

## Further specimens of the mud snake, *Gerarda prevostiana* (Homalopsidae) from Sri Lanka with insights from molecular phylogenetics

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**Abstract.** *Gerarda prevostiana* is an aquatic homalopsid snake distributed in the coastal brackish water habitats from Northwestern India, through peninsular Malaysia to the Philippines. In Sri Lanka, it appears to be a rare species known only from a few specimens. During a survey on the marine snake fauna of Sri Lanka, the authors found two specimens of *Gerarda prevostiana* as by-catch of fisheries from a coastal region in Northwestern Sri Lanka. Comparison of morphological data of the two individuals with available literature suggested little variation with their extralimital counterparts. However, moderate mitochondrial sequence divergence (~3%) between specimens from Sri Lanka and Singapore suggest a history of isolation between South Asian and Southeast Asian populations.

**Key words.** by-catch, Cytochrome b, genetic divergence, phylogeny, rear-fanged snakes, systematics

### INTRODUCTION

Oriental-Australasian rear-fanged snakes of the family Homalopsidae are distributed in the tropical regions from the Indus valley in Pakistan eastwards to the Philippines and Northern Australia, including the Micronesian island of Palau (Murphy, 2007). Currently, 53 species of homalopsids in 27 genera (Murphy & Voris, 2014), which underwent speciation mainly in the Southeast Asian region of the Indo-Australian Archipelago, are recognised (Alfaro et al., 2008). *Gerarda* Gray, 1849 is a monotypic genus of homalopsidae represented by *Gerarda prevostiana* (Eydoux & Gervais, 1837). This aquatic species is distributed in the coastal regions from India, Myanmar, through the Malayan Peninsula to the Philippines (Murphy, 2007; Vyas et al., 2013). Though the species is widespread in mainland Southeast Asia and Singapore (Murphy, 2007), it was only recently recorded from Borneo, the only Sunda island it is so far known from (Das et al., 2013). In South Asia, it is known from scattered populations in Bangladesh, Northwestern and Western India and Sri Lanka (Murphy, 2007; Vyas et al., 2013). This species is rare in Sri Lanka and known only from five specimens (Haly, 1886; Gyi, 1970; de Silva & de Silva, 1986–1987; Porej, 2001; Somaweera et al., 2006). Here, we report the collection of two more specimens from Sri Lanka and provide notes on their morphology, colouration and habitat. We have also collected fresh tissue samples for

DNA analysis to confirm the identity of the two collected specimens and compare mitochondrial sequence variation of Sri Lankan *Gerarda prevostiana* to their extralimital counterparts in Southeast Asia.

### MATERIAL & METHODS

Two dead specimens of *Gerarda prevostiana* were collected as by-catch of fisheries during an island-wide survey on the marine snakes of Sri Lanka, conducted by the authors from 2010 to 2012. Muscle tissue samples were extracted from the specimens and stored in 90% Isopropanol for molecular analysis. The two snakes were fixed in 10% formalin solution and later transferred to 70% Isopropanol solution. The specimens are deposited at the Zoology division in the National Museum of Sri Lanka, catalogued as 2013.18.01.NH and 2013.19.01.NH.

A total of 12 morphological characters were examined in the two specimens. The number of ventral scales were counted following Dowling (1951), and the number of costal scale rows were counted around neck and mid body. Morphometric data taken from the specimens were snout vent length (SVL) and tail length (TAL). The sum of SVL and TAL was used to obtain total length (TOTL). Measurements were taken using a measuring tape up to the nearest millimetre (mm).

To confirm the identity of Sri Lankan *Gerarda prevostiana* and examine population genetic divergence, we sequenced a ~1101 base pair fragment of the mitochondrial Cytochrome b (Cyt b) gene (Genbank accession numbers: KY206890-KY206891) and constructed a molecular phylogeny (Burbrink et al., 2000) including all the sequences available for homalopsids in Genbank (28 species in 16 genera). The Cyt b gene was used in this study because of its wide use in species identification and phylogeographic studies of snakes to examine the population genetic level variations and also

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due to the availability of reference sequences for most of the homalopsids in Genbank (Genbank: [www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)). *Xenochrophis vittatus* was used as an outgroup in the phylogenetic analyses following Alfaro et al., (2008).

The sequences were aligned in Geneious Pro 5.4 (Drummond et al., 2009) and then manually edited and refined by eye. The data was analysed using Bayesian and maximum likelihood (ML) methods. The Bayesian information criterion implemented in PartitionFinder ver. 1.0.1 (Lanfear et al., 2012) selected GTR+G, HKY+I+G and GTR+G respectively as the best-fit substitution models for the three codon positions (first, second and the third) for the dataset. Partitioned Bayesian analyses were done in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) with default priors and unlinked model parameters. The analysis was conducted employing 4 million generations with two independent runs and four chains sampling every 1,000 generations.

Convergence of the independent runs was assessed by examining split frequencies of clades across runs and effective sample sizes (ESS values) of the likelihood plots through time in Tracer v1.4.1 (Drummond & Rambaut, 2007) and the first 25% of sampled trees were discarded as burn-in. Partitioned maximum likelihood analyses were implemented in RAxML v7.2.6 (Stamatakis, 2006) by applying the GTR+G substitution model to the same partitions as the Bayesian analyses, and performing 200 independent ML searches. Branch support was estimated using 1,000 bootstrap pseudo-replicates. The number of fixed differences between *Gerarda prevostiana* sequences were estimated using DnaSP v5 (Librado & Rozas, 2009) and corrected genetic distances between specimens from Sri Lanka and Singapore were calculated in Geneious Pro 5.4 software (Drummond et al., 2009).

## RESULTS

The two specimens reported in this study were collected as fisheries by-catch in gill nets by local fishermen in the open ocean about 1 km off the coast of Vankale, Gulf of Mannar, Sri Lanka (08°53'36"N, 79°55'43"E) on 01 October 2011. Vankale is situated in the Northwestern coast of Sri Lanka, facing the Gulf of Mannar (Fig. 1). The depth of the sea in the area is generally less than 30 m and the substrate is sandy. The surrounding area has an estuary with mangrove habitats.

Both specimens have 17 costal scales around neck and mid-body, eight supralabial scales, one pre-ocular scale, two post-ocular scales, one anterior temporal scale, two posterior temporal scales, one loreal scale and one inter-nasal scale. Specimen 2013.18.01.NH has 154 ventral scales, 37 sub-caudal scales and seven infra-labial scales while specimen 2013.19.01.NH has 159 ventral scales, 33 sub-caudal scales and eight infra-labial scales. In both specimens the pre-anal scale is undivided, the anal scale is divided and the fourth supra-labial scale touches the eye. Scales on the head and body are generally smooth and are devoid of any apical pits in both specimens. The total lengths of the two specimens are

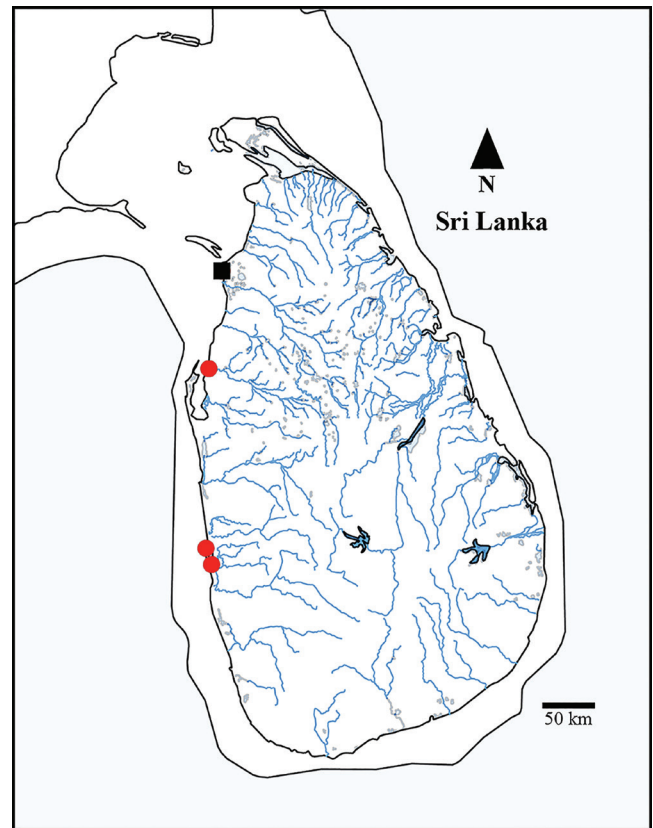


Fig. 1. Collection locality of the two specimens of *Gerarda prevostiana* during the marine snake survey (denoted by filled square) and the approximate locations of all the previous confirmed records of *G. prevostiana* in Sri Lanka (denoted by filled circles). The white coloured region around the coastline depicts the 200 m undersea contour line.

small (2013.19.01.NH: SVL–151 mm, TOTL–175 mm; and 2013.18.01.NH: SVL–169 mm, TOTL–196 mm) compared to the known average adult total length between 450–500 mm for the species. The smaller sizes of the two specimens indicate that they are juveniles, since the average total length of adults are almost twice this size.

Specimen 2013.19.01.NH had the typical colouration for this species, with olive colour upper labials; uniform dark brown head, trunk and tail and olive colour lateral stripe along each side of the body, olive colour lower jaw and a brownish venter (Fig. 2A, C, E, G, I, K). Contrastingly, the specimen 2013.18.01.NH had the characteristic dorsal and ventral colouration (Fig. 2B, G, H), however the upper labials and lower jaw were significantly darker than usual for this species (Fig. 2D, F, L).

Maximum likelihood and Bayesian analysis of the Cytochrome b gene yielded very similar branch lengths and topologies. The topologies of the phylogenetic trees were generally in agreement with the previously published phylogenies for homalopsids (Alfaro et al., 2008; Kumar et al., 2012). All sampled *G. prevostiana* was monophyletic (bootstrap support (BS) >70, posterior probability (PP) >0.9) with a sister species relationship with *Fordonia leucobalia* strongly supported (BS >70, PP >0.9), which is consistent with Alfaro et al. (2008). However, the four samples of

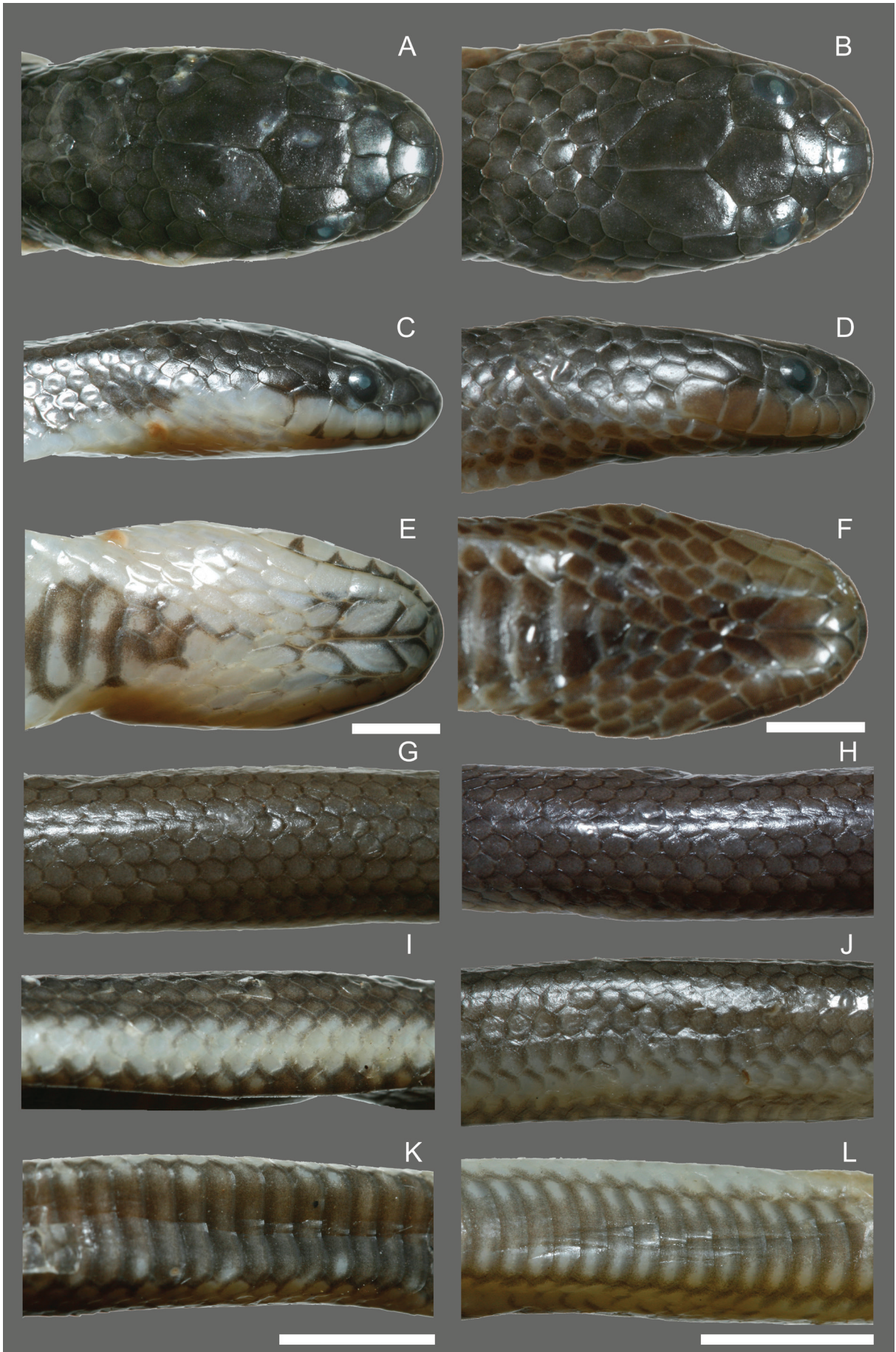


Fig. 2. The two *Gerarda prevostiana* specimens collected 1 km offshore from Vankale, Mannar, Sri Lanka. 2013.18.01.NH: A, C, E, G, I, K; 2013.19.01.NH: B, D, F, H, J, L. Scale bar is 3 mm. Dorsal (A, B), lateral (C, D) and ventral (E, F) aspect of the heads. Dorsal (G, H), lateral (I, J) and ventral (K, L) aspect of mid-body.

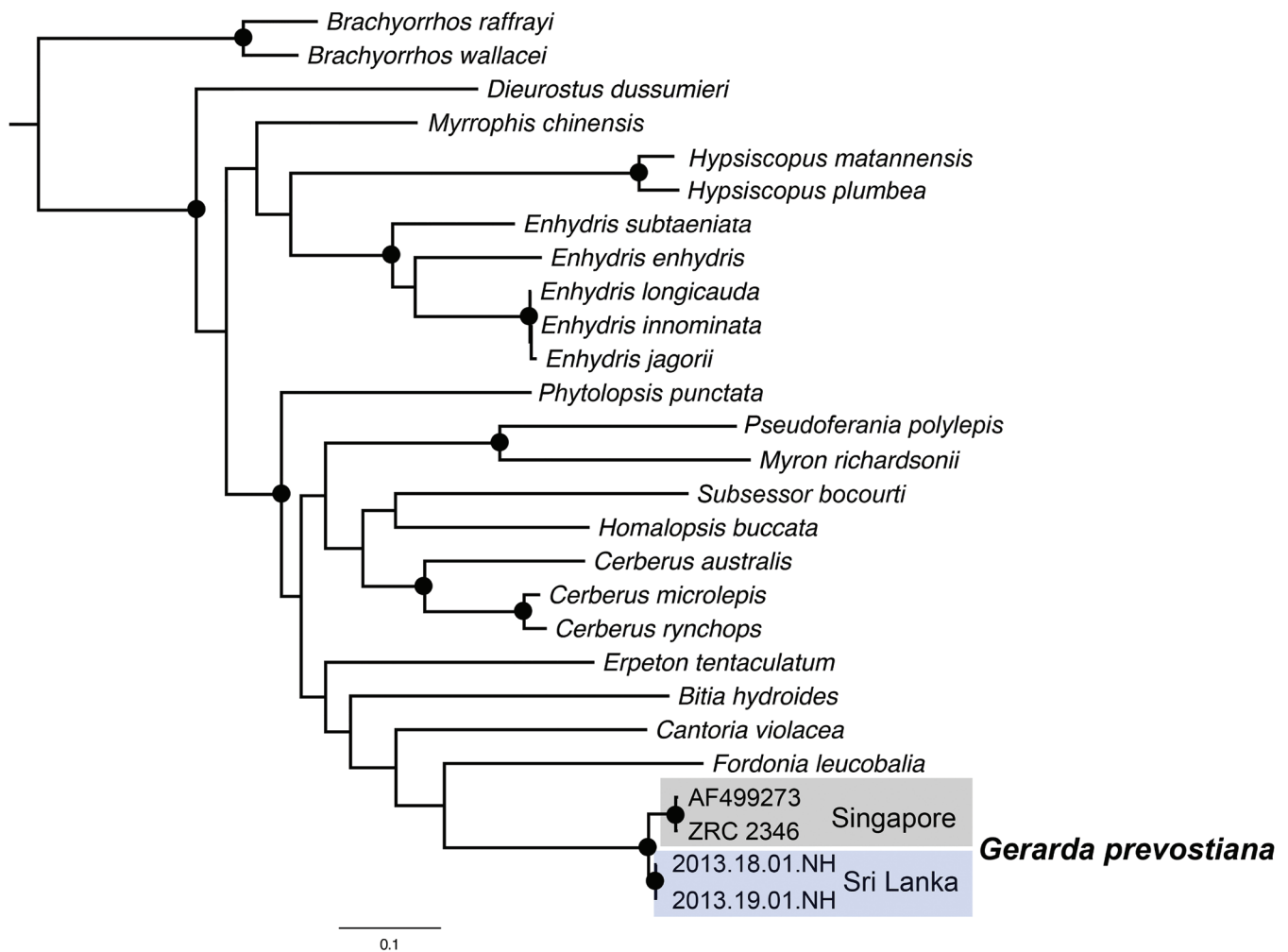


Fig. 3. Bayesian majority-rule consensus tree based on Cytochrome b gene showing the phylogenetic relationships of Sri Lankan *Gerarda prevostiana*. The scale bar indicates the number of substitutions per site. Black circles indicate node support of Bayesian posterior probability >0.9 and ML bootstrap support >70. Outgroup *Xenochrophis vittatus* is not shown.

*Gerarda prevostiana* formed two strongly supported (BS >70, PP >0.9) reciprocally monophyletic clades, which included the two Sri Lankan samples and another clade with the other two samples from Genbank that are from Singapore (Fig. 3). The average corrected (HKY) pairwise genetic distance between Sri Lankan and Singaporean *Gerarda prevostiana* samples were between 3.08–3.87%, with an average of 27.5 fixed substitutions between them.

### DISCUSSION

Our analysis of the mitochondrial Cytochrome b gene between Sri Lankan and Southeast Asian *Gerarda prevostiana* suggest moderate sequence divergence despite not having any observed morphological differences. We briefly discuss the significance of the present record and the molecular divergence observed in light of conservation and the biogeography of marine snakes from South and Southeast Asia.

The two specimens were caught as fisheries by-catch in the shallow open sea. Although *Gerarda prevostiana* is a mangrove specialist, it is known to occasionally venture to adjacent habitats (Murphy, 2007). Thus, specimens observed

here could be residents of the surrounding mangrove habitats that range to the open ocean. A previous study on the marine snakes of the Indian coasts of the Gulf of Mannar did not record this species, although the sympatric homalopsid *Cerberus rynchops* was commonly found (Lobo, 2006). In Sri Lanka, *Gerarda prevostiana* is apparently very rare and is considered endangered in the national Red List (Wickramasinghe, 2012). However, *Cerberus rynchops* is commonly found in coastal mangrove and brackish water habitats all over Sri Lanka. It is possible that *Gerarda prevostiana* is seldom recorded in Sri Lanka due to its nocturnal habits and habitation of mud lobster (*Thalassina* sp.) mounds (Murphy, 2007) that are rarely explored during reptile surveys. So far, all records of *Gerarda prevostiana* in Sri Lanka, including the present one, are from the West coast. Two recent specimens of *Gerarda prevostiana* have been located in mangrove habitats (Karativu and Eluvankulam, Puttalam district) of the coastal Northwestern Province (S. Goonewardene pers. comm.).

The collection of the two specimens in fishing nets in the open sea seems unusual for this mangrove-dwelling species. The authors have examined fisheries by-catch in many locations throughout Sri Lanka and have only observed *Acrochordus*

*granulatus*, *Cerberus rynchops* and species of viviparous sea snakes (Elapidae: Hydrophiini). The rarity of *Gerarda prevostiana* as by-catch indicates that fisheries are highly unlikely to be a significant threat to this species.

The morphological characters and colouration of the two new specimens reported here were generally in agreement with that for the previously reported specimens elsewhere (Murphy, 2007). However, the unusual upper labial colouration and lower jaw colouration of specimen 2013.18.01.NH is noteworthy and it is unlikely that it was caused by any physical damage to the specimen during its capture, since both the upper labials and the lower jaws have the same colouration. Hence, this could most likely be an individual variation that has not been reported before for this species. Thus, the lack of morphological divergence in other characteristics between the specimens from Sri Lanka and other regions may imply that there is little morphological variation in this species throughout its range. However, the ventral and subcaudal scale counts of the two specimens are at the higher end of the species' range (Murphy, 2007).

Our molecular analysis of *Gerarda prevostiana* from Sri Lanka and Singapore follows several previous studies that have attempted to understand the biogeographic relationships between aquatic snakes from the South Asian and Southeast Asian regions (Alfaro et al., 2004; Alfaro et al., 2008; Murphy et al., 2012; Ukuwela et al., 2014; Ukuwela et al., 2016). Southeast Asia supports higher species richness than South Asia for both homalopsid snakes (34 versus 10 species, respectively) and viviparous sea snakes (34 versus 21 species, respectively). Recent biogeographic reconstructions for viviparous sea snakes suggest that much of the South Asian viviparous sea snake fauna may have originated in Southeast Asia, but revealed substantial genetic divergence between the two regions indicative of cryptic lineage and species diversity (Ukuwela et al., 2014; Ukuwela et al., 2016). This suggests that isolation during Plio-Pleistocene glacial cycles may have been an important driver of population divergence in aquatic snakes. More than 3% sequence divergence in the Cytochrome b gene between *Gerarda prevostiana* from Sri Lanka and Singapore could also indicate such historical isolation of these populations. Not only sea snakes, previous studies on marine fish and invertebrates have similarly shown genetic divergence across the populations in the Indian Ocean and west Pacific (i.e. Southeast Asia) region due to historical isolation during the Pleistocene era (Carpenter et al., 2011). Sea levels as low as 120 m below current levels in the Southeast Asian region repeatedly connected the shallow Sunda continental shelf forming the Indo-Pacific biogeographic barrier (Voris, 2000). This phenomenon isolated the populations of marine or brackish water species on either sides, subsequently leading to genetic divergence. However, more samples across the range of *Gerarda prevostiana* are needed to test whether a pattern of Indo-Pacific divergence or just isolation by distance is responsible for the observed pattern of genetic divergence in this study.

The finding of this locally rare species of snake reported here highlights the importance of systematic surveys of vertebrate groups in Sri Lanka. Previous studies on other groups of vertebrates in Sri Lanka have rediscovered even species that were thought to be extinct (Meegaskumbura et al., 2012; Wickramasinghe et al., 2013). Future surveys might also shed light on the status of *Enhydris enhydris*, a species of homalopsid snake common throughout Southeast Asia but known only from a single specimen (Gyi, 1970; Murphy, 2007) in Sri Lanka, as well as nationally rare sea snakes such as *Hydrophis biturbiculatus*, *Hydrophis mammillaris* and *Hydrophis stokesi*.

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