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CONTENT

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UDC: 597.82(594)

Nia Kurniawan

Genetic divergence and geographic distribution of frogs in genus *Fejervarya* from Indonesia inferred from mitochondrial 16S rRNA gene analysis

TREUBIA, December 2014, Vol. 41, pp. 1–16.

The Indonesian archipelago is an ideal setting for the study of speciation and biogeography. This archipelago is divided into three island groups based on zoogeography: Sundaland, Wallacea and the Australian region. In this paper we used frogs in genus *Fejervarya* (Bolkay) to study biogeography and examine patterns of gene flow across proposed zoogeographic boundaries. Several molecular studies on *Fejervarya* species from Indonesia have been carried out, but comparative studies among members of the genus *Fejervarya* have yet to be performed. In order to elucidate genetic divergence and geographic distribution of these frogs, we conducted a molecular analysis of the mitochondrial 16S rRNA gene using 179 frogs from five *Fejervarya* species. In total we collected from 32 localities in Sumatra, Kalimantan (Indonesian part of Borneo), Java, Bali, Sulawesi and Lesser Sunda Islands in Indonesia. Molecular phylogenetic analysis recovered 35 haplotypes and showed that frogs in the genus *Fejervarya* were divided into two well-supported clades. The first group were of three species, *F. limnocharis*, *F. iskandari* and *F.* cf. *verruculosa* and the other group clade consisted of *Fejervarya cancrivora* and *Fejervarya* sp. (Sulawesi-type). The average sequence divergence among these four species ranged from 1.09 to 16.03% (mean = 11.29± 2.83%). The present results clearly show that there are five *Fejervarya* species in the Indonesian archipelago. *Fejervarya limnocharis* and *F. cancrivora* are widely distributed and sympatric in Sumatra, Borneo and Java. *Fejervarya iskandari* is not endemic to Java and also occurs in the Lesser Sundas. *Fejervarya* cf. *verruculosa* and *Fejervarya* sp. (Sulawesi-type) are endemic to Lesser Sunda and Sulawesi Island, respectively.

(Nia Kurniawan, Tjong Hon Djong, Tesri Maideliza, Amir Hamidy, Mahmudul Hasan, Takeshi Igawa and Masayuki Sumida)

Key words: *Fejervarya*, genetic divergence, geographic distribution, 16S rRNA gene

UDC: 595.78(594.53)

Djunijanti Peggie

Butterflies of Gunung Halimun-Salak National Park, Java, Indonesia, with an overview of the area importance

TREUBIA, December 2014, Vol. 41, pp. 17–30.

Data on the occurrence of butterfly species at Gunung Halimun-Salak National Park is presented based on collections and observations obtained in 2004, 2007, 2009 and 2010. In total, 161 butterfly species (10 Hesperiidae, 23 Lycaenidae, 86 Nymphalidae, 17 Papilionidae, 21 Pieridae, and 4 Riodinidae) were recorded. Of the total number of species, 133 were recorded from Gunung Halimun and 82 were recorded from Gunung Salak. The occurrence of butterflies at this national park was compared with data known from other localities in Java. The significance of Gunung Halimun-Salak NP in terms of the butterfly diversity is discussed.

(Djunijanti Peggie and Harmonis)

Key words: butterflies, endemic species, Gunung Halimun-Salak National Park, Java, occurrence

UDC: 595.34

Mulyadi

Taxonomic problems on four species of *Pontella* (Copepoda, Calanoida) described by A. Scott (1909) in Indo-Malayan waters

TREUBIA, December 2014, Vol. 41, pp. 31–50.

Four species of *Pontella,* i.e., *P. alata, P. cerami, P. denticauda*, and *P. forficula*, which were originally described by A. Scott (1909) were found from Indo-Malayan waters. Some misidentifications resulting in wrong species identity were discovered on *P. cerami* and *P. forficula. Pontella cerami* A. Scott, 1909, described based on two male

specimens from the Banda Sea, Indonesia is here recognised as the male of *P. alata.* Similarly, *P. forficula,* also known from two male specimens from the Sulu Sea, Philippine must be reassigned as the male of *Ivellopsis elephas* (Brady, 1883). Another Indo-Malayan *Pontella*, *i.e.*, *P. denticauda* A. Scott, 1909 must also be moved to the genus *Ivellopsis* Claus 1893, as *Ivellopsis denticauda* (A. Scott, 1909) by its having posterior corners of Pdg5 produced into rounded lobes in both sexes; particularly in the female, by (1) the genital double -somite with a large lateral process, (2) the CR asymmetrical with the right ramus longer than the left, and (3) the Re of P5 with 3 apical spines and with an acuminate Ri. The male has, (1) the CR asymmetrical with right ramus slightly longer than the left, and (2) the thumb of Re2 of right P5 is elongated, and (3) the Re2 of the left P5 bifurcate at apex.

Descriptions, measurements and figures of the four species are given, along with a review of their distribution and that of their species groups over Indo-West Pacific waters, together with taxonomic remarks and synonymies in each case.

(Mulyadi)

Key words: Copepoda, Indo-Malayan, *Pontella*, small islands, taxonomy

UDC: 599.323.4(594.2)

Anang Setiawan Achmadi

New records of two rarely encountered, endemic rats (Rodentia: Muridae: Murinae) from Gunung Gandangdewata, West Sulawesi Province

TREUBIA, December 2014, Vol. 41, pp. 51–60.

We collected specimens of Sommer's Sulawesi shrew-rat, *Sommeromys macrorhinos*, at three sites (1600, 2200, and 2600 m) and the Sulawesi small-bodied shrew-rat, *Crunomys celebensis*, at one site (1600 m) on Gunung Gandangdewata in the western block of the central core of Sulawesi during November 2011 and May 2012. Prior to 2011, *S. macrorhinos* was known only from the holotype, which was taken on 2 August 1973 at 2400 m near the summit of Gunung Tokala (upper montane forest). Previously, *C. celebensis* was known only from tropical lowland evergreen rain forest in the Danau Lindu valley and nearby upper drainage of the Sungai Miu in the northern portion of the west-central mountain block in Sulawesi's central core. The new specimens of *S. macrorhinos* and *C. celebensis* extend their known range of habitats to include the transition between lowland and montane forest. Because the original description of *S. macrorhinos* was based on a single specimen, we describe some external morphological features and provide measurements of new specimens as a supplement to the original description.

(Anang Setiawan Achmadi, Kevin C. Rowe and Jacob A. Esselstyn)

Key words: *Crunomys celebensis,* morphology, shrew-rat, *Sommeromys macrorhinos*

UDC: 598.2(594.25)

Frank E. Rheindt

New and significant island records, range extensions and elevational extensions of birds in eastern Sulawesi, its nearby satellites, and Ternate

TREUBIA, December 2014, Vol. 41, pp. 61-90.

The Wallacean Region continues to be widely unexplored even in such relatively well-known animal groups as birds (Aves). We report the results of an ornithological expedition from late Nov 2013 through early Jan 2014 to eastern Sulawesi and a number of satellite islands (Togian, Peleng, Taliabu) as well as Ternate, providing details on numerous first records of bird species outside their previously known geographic or elevational ranges observed or otherwise recorded during this expedition. We also document what appears to be a genuinely new taxon, possibly at the species level, of kingfisher from Sulawesi that has been overlooked by previous ornithologists. Our results underscore our fragmentary knowledge of the composition of the avifauna of eastern Indonesia, and demonstrate that there continues to be a high degree of cryptic, undescribed avian diversity on these islands.

(Frank E. Rheindt, Dewi M. Prawiradilaga, Suparno, Hidayat Ashari and Peter R. Wilton)

Key words: birds of eastern Sulawesi, elevational extensions, new island records, range extensions

GENETIC DIVERGENCE AND GEOGRAPHIC DISTRIBUTION OF FROGS IN GENUS *FEJERVARYA* FROM INDONESIA INFERRED FROM MITOCHONDRIAL 16S rRNA GENE ANALYSIS

Nia Kurniawan*[1], Tjong Hon Djong[2], Tesri Maideliza[2], Amir Hamidy[3], Mahmudul Hasan[4], Takeshi Igawa[4]and Masayuki Sumida[4]

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ABSTRACT

The Indonesian archipelago is an ideal setting for the study of speciation and biogeography. This archipelago is divided into three island groups based on zoogeography: Sundaland, Wallacea and the Australian region. In this paper we used frogs in genus *Fejervarya* (Bolkay) to study biogeography and examine patterns of gene flow across proposed zoogeographic boundaries. Several molecular studies on *Fejervarya* species from Indonesia have been carried out, but comparative studies among members of the genus *Fejervarya* have yet to be performed. In order to elucidate genetic divergence and geographic distribution of these frogs, we conducted a molecular analysis of the mitochondrial 16S rRNA gene using 179 frogs from five *Fejervarya* species. In total we collected from 32 localities in Sumatra, Kalimantan (Indonesian part of Borneo), Java, Bali, Sulawesi and Lesser Sunda Islands in Indonesia. Molecular phylogenetic analysis recovered 35 haplotypes and showed that frogs in the genus *Fejervarya* were divided into two well-supported clades. The first group were of three species, *F. limnocharis*, *F. iskandari* and *F.* cf. *verruculosa* and the other group clade consisted of *Fejervarya cancrivora* and *Fejervarya* sp. (Sulawesi-type). The average sequence divergence among these four species ranged from 1.09 to 16.03% (mean = 11.29±2.83%). The present results clearly show that there are five *Fejervarya* species in the Indonesian archipelago. *Fejervarya limnocharis* and *F. cancrivora* are widely distributed and sympatric in Sumatra, Borneo and Java. *Fejervarya iskandari* is not endemic to Java and also occurs in the Lesser Sundas. *Fejervarya* cf. *verruculosa* and *Fejervarya* sp. (Sulawesi-type) are endemic to Lesser Sunda and Sulawesi Island, respectively.

Key words: *Fejervarya*, genetic divergence, geographic distribution, 16S rRNA gene

INTRODUCTION

The Indonesian archipelago is an ideal setting for studying speciation and biogeography. The large number of islands and complicated geologic history of the region have produced a diverse biota. The archipelago is divided into three island groups, Sundaland, Wallacea and the Australian region. Among these islands, there are several zoogeographic boundaries that have been proposed. The border of Sundaland and Wallacea (also known as Wallace`s line) lies between Borneo and Sulawesi and also separates Bali

from the Lombok Islands. The border of Wallacea and the Australian region (also known as Weber`s line) lies between Sulawesi and the Moluccas islands and also separates the Lesser Sundas from Australia (Moss & Wilson 1998). The land masses of Sundaland are Sumatra, Borneo, Java, and Bali, while the Australian region contains the Moluccas and Papua Islands. Wallacea consists of the Lesser Sundas and Sulawesi Island (Moss & Wilson 1998, Hall 2001, Tingay *et al.* 2010).

Van Kampen (1923) suggested that three *Fejervarya* species, *F. cancrivora*, *F. limnocharis* and *F. verruculosa*, occur on several islands in the Indonesia archipelago. Those islands are Pulau Weh, Sumatra, Riau, Bangka, Natuna, Borneo, Pulau Laut, Java, Madura, Bali, Lombok, Sumbawa, Flores, Roti, Sumba, Timor, Selayar, Buton, Sulawesi and Wetar Islands. Presently, Indonesia is thought to be home to four *Fejervarya* species: *Fejervarya cancrivora*, *F. limnocharis*, *F. iskandari*, and *F. verruculosa*. Three of these species: *F. cancrivora*, *F. limnocharis*, *F. iskandari* were described from the type locality of West Java, while the type locality of *F. verruculosa* is Wetar, Iliwaki in Lesser Sunda (Iskandar 1998, Dubois & Ohler 2000, Veith *et al.* 2001). The species *F. cancrivora* and *F. limnocharis* are thought to be widely distributed in Indonesia from Sundaland as far as Lesser Sunda but *F. iskandari* has been reported as endemic to Java (Iskandar 1998, Frost 2009). Recently, *Fejervarya* sp. (Sulawesi-type) was found on Sulawesi Island (Kurniawan *et al.* 2010).

Although several molecular studies have been carried out on the genus *Fejervarya* from Indonesia (Veith *et al.* 2001, Djong *et al.* 2007a,b, Sumida *et al.* 2007, Kotaki *et al.* 2008, Kurniawan *et al.* 2010), an extensive comparison across Indonesian species has yet to be performed. Since 16S rRNA gene sequences were widely used for barcoding amphibians (Vences *et al.* 2005), we investigated genetic divergence and geographic distribution of the genus *Fejervarya* inhabiting Indonesia using this marker.

In the present study, we therefore sought to elucidate levels of genetic divergence and identify the geographic distribution of distinct evolutionary lineages in the genus *Fejervarya* from Indonesia.

MATERIALS AND METHODS

Molecular analysis

We used 179 individuals from five species that were collected from 32 localities in Indonesia. These individuals originated from 13 localities on Sumatra, nine on Java, four on Sulawesi, one on Borneo, one on Bangka, one on Bali, and three localities from the Lesser Sundas (Fig. 1; Table 1).

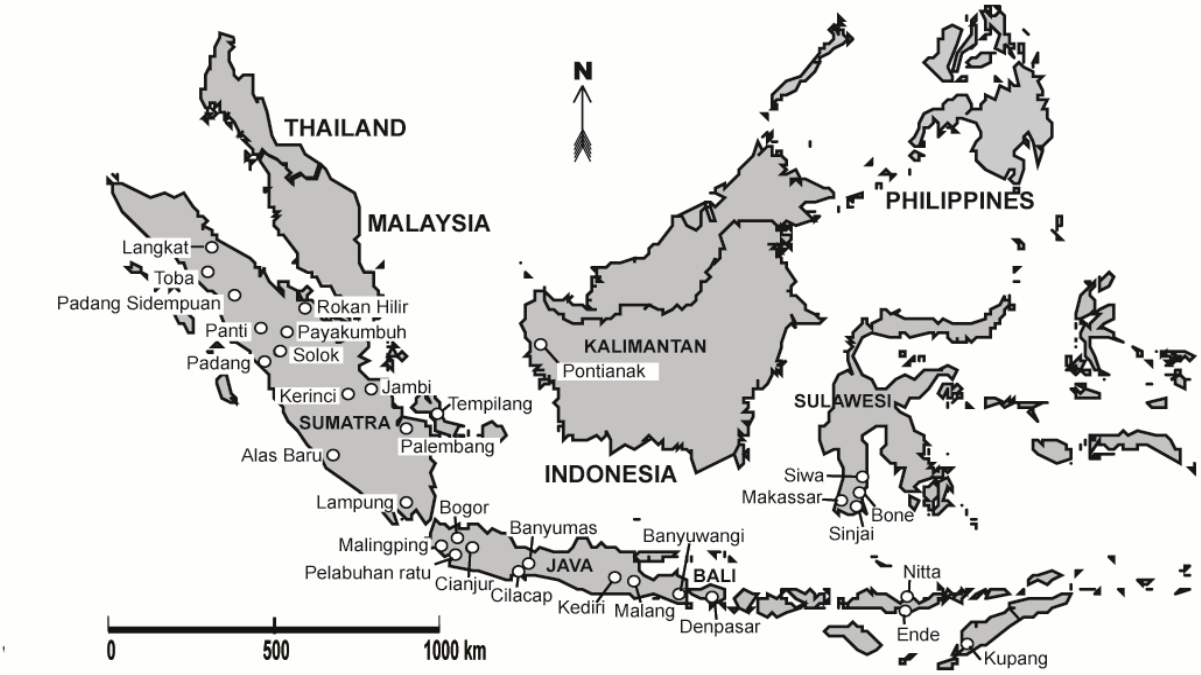

Figure 1. Map showing the sampling localities of *Fejervarya* used in the present study.

Table 1. List of species, haplotype name, accession number, locality and number of samples used in this study

Species	Haplotype Name	Access No.	Source	Locality	No. Indiv.
F. limnocharis	FL-1	AB570262	This study	Padang, Sumatra	2
	FL-2	AB570263	This study	Payakumbuh, Sumatra	3
	FL-3	AB570264	This study	Tempilang, Bangka	3
	FL-4	AB570265	This study	Jambi, Sumatra	2
	FL-4	AB570265	This study	Rokan Hilir, Sumatra	2
	FL-5	AB570266	This study	Alas Baru Bengkulu, Sumatra	3
	FL-5	AB570266	This study	Toba, Sumatra	1
	FL-6	AB570267	This study	Kerinci Jambi, Sumatra	3
	FL-6	AB570267	This study	Lampung, Sumatra	3
	FL-6	AB570267	This study	Langkat, Sumatra	2
	FL-6	AB570267	This study	Padang Sidempuan, Sumatra	3
	FL-6	AB570267	This study	Palembang, Sumatra	2
	FL-6	AB570267	This study	Panti, Sumatra	3
	FL-6	AB570267	This study	Solok, Sumatra	3
	FL-6	AB570267	This study	Toba, Sumatra	1
	FL-6	AB570267	This study	Pontianak, Kalimantan	2
	FL-6	AB570267	This study	Malimping, Java	1
	FL-7	AB277302	(Kotaki *et al*. 2008)	Bogor, Java	1
F. iskandari	FI-1	AB277303	(Kotaki *et al*. 2008)	Cianjur, Java	1
	FI-2	AB570268	This study	Malang, Java	1
	FI-3	AB570269	This study	Malang, Java	1
	FI-4	AB570270	This study	Malang, Java	1
	FI-5	AB570271	This study	Banyuwangi, Java	2
	FI-5	AB570271	This study	Malang, Java	8
	FI-5	AB570271	This study	Ende, Lesser Sunda	4
	FI-5	AB570271	This study	Kupang, Lesser Sunda	5
	FI-6	AB570272	This study	Banyuwangi, Java	14
F. cf. *verruculosa*	FCV-1	AB606420	This study	Nitta, Lesser Sunda	4
	FCV-1	AB606421	This study	Ende, Lesser Sunda	3
F. cancrivora	FC-1	AB444684	(Kurniawan *et al*. 2010)	Langkat, Sumatra	1
	FC-1	AB444684	(Kurniawan *et al*. 2010)	Tempilang, Bangka	1

Species	Haplotype Name	Access No.	Source	Locality	No. Indiv.
	FC-1	AB444684	(Kurniawan *et al*. 2010)	Bogor, Java	1
	FC-1	AB444684	(Kurniawan *et al*. 2010)	Cianjur, Java	1
	FC-1	AB444684	(Kurniawan *et al*. 2010)	Pelabuhan ratu, Java	1
	FC-2	AB444685	(Kurniawan *et al*. 2010)	Padang, Sumatra	1
	FC-2	AB444685	(Kurniawan *et al*. 2010)	Panti, Sumatra	1
	FC-2	AB444685	(Kurniawan *et al*. 2010)	Payakumbuh, Sumatra	1
	FC-3	AB570273	This study	Jambi, Sumatra	1
	FC-3	AB570273	This study	Lampung, Sumatra	1
	FC-3	AB570273	This study	Palembang, Sumatra	1
	FC-3	AB570273	This study	Pontianak, Kalimantan	6
	FC-3	AB570273	This study	Banyumas, Java	1
	FC-3	AB570273	This study	Banyuwangi, Java	1
	FC-3	AB570273	This study	Cilacap, Java	9
	FC-3	AB570273	This study	Kediri, Java	2
	FC-3	AB570273	This study	Malang, Java	2
	FC-3	AB570273	This study	Denpasar, Bali	6
	FC-4	AB570274	This study	Denpasar, Bali	1
	FC-5	AB570275	This study	Denpasar, Bali	1
	FC-6	AB570276	This study	Denpasar, Bali	1
	FC-7	AB570277	This study	Denpasar, Bali	1
Fejervarya sp (Sulawesi -type)	FS-1	AB444493	(Kurniawan *et al*. 2010)	Pelabuhan ratu, Java	1
	FS-1	AB444493	(Kurniawan *et al*. 2010)	Bone, Sulawesi	1
	FS-1	AB444493	(Kurniawan *et al*. 2010)	Sinjai, Sulawesi	1
	FS-1	AB444493	(Kurniawan *et al*. 2010)	Siwa, Sulawesi	1
	FS-2	AB570278	This study	Makassar, Sulawesi	3
	FS-3	AB570279	This study	Siwa, Sulawesi	1
	FS-4	AB570280	This study	Siwa, Sulawesi	1
	FS-5	AB570281	This study	Siwa, Sulawesi	1
	FS-6	AB570282	This study	Bone, Sulawesi	2
	FS-7	AB570283	This study	Makassar, Sulawesi	3
	FS-8	AB570284	This study	Makassar, Sulawesi	1
	FS-9	AB570285	This study	Makassar, Sulawesi	1
	FS-10	AB570286	This study	Makassar, Sulawesi	1
	FS-11	AB570287	This study	Makassar, Sulawesi	32
	FS-12	AB570288	This study	Makassar, Sulawesi	1
	FS-13	AB570289	This study	Makassar, Sulawesi	1
	FS-14	AB570290	This study	Makassar, Sulawesi	1

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from clipped toes using a DNA extraction kit (DNA easy Tissue Kit, QIAGEN) according to the manufacturer's instructions. One pair of primers (F51 and R51) (Sumida *et al.* 2002) was used for the amplification and sequencing of the 5′portion of the 16S rRNA gene, corresponding to positions 6189–6761 of the *F. limnocharis* complete mitochondrial (mt) genome (Liu *et al.* 2005). We used the following primer sequences: F51 (5′-CCC GCC TGT TTA CCA AAA ACA T-3′) and R51 (5′-GGT CTG AAC TCA GAT CAC GTA-3′). PCR mixtures were prepared using the TaKaRa Ex TaqKit in a final volume of 50 µL. The 16S rRNA gene fragment was amplified for 35 cycles, each cycle consisting of denaturation for 10 s at 98ºC, annealing for 30 s at 47.5ºC, and extension for 80s at 72ºC. Purified mtDNA gene fragments were

directly sequenced using the BigDye Terminator Cycle Sequencing Kit (ABI) equipped with an automated DNA Sequencer (3100-Avant, ABI). The sequences obtained were deposited in the DNA Data Bank of Japan (DDBJ, accession numbers: AB570262–AB570290).

Sequence data analysis

Alignment for nucleotide sequences was determined based on maximum nucleotide similarity using CLUSTAL W (Thompson *et al.* 1994). Gaps and ambiguous sites were excluded using default settings in the program GBlocks 0.91b (Castresana 2000). We used the dicroglossid *Limnonectes fujianensis* from China (Accession No. AY974191, Nie *et al*. unpublished) as an out group in all phylogenetic comparisons. Sequence divergence was calculated using the uncorrected "p" distance, while the phylogenetic relationships were estimated by maximum-parsimony (MP) and neighbor-joining (NJ) with 1000 bootstrap replicates by using PAUP* 4.0b10 (Swofford 2002). The MP tree was constructed using a heuristic search with 10 replicates that used simple sequence addition and tree bisection reconnection (TBR) criteria. Maximum-likelihood (ML) analyses were performed with 1000 bootstrap pseudoreplicates using Treefinder (Jobb *et al.* 2004). We used the program Kakusan 3.0 (Tanabe 2007) to identify the best-fitting model for ML and NJ analyses via likelihood scores generated using the Akaike Information Criterion (AIC). Mr. Bayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) was used for BI analyses. For BI analyses, the number of Markov chain Monte Carlo (MCMC) generations were three million with sampling occurring every 100 generations. We determined the appropriate number of burn-in generations by assessing convergence of -log likelihood (-ln*L*) and tree length against generation number using Tracer ver. 1.4 (Drummond & Rambaut 2007).This method resulted in 10% of trees being discarded (3000 trees). All MCMC runs were repeated twice to confirm consistent approximation of posterior parameter distributions. A haplotype network tree of 16S gene data was constructed using a median-joining network (Bandelt *et al.* 1999, Network 4.502 available at http://www.fluxus-engineering.com).

RESULTS

Molecular Data

The 16S rRNA gene sequenced from 179 individuals consisted of 368 bp, of which 124 were variable sites and 83 were parsimony informative. An alignment of these data revealed 35 haplotypes (Table 2). The substitution model selected for the data set was the

J2+G model with a gamma distribution shape parameter of 0.0489. For the ML analyses, the empirical base frequencies were T=0.2446, C=0.2575, A=0.2848, and G=0.2131. Our phylogenetic reconstructions suggest that there are well supported evolutionary lineages *Fejervarya* in Indonesia. Each of these lineages corresponds to a species that we sampled. Our topological results revealed two main clades: one clade includes three species; *F. limnocharis*, *F. Iskandari* and, *F.* cf. *verruculosa*, and the other clade includes *F. cancrivora* and *Fejervarya* sp. (Sulawesi-type). Within *F. limnocharis* we recovered seven haplotypes. For *F. iskandari* we recovered six *haplotypes*, while a single DNA sequence resulted from all sampled *F.* cf. *verruculosa* (Fig. 2). The *F. cancrivora* we sampled possessed seven haplotypes, and the *Fejervarya* sp. (Sulawesi-type) group were contain of 14 haplotypes (Fig. 2).The number of nucleotide substitutions between the *F. limnocharis* and *F. iskandari* and *F.* cf. *verruculosa*, *F. cancrivora*, and *Fejervarya* sp. (Sulawesi-type) sub clades was 34, 48, and 45 base pairs (bp), respectively (Fig. 4). The number of nucleotide substitutions between the *F. iskandari* and *F.* cf. *verruculosa* sub clade and the *F. cancrivora* and *Fejervarya* sp. (Sulawesi-type) sub clades was 42 and 35 bp, respectively (Fig. 4). The number of nucleotide substitutions between the *F. cancrivora* and *Fejervarya* sp. (Sulawesi-type) sub clades was 15 bp (Fig. 4).

Percent sequence divergence for the 16S rRNA gene within and between the five *Fejervarya* species is listed in Tables 2 and 3. The sequence divergence within *F. limnocharis*, *F. iskandari*, *F. cancrivora*, and *Fejervarya* sp. (Sulawesi-type) was 1.04 ±0.74% (0.30–2.40%), 0.74 ± 0.33% (0.27–1.09%), 0.65 ± 0.28% (0.27–1.09%), and 2.16 ± 1.49% (0.27–5.71%), respectively. Support values in the following section are reported in the following order: ML/MP/NJ/BI. A hypothesis of monophyly for the clade containing *F. limnocharis* group and *F. iskandari* and *F.* cf. *verruculosa* received moderate support 77/78/73/95. Within this clade, the *F. l imnocharis* we sampled clustered together with strong support: 100/100/100/100. The *F.* cf. *verruculosa* and *F. iskandari* haplotypes were recovered in the same clade which featured strong support (98/100/100/100), but little support was received to distinguish between *F. iskandari* and *F.* cf. *verruculosa* haplotypes (Fig. 2). In clade containing *F. cancrivora* and *Fejervarya sp*. (Sulawesi-type) received strong support: 95/99/99/100. Within this clade, the *F. cancrivora* individuals clustered together with strong support (99/100/100/76), while the clustering of *Fejerverya* sp. (Sulawesi-type) haplotypes received only moderate support : 82/64/68/83 (Fig. 2).

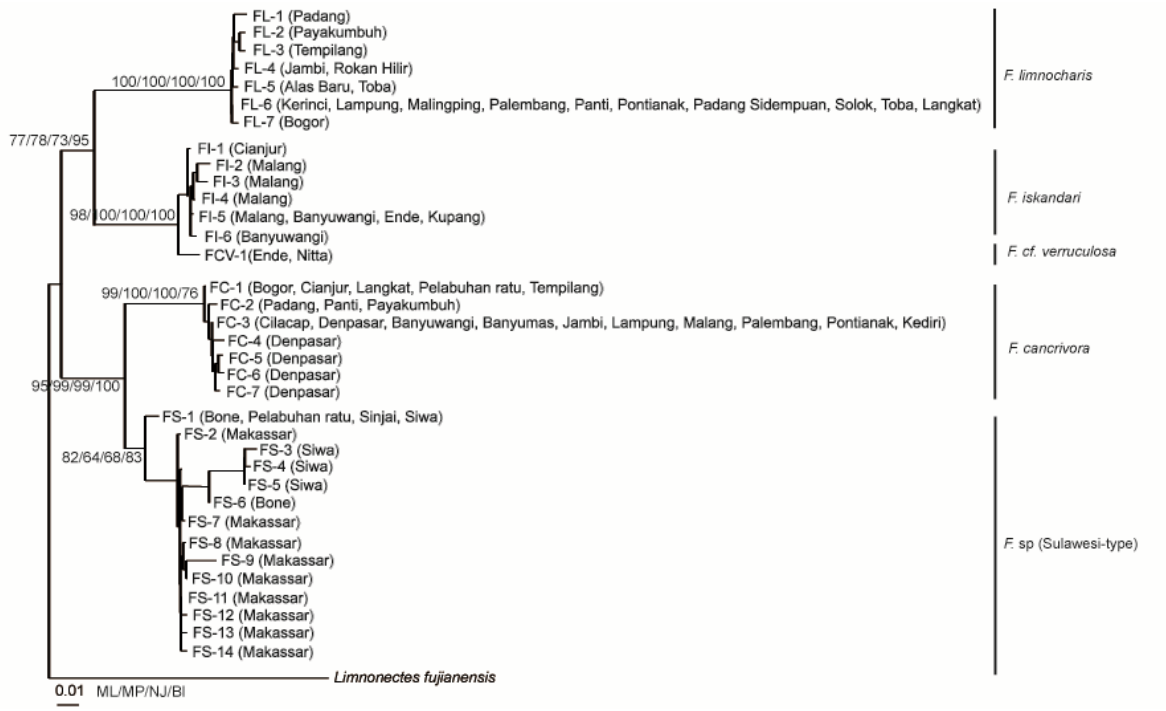

Figure 2. Phylogenetic tree constructed using the maximum-likelihood method based on a 368-bp segment of the mitochondrial 16S rRNA gene.

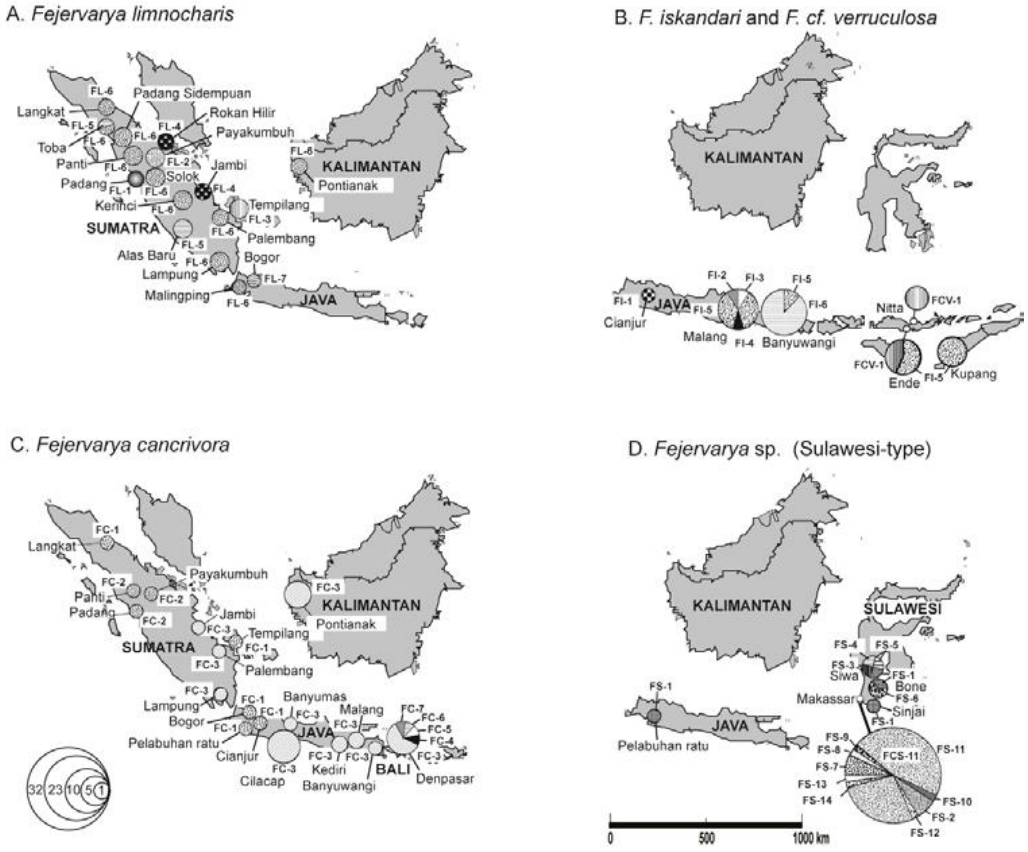

Figure 3. Frequencies and geographic distribution of mitochondrial haplotypes from the four *Fejervarya* lineages found in Sundaland.

Table 2. Percent sequence divergences estimated by uncorrected "p" distance based on the 368-bp fragment of 16S rRNA gene among the haplotypes of genus *Fejervarya*. FL = *Fejervarya limnocharis*, FI = *Fejervarya iskandari*, FCV = *Fejervarya* cf. *verruculosa* FC = *Fejervarya cancrivora* and FS = *Fejervarya* sp. (Sulawesi-type)

Haplotype	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
FL-1	1	-																																			
FL-2	2	2.2	-																																		
FL-3	3	2.2	0.5	-																																	
FL-4	4	1.9	0.8	0.8	-																																
FL-5	5	1.9	0.8	0.8	0.5	-																															
FL-6	6	2.2	0.5	0.5	0.3	0.3	-																														
FL-7	7	2.4	0.8	0.8	0.5	0.5	0.3	-																													
FI-1	8	12.0	10.6	10.6	10.3	10.3	10.1	10.3	-																												
FI-2	9	12.8	11.1	11.1	10.9	10.9	10.6	10.9	1.1	-																											
FI-3	10	12.8	11.1	11.1	10.9	10.9	10.6	10.9	1.1	1.1	-																										
FI-4	11	12.2	10.6	10.6	10.3	10.3	10.1	10.3	0.5	1.1	0.5	-																									
FI-5	12	12.0	10.3	10.3	10.1	10.1	9.8	10.1	0.3	0.8	0.8	0.3	-																								
FI-6	13	12.2	10.6	10.6	10.3	10.3	10.1	10.3	0.5	1.1	1.1	0.5	0.3	-																							
FCV-1	14	12.0	10.9	10.9	10.6	10.6	10.3	10.6	1.6	2.2	2.2	1.6	1.4	1.1	-																						
FC-1	15	15.2	14.1	14.1	13.9	13.9	13.6	13.9	11.7	12.5	12.5	12.0	11.7	12.0	12.5	-																					
FC-2	16	14.9	14.1	14.1	13.9	13.9	13.6	13.9	12.2	13.0	13.0	12.5	12.2	12.5	12.8	0.8	-																				
FC-3	17	14.9	13.9	13.9	13.6	13.6	13.3	13.6	12.0	12.8	12.8	12.2	12.0	12.2	12.8	0.3	0.5	-																			
FC-4	18	15.5	14.4	14.4	14.1	14.1	13.9	14.1	12.5	13.3	13.3	12.8	12.5	12.8	13.3	0.8	1.1	0.5	-																		
FC-5	19	15.5	14.4	14.4	14.1	14.1	13.9	14.1	12.5	13.3	13.3	12.8	12.5	12.8	13.3	0.8	1.1	0.5	1.1	-																	
FC-6	20	15.2	14.1	14.1	13.9	13.9	13.6	13.9	12.2	13.0	13.0	12.5	12.2	12.5	13.0	0.5	0.8	0.3	0.8	0.3	-																
FC-7	21	15.2	14.1	14.1	13.9	13.9	13.6	13.9	12.2	13.0	13.0	12.5	12.2	12.5	13.0	0.5	0.8	0.3	0.8	0.3	0.5	-															
FS-1	22	13.6	13.0	13.0	12.8	12.8	12.5	12.8	10.6	11.4	11.4	10.9	10.6	10.9	10.9	4.1	4.6	4.3	4.9	4.9	4.6	4.6	-														
FS-2	23	13.3	12.8	12.8	12.5	12.5	12.2	12.5	10.1	10.9	10.9	10.3	10.1	10.3	10.3	6.0	6.5	6.3	6.8	6.8	6.5	6.5	2.4	-													
FS-3	24	16.0	15.5	15.5	15.2	15.2	14.9	15.2	13.3	13.9	13.3	13.0	13.3	13.6	13.6	9.2	9.2	9.5	10.1	10.1	9.8	9.8	5.7	3.8	-												
FS-4	25	15.8	15.2	15.2	14.9	14.9	14.7	14.9	13.0	13.6	13.0	12.8	13.0	13.3	13.3	8.7	9.2	9.0	9.5	9.5	9.2	9.2	5.2	3.3	0.5	-											
FS-5	26	16.0	15.5	15.5	15.2	15.2	14.9	15.2	13.3	13.9	13.3	13.0	13.3	13.6	13.6	9.0	9.5	9.2	9.8	9.8	9.5	9.5	5.4	3.5	0.8	0.3	-										
FS-6	27	14.1	13.6	13.6	13.3	13.3	13.0	13.3	11.4	12.2	11.7	11.1	11.4	11.7	11.7	7.3	7.9	7.6	8.2	8.2	7.9	7.9	3.5	1.6	2.2	1.6	1.9	-									
FS-7	28	13.6	13.0	13.0	12.8	12.8	12.5	12.8	10.1	10.9	10.9	10.3	10.1	10.3	10.3	6.0	6.5	6.3	6.8	6.8	6.5	6.5	2.4	0.5	3.5	3.0	3.3	1.4	-								
FS-8	29	13.9	13.3	13.3	13.0	13.0	12.8	13.0	10.6	11.4	11.4	10.9	10.6	10.9	10.9	6.3	6.8	6.5	7.1	7.1	6.8	6.8	2.4	0.5	3.5	3.0	3.3	1.6	0.5	-							
FS-9	30	15.8	15.2	15.2	14.9	14.9	14.7	14.9	12.5	13.3	13.3	12.8	12.5	12.8	12.8	7.9	8.4	8.2	8.7	8.7	8.4	8.4	4.3	2.4	5.4	4.9	5.2	3.5	2.4	1.9	-						
FS-10	31	13.9	13.3	13.3	13.0	13.0	12.8	13.0	10.6	11.4	11.4	10.9	10.6	10.9	10.9	6.5	7.1	6.8	7.3	7.3	7.1	7.1	2.4	0.5	3.8	3.3	3.5	1.6	0.5	0.5	1.9	-					
FS-11	32	13.6	13.0	13.0	12.8	12.8	12.5	12.8	10.3	11.1	11.1	10.6	10.3	10.6	10.6	6.3	6.8	6.5	7.1	7.1	6.8	6.8	2.2	0.3	3.5	3.0	3.3	1.4	0.3	0.3	2.2	0.3	-				
FS-12	33	13.9	13.3	13.3	13.0	13.0	12.8	13.0	10.6	11.4	11.4	10.9	10.6	10.9	10.9	6.5	7.1	6.8	7.3	7.3	7.1	7.1	2.4	0.5	3.8	3.3	3.5	1.6	0.5	0.5	2.4	0.5	0.3	-			
FS-13	34	13.9	13.3	13.3	13.0	13.0	12.8	13.0	10.6	11.4	11.4	10.9	10.6	10.9	10.9	6.5	7.1	6.8	7.3	7.3	7.1	7.1	2.4	0.5	3.8	3.3	3.5	1.6	0.5	0.5	2.4	0.5	0.3	0.5	-		
FS-14	35	13.9	13.3	13.3	13.0	13.0	12.8	13.0	10.6	11.4	11.4	10.9	10.6	10.9	10.9	6.5	7.1	6.8	7.3	7.3	7.1	7.1	2.4	0.5	3.8	3.3	3.5	1.6	0.5	0.5	2.4	0.5	0.3	0.5	0.5	-	
Outgroup	36	18.5	17.9	17.7	17.4	17.7	17.4	17.7	17.4	18.5	18.5	17.9	17.7	17.9	17.9	17.7	17.4	17.4	17.9	17.9	17.7	17.7	16.6	16.3	18.2	18.5	18.2	17.7	16.8	16.6	17.9	16.8	16.6	16.8	16.8	16.8	-

Table 3. Average percent sequence divergences estimated by uncorrected "p" distance for 16S rRNA gene within and between five *Fejervarya* species

Species	***F. limnocharis***	***F. iskandari***	***F.* cf. *verruculosa***	***F. cancrivora***	***Fejervarya* sp. (Sulawesi-type)**
F. limnocharis	1.04 ± 0.74				
	(0.30-2.40)				
F. iskandari	10.77 ± 0.74	0.74 ± 0.33			
	(9.78-12.77)	(0.27-1.09)			
F. cf. *verruculosa*	10.83 ± 0.53	1.68 ± 0.44			
	(10.33-11.96)	(1.09 - 2.17)			
F. cancrivora	14.12 ± 0.52	12.55 ± 0.44	12.97 ± 0.30	0.65 ± 0.28	
	(13.32-15.49)	(11.69-13.32)	(12.5 0-13.32)	(0.27-1.09)	
Fejervarya sp. (Sulawesi-type)	13.7 ± 1.05	11.55 ± 1.14	11.53 ± 1.23	7.41 ± 1.37	2.16 ± 1.49
	(12.23-16.03)	(10.05-13.86)	(10.33-13.59)	(4.08-10.05)	(0.27-5.71)
Outgroup	17.74 ± 0.38	17.98 ± 0.44	17.94	17.66 ± 0.22	17.20 ± 0.73
	(17.66-18.48)	(17.39-18.48)		(17.39-17.94)	(16.30-18.48)

DISCUSSION

Genetic divergence within the genus *Fejervarya* in Indonesia

According to Veith *et al.* (2001), *F. limnocharis* and *F. iskandari* are sympatric species. Our results suggest that *F. limnocharis* has a close genetic relationship with *F. iskandari* and *F.* cf. *verruculosa* differing from these taxa by 34 and 40 nucleotide substitutions, respectively (Fig. 4). Based on the patterns recovered in our haplotype networks (Fig. 4), it appears that *F. limnocharis* and *F. cancrivora*are widely distributed species each with a predominant haplotype: FL-6and FC-3, respectively. The presence of FL-6 and FC-3 haplotypes at many localities coupled with high per site nucleotide divergence (Fig. 4) may suggest that the geographic distribution of these haplotypes is related an adaptative benefit. *Fejervarya limnocharis* was collected from 17 localities, and *F. cancrivora* from 18 localities in Indonesia. Both species had 48 nucleotide substitutions, and sympatrically in habited in 10 localities (Figs. 2 & 3). The results also indicate that *F. cancrivora* and *Fejervarya* sp. (Sulawesi-type) comprise different genetic clusters (Fig. 2). Kurabayashi *et al.* (2005) reported that the inter-species sequence divergence of the 16S rRNA gene between species of *Fejervarya* ranged from 4.69–19.58%. The sequence divergence between *F. cancrivora* and *Fejerverya* sp. (Sulawesi-type) ranged from 4.08–10.05% with a mean value of 7.41 ± 1.37%, suggesting that using the criterion of Kurabayashi *et al.* (2005), *Fejervarya* sp. (Sulawesi-type) is a distinct species. Based on preliminary morphological observation, *F. cancrivora* is larger than *Fejervarya* sp. According to Kurniawan *et al.* (2011), comparisons of *F. cancrivora* from Cianjur and *Fejervarya* sp. from Makassar

identified 11 and 16 characters (out of 31 total) that were significantly different at the 99% confidence level for males and females respectively. Kurniawan *et al.* (2011) also identified that the free flap in the outer of fifth toe is also useful in differentiating these taxa.

Within our sampling of the genus *Fejervarya*, the haplotypes of each species had variable distribution patterns in Indonesia. In *F. limnocharis* haplotypes, FL-6 was widely distributed, FL-1 and FL-2 were found only in West Sumatra, FL-3 was found only in Southern Sumatra, FL-4 was found only in East Sumatra, FL-5 was found in North and South Sumatra, and FL-7 was found only in West Java (Fig. 3). In *F. iskandari* haplotypes, FI-1 represented West Java and FI-2-6 represented East Java (Fig. 3). In *F. cancrivora* haplotypes, FC-3 was the most common, FC-1 represented West Java and Southern Sumatra, FC-2 represented West Sumatra, and FC-4-7 represented Bali (Fig. 3). In *Fejervarya*.sp. (Sulawesi-type) haplotypes, FS-1 and FS-3-6 represented the eastern part of Southwest Sulawesi, while FS-2 and FS-7-14 represented Southwestern Sulawesi (Fig. 3).

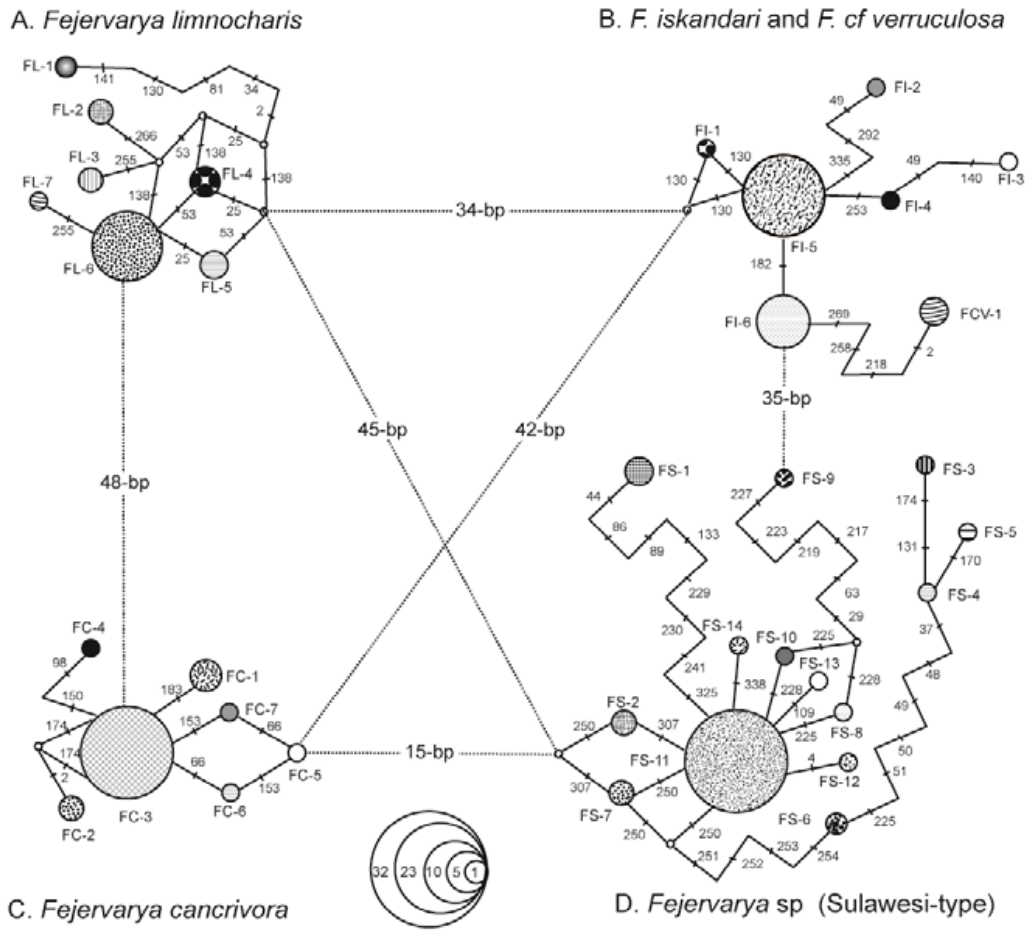

Figure 4. Haplotype network tree based on 35 haplotypes of the 16S rRNA gene sequence of 179 individuals of genus *Fejervarya*.

(a) *F. limnocharis*

According to Kurniawan (2005), *F. limnocharis* is found in Malang, East Java. Kusrini & Alford (2006) also reported that *F. limnocharis* occurs in West and East Java. Outside of Indonesia, Kotaki *et al.* (2008) reported that *F. limnocharis* occurs in the regions of Ranong, Khlong Saeng, Ratchaprabpha Dam, and Nakhon Si Thammarat in Thailand, and Kaki Bukit and Kuala Lumpur in Malaysia. This species was also found in Sabah, Malaysia (part of Borneo)(Kotaki *et al.* 2008). In the phylogenies we constructed, *F. limnocharis* from Bogor, Cianjur, and Malingping, West Java was closely related with *F. limnocharis* from Kota Kinabalu, Malaysia (part of Borneo) (Djong *et al.* 2007a). Djong *et al.* (2007b) also reported that based on a phylogenetic tree constructed from mitochondrial 16S rRNA and Cyt *b* genes, *F. limnocharis* from Kuala Lumpur, Malaysia (part of the Peninsula) and from Sabah, Malaysia clustered with *F. limnocharis* from Bogor and Malingping, West Java, Indonesia. According to Van Kampen (1923), *F. limnocharis* occurs on Lombok and Sumbawa Islands, and Iskandar (1998) mentioned that *F. limnocharis* occurs on Flores Island.

We found that FL-1 haplotype from Padang, West Sumatra is distinct relative to the other haplotypes. This haplotype is 1.9 to 2.4 percent divergent from other *F. limnocharis* samples, whereas comparisons among all other haplotypes from this species result in pair wise divergence levels below 1 percent (Table 2). Future research on individuals with this divergent haplotype is warranted since Inger (2009) described both *Rana rufipes* and *Rana parvaccola* based on morphological differences with *Rana chalconota* from Padang, West Sumatra.

(b) *F. iskandari* and *F.* cf. *verruculosa*

Iskandar (1998) reported that this species is endemic to Java and inhabits high altitude rice fields at approximately 700–1200 m above sea level. According to Djong (2007a,b), *F. iskandari* is also found at Bogor and Malingping. Iskandar (1998) reported that *F. iskandari* is found at Cianjur and Bandung. It is probable that *F. iskandari* and *F. limnocharis* shared a common ancestral species, and have remained on seperate evolutionary trajectories based on the high-altitude ecology of *F. iskandari*. However, this spatial distribution hypothesis remains in question since we found *F. iskandari* haplotypes on Flores and Timor islands (Figs. 2 & 3). Given our findings, we suspect that *F. iskandari* is not endemic to Java and also occurs in lowland rice fields found on the Lesser Sundas.

According to Van Kampen (1923), the zoogeographic boundary between the Indian and the Australian regions is not obvious in amphibians. Instead, the western part of the Indo-Australian Archipelago, including Sulawesi and the Lesser Sunda Islands, possesses a purely Indian amphibian fauna, while the eastern part, including the Moluccas and the Timor Archipelago, is a region of faunal transition where Indian and Australian communities have mixed.

Kurniawan *et al*. (2010) suggested that *F. cancrivora* was restricted to Sundaland in Southeast Asia, and that its distributional range covers the Malay Peninsula (to the south of the Isthmus of Kra), Sumatra, Borneo, Java, and Bali. These authors also demonstrated that *F. cancrivora* from Selangor, Malaysia was closely related to populations from Sumatra and Java, and also with a Borneo haplotype identified by Veith *et al.* (2001; accession number AF346810). Veith *et al.* (2000) reported that frogs exported for commerce (i.e., as frozen legs) to European countries from Indonesia that were documented as *Limnonectes macrodon, F. limnocharis, F. cancrivora*, and *Lithobates catesbeianus* were mostly *F. cancrivora*. The distribution of *F. cancrivora* in mangrove regions like southern Thailand and at Luzon (Philippines seashore) was dismissed after these populations were identified as a distinct species relative to the Sundaland lineage (Kurniawan *et al.* 2010). An individual matching the type locality and neotype of *F. cancrivora* was collected in Cianjur, West Java (Dubois & Ohler 2000). Using molecular and allozyme studies, Kurniawan (2010) matched this specimen to populations from Sundaland. *Fejervarya cancrivora* populations from Thailand were found to have a distinct free flap of skin on the outer side of the fifth toe, and metatarsal and toe webbing on their hind limbs (Kurniawan *et al.*, 2010). Taylor (1920) had previously described the Philippine form as *R.* (*Fejervarya*) *moodiei*.

In this study, we did not find evidence that *F. cancrivora* crosses Wallace's Line. However, Iskandar (1998) mentioned that *F. cancrivora* has been recently introduced to Sulawesi and Lesser Sunda. Furthermore, Van Kampen (1923) mentioned the occurrence of this species on several islands of Wallacea such as, Lombok, Sumbawa, Flores, Ombai, Sumba, Roti, Timor, Selayar, Buton, and Sulawesi Islands.

(d) *Fejervarya* sp. (Sulawesi-type)

Inger & Voris (2001) hypothesized that frogs in Sulawesi were mainly derived from Sundaland lineages. Inger (2005) proposed that *Fejervarya* sp. (Sulawesi-type) originated from Borneo and crossed Wallace's Line to Sulawesi via oceanic rafting. Based on our

results, neither theory seems appropriate. According to Kurniawan *et al.* (2010), *Fejervarya* sp. (Sulawesi- type) is distributed mostly in Southwestern Sulawesi and in Pelabuhan Ratu, West Java (Table 1). This species is thought to primarily inhabit the lowland rice fields close to the seashore. Kurniawan *et al.* (2010) further suggested that the lineage likely originated via allopatry since Southwestern Sulawesi was previously connected to Sundaland (Moss &Wilson 1998, Hall 2001, Tingay *et al.* 2010). Another explanation for this species enigmatic occurence in West Java (Pelabuhan Ratu) is that they were moved by human for use as food (Kurniawan *et al.* 2010).

Based on genetic divergence values within this group (Table 2), within *Fejervarya* sp. there are two groups. The first group is from Siwa, Bone and Sinjai localities and consists of the FS-1, FS-3, FS-4, FS-5, FS-6, and FS-9 haplotypes, which possess within group genetic diverence levels above 1.0 (ranged from 1.4 to 5.7). The second group is from Makassar (FS-2, FS-7, FS-8, FS-10, FS-11, FS-12, FS-13, and FS-14) and their within group genetic divergence levels were below 1.0 (ranged from 0.3 to 0.8) (Table 2). We note that Makassar is located on the opposite's side of Sulawesi from Siwa, Bone and Sinjai (Figs. 1 & 3). Given this geographical distance between the two lineages, a scenario of genetic isolation by distance or a complete break in gene flow is likely. Thus, we suspect that *Fejervarya* sp. (Sulawesi-type) originated from evolutionary processes associated with allopatric speciation.

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