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Length - weight relationship and molecular phylogenetic analysis to infer status of *Uroteuthis duvaucelii* (d'Orbigny 1835) population in the southern coastal region of Sri Lanka

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Abstract
Population status of Indian squid: *Uroteuthis* (*Photololigo*) *duvaucelii* of southern Sri Lanka was determined using length – weight relationship and genetic analyses. Growth performance and pattern was estimated by regression curves and growth coefficient constant of the male and female populations. The growth performance was significantly different between males and females ($p< 0.05$) within the population, but both sexes indicated negative allometric growth patterns. Genetic analyses were performed using partially amplified mitochondrial 16S rRNA (544 bp) and Cytochrome Oxidases subunit I (COI) (600 bp) gene regions. One and three haplotype were determined from two gene regions respectively. Southern Sri Lanka haplotypes show high divergence levels among other *U. duvaucelii* sequences for both gene regions which suggest the possibility of presence cryptic species. This suggestion is further confirmed by the formation of separate clade for southern Sri Lankan haplotypes in the phylogenetic trees of both gene regions. This morphologically and genetically distinct population within the genus *Uroteuthis* should be taken into consideration and prioritized when implimenting fisheries and conservation management programs.

Keywords: *Uroteuthis duvaucelii,* length – weight relationship, mitochondrial 16S rRNA, Cytochrome Oxidases subunit I.

1. Introduction
Indian squid, *Uroteuthis* (*Photololigo*) *duvaucelii* (d'Orbigny 1835), is a neritic species with a widespread distribution along the Indian Ocean from the west coast of Africa to the south-east Asian region including Red sea and the Arabian Sea [1, 2]. It is the most abundant squid species in the Indian Ocean [3]; thus, provides considerable importance to the fishery industry in many south and south-east Asian countries [2]. It has been reported that availability of morphologically different forms of *U. duvaucelii* in commercial fisheries [4]. With the aid of morphological and genetic data, Sin et al [5] indicated the availability of polymorphism within short geographic range and thus, suggested the presence of cryptic species within this species. Analysis of the length-weight relationship of a species could be used as a tool in shell fish biology, physiology, ecology and fisheries assessment population dynamic studies [6]. Length-weight relationships of squids are also important as it can be used in taxonomic purposes and as an indicator of stocks in stock assessment programs within species [7, 8]. Number of studies have been reported on the length weight relationship of *U. duvaucelii* from various parts of west and east coastal regions around Indian waters and have found growth patterns with significant variations among populations [9, 10, 11, 12].
Genetic differences of a geographically distributed species can be incorporated to identify a particular stock [13, 14]. The large ribosomal subunit of mitochondrial rRNA (16S rRNA) and Cytochrome C Oxidase I (COI) genes have been used in taxonomic studies of *Uroteuthis* sp. [5, 15, 16]. Both gene regions provided informative data sets for differentiation at different taxonomic levels [16]. The 5' end of COI gene region has been accepted as the standardized marker for metazoan known as the DNA barcode region and efficiently applied for the

Identification of cryptic species in many taxonomic groups [17, 18, 19] including squid species [16].

In the southern coastal belt of Sri Lanka, *U. duvaucelii* is the most abundant and commercially important squid species. It has been recorded the availability of few *Uroteuthis* species [1], however detailed studies have not been recorded on any species of this genus from Sri Lankan waters. Therefore, in order to clarify the status of the population of *U. duvaucelii* from the southern coastal belt of Sri Lanka, the present study compared growth performance derived from length -weight relationship data and genetic data for mitochondrial 16S rRNA and COI gene regions, with data available from previous studies on *U. duvaucelii* from other geographic locations.

2. Materials and Methods

A Total of 277 samples of *Uroteuthis duvaucelii* were collected from fish landing sites located in three districts of the southern coastal belt of Sri Lanka (Figure 1).

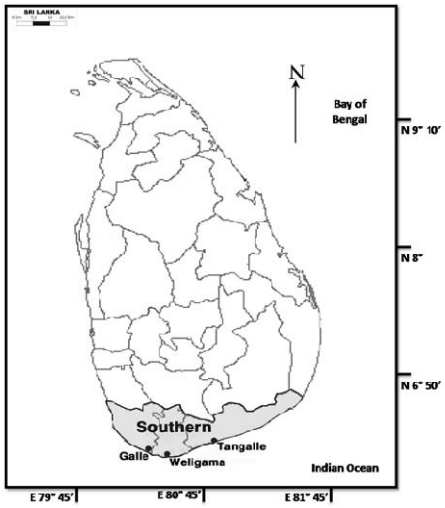

Fig 1: Map showing sampling localities of the current study in the southern Sri Lanka

Samples were collected from July to September 2012. Identification of the specimen was confirmed using the morphological characters as described in past studies [1, 2]. Upon sampling, individuals were cleaned to remove impurities adhered to the bodies and a reference number was given to each sample. The total weight of each individual was measured to the nearest 0.01g. Sex was determined observing the gonads (only individuals with mature ovaries or testes were considered for both morphological and genetic analyses) and the left ventral arm [2]. Dorsal mantle length (DML) of each mature individual was measured to the nearest 0.01centimeter. A tissue sample from the fin (5mm X 5mm) was collected from 10 individuals of each sampling location for molecular analysis. Then rest of the samples were preserved in ethanol as voucher specimen in the Research Laboratory, Department of Zoology, and University of Ruhuna, Matara, Sri Lanka for further examinations.

2.1 Analysis of growth performance

Length-weight measurements were analyzed for the two sexes separately. Logarithmic transformation of length-weight data was observed for normal distribution patterns. Pearson correlation of log weight and log length was calculated for two sexes. The relationship between total body weight (TW) and DML was estimated using the published equation [20, 21], where 'a' (intercept) and 'b' (regression coefficient or slope) are constants.

$TW = a\ DML^b$ and $Log\ TW = Log\ a + b\ Log\ DML.$

Regression lines were obtained using the simple least square regression method. The regression coefficient value obtained for the male and female groups were tested using student's t-test to see whether the regression coefficient values differ significantly from the value 3 or not for determination of growth pattern [22]. The significant difference of each regression line was analyzed using the general linear model analysis of covariance. The regression coefficient values derived from the analyses of length-weight relationship in the current study were compared statistically with the previously published data for this species using a student's test. MINITAB (Version 14) statistical software was used for morphological data analysis.

2.2 Analysis of molecular data

For molecular analyses, thirty individuals were selected representing ten individuals from each location. Total DNA was extracted from small fin tissue samples using DNEasy Extraction Kit (QIAGEN) following manufacturer's protocols. Mitochondrial 16S rRNA (~544 bp) and COI (~600 bp) gene regions were partially amplified using universal primers 16Sar/Sbr [23] and LCO1490/HCO2198 [24] respectively. For both gene regions, PCR amplifications were carried out in 25µL reaction mixture containing 1× Taq polymerase buffer, 1.5mM MgCl2, 0.4mM each dNTP, 0.2µm of each primer, 100ng of DNA template and 0.5U of Taq DNA polymerase. PCR conditions for COI gene region were used as 15 min. at 95 °C; 35 cycles of 94°C for 1min., 50 °C for 1min., 72 °C for 1min. and for 16S rRNA gene region were as 15 min at 95 °C; 35 cycles of 94 °C for 45 sec., 52 °C for 45 sec., 72 °C for 1 min. In both reactions, final extension was remained at 72 °C for 7 min. Amplified samples were sent for Macrogen Pvt. Ltd, Korea for purification and DNA sequencing by next generation sequencing method. Sequences resulted from this study were deposited in the Genbank.

For comparative purposes, selected 16S rRNA and COI sequences available in the Genbank for *U. duvencelli* were downloaded to construct the data set. Sequences of *U. chinensis* and *U. edulis* were also included in the data set to compare the divergence levels among *U. duvencelli* populations at the interspecific levels. *Sepioteuthis lessoniana* was used as an out group. Sequences were viewed and aligned using BioEdit (V.7.0.0) [25] and Masequit [26] programs. Suitable models for data sets of the two gene regions were estimated using jModelTest (v.0.1.1) software [27]. Phylogenetic analyses were performed using Mega program (v.6.0.6) [28]. Maximum Likelihood (ML) and Neighbor-Joining (NJ) phylogenies were constructed with 1000 bootstrap replicates. Analyses were carried out using the closest best-fit model when ML analysis was performed. Analyses for two gene regions were conducted separately as information available in the Genbank was not consistent for two gene regions.

3. Results

3.1 Analysis of growth performances

Analysis of length-weight relationship data in the current study indicated that resultant regression coefficient values were significantly different between males and females at a 5% significant level. Comparisons of regression coefficient values that have been published for male and female groups of *U. duvaucelii* from different geographical location are given in the Table 1.

Table 1: Derived growth coefficients of regression lines between dorsal mantle length and weight of *Uroteuthis duvaucelii* in different geographical locations.

Geographical Location	"b" values of regression lines		References
	Male	Female	
Gulf of Thailand, Thailand	1.78	2.04*	[29]
Madras Coast , India	2.38*	2.52*	[7]
Mumbai Coast , India	2.06	2.31*	[30]
Off Cochin waters, India	2.14	2.29*	[3]
Kerala, Maharasbtra, Gujarat, India	2.30*	2.50*	[31]
Mangalore, Southwest coast of India	1.95	2.33*	[9]
Tuticorin, Southeast Coast of India	2.19	2.30*	[11]
Mumbai Water, West Coast of India	2.16	2.28*	[10]
Goa, West coast of India	1.61*	1.62	[12]
Southern Sri Lanka	1.97	1.40	Present Study

* There is a significant difference between "b" values of present study and previous study.

Table 2: Data of descriptive statistics, logarithmic transformation between dorsal mantle lengths and weights (L: W) of male and female groups and the results of the students't test.

Results	Male	Female
Length	Mean DML**:** 12.19±2.67 cm Maximum: 25.70 cm Minimum: 7.70 cm	Mean DML**:** 10.75±1.63cm Maximum: 15.20 cm Minimum: 7.50 cm
Weight	Mean: 29.66 ±8.13g Maximum: 142.24 g Minimum: 11.78 g	Mean: 23.02 ±6.52g Maximum: 40.62 g Minimum: 10.74 g
Logarithmic transformation Length – weight relationship	Log W = - 0.694 + 1.97 Log ML $W = 0.202302ML^{1.97}$	Log W = - 0.089 + 1.40 Log ML $W = 0.814704ML^{1.40}$
Pearson correlation (Log L:Log W)	0.895 (P < 0.05)	0.747 (P < 0.05)
F value of regression	301.700	68.060
P value of regression	0.00	0.00

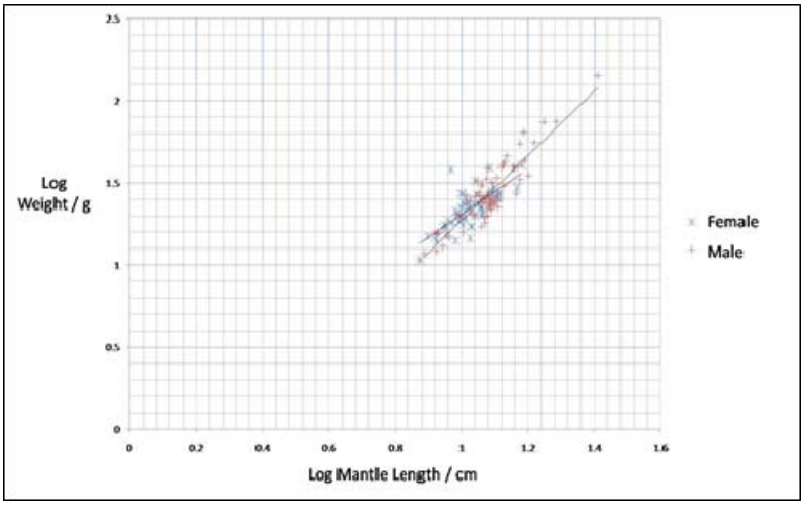

Fig 2: Scatter plot of log weight vs log mantle length for male and female populations of *Uroteuthis duvaucelii.*

The highest mean dorsal mantle length and mean weight are recorded for male group (Table 2). Results indicated that there is a relationship between mantle length and individual total weight (Figure 2). Correlation coefficients for males and females were significantly different ($P < 0.05$). The slopes of regression lines for male (1.97) and female (1.40) groups were lesser to the ideal value of 3 and results of the t-test indicated that regression coefficient values derived for male and female groups were significantly different from the ideal value of 3 at 5% significant level ($P<0.5$).

3.2 Analysis of molecular data

Details of the sequences downloaded from the Genbank are given in the Table 3. Descriptive information of the nucleotides data sets analyzed for two gene regions are given in the Table 4. Molecular analyses of Southern Sri Lankan sample produced one haplotype for 16S data set and three haplotypes for COI data set. Percentage sequence divergence (p distance) estimated for data sets of two gene regions are given in the Table 5 and Table 6. Percentage sequence divergence (p distance) among *U. duvencelli* sequences for 16S data set ranged from 0 to 5% while it was up to 13.2% for COI data set. As similar tree topologies derived from both ML and MP methods, only tree topologies derived from ML analysis are given in the Figure 3 and Figure 4 for 16S and COI data sets respectively.

Table 3: Accession numbers for *Uroteuthis duvaucelii* sequences for two gene regions downloaded from Genbank and the present study.

Species	16S	COI	Region	Study (Ref No)
Uroteuthis duvaucelii	KC959418 - KC959425	KC959454, KC959455, KC959458, KC959462, KC959463	Indonesia	32
	KC959426 - KC959438	KC959439, KC959445, KC959447, KC959448, KC959450, KC959457, KC959461, KC959464, KC959465, KC959470, KC959472	Vietnam	
	KC959405	KC959444	Philippine	
	HQ529576 - HQ529584	HQ529529 - HQ529536	China	16
	EU349492	EU349465	Shanghai China	5
	EU349490 EU349491	EU349463 - EU349464	Hong Kong, China	
	AF110092	AF075398 , AF075400	Andaman Sea	15
	AF110093		Gulf of Thailand	
		KC409385 - KC409386	India, Arabian Sea	Unpublished data in Genbank
	AJ000101		Unknown	Unpublished data in Genbank
	KF854044 – KF854046	KF854082 - KF854084	South America	33
	KF489893	KF489890 – KF489892	Southern Sri Lanka	Current study
Uroteuthis chinensis	EU349470	EU349433,	China	5
	EU234588	EU349443	Hong Kong	16
Uroteuthis edulis	EU349481	EU349448 EU349462	Japan China	5
Sepioteuthis lessoniana	HQ529588 HQ529591	EU349466	China	16

Table 4: Descriptive information of the nucleotides data sets analyzed for two gene regions.

	16S	COI
Number of nucleotides	544	600
Number of ingroup sequences	39	37
% of Nucleotide frequencies		
A	32.61	30.08
T	39.29	36.03
C	10.12	17.76
G	17.98	16.13
Best fit model for data set	TIM3+I	TIM2+I+G

Table 5: Percentage sequence divergence (p distance) estimated for data set of 16S rRNA gene region.

	Sample information	Percentage divergence value of P distance
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
1	Unk - AJ000101	-
2	And - AF110092	0.97
3	Tha - AF110093	3.14 2.89
4	Chi - EU349492	3.86 3.86 1.93
5	Hok - EU349491	3.86 3.86 1.93 0.00
6	Hok - EU349490	3.86 3.86 1.93 0.00 0.00
7	Chi - HQ529576	3.86 3.86 1.93 0.00 0.00 0.00
8	Chi - HQ529577	3.86 3.86 1.93 0.00 0.00 0.00 0.00
9	Chi - HQ529578	3.86 3.86 1.93 0.00 0.00 0.00 0.00 0.00
10	Chi - HQ529579	3.64 3.86 1.93 0.00 0.00 0.00 0.00 0.00 0.00
11	Chi - HQ529580	3.86 3.86 3.86 0.00 0.00 0.00 0.00 0.00 0.00 0.00
12	Chi - HQ529584	3.86 3.86 1.93 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
13	Chi - HQ529582	3.86 4.10 2.17 0.24 0.24 0.24 0.24 0.24 0.24 0.24 0.24 0.24
14	Chi - HQ529583	3.86 3.86 1.93 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.24
15	Srl - KF489893	4.83 4.34 3.38 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 4.12 3.86
16	Phi - KC959405	1.21 0.24 2.66 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.86 3.62 4.11
17	Ind - KC959418	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66
18	Ind - KC959419	1.21 0.24 2.66 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.86 3.62 4.59 4.83 2.66
19	Ind - KC959420	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66
20	Ind - KC959421	0.96 0.00 2.89 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 4.11 3.86 4.35 0.24 2.89 0.24 2.89
21	Ind - KC959422	3.38 3.14 0.24 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.42 2.17 3.62 2.89 0.24 2.89 0.24 3.14
22	Ind - KC959423	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66 0.00 2.89 0.24
23	Ind - KC959424	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66 0.00 2.89 0.24 0.00
24	Ind - KC959425	3.38 3.14 0.24 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.17 2.41 2.17 3.14 2.89 0.24 2.89 0.24 3.14 0.48 0.24 0.24
25	Sam - KF854044	0.97 0.00 2.89 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 4.11 3.86 4.35 0.24 2.89 0.24 2.89 0.00 3.14 2.89 2.89 3.14
26	Sam - KF854046	0.97 0.00 2.89 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 4.11 3.86 4.34 0.24 2.89 0.24 2.89 0.00 3.14 2.89 2.89 3.14 0.00
27	Sam - KF854045	0.97 0.00 2.89 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 3.86 4.11 3.86 4.35 0.24 2.89 0.24 2.89 0.00 3.14 2.89 2.89 3.14 0.00 0.00
28	Vie - KC959426	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.65 0.00 2.66 0.00 2.89 0.24 0.00 0.00 0.24 2.89 2.89 2.89
29	Vie - KC959427	3.38 3.14 0.24 2.17 2.17 2.17 1.93 2.17 2.17 2.17 2.17 2.17 2.41 2.17 3.62 2.89 0.24 2.89 0.24 3.14 0.48 0.24 0.24 0.48 3.14 3.14 3.14 0.24
30	vie - KC959428	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66 0.00 2.89 0.24 0.00 0.00 0.24 2.89 2.89 2.89 0.00 0.24
31	Vie - KC959429	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66 0.00 2.89 0.24 0.00 0.00 0.24 2.89 2.89 2.89 0.00 0.24 0.00
32	Vie - KC959430	3.38 3.14 0.24 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.93 1.69 3.14 2.89 0.24 2.89 0.24 3.14 0.48 0.24 0.24 0.48 3.14 3.14 3.14 0.24 0.48 0.24 0.24
33	Vie - KC959431	3.38 3.14 0.24 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.93 1.69 3.14 2.89 0.24 2.89 0.24 3.14 0.48 0.24 0.24 0.48 3.14 3.14 3.14 0.24 0.48 0.24 0.24 0.00
34	Vie - KC959432	3.14 2.89 0.00 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.66 0.00 2.66 0.00 2.89 0.24 0.00 0.00 0.24 2.89 2.89 2.89 0.00 0.24 0.00 0.00 0.24 0.24
35	Vie - KC959433	3.14 2.89 0.00 1.93 1.93 1.93 1.90 1.93 1.93 1.93 1.93 1.93 2.17 1.93 3.38 2.65 0.00 2.66 0.00 2.89 0.24 0.00 0.00 0.24 2.89 2.89 2.89 0.00 0.24 0.00 0.00 0.24 0.24 0.00
36	Vie - KC959434	3.86 3.86 1.93 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.24 0.00 3.86 3.62 1.93 3.62 1.93 3.86 2.17 1.93 1.93 2.17 3.86 3.86 3.86 1.93 2.17 1.93 1.93 1.69 1.69 1.93 1.93
37	Vie - KC959436	0.04 3.86 1.93 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.24 0.00 3.86 3.62 1.93 3.62 1.93 3.86 2.17 1.93 1.93 2.17 3.86 3.86 3.86 1.93 2.17 1.93 1.93 1.69 1.69 1.93 1.93 0.00
38	Vie - KC959437	3.38 3.14 0.24 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.69 1.93 1.69 3.62 2.89 0.24 2.89 0.24 3.14 0.48 0.24 0.24 0.48 3.14 3.14 3.14 0.24 0.48 0.24 0.24 0.48 0.48 0.24 0.24 1.69 1.69
39	Vie - KC959438	3.86 4.11 2.17 0.24 0.24 0.24 0.24 0.24 0.24 0.24 0.24 0.24 0.00 0.24 4.11 3.86 2.17 3.86 2.17 4.11 2.42 2.17 2.17 2.42 4.11 4.11 4.11 2.17 2.42 2.17 2.17 1.93 1.93 2.17 2.17 0.24 0.24 1.93
40	*Hok - EU349470*	6.76 6.28 4.83 4.83 4.83 4.83 4.83 4.83 4.83 4.83 4.83 4.83 5.07 4.83 5.31 6.04 4.83 6.04 4.83 6.28 5.07 4.83 4.83 5.07 6.28 6.28 6.28 4.83 4.59 4.83 4.83 5.07 5.07 4.83 4.83 4.83 4.83 5.07 5.07
41	*Chi - EU234588*	6.28 5.79 4.35 4.35 4.35 4.35 4.35 4.35 4.35 4.35 4.35 4.35 4.59 4.35 4.83 5.56 4.35 5.56 4.35 5.79 4.59 4.35 4.35 4.59 5.79 5.79 5.79 4.35 4.59 4.35 4.35 4.59 4.59 4.35 4.35 4.35 4.35 4.59 4.59 0.48
42	*Chi - HQ529588*	5.31 4.83 4.35 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.62 3.86 3.62 4.83 4.59 4.35 5.07 4.34 4.83 4.59 4.35 4.35 4.59 4.83 4.83 4.83 4.35 4.59 4.34 4.34 4.11 4.11 4.35 4.35 3.62 3.62 4.11 3.86 4.59 4.11
43	*Hok - EU349481*	5.31 5.07 3.86 3.38 3.38 3.38 3.38 3.38 3.38 3.38 3.38 3.38 3.62 3.38 4.58 4.80 3.86 5.31 3.86 5.07 4.11 3.86 3.86 4.10 5.07 5.07 5.07 3.86 4.11 3.86 3.86 3.62 3.62 3.86 3.86 3.38 3.38 3.62 3.62 4.58 4.11 1.21
44	*Outgroup - HQ529591*	8.93 8.69 7.00 6.52 6.52 6.52 6.52 6.52 6.52 6.52 6.52 6.52 6.76 6.52 8.93 8.45 7.00 8.69 7.00 8.69 7.24 7.00 7.00 6.76 8.69 8.69 8.69 7.00 7.00 7.00 7.00 6.76 6.76 7.00 7.00 6.52 6.52 6.76 6.76 9.66 9.42 8.21 7.49 -

1 – 39: *Uroteuthi. duvaucelii,* 40 – 41: *U. chinensis,* 42 – 43: *U. edulis,* 44: outgroup - *Sepioteuthis lessoniana*

Table 6: Percentage sequence divergence (p distance) estimated for data set of COI gene region.

	Sample information	Percentage divergence value of P distance
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43
1	Chi - HQ529535	_
2	Chi - HQ529533	0.00
3	Chi - HQ529531	0.00 0.00
4	Chi - 529529	0.18 0.18 0.18
5	Chi - HQ529536	0.00 0.00 0.00 0.18
6	Chi - HQ529534	0.00 0.00 0.00 0.18 0.00
7	Chi -HQ 529532	0.00 0.00 0.00 0.18 0.00 0.00
8	Chi - HQ529530	0.00 0.00 0.00 0.18 0.00 0.00 0.00
9	And - AF075400	5.38 5.38 5.38 5.19 5.38 5.38 5.38 5.38
10	And - AF075398	6.12 6.12 6.12 5.94 6.12 6.12 6.12 6.12 6.86
11	Hok - EU349463	0.00 0.00 0.00 0.19 0.00 0.00 0.00 0.00 5.38 6.12
12	Hok - EU349465	0.00 0.00 0.00 0.19 0.00 0.00 0.00 0.00 5.38 6.12 0.00
13	Hok - EU349464	0.00 0.00 0.00 0.19 0.00 0.00 0.00 0.00 5.38 6.12 0.00 0.00
14	Arb - KC409386	8.53 8.53 8.53 8.35 8.35 8.53 8.53 8.53 10.20 9.28 8.53 8.53 8.53
15	Arb - KC409385	8.53 8.53 8.53 8.35 8.53 8.53 8.53 8.53 10.20 9.28 8.53 8.53 8.53 0.00
16	Srl - KF489891	11.13 11.13 11.13 10.94 11.13 11.13 11.13 11.13 11.32 13.17 11.13 11.13 11.13 12.06 12.06
17	Srl - KF489892	10.76 10.76 10.76 10.58 10.76 10.76 10.76 10.76 10.95 12.80 10.76 10.76 10.76 11.69 11.69 0.37
18	Srl - KF489890	10.95 10.95 10.95 10.76 10.95 10.95 10.95 10.95 11.13 12.99 10.95 10.95 10.95 11.87 11.87 0.19 0.19
19	Phi - KC959444	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 0.74 7.05 5.19 5.19 5.19 10.02 10.02 10.76 10.39 10.58
20	Vie - KC959439	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12
21	Vie - KC959445	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00
22	Vie - KC959447	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.19 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00 0.00
23	Vie - KC959448	5.01 5.01 5.01 4.82 5.01 5.01 5.01 5.01 6.12 1.11 5.01 5.01 5.01 8.53 8.53 12.43 12.06 12.24 6.31 0.19 0.19 0.19
24	Vie - KC959450	0.19 0.19 0.19 0.37 0.19 0.19 0.19 0.19 5.57 6.31 0.19 0.19 0.19 8.35 8.35 11.32 10.95 11.13 5.38 5.38 5.38 5.38 5.19
25	Vie - KC959457	0.00 0.00 0.00 0.19 0.00 0.00 0.00 0.00 0.00 5.38 6.12 0.00 0.00 0.00 0.00 8.53 8.53 11.13 10.76 10.95 5.19 5.19 5.19 5.01 0.19
26	Vie - KC959461	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.19 0.93 5.19 5.19 5.19 8.34 8.35 12.24 11.87 12.06 6.12 0.00 0.00 0.00 0.19 5.38 5.19
27	Vie - KC959464	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 6.31 1.29 5.19 5.19 5.19 8.35 8.35 11.87 11.50 11.69 6.12 0.37 0.37 0.37 0.56 5.38 5.19 0.37
28	Vie - KC959465	0.19 0.19 0.19 0.00 0.19 0.19 0.19 0.19 5.19 5.94 0.19 0.19 0.19 8.35 8.35 10.95 10.58 10.76 5.01 5.01 5.01 5.01 4.82 0.37 0.19 5.01 5.01
29	Vie - KC959470	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00 0.00 0.00 0.19 5.38 5.19 0.00 0.37 5.01
30	Vie - KC959472	5.38 5.38 5.38 5.19 5.38 5.38 5.38 5.38 6.12 1.11 5.38 5.38 5.38 8.53 8.53 12.06 11.69 11.87 6.31 0.19 0.18 0.18 0.37 5.57 5.38 0.19 0.19 5.19 0.19
31	Ind - KC959454	4.82 4.82 4.82 4.65 4.82 4.82 4.82 4.82 0.56 6.68 4.82 4.82 4.82 9.65 9.65 10.76 10.39 10.58 0.37 5.75 5.75 5.75 5.94 5.01 4.82 5.75 5.75 4.64 5.75 5.94
32	Ind - KC959455	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00 0.00 0.00 0.19 5.38 5.19 0.00 0.37 5.01 0.00 0.19 5.75
33	Ind - KC959458	0.05 0.05 4.82 4.64 4.82 4.82 4.82 4.82 0.56 6.68 4.82 4.82 4.82 9.65 9.65 10.76 10.39 10.58 0.37 5.75 5.75 5.75 5.93 5.01 4.82 5.75 5.75 4.63 5.75 5.94 0.00 5.75
34	Ind - KC959462	0.05 0.05 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00 0.00 0.00 0.19 5.38 5.19 0.00 0.37 5.01 0.00 0.19 5.75 0.00 5.75
35	Ind - KC959463	5.19 5.19 5.19 5.01 5.19 5.19 5.19 5.19 5.94 0.93 5.19 5.19 5.19 8.35 8.35 12.24 11.87 12.06 6.12 0.00 0.00 0.00 0.19 5.38 5.19 0.00 0.37 5.01 0.00 0.19 5.75 0.00 5.75 0.00
36	Sam - KF854084	4.82 4.82 4.82 4.64 4.82 4.82 4.82 4.82 0.56 6.68 4.82 4.82 4.82 9.65 9.65 10.76 10.39 10.58 0.37 5.75 5.75 5.75 5.94 5.01 4.82 5.75 5.75 4.64 5.75 5.94 0.00 5.75 0.00 5.75 5.75
37	Sam - KF854083	4.82 4.82 4.82 4.64 4.82 4.82 4.82 4.82 0.56 6.68 4.82 4.82 4.82 9.65 9.65 10.76 10.39 10.58 0.37 5.75 5.75 5.75 5.94 5.01 4.82 5.75 5.75 4.64 5.75 5.94 0.00 5.75 0.00 5.75 5.75 0.00
38	Sam - KF854082	4.82 4.82 4.82 4.64 4.82 4.82 4.82 4.82 0.56 6.68 4.82 4.82 4.82 9.65 9.65 10.76 10.39 10.58 0.37 5.75 5.75 5.75 5.94 5.01 4.82 5.75 5.75 4.64 5.75 5.94 0.00 5.75 0.00 5.75 5.75 0.00 0.00
39	*Chi - EU349433*	12.80 12.80 12.80 12.61 12.80 12.80 12.80 12.80 14.10 14.84 12.80 12.80 12.80 14.66 14.66 16.51 16.14 16.33 13.91 13.91 13.91 13.91 13.73 12.99 12.80 13.91 13.91 12.62 13.91 14.10 13.54 13.91 13.54 13.91 13.91 13.54 13.54 13.54
40	*Chi - EU349443*	12.62 12.62 12.62 12.43 12.62 12.62 12.62 12.62 13.91 14.66 12.62 12.62 12.62 14.47 14.47 16.33 15.96 16.14 13.73 13.72 13.73 13.73 13.55 12.80 12.62 13.73 13.73 12.43 13.73 13.91 13.36 13.73 13.36 13.73 13.73 13.36 13.36 13.36 0.19
41	*Jap - EU349448*	13.34 13.36 13.36 13.17 13.36 13.36 13.36 13.36 14.66 13.36 13.36 13.36 13.36 15.39 15.39 15.96 15.58 15.77 15.03 12.43 12.43 12.43 12.24 13.54 13.36 12.43 12.62 13.17 12.43 12.62 14.66 12.43 14.66 12.43 12.43 14.66 14.66 14.66 13.73 13.54
42	*Chi - EU349462*	13.54 13.54 13.54 13.54 13.54 13.54 13.54 13.54 14.84 13.17 13.54 13.54 13.54 15.39 15.39 15.77 15.39 15.58 15.21 12.24 12.24 12.24 12.06 13.73 13.54 12.24 12.43 13.36 12.24 12.43 14.84 12.24 14.84 12.24 12.24 14.84 14.84 14.84 13.36 13.17 0.56
43	*Outgroup - EU349466*	17.07 17.07 17.07 17.07 17.07 17.07 17.07 17.07 17.99 18.92 17.07 17.07 17.07 20.22 20.22 19.48 19.11 19.29 17.63 17.99 17.99 17.99 17.81 17.25 17.07 17.99 17.99 17.07 17.99 18.18 17.63 17.99 17.63 17.99 17.99 17.63 17.63 17.63 17.99 17.81 19.11 18.92

1 – 38: *Uroteuthi. duvaucelii,* 39 – 40: *U. chinensis,* 41 – 42: *U. edulis,* 43: outgroup - *Sepioteuthis lessoniana*

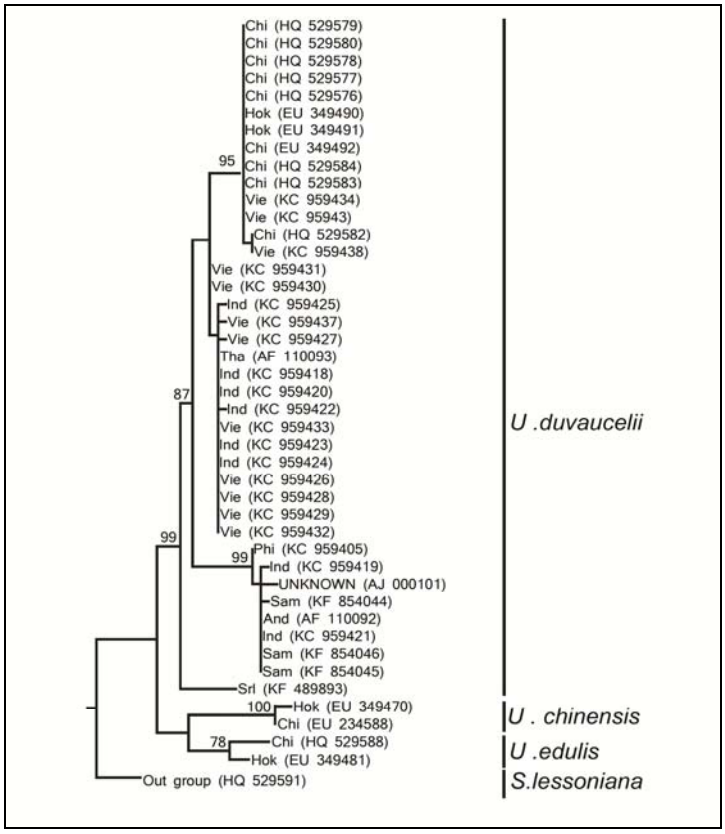

Fig 3: Maximum likelihood tree derived from analysis of mitochondrial 16S rRNA sequences. Bootstrap values more than 50% are indicated on branches of the tree.

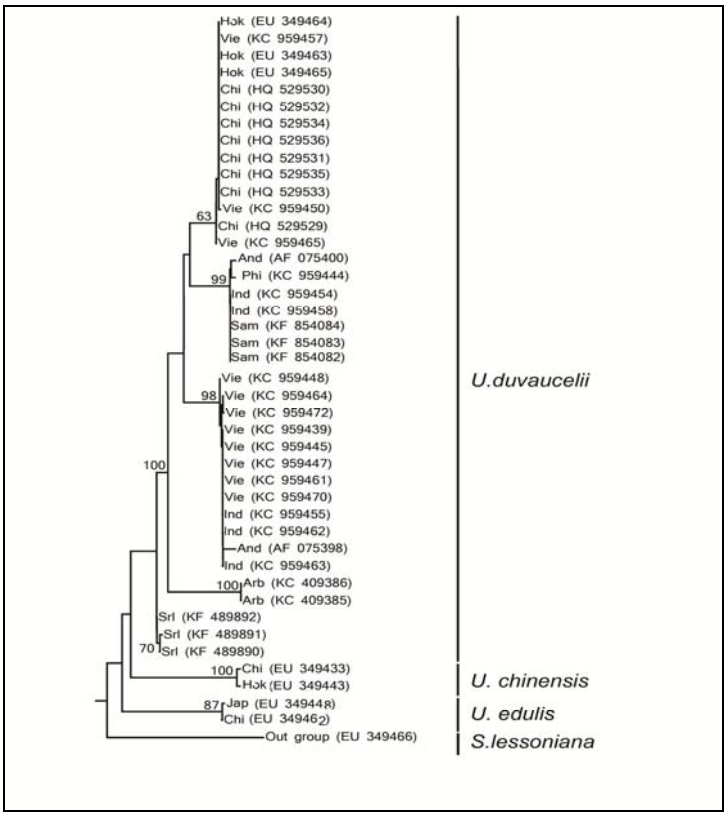

Fig 4: Maximum likelihood tree derived from analysis of mitochondrial COI sequences. Bootstrap values more than 50% are indicated on branches of the tree.

4. Discussion

4.1 Analysis of growth performance

Since the slopes of regression lines for male and female were lower to ideal value of 3, both populations in the current study indicated the negative allometric growth pattern [34]. When comparing length-weight relationships of *U. duvaucelii* populations, regression coefficient values derived from the current study showed slight difference from the previous studies (Table 1). The regression coefficient value of the Southern Sri Lankan male population (1.97) was significantly different from the male population of Goa, West coast of India, Kerala, Maharasbtra, Gujarat, India, Madras Coast, India [12, 3, 7], but not significantly different from regression coefficient values reported from other coastal regions of India.

Other populations demonstrated higher regression coefficient values except the Gulf of Thailand (1.78) [29] and Goa coastal waters, India (1.61) [12] which were lower compared to the southern coastal Sri Lankan population. However, the female population of southern Sri Lanka has reported the lowest regression coefficient value (1.40) which was significantly different when compared to other recorded populations in different geographical locations except Goa, west coastal region of India (1.62) [12]. Lowest regression coefficient implies lower size ranges in female group in the southern coastal region of Sri Lanka.

In general most of cephalopod species particularly *U. duvaucelii* exhibit negative allometric growth patterns [12, 3, 7]. Present study also confirmed a negative growth pattern for both sexes of *U. duvaucelii* populations in the southern coastal region of Sri Lanka. According to the results of the investigation, the growth coefficient was different among geographical location. The regression coefficient could be varied in various localities, since food availability, intra- and interspecific competition, characteristics of water and climate are different [35].

4.2 Analysis of molecular data

Percentage sequence divergence (p distance) among *U. duvencelli* populations of the 16S data set ranged from 0 to 5% which southern Sri Lankan haplotype shows the highest divergence level. This 5% divergence level also reported between some *U. duvencelli* sequences and *U. chinensis* and *U. edulis* sequences as well as between *U. chinensis* and *U. edulis* sequences (Table 5). The percentage sequence divergence (p distance) among three haplotyps derived from analyses of COI gene region of Sri Lankan sequences was ranged from 0.2% to 0.4% (Table 6). Sri Lankan sequences diverge from other *U. duvencelli* sequences with a range of 10.6% to 13.2%.while interspecific divergence levels ranged from 12.1% to 16.5%. 12.1% to 16.5%. However, recorded intra specific divergence levels for *U. duvencelli* in the current study are slightly higher when compared to the study conducted by Dai et al. (2012) [16] which ranged from 0 to 2.7 for 16S rRNA gene region and 0 to 6.8 for COI gene region (estimated under K2P distance).

Similar branching pattern could be observed in both phylogenetic trees (Figure 3 and 4) which Sri Lankan sequence has formed a separate clade in between clades derived for *U. duvencelli* sequences and *U. chinensis* and *U. edulis* sequences with the support of high bootstrap value. Phylogenetic tree derived for 16S data set grouped *U. duvencelli* sequences into three clades while in COI data set produced four clades. In both occasions patterns of grouping of sequences of different geographic locations are not consistent.

Sequence divergence level among COI sequences that recorded from Arabian Sea (Indian sequences) (KC409386 and KC409385) and southern Sri Lanka was ranged from 11.7% to 12.1%. It is interesting to observe that formation of separate clade for southern Sri Lankan samples in both phylogenetic trees. Sequences reported from India and Sri Lanka grouped into two distinct clades in the analysis of COI gene region which was supported with high bootstrap values (Figure 4).

When consider Indian and Sri Lankan species, although two geographic locations are in close proximity, significant difference between two locations can be observed by analyses both data sets of *U. duvaucelii* populations. Although marine organisms often show low levels of genetic differentiation even over large geographical ranges [36, 37] past studies reported high divergence levels and population subdivision in squid species due to the occurrence of geographical barriers to their distribution [38, 39]. Geographical barriers and environmental regimes lead to formation of isolated populations in the marine environment and contribute to the formation of cryptic species has been extensively reported for other marine fish species [40, 41, 42].

However, these isolated populations are more vulnerable to overfishing and environmental changes thus, are important for implementation of proper management and conservation strategies.

5. Conclusion

Sri Lanka is a hot spot with high biodiversity [43]. Most studies are paying attention and emphasized conservation of terrestrial and freshwater invertebrate species, while few have addressed on conservation strategies for marine invertebrate species. This is the first study to focus on the identification of the growth pattern and population status of *U. duvaucelii* in Sri Lankan waters.

Morphological and genetic analysis of the current study confirmed that southern Sri Lankan samples of *U. duvaucelii* represents a discrete population compared to other *U. duvaucelii* populations that recorded so far from the other geographical locations. Results suggest possibility of presence cryptic species of *U. duvaucelii* in Sri Lankan waters. However, more detailed study with samples representing wider geographic locations and data from both mitochondrial and nuclear gene regions is needed to confirm this suggestion.

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