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A survey of morphological variation in adult *Meristogenys amoropalamus* (Amphibia, Anura, Ranidae), with a description of a new cryptic species

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Abstract

Previous analyses of molecular and larval morphology have suggested that *Meristogenys amoropalamus* is composed of two cryptic species, but no diagnostic characters of their adult morphology have been reported. Here, we compared adult characters of these two species and found that they differed in iris colour (yellowish-green and sandy brown), tympanum size and relative limb length. Based on the results of analysis of DNA sequences of the type specimens and a discriminant analysis using 18 morphological variables, we conclude that the lineage with green irises is the true *M. amoropalamus*, and that the lineage with sandy brown irises is a new species, *M. dyscritus* sp. nov. In northern Sabah, *M. dyscritus* is distributed in altitudes lower than those of *M. amoropalamus*, but the distributional ranges of their larvae overlap in some streams. *Meristogenys amoropalamus* has larger and lighter-coloured ova, smaller clutch sizes and a more interstitial larval life than *M. dyscritus*. These differences suggest that *M. amoropalamus* has a more cryptic life during its larval period than *M. dyscritus*.

Key words: trade-off of reproductive traits, iris color, Borneo, cryptic species, *Meristogenys*

Introduction

In many phylogenetically related species of frogs, tadpoles are more difficult to separate than adults (Inger & Stuebing, 2005), but the reverse is true in some species such as *Bufo japonicus* and *B. torrenticola* (Matsui, 1976). The Bornean endemic ranid genus *Meristogenys* represents one such unusual case (Inger & Stuebing, 2005). Larvae of this genus are specialised for a life in strong currents, with a large mouth on the underside of the snout and a large abdominal sucker covering the abdomen. In contrast to adults, which usually have few notable inter-specific differences in external morphology, these larvae possess many taxonomically useful characters, such as their labial tooth raw formula (LTRF), jaw sheath shape, and presence or absence of surface projections and dermal glands (Shimada *et al*., 2007a).

This tendency is obvious in the two cryptic forms found in *Meristogenys amoropalamus* sensu lato. Shimada *et al*. (2007a, 2008) found two larval forms (morphotypes 1 and 3-a) in *M. amoropalamus* sensu lato that were differentiated by mitochondrial (mt) and nuclear DNA (nuDNA) sequence characters. These two forms were considered to represent distinct species, but no taxonomic decision was made because they were not easily differentiated by adult morphology and no evidence existed to determine which of the two forms is the true *M. amoropalamus*.

To resolve this taxonomic problem, we searched for diagnostic morphological characters that distinguish adult specimens. We also studied sequences of DNA fragments from the type specimens of *M. amoropalamus* and compared them with the larval sequences. Based on our results, we describe a new species and discuss the distribution and reproductive traits of the new form compared to those of *M. amoropalamus*.

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Material and methods

Specimen sampling. Following Matsui's (1986) description, we used 97 adult specimens (68 males and 29 females) morphologically identified as *M. amoropalamus*. However, not all specimens strictly fit Matsui's key (Matsui, 1986). For example, even when a specimen had more highly developed toe webbing than that described by Matsui (1986), it was identified as *M. amoropalamus* based on all the other characters diagnostic for this species. We followed the procedure of Shimada *et al*. (2007a) to fix and preserve specimens and to determine sex and maturity. Specimens were collected from five localities in Sabah (Kamborangah, Liwagu, Mahua, Mesilau, and Wario) and one locality in Sarawak (Bario) (Fig. 1). We also examined three type specimens of *M. amoropalamus* collected from the type locality [Sg. (Sungai, meaning "river") Pa Riman, Gn. (Gunung, meaning "mountain") Tapai Sia, Krayan County, East Kalimantan, Indonesia; alt. 1300 m] and Liwagu (Liwagu River, 1500 m, headquarters of Kinabalu National Park) (Matsui, 1986). For the larval description, we examined specimens from Bundu Tuhan, Liwagu, Mahua, Mesilau, Poring and Wario (Fig. 1). A part of these specimens had been molecularly identified to either of the two lineages of *M. amoropalamus* sensu lato by Shimada *et al*. (2008), based on DNA sequences extracted from tissue samples collected before formalin fixation.

Molecular identification. For all samples except for the type specimens, we extracted DNA, amplified approximately 440 base pair (bp) fragments of mitochondrial 12S rRNA using the primers L1091 and Hnew (Shimada *et al*., 2011) and determined the sequences following the protocol of Shimada *et al*. (2008).

Although a variety of methods has been proposed for the extraction of DNA from formalin-fixed specimens, some of them are known to have no effects, or even a harmful effects for the quality of extracted DNA (Gilbert *et al*., 2007). We adopt the phenol-chloroform extraction after the digestion with optimal temperature and long (48 h) duration, because this method was most similar to our original protocol among the methods which Gilbert *et al*. (2007) had confirmed the effectiveness. Under the allowance of the curator of Osaka Museum of Natural History (OMNH), we cut a small piece (3 mm x 10 mm) of muscle from the abdominal wall, and ground it with a plastic pestle in 600 µl of STE buffer (0.1M NaCl; 0.05M Tris-HCl, pH 7.5; 0.001M EDTA) in a 1.5 ml tube. After the addition of 60 µl of 10% SDS and 15 µl of 10 mg/ml proteinase K solution, we kept it in an incubator at 55 °C for 36–72 hours (we continued the digestion until the visible tissue completely disappears). During the digestion, we sometimes tapped the tube calmly, and added 15 µl of proteinase K solution twice. After the digestion, we added TE saturated phenol with the same amount of the sample solution, mixed it with a electric shaker for 10 min, and centrifuged them (7500 rpm, 10 min, room temperature). The upper layer (water layer) was washed once in phenol-chloroform-isoamylalcohol (PCI) and once in chloroform-isoamylalcohol (CIA) in the same way as the phenol. After the phenol-chloroform washes, we obtained a pellet of DNA through the ordinary ethanol precipitation (addition of 45 µl of 5M NaCl solution and 900 µl of 99.5% ethoanol; 10 min incubation; centrifuge with 14,000 rpm, 20 min, and 4 °C). The pellet was dissolved in 100 µl of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). We added two drops of sterile mineral oil to prevent the solution from evaporation. To examine the quality of extracted DNA, we applied 2 µl of the solution to an agarose gel, and compare the result of electrophoresis with others. As we found that the DNA extracted from formalin fixed specimens was shorter than others (data not shown), we divided the mtDNA region into four fragments, and prepared two primers for each of them (L1091, Hnew, L1173: AACCCAAAGGACTTGACGGT, L1264: TCAGTCTGTATACCTCCGTCG, L1371: AAGAAATGGGCTACAATTTCTA, H1243: GAGGTATACAGACTGATTAGG, H1352: TAGAAATTGTAGCCCATTTCTT, and H1471: TGACGGGCGGTGTGTACGCG). The polymerase chain reaction (PCR) cycle included an initial denaturation for 1 min at 94°C, 60 cycles of denaturation for 20 s at 94°C, primer annealing for 20 s at 45°C, extension for 40 s at 72°C and a last extension for 3 min at 72°C. As the PCR buffer, we used Ampdirect (Shimadzu Co.) for unpurified DNA. To avoid contamination, we separated all reagents, tubes, and extracted samples from those of other experiments, and conducted negative-control experiments for all procedures involving the type specimens. The sequences used in this paper were deposited in GenBank (AB359966–AB360009, AB360012–AB360018, AB360020–AB360043 and AB617705–AB617726).

We compared the adult sequences with those of known sequences of the larval morphotypes 1 and 3-a (AB262538 for morphotype 1 and AB262541 and AB262542 for morphotype 3-a). The genetic distances between these two morphotypes were 4.5%–4.7% [Kimura's two-parameter (K2p) distances] (Kimura, 1980) in this region of 12S rRNA (Shimada *et al*., 2007a). The larval morphotype 3-a is known to contain two distantly separate mtDNA lineages, but we treated them as a single taxon because no morphological or nuDNA differences between

them have been found (Shimada *et al*., 2008). We called adults of larval morphotype 1 "Group A" and those of morphotype 3-a "Group B". We considered a genetic (K2p) distance less than 1.1% as intra-specific because Shimada *et al*. (2007a) reported intra-specific genetic distances of *Meristogenys* to range from 0% to 1.1% in this fragment.

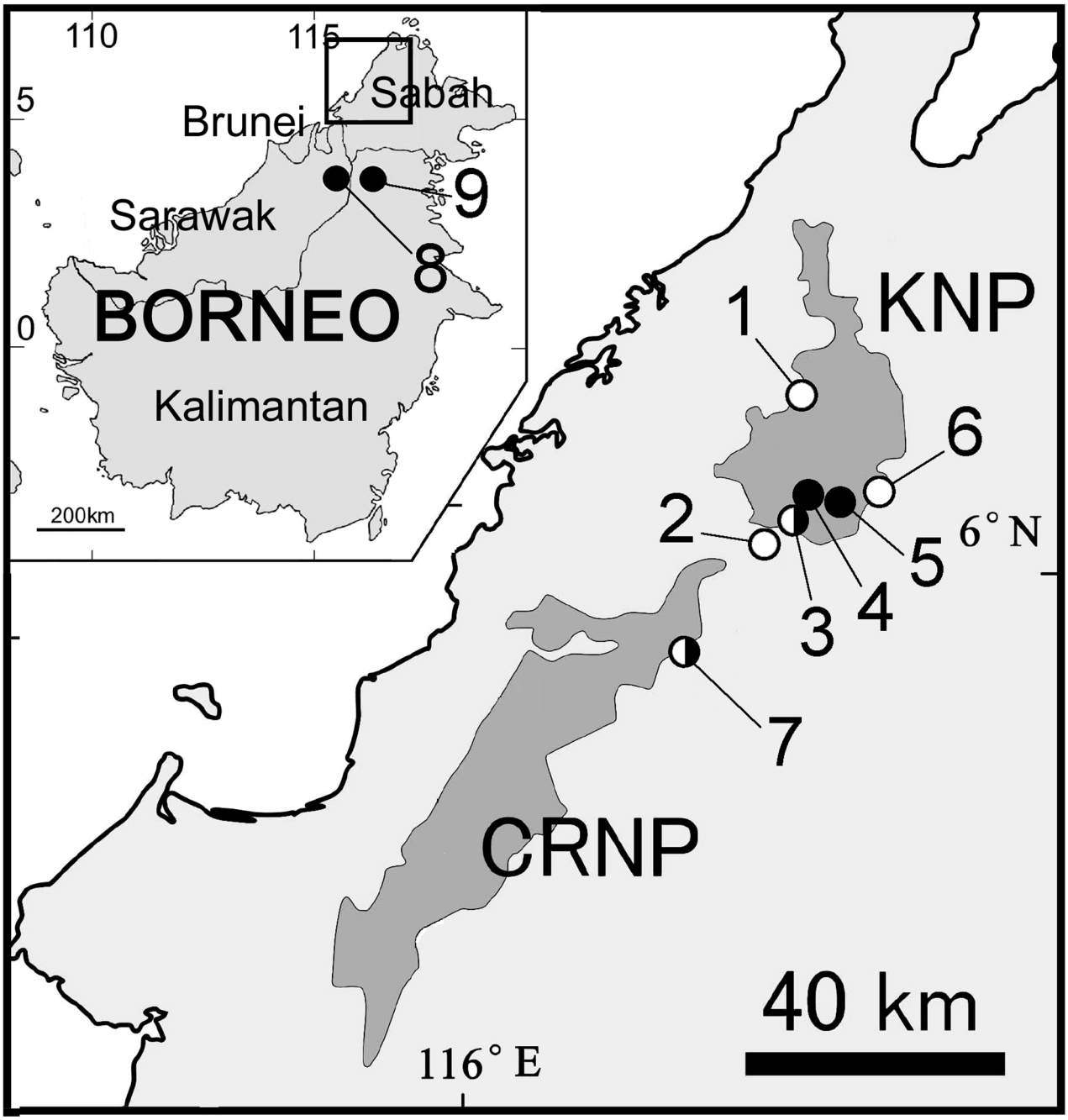

FIGURE 1. Map of Borneo showing the localities discussed in this study. 1, Wario; 2, Bundu Tuhan; 3, Liwagu; 4, Kamborangah; 5, Mesilau; 6, Poring; 7, Mahua; 8, Bario; 9, Gunung Tapai Sia. KNP and CRNP indicate Kinabalu National Park and Crocker Range National Park, respectively. Closed and open dots indicates the sampling localities of Group A (*Meristogenys amoropalamus*) and Group B (*M. dyscritus*), respectively. At half-closed dot localities, we collected larvae of both species.

Phylogenetic analysis. To elucidate the phylogenetic position of two cryptic species in *M. amoropalamus*, we reconstructed phylogenetic trees using GenBank data (AY158705, AB043889, AB526608-AB526701) of Shimada *et al*. (2011). We conducted an incongruence length difference (ILD) test (Farris *et al*., 1995) with 1000 replicates between nuDNA and mtDNA, and confirmed that there were no significant difference in phylogenetic information between them ($p>0.05$). Therefore, we combined these sequences and used approximately 9600 base pairs (bp)

including 12S rRNA (*12S*), 16S rRNA (*16S*), NADH dehydrogenase subunits 1 and 2 (ND1 and ND2), tRNAs (valin, leucine, isoleucine, glycine, methionine, and tryptophan), and cytochrome b (*cytb*) in mitochondrial DNA, and proopiomelanocortin A (POMC), recombination activating protein 1 (RAG-1), rhodopsin (RH1), solute carrier family 8 member 3 (SLC8A3), and sodium/calcium exchanger 1 (NCX1) in nuclear DNA. We subjected the data to three different methods of phylogenetic reconstruction: (1) the maximum parsimony (MP) analysis, with transitions and transversions given equal weighting; (2) the maximum likelihood (ML) analysis, based on the substitution model and phylogenetic parameters derived from a hierarchical likelihood ratio test (hLRT) in Modeltest 3.06 (Posada & Crandall, 1998); and (3) Bayesian analysis, with the model derived from an hLRT in MrModeltest (Nylander, 2002), with the run using 2,000,000 generations, sampling a tree every 100 generations, and discarding the initial 1000 trees as burn-in. We followed Matsui *et al*. (2006) for the MP and ML heuristic methods. Except for the Bayesian approach, which used MrBayes (Huelsenbeck & Ronquist, 2001), all analyses were conducted with PAUP 4.0b (Swofford, 2002). The confidential values of MP and ML trees were tested using bootstrap analyses (Felsenstein, 1985) with 1000 replicates for MP and ML (Hedges, 1992). Following Matsui *et al*. (2006), we considered bootstrap values of more than 70% and posterior probabilities of more than 95% to be statistically significant.

Morphological analysis. We took 20 measurements: snout-vent length (SVL) as a standard character, 12 characters of the head [head length (HL), snout-nostril length (S-NL), nostril-eyelid length (N-EL), snout length (SL), eye length (EL), tympanum-eye length (T-EL), vertical diameter of tympanum (TDv), horizontal diameter of tympanum (TDh), head width (HW), internarial distance (IND), interorbital distance (IOD), and upper eyelid width (UEW)]; and seven characters of the limbs [forelimb length (FLL), lower arm and hand length (LAL) from the elbow to the tip of the third finger, hand length (HAL), hind limb length (HLL), thigh length (THIGH), tibia length (TL), and foot length (FL)]. Dial callipers were used to make measurements to 0.1 mm. See Matsui (1984) for detailed definitions of each character. Because male and female *Meristogenys* differ greatly in body size, we treated them separately.

To delimit species groups and determine diagnostic characters, we compared the SVL and the ratio of each character to SVL, calculated on subsets of individuals from the five localities [Bario (males only), Liwagu, Mahua, Mesilau and Wario]. For the comparison of SVL, we used a one-way ANOVA with the Tukey–Kramer multiple comparison test (Zar, 1984). For each ratio variable, we applied the Kruskal–Wallis test with Dunn's multiple comparisons test (Zar, 1984). We further performed discriminant analyses (DA) calculated on the subsets of two molecular groups (Groups A and B) using only molecularly identified samples. Using the DA function, we attempted to classify the type specimens of *M. amoropalamus*. All analyses were conducted by R (R Development Core Team, 2005). Because of a significant heterogeneity in body size among samples (data not shown), we corrected the measurement differences using the allometric method of Thorpe (1976) prior to DA. In a comparative study of size-correction techniques, this method removed the size variation most efficiently without distorting shape information (Reist, 1985). As a size-adjusted, log-transformed value, we computed

$$K = \log[\log Y - b(\log X - \log \underline{X})]$$

where Y is the observed measurement, b is the common within-group regression slope of log(Y) on log(X), X is the SVL of the specimen, and $\underline{X}$ is the grand mean SVL of all individuals studied. Use of the common within-groups regression slope requires parallelism of the regression lines between localities for each variable; therefore, the parallelism of each log(var) on log(SVL) between localities was tested, and all variables satisfied the condition.

We also observed colour patterns on the head, body, and iris. We took photographs for iris colour before anaesthesia as long as possible, but for some specimens, only photos after anaesthesia were available.

For pregnant females, we measured the volume of the left ovary and counted the egg numbers to estimate the total clutch size. A female (SP 21500) laid eggs in captivity, and we estimated clutch size by counting released eggs and the remnants in the ovary. We also estimated the mean ovum volume by dividing ovary mass by ovum number. We tested the correlation between SVL and these parameters (Spearman's rank correlation test, $\alpha = 0.05$) (Kendall & Gibbons, 1990). We used the Mann–Whitney U-test to compare the clutch size and the mean ovum mass between species. From some pregnant females, we extracted several ova before fixation and preserved them in 10% formalin solution to measure precise diameters of the ova.

Recordings of calls were made in the field using a cassette tape recorder (Sony TC-D5) with an external microphone (Sony ECM-23F). Temperature measurements were made at the time of recording using a quick-recording thermistor thermometer (Takara A 600). The recorded calls were analysed using the computer programme Raven Lite Ver. 1.0.

Results

Molecular identification of adult specimens: Of 97 adults examined, 31 specimens (20 males and 11 females) from Bario, Mesilau and Kamborangah had 12S sequences similar to those of Group A (larval morphotype 1 in Shimada *et al*., 2007a), with genetic distances of D = 0%–0.5%. Sixty-three specimens (46 males and 17 females) from Liwagu, Mahua, and Wario had sequences similar to either of the two haplotypes seen in Group B (larval morphotype 3-a in Shimada *et al*., 2007a), with genetic distances of D = 0%–1.1%. Therefore, we identified these two groups as Groups A and B, respectively. In Table 1, we show the results of molecular identifications of adults (this study) and larvae (Shimada *et al*., 2008).

TABLE 1. Collection localities, altitudes, and the results of molecular identification. Sg = Sungai, meaning "river" in Malay. KNP, Kinabalu National Park; CRNP, Crocker Range National Park.

Location	Altitude (m a.s.l.)	Group A	Group B
Malaysia (Sabah)			
Kamborangah, Sg. Tibabar, KNP, Ranau District, Sabah	1800	Adults	
Mesilau, Sg. Mesilau, KNP, Ranau District, Sabah	1800	Adults & Larvae	
Liwagu, Sg. Liwagu, KNP, Ranau District, Sabah	1500	Larvae	Adults & Larvae
Mahua, Sg. Mahua, CRNP, Tambunan District, Sabah	1063	Larvae	Adults & Larvae
Bundu Tuhan, Sg. Liodan, Ranau District, Sabah	990		Larvae
Wario, Sg. Wario, KNP, Kota Belud District, Sabah	950		Adults & Larvae
Poring, Sg. Kipungit I, KNP, Ranau District, Sabah	500		Larvae
Malaysia (Sarawak)			
Bario, Kelabit Highland, Sarawak	1000	Adults	
Indonesia			
Gunung Tapai Sia, Sg. Pa Riman, East Kalimantan	1300	Adults	

The DNA sequences obtained from the holotype and the topotypic female paratype were identical and have not been detected previously in any other specimens extracted in our laboratory. Thus, we are convinced that the sequence is not derived from contaminated DNA. The sequence was similar to that of the larval morphotype 1, with a genetic distance of D = 0.5%. The male paratype collected from Liwagu had a haplotype similar to that of larval morphotype 3-a (D = 0.2%), and was completely identical to some samples from Liwagu.

Phylogenetic analysis. We obtained 9729 bp of DNA (5993 bp of mtDNA and 3736 bp of nuDNA) , of which 2788 bp were variable and 1506 bp were parsimony informative. The numbers of the aligned length, variable sites, and parsimony-informative sites of each region are shown in Table 2. The MP search recovered a most parsimonious tree of 5969 steps (*CI* =0.627; *RI* =0.329). The best substitution model derived from hLRT was the GTR + G + I evolutionary model (Rodriguez *et al*., 1990) for both the ML and Bayesian inferences. The likelihood values of the ML and Bayesian trees were identical: –ln *L* = 38 501.943. Although ML and Bayesian trees were completely identical, the results from ML and MP were slightly different, but the nodes that were significantly supported were completely identical (Fig. 2, only the Bayesian tree is shown).

In these analyses, all samples of *Meristogenys* formed a monophyletic group (with 100, 100, and 100% support in Bayesian posterior probability, ML bootstrap, and MP bootstrap values, respectively). The basalmost placement of *M. kinabaluensis* was well supported in all analysis (100, 100, and 100%, respectively). In the *M. jerboa* species group used in this study, *M. orphnocnemis* was proved to be a sister taxon of *M. dyscritus* (100, 99, and 99%, respectively). *Meristogenys maryatiae* made a monophyletic clade with these two species (100, 95, and 79%,

respectively). Although some of other clades were supported in Bayesian analysis, they were not supported by bootstrap values more than 70% in MP and ML (Fig. 2).

TABLE 2. The number of base pairs (bp), variable sites (vs), and parsimony-informative sites (pi) for DNA fragments examined in this study.

	bp	vs	pi
12S	931	297	154
16S	1607	521	262
ND1	973	451	306
ND2	1038	535	334
tRNAs	410	119	44
Pseudogene	74	5	2
cytb	960	408	303
POMC	583	97	22
RAG1	783	114	30
RH1	247	27	5
SLC8A3	1063	91	17
NCX1	1060	123	27

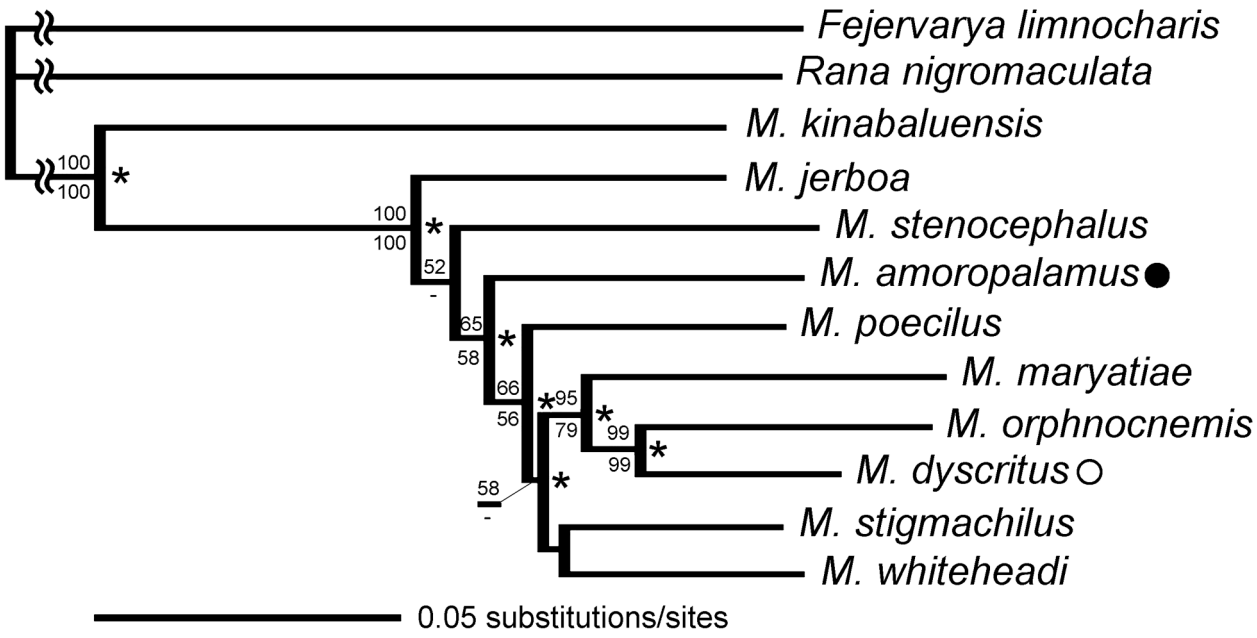

FIGURE 2. Bayesian trees of 9729-bp sequence of mtDNA and nuDNA for the species of *Meristogenys*. Numbers above or below branches represent bootstrap support with 1000 replicates for maximum likelihood (ML)/maximum parsimony (MP) inference. Nodes with asterisks indicate significant support (> 95%) by Bayesian inference.

Morphological analysis. Measurement data for 20 characters in seven populations of *M. amoropalamus* are shown in Table 3. Comparisons of localities with sufficient numbers of specimens showed significant differences in male SVL (ANOVA, $p < 0.05$), but not in female SVL (ANOVA, $p > 0.05$). A Tukey–Kramer test significantly showed males from Mahua to be smaller than those from Liwagu, Mesilau, and Wario. Of the latter three localities, the Mesilau males were significantly larger than those from Liwagu.

TABLE 3. Comparisons of snout-vent length (SVL: means ± 2SE, followed by ranges in parenthesis, in mm) and percentage ratios of each of the other character dimensions to SVL. (medians, followed by ranges in parenthesis) in *Meristogenys amoropalamus* and *Meristogenys dyscritus*. "Holotype" and "paratype" express the type series of *M. amoropalamus* in Matsui (1986).

	M. amoropalamus (Group A)					
	Male				Female	
	Bario	Kamborangah	Mesilau	Gn. Tapai Sia "holotype"	Mesilau	Gn. Tapai Sia "paratype"
	N=7	N=1	N = 12	N=1	N = 11	N=1
SVL	35.7 ± 0.8 (34.4–37.6)	36.8	37.8 ± 0.8 (35.8–40.1)	33.6	64.8 ± 0.8 (62.7–67.4)	61.1
HL	41.0 (39.1–42.5)	41.6	40.3 (37.6–42.7)	41.1	39.9 (37.5–41.5)	39.9
S-NL	6.7 (6.1–7.4)	7.1	6.7 (5.7–7.5)	7.1	6.9 (6.0–7.7)	6.5
N-EL	7.6 (7.4–8.2)	8.2	8.2 (7.7–8.6)	7.4	7.3 (6.5–8.3)	7.7
SL	15.4 (14.2–16.7)	16.0	16.3 (15.8–17.3)	14.6	15.7 (13.8–16.4)	15.4
EL	16.5 (16.0–17.4)	14.9	15.7 (14.2–16.8)	17.9	14.7 (13.4–16.8)	15.1
T-EL	1.5 (1.1–2.5)	3.3	2.1 (1.5–3.2)	1.5	3.6 (2.6–4.0)	2.9
TDv	12.5 (10.9–12.9)	11.4	11.5 (10.3–12.3)	13.4	7.7 (6.6–8.4)	7.9
TDh	12.8 (11.5–13.6)	12.0	11.8 (10.4–13.0)	14.0	6.9 (6.1–7.7)	7.9
HW	34.8 (33.1–36.4)	35.6	35.7 (34.2–37.7)	35.4	36.0 (33.7–38.1)	35.5
IND	11.6 (10.6–12.5)	12.5	11.8 (9.8–13.4)	13.1	11.0 (10.5–11.9)	11.6
IOD	9.1 (8.4–10.8)	9.2	9.7 (9.0–10.3)	9.8	8.9 (7.7–9.4)	9.3
UEW	10.2 (9.1–11.5)	9.2	9.4 (8.7–10.4)	10.4	9.6 (8.5–10.3)	8.5
FLL	69.4 (66.9–72.1	73.1	72.4 (67.3–76.2)	–*1	70.8 (68.7–73.4)	68.2
LAL	53.7 (50.1–54.1)	55.7	55.6 (53.8–59.1)	54.2	55.7 (53.9–56.7)	55.0
HAL	30.1 (29.6–32.4)	31.5	32.1 (29.4–33.2)	33.6	32.3 (30.7–33.2)	30.6
HLL	208.0 (199.2–212.5)	216.0	214.6 (202.8–223.7)	225.3	217.2 (209.7–218.7)	220.8
THIGH	59.2 (56.4–63.4)	62.2	61.3 (57.9–65.2)	63.7	62.3 (59.8–64.9)	64.3
TL	70.2 (66.4–72.3)	71.5	71.2 (68.5–74.4)	74.7	71.4 (70.4–74.4)	73.8
FL	55.8 (51.3–57.4)	60.1	59.2 (56.1–62.7)	60.4	59.9 (57.3–61.9)	57.4

continued.

	M. dyscritus (Group B)						
	Male				Female		
	Liwagu	Mahua	Wario	Liwagu "paratype"	Liwagu	Mahua	Wario
	N = 19	N=7	N = 20	N=1	N = 7	N=6	N = 4
SVL	35.9 ± 0.6 (32.9–38.3)	34.2 ± 0.9 (33.5–36.8)	36.8 ± 0.6 (33.9–39.5)	31.7	67.0 ± 2.2 (62.6–71.2)	64.3 ± 2.4 (61.0–69.4)	67.3 ± 2.9 (63.9–71.0)
HL	37.6 (37.6–41.6)	39.7 (38.0–41.1)	39.7 (38.0–42.3)	40.4	38.7 (38.7–41.7)	39.9 (39.6–41.2)	38.8 (37.9–39.4)
S-NL	6.5 (5.1–7.9)	6.0 (5.9–6.3)	6.6 (4.7–7.7)	7.6	6.0 (5.3–6.6)	6.4 (6.1–7.3)	5.7 (5.0–6.9)
N-EL	7.9 (7.1–9.2)	8.3 (7.2–9.0)	8.2 (7.6–9.0)	8.5	7.9 (7.4–8.5)	7.9 (7.6–8.1)	8.1 (7.2–8.5)
SL	16.1 (14.9–17.0)	15.5 (15.2–16.0)	15.8 (14.6–17.3)	16.4	15.4 (14.1–16.0)	15.3 (13.8–15.7)	15.0 (14.6–16.5)
EL	16.4 (14.4–18.1)	15.7 (14.1–15.8)	15.6 (15.0–17.1)	16.4	14.8 (13.2–15.4)	14.2 (13.6–15.1)	14.2 (12.9–14.9)
T-EL	2.2 (1.1–3.0)	2.7 (1.8–2.7)	2.4 (1.9–3.0)	2.5	4.2 (3.1–4.5)	4.2 (3.2–4.7)	4.4 (4.4–5.3)
TDv	11.0 (9.8–12.1)	10.7 (9.8–11.5)	10.9 (9.7–12.1)	11.0	7.2 (6.7–8.1)	6.8 (6.7–7.1)	6.6 (6.0–7.2)
TDh	11.1 (10.2–12.4)	10.7 (10.4–11.8)	10.8 (9.1–12.1)	10.7	6.8 (5.9–7.8)	7.5 (6.8–7.9)	6.5 (5.2–7.4)
HW	35.4 (32.6–36.8)	34.0 (32.6–35.6)	34.5 (33.1–37.2)	40.1	36.7 (34.7–37.4)	35.0 (34.1–38.6)	36.1 (34.6–36.3)
IND	11.5 (9.9–12.1)	11.9 (11.3–12.2)	11.4 (10.5–12.1)	13.6	10.6 (10.0–11.2)	11.0 (10.4–11.5)	10.2 (9.8–10.8)
IOD	9.4 (7.6–10.6)	9.5 (8.4–10.4)	9.1 (8.5–10.3)	9.5	8.1 (7.6–9.3)	8.1 (7.9–9.1)	8.4 (8.0–9.9)
UEW	10.1 (8.8–11.2)	9.6 (8.6–10.6)	10.5 (9.2–11.3)	10.7	10.0 (9.3–10.9)	9.9 (9.2–10.4)	9.0 (8.5–10.2)
FLL	73.7 (69.1–76.7)	72.7 (67.5–75.5)	72.0 (67.2–76.6)	76.3	68.9 (61.9–70.8)	67.3 (63.7–70.0)	66.3 (64.3–68.2)
LAL	54.9 (51.7–57.6)	55.4 (54.3–58.8)	55.1 (52.3–57.7)	60.3	53.6 (50.6–56.4)	53.7 (51.2–54.9)	51.8 (51.1–54.0)
HAL	29.5 (26.0–33.8)	30.4 (29.1–32.0)	30.5 (29.1–33.5)	34.7	29.7 (28.3–31.0)	30.2 (29.0–30.8)	28.6 (28.3–30.1)
HLL	207.7 (198.2–215.8)	218.5 (214.0–221.2)	208.5 (196.0–221.0)	219.9	201.8 (196.3–222.7)	213.4 (198.1–219.5)	206.6 (200.5–209.3)
THIGH	61.5 (57.6–64.0)	62.6 (61.7–64.5)	60.4 (55.5–63.6)	62.8	61.7 (57.4–66.0)	62.9 (58.4–63.6)	60.2 (59.8–61.1)
TL	68.5 (66.1–72.6)	72.6 (70.1–73.1)	69.6 (66.8–74.7)	72.9	69.5 (66.9–73.2)	69.7 (67.6–74.7)	68.9 (65.3–69.7)
FL	56.4 (53.7–59.6)	58.8 (58.2–60.9)	56.0 (53.9–61.2)	64.0	55.0 (52.9–58.6)	57.9 (54.8–59.8)	55.9 (55.1–57.1)

*1: We could not measure FLL of the holotype of *M. amoropalamus* because both humerus had been broken.

The Kruskal–Wallis test indicated significant heterogeneities in the six characters of female limbs (FLL, LAL, HAL, HLL, TL, and FL) (Table 4). Dunn's multiple comparisons showed that the Mesilau females significantly had limbs longer than those of the other females in four characters (FLL, LAL, HAL, and FL). Five female head characters (S-NL, N-EL, T-EL, TDv, and IND) proved to have significant heterogeneities in the Kruskal–Wallis test, but detecting general tendencies in the results of Dunn's multiple comparisons was difficult.

In males, the Kruskal–Wallis test indicated significant heterogeneities in 12 characters (N-EL, SL, EL, T-EL, TDv, TDh, UEW, FLL, LAL, HLL, TL, and FL) (Table 4), while in Dunn's multiple comparisons test, significant heterogeneities were found in 10 characters (SL, T-EL, TDv, TDh, UEW, FLL, LAL, HLL, TL, and FL). The results for TDv and TDh significantly indicated that Bario males had a larger tympanum than those found in other local samples. The results for LAL, HAL, HLL, TL, and FL significantly indicated that Mahua and Mesilau males had longer limbs than those of other local samples.

In some males of Groups A and B, tympanums were in close contact with eyes, and we could not measure T-EL. Additionally, we could not measure the FLL of the holotype of *M. amoropalamus* because both of its humeri had already been damaged. Therefore, for DA, we used 18 measurements, excluding these two characters. Discriminant functions classified 93.9% of males and 100% of females to the correct groups. Coefficients of linear discriminants are shown in Table 5. We also applied this function to the type specimens. The holotype and the female paratype (topotype) were identified as Group A, while the male paratype, collected from Liwagu, was included in Group B.

TABLE 4. Results of Kruskal–Wallis tests with multiple comparison tests for differences in means of each character's ratio to SVL among localities. Significant p-values are in bold. Ba, Bario; Li, Liwagu; Ma, Mahua; Me, Mesilau; Wa, Wario. For character abbreviations, see Material and methods.

	Males			Females		
Variable	X^2	p	multiple comparison	X^2	p	multiple comparison
Head measurements						
HL	5.050	0.282		6.642	0.084	
S-NL	6.129	0.190		11.738	**0.008**	Me>Li
N-EL	12.325	**0.015**		8.583	**0.035**	
SL	16.095	**0.003**	Me>Wa	2.880	0.411	
EL	15.257	**0.004**		2.548	0.467	
T-EL	14.445	**0.006**	Wa>Ba, Ma>Ba	13.202	**0.004**	Wa>Me
TDv	16.576	**0.002**	Ba>Li, Ba>Wa	11.403	**0.010**	Me>Wa
TDh	20.926	**<0.001**	Ba>Wa	4.547	0.208	
HW	8.450	0.076		2.170	0.538	
IND	7.769	0.100		9.708	**0.021**	
IOD	9.127	0.058		3.217	0.359	
UEW	15.774	**0.003**	Wa>Me	5.858	0.119	
Limb measurements						
FLL	11.240	**0.024**	Li>Ba	14.137	**0.003**	Me>Ma, Me>Wa
LAL	15.910	**0.003**	Ma>Ba, Me>Ba	12.219	**0.007**	Me>Wa
HAL	8.050	0.090		19.078	**<0.001**	Me>Li, Me>Wa
HLL	20.783	**<0.001**	Me>Li	9.029	**0.029**	
THIGH	8.057	0.090		4.038	0.257	
TL	20.678	**<0.001**	Ma>Li, Me>Li	11.027	**0.012**	
FL	25.621	**<0.001**	Ma>Ba, Me>Li Me>Ba, Me>Wa	14.743	**0.002**	Me>Li

Reproductive characters. Of 29 females examined, 21 specimens were pregnant (7 samples from Group A, 13 samples from Group B and the paratype of *M. amoropalamus*). Eggs of four females (two from Group A, one

from Group B and the paratype), however, had already been released from the ovary into the oviducts and were covered with jelly. After fixation, the jelly layer was elastic and harder than the eggs, making it difficult to separate them. Therefore, we could not estimate clutch size for these specimens.

TABLE 5. Coefficients of linear discriminants for males and females.

Variable	Males	Females
HL	-68.66	20.51
S-NL	-0.65	-11.38
N-EL	4.77	13.04
SL	21.46	74.39
EL	-13.53	-119.35
TDv	-19.18	-68.41
TDh	-31.02	22.86
HW	0.09	-25.79
IND	-12.44	68.56
IOD	2.70	-26.63
UEW	25.04	69.06
LAL	55.82	269.24
HAL	-35.25	-338.07
HLL	37.36	-188.07
THIGH	92.19	148.45
TL	-167.91	-59.87

Ova of Group A and the female paratype of *M. amoropalamus* were evidently larger and lighter in colour than those of Group B (Fig. 3). The relationships between female SVL and the reproductive characters (estimated clutch size and the ovum volume) are shown in Figures 4 and 5. The Group A females had significantly smaller clutch sizes (less than 300 ova per ovary, mean = 232) and larger ova (more than 5.0×10^{-3} mL per ova, mean = 6.4×10^{-3} mL) than those of the Group B females [clutch size more than 300 ova per ovary (mean = 495), ovum volume less than 4.0×10^{-3} mL (mean = 2.7×10^{-3} mL); Mann–Whitney U-test, $p < 0.05$]. The clutch size was significantly correlated with female SVL in Group B (Spearman's rank correlation test, $r = 0.68$, $p < 0.05$), but not in Group A. The ovum volume was not significantly correlated with female SVL in either group. The diameter of ova extracted from a few individuals before fixation was 2.27 ± 0.06 mm (2.05–2.61 mm; *N* = 20; KUHE 39461) in Group A, and 1.73 ± 0.02 mm (1.65–1.80 mm; *N* = 26; SP 26476 and 26477) in Group B.

Iris colour. Before anaesthesia, the Group A specimens had bi-coloured irises that were yellowish-green above and reddish-orange below (Fig. 6C). Group B also had bi-coloured irises, but they were sandy brown above (Fig. 6E). Some females of the latter type had a mono-coloured irises dark brown to reddish-brown in colour (Fig. 6F). Iris colour darkened soon after anaesthesia (Fig. 6B and 6D). Judged from a photograph of the female paratopotype taken after anaesthesia, the upper third of its iris seemed to be dark yellow (Fig. 6A), like the Group A iris after anaesthesia.

Meristogenys dyscritus sp. nov.

Amolops sp. A: Matsui, 1979, p. 340, figs. 26, 28A; *Amolops amoropalamus*: Matsui, 1986, p. 628–629 (part); *Meristogenys amoropalamus* (lineage 3, lineage 4): Shimada *et al*., 2007a, p. 173–189; Shimada *et al*., 2008, p. 24–34; Shimada *et al*., 2011, p. 158–177, Fig. 3–4, Appendix 2, *Meristogenys amoropalamus* (larval morphotype 3-a): Shimada *et al*., 2007b, p. 59–63.

Diagnosis. This species is a member of the genus *Meristogenys*, based on the morphology of the larval specimens molecularly assigned to it (larval morphotype 3-a in Shimada *et al.*, 2007a); these had diagnostic characteristics of

the genus: abdominal sucker; divided upper jaw sheaths; and ribs on the outer surface of both jaw sheaths. *Meristogenys dyscritus* is a small species of the *M. jerboa* species group (Matsui, 1986), with male SVL 31.7–39.5 mm, female SVL 61.0–71.2 mm; rear of thigh dark brown, dusted with small irregular light spots; web poorly developed leaving terminal two phalanges on fourth toe free of broad webbing; ova relatively small and heavily pigmented in the animal pole; upper third of iris sandy brown to yellowish brown.

FIGURE 3. Ova of *Meristogenys amoropalamus* (A) and *M. dyscritus* (B). Scale bar = 2 mm.

Etymology. The specific name is derived from dyskritos (Gr.), doubtful or indistinguishable, referring that the new species is difficult to distinguish from *M. amoropalamus* in adult morphology.

Holotype. Osaka Museum of Natural History (OMNH) Am8069, an adult male from Liwagu River, 1500 m, Headquarters of Kinabalu National Park, Sabah, Malaysia. Collected in March, 1979, by Masafumi Matsui.

Paratypes. Two males and a female from the type locality; Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK) 41644; Sabah Parks (SP) 26481, 21392.

Referred specimens. Forty-five males and 16 females from the type locality (Liwagu), Mahua, and Wario (See Appendix).

Description of Holotype (measurements in mm). Body moderately slender, SVL 31.7; head subtriangular, slightly longer (12.8) than wide (12.7); snout blunt, projecting slightly beyond lower jaw; eyes elevated; canthi

sharp, slightly concave; lores slightly oblique, concave; nostrils lateral, just below canthal edge, distinctly closer to tip of snout (2.4) than to eye (2.7); IND (4.3) wider than IOD (3.0); latter narrower than UEW (3.4); SL 5.2; pineal spot visible, slightly behind the line connecting anterior corners of orbits; tympanum distinct, TDv (3.5) and TDh (3.4), two-thirds of EL (5.2); T-EL (0.8) one-fourth of TDv and TDh; vomerine teeth obvious, in small oblique groups separated by the width of one group, groups on line connecting rear rims of choanae; tongue deeply notched, without papilla; paired subgular vocal sacs form gular pouches at corners of throat; vocal opening just inside commissures of jaws.

Fingers slender, first and second subequal, much shorter than third; tips expanded into disks having circummarginal grooves; disks of all fingers subequal in diameter, one-third diameter of tympanum; no fringes of skin along fingers; no supernumerary metacarpal tubercles; distinct nuptial pads covering dorsal and medial surfaces of the first finger from its base to subarticular tubercle.

Forelimb length unavailable; LAL 19.1; HAL 11.0; HLL 69.7; tibia long (23.1); heels overlapping when limbs are held at right angles to body; THIGH (19.9) and FL (20.3) much shorter than TL. Toe disks similar to those of fingers in shape and size; webbing not fully extending to disk on fourth toe, leaving two phalanges free of broad sheet; excision of web between fourth and fifth toes proximal to middle subarticular tubercle of fourth toe; a narrow fringe of skin along medial edge of first toe; inner metatarsal tubercle elliptical, shorter than distance between it and subarticular tubercle of first toe; a small round, raised outer metatarsal tubercle.

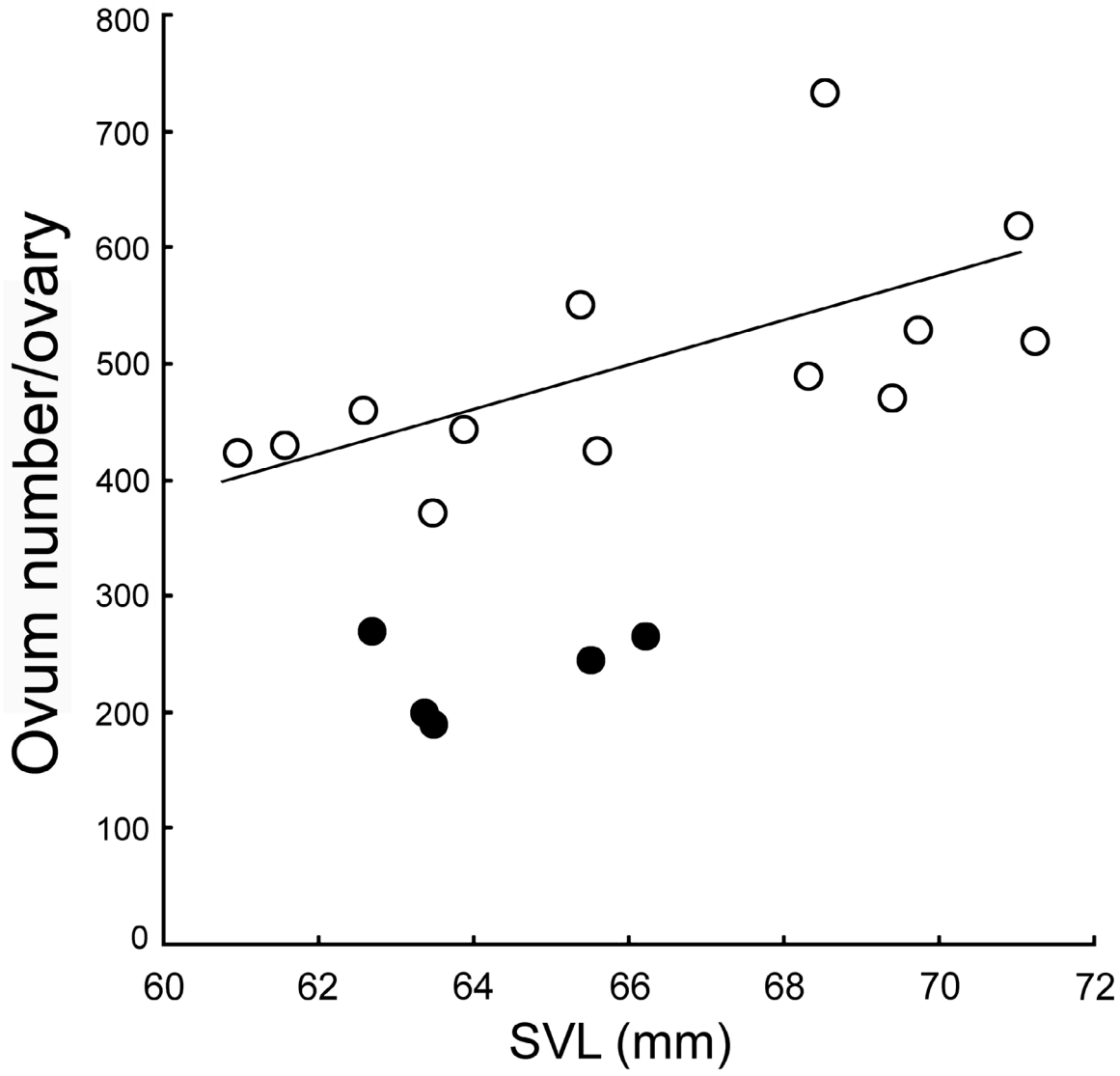

FIGURE 4. Relationship between female snout-vent length (SVL) and ovum numbers per ovary. Closed dots indicate *Meristogenys amoropalamus* (Group A), while open dots indicate *M. dyscritus* (Group B).

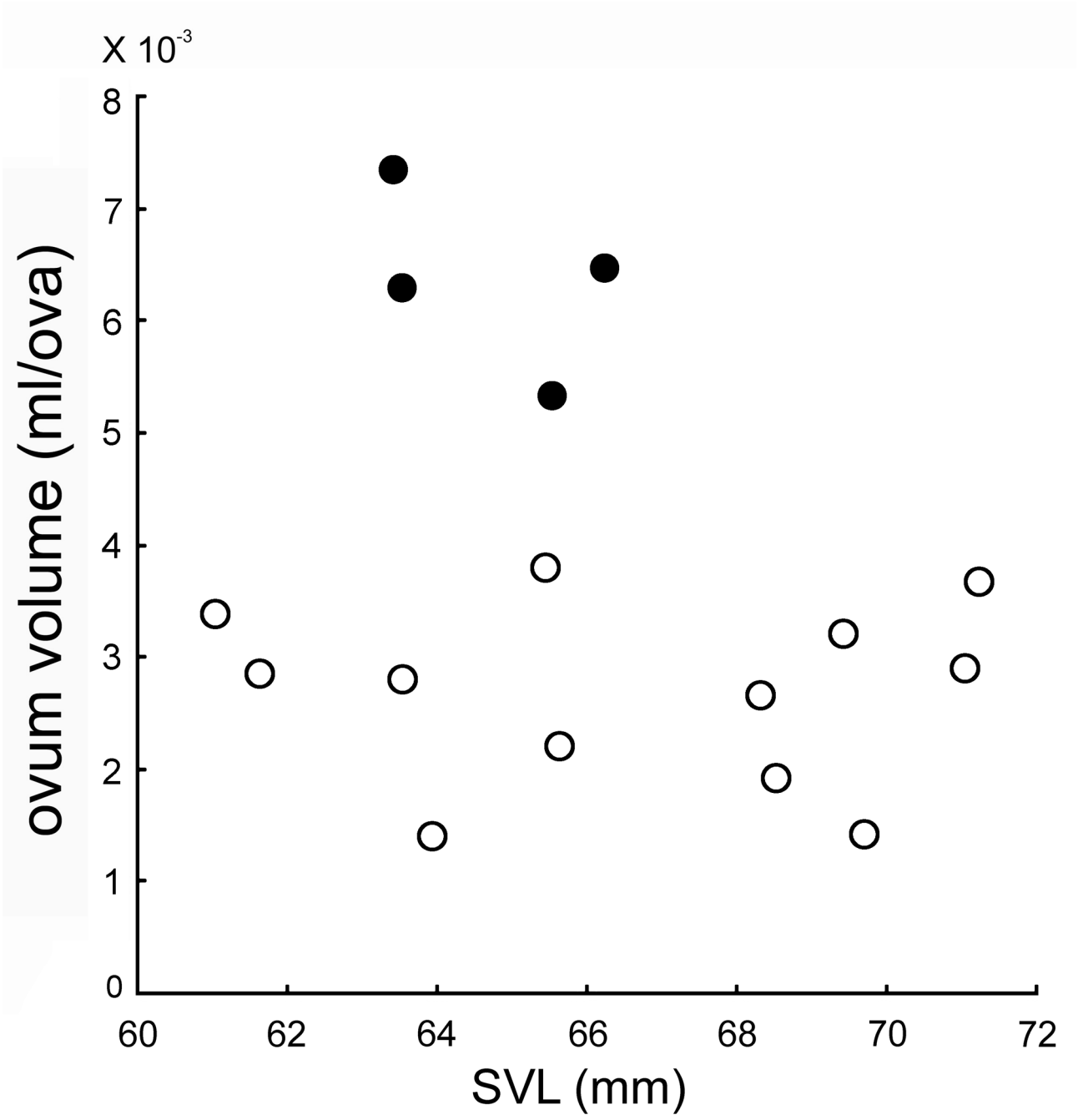

FIGURE 5. Relationship between female SVL and ovum volume (ovary volume divided by clutch size). Closed dots indicate *Meristogenys amoropalamus* (Group A), while open dots indicate *M. dyscritus* (Group B).

Skin of dorsum finely granular on head and trunk; a weak fold from above eye to axilla; a low dorsolateral glandular fold; side of trunk coarsely granular; limbs strongly rugose above; throat smooth; chest and abdomen weakly rugose.

Colour in life (See coloured pictures of Fig. 7). Dorsum light brown with small dark spots; lores with dark streak below canthus; upper lip light grey; a blackish brown band beginning behind eye bordering rear of the tympanum, diverging above the tympanum and nearly reaching the inguinal area, dorsal and ventral boundaries sharp; limbs marked dorsally with alternating light and dark brown crossbars, the darker ones narrower; a short dark streak ventrally at insertion of arm; rear of thigh light brown with scattered irregular light spots; throat and chest whitish with dots of melanophores; lower lip vaguely barred with blackish brown; ventral surface of tibia whitish, with heavy pigmentation of melanophores.

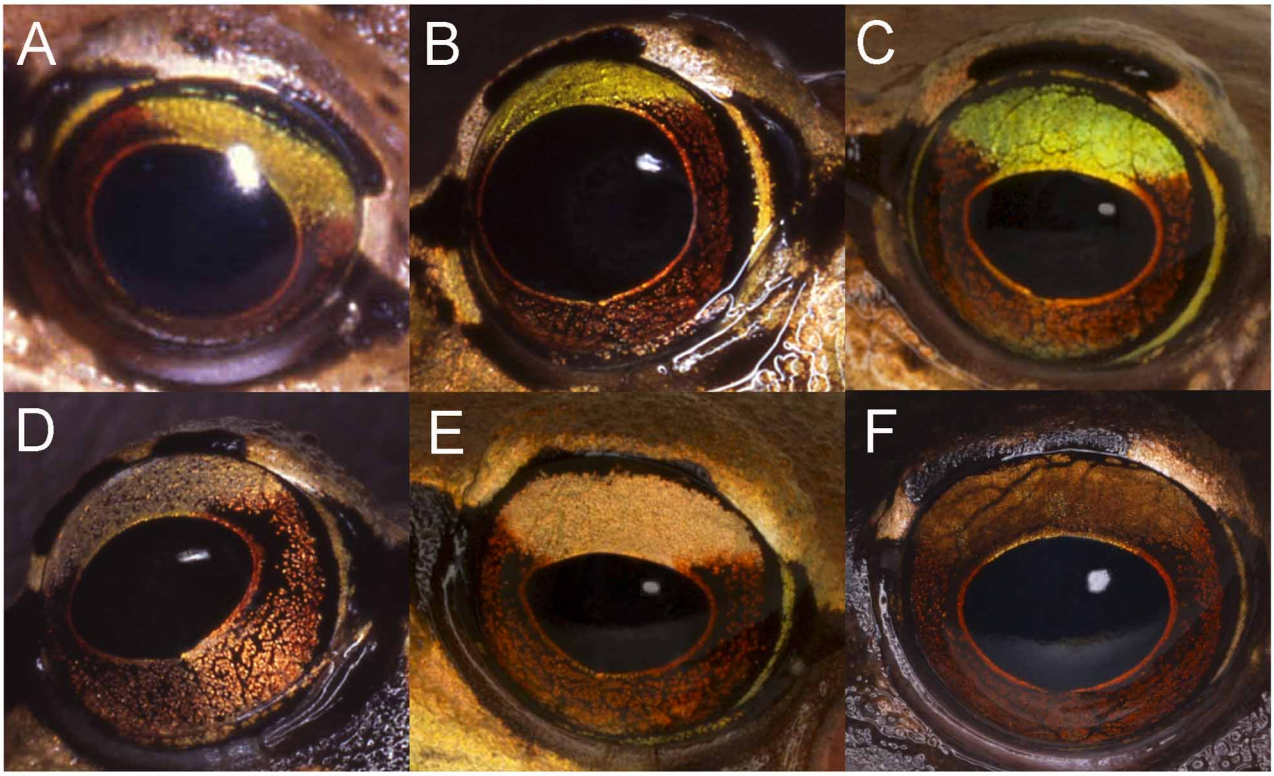

FIGURE 6. Eyes of *Meristogenys amoropalamus* (Group A) and *M. dyscritus* (Group B). A, the female paratopotype of *M. amoropalamus* from the type locality (Gunung Tapai Sia) after anaesthesia; B, C, *M. amoropalamus* from Mesilau after (B) and before (C) anaesthesia; D–F, *M. dyscritus* from Wario after anaesthesia (D) and *M. dyscritus* from Liwagu before anaesthesia (E, F). Specimen vouchers: A, OMNH Am8068; B, BORNEENSIS 22901; C, KUHE 39460; D, BOR 22970; E, SP 26481; F, SP 21514. For abbreviations, see Appendix.

Iris colour. Although iris colour of the holotype is unavailable, other specimens from the type locality (Liwagu) had the bicoloured iris, sandy brown above and reddish brown below.

Colour in alcohol. Color pattern has not been changed even after preservation in ethanol for several years, except for iris colour which disappeared soon after the fixation in formalin solution.

Eggs: Ova bicoloured, heavy pigmentations on the animal pole, cream white on the vegetal pole; clutch size 750–1500; ovum diameter 1.6–1.8 mm.

Larvae (See coloured pictures of Fig. 8): We examined 67 specimens of stage 26–41 of Gosner (1960) from Mahua. These specimens are identical to those used by Shimada *et al*. (2007a) to define their larval morphotype 3-a.

Head-body length (HBL) 10.0–16.8 mm. Head-body oval, broadly rounded at snout, flat below, head-body width (HBW) maximum at level of spiracle 61–71% (median=65.8%) of HBL; depth 49–64% (median=55.4%) of HBW; eyes dorsolateral, not visible from below, pointing outward, eyeball 13–15% (median=13.7%) of HBL; interorbital 123–169% (median=147.6%) of eye diameter; eye-snout distance 36–46% (median=41.1%) of HBL; nostril open, rim not raised, closer to eye than to tip of snout; internarial 57–80% (median=69.8%) of interorbital.

Oral disk ventral, width 57–73% (median=65.7%) of HBW; upper lip separated from snout by a groove; upper lip with short marginal papillae in lateral third, inframarginal papillae near corner; lower lip with uninterrupted row of short marginal papillae; labial tooth row formulae 6(4–6)/6(1) in three specimens, 6(4–6)/7(1) in 48 specimens, and 6(4–6)/8(1) in 13 specimens; jaw sheaths heavy completely black except for outer margins covered by thin film; upper sheath film thicker than the lower; outer surface of lower jaw sheaths with several weak ribs; margin finely serrate, 3–11 and 4–7 serrae on a half of upper and lower jaw sheaths, respectively; upper jaw sheaths M-shaped, lower V-shaped; both jaw sheaths divided; a large suctorial abdominal disk following oral disk; peripheral part of disk darkened and keratinized; length 40–52% (median=46.5%) of HBL; width 78–98% (median=87.9%) of HBW.

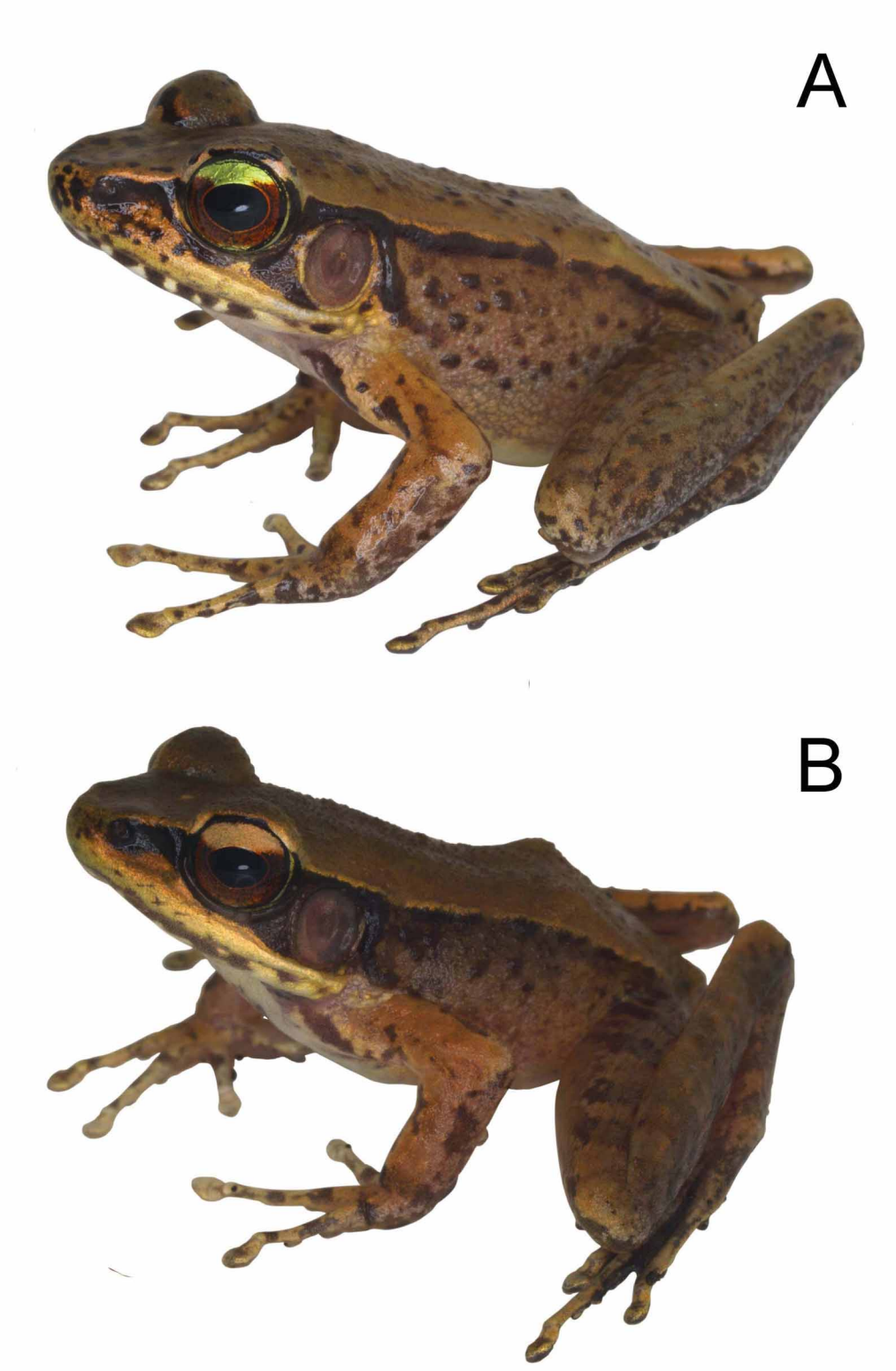

FIGURE 7. Dorsolateral views of *Meristogenys amoropalamus* (A, SP 26612) from Kamborangah and *M. dyscritus* (B, SP 26481) from Liwagu.

Spiracle sinistral; tube moderately long, length subequal to length of eyeball, pointing upward and backward, free of body wall for half its length; anal tube median, free of tail; tail heavily muscled, dorsal margin strongly convex, deepest before middle, tapering to slightly pointed tip; tail length 143–179% (median=164.7%) of HBL, maximum depth 23–33% (median=26.5%) of length; caudal muscle deeper than fins in basal half; dorsal fin origin behind body, fin deeper than ventral fin except in final fourth; ventral fin origin at end of proximal third of tail;

head-body with four pairs of glandular clusters; a postorbital cluster about an eye length behind eye, with 0–3 glands; a infraorbital at the base of snout, with 1–3 glands; a prespiracular cluster just anterior to spiracle, with 0–4 glands; a midlateral at the posterior end of body, with 0–8 glands; no ventral and dorsal fin glands; 0–16 ventral fin glands; head-body scattered dorsally with minute protuberances anterior to eye in developed larvae; the area occupied by spinules and their density increasing with stage of development; lateral line pores indistinct.

Head-body light brown dorsally and laterally, sometimes posterior half of lateral surface dark brown; caudal muscle light brown; translucent fins with scattered pigmentations.

Other ten larval specimens from Bundu Tuhan (st. 28), Liwagu (st. 32), Poring (st. 31), and Wario (st. 30 and 32) had characteristics similar to Mahua specimens, but all ten specimens had six rows of lower labial teeth.

Variation. Males from Mahua tended to be smaller than males from Liwagu and Wario in SVL (Table 3).

Although relatively weak web development is one of the diagnostic characters of this species, some females from Liwagu and Wario had developed broad webs, which reached to the toe disk. Individuals usually had a bicoloured iris, sandy brown above and reddish brown below, but some females from Liwagu had monocoloured reddish brown iris.

Larvae from Mt. Kinabalu tended to have fewer labial tooth rows in lower jaw than the specimens from Mahua (Crocker Range). In Mahua, only 4.7% of larvae had six rows of labial teeth on the lower jaw, and all other larvae had seven or eight rows, whereas all ten larvae collected around Mt. Kinabalu (Bundu Tuhan, Liwagu, Poring and Wario) had six rows.

Calls. We recorded calls of a male of *M. dyscritus* (SP 26472) at Liwagu at the midnight of 14 June 2007 (Fig. 9) at an air temperature of 17.5 C. The male perched on a low shrub, 50 cm above ground, and 1 m from the river. Many other males were also calling around there.

The call was emitted sporadically with several minutes intervals, and consisted of a short, unpulsed note, lasting about 0.11 sec. A call included three continuous phases, of which the first one had a marked frequency modulation; it descended quickly in frequency from 8000–10000 Hz down to about 6000 Hz. In the second phase, the frequency was kept stable around 6000 Hz. The third phase had a remarkable frequency modulation again; it ascended quickly in frequency from 6000 up to 9000 Hz. Dominant frequencies were traced at about 6000–10000 Hz ranges, and harmonic bands were often found between 12000–15000 Hz ranges.

Comparisons. Among 11 known species of *Meristogenys*, *M. kinabaluensis* (Inger, 1966), *M. poecilus* (Inger and Gritis, 1983), *M. stenocephalus* Shimada *et al*. 2011, *M. stigmachilus* Shimada *et al*. 2011, and *M. whiteheadi* (Boulenger, 1887) have a large body size (male SVL > 45 mm, female SVL> 70 mm) and are easily distinguished from *M. dyscritus*. From some small species, *M. dyscritus* is differentiated in the poorly developed toe webs [In *M. jerboa* (Gunther, 1872), *M. macrophthalmus* (Matsui, 1986), *M. maryatiae* Matsui *et al*., 2008, *M. orphnocnemis* (Matsui, 1986), and *M. phaeomerus* (Inger and Gritis, 1983), webs reach at the toe disk], heavy pigmentations on the ventral surface of tibia (*M. jerboa*, *M. maryatiae*, *M. orphnocnemis*, and *M. phaeomerus* have only scattered pigmentation), small irregular light spots on the rear of thigh (*M. jerboa* and *M. macrophthalmus* have large bright blotches), and black streaks around eyes, lores and tympanum (*M. jerboa*, *M. maryatiae*, *M. orphnocnemis*, and *M. phaeomerus* usually lack such colour patterns).

Meristogenys dyscritus resembles *M. amoropalamus* in adult morphology, but the typical individuals can be identified through morphological characters. The upper iris of *M. dyscritus* is usually sandy brown, but is yellowish green in *M. amoropalamus* (Fig. 6), although this character changes after anaesthesia, and there might be an intraspecific variation. Additionally, females of *M. dyscritus* have smaller (Fig. 5) ova and larger clutch size (Fig. 4) than *M. amoropalamus* (See "Reproductive characters" in Result). In females, *M. dyscritus* have longer hand (usually longer than 31% of SVL) than *M. amoropalamus* (usually less than 31% of SVL) (Fig. 10). Males of *M. amoropalamus* usually have larger tympanum (more than 13 mm^2) than those of *M. dyscritus* (less than 15 mm^2), although there is a range of overlap (Fig 11).

Contrary to the adult morphology, the larvae of *M. dyscritus* is quite different from those of *M. amoropalamus* in morphological characters. The larvae of *M. dyscritus* have six rows on upper jaws, while those of *M. amoropalamus* have seven. In the young developmental stages, the lower jaw sheaths of both larvae is divided, but only those of *M. amoropalamus* fuse into a large single jaw sheath before metamorphosis. Glands in a tail are limited to ventral fin in larval *M. dyscritus*, while they are present in both fins of larval *M. amoropalamus*. For the comparison between larval *M. dyscritus* and other *Meristogenys* larvae in Sabah, see the KEY TO *MERISTOGENYS* LARVAE FROM SABAH.

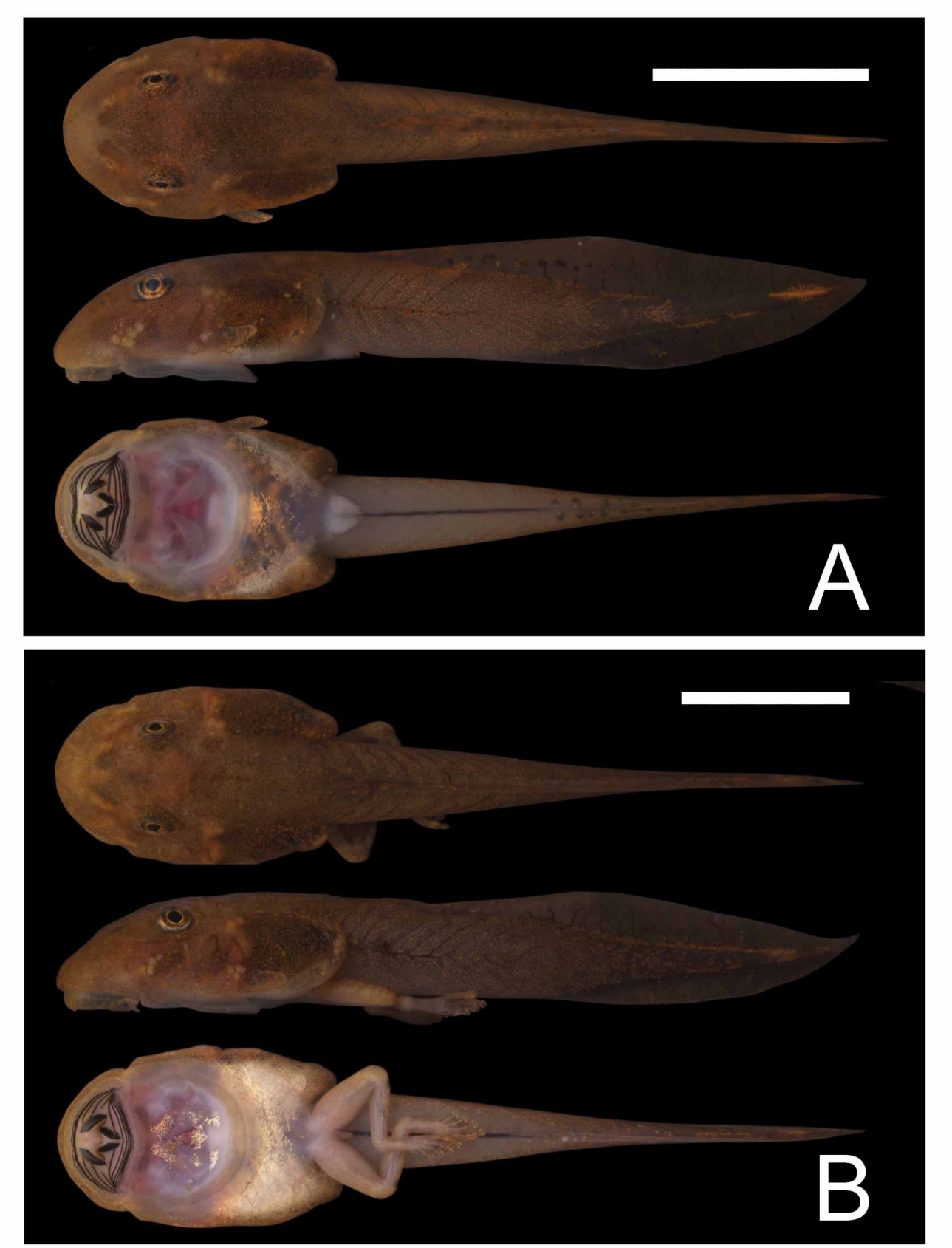

FIGURE 8. Larvae of two *Meristogenys* species treated in this study. A, *Meristogenys amoropalamus* (BORNEENSIS 05B247) from Mahua; B, *Meristogenys dyscritus* (BORNEENSIS 05B239) from Mahua. Scale bar = 10 mm.

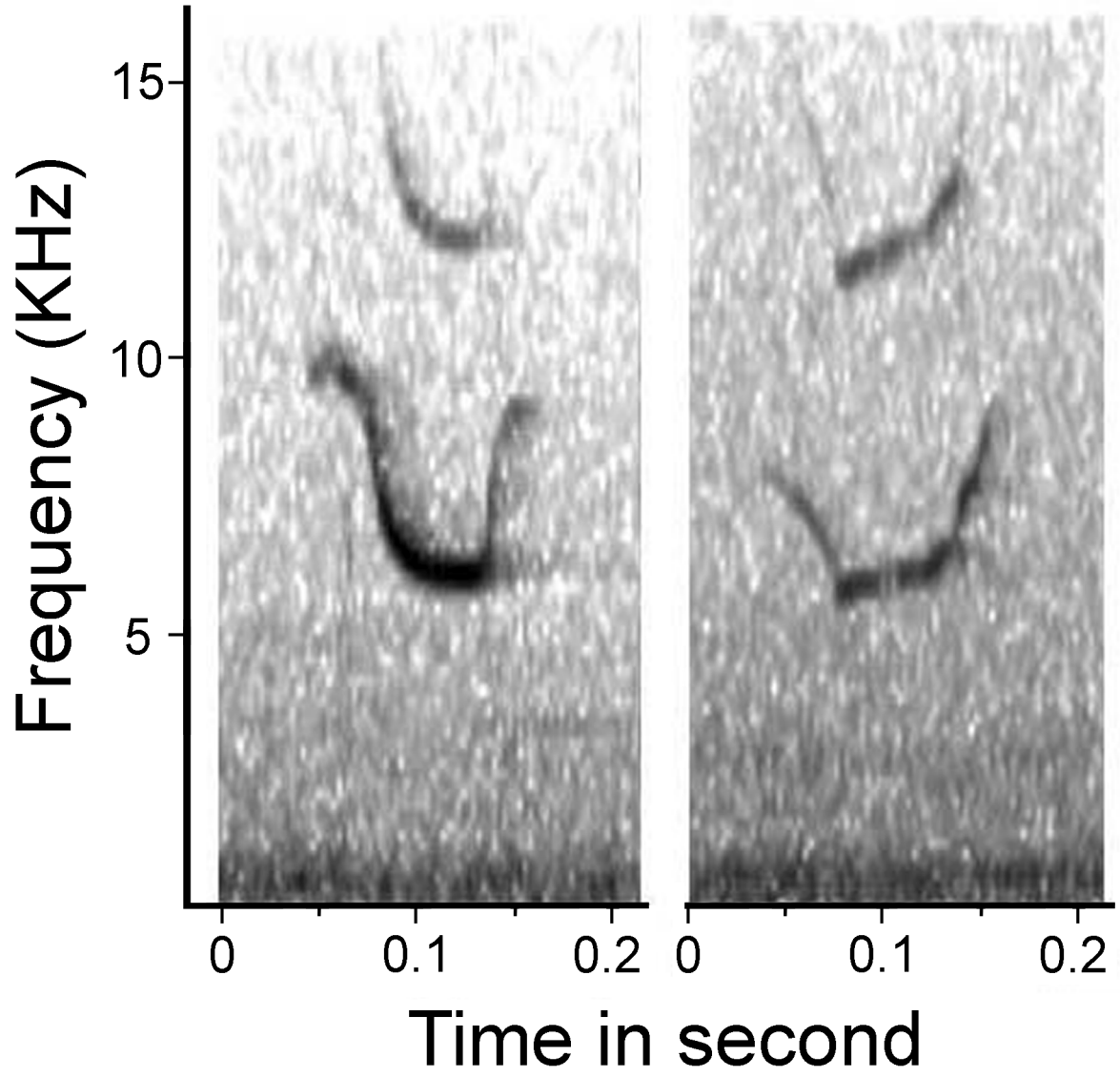

FIGURE 9. Sonograms of advertisement calls by *Meristogenys dyscritus* recorded at Liwagu.

The larvae of *M. dyscritus* share divided lower jaw sheaths, six rows of labial teeth on the upper jaw, and no glands on their dorsal fin or ventral surface with larval *M. orphnocnemis*. The larvae of *M. dyscritus*, however, have rectangular body shape (body oval in *M. orphnocnemis*), surface projections restricted to head (projections on head and body in advanced stage in *M. orphnocnemis*), and usually seven or eight rows of lower labial teeth (usually six rows in *M. orphnocnemis*; See Shimada *et al*., 2007a for details).

Range. Besides the type locality Liwagu, 1500 m a.s.l., adults of this species have been collected from Wario (900 m a.s.l.) in the Kinabalu National Park, and Mahua (1063 m a.s.l.) in the Crocker Range National Park, all in Sabah, Malaysia. The larvae of this species have also been collected from Bundu Tuhan (990 m a.s.l.) and Poring (500 m a.s.l.) in/around the Kinabalu National Park.

Natural history. Gravid females were collected in July (Liwagu), August (Mahua and Wario), November (Wario), and December (Mahua). Larvae collected in March (Liwagu and Mahua), August (Liwagu, Wario, Bundu Tuhan, and Mahua), and November (Poring) showed a wide range of developmental stages. Thus, there seems to be no particular reproductive seasons. Larvae were collected from rivers with a width of 5–10 m. At night, they make a mixed cluster with larvae of *M. kinabaluensis*, *M. orphnocnemis*, and *Huia cavitympanum* on top of flat rocks in the river.

Meristogenys amoropalamus (Matsui, 1986)

Amolops amoropalamus: Matsui, 1986, p. 628–629. figs. 1C, 1F, 2C, 2D; *Meristogenys amoropalamus* (larvae): Yang, 1991, p. 33–34; Malkmus *et al*., 2002, p. 149–150; *Meristogenys* sp. (lineage 1): Shimada *et al*., 2007a, pp. 173–189; *Meristogenys amoropalamus* (larval morphotype 1): Shimada *et al*., 2007b, pp. 59–63; *Meristogenys amoropalamus* (lineage 1): Shimada *et al*., 2011, p. 158–177, Fig. 3, 4, 6.

Iris colour. Iris bicoloured, yellowish green above and reddish brown below. The upper part became dark yellow after anaesthesia.

Eggs. Ova bicoloured, heavy pigmentations on the animal pole, cream white on the plant pole; clutch size 400–550; ovum diameter 2.1–2.6 mm.

Range. Besides the type locality (Gunung Tapai Sia; 1300 m a. s. l.), the adult of this species has been collected from Mesilau (1800 m) and Kamborangah (1800 m) in the Kinabalu National Park and Bario (1000 m) from Sarawak. The larvae of this species have also been collected from Mahua (1063 m), Liwagu (1500 m), and Trus Madi (Shimada *et al.*, 2007b; altitude unknown). According to Yang (1991), tadpoles similar to this species had been collected from Sg. Panataran [Melangkap; FMNH (Field Museum of Natural History) 229861], Sg. Mesilau (Mesilau; FMNH 229862), Sg. Bambangan (Mamut; FMNH 229863), Sg. Kipungit (Poring; FMNH 130893), and Sg. Pegalan (FMNH 228007; J. Ladonski, pers. comm.).

Discussion

A description of a new taxon, *Meristogenys dyscritus*. The two forms of *Meristogenys* discussed here share many characters such as small body size, incomplete toe webs, and pigmented ventral surfaces of the tibia, and have been identified as *M. amoropalamus*. However, the large genetic distances and differences in larval morphology between them (Shimada *et al*., 2007a) strongly suggest that they are distinct species. In the phylogenetic trees of *Meristogenys*, these two lineages did not make a monophyletic group (Fig. 2). We need more comparisons to judge whether the morphological similarities between these lineages are the results of homoplasy (convergence or linear evolution) or just ancestral characters, but anyway, it is clear that we should discover new morphological characters to differentiate these frogs.

Of these two forms, we concluded that Group A (larval morphotype 1) is the true *M. amoropalamus*, and that Group B (larval morphotype 3-a) is an unknown cryptic species based on several lines of evidence. First, the DNA sequences extracted from the holotype and the female paratopotype were much closer to those of Group A than to those of Group B. Second, a discriminant analysis using 18 characters placed the holotype in Group A. Third, ovum colour of the female paratopotype resembled that of Group A in its lack of heavy pigmentations. Last, iris colour of the female paratopotype (dark yellow) was also similar to that of Group A after anaesthesia. Thus, we described Group B as a new species, *M. dyscritus*.

Shimada *et al*. (2007a) noted that the number of larval forms (11) of *Meristogenys* reported by that time was larger than the number of species known through adult morphology (eight). In the present study, we resolved a part of this problem by describing *M. dyscritus* as a new species; although larvae of *M. amoropalamus* and *M. dyscritus* were treated separately in Shimada *et al*.'s (2007a) larval count (larval morphotypes 1 and 3-a), but these two species were treated as conspecifics at that time.

Until now, 12 species from this genus have been described (*M. amoropalamus*, *M. jerboa*, *M. kinabaluensis*, *M. macrophthalmus*, *M. maryatiae*, *M. orphnocnemis*, *M. phaeomerus*, *M. poecilus*, *M. stenocephalus*, *M. stigmachilus*, *M. whiteheadi*, and *M. dyscritus*), and larval morphologies of all species with the exception of *M. macrophthalmus* have been reported (Inger & Gritis, 1983; Shimada *et al*., 2007a, b, 2011; Inger & Stuebing, 2009; Matsui *et al*., 2009). However, three larval forms still remain to be associated with adult forms: Inger's (1966) larva C ("*Rana whiteheadi"* in Boulenger, 1893; *M. "whiteheadi"* in Yang, 1991 and Malkmus *et al*., 2002), Inger & Gritis' (1983) larva H (larva H in Inger, 1985) and Yang's (1991) "*M. orphnocnemis*". These larval forms possess unique characteristics that are uncommon in other larvae. For example, on the upper jaw, larva C and Yang's (1991) "*M. orphnocnemis*" have four or five rows of upper labial teeth, unlike most other known *Meristogenys* larvae, which have six or seven rows (Shimada *et al*., 2007a). Larva H is unique in having melanin-capped surface projections. We must add more specimens and conduct molecular analyses to verify whether these larval forms are unknown species or just exhibiting morphological variations of known species.

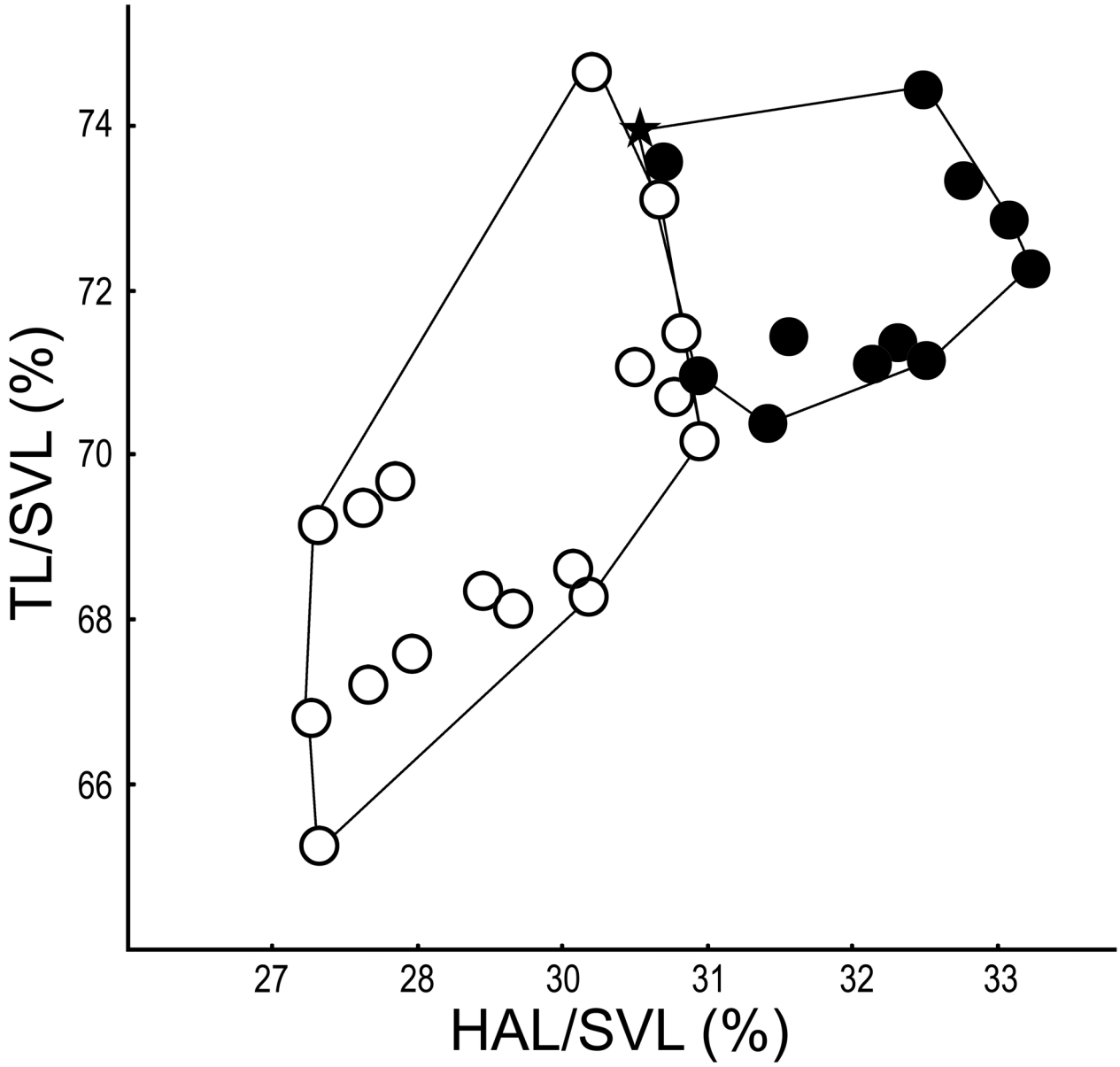

FIGURE 10. Plots of percentage ratios of HAL and TL to SVL in females. Closed dots indicate *Meristogenys amoropalamus* (the asterisk is the paratopotype of *M. amoropalamus*), while open dots indicate *M. dyscritus* (Group B).

Distribution of *M. amoropalamus* and *M. dyscritus.* Because larvae of *M. amoropalamus* and *M. dyscritus* were collected sympatrically at Mahua and Liwagu, these two species can co-exist at least during a part of their lives. However, in northern Sabah, the former mainly inhabits higher areas than the latter (Table 1). In the highest range, around 1800 m a.s.l. (Mesilau and Kamborangah), we only collected adults and larvae of *M. amoropalamus*, while in the middle range from 900 to 1500 m (Liwagu, Mahua, and Wario), we collected adults and larvae of *M. dyscritus*. In some low-mountain areas, such as Bundu Tuhan (990 m) and Poring (500 m), we collected several larvae of *M. dyscritus*, but never adults. These larvae might have flown from higher streams where their parents reside. Larval *M. amoropalamus* collected from Mahua (1063 m) and Liwagu (1500 m) may also have flown from higher streams because no adults of *M. amoropalamus* have been collected from there. In northern Sarawak (Bario) and Kalimantan (Gn. Tapai Sia), the distributional range of *M. amoropalamus* was lower compared to northern Sabah.

The collection sites of adult *M. amoropalamus* in northern Sabah (Mesilau and Kamborangah) are known as the highest distribution sites of *Meristogenys* (Smith, 1931; Inger, 1966, 1985; Matsui, 1979, 1986; Inger & Gritis, 1983; Malkmus *et al*., 2002; Shimada *et al*., 2007a, b, 2008; Matsui *et al*., 2009). From Mesilau, Malkmus *et al*.

(2002) reported the occurrence of *M. amoropalamus*, *M. kinabaluensis* and *M. whiteheadi*, but we were unable to ascertain the existence of the latter two species despite several field studies and surveys of specimen collections in Sabah Parks. From Kamborangah (on Kamborangah Road), Matsui (1979) collected a juvenile *Meristogenys* called "*Amolops* sp. B. Although this specimen lacked outer metatarsal tubercles, other characters did not contradict those of *M. amoropalamus*. These facts suggest that *M. amoropalamus* is the only species of *Meristogenys* inhabiting Mesilau and Kamborangah, and is probably the inhabitant of the highest areas for this genus.

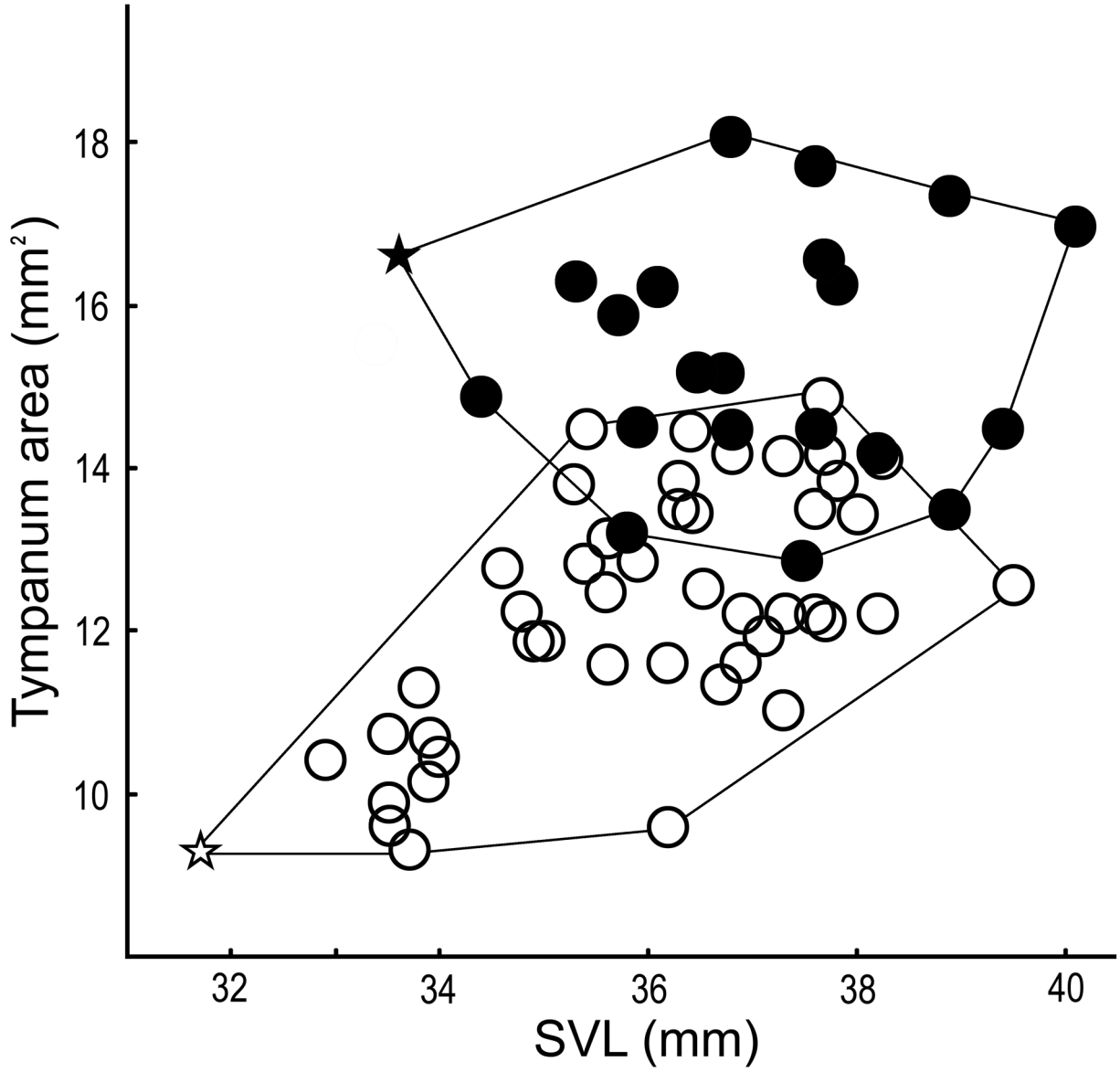

FIGURE 11. Relationship between SVL and the tympanum area (TDv*TDh*π/4) in males. Closed dots indicate *Meristogenys amoropalamus* (the asterisk is the holotype of *M. amoropalamus*), while open dots indicate *M. dyscritus* (the asterisk is the holotype of *M. dyscritus*).

Reproductive characters of *M. amoropalamus* and *M. dyscritus.* *Meristogenys dyscritus* and *M. amoropalamus* share many characteristics of adult external morphology, but differ significantly in reproductive characters. Although ovary volume did not differ between the two species (Mann–Whitney U-test, $p > 0.05$), *M. dyscritus* lays twice more eggs than *M. amoropalamus* (Fig. 4), while the ovum volume of *M. dyscritus* is half that of *M. amoropalamus* (Fig. 5) on average. This relationship seems to be a trade-off between the size and number of offspring (Bagon *et al*., 2005). Production of many small eggs, as in *M. dyscritus*, is more adaptive for high mortality, frequent disturbances or severe competition (r-selected species) (MacArthur & Wilson, 1967). In contrast, production

of small numbers of large eggs, as in *M. amoropalamus*, is preferred in stable environments with low mortality (K-selected species) (MacArthur & Wilson, 1967). Based on these views, larval *M. dyscritus* experiences higher mortality than larval *M. amoropalamus*.

We have no quantitative data for their natural history or larval mortality, but at Mahua, larval *M. amoropalamus* seemed to be more cryptic than larval *M. dyscritus*. At that site, we collected larval *M. dyscritus*, *M. kinabaluensis*, *M. orphnocnemis*, and *Huia cavitympanum* by scraping rock surfaces with a net (Shimada *et al*., 2007a). These larvae were found to form a mixed cluster during the night, but no larval *M. amoropalamus* were found in such clusters. To collect larval *M. amoropalamus*, we had to turn over rocks in the stream, holding a net below the rocks. Together with pebbles and gravel stirred up by turning rocks, larval *M. amoropalamus* sometimes entered the net. This observation indicates that larval *M. amoropalamus* tends to be interstitial in life and seldom appears on the upper surfaces of rocks.

The difference in egg colour might also reflect the variations in reproductive traits. While ova of *M. dyscritus* have pigmentation on the animal hemisphere as seen in other *Meristogenys* species, *M. amoropalamus* has creamy white ova without heavy pigmentation. The unpigmented ova, like those of *M. amoropalamus*, are known to be deposited in places not exposed to direct sunlight (Duellman & Trueb, 1994), such as underground streams (*Rana tagoi*) (Maeda & Matsui, 1990), the shade of rocks (*Rana narina* complex) (Matsui, 1994) and the inside of caves (*Onychodactylus fischeri*) (Park, 2006). Female *Meristogenys* are thought to usually lay their eggs on rock surfaces in rapidly flowing streams (Malkmus *et al*., 2002), but inter-specific variations have never been surveyed. From the reproductive characteristics clarified, *M. amoropalamus* appears to lay eggs in dark and cryptic sites in the stream. To ascertain this inference and elucidate the evolutionary significance of reproductive variations, we need to accumulate more knowledge of their reproductive sites, embryonic development and predators during early development.

Key to *Meristogenys* larvae from Sabah

1. Seven rows of upper labial teeth 2
- Six rows of upper labial teeth 5
2. Tail glands on both fins, head and body without surface projections . . . . . *M. amoropalamus*
- Tail glands absent or limited to ventral fin, head and body with surface projections 3
3. Lower jaw sheaths divided by a narrow vertical line or a crack . . . . . *M. maryatiae*
- Lower jaw sheath undivided. 4
4. Ventral tail glands usually less than six. . . . . *M. stenocephalus*
- Ventral tail glands usually more than six . . . . . *M. stigmachilus* . . . . . *M. whiteheadi*
5. Lower jaw sheath undivided, ventral glands present . . . . . *M. kinabaluensis*
- Lower jaw sheaths divided by a broad space, ventral glands absent 6
6. Head and body rectangular, usually seven or eight rows of lower labial teeth . . . . . *M. dyscritus*
- Head and body oval, usually six rows of lower labial teeth . . . . . *M. orphnocnemis*

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APPENDIX 1.

Specimens used in molecular and/or morphological analysis. BORNEENSIS: University Malaysia Sabah; KUHE: Graduate School of Human and Environmental Studies, Kyoto University; OMNH: Osaka Museum of Natural History; SP: Sabah Parks.

M. amoropalamus
Adults (n = 33)
Indonesia, East Kalimantan: OMNH Am8067 and Am8068 from Gunung Tapai Sia, Krayan County.
Malaysia, Sabah: SP 26612 from Kamborangah; BORNEENSIS 22901-22902, 22930, 22958-22959, KUHE 39288-39290, 39363-39369, 39458-39463, SP 21424-21425 from Mesilau.
Malaysia, Sarawak: KUHE 53059, 53061-53062, 53109-53112 from Bario.
Larvae (n = 78)
Malaysia, Sabah: BORNEENSIS 05B245-05B247 from Mahua, SP 3795, 3808 from Liwagu, BORNEENSIS 06B050 from Mesilau, uncatalogued specimens of BORNEENSIS from Trus Madi.

***M. dyscritus* sp. nov.**
Adults (n = 64)
Malaysia, Sabah: BORNEENSIS 8869, 12476, 12480, 12520, 12621, 12623, 12626-12628, SP 21545, 21605, 21624-21625 from Mahua, BORNEENSIS 22970, 22991, 23003, 23015, 23045, 23048-23049, 23341 23342, KUHE 39411-39423, 39427, 39447 from Wario, OMNH Am8069, SP 21392-21396, 21500, 21514, 26468-26486 from Liwagu.
Larvae (n = 75)
Malaysia, Sabah: BORNEENSIS 03B005, 03B023a (53 specimens), 03B202-03B203, 03B256, 03B343-03B344, 03B346-03B349, 03B350a, 05B046, SP 3810 (two specimens) from Mahua, BORNEENSIS 04B129 from Bundu Tuhan, BORNEENSIS 05B180-05B182 from Wario, BORNEENSIS 05B129, SP 3796 from Liwagu, BORNEENSIS 06B047-06B048 from Poring.