10th International Conference on Ecological Informatics
*24-28 September 2018, Jena, Germany*

# Beyond Allopatric Speciation: Testing for Genetic Homogeneity in *Duttaphrynus melanostictus* in Relation to Human-induced Dispersal

Siti N. Othman, Yi-Huey Chen, Desiree Andersen, Ming-Feng Chuang, Yikweon Jang, Amaël Borzée

Department of Life Sciences and Division of EcoScience, Ewha Womans University, Seoul, Republic of Korea

# THE MOST COMMON INVASIVE AMPHIBIANS

The Cane Toad, *Rhinella marina*

American bullfrogs, *Lithobates catesbeainus*

African Clawed Frog, *Xenopus laevis*

Asian Black Spined Toad, *Duttaphrynus melanostictus*, Toamasina Port, Madagascar, 2014

# GLOBAL CHALLENGE ON INVASIVE ASIAN BLACK-SPINED TOAD

**Madagascar toad invasion!**

Asian Black-spined Toad, *Duttaphrynus melanostictus*, Toamasina Port, Madagascar, 2014 (Kolby, 2014).

MENU

nature
International journal of science

Altmetric: 71 More detail »

Correspondence | Published: 28 May 2014

## Ecology: Stop Madagascar's toad invasion now

Jonathan E. Kolby

Asian common toads (*Duttaphrynus melanostictus*) have begun to invade Madagascar, threatening the biodiversity of its unique fau
short, so we are issuing an urgent call to the conservation
and to governments to prevent an ecological disaster.

The first reported sighting of *D. melanostictus* on Madagasc
March in Toamasina. We collected six adult toads from a sw
humid eastern region, six kilometres from Madagascar's larg
More were spotted nearby, suggesting that they arrived from
shipping containers, as they have elsewhere (see F. Kraus *Alie*

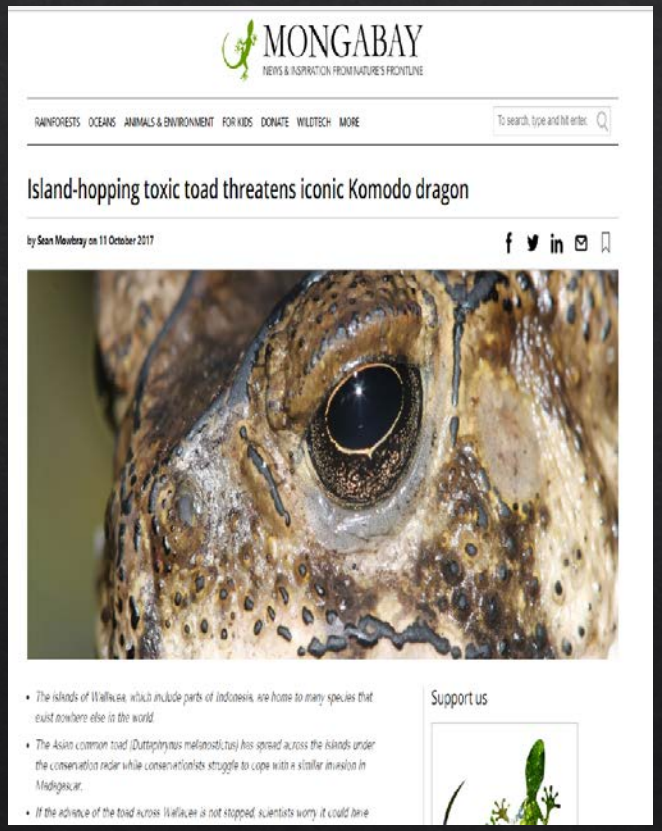
## Scientists warn of last chance to rid Madagascar of invasive toxic toad

**New report says there is a 'diminishing window of opportunity' to completely eradicate the Asian toad, which poses a threat to biodiversity, human health and the economy**

▲ The Asian toad (*Duttaphrynus melanostictus*) is a toxic and invasive species recently arrived in Madagascar. Experts fear ecological impacts similar or worse to those wrought by the cane toad in Australia. Photograph: James Reardon/ASA

The Guardian, 2016

# Wallacean islands Toad Invasion 2016


MONGABAY

NEWS & INSPIRATION FROM NATURE'S FRONTLINE

RAINFORESTS OCEANS ANIMALS & ENVIRONMENT FOR KIDS DONATE WILDTECH MORE

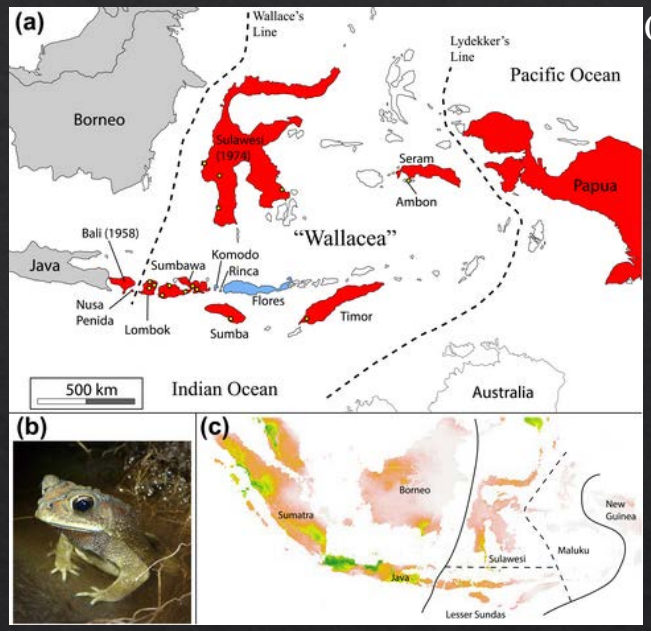
To search, type and hit enter

Island-hopping toxic toad threatens iconic Komodo dragon

by Sean Mowbray on 11 October 2017

- The islands of Wallacea, which include parts of Indonesia, are home to many species that exist nowhere else in the world.
- The Asian common toad (Duttaphrynus melanostictus) has spread across the islands under the conservation radar while conservationists struggle to cope with a similar invasion in Madagascar.
- If the advance of the toad across Wallacea is not stopped, scientists worry it could have

Support us





a) Map of insular Southeast Asia depicting the range of Duttaphrynus melanostictus. Gray-shaded islands represent native range of D. melanostictus, red-shaded islands represent known introduced areas (with the year of introduction for some), and blue-shaded islands represent the range of Varanus komodoensis. Yellow dots represent sampling localities for genetic analysis.

(b) A *D. melanostictus* from Lombok Island.

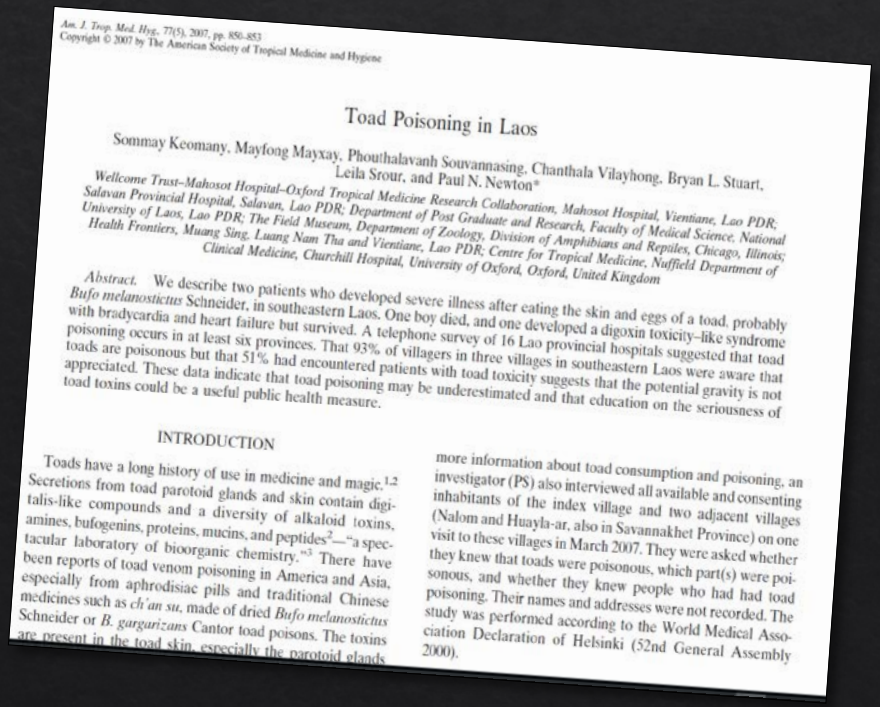
c) Range of spatial niche model from Reilly et al., (2017)

(d) A *V. komodoensis* from Komodo Island

# Fatality cases on human being

- A dramatic case of fatality was reported in Laos this species in 2006 (Keomany et al., 2007).

- The Asian Black spined Toad caused fatality to human being once again in Timor Leste in 2009 (Trainor, 2009).



Am. J. Trop. Med. Hyg., 77(5), 2007, pp. 850–853


Toad Poisoning in Laos

Sommay Keomany, Mayfong Mayxay, Phouthalavanh Souvannasing, Chanthala Vilayhong, Bryan L. Stuart, Leila Strour, and Paul N. Newton*

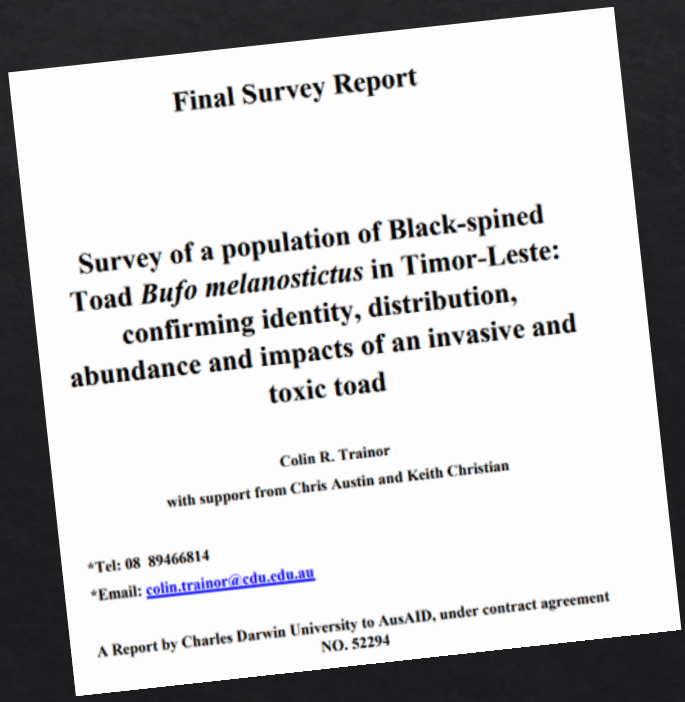
*Wellcome Trust–Mahosot Hospital–Oxford Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Lao PDR; Salavan Provincial Hospital, Salavan, Lao PDR; Department of Post Graduate and Research, Faculty of Medical Science, National University of Laos, Lao PDR; The Field Museum, Department of Zoology, Division of Amphibians and Reptiles, Chicago, Illinois; Health Frontiers, Muang Sing, Luang Nam Tha and Vientiane, Lao PDR; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Churchill Hospital, University of Oxford, Oxford, United Kingdom*

*Abstract.* We describe two patients who developed severe illness after eating the skin and eggs of a toad, probably *Bufo melanostictus* Schneider, in southeastern Laos. One boy died, and one developed a digoxin toxicity–like syndrome with bradycardia and heart failure but survived. A telephone survey of 16 Lao provincial hospitals suggested that toad poisoning occurs in at least six provinces. That 93% of villagers in three villages in southeastern Laos were aware that toads are poisonous but that 51% had encountered patients with toad toxicity suggests that the potential gravity is not appreciated. These data indicate that toad poisoning may be underestimated and that education on the seriousness of toad toxins could be a useful public health measure.

INTRODUCTION

Toads have a long history of use in medicine and magic.[1,2] Secretions from toad parotoid glands and skin contain digitalis-like compounds and a diversity of alkaloid toxins, amines, bufogenins, proteins, mucins, and peptides[2]—"a spectacular laboratory of bioorganic chemistry."[3] There have been reports of toad venom poisoning in America and Asia, especially from aphrodisiac pills and traditional Chinese medicines such as *ch'an su*, made of dried *Bufo melanostictus* Schneider or *B. gargarizans* Cantor toad poisons. The toxins are present in the toad skin, especially the parotoid glands

more information about toad consumption and poisoning, an investigator (PS) also interviewed all available and consenting inhabitants of the index village and two adjacent villages (Nalom and Huayla-ar, also in Savannakhet Province) on one visit to these villages in March 2007. They were asked whether they knew that toads were poisonous, which part(s) were poisonous, and whether they knew people who had had toad poisoning. Their names and addresses were not recorded. The study was performed according to the World Medical Association Declaration of Helsinki (52nd General Assembly 2000).



**Final Survey Report**

**Survey of a population of Black-spined Toad *Bufo melanostictus* in Timor-Leste: confirming identity, distribution, abundance and impacts of an invasive and toxic toad**

**Colin R. Trainor**

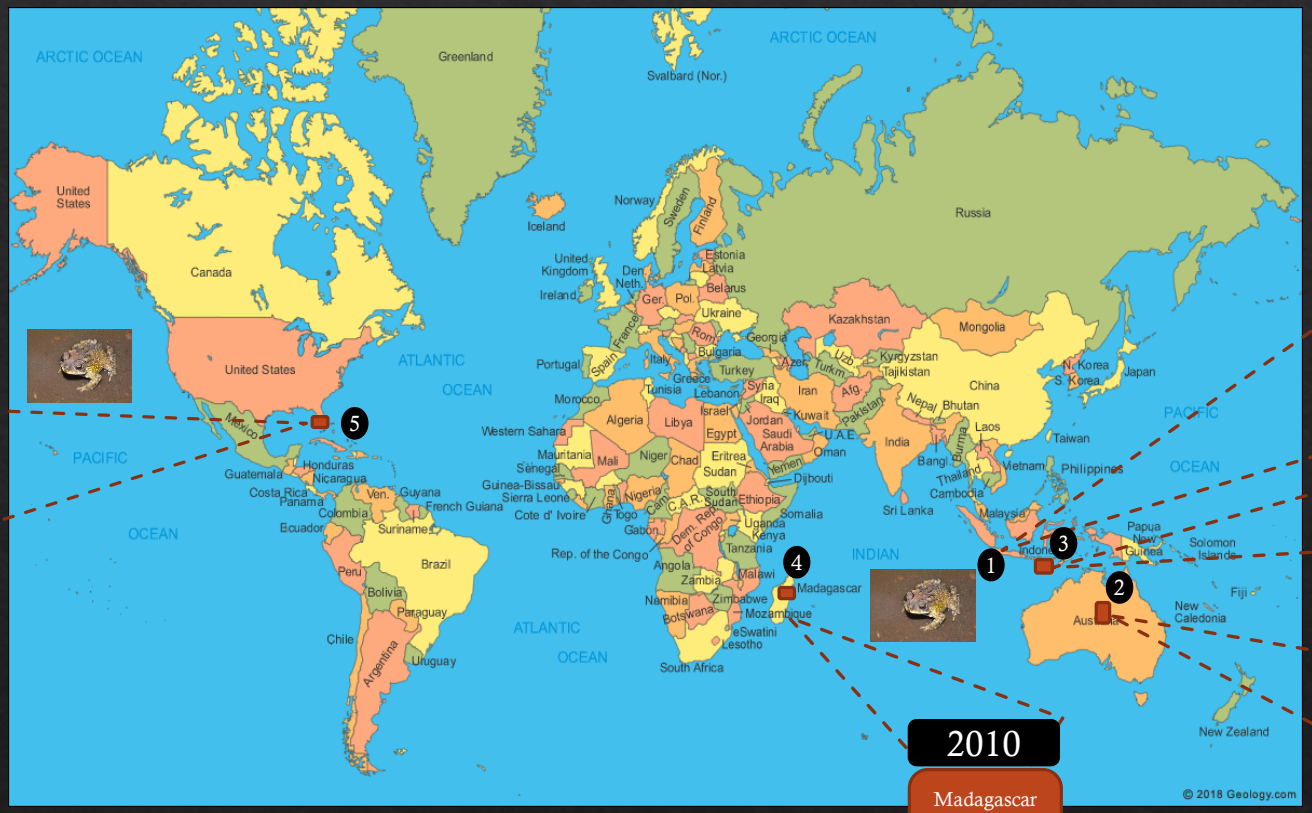
**with support from Chris Austin and Keith Christian**

***Tel: 08 89466814**

***Email: colin.trainor@cdu.edu.au**

**A Report by Charles Darwin University to AusAID, under contract agreement NO. 52294**

# HISTORY OF GLOBAL INVASION OF ASIAN BLACK-SPINED TOAD




**2012**
Florida, USA
Krysko et al, (2012)

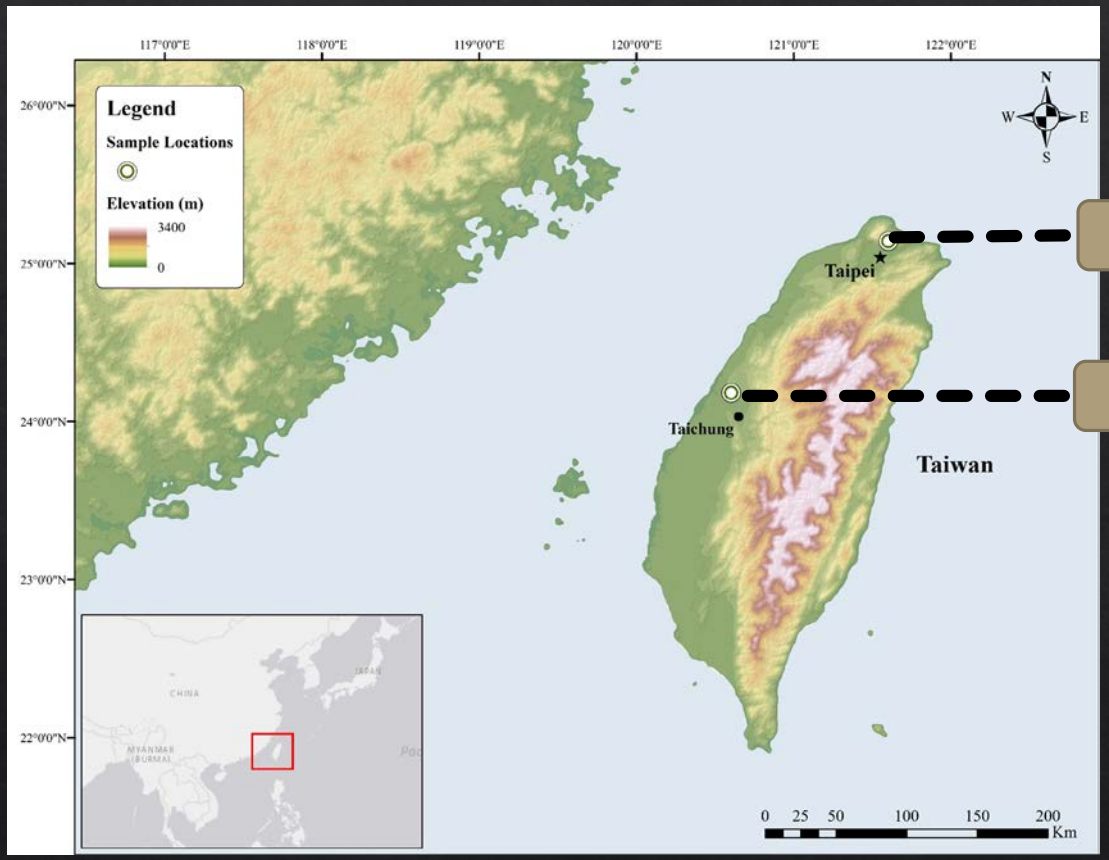
**1958**
Bali Church, (1960)

**2009**
Timor Leste
(Trainor, 2009)

**1999**
Australia
Mo et al, (2017)

**2010**
Madagascar
Moore et al, (2014)

# Sampling sites




19 individuals

3 individuals

22 individuals of D. *melanotictus* in total;

- 19 from Northern Taiwan
- 3 from Central West Taiwan

## PART A: TRACING THE ORIGIN OF *Duttaphrynus melanostictus* IN TAIWAN

- QUESTIONS:

- *Duttaphrynus melanostictus* in Taiwan is coming from which ancestor? Chinese clade or South East Asian clade?
- What is the Dispersal mechanism and pathway of *D. melanostictus* in Taiwan? Human-induced dispersal or natural dispersal?
- Is *D. melanostictus* invasive in Taiwan?

# MAJOR PORTS IN SOUTHEAST AND EAST ASIA

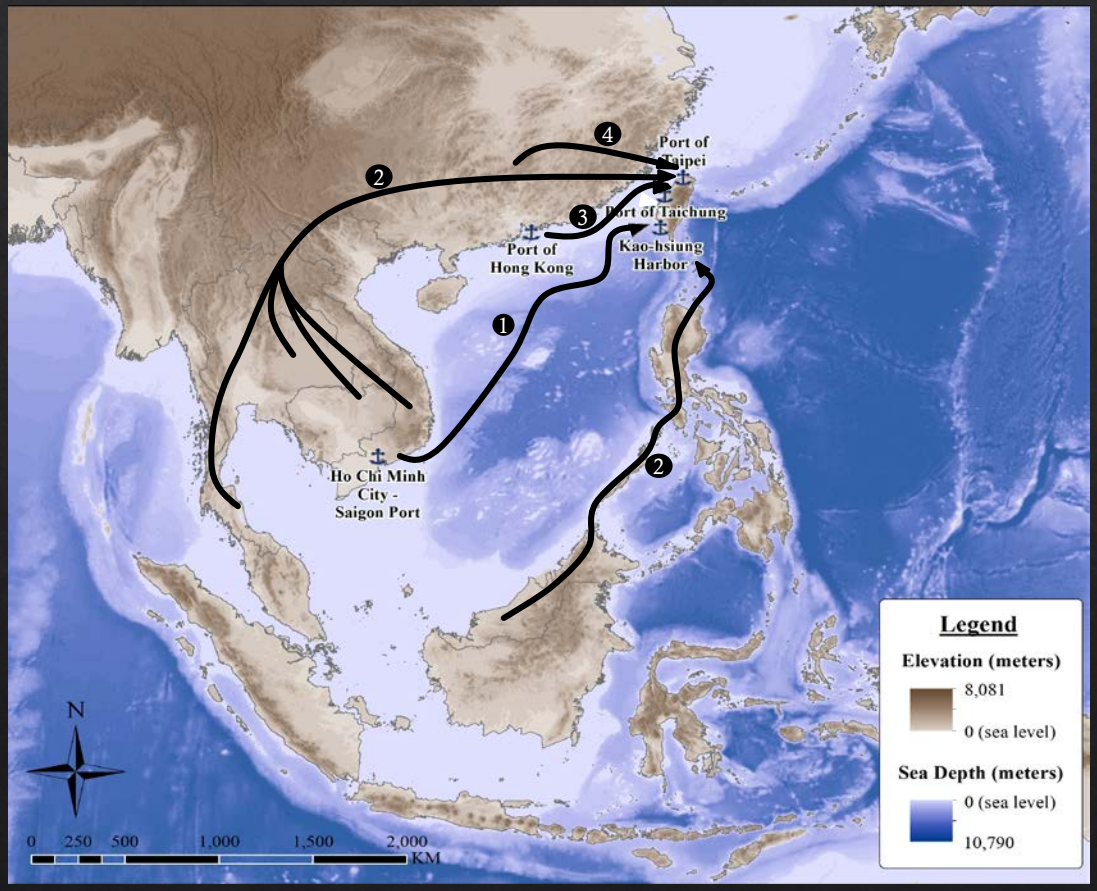

Hong Kong Port

Port of Kaohsiung, Taiwan


Saigon Port, Ho Chi Minh City of Vietnam

Taichung Port, Taiwan

# 4 major dispersal hypotheses of *D melanostictus*




1) The species originates from South East Asia (SEA) and it is invasive in Taiwan as a result of human-induced dispersal,

2) the species originates from South East Asia, and dispersed over land bridges,

3) the species comes from the Chinese mainland through human-induced dispersal,

4) the species originates from the Chinese mainland, and dispersed over land bridges during glacial maxima.

# MATERIAL AND METHODS

Field sampling

PCR – Isolation of *tRNA Gly-ND3* mtDNA genes


Purification of *COIII-ND3* mtDNA genes

Sequencing: ABI Platform

Part A

Part B

Haplotype networks

Phylogenetics inferences;
- Bayesian approach

Isolation by Distance (IBD) - Mantel Test

# Bioinformatics analyses

## Geneanology

Geneious ver 11.0.4
Clustal W2

We aligned our 22 sequences of *COIII-ND3* with 202 sequences from Wogan et al. (2016)

## Haplotype networks computation

Maximum parsimony approach:

DNA SP ver 5.0
PopART ver 1.7

## Nucleotides substitution Model test

Partition Finder 2.0
J Modeltest 2.1

| Loci | gene | Exon position | Intron position |
|---|---|---|---|
| mtDNA | *COIII* | 1-51 | - |
| | *ND3* | 52-332 | |
| NuDNA | *SOX9* | | |
| | *POMC* | | |

| Model | Parameters | lnL | AICc | AIC | BIC |
|---|---|---|---|---|---|
| GTR+I | 9 | -1077.47 | 2173.5 | 2172.94 | 2207.19 |
| GTR+G | 9 | -1078.75 | 2176.06 | 2175.5 | 2209.75 |
| GTR+I+G | 10 | -1078.92 | 2178.53 | 2177.85 | 2215.9 |
| HKY+G | 5 | -1094.3 | 2198.78 | 2198.59 | 2217.62 |
| HKY+I | 5 | -1095.09 | 2200.36 | 2200.17 | 2219.2 |

Best fitted substitutional model for ND3 alignment is GTR + I

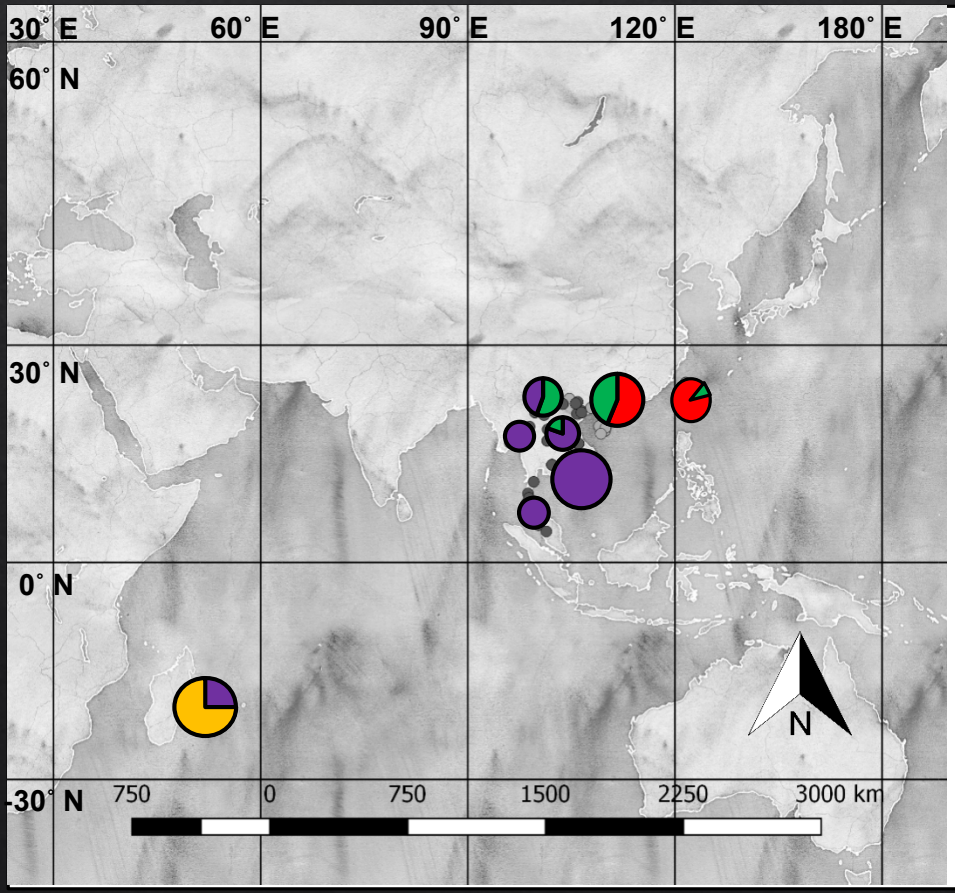
## Bayesian inference of phylogenetics

Mr. Bayes

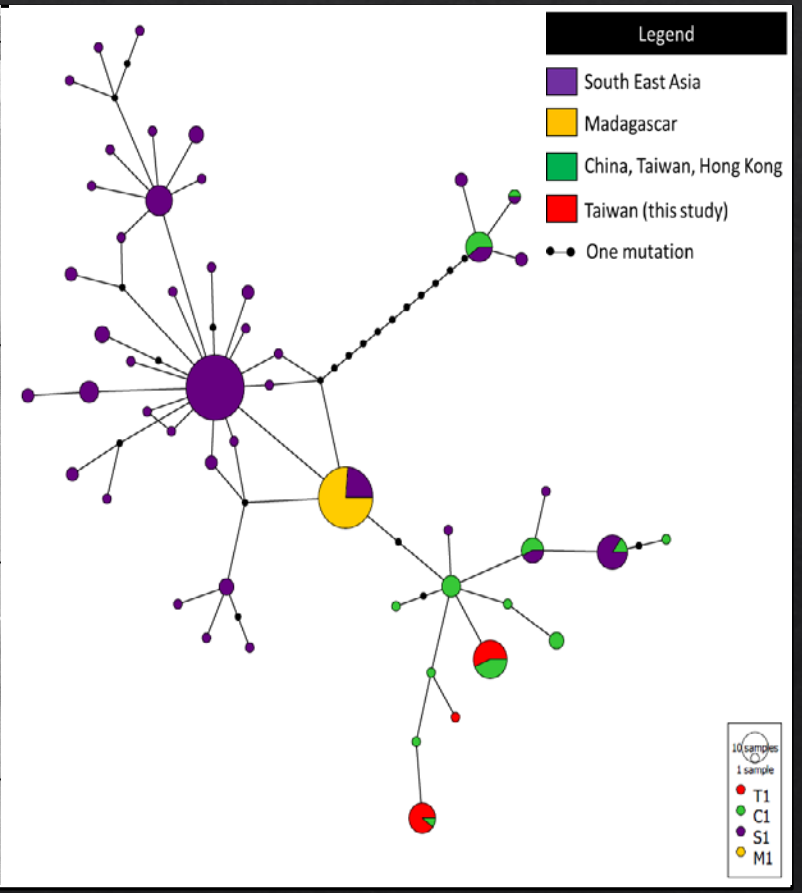
- Four chain ran for 10,000,000 generations using default priors.
- Tree sampled every 2,000 generations
- 25% of tree discarded as ‘burn in’.

# RESULTS AND DISCUSSIONS

## a) Haplotype networks

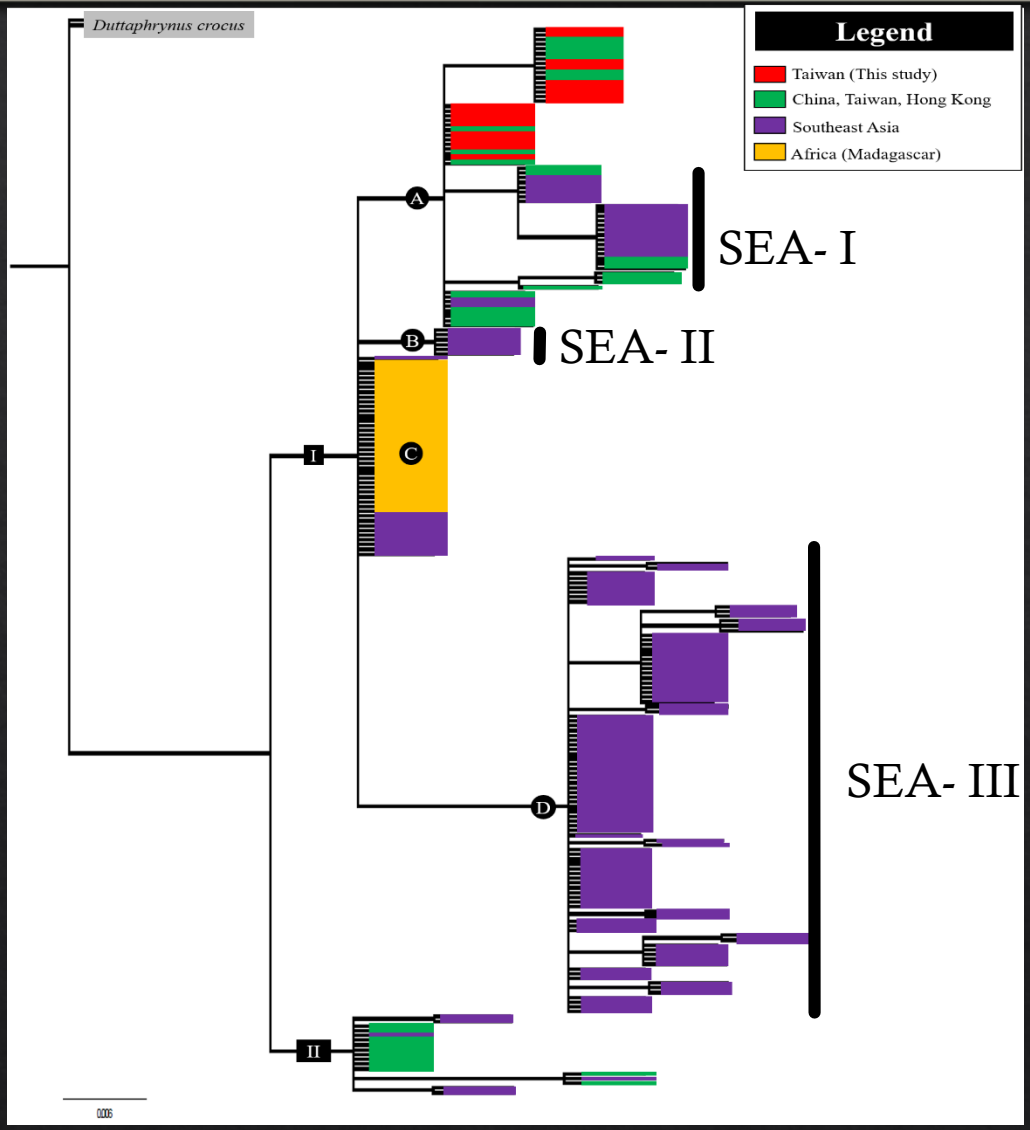



Haplotype networks based on localities



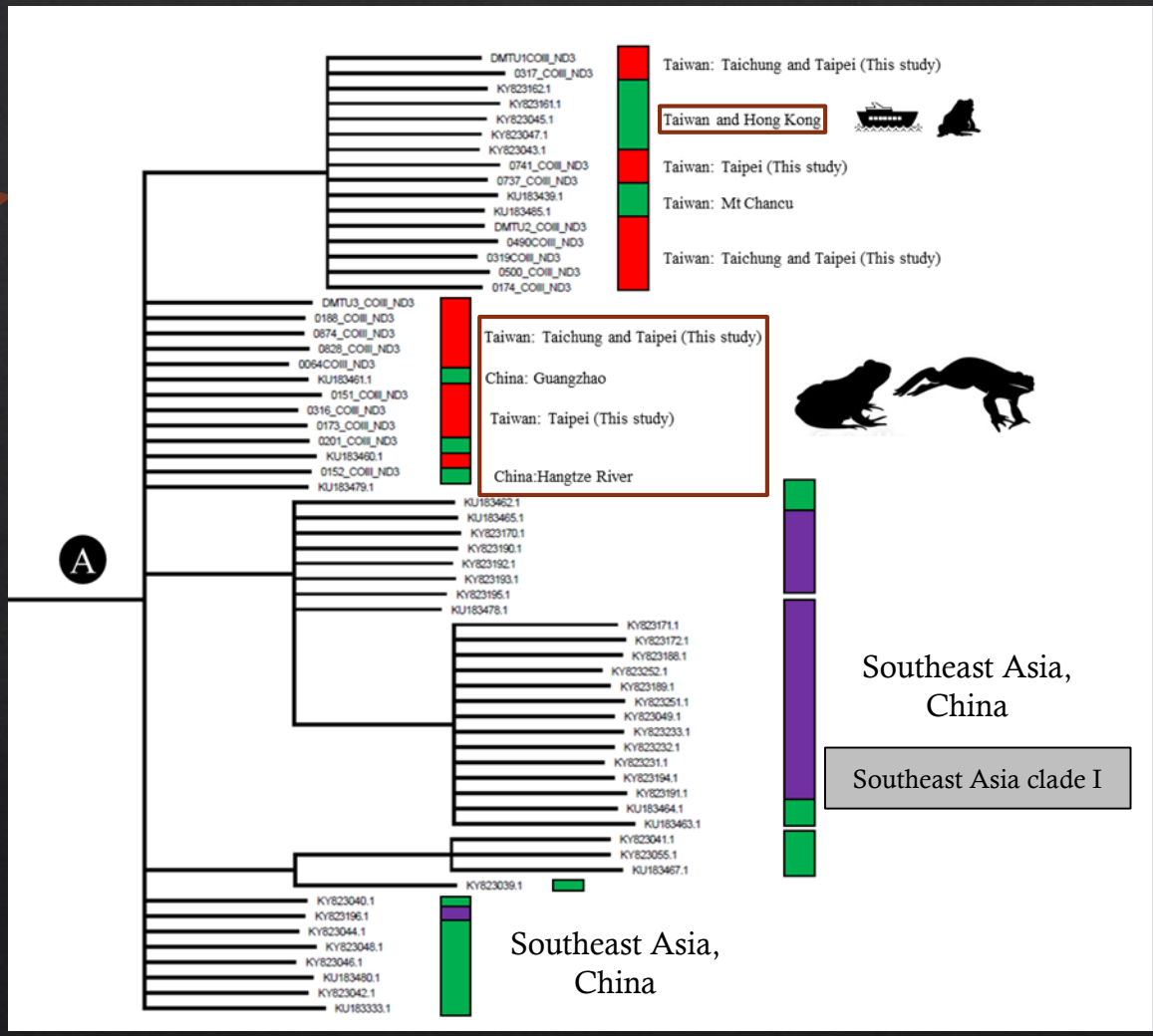

Median joining network of *tRNA Gly-ND3* mtDNA fragment from 221 individuals of *D,melanostictus.*

# PHYLOGENETIC ANALYSIS

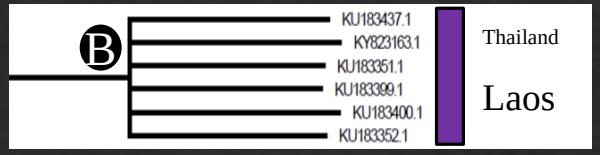


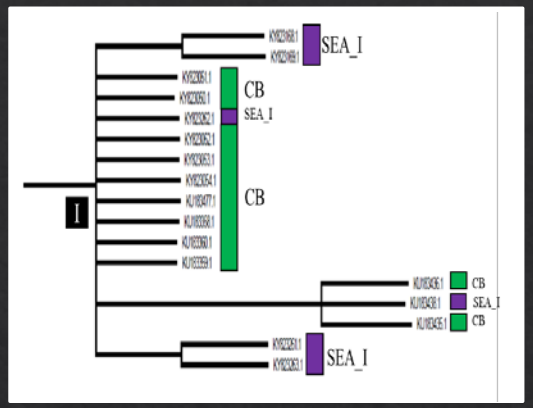

Bayesian tree inferred from *tRNA Gly-ND3* mtDNA fragments isolated from 224 individuals of *D. melanostictus*

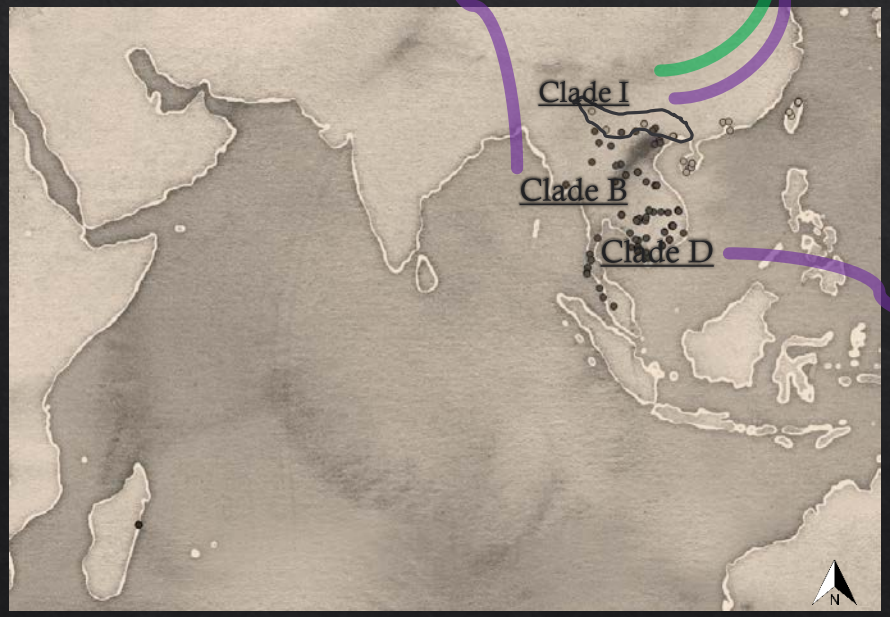
# Natural and unnatural dispersal of *D. melanostictus* from China and Hong Kong to Taiwan

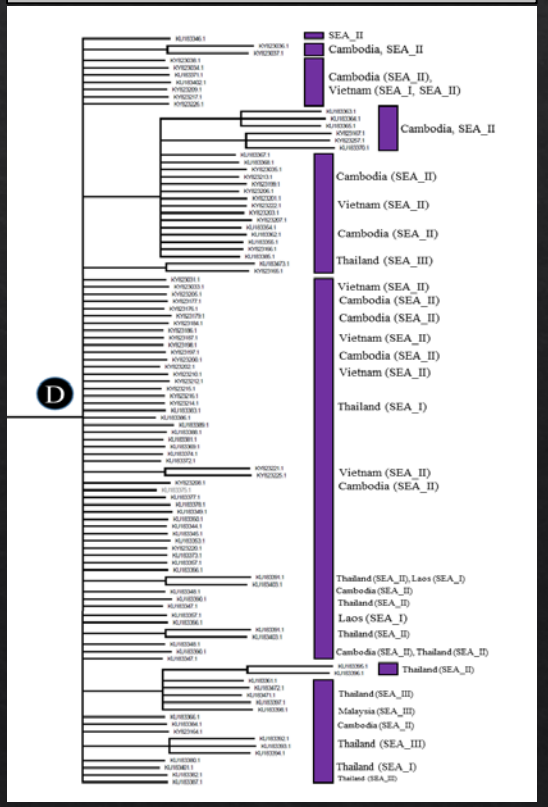
## Southeast Asia clade II



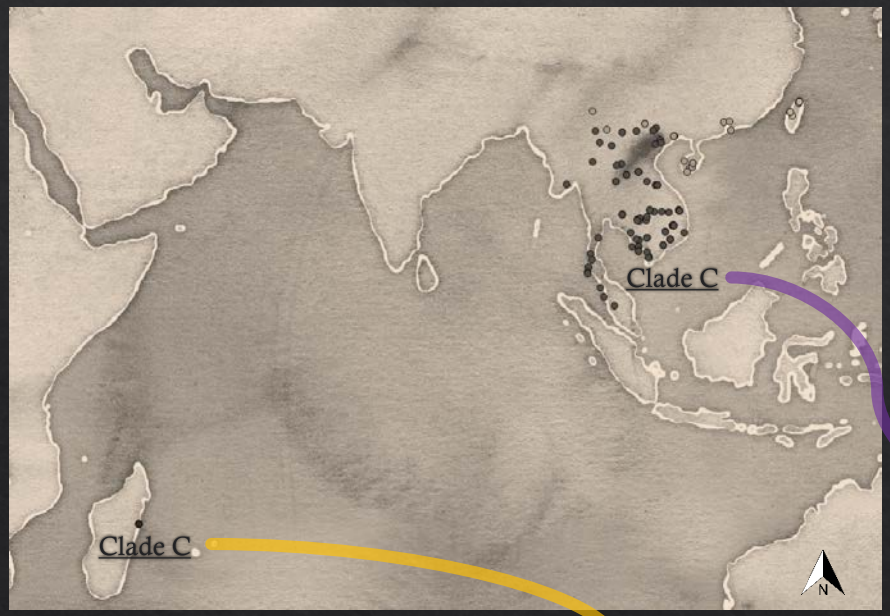



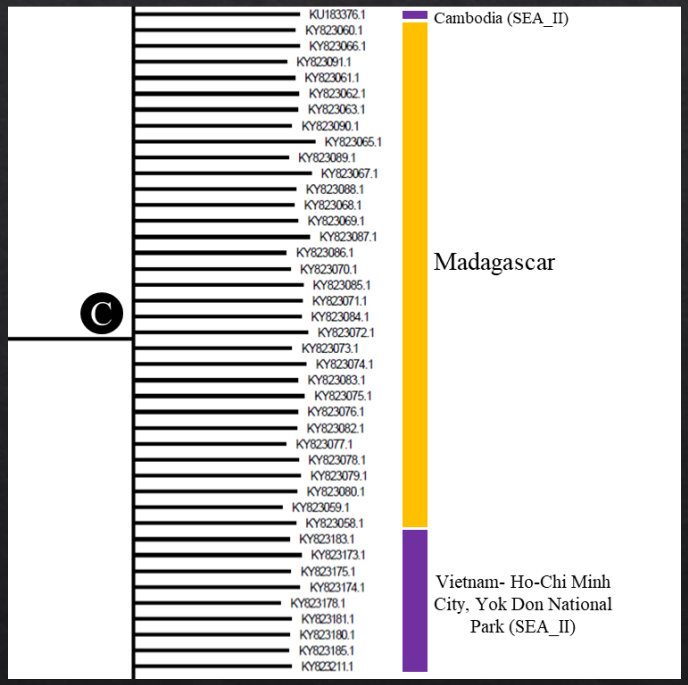




## Southeastasia clade III



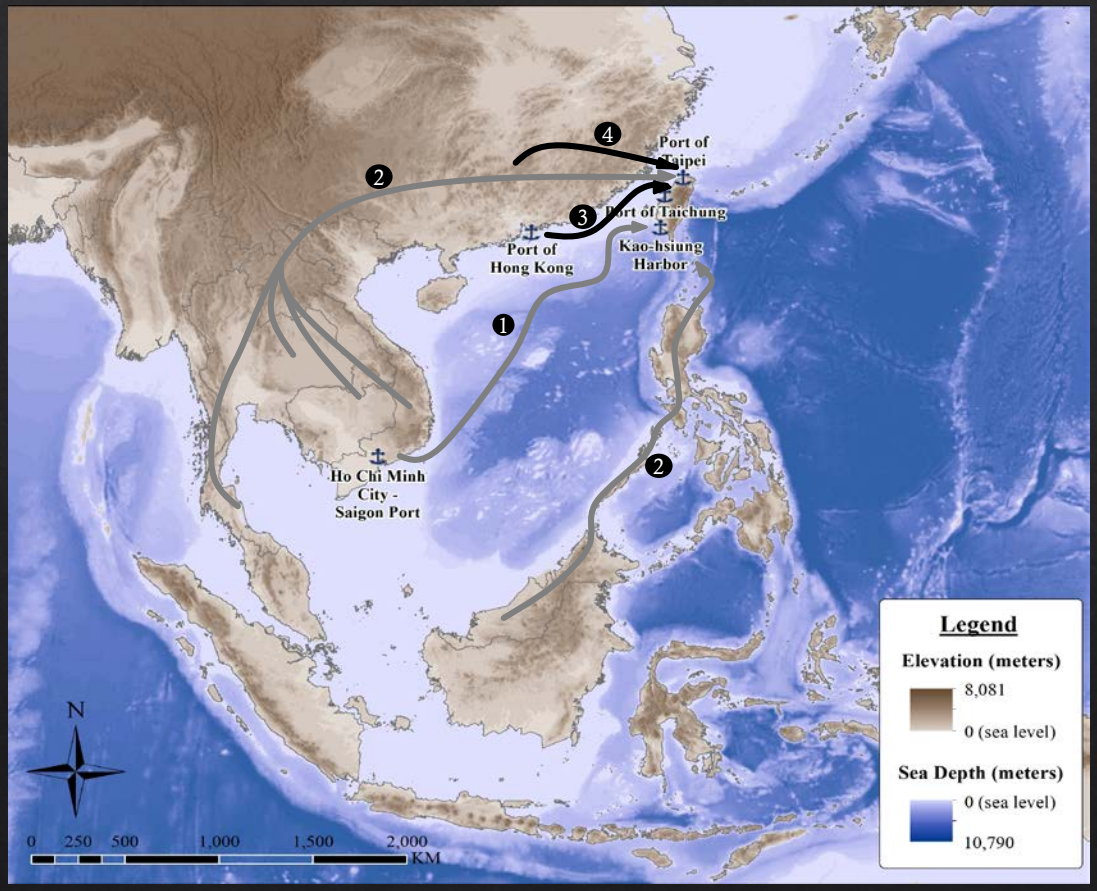
# Clade C




Africa (Madagascar) *D. melanostictus* invasion begins from a single population in Southern Vietnam and Cambodia population (Moore, 2014)

# Natural and unnatural dispersal mechanisms




1) The species originates from South East Asia (SEA) and it is invasive in Taiwan as a result of human-induced dispersal,

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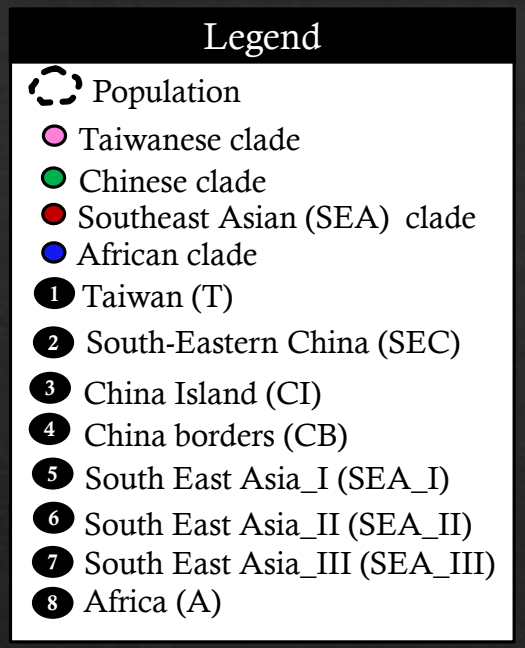
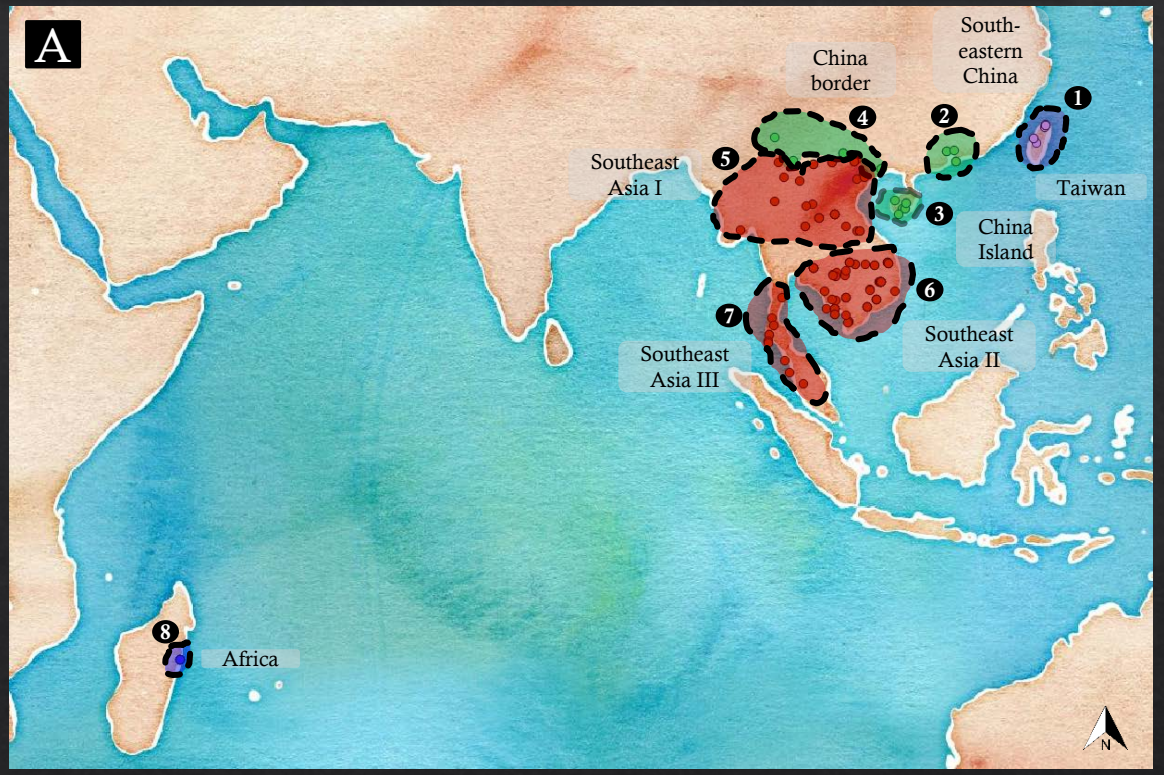
## PART B: TESTING ISOLATION BY DISTANCE (IBD)

QUESTIONS:

- Is there any influence of gene flow and Isolation by Distance (IBD) on genetic variability between 8 populations of *D. melanostictus*?
- Is geographical distance matters to the genetic structures of *D. melanostictus* populations?

# ISOLATION BY DISTANCE (IBD)



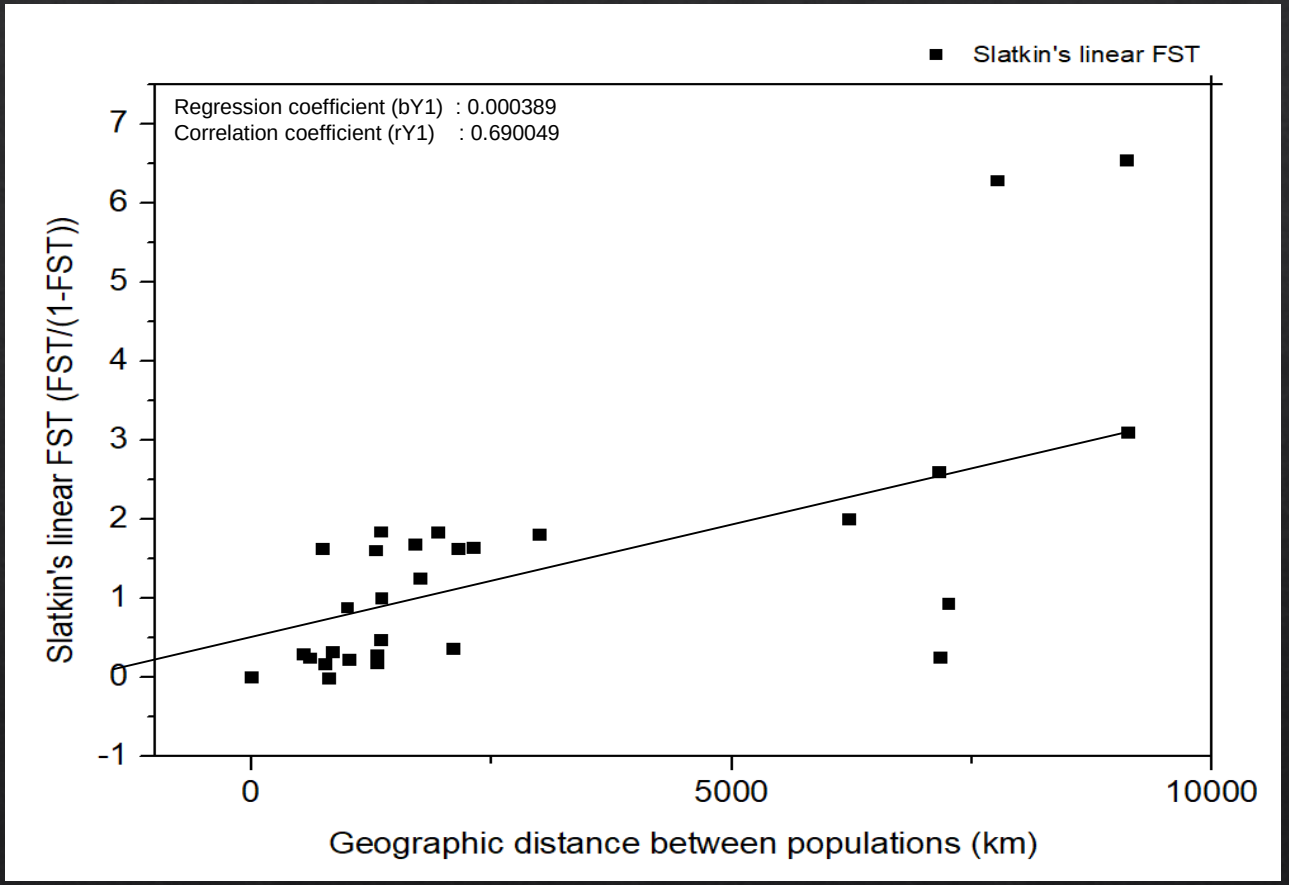




B

| | Taiwan | South-eastern China | China Island | China border | Southeast Asia-I | Southeast Asia-II | Southeast Asia-III | Africa |
|---|---|---|---|---|---|---|---|---|
| **Taiwan** | 0 | | | | | | | |
| **South-eastern China** | 807.46 | 0 | | | | | | |
| **China Island** | 1,346.71 | 601.81 | 0 | | | | | |
| **China border** | 2,145.94 | 1,353.06 | 995.67 | 0 | | | | |
| **Southeast Asia-I** | 2,093.73 | 1,298.79 | 767.60 | 537.00 | 0 | | | |
| **Southeast Asia-II** | 1,941.27 | 1,289.90 | 731.29 | 1,341.20 | 839.06 | 0 | | |
| **Southeast Asia-III** | 2,995.58 | 2,305.74 | 1703.72 | 1,751.58 | 1,309.24 | 1,014.40 | 0 | |
| **Africa** | 9,118.77 | 9,113.63 | 7,763.46 | 7,155.85 | 7,164.49 | 7,252.14 | 6218.86 | 0 |

The geographical distances (km) between 8 populations of *D. melanostictus*

# MANTEL TEST




P = 0.002

**p-value < 0.05**

Scatter plot of Slatkin's linear FST and Geographic distance. There is correlation between geographic distance and genetic variation for 8 populations of *D. melanostictus.* Nearby populations tend to be genetically similar than expected by chance and genetic distance increasing

# CONCLUSION

- Using Taiwan as case study, we trace human-mediated dispersal influence in the dispersal activity of *D. melanostictus.*
- Natural dispersal is still one of the main dispersal mechanisms of this species, only to prove how vigilant this nomadic toad.
- Under both scenarios, natural and human-induced dispersal, this toad invasiveness is hard to prevent.
- However, geographical distance **is still the main influence factor** for genetic variability of *D. melanostictus* in this study.
- We conclude the invasion status of this species in Taiwan as partial invasive.

# Future challenge: where is next…?


Asian Black-spined Toad (*D. melanostictus*), REPUBLIC OF KOREA: GYEONGGI: Gimpo (37.642314°N, 126.646957°E, WGS84; 3 m elev.)
6 October 2013.

## ACKNOWLEDGEMENT

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Korea Environmental Industry & Technology Institute

이화여자대학교 EWHA WOMANS UNIVERSITY


Frog team members of Laboratory of Animal Communication, EWHA

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3. S. Keomany *et al.*, "Toad poisoning in Laos," *Am. J. Trop. Med. Hyg.*, vol. 77, no. 5, pp. 850–853, 2007.

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