



**Threatened  
Species  
Recovery  
Hub**

National Environmental Science Programme



# Health Surveillance Survey of Christmas Island, Cocos (Keeling) Islands, and Taronga Reptile Populations

## Guiding Translocation Opportunities

November 2018



THE UNIVERSITY OF  
**SYDNEY**



**TARONGA**  
CONSERVATION SOCIETY AUSTRALIA  
**Australian Registry  
of Wildlife Health**



**Australian Government**  
Parks Australia



Health Surveillance Survey of Christmas Island, Cocos (Keeling) Islands, and Taronga Reptile Populations  
- Guiding Translocation Opportunities

National Environmental Science Program's Threatened Species Recovery Hub, School of BioSciences, University of Sydney.

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Front cover: Movement opportunities of Christmas Island critically endangered reptiles from their source locations (Taronga Zoo, Sydney and Christmas Island Captive Breeding Colony) to the proposed translocation site (Cocos Keeling Islands).

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## Summary

- This study forms part of an investigation into the feasibility of translocating the critically endangered Christmas Island Lister's gecko and blue tailed skink to the Cocos (Keeling) Islands, as insurance populations to reduce the risks of extinction.
- To assess the risks associated with infectious disease transmission and exposure resulting from the translocation of reptiles from Christmas Island and Taronga Zoo to the Cocos (Keeling) Islands, gross and microscopic examination of invasive species of lizards collected on Christmas Island and the Cocos (Keeling) Islands was performed. Additionally, the post mortem findings of Lister's geckos and blue tailed skinks that died spontaneously at the Taronga Zoo captive breeding colony were reviewed.
- Parasite infections were common in all three populations, but only one parasite, an uncharacterised amoeba, was confined to specimens from the Taronga Zoo captive breeding colony. Follow-up molecular testing of the Taronga colony for pathogenic *Entamoeba* species was negative.
- Fungal infections were not observed in any population.
- Bacterial infections occurred in all populations. Most appeared to be opportunistic infections from environmental sources. However, a novel *Enterococcus* sp. that is a known pathogen of both the blue tailed skink and the Lister's gecko was found affecting introduced geckos located in and around the blue tailed skink enclosures, with multiple infected blue tailed skink subsequently identified.
- A low prevalence of lesions that could have been caused by viruses was seen in all populations and inclusion bodies of potential, but unconfirmed viral origin were identified in animals from the Taronga breeding colony, at a low prevalence.
- Based on these findings, we did not detect diseases in the introduced animals collected on Cocos (Keeling) Islands to which the Christmas Island reptiles were not already exposed.
- It is recommended that, if translocation is undertaken, animals be sourced from the enclosures (and not exlosures) on Christmas Island, in addition to captive bred animals from Taronga Zoo. This translocation technique will act to enhance genetic diversity and resilience, whilst reducing the risk of inbreeding depression and genetic drift.



Photograph of a Christmas Island invasive four-clawed gecko (*Gehyra mutilata*) affected with the novel *Enterococcus* sp. bacterial disease. Swelling of the diseased tissue is evident at the crown of the head.  
Photo: Jess Agius

# Introduction

In order to ensure the viability and long-term conservation of the critically endangered Christmas Island Lister's geckos and blue tailed skinks <sup>1</sup>, it is proposed that insurance populations be established on Cocos (Keeling) Islands. Animals required for translocation will be sourced from the captive breeding colonies located on Christmas Island, or from those located at Taronga Zoo, or both.

An inherent risk associated with any translocation is the possibility of transferring a novel disease by means of the translocated animal to the new location, or having the translocated animals acquire a new disease from the endemic species at the translocation site <sup>2</sup>. Of particular concern is the novel species of *Enterococcus* that has been shown to be highly pathogenic in Lister's geckos (*Lepidodactylus listeri*), and has also been observed to cause disease in the blue tailed skink (*Cryptoblepharus egeriae*) <sup>3</sup>. This bacterial disease has only been identified on Christmas Island, and is not currently present in the Taronga collection. Infection with this novel bacterium has not reoccurred in Christmas Island Lister's geckos since the initial outbreak in 2014, however, it is currently prevalent in invasive common house geckos (*Hemidactylus frenatus*) and four-clawed geckos (*Gehyra mutilata*) at multiple locations across Christmas Island, and at the site of the captive breeding colony. These infected house geckos have been observed on the exterior of indoor enclosures, and within the outdoor enclosures housing the blue tailed skinks. The possibility of disease transfer from affected invasive reptiles to captive animals is evident, particularly considering the recent observation of a collection of captive blue tailed skinks located in enclosures presenting with characteristic lesions of the *Enterococcus* sp. infection.

An attempt to establish an insurance population of blue tailed skinks and Lister's geckos on Cocos (Keeling) Islands has been proposed. Reptiles have never been native to the Cocos (Keeling) Islands, however, the islands currently support populations of five introduced reptile species, including two species, the common house gecko and four-clawed gecko that are also found on Christmas Island. The disease status of these introduced species on the Cocos (Keeling) Islands is not known and there exists a possibility that they might harbour diseases not present in native and invasive reptiles on Christmas Island.

The primary objectives of this report are to assess:

1. The risk of a novel disease transfer from source populations (Taronga and/or Christmas Island captive animals) to the site of translocation (Cocos (Keeling) Islands).
2. The risk that a novel disease might be acquired by the translocated animals from endemic species on Cocos (Keeling) Islands.

To evaluate the risks associated with this translocation option, an island-wide health surveillance project was conducted to inform species conservation activities regarding translocation. Invasive specimens collected on Cocos (Keeling) Islands and Christmas Island in 2016 during an island-wide sampling effort were examined at a macroscopic and microscopic level to determine whether any apparent pathogenic disease processes of importance were occurring, and to identify external and internal parasites carried by these species. Specimens were again collected on Christmas Island in 2017 during an island-wide sampling collection, and were examined at a macroscopic level to identify gross changes indicative of disease processes and parasite burdens. To gain an appreciation regarding the health of Christmas Island endemic lizards, tissues from specimens that died or were euthanised at the Taronga Zoo captive colony were subjected to microscopic examination, faeces were additionally examined. Health surveillance data gathered from each of these efforts are reported herein to understand associated impacts, gain an appreciation for host-parasite relationships, and more importantly protect endemic biodiversity at the source and translocated sites. Knowledge of the overall health of these species is important to ensure that the proposed translocation of animals will result in no deleterious effects to either the translocated population, or endemic populations at the translocation site.

# Materials and Methods

## Study Site

Invasive reptiles free-ranging on Christmas Island and Cocos (Keeling) Islands were collected as part of an island-wide health surveillance project from multiple study locations during July to August in 2016. Six study sites were sampled on Christmas Island, which included Poon Saan (10°25'22.31"S, 105°40'47.37"E), Drumsite (10°26'00.0"S 105°40'00.00"E), Detention Centre (10°28'12.7"S 105°34'28.4"E), Pink House Research Station (10°29'30.50"S 105°38'49.60"E), Flying Fish Cove (10°25'31.02"S 105°40'20.07"E) and the Christmas Island Airport (10°27'02.86"S 105°41'23.68"E). Secondary sample collection occurred across multiple Cocos (Keeling) Islands - Pulu Panjang/West Island [Ferry Terminal (12°08'15.76"S 96°49'22.67"E), Rumah Baru Jetty (12°09'23.09"S 96°49'39.05"E), Township (12°11'01.98"S 96°49'29.62"E), Quarantine Station (12°11'17.54"S 96°49'43.96"E), Yacht Club (12°11'36.26"S 96°51'37.88"E), Scout Park (12°11'55.18"S 96°51'47.53"E), and Airport (12°11'12.74"S 96°49'45.50"E)], Pulu Selma/Home Island [Ferry Terminal (12°06'59.88"S 96°53'33.76"E)], Pulu Kembang/Horn Island (12°08'00.02"S 96°54'38.09"E), Pulu Blan/East Cay Island (12°11'50.57"S 96°52'44.98"E), and Pulu Blan Madar/Burial Island (12°12'03.96"S 96°52'45.46"E). A further collection of invasive reptiles occurred on Christmas Island during August to September 2017 at the six sites aforementioned, in addition to South Point (10°33'42.05"S 105°38'55.07"E), and at the Reptile Soft Release Site (10°29'43.16"S 105°36'55.27"E).

## Specimen collection & Euthanasia

Invasive reptiles were opportunistically collected from each 2016 Christmas Island and Cocos (Keeling) Islands sampling site independent of their health status. All living reptiles were euthanised via an overdose of anesthetic. Succeeding euthanasia, all reptiles were subjected to an external physical examination, inspected for any evidence of disease, and then opened for internal inspection. Succeeding examination, cadavers were fixed in 10% neutral buffered formalin and transported to the Australian Registry of Wildlife Health, Sydney, Australia for further analysis.

A minimum of sixty one invasive reptiles were opportunistically collected from each study site on Christmas Island in 2017, anaesthetised and humanely euthanised. All specimens were processed using the same procedures as during fieldwork conducted in 2016, with the addition of a more intensive internal inspection of organs for parasites and/or disease processes. The lungs, gall-bladder, stomach, and small and large intestines were also sectioned to expose internal contents.

## Taronga Zoo captive breeding colony

Lister's geckos and blue tailed skinks housed at the Taronga Zoo captive breeding colony that died or were euthanised due to veterinary or welfare concerns were collected between 2011 and 2017, and preserved in 10% neutral buffered formalin for histological examination.

## Histological preparation of reptile tissue

Longitudinal sections of the formalin-fixed specimens collected in 2016 from Christmas Island, and Cocos (Keeling) Islands, and Lister's geckos and blue tailed skinks housed at Taronga Zoo that died spontaneously, were paraffin-embedded, sectioned at 4µm, stained with Haematoxylin and Eosin and examined microscopically.

## Molecular analysis

Fecal samples preserved in 70% ethanol were collected from blue tailed skink and Lister's gecko enclosures at the Taronga captive breeding colony in 2018. A total of 14 samples, each containing more than 20 randomly collected fecal droppings were pooled according to sex and/or species (Table 1) and submitted for duplicate qPCR analysis for the detection of *Entamoeba ranarum/invadens* and an undescribed *Entamoeba* sp. found in cane toads (Shilton et al. 2018).

## Findings considered a potential risk

While many disease processes were found in the sampled animals from all locations, only those that were considered to be infectious in origin were included in the final analysis. Additionally, infectious diseases that were secondary to husbandry related problems and bacterial infections that appeared as a result of exposure to opportunistic environmental bacteria were not included in the final analysis. Infectious diseases were classified of concern if they were present in one population but not the other two.



# Results

## Geographic distribution

One hundred and three specimens were collected from six locations on Christmas Island during 2016. These specimens included of the common house gecko (n=89) and the four-clawed gecko (n=14). Secondary sample collections of invasive reptiles at eleven different locations across Cocos (Keeling) Islands during 2016 resulted in the collection of 202 specimens, which included the common house gecko (*Hemidactylus frenatus*) (n=166), Sri Lankan house gecko (*Hemidactylus parvimaclatus*) (n=3), flat-tailed house gecko (*Hemidactylus platyurus*) (n=3), four-clawed gecko (*Gehyra mutilata*) (n=24), and mourning gecko (*Lepidodactylus lugubris*) (n=6). Sampling on Christmas Island undertaken in 2017 resulted in the collection of the common house gecko (n=445), and four-clawed gecko (n=23). The proportions of species examined, and the associated collection localities on Christmas Island and Cocos (Keeling) Islands in 2016 are illustrated in Figures 1 and 2 respectively, and Figure 3 for 2017 Christmas Island collections. Additionally, a total of two hundred and twenty Christmas Island endemic Lister's geckos (n = 94) and blue tailed skinks (n = 126) housed at the captive breeding colony at Taronga Zoo, Sydney were examined.

## Macroscopic (gross) and microscopic (histology) parasite findings

Gross post-mortem examination of invasive reptiles sampled from Christmas Island and Cocos (Keeling) Islands revealed a number of parasitic species across various body systems. The presence and absence of parasites identified for Christmas Island and Cocos (Keeling) Islands invasive reptiles shown in Table 2. Presence and absence data on parasites were again collected on a microscopic level and presented in Table 3, which additionally included results from specimens collected from the Taronga Zoo captive population. An overall risk assessment for the classes/species of parasites detected across all sampling efforts is presented (Table 4).

## Molecular findings

All samples submitted for qPCR at the University Of Sydney Veterinary Pathology Diagnostic Services (VPDS) for the detection of pathogenic parasites *Entamoeba ranarum/invadens* and an uncharacterised *Entamoeba* sp. were negative.

## Additional macroscopic (gross) findings

A summary of the gross observations for Christmas Island 2016, Cocos (Keeling) Islands 2016, and Christmas Island 2017 are shown (Table 5). Granulomatous lesions of varying morphologies were common findings across multiple body systems during each sampling time-frame, particularly of the digestive system. Lesions consistent with the novel pathogenic *Enterococcus* bacterium were found in eight specimens during 2016, and five in 2017 on Christmas Island.

## Additional microscopic (histology) findings

Microscopic lesions recorded for Christmas Island 2016, Cocos (Keeling) Islands 2016, and Taronga Zoo 2011-2017 are shown (Table 6). Changes were classified as either consistent with bacterial or viral aetiologies. During gross and histological examination, no diseases of fungal origin were noted in any of the specimens examined.

## Enterococcus disease

Since its emergence in 2014, the novel *Enterococcus* sp. disease has affected a number of reptiles inhabiting Christmas Island. Four species of lizard have succumb to infection and include invasive common house geckos and four-clawed geckos, and the island endemic Lister's geckos and blue tailed skinks. Infection with this bacterium has been observed to be 100% fatal in all species except for in the blue tailed skink. The disease has not been observed in the Christmas Island giant gecko (*Cyrtodactylus sadleiri*) and invasive wolf snake (*Lycodon capucinus*) during limited health surveillance surveys, though it is possible that these reptiles are susceptible.

Affected lizards exhibited facial deformities characterised by distention of the eye, irregular swellings around the mouth, gingiva, and soft tissues of the head. Some affected individuals additionally developed subcutaneous nodules on the body and/or tail, however this was observed less frequently. Locations whereby affected lizards have been identified include the Pink House Research Station, South Point and Drumsite. Affected individuals have been found in close proximity to captive blue tailed skinks and Lister's geckos on the exterior of enclosures, and within enclosures.

Since discovery of this bacterium, systematic island-wide and opportunistic sampling efforts have been undertaken to gain an appreciation for disease prevalence. Specimens confirmed and suspected of the disease over various time-points on Christmas Island are presented (Table 7). Disease has not been found on the Cocos (Keeling) Islands or at Taronga.

# Figures

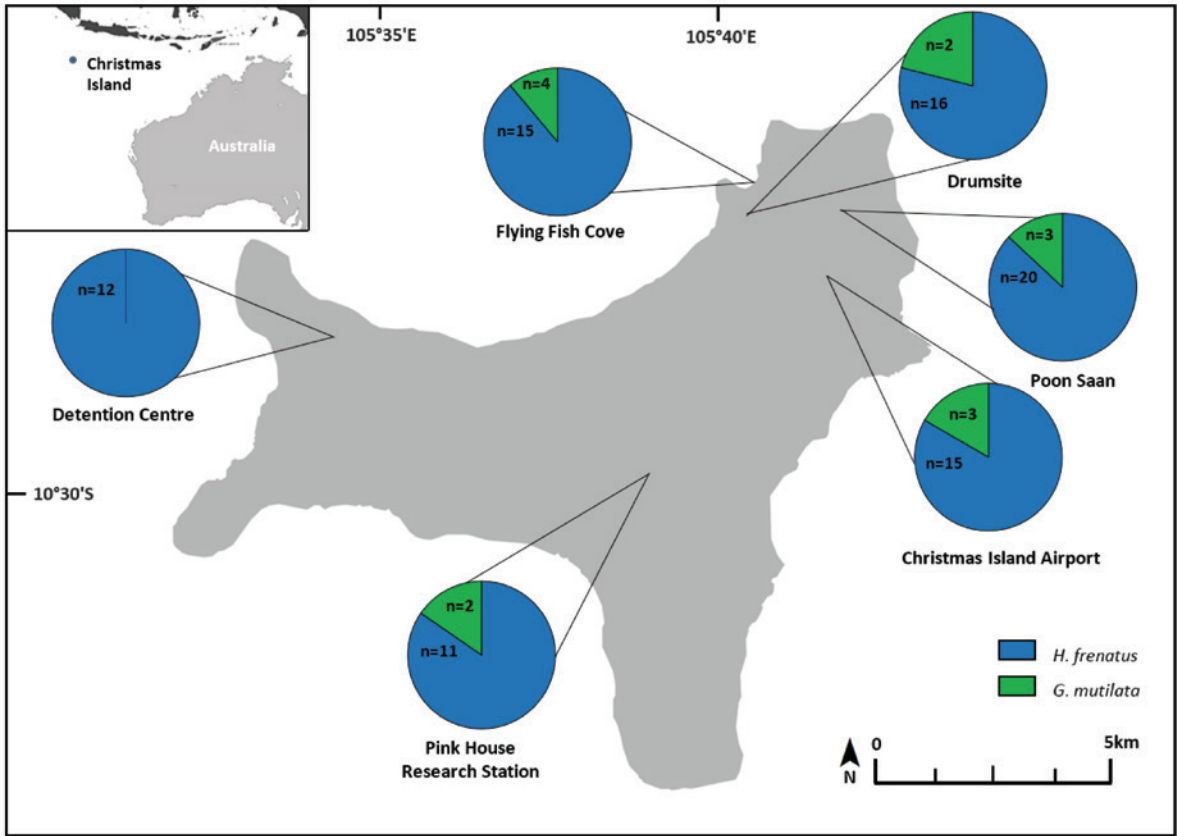


Figure 1: Distribution and relative proportions of invasive reptiles examined as part of a health surveillance project on Christmas Island in 2016 (n=103). The chart colours represent different reptile species, and numerical values indicate the quantity of each species examined.

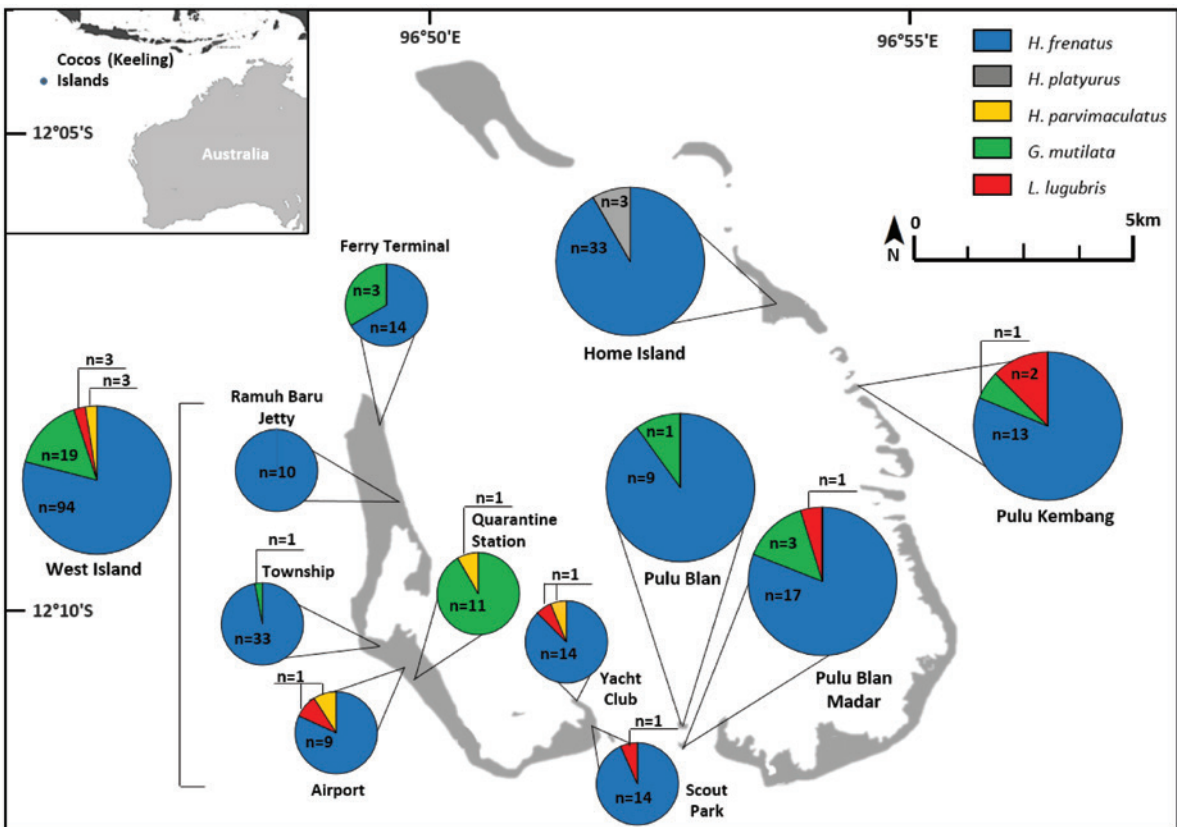


Figure 2: Distribution and relative proportions of invasive reptiles examined as part of a health surveillance project on Cocos (Keeling) Islands in 2016 (n=202). The chart colours represent different reptile species, and numerical values indicate the quantity of each species examined.



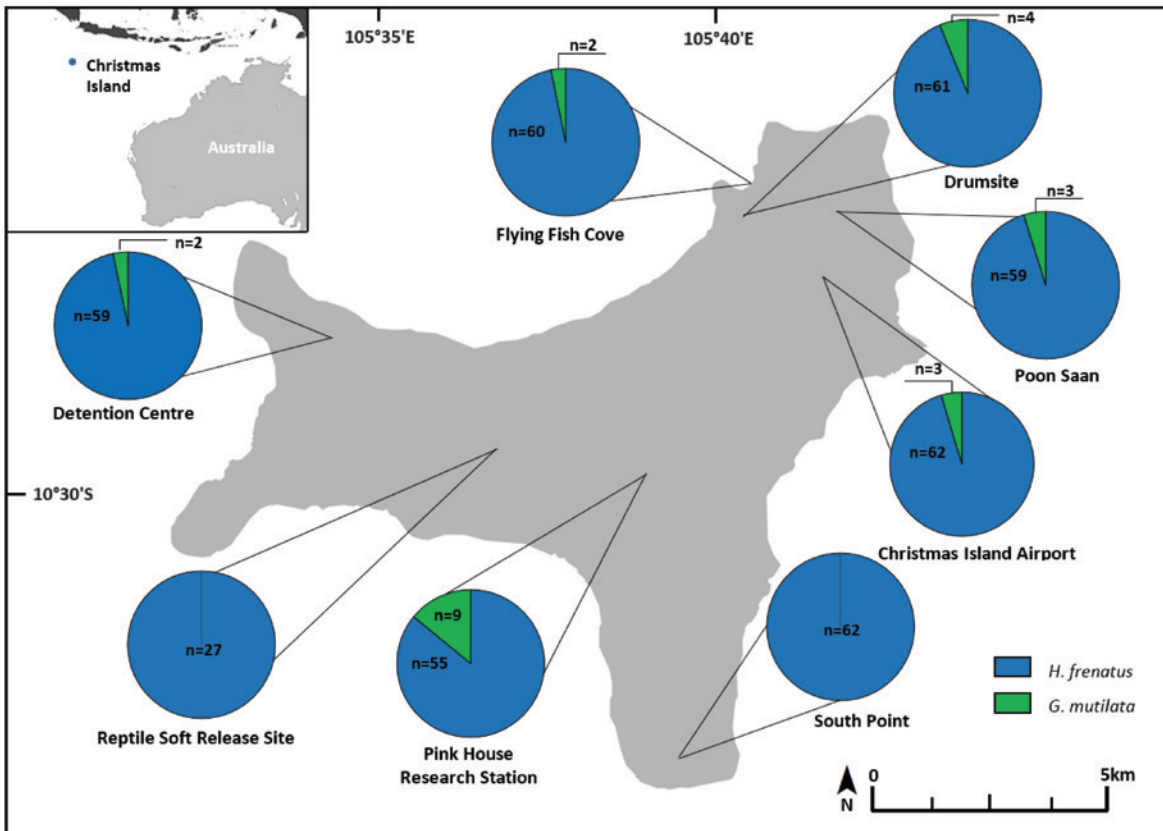
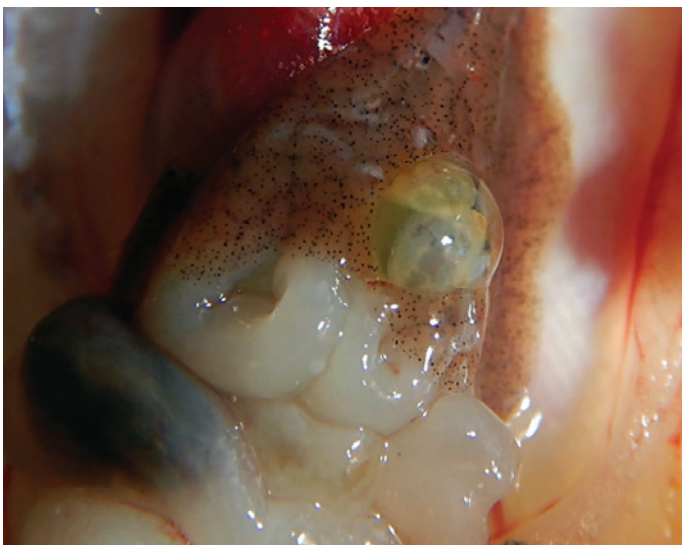


Figure 3: Distribution and relative proportions of invasive reptiles examined as part of a health surveillance project on Christmas Island in 2017 (n=468). The chart colours represent different reptile species, and numerical values indicate the quantity of each species examined.



Gall bladder of a Christmas Island invasive common house gecko (*Hemidactylus frenatus*) infected with trematode parasites. Photo: Jess Agius

## Tables

Table 1: Details of Taronga Zoo captive colony samples submitted for *Entamoeba* sp. detection.

Sample #	Tank ID	Species	Sex
1-2	A1 + A2	Blue tailed skink	Males
3-4	A3 + A4	Blue tailed skink	Females
5-6	B1 + B2	Blue tailed skink	Males
7-8	B3 + B4	Blue tailed skink	Females
9	B2 + B4	Blue tailed skink	Males + females
10	LG264	Lister's gecko	Females
11-14	LG4 + LG1000 + LG268 + LG256	Lister's gecko	Males

Table 2: Parasite presence and absence summary from gross examination of lizard species collected from all three sampling sites in 2016 and 2017.

Parasite	Type of Parasite	Christmas Island 2016	Cocos (Keeling) Islands 2016	Christmas Island 2017
<i>Geckobia bataviensis</i>	Ectoparasite Mite	✓	✓	✓
<i>Paradistomum geckonum</i>	Endoparasite Trematode	✓	✓	✓
<i>Oochoristica javaensis</i>	Endoparasite Cestode	✓	✓	✓
Coccidia spp.	Endoparasite Protozoan	✓	✓	✓
<i>Raillietiella frenatus</i>	Endoparasite Pentastome	✓	✓	✓
<i>Spauligodon hemidactylus</i>	Endoparasite Nematode			✓

Table 3: Parasite presence and absence summary from histological examination of lizard species collected from Christmas Island and Cocos (Keeling) Islands in 2016, and from captive Christmas Island endemic reptiles from Taronga Zoo from 2011 to 2017.

Parasite	Type of Parasite	Christmas Island 2016	Cocos (Keeling) Islands 2016	Taronga Zoo 2011-2017
<i>Geckobia bataviensis</i>	Ectoparasite Mite	✓	✓	
<i>Paradistomum geckonum</i>	Endoparasite Trematode	✓	✓	✓
<i>Oochoristica javaensis</i>	Endoparasite Cestode	✓	✓	✓
Coccidia spp.	Endoparasite Protozoan	✓	✓	✓
<i>Raillietiella frenatus</i>	Endoparasite Pentastome	✓	✓	
<i>Spauligodon hemidactylus</i> , <i>spirurid</i> spp. <i>strongylid</i> spp.	Endoparasite Nematode	✓	✓	✓
Protozoa (flagellates likely coccidian or amoeba)	Endoparasite Protozoan			✓
Amoeba spp.	Endoparasite Protozoan			✓



Photograph of a Christmas Island invasive common house gecko (*Hemidactylus frenatus*) parasitised by mites (*Geckobia bataviensis*). Photo: Jess Agius

Table 4: Brief risk-assessment of parasites identified in Christmas Island and Cocos (Keeling) Islands reptiles.

Parasite	Parasite-type	Pathology	Geographical location	Risks
<i>Spauligodon hemidactylus</i>	Nematode	<ul style="list-style-type: none"> <li>• Potential commensal relationship with host.</li> <li>• Benefits associated with breaking up faecal matter, reducing undigested material and preventing constipation.</li> <li>• High burdens can (but rarely) cause fatal impactions, diarrhoea, and anasarca.</li> </ul>	Christmas Island, Cocos (Keeling) Islands, Taronga colony.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low</li> </ul>
<i>Raillietella frenatus</i>	Pentastome	<ul style="list-style-type: none"> <li>• Direct damage to pulmonary lining which may result in pneumonia or secondary bacterial or fungal pathogens.</li> <li>• Can frequently be associated with inflammation as a result of antigenic stimulation.</li> <li>• Individuals with high burden likely more susceptible to predation.</li> <li>• Anaemia in individuals with high burdens.</li> <li>• Clinical disease more likely in captive, stressed reptiles.</li> </ul>	Christmas Island and Cocos (Keeling) Islands.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low to Moderate</li> </ul>
<i>Paradistomum geckonum</i>	Trematode	<ul style="list-style-type: none"> <li>• May cause partial to complete bile duct and urinary tract obstructions.</li> </ul>	Christmas Island, Cocos (Keeling) Islands, Taronga colony.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low</li> </ul>
<i>Oochoristica javaensis</i>	Cestode	<ul style="list-style-type: none"> <li>• May develop in extra-intestinal sites such as the liver, coelomic cavity, mesentery, and pancreas.</li> <li>• Inflammation is usually mild and localized, loss or replacement of normal cells and mechanical damage to parenchyma potentially leading to organ dysfunction.</li> </ul>	Christmas Island, Cocos (Keeling) Islands, Taronga colony.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low</li> </ul>
Coccidia spp.	Protozoa	<ul style="list-style-type: none"> <li>• Infection can sometimes be asymptomatic/ non-pathogenic.</li> <li>• May cause intestinal villi malformation, decreased GIT function and severe enteritis.</li> </ul>	Christmas Island, Cocos (Keeling) Islands, Taronga colony.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• General host specificity</li> <li>• OVERALL RISK = Low</li> </ul>
<i>Geckobia bataviensis</i>	Mite	<ul style="list-style-type: none"> <li>• Heavy infestations are unusual, however may cause skin irritations, anaemia, debility and occasionally mortality.</li> <li>• Can act as a potential haemoparasite vector.</li> </ul>	Christmas Island and Cocos (Keeling) Islands	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low to Moderate</li> </ul>
Amoeba	Protozoa	<ul style="list-style-type: none"> <li>• Can cause disturbances to gastrointestinal tract, and in serious cases haemorrhagic enteritis/colitis, marked anorexia and anaemia.</li> <li>• Depending on amoeba species and pathogenicity – infection can result in death.</li> </ul>	Christmas Island and Taronga colony.	<ul style="list-style-type: none"> <li>• Low pathogenicity</li> <li>• Low host specificity</li> <li>• OVERALL RISK = Low to Moderate</li> </ul>

Table 5: Presence and absence of gross necropsy findings in specimens collected from Christmas Island and Cocos (Keeling) Islands.

Gross findings	Christmas Island 2016	Cocos (Keeling) Islands 2016	Christmas Island 2017
Respiratory system			
Granulomas on the lung tissue	✓	✓	✓
Digestive/excretory system			
Granulomas on the liver	✓	✓	✓
Granulomas on the stomach			✓
Granulomas on the colon	✓		✓

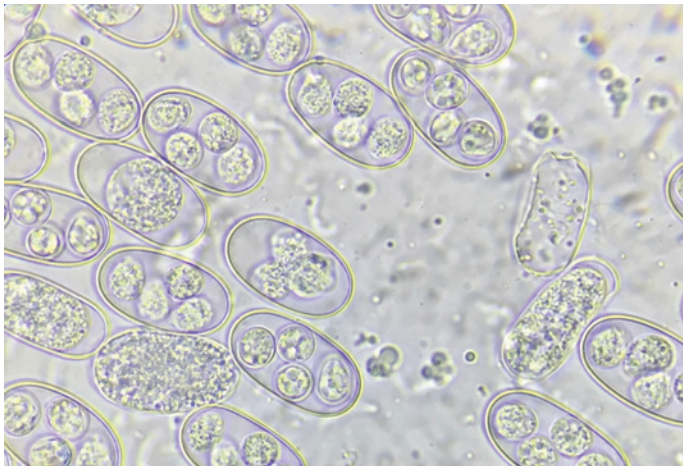
Table 6: Presence and absence of histology findings in specimens collected from Christmas Island, Cocos (Keeling) Islands and Taronga Zoo.

Histology findings	Christmas Island 2016	Cocos (Keeling) Islands 2016	Taronga Zoo 2011-2017
Bacterial origin			
Granulomas on the lung tissue	✓	✓	
Inflammation of the kidneys (nephritis)	✓	✓	✓
Inflammation of the liver (hepatitis)	✓	✓	✓
Inflammation of the liver and bile ducts (cholangiohepatitis)	✓		✓
Granulomas on the liver	✓	✓	✓
Necrosis of the liver tissue	✓	✓	
Inclusion bodies in liver cells (hepatocytes)			✓
Inflammation of the coelom (coelomitis)	✓	✓	✓
Inflammation of the stomach (enteritis)	✓	✓	✓
Granuloma of the mesentery	✓	✓	✓
Inflammation of the intestines and/or colon (colitis)	✓		✓
Viral origin			
Inflammation of the pancreas (pancreatitis)	✓		✓
Inflammation of the liver (hepatitis)	✓	✓	✓
Inclusion bodies in renal tubules			✓
Inclusion bodies in epidermal cells			✓

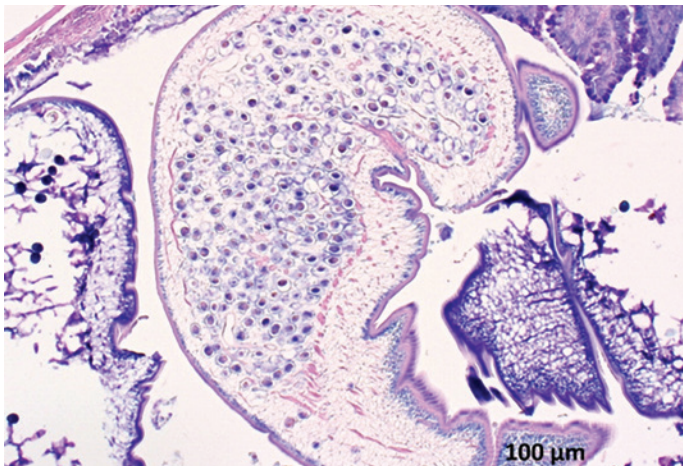


Table 7: Number of lizard specimens with enterococcal disease collected on Christmas Island over various time-points.

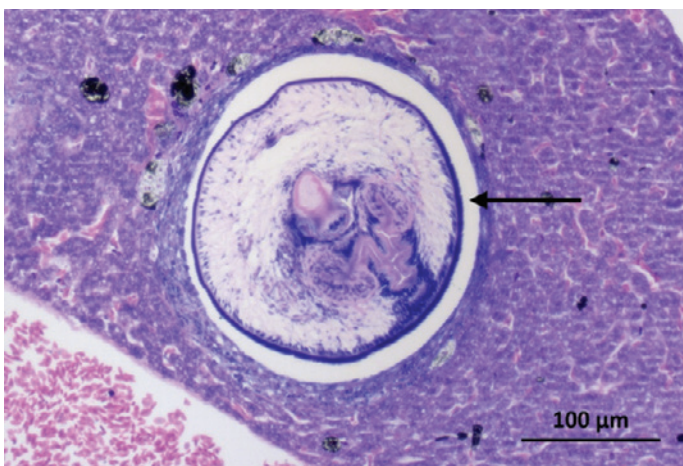
Year	Location	Number	Sampling status
2014	Christmas Island	36	Island-wide surveillance
2015	Christmas Island	19	Opportunistic
2016	Christmas Island	8	Island-wide surveillance
2017	Christmas Island	5	Island-wide surveillance
2018	Christmas Island	22	Opportunistic



Microscopic examination of wet mounts collected from the gall bladder of a Christmas Island invasive common house gecko (*Hemidactylus frenatus*) infested with coccidia. Photo: Jess Agius



Microscopic image of a gravid tapeworm found in the intestines of a Christmas Island invasive four-clawed gecko (*Gehyra mutilata*). Photo: Jess Agius



Microscopic image of a nematode encysted within the liver of a Christmas Island Lister's gecko (*Lepidodactylus listeri*). Photo: Jess Agius

## Discussion

The highest risk of disease transmission often comes from the chance or intentional introduction of confamilial or more closely-allied species that may carry pathogens and/or parasites to which their parent populations have developed high levels of immunity, but island populations have never been exposed<sup>5,6</sup>. To determine this risk, sample collections from Christmas Island endemics at the Taronga Zoo captive breeding colony, and invasive reptiles on Christmas Island and Cocos (Keelings) Islands were conducted, and the presence and absence of infectious diseases were compared. The overall objective of this research was to characterise the potential viral, bacterial, fungal, and parasitic diseases that might pose a threat to the Christmas Island native Lister's geckos and blue tailed skinks. Currently, an extensive amount of knowledge regarding parasites and the novel *Enterococcus* bacterium have been discovered, however there is limited information concerning other bacterial and viral diseases.

### Parasites

All parasites identified on Cocos (Keeling) Islands were also found in the invasive species on Christmas Island and, with the exception of protozoan flagellates and amoeba these parasites were also found in the Taronga captive breeding colony. The only parasites that met the criteria of posing a risk to translocation were the amoebas observed in observed in the cloaca and associated with cloacal inflammation of blue tailed skinks at the Taronga captive breeding colony. Further molecular analysis ruled out the existence of pathogenic amoeba (*Entamoeba*), therefore presenting no immediate risk to the proposed translocation effort. Whether these *Entamoeba* are occurring in animals from Christmas or Cocos (Keeling) Islands is not known. Only a limited amount of blue tailed skinks have been examined microscopically from the colony on Christmas Island.

### Bacterial and suspected bacterial infections

During the 2017 survey of feral reptiles on Christmas Island, up to 10% of each sampled population was found to have granulomatous lesions of the viscera. Impression smears of the diseased tissue and limited culture attempts suggested that these were caused by multiple different environmental or normally commensal bacteria. They were not caused by the more pathogenic novel *Enterococcus* sp. Although these organisms have not yet been fully characterised, they are not believed to represent a threat to the translocation plan.

Enteritis and coelomitis were identified in all three populations, colitis was absent from Cocos (Keeling) Islands. These lesions are suggestive of infection with salmonella. Salmonella is a bacteria which has been commonly isolated from a broad range of reptilian species<sup>7</sup>. Given that many salmonella are ubiquitous in the environment, it is assumed that all reptiles have some level of exposure to these species, and therefore it is not considered to be a risk to the translocation attempt.

### Lesions of possible viral origin

Changes associated with viral aetiologies were also documented across all localities. These included inflammatory lesions that were predominately lymphocytic and did not contain bacteria, fungi, or parasites. The presence of inclusion bodies in cells with or without associated necrosis or inflammation was also considered to be of possible viral origin. Inclusion bodies were only observed in three animals and all were in the Taronga collection.

Histopathology is a poor diagnostic tool for screening viral infections. Future studies are planned to incorporate advanced molecular techniques to more efficiently and accurately assess the viral status of invasive and captive bred reptiles on Christmas Island and the Cocos (Keeling) Islands. Until that time, it is not possible to adequately assess the risk that viruses may pose to the translocation process.

### The risk posed by the novel *Enterococcus* species present on Christmas Island

No evidence of the novel highly pathogenic *Enterococcus* was found in either the Taronga captive breeding program or in the introduced lizards from Cocos (Keeling) Islands. However, it is clear that this disease persists in the free-ranging invasive skinks on Christmas Island, and that blue tailed skinks in the enclosures are in constant contact with infected invasive species<sup>3</sup>. Therefore, preventing transmission of this infection to the Cocos (Keeling) Islands is critically important. Given that no diseased animals have been identified in the enclosures since quarantine procedures have been strictly adhered to, all animals used for the translocation should come from the enclosures on Christmas Island or the Taronga facility, no animals should be sourced from enclosures.



## Limitations of this study

Despite the extensive information on health surveillance data provided in this report, a number of limitations exist. (1) Classification of parasites was a difficult process, with gross and histological observation providing inadequate characterisation and differential power. When observed, parasites were tentatively identified to the highest ability based on their relative location, morphometrics and on existing literature. (2) The 2016 sampling of both islands involved a more descriptive approach in contrast to the 2017 sampling, and did not consist of the excision and sectioning of the gastrointestinal tract, and aspiration of gall bladder contents, which may explain the absence of detection of some gastrointestinal and gall bladder parasites. (3) The inability to correctly locate organisms or observe disease processes in tissues due to non-representative histological samples. (4) The lack of data provided for Christmas Island endemic blue tailed skinks and Lister's geckos housed at the Christmas Island and Taronga Zoo captive facilities. Specimens from these colonies are a rare finding, and when identified are often too decomposed to gather any valuable health data from. (5) The low sample size of examined endemic lizards over scattered time points can offer some useful information, yet cannot provide extensive evidence regarding population health. (6) The absence of baseline data from initial Lister's gecko and blue tailed skink founders. This lack of data means there is no plausible way to determine the origin of the documented parasites, if they occurred as a result of vertical transfer from initial founder populations through generations, or were acquired at the Taronga facility.

## Conclusions and recommendations

Based on the parasite survey, it appears that the invasive lizards on Christmas Island and the Cocos (Keeling) Islands have a similar complement of pathogens, and translocation of animals to the Cocos (Keeling) Islands and back has an overall low risk. There are, however caveats to this conclusion. The first is that it is essential that the novel pathogenic *Enterococcus* found on Christmas Island not be introduced to the Cocos (Keeling) Islands. To prevent this, animals will be sourced from the enclosures on Christmas Island or from the Taronga Zoo colony, and not from enclosures. These animals have been born into quarantine and housed in enclosures together with no movement (closed system), and are therefore considered as suitable candidates for translocation. Lastly, this is still a preliminary study and it cannot be fully ruled out that other infectious diseases, particularly viruses, might be present in one population, and not in the others'.

Currently, this translocation project has received (1) formal approval and support from the Shire of Cocos (Keeling) Islands to introduce blue tailed skinks to two islands (Pulu Blan and Pulu Kembang), and (2) animal ethics approval from the Taronga Conservation Society Australia with no conditions.

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**Further information:**

<http://www.nespthreatenedspecies.edu.au/>

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