Formosa Journal of Applied Sciences (FJAS)
Vol. 2, No. 10 2023: 2369-2382

Morphology and Karyotype of Two Frog Species from Irian Jaya

Rita Esterina
Fakultas Pasca Sarjana, Program Study MIPA, Universitas Indraprasta PGRI, Jakarta
Corresponding Author: Rita Esterina Rita.Esterina@gmail.com

A R T I C L E I N F O

Keywords: Morphology, Karyotype, Litoria Caerulea, Litoria Infrafrenata, Irian Jaya

Received : 24, August
Revised : 26, September
Accepted: 28, October

A B S T R A C T

This research aims to identify and compare the morphology and karyotype of *Litoria caerulea* and *Litoria infrafrenata*, which are useful as study materials to understand their patterns of relatedness and taxonomy. The results of this study indicate that the qualitative morphological characteristics of both frog species are different. *Litoria caerulea* has a chubby and short body with a distinctive feature of skin folds above the eyes that extend to the tympanum area, while *Litoria infrafrenata* has a slim and long body with a distinctive feature of a white line on the lower lip and along the front and hind limbs. And it was observed that the morphometry between the two frog species are significantly different ($P \leq 0.05$).

INTRODUCTION

Frogs are vertebrates classified under the class Amphibia and are terrestrial animals that spend their early lives in aquatic environments before undergoing a transformation into adult frogs through metamorphosis. Amphibia class consists of three orders: Urodela, Apoda, and Anura. In Indonesia, most amphibians belong to the order Anura. The island of Irian is located in the eastern part of the Indonesian archipelago and is bounded to the north by the 0°19' S latitude and to the south by the 10°34' S latitude. It is bounded to the west by the 130°45' E longitude and to the east by the 150°48' E longitude. The map island of Irian can be seen as resembling a giant bird on a map. Some might even perceive it as resembling a dinosaur, a reptile from the Mesozoic era that is now extinct. Approximately 47% of the island's territory, located in the western part, represents the head, neck, back, chest, and abdomen of the dinosaur mentioned above (Koentjaraningrat 1994), which is Irian Jaya. The remaining 53% of the territory belongs to the country of Papua New Guinea.

The wildlife in Irian Jaya shows distinctive characteristics. In Irian, there are over 200 different frog species categorized into six families: Bufonidae, Rhacophoridae, Leptodactylidae, Hylidae, Microhylidae, and Ranidae (Menzies 1975). The majority of Irian Jaya's territory is covered by rainforests, and its high humidity provides an ideal habitat for frogs. This explains why Irian Jaya is rich in frog species compared to other places in the world. Of the frog species known in Irian, 40% of them belong to the Hylidae family. Hylidae in Irian is grouped into two genera: *Litoria*, consisting of 56 species, and *Nyctimystes*, consisting of 23 species (Zweifel 1983). The genus *Litoria* is a large genus consisting of species with a diversity of body forms and behaviors. Members of the *Litoria* genus can be easily distinguished from *Nyctimystes* because *Litoria* has horizontal pupils (Cogger 1992).

Litoria caerulea and Litoria infrafrenata are species belonging to the genus *Litoria*. Both of them have relatively large body sizes and bright colors. *Litoria caerulea* is a native species of Australia that migrated towards the southern part of Irian, which occurred when Irian was connected to Australia. Its distribution in Australia is extensive, ranging from Sydney to Queensland. In Irian, it can be found in Merauke and Port Moresby. *Litoria infrarenata* is a native species of Irian that migrated to the northern part of Australia. In Australia, it can be found in the northern regions of Queensland, while in Indonesia, it ranges from the eastern part of Indonesia along the New Guinea archipelago to the Bismarck and Louisiade Islands. Although both species may appear similar, they can still be distinguished by their morphology. Both are large-sized and are the largest tree frogs in the world, but *Litoria infrarenata* has a longer body size (Menzies 1975).

Karyotype is a complete representation of both the number and morphology of chromosomes at the metaphase stage of a cell, arranged systematically and representing the pairs of normal diploid cells (Elridge 1985, as cited in Titrawani 1996). The techniques for preparing chromosome slides (karyotypes) can be performed in two ways: 1) processing chromosomes from cells of an animal's body organs and 2) through tissue culture.

The procedure for making chromosome preparations (karyotypes), according to Cook (1978, as cited in Titrawani 1996), involves several steps, including: 1) Preliminary treatment using an anti-mitotic substance (colchicine), 2) Treatment in a hypotonic solution, 3) Fixation, 4) Staining, and 5) Observation under a microscope. At the metaphase stage of the cell division process, the chromosomes of a particular organism can be easily observed. During this phase, all chromosomes are aligned along the equatorial plane, and to determine the karyotype, it is necessary to examine the length of the chromosome arms. This research aims to understand and compare the morphology and karyotype of *Litoria caerulea* and *Litoria infrafrenata.*

LITERATUR REVIEW

Fourteen measurements were made on each frog, following the methodology in GASCON et al. (1996). The fourteen variables are snout-vent length (SVL), nostril separation, eye width anterior, eye width posterior, head width, head length, eye to nostril distance, tympanum diameter (tympanum height of GASCON et al., 1996), eye length, thigh length (femur length of GASCON et al., 1996), shank length (tibia length of GASCON et al., 1996), foot length, maximum width of disk on third finger, and maximum width of disk on fourth toe. The working principle for identifying chromosomes is called karyotyping, namely observation chromosomes by utilizing staining techniques and using a microscope to observe the ride. Tissues that can be used for karyotyping include: embryos, fish larvae, fin or scale epithelium, leukocytes and ovaries and testes.

Karyotyping begins with preparing cells for the metaphase stage using a culture technique to stimulate cells to reach the metaphase stage, for example the use of colchicines. Evenly distributed Chromosomes in metaphase are the best time to count numbers chromosomes and comparing the size and morphology of the chromosomes and determining their number Chromosomes are taken from the highest frequency or mode. Similar things have been commonly done to 2 Melanoteania boasemani, M. patoti, and Oreochromis sp. (Carman et al., 1998) and Telmatherina ladigesi (Andriani, 2001). From other studies on the number of chromosomes based on mode, the number of diploid chromosomes was found to be 48 in the Atherian elymus fish studied by Arai and Fujiki in 1978, and in the fish Basichlichthys bonariensis studied by Arai and Koike in 1980. Different species have characteristic numbers of chromosomes. The range is huge extensive, from two in some flowering plants to several hundred in certain ferns.

The general procedure of chromosome analysis in outline can be described as follows: Step 1 Tissue or cells that divide quickly, Step 2 Treatment with colchicine (stops division at metaphase), Step 3 Treatment with hypotonic fluids (to enlarge cells), Step 4 Fixed to stop the reaction, Step 5 Application to slide and stain and Step 6 Analysis. For this observation, the Satmoko method (1986, cited in Yusmin 1998) was used with all steps.

METHODOLOGY

The samples used in this research consisted of 30 individuals, each of *Litoria caerulea* and *Litoria infrafrenata*. The chemicals employed included 0.005% colchicine (in distilled water), chloroform, ethanol, isotonic and hypotonic Ringer's solutions, distilled water, Carnoy's solution, and Giemsa stain. The equipment utilized in this study comprised an aquarium, basin, vernier caliper, scale, small surgical scissors, forceps, syringe needle, tissue grinder, laboratory glassware, centrifuge, spirit lamp, microscope, and writing tools.

Sampling

Adult *Litoria* frogs were randomly selected, with a total of 30 individuals per species. The captured frogs were separated by their species, and each species was collected and placed in an aquarium.

Morphology

For the morphological observations, characteristics were assessed following the criteria outlined by Van Kampen (1923) and Menzies (1975). Observations primarily focused on the major body parts, which include:

a. Head, including head shape, presence and type of teeth, the number of teeth, pupil positioning relative to the eye, eye color, and the clarity of the ear membrane;
b. Body, covering body shape, coloration of the upper and lower body parts, skin texture, and the presence of parotoid glands where applicable;
c. Limbs, involve the presence of webbing between the toes, the size of webbing, and the shape of toe tips.

The measurements of various frog body parts, as outlined by Menzies (1987), included:

1. Body length (SV), measured from the tip of the snout (nose) to the anus.
2. Tibia length (TL), measured from the heel to the outer side of the knee.
3. Tarsal length (TR).
4. Fourth toe length (base/ankle) (T4).
5. Third toe length (T3).
6. Fourth toe diameter (T4D).
7. Third finger length (F3).
8. Third finger diameter (F3D).

Head length (HL), measured from the tip of the snout (nose) to the posterior edge of the jaw angle.

1. Head width (HW) at the jaw junction.
2. Distance from the anterior edge of the eye to the nose (EN).
3. Nostril distance (IN).
4. Eye aperture diameter (EY), measured between the anterior and posterior edges.
5. Horizontal diameter of the tympanum (ear drum) (TY), including the circumference.
6. Interorbital distance (IO)

Measurements of the frog body parts are expressed in millimeters using vernier calipers with a sensitivity of 0.1 mm, while body weight is measured using a scale.

Karyotype

Before karyotype observations were conducted, the following solutions were prepared:

a. Colchicine solution, the quantity of which depends on the specimen's body weight. For the injection method, 5 mg of colchicine was dissolved in 100 ml of distilled water.
b. Hypotonic Ringer's solution, made from Ringer's solution 100% with a ratio of 1:4.
c. Carnoy's fixative solution, is made by mixing glacial acetic acid and methanol in a ratio of 1:3.
d. The 10% Giemsa stain solution was prepared by dissolving 1 part Giemsa in 9 parts of distilled water.

For this observation, the Satmoko method (1986, cited in Yusmin 1998) was used with the following steps: The frogs were weighed, and then they were injected with a 0.005% colchicine solution at a dose of 1/100 of their body weight (intra-abdominal). Subsequently, the frogs were placed in a basin for 2-3 hours. Afterward, the frogs were euthanized, and their left and right thigh bones were extracted. The thigh bones were placed in a watch glass containing isotonic Ringer's solution, and the tips of the thigh bones were cut, and the bone marrow was extracted by spraying it with hypotonic solution. The extracted bone marrow was collected in a watch glass containing hypotonic Ringer's solution, and then the bone marrow was ground using a tissue grinder. The previously ground bone marrow was transferred into a reaction tube, and a hypotonic solution was added until it reached a volume of 3 cc. It was left for 30-45 minutes, then centrifuged at 500 rpm for 4 minutes. The supernatant was discarded, and Carnoy's fixative solution (3 cc) was added. It was allowed to stand for 5 minutes, then centrifuged at 500 rpm for 4 minutes. The supernatant was discarded again, and a fresh fixative solution was added. It was centrifuged again at 500 rpm for 4 minutes. Afterward, 0.5 cc of fresh Carnoy's solution was added, mixed thoroughly, and then a smear slide was prepared. The slide was dried over a spirit lamp and left overnight. The next day, it was stained using a 10% Giemsa stain solution for 30 minutes, rinsed with distilled water, and allowed to dry. Subsequently, it was examined under a microscope. Good chromosome samples were photographed, the number of chromosomes was counted, and karyotypes were created.

The karyotype was created using the following steps: Good chromosome photos were cropped and enlarged. Then, each chromosome image was cut out, and random numbers were assigned to each chromosome. Measurements were taken for the chromosome arms using a millimeter block. The arm ratio was determined by dividing the length of the long arm by the length of the short arm. Total chromosome length was also measured by adding the lengths of the long and short arms. Chromosome pairs were determined using a scatter plot method, where the total length was plotted on the Y-axis, and the arm ratio was plotted on the X-axis. Chromosome pairs were identified based on two adjacent points, and if there were more than two adjacent points, chromosome pairs were determined based on the closest proximity. The karyotype was created by arranging chromosome pairs in order of decreasing arm ratio from largest to smallest.

Data Analysis

The collected morphometric data were used to calculate the mean values and standard deviations. Subsequently, statistical testing was performed using the T-test (Steel & Torrie 1991). Karyotype analysis involves the measurement of relative length (RL), centromere position (NVC), and chromosome type.

$$\text{RL} = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100\%$$

$$\text{NVC} = \frac{\text{Length of short arm}}{\text{Chromosome length}} \times 100\%$$

NVC (Normalized Centromere Position) categorization is as follows: 50.0 – 37.5%: Metacentric (m); 37.4 – 25.0%: Submetacentric (sm); 24.9 – 12.5%: Subtelocentric (st); 12.4 – 0%: Telocentric (t) (Nishioka et al. 1987, cited in Yusmin 1998).

DISCUSSION

A. Morphology

a. Litoria Caerulea

Qualitative morphological observations on 30 specimens of *Litoria caerulea* revealed the following characteristics: They have a large head with a blunt snout; have two vomerine teeth; have horizontally oriented pupils; have eye colors ranging from light brown to black; their ear membranes are clearly visible; they have a robust body with light green dorsal coloring and white ventral coloring, sometimes with white spots; their skin is thick and not rough; they feature distinctive skin folds above the eyes that extend to the tympanum, which is a distinguishing characteristic from other species; they lack parotoid glands; their toe and fingertips are disc-shaped, and they have webbed feet with incomplete webbing between the fingers and fully webbed toes.

The results of measurements on various body parts of adult *Litoria caerulea* frogs are presented in Table 1.

Table 1. Morphometrics of Adult *Litoria caerulea*, n = 30

No	Parameter	Mean ± SD	Range
1	BB (g)	49.32 ± 16.86	36.3 – 100.2
2	SV (mm)	75.29 ± 9.33	65.8 – 95.1
3	TL (mm)	35.39 ± 4.95	27.1 – 45.6
4	TR (mm)	22.60 ± 2.97	19.2 – 29.9
5	T4 (mm)	30.34 ± 4.28	23.8 – 38
6	T3 (mm)	24.40 ± 3.87	18.6 – 32.4
7	T4D (mm)	4.12 ± 0.92	3 – 6.1
8	F3 (mm)	21.01 ± 4.03	15 – 28.4
9	F3D (mm)	4.68 ± 1.12	3.1 – 7.2
10	HL (mm)	23.99 ± 2.93	19.8 – 29.7
11	HW (mm)	24.93 ± 5.30	18.3 – 38.3
12	EN (mm)	6.59 ± 0,71	5.3 – 8.1
13	IN (mm)	6,37 ± 0,96	4.8 – 8.3
14	EY (mm)	7,08 ± 0.62	6 – 8.3
15	TY (mm)	4.96 ± 0.69	3.9 – 6.4
16	IO (mm)	9.21 ± 2.02	5.7 – 13.1

Explanation:

1. BB = Body Weight
2. SV (Snout Vent length) = Body Length
3. TL = Tibial Length
4. TR = Tarsal Length
5. T4 = Fourth Toe Length
6. T3 = Third Toe Length
7. T4D = Fourth Toe Disc Diameter
8. F3 = Third Finger Length
9. F3D = Third Finger Disc Diameter
10. HL = Head Length
11. HW = Head Width
12. EN = Eye to Nostril Distance
13. IN = Internarial Distance
14. EY = Eye Diameter
15. IO = Interorbital Distance

b. Litoria Infrafrenata

Qualitative morphological observations on 30 specimens of *Litoria infrafrenata* revealed the following characteristics: They have a large head with a long and rounded snout at the tip; from the lower lip to the posterior tympanum, there is a distinctive white line that sets them apart from other species; they have two vomerine teeth; horizontally oriented pupils; eye colors ranging from dark yellow to brown; their ear membranes are clearly visible; they have a slender and elongated body with light green dorsal coloring and greenish-yellow on the sides, while the ventral side is white; their skin is thin and not rough; they lack parotoid glands; along the front and rear limbs, there are white lines; their toe and fingertips are disc-shaped, and they have incomplete webbing between the fingers and fully webbed toes.

The measurement results for various body parts of *Litoria infrafrenata* frogs can be seen in Table 2.

Table 2. Morphometry of adult *Litoria infrafrenata*, n = 30

No	Parameter	Mean ± SD	Range
1	BB (g)	34.77 ± 9.26	27.6 – 60.7
2	SV (mm)	84.60 ± 6.65	76.5 – 100.2
3	TL (mm)	44.82 ± 4.97	37 – 58
4	TR (mm)	27.06 ± 3.47	23.1 – 37.1
5	T4 (mm)	33.08 ± 3.96	26.6 – 43.4
6	T3 (mm)	26.05 ± 3.03	21.3 – 33.1
7	T4D (mm)	3.16 ± 0.47	2.5 – 4
8	F3 (mm)	23.99 ± 3.03	18.8 – 31
9	F3D (mm)	3.95 ± 0.75	2.8 – 5.4
10	HL (mm)	26.96 ± 2.46	22.9 – 32.2
11	HW (mm)	24.72 ± 2.74	20,7 – 32
12	EN (mm)	8.21 ± 0.83	6.8 – 10.2
13	IN (mm)	6.63 ± 068	5.6 – 8.2
14	EY (mm)	7.63 ± 0.91	6.5 – 10
15	TY (mm)	5.32 ± 0.38	4.7 – 6.1
16	IO (mm)	8.85 ± 0.10	7.1 – 10.5

Explanation: See the description in Table 1.

B. Karyotype

Tyler (1970, 1972, cited in King et al. 1979) suggested that Hylidae frogs have a close relationship with the genus *Cyclorana* (family Leptodactylidae) based on the similarity in chromosome morphology between the two.

Studies on Australian frogs' chromosomes have shown significant differences in the number of chromosomes between the Leptodactylidae and Hylidae families. Frogs belonging to the family Leptodactylidae have either 11 pairs of chromosomes (2n = 22) or 12 pairs of chromosomes (2n = 24), with none having 13 pairs of chromosomes (2n = 26). On the other hand, frogs belonging to the family Hylidae mostly have 13 pairs of chromosomes (2n = 26), with only one species having 12 pairs of chromosomes (2n = 24) (Morescalchi & Ingram 1974, 1978, as cited in King et al. 1979; Menzies & Tippett 1976).

In South America, the number of chromosomes in Hylidae frogs ranges from 9 pairs (2n = 18) to 15 pairs (2n = 30), and the number of chromosomes in Leptodactylidae frogs ranges from 9 pairs (2n = 18) to 18 pairs (2n = 36) (Bogart 1973, as cited in King et al. 1979). In this context, chromosome fusion and fission appear to have played a significant role in evolutionary history and are believed to be responsible for the variations that occurred (Bogart 1973; Cole 1974, as cited in King et al. 1979).

Out of the 52 known species of Australian Hylidae frogs, 36 of them have been cytologically studied (King, unpublished data). Most *Litoria* species have 13 pairs of chromosomes (2n = 26), with the exception of *Litoria infrafrenata*, which has 12 pairs of chromosomes (2n = 24) (Menzies & Tippett 1976). Out of the 28 known species of Papua (Irian) Hylidae frogs that have been studied, they have shown uniformity in chromosome numbers. This strengthens the argument that the original chromosome number for Australo-Papuan frog species is 2n = 26. One exception is *Litoria infrafrenata*, which has a reduced chromosome number of 2n = 24, derived from the more primitive 26 (Menzies & Tippett 1976).

The karyotype of the two frog species shows differences in the number and arrangement of chromosome types. *Litoria caerulea* has 13 pairs of chromosomes (2n = 26), with the longest chromosome having a relative length (RL) of 13.71%, while the shortest chromosome has a relative length (RL) of 3.91%. The average length of the chromosomes ranges from 1.4725 cm to 0.42 cm, while the average length of the short arms of the chromosomes ranges from 0.56 cm to 0.165 cm. NVC values range from 45.54% to 31.34%, indicating that *Litoria caerulea* frogs have only two types of chromosomes, namely metacentric and submetacentric.

Litoria infrafrenata has 12 pairs of chromosomes (2n = 24), where the longest chromosome has a relative length (RL) of 12.97%, while the shortest chromosome has a relative length of 4.35%. The average length of chromosomes ranges from 1.479 cm to 0.49583 cm, while the average length of short arms of chromosomes varies from 0.554 cm to 0.183 cm. The NVC ranges from 41.95% to 28.59%, indicating that *Litoria infrafrenata* only has two types of chromosomes, which are metacentric and submetacentric.

Although the two frog species have different numbers of chromosomes, the karyotype analysis results in Table 5 show that both frog species share 4 pairs of chromosomes with the same type, which are chromosomes number 1, 3, 6, and 7. Based on the karyotype analysis results, it is not possible to determine the close or distant relationship between the two species because a third species within the same genus is needed as a comparison.

Table 3. Mean Relative Length, Chromosome Length, Short Arm Length, Centromere Position Value, And Chromosome Type Of *Litoria Caerulea* In Metaphase. N = 10

No. Chro	Relative Length (RL) (%) ± SD	Chromosome Length (µm) ± SD	Short Arm Length (µm) ± SD	Numeric values of centromere positions	
				NVC (%)	**Type**
1	13.71 ± 1.07	0.98 ± 0.19	0.37 ± 0.09	38.03	M
2	11.41 ± 0.88	0.82 ± 0.23	0.31 ± 0.11	37.80	M
3	10.43 ± 0.77	0.75 ± 0.20	0.27 ± 0.14	35.71	Sm
4	9.29 ± 0.52	0.07 ± 0.16	0.26 ± 0.08	38.75	M
5	8.54 ± 0.29	0.61 ± 0.15	0.23 ± 0.06	38.42	M
6	7.80 ± 0.60	0.56 ± 0.16	0.17 ± 0.11	31.34	Sm
7	7.19 ± 0.65	0.51 ± 0.15	0.19 ± 0.05	35.99	Sm
8	6.59 ± 0.64	0.47 ± 0.13	0.18 ± 0.06	38.16	M
9	5.98 ± 0.49	0.43 ± 0.12	0.17 ± 0.05	38.52	M
10	5.54 ± 0.32	0.40 ± 0.12	0.17 ± 0.05	42.02	M
11	4.96 ± 0.43	0.35 ± 0.09	0.16 ± 0.05	45.54	M
12	4.66 ± 0.43	0.33 ± 0.09	0.12 ± 0.07	36.00	Sm
13	3.91 ± 0.37	0.28 ± 0.08	0.11 ± 0.03	39.29	M

Table 4. Mean relative length, chromosome length, short arm length, centromere position value, and chromosome type of *Litoria infrafrenata* in metaphase. n = 6

No. Chro	Relative Length (RL) (%) ± SD	Chromosome Length (µm) ± SD	Short Arm Length (µm) ± SD	Numeric values of centromere positions	
				NVC (%)	**Type**
1	12.97 ± 0.66	0.99 ± 0.11	0.37 ± 0.07	37.46	M
2	11.07 ± 0.42	0.84 ± 0.06	0.25 ± 0.03	29.07	Sm
3	10.44 ± 0.41	0.79 ± 0.06	0.27 ± 0.12	33.28	Sm
4	9.65 ± 0.41	0.73 ± 0.06	0.24 ± 0.05	32.55	Sm
5	9.28 ± 0.37	0.71 ± 0.06	0.21 ± 0.07	29.10	Sm
6	8.68 ± 0.41	0.66 ± 0.03	0.19 ± 0.16	28.59	Sm
7	8.15 ± 0.55	0.62 ± 0.07	0.20 ± 0.08	32.72	Sm
8	7.53 ± 0.27	0.57 ± 0.05	0.21 ± 0.05	36.93	Sm
9	6.73 ± 0.45	0.51 ± 0.05	0.17 ± 0.06	34.29	Sm
10	5.92 ± 0.27	0.45 ± 0.04	0.15 ± 0.13	34.52	Sm
11	5.26 ± 0.20	0.40 ± 0.04	0.12 ± 0.12	30.50	Sm
12	4.35 ± 0.34	0.33 ± 0.04	0.14 ± 0.01	41.95	M

Table 5. Comparison of chromosome types between *Litoria caerulea* and *Litoria infrafrenata*

No. Chro	*Litoria caerulea*	*Litoria infrafrenata*
1	M	**M**
2	M	Sm
3	Sm	Sm
4	M	Sm
5	M	Sm
6	Sm	Sm
7	Sm	Sm
8	M	Sm
9	M	Sm
10	M	Sm
11	M	Sm
12	Sm	**M**
13	M	

Explanation:

M = Metacentric
Sm = Submetacentric

CONCLUSION AND RECOMMENDATIONS

Litoria caerulea and *Litoria infrafrenata*, which may appear similar at first glance, can still be distinguished based on morphology. *Litoria caerulea* has a stout and short body with a distinctive feature of skin folds above the eyes that extend to the area around the tympanum. On the other hand, *Litoria infrafrenata* has a slender and long body with a characteristic white line on the lower lip and along the front and hind limbs. Body weight (BW), Body length (SV), Fourth toe diameter (T4D), Third finger diameter (F3D), Distance from the anterior edge of the eye to the nose (EN), Nostril distance (IN), Eye aperture diameter (EY), and Horizontal diameter of the tympanum (ear drum) (TY) between the two frog species showed significant differences ($p \leq 0.05$).

The karyotypes of *Litoria caerulea* and *Litoria infrafrenata* are different in the number and arrangement of their chromosome types. *Litoria caerulea* has 13 pairs of chromosomes (2n = 26), consisting of 9 pairs of metacentric chromosomes and 4 pairs of submetacentric chromosomes. On the other hand, *Litoria infrafrenata* has 12 pairs of chromosomes (2n = 24), consisting of 10 pairs of submetacentric chromosomes and 2 pairs of metacentric chromosomes. *Litoria caerulea* and *Litoria infrafrenata* share 4 pairs of chromosomes with the same type, which are chromosomes number 1, 3, 6, and 7.

ADVANCED RESEARCH

Advanced Research is needed to understand the diversity of *Litoria* species in the Irian Jaya region, including their ecology, distribution, and analysis of their sex chromosomes, protein patterns, and genetic relationships. A comparative study between *Litoria caerulea* and *Litoria infrafrenata* found in Indonesia and those found in Australia should be conducted, focusing on morphology, karyotype, and blood protein patterns.

ACKNOWLEDGMENT

In the name of Allah, the Most Gracious and Most Merciful, all praise is due to Him. I express my gratitude to the presence of Allah SWT, for it is by His grace and blessings that I have been able to complete the writing of this article. On this occasion, I would like to extend my heartfelt thanks to all those who have been involved and provided substantial assistance in the writing of this scientific work. The research was conducted at CV. BAYU, Sawangan-Depok, and in the Animal Biology Laboratory Inter-University Center (PAU), Faculty of Life Sciences, IPB. May this scientific work be of benefit to others.

REFERENCES

Bogart, J.P. 1973. Evolution of anuran karyotypes. In Evolutionary Biology of the Anurans. (Ed. J. L. Vial.) pp.337-49.

Cogger, G.H. 1992. *Reptiles and Amphibians of Australia*. Red books NSW.

Cole, C. J. 1974. Chromosome evolution in selected treefrogs, including casque-headed species *(Pternohyla, Triprion, Hyla, and Smilisca). Am. Mus. Novit.* No.2541.

Cook, P. C. 1978. Karyotypic analysis of the gobiid fish genus *Quietula* Jordan and Evermann. *J.Fish Biology* 12:229-244.

Elridge, F.E. 1985. *Cytogeneti of Livestock*. Avi Publishing Company Inc, Westport, Connecticut, USA.

K**ing, M., M. J. Tyler, M. Davies & D. King.** 1979. Karyotypic studies on *Cyclorana* and associated genera of Australian frogs. *Aust. J. Zool.*

Koentjaraningrat, H.W. Bachtiar, R.P. Soejono, M.A. Sutaarga, D.C. Ajamiseba. 1994. *Irian Jaya Membangun Masyarakat Majemuk.* Seri Etnografi Indonesia 5. Djambatan Anggota IKAPI, Jakarta.

Menzies, J.I. 1975. *Handbook of Common New Guinea Frogs.* WAU Ecology Institute Handbook no.1. Biology Department University of Papua New Guinea, Port Moresby.

Menzies, J.I. & J. Tippett. 1976. Chromosome numbers of Papuan hylids frogs and the karyotype of *Litoria infrafrenata* (Amphibia, Anura, Hylidae). *J. Herpetol.* 10: 167 – 173.

Menzies, J.I. 1987. A Taxonomic revision of Papuan *Rana* (Amphibia: Ranidae). National Museum and Art Gallery, Boroko, Papua New Guinea. *Aust. J. Zool.* 35: 373 – 418.

Morescalchi, A. & Ingram, G. J. 1974. New chromosome numbers in Australian *Leptodactylidae* (Amphibia, Salientia). *Experientia (Basel)* 30: 1134 – 5.

Morescalchi, A. & Ingram, G. J. 1978. Cytotaxonomy of myobatrachid frogs of the genus *Limnodynastes. Experientia (Basel)* 34: 584 – 5.

Nishioka, M., H. Okumoto, H. Ueda & M. Ryuzaki. 1987. Karyotypes of brown frogs are distributed in Japan, Korea, Europe, and North America. *Sci. Rep. Lab. Amphibian Biol.* 9: 165 – 212.

Satmoko, K. P. & N. Suhana. 1986. Biakan darah vena untuk analisis kromosom manusia. Lokakarya Sitogenetik, Biologi, FKUI, Jakarta.

Steel, R. G. D. & J. H. Torrie. 1991. *Prinsip dan Prosedur Statistika.* PT. Gramedia Pustaka Utama, Jakarta.

Titrawani. 1996. Biodiversiti kodok genus *Rana* ditinjau dari morfologi, kariotip dan pola protein di kodya Sawahlunto. Tesis. Jurusan Biologi FMIPA IPB, Bogor.

Tyler, M. J. 1968. *Papuan Hylids Frogs of the Genus Hyla*. Zoologische Verhandelingen.

Tyler, M. J. 1970. Patterns of distribution and the origins of the Papuan hylid frog fauna. *Search (Syd.)* 1: 246 – 7.

Tyler, M. J. 1972. Superficial mandibular musculaturale, vocal sacs, and the phylogeny of Australo – Papuan Leptodactylid frogs. *Rec. S. Aust. Mus.* 16: 1-20

Van Kampen, P. N. 1923. *The Amphibia of Indo-Australia Archipelago*. E. J. Brill, Leiden.

Yusmin, L. M. 1998. Morfologi, kariotip dan komponen darah pada beberapa jenis rusa di Indonesia. Tesis. Jurusan Biologi FMIPA IPB, Bogor.

Zweifel, R. G. 1983. Two new Hylid frogs from Papua New Guinea and a discussion of the *Nyctymystes* Papua species group. *Nat. Hist* 2759: 1–21.