

## Microhabitat partitioning of closely related Sarawak (Malaysian Borneo) frog species previously assigned to the genus *Hylarana* (Amphibia: Anura)

Ramlah ZAINUDIN<sup>1\*</sup>, Badrul Munir MD ZAIN<sup>2</sup>, Norhayati AHMAD<sup>2</sup>, Shukor M. NOR<sup>2</sup>

<sup>1</sup>Molecular Ecology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia

<sup>2</sup>School of Environment and Natural Resources Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

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**Abstract:** Microhabitats play an important role as resources that are partitioned between phylogenetically related or ecologically similar species (i.e., a guild). This hypothesis was tested by first elucidating phylogenetically closed Sarawak frog species via DNA sequencing of the 16S rRNA mitochondrial DNA gene, and later determining their microhabitat guild and partitioning via nonmetric dimensional scale. Mitochondrial 16S gene revealed 5 monophyletic groups consisting of *Hylarana erythraea* + *Amnirana nicobariensis*, *Chalcorana raniceps*, *Abavorana luctuosa*, *Pulchrana signata* + *P. picturata*, and *P. baramica* + *P. glandulosa* + *P. laterimaculata*. On the other hand, microhabitat utilization grouped the frogs into 5 ecological guilds consisting of semiarboreal species at the forest edge (*C. raniceps*), ground dwellers in an unforested region (*H. erythraea*), ground dwellers (rock) at the forest edge (*P. picturata*), ground dwellers on the forest floor and forest edge species (*P. signata*, *P. glandulosa*, *A. luctuosa*, *O. hosii*), and semiarboreal forest (riverine) species (*P. baramica*). Thus, the microhabitats used were not influenced by the proposed phylogenetic relationships. Partitioning of microhabitat utilization, on the other hand, was clearly seen in 2 closely related species in both vertical and substrate utilization. The study supports resource partitioning by closely related species reflected by their ecological guilds, and will be useful for conservation management of the Bornean fauna.

**Key words:** Ecological guilds, molecular phylogeny, 16S mtDNA, Sarawak frogs, monophyletic frog

### 1. Introduction

An ecological guild is a set of species, usually phylogenetically close, that have similar ecology and play a similar role (niche) in a community (Simberloff and Dayan, 1991). Species utilizing the same resources will tend to cluster together compared to species with different resource requirements (Webb et al., 2002). The phrase phylogenetically close, or closely related species, refers to species that share a most recent common ancestor or sibling species. Closely related species in the same area show that the differences between species (morphology and behavior) are related to differences in their ecological resources (Glor et al., 2003). Microhabitats often play an important role as resources that are partitioned between phylogenetically related or ecologically similar species (Luiselli, 2006; Elias et al., 2009). A study on leaf-litter herpetofauna (Whitfield and Pierce, 2005) indicated that the microhabitat provided by tree buttresses forms a site of generalized high abundance for the leaf-litter herpetofauna, and may contribute to localized high

abundance of at least 1 species. Thus, the availability of suitable microhabitats may be an important determinant of anuran distributions. Information on microhabitats of anurans can also be important information for conservation management. By studying microhabitat utilization of animals, which species are associated with specific microhabitats can easily be recognized; this is useful for conservation management (Chalmers and Loftin, 2006; Vitt et al., 2007; Hossein et al., 2009). If rapid changes in environmental factors occur, such as fire, heavy haze, and extreme winds, in tropical rain forests, anurans may behaviorally alter their microhabitat utilization in response to the changes; thus, which species are vulnerable to the changes can be predicted. For example, Doan (2004) showed that the cold winds of Amazon Friajes alter anuran vertical distributions by causing arboreal frogs to descend to lower levels of the tree trunk. However, Inger and Voris (1993) concluded that environmental factors (topography, rainfall, elevation, vegetation, and streams) did not account for all differences in overlaps between

\* Correspondence: [zramlah@unimas.my](mailto:zramlah@unimas.my)

communities of frogs within and among 8 localities in Sarawak. On the other hand, regional processes (isolation, speciation) might contribute to geographic restrictions of several species, leading to variation in overlap values.

Inger and Voris (2001) and Inger and Stuebing (2005) categorized the Sarawak frogs into 2 groups based on habitat utilization: i) town or village species (*Hylarana erythraea* (green paddy frog) and *Amnirana nicobariensis* (cricket frog), and ii) forests or forest edge species, which were further divided into inland hill species (category III: *P. signata*, *P. picturata*, *A. luctuosa*, *C. raniceps*) and species confined to coastal lowlands and swamp areas (Category II: *P. baramica*, *P. glandulosa*, and *P. laterimaculata*). However, there has been little study of microhabitat utilization by Sarawak frogs, except for studies on microhabitat utilization by Sarawak tadpoles by Inger (1985), and frogs of the Sarawak highlands (Zainudin et al., 2006).

Our main question is whether species that are closely related will partition their microhabitats to coexist. However, studies that relate phylogenetic relationships of a group of species to their ecological characteristics (resources) are still lacking. Tofts and Silvertown (2000) employed phylogenetic methods of analysis (PICs) based on species' traits to investigate predictions of competition theory (species in a community will be less similar than expected by chance) and environmental structuring hypothesis (greater similarity of species in a community). They correctly identified species in the pool but failed to predict absences of certain species. A recent study by Candioti and Altig (2010) also failed to correlate morphological traits with the trophic guilds in anuran larvae. The usage of mtDNA sequences successfully reveals genetic relationships within species (closely related species of Sarawak frogs [Zainudin et al., 2010]; human [Lim et al., 2010]; tarsier [Md-Zain et al., 2010]; sheep [Rane and Barve 2011]; Bali cattle [Syed-Shabthar et. al., 2013]; and Malayan Gaur [Rosli et al., 2016]). This paper aimed to look at the molecular phylogeny of adult Sarawak frogs from the previously assigned genus *Hylarana* and determine the ecological guilds of the species based on habitat and microhabitat used. Furthermore, it is postulated that phylogenetically close species (monophyletic) of Sarawak frogs with similar habitat and microhabitat requirements will partition their habitats and microhabitats in order for them to coexist.

## 2. Materials and methods

### 2.1. Sampling design

Sarawak frogs in this study (*Hylarana erythraea*, *Amnirana nicobariensis*, *Chalcorana raniceps*, *Abavorana luctuosa*, *Pulchrana signata*, *P. picturata*, *P. baramica*, *P. glandulosa*, and *P. laterimaculata*) are widely distributed and occupy various habitats; thus, it is highly recommended that

sampling localities represent most of their habitats. For this purpose, 7 study sites from Malaysian Borneo (Sarawak) were chosen for data collection, namely 4 national parks (Kubah/Matang [1] from 19 to 22 Sep 2005, Bako [3] from 28 Aug to 1 Sept 2005 and from 20 to 25 Nov 2005, Mulu [6] from 21 to 25 Aug 2006, and Similajau [5] from 27 Dec to 3 Jan 2006) and 3 unprotected areas (Borneo Height [2] from 18 to 20 May 2005 and from 13 to 15 Jan 2006, Sadong Jaya [4] from 24 to 27 Jul 2006, and Bario [7] from 18 to 22 Apr 2005).

### 2.2. Partitioning of microhabitat and ecological guild of Sarawak frogs

Adult frogs were collected via stream transect, forest floor quadrat, and forest transect. For each captured frog, ecological data such as locality, vegetation type, time and date of capture, and microhabitat were noted following procedures by Heyer et al. (1994). Four microhabitat attributes were chosen for the analysis: vegetation (habitat), horizontal position, vertical position, and substrates. Vegetation here was defined as the habitat of frogs at the study localities, while horizontal positions were defined as nearness (range of 5 m of both sides) to streams or ponds of the site occupied by Sarawak frogs. Vertical position, on the other hand, refers to subsurface, at exposed soil surface or in the water, and substrate was defined as the surface area that the frogs were sitting on at the time of capture, usually mineral soil, dead leaves, log, rock, or vegetation.

### 2.3. Statistical analysis

Similarity index of modified Morisita ( $MMc_{ij} = 2 \sum x_{ik} x_{jk} / [\sum x_{ik}^2 / N_i^2 + \sum x_{jk}^2 / N_j^2] N_i N_j$ ) (Krebs, 1989) was used to analyze data on microhabitat usage;  $x_{ik} x_{jk}$  = number of individuals of species *i* in samples *j* and *k*,  $N_i$  = total number of individuals in sample *j*, and  $N_j$  = total number of individuals in sample *k*. The index is then subjected to a hierarchical cluster analysis using UPGMA in the Multivariate Statistical Package (MVSP) v.3.13d (Kovach, 2002) to test species association based on microhabitat features. Horn's index of niche overlap and Levin's standardized niche breadth were calculated using EcoMath 6.1 (Krebs, 2002). Since the data were far from normal distributions, 2 nonparametric statistics were employed: a chi-square to see relationships of habitat and microhabitat utilizations within the frogs, and a nonmetric proximities multidimensional scale (proxscal NMDS) analysis. The NMDS was used for all species to identify significant microhabitats that the frogs occupied from the 7 study localities, with the addition of previous data collected in 1996 (Zainudin, 2003, 2005). It is assumed that habitat and microhabitat utilization of the Sarawak frogs includes the site they were occupying at the time of capture, the absence of co-occurrence with other animals, and that the utilization represents almost all habitats and microhabitats that the frogs occupy.

## 2.4. Molecular phylogenetic of Sarawak frogs (previously assigned as genus *Hylarana*)

Whole mitochondrial DNA (mtDNA) genome was extracted using Genispin Tissue DNA Kit (manufactured by BioSynTech, Subang Jaya) following the manufacturer's protocol. PCR was used to amplify fragments of the mitochondrial 16S rRNA genes. The primer used was 16Sar - L 3' CGCCTCTTGCTTAAAAACAT 5', and 16Sb - H 5' ATGTTTTTAAGCAAGAGGCG 3', following Palumbi et al. (1991). The components of a standard PCR protocol using *Taq* DNA polymerase followed Zainudin et al. (2010). Outgroups used in the phylogenetic reconstruction were based on other genera in the family Ranidae and most relatives of the frogs previously assigned as genus *Hylarana*. The chosen outgroups consist of *Fejervarya limnocharis*, *Limnonectes kuhlii*, *Meristogenys phaemoerus*, *Staurois natator* (now as *Staurois guttatus*), and *Odorrana hosii*. Sequences obtained were deposited in GenBank (accession numbers: DQ835318-DQ835354, DQ810283-810288, DQ861304-DQ861315). We followed the procedures of Matsui et al. (2005) and Zainudin et al. (2010) to align the sequences, obtain comparative data to reconstruct the phylogenetic tree, and assign a degree of confidence to the trees.

## 3. Results

### 3.1. Molecular phylogeny of the Sarawak frogs previously assigned as genus *Hylarana*

Phylogenetic analyses of the Sarawak frogs produced essentially the same tree topologies of maximum parsimony, neighbor-joining, Bayesian inferences (Figure 1), and maximum likelihood. The trees revealed paraphyletic relationships of the previously assigned *Hylarana* with respect to the outgroups *Limnonectes kuhlii* and *Fejervarya limnocharis* with high bootstrap supports (93% [MP], 69% [NJ], 92% [ML], and 99% [Bayesian PP]).

The following relationships were indicated by the 4 analyses with bootstrap P values and Bayesian posterior probabilities mentioned above as statistically reliable: i) the generalist and commensal species groups, comprising generalist frogs of *Chalcorana raniceps* (100% [MP], 99% [NJ], 100% [BPP], and 100% [ML]), and commensal species of frogs consisting of *Hylarana erythraea* and *Amnirana nicobariensis* (only supported by 99% of BPP); ii) the forest species, although not supported as shown by low values (70% [NJ] and 75% [BPP]), significantly comprise the inland hill species (98% [MP], 99% [NJ], 100% [BPP], and 84% [ML]), i.e. *Pulcharana picturata* and *P. signata*, and the species confined to coastal lowlands and swamps (93% [MP], 99% [NJ], 99% [BPP], and 86% [ML]), consisting of *P. baramica*, *P. glandulosa*, and *P. laterimaculata*.

In addition, phylogenetic trees also implied that *Pulcharana signata* is sister to *P. picturata* (98%, 99%,

100%, and 84% respectively), while *P. baramica* is sister to *P. laterimaculata* (supported only by MP [99%] and NJ [99%]). Both *P. baramica* and *P. laterimaculata* are also sisters to *P. glandulosa* (93%, 99%, 99%, and 86%, respectively). They also shared the last common ancestor and fall under the genus *Pulcharana*. The results also showed that *Amnirana nicobariensis* falls into the *Hylarana erythraea* group, which are species that are associated with humans.

*Chalcorana raniceps* showed intraspecific variation (high support of statistical analyses indicated by asterisks), in which 3 monophyletic groups were apparent. The first group consists of the fast-flowing stream dwellers from the Borneo Height/Matang populations. The second comprised the lowland/limestone dwellers of the Mulu/Sabah populations, and the last group includes the riverine swamp dwellers of the Bako and Similajau populations. The pattern of intraspecific variation by *C. raniceps* was congruent with biogeographical divisions of plants of Borneo, so that the Borneo Height and Matang populations were in the biogeographical division 'h', whereas the Mulu and western Sabah populations were in the biogeographical division 'e' (Figure 1). This finding also revealed that the Sarawak and Peninsular Malaysia populations of *C. raniceps* might consist of more than 1 lineage (cryptic species).

In conclusion, 16S rRNA of mitochondrial gene revealed 5 monophyletic groups (closely related groups) within Sarawak frog species consisting of: 1) *Hylarana erythraea* group [*H. erythraea* + *A. nicobariensis*], 2) *Chalcorana raniceps* (*Rana* [*Chalcorana*] *raniceps*), 3) *Abavorana luctuosa*, 4) *Pulcharana signata* group (*P. signata* + *P. picturata*), and 5) *Pulcharana baramica* group [*P. baramica* + *P. glandulosa* + *P. laterimaculata*].

### 3.2. Microhabitat utilization and partitioning by Bornean *Hylarana*

The results showed that vegetation used by the frogs differed significantly for all habitat types (Table 1) except for mixed dipterocarp forest (MDF) edge ( $\chi^2 = 2.333$ , df = 5, P = 0.9) and secondary forest ( $\chi^2 = 1.111$ , df = 6, P = 1.0), indicating that the Sarawak frogs occupied several types of vegetation and differ in terms of the type of habitat used, but are very common in MDF edge and secondary forest. Most species showed significant variation in terms of abundance in vegetation types, indicating species' preferences for specific habitat types. For example, some species are confined to specific vegetation; e.g., *P. baramica* ( $\chi^2 = 4.4$ , df = 3, P = 0.26) and *P. glandulosa* ( $\chi^2 = 9.8$ , df = 2, P = 0.01) occurred mainly in coastal lowland and swamp areas with riverine forest. *C. raniceps* is not significant in terms of differences in abundance among habitat types ( $\chi^2 = 5.4$ , df = 6, P = 0.58), and can be found in several types of vegetation. *C. raniceps*, however, was not found in

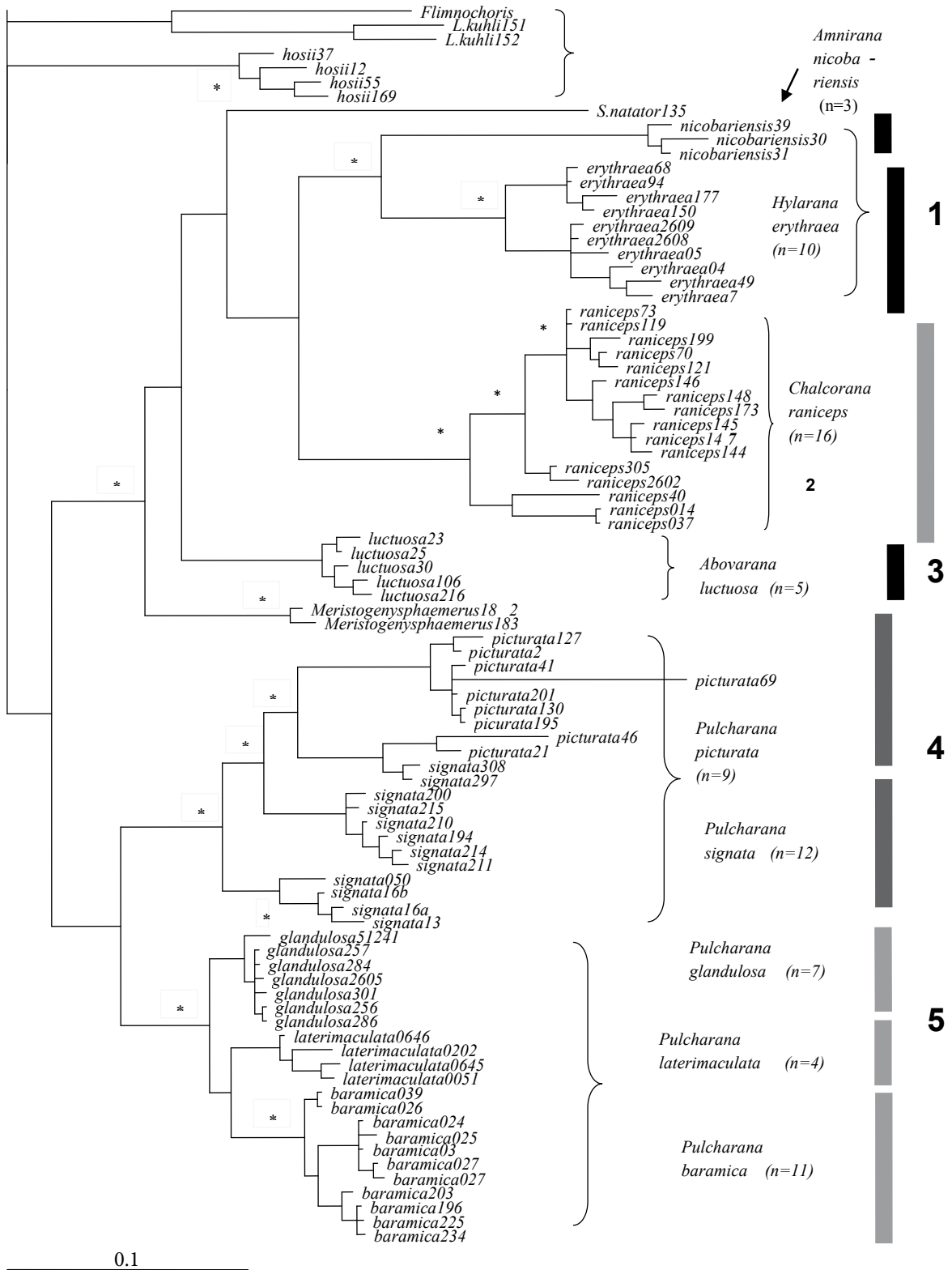


Figure 1. Bayesian inference of 16S mtDNA Sarawak frogs (\* represents 95% BPP).

**Table 1.** Vegetation occupied by Sarawak frogs.

	VA	VB	VW	VK	VRF	Vagr	VE	VG	VJ	VR	X <sup>2</sup>	Total Ind.
<i>P. baramica</i>	0	2	4	0	52	0	0	2	0	2	0.26*	62
<i>C. raniceps</i>	50	8	0	0	28	0	19	4	1	0	0.58*	110
<i>H. erythraea</i>	1	0	0	0	0	17	7	1	0	20	0.21*	46
<i>P. glandulosa</i>	0	0	0	0	9	0	1	0	0	0	0.01**	10
<i>O. hosii</i>	0	0	0	0	0	0	3	5	13	0	0.02**	21
<i>A. luctuosa</i>	16	0	0	0	0	0	0	0	0	0	0.02**	16
<i>A. nicobariensis</i>	0	0	0	0	0	5	0	1	0	0	0.01**	6
<i>P. picturata</i>	19	2	0	0	0	0	3	3	0	0	0.11*	27
<i>P. signata</i>	0	6	0	2	2	0	10	6	0	0	0.43*	26
X <sup>2</sup> P-value	0.15*	0.23*	0.04**	0.04**	0.15*	0.03**	0.91NS	1.0NS	0.03**	0.03**		
Total Inds.	86	18	4	2	91	22	43	22	14	22		324

X<sup>2</sup>\*exact significance of P value at P < 0.5; \*\*exact significance at P < 0.05; NS not significant; VA: primary rain forest, hilly; VB: primary rain forest, flat; VW: peat swamp; VRF: riverine forest; Vagr: agriculture; VE: edge mixed-dipterocarp forest (MDF); VG: secondary growth, immature, or regenerating forest; VJ: selectively logged forest; VR: oil palm plantation.

paddy fields (Bario) or oil palm plantations (Sadong). Yet the species was found in an *Acacia* plantation at Bintulu as well as populated areas.

This may be due to the type of streams (sand and gravel) occurring in the plantation or populated areas compared to the presence of only terrain and drainage in paddy fields and oil palm plantations, which are not suitable habitats for breeding sites of *C. raniceps*.

In horizontal positions, the abundance of the Sarawak frogs was significantly different among species (Table 2) except for the banks of permanent streams (HPC,  $\chi^2 = 2.667$ , df = 6, P = 0.95), which seems to be the most common horizontal position that the frogs used. The results indicate that the species occupy various horizontal

positions and differ significantly in terms of horizontal positions used, but are collectively very common on the banks of permanent streams. In addition, a species that is commensal with man, *H. erythraea*, uses the banks of plantation drainage as its horizontal position. On the other hand, 3 species (7.1% of total individuals), namely *P. baramica*, *P. glandulosa*, and *P. signata*, positioned themselves away from any body of water, mostly in riverine and kerangas forests lacking streams or ponds. Hence, more than 45% of the total frog individuals caught were occupying permanent streams, on banks, in midstream, or on overhanging vegetation.

The abundances of Sarawak frogs at their vertical positions were significantly different among species (Table

**Table 2.** Horizontal positions of Sarawak frogs.

	HPB	HPC	HPD	HPF	HPG	HPK	HPL	HPN	HPP	HPR	HPT	HPU	X <sup>2</sup>	Total Ind.
<i>P. baramica</i>	0	2	1	0	18	0	0	0	36	2	1	2	0.38*	62
<i>C. raniceps</i>	13	9	0	1	0	0	1	4	15	22	45	0	0.7NS	110
<i>H. erythraea</i>	0	0	0	0	0	18	0	3	0	2	3	20	0.02**	46
<i>P. glandulosa</i>	0	4	0	0	3	0	0	1	2	0	0	0	0.003**	10
<i>O. hosii</i>	13	8	0	0	0	0	0	0	0	0	0	0	0.002**	21
<i>A. luctuosa</i>	0	1	0	1	0	0	0	3	0	11	0	0	0.012**	16
<i>A. nicobariensis</i>	0	1	0	0	0	0	5	0	0	0	0	0	0.002**	6
<i>P. picturata</i>	25	1	0	0	0	0	0	0	0	0	1	0	0.012**	27
<i>P. signata</i>	5	15	0	0	2	0	0	0	0	0	4	0	0.003**	26
X <sup>2</sup> P-value	0.23*	0.95NS	0.04**	0.18*	0.05**	0.04**	0.03**	0.23*	0.05**	0.23*	0.58*	0.03**		
Total individuals	56	41	1	2	23	18	6	11	53	37	54	22		324

X<sup>2</sup>\*exact significance of P value at P < 0.5; \*\*exact significance at P < 0.05; NS not significant; HPB: permanent stream; midstream on bar, rock, or snag; HPC: permanent stream: on bank; HPD: Intermittent stream: in stream, actually in water; HPF: Intermittent stream, on bank; HPG: Distant from any body of water; HPK: Temporary pond, on bank; HPL: temporary pond, on vegetation; HPN: permanent pond; HPP: permanent swamp; HPR: permanent pond, on bank; HPT: permanent stream, on vegetation; HPU: permanent drainage, in plantation, on bank.

3), except for the surface of bare soil (VPF,  $\chi^2 = 2.333$ ,  $df = 5$ ,  $P = 0.91$ ) and on rock (VPH,  $\chi^2 = 2.667$ ,  $df = 6$ ,  $P = 0.98$ ), indicating that the species occupy various vertical positions and differ in terms of vertical positions used, but are very common on surfaces of bare soil and on rock.

Among the species, the Sarawak frogs showed significant variation in terms of abundance among vertical positions except for *Chalcorana raniceps* ( $\chi^2 = 4.8$ ,  $df = 8$ ,  $P = 0.86$ ), which seems to utilize almost all vertical positions. The results indicate that *C. raniceps* has broader vertical positions, with the majority (42% of total individuals) perching on seedlings and herbaceous plants.

*P. baramica* ( $\chi^2 = 7.0$ ,  $df = 5$ ,  $P = 0.26$ ) also has broader positions, mainly on shrubs (27% of total individuals). In contrast, its close relative, *P. glandulosa* ( $\chi^2 = 14.6$ ,  $df = 3$ ,  $P = 0.002$ ), mostly occupies the surface of bare soil (50% of total individuals), indicating specific preferences for vertical positions. It is noted that both *P. baramica* and

*P. glandulosa* seem to occupy similar vertical positions, but *P. baramica* was mostly abundant on shrubs, whereas *P. glandulosa* was abundant on the surface of bare soil. Partitioning of vertical positions, however, was clearly seen in the 2 phylogenetically closely related species of *P. signata* and *P. picturata*. *P. signata* ( $\chi^2 = 20.67$ ,  $df = 4$ ,  $P = 0.001$ ) occupied 5 positions vertically, with 65% of all individuals on rock (Table 3). *P. picturata* ( $\chi^2 = 19.2$ ,  $df = 2$ ,  $P = 0.000$ ), on the other hand, occupied only 2 vertical positions, being highly localized on rock (93% of all individuals), with the remaining individuals (7%) on the surface of leaf litter.

The results also indicate significant variation in substrates used (Table 4) except for bank mud (SG,  $\chi^2 = 2.33$ ,  $df = 5$ ,  $P = 0.9$ ) and bank rock (SJ,  $\chi^2 = 2.67$ ,  $df = 6$ ,  $P = 0.95$ ), thus implying that the species utilize various substrates but are common on bank mud (27%) and bank rock (25%).

**Table 3.** Vertical positions of Sarawak frogs.

	VPA	VPB	VPD	VPE	VPF	VPG	VPH	VPJ	VPK
<i>P. baramica</i>	0	0	11	3	5	4	5	0	3
<i>C. raniceps</i>	2	0	1	2	3	2	13	1	47
<i>H. erythraea</i>	0	0	0	0	20	0	0	0	0
<i>P. glandulosa</i>	0	0	2	1	5	0	0	0	0
<i>O. hosii</i>	0	0	0	0	1	0	19	0	1
<i>A. luctuosa</i>	0	1	3	1	2	8	1	0	0
<i>A. nicobariensis</i>	0	0	0	0	0	0	0	0	0
<i>P. picturata</i>	0	0	0	0	0	2	25	0	0
<i>P. signata</i>	0	0	0	1	5	0	17	0	1
X <sup>2</sup> P-value	0.04**	0.04**	0.15*	0.48*	0.91NS	0.23*	0.98NS	0.04**	0.23*
Total Ind.	2	1	17	8	39	16	80	1	52

**Table 3.** (Continued).

	VPL	VPM	VPN	VPO	VPQ	VPP	X <sup>2</sup>	Total Ind.
<i>P. baramica</i>	17	11	0	3	0	0	0.26*	62
<i>C. raniceps</i>	22	5	1	0	11	0	0.86NS	110
<i>H. erythraea</i>	3	2	0	0	18	3	0.001**	46
<i>P. glandulosa</i>	1	1	0	0	0	0	0.002**	10
<i>O. hosii</i>	0	0	0	0	0	0	0.001**	21
<i>A. luctuosa</i>	0	0	0	0	0	0	0.003**	16
<i>A. nicobariensis</i>	0	1	0	0	5	0	0.000**	6
<i>P. picturata</i>	0	0	0	0	0	0	0.000**	27
<i>P. signata</i>	2	0	0	0	0	0	0.001**	26
X <sup>2</sup> P-value	0.48*	0.58*	0.04**	0.04**	0.05**	0.04**		
Total Individuals	45	20	1	3	34	3		324

X<sup>2</sup> \*exact significance of P value at P < 0.5; \*\*exact significance at P < 0.05; NS not significant; VPA: Under surface of soil; VPB: In or under dead leaves; VPD: Under log; VPE: In log; VPF: On surface of bare soil; VPG: On surface of leaf litter or dead leaves; VPH: On rock; VPJ: On log; VPK: On seedling or herbaceous plant; VPL: On shrub or young sapling; VPN: On dead stump above ground; VPO: In crown of fallen dead shrub; VPQ: On grass blade; VPP: In grass.

**Table 4.** Substrates of Sarawak frogs.

	SA	SB	SC	SD	SF	SG	SH	SJ	X <sup>2</sup>	Total Ind.
<i>P. baramica</i>	4	3	16	11	14	8	0	6	1.00	62
<i>H. raniceps</i>	37	22	14	6	5	12	0	14	0.98	110
<i>H. erythraea</i>	1	2	1	1	0	41	0	0	0.85	46
<i>P. glandulosa</i>	0	0	1	1	3	5	0	0	0.85	10
<i>O. hosii</i>	1	0	0	0	0	0	1	19	0.14*	21
<i>A. luctuosa</i>	1	0	0	0	4	10	0	1	0.85	16
<i>A. nicobariensis</i>	0	0	0	1	0	5	0	0	0.14*	6
<i>P. picturata</i>	0	0	0	0	0	0	2	25	0.13*	27
<i>P. signata</i>	0	1	2	0	1	5	0	17	1.00	26
X <sup>2</sup> P-value	0.48*	0.15*	0.58*	0.48*	0.48*	0.90	NA@	0.95		
Total individuals	44	28	34	20	27	86	3	82		324

X<sup>2</sup>\*exact significance of P value at P < 0.5; \*\*exact significance at P < 0.05; NS not significant; @ not included for  $\chi^2$  analysis due to small sample size, N < 5; SA: Leaf of plant; SB: Stem or branch of herbaceous plant; SC: Twig or branch of woody plant; SD: Trunk of shrub or tree; SF: Under bark of log, stump, or tree; SG: Bank mud; SH: Bank sand or gravel; SJ: Bank rock.

For closely related species, *P. baramica* mostly used twigs of branches (26% of total individuals) and under the bark of logs (23%) as their preferred substrates, while the sister species, *P. glandulosa* ( $\chi^2 = 1.6$ , df = 3, P = 0.85), only occupies 4 types of substrates, with 50% of total individuals being found on bank mud (Table 4).

This suggests that both species have strong partitions for their substrate utilization, where *P. baramica* was semiarborescent while *P. glandulosa* seems to be a ground dweller (83% of total individuals). In another pair of closely related species, *P. signata* has broader substrate utilization (5 types) than sibling species *P. picturata* (2 types of substrates).

Sixty-five percent of total individuals of *P. signata* were found on bank rock, while the remaining individuals were found in other substrates. In contrast, the sister species *P. picturata* was found mainly on bank rocks (93% of total

individuals) (Table 4). Although both species preferred bank rocks as their substrate, *P. signata* seems to modify its ecological requirements by occupying more types of substrates than *P. picturata*, so that both can occur in sympatry. Both species were observed to be sympatric at the same stream at Matang National Park, where *P. signata* occupied leaves of plants of overhanging vegetation, whereas *P. picturata* was mostly caught on bank rocks.

### 3.3. Similarity of microhabitat utilization

The results showed that the Sarawak frogs have moderate sharing of microhabitats as shown by a moderate percentage of similarity of modified Morisita, ranging from 1.1% (*P. glandulosa* versus *P. picturata*) to 76.6% between *P. baramica* versus *P. glandulosa* (Table 5). Most species in this study seem to share specific microhabitats. For example, *P. baramica* and *P. glandulosa* live in riverine forest, on trees, or bare soil; *H. erythraea* and *A. nicobariensis* shared

**Table 5.** Pairwise comparison of similarity index, modified Morisita (below diagonal) of microhabitat utilization among the Sarawak frogs.

Species	<i>P. b</i>	<i>C. rani</i>	<i>H. eryth</i>	<i>P. glan</i>	<i>O. hosii</i>	<i>A. luc</i>	<i>A. nico</i>	<i>P. picturata</i>	<i>P. signata</i>
<i>P. baramica</i> ( <i>Pb</i> )	-	-	-	-	-	-	-	-	-
<i>C. raniceps</i> ( <i>Crani</i> )	43.0	-	-	-	-	-	-	-	-
<i>H. erythraea</i> ( <i>Heryth</i> )	11.5	15.3	-	-	-	-	-	-	-
<i>P. glandulosa</i> ( <i>Pglan</i> )	76.6	33.1	38.3	-	-	-	-	-	-
<i>O. hosii</i>	7.7	17.8	2.0	8.6	-	-	-	-	-
<i>A. luctuosa</i> ( <i>A. luc</i> )	12.1	41.1	32.1	37.9	31.1	-	-	-	-
<i>A. nicobariensis</i> ( <i>A. nico</i> )	7.4	10.8	58.8	22.0	3.7	24.5	-	-	-
<i>P. picturata</i>	6.9	34.6	1.4	1.1	53.5	33.0	0.8	-	-
<i>P. signata</i>	19.7	33.2	20.9	34.6	49.3	31.9	14.5	63.4	-

agricultural and populated areas; *O. hosii*, *P. signata*, and *P. picturata* shared rocks as their substrates.

Only *C. raniceps* lives in various microhabitats such as forest edge, swamp, kerangas, on the ground, or perching on vegetation. None of the frogs in the study shared 100% similarity of microhabitats, suggesting that there would be no exclusion of species if resources (microhabitats) become scarce, thus allowing coexistence of the species in certain habitats and microhabitats.

The dendrogram of the modified Morisita index (Figure 2) produced 2 main clusters: clade A, species associated with man (*H. erythraea* and *A. nicobariensis*); and clade B, forested frogs. The forested species group was further divided into 3 subclusters (Figure 2): subclade E, the swamp frogs (*P. baramica* and *P. glandulosa*); subclade D, the ground-dwelling frogs (*A. luctuosa* and *C. raniceps*); and subclade C, the rocky riparian frogs (*O. hosii*, *P. signata*, and *P. picturata*).

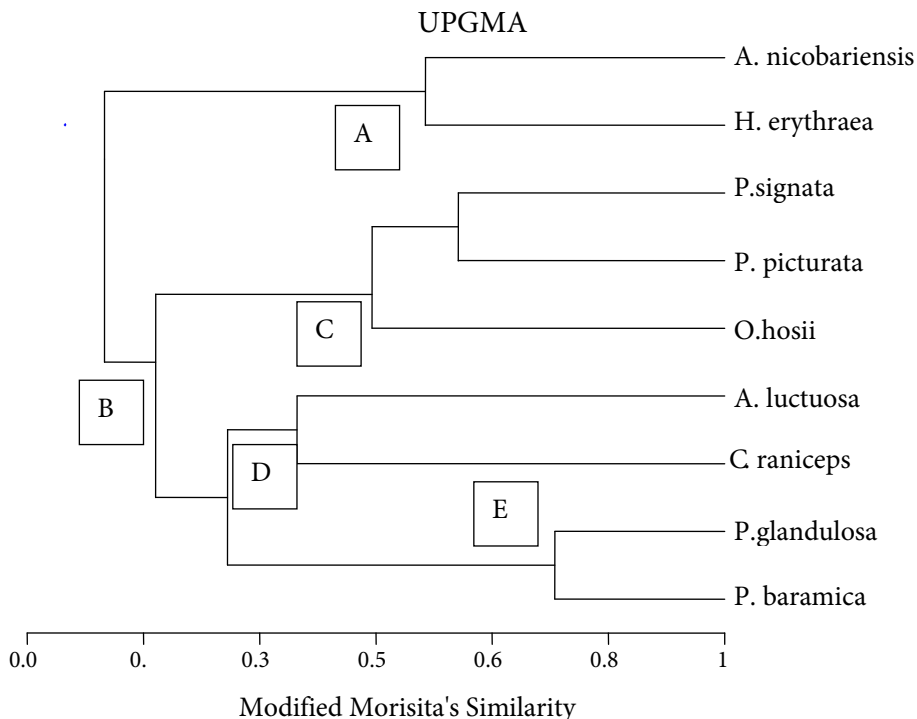
**3.4. Microhabitat preferences of the Sarawak frogs**

Among the meaningful variables, 6 groups were apparent in the 2 dimensions, with 5 positive, namely hill primary forest (VA), riverine forest (VRF), vegetation on permanent stream (HPT), seedlings or herbaceous plants (VPK), on leaves of plants (SA); and 1 negative loading (bank mud, SG) along dimension 1 (Figure 3a), while 4 negative—hill primary forest (VA), midstream at permanent stream

(HPB), on rock (VPH), rock (SJ)—and 2 positive (riverine forest [VRF] and permanent swamp [HPP]) loaded along dimension 2 (Figure 3b). The dominant characteristic along FCD 1 (dimension 1) is hilly primary rain forest, while high positive loading on the dimension 2 axis indicates riverine-forest-dominated habitat.

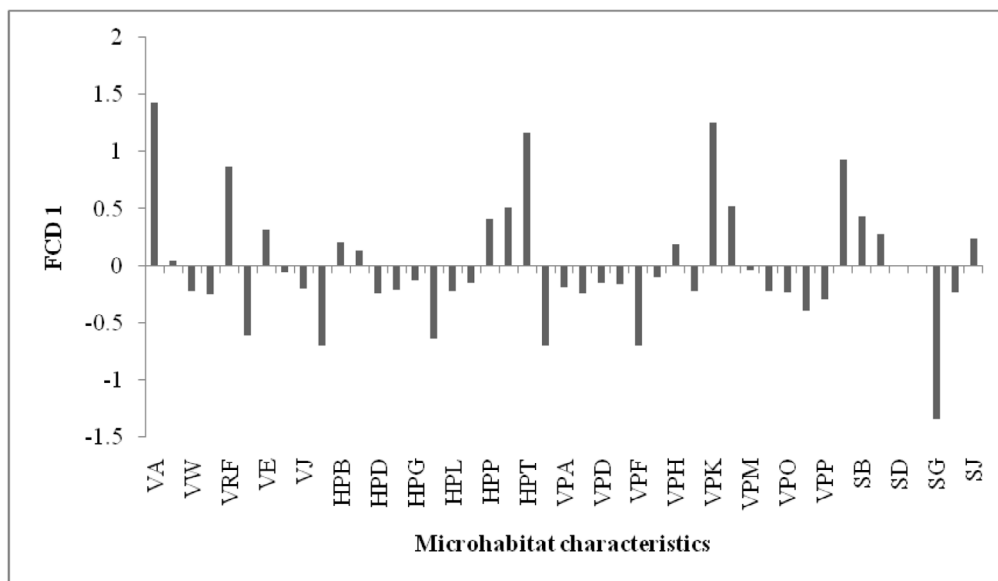
The results also showed that the 2 dimensions of the final coordinates have different domination in habitat and microhabitat usage, where habitat (vegetation) types (VA, VRF) were the major component that differentiated habitat and microhabitat utilization among species of Sarawak frogs.

Nevertheless, the results also indicate that most individuals of the Sarawak *Hylarana* and *Odorrana hosii* are dwellers of riverine forest (+ VRF) and vegetation of permanent streams (+ HPT), and are semi-arboreal as they used stems, branches, or leaves of plants (+ SA), saplings, and herbaceous plants (+ VPK) at their vertical positions and substrates at the time of capture. However, individuals of Sarawak frogs dislike utilizing bank mud (-SG), midstream or bars of rock in permanent streams (- HPB), and rock either as vertical positions (- VPH) or substrates (- SJ) at the time of capture (Figure 4). Estimation of niche overlap (Tables 6 and 7) among species revealed that some phylogenetically close species like *P. baramica* and *P. glandulosa* may have high

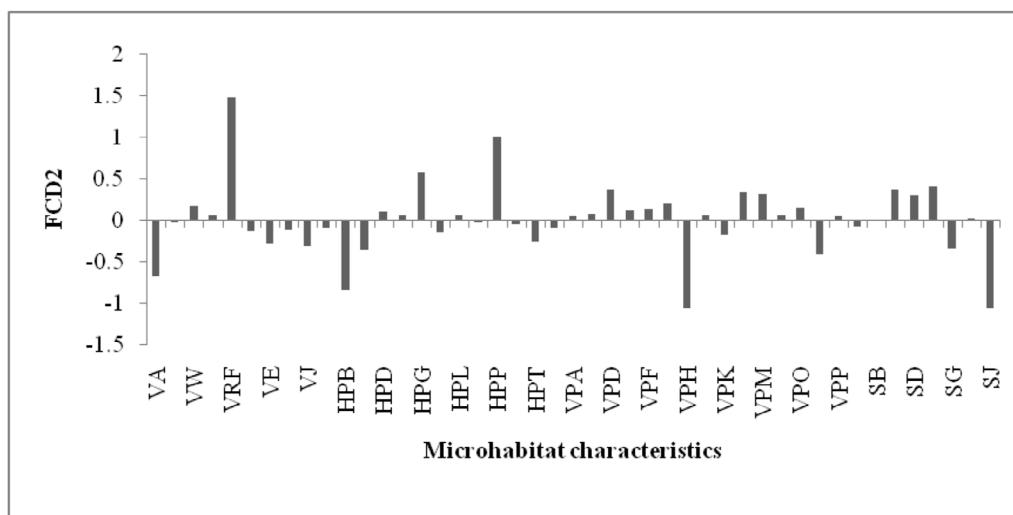


**Figure 2.** Dendrogram of Morisita's similarity resulting from average linkage clustering using the unweighted pair-group (UPGMA) method on data based on counts of individuals of frogs' species associated with habitats and microhabitats.





(A)

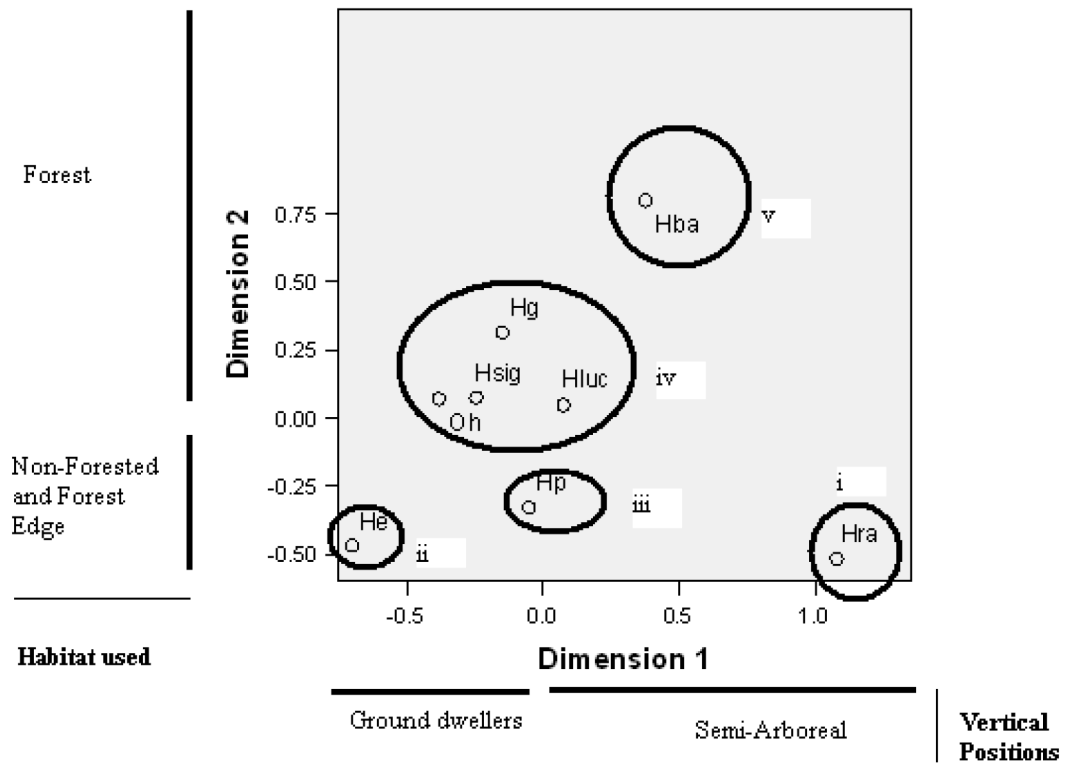


(B)

**Figure 3.** Final coordinate dimension (FDC) 1(A) and 2(B) of NMDS (PROXSCAL) of microhabitat characteristics of Sarawak frogs.

overlapping niches for habitat (Horn's index = 0.87, Table 6), and microhabitat used, i.e. substrates (Horn's index = 0.77, Table 7), horizontal position (Horn's index = 0.70, Table 6), and vertical positions (Horn's index = 0.70, Table 7), indicating that both species tend to utilize similar microhabitats. This might explain why we have a low abundance of *P. glandulosa* but a high abundance of *P. baramica*, as seen in Sedilo populations (Zainudin, 2002). Other sibling species such as *P. picturata* and *P. signata* also

have high overlapping niches for vertical positions (Horn's index = 0.77, Table 7) and substrates used (Horn's index = 0.77, Table 7), but overlap least on habitat (Horn's index = 0.47, Table 6) and horizontal positions (Horn's index = 0.54, Table 6), indicating that habitat and microhabitats are partitioned between these 2 sibling species. This might explain why both species were found to be sympatric, as observed in the Matang Ranges.



**Figure 4.** NMDS configuration showing ecological groupings from microhabitat characteristics of Sarawak frogs. Each point represents a species: Hba = *Pulchrana baramica* (N = 62 individuals), Hg = *Pulcharana glandulosa* (N = 10 individuals), Hsig = *Pulcharana signata* (N = 26 individuals), Hp = *Pulcharana picturata* (N = 27 individuals), Hra = *Chalcorana raniceps* (N = 112 individuals), He = *Hylarana erythraea* (N = 46 individuals), and Oh = *Odorrana hosii* (N = 21 individuals).

**Table 6.** Horn's index of niche overlap estimates of Sarawak frogs for habitat (below diagonal) and horizontal positions (above diagonal).

	<i>H. baramica</i>	<i>C. raniceps</i>	<i>H. erythraea</i>	<i>P. glandulosa</i>	<i>O. hosii</i>	<i>A. luctuosa</i>	<i>A. nicobariensis</i>	<i>P. picturata</i>	<i>P. signata</i>
<i>P. baramica</i>	1.00	0.42	0.15	0.70	0.08	0.14	0.06	0.06	0.27
<i>C. raniceps</i>	0.51	1.00	0.23	0.38	0.40	0.50	0.15	0.42	0.57
<i>H. erythraea</i>	0.11	0.25	1.00	0.08	0.00	0.22	0.00	0.05	0.09
<i>P. glandulosa</i>	0.87	0.57	0.12	1.00	0.39	0.27	0.25	0.09	0.61
<i>O. hosii</i>	0.07	0.27	0.20	0.12	1.00	0.13	0.24	0.84	0.79
<i>A. luctuosa</i>	0.00	0.67	0.07	0.00	0.00	1.00	0.24	0.05	0.15
<i>A. nicobariensis</i>	0.06	0.07	0.58	0.00	0.19	0.00	1.00	0.07	0.29
<i>P. picturata</i>	0.10	0.83	0.24	0.11	0.28	0.83	0.14	1.00	0.54
<i>P. signata</i>	0.33	0.57	0.28	0.37	0.46	0.00	0.19	0.47	1.00

In contrast, no real pattern was observed in habitat and microhabitat utilization among distantly related species; for example, between *Odorrana hosii* and the *Pulcharana*, niche overlap was highest between *O. hosii* and *P. picturata* or *P. signata* as they were rock-dwelling species, but no niche overlap occurred between ground- and rock-dwelling species such as *P. picturata* and *P. glandulosa* (Horn's index = 0.00, Table 7), as we never

found both species at 1 study location. Thus, regardless of phylogenetic relationships, the Sarawak frogs somehow partitioned their habitat and microhabitat utilizations in order to be syntopic and sympatric.

### 3.5. Ecological guilds

Ecological groups among 8 species of the Sarawak frogs defined by non-metric multidimensional scaling (NMDS) of habitat and microhabitat utilization for the entire

**Table 7.** Horn's index of niche overlap estimates of Sarawak frogs for vertical position (below diagonal) and substrates (above diagonal).

	<i>H. baramica</i>	<i>C. raniceps</i>	<i>H. erythraea</i>	<i>P. glandulosa</i>	<i>O. hosii</i>	<i>A. luctuosa</i>	<i>A. nicobariensis</i>	<i>P. picturata</i>	<i>P. signata</i>
<i>P. baramica</i>	1.00	0.80	0.46	0.77	0.29	0.63	0.44	0.23	0.62
<i>C. raniceps</i>	0.65	1.00	0.47	0.49	0.68	0.53	0.33	0.28	0.61
<i>H. erythraea</i>	0.36	0.40	1.00	0.74	0.03	0.78	0.91	0.00	0.44
<i>P. glandulosa</i>	0.71	0.34	0.61	1.00	0.00	0.83	0.77	0.00	0.47
<i>O. hosii</i>	0.31	0.42	0.11	0.12	1.00	0.22	0.00	0.97	0.77
<i>A. luctuosa</i>	0.55	0.25	0.21	0.49	0.24	1.00	0.72	0.17	0.56
<i>A. nicobariensis</i>	0.17	0.31	0.63	0.13	0.00	0.00	1.00	0.00	0.36
<i>P. picturata</i>	0.27	0.31	0.00	0.00	0.92	0.33	0.00	1.00	0.77
<i>P. signata</i>	0.50	0.49	0.34	0.44	0.89	0.36	0.11	0.77	1.00

assemblages are shown in Figure 4. On dimension 1, the dominant habitat and microhabitat characteristics determining high negative loadings are forest edge and agricultural or plantation regions (nonforested); high positive values indicated that the species predominantly utilizes forested regions, either riverine forest, primary forest, or secondary logged forest. On dimension 2, higher positive values indicate semiarborescent species where most individuals were found perching on vegetation, shrubs, saplings, or herbaceous trees and leaves of plants; negative values indicate ground dwellers where the species utilized rock, mud, dead leaves, or logs at the time of capture. These findings indicate that the meaningful variables of habitat and microhabitat characteristics of Sarawak frogs have grouped the species into 5 ecological guilds: i) semiarborescent species at the forest edge consisting of *C. raniceps*; ii) ground dwellers in the nonforested region consisting of *H. erythraea*; iii) ground dwellers (rock) at the forest edge consisting of *P. picturata*; iv) ground dwellers at forest and forest edge, consisting of *P. signata*, *P. glandulosa*, *A. luctuosa*, and *O. hosii*; v) semiarborescent forest (riverine) species consisting of *P. baramica*.

However, these guilds were not congruent with modified Morisita similarity classification (Figure 2), suggesting that classifying similarity using average linkage clustering with unweighted pair-groups (UPGMA) based on counts of individuals of frog species in a particular microhabitat cannot classify the Sarawak frogs into groups of organisms that have similar ecology and play similar roles in a community.

Overall, most of the Sarawak frogs show significant differences in terms of abundances among species and type of habitat and microhabitats used, with *C. raniceps* being a generalist (broader niche breadth for habitats and microhabitats used) compared to the remaining frogs in the study (Table 8). A summary of habitat and microhabitat characteristics of closely related (bold) and distinct species of Sarawak frogs is shown in Table 9.

**4. Discussion**

The reconstructed phylogeny of the previously assigned genus *Hylarana* is consistent with the newly assigned genera of *Pulcharana*, *Amnirana*, *Chalcorana*, *Hylarana*, and *Abavorana* by Frost (2016). A sister species relationship

**Table 8.** Levin's standardized niche breadth of microhabitat utilization of Sarawak frogs.

Species	Sample size (N)	Vegetation	Horizontal position	Vertical position	Substrates
<i>Pulcharana baramica</i>	62	0.10	0.23	0.65	0.75
<i>Chalcorana raniceps</i>	110	0.44	0.44	0.28	0.66
<i>Hylarana erythraea</i>	46	0.47	0.46	0.46	0.06
<i>Pulcharana glandulosa</i>	10	0.22	0.78	0.53	0.59
<i>Odorrana hosii</i>	21	0.59	0.89	0.11	0.11
<i>Abavorana luctuosa</i>	16	NA	0.31	0.44	0.39
<i>Amnirana nicobariensis</i>	6	0.38	0.38	0.39	0.38
<i>Pulcharana picturata</i>	27	0.30	0.08	0.16	0.16
<i>Pulcharana signata</i>	26	0.67	0.50	0.28	0.28



for *P. picturata* and *P. signata* was also supported by the reported morphological evidence (Inger, 1966; Malkmus et al., 2002; Inger and Stuebing, 2005), while *P. laterimaculata* with *P. baramica* as sibling species was also consistent with morphological and behavioral evidence (Leong et al., 2003). This study did not support the previous placement of *A. nicobariensis* in the genus *Ferjevarya* as in Frost et al. (2006). Recent placement of *Ferjevarya* (*Sylvirana*) *nicobariensis* into *Amnirana nicobariensis* (Chen et al., 2005; Che et al., 2007; Frost, 2008, 2016) is acceptable.

The pattern of intraspecific variation shown by *C. raniceps* was congruent with *H. erythraea* (Zainudin et al., 2010) in revealing that the landscapes of Borneo following the biogeographical divisions of Bornean plants (MacKinnon et al., 1996) might contribute to this variation. This finding was also consistent with those reported by Stuart et al. (2006) in revealing that *C. raniceps* of Sarawak and Peninsular Malaysia might consist of more than 1 species (cryptic species). This might lead to the fact that species adapt to their surroundings (habitats and microhabitats); if the subdivision is growing, this isolated population will eventually lead to speciation. This reflects the importance of microhabitats for frogs in determining their survival to the next generation.

The current study also showed that some Sarawak frogs commonly occupy edges of Dipterocarp and secondary forests. This is consistent with Vallan (2002) stating that the Raninae (including *Hylarana*, *Odorrana*, etc.) characterize degraded habitat. The occurrence of *P. baramica* and *P. glandulosa* in flat coastal lowland and swamp areas is consistent with the findings reported by Zainudin (2002) and Inger et al. (2005). However, the high percentage of Sarawak frogs occupying permanent streams was also consistent with Inger et al. (2000), who noted that more than 25% of total individuals of the species were confined to permanent streams as their horizontal position. The sharing of microhabitats by Bornean ranids was much lower than the similarity of microhabitat utilization shown by the bufonids *Phrynoidis juxtasper* versus *Ansonia longidigita* (100% of modified Morisita index), as reported by Zainudin et al. (2006). Grouping of microhabitats utilization was indeed in line with the classification of Sarawak frogs by Inger et al. (1985) into 2 groups: species associated with man and forest species.

A given species might occupy different niches in different places, so that 2 members of a guild can occupy the same niche space (Simberloff and Dayan, 1991). However, competition should ensure species within a guild do not occupy the same niche space within a single community (Simberloff and Dayan, 1991). If interspecific competition has been a strong force affecting the niches used within a community, then the members of a guild should not have heavily overlapping niches at any given

location (Simberloff and Dayan, 1991). The data of this study do not support this idea, indicating that interspecific competition was not a factor affecting the niches used by the Sarawak frogs. The results revealed high overlapping of microhabitat utilization of distantly related species belonging to the same ecological guild—for example, between *Odorrana hosii* (ecological guild = iv) and *Pulcharana signata* (ecological guild = iv).

Additionally, extensive overlapping niches were also observed in distant species from different ecological guilds as seen in the vertical positions and substrates used by *O. hosii* (ecological guild = iv) and *P. picturata* (ecological guild = iii). The same pattern was also observed in closely related species from different ecological guilds, as in *P. baramica* (ecological guild = v) and *P. glandulosa* (ecological guild = iv).

The findings are further supported by extensive niche overlap in populations of 3 riparian species of Sarawak frogs from the genus *Limnonectes* (previously assigned to genus *Rana*), i.e. *Limnonectes leporinus*, *Limnonectes ibanorum*, and *Limnonectes macrodon* at Nanga Tekalit, Sarawak (Inger and Greenberg, 1966), in which the researchers stated that *L. macrodon* is in the process of invading this part of Borneo, and maximum population levels for *L. leporinus* and *L. ibanorum* may be fixed by interspecific competition in order to maintain the coexistence of these related species.

These results indicate that closely related species from different ecological guilds, and distantly related species from the same or different ecological guilds, tend to partition their microhabitat utilization in order to coexist; this was also indicated in previous studies (Schoener, 1974; Toft, 1985; Vitt and Zani, 1996). Species that are closely related tend to partition their microhabitat utilization for them to coexist. Hence, the results imply that other factors such as availability of oviposition sites (horizontal positions) or exposure to direct sunlight (vertical positions) might affect the niches used within this community.

The phylogeny grouping, however, was not concordant with the grouping by habitat and microhabitat utilization (ecological guilds), which indicates that the evolution of habitat and microhabitat characteristics was not parallel with the molecular evolution of the 16S ribosomal mitochondrial DNA of the Sarawak frogs, thus suggesting that changes in habitat evolve quickly whereas 16S rRNA evolves slowly. Hence, the result is consistent for the European *Rana* (Van Buskirk, 2003) and the hylid frogs (Eterovick and Fernandes, 2001) on the contradiction of phylogenetic relationships and resources used.

Even with a limited set of variables, though, the patterns of microhabitat similarities are extremely useful in determining the shared characteristics of the species, and the patterns of ecologically similar groups have

previously been very useful in understanding patterns of herpetofauna diversity (Pawar et al., 2004) and bird diversity (Zakaria et al., 2009). Additionally, a study on spatial habitat partitioning among stream-breeding frog species in southeastern Sulawesi, Indonesia (Gillespie et al., 2004) found strong partitioning between species in habitat associations; partitioning of available habitat space was primarily associated with differences in proximity to stream features and height of perch sites. This scenario was also observed in the Sarawak frogs, especially at Kubah and Mulu National Parks, Sarawak. The clumped distribution of frogs along streams is probably a result of the heterogeneity of microhabitats (Kam and Chen, 2000). Knapp (2005) showed a strong association of the lentic amphibians with habitat characteristics, with elevation and water depth appearing to be important in influencing distributions. Furthermore, Nasim et al. (2007) found that the anuran populations in Markazy Province, Iran, were grouped according to their geographical and ecological conditions; this finding is concordant with the ranids of Sarawak within Malaysian Borneo.

Conclusively, the grouping of Sarawak frogs by habitat and microhabitat characteristics was not concordant with the genetic data. The genus is composed of 5 ecological groups based on their habitat and microhabitat utilizations, but this is contradicted by the observed molecular phylogeny. Closely related species of Sarawak frogs partitioned their habitat and microhabitats for coexistence.

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