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Two new species of shrub frogs (Rhacophoridae: *Pseudophilautus*) from Sri Lanka

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Abstract

Two new species of Sri Lankan shrub frogs of the genus *Pseudophilautus* are described. These species are diagnosed from their congeners on the basis of morphology, morphometrics and mitochondrial DNA sequence data. *Pseudophilautus schneideri*, new species, is distinguished from all Sri Lankan *Pseudophilautus* by its small size (< 22.8 mm SVL), distinct tympanum and supratympanic fold, sharp canthal edges, granular throat, chest and belly, and absence or presence of a vomerine ridge. *Pseudophilautus hankeni*, new species, is distinguished by its diminutive size (< 21.9 mm SVL), distinct tympanum, rounded canthal edges, tuberculated outer edge of lower arm, tuberculated dermal fold on outer edge of foot, granular throat, chest and belly, and the absence of a vomerine ridge. *Pseudophilautus schneideri* inhabits shrubs in open areas of the low to mid-elevations of the island's south-western 'wet zone' (rainfall > 2,000 mm•yr^{-1}), including anthropogenic habitats, while *P. hankeni* is found on shrubs in the understorey of montane forests of the highest peaks (*c*. 1,200–1,600 m elevation) of the Knuckles region. These descriptions bring the total number of valid species of Sri Lankan *Pseudophilautus* to 67, 48 of which are extant.

Key words: Rhacophorinae, taxonomy, molecular systematics, Knuckles Hills, conservation

Introduction

Following the discovery in Sri Lanka of a large radiation of Oriental tree frogs of the genus *Pseudophilautus* (Meegaskumbura *et al*. 2002), 39 new species have been described as part of an on-going effort to document this fauna (Manamendra-Arachchi & Pethiyagoda 2005; Meegaskumbura & Manamedra-Arachchi 2005; Meegaskumbura *et al*. 2007; Meegaskumbura *et al*. 2009). The review and description of 27 new species by Manamendra-Arachchi & Pethiyagoda (2005), though informed by a phylogeny, was based purely on morphology (given the unavailability of molecular data for the older type material). Meegaskumbura & Manamendra-Arachchi (2005), however, described eight more new species using the General Lineage concept (de Queiroz, 1998), where species are considered as independent evolutionary lineages based on multiple criteria (in this case molecular divergence, morphology, ecology and vocalization). Meegaskumbura *et al*. (2007) added two additional new but extinct species discovered in historical museum collections, again adopting a purely morphological approach. More recently Meegaskumbura *et al*. (2009) described two new species from the lowlands of Sri Lanka using molecular, morphological and morphometric data. The island's inventory of *Pseudophilautus* now stands at 67 species, of which 48 are extant. Surveys in Sri Lanka since the early 1990s suggest that 19 species, known only from museum specimens collected in the 19th and early 20th centuries, have since disappeared (Manamendra-Arachchi & Pethiyagoda 2005; Meegaskumbura *et al*. 2007); these extinctions, together with the high number of Critically Endangered (11) and Endangered (36) species, highlights the urgent need to describe and name the remaining new species of shrub frogs discovered by us in Sri Lanka, so that they can be included in the conservation planning process.

Here we continue to document the new species discovered in Sri Lanka as a result of our explorations on the island up to 2005. The species descriptions are based on morphological, morphometric and molecular data, in the context of the General Lineage concept of species.

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Material and methods

Field sampling and measurements were made as described in Manamendra-Arachchi & Pethiyagoda (2005), except as mentioned below.

Morphological analysis. The suite of characters and character states used by Manamendra-Arachchi & Pethiyagoda (2005) was analyzed for all individuals. Measurements were made to the nearest 0.1 mm using dial vernier calipers. Measurements with high coefficients of variation or low repeatability were omitted from the analysis, for which the following were used: distance between back of eyes (DBE); distance between front of eyes (DFE); length of disk (DL); width of disk (DW); eye diameter (ED); eye-to-nostril distance (EN); eye-to-snout length (ES); femur length (FEL); length of finger 1 (FLI); length of finger 2 (FLII); length of finger 3 (FLIII); length of finger 4 (FLIV); pes length (FOL); head length (HL); head width (HW); length of inner metatarsal tubercle (IML); internarial distance (IN); interorbital distance (IO); lower-arm length (LAL); posterior mandible-to-eye distance (MBE); least distance from mandible to anterior eye (MFE); least distance from mandible to nostril (MN); nostril-to-snout length (NS); palm length (PAL); snout–vent length (SVL); tibia length (TBL); length of toe 1 (TLI); length of toe 2 (TLII); length of toe 3 (TLIII); length of toe 4 (TLIV); length of toe 5 (TLV); diameter of tympanum (TYD); distance from tympanum to front of eye (TYE); length of upper arm (UAW); and width of upper eyelid (UEW). Illustration of the webbing pattern follows Manamendra-Arachchi & Pethiyagoda (2005). Measurements with high coefficients of variation or low repeatability were omitted from the PCA analysis, for which only the following were used: DBE, DFE, DL, DW, ED, EN, ES, FEL, FLI, FLII, FLIII, FLIV, FOL, HL, HW, IN, IO, LAL, MBE, MFE, MN, NS, PAL, SVL, TL1, TLII, TLIII, TLIV, TLV IML, TYE and TBL.

Principal components analysis (PCA) of the character correlation matrix was used to reduce dimensionality of the continuous morphological variables and to identify those variables that best discriminate between morphologically similar species (*P. schneideri* vs. *P. folicola* and *P. hankeni* vs. *P. schmarda*). Various axis rotations were tested and one selected for optimal interpretability of variation among the characters. For consistency, only mature males were used in this analysis. In several cases, the small sample sizes likely do not represent the full range of morphological variation; nonetheless, the analyses are sufficient to demarcate species and identify characters that contribute best to their diagnoses. SYSTAT (Version 11.00.01 for Windows XP) was used for statistical analysis.

Molecular analysis. A species, representing *P. hankeni* (WHT 6302) and two more undescribed species *P.* cf. *simba* WHT 5831 and *P. simba* WHT 6004 (WHT 3221 was called *P. simba* in Meegaskumbura *et al*. 2009, which is corrected here as *P.* cf. *simba*) were added to the data that were analyzed in Meegaskumbura *et al*. (2009; see Table 1); *P. schneideri* ('*Pseudophilautus* sp.' WHT 2667), was already included in that data set (Table 1). Only 12s rRNA and 16s rRNA partial sequences were used to construct the phylogenetic tree, as was done in Meegaskumbura *et al*. (2002), Meegaskumbura & Manamendra-Arachchi (2005) and Meegaskumbura *et al*. (2009). Cytochrome-*b* data was used, in addition, to determine the percentage divergences among sister taxa, and PCR amplification and alignment of sequences was done as explained in Meegaskumbura & Manamendra-Arachchi (2005).

The data was analyzed using Bayesian, Maximum Likelihood (ML) and Maximum Parsimony (MP) criteria. For brevity, we present only the Maximum Likelihood tree, which is identical to the Bayesian tree, together with one of the two equally parsimonious trees. We used Bayesian inference as implemented in MrBayes (Huelsenbeck & Ronquist 2001) to generate a phylogenetic hypothesis of relationships among the taxa and to estimate a general time-reversible model of sequence evolution with gamma-distributed rate variation among sites and a proportion of invariant sites (GTR+I+G). We ran four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains for 500,000 generations and the summed likelihood of the four chains converged on a stationary value by 100,000 generations (the burn-in time). We used the frequency of clades in trees that were sampled every ten generations from the last 250,000 generations as estimates of the posterior probabilities of those clades (Huelsenbeck *et al*. 2001). Uniform priors were used throughout and branch lengths, topology, and nucleotide substitution parameters were unconstrained. Maximum likelihood analysis used a GTR+I+G model of nucleotide substitution with parameters estimated from the Bayesian analysis. A single heuristic search with Tree Bisection and Reconnection (TBR) branch swapping was conducted using PAUP*4.0b10 (Swofford 2002). For tree searches under a Maximum Parsimony criterion we used 100 heuristic searches with TBR branch-swapping and random taxon addition as implemented in PAUP*4.0b10. Two equally parsimonious trees with tree scores of 1133 were recorded. A bootstrap analysis (1000 replicates, random stepwise addition with 100 reps.) to determine node support was also carried out within a maximum-parsimony framework.

TABLE 1. Reference numbers, and the Genbank accession numbers for the species used in the phylogenetic analysis.

Species	Reference number	Genbank Accession numbers	
		12s	16s
P. alto	WHT2723	AY141781	AY141827
P. asankai	WHT5107	FJ788141	FJ788160
P. auratus	WHT2792	AY141789	AY141835
P. caeruleus	WHT2511	AY141764	AY141810
P. cavirostris	WHT3299	FJ788137	FJ788156
P. cf. *sarasinorum*	WHT2484	AY141762	AY141808
P. cf. *sarasinorum*	WHT2489	AY141763	AY141809
P. cf. *simba*	WHT5831	GU593345	GU593347
P. cf. *simba*	WHT3221	FJ788148	FJ788167
P. cf. *sordidus*	WHT_H12	AY141791	AY141837
P. cf. *sordidus*	WHT_H15	AY141792	AY141838
P. charius	FB	AY141840	AY141794
P. decoris	WHT3271	FJ788144	FJ788163
P. femoralis	WHT2566	AY141771	AY141817
P. femoralis	WHT2772	AY141785	AY141831
P. frankenbergi	WHT2552	AY141768	AY141814
P. frankenbergi	WHT2555	AY141769	AY141815
P. hallidayi	WHT_H11	AY141793	AY141839
P. hankeni	WHT6302	GU593346	GU593348
P. hoffmanni	WHT3223	FJ788142	FJ788161
P. hoipolloi	WHT2675	AY141776	AY141822
P. limbus	WHT2690	AY141777	AY141823
P. limbus	WHT2700	AY141779	AY141825
P. lunatus	WHT3283	FJ788150	FJ788169
P. microtympanum	WHT2558	AY141770	AY141816
P. mittermeieri	KAN2	FJ788143	FJ788162
P. mooreorum	WHT3209	FJ788134	FJ788153
P. ocularis	WHT2887	FJ788145	FJ788164
P. pappilosus	WHT3284	FJ788151	FJ788170
P. pleurotaenia	WHT3176	FJ788146	FJ788165
P. poppiae	WHT5026	FJ788135	FJ788154
P. poppiae	WHT2779	FJ788136	FJ788155
P. popularis	WHT3191	FJ788149	FJ788168
P. procax	WHT2786	AY141788	AY141834
P. sarasinorum	WHT2481	AY141761	AY141807
P. schmarda	WHT2715	AY141780	AY141826
P. signatus	FB	AY141795	AY141841
P. simba	WHT6004	GQ204740	GQ204679
P. singu	WHT2658	AY141773	AY141819
P. sordidus	WHT2699	AY141778	AY141824
P. sp.	WHT2515	AY141765	AY141811

continued next page

TABLE 1. (continued)

Species	Reference number	Genbank Accession numbers	
		12s	16s
P. sp.	WHT2540	AY141767	AY141813
P. sp.	WHT2797	AY141790	AY141836
P. schneideri	WHT2667	AY141774	AY141820
P. sp.	WHT2669	AY141775	AY141821
P. sp.	WHT2525	AY141766	AY141812
P. sp.	WHT2774	AY141786	AY141832
P. sp.	WHT2729	AY141782	AY141828
P. sp.	WHT2731	AY141783	AY141829
P. steineri	WHT3210	FJ788138	FJ788157
P. stuarti	WHT3207	FJ788139	FJ788158
P. stuarti	WHT3208	FJ788140	FJ788159
P. tanu	WHT6343	FJ788152	FJ788171
P. viridis	WHT2627	AY141772	AY141818
P. viridis	WHT2766	AY141784	AY141830
P. wynaadensis	FB	AY141796	AY141842
P. zorro	WHT3175	FJ788147	FJ788166

As described in Meegaskumbura & Manamendara-Arachchi (2005), once we identified the divergent mtDNA lineages and their sister taxa using the 12S and 16S rRNA gene tree, and to facilitate comparisons with published summaries of mitochondrial divergence among vertebrate sister species (Johns & Avise 1998), we sequenced a 590 bp fragment of the mitochondrial cytochrome-*b* gene from the species described herein and their sister species. For this analysis, a ~ 590 base-pair fragment of the mitochondrial cytochrome-*b* gene was amplified using primers CB-J-10933, (5'- TATGTTCTACCATGAGGACAAATATC-3') and BSF4 (5'- CTTCTACTGGTTGTCCTCCGAT-TCA-3') (Bossuyt & Milinkovitch 2000) under standard PCR conditions: denaturation at 95° C for 40 s, annealing at 45° C for 40 s and extension at 72° C for 40 s, 35 cycles, with a final extension of 72° C for 5 min. Products were gel purified and sequenced on an ABI 377 or ABI 3100 automated sequencer following manufacturer's protocols. Sequences were aligned using translated amino acid sequences using Se-Al (ver. 2.0*a11*; Rambaut 1996).

Results

Morphometric analysis. *Pseudophilautus schneideri* and *P. folicola* (the species it morphologically resembles most closely) separate distinctly from each other in morphological space (Fig. 1, Table 2). Principal components analysis shows that the two species are distinguished by a combination of body dimensions and nostril to snout distance. The PC(1) axis, which explains 81 % of the variance, is a size axis (most of the variables load heavily and all variables have high, positive loadings on this axis; NS does not load heavily, but has a positive value of 0.275). The PC(2) axis represents 6 % of the variance, with NS (-0.802) and FLIII (-0.588) contributing most heavily. However, the two species only separate on the body size (i.e. the PC(1)) axis, but completely overlap on the PC(2) axis; *Pseudophilautus schneideri* is the smaller of the two species.

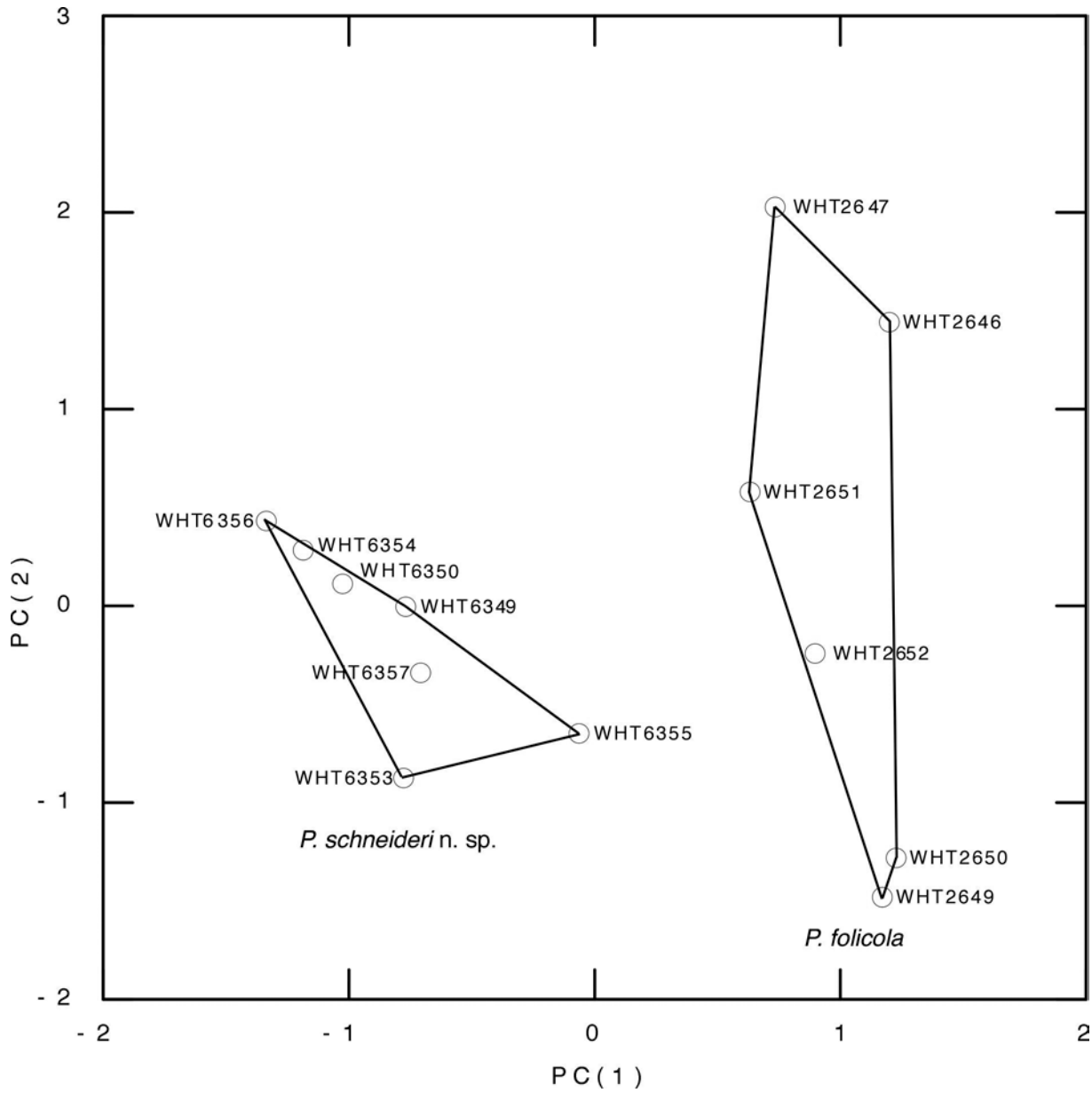

FIGURE 1. PC1 vs. PC2 factor scores of the principal components analysis of *Pseudophilautus schneideri,* **n. sp.** and *P. folicola*, show these species to separate well from each other in PC space. Most of the variation is explained by the PC1 axis, which relates mainly to body size (*P. schneideri* being smaller). The PC2 axis is mostly explained by nostril-to-snout length and length of finger 3; here, *P. schneideri* overlaps completely with *P. folicola*.

Pseudophilautus hankeni and *P. schmarda* also, separate from each other in morphological space (Fig. 2, Table 3). Principal components analysis shows that the two species are distinguished from each other by a combination of body dimensions, NS, MBE, MFE, and ED. The PC(1) axis, which explains 83 % of the variance, is a size axis (most variables load heavily and positively on this axis). PC(2) represents 7 % of the variance, with NS (0.507), MBE (-0.477) and MFE (-0.421) contributing most heavily. However, while these two species separate along the size (PC1) axis, they completely overlap on the PC(2) axis; *P. hankeni* has the smaller body dimensions.

Molecular phylogenetics. The final dataset contained 12S and 16S rRNA mitochondrial gene sequences from 58 putative haplotypes. Fifty-five of these represent Sri Lankan *Pseudophilautus*, while three represent Indian species (one, *P. wynaadensis,* is nested within the Sri Lankan clade, whereas the other two represent the sister group to the Sri Lankan and nested Indian *Pseudophilautus*: Meegaskumbura *et al*. 2002; Bossuyt *et al*. 2004). Hence, of the 939 nucleotide positions sequenced, 867 were clearly alignable and were included in this analysis.

TABLE 2. Component loadings for axes 1 and 2 of the principal component analysis, variance explained and percentage of total variance explained for *Pseudophilautus schneideri* and *P. folicola*.

	Axis 1	Axis 2
EN	0.989	0.090
HL	0.987	0.081
DBE	0.985	-0.049
SVL	0.978	0.038
FOL	0.973	-0.001
MFE	0.973	0.143
DFE	0.972	0.143
HW	0.971	0.113
ES	0.969	0.051
LAL	0.969	0.122
MN	0.961	0.107
IO	0.958	0.177
MBE	0.957	0.034
TBL	0.956	0.208
FEL	0.952	-0.109
TLV	0.934	0.088
TLI	0.928	0.068
FLIV	0.923	-0.003
IML	0.922	-0.070
TLII	0.919	-0.288
FLI	0.908	-0.185
ED	0.892	0.289
IN	0.891	0.118
FLII	0.839	-0.118
TLIII	0.837	-0.290
PAL	0.806	-0.446
TYE	0.794	0.215
TLIV	0.725	-0.103
FLIII	0.501	-0.588
NS	0.275	-0.802
Variance Explained by Components	24.344	1.762
Percent of Total Variance Explained	81.147	5.872

The Maximum Likelihood tree (from the Maximum Likelihood analysis) is shown in Fig. 3. The tree is rooted with two Indian taxa (*Pseudophilautus charius* and *P. signatus*) that represent the sister group to the Sri Lankan *Pseudophilautus* radiation (Meegaskumbura *et al*. 2002). For the Bayesian analysis, we ran 500,000 generations of the MCMCMC algorithm and the summed likelihood of the four chains reached stationarity by 85,000 generations. The posterior probabilities of clades shown at nodes in Fig. 3 represent the frequency of those clades in the 25,000 trees sampled from the last 250,000 generations, and clades with posterior probability of 50% or less were collapsed. The parameters of the nucleotide substitution model for the most likely tree were as follows. Rate matrix: R(G-T) = 0.0021, R(C-T) = 0.6815, R(C-G) = 0.0114, R(A-T) = 0.0308, R(A-G) = 0.2254, R(A-C) = 0.0486. Nucleotide frequency: A = 0.3243, C = 0. 0.2247, G = 0.1975, T = 0.2534. Rate variation: shape parameter for gamma distributed rate variation among sites (alpha) = 0.6458; proportion of invariant sites = 0.4020. The maximum likelihood tree found via a Tree Bisection and Reconnection branch-swapping heuristic search using the

above nucleotide substitution parameters in PAUP*v.4.0b10 had the same topology as the Bayesian tree, but had slight branch-length differences. A heuristic search using the Parsimony criterion, TBR branch swapping with 100 replicates with random taxon addition, and all characters unordered and weighted equally, gave two equally parsimonious trees with the maximum parsimony bootstrap values at nodes (bootstrap values less than 50 % collapsed, shown below nodes on ML tree; Fig. 3). Bootstrap values towards the base of the Sri Lankan radiation were low, which results in a basal polytomy. However, as expected, the values closer to the OTUs showed higher bootstrap values, and relationships of taxa within these better-supported clades were identical to those of the maximum likelihood analysis. The relationships of taxa of the clades from which the two new species are described were also identical to the relationships from the maximum likelihood analysis.

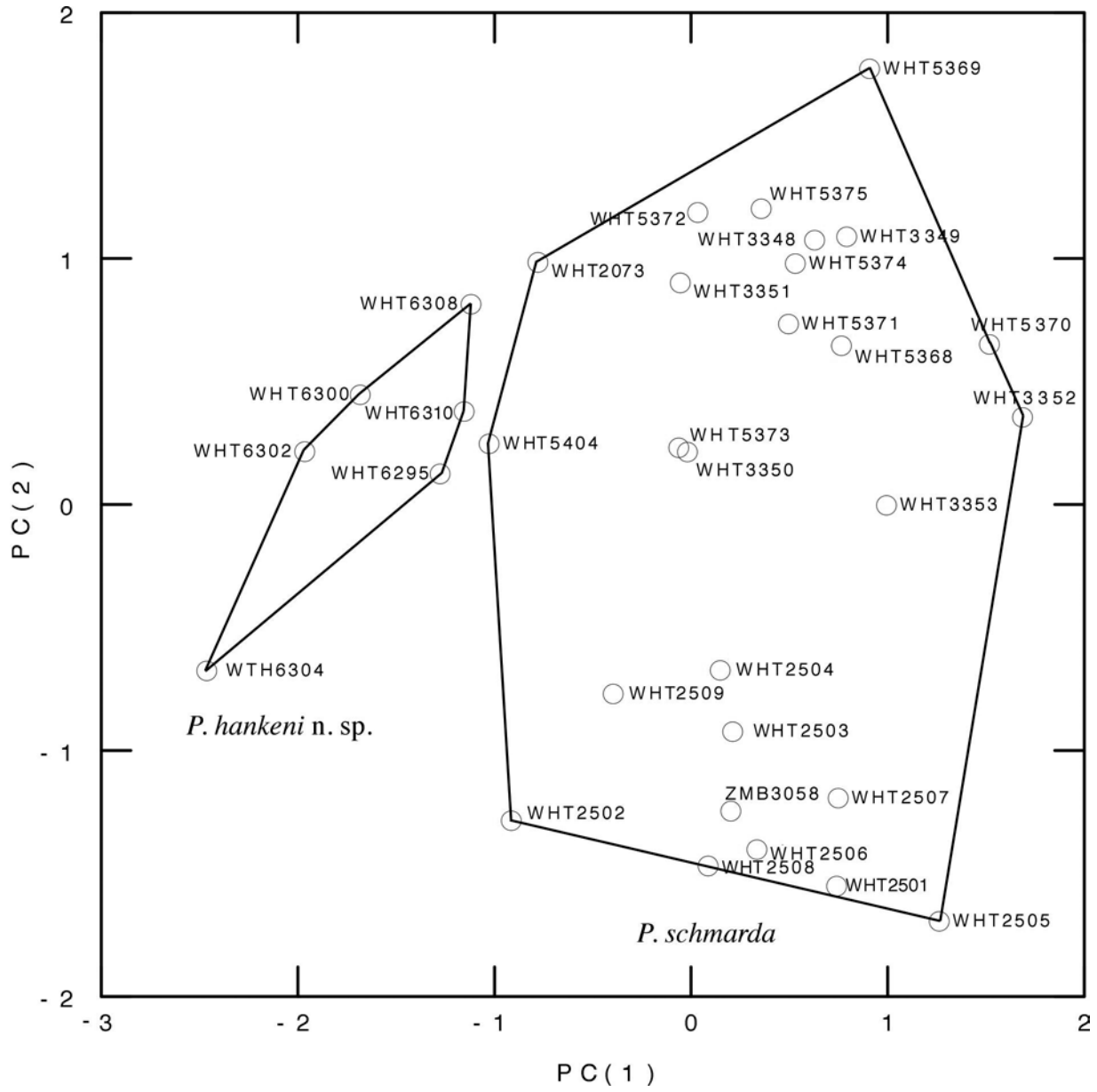

FIGURE 2. PC1 vs. PC2 factor scores of the principal components analysis of *Pseudophilautus hankeni,* **n. sp.** and *P. schmarda*, show these species to separate fairly well from each other in PC space. Most of the variation is explained by the PC1 axis, which relates mainly to body size (*P. hankeni* being smaller). The PC2 axis is mostly explained by NS, MBE and MFE (NS loads positively, and the other two negatively); here, *P. hankeni* overlaps completely with *P. schmarda*.

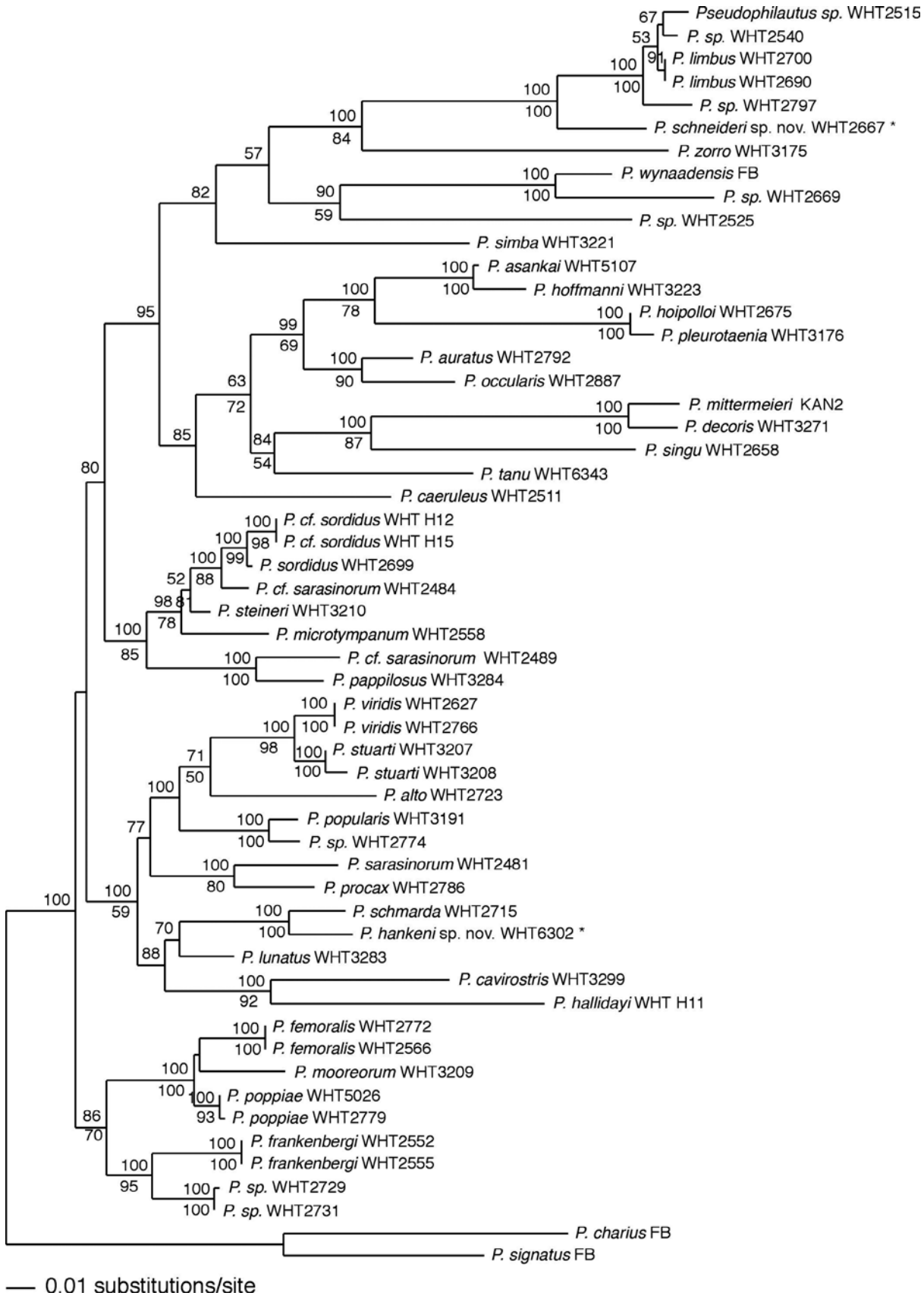

FIGURE 3. Maximum likelihood tree of 12s and 16s rRNA gene fragments, with posterior probabilities from the Bayesian analysis shown above nodes and Maximum Parsimony Bootstrap values shown below nodes. *Pseudophilautus schneideri* and *P. hankeni* are indicated by asterisks.

TABLE 3. Component loadings for axes 1 and 2 of the principal component analysis, variance explained and percentage of total variance explained for *Pseudophilautus hankeni* and *P. schmarda*.

	Axis 1	Axis 2
HL	0.979	-0.111
DBE	0.968	-0.085
MN	0.965	-0.230
ES	0.958	0.156
TBL	0.955	0.117
EN	0.942	0.210
FOL	0.937	0.250
HW	0.931	0.065
DFE	0.922	0.203
IN	0.917	0.137
SVL	0.915	0.004
ED	0.898	-0.308
MFE	0.874	-0.421
MBE	0.776	-0.477
NS	0.700	0.507
Variance Explained by Components	12.495	1.038
Percent of Total Variance Explained	83.195	6.922

***Pseudophilautus schneideri*, new species**
(Figs. 4–7)

Material examined. Holotype: mature male, 22.8 mm SVL, WHT6355, Kudawa, Sinharaja World Heritage Site, alt. 381 m (6°26'N, 80°25'E), coll. 9 June 1999; K.M.-A. & M.M.
Paratypes: 7, mature male, 19.9 mm SVL, WHT6354; mature male, 21.0 mm SVL, WHT6353; mature male, 21.0 mm SVL, WHT6357; mature male, 20.2 mm SVL, WHT6356; collection data same as holotype. Two mature males, 20.4 mm SVL, WHT6349; 20.7 mm SVL, WHT6350, Kanneliya Forest Reserve, alt. 41 m (6°15'N, 80°20'E), coll. 05 VI 1999, M.M. Mature female, 20.7 mm SVL, WHT2667, Elpitiya forest reserve, alt. 31 m (6°11'N, 80°11'E), coll. 14 September 1999, K.M.-A. & M.M.

Diagnosis. *Pseudophilautus schneideri* is assigned to the genus *Pseudophilautus* as they are well nested within the Sri Lankan monophyletic group (Figs. 1 & 2) of frogs (Meegaskumbura et al. 2002; Bossuyt et al. 2004; Yu *et al*. 2010) and are characterized by terrestrial direct development (Bossuyt and Dubois 2000). *Pseudophilautus schneideri* is distinguished from all other Sri Lankan congeners by a combination of the following characters: size small, mature individuals 19.9–22.8 (female 20.7 mm SVL) mm SVL; tympanum distinct; supratympanic fold distinct; canthal edges sharp; vomerine ridge absent or present; throat, chest and belly granular.

Description. (Based on Holotype WHT6355) Body elongate. Head laterally convex. Snout obtusely pointed in dorsal view, pointed in lateral view. Canthal edges sharp. Loreal region flat. Interorbital space convex. Internasal space flat. Nostrils oval. Pupil oval, horizontal. Tympanum distinct, oval, vertical. Pineal ocellus absent. Vomerine ridge present only on right side, bearing small teeth, between, anterior to and proximal to choanae, angled at about 45° to body axis. Tongue moderate, emarginate, not bearing a lingual papilla. Supratympanic fold distinct. Cephalic ridges absent. Co-ossified skin on head absent. Both upper and lower arms short. Fingers thin. Relative length of fingers, 1 < 2 < 4 < 3. Tips of fingers with discs, with circum-marginal grooves. Fingers lack a lateral dermal fringe. Webbing on fingers absent. Subarticular tubercles on fingers distinct, oval, single, some absent IV 2 (penultimate subarticular tubercle). Prepollex oval, distinct. Two palmar tubercles, oval, distinct. Supernumerary tubercles absent. Thigh slender. Shank slender. Toes thin. Relative length of toes, 1 < 2 < 3 < 5 < 4. Tips of toes with discs, with circum-marginal groves. Webbing present on toes. Subarticular tubercles on toes distinct, oval, sin-

gle, all present. Inner metatarsal tubercle distinct, oval. Outer metatarsal tubercle absent. Tarsal fold absent. Supernumerary tubercles present on toes and foot. Tarsal tubercle absent. Small tubercles with horny spinules on dorsal and lateral parts of head and body and dorsal side of flank. Lower flank granular. Dorsolateral fold absent. Dorsal and lateral parts of upper arm, lower arm, thigh, shank and foot smooth. Throat, chest and belly granular, underside of thigh smooth. Nuptial pad present on inner edge of 1st finger and on prepollex, creamy yellow, oval. Vocal sacs and internal vocal slits present.

FIGURE 4. *Pseudophilautus schneideri* (WHT 6127), in life, Sinharaja World Heritage Site.

Coloration in life. Head dorsally light brown, laterally dark brown. Supratympanic area, upper tympanum and interorbital area black. Mid-dorsum dark brown with tiny black and dark brown spots. Both upper and lower areas of flank pale brown with dark-brown spots. Inguinal area yellowish brown with dark-brown spots. Dorsal and lateral parts of limbs pale brown with dark-brown spots. Lower arm with 1, thigh with 3 and shank with 3 dark-brown crossbars. Fingers and toes pale yellowish brown with dark-brown pigments. Outer edge of ventral side of foot dark brown. Ventral side of foot pale yellowish brown with dark-brown patches. Throat, margins of throat, chest, belly, underside of thigh and webbing pale yellowish with dark-brown pigments.

Coloration in alcohol (description based on holotype, WHT6355). Snout dorsally pale brown, laterally dark brown. Supratympanic area, middle of tympanum and interorbital area dark brown. Mid-dorsum dark brown with tiny dark spots. Both upper and lower areas of flank pale brown with dark-brown spots. Inguinal zone yellowish pale brown. Both upper and lower lips dark brown. Dorsal and lateral parts of limbs pale brown with dark-brown spots. Lower arm with 1, thigh with 1 and shank with 2 dark-brown cross-bars. Fingers and toes pale yellowish brown with dark-brown pigments. Outer edge of ventral side of foot dark brown. Ventral side of foot pale yellowish brown with dark-brown patches. Throat, margins of throat, chest, belly, underside of thigh and webbing pale yellowish with dark-brown pigments. Paratype, WHT6354: A thin yellow line on mid dorsum from tip of snout to vent, on mid-thigh, on mid-flank and rear edge of foot.

Measurements of holotype (WHT6355, in mm): DBE, 8.2; DFE, 5.0; DL, 1.0; DW, 1.2; ED, 3.4; EN, 2.6; ES, 4.1; FEL, 10.7; FL I, 1.8; FL II, 2.3; FL III, 3.4; FL IV, 3.1; FOL, 14.4; HL, 9.7; HW, 8.9; IML, 1.0; IN, 2.3; IO, 2.3; LAL, 4.5; MBE, 2.9; MFE, 6.0; MN, 9.2; NS, 1.6; PAL, 6.6; SVL, 22.8; TBL, 11.4; TL I, 1.6; TL II, 2.0; TL III, 3.2; TL IV, 4.3; TL V, 3.4; TYD, 0.5; TYE, 1.5; UAW, 3.6; UEW, 2.3.

Etymology. The species name is a patronym in the genitive singular in honour of the evolutionary biologist and herpetologist Professor Christopher J. Schneider (Department of Biology, Boston University, USA).

Remarks. Morphologically, *P. schneideri* resembles *P. folicola*. It is distinguished from the latter, however, by possessing an obtusely pointed snout in lateral aspect (vs. snout rounded or truncate in lateral aspect); having the loreal region flat (vs. concave); lacking (vs. possessing) a lateral dermal fringe on fingers; lacking (vs. possessing) supernumerary tubercles on the palm; and having the underside of the thigh smooth (vs. granular).

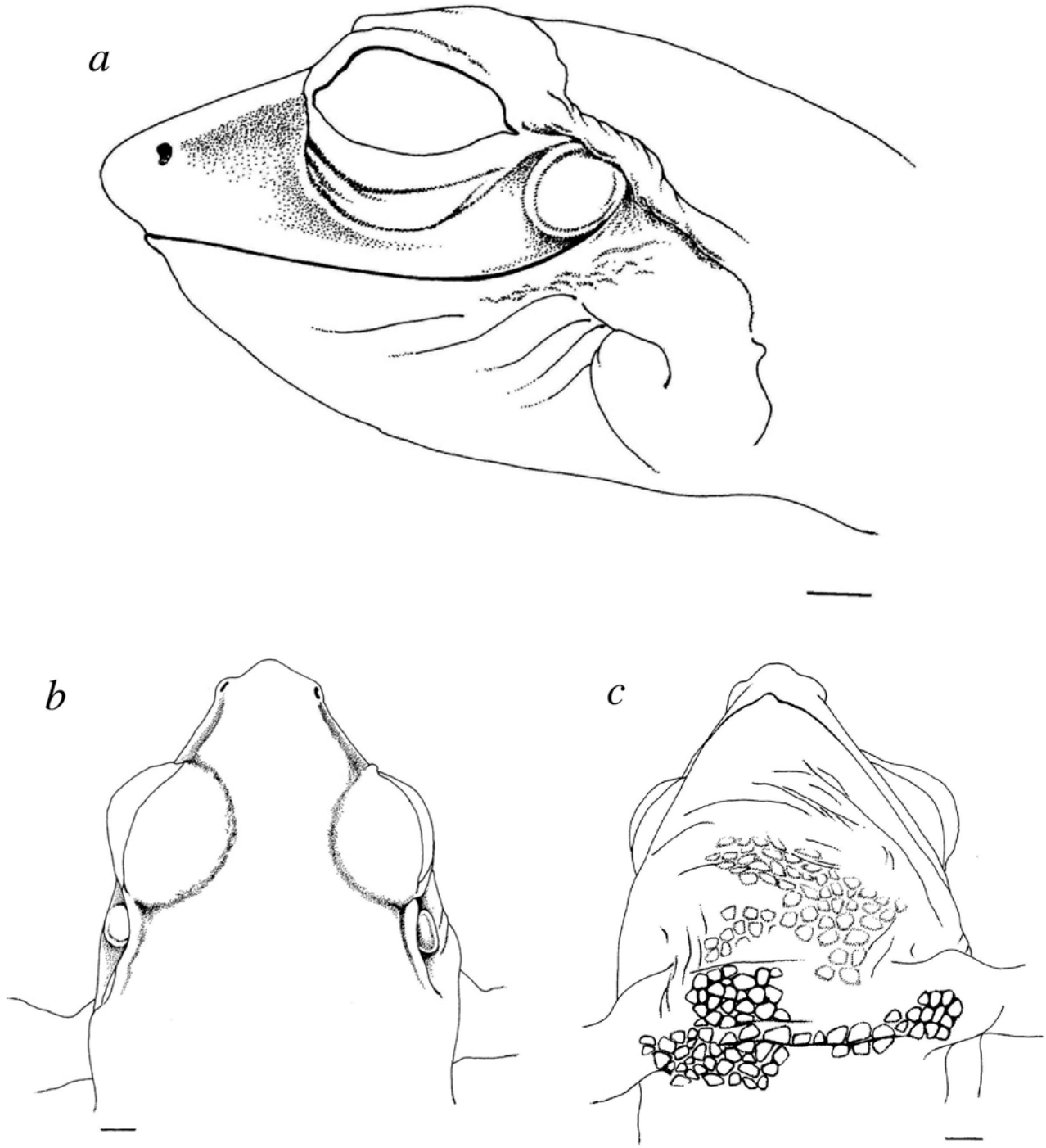

FIGURE 5. *Pseudophilautus schneideri* **n. sp.**: *a*, lateral; *b*, dorsal; and *c*, ventral aspects, respectively, of head of holotype, male, WHT 6355, 22.8 mm SVL. Scale bar: 1 mm.

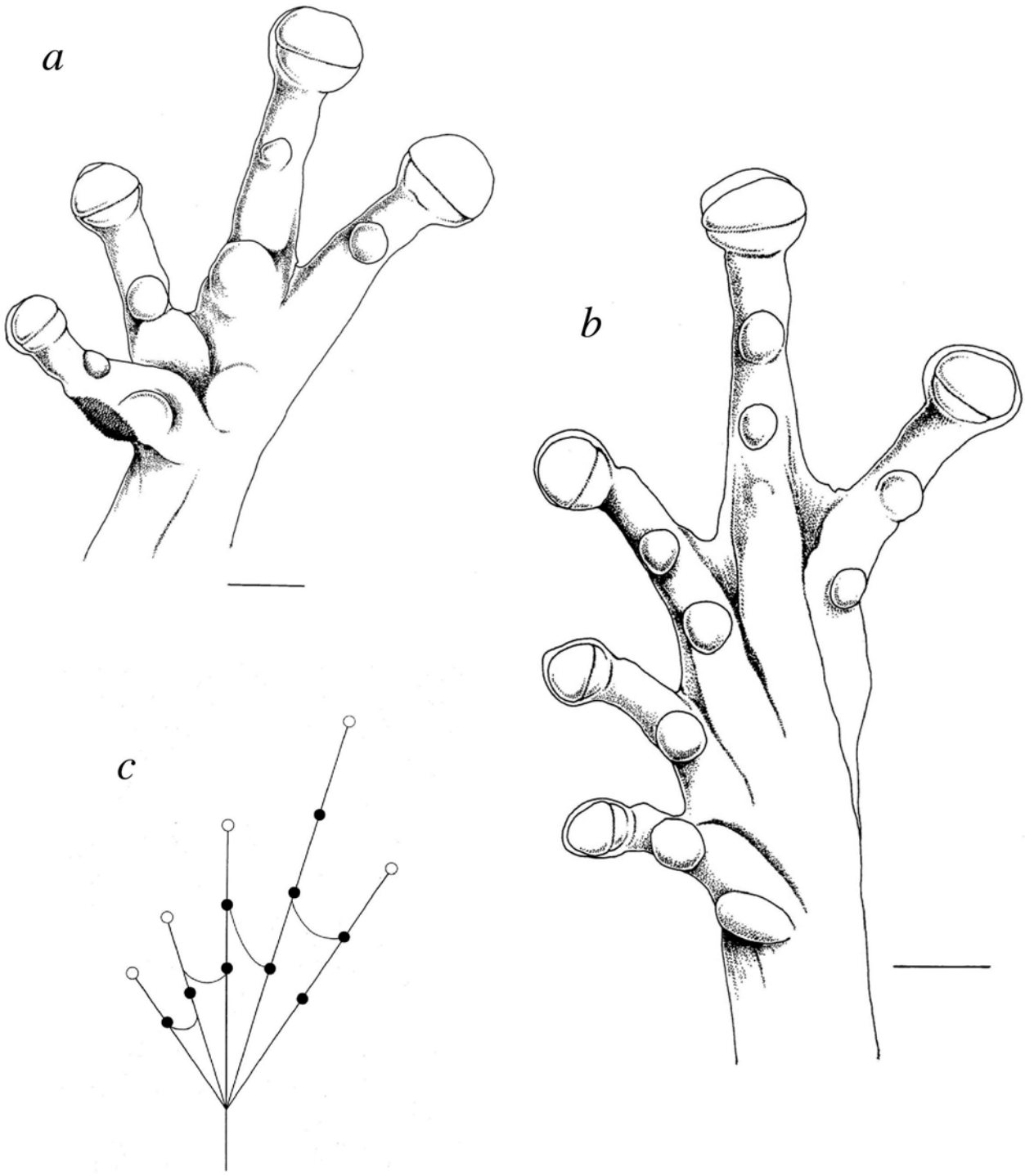

FIGURE 6. *Pseudophilautus schneideri* **n. sp.**: *a*, ventral aspect of left manus; *b*, ventral aspect of left pes; and *c*, semi-diagrammatic representation of the left-pes webbing pattern of the holotype, male,WHT 6355, 22.8 mm SVL. Scale bar: 1 mm.

Distribution. We observed males of *P. schneideri* perched on leaves of shrubs, 0.5–1.0 m above ground in open habitats (the species has hitherto not been observed in closed-canopy rainforest). Individuals were observed also in forest edges and in anthropogenic habitats such as home gardens and tea plantations. Most individuals were observed in the undergrowth of neglected tea plantations with extensive weedy undergrowth adjacent to rainforest (the species is apparently absent in plantations from which the undergrowth has been cleared. Considering its current distribution, however, the extent of available habitat for *P. schneideri* is relatively high: it may indeed have a much wider distribution than recorded here. Given that this species uses tea plantations as a surrogate for scrubland, intensive agro-chemical usage in this habitat may affect these populations.

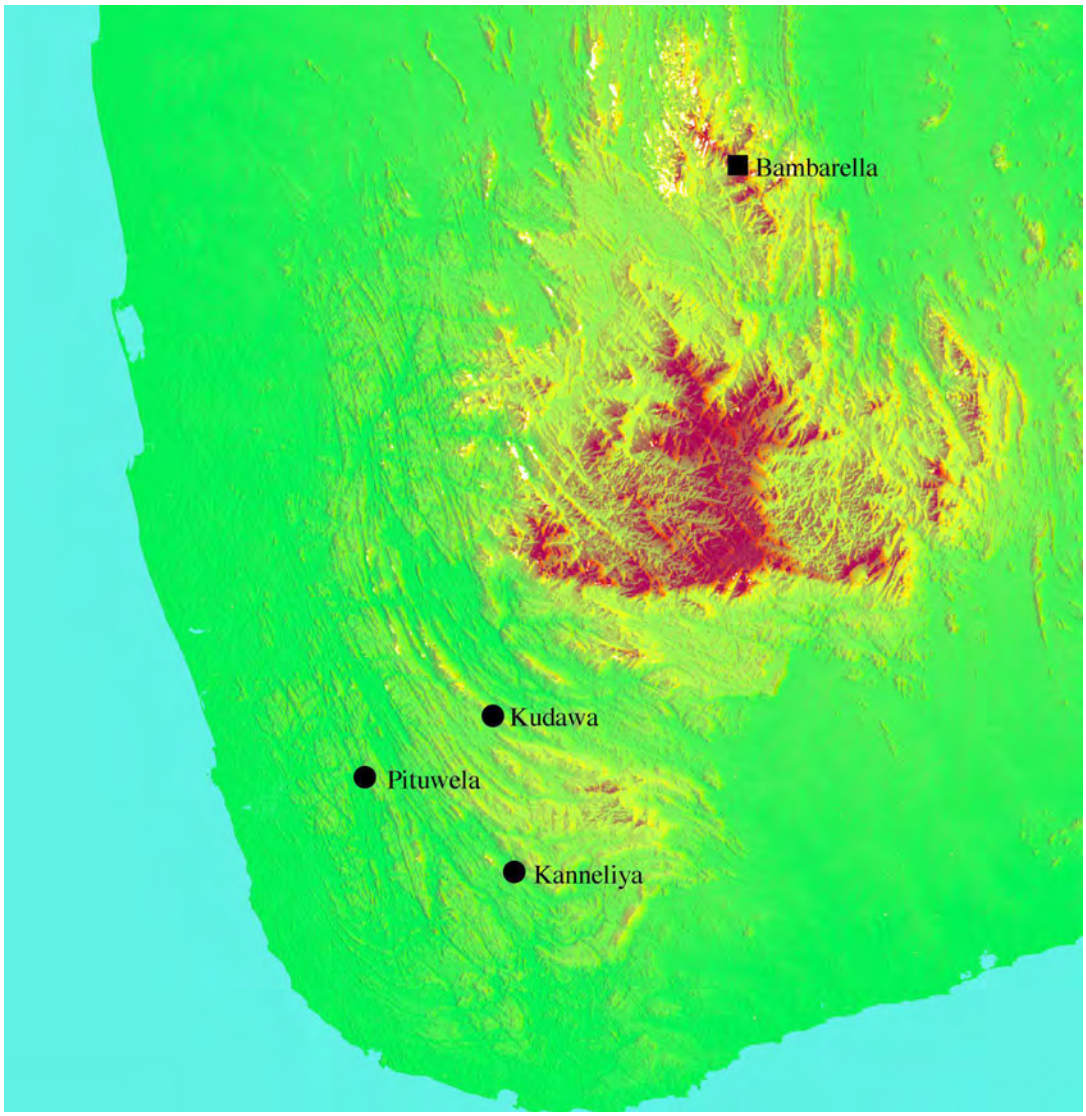

FIGURE 7. Distribution of *Pseudophilautus schneideri*, **n. sp.** (circles) and *Pseudophilautus hankeni*, **n. sp.**, (squares), in Sri Lanka.

***Pseudophilautus hankeni*, new species**
(Figs. 7–10)

Material examined. Holotype: mature male, 21.9 mm SVL, WHT6304, Bambarella, Knuckles, alt. 1490 m (7°26'N, 80°46'E), M.M., coll. 23 V 1999.
Paratypes: mature males, 19.0 mm SVL, WHT6310; 19.6 mm SVL, WHT6300; 20.7 mm SVL, WHT6302; 20.0 mm SVL, WHT6298; 21.4 mm SVL, WHT6295; 18.3 mm SVL, WHT6308; Bambarella, Knuckles, alt. 1600 m (7° 25'N, 80° 47'E), coll. 23 V 1999, M.M..

Diagnosis. *Philatus hankeni* is distinguished from all Sri Lankan congeners by the combination of the following characters: size small, mature individuals 18.3–21.9 mm SVL; tympanum distinct; canthal edges rounded; vomerine ridge absent; outer edge of lower arm and outer edge of foot with a tuberculated dermal fold; throat, chest and belly granular. (For diagnosis from its sister species, *P. schmarda*, see Remarks, below.)

Description. (based on holotype, WHT6304, and six paratypes, WHT6310, WHT6300, WHT6302, WHT6298, WHT6295, WHT6308). *Pseudophilautus hankeni* is assigned to the genus *Pseudophilautus* as they are well nested (Figs. 1 & 2) within the Sri Lankan monophyletic group of frogs (Meegaskumbura et al. 2002; Bossuyt et al. 2004) and are characterized by terrestrial direct development (Bossuyt and Dubois 2000). Body stout. Head

convex in lateral view. Snout rounded in dorsal view, pointed in lateral view. Canthal edges rounded. Loreal region concave. Interorbital space convex. Internasal space concave. Nostrils oval.

Pupil oval, horizontal. Tympanum distinct, oval, oblique, its outer rim indistinct. Pineal ocellus absent. Vomerine ridge absent. Tongue moderate, emarginate, not bearing a lingual papilla. Supratympanic fold distinct. Cephalic ridges absent. Co-ossified skin on head absent. Upper arm short, lower arm short, strong. Fingers thin. Relative length of fingers, 1 < 2 < 4 < 3. Tips of fingers with discs bearing circum-marginal grooves. Fingers without lateral dermal fringe. Outer edge of 4th finger and outer edge of lower arm with tuberculated dermal fold. Webbing on fingers absent. Subarticular tubercles on fingers prominent, oval, single, some absent IV 2 (penultimate subarticular tubercle). Prepollex oval, distinct. Two palmar tubercles, oval, distinct. Supernumerary tubercles present on palm. Thigh slender. Shank slender. Toes strong. Relative length of toes, 1 < 2 < 3 < 5 < 4. Tips of toes with discs, with circum marginal groves. Webbing present on toes (absent on toe I). Subarticular tubercles on toes prominent, oval, single, some absent IV 2. Inner metatarsal tubercle distinct, oval. Tarsal fold present. Outer metatarsal tubercle absent. Outer edge of 5th toe and outer edge of foot with a tuberculated dermal fold. Supernumerary tubercles present on foot. Tarsal tubercle present. Dorsal and lateral parts of head and body and upper part of flank with glandular warts bearing horny spinules. Lower part of flank granular. Dorsolateral fold absent. Dorsal and lateral parts of upper arm, lower arm, thigh, shank and foot with glandular warts with horny spinules. Nuptial pad absent, but pale yellow subdermal glands on inner edge of 1st finger and on prepollex. Throat, chest and belly granular. Underside of thigh and shank smooth. Vocal sacs and internal vocal slits present.

Coloration in life. Dorsal and lateral parts of head brown with symmetrical darker brown patches. Upper areas of eyelids and posterior area of interorbital with distinct black patches. Upper flank grayish brown, lower flank pale yellow-gray. Inguinal zone pale yellow with dark-brown patches. Loreal region brown. Tympanic region and tympanum brown with small darker brown patches. Both upper and lower lips pale brown. Dorsal and lateral parts of limbs brown. Lower arm with 2, thigh with 2, shank with 3 and foot with 4 wide, brown patches. Throat, margins of throat, chest and belly pale yellow or white. Underside of thigh and webbing pale yellow. Fingers and toes dorsally light brown.

FIGURE 8. *Pseudophilautus hankeni* (WHT 6295), in life, Bambarella.

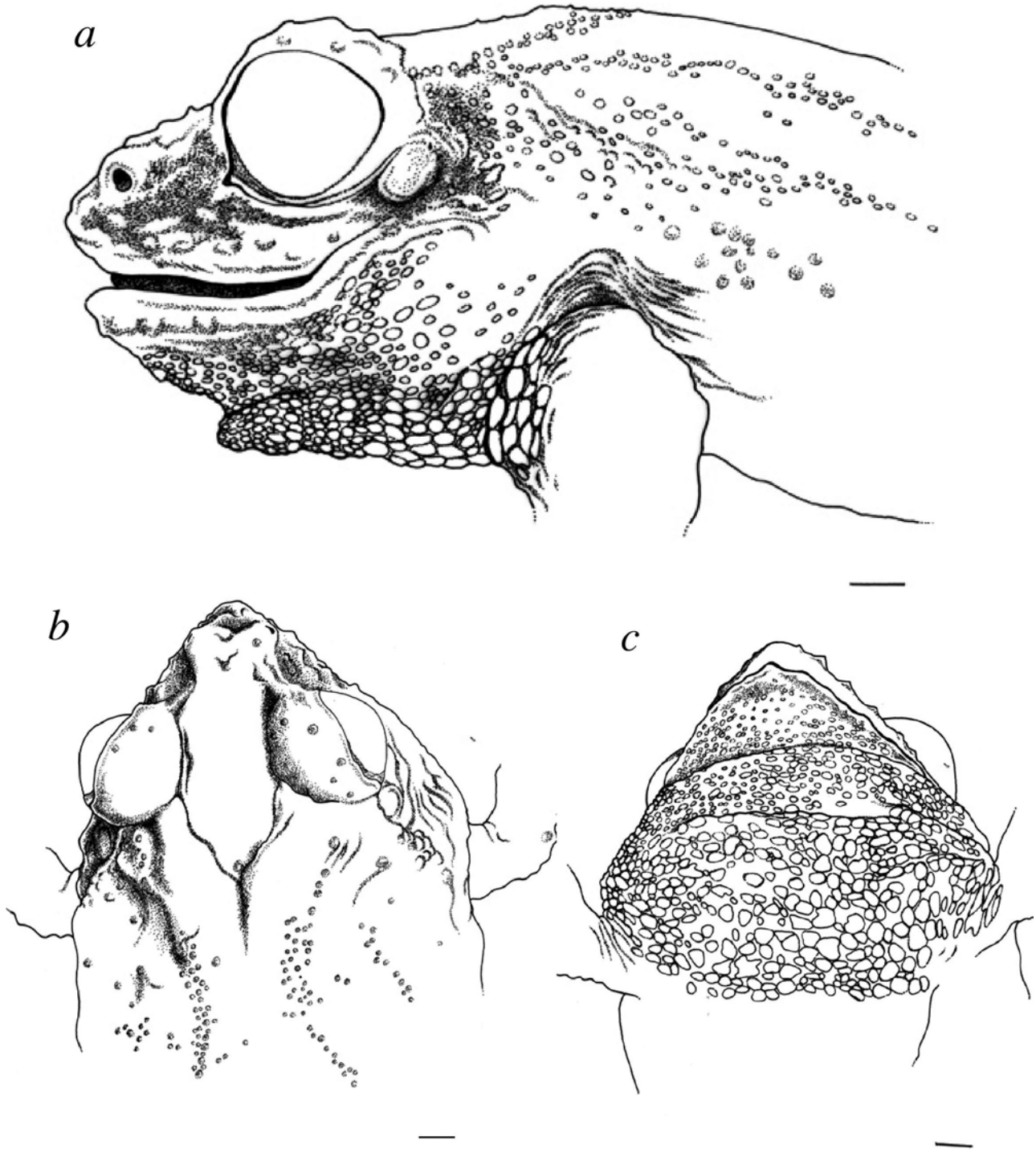

FIGURE 9. *Pseudophilautus hankeni* **n. sp.**: *a*, lateral; *b*, dorsal; and *c*, ventral aspects, respectively, of head of holotype, male, WHT 6304, 21.9 mm SVL. Scale bar: 1 mm.

Coloration in alcohol (based on holotype, WHT6304). Dorsal and lateral parts of head grayish brown with symmetrical brown patches. Upper areas of eyelids and posterior area of interorbital with distinct black patches. Upper part of flank grayish brown, lower part pale gray. Inguinal zone white with dark-brown patches. Loreal region gray. Tympanic region and tympanum grayish brown. Both upper and lower lips pale gray. Dorsal and lateral parts of limbs gray. Lower arm with 2, thigh with 2, shank with 3 and foot with 4 wide brown patches. Throat, margins of throat, chest and belly pale gray or white. Underside of thigh and webbing pale yellow. Fingers and toes dorsally pale gray.

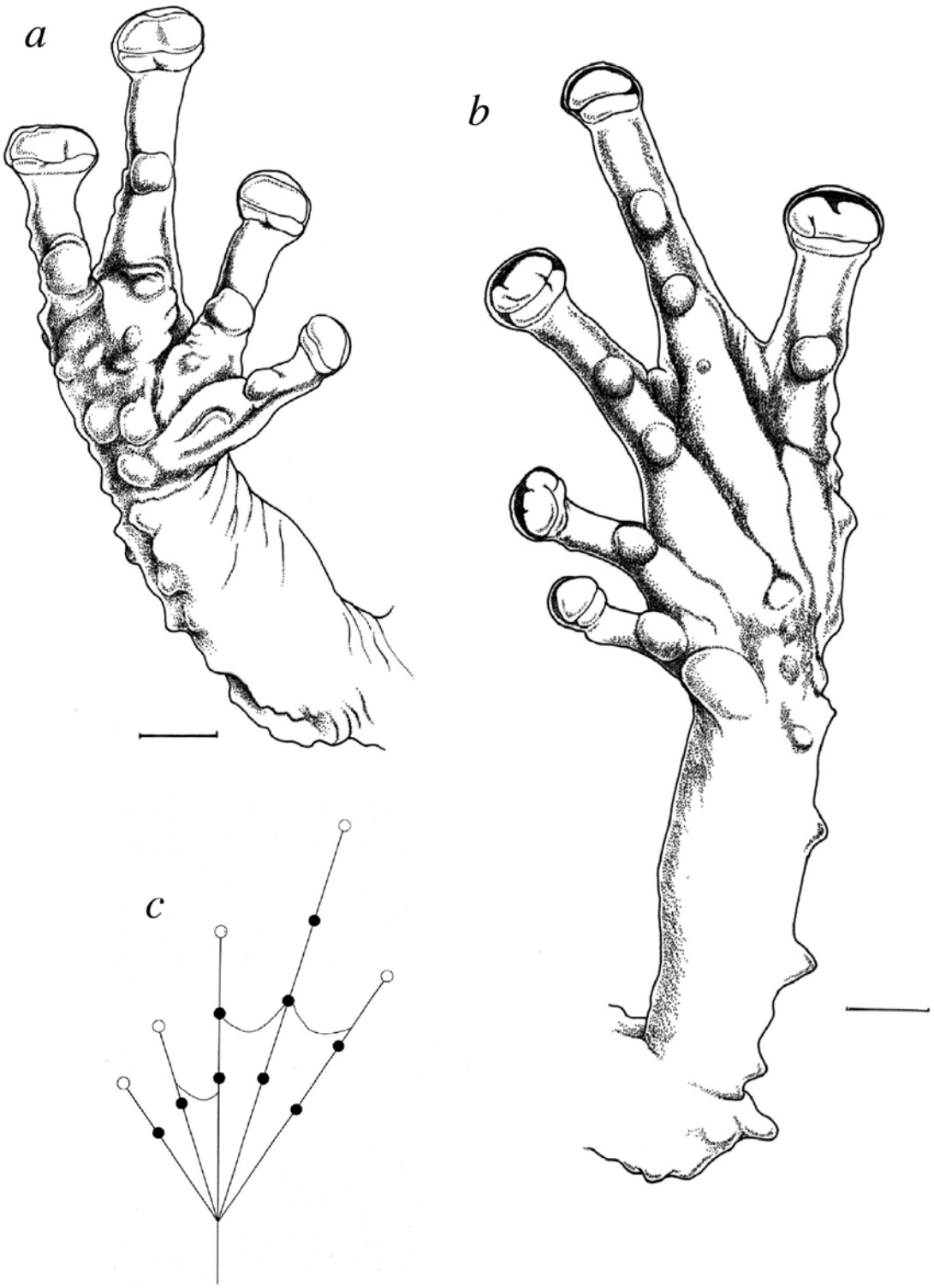

FIGURE 10. *Pseudophilautus hankeni* **n. sp.**: *a*, ventral aspect of left manus; *b*, ventral aspect of left pes; and *c*, semi-diagrammatic representation of the left-pes webbing pattern of the holotype, male, WHT 6304, 21.9 mm SVL. Scale bar: 1 mm.

Measurements of holotype (WHT6304, in mm): DBE, 7.4; DFE, 4.6; DL, 0.8; DW, 1.3; ED, 2.7; EN, 2.2; ES, 3.8; FEL, 9.6; FL I, 1.6; FL II, 2.2; FL III, 4.0; FL IV, 3.0; FOL, 13.1; HL, 8.4; HW, 8.5; IML, 0.9; IN, 2.0; IO, 2.1; LAL, 3.9; MBE, 2.8; MFE, 5.2; MN, 7.3; NS, 1.2; PAL, 6.4; SVL, 21.9; TBL, 10.1; TL I, 1.6; TL II, 2.0; TL III, 3.2; TL IV, 4.8; TL V, 3.3; TYD, 0.6; TYE, 1.1; UAW, 4.2; UEW, 1.8.

Etymology. The species name honors the developmental biologist, director of the Museum of Comparative Zoology (Harvard University, USA), and Alexander Agassiz Professor of Zoology, James Hanken. It is Latinized as a noun in the genitive singular case.

Remarks. *Pseudophilautus hankeni* resembles *P. schmarda* morphologically, but it can be distinguished from the latter species as follows: canthal edges rounded (vs. sharp in *P. schmarda*); toes with (vs. without) a lateral dermal fringe; rudimentary webbing present (vs. absent) on toes; and presence (vs. absence) of a subdermal nuptial pad.

Distribution. *Pseudophilautus hankeni* shows a very restricted distribution, being known hitherto only from its type locality at Bambarella and Riverston regions in Knuckles. The species seems to be restricted to the highest elevations of the Knuckles mountains (peaks over 1200 m elevation). It was observed on the leaves of shrubs (0.3–1.0 m above ground) under the cover of the montane cloud forest canopy. Given its restricted, high-elevation habitat, it may be at risk from climate warming, further encroachments into the forest from lower elevations, and agrochemical usage in nearby tea plantations.

Discussion

Given its extremely restricted range and specialized montane-forest habitat, *P. hankeni* is clearly at a high risk of extinction from habitat loss and change. Meegaskumbura & Manamendra-Archchi (2005) highlighted and discussed the conservation implications of six such *Pseudophilautus* species with similar upper montane distributions. *Pseudophilautus hankeni* also faces these same threats, such as further restriction of habitat due to future global warming, and given that it already occurs on the highest peaks—the unavailability of yet higher and cooler habitats. *Pseudophilautus schmarda,* the sister species of *P. hankeni,* lives on high elevation mountains of the island's Central Hills. The two species, however, are separated from each other by the deep (and therefore warmer) valley of the Mahaweli River, which apparently forms a barrier either is unlikely to cross. The available area of forest for the Endangered *P. schmarda*, however, is much greater than that for *P. hankeni,* making the latter more vulnerable to extinction.

Pseudophilautus schneideri is a forest-edge species, so far found only from the undergrowth of tea estates adjacent to secondary forests and also at night perched on shrubs along open trails. Because it has not been observed more than 100m away from the forests, it probably depends on the natural forest for increased humidity, lower temperature and other biological resources such as food species. However, given this frog's tolerance of secondary habitats, given suitable conditions, it may be able to traverse anthropogenic habitats and plantations. Future exploration is likely to show that the range of this species is substantially larger than is recorded at present.

Mascaro *et al*. (2008), show that native animals of a region could survive sometimes even in non-pristine environments. Such species may not be new recruits to the environment, but survivors ("relicts") of a past destruction of their original habitat. Clearly, this seems to be the case also for some of the Sri Lankan shrub frogs such as *P. schneideri* and *P. tanu*; but given increased risk, such as noted above, that these species face in a non-native habitat such as tea plantations, management and education of the public could play an important role in their conservation.

Pseudophilautus hankeni and *P. schmarda* are sister species (Figs. 3) but are separated from each other by substantial genetic distances. The combined 12s and 16s uncorrected percent genetic distance between *P. schamarda* and *P. hankeni* is 2.43, and for cytochrome-*b* it is 8.44. The closest species to WHT2667 (*P. schneideri*) are *P. zorro* and *P. limbus*; uncorrected percent genetic distance for the combined 12s and 16s gene fragment for *P. schneideri* and these species are 3.5–4.0; for cytochrome-*b* it is 15.9–21.8. These genetic distances are well over the 2% cytochrome-*b* genetic distance that indicates species-level divergence in several groups of mammals (Bradley & Baker 2001). Johns & Avise (1998) also indicate that 90% of the putative sister species across a wide range of vertebrate taxa showed more than 2% divergence in the cytochrome-*b* gene, adding confidence to our recognition of these taxa at the species level. The 16s rRNA gene has been recognized as a suitable barcoding gene for amphibians (Vences *et al*. 2005); for instance in Mantellidae, a wide range of inter-species genetic distances, ranging from 1–16.5%, has been recorded (Vences *et al*. 2005), and a 3% divergence has been proposed as a species-level threshold (Fouquet *et al*. 2007).

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