Zootaxa 4461 (4): 519–538
http://www.mapress.com/j/zt/
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Article

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

https://doi.org/10.11646/zootaxa.4461.4.4
http://zoobank.org/urn:lsid:zoobank.org:pub:BBC32EC4-F730-49C5-A597-3A9AACE09603

A new frog species from rapidly dwindling cloud forest streams of Sri Lanka—*Lankanectes pera* (Anura, Nyctibatrachidae)

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Abstract

The monotypic genus *Lankanectes,* considered an evolutionary long branch with India's *Nyctibatrachus* as its sister lineage, is represented by *L. corrugatus*, a species widely distributed within the wet zone of Sri Lanka up to 1500 m asl, where it inhabits a variety of lotic and lentic habitats. Here, following an integrative taxonomic approach using DNA-based phylogenies, morphology, morphometry, and ecological niche models, we describe a new species—*Lankanectes pera* **sp. nov.** The new species is distinguished from its sister species mainly by its tuberculated throat and absence of dark patches on venter, throat, manus and pes. The uncorrected genetic distances between the two *Lankanectes* species for a fragment of the non-coding mitochondrial *16S rRNA* gene is 3.5–3.7%. The new species has a very restricted climatic distribution with a total predicted area of only 360 km^2 (vs. 14,120 km^2 for *L. corrugatus*). Unlike *L. corrugatus*, which prefers muddy substrates and marshy areas, the new species is observed inhabiting only pristine streams flowing through canopy covered montane forests in the highest reaches of the Knuckles Mountain range. The specialized new species will need immediate conservation attention due to its restricted distribution (montane isolate), specialized habit of inhabiting clear mountain streams, and small population size.

Key words: Ecological niche models, General lineage concept, Knuckles Mountains, Montane-isolate

Introduction

Compared to mainland India, where endemic lineages at the level of higher taxa, such as *Nasikabatrachus, Micrixalus* and *Nyctixalus* abound (Biju & Bossuyt 2003; Roelants *et al*. 2004; Van Bocxlaer *et al*. 2011), only a few endemic anuran lineages dating to before the Cretaceous-Paleogene extinction event seem to have survived in Sri Lanka. The only Sri Lankan amphibian lineage that made it through this mass extinction event is *Lankanectes*, a monotypic endemic genus represented by *L. corrugatus* (Peters 1863). Its closest living relatives are considered to be the representatives of the family Nyctibatrachidae, which diverged prior to the Cretaceous-Paleogene extinction event, about 72–73.5 mya (Van Bocxlaer *et al*. 2011; Pyron & Wiens 2011, 2013).

Lankanectes is now accepted as being substantially different morphologically from *Euphlyctis, Limnonectus, Taylorana, Occidozyga, Phrynoglossus* and *Nyctibatrachus*, where it was previously placed (Peters 1863; Boulenger 1920; Deckert 1938; Dubois 1981; Dubois & Ohler 2001). Molecular studies have confirmed not only the distinctiveness of *Lankanectes*, but also its ancientness among South Asian anuran lineages (van der Meijden *et* *al*. 2004; Delorme *et al*. 2004; Roleants *et al*. 2004). This widely distributed species, with its conspicuously loud call, is found up to an elevation of about 1500 m asl, in submontane habitats in the southern, western and central parts of Sri Lanka, (Frost 2017; Manamendra-Arachchi & Pethiyagoda 2006).

During our field work in the streams of the cloud forests of the Knuckles mountain region, we discovered a population of *Lankanectes* that appeared to be morphologically distinct from *Lankanectes corrugatus*. Given that many of the species of *Nyctibatrachus* are montane endemics (Van Bocxlaer *et al*. 2011), the possibility of the existence of an undescribed species of Sri Lankan *Lankanectes* was high. Using an integrative taxonomic approach, in the sense of the General Lineage Concept (De Queiroz 1998), from a lineage that was thought to be on an evolutionary long branch with a single species, we show this population to be clearly distinct from *L. corrugatus* and describe it as a new species—*Lankanectes pera* **sp. nov.**

Materials and methods

Specimen collection, DNA barcoding, and Phylogenetic analyses. Field surveys for *Lankanectes* were conducted in Sri Lanka in the years 2012– 2016 (Table 1). Guided by calling males, sampling was carried out mostly at night. Animals (adults and tadpoles) were euthanized in tricaine methanesulphonate (MS-222). Thigh muscle tissue (~20 mg) from adults and a small part of the tail-fin of two tadpoles were stored in absolute ethanol at -20 °C for subsequent molecular studies in the Department of Molecular Biology & Biotechnology (DZ), University of Peradeniya. Adult specimens used in morphological studies were fixed in 4% formalin and later preserved in 70% ethanol. The type specimens of the new species are deposited in the Department of Molecular Biology & Biotechnology, University of Peradeniya.

Collected samples (Table 1) were DNA barcoded for the 16S ribosomal RNA (*16S rRNA*) mitochondrial gene fragment to ascertain species identity. DNA was extracted from ethanol-preserved tissue using a standard protocol (Sambrook *et al*. 1989). Portions of the mitochondrial *16S rRNA* gene were amplified by PCR and sequenced directly using dye-termination cycle sequencing. Primer sets, 16Sar and 16Sbr (Palumbi 1996) were used, which amplified a ca. 600 bp fragment of the *16S rRNA* gene. PCR conditions for amplification 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. Newly generated sequences were visualized and checked using 4peaks (v. 1.7.1).

Twenty-eight taxa (Table 1) representing closely related congeners of *Lankanectes* (according to recently published phylogenies—Van Bocxlaer *et al*. 2011) were also included in the dataset. Additionally, *Nasikabatrachus sahyadrensis* and *Micrixalus phyllophilus* representing closely related basal nyctibatrachids (Pyron & Wiens 2011) were used as an outgroup (Table 1). The compiled *16S rRNA* dataset was aligned using ClustalW as implemented in MEGA v. 7.0 (Kumar *et al*. 2016). Uncorrected pairwise distances were calculated using PAUP* 4.0b10 (Swofford 2002; Table 2). Regions that were highly variable were manually removed from the dataset; the final dataset consisted of 485 bps. The best-fitted model was chosen using jModeltest v. 2.1.4 (Posada & Crandall 1998). Maximum likelihood (ML) analysis was performed to infer relationships among the lineages and clades using the software GARLI (Zwickl 2006) on the CIPRES Science Gateway (Miller *et al*. 2010), using the best model (TIM2+I+G) parameters. Clade support was assessed using posterior probability (PP) and Maximum Parsimony (MP) bootstrapping values. Bayesian inference as implemented in MrBayes (v.3.1.2; Huelsenbeck & Ronquist 2001) was used to assess posterior probability (PP) values for each node with the parameters of the best-fitted model estimated as obtained from the jModelTest. Four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains were run for ten million generations. Burn-in of 5 million generations was estimated using Tracer v. 1.6 (Bouckaert *et al*. 2014). Bootstrapping was done in a MP framework using PAUP, where a full heuristic search was done, with 1000 replicates.

Morphology and Morphometrics. The suite of characters and character states used by Manamendra-Arachchi & Pethiyagoda (2006) was considered. Measurements were made to the nearest 0.1 mm using dial Vernier calipers. The following morphometric variables were measured: distance between back of eyes (DBE); distance between front of eyes (DFE); eye diameter (ED); eye-to-nostril distance (EN); eye-to-snout length (ES); femur length (FEL); length of finger 1 (FLI); length of finger 2 (FLII); length of finger 3 (FLIII); length of finger 4 (FLIV); pes length (FOL); head length (HL); head width (HW); length of inner metatarsal tubercle (IML); internarial distance (IN); interorbital distance (IO); lower-arm length (LAL); posterior mandible-to eye distance (MBE); least distance from mandible to anterior eye (MFE); least distance from mandible to nostril (MN); nostril-to-snout length (NS); palm length (PAL); snout–vent length (SVL); tibia length (TBL); length of toe 1 (TLI); length of toe 2 (TLII); length of toe 3 (TLIII); length of toe 4 (TLIV); and length of toe 5 (TLV); length of upper arm (UAW); and width of upper eyelid (UEW). Illustration of the webbing pattern follows Manamendra-Arachchi & Pethiyagoda (2006). All these measurements were used in the Principal Components Analysis (PCA). Abbreviations used in the study: DZ, Department of Molecular Biology and Biochemistry, Peradeniya, Sri Lanka; FR, Forest reserve; GS, Gayani Senevirathne; HUN, Hunnasgiriya (field collection numbers); KM-A, Kelum Manamendra-Arachchi; KNU, Knuckles (field collection numbers); MM, Madhava Meegaskumbura; NW, Nayana Wijayathilaka; WHT, Wildlife Heritage Trust of Sri Lanka, Colombo, Sri Lanka; ZMB, Zoological Museum of Berlin.

Principal Components Analysis of the character correlation matrix was used to reduce dimensionality of the continuous morphological variables and to identify those variables that best discriminate among morphologically similar forms. Various axis rotations were tested, and one was selected for optimal interpretability of variation among the characters. SYSTAT (Version 11.00.01) was used for statistical analysis.

Haplotype Network. Population genetic structure was determined by constructing haplotype networks using available sequences of the *16S rRNA* fragment as implemented in PopArt (http://popart.otago.ac.nz). Given the close relationships between populations and the two sister taxa, ambiguously aligned regions were absent among *Lankanectes* sequences, and the full dataset (560 bp) was used for this analysis.

Adult osteology. Osteological preparation and descriptions for *Lankanectes corrugatus*, which would serve as a description for the genus (type species for the genus), is carried out here based on cleared and stained postmetamorphic adults (*N* = 2; DZ 1397; DZ 1399). Neutral-buffered formalin preserved specimens were stained following the procedure by Taylor and Van Dyke (1985). Initial dehydration was done in 100% ethanol, followed by submersion in alcian blue for cartilage staining. Excessive musculature was digested using an infusion of borax and trypsin, and the specimens were subsequently stained in alizarin red for bone visualization. Preparations were photographed and scored for bones and cartilage within 2–3 days following the clearing and staining procedure. Osteological terminologies follow Duellman and Trueb (1986) and Senevirathne *et al*. (2016).

Niche modeling. We collected distribution records for *Lankanectes* both from the published literature (e.g. Manamendra-Arachchi & Pethiyagoda 2006) and our own field records. The program MaxEnt, version 3.3.3k (Philips *et al*. 2004) was used to predict the geographic distribution of each putative species. For this study, we used 24 presence locations for *L. corrugatus* and five for *L. pera* **sp. nov.** (Appendix 1). We downloaded 19 environmental variables and an altitude layer with a 30 arc-second (ca. 1 km^2) spatial resolution, from WorldClim dataset (www.worldclim.org). All layers were clipped to our study region bounded by 5.908° to 9.842 °N and 79.516° to 81.891 °E (which includes all of Sri Lanka). Highly correlated variables ($r \geq 0.8$ Pearson correlation coefficient) were eliminated from the analysis. Altogether, seven and four variables were selected to generate the predictive models of *L. corrugatus* and *L. pera* **sp. nov.**, respectively (Table 3). We used: the automatic mode with jackknife validation; random seed option for all sample points to train the model; and 25% of the records to test it. We ran 10 replicates using bootstrap function, and the average model was selected. The logistic method was used to obtain the values of habitat suitability, in which the probability values ranged from 0 to 1. Resulting values were then transferred to binary presence and absence values using Lowest Presence Threshold (LPT). Model performance was evaluated using area under the Receiving Operator Curve (AUC), in which the value ranges from 0 to 1 (Fielding & Bell 1997).

Results

DNA barcoding and phylogenetic analyses. The final dataset contained *16S rRNA* mitochondrial gene sequences from 41 putative species. Eleven of these represent Sri Lankan *Lankanectes* (9 tissues from adults and 2 tadpoles), while 28 represent *Nyctibatrachus*, the sister group of *Lankanectes*. The Maximum Likelihood (ML) tree (Fig. 1) is rooted using *Nasikabatrachus sahydrensis* and *Micrixalus phyllophilus*. TIM2+I+G was selected as the best-fitted model for our dataset. The parameters of the nucleotide substitution model for the most likely tree were as follows: rate matrix: shape parameter for gamma distributed rate variation among sites (alpha)= 0.5260; Tree length = 1.990892; R(A-C) = 9.0960; R(A-G) = 45.7402; R(A-T) = 15.2825; R(C-G) = 0.5040; R(C-T) = 142.4271; R(G-T) = 1.000000; Nucleotide frequencies: (A) = 0.3279; (C) = 0.2297; (G) = 0.2073; (T) = 0.2350; likelihood= -2631.6028. The ML tree had the same topology as the Distance, Bayesian, and Maximum Parsimony trees.

TABLE 1. GenBank Accession numbers and Voucher numbers of the taxa used in the study.

Species name	Accession number	Voucher number	Location
Lankanectes corrugatus	MH697874	DZ1396	Peradeniya
Lankanectes corrugatus	MH697875	DZ1397	Peradeniya
Lankanectes corrugatus	MH697876	DZ1409	Panwila
Lankanectes corrugatus (tadpole)	MH697877	GS2_32	Galle
Lankanectes pera **sp. nov.** (tadpole)	MH697873	DZ1320	Knuckles
Lankanectes pera **sp. nov.**	MH697872	DZ1307	Knuckles
Lankanectes pera **sp. nov.**	MH697871	DZ1290	Knuckles
Lankanectes corrugatus	DQ346971	X	Sri Lanka
Lankanectes corrugatus	AF215393	X	Sri Lanka
Lankanectes corrugatus	AY880445	X	Sri Lanka
Lankanectes corrugatus	DQ019603	X	Galle
Nyctibatrachus vrijeuni	JN644783	X	India
Nyctibatrachus sp. B	JN644784	X	India
Nyctibatrachus aliciae	JN644785	X	India
Nyctibatrachus poocha	JN644786	X	India
Nyctibatrachus minor	JN644787	X	India
Nyctibatrachus vasanthi	JN644788	X	India
Nyctibatrachus indraneili	JN644789	X	India
Nyctibatrachus karnatakaensis	JN644790	X	India
Nyctibatrachus deccanensis	JN644791	X	India
Nyctibatrachus petraeus	JN644792	X	India
Nyctibatrachus beddomii	JN644793	X	India
Nyctibatrachus sp. A	JN644794	X	India
Nyctibatrachus acanthodermis	JN644795	X	India
Nyctibatrachus deveni	JN644796	X	India
Nyctibatrachus minimus	JN644797	X	India
Nyctibatrachus sylvaticus	JN644798	X	India
Nyctibatrachus sanctipalustris	JN644799	X	India
Nyctibatrachus gavi	JN644800	X	India
Nyctibatrachus major	JN644801	X	India
Nyctibatrachus shiradi	JN644774	X	India
Nyctibatrachus dattatreyaensis	JN644775	X	India
Nyctibatrachus anamallaiensis	JN644776	X	India
Nyctibatrachus jog	JN644777	X	India
Nyctibatrachus humayuni	JN644778	X	India
Nyctibatrachus danieli	JN644779	X	India
Nyctibatrachus kempholeyensis	JN644780	X	India
Nyctibatrachus pillaii	JN644781	X	India
Nyctibatrachus grandis	JN644782	X	India
Nasikabatrachus sahyadrensis	AY364381	X	India
Micrixalus phyllophilus	KJ711349	X	India

TABLE 2. Uncorrected percentage pairwise divergences of *16S rRNA* between the *Lankanectes corrugatus* and *L. pera* **sp. nov.** species used in the study (refer Table 1 for the corresponding locations of the voucher numbers).

	DQ 346971	DZ 1396	DZ 1397	DZ 1409	GS 232	AF 215393	AY 880445	DQ 019603	DZ 1320	DZ 1307	DZ 1290
DQ346971	-										
DZ1396	0	-									
DZ1397	0	0	-								
DZ1409	0	0	0								
GS2 32	0.4	0.4	0.4	0.4							
AF215393	0.2	0.2	0.2	0.2	0.2						
AY880445	0.4	0.4	0.4	0.4	0.4	0.2					
DQ019603	0.0	0	0	0	0.4	0.2	0.4				
DZ1320	3.7	3.7	3.7	3.7	3.7	3.5	3.7	3.7			
DZ1307	3.7	3.7	3.7	3.7	3.7	3.5	3.7	3.7	0		
DZ1290	3.7	3.7	3.7	3.7	3.7	3.5	3.7	3.7	0	0	0

Maximum parsimony bootstrap values and PP values at the node of the *Lankanectes* lineage indicates that it is highly supported (Fig. 1). It results in two highly supported subclades: a widely distributed *Lankanectes corrugatus* and the new species from the Knuckles region. The relationships of the taxa of the *Lankanectes* clade were identical to the relationships from the Maximum Parsimony, Distance and Bayesian analyses. The uncorrected pairwise distance between *L. corrugatus* and *L. pera* **sp. nov.** is 3.5–3.7%. (Table 2).

Haplotype Network. The entire fragment (560 bp) was used to construct the haplotype networks for the 6 populations (Knuckles, Galle, Agra, Peradeniya, Panwila, Morningside), representing the two species of *Lankanectes* (Fig. 1). All haplotypes of *Lankanectes corrugatus* are shared between populations with 1–4 mutational changes. *Lankanectes pera* **sp. nov.** differs from *L. corrugatus* by 16 mutational steps with no sharing of haplotypes.

Adult osteology. Cranium (Fig. 5): Cranium of *Lankanectes corrugatus* has a height of 69% of its width. Frontoparietals are paired, quadrangular, invest the roof of the neurocranium dorsally, and posterolateral margins reach prootics fusing synostically. *Parasphenoid*, is azygous, ventral, and T-shaped; furcated cultriform process extends almost up to the palatines, but do not fuse with them. Perpendicular to the anterior cultriform process, alae extend laterally, overlaying prootics and exoccipitals, and end bluntly; medially, a depression is present where alae and cultriform process congregate. Vomers have four processes (dentary, anterior, prechoanal, and postchoanal) and are medially separated from one another; dentary processes of the vomers are edentate and reach the center region of the palatines; anterior process with a blunt terminal end extends towards the premaxillary-maxillary junction; prechonal and postchonal processes extend laterally from the anterior process, and the bifurcation between the two occurs at the base of the dentary process. Palatines are paired and invest the posterior margins of the planum antorbitale; lateral blunt ends reach up to medial face of the pars dentalis of maxillae, but do not articulate with maxillae. Anterior ramus of the pterygoids reaches the posterior margin of the planum antorbitale ventrally and is connected to the dentary process of the maxillae; posterior ramus, equal to the length of the anterior ramus, is directed towards the quadrate and has a cartilaginous epiphysis; medial ramus, shortest among the three rami, reaches the anterior margins of the otic capsule ventrally. Squamosals have three distinct rami; ventral ramus reaches up to the epiphysis of ventral ramus of the pterygoid; zygomatic ramus has a pointed terminus; otic ramus is blunt-ended.

Maxillary arcade: Premaxillae are separated from one another, but overlap with maxillae; alary process of the premaxilla is about half of the length of pars dentalis; pars palatina is bifurcated and extend from the posteroventral margin of dentate pars dentalis of premaxilla. Maxillae are composed of pars dentalis, pars facialis and pars palatina; pars dentalis bear 30/38 (upper/lower) blunt teeth; pars palatina of maxillae do not articulate with the pars palatina of premaxillae; posteriomost ends of maxillae articulate with the qudratojugals, which completes the maxillary arcade posteriorly; pars facialis is well ossified and forms the lateral walls of the nasal capsules. Qudratojugals are paired and complete the arcade laterally; they end with wide bases, which are connected to the ventral ramus of the squamosal.

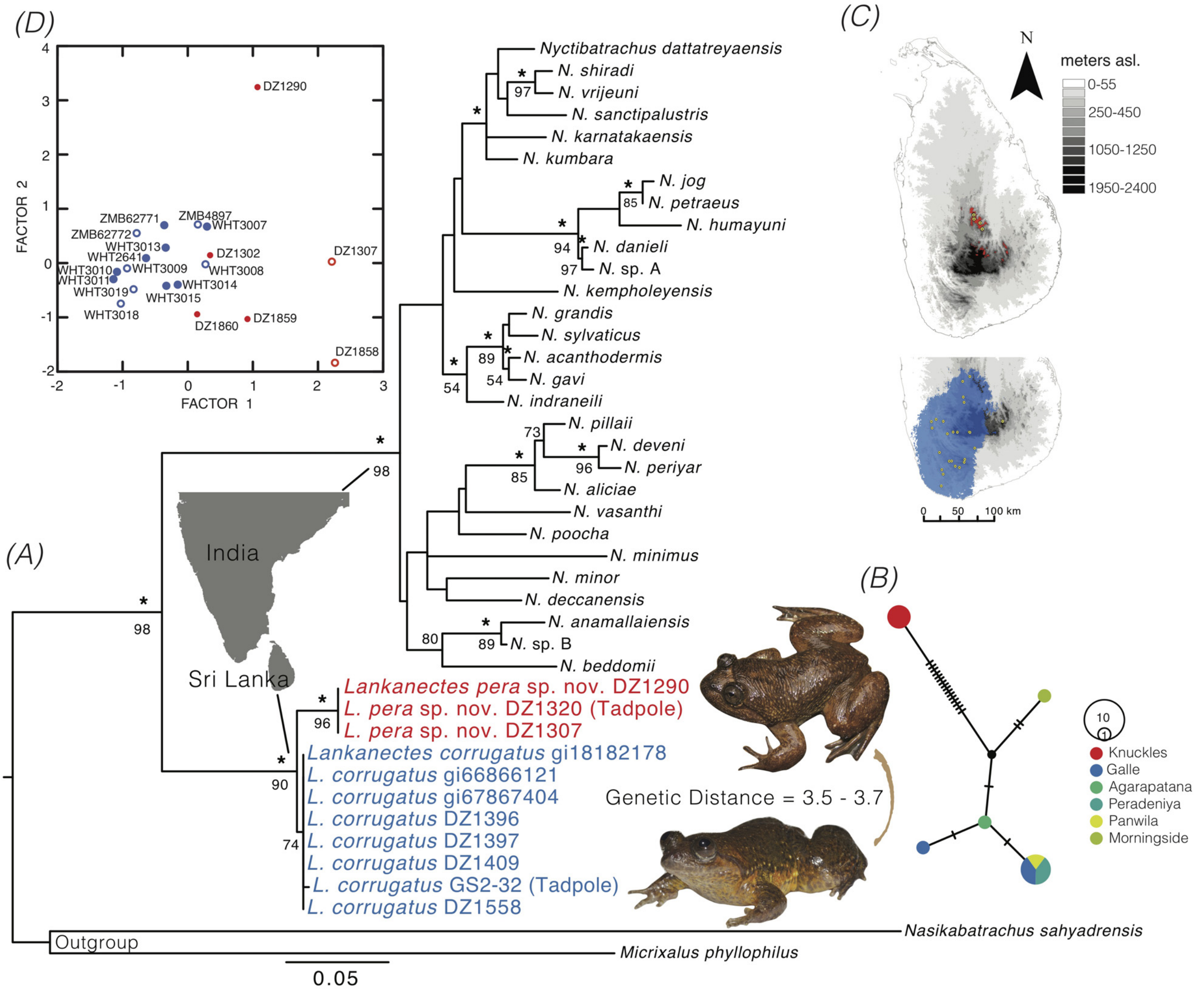

FIGURE 1. (A) *16S rRNA* Maximum-likelihood phylogram for 41 taxa with node support (posterior probabilities above nodes, with values >95 indicated by a *; MP bootstrap values below nodes, with values >50) showing two clearly-defined clades including *L. corrugatus* and *L. pera* (B) The uncorrected pairwise genetic distances range for *16S rRNA* 3.5–3.7%; Haplotype network analysis using the full dataset with 485 bp, shows all haplotypes shared across *L. corrugatus*; *L. pera* **sp. nov.** is different by more than 16 mutational steps from the *L. corrugatus* populations. (C) The predicted distributions for *L. corrugatus* is 360 km^2 and *L. pera* **sp. nov.** is 14120 km^2 on the northern hills of Sri Lanka. (D) Component loadings of the first two principal component axes (PC1 vs PC2) shows clear separation of the males, but with slight overlap of females; *L. pera* **sp. nov.** indicated in red, *L. corrugatus* in blue (filled circles denote females and open circles denote males).

Mandible: Cranial articulation of the manibular arch occurs via mineralized pars aricularis located at the posterolateral ends. Lateral margins of the mentomeckelians are attached to dentaries, elongated Meckel's cartilages and angulosplenials. Dentaries invest lateral and anterodorsal margins of the Meckel's cartilage.

Hyoid skeleton: Thin, mineralized hyoid plate houses U-shaped hyoglossal sinus, anterolateral processes, posterolateral (slightly longer than anterolateral processes), ossified posteromedial processes (longest) and a pair of curved hyales that allow attachment of hyoid skeleton to the cranium.

Postcranial skeleton: Eight presacral vertebrae, sacrum and urostyle form the axial skeleton. Transverse processes of vertebrae III and IV are posterolaterally directed and are same as the length as of the sacral diapophyses and end in cartilaginous epiphyses distally (Fig. 5). Rest of the transverse processes (II, V, VI, VII, VIII) are laterally oriented and are shorter in length. Hypochord is fussed with the coccyx and form the urostyle extending posteriorly, from the sacrum. Pectoral girdle has a firmisternal construct. Paired suprascapulae are flat, thin blade-like, with its proximal ends being cartilaginous. Anterior margins of suprascapulae are ossified to give rise to the paired cleithra. Ossified scapulae are connected together at the junction of glenoid fossa, via the articulation with clavicles and coracoids. Paired clavicles occupy the whole procoracoid cartilages except for a thin posterior margin. Epicoracoids connect each half of the pectoral girdle together. Epicoracoidal bridge is ossified. Sternum and omosternum are well developed, with expanded, ossified proximal ends. Proximal end of the omosternum is fused with the epicoracoidal bridge and the most distal cartilaginous end assumes a half-arched shape. Inverted v-shaped, proximal end of the sternum is attached to the cartilaginous epicoracoid cartilage and the wider distal part has irregular shaped margins, both anteriorly and posteriorly. Manus is composed of radiale, ulnare, carpals (2, 3-4-5), element Y, prepollex and prepollical element I. Metacarpals decrease in length as follows, 4> 3> 5> 2. Phalangeal digit formula is 3-3-4-4. Pes is composed of fused tarsals 2-3 (ossified), prehallical elements, metatarsals and phalange digits. Prehallux is completely ossified and base of the prehallical element 1 is ossified. Length of the metatarsals increase as; IV> V> III> II> I. Phalangeal formula is 3-3-4-5-4. Long ilia with ilial crests articulate with epiphyses of sacral diapophyses. Ischia and pubis are fused with each other.

TABLE 3. Bioclimatic variables used to predict the distribution models of the two species.

Code	Bioclimatic variables	*L. corrugatus*	*L. pera* sp. nov.
BIO1	Annual Mean Temperature	x	x
BIO2	Mean Diurnal Range	**1.7**	x
BIO3	Isothermality	**1.8**	x
BIO4	Temperature Seasonality	x	x
BIO5	Max Temperature of Warmest Month	x	x
BIO6	Min Temperature of Coldest Month	x	x
BIO7	Temperature Annual Range	x	x
BIO8	Mean Temperature of Wettest Quarter	x	x
BIO9	Mean Temperature of Driest Quarter	x	x
BIO10	Mean Temperature of Warmest Quarter	x	x
BIO11	Mean Temperature of Coldest Quarter	x	x
BIO12	Annual Precipitation	x	x
BIO13	Precipitation of Wettest Month	x	x
BIO14	Precipitation of Driest Month	**76.8**	x
BIO15	Precipitation Seasonality	**2.1**	x
BIO16	Precipitation of Wettest Quarter	x	x
BIO17	Precipitation of Driest Quarter	x	**0.9**
BIO18	Precipitation of Warmest Quarter	**7.7**	**0.1**
BIO19	Precipitation of Coldest Quarter	**0.5**	**42.3**
Alt	Altitude	**9.3**	**56.7**
Total number of variables used		7	4

Note. Highlighted variables selected through multi-collinearity test, values indicate the percentage contribution of the variable to build the model.

Niche modeling. The Maxent models for the two species provided satisfactory results, with higher AUC values; *Lankanectes corrugatus* = 0.959 and *L. pera* = 0.999. LPT values used for the two models of *L. corrugatus* and *L. pera* were 0.145 (20.1%) and 0.23 (28.7%), respectively. Precipitation of Driest Month (Bio 14) contributed most to the distribution model of *L. corrugatus*, whereas Altitude and Precipitation of Coldest Quarter (Bio19) contributed most to that of *L. pera* **sp. nov.** (Table 3). Even though *L. pera* is restricted to the Knuckles Mountain range, the predictive models show that the new species has suitable climatic conditions also in the northern high region of the central mountains (*L. pera* **sp. nov.** is restricted to the Knuckles mountain range). *Lankanectes corrugatus* is distributed throughout the wet-zone of the south-western quadrant of the island, extending also up the lower (< 1500m asl) slopes of the Central mountains (Fig. 1).

***Lankanectes pera*, sp. nov.**
(Figs. 2,3,4, Appendix 2)

Type Material. Holotype: mature male, 66.0 mm SVL, DZ1858 (KNU01), Knuckles Peak, alt. 1580 m, 7.4646 °N 80.7409 °E. Collected by MM, KM-A 10th August, 2012.

Paratypes: mature female, 51.0 mm SVL, DZ1859 (KNU02), Knuckles Peak, alt. 1580 m (7˚4646' N 80˚7409'E) collected by MM, KM-A 10th August, 2012; mature female, 42.4 mm SVL DZ1860 (HUN01), Dothalugala (Hunnasgiriya Peak), alt. 1420 m (7˚3206'N 80˚8568'E), MM, KM-A, NW 12th December, 2012; mature male, 68.7 mm SVL, DZ1307, Riverston Knuckles, alt 1330 m (7˚5233'N, 80˚7333'E), collected by MM, NW, GS 15th August, 2013; mature female, 55.8 mm SVL, DZ1290, Riverston Knuckles, alt. 1260 m (7˚5180'N, 80˚7375'E), collected by MM, NW, GS 15th August, 2013; mature female, 47.3 mm SVL, DZ1302, Riverston Knuckles, 1260 m (7˚5180'N, 80˚7375'E), collected by MM, NW, GS 15th August, 2013.

Diagnosis. *Lankanectes pera* **sp. nov.** can be distinguished from *L. corrugatus* by the following characters: ventrally greyish (*vs* white with dark brown patches in *L. corrugatus*); white tubercles on throat (*vs* smooth throat in *L. corrugatus*); edge of the upper lip uniform grey (*vs* white border with dark brown patches in *L. corrugatus*); inner edge of toes grey (*vs* inner edge of I, II, III and IV toes white in *L. corrugatus*); inner edge of foot grey (*vs* white in *L. corrugatus*); flank grey (vs. flank with dark brown and white patches in *L. corrugatus*).

FIGURE 2. *Lankanectes pera* **sp. nov.** in life, SVL 22.40 mm: (A) Dorsolateral view; (B) Dorsal view; (C) Habitat—clear water stream habitat under montane forest canopy cover.

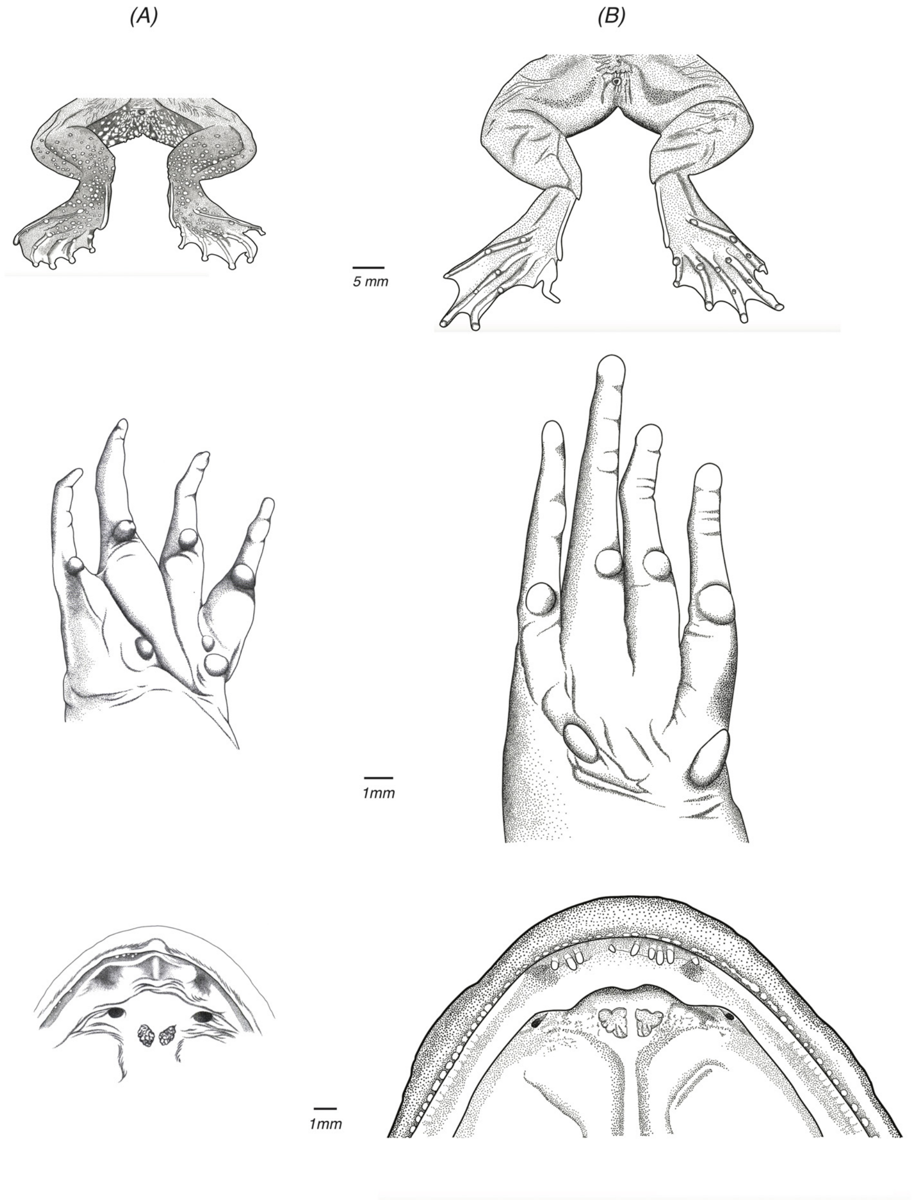

FIGURE 3. Illustrations of (A) *Lankanectes corrugatus*, ZMB4897, mature male, SVL 43.70 mm and (B) *L. pera* **sp. nov.**, DZ1858, mature male, SVL 66.0 mm, anal region (top); manus (middle) and maxilla (lower).

Description (based on the holotype, DZ 1858; Figs. 2,3,4**)**. Body stout. Head flat dorsally. Snout rounded when viewed dorsally and laterally. Canthal edges indistinct. Loreal region convex. Edges of upper lip with distinct tubercles; interorbital and internasal spaces convex. Nostrils oval; close to each other (19.3% of the width of the skull); placed dorsally on snout; edges fleshy. Tympanum absent; pineal ocellus absent. Vomerine teeth present; the vomerine teeth are tusk-like (more prominent in males), with an angle of 60º relative to body axis; less close to choanae than to each other. Tongue large; emarginated; not bearing a lingual papilla; two tubercles on posterior base of tongue. Two fang-like processes on the mandible. Internal vocal slits present, close to gape. Supratympanic fold absent. Parotid glands absent. Head wide. Cephalic ridges absent. Cephalic knob on head. Skin on head not co-ossified. Dorsal surface of head and body covered in numerous prominent dermal folds (corrugations) and glandular, white-tipped warts. Corrugations present also on ventral surface of head and throat. Manus robust.

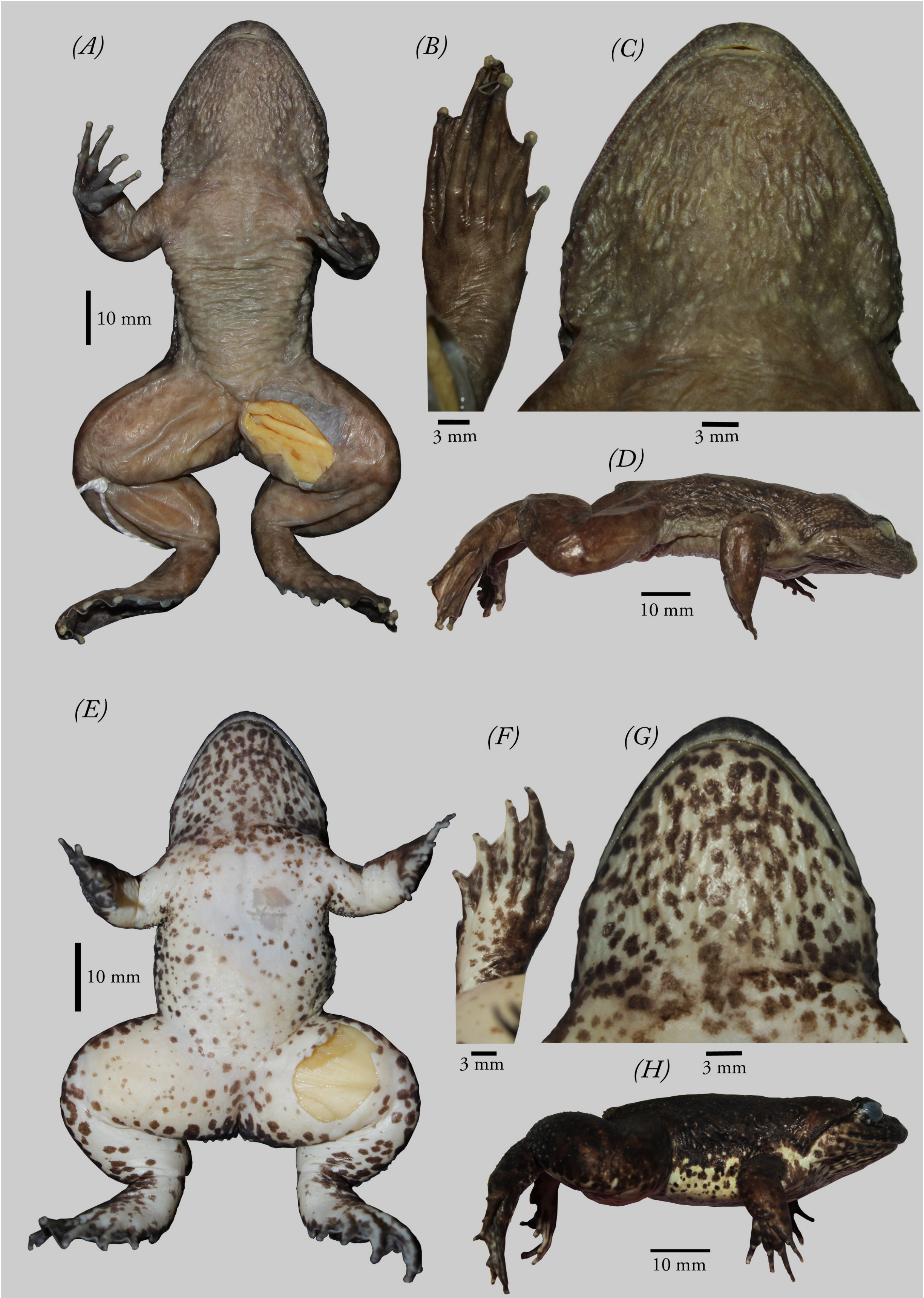

FIGURE 4. *Lankanectes pera* **sp. nov.**, SVL 68.69 mm, DZ1307 (A–D), in preservation. (A) ventral view; (B) right ventral foot; (C) ventral view of the head; (D) lateral view. *Lankanectes corrugatus*, SVL 55.28 mm, DZ1396 (E–H), in preservation. (A) ventral view; (B) right ventral foot; (C) ventral view of the head; (D) lateral view.

Forearms short, strong; fingers thin. Tips of fingers rounded, enlarged; discs absent; finger-tips not wide compared to finger width; no dermal fringe on inner or outer sides of fingers; no webbing on fingers; subarticular tubercles on fingers prominent, oval, single; prepollex distinct. Two palmar tubercles, oval, distinct, convex. Supernumerary tubercles on palm very small. Nuptial pad absent. Pes robust. Thigh and shank stout. Toes thin. Tips of toes rounded, enlarged, discs absent; tips of toes not wide compared to toe width. Toes fully webbed (see Figs 2B & 3B). Dermal folds present on inner edge of toe I and outer edge of toe V. Subarticular tubercles on toes prominent, rounded or oval, single. Inner metatarsal tubercle long, prominent, oval. Tarsal fold present. Outer metatarsal tubercle absent. Supernumerary tubercles on foot absent. Tarsal tubercle absent. Snout between eyes and side of head with folds and fine tubercles. Anterior and posterior part of back, and upper and lower flank with dermal folds. Dorsolateral fold absent on body. Corrugations and glandular warts present on dorsal surface of legs, but are less prominent; ventral surface of legs smooth. Lateral-line system present. Dorsal parts of forelimb and thigh with corrugations. Dorsal part of shank and tarsus with corrugations and tiny tubercles. Chest, belly and ventral part of thigh smooth. A cluster of macroglands (femoral glands) on inner surface of thigh. Possess vocal sacs and nuptial pads.

Sexual dimorphism. Head of females narrower than males (see Appendix 4 for measurements); cephalic knob and vocal slits absent.

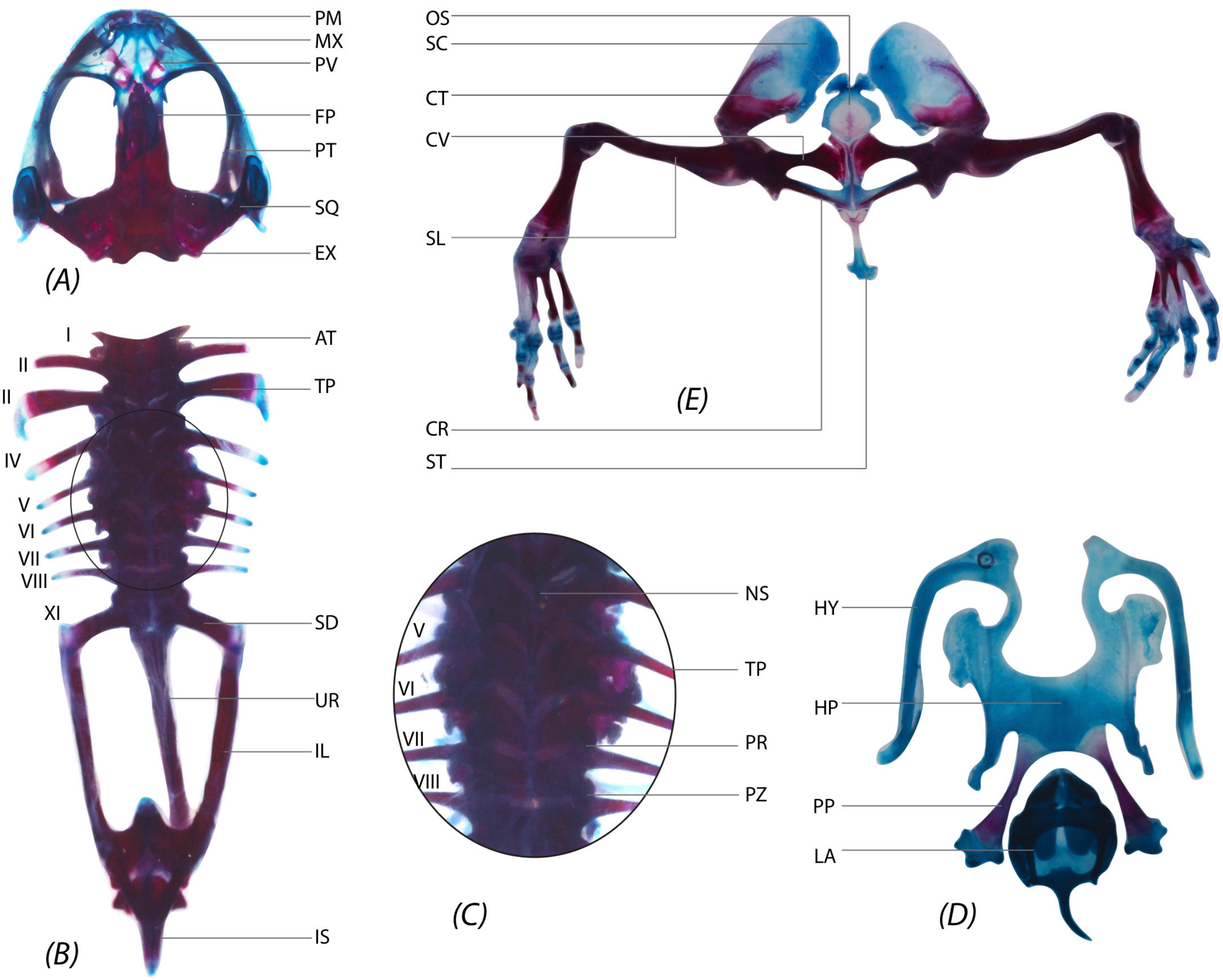

FIGURE 5. Cleared and stained adult specimen of *Lankanectes corrugatus* (DZ1397, SVL 45.02 mm). (A) Cranium, dorsal view. (B) Vertebral column, with the pelvic girdle attached to the sacral diapophysis via the cartilaginous epiphysis of ilia, dorsal view. (C) Close-up of vertebrae (D) Hyobranchial skeleton, ventral view. (E) Pectoral girdle and forelimbs, ventral view. Abbreviations: CR, coracoid; CT, cleithrum; CV, clavicle; EX, exoccipital; FP, frontoparietal; HP, hyoid plate; HY, hyale; IL, ilium; IS, ischium; MX, maxillae; OS, omosternum; PM, premaxilla; PP, posteromedial process; PR, prezygapophysis; PZ, postzygapophysis; PT, pterygoid; SC, suprascapula; SQ, squamosal; SL, scapula; ST, sternum; PV, vomer.

Coloration (in alcohol; Fig. 4)—Dorsally dark brown with unequal dark patches, edges of corrugations lighter in color, some pale spots on dorsum. A pale-yellow bar with dark edges on inter-orbital area. Flank, inguinal zone, loreal region and sides of back of head light brown, edges of corrugations pale. Throat, margin of throat and vocal sacs pale brown with lighter spots. Chest, belly, ventral sides of thighs and webbing light brown.

Color in life: Dorsally chocolate brown with unequal dark-brown patches. Ridges of the numerous prominent corrugations lighter in color, with interspersed light-brown spots. A light-brown bar edged with dark brown/black colors in the interorbital area. Flank, inguinal zone, loreal region and sides of back of head light brown. Throat, margin of throat and vocal sacs white with pale brown patches. Chest, belly ventral sides white. Ventral sides of thighs light brown, with white patches. Underside of webbing light brown. Disks and tubercles of pes and manus grey-brown.

Measurements of Holotype (DZ1858 in mm**)**. DBE, 17.2; DFE, 9.6; ED, 7.5; EN, 4.3; ES, 9.1; FEL, 29.7; FL I, 5.8; FL II, 6.0; FL III, 7.9; FL IV, 6.9; FOL, 42.0; HL, 27.3; HW, 25.7; IML, 3.3; IN, 3.6; IO, 5.6; LAL, 13.2; MBE, 12.6; MFE, 20.4; MN, 24.1; NS, 6.9; PAL, 15.8; SVL, 66.0; TBL, 28.4; TL I, 7.3; TL II, 9.0; TL III, 12.7; TL IV, 15.9; TL V, 12; UAW, 9.8; UEW, 3.0.

Etymology. The specific epithet *pera* is applied as a noun in apposition. It is a reference to the University of Peradeniya, Sri Lanka, affectionately referred to as "Pera" by its alumni.

Morphometrics. Unrotated principal components analysis separates the males of the two species on PC1, but slight overlap is seen for females (Fig. 1D). Of the total variance, 92 % is explained by PC1, which is a size axis (although the highest factor loading was for SVL, all other variables too, had high positive values); *Lankanectes pera* **sp. nov.** is larger in size than *L. corrugatus* (see Appendix 4 for all material studied and measurements). Only 2.6% of the total variance is explained by PC2, which reflects mostly in FLI (length of first finger) and NS (nostril to snout distance). This axis, however, is uninformative as the two species show nearly complete overlap (Fig. 1D, Appendix 4).

Distribution: *Lankanectes pera* **sp. nov.** is restricted to streams flowing through the montane forests on highest peaks of the Knuckles Mountain range—1100 m asl, in Dothalugala and Bamabarella and Riverston regions.

Ecological notes and natural history. This species has so far only been observed inhabiting pristine streams flowing through closed-canopy montane forests. These streams are characterized by clear, shallow and slow-flowing water, and sand and rock-strewn substrates. Males are found under rocks or rock-crevices in flowing water. Occasionally males call haltingly during daytime, but several males frequently vocalize in chorus at night, especially after light rain. Tadpoles of these frogs are large (total length of Gosner stage 35 tadpoles range between 42.00–45.14 mm, $N = 4$), and occur in deeper regions (0.5 m) where decaying vegetation gathers.

Discussion

All characters that typify the genus *Lankanectes* (Dubois and Ohler 2001) are present in *L. pera,* except for the femoral glands, which were thought to be absent. Here, we have shown femoral glands to be present both in *L. corrugatus* (Manamendra-Arachchci and Pethiyagoda 2006) and *L. pera.*

Lankanectes pera differs from *L. corrugatus* in all criteria (external morphology, genetics and climatic niche) on which they were evaluated. They differ from each other by 3.5–3.7% uncorrected genetic distances for *16S rRNA*, which is consistent with the range of species-level genetic distances commonly observed in amphibian sister taxa (Vences *et al*. 2005). *Lankanectes pera* differs from *L. corrugatus* in at least 16 mutational steps, with no sharing of haplotypes, indicating the reciprocal monophyly of the two clades; only between one and four mutational steps are observed within populations of *L. corrugatus*. Though we have data for only one mt-DNA gene fragment, *16S rRNA* is considered a conservative mitochondrial gene, and hence the patterns that are observed here are expected to hold also for other mt-DNA fragments.

In morphology, there are several, consistent, but somewhat subtle differences between the two species—tubercle distribution and several color and pattern related features (Figs. 2,3,4). However, in morphometry, in PCI, which is explained mostly by size, only separates the males, with a slight overlap of females, with *L. pera* being larger than *L. corrugatus.*

In contrast to *L. corrugatus*, which occurs commonly in muddy substrates, including marshes and rice paddies, where they burrow into soft mud and leaf litter, *L. pera* has so far only been found under more pristine conditions—sand and rock strewn clear-water mountain streams flowing under canopy cover, where they hide in rock crevices. This niche specialization partitioning between the two species can be an important factor that prevents the specialized *L. pera* from spreading more widely.

The large tadpole (maximum total length of ca. 45 mm) that was collected from the habitat of *L. pera* was DNA barcoded and confirmed to be of this species (Fig. 1). There seems to be resource partitioning between the adults and tadpole (stage 35) habitats—tadpoles are found in pockets of deeper pools with detritus, while adults prefer rocky and sandy regions. The tadpole of *L. corrugatus* has been described (Ukuwela & Bandara 2009), and in external morphology, the tadpole of *L. pera* is similar to that of *L. corrugatus*.

Ecological niche models suggest *Lankanectes pera* to be a montane isolate. Its predicted distribution is limited (360 km^2) due to its adaptations to high-altitude bioclimatic conditions. Though suitable climatic conditions are predicted by the niche model also to be present in the northern region of the central mountains, this area is climatically and ecologically isolated from the presently known range of the species. Due to its small area of occupancy and extent of occurrence (sensu IUCN Redlist Criteria 2001), together with the small population sizes observed during this study, *L. pera* can be evaluated as a Critically Endangered species. In contrast, the present area occupied by and predicted for *L. corrugatus* is much larger (14,120 km^2). Due to its wide distribution and large population size, *L. corrugatus* is considered as a Least Concern species.

Hence, *Lankanectes pera* is in need of immediate conservation attention due to its specialized habitat requirements and climatic conditions, which is predicted to deteriorate under the current predicted global climatic warming models. The Knuckles range is already highlighted as a mountain refuge for as many as eight micro-endemic frog species that are already considered to be critically endangered or endangered (Meegaskumbura & Manamendra-Arachchi 2005, Manamendra-Arachchi & Pethiyagoda 2006, MOE 2012, Senevirathne & Meegaskumbura 2015). Given that the habitat requirements of *L. pera* is different from that of the highly threatened micro-endemics highlighted so far, the conservation strategy for amphibians of the Knuckles mountains must consider this new knowledge, i.e. the conservation of streams of the mountains.

Given that *Lankanectes* being endemic to Sri Lanka, occupying a position on a phylogenetic long branch as two distinct species, in the absence of a common name to highlight these frogs, we propose calling them Corrugated Frogs, which describes the numerous and prominent transverse skin folds on of both *L. corrugatus* and *L. pera*.

Acknowledgements

We thank the following individuals and organizations: an anonymous reviewer and Miguel Vences for suggesting improvements to the manuscript; Department of Wildlife Conservation and Forest Department of Sri Lanka for research permits and logistical support at their respective reserves; National Research Council of Sri Lanka (NRC 11-124) for graduate student support; members of the EES lab for field support. GS acknowledges the Rufford Small Grant (No. 192721) and NW, the Nagao Natural Environment Foundation for funding part of this work.

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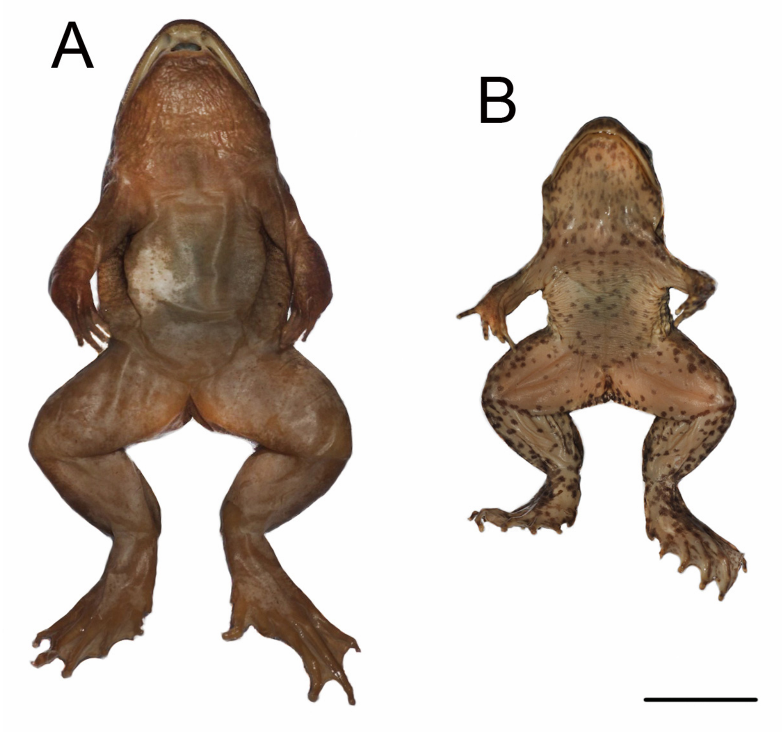
APPENDIX 1.
Presence locations used to make predicted distribution maps for the two species, *Lankanectes corrugatus* and *L. pera*

Species	District	Locality	Altitude	Coordinates	
			(m)	Latitude (ºN)	Longitude (ºE)
L. corrugatus	Nuwara-Eliya	Bogawanthalawa	1300	6.7977	80.6783
	Nuwara-Eliya	Bogawanthalawa	1300	6.7952	80.6865
	Nuwara-Eliya	Moray Estate	1430	6.7961	80.5238
	Ratnapura	Deniyaya	470	6.3380	80.5516
	Ratnapura	Kudawa	560	6.4201	80.4196
	Ratnapura	Dodammuluwa	410	6.4220	80.4498
	Ratnapura	Morningside Forest Reserve	1050	6.4068	80.6133
	Ratnapura	Suriyakanda	1100	6.4426	80.6199
	Ratnapura	Batadombalena	350	6.7747	80.3946
	Ratnapura	Ehaliyagoda	140	6.8501	80.1965
	Ratnapura	Mahawalathenna	540	6.5889	80.7494
	Ratnapura	Samanala Nature Reserve	1040	6.7986	80.4690
	Kandy	Doluwa	550	7.1832	80.6109
	Kandy	Peradeniya	520	7.2527	80.5997
	Kegalle	Deraniyagala	150	6.9368	80.3416
	Kegalle	Avissawella	70	6.9620	80.2485
	Galle	Kottawa	80	6.0985	80.3155
	Galle	Hiniduma	50	6.2515	80.3397
	Kaluthara	Athwelthota	80	6.5336	80.2927
	Matara	Diyaduwa	300	6.3544	80.4971
	Galle	Hiniduma	60	6.3096	80.3238
	Matale	Rattota	390	7.5183	80.6858
	Matale	Kumbiyangoda	500	7.4542	80.5925
	Colombo	Avissawella	30	6.9333	80.1876
L. pera **sp. nov.**	Matale	Knuckles F.R.	1330	7.5233	80.7333
	Matale	Knuckles F.R.	1260	7.5180	80.7375
	Matale	Knuckles F.R.	1580	7.4646	80.7409
	Kandy	Meemure	1100	7.4104	80.8240
	Kandy	Hunnasgiriya	1420	7.3209	80.8568



APPENDIX 2. Ventral view of *Lankanectes pera* (A, DZ1858) and *L. corrugatus* (B, DZ1399). The scale bar represents 20 mm.

APPENDIX 3. Component loadings and the variances explained by the loadings for the *Lankanectes* Principal Components Analysis.

Variables	Component Loading 1	Component Loading 2	Component Loading 3
SVL	0.996	0.048	-0.008
TL3	0.994	0.039	-0.007
PAL	0.994	-0.059	-0.055
FOL	0.993	0.077	-0.001
TL4	0.992	-0.012	0.062
TBL	0.992	0.067	-0.029
TL2	0.992	0.004	0.034
ES	0.991	-0.025	0.029
MN	0.990	-0.009	0.023
HW	0.989	0.065	-0.054
TL5	0.989	0.030	0.058
HL	0.988	0.010	0.015
FL3	0.987	-0.122	-0.055
MFE	0.987	-0.026	0.014
TL1	0.985	-0.090	-0.047
DFE	0.979	-0.010	-0.000
DBE	0.974	0.105	-0.140
FL2	0.973	-0.007	-0.138
FL4	0.969	-0.100	-0.171
MBE	0.964	0.044	0.043
LAL	0.954	0.120	0.091
ED	0.952	0.043	-0.189
IO	0.943	-0.145	0.084
EN	0.926	-0.109	0.252
FLI	0.924	-0.334	0.099
FEL	0.922	0.127	-0.140
IN	0.919	-0.284	0.041
NS	0.913	-0.324	0.027
IML	0.865	0.289	0.360
UAW	0.849	0.155	-0.210
UEW	0.808	0.519	0.046
Variance explained by components	28.51	0.810	0.414
% of total variance explained by components	91.96	2.60	1.34

APPENDIX 4. Variables used in morphometric analysis; measurements in mm.

Species	Voucher Number	DBE	DFE	ED	EN	ES	FEL	FLI	FL II	FL III	FL IV	FOL	HL	HW	IML	IN
L. corrugatus	WHT3007	11	6.7	4.6	3.4	6.6	19.1	3.7	3.9	5.2	3.6	27.7	18.9	17.1	3.5	2.5
L. corrugatus	WHT3008	11.3	7.1	4.9	3.4	6.9	17.6	3.8	3.5	5	4.2	27.2	18.2	17.5	3.3	3
L. corrugatus	WHT3009	8.1	4.8	3.8	2.7	5.1	10.6	2.3	2.7	3.7	2.8	18.8	12.8	11.7	2	2.2
L. corrugatus	WHT3018	7.6	4.7	3.5	2.4	4.6	13.2	2.8	2.8	3.9	3.2	18.2	11.8	11.8	1.8	2.2
L. corrugatus	WHT3019	9.6	5.7	3.9	2.4	5.1	14.7	2.8	2.9	4	3.3	19.3	13.6	13.2	2	2.3
L. corrugatus	WHT3013	10.3	5.8	4.6	2.9	5.7	16	2.8	3.3	4.4	3.6	22.9	15	15.2	2.1	2.5
L. corrugatus	WHT3014	10.5	6.1	4.9	2.7	6	18.3	3.3	3.6	4.9	4	25.1	16.3	15.2	2.3	2.7
L. corrugatus	WHT3015	11.1	5.8	4.7	2.7	5.7	16.1	3.1	3.2	4.6	4	22.6	16.5	15.1	2	2.6
L. corrugatus	WHT3010	8.4	5	3.8	2.4	4.7	12.7	2.4	2.4	3.7	3	17.5	11.5	11.4	1.8	2.2
L. corrugatus	WHT3011	7.7	5.1	3.5	2.4	4.5	11.9	2.3	2.4	3.5	2.5	17.9	11.2	11.4	1.8	2.1
L. corrugatus	WHT2641	9.2	5.2	4.3	2.7	4.8	13.6	3	2.7	4	3.3	22	14.2	13.5	2.5	2.3
L. corrugatus	ZMB4897	11.7	6.5	4.5	3.3	6.3	21.9	3.3	3.4	4.7	3.8	28.7	16.6	16.8	3.1	2.7
L. corrugatus	ZMB62772	8.9	5.4	3.8	2.6	4.9	14.7	2.7	2.7	3.8	3.2	20.8	12.7	12.2	2.6	2.2
L. corrugatus	ZMB62771	10.6	5.7	5.1	2.8	5.4	18	2.8	3.3	4.5	3.9	24	14.9	14	2.2	2.6
L. pera	DZ1858	17.23	9.6	7.5	4.28	9.09	29.7	5.8	6	7.9	6.9	42.03	27.35	25.71	3.32	3.6
L. pera	DZ1859	13.99	7.72	5.38	3.34	7.55	24.61	4.45	4.5	6.1	5.2	30.78	20.29	19.61	3.18	3.1
L. pera	DZ1860	11.14	6.76	5.23	3.01	6.23	18.81	3.9	4	5.4	4.7	26.12	16.9	16.65	2.4	2.8
L. pera	DZ1307	17.25	8.71	6.72	3.81	9.3	31.47	5.74	5.19	7.59	6.62	41.33	29.87	28.01	4.1	3.74
L. pera	DZ1290	16.02	8.1	6.5	3.1	7.53	28	3.07	5	5.92	5.32	35.7	22.48	22.86	3.62	2.5
L. pera	DZ1302	13.35	6.62	4.83	2.92	6.6	29.39	3.88	3.9	5.12	4.48	29.38	17.3	19.08	2.98	2.79

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APPENDIX 4. (Continued)

	SP	IO	LAL	MBE	MFE	MN	NS	**SVL**	TBL	TL I	TL II	TL III	TL IV	TL V	UAW	UEW	Locations
L. corrugatus	WHT3007	3.3	9.8	9.4	13.5	16.6	3.8	**44.9**	18.5	4.3	5.9	8.6	11.2	8.6	7.7	2.8	Morningside FR
L. corrugatus	WHT3008	3.4	8.4	8.2	12.1	15	3.5	**43.7**	19.5	4.5	5.9	8.4	10.9	8.2	7.2	2.8	Morningside FR
L. corrugatus	WHT3009	2.1	6.1	6.3	9.1	11.4	2.6	**28.6**	13.1	3.2	4.1	6	7.8	5.9	7.2	1.9	Morningside FR
L. corrugatus	WHT3018	2.3	6.1	5.8	8.6	10.4	2.3	**29.1**	12.3	2.9	3.6	5.5	7.4	5.4	6.7	1.6	Agra Elbadda, Agarapathana
L. corrugatus	WHT3019	2.3	6.8	6.1	9.1	10.7	2.8	**31**	13.3	3.1	3.9	6	7.5	5.3	5.8	1.9	Agra Elbadda, Agarapathana
L. corrugatus	WHT3013	2.9	7.2	7.2	10.8	12.9	3.1	**36.3**	16.1	3.6	4.8	7	9	6.9	9	2.5	Agra Elbadda, Agarapathana
L. corrugatus	WHT3014	2.6	6.9	8.4	12	14.5	3.3	**38.1**	17.4	4.4	5.2	7.4	9.8	7.1	5.6	2.5	Agra Elbadda, Agarapathana
L. corrugatus	WHT3015	2.2	7	8.5	12	14.1	3.4	**38.4**	16.7	3.6	4.7	6.8	8.8	6.3	6.9	2.3	Agra Elbadda, Agarapathana
L. corrugatus	WHT3010	2.1	5.3	4.8	7.8	9.8	2.4	**27.2**	12.3	3.1	4	5.8	7.7	5.8	5.5	2.1	Morningside FR
L. corrugatus	WHT3011	2.5	5.7	5.6	8.2	11.5	2.2	**27.5**	12.3	2.7	3.7	5.2	7.1	5	5.6	1.8	Morningside FR
L. corrugatus	WHT2641	2.7	6.5	6.5	10.4	12.8	2.3	**33.4**	15.3	3.3	4.7	6.7	9.2	6.4	5.6	2.2	Kottawa FR galle
L. corrugatus	ZMB 4897	3	9.6	8.9	12.7	15.3	3.4	**43.8**	19.1	3.9	5.4	8.3	10.9	7.7	7.4	2.7	Syntype, Ramboda
L. corrugatus	ZMB 62772	2	6.7	6.8	9.3	11.1	2.6	**32.3**	13.6	3.3	4.5	6	8.2	6.3	5.3	2.4	Syntype, Ramboda
L. corrugatus	ZMB 62771	2.3	8	7.2	11.1	13.3	2.8	**37.8**	15.8	3.6	4.5	7	9.1	7.1	6.4	2.9	Syntype, Ramboda
L. pera	DZ1858	5.59	13.22	12.64	20.38	24.14	6.88	**66**	28.44	7.3	9	12.7	15.9	12	9.82	3.05	Knuckles
L. pera	DZ1859	4.13	8.7	10.39	15.27	18.35	4.76	**51**	21.49	5.4	7	10.2	12.8	9.4	7.73	2.55	Knuckles
L. pera	DZ1860	3.07	7.78	8.79	12.42	14.88	3.43	**42.37**	17.83	4.6	5.5	8.5	10.8	7.9	7.61	2.23	Hunnasgiriya
L. pera	DZ1307	6.41	12.07	14.95	21.69	24.51	5.14	**68.69**	29.12	6.74	8.71	12.57	15.71	11.5	10.7	3.63	Riverston
L. pera	DZ1290	3.6	10.91	11.16	15.78	19.24	3.37	**55.81**	24.68	5.43	7.28	10.92	12.77	9.79	9.79	3.93	Riverston
L. pera	DZ1302	3.15	8.1	7.5	12.08	15.45	3.3	**47.3**	20.98	4.63	5.93	8.72	11.15	8.03	8.48	2.28	Riverston

APPENDIX 5.

Other material studied: mature female, 34.5 mm SVL, WHT1299B, Yogama; mature male, 36.8 mm SVL, WHT1299A; mature female, 38.7 mm SVL, WHT816, Kalatuwawa, Labugama; mature female, 37.1 mm SVL, WHT945, Koskulana, Panapola; mature male, 44.4 mm SVL, WHT912, Ambalamahena, Athwelthota; mature female, 59 mm SVL, WHT5132, Agra, Elbedda; mature male, 56.6 mm SVL, Agra, Elbedda; mature male, 56.6 mm SVL, Agra, Elbedda; mature male, 30.8 mm SVL, WHT875C, Kanneliya forest reserve; mature female, 33.3 mm SVL, WHT882, Devon ford estate; mature female, 44.6 mmm, Kanneliya FR, mature female, 25.7 mm SVL, WHT875B, Kanneliya FR; mature male, 26.4 mm SVL, WHT2474, Morningside FR; mature female, 22.1 mm SVL, WHT810, Namunukula, gonkale; mature male, 22.5 mm SVL, WHT3017, Agra, Elbedda; mature female, 50.0 mm SVL, WHT5130, Agra, Elbedda; mature male, 57.7 mm SVL, WHT 5129, Agra, Elbedda; mature male, 45.3 mm SVL, WHT5128, Agra, Elbedda; mature female, 34.4 mm SVL.