Zootaxa 4107 (3): 367–380
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4107.3.5
http://zoobank.org/urn:lsid:zoobank.org:pub:7791AAD7-2F1D-47EB-88A7-1B97C765BFFC

Phylogenetic relationships of geckos of the *Hemiphyllodactylus harterti* group, a new species from Penang Island, Peninsular Malaysia, and a likely case of true cryptic speciation

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Abstract

An integrative taxonomic analysis based on the mitochondrial gene ND2 and its flanking tRNAs, morphology, and color pattern indicates that a newly discovered gecko described herein as *Hemiphyllodactylus cicak* **sp. nov.** from Penang Hill on the Island of Penang, Peninsular Malaysia is a member of the *H. harterti* group. *Hemiphyllodactylus cicak* **sp. nov.** is most closely related to the clade composed of the sister species *H. harterti* from Bukit Larut, Perak in the Bintang Mountain Range and *H. bintik* from Gunung Tebu, Terengganu from the Timur Mountain Range. These three allopatric species form a monophyletic group that extends approximately 270 km across three isolated mountain ranges in northern Peninsular Malaysia. The molecular analysis also indicates that *H. titiwangsaensis* from the Titiwangsa Mountain Range is composed of three genetically distinct allopatric populations. The southern two populations from Fraser's Hill and Genting Highlands, Pahang have an uncorrected pairwise sequence divergence of 3.5% whereas these two populations have 12.4 and 12.8 % sequence divergences, respectively, from the northern population at Cameron Highlands, Pahang. Although the high sequence divergence clearly distinguishes the southern two populations from the former as a different species, all three populations are morphologically indistinguishable, leading to the hypothesis of a true, cryptic speciation event.

Key words: *Hemiphyllodactylus,* Malaysia, Penang, phylogeny, new species, cryptic speciation

Introduction

The gekkonid genus *Hemiphyllodactylus* Bleeker currently comprised of 25 species (Grismer *et al.* 2013, 2014,a,b, 2015; Ngo *et al.* 2014; Nguyen *et al.* 2013, 2014). Although the genus extends across a broad geographic range from the Mascarene Islands in the western Indian Ocean, eastward through southern Asia, Indochina, the Philippines, and the Indo-Australian Archipelago into much of Oceania as far east as Hawaii (Zug 2010), most species are geographically restricted to upland areas or islands (Grismer *et al*. 2013). Grismer *et al.* (2013) divided the genus into two monophyletic groups, the *harterti* group and the *typus* group. The *typus* group contains species that span the entire geographic distribution of the genus, whereas the *harterti* group contains only species endemic to the uplands of Peninsular Malaysia. The *harterti* group currently contains six species known from three major mountain ranges in Peninsular Malaysia: the Bintang Mountain Range in the west, the central Titiwangsa Mountain Range, and the Timur Mountain Range in the east (Fig. 1). During a recent expedition to Penang Island, Penang, Peninsular Malaysia, four individuals from a new population of *Hemiphyllodactylus* were collected from an upland region on Penang Hill. Although all have the diagnostic characters of a vestigial first digit on both the fore- and hind limbs, as well as a long slender body with widely splayed limbs that place them in the genus *Hemiphyllodactylus,* they also share a suite of morphological characters that differentiate them from all other

Accepted by A. Bauer: 29 Mar. 2016; published: 3 May 2016

congeners. Additionally, all were found to be genetically similar to one another but distinct from all other congeners and phylogenetically embedded within the *harterti* group. We, therefore, describe this population of *Hemiphyllodactylus* as a new species.

Grismer *et al.* (2013) demonstrated that *Hemiphyllodactylus titiwangsaensis* was composed of two reciprocally monophyletic lineages and noted that it formed a species complex. Bickford *et al.* (2007) considered two or more species that are morphologically indistinguishable and classified as a single nominal species as cryptic species. The pluralistic approach used in this study where both morphology and molecular genetics are taken into account reveals that genetically distinct populations cannot be separated on the basis of morphology and may represent true cryptic species. Grismer *et al.* (2014) noted that the numerous papers purporting to demonstrate that integrative taxonomic studies are revealing cryptic species are doing nothing of the sort. They are only revealing that the last papers dealing with the taxon in question did not look closely enough at the morphology. This is not the case with *Hemiphyllodactylus titiwangsaensis*.

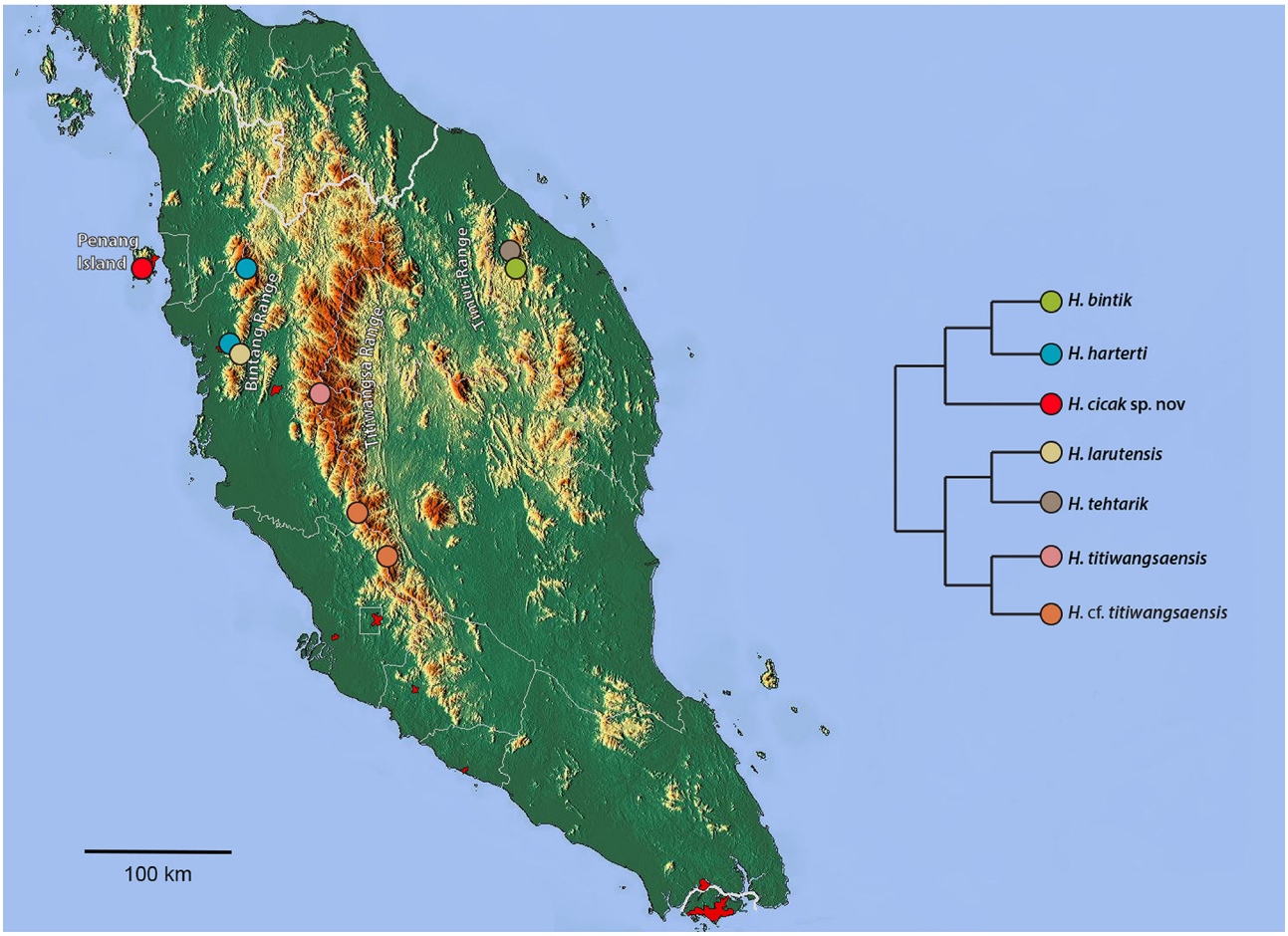

FIGURE 1. Distribution of the species of the *Hemiphyllodactylus harterti* group in Peninsular Malaysia.

Materials and methods

Phylogenetic analysis. A 1446 base pair fragment of the NADH dehydrogenase subunit 2 gene (ND2), including the flanking tRNA's (tRNAmet, tRNAtrp, tRNAala, tRNAsn, tRNAcys, tRNAtyr), was analyzed from 20 sequenced individuals. Three new samples of the new population from Penang Hill were sequenced for the same fragment along with three taxa used as outgroups (Table 1). Total genomic DNA was isolated from liver or skeletal muscle tissues stored in 95% ethanol using the Qiagen DNeasyTM tissue kit (Valencia, CA, USA). ND2 was amplified using a double-stranded Polymerase Chain Reaction (PCR) under the following conditions: 1.0 µl genomic DNA (~10–30 ng), 1.0 µl light strand primer (10 µM), 1.0 µl heavy strand primer (10 µM), 1.0 µl deoxynucleotide solution (1.5 µM), 2.0 µl 5x buffer (1.5 µM), 1.0 10x PCR buffer withMgCL2 (1.5 µM), 0.18 µl

Taq polymerase (5u/µl), and 7.5 µl H2O. PCR reactions were executed on an Eppendorf Mastercycler gradient thermocycler under the following conditions: initial denaturation at 95ºC for 2 min, followed by 31 cycles of denaturation at 95ºC for 35 s, annealing at 52ºC for 35 s, followed by a cycle extension at 72ºC for 35 s. All PCR products were visualized on a 1% agarose gel electrophoresis. Successful targeted PCR products were vacuum purified using MANU 30 PCR plates Millipore plates and purified products were re-suspended in sterile water. Cycle sequencing was performed on the purified PCR products using the ABI Big-Dye Terminator v3.1 Cycle Sequencing Kit in an ABI GeneAmp PCR 9700 thermal cycler. Cycle sequencing reactions were purified with Sephadex G-50 Fine (GE Healthcare) and analyzed on an ABI 3730xl DNA Analyzer at the BYU DNA Sequencing center.

TABLE 1. Taxon sampling for ingroup and outgroup, locality data, and Genbank accession numbers.

Voucher	Genus and species	Locality	GenBank accession numbers
AMB (n/a)	*Hemiphyllodactylus aurantiacus*	Tamil Nadu, Yercaud, India	JN393933
LSUHC 11216	*Hemiphyllodactylus bintik*	Gunung Tebu, Terengganu, Malaysia	KJ663757
LSUCH 9504	*Hemiphyllodactylus chiangmaiensis*	Chang Mai, Thailand	KF219782
LSUHC 11762	*Hemiphyllodactylus cicak* **sp. nov.**	Penang Hill, Penang, Malaysia	KU845548
LSUHC 11763	*Hemiphyllodactylus cicak* **sp. nov.**	Penang Hill, Penang, Malaysia	KU845549
LSUHC 11764	*Hemiphyllodactylus cicak* **sp. nov.**	Penang Hill, Penang, Malaysia	KU845550
LSUHC10384	*Hemiphyllodactylus harterti*	Bukit Larut, Perak, Malaysia	KF219761
LSUHC10383	*Hemiphyllodactylus harterti*	Bukit Larut, Perak, Malaysia	KF219760
LSUHC 11295	*Hemiphyllodactylus larutensis*	Bukit Larut, Perak, Malaysia	KJ663758
MVZ 239346	*Hemiphyllodactylus engganoensis*	Pulau Enggano, Sumatra	KF219776
LSUHC 6489	*Hemiphyllodactylus* cf. *titiwangsaensis*	Fraser's Hill, Pahang, Malaysia	KF219769
LSUHC 8092	*Hemiphyllodactylus* cf. *titiwangsaensis*	Fraser's Hill, Pahang, Malaysia	KF219774
LSUHC 10693	*Hemiphyllodactylus* cf. *titiwangsaensis*	Genting Highlands, Pahang, Malaysia	KF219763
LSUHC 10700	*Hemiphyllodactylus* cf. *titiwangsaensis*	Genting Highlands, Pahang, Malaysia	KF219764
LSUHC 10699	*Hemiphyllodactylus* cf. *titiwangsaensis*	Genting Highlands, Pahang, Malaysia	KF219765
LSUHC 10694	*Hemiphyllodactylus* cf. *titiwangsaensis*	Genting Highlands, Pahang, Malaysia	KF219766
LSUHC 10904	*Hemiphyllodactylus tehtarik*	Gunung Tebu, Malaysia	KF219784
LSUHC 7208	*Hemiphyllodactylus titiwangsaensis*	Cameron Highlands, Malaysia	JN393934
LSUHC 10717	*Hemiphyllodactylus titiwangsaensis*	Cameron Highlands, Malaysia	KF219785
LSUHC 10718	*Hemiphyllodactylus titiwangsaensis*	Cameron Highlands, Malaysia	KF219790

TABLE 2. Primers used for PCR amplification and sequencing reactions. Specific amplification conditions are presented in the materials and methods.

Primer name	Primer citation		Sequence
L4437b	(Macey & Schulte 1999)	External	5' –AAGCAGTTGGGCCCATACC –3'
CyrtintF1	(Siler *et al.* 2010)	Internal	5' –TAGCCYTCTCYTCYATYGCCC –3'
CyrtintR1	(Siler *et al.* 2010)	Internal	5' –ATTGTKAGDGTRGCYAGGSTKGG –3'
H5934	(Macey & Schulte 1999)	External	5' –AGRGTGCCAATGTCTTTGTGRTT –3'

Primers used for amplification and sequencing are presented in Table 2. The new sequences obtained from the Penang population were combined with the nexus file of Grismer *et al.* (2015). The nexus file was pruned, leaving only members of the *harterti* group and three different species (*Hemiphyllodactylus aurantiacus* Beddome, *H. engganoensis* Grismer, Riyanto, Iskandar, & McGuire, and *H. chiangmaiensis* Grismer, Wood, & Cota) for the

outgroups, following Grismer *et al.* (2015). A Maximum Likelihood (ML) analysis was run using RAxML version 8.0.0 (Stamatakis 2014) where ND2 was partitioned by codon using a GTR + GAMMA model of evolution, the tRNAs were treated as a separate partition, and 1000 bootstrap replicates were run to examine nodal support. A Bayesian inference (BI) analysis using the same partitioning scheme and model of evolution was carried out in MrBayes 3.2.3. on XSEDE (Ronquist *et al.* 2012) using CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller *et al.* 2010) employing default priors. Two simultaneous Markov Chain Monte Carlo (MCMC) runs were performed with four chains per run (three hot and one cold) using default priors. The analysis was run for 10 million generations, sampled every 1000 generations, and halted after the average standard deviation split frequency was below 0.01 and convergence was verified in Tracer v1.6 (Rambaut *et al.* 2014). The first 25% of the trees were discarded as burn–in. A 50% consensus tree was created using the sumt function. Nodes having ML bootstrap support values of ≥70 and BI posterior probabilities of ≥0.95 were considered significantly supported (Huelsenbeck *et al.* 2001; Wilcox *et al.* 2002). Uncorrected pairwise sequence divergences were calculated in MEGA v6.06 (Tamura *et al.* 2013). An ANOVA test was conducted on the *H. titiwangsaensis* and *H.* cf. *titiwangsaensis* populations using scale counts and measurements taken listed in Table 5 (excluding measurements that inform the presence or absence of a character).

Morphological analysis. For the descriptive work, color notes were taken using digital images of specimens prior to preservation. The terminology and methodology for the mensural and meristic characters follows Grismer *et al.* (2014b). Mensural data were taken with Mitutoyo dial calipers to the nearest 0.1 mm under a Nikon SMZ 1500 dissecting microscope on the left side of the body where appropriate: snout-vent length (SVL), taken from the tip of snout to the vent; tail length (TailL), taken from the vent to the tip of the tail, original or regenerated; trunk length (TrunkL), taken from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body; head length (HeadL), the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout; head width (HeadW), measured at the angle of the jaws; eye diameter (EyeD), the greatest horizontal diameter of the eyeball; snout-eye length (SnEye), measured from anteriormost margin of the eyeball to the tip of snout; nares-eye length (NarEye), measured from the anterior margin of the eye ball to the posterior margin of the external nares; and internarial width (SnW), measured between the nares across the rostrum. Meristic character states evaluated on the holotype and comparative material (see Appendix; Zug, [2010]) were the number of scales contacting the nares (circumnasal scales); the number of scales between the supranasals (postrostrals); the numbers of supralabial and infralabial scales counted from the largest scale immediately posterior to the dorsal inflection of the posterior portion of the upper jaw to the rostral and mental scales, respectively; the number of longitudinal ventral scales at midbody contained within one eye diameter; the number of longitudinal dorsal scales at midbody contained within one eye diameter; the number of subdigital lamellae wider than long on the first finger and toe; lamellar formulae determined as the number of U-shaped subdigital lamellae on the digital pads on digits 2–5 of the hands and feet; the total number of precloacal and femoral pores (*i.e.*, the contiguous or discontinuous rows of femoral and precloacal scales bearing pores); and the number of cloacal spurs. Color pattern characters evaluated were the presence or absence of dark pigmentation in the gonadal tracts and caecum; presence or absence of a dark postorbital stripe extending to at least the neck; and the presence of absence of a linear series of white postorbital spots above the dark postorbital stripe. Some of the information on character states and their distribution in other species was obtained from Zug (2010). LSUHC refers to the La Sierra University Herpetological Collection, La Sierra University, Riverside, California, USA; and LSUDPC refers to the La Sierra University Digital Photo Collection. Other acronyms follow Sabaj-Pérez (2010).

Results

Phylogenetic analysis

The ML and BI analyses of the 20 individuals resulted in phylograms with the same topology, showing that the Penang Hill population is embedded within the *harterti* group and is sister to the sister species *H. harterti* and *H. bintik* from Bukit Larut and Gunung Tebu, respectively (Fig. 2). These three allopatric species form a monophyletic group that spans a geographic range of approximately 270 km across three mountain ranges (Fig. 1). These

relationships are well-supported (1.0/100) and the Penang Hill population has an uncorrected pairwise sequence divergence of 8.07% from *H. harterti* and *H. bintik*. The analyses also showed that *H. titiwangsaensis* is a well-supported (1.0/0.99) lineage composed of distinct, northern and southern clades separated by approximately 90 km along the Titiwangsa Mountain Range between Fraser's Hill and Cameron Highlands, Pahang (Fig. 1). The uncorrected pairwise sequence divergences between the Cameron Highlands population and the Fraser's Hill and Genting Highlands populations are 12.4 and 12.8%, respectively.

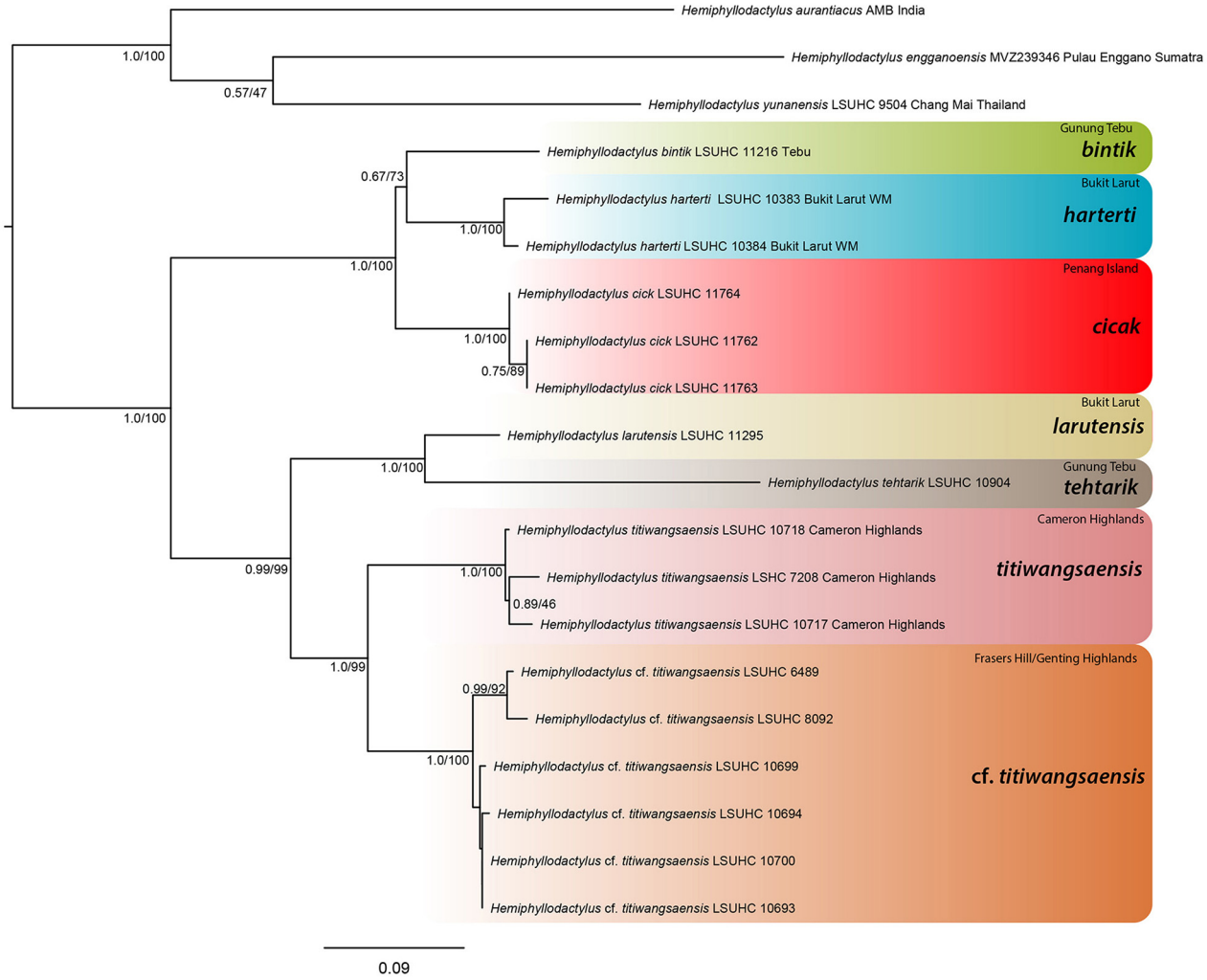

FIGURE 2. Maximum-likelihood phylogram of the *Hemiphyllodactylus harterti* group with Bayesian posterior probabilities followed by maximum likelihood bootstrap values.

Systematics

***Hemiphyllodactylus cicak* sp. nov.**
Penang Island Slender Gekko
Cicak Kerdil Pulau Pinang
Figs. 3,4

Holotype. Adult male (LSUCH 11762) collected by Evan Quah and L. Lee Grismer on 5 May 2014 at 600 m elevation from the living room of the old Ban Hin Lee Guest House on Penang Hill, Pulau Pinang, Peninsular Malaysia (5°25'23.14"N, 100°16'19.79"E) at approximately 1800 hrs during a heavy thunderstorm.

Paratypes. Three adult females (LSUHC 11763–65) are associated with the same collection data as the holotype.

FIGURE 3. Male Holotype (LSUCH 11762) of *Hemiphyllodactylus cicak* **sp. nov.** from Penang Hill, Penang Island, Penang, Peninsular Malaysia.

Diagnosis. *Hemiphyllodactylus cicak* **sp. nov.** can be separated from all other species of *Hemiphyllodactylus* by possessing the unique combination of characters, having a maximum SVL of 31.4 mm; three circumnasal scales; one or two scales between supranasals (=postrostrals); eight supralabials; eight infralabials; 10 or 11 longitudinally arranged ventral scales at midbody contained within one eye diameter; a series of 42 pore-bearing precloacal-femoral scales in the male; lamellar formula on hand 2–3–3–2; lamellar formula on foot 2–3–3–3; dorsal body pattern consisting of dark, transverse, paravertebral blotches coupled with white speckles; dark pre- and postorbital stripes; faint postorbital stripe extends along the flanks to the hind limbs. These characters, and other diagnostic characters, are scored across all species of *Hemiphyllodactylus* within the *harterti* group listed in Table 3.

Description of holotype. Adult male; head sub-triangular in dorsal profile, depressed, distinct from neck; lores and interorbital regions flat; rostrum moderate in length (NarEye/HeadL 0.23); prefrontal region flat to weakly concave; canthus rostralis smoothly rounded, barely discernable; snout moderate, rounded in dorsal profile; eye large; ear opening round, small; eye to ear distance greater than diameter of eye; rostral wider than high, partially divided dorsally, bordered posteriorly by small supranasals; one internasal (= postnasal); external nares bordered anteriorly by rostral, dorsally by supranasal, posteriorly by two postnasals, ventrally by first supralabial (= circumnasals 3R,L); 8 (R,L) square supralabials tapering to below posterior margin of orbit; 8 (R,L) square infralabials tapering to below posterior margin of orbit; scales of rostrum, lores, top of head, and occiput small, granular, those of rostrum largest and slightly raised; dorsal superciliaries flat, rectangular, subimbricate; mental triangular, bordered laterally by first infralabials and posteriorly by two large postmentals; each postmental bordered laterally by a single sublabial; one row of smaller scales extending transversely from juncture of second and third infralabials and contacting mental; seven chin scales; gular scales small, subimbricate, grading posteriorly into slightly larger, subimbricate, throat and pectoral scales which grade into slightly larger, subimbricate ventrals.

TABLE 3. Diagnostic characters of the species in the *Hemiphyllodactylus harterti* group.

	harterti	*tehtarik*	*larutensis*	*bintik*	*titiwangsaensis*	*cicak* **sp. nov.**
max SVL	39	40.4	52.2	36.6	52.2	31.4
chin scales	6–8	8	6–10	7	7–11	7
postmentals distinctly enlarged (1) or not (0)	1	1	1	1	1	1
circumnasal scales	2–5	5	3 –5	5	3 –4	3
scales between supranasals	3 –4	3	3	3	2 –4	1 –2
supralabial scales	10–11	11	9,10	11	5–8	8
infralabial scales	10–11	10	7–10	12	5–8	8
dorsal scales	14–19	18	13–20	17	17	16–18
ventral scales	6–14	12	7–13	7	8	10–11
lamellar formula on hand	3–3–3–3	3–3–3–3	/	2–4–4–3	3–4–4–4	2–3–3–2
lamellar formula on foot	3–3–4–3	3–4–5–4	/	3–4–4–4	4–5–5–5	2–3–3–3
subdigital lamellae on first finger	3	5	3,4	4	2	2
subdigital lamellae on first toes	4	5	3–5	5	2	2
Precloacal and femoral pores series separate (1) or continuous (0)	0	none	0	/	0	0
Precloacal and femoral pores	42–45	0	27–36	/	26–32	42
cloacal spurs	1,2	3	2,3	1	2–3	2
subcaudals enlarged, plate-like (1) or not (0)	0	/	0	0	0	0
dark postorbital stripe present (1) or absent (0)	1	1	1	1	1	1
light postocular or trunk spots (1) or absent (0)	1	0	1	0	1	1
dark dorsolateral stripe on trunk (1) or absent (0)	1,0	0	0	0	0	0
dorsal pattern unicolor (1) or not (0)	0	1	1	0	0	0
dark dorsal transverse blotches (1) or not (0)	0	0	0	0	1	0
longitudinal series of white (1) or yellow or red (0) dorsal spots	0	0	0	0	1	1
postsacral mark lacking anterior arms (1) or arms present (0)	0	1	1	0	1	1
caecum pigmented (1) or not (0)	1	0	0	1	0	0
gonads pigmented (1) or not (0)	0	0	0	0	0	0
Trunk/SVL	0.48–0.53	0.55	0.46–0.51	0.49	0.43–0.48	0.47–0.50
HeadL/SVL	0.22–0.24	0.2	0.21–0.24	0.23	0.15–0.27	0.25
HeadW/SVL	0.16–0.18	0.16	0.15–0.17	0.18	0.15–0.19	0.16–0.17
HeadW/HeadL	0.65–0.85	0.8	0.63–0.73	0.8	0.63–0.71	0.63–0.67
SnEye/HeadL	0.41–0.48	0.47	0.39–0.51	0.41	0.34–0.38	0.35
NarEye/HeadL	0.28–0.33	0.4	0.27–0.36	0.33	0.23–0.29	0.22–0.23
EyeD/HeadL	0.22–0.30	0.28	0.22–0.28	0.24	0.21–0.24	0.22
SnW/HeadL	0.15–0.22	0.16	0.11–0.15	0.17	0.14–0.21	0.16
EyeD/NarEye	0.81–1.00	0.72	0.66–0.90	0.74	0.84–0.94	0.95–1
SnV/HeadW	0.20 –0.32	0.2	0.18 –0.21	0.21	0.21 –0.31	0.19

Body somewhat elongate (Trunk/SVL 0.49), dorsoventrally compressed; ventrolateral folds absent; dorsal scales small, granular, 17 scales at midbody contained within one eye diameter; ventral scales, flat, subimbricate much larger than dorsal scales, ten scales contained within one eye diameter; enlarged, precloacal scales; 42 pore-bearing femoral and precloacal scales; forelimbs short, slender in stature, covered with flat, subimbricate scales dorsally and ventrally; palmar scales flat, subimbricate; all digits except digit I well-developed; digit I vestigial, clawless; distal, subdigital lamellae of digits II–V undivided, angular and U-shaped; lamellae proximal to these transversely expanded; lamellar formula of digits II–V 2–3–3–2 (R,L); two transversely expanded lamellae on digit I; claws on digits II–V well developed, unsheathed; distal portions of digits strongly curved, terminal joint free, arising from central portion of lamellar pad; hind limbs short, more robust than forelimbs, covered with flat, juxtaposed scales dorsally and by larger, flat subimbricate scales ventrally; plantar scales low, flat, subimbricate; all digits except digit I well-developed; digit I vestigial, clawless; distal, subdigital lamellae of digits II–V undivided, angular and U-shaped; lamellae proximal to these transversely expanded; lamellar formula of digits II–V 2–3–3–3 (R,L); two transversely expanded lamellae on digit I; claws on digits II–V well developed, unsheathed; distal portions of digits strongly curved, terminal joint free, arising from central portion of lamellar pad; tail rectangular in cross-section. Morphometric data are presented in table 4.

TABLE 4. Mensural and meristic data from the type series of *Hemiphyllodactylus cicak* **sp. nov.** from the LSUHC collection.

	11762	11763	11764	11765
Sex	m	f	f	f
Chin Scales	7	7	7	7
Postmentals distinctly enlarged (1) or not (0)	1	1	1	1
Circumnasal scales	3	3	3	3
Scales between supranasals	1	1	1	2
Supralabial scales	8	8	8	8
Infralabial scales	8	8	8	8
Dorsal scales	17	16	16	18
Ventral scales	10	10	10	11
Lamellar formula on hand	2–3–3–2	2–3–3–2	2–3–3–2	2–3–3–2
Lamellar formula on foot	2–3–3–3	2–3–3–3	2–3–3–3	2–3–3–3
Subdigital lamellae on first finger	2	2	2	2
Subdigital lamellae on first toes	2	2	2	2
precloacal and femoral pore series separate (0) or continuous (1)	0	/	/	/
# precloacal and femoral pores	42	/	/	/
dark postorbital strip present (0) or absent (1)	0	0	0	0
Number of cloacal spurs	2	1	1	1
Subcaudals enlarged, plate like (1) or not (0)	0	0	0	0
Dorsal pattern unicolor (1) or not (0)	0	1	1	0
Dark dorsal transverse blotches (1) or not (0)	0	0	0	0
Snout Vent Length	31.4	28.9	25.4	28.5
Trunk Length	15.6	14.04	11.9	14.5
Head Length	7.7	6.8	6.3	6.5
Head Width	4.9	4.7	4.2	4.6
Eye Diameter	1.7	1.6	1.4	1.6
Snout Eye Length	2.8	2.7	2.2	2.7
Nar Eye Internarial Width (SnW)	1.8	1.4	1.4	1.6

FIGURE 4. Ventral view of the type series *Hemiphyllodactylus cicak* **sp. nov.**

Coloration before preservation (Fig. 3). Top of head, body, and limbs beige with black spots and white speckles; dark pre- and postorbital and paired, paroccipital stripes present; dorsum overlain with paired, dark, slightly offset, squarish, paravertebral markings; ground color of the dorsum on the anterior portion of the tail gray while the ground color of the rest of the tail light beige; large, dark, lateral markings on anterior portion of tail that fade toward the posterior; flanks and dorsal surfaces of limbs darkly mottled; ventral surfaces of head, neck, body, and limbs whitish, semi –transparent with greyish brown speckling especially along the sides of the body; subcaudal region orange, especially bright on the underside of the tail (Fig. 4).

Distribution. *Hemiphyllodactylus cicak* **sp. nov.** is known only from the type locality of Penang Hill, Penang, Peninsular Malaysia (Fig. 1).

Natural history. All specimens of the type series were collected at night in the Ban Hin Lee Guest House during a heavy downpour. This species occurs in syntopy with various other species of geckos in the guesthouse those being *Hemiphyllodactylus typus* Bleeker, *Gehyra mutilata* Wiegmann, *Gekko monarchus* Schlegel, and *Hemidactylus frenatus* Duméril, Bibron. LSUHC 11764 was a gravid female. An additional gravid female (LSUHC 12488 not part of the type series) was collected late at night crossing a road near the guest house bordered by hill dipterocarp forest on the 30 July 2015. This indicates the reproductive biology of this species extends from at least early May through late July.

Etymology. This specific epithet "*cicak*" is the Malay word for lizard.

Variation (Fig. 4). The general color patterns of the paratypes closely match that of the holotype. LSUCH 11763 is an adult female that has a partially regenerated tail with a soft gray color. Differences in scales counts are presented in Table 4.

Comparisons. The molecular analysis indicates that *Hemiphyllodactylus cicak* **sp. nov.** is embedded within the *harterti* group. It can be distinguished from all other species in that group by having a SVL of 31.4 mm, which is smaller than all other species in the group (Table 3). *Hemiphyllodactylus cicak* **sp. nov.** has a manual lamellar formula of 2–3–3–2 as opposed to 3–3–3–3 in *H. harterti*, 2–4–4–3 in *H. tehtarik*, or 3–4–4–4 in *H. bintik* and *H. titiwangsaensis,* respectively, and a pedal lamellar formula of 2–3–3–3 as opposed to 3–3–4–3 in *H. harterti*,

3–4–5–4 in *H. tehtarik*, 3–4–4–4 in *H. bintik*, or 4–5–5–5 in *H. titiwangsaensis*, respectively. It also differs from all other species of the *harterti* group by having only one or two scales between the supranasals as opposed to three, four, or five. *Hemiphyllodactylus cicak* **sp. nov.** has eight supralabial scales, whereas *H. harterti* has 10 or 11, *H. tehtarik* has 11, *H. larutensis* has nine or 10, *bintik* has 11, and *H. titiwangsaensis* has 5–8. *Hemiphyllodactylus cicak* **sp. nov.** can be distinguished from *H. tehtarik* and *H. bintik* by having light postocular and trunk spots. *Hemiphyllodactylus cicak* **sp. nov**. lacks the dark transverse blotches present in *H. titiwangsaensis*, and lacks the unicolor dorsal pattern of *H. tehtarik* and *H. larutensis*. Additionally, the new species can be differentiated from *H. titiwangsaensis* and *H. larutensis* by having a precloacal and femoral pores series of 42, as opposed to 26–32 or 27–36 in these species, respectively. Although *H. cicak* **sp. nov**. is most closely related to *H. bintik* and *H. harterti* it has two lamellae on its first finger as opposed *H. harterti* which has three and *H. bintik* which has four. A similar difference can be seen on digit one of the foot where *H. harterti* has four lamellae and *H. bintik* has five, whereas the new species has two. There is an uncorrected pairwise sequence divergence of approximately 8.07% between *H. harterti* and *H. bintik.* Previously, Grismer *et al.* (2013) noted that a divergence of at least 5.0% in *Hemiphyllodactylus* was consistent with discrete, diagnostic, morphological differences delimiting species boundaries within gekkonids in general.

TABLE 5. Mensural and meristic data from the three different populations of *Hemiphyllodactylus titiwangsaensis*.

	Cameron Highlands	Genting Highlands	Fraser's Hill
Chin Scales	8–10	6–9	7–11
Postmentals distinctly enlarged (1) or not (0)	1	1	1
Circumnasal scales	3	3	3–4
Scales between supranasals	2–3	2–3	2–4
Supralabial scales	7	7–8	6–8
Infralabial scales	6–7	6–8	6–8
Dorsal scales	18–22	15–22	11–22
Ventral scales	7–12	7–9	6–14
Lamellar formula on hand	4–4–4–4	3–4–4–4	4–4–4–4
Lamellar formula on foot	4–4–4–4	4–4–4–4	4–4–4–4
Subdigital lamellae on first finger	2	2	3
Subdigital lamellae on first toes	2	2	2
Precloacal and femoral pore series separate (1) or continuous (2)	2	2	2
Number of precloacal and femoral pores	28	30–32	26–32
Dark postorbital strip present (1) or absent (2)	2	2	2
Number of cloacal spurs	3	3	3
Subcaudals enlarged, plate like (1) or not (0)	0	0	0
Dorsal pattern unicolor (1) or not (0)	0	0	0
Dark dorsal transverse blotches (1) or not (0)	1	1	1
Snout Vent Length	52.5–53.1	39.6–52.2	46.4–56.2
Trunk Length	21–25.3	20–25.2	19.9–27.2
Head Length	12.4–14.3	10.9–14.4	10.9–15.3
Head Width	8.4–9.8	7–9.5	6.9–10.9
Eye Diameter	2.6–3.1	2.2–3.4	2.3–3.7
Snout Eye Length	4.9–5.3	4.9–5.8	3.7–5.8
Nar Eye Internarial Width (SnW)	3.5–4.1	3.2–4.4	2.5–4.4

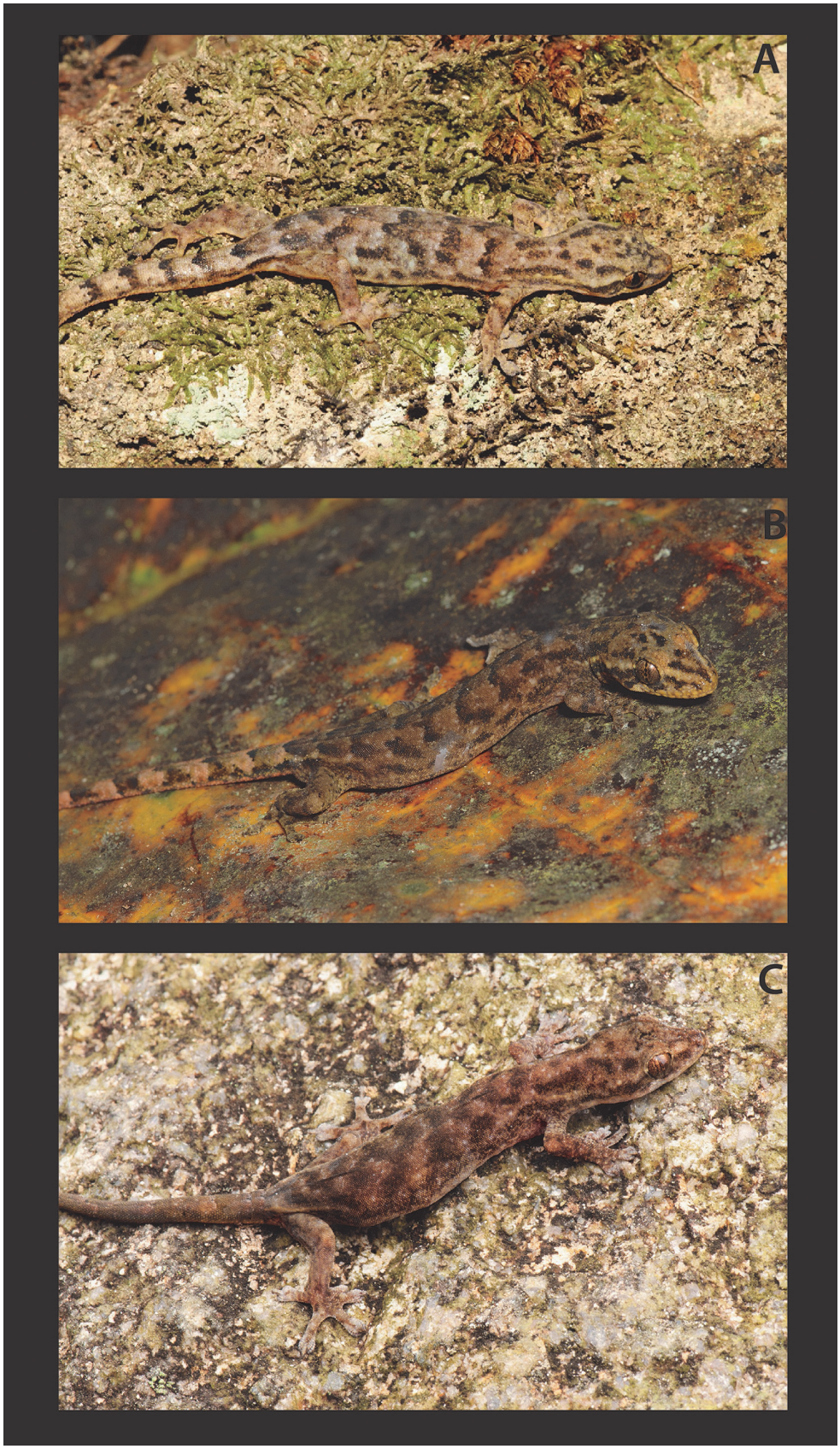

FIGURE 5. Upper: adult male *Hemiphyllodactylus titiwangsaensis* (LSUDPC 6708) from the type locality of Cameron Highlands, Pahang. Middle: adult male *H.* cf. *titiwangsaensis* (LSUDPC 2868) from Fraser's Hill, Pahang. Lower: adult male *H.* cf. *titiwangsaensis* (LSUDPC 6444) from Genting Highlands, Pahang.

Taxonomy of *Hemiphyllodactylus titiwangsaensis*

Hemiphyllodactylus titiwangsaensis was described by Zug (2010) based on a series of specimens from Gunung Brinchang in Cameron Highlands. Zug (2010) reported that members of this species could be found farther along the Banjaran Titiwangsa at Fraser's Hill 90 km to the south and Grismer (2011) reports an occurrence of this species even farther south at Genting Highlands, Pahang. Zug's (2010) description was based on morphological characters wherein he noted, that among the adults of the Cameron Highlands population, there was a broad range of variation in morphology (see Appendix; Zug, [2010]) but that the major diagnostic characters of *H. titiwangsaensis* were a non-pigmented caecum and gonadal ducts, a continuous precloacal-femoral pore series in males of 17–39 pore-bearing scales, an enlarged mental scale bordering chin scales, enlarged first infralabial scales, a digital lamellar formulae for the hand of normally 3–4–4–4, and for the foot of 4–4–5–5 or 4–5–5–5. Additionally, he noted the dorsal color pattern consisted of dark transverse bands. A series of 35 specimens of *H. titiwangsaensis* were re-examined here from all three localities (see Appendix Grismer *et al*., 2013). All measurements were retaken and additional morphological characters were added (Table 5). An ANOVA found no significant differences among any scale counts (P = 0.975; F = 0.0249; F *crit* = 3.219; Table 6), indicating there are no significant differences in these characters among the northern and southern populations. Additionally, we could find no consistent differences in color pattern between these populations. At this point in time, we consider *H. titiwangsaensis* to be a species complex in which the northern population from Cameron Highlands is morphologically similar to those from the southern two populations (Genting Highlands and Fraser's Hill), yet genetically very distinct (12.4 and 12.8%, respectively). Thus, we recognize the southern populations as *H.* cf. *titiwangsaensis* until additional morphological data separating them from *H. titiwangsaensis sensu stricto* can be obtained.

TABLE 6. ANOVA test performed on the mensural and meristic data collected from the 3 different populations of *Hemiphyllodactylus titiwangsaensis.*

SUMMARY

Groups	Count	Sum	Average	Variance
Cameron Highlands	15	170.265	11.351	172.2812
Genting Highlands	15	155.1667	10.34444	131.3763
Fraser's Hill	15	163.54	10.90267	155.4612

ANOVA

Source of Variation	SS	df	MS	F	P–value	F crit
Between Groups	7.628845	2	3.814422	0.024924	0.975398	3.219942
Within Groups	6427.661	42	153.0396			
Total	6435.29	44				

Discussion

Within the past five years, three new upland species of the *Hemiphyllodactylus harterti* group have been discovered in Peninsular Malaysia (Zug 2010; Grismer *et al.* 2013; this paper). All species in this group are small, cryptic, arboreal, non-vagile, upland endemics that are no larger than 55 mm in SVL and it is highly likely that additional species within this clade will be found on other mountain tops. We believe this is especially true for mountain tops in the Titiwangsa Mountain Range that are centrally located between *H. tehtarik* and *H. bintik* of the Timur Mountain Range and *H. larutensis* and *H. harterti* of the Bintang Range (Fig. 1).

What was believed to be the single species, *Hemiphyllodactylus titiwangsaensis*, that was distributed along the Titiwangsa Mountain Range, is now considered to be to be a species complex. Whereas current trends (and titles)

indicate that "cryptic species" are being revealed with molecular data sets, Grismer *et al.* (2014c) noted that what is actually being revealed are less than thorough morphological analyses in that subsequent examination of these "cryptic species" always reveals discrete diagnostic characters (*i.e.*, they really are not truly cryptic) that were previously overlooked. However, the *H. titiwangsaensis* species complex appears (at this point) to be a true case of cryptic speciation—rather than a putative case revealed retrospectively following a molecular analysis—in which the examination of all the populations with an expanded morphological data set and sample size found no diagnostic characters. As stated before, these geckos are non-vagile, which could give evidence of true species boundaries between the northern and southern populations that are supported by the significant genetic divergence between them. Although the genetic divergence may indicate that there is no gene flow between the northern and southern populations, it is possible *H. titiwangsaensis* acquired these similar morphologies prior to isolation and they have yet to be selected against. Conversely, another hypothesis is that, if females are sedentary and males are dispersing, nuclear gene flow may be occurring and maintaining the morphological similarity of these populations. Analyses using nuclear markers are currently in progress to test this hypothesis.

Acknowledgements

Funding for Shahrul Anuar comes from Universiti Sains Malaysia and Ministry of Higher Education Research Grants. Partial laboratory funding for generating molecular data and field work for PLW was from an NSF grant EF-1241885 to Jack W. Sites, Jr. at Brigham Young University. PLW would like to thank undergraduates in the Sites lab for help generating some of the molecular data used in this study. Partial funding to LLG comes from the National Geographic Society grant number 9722-15. We would like to thank Mr. Tan Choo Eng, the president of the Penang branch of the Malaysian Nature Society for helping arrange our accommodation on Penang Hill.

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