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Synchronous diversification of parachuting frogs (Genus *Rhacophorus*) on Sumatra and Java

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ABSTRACT

Geological and climatological processes can drive the synchronous diversification of co-distributed species. The islands of Sumatra and Java have experienced complex geological and climatological histories, including extensive sea-level changes and the formation of valleys between northern, central, and southern components of the Barisan Mountain Range, which may have promoted diversification of their resident species. We investigate diversification on these islands using 13 species of the parachuting frog genus *Rhacophorus*. We use both mitochondrial and nuclear sequence data, along with genome-wide SNPs to estimate phylogenetic structure and divergence times, and to test for synchronous diversification. We find support for synchronous divergence among sister-species pairs from Sumatra and Java ~9 Ma, as well as of populations of four co-distributed taxa on Sumatra ~5.6 Ma. We found that sister species diverged in allopatry on Sumatra and conclude that divergence on Sumatra and Java was affected by sea-level fluctuations that promoted isolation in allopatry.

1. Introduction

Biotic responses to climatological or geological changes often drive diversification on tropical islands (Esselstyn et al., 2009). Climatic fluctuations can accelerate diversification by isolating species into refugia or by expanding suitable habitat, thus promoting dispersal (Nater et al., 2015). Likewise, geological changes can initiate diversification by isolating populations in allopatry. The Sunda Shelf (Sumatra, Java, Borneo, and the Malay Peninsula) has experienced a turbulent geological and climatological history from the Miocene to present (Lohman et al., 2011). Sumatra in particular has experienced dynamic tectonic processes, volcanism, dramatic surrounding sea-level changes, and extensive connectivity with surrounding landmasses during the Pleistocene (Hall, 2001, 2002, 2009, 2011, 2012a, 2012b, Lohman et al., 2011). For most of the past 25 million years (Ma), highland habitats on Sumatra have remained tropical, while lowland forests were frequently inundated by marine incursions, and also experienced extensive cooling and drying (Hall, 2009, 2012a).

Although past studies have largely focused on the role of Pleistocene sea-level fluctuations on diversification on Sumatra and Java, few studies have investigated the role of Miocene-Pliocene sea-level changes, or of the formation of physical barriers during this time period (Inger & Voris, 2001; Leonard et al., 2015; Voris, 2000). During much of the Miocene, Sumatra was composed of several islands, with marine incursions serving as barriers to dispersal (van Bemmelen, 1949; Meijaard, 2004; Hall, 2012a). From the early Miocene to ~15 Ma, raised shorelines persisted on Sumatra, transforming volcanic peaks into small islands (Baumann, 1982; Haq et al., 1987; Batchelor, 1979; Anderson et al., 1993; Collins et al., 1995; Lourens & Hilgen, 1997; Barber et al., 2005). From 14 to 9 Ma sea levels receded, permitting dispersal between previously isolated volcanic islands (Batchelor, 1979; Baumann, 1982; Haq et al., 1987; Morley, 1998). This cycle continued, with sea levels rising from 8.5 to 6 Ma, receding from 5.8 to 5.4 My, and again rising from 5 to 4 My (Baumann, 1982; Haq et al., 1987; Krantz, 1991; Anderson et al., 1993; Van den Bergh et al., 2001).

Furthermore, van Bemmelen (1949) hypothesized the persistence of two transverse inland seaways on Sumatra from the early Miocene onward that divided Sumatra between the northern and central components of the Barisan Mountain Range (in the Padang Sidempuan Valley, just south of the Asahan High, which was an elevated region that ran transverse to the Sumatran mainland), and between the Gumai and Garba Mts (in the Pagar Alam Valley, Fig. 1). These seaways formed in the early Miocene, and completely subsided only in the middle Pliocene due to Barisan Mountain uplift (van Bemmelen, 1949). As

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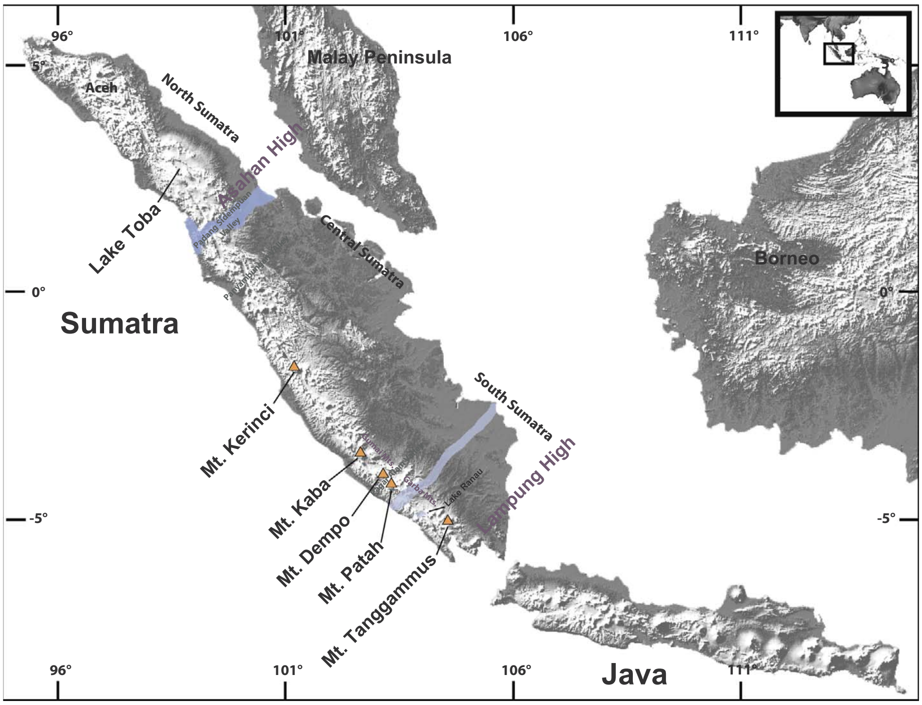

Fig. 1. Map of the islands of Sumatra and Java, showing their placement within the Sunda Shelf region. We also label historical and contemporary geological features on Sumatra referenced in this study. The two light blue waterways represent hypothesized marine incursions that may have promoted diversification of resident Sumatran taxa during the Miocene and early Pliocene.

such, Sumatra was composed of at least three large islands for much of its geologic history, and even at times of low sea levels (when marine incursions subsided), the persistence of the Padang Sidempuan and Pagar Alam Valleys likely maintained allopatric distributions of dispersal-limited species in the northern, central, and southern components of the Barisan Mountain Range (Meijaard, 2004). Equivalently, Java was composed of small volcanic islands from 10 Ma onward, and did not completely emerge above sea-level until ~5 Ma (Lohman et al., 2011). West Java may have been periodically connected to southern Sumatra via the Lampung High (elevated region similar to the Asahan High) as early as the Mid-Miocene, allowing early dispersal from southern Sumatra (van Bemmelen, 1949; Meijaard, 2004). Signals of these historical processes may be detected in the diversification histories, population structures and distributions of genetic diversity of extant biota (Weigelt et al., 2016; Portik et al., 2017; Xue & Hickerson, 2017). Under a comparative phylogeographic framework, shared diversification patterns between species can indicate synchronous responses to geological or climatological events (Hickerson et al., 2010; Bagley & Johnson, 2014; Smith et al., 2014; Prates et al., 2016).

We explore diversification processes on Sumatra and Java using species from the parachuting-frog genus *Rhacophorus*. This genus includes ~90 species distributed from the Indian peninsula to East and Southeast Asia (Frost, 2017). Sumatra and Java contain 16 described species of *Rhacophorus*, including *R. achantharrhena*, *R. barisani*, *R. bengkuluensis*, *R. bifasciatus*, *R. catamitus*, *R. cyanopunctatus*, *R. indonesiensis*, *R. margaritifer*, *R. modestus*, *R. nigropalmatus*, *R. norhayatii*, *R. pardalis*, *R. poecilonotus*, *R. prominanus*, *R. pseudacutirostris*, and *R. reinwardtii* (Harvey et al., 2002; Streicher et al., 2012, 2014a,b; Hamidy & Kurniati, 2015; O'Connell et al., 2018a). On Sumatra, some species distributions span the length of the island, whereas others are restricted to small geographic areas (Harvey et al., 2002; Streicher et al., 2012; Hamidy & Kurniati, 2015). Species of the genus *Rhacophorus* occupy a variety of niche spaces, and most species' ranges are partitioned by elevation and island region (Harvey et al., 2002). On Sumatra, up to four highland endemic species occur in sympatry across the Barisan mountain range (KAO, personal observation). Java contains two species: *R. margaritifer* and *R. reinwardtii* (Streicher et al., 2012; Frost, 2017, Fig. 2).

This study uses both mitochondrial and nuclear DNA sequence data, along with genome-wide SNPs, to pursue the following questions: (1) did species with similar geographic distributions respond synchronously to geological and climatological events on islands? (2) What historical processes promoted these diversification events?

2. Materials and methods

2.1. Sampling and molecular sequence generation

2.1.1. Taxonomic sampling

The taxonomy of several *Rhacophorus* species is currently under review; thus, we focused this study on 13 species (see discussion of *Rhacophorus* taxonomy in Appendix A). We extracted DNA from liver and thigh muscle tissue from 12 species from Sumatra and Java stored in SDS buffer or 70% ethanol. Our sampling included: *R. achantharrhena* (n = 8), *R. bengkuluensis* (n = 4), *R. catamitus* (n = 27), *Rhacophorus* sp. (n = 9), *R. cyanopunctatus* (n = 3), *R. margaritifer* (n = 5), *R. modestus* (n = 23), *R. nigropalmatus* (n = 1), *R. pardalis* (n = 3), *R. poecilonotus* (n = 25), *R. prominanus* (n = 5), *R. reinwardtii* (n = 4).

2.1.2. Molecular sequence data generation and alignments

We sequenced a 609 base pair (bp) fragment of the 16S ribosomal RNA gene following O'Connell et al. (2018a). To create a multi-locus concatenated alignment, we used brain derived neurotrophic factor gene (*BDNF*) sequence data from O'Connell et al. (2018b), and downloaded sequences from GenBank of all other available *Rhacophorus* (n = 56), at least one species of each genus within the family Rhacophoridae (n = 17), eight species of Mantellidae, and two outgroups (*Rana kukunoris*, and *Occidozyga lima*) following O'Connell et al. (2018b) and Li et al. (2013). Our dataset included sequences for 12S rRNA (n = 17), 16S rRNA (n = 180), Cytochrome oxidase c subunit I (*COI*, n = 23), Cytochrome *b* (*CYTB*, n = 29), *BDNF* (n = 30), pro-opiomelanocortin (*POMC*, n = 27), recombination-activating gene 1 (*RAG1*, n = 18), Rhodopsin (*RHOD*, n = 15) and Tyrosinase (*TYR*, n = 7). All information regarding specimens used and Genbank ID is presented in Table A1 (Appendix A). We aligned each locus individually using the Geneious aligner (global alignment with free end gaps and a Cost Matrix = 65% similarity, 5.0/−4.0).

To place Sumatran and Javan species within a broad phylogenetic