Parasite and gut-microbiota dynamics in the experimental translocation of an endangered lizard

by

Bonnie-Thaïs Derne

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Abstract

In this thesis, I aim to inform parasite risk assessment for the translocation of an endangered skink. Translocations are potentially useful wildlife conservation strategies, but entail parasite-related risks, such as loss of host fitness and population decline, but also loss of ecosystem function if parasites are unnecessarily eliminated. Novel host-symbiont associations may arise from translocations, and existing relationships may play out differently in new abiotic and biotic environments, where hosts are likely to be stressed and immunocompromised. Predicting and minimising parasite-related costs to the hosts and the ecosystem for translocation risk assessment requires a knowledge of parasite diversity, of host-parasite relationships, and how this may change under translocation conditions. Despite this need, much of the world's estimated parasite diversity remains undescribed, and the biology of most wildlife parasites poorly understood. This thesis helps address these knowledge gaps for the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) and its parasites and other symbionts; the mite *Ophiomegistus michaeli*, the nematode *Pharyngodon wandillahensis*, and its gut bacterial communities. Specifically, I examine the dynamics of parasites and gut microbiota, and the effect on resident and translocated host fitness following an experimental population augmentation of *T. adelaidensis* in a wild setting.

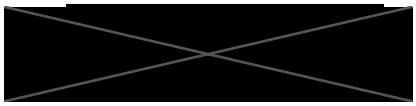
This thesis furthers our understanding of a wildlife host-parasite system by accompanying the taxonomic description of the parasitic mite *Ophiomegistus michaeli* sp. nov. with the first observations on the ecology of this mite and its relationship with *T. adelaidensis*. With its only known host being endangered, this parasite may be at risk of co-extinction. I examined inter-population genetic variation in parasite biota in this system, with implications for local adaptation and variable host-outcomes in a multi-population translocation context. Use of single nucleotide polymorphisms in mites and nematodes revealed genetic structure among isolated host populations. I used these genetic differences to identify transmission of allopatric parasites among lizards of three different population origins sharing habitat following translocation. These transmission events were few and occurred several months after translocation, suggesting slow and host-driven parasite dispersal. Transmission mechanisms were investigated but remain unclear. Gut microbiota in *T. adelaidensis* were also examined over the course of the translocation as likely influences of host health. No clear differences in detected bacterial species were found among hosts from different populations, nor did communities or strains clearly change in the two years following translocation. Finally, in models based on mark-recapture data, no difference was found between the survival

probabilities of translocated and resident *T. adelaidensis* individuals post-translocation. The lack of macroparasite spread, absence of microbial change, and unaffected survival probabilities together suggest that translocation is a relatively safe conservation intervention to undertake for the species in this respect.

This work has furthered knowledge on the host-parasite relationships in *T. adelaidensis* in a translocation context, and my findings suggest that parasite effects are not likely to threaten the viability of population augmentation as a conservation strategy for this species. Future research should be directed towards elucidating parasite lifecycles and transmission mechanisms, testing host fitness effects more precisely, and identifying functional roles of gut microbiota.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.



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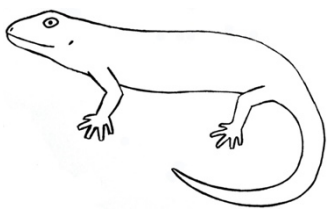

Details of co-authorship

Chapter 2

Published: Derne, B. (1), Hutchinson, M. (2), Weinstein, P. (3), Gardner, M. (4), Halliday, B. (5).

Parasite in peril? A new species of mite in the genus *Ophiomegistus* Banks (Parasitiformes: Paramegistidae) on an endangered host, the pygmy bluetongue lizard *Tiliqua adelaidensis* (Peters) (Squamata: Scincidae). *Austral Ecology*, *44*(3), pp.420-432.

The candidate was the primary author of the manuscript, conducted all specimen collection and host sampling. Authors 2,3 and 4 helped plan the study and critically reviewed the manuscript. Author 5 prepared the specimens, carried out taxonomic analysis, wrote the taxonomic description, taxonomic notes and diagnosis, and critically reviewed the manuscript.

Percentage contributions do NOT include the sections relating to the taxonomic description.

1: 80%, 2: 5%, 3: 5%, 4: 5%, 5: 5%

Chapter 3

Manuscript: Derne, B. (1), Hutchinson, M. (2), Weinstein, P. (3), Gardner, M. (4)

Mixing in moderation: allopatric macroparasites are genetically differentiated and minimally transmitted following population augmentation of an endangered lizard

The candidate was the primary author of the manuscript, conducted all field work and data analysis. Authors 2,3 and 4 helped conceive the study and critically reviewed the manuscript. Author 4 also provided advice on data analysis.

1: 85%, 2: 4%, 3: 4%, 4: 7%

Chapter 4

Manuscript: Derne, B. (1), Weinstein, P. (2), Hutchinson, M. (3), Gardner, M. (4)
Translocation does not appear to change the gut microbiota of an endangered lizard

The candidate was the primary author of the manuscript, conducted all field work, lab work and data analysis. Authors 2,3 and 4 helped conceive the study and critically reviewed the manuscript.

1: 85%, 2: 5%, 3: 5%, 4: 5%

Chapter 5

Manuscript: Derne, B. (1), Barnett, L. (2), Clive, L. (3), Weinstein, P. (4), Hutchinson, M. (5), Gardner, M. (6)
No home ground advantage: translocated lizards survive as well as residents in a population augmentation

The candidate was the primary author of the manuscript, conducted all field work in conjunction with author 3, who also critically reviewed the manuscript. Author 2 conceived the data analysis, and conducted it in conjunction with the candidate, and critically reviewed the manuscript. Authors 3, 4, 5 and 6 helped conceive the study and critically reviewed the manuscript.

1: 70%, 2:12%, 3:2%, 3:4%, 4:4%, 5:4%, 6: 4%

Preface

This thesis is comprised of four data chapters (Chapters 2–5) that communicate research contributing to the fulfillment of a doctoral program. These chapters are bookended by a general introduction (Chapter 1) containing research background, main aims and details about the overarching study conducted, and by a general discussion of key findings, their implications and future research directions (Chapter 6). The 'note to examiners' section preceding each data chapter details how the chapter relates to the others in this thesis.

Chapters 2-5 are each written and formatted to form a stand-alone research article and therefore each chapter features an abstract, its own bibliography, and supplementary materials in the case of Chapters 3 and 4. Some background overlap among chapters was therefore inevitable. Chapter 2 has been published in the journal Austral Ecology, whilst Chapters 3–5 are yet to be submitted to scientific journals for consideration (with Chapter 5 awaiting revision and resubmission). The input of external collaborators, sought to provide advice or undertake a small part of the work in this thesis, is outlined in the details of co-authorship (see preceding page of this thesis) for chapters 2 and 5. Although I conducted the majority of the work presented in this thesis, the formatting of the chapters as manuscripts acknowledges the contributions of the co-authors and therefore the pronoun "we" is used instead of the singular "I". Data collected and analysed in Chapters 3–5 can be made available upon request.

Chapter 1

Thesis Introduction

This thesis explores the relationship between an endangered host lizard species, *Tiliqua adelaidensis*, and its symbionts — two macroparasite species and its gut bacteria— in a translocation context. What follows is background on conservation-motivated translocation practice and the need for scientifically rigorous testing informed by a thorough understanding of the focal and interacting species' biology. I then focus on disease/parasite-related risks of translocations and the host-parasite-environment relationship underpinning translocation outcomes. After a brief overview of host-parasite studies in other members of the skink subfamily Egerniinae, I describe the species biology of *T. adelaidensis*, emphasising that extensive previous research suggests its suitability for translocation. The experimental population augmentation and associated monitoring, which I conducted with another student as part of a broader evaluation of translocation, is then outlined. This experiment provides the context for the studies in this thesis. Finally, the research aims and an overview of the subsequent chapters conclude this introductory chapter.

Background

Use of translocations in conservation management

The world's biodiversity continues to decline in the face of habitat degradation and loss, brought about by human activities and climate change impacts (Butchart et al., 2010; Opdam and Wascher, 2004). Declining wildlife numbers and habitat fragmentation results in once continuous species distributions being reduced to smaller populations which are ecologically and genetically isolated from each other (Schlaepfer et al., 2018). As a solution to biodiversity decline and fragmentation, wildlife translocations aim to conserve species (and associated genetic diversity and ecosystem function) by establishing and maintaining viable populations which can persist in the long term. In the last 50 years, translocations have become widely used as a conservation strategy (Armstrong and Seddon, 2008; Dodd and Seigel, 1991; Fischer and Lindenmayer, 2000; Griffith et al., 1989). Though the use of terminology varies within the animal biology literature (Armstrong and Seddon, 2008; Hällfors et al., 2014), 'translocation' as a broader term describes the intentional movement of individuals or populations from one location to another (International Union Conservation of Nature/Species Survival

Commission, 2013). There are three main types of translocations: 1) introductions, that aim to establish a population outside the historic distribution range of the species, 2) re-introductions, that aim to re-establish a population within the species historical range where it has become extirpated, and 3) augmentations, (also known as supplementation, reinforcements, re-stocking) that involve adding individuals to an existing conspecific population (IUCN/SSC, 2013).

Maintaining a population of a threatened species at the largest size possible by conservation management has important interacting ecological and genetic benefits, increasing its resilience to ecosystem perturbations and more gradual change. Small populations are less likely to persist over time as they are more vulnerable to stochastic population fluctuation, due to variation in reproductive success (Roughgarden, 1975), or environmental events such as predatory pressure, disease, habitat destruction or climatic events (MacArthur and Wilson, 1967; May, 1973; Shaffer, 1981). Increasing the density by population augmentation may result long-term population persistence following translocation by increasing encounters between reproductive partners, and conferring protection from predators by virtue of the dilution effect (Germano and Bishop, 2009; Zeisset and Beebee, 2013). Preserving a species within an ecosystem may also prevent the decline of ecosystem function and further biodiversity loss by virtue of other organisms that directly or indirectly rely on it (Heilpern et al., 2018; Strona and Lafferty, 2016).

Small populations are also vulnerable to outcomes associated with lowered genetic diversity, which may lead to lowered fitness in individuals and population decline (Frankham, 1995; Lacy, 1987; Lynch et al., 1995; Whitlock et al., 2000). Populations of low genetic diversity are more vulnerable to pathogens (O'Brien and Evermann, 1988; Spielman et al., 2004). Furthermore, genetic variation is a requirement for adaptation; small populations with low genetic diversity may have a diminished capacity to adapt to environmental change (Moritz, 1999; Templeton et al., 2001; Weeks et al., 2011), which is particularly important in the current context of rapid and widespread habitat alteration and climate change.

Improving translocation practice

Whilst translocations have several potential conservation benefits and present an intuitive solution to locally threatened animals, their success rates in the past have been low (Dodd and Seigel, 1991; Germano and Bishop, 2009; Griffith et al., 1989). Failures have occurred for a

variety of reasons, relating broadly to lack of scientific testing, and an inadequate understanding of the species' biology. Translocations have historically lacked extensive planning, *a priori* questions, testable hypotheses, and publication of negative results (Armstrong and Seddon, 2008; Chauvenet et al., 2013; Ewen et al., 2014; Fischer and Lindenmayer, 2000; Miller et al., 2014; Seddon et al., 2007; Sheean et al., 2012). Factors contributing to translocation success are complex and context-specific, necessitating an experimental approach, informed by modelling where appropriate (Germano and Bishop, 2009; Letty et al., 2007; Seddon et al., 2007). Clear indicators at the outset of the intervention should also be followed up by adequately long post-translocation monitoring (informed by focal species life-span), and results should be reported (Ewen et al., 2014; Ewen and Armstrong, 2007; Fischer and Lindenmayer, 2000; Germano and Bishop, 2009; Lindenmayer et al., 2020; Seddon et al., 2007; Sheean et al., 2012).

Sound empirical and modelling approaches to optimising translocation success require knowledge of the species' biology (Chauvenet et al., 2013; IUCN/SSC, 2013; Seddon et al., 2007). Evaluating the suitability of a species for translocation and optimising translocating success requires identification of the driver(s) of species decline (Griffith et al., 1989; Sheean et al., 2012), and an understanding of any limiting factors of ecology, genetics, physiology, and behaviour and their interrelationships (Batson et al., 2015; Besson and Cree, 2011; Dodd and Seigel, 1991; Johnson, 2000; Sullivan et al., 2004; Weeks et al., 2011). Prior knowledge of the species' biology can for example be used to minimise stress, which is common during and following translocations and can contribute to translocation failure (Dickens et al., 2010; Letty et al., 2007). Physiological effects of stress are compounded by post-release dispersal, which is likely a stress response (Aiello et al., 2014; Dickens et al., 2010; Massot et al., 1994), and can lead to increased predation, loss of body condition and movement into unsuitable habitat (Germano and Bishop, 2009; Griffith et al., 1989; Massot et al., 1994; Sullivan et al., 2004). Stress responses and low survivorship may also be driven by inadequate habitat provision (Germano and Bishop, 2009; Griffith et al., 1989), necessitating a detailed understanding of habitat needs of the focal species, as well as the consideration of the long-term drivers of habitat suitability at the release site (Ewen et al., 2014). Also required for planning effective translocation practice is an understanding of the possible ecological and genetic consequences of translocating individuals from one location to another. One potentially significant source of such effects is the burden imposed by parasites and pathogens, and this is what underlies the work in this thesis.

Despite the conferral of adaptive potential by increasing genetic diversity, translocation conversely may result in genotypes that are maladapted to local conditions (Savolainen et al., 2013). Furthermore, inter-population gene flow arising from translocation may result in outbreeding depression (where offspring of mixed lineage are less fit than their respective parents) and lead to population decline (Edmands, 2007; Storfer, 1999; Templeton et al., 2001). Vertebrate immune genes of the major histocompatibility complex (MHC) are involved in parasite recognition required for immune defence, and provide an example of where selection for certain genotypes within a certain host population are driven by local conditions (in this case, parasites present) (Eizaguirre et al., 2012; Hacking et al., 2018). The fitness advantage of a locally adapted genotype is likely to be lost when animals are translocated to a new location with different parasites. To optimally manage genetic diversity, translocation risk evaluation should involve consideration of the historical relationships of the populations in question, the respective habitat types and possible selective pressures of each population, and also quantitative estimates of gene flow (Storfer, 1999).

The need for prior knowledge of the species' biology also extends to predicting the effects of its introduction on other species and processes in the ecosystem (Seddon et al. 2007). Such forecasting may avoid ecosystem disruption by a novel species where existing biota have no pre-adaptation to its presence, and/or where suppression of other species result from competitive interactions (Mooney and Cleland, 2001; Ricciardi and Atkinson, 2004; Ricciardi and Simberloff, 2009). Population augmentations, where the focal species already exists in the ecosystem may pose risks by increasing the density of an established species. Increased density may not only have negative effects on its own long-term persistence by causing resource shortage, stress, and heightened disease transmission, but may also affect inter-specific interactions (Aiello et al., 2014; Ebrahimi and Bull, 2014a; Linklater and Swaisgood, 2008; Massot et al., 2007; Moseby et al., 2018; Tsurim et al., 2013). The risks associated with increased density must therefore be balanced with the potential benefits of increasing population density. Finally, parasites and other symbionts present another ecological guild that may both be affected by translocation and exert an altered effect on hosts (new or existing) as a result of translocation.

Disease risks associated with translocations

Disease-related risks are inherent to translocations (Kock et al., 2010; Northover et al., 2018). Translocated animals do not represent a single organism, but host a suite of micro-organisms such as bacteria, viruses and single-celled eukaryotes, and also macroparasites such as helminths and arthropods, adding further complexity to translocation contexts. Pathology and more subtle fitness loss caused by parasitic organisms, whilst not always obvious, can in many cases suppress wildlife populations, or cause outright decline, which may result in translocation failure and negate conservation gains (Daszak et al., 2000; Preece et al., 2017; K. F. Smith et al., 2009; Tompkins et al., 2011; Woodford and Rossiter, 1993). Translocations or biological invasions have often caused the emergence of disease as translocated or non-native individuals either became the source, or sink, of novel pathogens at the site of release (Kock et al., 2010; Northover et al., 2018). For example, the translocation of three black rhinoceros (*Diceros bicornis*) from the Kenyan highlands, where the tsetse fly (*Glossina spp.*), a vector of *Trypanosoma* spp. is absent, to tsetse-endemic lowlands. This resulted in the translocated individuals becoming anaemic from trypanosomiasis (Mihok et al., 1992). Similarly, pre-existing, sub-clinical infections can become pathological in stressful translocation conditions, as happened with the bacterium *Mycobacterium tuberculosis* in captive Arabian oryxes (*Oryx leucoryx*). Here, quarantine lasting for several months before translocation is thought to have caused the outbreak of pneumonia which occurred after release (Kock et al., 2010).

Despite this risk, pathogens and parasites have not been historically widely considered in translocations (Cunningham, 1996; Mathews et al., 2006; Viggers et al., 1993). Over time, however, their importance for translocation success been has been underlined (Armstrong and Seddon, 2008; Ewen and Armstrong, 2007; Fischer and Lindenmayer, 2000; Germano and Bishop, 2009). Recommendations to amend this oversight start with the consideration of disease/parasite risk in every translocation project. More concretely, identifying and mitigating risk of disease transmission in translocations can be achieved by conducting screening of individuals (and sympatric species) at the source, holding and recipient sites, effective quarantining of animals to be released, conditional treatment/vaccination of animals, and ongoing monitoring following release (Ewen et al., 2012; Kock et al., 2010; Mathews et al., 2006; Viggers et al., 1993).

In response to this need to better understand and manage the infectious disease risks of translocations, pathogen/parasite screening and monitoring and risk assessment has

increasingly become part of translocation practice (Gerhold and Hickling, 2016; Hartley and Sainsbury, 2017; Northover et al., 2019; Portas et al., 2020; Sainsbury et al., 2017; Sainsbury and Vaughan-Higgins, 2012; Smith et al., 2019). This recent work emphasises that host-parasite interactions and animal health outcomes are highly context-specific, requiring disease risk assessment in every case. At an early stage, disease risk assessment necessitates threat/hazard identification (Hartley and Sainsbury, 2017). This threat identification may prove difficult, since we generally lack knowledge both on the identity and distribution of parasites (Sainsbury and Vaughan-Higgins, 2012), and also on their prevalence and effects on wildlife individuals and populations (Baling et al., 2013; Preece et al., 2017; Viggers et al., 1993).

Host-parasite relationships

A parasite is defined as an organism that lives in or on another organism, where it derives nutrition from this host, shows structural adaptation to it, and causes it some degree of harm (Poulin, 2007). It is estimated that 30-50% of the world's living species are parasitic (Poulin, 2014). Parasitism falls along a gradual and often poorly delineated continuum of symbiotic relationships, organised by benefit to the host member of the closely associated organism pair (Leung and Poulin, 2008). Some organisms that are considered parasites, or their relatives, can under other circumstances, provide benefit to their host (Leung and Poulin, 2008). Alternatively there can be a shift from parasitism to commensalism, causing neither harm, nor benefit to their host (Leung and Poulin, 2008). At the other end of the spectrum, parasites are considered pathogens if they cause harm by producing pathology or disease, i.e. the abnormal function or change in the structure of an organ or system (Aiello et al., 2014), though pathogenicity is not necessarily a fixed characteristic of a parasitic organism (Méthot and Alizon, 2014). An example of variation within a taxon is the mutualistic amphibian pinworm *Gyrinicola batrachiensis* (Pryor and Bjorndal, 2005), contrasted with the lethal infection of the pinworm *Ozalaimus megatyphlon* in a captive green iguana (*Iguana iguana rhinolopa*), which is usually non-pathogenic in healthy, wild hosts (Loukopoulos et al., 2007). Examination of growth rates of spur-thighed tortoises (*Testudo graeca*) infested by oxyurid pinworms suggested that the effects of this symbiotic relationship ranged from parasitic to mutualistic depending on level of habitat disruption (Benítez-Malvido et al., 2019).

The intimate association between symbionts, such as parasites, and their hosts means that they may apply a major selective pressure to their host species by affecting key biological characteristics such as survival, reproduction, population size, behaviour, morphology, life

history and physiology (Anderson and May, 1982, 1979; Little, 2002; May, 1988; May and Anderson, 1990). Whilst there will be selection for the host's immune and other systems to minimise parasite-induced harm, the parasite will be under selective pressure to overcome any host defense mechanisms and to reproduce, theoretically resulting in a Red Queen dynamic (Decaestecker et al., 2007; May and Anderson, 1990; Sasal et al., 2000). The evolutionary effects of parasites on their hosts have been quantified in some systems (e.g. Brunner et al., 2017; Decaestecker et al., 2007; Fumagalli et al., 2011; Moritz et al., 1991), though in different systems may be difficult to disentangle from other processes (Decaestecker et al., 2007; Little, 2002).

The effects of parasites and other symbionts extend further than a single host or population. They may also indirectly influence community dynamics by affecting interspecific interactions of their host species, either by density mediated effects (when host survival and reproduction is affected), or trait-mediated effects (when host behaviour, morphology, life history or physiology is affected) (Dunn et al., 2012; Hatcher et al., 2006). The ability of parasites to change interspecific interactions relating to competition and trophic interactions are supported by theoretical and empirical studies (Brunner et al., 2017; Holt and Dobson, 2006; Holt and Pickering, 1985; Tompkins et al., 2011; Wood et al., 2007). Parasites also can contribute heavily to biomass and biodiversity within an ecosystem, and are a major component of stable trophic webs (Kuris et al., 2008; Lafferty et al., 2006). In light of their importance in ecosystem functioning, and their vulnerability to coextinction, parasite diversity has more recently started to become (at least theoretically) a conservation priority (Carlson et al., 2020; Jørgensen, 2014; Strona, 2015; Thompson et al., 2018; Windsor, 1995).

Whilst not always considered in the same disciplinary context as macroparasites and unicellular eukaryotic parasites (Poulin, 2007), non-eukaryotic microbe communities are associated with all animals and plants, and have potential to affect the fitness of hosts, therefore with implications for wildlife conservation management (Carthey et al., 2020). It should be noted that microparasites, especially viruses, with their short generation times, high evolutionary plasticity and consequent ability for host-switching, are considered to pose the largest risk of wildlife disease emergence (Dobson and Foufopoulos, 2001; Rideout et al., 2017). Cost-effective, high-throughput DNA sequencing technologies have seen the study of microbiota associated with humans and other animals increase dramatically in recent years, and the focus shift away from solely the culture of a single species of putative pathogen

(Douglas and Werren, 2016). Microbiota are defined as the assemblage of microorganisms, including bacteria, archaea, fungi, viruses and unicellular eukaryotes, present in a defined environment (Marchesi and Ravel, 2015). In contrast, 'microbiome' describes the microbiota, their genomes and the host environment (Marchesi and Ravel, 2015). The gut is the most microbe-rich site in the body, and the gut microbiota of humans and animal models have been implicated in a broad range of health and disease states (Lynch and Pedersen, 2016).

As animal gut microbiomes are increasingly characterised, we are also beginning to unravel their functional significance, both through empirical profiling, and functional gene analysis permitted by whole-genome sequencing (e.g. Riiser et al., 2019). The state of knowledge of wildlife-microbiome relationships lags behind our understanding of the human gut microbiome and that of model species however. Existing wildlife studies do show that microbiota include mutualistic taxa that provide essential or beneficial services such as digestion (Blyton et al., 2019; Dearing and Kohl, 2017) and parasite and pathogen regulation (Knutie et al., 2017; Murray et al., 2020). Other microbes in host-based communities appear to be commensal species, whilst some are pathogens or potential pathogens (Hall and Saito, 2008) — which may cause loss of fitness, host mortality and population decline (Preece et al., 2017). As such the term 'symbiont', which includes micro-organisms living in their host with mutualistic, commensal and parasitic/pathogenic roles, is useful for describing gut microbes. With many documented instances of mutualism between microbes and their host, and the discovery of a core group of microbes that appears consistent across conspecifics in some host species (Koskella and Bergelson, 2020; Shapira, 2016), there is debate as to whether microbiota together with their hosts can be considered to collectively form a unit of selection — as suggested by the concept of the holobiont (Douglas and Werren, 2016; Koskella and Bergelson, 2020; Simon et al., 2019). Regardless, the need for continued study of individual microbes and their interaction with the host, and other members of the microbial community, and the host environment has been pointed out (Koskella and Bergelson, 2020; Shapira, 2016). There is also growing recognition of the potential importance of understanding and maintaining microbiota-hosts relationships to enhance wildlife conservation practice (Carthey et al., 2020; Chong et al., 2019; Trevelline et al., 2019; West et al., 2019).

Host-parasite relationships during translocations

Pathology in a host caused by a parasitic symbiont can be considered a result of an (often complex) interaction between host, parasite and environmental factors, a conceptual model

known as the epidemiological triangle of disease causation (Frost, 1976). Poly-parasitism (co-infection of the host by multiple parasite taxa) also provides another dimension to the host-parasite-environment relationship, one that is poorly understood in wildlife, despite being the norm (Northover et al., 2018; Tompkins et al., 2011). It is expected that a host-parasite relationship will reach an equilibrium under a given set of conditions (May, 1988). Translocation of animal hosts, along with their symbionts, is likely to produce fundamental changes in host, symbiont and environment factors, leading to the disruption of any equilibria, and a change in infection outcome by parasites. If these changes lead to increased pathogenicity, either for the focal species being translocated, or other species in the recipient environment, population decline(s) and negative conservation outcomes may ensue. Specifically, the transfer of animals between ecosystems can result in the potentially negative parasite-related outcomes of: i) translocation-induced stress causing a clinical manifestation (and increased infectiousness) of a latent infection in the translocated animals, ii) translocated individuals transmitting a novel pathogen to immunologically naïve resident animals, and iii) translocated individuals being immunologically naïve to parasites acquired from resident animals (Kock et al., 2010). Generally the parasites that are most likely to cause pathology in translocation contexts are novel agents where pathogenicity is mediated by host stressors (Dobson and Foufopoulos, 2001). These stress-related and novel parasite-transfer outcomes of translocation are discussed below, and additional outcomes regarding altered host-parasite relationships in translocations are also considered.

Many endemic parasite infections do not cause obvious pathology in the host under conditions of equilibrium (Tompkins et al., 2011). However, stress leads to elevated glucocorticoid levels, in turn causing host immunosuppression. Stress associated with translocation may therefore increase susceptibility to either *de novo* infection by a parasite, or pathogenic effects of an already acquired, previously commensal parasite (Dickens et al., 2010; Hing et al., 2017; Teixeira et al., 2007). A further, possibly interactive, consequence of translocation-induced stress is that dispersal and movement of translocated animals at release site often increases, and may result in higher contact rates between individuals and thus increased disease transmission (Aiello et al., 2014).

The transfer of parasites between translocated animals and their recipient ecosystems, whether a new species or genotype, may change the host-parasite relationship and result in host pathology. The absolute or partial dependence on the host for survival and reproduction

inherent to parasitic organisms theoretically results in a close adaptive association between parasite and host (May and Anderson, 1990; Tompkins et al., 2011). This association may vary spatially, a concept consistent with the geographic mosaic of co-evolution model (Dybdahl and Lively, 1996). This model suggests that host-parasite relationships face different selection pressures and rates of gene flow, and therefore organisms evolve traits such as virulence and immune resistance differently, over space (Nuismer, 2006). Translocations can thus present a risk to hosts that lack immune adaptation to non-local parasites, even at fine spatial scales (Kock et al., 2010). Conversely, parasites are expected to hold an advantage in the evolutionary host-parasite arms race due to their shorter generation times, larger population sizes, and higher migration rates relative to their hosts, and thus have a high fitness in local hosts (Greischar and Koskella, 2007). However, local adaptation by parasites, where parasite fitness is highest in a local host, is common but not universal; a review and a meta-analysis found parasite success was greater on sympatric hosts only in some cases (Greischar and Koskella, 2007; Kaltz and Shykoff, 1998). Some studies found the reverse, providing support for the novel weapon effect, whereby a parasite is more successful in a host that is not adapted to it (Kaltz and Shykoff, 1998). Local adaptation is therefore not a given, emphasising the need to consider the possibility of increased parasite success on a non-local host when translocating animals and their parasites to allopatric conspecific populations.

Instances of translocated individuals introducing parasites into the recipient ecosystem were reviewed by Kock et al. (2010), and also later reported by Northover et al. (2019) for one mammal species, the marsupial woylie (*Bettongia penicillata*). No studies reporting the differential pathogenicity of parasites between translocated and recipient conspecifics following parasite introduction by translocated individuals specifically were found. Alternatively, there were reports of at least 16 mammal species and one bird species that became infected at their release site (Kock et al., 2010; Northover et al., 2019). The earlier example of trypanosomiasis emerging in translocated black rhinoceros (*Diceros bicornis*) from regions where the vector tsetse fly is absent to endemic regions, in contrast to latent infection in conspecifics at the recipient site (Mihok et al., 1992), highlights that pathology in translocated individuals may either occur due to stress, or due to their immunological naivety, or a combination of both. Matchett et al. (2010) demonstrated the role of acquired immunity in translocations by vaccinating re-introduced black-footed ferrets (*Mustela nigripes*) against the plague in conjunction with flea vector control, measures which increased ferret survival. Interestingly, when parasite infracommunities were monitored before and after woylie

population augmentations, the overall trend was for parasite biota of translocated individuals to converge with that of their resident conspecifics over the year following release (Northover et al., 2019).

Whether considering translocated animals as sources or sinks of allopatric parasites, the use of animals that have been born and raised in captivity, or have spent some time in captivity, is widely identified as high risk in translocations (Cunningham, 1996; Hare et al., 2012; Kock et al., 2010; Mathews et al., 2006; Viggers et al., 1993). Widespread mortality in wild animals following the introduction of a pathogen by captive conspecifics has been documented in tortoises infected by *Mycoplasma* sp. and in toads infected by chytrid fungus (Jacobson et al., 1991; Walker et al., 2008). In addition to heightened likelihood of exposure to pathogens from inter-specific sources, captive individuals are likely to show increased susceptibility to parasites they are exposed to at the release site, since the captive environment lacks selective pressures and makes for a population of lowered fitness (Hare et al., 2012; Kock et al., 2010).

Additionally, translocated individuals may theoretically lose their native parasite and undergo enemy release. Northover et al. (2019) noted that the prevalence of coccidia decreased in translocated woylies following release into a site where coccidia were apparently absent in resident conspecifics. This instance exemplifies a tendency for parasite prevalence within the introduced or invading population to be lower than in its original native habitat (Barnett et al., 2018; Torchin et al., 2003). No published studies documenting increased fitness due to enemy release in the context of conservation translocations were however found. Under enemy-release, a host will redirect resources from immunity towards activities such as reproduction and resource acquisition, and become more successful than it was in its native community, therefore potentially providing translocated individuals with a competitive advantage over resident conspecifics (or other related taxa), i.e. parasite-mediated competition. The enemy release hypothesis is partially supported by the results of biological invasion studies, where enemy-release from parasites may contribute to invader success (Torchin et al., 2003), though more studies are needed to comment on whether losing parasites truly translates to increased fitness (Colautti et al., 2004).

Parasite spill-back is a further possibility arising from the introduction of a new host species into an ecosystem and should be considered as a potential threat to native species (Kelly et al., 2013), though it does not appear to have been reported in the context of a conservation-

motivated translocation. Parasite spill-back occurs when introduced individuals provide highly competent hosts for parasites of native hosts, thus increasing parasite load for resident species. This dynamic arose with the introduction of the common brushtail possum (*Trichosurus vulpecula*) from Australia to New Zealand, where it became a highly competent reservoir and active disperser of bovine tuberculosis (*Mycobacterium bovis*) in cattle and deer (Viggers et al., 1993). An opportunity for parasite spillback created by the invasion of native Australian habitat by the exotic Asian house gecko (*Hemidactylus frenatus*) was also reported more recently by Barnett et al. (2018). This invasive lizard was observed in high densities and discovered to host a native gecko pentastome parasite (*Waddycephalus* sp.).

Finally, changes that translocations make to population structure and density during translocations must be considered in terms of parasite transmission and risk (Daszak et al., 2000; Lebarbenchon et al., 2006). Increased population densities are likely to increase contact between animals, either directly, or indirectly through resource sharing, and intra-specific contact may be further increased by translocation stress-induced movement (Aiello et al., 2014). For many parasites, increased rates of contact would increase parasite transmission and its reproductive rate (e.g. Aiello et al., 2014; Boyce et al., 2011; Page, 2013). Also, increased densities of one species may exclude other species and reduce species richness within a community, which can have implications for parasite transmission between species, and parasite load within a given species (Lebarbenchon et al., 2006). Concordantly, lowered biodiversity in an ecosystem has generally been associated with increased disease transmission (Ostfeld and Keesing, 2011).

Anticipating parasite-related consequences in translocations

There hence exists ample evidence that parasites can affect their hosts, and their wider ecosystems profoundly, including in translocation contexts. As with all ecological and evolutionary relationships, infection outcomes are infinitely variable and highly context-specific. Conducting useful disease risk assessment for translocation of a particular wildlife species to a particular habitat first requires hazard identification (Ewen et al., 2015; Sainsbury and Vaughan-Higgins, 2012). Such a process begins with identifying parasites that infect or may infect the focal and other species. A barrier to this is that much of the earth's parasite diversity remains to be discovered and described (Carlson et al., 2020). A useful description of a parasite species entails the taxonomy, but also its lifecycle and the relationship it has with its host(s), especially in the context of assessing the health costs imposed by the parasite to the

host. Following on from this, an understanding of how parasites behave at a host population level is required, that is screening of animals to determine presence and prevalence (Baling et al., 2013; Dalziel et al., 2017; Grange et al., 2017; Sainsbury and Vaughan-Higgins, 2012), and also an understanding how parasites are transmitted between hosts, and of the external factors that affect transmission (e.g. Aiello et al., 2014). When multiple host populations are involved in a translocation, an assessment of inter-population genetic diversity for both hosts and parasite may help predict the possibility of local adaptation or immunological naivety (Criscione et al., 2005). The use of molecular markers may be useful for all of these things, from parasite identification to surveillance, and elucidation of lifecycle, host choice, transmission dynamics, and evolutionary processes (Criscione et al., 2005).

The importance of *a priori* objectives in disease risk assessment and targeted monitoring has also been highlighted (Ewen et al., 2015; Wintle et al., 2010). Correctly anticipating processes and problems that may arise when defining objectives requires prior knowledge. This knowledge can be gained by empiricism, and if good-quality data are available from experiments or other sources, modelling approaches (Aiello et al., 2014; Seddon et al., 2007). Finally, since parasite transmission and effects on host health may take an extended period of time to arise, long term post-release monitoring in translocations is an essential part of answering questions and informing subsequent disease risk assessments (Sainsbury and Vaughan-Higgins, 2012).

This thesis hinges off of an experimental population augmentation of the endangered scincid lizard, the pygmy blue tongue (*Tiliqua adelaidensis*). I sought to learn more about the host-parasite relationships of this species in a multi-population translocation context, with the aim of evaluating the suitability of this species to translocation as a conservation strategy. The following sections provide background relevant to examining host-parasite relationships of *T. adelaidensis*. First, I provide a brief overview of host-parasite relationships in reptiles using lizard examples, with a focus on what is known of the egerniine skinks (the sub-family to which *T. adelaidensis* belongs). I then outline the biology of *T. adelaidensis*, including the known parasites of this species.

Host-parasite relationships of reptiles

Reptiles are infested by a taxonomically broad spectrum of micro- and macroparasites (Bower et al., 2019; Schumacher, 2006). Reptile parasite communities, their effect on the host — and

also that of mutualistic or commensal symbionts such as gut bacteria—are generally understudied, in spite of the current vulnerability to extinction of many reptile species, and the zoonotic potential of some of their parasites (Bower et al., 2019; Geyle et al., 2020; Gibbons et al., 2000; Jiang et al., 2017; Leung and Koprivnikar, 2019; Mendoza-Roldan et al., 2020). One recurring theme in the reptile parasite literature is the capability of the external environment to modulate the host-parasite relationship (Benítez-Malvido et al., 2019; Carbayo et al., 2019; Innis et al., 2009; Oppliger et al., 1998), which is pertinent to the context of human-induced habitat modification and conservation management activities.

Studies of host-parasite relationships in reptiles have documented a number of physiological, behavioural and genetic effects of parasitism (Bower et al., 2019). For example, high rates of mortality caused by a nidovirus in the skink *Tiliqua rugosa* have been observed (O'Dea et al., 2016). Sub-lethal physiological and developmental effects include anaemia and lowered running stamina caused by *Plasmodium mexicanum* haematozoans in the iguanid lizard *Sceloropus occidentalis* (Schall et al., 1982), and higher maternal investment and growth early in life for *Lacerta vivipara* lizards with high mite loads (Sorci and Clobert, 1995). Growth rates were observed to be outright lower in *Sceloporus virgatus* lizard males with higher mite loads (Cox and John-Alder, 2007). These parasite costs can select for certain genotypes within a population, as seen by the parasite-mediated selection of immune genes in the Australian agamid lizard *Ctenophorus decresii* associated with tick loads (Hacking et al., 2018). Mites may also be a driver of sexual reproduction in the gecko species complex *Heteronotia binoei*, where parthenogens had a higher susceptibility to mite infestation than sympatric sexually-reproducing conspecifics (Moritz et al., 1991). In contrast to these instances, costs of parasitism to the reptile host are not always evident (e.g. Barnett et al., 2018; Brown et al., 2006; Goldberg and Bursey, 1991; Schlaepfer, 2006) and more study is needed on determinants of parasite-induced fitness costs in reptiles (Bower et al., 2019; Fajfer, 2012; Norval et al., 2019). Symbiotic relationships may also help reptile hosts adapt to different environments, a possibility raised by a recent study of eastern water-dragons (*Intellagama lesueurii*) which observed a difference in gut microbiota between hosts in urban vs. wild habitats. This difference may have been related to the need to digest different food items (Littleford-Colquhoun et al., 2019). Whilst the effect of parasites and commensals on reptile hosts is variable and not always clear, the ability of disease to act synergistically on reptile populations should be a key consideration for conservation management (Bower et al., 2019; Gibbons et al., 2000; Tompkins et al., 2015).

Host-symbiont relationships of egerniine skinks

Like reptiles generally, egerniine skinks are associated with a broad taxonomic range of parasites and other symbionts. Species of this subfamily are found across a range of terrestrial, saxicolous and semi-arboreal environments primarily within Australia (Chapple, 2003). They are generally long-lived and occupy insectivorous, omnivorous and herbivorous dietary niches (Chapple, 2003; Gardner et al., 2016). The taxon's defining feature is that many species form stable social aggregations, exhibit high levels of social and genetic monogamy, and together cover a range of social structures (Chapple, 2003; Gardner et al., 2016), which has implications for disease transmission.

The recent discovery and partial genomic characterisation of a nidovirus in *Tiliqua rugosa* represented the first documentation of nidovirus in a lizard species (O'Dea et al. 2016). Wild lizards in southern Western Australia testing positive for this virus exhibited respiratory symptoms referred to as the bobtail flu, which caused high rates in mortality in the absence of treatment (O'Dea et al. 2016). However, 12% of wild, apparently healthy individuals tested in the region also tested positive, which suggests that infection can be asymptomatic (O'Dea et al. 2016).

A variety of enteric bacterial species have been recorded in *Tiliqua rugosa*, congeners and also in *Egernia stokesii* (Dodd, 2014; Iveson et al., 1969; Norval et al., 2019). In *Tiliqua rugosa* these include bacteria in genera from the Enterobacteriaceae family *Salmonella*, *Escherichia, Enterobacter, Citrobacter, Klebsiella* and *Proteus* (Bull et al., 2012; Gordon and Cowling, 2003; Iveson et al., 1969; Parsons et al., 2015). Other *Salmonella enterica* strains were also reported in *T. occipitalis* in Victoria and in *T. scincoides* in Western Australia, and in *Cyclodomorphus branchialis* in Western Australia (Iveson, 1969). Many of these taxa are highly-prevalent in across reptile species, with no apparent pathogenicity, though have been implicated with disease in reptiles (Corrente et al., 2004; Jacobson, 2007; Jho et al., 2011; Kumar and Sharma, 1978; Mathewson, 1979; Pees et al., 2007; Schumacher, 2006).

Salmonella can be transmitted through the faecal-oral route, the environment, and also vertically in some cases, as proven with infected turtle hatchlings (Jacobson, 2007). *Tiliqua rugosa* individuals who had social contacts with one another were most likely to be infected with the same genetic type of *Salmonella enterica*, suggesting that host-to-host contact was

more important than environmental transmission routes in this host species (Bull et al., 2012). A study of 207 *T. rugosa* lizards at the same locality over three activity seasons observed a high diversity of enteric bacterial strains (1140 strains belonging to 10 bacterial species) where *Salmonella enterica* and *Citrobacter freundii* both of which exhibited spatial structuring in similarity of strains (Parsons, 2004). The surrounding environment was also found to influence *S. enterica* subspecies inhabiting *T. rugosa* guts, suggesting the importance of habitat structure in enterobacteria persistence when in the external environment rather than the host gut (Parsons et al., 2015). Both *S. enterica* and *C. freundii* showed temporal variation in prevalence but tended to increase in spring then decline in summer to winter. It was inferred that the high diversity of bacterial strains and low extent of strain sharing were due to the importance of the environment-host transmission pathway (usually ingestion of contaminated food, primarily plant material) (Parsons et al. 2004), in contrast to the findings of Bull et al. (2012).

Gut protozoans, which have a direct lifecycle and are transmitted via the faecal-oral route, appear to be common in skinks, and five species from four phyla were observed in *T. rugosa* from unspecified locations following gut dissection (Johnston, 1932), and one coccidian *Eimeira* sp. in *Egernia stokesii* in captivity (Stein, 1999). The species *Eimeria tiliquae* was later described in *T. rugosa* by Yang et al. (2013) and was possibly associated with respiratory symptoms. Even though some intestinal parasitic protozoans are intra-cellular and cause cell death of the host (such as coccidians), most are not associated with pathology in their reptile hosts, though may decrease growth rates (Greiner, 2003; Wilson and Carpenter, 1996).

In contrast to gut protozoans, the coccidian blood protozoans, recorded in Australian skinks *Tiliqua rugosa* and *Egernia stokesii,* have indirect lifecycles, also relying on an arthropod vector such as a ticks, mosquitos or sandflies (Godfrey et al., 2009; Smallridge and Bull, 2000; Stein, 1999). In studies of infection of *E. stokesii* by *Hemolivia mariae*, *H. biplicata*, *Schellackia* sp., *Plasmodium mackerrasae*, *P. circularis* and *Hepatazoon* sp., Stein (1999) did not report any obvious parasitaemia. However, infection of *T. rugosa* by *H. mariae* (population prevalence 11.5%) was associated with lower body condition in male lizards (Smallridge and Bull, 2000), and also smaller home ranges — presumably due to lowered activity levels (Bouma et al., 2007). Lizard malaria has been known to cause aenemia, reduce running stamina and inhibit reproduction through hormonal and behavioural mechanisms in western fence lizards (*Sceloporus occidentalis*) (Dunlap and Schall, 1995; Schall et al., 1982). For the social *Egernia*

stokesii, lizards which shared rock crevice refuges with more conspecifics were found to have a higher chance of being infected by two or more species of blood protozoan (Godfrey et al. 2009), further exemplifying the role of social interactions in parasite transmission in this skink taxon.

Nematodes are the most commonly documented phylum of helminth parasite in *T. rugosa*, though trematodes, cestodes and an acanthocephalan were also observed (Johnston, 1932; Norval et al., 2019). The nematode parasites recorded in the egerniine skinks all belong to the order Oxyurida (Adamson, 1984, 1981; Fenner and Bull, 2008; Johnston, 1932; Norval et al., 2019; Stein, 1999), with the exception of the physalopterid nematode *Abbreviata antarctica* in some *Tiliqua* species (Norval et al., 2019). Oxyurid nematodes have a direct lifecycle that involves host ingestion of eggs in faeces (Adamson, 1989; Morand et al., 1996). Though obvious effects on body condition by reptile pinworms are not documented, groups of *Egernia stokesii* infested by *Pharyngodon tiliquae* spent less time basking and more time seeking refuge in rock crevices than groups which had been treated with anti-helminthic drugs, meaning that thermoregulation and reproductive success are affected (Fenner and Bull, 2008). Activity levels also differed with nematode load; treated lizards moved more frequently than untreated lizards (Fenner & Bull 2008). More broadly, oxyurids are common parasites of reptiles, and pathology appears more common in captive animals that are heavily infested and co-infected by additional parasites (Loukopoulos et al., 2007; Wilson and Carpenter, 1996). Fenner & Bull's (2008) study highlight the subtle, population level effects of nematode infestation and the need to study it in wild populations.

Several acarid ectoparasites have been recorded on egerniine hosts. The snake mite *Ophionyssus natricis*, a cosmopolitan species that can cause pathology in captive reptiles, was recently documented in wild *T. rugosa* individuals in South Australia, though not associated with obvious pathology (Norval et al., 2020). The reptile tick *Bothriocroton hydrosauri* is found in a range of Australian reptiles across Southern Australia, including several *Tiliqua* species and *Liopholis whitti* (Norval et al. 2019). Other haematophagus ixodid ticks parasitising egerniine lizards such as *T. rugosa* include *Ambylomma albolimbatum*, *A. limbatum*, *A. moreliae*, *A. triguttatum triguttatum* and *A. vikirri* (Norval et al. 2019). Additionally, the usually mammal-feeding soft tick *Ornithodoros gurneyi* recorded on *T. rugosa* is thought to be an opportunistic parasite (Norval et al., 2019; Sharrad and King, 1981).

With three on-host feeding stages alternating with free-living stages, environmental conditions are important for tick transmission. In *T. rugosa*, asynchronously overnighting in the same refuges as neighbouring conspecifics facilitates transmission of the *Ambylomma limbatum* and predicts tick load (Leu et al., 2010). Similarly, *E. stokesii* individuals that were connected to infested neighbours by refuge sharing were more likely to be infested by *A. vikirri* (Godfrey et al., 2009). Large numbers of ticks on a lizard host that is already weakened may cause irritation, aenemia, or exsanguination (Stein 1999). More subtle fitness costs of tick infestation have been observed in *Tiliqua rugosa.* In an experimental manipulations of tick loads, Main & Bull (2000) found that captive lizards with higher loads had lower sprint speeds and lower endurance. The same study also observed that in their natural habitat, lizards with higher tick loads had small home ranges, and spent more time basking, and less time moving than less heavily infested individuals. In a later study, *T. rugosa* males with higher tick loads were less likely to retain their mating partners from year to year. It was concluded that parasite load may influence pair-bond stability by the female seeking to minimise parasite transmission risk by avoiding highly infested partners (Bull and Burzacott, 2006).

In addition to being parasites in their own right, ticks can act as vectors for other parasites. For example, *Bothricroton hydrosauri* and *Ambylomma libatum* are both vectors for the haemogregarine blood parasite *Hemoliva mariae* in *Tiliqua rugosa* (Smallridge and Paperna, 1997). Similarly, the ticks *Ambylomma limbatum* and *A. vikirri* are vectors of various haemogregarines species in *Egernia stokesii* (Stein 1999). The bacteria *Rickettsia honeii* as well as an undescribed *Rickettsia* sp. has been found detected in *Bothriocroton hydrosauri* though not in *Tiliqua rugosa* itself (Stenos et al., 2003; Whiley et al., 2016).

The host study species: *Tiliqua adelaidensis*

Threats to *T. adelaidensis*

Species with narrow ecological niches are more vulnerable to decline and extinction since they are likely to have a small endemic range, and changes to their environment may preclude their persistence altogether. *Tiliqua adelaidensis* is restricted to mesic grasslands of the Mid-North region of South Australia, and relies on the vertical burrows dug by certain species of lycosid and mygalomorph spiders as thermal refuges and an ambush site for passing invertebrate prey (Milne et al., 2003a) (Figures 1.1–1.3). This region experiences a temperate climate, with distinctly dry and warm summers (BOM, 2020). Grasslands are amongst the most threatened ecosystems in southern Australia (Lunt, 1998) and accordingly, habitat fragmentation is a

major threat to the persistence of *T. adelaidensis* (Fenner et al., 2018). Changes in land use are especially pertinent threat to individual populations since they all occur on privately owned land thus management may be limited (A. L. Smith et al., 2009b).

Figure 1.1. An adult *Tiliqua adelaidensis* individual. Notes: The average snout-vent length for adults is 100 mm. Photo: P. Matejcic.

Models linking habitat suitability with stochastic demographic processes of *T. adelaidensis* populations have shown that in coming decades, climate change will negatively impact population abundance (Fordham et al., 2012). Population decline will occur regardless of whether temperature and rainfall changes act directly on habitat suitability, or indirectly by affecting the grassland plant species that characterise *T. adelaidensis* habitat. Extinction of the species is a likely outcome without managed relocations, even if the carrying capacity of selected sites is increased by the addition of artificial burrows. The most efficient criteria for source population translocation identified by Fordham et al. (2012) was dependent on the rate of change in habitat suitability; relocating individuals from small areas to large areas resulted in less population decline where the rate of change in habitat suitability was slow. When the rate of change was high, choosing a source population with low habitat stability and moving it to high-stability areas resulted in the best conservation outcomes.

Figure 1.2. Examples of mesic temperate grassland habitats of *Tiliqua adelaidensis* in the Mid-North region of South Australia. Notes: The Jamestown site (top) in November (austral spring) (photo: P. Matejcic) and the Burra site (bottom) in February (austral late summer) (photo: B. Derne). Both sites are used to graze sheep.

Figure 1.3. An example of a trapdoor spider (*Blakastonia* sp., Mygalomorphae) burrow, as preferred by *Tiliqua adelaidensis* as a refuge. Photo: B. Derne.

The biology of *Tiliqua adelaidensis* with implications for parasite transmission and translocations

Tiliqua adelaidensis has a lifespan of 9–10 years (Milne, 1999). It is active from September to March, whilst remaining in torpor in its burrow during the winter months (Milne et al., 2003a). During October to early November, males will leave their burrows to mate with females (Milne et al., 2003a; Schofield et al., 2012). The majority of litters consist of 2–4 live young, which are born in late January to early February, and may continue to mid–March (Milne et al., 2002). Some females will breed in consecutive years (Milne et al., 2002). Dispersal of young from the maternal burrow occurs in the weeks following birth, and burrow–sharing until then may present vertical parasite transmission opportunities. A study of dispersal by neonates found that 34.4% of litters observed had dispersed within 5-12 days of birth whilst 75.3 % had dispersed after 5 weeks, and in half of cases where juveniles remained, the mother had dispersed (Milne et al., 2002).

Scat analysis has revealed *T. adelaidensis* to have a largely arthropod-based, but omnivorous diet (Fenner et al., 2007). Common prey items included plague locusts (*Chortoicetes terminifera*), ants and spiders. As the season progresses, seeds and leaves from species such as

Medicago minima, represent a greater proportion of gut content (Fenner et al., 2007), consistent with observations of lizards re-entering their burrow with plant matter in their mouth (Milne et al., 2003a).

Extensive study of the ecology and genetics of *T. adelaidensis* has identified innate characteristics and optimal strategies that will maximise the viability of its conservation by translocation. Exclusive inhabitation of vertical burrows dug by lycosid and mygalomorph spider species is a defining feature of this species (Milne et al., 2003a), and several aspects of its ecology—distribution, social structure and dispersal — hinge on this burrow use. Burrow use is also likely to be important for parasite transmission. Burrows are an absolute requirement for this species because they provide a refuge from predators and thermal conditions, and as an ambush site for arthropod prey (Milne et al., 2003a). Individuals emerge partially from their burrows during the day to bask, and appear to leave the burrow completely only to feed, defecate, seek mating opportunities (in the case of adult males), or to find a new burrow (Milne et al., 2003a). Occupancy of a single burrow by an individual lizard is usually longer than three months (Milne et al., 2003a). The availability of deep spider burrows was found to be the most important habitat characteristic for presence of *T. adelaidensis* in an analysis of grassland patches across the species' range (Souter et al., 2007). Therefore, translocated individuals must have access to adequate spider burrows at their release site for survival. The provision of artificial burrows to supplement existing ones successfully increased *T. adelaidensis* population density during a one-season field study (Souter et al., 2004) (Figure 1.4). Furthermore, female lizards in artificial burrows were found to have a better body condition and produce larger offspring than females in natural burrows (Milne et al., 2003b). Artificial burrows are readily accepted by lizards if they are sufficiently deep, and individuals appear to prefer burrows of the narrowest width they can fit into (Milne and Bull, 2000), and additionally those that possess a basal chamber (Staugas et al., 2013).

Figure 1.4. A *Tiliqua adelaidensis* individual basking in its artificial hollow dowel burrow. Photo: B. Derne

Confinement to individually-occupied burrows is associated with a solitary, within stable colonies, social system in *T. adelaidensis* (Fenner and Bull, 2011). As a result of this solitary social system, direct-conspecific interactions are less likely to represent major parasite-transmission pathways, though adult-adult conspecific interactions do occur for mating and territory-defence purposes, which may lead to parasite transmission. Mating is a brief encounter in October-November (Fenner and Bull, 2009; Milne et al., 2003a), and burrow defence is thought to be the most likely cause of agonistic conspecific interactions (Fenner and Bull, 2011). *Tiliqua adelaidensis*-occupied burrow density in an area generally declines over the course of the activity season as lizard attrition occurs (Fellows et al., 2009) and unoccupied burrows degrade, however the deepest burrows remain occupied (Fenner and Bull, 2010). As a result of this dynamic, the same burrow may be shared asynchronously by different individuals, which may present a mechanism of parasite transmission.

In addition to reacting with burrow defense behaviour when other conspecifics are seen close to the burrow (Fenner and Bull, 2011), post-release dispersal by lizards translocated into captivity was increased by the perception of nearby conspecifics, particularly so at higher

conspecific densities, which also elicited more movement and agonistic interactions between conspecifics (Ebrahimi and Bull, 2014a, 2014b). Future translocations will therefore need to position artificial burrows at these lower densities in order to minimise stress and dispersal arising from burrow defence. Dispersal away from point of release was also affected by time of confinement when soft release techniques were used in *T. adelaidensis* translocations; lizards confined for one day post-release were less likely to subsequently disperse than those confined for four days (Ebrahimi and Bull, 2013).

Both field studies and genetic analysis of *T. adelaidensis* populations suggest that natural dispersal is generally low. High site fidelity and a tendency to disperse less than 20 m was observed during a mark and recapture study conducted over three activity seasons (Milne, 1999). Concordantly, mated partners were located no more than 100 m apart in a genetic study by Schofield et al. (2014), and genetic differentiation within populations occupying continuous habitat was observed at a fine-scale of less than 400m (A. L. Smith et al., 2009b). Dispersal from burrows occurs peaks during October and November, when adult males are moving around to seek mating opportunities, and also in February and March when neonates disperse from their maternal burrows, while adult females are the least mobile group (Bull et al., 2015; Schofield et al., 2012). A captive study has confirmed that as the activity season progresses, adult *T. adelaidensis* individuals move around their burrow entrance less, spend less time basking, engage in less conspecific agonistic interactions and are not as likely to disperse from the release point (Ebrahimi and Bull, 2014c). These seasonal dispersal and movement dynamics may have implications for when and how parasites are spread through a population. Furthermore, the risk of dispersal away from a release site following translocation may be minimised in this species by carrying out translocation late in the activity season, when natural dispersal and individual activity levels of adults are low (Ebrahimi and Bull, 2014c; Schofield et al., 2012).

Tiliqua adelaidensis engages in promiscuous, polygynous mating (Schofield et al., 2014), with some 75% of litters having multiple fathers (Schofield et al., 2014). This mating system may increase parasite transmission, not only due to increased mating interactions, but increased prospecting for mates relative to monogamous systems. Mate choice appears to not be influenced by level of relatedness and to be altogether random (Schofield et al., 2014). Indiscriminate mate choice is expected to be favourable for successful translocation, since translocation will not disrupt existing pair bonds and once established, translocated and

resident individuals should mate together readily. Furthermore, heterozygosity resulting from population mixing is expected to be beneficial, since levels of heterozygosity across 16 polymorphic microsatellite loci were found to be high amongst six populations of *Tiliqua adelaidensis* studied (A. L. Smith et al., 2009b). Genetic differentiation between these populations, whilst significant, was low between populations separated by up to 70 km, which suggests that gene flow across the area may have occurred in the past (A. L. Smith et al., 2009b). High heterozygosity and low inter-population genetic differentiation suggest that outbreeding depression is unlikely to occur when *T. adelaidensis* individuals from different populations interbreed, particularly if source and recipient populations are from the same genetic cluster, i.e. a nearby population (Allendorf et al., 2001).

Macroparasites of *T. adelaidensis*

Prior to work described in this thesis, only two species had been identified as parasitising *T. adelaidensis*; the ixodid reptile tick *Bothriocroton hydrosauri* and the oxyurid nematode *Pharyngodon wandillahensis* (Fenner et al., 2008; Fenner and Bull, 2007). *Bothriocroton hydrosauri* was reported at a very low prevalence of 0.33% in *T. adelaidensis* (in contrast to being common in larger congeners), and appears to be an opportunistic parasite to *T. adelaidensis* (Fenner and Bull, 2007). Tick infection status of *T. adelaidensis* individuals within a population was found to be positively correlated with proximity to adjacent burrows occupied by lizards who did not change burrows in the five month study period (Fenner et al., 2011).

In contrast to *B. hydrosauri*, the gut nematode *Pharyngodon wandillahensis* has a direct lifecycle and is only known to parasitise *T. adelaidensis*. Examination of lizard scats for nematode eggs of nine *T. adelaidensis* populations across the species' range detected the presence of *P. wandillahensis* in six populations, with an overall prevalence of 34% (Fenner et al., 2008). A later survey of three populations from the Burra region observed a similar prevalence of 30% (A. L. Smith et al., 2009a). Fenner et al. (2008) noted that infested lizards were in good physical condition. However, high prevalence of *Pharyngodon tiliquae* has been linked to reduced activity levels in the skink *Egernia stokesii* and other nematode species have been known affect their hosts in less immediately apparent ways, such as reduced fecundity, and by altered population dynamics (Fenner and Bull, 2008). In *T. adelaidensis* populations connectivity to 'disperser' conspecifics was found to be the most important factor determining *P. wandillahensis* infection status, that is, infected individuals had greater proximity to

neighbouring burrows occupied by lizards present within the study plot for two months or less (Fenner et al., 2011).

The most likely transmission route for *P. wandillahensis* is the accidental ingestion of eggs during substrate licking, feeding or tongue flicking aimed at scats (Fenner et al., 2008). Signalling to conspecifics through scats is common in skinks, as tongue flicks are used to deliver chemical signals from the environment to the highly developed vomeronasal sensory organ (Fenner and Bull, 2010). There is evidence that scats are used as social signals to conspecifics in *T. adelaidensis*, probably as a way to advertise their presence and avoid direct conflict over territory (Fenner and Bull, 2011, 2010). Individuals consistently position scat piles around their burrow (usually approximately 20 cm from the entrance) oriented in the direction of the nearest occupied conspecific burrow, a behaviour undeterred by the scat-pile destruction by rain or displaced by experimenters (Fenner and Bull, 2010). Further evidence that scat-piling serves a territory defence function is that individuals approach an empty burrow with a scat pile more cautiously than one without, and that males and females alike scat pile in this manner, regardless of the sex of their nearest neighbour (Fenner and Bull, 2010). Males scat pile closer to their burrow entrance as neighbour proximity increases, perhaps because they employ an alternative strategy in defending their burrows (Fenner and Bull, 2010). Presence of scat piles may additionally be used by *T. adelaidensis* as an indication of burrow suitability, since individuals preferentially occupy empty burrows with scat piles over empty burrows without scat piles (Fenner and Bull, 2010). In light of the social function of scat pile sniffing, lizards who move through populations and sniff scats from unfamiliar conspecifics at a higher frequency would therefore be expected to have a higher prevalence of faecal-orally transmitted parasites and in turn be more important sources of infection. This hypothesis is supported by the findings that *T. adelaidensis* individuals proximate to 'disperser' conspecifics are more likely to be infected by *P. wandillahensis* (Fenner et al., 2011). There is however a current lack of direct observational support for this scat sniffing behaviour in the field (Bull et al. unpublished data).

The experimental translocation of *Tiliqua adelaidensis*

The previous, extensive research into *Tiliqua adelaidensis* biology suggests that translocation is not only necessary for long term persistence of the species (Fordham et al., 2012), but likely to result in long term persistence of translocated individuals and their progeny. This viability is predicted because translocation is: i) unlikely to disrupt any social bonds between individuals or adaptive genetic structure within populations, ii) likely to meet habitat requirements (especially if the recipient site already contains conspecifics) and iii) likely to minimise stress and post-release dispersal (Ebrahimi and Bull, 2014c, 2013; Fenner et al., 2011; Milne et al., 2003b; Schofield et al., 2014; A. L. Smith et al., 2009b, 2009a). No parasites with obviously harmful effects are known to definitively parasitise *T. adelaidensis*, and its solitary social system may minimise the spread of any pathogens in a translocation context. However, host-symbiont relationships may change as a result of translocation, and there is a need to examine whether parasite transmission and changes in host-symbiont relationships are likely to compromise translocation success. To meet this need, I conducted a population augmentation of *T. adelaidensis.* This work was done in conjunction with another student, who examined the genetic and ecological consequences of the population augmentation (Clive, 2019). As a broad project, the aim was to comment on whether wild-wild translocation of *T. adelaidensis* was likely to have any genetic, ecological or parasite-related consequences that would compromise its viability as a conservation strategy.

This experimental population augmentation was conducted by selecting a large, established population of *T. adelaidensis* at the Nature Foundation of South Australia's 'Tiliqua' reserve near Burra township in the Mid North region of South Australia (Figures 1.2, 1.5, 1.6), and using it as a recipient (release) site, herein referred to as the 'Burra' site. This grassland area is also used to graze sheep, as is typical in the region. Since the movement of *T. adelaidensis* individuals is heavily focused on their home burrows and lizards will rarely move more than a few hundred metres, we were able to locate occupied burrows using an optiscope (Milne, 1999) and to build 30 m x 30 m, 30 cm high sheet-metal enclosures around them between activity seasons (during July – August 2015) (Figure 1.6). Use of enclosures made it possible to preclude immigration and emigration, and therefore to track the same individuals over time. Most importantly, enclosures made it possible to contain the lizards we later translocated and thus to limit any (unlikely) negative effects of the translocation to a small proportion of the wider local population.

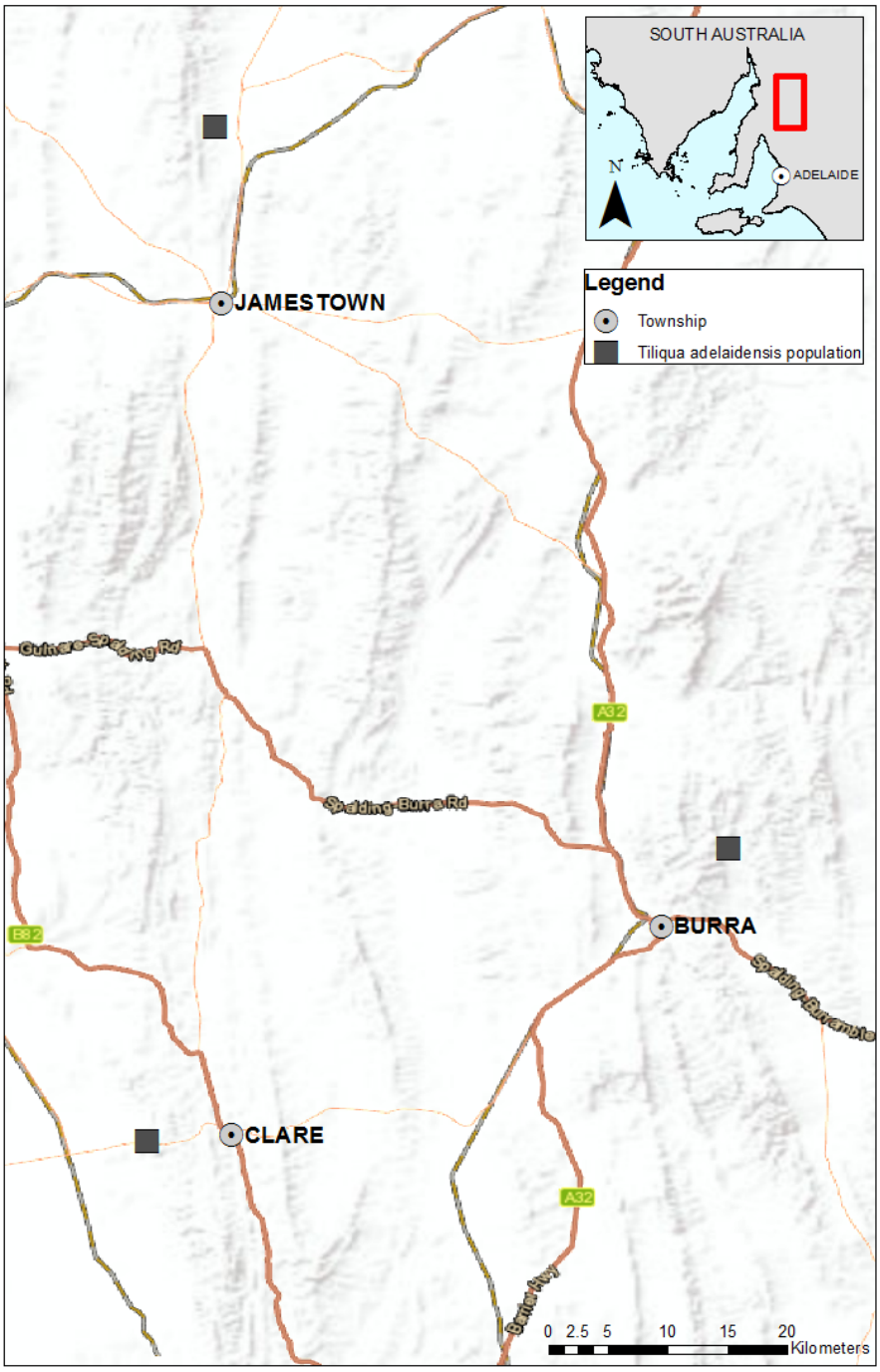

Figure 1.5. Approximate locations of source and recipient populations of *Tiliqua adelaidensis* populations involved in the experimental population augmentation at the Nature Foundation of South Australia's 'Tiliqua' reserve near Burra (Map: B. Derne).

Figure 1.6. Two adjoining 30 m x 30 m enclosures built of 30 cm high sheet metal, around occupied *Tiliqua adelaidensis* burrows at the Burra site, into which translocated individuals were released. Photograph taken in October (austral spring) (Photo: B. Derne).

Enclosures were built in three pairs; i.e. each 30 m x 30 m enclosure shared a wall with another enclosure, and enclosure pairs were located 120 –340 m from each other (Figure 1.7). These three pairs were intended to act as replicates, with three experimental enclosures, each adjoined to a control enclosure (Figure 1.7). Numbers of existing 'resident' *T. adelaidensis* lizards per enclosure ranged from 6 –23, reflecting the natural variability in density within a locality. In the spring of 2015, all lizard-occupied burrows within the enclosures were re-identified by thorough searching and were marked. At this time, 12 supplementary hollow dowel burrows measuring 30 cm in length, with a hole 18 mm in diameter, were vertically embedded throughout each enclosure. From October 2015 –March 2016, monthly monitoring was conducted of lizards in these enclosures. This monitoring involved re-identifying all occupied burrows, and capturing each lizard occupant (or the majority) using a tethered mealworm bait (Milne, 1999). Upon initial capture, each lizard was toe clipped using a unique sequence identifier, with the tissue retained for DNA analysis for another student's project. Each capture event resulted in the lizard being weighed and measured, with its home burrow recorded (using a 1 m^2 grid coordinates), and parasite samples being collected, before the animal was replaced in its burrow.

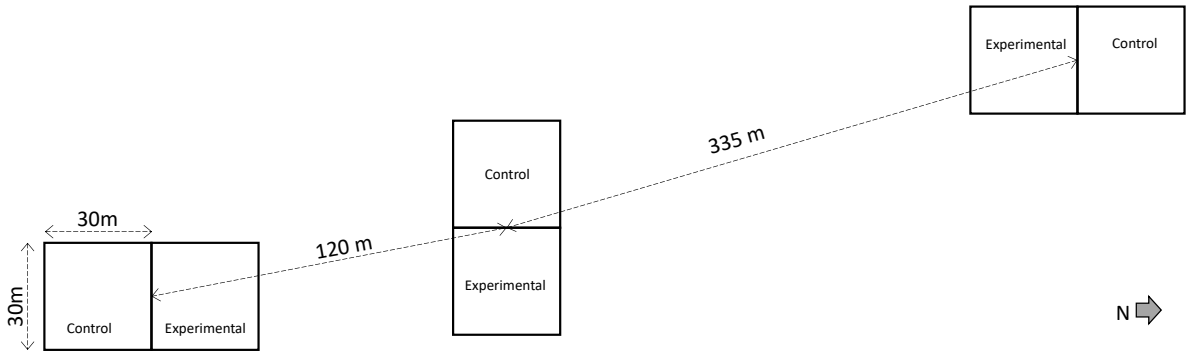

Figure 1.7. Schematic representation of 30m x 30m enclosures containing *Tiliqua adelaidensis* individuals that were part of the experimental population augmentation at the recipient site near Burra. Notes: 'Control' enclosures contained non-translocated Burra-resident lizards only, 'Experimental' enclosures contained non-translocated Burra residents, amongst which translocated individuals from Jamestown and Clare populations were later released. Distances are not drawn to scale.

In mid-February 2016, 11 *T. adelaidensis* individuals (adults and subadults) were located and captured from an isolated wild population west of the Clare township on pastoral land (Figure 1.5), and transported to the Burra recipient site (approximately 45 km away). Thirteen individuals from another wild population north of Jamestown on pastoral land approximately 72 km away (Figure 1.5) were also captured and transported to the Burra site 1 –2 days later.

One enclosure from each of three enclosure pairs at the recipient Burra site was designated as the experimental enclosure, and 3 –4 Clare lizards and 3 –4 Jamestown lizards were toe-clipped, measured and released into previously established dowel burrows (Milne and Bull, 2000) in each of the three experimental enclosures. Experimental enclosures therefore contained a mixture of translocated Clare and Jamestown lizards alongside resident Burra lizards, whilst adjoining enclosures served as control treatments containing only the original Burra resident lizards (Figure 1.7). Translocated lizards were confined to a one meter squared

area around their burrow and fed two mealworms twice in the week following release to minimise dispersal (Ebrahimi and Bull, 2013) before the soft release pens were removed.

In March 2016, one month after the translocation, monthly sampling involved capture, measurement and parasite sampling of all *T. adelaidensis* lizards in the enclosures. Lizards sampled thus comprised of Burra resident control lizards in the control enclosures, and the Burra experimental residents in the experimental enclosures, and translocated lizards originating from the Jamestown and Clare populations which now shared these experimental enclosures. Monthly sampling continued in the same manner over the next two austral spring-summer activity seasons; from October 2016–March 2017, and from October 2017–March 2018.

Research aims and chapter outline

In this thesis I examine aspects of the host-symbiont relationships of *Tiliqua adelaidensis* in the context of a conservation-motivated population augmentation, with a focus on two macroparasites and gut bacteria. My specific aims, which are further outlined below, are to:

1) Provide initial observations of a new parasite record for *T. adelaidensis*.
2) Determine whether there is inter-population genetic variation in macroparasites and also gut microbiota, at a sub-species level and at a community level respectively.
3) Use any inter-population differences in macroparasites or gut bacteria to comment on the extent and mechanisms of transmission following translocation, when lizards from different population origins are sharing the same habitat.
4) Examine lizard survival, as a measure of fitness crucial to translocation success, to determine if any treatment group was differentially affected by the translocation and possible parasite-related consequences.

To study host-symbiotic relationships, identifying symbiotic species is essential. Exploration of potential source populations for this translocation gave rise to the novel observation that some *T. adelaidensis* individuals at the Jamestown population appeared to have mites lodged underneath their scales (M. Hutchinson, pers comm.). In February 2016, prior to the translocation, mites were once again observed and collected from hosts at the Burra site, the Clare site and the Jamestown site. Taxonomic analysis (by B. Halliday) revealed these mites to be an undescribed species belonging to the genus *Ophiomegistus*, other members of which parasitise skinks and snakes in the Asia-Pacific region (Klompen and Austin, 2007). This discovery of a new parasite species and a new host-parasite relationship exemplifies the

importance of observation and studying the natural history of wildlife. As previously pointed out, the hazard identification phase of disease risk assessment in translocation requires a knowledge of which parasite species may parasitise the host or recipient community (Ewen et al., 2015). In Chapter 2, I contextualise the taxonomic description of *Ophiomegistus michaeli* by relating my field observations of this parasite to what is known about its only recorded host species. I use this information, as well as that from other systems, to make inferences about the life cycle of this mite and the nature of the host-parasite relationship. I also highlight the need to consider *O. michaeli* as a threatened species and conservation priority in itself.

Chapters 3 and 4 examine the dynamics of macroparasites and gut bacteria respectively, over time in a population augmentation context. First however, I determine whether there is a discernible difference between parasite biota between the isolated *T. adelaidensis* populations that are part of the experimental population augmentation. Inter-population variation in parasite species or intra-species variation may serve as a first indication that local adaptation (on the part of the host or the parasite) may exist, and that creating novel host-parasite interactions may alter existing host-parasite relationships. Such information may also shed light on the evolutionary history of the host and parasite species (e.g. Criscione et al., 2006). Chapter 3 consists of an intra-species examination of genetic variation (using single nucleotide polymorphisms as markers) for the two microparasite species observed across populations: the mite *Ophiomegistus michaeli* and the gut nematode *Pharyngodon wandillahensis*. Chapter 4 focuses on determining whether gut bacteria communities present in *Tiliqua adelaidensis* individuals from the three populations differ prior to translocation. This study joins a growing body of work which considers the possible fitness implications of changing gut microbiota by translocating hosts species to new environments.

Having examined the differences in macroparasite genetic structure and gut bacterial communities within and among host populations, Chapters 3 and 4 both examine how translocation changes parasite genotypes and biota in *T. adelaidensis* over time. In Chapter 3, I use the genetic differences in mites and nematodes to identify transmission events of non-local parasites within the experimental population augmentation, and then use network analysis to relate transmission to putative transmission mechanisms. One purpose of understanding the extent and mechanisms of transmission of these parasites is to contribute to informing risk assessment in any future population augmentations for this species. Chapter 4 examines whether gut microbiota change over time, both in resident and translocated hosts,

which may suggest transmission of gut bacteria from the local environment, food items, conspecifics or other interacting species. Again, insight into these processes may help avoid pitfalls in future translocation practice for this species.

The underlying question with any translocation is 'was it successful?'. The definition of success is of course variable, but there is general consensus that it involves the establishment of a self-sustaining population in the long term (Armstrong and Seddon, 2008; Sheean et al., 2012). Reproductive success of translocated and resident lizards in this population augmentation has been measured and reported by others elsewhere (Clive, 2019). In Chapter 5 I analysed mark-recapture data for individuals within the enclosure to comment on the other component of longer-term persistence—survival. Here the aim was to determine if survival probability differed between resident and translocated lizards over the first two years following translocation. This study did not include parasite infection data *per se*, since sample sizes for mites were small, and for the nematodes particularly definitively distinguishing infected from non-infected individuals would have required sampling of a much more invasive nature. These factors would have limited the meaningfulness of parasite infection status as a term in the models used to estimate survival probability. The results obtained do however provide an overall assessment of fitness of the different experimental treatments and serve as a point for further hypothesis generation.

In the final chapter, I discuss the collective results and their implications for the viability of translocation as a conservation strategy for *T. adelaidensis*. Future research directions are also considered.

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Chapter 2

Parasite in peril? A new species of mite in the genus *Ophiomegistus* Banks (Parasitiformes: Paramegistidae) on an endangered host, the pygmy bluetongue lizard *Tiliqua adelaidensis* (Peters) (Squamata: Scincidae)

Note to examiners

This chapter consists of the first observations of a newly documented parasite species for *Tiliqua adelaidensis,* the mite *Ophiomegistus michaeli* sp. nov. Information on its distribution, host attachment, prevalence combined with a discussion of relevant host biology begin to characterise the mite's ecology and the host-parasite relationship. These observations and inferences accompany a formal taxonomic description of *O. michaeli*, which was conducted and written by Dr. Bruce Halliday. Describing the taxonomy and biology of this newly observed parasite species provided a basis for monitoring for mites within lizard populations over the course of the experimental translocation, and for examining how genotypes varied among allopatric host populations and over time following translocation, which is reported in the following chapter. This chapter has been published in *Austral Ecology* and is therefore formatted according to the specifications of that journal

Abstract

Host parasite relationships are generally understudied in wild populations but have a potential to influence host population dynamics and the broader ecosystem, which becomes particularly important when the host is endangered. Herein we describe a new species of parasitic mite from the genus *Ophiomegistus* (Parasitiformes: Mesostigmata: Paramegistidae) of an endangered South Australian skink; the pygmy bluetongue lizard (*Tiliqua adelaidensis*). Adult mites were observed on lizard hosts in three different host populations, among which prevalence varied. No temporal trend in prevalence was evident over two spring-summer seasons of monitoring. We hypothesise that the reliance on burrows as refuges by *T. adelaidensis* may be essential for the completion of the mite life cycle and also for horizontal transmission. The conservation implications of not only its effect on the host but its potential status as an endangered species itself are considered.

Introduction

Parasites not of medical significance are generally understudied in wild animal populations (Strona, 2015; Thompson et al., 2010). This lack of attention belies their significant contribution to biomass, biodiversity (Kuris et al., 2008) and ecosystem function. Parasites are often key components of trophic webs, and may regulate host populations; either directly or by influencing their host's other biotic interactions (Dunn et al., 2012; Hatcher et al., 2006; Lafferty et al., 2006). In threatened hosts, parasites and the host-parasite relationship therefore acquire particular management significance for three reasons:

1) The parasite may cause host population decline by compounding the effects of small population sizes, poor habitat quality, chronic stress and loss of herd immunity commonly affecting vulnerable species (Lyles & Dobson 1993; Smith *et al.* 2009).
2) Parasites may promote ecosystem function (Kuris et al., 2008; Lafferty et al., 2006) in communities which are likely to be vulnerable alongside the host they support.
3) Parasites are at risk of co-extinction, further compounding biodiversity loss within the ecosystem (Dunn et al., 2009).

A current barrier to understanding how parasites interact with their host and the broader ecosystem is that most species remain uncharacterised (Nadler & Pérez-Ponce de León 2011). It is therefore important to describe new parasite species and attempt to understand their ecology when the opportunity arises.

The parasitic mite genus *Ophiomegistus* Banks (1914) (Parasitiformes: Mesostigmata: Paramegistidae) includes 20 described species from Australia, Papua New Guinea, Indonesia, Malaysia, and the Philippines, all of which are blood-feeding parasites of scincid lizards and snakes (Klompen & Austin 2007). Two species have been recorded from Australia (Domrow, 1988, 1978). *Ophiomegistus australicus* (Womersley, 1958) was described from four females collected from a lizard, "*Tiliqua* sp.", on Saint Francis Island, South Australia (Womersley, 1958), which is believed to be *Cyclodomorphus melanops.* This host has a similar size (SVL 132 mm) and body shape to *T. adelaidensis,* though shares no overlapping range, and is associated with grass hummocks (Wilson & Swan 2003). The other described Australian *Ophiomegistus* species, *O. clelandi* Womersley, 1958, is known from a single male from a snake collected in central Australia.

In this paper we describe a new ectoparasitic mite, *Ophiomegistus michaeli* sp. nov., from the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis* (Squamata: Scincidae). Moreover, we examine some aspects of its relationship to its host, particularly in the context of conservation management.

The host

Tiliqua adelaidensis is a medium-sized skink (adult SVL 88–105 mm) endemic to mesic grasslands in a limited area of South Australia, centered approximately 160 km north of the city of Adelaide (Hutchinson et al., 1994). *T. adelaidensis* populations form stable colonies of solitary individuals that exclusively occupy and defend vertical burrows dug by mygalomorph and lycosid spiders (Milne et al., 2003). These lizards display high site fidelity and limited dispersal (usually 20–70m), leaving burrows to either disperse from the natal burrow, seek a better quality burrow, or males to seek mating opportunities with females every spring (Milne et al., 2003; Schofield et al., 2012). Mating in *T. adelaidensis* is promiscuous within and between seasons, and mate choice appears to be influenced only by spatial proximity (Schofield et al., 2014). Aspects of the lizard's unique biology such as reliance on burrows, its low level of conspecific interaction and its low vagility are likely to heavily influence the mite's lifecycle, transmission ecology and genetic structure.

A small endemic range, and habitat fragmentation by cereal cropping and urbanisation, have driven the classification of *T. adelaidensis* as endangered (IUCN, 1996). Small, isolated populations (31 known) persist on land used to graze sheep. Modelling by Fordham *et al.* (2012) predicted that without managed translocations, climate change will drive many of these populations, and eventually the species, to extinction within several decades. Future human development, such as wind farms, may further reduce suitable habitat for this species (Fordham et al., 2012).

Methods

Collection and examination

Three *Tiliqua adelaidensis* populations were sampled for mite infestation during February 2016: one east of Burra (48 lizards sampled), one north of Jamestown (see specimen notes) (13 lizards sampled) and one west of Clare (11 lizards sampled) (Figure 2.1). The Burra population consisted of

lizards contained by six 30m x 30m enclosures 0–500m apart, which had been built around wild lizards established in burrows during the previous July as part of an ecological study. Each enclosure contained 6–18 lizards. This Burra group was also sampled monthly in March 2016, October 2016–March 2017, and October 2017–March 2018.

Lizards were captured by luring them from their burrows with a tethered mealworm bait (Milne, 1999). Lizards were restrained, ventral surface up, while mites were dislodged from under each scale. Mites were stored in 100% ethanol at 4°C until slide mounting for further examination.

Specimens temporarily stored in ethanol were cleared in hot Nesbitt's solution until they were sufficiently clear (approximately five minutes), and were mounted in Hoyer's medium (Krantz & Walter, 2009). Specimens were examined under a Zeiss compound photomicroscope, illustrated using a drawing tube, and measured with a graduated eyepiece. All measurements were recorded in micrometres. Measurements of the dorsal shield of the female are based on the holotype (in bold) and three paratypes (in parentheses); those of the male are based on the three paratypes. Chaetotaxy for the legs and palps follows Evans (1963a, 1963b). The holotype and paratypes of the new species are deposited in the Australian National Insect Collection, CSIRO, Canberra, Australia.

Data analysis

Mite prevalence between the three populations in February 2016 were compared using Fisher's exact test (two-tailed). Within the Burra population, mite prevalence between enclosures during October 2016 were also compared using Fisher's exact test (two-tailed), Both tests used a significance threshold of 0.05 and were conducted with R version 3.4.4 (R Core Team, 2020).

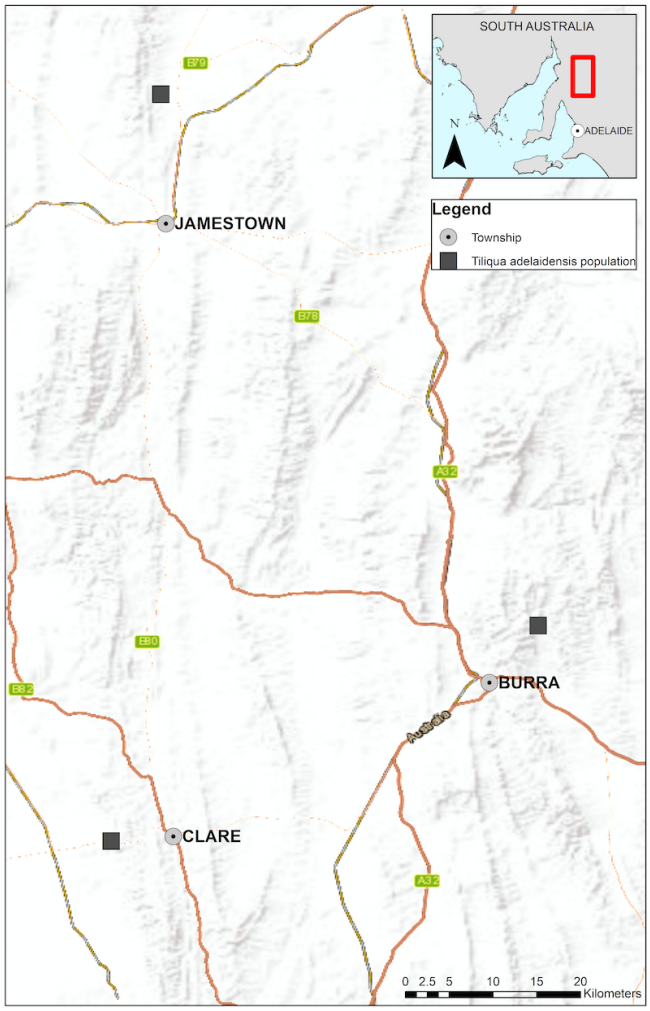

Figure 2.1. Locations of the three Tiliqua adelaidensis populations where *Ophiomegistus michaeli* sp. nov. mites were observed and sampled in the Mid North region of South Australia. Figure: B. Derne

Results

Description of the new mite species

Ophiomegistus Banks, 1914

Ophiomegistus Banks, 1914: 58.

Type species *Ophiomegistus luzonensis* Banks, 1914, by original designation.

***Ophiomegistus michaeli* Halliday sp. nov.**

Specimens examined. *Holotype* female, Mannanarie, 10 km north of Jamestown, South Australia, 33°03'S 138°59'E, 7 October 2016, on *Tiliqua adelaidensis*, pasture, B. Derne coll. (ANIC 51-006376). *Paratypes*, 3 females (ANIC 51-006377, ANIC 51-6378, ANIC 51-006379), 1 male (ANIC 51-006380), same data as holotype; 1 female (ANIC 51-006381), 1 male (ANIC 51-006382), same data as holotype except February 2016; 2 females (ANIC 51-006383, ANIC 51-006384), 1 male (ANIC 51-006385), same data except 15 October 2016; 3 females (ANIC 51-006386, ANIC 51-006387, ANIC 51-006388), Tiliqua property, Nature Foundation of South Australia, 8 km north-east of Burra, South Australia, 33°37'S 138°59'E, 15 October 2016, on *Tiliqua adelaidensis*, pasture, B. Derne coll.; 1 male (ANIC 51-006389), same data except February 2016.

Adult female

Dorsal idiosoma (Figure 2.2a). Dorsal shield oval, wider than long, length 651 (622–655), width 794 (752–823), completely covering idiosoma, uniformly ornamented with a faint pattern of fine crescentic cells. Surface of shield with about 60 minute setae, barely visible even at high magnification, and about 20 minute circular pores; arrangement of setae and pores uneven and asymmetrical. Margins of shield with 20 pairs of erect setae increasing in length and thickness from anterior (18) to posterior (90). These setae interspersed with 10 pairs of much longer setae (130), these appearing wavy in slide-mounted specimens, all setae smooth and pointed.

Ventral idiosoma (Figure 2.2b). Base of tritosternum with an expanded basal disc and elongate slightly roughened stalk (length 80–90), laciniae 105 long, densely covered with short spines. Jugular plates rectangular, with a strongly sclerotised diagonal ridge, two pairs of short pointed setae and one pair of circular pores. Soft integument behind jugular shields with a row of four small sclerotised platelets. Sternal shields approximately trapezoidal, anterolateral margin concave, with a strongly sclerotised curved anterolateral ridge, a smaller curved posterolateral ridge, four pairs of short pointed setae and one pair of small circular pores. Sternogynial shield divided longitudinally into two triangular plates, each slightly wider than long, width 100, length in midline 90, posterior margins with a strongly sclerotised ridge, surface with polygonal ornamentation throughout, each with a pore in the outer corner. Anterior margin of latigynial shields slightly sinuous, lateral margin concave, both with a strongly sclerotised parallel ridge,

posterior margin sinuous, without a sclerotised ridge, each shield with 9–11 short setae and a small circular pore. Metapodal shields crescentic, each with 3–4 setae in posterior half, and a row of 5–6 sclerotised cup-shaped invaginations along the posterior margin. Mesogynial-ventrianal shield large, length 340, width 390, surface smooth, its antero-lateral margins strongly concave. Shield with 34–44 short pointed setae in anterior and lateral regions, 54–75 flattened paddle-shaped setae in posterior region, and four pairs of pointed setae near the anus, post-anal seta absent. Peritrematal shields broad and sinuous, with an irregular row of sclerotised nodules along outer margin of peritreme. Unsclerotised integument between sclerites strongly striated, without setae.

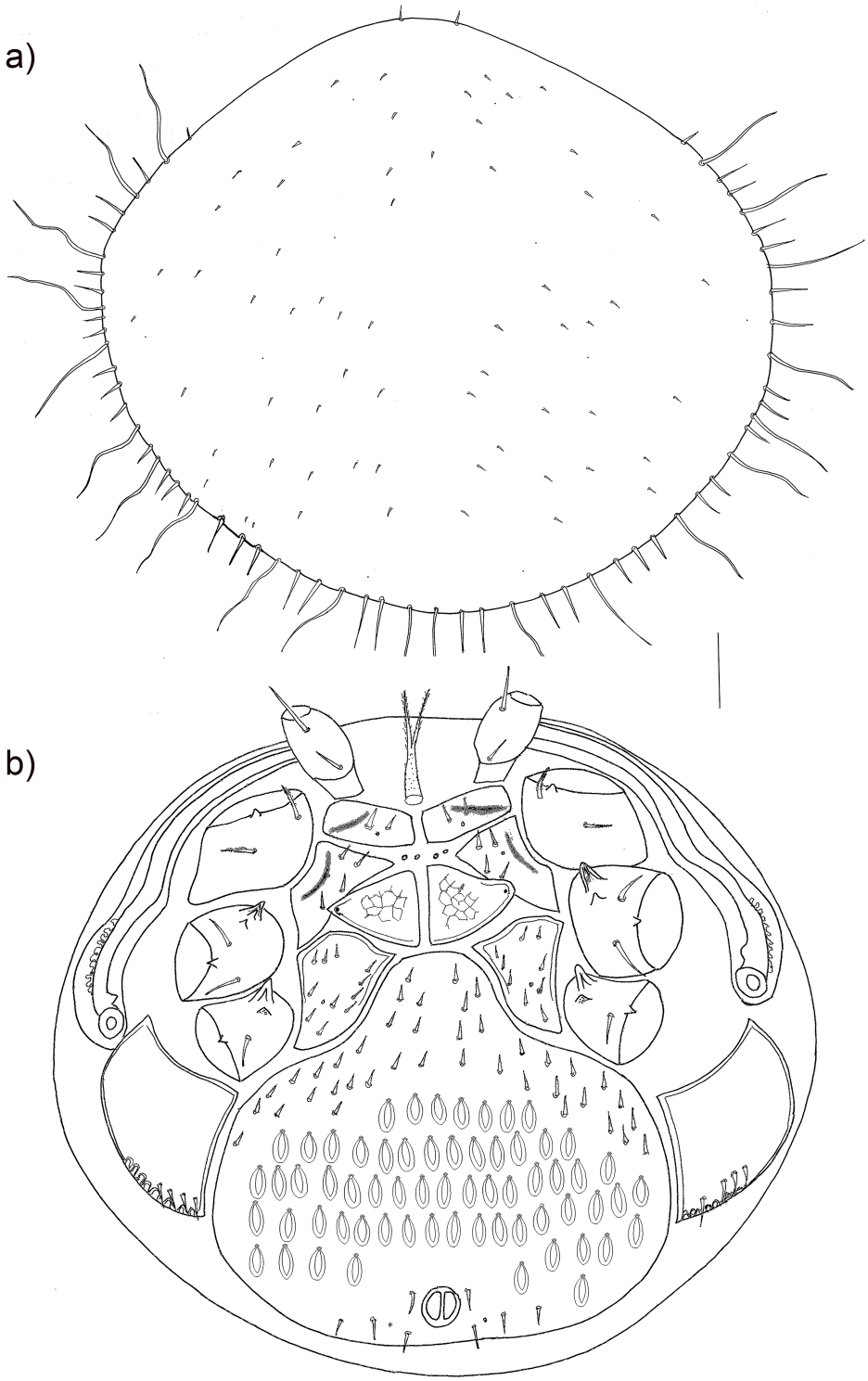

Figure 2.2. *Ophiomegistus michaeli* sp. nov., female. a) Dorsal idiosoma. b) Ventral idiosoma. Figure: B. Halliday

Gnathosoma. Hypostome (Figure 2.3a) with rostral setae *h1* very long (70), thick, finely spiculate, *h2* and *cx* shorter and finer (40), finely serrated, *h3* 60, thick, finely serrated. Deutosternal groove indistinct, with a few scattered denticles, flanked by three pairs of transverse lines. Corniculi broad and membranous, spiculate, internal malae very long and fine, lightly serrated. Hypostome enclosing a pair of large heavily sclerotised flask-shaped structures, apparently enlarged coxal glands with an opening at the anterior margin of the hypostome. Palp trochanter with two robust serrated setae, femur with five setae, all distally serrated, *pl* very long and conspicuous, *al* shorter; genu with six setae, all distally serrated, *pl* and *pd* very long and conspicuous; tibiotarsus terminating in a dense clump of sensory setae; palp tarsal claw 2-tined. Epistome broadly triangular, its margins sinuous (Figure 2.3b). Fixed digit of chelicera straight, edentate, length 75 (Figure 2.3c), dorsal lyrifissure distinct; dorsal seta 35 long, thick, prostrate, displaced ventrally. Movable digit straight, length 125, main shaft edentate, but with a membrane along its inner surface, membrane serrated and weakly sclerotised in proximal half, with a medial group of sclerotised teeth, distal one-third of membrane broad and smooth, with delicate serrations near tip.

Genital structures (Figure 2.3d). Genital opening underlain anteriorly by a pair of sclerotised bars, their outer ends fused to lateral corners of sternogynial shield, their inner ends connected by an undulating sclerotised bridge and embracing a pair of strongly sclerotised papillae. Anterior margin of mesogynial-ventrianal shield strongly sclerotised, arch-shaped, underlain by a complex sclerotised ridge surrounding a small oval-shaped genital operculum. Spermathecal structures not visible, most females containing two large eggs.

Legs. Leg I longest, 720, coxa and trochanter robust, femur–tarsus much thinner, antenniform; legs II–IV shorter and more robust. Chaetotaxy: Leg I: coxa 0 0/1 0/1 0, trochanter 2 1/1 01 1, femur 2 3/3 1/2 1, genu 2 3/1 3/1 1, tibia 2 3/2 2/2 2. Leg II: coxa 0 0/1 0/1 0, trochanter 1 0/2 0/1 1, femur 2 2/2 2/1 1, genu 2 3/2 3/1 1, tibia 2 2/2 2/1 1, tarsus 4 3/2 3/2 3 + *mv, md*. Leg III: coxa 0 0/1 0/1 0, trochanter 1 0/2 0/1 1, femur 1 2/1 2/1 0, genu 2 3/1 2/1 1, tibia 2 2/1 2/1 1, tarsus 4 3/2 3/2 3 + *mv, md*. Leg IV: coxa 0 0/0 0/1 0, trochanter 1 0/2 0/1 1, femur 1 2/1 2/1 1, genu 2 3/1 2/1 0, tibia 2 2/2 2/1 1, tarsus 4 3/3 3/3 3 + *mv, md*.

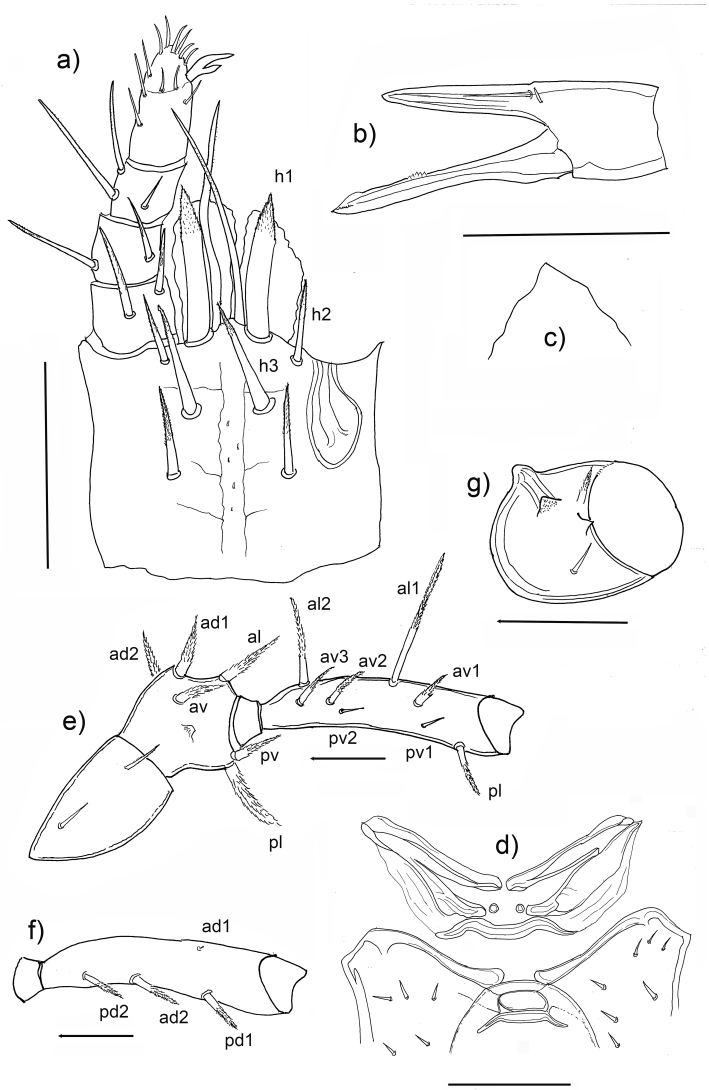

Figure 2.3. *Ophiomegistus michaeli* sp. nov., female. a) Hypostome. b) Chelicera. c) Epistome. d) Genital structures. e) Leg I, coxa, trochanter, femur, ventral aspect. f) Femur I, dorsal aspect. g) Coxa IV, ventral aspect. Figure: B. Halliday.

Adult male

Dorsal idiosoma. Dorsal shield length 580–600, width 660–685, structure and chaetotaxy as for female.

Ventral idiosoma. Jugular plates as for female, partly covering base of tritosternum. Sternal, sternogynial, latigynial and genito-ventrianal shields fused, genital opening at anterior edge of shield. Combined shields with 70–80 short pointed setae and 60–65 paddle-shaped setae, their arrangement irregular and asymmetrical (Figure 2.4a). Metapodal shields and peritremes as for female.

Gnathosoma. As for female, except chelicera with a long rod-shaped process extending from near the base of the fixed digit (Figure 2.4b).

Legs. As for female.

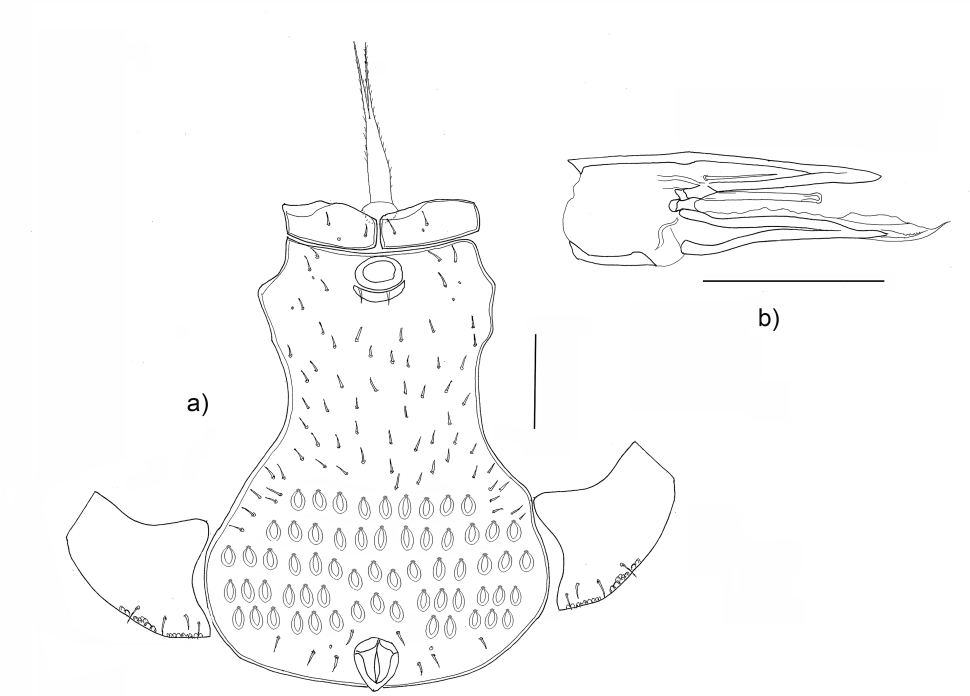

Figure 2.4. *Ophiomegistus michaeli* sp. nov., male. a) ventral idiosomal plates. b) chelicera. Figure: B. Halliday

Etymology

The new species is named in memory of our late friend and colleague Professor Mike Bull, in recognition of his outstanding contributions to herpetology and parasitology.

Diagnosis

Female metapodal plates each with 3–4 simple pointed setae; mesogynial-ventrianal plate with 34–44 simple pointed setae and 54–75 expanded leaf-like setae. Male with separate jugular plates, each bearing two setae and a small circular pore.

Taxonomic notes

In the most recent key to species of *Ophiomegistus* (Goff, 1980), *O. michaeli* is most similar to *O. clelandi* and *O. australicus*, in having only simple setae on the metapodal plates, and both simple and expanded setae on the mesogynial-ventrianal plate.

Ophiomegistus michaeli can be distinguished from these two Australian species by the morphology of both the male and female.

Ophiomegistus clelandi Womersley, 1958 is known only from the male. In the male of *O. clelandi* the jugular plates are fused into a single large shield with one pair of setae and one pair of lyriform pores. In the male of *O. michaeli* the jugular plates are separate, and each bears two setae and a small circular pore.

Womersley (1958) described *O. australicus* from South Australia, and Domrow (Domrow 1978, 1984) recorded specimens under this name from Moura, Mount Windsor, and Tamborine Mountain, all in Queensland. Specimens from these four populations differ from each other, and may not belong to a single species, so we compare them with *O. michaeli* separately. Males from Mount Windsor and Tamborine Mountain have a single jugular shield with one pair of setae, which distinguishes them from the males of *O. michaeli*. The female from Moura illustrated by Domrow (1978) is distinctive in having 25 pairs of setae on the latigynial shields, compared with 8–11 in *O. michaeli* and 11 in *O. australicus*. The male from Moura illustrated by Domrow (1984 Figure 30) and Domrow (1988, Figure 174) is excluded from our concept of *O. australicus*, and appears to belong to an undescribed species.

The type specimens of *O. australicus* cannot be located, but Womersley's illustration shows 84 expanded paddle-shaped setae on the mesogynial-ventrianal shield. We also examined three females from Flinders Island, South Australia, which we provisionally identify as *O. australicus*. These specimens have 87–102 paddle-shaped setae on the mesogynial-ventrianal shield. Our specimens of *O. michaeli* have only 54–75 such setae.

The legs of *O. michaeli* show some distinctive modifications. Tarsus I has a dense clump of distal sensory setae, five of which are long, thick, and usually twisted; the pre-tarsus is absent. Tarsi II–IV lack claws, but have a large pad-like membranous pre-tarsus. Most leg setae are thick and distally pilose. The chaetotaxy of leg I is difficult to interpret, because the leg is usually twisted and distorted. On femur I, setae *al1* and *al2* are very long, and pilose in their distal half; *av1*, *av2*, *av3*, *pl1*, *pd1*, *pd2* are short thick, and distally pilose; *pv1* and *pv2* are fine, short and smooth, and *ad1* is minute (Figures 2.3e, 2.3f). Coxae III and IV each have a large heavily sclerotised medially-directed spur on the antero-medial corner of the segment. The ventral surface of these coxae bears a small domed protuberance with a stippled surface. These two structures are connected by an internal duct, possibly of glandular function (Figure 2.3g). On tarsus IV, setae *av3* and *pv3* are inserted in a small intercalary sclerite between the basitarsus and telotarsus.

Host attachments

Adult mites were observed wedged under the ventral scales of *T. adelaidensis* hosts, as in *Ophiomegistus* spp. in other reptiles (Klompen & Austin 2007). The mites were often concentrated around the neck and pectoral regions (Figure 2.5), and around the pelvic region and the base of the tail. Observed mite loads ranged from 1–15 on a given host animal at one time. Although mite load was not formally quantified, only the Jamestown population sampled had mite loads exceeding ten mites per lizard. To our knowledge, this mite has not been recorded from any other host species.

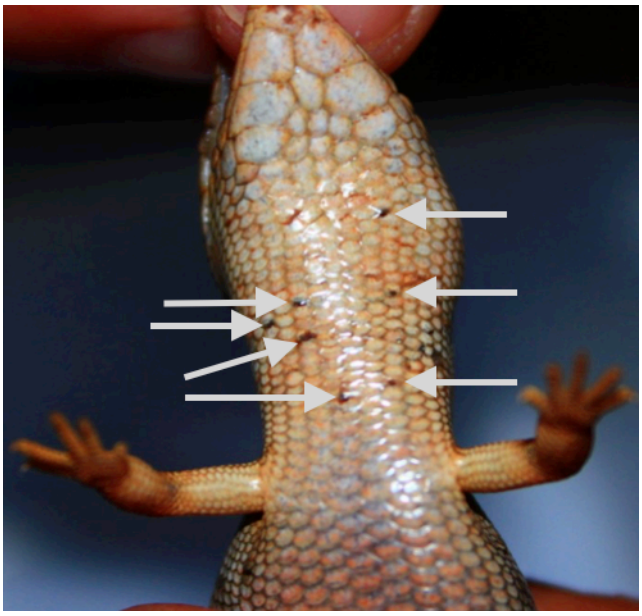

Figure 2.5. Infestation of *Tiliqua adelaidensis* by adult *Ophiomegistus michaeli* sp. nov. Note: Arrows indicate the location of mites. Photo: L. Clive

Prevalence

Prevalence of *O. michaeli* mites on *T. adelaidensis* hosts appears to vary both between populations, within populations and also temporally. In February 2016, a prevalence of 4.17% (2/48) was observed for lizards within the Burra population, compared with 18.18% (2/11) in the Clare population, and 46.15% (6/13) in the Jamestown population (Figure 2.6). In pairwise comparisons using Fishers exact test, only the Burra and Jamestown populations differed significantly in prevalence ($p<0.001$), whilst the three-factor Fisher's exact test was not significant ($p=0.53$).

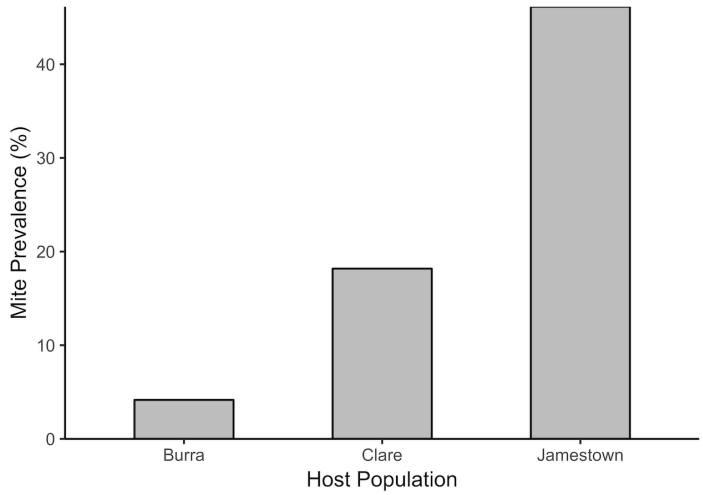

Figure 2.6. Prevalence of *Ophiomegistus michaeli* sp. nov. in three isolated populations of *Tiliqua adelaidensis* sampled in February 2016. Note: Number of lizards sampled were 48, 13 and 11 for Burra, Clare and Jamestown populations respectively.

Within the same enclosed subset of the Burra population, mite prevalence varied between months over two activity seasons without clear temporal trends (Figure 2.7). However, there was a decrease in prevalence between October and November 2016, from 41.18% (7/17) to 7.14 % (1/14). In contrast, within five other enclosures located 0–500m from this enclosure, containing similar numbers of *T. adelaidensis* that were sampled concurrently, the prevalence was 0% in 57/60 monthly enclosure samplings over the two activity seasons. During October 2016, where the aforementioned enclosure had a high mite prevalence of 41.18%, differences among mite prevalence in enclosures were significant (p=0.029).

Fifteen lizards in the Burra population infested with mites at one time point were recaptured over successive months. Seven of these 15 lizards did not apparently retain mites after initial infestation, whilst eight lizards were infested with mites at one or more later time points. All of these subsequent records of infestation were observed after an inactivity period of six cooler months (during which sampling was not conducted). The number of months between the first recorded instance of infestation on a lizard and the last ranged from 7–20 months (mean ± SD = 10 ± 4.24), though mites were only recorded in two or more consecutive months on three lizards.

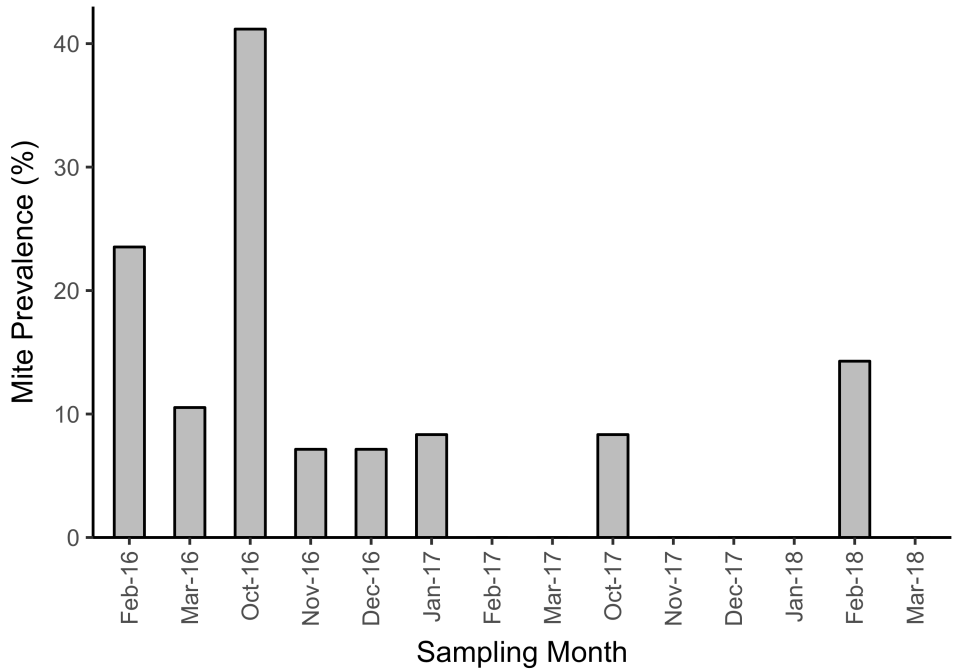

Figure 2.7. Prevalence of *Ophiomegistus michaeli* sp. nov. mites over time within a group of 6–17 *Tiliqua adelaidensis* individuals within a 30 m x 30 m enclosure in a wild population near Burra. Notes: Mites were first observed in February 2016. Monthly sampling was conducted during the host activity seasons from October to March, and not conducted during the austral winters between April and September.

Discussion

We aimed to help address the lack of documentation on parasite diversity and their role in threatened species conservation management by describing a new parasitic mite species of the endangered skink *T. adelaidensis*. Observations of *O. michaeli* thus far indicate that it may be host specific and that its prevalence may be variable in both time (Figure 2.7) and space, within and between host populations (Figure 2.6). The ecological significance of a new parasite species on an endangered host is further explored by our following speculation on how the host's biology and the environment shape its life history and prevalence. We also consider the fitness implications for a host, which is entering a phase of active conservation management, and question the mite's long-term persistence in light of a its reliance on an endangered host.

Host-parasite relationship

Parasites are generally adapted to exploit their host's biology, and mites that are closely associated with a single host species often have lifecycles synchronised to that of their host (Hunter & Rosario 1988). One of the defining characteristics of *Tiliqua adelaidensis*'s biology is its close association with spider burrows as refuges and ambush points for prey (Milne et al., 2003). A lizard spends the majority of its time in or immediately adjacent to its home burrow, defending it from takeover by conspecifics (Fenner & Bull 2011), and usually occupies the same burrow for months at a time (Milne, 1999). The stable, protected microenvironment of burrows may provide optimal conditions for the mite to carry out its lifecycle, and also for the adult stage to attach to the occupant lizard. Nothing is known about the immature stages of *Ophiomegistus* mites, though they are thought to be free-living (Klompen & Austin 2007). We speculate that the stable, protected microenvironment of burrows may provide optimal conditions for immature stages of *O. michaeli* to develop, and also for the adult stage to attach to the occupant lizard. Concordantly, the observation that some *T. adelaidensis* hosts showed infestation by adult mites over several, although not necessarily consecutive, months suggests that successive mite generations may infest the same individual.

The use of host burrows by the mites during non-parasitic stages of their lifecycle may also enable horizontal transmission within a host population. Good quality, sufficiently deep and vertical burrows are a limiting resource within a *T. adelaidensis* population, and high occupancy of deep burrows suggests take-over by new lizards if they become unoccupied (Fellows et al., 2009). A given burrow can be occupied by different lizards over time (unpublished data), providing the opportunity for a mite to attach to a different host than its parent. Tick transmission is associated with refuge sharing with conspecifics in related host species. For *Tiliqua rugosa,* the amount of asynchronous overnight refuge sharing an individual engaged in was positively correlated with tick load (Leu et al., 2010). Similarly, *Egernia stokesii* individuals which asynchronously sheltered in rock crevices used by more conspecifics were more likely to have ticks than individuals who sheltered in crevices used by a smaller number of conspecifics (Godfrey et al., 2009). Asynchronous burrow sharing in *T. adelaidensis* may similarly increase the risk of mite infestation.

Horizontal transmission of mites may also occur during close contact between lizards. Mating, which is promiscuous within seasons (Schofield et al., 2014), would be the main instance of

contact between *T. adelaidensis* adults. Ectoparasite transmission during mating has been proposed as likely in *Hemidactylus mabouia* geckoes with *Geckobia* mites (Rivera et al., 2003), and occurs for lice in ring-necked pheasants and mites in ladybirds (Hillgarth, 1996; Ryder et al., 2007). If *O. michaeli* survives in the grassland environment, spatial proximity to infected individuals may be sufficient for transmission, as is the case for the reptile tick *Bothriocroton hydrosauri,* an opportunistic parasite of *T. adelaidensis*. Lizards in burrows closer to conspecifics were more likely to be infested with tick larva than individuals living further away from conspecifics (Fenner et al., 2011).

Host specificity

Most *Ophiomegistus* species have been recorded on a single host species, though at least four species have been recorded on two or more species (Domrow 1978; Klompen & Austin 2007). Therefore, *O. michaeli* may parasitise other skinks or snakes co-occuring with *T. adelaidensis*. Putative host species include larger congeners *T. rugosa* and *T. scincoides*. The former is well-studied in the region, though they have large scales under which mites could wedge themselves unnoticed. Sympatric snake species include *Parasuta spectabilis* and the commonly observed *Pseudonaja textilis*, which preys on *T. adelaidensis*. It attempts to capture lizards by inserting its head and upper body into their burrows (Fenner et al., 2008), providing potential transmission opportunities for mites.

Ophiomegistus spp. adults live partially wedged under scales of their scincid or snake hosts (Klompen & Austin 2007) (Figure 2.5.). Host suitability may therefore depend on scale size, explaining why multiple hosts for a given mite species are less commonly observed. Neonate *T. adelaidensis* lizards, which share burrows with their mothers for one to five weeks following birth (Milne et al., 2002), have been extensively sampled at the Burra site and mites have not been observed on them. Apparent lack of infestation despite probable transmission opportunities may indicate that the small scale size of neonates does not present favourable host attachment sites for the adult mites. Similarly, mites have not been found on extensively surveyed sympatric small skinks, primarily *Menetia greyii* (SVL 38mm). Elucidation of genetic structure within and between mite populations may indicate how use of other host species, or lack thereof, is likely to have contributed to the observed patterns.

Spatial and temporal patterns

Prevalence of *Ophiomegistus michaeli* mites on *T. adelaidensis* appears variable, both between populations 40–80 km apart (Figures 2.1 & 2.6), and at a fine spatial scale of several metres. Further sampling of different host populations, or different subsets within the same population is required to further how prevalence varies over space. In other systems, mite prevalence and load on lizards may vary between different habitats within a locality. Habitat structure created by vegetation, such as leaf litter, seems to be an important determinant since it may affect exposure to desiccation or potential hosts and certain life stages (Talleklint-Eisen & Eisen 1999; Schlaepfer 2006; Corti *et al.* 2009; Ramirez-Morales *et al.* 2012). In addition to its influence on vegetation, climatic differences between localities also drive variation in abundance and prevalence by the direct effects of humidity, precipitation and temperature on the mites (Eisen et al., 2001; Klukowski, 2004; Krasnov et al., 2008; Zippel et al., 1996).

All three sites at which *O. michaeli* mites occur consist of mesic grassland with similar habitat structure. The Burra and Clare sites are dominated by wild oats (*Avena barbata*) and spear grass (*Austrostipa* sp.), whereas wallaby grass (*Austrodanthonia* sp.) is more common at the Jamestown site. Mean annual temperatures and humidity levels are similar between locations, though the Clare locality has a higher average annual precipitation (558 mm) than Burra (446 mm) and Jamestown (366mm). If, as we hypothesise, the more stable microclimate of burrows is important for mite survival, then external environmental variations may be less important in shaping local abundance and prevalence. Rather, soil type may drive fine scale spatial heterogeneity in mite abundance, since the water potential of the soil will influence burrow humidity levels, and possibly mite development. Alternatively, the restriction of mites to localised patches within a host population may reflect a transmission ecology based on host-host interactions (such as burrow sharing or mating) rather than dispersal into the environment.

In addition to spatial variation, the prevalence of mite infestation within the same group of *T. adelaidensis* appeared to vary over the spring-summer activity season, and also between activity seasons (Figure 2.7). Mite load of lizards quantified in several studies accordingly varies both within and between seasons by mite species, host and location (Goldberg & Bursey 1991; Bull & Burzacott 1993; Talleklint-Eisen & Eisen 1999; Eisen *et al.* 2001; Klukowski 2004; Lumbad *et al.* 2011). Changes in factors such as temperature and moisture levels over time, and space, drives mite

abundance in the environment, which often reflects prevalence and load of lizard hosts (Eisen et al., 2001; Klukowski, 2004). For *O. michaeli*, environmental conditions may be less of a constraint on abundance if their free-living stages shelter in more climatically stable burrows. Mite loads can also fluctuate due to seasonally-driven host factors such as activity levels, use of microhabitats, and testosterone levels (Eisen et al., 2001; Pollock et al., 2012). In the Burra *T. adelaidensis* population, decreases in mite prevalence between October and November 2016 (Figure 2.7) coincided with increased host shedding, which may play a role in reducing mite load in the short term.

Effect on the host

Infestation by *O. michaeli* mites does not visibly affect body condition in *T. adelaidensis*. However, minor scale damage often occurs on infested lizards, which may predispose them to secondary infections (Elkan & Cooper 1980). Haematophagous mites presumably impose fitness costs to their reptile hosts, which may have shaped host characteristics over evolutionary timeframes. Notably, the presence of specialized skin invaginations in several lizard species where trombiculid mite larvae attach (Arnold, 1986), and the maintenance of sexual reproduction by some populations of the Australian gecko *Heteronotia binoei* alongside parthenogenic conspecifics (Moritz et al., 1991) may minimise mite load and fitness costs borne by the host. However, clear direct effects on reptile host fitness by parasitic mites are lacking (Fajfer, 2012). The relatively few existing studies examining effects of mite ectoparasites on lizard fitness in wild populations report a range of often subtle and negative effects, though are biased towards the orders Prostigmata (e.g. trombiculid mites or chiggers) and Ixodida (ticks), rather than Mesostigmata (e.g. Sorci & Clobert 1995; Main & Bull 2000; Schlaepfer 2006; Barnett *et al.* 2018). Fitness costs of parasites may not be evident unless measured in a specific way, and in a large enough proportion of the population (Scott, 1988).

In contrast to wild populations, the fitness effects of mites are well known for captive reptiles. The snake mite *Ophionyssus natricis* is the most commonly reported species in snakes and lizards, causing debility, aenemia, dermatitis, behavioural changes and pathogen transmission (Wozniak & De Nardo 2000). Captive animals may be more severely affected by parasites than wild conspecifics for various reasons (Dickens et al., 2010; Kock et al., 2010; Spielman et al., 2004). The difference between the effects of parasitic mites on captive and wild reptile populations has implications for the conservation of *T. adelaidensis*, since the translocation of wild individuals into

both other wild populations and captivity are currently being evaluated. Understanding how *T. adelaidensis* is affected by their parasites under equilibrial conditions is necessary for identifying and mitigating any parasite-induced fitness costs associated with both management activities and wild populations at immediate risk of extirpation.

Co-extinction

The discovery of this new mite species on an endangered host species with an uncertain future raises the issue of co-extinction. Though often unnoticed, parasites that become extinct with their host are likely to account for much of the contemporary biodiversity loss that is occurring globally (Strona, 2015). Counter-intuitively, parasites may indirectly benefit their host and play an important role in the stability of their ecosystem. The benefits of parasites may operate at various spatial and temporal scales and include the maintenance of genetic diversity (Nunn *et al.* 2004; Sommer 2005), of ecological functioning (Hatcher et al., 2006); and of pathogen regulation (Strona, 2015). Recognition for parasites as an important component of biodiversity and functioning ecosystems is growing. For instance, the IUCN states that with appropriate risk assessment, recovery plans for a species should include the restoration of its parasites (IUCN/SSC, 2013).

We propose that this new mite species is at risk of extinction since it appears to be host-specific and rare in an endangered host with a fragmented population structure. Furthermore, climate and anthropogenic induced environmental change may lead to altered host behaviours which compromise a parasite's ability to find hosts and persist (Strona, 2015). Extinction risk may be further compounded if this mite species is arrhenotokous (as some mesostimatid mites are), by virtue of reduced allelic diversity typical of small populations (Zayed & Packer 2005). This point underlines the general need to understand parasite life cycles and population dynamics in order to properly assess their co-extinction risk (Strona, 2015).

Conclusion

In this study we identified and explored a model system at risk of co-extinction. The loss of such a system would jeopardise opportunities to increase our knowledge of this mite species and the wider genus. The extensive existing knowledge on and continued study of the host's biology should be capitalised upon to elucidate possible transmission mechanisms and other aspects of

the host-parasite relationship. In order to mitigate against future co-extinction and possible loss of ecosystem function, our case study highlights the need to understand both how parasites affect host fitness under various conditions, and how parasites respond to host population decline. Future work on the host-parasite relationship between *T. adelaidensis* and *O. michaeli* may contribute to not only optimising conservation strategies for the host, but also addressing the lack of documentation and empirical study of co-extinctions (Dunn et al., 2009).

Acknowledgements

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Chapter 3

Mixing in moderation: allopatric macroparasites are genetically differentiated and minimally transmitted following population augmentation of an endangered lizard

Note to examiners

The work presented in this chapter builds on the description and initial observations of the mite *Ophiomegistus michaeli* provided in the previous chapter and also on previous work by others on the gut nematode *Pharyngodon wandillahensis* to provide the first exploration of population genetics of these two species. I then use this genotyping to trace transmission of mites and nematodes from different population origins within the multi-lineage *T. adelaidensis* created by the experimental translocation, and attempt to identify a mode of transmission for these parasites. My examination of inter-population genetic variation and how translocation changes this in *T. adelaidensis* has been strengthened by comparing and contrasting the two macroparasite species, one an ectoparasite and one an endoparasite. I therefore chose to present this thematically and methodologically similar study of each parasite species in the same chapter.

Abstract

Translocations are necessary for mitigating biodiversity decline as they involve moving threatened organisms to more suitable habitat. However, pathology and loss of host fitness arising from parasites is a potential risk in translocations, particularly when hosts are stressed and exposed to novel parasites. Conversely, translocating parasites with their hosts may also be desirable since parasites can promote ecosystem function and may also require conservation. Genetic characterisation of parasites can help better elucidate host-parasite relationships, informing both disease risk assessment in wildlife translocations and parasite conservation. In this study, we examine genetic variation in parasites among isolated host populations of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). We also determined the extent and mechanism of transmission of non-local parasites after three host populations were combined in a wild-wild population augmentation. Genome-wide single nucleotide polymorphism (SNPs) markers were identified in two apparently host-specific macroparasites; the mite *Ophiomegistus michaeli* and the nematode pinworm *Pharyngodon wandillahensis*. Both ordination-based and Bayesian cluster analysis revealed genetic structure based on host population in these species; population structure was particularly evident for the nematode. Hosts mostly retained parasite genotypes congruent with their origin for two years following the translocation, though exceptions suggest transmission did occur over time. To determine modes of transmission, estimated relatedness between parasites on different hosts was compared to connectedness of respective hosts in networks based on proximity, asynchronous habitat use and potential mating events. None of these putative transmission mechanisms were clearly correlated with parasite relatedness, though this may have been due to small sample sizes. The minimal and slow nature of detected transmission of non-local mites and nematodes between translocated and resident host lizards suggests that these parasites are unlikely to pose a risk to hosts in a translocation context.

Introduction

Wildlife translocations are an increasingly used conservation tool as the world's biodiversity is threatened by habitat degradation and climate change (Armstrong and Seddon, 2008; Fischer and Lindenmayer, 2000; Fordham et al., 2012). In order to maximise chances of translocation success, the effects of parasites and pathogens need to be considered and adequately researched (Hartley and Sainsbury, 2017; IUCN/SSC, 2013). Transmission of parasites and microbial pathogens to susceptible hosts may occur in either direction between translocated animals and their recipient ecosystem. These infections may potentially result in pathology and reduced survival, therefore jeopardising conservation gains (Daszak et al., 2000; Kock et al., 2010). In the case of existing mild infections, conditions created by translocation may disrupt the previous host-parasite-environment equilibrium and produce more severe pathology and higher transmission rates (Aiello et al., 2014; Dickens et al., 2010; Lebarbenchon et al., 2006; Tompkins et al., 2011).

While parasites present a potential risk for translocated animals and their recipient communities, the paradigm that they are purely harmful for wildlife is proving to be an oversimplification. Growing evidence suggests that they are important, if understudied, components of functional ecosystems (Dunn et al., 2009; Kuris et al., 2008; Lafferty et al., 2006). To this effect, translocating parasites with their hosts may help preserve ecosystem function and evolutionary potential, and thus augment translocation success in the long term. For endangered hosts, host species-specific parasites are also likely to be threatened and themselves have intrinsic conservation value (Derne et al., 2019; Dunn et al., 2009; Strona, 2015), and should be translocated with their hosts if adverse effects are found to be unlikely following rigorous testing (IUCN/SSC, 2013). Avoiding further biodiversity loss during host-parasite translocations requires a deeper understanding of parasite diversity, and the host-parasite relationship under translocation conditions (Northover et al., 2019, 2018) — as well as how parasites are affected by host decline (Strona, 2015).

Determining how host-parasite relationships are affected by translocation requires the identification of parasites, and the tracing of their transmission between the introduced individuals and the recipient community following translocation. For population augmentations (reinforcements) or reintroductions involving multiple founder populations, this necessitates the ability to distinguish between conspecific parasites from allopatric source populations. Genetic markers provide a powerful tool for these purposes (Archie et al., 2009).

Single nucleotide polymorphisms (SNPs), neutral genome wide genetic markers, have become widely used for both model and non-model organisms with the expansion of high-throughput Next-Generation DNA sequencing (NGS) technology (Andrews et al., 2016) and enable confident population assignment of wildlife (e.g. Jenkins et al., 2019). For species-specific parasites with limited dispersal, genetic structure can be greater for the parasite (Cole and Viney, 2018; Falk and Perkins, 2013; Fricke et al., 2010; Mazé-Guilmo et al., 2016). As such, parasite genotyping can even be a useful method of identifying a host's provenance and better understanding the demographic history of both parasite and host populations, which can inform conservation strategies of both species (Carlson et al., 2020; Criscione et al., 2006; Whiteman and Parker, 2005).

A number of recent studies have genotyped bacteria to trace intra-population transmission (e.g. Balasubramaniam et al., 2019; Blyton et al., 2014; Bull et al., 2012; Proboste et al., 2019; VanderWaal et al., 2014) and others have genotyped macroparasites for transmission tracing within animal populations (e.g. Dharmarajan et al., 2010; Fricke et al., 2010; Lahmar et al., 2004; Neal et al., 2016). The use of SNP markers for parasite genotyping appears limited in the field of wildlife parasitology, being more common in parasites and vectors of human health significance (e.g. Amambua-Ngwa et al., 2019; Campos et al., 2017; Diawara et al., 2013) . Similarly, whilst there are studies looking at parasite communities or bacterial prevalence in translocations (e.g. Baling et al., 2013; Northover et al., 2019), there have been few studies using species-level parasite genotyping in the context of translocations, despite the potential of this approach (but see Grange et al., 2017, 2016).

In this study we conducted a wild-wild experimental population augmentation for an endemic, endangered scincid lizard, the pygmy bluetongue (*Tiliqua adelaidensis*) with the aim to examine interpopulation parasite differences and post-translocation transmission. This lizard species is restricted to fragmented populations within a small region of southern Australia (Fenner et al., 2018; Hutchinson et al., 1994) with low but significant genetic differentiation between isolated populations, particularly between southern and northern population groups more than 70 km apart (Smith et al., 2009b). Whilst anthropogenic activities have severely fragmented the grassland habitat of this species (IUCN, 1996; Lunt, 1998), the inter-population genetic differentiation observed may predate modern settlement (Smith et al., 2009b). Low vagility is presumed to drive genetic structure observed over distances greater than 400 m in even continuous habitat (Schofield et al., 2014; Smith et al., 2009b). The pygmy bluetongue is further threatened by climate change, making translocations a potential conservation strategy

(Fordham et al., 2012). This experimental population augmentation provides the first translocation in this species in a wild setting, and affords us the opportunity to elucidate parasite dynamics between hosts of different population origins when they are united by translocation.

Background

There are two macroparasite species for which *T. adelaidensis* is the only known host; an oxiuroid gut nematode, *Pharyngodon wandillahensis* and the paramegistid mite, *Ophiomegistus michaeli* (Derne et al., 2019; Fenner et al., 2008). Neither the nematode *P. wandillahensis* nor the mite *O. michaeli* have obvious fitness costs to their host (Derne et al., 2019; Fenner et al., 2008; Smith et al., 2009a), though these costs may be subtle and as yet unmeasured, as has been observed in related host-parasite systems (Fenner and Bull, 2008), or potentially may be increased in animals stressed by translocation or resource shortage (Benítez-Malvido et al., 2019; Dickens et al., 2010). Furthermore, understanding transmission of known parasites may also inform effective management of future disease outbreaks by pathogens. Transmission of *P. wandillahensis* presumably occurs by direct or indirect ingestion of faeces containing nematode eggs (Adamson, 1989), and prevalence within a population has been found to be 0- 34% (Fenner et al., 2008; Smith et al., 2009a). Fenner et al (2011) reported that individuals which had burrows close to 'disperser' individuals, i.e. ones that stayed in the study area for less than two months, had higher nematode prevalence than other lizards, and the authors hypothesised that the observed tongue-flicking at scats of unfamiliar individuals for social information (Fenner and Bull, 2011, 2010) may provide a transmission pathway. Prevalence of *O. michaeli* in *T. adelaidensis* varies considerably among populations and even within habitat patches less than 100 m, as well as with time of year (Derne et al., 2019). The non-adult life stages and transmission mechanisms of *O. michaeli* and congeners remain completely uncharacterised, though *T. adelaidensis* burrows may provide suitable conditions for any free-living life stages to persist and for new hosts to become infested (Derne et al., 2019).

Aims and hypotheses

In the likely absence of dispersal by other hosts, the parasites *Pharyngodon wandillahensis* and *Ophiomegistus michaeli* may be genetically differentiated between isolated host populations, as are their *T. adelaidensis* hosts. This genetic differentiation could be used to show the degree to which transmission of originally allopatric parasites occurs over time when *T. adelaidensis*

hosts from different populations become sympatric in the context of a population augmentation. Estimating the extent of allopatric parasite transmission and likely modes of transmission in a mixed host population would allow us to comment on whether any future population augmentations of this species are likely to be adversely affected by parasite transmission, and whether control measures may be necessary. To this end, our aims were to:

a) Determine if there is genetic differentiation between conspecific parasites based on population of origin.

b) Use any inter-population genetic differences to identify any transmission of allopatric parasites when allopatric hosts populations and their parasites are combined in a population augmentation.

c) Determine whether putative mechanisms of transmission — spatial proximity, refuge sharing and mating — were associated with the transmission patterns observed.

Methods

Translocation experiment and sample collection

A full description of experimental set-up and translocation that this study is based on is available in the introductory chapter of this thesis, and also in Clive et al. (2020). Briefly, three pairs of 30 m x 30 m enclosures were erected around established *T. adelaidensis* individuals in a wild population on a livestock grazed grassland approximately 8 km north east of Burra township, South Australia in July 2015 (Figure 3.1). One enclosure in each of the three pairs was designated as a control enclosure, whilst the other enclosure in the pair was designated as an experimental enclosure. In February 2016, 11 adult or subadult individuals were captured from another wild *T. adelaidensis* population west of Clare township (approximately 45 km south-east from the Burra site) and 13 individuals were captured from a wild population north of Jamestown (approximately 70 km north-west). Upon initial capture, any faecal pellets excreted were collected, and 1–2 mites were also collected when visually detected. For all infested lizards, 1–2 mites were also left in situ. These individuals were translocated to the Burra site and were released into artificial dowel burrows in the experimental enclosures, amongst the established Burra resident conspecifics. Each of these three 'mixed' experimental enclosures contained 11–18 resident Burra individuals, 3–4 translocated Clare individuals, and 4–5

Jamestown individuals. The three control enclosures each contained 13–23 Burra residents only; no translocated individuals were added to these.

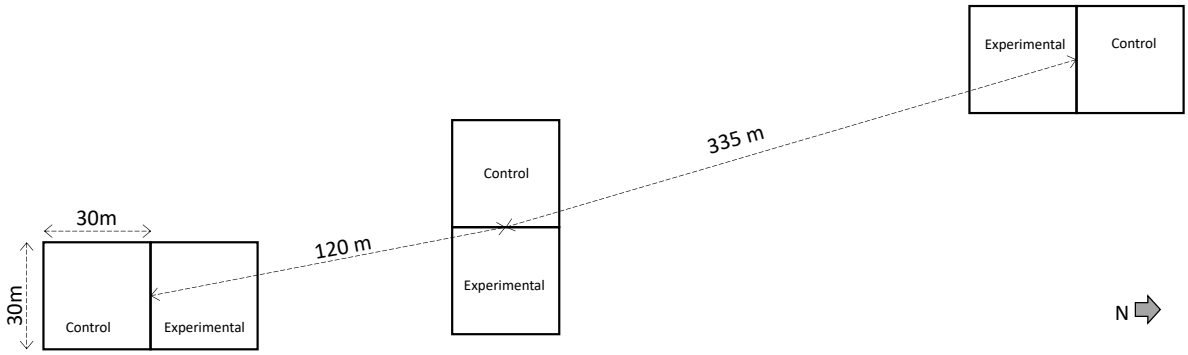


Figure 3.1. Schematic representation of 30 m x 30 m enclosures containing *Tiliqua adelaidensis* individuals that were part of the experimental population augmentation at the release site near Burra, South Australia. Notes: 'Control' enclosures contained non-translocated Burra resident lizards only, 'Experimental' enclosures contained non-translocated Burra residents, amongst which translocated individuals from Jamestown and Clare populations were later released. Distances are not drawn to scale.

Over one fortnight per month from October 2015–March 2016, October 2016–March 2017 and from October 2017–March 2018, the capture of all individuals in the six enclosures was attempted by identifying lizard-occupied burrows and luring lizards out with tethered bait (Milne, 1999). Typically, less than 10% of lizards in an enclosure remained uncaught for each month. Each burrow detected in an enclosure by thorough visual examination was given a set of coordinates based on a meter squared grid. Individual lizards were identifiable by the specific toe clip sequence given at first capture (toes were retained for DNA analysis as part of another study). Upon each monthly capture, the burrow coordinates of each individual were recorded, and a scat was collected if a lizard defecated in the approximately 10 minutes it was held. From February 2016 onwards, each lizard captured at Burra was also examined for adult mites. If mites were evident, at least one mite was collected from an individual per capture

occasion. Both scats and mites were immediately placed in 100% ethanol which was stored at 4°C from the end of each fieldwork day.

In addition to mites and nematodes from Burra resident lizards and the translocated lizards involved in the experimental population augmentation, mites and nematodes were also collected from non-translocated hosts in the Jamestown population between October 2016 and March 2018. Further sampling at the Clare population was not possible due to lack of on–going access to the private property. Between January and March 2018 nematodes were also collected from a *T. adelaidensis* population, which was not involved in the experimental translocation, located south of the Peterborough township, approximately 63 km north of the Burra population and 25km east of the Jamestown population.

After 1–6 months of storage in ethanol, each lizard scat was examined under a dissecting microscope and adult and subadult nematodes were separated from the other material and stored in 100% ethanol. Nematodes had the same macroscopic appearance (colour, shape, size range) and were assumed to be *Pharyngodon wandillahensis*, the only nematode species known be found in the gut of *Tiliqua adelaidensis*. Each individual mite and nematode had a host, location and date of collection associated with it. Subsets of 120 individual mites and 189 nematodes were selected for genotyping. These mites and nematodes represented all occasions where an infested host was captured in an enclosure at Burra, as well as most mites and nematodes from the Jamestown and Peterborough.

Single nucleotide polymorphism identification and analysis

The laboratory and data analyses are described in further detail in the supplementary material for this chapter, and are briefly outlined here. Extraction, digestion and sequencing of DNA from whole nematodes and mites was conducted using protocols developed by Diversity Array Technology Pty Ltd (DArTseq) (Kilian et al., 2012). Single nucleotide polymorphisms (SNPs) identified underwent a series of filtering steps carried out with the R package 'dartR' (Gruber et al., 2018).

Three analytical approaches were used to examine how mites and nematodes from various population origins were grouped according to genetic similarity: principal coordinates analysis (PCoA), discriminant analysis of principal components (DAPC), and Bayesian cluster analysis carried out with STRUCTURE. Genetic similarity between individual parasites from different

populations was examined using PCoA (Gower, 1966) in dartR. Another non-model based method for grouping individuals based on genetic similarity (which maximises between-group variation rather than the inter-individual variation of PCoA) was applied by performing cluster identification and DAPC with the R package 'adegenet' (Jombart et al., 2008).

The software STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to conduct Bayesian model-based cluster analysis, as an alternative method to examine whether or not SNP genotypes clustered by host population of origin, and to identify any evidence of allopatric parasite transmission between hosts of different origins within the translocation. The most likely number of groups was identified using the Evanno method (Evanno et al., 2005) in STRUCTURE HARVESTER (Earl and VonHoldt, 2012) after examination of the log-likelihood plot to ensure the real number of groups was not one. The estimated membership probabilities of different clusters for each individual were identified and visualised using CLUMPAK (Kopelman et al., 2015). When two or more likely clusters were identified by STRUCTURE, each cluster was further hierarchically analysed using the steps described above in order to identify all clusters rather than just those at the highest level of hierarchy (Coulon et al., 2008; Evanno et al., 2005; Janes et al., 2017).

Relatedness

Relatedness between mite individuals and between nematode individuals from the same lizard hosts (intra-host relatedness) and from different hosts (inter-host-relatedness) was calculated from SNP genotypes. Relatedness between all mite dyads and all nematode dyads in the datasets were reduced to a set of dyads between mites and nematodes found on hosts in the same enclosures.

Mite relatedness was calculated using the Dyad Maximum Likelihood estimator (Milligan, 2003), implemented by the program COANCESTRY 1.0.1.9 (Wang, 2011). This estimator was selected because it showed the highest correlation with the simulated dataset (Wang, 2011). The difference between mean relatedness of two groups (e.g. inter-host relatedness vs. intra-host relatedness on mites within a given enclosure was tested by bootstrapping in COANCESTRY.

Nematode relatedness was calculated using the Ritland's estimator of relatedness with Huang's correction (Huang et al., 2015). This was implemented in the program POLYRELATEDNESS 1.8

(Huang et al., 2014) in order to account for haplodiploidy exhibited by oxyurid nematodes (Adamson, 1989). Mean relatedness between two groups of three nematode dyads or more were compared by bootstrapping carried out in the R environment (R Core Team, 2020).

Network analysis

To investigate potential transmission mechanisms for *Pharyngodon wandillahensis* and *Ophiomegistus michaeli* among *Tiliqua adelaidensis* individuals, social networks which linked hosts within enclosures based on various measures of interaction were compared to networks based on pairwise relatedness of parasite individuals between hosts. Parasites on different hosts that were highly related to each other may indicate direct transmission by the putative transmission mechanism. Parasite relatedness networks included all hosts within an enclosure (for enclosures where there was a sufficient number infested lizards) from which a genotyped parasite had been collected over the 2.5 years of the field study. Using the 'igraph' package in R (Csardi and Nepusz, 2006; R Core Team, 2020), response variable networks were constructed where nodes consisted of hosts within an enclosure from which a genotyped parasite had been collected over the study, and edges were weighted by relatedness between their most highly related nematode or mite pair.

A series of predictor variable networks relating to lizard host interactions were constructed to examine if these were associated with parasite relatedness networks. These networks included ones where weighted edges reflected average inter-burrow distance between two hosts over the course of a season, networks where edges reflected asynchronous burrow sharing between a host pair, and networks where male lizards and female lizards were connected to each other as potential mated pairs. Each predictor variable network was compared to the response parasite-relatedness networks with multiple regression quadratic assignment procedure (MRQAP) with double semi partialing (DSP), (Dekker et al. (2007). This method for matrix regression of network data was implemented with the R package 'asnipe' (Farine, 2013; R Core Team, 2020).

Results

Ophiomegistus michaeli

DArTseq sequencing produced genotypes for 118 of 120 *Ophiomegistus michaeli* mite individuals, consisting of 6,736 binary SNPs with 42.7% missing data. Filtering steps resulted in 102 genotypes, with 458 SNPs and 10.68% missing data. Filtering for minor allele frequencies of less than 1% for relatedness analysis reduced the number of SNPs to 442 loci.

The genotype-based grouping patterns that were revealed by PCoA, DAPC and STRUCTURE analysis allowed us to determine whether or not genetic differences existed between mites of different host population origin. Population-based genetic structure was evident, which then allowed us to determine whether or not hosts sharing habitat with conspecifics from different population origins acquired parasites from a non-local origin following translocation.

The ordination from the PCoA of *O. michaeli* genotypes, with individual mites as entities and SNP loci as attributes, yielded 30 informative dimensions from 101 original dimensions. The total amount of variance explained by axis 1 was 13.1%; axes one and two combined explained 17.7%, whilst axes 1–3 combined explained 23.4% of the total variance (Supplementary material: Figure S3.1). The PCoA representing *O. michaeli* genotypes showed a distinct clustering of mites collected from Jamestown-originating hosts along PCoA axis one (explaining 13.1% of the variance) (Figure 3.2). Jamestown-originating hosts included both lizards translocated from Jamestown and released with conspecifics of other origins at the experimental enclosures in Burra ('Jamestown mixed'), and untranslocated Jamestown ('Jamestown') lizards at the Jamestown site. Twelve mites belonging to the 'Jamestown' group were collected from the Jamestown-originating translocated lizards immediately prior to translocation, and were grouped with mites from the untranslocated 'Jamestown' group at this timepoint. Following the translocation, mites collected from these translocated lizards were denoted as 'Jamestown mixed'. Most of these 'Jamestown mixed' mites were collected from these same host individuals two weeks to nine months following translocation, though two mites from this group were collected from a Jamestown-originating host 20 months after the translocation.

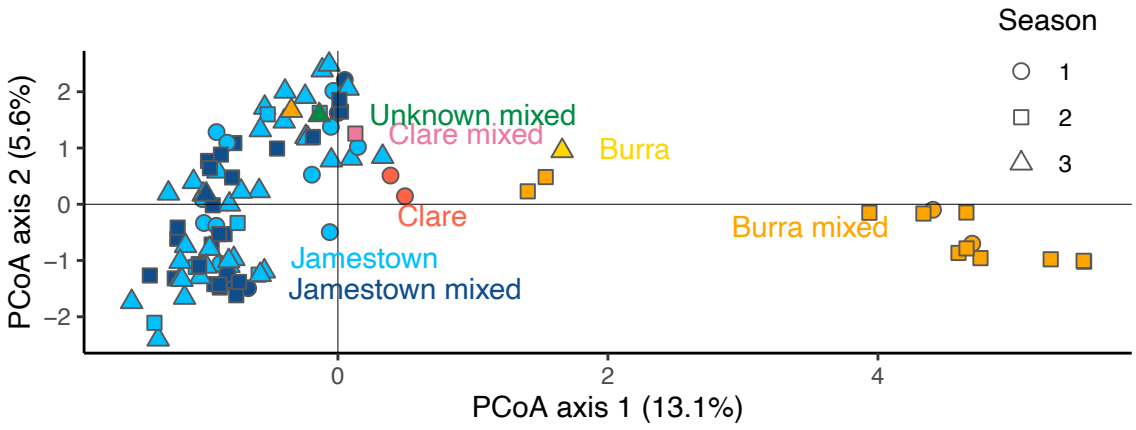


Figure 3.2. Principal Coordinates of Analysis plot representing genetic variation in 102 *Ophiomegistus michaeli* mites over three spring-summer seasons. Notes: Seasons are denoted by point shape, with colours corresponding to host population group. The single 'Burra' individual (shown in yellow) is from a Burra resident lizard in a control enclosure not exposed to translocated conspecifics. 'Jamestown' individuals (shown in pale blue) were collected from Jamestown lizards that were either not translocated to Burra, or translocated to Burra after parasite collection. 'Clare' mites (shown in red) were sampled from a Clare host immediately prior to translocation to Burra. 'Clare mixed' (pink), 'Jamestown mixed' (dark blue) and 'Burra mixed' (orange) refers to mites from experimental enclosures following the translocation, where lizard hosts from three populations were present. 'Unknown mixed' (shown in green) is one individual found in an experimental enclosure where host was not recorded.

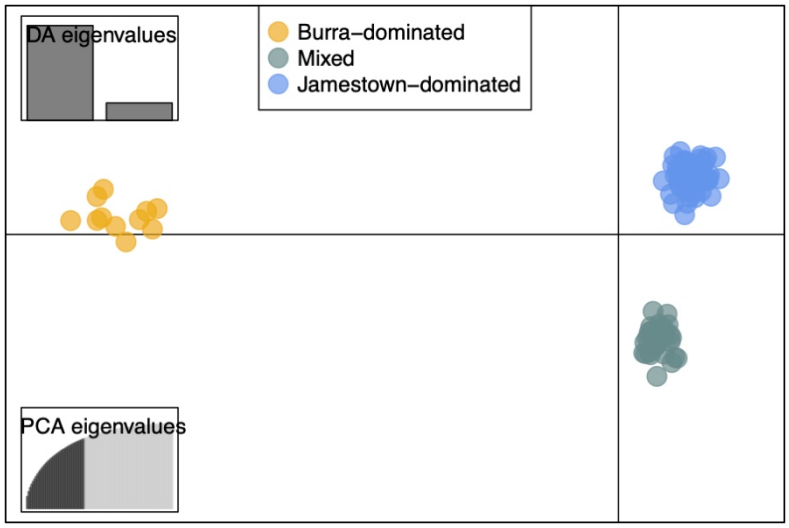
All the mites taken from Jamestown-originating translocated hosts fell within the same cluster regardless of whether they were collected in Burra or Jamestown, and regardless of the time period (Figure 3.2). The mites collected from the only infested Clare host immediately prior to translocation, and those from six Burra lizards did not cluster as distinctly along the axis 1 (13.1% of variance), though do show less spread along axis 2 (5.6% of total variance) (Figure 3.2). All 'Burra mixed' mites were collected from Burra residents the same 30m x 30m experimental enclosure (containing hosts of mixed population origin) clustered together, with the exception of two mites from one host individual, which are more similar to a mite

collected from the adjoining control enclosure (containing Burra resident-only). The two Clare mites are intermediate to these three Burra mites and the Jamestown cluster. The one mite collected from a Clare-originating translocated lizard ('Clare mixed'), 12 months after translocation, appears to fall into the Jamestown cluster. One mite collected from a Burra host in a mixed, experimental enclosure does not group with the other Burra mites, and falls within the Jamestown cluster. This mite was collected 20 months following translocation.

Cluster identification was conducted prior to DAPC of all *O. michaeli* SNP genotypes collected between the month of translocation and two years following it. All 101 principal components were retained (Supplementary material: Figure S3.2), and K=3 (BIC= 320.42) was chosen as the optimal number of clusters (Supplementary material: Figure S3.3). The BIC was the second lowest (k=4, BIC=320.23) but it changed only slightly with between K=3 and K=5. For the DAPC, the most informative 40 PCs and all 2 discriminant functions (k-1) were retained.

The DAPC revealed the three identified clusters to be distinct from one another, with a considerably greater distance in ordination space between one cluster than between the other two (Figure 3.3a). Cluster membership of individual mites showed some congruence with membership of groups based on location and/or the host's population of origin (Figure 3.3). The majority of mites collected from both Jamestown hosts and Jamestown-originating hosts after translocation belonged to the largest cluster, i.e. the Jamestown-dominated cluster. The majority of mites from Burra hosts belonged to the more distant Burra-dominated cluster. The remaining cluster, which was distinct from the Jamestown-dominated cluster though proximate to it, contained mites belonging to all host-based groups. The majority of mites in this mixed cluster were from Jamestown and Jamestown-originating translocated hosts, however it also contained the mites collected from Clare-originating hosts before and after translocation, two mites from a Burra host in an experimental enclosure with translocated lizards, and also the only genotyped mite from a Burra host in a non-mixed control enclosure. These clusters are largely consistent with those produced by PCoA (Figure 3.2), where 11 mites from Burra hosts in experimental, mixed enclosures group together in both analyses. However, the mites from Jamestown or Jamestown-originating hosts, which group in one larger cluster in the PCoA, are divided into two proximate clusters in the DAPC. The mites from Clare and Burra hosts which are proximate to the Jamestown-dominated cluster in the PCoA plot are grouped within the mixed cluster of the DAPC (Figure 3.3).

a)



b)

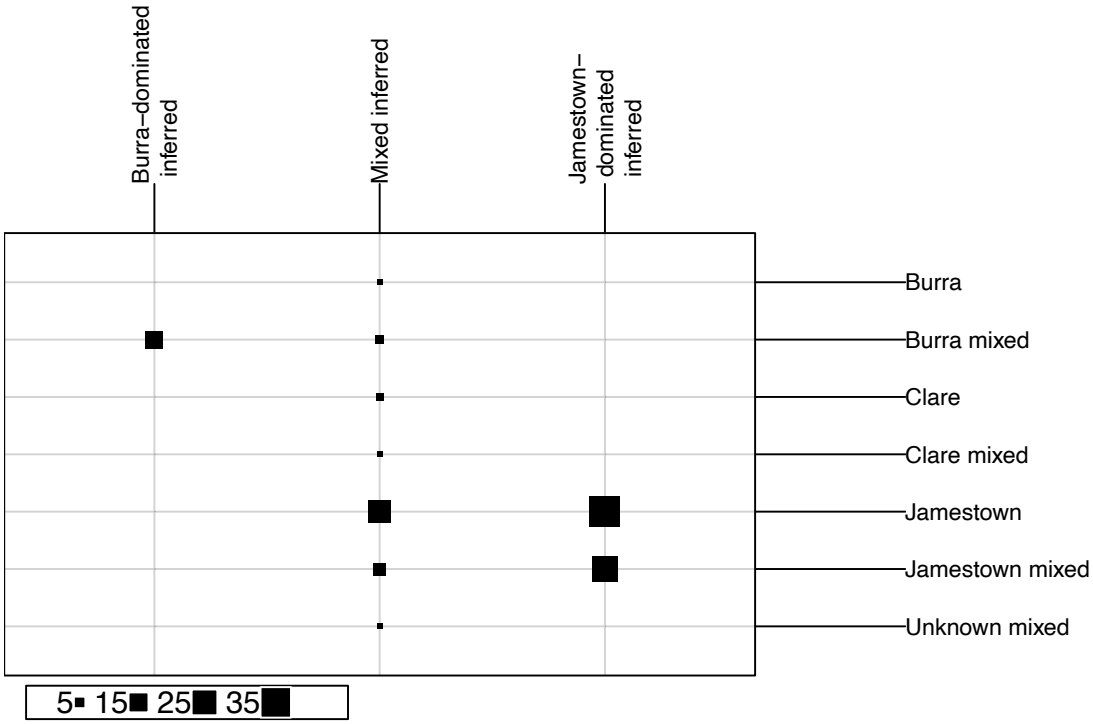


Figure 3.3. a) Discriminant Analysis of Principal Components (DAPC) of 102 genotyped *Ophiomegistus michaeli* mite individuals where k=3. Note: Cluster names indicate the population origin of the *Tiliqua adelaidensis* hosts**. b) Comparison of group membership based on host origin (rows) and the three clusters (columns) inferred by adegenet's cluster identification algorithm for 102 *Ophiomegistus michaeli* genotypes.** Note: Size of squares represents the number of mite individuals within the intersect of a given sampling group (based on host origin and treatment), and the cluster inferred by DAPC.

Three groups were identified from the initial STRUCTURE run of all 102 *O. michaeli* genotypes (Figure 3.4a), which is congruent with the PCoA and DAPC clusters (Figures 3.2 & 3.3). The largest subset (n=58, shown in Figure 3.4a in blue) and second-largest subset (n=28, shown in Figure 3.4a in purple) both consisted of mites collected from untranslocated Jamestown lizards in Jamestown, and translocated Jamestown-originating lizards in Burra. In addition to mites from Jamestown-originating hosts, the second-largest subset also contained a mite from a Burra host in an experimental enclosure (collected 20 months after translocation), one from a Clare host in an experimental enclosure (13 months after translocation), as also found by PCoA and DAPC analyses (Figures 3.2 & 3.3), and shown in Figure 3.5. The one mite collected from a Burra host in a non-mixed control enclosure 21 months after translocation had equal membership probability for this second-largest subset and the smallest subset (n=16, shown in Figure 3.4a in orange). This smallest subset largely consisted of mites that were collected from Burra hosts, though it also included the two mites collected from a host in the Clare population immediately prior to its translocation to Burra (Figure 3.4a).

At the next level of hierarchical STURCTURE analysis, the largest, Jamestown-dominated, subset (n=58) was separated into two most likely genetic groups, these both contained approximately equal proportions of mites from untranslocated and translocated Jamestown hosts (Figure 3.4b). That the Jamestown mites sampled cluster into two main groups regardless of translocation status is consistent with the Jamestown-dominated cluster and the mixed-inferred cluster from the DAPC (Figure 3.3).

The second largest STRUCTURE subset (n=28) was further split into four genetic groups (Figure 3.4c), which corresponded to members in the mixed inferred DAPC cluster (Figure 3.3), along with some of the members of the third STRUCTURE subset (shown in Figure 3.4d in orange). In this second STRUCTURE subset, mites from a Burra host and a Clare translocated host in an experimental, mixed enclosure grouped with a mite from a Jamestown translocated host in the same enclosure, an unknown host in an experimental enclosure (likely the same enclosure), and also with mites from a Jamestown untranslocated host (shown in Figure 3.4c in purple). Another group consisted only of mites from the same Jamestown translocated host at translocation and for 8 months after translocation (shown in Figure 3.4c in green). This same host also had a mite that grouped differently, with the largest group (n=10, shown in Figure 3.4c in blue), alongside two mites from another translocated Jamestown host and mites from an untranslocated Jamestown host. The fourth group (n= 6 shown in Figure 3.4c in orange) consisted of mites from three untranslocated Jamestown hosts collected at the same timepoint.

The third, Burra-dominated, STRUCTURE subset (n=16) was divided into two most likely genetic groups (Figure 3.4d) which reflected the grouping suggested by the PCoA analysis (Figure 3.2). The smaller of these groups included the mite from the Burra control enclosure, two mites from a Burra host in an experimental enclosure, and the two mites from the Clare population. The second of these groups consisted of the remaining mites collected from Burra resident hosts in the same mixed, experimental enclosure, consistent with the Burra-dominated inferred cluster of the DAPC (Figure 3.3).

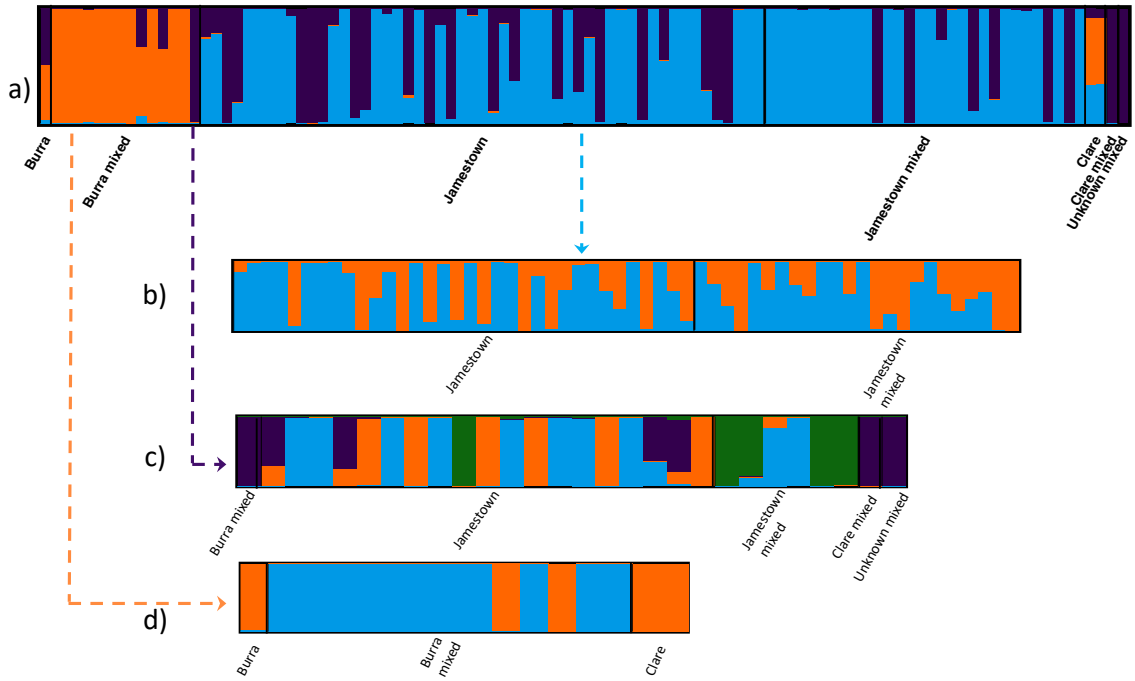


Figure 3.4. Cluster membership probabilities for genotyped *Ophiomegistus michaeli* mites assigned by STRUCTURE. Note: Labels indicate groups based on the *Tiliqua adelaidensis* host's population of origin and whether or host was in an experimental enclosure during the translocation ('Burra mixed', 'Jamestown mixed', 'Clare mixed', 'Unknown mixed') or was not translocated nor mixing with translocated conspecifics ('Burra', 'Jamestown', 'Clare'). a**) All genotyped individuals (n=102) are split into three most likely genetic groups** (k=3) (blue, purple and orange). **b) first subset** (n=58), (depicted in blue in a)) is further separated into two most likely groups (k=2) and; **c) second subset** (n=28), (depicted in purple in a)) is further separated into three most likely genetic groups (k=3) and; **d) third subset** (n=16) (depicted in orange in a)) is further separated into two most likely genetic groups (k=2).

Whilst these STRUCTURE subsets split individual mites at a finer resolution than the PCoA and DAPC (Figures 3.2 & 3.3), the three main clusters (Figure 3.4a) are consistent across analyses. Further evidence that mites from different populations are genetically differentiated was provided by comparison of relatedness of mites on hosts of the same origin vs. hosts of a different population origin within the experimental enclosures (Supplementary material: Figure S3.9).

Like the DAPC, subsequent levels STRUCTURE grouping also suggests a subset of individuals from different populations which are similar to each other (Figure 3.4c), and others that are more clearly from distinct clusters congruent with population origin of the host (Figures 3.4b & 3.4d). At further hierarchical levels of clustering analysis (not shown), sub-structure based on population of origin was not evident, as mites may have separated out into individual family groups.

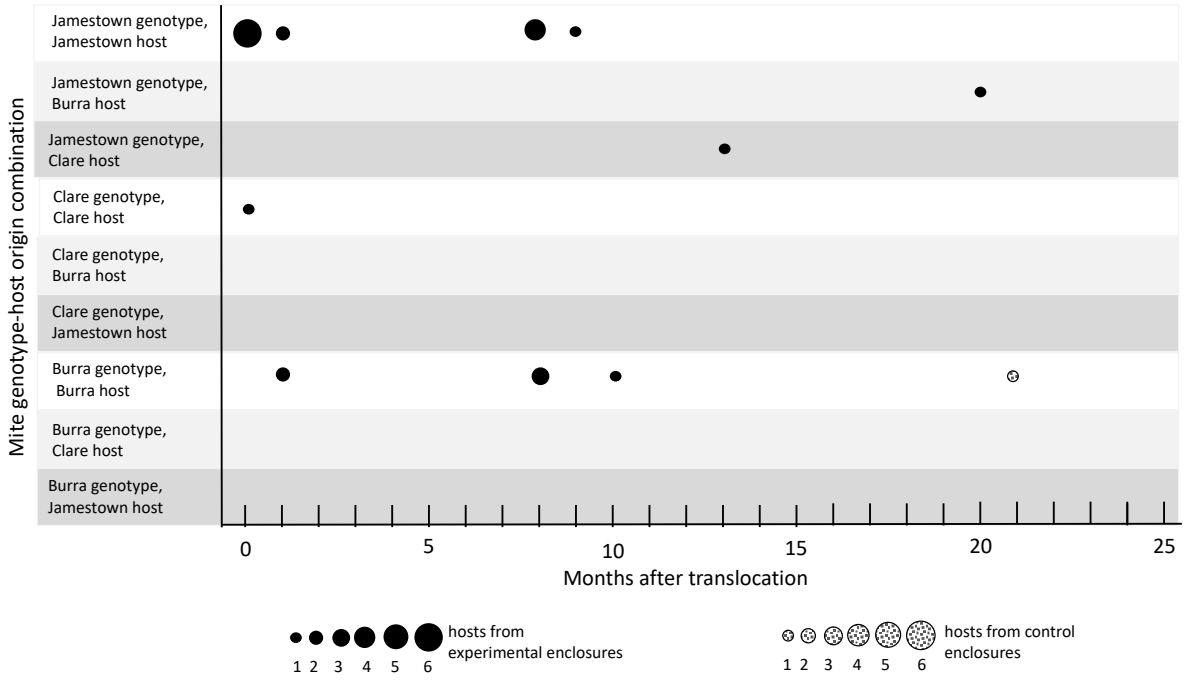


Figure 3.5. Summary of *Ophiomegistus michaeli* mites infesting *Tiliqua adelaidensis* hosts within experimental and control enclosures over time following translocation. Note: Infestation events are classified by inferred mite genotype-host origin combination.

Since grouping analyses suggested that some transmission of non-local mites was occurring following translocation (Figure 3.5), transmission mechanisms were investigated. To do this, the association between relatedness between mites on different hosts, and between hosts

networks reflecting three potential transmission mechanisms was examined. There was a significant, positive relationship between inter-host burrow proximity and the relatedness of their mites in one enclosure during the first spring-summer activity season (regression coefficient = 0.044, p=0.017). However, the distance between burrows predicted mite relatedness between hosts with low precision, as indicated by an adjusted r^2 value of 0.15. Burrow proximity during the first season in this enclosure was the only instance in which the association between mite-relatedness and burrow proximity could be examined, due to small sample sizes arising from low prevalence of mites, and lack of degrees of freedom in the MRQAP analysis precluding other models. Insufficient degrees of freedom also precluded the examination of any relationship between potential mates and inter-host mite relatedness, and also asynchronous burrow sharing and mite relatedness. In light of the lack of replication, the significant association between burrow proximity and mite relatedness may not be a reliable indication of a real effect.

Pharyngodon wandillahensis

Sequencing conducted by DArTseq produced 184 genotypes and 2,478 binary SNPs with 50.78% missing data for *Pharyngodon wandillahensis* nematodes. Filtering steps produced 147 genotypes with 395 SNPs and 3.18% missing data. Filtering for minor allele frequencies of less than 1% for relatedness analysis reduced the number of SNPs slightly, yielding genotypes with 358 loci.

As with the mite species, PCoA, DAPC and STRUCTURE analysis were used to determine whether *P. wandillahensis* nematodes collected from hosts of the same geographic origin were more genetically similar to each other than nematodes from non-local hosts. Exceptions to any such grouping patterns were also examined as evidence of transmission of non-local nematodes between hosts of different population origins following translocation. The ordination from the PCoA of *P. wandillahensis* genotypes yielded 34 informative dimensions from 146 original dimensions. The total amount of variance explained by axis one was 11.8%, axes 1 and two combined explained 21.7%, and axes 1–3 combined explained 26.7% of the total variance (Supplementary material: Figure S3.4).

The PCoA plot representing *P. wandillahensis* genotypes showed distinct clustering along both PCoA axis one (representing 11.8% of the total variance) and two (9.9% of variance) (Figure 3.6). Nematodes from Burra hosts, both from control and experimental, 'mixed' enclosures,

formed a distinct cluster. No nematodes from untranslocated Clare hosts were obtained, however nematodes from Clare-originating hosts, collected 1–19 months after translocation, also clustered together. The nematodes collected from Jamestown-originating translocated hosts ('Jamestown mixed'), collected 1–13 months after translocation, clustered with those collected from untranslocated 'Jamestown' lizards. The Jamestown cluster fell close in ordination space to the group of five nematodes genotyped from the Peterborough population, which was not involved in the translocation, but is the geographically closest population to Jamestown sampled in this study. These Jamestown and Peterborough nematodes were differentiated by variation in PCoA axis two rather than axis one. There were some exceptions to the host origin of these different genetic clusters. Seven nematodes within the Burra-dominated cluster were collected from three different Clare originating hosts, 9–20 months after translocation. Conversely, one nematode from the Clare cluster was found in a Burra host from an experimental enclosure 20 months after translocation. A further two nematodes that fell within the Jamestown cluster were found in a Burra host from a control (non-mixed) enclosure 22 months after the translocation.

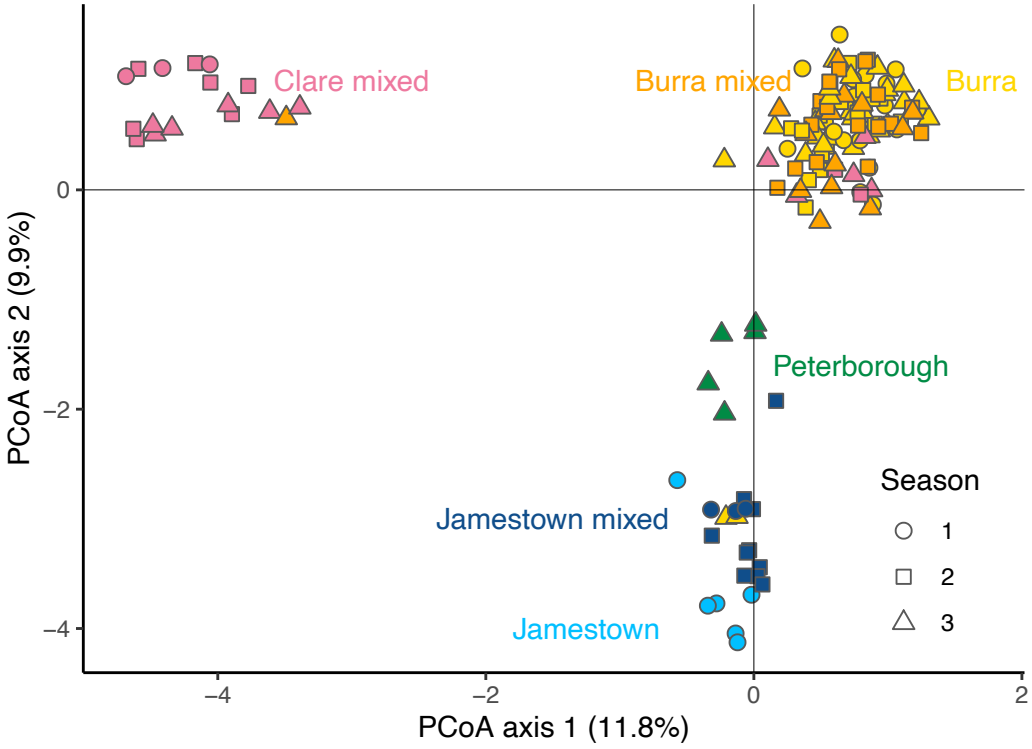
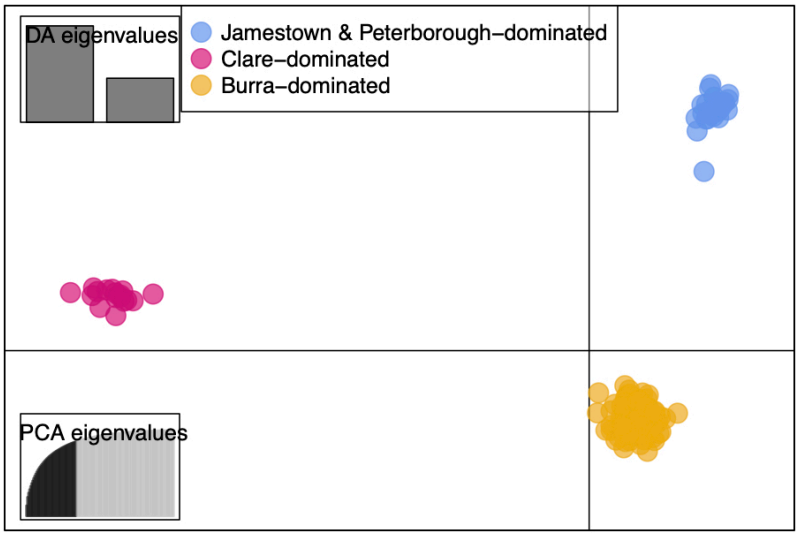


Figure 3.6. Principal Coordinates of Analysis plot representing genetic variation in 147 *Pharyngodon wandillahensis* nematodes over three spring-summer seasons. Notes: Seasons are denoted with by point shape, with colours corresponding to host population group. 'Burra' individuals (shown in yellow) are from Burra resident lizards not exposed to translocated conspecifics (either before the translocation and/or in control enclosures without translocated lizards). 'Jamestown' individuals (shown in pale blue) were collected from Jamestown lizards that remained in Jamestown, or that were translocated to Burra after parasite collection. 'Peterborough' nematodes (shown in green) were collected from the Peterborough population which was not involved in the translocation. 'Clare mixed' (pink), 'Jamestown mixed' (dark blue) and 'Burra mixed' (orange) refers to nematodes from experimental enclosures following the translocation where lizard hosts from three populations shared habitat.

All 146 principal components were retained for cluster identification preceding DAPC among *P. wandillahensis* genotypes (Supplementary material: Figure S3.5). A value of k=3 was selected, which corresponded to a BIC of 426.72 (Supplementary material: Figure S3.6). This BIC value was not the lowest, though changes in the BIC with increasing K were relatively small from k=3 onwards. The most informative 50 PCs and all 2 discriminant functions were retained for the DAPC (Supplementary material: Figure S3.5).

Nematodes grouped tightly within the identified clusters, which were well separated from each other in ordination space (Figure 3.7a). Inferred cluster membership was mostly associated with geographic origin of the host (Figure 3.7b). Nematodes from Burra hosts in both experimental ('Burra mixed') and control ('Burra') enclosures grouped together (Figure 3.7b). Similarly, the majority of nematodes from hosts translocated from Clare ('Clare mixed') formed a cluster, and nematodes from both translocated and untranslocated Jamestown ('Jamestown mixed' and 'Jamestown') hosts grouped with nematodes collected from the untranslocated Peterborough population (Figure 3.7b). The exceptions to host-origin and cluster congruence identified by the DAPC were consistent with the PCoA analysis (Figure 3.6); two nematodes from a Burra host were sorted to the Jamestown-dominated cluster, seven nematodes from Clare translocated hosts were in the Burra-dominated cluster, and one nematode from a Burra host was in the Clare-dominated cluster (Figure 3.7b).

a)



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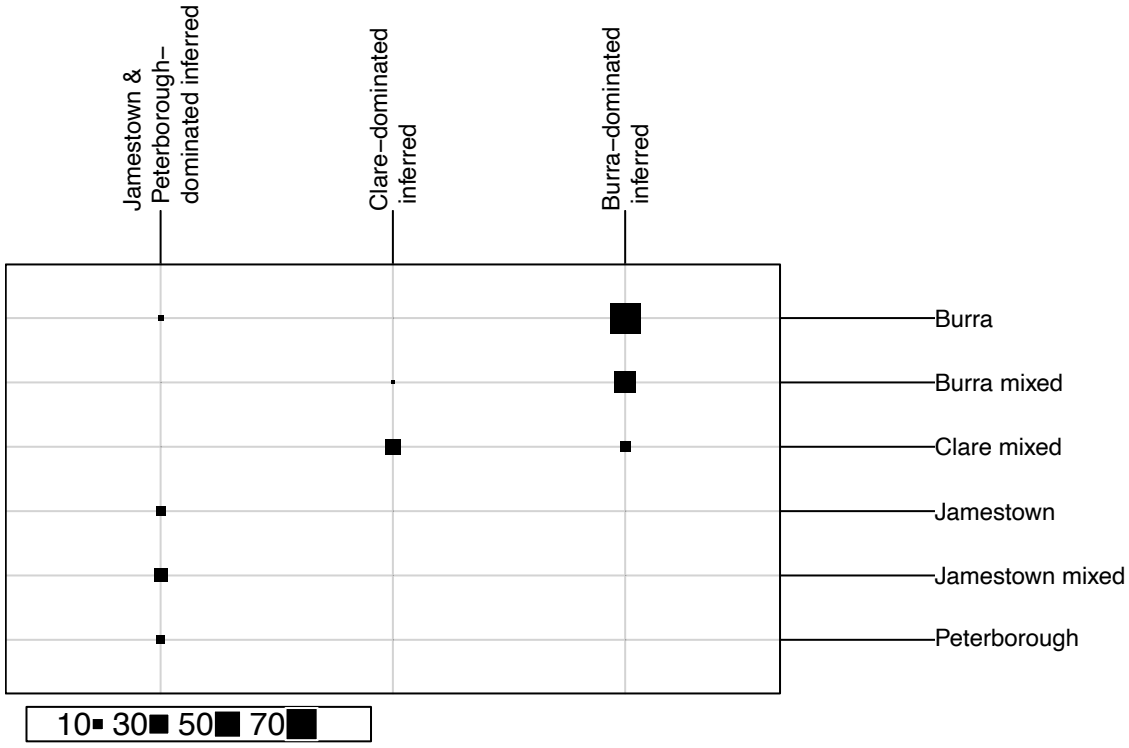

Figure 3.7. a) Discriminant Analysis of Principal Components of genotyped 147 *Pharyngodon wandillahensis* nematode individuals where k=3. Note: Cluster names indicate the population origin of the *Tiliqua adelaidensis* hosts. **b) Comparison of group membership based on host origin and the clusters inferred by adegenet's cluster identification algorithm for 147 *Pharyngodon wandillahensis* genotypes.** Note: Size of squares represents the number of individuals within the intersect of a given sampling group (based on host origin and treatment), and the cluster inferred by DAPC.

For comparison, the cluster identification and DAPC for *P. wandillahensis* genotypes was conducted using the same number of principal components but with a k value of 4 (BIC= 423.85). The difference between these two analyses was that the Burra-dominated cluster from the k=3 analysis (Figure 3.7a) was sub-divided into two overlapping clusters by the k=4 analysis (Supplementary material: Figure S3.7). Both of these overlapping clusters contained nematodes from Burra control, Burra experimental and Clare translocated hosts. The k=4 DAPC was deemed to be a less useful model than the k=3 DAPC on the basis of lack of differentiation between these two clusters and their similar host-based membership.

The initial STRUCTURE run of 147 genotyped *P. wandillahensis* nematodes yielded two groups (Figure 3.8a). The division between these two groups was between nematodes from Burra control hosts or Burra hosts from experimental, mixed enclosures (n=104, shown in blue), and those from hosts of other origins; Jamestown, Clare and Peterborough (n=43, shown in orange). This dichotomy was consistent with the Burra-dominated DAPC cluster and the Jamestown & Peterborough-dominated DAPC cluster (Figure 3.7a, b), whilst the Clare-dominated DAPC cluster corresponded with a Clare-dominated STRUCTURE cluster at subsequent levels of analysis (Figure 3.8c). Nematodes from both translocated Jamestown- and Clare-originating hosts tended to belong to this second cluster (Figure 3.8a). As in the PCoA and DAPC analyses (Figures 3.6 & 3.7), there were exceptions to this group membership; three nematodes collected from two Burra hosts 20-22 months following translocation belonged to the non-Burra-dominated cluster. Conversely, seven nematodes from Clare-originating translocated hosts did not cluster with others from the same host origin, but with the Burra-dominated group. These nematodes were collected from three hosts 9–20 months following the translocation. These apparent exceptions to group membership over time are summarised in Figure 3.9.

Subsequent analysis of the larger subset (n=104), which corresponded to the Burra-dominated cluster in the PCoA and DAPC (Figures 3.6 & 3.7), indicated further genetic division of nematodes from Burra and Clare originating hosts into three clusters (Figure 3.8b). All host groups (Burra control, Burra mixed and Clare mixed) contained nematodes that belong to these three likely clusters. All seven nematodes from Clare originating lizards in this Burra-dominated group were collected 9–20 months after the translocation.

Analysis of the smaller nematode STRUCTURE subset (n=43) shows clear clustering into two groups (Figure 3.8c); nematodes collected from untranslocated Jamestown hosts, Jamestown-

originating translocated hosts, and untranslocated Peterborough hosts fall into one group (shown in blue), and nematodes from Clare-originating translocated hosts into another (shown in orange). There are three nematodes from Burra hosts which provide exception to this clear geographic division: two nematodes found on a Burra host in a control enclosure 20 months after the translocation which groups with the Jamestown-dominated cluster, and a nematode collected from a Burra host in a mixed, experimental enclosure, collected 20 months after the translocation, which falls into the Clare-dominated cluster. These exceptions are also congruent with those identified by the PCoA and the DAPC (Figures 3.6 & 3.7) and are summarised in Figure 3.9.

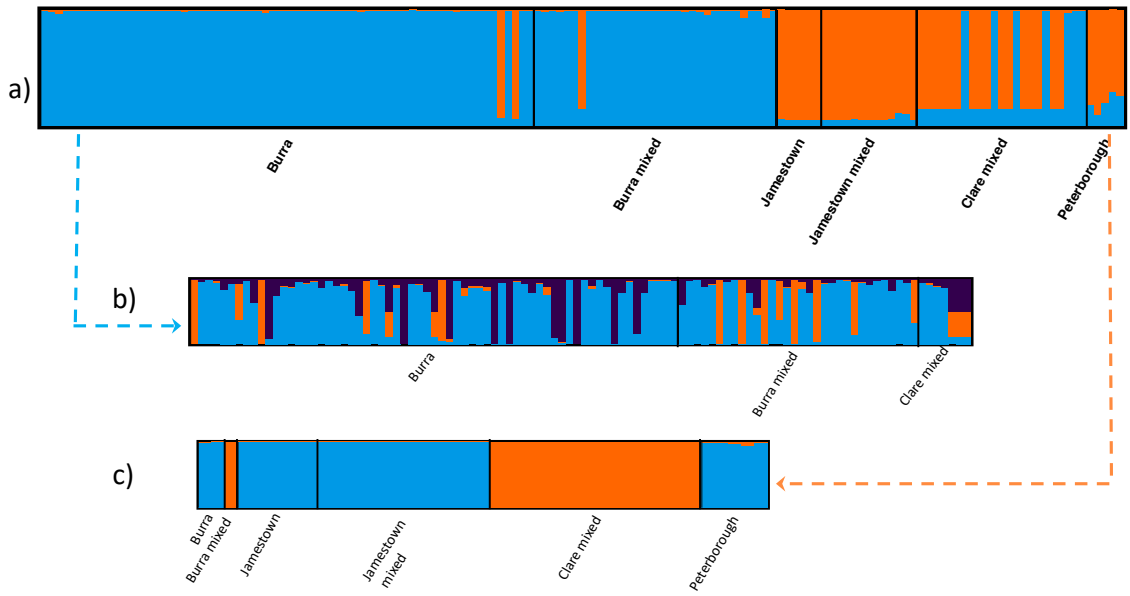

Figure 3.8. Cluster membership probabilities for genotyped *Pharyngodon wandillahensis* nematodes assigned by STRUCTURE. Note: Labels indicate groups based on the *T. adelaidensis* host's population of origin and whether or host was in an experimental enclosure during the translocation ('Burra mixed', 'Jamestown mixed', 'Clare mixed') or was not translocated nor mixing with translocated conspecifics ('Burra', 'Jamestown', 'Peterborough'). **a) All genotyped individuals n=147 are split into two genetic groups** (k=2) (blue and orange) b**) first subset** (n=104) (depicted in blue in a)) is further separated into three most likely groups (k=3) and; **c) second subset (**n=43) (depicted in orange in a)) is further separated into two most likely genetic groups (k=2).

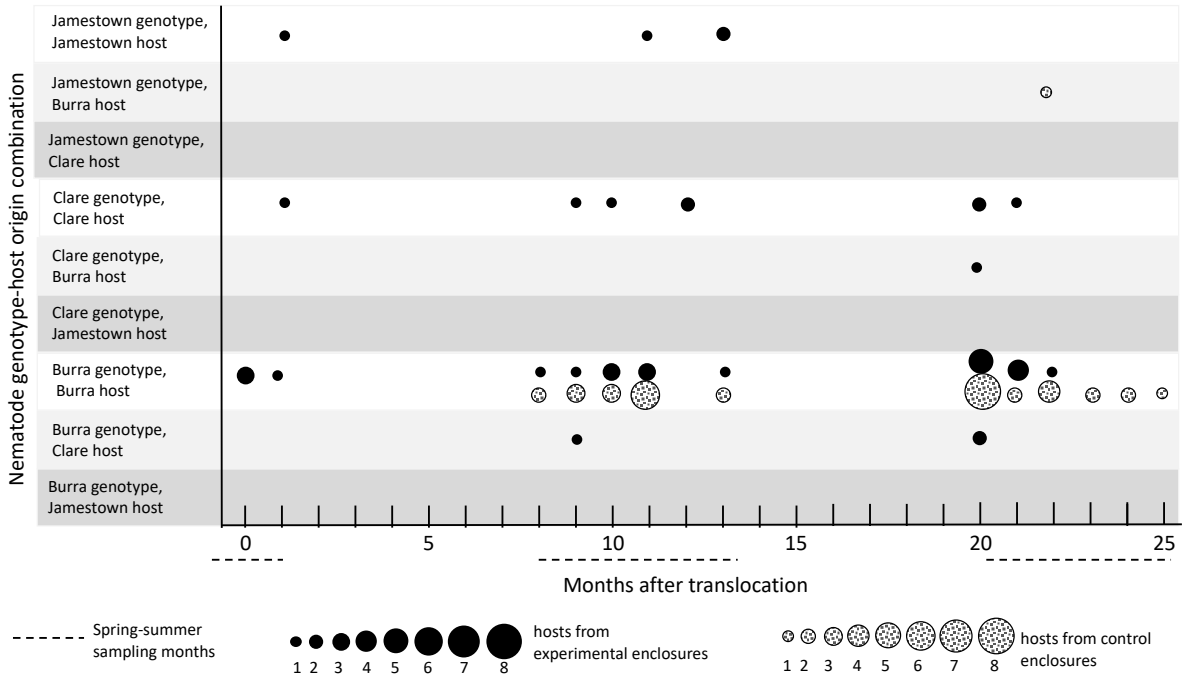

Figure 3.9. Summary of *Pharyngodon wandillahensis* infesting *Tiliqua adelaidensis* hosts within experimental and control enclosures over time following translocation. Note: Infestation events are classified by inferred nematode genotype-host origin combination.

Group membership at higher hierarchical levels (not shown) was not completely host-dependent, as subgroups contained nematodes from different hosts. Nematodes collected from the same host were often assigned to the same subgroup, especially when collected at the same timepoint, but there were also instances where nematodes from the same hosts were grouped differently at the subsequent levels of hierarchical analysis.

Having observed evidence of inter-host, non-local transmission of nematodes in the grouping analyses (Figure 3.9), the associations between inter-host nematode relatedness and between hosts networks reflecting three potential transmission mechanisms were examined. The comparison of nematode relatedness between hosts to host interactions yielded a lack of association in most cases (Table 3.1), regardless of whether nematode relatedness was compared to burrow proximity, burrow sharing or potential mates. An exception to this was a significant, positive association between burrow proximity and relatedness in one of the control enclosures (containing Burra originating hosts only) during the second season ($p=0.043$). Again, the adjusted r^2 value of 0.087 indicated low precision in burrow proximity as a predictor of nematode relatedness between hosts, and the lack of replication calls the presence of a biologically meaningful association into question.

Table 3.1. Regression analysis of social network matrices (using MRQAP) to test the effect of host-related predictor variables (asynchronous burrow sharing, inter-burrow distance, potential mating between males and females in an enclosure) on the relatedness of *Pharyngodon wandillahensis* nematodes in *Tiliqua adelaidensis* hosts. * denotes a statistically significant p value (α = 0.05)

Enclosure	Season	Predictor variable	Regression coefficient for predictor variable	p	Adjusted r^2
1	All	Burrow sharing	0.048	0.441	-0.004
3	All	Burrow sharing	-0.034	0.694	-0.007
1	1	Distance	-0.155	0.336	0.039
1	2	Distance	0.101	0.043*	0.087
1	3	Distance	0.028	0.531	-0.007
2	1	Distance	0.011	0.335	0.009
2	2	Distance	0.070	0.062	0.058
2	3	Distance	-0.091	0.227	0.012
3	1	Distance	-0.036	0.595	-0.010
3	2	Distance	0.085	0.392	-0.001
3	3	Distance	0.062	0.395	-0.009
5	1	Distance	-0.061	0.522	-0.067
5	2	Distance	-0.018	0.803	-0.050
5	3	Distance	-0.018	0.828	-0.050
6	2	Distance	0.004	0.954	-0.077
6	3	Distance	-0.027	0.736	-0.070
1	All	Male-Female	-0.001	0.989	-0.029
2	All	Male-Female	0.018	0.843	-0.028
3	All	Male-Female	-0.025	0.824	-0.022
5	All	Male-Female	0.055	0.472	-0.023
6	All	Male-Female	-0.047	0.711	-0.045

Discussion

Our aim was to examine whether the parasites *Pharyngodon wandillahensis* and *Ophiomegistus michaeli* were differentiated between isolated *Tiliqua adelaidensis* host populations, and to use any genetic differences to comment on the extent and modes of transmission within a translocated host group of mixed population origin. On the basis of the geographic isolation of host populations, and the fine-scale genetic structure of their only known hosts, we hypothesized that that genotyped parasites would cluster by geographical-origin based groups. Our results show considerable support for this hypothesis, particularly in the case of *P. wandillahensis*. Transmission of allopatric parasites once lizards from different origins were sharing the same habitat and interacting with one another following the population augmentation was predicted, and our results provide some evidence that this has occurred. We hypothesized that transmission would be driven by mechanisms such as spatial proximity of burrow refuges, asynchronous burrow sharing, or annual mating, and therefore that the relatedness of parasites among hosts would be correlated with these kinds of host-host interactions. However, network analysis conducted on small sample sizes did not strongly suggest any of these mechanisms affected parasite transmission. On the basis of the small number of allopatric parasite transmission events detected and their slow nature, rapid and extensive spread of these macroparasites seems unlikely to occur and pose a disease threat in future *T. adelaidensis* translocations.

Parasite genetic structure reflects host populations

Our results show support for genetic differentiation of both mites and nematodes among host populations across analyses. Small expected genome size (Gregory and Young, 2020; Kumar et al., 2012) and very small amounts of available DNA per individual meant that numbers of informative SNP markers confidently genotyped were relatively low. Parasite individuals genotyped per population were also low due to host and parasite rarity, and to logistical constraints of sample collection. However, genetic clusters obtained through all three cluster analysis methods grouped parasites in the same manner, which was consistent with population origin of the host. This clustering occurred for parasites from several hosts, and over timepoints several months apart. The congruence between genetic cluster and population origin was particularly evident for the nematode *P. wandillahensis*. Congruence was less clear for the genotyped *O. michaeli* mites, though STRUCTURE analysis does indicate that initial clusters of mixed population origin did separate out into geographic origin-based groups at

subsequent levels (Figure 3.4). Here, small sample size limits our ability to draw firmer conclusions about genetic structure in *O. michaeli* between host populations.

Genetic differentiation between allopatric populations of *P. wandillahensis* is consistent with the biology of oxyuriod nematodes, though there is little information beyond host association on the genus *Pharyngodon*. Species in the order Oxyurida tend to have single hosts, which limits gene flow over space, particularly if the host themselves have low vagility (Adamson, 1989; Falk and Perkins, 2013). Adding to their low dispersal ability is the fragile nature of the infective egg stage which are desiccation- and temperature-sensitive and thus may not persist outside of the host for long (Adamson, 1989). Haplo-diploidy exhibited by this group also contributes to high levels of inbreeding (Adamson, 1990, 1989). Similarly, little is known about the biology of the paramegistid genus that *O. michaeli* belongs to, though a few species have been recorded on more than one skink or snake host species (Klompen and Austin, 2007). It is speculated that scale size may preclude the mites from exploiting smaller skinks and snakes however, presumably increasing local host specificity somewhat (Derne et al., 2019).

Evidence of limited transmission for non-local parasite genotypes

When parasites of translocated lizards were examined, the majority remained within the same clusters as parasites from untranslocated hosts of the same origin, which suggests that translocation largely did not change parasite genotypes of a given host in the two years following translocation (Figures 3.5 & 3.9). The relatively few exceptions to this trend strongly suggest that transmission of non-local parasite genotypes following population augmentation occurred, albeit to a small extent. All instances of incongruence between host origin and the likely geographic origin of the mite or nematode genotype occurred several months after the translocation, which is a feasible time frame for transmission of parasites between hosts and development of subsequent generations (though lifecycle details for these species remain unknown). Furthermore, the parasites of untranslocated hosts from Jamestown or Peterborough had parasites which, without exception, always clustered in ordination space with other parasites of that location. This pattern suggests that the parasites not grouping with the clusters aligned with their host origin represented inter-population transmission events, and not merely a tendency for innate genetic variation within populations. The time frames and extent of transmission observed here are broadly consistent with that observed following a population augmentation of small marsupials where cestodes and coccidians appear to have been transmitted from translocated to resident conspecifics and vice versa, respectively, after

6–12 months (Northover et al., 2019). Although no wildlife examples were found, similarly, a correlation between genetic similarity and distance in *Plasmodium falciparum* isolates from humans was used to relate reported travel to long-distance malaria transmission events in The Gambia (Amambua-Ngwa et al., 2019).

Mechanisms of parasite transmission

Generally low rates of inter-host transmission are suggested by the longer time frames (9–22 months) taken for evidence of this to be observed, and by the small number of observed transmission events. This pattern may reflect parasite reliance on host animals for dispersal in contrast to active dispersal into the environment. Auto-infection may be a feature of either parasite's biology, which would result in the retention of parasite genotypes that we observed in Jamestown and Clare hosts following translocation. Some oxyuroid nematode species produce thinner walled eggs which remain in the host gut instead of passing out in faeces to be ingested by a new host individual (Adamson, 1989). We have hypothesised that the eggs and immature stages of the *O. michaeli* lifecycle may occur in the *T. adelaidensis* burrow and permit successive generations to attach to the same host (Derne et al., 2019), and these data provide support for this hypothesis. When intra- and inter-host parasite relatedness was measured, mean relatedness was higher among parasites on the same hosts compared to parasites on different hosts (Supplementary material: Figure S3.8 & S3.10) also suggesting an individual host-focused lifecycle.

The low number of clear inter-host transmission events indicated by transmission of allopatric parasite lineages over time is also consistent with the non-social nature of their hosts. *Tiliqua adelaidensis* individuals are known to avoid direct interaction with conspecifics, with the exceptions of mating and mother-offspring contact (Milne et al., 2002; Schofield et al., 2014). The lizards engage in behaviours such as use of scat piles as social signals possibly to reduce direct territorial conflict (Fenner and Bull, 2011). Our network analysis results did not clearly support any of the three hypothesized host-driven mechanisms for inter-host transmission. There was weak indication with both mites and nematodes that proximity of burrows was associated with inter-parasite relatedness and therefore could have represented a transmission pathway, though lack of replication across enclosures or seasons casts doubt on the effect of burrow proximity. For nematodes, this lack of effect may support findings by Fenner et al. (2011) that *T. adelaidensis* individuals are more or less likely to be infected based on their individual behaviour (i.e. propensity for dispersal) or their proximity to a disperser rather than

proximity to neighbours alone (White et al., 2017). Further network analyses exploring these factors would require larger sample sizes to overcome the low prevalence of the parasites and possible confounders. Longer follow-up monitoring may also help reveal what our data suggest are slow rates of transmission in a non-social host.

In the absence of broader patterns, individual-level occurrences of transmission may offer some additional insight into possible transmission mechanisms. The mite pair on different hosts with the highest relatedness (0.59, suggesting parent or sibling level relatedness) occurred between a Clare-originating male and a Burra female lizard in the same mixed enclosure 13 and 20 months after translocation respectively, suggesting that mites were transmitted from the male to the female. Whilst there were no genotyped offspring from this host pair during the 2016–2017 season, unsuccessful mating between the two may have occurred. Though the female later occupied the same burrow as the male, this asynchronous sharing occurred after the related mites were found on her, but a burrow sharing event prior to that may have gone unrecorded. For the nematodes, one documented instance of allopatric transmission to a Burra resident host from a control (non-mixed) enclosure not containing translocated lizards 20 months after translocation suggests that nematode eggs may have been transmitted by another, indirect, agent (researcher activities, insect prey, flies (Adenusi and Adewoga, 2013)). Our difficulty in identifying transmission mechanisms stems from a lack of understanding of basic parasite lifecycles, such as duration, and where and under which conditions off-host stages survive.

Implications for future translocations

The observed transmission of genetically differentiated parasites during a translocation raises the question of whether local adaptation — of the parasites, or of the hosts — occurs. Local adaptation by parasites may mean parasites impose a greater cost on sympatric hosts, which could be further aggravated if hosts undergo the stress of translocation. Conversely, local adaptation by the host may mean that allopatric parasites have a greater fitness effect for the host. A meta-analysis showed that local adaptation by parasites occurs some of the time (Greischar and Koskella, 2007), despite expectation that faster generation times and larger population sizes, and greater mutation rates will result in adaptation to the host that is faster than host adaptation to the parasite (Gandon and Michalakis, 2002). Also, selective pressures for host adaptation to parasites may be low in parasites that have little to no costs under conditions of equilibrium. Testing for local adaptation in this system to inform future

translocations would require deliberate cross-infection (e.g. Prugnolle et al., 2006) to provide adequate statistical power, and also targeted measures of host fitness/susceptibility (e.g. basking time as reported by Fenner and Bull, 2008).

Whether by virtue of host-driven transmission (most likely) or through possible local adaptation, our results suggest that these two parasite species are slow to spread through a host population. This bodes well for future translocations of *T. adelaidensis* where follow-up monitoring of animal health would afford the opportunity to identify pathogen emergence before it affected a large proportion of the population. Importantly, the slow nature of parasite transmission may also allow translocated individuals to recover from the shorter-term stress of translocation (i.e. the post-release effect *sensu* Armstrong et al., 2017) before being exposed to novel parasite genotypes, which may minimise fitness costs.

Whilst the transmission mechanisms of *P. wandillahensis* and *O. michaeli* remain unclear, we can use existing knowledge of host biology and also other systems to make recommendations for future *T. adelaidensis* translocations. Post-release dispersal and inadequate habitat provision is a common issue in reptile translocations generally (Germano and Bishop, 2009) and we infer that *T. adelaidensis* individuals are more likely to move around if burrow quality is poor (Bull et al., 2015; Fellows et al., 2009). Tongue-flicking of scat-piles, which may promote nematode egg ingestion, can occur for longer if conspecifics are unfamiliar, depending on the respective sexes of the interacting lizards (Fenner and Bull, 2011). In this system, we can't rule out that increased direct and indirect interaction (e.g. tongue flicking and asynchronous burrow sharing) with conspecifics would increase parasite transmission. Network modelling based on increased desert tortoise movements and contacts between conspecifics post translocation by Aiello et al. (2014) showed that disease outbreaks were more likely in a translocated population relative to a non-translocated one. In addition to the need for suitable burrow refuges (Milne et al., 2003; Souter et al., 2004), wild-to-captivity studies have shown that translocated *T. adelaidensis* individuals are less likely to disperse after release and less likely to have antagonistic interactions with conspecifics if factors such as seasonal timing, conspecific density and soft-release techniques are optimal (Ebrahimi and Bull, 2014b, 2014a, 2013). The consideration of parasite transmission therefore adds to the need to create conditions in a translocation which minimise stress-induced conspecific interactions.

Conclusion

With the potential to cause population decline, the consideration of parasites as part of wildlife translocations is paramount and provides a practical context in which to study host-parasite ecology and evolution. To our knowledge, this study is first to use genome-wide single nucleotide polymorphisms to trace parasite transmission in a wildlife translocation. For *T. adelaidensis,* transmission of mite and nematode parasites which show genetic structure across their range within a mixed-origin host population, does not appear to happen quickly, or to a large extent. These results do however highlight the need to better understand parasite life stages and parasite interactions with their hosts, including any fitness costs. Conservation managers should not seek to eliminate parasites by default, but should examine whether the potential stress of parasite infestation can be minimised by good animal handling practices and the provision of high quality release habitats.

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Supplementary material

Methods

Single nucleotide polymorphism identification by DArTSeq

Extraction of DNA from whole nematodes and mites was conducted using protocols developed by Diversity Array Technology Pty Ltd (DArTseq) (Kilian et al., 2012). Following optimisation, DNA was digested using the restriction enzymes PstI and SphI and then labelled with sample-specific adaptors and sequenced with short-read Next Generation Sequencing (Illumina) at a sequencing depth of 2.5 million reads, as per proprietary in-house pipelines of DArTseq (Kilian et al., 2012). One third of DNA samples were processed twice (with different adapters and independent allelic calls) as technical replicates to ensure reproducibility. A set of raw 'sequence tags' of approximately 75 bp in length were produced and quality filtered. Various proprietary filters were then applied in order to identify sequence tags that contained a reliable SNP marker.

Single nucleotide polymorphism filtering

These DArTseq data were imported into the R (R Core Team, 2020) package dartR (Gruber et al., 2018), converted to genlight format (Jombart et al., 2008) and filtered before subsequent analysis. Filtering consisted of first, individuals and loci for which the reproducibility (averaged over two allelic states) fell below 100% were removed, as were all monomorphic loci. Loci that had over 25% of missing data across individuals were removed, and then any secondary loci on sequence tags were removed. Next, individuals that had over 25% of missing data across loci were removed. Loci pairs with a Hamming distance of less than 0.2 were removed to reduce the possibility of sequencing error being confused with a different locus. Filters to remove any loci not in Hardy-Weinberg equilibrium were applied. Loci in linkage disequilibrium were retained in the interests of retaining the maximum number of SNPs for population-level differentiation. An additional filter to remove loci with minor allele frequencies less than 1% was applied for subsequent relatedness analysis.

Principal coordinates analysis (PCoA)

Genetic similarity between individual parasites from different populations was examined using principal coordinates analysis (PCoA) ordination (Gower, 1966) in dartR, where individuals were entities and SNP loci were the attributes. A plot placing each individual by the two most

informative dimensions – the loci which explained the greatest proportion of the total variation between individuals – was produced to visualise this ordination. Dimensions were considered informative when they explained more than the average of original variables.

Discriminant analysis of principal components (DAPC)

Another non-model based method for grouping individuals based on genetic similarity was applied by performing cluster identification and DAPC with the R package adegenet (Jombart et al., 2008). DAPC focuses on between-group variability of genetically related individuals, which may enable clearer identification of clusters, in contrast to principal component analysis (PCA) or PCoA, which summarise overall variability between individuals (Jombart et al. 2010). First, data were transformed by PCA and all principal components (PCs) were retained. Possible clusters were identified, and Bayesian information criterion (BIC) was used to choose an optimal value of K (a biologically plausible number of clusters that maximises variation). DAPC was then performed using only a subset of the most informative PCs in order to balance sufficient informativeness with overfitting and instability of the membership probabilities returned (Jombart and Collins, 2015). All discriminant functions were retained since the number of possible clusters were low.

Bayesian cluster analysis with STRUCTURE

The software STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to conduct Bayesian model-based cluster analysis, as an alternative way to examine whether or not SNP genotypes clustered by host population of origin, and to identify any evidence of allopatric parasite transmission between hosts of different origins within the translocation. Using GNU Parallel (Tange, 2018), 20 replicates for each value of k (number of populations) between 1–10 were run with different random seeds, as recommended by Evanno et al. (2005). Each run consisted of 100 000 burn-in iterations (as deemed sufficient by convergence of values of summary statistics (Porras-Hurtado et al., 2013) and 100 000 Markov Chain Monte Carlo (MCMC) repetitions. The population-specific prior was selected (POPALPHA=1) and alpha was set to 1/K, where K was the assumed number of populations (Wang, 2017). All other extra parameters were left at default options, notably Admixture models were used, and the correlated allele frequencies option was selected (Falush et al., 2003). In the absence of a genetic map, linkage models were not used.

The likelihood of each value of K, L(K), given the data, was averaged across replicates and plotted by Structure Harvester (Earl and VonHoldt, 2012). Unless the plot of L(K) clearly indicated a K value of 1 (i.e. there was no or little increase in likelihood with higher values of K), we used the complementary value of delta K to determine the most likely value of K outlined by Evanno et al. (2005). Where delta K was highest for k=2 or more, members of these identified sub-clusters were identified by CLUMPAK (Kopelman et al., 2015) using the greedy algorithm. An individual was considered a member of the cluster for which it had the highest estimated probability of membership, as compared to a using higher probability threshold (e.g. 0.6 used by Coulson et al (2008)). Delta K reliably detects the uppermost hierarchical level of population structure, though this may not reflect the true value of K (Evanno et al., 2005; Janes et al., 2017). Therefore, hierarchical structure analysis was conducted for each identified cluster (Coulon et al., 2008; Janes et al., 2017). This process involved further STRUCTURE runs and analysis of results, as outlined above for each identified sub-cluster. Sub-clusters of less than 4 individuals identified during hierarchical analysis were considered as one cluster and not further analysed (Shi et al., 2010). The estimated membership probabilities of different clusters for each individual were visualised using CLUMPAK (Kopelman et al. 2015).

Mite relatedness

Filtered SNP data for 102 genotyped mites were imported into the program COANCESTRY 1.0.1.9 (Wang, 2011) in order to estimate relatedness between individual mites. Simulations of mite genotypes using the allelic frequencies derived from the genotype data and missing data rates at each locus were compared to expected relatedness levels for each relationship class (e.g. parent-offspring, full-siblings, unrelated). The estimator providing the highest correlation between the estimated and expected relatedness values of dyads, in this case the Dyad Maximum Likelihood estimator (Milligan, 2003), was used to estimate relatedness between sampled individuals (herein referred to as relatedness).

Relatedness between all mites in the dataset were reduced to a set of dyads between mites found on hosts in the same enclosures. Relatedness between mites was considered by host individual; that is, the mean relatedness between mites found on the same host at the same time point and all other time points was calculated when sample size permitted, and referred to as intra-host relatedness. The mean relatedness between mites found on different hosts within the same enclosure was calculated and referred to as inter-host relatedness. In cases where there were sufficient numbers, inter-host relatedness was divided into host groups of

the same population origin and of different population origin, e.g. mean relatedness of mites from other Burra resident lizard hosts compared to mean relatedness of mites from translocated hosts. The difference between mean relatedness of two groups was tested by bootstrapping with 1000 samples, implemented in COANCESTRY. When the observed difference in mean relatedness fell outside of the 95%CI of the sampling distribution of the difference of mean relatedness between groups, relatedness between the two groups was considered significantly different. The correlation between relatedness and days elapsed between collection of a mite dyad was also investigated using the adjusted coefficient of determination (r^2) in a correlation between relatedness of mites found on the same host and the number elapsed between collection, and also for mites found on different hosts over time.

Nematode relatedness

Filtered SNP data were imported into the program POLYRELATEDNESS 1.8 (Huang et al., 2014) in order to estimate relatedness between individual nematodes as *Pharyngodon wandillahensis*, as members of the order Oxyurida, would be haplo-diploid (Adamson, 1989). Nematodes which were heterozygotes for two or less of 358 filtered loci were considered to be haploid individuals (23/147) and entered as such. Simulations of haplodiploid nematode genotypes using the allele frequencies derived from the empirical data showed that the Ritland's estimator of relatedness with Huang's correction (Huang et al., 2015) was most closely correlated with expected relatedness values for different relationship classes. This estimator was therefore used to estimate relatedness between sampled individuals. Unlike the Dyad ML estimator used for mite relatedness, this estimator had a scale range between 1 and -1, where negative relatedness indicated pairs that were less related than average. As with relatedness for mites, relatedness for nematodes pairs was sorted into enclosures, and intra-host and inter host relatedness was calculated for nematodes from each host. Mean relatedness between two groups of three nematode dyads or more were compared using bootstrapping resampling with replacement with 1000 samples and 95% confidence intervals, carried out in the R environment (R Core Team, 2020).

Network analysis

All networks were constructed as adjacency matrices using the 'igraph' package in R (Csardi and Nepusz, 2006; R Core Team, 2020). Parasite relatedness networks included all hosts within an enclosure from which a genotyped parasite had been collected over the 2.5 years of the field study. Host nodes were connected to all other hosts with a weighted edge that reflected the

relatedness between their respective parasites. Where multiple parasites occurred on the same host, the edge between two hosts reflected the relatedness between their most related parasites. Edges for parasite networks were undirected, since we could not be certain in which direction any transmission between hosts had occurred. As *P. wandillahensis* nematodes were collected from multiple hosts across all enclosures, nematode relatedness networks were created for all enclosures, whilst mite relatedness networks were created only for the two enclosures (both experimental, i.e. containing hosts from all origins) where multiple hosts were found to have *O. michaeli* mites.

To see if parasite relatedness was related to distance between home burrows of hosts, networks were constructed for each enclosure where undirected edges between each host lizard node were weighted by an inverse measure of Euclidean distance between burrows that two lizards inhabited (inverse distance measure = 10/inter-burrow distance (m)), so that higher burrow proximity would be more heavily weighted. Since lizard hosts did not always occupy the same burrow from month to month, the average distance between two host lizards per season (October–March with up to six monthly sampling occasions) was calculated and used, resulting in three burrow proximity-based networks per enclosure for hosts of each parasite type.

A weighted, directed network was also constructed for each enclosure over the entire study based on asynchronous burrow sharing by host lizards. Hosts were connected by edges when they had been caught from the same burrow at different times. Though burrow occupation was temporally sequential, these networks were left undirected in order to be compared with undirected parasite relatedness networks. Edge weights reflected the number of burrows that a pair of lizards had shared over time.

Mating between lizards in each enclosure were also examined as a potential transmission mechanism. Although we constructed networks based on lizards having mated and produced genotyped offspring together during the season 2 spring mating season, based on parentage analysis using microsatellite markers (Clive, 2019), we did not analyse these due to insufficient sample sizes. As a proxy measure of mating, we constructed networks where host lizards that we could confidently sex (see Chapter 5) were connected to lizards of the opposite sex as potential mates over the three mating seasons of the study. These networks were unweighted and undirected.

We examined the effects of inter-burrow proximity, asynchronous burrow-sharing and potential mating on the response variable of inter-host parasite relatedness using multiple regression quadratic assignment procedure (MRQAP) with double semi partialing (DSP), a method developed by Dekker et al. (2007) for matrix regression of network data. MRQAP calculations were implemented using the R package 'asnipe' with 1000 randomisations (Farine, 2013; R Core Team, 2020). Since matrices contained relatively few nodes (5-16), regression analyses performed with MRQAP were all univariable (one predictor variable per model) in order to avoid model overfitting.

Results

Principal coordinate analysis and discriminant analysis of principal components of *Ophiomegistus michaeli* and *Pharyngodon wandillahensis* SNPs

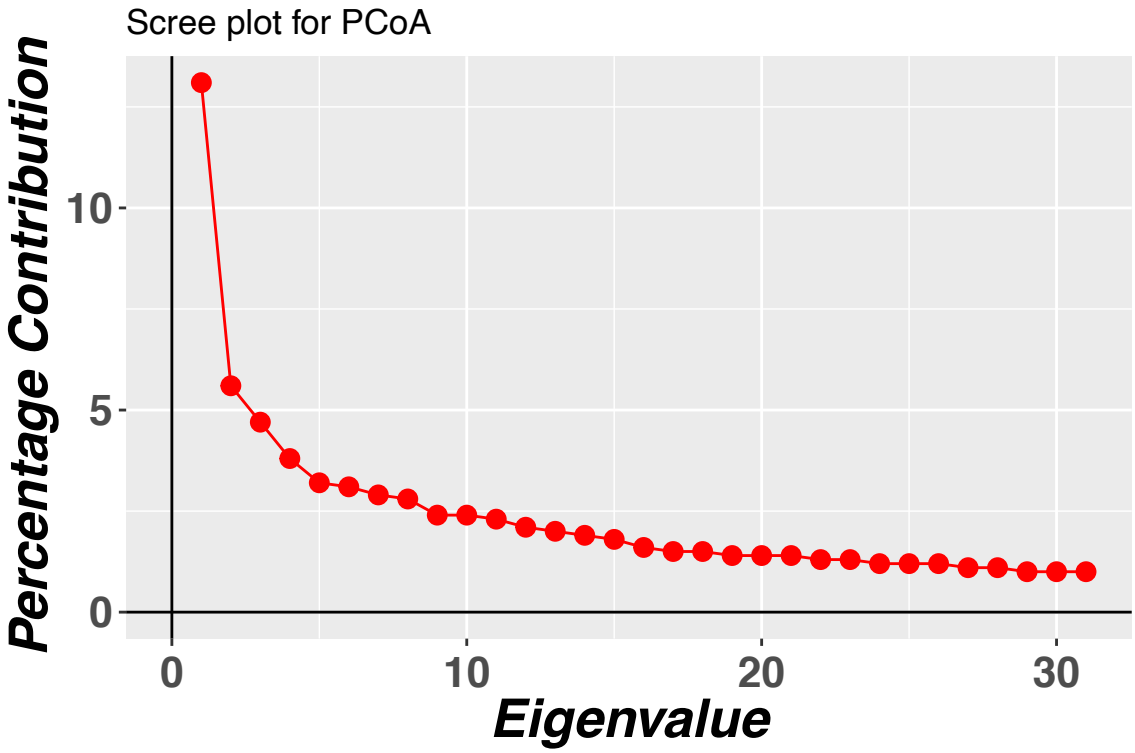


Figure S3.1. Percentage of total variance in *Ophiomegistus michaeli* SNPs represented by each dimension in the PCoA.

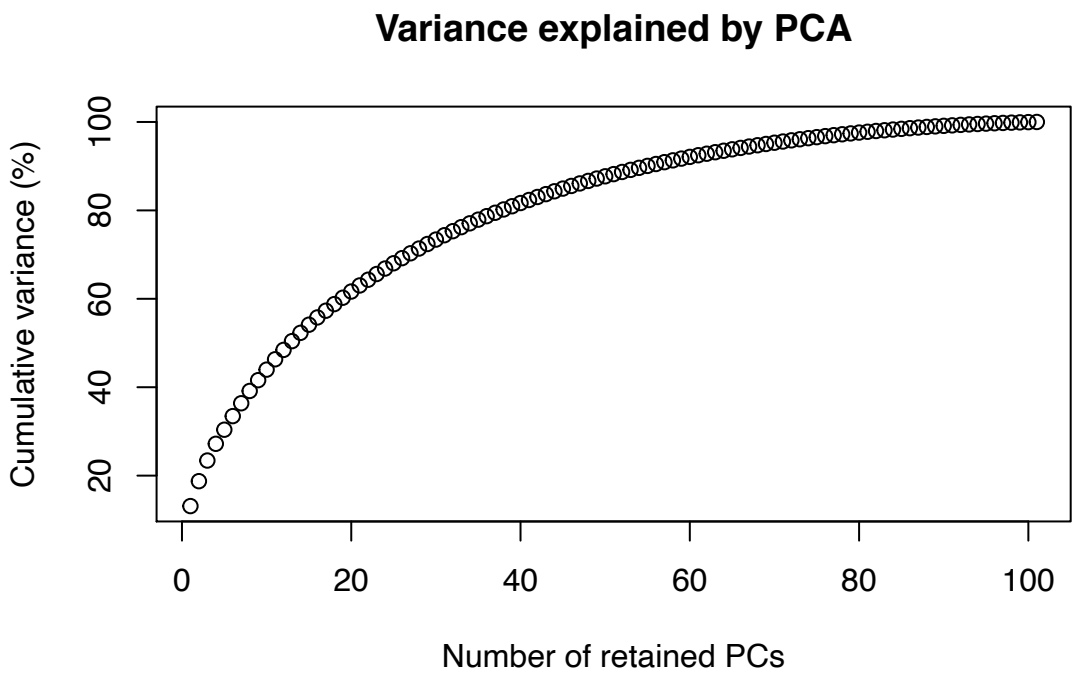


Figure S3.2. Informativeness of principal components (PCs) derived from PCA of *Ophiomegistus michaeli* genotype data.

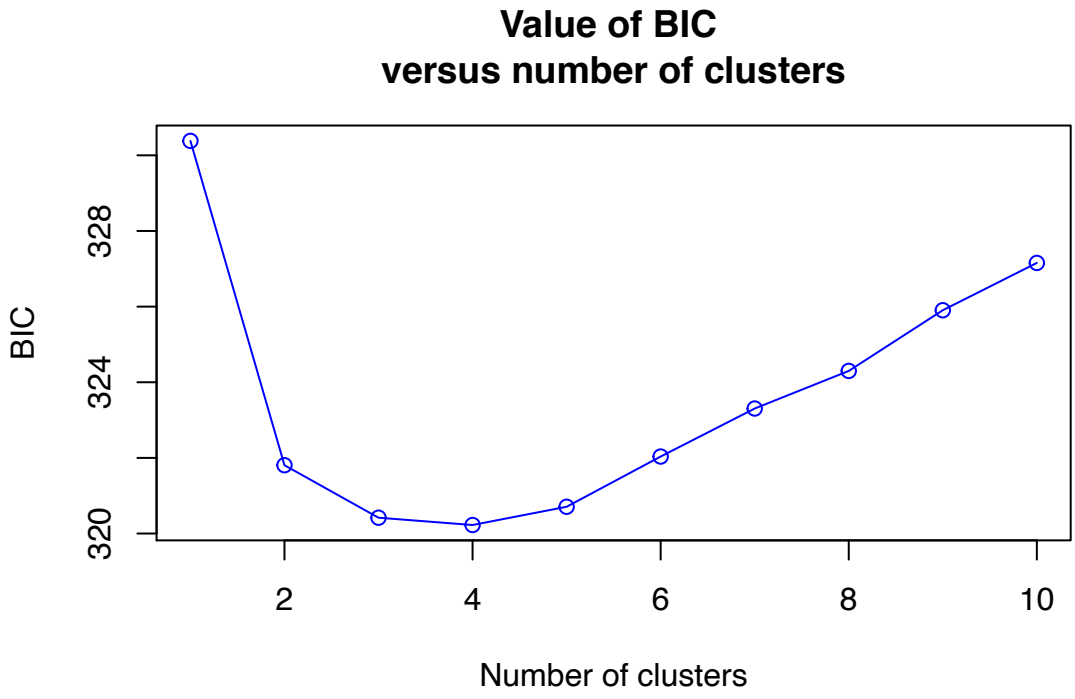


Figure S3.3. Bayesian information criterion for each value of K during cluster identification in *Ophiomegistus michaeli* genotypes preceding DAPC.

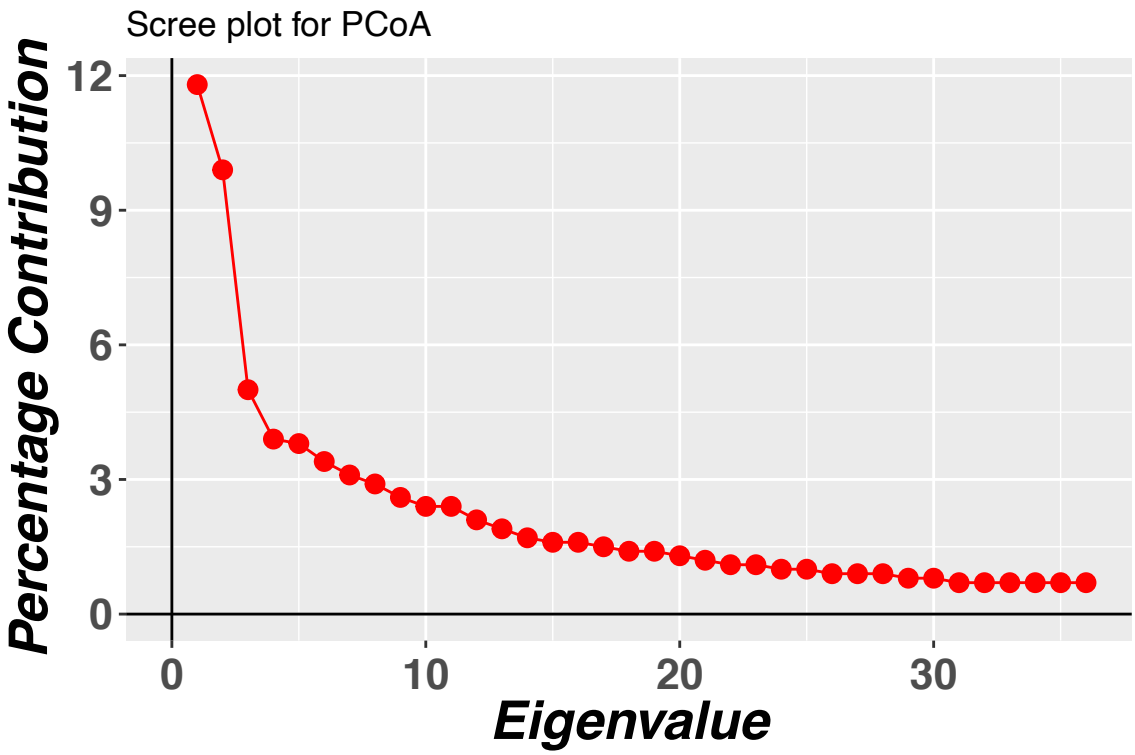


Figure S3.4. Percentage of total variance in *Pharyngodon wandillahensis* SNPs represented by each dimension in the PCoA.

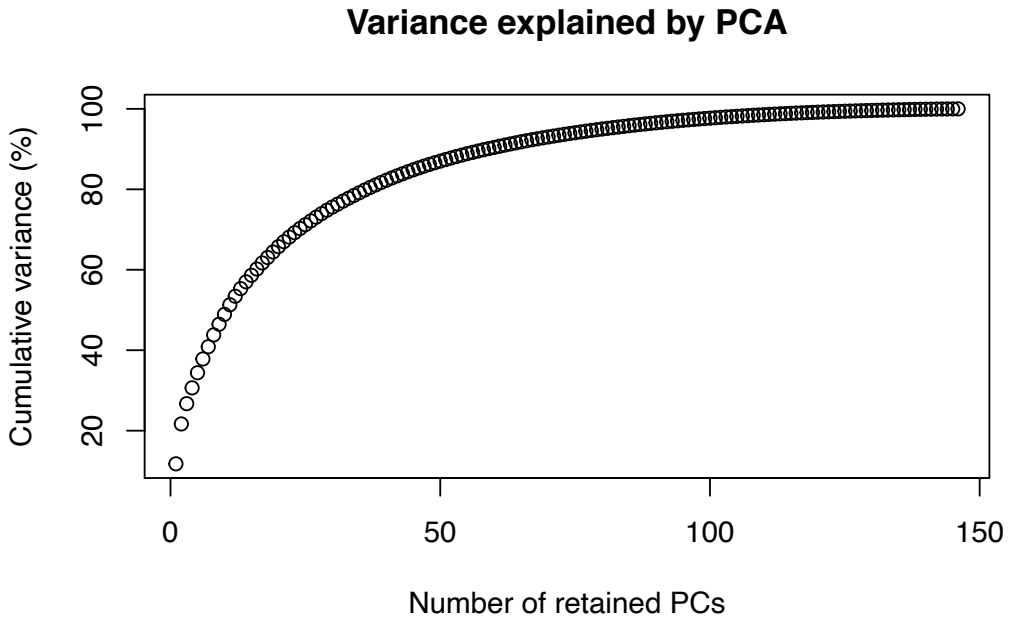


Figure S3.5. Informativeness of principal components (PCs) derived from PCA of *Pharyngodon wandillahensis* genotype data.

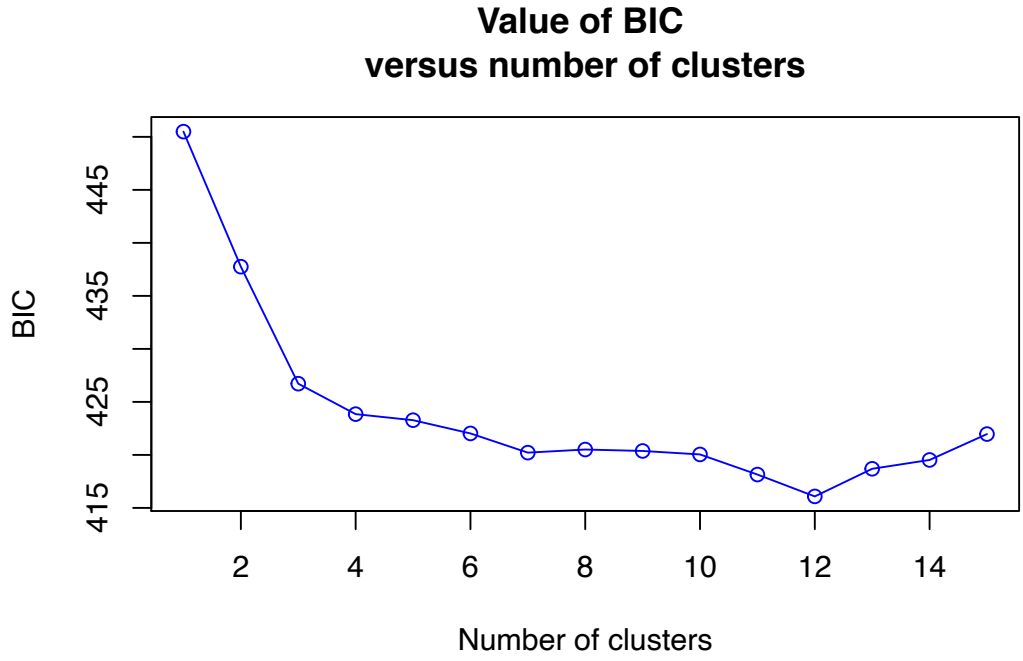


Figure S3.6. Bayesian information criterion for each value of K during cluster identification in *Pharyngodon wandillahensis* genotypes preceding DAPC. Note: K=3, BIC=426.7175, 423.8469, 422.5, k=10, BIC=416.9269

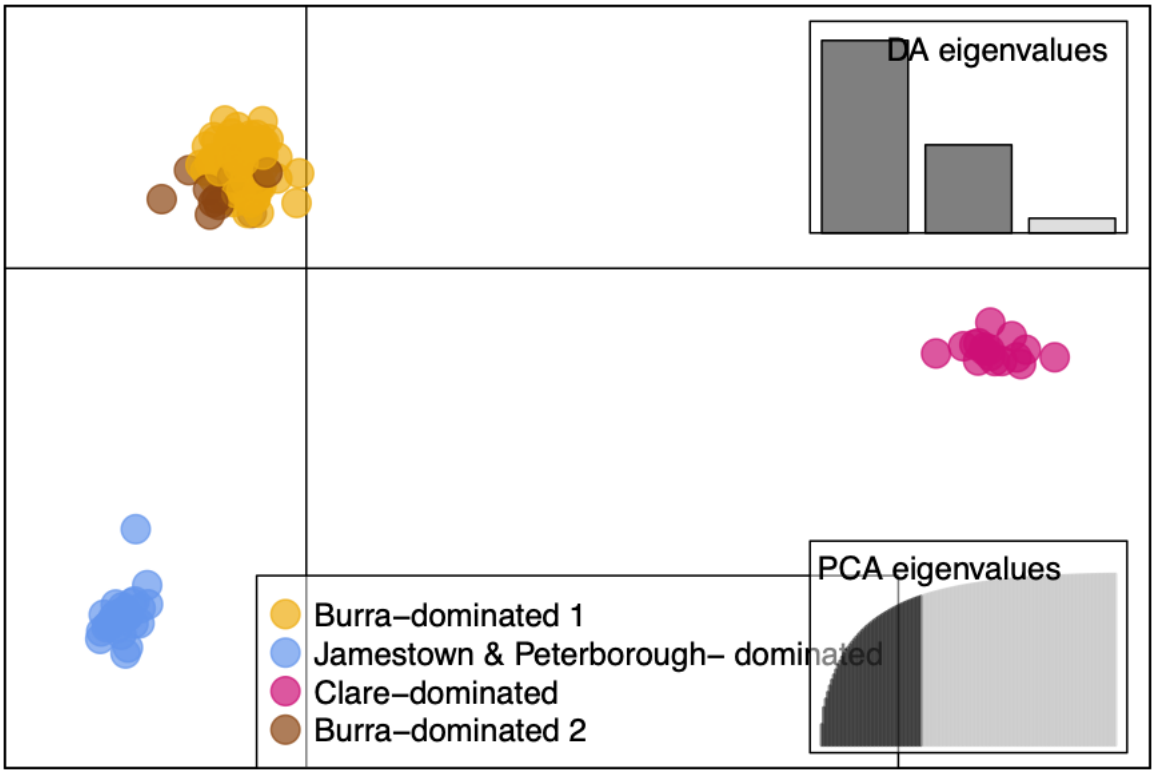


Figure S3.7. DAPC plot of genotyped *Pharyngodon wandillahensis* nematode individuals where k=4.

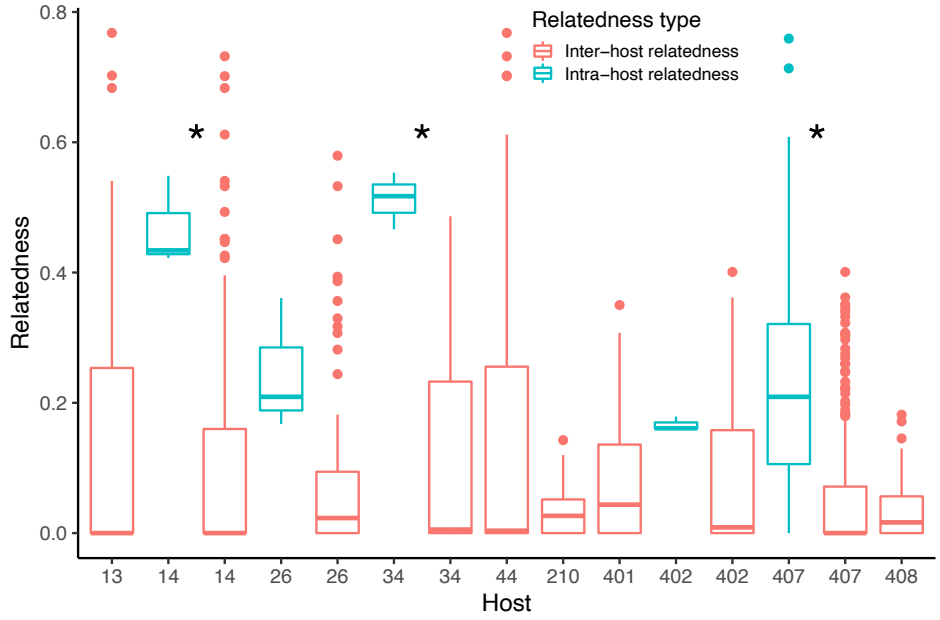
Relatedness estimates between *Ophiomegistus michaeli* and *Pharyngodon wandillahensis* individuals within the same enclosure

Mites

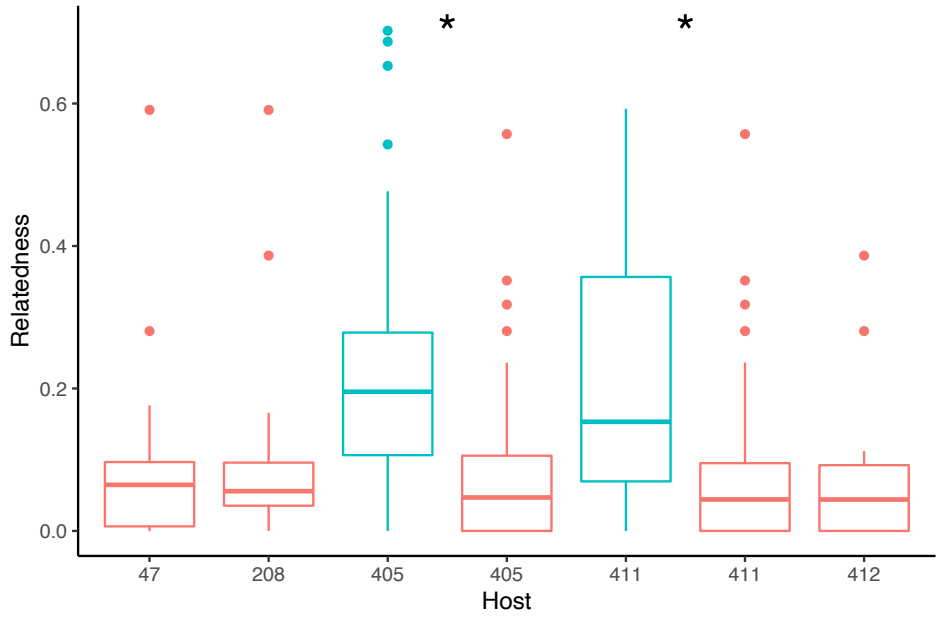
Only enclosure 2 and 5 had mites that were found on more than one lizard host (595 and 231 dyads respectively). Enclosure 2 had nine lizard hosts with a total of 35 mites and 2–13 mites per host (median=2.5), and enclosure 5 had five lizard hosts with a total of 22 mites and 1–11 mites per host (median=1).

Mean intra-host and inter-host mite relatedness was compared in seven hosts over two enclosures, and intra-host relatedness was found to be significantly higher in five out of seven hosts (Figure S3.8). As with the mean, median relatedness between mites found on the same host was higher than between mites found on different hosts. This difference between intra and inter-host median relatedness was smaller in the two hosts in enclosure 5 compared to those in enclosure 2 (Figure S3.9).

While this difference in mean relatedness was observed, there was no association between relatedness of mites and time elapsed between mite collection, both for mites on the same host and mites on different hosts. Adjusted coefficients of determination (r^2) were all between 0.1 and -0.1 (p values for correlation coefficients > 0.05), with the exception of one instance of intra-host relatedness over time having an adjusted r^2 of 0.167, which was a for a significantly positive relationship (p=0.001).



a)



b)

Figure S3.8. Median relatedness values between *Ophiomegistus michaeli* mites on the same *Tiliqua adelaidensis* host individuals (ID numbers as shown on the x-axis), compared to median relatedness values between mites on different host individuals. Asterisks denote a significant difference between the mean intra and inter-host relatedness, as determined by bootstrapping. **a) Enclosure 2, b) Enclosure 5.**

Inter-host relatedness was subdivided into mites from sympatric and allopatric hosts for eight out of nine hosts in enclosure 2, where there were four Burra resident lizards, one translocated lizard from Clare and four translocated lizards from Jamestown which hosted genotyped mites. Median inter-host relatedness was in all cases higher for mites on hosts from the same population origin (sympatric relatedness) than for mites on hosts of a different population origin (allopatric relatedness), with significant differences between the sympatric and allopatric relatedness means for all hosts (Figure S3. 9). In contrast, sympatric inter-host mean relatedness was not significantly different to allopatric inter-host mean relatedness for the mites on translocated hosts from Jamestown in enclosure 5 (these were the two hosts with enough mites to calculate this).

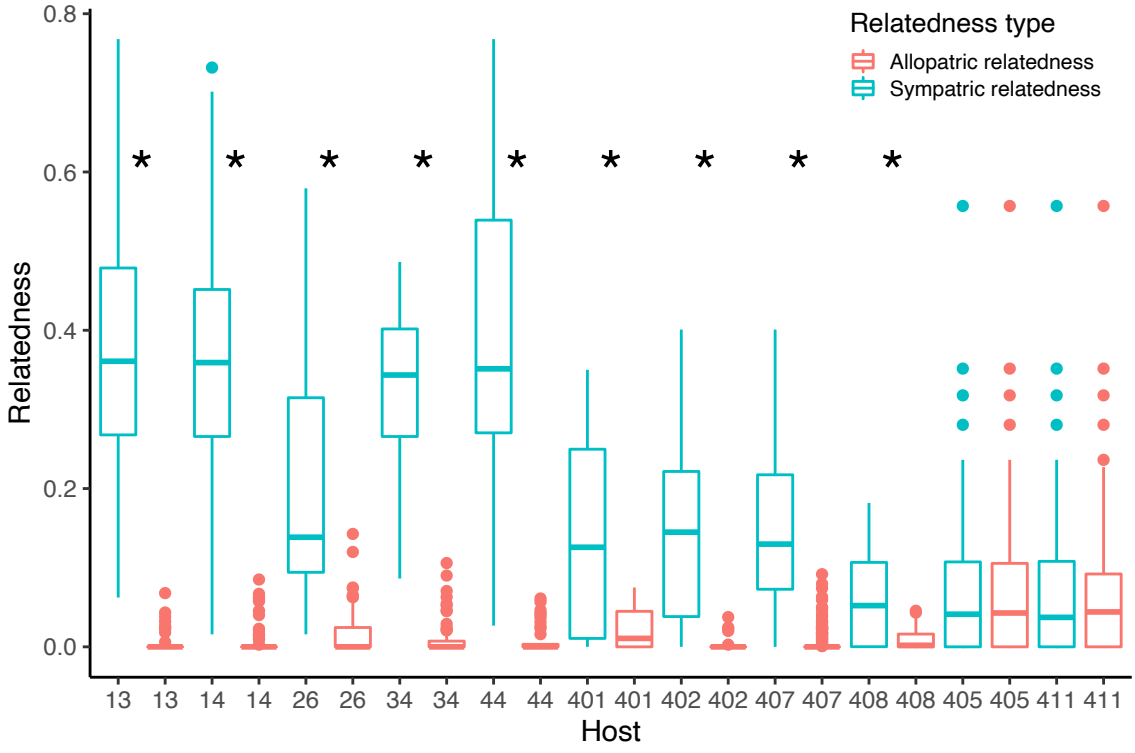


Figure S3.9. Median relatedness values between *Ophiomegistus michaeli* mites on *Tiliqua adelaidensis* host individuals (given ID numbers as shown on the x-axis) with the same population of origin, compared to mean relatedness values between mites on host individuals not from the same population of origin. Notes: All host individuals were in the same 30 m x 30 m enclosure (Enclosure 2), except individuals 405 and 411 which shared a second enclosure (Enclosure 5). All individuals with an ID number of less than 100 were Burra resident lizards and those with an ID number greater than 400 were translocated from Jamestown. Asterisks indicate a significant difference between mean sympatric inter-host relatedness and mean allopatric inter- host relatedness, as determined by bootstrapping methods.

Nematodes

Intra-host relatedness was compared to inter-host relatedness for 17 host animals found with 3 or more nematodes. In all hosts, mean intra-host relatedness was higher than mean inter-host relatedness, though none of the observed differences between intra- and inter-host relatedness means were statistically significant. Median intra-host relatedness values were higher than inter-host relatedness for nematodes in the same host only in experimental enclosures, i.e. where hosts from different populations shared the enclosure (Enclosures 2, 4 and 5) (Figure S3.10).

Inter-host relatedness was further divided into relatedness to nematodes on sympatric hosts compared to nematodes on allopatric hosts in 21 hosts from mixed, experimental enclosures. Mean sympatric inter-host relatedness was higher than allopatric inter-host relatedness in all hosts, though not statistically significantly so. Median relatedness values were also higher for sympatric inter-host relatedness than for allopatric inter-host relatedness (Figure S3.11).

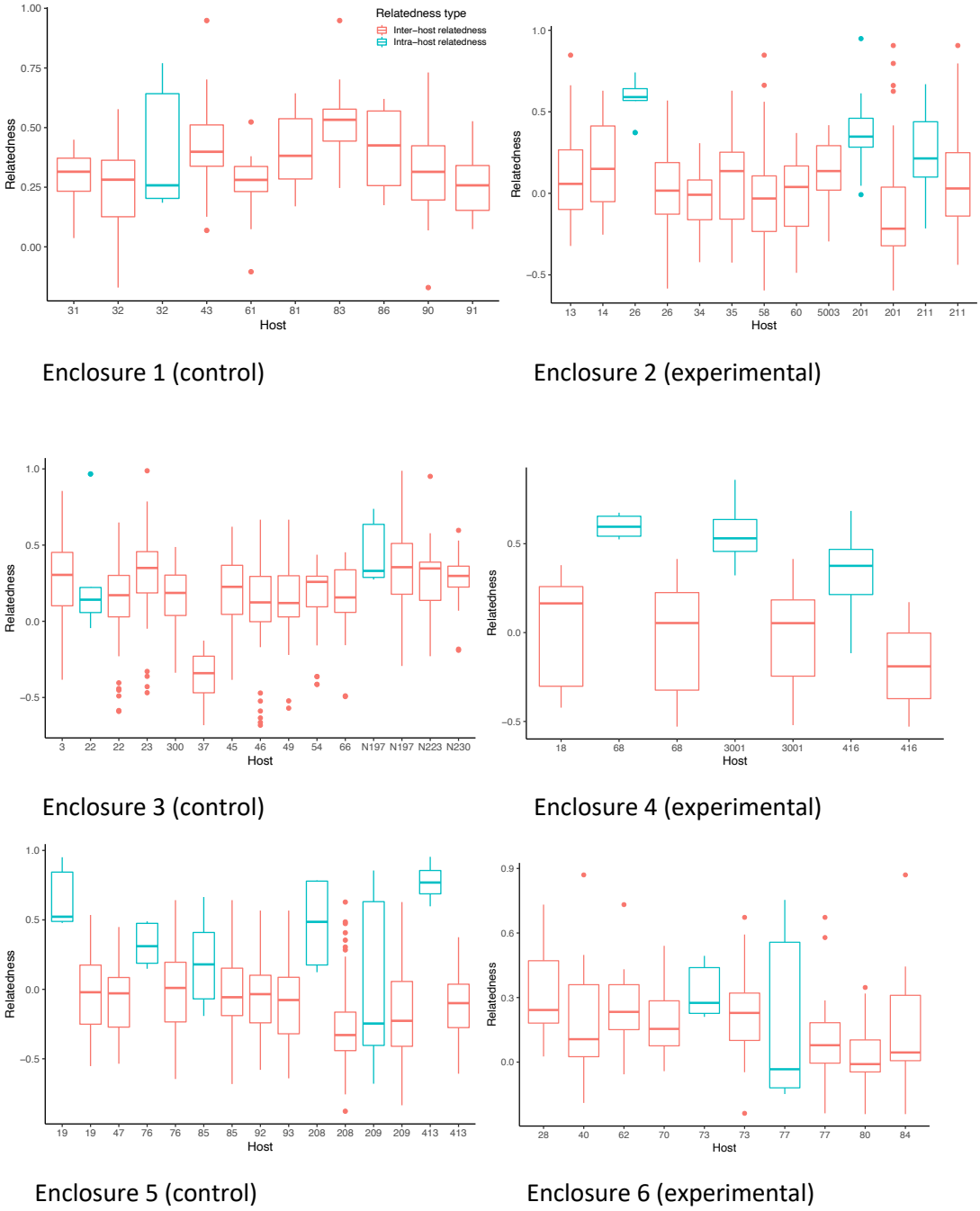


Figure S3.10. Relatedness values between *Pharyngodon wandillahensis* nematodes on the same *Tiliqua adelaidensis* host individuals (given ID numbers as shown on the x-axis), compared to relatedness values between nematodes on different host individuals. Note: Host individuals are shown by 30 m x 30 m enclosure. All individuals with an ID number of less than 100 or greater than 1000 were Burra resident lizards, those with an ID number of 201–211 were translocated from Clare, and those with an ID number greater 400 were translocated from Jamestown.

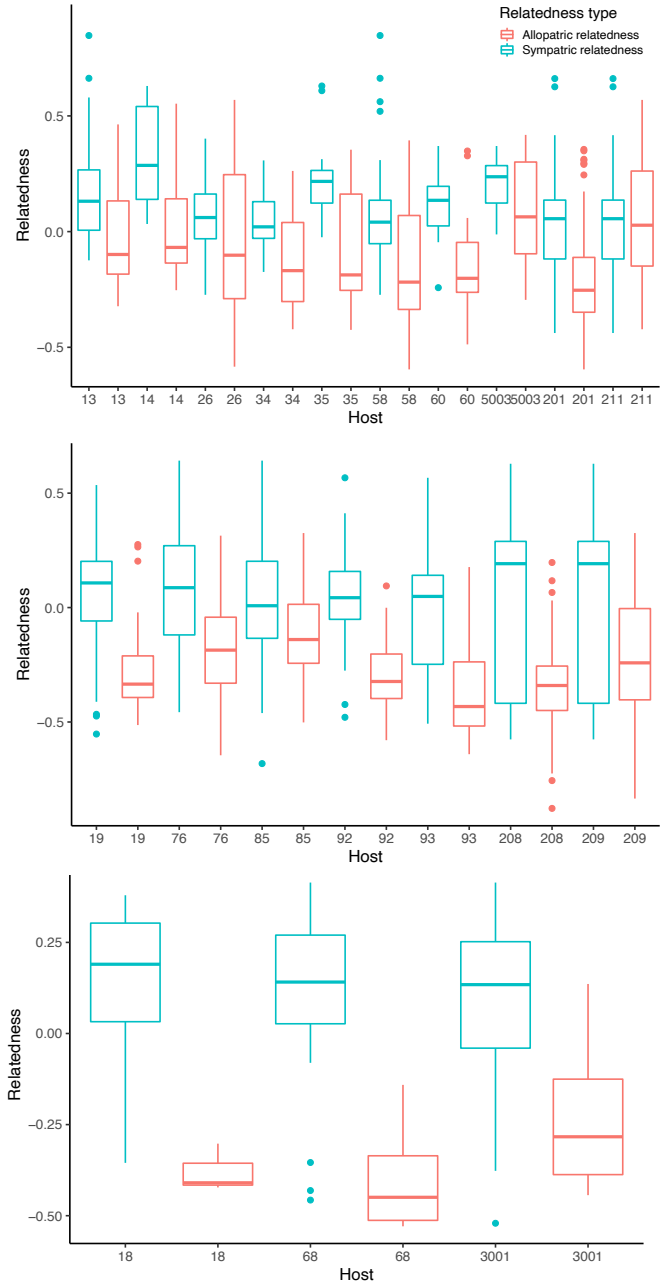


Figure S3.11. Median relatedness values between *Pharyngodon wandillahensis* nematodes from *Tiliqua adelaidensis* host individuals (given ID numbers as shown on the x-axis) with the same population of origin, compared to mean relatedness values between nematodes from host individuals not from the same population of origin. Notes: Host individuals are grouped by enclosure. All individuals with an ID number of less than 100 or greater than 1000 were Burra resident lizards, those with an ID number of 201–211 were translocated from Clare, and those with an ID number greater 400 were translocated from Jamestown.

The adjusted coefficients of determination (r^2) in the correlations examining relatedness between nematodes sampled from the same host over time were typically close to zero. The p value for the regression coefficient was significant ($p<0.05$) with an adjusted r^2 value of >0.2 in eight out of 71 correlations. These more highly correlated values included five out of 17 intra-host relatedness vs. time correlations which were calculated (29%), and two of 54 inter-host relatedness vs. time correlations (9%) which were calculated. These five intra-host relatedness vs. time correlations were all more highly associated than the two inter-host relatedness vs. time correlations, though these did include three correlations that had only 3–6 relatedness values to correlate with time and represented over-fitted models (Supplementary material: Table S3.1). Four out of five intra-host relatedness correlations revealed a lower relatedness between mites over time. The inter-host relatedness correlations showed one positive association between time and relatedness and also a negative association.

Table S3.1. Correlations between *Pharyngodon wandillahensis* relatedness (inter-host and intra-host) and number of days elapsed between collection of each individual. Note: Only correlations where $p<0.05$ for regression coefficient, and adjusted coefficient of determination (r^2) is higher than 0.2, are shown.

Enclosure	Host	Relatedness type	Number of dyads	Adjusted r^2	P value associated with regression coefficient	Association direction
1	32	Intra-host	3	0.98	6.43E-05	Negative
4	68	Intra-host	3	0.65	3.205E-02	Positive
5	208	Intra-host	6	0.98	2.00E-10	Negative
5	209	Intra-host	15	0.47	1.86E-05	Negative
5	47	Inter-host	18	0.24	2.43E-03	Positive
5	85	Intra-host	10	0.52	2.20E-4	Negative
5	93	Inter-host	18	0.21	4.57E-3	Negative

Chapter 4

Translocation does not appear to change the gut microbiota of an endangered lizard

Note to examiners

This chapter takes the overall aims of the previous chapter — to determine how parasites differ between host populations and to ascertain if inter-population transmission occurs during a population augmentation — and applies them to a different type of symbiont: the bacteria of the gut. Gut bacteria communities encompass both commensal species, some of which have the potential to be pathogenic, and also mutualistic species which perform important roles such as digestion or micronutrient synthesis. This study sought to track any change in gut bacteria taxa between pre- and post- translocation periods in lizards of different population origins and experimental groups in order to comment on whether this management strategy may have microbiota-mediated implications for host fitness.

Abstract

Gut microbiota have known health implications for their hosts, and the potential importance of microbiota in wildlife conservation has been highlighted. Environmental perturbations, including translocations — the intentional movement of animals from one location to another — may change a host animal's gut microbiota through various mechanisms, and reduce fitness. This study examined how gut microbiota are altered by the translocation in an endangered skink, *Tiliqua adelaidensis,* for which translocation is being evaluated as a conservation strategy. Individuals were taken from two isolated, wild source populations and released amongst a third, wild population, and both translocated lizards and residents were monitored for two years following translocation. Cloacal swabs were taken from these groups at the time of translocation, and at one and two years subsequently. Swabs underwent enrichment for Enterobacteriaceae, which include potential pathogens. Whole genome sequencing was used to identify the bacterial species and strains present in each animal at each timepoint. We predicted that some component of the microbiota communities would discernibly vary by population, and that translocated lizards would acquire the local microbiome of the recipient community over time. Our results did not support these hypotheses; no clear difference existed in either Enterobacteriaceal or non-Enterobacteriaceal communities detected in individuals from different populations at the time of translocation. Similarly, no geographic structure in strains of prevalent bacterial species was observed. Whilst there was some stochasticity over time, no group-specific temporal trends were evident over the two years. Taxa detected may have formed part of the species core microbiome, or there may have been little difference in the microbial environment between source and recipient locations. Gut microbiota changes arising from wild-wild translocation are likely to be negligible for *T. adelaidensis*, and these findings support the viability of translocation as a conservation strategy for the species.

Introduction

The microbe communities associated with animal hosts have emerged in recent years as an important influence of host health. The growing access to high-throughput DNA sequencing technology has enabled the characterisation of microbiota — the assemblage of bacteria, as well as archaea, fungi and viruses in a given environment — and their genomes, in a range of species, body regions and environmental contexts. Microbiota of humans and models such as rats have been particularly well characterised, and dysbiosis in the gut bacteria has been linked to a range of infectious and chronic diseases across body systems (Lynch and Pedersen, 2016; Vuong et al., 2017).

Certain gut bacteria have the potential to cause pathology and host mortality (Hall and Saito, 2008; Schumacher, 2006), which may lead to decline in animal populations (Daszak et al., 2000; Preece et al., 2017). Conversely, gut microbiota also perform essential roles for their hosts, such as the digestion of plant material (Dearing and Kohl, 2017) and the synthesis of micronutrients (LeBlanc et al., 2013). Gut microbiota community composition has also recently been associated with potential pathogen and parasite regulation in wild animals (DeCandia et al., 2020; Knutie et al., 2017; Murray et al., 2020). Further links to nutrition, behaviour and immunity are hypothesised to exist in wild animals, as is the case in humans and in rodent and primate models, but these have not been extensively studied (Amato, 2013; Bahrndorff et al., 2016; Carthey et al., 2020; Hanning and Diaz-Sanchez, 2015)(but see Amato et al., 2014, and Ingala et al., 2019). More broadly, a general lack of knowledge on bacterial prevalence and dynamics in wild animal populations has been identified (Baling et al., 2013; Colston and Jackson, 2016).

The influence of gut microbiota on host health begs the question of how these communities change in the face of perturbations that wildlife species commonly face due to anthropogenic land-use and climate change. Habitat fragmentation has been shown to drive changes in diet, which were associated with reduced microbiota in two species of wild primates (Amato, 2013; Barelli et al., 2015) and with more heterogenous microbiota in vampire bats (Ingala et al., 2019). Other primates appear to retain microbiota composition in the face of habitat degradation (Barelli et al., 2020; McCord et al., 2014). Human activity can also alter wildlife gut microbiota more directly by environmental contamination (Power et al., 2016).

In addition to unintentional perturbations, managed translocations — the intentional movement of animals between locations — represent perturbations for the animals involved and their recipient communities (Jones et al., 2018). In this context, exposure to a new environment, diet, conspecifics and also internal factors, such as stress may change an individual's microbiota (Amato, 2013; Chong et al., 2019; Noguera et al., 2018; van Leeuwen et al., 2020). Whilst translocations are necessary conservation interventions, the potential changes to microbiota and their genomes these perturbations cause need to be studied, and considered as a potential contributor to translocation success or failure (Carthey et al., 2020; Redford et al., 2012; Trevelline et al., 2019; West et al., 2019).

In this study we sought to examine the effects of translocation (specifically, population augmentation involving two source populations and a third recipient population) on the gut microbiota of the pygmy bluetongue lizard (*Tiliqua adelaidensis*). This endangered skink species is restricted to native grassland patches within a small region in South Australia, whose suitability as habitat are threatened by human activities and climate change (Fenner et al., 2018; Fordham et al., 2012; Hutchinson et al., 1994; Lunt, 1998; Souter et al., 2007). A specific aim of this study was to determine if *T. adelaidensis* gut bacterial communities differed in composition at a species or sub-species level among isolated host populations immediately prior to the translocation. We also sought to identify any changes over time in the two translocated groups and the resident recipient groups once individuals from three population origins were sharing habitat within the same experimental enclosures. In addition, comparison of gut microbiota between resident lizards exposed to translocated conspecifics with a control resident group (which did not share habitat with translocated conspecifics), aimed to disentangle any general temporal changes with those caused by translocation activities.

To characterise the gut microbiomes of individual lizards at given time, we focused on a culturable subset of gut bacteria, the Enterobacteriaceae family. Our objective with this choice was to comment on the presence of potential pathogens that are faecal-orally transmitted and common in reptile and other animal guts, including *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumonia* (Baling et al., 2013; Gordon and Cowling, 2003; Jacobson, 2007; Pees et al., 2007) at a higher taxonomic resolution than culture-independent methods might afford (Lau et al., 2016; Raymond et al., 2019). Bacteria with known pathogenic potential acquire added significance in a translocation context, since translocation can create host-related and environmental conditions whereby commensals may become pathogens (Méthot and Alizon, 2014; Noguera et al., 2018), an issue which has been raised for management of tuatara and

porpoises (Gartrell et al., 2007; Wan et al., 2016). Furthermore, study of usually commensal species may offer insights into more complete transmission pathways and inform disease management plans (Bull et al., 2012).

It was hypothesised that, whilst there might be a core microbiome for wild *T. adelaidensis* individuals common to most individuals regardless of geographic origin (West et al., 2019), some taxa would be unique to hosts of a given population of origin, creating a distinctive bacterial community signature for each population (Bird et al., 2019; Lankau et al., 2012). Over time, we predicted that differences in community composition would diminish as translocated lizards acquired local type microbiomes by virtue of environmental exposure and diet at the release site (Chong et al., 2019; Lankau et al., 2012). Due to the non-social nature of the species, we also hypothesised that the presence of translocated conspecifics would not be sufficient to drive a divergence in bacterial communities over time between resident lizards that were and were not sharing enclosures with translocated individuals.

Methods

Translocation & sample collection

We evaluated translocation as a conservation strategy of *T. adelaidensis* by performing an experimental population augmentation, where a total of 24 wild individuals from two donor populations were released amongst established conspecifics into a third, enclosed recipient population, and monitored regularly for two years following the translocation. The recipient site was established six months prior to the translocation at the Nature Foundation of South Australia's Tiliqua reserve, approximately five km east of Burra, South Australia, land that is also used to graze sheep. As outlined elsewhere (Chapter 1; Clive et al., 2020), three separate 30 m x 30 m enclosures pairs (each pair sharing an adjoining wall) were built out of 30 cm high sheet metal around an established *T. adelaidensis* population to prevent immigration and emigration of individuals. Enclosure pairs were 120–340 m apart. Numbers of existing 'resident' lizards per enclosure ranged from 6–23, reflecting the natural variability in density within a locality.

In mid-February 2016, 11 *T. adelaidensis* individuals (adults and subadults) were captured with a tethered mealworm bait (Milne, 1999) from an isolated wild population approximately 5 km west of Clare township on pastoral land, and transported to the Burra recipient site (approximately 45 km away). Thirteen individuals from another wild population approximately

10 km north of Jamestown on pastoral land approximately 72 km away were also captured and transported to the Burra site 1–2 days later. One enclosure from each of three enclosure pairs at the Burra site was designated as the experimental enclosure, and three to four Clare lizards and three to four Jamestown lizards were released into previously established dowel burrows (Milne and Bull, 2000) in each of the three experimental enclosures. Experimental enclosures therefore contained a mix of translocated Clare and Jamestown lizards alongside resident Burra lizards, whilst adjoining enclosures served as control treatments containing only the original Burra resident lizards (Figure 4.1).

All translocated lizards underwent cloacal swabbing, weighing and snout-vent length (SVL) measurement upon capture, using Copan aluminium shaft urethral swabs containing Amies agar gel for transport (Copan Italia S.p.A, Brescia, Italy). Resident Burra lizards had also been captured, measured, weighed and swabbed in the week before the translocation. Three to four weeks following the translocation, all translocated and resident lizards that could be caught within a two-week period within all enclosures were again measured, weighed and swabbed. This monthly sampling continued over the next two austral spring–summer activity seasons; from October 2016–March 2017 and from October 2017–March 2018. We used cloacal swabs to sample gut microbiota instead of faecal samples due to the limited opportunity to collect faecal samples; using cloacal samples greatly increased bacterial sampling opportunities.

Enrichment of bacterial samples

Used swabs were kept on ice for the remainder of the sampling day, before being stored at 4°C (or on ice during 2 hours of transport) until enrichment which occurred 1–6 days later in the laboratory. Swabs were opened in aseptic conditions and placed in 2ml of sterile Oxoid MacConkey's broth (Thermo Fisher Scientific Australia Pty Ltd, Scoresby, Australia) (made from powder at 40g/L) in a 20ml tissue flask. Swabs were then agitated for 18 hours at 37°C before a 1ml aliquot was centrifuged for 5 minutes at 3000rpm to form a cell pellet. The cell pellet was resuspended in a sterile mix of 70% Gibco Luria broth (Thermo Fisher Scientific Australia Pty Ltd, Scoresby, Australia) and 30% glycerol, left for 15 minutes, before long term storage at -80°C. These enrichment conditions targeted culture of the gram-negative Enterobacteriaceae family. Culture-enriched molecular profiling has shown to increase the number of OTUs detectable when compared to non-cultured metagenomic profiling in samples such as human faeces (Lau et al., 2016; Raymond et al., 2019).

Experimental design

Bacterial cultures from 23 of the 24 translocated lizards sampled at time of translocation (T_0) were selected for metagenomic analysis, alongside samples from 23 resident lizards from control enclosures and 22 resident lizards from experimental enclosures sampled during the week prior to the introduction of the translocated lizards (total n=67) (Figure 4.1). Samples from the same individuals that could be captured a year later (T_1) (n=43), and two years later (T_2) (n=23), were also selected for analysis.

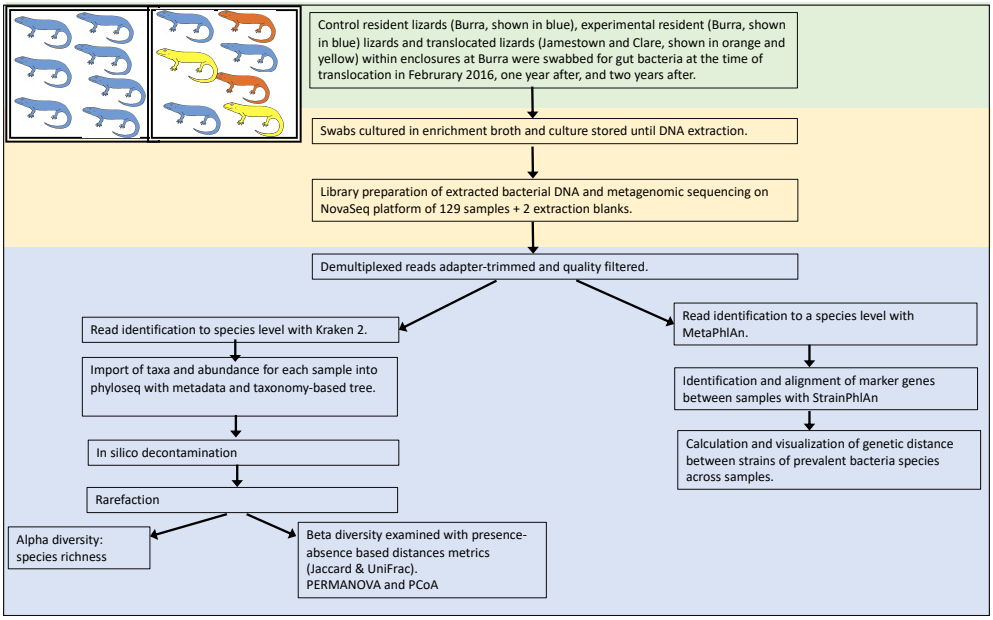


Figure 4.1. Experimental workflow for examining the effects of translocation and population augmentation on *Tiliqua adelaidensis* gut bacterial communities.

DNA extraction

Frozen cell cultures were warmed at 37°C and then re-pelleted for five minutes at 2000 rpm. DNA extraction methods were adapted from the Gentra® Puregene ® method for tissue (QIAGEN, 2014). Extractions were conducted in batches of 15–24. Extraction blank controls consisting of an empty tube were included for two batches as in Weyrich et al. (2018). Cell lysis buffer used consisted of 10mM Tris, 2% SDS, 0.1M EDTA at pH 8; and DNA hydration buffer used in the final step was TLE buffer. Extracted DNA was stored at 4°C until library preparation.

Library preparation

Samples of extracted DNA with Quantus ^{TM} Fluorometer (Promega, Madison, USA) -assessed concentrations of 15 ng/µl or higher were selected for library preparation for Illumina

sequencing using the method outlined by Meyer & Kircher (2010), which was adapted to the epMotion® 5075t liquid handling robot (Eppendorf South Pacific PTY Ltd., Macquarie Park, Australia). The "with-bead" method from Fisher et al. (2011) was used to minimise DNA loss associated with multiple elution steps, using Solid Phase Reversible Immobilisation (SPRI) with Agencourt AMPure XP magnetic beads (Beckman Coulter Australia, Lane Cove West, Australia) plus PEG as in Li et al. (2013). Sonication preceding blunt end repair and adapter ligation produced sheared DNA with a modal size of 400bp. Indexed samples were pooled in an equimolar manner. Three successive clean-ups of the pool were performed with the method adapted from Li et al. (2013) to ensure fragments were within an optimal size range. The first two clean-ups consisted of washing with 20% PEG with 0.8X Agencourt AMPure XP magnetic beads, then 0.5X AMPure with Tris and 0.1% Tween for elution. The library was then concentrated with centrivap for 10 min at 30°C, then the final clean with 1.2X AMPure with Tris buffer and 0.1% tween was conducted.

Sequencing

The cleaned library was paired-end sequenced (2X 250bp) on the Illumina NovaSeq 6000 platform (Illumina Inc. San Diego, USA) using an S Prime (SP) flowcell with 500 cycle SBS chemistry.

Pre-processing and classifying

Raw base call reads were demultiplexed for 129 experimental samples and two negative extraction controls and were converted to fastq format with bcl2fastq v2.20 (Illumina). Demultiplexed reads were adaptor-trimmed, bases with a quality score of 12 or lower were discarded, and PhiX contaminants were filtered using bbduk in the bbmap suite v.38.46 (Bushnell, 2019), before examination of reads with FASTQC (Andrews, 2010) (Figure 4.1). The DNA-DNA metagenomic classifier Kraken 2 (Wood et al., 2019) was then used to match trimmed and filtered paired reads (confidence= 0.05) to a database containing all bacteria in the Kraken 2 database.

Species-level analysis

Kraken reports were converted to BIOM-format with kraken-biom (Dabdoub, 2020) and imported into the R environment (R Core Team, 2020) for further analysis with the package 'phyloseq' (McMurdie and Holmes, 2013) (Figure 4. 1), alongside sample metadata and a taxonomy-based tree. This tree contained all bacterial taxa identified by Kraken 2 and was

constructed using NCBI's Taxonomy database Common Tree viewer (Federhen, 2012). Sample composition was examined with the R package 'decontam' (Davis et al., 2018) and likely contaminant taxa were identified using both frequency and prevalence-based criteria (threshold=0.5). Taxa that were identified as contaminants by either or both of these techniques were discarded from the phyloseq dataset. Following decontamination, these data were then split into two taxonomic groups: taxa belonging to the Enterobacteriaceae family and all other bacterial taxa (therein referred to as non-Enterobacteriaceae). The Enterobacteriaceae were examined separately to other taxa because these were targeted by the enrichment step and contain common commensal species which may become pathogens and their relatives. The decontaminated Enterobacteriaceae and non-Enterobacteriaceae datasets were then each rarefied to the lowest per-sample library size above 1,000 reads. Whilst rarefaction results in loss of information (McMurdie and Holmes, 2014), it has been deemed essential for subsequent presence/absence-based community composition analysis (Weiss et al., 2017).

Community analysis of species-level data

The alpha diversity of samples was quantified using species richness. Abundance was not considered in community composition analyses since the relative abundances of taxa within a sample were unlikely to reflect that of the biological system given the enrichment step (Pettengill et al., 2012). Furthermore, bias is inherent to all steps in even the culture-independent metagenomic workflow (McLaren et al., 2019). Beta diversity was compared using the presence-absence metrics unweighted UniFrac (Lozupone et al., 2011), which takes into account phylogenetic distance between taxa, and also Jaccard distance. Variation among samples were visualised with Principal Coordinates of Analysis (PCoA)(Gower, 1966).

The PERMANOVA+ extension of Primer 7 (Anderson et al., 2008) was used to conduct repeated measure permutational multivariate analyses of variance (PERMANOVA) with 9999 permutations on distance matrices of Enterobacteria and of non-Enterobacteria. Each of these two taxonomic groups were measured with UniFrac distance and Jaccard distance separately. Model design focused on the factors of population of origin (three levels), in order to comment on whether animals from Clare, Jamestown and Burra differed in community composition at time 0, and at 1- and 2-years following translocation. The other factor which was examined independently was treatment group (three levels). This factor was related to population of origin (partially nested), though examining differences between treatment groups specifically

sought to answer whether Burra residents showed a difference in bacterial community composition over time based on whether they were exposed to translocated conspecifics (experimental residents) or not (control residents). Potential confounding factors of sex (three levels: male, female, unknown), enclosure (six levels) and library batch (two levels) were evaluated by examining the change in model results when each term was added to the main model separately. These factors were included in the final model if their addition changed the significance of the original terms.

Prior to PERMANOVA testing, PERMDISP tests were used to examine dissimilarity between variances within factors of interest. For repeated measures of non-independent samples through time, non-significant PERMDISP results indicated that there was no artefact arising from sampling the same individuals through time.

Strain-level classification

In addition to species level classification by Kraken 2, trimmed and filtered reads were also classified by MetaPhlAn v.3.0 (Truong et al., 2015)(Figure 4.1). Within these classified reads, species-specific marker genes were identified by StrainPhlAn v 2.0 (Truong et al., 2017). StrainPhlAn then performed strain-level alignments of these marker genes for four of the most prevalent bacterial species for which marker genes were available (*Salmonella enterica*, *Klebsiella aerogenes*, *Escherichia coli* and *Enterococcus faecalis*) and called RAxML to build phylogenetic trees (Stamatakis, 2014). Genetic distances between strains characterised in different samples were calculated using the uncorrected method of the online application EMBOSS distmat (Carver, 2001). Phylogenetic trees were visualised using 'ggtree' (Yu et al., 2017) in the R environment (R Core Team, 2020). The two most prevalent species were examined for differences in genetic distances between host treatment and population groups using PERMDISP and PERMANOVA analysis, as with the species-level data described above.

Results

Sequencing and read pre-processing

Sequencing produced reads for all 129 bacterial cultures from *Tiliqua adelaidensis* cloacal swabs that underwent library preparation, as well as two extraction controls (subsequently referred to as controls). Raw read numbers ranged from 64,790–54,463,507 per sample, with a median read number of 4,580,419 reads (Supplementary material: Figure S4.1). After adapter trimming,

quality and PhiX contaminant filtering, numbers of reads ranged between 33,334–45,072,229 per sample, with a median of 3,987,636 reads (Supplementary material: Figure S4.1).

Read classification

Reads that were classified by Kraken 2 as bacterial DNA ranged from 21,457–43,890,168, reads per sample, with a median of 3,086,658 reads. In total, 3,901 bacterial taxa were identified across samples. Within these taxa, 1,364 were identified as likely contaminants and discarded, leaving 2,537 taxa. Of these 2,537 taxa, 117 belonged to the Enterobacteriaceae family. Rarefaction reduced Enterobacteriaceae sample libraries from as high as 43,286,539 to 11,242 reads per sample (Supplementary material: Figure S4.2). Non-Enterobacteriaceae taxa (2,406 taxa) accounted for up to 3,578,530 reads per sample, which were then rarefied to 1388 reads per sample (Supplementary material: Figure S4.3). One control and one experimental sample were excluded from the non-Enterobacteriaceae dataset, due to less than 1,000 reads being attributable to these taxa. After rarefaction, the Enterobacteriaceae dataset contained 112 taxa across all samples, and the non-Enterobacteriaceae dataset contained 1238 taxa. Samples grouped by host treatment group ('control residents' from Burra not mixing with translocated conspecifics, 'experimental residents' from Burra mixing with translocated conspecifics, and 'translocated' lizards from Clare and Jamestown mixing with experimental resident conspecifics) showed similar median species richness among groups, regardless of taxon (Enterobacteriaceae or non-Enterobacteriaceae) or whether rarefaction had been conducted (Supplementary material: Figures S4.4–S4.7).

Community analysis

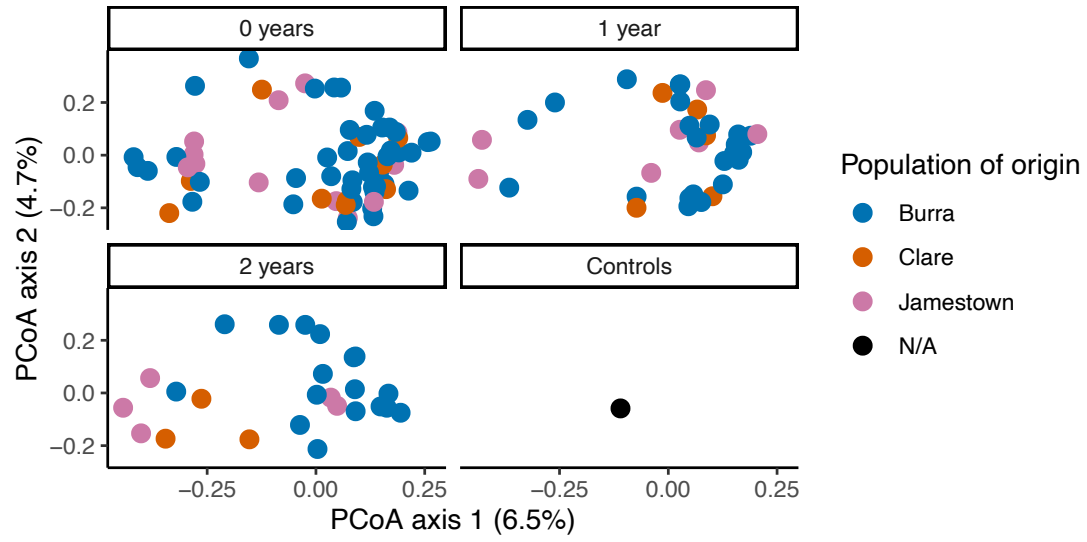
Differences in bacterial community composition at a species level did not appear to be driven by host population of origin at the time of translocation for any bacterial taxa that we cultured; PERMANOVA tests with repeated measures and Principal Coordinate Analysis did not show significant differences between samples from hosts with different population origins (Table 1, Figures 4.2a & 4.3, Supplementary material: Table S4.2). When samples were considered by host treatment group, there was some indication that treatment groups differed in non-Enterobacteriaceae community composition. Pairwise testing of a significant interaction between time and treatment for non-Enterobacteriaceal diversity, measured with phylogenetically weighted UniFrac distance, revealed differences between groups only at the two years post- translocation timepoint. At this timepoint, the pairwise tests indicated that translocated hosts had different communities compared to both Burra control residents, and

also to the Burra experimental residents that the translocated lizards shared habitat with (Table 4.1, Supplementary material: Table S4.3). When the same samples were measured with Jaccard distance, treatment approached significance as a PERMANOVA model term, though pairwise testing did not show any differences between specific pairs of timepoints (Table 4.1, Supplementary material: Table S4.3). PCoA ordination suggest that differences between treatment groups were small, since treatment-specific clustering was not evident (Figures 4.2b & 4.3, Supplementary material: Figure S4.10a).

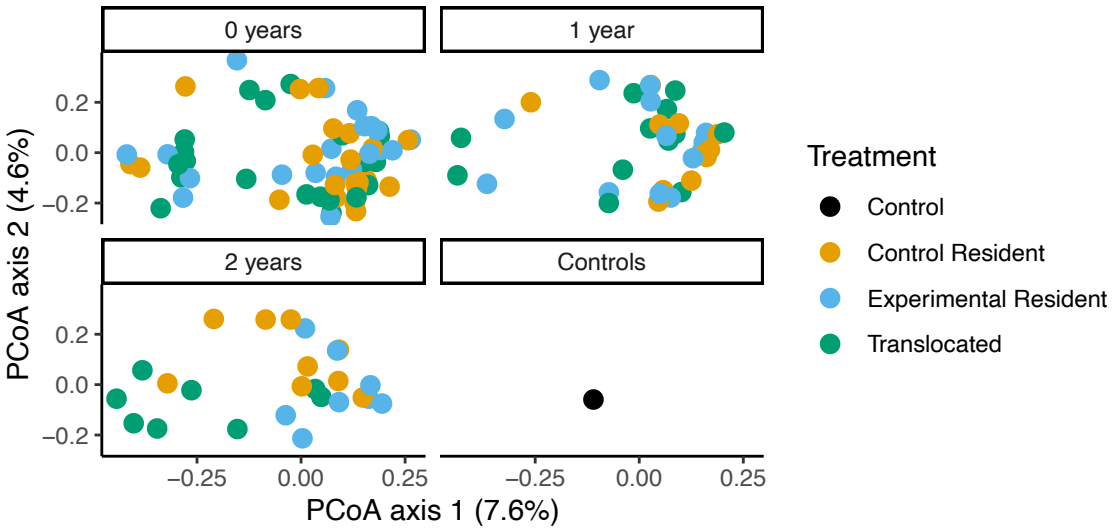
Enterobacteriaceal and non-Enterobacteriaceal diversity, when measured with phylogenetically weighted UniFrac distance, both showed some systemic variation over time, rather than population- or treatment- driven differences. For the non-Enterobacteriaceae with UniFrac distance, both the PERMANOVA models based on treatment group and the model containing population of origin revealed time to be a significant factor (Table 4.1). Pairwise testing suggested that these time differences were driven by differences in composition between one year and two years following translocation, rather than between the time of translocation and subsequent time points (Table 4.1, Supplementary material: Table S4.3). In contrast, temporal differences in Enterobacteriaceal diversity, when measured with UniFrac, were significant in the treatment-group based model between the point of translocation and two years after, and between one year after translocation and two years after. Despite the statistically significant effects, PCoA ordination suggests that whilst clusters decrease in size due to attrition of hosts over time, there was no clear difference between different time points (Figures 4.2 & 4.3, Supplementary material: Figure S4.10).

Table 1. Summary of PERMANOVA with repeated measures assessing the difference in bacterial community composition in *Tiliqua adelaidensis* individuals grouped by population origin or treatment group. See Supplementary material: Tables S4.1–S4.3 for full PERMANOVA results.

Question	Dataset	Permdisp test for time	Final PERMANOVA model	Significant effects of interest	Significant pairwise tests for significant effects
Are populations different over time?	Non-Enterobacteriaceae, UniFrac distance	F=0.11 p=0.92	Time x Population x Sex x Library prep. batch	Time, p=0.02	T1 vs. T2, p= 0.04
	Non-Enterobacteriaceae, Jaccard distance	F=1.84 p=0.23	Time x Population x Library prep. batch	-	-
	Enterobacteriaceae, UniFrac distance	F=2.125 p=0.176	Time x Population x Sex x Library prep. batch	-	-
	Enterobacteriaceae, Jaccard distance	F=5.51 p=0.026* Pairwise: T0 vs T2: t=3.16, p=0.022 T1 vs T2: t=2.99, p=0.006	Time x Population x Sex x Library prep. batch	-	-
Are treatment groups different over time?	Non-Enterobacteriaceae, UniFrac distance	F=0.11 p=0.92	Time x Treatment x Sex x Library prep. batch	Time, p=0.02 Time x Treatment: p=0.01	T2: Translocated vs. Control resident, p=0.02 Translocated vs. Experimental resident, p=0.01
	Non-Enterobacteriaceae, Jaccard distance	F=1.84 p=0.23	Time x Treatment x Library prep. batch	Treatment, p=0.05	-
	Enterobacteriaceae, UniFrac distance	F=2.125 p=0.176	Time x Treatment	Time: p=0.002	T0 vs. T2, p=0.01 T1 vs T2, p=0.03
	Enterobacteriaceae, Jaccard distance	F=5.51 p=0.026* Pairwise: T0 vs T2: t=3.16, p=0.022 T1 vs T2: t=2.99, p=0.006	Time x Treatment x Library prep. batch	-	-



a)



b)

Figure 4.2. PCoA ordination of non-Enterobacteriaceae taxa in *Tiliqua adelaidensis* individuals measured with UniFrac distance by host group over time a) hosts grouped by population of origin b) hosts grouped by treatment.

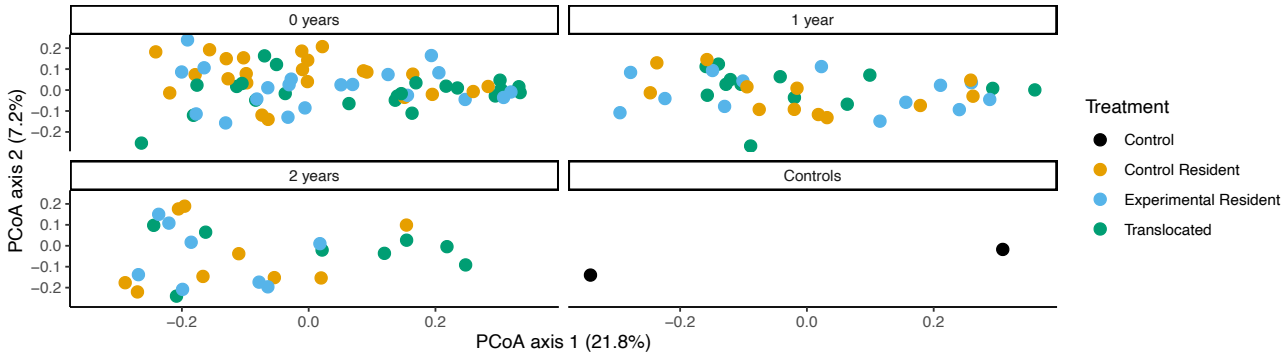


Figure 4.3. PCoA ordination of Enterobacteriaceae in *Tiliqua adelaidensis* individuals measured with UniFrac distance, by treatment group, over time.

Strain-level analysis

Variation in *Salmonella enterica* marker genes, identified in 80 of 129 *T. adelaidensis* samples, was not strongly associated with host population of origin at the time of translocation, nor was it at subsequent time points (Table 2, Supplementary material: Table S4.4 & Figure S4.11). In contrast, both time and the interaction between time and treatment were significant terms in the PERMANOVA model examining the effect of host treatment group over time on *S. enterica* strains (Table 4.2). These results suggest that the genetic distance between strains of *S. enterica* did vary over the three years, though pairwise tests between years did not reveal a significant difference (Table 4.2), nor did PCoA clusters or the phylogenetic relationships look clearly different between years (Figures 4.4 & 4.5). Pairwise testing of the time-treatment interaction indicates that differences in strains were significant between experimental Burra residents and translocated hosts they shared enclosures with at one- and two-years following translocation, but not at the time of translocation (Table 4.2). These treatment specific differences may be small as PCoA ordination does not show clear clustering of treatment groups at the one- and two-year post-translocation timepoints (Figure 4.4).

Marker genes for *Klebsiella aerogenes* were identified across 57 of 129 *T. adelaidensis* samples. Variation in *K. aerogenes* strains were not related to host population of origin or treatment group (Supplementary material Figures S4.12 & S4.12), and did not appear to vary in a predictable way through time (Table 4.2). Marker genes for *Enterococcus faecalis* and

Escherichia coli were identified in eight and six *T. adelaidensis* samples respectively, with these low numbers precluding further analysis of strain variation between host groups.

Table 4.2. Summary of PERMANOVA with repeated measures assessing the variation in bacterial strains of *Salmonella enterica* & *Klebsiella aerogenes* in PBT individuals grouped by population origin or treatment group. See Supplementary material: Tables S4.1–S4.3 for full PERMANOVA results.

Question	Bacterial species	PERMDISP tests for time	Final PERMANOVA model	Significant effects of interest	Pairwise tests for significant effects
Are populations different over time?	*Salmonella enterica*	F=0.29 p=0.88	Population x Time x Sex		
	Klebsiella aerogenes	F=1.25 p=0.46	Population x Time	-	
Are treatment groups different over time?	*Salmonella enterica*	F=0.29 p=0.88	Time x Treatment x Enclosure	Time, p=0.01 Treatment x Time, p=0.01	T1: Experimental Resident vs. Translocated, P=0.03 T2: Experimental Resident vs. Translocated, P=0.03
	Klebsiella aerogenes	F=1.25 p=0.46	Treatment x Time	-	-

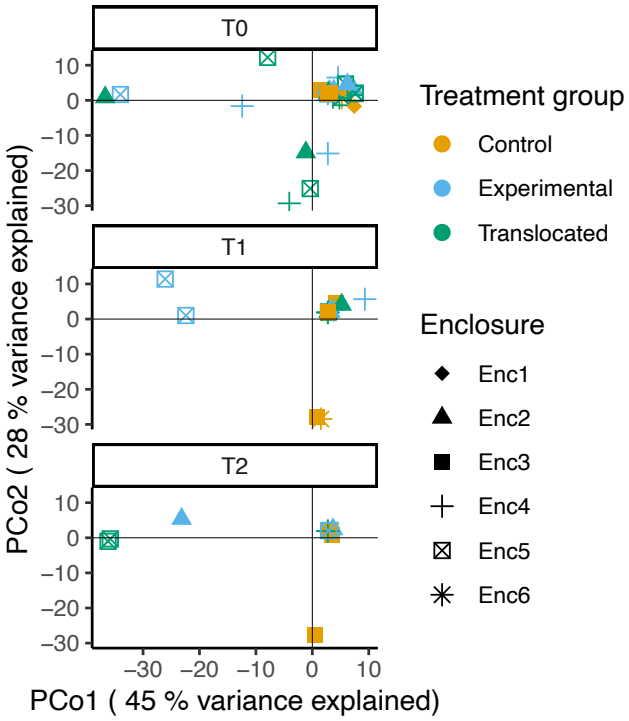


Figure 4.4. *Salmonella enterica* strain variation in *Tiliqua adelaidensis* individuals grouped by host treatment group and enclosure at 0, 1, and 2 years following translocation.

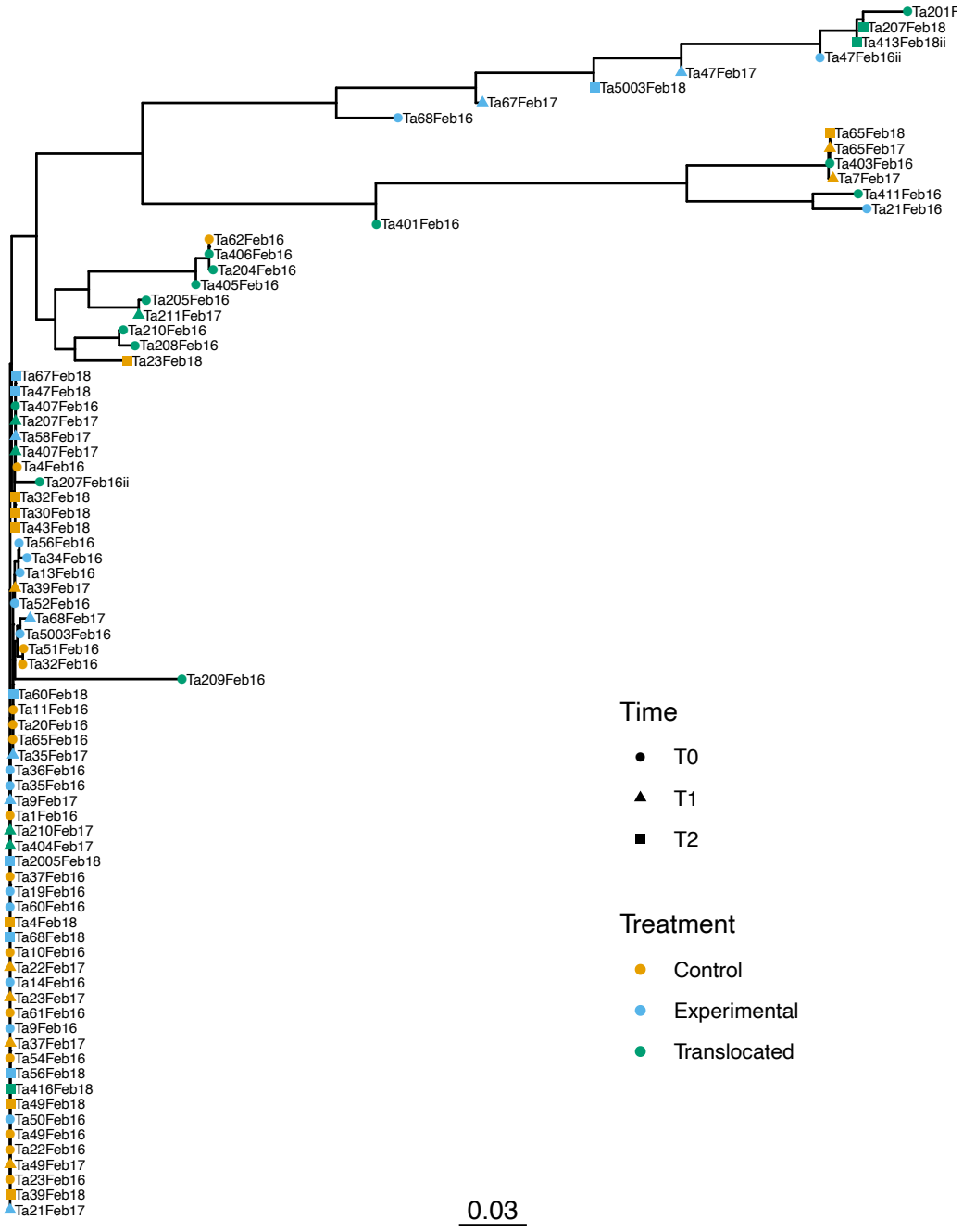


Figure 4.5. The phylogenetic relationship between *Salmonella enterica* strains by *Tiliqua adelaidensis* host treatment group and time point. Notes: T0 is at translocation in February 2016, T1 is one year after in February 2017, T2 is two years after in February 2018). Label names indicate host ID and collection date. Genetic distance measured in number of nucleotide base substitutions per 100.

Discussion

To assess the potential impact of translocation on species fitness, we examined gut microbiota variation among wild *Tiliqua adelaidensis* populations and sought to determine how translocation affects these microbiota. To date, very few studies have examined microbiota of wild, translocated individuals in a wild recipient setting (Chong et al., 2019). The Enterobacteriaceal and non-Enterobacteriaceal communities identified from *T. adelaidensis* cloacal samples showed little support for our hypothesis that community composition would differ at a species- and subspecies-level between isolated host populations. Samples collected immediately prior to the translocation from lizards from the Clare and Jamestown source populations and the recipient Burra population, show no consistent differences for either bacterial taxonomic group at a species level (Table 4.1 & Figure 4.2). Strain-level differences for *Salmonella enterica* and *Klebsiella aerogenes* were also inapparent between host populations at the time of translocation (Table 4.2, Figure 4.4, Supplementary material: Figure S4.12).

The lack of observed population-level difference in both Enterobacteriaceal and non-Enterobacteriaceal species and sub-species present at the time point of translocation may be due to having detected part of the 'core' gut microbiome, where all or most *T. adelaidensis* individuals host a consistent set of bacterial species regardless of location, as in some other species (Cahill et al., 2016; West et al., 2019). The gut microbiota of omnivores may be less influenced by diet than that of herbivores, as observed in iguanid lizards (Kohl et al., 2017). Similarly, the microbiota of baboons with omnivorous diets changed less between intact and fragmented habitats than that of sympatric, folivorous colobus monkeys (Barelli et al., 2020). Since *T. adelaidensis* eats a majority of insects to plant matter (Fenner et al., 2007), the exact food items it consumes may be less of an influence on gut microbiota and contribute to the ubiquity across populations that we observed. Also consistent with the lack of inter-population difference is the possibility that the microbial environment (including habitat and diet) an individual is exposed to does not vary largely across ecologically and climatically similar sites in the same region. Some *Salmonella enterica* subspecies sampled from *T. rugosa* guts were found by Parsons et al. (2015) to vary by micro-habitat type, such as amount of shade available. The grassland habitat of *T. adelaidensis* is, at least apparently, homogenous in habitat structure, and this may contribute to the observed lack of structure in *S. enterica* and *K. aerogenes* strains. Any relationship between geographical proximity and microbial community similarity is also likely to be influenced by the colonisation history of the host species (Lankau et al., 2012).

Sampling of *T. adelaidensis* gut microbiota at the time of translocation and at one and two years subsequent did not reveal any obvious temporal trends for any particular treatment- or population- based group, nor for the whole cohort. As such, there was no support for our hypothesis that gut microbiota of hosts from isolated populations became more similar to each other over time as they shared habitat at the recipient site. This lack of clear change over time despite the translocation again suggests that fine-scale environmental differences may play only a limited role in shaping the bacterial communities detected. In contrast, a previous study characterising wild-wild translocation gut microbiota changes in a carnivorous marsupial found that initially distinct translocated microbiomes came to more strongly resemble resident conspecifics over time, though with retention of some population- specific taxa (Chong et al., 2019). These shifts in the microbiome towards that of the resident conspecifics may have been driven by clear differences in environment and local diet prior to translocation (Chong et al., 2019).

An animal's microbial environment also entails interactions with conspecifics, since these often present transmission opportunities. The role of social interactions in shaping gut microbiota varies among and also within species (Sarkar et al., 2020). The lack of difference among populations and the negligible effect of translocation may be consistent with the solitary social system of *T. adelaidensis*, where conspecific interactions are not frequent or prolonged enough to drive changes in microbiota. By contrast, in the related, but more social host *Tiliqua rugosa*, relatedness of *Salmonella enterica* strains was found to reflect conspecific interactions more strongly than shared habitat (Bull et al., 2012). Similar results have been reported for *Escherichia coli* in highly social primates and giraffes (Balasubramaniam et al., 2019; Tung et al., 2015; VanderWaal et al., 2014). In less social primate systems, an individual's sociability had a smaller effect on overall gut microbiome composition than on individuals in more social primate species (Amato et al., 2017; Sarkar et al., 2020), and this may also be the case among *Tiliqua* species.

Despite the lack of clear shifts in gut bacteria taxa detected relating to our hypotheses, we did observe some small differences which may indicate the dynamic nature of bacterial communities. Temporal variation has been observed in the gut microbiota of other wildlife species. The time scale of change may range from a few hours for pythons that are fasting or feeding (Costello et al., 2010), over the course of weeks for wild squirrels (Bobbie et al., 2017), or seasons for other mammal species that experience changes in diet and social activity

(Maurice et al., 2015; Raulo et al., 2018). The sampling point two years after our *T. adelaidensis* translocation appears to have been slightly different from the preceding two years in terms of the Enterobacteriaceal and non-Enterobacteriaceal taxa detected (only detected when phylogenetic relationships were considered with UniFrac distance) (Table 4.1). This pattern may have been driven by external factors such as prey availability or climatic conditions. Local weather records indicate that whilst annual rainfall for those years ranged between 370 mm and 564 mm, the combined rainfall for January and February was lowest in the final year (16 mm compared to 51 mm and 64 mm) (BOM, 2020). Following up non-translocated individuals in a number of source populations over time may better elucidate temporal dynamics.

In addition to ubiquitous temporal variation, there was some evidence that changes in gut bacteria over time may be host-group specific. A difference in non-Enterobacteriaceae taxa between translocated individuals and both experimental and control Burra residents was evident at two years after the translocation (Table 4.1). Here, pooling Clare-originating and Jamestown-originating hosts may indicate that there were some population-based differences with very small effect sizes. Genetic distance in *Salmonella enterica* also varied slightly between translocated and experimental residents at one and two years following the translocation, though neither of these groups were significantly different to control residents (Table 4.2, Figure 4.5, Figure 4.7). These results suggest that the microbiota of lizards may be affected differently by stochastic changes in bacteria over time, based on their origin. Population of origin is associated with genetic differences in the lizard host (Smith et al., 2009), and perhaps also differences in epigenetic patterns or in an unmeasured aspect of their pre-translocation microbiome that could produce a time-population interaction. Genotype-microbiome interactions have been documented in humans and other animal and plant taxa (Goodrich et al., 2016; Kolde et al., 2018).

To ascertain whether the lack of differences in microbiota over geographical space and time are reflective of a broader range of bacterial taxa and also host populations, future studies would benefit from a number of features and techniques complementary to those used here. Firstly, sampling a larger number of host individuals from a larger number of isolated populations may detect what our results suggest would be small differences between gut microbiota communities. Future population augmentation studies should ideally also monitor non-translocated hosts at source populations over time, to elucidate temporal dynamics and any interaction with environment and genotype. Focusing on a culturable subset of bacteria may also have obscured broader differences between hosts or time points by not detecting

taxa, but also by not providing information on relative abundance — something culture-independent whole genome approaches would be best able to do. Finally, contamination remains a challenge in microbiota characterisation, with even sequencing from ultra-clean DNA laboratories detecting environmental contamination (Davis et al., 2018; Weyrich et al., 2019). Despite precautions taken and the in-silico decontamination performed, our two control samples (taken from the DNA extraction through the library preparation process) contained bacteria similar to the host samples (Figures 4.2–4.4), indicating that contamination occurred either from the laboratory environment or from other samples occurred. Here the sequencing of more control samples and sample spiking described by Zinter et al. (2019) would allow more accurate identification of contaminants. However, if contamination was obscuring any differences completely, we would expect to see inter-group differences reduce with sample size, which is not the case — two years post translocation had the most marked inter-group difference despite the smallest sample sizes.

Our results suggest that there are not enough detected differences in the gut microbiota among isolated host populations and within populations following translocation to have obvious implications for host fitness. Our collective understanding of the links between microbiota composition and specific fitness outcomes remains poor, though in this *T. adelaidensis* population augmentation, survival over the post translocation period of two years was not different between translocated, resident experimental or resident control animals (see Chapter 5), and reproductive output was consistent across groups (Clive, 2019). In the absence of targeted testing for associations between presence and differential abundance of specific taxa with finer resolution measures of fitness, this available evidence suggests that wild-wild translocation is unlikely to have microbiota fitness-related consequences for *T. adelaidensis*, and should be further considered as a conservation strategy for this species.

In addition to results favourable to translocation as a conservation strategy, this study does suggest that, like in the congener *T. rugosa* and many reptile species, potentially pathogenic bacteria such as *Salmonella enterica* are widespread in *T. adelaidensis* (Bull et al., 2012; Mitchell and Shane, 2001; Schumacher, 2006). Infection by *Salmonella spp.* are mostly asymptomatic, however all species and serovars are considered potential pathogens and can cause enteritis, nephritis, septicaemia, and other potentially fatal pathologies in reptiles, as well as presenting a zoonotic risk (Mitchell and Shane, 2001). Pathology is usually associated with host stress, provoked by environmental conditions such as overcrowding and unhygienic conditions in captivity (Mitchell and Shane, 2001; Schumacher, 2006). The mechanism by which elevated

cortisol levels can increase host *Salmonella* loads has been identified in mice (Verbrugghe et al., 2016).

The potential for stress-induced pathology from gut bacteria provides further reason to minimise stress to translocated wildlife. In reptiles, a common stress response to translocation is post-release dispersal (Germano and Bishop, 2009). This experimental translocation of *T. adelaidensis* was informed by several previous studies of the species biology, and sought to reduce stress-induced post-release dispersal by providing adequately spaced artificial dowel burrows at the recipient site (Ebrahimi and Bull, 2014a; Souter et al., 2004). Post-release dispersal was also reduced by performing the translocation at the end of the spring-summer activity season, well after breeding activities were completed, and by employing soft-release techniques (Ebrahimi and Bull, 2014b, 2013).

Conclusion

In this study, we have contributed to characterising the gut microbiome in the endangered skink *T. adelaidensis* in its native habitat, both over space and time. Our study takes a 'holobiont' approach to conservation management, in addition to helping address the lack of knowledge on microbial ecology in lizards and in wildlife generally (Colston and Jackson, 2016; Kohl et al., 2017). In a holobiont approach, an individual animal is accompanied by a suite of bacteria and other organisms that may be tightly ecologically and evolutionarily linked to its continued fitness and persistence in the ecosystem, thus requiring consideration during interventions such as translocations (Carthey et al., 2020). While wild-wild translocation does not appear to have disruptive effects on the *T. adelaidensis* gut microbiota, this work further underlines the continued importance of characterising wildlife microbiota and also better understanding its functional significance. In the absence of evidence of marked and potentially harmful changes the gut-microbiota translocating *T. adelaidensis* we recommend translocation be further considered as a conservation strategy for this species. Whilst a theoretical risk of pathogen transmission clearly remains, it is important to weigh the small effect suggested by our results against the risk of not acting to avoid extinction (Fordham et al., 2012; Meek et al., 2015; Scheele et al., 2018).

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Supplementary Material

Results

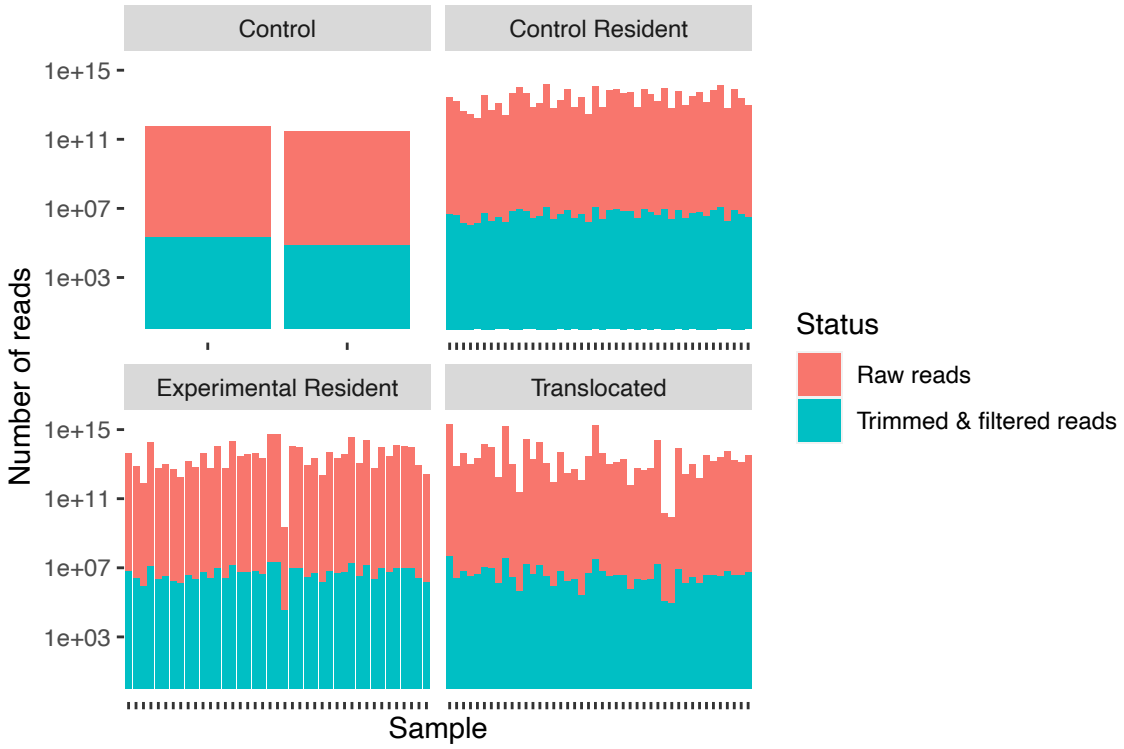


Figure S4.1. Numbers of reads generated by Illumina NovaSeq sequencing of *Tiliqua adelaidensis* cloacal bacterial cultures from individuals in different treatment groups.

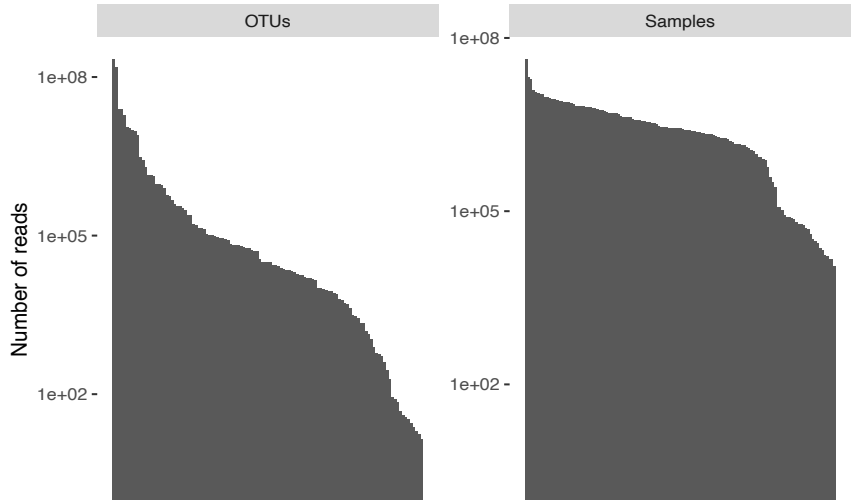


Figure S4.2. Number of sequencing reads from *Tiliqua adelaidensis* cloacal cultures attributed to the bacterial family Enterobacteriaceae by Operational Taxonomic Unit (OTU) and by sample, prior to rarefaction. Note: Taxonomic classification was performed on trimmed and filtered reads by Kraken 2.

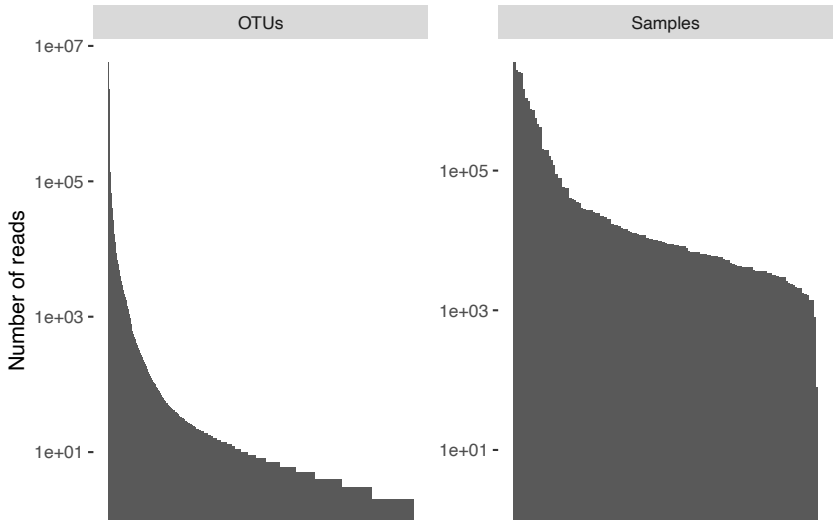


Figure S4.3. Number of sequencing reads from *Tiliqua adelaidensis* cloacal cultures attributed to the bacterial families other than Enterobacteriaceae by Operational Taxonomic Unit (OTU) and by sample, prior to rarefaction. Note: Taxonomic classification was performed on trimmed and filtered reads by Kraken 2.

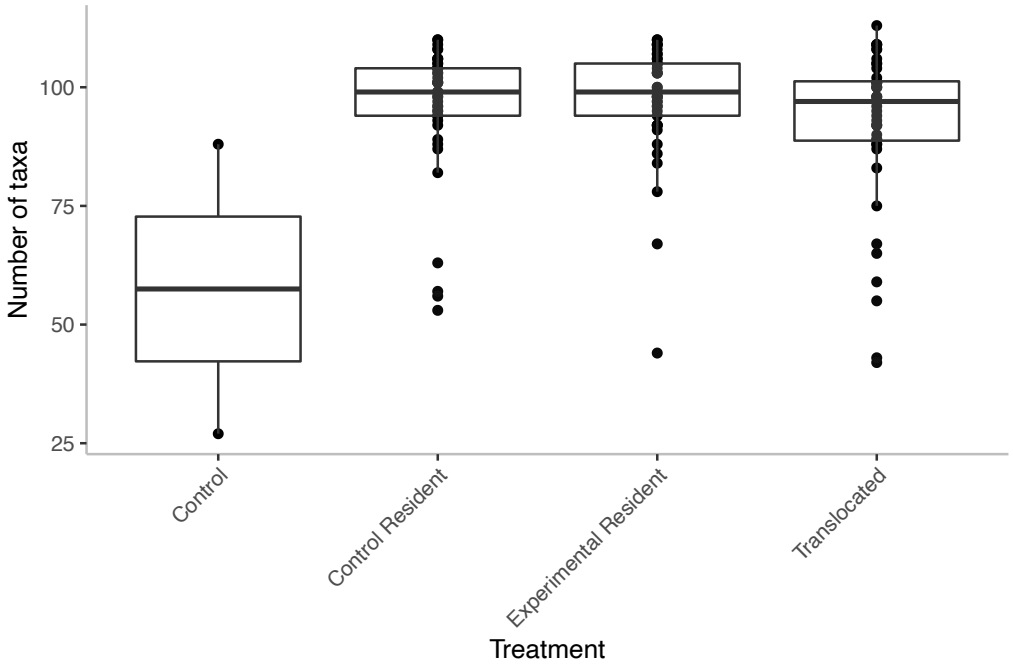


Figure S4.4. Number of Enterobacteriaceae taxa (species richness) of *Tiliqua adelaidensis* cloacal cultures, prior to rarefaction, by host treatment group.

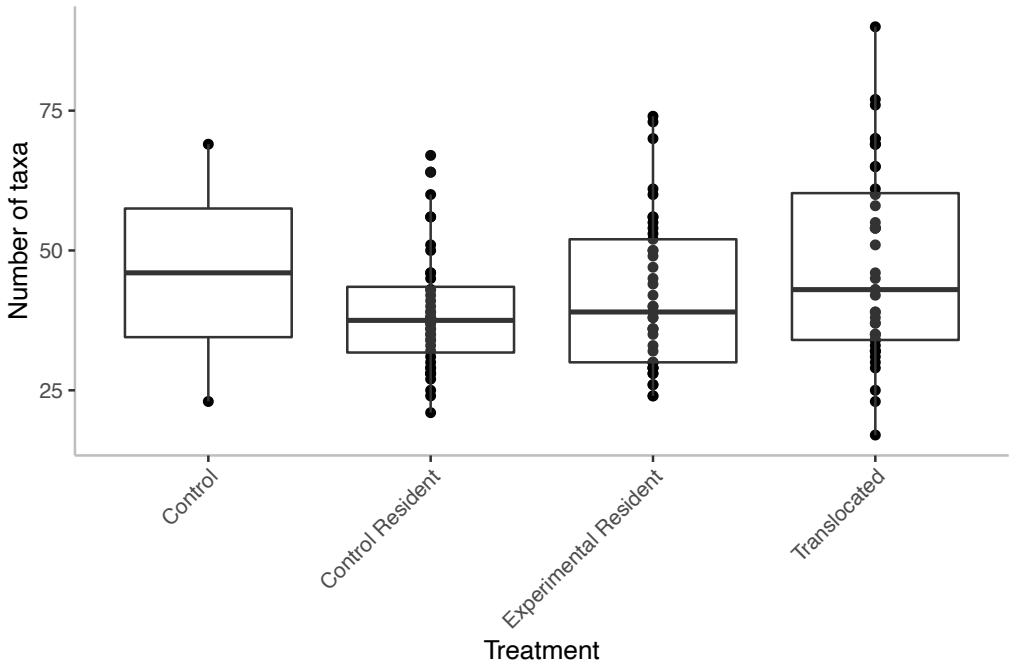


Figure S4.5. Number of Enterobacteriaceae taxa (species richness) of *Tiliqua adelaidensis* cloacal cultures, following rarefaction, by host treatment group.

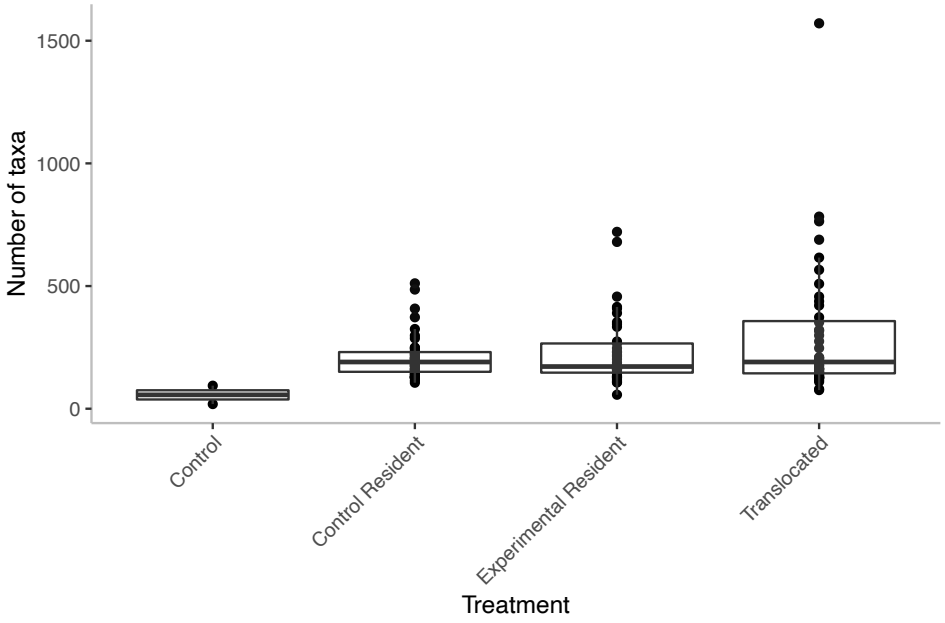


Figure S4.6. Number of taxa (species richness) outside of the Enterobacteriaceae family of *Tiliqua adelaidensis* cloacal culture samples, prior to rarefaction, by treatment group.

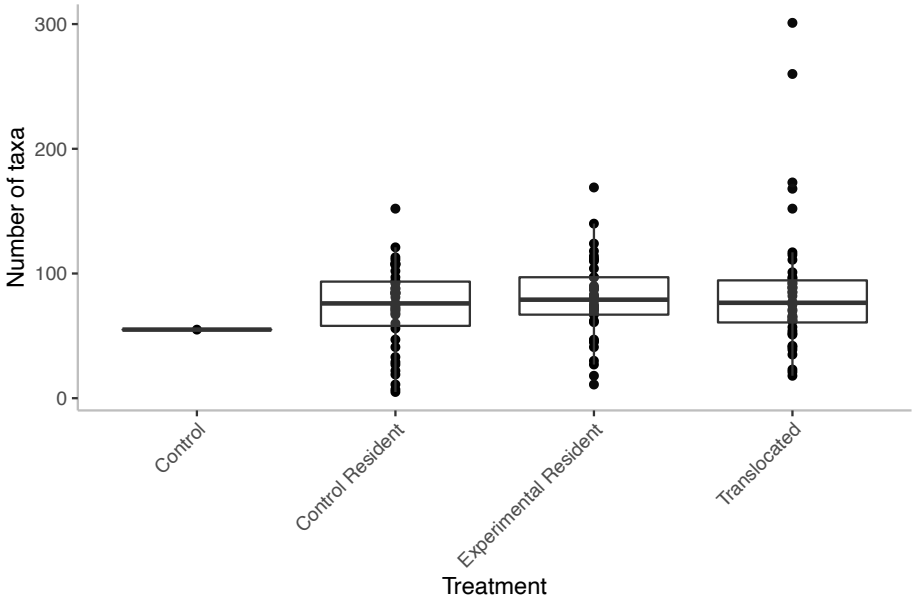


Figure S4.7. Number of taxa (species richness) outside of the Enterobacteriaceae family of *Tiliqua adelaidensis* cloacal culture samples, following rarefaction, by treatment group.

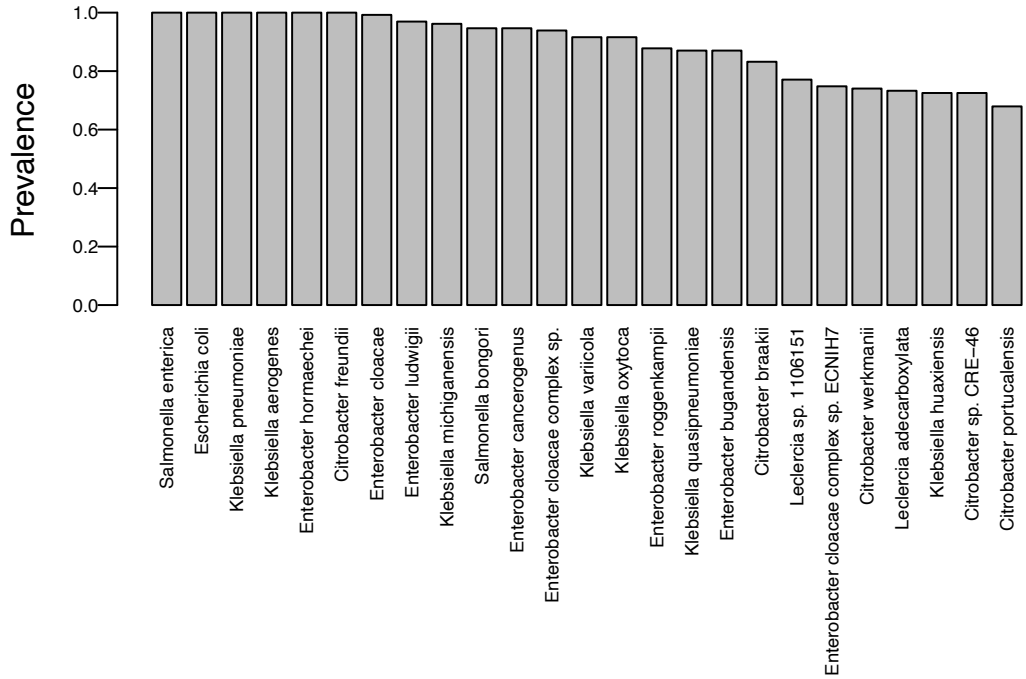


Figure S4.8. Prevalence of 25 most prevalent species belonging to the Enterobacteriaceae family across *Tiliqua adelaidensis* cloacal culture samples after rarefaction was performed.

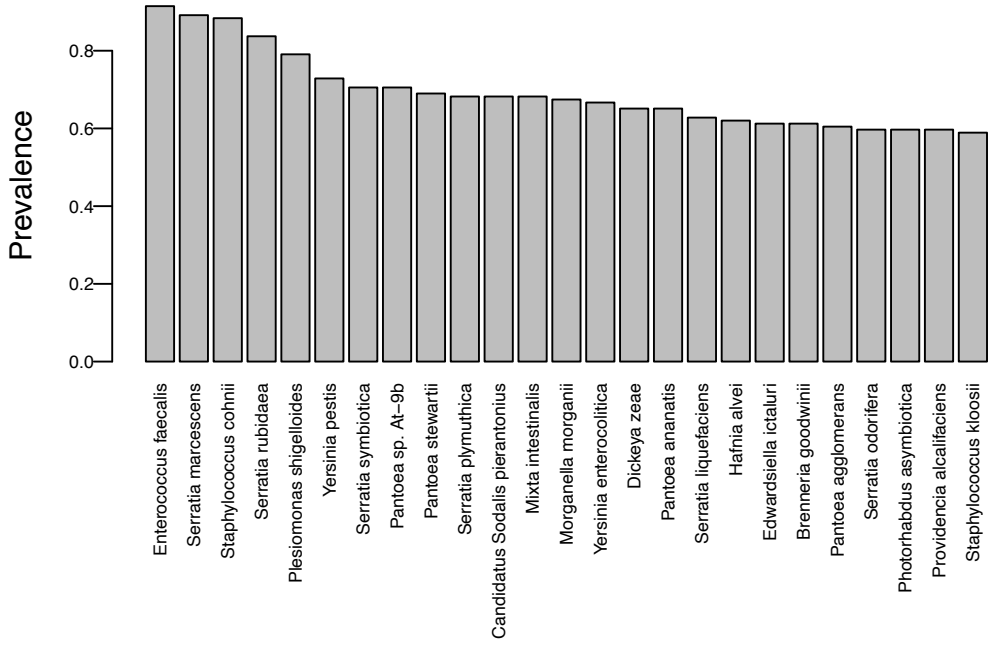


Figure S4.9. Prevalence of 25 most prevalent species belonging to bacterial taxa outside of the Enterobacteriaceae family across *Tiliqua adelaidensis* cloacal culture samples after rarefaction was performed.

Table S4.1. PERMANOVA models and results comparing Enterobacteriaceae and non-Enterobacteriaceae bacterial microbiomes in *Tiliqua adelaidensis* across host treatment groups.

Note: Two different beta diversity metrics are used for each of the two taxonomic group to measure community similarity: Jaccard distance and unweighted UniFrac. * denotes a significant p value of $p<0.05$.

Data	Final Model	Model term	Degrees of Freedom	Sums of squares	Mean of Squares	Pseudo-F	p	Unique perm-utations
Entero-bacteriaceae, Jaccard distance	Time x Treatment x Library batch	Time	2	1.07	0.53	1.85	0.06	9923
		Treatment	2	0.61	0.31	1.07	0.43	9789
		Library batch	1	0.59	0.59	1.59	0.27	360
		Time x Treatment	4	1.14	0.28	0.98	0.46	9905
		Time x Library batch	2	0.75	0.37	1.29	0.21	9939
		Treatment x Library batch	2	0.91	0.46	1.58	0.11	9915
		Residual	115	33.30	0.29			
		Total	128	39.41				
Entero-bacteriaceae, UniFrac distance	Time x Treatment	Time	2	0.60	0.30	2.07	0.00*	998
		Treatment	2	0.40	0.20	1.32	0.25	997
		Time x Treatment	4	0.61	0.15	1.06	0.31	996
		Residual	120	17.30	0.14			
		Total	128	18.91				
Non-Entero-bacteriaceae, Jaccard distance	Time x Treatment x Library batch	Time	2	0.51	0.25	0.87	0.59	9960
		Treatment	2	0.56	0.28	0.92	0.50	9830

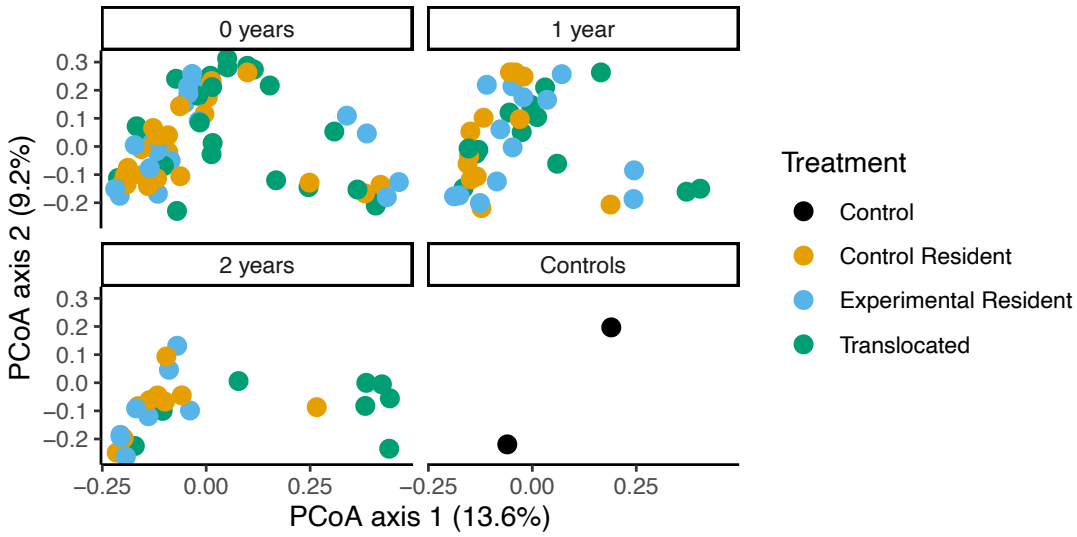
		Library batch	1	0.68	0.68	0.86	0.46	360
		Time x Treatment	4	1.23	0.31	1.05	0.44	9927
		Time x Library batch	2	1.59	0.80	2.72	0.00*	9952
		Treatment x Library batch	2	0.39	0.19	0.66	0.73	9939
		Residual	114	33.35	0.29			
		Total	127	38.94				
Non- Entero-bacteriaceae, UniFrac distance	Time x Treatment x Sex x Library batch	Time	2	0.82	0.41	1.36	0.02*	9782
		Treatment	2	0.74	0.37	1.07	0.44	9811
		Sex	2	0.63	0.32	0.91	0.56	9800
		Library batch	1	0.74	0.74	1.49	0.22	360
		Time x Treatment	4	1.55	0.39	1.29	0.01*	9673
		Time x Sex	4	1.43	0.36	1.18	0.05	9724
		Time x Library batch	2	1.52	0.76	2.52	0.00*	9782
		Treatment x Sex	4	1.14	0.28	0.94	0.72	9706
		Treatment x Library batch	2	0.62	0.31	1.02	0.41	9786
		Sex x Library batch	2	0.73	0.36	1.21	0.08	9805
		Residual	102	30.81	0.30			
		Total	127	42.08				

Table S4.2. PERMANOVA models and results comparing Enterobacteriaceae and non-Enterobacteriaceae bacterial microbiomes in *Tiliqua adelaidensis* across host population of origin groups. Notes: Two different beta diversity metrics are used for each of the two taxonomic group to measure community similarity: Jaccard distance and unweighted UniFrac. * denotes a significant p value of p<0.05.

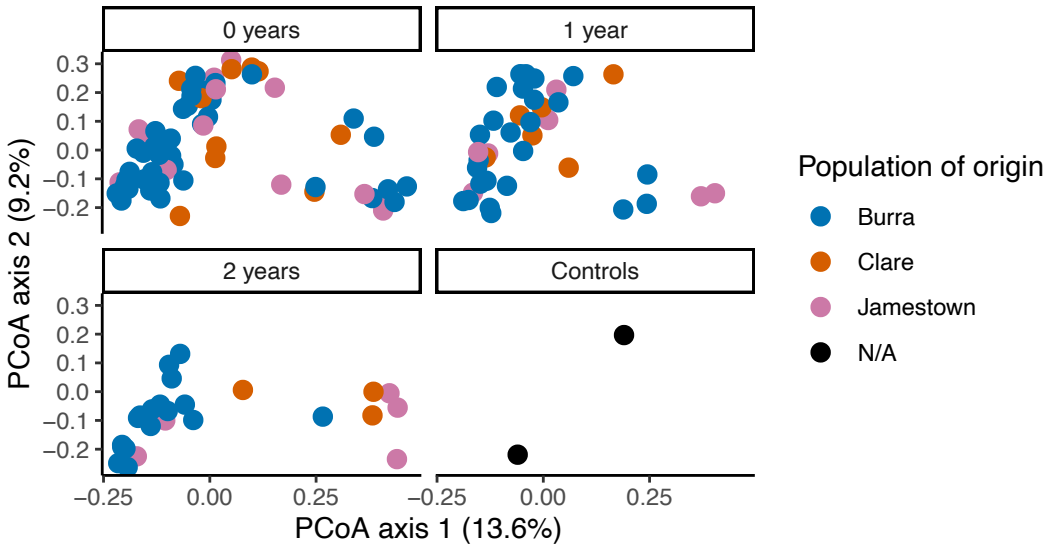
Data	Final Model	Model term	Degrees of Freedom	Sums of squares	Mean of Squares	Pseudo-F	p	Unique perm-utations
Entero-bacteriaceae, Jaccard distance	Population x Time x Sex x Library batch	Time	2	0.63	0.31	1.07	0.38	9930
		Population	2	0.94	0.47	1.58	0.19	9834
		Sex	2	0.37	0.19	0.74	0.70	9796
		Library batch	1	0.72	0.72	2.32	0.19	360
		Time x Population	4	1.22	0.30	1.04	0.37	9905
		Time x Sex	4	0.87	0.22	0.75	0.77	9902
		Time x Library batch	2	0.74	0.37	1.28	0.23	9938
		Population x Sex	4	1.70	0.43	1.46	0.09	9918
		Population x Library batch	2	0.71	0.36	1.22	0.25	9920
		Sex x Library batch	2	0.21	0.10	0.36	0.98	9928
		Residual	103	29.99	0.29			
		Total	128	39.41				
Entero-bacteriaceae, UniFrac distance	Population x Time x Sex x Library batch	Time	2	0.35	0.18	1.26	0.14	9886

		Population	2	0.18	0.09	0.64	0.83	9792
		Sex	2	0.29	0.14	0.99	0.52	9782
		Library batch	1	0.20	0.20	1.37	0.30	360
		Time x Population	4	0.58	0.15	1.04	0.39	9848
		Time x Sex	4	0.60	0.15	1.06	0.34	9830
		Time x Library batch	2	0.32	0.16	1.13	0.27	9895
		Population x Sex	4	0.37	0.09	0.67	0.99	9870
		Population x Library batch	2	0.41	0.20	1.45	0.06	9885
		Sex x Library batch	2	0.26	0.13	0.93	0.57	9884
		Residual	103	14.49	0.14			
		Total	128	18.91				
Non-Entero-bacteriaceae, Jaccard distance	Population x Time x Library batch	Time	2	0.87	0.43	1.51	0.18	9951
		Population	2	1.54	0.77	2.45	0.09	9815
		Library batch	1	0.74	0.74	1.34	0.23	360
		Time x Population	4	1.31	0.33	1.15	0.35	9939
		Time x Library batch	2	1.76	0.88	3.07	0.00*	9950
		Population x Library batch	2	0.84	0.42	1.46	0.19	9945
		Residual	114	32.58	0.29			
		Total	127	38.94				
Non- Entero-bacteriaceae, UniFrac distance	Population x Time x Sex x	Time	2	0.83	0.41	1.37	0.02*	9807

	Library batch							
		Population	2	0.78	0.39	1.22	0.29	9799
		Sex	2	0.65	0.32	0.98	0.53	9805
		Library batch	1	0.46	0.46	1.09	0.34	360
		Time x Population	4	1.39	0.35	1.15	0.06	9720
		Time x Sex	4	1.41	0.35	1.17	0.06	9718
		Time x Library batch	2	1.64	0.82	2.72	0.00*	9784
		Population x Sex	4	1.08	0.27	0.90	0.86	9695
		Population x Library batch	2	0.65	0.33	1.08	0.25	9774
		Sex x Library batch	2	0.73	0.36	1.21	0.07	9766
		Residual	102	30.71	0.30			
		Total	127	42.08				



a)



b)

Figure S4.10. PCoA ordination of all detected bacteria taxa (Enterobacteriaceae and non-Enterobacteriaceae combined) in *Tiliqua adelaidensis* individuals measured with UniFrac distance, a) coloured by treatment group, over time and b) coloured by host population of origin.

Table S4.3. PERMANOVA pairwise tests for statistically significant terms of interest in the models listed in Tables S4.1 & S4.2. Notes: These include pairwise tests conducted for two taxonomic bacterial subsets: Enterobacteriaceae and non- Enterobacteriaceae, each measured with two distance metrics, Jaccard and Unifrac. Models tested host individuals grouped by either treatment or population of origin. * denotes a significant p value of $p<0.05$.

Data set, factor of interest & significant model term				
Enterobacteriaceae, UniFrac distance, Treatment				
Time	Groups	t	P(perm)	Unique permutations
	T0, T1	1.09	0.23	9903
	T0, T2	1.80	0.00	9895
Non-Enterobacteriaceae, UniFrac distance, Treatment				
Time x Treatment	Within level 'T0' of factor 'Time'			
	Groups	t	P(perm)	Unique permutations
	Translocated, Control Resident	1.0969	0.15	9846
	Translocated, Experimental Resident	1.0468	0.28	9855
	Control Resident, Experimental Resident	0.96046	0.60	9857
	Within level 'T1' of factor 'Time'			
	Groups	t	P(perm)	Unique permutations
	Translocated, Control Resident	No test, df = 0		
	Translocated, Experimental Resident	1.204	0.03	9861
	Control Resident, Experimental Resident	No test, df = 0		
	Within level 'T2' of factor 'Time'			
	Groups	t	P(perm)	Unique permutations
	Translocated, Control Resident	1.3587	0.02	9917
	Translocated, Experimental Resident	1.4806	0.01	9927

	Control Resident, Experimental Resident	1.1127	0.25	9919
	T1, T2	1.35	0.03	9918
Non- Enterobacteriaceae, Jaccard distance, Treatment				
Treatment	Groups	t	P (perm)	Unique permutations
		1.5238	0.21	3600
		1.0657	0.33	3360
		0.20752	0.79	3600
Treatment x Library batch	Within level 'First' of factor 'Library batch'			
	Groups	t	P (perm)	Unique permutations
	Translocated, Control Resident	1.6807	0.18	3600
	Translocated, Experimental Resident	Negative		
	Control Resident, Experimental Resident	1.1476	0.43	3600
	Within level 'Second' of factor 'Library batch'			
	Groups	t	P (perm)	Unique permutations
	Translocated, Control Resident	1.10	0.34	1800
	Translocated, Experimental Resident	0.99724	0.44	1800
	Control Resident, Experimental Resident	0.87806	0.25	3360
Non- Enterobacteriaceae, UniFrac distance, Population				
Time	Groups	t	P (perm)	Unique permutations
	T0, T1	1.167	0.05	9850
	T0, T2	1.1064	0.13	9871
	T1, T2	1.1948	0.04	9854

Table S4.4. PERMANOVA models and results testing differences between *Salmonella enterica* and *Klebsiella aerogenes* strains across *Tiliqua adelaidensis* host groups based on treatment and based on population of origin.

Data	Final Model	Model term	Degrees of Freedom	Sums of squares	Mean of Squares	Pseudo-F	p	Unique perm-utations
Salmonella enterica	Time x Treatment x Enclosure	Treatment	1	688.71	688.71	1.11	0.45	4795
		Time	1	744.21	744.21	4.03	0.01*	9928
		Enclosure	2	1300.3	650.15	2.30	0.05*	9940
		Treatment x Time	2	1377.2	688.61	3.72	0.00*	9947
		Treatment x Enclosure	2	873.57	436.79	2.36	0.03*	9947
		Time x Enclosure	6	1710.9	285.15	1.54	0.10	9916
		Residual	59	10909	184.89			
		Total	79	17732				
Klebsiella aerogenes	Treatment x Time	Treatment	2	421.00	210.50	0.48	0.82	9841
		Time	2	613.41	306.70	0.87	0.55	9947
		Treatment x Time	4	1777.60	444.40	1.26	0.20	9924
		Residual	46	16287.00	354.08			
		Total	54	19348.00				
Salmonella enterica	Population x Time x Sex	Population	2	746.19	373.09	1.48	0.30	9838
		Time	2	901.07	450.53	2.27	0.07	9952
		Sex	2	429.24	214.62	0.91	0.61	9820
		Population	4	1115.4	278.85	1.41	0.24	9959
		Population x Sex	4	587.11	146.78	0.87	0.57	9957
		Time x Sex	4	1069.9	267.48	1.35	0.25	9944
		Residual	59	11362	198.4			
		Total	79	17388				
Klebsiella aerogenes	Population x Time	Population	2	790.91	395.46	1.67	0.12	8787
		Time	2	493.78	246.89	0.68	0.74	9929
		Population x Time	3	631.71	210.57	0.58	0.88	9913
		Residual	47	17169.00	365.29			
		Total	54	19348.00				

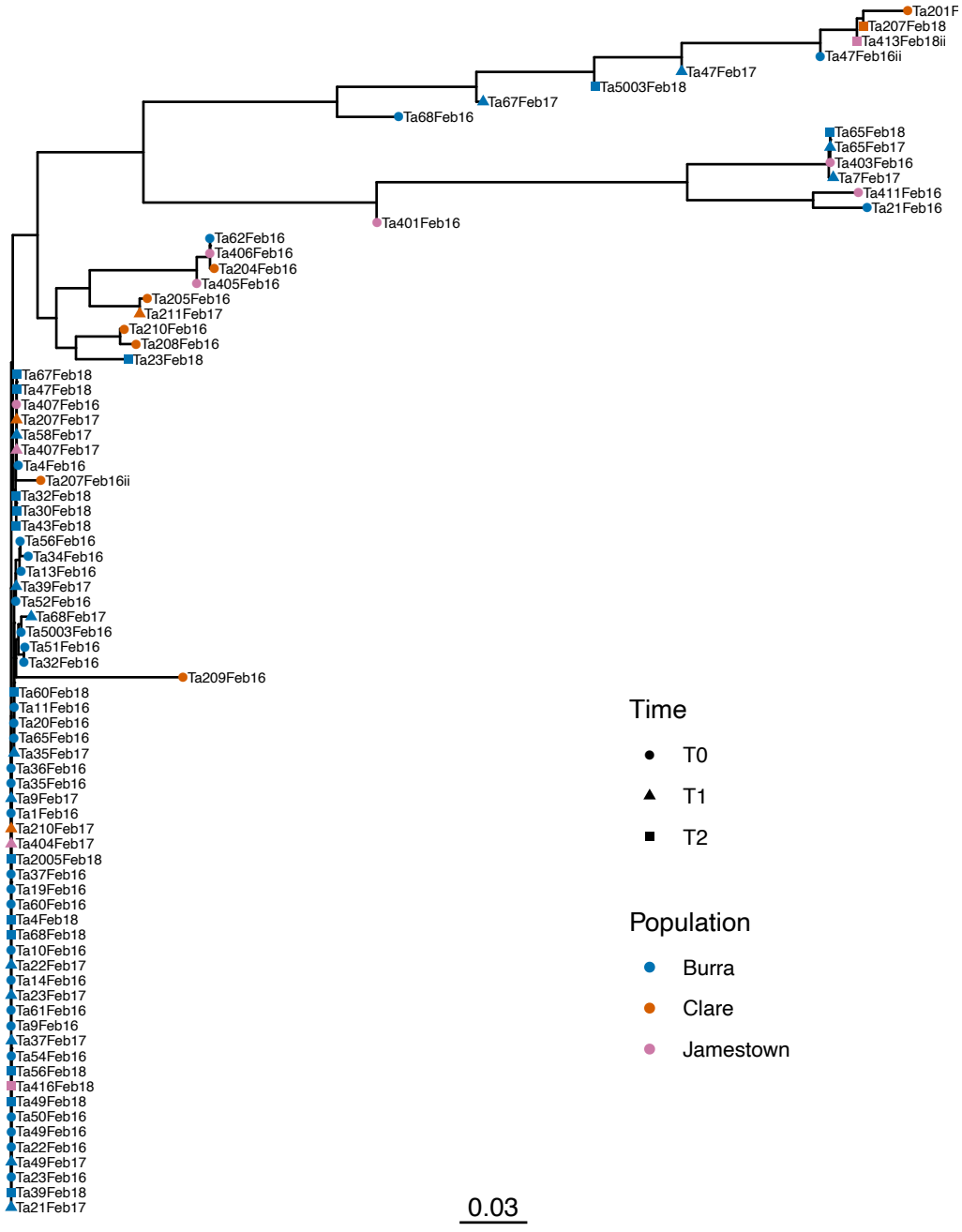


Figure S4.11. The phylogenetic relationship between Salmonella enterica strains by *Tiliqua adelaidensis* host population of origin and time point. Notes: T0 is at translocation in February 2016, T1 is one year after in February 2017, T2 is two years after in February 2018). Label names indicate host ID and collection date. Genetic distance measured in number of nucleotide base substitutions per 100.

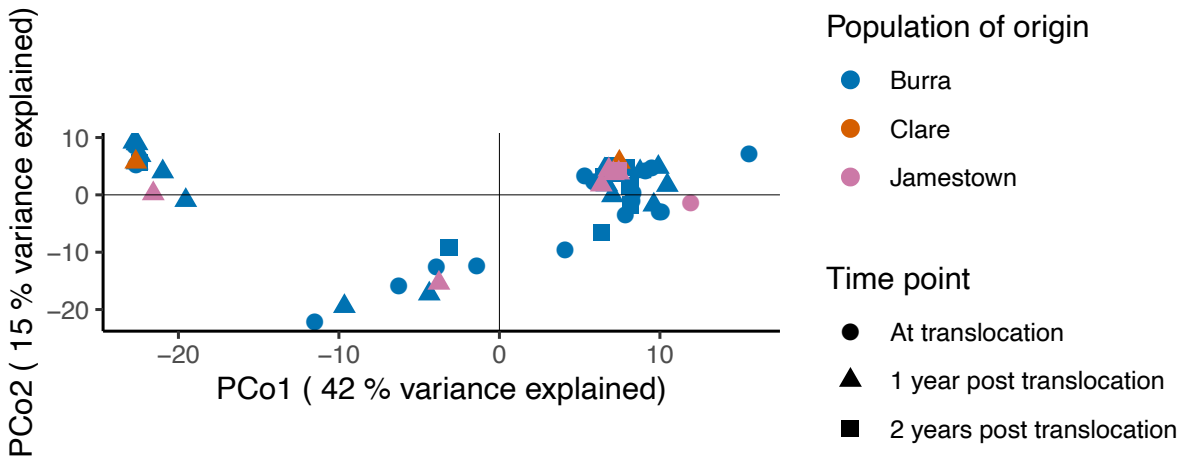
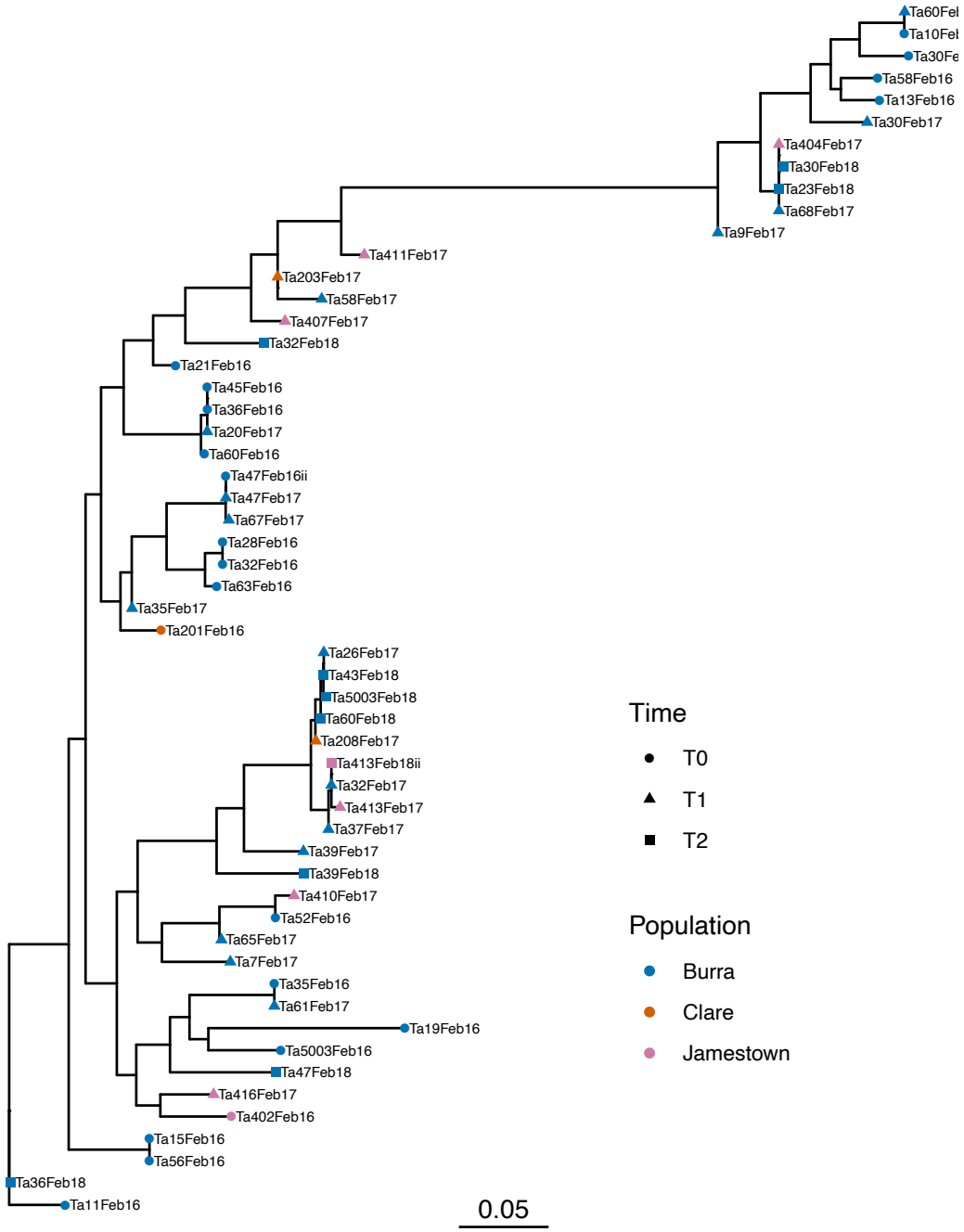
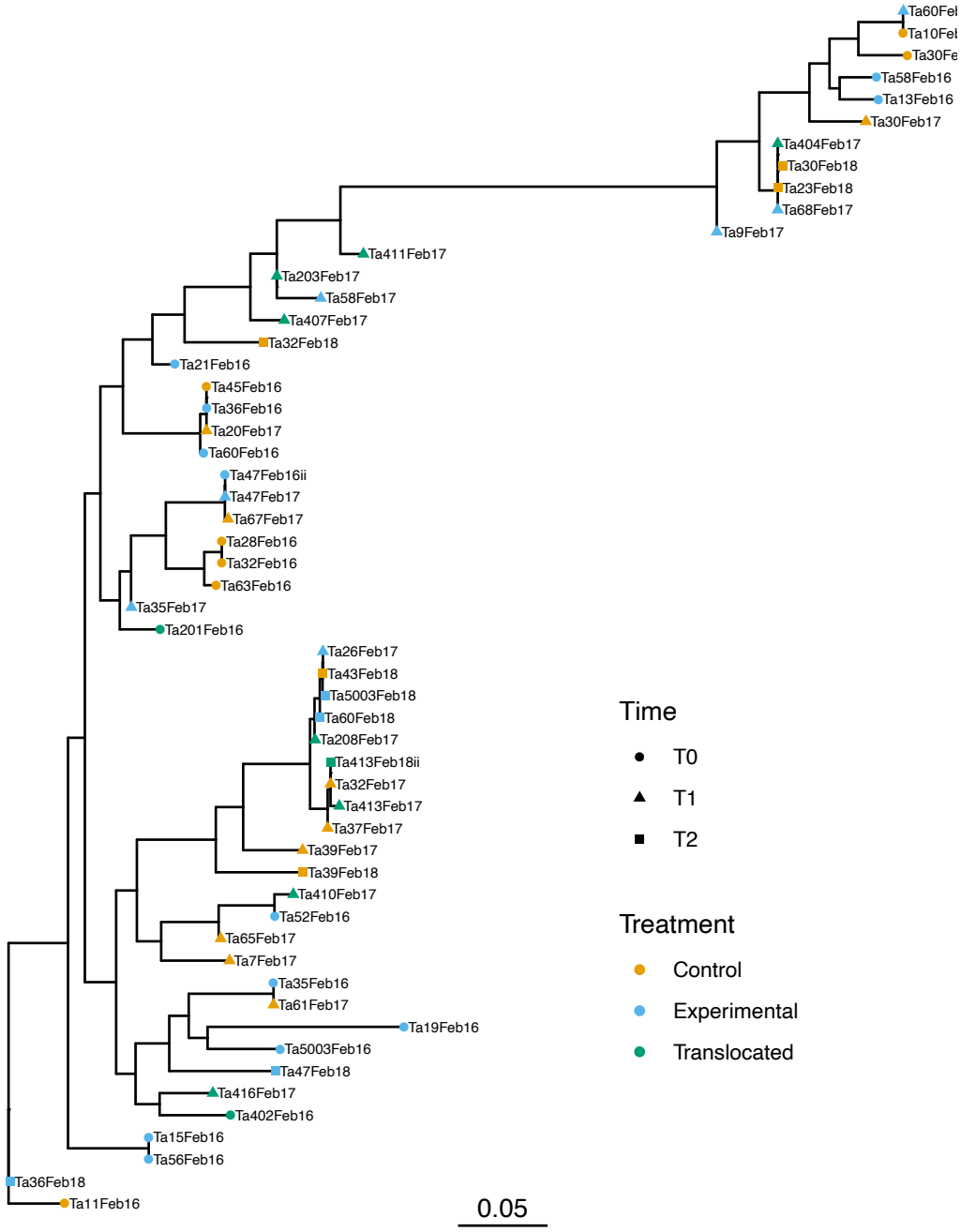


Figure S4.12. PCoA ordination of *Klebsiella aerogenes* variation by *Tiliqua adelaidensis* population of origin and time point


Ta60Fel
Ta10Fel
Ta30Fe
Ta58Feb16
Ta13Feb16
Ta30Feb17
Ta404Feb17
Ta30Feb18
Ta23Feb18
Ta68Feb17
Ta9Feb17
Ta411Feb17
Ta203Feb17
Ta58Feb17
Ta407Feb17
Ta32Feb18
Ta21Feb16
Ta45Feb16
Ta36Feb16
Ta20Feb17
Ta60Feb16
Ta47Feb16ii
Ta47Feb17
Ta67Feb17
Ta28Feb16
Ta32Feb16
Ta63Feb16
Ta35Feb17
Ta201Feb16
Ta26Feb17
Ta43Feb18
Ta5003Feb18
Ta60Feb18
Ta208Feb17
Ta413Feb18ii
Ta32Feb17
Ta413Feb17
Ta37Feb17
Ta39Feb17
Ta39Feb18
Ta410Feb17
Ta52Feb16
Ta65Feb17
Ta7Feb17
Ta35Feb16
Ta61Feb17
Ta19Feb16
Ta5003Feb16
Ta47Feb18
Ta416Feb17
Ta402Feb16
Ta15Feb16
Ta56Feb16
Ta36Feb18
Ta11Feb16
0.05
Time
T0
T1
T2
Population
Burra
Clare
Jamestown

a)



b)

Figure S4.13. The phylogenetic relationship between *Klebsiella aerogenes* strains by *Tiliqua adelaidensis* host group and time point. Notes: T0 is at translocation in February 2016, T1 is one year after in February 2017, T2 is two years after in February 2018). Label names indicate host ID and collection date. Genetic distance measured in number of nucleotide base substitutions per 100. **a) hosts coloured by population origin b) hosts coloured by treatment group.**

Chapter 5

No home ground advantage: translocated lizards survive as well as residents in a population augmentation

Note to examiners

This final data chapter examines a measure of host fitness — survival probability — in translocated and resident lizards for two years following the translocation. It indirectly comments on whether parasites may affect fitness during a translocation of *Tiliqua adelaidensis* by answering the preliminary question of whether there was differential survival in experimental groups of lizards that may indicate that factors such as parasite virulence were at play. Parasite infestation status of individual lizards was not included in the mark-recapture models examined in this study, since the already small cohort of animals was considerably reduced when visibly mite- or nematode- infested animals were considered. Furthermore, it would have been difficult to definitively distinguish between lizards infested by gut nematodes and those that were not without invasive sampling. These factors would have limited the meaningfulness of parasite infection status as a term in the models used to estimate survival probability. This chapter has been submitted for publication to the journal *Animal Conservation*. The manuscript will be resubmitted for consideration to the same journal once reviewer comments have been addressed.

Abstract

Post-release survival of animals involved in a translocation is essential for the establishment of a self-sustaining population in the long term and is thus an important measure of translocation success. Disentangling translocated-related mortality from background attrition becomes possible by comparing survival of translocated animals (or conspecifics otherwise affected by the translocation) to a non-translocated control group at the recipient or source site. We evaluated translocation as a conservation strategy for the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) — a skink endemic to a restricted region of temperate native grasslands in South Australia — by performing an experimental population augmentation and estimating survival of the different groups involved. Wild lizards from two source populations were added to an existing wild, recipient population within enclosures. Cormack-Jolly-Seber capture-mark-recapture models were used to estimate recapture and survival probabilities of resident lizards alone, resident lizards co-habiting with translocated individuals, and the translocated individuals. We also included models that accounted for survival differences between sexes within and outside the spring mating season. The model with the strongest information-theoretic support ($wAIC_c$ =0.99) indicated that an interaction of sex with mating season best predicted survival probability, whilst models including experimental treatment as a grouping variable had very little support ($wAIC_c$<0.01). These findings corroborate previous studies of *T. adelaidensis* identifying male-biased movement during spring and suggest that neither the survival of translocated nor resident lizards was adversely affected by the translocation. On this basis, translocation appears to be a viable conservation strategy that should be adopted for this species (or further explored if resources allow). We urge conservationists conducting translocations to assess and report survival for non-translocated control groups where possible in order to more accurately identify and mitigate translocation-related mortality.

Introduction

Habitat unsuitability brought about by anthropogenic land use and climate change poses a major threat to biodiversity (Butchart et al., 2010; Opdam and Wascher, 2004). Species with small endemic ranges and low vagility are particularly vulnerable; and decline, extirpations and extinction may result (Opdam and Wascher, 2004). Translocation — the intentional movement of organisms from one location to another — may mitigate such problems by a) directly preventing the loss of individuals by moving them into a more suitable habitat and b) benefiting a recipient population of conspecifics by increasing effective population size, genetic diversity and adaptive capacity (Weeks et al., 2011). Conservation-motivated translocations have accordingly increased over recent decades, but have experienced high failure rates, having often been performed in an ad-hoc manner, and lacking experimental testing, sufficient understanding of the species' biology, and ongoing monitoring of the translocated and resident population (Armstrong and Seddon, 2008; Dodd and Seigel, 1991; Ewen et al., 2014; Germano and Bishop, 2009; Letty et al., 2007; Sheean et al., 2012). Furthermore, unsuccessful translocations are not always published in the scientific literature (Miller et al., 2014), making it difficult to learn from past mistakes.

Meaningful evaluation of translocation attempts become essential in a bid to avoid future translocation failure and to develop best practice, and necessitates the *a priori* definition of what constitutes success (IUCN/SSC, 2013). The most widely agreed definition of translocation success in the literature is the establishment of a population that can sustain itself beyond the lifetime of the founder individuals (Armstrong and Seddon, 2008; Dodd and Seigel, 1991; Sheean et al., 2012). To achieve this, translocated individuals must first survive and reproduce (Miller et al., 2014), and survival during the establishment phase immediately following translocation is a major determinant of translocation success (Armstrong and Seddon, 2008). Interacting factors such as chronic stress, dispersal, disease, and predation may lower survival of translocated animals (Aiello et al., 2014; Dickens et al., 2010; Germano and Bishop, 2009). Studies that report survival of translocated animals following release are valuable if these factors are to be identified and controlled in future translocations.

Reptiles and amphibians represent an increasing proportion of published translocation attempts over time (Dodd and Seigel, 1991; Fischer and Lindenmayer, 2000; Germano and Bishop, 2009; Seddon et al., 2007), and these have started to yield insights into why failures

occur. Common reasons for failure include post-release dispersal, and inadequate habitat provision (Germano and Bishop, 2009, Sullivan et al. 2004, Tuberville et al. 2008), particularly given the often complex microhabitat requirements of reptiles for thermoregulation (McCoy et al., 2014; Pernetta et al., 2005; Platenberg and Griffiths, 1999). The post-release survival of either wild or captive-reared reptiles in translocations has been reported in several studies (e.g. (Bell and Herbert, 2017; Christie et al., 2011; Dickinson and Fa, 2000; Field et al., 2007; Hare et al., 2012; McCoy et al., 2014; Norbury et al., 2014; Platenberg and Griffiths, 1999). While these survival rates are informative in their own right, survival should be compared to that of non-translocated conspecifics, where applicable, to identify specific effects of the translocation, enabling mitigation or optimisation of these effects in the future. Comparison of survival between translocated and non-translocated individuals at either the source or recipient site has been done in a subset of reptile translocations (e.g. Attum et al., 2013; Brown et al., 2008; Holding et al., 2014; Nelson et al., 2002; Plummer and Mills, 2000; Reinert and Rupert, 1999; Tuberville et al., 2008)), few of which involve lizards (e.g. Massot et al., 1994; Santos et al., 2009).

Here we sought to overcome some specific limitations commonly associated with translocations by conducting an experimental population augmentation of the pygmy bluetongue lizard (*Tiliqua adelaidensis*), where dispersal was precluded by enclosures, knowledge of the species biology was considerable, and follow-up monitoring was conducted. *Tiliqua adelaidensis* is a relatively long-lived (up to 10 years) Australian skink (average SVL 100 mm) that occupies abandoned spider burrows in the mesic grasslands of the Mid North region of South Australia (Hutchinson et al., 1994; Milne, 1999). Fragmented populations continue to be threatened by cereal cropping and urbanisation, and the species is consequently endangered (IUCN 1996). Population viability modelling by Fordham et al. (2012) predicted that without managed translocations climate change would drive northern populations — and eventually the species — to extinction in the next 100 years.

Our objective for this study was to focus on the initial establishment and survival of a translocated group among an existing wild population of conspecifics. We aimed to determine whether survival rates differed between resident and translocated lizards over a two-year post-release monitoring program, and also to compare the effects of treatment to that of putatively important factors affecting survival; sex and mating season (Bull et al., 2015). Dispersal away from the study site, which is often identified as a potential driver for apparently low post-translocation survival, was precluded by use of enclosures. Nonetheless, we hypothesise that

translocated *T. adelaidensis* individuals may have a lower survival than residents initially as they are more likely to move around between burrows post release (Ebrahimi and Bull, 2014, 2013) and thus be at higher risk of predation (Fenner et al., 2008a).

Methods

Recipient site establishment and monitoring

The recipient site for the translocation was the Nature Foundation of South Australia's Tiliqua reserve, approximately 8 km north east of Burra township. This grazed grass land hosts a large natural population of *T. adelaidensis*. In July 2015, individuals overwintering in their burrows were located and three pairs of 30 m x 30 m, 30 cm high enclosures erected around them — precluding immigration and emigration of *T. adelaidensis* from the enclosures, but not excluding snake and avian predators. Each pair of enclosures shared an adjoining wall, and the three pairs of enclosures were located 100–500 m apart.

The natural density of *T. adelaidensis* individuals found in each 30 m x 30 m enclosure ranged between six and 23, reflecting the patchiness in even the fine scale distribution of the species, reliant on the presence of suitable spider burrows (Souter et al., 2007). In addition to the existing natural burrows within the enclosures, 12 artificial burrows (consisting of a 30 cm length of hollow hardwood dowel with an internal diameter of 18 mm hammered into the ground) were established in each of 6 enclosures in October 2015. Such burrows were shown by Milne and Bull (2000) to be utilised by *T. adelaidensis* and to increase recruitment into an area.

Monthly monitoring of lizards within the enclosures began in October 2015 and consisted of locating lizards in marked burrows using an optiscope and capturing them by luring them out of their burrows with a tethered mealworm bait (Milne, 1999). Upon first capture, each lizard was toe clipped with a specific sequence as a unique identifier and toes were retained for DNA analysis. Toe-clipping has been found to not significantly increase corticosterone levels in the skink *Eulamprus heatwolei,* in contrast to microchip implantation (Langkilde and Shine, 2006). Furthermore, in *T. adelaidensis,* other forms of permanent marking are precluded by small body size and shedding of skin. At first and subsequent captures of each lizard, the lizard's home burrow was marked as captured, and capture of a particular lizard was not attempted again till the following month. Each month, capture attempts continued until as many optiscope- located lizards as possible in an enclosure had been captured over a 3–15 day period

(as dictated by logistical constraints), with inter-sampling period intervals ranging between 14–28 days. A small proportion of lizards typically remained uncaught during each sampling period. Monthly monitoring was conducted in this fashion from October 2015–March 2016, from October 2016–March 2017, and from October 2017–2016 (the two inter-season intervals lasted 200 and 214 days). At the end of the 2018 monitoring season, catch records for each individual were reviewed to assign a sex to each adult (>82 mm SVL) in the cohort. Sexing *T. adelaidensis* on external morphology alone is unreliable. Sex was positively identified when the individual had given birth to young, or when everted hemipenes were observed during handling. In other cases, maximum SVL and burrow movement patterns were used to infer sex, since females typically achieve a larger maximum SVL than males (90–110 mm c.f. 80–100 mm) (Milne, 1999) and change home burrows less frequently in the spring mating season than males (Bull et al., 2015). Some individuals were not assigned a sex at all due to lack or ambiguity of data.

Translocation

Two other *Tiliqua adelaidensis* populations served as source populations for the augmentation, one approximately five km west the township of Clare and one approximately 15 km north of Jamestown township. In February 2016, for each of these two populations, 5–6 adult lizards (SVL >82 mm) per day for two consecutive days were captured and transported to the recipient site (enclosures at Tiliqua reserve, approximately one hour away).

Following weighing, measurement and toe clipping, each translocated lizard was randomly allocated to one of three experimental enclosures at Tiliqua reserve. Experimental enclosures consisted of an enclosure from each of three enclosure pairs, where the adjoining enclosure was the control enclosure that contained 8–23 resident lizards only. Each experimental enclosure thus contained a mixture of resident lizards (the number dependent on the existing density and ranging from 12–18), 4–5 translocated lizards from Jamestown, and 3–4 translocated lizards from the Clare population. Translocated lizards were each released into an artificial burrow, which was surrounded by a ring 50 cm in diameter of 30 cm high plastic garden edging for seven days following release. Soft release was employed because it reduced post-release dispersal in wild to captivity translocations (Ebrahimi and Bull, 2013). Translocated lizards were also fed two meal worms (commercially bred *Tenebrio molitor* larvae) every second day for the first week following release.

One month following release, all 24 translocated lizards were located, with only one having moved from the burrow it was released into. From this point, translocated lizards were recaptured monthly in the same manner as the resident conspecifics. Offspring were born in January-February 2016, 2017 and 2018 to mothers of all three population origins (Clive, 2019). These offspring were all excluded from the current study, and in most cases were removed from the enclosures.

Data analysis

Capture histories

Capture histories were constructed from mark-recapture data for the enclosed *T. adelaidensis* individuals from the month of translocation- February 2016, until March 2018, spanning over the end of the first activity season (February 2016-March 2016), and then two subsequent October-March activity seasons. Each monthly sampling period (lasting 3–15 days) was classified as a capture occasion, because every month only the first capture of an individual was recorded. Inter-sampling period intervals were calculated as a ratio of 39 days (the mean number of days between sampling periods).

Covariates included in the models

Two sets of candidate Cormack-Jolly-Seber mark-recapture models were constructed in program MARK (White and Burnham, 1999), which was accessed through the R (R development core team 2018) package, 'RMark' (Laake, 2013). In order to evaluate any effect of translocation on survival probability, animals were grouped by treatment; they comprised of controls (animals from Burra not mixing with translocated conspecifics), resident mixed (animals from Burra mixing with translocated conspecifics), and translocated mixed (animals translocated from Jamestown and Clare mixing with Burra conspecifics) (Table 5.1). Sex and mating season were also included as biological factors likely to influence survival (Table 5.1), since previous studies show that males disperse during the spring mating season (e.g. (Bull et al., 2015; Schofield et al., 2012), making them vulnerable to predation and exposure. Including these variables allowed us to identify confounding effects on survival, and also to determine if there was any evidence of sex or mating season affecting the survival of the three treatment groups differently.

Table. 5.1. List of single-factor, interactive and additive factor for survival probability (Φ) in models tested in the first candidate model set for the *Tiliqua adelaidensis* translocation mark-recapture study, where recapture probability (p) was either 'time' (a time dependent co-variate reflecting capture occasion) or null (one parameter for all capture occasions). Note: $\Phi_{treatment*sex*matingseason*time}$ P_{time} was the global, most parameterised model. The second model set including individuals of unknown sex was identical to the first set except that it excluded sex as a grouping variable.

Φ single	Φ interactive	Φ additive
Φ_{time}	$\Phi_{treatment*sex*}$ mating season*time	$\Phi_{time + treatment}$
$\Phi_{treatment}$	$\Phi_{treatment*sex}$	$\Phi_{time + sex}$
Φ_{sex}	$\Phi_{treatment*}$ mating season	$\Phi_{treatment +sex}$
$\Phi_{mating\ season}$	$\Phi_{treatment*time}$	$\Phi_{treatment + mating\ season}$
Φ_{null}	Φ_{sex*} mating season	$\Phi_{mating\ season+ sex}$
	$\Phi_{sex*time}$	

Sex as a grouping variable included an additional 'unknown' category when we were not able to confidently sex an individual. Lizards of an unknown sex represented 35 of 125 individuals in our study (28%). Our first candidate model set excluded these individuals as the variable sex would unduly affect survival, since individuals of unknown sex were most often animals with a shorter capture history that precluded accurate sexing. Our second candidate model set, which did not include sex as a grouping variable, included these individuals of unknown sex in order to maximise sample size.

Sampling effort, though not recorded, varied between occasions (due to variable person-catch hours/volunteer availability). We therefore used capture occasion as a time-varying covariate for estimating recapture probability (p) instead of sampling effort, as represented by 'time'.

Goodness of fit

We confirmed that the global model $\Phi_{treatment*sex*mating\ season*time}$ P_{time} adequately fit the data by goodness of fit testing using the global test in the program U-CARE (Choquet et al., 2009). This was repeated with the dataset including the individuals of unknown sex.

Model fitting and survival estimation.

Relative model fit was ranked using Akaike's Information Criterion for small sample sizes (AIC_C), and the normalised Akaike weight ($wAIC_C$) (Burnham and Anderson, 2002). Average survival probability was estimated from the top-ranking model, where there was a delta AIC_C value >2 between the top ranked model and subsequent models. A delta AIC_C value of this size indicates considerable support for there being a real difference between models, while a delta AIC_C of <7 strongly supports a difference (Burnham and Anderson, 2004), thus model averaging was not used.

Lastly, we examined β-estimates (i.e. estimates of the slope parameters) from the model with the most support. The 95% confidence intervals of the effect sizes for each grouping variable (e.g. sex or treatment, represented by $\beta_{2\text{-}i}$) in the linear model were used to assess whether survival estimates for different groups were statistically significantly different from each other (Nakagawa and Cuthill, 2007). If the confidence interval of an effect size include 0, then the effect was not statistically significant (Cooch and White, 2019; Nakagawa and Cuthill, 2007), and the difference in survival of that group was not considered significantly different from groups without that effect.

Results

One hundred and twenty-five *T. adelaidensis* individuals were captured and marked within the enclosures over the three spring-summer seasons (February 2016 –March 2018) for which monitoring was conducted, representing 828 capture events over 14 or less sampling occasions. Mean number of recaptures for 24 translocated individuals, 45 experimental residents, and 56 control residents were 6.7, 5.8 and 6 respectively. There were 31 male individuals, 50 female individuals and 35 of unknown sex. A two-tailed Fisher's exact test of individual counts revealed no association between sex and treatment (p=0.95).

Goodness of fit testing indicated that the mark-recapture data for the *T. adelaidensis* in this study adequately fit the global model phi sex*treatment*time p time (p=0.94 for first model set, and p=0.99 for second set). Our data were found to not be over-dispersed with a $\hat{c}$ value of 0.75 (0.64 when all 125 individuals were included in the second model set).

The model with the highest information-theoretic support for our first candidate model set indicated that an interaction between sex and mating season best predicted survival

probability (Φ) in this mark-recapture study (Table 5.2). This model's sample size corrected Akaike's information criterion weight ($wAIC_C$) was 0.99; 226 times larger than the next ranked model, in which mating season was the sole predictor of survival. The delta AIC_C between these neighbouring models is 11.03, indicating strong support for there being a real difference between the two (Burnham and Anderson, 2004). The highest-ranking model which included experimental treatment as a predictor of survival (treatment additive to mating season) showed very little support in the data, with a delta AIC_C of 14.6 and a $wAIC_C$ over 1200 times smaller than the model with the most support (Table 5.2). All models where encounter probability (p) was time- dependent were better supported than the null model, where the parameter for p estimation was held constant across all capture occasions.

Table 5.2. Comparison of five best supported Cormack-Jolly-Seber capture-mark-recapture models from first model set estimating survival and recapture probability for enclosed groups of *Tiliqua adelaidensis* individuals that were part of an experimental translocation near Burra, South Australia. Note: Individuals were grouped by experimental treatment and sex, and a time varying covariate of mating season (MS) was applied.

Model	Number of parameters	AIC_C	Delta AIC_C	AIC_C weights
Φ (Sex * MS)p(time)	17	856.32	0.0	0.99
Φ (MS)p(time)	15	867.86	11.03	<0.01
Φ (Sex + MS)p(time)	16	868.26	11.93	<0.01
Φ (time)p(time)	26	870.36	14.04	<0.01
Φ (Treatment + MS)p(time)	17	870.96	14.86	<0.01

During the spring mating season (October and November), the model with the highest support estimated survival probability to be 0.99 (95 % CI: 0.79–1.00) for female lizards, 21% higher than 0.78 (95% CI: 0.64–0.88) for male lizards (Figure 5.1). During the rest of the activity season (December-March), average survival probability was 0.97 (95% CI: 0.95–0.98) for female lizards, which was 2% lower than females during the mating season. For male lizards outside of the mating season however, average survival probability (0.99, 95% CI: 0.96–99) was significantly higher (by 21%) than during the mating season, and 0.5% higher than that of females outside the mating season (Figure 5.1). Logit scale β-estimates from the linear

model corresponding to the top-ranked model (Table 5.3), show that only the effect size of the interaction between being both male and in mating season had a statistically significant effect on survival probability, since the 95% confidence interval did not include 0.

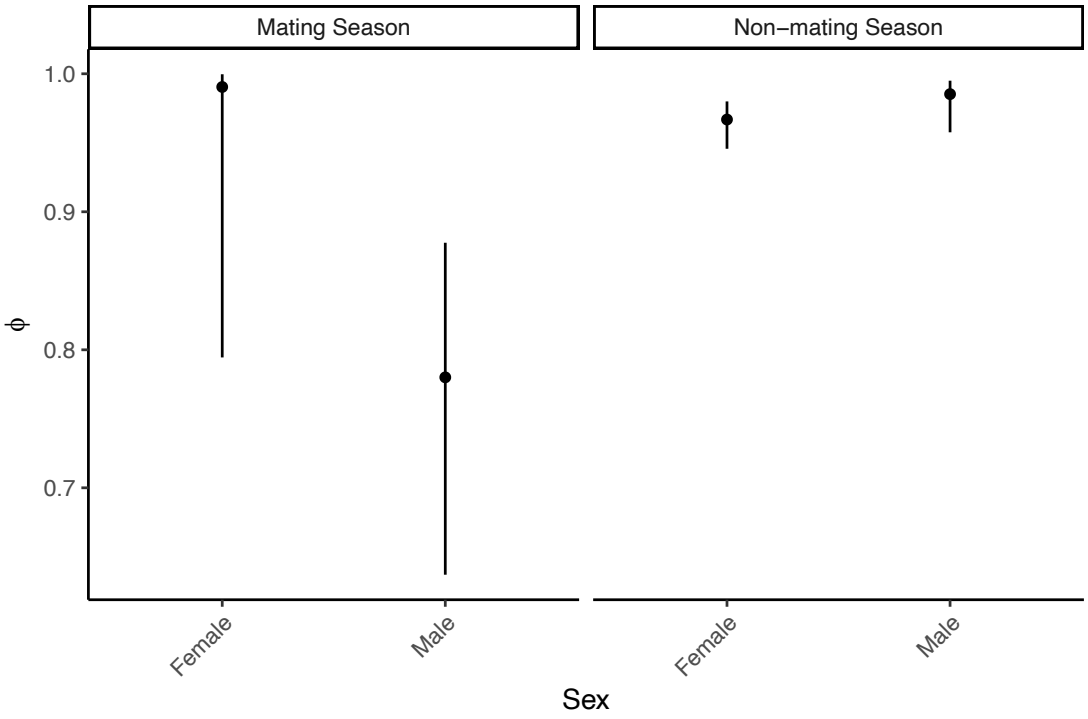


Figure 5.1. Average survival probability (Φ) estimate based on the top-ranked model $\Phi_{sex*season/}p_{time}$ where *Tiliqua adelaidensis* individuals within experimental enclosures were grouped by sex within and outside of the mating season for 14 capture occasions over 25 months.

Table 5.3. β- estimates from the linear model for survival probability (φ) corresponding to the top ranked model φ(Sex*mating season) for *Tiliqua adelaidensis* individuals in the experimental population augmentation. Notes: $\widehat{\varphi} = \widehat{\beta 1} + \widehat{\beta 2} + \widehat{\beta 3} + \widehat{\beta 4}$. The effect sizes of sex, mating season and the interaction between the two was calculated on the probability scale (bound by 0 and 1) using the formula $\varphi = (e^{\beta i}/1 + e^{\beta i}) - (e^{\beta 1}/1 + e^{\beta 1})$. Note that $\varphi = \beta_1$ is equal to the survival probability of female lizards outside of breeding season.

Model parameter	**β_1 (intercept)**	**β_2 (effect size of being male)**	**β_3 (effect size of mating season)**	**β_4 (effect size of being male and within mating season)**
Logit scale	3.37 (95%CI: 2.85–3.89)	0.83 (95%CI: -0.37–2.03)	1.27 (95%CI: -2.12 – 4.6)	-4.21 (95%CI: -7.85 – -0.56)
Probability scale	0.97 (95%CI: 0.94–0.98)	0.02 (95%CI: -0.01–0.03)	0.02 (95%CI: -0.17–0.03)	-0.19 (95%CI: -0.33– -0.09)

The second model set, where data included an additional 35 animals of unknown sex (and where the grouping variable sex was not included), showed that mating season alone was the best-supported predictor of survival, with a $wAIC_C$ of 0.65 (Table 5.4). In the absence of sex, the next most supported model included the additive effects of mating season and treatment. However, the $wAIC_C$ for this was 4.2 times smaller than the top ranked model, and there was considerable support for a real difference between the two, with a delta AIC_C of 2.85 (Table 5.4). Control, resident mixed and translocated mixed lizards showed no statistically significant differences in survival from each other — both within and outside of the October-November mating season.

Table 5.4. Comparison of five best supported Cormack-Jolly-Seber capture-mark-recapture models from the second model set estimating survival and recapture probability for enclosed groups of *Tiliqua adelaidensis* that were part of an experimental translocation near Burra, South Australia. Note: Individuals were grouped by experimental treatment, and a time varying covariate of mating season was applied.

Model	Number of parameters	AIC_C	Delta AIC_C	AIC_C weights
Phi(MS)p(time)	15	1161.10	0.00	0.64
Phi(Treatment + MS)p(time)	17	1163.96	2.85	0.15
Phi(time)p(time)	26	1164.35	3.25	0.12
Phi(Treatment + time)p(time)	28	1166.56	5.46	0.04
Phi(Treatment * MS)p(time)	19	1167.21	6.11	0.03

Discussion

Our aim for this study was to identify any translocation-specific effects on survival by comparing the post-release survival of translocated *Tiliqua adelaidensis* individuals to that of resident conspecifics at the release site. Our hypothesis that survival would be lower for translocated individuals was not supported, since the model with by far the most support in the data was the one that grouped animals by sex within and outside of the spring mating season. This result suggests that sex during mating season most strongly influenced survival during this experimental translocation. In contrast, the model grouping individuals by experimental treatment was poorly supported. No significant differences in average survival were observed between control lizards, the resident lizards mixing with translocated conspecifics in the experimental enclosures over the 2 years following translocation. Similarly, body condition measured during capture occasions over this time showed no significant difference between treatment groups (Clive et al., 2020). These results suggest that the lizards were not detrimentally affected by either being translocated, nor by cohabiting with translocated individuals — at least in terms of initial survival and establishment probability. They therefore provide some assurance that population augmentation may be a viable strategy for small/medium sized lizards.

Average survival estimates from the best-supported model indicate that male survival is significantly lower than that of females during the mating period of October-November, with little difference between December and March. This finding is consistent with those from a three year pitfall trapping study, where the majority of *T. adelaidensis* adults moving away from their burrows during spring were male (Schofield et al., 2012), and also the results of a two year observational study where males were more likely to disperse from their burrows than females (Bull et al., 2015). Male movement in spring is presumably largely to find females in their burrows for mating opportunities (Ebrahimi et al., 2014; Fenner and Bull, 2009). Genetic analysis of neonates showed that males travelled a mean distance of 27 metres to mate with a female, and at least 10% of males mated with more than one female in a season (Schofield et al. 2014). Lizards out of their burrows are known to be vulnerable to predation from raptors and snakes (Fenner et al., 2008a; Fenner et al., 2008b). Furthermore, sex-biased mortality associated with seasonal mate-searching activity by males has been observed in several reptile and non-reptile species (Bonnet et al., 1999; Magnhagen, 1991; Sperry and Weatherhead, 2009). The congruence between the observational studies of *T. adelaidensis* and our top- ranked capture-mark-recapture model suggests that this model is able to predict survival with some accuracy, which validates the lack of support for experimental treatment as an important predictor of survival.

Our findings complement previous work that examined the effects of translocation on survival, and they contribute to the development of best practice in future reptile translocations. Studies of reptile translocations comparing the survival of translocated animals to that of established residents at the recipient sites have yielded mixed results. A salient point from these studies, regardless of whether they report lowered or similar survival rates of translocated animals relative to their resident conspecifics, is higher movement of translocated individuals (Brown et al., 2008; Plummer and Mills, 2000; Reinert and Rupert, 1999; Santos et al., 2009). In a translocation of the lacertid lizard *Pssammodromus algirus*, fitness costs of heightened movement may have been offset by the better initial body condition of translocated individuals, and survival rates between translocated and resident lizards were similar (Santos et al., 2009). However, increased movement following translocation is thought to have increased predation risk and resulted in lower survival in hog-nosed snakes (*Heterodon platirhinos*) (Plummer and Mills, 2000). In some cases, movement takes the form of permanent dispersal from release area, lowering apparent survival relative to established conspecifics (Massot et al., 1994; Tuberville et al., 2008), and increasing the chance of encountering

unsuitable habitat. Supporting this, where dispersal away from release site was prevented by the fragmented nature of wetland habitat, survival of translocated musk turtles was similar to that of residents (Attum et al., 2013). These studies highlight the importance of minimising post release dispersal, which has been identified as an issue in the translocation of herpetofauna more broadly (Butler et al., 2005; Germano and Bishop, 2009; Sullivan et al., 2004). In addition to physical barriers to dispersal, encouraging site fidelity for translocated animals calls for a sound understanding of the species biology, including how to provide good quality habitat (Germano and Bishop, 2009). For example, failure to find any or adequate quality hibernacula may have driven lowered survival rates of translocated timber rattlesnakes (*Crolatus horridus*) in their new environment (Reinert and Rupert, 1999). The provision of adequate habitat and resources may be particularly important when familiarity with the environment is low, and there is potential for competition from resident conspecifics (Massot et al., 1994). Reducing long-distance dispersal and providing adequate habitat have thus been shown to be requirements for reptile translocation success, and these factors may largely explain the lack of difference between translocated and resident lizards in our study.

In this experimental population augmentation of *Tiliqua adelaidensis*, we employed both physical means (i.e. enclosures) to reduce long-distance dispersal, and prior knowledge of the species biology to minimise translocation-related dispersal. Firstly, immediate dispersal by translocated individuals was reduced by timing the translocation towards the end of the activity season, and by employing a soft release method, as previously shown to be effective by Ebrahimi et al. (2014, 2013). Furthermore, we endeavoured to address the common issue of inadequate habitat quality in translocations (Germano and Bishop, 2009; Griffith et al., 1989; McCoy et al., 2014) by releasing translocated lizards into artificial dowel burrows, which supplemented natural burrows. This supplementation has been shown to increase *T. adelaidensis* survival and recruitment (Milne, Bull, & Hutchinson, 2003; Souter, Bull, & Hutchinson, 2004), and individuals may have had reduced incentive to disperse and spend time seeking good quality burrows on the surface, thus decreasing vulnerability to predators. Provision of extra burrows may have also reduced intra-specific competition between residents and translocated individuals since good quality natural burrows are a limiting resource in *T. adelaidensis* populations and individuals are known to defend their burrows against conspecifics (Fellows et al., 2009; Fenner and Bull, 2011). Lack of familiarity with the environment may not be a disadvantage to translocated males seeking mating opportunities in a promiscuous species where spatial proximity appears to be the most important correlate of mate choice (Schofield et al., 2014). Locating mates may be reliant largely on sensing chemical

trails laid by females, as supported by behavioural observations by Ebrahimi et al. (2014), rather than prior knowledge of conspecific locations.

Small sample sizes, particularly of the translocated experimental group, were a limitation of this study. Whilst there was a clear difference between estimated survival between male lizards during mating season and other groups, the smaller number of capture occasions made precise estimation of survival difficult (Figure 5.1). It has been previously acknowledged in the translocation literature that the number of individuals taken from a source population may be smaller than ideal for an endangered species (Nelson et al., 2002; Towns and Ferreira, 2000). Good outcomes for post-release establishment phase are usually most strongly associated with overall translocation success (Armstrong and Seddon, 2008). While our results for this critical post-release establishment phase of translocated *T. adelaidensis* were positive, we acknowledge that following up individuals and assessing survival, in addition to other measures of fitness, for several subsequent years would improve our ability to comment on translocation success (e.g. Hare et al., 2020). Translocation of animals into a new environment alters the environment-genotype/phenotype interactions between individuals and their habitat, and this may impact on their fitness (Niewiarowski and Roosenburg, 1993). Disentangling these effects from the direct, short term effects of translocation requires the initial comparison of translocated and resident animals at the recipient site, such as we have conducted, to be built upon with long-term post-release monitoring and modelling analysis (Armstrong et al., 2017). Here we note that a detailed, empirically-based understanding of best translocation practice for a species must be balanced with the need to act quickly in the face of urgent conservation objectives and low resource availability (Meek et al., 2015; Scheele et al., 2018).

Recommendations and conclusions

The high average survival rates and lack of difference in both survival and body condition between treatment groups observed during this establishment phase support the viability of translocation as a future conservation strategy for *Tiliqua adelaidensis*. Recommendations would therefore be to adopt population augmentation as a conservation strategy for this species, whilst continuing to a) optimise outcomes with further testing involving larger sample sizes and longer follow-up periods (resources permitting); and b) trial other forms of translocation, i.e. re-introduction into areas previously part of the species' range. The caveats here are to limit post release dispersal (by optimising release conditions and using physical barriers if feasible); and to provide an abundance of suitable burrows in order to reduce above-

ground searching for new home burrows (particularly during mating season). Balancing sex ratios for translocated cohorts is also recommended; if males suffer greater rates of attrition, selecting at least 50 percent males to translocate may ensure adequate reproductive output. Recent elucidation of a reliable sex marker for egerniine skinks will render propagule selection on the basis of sex possible (Stuart, 2019). Accurate sexing of individuals will also allow strategic placement of individuals into new home burrows, which may increase male survival by reducing above-ground distance to mates.

In this study we have demonstrated the benefits of preceding translocation attempts with extensive research into the biology of the study species, particularly with regards to adequate habitat provision and minimising stress-induced dispersal immediately following release. Following the translocation event, future projects should report survival of released individuals for as long as logistically possible, and also monitor reproductive success over subsequent generations (Hedrick and Fredrickson, 2010). Furthermore they should endeavour to contextualise survival rates and other fitness measures by using comparison groups - whether non-translocated individuals at the recipient site in population augmentations, or animals at the source site, at environmentally similar locations, or model estimates (e.g. Towns and Ferreira, 2000) in order to identify and mitigate drivers of undue mortality as swiftly as possible.

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Chapter 6

Thesis Discussion

The work in this thesis explored the intersection between two important issues in conservation biology — parasites and translocations — with the over-arching aim of informing risk assessment for population augmentation as a conservation measure for *Tiliqua adelaidensis*. Parasites can influence conservation outcomes by affecting their hosts in a variety of ways, with potential population-wide impacts of chronic suppression or outright decline (Daszak et al., 2000; Papkou et al., 2016; Preece et al., 2017; Tompkins et al., 2011). Conversely, there is mounting evidence that parasites are necessary for maintaining ecosystem function, and should not and, in any case, cannot necessarily be completely eradicated from wildlife populations under management (Carlson et al., 2020; IUCN/SSC, 2013; Kuris et al., 2008; Miller, 2007; Northover et al., 2019, 2018; Wood et al., 2007). Whilst translocations are a necessary conservation tool for many species, the fitness cost of parasites can be increased in these situations, where stressed hosts are exposed to new environments and potentially to novel parasites (Dickens et al., 2010; Kock et al., 2010; Northover et al., 2018). Despite this general-level understanding, our ability to assess disease/parasite related risks and overall viability in future translocations in specific systems is hampered by a lack of knowledge of parasite diversity in wildlife, how parasites interact with their particular hosts under conditions of equilibrium, and how this can change in a context such as translocation.

In this thesis, I have addressed knowledge gaps relating to the host-parasite relationships of an endangered reptile, *Tiliqua adelaidensis*, with a focus on how these dynamics behave when hosts from allopatric populations share habitat following a population augmentation. This work was needed to inform the risk assessment of wild-wild translocation as a conservation strategy, and has subsequently been applied in a translocation plan that is being adopted for the next phase of the conservation of this species. I have used variety of established molecular and non-molecular based methods to comment on variation in both space and time in an ectoparasitic mite, an endoparasitic helminth, and also enteric bacteria as potential pathogens or mutualists, as well as examining a measure of host fitness over time. How this work addresses the specific research aims within this broad objective are discussed below.

Key research findings

The host-parasite relationship of a newly discovered parasite

The discovery of *Ophiomegistus michaeli* as a new mite species and parasite record for *T. adelaidensis* arising from our translocation monitoring was not an *a priori* objective of the project but fulfilled the aim of better characterising host-parasite relationships of this endangered host. Its discovery relates directly to the hazard identification phase of disease risk assessment in translocation (Ewen et al., 2015), since the potential host fitness costs and effect on translocation success cannot be estimated for a parasite species not known to exist. Field observations I made provided the first step in characterising the ecology and life history of this mite. I found it to occur across at least some of the host's range, with prevalence on hosts appearing to vary among different populations. Inter-population variation in mite abundance and prevalence in host lizards across different habitat types appears common (Klukowski, 2004; Ramirez-Morales et al., 2012; Schlaepfer, 2006). In this system however, the drivers of mite abundance are unclear, since vegetation structure and climatic conditions are broadly consistent across sites sampled. Further sampling of more host populations is needed to further comment on prevalence and its drivers. Distribution of *O. michaeli* on host individuals was also highly spatially variable at a local scale, which suggests that off-host mite dispersal into the environment is limited. The recurring observation of the same individuals with mites several months apart would be expected if subsequent generations of mites infect the same host, unlike eutrombiculid 'chigger' mites which are free-living as adults. Mites were observed throughout the spring-summer activity season, without showing clear seasonality. This lack of seasonality contrasts with other systems in which lizard mites and ticks may be exposed to and affected by environmental conditions, whereas *O. michaeli* mites that may be sheltered in host burrows (Bull and Burzacott, 1993; Lumbad et al., 2011; Talleklint-Eisen and Eisen, 1999). Insights into the biology of *O. michaeli* were further provided by examining genetic markers in mites from the three host populations they were observed in.

Inter-population genetic variation of parasites and other symbionts

One of the primary aims of this project was to determine whether parasite biota differed across geographically and genetically isolated *T. adelaidensis* host populations. Whether or not inter-population variation existed was particularly relevant to translocations in which non-local hosts and their parasites would share habitat, since this variation may underly local adaptation (Sasal et al., 2000). Local adaptation by hosts would imply they are more susceptible to any detrimental effects of parasites when non-local parasites represent a novel species or genotype.

Using genome-wide, neutral single nucleotide polymorphism (SNP) markers in *O. michaeli* and the nematode *Pharyngodon wandillahensis* I was able to show that there was indeed genetic differentiation among both mites and nematodes from different host populations across the range of *T. adelaidensis* (Chapter 3). This inter-population level genetic structure was clearest for the nematode, but the smaller sample sizes in *O. michaeli* also indicated that mites from the same population were more genetically similar than mites from allopatric host populations. This work was the first to investigate the population genetics of these two macroparasite species, an avenue of research that has been highlighted as necessary for the conservation of parasites and the ecosystem structure and function this guild contributes to (Carlson et al., 2020). The genetic structure observed among the three host populations suggests that, like the host, gene flow between populations is limited or non-existent (Smith et al., 2009b). Lack of gene of gene flow is consistent with the hypothesis *P. wandillahensis* and *O. michaeli* may be host-specific to *T. adelaidensis*. This host-specificity seems especially likely in *P. wandillahensis*, given that other species in the order are monoxenous (Anderson, 2000). Without gene-flow from other fragmented populations, local maladaptation by the parasite is more likely to arise in a host-parasite system, whereby non-local parasite genotypes may show greater virulence in hosts (Gandon and Michalakis, 2002; Gandon and Nuismer, 2009).

Genetic characterisation using SNPs also suggested that *P. wandillahensis* exhibits haplodiploidy as a reproductive system, as oxyuroid nematodes are known to (Adamson 1989). Similarly, while haplodiploidy has evolved in the same order as *O. michaeli* (Mesostigmata) (Blackmon et al. 2015, Cruikshank & Thomas 1999), no records of haplodiploid species within the same family (Paramegistidae) were found, and my results concordantly indicate a diploid, sexual reproductive system for *O. michaeli*.

Inter-population variation was also examined at a species and sub-species level with gut bacteria of *T. adelaidensis* (Chapter 4). Gut microbiota contain potential pathogens, as well as preforming a number of essential services relating to nutrition and immunity (Amato, 2013; Dearing and Kohl, 2017). As such, providing an assessment of the diversity of bacteria, and whether these communities stood to change as a result of translocation was particularly pertinent. I did not detect any population-level differences in the subset of bacteria that enrichment conditions favoured (bacteria from within and also outside of the Enterobacteriaceae family). These communities may have represented part of a core bacterial community for the host species that does not vary between habitats, as has been observed for

microbiota in other species (Cahill et al., 2016; West et al., 2019). Furthermore, some wildlife studies suggest that the gut microbiota of herbivores may be more influenced by locality-driven dietary differences than that of omnivores (Barelli et al., 2020; Kohl et al., 2017). Other taxa not detected by the methods I used may be sensitive to more subtle differences between environments at ecologically similar sites. Examination of two prevalent potential pathogens, *Salmonella enterica* and *Klebsiella aerogenes,* at a strain level also revealed no clear structure in strain relatedness among the three host populations involved. This lack of structure at strain level between populations may be consistent with findings by Parsons et al. (2015) where some *Salmonella enterica* subspecies in *Tiliqua rugosa* within a locality varied by the micro-habitat (e.g. by amount of tree shade). The grassland environment of *T. adelaidensis* has a more homogenous vegetation structure than the mallee and salt-bush plain environment of the *T. rugosa* study.

Transmission in an augmented population

A further aim of this thesis was to comment on the parasite transmission dynamics between the *T. adelaidensis* host individuals which were involved in the population augmentation, either as translocated or resident individuals. Specifically, the focus was to ascertain whether previously allopatric parasites were transmitted to hosts following translocation. The genetic differentiation between populations of *O. michaeli* and *P. wandillahensis* made it possible to identify a number of these allopatric genotype transmission events, which occurred 9–22 months after translocated lizards were released among resident conspecifics at the recipient site (Chapter 3). Despite a small number of allopatric transmission events detected by parasite sampling, there was an overall trend to retain parasite genotypes from the population of origin. These results suggest that parasite transmission between hosts is slow. Similarly, transmission of cestode parasites was observed from resident woylies to translocated conspecifics one year after release, and transmission of strongyle nematodes from translocated individuals to residents was observed after 6–9 months by Northover et al. (2019). Such slowly-transmitted parasites would afford the opportunity for conservation managers to limit the spread through a host population should pathogenic effects be observed (e.g. Page et al., 2011).

In chapter 3, I also addressed the aim to better characterise mite and nematode transmission dynamics in a *T. adelaidensis* population. To do this, I compared transmission patterns suggested by relatedness of parasite individuals between hosts to host networks reflecting putative transmission mechanisms. The small number of allopatric parasites found on hosts

over the two years following translocation suggested that transmission was host-individual focused. A previous study using network analysis by Fenner et al. (2011) suggested that home burrow proximity to frequently moving 'disperser' individuals made infestation by *P. wandillahensis* nematodes more likely. The presence of contact heterogeneity between individuals in wildlife populations, and its importance in accurately modelling disease spread has been demonstrated by several studies (reviewed by White et al., 2017). In chapter 3, I found no strong evidence that *T. adelaidensis* individuals that lived closer to each other were more likely to share highly related nematodes, nor individuals that occupied the same home burrow asynchronously, nor male-female pairs that may have mated. This finding may provide support for the hypothesis that exploratory behaviour leads to increased conspecific scat-sniffing and increased transmission (Fenner et al., 2011). The potential role of prospecting behaviour (for new habitat or breeding opportunities) in shaping parasite transmission dynamics has been highlighted for systems with much larger spatial extents, i.e. seabirds visiting islands (Boulinier et al., 2016), but may need consideration in *T. adelaidensis* populations. In contrast to my findings, asynchronous refuge sharing has been implicated in the transmission of ticks in related lizard systems (Godfrey et al., 2010; Leu et al., 2010). Regardless of the factors at play, low host numbers within the experiment presented in chapter 3 are unlikely to have revealed any relationships with small effect sizes. Similarly, no patterns were evident for mite transmission, though a non-replicated result indicated that host burrow proximity may play a part in transmission.

Examining transmission of gut bacteria in translocated *T. adelaidensis* hosts was not possible, since the lack of clear difference in gut microbial communities between individuals from different populations made any transmission impossible to identify. In chapter 4 however, I report that this consistency among populations persisted one year following translocation, suggesting that translocation had no obvious short to medium-term effect on the gut microbiota of translocated and resident lizards, and that these communities were not dynamic enough to be significantly different between all years. There were, however, small but significant differences between microbiotas at two years following translocation and previous timepoints. At this two-year time point, translocated lizards and resident lizards also had small but significant differences between their detected microbiota. These results suggest that some of the gut microbiota are prone to change over time, and temporal processes or stochasticity may interact with genotype and be group-specific (Goodrich et al., 2016). Temporal variation in gut microbiota have been observed in various wildlife species. Examples include presumably diet- and activity-driven seasonal variation in lemurs and wood mice (Maurice et al., 2015;

Raulo et al., 2018), and differences over 2–4 weeks in squirrels (Bobbie et al., 2017). The fasting status of reptiles may also drive temporal differences in gut microbiota (Costello et al., 2010). However, larger studies of *T. adelaidensis* are required to further comment on the temporality observed, as these between-year differences may have been affected by the reduction of individuals sampled at each successive timepoint.

Effects of translocation on host fitness

Assessing the impact of translocation and any related parasite dynamics on host fitness was the final main objective of this work. With relatively small numbers of hosts for mites and nematodes identified (definitively confirming lack of nematode infestation would be difficult to do without gut dissection), I chose to instead assess the survival probability of all groups of lizards involved in the population augmentation: translocated lizards from two source populations, resident lizards sharing habitat with them, and control residents not sharing habitat with them. Survival of translocated and resident individuals is a necessary prerequisite to the long-term persistence of a population, which is the ultimate aim of a translocation (Armstrong and Seddon, 2008; Sheean et al., 2012). Including all lizards in the mark-recapture models shifted the focus to assessing the early success of the translocation itself rather than merely evaluating any effects by parasites on survival. The latter approach was likely to have been unsuccessful, as the effect size would have been small and sample sizes would have been insufficient. Evaluating survival in the whole cohort nonetheless provides a first step for assessing any parasite effects on survival, since differences in a particular group of lizards may have indicated that they were being affected differentially by a cause of mortality, which would have invited further investigation. I did not, however, find any differences in survival between translocated, co-habiting residents and non-co-habiting residents, suggesting that our experiment had not affected survival detrimentally. The model with the most support in the data suggested rather that there was an interactive effect between sex and time of year on survival. These findings are entirely consistent with male lizards being more likely to disperse during spring in order to seek mates and new burrows (Bull et al., 2015; Schofield et al., 2012). Similarly, male-biased mortality as a result of seasonal mate-searching activity has been reported in a diverse range of invertebrate and vertebrate species, including several snakes (Bonnet et al., 1999; Magnhagen, 1991; Sperry and Weatherhead, 2009).

Research implications

The results of the studies in this thesis collectively point towards the viability of translocation as a conservation strategy for *Tiliqua adelaidensis*. Like all vertebrates, this lizard species hosts parasitic symbionts, and we need to consider these when taking animals from one habitat and releasing them into another. This work is part of a small but growing number of studies (at least in the published literature) that assess disease risk or examine parasite dynamics in conservation-motivated reptile translocations (e.g. Aiello et al., 2014; Baling et al., 2013; Bobadilla Suarez et al., 2017). Both macroparasite species studied here appear to be host-specific and to not have obvious effects on host health (Fenner et al., 2008; Smith et al., 2009a, Chapter 2). These presumably stable host-parasite relationships do not appear to be disrupted by translocation, in the sense that translocated and resident lizards retained local-type parasites rather than acquiring a larger proportion of non-local parasites, and also retained symbiotic gut bacteria communities for several months following translocation (Chapters 3 and 4). Furthermore, no difference in survival was observed between lizards involved in the translocation and those that were not (Chapter 5). Similarly, a parallel study did not report differences in body condition or reproductive success between these groups (Clive, 2019; Clive et al., 2020). Low transmission rates of mite and nematode observed by genotyping these parasites suggests that translocating parasites with their hosts is not likely to result in a rapid spread of new parasite genotypes to immunologically naïve hosts. Furthermore, the lack of systematic differences and changes in gut bacteria over time do not suggest that microbiota changes are likely to pose a threat to individual health and translocation success. The presence of potential pathogenic species such as *Salmonella enterica*, *Escherichia coli* and *Klebsiella aerogenes* in *T. adelaidensis* guts do however highlight the potential for stressful conditions to induce pathology in their hosts (Schumacher, 2006; Verbrugghe et al., 2016).

The results obtained throughout these studies suggest that the experimental augmentation was conducted in a way that appeared to minimise stress to hosts and maximise post-translocation survival. Post-release dispersal appears to be a common stress response in reptile translocations (Aiello et al., 2014; Dickens et al., 2010; Massot et al., 1994) and has reduced body condition and survival (Germano and Bishop, 2009; Griffith et al., 1989; Massot et al., 1994; Sullivan et al., 2004). Prior studies assessing *T. adelaidensis* behaviour following wild-captivity translocation (Ebrahimi and Bull, 2014a, 2014b, 2013) and also the effect of artificial burrow provision (Milne et al., 2003a; Souter et al., 2004), and social structure (Schofield et al., 2014) informed measures we took in this experimental population augmentation to provide

adequate habitat, minimise stress and stress-induced dispersal. My findings show that inherent spring-time dispersal by male individuals does reduce survival probability in *T. adelaidensis*, but the lack of difference between treatment groups indicates that translocation did not increase dispersal enough to reduce survival. Increased post-release movement in other reptiles has been shown to increase interaction between conspecifics and to increase disease risk (Aiello et al., 2014) (though see Lafferty and Holt, 2003). The slow and minimal extent of transmission of non-local macroparasites I observed between translocated and resident lizards may have been partially due to individuals having minimal contact with conspecifics, as a result of a reduced tendency to move around in search of new home burrows. The consistency in gut bacterial taxa across sites and timepoints also suggest that microbiota were not overly perturbed by this experiment (Chapter 4). I propose that this experimental translocation provides an example of where extensive prior study into the biology of the focal species has been possible and executed (a requirement that has been pointed out in the translocation literature (Batson et al., 2015; Besson and Cree, 2011; Dodd and Seigel, 1991; Sheean et al., 2012; Weeks et al., 2011), and has enhanced translocation outcomes. On the basis of the likely relationship between parasite virulence — its ability to infect and damage a host — and the various effects of stress on the host, parasite prevalence and its change over time may provide an additional indicator of host animal health and translocation success.

This thesis examined the dynamics of parasites and commensal gut bacteria which do not produce obvious pathology or loss of fitness in their host, and presumably share evolutionary history with *T. adelaidensis.* It is however worth noting that high virulence and co-evolutionary history are not mutually exclusive (Alizon et al., 2009; Read, 1994). The observations made in this thesis may be useful in considering the risk posed by parasites novel to *T. adelaidensis* with pathogenic potential. Particularly relevant are recent discoveries of a nidovirus that causes an often-fatal respiratory syndrome in the congener *Tiliqua rugosa* (O'Dea et al., 2016) (with microparasites most likely to pose a risk in translocations, Rideout et al., 2017), and also the occurrence of the introduced snake mite *Ophionyssus natricis* in wild *T. rugosa* individuals in a locality nearby to the *T. adelaidensis* Burra population (Norval et al., 2020). This cosmopolitan mite species is a known cause of pathology in captive reptiles (Wozniak and De Nardo, 2000), and generalist parasites are deemed more likely than specialists to pose a risk in wildlife translocations (Rideout et al., 2017). My findings suggest that parasites are slow to spread in *T. adelaidensis*, a likely result of its solitary social system and somewhat sessile lifestyle (Fellows et al., 2009; Hutchinson et al., 1994; Milne et al., 2003b), in contrast to the more social and vagile *T. rugosa* (Leu et al., 2011). These characteristics could have a protective

effect by slowing and reducing the extent of disease transmission. However, parasites that can persist in the open grassland or burrow environment, or are vertically transmitted, may pose a non-negligible threat for *T. adelaidensis*.

In the absence of evidence that they parasitise other host species, *O. michaeli* and *P. wandillahensis* provide examples of parasites that are vulnerable to co-extinction. Though documented co-extinction events have been few, modelling approaches predict them to be common (Dunn et al., 2009) and as such, co-extinctions are predicted to be a major driver of global-scale biodiversity loss (Carlson et al., 2017; Strona, 2015). In the ixodid ticks of reptiles alone, some 20 species were estimated to be co-endangered with their hosts by Mihalca et al. (2011). Parasites of hosts that experienced relative population stability over evolutionary time frames, but have been threatened by anthropogenic factors are considered at heightened risk of co-extinction (Strona, 2015), as seems likely for *T. adelaidensis*. In addition to representing intrinsic biodiversity loss, parasite co-extinctions are also likely to reduce the ecological robustness of biotic communities (Hatcher et al., 2006; Lafferty et al., 2006; Wood et al., 2007). At a host population level, parasites may exert selective pressures that help maintain evolutionary fitness (Nunn et al., 2004; Sommer, 2005). There is also generally a lack of understanding of co-infection and poly-parasitism in wildlife to confidently predict the effect of parasite eradication or co-extinction (Northover et al., 2018; Thompson et al., 2018; Trevelline et al., 2020); loss of parasites may subject the host to heightened virulence of another. Taxonomic description provides the first step towards understanding the ecological role of wildlife parasites, and towards conserving them (Carlson et al. 2020). The work presented here has thus contributed to this effort, as well as furthering our understanding on the ecology of its endangered host and the factors that could affect its fitness in wild and captive settings. A nomination that I made for *O. michaeli* to be considered as a flagship species for invertebrate conservation in its bioregion (leading to listing under the Commonwealth Environmental Protection and Biodiversity Conservation 1999 Act) is included in appendix 1 of this thesis (see Taylor et al., 2018).

Future research directions

The research described in this thesis contributed directly to the evaluation of wild-wild translocation as a conservation strategy for *Tiliqua adelaidensis*. It was not however, a formal disease risk assessment (*sensu* Ewen et al., 2015), but rather will serve to inform future translocation risk assessments in this species. My work has furthered knowledge on the host-

parasite relationships of this endangered host, and adds to the extensive prior study which has contributed heavily to the apparent success (at least in the short-term) of this experimental population augmentation. Future *T. adelaidensis* translocation evaluation will include re-introductions to further south of the current range of the species, where climatic conditions are cooler and wetter (Duffy et al., 2012). These trials will test a more future-proof strategy for pre-empting predicted climate-change- driven population declines (Delean et al., 2013; Fordham et al., 2012). These future translocations should not look to necessarily eradicate macroparasites such as *O. michaeli* and *P. wandillahensis,* nor take specific measures to change gut microbiota. They should however continue to monitor parasite dynamics alongside routine post-release monitoring, including surveillance for pathologies which may be caused by novel or previously unknown parasites.

The unanswered questions my work has uncovered in this host-parasite system, reflect those concerning wildlife parasites generally. The lifecycle of the mite *Ophiomegistus michaeli* remains uncharacterised, as nothing is known about non-adult life stages in any species of this genus (Halliday and Grimm-Seyfarth, 2019; Klompen and Austin, 2007). The unsuccessful attempts in this study to identify a likely transmission mechanism for it exemplify that such knowledge is not only innately interesting, but has application in understanding host-parasite dynamics and thus conservation management of both the host, and also the mite itself. Similarly, the exact behavioural/environmental context in which ingestion of nematode eggs occurs in *T. adelaidensis* remains unknown, and this gap has implications for any control measures that may be needed for this species in future circumstances, or other faecal-orally transmitted pathogens, such as pathogenic gut bacteria. Such a lack of understanding appears to be typical of wildlife parasite species (Doña et al., 2019; Spratt and Beveridge, 2019; Thompson et al., 2010). Future research ideally needs to describe a larger portion of the earth's enormous parasite diversity (Carlson et al. 2020) and to focus effort towards elucidating their lifecycles, host specificity and transmission mechanisms (Dunn et al. 2009). The rapid advancement of DNA-based techniques such as genome wide sequencing provide the tools necessary to complement traditional taxonomy in classifying new and perhaps cryptic parasite species. These tools will also be useful to identify life stages and host species, to help elucidate transmission mechanisms, and to understand population history and inform parasite conservation (Adlard et al., 2015; Archie et al., 2009; Carlson et al., 2020; Spratt and Beveridge, 2019; Tompkins et al., 2011). Additional areas requiring research are the co-infection dynamics in this system. These dynamics may occur between macroparasites, between microparasites and commensal/mutualistic symbionts, or between macroparasites and microbiota e.g.

DeCandia et al. (2020). The possible relationship between *P. wandillahensis* and gut microbiota would particularly be worth investigating. Gut nematode-microbiota relationships are exemplified by the gut nematode *Heligmosomoides polygyrus*. Nematode infection was found to be related to the presence of certain gut bacteria in wild wood mice (*Apodemus sylvaticus*)(Maurice et al., 2015). Excretory and secretory products from this nematode species were found in another study to exhibit antimicrobial activity against certain bacteria, and nematode fitness may be increased by microbiota-driven immune regulation in the lab mouse host (Rausch et al., 2018).

More extensive knowledge of parasite ecology would also underpin studies on how, and under what conditions, hosts are affected by their parasites at scales ranging from cells, organs, organisms to populations and the broader ecosystem. This understanding will be increasingly necessary as wildlife populations are further stressed by habitat destruction and deterioration (Benítez-Malvido et al., 2019; Bower et al., 2019). Parasites may have effects on their hosts other than reducing reproduction and survival directly (Papkou et al., 2016; Tompkins et al., 2011). Examples of these more subtle effects can be found in other mite/tick-lizard systems, and also the related *Egernia stokesii -Pharyngodon tiliquae* host-parasite system (Fenner and Bull, 2008; Main and Bull, 2000; Sorci and Clobert, 1995). The exact fitness costs (if any) of *O. michaeli* and *P. wandillahensis* remain to be further investigated by using targeted tests of fitness (e.g. Main and Bull, 2000). Parasites may actually be inaccurately classified commensals or mutualists (Doña et al., 2019). *Pharyngodon wandillahensis* particularly may play some role in digestion and be mutualistic when hosts are in good-quality habitat (Benítez-Malvido et al., 2019).

Gaining a deeper understanding of how hosts are affected by their parasites may help us predict and test whether or not parasites exert selective pressure, and whether local adaptation is present, with a higher potential for translocation related consequences. Characterisation of parasite and host population genetics may also complement empirical cross infection studies (e.g. Prugnolle et al., 2006) by identifying specific genes under selection in both parties. Resulting genotyping approaches could inform host propagule selection in future translocations. In the case of *T. adelaidensis*, genotyping as many individuals and their parasites from as many populations as possible across the species range would be required to build on this and previous work (Smith et al., 2009b, 2009a) and to test for gene loci under selection — research that will be enhanced by sequencing of a genome of the congener *T. rugosa* planned in the OzARG genome consortium. Genotyping parasites may also

complement *T. adelaidensis* population genetics in elucidating the demographic history of this skink species (Whiteman and Parker, 2005).

Chapter 4 provided an assessment of some of the gut microbiota of *T. adelaidensis* and examined how host translocation changed the gut bacterial taxa present. This was one of the first studies to characterise microbiota in animals throughout the course of a translocation and the post-release period (but see Chong et al., 2019). Continued characterisation of microbial diversity and function in wildlife (Colston and Jackson, 2016) and in translocation contexts provides an avenue for future research, enabled by growing accessibility to high-throughput DNA sequencing of metagenomes. As the functions of bacterial genes are better characterised through whole-genome sequencing, understanding which microbial profiles confer fitness benefits to wildlife hosts such as *T. adelaidensis* will grow. An example of where this understanding of functional roles of gut bacteria has been applied to manipulate the host microbiome and enhance host success in a new environment already exists, where faecal inoculations broadened the dietary niche of koala individuals (*Phascolarctos cinereus*, Blyton et al., 2019). More generally, screening the microbiota of individuals for certain taxa (e.g. absence of potential pathogens or presence of mutualists) may inform optimal propagule selection for translocations.

Conclusion

This thesis has helped address the general need to consider parasites and other symbionts when translocating wildlife for conservation purposes, by examining host-parasite relationships and the effects of translocation on these in an endangered reptile species. The results obtained suggest that these now better-characterised host-parasite relationships are not altered or disrupted by wild-wild translocation, and that host survival remained high following translocation. On the basis of these findings, we should continue testing translocation as a conservation strategy for *T. adelaidensis*, whilst seeking to better understand symbiont biology, and their effects on hosts and on conservation outlook. It is hoped that work such as this will contribute to devising conservation management that minimises host fitness loss due to parasites, but also maintain parasite diversity and the ecosystem roles that this guild performs, making for more biodiverse, robust ecosystems.

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Appendix 1.

Nomination of *Ophiomegistus michaeli* to the Australian Entomological Society Conservation Committee's invertebrate conservation initiative.

This nomination was submitted by B. Derne, B. Halliday and P. Weinstein in April 2020.

Australian Entomological Society

AES Conservation Committee

Species Nomination

Taxonomy

[List scientific name, author, date, synonymies; higher classification (Order: Family); and common name (provide one if not available)]

Scientific name: *Ophiomegistus michaeli* Halliday (in Derne *et al*. 2019)
(Parasitiformes: Paramegistidae)

Common name: Pygmy bluetongue mite

Description

[Provide a brief description, giving diagnostic features for identification; how it is distinguished from similar species; and an image of species]

Ophiomegistus michaeli is a large mite, dark brown in colour, and dorso-ventrally flattened (Fig. 1). The dorsal surface is covered by a single large shield that has a sparse covering of minute pointed setae, while the ventral surface bears a combination of short pointed setae and others modified into a flat paddle-shape. The genital opening of the female is on the ventral surface, protected by a complex series of sclerotised plates. The male is generally similar to the female but smaller, and its genital opening is small and circular. The anterior legs are slender and flexible, and have a mainly sensory function. The other three pairs of legs are shorter and more robust, and carry membranous pads that assist in adhesion to the host. The mouthparts are elongate and pointed, adapted for piercing the skin of the host.

Male: Dorsal shield length 580-600 micrometres, width 660-685 micrometres.

Female: Dorsal shield oval, wider (794 micrometres) than long (651 micrometres).

Similar species: The genus *Ophiomegistus* includes about 25 species that occur in Australia and southeast Asia. Different species are distinguished from each other by the number, structure, and arrangement of setae on the ventral surface. Their degree of host specificity has not yet been determined.

A full species description of *O. michaeli* can be found in Derne *et al.* (2019).

Figure 1. Ventral surface of an *Ophiomegistus michaeli* female. (Image: B. Halliday)

Distribution

[Provide an overview of the species' known or estimated current and past distribution; IBRA region; land tenure, especially for sites protected within the reserve system; and spatial map]

IBRA region: Flinders Lofty Block (FLB)

Distribution: Occurrence records for this newly described species are currently restricted to three sites within the Mid North region of South Australia (Fig. 2), one north of Jamestown, one west of Clare and one east of Burra (Derne *et al*. 2019)**.** Distribution is likely to reflect that of its only known host, the pygmy bluetongue lizard (*Tiliqua adelaidensis*) which inhabits natural grasslands in the Mid North Region of South Australia (T. Milne 1999; Souter *et al*. 2007) over an approximate area of 7000 km^2 (Delean *et al*., 2013). This lizard range once extended to the Adelaide region (Duffy *et al*. 2012).

Land tenure: Occurrence records are on privately owned land, including the Nature Foundation of South Australia's Tiliqua property and two privately owned sheep farms (north of Jamestown and west of Clare townships).
The 31 known populations of *T. adelaidensis* occur on privately owned land used to graze sheep (Duffy *et al*. 2012).

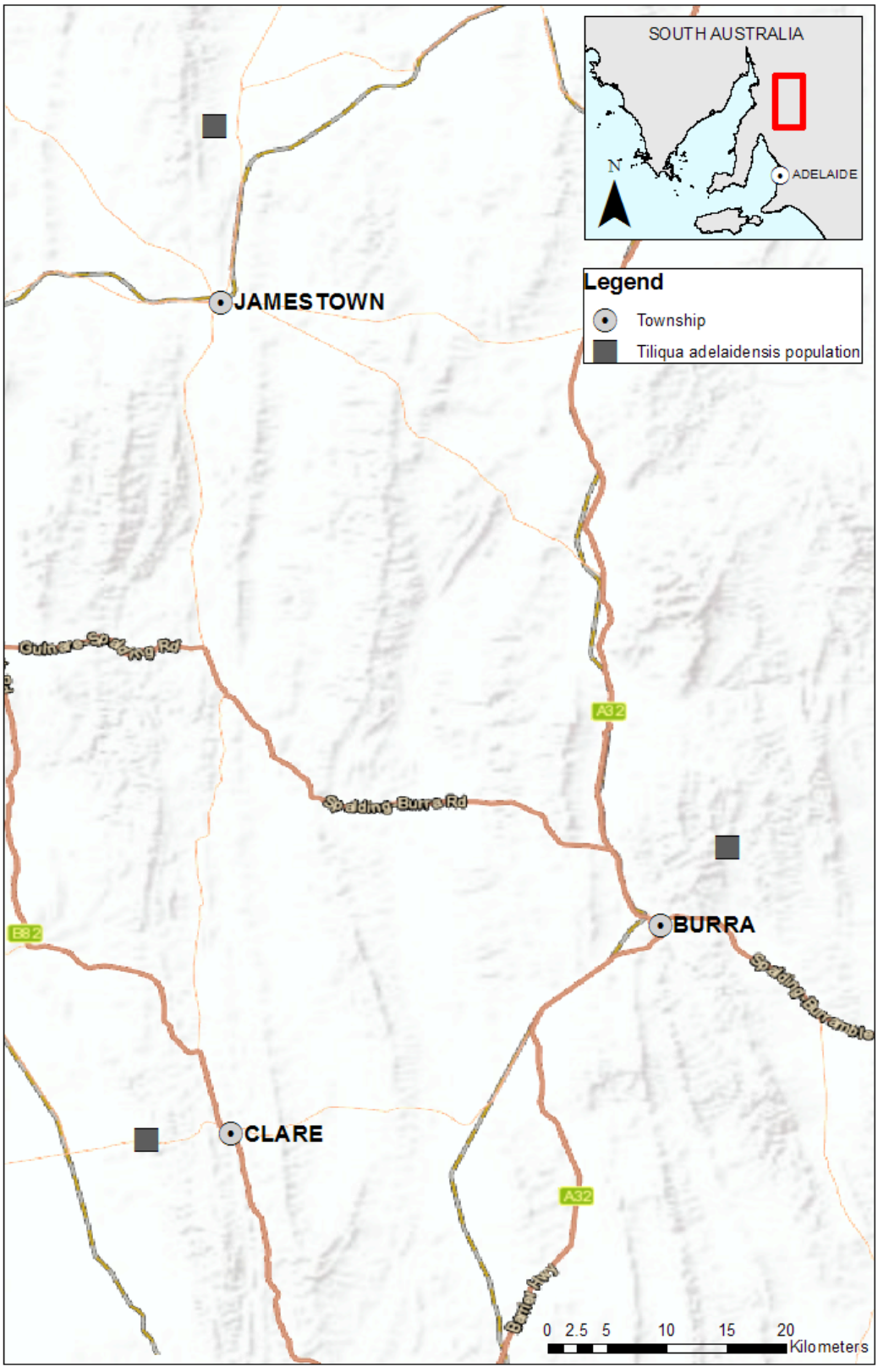

Figure 2. Approximate locations of known *Ophiomegistus michaeli* populations, reflecting that of their lizard host *Tiliqua adelaidensis* in the Mid-North region of South Australia, part of the Flinders Lofty Block IBRA region. (From Derne *et al.* (2019).

Biology

[Summarise what is known about the life history, seasonality and life cycle]

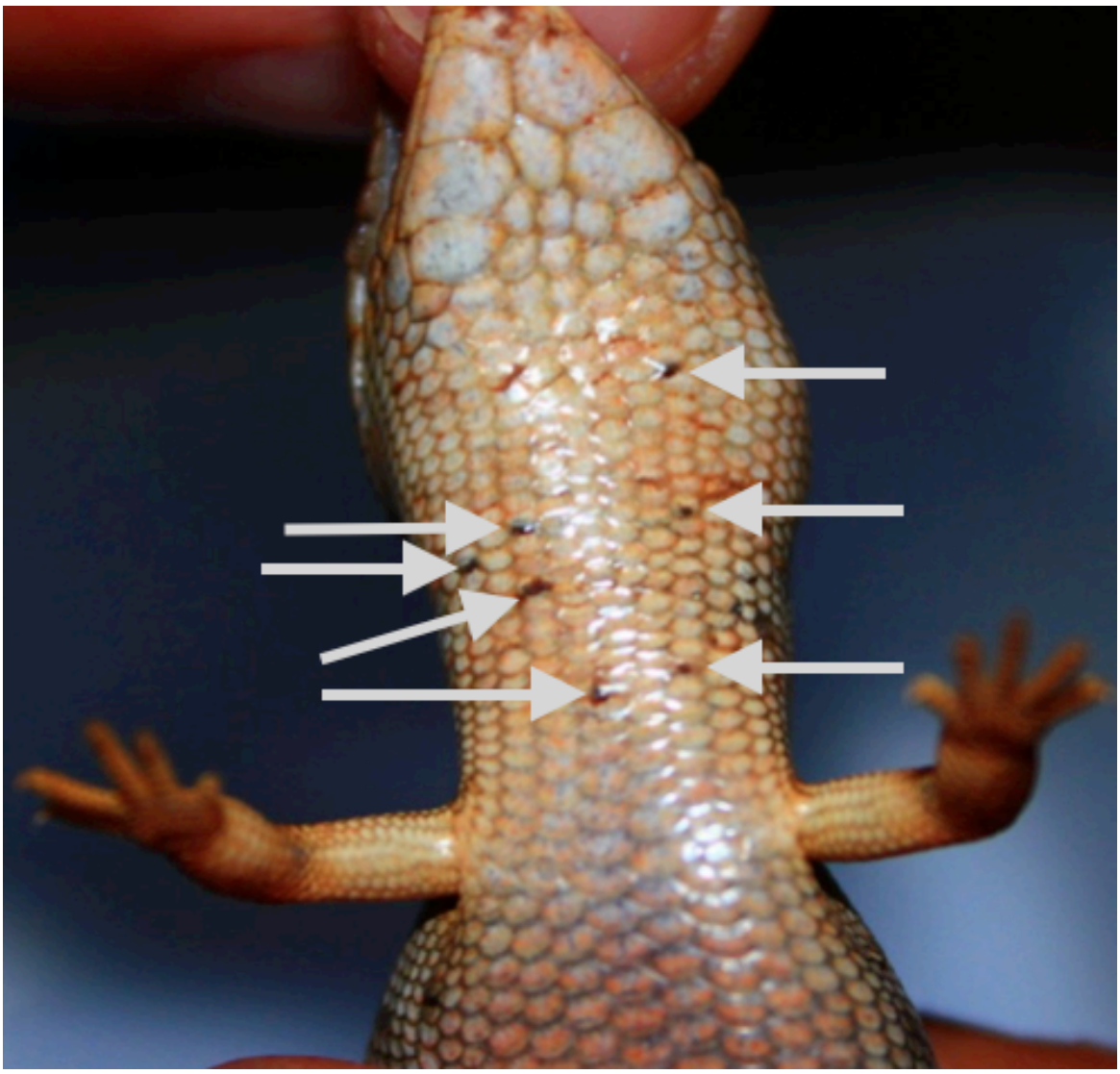

Figure 3. *Ophiomegistus michaeli* adult mites attached to ventral surface of its host, *Tiliqua adelaidensis* (Derne *et al*. (2019).

Adult mites in the genus *Ophiomegistus* have been observed attached to snake or skink hosts, in the case of *O. michaeli*, that of the pygmy bluetongue lizard (*Tiliqua adelaidensis*), a medium sized skink. The mite lodges itself under the scales of its host and pierces the skin with specialised chelicerae, presumably to obtain a blood meal (Derne *et al*. 2019) (Fig. 3). Free living *O. michaeli* have not been observed and nothing is currently known about the

immature stages of this species, or indeed any other species in the genus (Klompen & Austin 2007). We believe that the immature stages (eggs, larvae and nymph) may occur in the disused spider burrows occupied by its host. They are probably not parasitic and may feed on other small invertebrates, but that is a subject for future research. Adult mites have been observed on lizards all months between October and February, coinciding with when the host species is active and studied. No discernible seasonal patterns in the prevalence of the mite on its host species has been observed (Derne *et al*. 2019).

Ecology

[List any ecological interactions (e.g. food plants, hosts, predators)]

Adult *O. michaeli* mites attach to their skink host, *Tiliqua adelaidensis.* They have not been observed on other reptile hosts, nor free-living in the environment. They are presumed to be host specific.

Critical habitat

[Summarise breeding habitat or ecological community]

Ophiomegistus michaeli is a host specific parasite of *Tiliqua adelaidensis*, therefore host populations are essential for its survival. *Tiliqua adelaidensis* requires natural temperate grassland habitats where appropriate vertical spider burrows are present for use as refugia from thermal extremes and predators (Hutchinson *et al*. 1994, Milne *et al*. 2003, Fellows *et al*. 2009). These burrows are dug by a number of species of spider belonging to two families: Lycosidae, such as *Lycosa stirlingi* and *L. gilberti*; and Mygalomorphidae, namely *Blakistonia aurea* (Hutchinson *et al*. 1994, Fellows *et al*. 2009). Appropriate soil composition and depth are important constraints for *T. adelaidensis*-suitable spider burrows; these require unploughed native grasslands on lower footslopes and hill flanks (Souter *et al*. 2007). Native grasses which are typically found in *T. adelaidensis* habitat include brushwire grass (*Aristida behriana*), and wallaby grasses (*Austrodanthonia carphoides* and *Austrodanthonia eriantha*) (Delean *et al*. 2013), although non- native species such as wild oats (*Avena barbata*), spear grass (*Austrostipa* sp.) and Salvation Jane (*Echium plantagineum*) have also been commonly observed.

Tiliqua adelaidensis feeds on arthropods such as locusts, spiders, beetles, ants, cockroaches and also a small amount of plant matter (Fenner *et al*. 2007). Natural predators of *T. adelaidensis* include raptors, and snakes such as the Eastern Brown Snake (*Pseudonaja textilis*) (Fenner *et al*. 2008).

Key threatening processes

[If known, list evidence of decline; past, current and potential future threats and their impact]

Evidence of decline: *Tiliqua adelaidensis* is the only known host for *O. michaeli* and is listed as Endangered by the IUCN (Fenner *et al*. 2018). The following threats have been identified for this lizard host:

Past threats: Native temperate grasslands which the lizard host exclusively inhabits have undergone extensive clearing and fragmentation in South Australia (Duffy *et al*. 2012). Clearing and ploughing of grassland habitats in the Adelaide plain and the mid-north region for agriculture and urban development has resulted in the direct destruction of *T. adelaidensis*, and also the destruction of the spider burrows needed for its persistence (Hutchinson *et al*. 1994). In the case of grassland fragments that have not been ploughed or cleared, inappropriate livestock grazing regimes have furthermore been identified as a threat to the lizard's persistence.

Current threats: Current and future threats to *T. adelaidensis* and *O. michaeli* populations continue to be any form of landuse change that disrupts the grassland habitat and precludes lizards and burrowing spiders. Recently, the development of windfarms and telecommunication infrastructure and associated roads have been an important threat to *T. adelaidensis* habitat. More generally, small, isolated populations created by habitat fragmentation are furthermore vulnerable to decline due to stochastic events such as climatic and disease events (May 1973).

Potential future threats: Modelling suggests that future climate change will cause decline in population abundances of *T. adelaidensis*, and that extinction of the species is likely in the long term unless managed translocations are undertaken (Fordham *et al*. 2012).

Community engagement and conservation management

[Identify relevant stakeholders; and any management plans or recovery teams overseeing threat abatement/mitigation actions, either underway or proposed]

Community engagement: Community engagement has been encouraged and facilitated by the recovery team of the host species *Tiliqua adelaidensis* (Duffy *et al*. 2012). Initiatives include the formation of the Pygmy Bluetongue Community Conservation Committee, the Nature Foundation of South Australia's annual lizard crawl event, and the publication of a children's book on the lizard, with associated school visits. Captive breeding programs of *T. adelaidensis* have also been undertaken by Zoos South Australia. Residents in the township of Burra and surrounding towns are generally well aware of *T. adelaidensis* and its local endemism. *Ophiomegistus michaeli* itself featured as a local example of parasitism at the South Australian Museum's 'Parasites: Life Undercover' exhibition (2018-2019).

Conservation management and actions: *Tiliqua adelaidensis* was listed as Endangered on the IUCN Red List in 1996 (IUCN 1996, Fenner *et al*. 2018). *Tiliqua adelaidensis* has been the subject of a recovery actions since 1992. The South Australian Government's Recovery Plan for the lizard, with associated recovery team, was implemented in 2012 (Duffy *et al*. 2012). The plan's overarching objectives are to research the distribution, habitat, ecology (including parasites) and management requirements of *T. adelaidensis*, as well as to raise awareness and provide evidence-based guidelines for land management.

Conservation status

[If known or evaluated, give the current listing and relevant conservation schedule or Act]

International (IUCN Red List): Not listed. Only known host, *Tiliqua adelaidensis* is listed as Endangered (Fenner *et al*. 2018).

National (EPBC): Not listed. Only known host, *Tiliqua adelaidensis* is Endangered (Duffy, Pound, & How, 2012).

State: Not listed. Only known host, *Tiliqua adelaidensis* is listed as Endangered under the Government of South Australia *National Parks and Wildlife Act 1972* (Duffy *et al*. 2012).

Proposed conservation status evaluation

[If recommendations are to be made for threatened status and listing provide justification based on IUCN Red List Criteria. For example, Criterion 2: geographic range is precarious for either extent of occurrence (EOO) and/or area of occupancy (AOO)]

We propose that *Ophiomegistus michaeli* be accorded the same conservation status as its only known host species, *Tiliqua adelaidensis*. Since parasite prevalence will rarely be 100% in a wild host population, *O. michaeli* is likely to be less common and less widely distributed than the host species.

EOO: Unknown for *O. michaeli*, though Atlas of living Australia contemporary records for the host *T. adelaidensis* are restricted to a small endemic range, approximately bound by the South Australian townships of Peterborough (North), Kapunda (South), Eudunda (East) and Bute (West). Within this range estimated extent of occupancy of some 7000 km^2, host populations occur on isolated fragments of native grassland (Delean *et al*. 2013) which are subject to a number of threatening processes, as described in previous sections.

AOO: Unknown, although estimated area of occupancy for *T. adelaidensis* is less than 500 km^2, on habitat that is severely fragmented and is subject to observed and projected continuing decline in extent and or quality (Duffy *et al*. 2012).

Scientific and/or social value

[e.g. relictual, phylogenetically distinct, keystone species, aesthetic, mediagenic, cultural, entomophagy, biophilia, economic, ecotourism]

Parasitic species are widely perceived as purely harmful to their hosts, and those not of medical significance are generally understudied. However, parasites are increasingly being recognised as key components of ecosystems in terms of biodiversity, biomass and agents of population regulation (Lafferty *et al.* 2006, Kuris *et al*. 2008, Dunn *et al*. 2009). As such, parasitic species depending on vulnerable host species are increasingly considered as vulnerable species in need of conservation themselves. Co-extinctions such as those occurring by host-parasite relationships have been identified as a major driver of biodiversity loss (Strona 2015). *Ophiomegistus michaeli*, as a host-specific parasite of an endangered species

such as *Tiliqua adelaidensis* therefore appear to be likely candidates for co-extinction. The only recent discovery and description of *O. michaeli* supports the notion that our documentation and understanding of parasite diversity is nascent, and is threatened by the widespread decline of host species and their ecosystems. We advocate that efforts be made to conserve and study such parasites and the intimate relationship they have with their host.

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[Cite all relevant information]

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Nominator/s

[Provide your contact details, including name, address and email]

Bonnie T. Derne

Flinders University, South Australia

Email: [redacted]

Bruce Halliday

Australian National Insect Collection, CSIRO

Email: [redacted]

Philip Weinstein

University of Adelaide, South Australia.

Email: [redacted]