



Candoia paulsoni in a defensive posture

INTRODUCTION

Wallacea

Biodiversity is not evenly distributed throughout the world. The majority of vascular plant species and vertebrates can be found in just 25 biodiversity "hotspots" covering only 1.4% of the earth's land surface (Myers *et al.* 2000). Indonesia has 2 of these hotspots – Sundaland in the west and Wallacea in the center. The region of Wallacea is defined as the island of Sulawesi, the Moluccas, and the Lesser Sunda Islands. The uniqueness of this area derives from a complex geological history which permitted the mixture of Asian and Australian species to occur. Several authors such as A.R. Wallace, R. Lydekker and T.H. Huxley have described this unique interface (Fig. 1).

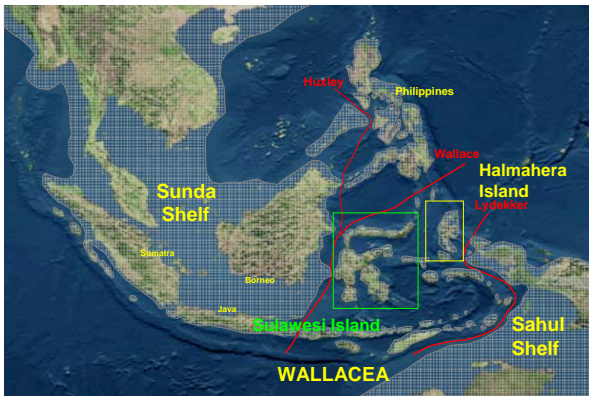


Fig.1 The study site location of Halmahera and Sulawesi Island, Indonesia. Also indicated are Wallace's, Huxley's, and Lydekker's Lines, which demarcate the interface of the Asian and Australian faunal zones.

Wallacea, Halmahera and Sulawesi Island are the largest islands of Wallacea. Existing records show that there are between 6 to 11 species of amphibians in Halmahera, whereas Sulawesi has 26-32 species. However, due to the paucity of field work on this island, it has likely that these islands are more diverse than this (van Kampen, 1923; Monk *et al.* 1997; Whitten *et al.* 2006; Frost, 2006; Global Amphibian Assessment, 2006).

Objective

The objective of this research is to identify areas of genetic endemism (AGEs) in the biodiversity hotspot of Wallacea, with a focus on Halmahera and Sulawesi islands. We will test for the presence of AGEs using molecular data from multiple amphibian and reptile groups on Halmahera & Sulawesi.

Hypotheses

Halmahera was formed by the collision of two paleoislands (Hall 1996, 1999, Monk *et al.* 1997), that now comprise the Western and Eastern portions (Fig 2). Sulawesi was also formed from multiple paleoislands, and parts of them were periodically submerged by sea (Hall 1996, 1999; Whiten *et al.* 2002). We aim to test how and whether this geological history impacted the distribution of diversity on these island.

Our null hypothesis for each species is that of panmixia (random mating). Alternatively another demographic model, such as isolation by distance (IBD) or fragmentation (F) might better account for the distribution of genetic variation on this island. A second question asks whether the same demographic models (IBD or F) can be applied across multiple groups on Halmahera and Sulawesi.

METHODS

Genetic Samples. We sampled along transects extending through all peninsulas of Halmahera & Sulawesi island. Field collection were performed at night by walking up rivers and searching for amphibians and reptiles with flashlights. Sampling locations depended to some degree on accessibility. On each locality we spent about three collection nights for genetic as well as whole specimens. Here we report results of our analysis of eight co-distributed species (Fig. 2) in Halmahera and result of one genus from Sulawesi (Fig.3).
Molecular Data (Barcoding). As a first step, we have generated a DNA barcode (www.barcodinglife.org) for all of our samples: 160 samples from 8 species (Halmahera), and 420 samples of *Limnocyathus* (Sulawesi). DNA barcodes are based on about 650 base pairs of DNA sequence of the mitochondrial cytochrome c oxidase I gene. In addition to barcoding, we have also collected sequences from the nuclear gene Rag 1 for 100 *Limnocyathus* samples from Sulawesi.
Phylogenetic analysis. Genealogies were estimated using Bayesian statistics. We also used statistical parsimony to construct networks within each species.
Partial correlation tests. We used a partial correlation test to test the null hypothesis of no correlation between Halmahera paleoislands and genetic distance, while holding the effect of geographic distance constant.
Species concept. We employed a genealogical species concept, where different species are defined as an evolutionary lineage with divergent clades of mtDNA and nDNA.

RESULTS

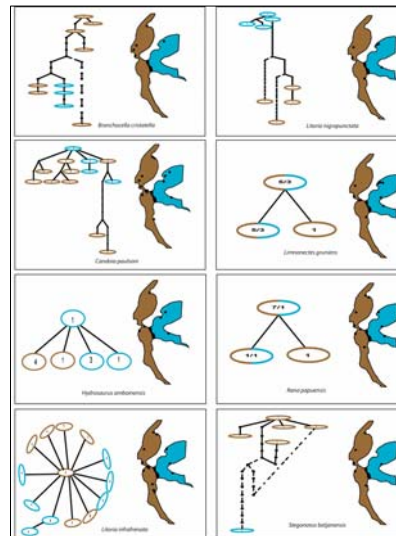


Figure 2. Network of eight amphibian and reptile taxa in Halmahera. The colors blue and brown represent the division of Halmahera based on geological origins. The numbers inside the network ellipses represent the number of individuals for that haplotype.

Four out of eight of the Halmahera species show an individually significant partial correlation between genetic distance and area of endemism after holding the effect of distance constant. Two of these (*Litoria nigropunctata* and *L. infrafrenata*) are also significant after correcting for multiple tests. The P values of the partial correlation tests for *Bronchocella cristatella*, *Candoia paulsoni*, *Hydrosaurus amboinensis*, *Litoria infrafrenata*, *L. nigropunctata*, *Limnocyathus gruniens*, *Rana pappuensis*, and *Stegonotus batjanensis* are 0.1615666, 0.010965, 0.078784, 0.004701, 0.00145, 0.34464, 0.38275, and 0.01944 respectively. However networks of the mtDNA sequences illustrate that in some species, molecular variation is shared across the two paleoislands (Fig. 2) contrary to the fragmentation hypothesis.

In contrast on Sulawesi, our results identified 10 species whose mtDNA haplotypes are mostly

congruent with paleoisland boundaries (Fig.3). This pattern is echoed in other endemic Sulawesi species such as macaque monkeys, toads, flying lizards and carpenter bees (McGuire, 2002; Whitten *et al.*, 2002; Evans *et al.*, 2003)

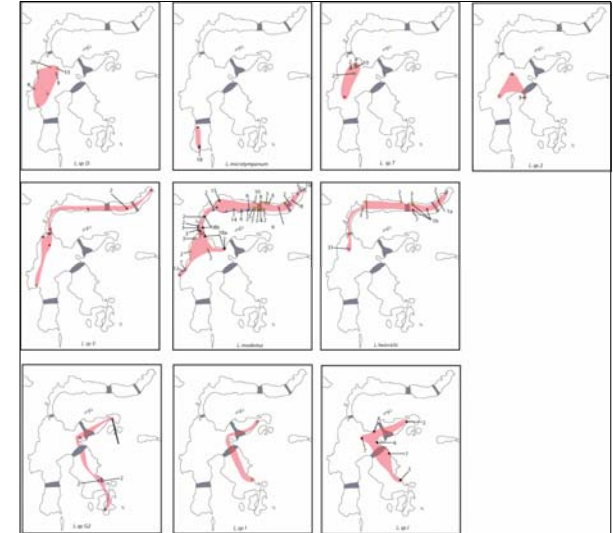


Figure 3. Distribution of 10 *Limnocyathus* haplotypes in the island of Sulawesi. Gray lines are boundaries of paleoislands. The numbers on each point refers to the number of specimens collected on each site. Different colors represent different haplotypes.

CONCLUSIONS

Although four out of eight of the groups in Halmahera have an individual significant partial correlation test of paleoislands and genetic distance, only two of them are significant after correction for multiple tests. Based on these results, we conclude that there is not strong support for a demographic hypothesis of multi-taxon fragmentation in Halmahera. However on Sulawesi, our results supports a hypothesis of fragmentation, and indicate that there are 6 additional cryptic species in the *Limnocyathus* group.

An implication of these findings for biodiversity conservation is that steps should be taken to ensure representation of conservation areas to best preserve historical patterns of gene flow among Halmahera & Sulawesi. We aim to expand these analyses with further sampling and with additional data from nuclear genes.

REFERENCES

- Evans BJ, Supriatna J, Andayani N, Setiadi MI, Cannatella D, Melnick DJ (2003) Monkeys and toads define areas of endemism on the island of Sulawesi.
 Hajibabaei M, deWaard JR, Ivanova NV *et al.* (2005) Critical factors for the high volume assembly of DNA barcodes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360, 1959–1967.
 Hall, R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. in: Hall, R. & J.D. Holloway (eds.), 1998. *Biogeography and Geological Evolution of SE Asia*. Backhuys Publishers, Leiden.
 Global Amphibian Assessment. www.globalamphibians.org/index, accessed February 2006
 Monk, K.A., Y. de Fretes & G. Reksodihardjo-Lilley. 1997. *The ecology of Nusa Tenggara & Maluku*. Periplus, Hongkong.
 McGuire, J. A. 2002. Bayesian phylogenetics and biogeography of the *Dracolineatus* group. P. 212 in Program book and abstracts of the joint meeting of ichthyologists and herpetologists. 3–8 July, Kansas City, KS.
 Myers, N. *et al.* (2000). Biodiversity hotspots for Conservation priorities. *Nature* 403, 853-858.
 van Kampen, P.N., 1923. *The Amphibians of the Indo-Australian Archipelago*. E. J. Brill Ltd. Leiden
 Whitten, A.J., M. Mustiata, & G. S. Henderson. 2002. *The ecology of Sulawesi*. Periplus, Hongkong.