



Australian Government

Department of the Environment, Water, Heritage and the Arts

**Marine and Tropical Sciences Research Facility (MTSRF)
Final Milestone Report 2006-2007**

Project 4.8.7 Forecasting risk of exposure to irukandji

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Summary

Projected milestones were as follows:

- Collect samples of *C. fleckeri* and *C. barnesi*
- Report on microsatellite DNA markers in existence for *C. fleckeri*, applicability for use with *C. barnesi*, including any laboratory testing, and approach to developing microsatellite library of *C. barnesi*.
- Summary/provision of any communication activities/articles undertaken to date, including minutes of meetings by investigators and half day workshop if applicable. Information to be disseminated through the stinger newsletter.

All of these items have been delivered without matching funds (this does not include the in-kind contributions of the investigators).

the extent to which we can deliver these components of the work will be limited without matching funds

For reference: Milestone extracted from Project Schedule

Date: June 2007

Description

Milestones will be delivered by June 2007.

Project Results

Description of the results achieved for this milestone

Field trips

Time	Location	Species	Number	Notes
Oct-Nov 2006	Weipa	<i>Chironex fleckeri</i>	40 to 50	Specimens for genetics and statolith chemistry
Dec. 2006	Low Isles	<i>Carukia barnesi</i>	~30	Venom extracted, specimens to Madeleine van-Oppen, specimens for statolith chemistry
Dec. 2006	Double Island	<i>Carukia barnesi</i>	40 to 50	Venom extracted, specimens to Madeleine van-Oppen, specimens for statolith chemistry
Jan.07	Hamilton Is.	<i>Carukia barnesi</i>	~30	Specimens for genetics and statolith chemistry
Jan.07	Airlie Beach	<i>Carukia barnesi</i>	3	Specimens for genetics and statolith chemistry
April.07	Fraser Island	<i>Carukia barnesi</i>	7	Specimens for genetics and statolith chemistry
April.07	Broome, WA	<i>Chironex fleckeri</i>		Specimens for genetics and statolith chemistry
May.07	Fannie Beach, Darwin	<i>Chironex fleckeri</i>	10	Specimens for genetics and statolith chemistry

We also have about 300 *Carukia barnesi* that were collected and stored in alcohol in 2005. We will examine the DNA and statoliths of these specimens,

A **microsatellite library** is in progress for both *Chironex* and *Carukia*.

Testing of primers developed by Coughlan *et al.* 2005 for *Chironex fleckerii* commenced on 3rd October, 2006. The 7 primer pairs were tested on 2 test DNA samples from *Carukia barnesi* and 2 control DNA samples from *Chironex fleckeri*, which had been held at AIMS from a previous study. The quality of these DNA samples was verified by visualisation on a 1% agarose gel. The primer pairs used in this trial were Cfl_17, Cfl_77 and Cfl_83. These reactions were unsuccessful, showing no amplification in the *Chironex fleckeri* samples and a multiple banded smear in only one of the *Carukia barnesi* samples.

Several attempts were made to optimise the PCRs. Annealing temperature gradient, and sample dilution trials were undertaken for the above 3 primer pairs. The results of the trials showed altering the temperature made a marginal improvement for locus Cfl-77, but loci Cfl_17 and Cfl_83 still amplified poorly, if at all, across the examined range of temperatures.

As temperature changes had little effect on the PCR, sample dilutions were tested for improvement on the remaining 4 loci. Of these 4 loci, Cfl_4 did not amplify at all, Cfl-16, Cfl_26 and Cfl_31 produced multiple banded smears for samples at all dilutions.

It was considered that despite appearing to be of acceptable quality, the DNA itself could be the problem. New *Chironex fleckeri* samples collected from 2 locations at Weipa were delivered at AIMS on the 4th December 2006. DNA was extracted from 20 samples from each of the Weipa locations so the markers could be tested on DNA extracts from new tissue. PCR for the 7 loci was set up as described by Coughlan *et al.* 2005, using undiluted template and a 1/10 dilution of template. Amplification was non-specific and inconsistent among the samples for Cfl_77, Cfl_26 and Cfl_31 and failed completely for the remaining Loci.

Work to develop new microsatellite loci commenced on the 9th January, 2007. The total genomic DNA used for library construction was isolated from individual ethanol preserved *Chironex fleckeri* and *Carukia barnesi* samples, provided by Jamie Seymour on the 18th November and the 12th December 2006 respectively.

Two DNA isolation approaches were used to isolate high molecular weight (HMW) total genomic DNA for library construction. Initially a simple in-house salt precipitation DNA isolation method (Wilson *et al.*, 2002) produced high yields of good quality gDNA for *Chironex fleckeri*, but less than adequate yields from *Carukia barnesi*. The DNeasy method produced over 10ug of HMW DNA from one of the three *Carukia* samples. The other *Carukia* samples and the *Chironex* sample yielded only limited amounts of degraded DNA. Quality and quantity of the DNA preparations was determined by visualisation on 1% agarose gels and spectrometer absorbance readings.

The libraries were constructed using the initial in-house DNA preparation for *Chironex fleckeri*, and the DNeasy DNA preparation for the *Carukia barnesi*. To create the libraries, total genomic DNA was digested and fragments containing di, tri and tetranucleotide microsatellite repeats were selected using the hybridisation capture procedures outlined by Glenn and Schable (2005). Microsatellite enriched DNA fragments were ligated into Promega's pGem T Easy vector and the ligations were sent to the Australian Genome Research Facility (AGRF), on the 14th of February, for transformation, colony screening and sequencing of 192 putative positive clones for each species.

The AGRF returned the sequence data in two batches. The first batch of 96 sequences from each library was returned on the 1st of March, the second batch on the 19th March. On receiving the data, the sequence quality was checked, vector and linker sequences were removed and the edited sequences were aligned to identify redundancies using Sequencher software. Microsatellites in the edited sequences were detected using the freeware tandem repeat finding programme TRF. Thirty-four were detected among the *Carukia* clone sequences and 28 in the *Chironex* clones. Of these it was possible to design primers, using the Primer 3 programme, for 33 *Carukia* and all 28 of the *Chironex* sequences. PCR product length was a consideration in primer design and a range of 95bp to 350bp was chosen. The primers were checked against all clone sequences and were excluded if they bound to multiple sites on the sequences and alternative primers could not be designed because of limited flanking regions surrounding the microsatellites. This left 19 *Carukia* and 14 *Chironex* primer pairs for further testing. The forward primers from each locus were modified by the addition of a universal M13 tail to the 5' end to facilitate fluorescent labelling of the resulting PCR products and enable Gelscan analysis of the loci for polymorphism.

The DNA from which the libraries were developed was used to optimise PCR for the new primers. Thirteen of the 17 *Carukia* primers and 11 of the 13 *Chironex* primers tested to date, amplify well. Results were confirmed by repeating PCRs on the original DNA and adding 2 other DNA samples for each species from a previous study.

DNA from 23 *Chironex fleckeri* samples from Weipa, provided by Jamie Seymour, have been screened for polymorphism for 7 of the *Chironex* loci that produced satisfactory PCR product. All 7 loci were polymorphic, with each locus showing a minimum of 6 alleles; the presence of strong stutter bands made scoring difficult for one locus.

DNA was extracted from 23 *Carukia* samples from Lizard Island, provided by Jamie Seymour, and screened for polymorphism for 7 of the *Carukia* loci. Two loci were abandoned because they were too difficult to score. The remaining 5 loci were polymorphic, showing a minimum of 4 alleles per locus.

In the year 2007-08, we'll continue development of microsatellite markers as follows:

***Carukia barnesi*:** test remaining 4 primer pairs for PCR success
Check remaining successful loci for polymorphism
Check 5 *Chironex* loci on *Carukia* samples for polymorphism
Sequence allele representative for each locus to confirm microsatellite presence.

***Chironex fleckeri*:** test remaining 2 primer pairs for PCR success
Check remaining successful loci for polymorphism
Sequence allele representative for each locus to confirm microsatellite presence.

References.

Glenn, T.C. and Schable, N.A. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* 395:202-222.

Wilson KJ, Li Y, Whan V, Lehnert SA, Byrne K, Moore SS, Pongsomboon S, Tassanakajon A, Rosenberg G, Ballment E, Fayazi Z, Swan J, Kenway MJ and Benzie JAH (2002) Genetic mapping of the black tiger shrimp *Penaeus monodon* with Amplified Fragment Length Polymorphisms. *Aquaculture*. 204: 297-309.

Communications, major activities or events

During milestone reporting period

Meetings

- 11-October-06 Van Oppen and Kingsford – sampling priorities
- 16-Feb-07 Seymour and Kingsford – meeting milestones and planning trips.
- Two Honours students will join in with the collaboration in September 2007 (Chris Mooney and Shelly Templeman).
- April 6th meet with Jamie Seymour
- May 15th meet with Madeleine van Oppen

Workshops over last 12 months

Date	Present	Item
11-Oct-06	Seymour, Van Oppen and Kingsford	Sampling priorities

Next work shop August – September 2007

Other

We regularly communicate on e-mail.

During next milestone reporting period

We have met all proposed milestones 2006 to June 2007.