Project Update: January 2019

Covering period of this report: August 2018 – December 2018

Progress during period:

A. Field survey and sample collection

Field surveys to collect the morphometric measurements and tissue samples of *Lankascincus* skink species were continued. The sampling was extended to collect 12 tissue samples of *Lankascincus* species in various parts of the country. The surveys were carried out within the existing forest trails under the supervision and guidance of the respective forest and wildlife officers. Sampling was carried out according to the following table (Table 1).

Table 1: Sampling of *Lankascincus* tissue samples

Species	Number of samples
L. gansi	3
L. fallax	3
L. greeri	1
L. dorsicatenatus	2
L. taylori	3

So far, 12 tissue samples out of 30 have been collected. Collection of other required samples and morphometric measurements will be continued during the next phase of the project.

B. Laboratory analysis

B.1: DNA extraction and PCR amplification

Genomic DNA from all collected samples was extracted using commercially available DNA extraction kit (Blood & Tissue DNA Extraction Kit, Qiagen). The concentrations of the extracted DNA were sufficient for further analysis (50 – 120 ng/µl).

B.2: PCR amplification

The DNA was amplified for three gene regions; *Cmos-2*, *12S* and *16S*.
For amplifying the Cmos-2 gene, the 20µl reaction mixture was consisted with 4µl PCR master mixture including 1.5mM $MgCl_2$, 200 µM dNTPs, 2.50 units of Taq DNA polymerase (FIREPol® and 0.2µl of each primers. The cyclic conditions were initial denaturation at 94°C for 3 min., 35 cycles of denaturation (94°C for 30 sec), annealing (54°C for 40 sec) and extension (72°C for 45 min) followed by a final extension of 5 min at 72°C.

The expected band size amplified for *Cmos*-2 gene was observed around 300 bp for all samples.

The reaction and cyclic conditions for 12S and 16S PCRs were as in the Table 2. The expected band sizes for 12S and 16S products were observed around 300 bp and 400 bp respectively (Figure 2).

Table 2: The reaction and cyclic conditions for *12S* and *16S* PCR

PCR	Reaction conditions	Cyclic conditions
12S	PCR Master Mix (1X) Forward Primer(0.1uM) Reverse Primer(0.1uM) Template DNA(68.6ng/ µl) PCR Graded water	Initial denaturation- 94^0C- 3min Denaturation- 94^0C- 30 sec Annealing- 57^0C- 40sec Extension- 72^0C- 45sec Final extension- 72^0C- 5min
16S	PCR Master Mix (1X) Forward Primer(0.1uM) Reverse Primer(0.1uM) Template DNA(68.6ng/ µl) PCR Graded water	Initial denaturation- 94^0C- 3min Denaturation- 94^0C- 30 sec Annealing- 57^0C- 40sec Extension- 72^0C- 45sec Final extension- 72^0C- 5min

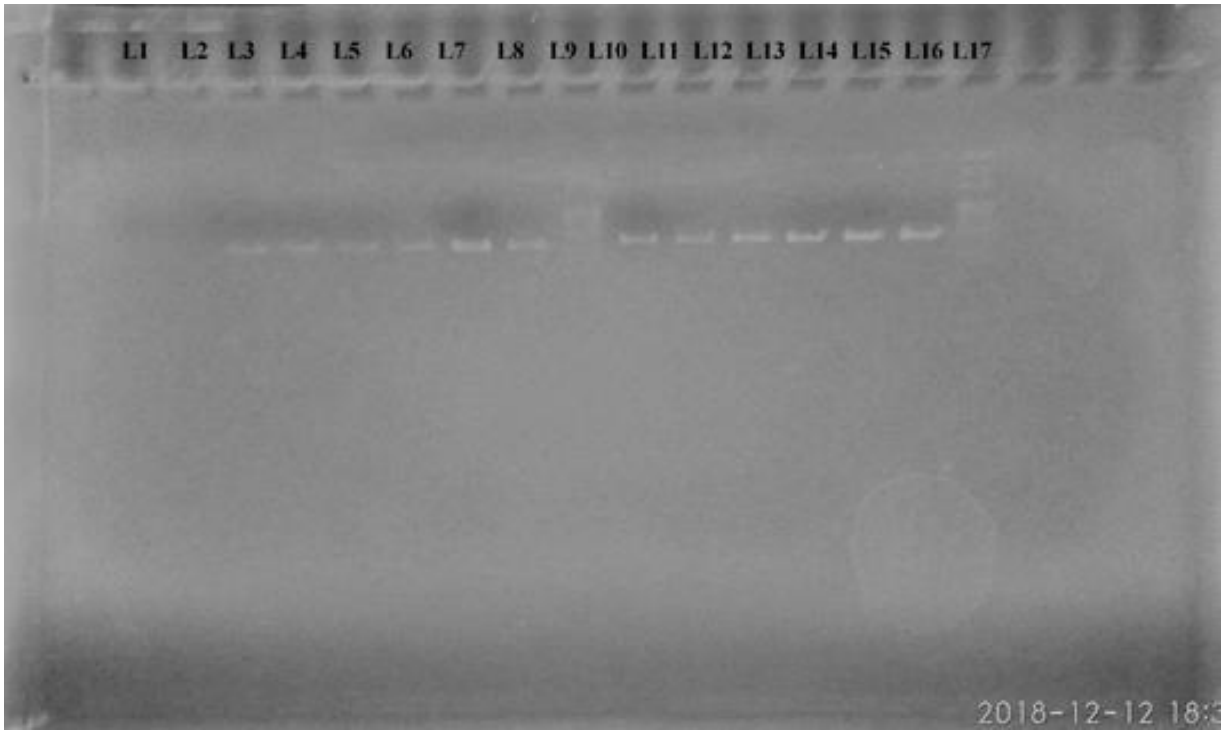

Figure 1: L1,L2 – Negative control, L3-L8 – samples amplified 12S gene, L8 and L17- 100bp size fractionated DNA ladder, L9-L16 - samples amplified 16S gene

C. Conservation awareness programmes

A conservation awareness seminar was conducted for the staff, who are not aware of the reptile species of the Institute for Research and Development (IRD). A questionnaire was distributed pre- and post-seminar to evaluate the knowledge of the audience on skinks. After conducting the seminar, a leaflet prepared to disseminate the knowledge on *Lankascnincus* skinks was distributed.

Figure 2: Moments of conducting seminar and discussions with the participants

Work plan for the next 3 months:

Collection of other required samples and morphometric measurements will be continued for the next phase of the project. Extraction of genomic DNA from all collecting samples will be carried out. The PCR products will be sequenced using the sequencing service at University of Colombo, Sri Lanka. Sequence analysis and phylogenetic reconstruction will be proceeded as the final step of the molecular analysis. Then the morphometric data of all species will be analyzed using appropriate software.

The nNext conservation seminar is scheduled to be conducted for schoolchildren in a rural village. Similar conservation awareness seminars will be conducted within the next phase of the project.

Publications/ Communications arising from the project during the reporting period:

Dhanushka, A.D., Kanishka, S.K., Cooray, R., Hapuarachchi, N.S., Warnakula, L., Harischandra, I.N. (2018) Review of Taxonomy, Molecular Systematics and Conservation Status of Endemic Skink Genus Lankascincus (Squamata: Scincidae). Proceeding of the Open University International Research Sessions (iOURS 2018), proceeding page 103.

Skink leaflet distributed in awareness programs (in local language)