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MORPHOMETRY OF STRIPED TREE FROGS, *Polypedates leucomystax* (GRAVENHORST, 1829) FROM INDONESIA WITH DESCRIPTION OF A NEW SPECIES

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We examined patterns of geographic variation within *Polypedates leucomystax* using multivariate statistical analyzes of 14 morphological characters measured from 146 specimens obtained from Sumatra, Java, Kalimantan, and Sulawesi. Discriminant analysis revealed ten morphological characters and found that the Sulawesi population is morphologically distinct from all others considered. Following the Evolutionary Species Concept, we describe the Sulawesi population as a new species in *P. leucomystax* species complex.

Keywords: geographic variation, *Polypedates leucomystax*, multivariate, Sulawesi, new species.

INTRODUCTION

The genus *Polypedates* (Anura: Rhacophoridae) includes 11 species in Southeast Asia, four of which occur in Indonesia, namely *P. colletti*, *P. leucomystax*, *P. macrotis*, and *P. otilophus* (Frost et al., 2006). *Polypedates leucomystax* (Gravenhorst, 1829) is widely distributed in Asia, with a geographic range that includes India, southern China, the Philippines, the Malay Peninsula, Sumatra, Java, Borneo, Sulawesi, Bali, and Lombok (van Kampen, 1923; Inger, 1966; Iskandar, 1998; Iskandar and Colijn, 2000; Kamsi, 2003; Marquez and Eekhout, 2006).

Many wide ranging species in South-East Asia may in fact represent species complexes, as has been shown recently for *Rana chalconota* (Inger et al., 2009), *Varanus indicus* (Koch et al., 2009), and *Dendrelaphis pictus* (van Rooijen and Vogel 2008). Thus, we suspect that *Polypedates leucomystax* may fit this description as well. We correspondingly opted to evaluate *P. leucomystax* populations from several major islands and biogeographical boundaries to determine if morphometric variation could be identified suggesting cryptic speciation. Our sample includes representatives from the Sunda Shelf Island of Borneo, Sumatra, and Java, as well as the Wallacean island, Sulawesi. We hypothesized that our sample of *P. leucomystax* would represent up to three cryptic species, with one on the Sunda Shelf islands of Borneo and Sumatra, a second on Java, and third on Sulawesi. We based this hypothesis on the observation that many Sunda Shelf clades include Javan endemics, as well as the expected species barrier spanning Wallace's Line (between the Sunda Shelf and Sulawesi).

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MATERIAL AND METHODS

Sampling. A total of 146 specimens from Sumatra, Java, Kalimantan (Indonesian Borneo) and Sulawesi were examined and measured. Measurements were taken using digital calipers to the nearest 0.01 mm (Table 1). Measurements follow Menzies (1987) as follows: snout vent length (SV, from tip of snout to vent); tibia length (TL, distance between knee and heel); tarsal length (TR, distance between tarsus and knee); fourth toe length (T4); third toe length (T3); fourth toe disc diameter (T4D); third finger length (F3); third finger disc diameter (F3D); head width (HW); eye-naris distance (EN); internarial distance (IN); eye diameter (EY); tympanic diameter (TY) and interorbital distance (IO). Comparative materials examined are listed in appendix.

Statistical analyses. Each sample was classified a priori into four groups recognized as island population

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TABLE 1. Summary of Morphometric Measurements for the *Polypedates leucomystax* Species Complex

Population		TL/SVL	TR/SVL	T4/SVL	T3/SVL	T4D/SVL	F3/SVL	F3D/SVL	HL/SVL	HW/SVL	EN/SVL	IN/SVL	EY/SVL	TY/SVL	IO/SVL
Sulawesi	mean	0.582	0.330	0.458	0.334	0.046	0.312	0.054	0.405	0.352	0.113	0.096	0.121	0.082	0.129
	S.D.	0.033	0.027	0.032	0.018	0.006	0.020	0.006	0.018	0.018	0.007	0.006	0.009	0.007	0.009
	min	0.522	0.246	0.335	0.304	0.029	0.262	0.038	0.375	0.322	0.099	0.083	0.105	0.066	0.109
	max	0.692	0.458	0.546	0.389	0.059	0.351	0.068	0.465	0.399	0.129	0.109	0.144	0.096	0.165
	n	57	57	57	57	57	57	57	57	57	57	57	57	57	57
Kalimantan	mean	0.551	0.305	0.436	0.314	0.037	0.299	0.048	0.355	0.320	0.114	0.079	0.108	0.086	0.125
	S.D.	0.015	0.010	0.022	0.020	0.005	0.010	0.003	0.013	0.014	0.005	0.004	0.008	0.010	0.012
	min	0.529	0.290	0.388	0.291	0.028	0.285	0.042	0.330	0.295	0.107	0.071	0.095	0.074	0.107
	max	0.575	0.318	0.466	0.351	0.044	0.316	0.053	0.374	0.120	0.120	0.088	0.125	0.145	0.145
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Java	mean	0.538	0.301	0.426	0.311	0.038	0.295	0.045	0.350	0.325	0.102	0.083	0.105	0.077	0.120
	S.D.	0.030	0.015	0.026	0.018	0.004	0.019	0.005	0.015	0.016	0.007	0.007	0.009	0.007	0.011
	min	0.430	0.271	0.371	0.276	0.027	0.249	0.032	0.302	0.279	0.089	0.064	0.084	0.063	0.096
	max	0.625	0.337	0.484	0.354	0.047	0.361	0.056	0.375	0.362	0.116	0.096	0.128	0.096	0.152
	n	52	52	52	52	52	52	52	52	52	52	52	52	52	52
Sumatra	mean	0.546	0.298	0.414	0.300	0.032	0.296	0.041	0.361	0.314	0.109	0.081	0.115	0.072	0.115
	S.D.	0.031	0.014	0.019	0.013	0,006	0.019	0.005	0.013	0.018	0.006	0.005	0.013	0.006	0.008
	min	0.498	0.265	0.373	0.275	0.018	0.262	0.030	0.338	0.283	0.099	0.072	0.087	0.058	0.104
	max	0.655	0.336	0.465	0.336	0.043	0.331	0.051	0.383	0.364	0.119	0.090	0.144	0.083	0.133
	n	27	27	27	27	27	27	27	27	27	27	27	27	27	27

(Sumatra, Java, Kalimantan and Sulawesi). The mean ± 2 standard deviations were applied as standard univariate descriptive statistics were calculated for each group. All measurement data were standardized by dividing by snout vent length and then transformed into arcsines.

The effect of sex and island on each character was examined using two-way ANOVAs for those islands. Significant level is reported as follows: *0.05 > P > > 0.01; **0.01 > P > 0.001; and ***P < 0.001. From our original set of fourteen characters, we only used ten characters were combined males and females in the multivariate analyses because not influenced by sex or interaction between sex and island. So, for three characters (TL, F3, and T3) that were influenced by sex were analyses separately between males and females, and one character (IO) that were influenced by interaction between sex and island were not used in multivariate analyses. We ran stepwise discriminant function analysis (DFA) to investigate differences among prespecified groups and to determine which variables contributed the most variation to group dispersion (Smith et al., 2008). The best variable for discrimination on the basis of minimized value of Wilk's lambda. All analyses were calculated using SPSS ver. 15.0.

RESULTS

Univariate statistics. Mean, standard deviation, minimum and maximum values, and sample size for Su-

TABLE 2. Multiple Regressions on Sex and Island of *P. leucomystax* Species Complex for Morphometric Characters

Character	Sex	Island/Locality	Interaction
TL/SVL	5.544*1	9.100*2	0.539
TR/SVL	1.504	6.936*2	0.200
T4/SVL	2.528	6.520*2	0.246
T3/SVL	6.149*1	7.809*2	0.596
T4/SVL	0.143	14.187*2	0.490
F3/SVL	4.375*1	1.939	1.111
F3D/SVL	0.214	8.456*2	1.336
HL/SVL	0.377	22.162*2	0.714
HW/SVL	1.975	8.088*2	0.391
EN/SVL	0.874	12.118*2	2.011
IN/SVL	0.454	19.698*2	1.630
EY/SVL	0.373	10.162*2	2.512
TY/SVL	0.460	8.551*2	0.083
IO/SVL	0.298	4.135	3.646*1

Notes. F values are presented for the main effects. For the explanation of character codes see *Materials and Methods*. Significance levels are: *1 0.05 > P > 0.01; *2 P < 0.001.

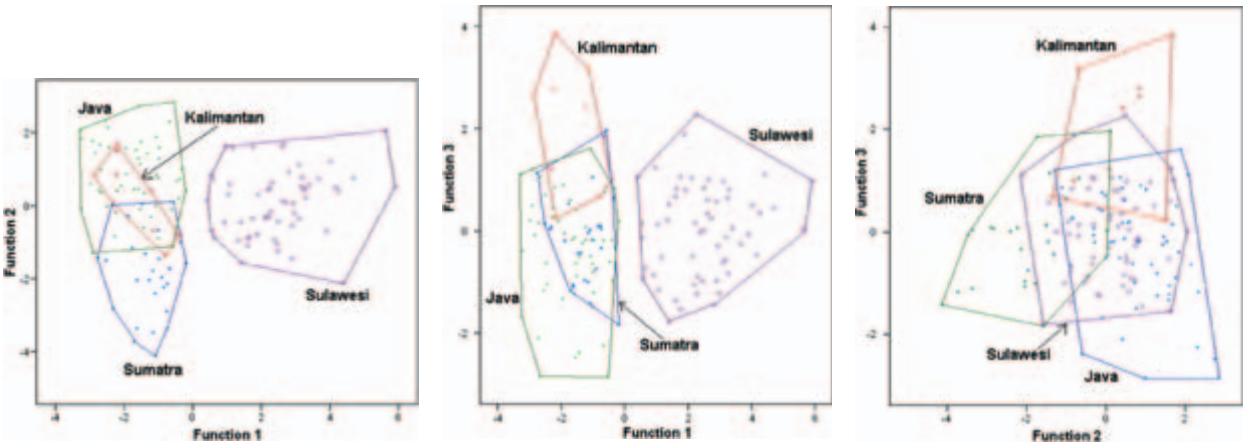

Fig. 1. Plot of function 1, 2 and 3 from Canonical Variate Analysis on four island groups based on ten morphometric characters (HL/SVL, IN/SVL, F3D/SVL, HW/SVL, TR/SVL, EY/SVL, T4/SVL, T4D/SVL, EN/SVL and TY/SVL).

matra, Java, Kalimantan and Sulawesi are presented in Table 1 for all characters examined.

Two-way ANOVAs. Two-way ANOVAs were run for all characters. Three characters (TL/SVL, T3/SVL and F3/SVL) were significantly influenced by sex and one character (IO/SVL) was influenced by interaction between sex and islands. All characters except F3/SVL were influenced by island (Table 2).

Multivariate analyses. Males and females were combined in the following analyses for 10 characters that were not significantly influenced by sex or interaction between sex and island. DFA was run with all islands as a priori groups. Four groups of populations were recognizable from the analysis, i.e., Java, Sumatra, Kalimantan and Sulawesi; with the Sulawesi population found to be distinct from the others using ten characters.

A DFA run on a ten set characters (see Table 3), selected from the above analysis, based on these above four broad groups, extracted three very significant functions ($\chi^2 = 357.796$; $df = 30$; $P = 0.0001$), ($\chi^2 = 134.465$; $df = 18$; $P = 0.0001$) and ($\chi^2 = 45.346$; $df = 8$; $P = = 0.0001$). Function 1 explained 75.7% of the variance, Function 2 (17%), and Function 3 (7.3%). A total of 90.4% of included individuals were classified to their correct group.

The Sulawesi group separated from the other group on Function 1 (Fig. 1). The DFA run on ten characters based on two groups (Sulawesi and Non Sulawesi samples), extracted a significant function ($\chi^2 = 223.640$; $df = 10$; $P = 0.0001$). Total of 100% individuals were classified to their correct group (Fig. 2).

Taxonomic interpretation. Some researchers (Devitt et al., 2008; van Rooijen and Vogel, 2008; Koch

TABLE 3. Canonical Variate Function Coefficients for 10 Morphometric Characters from DFA between Four Island Groups (Sumatra, Java, Kalimantan and Sulawesi).

Characters	Function 1	Function 2	Function 3
HL/SVL	0.949 (54.957)	–0.821 (–47.575)	–0.182 (–10.550)
IN/SVL	0.504 (81.870)	0.290 (47.219)	–0.352 (–57.250)
F3D/SVL	0.411 (77.088)	0.322 (60.432)	0.349 (65.470)
HW/SVL	–0.164 (–9.155)	0.521 (29.028)	–0.561 (–31.229)
TR/SVL	–0.149 (–7.014)	0.294 (13.815)	0.171 (8.060)
EY/SVL	–0.160 (–15.980)	–0.435 (–43.388)	0.148 (14.781)
T4/SVL	–0.076 (–2.514)	0.017 (0.572)	0.099 (3.260)
T4D/SVL	0.026 (4.863)	0.355 (66.947)	0.018 (3.343)
EN/SVL	0.071 (10.748)	–0.263 (–39.668)	0.591 (89.018)
TY/SVL	–0.521(-34.644)	0.456 (62.859)	0.677 (93.365)
Constant	–22.238	–1.659	–6.346
Variation explanation, %	75.7	17	7.3

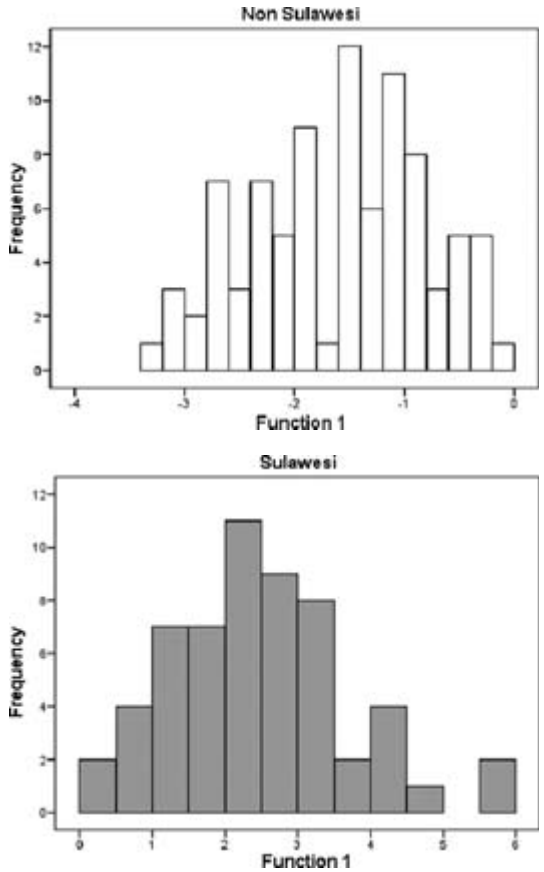

Fig. 2. Histogram of Function 1 from Canonical Variate Analysis based on ten morphometric characters (HL/SVL, IN/SVL, F3D/SVL, HW/SVL, TR/SVL, EY/SVL, T4/SVL, T4D/SVL, EN/SVL, and TY/SVL) from non-Sulawesi and Sulawesi groups.

et al., 2009) demonstrated that the application of advanced multivariate methods for taxonomic issues has greatly improved the possibilities for sophisticated discriminant analyses of cryptic species complexes. We follow the Evolutionary Species Concept (ESC) proposed by Simpson (1961) and later modified by Wiley (1978) that a species is a single lineage of ancestral descendent population of organisms which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate. We also agree with Devitt et al. (2008) in that we view morphological differences corroborated by genetic divergence between populations in combination with geographic distribution as sufficient evidence for recognizing separate evolutionary species. Diagnosibility and geographic isolation serve as the determining criteria for recognition and definition of separated species (McGuire et al., 2007; Vogel and van Rooijen, 2008; Koch et al., 2009).

Fig. 3. Holotype MZB.Amph. 13792 of *Polypedates iskandari* sp. nov.; dorsal view.

The population of Sulawesi is here described as a new species in the *P. leucomystax* species complex.

DESCRIPTION OF TAXA

***Polypedates iskandari* sp. nov.**
(Figs. 3 – 5)

Holotype. MZB.Amph. 13792 (field number BSI 2068), an adult male, collected from Desa Tomado, Kecamatan Kulawi, Kabupaten Donggala, Propinsi Sulawesi Tengah, Sulawesi Island, Indonesia (0°19′39″ S 120°3′3″ E, elevation 1008 m) on 11 November 2004 by Rafe M. Brown, Christopher Hayden, Ferdi Rangkuti, Iqbal Setiadi, and Abdul Rosyid.

Paratype. MZB.Amph. 13781, adult male, collected from Desa Wuasa, Kecamatan Lore Utara, Kabupaten Poso, Sulawesi Tengah (1°26′ S 120°18′29″ E) on 25 November 2004 by Freddy Chain, Umi Laela and Abdul Rosyid; MZB.Amph. 13790 – 91, adult males, same data as holotype; MZB.Amph. 13793, 13795, adult males, collected from Desa Wuasa, Kecamatan Lore Utara, Kabupaten Poso, Sulawesi Tengah (1°26′ S

Fig. 4. Holotype MZB.Amph. 13792 of *Polypedates iskandari* sp. nov.; ventral view.

Fig. 5. Holotype MZB.Amph. 13792 of *Polypedates iskandari* sp. nov.; ventral view of right foot, red arrow showing inner web of fourth toe start on first subarticular tinny longed to second and then widen.

120°18′29″ E) on 22 November 2004 by Freddy Chain, Umi Laela and Abdul Rosyid.

Diagnosis. *Polypedates iskandari* sp. nov. can be distinguished from *P. leucomystax* by the following relatively longer or wider combination of characters: (1) head length, ratio of HL/SVL about 0.373 – 0.465 (x = = 0.405, S.D. = 0.018, n = 57); (2) internarial distance, ratio IN/SVL about 0.083 – 0.109 (x = 0.096, S.D. = = 0.006, n = 57); (3) diameter of the third finger, ratio F3D/SVL about 0.038 – 0.068 (x = 0.054, S.D. = 0.006, n = 57); (4) head wide, ratio of HW/SVL about 0.322 – 0.399 (x = 0.352, S.D. = 0.018, n = 57); (5) tarsal length, ratio TR/SVL about 0.246 – 0.458 (x = 0.330, S.D. = = 0.027, n = 57); (6) eye diameter, ratio of EY/SVL about 0.105 – 0.144 (x = 0.121, S.D. = 0.009, n = 57); (7) fourth toe length, ratio of T4/SVL about 0.335 – 0.546 (x = 0.458, S.D. = 0.032, n = 57); (8) fourth toe disc diameter, ratio of T4D/SVL about 0.029 – 0.059 (x = 0.046, S.D. = 0.006, n = 57); (9) eye-naris distance, ratio of EN/SVL about 0.099 – 0.129 (x = 0.113, S.D. = = 0.007, n = 57) to Javan and Sumatran populations but relatively same to Kalimantan population; (10) tympanic diameter, ratio of TY/SVL about 0.066 – 0.096 (x = 0.082, S.D. = 0.007, n = 57) to Javan and Sumatran populations but relatively smaller to Kalimantan population; (11) inner web of fourth toe start on the first subarticular tinny longed to second and then wide.

Description of holotype. Adult male, small species of *Polypedates*, SVL 44.46 mm; head moderately wide (HW/SVL 0.326), as wide as body, and elongated (HL/SVL = 0.382, HL/HW = 1.175); nostril slightly compressed, oblique, opened up-backward, closer to tip of snout than to eye; internarial distance almost equal to distance from external naris to eye (EN/IN = 1.185, IN/SVL = 0.101, EN/SVL = 0.120); canthus rostralis concave loreal region; snout long (Snout/SVL = 0.189); eye large (EY/SVL 0.113); tympanum distinct, rounded, horizontal diameter as long as half width of eye (TY/EY = 0.647); teeth at upper labial present; vomerine teeth present; skin of head co-ossified with skull; a distinct fold running horizontally from above tympanum to behind arm.

Disc of fingers truncate or spatulate with sircummarginal, relative length of fingers (longest to shortest) is 3 > 4 > 2 > 1; nuptial pad white (in alcohol), visible from above; toes webbed with formula 1(0) 2(1/0)

3(1/0.5) 4(1/1) 5(0), inner web of fourth toe start on first subarticular tinny longed to second and then wide (Fig. 5). Relatively long leg (TL/SVL = 0.554); relative length of toes (longest to shortest) is 4 > 5 > 3 > 2 > 1; subarticular tubercles well developed, single; small elliptical inner metatarsal, outer metatarsal very small. Dorsal surface is with small granular; ventral surfaces granular, throat granular smaller than ventral.

Coloration (in preservation). Dorsal is dark with very few tinny white dots; lateral of the body with some white dots; ventral whitish; tight with five dark bars; throat with dark blotches.

Measurement of holotype (mm). SVL 44.46, TL 24.62, TR 14.07, T4 20.09, T3 14.56, T4D 1.92. F3 13.52, F3D 2.26, HL 17, HW 14.47, EN 5.32, IN 4.49; EY 5.02, TY 3.25, IO 5.19.

Etymology. The specific epithet *iskandari* is non-latinized in honor to Djoko T. Iskandar, the world's authority on the herpetology of Indonesia, in recognition of his tremendous contribution to our knowledge of herpetology of this region.

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APPENDIX
Specimen examined

SUMATRA: MZB.Amph. 6895, male, Batangtoru, Sumatra Utara; MZB.Amph. 6896, female, Batangtoru, Sumatra Utara; MZB.Amph. 7077, female, Bukit Tiga Puluh, Jambi; MZB.Amph. 7078 – 79, (2 ex), males, Bukit Tiga Puluh, Jambi; MZB.Amph. 7081, male, 7082 – 83, (2 ex), females, Bukit Tiga Puluh, Jambi; MZB.Amph. 7085, female, Bukit Tiga Puluh, Jambi; MZB.Amph. 7086, male, Bukit Tiga Puluh, Jambi; MZB.Amph. 7087, female, Bukit Tiga Puluh, Jambi; MZB.Amph. 8168, male, Bukit Dua Belas, Jambi; MZB.Amph. 8169 – 71, (3 ex), males, Jambi; MZB. 8075 – 76, (2 ex), females, Sorolangun, Jambi; MZB.Amph. 11158, 11160 – 62, 11166, 11168, 11173, (7 ex), males, Martabe, Sumatra Utara; MZB.Amph. 14538 – 40, (3 ex), males, Bengkalis, Riau.

KALIMANTAN: MZB.Amph. 2524A-E, (5 ex), adult males, from Gunung Niut; MZB.Amph. 2617, male, Tabalong, Kalimantan Selatan; MZB.Amph. 2915B, male, Sambas, Kalimantan Barat; MZB.Amph. 5962, male, Kalimantan Selatan; MZB.Amph. 6148, female, Kalimantan Selatan; MZB.Amph. 9264, female, Sambas, Kalimantan Barat.

JAVA: MZB.Amph. 2125, female, Jawa Timur; MZB.Amph. 3552, female, Cibinong, Jawa Barat; MZB.Amph. 3809C-D, (2 ex), males, Mount Halimun, Jawa Barat; MZB.Amph. 3809C-D, (2 ex), males, Mount Halimun, Jawa Barat; MZB.Amph. 4639, 41, (2 ex), females, Mount Halimun, Jawa Barat; MZB.Amph. 5741 – 42, (2 ex), males, Mount Halimun, Jawa Barat; MZB.Amph. 6305, female, Mount Halimun, Jawa Barat; MZB.Amph. 6337, female, Yogyakarta; MZB.Amph. 6517, 21, 24 – 25, (4 ex), males, Depok, Jawa Barat; MZB.Amph. 6518, 6523, 26 – 27, (4 ex), females, Depok, Jawa Barat; MZB.Amph. 6668, female, Cibinong, Jawa Barat; MZB.Amph. 6979 – 80, (2 ex), males, Mount Halimun, Jawa Barat; MZB.Amph. 7363, male, Nanggung, Bogor, Jawa Barat; MZB.Amph. 10978, 10980, (2 ex), females, Cibinong, Jawa Barat; MZB.Amph. 10979, male, Cibinong, Jawa Barat; MZB.Amph. 12362, male, Pacitan, Jawa Timur; MZB.Amph. 12864, male, Merubetiri, Jawa Timur; MZB.Amph. 12867, 12869, (2 ex), males, Yogyakarta; MZB.Amph. 12992. male, Alas Purwo, Jawa Timur; MZB.Amph. 12866, 12868, 12870 – 71, (4 ex), females, Yogyakarta; MZB.Amph. 15182, 15186 – 90, (6 ex), males, Ujung Kulon, Jawa Barat; MZB.Amph. 15183, 15185, (2 ex), females, Ujung Kulon, Jawa Barat; MZB.Amph. 3809C, D, (2 ex), males, Mount Halimun, Jawa Barat; MZB.Amph. 14516, male, Pringsurat, Jawa Tengah; MZB.Amph. 14772 – 73, (2 ex), females, Cibinong, Jawa Barat; MZB.Amph. 15114, female, Mount Halimun, Jawa Barat; MZB.Amph. 15308 – 09, (2 ex), females, Banyumas, Jawa Tengah; MZB.Amph. 15317, male, Mount Slamet, Jawa Tengah; MZB.Amph. 15326, female, Banyumas, Jawa Tengah; MZB.Amph. 15114, female, Mount Halimun, Jawa Barat; MZB.Amph. 15182, male, Ujung Kulon, Jawa Barat; MZB.Amph. 15183, 15185, (2 ex), females, Ujung Kulon, Jawa Barat; MZB.Amph. 15186 – 90, (5 ex), males, Ujung Kulon, Jawa Barat; MZB.Amph. 16019 – 20, (2 ex), males, Mount Ciremai, Jawa Barat.

SULAWESI: MZB.Amph. 13730 – 34, (5 ex), males, Minahasa, Sulawesi Utara; MZB.Amph. 13741 – 43, (3 ex), males, Mamuju, Sulawesi Barat; MZB.Amph. 13745, female, Gowa, Sulawesi Selatan; MZB.Amph. 13746 – 50, (5 ex), males, Gowa, Sulawesi Selatan; MZB.Amph. 13751 – 53, 13755, 13757, (5 ex), males, Mamuju, Sulawesi Barat; MZB.Amph. 13758 – 59, (3 ex), males, Gowa, Sulawesi Selatan; MZB.Amph. 13764 – 66, 13768, 13771 – 75, (9 ex), males, Gorontalo; MZB.Amph. 13767, female, Gorontalo; MZB.Amph. 13779 – 80, (2 ex), males, Donggala, Sulawesi Tengah; MZB.Amph. 13781 (paratype), male, Poso, Sulawesi Tengah; MZB.Amph. 13783, male, Donggala, Sulawesi Tengah; MZB.Amph. 13784, male, Bone, Sulawesi Barat; MZB.Amph. 13786 – 88, (3 ex), males, Mamuju, Sulawesi Barat; MZB.Amph. 13790 – 91 (paratypes), (2 ex), males, Donggala, Sulawesi Tengah; MZB.Amph. 13792 (type), male, Donggala, Sulawesi Tengah; MZB.Amph. 13793, 13795 (paratypes), (2 ex), males, Poso, Sulawesi Tengah; MZB.Amph. 13799, 13800 – 03, (5 ex), males, Mamuju, Sulawesi Barat; MZB.Amph. 13804, male, Mamuju, Sulawesi Barat; MZB.Amph. 13806 – 07, (2 ex), males, Poso, Sulawesi Tengah; MZB.Amph. 13809 – 12, (4 ex), males, Poso, Sulawesi Tengah.