THE RAFFLES BULLETIN OF ZOOLOGY 2005 Supplement No. **12**: 305–338

DESCRIPTION OF EIGHT NEW SPECIES OF SHRUB FROGS (RANIDAE: RHACOPHORINAE: *PHILAUTUS*) FROM SRI LANKA

Madhava Meegaskumbura
Department of Biology, Boston University, 5 Cummington Street, Boston, MA, 02215, USA
Wildlife Heritage Trust, 95 Cotta Road, Colombo 8, Sri Lanka
Email: madhava@bu.edu

Kelum Manamendra-Arachchi
Wildlife Heritage Trust, 95 Cotta Road, Colombo 8, Sri Lanka
Email: kelum@slt.lk

ABSTRACT. – Eight new species of Sri Lankan frogs of the genus *Philautus* are described (*P. mooreorum, P. poppiae, P. hoffmanni, P. mittermeieri, P. frankenbergi, P. hallidayi, P. steineri and P. stuarti*). The species are diagnosed on the basis of mitochondrial DNA sequence, morphological features and, in two cases, bioacoustics data. Six of the eight species are confined to high elevation cloud forest isolates on the three main mountain ranges of central Sri Lanka. Because of their limited geographic distribution and small extent of remaining habitat, these species are classified as Endangered under the IUCN Red List criteria. These descriptions bring the total number of Sri Lankan *Philautus* to 61 species, 44 of which are extant.

KEY WORDS. – Rhacophoridae, taxonomy, montane, bioacoustics, evolutionary lineage, conservation.

INTRODUCTION

The high endemism and diversity of the direct-developing rhacophorine frogs of the genus *Philautus* Gistel, 1848 of Sri Lanka is now well established (Meegaskumbura et al., 2002; Bossuyt et. al., 2004; Manamendra-Arachchi & Pethiyagoda, 2005), but many species remain to be described. Molecular phylogenetic analyses have played an important role in the recognition and diagnosis of species as well as in the higher-level systematics of the Sri Lankan radiation. We believe that cautious description supported by phylogenetic analyses, utilizing also data on the biology of the species involved, represents the best approach to the recognition of species. Otherwise, hypotheses to explain patterns of diversity and distribution in the Sri Lankan radiation of *Philautus* risk being no more than speculative (e.g., Manamendra-Arachchi & Pethiyagoda, 1998; Meegaskumbura et al., 2002; Dubois, 2004). While we strive for a complete documentation of the anuran diversity of Sri Lanka, made particularly urgent by the Globally Threatened status of many of the recently-discovered species (Stuart et al., 2004), we hope the naming of these species will facilitate their conservation and further research on their biology.

Following almost a decade of fieldwork, museum visits to study type material and molecular analyses (Pethiyagoda & Manamendra-Arachchi, 1998; Meegaskumbura et al., 2002) and the taxonomic report of Bossuyt & Dubois (2001), Manamendra-Arachchi & Pethiyagoda (2005) reviewed the Sri Lankan *Philautus*. The latter authors re-described the name-bearing types, provided descriptions of 27 new species, recognized 17 species as extinct, and provided a key to all the valid Sri Lankan species of *Philautus*. This served to increase the total number of *Philautus* in Sri Lanka from six (Kotagama et al., 1981) to 53 species. The present work furthers this objective, but it differs from the purely morphological approach of Manamendra-Arachchi & Pethiyagoda (2005) by doing so within a phylogenetic framework. Focusing largely on montane isolates, we provide a hypothesis of phylogeny based on mitochondrial DNA (with a more complete phylogenetic analysis of mitochondrial and other data to follow) and describe eight new species. This brings the total number of Sri Lankan *Philautus* to 61.

Based on a “morpho-species” assessment in their original announcement of this hitherto unsuspected diversity, Pethiyagoda & Manamendra-Arachchi (1998) suggested that there could be ~ 200 species of *Philautus* in Sri Lanka. This estimate was revised downward when phylogenetic analysis of mitochondrial DNA (mtDNA) sequences from putative species suggested that the actual number of distinct lineages was in fact about half of that originally estimated

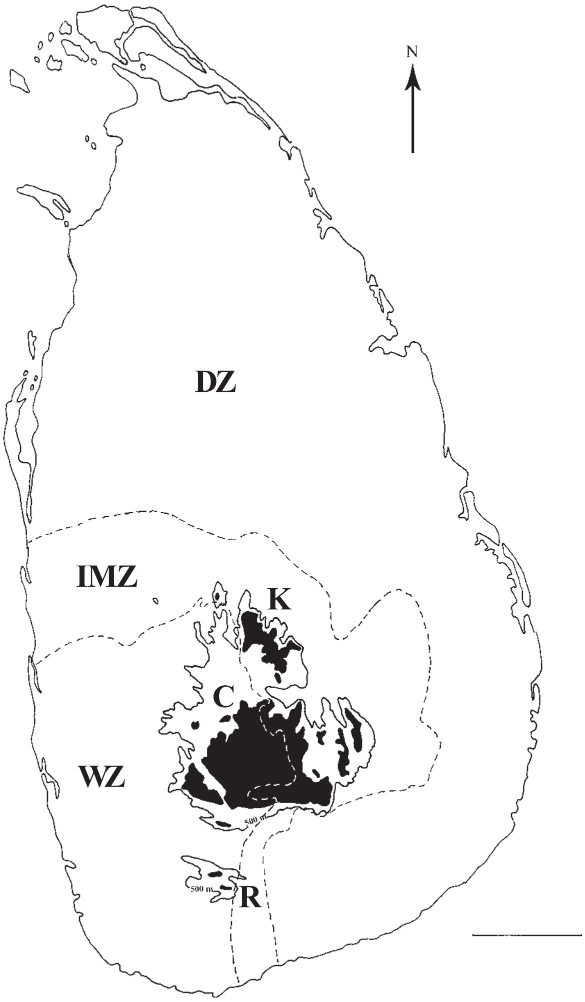

Fig. 1. Map of Sri Lanka depicting the wet zone (WZ), intermediate zone (IMZ) and the dry zone (DZ), and the three mountain ranges referred to in this study: Knuckles hills (K), Central hills (C) and the Rakwana hills (R). The 500 m and 1,000 m contours are shown. The Central hills have the largest area above 1,000 m and Rakwana Hills have the smallest. The valley separating Central hills and Knuckles has a maximum elevation of ~ 550 m and that separating the Central and Rakwana hills has a maximum elevation of ~ 450 m. Scale bar: 50 km.

(Meegaskumbura et al., 2002). In addition, the mtDNA phylogeny provided a phylogenetic framework that facilitated the identification of apomorphic morphological characters that were diagnostic of clearly defined mtDNA lineages. Most of these lineages had already been recognized as potential new species by Manamendra-Arachchi & Pethiyagoda (2005) using classical, morphological taxonomy and were not discovered as a consequence of the molecular analysis alone contra Mace et al. (2003). While the descriptions and key presented in Manamendra-Arachchi & Pethiyagoda (2005) do not explicitly reference the mtDNA tree, both were informed by patterns of morphological apomorphy implied by the mtDNA tree and are thus consistent with patterns of morphological character state distribution in it (a detailed analysis of morphological evolution in Sri Lankan *Philautus* will be presented elsewhere). In addition to informing the diagnosis of species of Sri Lankan *Philautus*, the phylogenetic analysis revealed several distinct mtDNA lineages, which were represented, however, only by relatively small series of specimens. These show sufficient morphological and other differentiation to allow their recognition as species; here we describe eight such species.

Species Concept. – We consider it important in taxonomic studies to state explicitly as a testable hypothesis the species concept used, so as to prevent definition of species in a post-hoc, arbitrary manner (Sites & Crandall, 1997). More than 25 species “concepts” are recognized in the literature (reviewed by de Queiroz, 1998; Coyne & Orr, 2004), each with its own limitations (Hey, 2001). As in our previous treatments of the Sri Lankan rhacophorine fauna (Meegaskumbura et al., 2002; Manamendra-Arachchi & Pethiyagoda, 2005), we here adopt the General Lineage Concept of species (de Queiroz, 1998). This defines species as independent evolutionary lineages that are diagnosed by multiple criteria. We find the General Lineage Concept to be useful and at the same time inclusive of many of the current species “concepts” in that species are recognized as segments of evolving lineages which, based on multiple criteria, appear to be independent from other such lineages (see de Queiroz, 1998, for a fuller discussion). Furthermore, the incorporation of multiple criteria for diagnosing species recognizes the multi-dimensional nature of species as ecological, morphological and behavioural entities as well as reproductive and historical entities.

In this analysis, we used mitochondrial DNA (mtDNA) sequences, external morphology, ecology and, in some instances, bioacoustics data for diagnosing species. We consider sets of populations to be evolutionarily independent species when they are consistently divergent in at least two, and preferably three or more, of these criteria.

The conservation status of many of the Sri Lankan *Philautus* species is precarious, mostly because of habitat loss and the fragmentation of remaining habitats. Of the 36 extant species recognized by Manamendra-Arachchi & Pethiyagoda (2005), seven were classified as Critically Endangered and 17 as Endangered in terms of the IUCN (1996) criteria for global red listing. This underlines the urgency to describe and document the remaining diversity. Six of the eight species described in this paper are classic montane isolates, restricted to cloud-forest habitats at high altitude (more than 1,000 m elevation) in three major mountain ranges: the Central Hills, Rakwana Hills, and Knuckles Hills (Fig. 1). We have focused on these especially since the long-term persistence of these montane isolates is uncertain given that cloud forest habitats, and the species that rely on them, may be highly susceptible to the effects of global climate change (Pounds & Puschendorf, 2004) and also local pressures from habitat loss and degradation.

MATERIALS AND METHODS

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

Morphological analysis. – The suit of characters (continuous and discrete) used by Manamendra-Arachchi & Pethiyagoda (2005) were analysed for all individuals.

For continuous characters, measurements were made to the nearest 0.1 mm using dial vernier callipers. All 32 measurements listed by Manamendra-Arachchi & Pethiyagoda (2005) were made for the species described herein, but those with high coefficients of variation or low repeatability were omitted from the analysis. The following measurements were used for the current analysis: distance between back of eyes (DBE); distance between front of eyes (DFE); eye diameter (ED); eye-to-snout length (ES); femur length (FEL); length of finger 3 (FLIII); pes length (FOL); head length (HL); head width (HW); inter-narial distance (IN); inter-orbital 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); and length of toe 4 (TLIV).

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 species. Various axis rotations were tested and one selected for optimal interpretability of variation among the characters. In almost all cases, the first two principal components explained more than 90% of the variance. Sample sizes from most populations were small and biased toward calling males. For consistency, therefore, only mature males were used in the multivariate morphological analysis. The single exception was *P. hallidayi*, for which only a single male was collected. In this case, mature females were used in the analysis. In several cases, the small sample sizes likely do not represent the full range of morphological variance; nonetheless, the analyses are sufficient to demarcate species and identify characters that contribute best to species diagnoses. SYSTAT (Version 11.00.01 for WindowsXP) was used for statistical analysis.

Molecular analysis. – DNA was extracted from ethanol-preserved tissues using Qiagen tissue extraction kits and manufacturer's protocols. Mitochondrial 12S and 16S ribosomal RNA gene fragments were amplified using standard PCR conditions and primers 12Sa and 12Sb (Palumbi, 1996) which amplified ~ 380bp of the 12S rRNA gene, and 16Sar and 16Sbr (Palumbi, 1996) which amplified ~ 550bp of the 16S rRNA gene. PCR conditions were as follows: 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 Clustal X (Jeanmougin et al., 1998) and adjusted by eye using Se-Al (ver. 2.0a9; Rambaut, 1996). Positions which were difficult to align and in which we had low confidence in positional homology were excluded from subsequent analyses.

Phylogenetic analysis utilized the dataset for the Sri Lankan *Philautus* from Meegaskumbura et al. (2002) with 17 additional sequences. The tree was rooted using two species of Indian *Philautus* which form the sister group to the Sri Lankan *Philautus* (Meegaskumbura et al., 2002). We removed all other taxa from the analysis of Meegaskumbura et al. (2002) since supraspecific taxonomy was not an aim of this study. The data were analyzed using Bayesian, Maximum Likelihood (ML) and Maximum Parsimony (MP) criteria. For brevity, we present only the Bayesian tree, which is identical to the Maximum Likelihood tree and one of the three 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 80,000 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, 1998). 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.

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'-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. 2.0a9; Rambaut, 1996).

We used the observed molecular distances (of cytochrome-b gene fragments) between the new species and their sister species to estimate molecular divergence times. Since there is no reliable rate calibration for *Philautus* (or Rhacophorine frogs), a crude estimate of the ranges of divergence times for the sister species pairs was derived using published low and a high estimates of divergence rates. Since some of the hypervariable regions of the ribosomal genes were removed from this analysis, only the cytochrome-b fragment was used. The two rates are 0.69% divergence rate per million years for all substitutions, which was estimated using frogs (Martin & Palumbi, 1993) and 1.0% divergence rate per million years for all substitutions, which was estimated using newts (Tan & Wake, 1995). A more complete divergence time analysis will be made with a more complete phylogeny of the Sri Lankan *Philautus*.

Bioacoustics analysis. **–** Bioacoustics data were collected in the field using a Sony analogue Walkman (WM-D6C) with a Sennheiser K6 shotgun microphone or a Sony digital Walkman (TCD-D100) with a Sony ECM-717 microphone. Ambient relative humidity and temperature were noted during recordings. Recorded calls were transferred to an Apple G4 iBook using a Griffin iMic USB audio input device. Calls were analyzed using Raven (ver. 1.4 for Mac OS X).

Since the call terminology is confusing due to the diversity of signal structures it is necessary explicitly to define the terms used (Gerhardt & Huber, 2002). Here we use call length (time in seconds from beginning to end of a single call), pulse rate ((total number of pulses -1)/time, for each call), dominant frequency (frequency in call that contains the greatest energy), fundamental frequency (the harmonic with the lowest frequency), pulse length (time from beginning to end of one pulse from mid-call), call type (the absence or presence of frequency modulation within a call), pulse type (absence or presence of frequency modulation within pulse) and the number of pulses per call, as described by Cocroft & Ryan (1995). These characters are potentially phylogenetically informative and also thought to be important in mate recognition and sexual selection (Cocroft & Ryan, 1995; McCraken & Sheldon, 1997; Sullivan, 1992). Some of these call characters have also been used to distinguish among anuran species in morphologically cryptic species (eg. Vences et al., 2000).

Etymological note. **–** For nearly two decades, the enigmatic and steady worldwide decline of amphibian populations has been watched with concern and studied by scientists across the world. During this time, dozens of species have disappeared, and hundreds of others are on the brink. Solutions to this crisis have been scarce, with each result of ongoing research revealing fresh, hitherto unsuspected complexity in this problem. Two initiatives have sought to turn this dismal tide, not just at the level of nations or regions, but on a global scale: the Declining Amphibian Populations Task Force (DAPTF) and the Global Amphibian Assessment (GAA). For more than a decade, the DAPTF has served to network scientists and conservation managers across the world, helping to build capacity where it is needed, and to share information and experience. Over a shorter time frame, the GAA, through worldwide collaboration perhaps without precedent in conservation science, succeeded in assessing the status of every amphibian species (Stuart et al., 2004). There can be no doubt that both these initiatives have succeeded only because of the extraordinary zeal, dedication and resolve of the small number of conservation scientists who have given of their time, and the handful of private donors who have given of their wealth, to help save these wonderful animals.

The results of the GAA focus attention on the fact that with 17 species extinct and a further 44 threatened with extinction, Sri Lanka's amphibians are in dire straits. Indeed, we cannot escape the concern that even the species we describe here may soon disappear forever. However, the DAPTF and GAA have given fresh hope that amphibian conservation programmes on a truly global scale are not just possible, but realistic. The flow of resources for conservation has necessarily been from developed countries to those tropical, developing nations in which the vast majority of amphibian species dwells, with so few opportunities for workers in the latter to acknowledge those who make these resources available. We are grateful therefore, to Rohan Pethiyagoda (pers. comm.) for his proposal that we recognize eight of the people who have made the DAPTF and the GAA possible by naming these new species in their honour. We would like our action to be viewed as one of the ways in which herpetologists in developing countries can acknowledge those who disinterestedly have found the will and the commitment to support amphibian conservation world-wide.

RESULTS

Molecular phylogenetics. **–** The final dataset contained mitochondrial 12S and 16S rRNA gene sequences from 53 individuals (36 from the dataset analyzed by Meegaskumbura et al. (2002) plus seventeen in addition). Fifty individuals represent Sri Lankan *Philautus*; 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*: Fig. 2: Meegaskumbura et al., 2002). As most of the divergent genera that were used by Meegaskumbura et al. (2002) in their earlier analysis were removed, alignment of the Indian and Sri Lankan *Philautus* sequences was less problematic, resulting in the inclusion of some sequence regions that were excluded in the previous analysis. Hence, of the 939 nucleotide positions sequenced, 895 were clearly allignable and were included in this analysis, whereas only 802 positions were included in the earlier analysis.

The tree with the highest likelihood resulting from the Bayesian analysis is shown in Fig. 2. The tree 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). We ran 500,000 generations of the MCMCMC algorithm and the summed likelihood of the four chains reached stationarity by 80,000 generations. The posterior probabilities of clades shown in Fig. 2 represent the frequency of those clades in the 25,000 trees sampled from the last 250,000 generations and clades with posterior probability of fifty percent or less were collapsed. The parameters of the nucleotide substitution

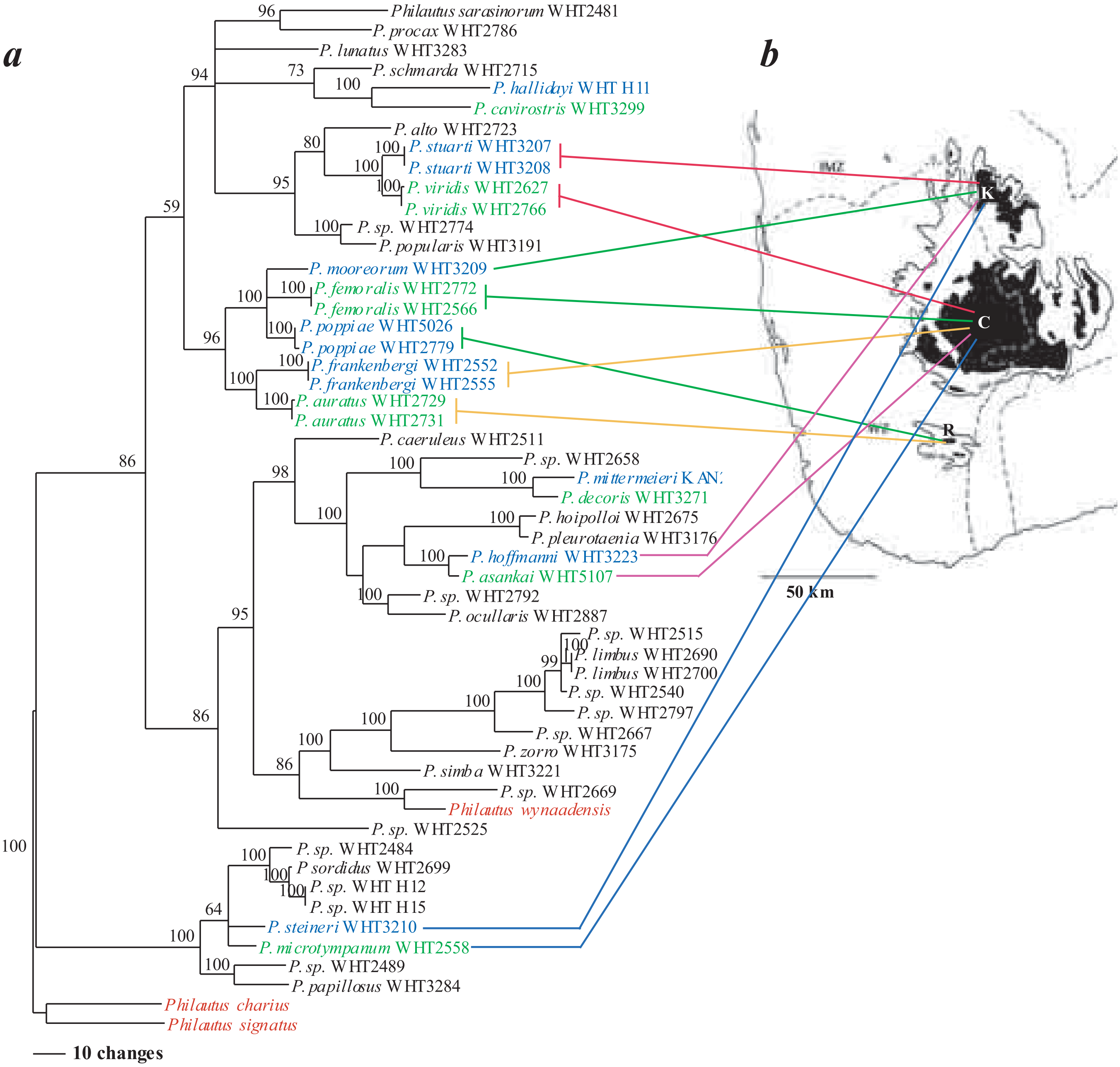

Fig. 2. *a*, The Bayesian tree from 895 base pairs of 12s and 16s rRNA genes of the Sri Lankan and Indian *Philautus* species. Each included Sri Lankan sister group is shown in blue and green (new species in blue, their sister species in green). The Indian species, including the outgroup species, are shown in red. The numbers on the branches depict the Bayesian posterior probabilities (nodes having a posterior probability of less than 50 have been collapsed). *b*, Lines indicate distribution of species that are isolated on mountain tops over 1000 m altitude (red: *P. stuarti* and *P. viridis*; light green: *P. femoralis*, *P. mooreorum* and *P. poppiae*; yellow: *P. auratus* and *P. frankenbergi*; light blue: *P. steineri and P. microtympanum*; purple: *P. hoffmani* and *P. asankai*). The three major mountain ranges are Kunckles hills (K), Central hills (C) and Rakwana hills (R).

model for the most likely tree were as follows. Rate matrix: R(G-T) = 1, R(C-T) = 78.3408, R(C-G) = 1.2399, R(A-T) = 5.5206, R(A-G) = 34.0613, R(A-C) = 6.1740. Nucleotide frequency: A = 0.3162, C = 0. 0.2502, G = 0.1877, T = 0.2457. Rate variation: shape parameter for gamma distributed rate variation among sites (alpha) = 0.745; proportion of invariant sites = 0.382. As expected, 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 (tree not shown). 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 three equally parsimonious trees (trees not shown), one of which matched the topology of the Bayesian and ML tree. The other two MP trees differed only slightly from the Bayesian and ML trees.

The 22 sequences representing previously described species are all well separated by relatively long branch lengths on the tree (see branch lengths for *Philautus* *sarasinorum, P. procax, P. schmarda, P. cavirostris, P. alto, P. viridis, P. femoralis, P. auratus, P. sordidus, P. microtympanum, P. caeruleus, P. decoris, P. hoipolloi, P. asankai, P. ocularis, P. zorro, P. pleurotaenia, P. popularis, P. simba, P. lunatus, P. papillosus and P. limbus*: Fig. 2). However, there are also several unnamed, yet distinct mitochondrial lineages on the tree that need further investigation (WHT 2774; WHT 2489; WHT 2484; WHT 2658; WHT 2792; WHT 2797; WHT 2667; WHT 2525; and WHT 2669: Fig. 2). We selected eight such lineages (labelled with their new species epithets, *P. mooreorum*, *P. poppiae, P. hoffmanni, P. frankenbergi, P. hallidayi, P. steineri, P. mittermieiri* and *P. stuarti,* shown in blue in Fig. 2) that had adequate sample sizes of voucher specimens for further investigation. Each of the mtDNA lineages that we selected for further scrutiny are robustly placed (posterior probability of 1.00) as closely related sister lineages to one of the previously recognized species (*P. femoralis, P. asankai, P. auratus, P. cavirostris, P. microtympanum, P. decoris* and *P. viridis*) (Fig. 2, Table 1), except for a single instance in which the sister relationship of *P. steineri* is not well resolved, although it is placed in a clade together with *P. microtympanum* and *P. sordidus,* which was moderately supported with a posterior probability of 0.64. The uncorrected molecular distance between the new species and their sister species, for the combined 12S and 16S gene fragment, ranged between 1% and 8% (Table 1).

We further tested the robustness of the mitochondrial lineages that were selected as potential species by sequencing a 590 bp fragment of the mitochondrial cytochrome-b gene, which has been widely used in systematics and taxonomy across vertebrate taxa (Johns & Avise, 1998). We generated 21 sequences representing the eight new species described herein and their immediate sister taxa (the same individuals sequenced for the 12S and 16S fragments were sequenced for the cytochrome-b fragment). An aim of this analysis was to compare how the eight putative new *Philautus* species compare with trends in molecular divergence among other vertebrate sister species. Johns & Avise (1998) showed that 90% of putative vertebrate species, including amphibians, differ by more than 2% genetic distance in the cytochrome-b gene (uncorrected for unobserved multiple substitutions). The uncorrected genetic distance between the eight new species described herein and their respective sister species ranges from 5.8–15.7% (Table 2), which are moderate to large mtDNA distances consistent with the lineages representing different species. This conclusion is supported by analysis of morphological variation within and among these sister lineages.

Table 1. Percent pairwise uncorrected molecular distance between the various sister-species groups using a 895 bp fragment of the 12s and 16s gene, analyzed using PAUP (v. 4b10). Montane isolates found above 1,000 m altitude are indicated with an asterisk (*).

sister species	uncorrected pairwise molecular distance (%)
P. asankai – P. hoffmanni*	1.01
P. auratus – P. frankenbergi*	3.00 – 3.13
P. decoris – P. mittermeieri	2.38
P. viridis – P. stuarti*	1.45 – 1.56
P. microtympanum – P. steineri*	2.34
P. femoralis – P. mooreorum*	3.15
P. femoralis – P. poppiae*	2.56 – 2.78
P. mooreorum – P. poppiae*	2.56 – 2.80
P. cavirostris – P. hallidayi	7.90

Table 2. The percent pairwise uncorrected molecular distance between the sister species groups using a 590 bp fragment of the cytochrome-b gene, analyzed in the program PAUP (v. 4b10) and the time of divergence of the sister species estimated using 0.69 – 1.00% rate of change per million years. Montane isolates found above 1,000 m are indicated with an asterisk (*).

sister species	pairwise uncorrected molecular distance (%)	molecular divergence estimate (mya)
P. asankai – P. hoffmanni*	6.04	4.16 – 6.04
P. auratus – P. frankenbergi*	11.22 –11.97	7.74 –11.97
P. decoris – P. mittermeieri	5.80	4.00 – 5.80
P. viridis – P. stuarti*	6.34 – 6.93	4.37 – 6.93
P. microtympanum – P. steineri*	11.06	7.63 –11.06
P. femoralis – P. mooreorum*	8.0	5.52 – 8.0
P. femoralis – P. poppiae*	8.9	6.14 – 8.90
P. mooreorum – P. poppiae*	7.7	5.31 – 7.70
P. cavirostri – P. hallidayi	15.7	10.83 –15.70

Morphological analysis. **–** For each of the novel species, there is a combination of consistent morphological character states that defines the species and that does not vary within a given population. These include shagreened, horny, or smooth body surfaces; snout-angle categories; distinctness of the tympanum; shape of inter-orbital space; shape of snout in lateral aspect; presence or absence of glandular warts on skin; presence or absence of sheath-like undulating membranes on the posterior margins of limbs; presence or absence of vomerine ridges; cross-bars on limbs; shapes of canthal edges; webbing pattern on toes; angle of vomerine ridge to body axis; presence or absence of calcar on tibiotarsal fringe; shape of loreal region; presence or absence of dermal fringes on fingers; distinctiveness of supratympanic fold; consistent markings on body; presence or absence of lingual papilla; and differences in colour. Diagnostic character state sets and differences among species are discussed in the species descriptions below.

All the species recognized were effectively differentiated using principal components analysis (Figs. 5a, 5b, 16, 20, 24, 28, 32). Most of the species pairs differed most strongly on the first principal component, which in all cases represented a size axis (snout-vent length). However, in one group (the *P. femoralis, P. mooreorum* and *P. stuarti* clade), *P. femoralis* differed from the other species on the second principal component as well, which represented differences in the proportional size of hands and feet. In contrast to all others, *P. hallidayi* and *P. cavirostris* differed in head dimensions (represented on the second principal components axis), as well as size. The clear discrimination of the new species from their sister species in multivariate morphological space further supports our contention that these species represent independent evolutionary lineages that warrant formal recognition. Below we detail the comparisons of morphometric variation among sister species.

Principal components analysis with unrotated axes on the correlation matrix of continuous characters from *Philautus mooreorum* from Knuckles Hills, *P. femoralis* from the Central Hills and *P. poppiae* from the Rakwana Hills showed clear separation of the three species on two axes (Fig. 5a). Of the total variance, 79.5% was explained by PC1, which represents a size axis, with body size (snout–vent length) having the highest loading and with all other variables having high positive loadings. Manus and toe dimensions (which load negatively, but with relatively large absolute values) explain 7.3% of the total variance. All three species separate out well on the first principal component axis (PC1), with size ascending in the following order: *P. poppiae*, *P. femoralis*, *P. mooreorum*. *Philautus femoralis* separates well from *P. poppiae* and *P. mooreorum* on the second principal component axis (PC2) as well, with *P. femoralis* having relatively larger palms, fingers and toes, for its body size when compared to the other two species. *Philautus poppiae* and *P. mooreorum* almost completely overlap on PC2 and hence are indistinguishable on this axis.

The name-bearing type specimen of *P. femoralis* (BMNH1947.2.26.89) is a female and is not accompanied by sufficient information to determine its collection locality within Sri Lanka. However, based on morphometric data, it appears to be a member of the Central Hills population (Fig. 5b). Principal components analysis including the holotype of *P. femoralis* with females and males from the three prominent mountain ranges revealed that the holotype overlaps with individuals from the Central Hills in size (and, like the Central Hills specimens, it is distinct from those of the Rakwana and Knuckles mountains). However, the finger and palm dimensions are small in BMNH1947.2.26.89, which distinguishes it from the other three species, including the Central Hills population of *P. femoralis,* on PC2. Smaller hands and feet may be the result of shrinkage in preservative, but until further evidence is available, we follow Manamendra-Arachchi & Pethiyagoda (2005) in regarding the Central Hills population to be *P. femoralis* sensu stricto.

Unrotated principal components analysis effectively separates *Philautus mittermeieri* and *P. decoris* on a single axis (PC1; Fig. 16). Of the total variance, 69.35% is explained by PC1, which is a size axis (the highest factor loading was for SVL and all other variables had high positive values). *Philautus mittermeieri* and *P. decoris* separate well on PC1 (size), *P. decoris* being larger than *P. mittermeieri*. Of the total variance, 9.1% is explained by PC2, which reflects variance in eye diameter and distance between eyes. This result, however, is uninformative as the two species show nearly complete overlap on this axis.

Philautus frankenbergi and *P. auratus,* with no rotation (Fig. 20), separate along a single PC axis. Of the total variance, 90.1% was explained by PC1, which again is a size axis. PC2, which represents variance in inter-narial distance, explains 3.9% of the total variance. *Philautus frankenbergi* and *P. auratus* separate out well on PC1, with *P. frankenbergi* having a larger body size relative to *P. auratus*. There is nearly complete overlap on PC2, which is uninformative with regard to species diagnosis.

Philautus hallidayi and *P. cavirostris* also separate on a single axis (Fig. 24). With a varimax rotation of axes, 36% of the total variance is explained by PC1which represents limb dimensions (length of femur, tibia, foot, lower arm, palm and finger load positively and most heavily). However, the two species show nearly complete overlap on PC1 and hence are not diagnosable by limb dimensions. In contrast, the two species separate well on PC2, which explains 33% of the variance and represents variation in head-eye dimensions (distance between front of eyes, eye-to-snout distance and inter-orbital distance load positively on PC2). Thus, *P. hallidayi* can be distinguished from *P. cavirostris* by having a smaller eye-to-snout distance, lesser distance between front of eyes, and lesser inter-orbital distance.

Philautus steineri and *P. microtympanum* also separate well in PC space along a size axis (PC1; Fig. 28), with *P. steineri* being larger than *P. microtympanum.* With unrotated axes, 94.3% of the total variance was explained by the PC1 (size). Only 2.2% of the total variance was explained by PC2, which represented palm length (negative loading), finger 3 length (negative loading) and lower-arm length. *Philautus steineri* and *P. microtympanum* overlapped almost completely on PC2.

Philautus stuarti and *P. viridis* also show separation by body size, with *P. viridis* being larger than *P. stuarti* (Fig. 32). With unrotated axes, 74.0% of the total variance is explained by variance along PC1, which again represents body size. Variance along PC2 explains 7.0% of the total variance, representing eye diameter, distance between front of eyes (negative loading) and inter-orbital distance (negative loading), but is largely uninformative with regard to species differences.

Bioacoustics analysis. – Bioacoustic data were limited in that we were able to obtain good-quality recordings from multiple males only from the *P. viridis* (n=4) */ P. stuarti* (n=3), and *P. hoffmanni* (n=3) */ P. asankai* (n=3) species pairs. For these pairs, bioacoustics analysis showed clear differences in call characteristics between the sister species (Table 3). The calls of both *P. hoffmanni* and *P. asankai* comprised a series of short whistles. These calls differed in call length, pulse rate, dominant frequency, fundamental frequency, pulse length, and the number of pulses per call (Fig. 12). *Philautus hoffmanni* has a greater call length than *P. asankai* (0.0638 – 0.692 s vs. 0.385 – 0.409 s), greater pulse rate (12.52 – 12.60 s^{-1}, vs.11.62 – 11.68 s^{-1}), lower fundamental and dominant frequency (2,386 – 2,399 Hz, vs. 2,859 – 2,870 Hz), shorter pulse length (0.048 – 0.051 s, vs. 0.069 – 0.071 s) and a greater number of pulses per call (9 pulses, vs. 3–4 pulses). For these

Table 3. Bioacoustics characters for two pairs of sister taxa: *P. stuarti* – *P. viridis* and *P. asankai* – *P. hoffmanni*.

character	*P. stuarti* (n=3)	*P. viridis* (n=4)	*P. asankai* (n=2)	*P. hoffmanni* (n=3)
Call length (s)	1.713 – 1.805	1.018 – 1.508	0.385 – 0.409	0.638 – 0.692
Pulse rate (s^{-1})	3.00 – 3.09	2.09 – 2.12	11.62 – 11.68	12.52 – 12.60
Dominant frequency (Hz)	2,520 – 2,539	2,315 – 2,328	2,859 – 2,870	2,386 – 2,399
Fundamental frequency (Hz)	2,520 – 2,539	2,315 – 2,328	2,859 – 2,870	2,386 – 2,399
Pulse length (s)	0.051 – 0.059	0.077 – 0.080	0.069 – 0.071	0.048 – 0.051
Frequency modulation within call	No	No	No	No
Frequency modulation within pulse	No	No	No	No
Number of pulses per call	6	3 – 4	3 – 4	9

two species, there was no frequency modulation within the call or a given pulse and the fundamental frequency is also the dominant frequency (Fig. 12). Temperature and relative humidity at which the calls were recorded were 16° C and 85% for *P. asankai* and 19° C and 87% for *P. hoffmanni*.

The calls of both *P. stuarti* and *P. viridis* comprised a series of terse ticks. These calls differed in call length, pulse rate, dominant frequency, fundamental frequency, pulse length, and the number of pulses per call (Fig. 33). *Philautus stuarti* has a greater call length than *P. viridis* (1.713 – 1.805 s vs. 1.018 – 1.508 s), greater pulse rate (3.00 – 3.09 s^{-1}, vs. 2.09 – 2.12 s^{-1}), greater fundamental and dominant frequency (2,520 – 2,539 Hz, vs. 2,315 – 2,328 Hz), shorter pulse length (0.051 – 0.059 s, vs. 0.077 – 0.080 se and a greater number of pulses per call (6 pulses vs. 3–4 pulses). For these two species, there was no frequency modulation within the call or a given pulse and the fundamental frequency is also the dominant frequency (Fig. 33). Temperature and relative humidity at which the calls were recorded were 16° C and 90% for *P. viridis* and 18° C, 87% for *P. stuarti.*

Call characteristics are affected by temperature and body size (Ryan, 1985; Duellman & Trueb, 1986; Sullivan, 1992). However, Sullivan (1992) has shown that the characteristic frequency of a call and call rate do not change significantly over a 10° C temperature range. The calls analyzed here were recorded at temperatures within 3° C of each other and at similar levels of relative humidity. We therefore discount the possibility that the distinctive vocalizations of these species are the result of differences in temperature.

TAXONOMY

Philautus mooreorum, new species
(Figs. 3, 4)

Material examined. – Holotype - male, 30.3 mm SVL, WHT 5862, Hunnasgiriya (Knuckles), elevation 1,100 m (07º23' N, 80º41' E), coll. 17 Oct.2003.

Paratypes - females, 35.0 mm SVL, WHT 2477, Corbett's Gap (Knuckles), 1,245 m (07º 22' N, 80º 51' E) coll. 6 Jun.1999; 33.8 mm SVL, WHT 6124, Corbett's Gap (Knuckles), 1,245 m (07º 22' N, 80º 51' E) coll. 16 Jun.2004; males, 30.3 mm SVL, WHT 5868, Hunnasgiriya, same data as holotype, coll. 16 Oct.2003; 31.3 mm SVL, WHT 3209, Corbett's Gap (Knuckles), coll. 29 Jun.2001; 29.4 mm SVL, WHT 5869, same data as holotype.

Fig. 3. *Philautus mooreorum,* new species, WHT 5862, holotype male, 30.3 mm SVL.

Diagnosis. – (Figs. 4, 5). *Philautus mooreorum* is distinguished from all other Sri Lankan congeners by a combination of the following characters: mature males 29.4–31.3 mm SVL; tympanic membrane absent; snout-angle category 7–9; canthal edges rounded; supratympanic fold, lingual papilla, vomerine teeth, supernumerary tubercles and calcar absent; limbs dorsally shagreened; toes medially webbed; male without nuptial pads; dorsally 'luminous green' in life.

Description. – (Figs. 3–5). Mature males 29.4–31.3 mm SVL; mature female 33.8–35.0 mm SVL. Body stout. Head dorsally flat. Snout-angle category 7–9 (angle of snout 108º–115º); snout rounded in lateral aspect. Canthal edges rounded. Loreal region concave. Interorbital space flat. Internasal space concave. Tympanic membrane absent, tympanic rim absent. Supratympanic fold indistinct or absent. Pineal ocellus, vomerine ridge, cephalic ridges, calcar, lingual papilla and co-ossified skin on skull absent. A lateral dermal fringe present on fingers. Toes webbed. Tarsal folds absent. Snout, interorbital space, side of head and dorsum, and upper part of flank with horny spinules in males. Lower flank smooth. Dorsal part of forelimb, thigh, shank and pes shagreened. Throat, chest and belly granular; underside of thigh granular. Inner vocal slits present in males, nuptial pad absent (but yellow subdermal glands present on finger 1). Dorsum finely granular or shagreened in female.

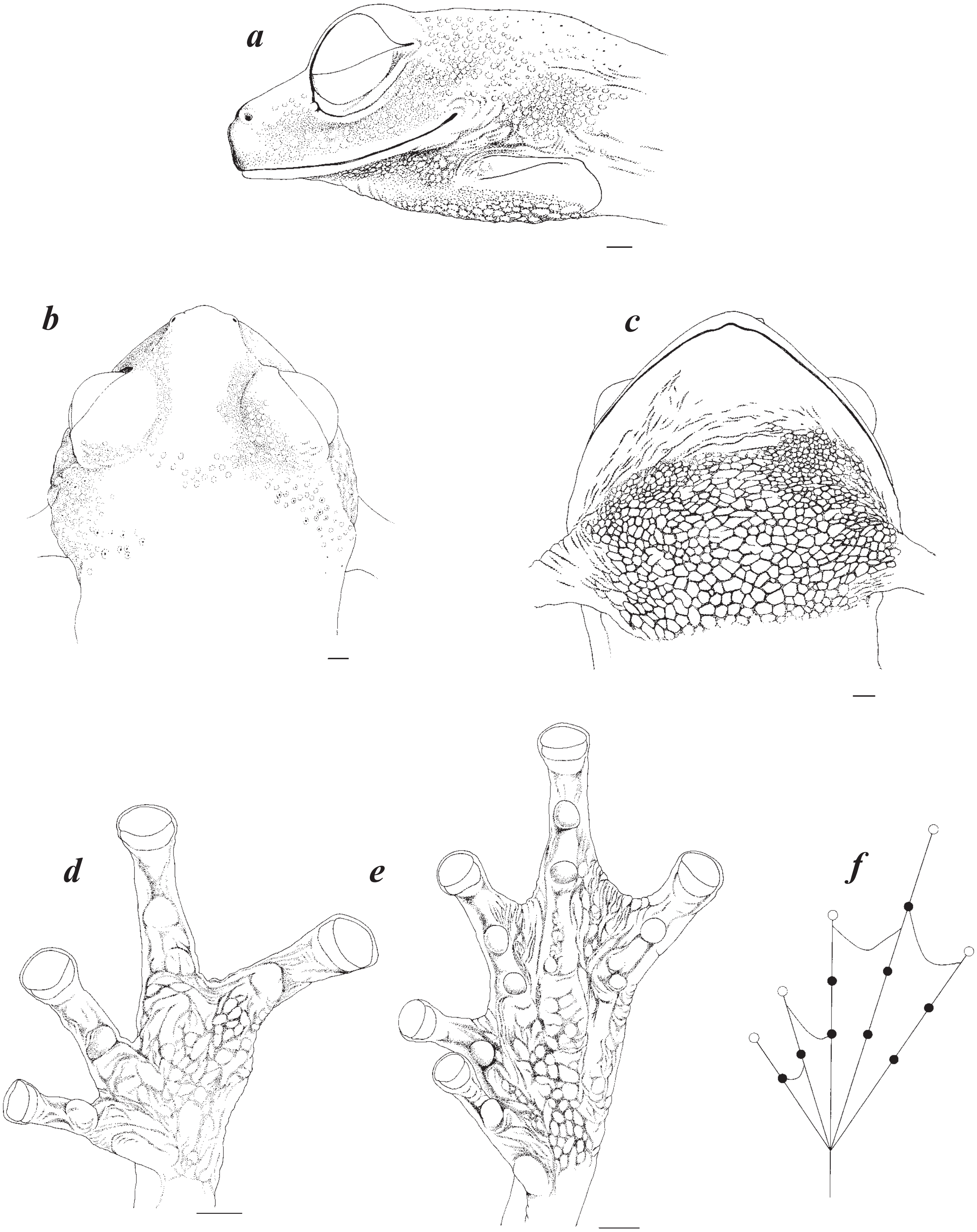

Fig. 4. *Philautus mooreorum,* new species: *a–c*, lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left hand; *e*, ventral aspect of left foot; and *f*, semi-diagrammatic representation of the left-foot webbing-pattern of holotype, male, WHT5862, 30.3mm SVL. Scale bar: 1 mm.

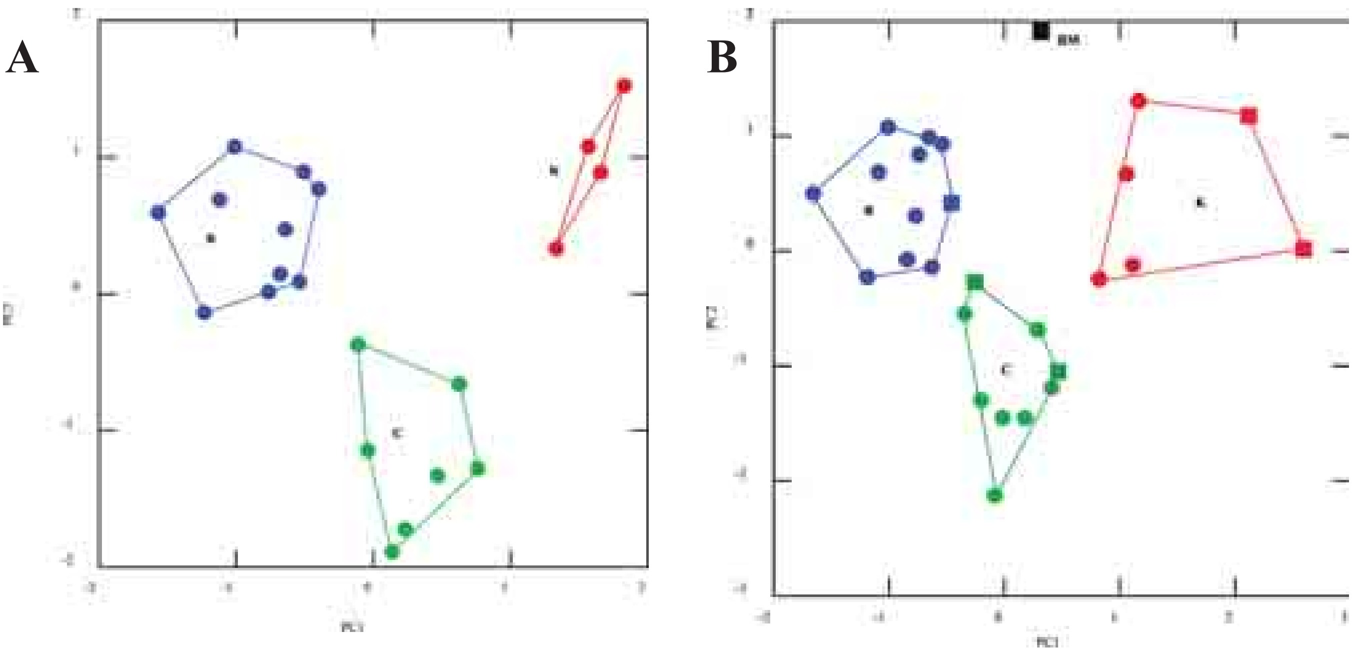

Fig. 5. A, PC1 vs. PC2 factor scores of the principal components analysis of male *Philautus mooreorum* (Knuckles hills, K), *P. femoralis* (Central ills, C) and *P. poppiae* (Rakwana hills, R). Most of the variation is explained by body size, manus and toe dimensions. All three species separate well on the PC1 axis in relation to body size (*P. mooreorum* is the largest and *P. poppiae* is the smallest). *Philautus mooreorum* separates out well on the PC2 axis in having palms, fingers and toes relatively larger in comparison to the other two species. *Philautus poppiae* and *P. mooreorum* almost completely overlap on the PC2 axis. B, PC1 vs. PC2 factor scores of the principal components analysis of female *Philautus mooreorum* (Knuckles hills, K), *P. femoralis* (Central hills, C) and *P. poppiae* (Rakwana hills, R) and the holotype of *P. femoralis* BMNH1947.2.26.89 (BM). Most of the total variation is explained by the PC1 axis, which relates mostly body size (*P. mooreorum* is the largest and *P. poppiae* the smallest; *P. femoralis* and the BMNH holotype overlap in size). *Philautus femoralis* and its holotype separate out from the other two species on the PC2 axis, which relates mostly to manus dimensions; the type and the central hills population do not, however, overlap in this respect. The males are represented by circles, the females by squares.

Colour in life. **–** (Fig. 3). Head dorsally and laterally bright 'luminous' green. Upper flank yellow and white, lower flank white. Margins of both lips white. Limbs dorsally green. Outer edge of lower arm and pes white. Dorsal area of upper arm white. Fingers and toes dorsally white or pale green. Disks white. Venter white.

Colour in alcohol. **–** Dorsally and laterally brownish dark pink, with or without white pigments. Lower flank, venter, upper lip and inguinal zone yellow.

Measurements of holotype. **–** (WHT 5862, in mm) DBE, 11.4; DFE, 6.4; DL, 1.2; DW, 1.5 ED, 4.2; EN, 3.5; ES, 5.1; FEL, 16.0; FL I, 2.4; FL II, 3.1; FL III, 5.0; FL IV, 4.1; FOL, 20.4; HL, 12.3; HW, 13.6; IML, 1.1; IN, 2.6; IO, 3.8; LAL, 6.7; MBE, 4.5; MFE, 8.4; MN, 11.2; NS, 1.5; PAL, 9.1; SVL, 30.3; TBL, 15.5; TL I, 2.4; TL II, 2.6; TL III, 4.7; TL IV, 6.7, TL V, 5.3; TYD, – (tympanum indistinct); TYE, – (tympanum indistinct); UAW, 6.4; UEW, 2.8.

Distribution. **–** (Fig. 6) *Philautus mooreorum* is currently known from the type locality, Corbett's Gap, and from Hunnasgiriya, both in the Knuckles hills.

Etymology. **–** The species epithet is an eponym in the Latin genitive plural honouring the benefactors of the Moore Foundation, Dr. Gordon and Betty Moore (b. California, 1929 and 1928, respectively), in appreciation of their support of the Global Amphibian Assessment and decades of philanthropic work in science and conservation.

Remarks. **–** The *P. femoralis* group comprises three species: *P. femoralis* (Günther, 1864), *P. mooreorum* and *P. poppiae* (Figs. 2, 5), which are separated from each other by a 12S and 16S sequence divergence of 2.56–3.15% and cytochrome-b sequence divergence of 7.7–8.9% (Tables 1, 2).

Philautus mooreorum keys out as *P. femoralis* according to the morphological key of Manamendra-Arachchi & Pethiyagoda (2005). It may be distinguished from *P. femoralis* however, by having the limbs dorsally shagreened (vs. limbs dorsally smooth in *P. femoralis*), and its large size (adult male snout-vent length to 31.3 mm, vs. 27.5 mm in *P. femoralis*).

Philautus mooreorum is further distinguished from *P. poppiae*, by having the head dorsally flat (vs. head dorsally convex in *P. poppiae*); limbs dorsally shagreened (vs. limbs dorsally horny and spinulated); no black dots on dorsum (vs. black dots present on dorsum); and snout-angle category of 7–9 (vs. snout-angle category of 6 or 7). We note that unusually for Sri Lankan *Philautus, P. mooreorum* shows significant variation in snout-angle, ranging from 108°–115°.

Principal components analysis of metric data (Fig. 5a) shows clear separation of the three species in body size (snout–vent length). *Philautus mooreorum* may further be

Fig. 6. Distribution of *Philautus mooreorum,* new species, in Sri Lanka.

distinguished from *P. femoralis* by having relatively smaller palm, fingers and toes.

Philautus mooreorum is the largest of the species in the *P. femoralis* group, with a male snout–vent length range of 29.4 –31.3 mm; and *P. poppiae* the smallest (male snout–vent length 21.3–24.7 mm). *Philautus femoralis* is intermediate in size, with a male snout–vent length range of 26.3–28.8 mm.

The holotype of the *P. femoralis* (BMNH 1947.2.26.89) is a female. In principal components analysis this specimen clearly separates from the other members of the group (Fig. 5b). Though it may be distinguished from the other species by its relatively smaller manus dimensions, it overlaps with *P. femoralis* in SVL. Until further evidence is available, we follow Manamendra-Arachchi & Pethiyagoda (2005) in considering the Central Hills population as conspecific with *P. femoralis* sensu stricto.

Though *P. mooreorum* has been recorded from elevations of 1,100–1,245 m, we expect it to be present on the higher peaks of the Knuckles mountain range where suitable cloud forest habitat persists. *Philautus femoralis* occurs only in the higher parts (above 1,000 m) of the Central Hills, whereas *P. poppiae* is restricted to the Rakwana Hills, at elevations between 1,060 and 1,270 m. These species are isolated from one other by the deep valleys that separate these mountain ranges.

Specialised to the forest sub-canopy and shrubs in the understorey of closed-canopy cloud forest (though occurring also in areas under-planted with cardamom), the three species of the *P. femoralis* group occupy similar microhabitats. Males vocalize 1–3 m above ground, perched on leaves (the species is strictly arboreal). We noted that the density of all three species is greater in somewhat marshy habitats, possibly because these frogs depend for reproduction on environments with high relative humidity (Bahir et al., 2005).

Fig. 7. *Philautus poppiae,* new species, WHT 3285, holotype male, 24.7 mm SVL.

Conservation status. **–** (Fig. 5). The limited Extent of Occurrence (~10 km^2, in the Corbett's Gap and Hunnasgiriya region of the Knuckles mountain range), suggests that the species should be considered Endangered (criteria B1 a, b(iii)). Restriction to a single forest site, low abundance and strictly arboreal behaviour renders these frogs susceptible to habitat modification (forest clearing) and stress during periods of drought, with chemicals used in cardamom agriculture also posing a potential threat. We advocate periodic monitoring to assess population trends and address possible negative impacts.

Philautus poppiae, new species
(Figs. 7, 8)

Material examined. **–** Holotype - male, 24.7 mm SVL, WHT 3285, Handapan Ella Plains (near Suriyakanda), elevation 1,270 m (06º26'42"N, 80º36'35"E), coll. 5 Jul.2001.

Paratypes - males, 22.7 mm SVL, WHT 2030, coll. 5 Aug.1997; 21.3 mm SVL, WHT 2029, coll. 5 Aug.1997; 23.9 mm SVL, WHT 2475, coll. 29 May.1999; 24.0 mm SVL, WHT 2778, coll. 24 Jul.1999; 23.0 mm SVL, WHT 2781, coll. 24 Jul.1999; 22.5 mm SVL, WHT 3533; 22.5 mm SVL, WHT 3534; 24.1 mm SVL, WHT 3535; 24.3 mm SVL, WHT 3536, coll. 14 Jan.1999, Morningside (near Rakwana), elevation 1060 m (06º24' N, 80º38' E).

Others - female, 26.0 mm SVL, WHT 3543, Morningside (near Rakwana), elevation 1060 m (06º24' N, 80º38' E), coll. 22 Feb.1996. Juvenile, 18.6 mm SVL, WHT 3274, Morningside (near Rakwana), elevation 1060 m (06º24' N, 80º38' E), coll. 14 Jul.2001.

Diagnosis. **–** (Fig. 8). *Philautus poppiae* is distinguished from all other Sri Lankan congeners by a combination of the

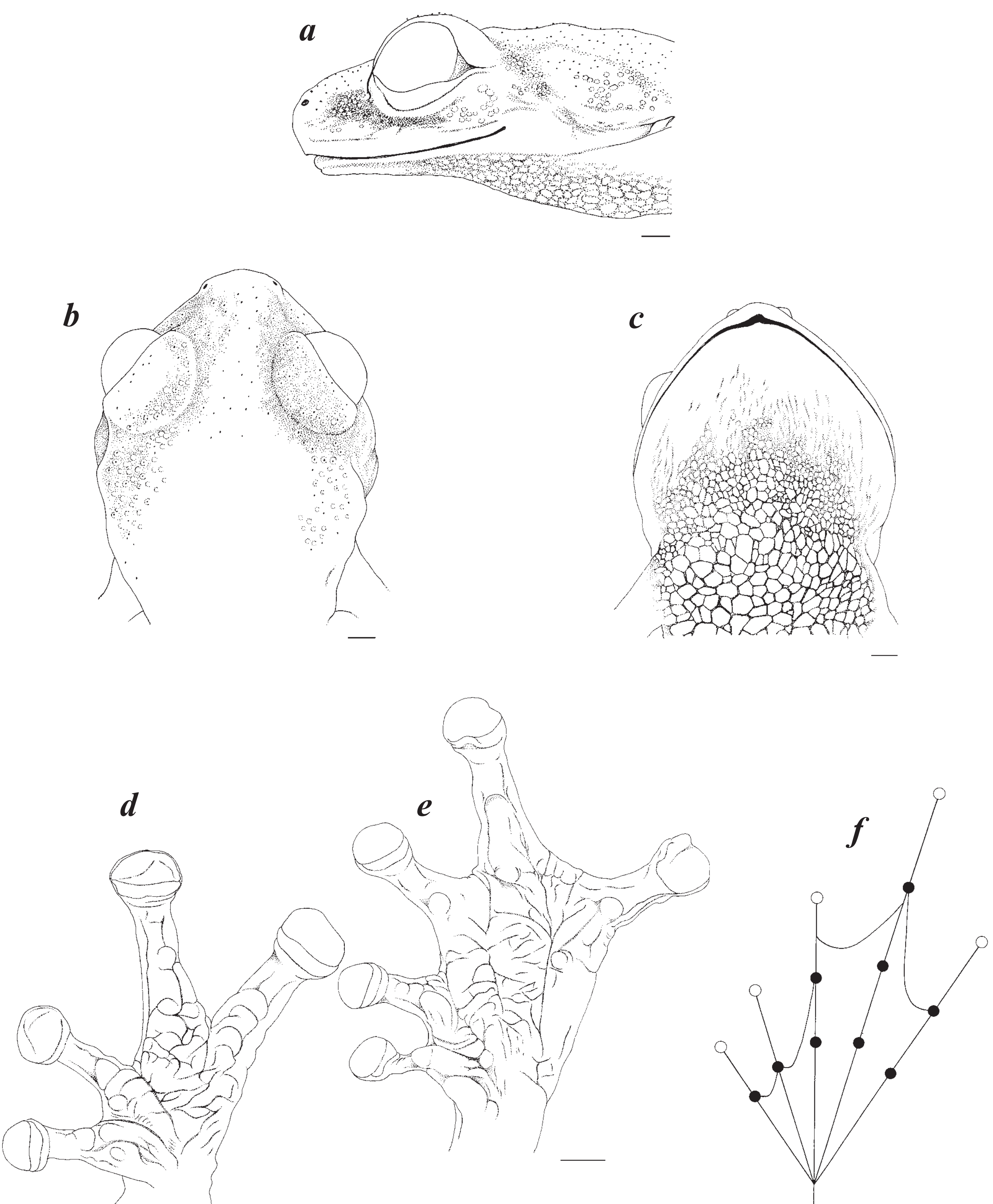

Fig. 8. *Philautus poppiae,* new species: *a–c*, lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus; *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT3285, 24.7 mm SVL. Scale bar: 1 mm.

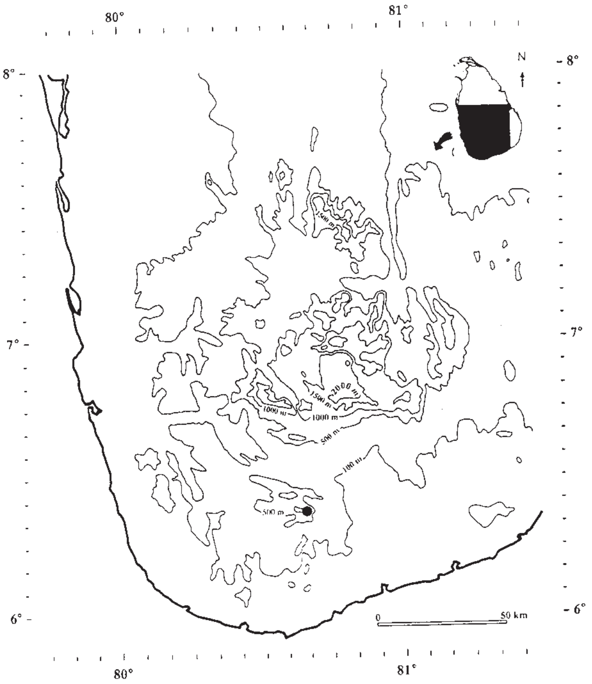

Fig. 9. Distribution of *Philautus poppiae,* new species, in Sri Lanka.

following characters: mature males 21.3–24.7 mm SVL; tympanum membrane absent; lower tympanic rim distinct; snout-angle category 6 or 7; dorsal surface of body with horn-like spinules in males; canthal edges rounded; supratympanic fold present; lingual papilla, vomerine teeth, supernumerary tubercles and calcar absent; toes medially webbed; male without nuptial pads; belly granular; dorsally 'luminous green' and dorsum with black dots in life.

Description. – (Figs. 6, 7). Mature males 21.3–24.7 mm SVL; mature female 26.0 mm SVL. Body stout. Head dorsally convex. Snout-angle category 6 or 7 (angle of snout 100º–108º); snout rounded in lateral aspect. Canthal edges rounded. Loreal region concave. Interorbital space flat. Internasal space concave. Tympanic membrane absent, lower tympanic rim distinct. Supratympanic fold present. Pineal ocellus, vomerine ridge, cephalic ridges, calcar, lingual papilla and co-ossified skin on skull absent. A lateral dermal fringe present on fingers. Toes webbed. Tarsal folds absent. Snout, interorbital space, side of head and dorsum, upper part of flank, dorsal area of forelimb, thigh, shank and pes with horny spinules in males. Lower flank smooth. Throat, chest and belly granular; underside of thigh smooth. Inner vocal slits present in males; nuptial pad absent, but yellow subdermal glands present on finger 1. Dorsum finely granular or shagreened in female.

Colour in life. – (Fig. 7). dorsal and lateral parts of head and dorsum bright 'luminous' green; some specimens with yellow or red spots on dorsum. Dorsum spotted with black. Flank yellow. Inguinal zone and anterior thigh brownish-yellow. Edges of both lower and upper lips yellow or white. Upper arm yellow or greenish-yellow dorsally. Outer edge of lower arm with a longitudinal white band. Inner side of both upper and lower arms yellow. Outer edges of shank, pes and toe 5 white. Pes ventrally white. Venter pale yellow.

Colour in alcohol. – Dorsum and flanks ashy pink with whitish yellow (rarely with red) patches and scattered black dots. Lower flank yellow. Upper lip yellow. Limbs dorsally ashy pink with scattered black dots. Posterior thigh yellow. Venter pale yellow.

Measurements of holotype. – (WHT 3285, in mm) DBE, 9.2; DFE, 5.0; DL, 1.1; DW, 1.5 ED, 3.6; EN, 3.0; ES, 4.2; FEL, 10.9; FL I, 2.1; FL II, 2.5; FL III, 4.5; FL IV, 4.3; FOL, 16.6; HL, 10.3; HW, 10.1; IML, 1.1; IN, 2.1; IO, 3.1; LAL, 5.4; MBE, 3.7; MFE, 6.5; MN, 9.2; NS, 1.2; PAL, 7.7; SVL, 24.7; TBL, 12.2; TL I, 2.2; TL II, 2.6; TL III, 3.9; TL IV, 5.7, TL V, 4.0; TYD, – (tympanum indistinct); TYE, – (tympanum indistinct); UAW, 4.4; UEW, 2.2.

Distribution. – (Fig. 9). *Philautus poppiae* shows a very restricted distribution, being known only from the type locality (Handapan Ella, elevation 1,270 m; 06º26'42"N, 80º36'35"E) and Morningside Forest, near Rakwana (elevation 1,060 m; 06º24' N, 80º38' E, about 10 km from the type locality).

Etymology. – The species epithet is an eponym, Latinized in the feminine genitive singular, for Poppy Valentina Meyer (b. 27 Nov.2003), in honor of her parents, George A. Meyer (b. Pennsylvania, 1956) and Maria Semple (b. California, 1964), for their support of the Global Amphibian Assessment and ongoing commitment to amphibian conservation around the world.

Remarks. – According to the key of Manamendra-Arachchi & Pethiyagoda (2005), *P. poppiae* keys out as *P. femoralis* (Günther, 1864). *Philautus poppiae* may be distinguished from *P. femoralis* by having dorsal surface of head flat (vs. dorsal surface of head convex in *P. femoralis*); limbs dorsally horny, spinulated (vs. limbs dorsally smooth); black dots present on dorsum (vs. black dots absent on dorsum); and snout-angle category of 6 or 7 (vs. snout-angle category 8). It may be distinguished from *P. mooreorum* by having the head dorsally convex (vs. head dorsally flat in *P. mooreorum*); limbs dorsally horny and spinulated (vs. limbs dorsally shagreened); black dots present on dorsum (vs. black dots absent on dorsum); snout angle category 6 or 7 (vs. snout angle category of 7–9).

Philautus poppiae is the smallest of the three species in the *P. femoralis* group, with a male snout–vent length up to 24.7 mm (see also Remarks in account of *P. mooreorum*). While the manus and pes dimensions of *P. poppiae* are similar to those of *P. mooreorum,* they are distinctly less than those of *P. femoralis* (Fig. 5).

Philautus poppiae occurs only in the montane cloud forests in the Rakwana mountains, above 1,060 m elevation, while *P. mooreorum* and *P. femoralis* are restricted to the cloud forests of the Knuckles and Central Hills, respectively. Only a very small part of the Rakwana Hills exceed an elevation of 1,000 m, from which we conclude that the area of occupancy of *P. poppiae* is extremely small, even if it were to occur on peaks as yet unexplored.

Conservation status. **–** (Fig. 9). Extent of Occurrence, c. 10 km^2: Morningside and Handapan Ella region of the Rakwana Hills. Outcome: Endangered (criteria B1 a, b(iii)). Restriction to a single forest site, low abundance and strictly arboreal behaviour renders these frogs susceptible to habitat modification (forest clearing) and stress during periods of drought, with chemicals used in cardamom agriculture also posing a potential threat. We advocate periodic monitoring of this population to assess trends and address negative impacts.

Philautus hoffmanni, new species
(Figs. 10, 11)

Material examined. **–** Holotype - male, 23.4 mm SVL, WHT 6120, Corbett’s Gap (Knuckles Hills), elevation 1,245 m (07º22’ N, 80º51’ E), coll. 15 Jan.2004.

Paratypes - males, 21.2 mm SVL, WHT 3542; 21.7 mm SVL, WHT 3223; 23.1 mm SVL, WHT 3222, coll. 29 Jun.2001, all from type locality.

Diagnosis. **–** (Fig. 11). *Philautus hoffmanni* is distinguished from all other Sri Lankan congeners by a combination of the following characters: mature males 21.2–23.4 mm SVL; tympanum present, distinct; snout-angle category 6 or 7; snout rounded in lateral aspect; interorbital space flat; fine, horny spinules absent on lower flank; canthal edges rounded; vomerine ridge and lingual papilla absent; nuptial pads absent in males.

Description. **–** (Figs. 10, 11). Mature males 21.2–23.4 mm SVL. Body stout. Head dorsally flat. Snout-angle category 6 or 7 (angle of snout 103º–107º); snout rounded in lateral aspect. Canthal edges rounded. Loreal region flat. Interorbital space flat. Internasal space flat. Tympanum distinct. Supratympanic fold indistinct. Pineal ocellus, vomerine ridge, cephalic ridges, lingual papilla and co-ossified skin on skull absent. A lateral dermal fringe present on fingers. Toes webbed. Tarsal folds and calcar absent. Snout, interorbital space, side of head, dorsum and upper flank smooth; lower flank glandular. Dorsal part of forelimb, thigh, shank and pes smooth. Throat, chest and belly granular; under thigh smooth. Inner vocal slits present in males and nuptial pad absent.

Colour in life. **–** (Fig. 10) (based on WHT 3222). Dorsally ash and brown. Loreal region brown. Both upper and lower lips ash. Limbs dorsally ashy brown. Digits dorsally bright yellow and ash. Flank ash. Inguinal zone, posterior and anterior thigh ash with golden-yellow dots. Ventral aspect of digits, limbs and body golden yellow.

Colour in alcohol. **–** Dorsal parts of head and body greyish-brown with brown dots. Upper flank brown and lower flank yellow. Inguinal zone brown. Loreal region greyish-brown, tympanic region and tympanum brown. Limbs dorsally greyish brown. Venter yellow.

Measurements of holotype. **–** (WHT 6120, in mm), DBE, 8.5; DFE, 4.8; DL, 1.1; DW, 1.5; ED, 3.0; EN, 2.5; ES, 4.0; FEL, 10.5; FL I, 1.8; FL II, 2.4; FL III, 3.8; FL IV, 3.1; FOL, 14.7; HL, 9.5; HW, 9.7; IML, 1.1; IN, 2.2; IO, 2.8; LAL, 4.4; MBE, 3.1; MFE, 5.7; MN, 8.2; NS, 1.6; PAL, 7.2; SVL, 23.4; TBL, 11.2; TL I, 1.9; TL II, 2.1; TL III, 3.5; TL IV, 5.4, TL V, 3.9; TYD, – outer rim of tympanum indistinct; TYE, –outer rim of tympanum indistinct; UAW, 3.6; UEW, 2.1.

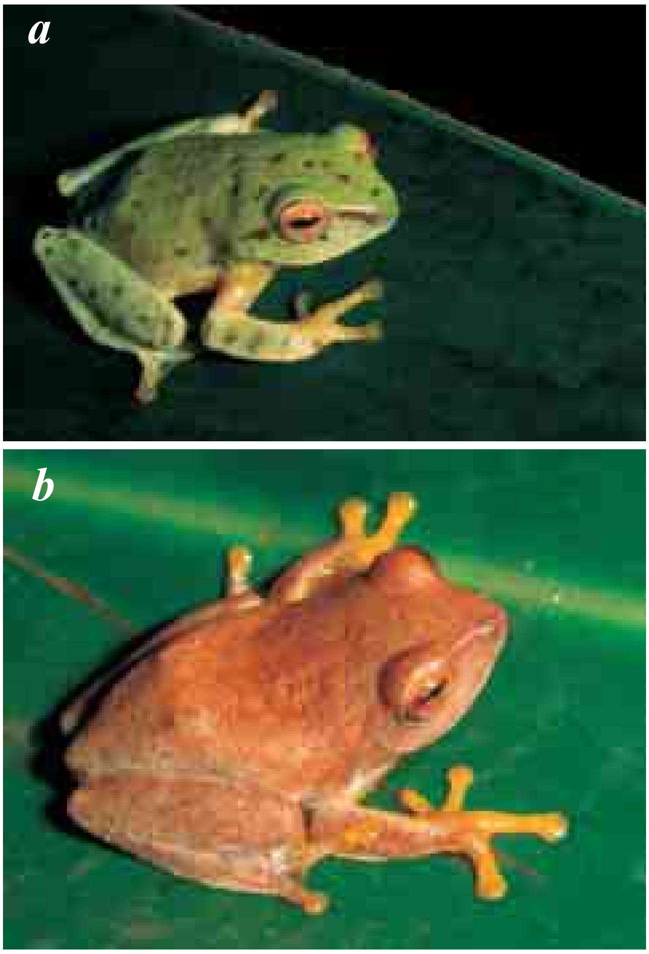

Fig. 10. *Philautus hoffmanni,* new species, *a,* WHT 6120, holotype male, 23.4 mm SVL; *b,* WHT 3542, paratype male, 21.2 mm SVL; WHT 3222, paratype male, 23.1 mm SVL.

Distribution. **–** (Fig. 13). The species was recorded only from Corbett’s Gap (Knuckles hills), elevation 1,245 m (07º22’ N, 80º51’ E).

Etymology. **–** The species name is an eponym in the Latin genitive singular honouring Luc Hoffmann (b. Switzerland, 1923), Honorary President of World Wildlife Fund – France and Director Emeritus of Wetlands International who, as benefactor to the MAVA Foundation, generously supported the Global Amphibian Assessment.

Remarks. **–***Philautus hoffmanni* is the sister species of *P. asankai* Manamendra-Arachchi & Pethiyagoda, 2005 (Fig. 2). These two species are separated from each other by a 12S and 16S sequence divergence of 1.01% and a cytochrome-b divergence of 6.04% (Tables 1, 2). *Philautus hoffmanni* keys out as *P. asankai* according to the key of Manamendra-Arachchi & Pethiyagoda, 2005, but can be distinguished from that species by having the interorbital space flat (vs.

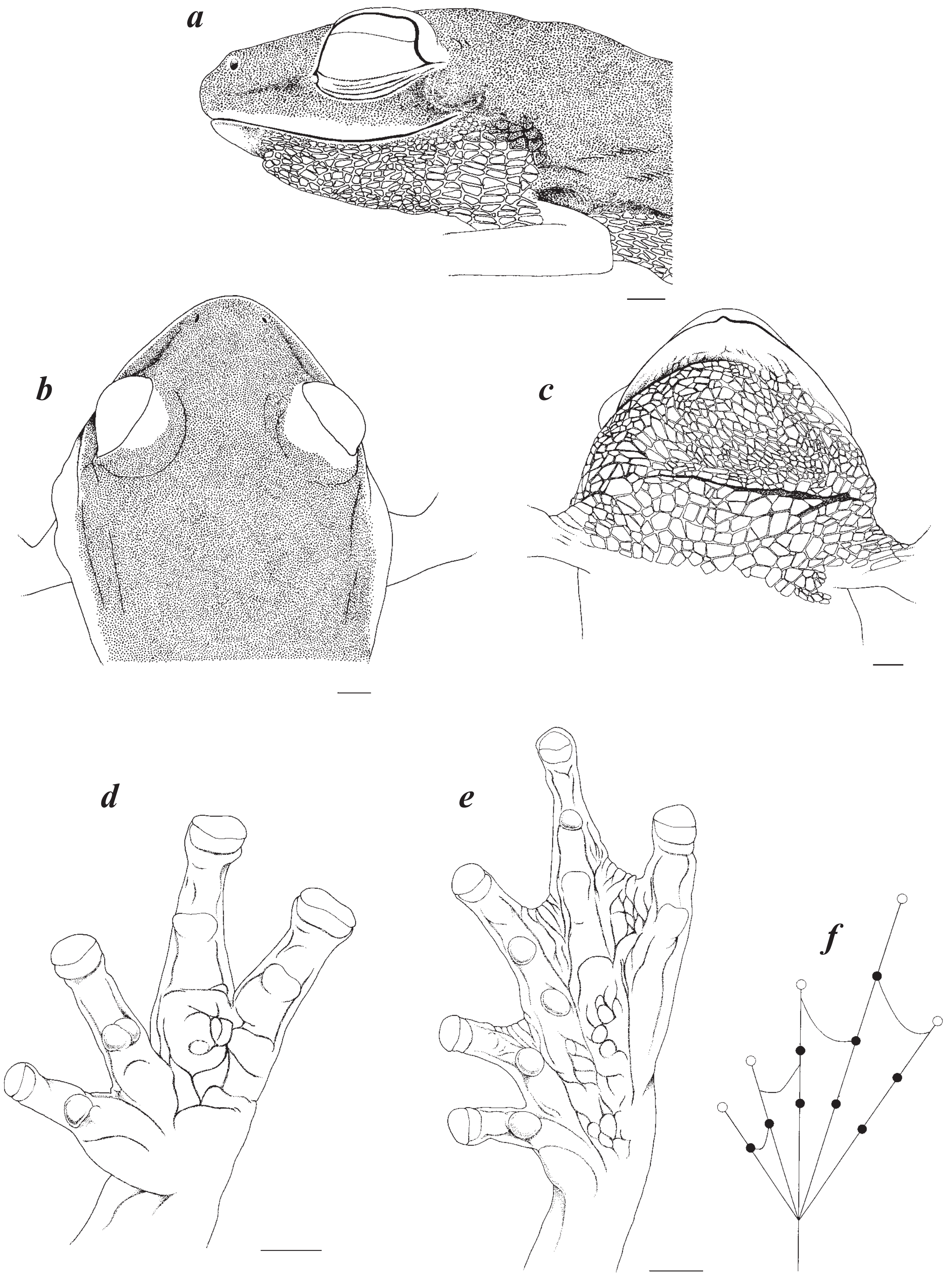

Fig. 11. *Philautus hoffmanni,* new species: *a–c*: lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus; *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT6120, 23.4 mm SVL. Scale bar: 1 mm.

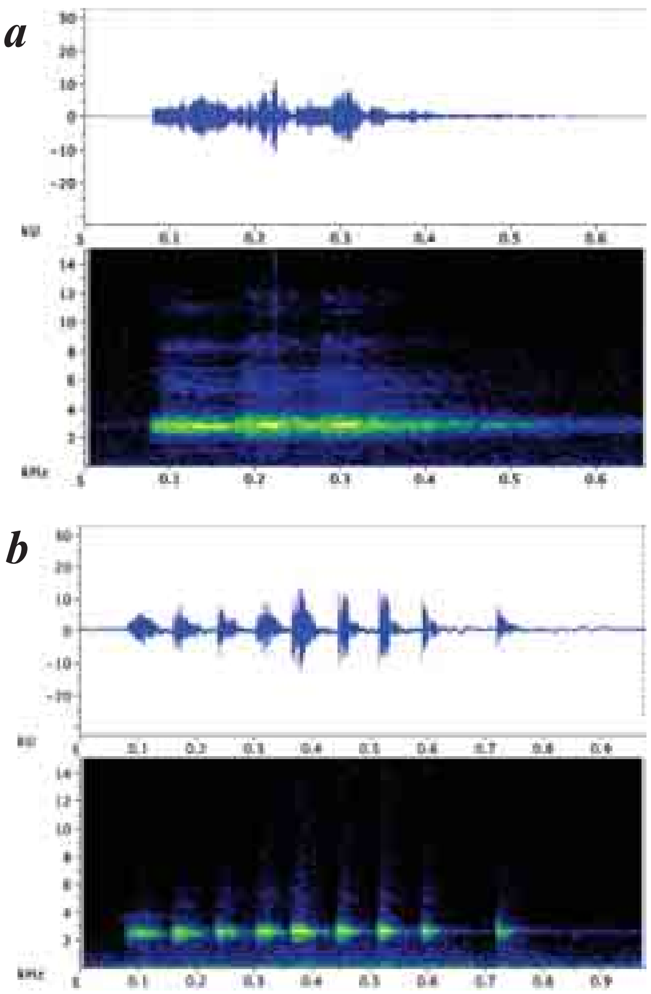

Fig. 12. Uncalibrated waveform envelope and spectrogram of the whistle-like advertisement calls of *a, Philautus asankai* and *b, P. hoffmanni.* The advertisement call of *P. asankai* may be distinguished from that of *P. hoffmanni* by the shorter call length (0.385–0.409 s, vs. 0.638–0.692 s), lower pulse rate (11.62–11.68 s^{-1}, vs. 12.52 – 12.60 s^{-1}), higher dominant and fundamental frequencies (2,859–2,870 Hz, vs. 2,396–2,399 Hz), greater pulse length (0.069–0.071 s, vs. 0.048–0.051 s) and smaller number of pulses per call (3–4, vs. 9). There is no frequency modulation within a call or within a pulse.

interorbital space convex); tympanum distinct (vs. tympanum indistinct); fine, horn-like spinules absent on lower flank (vs. fine, horn-like spinules present on lower flank).

The vocalizations (Table 3; Fig 12) of *Philautus hoffmanni* and *P. asankai* consist of a series of short whistles. The advertisement call of *P. asankai* may be distinguished from that of *P. hoffmanni* by the shorter call length (0.385 – 0.409 s, vs. 0.638 – 0.692 s), lower pulse rate (11.62 – 11.68 s^{-1}, vs. 12.52 – 12.6.60 s^{-1}), higher dominant and fundamental frequencies (2,859 – 2,870 Hz, vs. 2,396 – 2,399 Hz), greater pulse length (0.069 – 0.071 s, vs. 0.048 – 0.051 s) and the smaller number of pulses per call (3 or 4, vs. 9).

Both species are restricted to montane regions: *P. asankai* occurs only in the highest peaks (1,300–1,900 m elevation) of the Central Hills, while *P. hoffmanni* is restricted to the type locality, Corbett's Gap, elevation 1,245 m (07º22' N, 80º51' E).

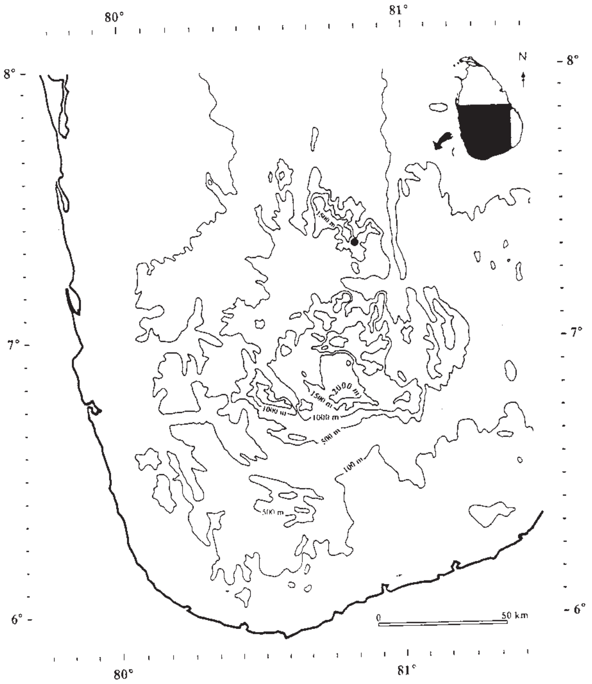

Fig. 13. Distribution of *Philautus hoffmanni,* new species, in Sri Lanka.

Future sampling may show that *P. hoffmanni* occurs also in other cloud-forest areas of the Knuckles Hills.

Philautus hoffmanni and *P. asankai* utilize similar microhabitats, inhabiting shrubs in gaps within closed-canopy cloud forests, and cardamom plantations within these. The ability to live in gaps has enabled *P. asankai* to persist in anthropogenic habitats. *Philautus hoffmanni,* however, was observed only in close proximity to relatively undisturbed montane forests. Males of both species vocalize while perched on leaves ~0.3–1 m above ground. The diurnal resting habitat of these frogs is under a leaf, or on a leaf axil, often in well-illuminated habitats.

Conservation status. – (Fig. 13). The limited Extent of Occurrence (~10 km^2 in the Corbett's Gap region of the Knuckles mountain range) suggests that the species should be considered Endangered (criteria B1 a, b(iii)). Its restriction to a single forest site, low abundance, and dependence on a closed canopy forest, makes this frog susceptible to habitat modification (clearing) and stress during periods of drought. Continuous population monitoring is recommended.

***Philautus mittermeieri,* new species**
(Figs. 14–15)

Material examined. – Holotype - male, 18.4 mm SVL, WHT 3522, Kottawa (Galle), elevation 60 m (06º'06' N, 80º20' E), coll. 22 May 2004.

Paratypes - males, 17.8 mm SVL, WHT 2668, Beraliya forest (Elpitiya), elevation 150 m (06º16' N, 80º11' E), coll. 14 Sep.1999; 16.3 mm SVL, WHT 3523; 17.7 mm SVL, WHT 3524; 17.7 mm SVL, WHT 3525; 17.2 mm SVL, WHT 3526, all from type locality.

Diagnosis. – (Figs. 14, 15). *Philautus mittermeieri* is distinguished from all other Sri Lankan congeners by a

Fig. 14. *Philautus mittermeieri,* new species, WHT 3522, holotype male, 18.4 mm SVL.

combination of the following characters: mature males 16.3–18.4 mm SVL; tympanum distinct; snout-angle category 4 or 5; snout pointed in lateral aspect; canthal edges rounded; vomerine ridge absent' tarsal folds and calcar present; dorsal surface of forelimb, thigh, shank and pes smooth; a prominent sheath-like undulating fringe present on posterior margin of pes and lower arm; nuptial pads absent in male.

Description. – (Figs 13, 14). Mature males 16.3–18.4 mm SVL. Body slender. Head dorsally convex. Snout-angle category 4 or 5 (angle of snout 90º–95º); snout pointed in lateral aspect. Canthal edges rounded. Loreal region concave. Interorbital space concave. Internasal space concave. Tympanum distinct, vertically orientated. Supratympanic fold distinct. Pineal ocellus, vomerine ridge, cephalic ridges, lingual papilla and co-ossified skin on skull absent. A lateral dermal fringe present on fingers. Toes webbed. Tarsal folds and calcar present. Snout, interorbital space, side of head and dorsum, both upper and lower flank with glandular warts. Dorsal part of forelimb, thigh, shank and pes smooth. Throat, chest, belly granular and underside of thigh granular. Inner vocal slits present in males; nuptial pad absent.

Colour in life. – (Fig. 14). Mid-dorsum dark ashy-olive. Tubercles on head orange. Posterior part of back with orange pigments. Side of head ashy brown. Supratympanic fold orangish-light brown. Lower lip ash. Inguinal area dark brown. Flank ashy-yellow with dark-brown patches. Anterior thigh with dark-brown cross markings. Posterior thigh and tibia dark brown. Throat, chest, and upper abdomen ash; lower abdomen ashy-yellow. Fingers ventrally ash. Lower arm with 4, thigh with 3 and shank with 3 dark, ashy-brown crossbars. Ventral area of thigh, shank, pes and toes dark brown. Venter yellow.

Colour in alcohol. – Dorsal parts of head and body grey with brown patches. Both upper and lower flanks grey with brown pigments. Upper lip and loreal region grey. Inguinal zone dark brown. Tympanic region and tympanum grey with brown pigments. Limbs dorsally grey, with indistinct crossbars; posterior part of thigh brown; area around vent yellow. Venter pale yellow.

Measurements of holotype. – (WHT 3522, in mm) DBE, 7.1; DFE, 4.6; DL, 0.8; DW, 1.1; ED, 3.3; EN, 2.5; ES, 3.7; FEL, 8.6; FL I, 1.3; FL II, 1.6; FL III, 2.6; FL IV, 2.1; FOL, 11.7; HL, 8.3; HW, 7.8; IML, 0.9; IN, 2.0; IO, 2.2; LAL, 3.6; MBE, 2.9; MFE, 5.1; MN, 7.3; NS, 1.3; PAL, 5.1; SVL, 18.4; TBL, 9.5; TL I, 1.1; TL II, 1.6; TL III, 2.6; TL IV, 4.1, TL V, 2.5; TYD, 0.5; TYE, 1.1; UAW, 3.4; UEW, 2.1.

Distribution. – (Fig. 17) *Philautus mittermeieri* is a lowland (60–150 m) species that has been observed in two widely spaced localities, Kottawa (60 m elevation, 06º06' N, 80º20' E); and Beraliya Forest, Elpitiya (150 m elevation, 06º16' N, 80º11' E).

Etymology. – The species name is a patronym in the Latin genitive singular, in honour of Russell Mittermeier (b. New York, 1949), President of Conservation International, for his special commitment to the Global Amphibian Assessment and for continuing support of amphibian conservation worldwide. Dr. Mittermeier's lifetime focus on protection of threatened species has resulted in effective conservation programmes in many parts of the world.

Remarks. – The sister species of *P. mittermeieri*, is *P. decoris* Manamendra-Arachchi & Pethiyagoda, 2005 (Fig. 2). The two species are separated from each other by a 12S and 16S divergence of 2.38% and a cytochrome-b sequence divergence of 5.80% (Tables 1, 2).

Philautus mittermeieri keys out as *P. decoris* according to the morphological key of Manamendra-Arachchi & Pethiyagoda (2005). It may, however, be distinguished from *P. decoris* by having the snout obtusely pointed in lateral aspect (vs. snout sharply pointed); glandular warts present on dorsal surface of forelimb, thigh, shank and pes (vs. dorsal surface of forelimb, thigh, shank and pes smooth); and a prominent sheath-like undulating fringe present on posterior margin of pes and lower arm (vs. a feebly-defined sheath-like undulating fringe present on posterior margin of pes and lower arm).

Principal components analysis shows clear separation of the two species by size, with *P. mittermeieri* being the smaller species (up to 18.4 mm SVL in *P. mittermeieri,* vs. up to 20.7 mm in *P. decoris*; Fig. 16).

Philautus decoris occurs only on the highest peaks (above 1,000 m elevation) of the Rakwana Hills (Morningside Forest), *P. mittermeieri* being a lowland species (60–150 m elevation). The two species are thus isolated from each other also by altitudinal constraints.

Philautus mittermeieri is a habitat specialist inhabiting the shrub understorey of closed-canopy lowland rainforests. At night, males vocalize in chorus from leaves, up to ~ 1 m above ground. The calling site of *P. mittermeieri* is similar to that of *P. decoris,* and calling behaviour similar in the two species in that they are group callers, where several males call in chorus.

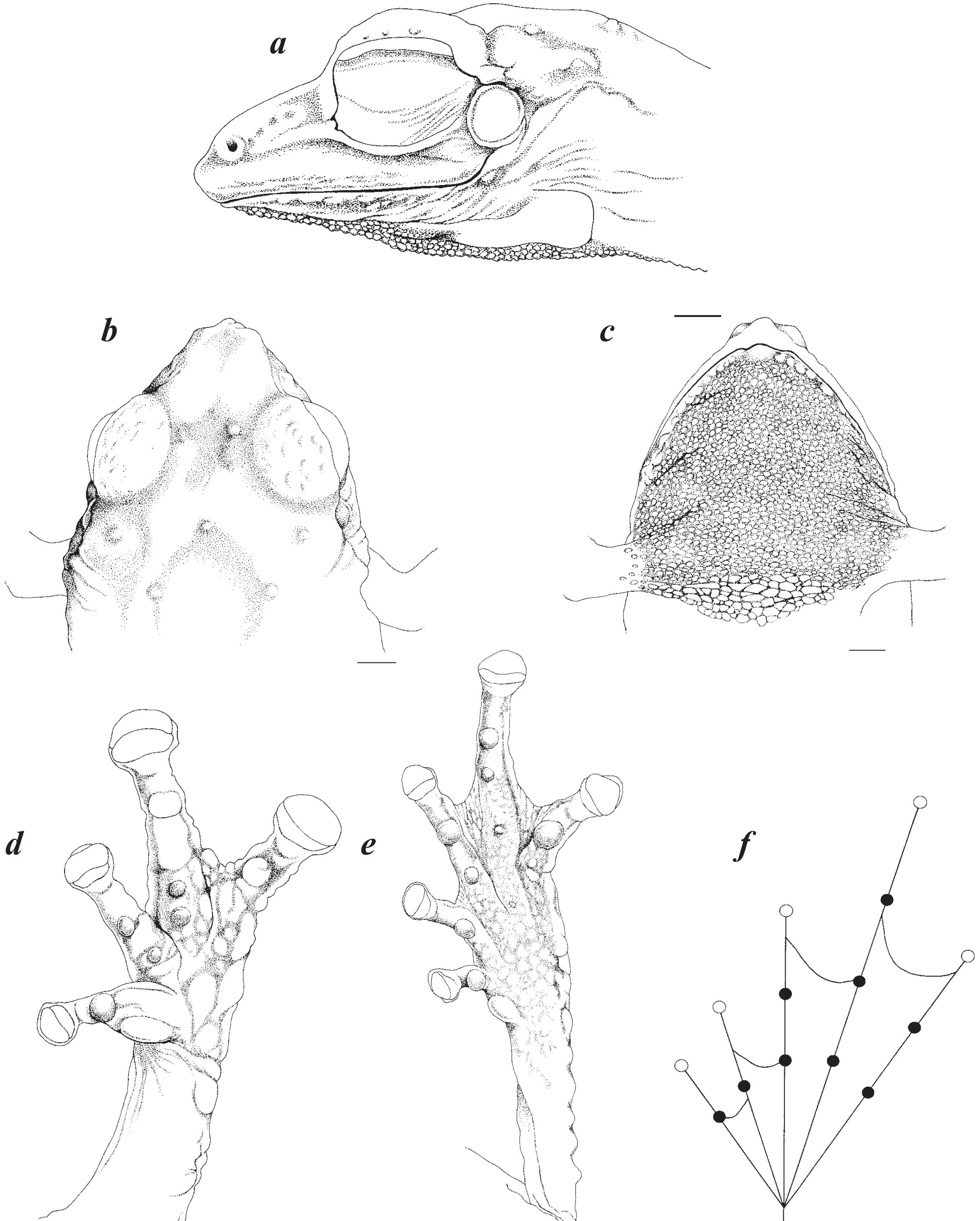

Fig. 15. *Philautus mittermeieri,* new species: *a–c*, lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus; *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT3522, 18.4 mm SVL. Scale bar: 1 mm.

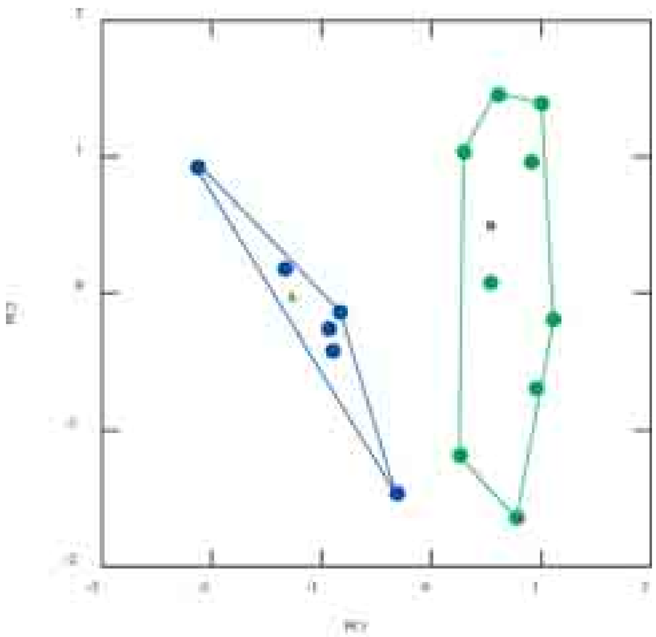

Fig. 16. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus mittermeieri,* new species, (wet-zone lowlands, L) and *P. decoris* (Rakwana hills, R). Most of the variation is explained by the PC1 axis, which relates mostly to body size (*P. decors* is the larger species). The two species overlap on the PC2 axis, which relates mostly to eye diameter and distance between eyes.

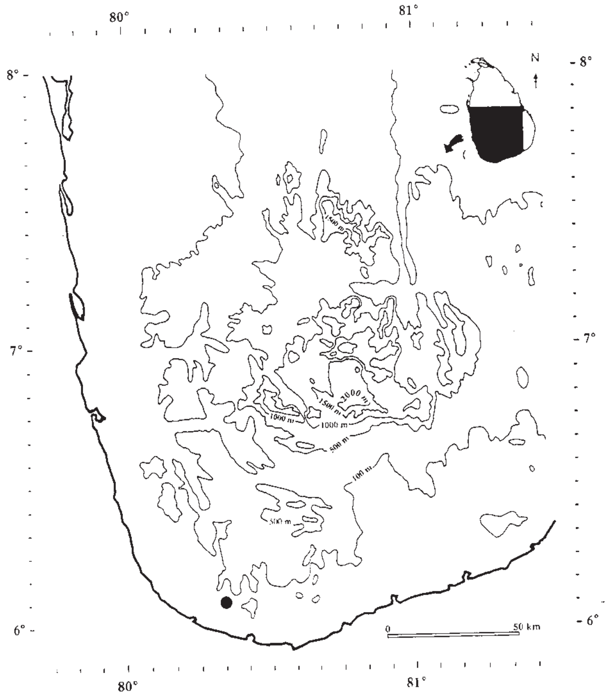

Fig. 17. Distribution of *Philautus mittermeieri,* new species, in Sri Lanka.

Conservation status. **–** (Fig. 17). Extent of Occurrence: 2,000 km^2: Kottawa and Beraliya. Some parts of the range of *P. mittermeieri* are subject to rapid land-use change. Outcome: Vulnerable (criteria B1 a, b(iii)).

Philautus frankenbergi, new species

(Figs. 18, 19)

Material examined. **–** Holotype - male, 29.3 mm SVL, WHT 2554, Namunukula Peak, elevation 1,850–1,980 m (06º56' N, 81º07' E), coll. 2 Sep.1999.

Paratypes - males, 27.4 mm SVL, WHT 2726; 26.7 mm SVL, WHT 2727; 29.2 mm SVL, WHT 2728; Horton Plains National Park, elevation 2135 m (06º46' N, 81º07' E), coll. 20 Sep.1999. Males, 27.4 mm SVL, WHT 2551; 28.7 mm SVL, WHT 2552; 28.7 mm SVL, WHT 2555; 27.5 mm SVL, WHT 2556 (all from type locality).

Diagnosis. **–** (Fig. 19). *Philautus frankenbergi* is distinguished from all other Sri Lankan congeners by a combination of the following characters: mature males 26.7–29.3 mm SVL; snout-angle category 5–7 (97º–106º); vomerine ridge absent; lateral dermal fringe present on fingers; canthal edges sharp; horny spinules on dorsum; limbs with distinct cross-bars; supernumerary tubercles absent on pes; nuptial pad absent in males.

Description. **–** (Figs. 18, 19). Mature males 26.7–29.3 mm SVL. Body stout. Head dorsally flat. Snout-angle category 5–7 (angle of snout 97º–106º), rounded in lateral aspect. Canthal edges sharp. Loreal region concave. Interorbital and internasal spaces flat. Tympanum distinct, oval, vertically orientated. Vomerine ridge absent. Pineal ocellus, lingual papilla, cephalic ridges, tarsal tubercle, tarsal fold and co-ossified skin on skull absent. Supratympanic fold distinct. Lateral dermal fringe present on fingers. Rudimentary webbing present on fingers. Supernumerary tubercles present or absent on palm and absent on pes. Toes webbed. Snout, interorbital space, dorsum and upper flank shagreened; side of head with glandular warts; lower flank granular. Dorsal part of forelimb and shank with glandular warts; dorsal parts of thigh and pes smooth. Horny spinules scattered on dorsum. Throat, chest, belly and underside of thigh granular. Inner vocal slits present in males, nuptial pad absent but subdermal nuptial glands present on inner surface of prepollex.

Colour in life. **–** (Fig. 18) (based on holotype, WHT 2554). Dorsum uniform ashy brown. Canthal edges, loreal and temporal regions dark brown. Lower half of tympanum light brown. Inguinal zone marbled in black and white. Flank ashy brown with white patches. Discs dorsally ashy yellow. Limbs dorsally pale brown with dark-brown crossbars. Some specimens (e.g., paratype WHT 2551) have dark-brown dorsal markings, pale reddish-brown dots on dorsum, a dark-brown interorbital bar, and rarely, white patches on dorsum.

Colour in alcohol. **–** (based on holotype, WHT 2554). Dorsally ashy brown. Both upper and lower flanks white with dark-brown patches. Inguinal zone with white and dark-brown patches. Loreal region, tympanic region and tympanum ashy brown. Upper lip ashy brown. Limbs dorsally ashy brown with indistinct crossbars. Thigh dorsally ashy brown with white patches and indistinct crossbars. Venter and webbing pale yellow with brown pigments.

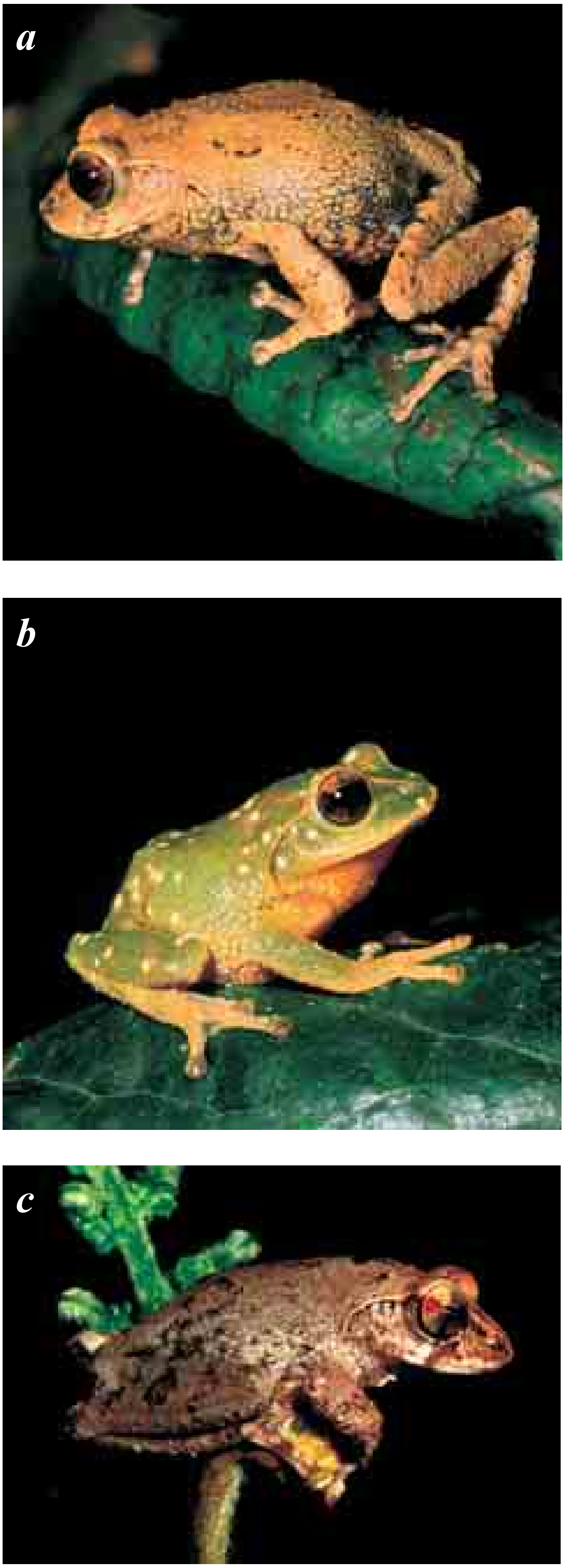

Fig. 18. *Philautus frankenbergi,* new species, *a,* WHT 2554, holotype male, 29.3 mm SVL; *b,* WHT 2726, paratype male, 27.4 mm SVL; and *c*, WHT 2551, paratype male, 27.4 mm SVL.

Measurements of holotype. **–** (WHT 2554, in mm) DBE, 11.2; DFE, 6.4; DL, 1.4; DW, 1.7, ED, 4.7; EN, 3.1; ES, 5.0; FEL, 12.8; FL I, 2.3; FL II, 3.3; FL III, 5.1; FL IV, 4.7; FOL, 19.2; HL, 12.4; HW, 12.4; IML, 1.4; IN, 2.5; IO, 3.3; LAL, 6.0; MBE, 4.3; MFE, 8.0; MN, 10.8; NS, 2.0; PAL, 9.3; SVL, 29.3; TBL, 13.5; TL I, 2.4; TL II, 2.9; TL III, 4.8; TL IV, 7.1, TL V, 5.3; TYD, 0.6; TYE, 1.8; UAW, 6.7; UEW, 3.7.

Distribution. **–** (Fig. 21). *Philautus frankenbergi* is restricted to the highest peaks of the Central Hills: Horton Plains National Park (06º46’ N, 81º07’ E, elevation 2,135 m) and Namunukula Peak (06º56’ N, 80º47’ E, alt. 1,850–1,980 m).

Etymology. **–** The species name honours the late Regina Bauer Frankenberg (USA, 1908–1991), a resident of New York City, who was passionately devoted to the welfare of animals. Through her will, Ms. Frankenberg established a foundation devoted exclusively to animal welfare, including the protection of threatened species through conservation and research. The Regina Bauer Frankenberg Foundation supported the involvement of NatureServe in carrying out the Global Amphibian Assessment.

Remarks. **–** The sister species of *P. frankenbergi*, is *P. auratus* Manamendra-Arachchi & Pethiyagoda, 2005 (Fig. 2). These two species are separated from each other in 12S and 16S sequence divergence of 3.01–3.13% and a cytochrome-b sequence divergence of 11.6% (Tables 1, 2).

Philautus frankenbergi keys out as *P. auratus* in the key of Manamendra-Arachchi & Pethiyagoda (2005), from which species it differs by having the canthal edges sharp (vs. canthal edges rounded in *P. auratus*); vomerine ridge absent (vs. vomerine ridge present); horny spinules present on dorsum (vs. horny spinules on absent dorsum); and limbs with distinct crossbars (vs. limbs with indistinct crossbars).

Principal components analysis reveals that the two species separate well by size, with *P. frankenbergi* being the larger species (male snout–vent length up to 29.3 mm, vs. male snout-vent length up to only 23.3 mm in *P. auratus*) (Fig. 20).

The ranges of the two species are separated by a 1,450 m-deep valley. *Philautus frankenbergi* occurs only on the highest peaks (above 1,850 m elevation) of the Central Hills (including the Namunukula peak area), whereas *P. auratus* occurs only in Rakwana Hills, at elevations of about 1,000 m.

Philautus frankenbergi and *P. auratus* occupy similar microhabitats in the sub-canopy of closed-canopy montane forests and cardamom plantations within these. Males of both species call from about 1–3 m above ground, while perched on branches or large leaves.

Conservation status. **–** (Fig. 21). The limited Extent of Occurrence of *Philautus frankenbergi* (~ 100 km^2, in the highest peaks of the Central Hills and Namunukula mountain) suggests that the species should be considered Endangered (criteria B1 a, b(iii)). Dependence on relatively undisturbed closed-canopy cloud forest renders this frog susceptible to

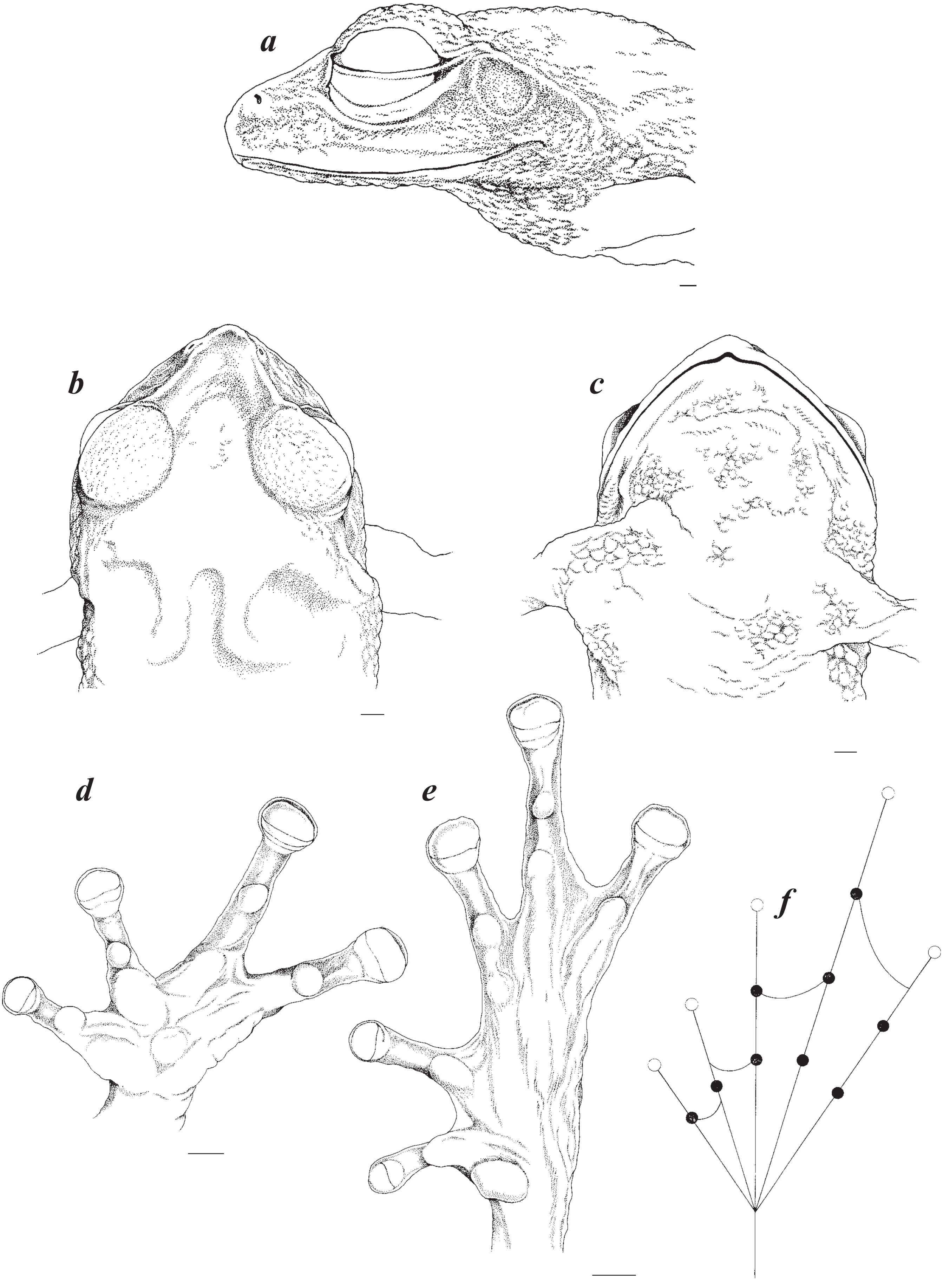

Fig. 19. *Philautus frankenbergi,* new species: *a–c,* lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of right manus; *e*, ventral aspect of right pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT2554, 29.3 mm SVL. Scale bar: 1 mm.

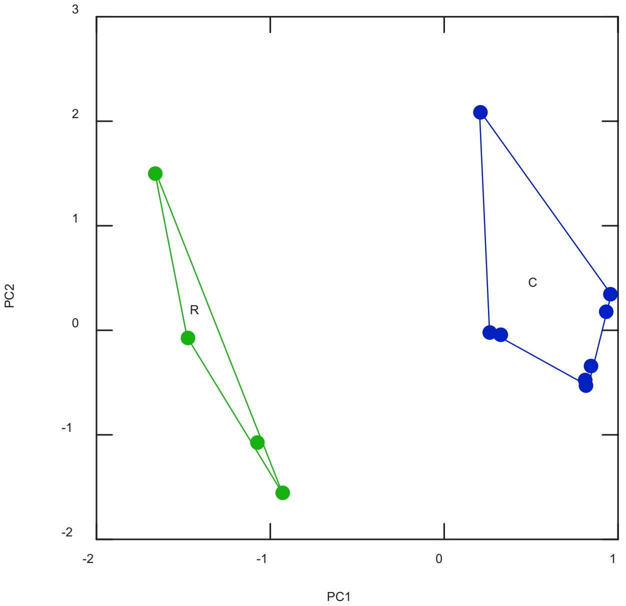

Fig. 20. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus frankenbergi,* new species (Central hills, C) and *P. auratus* (Rakwana hills, R). Most of the total variation is explained by the PC1 axis, which relates mainly to body size (*P. frankenbergi* is the larger species). These two species overlap on the PC2 axis, which relates mostly to inter-narial distance.

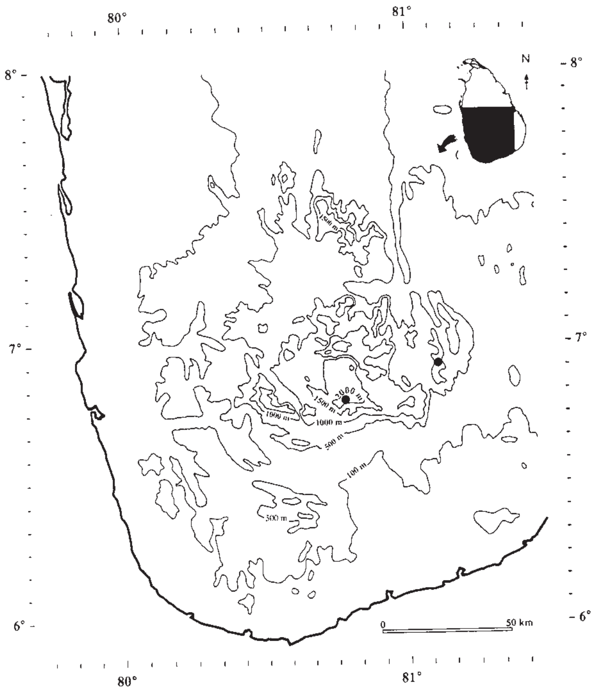

Fig. 21. Distribution of *Philautus frankenbergi,* new species, in Sri Lanka.

habitat modification (land-use change) and stress during periods of drought. Continuous population monitoring is recommended.

Philautus hallidayi, new species
(Figs. 22, 23)

Material examined. – Holotype - female, 42.9 mm SVL, WHT 3575, Hanthana range, Kandy, elevation 510–800 m (07º15' N, 80º34' E), coll. 24 May 2003.

Paratypes - females, 38.8 mm SVL, WHT 3577; 36.7 mm SVL, WHT 3576, from type locality. Male, 32.9 mm SVL, WHT 6072, Tonacombe Estate, Namunukula, elevation 1320 m (06º52' N, 81º07' E), coll. 23 Apr.2004; 37.9 mm SVL, WHT 3573, type locality, 6 Sep.1999.

Diagnosis. – (Figs. 22, 23). *Philautus hallidayi* is distinguished from all other Sri Lankan congeners by a combination of the following characters: snout-angle category 5 or 6; vomerine ridge absent; lateral dermal fringe present on fingers; supernumerary tubercles present on pes; canthal edges rounded; toes 1–3 not fully webbed; toe 5 fully webbed only in a single specimen; angle of vomerine ridge to body axis ~ 70º; tuberculated fringe on posterior margin of lower arm and pes absent; calcar absent; nuptial pad present in males.

Description. – (Figs. 22, 23). Mature male 32.9 mm SVL, females 36.7–42.9 mm SVL. Body stout. Head dorsally convex or flat. Snout-angle category 5 or 6 (angle of snout 97º–103º); snout rounded in lateral aspect. Canthal edges rounded. Loreal region concave. Interorbital space flat. Internasal space flat or concave. Tympanum rather distinct, rounded. Vomerine ridge present (left ridge absent in holotype), bearing about 3 or 4 small teeth, angled at about 70º relative to body axis. Pineal ocellus, lingual papilla, cephalic ridges, tarsal tubercle, tarsal fold and co-ossified skin on skull absent. Supratympanic fold distinct. Lateral dermal fringe present on fingers. Discs on fingers and toes oval. Distinct glandular warts present on palm (including outer edge of palm) and outer edge of lower arm. Supernumerary tubercles present on palm, absent on pes. Toes webbed. Distinct glandular warts present on outer edge of pes and on tibio-tarsal articulation. Snout, interorbital space, side of head, dorsum and upper flank with glandular warts; lower flank granular. Snout, interorbital space, dorsum and upper flank with horny spinules in males. Dorsal part of forelimb, thigh, shank and pes with glandular warts. Throat

Fig. 22. *Philautus hallidayi,* new species, WHT 3575, holotype female, 42.9 mm SVL.

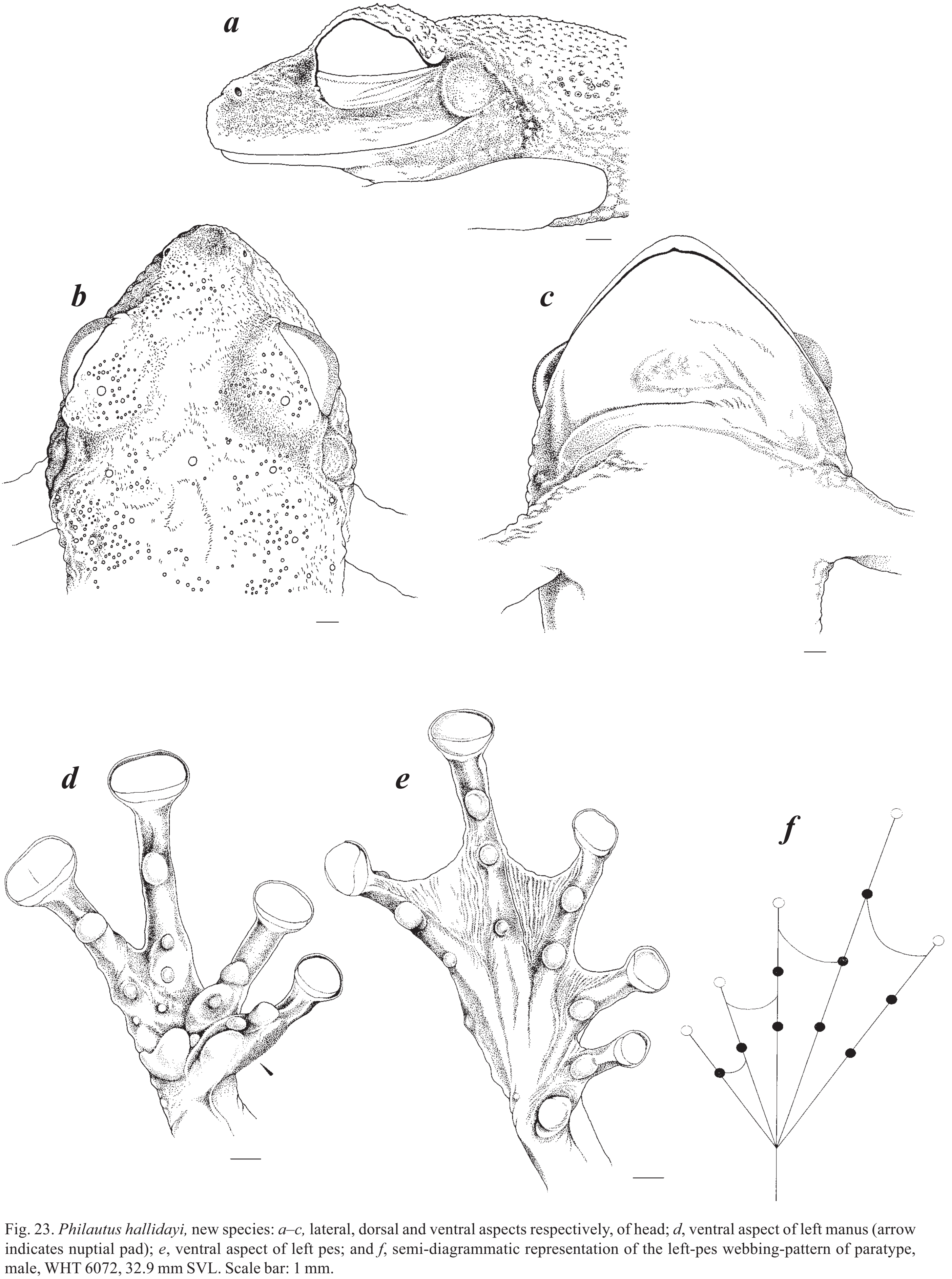

Fig. 23. *Philautus hallidayi,* new species: *a–c,* lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus (arrow indicates nuptial pad); *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of paratype, male, WHT 6072, 32.9 mm SVL. Scale bar: 1 mm.

and chest smooth, belly and underside of thigh granular. Throat and chest granular in males. Inner vocal slits and nuptial pad present in males.

Colour in life. **–** (Fig. 22) (based on holotype, WHT 3575). Dorsum and lateral head brown, with symmetrical, dark-brown markings and white patches; both upper and lower flanks brown with dark-brown and white patches. Inguinal zone dark brown with white markings. Limbs dorsally brown with dark-brown crossbars and white dots. Posterior thigh dark brown. Venter pale brown; webbing dark brown.

Colour in alcohol. **–** (based on holotype, WHT 3575). Dorsally brown with symmetrical dark-brown markings. Interorbital bar dark brown. Both upper and lower flanks brown with dark-brown and white patches. Inguinal zone dark brown with white markings. Loreal region and tympanic region brown with dark-brown patches; tympanum yellowish brown, its outer rim brown; mid-tympanum dark brown. Upper lip brown with white patches. Limbs dorsally brown with dark-brown crossbars and white dots. Posterior part of thigh dark brown. Venter pale yellowish brown; webbing dark brown.

Measurements of holotype. **–** (WHT 3575, in mm) DBE, 15.3; DFE, 9.4; DL, 1.5; DW, 2.7, ED, 5.7; EN, 5.0; ES, 7.3; FEL, 24.5; FL I, 4.4; FL II, 4.9; FL III, 7.5; FL IV, 5.6; FOL, 30.6; HL, 18.5; HW, 18.4; IML, 1.5; IN, 3.8; IO, 3.9; LAL, 8.7; MBE, 6.3; MFE, 10.9; MN, 16.2; NS, 2.4; PAL, 13.4; SVL, 42.9; TBL, 24.4; TL I, 3.4; TL II, 4.1; TL III, 6.7; TL IV, 9.5, TL V, 7.0; TYD, 0.8; TYE, 2.4; UAW, 7.4; UEW, 3.4.

Distribution. **–** (Fig. 25). *Philautus hallidayi* is known only from two locations in Sri Lanka's wet-zone: Hanthana Range, Kandy and Tonacombe Estate, Namunukula.

Etymology. **–** The species name, in the Latin genitive singular, is a patronym honouring Timothy Richard Halliday (b. England, 1945), since 1994 International Director of the IUCN/SSC Task Force on Declining Amphibian Populations (DAPTF), recognizing also his three decades of research on amphibians and his exceptional commitment to advancing our understanding of the global amphibian decline crisis.

Remarks. **–** The sister species of *P. hallidayi*, is *P. cavirostris* (Günther, 1869) (Fig. 2). These two species are separated from each other by a 12S and 16S divergence of 7.9% and a cytochrome-b sequence divergence of 15.7% (Tables 1, 2).

Philautus hallidayi keys out as *P. cavirostris* (Günther, 1869) in the key of Manamendra-Arachchi & Pethiyagoda (2005), but may be distinguished from that species by having canthal edges rounded (vs. canthal edges sharp); toes 1, 2, 3 and 5 not fully webbed (vs. toes 1, 2, 3 and 5 fully webbed); angle of vomerine ridge to body axis ~ 70º (vs. angle of vomerine ridge to body axis ~ 45º); absence of tuberculated fringe on posterior margin of lower arm and pes (vs. presence of a tuberculated fringe on posterior margin of lower arm and pes); and absence of calcar on tibio-tarsal articulation (vs. presence of calcar on tibio-tarsal articulation).

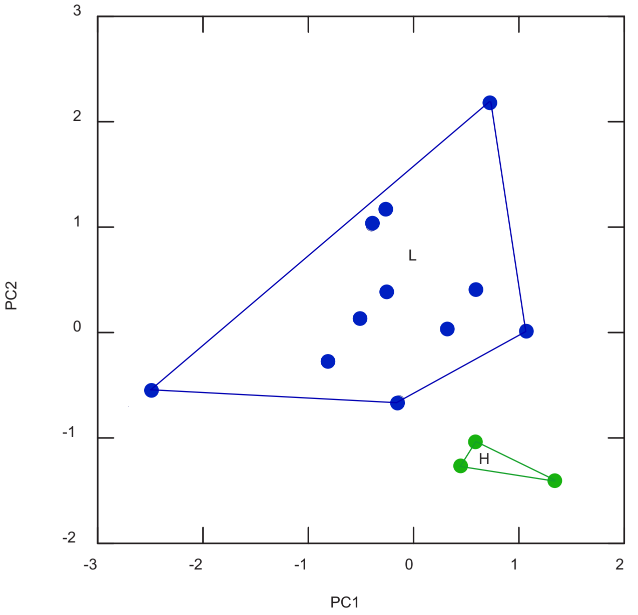

Fig. 24. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus hallidayi,* new species (Hantana, Kandy, H) and *P. cavirostris* (lowlands and mid-elevations, L). About one-third of the variation is explained by the PC1 axis, which relates to pes and manus dimensions; there is, however, some overlap of these characters. Another third of the variation is explained by the PC2 axis, which relates to distance between front of eyes, eye-to-snout distance and inter-orbital distance (these dimensions are all relatively smaller in *P. hallidayi* than in *P. cavirostris*).

Fig. 25. Distribution of *Philautus hallidayi,* new species, in Sri Lanka.

Principal components analysis of *Philautus hallidayi* and *P.* shows that the two species may be distinguished by distance between front of eyes, eye-to-snout distance and interorbital distance (Fig. 24). *Philautus hallidayi* may be distinguished by having a smaller eye-to-snout distance (up to 7.3 mm, vs. up to 9.5 mm); smaller distance between the front of eyes (up to 9.4 mm, vs. up to 12.1 mm); and smaller inter-orbital distance (up to 4.0 mm, vs. up to 5.1 mm), compared to *P. cavirostris*.

Microhabitat utilization by *P. hallidayi* differs from that of *P. cavirostris*. *Philautus hallidayi* is a habitat specialist usually observed perched on large boulders within closed-canopy habitats, often in proximity to large streams (boulders are common in the vicinity of streams in the mid-elevations of Sri Lanka, and this apparent correlation could in fact be a coincidence). *Philautus cavirostris,* on the other hand, is a shrub and sub-canopy species usually found 0.3–2 m above ground, perched on branches in closed-canopy forests.

Conservation status. **–** (Fig. 25). Though this species has a large range, it is only common where optimal habitat conditions (mentioned above) are present. It may also be at risk from water-borne pollutants (agricultural effluents). Outcome: Vulnerable (criteria B1 a, b(iii)).

Philautus steineri, new species
(Figs. 26, 27)

Material examined. **–** Holotype - male, 31.2 mm SVL, WHT 3210, Corbett's Gap (Knuckles Hills), elevation 1245 m (07º22' N, 80º51' E), coll. 29 Jun.2001.

Paratypes - (all from type locality), male, 30.5 mm SVL, WHT 3519; male, 30.2 mm SVL, WHT 3521; female, 41.6 mm SVL, WHT 3520, coll. 5 Jun.1999. Male, 30.6 mm SVL, WHT 6116, coll. 17 Oct.2003; female, 30.4 mm SVL, WHT 3518, coll. 1998.

Diagnosis. **–** (Figs. 26, 27). *Philautus steineri* is distinguished from all other Sri Lankan congeners by a combination of the following characters: snout-angle category 5 or 6; vomerine ridge present; lateral dermal fringe absent on fingers; an angle of about 85° between vomerine ridge and body axis; supernumerary tubercles present on both palm and on pes; posterior margin of thigh pale brown; nuptial pad present in males.

Description. **–** (Figs. 26, 27). Mature males 30.2–31.2 mm SVL; mature female 30.4–41.6 mm SVL. Body stout. Head dorsally flat. Snout-angle category 5 or 6 (angle of snout 98º–103º); snout rounded in lateral aspect. Canthal edges sharp. Loreal region concave. Interorbital space flat. Internasal space concave. Tympanum distinct, oval, oblique. Vomerine ridge present, bearing about 5 small teeth, angled at about 85º relative to body axis, shorter than the distance between them. Pineal ocellus present or absent. Lingual papilla, cephalic ridges, calcar, and co-ossified skin on skull absent. Supratympanic fold distinct. A lateral dermal fringe present on fingers. Supernumerary tubercles present on both palm and pes. Toes webbed. Tarsal folds absent. Snout, interorbital space, dorsum and upper flank with glandular warts and horny spinules (females lack horny spinules on dorsum); side of head smooth or with glandular warts; lower flank granular. Dorsal part of forelimb, thigh, shank and pes smooth with scattered glandular warts. Throat, chest, belly and underside of thigh granular. A feebly-defined dermal fringe mid-extends from tip of snout to posterior dorsum. Inner vocal slits and nuptial pad present in males.

Fig. 26. *Philautus steineri,* new species, WHT 3210, holotype male, 31.2 mm SVL.

Colour in life. **–** (Fig. 26) (based on WHT 3210). Mid-dorsum brown; dorso-lateral area light green; upper flank green with black patches, lower flank ashy green. A dark brownish-black stripe present on upper flank. Interorbital bar dark brown. Posterior head and mid-back brownish-green. Loreal and tympanic regions and tympanum dark brown with light-green stripes. Upper edge of supratympanic fold and canthal edges light brown; lower lip white with brown bands. Chin, chest, abdomen and limbs ventrally pale brown with dark-brown patches. Limbs dorsally brown with dark-brown crossbars. Digits pale-ashy brown. Posterior thigh green.

Colour in alcohol. **–** Mid-dorsum dark brown; flank and inguinal zone ashy brown. Loreal region tympanic region and tympanum dark brown. Limbs dorsally brown or ashy brown; posterior thigh pale brown. Venter and webbing yellowish brown.

Measurements of holotype. **–** (WHT 3210, in mm) DBE, 12.7; DFE, 6.8; DL, 1.1; DW, 1.9, ED, 4.7; EN, 3.3; ES, 5.4; FEL, 15.6; FL I, 2.6; FL II, 3.3; FL III, 5.9; FL IV, 4.6; FOL, 22.5; HL, 13.4; HW, 13.0; IML, 1.4; IN, 3.2; IO, 3.7; LAL, 7.2; MBE, 5.2; MFE, 8.5; MN, 11.7; NS, 2.3; PAL, 9.5; SVL, 31.2; TBL, 16.4; TL I, 2.2; TL II, 3.1; TL III, 5.1; TL IV, 7.7, TL V, 5.2; TYD, 2.0; TYE, 1.6; UAW, 5.0; UEW, 3.7.

Distribution. **–** (Fig. 29) *Philautus steineri* has been recorded only from the type locality, the Corbett's Gap region in the Knuckles mountain range (elevation 1,245 m; 07º22' N, 80º 51'E).

Etymology. **–** The species epithet, in the Latin genitive singular, honours Achim Steiner (b. Brazil, 1961), Director General (2001–) of IUCN, The World Conservation Union, a champion of the Global Amphibian Assessment.

Remarks. **–** The sister species of *P. steineri*, is *P. microtympanum* (Günther, 1859) (Fig. 2). These two species are separated from each other by a 12S and 16S sequence divergence of 2.34% and cytochrome-b sequence divergence of 11.6% (Tables 1, 2).

Principal components analysis of morphological variables reveals that *P. steineri* and *P. microtympanum* separate well

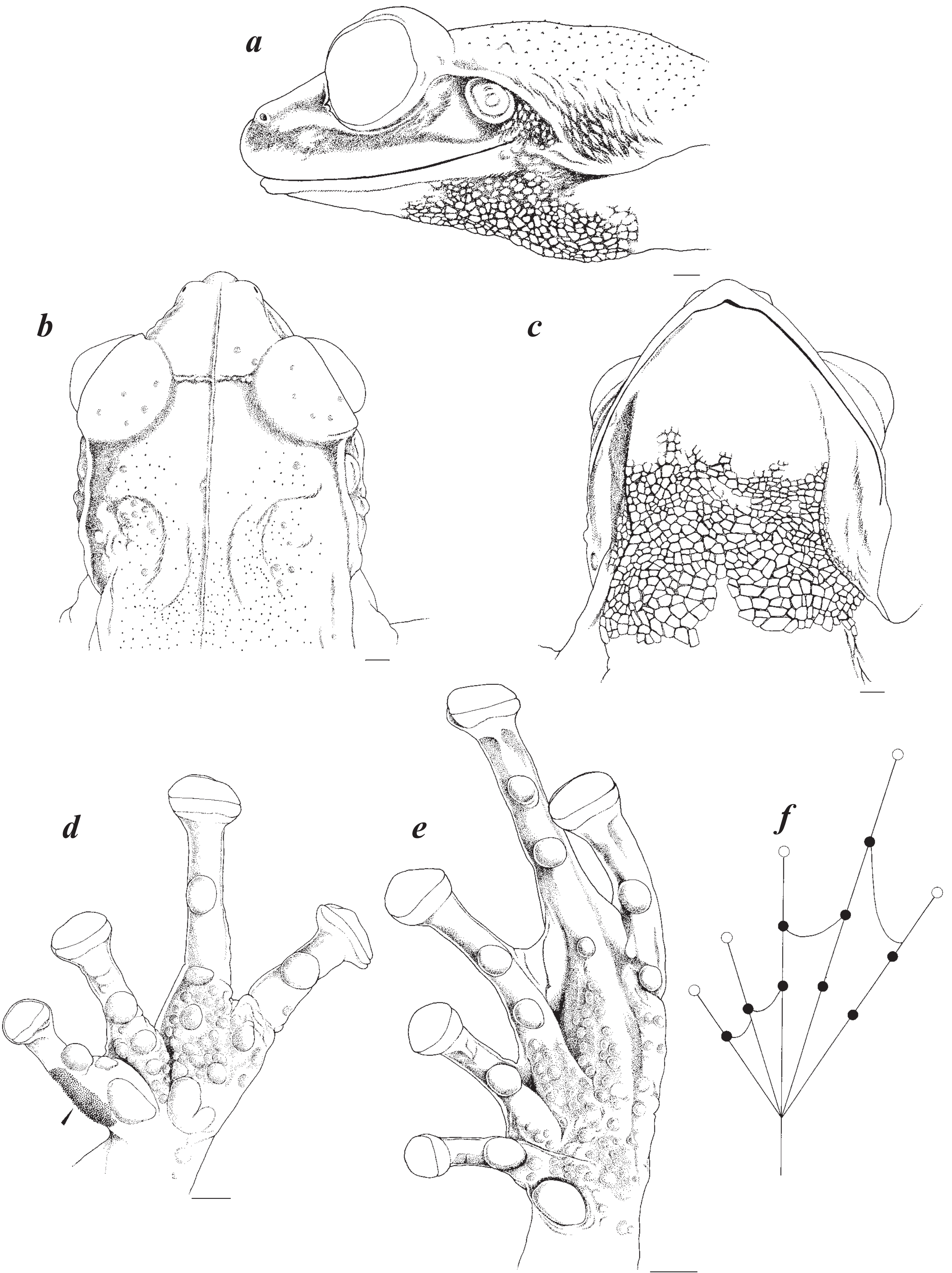

Fig. 27. *Philautus steineri,* new species: *a–c,* lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus (arrow indicates nuptial pad); *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT3210, 31.2mm SVL. Scale bar: 1 mm.

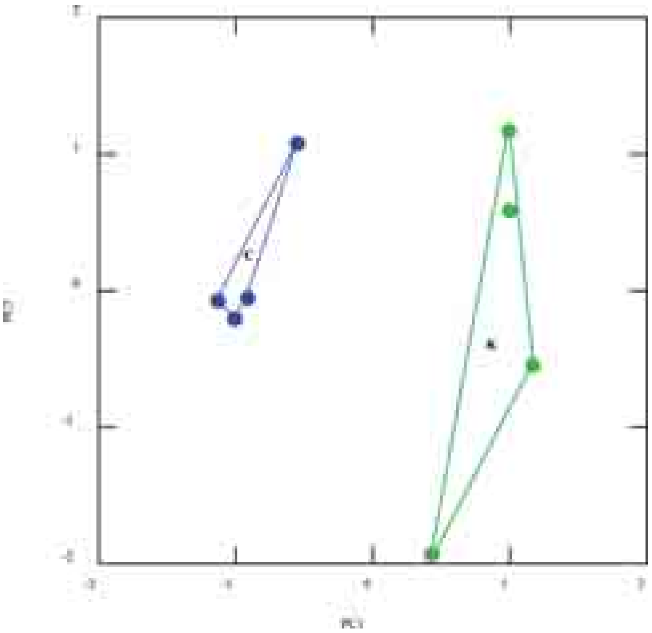

Fig. 28. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus steineri,* new species, (Knuckles hills, K) and *P. microtympanum* (Central hills, C). Most of the variation is explained by the PC1 axis, which relates mostly to body size (*P. steineri* is the larger species). The two species overlap completely on the PC2 axis, which relates mostly to palm length, finger 3 length and lower-arm length.

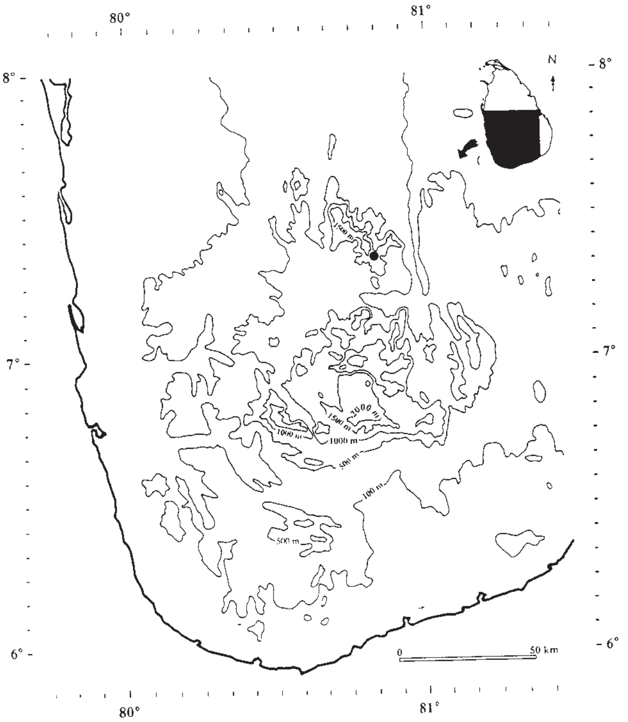

Fig. 29. Distribution of *Philautus steineri,* new species, in Sri Lanka.

by size (Fig. 28). *Philautus steineri* is the larger species (male SVL to 31.2 mm), compared to *P. microtympanum* (male SVL to 25.6 mm).

According to the key of Manamendra-Arachchi & Pethiyagoda (2005), *P. steineri* keys out as *P. microtympanum* (Günther, 1869) and can be diagnosed morphologically by having snout-angle category 5 or 6 (vs. snout-angle category 7); an angle of 45° between vomerine ridge and body axis (vs. angle of 85° between vomerine ridge and body axis); pale brown posterior margin of thigh (vs. dark-brown posterior margin of thigh); and pale-brown blotches absent on posterior margin of thigh (vs. pale-brown blotches present on posterior margin of thigh).

Philautus steineri is restricted to the highest peaks (~ 1,245 m) of the Knuckles Hills, while *P. microtympanum* occurs only in the Central Hills, above 1,555 m. The two populations are separated from each other by a distance of ~ 100 km and the Mahaweli River valley, which descends to about 500 m at Kandy (Figs. 1, 29).

Philautus steineri is a habitat generalist, inhabiting the leaf litter, shrubs and sub-canopy trees, both in open and closed-canopy habitats. Males of both species call from 0.3–3 m above ground, perched on branches.

Conservation status. – (Fig. 29). The Extent of Occurrence of *P. steineri* is approximately 10 km^2 in the Corbett's Gap region at the southern end of the Knuckles mountain range. The species is considered Endangered (criteria B1 a, b(iii)). Its restriction to a single forest site and relatively low abundance make this frog susceptible to habitat modification (forest clearing). Periodic population monitoring is recommended.

***Philautus stuarti*, new species**

(Figs. 30, 31)

Material examined. – Holotype - male, 25.3 mm SVL, WHT 3208, Corbett's Gap (Knuckles Hills), elevation 1,245 m (07º22' N, 80º 51' E), coll. 29 Jun.2001.

Paratypes - (all from type locality), male, 24.2 mm SVL, WHT 3207; male, 25.2 mm SVL, WHT 3206; female, 32.4 mm SVL, WHT 3218, coll. 29 Jun.2001. male, 25.0 mm SVL, WHT 3527, coll. 1998; male, 25.1 mm SVL, WHT 3574, coll. 1998.

Diagnosis. – (Fig. 31). *Philautus stuarti* is distinguished from all other Sri Lankan congeners by a combination of the

Fig. 30. *Philautus stuarti,* new species, WHT 3208, holotype male, 25.3 mm SVL.

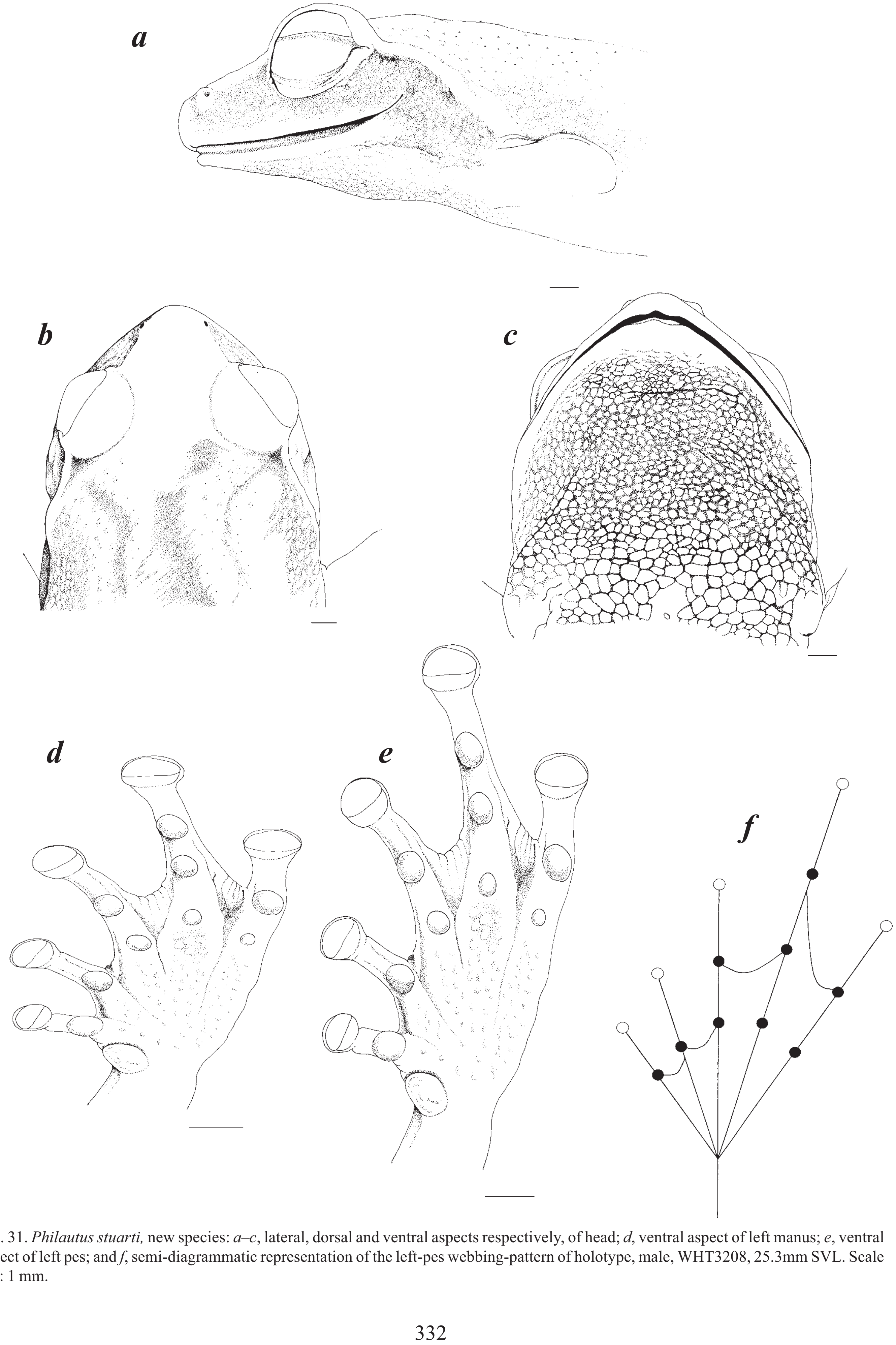

Fig. 31. *Philautus stuarti,* new species: *a–c*, lateral, dorsal and ventral aspects respectively, of head; *d*, ventral aspect of left manus; *e*, ventral aspect of left pes; and *f*, semi-diagrammatic representation of the left-pes webbing-pattern of holotype, male, WHT3208, 25.3mm SVL. Scale bar: 1 mm.

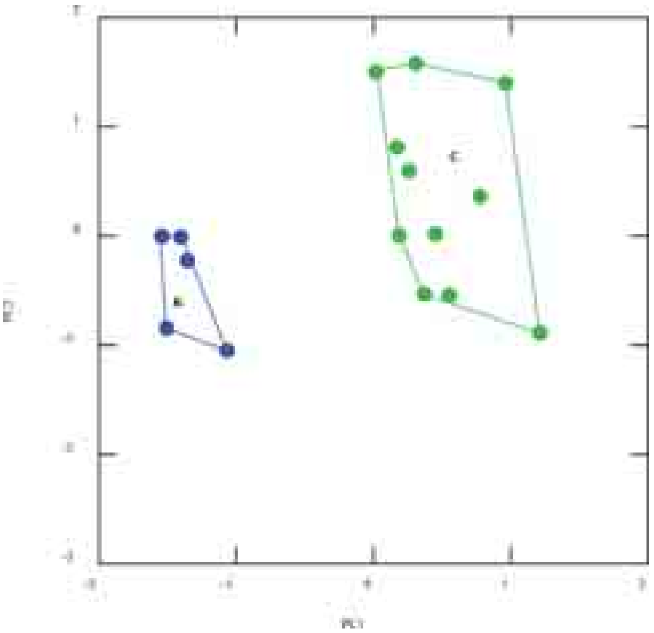

Fig. 32. PC1 vs. PC2 factor scores of the principal components analysis of *Philautus stuarti,* new species (Knuckles hills, K) and *P. viridis* (Central hills, C). Most of the variation is explained by the PC1 axis, which relates mainly to body size (*P. viridis* is the larger species). The two species overlap on the PC2 axis, which relates mainly to inter-orbital distance and distance between front of eyes.

following characters: snout-angle category 7; vomerine ridge absent; lateral dermal fringe absent on fingers; canthal edges sharp; supernumerary tubercles present on both palm and pes; nuptial pad absent in males.

Description. **–** (Figs. 30, 31). Mature males 24.2–25.3 mm SVL; mature female 32.4 mm SVL. Body stout. Head dorsally flat. Snout-angle category 7 (angle of snout 105º–107º); snout rounded in lateral aspect. Canthal edges sharp. Loreal region concave. Interorbital and internasal spaces flat. Tympanum distinct, vertically orientated, crescentic. Vomerine ridge absent. Pineal ocellus, lingual papilla , cephalic ridges, calcar, and co-ossified skin on skull absent. Supratympanic fold prominent. Lateral dermal fringe absent on fingers. Supernumerary tubercles present on both palm and on pes. Toes webbed. Tarsal folds absent. Snout, interorbital space, and both anterior and posterior dorsum with horny spinules; side of head, both upper and lower flanks smooth. Dorsal part of forelimb, thigh, shank and pes smooth. Throat, chest, belly and underside of thigh granular. Females lack horny spinules on dorsum. Inner vocal slits present, nuptial pad absent in males.

Colour in life. **–** (Fig. 30) (based on WHT 3208 and WHT 3206). Dorsally light green with ashy green, ashy yellow or black dots. Loreal region, tympanic region and tympanum green. Canthal edges, supratympanic fold and both upper and lower lips yellow (lips white or brown in some specimens). Digits dorsally yellow. Mid-flank yellow. Chin and chest yellow; abdomen ashy flesh colour, granules on abdomen white; area around vent ash. Limbs and digits ventrally ashy yellow; anterior and posterior thigh brown.

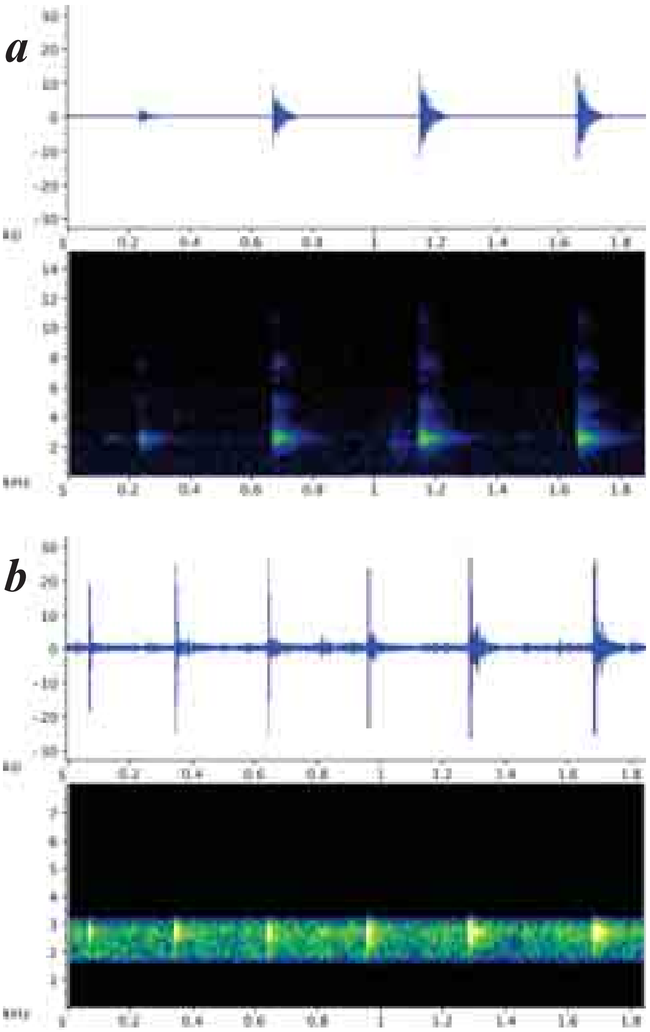

Fig. 33. Uncalibrated waveform envelope and spectrogram of the advertisement calls of *a, Philautus stuarti* and *b, P. viridis.* The call of *P. stuarti* may be distinguished from that of *P. viridis* by the greater call length (1.731–1.805 s, vs. 1.018–1.508 s), greater pulse rate (3.00–3.09 s^{-1}, vs. 2.09–2.12 s^{-1}), lower dominant and fundamental frequency (2,520–2,539 Hz, vs. 2,315–2,328 Hz), smaller pulse length (0.051–0.059 s, vs. 0.077–0.080 s) and the greater number of pulses per call (6, vs. 3 or 4). There is no frequency modulation within a call or within a pulse.

Colour in alcohol. **–** (Based on WHT 3208). Dorsal and lateral parts of head and body ashy light blue; upper flank ashy blue with black patches; lower flank white, inguinal zone dark brown. Loreal region, tympanic region and tympanum ashy blue. Upper lip grey. Dorsal parts of limbs ashy blue; posterior thigh brown. Throat, margin of throat and vocal sacs yellow with brown dots; chest and belly yellow; ventral thigh and web yellow with brown dots.

Measurements of holotype. **–** (WHT 3208, in mm) DBE, 10.1; DFE, 6.0; DL, 1.0; DW, 1.4, ED, 3.7; EN, 2.6; ES, 4.6; FEL, 13.1; FL I, 1.9; FL II, 2.7; FL III, 4.3; FL IV, 3.1; FOL, 17.3; HL, 10.4; HW, 11.4; IML, 1.1; IN, 2.6; IO, 3.2; LAL, 5.1; MBE, 4.0; MFE, 6.6; MN, 9.0; NS, 1.9; PAL, 7.5; SVL, 25.3; TBL, 13.3; TL I, 1.8; TL II, 2.3; TL III, 4.1; TL IV, 5.9, TL V, 4.3; TYD, 0.8; TYE, 1.4; UAW, 4.1; UEW, 2.4.

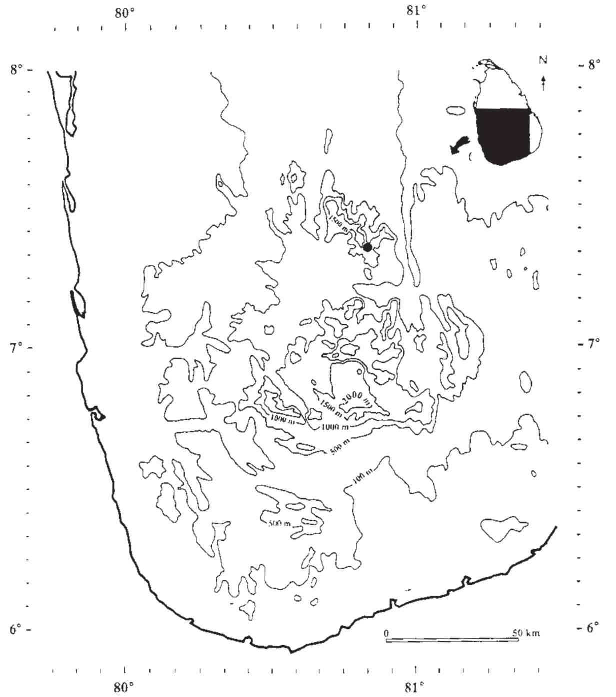

Fig. 34. Distribution of *Philautus stuarti,* new species, in Sri Lanka.

Distribution. **–** (Fig. 34). *Philautus stuarti* is currently known only from a singe location, Corbett's Gap, in the southern end of the Knuckles Hills (07º22' N, 80º51' E), 1,245 m elevation.

Etymology. **–** The species epithet is a patronym in the Latin genitive singular in honour of Simon Nicolas Stuart (b. England, 1956). A former Acting Director General of IUCN (2000–2001), Dr. Stuart has a legacy of establishing international conservation priorities through global biodiversity studies and enabling conservation projects for threatened species worldwide. As Head of program of the Species Survival Commission (1991–2000), he led the development of the new IUCN Red List categories. As current Senior Director of the IUCN SSC/CABS Biodiversity Assessment, Dr. Stuart continues to enhance the Red List of threatened species by leading global species assessments.

Remarks. **–** The sister species of *P. stuarti*, is *P. viridis* Manamendra-Arachchi & Pethiyagoda, 2005 (Fig. 2). These two species are separated from each other by a mtDNA sequence divergence of 6.6% and from all other Sri Lankan *Philautus* in mtDNA sequence divergence of more than 13.8% (Table 1, 2).

Principal components analysis of morphological variables show that *P. viridis* and *P. stuarti* separate well in morphological space by size (Fig. 32). *Philautus stuarti* is the smaller species when compared to *P. viridis*: males reach 25.3 mm SVL (cf. 29.6 mm in *P. viridis*).

According to the key of Manamendra-Arachchi & Pethiyagoda (2005), *P. stuarti* keys out as *P. viridis* from which species it may be distinguished, by having snout-angle category 7 (vs. snout-angle category 8) and canthal edges sharp (vs. canthal edges indistinct).

The vocalisations of these two species (Table 3; Fig. 33) consist of a series of terse ticks. The advertisement call of *P. stuarti* may be distinguished from that of *P. viridis* by the greater call length (1.731 – 1.805 s, vs. 1.018 – 1.508 s), greater pulse rate (3.00 – 3.09 s^{-1}, vs. 2.09 – 2.12 s^{-1}), lower dominant and fundamental frequencies (2,520 – 2,539 Hz ,vs. 2,315 – 2,328 Hz), smaller pulse length (0.051 – 0.059 s, vs. 0.077 – 0.080 s) and the greater number of pulses per call (6, vs. 3 or 4).

Philautus viridis occurs only on the highest peaks of the Central Hills (above 1,000 m), *P. stuarti* being restricted to the Knuckles Hills at about 1,245 m. Populations of *P. stuarti* and *P. viridis* are separated from each other by the Mahaweli River valley, which separates the Central Hills from the Knuckles Hills (Figs. 1, 34), descending to an altitude of about 500 m at Kandy.

The microhabitat utilized by *P. stuarti* is similar to that of *P. viridis:* both species are habitat specialists inhabiting the understorey of closed-canopy montane forests, including cardamom plantations within these. Males of both species call from 2–3 m above ground, while perched on leaves.

Conservation status. **–** (Fig. 34). Extent of Occurrence ~10 km^2. The species is known from only a single population. Its low abundance and the susceptibility of its habitat to change as a result of agricultural expansion lead to it being considered Endangered (criteria B1 a, b(iii)). Population monitoring is recommended.

DISCUSSION

Phylogenetic analysis of mitochondrial 12S and 16S ribosomal sequences revealed at least 17 distinct lineages that may represent undescribed species (Fig. 2). Close examination of eight of these revealed diagnostic morphological characters which, when combined with the separation of these taxa in multivariate morphological space, differences in advertisement calls for some species, and differences in ecology for some, support their recognition as distinct species at this time.

While we used genetic analyses to help identify distinct evolutionary lineage, we emphasize that there is no genetic benchmark to designate species. At a minimum, factors such as the rate and pattern of sequence variation in the taxon under investigation and the gene region addressed have to be considered in interpreting genetic distance data for species delimitation. This is particularly true for organellar DNA which may have a history distinct from the organismal phylogeny. Nonetheless, based on experience or review of published data, some workers have suggested various amounts of genetic divergence in mitochondrial DNA (mtDNA) as benchmarks for species-level differences. For example, Bradley & Baker (2001) suggested that more than 2% genetic divergence of the cytochrome-b gene indicates the possibility of species-level divergence in several groups of mammals. Johns & Avise (1998) found that 90% of putative sister species across a wide range of vertebrate taxa showed more than 2% molecular divergence in the mitochondrial cytochrome-b gene. However, well-known radiations such as the cichlid fauna of the African rift lakes have virtually no mtDNA differences between closely related species that have nevertheless been demonstrated to be robust ecological and sexual entities (Moran et al., 1994; Bowers et al., 1994; Reinthal & Meyer, 1997). Here we have used a portion of the mitochondrial 12S and 16S gene fragment to integrate the current analysis with previously published data (Meegaskumbura et al., 2002), because it is extensively used in molecular systematic studies of frogs (de Sá & Hillis, 1990; Richards & Moore, 1998; Emerson et al., 2000; Vences et. al., 2004) and because it has been used to differentiate species that are difficult to diagnose morphologically in a wide range of frogs (Dawood et al., 2002; Chek et al., 2001; Donnellan et al., 1999; Mahony et al., 2001; Knowles et al., 2004). All species described here are distinguished by 1–8% 12S and 16S mitochondrial ribosomal gene sequence divergence between sister species.

Investigation of cytochrome-b sequence divergence among the new species described here and their respective sister species showed that they differ substantially (5.8–15.7%) and thus fit the pattern of greater than 2% cytochrome-b divergence that is common among vertebrate species (Johns & Avise, 1998). Some taxonomists advocate that genetic distance alone should not be used as a criterion to designate species (Ferguson, 1998), especially if it is low; Hebert et al. (2004) strongly recommended coupling analysis of molecular data with traditional taxonomic tools to distinguish between species. We agree, and think it important to consider multiple sources of data in diagnosing independent evolutionary lineages, especially when the molecular data are derived from organellar gene sequences.

The new species separate from their sister species in multivariate morphological space largely by size but also, in some cases, by combinations of other characters. As has been shown in many disparate frog taxa, the anuran body plan is conservative (Richards & Moore, 1996; Liu et al., 2000; Check et al., 2001). Though superficially more or less similar, all the frog species described here are diagnosable on the basis of both morphological and molecular characters, with each species possessing a suite of diagnostic morphological characters that distinguished it from its congeners. Many of these same characters have also been used to diagnose other species in the genus *Philautus* (Biju & Bossuyt, 2005; Bossuyt & Dubois, 2001; Das & Chanda, 1998; Inger & Stuebing, 1996; Ohler et al., 2002; Stuebing & Wong, 2000).

Some of the characters that distinguish the sister species discussed in this analysis, such as body size and call characteristics, have been shown to be important in mate choice and sexual selection (Ryan, 1985; Gerhardt, 1991; Giacoma & Castellano, 2001). In many groups of frogs, size-assorted mating preference is pronounced (Tsuji, 2004; Boll & Linsenmair, 1998). In addition, body size affects other characters such as the tone of the call and the call rate (Smith & Roberts, 2003; Tarano & Herrera, 2003). The significant body size and call differences that exist between the sister species described here suggests that these species are different reproductive entities as well. Thus, by multiple criteria, the new species described here represent independent evolutionary lineages that warrant recognition as species.

Conservation must aim to preserve the processes that generate and sustain biodiversity (Crandall et al., 2000; Cowling & Pressey, 2001; Pressey et al., 2003; Rodrigues, et al., 2004). Most of the new species described here, along with their sister species, show a repeated biogeographic pattern of sister species being isolated on adjacent mountain ranges at high altitudes, among the three main mountain ranges in Central Sri Lanka (Fig. 2). For example, each of the three species of the *P. femoralis* group (*P. femoralis, P. mooreorum,* and *P. poppiae*) is isolated in cloud forests of one of the three prominent mountain ranges: the Knuckles, Central and Rakwana Hills. This same pattern is seen in all other cloud forest sister species described here (*P. viridis* Central Hills and *P. stuarti* Knuckles Hills; *P. microtympanum* Central Hills and *P. sterneri* Knuckles Hills; *P. auratus* Rakwana Hills and *P. frankenbergi* Central Hills; *P. asankai* Central Hills and *P. hoffmanni* Knuckles Hills). This is also consistent with the geographic predicted by the montane isolation hypothesis proposed by Moreau (1966), which postulates that cool, wet adapted species were pushed to mountaintops during Pleistocene interglacials. However, the mtDNA distances among the sister species treated here suggests divergence that antedates the Pleistocene (Table 2).

Mitochondrial DNA divergence among sister species isolates suggests divergence times in the Late Miocene to Early Pliocene (12 – 4 mya). However, these are crude estimates at best; calculating divergence times from molecular data is fraught with difficulty, particularly given the lack of calibration points with which to estimate nucleotide substitution rates within the Sri Lankan radiation. Even with good calibration, stochastic variance in the substitution process and sampling variance introduced by limited sampling of both taxa and nucleotides is likely to increase the variance associated with any estimate.

It is difficult to identify the initial barrier that separated the montane species but the current factors that separate these species are low altitude saddles, which are substantially warmer and drier than the cloud forest habitats in which the montane species are found. The valleys that separate the three prominent mountain massifs dip to an elevation of at most 550 m, and apparently present a significant barrier to dispersal for species that are adapted to the cool wet cloud forest habitats found above 1,000 m elevation in the three mountain ranges. While it is difficult to infer the process of speciation from geographic distribution alone, the repeated pattern displayed by the taxa described here strongly suggests a similar response by several taxa to external forcing factors, likely past climate change (Schneider et al., 1998; Foster, 2001; Pounds & Puschendorf, 2004). We do not have sufficient information on paleoclimate in Sri Lanka to make strong inference of causality, but the levels of mtDNA divergence among species on mountain tops suggests that their origin lies with climate change in the Late Miocene - Pliocene which resulted in the retreat of cool, wet habitats to higher elevations in response to climate warming and perhaps drying

(Premathilake & Risberg, 2003; Manamendra-Arachchi et al., 2005). Interestingly, this scenario may be repeated in the agamid lizards of Sri Lanka (Schulte et al., 2002). The isolation of populations in cool, wet montane habitats makes them vulnerable to the effects of global warming and we are concerned that many of these species will lose their optimal habitats, and thus face extinction, as a consequence of climate warming over the next several decades.

ACKNOWLEDGEMENTS

We thank Rohan Pethiyagoda, Christopher J. Schneider and James Hanken for suggestions to improve this manuscript and financially supporting our work; Mohomed M. Bahir for comments on the manuscript and extensive field work associated with this study; Sudath Nanayakkara for field work and support at WHT's Agrapatana field station; Suyama Meegaskumbura for comments on the manuscript and assistance with field work; and Sudesh Batuwita for field work. We are very grateful for the critical comments of an anonymous reviewer, Robert F. Inger (Field Museum of Natural History, Chicago), and Don Church (Conservation International), to improve this paper. We also thank Mark Wilkinson, Barry Clarke and David Gower (BMNH) for facilities to work in their institution, hospitality and much valuable assistance. We are grateful to the Forest Department of Sri Lanka for research permits to work in their reserves, enthusiastic encouragement and support, and accommodation during visits; and the Department of Wildlife Conservation Sri Lanka for collection and export permits. This study was supported financially by a grant from the Nagao Environmental Foundation to KM-A; National Science Foundation (grant No. DEB 0345885); National Geographic Society (Grant No. 7612-04); a Declining Amphibian Populations Task Force seed grant to Rohan Pethiyagoda; the Society of Systematic Biologists and SigmaXi (graduate student awards to MM); and Boston University.

LITERATURE CITED

Bahir, M. M., M. Meegaskumbura, K. Manamendra-Arachchi, C. J. Schneider & R. Pethiyagoda, 2005. Reproduction and terrestrial direct development in Sri Lankan shrub frogs (Amphibia: Ranidae: Rhacophorinae: *Philautus*). In: Yeo, D. C. J., P. K. L. Ng & R. Pethiyagoda (eds.), Contributions to biodiversity exploration and research in Sri Lanka. *The Raffles Bulletin of Zoology,* Supplement No. **12**: 339–350.

Biju. S. D. & F. Bossuyt, 2005. A new species of frog (Ranidae, Racophorinae, *Philautus*) from the rainforest canopy of the Western Ghats, India. *Current Science*, **88**(1): 175–178.

Boll, S. & K. E. Linsenmair, 1998. Size-dependent male reproductive success and size-assortative mating in the midwife toad *Alytes obstetricans*. *Amphibia-Reptilia*, **19**(1): 75–89.

Bossuyt, F. & M. C. Milinkovitch, 2000. Convergent adaptive radiation in Madagascan and Asian Ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences of the United States of America*, **97**(12): 6585–6590.

Bossuyt, F. & A. Dubois, 2001. A review of the frog genus *Philautus* Gistel, 1848 (Amphibia, Anura, Ranidae, Rhacophorinae). *Zeylanica*, **6**: 1–112.

Bossuyt, F., M. Meegaskumbura, N. Beenaerts, D. J. Gower, R. Pethiyagoda, K. Roelants, A. Mannaert, M. Wilkinson, M. M. Bahir, K. Manamendra-Arachchi, P. K. L. Ng, C. J. Schneider, O. V. Oommen & M. C. Milinkovitch, 2004. Local endemism within the Western Ghats-Sri Lanka biodiversity hotspot. *Science*, **306**: 479–481.

Bowers N., J. R. Stauffer & T. D. Kocher, 1994. Intra- and interspecific mitochondrial DNA sequence variation within two species of rock dwelling cichlids. *Molecular Phylogenetics and Evolution*, **3**: 7582

Bradley, R. D. & R. J. Baker, 2001. A test of the genetic species concept: Cytochrome-b sequences and mammals. *Journal of Mammalogy*, **82**(4): 960–973.

Chek, A. A., S. C. Lougheed, J. P. Bogart & P. T. Boag, 2001. Perception and history: Molecular phylogeny of a diverse group of neotropical frogs, the 30-chromosome *Hyla* (Anura: Hylidae). *Molecular Phylogenetics and Evolution*, **18**(3): 370–385.

Cocroft, R. B. & M. J. Ryan, 1995. Patterns of advertisement call evolution in toads and chorus frogs. *Animal Behaviour*, **49**: 283-303.

Cowling, R. M. & R. L. Pressey, 2001. Rapid plant diversification: Planning for an evolutionary future. *Proceedings of the National Academy of Sciences of the United States of America*, **98**(10): 5452–5457.

Coyne, J. A. & H. A. Orr, 2004. *Speciation*. Sinauer Assocites, Massachusetts. 545 pp.

Crandall, K. A., R. P. Olaf, Bininda-Emonds, G. M. Mace & K. Robert, 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, 15(7): 290–295.

Das, I. & S. K. Chanda, 1998. A new species of *Philautus* (Anura: Rhacophoridae) from the Eastern Ghats, south-eastern India. *Journal of South Asian Natural History*, **3**(1) 103–112.

Dawood, A., A. Channing, & J. P. Bogart, 2002. A molecular phylogeny of the frog genus *Tomopterna* in southern Africa: Examining species boundaries with mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution*, **22**(3): 407–413.

de Sá, R. O. & D. M. Hillis, 1990. Phylogenetic relationships of the pipid frogs *Xenopus* and *Silurana*: an integrating of ribosomal DNA and morphology. *Molecular Biology and Evolution,* **7**: 365–376.

Donnellan, S. C., K. McGuigan, R. Knowles, M. Mahony & C. Moritz, 1999. Genetic evidence for species boundaries in frogs of the *Litoria citropa* species-group (Anura: Hylidae). *Australian Journal of Zoology*, **47**(3): 275–293.

Dubois A., 2004. Developmental pathway, speciation and supraspecific taxonomy in amphibians, 1: Why are there so many frog-species in Sri Lanka? *Alytes,* **22**(1–2): 19–27.

Duellman W. E. & L. Trueb, 1986. *Biology of Amphibians*. McGraw-Hill, New York. 696 pp.

Emerson, S. B., R. F. Inger, & D. Iskandar, 2000. Molecular systematics of the fanged frogs of Southeast Asia. *Molecular Phylogenetics and Evolution,* **16**: 131–142.

Foster, P., 2001. The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews*,

55(1–2): 73–106.

Gerhardt, H., 1991. Female mate choice in treefrogs. Static and dynamic acoustic criteria. *Animal Behaviour*, **42**: 615–635.

Gerhardt, C. H. & F. Huber, 2002. *Acoustic communication in insects and anurans*. The University of Chicago Press, Chicago. 542 pp.

Giacoma, C. & S. Castellano, 2001. Advertisement call variation and speciation in the *Bufo viridis* complex. In: Ryan, M. J. (ed.), *Anuran communication*. Smithsonian Institution Press, Washington & London. pp. 205–219.

Gistel, J., 1848. *Naturgeschichte des Thierreichs für höhere Schulen*. Hoffmann, Stuttgart. xi+216+iv pp., 32 pls.

Günther, A., 1859. *Catalogue of the Batrachia Salientia in the collection of the British Museum*. Taylor & Francis, London. xvi+160 pp, 12 pls.

Günther, A., 1864. *The reptiles of British India*. Ray Society, London. xxvii+452 pp, 26 pls.

Günther, A., 1869. First account of species of tailless batrachians added to the collection of the British Museum. *Proceedings of the Zoological Society of London*, **1868**: 478–490, pls. 37–40.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen & W. Hallwachs, 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, **101**(41): 14812–14817.

Hey, J., 2001. *Genes, categories and species*. Oxford University Press, Oxford. 217 pp.

Huelsenbeck, J. P. & F. Ronquist, 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**(8): 754–755.

Huelsenbeck, J. P., F. Ronquist, R. Neilsen & J. P. Bollback, 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**: 2310–2314.

Inger, R. F. & R. B. Stuebing, 1996. Two new species of frogs from Southern Sarawak. *The Raflles Bulletin of Zoology,* **44**(2) 543–549.

Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins & T. J. Gibson, 1998. Multiple sequence alignment with Clustal x. *Trends in Biochemical Sciences*, **23**(10): 403–405.

Kotagama, S. W., K. D. Arudpragasam & I. Kotalawala, 1981. *A check list of Amphibia of Sri Lanka.* National Science Council, Colombo. 8 pp.

Liu, W. Z., A. Lathrop, J. Z. Fu, D. T. Yang & R. W. Murphy, 2000. Phylogeny of east Asian bufonids inferred from mitochondrial DNA sequences (Anura: Amphibia). *Molecular Phylogenetics and Evolution*, **14**(3): 423–435.

Mace, G. M., J.L. Gittleman & A. Purvis, 2003. Preserving the Tree of Life. *Science* **300**(5626): 1707–1709.

Mahony M., R. Knowless, R. Foster & S. Donnellan, 2001. Systematics of the *Litoria citropa* (Anura: Hylidae) complex in Nothern New South Wales and Southern Queensland, Australia, with the description of a new species. *Records of the Australian Musuem*, **53**: 37–48.

Manamendra-Arachchi, K. & R. Pethiyagoda, 2005. The Sri Lankan shrub-frogs of the genus *Philautus* Gistel, 1848 (Ranidae: Rhacophorinae), with description of 27 new species. In: Yeo, D. C. J., P. K. L. Ng & R. Pethiyagoda (eds.), Contributions to biodiversity exploration and research in Sri Lanka. *The Raffles Bulletin of Zoology,* Supplement No. **12**: 163–303.

Manamendra-Arachchi, K., R. Pethiyagoda, R. Dissanayake & M. Meegaskumbura, 2005. An extinct tiger from the Late Quaternary of Sri Lanka. In: Yeo, D. C. J., P. K. L. Ng & R. Pethiyagoda (eds.), Contributions to biodiversity exploration and research in Sri Lanka. *The Raffles Bulletin of Zoology,* Supplement No. **12**: 423–434.

Martin, A. P. & S. R. Palumbi, 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Science USA,* **90**: 4087 – 4091.

McCraken K. G. & F. H. Sheldon, 1997. Avian vocalizations and phylogenetic signal. Proceedings of the National Academy of Sciences of theUSA*,* **94**(8): 3833–3836.

Meegaskumbura, M., F. Bossuyt, R. Pethiyagoda, K. Manamendra-Ararchchi, M. Bahir, M. C. Milinkovitch & C. J. Schneider, 2002. Sri Lanka: an amphibian hotspot. *Science*, **298**: 379.

Moran P., I. Kornfield & P. Reinthal, 1994. Molecular systematics and radiation of the haplochromine cichlids (Teleostei: Perciformes) of Lake Malawi. *Copeia*, **1994**: 274–288.

Moreau, R. E., 1966. *The bird faunas of Africa and its islands*. Academic Press, New York. 424 pp.

Palumbi, S. R., 1996. Nucleic Acids II: The polymerase chain reaction. In: Hillis, D. M., C. Moritz & B. K. Mable (eds.), *Molecular Systematics*. Sinauer Associates Inc., Sunderland, Massachusetts. pp. 205–248.

Ohler, A., S. R. Swan & J. C. Daltry, 2002. A recent survey of the amphibian fauna of the Cardamom Mountains, Southwest Cambodia with descriptions of three new species. *The Raffles Bulletin of Zoology,* **50**(2): 465–481.

Pethiyagoda, R. & K. Manamendra-Arachchi, 1998. Evaluating Sri Lanka's amphibian diversity. *Occasional Papers of the Wildlife Heritage Trust,* **2**: 1–12.

Pounds, J. A. & R. Puschendorf, 2004. Ecology — Clouded futures. *Nature*, **427** (6970): 107–109.

Premathilake, R. & J. Risberg, 2003. Late Quaternary climate history of the Horton Plains, central Sri Lanka. *Quaternary Science Review,* **22**: 1525–1541.

Pressey, R. L., R. M. Cowling & M. Rouget, 2003. Formulating conservation targets for biodiversity pattern and process in the Cape Floristic Region, South Africa. *Biological Conservation*, **112**(1–2): 99–127.

Queiroz, K. de, 1998. The General Lineage Concept of species. In: Howard, D. J. & S. H. Berlocher (eds.), *Endless forms: species and speciation*. Oxford University Press, Oxford. pp. 57–75.

Rambaut, A., 1996. Se-Al: Sequence Alignment Editor. *Available at http://evolve.zoo.ox.ac.uk/.*

Reinthal, P. & A. Meyer, 1997. Molecular phylogenetic tests of speciation models in African cichlid fishes. In Givinish, T. & K. Sytsma (eds.), *Molecular Phylogenetics of Adaptive Radiations*. Cambridge University Press, Cambridge and Boston. pp 375–390.

Richards, C. M. & W. S. Moore, 1996. A phylogeny for the African treefrog family Hyperoliidae based on mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **5**: 522–532.

Richards, C. M. & W. Moore, 1998. A molecular phylogeny of the

Old World tree frog family Racophoridae. *Journal of Herpetology,* **8**: 41–46.

Rodrigues, A. S. L., S. J. Andelman, M. I. Bakarr, L. Boitani, T. M. Brooks, R. M. Cowling, L. D. C. Fishpool, G. A. B. da Fonseca, K. J. Gaston, M. Hoffmann, J. S. Long, P. A. Marquet, J. D. Pilgrim, R. L. Pressey, J. Schipper, W. Sechrest, S. N. Stuart, L. G. Underhill, R. W. Waller, M. E. J. Watts & X. Yan, 2004. Effectiveness of the global protected area network in representing species diversity. *Nature*, **428**(6983): 640–643.

Ryan M. J., 1985. *The Tungara Frog: a study in sexual selection and communication*. University of Chicago Press, Chicago. 246 pp.

Schneider C.J., M. Cunningham & C. Moritz, 1998. Comparative phylogeography and the evolution of reptiles and amphibians endemic to the Wet Tropics rainforests of Australia. *Molecular Ecology*, **7**: 487–498

Schulte, J. A., J. R. Macey, R. Pethiyagoda & A. Larson, 2002. Rostral horn evolution among agamid lizards of the genus *Ceratophora* endemic to Sri Lanka. *Molecular Phylogenetics and Evolution*, **22**(1): 111–117.

Sites, J. W. & K. A. Crandall, 1997. Testing species boundaries in biodiversity studies. *Conservation Biology* **11**(6): 1289–1297.

Smith M. J., & J. D. Roberts, 2003. Call structure may affect male mating success in the quacking frog *Crinia georgiana* (Anura: Myobatrachidae). *Behavioral Ecology and Sociobiology*, **53**(4): 221–226.

Stuart, S., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman & R. W. Waller, 2004. Status and trends of amphibian declines and extinctions worldwide. *Science,* **306**: 1783–1786.

Stuebing, R. B. & A. Wong, 2000. A new species of frog, *Philautus erythrophthalmus* (Rhacophoridae) from southwestern Sabah, Malaysia. *The Raffles Bulletin of Zoology,* **48**(2): 293–296.

Sullivan, B. K., 1992. Sexual selection and calling behavior in the American toad (*Bufo americanus*). *Copeia*, **1992** (1): 1–7.

Swofford, D. L., 2002. PAUP, phylogenetic analysis using parsimony (and other methods), v. 4b10. Sinauer Associates, Sunderland, MA.

Tan, A. M. & D. Wake, 1995. MtDNA phylogeography of the California newt *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution,* **4**: 383–394.

Tarano, Z. & E. A. Harrera, 2003. Female preference for call traits and male mating success in the neotropical frog *Physalaemus enesefae*. *Ethology*, **109**(2): 121–134.

Tsuji, H., 2004. Reproductive ecology and mating success of male *Limnonectes kuhlii*, a fanged frog from Taiwan. *Herpetologica*, **60**(2): 155–167.

Vences, M., F. Glaw, J. Riccardo & G. Schimmenti, 2000. A new species of *Heterixalus* (Amphibia, Hyperoliidae) from western Madagascar, *African Zoology,* **35**(2) 269–276.

Vences, M., J. Kosuch, S. Lötters, A. Widmer, K. H. Jungfer, J. Köhler & M. Veith, 2000. Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal gene sequences. *Molecular Phylogenetics and Evolution,* **15**(1): 34–40.