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Ecology and colour variation of *Oreophryne monticola* (Anura: Microhylidae) with reference to vocalisation and predicted habitat suitability

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ABSTRACT - The Lombok cross frog (*Oreophryne monticola*) is endemic to the islands of Bali and Lombok. We describe the colour variation and associated microhabitats of this endangered species, and provide some notes on its call characteristics and predictions of its distribution in Bali and Lombok. We surveyed sites on both islands with similar altitude but slightly different microhabitats. The frogs were more likely to be found in forests dominated by dipterocarp trees, many epiphytic ferns, and ground covered with leaf litter. Possible differences in colour variation and call suggest that there may be cryptic species within *O. monticola* that could be resolved by morphological and molecular study. The predicted distribution suggests nine isolated areas at around 1100 m a.s.l. on both islands that may be home to this frog.

INTRODUCTION

The islands of Bali and Lombok are part of the Lesser Sunda region known to be home to a rich biodiversity with several endemic species that are threatened with extinction (Reilly, 2016; Reilly et al., 2019a; Tänzler et al., 2016). A major threat to the fauna is habitat loss and disturbance from infrastructure and tourism since both islands are popular tourist destinations (Masseti, 2009; Simay et al., 2009).

One threatened amphibian species, the Lombok cross frog *Oreophryne monticola* (Boulenger, 1897), is endemic to both islands even though Bali lies to the west of Wallace's line and Lombok to the east. All other species of the genus *Oreophryne* are to the east of the line, suggesting that they are of Australasian rather than Asian origin (Reilly et al., 2019b). The islands are separated from each other by very strong ocean currents so that the two populations of *O. monticola* are isolated (Evans et al., 2003; Reilly et al., 2019b). Such isolation, may give rise to specific variations of perhaps colour, vocalisation and other characters (Kurniati, 2013; Amezquita et al., 2009).

The Lombok cross frogs is categorised as an endangered species (IUCN SSC Amphibian Specialist Group, 2018; Iskandar & Mumpuni, 2004), occupying a land area of less than 5,000 km^2 (Iskandar & Mumpuni, 2004), and restricted to elevations from 1000 to 1400 m a.s.l. (Iskandar, 1998). This small frog, never usually more than 25 mm long, has unusual reproductive habits as like others of the same genus its tadpoles are not free living but remain within the egg from which the froglet emerges.

In this study, we have investigated the frog on both Bali and Lombok with the objective of adding to the knowledge of its ecology, colour variation, and call characteristics. In addition, as a contribution to conservation planning, we have estimated its likely distribution on the two islands.

MATERIALS AND METHODS

Study sites and surveys

In 2017, single night time surveys were undertaken on Bali (Batu Karu, 1000-1450 m a.s.l.) on 28th January and Lombok (Sembalun, 1000-1480 m a.s.l.) on 3rd February; each study site was approximately 0.5 km^2 (Fig. 1). From 18.00h to midnight at each site, four surveyors used the visual encounter survey (VES) methods (Campbell & Christman, 1982; Pradhan et al., 2014) to search for frogs in forest litter, leaves, bushes, twigs, debris, ferns, and decayed tree trunks. To increase the chances of finding *O. monticola*, any sound of the frog's call was pursued. The location of each frog was recorded using a GPS Garmin 64s and descriptions made of the habitats and microhabitats occupied. The snout-vent-length (SVL) of each frog was measured using a caliper with 0.01 mm accuracy and frogs' body colour was recorded by photographing the dorsal and ventral surfaces using a DSLR Canon 70D camera. These photographs and descriptions were compared with the existing literature (Iskandar 1998; Iskandar & Mumpuni, 2004; McKay, 2006). All the captured individuals were then released back into the habitat where they had been found.

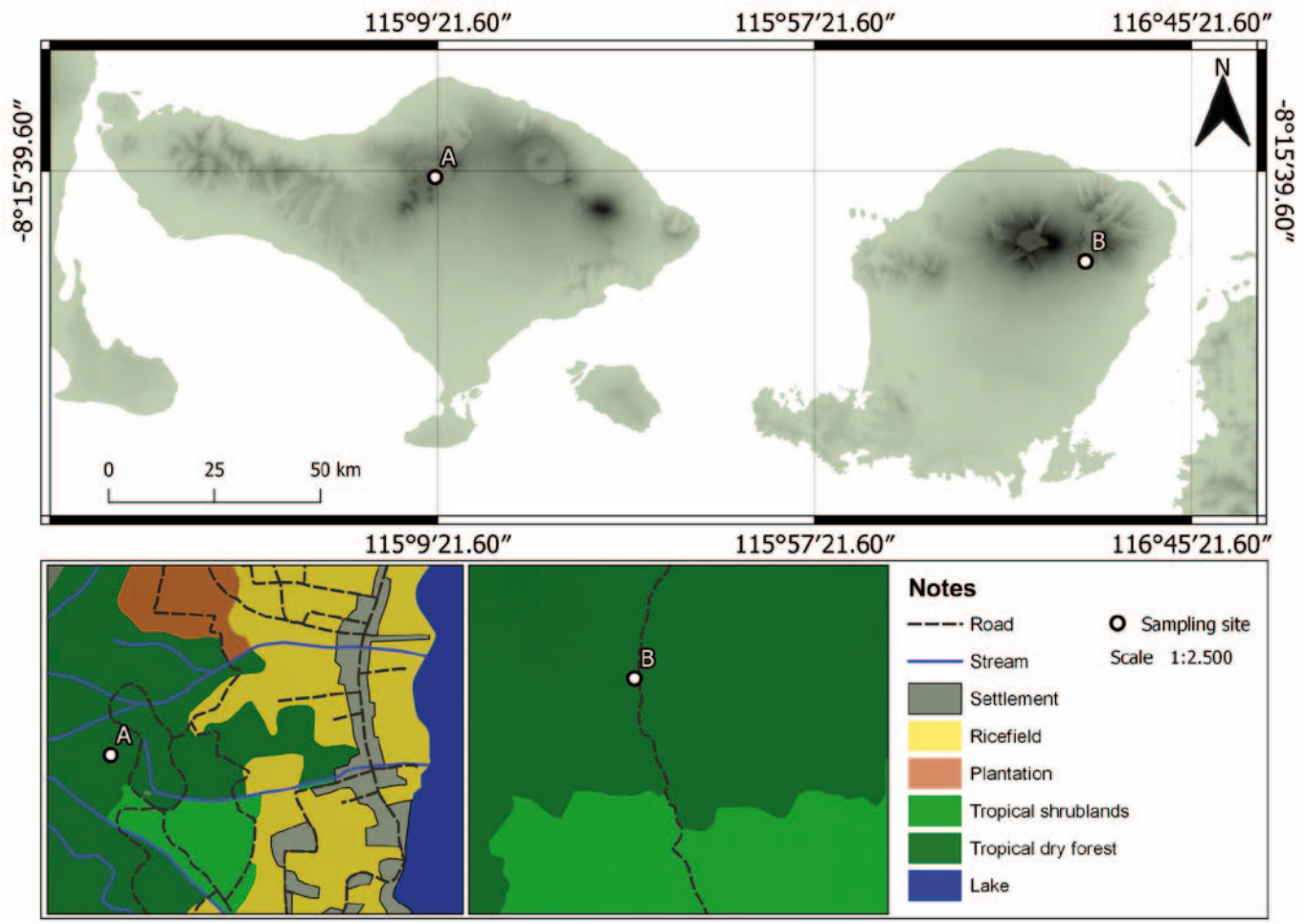

Figure 1. Map showing *O. monticola* sampling sites on the two islands (above), and the center point of sampled sites with the surrounding land use (below): **A)** Batu Karu (Bali), **B)** Sembalun (Lombok)

Call characteristics

Attempts were made to record frog calls at both sites but only on Lombok were calls successfully recorded. The surveyors got as close as possible to the source of frog calls and then made a recording with a SONY ICD-PX240 using WAV format, with a note of the time of day. Calls were analyzed using Adobe Audition version 3.0 software. The call characteristics are based on variation in several parameters including pulse duration, pulse period, dominant frequency, and bandwidth. For each of these parameters, the coefficient of variation (CV) was determined in order to categorize the calls. To make comparisons between the frog calls between the two islands, we used calls from Bali analyzed during a previous study by Kurniati & Hamidy (2014).

Distribution probability analysis

To estimate the likely distribution of *O. monticola*, we used MaxEnt v.3.4.1 (Phillips et al., 2017) species distribution modeling system (Sarker et al., 2019), maximum entropy approach. Several studies have shown that this model can provide meaningful results with as few as three GPS records (Proosdij et al., 2016). We modeled the potential distribution based on the GPS records of four individuals, two individuals from each island. The analysis was supported with 19 environmental variables provided by WorldClim v2.0 (http://worldclim.org/version2) datasets containing average monthly climate data, temperature, and precipitation (Fick & Hijmans, 2017), and 27 arc-second digital elevation modeling data points (DEM) from Badan Informasi Geospasial (http://tides.big.go.id/DEMNAS). The spatial data were masked to the shapefile from (http://tanahair.indonesia.go.id) using ArcGIS (ArcGIS v10.3; ESRI, Redlands, CA). The auto-co-linearity was not analyzed, because we were limited to predicting the probability of current range distribution without a clear understanding of ecological factors and the natural history of this species (Braunisch et al., 2013). The Jackknife method was used to asses best predictor variables for the distribution. We used 25 % of data for test and the remainders for training following Hu et al. (2016). Default regularisation was performed following Phillips et al. (2006). Four replicates were generated by subsample replication with a maximum of 1000 iterations for each replication. We present the data under the receiver operating characteristic (ROC) curve (by the AUC value). The closer the AUC value is to 1.0 the higher is the probability of the model predicting the distribution (Phillips & Dudik, 2008).

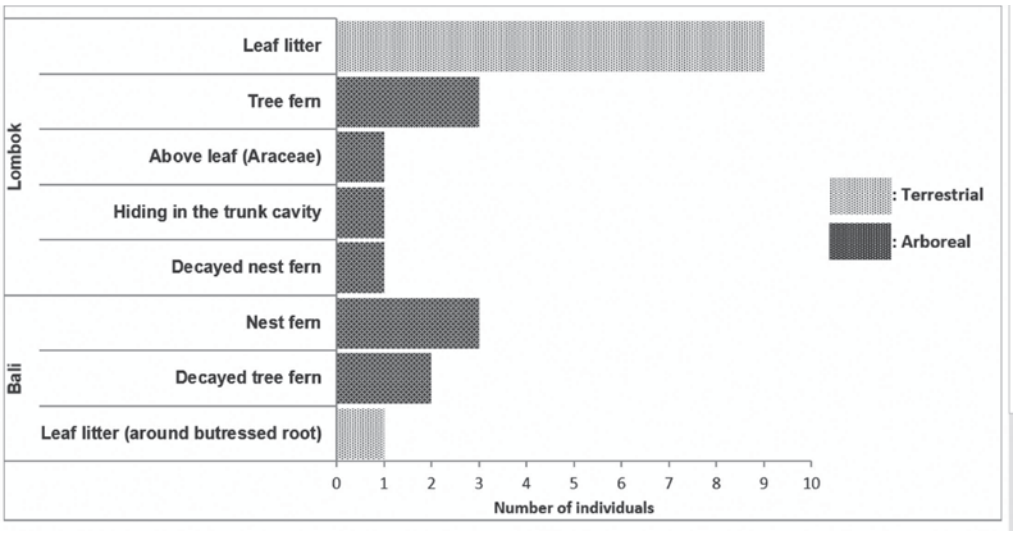

Figure 2. The frequency of *O. monticola* encountered in a variety of terrestrial and arboreal microhabitats

RESULTS

Microhabitat characteristics of *O. monticola*

On Bali, we found six frogs (one female and five males) located close to each other at an elevation of 1400 m to 1410 m a.s.l.. The female was found in terrestrial habitat among leaf litter, which was 30-40 mm deep around the buttress roots of a dipterocarp tree (Fig. 2). In contrast, all the males were found 0.8 to 3 meters above the ground either among the rhizomes and fronds of nest ferns (*Asplenium* sp.), which grow as epiphytes on dipterocarp trees (Fig. 3A), or inside the holes in the midrib of decaying tree ferns (*Cyathea* sp.). Overall, the site sampled on Bali can be categorised as tropical dry forest with steeply sloping open areas (±20˚) with or without leaf litter but with ferns in several locations, with trees about 5-7 meters apart but no water sources nearby. We suspect that the breeding sites of *O. monticola* were on the rhizome of the epiphytic nest ferns (*Asplenium* sp.).

On Lombok, 15 individuals (nine males and six females) were found at elevations ranging from 1447 m to 1475 m a.s.l.. Individuals were gathered in groups of 2 to 6, each individual about 2–8 meters apart, and each group was more than 100 m from others. All individuals, whether male or female, were found in a range of microhabitats which were either terrestrial, hiding amidst leaf litter at the base of tree trunks, or arboreal on nest ferns (1.5 m above the ground), decayed nest fern, or above the leaves of Araceae plants (Fig. 3B). The site sampled in Lombok was a tropical dry forest, more gently sloping than in Bali. It was dominated by dipterocarp trees and several gymnosperms with a leaf litter thickness of 50-60 mm; a greater depth than Bali. The environment was more humid and dense than on Bali with diverse vegetation such as perennial plant, shrubs, nest fern, abundant wet mosses. There were masses of *O. monticola* eggs embedded in a longitudinal strand of mucous observed on three substrates (nest fern, tree trunk, leaf litter) where they were guarded by the parents. On the nest fern, the mucous resembled water droplets hanging 20-30 cm from the fronds.

Figure 3. Preferred microhabitats of *O. monticola*: **A.** Batu Karu (Bali), dipterocarp tree with attached nest fern (*Asplenium* sp.), **B.** Sembalun (Lombok) a more humid environment with more dense and diverse vegetation

Body colour variation of *O. monticola*

The species is highly variable in pattern and coloration (Fig. 4). Dorsally, individuals from Bali were dark or light brown or red-brown (Fig. 4 A to F); the light brown frog had a darker broad mid-dorsal marking extending to the vent (Fig. 4B). Likewise from Lombok, there was great colour variation (Fig. 4 G to U). Some striking coloration was observed including uniformly brick red with black-spots (Fig. 4H); light to dark brown with striking nodules dorsally (Fig. 4 I & N); dorsum predominant

grey with thin longitudinal mid-dorsal line from snout tip extending to the back of the femur (Fig. 4J); dorsum uniformly brackish brown (Fig. 4K); and dominant yellow mottled with black spots dorsally with yellow longitudinal mid-dorsal line, a dense light yellow from snout tip to interorbital on the head dorsally with 2 bold transversal yellow bars across the tympanum (Fig. 4 L & R). The venter colour of the Bali and Lombok frogs are similarly highly diverse, varying from dark brown and orange with white spot on the throat and belly, predominantly brick orange with light yellow on part of the belly, to uniformly black.

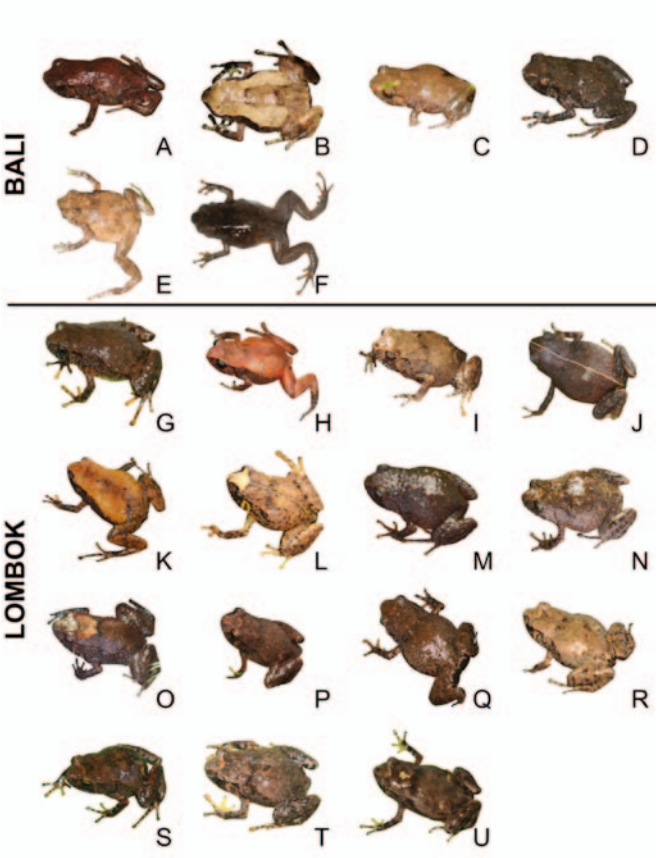

Figure 4. Dorsal colour variation in *O. monticola* from Bali and Lombok. Frogs are shown slightly smaller than life size (x0.9)

Call characteristics of *O. monticola*

The only call type we recorded on Lombok was a simple pulse repetition which covered a narrow spectral band with a dominant frequency in the range of 2147 – 2744 Hz giving a 597 Hz bandwidth (Fig. 5) (Table 1). The characteristics of the calls are static indicated by dominant frequency (CV: 7 %), maximum dominant frequency (CV: 3 %), minimum dominant frequency (CV: 3 %), and bandwidth (CV: 11 %) (Table 1). The call of *O. monticola*, recorded previously on Bali by Kurniati & Hamidy (2014), also covered a narrow spectral band with a dominant frequency of approximately 3000 – 3937 Hz and 937 Hz bandwidth (Table 1). However, the calls are more dynamic, with quite variable pulse period (CV: 26 %) and duration (CV: 47 %) (Kurniati & Hamidy, 2014) (Table 1).

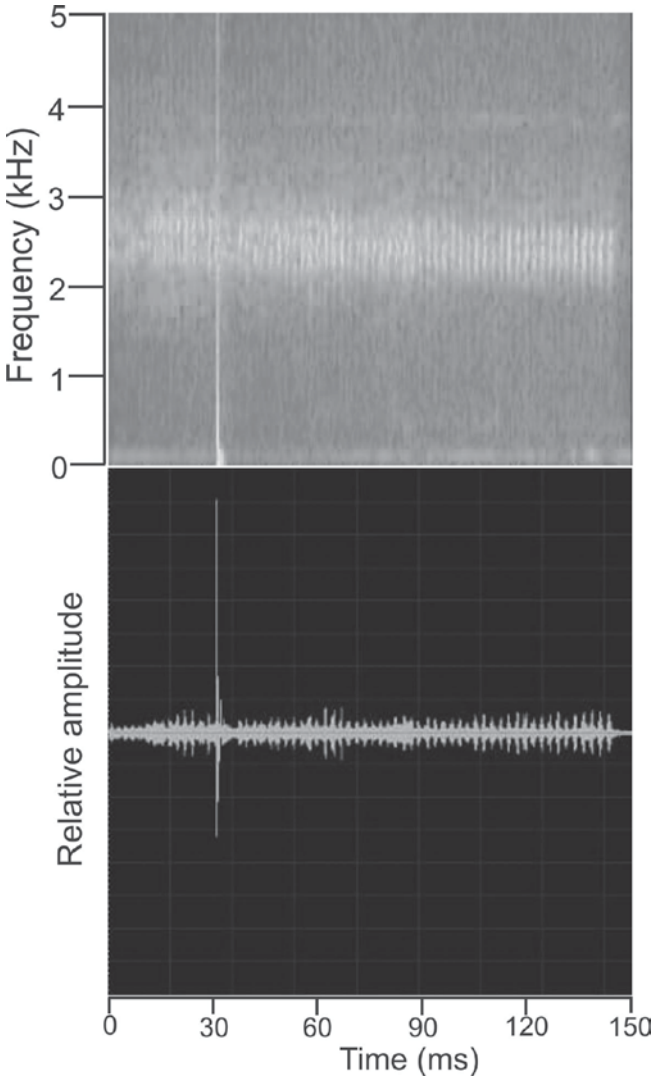

Figure 5. Spectrogram (above) and oscillogram (below) of a call of *O. monticola* from Sembalun (Lombok) based on our field recording

Table 1. Call parameters for *O. monticola* on Bali (from Kurniati & Hamidy, 2014) and on Lombok

Call parameters	Bali	Lombok
Pulse duration	1.91 ms (CV*: 47 %)	-
Pulse period	7.49 ms (CV: 26 %)	-
Dominant frequency	-	2476 Hz (CV: 7 %)
Maximum dominant frequency	3937 Hz (-)	2744 Hz (CV: 3 %)
Minimum dominant frequency	3000 Hz (-)	2147 Hz (CV: 3 %)
Bandwidth	937 Hz (-)	597 Hz (CV: 11 %)

*CV = coefficient of variation

Distribution probability and elevational distribution of *O. monticola*

The average test AUC score for four replicate runs was 0.965, with a standard deviation of 0.0001. Our jackknife

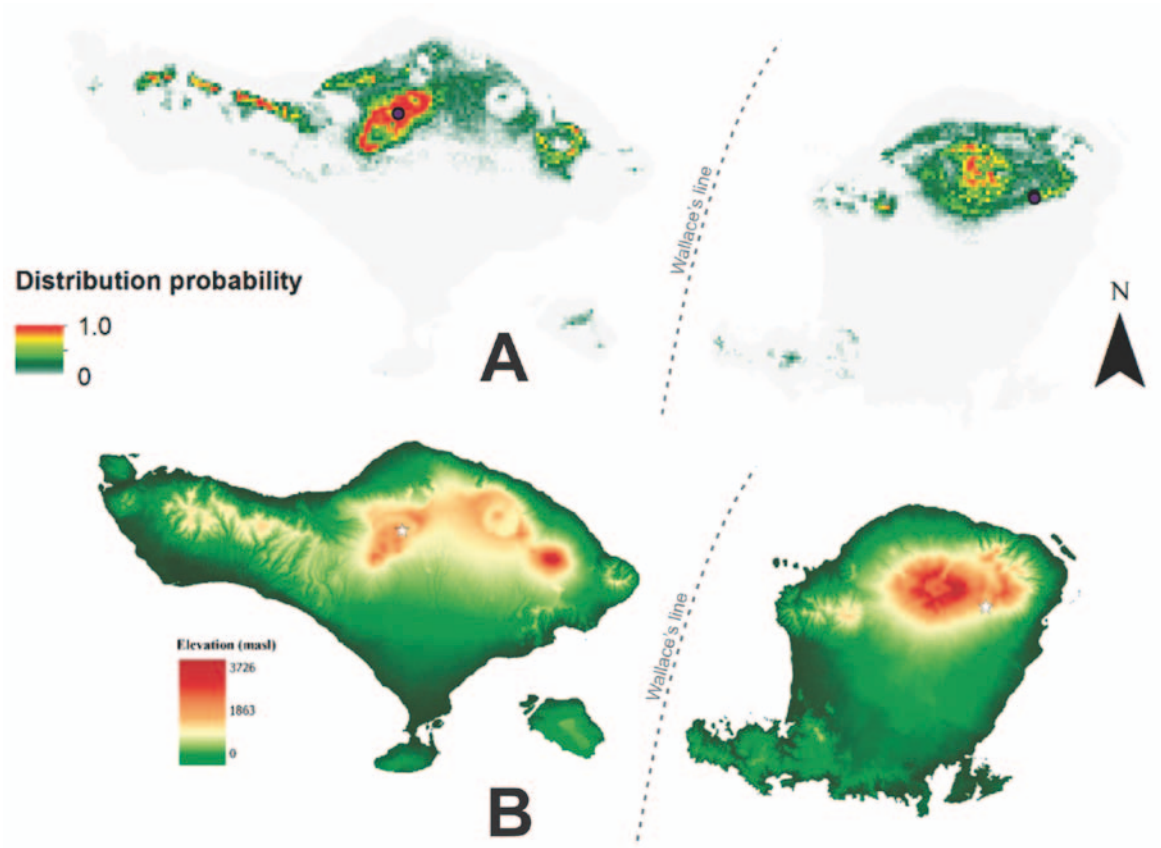

Figure 6. A) Map showing the probability of the presence of *O. monticola* on Bali (left) and Lombok (right) by maximum entropy approach (purple dots = study sites), **B)** elevational distribution of *O. monticola* encountered in Bali (left) and Lombok (right) (white stars = study locations)

variable contribution test shows that among all the variables used for the modeling, the maximum temperature of the warmest month (28.9 %) contributed most significantly, followed by isothermality (mean diurnal range/temperature annual range) (25.7 %), and elevation (22.7 %). Our model predicted many isolated and fragmented environmental niche envelopes especially on Bali (Fig. 6A), at somewhat lower elevations than we encountered the species (Fig. 6B).

DISCUSSION

On both Bali and Lombok, *O. monticola* was found at a similar altitude but in slightly different microhabitats, more likely to be found in a forest dominated with dipterocarp trees, many epiphytic ferns, and ground covered with leaf litter. Compared to our findings on Bali, the frogs on Lombok were more evenly distributed between terrestrial and arboreal habitats. Ferns have previously been shown to create small-scale temperature and moisture refuges that buffer sensitive ectotherms, protecting not only the frogs but also many of their prey species from excessive heat and desiccation (Scheffers, 2014; Ellwood & Foster, 2004; Beaulieu et al., 2010). Ferns also offer suitable breeding habitat. We also found the eggs of *O. monticola* placed on nest ferns (*Asplenium* sp.) suggesting that they may present a more suitable microhabitat for arboreal frog breeding than other epiphytic plants (Inger, 1954). Other congeneric species such as *O. furu* (Gunther et al., 2009), *O. sibilans*, *O. unicolor*, and *O. clamata* (Gunther, 2003), also use ferns as breeding sites. Leaf litter is also one of the most important microhabitats for terrestrial frogs by providing refuge against predators, great densities of arthropod prey, as well as adequate conditions for egg-laying (Stoler & Rick, 2011; Sluys et al., 2007). *Oreophryne monticola* has been categorised as a terrestrial breeder (IUCN SSC Amphibian Specialist Group, 2018), however, we doubt that leaf litter is the main microhabitat for *O. monticola*. Although leaf litter can be sufficiently humid, *O. monticola* still uses nest ferns as breeding sites, indicating arboreal adaptations. In other amphibians, placing the eggs in between the canopy and ground vegetation is an attempt to avoid both arboreal and ground-dwelling predators (Stewart, 1985). These findings suggest *O. monticola* is a semi-arboreal frog.

In our survey, we found the species at about only 1400 m a.s.l. (Figure 6B). A previous rainy season survey in Lombok (Rinjani mountain) recorded seven *O. monticola* at an altitude of 1250 m a.s.l. (Septian, 2016). This species was previously found as low as 1000 m a.s.l. (Iskandar, 1998), and we heard the calls of this frog at 1000 m a.s.l. in sampled sites in Lombok. This indicates that if suitable forest exists at lower elevations then the species can persist there.

The call recordings of *O. monticola* from the two islands showed differences in dominant frequency, although this may be significantly affected by variables such as altitude, humidity, and temperature (Goutte, 2013). There was also a difference in bandwidth which may result from differences in

body size of the calling frogs (Kime, 2004). Call characteristics can be defined by the coefficient of variation (CV), where the static type has a CV of less than 12 % while dynamic type has a CV of greater than 12 % (Gerhardt, 1991). Based on the CV value, male individuals from Lombok may just be engaging with other males, while males in Bali were probably calling to attract females to make a mate choice (advertisement calls). These differences add urgency for further call recordings to compare these populations to ensure the vocalisations are comparable given the same behavioral context.

The body colour variations and vocalisations of *O. monticola* suggest the possible presence of cryptic species. The populations of the two islands have most likely be isolated for a long time since the two islands have been separated for ~12.7-24.4 million years, according to the biogeographical history of Limnonectes in Lesser Sunda isles (Reilly et al., 2019b). Further comprehensive assessment of morphological and molecular characteristics may help to resolve this issue.

According to our spatial analysis prediction, there are 9 isolated areas at around 1100 m a.s.l. on both islands that may be home to *O. monticola* provided that they have not already been extirpated since fragmented and isolated populations are prone to terminal decline. Further studies are required to confirm the predicted distribution and the results of such study will improve the understanding of this species' conservation status and what steps should be taken to secure its future.

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