A NEW SPECIES OF NARROW-MOUTHED FROG OF THE GENUS KALOULA FROM EASTERN INDOCHINA

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ABSTRACT: We describe a new species of frog of the genus Kaloula (family Microhylidae) from Vietnam, Laos, and Cambodia based on morphological evidence. The new species has previously been mistaken with Kaloula baleata, which it most closely resembles. Kaloula indochinensis sp. nov. can be distinguished from its congeners by the following combination of characters: maximum snout–vent length 53.7 mm; finger tips expanded into wide discs; the majority of specimens with two subarticular tubercles on the fourth toe; inner and outer metatarsal tubercle slightly raised, inner metatarsal tubercle shorter than first toe; absence of dorsolateral stripe; and large, bright, orange-yellow axillary and inguinal spots.

Key words: Amphibia; Anura; Linear discriminant analysis; Principal component analysis; Southeast Asia; Systematics

THE MICROHYLID frog genus Kaloula Gray 1831 currently contains 15 recognized species that collectively range from the Indian subcontinent (Dutta and Manamendra-Arachchi, 1996; Das et al., 2005), eastward to Korea (Lee et al., 2000) and China (Zhao and Adler, 1993), and southward through Southeast Asia (Taylor, 1962; Berry, 1975; Manthey and Grossmann, 1997; Teiñié et al., 2004; Neang and Holden, 2008; Nguyen et al., 2009) including the Philippines (Taylor, 1920; Inger, 1954; Diesmos et al., 2002). Of these, the widespread K. baleata (Miller, in Van Oort and Müller, 1836) typically occurs south of the Isthmus of Kra, through Peninsular Malaysia (Chan et al., 2010), Indonesia (Iskandar, 1998), Borneo (Inger and Stuebing, 2005; Das and Kraus, 2007), and Palawan Island in the Philippines (Taylor, 1920; Brown, 2007; Diesmos and Brown, 2011) but with disjunct records in Gia Lai Province, Vietnam and Khammouan Province, Laos to topotypes from Java, Indonesia (including syntypes) and demonstrate that the Vietnam and Laos specimens are morphologically distinctive and therefore warrant taxonomic recognition.

MATERIALS AND METHODS

Specimens were examined in the holdings of the Royal Ontario Museum (ROM-HERP), Field Museum of Natural History (FMNH), Natural History Museum, London (BMNH), University of Kansas Museum of Natural History (KU), and Muséum National d’Histoire Naturelle, Paris (MNHN). Morphometric

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data for species comparisons were obtained from examined specimens (see Appendix) and data published in Das et al. (2005).

The following characters were measured with a Mitutoyo digitmatic caliper to the nearest 0.1 mm and on the left side of the body for symmetrical characters: snout–vent length (SVL), from tip of snout to vent; head length (HL), from posterior margin of mandible to tip of snout; head width (HW), measured at the level of the jaw articulation; snout length (SNL), from anterior corner of eye to tip of snout; eye diameter (ED), length between anterior and posterior corners of eye; interorbital diameter (IOD), distance between medial margins of palpebrae at its closest point; internarial distance (IND), measured from medial, inner margins of nostrils; third finger disc width (Fin3DW), widest horizontal diameter of third finger disc; femur length (FL), measured from the midventral line to the knee articulation in line with femur; crus length (CL), from knee inflection to tarsal inflection; and inner metatarsal tubercle length (MTTL), from base to tip of inner metatarsal tubercle. Only specimens judged to be sexually mature adults were assessed. All data were taken by the first author except for those from the *K. baleata* syntypes, which were taken by BLS.

**Fig. 1.**—Morphometric analysis of *Kaloula indochinensis* and *K. baleata*: (A) Plot of size (SVL) vs. relative third finger disc width (Fin3DW / SVL); (B) plot of size (SVL) vs. relative outer metatarsal length (MTTL / SVL); (C) ordination of *Kaloula* populations along PC I and PC II; (D) ordination of *Kaloula* populations along PC II and PC III.
Multivariate analyses were conducted in the statistical software environment R, version 2.15.1 (R Development Core Team, 2012). A principal component analysis (PCA) was performed to find the best low-dimensional representation of morphological variation in the data and to determine whether the variation could form the basis of distinct, detectable group structure. Components with eigenvalues above 1.0 were retained in accordance to Kaiser’s criterion. We then incorporated into a separate LDA to demonstrate distinctness of *K. baleata* from the new species.

### RESULTS

Standard morphometric statistics are presented in Table 1 with localities grouped together as operational taxonomic units (OTUs). A statistically informative Mann–Whitney *U*-test could not be performed on separate sexes because of the lack of female specimens and was therefore performed on combined sexes of *K. baleata* (Java) and the new species (Vietnam and Laos combined). Results of this test show statistically significant values for the characters HL, ED, IOD, Fin3DW, and MTTL, with the former three characters having *P*-values < 0.05 and the latter two with *P*-values < 0.0001. Scatterplots of Fin3DW/SVL and MTTL/SVL against SVL show distinct separation between *K. baleata* (Java) and the new species whereas no distinguishable separation could be detected between Vietnam and Laos OTUs (Fig. 1A, B).

**Table 1.—** Morphometric variation between *Kaloula baleata* from Java and *K. indochinensis* sp. nov. from Vietnam/Laos. A Mann–Whitney *U*-test was performed on combined sexes using the nominal variables Java and Vietnam/Laos (grouped together). Values indicate means (+ standard deviations) and ranges.*

<table>
<thead>
<tr>
<th></th>
<th>Java</th>
<th>Vietnam</th>
<th>Laos</th>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
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<td>Female</td>
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<tr>
<td>SVL</td>
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<td>HL</td>
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<td>HW</td>
<td>11.9 ± 1.2</td>
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<td>10.1–12.9</td>
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<td>HL</td>
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<td>SNL</td>
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<td>IOD</td>
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<td>FL</td>
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<tr>
<td>CL</td>
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<tr>
<td>MTTL</td>
<td>1.7 ± 2.1</td>
<td>15.3 ± 0.9</td>
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<td>2.3–2.9</td>
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</tbody>
</table>

* SVL = snout–vent length; HL = head length; HW = head width; SNL = snout length; ED = eye diameter; IOD = interorbital diameter; IND = internarial distance; FL = femur length; CL = crus length; Fin3DW = third finger disc width; MTTL = metatarsal tubercle length.
We retain and discuss the first three principal components which accounted for 67.9% of the total variance and had eigenvalues above 1.0. Loadings for PC I were all negative except for FL and Fin3DW and were most heavily loaded on SVL, HL, HW, and CL, demonstrating that PC I accounted for variation in body size and representing 38.2% of the variance (Table 2). No differentiation could be detected along the PC I axis between Vietnam and Laos OTUs or between Vietnam–Laos and Java. The second principal component (PC II) accounted for 18.8% of the total variance and loaded heavily and positively on MTTL and negatively on Fin3DW, indicating a strong negative correlation between these characters. Variation from these characters distinctly separated K. baleata (topotypic, Java) from Vietnam and Laos OTUs along the PC II axis (Fig. 1C). Loadings of PC III were heavy on IND and Fin3DW but no clear separation could be observed along this axis (Fig. 1D).

Results from the LDA showed similar ordinations but with strong loadings on Fin3DW and MTTL for LD I which accounted for 91.4% of the total variance (Table 2). The second linear discriminant (LD 2), on the other hand, accounted for 8.6% of the total variation and was most heavily loaded on HW. Complete separation was achieved for LD 1 between Java and Vietnam/Laos populations (Fig. 2A). A separate LDA incorporating K. baleata syntypes showed that the Java OTU is morphologically indistinguishable from the syntypes and also demonstrated complete separation from Vietnam and Laos OTUs (Fig. 2B). In summary, results from the PCA and LDA demonstrated that Laos and Vietnam Kaloula are indistinguishable from each other in morphospace and are phenotypically distinguishable from true K. baleata from Java. Based on phylogenetic evidence (Blackburn et al., 2013), morphological differences, and discrete separation in morphospace of Indochinese populations, the specimens from Indochina are concluded to be not conspecific with those from Java Island and are described here as a new species.

**SYSTEMATICS**

Kaloula indochinensis sp. nov.

(Fig. 3)

Kaloula baleata Orlov et al., 2002:99; Teynié et al., 2004:35; Nguyen et al., 2009:94.

Holotype.—ROM-HERP 32935, adult male, SVL 50.3 mm, collected on 22 September 1997 at Krong Pa Village, K’Bang District, Gia Lai Province, Vietnam (14.341389°N 108.4794444°E; ~850 m elevation, WGS84 datum) by R.W. Murphy, N. Orlov, A. Lathrop, C. T. Ho, and L. Lowcock.
Paratypes.—ROM-HERP 32924, 32927, 32931, 32940, adult males, and ROM-HERP 32945, adult female, collected on 28 February 2007 at Nakai District, Khammouan Province, Laos (17.5840167°N, 105.1717917°E, 540 m elevation) by D.A. Emmett. Measurements of the type series are presented in Table 3.

Referred material.—MNHN 2003.1170, Ban Kiatngong (14.5667°N, 106.2°E), Xe Pian National Protected Area, Pathomphon District, Champasak Province, Laos.

Diagnosis.—The new species differs from all other congeners by the following combination of unique characters: SVL 44.1–53.7 mm ($\bar{X} = 48.4 \pm 2.5$) in males and SVL 39.4–53.7 mm ($\bar{X} = 45.4 \pm 6.7$) in females; finger tips expanded into large, transversely expanded discs, Fin3DW 2.6–3.6 mm ($\bar{X} = 3.0 \pm 0.23$) in males, 2.5–3.7 mm ($\bar{X} = 3.0 \pm 0.5$) in females; the majority of individuals with two subarticular tubercles on fourth toe; inner metatarsal tubercle elongate, slightly raised, short than first toe, MTTL 2.1–2.9 mm ($\bar{X} = 2.5 \pm 0.2$) in males, 2.2–3.0 mm ($\bar{X} = 2.6 \pm 0.4$) in females; orange-yellow patch on either side of the neck posterior to eyes; large, bright, orange-yellow axillary and inguinal patch present.

*Kaloula indochinensis* most closely resembles *K. baleata* in overall morphology but can be distinguished by having larger finger disc width (Fin3DW 2.5–3.7 mm in *K. indochinensis* vs. 1.4–2.5 mm in *K. baleata*); smaller inner metatarsal tubercle length (MTTL 2.1–3.0 mm in *K. indochinensis* vs. 3.0–3.7 mm in *K. baleata*); inner and outer metatarsal tubercles raised slightly (in *K. indochinensis*) vs. strongly (in *K. baleata*); two subarticular tubercles on fourth toe in 95% of *K. indochinensis* specimens (remaining 5% had a small, weakly developed basal subarticular tuberecle) vs. three well-developed tubercles in *K. baleata*.

*Kaloula indochinensis* further differs from congeners by the following opposing characters: *K. assamensis*, distribution in northeastern India, SVL up to 38.0 mm, three subarticular tubercles on fourth toe, dorsum pale brown with bright yellow vertebral stripe and broad dark brown lateral stripes, axillary and inguinal spots absent, and stratified coloration on flanks and thighs; *K. aureata*, distribution in southern peninsular Thailand.
(Nakhon Si Thammarat Province), SVL up to 65.0 mm, and dorsum golden with dark brown reticulations and dorsolateral stripe; *K. borealis*, distribution in eastern China and Korea, finger tips slightly dilated but not forming wide discs, inner and outer metatarsal tubercle distinctly large and raised, inner metatarsal tubercle longer than first toe, dorsum gray-brown with dark blotches forming a distinct network on the flanks; *K. conjuncta*, distribution in Philippines, SVL up to 47.0 mm, web reaching disc of fourth toe, stratified lateral flank coloration, and weak outer metatarsal tubercle; *K. kalingensis*, distribution in Luzon, Polillo, and Palau islands, Philippines, SVL up to 39.3 mm, dorsal tubercles absent, weak outer metatarsal tubercle, axillary and inguinal spots absent, and light pericloacal ring present; *K. kokacii*, distribution in Catanduanes Island, Bicol Peninsula of Luzon Island, Philippines, SVL up to 44.3 mm, dorsal tubercles, axillary and inguinal spots absent, and light pericloacal ring present; *K. mediolineata*, distribution in mainland Thailand and Laos, SVL up to 63.0 mm, finger tips slightly dilated but not forming wide discs, web reaching disc of first toe and disc of postaxial side of second and third toe, inner and outer metatarsal tubercle extremely large and raised, inner metatarsal tubercle longer than first toe, and distinct dorsolateral and sacral stripes; *K. picta*, distribution in Philippines, SVL up to 56.1 mm, finger tips slightly dilated but not expanded into wide discs, three subarticular tubercles on fourth toe, inner metatarsal tubercle longer than first toe, dorsolateral stripes present, and stratified lateral flank coloration; *K. pulchra*, distribution in northeastern India, southern China, and Indo-
Malaya, SVL up to 75.0 mm, inner metatarsal tubercle large, raised, and longer than first toe, dorsolateral stripes present, and axillary and inguinal spots absent; *K. rigidia*, distribution in Luzon Island, Philippines, SVL up to 56.1 mm, finger tips slightly dilated but not expanded into wide discs, majority with three subarticular tubercles on fourth toe, stratified lateral flank coloration, dorsolateral stripes present, and axillary and inguinal spots absent; *K. rugifera*, distribution in central and south-central China, dorsum olive brown with yellowish-olive chin and throat, and axillary and inguinal spots absent; *K. verrucosa*, distribution in southeastern China, SVL up to 61.0 mm, finger tips slightly dilated but not expanded into wide discs; outer metatarsal tubercle absent or indistinct, stratified lateral flank coloration, and axillary and inguinal spots absent.

**Description of holotype.**—Fig. 4. Adult male; habitus robust; head wider than long (HW/HL = 1.28; HW/SVL = 0.29); snout projecting beyond lower jaw, truncated in dorsal view, rounded and caudoventrally sloping in lateral view; eyes large, slightly protruding beyond labials in dorsal view, diameter less than snout length (ED/SNL = 0.61) and interorbital distance (ED/IOD = 0.59), pupils circular; canthus rostralis rounded, barely evident; lores oblique, slightly concave; nares open laterally, slightly below canthus, nearly terminal on snout (IND/SNL = 0.59); labial region not swollen; interorbital region flat; dorsal rostrum slightly convex; tympanum barely visible; supratympanic fold present, extending from posterior edge of eyelid to just above and anterior of forelimb insertion and continuous with a distinct supraaxillary fold; choanae transversely oriented, tapered at both ends, separated by distance almost equal to their diameter; vomerine teeth absent; tongue wide, spatulate with a small posterior notch; vocal slits posterior to rictus.

Skin on dorsal surfaces smooth, infused with low, well-spaced, rounded tubercles that are

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sex</th>
<th>SVL</th>
<th>HL</th>
<th>HW</th>
<th>SNL</th>
<th>ED</th>
<th>IOD</th>
<th>IND</th>
<th>FL</th>
<th>CL</th>
<th>Fin3DW</th>
<th>MTTTL</th>
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</thead>
<tbody>
<tr>
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<td>Males</td>
<td>50.3</td>
<td>12.5</td>
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</tbody>
</table>

*ROM-HERP* = Royal Ontario Museum; *FMNH* = Field Museum of Natural History; *SVL* = snout–vent length; *HL* = head length; *HW* = head width; *SNL* = snout length; *ED* = eye diameter; *IOD* = interorbital diameter; *IND* = internarial distance; *FL* = femur length; *CL* = crus length; *Fin3DW* = third finger disc width; *MTTTL* = metatarsal tubercle length.

Table 3.—Morphological variation in the type series of *Kaloula tanksiensis*.a

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Fig. 4.—(A) Manus of holotype of *Kaloula indochinensis*; (B) pes of holotype; (C) dorsal view of holotype; (D) ventral view of holotype.
most prominent on the temporal region; ventral surfaces slightly granular; throat distinctly granular; loose skin overlying median subgular vocal sac forming a sternal fold; nuptial pad absent; forelimbs relatively long and robust; order of digits from shortest to longest: I<<II<III<IV<; no webbing between digits; distal ends of fingers expanded into wide, transversely expanded discs lacking circummarginal grooves; subarticular tubercles distinct, large, round, numbering one on digits I and II, two on digits III and IV; proximal subarticular tubercles larger than distal ones; inner metacarpal tubercle elongate, larger than subarticular tubercles; outer metacarpal tubercle oval, flat, indistinctly divided, smaller than inner (Fig. 4A).

Hindlimbs robust, relatively short (CL/SVL = 0.33); order of digits from shortest to longest: I<II<III<IV<V; tip of toes slightly expanded into small, round discs; subarticular tubercles distinct, round, numbering one beneath digits I and II and two beneath digits III, IV, V; basal subarticular tubercle absent on digit IV; basal webbing between digits I–II, II–III, IV–V; webbing between digits III and IV extends from distal margin of distal subarticular tubercle of digit III to proximal margin of proximal subarticular tubercle of digit IV; inner metatarsal tubercle elongate, slightly raised, larger than subarticular tubercles; outer metatarsal tubercle round, slightly raised, smaller than inner (Fig. 4B).

Color in preservative.—Dorsal surfaces grayish brown, infused with indistinct, fine white spots; clusters of irregular dark brown patches between orbits, shoulders, sacral region, front and hindlimbs; creamy patch on proximodorsal portion of brachium and anteroproximal portion of thigh; irregular creamy reticulations on the wrist, hindlimbs, and cloacal region forming a light percloacal ring (Fig. 4C); ventral surfaces a lighter shade of brown than dorsum, scattered with fine white spots; gular dark grayish-brown (Fig. 4D).

Color in life.—Dorsum chocolate to dark grayish-brown, covered with fine white spots corresponding to tip of tubercles which are most prominent and dense on the temporal region, flanks, vent, and dorsal surfaces of limbs; orange-yellow patch on either side of the neck posterior to eyes; clusters of irregular blackish patches between the orbits, shoulders, sacral region, front and hindlimbs; orange-yellow patch on dorsoproximal portion of brachium and anteroproximal portion of thigh with similar-colored reticulations on wrist, elbow, crus, and tarsus; white reticulations on dorsal surfaces of manus, pes, cloacal region, proximal portion of crus, and tarsus; venter chocolate brown, covered with small, distinct white spots; gular region black with fine white spots.

Variation in paratypes.—Paratypes closely resemble the holotype in overall morphology and color pattern. ROM-HERP 32924, 32927, 32931, 32944 have darker dorsal surfaces and consequently less distinct dorsal patches; the female paratype ROM-HERP 32945 has a brown and significantly less-granular gular; axillary and inguinal spots on FMNH 270359 are small and indistinct; dorsal tubercles are more pronounced in FMNH 270363.

Sexual dimorphism.—Coloration differences are minor but include males having a black and distinctly granular throat in contrast to females, in which this region is brownish and much-less granular. No significant differences in body proportions were detected between sexes.

Distribution.—The new species is known to occur at five localities from southern Vietnam through eastern Cambodia to central Laos (Fig. 5). The record from Cambodia is based on a photograph of an individual from Phnom Prich Wildlife Sanctuary, Mondulkiri Province, Cambodia (Fig. 3D).

Etymology.—The specific epithet refers to the new species’ distribution in Indochina, a geographic area containing the modern-day countries of Vietnam, Laos, and Cambodia.

Natural history.—Kaloula indochinensis occurs at a wide range of elevations with records at 100–150 m at Cat Tien, Vietnam (E. Galoyan, personal communication), 90–300 m at Ban Kiatngong, Champasak Province, Laos (Teynié et al., 2004), 540 m at Nakai District, Khammouan Province, Laos (this study), and ~850 m at Krong Pa Village, K’Bang District (type series) and 900–1000 m at the Tay-Nguyen plateau, An Khe district (Orlov et al., 2002), both in Gia Lai Province, Vietnam. In Vietnam most specimens were
collected at night in small, ephemeral ponds, some of which appeared to be depressions in the forest floor resulting from old bomb craters (Fig. 6). Specimens were located at night by collectors who were initially attracted to the loud choruses produced by numerous males of the new species calling in these pools. Some pools were located in patches of tall, dense grass, which is typical of open areas created by application of the defoliant Agent Orange during the Viet Nam–American conflict. In Laos, frogs were only observed at night during heavy rains, where they were found on the ground alongside temporary watercourses at approximately 550–650 m elevation. Frogs were also seen calling from raised positions on rocks and from trees, where they were observed climbing to approximately 2 m above the ground. Amplexus in leaf litter beside temporary watercourses was observed during rainstorms. Searches of the same locations by day and on nights without rain produced no encounters with the species. The Cambodian individual was climbing bamboo at the transition of deciduous and lowland evergreen forest when photographed (Fig. 3D).

**Discussion**

The known distribution of *K. indochinensis* is restricted to eastern Indochina. Other co-occurring congeners in this region include *K. mediolineata* from Dac Lac Province, Vietnam (Nguyen et al., 2009), *K. aureata* from southern Peninsular Thailand (Nutphund, 2001), and *K. pulchra*, which is ubiquitous throughout disturbed habitats in Asia (Frost, 2011). Based on geographic proximity, it is possible that *K. indochinensis* occurs in sympathy with *K. pulchra* and *K. mediolineata*.

Results from this and a related study (Blackburn et al., 2013) suggest that other populations of *K. baleata* may also require reexamination and taxonomic reappraisal. Populations from Peninsula Malaysia, Borneo, Sulawesi, and Palawan Island, Philippines may not be conspecific with true *K. baleata*. For some of these populations clear morphological differences are immediately apparent. For example, fully mature Palawan specimens are much smaller than Javanese *K. baleata* (averaging 41.6 mm, \( n = 5 \), SVL as opposed to 47.2 mm for *K. baleata*, \( n = 12 \)) and also inhabit tree holes, where they call and breed year round. This distinct microhabitat preference and activity pattern is shared exclusively with the Philippine *K. kalingensis* group, to the exclusion of the ephemeral pool-breeding, explosive rainy season breeding patterns exhibited by *K. baleata* on Java and Sulawesi (RMB, personal observations). A comprehensive review of Philippine *Kaloula* will be required to determine the taxonomic status of the Palawan lineage as well as several additional distinctive populations from other parts of the archipelago.

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APPENDIX

Specimens Examined


Kaloula mediolinata Smith, 1917; Thailand, Nakhon Ratbasima. KU 328280–85.

Kaloula picta (Duméril and Bibron, 1841): Philippines, Luzon Island, Manila: KU 4473; Barangay Poblacion: KU 305855–92, 305918, 305920–22, 305924; Barangay Casapisan: KU 322416–17; Barangay Batong Malaké: KU 326266–67; Barangay Malinoa Ilaya: KU 326265; Barangay Sanil: KU 327338; Barangay Palola: KU 328507; 10

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Kaloula pulchra Gray, 1831: Thailand, Surat Thani, Mountain View Bungalows: KU 328271–72, Seashore Bungalow: KU 328275; Bangkok: KU 328273; Phattalung Province, Near Khao Bantad: KU 328274; Nakhon Si Thammarat, Khao Luang National Park: KU 328276; Nakhon Ratchisma Province: KU 328227–79.
