Genetic Relationships and Phylogeny of Papua New Guinean Hylid Frogs Elucidated by Allozyme Analysis

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Abstract: To elucidate the evolutionary relationships of Papua New Guinean hylid frogs, we conducted an allozyme analysis using a total of 70 individuals from nine species of *Litoria* and two species of *Nyctimystes*. Fourteen enzymes extracted from skeletal muscles and livers were analyzed by starch-gel electrophoresis. These enzymes were encoded by genes at 20 presumptive loci. There were 3–11 phenotypes produced by 3–9 alleles at these loci. The mean proportion of heterozygous loci per individual, mean proportion of polymorphic loci per population, and mean number of alleles per locus in 11 species were 4.5%, 12.5%, and 1.14 on average, respectively. Genetic distances were 0.036 between two populations of *L. infrafrenata*; 0.248–1.849 ($\bar{x}=1.135$) between nine species of the genus *Litoria*; and 1.703 between two species from the genus *Nyctimystes*. The intergeneric genetic distances were 1.551–2.877 ($\bar{x}=1.944$) between *Litoria* and *Nyctimystes*. The UPGMA dendrogram and NJ tree showed the primary dichotomy of the Papua New Guinean hylids *Nyctimystes* and *Litoria*. Our distance data confirmed the presence of several sibling species of *Litoria*, which are similar to each other in external morphology; i.e., *L. arfakiana* and *L. wollastoni*, and *L. contrastens* and *L. bicolor* group sp.

Key words: Allozyme analysis; Anura; Hylidae; *Litoria*; *Nyctimystes*; Phylogeny; Papua New Guinea

Six families of frogs are known to inhabit Papua New Guinea (Menzies, 1976). One of these (Bufonidae) contains only a single species, *Bufo marinus*, which was introduced from Hawaii in 1937, and another (Rhacophoridae) is known only from two specimens of *Rhacophorus* of possible artificial origin collected near Jayapura in 1926. Of the four remaining families, Myobatrachidae and Hylidae have affinities only with Australia, whereas Ranidae and Microhylidae are virtually absent from Australia and are obviously derived from the Oriental Region (Menzies, 1976).

According to Zweifel and Tyler (1982), our knowledge of the systematics of the New Guinean frog fauna is far from being complete, but is sufficient to establish the relative diversity within the anuran families and, hence, of the fauna as a whole. They listed 184 species of frogs for the New Guinean region. The Hylidae comprise more than 40% of the known species from New Guinea and are outnumbered only by the Microhylidae (Zweifel and Tyler, 1982). Two genera of Hylidae are recognized in New Guinea: *Litoria* with 52 species and *Nyctimystes* with 19 species (Zweifel, 1983). In an examination of the

phylogenetic relationships and evolution of *Nyctimystes* by skeletal characteristics, Tyler and Davies (1979) concluded that the genus probably derived from a montane *Litoria* stock resembling *Litoria eucnemis*.

An allozyme analysis of New Guinean frogs has resolved genetic relationships among several species within the genus *Litoria* (Dessauer et al., 1977). Dessauer et al. (1977) studied biochemical variation among 13 New Guinean species of *Litoria*, and recognized two major groups: one containing six species, and the other containing seven species. However, no *Nyctimystes* were included in the samples they analyzed, and the genetic relationships between genus *Litoria* and genus *Nyctimystes* have still not been reported by allozyme analysis.

In the present study, an allozyme analysis was carried out using 11 species of *Litoria* and *Nyctimystes* to elucidate their genetic relationships at the genus, species, and population levels.

MATERIALS AND METHODS

A total of 70 specimens from 11 species of *Litoria* and *Nyctimystes* were used (Table 1, Figs. 1 and 2). Of these, specimens of *L. pratti* group sp. and *L. bicolor* group sp. (Figs. 1E, F and 2C) possibly represent undescribed species

TABLE 1. Specimens of a total of 70 Papua New Guinean hylid frogs used in the present study.

Species	Locality	Sample size	Body length (mm)		Date of collection	Individual number ^{a)}
			Range	Mean		
<i>Litoria arfakiana</i>	Wau	12	39.4–51.0	46.3	Nov 1982, Jan 1985	6508, 6509, 6532–6534, 6638, 6654, 6656–6658, 6660, 6694
<i>Litoria wollastoni</i>	Wau	9	35.6–40.2	37.5	Nov 1982, Jan 1985	6524, 6525, 6536, 6541–6544, 6596, 6640
<i>Litoria angiana</i>	Wau	4	53.0–80.8	67.3	Jan 1985	6531, 6611, 6624, 6653
<i>Litoria pratti</i> group sp.	Wau	2	33.0–48.0	40.5	Nov 1982	6506, 6507
<i>Litoria thesaurensis</i>	Wau	4	38.0–42.4	40.6	Nov 1982, Jan 1985	6539, 6606, 6637, 6739
<i>Litoria infrafrenata</i>	Wau	8	81.0–108.0	92.7	Nov 1982	6520–6523, 6590, 6696, 6776, 6777
<i>Litoria infrafrenata</i>	Port Moresby	5	88.8–135.0	106.8	Jan 1985	6636, 6698, 6702, 6743, 6750
<i>Litoria contrastans</i>	Wau	2	28.5–30.0	29.3	Jan 1985	6599, 6639
<i>Litoria bicolor</i> group sp.	Port Moresby	3	23.6–25.8	24.6	Jan 1985	6731–6733
<i>Litoria</i> sp.	Lae	3	20.2–40.8	27.1	Dec 1984	6605, 6608, 6735
<i>Nyctimystes forficula</i>	Wau	16	34.4–52.0	37.2	Nov 1982, Jan 1985	6504, 6528–6530, 6537, 6625–6635
<i>Nyctimystes kubori</i>	Wau	2	49.4–58.0	53.7	Nov 1982	6535, 6540

^{a)} Catalogued specimens are stored at the Laboratory for Amphibian Biology.

(Menzies, 1976). Three specimens of another seemingly undescribed species (*L. sp.*) were collected in the vicinity of Lae. These frogs were dark brown, blotched with a considerable amount of yellow, and had a light line connecting the upper eyelids (Fig. 2A). *Litoria infrafrenata*, which was shown to contain highly diverged populations (Dessauer et al., 1977), was collected from two different localities, Wau and Port Moresby, and these two local populations were treated as different OTUs. The other seven species were collected in the vicinity of Wau (Table 1).

Fourteen enzymes extracted from muscles and livers were analyzed by horizontal starch-gel electrophoresis following the method of Nishio-ka et al. (1980, 1992) (Table 2). The detection of each enzyme was carried out following the method outlined by Harris and Hopkinson (1976). The genetic distances were calculated following Nei (1972), and phenetic relationships were assumed by constructing dendrograms on the basis of the UPGMA method (Nei, 1975, 1987; Sneath and Sokal, 1973) and the NJ method (Saitou and Nei, 1987). In the NJ analysis, the root was assumed at the midpoint of the longest path.

RESULTS

The electrophoretic patterns of 14 enzymes analyzed were presumed to be encoded by genes at 20 loci. Electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, etc., in the order of greater mobility, and alleles were indicated by a, b, c, etc. The CK locus was least variable; three phenotypes were produced by three alleles. The IDH-1 locus was the most polymorphic; 10 phenotypes were produced by nine alleles. At the 20 loci examined, there were 6.5 phenotypes produced by 5.3 alleles on average (Table 3). Table 4 presents gene frequencies for each species at 20 loci. At seven loci (AAT-1, IDH-1, LDH-B, MDH-1, ME-1, PGM, and SOD), all alleles were specific to each genus.

The mean proportion of heterozygous loci per individual, mean proportion of polymorphic loci per population, and mean number of alleles per locus in 11 species were 0–11.0% ($\bar{x}$ = 4.5%), 0–40.0% ($\bar{x}$ = 12.5%), and 1.00–1.40 ($\bar{x}$ = 1.14), respectively (Table 5). There were no obvious differences between the actual and expected values of the first measure of genetic variability.

Genetic distances among the 11 species examined are shown in Table 6. The distances were 0.036 between two populations of *Litoria in-*

FIG. 1. Papua New Guinean hylid frogs. Scale bars equal 5 mm. A, B: *Litoria arfakiana*. C, D: *Litoria wollastoni*. E, F: *Litoria pratti* group sp. The head of *L. pratti* group sp. is broad and its snout is angular and projected (E, F), but usually not so acutely as in members of the *L. arfakiana* group (A-D). There are spikes on the heels of frogs of the *L. arfakiana* group (A-D), whereas there are no spikes on the heels of the frogs of *L. pratti* group sp. (E, F).

fratrenata, and 0.248–1.849 ($\bar{x}=1.135$) between nine species of the genus *Litoria*. Of the latter, the distance value was smallest between *L. wollastoni* and *L. arfakiana* (0.248), the second smallest between *L. contrastens* and *L. bicolor* group sp. (0.357), and the greatest between *L. arfakiana* and *L. sp.* from Lae (1.849). Genetic distance was 1.703 between two *Nyctimystes* species examined. Between *Litoria* and *Nyctimystes*, genetic distances were 1.551–2.877 ($\bar{x}=1.944$).

The primary divergence expressed in the UPGMA dendrogram (Fig. 3A) was concordant

with the generic classification of the present samples. The genus *Litoria* was divided into two subgroups; one containing *L. arfakiana*, *L. wollastoni*, *L. angiana*, and *L. pratti* group sp., and the other containing *L. contrastens*, *L. bicolor* group sp., *L. thesaurensis*, *L. infrafratrenata*, and *L. sp.* from Lae. Two species of *Nyctimystes* were remotely related to each other. The NJ tree (Fig. 3B) also showed that the New Guinean hylids primarily diverged into two genera, *Nyctimystes* and *Litoria*, but then the latter gradually diverged into several species groups. In contrast to the UPGMA dendro-

FIG. 2. Papua New Guinean hylid frogs. Scale bars equal 2.5 mm. A: *Litoria* sp. collected at Lae. B: *Litoria contrastens*. C: *Litoria bicolor* group sp. *Litoria contrastens* is very similar to *L. bicolor* group sp. in external morphology and coloration.

gram, the NJ tree showed that *Litoria infrafrenata* was first differentiated from the other *Litoria* species.

DISCUSSION

According to Zweifel and Tyler (1982) and Frost (1985), all hylid frogs of the New Guinean region (71 species) belong to only two genera: *Nyctimystes* and *Litoria*. The former lives predominantly in montane habitats and centers upon the mainland, whereas the latter ranges throughout New Guinea and is the most diverse group of frogs in the region. Tyler and Davies (1979) examined the phylogenetic relationships and evolution of *Nyctimystes*, and suggested that this genus originated from a montane *Litoria* stock resembling *L. eucnemis*. Examining 22

Litoria and 6 *Nyctimystes* species from Papua New Guinea, Menzies and Tippet (1976) reported that the chromosome number of Papuan hylids is $2n=26$, with the exception of *L. infrafrenata*, which has $2n=24$ chromosomes. Kuramoto and Allison (1991) also reported the karyotypes of three species of *Litoria* (*L. arfakiana*, *L. thesaurensis*, *L. wollastoni*) and two species of *Nyctimystes* (*N. foricula*, *N. pulchra*) from Papua New Guinea, and suggested that the karyotype of *Nyctimystes* was derived from that of a group of *Litoria*. Our results showed that the New Guinean hylids examined in this study are largely divided into two groups, *Litoria* and *Nyctimystes*. The genetic distances between the two genera, 1.551–2.877 ($\bar{x}=1.944$), were large compared with those within each of

TABLE 2. Enzymes analyzed in the present study.

Enzyme	Abbreviation	E.C.No. ^{a)}	Tissue	Buffer system ^{b)}
Aspartate aminotransferase	AAT	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenylate kinase	AK	2.7.4.3	Skeletal muscle	T-C pH 7.0
Creatine kinase	CK	2.7.3.2	Skeletal muscle	T-B-E pH 8.0
α -Glycerophosphate dehydrogenase	α -GDH	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	Skeletal muscle	T-B-E pH 8.0
Isocitrate dehydrogenase	IDH	1.1.1.42	Skeletal muscle	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	Skeletal muscle	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	Skeletal muscle	T-C pH 6.0
Malic enzyme	ME	1.1.1.40	Skeletal muscle	T-C pH 7.0
Mannose phosphate isomerase	MPI	5.3.1.8	Skeletal muscle	T-C pH 7.0
Peptidase	PEP	3.4.3.1	Liver	T-B-E pH 8.0
Phosphogluconate dehydrogenase	PGD	1.1.1.44	Skeletal muscle	T-C pH 7.0
Phosphoglucomutase	PGM	2.7.5.1	Skeletal muscle	T-B-E pH 8.0
Superoxide dismutase	SOD	1.15.1.1	Skeletal muscle	T-B-E pH 8.0

^{a)} Enzyme Commission numbers (Nomenclature Committee of Biochemistry, 1992).

^{b)} T-C = Tris-citrate buffer; T-B-E = Tris-borate-EDTA buffer.

TABLE 3. The Numbers and kinds of alleles and phenotypes at 20 enzyme loci in 11 species belonging to two genera of the family Hylidae in Papua New Guinea.

Loci	Alleles		Phenotypes	
	N	Kind	N	Kind
AAT-1	7	a-g	9	BB, CC, DD, EE, FF, GG, AC, BC, DF
AAT-2	5	a-e	6	AA, BB, CC, DD, EE, AD
AK	4	a-d	6	AA, BB, CC, DD, BC, CD
CK	3	a-c	3	AA, BB, CC
α -GDH	3	a-c	4	AA, BB, CC, AB
GPI	5	a-e	8	AA, BB, CC, DD, EE, AC, BD, BE
IDH-1	9	a-i	10	AA, BB, CC, DD, EE, FF, GG, HH, II, EH
IDH-2	4	a-d	5	AA, BB, CC, DD, BD
LDH-A	4	a-d	4	AA, BB, CC, DD
LDH-B	7	a-g	8	AA, BB, CC, DD, EE, FF, GG, CE
MDH-1	5	a-e	5	AA, BB, CC, DD, EE
MDH-2	5	a-e	6	AA, BB, CC, DD, EE, AE
ME-1	5	a-e	5	AA, BB, CC, DD, EE
ME-2	4	a-d	5	AA, BB, CC, DD, AC
MPI	7	a-g	11	BB, CC, DD, EE, FF, GG, AG, BE, BG, CG, DG
PEP-A	5	a-e	5	AA, BB, CC, DD, EE
PEP-D	5	a-e	6	AA, BB, CC, DD, EE, AC
PGD	6	a-f	9	AA, BB, CC, DD, EE, FF, BD, CD, DE
PGM	4	a-d	4	AA, BB, BC, BD
SOD	9	a-i	10	AA, BB, CC, EE, FF, GG, HH, II, CH, DG
Average	5.3		6.5	

them (0.248–1.849, $\bar{x}$ = 1.135). Both the UPGMA dendrogram and the NJ tree showed a primary dichotomy of the New Guinean hylids *Nyctimystes* and *Litoria*. This result contradicts the hypothesis of Tyler and Davies (1979), who assumed that the numerous points of morphological and reproductive resemblance of *Nyctimystes* to montane species of *Litoria* reflect a common origin of *Nyctimystes* and the montane *Litoria*. Of 11 species examined in the present study, only *L. infrafronata* and *L. bicolor* group sp. inhabit the lowlands of New Guinea, and the other nine species are found in hilly forests or the mountains (Menzies, 1976; Tyler and Davies, 1979). The present data showed no close relationships between *Nyctimystes* and montane *Litoria*. Thus, it is probable that after the common ancestor(s) diverged into the two genera, *Litoria* and *Nyctimystes*, they have independently acquired montane adaptations.

Dessauer et al. (1977) indicated a wide divergence in 16 proteins among 13 species of *Litoria* that was in concordance with morphological divergence. Based on this, they suggested the presence of two distinct lineages in the species they examined. Their biochemical data, while supporting the recognition of two pairs of sibling species (*L. arfakiana* and *L. wollastoni*,

and *L. modica*-like frogs from two localities), suggested that a third cryptic species may exist in the former pair. According to Menzies and Zweifel (1974), *Litoria arfakiana* and *L. wollastoni* cannot be clearly distinguished from each other morphologically (although there is an average size difference: see Menzies and Zweifel, 1974), but do differ in mating calls. The present study showed that, although the genetic distance between these two species is relatively small (0.248), they are clearly distinguished by diagnostic alleles at four loci (IDH-1, PEP-A, PEP-D and SOD). This confirms the taxonomic conclusion by Dessauer et al. (1977), which was made on the basis of very few specimens for each species. It is interesting to note that no genetic variability was observed in *L. wollastoni*, whereas comparatively high genetic variability was found in *L. arfakiana*. These results may reflect the differences between ecological and habitat characteristics of these two species (Dessauer et al., 1975). On the other hand, *Litoria contrastens* is very similar to members of the *L. bicolor* group in external morphology and coloration (Fig. 2B, C). The genetic distance between *L. contrastens* and *L. bicolor* group sp. was comparatively small (0.357), but they were distinguished by diagnostic alleles at six loci

TABLE 4. Gene frequencies at 20 enzyme loci in 11 species belonging to two genera of the family Hylidae.

Species	Sample size	Loci									
		AAT-1	AAT-2	AK	CK	α -GDH	GPI	IDH-1	IDH-2	LDH-A	LDH-B
<i>Litoria arfakiana</i>	12	b (0.58)	c (0.42)	c (0.96)	d (0.04)	b (0.75)	a (0.25)	c (0.83)	g (0.04)	b (0.96)	d
<i>Litoria wollastoni</i>	9	b	d	c	b	a	a	i	d	d	d
<i>Litoria angiana</i>	4	c	e	c (0.38)	d (0.63)	a	a	c (0.13)	g (0.88)	d	h
<i>Litoria pratti</i> group sp.	2	d	c	b	b	a	a	f	a	d	g
<i>Litoria thesaurensis</i>	4	d (0.50)	f (0.50)	c	a	a	b (0.50)	d (0.50)	a	a	b
<i>Litoria infrafenata</i> (Wau)	8	c	d	b	a	a	a	d	d	a	c
<i>Litoria infrafenata</i> (POM)	5	a (0.10)	b (0.20)	c (0.70)	b	a	e	d	d	a (0.80)	c (0.20)
<i>Litoria contrastens</i>	2	c	d	c	a	a	a	c	b	b	a
<i>Litoria bicolor</i> group sp.	3	c	c	c	a	a	b	c	b	a	a
<i>Litoria</i> sp. (Lae)	3	c	a	b	c	a	b	b	b	d	b
<i>Nyctimystes foricula</i>	16	e	b	b (0.03)	c (0.97)	c	b (0.50)	e (0.50)	h (0.09)	a	f
<i>Nyctimystes kubori</i>	2	g	c	a	a	a	b	e	c	c	f

TABLE 4. Continued.

Species	Sample size	Loci									
		MDH-1	MDH-2	ME-1	ME-2	MPI	PEP-A	PEP-D	PGD	PGM	SOD
<i>L. arfakiana</i>	12	d	e	d	a c (0.13)(0.88)	d	a	a c (0.92)(0.08)	d	b c (0.96)(0.04)	b
<i>L. wollastoni</i>	9	d	e	d	c	d	c	b	d	b	f
<i>L. angiana</i>	4	e	e	c	c	f	c	c	d	b	b
<i>L. pratti</i> group sp.	2	e	e	d	a	f	b	c	d	b	c
<i>L. thesaurensis</i>	4	b	c	d	a	d g (0.50)(0.50)	c	e	c d (0.25)(0.75)	b	c
<i>L. infrafrenata</i> (Wau)	8	b	e	a	d	c g (0.63)(0.38)	c	d	d	b	c h (0.69)(0.31)
<i>L. infrafrenata</i> (POM)	5	b	d	a	d	c g (0.90)(0.10)	c	d	b d (0.40)(0.60)	b	c h (0.30)(0.70)
<i>L. contrastens</i>	2	e	d	d	d	b	d	c	c	b	e
<i>L. bicolor</i> group sp.	3	e	d	d	c	c	d	a	c	b	e
<i>L. sp.</i> (Lae)	3	e	c	a	a	c	c	d	a	b d (0.67)(0.33)	i
<i>N. foricula</i>	16	c	a e (0.31)(0.69)	e	b	a b e g (0.03)(0.44)(0.47)(0.06)	e	d	d e (0.34)(0.66)	a	a
<i>N. kubori</i>	2	a	b	b	d	d	c	b	f	a	d g (0.25)(0.75)

TABLE 5. Genetic variabilities at 20 enzyme loci in 11 species belonging to two genera of the family Hylidae.

Species	Sample size	Mean proportion (%) of heterozygous loci per individual ^{a)}	Mean proportion (%) of polymorphic loci per population	Mean number of alleles per locus
<i>Litoria arfakiana</i>	12	7.5(8.8)	40.0	1.40
<i>Litoria wollastoni</i>	9	0 (0)	0	1.00
<i>Litoria angiana</i>	4	5.0(3.4)	10.0	1.10
<i>Litoria pratti</i> group sp.	2	0 (0)	0	1.00
<i>Litoria thesaurensis</i>	4	10.0(9.4)	20.0	1.20
<i>Litoria infrafrenata</i> (Wau)	8	5.6(4.5)	10.0	1.10
<i>Litoria infrafrenata</i> (POM)	5	11.0(11.7)	30.0	1.35
<i>Litoria contrastens</i>	2	0 (0)	0	1.00
<i>Litoria bicolor</i> group sp.	3	0 (0)	0	1.00
<i>Litoria</i> sp. (Lae)	3	3.3(2.2)	5.0	1.05
<i>Nyctimystes foricula</i>	16	8.8(11.0)	30.0	1.40
<i>Nyctimystes kubori</i>	2	2.5(1.9)	5.0	1.05
Average	5.8	4.5(4.4)	12.5	1.14

^{a)} Parentheses show an expected value.

TABLE 6. Nei's (1972) genetic distance (D) among 11 species belonging to the genera *Litoria* (L.) and *Nyctimystes* (N.).

Species	Species										
	1	2	3	4	5	6	7	8	9	10	11
1 <i>L. arfakiana</i>											
2 <i>L. wollastoni</i>	0.248										
3 <i>L. angiana</i>	0.519	0.651									
4 <i>L. pratti</i> group sp.	0.781	0.916	0.592								
5 <i>L. thesaurensis</i>	1.275	1.114	1.512	1.288							
6 <i>L. infrafrenata</i> (Wau)	1.301	1.181	1.164	1.586	0.892						
7 <i>L. infrafrenata</i> (POM)	1.435	1.247	1.248	1.653	0.910	0.036					
8 <i>L. contrastens</i>	1.268	1.386	1.273	1.386	1.114	1.027	1.093				
9 <i>L. bicolor</i> group sp.	1.172	1.386	1.296	1.386	0.899	1.082	1.046	0.357			
10 <i>L. sp.</i> (Lae)	1.849	1.685	1.232	1.250	0.835	0.975	0.885	1.444	1.087		
11 <i>N. foricula</i>	1.821	1.839	2.047	2.877	1.969	1.554	1.555	2.596	2.034	1.998	
12 <i>N. kubori</i>	2.381	1.600	2.276	2.293	1.551	1.577	1.538	1.888	1.600	1.876	1.703

(AAT-2, GPI, LDH-A, ME-2, MPI, and PEP-D). This confirms their distinctiveness at the species level.

Litoria infrafrenata in New Guinea appears to be split into several geographic populations (Tyler, 1994). Dessauer et al. (1977), for example, showed distinct biochemical differentiation between two populations, the Daru and Garaina populations, of *L. infrafrenata*. According to them, this widely ranging species probably includes some populations which are at least in the early stage of speciation. However, no genetic differentiation was evident between the Wau and Port Moresby populations of this species in the present study (0.036 in genetic distance), despite their distinct geographical isolation by high mountain ranges such as the Owen Stanley.

According to Menzies (1976), all torrent breeding species of the New Guinean *Litoria* other than *L. angiana* and members of the *L. arfakiana* group (*L. arfakiana*, *L. wollastoni* and *L. oenicolen*), could be included in the *L. pratti* group. In the *L. pratti* group, the snout-vent length of males does not grow longer than 38 mm (often much less), and the dorsal color is mostly brown (Menzies, 1976). Their heads are broad, and their snouts are angular and projecting, but usually not so acutely as in members of the *L. arfakiana* group. The fingers are unwebbed and there are no spikes on the heels in the *L. pratti* group (Fig. 1). This last trait serves to distinguish them from frogs of the *L. arfakiana* group, whereas the small size distinguishes them from *L. angiana*. However, the

FIG. 3. UPGMA dendrogram (A) and NJ tree (B) for 11 species of Papua New Guinean hyliid frogs belonging to two genera of the family Hyliidae based on Nei's (1972) genetic distance.

present study revealed that four torrent breeding species, *L. arfakiana*, *L. wollastoni*, *L. angiana*, and *L. pratti* group sp., are relatively closely related to each other, constituting a single cluster

(0.248–0.916, $\bar{x}$ = 0.618 in genetic distances) (Fig. 3).

A possible undescribed species from Lae (Fig. 2A) formed a cluster with *L. thesaurensis*,

although this species was distinctly differentiated from the latter and other congeneric species (0.835–1.998, $\bar{x}$ = 1.374 in genetic distances). Available specimens representing this problematic species are all basically dark brown and blotched with a considerable amount of yellow, and there is a light line connecting the upper eyelids (Fig. 2A). Their heads are broad, and the snouts are angular. Tyler et al. (1986) pointed out the existence of a large number of undescribed species in the Papua New Guinean hylids. This species is likely to be recognized as a valid new species of genus *Litoria*, although further examination is needed to describe this species.

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要旨 アロザイム分析によるパプアニューギニア産アマガエル科のカエル類の遺伝的關係と系統

住田正幸・アレン＝アリソン・西岡みどり

パプアニューギニア産アマガエル科の *Litoria* 属 9 種と *Nyctimystes* 属 2 種の計 70 個体を用いて、肝臓および骨格筋の 14 酵素をデンブングル電気泳動法によって分析した。これらの酵素には 20 の遺伝子座が関与し、各遺伝子座にはそれぞれ 3-9 の対立遺伝子によって生じる 3-11 の表現型が観察された。各種における平均ヘテロ接合体率は 4.5%，多型的遺伝子座の割合は 12.5%，遺伝子座当たりの平均対立遺伝子数は 1.14 であった。Nei の遺伝距離は *L. infrafrenata* の 2 集団間では 0.036，*Litoria* 属内の 9 種間では 0.248-1.849（平均 1.135），*Nyctimystes* 属内の 2 種間では 1.703，*Litoria* と *Nyctimystes* の 2 属間では 1.551-2.877（平均 1.944）であっ

た。UPGMA 法および NJ 法による系統分析の結果、ニューギニア産アマガエル科は *Litoria* 属と *Nyctimystes* 属の 2 つに大きく分岐することが分かった。また本結果により、*Litoria* 属内の形態的によく似たいくつかの種（*L. arfakiana* と *L. wollastoni*, *L. contrastens* と *L. bicolor* 種群未記載種）が明瞭に識別できることが確認された。

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