NICHE CHARACTERIZATION AND STUDY OF GENETIC VARIABILITY IN PANAX SPECIES COMPLEX OF NORTH-EAST INDIA

(ABSTRACT)

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THESIS SUBMITTED
IN FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

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Botany

Ginseng, the members of the genus *Panax* are known as 'King of Herbs'. It has been highly valued for its mystical properties and has a 5000-year long history as a traditional herbal medicine originating from ancient China. The ginseng genus, belonging to the family Araliaceae

is one of the approximately 120 genera of flowering plants with an eastern Asian and eastern North American disjunct distribution. *Panax* consists of approximately 18 species, of which 16 are from eastern Asia and two from eastern North America. In India, Araliaceae is represented by 15 genera distributed mostly in north and northeastern region. The genus *Panax* in India comprises of *Panax assamicus* Ban., *P. bipinnatifidus* Seem., *P. pseudoginseng* Wall., *P. sokpayensis* Sharma and Pandit., and *P. sikkimensis* Ban. In India, *Panax* species are found only in north-eastern region and Darjeeling hills of West Bengal. Among the north-eastern states, the species have been reported from Arunachal Pradesh, Meghalaya, Manipur, Nagaland and Sikkim.

Several Himalayan species of *Panax* have been debatable due to sympatry of morphologically distinct taxa and the existence of occasional morphological intermediates. The Himalayas, central and Western China are considered as the centre of diversity of *Panax.* High medicinal value of *Panax* is responsible for their extraction from the wild, which has reduced the sizes of most of their natural populations. The illegal trade in wild ginseng has caused a drastic decline in their population sizes in the wild during the recent years. This has made at least two *Panax* species as threatened. The Himalayan *Panax* species are already enlisted as vulnerable in the Red Data Book of India. In CAMP (2003), *P. wangianus* (present day identified as *P. assamicus*) was classified as an endangered species. Because of deficient data, other species of Himalayan *Panax* are yet to be classified under IUCN criteria (IUCN, 2010).

The study was conducted in Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Sikkim. In Arunachal Pradesh, *Panax* species are found in the temperate forest of Tawang district and subtropical broadleaved forest of West Kameng district. In Manipur, *Panax* species are found in temperate forests of Senapati and Ukhrul districts at an altitude of 1,800 to 2,400 m asl where Oaks are the dominant trees. In Meghalaya, *Panax* populations are found in subtropical broadleaved forest which occurs above 1,200 m asl. In Nagaland, the species are found mainly in the wet temperate forests classified under Naga hills wet temperate forest which is distributed along the Assam/Burma border from 1,800 m asl. In Sikkim, *Panax* species are found in the temperate forest and subtropical or lower montane forests. Thus, all *Panax* species were at an altitudinal range of 1,490-3,306 m asl in north-east India.

Panax species recorded in the present study are, *P. assamicus, P. bipinnatifidus, P. pseudoginseng, P. variabilis* and one unidentified species (referred as *Panax* sp.). The *Panax* species in the present study consists of: (i) horizontally elongated rhizomes with thick and short internodes which includes *P. assamicus, P. variabilis*, *P. pseudoginseng*, and *Panax* sp. (unidentified), and (ii) horizontally elongated rhizomes with slender and elongated internodes and subglobose nodes that includes *P. bipinnatifidus*.

The present study was undertaken with the following objectives:

1. Study the distribution pattern, niche and population characteristics of *Panax* species complex in north-eastern India;
2. Resolve the taxonomic ambiguity of Himalayan *Panax* species complex using molecular phylogeny approach; and
3. Quantify the ginsenoside contents for Himalayan *Panax* species complex and correlate it with the ecological conditions of the species.

Ecological niche characterization

Each species had a different range of occurrence with respect to different edaphic and climatic variables. The range of air temperature where *Panax assamicus* grows was 18-28°C. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng, P. variabilis* and *Panax* sp. were 13.1-14°C, 20- 24.9°C, 18-18.6°C and 23.2-26.6°C, respectively. The range of relative humidity for *Panax assamicus* was 58.5-94.9% while that for *P. bipinnatifidus* it was 52-60%. The respective ranges for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. were 76-99 %, 86-96% and 62-79.4%, respectively. The range of light intensity for *P. assamicus* was 800-2,500 lux while that for *P. bipinnatifidus* and *P. variabilis* it was 700-1,300 lux. *P. pseudoginseng* occurred in the range of 650-2,900 lux and *Panax* sp. grew in the range of 600-1,640 lux. The range of soil temperature for *P. assamicus* was 14.8-21.1°C while that for *P. bipinnatifidus* it was 10.2-11.4°C. *P. pseudoginseng* had a range of 15.9-18.4°C, *P. variabilis* 14.8-15.6°C and *Panax* sp. grew in the soil temperature range of 16.7-19.2°C. The texture of the soils for *Panax assamicus, P. pseudoginseng* and *Panax* sp. showed a range of textural class from loamy sand to sandy . In *P. variabilis* and *P. bipinnatifidus* the soil texture was sandy. The range of water holding capacity for *P. assamicus* was 31-76.8% while that for *P. bipinnatifidus* it was 46.8-53.8%. *P. pseudoginseng* had a range of 31.3-51.9%, *P. variabilis* 43.3-51.2% and *Panax* sp. grew in the range of 28.6-51.7%. The soil bulk density ranges for *P. assamicus*, *P. bipinnatifidus, P. pseudoginseng,* and *P. variabilis* and *Panax sp.,* were 0.4-0.8 g cm-3, 0.7-1.2 g cm^{-3} 0.3-1 g cm^{-3}, 0.5-1g cm^{-3} and 0.5-0.6 g cm^{-3}. The range for soil porosity in *P. assamicus* was 69.1-86.2%, while that for *P. bipinnatifidus* was 55.3-75.6%. The range for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 62.4-87.5%, was76.3-81.3% and 62.9-81% respectively. The soil moisture content range for soils of *P. assamicus* growing areas was 22.1-

47.7%. The corresponding range for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax sp.*, were 29.5-31.45%, 31.1-46%, 44.1-46.7% and 30.6-47.5%. The range of soil pH for *P. assamicus* was 4.4-6.0 while that for *P. bipinnatifidus* was 4.3 to 4.8. *P. pseudoginseng*, *P. variabilis* and *Panax* sp. grows in the range of 4.9-7.1, 4.8-5.9 and 5.2-7.3. The range of total kjeldahl nitrogen for *P. assamicus* was 0.4-1.0% while that for *P. bipinnatifidus* was 0.4-0.5%. *P. pseudoginseng* had a range of 0.4-1.0%, *P. variabilis* with 0.6-0.7% and *Panax* sp. with 0.5-1.25%. The range of soil organic carbon for *P. assamicus*, *P. bipinnatifidus* *P. pseudoginseng* *P. variabilis* and *Panax* sp. was 1-5.8% , 4.1-4.2%, 3.9-5.5%, 5.2-6.2% and 2.5-4.4%. The range of available phosphorus for *P. assamicus* was 16-25.9 μg g^{-1} while that for *P. bipinnatifidus* was 17.3-18 μg g^{-1}. *P. pseudoginseng* *P. variabilis* and *Panax* sp. grow in the range of 23.8-78 μg g^{-1}, 59-65.2 μg g^{-1} and 22.8-143.3 μg g^{-1}. The range for exchangeable potassium for *P. assamicus* was 78.9-362.5 μg g^{-1} while that for *P. bipinnatifidus* was 165.5-178 μg g^{-1}. The respective ranges for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 192.5-360 μg g^{-1}, 135-275 μg g^{-1} was 138-440 μg g^{-1} respectively. The range of total magnesium for *P. assamicus* was 1.7-11 mg g^{-1} while that for *P. bipinnatifidus* was 3.2-3.6 mg g^{-1}. *P. pseudoginseng* had a range of 2.9-4.9 mg g^{-1}, *P. variabilis* was 2.7-3.6 mg g^{-1} and *Panax* sp. grow in the range of 3.1-5.7 mg g^{-1}. The range of total calcium for *P. assamicus* was 0.03-11.09 mg g^{-1}. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 0.1-0.2 mg g^{-1}, 0.01-0.6 mg g^{-1}, 0.1-0.2 mg g^{-1} was 0.05-1 mg g^{-1}. The range for total iron in *P. assamicus* was 27.3-83.6 mg g^{-1} while that for *P. bipinnatifidus* was 27.7-28.6 mg g^{-1}. *P. pseudoginseng* grows in the range of 24.5-37 mg g^{-1}, *P. variabilis* with 23.5-31.6 mg g^{-1} and *Panax* sp. with 29.5-59.2 mg g^{-1}. The range for total manganese in *P. assamicus* was 0.1-1.4 mg g^{-1}. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 0.1-0.3 mg g^{-1}, 0.2-0.3 mg g^{-1} and 0.2-0.5 mg

g^{-1}. The corresponding ranges for total zinc in *P. assamicus, P. bipinnatifidus, P. pseudoginseng, P. variabilis* and *Panax* sp. was 40.4-101 μg g^{-1}, 70.7-73.8 μg g^{-1}, 55.8-93.6 μg g^{-1}, 42.8-53.8 μg g^{-1} and 53.9-69.5 μg g^{-1}. The range for total copper in *P. assamicus* was 23.53-48.05 μg g^{-1} while that for *P. bipinnatifidus* was 29.2-32.4 μg g^{-1}. *P. pseudoginseng, P. variabilis* and *Panax* sp. was 16.9-47.2 μg g^{-1}, 27.8-28.2 μg g^{-1} was 32.3-52.4 μg g^{-1}.

Niche breadth and Niche differentiation

The mean Levin's niche breadth for all the environmental parameters clearly showed that *P. assamicus* had the broadest niche breadth of 2.2 followed by *P. pseudoginseng* with a niche breadth of 2.0. *Panax* sp. had an intermediate niche breadth of 1.7 and both *P. bipinnatifidus* and *P. variabilis* showed the same niche breadth of 1.0.

The scatter plot, an output of PCA with the species and the corresponding environmental parameters revealed the distinct niches (environmental requirements) of each species, although three species viz., *P. assamicus, P. pseudoginseng* and *Panax* sp. showed overlapping but stretched out group indicating the wide range of species. However, *P. variabilis* and *P. bipinnatifidus* were clumped which shows that these species have a narrow range of variation.

Although the distribution of different *Panax* species was correlated with different environmental factors, available phosphorus, soil moisture content, total Kjeldahl nitrogen, copper and manganese, porosity, relative humidity, air temperature, exchangeable potassium, soil organic carbon, soil pH and iron were important factors responsible for their distribution/occurrence.

Ecological niche modeling

The potential distribution area of each *Panax* species was modeled using Ecological Niche Modelling. The most important factor which determined the distribution of the potential habitats of *P. assamicus* (Manipur), *P. bipinnatifidus, P. pseudoginseng, P. variabilis* and *Panax* sp. was Normalized Difference Vegetation Index (NDVI) but for *P. assamicus* from Meghalaya, Digital Elevation Model (DEM) was the key factor. The contribution of NDVI for the former set of species ranged between 88.4 and 94.6%. *P. bipinnatifidus* had the maximum value with a contribution of 94.6%. For *P. assamicus* from Meghalaya, DEM contributed about 64% to the model. The greater contribution of NDVI than the other factors to the potential distribution models of most species revealed the importance of NDVI layer in defining habitat suitability which is related to the phenological cycle of the vegetation of that particular area. The relatively high importance of vegetation parameters such as herbaceous cover, NDVI and EVI in predicting distribution of species was in conformity with the distribution models developed by several earlier workers (Stohlgren *et al.*, 2001; Osborne *et al.*, 2001; RouraPascual *et al.*, 2006; Bino *et al.*, 2008).

The distribution models developed for different species were quite accurate as reflected in the high AUC values (AUC>0.9) for all the species of *Panax*. The values ranged between 0.97 and 0.99 for *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. The models with AUC>0.95 but <1.0 are considered very good (Thuiller *et al.*, 2005). The high AUC values for different species revealed that the habitat distribution models generated by MaXent have the ability to differentiate between suitable and unsuitable habitats of *Panax* species. With the AUC values very close to 1.0, the models exhibit their capability for strong prediction. The niche overlap according to Schoener's D statistic (Schoener, 1968) in all the

Panax species ranged from limited to moderate overlap. This indicates that although these species are different, they do share many environmental variables.

The importance of each environmental predictor variable was assessed using jackknife operation (Yost *et al.*, 2008) and showed the environmental variable with highest gain when used in isolation but when omitted, decreases the gain the most was NDVI values of January, March, July and December, and elevation. These variables contributed the most to the Maxent models in *Panax* species.

The potential distribution maps generated through ENM indicates that *P. assamicus* is distributed in Meghalaya and Manipur. The distribution of the species was also predicted in Tuensang district of Nagaland bordering Myanmar. However, it could not be located in Tuensang. *P. bipinnatifidus* was predicted with continuous distribution from Sikkim extending to Bhutan and Arunachal Pradesh, Myanmar, West Bengal and Sikkim. *P. pseudoginseng* was predicted in Nagaland, Manipur, Bhutan and Myanmar. *P. variabilis s*howed a restricted distribution confined only to the northern part of Senapati district of Manipur and areas in southern part of Kohima district of Nagaland bordering Senapati. A continuous distribution pattern of *Panax* sp. was predicted in Arunachal Pradesh spreading to Tuensang district of Nagaland, and also in southern part of Bhutan, Tibet, Myanmar, Sikkim, West Bengal and Nepal.

Establishing taxonomic identity of *Panax* species from north-eastern India

Morphometric analysis

Cluster Analysis using the 15 morphological characters of 56 specimens produced two major clusters in the dendrogram. The first cluster comprised of *P. assamicus* with a few individuals at lower distance measure. The second cluster was formed at greater distance and divided into sub-clusters which included, *P. assamicus, P. bipinnatifidus, P. variabilis, P. pseudoginseng* and *Panax* sp. *P. bipinnatifidus* formed a subcluster of its own but all the other specimens were intermixed with each other and did not give a clear distinction in all the species. However, there was significant difference among the species and also among the populations in the morphological characters.

PCA scatter plot showed that *Panax* in north-east formed five distinct groups i.e. *P. assamicus, P. pseudoginseng, Panax sp., P. variabilis and P. bipinnatifidus.* The samples of *P. bipinnatifidus* and *Panax sp.* showed overlapping and more condensed indicating less variation. *P. variabilis* and *P. pseudoginseng* also showed overlapping but with a broad ellipse indicating a wide range of variation. *P. assamicus* on the other hand showed overlapping with *P. variabilis* and *P. pseudoginseng* and is broadly stretched out indicating a wide range of variation. *P. bipinnatifidus* is narrowly stretched .

Molecular phylogeny

Approximately 21 kb genomic DNA bands were observed for all *Panax* species. The best amplification result was obtained at annealing temperature of 67°C with a total band size of 1.8Kb for 18S rRNA gene. For 18S-28S rDNA ITS region, the best amplification result was obtained at annealing temperature of 57.5°C where a single band of approximately 700 bp was amplified.

Nucleotide sequence analysis: The BLAST for the *18S rRNA* gene showed a high degree of homology (99%) with *P. ginseng, P. quinquefolius, P. notoginseng, P. japonicus, P. vietnamensis, P. stipuleanatus, P. pseudoginseng* subsp. *himalaicus, P. bipinnatifidus, P. notoginseng.* For 18S-28S rDNA ITS region, BLAST analysis showed 100% identity of *P. assamicus, P. bipinnatifidus* and *P. variabilis. Panax sp.* collected from Nagaland and Arunachal Pradesh showed 99% identity with *P. japonicus, P. quinquefolius, P.variablis* and *P. vietnamensis.*

Sequence characteristics: A total of 18 sequences of *Panax* species were used including the 8 sequences of the studied species for the 18S rRNA gene. The sequence data set ranged from 1692-1694. Out of 1694 characters analysed, 11 were variable/polymorphic sites, 1680 were invariable/monomorphic sites, 10 were autapomorphic sites, 1 site was parsimony informative, GC (%) content was 49.65% and transition/tranversion ratio was 0.95.The total number of indel sites analysed was 3 with an average indel length of 1. Indel diversity k (i) was 0.53 Tajima'D test was 1.09 with p> 1.0 which was not significant.

A total of 41 sequences of *Panax* species were used including the 8 sequences of the studied species for the 18S-28S rDNA ITS region. The sequence data set ranged from 602-617. Out of 620 characters analysed 113 were variable/polymorphic sites, 480 were invariable/monomorphic sites, 68 sites were parsimony informative, 45 were autapomorphic sites, GC (%) content was 60.42% and transition/tranversion ratio was 2.94. The total number of indel sites analysed were 27 with an average indel length of 1.22. Indel diversity k (i) was 1.45 and Tajima'D test was 2.53 with $p<0.001$ which was significant.

Phylogenetic analysis of 18S rRNA gene: The phylogenetic tree constructed through NJ and ML methods revealed three clades. The first clade included *P. assamicus, P. bipinnatifidus Panax sp.*(new species in present study), *P. ginseng, P. japonicus, P. japonicus var. bipinnatifidus, P. pseudoginseng subsp. himalaicus, P. variabilis and P. zingiberensis* supported by 100% bootstrap value. *P. pseudoginseng* and *P. stipuleanatus* formed a separate clade. *P. quinquefolius, P. notoginseng* and *P vietnamensis* formed together one clade (BS=62%). The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G model showed the support for relationships between the taxa with posterior probability values.The tree topology was congruent with that of the Maximum Likelihood tree. The first clade was supported with maximum posterior probability value (100%). *P. pseudoginseng* and *P. stipuleanatus* were grouped together with 69% posterior probability value. The third clade which grouped together *P. quinquefolius, P. notoginseng* and *P vietnamensis* was supported by 1 % posterior probability.

Phylogenetic analysis of the 18S-28S rDNA region: The Neighbour joining tree yielded more or less similar results as obtained with the Maximum likelihood method. The Maximum likelihood tree revealed that the sampled populations of *P. assamicus* from Meghalaya and Manipur grouped together (BS=93%) and showed a close relationship with *P. bipinnatifidus* clade (BS=92%). The populations of *P. bipinnatifidus* from Arunachal Pradesh were grouped together in one clade with the other populations from China. *P. variabilis* from Manipur was grouped together with *P. variabilis* from China (BS=88%). *Panax sp.* populations grouped together (BS=98%) and formed a separate clade. This clade showed close relationship with the basal clade i.e., *P. notoginseng* (BS=51%) which in turn was related to the *P. pseudoginseng-P. stipuleanatus* clade (BS=99%). The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G+I model showed strong support for relationships between the taxa with posterior probability values (Fig 6.17). The tree topology was congruent with that of the Neighbour Joining and Maximum Likelihood tree.

***Secondary structure of 5.8S rRNA*:** The secondary structures of *P. assamicus* from both the populations in Meghalaya matched the secondary structure in *P. assamicus* from GenBank. *P. assamicus* from Manipur population had similar structure with another population from Manipur which was retrieved from GenBank (HQ141404). The populations of *P. bipinnatifidus* from Arunachal Pradesh had the same structure with that of *P. bipinnatifidus* from Nepal. *Panax sp.* from both the populations i.e. Arunachal Pradesh and Nagaland had similar structures but did not match with any of the structures in GenBank.

PCR –restriction fragment length profile (pcr-rflp)/ amplicon restriction pattern (arp): The PCR-RFLP using the endonuclease *BstN*1 and *Psp*G1 produced two different profiles The first profile was obtained in all the populations of *P. assamicus* from Meghalaya and Manipur and the second profile was obtained for *P. bipinnatifidus, Panax sp.* and *P. variabilis*. Restriction enzyme *Msp*I generated two profiles, PM1 profile was obtained for *P. assamicus* populations and *P. bipinnatifidus* populations. Profile PM2 was obtained for *P. variabilis* population and *Panax sp.* populations from Arunachal Pradesh and Nagaland.

Cluster dendrogram constructed by using the combined PCR-RFLP profiles of the 18S-28S rDNA ITS region of all the *Panax* species revealed two major clusters, I and II. Cluster I comprises all the sub-populations of *P. assamicus* from Meghalaya and Manipur. Cluster II comprised of two sub-clusters, the first cluster (IIA) consists of *P. bipinnatifidus* populations from Arunachal Pradesh and the second cluster (IIB) includes *P. variabilis* from Manipur population and *Panax sp.* populations from Arunachal Pradesh and Nagaland.

Scale developed for identification of Panax species: The comparison of sequence homologies of the different genera under Araliaceae, different species of *Panax* genus and members of same species collected from different geographical locations revealed that the 18S-28S rDNA ITS sequence homology of members belonging to the *Panax* genus but different species ranged from 92-98%. Species from different geographical locations exhibited homology of 98-99%. The unidentified species showed 94-99% homology with the other species of *Panax*. However, it showed 99% homology with *P. quinquefolius* and *P. vietnamensis* which indicated that despite morphological dissimilarity that this species is genetically close to these two species.

Assessing the impact of disturbance on *Panax assamicus* metapopulation

Demographic stages: *Panax assamicus* have 8 stages i.e., seedling stage and seven other leaf-stages from 1-leaved to 7-leaved stage. 3 and 4-leaved stage are reproductive individuals.

Spatial Characteristics: *Panax assamicus* metapopulation had only 5 populations. The mean patch size was 89.6 ± 42.1 ha. Total plant density was 27 ±11.61/ha and the mean patch isolation was 12.28 ± 4.7.

Finite Rate of Increase (λ): Deterministic analysis yielded the finite rate of increase (λ) which revealed that all the populations of *Panax assamicus* exhibited discrete population growth pattern and the growth rate declined (λ <1) over 3 years of study period i.e. 2009-2011. The growth rate (λ) in all the five populations remained within 1.0 which depicts a general decline in population. The population growth rate was in the order Upper Shillong>Laitkseh>Laitkor >Tyllang and Nongkrem. The total metapopulation level growth rate (λ) also remained negative (λ=0.898) indicating a net decline in the total population size of the species.

Elasticity: The contribution of all these demographic processes to overall growth of the population in the life cycle of *P. assamicus* was represented by the relative sensitivity of survival, growth and fecundity to λ. Survival growth contributes the maximum towards the growh rate. Contribution of growth to elasticity was highest in Upper Shillong which is near to 0.1 followed by Tyllang population. However, in Laitkor and Nongkrem populations, the elasticity values fall below 0.001. There was no contribution of growth in Laitkseh population. Fecundity had the least elasticity which shows it had relatively less contribution to λ. In Laitkseh and Nongkrem populations, fecundity did not have any contribution to *P. assamicus* and demonstrated the actual behaviour of a typical iteroparous forest herb where survival had the highest elasticity compared to growth and fecundity.

Stochastic Risk Analyses: A large decline was expected in all the populations of *P. assamicus* within 40 years except for Upper Shillong population where the decline was expected after 60 years in both the M1 and M2 scenarios. After a sharp decline the trajectories stabilized in both the scenarios which indicated an almost deterministic decline in the future. However, the summary on the meatpopulation size declines in both M1 and M2 scenario which leads to an extremely low abundance. In M1 scenario, the metapopulation size after a sharp decline becomes stable after 65 years and in M2 scenario the population becomes stable after 40 years.

Metapopulation viability: The occupied populations in *P. assamicus* depicted a sharp decline through time and only 50% of these would still remain within 24 years. The remaining sub-populations are from Upper Shillong under M1 scenario. The metapopulation occupancy in M2 scenario showed a similar trend and approximately 50% of the population would be extant by 16th year and the populations which will remain are from Upper Shillong.

Probability of extinction: The extinction curves for all the sub-populations approached extinction probability 1.0 much before 100 years except for Upper Shillong sub-population. The extinction probability curves revealed identical curves in 3 of the subpopulations. Laitkseh population showed overlapping in both the scenarios but Upper Shillong sub-population showed different extinction curve in M1 and M2 models.

Management intervention: Threat status of the study species was determined and *Panax assamicus* was categorized as vulnerable. Management options was analysed and introduction of approximately 400 1-leaved stage plants is required to bring down the risk of *Panax assamicus* from vulnerable category. To lower the risk, an estimate of 5,520 plants is required to be introduced to reach its Minimum Viable Population (MVP) size.

Inter-population variation in ginsenoside contents

The retention time for ginsenoside Rb1 was 9.13 mins and for ginsenoside Rg2, it was 8.68 mins. The retention time for Rb1 in all the samples ranged from 8.93-9.17 minutes. Rb1 content (%) was highest in *P. assamicus* and the trend was *P. assamicus* > *P. bipinnatifidus* >*P. pseudoginseng* >*Panax* sp. > *P. variabilis*. The highest content was recorded for *P. assamicus* population at Laitkseh (3.05%) and lowest in *P. variabilis* population (0.45). There was significant variation in Rb1 ginsenoside content in all the populations and even among the species. The retention time for Rg2 in all the samples ranged from 8.4-8.8 minutes. Rg2 content (%) was highest in *P. assamicus* and the trend was *P. assamicus* > *P. pseudoginseng* > *P. bipinnatifidus*, *P. variabilis* > *Panax* sp. The highest content was recorded for *P. assamicus* population at Laitkseh (= 0.55) and lowest in *Panax* sp. population (0.006). There was significant variation in Rg2 ginsenoside content in all the populations and even among the species. The total ginsenoside content ranged from 3.60 to 0.19 % . The Rg2/Rb1 ratio for *P. assamicus* population ranged from 0.05-0.18, for *P. bipinnatifidus* it was 0.05. The lowest Rg2/Rb1 ratio was lowest in *Panax sp.* with 0.03, *P. pseudoginseng* had the highest range of 0.11-0.37 and for *P. variabilis* it was reported at 0.16.

The ginsenoside Rb1 was positively correlated with soil organic carbon, calcium, zinc, air temperature, light intensity, and iron and negatively correlated with available phosphorus, altitude and copper. The ginsenoside Rg2 was positively correlated with soil organic carbon, soil temperature and magnesium and negatively correlated with altitude, available phosphorus and copper.

Morphometric analyses could separate *P. assamicus, P. bipinnatifidus, P. variabilis* and *Panax* sp. into distinct species. Ecological niche characterization revealed that *P. bipinnatifidus* and *P. variabilis* have two distinct niches. However, *P. assamicus* and *Panax* sp. showed overlapping niches. Ecological niche modeling could segregate all the species distinctly. Molecular analysis through phylogeny of the 18S-26S rDNA ITS region, could separate all the species clearly. Biochemical marker could also separate the species based on the Rg2/Rb1 ratio. It was clear for *Panax* species with a value of 0.03. However, for other species, it was overlapping, hence needs further investigation with more populations.

Thus, the present study on *Panax* species complex could reaffirm the identity of most of the species of Himalayas. The extensive field survey in the entire north-eastern region provided a holistic distribution pattern of different *Panax* species of Himalayas. During the process, the study could locate a morphologically divergent species that might be a new species. The study has also proved the efficiency of ENM in predicting the potential distribution areas of a species. The study has demonstrated that based on niche breadth and fundamental niche of the species, it is possible to segregate the species. Thus, the study concludes that a combined approach encompassing morphometric, ecological niche characterization, biochemical marker and molecular phylogeny would yield better result in establishing species identity than following any of these approaches individually.