

Appendix 4 : Scientific Meeting Abstract , Award and Related Publication.

4.1 Polymerase Chain Reaction (PCR) Optimization for Mitochondria Cyt-b and D-loop Gene Fragment of Malaysian Slow Loris (*Nycticebus coucang coucang*).

POLYMERASE CHAIN REACTION (PCR) OPTIMIZATION FOR CYT-B GENE FRAGMENT OF MALAYSIAN SLOW LORIS (*Nycticebus coucang coucang*)

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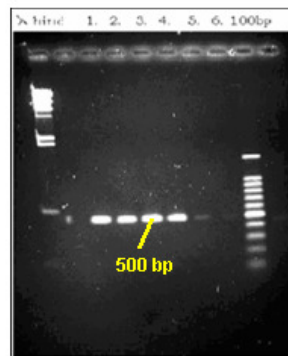
3 Jabatan PERHILITAN, Cheras Kuala Lumpur

At present, little work has been done on the molecular systematics of the Loridae. Therefore, very limited inferences can be made on the basis of molecular data of the Malaysian Slow Loris. The mitochondrial cytochrome b (cyt-b) gene is widely used in systematic studies to resolve divergences at many taxonomic levels. The present preliminary study focuses mainly on identifying the best amplification conditions for cyt-b gene fragment of Slow loris (*Nycticebus coucang coucang*) as a molecular marker for future phylogenetic relationship studies of this prosimian species in Peninsular Malaysia, DNA was extracted from a total of six individuals captivity in the Malacca Zoo.

Polymerase chain reaction (PCR) was used to amplify the complete sequence of the cyt-b mitochondrial gene, PCR reactions were carried out in 50 µl reaction containing 1.0 µl dNTP (1 mM), 5 µl (5x) reaction buffer, 5 µl (50mM) MgCl₂, 0.5 µl (10 mM) each primer, and 0.5 µl Tag DNA polymerase. PCR cycles for amplifications were performed using the following conditions 35 cycles of denaturation at 94 °C for 1 min, annealing at 55°C for 1 min. A final extension at 72°C for 10 min was performed to completely extend the amplified product. The PCR product was purified with the QIAquick (QUIAGEN) kit. The estimated PCR product size for this gene fragment was approximately 500 basepairs

Sequencing primers for Cyt-b gene (Kocher et al 1989)

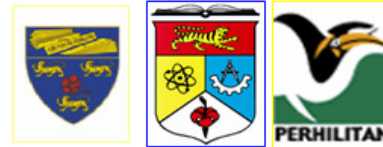
L14724:
5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' &
h18918
5'-AACTGCAGTCATCTCCGTTTACAAGAC-3'



Cyt-b DNA PCR product after purification

Marker λ -Hind III;
1. Individual No. 1
2. Individual No. 2
3. Individual No. 3
4. Individual No. 4
5. Individual No. 5
6. Individual No. 6
Marker 100bp.

*Kocher, TD, Thomas, WK, Meyer, A, Edwards, SV, Paabo, S, Villablanca, FX, & Wilson, AC (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86: 8196-8200.



ACKNOWLEDGEMENT

We appreciate genetic tissue sampling assisted by Jabatan PERHILITAN and Malacca Zoo's. The laboratory work has supported by the government Malaysia through the IRPA grants 04-02-02 0019 EA 301

4.2 Cross-Species Amplification of 12 Primates microsatellites markers previously developed using samples from the Pro-primate Slow Loris (*Nycticebus coucang coucang*)

POLYMERASE CHAIN REACTION (PCR) OPTIMIZATION OF 12 MICROSATELLITE MARKERS DEVELOPED ON THREE SPECIES OF MONKEYS FOR AMPLIFICATION ON THE MALAYSIAN SLOW LORIS (*NYCTICEBUS COUCANG COUCANG*)

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INTRODUCTION

Microsatellite DNA markers are simple sequence repeats (Tautz et al., 1996) distributed along the genome (Litt and Luty, 1999) that have been used to assess family structure, and to study ecological/behavioral characteristics such as mating system and dispersal patterns.

Microsatellite markers are powerful research tools but their development is labor intensive and costly. Consequently, researchers have tried to use microsatellite markers developed in one species for analysis of another "cross-species" (Moore et al., 1991).

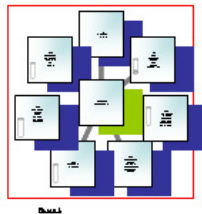
OBJECTIVE

The present preliminary study focuses mainly on testing the ability of markers previously developed for *Macaca* Monkeys and *Lemurs* to amplify on *Slow Loris* as a molecular tool for future studies of paternity and relatedness among one population of wild slow lorics tracked in Pahang, Malaysia.

METHODOLOGY

Blood samples were collected from 27 individuals *Slow lorics* and stored on FTA Cards at room temperature. Twelve cross-species microsatellite markers were screened for their utility in the cross-species amplification of *Slow lorics* derived from blood.

DNA EXTRACTION: Chelex-100 protocol (Walsh, 1991) was used to do this extraction. To make 10 ml of 5% chelex 100 in 12 g TRIzol. 5g CHELEX 100 ml H₂O (Figure 1).



PCR reactions were carried out in 25 µl reaction containing 4.0 µl dH₂O (50 mM Tris-HCl, 2.5 µl (10x) reaction buffer, 1 µl (50 mM) MgCl₂, 1 µl (10 mM) each primer, and 0.3 µl Taq DNA polymerase. PCR cycles for amplifications were performed using the following conditions: 33 cycles of denaturation at 95°C for 3 min, annealing at 50°C for 1 min. A final extension at 72°C for 5 min was performed to completely extend the amplified product. Agarose gel electrophoresis of 5 µl of the reaction and visualization using ethidium bromide was used to evaluate whether the reactions were successful.

RESULTS

We attempted to amplify 12 microsatellite markers that were previously characterized in three species of primates. Thirty-three percent (4/12, 33%) of these markers from primates successfully amplified *slow lorics* DNA.

CONCLUSION

Of the 12 markers tested in this preliminary study, 4 (33%) fulfilled the criteria of amplifying well. This is a surprising result given that *slow lorics* is not closely related to those primates tested.

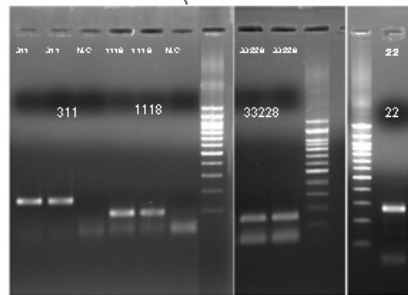
Furthermore, by using cross-species markers one can save time and money rather than devote significant resources to a few species-specific primers. We can suggest as well that the number of cross-species markers that positively amplify might improve tremendously by screening a larger number.

Nevertheless, in spite of having successfully amplified 4 cross-species markers the question remains: whether they exhibit levels of polymorphism and produce reproducible genotypes to determine relatedness between individuals.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. Zulqarnain and his group for their broadmindedness and trust in helping to further the objectives of an unknown foreigner.

PCR PRODUCT MARKERS AMPLIFICATION



Leuca Pteropus
 2128
 CTCTGAGG-AGAGG-GTC
 Z
 ATTCCTCTGAGG-GTC
 2128
 F
 AGTCTTAACTTAACTTAACT
 R
 AGCTTAACTTAACTTAACT

Microlepus reevesi



Webb's Monkey



Cebus olivaceus



1000 bp
 500 bp
 250 bp
 125 bp
 62.5 bp
 31.25 bp
 15.625 bp
 7.8125 bp
 3.90625 bp
 1.953125 bp
 0.9765625 bp



Who is Related to whom???



4.3 Conservation and Awareness of Slow Loris. Trailing the Slow Loris, The Star newspaper Kuala Lumpur Malaysia (17 October 2006)



Tuesday 17 October 2006

T2 Environment

STARTWO

Star **StarTwo**

cook's nook

Weekend brunch 6



Try some quick and easy recipes for brunch!

lifestyle

On the run 8

Runner Khairul Azwar Hashim talks about how he got into running.

starchild

Birthday joy 12



What's in a birthday party? Good food, lots of games, and presents!

books

Peter Pan 2 14

Few tales raise stronger emotions than *Peter Pan*, so a sequel to J.M. Barrie's classic is bound to raise hackles.

entertainment

Fender bows out 17

Freddie Fender, the 'Bebo Kid' of the Texas-Mexico border, died on Saturday.



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Supremely suited to its arboreal life, the slow loris uses all four limbs in a way that would put the most agile gymnast to shame. — Pictures by SIA HONG KIAU

Stories by LEE TSE LUNG

In a lonely forest

IT IS possibly the loneliest, most depressing sound in the forest tonight: a small "pip...pip...pip", pitiful in the dripping dark. Hardly the reassuring "beep...beep...beep" we are listening for, that monotone music that would herald the presence of our nocturnal radio-collared quarry.

It is 11 pm, and the three of us — Columbian research student Jaime Castillo Garcia, a photographer and I — have been tramping in the orchards and secondary forests near Lanchang, Pahang for the last four hours. As we negotiate yet another barbed wire fence lurking in the undergrowth, I cannot help but think this is not what I imagined radio tracking would be. Nowhere on TV do they mention the need to triangulate repeatedly, to effectively spiral in on your target.

On a hill. In the dark. Under incessant rain. Just as my spirits start to sink into a deeper gloom than the one that surrounds us, Jaime stops short, head cocked to one side, antenna held aloft. He bounds off between the trees, clearly excited at the prospect of seeing Hermosa, Linda or Timide again. His enthusiasm is infectious, and we find the energy to scramble after his receding back, yet again.

Hermosa means "gorgeous" in Latin. Linda means "pretty" and Timide "shy". All three are female adult slow lorises. Aggre (short for Aggressive), an adult male, also showed up, invisible despite the signal that

told us we were right under his nose.

Like many vertebrates and most nocturnal animals, the slow loris has a super-reflective layer called the *tapetum lucidum* behind its retina, which helps its night vision by increasing the quantity of light caught by the retina. It also helps humans see them better at night, by scanning the treetops with a flashlight for this "eyeshine".

While we managed to pinpoint their individual locations down to 3m of accuracy, we saw neither eyeshine, nor hair nor hide of them. Save for Hermosa, who wandered out of the trees onto a telephone wire before scuttling on detecting our approach. That is where the locals usually spot them — crawling along the wires, which act like express lanes between the trees that line the kampung roads.

Hermosa moves the way all slow lorises move — eyes constantly scanning for footholds and threats, steps careful, deliberate and as fluid as the foliage nodding in the breeze. Hermosa's gait hid her well. Take your eyes off her for a

moment and her furry grey-brown behind melts away, lost to sight. It is not hard to see why villagers believe the slow loris or *kongkang*, has special powers. Should a kongkang find itself in a tight spot, says Kamal Ahmad from nearby Kampung Chempaka, it can use its *ilmu*, or magical knowledge, to disappear into thin air.

Fortunately, the 10 lorises which Garcia has captured over the last few months with the help of Kamal and his friends did not employ this trick. Five have not been released and remain under his care: Manso ("tame", adult male), Gent (short for Gentle, a sub-adult male), Peq (short for Pequeno or "small", a juvenile) and Bonita ("beautiful", an adult female) and her baby.

By radio "The animal is rare and very difficult to observe, so there is no other way besides using a radio collar or keeping it in captivity if you want to study them," said Garcia, as he attached and checked Bonita's latest accessory, a 12g radio collar.

17 October 2006 Tuesday

Environment T3

STARTWO

Trapping one

TO say that trapping slow lorises is difficult is an understatement, heaves Jaime Castillo Garcia. And without help, it is impossible.

"Whenever I saw one, I would call the village people for help. We would spend two, three hours getting it down. Seven people, all big guys, trapping this small *kongkang!* It moves slowly, but they climb one tree it moves to another, and when they climb that tree, it moves to another."

Prior to his arrival in Lanchang a little over four months ago, Garcia was in the primal forest of the Krau Wildlife Reserve further north in Pahang. There, he worked on three transects – one at the peak of Bukit Tapah, another on the foothills of Bukit Rengit and the last at sea level along Sungai Lantai – to determine the population density of local loris communities.

He also tried trapping in nearby Kuala Gandah for a month but his collapsible traps baited with food, urine and faecal samples from captive lorises yielded nothing more exotic than rats and squirrels. Even the Orang

Asli hunters he enlisted eventually gave up the ghost.

"I decided this wasn't working, so I offered to pay the kampung people if they trap the kongkang for me."

According to Kamal Ahmad, 39, from Kampung Chempaka, while the slow loris is sought after by bomohs in Indonesia for charms, the local attitude here is "if it doesn't disturb us, we won't disturb it".

Along with the other villagers helping Garcia, he feels proud that their little animal is the subject of so much interest, and that Garcia is sharing his knowledge of it with them.

Garcia believes the slow loris population in Lanchang is doing well because trees which villagers grew in between their orchards have formed wildlife corridors, so the animals are not isolated but could move between forest patches.

"They like it because the trees look good in front of their house but without knowing it, they're conserving the place."



Jaime Garcia releasing the radio-collared adult female and baby where he originally trapped them.

SLOW loris populations were assessed by the World Conservation Union-IUCN in 1996 and in 2000. On both occasions, the populations were categorised as "lower risk/least concern".

In Indonesia, the slow loris faces habitat destruction, illegal trade and hunting. The animals, which are sold openly at markets, are used in traditional medicines and kept as charms.

The Loris and Potto Conservation Database, compiled by biologist Helga Schulze from the Ruhr University Bochum in Germany, says a 1987 study estimated 150,000 animals exist in Indonesian reserves, but cannot be regarded as well-protected.

In Indochina, the species is threatened not only by demand for traditional medicines but for exotic meat as well.

There is little data available on slow loris numbers in Malaysia, although the Loris Database observes that "in Malaysia the protection of lorises seems to be relatively efficient". The Universiti Malaysia Lorisidae Primate Research Group hopes to fill this information gap.

Global Positioning System (GPS) transmitters, he pointed out, while easier to track, are expensive and too heavy for smaller animals like the slow loris, which each measures between 30cm and 40 cm in length and typically weighs less than 2kg.

It was the afternoon before our night trek and while Bonita lay sedated, Jaime photographed her and took detailed measurements as well as blood and hair samples for the DNA fingerprinting work that would make up the final component of his research project.

Garcia, 33, is reading for a Masters of Science at University Malaya, under the recently formed Lorisidae Primate Research Group. He is focusing on the ecology of the Malayan slow loris and hopes to shed more light on the animal's ranging patterns and the list of plants which the animal uses.

"We want to understand things like how much the animal moves daily and how the movement relates with other animals. We also want to capture dispersal... how young individuals move and establish an area a little bit further away and how they use sleeping sites. I also want to develop a biomap (a map of biological features related to the animal's ranging patterns)."

To do all of this, Garcia needs to obtain data by tracking his collared animals via radio telemetry and recording their movements using the GPS. This he does daily between 7pm and 7am. Once he has that, he can use a Geographical Information System (GIS) to create layers representing movement patterns for each slow loris. By layering the various elements together, he

The data

The Slow Loris:

- Belongs to the suborder of Prosimii or "primitive apes"

- A totally protected species, and so cannot be trapped or kept as pets

- Nocturnal (active at night) and arboreal (lives in treetops)

- Has a poisonous bite

- Distributed over the Indian sub-continent, southern China and South East Asia.

- Is grouped into two species, *Nycticebus bengalensis* and *Nycticebus coucang*. The Malayan slow loris is considered a subspecies, *Nycticebus coucang coucang*, along with *Nycticebus coucang menagensis* (found in Borneo and the Philippines) and *Nycticebus coucang javanicus* (found in Java).

- The Loris and Potto Conservation Database can be found at: www.loris-conservation.org

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4.4 Wild Asia Seed Grant Recipients and Awareness, The Star newspaper Kuala Lumpur Malaysia (17 October 2006)

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Saturday September 16, 2006

Wild Asia seed grants



When Jaime Castillo Garcia (above) of Colombia arrived in Malaysia to do his masters programme at Universiti Malaya, he applied for grants to fund his research. "I sent out 23 letters to different organisations but it was difficult," says Garcia who is doing his Masters in Science. But after surfing the internet, he stumbled on Wild Asia's Seed Grant programme.

WA's seed grants are basically small amounts of money (from RM2,000-RM4,000) given to people who want to be involved in conservation, either for research or to run projects, explains WA's director Dr Reza Azmi.

"It's for people who find it difficult to source for grants," says Reza who introduced the programme in 2005. "These grants complement funding from bigger organisations like WWF. They are easy to apply for with no complicated forms to fill."

So far, WA has given out grants to four projects. Funds were given to a community in Klias Peninsula in Sabah to buy natural history guide books. The community is running nature trails for tourists and needed reference material to enhance their knowledge.

A University of Rhode Island researcher working on an Asian Elephant conservation project in Taman Negara also received WA's grant to fund her research into the movement of translocated elephants.

One of the three researchers of the Lorisidae Primate Research Group, Universiti Malaya, Garcia's studying the nocturnal slow loris.

Based in Sg Bolok, near Lanchang, Pahang, Garcia, with the help of local guides, catches slow lorises, collars them and then releases them back into the wild.

"I need the grant for guide fees, data collection and transport expenses," says Garcia, who also receives assistance from WWF.

This year's Wild Ride event managed to raise about RM6,000, and Garcia is the latest recipient of the grant. Currently, the grant is sourced from leftover funds from WildAsia and sales of merchandise like WildAsia T-shirts.

"But we're looking at a long-term plan. Perhaps we'll use the grant as a channel for Corporate Social Responsibility (CSR) funding," says Reza.

— By **LEONG SIOK HUI**

For more information on Wild Asia and its Seed Grants, visit www.wildasia.net, e-mail: info@wildasia.net or call (03) 6201 2150.

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4.5 Department of Veterinary Services of Perak, Malaysia, Raising Awareness of Slow Loris (Trapping One)

Harga Daging Dan Ikan Tidak Dikawal

Department of Veterinary Services
of Perak, M A L A Y S I A

Trapping one

The Star, 17 Oct 2006

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