The Journal of Agricultural Sciences - Sri Lanka
Vol 16, No. 1, January, 2021. Pp 1-18
http://doi.org/10.4038/jas.v16i1.9179

Potentila indica (Andr.) nov. (wild strawberry) in Sri Lanka is Restricted to a Small Climatic Envelop Urging Strict Conservation

L.T. Ranaweera[1], H.A.C.R. Perera[1,2], W.W.M.U.K. Wijesundara[1], R.M.S.K. Rathnayake[1], W.M.D.A. Wijesundara[1], H.M.T.N. Senavirathna[1], R.W.K.M. Senevirathna[1], C.K. Weebadde[3], J.F. Hancock[4] and S.D.S.S. Sooriyapathirana[1,2*]

Date Received: 26th February 2020 / Date Accepted: 14th August 2020

ABSTRACT

Purpose : *The nomenclature of the wild strawberries inhabited in Sri Lanka is ambiguous. In Sri Lanka, this species is still named Duchesnea indica which needs a revision. Wild strawberries grow well in natural habitats of upcountry in Sri Lanka. Since the commercial strawberry cultivations gain a popularity in upcountry, the studies on wild strawberry is essential for crop improvement and management.*

Research Method : *In the present study, we conducted extensive field sampling followed by a phylogenetic analysis with the DNA barcoding markers ITS and, trnL-F by using a representative sample of wild strawberry plants in Sri Lanka. The distribution of the species was identified using maximum entropy modeling approaches.*

Findings : *Sri Lankan wild strawberry got placed at subtribe: Potentilla, and clade: Reptans and show a shallow divergence with the species Potentilla indica reported. Thus, we reposition the genus of wild strawberries in Sri Lanka from Duchesnea to Potentilla and hereafter name it as P. indica. The niche model analysis predicted a highly restricted distribution of Sri Lankan wild strawberry in Nuwara-Eliya district over an area of 166.36 km^2 in the altitude range of 1546 - 2524 m in a small climatic envelop highlighting the need for urgent conservation measures.*

Research Limitations : *The pop-set for available in literature of P. indica is limited for comparison. Extensive studies based on DNA sequencing is needed for further validation.*

Originality / Value : *Taxonomy, narrow distribution, need of conservation, and phylogenetic distance to Fragaria chiloensis, a progenitor species of cultivated strawberry, are defined for Sri Lankan wild strawberries.*

Keywords: *Duchesnea indica, ecological niche modeling of Potentilla, Indian strawberry, Reptans, wild strawberry in Sri Lanka*

INTRODUCTION

In Sri Lanka, the wild strawberries (also known as Indian or mock strawberries) are named under the genus *Duchesnea* (Focke, 1888) in the *Revised Handbook to the Flora of Ceylon* (Wadhwa *et al*., 2000). The genus of the wild strawberries in Sri Lanka was later changed to *Fragaria* (Andrews, 1807) and then to *Potentilla* (Wolf, 1908). However, later the taxonomists have agreed to keep wild strawberries in Sri Lanka in the genus *Duchesnea* which is considered more of a transitional group between *Fragaria* and

---

[1] *Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, 20400, Peradeniya, Sri Lanka.*

sunethss09@gmail.com

[2] *Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.*

[3] *Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan, USA.*

[4] *Department of Horticulture, Michigan State University, East Lansing, Michigan, USA.*

http://orcid.org/0000-0002-5592-1742

Potentilla (Wadhwa *et al*., 2000). However, the current nomenclature of wild strawberries in Sri Lanka (*Duchesnea indica*) is solely dependent on morphology, and no molecular systematic studies were conducted to support the taxonomic status reported in *Revised Hanbook to the Flora of Ceylon* (Wadhwa *et al*., 2000).

Potentilleae is one of the taxonomically ambiguous tribes in family Rosaceae. The taxonomy of Potentilleae has been consistently changing over the recent years in world-wide studies (Feng *et al*., 2017). In the study conducted by Eriksson *et al.* (1998), described *Potentilla* as a non-monophyletic genus, combining previously recognized genera such as *Duchesnea*, *Horkelia* and *Ivesia* under *Potentilla*. The most recent systematic studies using molecular data (Potter *et al*., 2007; Dobeš and Paule, 2010; Töpel *et al*., 2011) support the monophyly of the tribe Potentilleae as defined by a study done by Eriksson *et al.* (2003) and have recovered three main clades within this tribe. Töpel *et al.* (2011) compared a nuclear and chloroplast gene-based tree to identify topological incongruences that may indicate the hybridization events within the genus *Potentilla*. The recent phylogenetic studies on Potentilleae were carried out using combined analysis of chloroplast and nuclear markers to further resolve the taxonomic ambiguities (Wadhwa *et al*., 2000). Once the taxonomic ambiguities are resolved, it is essential to assess and demarcate the conservation priorities of the available germplasm.

The prediction of the ecological distribution of a species is one of the critical aspects when setting up the conservation priorities for a species (Faith, 1996; Geneletti, 2004). The occurrence of a species can be modeled and predicted using an array of methods (Peterson, 2003; Wiens and Graham, 2005; Elith *et al*., 2006; Kozak *et al*., 2008; Peterson *et al*., 2011). The niche modeling using the species-occurrence/location data is popular as the locations of presence are well documented or easy to record for a particular species (Elith *et al*., 2006; Hernandez *et al*., 2006; Phillips and Dudík, 2008; Wisz *et al*., 2008). In the model calibration, we can use presence-locations and set of environmental variables. However, when using the presence-locations of species, some areas could be oversampled or under-sampled leaving a bias (Hortal *et al*., 2008; Anderson and Gonzalez, 2011). Thus the over-fitting of the models could happen when the model is incorporated with environmental variables (Anderson and Gonzalez, 2011). Another common problem is the over-fitting of model to noise when the number of predictor variables become too high. This confounds the ultimate niche of the species from the predicted niche. Such issues can be avoided using maximum entropy modeling methods (Phillips *et al*., 2004; Phillips *et al*., 2006). Through identification of the niche of a species; the distribution pattern, as well as the climatic factors supporting the distribution can be tested (Geneletti, 2004). Currently distribution modeling is used as a versatile tool to identify the populations and species that are climatically restricted to device the conservation priorities (Senevirathne *et al*., 2018; Wijayathilaka *et al*., 2018).

In the present study, we aimed to determine the phylogenetic position of wild strawberries inhabited in Sri Lanka. Since the morphology of wild strawberries has been well described, we conducted a molecular systematic analysis using nuclear genome specific *ITS* and chloroplast genome specific *trnL-trnF* markers to find the correct phylogenetic position in comparison to other species in the tribe Potentilleae. Then we conducted an extensive field survey to determine the distribution of wild strawberries to get an understanding of the niche dimensions using maximum entropy modeling and introduce conservational priorities.

MATERIALS AND METHODS

Field Survey and Sample collection

The species range and the habitat information were collected from the revised handbook to the Flora of Ceylon [Wadhwa *et al*., 2000] and Royal Botanical Garden, Peradeniya, Sri Lanka. According to the previous species occurrence records, the species was concentrated on central hills of Sri Lanka. Thus, numerous field surveys were conducted across the central hills in Sri Lanka. Once the species was found in a particular location, the GPS coordinates were recorded, and three to five leaf samples were collected. The collected leaf samples were stored in -20 °C until the DNA extraction.

PCR and DNA sequencing

The DNA sequencing was performed for two DNA markers namely *ITS* (*ITS1*-TCCGTAGGTGAACCTTGCGG, *ITS4*-TCCTCCGCTTATTGATATGC) (White *et al*., 1990) and *trnL-trnF* (*F-CGAAATCGGTAGACGCTACG, R-ATTTGAACTGGTGACACGAG*) (Taberlet *et al*., 1991). The PCR was carried out in 50 µL of total reaction mixture comprised of 25 µL of Go Taq® Green Master Mix, 2× Green Go Taq® Reaction Buffer (pH 8.5), 400 µL dATP, 400 µL dGTP, 400 µL dCTP, 400 µL dTTP, 3 mM $MgCl_2$, 1 µL of forward primer, 1 µL of reverse primer, template DNA, and nuclease free water. The PCR cyclic process conducted in the Thermal Cycler (Takara, Otsu Shiga, Japan) was consisted of initial denaturation at 94 °C for 5 mins, followed by 35 cycles including 30 sec of denaturation at 94 °C, 1 min annealing at 55 °C and 2 mins extension at 72 °C followed by final extension of 10 mins at 72 °C (Senavirathna *et al*., 2020). The PCR products were visualized using 1 % agarose gel electrophoresis and purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany). The purified products were subjected to cycle sequencing using ABI Genetic analyzer 3500 (Applied Bio Systems®).

Phylogenetic analysis

The raw sequence reads were edited, and consensus sequences were constructed using the MEGA software v.7 (Kumar *et al*., 2016). All the consensus sequences generated in the present study were submitted to the GenBank under the accession numbers MK605458-MK60573 and MK587724-MK587739. To examine the precise phylogenetic position of the study species, we adapted the phylogeny constructed in a study by Feng *et al*. (2017) (Table 01) using both nuclear and plastid genetic markers. We attempted to reconstruct the tribe: Potentilleae phylogeny using the sequences generated in the present study and sequences reported in previous studies (Feng *et al*., 2017). We constructed the multiple sequence alignments separately for *ITS* and *trnL-trnF* in MEGA v7. A partition homogeneity (ILD) test (Planet, 2006) was carried out to check the phylogenetic concordance of *ITS*, *trnL-trnF*, and *ITS*+*trnL-trnF* (combined) datasets. To define the best partition scheme and the best model of evolution for each data matrix, we implemented the analysis in PartitionFinder 2 (Lanfear *et al*., 2016). In PartitionFinder 2, we implemented the corrected Akaike information criteria (Cavanaugh, 1997) (AICc) using hcluster (Lanfear *et al*., 2014) and Kmeans (Frandsen *et al*., 2015) algorithms for model selection.

We carried out the tree search in both Maximum Likelihood (ML) and Bayesian frameworks. The ML tree search was implemented using the rapid bootstrap algorithm (Stamatakis *et al*., 2008) for 1000 iterations in RAxML-VI-HPC workflow (Stamatakis, 2006) using CIPRES supercomputer (Miller *et al*., 2010). We used the GTRGAMMA model to assess the evolutionary process of the partitions defined in the analysis. Using the bipartition option of the RAxML, we performed a consensus algorithm to conclude all the bootstrap bipartitions into a single tree topology. The best tree resulted with highest –log likelihood value was used as the tree topology to imprint the bootstrap values. We also constructed a phylogenetic tree based on the Bayesian framework by employing MrBays (Huelsenbeck and Ronquist, 2001) in the CIPRES platform (Miller *et al*., 2010). During this tree run, differences were assessed separately, which were achieved by the partition log file that contains the model information and partition criteria. Two hot and cold chains of Markov chain Monte Carlo (MCMC) were run for 60 million generations to probe the trees in tree-space. The final 50% majority rule consensus tree was drawn using the trees probed after maximum chain convergence. However, the initial 25% of the trees were discarded as burn-in. The chain convergence and independent tree sampling were checked by assessing Effective Sample Size (ESS) in TRACER v1.4 (Rambaut and Drummond, 2007). Finally, all the trees constructed in the study were visualized and edited using FigTree v1.4.3 (Rambaut, 2014).

Table 01: The metadata of the sequences used for the phylogenetic analysis

Taxa	Voucher	Accession Number		Reference
		ITS	*trnL/trnF*	
Potentilla indica	DMB61	MK587724	*MK605458*	This study
Potentilla indica	DMB62	MK587725	MK605459	
Potentilla indica	DMB63	MK587726	MK605460	
Potentilla indica	DMB64	MK587727	MK605461	
Potentilla indica	DMB65	MK587728	MK605462	
Potentilla indica	DMB66	MK587729	MK605463	
Potentilla indica	DMB67	MK587730	MK605464	
Potentilla indica	DMB68	MK587731	MK605465	
Potentilla indica	DMB69	MK587732	MK605466	
Potentilla indica	DMB70	MK587733	MK605467	
Potentilla indica	DMB71	MK587734	MK605468	
Potentilla indica	DMB72	MK587735	MK605469	
Potentilla indica	DMB73	MK587736	MK605470	
Potentilla indica	DMB74	MK587737	MK605471	
Potentilla indica	DMB75	MK587738	MK605472	
Potentilla indica	DMB76	MK587739	MK605473	
*Alchemilla_cryptantha*_Steud._ex_A._Rich	T._Eriksson_914_(S)	FJ356153	FJ422283	Feng *et al.* (2017)
*Alchemilla_mollis*_(Buser)_Rothm.	T._Eriksson_s.n.(S)	AJ511769	AJ512218	
*Alchemilla_pentaphyllea*_L.	B._Gehrke_BG-E400_(ZH)	FJ356154	FJ422284	
*Argentina_anserina*_(L.)_Rydb.	Eriksson_&_Smedmark_44_(SBT)	FN430824	FN561752	
*Argentina_glabriuscula*_(T.T._Y€u_&_C.L.Li)_Sojak_53	Feng_53_(HIB)	KF954763	KJ020639	
*Argentina_glabriuscula*_(.T._Y€u_&_C.L.Li)_Sojak_57	Feng_57_(HIB)	KF954764	KJ020640	
*Argentina_leuconota*_(D._Don T)_Sojak	Feng_108_(HIB)	KF954771	KJ020641	
*Argentina_lignosa*_(Willd._in_D.F.K.Schltdl.)_Sojak	M._Topel_MA132_(GB)	FJ356171	FJ422299	
*Argentina_micropetala*_(D._Don)_Sojak	Feng_8_(HIB)	KF954771	KJ020641	
*Argentina_microphylla*_(D._Don)_Sojak	MA_144_(GB)	FN430809	FN556412	
*Argentina_peduncularis*_(D._Don)_Sojak	MA_173_(GB)	FN430820	FN561742	
*Argentina_phanerophlebia*_(Yu_&_Li)_Feng_&_Wang	Feng_6_(HIB)	KF954770	KJ020642	
*Argentina_songzhuensis*_T._Feng_&_H.Wang	Feng_58_(HIB)	KF954766	KJ020638	
*Argentina_stenophylla*_(Franch.)_Sojak	KGB_299_(GB)	FN555607	FN561738	
*Argentina_tapetodes*_(Sojak)_Sojak	Feng_93_(HIB)	KF954769	KP875330	
*Argentina_turfosa*_(Hand.-Mazz.)_Sojak	Feng_55_(HIB)	KF954768	KP875331	
*Chamaecallis_perpusilloides*_(W.W._Sm.) Smedmark	Feng_52_(HIB)	KP875287	KP875336	
*Chamaecallis_perpusilloides*_(W.W._Sm.) Smedmark	Feng_68_(HIB)	KP875288	KP875335	
*Chamaerhodos_mongholica*_Bunge	E._Rosenius_1028_(S)	FJ356155	FJ422285	
*Dasiphora_davurica*_(Nestl.)_Kom._&_Aliss	M._Lundberg_24_(S)	FJ356159	FJ422287	
*Dasiphora_fruticosa*_(L.)_Rydb.	Feng_103_(HIB)	KP875290	KP875337	
*Dasiphora_glabra*_(G._Lodd.)_Sojak	Feng_120_(HIB)	KP875289	KP875338	
Dasiphora_parvifolia(Fisch._ex_Lehm.)_Juz.	Feng_119_(HIB)	KF954762	KJ020646	
*Dasiphora_phyllocalyx*_Juz.	T._Eriksson_757_(S)	FJ356160	FJ422288	
*Drymocallis_corsica*_(Soleirol_ex_Lehm.) Kurtto	M._Lundberg_13_(S)	FJ356161	FJ422290	
*Drymocallis_glutinosa*_Rydb.	M._Lundberg_5_(S)	FJ356162	FJ42229	
*Drymocallis_rupestris*_(L.)_Sojak	M._Lundberg_6_(S)	FJ356163	FJ422292	
*Fragaria_chiloensis*_(L.)_Mill.	M._Lundberg_14_(S)	FJ356164	FJ422293	
*Fragaria_orientalis*_Losinsk.	Feng_107_(HIB)	KP875292	KP875334	
*Fragaria_viridis*_Weston	M._Lundber_16_(S)	FJ356166	FJ422295	

*Horkelia_bolanderi*_A._Gray	Eriksson_s.n._(SBT)	FN430789	FN556395	Feng *et al.* (2017)
*Ivesia_kingii*_S._Watson	J._L._Reveal_et_al.#4782_ (GB)	FN430787	FN561735	
*Potaninia_mongolica*_Maxim	Norlindh_&_Ahti_10348_(S)	AM286742	AM286743	
*Potentilla_alba*_L.	MA_122_(GB)	FN430774	FN556379	
*Potentilla_alchemilloides*_Lapeyr.	A._&_A-L._Anderberg_26_ (S)	FJ356168	FJ422297	
*Potentilla_argentea*_L.	MA_143_(GB)	FN430808	FN561750	
*Potentilla_biflora*_Willd._ex_Schltdl._102	Feng_102_(HIB)	KP875301	KP875329	
*Potentilla_caulescens*_L.	MA_133_(GB)	FN430819	FN556399	
*Potentilla_chinensis*_Ser.	Feng_110_(HIB)	KP875298	KP875319	
*Potentilla_clandestina*_Sojak	Feng_25_(HIB)	KP875308	KP875327	
*Potentilla_conferta*_Bunge	Feng_127_(HIB)	KP875296	KP875320	
*Potentilla_discolor*_Bunge	Feng_118_(HIB)	KP875299	KP875321	
*Potentilla_griffithii*_Hook._f.	Feng_44_(HIB)	KP875293	KP875316	
*Potentilla_indica*_(Andrews)_Wolf	Feng_138_(HIB)	KP875300	KP875314	
*Potentilla_kleiniana*_Wight_&_Arn.	Feng_139_(HIB)	KP875294	KP875315	
*Potentilla_multifida*_(Tausch)_Wolf	Feng_124_(HIB)	KP875295	KP875318	
*Potentilla_purpurea*_(Royle)_Hook._f.	Feng_64_(HIB)	KP875307	KP875326	
*Potentilla_reptans*_L.	MA_131_(GB)	FN430815	FN561728	
*Potentilla_sischanensis*_Bunge_ex_Lehm.	Feng_112_(HIB)	KP875297	KP875322	
*Potentilla_stolonifera*_Lehm._ex_Ledeb	BE_1382:_1_(GB)	FN430814	FN556420	
*Potentilla_suavis*_Sojak	Feng_37_(HIB)	KP875305	KP875323	
*Potentilla_tenuis*_(Hand-Mazz.)_Sojak	Feng_26_(HIB)	KP875306	KP875325	
*Potentilla_tetrandra*_(Hook._f.)_Bunge_89	Feng_89_(HIB)	KP875303	KP875328	
*Potentilla_tetrandra*_(Hook._f.)_Bunge_97	Feng_97_(HIB)	KP875304	KP875324	
*Rosa_majalis*_Herrm.	T._Eriksson_641_(GH,_S)	U90801	AJ512229	
*Sibbaldia_parviflora*_Willd.	M._Lundberg_4_(S)	FJ356174	FJ422302	
*Sibbaldia_procumbens*_L.	Feng_131_(HIB)	KP875310	KP875339	
*Sibbaldia_procumbens*_L	Feng_S4_(HIB)	KP875309	KP875341	
*Sibbaldia_semiglabra*_C._A._Mey.	J._Klackenberg82062-11_(S)	FJ356175	FJ422303	
*Sibbaldianthe_adpressa*_(Bunge)_Juz.G11	V.A._Gusev_391_(S)	FJ356176	FJ422304	
*Sibbaldianthe_sericea*_Grubov	Feng_122_(HIB)	KP875312	KP875333	
*Sibbaldiopsis_cuneifolia*_(Bertol.)_Sojak_G5	M._Lundberg_39_(S)	FJ356169	FJ422298	
*Sibbaldiopsis_cuneifolia*_(Bertol.)_Sojak_48	Feng_48_(HIB)	KP875313	KP875340	

Ecological niche modeling (ENM)

We modeled the predicted niche of wild strawberries in Sri Lanka using maximum entropy modeling approach methods (Phillips *et al.*, 2004; Phillips *et al.*, 2006). We used Maxent version 3.3.3 k program (Phillips *et al.*, 2006) to model the species distribution. Maxent only uses presence-only data for entropy modeling. Thus we used 162 presence-localities taken during our field visits (Table 02). We also used 19 bioclimatic variables as the environmental layers for our analysis (Table 03). Maxent often deals with the model overfitting errors, thus to obtain optimum maxent model, we used "species-specific tuning" approach (Anderson and Gonzalez, 2011; Elith *et al.*, 2011). We smoothened our model by choosing different feature classes [Auto, Linear (L), Quadratic (Q), Product (P), Threshold (T), Hing (H)] between different β regulation parameters (0.01, 0.1, 0.5, 1, 2, 3, 5). The model performance evaluated under three approaches. Initially, we assessed the model performance using threshold dependent manner, where we implemented different feature classes to achieve the best-tuned model. The linear and quadratic features are more informative when the number of occurrence points is 20 or less (Anderson and Gonzalez, 2011). Thus we evaluated our model using P, T, H, L+Q+P, L+Q+P+T, and L+Q+P+T+H. Then we implemented the threshold-independence of the method by assessing Area Under Curve (AUC) value of the Receiver Operation Curves (ROC). Finally, we visualized the model performance by checking the graphical outputs generated during the model building. We ran the program for 5000 iterations, and variable importance was measured using jackknifing. We set 10% of the occurrence data as the test data and kept other parameters as default. The best model was further edited in ArcGIS v10.4. A 20% threshold was set for the species distribution. We overlaid predicted species distribution into a 3-D map in ArcScene v 10.4 using 30-meter Digital Elevation Map (DEM) profile available in Jet Propulsion Laboratory (JPL) of California Institute of Technology. Finally, we used Google Earth Pro software to overlap our predicted niche with high-resolution satellite image of the predicted niche.

Table 02: The geographical locations of the *Potentilla indica* presence recorded during this study

Sample	Longitude	Latitude	Location
R1	7.084561	80.724723	Chariot path mountain
R2	7.083933	80.722792	
R3	7.082719	80.724079	
R4	7.082442	80.726257	
R5	7.081324	80.731707	
R6	7.083134	80.738509	
R7	7.083847	80.736599	
R8	7.083293	80.739592	
R9	7.083229	80.741223	
R10	7.082249	80.743101	
R11	7.081408	80.740912	
R12	7.081163	80.73823	
R13	7.02571	80.762499	Piduruthalagala mountain
R14	7.02703	80.770513	
R15	7.019342	80.77489	
R16	7.017372	80.786584	
R17	7.006777	80.786938	
R18	7.006777	80.786938	
R19	7.006553	80.789223	
R20	6.999376	80.787603	
R21	6.993445	80.790425	
R22	6.990767	80.789787	
R23	6.999307	80.771398	
R24 (DMB71)	6.942364	80.715941	Radella
R25	6.941901	80.716665	
R26 (DMB72)	6.941017	80.715528	
R27	6.939611	80.719358	
R28 (DMB73)	6.938706	80.716429	
R29	6.938812	80.714648	
R30	6.933966	80.716322	
R31	6.93338	80.716499	
R32	6.932342	80.716617	
R33	6.931799	80.716306	
R34	6.931022	80.716703	
R35	6.930287	80.718817	
R36	6.93158	80.718412	Radella (Black Pool junction)
R37	6.929439	80.719356	
R38 (DMB74)	6.926803	80.720761	
R39	6.925184	80.719774	
R40	6.924183	80.72031	
R41	6.924023	80.720664	

R42	6.921446	80.725889	Radella (Black Pool junction)
R43 (DMB75)	6.919348	80.729494	
R44	6.915418	80.734451	
R45	6.912297	80.739118	
R46	6.915737	80.739762	
R47	6.922575	80.735857	
R48 (DMB76)	6.932017	80.737423	
R49	6.939259	80.742659	
R50	6.949238	80.771069	
R51 (DMB66)	6.948583	80.775189	Meepilimana
R52	6.947699	80.774417	
R53	6.946613	80.773612	
R54	6.944105	80.775575	
R55	6.944243	80.776487	
R56	6.943892	80.777485	
R57 (DMB67)	6.94304	80.778021	
R58	6.942508	80.777624	
R59	6.941965	80.776605	
R60	6.941454	80.775747	
R61	6.940677	80.77609	
R62	6.939761	80.774749	
R63	6.937705	80.774449	
R64	6.936118	80.774674	
R65	6.933977	80.775414	
R66	6.933444	80.776465	
R67	6.933636	80.77742	
R68	6.932507	80.77771	
R69 (DMB68)	6.932156	80.778997	
R70	6.931197	80.778214	
R71	6.928183	80.776251	
R72	6.927821	80.7758	
R73	6.928396	80.775693	
R74	6.927927	80.775414	
R75	6.927607	80.775671	
R76	6.927287	80.775317	
R77	6.928096	80.774834	
R78 (DMB69)	6.927287	80.776036	
R79	6.926861	80.776165	
R80	6.927713	80.77624	
R81	6.92718	80.776809	
R82	6.927169	80.777592	
R83	6.928021	80.777292	
R84	6.928436	80.777946	
R85	6.927179	80.777882	
R86	6.927307	80.774063	
R87	6.929341	80.7749	
R88	6.929607	80.777636	
R89	6.928435	80.778119	
R90 (DMB70)	6.927849	80.777615	

R91	6.90111	80.802527	Ambewela
R92	6.899555	80.803332	
R93	6.89915	80.802356	
R94	6.898479	80.802249	
R95	6.897712	80.802807	
R96	6.897446	80.803579	
R97	6.896019	80.802066	
R98	6.889798	80.799513	
R99	6.891205	80.801068	
R100	6.890555	80.801358	
R101	6.89079	80.797774	
R102	6.890588	80.800113	
R103	6.889789	80.800928	
R104	6.889074	80.801079	
R105	6.889362	80.800167	
R106	6.889936	80.800489	
R107	6.888626	80.800167	
R108	6.888551	80.799169	
R109	6.888157	80.800242	
R110	6.888338	80.80124	
R111	6.853043	80.83038	World's end road, Horton Plains National park
R112	6.853032	80.828996	
R113	6.852467	80.827108	
R114	6.851881	80.825595	
R115	6.851498	80.824705	
R116	6.851466	80.821615	
R117	6.850124	80.820424	
R118	6.850177	80.816744	
R119	6.847812	80.813794	
R120	6.844275	80.814298	
R121	6.840089	80.812335	Entrance of the Horton Plains National park
R122	6.840078	80.811198	
R123	6.839439	80.811101	
R124	6.839492	80.811744	
R125	6.839727	80.811787	
R126	6.839662	80.811047	
R127	6.839305	80.812522	
R128	6.839917	80.810499	
R129	6.83997	80.812913	
R130	6.839959	80.813396	
R131	6.839426	80.813085	
R132	6.83916	80.812388	
R133	6.838915	80.812839	
R134	6.838755	80.813397	
R135	6.841248	80.81227	
R136	6.839544	80.813901	
R137	6.839768	80.812324	
R138	6.839442	80.811989	
R139	6.839503	80.811681	
R140	6.839242	80.812228	

R141 (DMB61)	6.839959	80.813396	Nuwara-Eliya
R142	6.982601	80.751725	
R143	6.9839	80.75209	
R144	6.985125	80.7512	
R145	6.987127	80.750846	
R146 (DMB62)	6.993112	80.746855	
R147	6.994198	80.747745	
R148	6.995029	80.748571	
R149	6.995806	80.747831	
R150	6.995881	80.74679	
R151	6.997063	80.74487	
R152	6.99816	80.744484	
R153	6.999033	80.744012	
R154	6.999241	80.743282	
R155	6.998858	80.742826	
R156	6.998949	80.741388	
R157 (DMB63)	7.000056	80.741211	
R158	7.000471	80.74164	
R159	7.000854	80.741012	
R160 (DMB64)	7.00103	80.740771	
R161	7.00136	80.740326	
R162	7.001839	80.740278	
R163 (DMB65)	7.002371	80.740283	

Table 03: **The Bioclimatic variables and their parentage contribution for occurrence of the species assessed.**

Bio Climatic Variable	Percentage contribution (%)
Mean Temperature of Coldest Quarter	24.0
Mean Temperature of Warmest Quarter	17.6
Precipitation Seasonality [Coefficient of Variation]	11.2
Min Temperature of Coldest Month	11.0
Annual Mean Temperature	10.1
Precipitation of Coldest Quarter	6.9
Annual Precipitation	4.4
Max Temperature of Warmest Month	3.3
Temperature Seasonality	2.4
Precipitation of Wettest Quarter	1.9
Mean Temperature of Wettest Quarter	1.5
Precipitation of Driest Month	1.2
Precipitation of Wettest Month	1.2
Precipitation of Driest Quarter	1.0
Precipitation of Warmest Quarter	1.0
Isothermality	0.9
Temperature Annual Range	0.4
Mean Diurnal Range [Mean of monthly (max temp - min temp)]	0.0
Mean Temperature of Driest Quarter	0.0

RESULTS

Phylogenetic position of Sri Lankan wild strawberries

The phylogenetic analysis carried out in both ML and Bayesian frameworks resulted almost congruent tree topologies. The ML analysis produced a tree with higher -log-likelihood value with well-supported clades. The MCMC chains in the Bayesian analysis reached maximum convergence at initial 50,000 generations thus trees probed up to that point were discarded as burn-in. The ESS values (<200) indicated the independent sampling of trees after maximum chain convergence. Thus the 50 million chain runs were enough to obtain a robust phylogeny. The 50% majority rule consensus tree constructed in the Bayesian criteria also had well supported and well-resolved branches. Since both ML and Bayesian trees had almost similar branching patterns, we only present the ML tree with ML bootstrap values and Bayesian posterior probabilities imprinted on each node.

As depicted in previous studies (Feng *et al*., 2017), our phylogeny constructed for the tribe Potentilleae had three main clades separating the sub tribes *Potentilla*, Argentina and Fragariinae with the PP of 100 and the bs of 100 support values. The combined analysis of plastid and nuclear DNA markers also recovered the clade topology where sub tribe *Potentilla* clade sister to the sub tribe Argentina (bs=99, PP=96) parallel with the findings of a previous study (Töpel *et al*., 2011). In congruence with previous studies ((Töpel *et al*., 2011; Feng *et al*., 2017), the ML tree also resolved the five lineages within subtribe *Potentilla* with high node support values (Figure 01). Similarly, we observed a similar branching pattern for subtribes Argentina and Fragariinae indicating the accuracy of the phylogenetic analysis. The wild strawberry samples sequenced during the present study included under Reptans clade of subtribe *Potentilla* with a slight divergence to *Potentilla indica* (bs=100, pp=100) (Figure 01). The topology of *P. indica* did not recover any reciprocal monophyly between the samples collected from *Nuwara-Eliya*, *Radella*, and *Mipillimana* populations of the *Nuwara-Eliya* District.

Predicted distribution of Sri Lankan wild strawberries

We assessed the niche model performances using three methods. Initially, we evaluated the model performance using threshold-independent criteria, where we checked the AUC values of the ROC curves. For Auto and H threshold rule features, the AUC curves functioned similarly with the highest AUC value of 0.998 to the lowest AUC value of 0.995 (Figure 02 A and B). The optimum β regulation parameters for these features were 0.01 and 0.1. However, for P threshold rule feature, H feature, and L+Q+P features, the AUC was constant at 0.995 at all the regulation parameters (Figure 02 C, D and E). The AUC curves functioned similarly for L+Q+P+T features and L+Q+P+T+H features where the highest AUC values of 0.998 to the lowest AUC value of 0.995 (Figure 02 F and G). The optimum β regulation parameters for these features were 0.01 and 0.1. Next, we chose the best models which had the highest AUC values and checked the model performance by analyzing omission and commission graphs. We tested the best performance at the omission of training samples concerning the predicted omission. The best performance was observed for $\beta = 0.01$ and 0.1 where the L+Q+P+T and L+Q+P+T+H threshold features were applied. Finally, we checked the visual outputs to select the final model that best fit for our occurrence data of wild strawberries. After tuning our model, we chose the model which applied L+Q+P+T+H threshold features at $\beta = 0.01$ (Figure 02).

According to the ENM, the most important environmental variable for species occurrence was the mean temperature of the coldest quarter (24% contribution). However, the mean temperature of the warmest quarter (17.6% contribution), seasonality precipitation (11.2% contribution), the minimum temperature at the coldest quarter (11.2% contribution) and annual mean temperature (10.1% contribution) were also crucial for the species occurrence. According to the ENM, the predicted distribution was restricted to *Nuwara-Eliya* District at an area of 166.36 km^2 (Figure 03A, B, C, D). The predicted distribution also overlaps with our sampling scheme, where we found a restricted occurrence of wild strawberry in the *Nuwara-Eliya* District (Figure 03. E and F). Also, the species occurs in Sri Lanka in the altitude of 1546 m - 2524 m (Figure 03G).

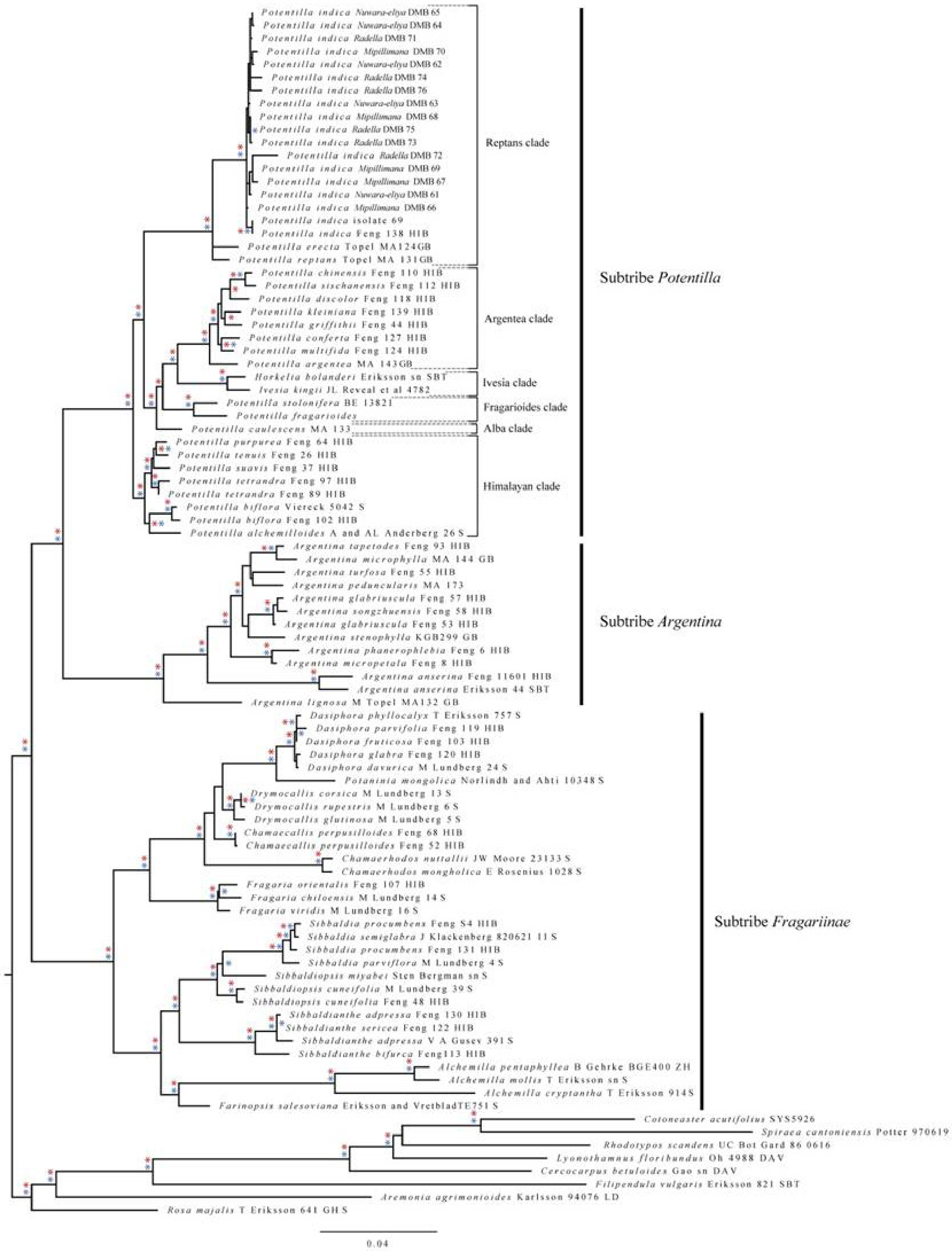

Figure 01: **The majority rule consensus tree constructed in Maximum likelihood framework for combined datasets of *ITS* and trn*L-F*. The red * indicates the nodes that are having a posterior probability higher than 90. The blue * indicates the nodes that are having a bootstrap value higher than 80. The subtribes and lineages are given in front of the respective clade.**

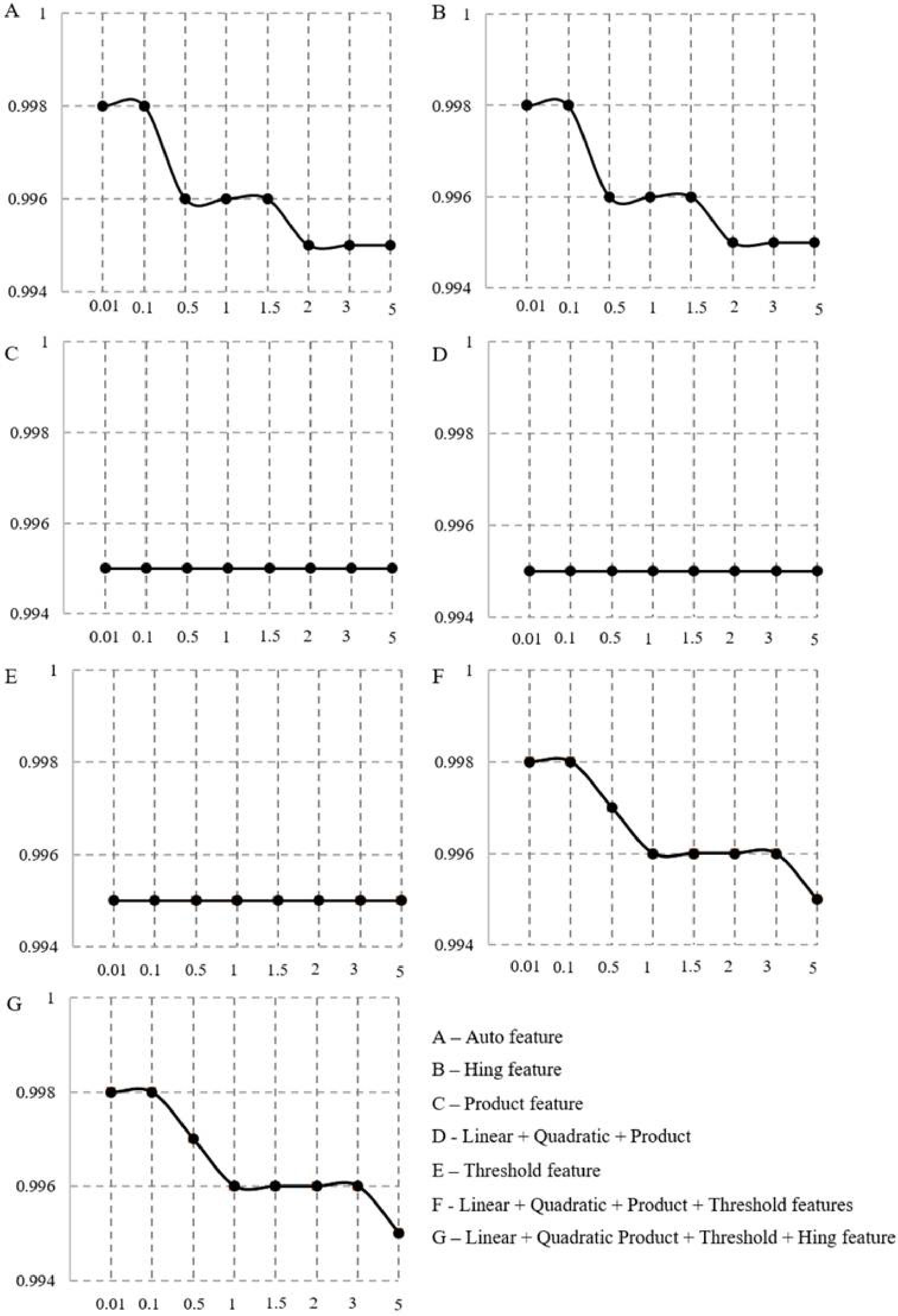

Figure 02: **The Receiver Operation Curves (ROC) showing the behavior of AUC values with respect to the β regulation parameter under different threshold rules. The X axis represent the AUC value and the Y axis represent the value for the β parameter.**

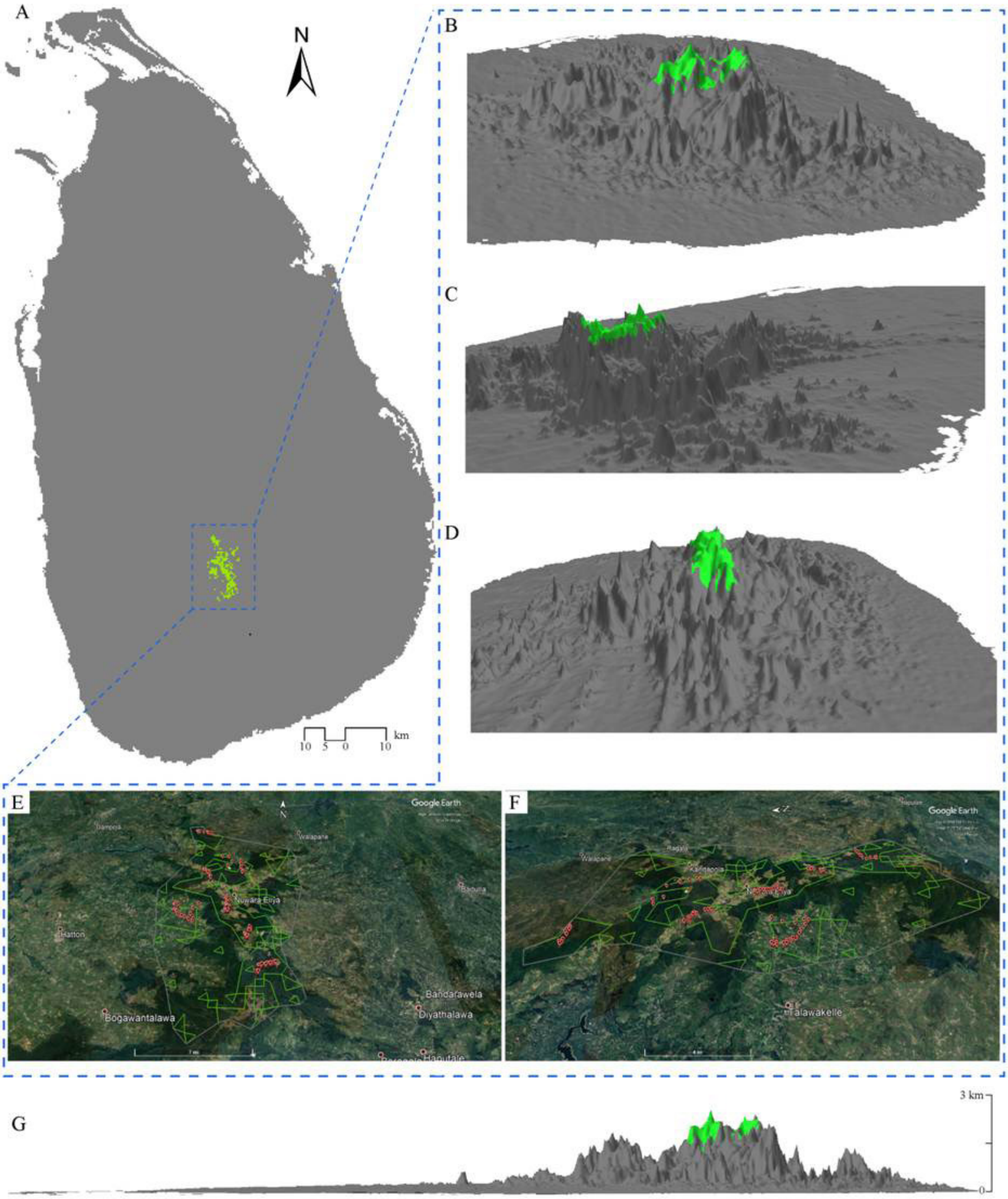

Figure 03: **The predicted distribution of the study species. A: The expected distribution overlaid to map of Sri Lanka. B: 3-D visualization of Sri Lanka showing the predicted niche from the western slope of Central hills. C: 3-D visualization of Sri Lanka showing the predicted niche from Northeastern slope of Central hills. D: 3-D visualization of Sri Lanka showing the predicted niche from the southern slope of Central hills. E and F: The satellite image of the area of the predicted niche showing geographical features, predicted niche (green lines), the overall area of the predicted niche (grey line), and the presence-localities used for distribution prediction. G: Elevational profile of Sri Lanka showing the climatic envelop of *P. indica*.**

DISCUSSION

In the present study, we aim to identify the phylogenetic position of wild strawberry (Indian strawberry or mock strawberry) through a phylogenetic analysis of nuclear and plastid markers. We also carried out an extensive sampling scheme and a distribution prediction using niche model approach to re-locate the full distribution of wild strawberry. A combined phylogeny of *trnL-trnF* and *ITS* markers resolved congruent to previously published data (Feng *et al*., 2017; Dobeš and Paule, 2010; Töpel *et al*., 2011). Our adapted phylogeny from Feng *et al*. (2017) also resolved some of the polytomic positions reported in previous studies. The employment of data partition matrices to capture the differential evolutionary processes of chloroplast and nuclear markers resulted more robust phylogeny with higher node support values. The phylogenetic positions of the samples sequenced during the present study cladded under sub-tribe *Potentilla*, lineage Reptans and the clade (species) *Potentilla indica*. Although this species is previously named as *Duchesnea indica* in the Sri Lanka descriptors (Wadhwa *et al*., 2000), now it is possible to revise the generic name as *Potentilla indica* as observed in our phylogeny. The *P. indica* (*nov.*) inhabited in Sri Lanka shows a uniform genetic structure for the *P. indica* sequences reported in previous studies. Although we sequenced samples from three distinct populations, no variations were identified between populations.

Since *P. indica* is a runnering plant, it is possible to have less variation within a population. However, due to the polyploid nature of *Potentilla*, there could be variations among the plants that arise from seeds. The clade structure with slight divergence must have been occurred due to the polyploid genetics. We identified a high gene flow among the populations of *P. indica* in *Radella*, Nuwara-Eliya, and *Mipillimana*. We believe that the dispersal of *P. indica* is mainly occurring through birds maintaining higher gene flow among the populations. In our sampling, we found wild strawberries abundantly in Nuwara-Eliya district in disturbed and opened habitats with high exposure to the sun. Despite higher abundance in Nuwara-Eliya district, we did not find wild strawberry in other higher elevations, mountain ranges such as Knuckles and *Rakwana* hills. Moreover, our niche model analysis predicted a similar distribution pattern in line with our field sampling (Figure 03E and 03F). The predicted distribution of *P. indica* is restricted to the about 166.36 km^2 area in *Nuwara-Eliya* District (Figure 03A). Our predicted distribution also shows the occurrence of *P. indica* exclusively restricted to high altitudes (Figure 03B, C, and D), starting from 1546 m to 2524 m (Figure 03G). Thus it is apparent that *P. indica* in Sri Lanka is highly restricted by a climatically enveloped structure. According to our entropy modeling, the most important climatic parameter for the occurrence of *P. indica* is the mean temperature of the coldest quarter (24 % contribution) (Table 03).

According to our results, it is apparent that the distribution of *P. indica* in Sri Lanka is directly correlated with the temperature variation in highland and seasonality of the precipitation. Although *P. indica* has the capability of distributing into other mountain ranges, due to the climatic envelope, the growth and dispersal are highly governed by the climatic factors. Thus we identified a mountain isolation in which habitat shrinkage could easily be resulted with the global climate change. With the rising environmental pollution, one of the most common problems for floral and faunal populations is the mountain isolation (Busby, 1988; Hamilton, 1995; Foste, 2001; Peñuelas and Boada, 2003; Feng *et al*., 2016). With the drastic increment of global warming, the floral and faunal species that prefer the coldest environmental conditions tend to confine into higher altitudes. Since the *P. indica* germplasm in Sri Lanka is restricted with climatic envelop, we believe it is currently at a threat and requires a conservation priority demanding urgent conservation measures.

CONCLUSIONS

In the present study, we carried out a systematic revision and species distribution prediction to identify the conservation needs of wild (Indian or mock) strawberries inhabited in Sri Lanka. Through a phylogenetic analysis, we revealed that Sri Lankan wild strawberry germplasm clades with subtribe: *Potentilla*, clade: Reptans

(PP=100, bs =100). The shallow divergence of the study species with *Potentilla indica* species indicates the need of taxonomic repositioning of this genus. Thus we reposition the genus of study species from *Duchesnea* to *Potentilla* and hereafter name wild strawberries in Sri Lanka as *P. indica*. After smoothening the maximum entropy model, we chose the model which applied L+Q+P+T+H threshold features at β = 0.01 that had a high AUC value (0.998). The predicted niche indicated that *P. indica* (nov.) has a highly restricted distribution. The *P. indica* was only dispersed in *Nuwara-Eliya* District over 166.36 km^2 of the area found in the altitude range of 1546 m - 2524 m. Since the *P. indica* germplasm in Sri Lanka is restricted within a climatic envelop, we believe that it is under threat and requires a conservation priority.

ACKNOWLEDGEMENTS

Authors wish to thank site-owners of the plants for granting permission to access and collect samples.

Data Availability Statement

The nucleotide dataset generated during and/or analyzed during the current study are available in the GenBank, and https://www.ncbi.nlm.nih.gov/nuccore (*ITS*: MK587724-MK587739; *trnL-trnF*: MN605458-MK605473; Table 01).

The other datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

Anderson, R.P. and Gonzalez Jr, I. (2011) Species-specific tuning increases robustness to sampling bias in models of species distributions: an implementation with Maxent. *Ecological Modelling*. 10(15), pp.2796-2811. DOI: https://doi.org/10.1016/j.ecolmodel.2011.04.011.

Andrews, H.C. (1807). Botanists repository. London. 7pp.

Busby, J.R. (1988). Potential impacts of climate change on Australia's flora and fauna. Commonwealth Scientific and Industrial Research Organisation: MelbourneFL: USA.

Cavanaugh, J.E. (1997) Unifying the derivations for the Akaike and corrected Akaike information criteria. *Statistics and Probability Letters*. 33(2), pp.8-201. DOI: https://doi.org/10.1016/S0167-7152(96)00128-9.

Dobeš, C. and Paule, J. (2010). A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): implications for its geographic origin, phylogeography and generic circumscription. *Molecular Phylogenetics and Evolution*. 56, pp.156-175. DOI: https://doi.org/10.1016/j.ympev.2010.03.005.

Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A. and Li, J., Overton, Peterson, A.T, Phillips, S.J., Richardson K.S., Scachetti-Pereira, R., Schapire, R.E., Soberón, J., Williams, S., Wisz, M.S. and Zimmermannet, N.E. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*. 29, pp.129–151. DOI/; https://doi.org/10.1111/j.2006.0906-7590.04596.x.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. and Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists, *Diversity and Distributions*. 17(1), pp.43-57. DOI: https://doi.org/10.1111/j.1472-4642.2010.00725.x.

Eriksson, T., Donoghue, M.J. and Hibbs, M.S. (1998). Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal *internal transcribed spacers* (*ITS*), and implications for the classification of Rosoideae (Rosaceae). *Plant Systematics and Evolution.* 211, pp.155-179. DOI: https://doi.org/10.1007/BF00985357.

Eriksson, T., Hibbs, M.S., Yoder, A.D., Delwiche, C.F. and Donoghue, M.J. (2003). The phylogeny of *Rosoideae* (Rosaceae) based on sequences of the *internal transcribed spacers (ITS)* of nuclear ribosomal DNA and the trnL/F region of chloroplast DNA. *International Journal of Plant Sciences*. 164, pp.197-211. DOI: https://doi.org/10.1086/346163.

Faith, D.P. (1996). Conservation priorities and phylogenetic pattern. *Conservation Biology*. 10, pp.1286-1289. DOI: https://doi.org/10.1046/j.1523-1739.1996.10041286.x.

Feng, B., Zhao, Q., Xu, J., Qin, J. and Yang, Z.L. (2016). Drainage isolation and climate change-driven population expansion shape the genetic structures of Tuber indicum complex in the Hengduan Mountains region. *Scientific Reports*. 6, pp.21811. DOI: https://doi.org/10.1038/srep21811.

Feng, T., Moore, M.J., Yan M.H., Sun, Y.X., Zhang, H.J., Meng, A.P., Li, X.D., Jian, S.G., Li, J.Q. and Wang, H.C. (2017). Phylogenetic study of the tribe Potentilleae (Rosaceae), with further insight into the disintegration of Sibbaldia. *Journal of Systematics and Evolution*. 55, pp.177-191. DOI: https://doi.org/10.1111/jse.12243.

Focke, W.O. (1888).Rosaceae in Engler, A. Natürliche Pflanzenfamilien, Leipzig.

Foster, P. (2001). The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews*. 55(1-2), pp.73-106. DOI: https://doi.org/10.1016/S0012-8252(01)00056-3.

Frandsen, P.B., Calcott, B., Mayer C. and Lanfear, R. (2015). Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. *BMC Evolutionary Biology*. 15(1), pp13. DOI: https://doi.org/10.1186/s12862-015-0283-7.

Geneletti, D.A. (2004). GIS-based decision support system to identify nature conservation priorities in an alpine valley. *Land Use Policy*. 21, 149-160. DOI: https://doi.org/10.1016/j.landusepol.2003.09.005.

Hamilton, L.S. (1995). Mountain cloud forest conservation and research: a synopsis. *Mountain Research and Development*. 1, pp.259-266. DOI: 10.2307/3673933

Hernandez, P.A., Graham, C.H., Master, L.L. and Albert, D.L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*. 29, pp.773–785. DOI: https://doi.org/10.1111/j.0906-7590.2006.04700.x.

Hortal, J., Jiménez-Valverde, A., Gómez, J.F., Lobo, J.M. and Baselga, A. (2008). Historical bias in biodiversity inventories affects the observed environmental niche of the species. *Oikos*. 117(6), pp.847-858. DOI: https://doi.org/10.1016/j.ecolmodel.2005.03.026.

Huelsenbeck, J.P. and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, pp.754-755. DOI: 10.1093/bioinformatics/17.8.754

Kozak, K.H., Graham, C.H. and Wiens, J.J. (2008). Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology and Evolution*. 23:, pp.41–48. DOI: https://doi.org/10.1016/j.tree.2008.02.001.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. 33(7), pp.1870-1874. DOI: https://doi.org/10.1093/molbev/msw054.

Lanfear, R., Calcott, B., Kainer, D., Mayer C. and Stamatakis, A. (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology*. 14(1), pp.82. DOI: https://doi.org/10.1186/1471-2148-14-82.

Lanfear, R., Frandsen, PB., Wright, AM., Senfeld, T. and Calcott, B. (2016). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*. 34(3), pp.772-773. DOI: https://doi.org/10.1093/molbev/msw260.

Miller, M.A., Pfeiffer, W. and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: gateway computing environments workshop (GCE). 1-8.

Peñuelas, J. and Boada, M. (2003). A global change induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology*. 9(2), pp.131-140. DOI: https://doi.org/10.1046/j.1365-2486.2003.00566.x.

Peterson, A.T. (2003). Predicting the geography of species' invasions via ecological niche modeling. *The Quarterly Review of Biology.* 78, pp.419-433. DOI: https://doi.org/10.1086/378926.

Peterson, A.T., Soberón, J., Pearson, R.G., Anderson, R.P., Martínez-Meyer, E., Nakamura M. and Araújo, M.B. (2011). Ecological niches and geographic distributi.ons. Princeton University Press: Princeton.

Phillips, S.J. and Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*. 31, 161-175. DOI: https://doi.org/10.1111/j.0906-7590.2008.5203.x.

Phillips, S.J., Anderson, R.P. and Schapire, R.E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*. 190(3-4), pp.231-259.

Phillips, SJ., Dudík, M. and Schapire, R.E. (2004). A maximum entropy approach to species distribution modeling. In Proceedings of the twenty-first international conference on Machine learning. DOI: 10.1145/1015330.1015412.

Planet, P.J. (2006). Tree disagreement: measuring and testing incongruence in phylogenies, *Journal of Biomedical Informatics*. 39(1), pp.86-102. DOI: https://doi.org/10.1016/j.jbi.2005.08.008.

Potter, D., Still, S.M., Grebenc, T., Ballian, D., Božič, G., Franjiæ, J. and Kraigher, H. (2007). Phylogenetic relationships in tribe Spiraeeae (Rosaceae) inferred from nucleotide sequence data. *Plant Systematics and Evolution*. 266, pp.105-118. DOI: https://doi.org/10.1007/s00606-007-0544-z.

Rambaut, A. and Drummond, A. (2007). Tracer v1. 4. http://beast. bio. ed. ac. uk/Tracer. 15.12.2018.

Rambaut, A. (2014). FigTree, a graphical viewer of a phylogenetic trees. http://tree. bio. ed.ac. uk/software/figtree. 20.12.2018.

Senavirathna, H.M.T.N., Ranaweera, L.T., Mudannayake, M.M.A.W.P., Nawanjana, P.W.I., Wijesundara, W.M.D.A., Jayarathne, H.S.M., Ratnasuriya, M.A.P., Weebadde, C.K. and Sooriyapathirana, S.D.S.S. (2020). Assessment of the taxonomic status of the members of genus *Artocarpus* (Moraceae) in Sri Lanka. *Genetic Resources and Crop Evolution*. 67, pp. 1163–1179. DOI: https://doi.org/10.1007/s10722-020-00902-x

Senevirathne, G., Samarawickrama, V.A., Wijayathilaka, N., Manamendra-Arachchi, K., Bowatte, G. and Samarawickrama, D.R. (2018). A new frog species from rapidly dwindling cloud forest streams of Sri Lanka-*Lankanectes pera* (Anura,Nyctibatrachidae), *Zootaxa*. 23, pp.519-538. DOI: http://dx.doi.org/10.11646/zootaxa.4461.4.4.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 22, pp.2688–2690. DOI: https://doi.org/10.1093/bioinformatics/btl446.

Stamatakis, A., Hoover P. and Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*. 57, pp.758-771. DOI: https://doi.org/10.1080/10635150802429642.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*. 17(5), pp.1105-1109. DOI: https://doi.org/10.1007/BF00037152.

Töpel, M., Lundberg, M., Eriksson, T. and Eriksen, B. (2011). Molecular data and ploidal levels indicate several putative allopolyploidization events in the genus *Potentilla* (Rosaceae). *PLoS Currents*. 3. DOI: 10.1371/currents.RRN1237.

Wadhwa, B.M. (2000). *Duchesnea*, In: A revised handbook to the Flora of Ceylon. (Dassanayake, M.D. and Clayton, W.D. Eds.). Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, India. 361- 364.

White, T.J., Bruns, T., Lee, S. and Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. Eds). San Diego (CA): Academic Press. 315-322.

Wiens, J.J. and Graham, C.H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*. 36, 519-539. DOI: https://doi.org/10.1146/annurev.ecolsys.36.102803.095431.

Wijayathilaka, N., Senevirathne, G., Bandara, C., Rajapakse, S., Pethiyagoda, R and Meegaskumbura, M. (2018) Integrating bioacoustics, DNA barcoding and niche modeling for frog conservation– The threatened balloon frogs of Sri Lanka. *Global Ecology and Conservation*. 1, pp.16. DOI: https://doi.org/10.1016/j.gecco.2018.e00496.

Wisz, M.S., Hijmans, R.J., Li, J., Peterson, A.T., Graham, C.H. and Guisan, A. (2008). NCEAS Predicting Species Distributions Working Group, Effects of sample size on the performance of species distribution models. *Diversity and Distributions*. 14, pp.763–773. DOI: https://doi.org/10.1111/j.1472-4642.2008.00482.x.

Wolf, F.T. (1908). Monographie der Gattung *Potentilla*. *Bibliotheca Botanica*. 16, pp.1-714.