



Peninsular Malaysia's first limbless lizard: a new species of skink of the genus *Larutia* (Böhme) from Pulau Pinang with a phylogeny of the genus

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Abstract

A new species of the scincid genus *Larutia*, *L. penangensis* **sp. nov.**, from Pulau Pinang, Peninsular Malaysia is separated from all other congeners by having the unique characteristics of the complete absence of limbs, four supralabials, large body scales, and no banding or striping pattern. Additionally, it has the following unique combination of characters that further separates it from all congeners: a single presubocular; separated nasals; paired frontoparietals; small, widely separated prefrontals; no supranasals or postnasal; two postsupralabials; and large, posterior chinshields that contact the infralabials. A molecular phylogeny based on one mitochondrial and two nuclear genes from all species of *Larutia* from Peninsular Malaysia indicates *L. penangensis* **sp. nov.** is most basal and that *L. seribuatensis* is the sister lineage to a clade containing *L. miodactyla* and the sister species *L. trifasciata* and *L. larutensis*. Consistencies and inconsistencies between this phylogeny and a previously proposed morphological phylogeny are discussed.

Key words: *Larutia*, *Larutia penangensis*, Malaysia, new species, Penang, Pulau Pinang, Scincidae, taxonomy, phylogeny

Introduction

Pulau Pinang (= Penang Island) is the largest island along the west coast of Peninsular Malaysia, encompassing approximately 330 square kilometers in area and reaching 830 meters in elevation at Penang Hill in the central ranges of Bukit Bendera. In 1786, it became the first British acquisition on the Malay Peninsula and for well over a century, the cool air of Penang Hill served as a retreat where Penang Island's colonialists could escape the hot, humid, coastal lowlands. During such retreats, a number of early naturalists (*i.e.* Cantor 1847; Flower 1896, 1899; Stoliczka 1870a,b,c, 1873) collected amphibians and reptiles and thus, Penang Hill became the type locality for a significant portion of Peninsular Malaysia's herpetofauna. Despite this auspicious historical legacy, Penang Hill has frustrated herpetologists for many years by harboring a number of species known only from one or two specimens collected well over a century ago. Notable among these are the skinks *Eutropis novemcarinata* (Anderson) (not seen on the island since the report of Flower 1896), *Lygosoma albopunctatum* (Gray) (first reported by Cantor 1847 but not confirmed until 1956 and not seen since then), and *Sphenomorphus anomalopus* (Boulenger) (not seen since its collection by Dr. J. G. Fischer around the year 1889). Therefore, it is not surprising that a new species of semi-fossorial skink recently found on Penang Hill has escaped detection for so long. Being that the specimen collected has an elongate, snake-like body; paired prefrontals; two loreals; separated nasals; three supraoculars; and

the last supralabial being horizontally divided (or two post supralabials) places it in the genus *Larutia* Böhme (Greer 1997). Currently, *Larutia* is composed of six, generally upland, Sundaland species that extend from northern Peninsular Malaysia southward to Sumatra and Borneo (J. Grismer *et al.* 2003; Fig. 1). All have elongate, snake-like bodies, long tails, and extremely reduced limbs bearing zero to two digits. Additionally, the four species from Peninsular Malaysia have stripping and/or banding patterns (J. Grismer *et al.* 2003). Being that the specimen from Pulau Pinang has no trace of limbs, stripping, or banding it cannot be ascribed to any known species. Additionally, a re-evaluation of the phylogenetic relationships within *Larutia* based on one mitochondrial and two nuclear genes indicates that the Pulau Pinang specimen is the basal lineage of a monophyletic group containing the remaining Malaysian taxa. Therefore, these data indicate this skink is a new species of *Larutia*.

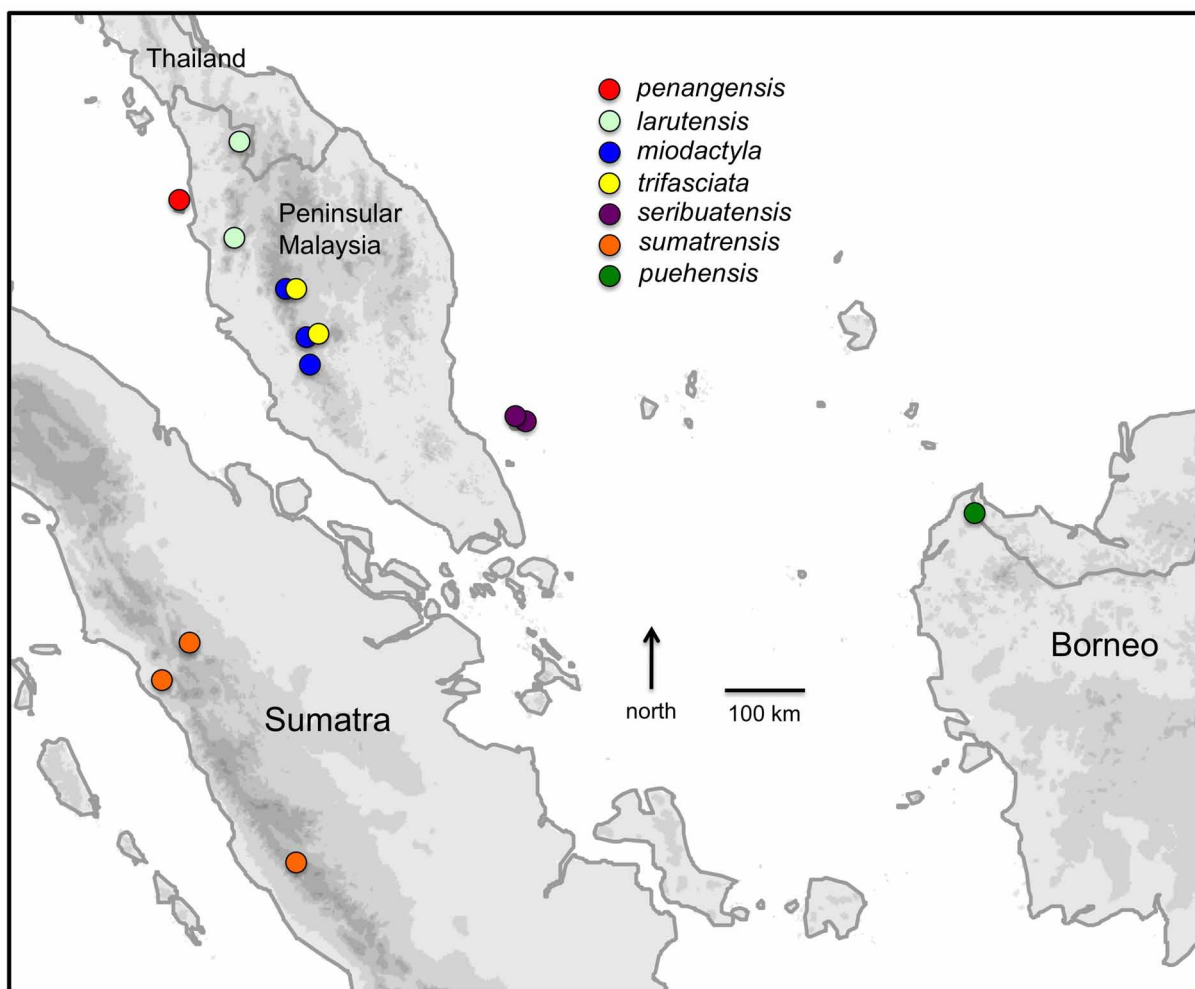


FIGURE 1. Distribution of the species of the genus *Larutia*.

Material and methods

Scale terminology follows J. Grismer *et al.* (2003) and Lim (1998). All measurements were made with Mitutoyo digital calipers to the nearest 0.1 mm. Scale counts were made on the right side of the body with a Nikon SMZ 1500 dissecting microscope. Measurements and scale counts used are snout-vent length (SVL) measured from the tip of the rostral scale to the vent; tail length (TailL) measured from the end of the broken tail to the vent; midbody scale rows counted as the number of longitudinal scale rows encircling the body at a point midway between the limb insertions; paravertebral scale rows counted as the number of scales in a line from the parietal scales to a point on the dorsum opposite the vent; and ventral scale rows counted as a row of scales between the postmentals and the anal plate. Other standard counts include supraoculars, suboculars, loreals, supralabials, and infralabials. The material examined is listed in Appendix I.

Institutional acronyms follow Leviton *et al.* (1985) and the following: LSUHC—La Sierra University Herpetological Collection, La Sierra University, Riverside, California, U.S.A. and ZRC—Zoological Reference Collection in the Raffles Museum of Biodiversity Research, National University of Singapore, Singapore.

Taxon sampling and outgroup selection for phylogenetic analyses. Our primary goal was to estimate phylogenetic relationships among species in the genus *Larutia* for which we had samples (Appendix II). A total of seven ingroup samples were used including representatives of *Larutia penangensis* **sp. nov.**, *L. larutensis* (Boulenger), *L. miodactyla* (Boulenger), *L. seribuatensis* Grismer, Leong & Norsham, and *L. trifasciata* (Tweedie). To assess the monophyly of the genus as well as investigate appropriate outgroup taxa, scincid species from the subfamilies Lygosominae and Scincinae were included, as well as a single outgroup sample from the family Lacertidae (Appendix II). For all 16 samples, complete sequences were collected for the mitochondrial NADH Dehydrogenase Subunit 1 (ND1) gene and two nuclear loci, R35 and PTGER4, were completely sequenced for all ingroup samples and many of the outgroup samples (Appendix II). All sequences were deposited in GenBank (Appendix II).

DNA extractions, purifications, and amplification. Genomic DNA was extracted from liver tissues and stored in 95.0–100.0% ethanol following the guanidine thiocyanate method of Esselstyn *et al.* (2008). All primers and thermal cycler profiles used to amplify the target fragment using the polymerase chain reaction (PCR) follow the methods of Siler *et al.* (in press). Amplified products were visualized on 1.0% agarose gels and then purified with 1 μ L of a 20.0% diluted solution of ExoSAP-IT (US78201, Amersham Biosciences, Piscataway, NJ) for 31 min at 37°, and 15 min at 80°. We cycle sequenced in both directions with ABI Prism BigDye Terminator chemistry (Ver. 3.1; Applied Biosystems, Foster City, CA), and purified sequence reactions with Sephadex (NC9406038, Amersham Biosciences, Piscataway, NJ) in Centri-Sep 96 spin plates (CS-961, Princeton Separations, Princeton, NJ). Targeted gene regions were analyzed with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). Consensus gene sequences were assembled and edited in Sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI).

Alignment and phylogenetic analyses. Initial alignments were produced using MUSCLE (Edgar 2004) and manual adjustments made in Se-Al Sequence Alignment Editor, version 2.0a11 (Rambaut 2002). To assess phylogenetic congruence between the mitochondrial and nuclear data, we inferred the phylogeny for each subset independently using likelihood and Bayesian methods. Following the observation of no statistically significant incongruence between datasets, we chose to conduct all subsequent analyses on a concatenated ND1 + PTGER4 + R35 dataset. Exploratory analyses of this combined dataset for all 16 individuals (including four outgroup samples lacking ND1, PTGER4, or both) and a reduced dataset of 12 individuals (no missing data) supported identical relationships; we therefore chose to include all available data (16 individuals) for subsequent analyses.

Partitioned Bayesian analyses were conducted with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) for the combined datasets. As much of this dataset is a subset of the Siler *et al.* (in press) dataset, we followed the same partitioning strategy. The mitochondrial dataset was partitioned by codon position for the protein-coding region of ND1. The Akaike Information Criterion (AIC), as implemented in jModeltest v0.1.1 (Guindon & Gascuel 2003; Posada, in press), was used to select the best model of nucleotide substitution for each partition (Table 1). The best-fit model for each of the three subsets of mitochondrial data, as well as the nuclear data, was the general time reversible (GTR) model with gamma distributed rate variation among sites (Γ). A rate multiplier model was used to allow substitution rates to vary among subsets, and default priors were used for all model parameters. We ran four independent Metropolis-coupled MCMC analyses, each with four chains and the default heating scheme (temp = 0.2). All analyses were run for 20 million generations, sampling every 5000 generations. To assess stationarity, all sampled parameter values and log-likelihood scores from the cold Markov chain were plotted against generation time and compared among independent runs using Tracer v1.4 (Rambaut & Drummond 2007). Furthermore, we plotted the cumulative and non-overlapping split frequencies of the 20 most variable nodes, and compared split frequencies among independent runs using Are We There Yet? [AWTY (Wilgenbusch *et al.* 2004)]. All samples showed patterns consistent with stationarity after five million generations, hence the first 50.0% of samples were discarded as burn-in for all three analyses.

Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis 2006) for the combined dataset under the same partitioning strategy as for the Bayesian analysis. The model (GTR + Γ) was used for all subsets, and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree, and employed the rapid hill-climbing algorithm (Stamatakis *et al.* 2007). Clade confidence was assessed with 1000 bootstrap pseudoreplicates. In all analyses, nodes receiving $\geq 95\%$ Bayesian

posterior probability or $\geq 70\%$ maximum likelihood bootstrap support were considered significantly supported (Hillis & Bull 1993).

TABLE 1. Models of evolution selected by AIC and applied for partitioned, model-based phylogenetic analyses.

| Partition | AIC Model | Model Applied | Number of Characters |
|--|-------------------|----------------|----------------------|
| NADH 1, 1 st codon position | TIM1 + Γ | GTR + Γ | 322 |
| NADH 1, 2 nd codon position | TPM3uf + Γ | GTR + Γ | 322 |
| NADH 1, 3 rd codon position | TPM3uf + Γ | GTR + Γ | 322 |
| PTGER4 | TPM3uf + Γ | GTR + Γ | 490 |
| R35 | TVM + Γ | GTR + Γ | 689 |

Results

Phylogeny and genetic divergences. Maximum likelihood and Bayesian inferences yield the same interspecific topology, with minor intraspecific topological changes (Fig. 2). All analyses of the combined dataset recovered five lineages within the genus *Larutia*. The combined analyses strongly support all species-level relationships except for the placement of *Brachymeles taylori* (Fig. 2). The outgroup species *Lipinia pulchella pulchella* (Gray) and *Scincella reevesii* (Gray) are strongly supported to be part of a clade sister to the genus *Larutia*.

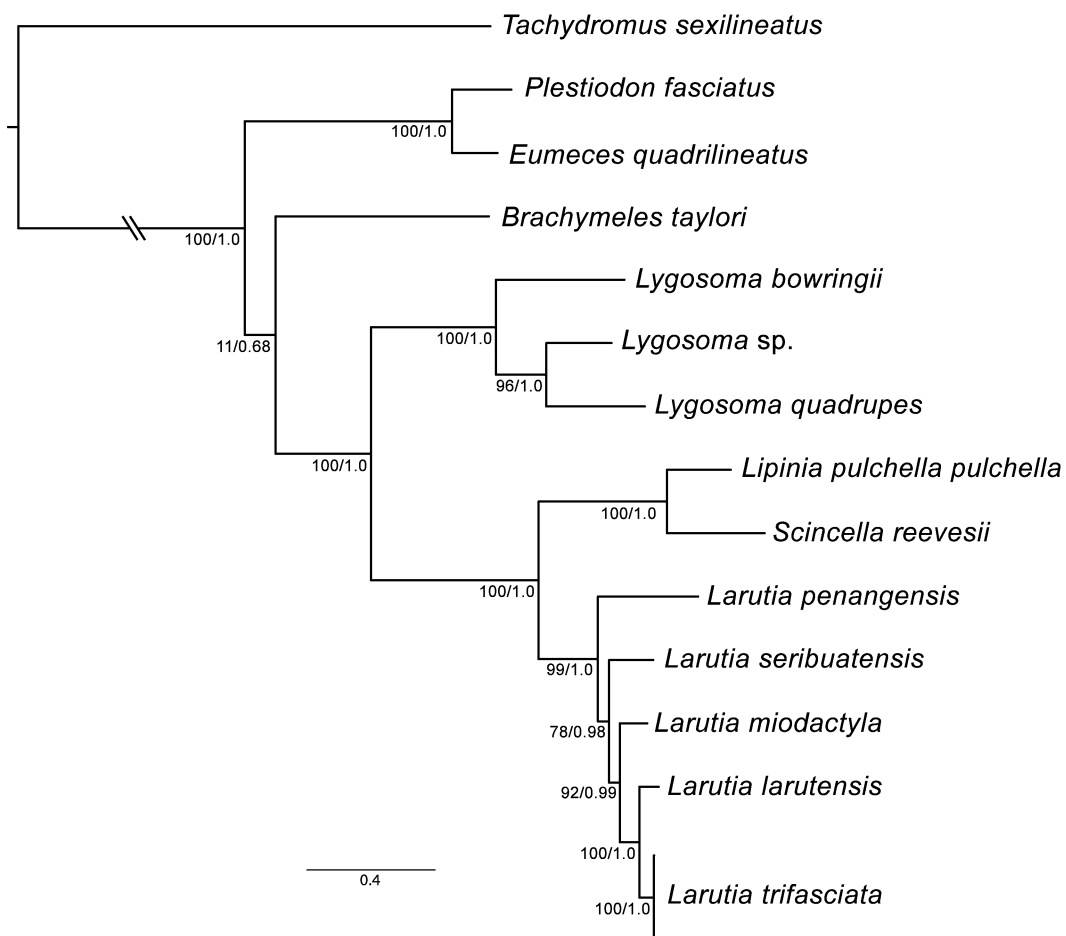


FIGURE 2. Maximum clade credibility tree from a partitioned phylogenetic analysis of the data (-lnL 8976.934287). Nodes shown with numerical values corresponding to MLBP, and Bayesian PP support values respectively. Terminals are labeled with taxonomic names.

The five lineages of *Larutia* correspond to four currently recognized species and one morphologically distinct new species from Pulau Pinang. Each species clade defined in our analysis is separated from the remaining lineages by 5.9–15.6% uncorrected mitochondrial sequence divergence (Table 2). The new, limbless species of *Larutia* from Pulau Pinang is sister to all other sampled species within the genus and all three species currently recognized to occur on Peninsular Malaysia (*L. larutensis*, *L. miodactyla*, and *L. trifasciata*) were recovered as a well-supported clade sister to the insular *L. seribuatensis* from the Seribuat Archipelago (Figs. 1,2).

Systematics. The phylogenetic analyses and external morphological data clearly demonstrate that the new population from Pulau Pinang belongs in the genus *Larutia* but that it can not be ascribed to any of the known species, and is thus described herein as new.

TABLE 2. Uncorrected pairwise sequence divergence (%) for mitochondrial data (below diagonal), nuclear data (above diagonal) for *Larutia penangensis* **sp. nov.**, *L. larutensis*, *L. miodactyla*, *L. seribuatensis*, and *L. trifasciata* (Fig. 2).

| | <i>penangensis</i> | <i>larutensis</i> | <i>miodactyla</i> | <i>seribuatensis</i> | <i>trifasciata</i> |
|----------------------|--------------------|-------------------|-------------------|----------------------|--------------------|
| <i>penangensis</i> | – | 1.5 | 1.4 | 1.1 | 1.1 |
| <i>larutense</i> | 14.7 | – | 1.0 | 0.9 | 0.7 |
| <i>miodactyla</i> | 14.8 | 10.2 | – | 0.7 | 0.6 |
| <i>seribuatensis</i> | 15.6 | 10.8 | 10.9 | – | 0.4 |
| <i>trifasciata</i> | 15.3 | 5.9 | 9.9 | 11.7 | – |

Larutia penangensis **sp. nov.**

Figures 3,4

Holotype. Juvenile of unknown sex (ZRC 2.6918) collected by Evan Quah, on 13 June 2010 at Penang Hill, Pulau Pinang, Penang (N.05.43825°, E100.28200°, ±5m; 308m asl.), Peninsular Malaysia.

Diagnosis. Body elongate, snake-like; dorsal scales smooth; limbs absent; lower eyelid bearing large, transparent scales, central scale largest; four supraoculars; nasals separated; frontoparietals paired; prefrontals small, widely separated; supranasals absent; postnasal scale absent; last supralabial horizontally divided (or two post supralabials); large, posterior chinshields not separated from the infralabials by smaller scales; 120 paravertebral scale rows; 132 ventral scale rows; 18 longitudinal scale rows around midbody; caudal and body scales undifferentiated; body unicolor dark brown; no yellow nuchal bands or pale, yellow spot on frontoparietals, supraoculars, or rostrum. These characters are summarized across all species in Table 4.

Description of holotype. Head small, scarcely distinct from neck, rounded, triangular in dorsal profile; head scales smooth; rostral wider than long, in broad contact with frontonasal; frontonasal wider than long; prefrontals small, not contacting on midline; frontal wide, diamond-shaped, in contact with first supraocular; three supraoculars; frontoparietals contacting all supraoculars anteriorly and parietals and interparietal posteriorly; interparietal diamond-shaped, large, slightly projecting posteriorly, no parietal eyespot; parietals large, in medial contact posterior to interparietal, contacting posterior margin of third supraocular anteriorly; enlarged, differentiated nuchals absent; nasals moderately large, separated, trapezoidal, contacting rostral anteriorly, frontonasal dorsally, first loreal posteriorly, first supralabial ventrally; nostril in central portion of nasal scale; supranasals absent; anterior loreal nearly same size as posterior loreal; two preoculars in contact with second loreal; dorsal preocular much smaller than ventral preocular; four supraciliaries, fourth supraciliary enlarged; two pretemporals; five suboculars; single presubocular; four supralabials; first supralabial large and triangular; two postsupralabials; two twmporals; two primary temporals; two secondary temporals, uppermost contacting parietal; granular scales at anterior corners of eye; lower eyelid bearing large, transparent scales, central scale largest; mental wider than long; single large, rectangular postmental contacting first infralabials; two enlarged pairs of chinshields following postmental, anterior pair contacting medially, posterior pair separated posteriorly by two gular scales; all chinshields contacting infralabials; three similarly sized infralabials; no external ear opening.

Body elongate, snake-like; body scales smooth, cycloid, imbricate; flank, ventral, and dorsal scales equal in size; 18 longitudinal scale rows around midbody; 120 paravertebral scale rows; 132 ventral scale rows; two enlarged, medial, preanal scales; limbs absent; tail robust, cylindrical; caudal scales equal in size, indiscernable from body scales. Measurements are SVL 51.4 mm; TL (broken) 17.6 mm.

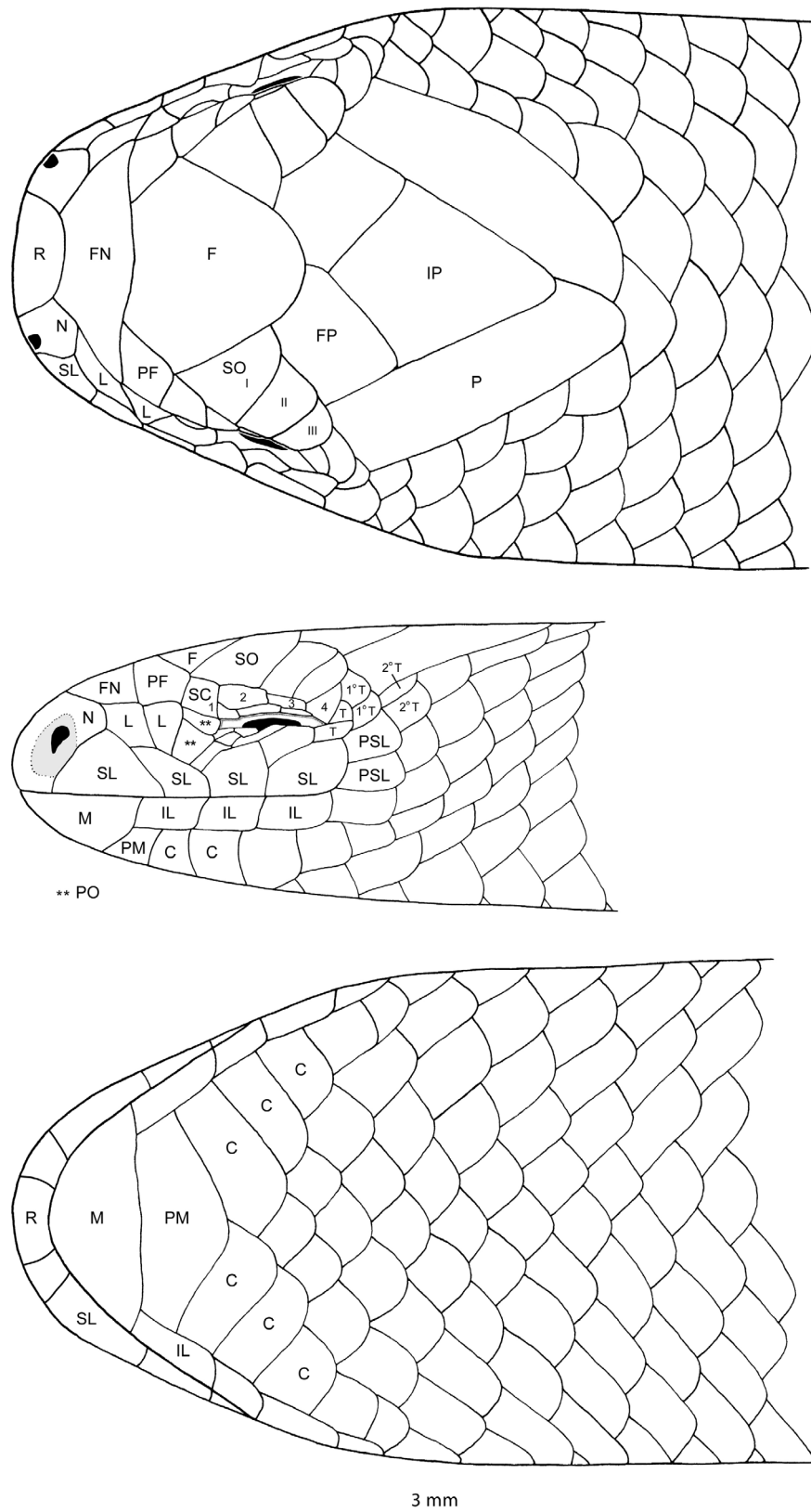


FIGURE 3. Illustrations of head of juvenile holotype *Larutia penangensis* sp. nov. (ZRC 2.6918) in dorsal, lateral, and ventral views. Taxonomically diagnostic head scales are labeled as follows: C, chinshield; F, frontal; FN, frontonasal; FP, frontoparietal; IL, infralabial; IP, interparietal; L, loreal; M, mental; N, nasal; P, parietal; PF, prefrontal; PM, postmental; PN, postnasal; PO, preocular; PSL, postsupralabial; R, rostral; SC, supraciliary; SL, supralabial; SO, supraocular; T, temporal; 1°T, primary temporal; and 2°T, secondary temporal. Roman numerals indicate scales in the supraocular series, with Arabic numbers indicating scales in the supraciliary series (Illustrations by CDS).



FIGURE 4. Upper: holotype of *Larutia penangensis* sp. nov. ZRC 2.6918 (photo by EQSH). Lower: habitat at type locality on Penang Hill, Pulau Pinang, Penang, Malaysia (photo by LLG).

Coloration. Dorsal surface of head, body, and tail uniform dark brown; nuchal bands absent; no striping on body; ventral surface slightly lighter than dorsal surface (Fig. 4), rostral, first supralabial, nasal mental, postmental, and first infralabial scales opaque.

Distribution. *Larutia penangensis* sp. nov. is known only from the lower elevations of Penang Hill, Pulau Pinang, Peninsular Malaysia (Fig. 1). It is expected to occur much more widely across the island in appropriate habitats.

Natural history. The specimen was found crawling across an open dirt path surrounded by lowland dipterocarp forest (Fig. 4). All other species of *Larutia* are fossorial to semi-fossorial and found in loose soils beneath surface objects. This species is expected to be no different.

Etymology. The specific epithet *penangensis* is in reference to the type locality, Penang Island. The suffix *ensis* is a derivation meaning “from” or “inhabiting.” It renders the specific epithet an adjective that must be in grammatical accord with the gender of *Larutia*.

Comparisons. *Larutia penangensis* sp. nov. is clearly separated from all other species of *Larutia* by its complete lack of limbs (Fig. 4); having fewer supralabials (4 vs. 5–7; Fig. 3); having proportionately larger body scales (18 vs. 20–30 scales around midbody); and its unique, unicolor, color pattern (vs. nuchal bands and/or striping on the body; Figs. 4,6). The new species is further separated from all other species except some specimens of *L. miodactyla* (see J. Grismer *et al.* 2003), in having fewer infralabials (3 vs. 4 or 5; Fig. 3); from all other species except *L. larutensis* and *L. trifasciata*, in that the second pair of chin shields is separated by two gular scales (vs. separated by one scale; Fig. 3); from *L. larutensis* and *L. miodactyla*, in that the first two pairs of chin shields contact two infralabials (vs. contacting only one infralabial; Fig. 3); from all other species except *L. sumatrensis* (Bleeker) in the absence of linearly arranged yellow spots on the body (Figs. 4,6); from *L. larutensis* (in juveniles only), *L. seribuatensis*, and *L. trifasciata*, in the absence of nuchal bands (Figs. 4,6); from *L. seribuatensis* and some *L. trifasciata* in the absence of yellow spots on the head (Figs. 4,6); and from all species except *L. larutensis*, *L. sumat-*

rensis, and *L. trifasciata* in having opaque scales on the snout (vs. the absence of such scales; Figs. 4,7). These character states are summarized across all species in Table 3.

TABLE 3. Diagnostic scale counts and color pattern characteristics of the species of the genus *Larutia*. += character state present; 0 = character state absent.

| | <i>larutensis</i> | <i>miodactyla</i> | <i>puehensis</i> | <i>seribuaten-</i> <i>sis</i> | <i>sumatrensis</i> | <i>trifasciata</i> | <i>penangen-</i> <i>sis</i> sp. nov. |
|---|-------------------|-------------------|------------------|----------------------------------|--------------------|--------------------|--|
| Suproculars | 4 | 4 | 4 | 4 | 3 or 4 | 4 | 3 |
| Supralabials | 6 or 7 | 5 | 5 | 5 or 6 | 5 or 6 | 6 | 4 |
| Infralabials | 4 | 3 or 4 | 5 | 5 | 4 | 5 | 3 |
| Midbody scales | 25 or 26 | 20–22 | 23 | 24 or 25 | 22 | 29 or 30 | 18 |
| Laterally compressed rostrum | 0 | 0 | 0 | 0 | + | 0 | 0 |
| No. of scales between 2 nd pair of chinshields | 2 | 1 | 1 | 1 | 1 | 2 | 2 |
| No. of infralabials contacted by first 2 pairs of chinshields | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| Limbs present | + | + | + | + | + | + | 0 |
| Linearly arranged light spots | + | + | + | + | 0 | + | 0 |
| Nuchal bands | + | 0 | 0 | + | 0 | + | 0 |
| Yellow spots on head | 0 | 0 | 0 | + | 0 | 0/+ | + |
| Opaque snout scales | + | 0 | 0 | 0 | + | + | + |
| Sample size | 4 | 6 | 1 | 8 | 4 | 10 | 1 |

Discussion

Given the secretive nature of many skinks in general and fossorial skinks in particular, it is not too surprising to find a new species of *Larutia* in Peninsular Malaysia. Not only does *L. penangensis* **sp. nov.** represent a new record of yet another poorly known skink from Pulau Pinang, it is Peninsular Malaysia's first completely legless lizard (Grismer 2008). J. Grismer *et al.* (2003) presented a morphological phylogeny (Fig. 5) of *Larutia* wherein they hypothesized that the light yellow nuchal bands and opaque scales on the rostrum were derived character states grouping *L. larutensis*, *L. trifasciata*, and *L. seribuatensis* to the exclusion of *L. miodactyla*, *L. sumatrensis*, and *L. puehensis* Grismer, Leong & Norsham. They further hypothesized that *L. trifasciata* and *L. seribuatensis* were sister species based on them having three nuchal bands and yellow spots on the head (Fig. 6). J. Grismer *et al.* (2003) also hypothesized that extreme limb reduction (i.e. complete loss of digits and movable wrist and ankle joints) in *L. miodactyla*, *L. puehensis*, and *L. sumatrensis* was evidence supporting their monophyly. The results of the molecular phylogenetic analyses (Fig. 2) do not completely agree with some of the relationships proposed by J. Grismer *et al.* (2003). The molecular analyses recover *L. larutensis* and *L. trifasciata* as sister species, and places *L. miodactyla* as the sister lineage to them to the exclusion of *L. seribuatensis*. Unfortunately tissues of *L. sumatrensis* and *L. puehensis* were not available and thus these species can not be evaluated. The molecular analyses also recover *L. penangensis* **sp. nov.** basal to all other species.

Our phylogenetic analyses suggest that extreme limb reduction may be occurring independently within different lineages of *Larutia* (Fig. 2). Unlike the morphological phylogeny that grouped *L. miodactyla*, *L. puehensis*, and *L. sumatrensis* on the basis of extreme limb reduction (Fig. 5), the molecular analysis nests *L. miodactyla* within a clade containing *L. larutensis*, *L. trifasciata*, and *L. seribuatensis* and does not place it as being closely related to the limbless *L. penangensis* **sp. nov.** The degree of limb reduction in *L. miodactyla* is so variable (individuals having 0–2 digits) that J. Grismer *et al.* (2003) and Grismer (2011) suggest this species is likely to be a species complex. The specimen used in the molecular analysis had two digits on each limb which is the condition seen in *L. larutensis*, *L. trifasciata*, and *L. seribuatensis* with whom it was shown to be related (Fig. 2). The systematics and relationships of *L. miodactyla* that lack digits remain to be investigated.

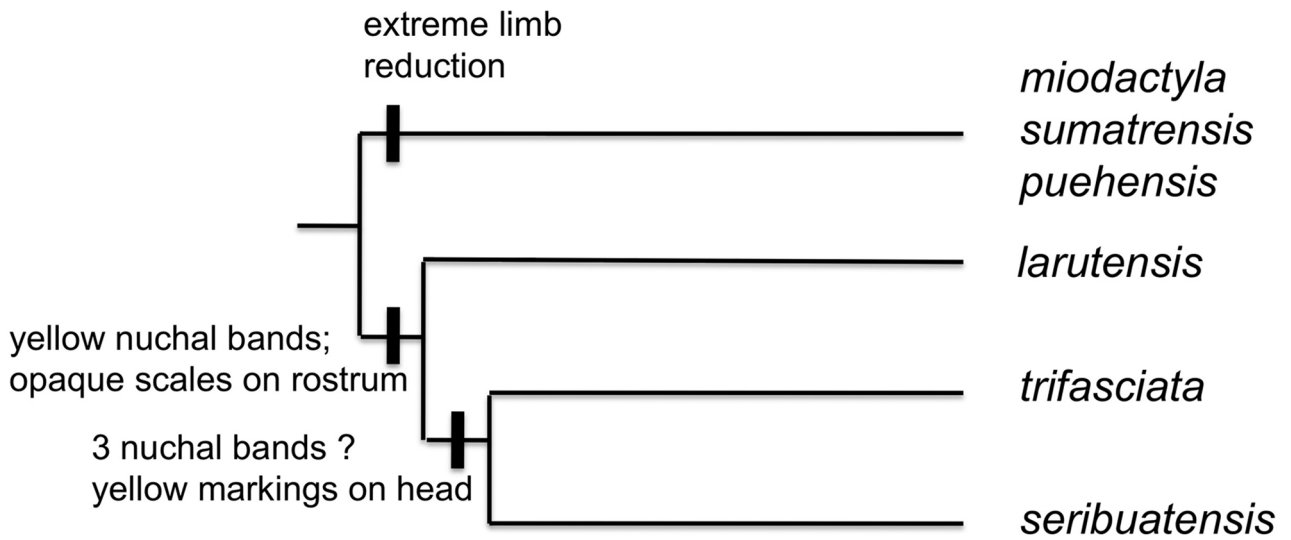


FIGURE 5. Morphological phylogeny of *Larutia* proposed by J. Grismer *et al.* (2003).



FIGURE 6. Upper: *Larutia miodactyla* from Fraser's Hill, Pahang (photo by LLG). Lower: *Larutia trifasciata* from Cameron Highlands, Pahang (photo by LLG).



FIGURE 7. Head of *Larutia larutensis* from Bukit Larut, Perak showing the opaque scales on the rostrum (photo by LLG).

The molecular phylogeny also does not support the hypothesis that the putatively derived (J. Grismer *et al.* 2003) acquisition of opaque scales on the rostrum (Fig. 7) has utility in delimiting monophyletic subgroups within *Larutia* being that opaque scales occur in the most basal species *L. penangensis* **sp. nov.** and *L. seribuatensis* as well as the most derived species, *L. larutensis* and *L. trifasciata* but are absent in *L. miodactyla* which is nested between these lineages (Fig. 2). This would suggest that *L. miodactyla* may have the autapomorphic state of losing opaque scales and that their presence in the other species is plesiomorphic.

J. Grismer *et al.* (2003) also hypothesized that *L. trifasciata* and *L. seribuatensis* were sister species based on the presence of three nuchal bands (Fig. 5) and head spotting whereas the molecular analyses (Fig. 2) place *L. larutensis* as the sister species to *L. trifasciata* and *L. seribuatensis*, all basal to a clade containing *L. larutensis*, *L. trifasciata*, and *L. miodactyla*. The examination of additional specimens of each species indicates that the number of nuchal bands and degree of head spotting can be quite variable, and that these character states may be unreliable as indicators of relationship. What the molecular phylogeny also suggests is that nuchal banding occurred in the ancestor of *L. seribuatensis*, *L. miodactyla*, *L. larutensis* and *L. trifasciata* and was independently lost in *L. miodactyla* or that it evolved independently in *L. seribuatensis* and the ancestor of *L. larutensis* and *L. trifasciata* and was never present in the *L. miodactyla* lineage. We prefer the former hypothesis on the basis that in *L. larutensis*, nuchal banding only occurs in juveniles and is lost in adulthood, indicating that this is a character that is subject to loss. There are no independent data suggesting this character can evolve independently. With the acquisition of tissues from *L. puehensis*, *L. sumatrensis* and especially *L. miodactyla*, we will be able to more adequately discuss aspects the evolution of limb loss within and among the various lineages of *Larutia* as well as the historical biogeography of this group within the context of that of other Sundaland taxa.

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APPENDIX I.

The following specimens were examined.

Larutia larutensis. MALAYSIA: Perak; Maxwell's Hill, LSUHC 9073, 9844, 9927, ZRC.2.1861.

Larutia miodactyla. MALAYSIA: Pahang, Cameron Highlands, BM 1974. 3851, ZRC 2.1584, 2.1588, 1591–92; Fraser's Hill 9932. Selangor; Genting Highlands, BM 1974.3852

Larutia trifasciata. MALAYSIA: Pahang; Cameron Highlands, BM 1974 3853, LSUHC 9077–80, 9101–03, ZRC 2.1862, 1867

Larutia sumatrensis. SUMATRA: locality unknown, BM 1946. 8.16.89. Fort de Kock, BM 1926 2.18.5. Lolo BM 2.18.6. Agam BM 9.25.5.

Larutia seribuatensis. MALAYSIA: Johor: Pulau Tulai, LSUHC 3915, 3917–18, 4783, 4795. Pahang; Pulau Tioman, LSUHC 5168, 6170, 7074.

Larutia puehensis. MALAYSIA: Sarawak; Gunung Berumput, BM 8.112.

APPENDIX II.

Summary of specimens corresponding to genetic samples included in the study. KU = University of Kansas Natural History Museum; LSUHC = La Sierra University Herpetological Collections; FMNH = Field Museum of Natural History Herpetological Collections; * = currently uncataloged specimen, deposited in the National Museum of the Philippines.

| Species | Voucher | Locality | Genbank Accession Numbers |
|------------------------------------|-------------|---|---------------------------|
| <i>Brachymeles taylori</i> | KU 320841 | Philippines, Negros Island, Municipality of Valencia, Mt. Talinis | |
| <i>Eumeces quadrilineatus</i> | KU 311490 | China, Guangxi State, Shiwan Dashang Nature Reserve | |
| <i>Larutia larutensis</i> | LSUHC 9703 | West Malaysia, Perak, Bukit Larut | |
| <i>Larutia miodactyla</i> | LSUHC 9932 | West Malaysia, Pahang, Fraser's Hill | |
| <i>Larutia seribuatensis</i> | LSUHC 5168 | West Malaysia, Pahang, Pulau Tioman | |
| <i>Larutia penangensis</i> | ZRC 2.6918 | Penang Hill, Pulau Pinang, Penang | |
| <i>Larutia trifasciata</i> | LSUHC 9077 | West Malaysia, Pahang, Cameron Highlands | |
| <i>Larutia trifasciata</i> | LSUHC 9078 | West Malaysia, Pahang, Cameron Highlands | |
| <i>Larutia trifasciata</i> | LSUHC 9079 | West Malaysia, Pahang, Cameron Highlands | |
| <i>Lipinia pulchella pulchella</i> | RMB 1079* | Philippines, Bohol Island, Municipality of Carmen | |
| <i>Lygosoma bowringii</i> | LSUHC 6998 | West Malaysia | |
| <i>Lygosoma</i> sp. | LSUHC 6931 | West Malaysia | |
| <i>Lygosoma quadrupes</i> | LSUHC 8403 | West Malaysia | |
| <i>Plestiodon fasciatus</i> | KU 289462 | United States, Texas, Smith County | |
| <i>Scincella reevesii</i> | FMNH 255540 | Lao PDR, Khammouan Prov, Thakhek District | |
| <i>Takydromus sexlineatus</i> | KU 311512 | China, Guangxi State, Shiwan Dashang Nature Reserve | |