

Molecular identification of rufous horseshoe bat, *Rhinolophus rouxii* from the Wellawaya Wavulgalge cave in Sri Lanka

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The rufous horseshoe bat, *Rhinolophus rouxii* (family Rhinolophidae) has a wide distribution and has been recorded from China, India, Myanmar, Nepal, Vietnam, and Sri Lanka. It is a medium sized bat (11-13g), and shows enormous cryptic diversity. Recent research has shown that *R. rouxii* contains many morphologically diverse, species complex and fur color as well as the echolocation call frequency varies in different populations. We have used molecular techniques for establishing the accurate identity of *R. rouxii*, recorded in one of the largest cave populations in Sri Lanka. Bats were caught using a hand net and saliva samples (n=21) were collected non-invasively from each bat using a sterile oral swab. Nucleic acids were extracted from oral swab samples following the QIAmp Viral RNA Mini kit (extract both DNA and RNA) protocol. Mitochondrial cytochrome b gene was amplified using cytochrome b, RrFP and RrRP primers. PCR products were visualized using gel electrophoresis. Purified PCR products were sequenced using Sanger sequencing. Sequence analyses were carried out using the GeneiousPrime application. FASTA sequences of each bat obtained from sequencing were trimmed and assembled to form the consensus sequences. Aligned sequences were compared with sequences in the database at National Center for Biotechnology Information Support Center (NCBI). Cytochrome b gene sequences of *Rhinolophus rouxii* bats were downloaded from NCBI database and aligned with the consensus sequences using the Geneious alignment tool. The phylogenetic tree was constructed using the Neighbor-Joining method. This study identified the *Rhinolophus* species recorded in Wellawaya cave as *Rhinolophus rouxii* with an average identity of 98.40%. Phylogenetic analysis showed it is closely related to the same species identified in Karnataka district in India. This study reports the first molecular confirmation carried out on *Rhinolophus rouxii* in Sri Lanka. This will provide the basis for a genetic database for Sri Lankan bats.

Key words: Horseshoe bats, molecular identification of bats, Rhinolophid bats, *Rhinolophus rouxii*, Wavulgalge

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