



A new insular, endemic frog of the genus *Kalophrynus* Tschudi, 1838 (Anura: Microhylidae) from Tioman Island, Pahang, Peninsular Malaysia

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Abstract

A new insular, endemic species of microhylid frog of the genus *Kalophrynus* is described from Tioman Island, off the southeastern coast of Pahang, Peninsular Malaysia. *Kalophrynus tiomanensis* **sp nov.** can be differentiated from its congeners by the following combination of characters: SVL 21.4–26.3 mm; reduced webbing on toes; outer metatarsal tubercle absent; large, black inguinal spot and unique markings on dorsum. This discovery increases the number of endemic species of amphibians on Tioman Island to at least three.

Key words: Amphibia; Conservation, Herpetofauna, Microhyla, Morphology, reduced webbing, Species complex

Introduction

Microhylid frogs of the genus *Kalophrynus* collectively range from northeastern India (Ohler & Grosjean 2005) and southern China (Yang & Su 1980), through Indochina (Ohler & Grosjean 2005), Peninsular Malaysia (Berry 1975; Matsui 2009; Chan *et al.* 2010a), Borneo (Das & Haas 2003; Inger & Stuebing 2005), Java, and Sumatra (Iskandar 1998) to the Philippines (Frost 2011). Four species occur in Peninsular Malaysia *i.e.* *Kalophrynus pleurostigma* Tschudi, *K. palmatissimus* Kiew, *K. robinsoni* Smith and *K. yongi* Matsui (Chan *et al.* 2010a). *Kalophrynus yongi* is an upland species endemic to the mossy forest on Cameron Highlands, Pahang (Matsui 2009), whereas *K. robinsoni* is an upland species known only from the type series from Gunung Tahan, Pahang (Smith 1922). The most common and widely distributed species, *K. pleurostigma*, ranges throughout lowland dipterocarp forests of the Malay Peninsula (Grandison 1972; Berry 1975; Dring 1979; Norhayati *et al.* 2005; Wood *et al.* 2008; Chan *et al.* 2010b) including the offshore island of Tioman, Pahang off the southeastern coast of Peninsular Malaysia (Grismer 2011; Fig. 1). Formerly considered a single, widespread species, *K. pleurostigma* has been relatively recently split into several species including *K. interlineatus* (Blyth), *K. minusculus* Iskandar and *K. palmatissimus* (Kiew 1984; Matsui 1996; Iskandar 1998). In addition, recent molecular work (Matsui *et al.* 2011) has confirmed our studies in progress that *Kalophrynus pleurostigma* from Peninsular Malaysia is both morphologically and genetically distinct from *K. pleurostigma* from the type locality in Sumatra, Indonesia (*vide* Miracle *et al.* 2007) and deserves distinct species recognition (Chan *et al.* in prep). Therefore, subsequent reference to Peninsular Malaysian populations of *K. pleurostigma* in this paper will be referred to as *Kalophrynus cf. pleurostigma*. To further highlight the complexity of this group, we describe another new species that was previously considered as *K. pleurostigma* (Escobar *et al.* 2003; Grismer 2011) from Tioman Island, Pahang based on several distinct morphological differences which clearly separate it from all other species of *Kalophrynus*.

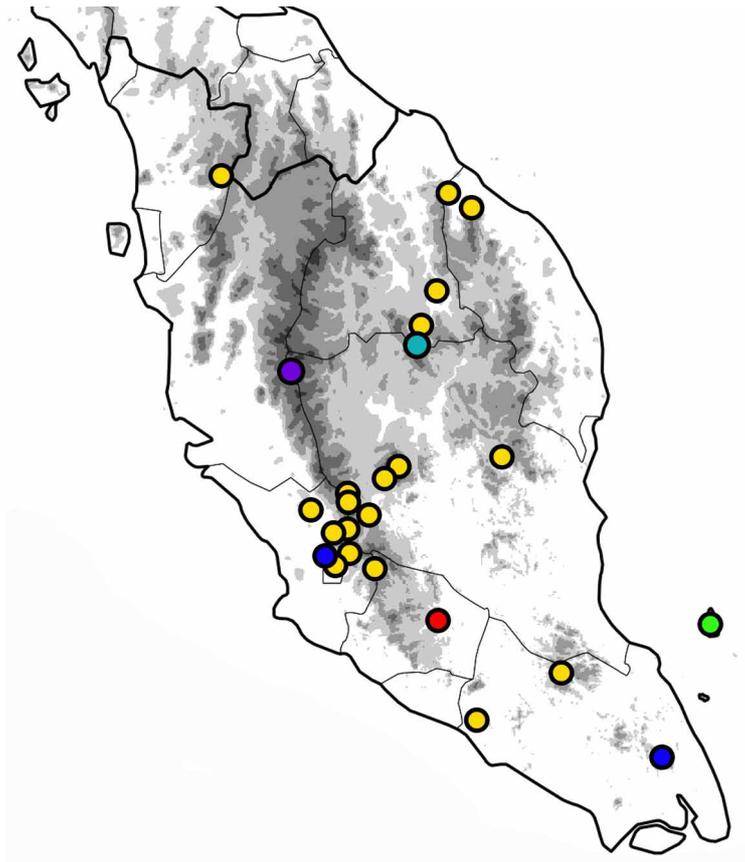


FIGURE 1. Known localities of *Kalophrynus* in Peninsular Malaysia. Yellow: *Kalophrynus* cf. *pleurostigma*; Purple: *K. yongi*; Turquoise: *K. robinsoni*; Red: Type locality of *K. palmatissimus*; Blue: *K. palmatissimus* sympatric with *Kalophrynus* cf. *pleurostigma*; Green: *K. tiomanensis*

Materials and Methods

Fieldwork was conducted along the Tekek-Juara Trail during July 2002 and 2003 and at Gunung Kajang during August 2002, March 2003 and 2004. Specimens were photographed and liver tissue taken prior to preservation and stored in 95% alcohol. Specimens were fixed in 10% formalin and stored in 70% ethanol. Sex was determined by the presence of nuptial excrescences on the base of the dorsal surface of the second and third fingers. The following measurements were taken to the nearest 0.1 mm: Snout-vent-length (SVL) from tip of snout to anus; head length (HL) from posterior margin of lower jaw to tip of snout; head width (HW), taken posterior to eyes at level of tympanae; snout length (SL), from anterior corner of eye to tip of snout; internarial distance (IND), measured from medial inner margins of nostrils; eye diameter (ED), distance between anterior and posterior corners of upper and lower eyelids; tympanum diameter (TD), maximum diameter of tympanum; upper eyelid width (UEW), from base of upper eyelid to lateral edge at its widest point; interorbital distance (IOD), distance across top of head between medial margins of orbits at their closest points; eye-nostril distance (END), from anterior corner of eye to posterior margin of nostril; forearm length (FL), from elbow inflection to wrist inflection; tibia length (TL), from knee inflection to ankle inflection. Toe webbing formula follows Savage & Heyer (1997). All specimens have been deposited at the La Sierra University Herpetological Collection (LSUHC), La Sierra University, Riverside, California, USA. Museum abbreviations are as follow: FMNH, Field Museum of Natural History, Chicago, USA; ZRC, Zoological Reference Collection, National University of Singapore.

Systematics

Kalophrynus tiomanensis sp nov.

Fig. 2, 3, 4A-B

Kalophrynus pleurostigma Escobar *et al.* 2003:317; Grismer *et al.* 2006:160; Grismer 2011:53

Diagnosis

We assign the new species to the genus *Kalophrynus* based on the following characters: One or more transverse folds across the palate in front of the oesophagus; finger and toe tips not dilated; snout short, less than twice the eye diameter; inner metatarsal tubercle low, not shovel-like; tympanum visible; skin thick and glandular (Parker 1934). The new species can be differentiated from its congeners by the following combination of characters: adult males 25.8–26.3 mm SVL, adult females 21.4–25.8 mm SVL; fingers with distinct subarticular and palmar tubercles; toe webbing reduced; outer metatarsal tubercle absent; single, large, black inguinal spot on both sides; distinct hour-glass mark between eyes to scapular region or when absent, a series of spots forming a chevron at the scapular region; lower back spotted.

Comparisons

The following is a morphological comparison between *Kalophrynus tiomanensis* and all other congeners: *K. tiomanensis* has a smaller SVL [21.4–26.3 mm (n=5)] compared to *Kalophrynus* cf. *pleurostigma* [33.7–47.3 mm (n=16)], *K. palmatissimus* [31.2–38.8 mm (n=19): Kiew 1984] and *K. yongi* [28.8–31.0 mm (n=3): Matsui 2009] but is larger than *K. robinsoni* (<20 mm : Smith 1922). It further differs from *Kalophrynus* cf. *pleurostigma* and *K. palmatissimus* in having reduced webbing on the second through fifth toes (Fig. 4B,C, D) and from *K. yongi* in having more webbing (formula: I 0 – 3 II 1½ – 3 III 1¾– 3¾ IV 3¾ – 1½ V vs. I 2 – 2⁺ II 2 – 3⁺ III 3 – 4⁺ IV 4 – 2 V). The inguinal spots are relatively larger in *K. tiomanensis* compared to *Kalophrynus* cf. *pleurostigma* and *K. palmatissimus* when present (see discussion). *Kalophrynus tiomanensis* also has a distinct hour-glass shaped marking that extends from between the eyes to the scapular region or if absent, there is a series of spots forming a chevron shaped marking in the scapular region and numerous small, dark spots throughout the lower back, characters that distinguishes it from all other Peninsular Malaysian congeners (Fig. 2, 3).



FIGURE 2. *Kalophrynus tiomanensis* paratype female LSUHC 4682

Kalophrynus tiomanensis is smaller in SVL (21.4–26.3 mm) than *K. interlineatus* (40.4–43.4 mm: Ohler & Grosjean 2005), *K. intermedius* Inger 1966 (38–41 mm), *K. minusculus* (32.2 mm: Iskandar 1998; Matsui 2009), *K. baluensis* Kiew 1984 (34.8–39.0 mm) and *K. calciphilus* Dehling 2011 (male 29.7–30.1 mm, female 35.5–38.8 mm). It further differs from *K. baluensis* in having black vs. yellow ocelli in inguinal region and from *K. calciphilus* in having as opposed to lacking inguinal ocelli. *Kalophrynus tiomanensis* can be distinguished from *K. nubicola* Dring 1983 in having distinct subarticular and metacarpal tubercles on fingers and toes vs. very low, reduced toe pads of thickened skin under the digits and no metacarpal tubercles; distinct tympanum and toe webbing vs. concealed tympanum and no toe webbing in *K. menglienicus* Yang & Su 1980; fourth finger distinct, bearing one subarticular tubercle vs. knob-like fourth finger lacking subarticular tubercle in *K. bunguranus* (Günther 1895); two tubercles under the fourth finger between the palmar tubercle and the tip of fourth finger as opposed to one in *K. subterrestris* Inger 1966 and *K. eok* Das & Haas 2003; one subarticular tubercle on fourth finger vs. none in *K. heterochirus* and *K. subterrestris*; fifth toe not projecting beyond third vs. fifth toe equal or longer than third in *K. punctatus* Peters 1871. *Kalophrynus tiomanensis* further differs from *K. punctatus* and *K. subterrestris* in having as opposed to lacking a dorsolateral stripe and from *K. heterochirus* in having a black inguinal spot in a light area as opposed to a light spot(s) in a dark area.

Holotype

Adult male (LSUHC 5024) from outside Gua Tengku Air (=Tengku Air Cave), Gunung Kajang, Tioman Island, Pahang at 810 m elevation (2°46'12.22"N 104° 9'15.75"E), collected on 9 August 2002 by J. Grismer, P. Wood, Jr., T. Youmans and L. Grismer.

Paratypes

Adult male (LSUHC 6147) and subadult female (LSUHC 5154) collected by the same collectors and locality as the holotype on 20 March 2004 and 19 March 2003 respectively; two adult females (LSUHC 4682, 5558) collected by the same collectors from Tekek-Juara trail on 17 July 2002 and 21 July 2003 respectively.

Description of holotype (measurements in mm)

SVL 26.3; habitus moderately robust; head slightly wider (8.7) than long (8.2); vomerine teeth absent; tongue spatulate; snout sharply pointed, slightly projecting beyond lower jaw; horizontal diameter of eye (3.0) slightly less than snout length (3.6); canthus distinct but not sharp; lores vertical, flat; nostrils lateral, in line with canthus, distinctly closer to tip of snout than to eye; tympanum distinct, in contact with eye; maximum diameter of tympanum (2.3) less than horizontal diameter of eye; upper eyelid width (1.9) almost half of interorbital distance (4.0).

Front limbs moderately long and robust; fingers short with rudimentary webbing; first and second fingers almost equal in length, slightly longer than fourth, third finger longest; finger tips rounded, slightly dilated; subarticular tubercles distinct, rounded, numbering one on the first, second and fourth fingers, two on the third finger; one large, round palmar tubercle at base of outer side of palm bordered anteriorly by four smaller, round tubercles; translucent nuptial excrescences on base of second and third fingers.

Hind limbs relatively short and robust; tibio-tarsal articulation reaches level of tympanum when adpressed against body; relative length of toes from shortest to longest, $1 < 2 < 5 < 3 < 4$; toe tips rounded, slightly dilated; webbing confined to bases of toes, formula: I 0 – 3 II $1\frac{1}{2}$ – 3 III $1\frac{3}{4}$ – $3\frac{3}{4}$ IV $3\frac{3}{4}$ – $1\frac{1}{2}$ V; subarticular tubercles distinct, rounded, numbering one on first and second toes, two on third, three on fourth and none on fifth toe; inner metatarsal tubercle distinct, oval; outer metatarsal tubercle absent.

Skin on dorsal surfaces smooth, infused with fine tubercles that are more apparent on upper eyelids and flanks; dorsolateral fold indistinct but lined with tubercles; supratympanic fold absent; scapular swellings present; belly and underside of thighs covered with large, flat granules; similar but less distinct granules on gular region and chest; underside of front limbs, tibia and tarsus smooth.

Additional measurements are summarized across all types in Table 1.

Color in life

Dorsum yellowish brown; dark brown hour-glass mark extends from between eyes to scapular region; numerous irregularly shaped dark brown spots throughout lower back and sacral region posterior to the hour-glass mark; single large, black, round ocellus on both sides of the inguinal region; pale dorsolateral stripe extends from tip of

snout, through outer margins of upper eyelids and obliquely down flanks towards groin; area below dorsolateral stripe dark brown and fades ventrally; single crossbar on wrist; hind limbs with indistinct crossbars; venter light brown; chest and belly scattered with irregular white spots; underside of hind limbs with dark brown stippling.

TABLE 1. Measurements for the type series in mm. See Materials & Methods for abbreviations

	Holotype LSUHC 5024	Paratype LSUHC 6147	Paratype LSUHC 4682	Paratype LSUHC 5154	Paratype LSUHC 5558
Sex	m	m	f	f	f
SVL	26.3	25.8	24.7	21.4	25.8
HL	8.2	8.1	8.1	7.3	8
HW	8.7	8.6	8.4	7.3	8.5
IND	2.3	2.3	2.4	2	2.4
SL	3.6	3.2	3.4	2.7	3.5
ED	3	3	3	2.5	2.9
TD	2.3	2.3	2.3	2	2.3
UEW	1.9	1.9	1.8	1.8	1.9
IOD	4	3.8	3.9	3	3.8
END	2.3	2.3	2	1.7	2.1
FL	7.9	7.5	7.6	5.6	7.7
TL	10.4	10.3	10.5	9.6	10.4

Variation in paratypes

The dorsal tubercles of adult female LSUHC 4682 are more distinct, the base coloration is darker and it has slightly more webbing on the fifth toe; adult females LSUHC 5558 and LSUHC 5154 have a supratympanic fold extending to the axilla; subadult female LSUHC 5154 and adult male LSUHC 6147 lack the dark hour-glass marking on the dorsum but have a series of three small spots between the orbits and another series towards the middle of the back forming an irregular chevron (Fig. 3). Measurements for the paratypes are presented in Table 1.



FIGURE 3. Top row: Type series of *Kalophrynus tiomanensis*; Bottom row: *K. palmatissimus* (left three specimens), *Kalophrynus cf. pleurostigma* (right two specimens)

Sexual dimorphism

Based on the type series, no sexual dimorphism can be detected. Like many other *Kalophrynus*, females may attain larger SVL compared to males, but this can only be ascertained with the acquisition of additional adult specimens.

Distribution

Kalophrynus tiomanensis is endemic to Tioman Island, Pahang where it is known from Gua Tengkok Air near the top of Gunung Kajang and along the Tekek-Juara trail. Further fieldwork may reveal a wider distribution on the island (Fig. 1).

Etymology

The specific epithet is in reference to the type locality of the new species to which it is endemic.

Natural History

Based on *in situ* observations of the type series at the time of capture, *Kalophrynus tiomanensis* occurs in lowland dipterocarp forests from approximately 300 m in elevation up through hill dipterocarp forest to at least 810 m in elevation. This species is a leaf litter inhabitant and is generally most active at night. The paratypes, however, were all found between 1100 hrs and 1400 hrs at the base of large trees under an overcast sky. The holotype was collected at night sitting on a leaf 0.5 m above the forest floor.

Discussion

The discovery of *Kalophrynus tiomanensis* increases the number of endemic frogs on Tioman Island to three species (the other two being *Leptotalax kajangensis* Grismer, Grismer & Youmans and *Ansonia tiomanica* Hendrickson) and the total number of endemic reptiles and amphibians to 17 species (Grismer 2011), once again highlighting the importance of offshore islands as key areas for conservation and biodiversity. Unsurprisingly, *K. tiomanensis* was previously identified as *K. pleurostigma*, as is often the case with many *Kalophrynus* due to the high variability within *K. pleurostigma sensu stricto* (Bourret 1942; Inger 1954, 1966).

On the distribution and variation of *Kalophrynus cf. pleurostigma* and *K. palmatissimus* in Peninsular Malaysia

Kalophrynus cf. pleurostigma has often been confused with *K. palmatissimus* due to a number of inconsistent characters used to diagnose both species, and this has resulted in inaccurate distribution records. We examined a large sample size of both species and hereby provide some observations on the distribution, identification and variation of the two species.

In Peninsular Malaysia, *Kalophrynus cf. pleurostigma* is much more abundant and widespread, occurring in the northern state of Kedah at Weng, Ulu Muda (Norhayati *et al.* 2005) as well as Gunung Lawit (Dring 1979) and Lata Tembakah in the eastern state of Terengganu, and in various parts in the central and southern states of Pahang, Selangor (Berry 1975), Negeri Sembilan and Johor (Wood *et al.* 2008; Chan *et al.* 2010b). *Kalophrynus palmatissimus* on the other hand, has only been verified from three localities; Pasoh, Negeri Sembilan (type series: Kiew 1984); Engkabang trail (ZRC 1.11000) and Kruing trail (ZRC 1.7530) at the Forest Research Institute Malaysia (FRIM), Selangor; and Bunker trail at the Gunung Panti Forest Reserve, Johor (ZRC 1.11801, 1.10349, 1.10348; Fig. 1). Kiew (1984) stated that *K. palmatissimus* differs from *Kalophrynus cf. pleurostigma* in being smaller, having a more pointed snout, shorter head, smaller tympanum, shorter arm, and more webbing on the feet and concluded that its most distinctive features are the degree of webbing on its feet and its more pointed snout. We examined 46 specimens of *Kalophrynus* from various localities throughout Peninsular Malaysia (appendix) and conclude that the most reliable character is the degree of webbing on the foot and size of adult females. The webbing formula for *K. palmatissimus* is I 0.5 – 2 II 0.5 – 2 III 0 – 2 IV 2 – 0 V (Fig. 4D) and the formula for *Kalophrynus cf. pleurostigma* is I 0.5 – 2 II 0.5 – 3 III 1.5 – 3.5 IV 3.5 – 1 V (Fig. 4C). Webbing in *Kalophrynus cf. pleurostigma* may vary slightly on the first three toes but always has at least three phalanges free of web on the fourth toe and at least half a phalanx free on the fifth toe as opposed to *K. palmatissimus* which always has webbing extending beyond the medial subarticular tubercle on the fourth toe and to the tip of the disc on the fifth toe. We did not observe any quantifiable difference for the shape of the snout but noted that some specimens have more blunt snouts due to the rubbing and compression of specimens in storage jars.

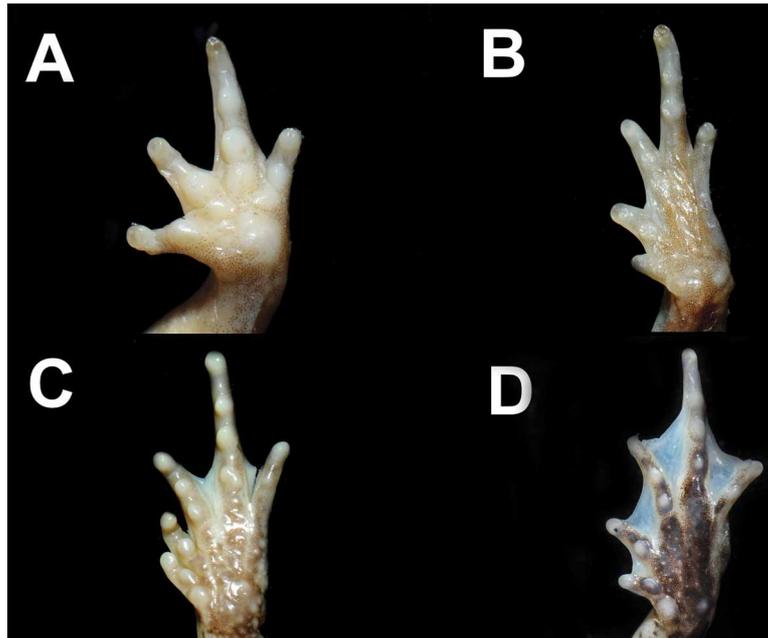


FIGURE 4. Ventral view of **A.** *Kalophrynus tiomanensis* left hand; **B.** *Kalophrynus tiomanensis* left foot; **C.** *Kalophrynus* cf. *pleurostigma* left foot; **D.** *Kalophrynus palmatissimus* left foot

Kiew (1984) gave a size range of 31.2–38.8 mm SVL ($n=19$) for *K. palmatissimus* without stating the sex. The specimens of *K. palmatissimus* we examined fell within this range whereas males of *Kalophrynus* cf. *pleurostigma* ranged from 33.7–38.1 mm SVL ($n=7$) and females from 41.1–47.3 mm SVL ($n=10$). *Kalophrynus pleurostigma* from Borneo is significantly larger than *Kalophrynus* cf. *pleurostigma*. Inger & Stuebing (2005) reported males at 37–50 mm and females at 35–57 mm SVL. We examined a series of six adult specimens from Borneo that measured 40–49.3 mm for males ($n=3$) and 48.3–50.7 mm for females ($n=3$). Furthermore, all Bornean *K. pleurostigma* have one large, black inguinal spot on each side and males have conspicuous, spinose tubercles on the dorsum. Only three out of seven males of *Kalophrynus* cf. *pleurostigma* we examined had spinose dorsal tubercles.

Another character frequently used to diagnose these two species is the presence of inguinal spots. According to Ohler & Grosjean (2005), a distinct inguinal spot is always present in *K. pleurostigma*. We assessed this character in 41 specimens of *Kalophrynus* cf. *pleurostigma* from Peninsular Malaysia and found only three specimens with distinct spots on both sides, 20 specimens with no inguinal spots, five specimens with a spot only on one side of the groin, and other specimens with varying degrees of intermediate spotting. Some have very tiny spots, one big spot and one small spot on each side, multiple small spots, or spots which appear more like streaks extending from the dorsal markings. The location of these spots varies as well with some located more dorsally or anteriorly with regard to the groin. Similarly, Kiew (1984) diagnosed *K. palmatissimus* as lacking inguinal spots but we examined two specimens from the Bunker trail, Johor (ZRC 1.11801, 1.10348) with multiple small spots. At this point, we hypothesize that inguinal spotting is population instead of species specific. However, we are not dismissing Ohler & Grosjean's (2005) postulation that a distinct inguinal spot is always present in *K. pleurostigma* since populations in Peninsular Malaysia are not conspecific.

It is worthy to note that *K. palmatissimus* is sympatric with *Kalophrynus* cf. *pleurostigma* in two localities i.e. Engkabang trail at the Forest Research Institute Malaysia (FRIM), Selangor and at Bunker trail, Gunung Pantu Forest Reserve, Johor. The implications of sympatric lineages are still unclear and pose interesting questions regarding niche partitioning, competition, cryptic species and potential hybridization.

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Appendix: Material examined

Kalophrynus baluensis ZRC 1.10869 Kinabalu Park, Sabah

Kalophrynus heterochirus ZRC 1.11258–59 Kalimantan, Indonesia

Kalophrynus interlineatus ZRC 1.9922 Nam Cat Tien National Park, Vietnam

Kalophrynus palmatissimus ZRC 1.9113, 1.1100 Forest Research Institute Malaysia, Selangor

Kalophrynus pleurostigma FMNH 125765 Kuala Lumpur Mts, near km13 on Pahang Rd; FMNH 143674, 143675, 143676 Bukit Lagong Forest Reserve, Selangor; FMNH 185810–11, 185813–15 Bukit Lanjan, Kuala Lumpur; FMNH 185812 Kuala Lompat, Pahang; FMNH 218951, 218954–56, 218961, 218963, 218967–70 Muar town, on road to sea that starts ca 2 mi North of Muar River bridge; ZRC 1.10797 Ulu Muda; ZRC 1.7532, 1.10194, 1.9113, LSUHC 4002, 4006, 4831, 4837 Forest Research Institute Malaysia, Selangor; LSUHC 7660 Endau-Rompin National Park (Peta), Johor; LSUHC 8214, 8216 Endau-Rompin National Park (Selai), Johor; LSUHC 8915–16 Bunker Trail, Gunung Panti Forest Reserve, Johor; LSUHC 8828 Lata Tembakah, Terengganu; ZRC 1.8021–22, 1.10510 Bukit Timah Nature Reserve, Singapore; FMNH 77266 Brantian River Estate, Tawau, Sabah; FMNH 138061, 145566–67 Nanga Tekalit, Mengiong River, Kapit, Sarawak; FMNH 150430 Sungai Seran, FMNH 157673, 157680–81 Sungai Pesu, Bintulu, Sarawak; FMNH 230845, 230849, 231267, 231268, 244773–74 Danum Valley Research Center, Sabah; FMNH 50718–23, 50728–29, 50731–32 Davao Province, Mindanao, Philippines
Kalophrynus subterrestris ZRC 1.3172 Bako National Park, Sarawak