# DIET OF *MICROHYLA BUTLERI* AND *M. HEYMONSI* FROM SON LA PROVINCE, NORTHWESTERN VIETNAM

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*Abstract.*—We provide the first data on the diet of Butler's Rice Frog (*Microhyla butleri*) and Heymon's Rice Frog (*M. heymonsi*) based on the stomach content analysis of 105 frog individuals (57 *M. butleri*; 48 *M. heymonsi*) from three sites in Son La Province, Vietnam. We found 11 distinct prey categories in the stomachs of *M. butleri* and nine in the stomachs of *M. heymonsi*. The diet composition of the two species was similar to each other with a trophic niche overlap of 0.97. Formicidae and Rhinotermitidae were the most important prey types for both species (Index of Relative Importance for these two taxa totaled 76% for *M. butleri* and 90% for *M. heymonsi*). The physical dimensions for the diet of *M. butleri* differed significantly from that for *M. heymonsi* by greater average food volume of each individual (36.62 ± [standard deviation] 72.98 mm<sup>3</sup> and 34.17 ± 98.92 mm<sup>3</sup>, respectively), greater average volume of each food item (4.08 ± 19.14 mm<sup>3</sup> and 2.51 ± 2.89 mm<sup>3</sup>, respectively), larger mean length of food item (2.61 ± 1.49 mm and 2.06 ± 0.88 mm, respectively), and smaller mean width of food item (1.17 ± 0.61 mm and 1.29 ± 0.51 mm, respectively). Frog mouth width was significantly positively correlated with prey length, width, and volume for *M. butleri*, and with prey length and volume for *M. heymonsi*. This is consistent with the gape limitation hypothesis that gape size limits prey size.

Key Words.—Anura; feeding ecology; food composition; frog; prey; stomach contents

### INTRODUCTION

Understanding the natural history of amphibians is essential for successful conservation and management programs (Bury 2006). Most anuran species are known to be generalist predators, consuming mainly invertebrates, with some vertebrates being ingested by large frogs (Duellman and Trueb 1994; Ngo et al. 2014). There have been many descriptive studies of microhabitat use and feeding habits of frogs in local assemblages, but there are still limited studies describing ecological differences between two or more, often closely related, species that comprise only part of a local community (Wells 2007). Food is an important niche axis for partitioning among coexisting anurans (Toft 1985) and food niche overlap has been used to hypothesize potential interactions between coexisting species (Hirai and Matsui 2001).

The genus *Microhyla* currently contains 46 species with a wide distribution in Asia from India eastwards to the Ryukyu Islands of Japan, throughout Southeast Asia, and southwards to Sumatra (Frost, D.R. 2021. Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available from http://research. amnh.org/herpetology/amphibia/index.html. [Accessed 31 December 2019]). Among those species, Butler's Rice Frog (Microhyla butleri) and Heymon's Rice Frog (*M. heymonsi*) have highly overlapping distributions; they are sympatric in Asia from northeastern India and southern China throughout Indochina and southwards to the Malay Peninsula and Sumatra (Nguyen et al. 2009; Frost op. cit.). In Vietnam, these species have been recorded from Lai Chau and Ha Giang provinces in the north to Ho Chi Minh City and Ca Mau Province in the south (Nguyen et al. 2009). There is some information on the feeding ecology of *M. butleri* and *M. heymonsi* from west Java, southwestern Taiwan, northern Peninsular Malaysia, and Singapore (Berry 1963; Erftemeijer and Boeadi 1991; Norval et al. 2014; Hui 2015). Specifically, insects in the orders Collembola, Orthoptera, Blattidae, Isoptera, Hemiptera, Hymenoptera (Formicidae),

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Coleoptera, Lepidoptera, Plecoptera, Phthiraptera, Dermaptera, and Diptera, as well as arthropods in the taxa Arachnida, Crustacea, Diplopoda, Chilopoda, and Oligochaeta have been found in the diet of M. butleri (Berry 1963; Hui 2015). In M. heymonsi, an even wider variety of prey have been consumed including Mollusca, Nematoda, Acari, Arachnida, Myriapoda, Collembola, Blattidae, Thysanoptera, Coleoptera, Hymenoptera, Isoptera, Formicidae, Lepidoptera, Neuroptera, Diptera, Dermaptera, Ephemeroptera, Hemiptera, Plecoptera, Chilopoda, Crustacea, and Diplopoda (Berry 1963; Erftemeijer and Boeadi 1991; Norval et al. 2014; Hui 2015). Although there were slight differences in prey categories between M. butleri and M. heymonsi, Formicidae represented the most important prey category for both species (see Berry 1963; Erftemeijer and Boeadi 1991; Norval et al. 2014; Hui 2015).

We present the first investigation of the dietary composition of M. butleri and M. heymonsi from Vietnam. We compared the diet of three populations of M. butleri and M. heymonsi to examine variation in the dietary composition of these two species. For anurans with small body sizes such as these species, prey size often correlates with mouth width or snout-vent length of frogs (Wells 2007). This relationship is often viewed as evidence for the gape limitation hypothesis that prey size is limited by gape size (Arim et al. 2011). Thus, we expected prey size (length, width, and volume) to correlate with body size (i.e., snout-vent length and mouth width). We present data that address the following questions: (1) What are the similarities and differences in the diets of the two species?; (2) What are the important previtems in the diets of the two species?; (3) How do the diets of the two species in Vietnam compare with the diets of these species elsewhere?; and (4) Does prey size correlate positively with frog body size, particularly mouth width, which would be consistent with the gape limitation hypothesis?

#### MATERIALS AND METHODS

Field surveys took place at three sites in Son La Province, northwestern Vietnam: (1) Son La City (21°18'46"N, 103°45'46"E, 680 m elevation); (2) Phong Lai forest, Thuan Chau District (21°36'32"N, 103°34'54"E, 720 m elevation); and (3) Copia Nature Reserve, Thuan Chau District (21°19'62"N, 103°35'09"E, 1610 m elevation; Table 1; Fig. 1). We collected frogs by hand between 2,100–2,400 elevation in meadows, small puddles, near small streams, on forest paths, and in the leaf layer at the edge of the forest (Fig. 2). We measured snout-vent length (SVL) and mouth width (MW) with a digital caliper to the nearest 0.01 mm. Taxonomic identification was based on morphological characters. We assigned individuals to *M. butleri* or *M. heymonsi* based upon characters as given by Hecht et al. (2013) and Poyarkov et al. (2014; Fig. 3).

We used the stomach-flushing method to obtain stomach contents without sacrificing frogs and we preserved the stomach contents in 70% ethanol for further analysis (Griffiths 1986; Leclerc and Courtois 1993). We used different sizes of soft catheter tubes (2- or 3-mm inner diameter) with appropriately sized syringes and different amounts of clean water (60 or 120 ml) for frogs of different SVL ( $\leq 20$  mm and >20 mm, respectively). Each frog was stomach-flushed only once following the guidelines approved by the American Society of Ichthyologists and Herpetologists for animal care (Beaupre et al. 2004). We deposited stomach samples at the Department of Biology, Faculty of Natural Science and Technology, Tay Bac University, Son La Province, Vietnam.

In the laboratory, we identified prey items under a microscope (model SZ61TR; Olympus, Tokyo, Japan) to the lowest possible taxonomic level (mostly family or order) based on literature (i.e., Thai 2003; Johnson and Triplehorn 2005). We measured maximum length (L) and maximum width (W) of each prey item to the nearest 0.01 mm using either a caliper or a calibrated ocular micrometer fitted to a microscope. In some cases, we made a best estimation of sizes for incomplete items. We considered materials such as sand, stones, and plastic parts to have been ingested accidentally and we excluded these from the analyses. The volume (V, mm<sup>3</sup>) of prey items was calculated using the formula for a prolate spheroid (Magnusson et al. 2003):

$$V = 4\pi/3 \times (L/2) \times (W/2)^2$$

**TABLE 1**. The number of individual Butler's Rice Frog (*Microhyla butleri*; n = 57) and Heymon's Rice Frog (*M. heymonsi*; n = 48) collected at each site and each survey time from Son La Province, northwestern Vietnam.

		Number of individuals		
Survey sites	Survey times	M. butleri	M. heymonsi	
Son La City	10-14 April 2015	1	2	
	15-20 May 2015	2	2	
	5-10 May 2016	2	4	
	5–9 July 2016	3	4	
	2-7 October 2016	0	2	
Phong Lai Forest	17-20 April 2016	9	2	
	11-17 June 2016	18	5	
	19-24 October 2016	6	1	
	22-28 April 2017	7	1	
Copia	24–28 April 2015	1	5	
Nature Reserve	6-11 May 2015	2	4	
	16–21 May 2016	2	8	
	17-22 July 2016	4	8	

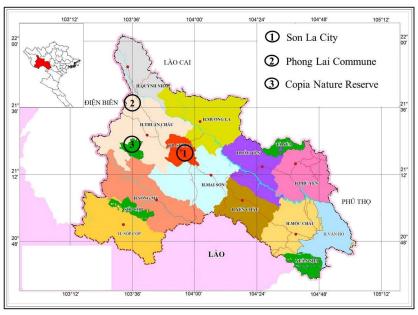


FIGURE 1. Map showing the survey sites for Butler's Rice Frog (*Microhyla butleri*) and Heymon's Rice Frog (*M. heymonsi*) in Son La Province, northwestern Vietnam.

We determined the frequency of occurrence of each prey category (F) among stomach samples and the total number of prey items (N) in the stomachs of each individual. We used the Index of Relative Importance (IRI) to determine the importance of each food category. This index provides a more informed estimation of food item consumption than any of the three components alone by using the following formula:

$$IRI = (\%F + \%N + \%V)/3$$

where %F is occurrence percentage, %N is numeric percentage, and %V is volumetric percentage (Lee and McCracken 2005; Caldart et al. 2012).

We used the reciprocal Simpson's Heterogeneity Index, 1/D, to calculate dietary heterogeneity:

$$D = \sum [n_i (n_i - 1)] / [N (N - 1)]$$

where  $n_i$  is the number of food items in the i<sup>th</sup> taxon category and N is the total number of prey items (Krebs 1999). We used Shannon's Index of Evenness to estimate prey evenness. The evenness index is calculated from the equation:

$$J' = H'/H_{max} = H'/lnS$$

where  $H_{max}$  is the maximum diversity that would occur in a situation where all prey taxa had equal abundances, where S is the total number of prey taxa. and H' is the Shannon-Weiner Index of Diversity. The value of H' is calculated from the equation:

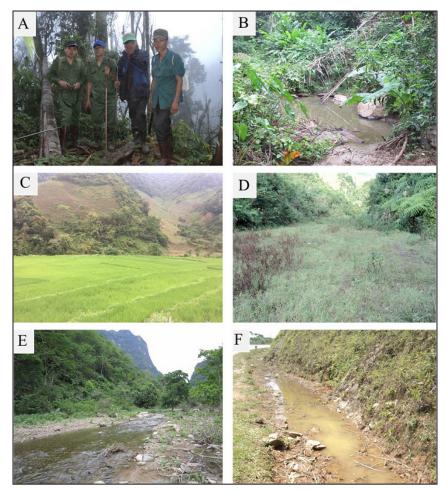
$$H' = -\Sigma(p_i \times \ln p_i)$$

where the quantity  $p_i$  is the proportion of food items belonging to the i<sup>th</sup> taxon for the total food items of the sample (Magurran 2004; Muñoz-Pedreros and Merino 2014). For the analysis of overlap in trophic niche dimensions and/or the degree of similarity between the diets of species pairs, we used Pianka's Niche Overlap Index (O<sub>jk</sub>; Pianka 1973), with values ranging from 0 (no overlap) to 1 (complete overlap; Krebs 1999). This overlap is calculated by the following expression:

$$\mathbf{O}_{jk} = \frac{\sum_{i}^{n} \mathbf{p}_{ij} \mathbf{p}_{ik}}{\sqrt{\sum_{i}^{n} \mathbf{p}_{ij}^{2} \sum_{i}^{n} \mathbf{p}_{ik}^{2}}}$$

where  $O_{jk}$  represents the index of overlap of Pianka's niche between species j and k;  $p_{ij}$  is the proportion of prey i<sup>th</sup> in the total prey categories used by species j;  $p_{ik}$  is the proportion of prey i<sup>th</sup> in total prey categories used by species k; and n is the total number of prey categories for species j and k.

We analyzed data statistically using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) software and set the significance level to  $\alpha = 0.05$  for all analyses. Data are presented as mean  $\pm$  standard deviation. We used Oneway Analysis of Variance (ANOVA) to compare the number of prey items and prey size (length, width, and volume) between *M. butleri* and *M. heymonsi*. If size of prey differed significantly between *M. butleri* and *M. heymonsi*, we used Analysis of Covariance (ANCOVA)



**FIGURE 2.** Habitat of Butler's Rice Frog (*Microhyla butleri*) and Heymon's Rice Frog (*M. heymonsi*) at sampling sites in Son La Province, northwestern Vietnam. (A) Survey team in the Copia Nature Reserve, Thuan Chau District; (B) A small puddle in Phong Lai forest, Thuan Chau District; (C) Rice field near the secondary forest in Son La City; (D) Meadows at Phong Lai forest, Thuan Chau District; (E) A stream in the secondary forest of Copia Nature Reserve, Thuan Chau District; and (F) A small puddle in the road of Chieng Xom Communute, Son La City. (Photographed by Anh V. Pham).

to test prey size (length, width, and volume) with sampled localities as a covariate. We used Linear Regression to test for relationships between mouth width (MW) and prey size, and between snout-vent length (SVL) and prey size. In the field, we could not determine the sex for each species because external morphological characters of males and females are similar. Thus, we did not analyze the diet composition according to sex, seasons, and habitats. For each analysis, when necessary to meet normality assumptions and homogeneity of variance, variables of body and prey sizes were log<sub>10</sub>-transformed prior to the analyses (Zar 2010).

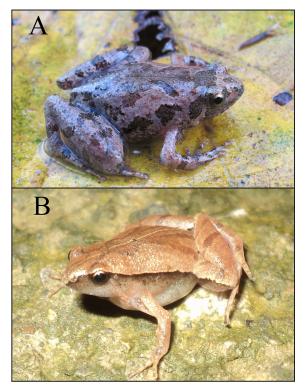
### RESULTS

We collected 105 stomachs of *M. butleri* (n = 57) and *M. heymonsi* (n = 48) from the three sites in Son La Province for stomach analyses (Table 1). *Microhyla* 

*butleri* averaged significantly larger than *M. heymonsi* (SVL;  $F_{1,103} = 8.59$ ; P = 0.004; Table 2). The largest *M. butleri* was 28.92 mm SVL and the largest *M. heymonsi* 26.51 mm SVL (Table 2). By contrast, MW did not differ significantly between the two species ( $F_{1,103} = 0.47$ ; P = 0.494). When SVL was included in analysis as a covariate, the slope of the relationship between mouth

**TABLE 2.** Mean ( $\pm$  standard deviation) and range (in parentheses) of snout-vent length (SVL; in mm) and mouth width (MW; in mm) of Butler's Rice Frog (*Microhyla butleri*; n = 57) compared to Heymon's Rice Frog (*M. heymonsi*; n = 48) from Son La Province, northwestern Vietnam using Analysis of Variance to compare traits.

Trait	M. butleri	M. heymonsi	F	Р
SVL (mm)	$22.53 \pm 3.01$ (15.61–28.92)	$21.04 \pm 2.13$ (16.32-26.51)	8.59	0.004
MW (mm)	$6.16 \pm 0.64$ (4.82–7.71)	$\begin{array}{c} 6.08 \pm 0.57 \\ (4.72  7.51) \end{array}$	0.47	0.494



**FIGURE 3.** (A) Butler's Rice Frog (*Microhyla butleri*) and (B) Heymon's Rice Frog (*M. heymonsi*) from Son La Province, northwestern Vietnam. (Photographed by Anh V. Pham).

width and SVL was significantly greater for *M. butleri* than for *M. heymonsi* ( $F_{1,102} = 121.17$ ; P < 0.001).

We found 1,074 food items, comprising 1,056 items of invertebrates and 18 unidentified items belonging to 13 prey categories, in the stomachs of the two species. Seven of the 105 stomachs (6.67%) were empty (one for M. butleri and six for M. heymonsi). Stomachs of Microhyla butleri contained 11 prey categories with 502 items and stomachs of M. heymonsi had nine categories with 572 items (Table 3). The average number of prey items per stomach in M. butleri was  $8.96 \pm 10.04$ items (range, 1–38) and in M. heymonsi was  $13.62 \pm$ 21.19 items (range, 1-87). The total number of prey items found in each stomach did not differ significantly between *M. butleri* and *M. heymonsi* ( $F_{1,96} = 2.08$ ; P =0.152). The reciprocal Simpson's Heterogeneity Index in *M. butleri* (1/D = 2.04 with an evenness index of J' = 0.38) was higher than that in *M. heymonsi* (1/D = 1.56)with an evenness index of 0.28). The trophic niche overlap index  $(O_{\mu})$  showed an overlap of 97% between the two species.

The most important prey items for *M. butleri* and *M. heymonsi* were ants (Formicidae; IRI = 43.25% for *M. butleri* and 70.16% for *M. heymonsi*) and termites (Rhinotermitidae; IRI = 35.73% for *M. butleri* and 19.48% for *M. heymonsi*; Table 3). We found three additional taxa in the diet of both species: Carabidae, Coccinellidae, and Elateridae. We found Empididae,

**TABLE 3.** Dietary composition of Butler's Rice Frog (*Microhyla butleri*; n = 57 stomach contents) and Heymon's Rice Frog (*M. heymonsi*; n = 48 stomach contents) sampled in Son La Province, northwestern Vietnam. Data for each prey category are occurrence percentage among stomachs (%F), numeric percentage among stomachs (%N), volume percentage among stomachs (%V), and the index of relative importance (IRI). The IRI = (%F + %N + %V)/3.

	M. butleri				M. heymonsi			
Prey category	%F	%N	%V	IRI	%F	%N	%V	IRI
Araneae	_	_	_	_	1.96	0.17	0.53	0.89
Coleoptera								
Carabidae	10.26	1.79	3.58	5.21	5.88	0.52	0.44	2.28
Coccinellidae	1.28	0.20	0.02	0.50	1.96	0.17	0.47	0.87
Elateridae	1.28	0.20	1.38	0.95	1.96	0.17	0.86	1.00
Lucanidae	1.28	0.20	0.31	0.60	_	_	_	—
Staphylinidae	1.28	0.20	0.43	0.64	3.92	0.52	1.09	1.85
Diptera								
Empididae	1.28	0.20	0.05	0.51	_	—	_	_
Hymenoptera								
Diapriidae	_	—	_	—	1.96	0.17	0.07	0.74
Formicidae	51.28	62.35	16.11	43.25	58.82	77.80	73.87	70.16
Isoptera								
Rhinotermitidae	17.95	31.87	57.38	35.73	17.65	19.23	21.55	19.48
Odonata	1.28	0.20	0.71	0.73	_	_	_	—
Insect larvae	3.85	0.60	0.38	1.61	_	_	—	_
Unidentified items	8.97	2.19	19.65	10.27	5.88	1.22	1.13	2.74

	<i>M. butleri</i> (n = 502)			M. heymonsi (n = 572)		
Value	L (mm)	W (mm)	V (mm <sup>3</sup> )	L (mm)	W (mm)	V (mm <sup>3</sup> )
Mean $\pm$ SD	$2.61 \pm 1.49$	$1.37\pm0.61$	$4.08 \pm 19.14$	$2.06\pm0.88$	$1.29\pm0.51$	$2.51\pm2.89$
Range	0.91-12.11	0.5-8.11	0.21-16.28	0.91-6.51	0.51-2.91	0.21-16.28

**TABLE 4.** Summary of the mean  $\pm$  standard deviation (SD) and range for food items (length [L], width [W], and volume [V]) consumed by Butler's Rice Frog (*Microhyla butleri*) and Heymon's Rice Frog (*M. heymonsi*) from Son La Province, northwestern Vietnam.

Lucanidae, Odonata, and insect larvae exclusively in the diet of *M. butleri*, whereas we found Araneae and Diapriidae only in the diet of *M. heymonsi* (Table 3).

The average total food volume of each stomach of M. *butleri*  $(36.62 \pm 72.98 \text{ mm}^3; \text{ range}, 0.5-430.77 \text{ mm}^3; \text{ n}$ = 56) was significantly larger than that of *M. heymonsi*  $(34.17 \pm 98.92 \text{ mm}^3; \text{ range}, 0.39-596.72 \text{ mm}^3; n = 42;$  $F_{1.96} = 7.41$ ; P = 0.008). The average prey length for M. butleri was significantly larger than that for M. heymonsi  $(F_{1,1072} = 56.16; P < 0.001;$  Table 4). The mean width of food items in M. butleri was significantly larger than that in *M. heymonsi* ( $F_{1,1072} = 12.31$ ; P < 0.001; Table 4). The average prey volume in *M. butleri* was significantly larger than that in M. heymonsi  $(F_{11072} = 3.78; P = 0.050;$ Table 4). When sampling localities were taken into account as a covariate, size of prey was significantly greater in *M. butleri* (n = 502) than in *M. heymonsi* (n = 572; prey length,  $F_{1,1071} = 13.57$ , P < 0.001; prey width,  $F_{1,1071} = 5.22$ ; P = 0.022; prey volume,  $F_{1,1071} = 4.75$ ; P =0.030). Mouth width (MW) was significantly positively related to prey size (length, width, and volume) in M. butleri, and to prey length and volume in M. heymonsi, but was not significantly related to prey width in M. heymonsi (Table 5). Snout-vent length was significantly positively related to prey length, width, and volume in *M. heymonsi*, and to prey width in *M. butleri*, but was not significantly related to prey length and volume in *M. butleri* (Table 5).

#### DISCUSSION

There may be minor ecological differences among co-occurring species along one or more resource axes, and such differences sometimes have been interpreted as the result of interspecific competition (Wells 2007). In Son La Province, Vietnam, both M. butleri and M. heymonsi often hide in the leaf litter during the day, but usually forage on vegetation above the forest floor at night. They live in similar habitats and ecological situations such as grasslands, shrubs in secondary forest habitats and fields, and small ponds. Such similarities in where they occur may account for the high degree of overlap in the trophic niche of the two species. Differences between the species that were significant, although slight in magnitude, were that M. buterli fed on larger volume prey that were significantly longer and narrower than those for M. heymoni.

The dominant prey items of *M. butleri* and *M. heymonsi* were Formicidae and Rhinotermitidae (assessed by IRI). These results are similar to previous

**TABLE 5.** Regression results for relationship between mouth width (MW) and prey size (length, width, volume) and between snout-vent length (SVL) and prey size for Butler's Rice Frog (*Microhyla butleri*) and Heymon's Rice Frog (*M. heymonsi*) from Son La Province, northwestern Vietnam. All variables have been log<sub>10</sub>-transformed. Significant *P*-values in bold.

Prey size	F	Р	Coefficient $(r^2)$	Regression equation
Microhyla butleri				
Mouth width (M	IW)			
Length	$F_{1.500} = 12.23$	0.001	0.024	$\log_{10}[L] = 0.71 \log_{10}[MW] - 0.194$
Width	$F_{1,500} = 52.88$	< 0.001	0.096	$\log_{10}[W] = 1.15 \log_{10}[MW] - 0.874$
Volume	$F_{1,500} = 38.47$	< 0.001	0.071	$\log_{10}[V] = 3.01 \log_{10}[MW] - 1.621$
Snout-vent lengt	th (SVL)			
Length	$F_{1,500} = 0.14$	0.707	0.001	$\log_{10}[L] = -0.08\log_{10}[SVL] + 0.469$
Width	$F_{1,500} = 7.56$	0.006	0.015	$\log_{10}[W] = 0.46\log_{10}[SVL] - 0.591$
Volume	$F_{1,500} = 2.74$	0.099	0.005	$\log_{10}[V] = 0.84 \log_{10}[SVL] - 0.391$
Microhyla heymor	nsi			
Mouth width (M	IW)			
Length	$F_{1.570} = 66.82$	< 0.001	0.105	$\log_{10}[L] = 1.23 \log_{10}[MW] - 0.661$
Width	$F_{1,570} = 2.79$	0.095	0.052	$\log_{10}[W] = 0.23 \log_{10}[MW] - 0.093$
Volume	$F_{1,570} = 17.95$	< 0.001	0.031	$\log_{10}[V] = 1.68 \log_{10}[MW] - 0.528$
Snout-vent lengt	th (SVL)			
Length	$F_{1,570} = 51.94$	< 0.001	0.084	$\log_{10}[L] = 1.25 \log_{10}[SVL] - 1.371$
Width	$F_{1,570} = 27.63$	< 0.001	0.046	$\log_{10}[W] = 0.81g_{10}[SVL] - 0.979$
Volume	$F_{1.570} = 41.26$	< 0.001	0.067	$\log_{10}[V] = 2.85 \log_{10}[SVL] - 3.011$

findings for these two species in west Java, southwestern Taiwan, northern Peninsular Malaysia, and in Singapore (Berry 1963; Erftemeijer and Boeadi 1991; Norval et al. 2014; Hui 2015). Formicidae has been reported as an important prey category in the diet of many anurans (Duellman and Trueb 1994; Wells 2007); however, for ant-specialist species such as *M. butleri* and *M. heymonsi* (i.e., IRI > 40 for both species), their diet would have little relation to the abundance of other prey in the environment (Norval et al. 2014; Hui 2015). Moreover, *M. butleri* and *M. heymonsi* have very narrow mouths (6.08–6.16 mm), a trait that is more typical of terrestrial ant-eating microhylids than of hylids, which usually have generalized diets (Wells 2007).

Body size and prey size were generally correlated for both species. Mouth width of frogs was significantly positively correlated with prey size (length, width, and volume) in *M. butleri* and with length and volume in *M. heymonsi*, and SVL was significantly positively correlated with prey length, width, and volume in *M. heymonsi* and with width in *M. butleri*. These findings are consistent with the gape limitation hypothesis that gape size limits prey size.

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