An evolutionary approach to the biological management of invasive Brown Treesnakes (*Boiga irregularis*) on Guam

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Gran Canaria
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Bulisa Iova: PNG National Museum

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Alaska
United States
Midway Islands
Northern Mariana Islands
Wake Island
Hawaiian Islands
Guam
Johnston Atoll
Howland Island
Palmyra Atoll
Kingman Reef
Baker Island
Jarvis Island
American Samoa
Navassa Island
Puerto Rico and the U.S. Virgin Islands

Guam Buildup

Guam population will increase from 160K in 2009 to projected 240K by 2014

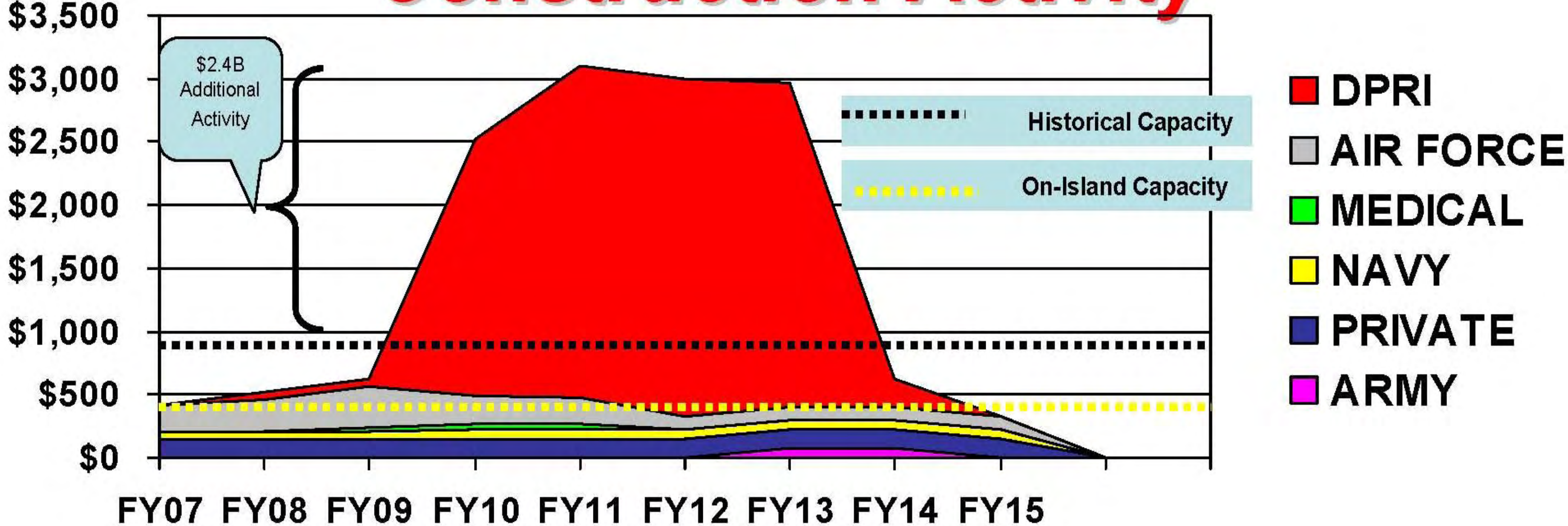
Guam build-up will cost $20B

Largest military relocation since WWII

Likelihood of continental US invasion of Brown Treesnakes

Brown Treesnakes have three major impacts: Economic, Human Health, and Ecological

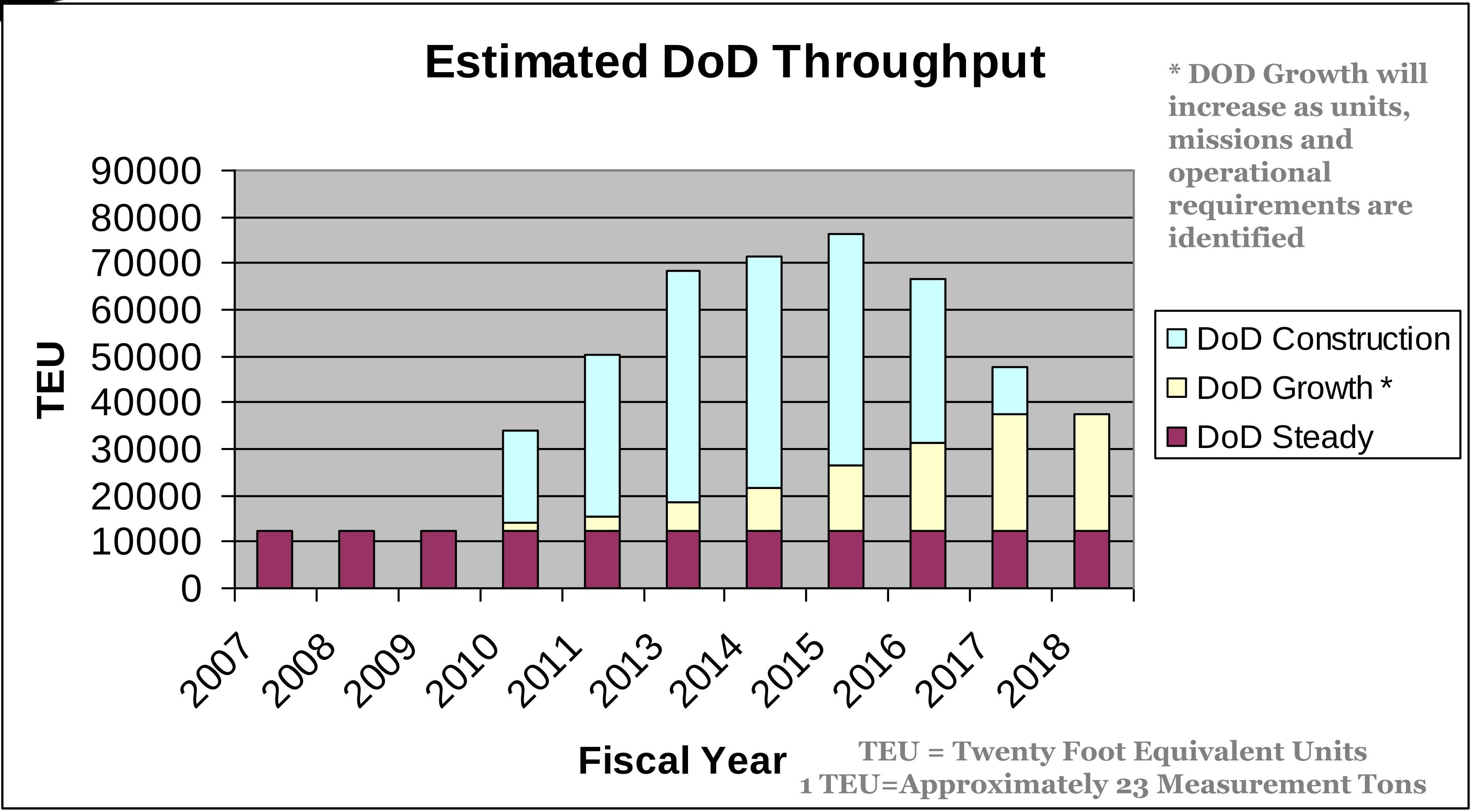
Notional Projected Guam Construction Activity



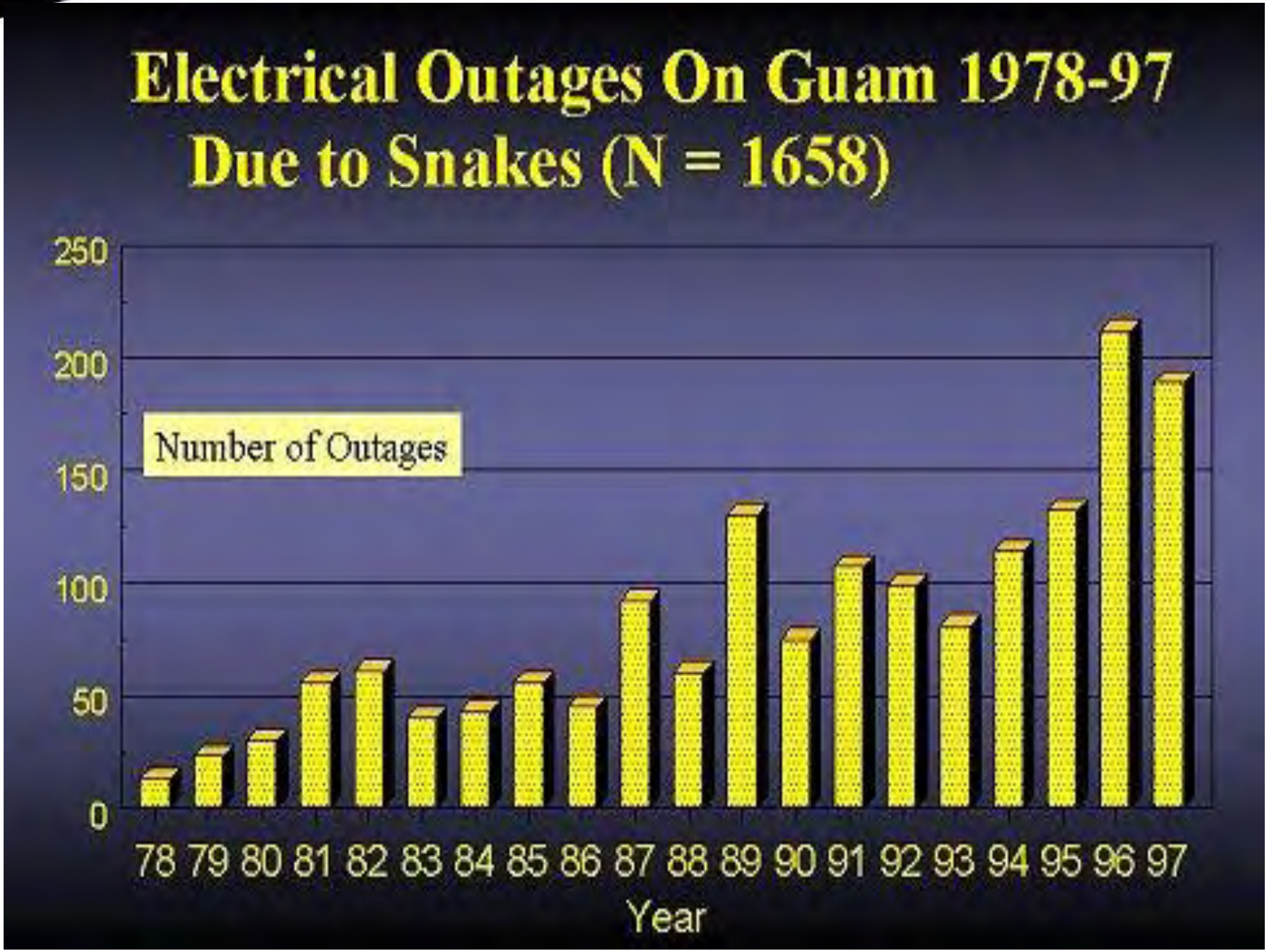
Construction Capacity

- Historical construction capacity approximately $800M/yr
- FY14 completion will require excess of $3B/year capacity
- 2007 NDAA repeals a ban against foreign laborers working on military construction projects on Guam.

Commercial Port Requirements



Volume of DOD passengers, cargo & vehicles is also needed for commercial airport, COMNAVMARIANAS & AAFB for planning purposes.

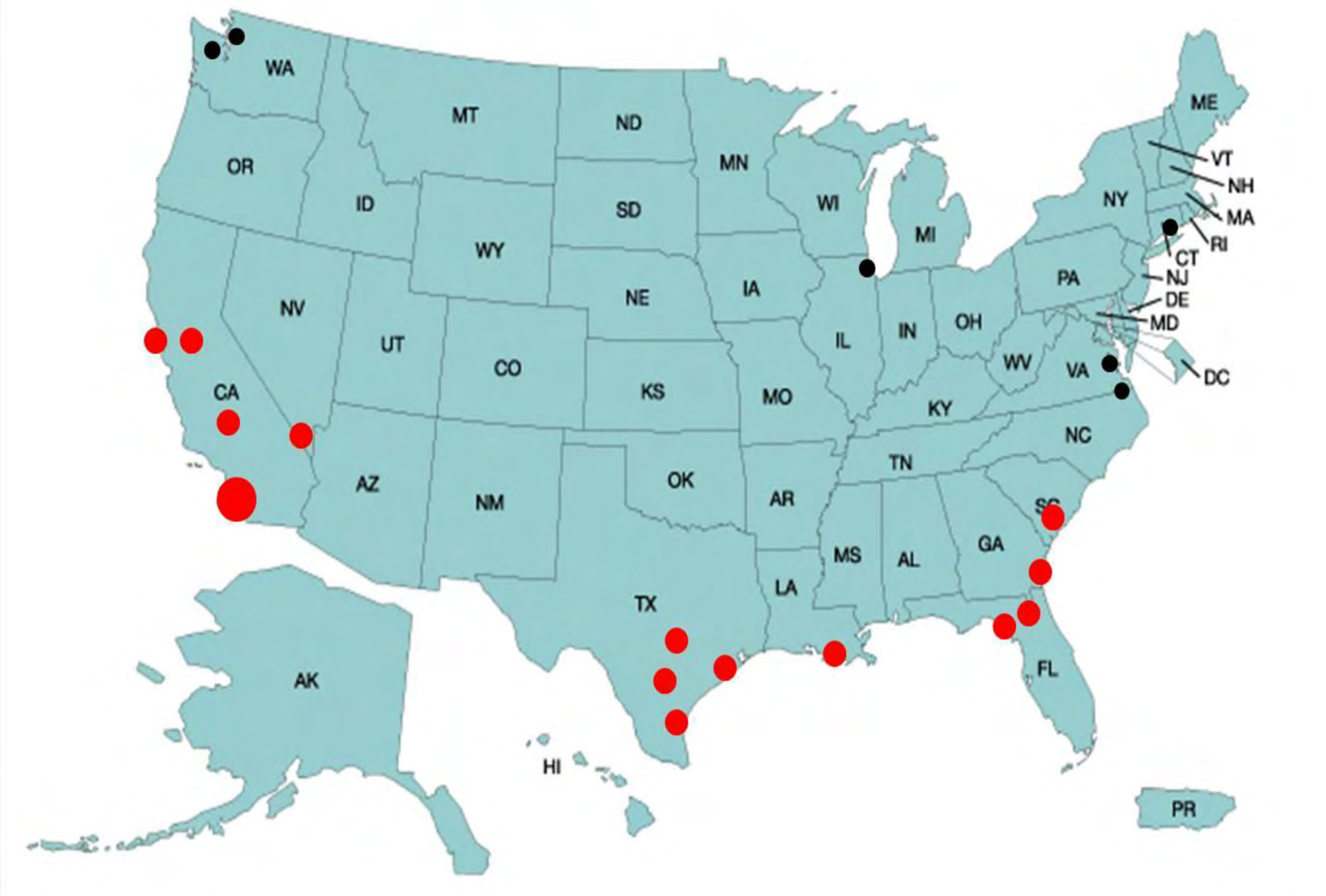


Estimate it would cost $1.7B per year in Hawaii

Rank	Shipping Only (2006-2008)	Risk: Native Range	Risk: Guam (Invaded Range)
1.	San Diego, CA	Pensacola, FL	San Diego, CA
2.	Benicia, CA	San Diego, CA	Norfolk, VA
3.	Norfolk, VA	Norfolk, VA	Benicia, CA
4.	Jacksonville, FL	Jacksonville, FL	Pensacola, FL
5.	Oak Harbor, WA	Kings Bay, GA	Kings Bay, GA
6.	Virginia Beach, VA	Houston, TX	Jacksonville, FL
7.	Pensacola, FL	Benicia, CA	Virginia Beach, VA
8.	Groton, CT	Mayport, FL	Charleston, SC
9.	Bremerton, WA	Virginia Beach, VA	Groton, CT
10.	Great Lakes, IL	Charleston, SC	Mayport, FL

Brown Tree Snake Invasion Risk

Red are high invasion risk; SD has 3X more shipping then elsewhere



Objectives

- **Statement of Need**
  - Development of a novel and innovative technique for managing or eradicating the invasive treesnake (*Boiga irregularis*) population on Guam.

- **Specific objective** – to develop and explore the prospects for biological management
  - Genetically identify the source population(s) for *B. irregularis* on Guam.
  - Provide preliminary phylogenetic data upon which to base decisions for parasite prospecting.
  - Test the presumption that invasive *B. irregularis* are parasite free and assess whether parasites from the native range persist on Guam.
  - Provide an initial characterization of metazoan and protozoan parasites in *B. irregularis* populations within its native range.

Objectives

- **Hypotheses**
  - Invasive snakes on Guam likely have a reduced immunogenetic variability due to a severe founder effect.
  - Parasites and pathogens acting as natural population controls in the snake's native range can be used as biological agents to manage the invasive snakes on Guam.
  - Host phylogenetics can be used as an evolutionary map to guide parasite prospecting

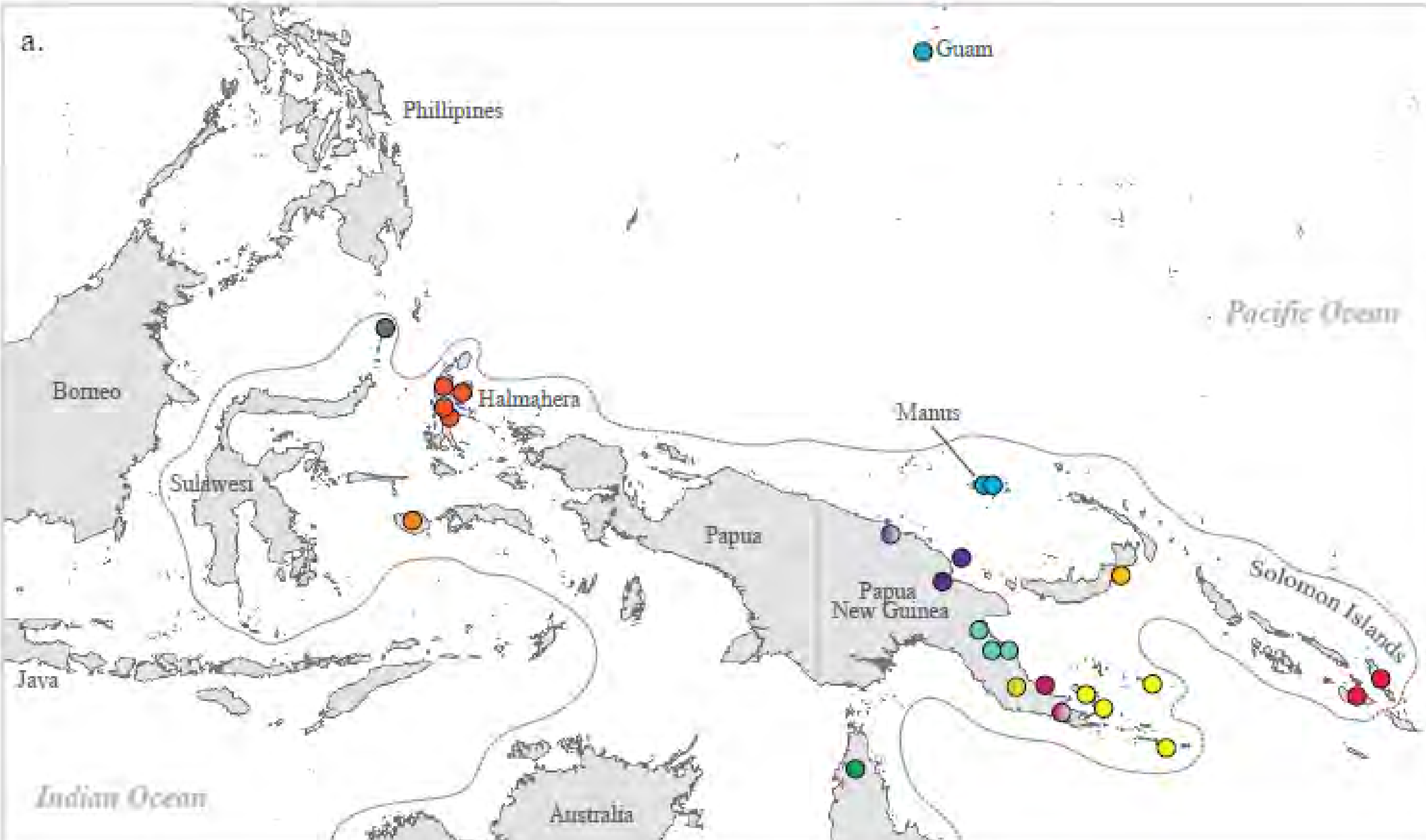
- **Specific technical issue reduction step**
  - Were treesnakes introduced from multiple sources, and where exactly did they come from?
  - Has the invasive population been purged of its native parasites?
  - Can snakes be captured in sufficient numbers in the native range to obtain adequate representation of parasites and pathogens from a given area?

Materials and Methods

- **Molecular approach**
  - Obtain as many tissue samples as possible from representative populations across the snake's native range and on Guam.
  - Use DNA sequence data from a series of mitochondrial and autosomal genes to estimate a statistically well-supported phylogenetic tree for *B. irregularis*.
  - Provide preliminary estimates of genetic diversity, comparing PNG mainland and island populations (including the source) with the Guam population.
  - Develop a genomic library for isolating microsatellites and other loci that are functionally associated with the immune response.

Materials and Methods

- **Parasite surveys**
  - Harvest parasites from *B. irregularis* on Guam and from two locations in Papua New Guinea (one ‘mainland’ and one island).
  - Obtain measurements of age, sex, and body condition.
  - Prepare thin blood smears and blood blots for haemoprotozoan species identifications (morphological and molecular).
  - Harvest and identify all internal and external metazoan parasites.


a.
Guam
Phillipines
Pacific Ocean
Borneo
Halmahera
Manus
Sulawesi
Papua
Papua
New Guinea
Solomon Islands
Java
Indian Ocean
Australia

Materials and Methods

- **How is our approach innovative?**
  - Biological management has received almost no attention as a control technique on Guam – “Magic Tools”
  - None of the limited, previous studies have used host phylogenetics to guide efforts for isolating candidate biological agents.
  - Our goal is to implement population controls that are evolutionarily ‘tried and true’, not only because (**1**) they have presumably proven effective in nature, but (**2)** because they would minimize the possibility of creating new environmental problems on Guam.

Results: Field captures

- Dec 2009: 48 snakes captured on Guam
  - (mean SVL = 864.5 mm ± 225.9, range = 477.0 – 1510.0 mm)
- May 2010: Four snakes captured at Kamiali
  - (mean SVL = 772.3 ± 50.5, range SVL = 712.0 – 821.0)
- May-June 2010: 12 snakes captured on Manus Island
  - (mean SVL = 1100.7 ± 249.5, range = 573.0 – 1548.0)
- April 2011: 16 snakes captured on Guam
  - (mean SVL = 870.3 mm ± 138.6, range = 638.0 – 1155.0 mm)

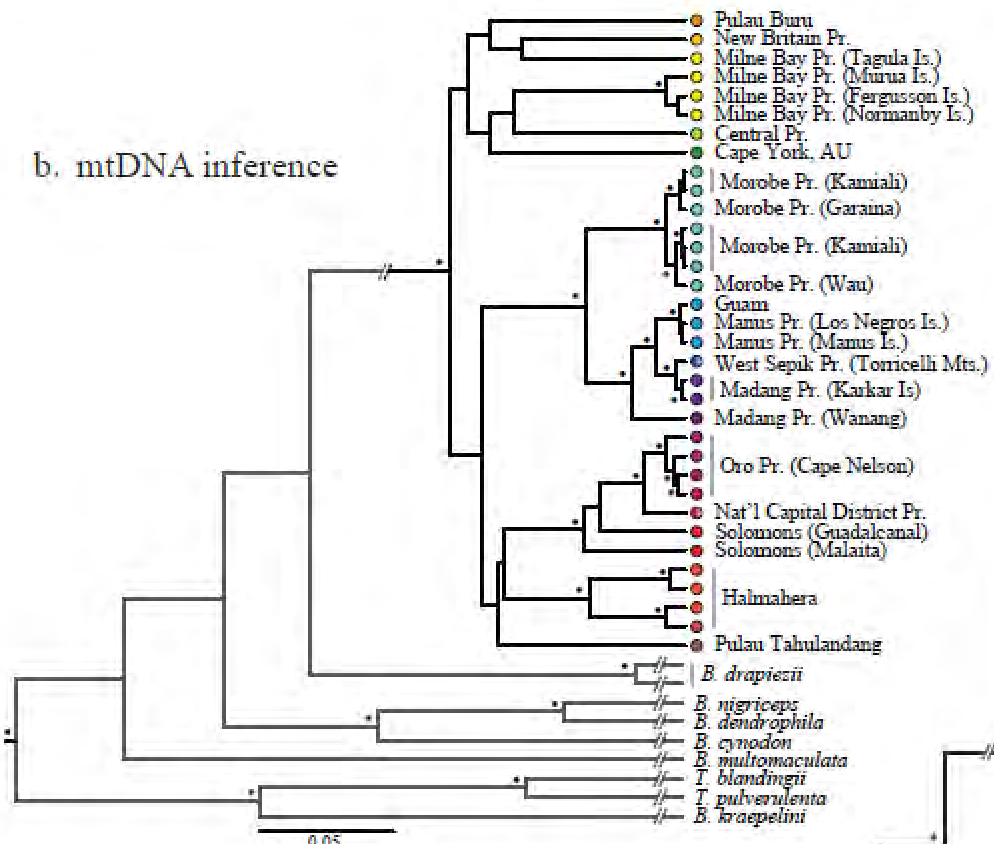
- *Also collected tissue samples and parasite data from six additional snake species to examine host specificity.*

Results: Molecular study

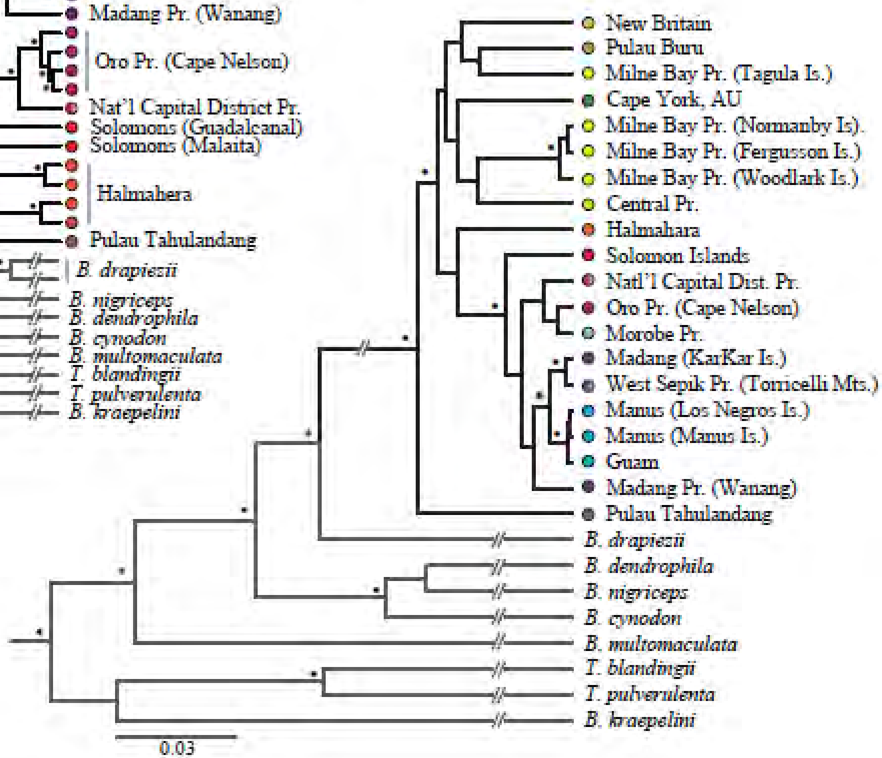
- Definitive genetic identification of a source population in the Admiralty Islands of Papua New Guinea.
- The estimated 1 x 10^{6+} snakes currently on Guam were founded by an extremely small number of individuals, as evidenced by the complete absence of any mtDNA variability across the island.
- Both the mtDNA and combined mtDNA/autosomal tree identify monophyletic groups of populations from that are intermediate and distantly related to the Guam population, making them good targets for future parasite prospecting.

Results

b. mtDNA inference

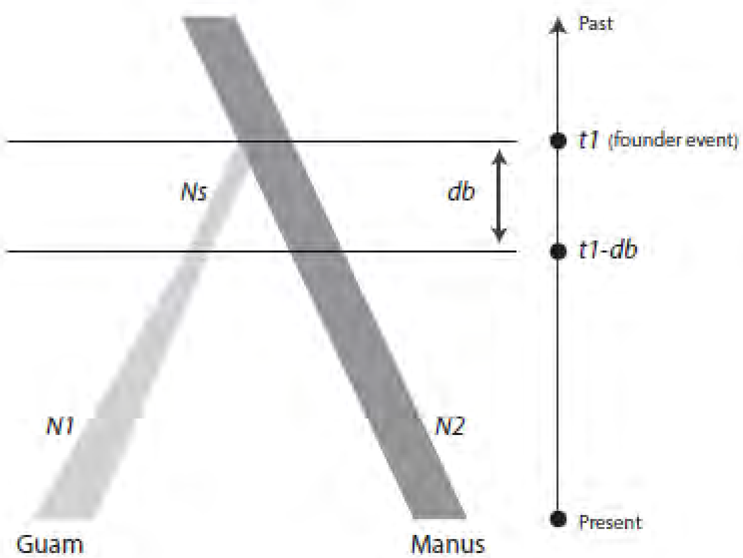


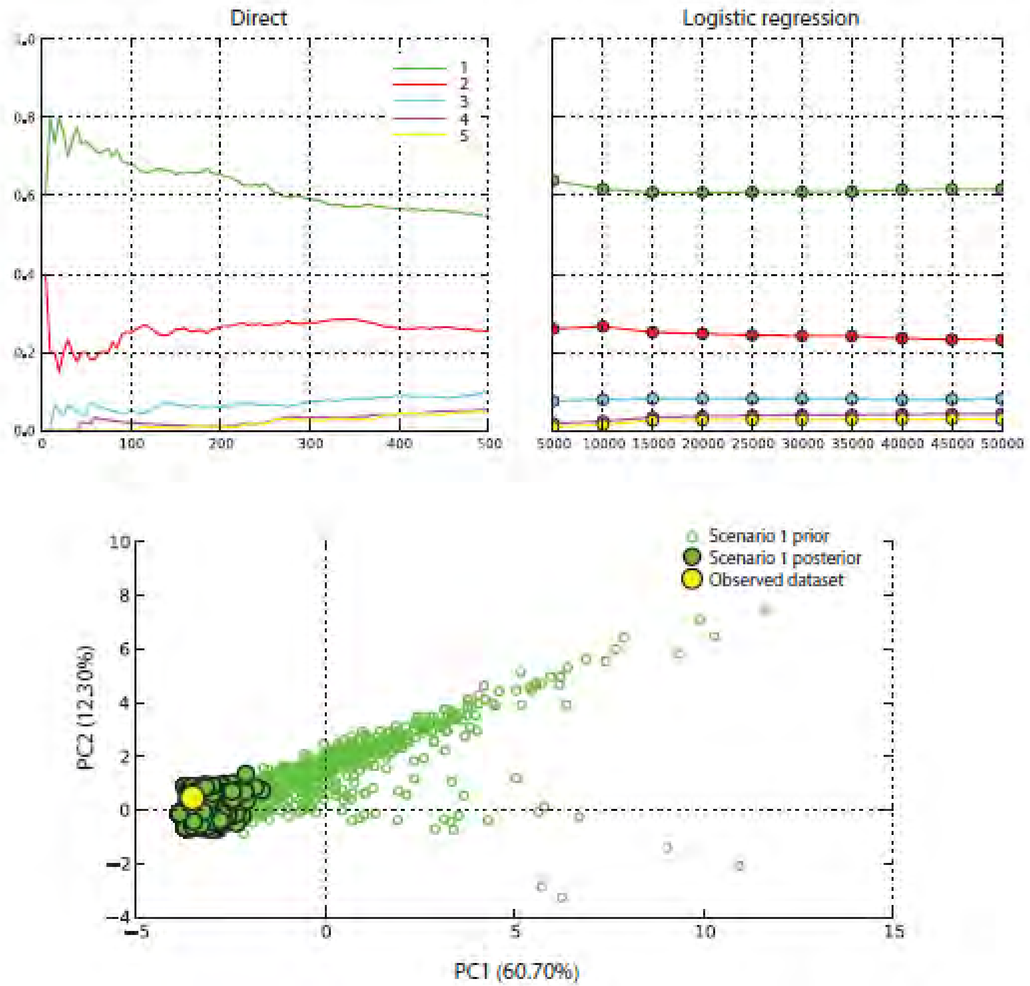
C. Coalescent-based inference



Results

< 10 founders, no evidence of serial invasions





Results: Molecular study

- Specific risk reductions steps for the molecular study
  - ♦ Ruled out the possibility of introductions from multiple source populations.
  - ♦ Provided evidence that the Guam population is genetically depauperate relative to the source and other native areas.

These results suggest that treesnakes on Guam have a limited genetic repertoire for defending against pathogens and parasites.

  - ♦ Data allow us to identify intermediate and distantly related groups of populations, as well as pinpoint places in the native range where substantial genetic variation exists over relatively small areas.

By targeting these locations in our follow-up work, we can potentially increase our ability to recover a wide breadth of parasites and pathogens, given that these genetically diverse host populations may also harbor some of the more diverse parasite faunas.

Results: Metazoan parasites

- Guam
  - *B. irregularis* from Guam do in fact have parasites, although prevalence (24%) and species diversity was low.
  - Most prevalent helminth was a cestode in the genus *Oochoristica.*
  - Detected species are all generalists, with some if not all acquired from the diet.
  - More recently *Spirometra* is clearly spreading
- Papua New Guinea
  - Prevalence (88%) and species diversity was high at both Kamiali and in the Admiralties.
  - Most prevalent species were tongue worms in the genus *Kiricephalus* – *Kiricephalus tortus* is host specific.
  - None of the helminth species detected in *B. irregularis* in the native range were recovered in any snakes on Guam.

Figure 4A. Exposed intestinal lumen of a *B. irregularis* infected with cestodes in the genus *Oochoristica*; this individual was collected in the Guam National Wildlife Refuge (intestine removed from the snake). **B.** Adult pentastomid tongue worms (*Kiricephalus tortus*; n = 6) infecting the lung tissue of a *B. irregularis* from Manus Island. The snake was opened by a ventral incision (head off to the left of the photo), and the lung is cut open to expose the worms. Note that the head of each worm (indicated by the arrow) penetrates the lung tissue and attaches to the body wall, while the remainder of the worm's body lies within the lung.

Table 3. Parasite identity, site of infection, prevalence (proportion of infected hosts among all hosts examined), and mean intensity (number of helminths per infected individual). Values are distinguished for Guam and Papua New Guinea (PNG). Light blue rows indicate helminths that were detected in snake species other than *Boiga* in Papua New Guinea. Note that some snakes were parasitized by more than one helminth species. Prevalence and intensity for *L. intestinalis* in Guam snakes are presented as unknown (?) because only a few worms were isolated from three snakes subsequent to this study.

Helminth species	Site of Infection	Prevalence (Guam)		Intensity (Guam)	Prevalence (PNG)		Intensity (PNG)
		Number	(%)	Mean ± SD	Number	(%)	Mean ± SD
Trematoda (flukes)							
Allopharynx macallisteri [1]	Intestine	1	1.6	2	0	0.0	0
Paradistomum mutabile [1]	Intestine	1	1.6	3	0	0.0	0
Unidentified trema	Intestine	0	0.0	0	5	31.3	125.0 ± 129.3
Cestoda (tapeworms)							
Oochoristica sp.	Intestine	9	14.5	2.6 ± 2.8	2	12.5	1.5 ± 0.7
Ligula intestinalis [2] (plerocercoid larvae)	Subcutaneous/Coelom	?	?	? ?	0	0.0	0
Nematoda (round worms)							
Kalicephalus viperae chunkingensis	Intestine	3	4.8	3.0 ± 2.6	0	0.0	0
Kalicephalus novae-brittanniae	Trachea	0	0.0	0	2	12.5	2.0 ± 1.4
Kalicephalus costatus indicus	Intestine	0	0.0	0	1	6.2	1
Kalicephalus posterovulvus		–	–	– –	1	7.7	2
Meteterakis sp.	Intestine	1	1.6	1	0	0.0	0
Ascarididae sp.	Intestine	0	0.0	0	1	6.2	1
Abbreviata sp.	Stomach	–	–	– –	2	15.4	5.0 ± 1.4
Pentastomida (tongue worms)							
Kiricephalus tortus	Lung/Coelom/Intestine	0	0.0	0	12	75.0	5.8 ± 7.1
Parasambonia bridgesi	Lung/Coelom	–	–	– –	2	15.4	1.0 ± 0.0
Waddycephalus punctulatus	Lung/Coelom	–	–	– –	2	15.4	7.0 ± 8.5

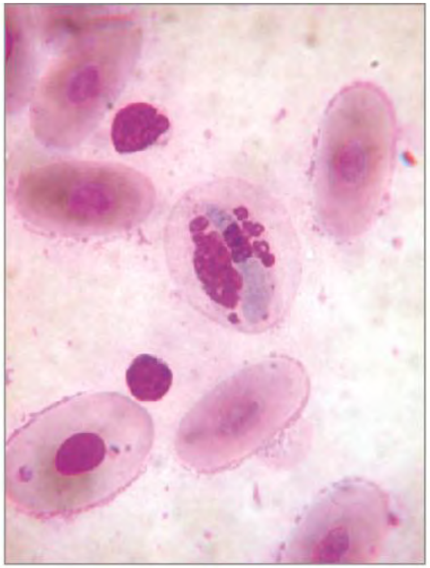
[1] Likely acquired from the diet, as lizards are the typical hosts.

[2] Morphological characters are consistent with *L. intestinalis*; however, we consider this a tentative identification because snakes are not a known host for this worm.

Results: Protozoan parasites

- Guam
  - ♦ Protozoan blood parasites were not detected in any snakes collected on Guam.

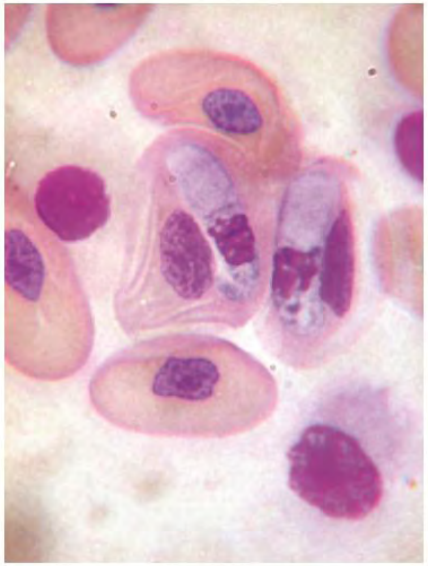
- Papua New Guinea
  - ♦ High prevalence of haemegregarines at both Kamiali and the Admiralty islands (75%); infection intensity was variable across individuals.
  - ♦ Based on morphology, the haemegregarines detected in our samples are species of the genus *Hepatozoon.*
  - ♦ Up to four *Hepatozoon* species were detected among all snakes that were positive for infections.



Hepatozoon spp. 1

Hepatozoon spp. 2

Hepatozoon spp. 3



Hepatozoon spp. 4

Results: Parasite study

- Specific risk reductions steps for parasite exploration:
  - Snakes can be captured in sufficient numbers in the native range to obtain adequate representation of parasites and pathogens for any given area.
  - Guam treesnakes do have parasites, but most are innocuous and none were transported from the source population.
  - Several parasite species were detected that meet specific criteria for a candidate biocontrol agent.
  - At least one potential biological agent may already exist on Guam, and might only require dispersal to other parts of the island.

These results suggest that B. irregularis *on Guam have been purged of their former parasites for approximately 70+ years.*

Conclusions and Implications for Future Research

- **Major results and conclusions**
  - *B. irregularis* on Guam were introduced from a single source population in the Admiralty Islands of Papua New Guinea
  - Current data show that the Guam population is genetically depauperate compared to others in the native range.
  - Guam treesnakes harbor helminth parasites, but none were transported from the native range – this provides strong evidence that the release from co-evolved parasites may be an important factor contributing to their ecological success on Guam.
  - The absence of parasites in Guam treesnakes for 70+ years indicates that immunological defenses against former parasites may be severely weakened.
  - Parasite prevalence is high in the native range - the fact that *B. irregularis* persist on small islands in the Admiralty Archipelago with apparently unlimited prey resources suggests that parasitism may be important in controlling population densities.

Conclusions and Implications for Future Research

- Research objectives that were met
  - Ruled out the possibility that multiple source populations have become established on Guam.
  - Established a phylogenetic ‘blueprint’ that serves as a guide for future parasite prospecting in native populations.
  - Collected snakes in appropriate numbers for characterizing the parasite faunas of native *B. irregularis* populations.
  - Identified parasites that meet some of the criteria necessary for effective biological management.

- Research objectives that still needing completion
  - Molecular identification of *Hepatozoon* species

Conclusions and Implications for Future Research

- How did this research resolve particular knowledge gaps?
  - No previous work had genetically verified a source population or tested for repeated introductions from different areas.
  - We resolved the longstanding issue of whether *B. irregularis* on Guam have parasites, and showed that all parasites currently infecting snakes were acquired after their introduction.
  - We showed that while parasite prevalence and species diversity is low on Guam, *B. irregularis* from Papua New Guinea are heavily infected with a variety of parasites.
- How did this research identify remaining gaps?
  - A biological agent may already be in play on Guam.
  - What controls population density on small islands where prey resources are apparently unlimited?

Conclusions and Implications for Future Research

- Proposed follow-on **genetic research** will address the following questions:
  - Based on DNA sequence data, what haemogregarine species infect *B. irregularis*? Are any host-specific to *B. irregularis*? Is there evidence for *Plasmodium* infection?
  - How genetically depauperate is the Guam population relative to the source population, and is there evidence of a genome wide bottleneck? Does the Guam population have limited variation in genes that control the upstream regulation of acquired immunity?
  - Do Guam treesnakes constitute one panmictic population of randomly mating individuals, or is there detectable population structuring on the island? What is the effective population size? How much gene flow is there among different areas on the island?

Conclusions and Implications for Future Research

- Proposed follow-on **parasite research** will address the following questions:
  - ♦ What bacterial and viral pathogens infect native *B. irregularis* populations? Besides blood protozoans, what additional protozoans, specifically amoebas and coccidia, infect the gastrointestinal tracts of *B. irregularis*?
  - ♦ How common is *Spirometra*on Guam and what are the other intermediate hosts? How does this cestode affect treesnake condition? How localized are *Spirometra* infections on Guam?
  - ♦ What factors serve as natural population controls on small islands in the native range? Are parasites and pathogens more of a limiting factor to *B. irregularis* population growth than prey resources?

Publications

- Publications in prep:
  - ♦ The use of phylogenetic data in the strategic planning of biological management: A case study on the invasive Brown Treesnake (*Boiga irregularis*) on Guam
  - ♦ Evidence for the enemy release hypothesis as a determinant of ecological success in the invasive Brown Treesnake (*Boiga irregularis*) on Guam
- Planned publications:
  - ♦ Molecular identification of haemogregarine protozoans in the Brown Treesnake (*Boiga irregularis*)

Backup Slides