December 2018. Two Diplodactylid gecko species (the northern spotted velvet gecko, *Oedura coggeri*, and the giant tree gecko, *Pseudothecadactylus australis*) were used to determine whether clinging ability imparted by geckos would decline monotonically with roughness or vary nonlinearly across substrates. Ten adult individuals (three males and seven females) of *O. coggeri*, a saxicolous species, were collected exclusively from rocky microhabitats around Paluma Range National Park, Queensland, Australia (GPS coordinates: −18.982772, 146.038974; datum = WGS84; 10 km radius), and housed at the James Cook University, Townsville Campus. Similarly, ten adult individuals (six males and four females) of *P. australis*, an arboreal species, were collected from tree bark and bamboo in Iron Range National Park, Queensland, Australia (GPS coordinates: −18.054768, 143.322002; 10 km radius), and were tested at a field station prior to release at their site of capture.

ECOLOGICAL RELEVANCE OF SUBSTRATES

To select test substrates offering similar ecological challenges (at least in terms of peak-to-valley heights) to those faced by *O. coggeri* and *P. australis* in nature, we measured the peak-to-valley heights of natural substrates used by geckos (rock, tree bark, and bamboo samples collected at gecko capture sites). To quantify gecko clinging ability on surfaces at least partially representative of natural surfaces, we used coarse (P40) and fine (P400) sandpaper with similar peak-to-valley heights as test surfaces in this study (Figure II-2). Additionally, glass was used as a test substrate as it is a smooth substrate, commonly used in gecko performance studies. Average peak-to-valley heights were measured using a surface profile gauge (Landtek Srt-6223 Surface Profile Gauge, accuracy: ±5 µm; resolution: 0.1 µm /1 µm; range: 0–800 µm). Peak-to-valley heights were measured at 10 random points, within 10 cm of each other, in the laboratory for coarse and fine sandpaper, and from collected samples of rocks used by O. coggeri. Bamboo and bark substrates used by *P. australis* were measured in the field, using similar methodology. The surface profile gauge was calibrated prior to each measure using supplied standard glass exhibiting peak-to-valley heights of 0 µm. Differences in mean peak-to-valley heights (µm) among the substrate types were quantified using a Kruskal–Wallis test followed by a pairwise Wilcoxon post hoc analysis.

CLINGING ABILITY

We used three artificial surfaces (instead of using the natural surfaces used by the geckos) to ensure that the roughness characteristics and surface chemistry of the rougher surfaces were uniform. This approach allowed meaningful comparisons between species and surfaces, while providing measurements on substrates with peak-to-valley heights similar to those of natural substrates.

Prior to recording clinging ability, mass was measured once for each individual, using a digital scale (resolution: 0.01 g). To measure the surface area of toe pads, the ventral aspect of the hands and feet of all individuals was photographed through glass against a uniform dark background with a scale in each image. Lightroom CC (Adobe Systems Incorporated, 2019) was used to adjust the contrast of images to ensure that the emphasis was on the toe pads only. The thresholding feature in ImageJ (version 1.52a; Gillies & Fearing, 2014; Schneider, Rasband, & Eliceiri, 2012) was then used to select these toe pads by saturation, as they contrasted highly with the rest of the image. Measurements were calibrated using the scale incorporated in every image. We calculated the attachment area for each gecko on all five toes on the right hand (manus) and right foot (pes) of all geckos and doubled these measures to calculate the total attachment area for each individual. Each toe was measured once.

To record the clinging ability of geckos when attached to a surface, we attached a force gauge (Extech 475040; resolution: 0.01 Newtons; maximum: 49 N ± 0.4% accuracy, Extech Equipment Pty Ltd) to the inguinal region of the gecko using a harness (Niewiarowski, Lopez, Ge, Hagan, & Dhinojwala, 2008) of fishing line (13.61 kg breaking strength; 0.5 mm diameter). Each gecko was permitted to take one step with each of its four feet on the testing substrate (P40, or P400 grit sandpaper, or glass), thereby ensuring that the natural adhesive system of the gecko was engaged (Collins, Russell, & Higham, 2015; Niewiarowski et al., 2008; Stark et al., 2015). Geckos were then pulled horizontally backward at an angle of 0° relative to the tabletop, using a constant velocity (~0.5 cm/s, calibrated using a 30-cm ruler and stopwatch; Crandell, Herrel, Sasa, Losos, & Autumn, 2014; Irschick et al., 2005; Tulli, Abdala, & Cruz, 2011; Zani, 2000). Each individual lizard was tested three times on each surface (three measures per individual: Cole et al., 2005; McKnight et al., 2019; Tulli, Cruz, Herrel, Vanhooydonck, & Abdala, 2009) using all 10 individuals of each species. Order of testing on each surface type was randomized; therefore, we minimized the likelihood of damage caused to the adhesive apparatus by one substrate negatively influencing performance on another substrate. To reduce variation, the "toe pad engagement" of geckos was scored based on their level of attachment from a scale of 1–3 (highest to lowest attachment), and trials with scores higher than 3 were not included in this study (e.g., if a gecko tried to escape, or it did not appear to actively adhere the substrate, it received a higher score and the trial was excluded; Figure II-3). Only one investigator (RP) conducted clinging ability trials to ensure consistency (Tulli et al., 2011). One measure of performance by *P. australis* on glass substrates was identified as an outlier (much >3 standard deviations from the mean) and was excluded from all further analysis.

Linear mixed-effects models were used to quantify the differences in shear force exerted by both species on coarse and fine sandpaper and glass, in the R package *lme4* (Bates, Maechler, Bolker, & Walker, 2015). We constructed nine candidate models with three measures per individual on each substrate as our response variable. To account for intraindividual variation, all models included individual gecko IDs as random effects. Toe pad area is positively correlated with body size (mass; Collette, 1962; Irschick et al., 1996), and larger toe pads are more likely to have a larger setal field area, producing increased shear forces, which increase clinging ability (Irschick et al., 1996; Johnson & Russell, 2009; Russell & Johnson, 2014; Webster, Johnson, & Russell, 2009). Hence, the attachment force generated by the adhesive system on a substrate increases proportionally with an increase in toe pad area and with mass (Irschick et al., 1996). The species in our study had very different body sizes and toe pad areas (*O. coggeri*: mean mass = 7.48 g, whole animal mean toe pad area = 55.28 mm^2; *P. australis*: mean mass = 20.21 g, whole animal mean toe pad area = 154.33 mm^2); therefore, to account for the influence of mass and toe pad area on absolute force generated, we also included mass and toe pad area as fixed effects in all models, to control for their effects on clinging ability. Shear force, mass, and toe pad area were log-transformed in all models (Table II-1). Model selection was conducted using Akaike's information criterion (AIC) in the R package *AICcmodavg* (Mazerolle, 2019) to identify the model of best fit ($\Delta AIC < 2$). We conducted post hoc analyses on the best-fit model to identify differences within the fixed effects using the R package *emmeans* (Lenth, 2019). Results are reported as mean ± 1 standard error. All statistical analyses were conducted in R Studio (version 1.1.383, RStudio Team, 2016).

Results

There was a significant difference in the mean peak-to-valley height of substrates (Kruskal–Wallis test: $p < .001$). The peak-to-valley heights of the coarse sandpaper (P40 grit) were not significantly different from those of rock or tree bark substrates used by *O. coggeri* and *P. australis* in nature (pairwise Wilcoxon test: tree bark: $p = 0.14$; rock: $p = 0.12$). The peak-to-valley heights of the coarse sandpaper (P40 grit) were significantly different from bamboo substrates used by *P. australis* (pairwise Wilcoxon test: $p < 0.001$). Peak-to-valley heights of bamboo substrates used by *P. australis* in nature were not significantly different from fine sandpaper (P400 grit; pairwise Wilcoxon test: $p = 0.26$). Glass had lower peak-to-valley heights than all other substrates (pairwise Wilcoxon test: bamboo: $p < 0.001$; bark: $p < 0.001$; rock: $p < 0.001$; coarse sandpaper: $p < 0.001$; Figure II-4).

The best model (ΔAIC < 2) predicting shear force exerted included substrate, mass, and toe pad area as fixed effects, with individual gecko IDs as random effects (conditional *R*2 = 0.59, marginal *R*2 = 0.64; Table II-2 and II-3). Shear force exerted by both species was significantly greater on glass (*O. coggeri*: 2.13 ± 0.64 N; post hoc comparison: $p < 0.001$; *P. australis*: 1.06 ± 0.20 N; post hoc comparison: $p < 0.001$) and coarse sandpaper (*O. coggeri*: 1.72 ± 0.51 N; post hoc comparison: $p < 0.001$; *P. australis*: 0.86 ± 0.16 N; post hoc comparison: $p < .001$) compared to fine sandpaper (*O. coggeri*: 0.72 ± 0.21 N; *P. australis*: 0.36 ± 0.07 N). Shear force exerted on glass and coarse sandpaper was not significantly different in either species (post hoc comparisons, *O. coggeri*: $p = 0.18$; *P. australis*: $p = 0.18$, Figure II-5). Thus, shear forces did not decline in a linear fashion with roughness, as predicted in Figure II-1a, but instead varied among substrates in a nonlinear trajectory, consistent with Figure II-1b.

Discussion

Both *P. australis* and *O. coggeri* exerted significantly higher shear forces on glass and coarse sandpaper than on fine sandpaper. Therefore, we did not observe a monotonic decline in performance with increasing peak-to-valley heights, which contrasts with findings of studies in which performance diminished considerably with increasing levels of roughness (Cole et al., 2005; Vanhooydonck et al., 2005). Shear force exerted on coarse substrates was not significantly different from that on glass in either species; thus, our results showed a nonlinear relationship between peak-to-valley heights and shear forces on the continuum of surfaces we used, consistent with studies by Huber et al. (2007; on a scale of single spatula), and Naylor and Higham (2019). Gecko adhesive systems have been well studied on a range of artificial substrates that have revealed the form and function of the adhesive apparatus in this taxon; however, our findings further highlight the need for gecko adhesion studies under more ecologically relevant conditions (Collins et al., 2015; Higham & Russell, 2010; Higham et al., 2019; Russell & Delaugerre, 2017). More comparative studies examining gecko attachment on different substrates are needed to elucidate the potentially context-specific nature of gecko attachment.

The shear force that can be generated by geckos is thought to be impacted by surface topology because topology determines the area available for attachment at the scale of the setal fields and also the degree to which claws can be effective. Natural substrates have microtopographies that are unpredictable and nonuniform compared to glass and other artificially smooth substrates (Russell & Johnson, 2007, 2014), highlighting the importance of overall structural considerations of locomotory substrates in gecko adhesion studies (Higham et al., 2019). The peak-to-valley heights of the coarse sandpaper we used to measure gecko clinging ability were similar to those of the rock and bark microhabitats used by *O. coggeri* and *P. australis*, respectively. Additionally, the fine sandpaper used in our study was similar in peak-to-valley height to bamboo surfaces used by *P. australis* in nature. There are, however, a range of other characteristics of rough surfaces that may influence attachment, such as variation in amplitude, wavelength (Gillies et al., 2014), spacing (Zhou, Robinson, Steiner, & Federle, 2014), and microstructuring of surface asperities, which could affect conformity of the adhesive apparatus or the attachment of claws. Additionally, the chemistry of the surfaces could influence interaction strength (Prüm, Bohn, Seidel, Rubach, & Speck, 2013), although we controlled for surface chemistry on both our rough surfaces by using the same brand of sandpaper, instead of using natural substrates. More research is required to determine the importance of exact topography and chemistry in replicating characteristics of natural substrates and to address the challenges of describing and quantifying surface roughness (Higham et al., 2019; Persson, Tiwari, Valbahs, Tolpekina, & Persson, 2018). Future research should incorporate carefully described and quantified, realistic surfaces in laboratory studies of attachment (Higham et al., 2019; Langowski et al., 2018).

We found that shear forces exerted by both *P. australis* and *O. coggeri* were greater on glass compared to on fine sandpaper. The gekkotan adhesive system is often characterized as most efficient on smooth substrates (Russell, Baskerville, Gamble, & Higham, 2015). High performance on glass, observed in our study, was consistent with previous studies that have tested clinging ability on artificial smooth substrates (Autumn et al., 2006, 2000; Huber et al., 2007; Irschick et al., 1996; Mahendra, 1941; Naylor & Higham, 2019). Smoother surfaces provide an increased area onto which fields of setae can make simultaneous contact, and generate substantial force (Russell & Johnson, 2007). Both species exhibited their highest clinging ability on glass. Our findings were consistent with the findings of previous studies in which instantaneous acceleration (40 m/s2 on wood with 98% surface area available for attachment; Vanhooydonck et al., 2005) and maximum clinging ability (~2.5 N on acrylic with 0.0 root mean square height Sq [μm]; Naylor & Higham, 2019) were highest on substrates that provided high surface area for attachment.

In our study, shear forces exerted on coarse substrates were not significantly different from those on glass, showing that the gekkotan attachment system also attaches efficiently to rough substrates. The question remains, however, what is the source of this effective attachment? Studies examining attachment systems consisting of claws and adhesive hairs in geckos (Naylor & Higham, 2019) and other taxa (rove beetles: Betz, 2002; dock beetle: Bullock & Federle, 2011; leaf beetles: Voigt, Schweikart, Fery, & Gorb, 2012) have demonstrated that claws are a critical aspect of clinging in nature, and suggest that there may be a synergistic relationship between claws and setae. They propose that greater attachment is achieved on surface topographies onto which both components can attach (Song, Dai, Wang, Ji, & Gorb, 2016). In our study, the nonlinear relationship of adhesion with roughness may have occurred because setal fields could maximize contact on smooth surfaces compared to fine-grained substrates. The lower generation of shear forces on fine-grained substrates was possibly because the opportunity for mechanical interlocking of claws was reduced on the finer-grained sandpaper. Fine-grained substrates are less likely to permit claws to attach compared to coarse substrates, producing the lowest generation of shear forces on fine-grained substrates in our study. On coarse surfaces, claws could mechanically interlock, compensating for the lack of effectiveness of setae on such surfaces and increasing overall shear forces. Other studies suggest that rough surfaces provide plenty of purchase for the setal system alone (Russell & Johnson, 2014). For example, the African geckos *Rhoptropus* cf. *biporosus* attached well to sandstone substrates, even though they lack tractive claws (Russell & Johnson, 2014). Additionally, Langowski et al. (2019) also report a similar trend in tree frogs, which lack claws entirely. Such observations suggest that the nonlinear performance graph we observed may not be driven solely by the relative role of claws in the adhesive apparatus of geckos. Experiments disabling setal fields or claws, while determining the role of the other part of the clinging apparatus on surfaces of various roughnesses, are required to further examine the hypotheses raised by these observations.

Pseudothecadactylus australis uses bamboo substrates in nature, but they exerted lower shear forces on fine sandpaper with peak-to-valley heights similar to bamboo substrates. Our field observations show that *P. australis* used bamboo substrates less often than tree bark (one observation on bamboo and 25 observations on tree bark). Possibly, bamboo substrates do not permit sufficient setal contact nor do they provide the undulance required for mechanical interlocking of claws, and so they are not preferred substrates for these geckos. Further studies should record microhabitat selection and investigate clinging ability in relation to preferred microhabitats.

Our results show that gecko clinging performance did not decline monotonically with increasing peak-to-valley heights of substrates. Instead, performance was lowest on the substrate with intermediate peak-to-valley heights and was similar on glass and coarse sandpaper. Our findings demonstrate that gecko attachment forces can be context-dependent and provide a basis for further studies examining the role of substrate and the different elements (claws and setae) in gecko attachment. Further, our study showed: (a) complex mechanisms promoting gecko attachment on multiple substrates with different microtopography, and illustrated that geckos can cling well to rough substrates thought to offer limited accommodation for the adhesive apparatus of geckos (Naylor & Higham, 2019; Russell & Johnson, 2007, 2014); and (b) that measuring performance using substrates with ecologically relevant roughness enables the quantification of clinging ability within a range that is biologically and evolutionarily meaningful (Bartholomew, 2005; Hagey et al., 2014; Higham et al., 2019; Langowski et al., 2018; Niewiarowski, Stark, McClung, Chambers, & Sullivan, 2012; Peattie, 2007; Russell & Johnson, 2007, 2014).

Figures

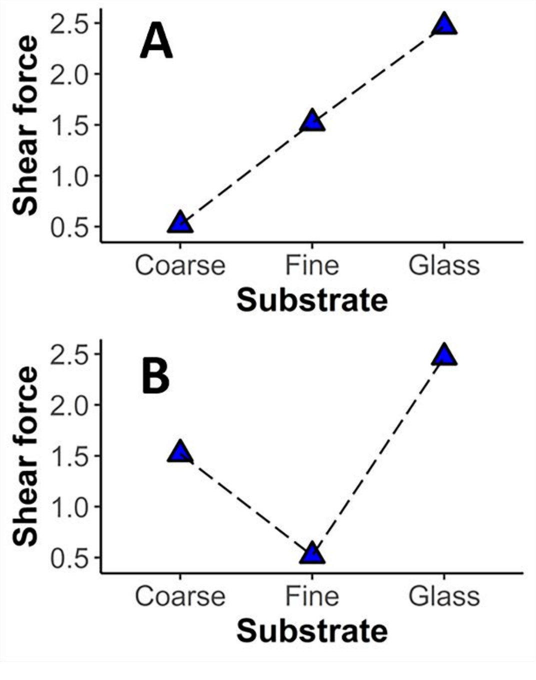

Figure II-1 Conceptual model in which substrates are ordered by decreasing roughness (coarse sandpaper, fine sandpaper, and glass), suggesting **(a)** declining shear force with increasing roughness or **(b)** a nonlinear performance curve in relation to roughness. Points are joined to illustrate the expected shape of trends.

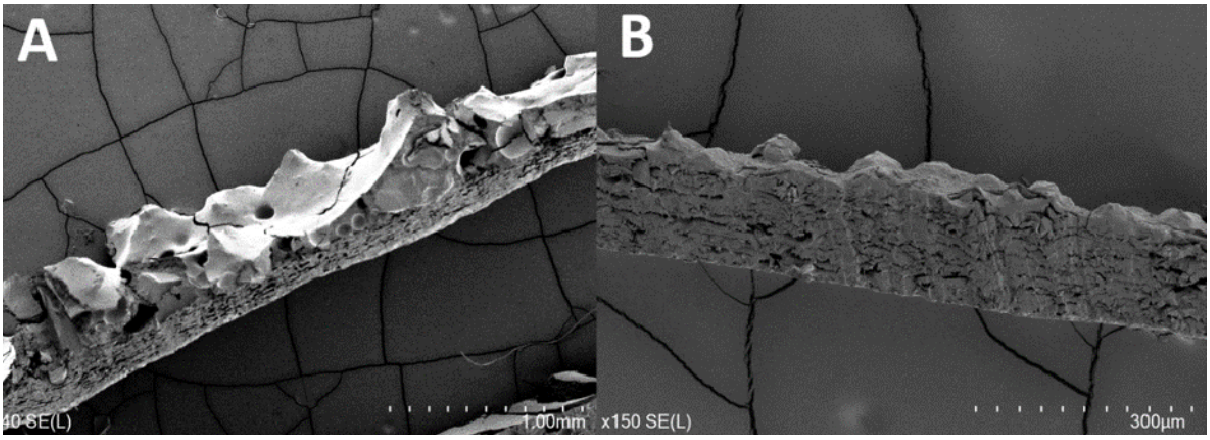

Figure II-2 Lateral view of surface microtopography of sandpaper with peak-to-valley heights similar to natural substrates used to measure clinging ability in My study. **(a)** Cross section of coarse sandpaper (P40; 40× magnification); **(b)** cross section of fine sandpaper (P400; 150× magnification).

Figure II-3 View from above of experimental setup used to measure shear force in the geckos Pseudothecadactylus australis and *Oedura coggeri.*

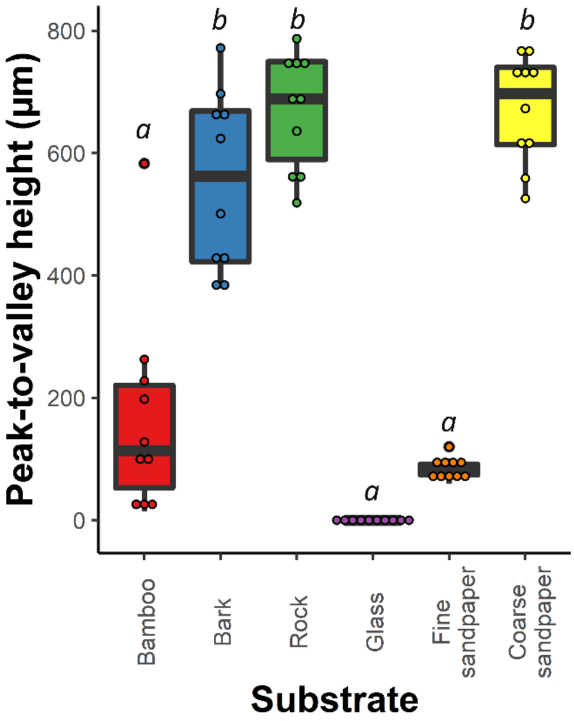

Figure II-4 Peak-to-valley heights of substrates (µm). Substrates include natural surfaces used by *Pseudothecadactylus australis* and *Oedura coggeri* in nature (bark, rock, and bamboo) and test surfaces (coarse and fine sandpaper, and glass) used in this study. The artificial substrates were used to approximate the peak-tovalley heights of natural substrates used by geckos. Rocks used by the northern velvet geckos (*O. coggeri*) and bark used by giant tree geckos (*P. australis*) had similar average peak-to-valley height to coarse sandpaper. Bamboo, used by *P. australis*, had similar average peak-to-valley height to fine sandpaper. Significant differences between peak-to-valley heights of substrates are indicated by different italicized letters (Kruskal–Wallis test; pairwise Wilcoxon test; *α* = .05)

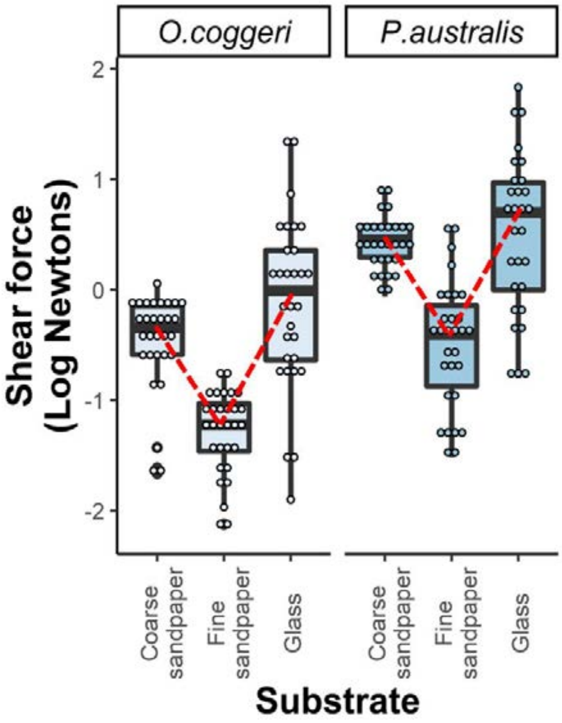

Figure II-5 Clinging ability (log (Newtons)) of the northern spotted velvet gecko (*Oedura coggeri*) and giant tree gecko (*Pseudothecadactylus australis*) on glass, coarse sandpaper (P40 grit), and fine sandpaper (P400 grit). Both species performed significantly better on glass and coarse sandpaper than on fine sandpaper, producing a nonlinear trajectory, consistent with the prediction in Figure 1b (trend shape indicated with red dotted line)

Tables

Table II-1. Mixed effects models used to analyse shear forces exerted by the geckos *Pseudothecadactylus australis* and *Oedura coggeri.*

Model number	Fixed effects	Random effects	Response variable
1	Substrate + log (toe pad area)	Individual gecko ID	Log (Shear force)
2	Substrate + log (mass)	Individual gecko ID	Log (Shear force)
3	Substrate + log (mass) + log (toe pad area)	Individual gecko ID	Log (Shear force)
4	Species + log (toe pad area)	Individual gecko ID	Log (Shear force)
5	Species + log (mass)	Individual gecko ID	Log (Shear force)
6	Species + log (toe pad area) + log (mass)	Individual gecko ID	Log (Shear force)
7	Substrate*Species + log (toe pad area)	Individual gecko ID	Log (Shear force)
8	Substrate*Species + log (mass)	Individual gecko ID	Log (Shear force)
9	Substrate*Species + log (toe pad area)	Individual gecko ID	Log (Shear force)

Table II-2. Models included in selection using Akaike's information criterion, to analyse shear forces exerted by the geckos *Pseudothecadactylus australis* and *Oedura coggeri*. The best model (ΔAIC < 2) included substrate, mass and toe pad area as fixed effects. Models are arranged in increasing order of ΔAIC values. df = degrees of freedom.

Fixed effects	ΔAIC	df	Weight	Residual deviance
Substrate + log(mass) + log (toe pad area)	0.0	7	0.449	269.1
Substrate*Species + log(mass)	1.2	10	0.247	264.3
Substrate + log(mass)	1.3	6	0.237	272.4
Substrate*Species + log (toe pad area)	3.8	9	0.066	269.0
Substrate + log (toe pad area)	18.7	6	<0.001	289.8

Substrate*Species + log (toe pad area)	19.0	9	<0.001	284.2
Species + log(mass)	109.5	5	<0.001	382.6
Species + log (toe pad area) + log(mass)	109.9	6	<0.001	381.1
Species + log (toe pad area)	117.2	5	<0.001	390.4

Table II-3. Fixed-effects coefficient estimates of the linear mixed-effects model for the differences in shear force on substrates with different peak-to-valley heights. Standard error (SE), degrees of freedom (DF), t-statistic (*t*), p-value (*P*). *Shear force on coarse sandpaper

	Estimate	SE	DF	*t*	*P*
Intercept*	-2.37	0.37	173.13	-6.365	>0.001
Fine sandpaper	-0.87	0.09	168.92	-9.609	>0.001
Glass	0.21	0.09	168.99	2.344	>0.050
log(mass)	0.54	0.12	135.02	4.69	>0.001
log(toe pad area)	0.23	0.12	152.61	1.83	0.060

Authors contributions

RP, EN, JR, and LS conceived the ideas and designed the methodology; RP collected the data; RP, EN, JR, and LS analysed the data; and RP led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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