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BIOLOGY AND CONSERVATION OF TROPICAL ASIAN AMPHIBIANS

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Edited by
Indraneil Das, Alexander Haas and Andrew Alek Tuen

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# BIOLOGY AND CONSERVATION OF TROPICAL ASIAN AMPHIBIANS

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# SYSTEMATICS OF *FEJERVARYA CANCRIVORA* COMPLEX FROM INDONESIA AND OTHER ASIAN COUNTRIES BASED ON ALLOZYME AND MOLECULAR TECHNIQUES, MORPHOLOGICAL OBSERVATIONS AND CROSSING EXPERIMENTS

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(with 18 text-figures)

**ABSTRACT.– The crab-eating frog, *Fejervarya cancrivora* is widely distributed in Asia, thus cryptic species might occur in this group. In order to elucidate the genetic divergence, evolutionary relationship and taxonomic status in *F. cancrivora*, allozyme and molecular analyses were carried out from 24 populations of frogs in Indonesia, Thailand, Bangladesh, Malaysia and the Philippines. Five populations of *F. cancrivora* from Selangor, Cianjur, Trat, Khulna and Makassar were examined morphologically and subjected to crossing experiments. Allozyme and molecular analysis of 16S rRNA and Cyt *b* genes revealed three groups of *F. cancrivora,* here dubbed large-type, mangrove-type and Pelabuhan ratu/Sulawesi-type. In addition, PCA and clustering analyses revealed that the five populations can also be separated into three groups: large-type, mangrove-type and Sulawesi-type. The limited crossing experiments showed that the hybrids between Selangor females and Cianjur and Trat males developed normally, whereas the hybrids between Selangor females and Khulna males showed incomplete gametic isolation. It was concluded that each of three identified types represents a distinct species that comprised the large-type *F. cancrivora*, the mangrove-type *F. moodiei*, and a yet undescribed species of Sulawesi-type.**

**KEY WORDS.– *Fejervarya cancrivora*, Anura, Amphibia, allozyme, mtDNA, morphometry, crossing experiments.**

## INTRODUCTION

Since the first description of the crab-eating frog, *Rana cancrivora* Gravenhorst, 1829, the name has been applied to distinguish large-sized frogs that resemble *R. limnocharis*. According to Gravenhorst's analysis, *R. cancrivora* was "larger" than *R. limnocharis* and has therefore been consistently applied to large individuals of the *R. limnocharis* complex occurring in Java and neighbouring regions. The holotype of *R. cancrivora* was originally deposited in the Breslau Museum and is considered lost. A specimen collected from a rice field in Cianjur (06°49'S, 107°08'E, West Java, Indonesia), was subsequently designated neotype (Dubois and Ohler, 2000). Dubois (1987) classified this taxon and its allies as the subgenus *Fejervarya* and proposed to remove it from the genus *Rana*. Fei et al. (1991) and Ye et al. (1993) subsequently elevated the subgenus to the rank of a genus. *Rana cancrivora* (Gravenhorst, 1829; Annandale, 1918; Boulenger, 1920; Dunn, 1928) has been referred to as *Fejervarya cancrivora* (see Iskandar, 1998; Dubois and Ohler, 2000).

*F. cancrivora* is one of the most widely distributed frog species in the Asian region, with populations extending from Guangxi and the north-eastern coast of Hainan Island, China, through Vietnam, the Andaman and Nicobar Islands (India), Peninsular Thailand, Peninsular Malaysia, Singapore, the Greater Sundas, the Philippines, and the Lesser Sundas, to as far as Flores (Frost, 2011), and also including Bangladesh (Islam et al., 2008a). Nutphund (2001) and Taylor (1962) reported that *F. raja* inhabits southern Thailand, whereas Iskandar (1998) asserted that *F. raja* from Thailand could be described as an extra large-sized specimen of *F. cancrivora*. In fact, the possibility exists that a number of the records of *F. cancrivora* in Peninsular Malaya actually refer to *F. raja*.

It has been proposed that *F. cancrivora* on Sulawesi may have been introduced from Kalimantan (Indonesian part of Borneo Island) crossing Wallace's Line (Inger, 2005), although the available data are insufficient to corroborate this speculation. *F. cancrivora* from Thailand and the Philippines has been studied using allozyme and mitochondrial gene markers (Nishioka and Sumida, 1990; Sumida et al., 2002). A preliminary study based on *F. cancrivora* isolates from Thailand, the Philippines, and the type locality in Indonesia revealed a significant divergence between these populations (Kurniawan, 2008), which implies the possibility that *F. cancrivora* includes several cryptic species that are difficult to differentiate morphologically. To date, no study has been performed to elucidate the genetic divergence among the wide-ranging populations of this species.

Investigations using allozyme analysis continue to provide valuable information on the relatedness and degree of genetic variability within and between populations (Bader, 1998; Islam et al., 2008a). 16S rRNA gene sequences are now widely used for the barcoding of vertebrates and frogs in particular (Vences et al., 2005), Cyt *b* gene sequences have proven being useful in resolving relationships among closely related taxa and are widely used in phylogenetic (Sumida et al., 1998; Parson et al., 2000; Igawa et al., 2006; Djong et al., 2007b) and phylogeographic studies (Fouquet et al., 2007; Gamble et al., 2008).

Morphological differences among species of *Fejervarya* are often small, primarily involving differences in body proportions (Veith et al., 2001; Djong et al., 2007a, b). The toe webbing and free flap in the hindlimb, however, are considered to be important traits for distinguishing *F. cancrivora* from *F. limnocharis* and *F. iskandari*, respectively (Inger, 1954, 1966; Berry, 1975; Dubois and Ohler, 2000). In West Java, Indonesia, these three *Fejervarya* species are sympatrically distributed, where *F. cancrivora* (large-type) can be reliably differentiated from *F. limnocharis* and *F. iskandari* by examining toe webbing (Iskandar, 1998). Inger (1954) reported that the Philippine population of *F. cancrivora* possessed a distinct free flap of skin on the outside of the fifth toe and metatarsal, and later described that a population from Borneo also show a less distinct flap (Inger, 1966).

The aims of the present study were to elucidate the genetic divergence, phylogenetic relationship, and taxonomic status of *F. cancrivora* from Indonesia and other Asian countries using allozyme, mitochondrial gene markers, morphological variation and cross-breeding experiments. Our results show the possible existence of several cryptic species in this group that need further corroboration.



## MATERIALS AND METHODS

Allozyme analysis.– Specimens were collected from eight localities of Indonesia, the Philippines, Malaysia, Thailand, and Bangladesh (Fig. 1). Ninety-two frogs, consisting of 40 males, 38 females, and 14 immature frogs were used in the present allozyme study (Table 1). Three distinct types were found among specimens based on SVL (snout vent length) and ecological characteristics. The first type has an average SVL of 54.1 mm in males and 68.0 mm in females, and mainly inhabits brackish waters such as shrimp ponds or mangroves. The second type has an average SVL of 68.1 mm in males and 86.7 mm in females, and mainly inhabits rice fields. The third type has an average SVL of 43.5 mm in males and 50.7 mm in females, and inhabits rice fields. The first, second, and third types are tentatively referred to in the following as the large-, mangrove-, and Pelabuhan ratu-types, respectively. *Fejervarya iskandari* was used as outgroup taxon. Seventeen enzymes extracted from skeletal muscles were analyzed by means of starch-gel electrophoresis (Table 2). Horizontal starch-gel electrophoresis was carried out using Sigma starch at a concentration of 12.5% by a procedure previously described by Nishioka et al. (1980, 1992). Each locus was detected using the agar-overlay method outlined by Harris and Hopkinson (1976), with a slight modification (Nishioka et al., 1992). Genetic distance (D) and genetic identity (I) were calculated following Nei (1972) using the software POPGENE (Yeh et al., 1997). A neighbour-joining (NJ) tree was constructed based on Nei's genetic distances to infer phylogenetic relationships among populations (Saitou and Nei, 1987). Bootstrap values were calculated from 1,000 pseudoreplicates using PHYLIP 3.65 (Felsenstein, 2005).

Molecular analysis.– Tissue samples for molecular analysis are in Table 1. Neotypes specimens from Cianjur, Indonesia (06°49'S, 107°08'E) were also included in the analysis. One individual *F. iskandari* from Cianjur, Indonesia, was used as the outgroup. In addition five additional 16S rRNA gene sequences from the GenBank were also used in the study (Table 3).

DNA extraction, PCR and sequencing.– Total genomic DNA was extracted from clipped toes using a DNA extraction kit (DNeasy Tissue Kit, QIAGEN) according to the manufacturer's instructions. Two sets of primer pairs, F51-R51 (Sumida et al., 2002) and Fow 1-1-Rev-1, were used for the amplification and sequencing of the 5' portion of the 16S rRNA and Cyt *b* genes, corresponding to positions 6189–6761 and 16662–17491, respectively, in *Fejervarya limnocharis* (Liu et al., 2005). The primer sequences were F51 (5'-CCC GCC TGT TTA CCA AAA ACA T-3'), R51 (5'-GGT CTG AAC TCA GAT CAC GTA -3'), Fow 1-1 (5'-ACM GGH YTM TTY YTR GC ATR CAY TA -3'), and Rev-1 (5'-TAD GCR AAW AGR AAR TAY CAY TCN GG-3'). PCR mixtures were prepared by using the TaKaRa Ex Taq™ Kit at a final volume of 50 µl. The 16S rRNA and Cyt *b* gene fragments were amplified by 35 cycles, each cycle consisting of denaturation for 10 s at 98ºC, annealing for 30 s at 47.5ºC, and extension for 80 s at 72ºC. Purified mtDNA gene fragments from PCR cycles 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) database (Accession Nos. AB444684 – AB 444710).

Sequence data analysis.– Alignments for DNA sequences were determined based on maximum sequence similarity using CLUSTAL W (Thompson et al., 1994). Gaps and ambiguous sites were excluded using GBlocks 0.91b (Castresana, 2000) at the default settings. Gaps were present only on the 16S rRNA gene sequences. Two sets that consist of 16S rRNA and Cyt *b* genes sequence alignments were used for phylogenetic analyses of *F. cancrivora* from 24 populations and *F. iskandari* from Cianjur, Indonesia as an outgroup. Pair-wise sequence divergences were calculated using the uncorrected "p" distance, while phylogenetic relationships were estimated by maximum-likelihood (ML), Bayesian inference (BI), maximum-parsimony (MP), and neighbour-joining (NJ) methods. The nucleotide substitution models in the ML, Bayesian, and NJ methods were selected based on the Akaike Information Criterion using the program Kakusan 3.0 (Tanabe, 2007) for both the 16S rRNA and Cyt *b* genes.

The ML analyses were performed with 1,000 bootstrap replicates by using Treefinder (Jobb et al., 2004). MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003) was used for BI analyses. For BI analyses, the number of Markov chain Monte Carlo (MCMC) generations was set to one million, and the sampling frequency to 100. The number of generations of MCMC and the burn-in sizes for both 16S rRNA and Cyt *b* genes data were determined by checking convergence of -log likelihood (-ln*L*) and tree length against generation number using Tracer ver. 1.4 (Drummond and Rambaut, 2007). The first 100,000 generations were discarded. All MCMC runs were repeated twice to confirm consistent approximation of posterior parameter distribution. The MP and NJ analyses were performed with 1,000 bootstrap replicates by using PAUP* 4.0b10 (Swofford, 2003). A haplotype network tree of Cyt *b* data was constructed with the median-joining network

**TABLE 1:** Samples used in the present study.

| Type | Collecting Station | | | Number of frogs | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Allozyme | | | | | Molecular |
| | Country | Locality | (Population) | Male | (SVL; mean) (mm) | Female | (SVL; mean) (mm) | Immature | Work |
| Mangrove | Philippines | Manila | (Mani) | 3 | (45.0-55.0; 50.3) | 5 | (49.0-68.0; 55.4) | 0 | 3 |
| | Thailand | Bangkok | (Bang) | 6 | (48.0-60.0; 53.3) | 4 | (59.0-71.0; 65.0) | 0 | 2 |
| | | Trat | (Trat) | 10 | (47.3-62.4; 55.5) | 7 | (51.3-78.3; 66.0) | 14 | 1 |
| | | Chantaburi | (Chan) | 0 | | 1 | (74.2) | 0 | 1 |
| | Bangladesh | Khulna | (Khul) | 7 | (46.6-60.6; 54.5) | 6 | (46.6-83.6; 70.5) | 0 | 3 |
| Large | Malaysia | Selangor | (Sela) | 3 | (59.3-71.2; 64.9) | 6 | (58.0-87.5; 73.4) | 0 | 4 |
| | Indonesia | Cianjur (A)[1], Java | (Cian-A) | 10 | (62.7-76.2; 69.7) | 7 | (87.6-112.0; 98.6) | 0 | 2 |
| | | Cianjur (B)[2], Java | (Cian-B) | 0 | | 0 | | 0 | 1 |
| | | Pelabuhan ratu (B)[3], Java | (Pela-B) | 0 | | 0 | | 0 | 3 |
| | | Bogor, Java | (Bogo) | 0 | | 0 | | 0 | 2 |
| | | Banyumas, Java | (Bany) | 0 | | 0 | | 0 | 2 |
| | | Langkat, Sumatra | (Lang) | 0 | | 0 | | 0 | 3 |
| | | Padang, Sumatra | (Pada) | 0 | | 0 | | 0 | 3 |
| | | Payakumbuh, Sumatra | (Paya) | 0 | | 0 | | 0 | 2 |
| | | Panti, Sumatra | (Pant) | 0 | | 0 | | 0 | 3 |
| | | Jambi, Sumatra | (Jamb) | 0 | | 0 | | 0 | 4 |
| | | Palembang, Sumatra | (Pale) | 0 | | 0 | | 0 | 2 |
| | | Lampung, Sumatra | (Lamp) | 0 | | 0 | | 0 | 2 |
| | | Tempilang, Bangka | (Temp) | 0 | | 0 | | 0 | 1 |
| Pelabuhan ratu/ Sulawesi | Indonesia | Pelabuhan ratu (A)[4], Java | (Pela-A) | 1 | (43.5) | 2 | (48.7-52.7; 50.7) | 0 | 3 |
| | | Makassar, Sulawesi | (Maka) | 0 | | 0 | | 0 | 3 |
| | | Sinjai, Sulawesi | (Sinj) | 0 | | 0 | | 0 | 2 |
| | | Siwa, Sulawesi | (Siwa) | 0 | | 0 | | 0 | 2 |
| | | Bone, Sulawesi | (Bone) | 0 | | 0 | | 0 | 1 |
| Total | | | | 40 | | 38 | | 14 | 55 |

[1] collected by Nia Kurniawan in 2007

[2] collected from type locality by Nia Kurniawan in 2008

[3] collected by Nia Kurniawan in 2008

[4] collected and provided by H. Ota in 1996



**TABLE 2:** Enzymes analyzed and number of phenotypes and alleles at each locus in the present study.

| Enzyme | Abbreviation | E.C. No. | No. of loci | Locus | Sample | Buffer system | Number of phenotypes* | Number of alleles* |
|---|---|---|---|---|---|---|---|---|
| Aspartate aminotransferase | AAT | 2.6.1.1 | 2 | AAT-1 | Muscle | T-C pH 7.0 | 2 | 2 |
| | | | | AAT-2 | Muscle | T-C pH 7.0 | 1 | 1 |
| Adenosine deaminase | ADA | 3.5.4.4 | 1 | ADA | Muscle | T-C pH 7.0 | 3 | 3 |
| Adenylate kinase | AK | 2.7.4.3 | 1 | AK | Muscle | T-C pH 7.0 | 3 | 3 |
| Aldolase | ALD | 4.1.2.13 | 1 | ALD | Muscle | T-C pH 7.0 | 3 | 2 |
| Creatine kinase | CK | 2.7.3.2 | 1 | CK | Muscle | TEB pH 8.0 | 1 | 1 |
| Fumarase | FUM | 4.2.1.2 | 2 | FUM-1 | Muscle | TEB pH 8.0 | 3 | 3 |
| | | | | FUM-2 | Muscle | TEB pH 8.0 | 1 | 1 |
| α-Glycerophosphate dehydrogenase | α-GDH | 1.1.1.8 | 1 | α-GDH | Muscle | T-C pH 6.0 | 2 | 2 |
| Glucose-6-phosphate isomerase | GPI | 5.3.1.9 | 1 | GPI | Muscle | TEB pH 8.0 | 4 | 3 |
| Isocitrate dehydrogenase | IDH | 1.1.1.42 | 2 | IDH-1 | Muscle | T-C pH 7.0 | 3 | 3 |
| | | | | IDH-2 | Muscle | T-C pH 7.0 | 1 | 1 |
| Lactate dehydrogenase | LDH | 1.1.1.27 | 2 | LDH-1 | Muscle | T-C pH 6.0 | 5 | 4 |
| | | | | LDH-2 | Muscle | T-C pH 6.0 | 2 | 2 |
| Malate dehydrogenase | MDH | 1.1.1.37 | 2 | MDH-1 | Muscle | T-C pH 6.0 | 4 | 4 |
| | | | | MDH-2 | Muscle | T-C pH 6.0 | 2 | 2 |
| Malic enzyme | ME | 1.1.1.40 | 2 | ME-1 | Muscle | T-C pH 7.0 | 5 | 4 |
| | | | | ME-2 | Muscle | T-C pH 7.0 | 4 | 3 |
| Mannose-6-phosphate isomerase | MPI | 5.3.1.8 | 1 | MPI | Muscle | T-C pH 7.0 | 8 | 5 |
| Peptidase | PEP | 3.4.3.1 | 4 | PEP-A | Muscle | TEB pH 8.0 | 1 | 1 |
| | | | | PEP-B | Muscle | TEB pH 8.0 | 5 | 4 |
| | | | | PEP-C | Muscle | TEB pH 8.0 | 2 | 2 |
| | | | | PEP-D | Muscle | TEB pH 8.0 | 2 | 2 |
| 6-Phosphogluconate dehydrogenase | 6-PGD | 1.1.1.44 | 1 | 6-PGD | Muscle | T-C pH 7.0 | 5 | 4 |
| Phosphoglucomutase | PGM | 5.4.2.2 | 1 | PGM | Muscle | TEB pH 8.0 | 3 | 3 |
| Superoxide dismutase | SOD | 1.15.1.1 | 1 | SOD | Muscle | TEB pH 8.0 | 4 | 4 |

T-C, Tris Citrate buffer; TEB, Tris-EDTA-Borate buffer; E.C., Enzyme Commission

*considering both ingroup and outgroup taxa

**TABLE 3:** List of genes, types, haplotype names, populations and accession numbers used in this study[1].

| Type | Haplotype name | | Population | Accession number[2] | | Source | |
|---|---|---|---|---|---|---|---|
| | 16S | Cyt *b* | | 16S | Cyt *b* | 16S | Cyt *b* |
| Mangrove | M-I | M-1a | Mani | (AB070738) | AB444706 | (Sumida et al., 2002) | This study |
| | | M-1b | Bang | AB444691 | AB444707 | This study | This study |
| | | – | Negros Island (Philippines) | (AF206473) | – | (Chen et al., 2005) | – |
| | | – | Hainan island (China) | (DQ458252) | – | (Che et al., 2007) | – |
| | M-II | M-2 | Trat, Chan | AB444692 | AB444708 | This study | This study |
| | M-III | M-3 | Khul | (AB372018) | (AB372070) | (Islam et al., 2008) | (Islam et al., 2008) |
| | M-IV | – | Orissa (India) | (AY841754) | – | (Guha et al., unpublished) | – |
| Large | L-I | L-1a | Bogo | AB444689 | AB444702 | This study | This study |
| | | L-1b | Cian-A | AB444684 | AB444695 | This study | This study |
| | | L-1c | Cian-B, Pela-B-1, Sela-1 | AB444684 | AB444696 | This study | This study |
| | | L-1d | Lang, | AB444684 | AB444694 | This study | This study |
| | | L-1e | Temp | AB444684 | AB444698 | This study | This study |
| | L-II | L-1e | Pale | AB444686 | AB444698 | This study | This study |
| | | L-2a | Lamp | AB444686 | AB444699 | This study | This study |
| | | L-2b | Jamb | AB444687 | AB444700 | This study | This study |
| | | L-2c | Bany | AB444690 | AB444703 | This study | This study |
| | L-III | L-3 | Pada, Paya, Pant | AB444685 | AB444697 | This study | This study |
| | L-IV | L-4 | Sela-2 | AB444688 | AB444701 | This study | This study |
| | L-V | – | Kalimantan Island (Indonesia) | (AF346810) | – | (Veith et al., 2001) | – |
| | L-VI | – | Jiadong (Taiwan) | (EU365387) | – | (Hsu et al., unpublished) | – |
| | – | L-5 | Paya-2 | – | AB444704 | – | This study |
| | – | L-6 | Pela-B-2 | – | AB444705 | – | This study |
| Pelabuhan ratu/Sulawesi | PS-I | PS-1a | Pela-A, Maka | AB444693 | AB444709 | This study | This study |
| | | PS-1b | Bone, Sinj, Siwa, | AB444693 | AB444710 | This study | This study |

[1]We used *F. iskandari* for outgroup, with accession number AB277303 (Kotaki et al., 2008) for 16S rRNA gene and accession number AB296085 (Djong et al., 2007) for Cyt *b* gene.

[2]Accession numbers in parenthesis were retrieved from Genbank.

– No Data

**TABLE 4:** Allele frequencies at 26 loci in eight populations of *F. cancrivora* and one population of *F. iskandari* as outgroup.

| Locus | Mangrove type | | | | Large type | | Pelabuhan ratu type | Outgroup |
|---|---|---|---|---|---|---|---|---|
| | Mani | Bang | Trat | Chan | Khul | Sela | Cian-A | Pela-A | Fisk |
| AAT-1 | b | b | b | b | b | b | b | a | a |
| AAT-2 | a | a | a | a | a | a | a | a | a |
| ADA | c | c | c | c | c | a | a | a | b |
| AK | a | a | a | a | a | b | b | b | c |
| ALD | b | b | a(0.02) b(0.98) | b | b | a(0.12) b(0.88) | b | b | a |
| CK | a | a | a | a | a | a | a | a | a |
| FUM-1 | a(0.19) c(0.81) | c | a(0.02) c(0.98) | a(0.50) c(0.50) | c | c | c | c | b |
| FUM-2 | a | a | a | a | a | a | a | a | a |
| α-GDH | b | b | b | b | b | b | b | b | a |
| GPI | a | a | a(0.95) b(0.05) | a | a | b | b(0.88) c(0.12) | b | a |
| IDH-1 | b | b | a(0.02) b(0.98) | b | b | b | b | b | c |
| IDH-2 | a | a | a | a | a | a | a | a | a |
| LDH-A | a | a | a | a | a | a | a | a | b |
| LDH-B | d | d | c(0.02) d(0.98) | d | d | c | c | c | a(0.33) b(0.67) |
| MDH-1 | d | d | d | d | d | d | d | c | a(0.67) b(0.33) |
| MDH-2 | b | b | b | b | b | b | b | b | a |
| ME-1 | a(0.13) b(0.87) | b | a(0.02) b(0.98) | b | b | c(0.78) d(0.22) | c | c(0.84) d(0.16) | b |
| ME-2 | a(0.75) b(0.25) | a | a(0.98) b(0.02) | a | a(0.96) b(0.04) | b | b | a(0.17) b(0.83) | c |
| MPI | c(0.38) d(0.62) | d(0.95) e(0.05) | d(0.92) e(0.08) | d | d | b(0.61) c(0.27) d(0.12) | b(0.35) c(0.23) d(0.42) | d | a(0.17) b(0.50) c(0.33) |
| PEP-A | a | a | a | a | a | a | a | a | a |
| PEP-B | c(0.13) d(0.87) | d | d | d | d | c | c | b | a |
| PEP-C | b | b | b | b | b | b | b | b | a |
| PEP-D | b | b | b | b | b | b | b | b | a |
| 6-PGD | b | b | a(0.08) e(0.92) | b | c | d | d | c | a |
| PGM | c | c | c | c | c | b | b | b | a |
| SOD | d | d | d | d | d | b | a(0.03) b(0.97) | b | c(0.50) d(0.50) |

**TABLE 5:** Nei's genetic distance (D) (below diagonal) and genetic identity (I) (above diagonal) based on allozyme data among eight populations of *F. cancrivora* and one population of *F. iskandari* as outgroup. See Table 1 for population abbreviation.

| Type | No. | Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mangrove | 1 | Mani | – | 0.992 | 0.992 | 0.986 | 0.952 | 0.617 | 0.626 | 0.552 | 0.316 |
| | 2 | Bang | 0.008 | – | 1.000 | 0.990 | 0.961 | 0.590 | 0.604 | 0.550 | 0.301 |
| | 3 | Trat | 0.008 | 0.000 | – | 0.990 | 0.964 | 0.598 | 0.611 | 0.556 | 0.305 |
| | 4 | Chan | 0.014 | 0.010 | 0.010 | – | 0.951 | 0.575 | 0.590 | 0.537 | 0.303 |
| | 5 | Khul | 0.049 | 0.040 | 0.037 | 0.050 | – | 0.592 | 0.606 | 0.592 | 0.301 |
| Large | 6 | Sela | 0.483 | 0.527 | 0.514 | 0.553 | 0.525 | – | 0.994 | 0.815 | 0.223 |
| | 7 | Cian-A | 0.468 | 0.505 | 0.493 | 0.528 | 0.501 | 0.006 | – | 0.829 | 0.213 |
| Pelabuhan ratu | 8 | Pela-A | 0.595 | 0.598 | 0.588 | 0.623 | 0.525 | 0.205 | 0.188 | – | 0.243 |
| Outgroup | 9 | Fisk | 1.153 | 1.201 | 1.187 | 1.194 | 1.202 | 1.501 | 1.545 | 1.416 | – |

**TABLE 6:** Average Nei`s genetic distance (D) based on allozyme data within and between three *F. cancrivora* types and *F. iskandari* as outgroup.

| Type | No. | 1 | 2 | 3 |
|---|---|---|---|---|
| Mangrove | 1 | 0.023±0.019 | | |
| Large | 2 | 0.510±0.025 | 0.006 | |
| Pelabuhan ratu | 3 | 0.586±0.036 | 0.197±0.012 | – |
| *F. iskandari* | 4 | 1.187±0.02 | 1.153±0.031 | 1.416 |



**TABLE 7:** Percent sequence divergence estimated by uncorrected "p" distance based on the 389-bp fragment of 16S rRNA gene among *F. cancrivora* types and *F. iskandari* haplotypes.

| Type | No. | Haplotype | Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mangrove | 1 | M-I | Mani, Bang, Negros Isl.(Philippines), Hainan Isl.(China | – | | | | | | | | | | | |
| | 2 | M-II | Trat, Chan | 0.26 | – | | | | | | | | | | |
| | 3 | M-III | Khul | 1.54 | 1.29 | – | | | | | | | | | |
| | 4 | M-IV | Orissa (India) | 1.80 | 1.54 | 0.26 | – | | | | | | | | |
| Large | 5 | L-I | Cian-A, Cian-B, Lang, Sela-1, Pela-B, Temp, Bogo | 9.00 | 9.00 | 9.25 | 9.51 | – | | | | | | | |
| | 6 | L-II | Pale, Lamp, Jamb, Bany | 8.74 | 8.74 | 9.00 | 9.25 | 0.26 | – | | | | | | |
| | 7 | L-III | Pada, Paya, Pant | 8.74 | 8.74 | 9.00 | 9.25 | 0.51 | 0.26 | – | | | | | |
| | 8 | L-IV | Sela-2 | 9.00 | 9.00 | 9.25 | 9.51 | 0.51 | 0.26 | 0.51 | – | | | | |
| | 9 | L-V | Kalimantan Isl. (Indonesia) | 9.00 | 9.00 | 9.25 | 9.51 | 0.51 | 0.26 | 0.51 | 0.51 | – | | | |
| | 10 | L-VI | Jiadong (Taiwan) | 9.00 | 9.00 | 9.25 | 9.51 | 0.51 | 0.26 | 0.51 | 0.51 | 0.51 | – | | |
| Pelabuhan ratu/Sulawesi | 11 | PS-I | Pela-A, Maka, Bone, Sinj, Siwa | 10.03 | 10.03 | 10.28 | 10.54 | 5.40 | 5.66 | 5.91 | 5.91 | 5.91 | 5.91 | – | |
| Outgroup | 12 | *F. iskandari* | | 15.68 | 15.68 | 15.94 | 16.20 | 14.65 | 14.40 | 14.40 | 14.65 | 14.65 | 14.14 | 14.14 | – |



**TABLE 8:** Average percent sequence divergence estimated by uncorrected "p" distance for 16S rRNA gene within and between three *F. cancrivora* types and *F. iskandari* haplotypes.

| Type | No. | 1 | 2 | 3 |
|---|---|---|---|---|
| Mangrove | 1 | 1.12±0.68 | | |
| Large | 2 | 9.10±0.25 | 0.43±0.12 | |
| Pelabuhan ratu/Sulawesi | 3 | 10.22±0.24 | 5.78±0.21 | – |
| *F. iskandari* | 4 | 15.88±0.25 | 14.48±0.21 | 14.14 |

**TABLE 8:** Percent sequence divergence estimated by uncorrected "p" distance for Cyt *b* gene among *F. cancrivora* types and *F. iskandari* haplotypes.

| Type | No. | Haplotype | Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mangrove | 1 | M-1a | Mani | – | | | | | | | | | | | | | | | | | | |
| | 2 | M-1b | Bang | 0.54 | – | | | | | | | | | | | | | | | | | |
| | 3 | M-2 | Trat, Chan | 2.33 | 1.80 | – | | | | | | | | | | | | | | | | |
| | 4 | M-3 | Khul | 3.77 | 5.03 | 3.23 | – | | | | | | | | | | | | | | | |
| Large | 5 | L-1a | Bogo | 14.36 | 15.62 | 14.18 | 14.54 | – | | | | | | | | | | | | | | |
| | 6 | L-1b | Cian-A | 14.54 | 15.80 | 14.36 | 14.72 | 0.18 | – | | | | | | | | | | | | | |
| | 7 | L-1c | Cian-B, Pela-B-1, Sela-1 | 14.54 | 15.80 | 14.36 | 14.72 | 0.18 | 0.36 | – | | | | | | | | | | | | |
| | 8 | L-1d | Lang | 14.90 | 16.16 | 14.72 | 15.08 | 0.54 | 0.72 | 0.72 | – | | | | | | | | | | | |
| | 9 | L-1e | Temp, Pale | 14.00 | 15.08 | 13.82 | 14.18 | 1.08 | 1.26 | 1.26 | 1.62 | – | | | | | | | | | | |
| | 10 | L-2a | Lamp | 14.18 | 15.44 | 14.00 | 14.36 | 0.36 | 0.54 | 0.54 | 0.90 | 1.08 | – | | | | | | | | | |
| | 11 | L-2b | Jamb | 14.00 | 15.08 | 13.82 | 14.18 | 0.72 | 0.90 | 0.90 | 1.26 | 0.72 | 0.72 | – | | | | | | | | |
| | 12 | L-2c | Bany | 14.36 | 15.44 | 14.18 | 14.54 | 0.72 | 0.90 | 0.90 | 1.26 | 0.72 | 0.72 | 0.36 | – | | | | | | | |
| | 13 | L-3 | Pada, Paya-1, Pant | 14.18 | 15.26 | 14.00 | 14.36 | 0.54 | 0.72 | 0.72 | 1.08 | 0.54 | 0.54 | 0.18 | 0.18 | – | | | | | | |
| | 14 | L-4 | Sela-2 | 14.18 | 15.08 | 14.00 | 14.36 | 0.90 | 1.08 | 1.08 | 1.44 | 0.90 | 0.90 | 0.36 | 0.54 | 0.36 | – | | | | | |
| | 15 | L-5 | Paya-2 | 14.36 | 15.44 | 14.18 | 14.54 | 0.72 | 0.90 | 0.90 | 1.26 | 0.72 | 0.72 | 0.18 | 0.36 | 0.18 | 0.54 | – | | | | |
| | 16 | L-6 | Pela-B-2 | 14.54 | 15.80 | 14.36 | 14.72 | 0.54 | 0.36 | 0.72 | 1.08 | 1.26 | 0.54 | 0.72 | 0.90 | 0.72 | 1.08 | 0.90 | – | | | |
| Pelabuhan ratu/ | 17 | PS-1a | Pela-A, Maka | 16.16 | 17.06 | 15.98 | 15.98 | 12.93 | 13.11 | 13.11 | 13.47 | 12.75 | 12.93 | 12.57 | 12.75 | 12.57 | 12.39 | 12.75 | 13.11 | – | | |
| Sulawesi | 18 | PS-1b | Bone, Sinj, Siwa | 16.34 | 17.24 | 16.16 | 16.16 | 12.93 | 13.11 | 13.11 | 13.47 | 12.75 | 12.93 | 12.57 | 12.75 | 12.57 | 12.39 | 12.75 | 13.11 | 0.36 | – | |
| Outgroup | 19 | *F. iskandari* | | 21.19 | 21.90 | 21.01 | 20.47 | 19.57 | 19.75 | 19.75 | 19.57 | 19.93 | 19.75 | 19.75 | 19.57 | 19.75 | 19.75 | 19.93 | 20.11 | 21.19 | 21.19 | – |



**TABLE 10:** Average percent sequence divergence estimated by an uncorrected "p" distance for Cyt *b* gene within and between three *F. cancrivora* types and *F. iskandari* haplotypes.

| Type | No | 1 | 2 | 3 |
|---|---|---|---|---|
| Mangrove | 1 | 2.78±1.58 | | |
| Large | 2 | 14.64±0.59 | 0.76±0.33 | |
| Pelabuhan ratu/Sulawesi | 3 | 16.38±0.49 | 12.88±0.28 | 0.36 |
| *F. iskandari* | 4 | 21.14±0.59 | 19.78±0.17 | 21.9 |



**TABLE 11:** Locality and number of samples used in the present study.

| Country | Locality | Population abbreviation | Morphometry | | Crossing experiments | |
|---|---|---|---|---|---|---|
| | | | ♂ | ♀ | ♂ | ♀ |
| Indonesia | Cianjur, West Java | Cian | 11 | 12 | 3 | 0 |
| Malaysia | Selangor, Peninsular Malaysia | Sela | 10 | 11 | 3 | 5 |
| Thailand | Trat, Eastern Thailand | Trat | 10 | 10 | 2 | 0 |
| Bangladesh | Khulna, Southern Bangladesh | Khul | 7 | 6 | 2 | 0 |
| Indonesia | Makassar, South Sulawesi | Maka | 10 | 10 | – | – |
| Total | | | 48 | 49 | 10 | 5 |

**TABLE 12:** Measurements of morphological characters in males and females of five populations of *F. cancrivora.*

| Morphological character | Cianjur, Indonesia | | Selangor, Malaysia | | Trat, Thailand | | Khulna, Bangladesh | | Makassar, Indonesia | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| | n=11 | n=12 | n=10 | n=11 | n=10 | n=10 | n=7 | n=6 | n=10 | n=10 |
| SVL | 69.5±3.9 (62.7-76.2) | 95.7±5.3 (89.5-106) | 65.0±4.1 (59.3-71.2) | 76.0±12.5 (60.7-98.3) | 55.5±5.3 (47.3-62.4) | 67.4±9.6 (51.3-78.3) | 54.5±5.4 (46.6-60.6) | 70.4±12.9 (46.6-83.6) | 58.2±4.5 (48.1-63.9) | 70.9±8.6 (63.2-85.7) |
| HL | 22.4±1.5 (19.7-24.1) | 34.4±1.5 (32.3-37.6) | 21.6±2.3 (18.0-24.4) | 25.8±4.0 (21.0-33.0) | 18.9±1.5 (16.6-21.2) | 22.5±2.7 (18.5-25.8) | 18.3±1.5 (16.6-20.0) | 23.2±3.9 (15.8-26.2) | 23.6±1.1 (21.6-25.3) | 28.1±3.8 (24.1-33.8) |
| HW | 24.9±1.6 (22.6-27.6) | 35.4±1.9 (32.1-38.8) | 22.9±1.6 (19.5-25.0) | 26.3±5.2 (20.2-34.7) | 17.7±1.9 (15.9-20.9) | 21.6±3.8 (16.3-26.7) | 18.3±1.7 (15.8-20.9) | 24.8±4.7 (15.8-28.2) | 23.3±1.4 (20.8-25.6) | 28.3±3.3 (24.8-33.4) |
| STL | 17.0±1.9 (13.3-20.0) | 26.0±2.0 (24.1-31.3) | 16.8±2.3 (13.5-19.4) | 21.4±3.8 (16.8-28.4) | 14.8±1.3 (12.3-16.3) | 8.2±2.2 (14.9-21.4) | 14.2±1.4 (12.1-15.5) | 18.3±3.6 (11.7-21.4) | 16.8±1.2 (14.5-19.2) | 20.6±2.3 (18.6-24.6) |
| MSL | 19.1±3.6 (15.4-27.9) | 27.7±2.8 (25.0-35.1) | 18.0±2.5 (13.5-21.3) | 23.3±3.4 (19.0-30.2) | 16.5±1.6 (14.4-19.2) | 19.7±2.3 (16.1-22.2) | 16.7±1.6 (14.2-19.2) | 19.5±4.1 (12.8-25.0) | 18.3±1.2 (15.9-20.5) | 21.9±2.6 (19.6-27.1) |
| NS | 4.3±1.4 (2.6-7.4) | 6.6±0.2 (6.2-7.0) | 4.0±1.0 (2.5-5.2) | 5.0±0.9 (4.0-6.6) | 3.6±0.5 (2.8-4.3) | 4.4±0.5 (3.5-5.3) | 3.7±0.4 (3.1-4.1) | 3.7±0.6 (2.7-4.3) | 4.0±0.4 (3.4-4.5) | 4.8±0.8 (3.9-6.2) |
| SL | 9.0±1.2 (7.2-10.6) | 13.2±0.7 (12.2-14.6) | 8.5±1.3 (6.0-10.5) | 11.1±1.8 (9.0-14.5) | 8.4±0.7 (6.9-9.0) | 9.7±1.3 (7.4-11.2) | 7.7±0.8 (6.7-8.7) | 9.6±1.8 (6.7-12.3) | 8.6±0.5 (7.7-9.4) | 10.6±1.5 (8.8-12.8) |
| NTL | 15.1±1.6 (12.9-16.9) | 20.9±0.7 (19.8-22.1) | 14.4±1.0 (12.5-15.8) | 16.7±2.6 (13.4-20.5) | 12.3±0.7 (11.3-13.5) | 14.7±1.8 (12.0-17.1) | 12.2±0.9 (11.0-13.4) | 15.1±2.2 (11.0-16.8) | 14.0±1.1 (11.8-16) | 16.8±1.7 (15.1-19.7) |
| EN | 6.6±1.3 (4.2-8.4) | 10.6±0.8 (9.5-12.0) | 5.9±0.7 (4.6-6.8) | 7.8±2.0 (5.6-12.6) | 5.4±0.6 (4.0-6.2) | 6.4±0.9 (4.9-7.2) | 5.9±0.9 (5.3-7.9) | 6.6±1.2 (4.5-8.0) | 5.7±0.4 (5.0-6.4) | 7.2±0.7 (6.4-8.4) |
| TEL | 3.5±0.9 (2.5-4.8) | 5.8±0.3 (5.3-6.4) | 2.9±0.5 (2.5-4.1) | 3.5±0.9 (2.4-5.9) | 2.8±0.4 (2.3-3.4) | 3.3±0.6 (2.3-3.8) | 2.6±0.3 (2.2-2.9) | 3.5±0.6 (2.5-4.0) | 3.7±0.4 (3.2-4.7) | 4.5±0.4 (3.9-5.2) |
| TD | 4.7±0.4 (4.0-5.4) | 6.2±0.3 (5.6-6.8) | 4.4±0.5 (3.9-5.3) | 4.7±0.6 (4.0-5.7) | 4.1±0.5 (3.3-4.6) | 4.4±0.7 (3.4-5.4) | 4.1±0.5 (3.3-4.6) | 4.7±0.9 (3.3-5.8) | 4.5±0.2 (4.0-4.8) | 5.2±0.7 (4.3-6.5) |
| MN | 23.3±2.2 (19.7-26.7) | 33.0±1.0 (31.2-35.0) | 21.3±1.7 (19.0-23.4) | 26.2±5.0 (20.5-34.8) | 18.2±2.3 (15.2-22.0) | 22.3±3.7 (17.3-26.8) | 18.2±2.1 (14.9-20.5) | 21.5±3.9 (14.9-24.8) | 20.3±1.3 (17.9-22.5) | 24.6±2.7 (22.1-29.1) |
| MFE | 17.3±2.2 (14.2-20.3) | 25.2±1.1 (23.7-27.0) | 16.1±2.0 (12.7-19.8) | 19.5±3.0 (15.2-24.3) | 14.4±2.8 (11.0-19.4) | 16.9±3.1 (12.9-21.4) | 13.8±1.6 (11.2-15.8) | 16.6±3.4 (11.2-19.6) | 14.9±0.8 (13.6-15.9) | 18.0±2.2 (15.8-21.3) |
| MBE | 10.2±2.1 (7.0-13.3) | 16.5±2.1 (12.2-19.9) | 10.3±1.1 (8.5-12.3) | 12.3±2.9 (8.0-18.4) | 9.4±2.3 (6.5-14.0) | 10.7±2.4 (7.5-15.3) | 8.0±1.3 (6.3-9.4) | 10.2±2.2 (7.2-12.7) | 10.4±0.9 (9.2-12.1) | 12.6±1.5 (10.5-14.7) |
| IN | 4.0±1.1 (2.1-5.6) | 5.9±1.1 (4.5-8.0) | 4.2±0.4 (3.4-4.8) | 4.9±0.9 (4.0-6.7) | 3.6±0.4 (3.2-4.5) | 4.3±0.5 (3.5-5.0) | 3.6±0.2 (3.2-4.0) | 4.3±0.6 (3.2-5.1) | 3.2±0.3 (2.8-3.8) | 3.9±0.6 (3.1-4.8) |
| EL | 7.3±0.5 (6.4-8.2) | 10.2±1.3 (8.8-13.5) | 7.4±0.7 (6.0-8.4) | 8.3±1.2 (6.9-10.9) | 6.4±0.7 (5.0-7.9) | 7.3±1.0 (6.2-8.8) | 6.5±0.9 (4.7-7.5) | 6.9±1.1 (4.7-7.8) | 6.0±0.5 (5.5-7.0) | 6.4±0.6 (5.7-7.4) |
| IOD | 4.0±0.8 (3.0-5.5) | 5.5±1.4 (3.6-9.2) | 3.3±0.6 (2.4-4.7) | 4.5±0.6 (4.0-6.2) | 3.2±0.5 (2.5-4.0) | 3.1±0.7 (2.1-4.4) | 3.3±0.5 (2.3-4.0) | 4.5±0.9 (3.0-5.3) | 3.7±0.2 (3.3-4.0) | 4.6±0.4 (4.2-5.2) |
| UEW | 5.6±0.5 (5.2-6.7) | 7.8±1.5 (6.3-11.7) | 5.5±0.8 (4.8-7.4) | 6.1±0.6 (5.1-7.1) | 4.5±0.6 (3.6-5.5) | 5.3±0.4 (4.7-5.8) | 4.3±0.6 (3.5-5.1) | 4.2±0.7 (3.0-5.0) | 4.7±0.3 (4.1-5.1) | 5.7±0.6 (4.8-6.5) |
| HAL | 15.0±1.3 (13.1-16.6) | 22.2±2.2 (19.9-25.9) | 14.5±1.0 (12.5-15.7) | 16.6±1.9 (14.4-20.6) | 11.5±1.5 (9.0-13.3) | 13.5±2.3 (10.3-16.2) | 11.8±0.9 (10.5-13.0) | 14.5±2.3 (10.5-16.9) | 13.7±0.8 (11.9-14.6) | 16.7±2.2 (13.8-21.2) |
| FLL | 16.0±1.1 (14.2-17.9) | 22.1±1.6 (20.0-25.4) | 16.0±1.0 (14.5-18.1) | 16.6±2.8 (13.3-22.4) | 13.3±1.6 (11.0-15.9) | 14.0±2.6 (9.9-17.2) | 13.4±1.7 (11.2-15.3) | 15.9±2.4 (11.2-17.8) | 14.3±0.9 (12.6-15.3) | 17.1±1.9 (15.2-19.9) |
| LAL | 20.7±1.9 (17.0-23.1) | 27.9±1.9 (25.1-31.8) | 19.6±0.9 (18.5-20.9) | 22.0±3.9 (18.2-28.9) | 15.9±1.7 (12.5-19.0) | 17.3±3.2 (11.8-22.0) | 15.8±2.6 (12.4-19.4) | 18.1±3.5 (12.5-22.0) | 12.4±0.3 (11.9-12.8) | 15.3±1.9 (13.3-18.6) |
| HLL | 103.7±6.3 (93.7-116.3) | 142.6±7.1 (129.7-155.9) | 96.0±7.0 (85.1-103.5) | 112.8±20.5 (87.0-151.2) | 76.5±7.2 (63.0-84.1) | 85.5±13.3 (66.9-100.0) | 77.2±7.3 (64.0-85.9) | 97.6±19.0 (64.0-113.7) | 97.5±7.0 (81.6-107.9) | 118.7±11.9 (106.9-137.9) |
| THIGHL | 30.6±2.3 (26.3-33.5) | 42.5±3.3 (37.5-48.5) | 29.0±3.5 (23.5-35.3) | 30.8±5.1 (26.0-43.0) | 22.4±1.8 (20.8-25.9) | 25.0±5.0 (18.6-33.1) | 23.3±2.2 (20.1-25.7) | 29.4±6.1 (18.1-33.7) | 29.8±1.9 (26.3-33.4) | 36.7±4.8 (31.4-44.0) |
| TL | 36.5±2.3 (32.6-41.6) | 47.1±3.1 (41.6-51.4) | 33.4±1.7 (31.0-36.0) | 33.8±7.6 (24.2-49.2) | 24.9±2.5 (21.0-29.5) | 27.9±5.6 (20.2-34.5) | 25.1±1.7 (21.8-27.3) | 32.5±7.2 (21.8-40.8) | 31.5±2.1 (26.9-35.2) | 38.9±4.7 (33.7-47.0) |
| FOL | 35.4±2.9 (31.0-40.9) | 44.1±6.7 (28.0-50.7) | 33.1±1.8 (29.8-36.1) | 36.5±7.2 (26.7-50.3) | 25.2±2.9 (21.1-29.1) | 30.2±5.5 (23.5-37.8) | 25.5±3.4 (21.4-29.5) | 34.4±7.4 (21.4-40.6) | 31.6±2.3 (27.2-34.5) | 38.3±4.0 (33.7-43.8) |
| TFOL | 51.3±3.6 (45.2-57.2) | 69.9±2.5 (66.4-75.2) | 47.3±1.8 (44.5-49.8) | 55.1±10.5 (42.9-70.0) | 36.3±3.9 (29.0-42.0) | 43.1±8.1 (33.5-54.8) | 36.3±4.4 (30.2-41.4) | 51.1±10.5 (32.4-60.4) | 47.2±3.7 (39.3-51.8) | 56.7±5.8 (50.0-66.7) |
| 3FL | 12.3±2.5 (9.5-17.0) | 12.4±1.3 (10.5-13.9) | 10.4±1.5 (8.9-13.0) | 10.0±1.3 (8.0-12.7) | 7.4±0.8 (6.0-8.2) | 7.6±0.9 (5.9-8.5) | 7.3±0.7 (6.4-8.3) | 8.8±1.9 (5.6-11.1) | 8.1±0.4 (7.3-8.7) | 9.8±1.0 (8.4-11.3) |
| 1FL | 11.1±1.4 (8.3-12.8) | 13.6±0.6 (12.8-14.6) | 10.1±0.9 (9.1-11.9) | 10.5±1.6 (8.7-13.8) | 6.5±1.0 (5.0-8.2) | 8.0±1.9 (4.9-10.1) | 6.0±0.7 (5.0-7.2) | 8.5±2.0 (5.0-10.6) | 7.4±0.6 (6.4-8.3) | 9.3±0.8 (8.1-10.3) |
| 4TL | 26.4±3.9 (21.7-35.3) | 34.0±2.5 (28.8-37.7) | 24.1±4.2 (20.9-35.2) | 26.1±2.2 (22.6-29.3) | 18.8±1.2 (16.9-21.3) | 20.7±2.8 (16.0-24.1) | 19.2±2.0 (15.9-21.3) | 23.0±3.4 (16.5-26.1) | 22.1±2.1 (18.3-25.6) | 28.1±3.5 (24.1-33.7) |
| IMTL | 4.2±0.7 (2.7-5.0) | 5.8±0.3 (5.3-6.3) | 3.9±0.5 (2.9-4.5) | 4.9±1.0 (3.3-6.3) | 3.2±0.4 (2.6-3.7) | 3.6±0.8 (2.4-5.0) | 3.2±0.2 (3.0-3.5) | 4.1±0.7 (3.0-4.7) | 3.1±0.2 (2.8-3.4) | 3.8±0.5 (3.1-4.8) |
| ITL | 9.1±1.3 (7.4-11.4) | 12.2±0.7 (11.4-13.9) | 8.6±0.9 (7.2-9.8) | 10.1±1.1 (8.5-11.9) | 6.7±1.0 (5.8-9.1) | 7.4±0.9 (6.2-8.5) | 7.0±1.3 (5.6-8.9) | 7.6±1.1 (5.3-8.1) | 7.6±0.6 (6.5-8.5) | 9.1±1.0 (7.8-10.5) |

Data are shown as the mean and standard deviation, followed by the range in parentheses.
All measurements are shown in mm.



**TABLE 13:** Factor loading on the first two components extracted from the correlation matrix of 31 characters for males and females of *F. cancrivora.*

| Character | Male | | Female | |
|---|---|---|---|---|
| | PC 1 | PC 2 | PC 1 | PC 2 |
| SVL | 0.0437 | 0.3013 | 0.0490 | -0.1638 |
| HL | -0.2852 | -0.1636 | 0.2545 | 0.0668 |
| HW | -0.3231 | 0.0938 | 0.2531 | 0.1229 |
| STL | -0.2052 | -0.2373 | 0.1642 | -0.0935 |
| MSL | -0.1542 | -0.2884 | 0.1281 | -0.1688 |
| NS | -0.0621 | -0.2038 | 0.1121 | -0.2817 |
| SL | -0.1036 | -0.3064 | 0.0954 | -0.0592 |
| NTL | -0.2027 | -0.0778 | 0.1742 | 0.0578 |
| EN | -0.0438 | -0.1922 | 0.1300 | -0.2270 |
| TEL | -0.1997 | -0.0921 | 0.1929 | 0.0549 |
| TD | -0.0615 | -0.1400 | 0.1533 | 0.1935 |
| MN | -0.1975 | -0.0674 | 0.1741 | -0.1935 |
| MFE | -0.0602 | -0.1664 | 0.1189 | -0.2474 |
| MBE | -0.1140 | -0.1411 | 0.1644 | -0.1118 |
| IN | 0.0867 | -0.1925 | -0.1099 | -0.2637 |
| EL | 0.1058 | -0.1947 | -0.1174 | -0.3362 |
| IOD | -0.0883 | -0.0409 | 0.1502 | 0.0328 |
| UEW | -0.0010 | 0.0415 | 0.0817 | -0.2647 |
| HAL | -0.2301 | 0.0020 | 0.2345 | -0.0935 |
| FLL | -0.0940 | -0.0726 | 0.2133 | 0.0235 |
| LAL | 0.2410 | 0.0737 | -0.0725 | -0.3699 |
| HLL | -0.3163 | 0.1154 | 0.2714 | 0.0435 |
| THIGHL | -0.3059 | 0.0249 | 0.2685 | 0.1292 |
| TL | -0.2631 | 0.2573 | 0.2520 | 0.1047 |
| FOL | -0.2754 | 0.1915 | 0.1669 | 0.1652 |
| TFOL | -0.2856 | 0.1888 | 0.2376 | 0.1005 |
| 3FL | 0.0010 | 0.3151 | 0.2181 | -0.0420 |
| 1FL | -0.0195 | 0.3177 | 0.1614 | -0.2357 |
| 4TL | -0.1412 | 0.1279 | 0.2539 | 0.0145 |
| IMTL | 0.0236 | 0.0179 | 0.0246 | -0.2117 |
| ITL | -0.0466 | 0.1305 | 0.1765 | -0.2407 |
| **Eigen values** | 7.6247 | 5.1981 | 11.2945 | 4.0814 |
| **Variance explained (%)** | 24.5957 | 16.7680 | 36.4338 | 13.1658 |
| **Cumulative explained (%)** | 24.5957 | 41.3637 | 36.4338 | 49.5997 |

**TABLE 14:** Comparisons among adult males of five populations of *F. cancrivora* from Asian countries by Dunn's multiple comparision test.

| Character | Cian-Sela | Cian-Trat | Cian-Khul | Cian-Maka | Sela-Trat | Sela-Khul | Sela-Maka | Trat-Khul | Trat-Maka | Khul-Maka |
|---|---|---|---|---|---|---|---|---|---|---|
| SVL | 0.0532 | 0.0532** | 0.0588** | 0.0532** | 0.0544** | 0.0600** | 0.0544* | 0.06 | 0.0544 | 0.06 |
| HL | 0.038 | 0.038 | 0.0421 | 0.0380** | 0.0389 | 0.0429 | 0.0389** | 0.0429 | 0.0389** | 0.0429** |
| HW | 0.0361 | 0.0361** | 0.04 | 0.0361** | 0.0370** | 0.0407 | 0.0370** | 0.0407 | 0.0370** | 0.0407** |
| STL | 0.0499 | 0.0499 | 0.0552 | 0.0499** | 0.0511 | 0.0563 | 0.0511** | 0.0563 | 0.0511 | 0.0563 |
| MSL | 0.0632 | 0.0632 | 0.0699 | 0.0632** | 0.0647 | 0.0713 | 0.0647* | 0.0713 | 0.0647 | 0.0713 |
| NS | 0.1304 | 0.1304 | 0.1443 | 0.1304 | 0.1335 | 0.1471 | 0.1335 | 0.1471 | 0.1335 | 0.1471 |
| SL | 0.0635 | 0.0635** | 0.0703 | 0.0635* | 0.0650** | 0.0717 | 0.0650* | 0.0717 | 0.065 | 0.0717 |
| NTL | 0.0474 | 0.0474 | 0.0525 | 0.0474* | 0.0485 | 0.0535 | 0.0485 | 0.0535 | 0.0485 | 0.0535 |
| EN | 0.0929 | 0.0929 | 0.1028 | 0.0929 | 0.0951 | 0.1048 | 0.0951 | 0.1048 | 0.0951 | 0.1048 |
| TEL | 0.1047 | 0.1047 | 0.1158 | 0.1047** | 0.1071 | 0.118 | 0.1071** | 0.118 | 0.1071** | 0.1180** |
| TD | 0.0595 | 0.0595 | 0.0659 | 0.0595* | 0.061 | 0.0672 | 0.0610* | 0.0672 | 0.061 | 0.0672 |
| MN | 0.0394 | 0.0394 | 0.0436 | 0.0394 | 0.0403 | 0.0444 | 0.0403 | 0.0444 | 0.0403 | 0.0444 |
| MFE | 0.0621 | 0.0621 | 0.0687 | 0.0621 | 0.0636 | 0.0701 | 0.0636 | 0.0701 | 0.0636 | 0.0701 |
| MBE | 0.0961 | 0.0961 | 0.1064 | 0.0961* | 0.0984 | 0.1084 | 0.0984 | 0.1084 | 0.0984 | 0.1084 |
| IN | 0.1111 | 0.1111 | 0.123 | 0.1111 | 0.1138 | 0.1254 | 0.1138 | 0.1254 | 0.1138 | 0.1254 |
| EL | 0.0582 | 0.0582 | 0.0644* | 0.0582 | 0.0596 | 0.0657 | 0.0596 | 0.0657 | 0.0596 | 0.0657* |
| IOD | 0.106 | 0.106 | 0.1173 | 0.106 | 0.1085 | 0.1195 | 0.1085* | 0.1195 | 0.1085 | 0.1195 |
| UEW | 0.0733 | 0.0733 | 0.0811 | 0.0733 | 0.075 | 0.0826 | 0.075 | 0.0826 | 0.075 | 0.0826 |
| HAL | 0.0465 | 0.0465 | 0.0514 | 0.0465 | 0.0476 | 0.0524 | 0.0476 | 0.0524 | 0.0476** | 0.0524 |
| FLL | 0.0374 | 0.0374 | 0.0414 | 0.0374 | 0.0383 | 0.0422 | 0.0383 | 0.0422 | 0.0383 | 0.0422 |
| LAL | 0.0547 | 0.0547 | 0.0605 | 0.0547** | 0.056 | 0.0617 | 0.0560** | 0.0617 | 0.0560** | 0.0617** |
| HLL | 0.0335 | 0.0335** | 0.0371 | 0.0335** | 0.0343* | 0.0378 | 0.0343** | 0.0378 | 0.0343** | 0.0378** |
| THIGHL | 0.0493 | 0.0493 | 0.0545 | 0.0493** | 0.0504 | 0.0556 | 0.0504** | 0.0556 | 0.0504** | 0.0556** |
| TL | 0.0252 | 0.0252** | 0.0279** | 0.0252 | 0.0258** | 0.0284** | 0.0258* | 0.0284 | 0.0258** | 0.0284** |
| FOL | 0.0408 | 0.0408** | 0.0452 | 0.0408 | 0.0418** | 0.0461 | 0.0418 | 0.0461 | 0.0418** | 0.0461** |
| TFOL | 0.0335 | 0.0335** | 0.0371** | 0.0335** | 0.0343** | 0.0378** | 0.0343** | 0.0378 | 0.0343** | 0.0378** |
| 3FL | 0.1057 | 0.1057** | 0.1169** | 0.1057* | 0.1082 | 0.1192 | 0.1082 | 0.1192 | 0.1082 | 0.1192 |
| 1FL | 0.0675 | 0.0675** | 0.0747** | 0.0675** | 0.0691** | 0.0761** | 0.0691** | 0.0761 | 0.0691 | 0.0761 |
| 4TL | 0.0652 | 0.0652 | 0.0722 | 0.0652 | 0.0668 | 0.0736 | 0.0668 | 0.0736 | 0.0668 | 0.0736 |
| IMTL | 0.0798 | 0.0798 | 0.0883 | 0.0798 | 0.0817 | 0.09 | 0.0817 | 0.09 | 0.0817 | 0.09 |
| ITL | 0.0683 | 0.0683 | 0.0756 | 0.0683 | 0.0699 | 0.0771 | 0.0699 | 0.0771 | 0.0699 | 0.0771 |

*significance level p <0.05; ** significance level p <0.01.



**TABLE 15:** Comparisons among adult females of five populations of *F. cancrivora* from Asian countries by Dunn's multiple comparision test.

| Character | Cian-Sela | Cian-Trat | Cian-Khul | Cian-Maka | Sela-Trat | Sela-Khul | Sela-Maka | Trat-Khul | Trat-Maka | Khul-Maka |
|---|---|---|---|---|---|---|---|---|---|---|
| SVL | 0.088** | 0.0903** | 0.1055** | 0.0903** | 0.0922 | 0.107 | 0.0922 | 0.1089 | 0.0943 | 0.1089 |
| HL | 0.021** | 0.0215** | 0.0251** | 0.0215** | 0.022 | 0.0255 | 0.022** | 0.026 | 0.0225** | 0.026** |
| HW | 0.0274** | 0.0281** | 0.0329 | 0.0281** | 0.0287** | 0.0334 | 0.0287** | 0.0339** | 0.0294** | 0.0339** |
| STL | 0.0263 | 0.0269 | 0.0314 | 0.0269** | 0.0275 | 0.0319** | 0.0275 | 0.0325 | 0.0281** | 0.0325** |
| MSL | 0.0364 | 0.0373 | 0.0436 | 0.0373 | 0.0381 | 0.0443** | 0.0381 | 0.045 | 0.039 | 0.045** |
| NS | 0.042 | 0.0431 | 0.0503** | 0.0431 | 0.0439 | 0.051** | 0.0439 | 0.0519** | 0.045 | 0.0519** |
| SL | 0.0265** | 0.0272 | 0.0318 | 0.0272** | 0.0277 | 0.0322* | 0.0277 | 0.0328 | 0.0284 | 0.0328** |
| NTL | 0.032 | 0.0329 | 0.0384 | 0.0329** | 0.0335 | 0.0389 | 0.0335* | 0.0396 | 0.0343** | 0.0396** |
| EN | 0.0427* | 0.0438** | 0.0512** | 0.0438 | 0.0447 | 0.0519 | 0.0447 | 0.0528 | 0.0458 | 0.0528 |
| TEL | 0.0595** | 0.0611** | 0.0713** | 0.0611 | 0.0623 | 0.0724 | 0.0623** | 0.0736 | 0.0638** | 0.0736** |
| TD | 0.0426 | 0.0437 | 0.051 | 0.0437** | 0.0445 | 0.0517 | 0.0445** | 0.0526 | 0.0456** | 0.0526* |
| MN | 0.0308 | 0.0316 | 0.0369** | 0.0316 | 0.0323 | 0.0375** | 0.0323 | 0.0381 | 0.033 | 0.0381** |
| MFE | 0.0341 | 0.035 | 0.0408** | 0.035 | 0.0357 | 0.0415* | 0.0357 | 0.0422 | 0.0365 | 0.0422 |
| MBE | 0.0631 | 0.0647 | 0.0755** | 0.0647 | 0.066 | 0.0767 | 0.066 | 0.078 | 0.0676 | 0.078** |
| IN | 0.0602 | 0.0617 | 0.0721 | 0.0617 | 0.063 | 0.0732 | 0.063** | 0.0745 | 0.0645* | 0.0745 |
| EL | 0.0504 | 0.0517 | 0.0604 | 0.0517** | 0.0528 | 0.0613* | 0.0528** | 0.0624 | 0.054** | 0.0624 |
| IOD | 0.0879 | 0.0902* | 0.1053 | 0.0902 | 0.092** | 0.1069 | 0.092 | 0.1088** | 0.0942** | 0.1088 |
| UEW | 0.068 | 0.0698 | 0.0815** | 0.0698 | 0.0712 | 0.0827** | 0.0712 | 0.0842** | 0.0729 | 0.0842** |
| HAL | 0.0382 | 0.0392** | 0.0458** | 0.0392 | 0.04** | 0.0465 | 0.04 | 0.0473 | 0.041** | 0.0473** |
| FLL | 0.0366 | 0.0375** | 0.0438 | 0.0375 | 0.0383 | 0.0445 | 0.0383** | 0.0453* | 0.0392** | 0.0453 |
| LAL | 0.0425 | 0.0436** | 0.0509** | 0.0436** | 0.0445** | 0.0516** | 0.0445** | 0.0525 | 0.0455** | 0.0525** |
| HLL | 0.0284 | 0.0291** | 0.034* | 0.0291** | 0.0297** | 0.0345* | 0.0297** | 0.0351** | 0.0304** | 0.0351** |
| THIGHL | 0.0368** | 0.0377** | 0.0441 | 0.0377** | 0.0385** | 0.0447 | 0.0385** | 0.0455** | 0.0394** | 0.0455** |
| TL | 0.0394** | 0.0404** | 0.0472 | 0.0404** | 0.0412 | 0.0479 | 0.0412** | 0.0487** | 0.0422** | 0.0487** |
| FOL | 0.0586 | 0.0601 | 0.0702 | 0.0601** | 0.0613 | 0.0712 | 0.0613* | 0.0725 | 0.0628** | 0.0725 |
| TFOL | 0.0386 | 0.0396** | 0.0462 | 0.0396** | 0.0404** | 0.0469 | 0.0404** | 0.0477** | 0.0413** | 0.0477* |
| 3FL | 0.0388 | 0.0398** | 0.0465 | 0.0398 | 0.0406** | 0.0472 | 0.0406 | 0.048 | 0.0416** | 0.048* |
| 1FL | 0.0562 | 0.0577** | 0.0674** | 0.0577 | 0.0589** | 0.0684* | 0.0589 | 0.0696 | 0.0603* | 0.0696 |
| 4TL | 0.046 | 0.0472** | 0.0551 | 0.0472** | 0.0482** | 0.056 | 0.0482** | 0.0569 | 0.0493** | 0.0569** |
| IMTL | 0.0488 | 0.05** | 0.0584 | 0.05** | 0.0511** | 0.0593 | 0.0511** | 0.0603 | 0.0523 | 0.0603 |
| ITL | 0.0448 | 0.046** | 0.0537** | 0.046 | 0.0469** | 0.0545** | 0.0469 | 0.0554 | 0.048** | 0.0554** |

*significance level p <0.05; ** significance level p <0.01

**TABLE 16:** Developmental capacity and sex of the hybrids among four populations of *F. cancrivora* and the controls.

| Parent | | No. of eggs | No. of normally cleaved eggs (%) | No. of normal tail-bud embryos (%) | No. of normally hatched tadpoles (%) | No. of normally feeding tadpoles (%) | No. of normal 30-day-old tadpoles (%) | No. of metamorphosed frogs (%) | Matured frogs | |
|---|---|---|---|---|---|---|---|---|---|---|
| Female | Male | | | | | | | | ♂ | ♀ |
| Sela.1 | Sela.1 | 507 | 471 (93) | 449 (89) | 450 (89) | 418 (82) | 146 (29) | 129 (25) | 0 | 0 |
| Sela.2 | Sela.1 | 465 | 405 (87) | 154 (33) | 149 (32) | 67 (14) | 67 (14) | 62 (13) | 7 | 5 |
| Sela.3 | Sela.1 | 468 | 425 (91) | 332 (71) | 273 (58) | 219 (47) | 185 (40) | 145 (31) | 0 | 0 |
| Sela.4 | Sela.2 | 791 | 791 (100) | 669 (85) | 603 (76) | 578 (73) | 570 (72) | 487 (62) | 0 | 0 |
| Sela.5 | Sela.3 | 653 | 625 (96) | 507 (78) | 323 (49) | 304 (47) | 233 (36) | 193 (30) | 0 | 0 |
| Total | | 2884 | 2750(95) | 2111(73) | 1798(62) | 1586(55) | 1201(42) | 1016 (35) | 7 | 5 |
| Sela.1 | Cian.1 | 531 | 364 (69) | 278 (52) | 278 (52) | 264 (50) | 250 (47) | 229 (43) | 0 | 0 |
| Sela.2 | Cian.1 | 541 | 391 (72) | 324 (60) | 295 (55) | 120 (22) | 104 (19) | 102 (19) | 8 | 3 |
| Sela.3 | Cian.1 | 393 | 378 (96) | 310 (79) | 279 (71) | 197 (50) | 184 (47) | 144 (37) | 0 | 0 |
| Sela.4 | Cian.2 | 736 | 699 (95) | 653 (89) | 294 (40) | 284 (39) | 268 (36) | 227 (31) | 0 | 0 |
| Sela.5 | Cian.3 | 90 | 84 (93) | 64 (71) | 59 (66) | 51 (57) | 51 (57) | 50 (56) | 0 | 0 |
| Total | | 2291 | 1916 (84) | 1629 (71) | 1205 (53) | 916 (40) | 857 (37) | 752 (33) | 8 | 3 |
| Sela.1 | Khul.1 | 624 | 5 (1) | 2 (0) | 2 (0) | 2 (0) | 2 (0) | 2 (0) | 0 | 0 |
| Sela.2 | Khul.1 | 687 | 389 (57) | 260 (38) | 170 (25) | 119 (17) | 110 (16) | 62 (9) | 5 | 0 |
| Sela.3 | Khul.2 | 250 | 4 (2) | 3 (1) | 2 (1) | 2 (1) | 0 (0) | 0 (0) | 0 | 0 |
| Sela.4 | Khul.2 | 1187 | 729 (61) | 561 (47) | 555 (47) | 538 (45) | 484 (41) | 293 (25) | 0 | 0 |
| Total | | 2748 | 1127 (41) | 826 (30) | 729 (27) | 661 (24) | 596 (22) | 357 (13) | 5 | 0 |
| Sela.4 | Trat.1 | 812 | 812 (100) | 610 (75) | 489 (60) | 429 (53) | 400 (49) | 362 (45) | 0 | 0 |
| Sela.5 | Trat.2 | 104 | 90 (87) | 79 (76) | 75 (72) | 68 (65) | 64 (62) | 63 (61) | 0 | 0 |
| Total | | 916 | 902 (98) | 689 (75) | 564 (62) | 497 (54) | 464 (51) | 425 (46) | 0 | 0 |



**TABLE 17:** Numbers of meiotic spreads differing in number of univalents in male hybrids between the Selangor and Khulna populations of *F. cancrivora* and the control.

| Parent | | No. of meioses | No. of univalents (%) | | | | | | Mean no. of univalents per spermatocyte |
|---|---|---|---|---|---|---|---|---|---|
| Female | Male | | 0 | 2 | 4 | 6 | 8 | 10 | |
| Selangor | Selangor | 248 | 248 (100) | | | | | | 0.00 |
| Selangor | Khulna | 237 | 196 (82.7) | 31 (13.1) | 13 (5.5) | 2 (0.8) | 0 | 0 | 0.53 |



**TABLE 18:** Numbers of the ring- and rod-shaped bivalents in male hybrids between the Selangor and Khulna populations of *F. cancrivora* and the control.

| Parent | | Total no. of bivalents | Large chromosome | | Small chromosome | | Total | | Mean no. of bivalents per cell |
|---|---|---|---|---|---|---|---|---|---|
| Female | Male | | Ring (%) | Rod (%) | Ring (%) | Rod (%) | Ring (%) | Rod (%) | |
| Selangor | Selangor | 248 | 1240 (100) | 0(0) | 1984(100) | 0(0) | 3224(100) | 0(0) | 13.00 |
| Selangor | Khulna | 237 | 667(61.9) | 411(38.1) | 1270(78.2) | 354(21.8) | 1937(71.7) | 765(28.3) | 11.96 |

**TABLE 19:** Comparison of snout-vent length of *F. cancrivora* in the literature.

| No. | Locality | Country | SVL (mm) | Source |
|---|---|---|---|---|
| 1 | Borneo | Malaysia | ♂: 58.80 (51.00-70.90, n=25) | Inger (1966) |
| | | | ♀: 68.60 (52.90-82.00, n=19) | |
| 2 | Cianjur, Java (neotype) | Indonesia | ♂ : 68.2 | Dubois and Ohler (2000) |
| 3 | Bogor, Java | Indonesia | ♂ : 72.4 (n=80) | Premo and Atmowidjojo (1987) |
| | | | ♀: 87.5 (n=93) | |
| 4 | Jakarta, Java | Indonesia | ♂: 74-82 (61-93, n=227) | Church (1960) |
| | | | ♀ : 101-105 (64-132, n=1.098) | |
| 5 | West and East Java | Indonesia | ♂: 65.54 (50.20-86.20, n=183) | Kusrini and Alford (2006) |
| | | | ♀: 75.10 (40.00-162.00, n=367) | |
| 6 | Java | Indonesia | (Sex n/a)*: 100-120 | Iskandar (1998) |
| 7 | Java | Indonesia | ♂: 77 | Smith (1930) |
| | | | ♀ : 75 | |
| | Bangkok | Thailand | ♂: 60 | |
| | | | ♀ : 73 | |
| | Philippines | Philippines | ♂: 69 | |
| | | | ♀ : 67 | |
| 8 | Luzon and adjacent islands | Philippines | ♂: 55.30 (44.50-74.90, n=138) | Inger (1954) |
| | | | ♀: 64.20 (46.10-79.00, n=116) | |
| 9 | Samut Prakan, Chon Buri and Songkla | Thailand | (Sex n/a): 45 | Nutphund (2001) |
| 10 | Chon Buri | Thailand | ♀ : 68 | Taylor (1962) |
| 11 | Bangkok | Thailand | ♂: 53.30 (48.00-60.00; n=6) | Kurniawan et al. (2010) |
| | | | ♀: 65.00 (59.00-71.00; n=4) | |
| | Manila | Philippines | ♂: 50.30 (45.00-55.00; n=3) | |
| | | | ♀ : 55.40 (49.00-68.00; n=5) | |
| 12 | Negros island | Philippines | ♀: 67 | Alcala (1962) |

*Sex n/a = Sex information was not available



(Bandelt et al., 1999; Network 4.502 available at http ://www.fluxus-engineering. com).

Morphometry.– Morphological analyses were performed on 97 live individuals from various countries (Fig. 10, Table 11). These specimens were deposited in the Institute for Amphibian Biology, Hiroshima University (IABHU) (Appendix 1).

Morphological measurements were conducted using characters previously described by Djong et al. (2007b) and Islam et al. (2008a) (Fig. 11). The following 31 characters were measured with calipers to the nearest 0.1 mm: snout-vent length (SVL), head length (HL), head width (HW), snout tympanum length (STL), mouth angle-snout length (MSL), distance from nostril to tip of snout (NS), distance from front of eye to tip of snout (SL), nostril tympanum length (NTL), distance from eye to nostril (EN), tympanum-eye distance (TEL), tympanum diameter (TD), distance from back of mandible to nostril (MN), distance from back end of mandible to front of eye (MFE), distance from back of mandible to back of eye (MBE), inter-narial space (IN), eye length (EL), inter-orbital distance (IOD), maximum width of upper eyelids (UEW), hand length (HAL), forelimb length (FLL), lower arm length (LAL), hindlimb length (HLL), thigh length (THIGHL), tibia length (TL), foot length (FOL), length of tarsus and foot (TFOL), third finger length (3 FL), first finger length (1FL), fourth toe length (4TL), length of inner metatarsal tubercle (IMTL), and inner toe length (ITL).

To standardize the different body sizes among the specimens, each measurement was divided by the SVL and converted to a percentage. The converted data were transformed into $\log_{10}$ values before being subjected to principal component analysis (PCA) and clustering analysis using R-2.4.1 software. The morphological differences among populations were examined using Dunn's multiple comparison test at a significance level of 5%.

Crossing Experiments.– Crossing experiments were performed by artificial insemination (Kawamura et al., 1980). During the breeding season (May to August) of 2007, using 15 *F. cancrivora* individuals, consisting of five females and three males from Selangor (Malaysia), three males from Cianjur (Indonesia), two males from Trat (Thailand), and two males from Khulna (Bangladesh) were used (Table 11).

Sperm suspensions were prepared by crushing a single testis removed from each male in 2–3 ml of distilled water. Ovulation was accelerated by the injection of bullfrog pituitary extracts into the body cavity, and the released eggs were stripped from the females and placed on glass slides. After sperm motility was confirmed by visual examination under a microscope, eggs were inseminated with the sperm suspension, transferred into glass Petri dishes containing 400–450 ml of tap water, and observed to confirm normal development. Tadpoles were fed boiled spinach and metamorphosed frogs were fed a diet of crickets. Viability was calculated as the rate of normal development among the total eggs at each of the following developmental stages: normal cleavage, tail-bud embryo, hatched tadpole, feeding tadpole, 30-day-old tadpole, and metamorphosed frog. The ploidy of the hybrids was checked by observing the number of chromosomes in metaphase spreads of cells derived from the tail tips.

Histological and spermatogenesis observations.– Testes of the mature hybrids and control frogs were examined histologically and spermatogenesis was microscopically examined. Among the mature hybrid and control individuals, four hybrids between Selangor females and Khulna males and two controls were subjected to histological and spermatogenesis examinations. For each individual, one testis was fixed in Navashin's solution, embedded in paraffin, sectioned at 10 µm, and stained with Heidenhain's iron hematoxylin. The respective other testis was used for the chromosome preparation. Meiotic chromosomes were prepared according to the technique described by Schmid et al. (1979) with slight modifications. The chromosomes were stained with a 2% Giemsa solution for 5 min. The chromosome analysis was performed using only diploid cells at the diakinesis and metaphase stages of the first reduction division as bivalent and univalent chromosomes could be easily distinguished from each other. Bivalent chromosomes appeared identical to normal chromosomes, which adopt a thick, symmetrical form, whereas univalent chromosomes were identical to abnormal chromosomes, which are asymmetric and thin (Kawamura et al., 1980, 1981; Kuramoto, 1983; Sumida et al., 2003).

## RESULTS

Allozyme data.– Based on the electrophoretic patterns, 17 enzymes were presumed to be encoded by genes at 26 loci. One to eight phenotypes (mean 3.0) were produced by one to five alleles (mean 2.7) (Table 2). The AAT-2, CK, FUM-2, IDH-2 and PEP-A loci were monomorphic, and *F. cancrivora* and *F. iskandari* shared the same phenotype. The MPI locus was most polymorphic 5 alleles gave rise to eight phenotypes.

The allele frequencies for each population of *F. cancrivora* and outgroup *F. iskandari* were

represented at all 26 loci (Table 4). The alleles for distinguishing *F. cancrivora* populations from the *F. iskandari* were at 14 loci: ADA, AK, FUM-1, α-GDH, IDH-1, LDH-A, LDH-B, MDH-1, MDH-2, ME-2, PEP-B, PEP-C, PEP-D and PGM (Table 4).

At the ADA locus, allele *a* was present in the large-type and the Pelabuhan ratu-type, allele *c* was present in the mangrove-type, and allele *b* was exclusively present in *F. iskandari* (Fig. 2). The same pattern was found at two more loci: AK and PGM (Table 4). An almost complete separation of *F. cancrivora* into three clusters was observed at the PEP-B locus, where allele *d* dominated in the mangrove-type, allele *c* was present in the large-type, allele *b* was present in the Pelabuhan ratu-type, and allele *a* characterized *F. iskandari*. The Manila population was partly differentiated from the other populations of the mangrove-type by the addition of allele *c* at the PEP-B locus (Fig. 3). At the MDH-1 locus, the Pelabuhan ratu-type possessed allele *c*, while the mangrove-type and the large-type had allele *d*, and *F. iskandari* possessed alleles *a* and *b*. At the MPI locus, the Cianjur and Selangor populations (large-type) shared the alleles, *b*, *c*, and *d*. The other populations of that type were dominated by allele *d*. Allele *d* of the MPI locus was dominant in the mangrove-type and the Pelabuhan ratu-type, while in *F. iskandari* by allele *b* dominated in addition to alleles *a* and *c* (Fig. 4). The ALD locus almost completely separated *F. cancrivora* populations from *F. iskandari*: allele *b* dominated in the *F. cancrivora* populations, while allele *a* dominated in the *F. iskandari* (Table 4). At the AAT-1 locus, allele *a* was present in the Pelabuhan ratu-type and the outgroup, and allele *b* was present in the mangrove-type and the large-type. At the GPI locus, *F. iskandari* and the mangrove-type had allele *a*, while the Trat population belonging to the mangrove-type was dominated by allele *a* in addition to allele *b*. The large-type and the Pelabuhan ratu-type had allele *b*, while the Cianjur population corresponding to the large-type was dominated by allele *b* in addition to allele *c* at the GPI locus (Table 4). At the ME-1 locus, the mangrove-type had allele *b*, while the Manila and Trat populations (mangrove-type) were dominated by allele *b* in addition to allele *a*. The large-type had allele *c*, while the Selangor population (large-type) was dominated by allele *c* in addition to allele *d*. The Pelabuhan ratu-type was also dominated by allele *c* in addition to allele *d* at the ME-1 locus (Table 4).

Within *F. cancrivora* types, we conclude that for distinguishing the mangrove-type from the large- and Pelabuhan ratu/Sulawesi-types, respectively, clear difference in allele frequencies were found at the ADA, AK, GPI, LDH-B, ME-1, PGM and SOD loci. For distinguishing the Pelabuhan ratu/Sulawesi-type from the mangrove- and large-types, clear differences in allele frequencies were found at the AAT-1 and MDH-1 loci; and for distinguishing each type from *F. iskandari*, clear differences were present at the PEP-B locus (Table 4).

Nei's genetic distance (D) and genetic identity (I) between each the populations of *F. cancrivora* and *F. iskandari* respectively are shown in Table 5. *Fejervarya cancrivora* was divided into two groups, the mangrove-type and the large- plus Pelabuhan ratu-types. The genetic distance (D) within the mangrove-type populations (mean, 0.023 ± 0.019) was higher than the large-type populations (0.006) (Table 6). The genetic distance (D) between the mangrove-type and large-type (mean, 0.510 ± 0.025) and between the mangrove-type and Pelabuhan ratu-type (mean, 0.586 ± 0.036) was almost the same value, while between the large-type and Pelabuhan ratu-type (mean, 0.197 ± 0.012) had smaller value (Tables 5 and 6). The genetic distances within the ingroup *F. cancrivora* were smaller than those between *F. cancrivora* and outgroup *F. iskandari* (Tables 5 and 6).

In the NJ tree based on Nei's genetic distances (Fig. 5), *F. cancrivora* was divided into two clades, the mangrove-type (BP = 86.5%) and the large- plus Pelabuhan ratu-type (BP = 88.5%), the latter was further subdivided into two subclades, the large-type (BP = 97.8%) and the Pelabuhan ratu-type, respectively.

DNA sequence data.– The alignment data from nucleotide sequences revealed 10 haplotypes for a 497-bp segment of the 16S rRNA gene and 17 haplotypes for a 557-bp segment of the Cyt *b* gene, respectively (Table 3). In the 16S rRNA gene analysis, we used these 10 haplotypes and seven additional haplotypes retrieved from GenBank. The new alignment, 389-bp in length, revealed 12 haplotypes including the outgroup (Table 7). The 389-bp segment of the 16S rRNA gene data set, contained 83 variable sites, of which 42 were parsimony informative. The 557-bp-segment of the Cyt *b* gene contained 180 variable sites, of which 125 were parsimony informative. In our substitution model for the 16S rRNA gene data set, Kakusan 3.0 (Tanabe, 2007) suggested J2+G model as the best-fitting model, with a Gamma distribution shape parameter (G) of 0.2393. The J2+G was used as substitution model to carry out the ML, BI and NJ analyses. For the ML analyses, the empirical base frequencies were T = 0.2340; C = 0.2705; A = 0.2929; G = 0.2026. In our substitution model for the Cyt *b* gene data set, Kakusan 3.0 (Tanabe, 2007) suggested a Hasegawa Kishino and Yano (HKY+G) model as the best fitting model, with a Gamma distribution




**FIGURE 1:** Map showing the localities of *Fejervarya cancrivora* examined in the present study. Allozyme samples were collected from asterisked localities.


**FIGURE 2:** Geographical distribution of ADA alleles in the specimens of *Fejervarya cancrivora* and *F. iskandari* examined. The large-type (Selangor and Cianjur) shared the same allele with the Pelabuhan ratu-type.


**FIGURE 3:** Geographical distribution of PEP-B alleles in *Fejervarya cancrivora* and *F. iskandari*. With the exception of Manila, where a small percentage of allele *c* was present, exclusive allele were found in all other localities (i.e., *F. iskandari* = allele *a*, Pelabuhan ratu-type = allele *b*, Large-type = allele *c*, Mangrove-type = allele *d*).


**FIGURE 4:** Geographical distribution of MPI alleles in *Fejervarya cancrivora* and *F. iskandari* from Indonesia and other Asian countries.



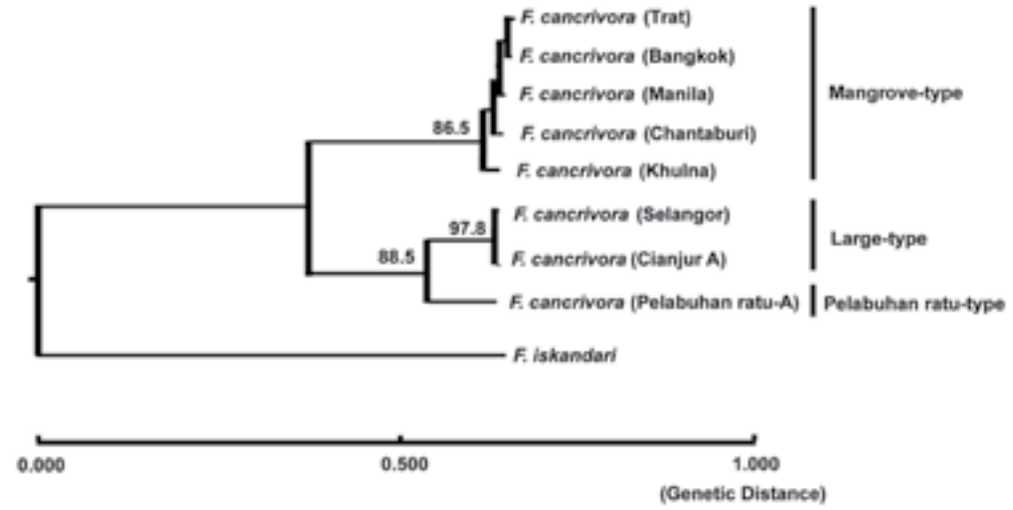


**FIGURE 5:** Phylogenetic tree constructed using neighbor-joining algorithm. It was based on Nei`s genetic distances (Saitou and Nei, 1987) as calculated from allelic frequencies at 26 loci from eight populations of *F. cancrivora* and one population of *F. iskandari* as an outgroup. Values above branches are bootstrap values (>50%), from 1000 pseudoreplicates. The scale bar represents branch length represented by Nei's genetic distance.


**FIGURE 6:** Phylogenetic tree inferred from maximum-likelihood analysis based on a 389-bp segment of the mitochondrial 16S rRNA gene from 17 haplotypes of *Fejervarya cancrivora*, and one haplotype of *F. iskandari* as outgroup. Bootstrap supports are given in order for ML/MP/NJ/BI analyses, respectively.


**FIGURE 7:** Phylogenetic tree constructed by the maximum-likelihood method based on a 557-bp segment of the mitochondrial Cyt *b* gene from 18 haplotypes of *Fejervarya cancrivora*, using *F. iskandari* as an outgroup. The bootstrap supports are given in order for ML/MP/NJ/BI analyses.

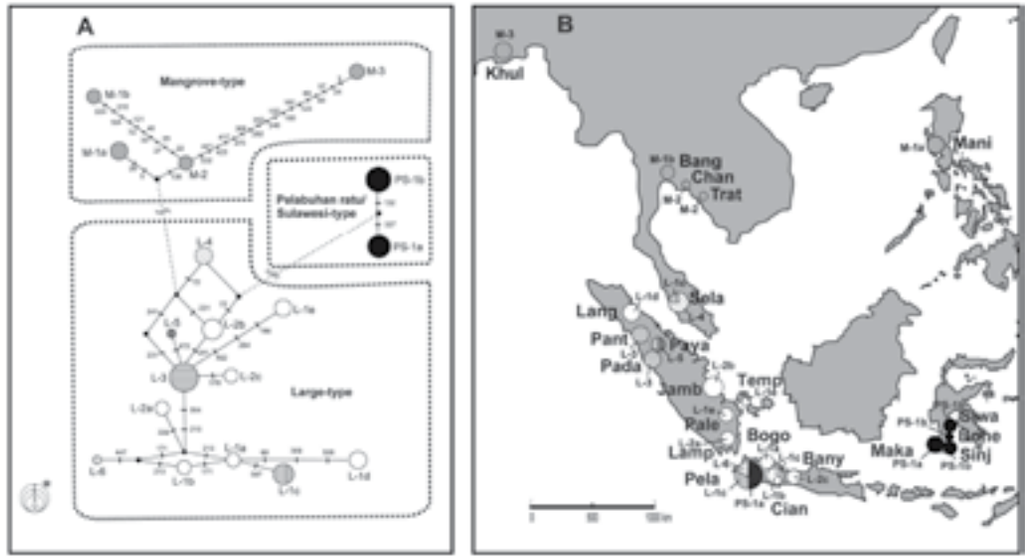


**FIGURE 8:** A. Haplotype network tree based on 18 haplotypes of the Cyt *b* gene sequence of 55 individuals of *Fejervarya cancrivora*. The haplotypes are represented by circles, and the numbers of individuals are represented by different sizes in asterisked circles. Asterisked circles comprise sample sizes of 1, 3, 5, and 7 individuals from inside. The substitution sites in the alignment data of Cyt *b* gene sequences between each haplotype are represented by transversal lines and figures on branches. Each haplotype is abbreviated based on the name in Table 3: e.g., Mangrove-type = "M"; Large-type = "L"; and Pelabuhan ratu/Sulawesi-type = "PS."

B. Geographic distribution and frequency of the haplotypes of Cyt *b* gene sequences. Pie charts indicate ratios of haplotype in total numbers of individuals within respective populations.




**FIGURE 9:** Geographic distribution of *Fejervarya cancrivora* types in Asia based on molecular data presented here.

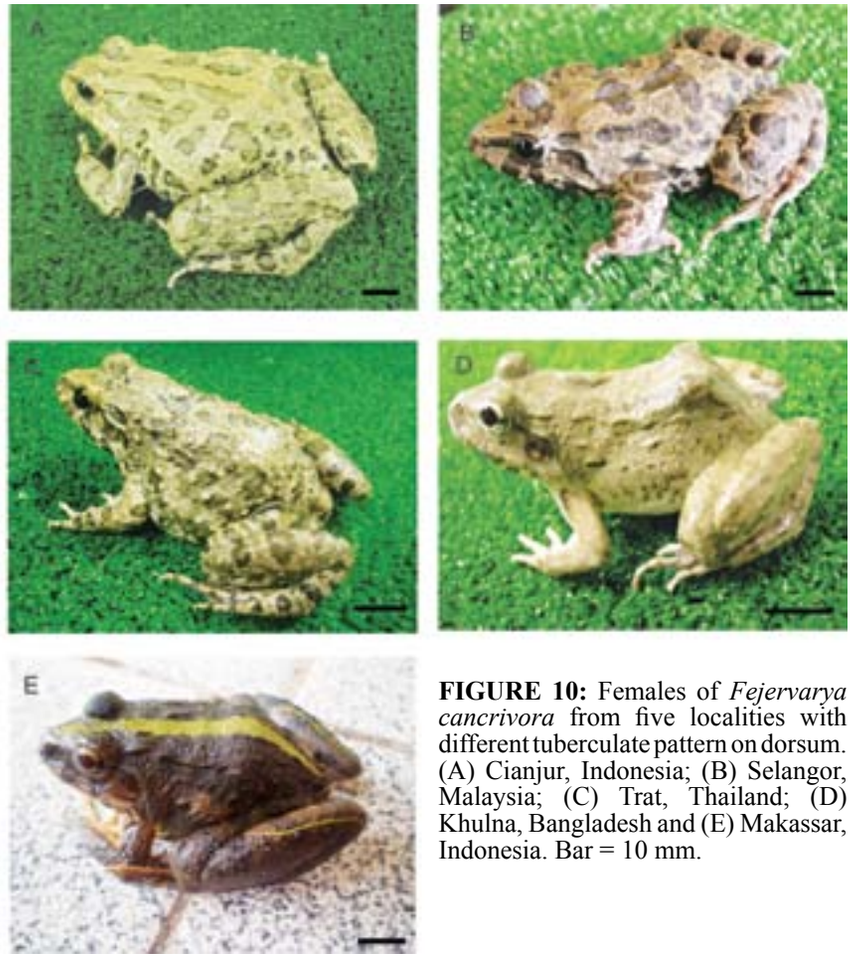


**FIGURE 10:** Females of *Fejervarya cancrivora* from five localities with different tuberculate pattern on dorsum. (A) Cianjur, Indonesia; (B) Selangor, Malaysia; (C) Trat, Thailand; (D) Khulna, Bangladesh and (E) Makassar, Indonesia. Bar = 10 mm.

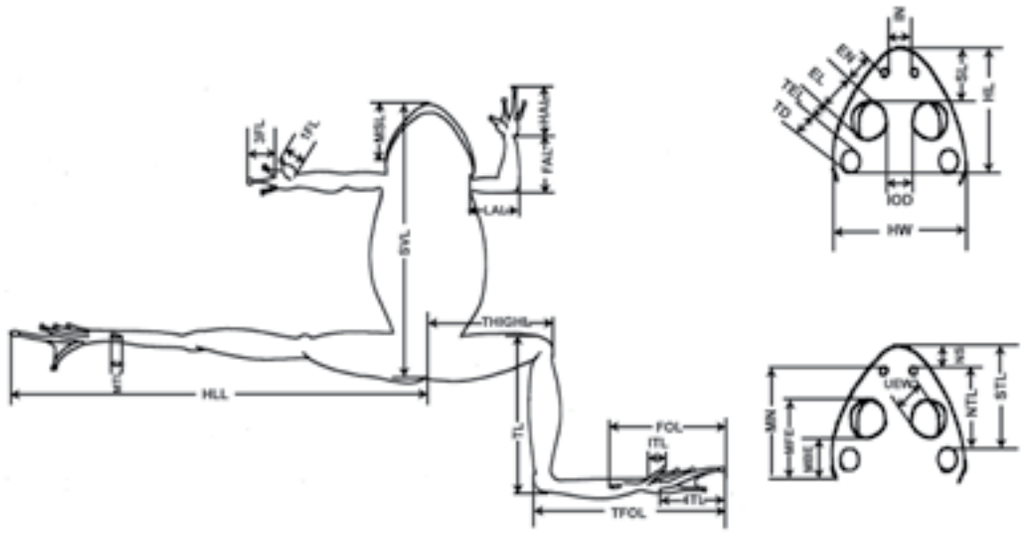


**FIGURE 11:** Morphological characters measured in the current study.

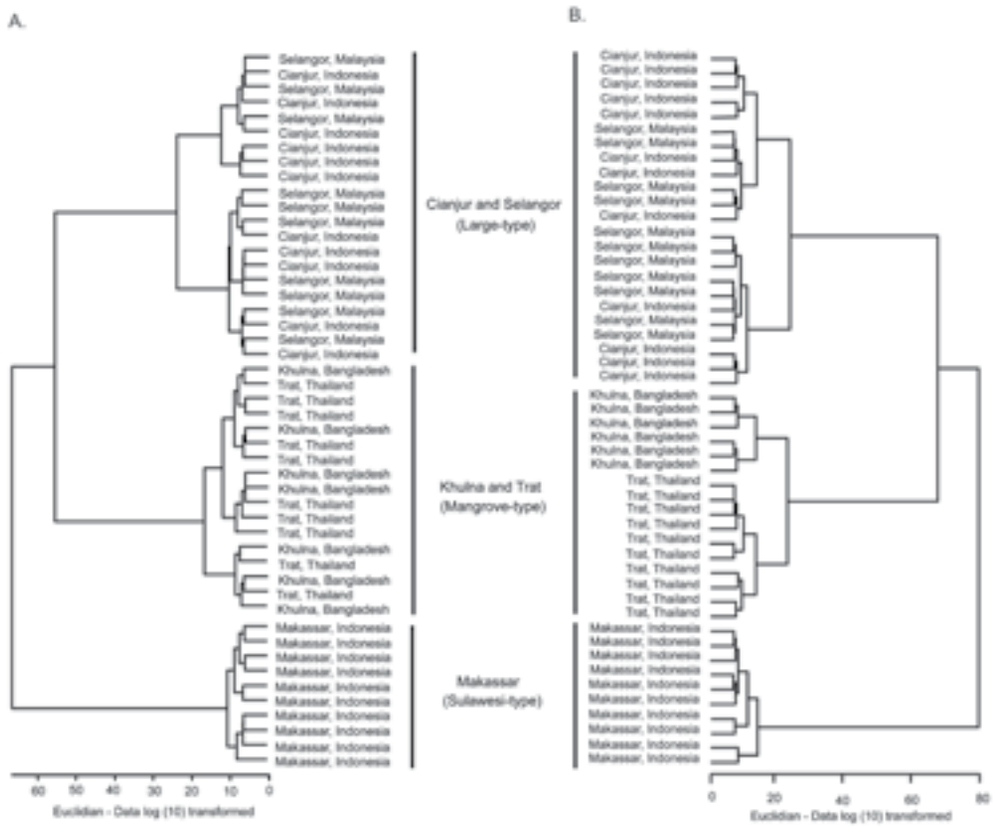


**FIGURE 12:** UPGMA dendrogram based on morphological characters of females and males in five populations of *Fejervarya cancrivora*. (A) Males and (B) Females.



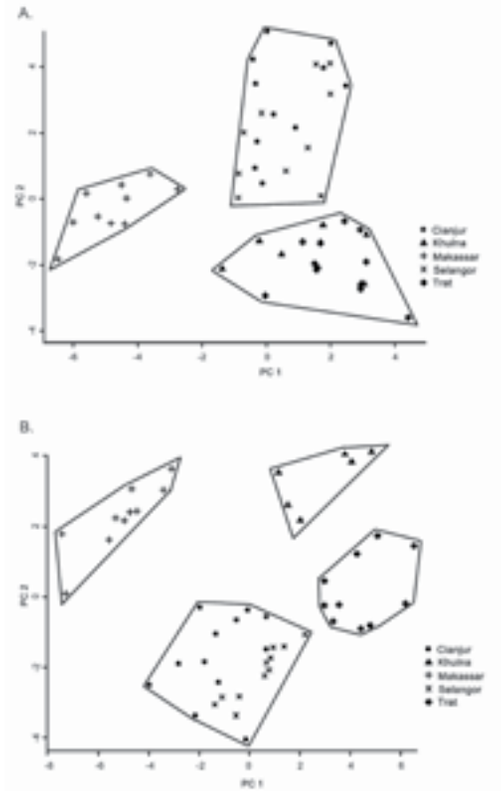


**FIGURE 13:** Plot of principal component 1 (PC1) versus principal component 2 (PC2) for the principal component analysis of females and males in five populations of *Fejervarya cancrivora*. (A) Males and (B) Females.

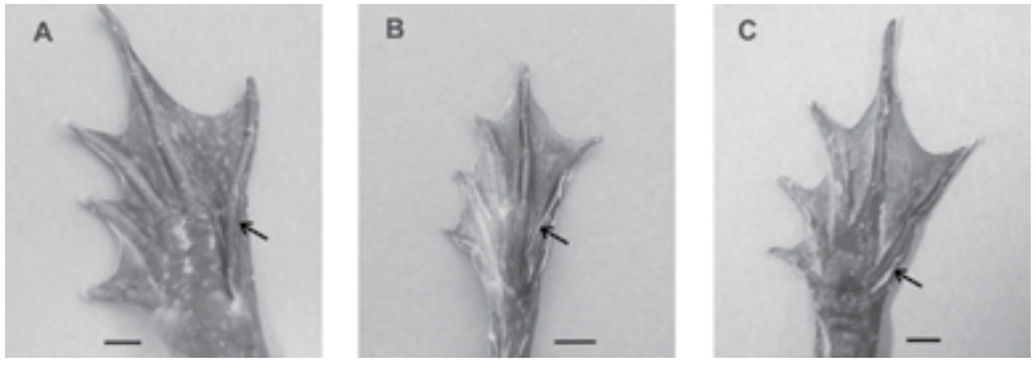


**FIGURE 14:** Hindlimb toe webbing and free flap in three types of *Fejervarya cancrivora*. (A) Hindlimb toe webbing of the Selangor population (Large-type female); (B) Hindlimb toe webbing of the Trat population (Mangrove-type female) and (C) Hindlimb toe webbing of the Makassar population (Sulawesi-type female). Bar = 5 mm. Arrow indicates the free flap.

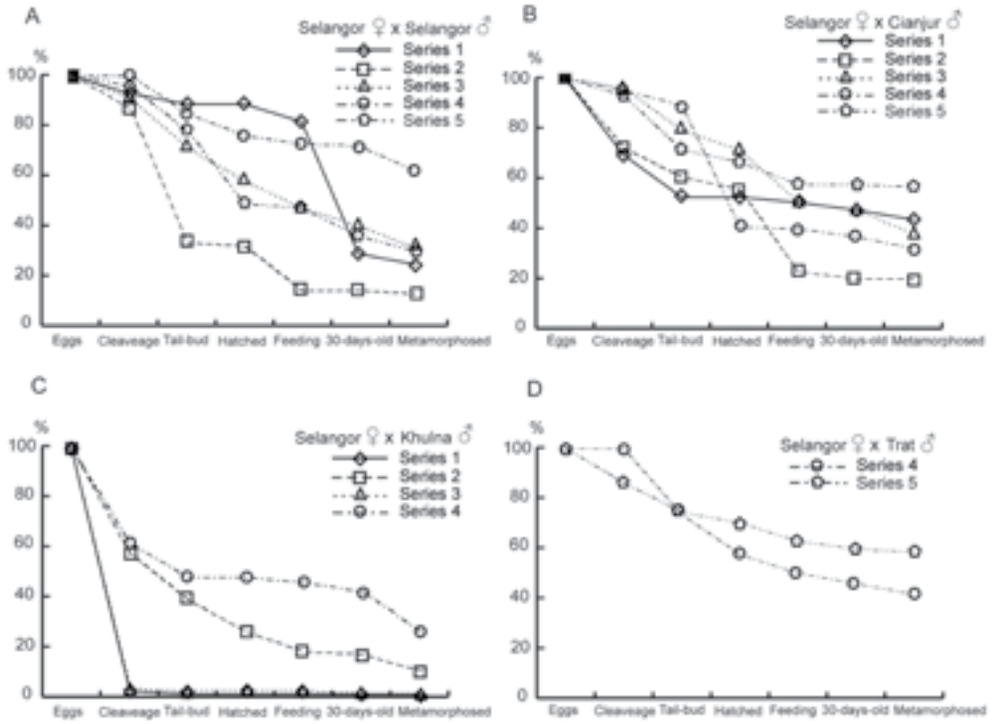


**FIGURE 15:** Survival curves of the hybrids among four populations of *Fejervarya cancrivora* and the control. (A) Control; (B) Hybrid Selangor female x Cianjur male; (C) Hybrid Selangor female x Khulna male and (D) Hybrid Selangor female x Trat male.

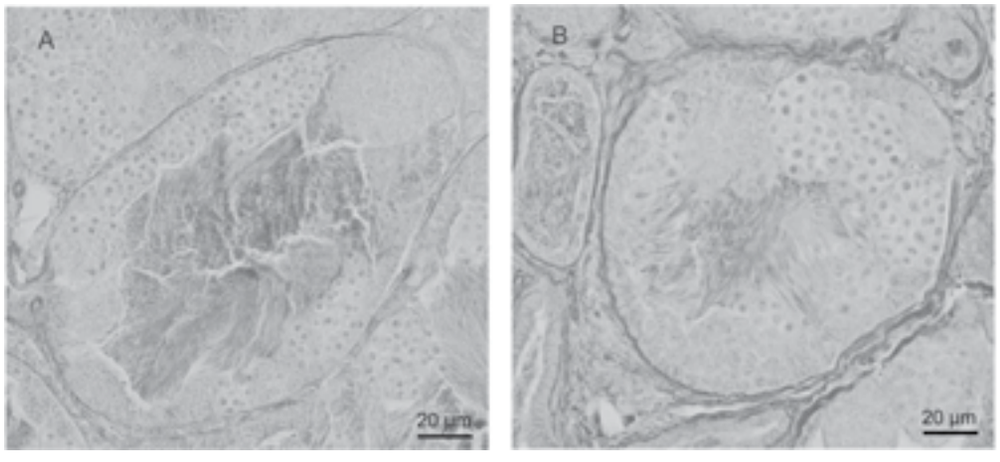


**FIGURE 16:** Histological cross-sections of seminiferous tubules in the testes of the control and the hybrid *Fejervarya cancrivora* Selangor female x Khulna male. (A) Control and (B) Hybrid between Selangor female and Khulna male.



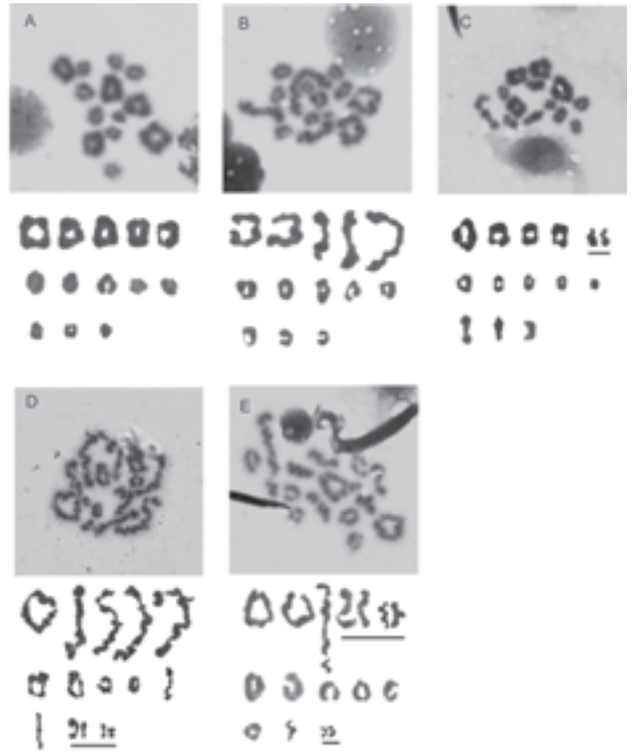


**FIGURE 17:** Spermatocytes at the first meiosis and chromosome complements in the control and the hybrid *Fejervarya cancrivora* Selangor female x Khulna male. (A) Control containing 13 bivalents, which are all ring-shaped and (B) Hybrid containing 13 bivalents, which are ring- or rod-shaped. (C-E) Hybrids contained 2-6 univalents.

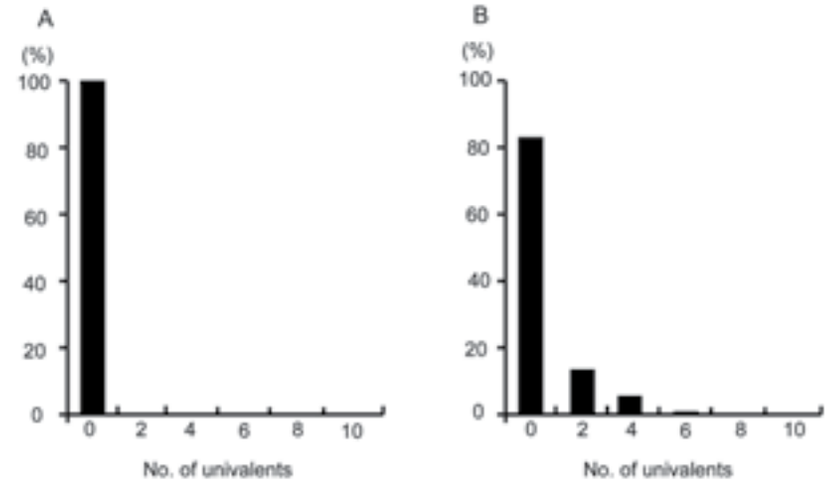


**FIGURE 18:** Frequencies of meiotic spreads differing in the number of univalents in the of *Fejervarya cancrivora* males of the control and the hybrid Selangor female x Khulna male. (A) Control and (B) Hybrid Selangor female x Khulna male.

shape parameter (G) of 0.5244. The HKY+G substitution model was used for the ML and NJ analyses. For the ML analyses, the empirical base frequencies were T = 0.3094; C = 0.2778; A = 0.2460; G = 0.1668.

In the 16S rRNA gene, the sequence divergences between *F. iskandari* and 11 haplotypes of *F. cancrivora* ranged from 14.14% to 16.20% (mean, 14.96 ± 0.76%) (Tables 7 and 8). The sequence divergences within the mangrove-type haplotypes ranged from 0.26% to 1.80% (mean, 1.12 ± 0.68%), while the sequence divergences within the large-type haplotypes ranged from 0.26% to 0.51% (mean, 0.43 ± 0.12%). The sequence divergences between the mangrove-type and large-type haplotypes ranged from 8.74% to 9.51% (mean, 9.10 ± 0.25%) (Tables 7 and 8). The sequence divergences between the mangrove-type and the Pelabuhan ratu/Sulawesi-type haplotypes ranged from 10.03% to 10.54% (mean, 10.22 ± 0.24%) (Tables 7 and 8). The sequence divergences between the large-type and Pelabuhan ratu/ Sulawesi-type haplotypes ranged from 5.40% to 5.91% (mean, 5.78 ± 0.21%) (Tables 7 and 8). In the 16S rRNA gene ML tree, *F. cancrivora* formed two main clades, the mangrove-type clade and the large- and Pelabuhan ratu/Sulawesi-type clades (Fig. 6). The mangrove-type population formed one clade consisting of four haplotypes, and the large-type samples formed a clade consisting of six haplotypes. The Pelabuhan ratu/Sulawesi-type had one haplotype (Table 3 and Fig. 6).

In the mangrove-type (M), M-I haplotype from Manila, Bangkok, Negros Island and Hainan Island joined with M-II haplotype from Trat and Chantaburi, while M-III haplotype from Khulna joined with M-IV haplotype from Orissa. The M-I and M-II haplotypes diverged from M-III and M-IV haplotypes with strongly supported bootstraps (98/100/100/100) (Fig. 6). In the large-type (L), L-I haplotype from Cianjur, Langkat, Selangor, Pelabuhan ratu, Tempilang and Bogor was separated from other haplotypes with moderate bootstraps (81/99/100/96). The other five haplotypes in the large-type were L-II from Palembang, Lampung, Jambi and Banyumas; L-III from Padang, Payakumbuh and Panti; L-IV from Selangor; L-V from Kalimantan; L-VI from Jiadong (Fig. 6). The Pelabuhan ratu/Sulawesi-type contained PS-I haplotype from Pelabuhan ratu, Makassar, Bone, Sinjai and Siwa. PS-I haplotype joined with the large-type with poorly supported bootstraps (65/63/82/68) (Fig. 6).

In the Cyt *b* gene (Tables 9 and 10), sequence divergences between the outgroup *F. iskandari* and 18 haplotypes of *F. cancrivora* ranged from 19.57% to 21.90% (mean, 20.24 ± 0.73%). Sequence divergences among mangrove-type haplotypes ranged from 0.54% to 5.03% (mean, 2.78 ± 1.58%); those among large-type haplotypes from 0.18% to 1.62% (mean, 0.76 ± 0.33%); and among Pelabuhan ratu/Sulawesi-type haplotypes 0.36% (Tables 9 and 10). The sequence divergences between the large-type and Pelabuhan ratu/Sulawesi-type haplotypes ranged from 12.39% to 13.47% (mean, 12.88 ± 0.28%). The sequence divergences between the mangrove-type and Pelabuhan ratu/Sulawesi-type haplotypes ranged from 15.98% to 17.24% (mean, 16.38 ± 049%) (Tables 9 and 10).

In the ML tree of the Cyt *b* gene fragment (Fig. 7), *F. cancrivora* formed two main clades, the strongly supported mangrove-type (BP = 98/100/100/100) and the less supported large- plus Pelabuhan ratu/Sulawesi-types clades (BP = 66/67/78/80), made of the strongly supported large-type subclade (BP = 85/99/100/100) and the strongly supported Pelabuhan ratu/Sulawesi-type subclade (BP = 100/100/100/100). In the mangrove-type clade, haplotypes M-1a, M-1b and M-2 formed a subclade (BP = 87/95/99/97) (Fig. 7).

The haplotype network tree based on 18 haplotypes of the Cyt *b* gene sequence of 55 individuals (Fig. 8A) revealed three haplogroups of *F. cancrivora* corresponding to three subclades observed in phylogenetic trees, based on allozyme and molecular data (Figs. 5, 6, and 7). The mangrove-type haplogroup was separated from the large-type haplogroup and from the Pelabuhan ratu/ Sulawesi-type haplogroup by 44 and 69 nucleotide substitutions, respectively. In the mangrove haplogroup, each population had only one haplotype: the Manila, Bangkok and Khulna populations had M-1a, M-1b and M-3, respectively, and the Trat and Chanthaburi populations had M-2 (Figs. 7 and 8). In 17 populations of the large and Pelabuhan ratu/Sulawesi haplogroups, 13 populations had only one haplotype each, whereas the other four populations had two or three haplotypes (Fig. 8 B). Among them, the Pelabuhan ratu population had three haplotypes, PS-1a, L-1c and L-6, in the Pelabuhan ratu/Sulawesi haplogroup, and the Selangor, Payakumbuh, and Cianjur populations each had two haplotypes, L-1c and L-4; L-3 and L-5; and L-1b and L-1c, respectively, in the large haplogroup (Fig. 8 B).

Morphological observations.– (a) Measurements: Figure 10 compares representative female frogs from the five *F. cancrivora* populations examined, with different tubercles line pattern on dorsal skin. The large-type and Sulawesi-type had ridges of elongated tubercles, while the mangrove-type had rounded tubercles on the dorsum. In the large-type group, female SVL ranged from 60.7–106



mm, whereas male SVL ranged from 59.3–76.2 mm (Table 12). Among the mangrove-type group, female SVL was 46.6–83.6 mm, both extremes found in the Khulna, Bangladesh population. The smallest (46.6 mm) and largest (62.4 mm) males were from the Khulna, Bangladesh and Trat, Thailand populations, respectively (Table 12). For the Sulawesi-type group, female size ranged from 63.2–85.7 mm, and males from 48.1–63.9 mm, respectively (Table 12).

The dendrograms based on Euclidean distance showed that the five populations of *F. cancrivora* clustered into two groups, the Sulawesi-type and the mangrove-plus-large-types, and that the latter cluster could be further split into two subclusters, namely, the mangrove- and large-types (Fig. 12). This clustering was based on the similarity and dissimilarity of transformed data derived from 31 morphometric variables. Among the males and females of the large-type group, the Cianjur population from Indonesia could not be distinguished from the Selangor population from Malaysia. In the mangrove-type group, although the females of the Trat population from Thailand were distinguishable from those of the Khulna population from Bangladesh, mangrove-type males were indistinguishable between these two populations (Fig. 12).

The PCA based on the 31 selected morphometric characters showed that the males of the five populations cluster into the large-, mangrove-, and Sulawesi-type groups. The PCA for females produced nearly identical results to those obtained for males, with the exception of the Trat population from Thailand and the Khulna population from Bangladesh, which could be considered separate groups (Fig. 13). In the PCA analysis, two components were extracted with eigenvalues of greater than 1.0, and this explained 24.60% and 36.43% (first component) and 16.77% and 13.17% (second component) of all observed morphometric variation in males and females, respectively (Table 13). Hindlimb (HLL, THIGHL, TFOL, and FOL) and head (HW and HL) characters were dominant in the PC1 for both males and females (Table 13), whereas forelimb characters (3FL, 1FL, and LAL) and eye size (EL) were dominant in the PC2 for both sexes (Table 13). When the morphological data of the adult specimens among the five populations were analyzed by Dunn's multiple comparison test, significant differences were found among 21 morphometric characters in males and 24 females, respectively (Tables 14 and 15).

In the morphological comparison of adult males, no significant differences between the Cianjur and Selangor populations or between the Trat and Khulna populations were observed in any examined character (Table 14). In contrast, the Makassar population showed significant differences from both the Trat and Khulna populations in 10 characters and from the Cianjur and Selangor populations in 16 and 15 characters, respectively (Table 14). In the adult female comparisons, significant differences were observed in eight characters between the Cianjur and Selangor populations, and in nine characters between the Trat and Khulna populations (Table 15). As was the case in males, the Makassar population showed the highest variability from the other populations, with significant differences in 21 and 16 characters between both the Trat and Khulna populations and Cianjur and Selangor populations, respectively (Table 15).

(b) Toe webbing and free flap of the hindlimb: The hindlimb toe webbing of a Selangor female (large-type, SVL 82.1 mm) of *F. cancrivora* was moderate, terminating short of the tips (Fig. 14A), and the free flap of Toe V extended until the first segment of the toe, although it gradually narrowed from the base to the first toe segment (free flap width 1.0 mm, length 24 mm). In contrast, the toe webbing of the Trat female (mangrove-type, SVL 70.3 mm) extended to the tips (Fig. 14B), with the free flap of Toe V extending to the tip of the toe and gradually narrowing from the bottom to the tip of the toe width 1.5 mm, length 21 mm). The hindlimb toe webbing of the Makassar female (Sulawesi-type, SVL 84.2 mm) was moderate, stopping short of the tips, as was observed in the Selangor female (Fig. 14C). The free flap of the fifth toe of this female extended up to the second segment of the toe and had a width and length of 1.0 mm and 20 mm, respectively.

Crossing experiments.– (a) Developmental capacity of the hybrids: As differences exist in the breeding seasons among populations of *F. cancrivora*, matured eggs could not always be obtained from a target population at the required time for the crossing experiments. Thus, crossing experiments were performed in limited combinations using individuals from the Selangor (Malaysia), Cianjur (Indonesia), Trat (Thailand), and Khulna (Bangladesh) populations. The developmental capacity and survival curves of the hybrid and control frogs from a portion of the possible combinations are shown in Table 16 and Fig. 15.

In the five control matings between individuals of the Selangor population, approximately 95% of the total number of eggs cleaved normally and in the end of tadpole phase, around 35% metamorphosed normally (Table 16). In the five hybrid matings conducted between Selangor females and Cianjur males, slightly lower values than those of the Selangor control matings were observed. In these mating combinations, approximately 84% of the total number of eggs cleaved

normally, then around 33% of tadpoles were metamorphosed normally (Table 16). In two of the four hybrid matings between Selangor females and Khulna males, approximately 60% of the total number of eggs cleaved normally and in the end of tadpole phase, around 19% of tadpoles were metamorphosed normally. While in two hybrid matings between Selangor females and Trat males, around 98% of the total number of eggs cleaved normally and in the end of tadpole phase, around 46% of tadpoles were metamorphosed normally (Table 16).

(b) Sexes of the hybrids: The matured frogs from the Selangor population control matings were 41.7% female and 58.3% male. However, the hybrids between the Selangor females and Cianjur males were 27.3% female and 72.7% male. The disparity between the sexes was even more striking for the hybrids between the Selangor females and Khulna males as only males were observed (Table 16).

(c) Histological examination of the testes: To further clarify the relationships among these populations, the inner structures of the testes from matured male hybrids between Selangor females and Khulna males and the controls were examined by histological and spermatogenesis observations. The inner structures of the testes of the control males were completely normal, with seminiferous tubules filled with compact bundles of normal spermatozoa (Fig. 16A). In contrast, the testes of the hybrids were slightly abnormal, with seminiferous tubules containing pycnotic nuclei in addition to normal bundles of spermatozoa (Fig. 16B).

In the controls, 248 meiotic spreads were analyzed from two males, and all of them contained 13 bivalents. In the hybrids, of the 237 meiotic spreads that were analyzed from four hybrid males, 196 (82.7%) contained 13 bivalents, 31 (13.1%) contained 12 bivalents and two univalents, 13 (5.5%) contained 11 bivalents and four univalents, and the remaining two (0.8%) contained 10 bivalents and six univalents (Table 17, Figs. 17, 18). The mean number of univalents per spermatocyte was 0.53 and the proportion of univalents among all chromosomes was 4.1% (Table 17). In a comparison of the proportion of ring- and rod-shaped bivalents in the controls and hybrids (Table 18), the ring-shaped bivalents outnumbered the rod-shaped bivalents overwhelmingly in the controls, whereas the ring-shaped bivalents decreased and the rod-shaped bivalents increased in the hybrids in both the large and small chromosomes (Table 18). In total, 3224 (100%) of the bivalents were ring-shaped in the controls, whereas 1937 (71.7%) and 765 (28.3%) of both the large and small bivalent chromosomes were ring- and rod-shaped, respectively, in the hybrids (Table 18). The mean number of bivalents per spermatocyte in the controls was 13.00, while that in the hybrids was 11.96 (Table 18).

## DISCUSSION

Genetic distances and divergences among three types of *F. cancrivora*.– The genetic distances and percent sequence divergences of the 16S rRNA and Cyt *b* genes among the three types of *F. cancrivora* were determined (Tables 6, 8, 10). Between the mangrove and large types, these values were 0.510, 9.10%, and 14.64%, respectively. The values between the mangrove and Pelabuhan ratu/Sulawesi types were 0.586, 10.22%, and 16.38%, respectively, and those between the large and Pelabuhan ratu/Sulawesi types were 0.197, 5.78%, and 12.88%, respectively.

Genetic distance and divergence have been examined at the species level of numerous types of frogs. Based on data from 116 crosses involving 46 frog species, Sasa et al. (1998) suggested a lower threshold of Nei's genetic distance (D = 0.30) for the evolution of hybrid inviability. In a report by Vences et al. (2004), differentiation among conspecific populations in African Malagasy frogs never exceeded 2.0% for the 16S rRNA gene. Sumida et al. (1998) found that sequence divergence of the Cyt *b* gene in Japanese pond frogs (comprising *Rana nigromaculata*, *R.porosa porosa* and *R. p. brevipoda*) ranged from 10.4% to 12.4% at the species level and from 3.68% to 4.62% at the subspecies level. In Palearctic pond frogs (comprising *Rana nigromaculata, Rana brevipoda, Rana plancyi, Rana lessonae, Rana esculenta* and *Rana ridibunda*), Cyt *b* gene sequence divergences ranged from 9.50% to 20.45% at the species level (Sumida et al., 2000). Bradley and Baker (2001) mentioned that sequence divergence of the Cyt *b* gene between 2.0% and 11.0% would merit additional study concerning species status, and values greater than 11.0% would indicate recognition of a species.

Within the *F. limnocharis* species complex, Toda et al. (1998) first recognized two syntopically occurring and genetically divergent species (Nei's genetic distance = 0.458) within this complex in Java. Later, corroborating these two species in sympatry in Java, Veith et al. (2001) described a new taxon known exclusively from Java, *F. iskandari*. Although *F. limnocharis* and *F. iskandari* are nearly identical morphologically, these two species show substantial genetic differentiation, with a Nei's genetic distance of 0.316 and a 16S rRNA sequence divergence of 13.5% (Veith et al., 2001). Djong et al. (2007a, b) found that these two species were isolated by complete hybrid



inviability at the tadpole stage. They detected a genetic distance of 0.628–0.749 and sequence divergences of 10.8%–11.0% and 18.6%–18.8% in 16S rRNA and Cyt *b,* respectively.

Islam et al. (2008a, b) observed three morphologically divergent species in sympatry in Mymensingh, Bangladesh, which exhibited substantial genetic divergence (Nei's genetic distance of 0.739–1.628; sequence divergences of 5.5%–17.1% and 18.0%–25.0% in the 16S rRNA and Cyt *b* genes, respectively). These species were reproductively isolated by either hybrid inviability or hybrid sterility. In a study on *Fejervarya*, Sumida et al. (2007) identified that members of the genus *Fejervarya* had diverged into: the south Asian and east-south-east Asian groups (Nei's genetic distance of 1.185–1.898, 16S and 12S rRNA gene sequence divergences of 19.3%–21.9%). There was complete reproductive isolation between the two groups, by complete hybrid inviability at the embryonic stage.

Based on the current knowledge on *Fejervarya* species, we can tentatively conclude that a Nei's genetic distance of greater than 0.3, and sequence divergences of greater than 5.5% for 16S rRNA and 9.50% for Cyt *b* sequence, are indicators for the recognition of species within the genus.

Morphological differentiation and taxonomic status of three types of *F. cancrivora*.– In this study, we could show that the Selangor and Cianjur, Trat and Khulna, and Makassar populations cluster into three groups congruent with our defined large-, mangrove-, and Sulawesi-type groups. These three groups exhibited significant morphological differentiation in SVL, HW, TL, TFOL, and IFL in males, and in HW, LAL, HLL, THIGHL, TL, and TFOL in females. Within the mangrove-type group, several of the female traits from the Trat and Khulna populations were also differentiated (Table 15, Fig. 13). Kurniawan et al. (2010) reported slight genetic differentiation between the Trat and Khulna populations, and between these two populations and a Philippine population of mangrove-type *F. cancrivora*. As mangrove-type frogs typically inhabit seashore areas, the natural overseas dispersal proposed by Toda et al. (1997) may explain the differentiation observed among populations of mangrove-type *F. cancrivora,* although the time of divergence for this group remain to be estimated. Further studies are required to precisely elucidate the evolutionary time frame and degree of differentiation among populations of the mangrove-type group.

The specimens examined show that the large-type group of *F. cancrivora* has a longer SVL than the mangrove- and Sulawesi-type groups, and that the latter two groups show similar SVL. Other studies have demonstrated that *F. cancrivora* frogs from Indonesia are comparatively larger than those from Thailand and the Philippines (Table 19). Therefore, based on the present measurement data and the distribution areas reported previously (Kurniawan et al., 2010), the Indonesian frogs and those from Thailand and the Philippines are assigned to the large- and mangrove-type groups, respectively.

In our present morphological characterizations, toe webbing and free flap are clearly differentiated among the three *F. cancrivora* types with respect to size and shape. The toe webbing and free flap of the hindlimb are extensive in the mangrove type and only moderate in the large and Sulawesi types. As mangrove-type frogs inhabit mangrove areas near the seashore, the extensive toe webbing and well-developed free flap may be regarded as traits adapted to these coastal (seashore) habitats.

Large type as a nominal species of *F. cancrivora*.– The type specimen of *F. cancrivora* (Gravenhorst, 1829) originally stored in the Breslau Museum is considered lost. Another specimen collected from Cianjur by Michael Veith in 1993 was designated as the neotype (adult male, 68.2 mm SVL) (Dubois and Ohler, 2000). If we compare the neotype with the large type, we find that the body size of the former (68.2 mm) is close to the average male body size of the latter (69.7 and 64.9 mm for the Cianjur and Selangor populations, respectively) (Table 1). Iskandar (1998) reported that *F. cancrivora* from the Java locality reached a SVL of up to 120 mm, but typically displayed a SVL of ca. 100 mm. From our data on the large-type frogs from the Cianjur population, the female body size ranged from 87.6–112.0 mm (Table 1). In addition, the skin texture of the large-type isolates was similar to that described for the neotype, which show glandular folds and glandular warts on the back and upper parts (Dubois and Ohler, 2000). This shared characteristic may be explained by the fact that one of our large-type populations was collected from a rice field in the same region of Cianjur, West Java, where the neotype had been collected (Dubois and Ohler, 2000). Based on comparisons of the morphological characteristics, habitat, and locality, we conclude that the *F. cancrivora* neotype belong to the same evolutionary and taxonomical unit as the large-type *F. cancrivora* in the present work.

Possible synonymy of *F. raja* with *F. cancrivora*.– Iskandar (1998) provided information on the distribution of *F. raja* in Thailand, describing it based on an extra-large specimen of *F. cancrivora*. According to Nutphund (2001), the natural habitat of *F. raja* were rice fields distributed in the localities of Patthalung, Satun, and Songkhla Provinces in southern Thailand, and on the Malay

Peninsula. Taylor (1962), however, reported that the distribution of *F. raja* was in Pattani, Songkla, and Phatthalung in Thailand, and in Kuala Lumpur in Malaysia. The large-type *F. cancrivora* of the Cianjur and Selangor populations also live in rice fields. Selangor is situated near Kuala Lumpur in the Malay Peninsula, in what is considered to be within the range of *F. raja*.

Nutphund (2001) reported that *F. raja* had a total body length of 120 mm, and Taylor (1962) also described *F. raja* as a large species. Among specimens in The Natural History Museum, London, the SVL of the largest *F. raja* paratypes reach 62.0 mm in males and 121.0 mm in females. The SVLs of the other two paratypes of *F. raja* were 84.0 mm and 88.0 mm in males, and 98.0 mm to 119.5 mm in females (Taylor, 1962). In our study, among the large-type frogs, the largest females reached 112.0 mm (from Cianjur, Indonesia) (Table 1), which were within the size range of *F. raja* female paratypes. The SVL of the large-type frogs from Cianjur and Selangor populations were 59.3–76.2 mm (mean, 68.6 ± 5.4 mm) in males and 58.0–112.0 mm (mean, 86.9 ± 16.2 mm) in females, which were larger than those of the mangrove- and Pelabuhan ratu/Sulawesi-type groups. Given that the large-type frog was morphologically identical to the neotype of *F. cancrivora*, inhabited rice fields, and was comparable to *F. raja* in body size, it appears likely that the large-type frog is the true *F. cancrivora*, and that *F. raja* may be a synonym of *F. cancrivora*.

Mangrove type.– Taylor (1962) described *F. cancrivora* based on the examination of an individual female (EHT No. 34294) with an SVL of 68 mm, collected from the seashore at Anghin, Chon Buri, Thailand. This female, which displayed a strong tolerance for salt water, had numerous glandular warts on the skin, with several forming elongated ridges and others forming small tubercles (Taylor, 1962). Nutphund (2001) reported that *F. cancrivora* from mangrove swamps and brackish areas had a smooth covering of skin over the chin, venter, and underside of the thighs. In view of its smaller body size and confinement to mangrove habitats, we conclude that Taylor's *F. cancrivora* does not belong to *F. cancrivora* sensu Dubois and Ohler (2000). We conclude that the mangrove-type defined herein is close to Taylor's *F. cancrivora* (1962) and distinct from the *F. cancrivora*. Given the significant differences between the morphology and ecology of the mangrove-type and neotype, we speculate that the mangrove-type and *F. cancrivora* neotype in fact represent two evolutionary and taxonomic units.

Taylor (1920) described the Philippine population of *F. cancrivora* as a distinct species, *Rana moodiei* (currently *Fejervarya moodiei*), with the type specimen (73 mm SVL) from Manila, Luzon. Taylor (1923) maintained that this species has a distinct flap of skin on the outer face of Toe V and metatarsus, He mentioned that the species was common to the Philippine islands of Mindanao, Luzon, and Negros, and likely occurred on numerous other islands. *Fejervarya vittigera* found in sympatry in these regions does not possess a free skin fringe on Toe V. However, Smith (1927) concluded that *F. moodiei* could not be distinguished from "typical" *F. cancrivora* using this characteristic, Inger (1954) reported that the differences between the frog populations of Luzon and those from the Greater Sundas were not pronounced. Inger (1954), considered that the distinctions too insufficient to warrant the designation of any Philippine population of *F. cancrivora* as a separate subspecies. However, our data demonstrate that the Philippine population is distinct from the Cianjur and Malaysia populations both genetically and morphologically (see also Kurniawan et al., 2010; 2011) As the large-type group of the Cianjur and Malaysia populations is considered to be true *F. cancrivora*, the mangrove-type group of the Philippine and China populations must be assigned to the available name and taxonomic unit *F. moodiei*.

Pelabuhan ratu/Sulawesi type.– Based on genetic distance and sequence divergences (Tables 6, 8, and 10), the Pelabuhan ratu/Sulawesi type was more closely related to the large-type than to the mangrove type. The relative closeness of the Pelabuhan ratu/Sulawesi and large-type groups was also evidenced by the moderate bootstrap values (Figs. 5, 6, and 7) and the similar morphological characteristics of skin texture. Additional morphological examinations indicated that the Pelabuhan ratu/Sulawesi type had mean SVL of 43.5 and 50.7 mm in male and females, respectively, but these values were based on a small sample. These limited data suggest that this type is smaller than the large- and mangrove-type groups. According to H. Ota (pers. comm.), the Pelabuhan ratu/Sulawesi-type frogs inhabit rice fields near the seashore of Pelabuhan ratu, suggesting that this type may also fail to qualify as a true *F. cancrivora*, and may be a distinct species related to the large type.

With respect to geographic distribution and haplotype frequency (Figs. 8 and 9), the PS-1a haplotype of the Pelabuhan ratu/Sulawesi-type group was identified in two localities separated by sea: in Pelabuhan ratu, Java and in Makassar, Sulawesi. Iskandar and Colijn (2000) reported that *F. cancrivora* was introduced into Sulawesi, Ambon, and Papua, whereas Inger (2005) presumed that *F. cancrivora* was introduced into Sulawesi from Borneo. In contrast, the present study shows that the haplotypes of southern Sulawesi differed genetically from those of Sunda Land, including



those of Borneo (Fig. 9), indicating that *F. cancrivora* from southern Sulawesi was not introduced from Borneo. As four localities from southern Sulawesi prove the existence of the Pelabuhan ratu/Sulawesi type (Figs. 8 and 9), we rather assume that this type originated from Sulawesi. Given that the Pelabuhan ratu and Makassar haplotypes are genetically identical, we consider that the frogs of Makassar could have been artificially introduced to Pelabuhan ratu. Our contention that this type was introduced to Pelabuhan ratu, Java from Sulawesi is supported by the fact that both Makassar and Pelabuhan ratu have long been port cities associated with the fishery industry. To this day, fishermen from both cities often visit each other to engage in fish trade. Although these frogs were likely to have been found accidentally at Pelabuhan ratu in our 1996 sampling, but we could not locate them in the 2008 sampling at the same locality.

Reproductive isolation.– Evidence that two populations are reproductively isolated can be obtained from direct observations, experiments related to mating properties, or from the examination of the viability and sterility of hybrids produced in the laboratory (Futuyma, 1986). In amphibians, it is not difficult to determine reproductive isolation among allopatric populations through hybridization experiments. For example, Kawamura et al. (1981, 1985) and Sumida et al. (2003) conducted a series of artificial crossing experiments to investigate the reproductive isolation mechanisms among brown frog species distributed in the Palearctic region and North America. These studies revealed that these species are reproductively isolated from one another by either gametic isolation, hybrid inviability, or hybrid sterility, and that all viable interspecific hybrids were completely sterile males.

In the present crossing experiments, we were only able to examine a portion of all possible combinations of reciprocal crosses due to problems encountered with maintaining the eggs and frogs of certain populations in the laboratory. For example, we were unable to use the eggs from the mangrove-type females of the Trad and Khulna populations because the eggs swelled and ruptured after being placed into distilled water. As Uchiyama et al. (1990) reported that breeding experiments using parental *F. cancrivora* from the Thailand mangrove region require saline medium consisting of 10% seawater, a similar medium should be used for future crossing experiments using eggs from mangrove-type frogs. It was also not possible to obtain data related to the Sulawesi-type group, as many frogs died before the crossing experiments could be conducted.

From the limited data obtained from the crossing experiments, we found no hybrid inviability among the large-type females and the mangrove-type males of *F. cancrivora*. However, incomplete gametic isolation and abnormal spermatogenesis was observed in the hybrids between the Selangor and Khulna populations, which involved the crossing of four Selangor females and two Khulna males. Incomplete gametic isolation occurred in half of the hybrid matings between two Selangor females and the two Khulna males, whereas the hybrids from the other two matings developed normally. However, the viable $F_1$ hybrids produced from the latter combinations showed abnormal spermatogenesis, including an increase in univalents and rod-shaped bivalents in the meiotic metaphases of testes, whereas the controls were characterized by the occurrence of ring-shaped bivalents without univalents in meiotic metaphase. The occurrence of univalents and rod-shaped bivalents in the meiotic metaphases of testes could therefore serve as an indication of the degree of abnormality of $F_1$ hybrids (Kawamura et al., 1980, 1981; Kuramoto, 1983; Sumida, 1994, 2003). Numerous studies have examined the behaviour of meiotic chromosomes in the spermatogenesis of $F_1$ hybrids between species, subspecies, and races of amphibians in which chromosome structures were expected to differ (Callan and Spurway, 1951; Spurway and Callan, 1960; Günther, 1975; Okumoto, 1980; Callan et al., 1991; Sumida, 1994; Djong et al., 2007a, b).

The meiotic chromosomes of the hybrids in these studies displayed drastic reductions in chiasma frequency, restriction of the chiasmata to the chromosome ends, and increases in univalent chromosomes, suggesting that the degree of meiotic aberration is related to the difference in the taxonomic level of the crossed individuals. In the present study, we found that among the hybrids between the Selangor females and Khulna males, the mean number of univalents per spermatocyte was 0.53, the proportion of univalents to all chromosomes was 4.1%, and the rod-shaped bivalents increased by 28.3%. This trend of increasing univalents and rod-shaped bivalents is similar to that observed in the hybrids generated between several other frog populations, including the Malaysian and Indonesian populations of *F. limnocharis* (Djong et al., 2007b), the Yaeyama and Hiroshima populations of *F. limnocharis* (Sumida et al., 2006), the Amami and Okinawa populations of *Odorrana ishikawae* (Sumida et al., in preparation), and the Amami and Okinawa populations of *Rana "okinavana"* (Matsui, 2007; Iwanari et al., 2009). All of these hybrids are regarded to be interspecific, which indicates that both parental populations in each case represent distinct species.

Sasa et al. (1998) proposed a lower threshold of genetic distance (D) of 0.30 for speciation, which was based on data from 116 crosses involving 46 frog species. Vences et al. (2004) found that the differentiation among conspecific populations of African Malagasy frog species never exceeded 2.0% for the mitochondrial 16S rRNA gene. Based on allozyme and mtDNA analyses (Kurniawan et al., 2010), the D value and sequence divergence of the 16S rRNA gene between the Khulna and Selangor populations were 0.525 and 3.0%, respectively. These values, together with the spermatogenetic aberration observed in the hybrids described above, suggest that the Khulna and Selangor populations of mangrove- and large-type frogs can be regarded as distinct species. As the neotype of *F. cancrivora* from Cianjur, West Java, Java (Indonesia) (Dubois and Ohler, 2000) belongs to the large type (Veith et al., 2001; Kurniawan et al., 2010), we propose that the mangrove type should be reclassified as a different species, namely, *F. moodiei.*

From the data obtained in our present study, we can conclude that the genetic distances, percent sequence divergences, and morphological observations among the three types of *F. cancrivora* typically exceed the above criteria for the recognition of species differentiation. The reproductive isolation results provide further support that the large- and mangrove-type groups are separate species. We proposed that the large- and mangrove-type groups represent real *F. cancrivora* and *F. moodiei*, respectively, whereas the Sulawesi-type group likely represents an undescribed species.

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## APPENDIX 1:

Specimens used in study. For institution abbreviations, refer to text.

Indonesia: Cianjur (IABHU 18723–18729, IABHU 18737–18738, IABHU 18740–18741, IABHU 18748, IABHU 18753–18754, IABHU 18757, IABHU 18794–18796, IABHU 18802, IABHU 18852, IABHU 18884, IABHU 19094–19095); Makassar (IABHU 19048–19057, IABHU 19071–19080).

Malaysia: Selangor (IABHU 18843, IABHU 18846, IABHU 18849–18850, IABHU 18866, IABHU 18875, IABHU 18877–18879, IABHU 18886, IABHU 19011–19016, IABHU 19028, IABHU 19091–19093, IABHU 19113).

Thailand: Trat (IABHU 18818–18828, IABHU 18845, IABHU 18847–18848, IABHU 18851, IABHU 18870–18871, IABHU 18874, IABHU 18881, IABHU 19090).

Bangladesh: Khulna (IABHU 3432, IABHU 3516–3517, IABHU 3526–3531, IABHU 3539–3540, IABHU 3545, IABHU 3560).



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# ANCIENT POLYMORPHISM WITHIN *HYLARANA SIGNATA* (AMPHIBIA: ANURA: RANIDAE) LINEAGES OF WEST (PENINSULAR) AND EAST (SARAWAK, BORNEO) MALAYSIA

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(with four text-figures)

**ABSTRACT.– The *Hylarana signata* group in Malaysia, as currently construed, comprises two species- *Hylarana signata* and *H. picturata.* Both are similar in morphology and habits, but differ in colouration (chiefly, the presence or absence of a dorsolateral line). It has been suggested recently that Malaysian *H. signata* (from both Peninsular and Borneo) is different from *H. signata* of Philippines and may be non-conspecific, also from what is currently referred to this taxon on Malaysian Borneo. Specimens of the *Hylarana signata* group were sequenced to detect genetic variation among species and to confirm the existence of cryptic species, via 16S rRNA gene. Seven study sites in Sarawak were chosen for data collection, namely four National Parks (Matang/Matang, Bako, Mulu and Similajau), and three unprotected areas (Borneo Heights, Sadong Jaya and Bario). Data from Tasik Chini, Pahang, West (Peninsular) Malaysia were included in our molecular analysis, to infer relationships within the species group. PCR amplification and direct sequencing of partial 16S rRNA mitochondrial DNA was used to infer the phylogeny presented. The study revealed phylogenetic complexity within Malaysia *Hylarana signata* group due to the occurrence of cryptic species or ancient polymorphism of the lineages. The results obtained underscore the need for a complete sequence of DNA regions or multigenes of the same rate of evolution in order to elucidate the phylogenetic relationship in the group through more extensive samplings spanning wider geographical ranges.**

**KEYWORDS.– Phylogeny, *Hylarana signata* group, 16S mtDNA, cryptic species.**

## INTRODUCTION

*Hylarana signata* is a small frog, with males less than 50 mm and females less than 70 mm. The species was described as *Polypedates signatus* Günther, 1872 (type locality Matang Sarawak), and reallocated to *Rana* by Boulenger (1882), which name was used by Boulenger (1920), van Kempen (1923) and Inger (1954). Dubois (1992) referred the species as *Rana* (*Pulchrana*) *signata,* basing his reclassification of ranoid frogs on morphological characters, and was later referred to *Pulchrana signata,* based on molecular and morphological data by Frost et al. (2006). More recently, the species has been classified as *Hylarana signata* (Che et al., 2007; Frost 2008). *Hylarana picturata* was treated as a synonym of *H. signata* by Inger (1954). However, *Hylarana picturata* was removed from the synonymy of *H. signata* without discussion by Inger and Tan (1996) and stated to have a distribution of Brunei, Kalimantan, Sabah, and Sarawak in Borneo. The species is now assigned as *Pulchrana picturata* (Frost et al. 2006), and later, as *H. picturata* by Frost (2007) and Che et al. (2007) by implications. Distribution of *H. signata* includes Peninsular Thailand and Malaya, Sumatra (Indonesia); Sabah and Sarawak, Malaysian Borneo (Inger and Stuebing, 1997). On the other hand, *H. picturata* was distributed throughout Borneo (Brunei, Kalimantan, Sabah, and Sarawak), and Peninsular Malaysia. Brown and Guttman (2002) noted