

TESTING BIOGEOGRAPHIC PREDICTIONS DERIVED FROM QUATERNARY ISLAND
CONNECTIVITY USING GENOMIC DATA FROM MAMMALS IN THE SOUTHERN
PHILIPPINES

BY

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Abstract

Island archipelagos have long piqued the attention of evolutionary biologists interested in biogeographical patterns implying processes of diversification in terrestrial vertebrates. One model island archipelago, the Philippines, has emerged as an ideal geographical theater for testing hypotheses related to a 30 year biogeographical paradigm, derived from climatic oscillations of the Pleistocene and species pump expectations. To test predictions from the Pleistocene Aggregate Island Complex (PAIC) Paradigm, we generated the first ever Rad-seq datasets of thousands of genomewide loci from forest mammals restricted to the southern portions of the archipelago. We used genomic population-level statistics and demographic model selection techniques to test assumptions of the PAIC Paradigm and predicted differences among focal taxa based on life history and ecological preferences. Predictably, our analyses revealed pronounced differences in historical population demographics of forest rodents and understory bats in terms of geographical structure of populations and gene flow among populations, suggesting taxon-specific response to climatic and environmental variation across recent evolutionary timescales. In contrast to previous inferences derived from single-locus phylogeography of the same populations, our new genomic data warn against uncritical assumptions of highly divergent, geographically structured island populations. They also suggest that individual, species-specific, idiosyncratic response of small forest mammal lineages to the geographical and environmental template may become more prevalent in structured island systems, in strong contrast to the popularly hypothesized shared mechanisms of diversification that have been so prevalent over the last century.

Acknowledgements

I would first like to thank my undergraduate advisor David Reed. He personally trained me in laboratory and field collecting techniques and provided me all the tools and opportunities to succeed in graduate school. From the start, he treated me as colleague even though I was an undergraduate researcher. Dr. Reed is an exceptional mentor and the reason I was prepared to continue my education.

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TABLE OF CONTENTS

CHAPTER 1. MINDANAO PAIC PHYLOGEOGRAPHY OF <i>HAPLONYCTERIS</i> AND <i>BULLIMUS</i> ...	1
Introduction	1
PAIC Model of Diversification	1
Hypotheses of the within-PAIC model & their predictions	2
Limitations	3
What we still don't know	4
Model system	4
Materials and Methods	6
Genomic Library Preparation	6
Read and Parsing Assembly	7
Phylogenetic Analyses	8
Population Genomic Analyses	8
Demography Analyses	9
Results	11
Genomic Library Preparation & Read and Parsing Assembly	11
Phylogenetic Analyses	11
Population Genomic Analyses	15
Demography Analyses	17
Discussion	18
REFERENCES CITED	22

CHAPTER 1
MINDANAO PAIC PHYLOGEOGRAPHY OF *HAPLONYCTERIS* AND *BULLIMUS*

INTRODUCTION

PAIC model of diversification.

Southeast Asia has been dramatically impacted over the past few million years by glacial cycles that have shifted sea levels resulting in major fluctuations in emergent land area and connectivity (Haq et al., 1988; Rohling et al., 1998; John et al., 2001; Siddall et al., 2003). Because geographic isolation is an important contributing factor in population divergence and, potentially, speciation (via vicariance and/or dispersal over a barrier, colonization, and eventual isolation and divergence), the ‘species pump’ action of repeated island isolation-connectivity cycles have been hypothesized to be a mechanism of diversification, generating biodiversity and endemism throughout Southeast Asia and other island systems around the world (Heaney 1985).

This climate-driven, sea-level oscillation framework has been widely promoted as a driver of land vertebrate diversity based on patterns of the distribution and partitioning of terrestrial biodiversity (primarily species distributions) within and among island groups (Heaney 1985; Peterson et al., 2005; Papadopoulou and Knowles, 2015, Papadopoulou and Knowles, 2015, but see Oaks et al., 2013). Landmasses that became connected during multiple periods of lower sea levels, the so-called “Pleistocene Aggregate Island Complexes” (PAICS; Brown and Diesmos 2009) have been celebrated as the major centers of Philippine vertebrate biodiversity (Heaney 1985; Heaney and Regalado 1998; Brown and Diesmos 2009).

Two PAIC-specific patterns inspired the present study. First, the well-characterized Philippine PAICs (e.g., the Luzon PAIC; (Brown et al. 2013b) are often noted for high levels of

endemism taxonomic endemism (globally unique species-, and/or genus-level faunal elements), and these former landmass amalgamations often possess communities that are highly distinct from other similar, nearby PAICs. Furthermore, in the few cases where genetic studies involving PAIC level sampling (Peterson et al. 2005; citations), taxonomic endemism and shared genetic variation can be higher within PAICs than islands that were not conjoined during Pleistocene glacial maxima / reduced global sea levels (citations). Second, a variety of population genetic and phylogeographic studies have demonstrated how species tend to be genetically structured by the current island boundaries (Esselstyn et al., 2009; Siler et al., 2010). This tendency in some systems towards more finely partitioned terrestrial biodiversity suggests that current water barriers (channels between land-bridge islands), although historically intermittent, may be a more predominant wholesale isolating mechanisms than deep-water barriers between PAICs.

Hypotheses of the within PAIC model & their predictions.

Although simple, the PAIC diversification model holds great exploratory potential because it offers a number of simple hypotheses with testable predictions (Brown and Diesmos, 2009; Esselstyn et al., 2009; Brown et al., 2013a). Support for the fission/fusion action dynamics of PAIC formation and emphasis on the importance of the phenomenon for explaining diversification within Philippine faunal regions, however, are currently mixed (Oaks et al., 2013; Brown et al., 2013a). Whether or not diversification is maintained during times of land-positive island connectivity and, to what degree is this maintain held is still unresolved. Additionally, (Hosner et al., 2014; and Papadopoulou and Knowles, 2015) have indicated that these two scenarios are not mutually exclusive for all taxa, calling into question the universality of the shared mechanism for diversification (Brown et al. 2013a). More recent studies have used

considerations of ecology and paleodistribution modeling of habitats to demonstrate that even with land bridge exposure between islands, isolation-limited (i.e., habitat unsuitability), dispersal-limited (i.e., other dispersal barriers) or even life history traits could all impede gene flow in an idiosyncratic fashion, specific to some taxa, but not necessarily involving all members of an island land vertebrate community (Oaks et al., 2013; Hosner et al., 2014; Papadopoulou and Knowles, 2015; Brown 2016).

Limitations.

Explicit testing of the PAIC model are limited for several reasons (review: Brown et al., 2013a). Although, these studies show support for divergences among PAIC (Peterson et al., 2005; Brown and Esselstyn 2009; Siler et al., 2010, 2011), additional within-PAIC patterns emerged, some of which conflicted with simple predicted PAIC-structured geography (Heaney 1985). Many of the studies to date have used single or only a few genes to evaluate the PAIC Paradigm common mechanism of diversification; some have used coalescence-based simulation to predict the effect of drift backwards through time (see Oaks et al., 2013 for discussion). However, such an approach and/or limited single locus datasets can produce biased or incorrect interpretations because of incomplete lineage sorting -- deep coalescence, lineage sorting, gene tree vs. species tree (Siler et al., 2010; Alexander et al., 2016; Brown et al., 2016), loci under selection, secondary contact. Additionally, coalescence-based models assume no gene flow and no population structure, among other limitations (citations). To test whether these oscillations in geographic connectivity through time have an effect on genetic diversity, it would be advantageous to include tests that could account for gene flow between populations. (Esselstyn and Brown 2009; Siler et. al., 2010; Brown et al., 2013a).

What do we still not know?

In this study we sought resolution of more recent demographic histories of populations within PAICs through a genomic approach. Recent developments in sequencing technology, allow genome wide polymorphism data to be collected with the advent of Next Generation Sequencing Technology (NGS) (Emerson et al., 2010; Peterson et al., 2012). Unlike, previous tests of PAIC model predictions, which used only one or few loci, we are able to test alternative models of diversification with genomic data and thousands of loci, in an attempt to test model predictions at shallow, population-level divergences, involving recent demographic history and finely-partitioned genetic variation.

Model system.

Island archipelagos have played a fundamental role towards understanding the importance of geographic isolation in driving population divergence (Lomolino et al., 2016; Brown, 2016). The Philippine Islands, since the Pleistocene, have undergone many submersions and emersions making them a well-suited system to study the effects of geological and climatic changes on species divergences (Delacour and Mayr, 1946; Dickerson, 1928; Heaney, 1985; Inger, 1954; Kloss, 1929; Simpson, 1977; Voris, 2000). During times of positive land exposure, sea levels fell 100–140 m from current day shorelines (Rohling et al., 1998) thus grouping present day islands into five PAICs. These cyclical events have been linked to the high levels of biodiversity found in the Philippines, making it one the most diverse archipelagos, and globally significant biodiversity conservation hotspot (Brown and Diesmos, 2009). Of the native terrestrial mammals, 29% of the genera and 64% of the species are island endemics, or restricted

to single PAICs, implying *in situ* diversification (Heaney et al., 1998; Heaney & Rickart, 1990; Heaney, 2000; Stepan et al., 2003).

Here, we examine and test population level demographic predictions of *in situ* diversification in two endemic mammals from the southern portions of the archipelago (the Mindanao PAIC). We selected a bat (*Haplonycteris fischeri*) and a forest rodent (*Bullimus bagobus*) because of their variable life history and predicted differences in relative dispersal abilities. Both of the species inhabit mostly primary rain forest habitat (Heaney et al., 2010). *Haplonycteris fischeri*, the sole member of a monotypic genus, has been documented to occur widely distributed throughout the archipelago, on all five PAICs (citation). In contrast *B. bagobus*, one of only three species in this endemic Philippine genus, is restricted only to a few islands of the Mindanao PAIC. The only previous genetic/phylogenetic study including *Bullimus* was a single higher level phylogenetic analysis demonstrating the phylogenetic affinities of the genus (Janse et al., 2006). In contrast, *Haplonycteris* has been the focus of several previous studies employing genetic data. These included starch gel electrophoretic characterization of allozyme data (Heaney et al., 2005) and a single-locus DNA sequence data phylogeographic studies (Roberts 2006a; Catibog-Sinha and Heaney, 2006).

In this study we utilized two novel empirical genomic datasets for a comparative study of diversification in nonvolant forest rodents versus more dispersive, but presumably geographically structured, rainforest fruit bats. Where as *Bullimus* forest rodents are strongly geographically structured along a south-to-north gradient (Mindanao vs Samar-Leyte), and show a distinct absence of gene flow between geographically restricted genotypes, *Haplonycteris* forest fruit bats are characterized by the absence of geographical structure, high levels of gene flow between geographic types, lack of support for geographically restricted clades, and possible

evidence of recent migration (misplaced haplotypes across marine and PAIC boundaries). Together these data inform us about additional exceptions to near-textbook predictions of a prevailing 30-year PAIC Paradigm for Philippine biodiversity (Heaney et al., 1985; Brown et al., 2013a), they warn against deriving strong conclusions from single-locus phylogeographic studies (see also Roberts, 2006a,b; Esselstyn et al. 2012), and they reinforce the explanatory value of cross-genome inference in studies of diversification at finer scales at which these occur. In this study we highlight findings from the first RAD-seq dataset applied to Philippine mammal diversification and demonstrate cautionary lessons for interpretation of phylogeographic data for forest vertebrates from island archipelagos.

MATERIAL AND METHODS

Genomic library preparation.

Individuals were sampled from populations across each species range with a focus on populations found in the Mindanao PAIC (see Fig. 1). Genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit, quantified using a Qubit 2.0 Fluorometer and diluted to 5 ng/ μ L. A multiplexed shotgun genotyping (MSG) protocol was used, modified version of RAD-Seq (Andolfatto et al., 2011), to generate two separate genomic libraries of each species, a total of 96 (80 individuals within Mindanao PAIC) individuals of *Haplonycteris fischeri* and 53 (31 individuals within Mindanao PAIC) individuals of *Bullimus bagobus*. Each DNA sample was digested with NdeI restriction enzyme (New England BioLabs, Ipswich, MA, USA) and each individual was ligated with a unique adapter. Each library was ‘size selected’ to ensure capture of the ligated adapter selected size fragment. We selected base pair size fragment of 248–302

using Pippin Prep (Sage Science, Beverly, MA). Size fragments were confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). To get sequenceable quantities for each library we used Phusion High-Fidelity PCR Master Mix each library. Each library was then sequenced on separate lanes of an Illumina HiSeq 2500 sequencer using a single-end 100-bp read protocol.

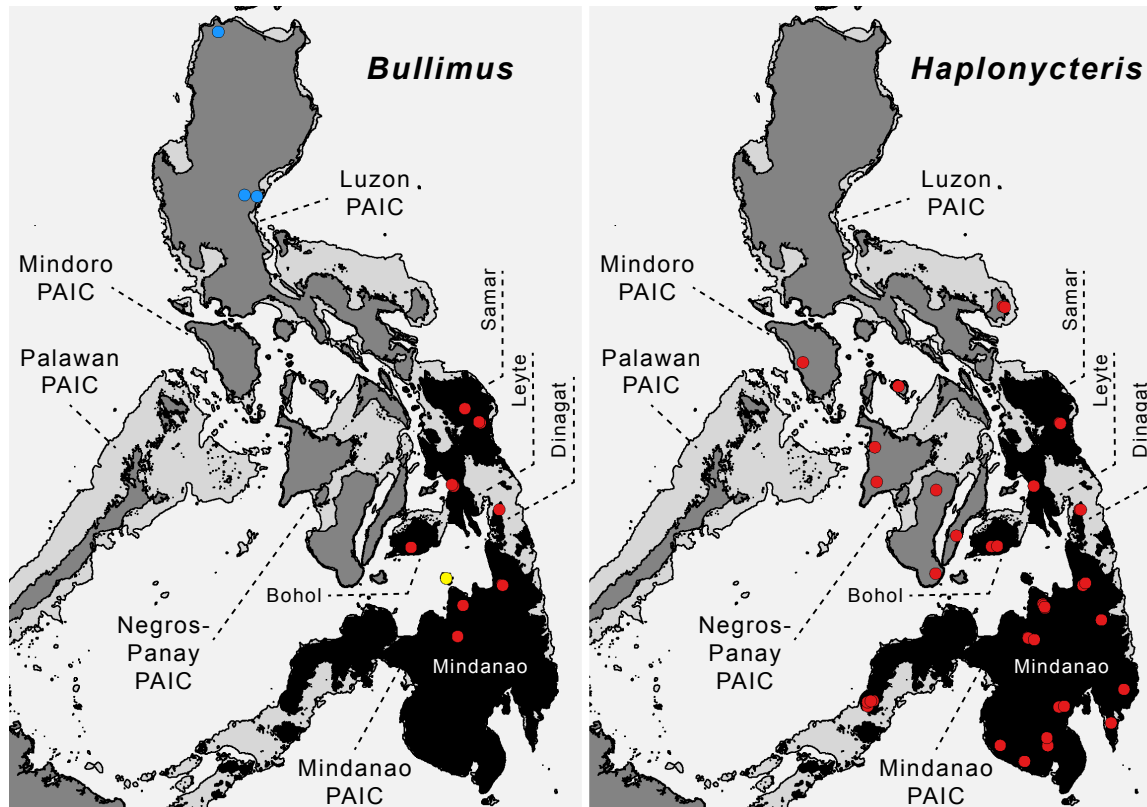


Figure 1 Sampling localities of *Bullimus* and *Haplonycteris*. Representatives for each species range were included in Phylogenetic analyses. Blue dots represent *B. luzonicus*, the yellow dot is *B. gamay* and red dots for *B. bagobus*.

Read and parsing assembly.

Because there is no reference genome of any closely related species, all data were *de novo* assembled using PyRAD v3.0.66 pipeline (Eaton, 2014). PyRAD filters and clusters RAD

sequences to identify putatively orthologous loci. Each individual was demultiplexed using their unique adapter sequences and barcode. Any site with a Phred quality under 99% (Phred score = 20) was changed to “N” character, and reads with N’s $\geq 10\%$ were discarded. Filter reads were then clustered using VSEARCH v2.1.2_linux_x86_64 and aligned using MUSCLE v3.8.31, to establish homology among reads within a species. A clustering threshold of 90% was used as the similarity threshold for VSEARCH. Consensus sequences with coverage of <10 reads, from base calls at each site within a cluster, were discarded. To detect for potential paralogs, 10% of shared polymorphic sites in a locus were kept. These filtered consensus sequences were then assembled to include a minimum of 60% of taxa per locus. Because there is no current consensus regarding treatment of RAD-seq within-sample missing data, final analyses were conducted after removing individuals possessing >35% missing data from their structure output consensus.

Phylogenetic analyses.

Historical relationships inferred from species-level phylogenetic analyses were estimated in SVDQuartets (Chifman and Kubatko 2014) and implemented in PAUP*v4.0a150 (Swofford, 2002). This method uses a coalescent model to infer topology among randomly sampled quartets of individuals.

Population genomic analyses.

Population genomic statistics were generated using PYRAD, GENODIVE (Meirmans et al 2004) and Arlequin (Excoffier et al 2010). Custom R scripts were used to obtain nucleotide diversity (Alexander, *unpublished*). Population structure was estimated using SNMF in R (Frichot & François 2015). This approach in SNMF implements sparse nonnegative matrix

factorization (sNMF) and least-squares optimization algorithms to estimate individual admixture coefficients (Lee & Seung 1999; Kim & Park 2007, Kim & Par 2011). One hundred independent runs of each value of possible number of ancestral gene pools (K). The optimal K is chosen by selecting the lowest cross-entropy criterion value. This cross-validation technique measures the capability of the ancestry estimate algorithm to evaluate the prediction error. Unlike Structure (Pritchard 2000), this approach does not make equilibrium population genetic assumptions, such as, Hardy-Weinberg and linkage disequilibrium (Frichot 2014). Analysis of molecular variance (AMOVA) analyses were conducted in Arlequin 3.1 with 1000 permutation to assess genetic diversity within the Mindanao PAIC, and more specifically the northern portion. Data were partitioned by islands and groups of islands within the northern and southern portion of the Mindanao PAIC.

Demographic analyses.

Divergence times and migration events were estimated using fastsimcoal2 (Excoffier and Foll 2011) to estimate demographic parameters using the site frequency spectrum (SFS). These SFS files were obtained using $\delta a \delta i$ (Diffusion Approximations for Demographic Inference) (Bustamante et al 2009), to simulate joint site frequency spectrum using a diffusion (neglects linkage, so we bootstrap to account for this) approximation. The diffusion approach is continuous approximation of population genetics of a distinct group of individuals evolving in distinct generations. Since Single Nucleotide Polymorphism (SNP) data do not contain flanking bases, a folded SFS was used, in which entries relate to the frequency of the minor allele. To maximize the number of segregating site, each SNP was projected down to remove all monomorphic sites. In fastsimcoal2 we used the following operations: -N (maximum number of

simulations), -L 40 (maximum number of EN cycles), -M 0.001 (stop criterion of relative differences between iterations). The best fitting model was identified on the basis of Akaike's information criterion (AIC) score (Akaike 1974). To estimate parameter uncertainties, we performed 100 parametric bootstraps (fitting simulated data sets) by simulating SFS with the same number of SNPs from maximum likelihood estimates, while reestimating parameters for each run.

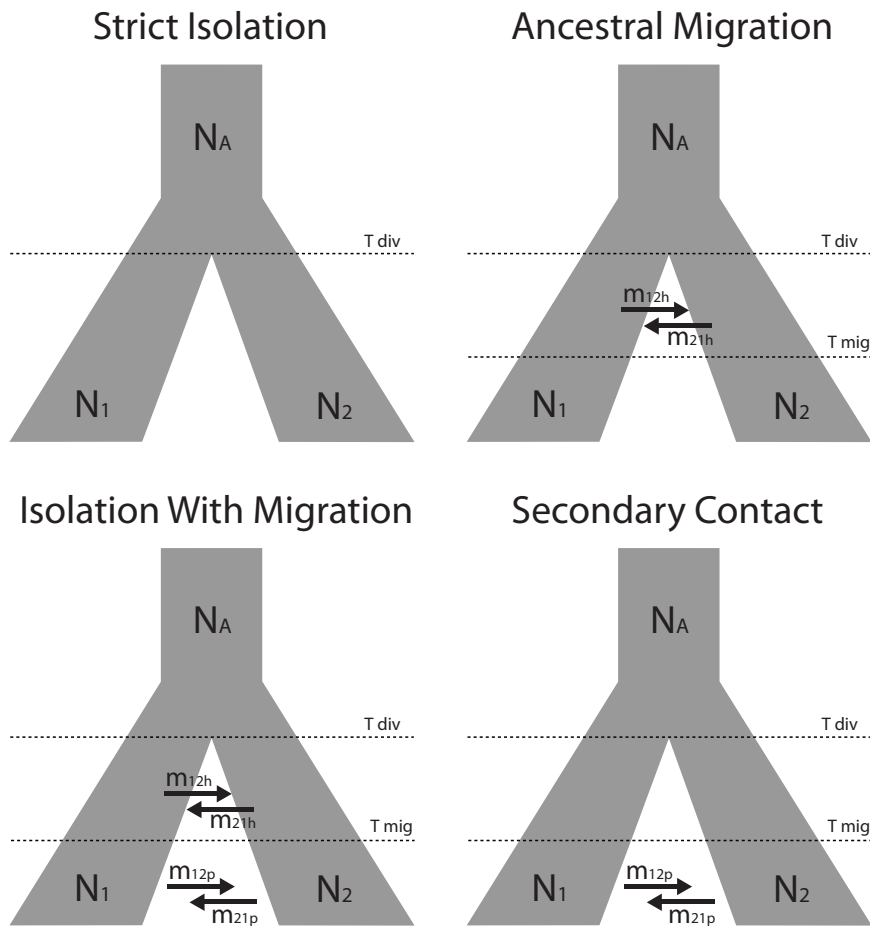


Figure 2 Model Test. Effective population size estimates of the northern (N_1), southern (N_2) PAIC as well as ancestral divergence (N_A) are indicated. Divergence times (T_{div}) represent the number of generations backward in time until coalescence between populations. Divergence to

migration (T_{mig}) were calculated from half the time between present time and ancestral divergence (N_A). Migration (m) rates are in units of backward in time, per generation.

RESULTS

Genomic library preparation & Read and parsing assembly.

Sequencing of each genomic library resulted in approximately 167 million reads for *Haplonycteris* and 122 million reads for *Bullimus*. More complete data sets, including sampling for each species range, was used for species relationship analyses, SVDQuartets (see map). These data sets were filtered and processed in Pyrad and resulted in 17,206 unlinked biallelic SNPs loci for *Haplonycteris* and 12,773 for *Bullimus*. Additional data sets, only containing individuals found within the Mindanao PAIC and with at least 2 individuals per locality were analyzed with Pyrad with identical filtering steps as above. *H. fischeri* and *B. bagobus* localities were further reduced to match similar geographic sampling for each species. These filtering steps resulted in 14,319 unlinked loci for *H. fischeri* and 9,196 unlinked loci for *B. bagobus*.

Phylogenetic analyses.

Species relationships for *Haplonycteris* and *Bullimus* ranges were resolved using SVDQuartets. Previous phylogenetic work, for *Haplonycteris*, show a monophyletic clade within the Mindanao PAIC using two mitochondrial genes and showed high level of population differentiation within the Mindanao PAIC (Roberts 2006). Our results also show a monophyletic clade within the Mindanao PAIC but little differentiation within the clade. To date there has been no phylogenetic work on *Bullimus*. Rickart et al. (2002) described a new species, *B. gamay*, from

the island of Camiguin, a small off the coast of Mindanao, on the basis of morphological traits. Our phylogenetic relationships of *Bullimus* find, *B. gamay* to be sister to *B. bagobus*, and *B. luzonicus* sister to both clades. We used *Rattus everetti* the outgroup in initial analyses. Previous Muridea phylogenies place *B. bagobus* and *B. luzonicus* as sister to *B. gamay* (Jansa et al. 2006). We also find that *Bullimus* has more structure, deeper branch lengths than that of *Haplonycteris* within the Mindanao PAIC.

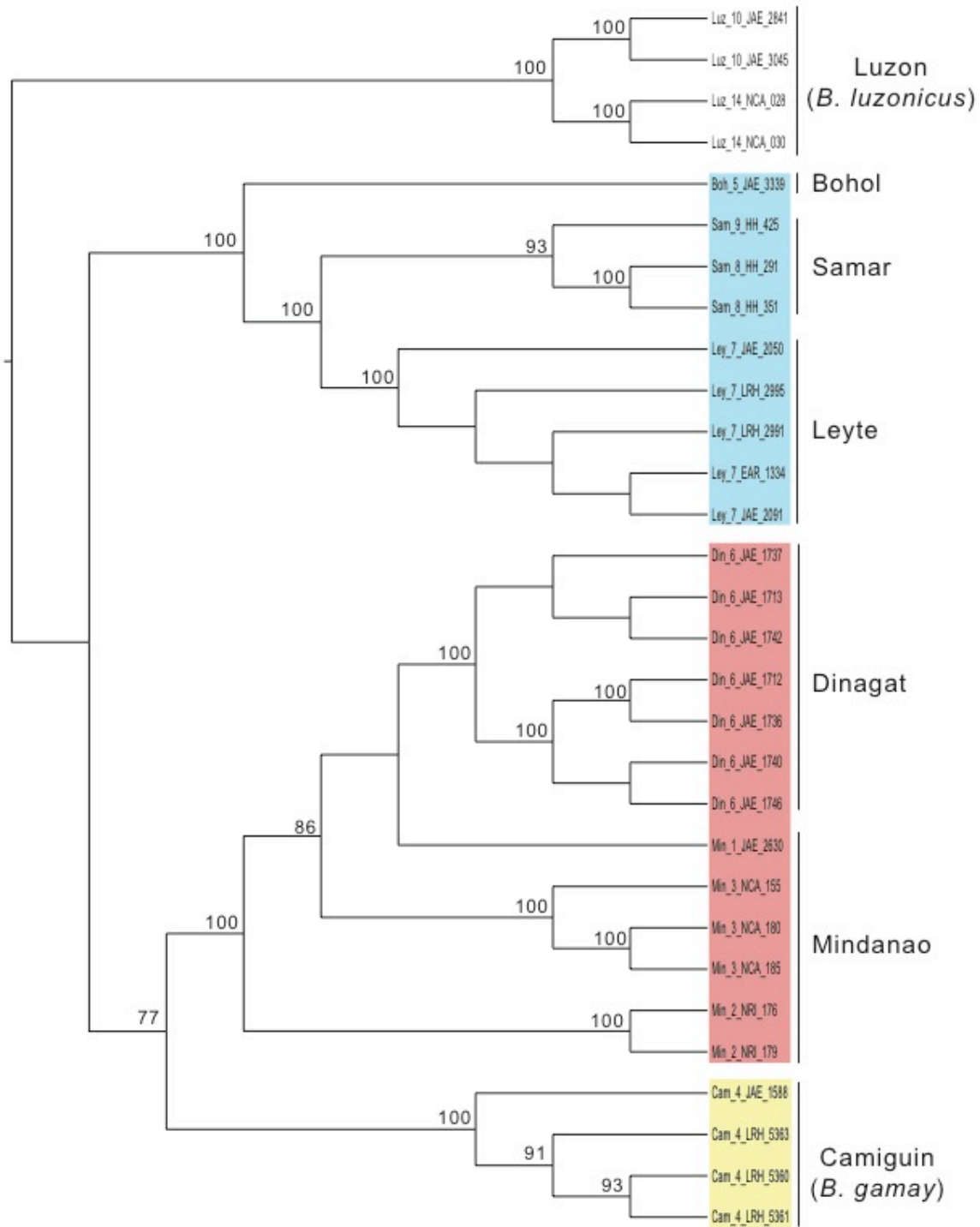


Figure 3.1. Phylogenetic analyses from SVDQuartets for *Bullimus* was rooted with *Rattus everetti* (not shown on tree).

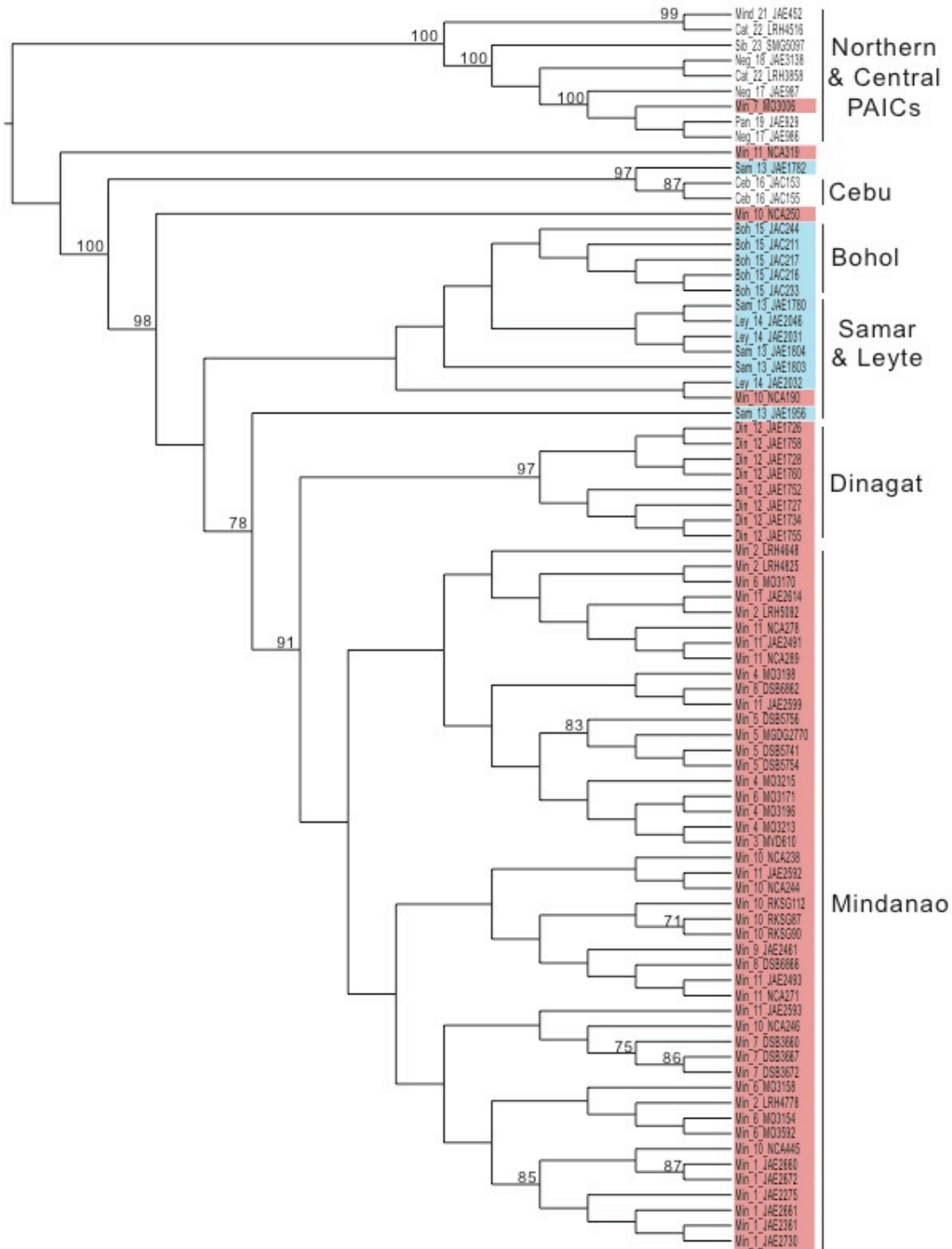


Figure. 3.2. Phylogenetic analyses from SVDQuartets for and *Haplonycteris* was rooted by the *H. fischeri* Luzon clade.

Population genomic analyses.

LEA analyses of ancestry coefficients, within the Mindanao PAIC, produced estimates of ancestry structure for each species. Population structure for *Bullimus bagobus* exhibited a $K=2$ solution of two lineages, one consisting of individuals from Samar and Leyte islands, at the northern end of the Mindanao faunal region PAIC; and the other individuals from Dinagat and Mindanao islands, at the southern extent of the same PAIC (cross-entropy value of 0.6278773). For *Haplonycteris fischeri*, a $K=1$ was selected, based on a cross-entropy value of 0.2991402; indicating that these populations are best viewed not as a highly structured series of divergent lineages but, instead, a population of single-lineage ancestry.

Population genetic analyses were calculated by grouping northern islands (Samar and Leyte) and southern islands (Dinagat and Mindanao) to make comparisons between islands previously connected during the Pleistocene. *Bullimus bagobus* had higher genetic differentiation (F_{st} value of 0.512), than *Haplonycteris fischeri* (F_{st} value of 0.056). Nucleotide diversity (π) within northern and southern islands of the PAIC were higher for *B. bagobus* (~0.040 for northern and ~0.037 for southern island groups). *Haplonycteris fischeri* had lower nucleotide diversity (~0.0016 for southern and ~0.0015 for northern islands groups). Observed heterozygosity for *B. bagobus* was lower than the expected heterozygosity, whereas *H. fischeri*'s observed heterozygosity was close to its expected heterozygosity. Our inbreeding coefficient, G_{is} , was found to higher in *B. bagobus*.

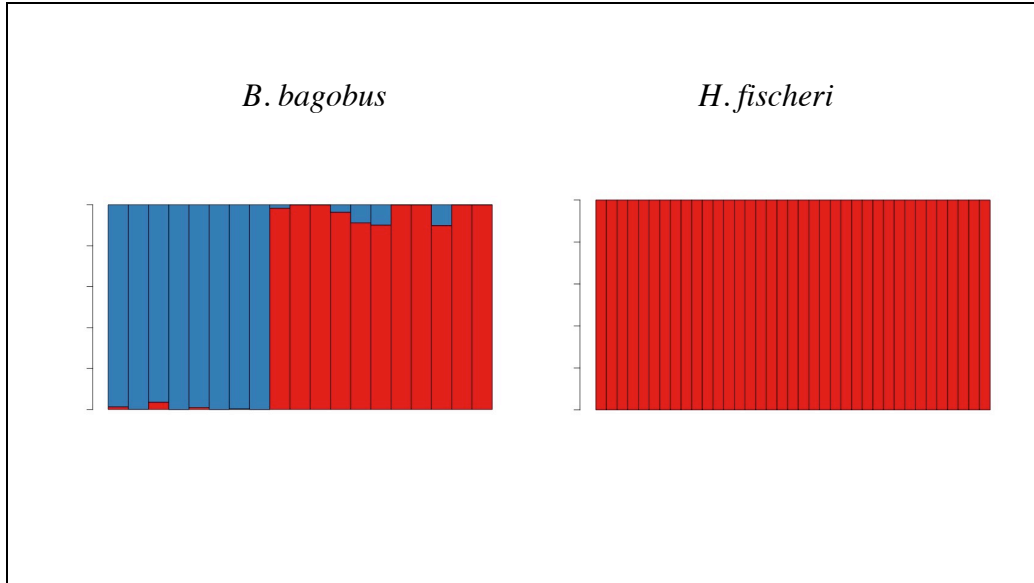


Figure 4. Genetic grouping of population structure analyses for *Bullimus bagobus* and *Haplonycteris fischeri* estimates of ancestry coefficients, implemented in LEA. The mostly likely $K=2$ was chosen for *B. bagobus*, and blue represents shared alleles for individuals found on the northern PAIC (Samar and Letye) whereas red represents shared alleles for individuals found on the southern (Dinagat and Northern Mindanao) portion of the Mindanao PAIC. The mostly likely $K=1$ was chosen for *H. fischeri*.

	<i>B. bagobus</i>		<i>H. fischeri</i>	
	North	South	North	South
N	8	11	8	29
H_{obs}	0.05	0.056	0.044	0.031
H_{exp}	0.106	0.108	0.052	0.037
G_{is}	0.528	0.481	0.148	0.156
F_{st}	0.512		0.056	
π	0.004079103	0.003400588	0.001617058	0.0015806

Table. 1. Population statistics for both focal taxa from the northern and southern Mindanao PAIC; N = sample size. Inbreeding (G_{is}) coefficients, observed and expected heterozygosity were calculated using 8 randomly selected individuals from each population. Nucleotide diversity (π) is indicated below.

	<i>B. bagobus</i>	<i>H. fischeri</i>
Among North & South	49.46	2.33
Among Islands within N & S	10.42	1.51
Within Islands	40.13	96.16

Table. 2. Analysis of molecular variance (AMOVA) for both focal taxa within the Mindanao PAIC. Values represent the percentage of variation at each level of geographical organization.

Demographic analyses.

Based on AIC values, the secondary contact model was chosen for both *Haplonycteris fischeri* and *Bullimus bagobus*, with a w_i probability of ~ 1 . The second model chosen was Isolation with Migration, for both species, and had a w_i probability 7.66×10^{-123} number (see sup material). Population sizes were similar across both species with the exception of *B. bagobus*'s population on Samar and Leyte islands, which was smaller. Divergence times, represent the number of generations backward in time until coalescences of both populations, were also similar for both species. However, actual reproductive rates and generation times for *B. bagobus*

and *H. fischeri* are unknown and would be required in future studies to gain accurate estimates of real divergence times for both species. Migration rates did differ between species. *Haplonycteris fischeri* has approximately two orders of magnitude more migrants, per generation backward in time than *B. bagobus*.

Species	Loci	N_1	N_2	T_{div}	$2N_1m_{12}$	$2N_2m_{21}$
<i>B. bagobus</i>	8,142	57,716	91,876	461,303	1.678	2.672
<i>H. fischeri</i>	10,742	94,612	93,915	442,046	432.888	429.699

Table 3. Demographic parameter estimates of the secondary contact model. The number of loci used for the SFS and effective population size estimates of the north (N_1) and south (N_2) are indicated. N_1 was held constant for bootstrap analyses. Divergence times (T_{div}) represent the number of generations backward in time until coalescence between populations. Migration (m) rates are in units of $2Nm$, backward in time, per generation.

DISCUSSION

The role of Pleistocene sea level fluctuations have been hypothesized widely to have promoted the diversification, partitioning, maintenance, and endemism in land vertebrate terrestrial biodiversity of island systems (Heaney 1985; Knowles lab pubs; Lomolino et al. 2014; review Brown et al., 2012). Previous research has focused on resident, endemic faunal region land vertebrate biodiversity *differences* among and between Pleistocene landmass

amalgamations; the majority of these studies have focused on species-level relationships (e.g., Stepan et al., 2003; Peterson et al. 2005; cite some newer Heaney & Rickart, Balet, & colleagues papers phylogeny of Rynchomys, Crotomys, etc), nearly all have utilized single locus datasets or datasets with multiple markers, but with relatively few loci (but see Esselstyn et al., 2010; and the recent sys bio shrew paper). Only one recent work utilized multiple taxa to test the hypothesis that PAIC fragmentation and sea-level vicariance might drive diversification (Oaks et al. 2013).

Our work has focused on the effect of sea level fluctuations within a single PAIC, specifically elucidating population genetics level phenomena independently in two endemic and unrelated, but co-distributed, species. Given that their specific forest-obligate habitats potentially were connected across a wide land bridge that formed repeatedly at multiple periods during the Pleistocene (Fig. 1), we can make reasonable predictions regarding demographic and population genetic processes contributing to—or perhaps overcoming through reticulation—divergence of northern versus southern Mindanao PAIC mammals. Although phylogenetic analyses, inferred with thousands of presumably neutral genomic loci, confirm that both *Bullimus bagobus* and *Haplonycteris fischeri* form monophyletic clades within the Mindanao PAIC, our analyses revealed the presence of several individuals (presumably recent migrants) on Mindanao, but with genotypes otherwise restricted to populations in the central or northern portions of the archipelago. This discrepancy could be caused by high levels of widely-ranging non-residents, large, panmictic populations (absence of local geographic structure), extensive regional gene flow, recent migration events, storm-mediated colonization, and/or ancestral polymorphism within the Mindanao faunal region.

More than 96% nucleotide variation for *Haplonycteris fischeri* was explained by within-island variation (versus an alternative of between northern vs. southern population). This variation could also be explained by mutations, large population size, or migration. However, considering no ancestral structure was observed between north and south portions of the Mindanao PAIC, coupled with high migration rates and large effective population sizes, one might hypothesize that Pleistocene sea level fluctuations have had little effect on the diversity of *H. fischeri*. Although not entirely surprising/unexpected (e.g., bats might be predicted to be able to fly across and overcome marine barriers to dispersal), all available field-based empirical natural history studies have shown *H. fischeri* to be a strict understory, canopy-limited forest feeding resident bat lineage (citations). Although common in understory forested habitats at mid elevations (Heaney et al. 2005; Roberts 2006a), *H. fischeri* has seldom been collected in agroecosystems or forest edges, and is virtually never observed or captured in open areas, coastal habitats, mangroves or heavily disturbed residential areas surrounding human habitats. The fundamentals of wide-ranging movements, long-distance flight, migration, and within-island gene flow remains entirely unstudied in this poorly known Philippine endemic—yet our data make it clear that these heretofore undocumented phenomena do occur in *H. fischeri*.

In contrast, our results demonstrate that Pleistocene sea level fluctuations did have a pronounced effect on *Bullimus bagobus* diversity. Ancestral, geographically-based population genomic structure was identified, along with deeper divergence times, and limited gene flow. Additionally, ~50% nucleotide diversity was explained by the north versus south partitioning of the Mindanao PAIC faunal region. This suggests that although connected by broad, land-positive terrestrial habitats, ecological factors (habitat specificity) may have maintained and reinforced population separation and lineage divergence in forest rodents of the genus *Bullimus* (Steppan et

al., 2003; Hosner et al., 2014).

Finally, a model of present migration only was chosen for both species. Our preferred model suggests that migration may have occurred at more recent times, between the present and a time *after* divergence occurred. Results for both species, despite their ecological, demographic, and life history differences suggests recent migration has been more significant or impactful in structuring for partitioning modern day genetic variation in unrelated forest taxa. This surprising finding contradicts the implied and presumed importance of even the most recent land bridge formation connecting insular components of the southern Philippine faunal region Mindanao PAIC, calling into question the significance of population fragmentation cycles assumed to drive diversification in forest vertebrates, and underlying Philippine archipelago megadiversity (Oaks et al., 2013; Brown et al., 2013a).

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