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Two new species of shrub frogs (Rhacophoridae: *Philautus*) from the lowlands of Sri Lanka

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Abstract

Two new species of Sri Lankan frogs of the genus *Philautus* are described. Species diagnoses are based on morphology, morphometrics and mitochondrial DNA sequence data. *Philautus tanu* sp. nov. inhabits shrubs in open areas of the lowland wet zone, while *P. singu* sp. nov. is found on shrubs in the understory of lowland and mid-elevation rainforests. These descriptions bring the total number of valid Sri Lankan *Philautus* to 65 species, of which 46 are extant.

Key words: Rhacophorinae, taxonomy, molecular systematics, new species, conservation

Introduction

Following the discovery in Sri Lanka of a large radiation of Oriental tree-frogs of the genus *Philautus* (Meegaskumbura *et al.* 2002), 37 new species have up to now 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). The review and description of 27 new species by Manamendra-Arachchi and Pethiyagoda (2005) though informed by a phylogeny, was based purely on morphology (given the unavailability of molecular data for historical type material). However, Meegaskumbura & Manamendra-Arachchi (2005) described eight additional new species using the General Lineage concept (de Quieroz, 1998), according to which species are regarded as independent evolutionary lineages based on multiple criteria, such as genetic divergence, morphology, ecology and vocalization. Meegaskumbura *et al*. (2007) added two new but extinct species discovered in historical museum collections, again adopting a purely morphological approach. The island's inventory of *Philautus* now stands at 63 species, of which 44 are extant. Surveys in Sri Lanka since the early 1990s have shown that 19 of these, known today only from museum specimens collected in the 19th and early 20th centuries, have since disappeared (Manamendra-Arachchi & Pethiyagoda 2005; Meegaskumbura *et al.* 2007).

Here we continue to document the new species discovered in Sri Lanka as a result of exploratory work, based on morphological, morphometric and molecular data, in the context of the General Lineage concept of species.

Materials and methods

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

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Morphological analysis. The suite of characters and character states used by Manamendra-Arachchi & Pethiyagoda (2005) was analysed for all individuals. Measurements were made to the nearest 0.1 mm using dial vernier calipers. These were 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); and 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 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 and TBL.

Principal components analysis of the character correlation matrix was used to reduce dimensionality of the continuous morphological variables and to identify those variables that best discriminate among morphologically similar species (*P. singu* and *P. tanu* were compared to *P. decoris* and *P. mittermeieri*). 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. SYSTAT (Version 11.00.01 for Windows XP) was used for statistical analysis.

Molecular analysis. One of the species described here is included in the phylogenetic analysis of Meegaskumbura *et al*. (2002) and Meegakumbura & Manamendra-Arachchi (2005; reference number WHT 2658). A further species (WHT 6343) was added to the latter phylogeny. Only 12s rRNA and 16s rRNA partial sequences were used to construct the phylogenetic tree, as was done in the above-mentioned works (see Table 1 for details on species and their genbank accession numbers). Cytochrome-*b* data were used, in addition, to determine the percentage divergences among sister taxa, and PCR amplification and alignment of sequences were done as explained in Meegaskumbura & Manamendra-Arachchi (2005).

Data were analyzed using Bayesian, Maximum Likelihood (ML) and Maximum Parsimony (MP) criteria. Here, 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 the 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 1075 were recorded. A bootstrap analysis (1000 replicates, random stepwise addition with 100 repetitions) to determine node support was also carried out within a maximum-parsimony framework.

Once we identified the divergent mtDNA lineages and their sister taxa using the 12S and 16S rRNA gene tree, to facilitate comparisons with published summaries of mitochondrial divergence among vertebrate sister species (Johns & Avise 1998), we sequenced a fragment of the mitochondrial cytochrome-*b* gene from the

species described herein and their sister species (Meegaskumbura & Manamendara-Arachchi 2005). A ~ 590 base-pair fragment was amplified using primers CB-J-10933, (5'- TATGTTCTACCATGAGGACAAATATC-3') and BSF4 (5'- CTTCTACTGGTTGTCCTCCGATTCA-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).

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. *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. 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. ocullaris	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

to be continued.

TABLE 1. (continued)

Species	Reference number	Genbank Accession numbers	
		12s	16s
P. schmarda	WHT2715	AY141780	AY141826
P. signatus	FB	AY141795	AY141841
P. simba	WHT3221	FJ788148	FJ788167
P. singu	WHT2658	AY141773	AY141819
P. sordidus	WHT2699	AY141778	AY141824
P. sp.	WHT2515	AY141765	AY141811
P. sp.	WHT2540	AY141767	AY141813
P. sp.	WHT2797	AY141790	AY141836
P. sp.	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

Results

Morphometric analysis. *Philautus tanu*, *P. singu*, *P. mittermeieri* and *P. decoris* separate distinctly from each other in morphological space (Fig. 1, Table 2). Principal components analysis shows that the four species are distinguished by a combination of body size, finger lengths, and head dimensions. The PC(1) axis, which explains 82 % of the variance, is a size axis (SVL loads most heavily and FLII least heavily, but all variables have high, positive loadings on this axis; component loadings range from 0.879–0.765, suggesting that the variation relates mostly to size). The PC(2) axis represents 8 % of the variance, with FLII (-0.570), FLIII (-0.505), IO (0.359), TLII (-0.377), ES (0.359), FLI (-0.353), MBE (0.321), and ED (0.311) loading most heavily. Three of the four species separate well on the PC(1) axis, *P. decoris* being the largest and *P. tanu* being the smallest (*P. singu* and *P. mittermieri* overlap on this axis). *Philautus singu* separates from all other species on the PC2 axis, while *P. tanu* separates from *P. mittermieri*, but not *P. decoris*, on this axis.

Molecular phylogenetics. The final dataset contains 12S and 16S rRNA mitochondrial gene sequences from 54 putative species, 53 from the dataset analyzed by Meegaskumbura and Manamendra-Arachchi (2005); plus one additional species. Fifty-one of these represent Sri Lankan *Philautus*, 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 *Philautus*: see Fig. 2 in Meegaskumbura *et al.* 2002; Bossuyt et al. 2004). Out of the 939 nucleotide positions sequenced, 867 were clearly allignable 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 *Philautus tanu, P. singu, P. mittermeieri* and *P. decoris.*

	Axis 1	Axis 2
SVL	0.979	0.060
FEL	0.976	-0.056
HW	0.975	0.142
FOL	0.969	-0.118
EN	0.964	0.136
DBE	0.963	0.173
LAL	0.960	0.028
HL	0.957	0.212
MN	0.955	0.257
TBL	0.949	-0.041
PAL	0.942	-0.223
DFE	0.941	0.261
TLIII	0.938	-0.275
MFE	0.933	0.278
TLIV	0.918	-0.151
FLIV	0.908	-0.275
MBE	0.890	0.321
DW	0.885	-0.106
FLI	0.872	-0.353
TLII	0.870	-0.377
TLV	0.867	-0.216
ES	0.849	0.359
IO	0.824	0.490
IN	0.810	0.111
ED	0.796	0.311
FLIII	0.770	-0.505
FLII	0.765	-0.570
Variance explained by components	22.209	2.058
Percent of total variance explained	82.254	7.622

The Maximum Likelihood tree (from the Maximum Likelihood analysis) is rooted with two Indian taxa (*Philautus charius* and *P. signatus*) that represent the sister group to the Sri Lankan *Philautus* radiation (Meegaskumbura et al. 2002; Fig. 2). 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. Posterior probabilities of clades shown at nodes in Fig. 2 represent the frequency of those clades in the 25,000 trees sampled from the last 250,000 generations; clades with posterior probability of 50% or less were collapsed. Parameters of the nucleotide substitution model for the most likely tree are as follows. Rate matrix: R(G-T), 0.0078; R(C-T), 0.6649; R(C-G), 0.0152; R(A-T), 0.0368; R(A-G), 0.2356; R(A-C), 0.0395. Nucleotide frequency: A, 0.3493; C, 0. 0.2204; G, 0.1906; T, 0.2394. Rate variation: shape parameter for gamma distributed rate variation among sites (alpha) = 0.745; proportion of invariant sites = 0.382. 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 has the same topology as the Bayesian tree, but

has slightly different branch-lengths. 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. One of these is shown (Fig. 3) with the maximum parsimony bootstrap values at nodes (with nodes having bootstrap values less than fifty percent collapsed). Bootstrap values towards the base of the Sri Lankan radiation are low, which results in a basal polytomy. However, as expected, values closer to the OTUs show higher bootstrap values, and relationships of taxa within these better-supported clades are identical to those of the maximum likelihood analysis. The relationships of taxa of the clade from which the two new species are described are also identical to the relationships from the maximum likelihood analysis.

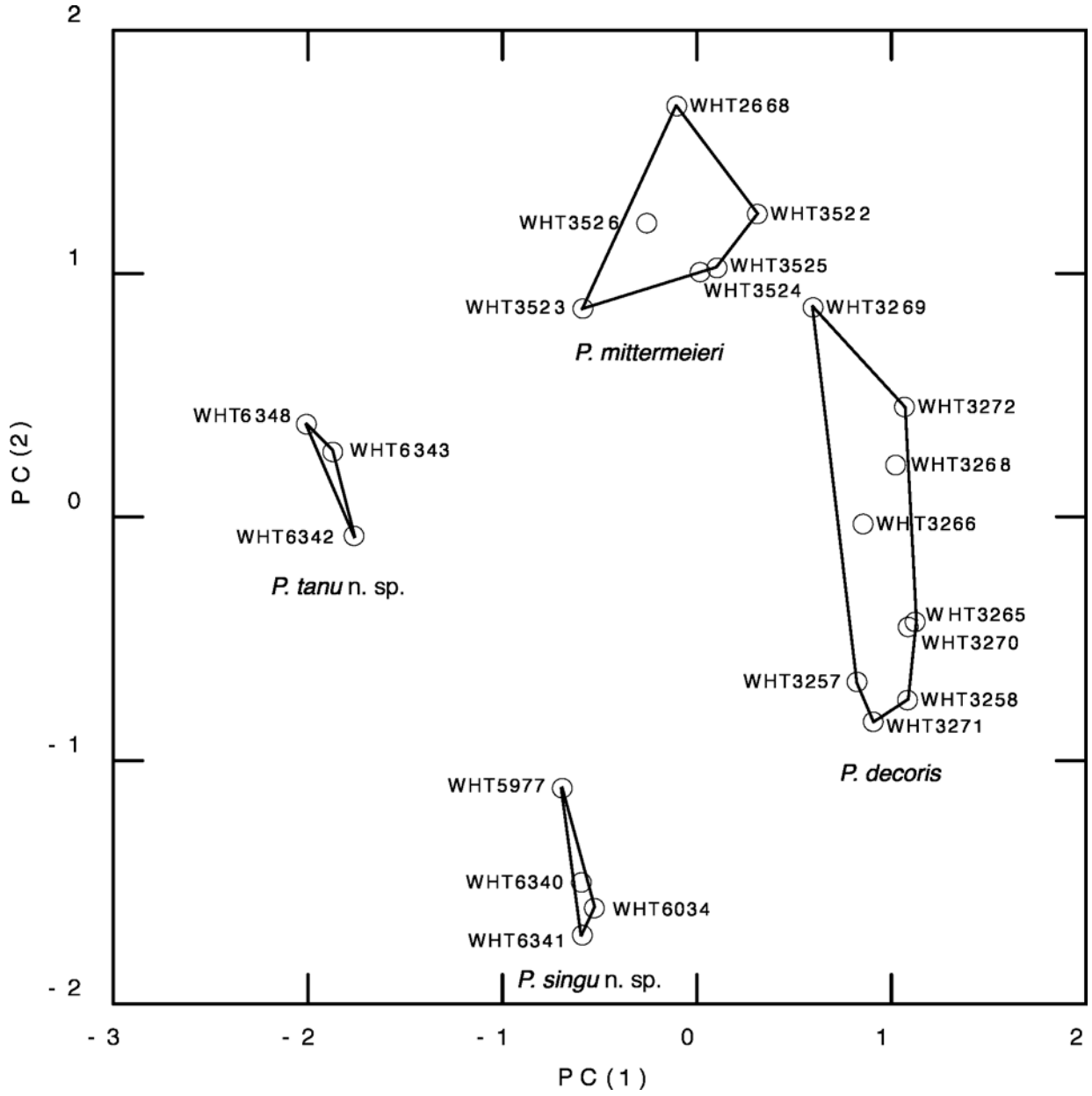

FIGURE 1. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus tanu,* n. sp., *P. singu* n. sp., *P. mittermeieri* and *P. decoris*, show these four species to separate well from each other in PC space. Most of the variation is explained by the PC1 axis, which relates mainly to body size (*P. decoris* is the largest and *P. tanu* the smallest; however, *P. mittermeieri* and *P. singu* overlap on this axis). The PC2 axis is mostly explained by finger, toe and head dimensions; here, *P. tanu* overlaps completely with *P. decoris,* but *P. singu* and *P. mittermeieri* do not.

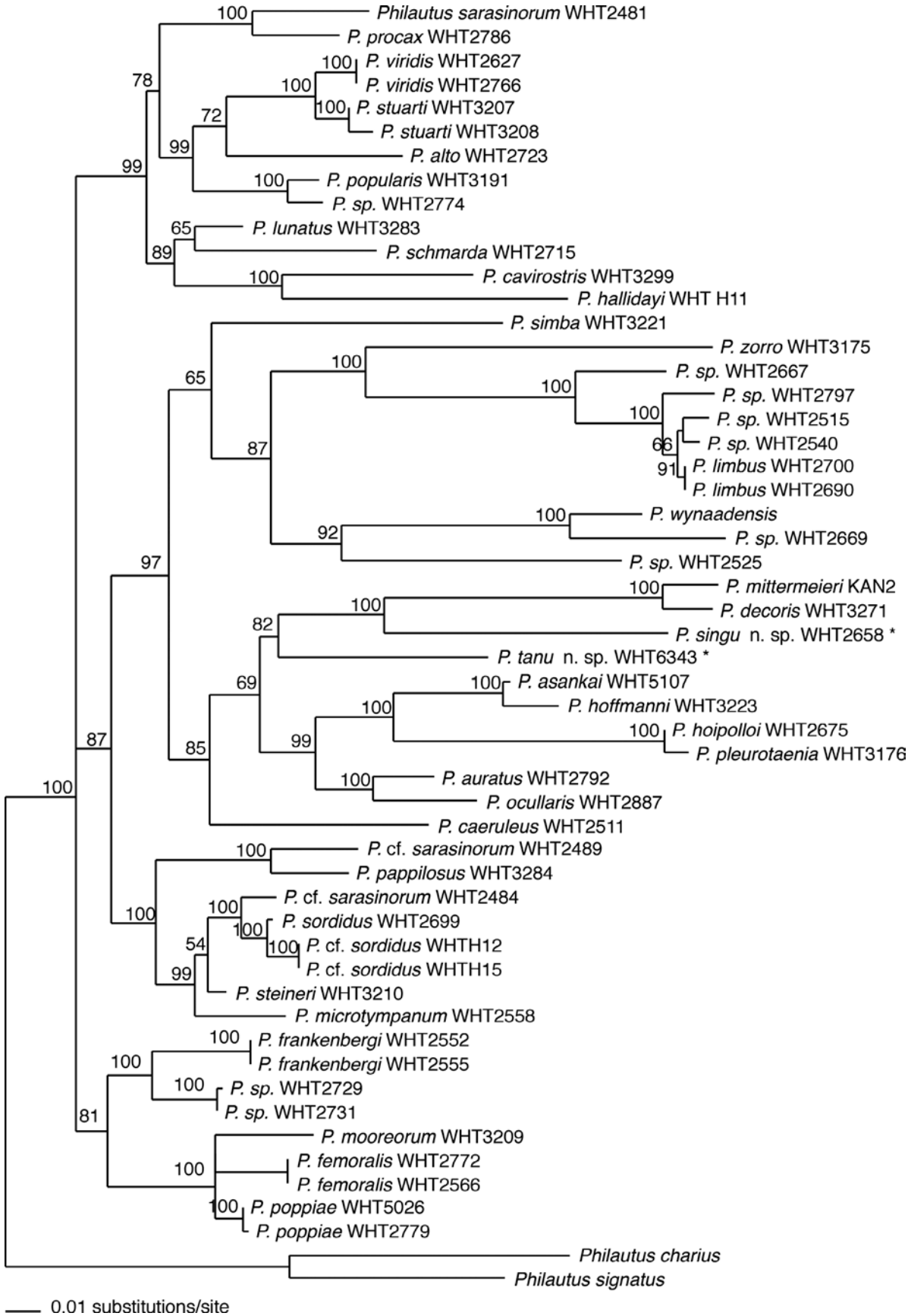

FIGURE 2. Maximum likelihood tree of 12s and 16s rRNA gene fragments, with posterior probabilities from the Bayesian analysis shown at nodes. The two new species, *Philautus tanu* and *P. singu* (indicated by asterisks), form a clade with *P. mittermeieri* and *P. decoris.*

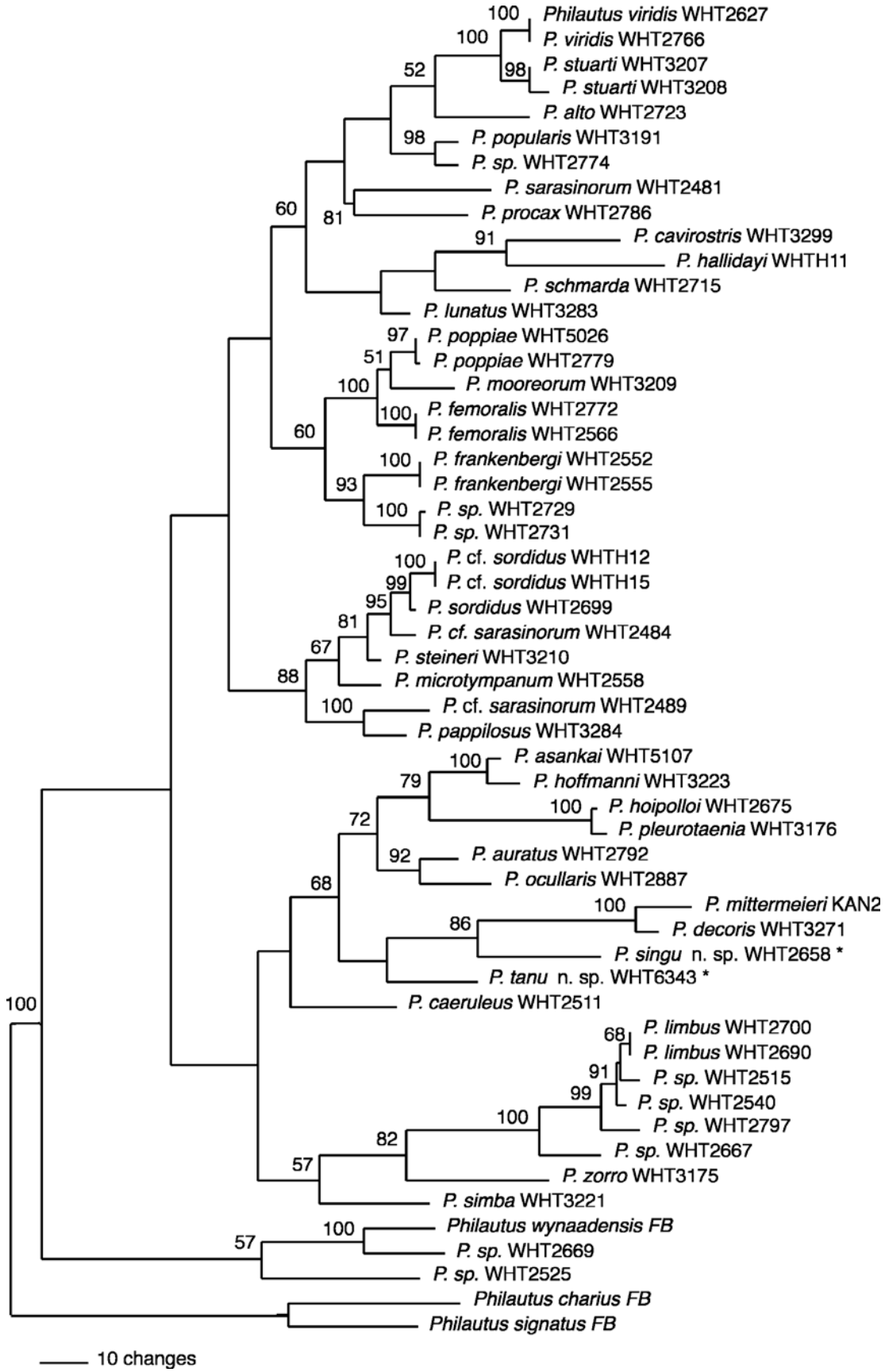

FIGURE 3. Unweighted Maximum Parsimony tree of 12s and 16s rRNA gene fragments, with maximum parsimony bootstrap values shown at the nodes. The two new species, *Philautus tanu* and *P. singu* (indicated by asterisks), form a clade with *P. mittermeieri* and *P. decoris.*

***Philautus singu*, new species**
(Figs. 4–7)

Material examined. Holotype: mature male, 16.2 mm SVL, WHT6034, Kitulgala, alt. 101 m (6°59'N, 80°20'E), coll. 22 February 1999.

Paratypes: mature males, 16.6 mm SVL, WHT6340, Kottawa Forest Reserve (Galle), alt. 60 m (06°06'N, 80°20'E), coll. 3 June 1999; 16.1 mm SVL, WHT5977, Sinharaja World Heritage Site (near Kudawa), alt. 513 m (06°25'N, 80°25E), coll. 25 January 1999; 16.1 mm SVL, WHT6341, Kottawa Forest reserve (Galle), alt. 60 m (6° 5'N, 80°18'E), coll. 3 June 1999.

Diagnosis. *Philautus singu* is distinguished from all its Sri Lankan congeners by the following combination of characters: size small, mature males 16.1-16.6 mm SVL; a prominent tubercle present on upper eyelid; tympanum distinct; supratympanic fold distinct; canthal edges rounded; vomerine ridge absent; throat, chest and belly granular.

FIGURE 4. *Philautus singu* (WHT 6340), in life, Kottawa Forest Reserve, Galle.

Description. Body slender. Head laterally convex above. Snout obtusely pointed in dorsal view, rounded 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 distinct. Pineal ocellus absent. Vomerine ridge absent. Tongue moderate, emarginate, sometimes (n = 1) bearing a rounded lingual papilla. Supratympanic fold distinct. Cephalic ridges absent. Co-ossified skin on head absent. Upper and lower arms short, thin. Fingers slender. Relative length of fingers, 1 < 2 < 4 < 3. Tips of fingers with discs bearing circum-marginal grooves. Webbing and lateral dermal fringe absent on fingers. Subarticular tubercles on fingers prominent, oval, single, sometimes absent on fingers III (n = 2) and IV (n = 2). Prepollex oval, distinct. Two palmar tubercles, oval, distinct; outer tubercle bifid. Supernumerary tubercles present. Thigh, shank slender. Toes thin, relative length 1 < 2 < 3 = 5 < 4 (holotype), (1 < 2 < 5 < 3 < 4, WHT6340), (1 < 2 < 3 < 5 < 4, WHT5977, WHT6341). Tips of toes with discs, with circum-marginal groves. Webbing present on

toes. Subarticular tubercles on toes prominent, oval, single, sometimes absent on toe V (n = 2). Inner metatarsal tubercle distinct, oval. Outer metatarsal tubercle absent. Tarsal fold absent; some dermal tubercles on outer edge of foot. Supernumerary tubercles on toes absent. Tarsal tubercle absent. Dorsal and lateral parts of snout, between eyes, side of head, anterior part of back, posterior part of back, both upper and lower flanks with scattered, glandular tubercles. Dorsolateral fold absent. Dorsal and lateral parts of upper arm, lower arm, thigh, shank and foot with scattered glandular tubercles. Throat, chest, belly and ventral side of thigh granular; underside of thigh smooth. Nuptial pad absent. Internal vocal slits present.

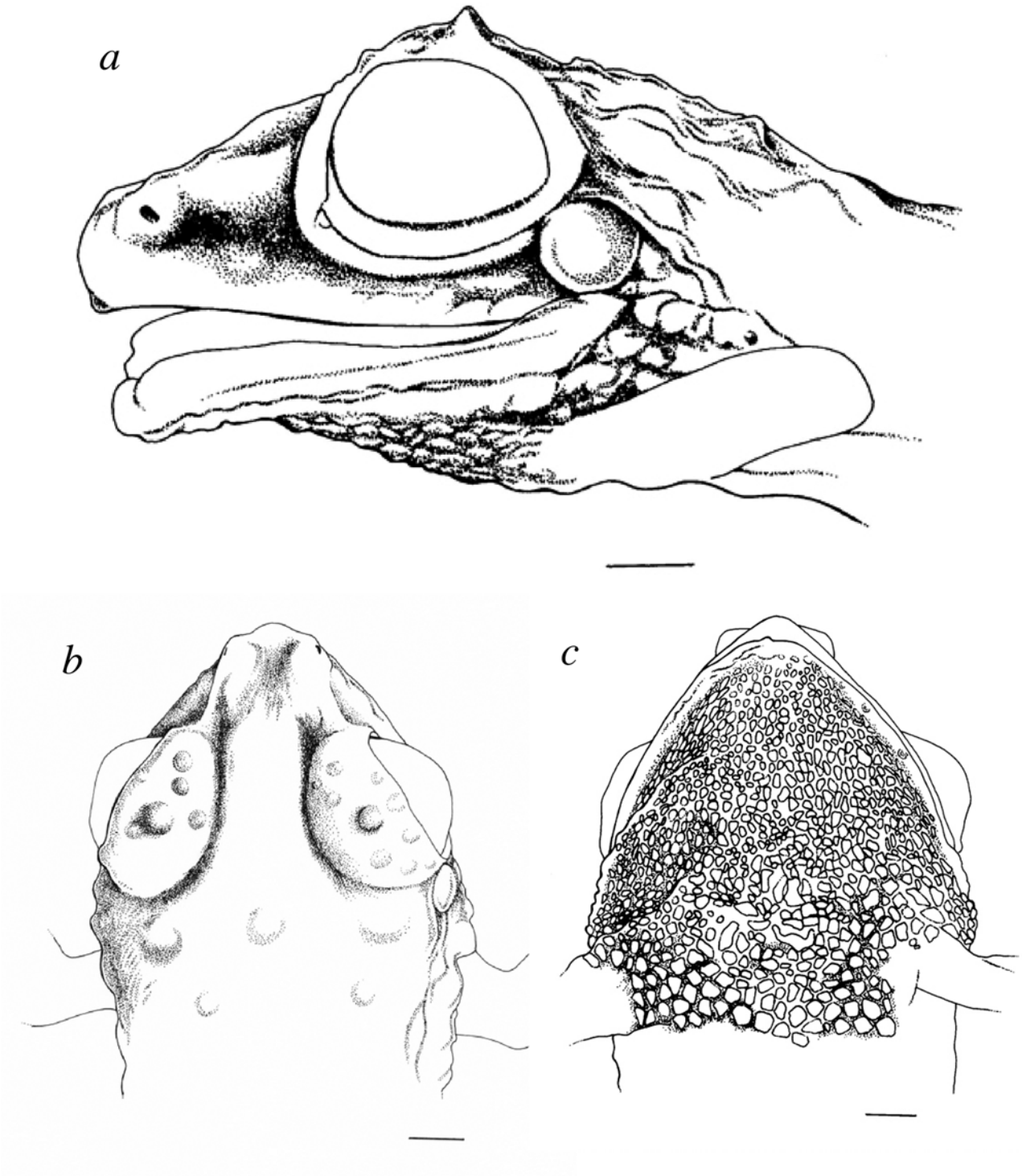

FIGURE 5. *Philautus singu*: *a*, lateral; *b*, dorsal; and *c*, ventral aspects, respectively, of head of holotype, male, WHT 6034, 16.2 mm SVL. Scale bar: 1 mm.

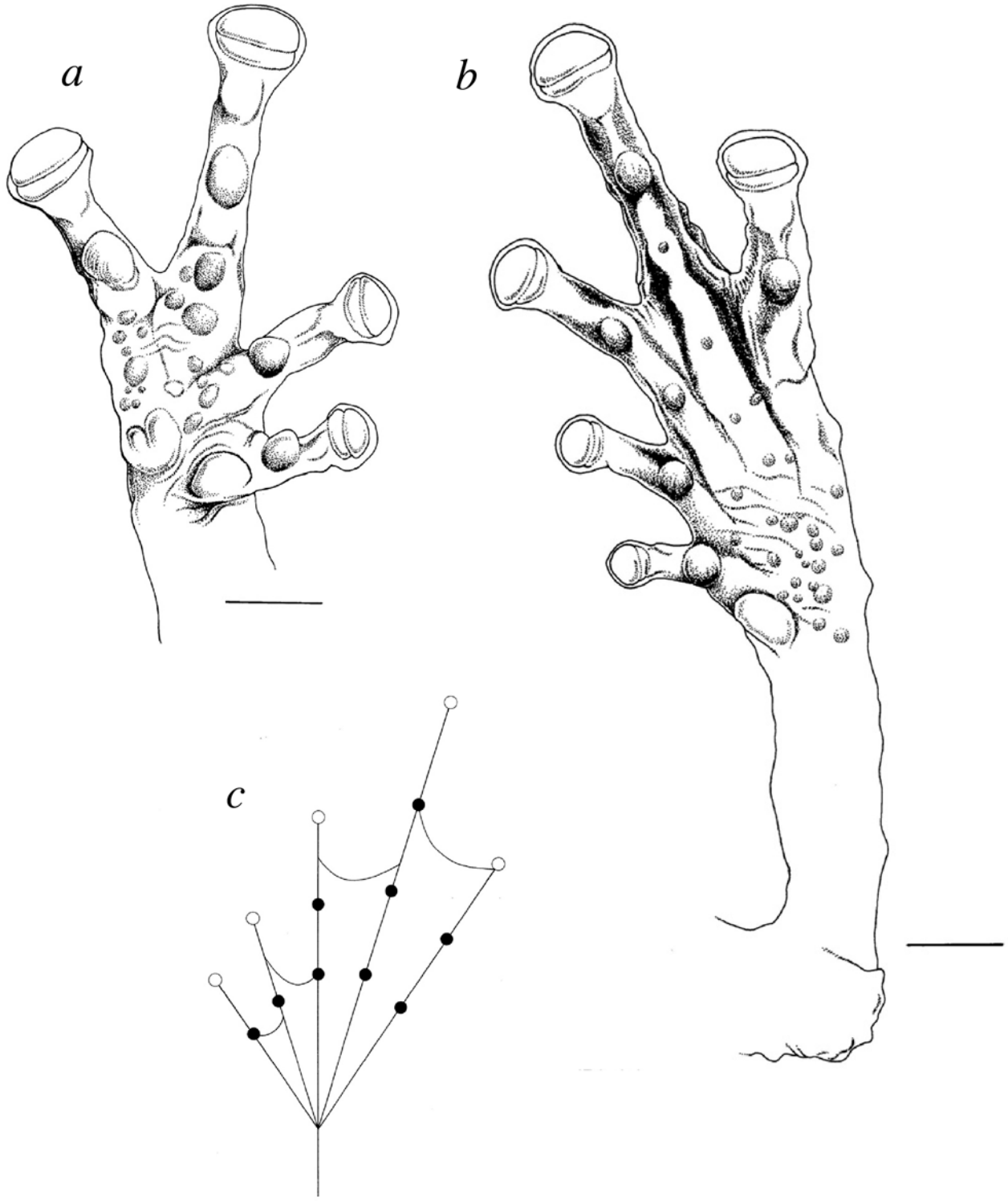

FIGURE 6. *Philautus singu*: *a*, ventral aspect of left manus; *b*, ventral aspect of left pes; and *c*, semi-diagrammatic representation of left-pes webbing pattern of the holotype, male,WHT 6034, 16.2 mm SVL. Scale bar: 1 mm.

Coloration in life. Dorsal and lateral parts of head and body brown (Fig. 4). Interorbital area dark brown. A dark-brown "W"-shaped marking on mid-back. Upper flank brown, lower flank yellow with brown pigments. Inguinal zone pale yellowish brown. Loreal region dark brown. Tympanic region blackish brown. Upper half of tympanum dark brown, lower half pale yellowish light brown. Upper lip brown with pale-yellow patches. Dorsal and lateral parts of forelimb pale yellow with brown pigments, lacking distinct

crossbars. Thigh and shank pale brown with three dark-brown crossbars. Dorsal side of foot pale brown with darker patches. Posterior thigh pale brown. Throat and margin of throat with dark-brown pigments on a pale-yellow background. Chest, belly, thigh and webbing on toes with dark-brown pigments and pale-yellow patches.

Coloration in alcohol (based on holotype, WHT6034). Dorsal and lateral parts of head and body brown. Interorbital area dark brown. A dark-brown "W"-shaped marking on mid-back. Upper flank brown, lower flank yellow with brown pigments. Inguinal zone pale yellowish brown. Loreal region brown. Tympanic region dark brown. Upper half of tympanum dark brown, lower half pale yellowish light brown. Upper lip brown with pale-yellow patches. Dorsal and lateral parts of forelimb pale yellow with brown pigments; no distinct crossbars. Thigh and shank pale brown with three dark-brown crossbars. Dorsal side of foot pale brown with dark-brown patches. Posterior side of thigh pale brown. Throat and margin of throat with dark-brown pigments on a pale-yellow background. Chest, belly, thigh and webbing on toes with dark-brown pigments and pale-yellow patches.

Measurements of holotype (WHT6034, in mm): DBE, 6.3; DFE, 3.6; DL, 0.7; DW, 1.0; ED, 2.5; EN, 2.0; ES, 2.3; FEL, 7.7; FL I, 1.2; FL II, 1.7; FL III, 3.0; FL IV, 2.2; FOL, 11.1; HL, 6.4; HW, 6.2; IML, 0.9; IN, 1.8; IO, 1.6; LAL, 3.2; MBE, 2.2; MFE, 4.0; MN, 5.7; NS, 1.2; PAL, 5.1; SVL, 16.2; TBL, 8.2; TL I, 1.3; TL II, 1.7; TL III, 2.8; TL IV, 3.9; TL V, 2.8; TYD, 0.7; TYE, 0.7; UAW, 3.6; UEW, 2.2.

Etymology. The species name is Sinhala for horn, an allusion to the horn-like tubercles on the upper eyelids of this frog; applied as a noun in apposition.

Remarks. Morphologically, *Philautus singu* is reminiscent of *P. decoris, P. mittermeieri* and *P. tanu*, new species. It is distinguished from *P. decoris* and *P. mittermeieri*, however, by the presence of a prominent tubercle on the upper eyelid (absent in *P. mittermeieri, P. decoris* and *P. tanu*); by having the snout rounded in lateral aspect (pointed in *P. mittermeieri,* and obtusely pointed in both *P. decoris* and *P. tanu*); by the absence of a tarsal tubercle (present in *P. mittermeieri* and *P. decoris*); absence of a lateral dermal fringe and webbing on fingers (present in *P. mittermeieri* and *P. decoris*); and by the absence of a tarsal fold (presenct in both *P. mittermeieri* and *P. decoris*).

Distribution. We observed males of *P. singu* perched on leaves of shrubs, 0.5-1.5 m above ground level, in the rainforest understory. Although we recorded the species only from the Kottawa and Kitulgala Forest Reserves, it probably occurs also in other rainforest patches in the wet-zone lowlands of Sri Lanka.

***Philautus tanu*, new species**
(Figs. 7–10)

Material examined. Holotype: mature male, 13.5 mm SVL, WHT6348, Kanneliya Forest Reserve (near Galle), alt. 45 m (6°15'N, 80°20'E), coll. 5 May 1999.

Paratypes: mature males, 13.6 mm SVL, WHT6343; 13.9 mm SVL, WHT6342, Pituwala (Galle), alt. 24 m (6°16'N, 80°12'E), coll. 4 March 1999.

Diagnosis. *Philatus tanu* is distinguished from all other Sri Lankan congeners by the following combination of characters: small size, mature individuals 13.5–13.9 mm SVL; canthal edges rounded; tympanum distinct; vomerine ridge absent; supratympanic fold absent; a very narrow dermal fold along mid-dorsum, from tip of the snout to vent; venter granular; nuptial pads absent; vocal sac indistinct.

Description. Body stout. Head laterally convex. Snout obtusely pointed in both dorsal and lateral aspect. Canthal edges rounded. Loreal region flat. Interorbital space flat. Internasal space flat. Nostrils oval. Pupil rounded or (horizontally) oval. Tympanum distinct, oval, vertical. Pineal ocellus absent. Vomerine ridge absent. Tongue moderate, emarginate, not bearing a lingual papilla. Supratympanic fold absent. Cephalic ridges absent. Co-ossified skin on head absent. Both upper and lower arms slender. Fingers slender. Relative length of fingers, 1 < 2 < 4 < 3. Tips of fingers with discs bearing circum-marginal grooves. Fingers without lateral dermal fringe. Webbing on fingers absent. Subarticular tubercles on fingers prominent, oval, single

(absent on finger IV; n = 2). Prepollex oval. Two palmar tubercles, oval, flat. Supernumerary tubercles present on fingers I–III and on palm. Thigh and shank slender. Toes thin. Relative length of toes 1 < 2 < 5 < 3 < 4 or 1 < 2 < 3 < 5 < 4 (WHT6343, WHT6342). Tips of toes with discs bearing circum-marginal grooves. Webbing on toes present. Subarticular tubercles on toes prominent, oval, single. Inner metatarsal tubercle distinct, oval. Outer metatarsal tubercle absent. Tarsal fold absent. Supernumerary tubercles present on toes and on foot. Tarsal tubercle absent. Dorsal and lateral parts of head and body shagreened. Both upper and lower parts of flank granular. Dorsolateral dermal fold absent. Dorsal and lateral parts of upper arm, lower arm, thigh, shank and foot smooth. A very narrow dermal fold present on mid dorsum, extending from tip of snout to vent. Ventral parts of throat, chest, abdomen, both upper and lower arms, anterior thigh, shank, and foot granular. Nuptial pad absent. Vocal sacs indistinct. Internal vocal slits present.

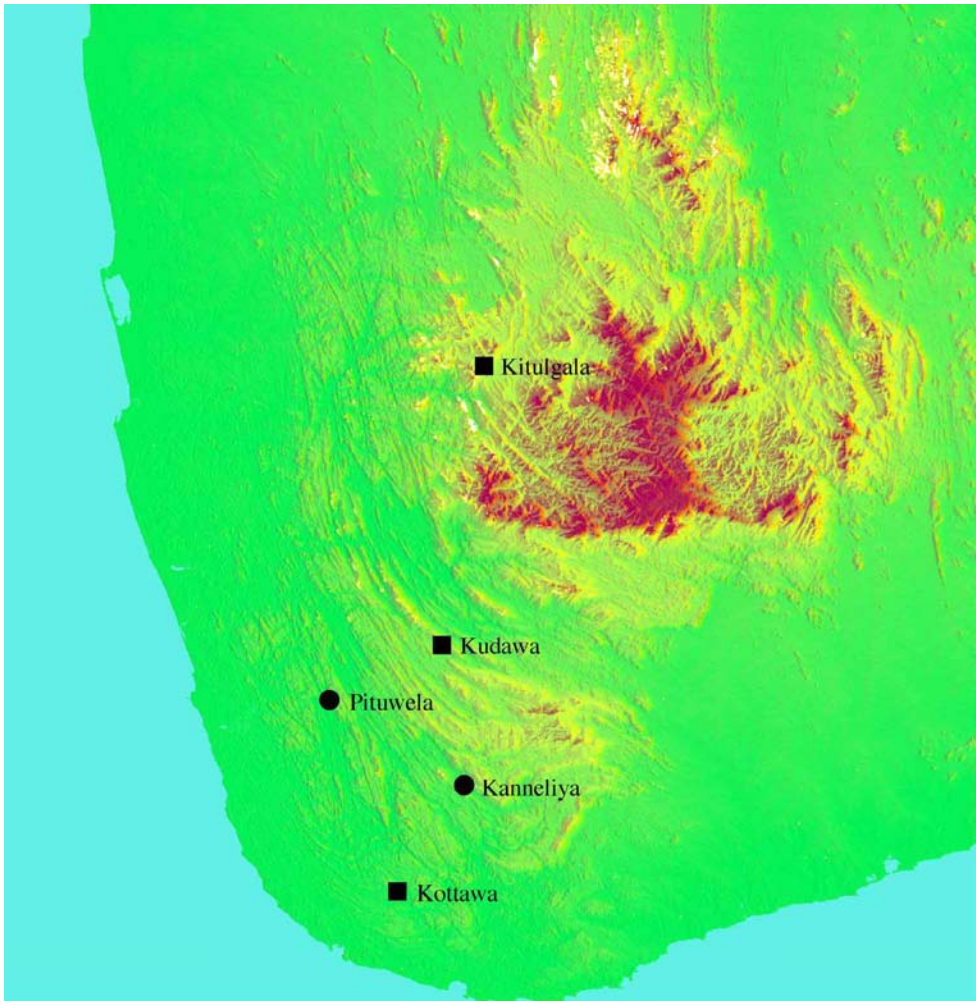

FIGURE 7. South-western Sri Lanka, showing the distribution of *Philautus singu* (squares) and *P. tanu* (circles), and place-names cited in the text.

Coloration in life. Dorsal parts of head and body pale brown (Fig. 8). A dark-brown stripe about as wide as pupil extends backwards from snout, fading away on mid-flank. About eight dark-brown stripes of varying width on dorsum. Ground color of body creamy-light brown. A narrow creamy-brown stripe extends from snout, over eye to flank. Lower flank pale yellow or white. Inguinal zone white with light brown pigments. Loreal and tympanic regions dark brown. Both upper and lower lips light brown with dark-brown pigments. Dorsal and lateral parts of both upper and lower arms, fingers, thigh, shank, foot and toes pale brown. Ventral parts of head, body, upper and lower arms, fingers, thigh, shank, foot and toes white with scattered brown pigments. Posterior edge of orbit light blue.

FIGURE 8. *Philautus tanu* (WHT 6342), in life, Beraliya Forest Reserve, Pituwela.

Coloration in alchohol (description based on holotype, WHT6348). Dorsal and lateral parts of head and body pale brown. Anterior half of upper flank dark brown, posterior half yellow or white. Lower flank pale yellow or white. Inguinal zone white with light-brown pigments. Loreal and tympanic regions dark brown. Both upper and lower lips white with dark-brown pigments. Dorsal and lateral parts of both upper and lower arms, fingers, thigh, shank, foot and toes pale brown. Ventral parts of head, body, upper and lower arms, fingers, thigh, shank, foot and toes white with scattered brown pigments.

Measurements of holotype (WHT6348, in mm): DBE, 4.6; DFE, 3.1; DL, 0.6; DW, 0.7; ED, 2.3; EN, 1.7; ES, 2.6; FEL, 6.0; FL I, 0.8; FL II, 1.3; FL III, 2.1; FL IV, 1.6; FOL, 8.7; HL, 5.2; HW, 5.2; IML, 0.6; IN, 1.4; IO, 1.6; LAL, 2.2; MBE, 1.6; MFE, 3.1; MN, 4.6; NS, 1.0; PAL, 3.7; SVL, 13.5; TBL, 6.8; TL I, 0.9; TL II, 1.1; TL III, 1.8; TL IV, 2.9; TL V, 1.7; TYD, 0.4; TYE, 0.7; UAW, 1.8; UEW, 1.4.

Etymology. The species name is Sinhala for 'slender,' a reference to the habitus of *P. tanu*; applied as a noun in apposition.

Remarks. *Philautus tanu* morphologically resembles *P. decoris, P. mittermeieri* and *P. singu* new species, but it can be distinguished from them as follows: no prominent tubercle on the upper eyelid (present in *P. singu*); snout obtuse in lateral aspect (pointed in *P. mittermeieri*); tarsal tubercle absent (present in both *P. mittermeieri* and *P. decoris*); no lateral dermal fringe or webbing on fingers (present in both *P. mittermeieri* and *P. decoris*); no tarsal fold (present in both *P. mittermeieri* and *P. decoris*).

Distribution. We observed males of *P. tanu* sitting on leaves of shrubs, 0.5-1.0 m above ground level, in shrubs adjacent to (10-25 m from) the canopy covered forests. *Philautus tanu* was recorded from Kanneliya (6°15'N, 80°20'E) and Pituwela (6°16'N, 80°12'E) Forest Reserves, and probably occurs also in other open habitats close to patches of rainforest elsewhere in the wet-zone lowlands of Sri Lanka.

Discussion

Philautus tanu, a widespread but strictly lowland species, is basal to two other lowland species, *P. singu,* and *P. mittermeieri*, and to a high elevation species, *P. decoris*, suggesting that the diversification in this clade

occurred in the lowlands. Furthermore, *P. tanu* inhabits open shrub habitats and may be able to disperse freely through secondary-forest corridors and suitable anthropogenic habitats. Thus, given the availability of conducive habitat, *P. tanu* is likely to have a wider distribution than currently recorded. *Philautus tanu* appears to be a highly cryptic species, usually calling from deep within shrubs, which makes these frogs difficult to localize. This trait may represent an adaptation high predation pressure in its relatively open habitat.

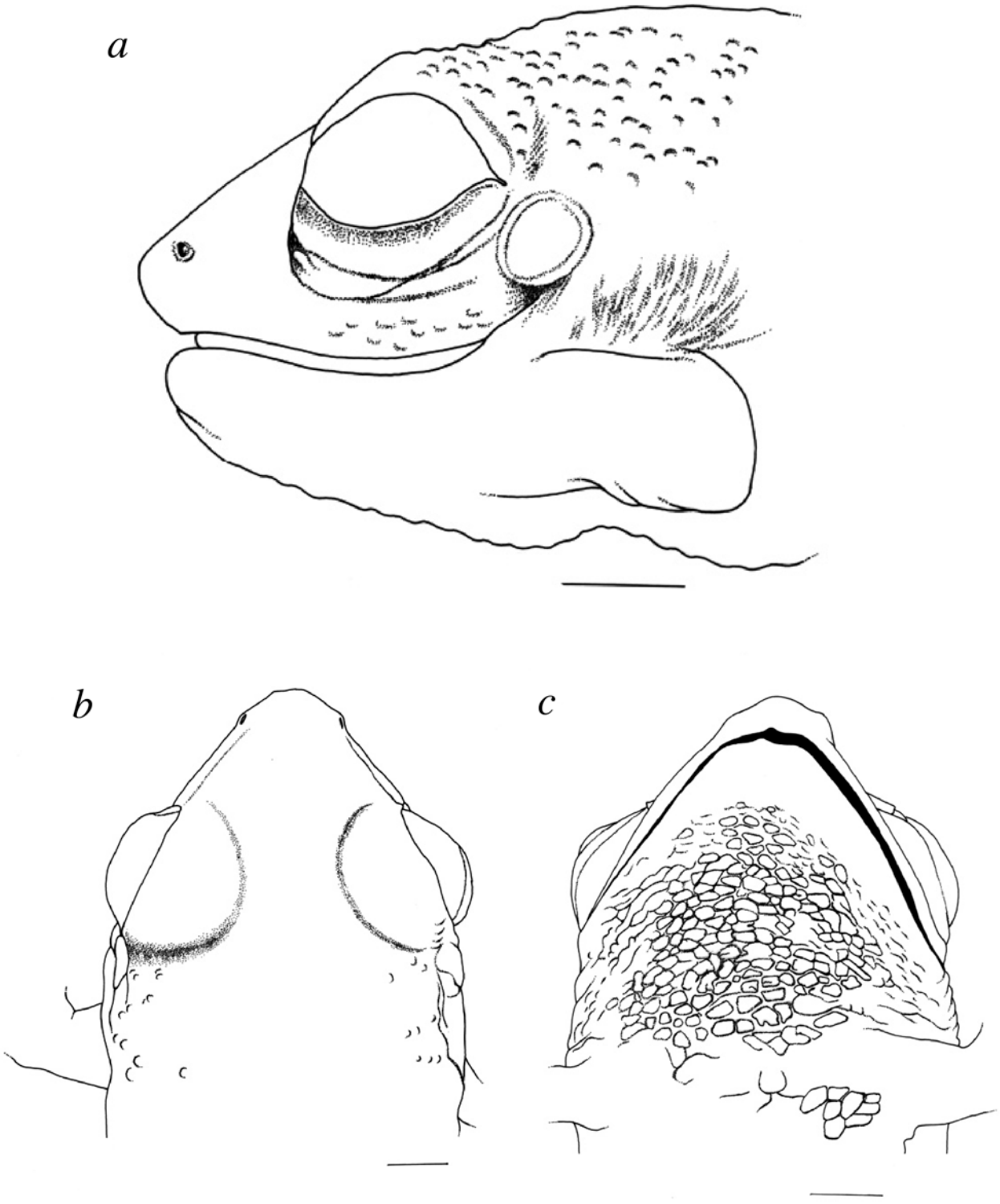

FIGURE 9. *Philautus tanu:* *a*, lateral; *b*, dorsal; and *c*, ventral aspects, respectively, of head of holotype, male, WHT 6348, 13.5 mm SVL. Scale bar: 1 mm.

Philautus singu is distributed widely both in the lowlands and the mid-hill region. Its range overlaps partly with that of *P. mittermeieri*, one of its sister species. Neither of these frogs is a strictly leaf litter dweller; they do climb on to shrubs at night to vocalize. *Philautus singu*, like *P. mittermeieri*, is restricted to primary and secondary forests with extensive canopy cover; we did not record either species from open habitats. Interestingly, where they are syntopic, *P. singu* occupies a separate microhabitat from *P. mittermeieri*: 0.5–1.5 m above ground, and less than 0.5 m above ground, respectively. Thus, the two species may partition resources to some extent.

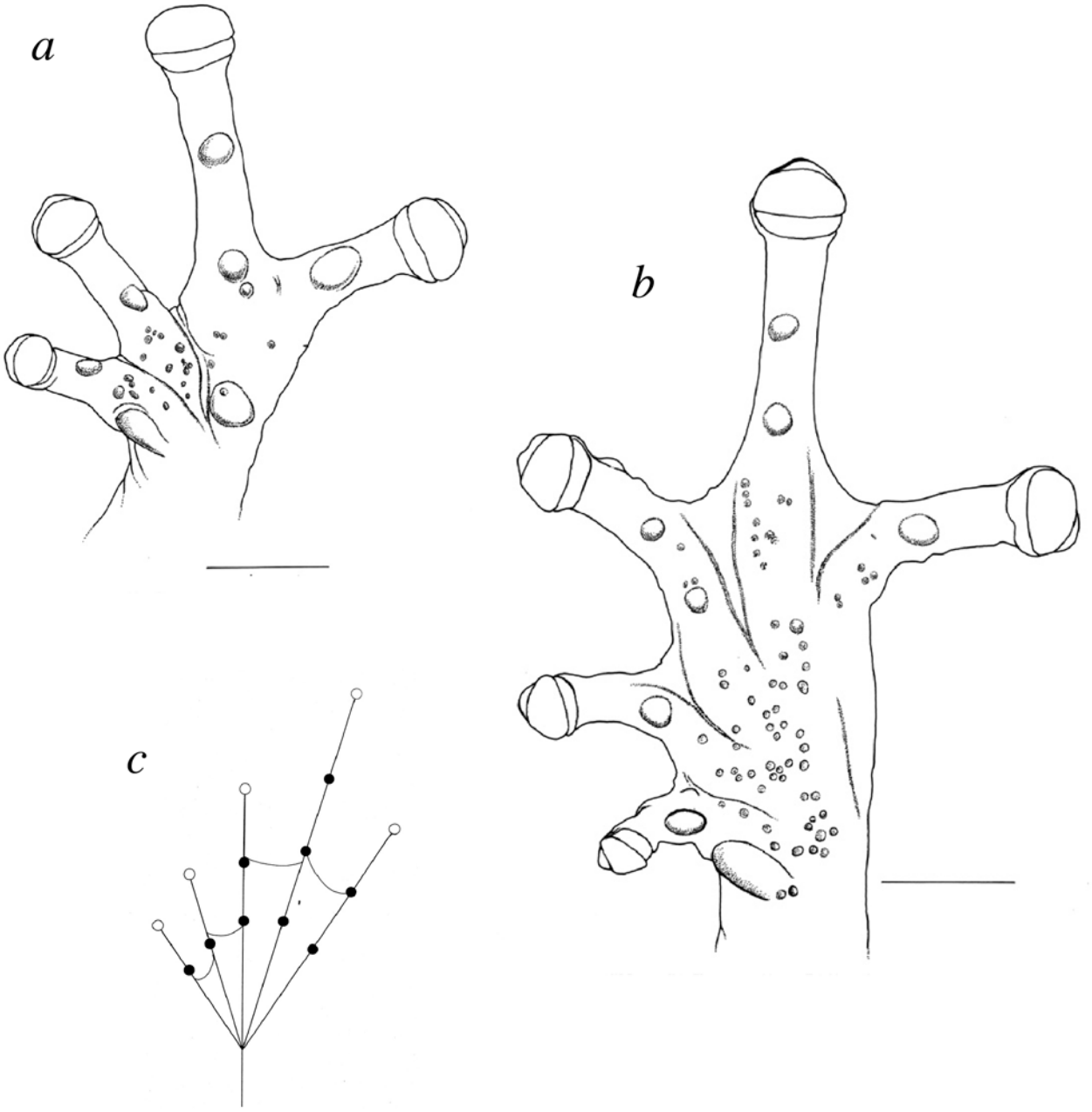

FIGURE 10. *Philautus tanu*: *a*, ventral aspect of left manus; *b*, ventral aspect of left pes; and *c*, semi-diagrammatic representation of the left-pes webbing pattern of the holotype, male, WHT 6348, 13.5 mm SVL. Scale bar: 1 mm.

Philautus tanu, *P. singu*, *P. decoris* and *P. mittermeieri* form a single clade (Figs. 2, 3), but the new species are well differentiated genetically from the others (Tables 2, 3). *Philautus singu* is distinct from the three other species by uncorrected percent genetic distances of 7.7–8.4 (combined 12s and 16s gene fragments) and 17.1–18.5 (cytochrome-*b* gene fragment). Similarly, *Philautus tanu* is distinct by uncorrected percent genetic

distances of 7.9–8.7 (combined 12s and 16s gene fragments) and 18.2–22.6 (cytochrome-*b* gene fragment) (Tables 3 & 4). These values are well over the 2% cytochrome-*b* genetic distance that indicates species-level divergence in several groups of mammals (Bradley & Baker 2001); and that is exceeded by 90% of putative sister species across a wide range of vertebrate taxa (Johns and Avise 1998), which adds 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 Mantellidae, a wide range of divergence among species, ranging from 1-16.5% has been highlighted (Vences *et al.* 2005), and a 3% divergence has been proposed as a species level threshold (Fouquet *et al.* 2007)

TABLE 3. Matrix of pairwise uncorrected percent divergences for 12s and 16s rRNA gene fragments among *P. mittermeieri, P. decoris, P. singu* and *P. tanu.*

	P. mittermeieri	*P. decoris*	*P. singu*
P. decoris	2.0		
P. singu	8.4	7.7	
P. tanu	8.2	8.7	7.9

TABLE 4. Matrix of uncorrected percent divergences for cytochrome-*b* gene fragments among *P. mittermeieri, P. decoris, P. singu* and *P. tanu.*

	P. mittermeieri	*P. decoris*	*P. singu*
P. decoris	5.5		
P. singu	17.1	18.5	
P. tanu	21.3	22.6	18.2

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