

TARUGA (ANURA: RHACOPHORIDAE), A NEW GENUS OF FOAM-NESTING TREE FROGS ENDEMIC TO SRI LANKA

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ABSTRACT

Phylogenetic relationships among foam-nesting clades of Old World tree frogs are analyzed using both nuclear and mitochondrial DNA data, with particular focus on Sri Lankan members of the genus *Polypedates*. A distinctive, highly supported endemic Sri Lankan clade is identified, and recognized as a new genus, *Taruga*. This clade, which had previously been assigned to the genus *Polypedates*, includes *P. eques*, *P. fastigo* and *P. longinasus*. A combination of characters distinguishes adult *Taruga* from *Polypedates*: *Taruga* possess a dorsolateral glandular fold that extends from the posterior margin of the upper eyelid to the mid-flank (vs. a supratympanic fold that curves over the dorsal margin of the tympanic membrane in *Polypedates*); a prominent calcar at the distal end of the tibia (absent in most *Polypedates*); a more acutely pointed snout; and 6–10 prominent conical tubercles surrounding the cloaca (absent in *Polypedates*). Tadpoles of *Taruga eques* and *Polypedates cruciger* are distinguished by several characters, including features of the buccal cavity and the form of the vent tube. *Taruga* is the sister group of the remaining *Polypedates* sensu stricto.

Key words: *Polypedates*, Rhacophorinae, systematics, tadpole morphology, molecular phylogenetics

INTRODUCTION

Foam nesting is a reproductive mode that occurs in many species of anuran amphibians. Typically, eggs are laid in a foamy mass that overhangs a pool of water, into which late-stage tadpoles fall and undergo further development. Foam nesting occurs in several phylogenetically disparate frog lineages, including Leptodactylidae, Hyperoliidae, Leiuperidae, Myobatrachidae and Rhacophoridae (Wells, 2007). Within each lineage, entire clades, genera or single species (as in Hyperoliidae) practice foam nesting (Duellman and Trueb, 1986; AmphibiaWeb, 2011).

The Rhacophoridae, a well-supported monophyletic group (Frost *et al.*, 2006), contains three well recognized foam-nesting genera: *Rhacophorus*, *Polypedates* and *Chiromantis* (*Chirixalus*) (Meegaskumbura *et al.*, 2002; Wilkinson and Drewes, 2000). Whereas each genus in turn constitutes a distinct, monophyletic group (Richards and Moore, 1998;

Meegaskumbura *et al.*, 2002; Wilkinson *et al.*, 2002; Frost *et al.*, 2006), phylogenetic relationships amongst these genera are not yet fully resolved (Meegaskumbura, 2007).

Five foam-nesting species of anurans from Sri Lanka have previously been assigned to the genus *Polypedates*: *P. maculatus*, *P. cruciger*, *P. eques*, *P. fastigo*, and *P. longinasus* (Meegaskumbura *et al.*, 2002; Manamendra-Arachchi and Pethiyagoda, 2005). Four of these species are endemic to the island, whereas *P. maculatus* also occurs on the Indian Subcontinent. Sri Lankan *Polypedates*, however, possess two different body forms, both as adults and as tadpoles. Moreover, each body form displays a distinct pattern of distribution: slender-bodied, sharp-snouted species with small ranges (*P. eques*, *P. fastigo* and *P. longinasus*; Figs. 1, 2, 3A and 4B); and stouter, blunt-snouted species with wider distributions (*P. cruciger* and *P. maculatus*, Figs. 3B and 3A), respectively. The tadpoles of these two groups, too, are distinctive (Fig. 4).

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Figure 1. A foam nest being made by *Polypedates cruciger*. Several males may be involved in fertilizing the eggs. The female deposits her eggs in a foamy mass overhanging water. The eggs undergo development within the foam nest for 5-6 days; tadpoles of Gosner stage 23 fall into water to undergo further development.



Figure 2. *Taruga fastigo*. Note the prominent calcar on the tibia, tubercles around vent (two of these visible in this photo), and a glandular fold extending from the back of the eye to the mid-flank region. This male was photographed in the type locality for the species, at Morningside Estate, Eastern Sinharaja (1060 m asl).

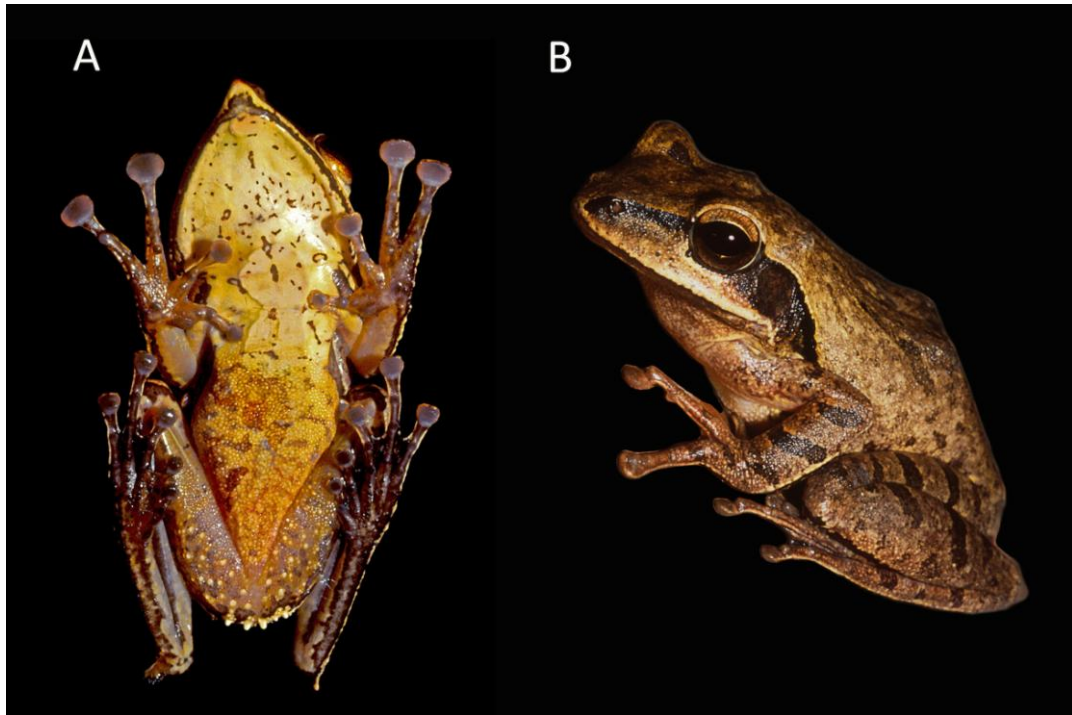


Figure 3. (A) *Taruga longinasus*, ventral aspect, showing the papillae in the region of the cloaca, calcar on tibia and the pointed snout. (B) *Polypedates maculatus*, showing the rounded snout, supratympanic membrane that curves around tympanum and ends anterior to the mid-flank.

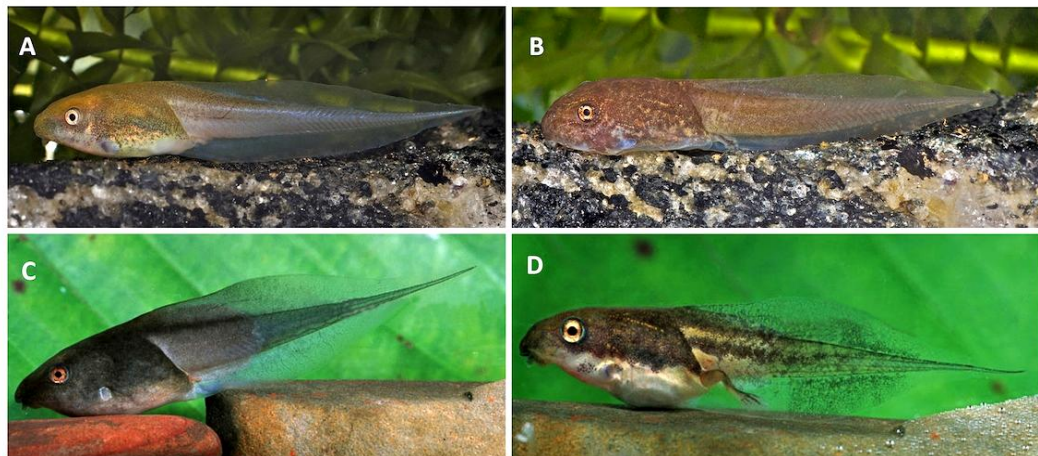


Figure 4. Tadpoles of Gosner stage 32-37 (A) *Polypedates cruciger*, (B) *Taruga eques*, (C) *P. ottilophus* and (D) *P. leucomystax*. Tadpoles of *Taruga eques* differ from all others in having an acute tail fin tip, position of eye, distinct body shape, and having a pronounced extension of the connective tissue covering the proximal tail musculature about one-third along the length of the tail. (Photographs (C) and (D) courtesy of Alexander Haas).

Here we evaluate the genus-level relationships among these frogs through a phylogenetic analysis that uses both mitochondrial and nuclear DNA markers and includes representatives of all known foam-nesting genera within Rhacophoridae. Our analysis reveals a hitherto unrecognized lineage

of foam-nesting tree frogs endemic to Sri Lanka, which we recognize as a new genus. We also describe the morphological characters that distinguish adults and tadpoles of this genus from those of *Polypedates*.

Table 1. Taxonomic information, collection reference numbers, country, gene fragments for which sequences are not available (X) and Genbank reference numbers of the species included in this study. (FMNH – Field Museum of Natural History Chicago; ZRC – Zoological Reference Collection, Singapore; WHT – Wildlife Heritage Trust, Sri Lanka; MVZ – Museum of Vertebrate Zoology, University of California Berkeley; MCZ – Museum of Comparative Zoology, Harvard University)

Genus	Species	Reference number	Country	Gene fragments and Genbank reference numbers					
				12s	16s	cyt-b	Rag-1	BDNF	Rhod
<i>Polypedates</i>	<i>cruciger</i>	WHT 2640	Sri Lanka	X	GQ204687	GQ204504	GQ204570	GQ204446	GQ204632
	<i>cruciger</i>	WHT Kuru3	Sri Lanka	GQ204746	GQ204692	GQ204508	X	X	X
	<i>maculatus</i>	WHT Kuru2	Sri Lanka	GQ204747	X	GQ204510	GQ204575	GQ204451	X
	<i>maculatus</i>	WHT Kantalai	Sri Lanka	X	GQ204694	GQ204511	GQ204576	GQ204451	GQ204637
	<i>leucomystax</i>	ZRC 1.15269	Java	X	GQ204693	GQ204509	GQ204574	GQ204450	GQ204636
	<i>colletti</i>	FMNH 242765	Malaysia	GQ204750	GQ204697	GQ204514	GQ204579	GQ204454	X
	<i>macrodis</i>	FMNH 239119	Malaysia	GQ204748	GQ204695	GQ204512	GQ204577	GQ204452	GQ204638
	<i>otilophus</i>	FMNH 239147	Malaysia	GQ204749	GQ204696	GQ204513	GQ204578	GQ204453	GQ204639
	<i>leucomystax</i> var. A	FMNH 253086	Vietnam	GQ204752	GQ204699	GQ204516	GQ204581	GQ204456	X
	<i>leucomystax</i> var. B	FMNH 253029	Vietnam	GQ204751	GQ204698	GQ204515	GQ204580	GQ204455	X
	<i>leucomystax</i>	FMNH 255296	Laos	GQ204753	GQ204700	GQ204517	GQ204582	GQ204457	X
	<i>leucomystax</i>	FMNH 256451	Laos	GQ204754	GQ204701	GQ204518	GQ204583	X	X
<i>Taruga</i>	<i>eques</i>	WHT 2741	Sri Lanka	X	GQ204689	GQ204505	GQ204571	GQ204447	GQ204633
	<i>eques</i>	WHT 2714	Sri Lanka	X	GQ204688	X	X	X	X
	<i>fastigo</i>	WHT 2783	Sri Lanka	X	GQ204690	GQ204506	GQ204572	GQ204448	GQ204634
	<i>longinasus</i>	WHT KAN1	Sri Lanka	GQ204745	GQ204691	GQ204507	GQ204573	GQ204449	GQ204635
<i>Rhacophorus</i>	<i>anamensis</i>	FMNH 253934	Vietnam	GQ204768	GQ204717	GQ204534	GQ204598	GQ204470	GQ204653
	<i>bipunctatus</i>	FMNH 253114	Vietnam	GQ204767	GQ204716	GQ204533	X	GQ204469	GQ204652
	<i>calcaneus</i>	FMNH 256465	Lao	GQ204770	GQ204719	GQ204536	GQ204600	X	GQ204655
	<i>chenfui</i>	FMNH 232964	China	GQ204763	GQ204712	GQ204529	GQ204594	GQ204467	GQ204648
	<i>dulitensis</i>	FMNH 235741	Malaysia	GQ204766	GQ204715	GQ204532	GQ204597	X	GQ204651

	<i>gauni</i>	FMNH 235047	Malaysia	GQ204765	GQ204714	GQ204531	GQ204596	X	GQ204650
	<i>nigropalmatus</i>	FMNH 230902	Malaysia	GQ204761	GQ204710	GQ204527	GQ204592	GQ204465	GQ204646
	<i>pardalis</i>	FMNH 231366	Malaysia	GQ204762	GQ204711	GQ204528	GQ204593	GQ204466	GQ204647
	<i>reinwardtii</i>	ZRC 1.1.5273	Java	GQ204771	GQ204720	GQ204537	GQ204601	GQ204472	GQ204656
	<i>reinwardtii</i>	FMNH 235034	Malaysia	GQ204764	GQ204713	GQ204530	GQ204595	GQ204468	GQ204649
	sp.	FMNH 255280	Laos	GQ204769	GQ204718	GQ204535	GQ204599	GQ204471	GQ204654
Chiromantis (<i>Chirixalus</i>)	<i>doriae</i>	FMNH 255215	Laos	GQ204772	GQ204721	GQ204538	GQ204602	GQ204473	GQ204657
	<i>nongkhorensis</i>	FMNH 255378	Laos	GQ204774	GQ204723	GQ204540	GQ204604	GQ204475	GQ204659
	<i>vittatus</i>	FMNH 255217	Laos	GQ204773	GQ204722	GQ204539	GQ204603	GQ204474	GQ204658
Chiromantis	<i>rufescens</i>	CAS	Africa	GQ204775	GQ204724	GQ204541	GQ204605	GQ204476	GQ204660
	<i>xerampelina</i>	MVZ 234606	Africa	GQ204785	GQ204734	GQ204551	X	X	X
	<i>petersi</i>	MVZ 234168	Africa	GQ204784	GQ204733	GQ204550	X	X	X
Theloderma	<i>asperum</i>	ZRC 1.1.9321	Malaysia	GQ204776	GQ204725	GQ204542	GQ204606	X	GQ204661
Nyctixalus	<i>pictus</i>	FMNH 231094	Malaysia	GQ204777	GQ204726	GQ204543	GQ204607	GQ204477	X
	<i>pictus</i>	MVZ 239460	Indonesia	GQ204783	GQ204732	GQ204549	GQ204613	GQ204483	GQ204666
Buergeria	<i>oxycephalus</i>	MVZ 230425	China	GQ204781	GQ204730	GQ204547	GQ204611	GQ204481	GQ204665
	<i>oxycephalus</i>	MVZ 230426	China	GQ204782	GQ204731	GQ204548	GQ204612	GQ204482	X

MATERIALS AND METHODS

Tissue extraction, PCR amplification and DNA sequencing

We obtained DNA sequence data from 40 individuals of 31 putative rhacophorid species, which represent all currently recognized genera of foam-nesting species within Rhacophoridae as well as several basal rhacophorid outgroup species (Table 1). Tissues of Sri Lankan species (8 individuals, 5 species) were collected in the field; the rest (32) were obtained from natural-history museum collections.

DNA was extracted from ethanol-preserved tissues using Qiagen tissue-extraction kits following manufacturers' protocols. A total of 3730 base pairs (bp) were sequenced, including three mitochondrial gene fragments with a total of ~ 1500 bp and three nuclear gene fragments with a total of ~ 2230 bp. Portions of the mitochondrial 12S and 16S ribosomal RNA (12S and 16S, respectively) and cytochrome *b* (*cyt-b*) genes were amplified by PCR and sequenced directly using dye-termination cycle sequencing. The following primer sets were used for both PCR and sequencing of mitochondrial genes: 12Sa and 12Sb (Palumbi, 1996), which amplified a ~ 380 bp fragment of the 12S rRNA gene; 16Sar and 16Sbr (Palumbi, 1996), which amplified ~ 550 bp of the 16S rRNA gene; and CBJ10933 and BSF4 (Bossuyt and Milinkovitch, 2000), which amplified a 567 bp region of the *cyt-b* gene. PCR conditions for amplification of all three fragments were as follows: denaturation at 95° C for 40 s, annealing at 45° C for 40 s, and extension at 72° C for 50 s, 35 cycles, with a final extension of 72° C for 5 min.

Nuclear Rag-1 (recombination activating gene-1), BDNF (brain-derived neurotrophic factor) and Rhod (rhodopsin exon-1) partial gene fragments were amplified by PCR. Two sets of primers were used to amplify two fragments of the Rag-1 gene for a total sequence length of 1403 bp: Amp-Rag1F (5'-AGC TGC AGY CAR TAC CAY AAR ATG TA - 3') and Amp-Rag1R1 (5'-AAC TCA GCT GCA TTK CCA ATR TCA CA-3'); and Amp-Rag1F1 (5'-ACA GGA TAT GAT GAR AAG CCT GT-3') and Amp-Rag1R (5'-TTR GAT GTG TAG AGC CAG TGG TGY TT-3') (Mauro *et al.*, 2004). Primers BDNFF (5'-CAT CCT TTT CCT TAC TAT GGT T-3') and BDNFR (5'-TTC CAG TGC CTT TTG TCT ATG-3') (Murphy *et al.*, 2001), were used to amplify 550 bp of the BDNF gene; and Rhod.ma (5'-AAC GGA ACA

GAA GGY CC-3') and Rhod.md (5'-GTA GCG AAG AAR CCT TC-3') (Hoegg *et al.*, 2004), were used to amplify 281 bp of Rhodopsin exon. PCR conditions for amplification of Rag-1 were denaturation at 95° C for 45 s, annealing at 52–58° C for 45 s, and extension at 72° C for 60 s, 35–38 cycles, with a final extension at 72° C for 10 min. PCR parameters for amplification of BDNF and rhodopsin gene fragments were the same as for Rag-1 amplification except for the annealing temperatures, which were 50° C and 45° C, respectively. All products were gel purified and sequenced on an ABI 3100 automated sequencer following manufacturers' protocols.

Alignment of sequences and phylogenetic analyses

Chromatograms were edited using Codon Code Aligner (v. 1.5.2). The program Se-Al (v. 2.0a; Rambaut, 1996) was used to align the coding regions of *cyt-b*, Rhodopsin, BDNF and Rag-1 using translated amino acid sequences. The 12S and 16S rRNA gene sequences were aligned visually. Positions that were difficult to align, and in which we had low confidence in positional homology, were excluded from the phylogenetic analyses.

The Partition Homogeneity Test as implemented in PAUP* (v. 4.0b10) was used to assess the congruence of the six gene fragments. All available species (40 taxa) were included in a single dataset. A non-partitioned Bayesian analysis, and an equal-weights unordered Maximum Parsimony Analysis were performed.

Modeltest 3.06 (Posada and Crandall, 1998) was used to choose the model of nucleotide substitution that best fit the datasets. The General Time Reversible model with invariant sites and gamma distribution (GTR+I+G) yielded the best-fit for all datasets (using the Akaike information criterion: Akaike, 1974). This was implemented as a non-partitioned Bayesian analysis, with all parameters estimated during the run.

The tree was rooted using *Buergeria oxycephalus* (China), *Theloderma asperum* (Malaysia) and *Nyctixalus pictus* (Malaysia and Indonesia). Previous studies suggest that these taxa represent the basal clades of Rhacophoridae and hence can be justified as outgroups to root the tree of the foam-nesting species (Meegaskumbura *et al.* 2002; Bossuyt *et al.*, 2006; Frost *et al.*, 2006; Meegaskumbura, 2007).

Bayesian inference as implemented in MrBayes (v. 3.1: Huelsenbeck and Ronquist, 2001) was used to generate a phylogenetic hypothesis of relationships among the taxa with the parameters of a general time-reversible model of sequence evolution with gamma-distributed rate variation among sites and a proportion of invariant sites (GTR+I+G) estimated during the run. Four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains were run for one million generations and the summed likelihood of the four chains converged on a stationary value by 250,000 generations (burn-in time). Burn-in time was determined using the program AWTY (Wilgenbusch *et al.*, 2004). The frequency of clades in trees that were sampled every ten generations from the last 250,000 generations (a total of 25,000 trees) was used as an estimate of the posterior probabilities of those clades (Huelsenbeck *et al.*, 2001). Uniform priors were specified, and branch lengths, topology and nucleotide substitution parameters were unconstrained. For the Maximum likelihood analysis, we used the GTR+I+G model of nucleotide substitution with the parameters estimated from the Bayesian analysis. A single heuristic search with Tree Bisection and Reconnection (TBR) branch swapping was conducted using PAUP*4.0b10 (Swofford, 2002). Tree searches under a Maximum Parsimony criterion used heuristic searches with TBR branch-swapping and 100 replicates with random taxon addition as implemented in PAUP*4.0b10. A bootstrap analysis (100 replicates, random stepwise addition with 100 reps) to determine node support was also carried out within a maximum parsimony framework.

Adult morphology

The distinguishing external morphological features of all *Polypedates* species in Sri Lanka were evaluated. Characters used by Manamendra-Arachchi and Pethiyagoda (2005) were noted for all individuals. The following characters were measured to the nearest 0.1 mm using vernier calipers: eye diameter (ED); eye-to-nostril distance (EN); eye-to-snout length (ES); distal end of tibia length (FEL); length of third finger (FLIII); pes length (FOL); head length (HL); head width (HW); internarial distance (IN); interorbital distance (IO); nostril-to-snout length (NS); snout-vent length (SVL); tibia length (TBL); and length of fourth toe (TLIV).

X-ray computed tomography (CT) scans of skulls of *Taruga eques* (BMNH 1947.2.27.56), *P. cruciger* (BMNH 1875.2.27.8) and *P. leucomystax* (MCZ A-135392) were prepared by the Digimorph imaging facility at University of Texas Austin (BMNH: Natural History Museum, London; MCZ: Museum of Comparative Zoology, Harvard University.). All material examined are listed in Appendix 1.

Tadpole morphology

Tadpoles of *Taruga eques* were collected from a pond in a tea estate at Agarapatana (1550 m elevation) and *P. cruciger* from a man-made pond at the University of Peradeniya. They were fixed in 10% neutral-buffered formalin (NBF) and preserved in a 1:1 mixture of 70% ethanol and 10% NBF. Measurements were made to the nearest ± 0.01 mm using a digital caliper and a graduated microscope attachment (Motic K-400) fitted with a drawing tube. Terminology used to describe external features follows McDiarmid and Altig (2000); terminology for internal oral features follows Wassersug (1976). Only Gosner (1960) stage-38 tadpoles were evaluated.

RESULTS

Molecular phylogenetics and systematics

The complete dataset initially consisted of DNA sequences of 3730 bp length. A total of 3404 bp remained after removing regions of the mitochondrial 12S and 16S rRNA genes for which alignment was ambiguous and confidence of positional homology was low, but retaining all positions of the *cyt-b* and nuclear genes. The Partition Homogeneity test showed that the data are congruent ($p > 0.01$) and that the combined analysis of data is justified. Model test results show that GTR+I+G is the best fit (based on AIC) of the 56 models considered for the dataset.

We chose as our best tree the one with the highest likelihood from the unpartitioned Bayesian analysis. All nodes are well supported, with high posterior probabilities ($> 95\%$; Fig. 5). Maximum parsimony analysis yields two equally parsimonious trees (tree length 4161) and these trees agreed in major clade arrangement to a great degree with the clade arrangement of the Bayesian tree (the two maximum parsimony trees are not shown).

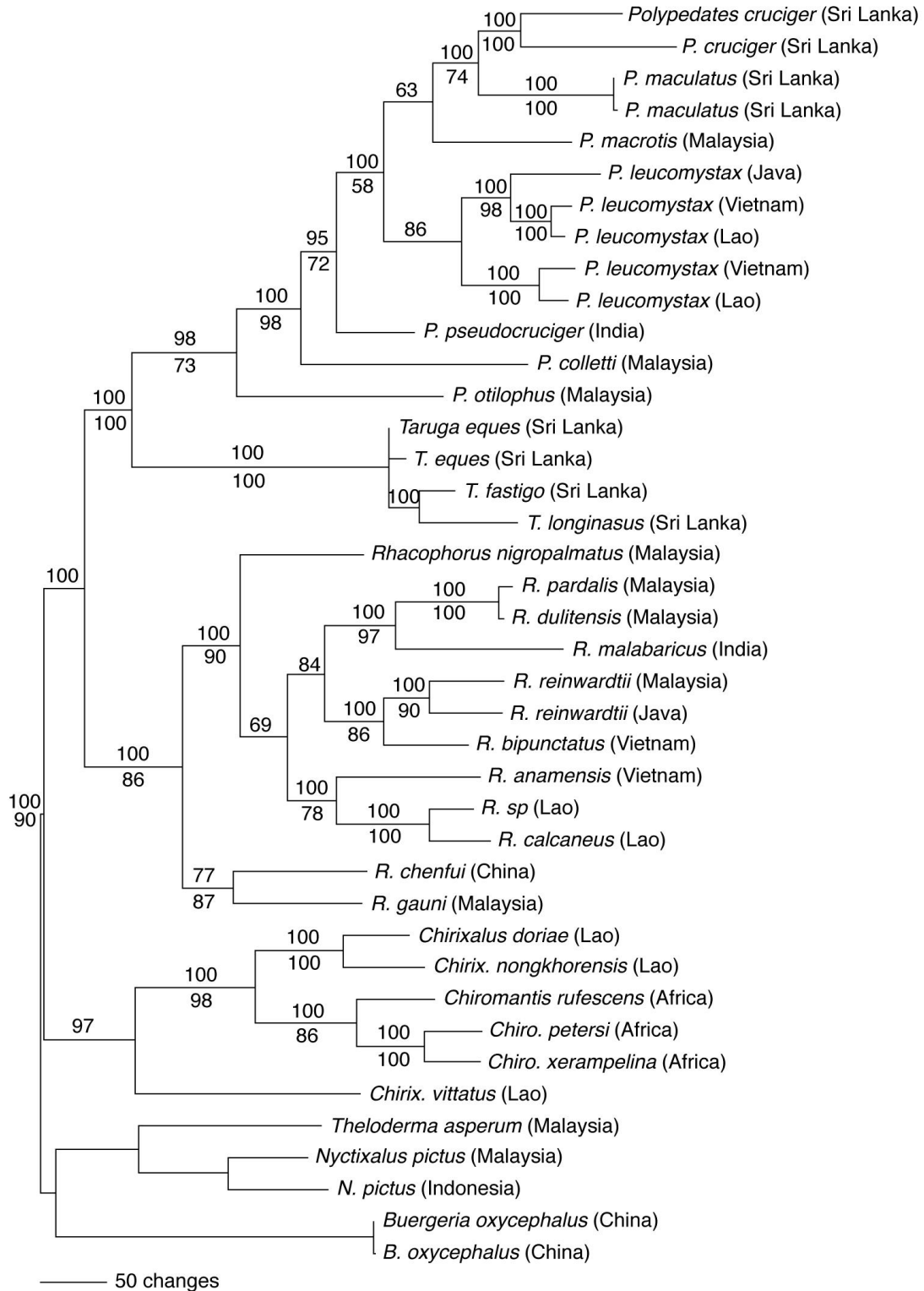


Figure 5. Bayesian phylogram with posterior probabilities shown above nodes, parsimony bootstrap values shown below nodes. All the major nodes that distinguish foam nesting rhacophorid genera have high support; *Taruga*, new genus, is a highly supported and is the basal sister taxon to *Polypedates*.

Major phylogenetic conclusions, all of which receive high clade support in the Bayesian tree (Fig. 5) and the parsimony trees, are: (1) all Sri Lankan species of *Polypedates* are contained within two distinct clades: a basal, endemic Sri Lankan clade, which includes *P. eques*, *P. fastigo* and *P. longinasus*; and a clade which contains two widely-distributed species, *P. cruciger* and *P. maculatus*, and which is nested within Southeast Asian and Indian taxa; (2) the genus *Rhacophorus* and the endemic Sri Lankan clade are reciprocally monophyletic; and (3) *Chiromantis* is nested within the *Chirixalus* group.

Taruga, new genus

Type species:

Polypedates fastigo Manamendra-Arachchi and Pethiyagoda, 2001. Type series (including the Holotype WHT2352) is deposited in the National Museum of Sri Lanka.

Included species:

Polypedates eques Günther, 1858

Rhacophorus longinasus Ahl, 1927

Etymology. *Taruga* in Sanskrit and early Sinhala refers to “tree climber,” a reference to the arboreal habitat of these frogs. Gender masculine.

Diagnosis. Several adult characters and character states distinguish *Taruga* from Sri Lankan *Polypedates* (Figs. 2, 3A and 6; Figs. 1 and 3B). *Taruga* have a straight or slightly curved dorsolateral glandular fold that extends from the back of the upper eyelid to the mid-flank, whereas *Polypedates* have a supratympanic fold that curves around the dorsal margin of the tympanum. Additionally, *Taruga* possess a prominent calcar at the distal end of the tibia (absent in most *Polypedates*); 6–10 prominent conical tubercles around the cloaca (absent in *Polypedates*); and a pointed snout in both dorsal and lateral aspects (vs. a shorter, more rounded snout in *Polypedates*) (Fig. 6). Snout acuteness differs markedly between the two genera; snout angle in *Taruga* varies between 58° and 70° (*T. longinasus*, 58°; *T. eques*, 70°; *T. fastigo*, 67°) whereas in Sri Lankan *Polypedates* it varies between 76° and 92° (*P. cruciger*, 77 to 92°; *P. maculatus*, 76°): data from Manamendra-Arachchi and Pethiyagoda (2005).

The cranium of *T. eques* differs from that of *P. cruciger* and *P. leucomystax* in several

respects (Fig. 7). The skull of *T. eques* is narrow and long in dorsal view; skull length/width ratio 0.90, frontoparietal length/width ratio 1.57 (the skull is wide and short in *P. cruciger* and *P. leucomystax*; skull length/width 1.03 and 1.12, frontoparietal length/width 1.40 and 1.25 in the two genera, respectively). The dorsal surface of the cranium is smooth in *Taruga* (with bony ridges in *P. cruciger* and *P. leucomystax*). The other features by which the two genera differ are: posterior margin of frontoparietal blunt in *Taruga* (with two pointed bony ridges in *P. cruciger*, concave in *P. leucomystax*); the anterior margin of the frontoparietal is convex in *Taruga* (concave in *P. cruciger*, straight in *P. leucomystax*); pterygoid strongly curved in *Taruga* (slightly curved in *P. cruciger* and *P. leucomystax*); and orbit dorsally long and narrow, length/width ratio 2.1 in *Taruga* (short and wide, length/width equals 1.6 in *P. cruciger*, 1.8 in *P. leucomystax*).

Tadpoles of *T. eques* and *P. cruciger* differ markedly in external morphology (Table 2; Figs. 4 and 8). *Taruga eques* has a pointed snout in dorsal view (rounded in *P. cruciger*), dorsally-directed eyes (laterally directed in *P. cruciger*) and a thin dorsal lip and groove at the base of the tail (absent in *P. cruciger*). It also lacks a marked, whip-like flagellum at the tip of the tail (present in *P. cruciger*).

In lateral view (Figs. 4 and 8), tadpoles of *T. eques* feature a sinistral spiracle attached to the trunk along most of its length, its inner wall free and formed such that the aperture opens laterally instead of posteriorly (a sinistral and cylindrical spiracle, centripetal, its distal end is directed posteriorly); the ventral tail fin originates at the caudal end of the trunk (originates from the trunk's ventral surface in *P. cruciger*); the dorsal fin originates at the caudal end of the trunk (originates from the trunk's dorsal surface in *P. cruciger*); and the vent opens as a dextral aperture between the hind limb and tail and does not form a tube (a distinct conical dextral vent tube, open at the free end, originates between the hind limb and the tail in *P. cruciger*; Fig. 9). The configuration of the vent in *Taruga* is a derived and possibly unique character-state in anuran larvae, which typically possess a vent tube (McDiarmid and Altig, 2000).

The larval buccal cavity also differs in the two species (Table 3 and Fig. 10). Characteristic features of the buccal floor in *Taruga eques* are; heart-shaped prelingual arena (U-shaped in *P. cruciger*); absence of prelingual-arena papillae

(one pair in *P. cruciger*); three major digitations of the anterior prelingual arena palps (two in *P. cruciger*); flat and broad, multifurcated, posteriorly-directed posterior prelingual arena palps (long and medially directed with projections in *P. cruciger*); absence of papillae on the anterior wall of the buccal cavity (4–5 pairs in *P. cruciger*); tongue rounded (slightly conical in *P. cruciger*); a single pair of lingual papillae (two unequally-sized pairs in *P. cruciger*); lingual pigmentation present (absent in *P. cruciger*); tongue attached posteriorly with indiscernible buccal floor musculature, lacking a notch (tongue fully attached to prominent musculature, in a notch, in *P. cruciger*); a U-

shaped, depressed buccal floor arena (BFA; triangular and elevated in *P. cruciger*); fewer than 50 BFA pustulations (more than 50 in *P. cruciger*); and 19–21 BFA papillae (11–13 in *P. cruciger*).

Characteristic features of the buccal roof in *Taruga* (Table 3 and Fig. 10) are; broad, V-shaped prenarial arena (U-shaped in *P. cruciger*); a broad, V-shaped anterior transverse ridge (crescentic in *P. cruciger*); absence of an anterior projection on the narial papilla (present in *P. cruciger*); and five pairs of arena papillae (four pairs in *P. cruciger*).

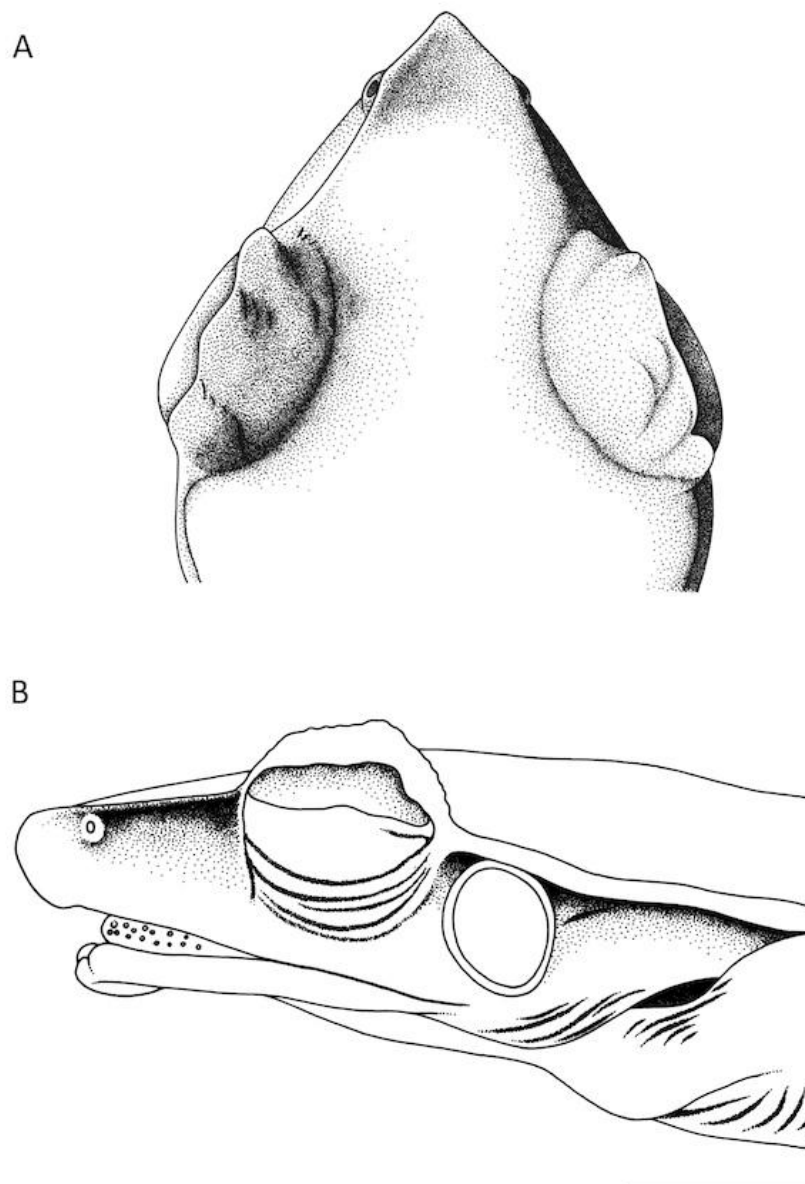


Figure 6. Head of *Taruga eques*, (A) dorsal and (B) lateral aspects, showing the pointed snout and the straight glandular fold extending from back of eye to mid-flank. (Scale bar, 5 mm)

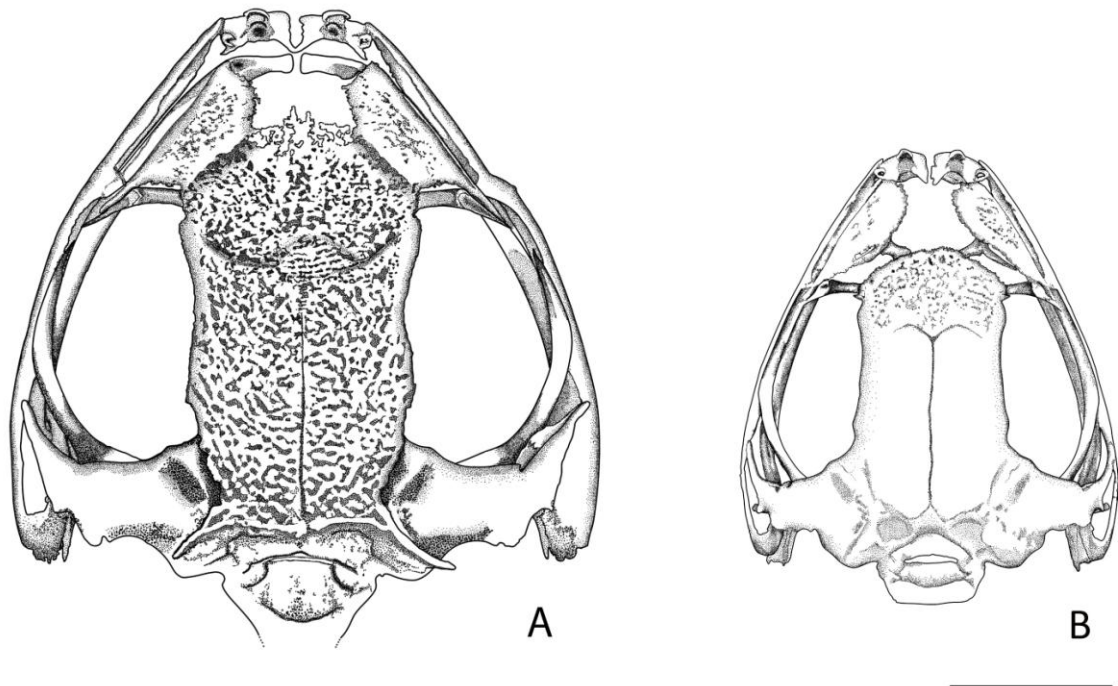


Figure 7. Dorsal aspect of the skull of (A) *Polypedates cruciger* (BMNH 1875.2.27.8; and (B) *Taruga eques* (BMNH 1947.2.27.56; drawings based on CT images. The two species differ from each other in the shape of the skull, frontoparietal and orbit; rugosity and shape of the anterior and posterior edges of the frontoparietal; and the shape of the pterygoid. (Scale bar, 5 mm)

Table 2. Differences in the external morphology of tadpoles of *Polypedates cruciger* and *Taruga eques*.

Tadpole external morphology	<i>Polypedates cruciger</i>	<i>Taruga eques</i>
Dorsal view		
Snout shape	rounded	pointed
Position of eyes	mostly lateral	mostly dorsal
Tail end	with a whip-like flagellum	no whip-like flagellum on tail end
Point of origin of tail	dorsal skinny lip and groove absent	dorsal skinny lip and groove present
Lateral profile		
Spiracle characteristics	cylindrical, sinistral, centripetal; end directed posteriorly	sinistral, mostly attached to body; inner wall free, aperture opens laterally
Origin of ventral tail fin	begins under body	begins at end of trunk
Origin of dorsal tail fin	dorsal tail fin begins on the trunk	dorsal fin begins at the end of trunk
Shape and position of vent	between hind limb and tail; forms a distinct conical tube with opening at the end	vent opens as an aperture, not forming a tube; aperture dextral between hind limb and tail

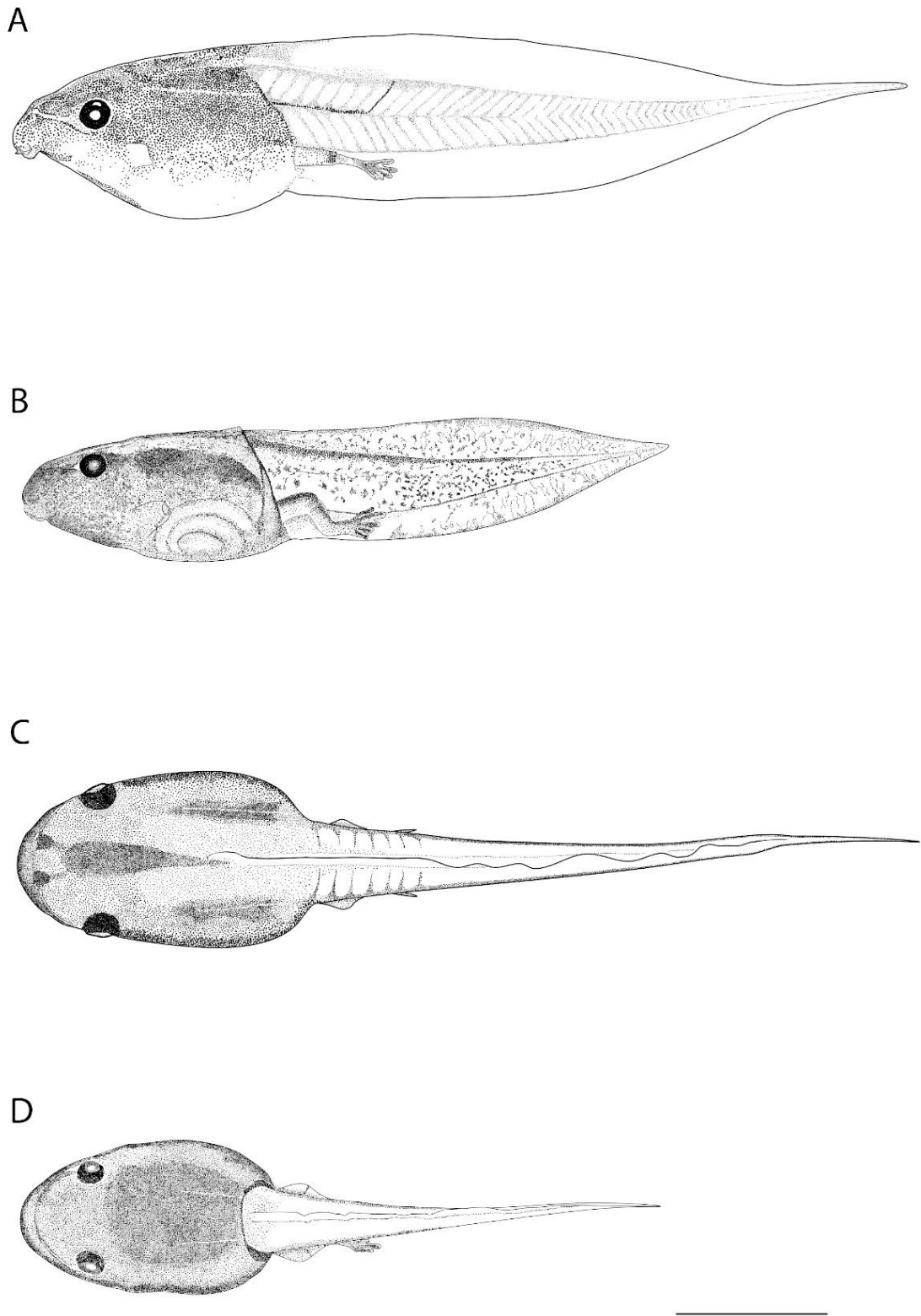


Figure 8. External features of *Polypedates cruciger* (A) left profile; (C) dorsal profile and *Taruga eques* (B) left profile; (D) dorsal profile. The two taxa differ in size and form, existence of tail-end flagellum, shape and form of the spiracle, and the point of origin of dorsal and ventral tail fin. (Scale bar 10 mm)

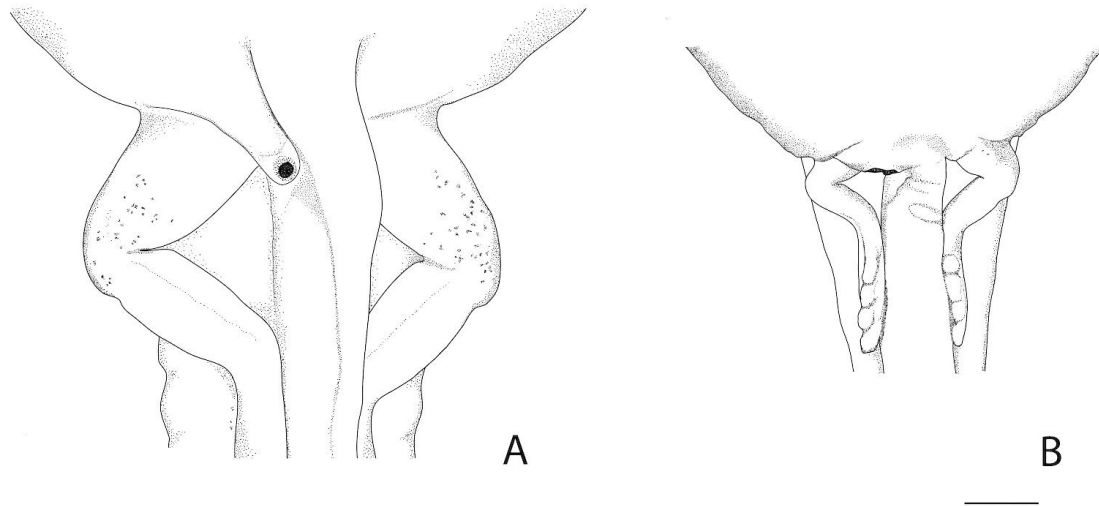


Figure 9. (A) *Polypedates cruciger* and (B) *Taruga eques* also differ in the form of the vent. While *P. cruciger* has a vent tube, *T. eques* has only a vent aperture. (Scale bar, 1 mm)

Table 3. Differences in buccal morphology between tadpoles of *Polypedates cruciger* and *Taruga eques*.

Character	<i>Polypedates cruciger</i>	<i>Taruga eques</i>
Ventral buccal		
Shape of prelingual arena	U-shaped (lunate)	heart-shaped (cardioid)
Prelingual arena papillae	one pair	absent (only a pair of pustulations)
Anterior prelingual arena palps	two major digitations	three major digitations
Posterior prelingual arena palps	long, with projections, medially directed	flat and broad, multifurcated, posteriorly directed
Papillae on anterior wall of buccal cavity	4-5 pairs	absent
Tongue shape	slightly conical	rounded
Lingual papillae	two unequally sized pairs	a single pair
Tongue pigmentation	absent	present
Tongue attachment	fully attached to prominent buccal floor musculature, in a notch	posteriorly attached, musculature indiscernible, notch absent
BFA	triangular, elevated	U-shaped, depressed
BFA pustulations	more than 50	fewer than 50
BFA papillae	11-13	19-21
Dorsal buccal		
Prenarial arena	broad, U-shaped	broad, V-shaped
Anterior transverse ridge	crescentic	broad, V-shaped
Anterior projection on narial papillae	present	absent
BRA papillae	4 pairs	5 pairs

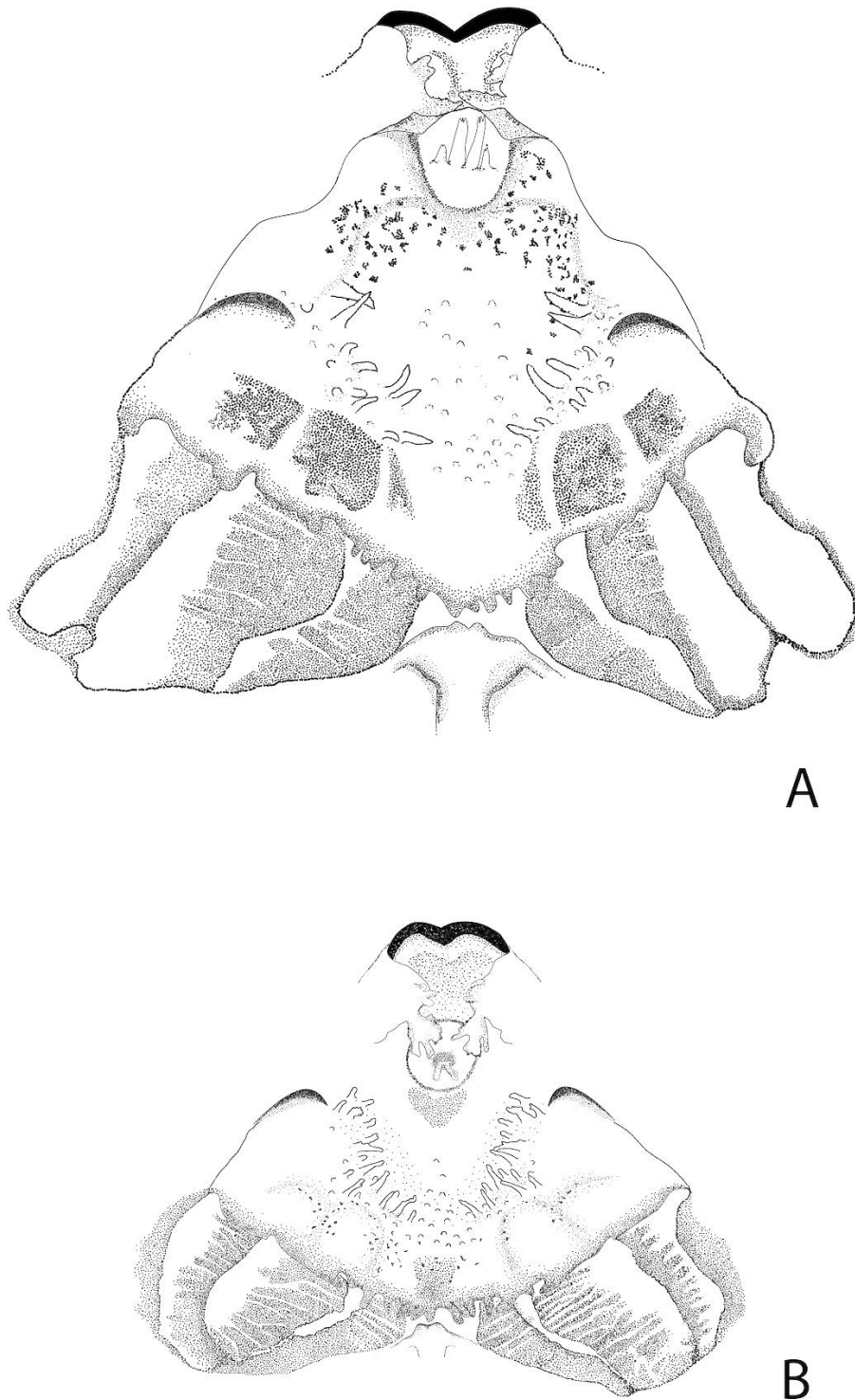


Figure 10. Ventral buccal region of (A) *Polypedates cruciger* and (B) *Taruga eques*. Presence of prelingual papillae and papillae of the anterior wall of buccal cavity, shape of tongue, attachment surface of tongue, features of the buccal floor arena, and number of buccal-floor arena papillae differ between the two taxa. (Scale bar, 1mm)

DISCUSSION

Our phylogenetic results (Fig. 5) confirm those of several earlier less-well supported studies that identified *Polypedates* as the sister taxon of *Rhacophorus*. Liem (1970) and Wilkinson and Drewes (2000) recovered *Rhacophorus* and *Polypedates* as sister taxa, although Channing (1989) did not. Most previous mtDNA-based phylogenies failed to recover this sister-clade relationship (Richards and Moore, 1998; Meegaskumbura *et al.*, 2002; Wilkinson *et al.*, 2002), but intergeneric relationships are only weakly supported in those studies. The phylogeny of Frost *et al.* (2006), which is based on mtDNA and some nuclear DNA sequence data, recovers *Chiromantis*/*Chirixalus* as the sister taxon of *Polypedates*, and *Rhacophorus* as the sister taxon of a clade containing *Chiromantis*/*Chirixalus* + *Polypedates*.

There also is ambiguity in the morphological diagnoses of some species of *Polypedates* and *Rhacophorus* (Bossuyt and Dubois, 2001). Wilkinson *et al.* (2002), for example, show *P. dennysi* nested within *Rhacophorus* and allocate it to that genus (see Frost *et al.*, 2006). By including in the present study the type species of *Rhacophorus* (*R. reinwardtii*) and *Polypedates* (*P. leucomystax*) from Java, the type locality of each species, we seek to stabilize the identity of both genera.

A basal Sri Lankan clade can be distinguished within what was previously referred to as *Polypedates*. This endemic clade, for which we erect the new genus *Taruga*, is molecularly distinct (i.e., both well supported and deeply divergent) from remaining *Polypedates* species, including the two Sri Lankan species, *P. maculatus* and *P. cruciger*. As noted above, it also is differentiated morphologically from *Polypedates*. As presently understood the clade comprises three named species: *T. fastigo*, *T. eques* and *T. longinasus*. However, our molecular results also suggest that *Polypedates* is more speciose than currently understood, as two *P. cruciger* species used in the current analysis are widely different from each other; we are currently in the process of describing this new form.

Based on examination of the species included in the present study, snout angles of *Taruga* and *Polypedates* do not overlap. However, this character may be sexually dimorphic, at least in some species (males tend

to have more acutely-pointed snouts than females), hence we recommend that the sexes be compared separately when evaluating this character for taxonomic purposes.

Chiromantis occupies a well-supported basal position within the monophyletic foam-nesting group (Fig. 5). This arrangement agrees with the morphology-based phylogeny of Wilkinson and Drewes (2000), but not with those of either Liem (1970), who considered *Chiromantis* the sister group of *Philautus*, or Channing (1989), who considered *Chiromantis* as basal to all *Rhacophoridae* + *Mantellidae*, excluding *Buergeria*. Our result also differs from the mtDNA-based phylogenies of Richards and Moore (1998), Meegaskumbura *et al.* (2002) and Wilkinson *et al.* (2002), in which *Chiromantis* forms the sister group of *Polypedates*, although with only weak support. The present result also conflicts with the analysis of Richards *et al.* (2000), in which *Chiromantis* comprises a sister group of *Rhacophorus*, but again with only weak support. Our result reinforces the conclusions of Wilkinson *et al.* (2002) and Frost *et al.* (2006) that *Chiromantis* is nested within the *Chirixalus* clade, which, in our analysis, includes the type species of the genus, *Chirixalus doriae* (albeit from Laos the type locality is in Burma). We concur with Frost *et al.*'s (2006) recommendation that *Chirixalus* Boulenger, 1893, be regarded as a synonym of *Chiromantis* Peters, 1854.

Taruga is the sister group of the remaining species of *Polypedates* sensu stricto. The position of the *Taruga* + *Polypedates* clade as sister to Asian *Rhacophorus* and *Chirixalus* suggests an Asian origin of their common ancestor, with an ancient dispersal to Sri Lanka. Presence in Sri Lanka of *Polypedates cruciger* and *P. maculatus* indicates a second, more recent dispersal from mainland Asia to Sri Lanka. Since *Taruga* forms an isolated clade and is species depauperate, we regard it as a relict taxon. The biogeography of *Taruga* will be considered in a subsequent work.

Two genera of foam-nesting rhacophorid tree frogs are present in Sri Lanka: (1) *Polypedates*, with several species in Asia but just two in Sri Lanka (*P. maculatus*—widely distributed in South Asia; and *P. cruciger*—endemic to Sri Lanka, albeit widely distributed); and (2) *Taruga*, a Sri Lankan endemic, whose three constituent species (*T. eques*, *T. fastigo* and *T. longinasus*) each have restricted distributions mostly in the forested highlands.

External morphology of tadpoles of *T. longinasus* was reported by Fernando and Dayawansa (1995) and Fig. 11. Their results agree with ours for *T. eques* in showing that *T. longinasus* lacks a tail-end flagellum, that the dorsal and anal fins seem to commence at the posterior end of body, and that the spiracle appears to have a similar form to the one we observe in *T. eques*. Additional studies on the morphology of both *T. fastigo* and *T. longinasus*, as well as species of the *Polypedates* and the Rhacophoridae are needed to confirm whether the derived characters and character states we observe in *T. eques* are synapomorphic in the genus.

All three species of *Taruga* show a restricted distribution within Sri Lanka, whereas *Polypedates* is widely distributed through South Asia. *Taruga eques* and *T. fastigo* are restricted to submontane and montane regions: *T. eques*

occurs in the Central Hills and Knuckles Mountains; *T. fastigo* is restricted to the highest peaks of the Rakwana Hills (e.g., Morningside). *Taruga longinasus* is found in the mid-hill regions (of Rakwana Hills, and south and southwestern Central Hills) up to an elevation of about 600 m, and in the lowland wet zone. *Polypedates cruciger*, on the other hand, is widely distributed in the lowlands of the southwestern “wet zone” (annual rainfall > 2500 mm), extending to an elevation of about 1500 m. It also occurs in the “intermediate zone” and the island’s relatively dry south-eastern region, extending into the dry zone (annual rainfall < 2000 mm). *Polypedates maculatus* is abundant in the dry zone lowlands and occurs also in the lowlands of the wet zone, especially along the coastal floodplain. It usually does not occur at elevations above 500 m.

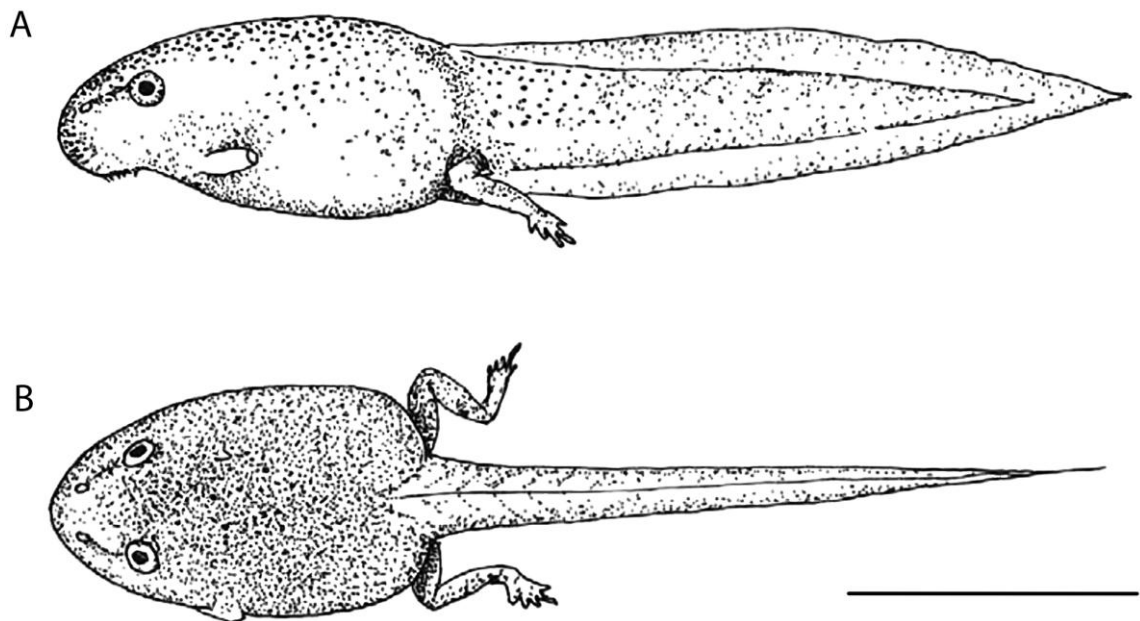


Figure 11. (A) Left profile and (B) dorsal profile of the tadpoles of *T. longinasus*, showing the absence of a flagellum. The body shape is similar to that of *T. eques* tadpoles. (Adapted from Fernando and Dayawansa, 1995). (Scale bar, 10 mm)

Table 4. Percent uncorrected pairwise genetic distances among foam-nesting genera of Rhacophoridae for the six gene fragments combined. Genetic distances to *Taruga* mostly overlap distances between the other pairs of foam-nesting genera.

% Uncorrected genetic distances				
Genera	<i>Taruga</i>	<i>Polypedates</i>	<i>Rhacophorus</i>	<i>Chiromantis</i>
<i>Taruga</i>	*	22-28	23-31	24-31
<i>Polypedates</i>	-	*	25-30	24-30
<i>Rhacophorus</i>	-	-	*	23-31
<i>Chiromantis</i>	-	-	-	*

Bossuyt *et al.* (2004) presented evidence that the fauna of Sri Lanka and India show clade-level endemism despite the multiple land-bridge connections between the island and the mainland during the past 50 million years, most recently up to about 10,000 years. *Taruga* appears to be another such a deeply divergent clade that Sri Lanka does not share with India.

Although genus-level genetic distances in Rhacophoridae have hitherto not been discussed comparatively, comparisons between pairs of other foam-nesting genera may be informative in validating *Taruga* as a distinctive genus. The uncorrected genetic distances (for all 6 gene fragments, both nuclear and mitochondrial, used in this study) between *Chiromantis*, *Polypedates*, *Rhacophorus* and *Taruga* varies in the range 22–31% (Table 4), that between *Taruga* and *Polypedates* being comparable to the divergences between the other genera.

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APPENDIX 1

Material Examined

***Taruga fastigo*.** Holotype, male, 37.3 mm SVL, WHT 2352; Morningside Estate (near Rakwana), alt. 1060 m (06°24'N, 80°38'E), coll. M.M. Bahir & S.V. Nanayakkara, 14 I 1999. Paratypes (all from type locality.), females, 63.9 mm SVL, WHT 2027; 59.9 mm SVL, NMSL ARH14(a), coll. M.M. Bahir & S.V. Nanayakkara, 05 VIII 1997. Females, 54.8 mm SVL, NMSL ARH14(b); 61.2 mm SVL, WHT 2343, coll. D. Gabadage & M.M. Bahir, 05 IX 1996. Males, 36.0 mm SVL, WHT 2335; 39.5 mm SVL, WHT 2340; 36.0 mm SVL, WHT 2341; 36.4 mm SVL, WHT 2347; 35.3 mm SVL, WHT 2348; 39.1 mm SVL, WHT 2349, coll. M.M. Bahir & S.V. Nanayakkara, 12 I 1999. Tadpoles, WHT 2902, SVL 17.0 mm, stage 44; WHT 2903, SVL 17.9, stage 43; WHT 2904, SVL 16.9, stage 43; WHT 2905, 17.0 mm SVL, stage 43; coll. M.-Arachchi & S.V. Nanayakkara, 30 VI 2000.

***Taruga eques*.** Syntypes (6 ex.). All males, 39.1 mm SVL, BMNH 1947.2.27.54; 36.9 mm SVL, BMNH 1947.2.27.55; 36.7 mm SVL, BMNH 1947.2.27.56; 36.6 mm SVL, BMNH 1947.2.27.57; 36.0 mm SVL, BMNH 1947.2.27.58; 35.1 mm SVL, BMNH 1947.2.27.59, Ceylon; H. Cuming. Female, 71.0 mm SVL, WHT 968, Horton Plains, alt. 2135 m (06°46'N, 80°47'E), coll. D. Gabadage & J. Karunaratne, 04 III 1995. Two ex. males, 32.7 mm SVL; 33.8 mm SVL, WHT 1238, Haputale, alt. 1525 m (06°46'N, 80°52'E), coll. D. Gabadage, 01 V 1996. Female, 59.2 mm SVL, WHT 1313, Pattipola, alt. 1890 m (06°51'N, 80°50'E), coll. D. Gabadage, 24 VI 1996. Two ex. males, 36.0 mm SVL; 37.5 mm SVL, WHT 1707, Hakgala (near Nuwara Eliya), alt. 1830 m (06°55'N, 80°49'E), coll. M.M. Bahir & D. Gabadage, 11 VI 1996. Female, 61.4 mm SVL, WHT 2316, Agra Arboretum, Agarapatana, alt. 1555 m (06°51'N, 80°41'E), coll. M.M. Bahir & S.V. Nanayakkara, 05 X 1998. Nine ex., 33.1-38.3 mm SVL, NMSL ARH 3 (a), Lindula, alt. 1265 m (06°55'N, 80°41'E). Male, 42.5 mm SVL, WHT 2937, Moray Est., Maskeliya, alt. 1370 m (06°48'N, 80°31'E), coll. K. Manamendra-Arachchi, 04 V 2000.

***Taruga longinasus*.** Holotype (holotype of *Ixalus nasutus* Günther, 1868), female, 58.5 mm SVL, BMNH 1947.2.8.61, Southern Ceylon; coll. Higgins. Female, 55.2 mm SVL, WHT 778, Parawalatenna (Kitulgala), alt. 150 m (06°59'N, 80°24'E), coll. M.M. Bahir & D. Gabadage, 21 XII 1995. Two ex. male, 44.0 mm SVL; female, 58.6 mm SVL, WHT 1176, Koskulana (near Panapola), alt. 460 m (06°25'N, 80°27'E), coll. M.M. Bahir & D. Gabadage, 23 V 1996. Female, 57.7 mm SVL, WHT 1743, Kudawa (near Weddagala), alt. 460 m (06°26'N, 80°25'E), coll. M. Wijesinghe, 04 VIII 1997. Four ex., males, 43.2 mm SVL, WHT 2304; 45.8 mm SVL, WHT 2305; 42.0 mm SVL, WHT 2306; 40.5 mm SVL, WHT 2307; Kanneliya (near Galle), alt. 150 m (06°15'N, 80°20'E), coll. M.M. Bahir, M.M. Bopage & S. Udayanga, 20 X 1998. Male, 45.5 mm SVL, WHT 2353, Kanneliya (near Galle), alt. 150 m (06°15'N, 80°20'E), coll. S. Batuwita & M.M. Bopage, 13 VII 1998.

***Polypedates maculatus*.** Female, 38.6 mm SVL, WHT 965 Mahiyanganaya, alt. 90 m (07°19'N, 80°59'E), coll. D. Gabadage, 22 III 1995. Female, 52.9 mm SVL, WHT 969, Ritigala, alt. 200 m (08°07'N, 80°40'E), coll. D. Gabadage & S. Dharmasiri, 06 X 1994. Female, 58.3 mm SVL, WHT 973, Piliyandala, alt. 12 m (06°48'N, 79°55'E), colle. S. Dharmasiri, 06 XII 1994. Male, 42.3 mm SVL, WHT 974, Palatupana (Kirinda), alt. 3 m (06°16'N, 81°24'E), coll. R. Pethiyagoda, 1994. Female, 38.6 mm SVL, WHT 1098, Kottawa (Galle), alt. 60 m (06°06'N, 80°20'E), coll. M.M. Bahir, 07 V 1995. Female, 46.6 mm SVL, WHT 1301, Polonnaruwa, alt. 55 m (07°56'N, 81°00'E), coll. D. Gabadage & M.M. Bahir, 02 I 1996. Female, 62.7 mm SVL, WHT 2350, Yakkala, alt. 30 m (07°05'N, 80°02'E), coll. M.M. Bahir, 1999. Female, 42.53 mm SVL, WHT 1086, Modera (Colombo), alt. 4 m (06°58'N, 79°52'E), coll. M.M. Bahir, 28 IX 1995.

***Polypedates cruciger*.** Syntypes (4 ex.), male, 53.5 mm SVL, ZSI A 10178; female, 78.1 mm SVL, ZSI A 10179; female, 78.9 mm SVL, ZSI A 10177; female, 80.1 mm SVL, ZSI A 10176, Ceylon; E.F. Kelaart. Male, 59.8 mm SVL, WHT 202, Lihinigala- Yattapatha (Moragala near Agalawatta), alt. 150 m (06°23'N, 80°17'E), coll. K.M.-Arachchi, 24 XI 1993. Female, 73.1 mm SVL, WHT 1090, Monaragala, alt. 150 m (06°52'N, 81°20'E), coll. K.M.-Arachchi & D. Gabadage, 17 X 1994. Female, 81.0 mm SVL, WHT 1092, Navinna (Galle), alt. 15 m (06°04'N, 80°12'E), coll. M.M. Bahir & M.M. Bopage, 6 IX 1995. Female, 75.6 mm SVL, WHT 1093, Galge (between Kataragama and Buttala), alt. 90 m (06°33'N, 81°19'E), coll. M.M. Bahir, May 1995. Male, 54.3 mm SVL, WHT 2351, Ambalangoda, alt. 15 m (06°14'N, 80°03'E), coll. A.I. Alagiyawadu, 1999.

***Polypedates biscutiger*.** Holotype, male, 55.2 mm SVL, ZMB 3049; from Ceylon.