

RESEARCH ARTICLE

## Phylogeographic and phylogenetic analyses of selected set of wild and naturalized *Solanum* spp. in Sri Lanka

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**Abstract:** *Solanum* spp. encompass a greater share of the biodiversity in the world. Sri Lanka, one of the biodiversity hotspots in the world, is the home for wide range of *Solanum* spp. which have been named and morphologically characterized. However, their origins and times of origin have not yet been resolved. Hence, this study was conducted to identify the origins and origination events of 13 wild and naturalized *Solanum* spp. found in Sri Lanka, using DNA barcoding locus *matK* in comparison to worldwide *Solanum* spp. with phylogenetics and divergence dating approaches. In this study, 13 Sri Lankan *Solanum* spp. were separated into four defined phylogenetic groups viz., Old world, Morelloids, Acanthophora and Trova. The studied Sri Lankan *Solanum* spp. would have been originated in Africa 2.007 million years ago (MYA) in the Pleistocene epoch through mammalian migration from Mediterranean land bridges. The Australian relatives of Sri Lankan *Solanum* spp. would have been colonized from South Asia through South East Asia. The floristic connectivity in Pleistocene epoch may have introduced Asian *Solanum* spp. to South East Asia where mid Miocene collisions between Australian and Asian plates as well as over water Long Distance Dispersal (LDD) may have caused these species to colonize in Australia. Our analysis demonstrated that most of the *Solanum* spp. found in Sri Lanka were introduced from India during the Pleistocene ice age. We suggest that Pre Pleistocene migrations of *Solanum* spp. such as *S. nigrum* may have occurred through overwater LDD.

**Keywords:** *matK*, Solanaceae, nightshades, evolution and geo-dispersal of *Solanum*, over water Long Distance Dispersal (LDD).

### INTRODUCTION

Solanaceae (nightshades) is one of the most cosmopolitan and economically important families within angiosperms (Samuels, 2009). It contains around 2500 species within 100 genera composed of 14 main clades (Knapp, 1991; Olmstead *et al.*, 2008). Solanaceae contains major vegetable species such as eggplant (*Solanum melongena*), chili pepper (*Capsicum* spp.), potato (*S. tuberosum*) and tomato (*S. lycopersicum*) and nearly 180 nightshade species are also grown as crops in diverse countries (Samuels, 2015). *Solanum* is the largest genus in the Solanaceae with 1,250 to 1,750 species distributed in many parts of the world (Frodin, 2004). In general, only 7% of species

in Solanaceae are considered as established crop species although there are many other underutilized Solanaceous species available with edible parts (Samuels, 2015).

Sri Lanka is one of the biodiversity hot spots in the world (Bossuyt *et al.*, 2004) and is home to 15 wild / naturalized and 13 exotic *Solanum* species (Dassanayake, 1987) and many of them are found in abundance in nearby India. These *Solanum* species possess valuable economic potential as edible and medicinal plants (Jeyakumar *et al.*, 2016). Different parts of the wild *Solanum* plants exhibit important medicinal properties including anti-diabetic (Gupta *et al.*, 2005), anticonvulsant (Adesina, 1985), anticancer (Jain *et al.*, 2011), antibacterial (Salar, 2009; Doss *et al.*, 2009), and wound healing properties (Huang *et al.*, 2008) as well as larvicidal activity against mosquitoes (Bansal *et al.*, 2009). Although numerous taxonomic studies have been undertaken, phylogenetic analyses to decipher possible origins and divergence times of the species, bioprospecting programs to characterize important bio-active compounds and biotechnological attempts to utilize these values in economic development are limited for the *Solanum* species present in Sri Lanka.

History of the geographical distribution of plant species around the world is considered a topic of interest to researchers (Bell *et al.*, 2017; Baldwin and Wagner, 2010; Davis *et al.*, 2002). The Gondwana fragmentation followed by the continental drift is one of the key hypotheses that explains the colonization of the biota in various regions (Yuan *et al.*, 2005; Balme, 1980; Conti *et al.*, 2004; Macey *et al.*, 2000). However, the divergence time for most of the present angiosperms date below 75 million years (Renner, 2004) whereas, Gondwana fragmentation occurred 80 – 160 million years ago (MYA) (Hall, 1998; Chatterjee and Scotese, 1999). Although fragmentation of Gondwanaland into major continents is considered the first reason for geographic distribution of plants, the wide distribution of plant families such as nightshades can only be addressed through two other hypotheses: land to land distribution through continental connections (Wolfe, 1975; Tiffney, 1985) and over water long distance dispersal (LDD) (Renner, 2004). The formation and breakdown of the central and regional bridges and the occurrence of discrete ice ages might have connected the floristic continuity

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across the countries (Wolfe, 1975; Tiffney, 1985). Over water LDD is also considered as a prime causative agent for higher rates of dispersal events of sessile flora (Baldwin and Wagner, 2010; Booth, 2017; Gillespie *et al.*, 2012; Renner, 2004). Primarily three means of LDD are proposed in Gillespie *et al.*, (2012) namely, wind dispersal, dissemination *via* oceanic currents or floating rafts and migratory bird mediated distribution. These three mechanisms are considered as regional distribution events and many studies suggest that they were more instrumental in recent floral diversifications events than the effect of Gondwana fragmentation.

The Solanaceae phylogenies, cladistic relationships and the overall evolution are well studied (Knapp, 1991; Weese and Bohs, 2007; Särkinen *et al.*, 2013; Bohs and Olmstead, 1997; Volkov *et al.*, 2001). Species in the genus *Solanum* consist of many diverse origins such as Eurasian, South American, North American, Asian, Australian and African and moreover, some of these species are endemic to the original geographical ranges (i.e. native ranges). Many studies have used plastid barcoding markers such as *matK*, *ndhF*, *trnS-G* and *trnL-F* (Särkinen *et al.*, 2013; Bohs and Olmstead, 1997; Weese and Bohs, 2007), nuclear barcoding markers such as *waxy*, *ITS* and *5s rDNA* (Särkinen *et al.*, 2013; Volkov *et al.*, 2001) to study these species. However, none of these published studies have considered *Solanum* spp. in Sri Lanka causing a significant knowledge gap in the phylogenetic grouping, origins and origination events of the species present in the country.

The establishment of phylogroups and origination events of all the Solanaceae species in Sri Lanka is an uphill task, although they would provide a strong platform for germplasm conservation and, cataloging the Sri Lankan species in world wide diversity structures and for application in plant breeding, bio-prospecting and biotech industries studies in order to utilize this wealth of genomic diversity for the economic development. If molecular phylogenetic trees could be established for at least prominent and economically important Solanaceae species, molecular geneticists would enable characterizing the orthologs of the important genes present in the clades and sister-groups. Therefore, the objective of the present study was to establish the molecular phylogenetic relationships, origins and the origination events of a selected group of economically important *Solanum* spp. in Sri Lanka.

## MATERIALS AND METHODS

### DNA sequencing

The purified DNA isolated from 13 wild and naturalized *Solanum* spp. in Sri Lanka were PCR amplified using *matK* primers (forward primer: 5' CGA TCT ATT CAT TCA ATA TTT C 3' and reverse primer: 5' TCTAGCACACGAAAGTCGAAGT3') (Jeyakumar *et al.*, 2016), the standard reagents, conditions and primer annealing temperature of 48 °C. The purified PCR products were subjected to Sanger DNA sequencing using ABI 3500 Genetic Analyzer Version 4405186. The obtained *matK* DNA sequences of these 13 species were

published in Jeyakumar *et al.*, (2016) in comparison to the morphogenetic diversity structure of the species (Table 1, GenBank Accession Numbers KX258741 to KX258754). The same dataset was used in the present analysis to estimate the origination events of these species.

### Phylogenetic analysis

A set of Basic Local Alignment Search Tool (BLAST) analysis was performed for 13 *matK* sequences to fetch all the orthologous *Solanum matK* sequences available in the GenBank (Table 1). A total of 59 sequences identified through BLAST searches were aligned manually with the sequences of 13 wild and naturalized *Solanum* species in Sri Lanka using the software MEGA 7 (Kumar *et al.*, 2016). A species from sister group of *Solanum* clade, *Jaltomata bicolor*, was used as the out-group to root all the trees in the phylogenetic analysis. The bootstrap+consensus algorithm was executed in Maximum Likelihood framework for 1000 replicates in RAxML-HPC2 Workflow (Stamatakis, 2006) in Cipres Science gateway (Miller *et al.*, 2010). The GTRCAT was used as the nucleotide substitution model to get an accurate and rapid estimation of substitution rate at each site. A Heuristic tree search was initially run in Maximum Parsimony criterion in PAUP V4 (Swofford, 2001) where the strict consensus tree was used as the starting topology in RAxML. The resulting consensus tree was further modified using FigTree v1.4.3 (Rambaut, 2014).

### Calibration of divergence time

The software jModel Test V.2 (Posada, 2008) was initially implemented on Cipres Science gateway (Miller *et al.*, 2010) to measure the best fitting nucleotide substitution model in Akaike information criterion (AIC) (Akaike, 1974) for the data set. The software BEAST 2.0 (Drummond and Rambaut, 2007) was used to carry out the divergence time estimation in Bayesian framework. According to the selected model, TVM+I+G, the rate (AC= 1.147, AG=1.057, AT=-0.216, CG=0.729, CT=1.057 and GT=1.0) and shape parameters were assigned (Zharkikh, 1994). The uncorrelated log normal clock (Drummond *et al.*, 2006) was used to compensate for the variation of evolutionary rates across the lineages using a lognormal prior with 0.001 mean and 1 standard deviation as well as Birth death model as tree-prior to account for speciation and extinction. A total of eight *S. dulcamara* seed fossils (7.3- 2.6 MYA) from Pliocene epoch (Mai, 1988; Reid and Reid, 1907; Szafer, 1946) and a seed fossil *S. nigrum* (5.3 – 11.6 MYA) (van der Beek and van der Burgh, 1987) were used to calibrate two nodes of the time tree developed in the present study. The Time to Most Recent Common Ancestor (TMRCA) for crown of the tree was calibrated using a lognormal prior with 0.001 mean, 0.1 standard deviation and 13.3 MYA offset. The TMRCA was calibrated for root of the tree to be 17 MYA using a lognormal prior. All the node calibration except the fossil data were done as described in Särkinen *et al.* (2013). The Markov Chain Monte Carlo (MCMC) was performed for 100 million generations with a 10% burn-in to achieve maximum chain convergence. The resulting log file was analyzed to assess the Effective Sample Size

**Table 1:** The matk sequence data used in this study.

Accession No.	Species name	Sequence length	Reference
NC_030207*	<i>Solanum melongena</i>	1521	NCBI**
KX258741	<i>S. hispidum</i> V. Gonabatu	936	Jeyakumar et al., 2016
KX258742	<i>S. virginianum</i> V. Katuwelbatu	936	Jeyakumar et al., 2016
KX258744	<i>S. nigrum</i> V. Small Kalukenweriya	936	Jeyakumar et al., 2016
KX258745	<i>S. torvum</i> V. Thibbatu	936	Jeyakumar et al., 2016
KX258747	<i>S. melongena</i> V. Large Elabatu	936	Jeyakumar et al., 2016
KX258746	<i>S. trilobatum</i> V. Welthibbatu	936	Jeyakumar et al., 2016
KX258749	<i>S. melongena</i> V. Round Elabatu	936	Jeyakumar et al., 2016
KX258748	<i>S. melongena</i> V. Long Elabatu	936	Jeyakumar et al., 2016
KX258750	<i>S. melongena</i> V. Round Elabatu	936	Jeyakumar et al., 2016
KX258751	<i>S. melongena</i> V. Wambatu	936	Jeyakumar et al., 2016
KX258752	<i>S. melongena</i> V. Wambatu	936	Jeyakumar et al., 2016
KX258753	<i>S. violaceum</i> V. Thithathibbatu	936	Jeyakumar et al., 2016
KX258754	<i>S. pubescens</i> V. Walthibbatu	936	Jeyakumar et al., 2016
EU983552	<i>S. tudununggae</i>	1530	Martine et al., 2009
EU983553	<i>S. dioicum</i>	1530	Martine et al., 2009
EU983554	<i>S. dioicum</i>	1530	Martine et al., 2009
EU983555	<i>S. petraeum</i>	1530	Martine et al., 2009
EU983556	<i>S. carduiforme</i>	1530	Martine et al., 2009
EU983557	<i>S. carduiforme</i>	1530	Martine et al., 2009
EU983568	<i>S. sejunctum</i>	1530	Martine et al., 2009
EU983558	<i>S. carduiforme</i>	1530	Martine et al., 2009
EU983559	<i>S. petraeum</i>	1530	Martine et al., 2009
EU983560	<i>S. leopoldense</i>	1530	Martine et al., 2009
EU983561	<i>S. spLongini</i>	1530	Martine et al., 2009
EU983562	<i>S. chippendalei</i>	1530	Martine et al., 2009
EU983563	<i>S. diversiflorum</i>	1530	Martine et al., 2009
EU983564	<i>S. clarkiae</i>	1530	Martine et al., 2009
EU983565	<i>S. melanospermum</i>	1530	Martine et al., 2009
EU983566	<i>S. oedipus</i>	1530	Martine et al., 2009
EU983567	<i>S. heteropodium</i>	1530	Martine et al., 2009
EU983569	<i>S. sejunctum</i>	1530	Martine et al., 2009
EU983570	<i>S. asymmetriphyllum</i>	1530	Martine et al., 2009
EU983572	<i>S. stupefactum</i>	1530	Martine et al., 2009
EU983573	<i>S. aculeastrum</i>	1530	Martine et al., 2009
EU983574	<i>S. linnaeanum</i>	1530	Martine et al., 2009
EU983576	<i>S. elaeagnifolium</i>	1530	Martine et al., 2009
EU983571	<i>S. hoplopetalum</i>	1530	Martine et al., 2009
EU983575	<i>S. cinereum</i>	1530	Martine et al., 2009
AM087200	<i>S. lycopersicum</i>	1530	Kahlau et al., 2006
DQ231562	<i>S. tuberosum</i>	1530	Chung et al., 2006
DQ347958	<i>S. bulbocastanum</i>	1530	Daniell et al., 2006
DQ347959	<i>S. lycopersicum</i>	1530	Daniell et al., 2006
DQ386163	<i>S. tuberosum</i>	1530	Gargano et al., 2005
KM489054	<i>S. commersonii</i>	1530	NCBI
JF772170	<i>S. tuberosum</i>	1530	Xuet et al., 2011
JF772171	<i>S. tuberosum</i>	1530	Xuet et al., 2011
KC535796	<i>S. clavatum</i>	1282	NCBI

Accession No.	Species name	Sequence length	Reference
KC535797	<i>S. nigrum</i>	1282	NCBI
KC535798	<i>S. aculeatissimum</i>	1282	NCBI
KC535799	<i>S. sisymbriifolium</i>	1282	NCBI
KC535801	<i>S. aethiopicum</i>	1281	NCBI
KC535802	<i>S. torvum</i>	1282	NCBI
KT176602	<i>S. carolinense</i>	1530	Aust <i>et al.</i> , 2015
KT176603	<i>S. rostratum</i>	1530	Aust <i>et al.</i> , 2015
KT176604	<i>S. triflorum</i>	1530	Aust <i>et al.</i> , 2015
KY419708	<i>S. berthaultii</i>	1530	NCBI
Z11741	<i>S. tuberosum</i>	1530	NCBI
KU682719	<i>S. melongena</i>	1521	NCBI
KU679363	<i>S. pyracanthu</i>	1520	NCBI
FJ395444	<i>S. dulcamara</i>	851	NCBI
JN894601	<i>S. dulcamara</i>	859	NCBI
FN668838	<i>S. dulcamara</i>	846	NCBI
KP117020	<i>S. cheesmaniae</i>	1530	Wu, 2016
KP117021	<i>S. chilense</i>	1530	Wu, 2016
KP117022	<i>S. galapagense</i>	1530	Wu, 2016
KP117023	<i>S. habrochaites</i>	1530	Wu, 2016
KP117024	<i>S. lycopersicum</i>	1530	Wu, 2016
KP117025	<i>S. neorickii</i>	1530	Wu, 2016
KP117026	<i>S. peruvianum</i>	1530	Wu, 2016
KP117027	<i>S. pimpinellifolium</i>	1530	Wu, 2016
KP331414	<i>S. lycopersicum</i>	1530	NCBI
KP747439	<i>Jaltomata bicolor</i>	1530	Carrizo Garcia <i>et al.</i> , 2016

\* Reference sequence

\*\*NCBI: Published in National Center for Biotechnology Information

(ESS) and chain convergence in Tracer v1. 4 (Rambaut and Drummond, 2007). The maximum clade credibility (MCC) tree was visualized and further edited in FigTree v1.4.3 (Rambaut, 2014).

## RESULTS AND DISCUSSION

### Phylogenetic Analysis

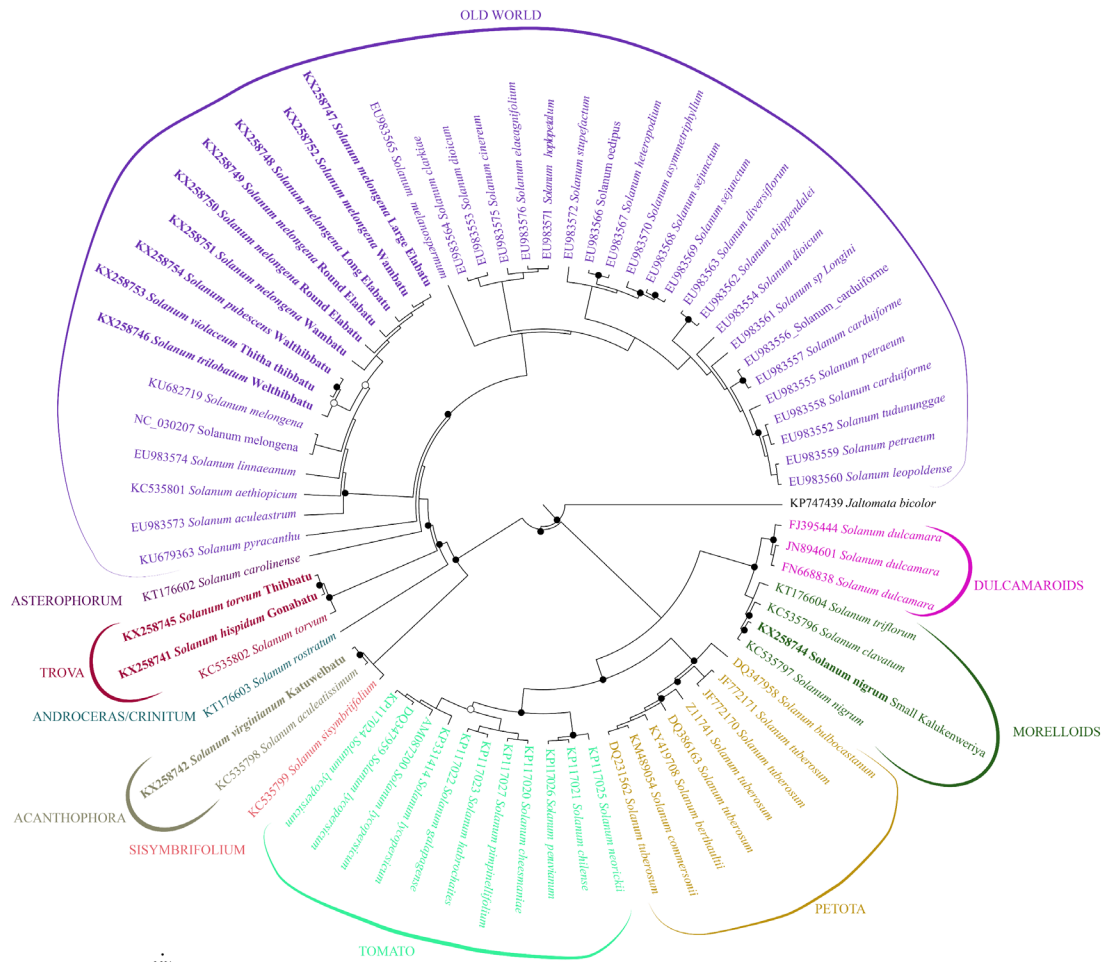
All the *Solanum matK* sequences obtained from BLAST searches (E value greater than 98) with Sri Lankan *Solanum* spp. were included in this analysis (Table 1). The reading frame of *matK* locus contained 1530 base pairs (bp). The sequences less than 50% missing bp were excluded from the analysis as Maximum Likelihood (ML) inference can be deluded under the presence of large number of missing data in a non-random passion (Xi *et al.*, 2015). Although some of thesequences included in the analysis contained missing data, both ML majority rule consensus tree and Bayesian Maximum Clade Credibility (MCC) tree branched out similarly at major nodes (Figures 1 and 2). The posterior probability (PP) values and bootstrap (bs) values were higher at these nodes while few nodes towards the tips that were present in the ML collapsed in MCC tree, yielding low PP values even though they had higher bs values.

The initial Maximum Parsimony tree had a well resolved

topology given by the Heuristic search. The resolution of the tree was enough to use it as the starting tree topology in RAxML. The ML tree separated the analyzed *Solanum* species (Table 1) into a total of 10 phylogenetic groups and they were congruent with phylogenetic constructions obtained using different genes in previously published work (Weese and Bohs, 2007; Särkinen *et al.*, 2013; Bohs and Olmstead, 1997). The 13 Sri Lankan *Solanum* species were separated into four phylogenetic groups namely Old world, Morelloids, Acanthophora and Trova (Olmstead and Bohs, 2006).The robustness of these clusters was displayed by higher node support with PP values larger than 95% and bs values of 100 (Figure 1). Since our analysis missed some of the phylogenetic groups of *Solanum* genus, topology at the clade connection was not well resolved. Thus sequences of the other DNA barcoding loci as well as the *matK* sequences for more *Solanum* spp. must be incorporated in future studies to identify the robust relationships with higher resolution. However, in the present analysis, major relationships between prominent sister clades remained constant (Särkinen *et al.*, 2013).

### Calibration of Divergence Time and Origination Events

The MCMC chains ran in the tree search converged maximally at 10% burn-in value. The initial 100,000 trees



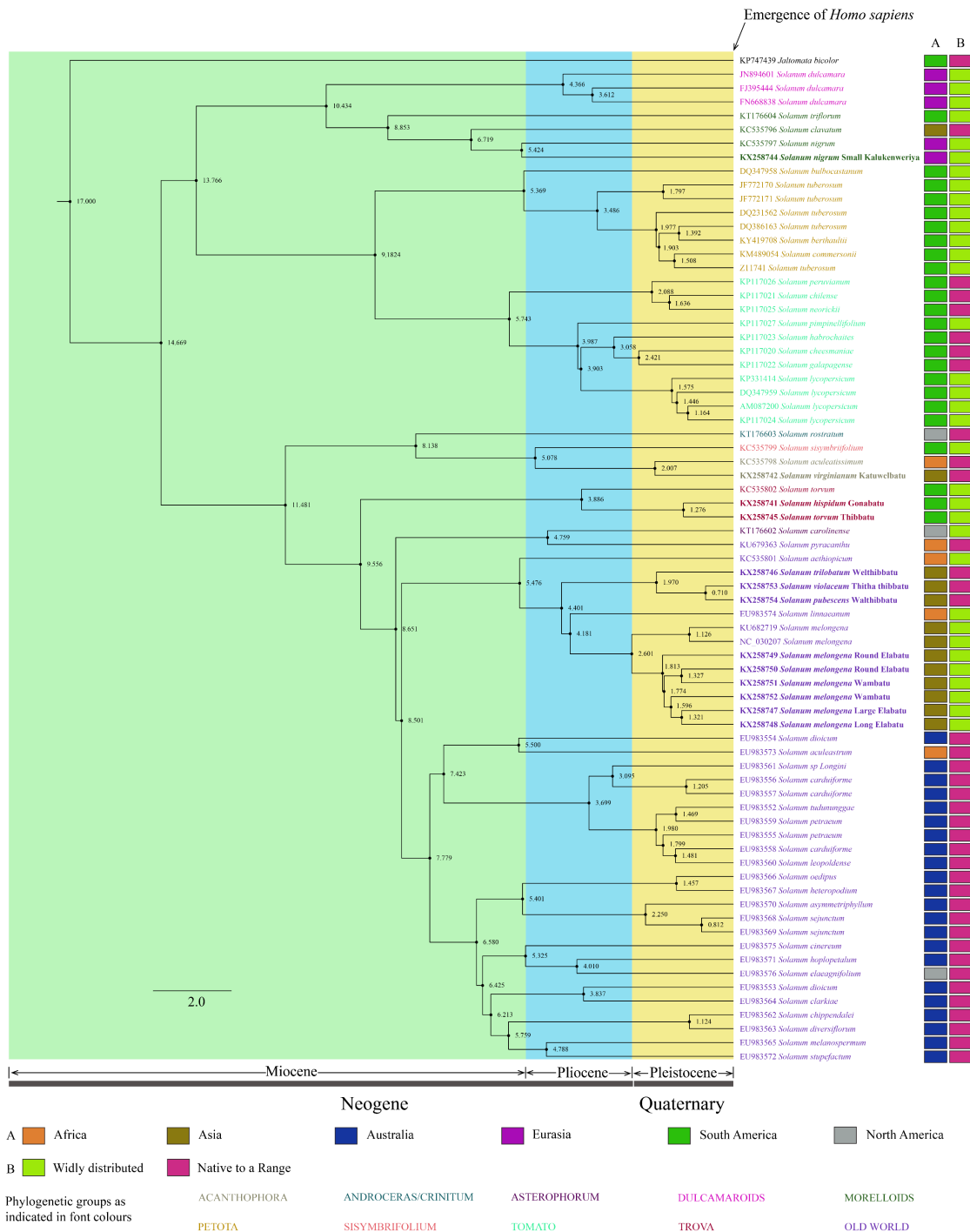
**Figure 1 :** The majority rule consensus tree constructed on Maximum Likelihood (ML) framework. The major phylogenetic groups of *Solanum* spp. included in this analysis are presented. The node support values are given on its respective nodes indicated either in black dots (over 95 posterior probability and 100 bootstrap value) or white dots (over 85 posterior probability and 95 bootstrap value). The nodes containing posterior probabilities lower than 85 and bootstrap values less than 90 are not shown as dots. The Operational Taxonomic Units of Sri Lankan *Solanum* spp. are indicated in bold letters.

were discarded as burn-in. The trees were probed from stationary distribution in tree space as all the ESS values calculated were higher than 200, thus inferring our MCMC chains were run long enough (10 million runs) to sample independently and to avoid poor mixing. The divergence time for each node was calibrated according to the TMRCAs calibrations.

The three wild *Solanum* species in Sri Lanka, *S. pubescens*, *S. violaceum* and *S. trilobatum*, formed a monophyletic group which was a sister to *S. melongena* group. This group clustered together with *S. linnaeanum* forming a well resolved monophyletic group with clades that are saturated with native Australian *Solanum* spp. The age of the recent common ancestor of this monophyletic group was calculated as 7.779 MYA [95% highest posterior density (HPD) at 3.158- 10.236 MYA]. Interestingly Sri Lankan *S. hispidum* and *S. torvum* together formed a sister clade with Indian *S. torvum*. The divergence time between these two sister *Solanum* spp. and their Indian descendent was calculated to be 3.886 MYA (95% HDP values of 0.148 – 8.621 MYA). The uncorrelated relaxed molecular clock estimated that *S. nigrum* inhabiting in Sri Lanka was descendent from an Indian *S. nigrum* 5.424 MYA (95% HPD value of 5.30– 5.88 MYA). All *S. melongena*, *S. pubescens*

*Vern. wal-thibbatu*, *S. violaceum* Vern. thibtha thibbatu and *S. trilobatum* Vern. wel-thibbatu were clustered under the phylogenetic group Old world. This group is closely related to the phylogenetic group *Sisymbriifolium* sharing a common ancestor 1.556 MYA before in Miocene epoch.

*S. virginianum* vern. Katuwelbatu which is native to South Asian region (Pandey et al., 2008) was cladded with *S. aculeatissimum* which is known to be a wild *Solanum* species in India. However, the native range of *S. aculeatissimum* is believed to be from Africa (Hepper and Jaeger, 1986). It is permissible to argue that the mammalian distribution due to late neogenic climatic changes (Cerling et al., 1997) and formation of land connections (Qiu et al., 2001) may have introduced African floral species to Asia. Especially extinct herbivore mammals such as *Stegodon* were known to have migrated from Africa to Asia 4 MYA (Ao et al., 2016), which might have been a perfect vector for *Solanum* species to distribute from Afrotropical region to Oriental region. The present analysis confirms that the African *S. aculeatissimum* diverged 2.007 MYA from its Asian sister taxa in Pleistocene epoch which is in parallel to mammalian migration events (Cerling et al., 1997). After introduction of *S. aculeatissimum* to Oriental region, it might have radiated into Asian region giving rise to *S. virginianum*. The species



**Figure 2 :** The Maximum Clade Credibility Tree (MCC) showing the divergence time and geographic distribution of tips (operational taxonomic units). The geological time periods parallel to our divergence dating are given below the tree and tree is colored respectively. The color key below the tree indicates the native range (A) and the current state of distribution (B) of the respective *Solanum spp.* in the world. The nodes of the tree are labeled with the divergence time (in Million years ago). The Operational Taxonomic Units of Sri Lankan *Solanum spp.* are indicated in bold letters

*S. nigrum* Vern. Small Kalukenweriya was claded with an Indian *S. nigrum*, however, they got split from each other 5.42 MYA in Miocene epoch. There were frequent sea level fluctuations in Pleistocene epoch (*i.e.* ice age) to open up gateways to connect Sri Lanka with India (Rohling *et al.*, 1998) however according to the present analysis *S. nigrum* Vern. Small Kalukenweriya would have been introduced to Sri Lanka before Pleistocene ice age probably through

over water LDD. The mixing of *S. nigrum* population from India with the Sri Lankan population has not occurred since this was a very early divergent event. Moreover, the phylogenetic group Trova contained three species in the current analysis. Out of these three species, the two Sri Lankan species, *S. hispidum* Vern. Gonabatu and *S. torvum* Vern. Thibbatu were found to be sister taxa diverged 2.097 MYA in Pliocene epoch. This group was claded with *S.*

*torvum* (KC535802) isolated from India implying that *S. hispidum* and *S. torvum* were split from a common ancestor in India and then colonized to Sri Lanka. *S. hispidum* would have been evolved from *S. torvum* due to a major speciation event in Pleistocene epoch. It is logical to argue that *S. hispidum* is a more recent species in nightshade family. Similar pattern is shown in Sri Lankan endemic vertebrate and invertebrate species where they share similar morphological traits although they keep endemic genetic structures by maintaining unique haplotypes within the island irrespective of numerous chances to mix up due to the frequent formations of land bridges at low sea levels (Bossuyt et al., 2004). It provides insights into the fact that these two *S. nigrum* species evolving into two new species in India and Sri Lanka, but further morphological and molecular phylogenetic analyses are required to resolve their taxonomic status.

It is evident from the present analysis that the diversity of the Sri Lankan *Solanum* species may also be closely aligned to Australian *Solanum* spp. All *S. melongena*, *S. pubersena*, *S. violaceum*, *S. trilobatum*, *S. torvum*, *S. hispidum* and *S. virginianum* of old world clade (Särkinen et al., 2013; Olmstead et al., 2008) deeply rooted with a clade that is saturated with numerous native Australian *Solanum* species implying that they would have been distributed across Lydekker's and Wallace's Lines explained in Toussaint et al., (2015). Although as described in "out of India" hypothesis the Gondwana fragmentation has moved many taxa from Africa, through India to South East Asia (Ali et al., 2013), the common ancestor of this Australian-Asian clade dates back to more recent ages. Thus, the only passageway to mix Asian flora with Australian flora is through South East Asia. The Southeast Asian floral diversity is known to originate from east Eurasia and Australia. Large migratory events have been recorded in mid Miocene epoch soon after the Sunda plate collided with Australian plate (Morley, 1998). Plant dispersal LDD by the migrating birds from routes such as East Asian-Australasian Flyway is also possible. Moreover the dispersal routes in late Neogene may have opened up passage ways for animal distribution from South Asia to South East Asia.

## CONCLUSION

The current analyses for the first time describes the phylogenetic positions, origin and origination events of 13 Sri Lankan wild and naturalized *Solanum* spp. The *Solanum* diversity in Sri Lanka is strictly connected with the Indian *Solanum* diversity although the origins of these clades are largely differ from each other. With the molecular dating results we identified two major means of diversification events namely through land bridges of Pleistocene ice age and overwater LDD. Although same species name is given the *Solanum* species in India and Sri Lanka, the geographical isolation events were dated back to Pleistocene epoch and before, thus a comprehensive systematic and morphological revision is needed to distinguish between Indian and Sri Lankan *Solanum* spp. The worldwide mixing of Solanaceae germplasm

due to continental and regional bridges and migratory birds would have played a significant role in the groups' history. The disturbance to the migratory behavior of birds caused by developmental activities and environmental pollution, habitat fragmentation, global warming may therefore hinder the further reshaping of these germplasm distribution patterns. In the future if these dispersive forces are greatly weakened, the natural evolution of these species would slow down dramatically and get restricted to smaller conservation patches in bio-geographical regions.

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