Taxonomy of the salt lake gastropod, *Coxiella*: can genetic data be extracted from dried shells?

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Abstract

The fauna of salt lakes in Western Australia are currently under threat from climate change, secondary salinity and mining practices. Knowledge gaps still exist in our understanding of the ecologies of many groups including the unique halophilic gastropod genus *Coxiella*. The taxonomy of the genus is currently regarded as insufficient and in need of revision due to the unreliable nature of the morphological characters currently used to differentiate species. However, collecting live specimens for analysis is logistically constraining as *Coxiella* are generally only active for a limited period of time each year when salt lakes are at reasonably low salinities. Alternatively, the shells of dead *Coxiella* are abundant and are easily collected on the shores of salt lakes and may offer as substitute for studying live specimens. Given the current unreliability of the morphological taxonomy, DNA barcoding may offer a more consistent method for species identification, but first, a methodological approach for extraction and amplification of DNA from dried shells must be established. In this study, DNA of high molecular weight was successfully extracted from dried *Coxiella* shells, but the identity of such DNA was not verified due to substantial issues encountered with the polymerase chain reaction (PCR). Seven out of 20 PCRs yielded enough product that could be visualised on a stained agarose gel, but no PCR produced any amplicon suitable for Sanger sequencing. Six of the successful amplifications were using Universal primers that are designed to amplify a ~650 bp fragment of the *cytochrome oxidase 1* gene for a wide range of metazoans and thus could not be confirmed to be *Coxiella*. One PCR using *Coxiella* specific primers produced a ~350 bp amplicon suspected to be *Coxiella,* but further optimisation of the PCR protocol is required before sequencing would be possible. The lack of successful amplification this project was attributed to inappropriate primer design and the presence of PCR inhibitors that could not be resolved using the methodologies attempted here.

Contents

Introduction

Australian salt lakes

Salt lakes are found on every continent on earth and are common in warm, arid climates such as those that dominate much of Australia (Hammer, 1986; Williams, 1981). Salt lakes can be defined as an enclosed body of water disconnected from the marine environment with total dissolved solids greater than 3 gL^{-1} that may form at the termini of drainage systems or in depressions where a balance of surface/subsurface inflows and outflows from evaporation and seepage into sediments allows the persistence of water (Bayly & Williams, 1966; Williams, 1964; Williams, 2002). Lakes exist either as permanent forms holding water throughout the year, seasonally (ephemerally) or episodically, where filling is irregular, and lakes may go years without water (Williams, 1998, 2002).

Threats to salt lakes

Salt lakes across the Australian continent are currently impacted by a diverse range of threats currently dominated by changes to hydrological budgets or direct degradation from mining practices (Timms, 2005; Williams, 2002).

Changes to hydrological budgets

The hydrological budgets of salt lakes are an equilibrium between inflows from surface runoff, subsurface flows, direct precipitation and outflows from evaporation and seepage into sediments (Timms, 2005; Williams, 2002). Salt lakes are therefore sensitive to changes to their hydrological budgets given that changes to the physiological chemistry of lakes have a large influence on the faunal compositions of lakes and that generally, biodiversity is inversely correlated with salinity (Williams, 1998).

Anthropogenic climate change is attributed with the declines in rainfall that are being experienced across much of southern Australia including declines being experienced in both the south-western (Hughes, 2003) and south-eastern (Kirono et al., 2012) portions of the continent. Ephemeral lakes are most affected by reductions in rainfall as they rely on seasonal (winter-spring) precipitation to fill (Timms, 2005). Lower rainfall results in higher salinities that can only be occupied by a restricted community of fauna (Williams, 1998). Alternatively, lakes may not fill at all, and although the biota of ephemeral lakes are adapted to surviving through periods when lakes dry up, the viability of these phases is finite and extended dry periods may result in localised extinction (Timms, 2005).

Secondary salinisation can also alter the salinity, water period and pH of naturally saline lakes with these impacts acting synergistically to restrict biodiversity to a few species with broad environmental tolerances (Halse et al., 2003; Stewart et al., 2009; Timms, 2005). Secondary salinisation occurs as a result of clearing of deep-rooted native vegetations that enables the water table to rise and mobilises the salts stored in the soil profile bringing them to the surface inducing waterlogging of saline water (Cramer & Hobbs, 2002). The Wheatbelt of Western Australia is an area that has been extensively cleared and experienced significant levels of secondary salinisation (Halse et al., 2003). Although an increase of saline aquatic habitats may logically benefit salt lake invertebrates by providing more habitat or changing some lakes from ephemeral to permanent, secondary saline lakes have been found to have more homogenous faunal compositions and also to be limited in their diversity when compared to naturally saline lakes (Halse et al., 2003; Pinder et al., 2004). It has been proposed that hyposaline invertebrate groups have adapted to the hydrological fluctuations in ephemeral lakes require the seasonal drying and periods of low salinity to initiate lifecycle cues (Pinder et al., 2004). For example, some species of the endemic genus *Parartemia* are considered threatened due to the increased salinity and waterlogging of naturally saline wetlands as

a result of secondary salinity (Timms et al., 2009). Therefore, permanent saline water associated with the rise of a shallow water table may affect the ability of invertebrates to develop and maintain populations in lakes affected by secondary salinity (Pinder et al., 2005).

Additionally, much of the groundwater in the Wheatbelt is naturally acidic and can alter the pH of wetlands when affected by secondary salinity (Timms, 2009a). Deep drains aimed at reducing the effect of waterlogging on agricultural land have also resulted in dumping acidic groundwater into rivers and wetlands resulting in losses in biodiversity (Stewart et al., 2009). The low pH allows for the mobilisation of metals and other elements that are harmful to biota that is reflected in their low faunal diversity (Timms, 2009a).

Mining practices also impact salt lakes in a variety of ways, including direct degradation from mining the lake itself for mineral extraction or as waste dumps for rocks and highly saline groundwater (Timms, 2005). The dewatering of hypersaline groundwater on the surface of the lake has been highlighted as of particular concern, as the extra salt load may affect the capacity for fauna to regenerate after a filling event as salinities may not become dilute enough (Timms, 2005). A study from Lake Carey Western Australia that has a history of localised mining practices suggested that although dewatering of hypersaline water from mining practices did affect the diversity of fauna within immediate proximity, due to the lakes size the overall impact was limited (Gregory et al., 2009). In addition, after significant deluges from cyclonic rainfall events caused a lowering in salinities as salts were flushed from the system (Gregory et al., 2009).

Why is it important to document invertebrate biodiversity?

Despite the threats, management interventions for the conservation of salt lake environments in southern Australia are restricted by gaps in our understanding of the biology of these species. Salt lake invertebrates in south-western Australia are highly diverse but often have naturally restricted distributions (Pinder et al., 2004). However, for taxa such as the gastropod genus *Coxiella*, even basic information about the number and distribution of species is lacking (see below).

The number and distribution of species is fundamental information for the conservation of any group for two reasons; (1) species are the unit of analysis that is used in conservation science crucially when protective management interventions are being considered (Sites & Crandall, 1997); and (2) the distribution of a species is also fundamental to its vulnerability to disturbance i.e. a broadly distributed species is less vulnerable to extinction than a short-range endemic (Ponder & Colgan, 2002).

Difficulties of adequately sampling salt lake invertebrates

Sampling difficulties are a part of the reason that the fauna of Australian salt lake environments is generally poorly studied. The salt lakes are numerous and spread over an enormous area (De Deckker, 1983), making it difficult and expensive to sample these environments adequately. Further, sampling the entire suite of species present in a salt lake at any one time is hindered by the stochastic nature of the environment (Halse et al., 2002). Also, the species composition changes with salinity (Pinder et al., 2004), typically starting from hyposaline fauna and progressing to a restricted hypersaline fauna as the lake evapoconcentrates (Williams, 1985). The sampling of lakes is also limited by their ephemeral nature as many, are dry for much of the year. The window for sampling is then

restricted and is a logistical constraint on attempts to better understand the fauna of these systems.

Coxiella

Coxiella is a genus of halophilic gastropods that inhabit the salt lakes in Western Australia, South Australia, Victoria, Tasmania and Northern Queensland (Bayly et al., 1966; De Deckker & Williams, 1982; MacPherson, 1957; Timms, 1993; Timms, 2007). Western Australia is the most diverse area for *Coxiella* with seven of the nine species described including five endemic species (MacPherson, 1957; Pinder et al., 2002).

Information on the ecology of *Coxiella* is limited (due to taxonomic insufficiencies, see below) but one study of the *Coxiella* from Lake Tallina South Australia suggested that the species studied did not possess high tolerances to desiccation, temperatures or salinities but instead has behavioural adaptions which do not expose it to the environmental extremes of salt lakes (Williams & Mellor, 1991). During periods where water levels begin to drop, and temperatures and salinities begin to rise, *Coxiella* shelters in areas of high humidity such as under algal mats or within mud cracks and retract its operculum to prevent desiccation (Williams, 1985)

Upper salinity tolerances for active specimens of *Coxiella striata* have been reported up to 124 gL^{-1} (De Deckker & Geddes, 1980) although the majority of *Coxiella* populations are found in hyposaline to mesohaline permanent and ephemeral salt lakes (Geddes et al., 1981; Halse, 1981; Timms, 1983; Williams, 1995). Although the precise ecological factors limiting the distribution of *Coxiella* have not yet been determined, hydroperiod, salinity and substrate composition appear influential (Doupe & Horwitz, 1995; Pinder & Quinlan, 2015; Pinder et al., 2002; Timms, 2007, 2009b). *Coxiella* does have some resistance to desiccation and is found in ephemeral lakes in central Australia (Timms,

2007), but is not found in episodic lakes such as Lake Eyre which suggests that these resistances are limited (Williams & Kokkinn, 1988).

The nine morphospecies of *Coxiella* recognised are differentiated by a number of shell and operculum characters (MacPherson, 1957). However, these characteristics are widely regarded as insufficient for distinguishing between all *Coxiella* species (Cale et al., 2004; De Deckker et al., 1980; Pinder et al., 2015; Williams et al., 1991) and several undescribed species have been reported in ecological surveys of salt lakes in south-western Australia (Pinder et al., 2002; Timms, 2009a). There is a strong need for additional taxonomic studies of *Coxiella*, especially as some populations have already gone extinct (Timms, 2009b; Williams, 1995). Utilising DNA based methods to resolve the taxonomic issues for *Coxiella* is essential due to the unknown suitability for morphology to reliably differentiate species with the benefits of genetic methods for recognition of putative species boundaries now well established (Haase & Zielske, 2015; Zou & Li, 2016).

Natural history specimens

Obtaining DNA from dry or natural history samples is particularly important for material from endangered or critically endangered organisms where retrieving tissue samples from live specimens is either impractical or impossible (Geist et al., 2008; Phillips et al., 2009). For example, although *Coxiella* persists through unfavourable environmental periods by burying themselves in mud (Williams et al., 1991), finding and collecting these aestivating individuals is difficult and time-consuming. However, DNA extractions using the shells of dead gastropod has previously given researchers the opportunity to study specimens after the animal itself was absent (Vogler et al., 2016). Although extractions

from gastropod shells is a difficult process and often met with limited success, it has also allowed for the identification of species where the reliability of morphological characters to identify species is questionable (Caldeira et al., 2004).

Empty Coxiella shells?

Empty (dead) *Coxiella* shells are often very abundant on or at the edges of dry salt pans (Bayly et al., 1966), even when live and active snails are not (Geddes et al., 1981). These empty shells are easy to collect and potentially represent an important source of samples for taxonomic and other biological studies of *Coxiella*, although the quality and quantity of information that can be obtained from these shells are not known.

Although some *Coxiella* species can be differentiated from each other by some shell characters, i.e. the presence or absence of fine shell decoration (striations or lirae), the presence or absence of rugged shell growth lines, number of whorls and to some extent shell size, a fundamental aspect of the current species identification (however questionable) is the ornamentation of the operculum, which is absent for the majority of dried shells (MacPherson, 1957). In addition, dried shells with or without opercula have been previously regarded as "quite valueless for critical taxonomic studies" (Williams et al., 1991). Therefore, the value of extracting DNA from dried *Coxiella* shells is then apparent as this may serve to improve the identification of species.

Aims

The overall objective of this study is to test the utility of empty/dead shells as a source of samples in taxonomic studies of *Coxiella*. The specific aims are to determine: (1) whether it is possible to obtain DNA from empty shells consistently; and (2) whether a genetic

marker (a portion of the *cytochrome oxidase 1* gene) can be consistently PCR amplified and sequenced from the DNA obtained from the empty shells.

Methods

Sampling

Samples of *Coxiella* were collected from six, ephemerally dry saline lakes and one permanent lake (Metro 1) across south-western Australia in December of 2018 (Figure 1). All samples consisted of empty or dead shells (shell not sealed by operculum) and were collected by hand from either the lake surface or retrieved from mud.

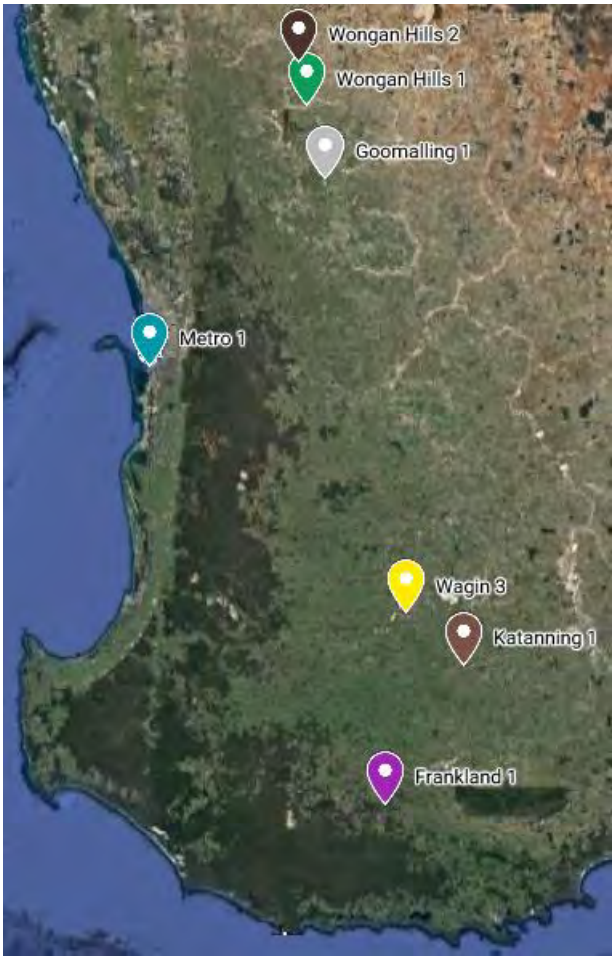

Figure 1: Map of the south-west of Western Australia (Google Earth) showing the relative positions of the seven lakes that *Coxiella* specimens were collected from during this study.

DNA extraction

Extractions were carried out in a laboratory where no previous work had been done with *Coxiella* snails inside a sterilised laminar flow to prevent carry-over contamination and to minimise the chance of false-positives (Mulligan, 2005). Whole individual snails that were selected *ad hoc* were first washed in autoclaved water before undergoing total genomic DNA extraction (Figure 2).

Figure 2: Example of the condition of *Coxiella* shells (Wongan Hills 2) were in before undergoing DNA extraction.

Two DNA extraction kits were trialled throughout this study. A Masterpure™ Complete DNA and RNA Purification Kit (Epicentre ®) was used essentially following the manufactures instructions with an incubation (at 65 C°) period of four hours to maximise DNA yield. The DNeasy Blood and Tissue Kit (Qiagen) was also trialled following the manufactures instructions. Each round of extractions had one assay that purposefully did not contain any DNA that was used as a DNA negative to check for contamination throughout the extraction process. Successful extractions were confirmed by gel electrophoresis on a 2% agarose gel stained with SYBR Safe.

Agarose gel purification

Twenty microlitres of DNA product (from the Masterpure extractions) were loaded into a 1 % agarose gel stained with SYBR Safe and physically isolated under orange light. DNA was then purified using a QIAquick Gel Extraction Kit (Qiagen) following the manufactures instructions for six samples between two populations (Goomalling 1 and Wongan Hills 2). The viability of this method was first established using DNA from positive samples (DNA previously extracted from live *Coxiella*) with resulting DNA successfully PCR amplified.

Polymerase chain reaction

Polymerase chain reactions (PCR) were done in a physically separate laboratory to that of the DNA extractions to minimise the chance of aerosol contamination between labs.

Three sets of primers were used including the Universal primers LCO1490 and HCO2198 (Table 1) as well as to two *Coxiella* specific primers (Table 1) designed for the amplification of a fragment of the mitochondrial *cytochrome oxidase 1* (Vrijenhoek, 1994). The viability of all primer pairs were tested on positive samples before confirming that these primers could reliably amplify *Coxiella* DNA.

Table 1: All six primers used throughout this study all of which had previously successfully amplified DNA from positive *Coxiella* DNA samples.

Primer	Sequence (5' – 3')	Amplicon size
Universal primer (LCO1490)	GGTCAACAAATCATAAAGATATTGG	~650 bp
Universal primer (HCO2198)	TAAACTTCAGGGTGACCAAAAAATCA	~650 bp
Primer set 1 (34F)	GGTCTGGACTAGTAGGAACAGC	~600 bp
Primer set 1 (625R)	ATTGGATCCCCTCCTCCAGC	~600 bp
Primer set 3 (74F)	CTGAATTAGGTCAACCTGGAGC	~350 bp
Primer set 3 (472R)	AATTGCATCCCTCGCCATCG	~350 bp

Reaction volumes were 25 µL consisting of 2.5 µL *Taq* Buffer (ROCHE), 1.25 µL of dNTPs (from a 10 mM per nucleotide solution), 0.05 µL of *Taq* DNA polymerase (from a 5 µg/µL solution; ROCHE) 1.0 µL of the forward and reverse primers (from 10 µM solutions), a range of volumes of bovine serum albumin (BSA) experimented with either 0.36 µL, 0.75 µL, 1.125 µL or 1.50 µL (from a 10 µg/µL solution), 1.0 µL DNA (usually diluted by factors of 1:5, 1:10, 1:20) and then adjusted to the final volume with polymerase chain reaction (PCR) grade water.

Negative assays containing no DNA were run in all PCRs to check for cross-contamination while positive assays (to confirm the viability of reaction conditions) were run in half of the reactions. Positives were assays containing DNA from live *Coxiella* that were added separately to all other samples with a different set of pipettes to prevent contamination.

Cycling conditions were either at a fixed annealing temperature or touchdown PCR. The fixed temperature PCRs consisted of an initial denaturation period of 4 minutes at 94 C° then 51 cycles of denaturation at 94°C for 45s, annealing at 55°C for 60s and extension

at 72°C for 30s followed by a final extension at 72 C° for 20 minutes. The touchdown PCR protocol was an initial denaturation period of 4 minutes at 94 C° then 50 cycles of denaturation at 94°C for 45s, annealing starting at 60 C° for 60s and declining by 0.5 C° every cycle with an extension at 72°C for 30s followed by a final extension of 20 minutes at 72 C°. Successful amplifications were confirmed by gel electrophoresis on a 2% agarose gel stained with SYBR Safe.

Results and discussion

DNA extractions

DNA extractions from all but one population (Wongan Hills 1) yielded DNA that could be visualised on a stained agarose gel (Figure 3) with most samples appearing to have high molecular weight DNA when compared to a lambda standard (e.g. wells 1 – 3 of Figure 3). However, whether the DNA visualised was from *Coxiella* or some exogenous source (i.e. from microbe living in/on the shell or from some other environmental sources) was not clear. This issue will be discussed below.

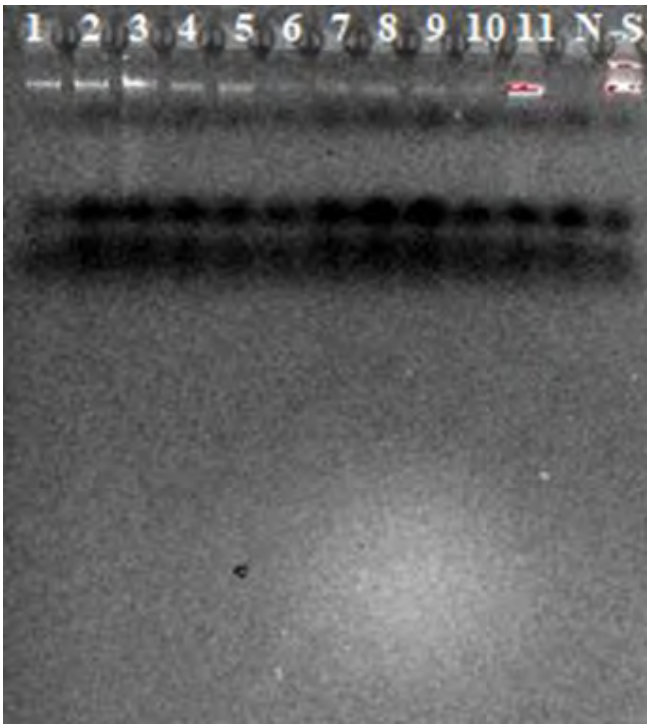

Figure 3: Example of a 2% agarose gel stained with 2 µL of SYBER Safe showing the results of DNA extraction from 11 shells and a DNA negative (N) from Frankland 1 compared with 6 µL of lambda standard (S). Wells 1 – 5 and 11 have DNA with relatively high molecular weight DNA while 6 – 10 at least have some DNA visible.

PCR amplification

Significant difficulties were encountered when DNA was PCR amplified with only 7 out of 20 reactions producing enough product that could be visualised on an agarose gel but not enough that would allow for sequencing (e.g. wells 1 – 3, 5 and 6 of Figure 4). Of the PCRs that had any successful amplification(s), six were using Universal primers with one using *Coxiella* specific primers. Of the individuals that amplified with Universal primers, follow up PCRs were undertaken to attempt to amplify these samples with *Coxiella* specific primers (both set 1 and 3) but none were successful. These PCRs were necessary as the amplicons generated by Universal primers were not necessarily of *Coxiella* DNA, given that these primers are broadly used for the amplification of metazoans (Hebert et al., 2003). Therefore, it is not possible to conclude whether these amplicons were of *Coxiella* but given that these samples did amplify with the Universals (suggesting that limited inhibitors were present) but not with species-specific primers suggests that the amplicons observed may not been of the targeted taxon.

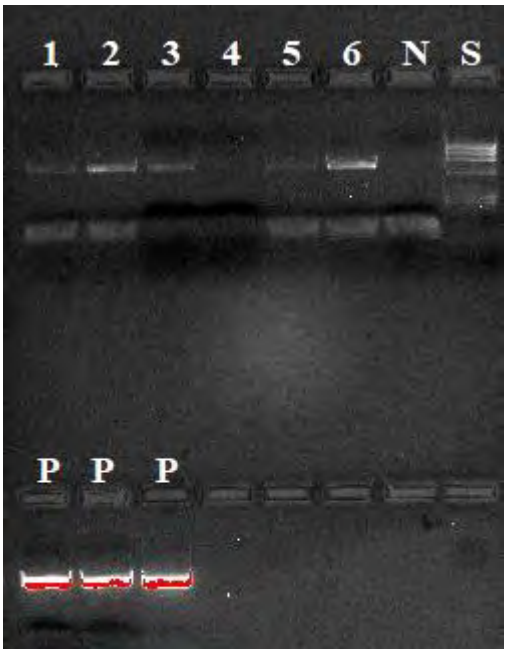

Figure 4: Example of the extent to which samples PCR amplified during this project. The gel consists of 2 % agarose stained with 2 µL SYBER Safe. 10 µL of Phi174/Hae III (S)

is used as a standard both to estimate amplicon size and concentration. 10 µL of the PCR product is loaded into wells (1 – 6). N and P are PCR negative and positive controls respectively each containing 10 µL of PCR product.

One individual did amplify using the designed primer set 3, that was targeting a ~350 bp section of the COI gene. It is worth noting that a follow-up PCR for this individual attempting to optimise the PCR conditions for sequencing were unsuccessful and but not pursued further due to time restrictions. Regardless, the amplification of a ~350 bp section of COI from a dried shell of *Coxiella* is significant as it suggests that it may be possible to amplify *Coxiella* DNA extracted from some individuals. However, the PCR conditions still need to be optimised further so that the amplicon can be sequenced to be used for species identification.

Limitations and future studies

The work conducted here found little success in terms of the ability to amplify DNA from dried *Coxiella* shells. The methodological approach taken is likely the key attributable reason for such limited success as opposed to the idea that the aims of this project were not achievable. The flaws in the approach of this project, however, offer valuable learning opportunities for the methodologies that future researchers could use to answer the question of whether DNA barcoding for dried *Coxiella* shells is viable. The two key issues that governed the success of this project (rather the lack of success) were primers targeting inappropriately sized regions of DNA and the presence of PCR inhibitors which will be discussed below.

Primers

The expectation when working with natural history specimens such as the dried *Coxiella* shells used here is that the DNA remaining will be both degraded and in a low abundance (Jaksch et al., 2016). Studies attempting to amplify such DNA fragments typically use primers targeting small sections of DNA (i.e. < 300 bp) (Andree & López, 2013; Jaksch et al., 2016; Villanea et al., 2016). However, even the smallest primer set used here (i.e. set 3, Table 1) was attempting to amplify a fragment of ~350 bp. The decision to use primers targeting relatively large fragments was in part undertaken given the high molecular weight of DNA observed after the initial extractions (e.g. Figure 3), and it was assumed that these bands of DNA would be of *Coxiella* DNA. However, given the tendency for Universal primers to amplify successfully without the success of the *Coxiella* specific primers on the same samples suggests that the high molecular weight of DNA observed may not be *Coxiella*. To understand whether any DNA in the samples is, in fact, *Coxiella* and to account for the potentially degraded nature of such DNA, specific primers targeting a much shorter sequence of *Coxiella* DNA (~100 bp) should be designed. Targeting a smaller fragment will loosen the inherent requirements on (if present) *Coxiella* DNA for amplification and thus hopefully improve the rate of success of amplification. Such primers would have been designed in this study, but time had become a limiting factor.

Inhibition

The lack of amplification despite having seemingly high molecular weight DNA of reasonable concentration in most cases (e.g. Figure 3) and using Universal primers that are extensively utilised in the amplification of a range metazoans suggests that some inhibitor was present in the majority of samples. It should be noted that no test to prove

the presence of inhibitors was carried out successfully, however, the assumption is made that given the promising appearance of the DNA extracted in this study, more samples would have been expected to amplify at least with the Universal primers.

Under the assumption that inhibitors were at least in part affecting the PCR, dilutions, bovine serum albumin (BSA) and agarose purification were employed to remove the effect of the inhibitor. The combination of the dilution of DNA assays and addition of BSA proved to be crucial in all successful amplifications observed, while the agarose purification was ineffective. All successfully amplified assays either had DNA added at a dilution of 1:5, 1:10 or 1:20 and a BSA amount of 7.5 - 15 µg (in a 25 µL reaction). Although, both dilutions and BSA are common strategies for reducing the effect of inhibitors in PCR (Fulton & Stiller, 2012) and their inclusion did increase the proportion of successful amplifications in this study, DNA dilutions and BSA did not comprehensively resolve the inhibition problem.

Additional troubleshooting methods were trialled throughout this study to reduce the effect of inhibitors including the agarose purification of DNA samples. This method uses gel electrophoresis to physically separate the contents of a sample based on size with the smaller inhibitory molecules diffusing out faster than the larger DNA molecules allowing for their isolation (Kemp et al., 2006). The efficacy of this method was first tested on DNA from live *Coxiella* samples (known to be inhibited) that produced PCR product of very high yield, but no amplification success was found with DNA from the dried shell material. Agarose purification was only trialled for six samples from two populations (using potentially unsuitable primers, see above) and therefore no strong conclusions of the efficacy of this method can be made. Given the success of positive samples, it is recommended that this method is investigated further but with primers targeting a smaller fragment of DNA.

It was briefly investigated here whether a different DNA extraction technique (i.e. the salt precipitation of the Masterpure kit versus a silica spin column extraction of the Qiagen kit) would have an effect on the presence or number of inhibitors present and that one might produce 'cleaner' DNA assays over the other. However, this was only tested in one PCR where a positive was not run, so it is unknown whether the PCR was unsuccessful due to the presence of inhibitors or some other unrelated reason (i.e. pipetting error). Follow up PCRs were not attempted due to time restrictions but would have included positive controls. However, previous studies comparing the efficacy of different DNA extraction kits for the amplification of degraded DNA from mollusc material have found that the unique preservation history of a sample (how likely a sample is to have retained viable DNA) is more important than the DNA extraction method used (Jaksch et al., 2016).

Conclusions

This projected aimed to determine whether genetic data could be yielded from dry *Coxiella* shells. Initially, results were promising with high molecular weight DNA retrieved from the DNA extractions, but given the issues with PCR optimisation, it was not possible to conclude whether this was *Coxiella* DNA. Given the methodologies used here, it is difficult to comment on whether it is viable or cost-effective to identify *Coxiella* species using DNA extracted from dried shells, but this question is worth further investigation. The benefits for being able to identify *Coxiella* shells using genetic methods are substantial for the management of this genus given that it is currently facing a suite of imperilling factors. Studies attempting to retrieve taxonomically significant information from dead shells are important as finding live, active specimens may become

increasingly difficult or impossible, and therefore, shells may become the only material available for studying *Coxiella* if live populations have become extinct.

References

Andree, K. B., & López, M. A. (2013). Species identification from archived snail shells via genetic analysis: a method for DNA extraction from empty shells. *Molluscan Research, 33*(1), 1-5.

Bayly, I., & Williams, W. (1966). Chemical and biological studies on some saline lakes of south-east Australia. *Marine and Freshwater Research, 17*(2), 177-228.

Caldeira, R. L., Jannotti-Passos, L. K., Lira, P. M., & Carvalho, O. S. (2004). Diagnostic of *Biomphalaria* snails and *Schistosoma mansoni*: DNA obtained from traces of shell organic materials. *Memorias do Instituto Oswaldo Cruz, 99*(5), 499-502.

Cale, D., Halse, S., & Walker, C. (2004). Wetland monitoring in the Wheatbelt of south-west Western Australia: site descriptions, waterbird, aquatic invertebrate and groundwater data. *Conservation Science Western Australia, 5*(1), 20-136.

Cramer, V., & Hobbs, R. (2002). Ecological consequences of altered hydrological regimes in fragmented ecosystems in southern Australia: impacts and possible management responses. *Australian Ecology, 27*(5), 546-564.

De Deckker, P. (1983). Australian salt lakes: their history, chemistry, and biota—a review. *Hydrobiologia, 105*(1), 231-244.

De Deckker, P., & Geddes, M. (1980). Seasonal fauna of ephemeral saline lakes near the Coorong Lagoon, South Australia. *Marine and Freshwater Research, 31*(5), 677-699.

De Deckker, P., & Williams, W. (1982). Chemical and biological features of Tasmanian salt lakes. *Marine and Freshwater Research, 33*(6), 1127-1132.

Doupe, R., & Horwitz, P. (1995). The value of macroinvertebrate assemblages for determining priorities in wetland rehabilitation: a case study from Lake Toolibin, Western Australia. *Journal of the Royal Society of Western Australia, 78*, 33-38.

Fulton, T. L., & Stiller, M. (2012). PCR amplification, cloning, and sequencing of ancient DNA. In *Ancient DNA* (Vol. 840, pp. 111-119): Springer.

Geddes, M., De Deckker, P., Williams, W., Morton, D., & Topping, M. (1981). On the chemistry and biota of some saline lakes in Western Australia. *Hydrobiologia, 81*(1), 201-222.

Geist, J., Wunderlich, H., & Kuehn, R. (2008). Use of mollusc shells for DNA-based molecular analyses. *Journal of Molluscan Studies, 74*(4), 337-343.

Gregory, S., Ward, M., & John, J. (2009). Changes in the chemistry and biota of Lake Carey: a large salt lake impacted by hypersaline discharge from mining operations in Western Australia. *Hydrobiologia, 626*(1), 53-66.

Haase, M., & Zielske, S. (2015). Five new cryptic freshwater gastropod species from New Caledonia (Caenogastropoda, Truncatelloidea, Tateidae). *ZooKeys*(523), 63-87.

Halse, S. (1981). Faunal assemblages of some saline lakes near Marchagee, Western Australia. *Marine and Freshwater Research, 32*(1), 133-142.

Halse, S., Cale, D., Jasinska, E., & Shiel, R. (2002). Monitoring change in aquatic invertebrate biodiversity: sample size, faunal elements and analytical methods. *Aquatic Ecology, 36*(3), 395-410.

Halse, S., Ruprecht, J., & Pinder, A. (2003). Salinisation and prospects for biodiversity in rivers and wetlands of south-west Western Australia. *Australian Journal of Botany, 51*(6), 673-688.

Hammer, U. T. (1986). *Saline lake ecosystems of the world* (Vol. 59). Netherlands: W. Junk.

Hebert, P. D., Cywinska, A., & Ball, S. L. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1512), 313-321.

Hughes, L. (2003). Climate change and Australia: trends, projections and impacts. *Austral Ecology, 28*(4), 423-443.

Jaksch, K., Eschner, A., Rintelen, T. V., & Haring, E. (2016). DNA analysis of molluscs from a museum wet collection: a comparison of different extraction methods. *BMC research notes, 9*(1), 348.

Kemp, B. M., Monroe, C., & Smith, D. G. (2006). Repeat silica extraction: a simple technique for the removal of PCR inhibitors from DNA extracts. *Journal of archaeological science, 33*(12), 1680-1689.

Kirono, D. G., Kent, D., Jones, R. N., & Leahy, P. (2012). Assessing climate change impacts and risks on three salt lakes in western Victoria, Australia. *Human and Ecological Risk Assessment: An International Journal, 18*(1), 152-167.

MacPherson, J. (1957). A review of the genus *Coxiella* Smith, 1894, sensu lato. *Western Australian Naturalist, 5*, 191-204.

Mulligan, C. J. (2005). Isolation and analysis of DNA from archaeological, clinical, and natural history specimens. In *Methods in Enzymology* (Vol. 395, pp. 87-103): Elsevier.

Phillips, N., Chaplin, J., Morgan, D., & Peverell, S. (2009). Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics. *Pacific Conservation Biology, 15*(2), 128-134.

Pinder, A., & Quinlan, K. (2015). Aquatic invertebrate communities of wetlands along the Jurien coast of Western Australia. *Journal of the Royal Society of Western Australia, 98*, 69-88.

Pinder, A. M., Halse, S., McRae, J., & Shiel, R. (2004). Aquatic invertebrate assemblages of wetlands and rivers in the wheatbelt region of Western Australia. *Records of the Western Australian Museum Supplement, 67*(7), 7-37.

Pinder, A. M., Halse, S. A., McRae, J. M., & Shiel, R. J. (2005). Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia, 543*(1), 1-24.

Pinder, A. M., Halse, S. A., Shiel, R. J., Cale, D. J., & McRae, J. M. (2002). Halophile aquatic invertebrates in the wheatbelt region of south-western Australia. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 28*(4), 1687-1694.

Ponder, W., & Colgan, D. (2002). What makes a narrow-range taxon? Insights from Australian freshwater snails. *Invertebrate Systematics, 16*(4), 571-582.

Sites, J. W., & Crandall, K. A. (1997). Testing species boundaries in biodiversity studies. *Conservation Biology, 11*(6), 1289-1297.

Stewart, B., Strehlow, K., & Davis, J. (2009). Impacts of deep open drains on water quality and biodiversity of receiving waterways in the Wheatbelt of Western Australia. *Hydrobiologia, 619*(1), 103-118.

Timms, B. V. (1983). A study of benthic communities in some shallow saline lakes of western Victoria, Australia. *Hydrobiologia, 105*(1), 165-177.

Timms, B. V. (1993). Saline lakes of the Paroo, inland New South Wales, Australia. *Hydrobiologia, 267*(1-3), 269-289.

Timms, B. V. (2005). Salt lakes in Australia: present problems and prognosis for the future. *Hydrobiologia, 552*(1), 1-15.

Timms, B. V. (2007). The biology of the saline lakes of central and eastern inland of Australia: a review with special reference to their biogeographical affinities. *Hydrobiologia, 576*(1), 27-37.

Timms, B. V. (2009a). Study of the saline lakes of the Esperance Hinterland, Western Australia, with special reference to the roles of acidity and episodicity. *Natural Resources and Environmental Issues, 15*(1), 44.

Timms, B. V. (2009b). A study of the salt lakes and salt springs of Eyre Peninsula, South Australia. *Hydrobiologia, 626*(1), 41-51.

Timms, B. V., Pinder, A. M., & Campagna, V. S. (2009). The biogeography and conservation status of the Australian endemic brine shrimp *Parartemia* (Crustacea, Anostraca, Parartemiidae). *Conservation Science Western Australia, 7*(2), 413-427.

Villanea, F. A., Parent, C. E., & Kemp, B. M. (2016). Reviving Galápagos snails: Ancient DNA extraction and amplification from shells of probably extinct endemic land snails. *Journal of Molluscan Studies, 82*(3), 449-456.

Vogler, R. E., Beltramino, A. A., Strong, E. E., Rumi, A., & Peso, J. G. (2016). Insights into the evolutionary history of an extinct South American freshwater snail based on historical DNA. *PloS one, 11*(12), e0169191.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.

Williams, W. (1964). A contribution to lake typology in Victoria, Australia. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 15*(1), 158-168.

Williams, W. (1981). 19. The limnology of saline lakes in Western Victoria. *Hydrobiologia, 81*(1), 233-259.

Williams, W. (1985). Biotic adaptations in temporary lentic waters, with special reference to those in semi-arid and arid regions. *Hydrobiologia, 125*(1), 85-110.

Williams, W., & Kokkinn, M. (1988). The biogeographical affinities of the fauna in episodically filled salt lakes: a study of Lake Eyre South, Australia. In *Saline Lakes* (pp. 227-236): Springer.

Williams, W., & Mellor, M. (1991). Ecology of *Coxiella* (Mollusca, Gastropoda, Prosobranchia), a snail endemic to Australian salt lakes. *Palaeogeography, Palaeoclimatology, Palaeoecology, 84*(1-4), 339-355.

Williams, W. D. (1995). Lake Corangamite, Australia, a permanent saline lake: conservation and management issues. *Lakes & Reservoirs: Research & Management, 1*(1), 55-64.

Williams, W. D. (1998). Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia, 381*(1-3), 191-201.

Williams, W. D. (2002). Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Environmental Conservation, 29*(2), 154-167.

Zou, S., & Li, Q. (2016). Pay attention to the overlooked cryptic diversity in existing barcoding data: the case of mollusca with character-based DNA barcoding. *Marine Biotechnology, 18*(3), 327-335.