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Two new species of *Kalophrynus* Tschudi, 1838 (Anura: Microhylidae) from the Annamite mountains in southern Vietnam

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Abstract

We describe two new mountain-dwelling microhylid species of the genus *Kalophrynus* from the southern part of the Annamite Mountains in Vietnam. The two new species differ from all known congeners in morphological characters and mtDNA; phylogenetically, they form a sister clade to the large-bodied *K. interlineatus* (1009 bp, 16S rRNA gene, mtDNA). Both species share the following characteristics: snout pointed in dorsal and lateral views, slightly sloping in profile; tympanum distinct, smaller than eye in diameter; toe webbing moderate; outer metatarsal tubercle present; light dorsolateral line absent. *Kalophrynus cryptophonus* **sp. nov.** from Loc Bao, Lam Dong Province is a small-sized species distinguishing from its congeners by a combination of: SVL 27.9–30.4 mm in males, 23.4 mm in female; canthus rostralis indistinct; males with large sharp conical spines on the skin covering mandible margins and finely asperous nuptial pads on the dorsal surface of fingers I–III; dark ocelli in the inguinal region usually present, small, without a light border; anterior palatal dermal ridge short, restricted to medial part of palate. *Kalophrynus honbaensis* **sp. nov.** from Hon Ba, Khanh Hoa Province is a medium-sized *Kalophrynus*, distinguishing from its congeners by a combination of: SVL 26.7–36.8 mm in males; canthus rostralis distinct; males without distinguishable spines on the mandible margins nor the nuptial pads; dark ocelli in the inguinal region present, large, without a distinct light border, anterior palatal dermal ridge developed, parallel to posterior one. *Kalophrynus cryptophonus* **sp. nov.** reproduces in hollow bamboo stems; we describe larval morphology and bioacoustics of this species in relation to phytotelm breeding. A review of the distribution of the genus *Kalophrynus* in Indochina is provided.

Key words: Indochina, taxonomy, 16S rRNA, advertisement call, tadpole, phytotelm breeding, *Kalophrynus cryptophonus* **sp. nov.**, *Kalophrynus honbaensis* **sp. nov.**

Introduction

The genus *Kalophrynus* Tschudi, 1838, or sticky frogs, is the only representative of the subfamily Kalophryninae Mivart, 1869 and is recovered as a phylogenetically divergent group of Microhylidae, showing affinities to Melanobatrachinae and Asterophrynae (Van Bocxlaer *et al.* 2006; Frost *et al.* 2006; Pyron & Wiens 2011). The assumed Mesozoic origin of the group allow some authors to regard it as a distinct family of anurans (Bossuyt & Roelants 2009). At present, the genus *Kalophrynus* encompasses 19 species of small to medium sized litter frogs widely distributed in Southeast Asia, from northeastern India (Assam State) through Myanmar, southern China, Indochina (Vietnam, Cambodia and Laos) and southwards to Thailand, Malaysia, Indonesia, and the Philippines (Frost 2014; AmphibiaWeb 2014).

The species diversity of *Kalophrynus* is found in Peninsular Malaysia and on Borneo (Inger 1966; Ohler & Grosjean 2005; Matsui 2009). The Indochina and neighboring areas are notably depauperate in *Kalophrynus* diversity, with only two of 19 recognized species of this genus being recorded from this region. One of these, the

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widely distributed large-sized *Kalophrynus interlineatus* Blyth, 1855, typically inhabits relatively low elevations from southern China (Yunnan, Guangxi, Hainan, and Guangdong provinces) to southern Vietnam, Laos, southern Cambodia, southeastern Myanmar to eastern and northwestern Thailand, Assam, northeastern India, and Bangladesh (Nguyen *et al.* 2009; Matsui *et al.* 2012). The second smaller species is *Kalophrynus menglienicus* Yang & Su 1980, distributed mainly in southern China (Menglien, southern Yunnan Province) and according to Ohler & Grosjean (2005), also recorded from Lang Son, Cao Bang Province in northern Vietnam. However, this record was not confirmed and probably is based on a misidentification, thus *K. menglienicus* is not included in the latest list of the Vietnamese herpetofauna (Nguyen *et al.* 2009).

During fieldwork in the southern part of the Annamite Mountains in 2013, we collected specimens of two *Kalophrynus* species of unclear taxonomic status which clearly differ from *K. interlineatus*, the only *Kalophrynus* species confirmed from Vietnam to date (Nguyen *et al.* 2009). Further investigation of morphological variation and mtDNA sequences, as well as the study of reproductive biology and advertisement call of one of those species, confirmed that both populations represent unknown *Kalophrynus* taxa; we describe them herein as new species.

Material and methods

Sample collection. All specimens were collected during fieldwork in southern Vietnam, in April 2013 within Loc Bao Forest Enterprise (Lam Dong Province, Bao Lam District, approximate coordinates 11° 44' N, 107° 42' E, nearly 800 m a.s.l.), and in June 2013 in Hon Ba Nature Reserve (Khanh Hoa Province, Cam Lam District, approximate coordinates 12°07' N, 108° 56' E, nearly 1500 m. a.s.l.). The geographic position of the surveyed localities and the distribution of *Kalophrynus* species in Annamite Mountains (Truong Son) and adjacent regions (central and southern Vietnam, southern Laos, eastern Cambodia) are shown in Figure 1.

DNA isolation, PCR and Sequencing. For molecular analysis, total genomic DNA was extracted from ethanol-preserved muscle or liver tissues using standard phenol-chloroform extraction procedures (Hillis *et al.* 1996) followed with isopropanol precipitation. The isolated total genomic DNA was visualized in agarose electrophoresis in presence of ethidium bromide. The concentration of total DNA was measured in 1 μl using NanoDrop 2000 (Thermo Scientific), and consequently adjusted to ca. 100 ng DNA / μL. We amplified two mtDNA fragments of 16S rRNA mtDNA gene, which altogether comprised 1009 bp-length fragment of the gene. 16S rRNA is a molecular marker widely applied for biodiversity surveys in amphibians (Vences *et al.* 2005a; 2005b; Vieites *et al.* 2009), and proved to be particularly useful in studies of Microhylidae cryptic diversity (Matsui 2011; Matsui *et al.* 2011; Shimada et al., 2011; Li et al., 2012).The obtained fragments were sequenced in both directions for each sample, and a consensus sequence was generated. Amplification was performed in 25 μl reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl 2, Taq PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl 2 and 0.01% gelatine) and 1 U of Taq DNA polymerase. Primers used in PCR and sequencing followed Matsui *et al.* (2011) and are summarized in Table 1. The PCR conditions followed Matsui *et al.* (2011) and included an initial denaturation step of 5 min at 94°C and 33cycles of denaturation for 30 s at 94°C, primer annealing for 30 s at 48–50°C, and final extension step for 1 min 30 s at 72°C.

TABLE 1. Primers used in this study.

Direction	Primer name	Primer sequence	Reference
F	L1879	CGTACCTTTTGCATCATGGTC	Matsui *et al.* (2010)
F	L2188	AAAGTGGGCCTAAAAGCAGCCA	Matsui *et al.* (2006)
F	16L-1	CTGACCGTGCAAAGGTAGCGTAATCACT	Hedges (1994)
R	H1923	AAGTAGCTCGCTTAGTTTCGG	Matsui *et al.* (2010)
R	H2317	TTCTTGTTACTAGTTCTAGCAT	Shimada *et al.* (2011)
R	Will6	CCCTCGTGATGCCGTTGATAC	Wilkinson *et al.* (2002)

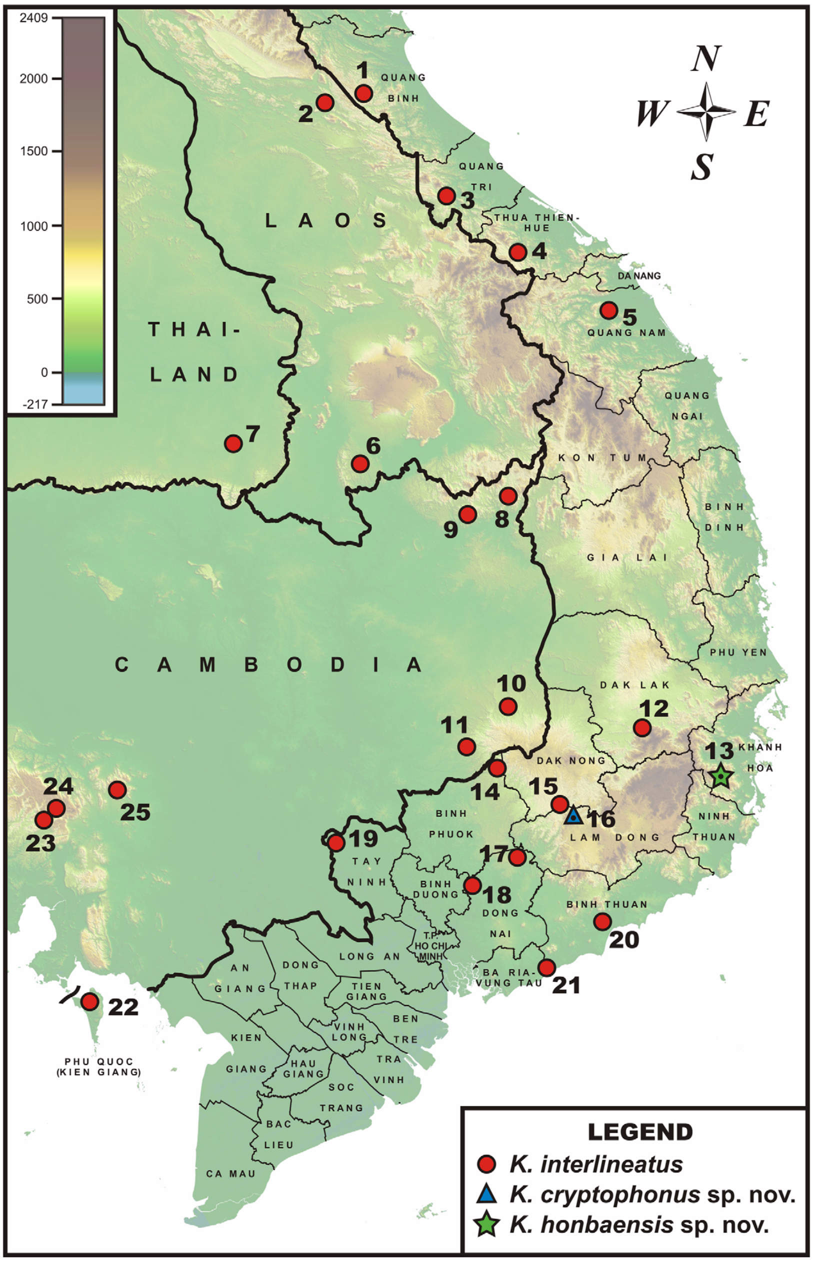

FIGURE 1. Distribution of *Kalophrynus* species in southern Indochina: Annamite Mountains and adjacent regions. Black dot in the center of an icon indicate the type locality of new species. **Locality information.** ***K. interlineatus*****: Vietnam:** 1—Quang Binh Prov., Phong Nha—Ke Bang NP (Nguyen *et al.* 2009); 3—Quang Tri Prov., no exact locality (Orlov & Anajeva 2007); 4—Thua Thien—Hue Prov., no exact locality (Orlov & Anajeva 2007); 5—Quang Nam Prov., 2.5 mi. NW. of Hoa Phat (CAS-HERP115821); 12—Dak Lak Prov., Chu Yang Sin NP (BirdLife International 2010); 14—Binh Phuoc Prov., Bu Gia Map NP (this paper); 15—Lam Dong Prov., Bao Lam Distr., Loc Bao forest, near the border with Dak Nong Prov. (this paper); Dong Nai Prov.: 17—Nam Cat Tien NP (Nguyen *et al.* 2009; this paper); 18—Ma Da forest (Vinh Cuu Reserve) (Nguyen *et al.* 2009; this paper); 19—Tay Ninh Prov., Lo Go—Xa Mat NP (this paper); 20—Binh Thuan Prov., Ta Kou NR (Duc Minh Hoang pers. comm., this paper); 21—Ba Ria—Vung Tau Prov., Binh Chau—Phuoc Buu NP (this paper); 22—Kien Giang Prov., Phu Quoc NP (Nguyen *et al.* 2009; this paper). **Laos:** 2—Khammouan Prov. (FMNH255124; Stuart 2005); 6—Champassak Prov., Xepian NBCA (Teynié *et al.* 2004). **Thailand:** 7—Ubon Ratchatani Prov. (FMNH265846-265847). **Cambodia:** Ratanakiri Prov.: 8—Ta Veng Distr., Virachey NP (MVZ-HERP-258069); 9 –Veunsai Distr., Virachey NP (MVZ-HERP-258070); Mondol Kiri Prov.: 10—Pechrad [Pichrada] Distr., Pnom Nam Lyr Mt. (Stuart *et al.* 2006); 11—Kaev Seima [Keo Seima] Distr. (Stuart *et al.* 2006); Kaoh Kong Prov.: 23—Thmar Baing Distr., Areng Valley (Stuart & Emmett 2006); 24—Thmar Baing Distr., Tatai Leu (Stuart & Emmett 2006); 25—Kampong Speu Prov.,Pnom Aural Mt. (Ohler *et al.* 2002). ***K. honbaensis*** **sp. nov.**: 13—Khanh Hoa Prov., Hon Ba Mt. (this paper). ***K. cryptophonus*** **sp. nov.**: 16—Lam Dong Prov., Bao Lam Distr., Loc Bao forest, near the border with Dak Nong Prov. (this paper).

PCR products were loaded onto 1% agarose gels, stained with GelStar gel stain (Cambrex), and visualized in a Dark reader transilluminator (Clare Chemical). If results were satisfying, products were purified using 2 µl, from a 1:4 dilution of ExoSapIt (Amersham), per 5 µl of PCR product prior to cycle sequencing. A 10 µl sequencing reaction included 2 µL of template, 2.5 µl of sequencing buffer, 0.8 µl of 10 pmol primer, 0.4 µl of BigDye Terminator version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 µl of water. The cycle-sequencing reaction was 35 cycles of 10 sec at 96° C, 10 s at 50° C and 4 min at 60° C. Cycle sequencing products were purified by ethanol precipitation. Sequence data collection and visualization were performed on an ABI 3730xl automated sequencer (Applied Biosystems). The obtained sequences are deposited in GenBank under the accession numbers KJ418711–KJ418722 (see Table 2).

Phylogenetic analysis. In total, 16S rRNA partial sequences of 27 specimens (26 representatives of *Kalophrynus* and 1 outgroup *Kaloula pulchra*) with total length up to 1009 bp were included in the final alignment and subjected to phylogenetic analyses. Sequences were also submitted to a BLAST search in GenBank to confirm that the required sequences had been amplified. Nucleotide sequences were checked by eye using Chromas Pro software (Technelysium Pty Ltd., Tewntin, Australia), aligned using ClustalX 1.81 (Thompson *et al.* 1997) with default parameters, and then carefully optimized in BioEdit 7.0.5.2 (Hall 1999). Phylogenetic analyses were conducted in Treefinder (Jobb *et al.* 2004), PAUP version 4.0b4a (Swofford 1998) and MEGA 5.0 (Tamura *et al.* 2011). Uncorrected genetic distances (*p*-distance) between sequences were calculated in MEGA 5.0. Optimal Neighbour-Joining (NJ) trees were constructed in MEGA 5.0. NJ trees were calculated based on evolutionary distances obtained using the Maximum Composite Likelihood method (Nei & Kumar 2000). Maximum Likelihood (ML) analysis was run in Treefinder (Jobb *et al.* 2004). Transitions and transversions were equally weighted, and gaps were treated as missing data. MODELTEST v.3.06 (Posada & Crandall 1998) was used to estimate the optimal evolutionary models to be used for the data set analysis. A general time-reversible (GTR) model of DNA evolution with a gamma shape parameter (G) was identified as the best-fitting model according to Akaike Information Criterion (AIC). We performed a heuristic ML search (Felsenstein 1985a) with ten random-addition sequence replicates and TBR branch swapping. The Maximum Parsimony (MP) analysis was conducted with the program PAUP* version 4.0b4a (Swofford 1998), using a heuristic search and the closest step-wise sequence addition algorithm. Most-parsimonious trees were generated with 100 random-addition sequences and the tree-bisection-reconnection (TBR) algorithm, for branch swapping. For the maximum parsimony (MP) analysis we preformed ten random-addition replicates. Transitions and transversions were equally weighted. Confidence in tree topology was assessed by non-parametric bootstrap analysis (Felsenstein 1985b) with 1000 replicates for all types of the analyses. We *a priori* regarded tree nodes with bootstrap values 75% or greater as sufficiently resolved, those between 75% and 50% were regarded as tendencies, those below 50% were considered to be non-resolved (Huelsenbeck & Hillis 1993).

We compared the obtained sequences of Vietnamese *Kalophrynus* species with the data available in GenBank. We used sequences of *Kaloula pulchra* (JX678902) as an outgroup, since *Kalophrynus* was shown to be quite distant in respect to Microhylinae in the analysis of Matsui *et al.* (2011). The data on voucher specimens and GenBank sequences used in phylogenetic analyses are summarized in Table 2.

Adult morphology. The collected specimens were photographed in life and tissue samples for genetic analysis were taken and stored in 96% ethanol prior to specimen preservation All other specimens were fixed and stored in 75% ethanol except for ZMMU A-4944 and A-4859, which were fixed in 10% formalin and then transferred to 75% ethanol for storage. Gender was determined by dissection. Webbing formulae are given following Savage (1975). All measurements were taken using digital caliper under the light dissecting microscope to the nearest 0.1 mm; morphometrics mainly follow Matsui (2009) and Dehling (2011): snout-vent length (SVL), head length (HL, from posterior end of mandible to snout tip), head width (HW, at the level of jaw articulations), snout length (SL, from anterior edge of eye to snout tip), snout-nostril distance (SND, from center of nostril to snout tip), eye-nostril distance (END, from anterior edge of eye to center of nostril), eye horizontal diameter (ED), upper eyelid width (UEW), interorbital distance (IOD, shortest distance between upper eyelids), internarial distance (IND, between centers of nostrils), tympanum horizontal diameter (TD), inguinal spot diameter (ISD), forearm–hand length (FHL, length of straightened forearm from elbow to tip of finger III), humerus length (HumL, from forelimb insertion to elbow), total forelimb length (FLL), length of fingers I–IV (1FL, 2FL, 3FL= hand length, 4FL, from proximal edge of palmar tubercle to tips of fingers I–IV, respectively), thigh length (ThL), tibiofibula length (TFL, from knee to tibio-tarsal articulation), tarsus+foot length (TarFL, from tibio-tarsal articulation to the tip of toe IV), foot length

TABLE 2.Specimens and GenBank sequences used in molecular analyses. AN—Accession number.

No.	Species	Locality	Voucher ID	GenBank AN	Reference
1	*K. baluensis*	Malaysia	VUB-0617	EF017953	Van Bocxlaer *et al.* 2006
2	*K. heterochirus*	Malaysia, Sabah, Crocker Range Park	BORN-8089	AB634697	Matsui *et al.* 2011
3	*K. interlineatus*	Myanmar, Chatthin	USNM-GZ-33787	AB634698	Matsui *et al.* 2011
4	*K. interlineatus*	Pet trade	no voucher	AB611916	Kurabayashi *et al.* 2011
5	*K. interlineatus*	Vietnam, Lam Dong, Loc Bao	ZMMU-NAP-02829	KJ418719	this paper
6	*K. interlineatus*	Vietnam, Lam Dong, Loc Bao	ZMMU-NAP-03403	KJ418720	this paper
7	*K. interlineatus*	Vietnam, Kien Giang, Phu Quoc	ZMMU-NAP-03755	KJ418721	this paper
8	*K. interlineatus*	Vietnam, Kien Giang, Phu Quoc	ZMMU-NAP-03810	KJ418722	this paper
9	*K. interlineatus*	Vietnam, Dong Nai, Nam Cat Tien	ZMMU-NAP-02434	KJ418717	this paper
10	*K. interlineatus*	Vietnam, Ba Ria–Vung Tau, Binh Chau	ZMMU-NAP-03071	KJ418718	this paper
11	*K. interlineatus*	Southern China	KIZHERP0169	JX678906	Li *et al.* 2012
12	*K. intermedius*	Malaysia	VUB-0617	EF017952	Van Bocxlaer *et al.* 2006
13	*K. limbooliati*	Malaysia, Joho, Pulai	KUHE 53284	AB634701	Matsui *et al.* 2011
14	*K. palmatissimus*	Malaysia, Pahang, Temerloh	KUHE-52454	AB634699	Matsui *et al.* 2011
15	*K. pleurostigma*	Indonesia, Sumatra, Lampung	MZB-Amp-15295	AB634700	Matsui *et al.* 2011
16	*K. pleurostigma*	Peninsular Malaysia	VUB-0601	AY948733	Roelants *et al.* 2007
17	*K. pleurostigma*	Malaysia, Sabah, Tawau Hills NP	ZMH A10025	GU154883	Haas & Das 2010
18	*K.* cf. *stellatus*	Pet trade (Philippines?)	KUHE-35230	AB634702	Matsui *et al.* 2011
19	*K. subterrestris*	Malaysia, Sarawak, Tubau	KUHE-53145	AB634703	Matsui *et al.* 2011
20	*K. yongi*	Malaysia, Pahang, Cameron	KUHE-15531	AB634704	Matsui *et al.* 2011
21	*K. cryptophonus* **sp. nov.**	Vietnam, Lam Dong, Loc Bao	ZMMU-ABV-00136	KJ418711	this paper
22	*K. cryptophonus* **sp. nov.**	Vietnam, Lam Dong, Loc Bao	ZMMU-ABV-00222	KJ418712	this paper (**holotype**)
23	*K. cryptophonus* **sp. nov.**	Vietnam, Lam Dong, Loc Bao	ZMMU-ABV-00223	KJ418713	this paper
24	*K. cryptophonus* **sp. nov.**	Vietnam, Lam Dong, Loc Bao	ZMMU-ABV-00226	KJ418714	this paper
25	*K. honbaensis* **sp. nov.**	Vietnam, Khanh Hoa, Hon Ba	ZMMU-ABV-00302	KJ418715	this paper
26	*K. honbaensis* **sp. nov.**	Vietnam, Khanh Hoa, Hon Ba	ZMMU-ABV-00320	KJ418716	this paper (**holotype**)
27	*Kaloula pulchra*	Southern China	KIZHERP3003	JX678902	Li *et al.* 2012

(FL, from proximal end of inner metatarsal tubercle to IV toe tip), total hind limb length (HLL, length of fully extended leg at right angle to body from vent to IV toe tip), inner metatarsal tubercle length (IMTL), outer metatarsal tubercle length (OMTL), toe I length (1TL, from proximal end of inner metatarsal tubercle to the tip of toe I).

For comparison we examined voucher specimens of *Kalophrynus interlineatus* Blyth from southern Vietnam and Laos, and *Kalophrynus pleurostigma* Tschudi from southern Thailand (see Appendix).

The diagnosis of the genus *Kalophrynus* and morphological characters chosen for comparison were taken from Boulenger (1900), Stejneger (1908), Smith (1922), Parker (1934), Bourret (1942), Inger (1966), Yang & Su (1980), Dring (1983), Manthey & Grossmann (1997), Iskandar (1998), Das & Haas (2003), Matsui (2009), Dehling (2011), Matsui & Nishikawa (2011), Chan *et al.* (2011), and Matsui *et al.* (2012).

Larval morphology. Collected tadpoles (ZMMU A-4986; field number ABV-00234) were fixed in 5% formalin and staged accordingly to Gosner (1960). Tadpole gross morphology was examined using a LEICA EZ4 dissecting stereo microscope with digital photo attachment. Original drawings were done by A.B. Vassilieva based on a series of photographs. Tadpole morphometrics mainly follow Bowatte & Meegaskumbura (2011) and included the following measurements (taken with the use of digital caliper and microscopic scale bar to the nearest 0.01 mm): total length (TL), body length (BL), maximal body width (BW), maximal body height (BH), snout-vent length (SVL), snout-spiracle length (SSp), interpupilar distance (IPD), snout-eye distance (SED), eye diameter (ED), oral disk width (ODW), tail length (TaL) and maximal tail height (TH).

Acoustic analyses. Advertisement calls of the described *Kalophrynus* species were recorded in the habitat on 20th April 2013 from 22:30 to 23:36 at 25°C, using a Nikon D 600 digital SLR camera (Nikon Corporation, Japan) in video mode; in total, 105 advertisement calls from 11 individuals were recorded. The audio track was removed from video recording using Avisoft SASLab Pro software v. 5.2.05 (Avisoft Bioacoustics, Germany) with a 22.05 kHz sampling frequency and 16-bit precision.

Advertisement calls of *Kalophrynus interlineatus* (36 calls from two individuals) were recorded in the habitat in Cat Tien National Park on 8th September 2011 from 21:53 to 21:55 at24°C, using a Marantz PMD660 digital tape recorder (D&M Professional, Japan) at a sampling rate of 48 kHz in mono mode with the high-sensitivity Sennheiser K6 ME66 cardioid electret condenser microphone (Sennheiser Electronic, Germany).

Calls were analyzed using Avisoft SASLab Pro software v. 5.2.05; spectrograms for analysis were created using Hamming window, FFT-length 512 points, frame 100%, and overlap 87.5%. These settings provided a bandwidth of 56 Hz, frequency resolution of 43 Hz and time resolution of 2.9 ms. Calls for figure spectrograms were digitized with a 11025 kHz sampling frequency. Figure spectrograms were created using Hamming window, FFT-length 512 points, frame 50%, and overlap 87.5% or 93.75%. We measured the duration of each call, the interval between successive calls, the pulse period, the initial and final fundamental frequency and the frequency of maximum amplitude. The low-pass filter (up to 0.55 Hz) was applied to avoid low-frequency noise.

Types and referring material are stored in the herpetological collection of the Zoological Museum of Moscow State University (ZMMU) in Moscow, Russia. Advertisement calls were deposited at the Fonoteca Zoologica (http://www.fonozoo.com; accession numbers: 9122 to 9127).

Results

Phylogenetic analysis of the genus *Kalophrynus*

Sequence and statistics: The studied 16S rRNA mtDNA fragment consisted of 1009 sites, where 770 sites were conserved and 233 sites were variable, of which 151 were found to be parsimony-informative. The transition–transversion bias (R) was estimated as 1.91 (all data given for ingroup only). Substitution rates were estimated under the Kimura (1980) 2-parameter model (+G+I). Nucleotide frequencies were A = 34.9%, T = 25.8%, C = 22.3%, and G = 17.0%.

Phylogenetic relationships of Vietnamese *Kalophrynus*: The fragment of the 16S rRNA gene in this study partially resolved phylogenetic relationships between the studied species of *Kalophrynus*, with several nodes in the tree reflecting basal splits within the genus having low (BS < 75%) or insignificant levels (BS < 50%) of BS-support. However, the monophyly of species-level groups was significantly supported (BS > 90% for all types of analysis). ML and NJ analyses resulted in similar topologies (see Fig. 2). The MP-trees differed from ML and NJ topologies only in associations at poorly supported nodes. An overall assessment of the phylogenetic relationships among *Kalophrynus* species was not possible with available data, however, certain groups received significant support. The ML tree of the (Fig. 2) infered the following set of phylogenetic relationships among studied *Kalophrynus* species:

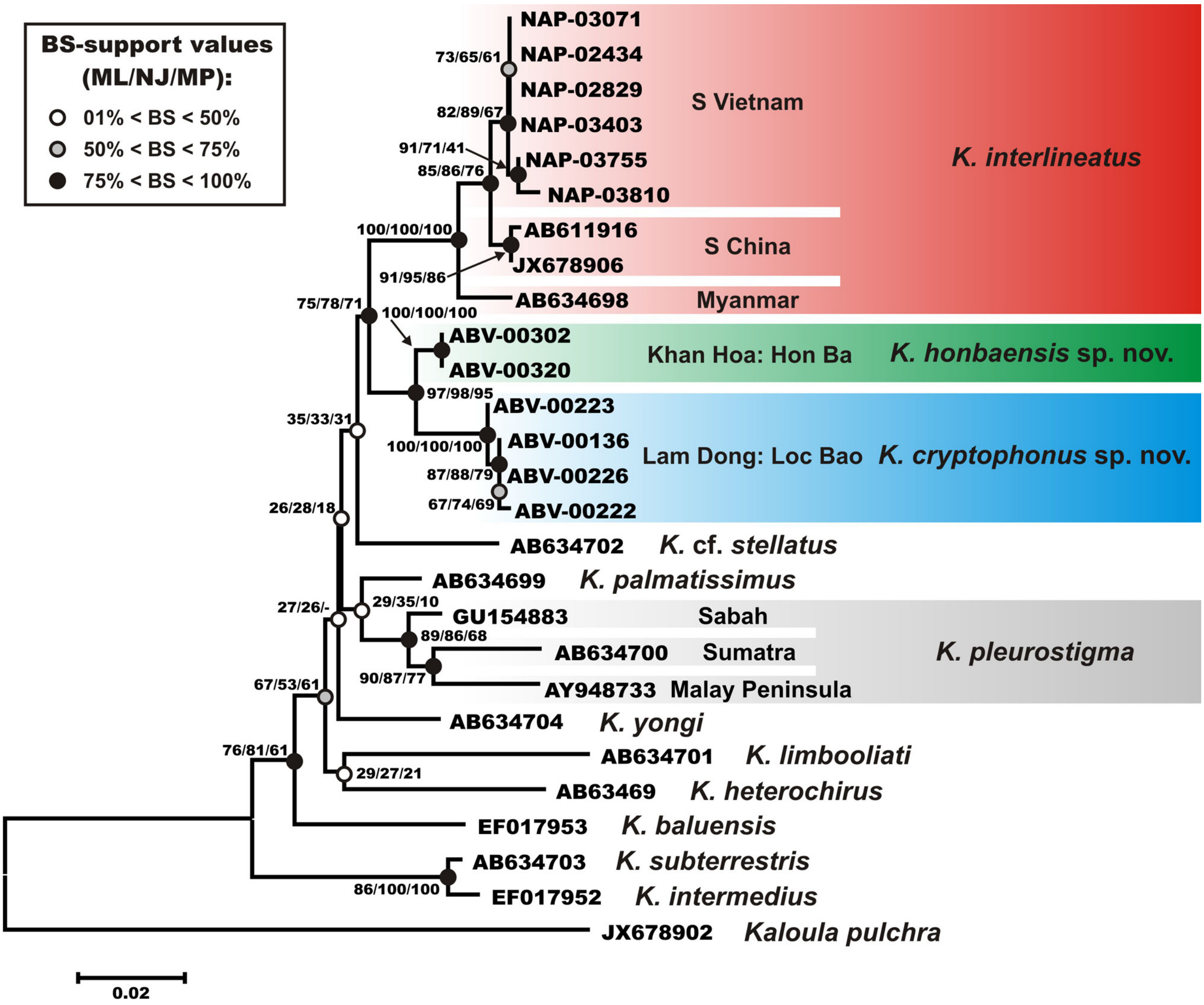

FIGURE 2. Maximum likelihood dendrogram of *Kalophrynus* derived from the analysis of 1009 bp of 16S rRNA mtDNA gene. Voucher sample and GenBank accession numbers are given in Table 2. *Kaloula pulchra* was used as an outgroup. Numbers near branches represent bootstrap support values (1000 replicates) for ML/NJ/MP inferences respectively. Node color reflects node support values (see Legend).

The most basal lineages of *Kalophrynus* were two lowland species from Sarawak, Borneo; *K. subterrestris* and *K. intermedius* formed a monophyletic group (86/100/100; hereafter BS values were given for ML/NJ/MP analyses respectively) which was recovered as a sister clade to all other studied *Kalophrynus* species in all analyses (BS 76/81/61).

K. baluensis, an endemic of Gunung Kinabalu, Sabah, Borneo, with poor yet significant support (BS 67/53/61), formed a sister clade in respect to all other *Kalophrynus* species; phylogenetic relationships among them were sufficiently unresolved.

The three specimens identified as *K. pleurostigma* from Borneo, Sumatra and Peninsular Malaysia formed a monophyletic group (BS 89/86/68), the specimens from Sumatra and Malay Peninsula were the most closely related to each other and formed a clade (BS 90/87/77).

TABLE 3. Uncorrected *p*-distance (percentage) between 16S rRNA sequences of *Kalophrynus* species included in phylogenetic analyses(below the diagonal) and standard error estimates (above the diagonal). The ingroup mean uncorrected *p*-distances are shown on the diagonal and shaded with grey.

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	*K. interlineatus*	**0.98**	0.87	0.74	0.77	0.81	0.82	0.87	1.04	0.90	0.92	1.20	1.20	1.47
2	*K. cryptophonus* **sp. nov.**	4.91	**0.21**	0.52	0.84	0.88	0.80	0.88	1.18	0.97	1.13	1.12	1.14	1.49
3	*K. honbaensis* **sp. nov.**	3.26	1.69	**0.00**	0.74	0.82	0.66	0.78	1.20	0.83	0.94	1.00	1.04	1.47
4	*K.* cf. *stellatus*	5.06	4.53	3.70	**-**	0.85	0.78	0.74	1.22	1.05	1.15	1.24	1.27	1.48
5	*K. yongi*	4.73	4.53	3.29	5.14	**-**	0.69	0.77	1.11	0.83	0.92	1.03	1.06	1.44
6	*K. palmatissimus*	4.48	3.92	2.68	3.92	2.89	**-**	0.78	1.08	0.89	0.97	1.01	1.08	1.46
7	*K. pleurostigma*	6.06	5.62	4.39	5.08	4.73	3.78	**3.57**	1.21	0.97	1.08	1.10	1.17	1.43
8	*K. limbooliati*	7.16	7.84	7.01	6.60	7.01	5.58	7.97	**-**	1.01	1.10	1.33	1.37	1.60
9	*K. heterochirus*	6.19	6.80	5.15	6.80	5.36	5.79	7.70	7.44	**-**	1.06	0.99	1.12	1.60
10	*K. baluensis*	6.61	6.58	5.35	6.58	5.14	5.57	7.00	7.22	6.60	**-**	1.06	1.06	1.47
11	*K. subterrestris*	8.16	7.22	5.98	8.45	6.80	6.61	8.25	9.71	7.02	7.22	**-**	0.47	1.56
12	*K. intermedius*	8.52	7.61	6.38	8.85	7.00	6.80	8.50	10.10	7.84	7.61	1.24	**-**	1.55
13	*Kaloula pulchra*	16.28	15.23	15.43	15.23	15.43	14.02	15.02	16.91	17.73	15.43	14.85	15.23	**-**

The phylogenetic position of the other *Kalophrynus* species sampled from Borneo and Malay Peninsula remained unclear. The specimen provisionally identified as *K. stellatus* in the work of Matsui *et al.,* 2011 (assumingly, from the Philippines; specimen obtained through pet trade and no locality is given in the original publication), was clearly distinct from *K. pleurostigma*, which contradicts the synonymy by Parker (1934) and Ohler & Grosjean (2005).

The Indochinese species of *Kalophrynus* in our analyses formed a significantly supported monophyletic group (BS 75/78/71). This group consisted of two major subclades. The first subclade included all large-bodied populations from southern China, Vietnam and Myanmar, identified as *K. interlineatus* (monophyly strongly supported: BS 100/100/100).The second subclade included two populations of small- to medium-sized montane *Kalophrynus* from the Southern part of the Annamite Mountains in Vietnam and is also significantly monophyletic (BS 97/98/95).

Within *K. interlineatus,* the specimen from Myanmar formed a sister clade to the south Vietnamese (BS 82/74/ 67) and south Chinese (BS 91/95/86) groups, which are significantly monophyletic (see Fig. 2).

The mountain-dwelling *Kalophrynus* from southern Annamites formed two clearly distinct monophyletic subclades corresponding to the medium-sized Hon Ba and small-bodied Loc Bao populations (in both cases BS 100/100/100), described below as the new species.

Sequence divergence: The uncorrected genetic *p*-distances among and within 16S rRNA gene fragment sequences of the studied *Kalophrynus* taxa are shown in the Table 3. Interspecific variation was from $p = 0\%$ in *Kalophrynus* sp. from Hon Ba to $p = 0.21\%$ in *Kalophrynus* sp. from Loc Bao.

The genetic distance between the *Kalophrynus* species in our study and the outgroup (*Kaloula pulchra*) varied from $p = 14.02\%$ (*K. palmatissimus*) to $p = 17.73\%$ (*K. heterochirus*). The observed intraspecific distances within the genus *Kalophrynus* based upon samples in our analysis varied from $p = 1.24\%$ to 10.10%, thus values of genetic distances in ingroup and outgroup comparisons did not overlap. The least intraspecific *p*-distance was found between the two closely related Bornean species from Sarawak: *K. intermedius* and *K. subterrestris* ($p = 1.24\%$), and the greatest *p*-distance observed was between *K. intermedius* and *K. limbooliati* from Malay Peninsula ($p = 10.02\%$).

Interspecific distances within *Kalophrynus* species in our analysis varied from $p = 0.0\%$ to 3.57% in *K. pleurostigma* complex; we assume that the overlap between inter- and intraspecific distances in the latter case was the result of the insufficient taxonomy of the group. The genetic variation within the *K. interlineatus* complex was less than $p = 1\%$, with the most distant specimen from Myanmar $p = 0.98\%$ divergent to the other populations of this species.

The two populations of *Kalophrynus* from the Annamite mountains which were found to be closely related to *K. interlineatus*, showed genetic distances to *K. interlineatus* of $p = 3.26\%$ for the Hon Ba population and $p = 4.91\%$ for the Loc Bao population. However, both species also showed low values of *p*-distance in comparisons with *K. palmatissimus* ($p = 2.68\%$ and 3.92%, respectively). The Hon Ba and Loc Bao populations were found to be genetically comparatively close to each other with the genetic distance between them equal $p = 1.69\%$ (see Table 3).

Taxonomic status of the Annamite lineages: The two small and medium-sized mountain populations of *Kalophrynus* from the Annamites are genetically distinct from other species of the genus, including their putative closest relative, the widespread Indochinese *K. interlineatus*. The genetic distance between these populations ($p = 1.69\%$) is not high, although it is notably higher than the smallest intraspecific distance for *Kalophrynus* reported between *K. intermedius* and *K. subterrestris* ($p = 1.24\%$), and comparable with genetic distances in other complexes of closely related species in Microhylidae, like *Kaloula verrucosa – Kaloula rugifera* group (p = 1.81%–2.02%; Li *et al.* 2012; our data). Vieites *et al.* (2009) reviewed application of 16S rRNA for studies of cryptic diversity in amphibians and proposed the 3% value of *p*-distance as a suggested threshold for candidate new species recognition. However, these authors also noted that in some cases the intraspecific distances can be below 2% or even 1% of substitutions. In these cases, taxonomic recognition is recommended when reproductive isolation is confirmed or when "a diagnostic morphological difference in a character that in the respective group of animals is known to be of low intraspecific variability and of high value to discriminate species" exists (Vieites *et al.* 2009). Our detailed morphological examination of the specimens in hand revealed a number of key characters important in *Kalophrynus* taxonomy, such as body size, the presence of skin spines on the head and nuptial pads on fingers in breeding males, form and size of palatal dermal ridges, form of canthus rostralis, degree of foot webbing, and size of inguinal spots. We assume that these populations, which are likely geographically isolated (see discussion), display correlated differences in morphology and mtDNA variation and describe them below as new species.

Systematics

Kalophrynus cryptophonus sp. nov.

Holotype. ZMMU A-4944 (field number ABV-00222), adult male from Loc Bao, Lam Dong Province, Vietnam (coordinates 11°44' 17" N, 107° 42' 25" E, elevation 800 m. a.s.l.), collected by E.A. Galoyan and A.B. Vassilieva on 22 April 2013.

Paratypes. ZMMU A-4858, one adult female (individual field number ABV-00136) collected on 12 of April 2013, and three adult males (individual field numbers ABV-00224, ABV-00225, ABV-00226) collected on 22 April 2013 in the same area as holotype; ZMMU A-4859, one adult male (individual field number ABV-00223), collected on 22 April 2013 in the same area as holotype.

Diagnosis. The species is allocated to *Kalophrynus* based on the following characters considered diagnostic for the genus (Parker 1934; Bourret 1942; Inger 1966): one or more transverse dermal ridges across the palate anteriorly to the oesophagus; tips of digits not dilated to disks; no spine-like projections at heel or elbow; snout short, pointed; tympanum visible; pupil horizontal; inner metatarsal tubercle low, not shovel-like; skin thick and glandular. The new species is distinguishable from its congeners by a combination of the following features: (1) small size, SVL 27.9–30.4 mm in males, 23.4 mm in female; (2) snout pointed, slightly sloping in profile; (3) canthus rostralis indistinct; (4) males with large sharp conical spines on the skin covering the jaws; (5) males with finely asperous nuptial pads on the dorsal surface of the fingers I–III; (6) tympanum distinct, smaller than eye in diameter; (7) toe webbing moderate; (8) outer metatarsal tubercle present; (9) light dorsolateral line absent; (10) dark ocellus in the inguinal region usually present, small, without light bordering; (11) anterior palatal dermal ridge short, restricted to medial part of the palate.

Description of holotype. Adult male (Fig. 3AB) with well-developed oblong testes; measurements are given in Table 4.

Habitus stout, with body widest in lumbar area. Head relatively short (HL/SVL 0.31), slightly wider than long (HL/HW 0.88), triangular. Snout pointed, short (SL/HL 0.36), sloping in profile (Fig. 3C) and distinctly projecting beyond lower jaw; canthus rostralis indistinct, smooth; nostrils rounded, situated closer to snout tip than to eye (SND/END 0.65) and oriented rostrolaterally. Eyes bulging, medium-sized (ED/SVL 0.11), lateral, well visible from below, with oval horizontal pupil; upper eyelid with fine tubercles on margin, UEW/IOD 0.45. Tympanum very close to eye, smaller than eye (TD/ED 0.69, TD/SVL 0.08), roughly round, distinct, bordered from above by sharply prominent supratympanic fold running from posterior corner of eye along upper edge of tympanum and then down to the point of forelimb insertion. Maxillary and vomerine teeth absent; tongue with even rounded edge posteriorly; transversal crenulated dermal ridge on palate posterior to eyes level, short low dermal fold anterior to it, restricted to medial part of palate; postchoanal dermal ridges distinct. Small notch in the rostral part of skin covering of upper jaw and small prominence on the rostral part of lower jaw, fitting together. Single subgular vocal sac; wide slit-like vocal openings between jaw articulation and tongue insertion.

Numerous tubercles with wide base and sharp, conical, semitransparent apical spine being clearly distinct on skin covering maxilla and mandible (Fig. 3D). Spines on mandible largest, bordering the lower jaw in one to three rows, from rostral end to mouth corner and spreading on the area below and behind tympanum; on upper jaw spines smaller and occupying the entire area below snout tip, nostrils and eyes. Several tiny, hardly distinct similar spines present on each tympanum. Small scattered spines spread on axillar area, on anterior forelimb surface and on breast between forelimbs, but not on gular region.

Forelimbs slender, more than half of body length (FLL/SVL 0.71); hands with rudimental webbing. Tips of fingers rounded, without dilatation. Relative length of fingers I<IV<II<III; palmar tubercle moderately large, prominent, oval in shape; subarticular tubercles prominent, rounded; one on each fingers I, II and IV and two on finger III; smaller additional metacarpal tubercles between palmar tubercle and each finger base (Fig. 4A).

Nuptial pads on dorsal hand well-defined, finely asperous, thickened areas of skin covering fingers I–III from base to proximal end of terminal phalange (Fig. 4C).

TABLE 4. Morphometric features in three *Kalophrynus* species occurring in southern Vietnam. For abbreviations see Material and methods. All measurements are given in mm.

Specimen	Sex	SVL	HL	HW	SL	SND	END	ED	UEW	IOD	IND	TD	ISD
***Kalophrynus cryptophonus* sp. nov.**													
ABV-00136	f	23.4	6.9	8.4	2.7	1.0	1.7	2.9	1.7	3.8	2.0	1.6	1.1
holotype ABV-00222	**m**	**28.3**	**8.9**	**10.1**	**3.2**	**1.3**	**1.9**	**3.1**	**2.1**	**4.7**	**2.4**	**2.2**	**1.7**
ABV-00223	m	28.2	8.0	9.8	3.1	1.2	2.0	3.2	2.2	4.8	2.6	2.2	1.7
ABV-00224	m	27.9	8.6	9.0	3.1	1.0	2.1	3.3	2.0	4.4	2.1	1.8	1.5
ABV-00225	m	30.4	9.1	9.7	3.2	1.3	1.9	3.3	2.2	4.7	2.2	1.9	1.5
ABV-00226	m	28.8	8.8	10.1	3.2	1.2	2.0	3.4	1.9	4.9	2.4	2.2	1.6
***Kalophrynus honbaensis* sp. nov.**													
ABV-00302	m	26.7	8.6	11.1	3.1	1.0	2.2	2.9	2.1	5.4	2.5	2.2	3.1
holotype ABV-00320	**m**	**36.8**	**11.1**	**13.5**	**3.7**	**1.4**	**2.3**	**4.1**	**2.7**	**6.3**	**3.5**	**3.2**	**4.9**
Kalophrynus interlineatus (southern Vietnam)													
ABV-00405	m	42.8	12.0	15.1	4.4	1.4	3.0	4.3	3.2	8.0	3.5	3.2	4.5
ABV-00406	m	44.5	11.6	14.7	4.3	1.4	2.9	3.9	3.5	7.9	3.3	3.0	4.5
ABV-00407	m	38.6	10.3	14.1	4.5	1.5	3.0	4.0	3.2	7.3	3.2	2.8	6.2
ABV-00408	f	40.2	12.7	14.3	4.5	1.4	3.1	4.2	3.3	7.1	3.1	2.9	3.8
ABV-00409	f	45.4	12.8	13.6	4.6	1.6	3.0	4.6	2.9	7.8	3.3	2.5	3.9

……*continued on the next page*

TABLE 4 (continued).

Specimen	FHL	HumL	1FL	2FL	3FL	4FL	ThL	TFL	TarFL	FL	HLL	IMTL	OMTL	1TL
Kalophrynus cryptophonus **sp. nov.**														
ABV-00136	10.3	5.3	2.5	3.2	4.6	3.1	10.5	9.5	13.7	7.3	30.1	0.5	0.3	2.2
holotype ABV-00222	**14.1**	**5.8**	**3.4**	**4.4**	**6.4**	**4.0**	**12.3**	**11.9**	**18.0**	**11.0**	**38.5**	**1.2**	**1.0**	**3.2**
ABV-00223	13.1	6.8	2.8	4.1	6.4	3.7	12.8	11.4	17.1	10.9	38.7	1.2	0.9	3.1
ABV-00224	12.4	6.4	3.4	4.3	6.1	3.6	12.7	11.6	16.5	10.0	38.5	1.3	0.7	3.1
ABV-00225	14.2	7.6	3.9	4.7	6.7	4.0	13.1	12.1	18.4	11.3	39.6	1.3	0.8	3.6
ABV-00226	14.2	6.4	3.1	4.4	6.5	3.8	12.5	12.3	17.6	10.8	40.4	1.2	1.0	3.0
Kalophrynus honbaensis **sp. nov.**														
ABV-00302	12.0	5.8	2.7	3.8	5.7	3.5	10.8	10.5	15.3	9.0	34.4	1.1	0.6	2.7
holotype ABV-00320	**17.1**	**8.4**	**4.6**	**6.0**	**8.5**	**5.5**	**15.1**	**14.4**	**22.7**	**14.2**	**49.6**	**1.2**	**0.6**	**3.6**
Kalophrynus interlineatus (southern Vietnam)														
ABV-00405	19.4	9.4	5.4	6.2	9.3	5.9	15.5	15.4	22.1	13.7	50.1	1.6	1.0	3.8
ABV-00406	19.6	10.4	5.7	6.7	9.5	6.0	15.5	15.6	22.2	14.6	51.2	1.3	1.0	4.2
ABV-00407	16.6	8.1	5.6	6.0	8.4	5.3	14.9	13.9	20.2	13.0	44.3	1.2	0.8	3.9
ABV-00408	19.5	9.9	6.3	7.0	9.7	6.3	16.7	16.1	22.5	14.6	50.8	1.4	1.2	3.4
ABV-00409	20.2	9.3	6.1	7.0	10.4	6.2	15.6	15.4	22.7	14.8	48.9	1.3	0.9	4.1

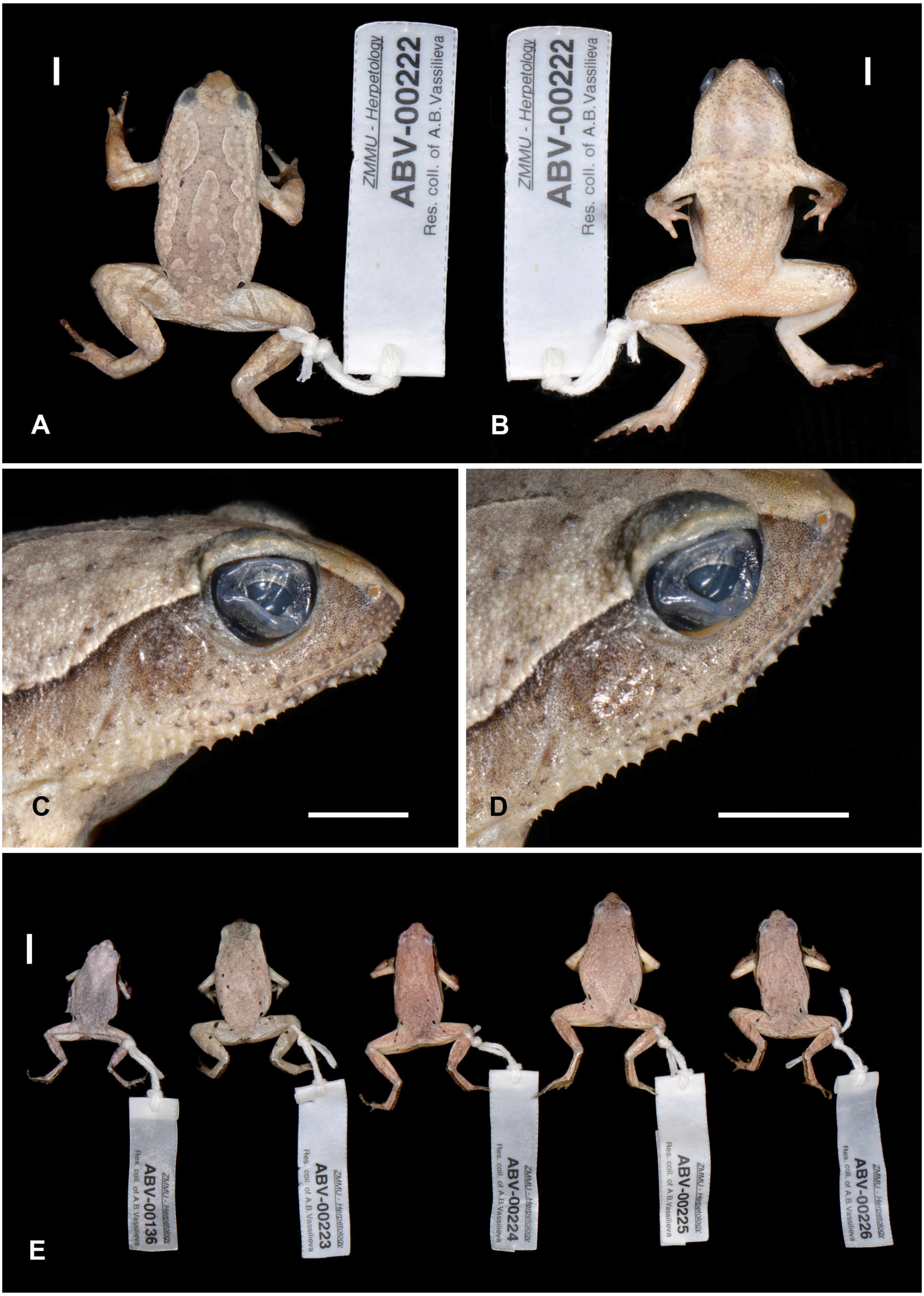

FIGURE 3. Holotype of *Kalophrynus cryptophonus* **sp. nov.** (ZMMU A-4944) in preservative: (A) dorsal view, (B) ventral view (C) head, lateral view, (D) head, dorsolateral view, with clearly visible skin spines on the jaws; (E) paratypes of *Kalophrynus cryptophonus* **sp. nov.** in preservative. Scale bar 3 mm. Photo by V. Trounov.

FIGURE 4. Holotype of *Kalophrynus cryptophonus* **sp. nov.**(ZMMU A-4944) in preservative: (A) palmar view of the right hand, (B) plantar view of the right foot, (C) nuptial pads on fingers I–III. Scale bar 3 mm. Photo by V. Trounov.

Hind limbs slender, relatively short (HLL/SVL 1.36); when legs adpressed to body laterally, heel reaches interval between eye and tympanum. Tibia slightly shorter than thigh (TFL/ThL 0.96) and significantly shorter than tarsus+foot (TFL/TarFL 0.66). Toe tips rounded, without dilatation; relative length of toes I<II<V<III<IV; foot webbing formula **I** 1–2½ **II** ½–2½ **III** 1½ –3½ **IV** 3¾–1½ **V**; subarticular tubercles moderately prominent on toes I–IV, oval in shape; one on each toe I and II , two on toe III and three on toe IV; distal subarticular tubercle on toe V distinct, proximal one less conspicuous, rudimentary; inner metatarsal tubercle prominent, oval; outer metatarsal tubercle smaller than inner one (OMTL/IMTL 0.8), low, rounded (Fig. 4B).

Skin on dorsum finely shagreened from interorbital level to vent, with singular small tubercles topped with tiny apical spine; smooth on the snout; roughly granular on belly, lower flanks, thighs undersides and in cloacal region, smooth on gular area, inner leg surfaces and forelimbs except for scarce, tiny spines in outer and axillar surfaces. Two pairs of whitish glandular tubercles on the pectoral region, each with tiny sharp spines on apex. Dorsolateral boundary sharp, finely glandular.

FIGURE5. Holotype of *Kalophrynus cryptophonus* **sp. nov.** (ZMMU A-4944) in life: (A) dorsolateral view, (B) ventral view; female *Kalophrynus cryptophonus* **sp. nov.** (paratype ZMMU A-4858) in life: (C) dorsolateral view, (D) ventral view; (E) male *Kalophrynus cryptophonus* **sp. nov.** *in situ*, inside bamboo stem. Photo by E. Galoyan.

FIGURE 6. Natural habitat of *Kalophrynus cryptophonus* **sp. nov.** in Loc Bao woodland, Lam Dong Province: (A) bamboo (*Phyllostachys* sp.) tangles, and (B) decaying bamboo stems as typical breeding site. Photo by E. Galoyan.

Coloration. In life,(Fig. 5AB) ground coloration on upper head, trunk and limb surfaces variable, ranging from chocolate-brown at night to pinkish-beige during the day time. Sharp edge of dorsal coloration extending from snout tip through upper eyelid to groin contrasted by darker, pinkish-brown flanks and head sides. Diffuse pinkish-gray pattern in the shape of inverted “Y” on the dorsum lasting from interorbital area towards inguinal region and edged with light-beige color. "Y"-shaped pattern followed laterally by three less distinct, parallel, intervening stripes forming somewhat reticulate pattern. Pale rounded spot visible on parietal area, similar diffuse spots scattered on dorsum. Three faint brownish crossbands on thigh and shin; brown marmorate pattern on elbow and knee, on foot underside and on anterior surface of lower forearm and hand. Cloacal region brownish, separated from dorsal surface by thin pale edging line. Inguinal ocelli represented by small (smaller than tympanum, ISD/TD 0.77) rounded black spot in each inguinal area, without light edges; three asymmetric small dark dots on left side of back. Venter pale, yellowish-pink, unpatterned except for gular, chest and upper belly areas displaying diffuse brownish-gray spotting; palmar and plantar surfaces pink. Iris golden.

In preservative coloration fades to gray on dorsum and flanks and yellowish-gray on venter; other features remain without significant change.

Variation. Individuals of the type series are similar in morphology, body size and proportions (Table 4; Fig. 3E), except for the female that is smaller in size and lacks the spines and the asperous patches on the hand. The spines in males vary slightly in abundance and size; in all individuals they are the largest on mandible margins, especially in the subtympanal area, and may be less conspicuous on the upper jaw. Nuptial pads on fingers I–III are similar in all males. Variations in the coloration include the inverted “Y” or hourglass-shaped pattern on dorsum, which may be almost indistinct; three paratypes have a uniform stellar, slightly paler pattern on back. The inguinal ocelli vary in size, but are always smaller in diameter than tympanum (ISD/TD 0.77±0.05); in the female this spot is present only on the left side of body. Supernumerary asymmetric small dark spots may occur in the inguinal area, on back and on upper thigh and shin surface. The female (Fig. 5CD) additionally differs in undersides coloration by having a more pronounced dark marbling or spotting on the upper belly and lower part of flanks, and by having a light medial band on the gular surface bordered with diffuse brown blotches. The ovaries of the female contain relatively small quantity (50–60 per ovary) of developing unpigmented eggs variable in size (diameter 0.3–0.9 mm).

Natural history. All specimens were collected at night time on the limited parcel of secondary, disturbed mountain high polydominant evergreen tropical forest with the abundance of bamboo *Phyllostachys* sp. (Poaceae) (Fig. 6), where *Kalophrynus cryptophonus* **sp. nov.** occurs syntopically with *Kurixalus* sp., *Theloderma bambusicola* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Sang & Geissler, *Microhyla fissipes* Boulenger, *Limnonectes limborgi* (Sclater), and *Ingerophrynus galeatus* (Günther). The female was found on the ground; all males were collected while calling from inside dead bamboo stems with diameters of about 30–40 mm (Fig. 5E), sometimes as close to each other as 5–10 m; up to four males were heard simultaneously from one point. Tadpoles were collected on 22 April 2013 in a decaying bamboo stem with diameter about 35 mm. In total, 11 early-stage larvae (Gosner Stage 25) were recorded in one partly perforated internode, containing 70–80 ml of rainwater. A calling male was found in the adjacent internode of the same stem.

Tadpole description. Tadpoles were allocated to the species based on the following evidence: (1) morphological features characteristic for microhylid larvae in general and other phytotelm breeding *Kalophrynus* species in particular (see Discussion); (2) specific site of collection (water-filled decaying bamboo internode) which was close to calling male found in the same bamboo stem; (3) all other phytotelm breeding frogs known from the region (members of the genera *Theloderma* and *Rhacophorus*, Rhacophoridae) differ in tadpole morphology and cannot be mistaken with larvae of the new species.

Standard tadpoles measurements (mean±SD, n=4, Stage 25): TL 9.14±0.77; BL 2.50±0.05; BW 1.47±0.03; SVL 3.02±0.16; SSp 1.89±0.15; IOD1.05±0.03; SED 0.98±0.03; ED= 0.16±0.01; ODW= 0.94±0.06; TaL= 6.6±0.72; TH= 0.89±0.1.

In dorsal view (Fig. 7A), body oval or slightly pyriform, with maximum width at eye level (BW/BL 0.59±0.01); snout long (SED/BL 0.39±0.01), broad, blunt. In lateral view (Fig. 7B), body compressed dorsoventrally (BH/BW 0.66±0.05), especially anteriorly, flattened above and convex below. Tail long (TaL/BL2.70±0.25), lanceolate; almost equal in height along its length; tail tip bluntly acuminate, without terminal filament; muscular part moderately developed; at vent level, tail muscular portion about three times higher than lower fin. Upper fin originating slightly behind body-tail junction, much shorter than the lower fin proximally and nearly equal in height to it distally. Eyes dorsolateral, not visible from below, small (ED/BL 0.06±0.002), pupils oriented dorsolaterally. Nostrils not opened at the stages under description. Spiracle ventral, medial, with short free flap, opening at 0.80±0.06 of distance from snout tip to body end. Vent tube medial, long, directed obliquely backward, with opening at ventral edge of lower fin. Lateral line system not visible. Mouth terminal, oral disk wide (ODW/BW 0.64±0.05), without keratinized elements and obvious papillae. Upper labium widely arched, slightly overhanging Ω-shaped lower labium.

Live tadpoles appeared almost unpigmented, whitish, with darker gut and reddish gills clearly visible through translucent skin. In preservative, scattered melanophores visible on the dorsal surface of body and tail. Belly and tail fins totally transparent. Eyes black. Gut forming three wide loops.

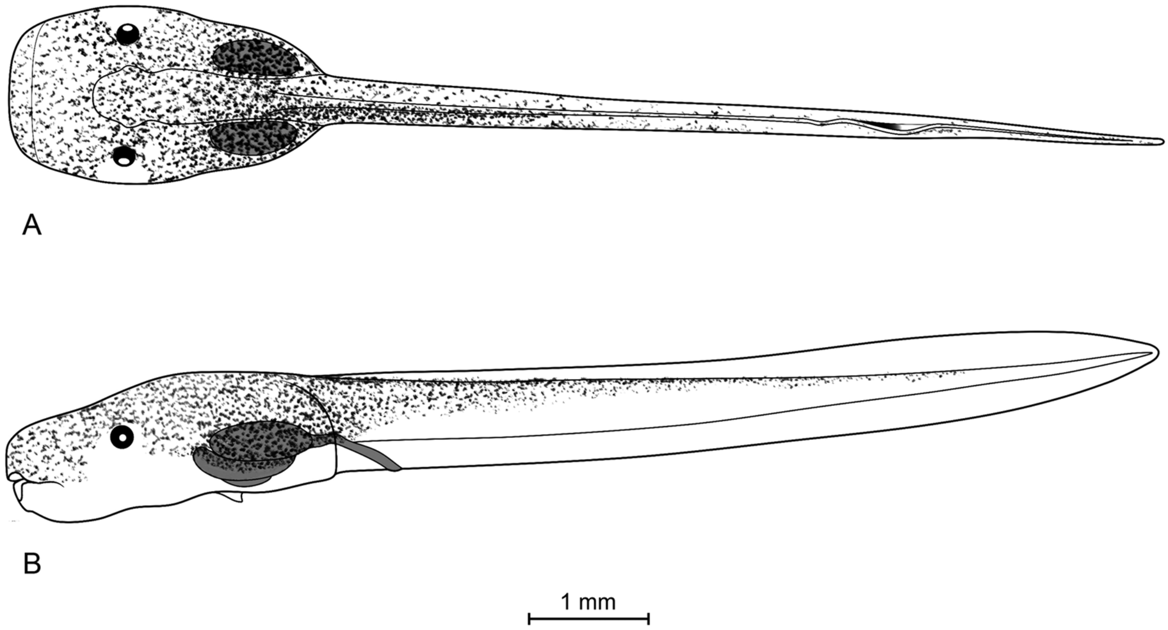

FIGURE7. External morphology of *Kalophrynus cryptophonus* **sp. nov.** tadpole (Gosner Stage 25): (A) dorsal view, (B) lateral view.

Call characteristics. The advertisement call of *Kalophrynus cryptophonus* **sp. nov.** recorded at 25°C is represented by rhythmic, non-modulated, tonal calls (Fig. 8A).

Call duration varied from 42 to 114 ms (79±13ms, n=105). The interval between successive calls in one individual varied from 5.91 to 25.57 s (11.76±6.57 s, n=10). In two individuals calling simultaneously at the distance 5 m apart from each other the interval between calls uttered by different males varied from 0.009 to 2.37 s (1.06±0.56 s, n=31). The call repetition rate in individuals (n=11) varied from 0.016 to 0.42 calls per second (0.13±0.12, n=105 calls). The call repetition rate in choruses (n=3) consisting of 4–5 individuals varied from 0.21 to 0.74 calls per seconds (0.44±0.27). The initial (1020±100 Hz) and final fundamental frequency in a single call (920±130 Hz) were almost equal (n=38). Two lower harmonics were distinguishable on the majority of call spectrograms. The maximum amplitude frequency in individual calls (1100±370 Hz, n=38) coincided with the fundamental frequency. The entire call was pulsed, with2–5 pulses (3.270.69, n=52) per call and a mean pulse period of 24±0.3 ms. In all calls recorded the pulse rate varied from 30.3 to 55.6 pulses per second (42.51±6.29 pulses/s, n=52).

Comparison. In southern Vietnam, *Kalophrynus cryptophonus* **sp. nov.** co-occurs with *Kalophrynus interlineatus*, which inhabits diverse kinds of forested biotopes at elevations up to 900 m a.s.l. The latter was reliably recorded from the same province and district as *Kalophrynus cryptophonus* **sp. nov.,** and both species may potentially occur syntopically with each other. From southern Vietnamese *K. interlineatus*, the new species differs morphologically by the following features: a smaller size (SVL 23.4–30.4 mm vs. 38.6–45.4 mm); the presence of skin spines on head and nuptial pads on fingers in males (absent in *K. interlineatus*); smooth canthus rostralis (vs.

sharp in *K. interlineatus*); skin on gular area smooth (roughly tuberculated in *K. interlineatus*); longer legs (HLL/SVL 1.35±0.05 vs. 1.16±0.07 in *K. interlineatus*), in adpressed leg heel reaching tympanum (vs. not reaching tympanum in *K. interlineatus*); the presence of rudimental webbing on fingers (absent in *K. interlineatus*) and slightly less extensive webbing on toes (webbing formula **I** 1–2½ **II** ½–2½ **III** 1½ –3½ **IV** 3¾–1½ **V** vs. **I** ½ –2 **II** 1–2½ **III** 1–3 **IV** 3¾–1 **V** in *K. interlineatus*, Fig. 10CD), and reduced anterior palatal dermal ridge (well developed, arch-shaped in *K. interlineatus*). In coloration, *Kalophrynus cryptophonus* **sp. nov.** differs from *K. interlineatus* by having a very small, rudimentary black spot without light edging in inguinal area (smaller than tympanum, ISD/TD 0.77±0.05 vs. larger than tympanum, ISD/TD 1.60±0.36), and white-edged inguinal ocellus in *K. interlineatus*. In reproductive characteristics, *Kalophrynus cryptophonus* **sp. nov.** differs from *K. interlineatus* by having unpigmented eggs (vs. pigmented in *K. interlineatus*). In vocalization, *Kalophrynus cryptophonus* **sp. nov.** differs from *K. interlineatus* by calling from inside bamboo stems (vs. from forest floor, often in dense choruses around breeding ponds), as well as call duration, inter-call interval, frequency and other acoustic parameters (Fig. 8AB, Table5).

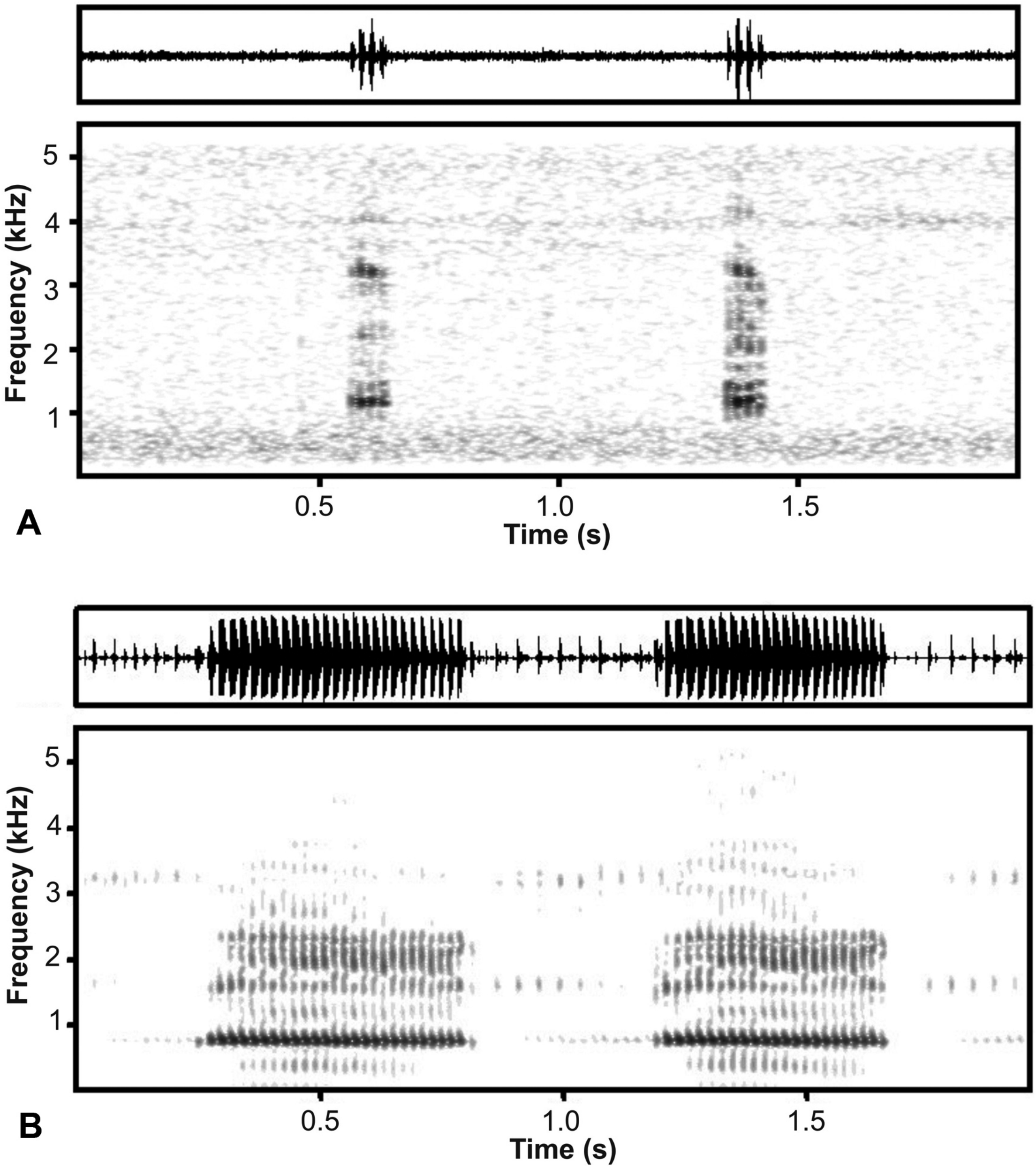

FIGURE 8. Oscillogram (top) and sonogram (bottom) of the male advertisement calls of (A) *Kalophrynus cryptophonus* **sp. nov.** (ZMMU A-4859), recorded at 25ºC, and (B) *Kalophrynus interlineatus* (not collected), recorded at 24ºC.

TABLE 5. Structural and frequency characteristics ($\bar{x}$±SD, min–max) of male advertisement call in the representatives of the genus *Kalophrynus*. Sources are given in footnotes; "–" implies missing data.

Species	t °C	Calls per sec	Call duration (ms)	Interval between consecutive calls (ms)	Pulses per call	Pulse repetition (pulses per sec)	Fundamental frequency (Hz)	Visible harmonics	Dominant frequency (Hz)	Frequency modulation
K. baluensis[1]	19	–	125	8000–10000	–	–	ca. 1300	1–2 or more	2600	absent?
K. barioensis[2]	24.6	0.28±0.04 0.21–0.34	72.9±2.7 68–77	3610±560 2900–5600	–	–	1907.7±53.4 1800–1950	1–6	ca. 3600–3900	present
K. calciphilus[3]	19–25	0.37±0.12 0.18–0.53	161±4	–	7 (rarely 8)	43 38–67	1500–1700	1–2	2250–2650	absent
K. cryptophonus **sp. nov.**[4]	25	0.13±0.12 0.016–0.42	79±13 42–114	11760±6570 5910–25570	3.27±0.69 2–5	42.51±6.29 30.3–55.6	1020±100 900–1200	1–3	1020±100 900–1200	absent
K. interlineatus[4]	24	0.46±0.23 0.30–0.61	525±36 367–572	1210±1790 370–9030	25.1±1.63 18–28	47±3.83 35.7–62.5	760±30 650–840	1–3	755.15±30 650–840	absent
K. interlineatus[5]	22.2	0.54±0.11 0.35–0.74	843±109.6 618–1145	1095±443.2 558–2007	30.7±4 23–41	36.37±0.54 35.5–37.3	743±18.9 660–820	1 and 3	743±18.9 660–820	absent
K. interlineatus[6]	25	0.57±0.07 0.47–0.68	682±80.1 553.8–1055	1919±863 403–3467	30.7±3 25–46	45.2±1.8 36.5–47.8	685–747	1 and 3	685–747	absent
K. limbooliati[7]	24–27	3.72±0.3 3.31–4.2	70±6 61–76	271.7±25.4 238–310	–	–	1909±156 1632–2008	1–3	1909±156 1632–2008	present
K. nubicola[8] (type one, male)	20	–	80	–	10	130	80–1200	1–2	2800–3400	weak
K. pleurostigma (Sabah)[5]	27.5	2.50±0.27 2.1–2.99	231.5±45.8 158–285	172.5±24 138–210	–	–	500.7±30 450–550	1–5	500.7±30 450–550	present
K. pleurostigma (Sarawak)[5]	25	0.87±0.08 0.70–0.97	487.1±16.9 477–518	676.8±115.4 518–961	–	–	462.1±11.4 450–475	1–3	462.1±11.4 450–475	present
K. yongi[9]	13–14.5	–	174.7±37.5 134–288	4410±1080 3300–7000	–	–	1300–1800	1–7	1300–1650	present

[1] Malkmus & Riede (1996); [2] Matsui & Nishikawa (2011); [3] Dehling (2011); [4] Our data; [5] Matsui *et al.* (1996); [6] Ohler & Grosjean (2005); [7] Matsui *et al.* (2012); [8] Dring (1983); [9] Matsui (2009)

From its other congener, described herein from southern Vietnam, *Kalophrynus honbaensis* **sp. nov.** (see below), *Kalophrynus cryptophonus* **sp. nov.** differs morphologically by having a smaller body size in males (SVL 27.9–30.4 mm vs. 26.7–36.8 mm in *Kalophrynus honbaensis* **sp. nov.**); large skin spines on head (vs. only microscopic spines in *Kalophrynus honbaensis* **sp. nov.**), nuptial pads on fingers in males present (absent in males *Kalophrynus honbaensis* **sp. nov.**); a smooth canthus rostralis (vs. sharp in *Kalophrynus honbaensis* **sp. nov.**); reduced anterior palatal dermal ridge (vs. well developed, parallel to posterior ridge in *Kalophrynus honbaensis* **sp. nov.**), and more extensive webbing on toes (webbing formula **I** 1–2½ **II** ½–2½ **III** 1½ –3½ **IV** 3¾–1½ **V** vs. **I** 1–2 **II** 1 ½ –3 **III** 2–3 ¾ **IV** 4–2 **V**). In coloration, *Kalophrynus cryptophonus* **sp. nov.** differs from *Kalophrynus honbaensis* **sp. nov.** by having a very small, rudimentary black spot in inguinal area (smaller than tympanum, ISD/TD 0.77±0.05, vs. larger than tympanum, ISD/TD 1.47±0.09).

Kalophrynus cryptophonus **sp. nov.** differs from other congeners distributed in South Asia, China, the Malay Peninsula and the Malay Archipelago by the presence of sharp prominent spines on the head skin (not recorded in any other species) in males. Moreover, *Kalophrynus cryptophonus* **sp. nov.** has substantially smaller body size (SVL 23.4–30.4 mm) than *K. baluensis* Kiew, 1984 (SVL 34.8–39 mm), *K. intermedius* Inger, 1966 (SVL 35–40 mm in females), *K. orangensis* Dutta, Ahmed & Das, 2000 (SVL up to 38 mm), *K. palmatissimus* Kiew, 1984 (SVL 31–39 mm) and *K. pleurostigma* Tschudi, 1838 (SVL 35–50 mm in males, 38–58 mm in females). Additionally, from these species *Kalophrynus cryptophonus* **sp. nov.** differs by having a single, small black inguinal ocellus not edged in white on each side of sacrum (vs. one or two yellow ocelli in *K. baluensis*, absence of inguinal spot in *K. intermedius*, white-edged black ocelli in *K. pleurostigma*), the presence of rudimental webbing on fingers (absent in *K. orangensis*) and moderate toe webbing (vs. extensive webbing in *K. palmatissimus*), and from *K. baluensis* and *K. pleurostigma* by call characteristics (i.e. call duration, dominant frequency and modulation) (Table 5).

Kalophrynus cryptophonus **sp. nov.** differs from *K. barioensis* Matsui & Nishikawa, 2011 (SVL 17.5–19.8 mm in males, 20.5 mm in females), *K. nubicola* Dring, 1983 (SVL 14.4–24.4 mm in males) and *K. robinsoni* Smith, 1922 (SVL 17 mm in males, 18 mm in females) in having a significantly larger body size (SVL 27.9–30.4 mm in males, 23.4 mm in female); additionally, from these species *Kalophrynus cryptophonus* **sp. nov.** differs by the presence of finely asperous nuptial pads on fingers (absent in *K. barioensis* and *K. nubicola,* few large spines in *K. robinsoni*), presence of outer metatarsal tubercle (vs. absent in *K. barioensis* and *K. nubicola*), coloration features (presence of a light lateral stripe in *K. barioensis*, no inguinal ocellus in *K. nubicola* and *K. robinsoni*), and from *K. barioensis* and *K. nubicola* by call characteristics, namely a lower dominant frequency (Table 5).

From *Kalophrynus* species, which are similar in size, *Kalophrynus cryptophonus* **sp. nov.** clearly differs by the following important features: from *K. bunguranus* Günther, 1895 (SVL 22.2–23.4 mm in males, 25.5–26.7 mm in females) by having small inguinal spots not edged in white (vs. dark ocelli surrounded by a light area in *K. bunguranus*); from *K. calciphilus* Dehling, 2011 (SVL 29.7–30.1 mm in males, 35.5–38.8 mm in females) by the absence of dentition on the upper jaw (vs. weak dentition present in *K. calciphilus*), by finger IV being longer than I (vs. IV shorter than I in *K. calciphilus*), by coloration features (black dorsum and chest, white dorsolateral line, red iris in *K. calciphilus*), by call characteristics, in particular call duration and dominant frequency (Table 5); from *K. eok* Das & Haas, 2003 (SVL 26.3 mm in male), by coloration features (dark interorbital bar and chevron on shoulders, inguinal spots absent in *K. eok*); from *K. heterochirus* Boulenger, 1900 (SVL 26 mm in males, 30 mm in female) by normally developed fingers (vs. strongly shortened fingers I, II and IV in *K. heterochirus*) and small black inguinal spot (vs. few large round yellowish-white spots in the lumbar region in *K. heterochirus*); from *K. limbooliati* Matsui, Nishikawa, Belabut, Norhayati & Yong, 2012 (SVL 26.2–28.7 mm in males) by the presence of subarticular tubercles under toe V and the outer metatarsal tubercle (absent in *K. limbooliati*), the absence of light dorsolateral line (present in *K. limbooliati*) and call characteristics, in particular dominant frequency and frequency modulation (Table 5); from *K. menglienicus* Yang & Su, 1980 (SVL 19.75–23.4 mm) by moderate webbing on toes (vs. foot webbing absent in *K. menglienicus*) and the absence of horny granules on back and belly (present in *K. menglienicus*); from *K. minusculus* Iskandar, 1998 (SVL 25 mm in males, 35 mm in females) by coloration features (brownish black coloration with black banded pattern in *K. minusculus*); from *K. punctatus* Peters, 1871 (SVL 22–27 mm in males) by the presence of outer metatarsal tubercle (absent in *K. punctatus*); from *K. stellatus* Stejneger, 1908 (SVL 24 mm) by reduced anterior palatal ridge (vs. developed arched ridge in *K. stellatus*) and inguinal spots smaller than tympanum and not edged in white (vs. black inguinal spots as large as tympanum and surrounded with light dots in *K. stellatus*); from *K. subterrestris* Inger, 1966 (SVL 23 mm in males, 27 mm in females) by indistinct canthus rostralis (distinct in *K. subterrestris*), reduced anterior palatal ridge (vs. developed angular, strongly notched ridge in *K. subterrestris*) and subarticular tubercles on toe V present (absent in *K. subterrestris*); from *K. tiomanensis* Chan, Grismer & Grismer, 2011 (SVL 25.8–26.3 mm in males, 21.4–25.8 mm in females) by the presence of outer metatarsal tubercle (absent in *K. tiomanensis*), small inguinal spot (vs. large spot in *K. tiomanensis*) and other coloration features (spotted lower back in *K. tiomanensis*); from *K. yongi* Matsui, 2009 (SVL 28.8–31.0 mm in males) by the absence of strongly developed humeral spine (present in males *K. yongi*), finely asperous nuptial pads on fingers I–III (vs. sharp white asperities on nuptial pads in males *K. yongi*), tadpole features (see Discussion) and call characteristics, namely call duration, dominant frequency and modulation (Table 5).

Distribution. To date the species is known only from type locality, the Loc Bao Forestry Enterprise in Lam Dong Province.

Etymology. The species name is a noun in the nominative case, derived from the Greek *cryptos,* meaning "to hide", "mystery", "cryptic", and *phonus* meaning "voice" in reference to our history of discovering the species. During the expedition of the JRVTRTC in April–May, 2013 in Loc Bao forest, Lam Dong Province, we recorded unusual frog calls which were hard for us to locate. After two weeks of searching we found a male *Kalophrynus cryptophonus* **sp. nov.** calling from an empty bamboo stem.

Recommended vernacular name: The recommended common name in English is Bamboo sticky frog, referring to the unusual breeding habitat of the new species.

Kalophrynus honbaensis sp. nov.

Holotype. ZMMU A-4941 (field number ABV-00320), adult male from the vicinity of the Yersin station on Hon Ba Mountain, Hon Ba Nature Reserve, Cam Lam District, Khanh Hoa Province, Vietnam (coordinates 12°07' 16" N, 108° 56' 55" E, elevation 1500 m a.s.l.), collected by A.B. Vassilieva on 19 June 2013.

Paratype. ZMMU A-4943 (field number ABV-00302), adult male collected by A.B. Vassilieva on 16 of June 2013 in the same area with holotype.

Diagnosis. The species is allocated to *Kalophrynus* based on the following characters considered diagnostic for the genus (Parker 1934, Bourret 1942, Inger, 1966): one or more transverse dermal ridges across the palate anteriorly to the oesophagus; tips of digits not dilated to disks; no spine-like projections at heel or elbow; snout short, pointed; tympanum visible; pupil horizontal; inner metatarsal tubercle low, not shovel-like; skin thick and glandular. The new species is distinguishable from its congeners by a combination of the following features: (1) SVL 26.7–36.8 mm in males; (2) snout pointed, slightly sloped ventrally; (3) canthus rostralis distinct; (4) males without distinguishable spines on the mandible margins; (5) males without nuptial pads on fingers; (6) tympanum distinct, smaller than eye in diameter; (7) toe webbing moderate; (8) outer metatarsal tubercle present; (9) light dorsolateral line absent; (10) dark ocelli in the inguinal region present, large, without distinct light bordering; (11) anterior palatal dermal ridge on the palate developed, parallel to posterior palatal dermal ridge.

Description of holotype. Adult male (Fig. 9AB) with well-developed oblong testes; measurements are given in the Table 4.

Habitus stout, with body widest in lumbar area. Head relatively short (HL/SVL 0.3), wider than long (HL/HW 0.82), triangular. Snout pointed, short, about one third of head length (SL/HL 0.33), sloping in profile (Fig. 9C) and distinctly projecting beyond lower jaw; canthus rostralis distinct, finely tuberculate; nostrils rounded, situated closer to snout tip than to eye (SND/END 0.58) and oriented rostrolaterally. Eyes bulging, medium-sized (ED/SVL 0.11), lateral, well visible from below, with oval horizontal pupil; upper eyelid with prominent tubercles on margin, UEW/IOD 0.43. Tympanum very close to eye, smaller than eye (TD/ED 0.79; TD/SVL 0.09), roughly round, distinct, bordered from above by sharply prominent supratympanic fold running from posterior corner of eye along upper edge of tympanum and then down to the point of forelimb insertion. Maxillary and vomerine teeth absent; tongue with even rounded edge posteriorly; two transversal dermal ridges on palate posterior to eye level, in close proximity and parallel to each other: anterior one smaller and smooth, posterior ridge more prominent and crenulated; postchoanal ridges indistinct. Small notch in the rostral part of skin covering upper jaw and small prominence on the rostral part of lower jaw, fitting together. Single subgular vocal sac; wide slit-like vocal openings between jaw articulation and tongue insertion.

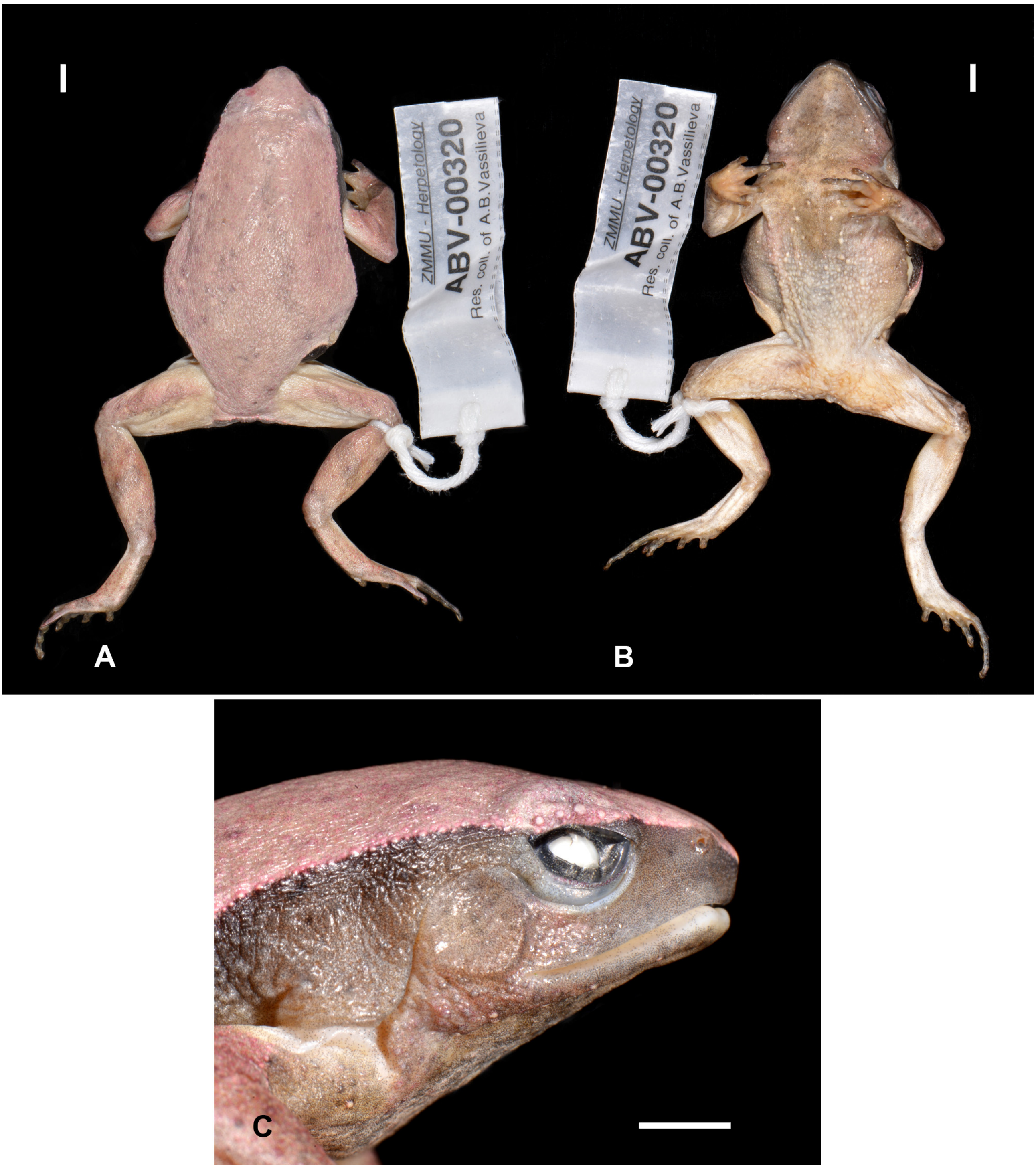

FIGURE 9. Holotype of *Kalophrynus honbaensis* **sp. nov.** (ZMMU A-4941) in preservative: (A) dorsal view, (B) ventral view, (C) profile view. Scale bar 3 mm. Photo by V. Trounov.

Prominent blunt tubercles on subtympanal and axillar areas and on ventral surface of mandible in its posterior part; some tubercles topped with extremely tiny, microscopic sharp spines, distinguishable only under strong magnification.

Forelimbs slender, more than half of body length (FLL/SVL 0.69); hands with rudimentary webbing. Tips of fingers rounded, without dilatation. Relative length of fingers I<IV<II<III; palmar tubercle moderately large, prominent, oval in shape; subarticular tubercles prominent, rounded; one on each fingers I, II and IV and two on finger III; smaller additional metacarpal tubercles between palmar tubercle and each finger base (Fig. 10A).

FIGURE 10. Palmar views of the right hand of the holotype *Kalophrynus honbaensis* **sp. nov.** (ZMMU A-4941) (A) and *Kalophrynus interlineatus* (C), and plantar views of the right foot of the holotype *Kalophrynus honbaensis* **sp. nov.** (B) and *Kalophrynus interlineatus* (D) in preservative. Scale bar 3 mm. Photo by V. Trounov.

FIGURE 11. Holotype of *Kalophrynus honbaensis* **sp. nov.** (ZMMU A-4941) in life: (A) dorsolateral view, (B) ventral view; male *Kalophrynus honbaensis* **sp. nov.** (paratype ZMMU A-4943) in life: (C) dorsolateral view, (D) ventral view. Photo by V. Trounov.

Hind limbs slender, relatively short (HLL/SVL 1.35); when legs adpressed to body laterally, heel reaches posterior end of tympanum. Tibia slightly shorter than thigh (TFL/ThL 0.95) and significantly shorter than tarsus+foot (TFL/TarFL 0.63). Toe tips rounded, slightly dilated; relative length of toes I<II<V<III< IV; foot webbing formula **I** 1–2 **II** 1 ½ –3 **III** 2–3 ¾ **IV** 4–2 **V**; subarticular tubercles moderately prominent on toes I–IV, oval in shape; one on each toe I and II , two on toe III and three on toe IV; distal subarticular tubercle on toe V distinct, proximal rudimentary, almost absent; inner metatarsal tubercle prominent, oval; outer metatarsal tubercle about half the size of the inner (OMTL/IMTL 0.49), low, rounded (Fig. 10B).

Skin on dorsum finely shagreened from snout to vent, with singular small rounded tubercles; roughly granular on belly, lower flanks, underside of thighs and the cloacal region; smooth on chest area, inner leg surfaces and forelimbs; finely granular on gular area. About ten asymmetric whitish glandular tubercles on chest. Dorsolateral boundary sharp, glandular, with prominent tubercles.

Coloration. In life (Fig. 11AB) ground coloration on dorsal surfaces of head, trunk and limb orange-yellow, unpatterned, with tiny reddish dots on skin tubercles. Inguinal and axillar areas and inner leg surfaces yellow. Lateral surfaces of head and flanks brown, contrasting with dorsal coloration. Faint brownish transversal bands running across thigh, shin, foot and forearm; underside of foot, lower forearm and knee with brown marbled pattern. Cloacal region brownish, separated from dorsal surface by thin pale edging line. Inguinal ocellus represented by large (larger than tympanum, ISD/TD 1.53) rounded, black spot in each inguinal area, with faintly distinct lighter bordering. Venter yellowish-pink, with brown marbled pattern on chest; gular area brownish, darkening to rostral mandible part; palmar surfaces pink, plantar mostly brown. Iris copper-brown.

In preservative coloration fades to grayish-pink on dorsum, dark gray on flanks and yellowish-gray on venter; other features remain without significant change.

Variation. Paratype male is mostly similar to the holotype in morphology and body proportions, but smaller in size (Table 1; Fig. 12); microscopic spines on mandible are absent. In life coloration, paler diffuse blotches occur on orange-yellow ground on dorsum; lower belly and thigh undersides display slightly denser marbled pigmentation (Fig. 11CD).

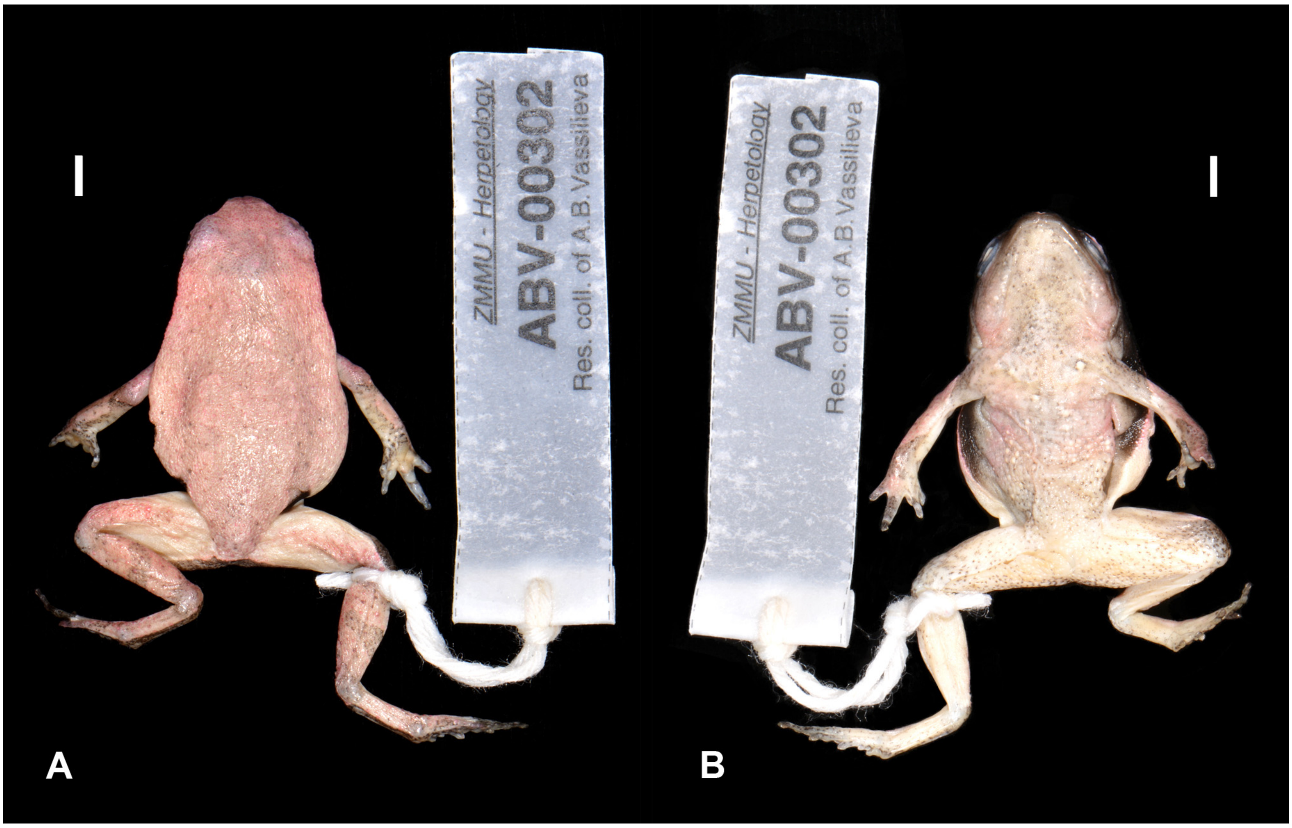

FIGURE 12. Male *Kalophrynus honbaensis* **sp. nov.** (paratype ZMMU A-4943) in preservative: (A) dorsolateral view, (B) ventral view. Photo by V. Trounov.

Natural history. Both specimens were collected on the ground at night time after rain, on the limited parcel of primary montane polydominant evergreen forest on the top of mountain ridge at elevation ca. 1500 m a.s.l., with the predominance of Fagaceae (*Lithocarpus, Quercus*), Elaeocarpaceae (*Elaeocarpus*), Theaceae, Lauraceae, Araliaceae and Rutaceae, and abundance of large granite rocks (Fig. 13), where *Kalophrynus honbaensis* **sp. nov.** occurs syntopically with *Raorchestes gryllus* (Smith), *Hylarana* cf. *montivaga* (Smith), *Leptobrachium leucops* Stuart, Rowley, Tran, Le & Hoang, and *Ingerophrynus galeatus*.

Comparison. In southern Vietnam *Kalophrynus honbaensis* **sp. nov.** co-occurs with two congeners: the mostly lowland *K. interlineatus*, and *Kalophrynus cryptophonus* **sp. nov.** From southern Vietnamese *K. interlineatus*, the new species differs morphologically by following features: smaller body size in males (SVL 26.7–36.8 mm in *Kalophrynus honbaensis* **sp. nov.** vs. 38.6–44.5 in *K. interlineatus*); finely granular skin on gular area (vs. roughly tuberculated in *K. interlineatus*); relatively longer legs (HLL/SVL 1.32±0.04 vs. 1.16±0.07 in *K. interlineatus*), in adpressed leg heel reaching tympanum (not reaching tympanum in *K. interlineatus*); inner metatarsal tubercle twice as long as the outer metatarsal tubercle (average OMTL/IMTL 0.5 vs. 0.74 in *K. interlineatus*); anterior palatal dermal ridge straight, entire, parallel to posterior palatal ridge (vs. arch-shaped or angular, often with a notable medial gap in *K. interlineatus*); single subarticular tubercle on toe V (vs. two in *K. interlineatus*); rudimentary webbing on fingers present (absent in *K. interlineatus*) and less extensive webbing on toes (webbing formula **I** 1–2 **II**1 ½ –3 **III** 2–3 ¾ **IV** 4–2 **V** vs. **I** ½ –2 **II** 1–2½ **III** 1–3 **IV** 3¾–1 **V** in *K. interlineatus*, Fig. 10CD).

From *Kalophrynus cryptophonus* **sp. nov.** the new species differs morphologically by having a larger body size in males (SVL up to 36.8 mm vs. up to 30.4 mm in *Kalophrynus cryptophonus* **sp. nov.**); only microscopic spines on head (vs. large skin spines in *Kalophrynus cryptophonus* **sp. nov.**), the absence of nuptial pads in males (present in *Kalophrynus cryptophonus* **sp. nov.**); having canthus rostralis distinct, sharp (vs. indistinct, smooth in *Kalophrynus cryptophonus* **sp. nov.**); well developed anterior palatal dermal ridge (reduced in *Kalophrynus* *cryptophonus* **sp. nov.**), and less extensive webbing on toes (webbing formula **I** 1–2 **II** 1 ½ –3 **III** 2–3¾ **IV** 4–2 **V** vs. **I** 1–2½ **II** ½–2½ **III** 1½ –3½ **IV** 3¾–1½ **V**). In coloration, *Kalophrynus honbaensis* **sp. nov.** differs from *Kalophrynus cryptophonus* **sp. nov.** by having a black inguinal ocelli being larger than tympanum (ISD/TD 1.47±0.09) vs. smaller than tympanum (ISD/TD 0.77±0.05).

FIGURE 13. Natural habitat of *Kalophrynus honbaensis* **sp. nov.** in Hon Ba Nature Reserve, Khanh Hoa Province. Photo by A. Vassilieva.

Kalophrynus honbaensis **sp. nov.** differs from other congeners distributed in Southern Asia, China, the Malay Peninsula and the Malay Archipelago by a number of morphological and coloration features. In particular, *Kalophrynus honbaensis* **sp. nov.** differs by having a substantially larger body size (SVL 26.7–36.8 mm in males) than *K. barioensis* (SVL 17.5–19.8 mm in males), *K. bunguranus* (SVL 22.2–23.4 mm in males), *K. menglienicus* (SVL 19.75–23.4 mm), *K. nubicola* (SVL 14.4–24.4 mm in males), *K. robinsoni* (SVL 17 mm in males), *K. subterrestris* (SVL 23 mm in males), and *K. stellatus* (SVL 24 mm). Additionally, from these species *Kalophrynus honbaensis* **sp. nov.** differs by the presence of subarticular tubercles under finger IV and toe V and an outer metatarsal tubercle (absent in *K. barioensis* and *K. nubicola*); spines on back and upper arms absent (vs. present in males *K. bunguranus*); moderate webbing on foot (vs. foot webbing absent in *K. menglienicus*), normally developed fingers (vs. shortened finger IV in *K. nubicola*); spiny nuptial pads absent (vs. present in males *K. robinsoni*); anterior palatal dermal ridge straight (vs. angular in *K. subterrestris*, arched in *K. stellatus*); inguinal ocelli present, larger than tympanum (vs. absent in *K. nubicola*, equal to tympanum in *K. stellatus*).

From other congeners *Kalophrynus honbaensis* **sp. nov.** differs by the following features: from *K. baluensis* by having a single black inguinal ocellus on each side of sacrum (vs. one or two yellow ocelli in *K. baluensis*); from *K. calciphilus* by the absence of dentition on the upper jaw (weak dentition present in *K. calciphilus*), by having finger IV longer than I (vs. finger IV shorter than I in *K. calciphilus*) and by coloration features (black dorsum and chest, white dorsolateral line, red iris in *K. calciphilus*); from *K. eok* by the presence of inguinal spot (absent in *K. eok*) and other coloration features (dark interorbital bar and chevron on shoulders in *K. eok*); from *K. heterochirus* by having normally developed fingers (vs. strongly shortened fingers I, II and IV in *K. heterochirus*) and one black inguinal spot on each side of sacrum (vs. few large round yellowish-white spots in the lumbar region in *K. heterochirus*); from *K. intermedius* by the presence of inguinal ocelli (absent in *K. intermedius*) and moderate webbing on toes (vs. extensive webbing in *K. intermedius*); from *K. limbooliati* by the presence of subarticular tubercles under toe V and outer metatarsal tubercle (absent in *K. limbooliati*) and the absence of a light dorsolateral line (present in *K. limbooliati*); from *K. minusculus* by coloration features (brownish black coloration with black banded pattern in *K. minusculus*); from *K. orangensis*by the presence of rudimental webbing on fingers (absent in *K. orangensis*); from *K. palmatissimus* by moderate toe webbing (vs. extensive webbing in *K. palmatissimus*); from *K. pleurostigma* by a smaller body size (SVL 35–50 mm in males *K. pleurostigma*) and the absence of nuptial pads on fingers (present in males *K. pleurostigma*); from *K. punctatus* by a slightly larger body size (SVL 22–27 mm in males *K. punctatus*), the presence of an outer metatarsal tubercle (absent in *K. punctatus*) and relatively larger tympanum (about ¾ of eye diameter vs. ½ of eye diameter in *K. punctatus*); from *K. tiomanensis* by the presence of an outer metatarsal tubercle (absent in *K. tiomanensis*) and the absence of dorsal pattern (vs. spotted lower back in *K. tiomanensis*); from *K. yongi* by the absence of a strongly developed humeral spine and nuptial pads (present in males *K. yongi*).

Distribution. To date, the new species is known only from the type locality, Hon Ba Mountain, Cam Lam District, Khanh Hoa Province, Vietnam.

Etymology. The species name is a toponymic adjective derived from the name of the type locality of the new species, the Hon Ba Mountain in Khanh Hoa Province.

Recommended vernacular name: The recommended common name in English is Hon Ba sticky frog.

Discussion

Discovery of new species of *Kalophrynus* in southern Vietnam. Our molecular analysis did not fully resolve the phylogenetic relationships between the 12 studied *Kalophrynus* species and application of additional mtDNA and nuclear genetic markers is required for a better understanding of the evolutionary history of the genus. However, we presented a phylogeny for the maximal number of species published up to date (see Matsui *et al.* 2011). Our study indicates the highest divergence between Bornean species of the genus *Kalophrynus* and also suggests the monophyly of the Indochinese species of the genus. In Indochina, we recorded the presence of two major evolutionary lineages, one of them corresponding to large-bodied, lowland *K. interlineatus* and the second lineage containing two, comparatively smaller, mountain-dwelling populations from the southern Annamite Mountains.

The Annamite, or Truong Son Mountains are recognized as a herpetological biodiversity hotspot that remains insufficiently studied (Orlov & Ananjeva 2007; Bain & Hurley 2012 and references therein). The Da Lat, or Lang Bian Plateau is one of the most poorly investigated parts of the Annamites with many species of amphibians and reptiles being described from this area during the last three years (Rowley *et al.* 2010; 2011a; 2011b; Stuart *et al.* 2011; Orlov *et al.* 2012; Nazarov *et al.* 2012). The discovery of the two new species of mountain dwelling *Kalophrynus* significantly increases the diversity of the genus in the Indochinese region and confirms the key role of the Truong Son Mountains in shaping endemism and species richness patterns in the herpetofauna of Indochina.

Ohler & Grosjean (2005) restricted use of the name *Kalophrynus interlineatus* to populations from India to Laos, with tentative application to Vietnamese and south Chinese populations. Our preliminary phylogenetic data indicate relatively small genetic variation within *K. interlineatus* and do not contradict application of this name to the Indochinese populations, though further studies of genetic variation of this widespread *Kalophrynus* species are needed.

Distribution of *Kalophrynus* in Indochina. The known distribution of the genus *Kalophrynus* in Indochina based on published data and our records is shown in Fig. 1. Distribution of the genus *Kalophrynus* in Vietnam and adjacent territories is wider than previously thought (Orlov & Ananjeva 2007; Nguyen *et al.* 2009). The most widespread species is the lowland large-bodied *K. interlineatus*. In Indochina it is known from southern Vietnam and several provinces in the north (not shown in Fig. 1; see Nguyen *et al.* 2009). Our study provides the first record of this species for several provinces in the south of Vietnam (Dak Lak, Lam Dong, Binh Phuoc, Binh Thuan, Ba Ria – Vung Tau, Tay Ninh). The species is also occasionally found in the central Vietnamese provinces from Quang Nam to Quang Binh (Orlov & Ananjeva 2007), is known from several localities in Laos (Teynié *et al.* 2004; Stuart 2005), and is thought to have a wider distribution throughout Cambodia (Neang & Holden 2008), with localities reported from Ratanakiri (see Fig. 1) and Mondul Kiri (Stuart *et al.* 2006) Provinces, as well as from Cardamom Mountains (Ohler *et al.* 2002; Stuart & Emmett 2006) and Phu Quoc island (Vietnam; Nguyen *et al.* 2009, our data).

Our specimen of *K. interlineatus* from Loc Bao Forestry Enterprise was collected in disturbed polydominant tropical monsoon forest on a gentle hill slope at an altitude around 600 m a.s.l. and was found approximately 13 km from the type locality of *Kalophrynus cryptophonus* **sp. nov.** The sympatric distribution of both species named is quite probable but not confirmed yet; this issue requires further investigation.

Whereas *K. interlineatus* is usually found in lowland monsoon tropical forests at altitudes below 500–600 m a.s.l., the two new mountain-dwelling *Kalophrynus* inhabit forest at higher altitudes. The ranges of *Kalophrynus cryptophonus* **sp. nov.** and *Kalophrynus honbaensis* **sp. nov.** are located on the opposite edges of the Lang Bian Plateau with quite different climatic conditions. *Kalophrynus honbaensis* **sp. nov.** is found on the top of Hon Ba Mountain, an isolated mountain massif which forms the easternmost part of the Lang Bian Plateau with wet maritime climate, and inhabits wet, mossy montane forest. *Kalophrynus cryptophonus* **sp. nov.** inhabits the western slope of the Lang Bian Plateau with notably drier climate and is associated with a specific habitat in bamboo tangles. Thus, we assume that the ranges of the two new species are likely isolated from each other due to different altitudinal preferences and the isolated nature of the mountain massifs. However, further studies are needed to determine whether the new species have allopatric distributions; discoveries of new *Kalophrynus* spp. populations in Lang Bian plateau are anticipated.

Life history. Members of the genus *Kalophrynus* differ substantially in their reproductive modes. Some species breed in still water, mainly in temporary ponds, like the majority of Asian microhylids. This is especially the case for *K. interlineatus* (Orlov & Ananjeva 2007; our observations) and *K. palmatissimus* (Manthey & Grossmann 1997), which spawn en masse in rainy ponds after heavy cloudbursts. In contrast, some small species encountered in the Malay Peninsula and Borneo tend to reproduce in small, secluded water-filled cavities. For example, *K. yongi* is a phytotelm-breeder, whose tadpoles develop in the cups of pitcher plants (*Nepenthes* spp.), water-filled internodes of bamboo stems or holes in the tree trunks or stumps (Matsui 2009). Male *K. eok* have also been found calling in a water-filled bamboo internode (Das, Haas 2003), potentially indicating phytotelm breeding in this species. Additionally, *K. calciphilus* is presumed to spawn underground in karst holes (Dehling 2011). For some recently described species (*K. barioensis, K. limbooliati*) their reproductive mode is unknown, however, given the absence of any waterbodies in the proximity of calling frogs, authors presume they are also phytotelm breeders (Matsui & Nishikawa 2011; Matsui *et al.* 2012). This is also likely the case of *Kalophrynus honbaensis* **sp. nov.**, since this species was discovered on the top of narrow mountain ridge with abrupt slopes, where any kinds of open ponds were absent even at the height of rainy season. Large-stem bamboos are absent on Hon Ba Mountain at altitudes around 1500 m a.s.l., so the breeding habitat of *Kalophrynus honbaensis* **sp. nov.** remains unclear. The species *K. pleurostigma* is rather opportunistic in this concern and uses a variety of microhabitat for breeding, namely temporary rainy pools, road ruts, bamboo stems, holes or pitcher plant cups (Inger 1966; Berry 1972; Lim & Ng 1991; Manthey & Grossmann 1997; Leong & Chou 1999).

The diversity of *Kalophrynus* larvae is rather poorly studied; only tadpoles of *K. pleurostigma*, *K. palmatissimus* and *K. yongi* have been described to date (Berry, 1972; Kiew, 1984a; Inger, 1985; Lim & Ng, 1991; Leong & Chou 1999; Matsui 2009). Apart from some characteristics common for microhylid larvae in general, such as medial spiracle and absence of keratinized mouthparts, tadpoles of *Kalophrynus cryptophonus* **sp. nov.** share with other *Kalophrynus* some peculiarities of oral morphology (terminal mouth without papillae), but differ significantly in other features. In particular, they differ substantially from tadpoles of *K. palmatissimus* and *K. pleurostigma* by tail length and shape. In pond-breeding *K. palmatissimus,* the tail of the tadpole is moderately long, with high fins and a terminal filament (Kiew 1984a), like many other lentic microhylid larvae (Chou & Lin 1997); in *K. pleurostigma* with variable breeding ecology and presumably endotrophic tadpoles, the larval tail may vary significantly in length and shape (Inger 1966; 1985; Berry 1972; Lim & Ng 1991; Leong & Chou 1999), but is usually widely lanceolate with relatively high fins. In contrast, in phytotelm-breeding *K. yongi* tadpoles have a very long tail (in average 2.8 times longer than body length) and low tail fins with straight margins (Matsui 2009). Tadpoles of *Kalophrynus cryptophonus* **sp. nov.** are similar to *K. yongi* larvae in tail features and general appearance, but differ from them by wider and markedly longer snout (mean SED/SVL 0.39 mm vs. 0.27 in *K. yongi*). The very scarce pigmentation in almost colorless *Kalophrynus cryptophonus* **sp. nov.** tadpoles possibly is related to their habitation of closed microhabitats with weak lightning. The morphology of *Kalophrynus cryptophonus* **sp. nov.** larvae therefore likely reflects the specialization of this species to phytotelm breeding.

Vocalization. To date, the advertisement calls of eight *Kalophrynus* species have been described (see Table 5); they can be divided in two main groups: the relatively long-duration and low-frequency calls like those of *K. interlineatus* and *K. pleurostigma*, and the short-duration, and higher frequency calls of *K. barioensis*, *K. baluensis*, *K. calciphilus*, *K. limbooliati*, *K. nubicola, K. yongi*, and *Kalophrynus cryptophonus* **sp. nov.** Other acoustic features, such as presence and number of pulses, as well as the occurrence of frequency modulation, vary between species. Since the temporal acoustical properties in amphibians are temperature-dependent (Gerhardt, Huber 2002), the difference between the species groups may be explained partially by the environmental factors; however, the frequency of calls in anurans is known to be correlated mainly with body size (see review in Wells, 2007). *K. interlineatus* and *K. pleurostigma* are among the largest representatives of the genus, and other acoustically studied species belong to the group of relatively small *Kalophrynus*, including *Kalophrynus cryptophonus* **sp. nov.**

The calling strategy in *Kalophrynus* also may differ; for example, males *K. interlineatus* vocalize in dense and loud, easy to localize choruses near the breeding ponds (Orlov & Ananjeva 2007; our observations), and small *Kalophrynus* call in rather loose choruses or singularly, often from diverse hides, and are rather hard to localize (Matsui 2009; Dehling 2011; Matsui *et al.* 2012; this study). Among small species the advertisement calls of *Kalophrynus cryptophonus* **sp. nov.** and *K. yongi* are the most similar in call duration, call repetition rate and frequency. Given that both these species are phytotelm breeders with similar male calling strategy (they call while hidden near the breeding sites), their acoustic features may be related to their reproductive ecology. Such sites (e.g. burrows and tree holes) may perform important acoustic functions, acting as resonators enhancing the frequency of maximal amplitude (Bailey & Roberts 1981; Penna & Solis 1999) or increasing sound propagation. The hollow bamboo stems are obviously good resonators, and to the human ear the calls of the males vocalizing from their natural hides sounded markedly louder than the same calls uttered inside the collector containers.

Phytotelm breeding provides a specific ecological niche for anurans, which appears relatively important for mountain-dwelling taxa in the region. Recent field studies in mountainous areas of southern Vietnam indicate that not only do typical phytotelm breeding groups of frogs, such as the rhacophorid genus *Theloderma*, reproduce in water-filled tree hollows (*T. palliatum* Rowley, Le, Hoang, Dau & Cao, *T. nebulosum* Rowley, Le, Hoang, Dau & Cao, *T. stellatum* Taylor; see Rowley *et al.* 2011a; Orlov *et al.* 2012) or bamboo internodes (*T. bambusicola*, see Orlov *et al.* 2012), but other anuran taxa that typically breed in stream or ponds show remarkable adaptations to phytotelm breeding (e.g. *Rhacophorus vampyrus* Rowley, Le, Thi, Stuart & Hoang; see Rowley *et al.* 2010; 2012; Vassilieva *et al.* 2013). The present paper is the first report of phytotelm breeding Microhylidae for Vietnam.

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APPENDIX. Material examined.

Kalophrynus interlineatus (5 specimens): southern Vietnam (Cat Tien National Park, Dong Nai Province), approximate coordinates 11°26' N, 107° 25' E, nearly 120 m a.s.l. (ZMMU A-4606; individual field numbers from ABV-00405 to ABV-00409); (5 specimens): Laos (Khammouane Province) (private collection of Nikolai L. Orlov, Zoological Institute of Russian Academy of Sciences, St. Petersbourg)

Kalophrynus pleurostygma (2 specimens): Thailand (Hala-Bala Wildlife Sanctuary, Narathiwat Province) (Herpetological collection of the Prince of Songhkla University, Hat Yai; no numbers).

Kalophrynus cryptophonus sp. nov. (6 specimens, adults): southern Vietnam (Loc Bao, Lam Dong Province), coordinates 11°44' 17" N, 107° 42' 25" E, elevation 800 m. a.s.l. (ZMMU A-4944; ZMMU A-4858; ZMMU A-4859; individual field numbers ABV-00136, and from ABV-00222 to ABV-00226); tadpoles, from the same locality as adults (ZMMUA-4986; field number ABV-00234).

Kalophrynus honbaensis sp. nov. (2 specimens): southern Vietnam (vicinity of the Yersin station on Hon Ba Mountain, Hon Ba Nature Reserve, Cam Lam District, Khanh Hoa Province), coordinates 12°07' 16" N, 108° 56' 55" E, elevation 1500 m a.s.l. (ZMMU A-4941, ZMMU A-4943; individual field numbers ABV-00302 and ABV-00320).