

ECOLOGICAL NICHE MODELING PREDICTS THE HABITAT SUITABILITY AGAINST THE FUNCTIONAL TRAITS IN DIFFERENT ECOTYPES OF *NOTHAPODYTES NIMMONIANA* (GRAHAM) MABB.

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ABSTRACT

Ecological niche models serve as one of the implicit tools to analyse the adaptive landscape traits of the species, indicating that the habitat predicted as highly suitable would support maximum fitness. In this study, we have used Maximum Entropy method to predict the habitat suitability of ecotypes of *Nothapodytes nimmoniana*. For the first time, the observed synonymised species of *N. nimmoniana* from Western Ghats were considered as five different ecotypes and were evaluated independently. The predicted habitat suitability was tested with the functional plant traits such as fluctuating leaf asymmetry (FLA) and specific leaf weight (SLW), towards the population fitness. The results show that the ecotypes in the highly suitable sites exhibited significantly low FLA and high SLW compared to ecotypes that are present in the poorly suitable habitats. This study also provides valuable insights into selection of highly suitable ecotypes for conservation and restoration against the indiscriminate wild extraction.

Keywords: *Ecological Niche Modeling, MaxEnt, Ecotypes, functional traits*

I. INTRODUCTION

With an ever increasing global pharmaceutical demand for medicinal plants, there has been a scrupulous loss of many species from their natural habitats apart from the impact of the global climate change, thereby effecting the structure and the functioning of the ecosystem (Parmesan & Yohe, 2003; Feeley et al., 2012). Plants during their growth and development are in constant interaction with the abiotic and biotic

components of their ecosystem (Hutchinson, 1959). The set of ecological conditions that support the survival, growth, reproduction and population density of the species is referred to as “ecological niche of a species” (Grinnell, 1917). These environmental conditions play a key role in governing the geographic distribution and growth performances of the plant species (Shivaprakash et al., 2013; Silva et al., 2016).

Ecological niche models (ENM) help in predicting species specific habitats where it can thrive best on range of probability scale from low suitable to highly suitable habitats (Joseph & Stockwell, 2002; Hirzel & Lay, 2008; Pearman et al., 2008). These predictions serve as a platform to locate rare and endangered species, prospecting for habitats for species restoration, predicting spread of invasive species etc. (Hijmans & Spooner, 2001; Craig & Robertson, 2011; Shivaprakash et al., 2013; Peterson & Vieglais, 2001; Peterson, 2003; Sarma et al., 2015). According to Silva et al. (2016) the plants growing in stressful environments show developmental instability pertaining to their growth, physiology and metabolism. Thus reflecting the fact that the species growing in a niche predicted as good by ENM show higher fitness compared to their con-specific populations in poorly suitable sites (Shivaprakash et al., 2013).

In plants, leaves serve as the main seats of photosynthesis that show case vivid plastic responses in fluctuating growth conditions like varying ranges of lamina morphology, mesophyll content and physiological characteristics (Jurik, 1986). Specific leaf weight (SLW) is the leaf weight per unit leaf area, which is basically a measure of the mesophyll development (Shivaprakash et al., 2013). It helps to determine the plant nitrogen status, carbon content, light and several other stresses (Field & Mooney, 1986; Grossman et al., 1996). When the plant is in an ambient environment the plant shows an increased mesophyll layer development adding to the leaf thickness (Turrel, 1936; Nobel et al., 1975). The dense packing of the chloroplast indicates increased photosynthetic efficiency thereby enhancing food resource supply for the better growth and reproduction thereby indicating a positive correlation with the fitness of the plants (Shivaprakash et al., 2013).

Fluctuation leaf asymmetry (FLA) is performed in plants to assess the developmental stability (Palmer & Strobeck, 1986). During plant growth and development, the ability of the leaf blades to attain equal development on both the sides of the midrib in any given environmental condition shows the species is developmentally stable (Zakharov, 1992). According to Stige (2004) the developmental stability is exhibited by organisms with fit genetic makeup and those that grow in a less stressed environment. Similarly plants that are being exposed to abiotic and biotic stresses show developmental instability where the impaired metabolism and physiology are manifested in the form of retarded growth and development (Hódar, 2002; Cornelissen & Stiling, 2011; Alves-Silva & Del-Claro, 2013; Choudhury et al., 2014). Several studies have pointed out that plants growing in stressful conditions like pollution (Kozlov et al., 1996), varying light conditions (Puerta-Piñero et al., 2008, Silva et al., 2016), herbivory (Cornelissen & Stiling, 2011), varying nutrient concentration (Santos et al., 2013) etc. show high FLA. Hence FLA can be used as a biological quality check for species as well as environmental pointer to help identify habitat suitability (Mal, 2002).

Nothapodytes nimmoniana is a small native tree seen widely growing in the warmer regions of the Indian subcontinent (Suhas et al., 2007). The plant known worldwide for its anti-cancer compound - camptothecin (CPT) has been mercilessly extracted from its natural habitat in India and is exported globally pushing it to the vulnerable status (Kumar & Ved, 2000). The survey of literature and field observations have supported the fact that the populations of *N. nimmoniana* exhibited high degree of intra-specific variations (Hombegowda et al., 2002; Shivaprakash et al., 2014; Suhas et al., 2007). Such variations are observed mainly due to the existence of five different synonymised species within this region i.e. *Mappia foetida*, *M. oblonga*, *M. ovata*, *M. tomentosa* and *M. wightiana*. This synonymy was mainly due to the transfer of old world *Mappia* species to a single Asian genus *Nothapodytes* by Howard (1942).

In the present study the rationale was to analyse the five synonymised species of *N. nimmoniana* as different ecotypes against their habitat suitability using the ENMs. These predictions were correlated with functional plant traits like SLW and FLA which would help in identifying a fit ecotype among the populations. Thus this study would help unravel the elite ecotype that could be reintroduced in suitable habitats and can also be exploited for cultivation, conservation and capitalization purposes.

II. MATERIALS AND METHODS

2.1. Sample collection

Leaves of five ecotypes were collected based on their type localities from different parts of Western Ghats (Figure 1). The distribution of the ecotypes was mapped using primary data which was recorded using Global Positioning System (GPS) Garmin GLX-12 during the field visits from 2014 to 2017. The spatial map was generated using the GIS software MapInfo Professional v7.1 (Ganeshaiyah et al., 2003, Yethish 2006). The fitness surrogates like FLA and SLW were measured for each ecotype respectively.

2.2. Fluctuating leaf asymmetry (FLA)

Ten mature leaves were randomly collected from five trees for each ecotype. Each leaf was taken and the mid-point of the leaf was found using the leaf length position. The distance between the midrib (from the mid-point) and the right side half of the lamina was measured and similar measurements were carried out for the left side half of the lamina (Kozlov et al., 1996). Fluctuating leaf asymmetry was then calculated as: $FLA = (L-R)/Size$, where L = width of the left side, R = width of the right side; $Size = (L+R)/2$ (Palmer and Strobeck, 1986). Further, the frequency distribution of fluctuating leaf asymmetry was tested and the significance evaluated.

2.3. Specific Leaf Weight (SLW)

Randomly five fully matured leaves were collected from three trees of each ecotype. Care was taken to collect healthy ones. Five circular leaf discs were bored in the middle of the leaf lamina using cork borer of 1 cm diameter. The leaf discs were weighed directly before they get dried to avoid the loss of water content. SLW was calculated as follows:

$$SLW = \text{Weight of leaf disc in mg/area of hole-punch in mm}^2$$

The mean difference in specific leaf weight was compared and statistically evaluated based on one tailed t-test. Further, the frequency distribution of specific leaf weight was calculated.

2.4. Environmental data

For developing Ecological Niche Models, 19 Bioclimatic variables were used from Worldclim database version 1.4 (Table 1, www.worldbioclim.org). The data was downloaded from Intergovernmental Panel for Climate Change (IPCC) 3rd Assessment (www.ccafs-climate.org/data). The Worldclim data are at a spatial resolution of 1 km² (30 arc seconds). From the ENM analysis using the presence data a receiver operating characteristic (ROC) curve was derived with mean and standard deviation of the Bioclimatic variables for the ecotypes of *N. nimmoniana*.

2.5. Ecological niche modeling

Based on a number of field surveys undertaken over a period of three years (2014 to 2017) in the Western Ghats, the latitude and longitude of 25 sites of the ecotypes were recorded using GPS. Primary presence data was used for modeling the species distribution using Maxent (Ganeshiah et al., 2003).

Maxent (Maximum Entropy; version 3.3.2) - which included 19 Bioclimatic variables of Hijmans et al. (2005) were used for niche modeling. These variables represent the fundamental requirements for the species survival which is a combinations of temperature and precipitation (Ganeshiah et al., 2003). Species occurrence data was obtained from both literature and field survey. But the presence data (25 sites) was only used for calibration of model and the other set for evaluation of model performance. The default setting were used while training the data in Maxent. The continuous probability scale and the measures were used from the output grid to derive the HSI values.

The fitness measures were evaluated based on the model predictions representing different habitat suitability indices for the five ecotypes. For the purpose of analysis, 25 sites were taken as a continuum of habitat suitability indices. The sites are separated geographically ranging from 10 to 1500 km in the Western Ghats from Kanyakumari (Tamil Nadu) to Amboli Ghat (Maharashtra).

III. RESULTS

3.1. ENM predictions for *N. nimmoniana*

A spatial map was generated prior to ENM using 75 sites that includes 25 primary collections sites for five different ecotypes across Western Ghats (Fig. 1). Niche modeling was performed using the above points for 100 bootstrap replicates and summarized into an average as a grid file (Fig. 2a). Then, the grid was imported into DIVA-GIS where the percentiles of Maxent output were combined together with an equal intervals (of percentile of 20) and the habitat suitability were represented in five colours: Red - Orange (Highly suitable habitat), Yellow (Medium) and light green - green (poorly suitable habitats) (Fig. 2b & 2c).

Furthermore, the percentile values of the habitat suitability for respective ecotypes were derived from the pixels. (Fig. 2c)

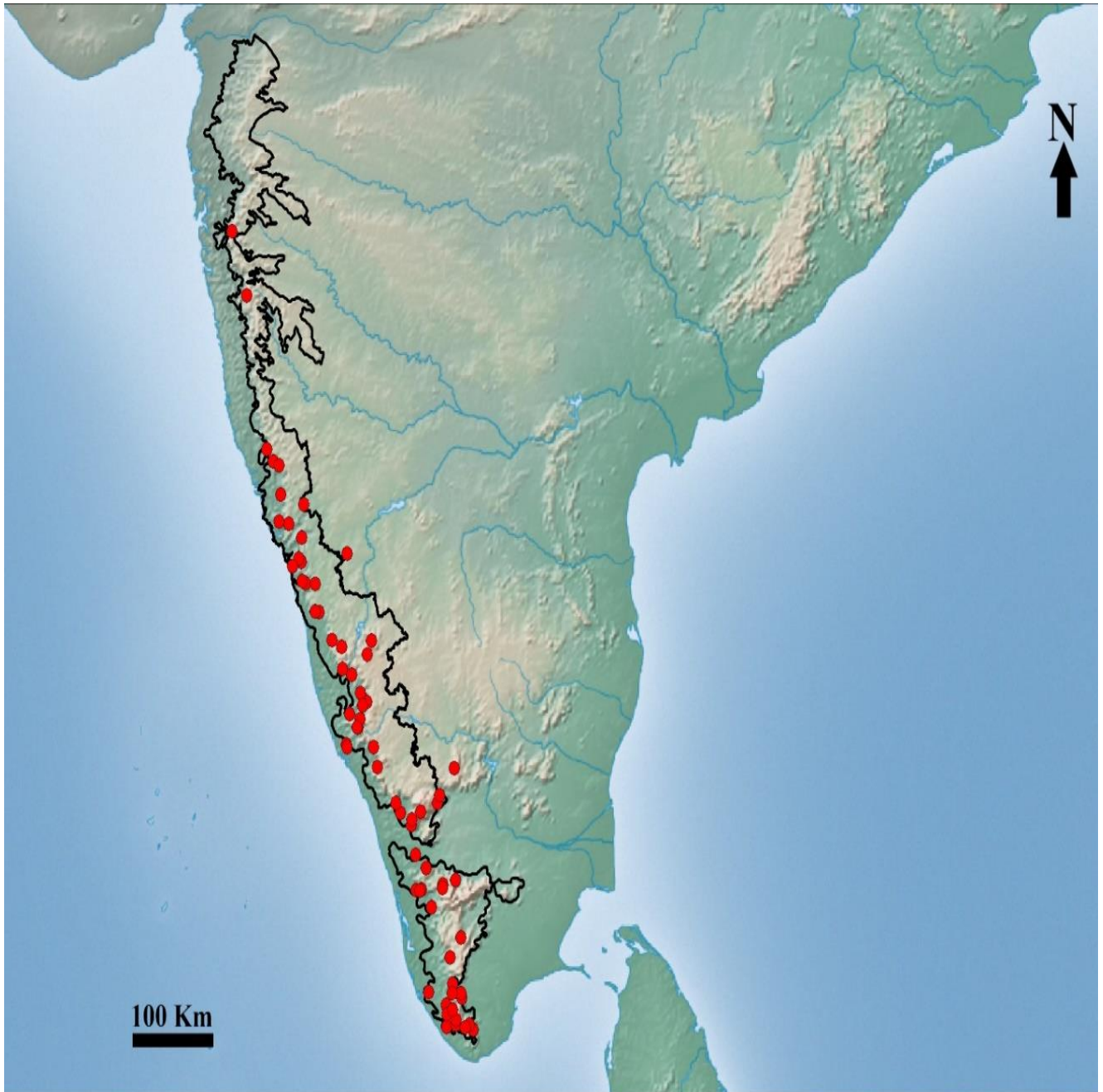


Figure 1. Map shows the spatial occurrence of *Nothapodytes nimmoniana* from Western Ghats

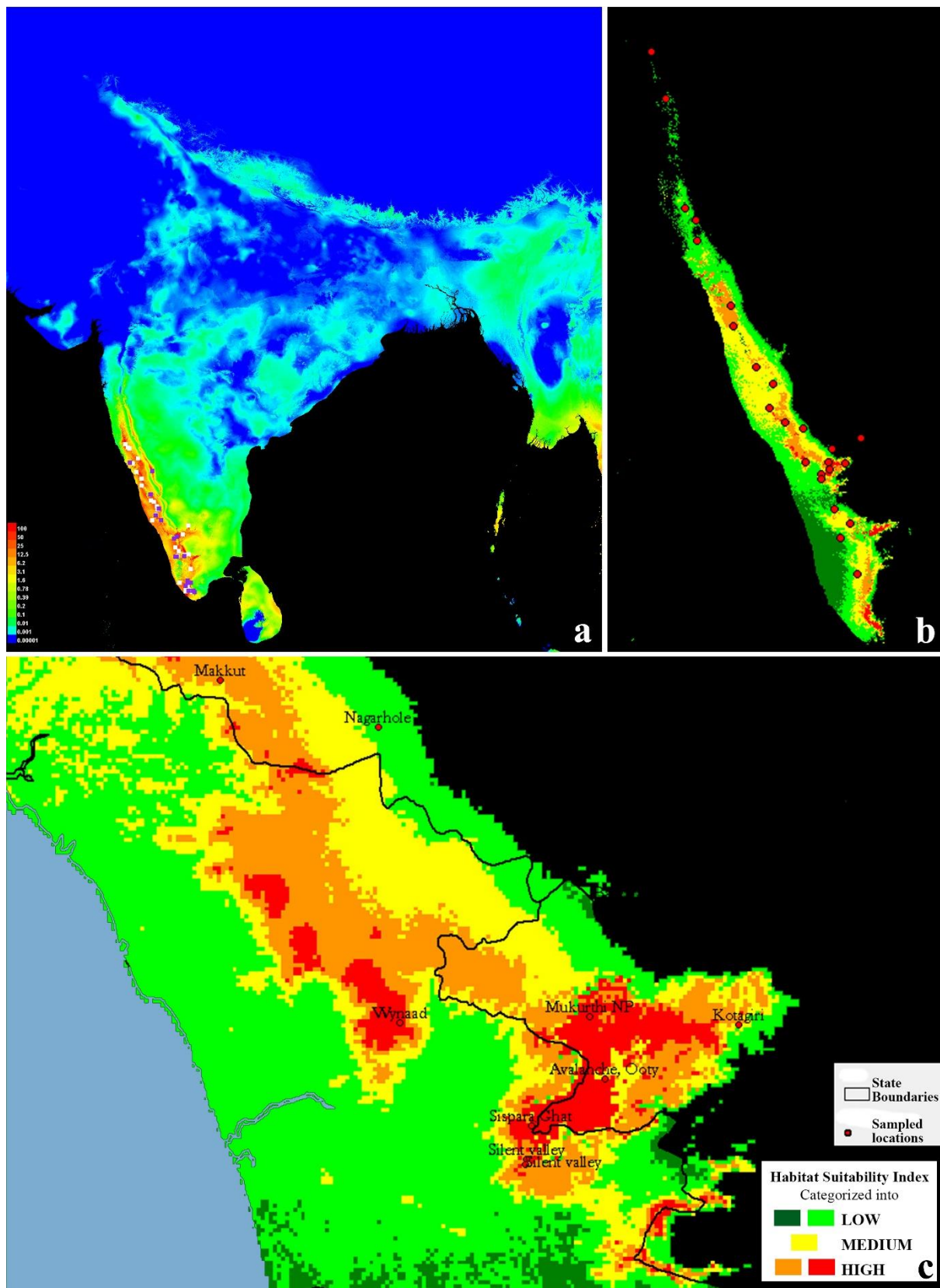


Figure 2. Ecological niche modeling predictions using Maximum Entropy Method (Maxent) showing Habitat suitability Index (HSI) for different ecotypes *N. nimmoniana* from Western Ghats.

3.2. Calibration of models

The model calibration test for *N. nimmoniana* yielded satisfactory results (AUC train = 0.997 ± 0.002 and AUC test = 0.992 ± 0.015 ; Fig. 3). Amongst the input environmental variables, temperature seasonality (BIO4), precipitation of the coldest quarter (BIO19) and Isothermality (BIO3) were the most influential and contributed 78.8% in Maxent model. The rest of sixteen layers of Bioclim collectively contributed 21.2% to the habitat model of the species of which annual precipitation had maximum contribution (3.7%), while the layers from BIO13 to BIO16 (of precipitation) collectively contributed to 10.5% (Table 1). Considering the permutation importance, precipitation of both the month (BIO13) and the quarter (BIO16) have influence on the habitat model and contributed to 85.7%, while BIO3, 4 and 19 together contributed to 6.7% (Table 1).

Table 1. Analysis of environmental variables for ecotypes of *N. nimmoniana*

Environmental variable	Percent contribution	Permutation importance
Temperature seasonality (Bio4)	51.6	1.8
Precipitation of coldest quarter (Bio19)	20.3	2.1
Isothermality (Bio3)	6.9	2.8
Annual precipitation (Bio12)	3.7	1.9
Precipitation of driest month (Bio14)	2.9	1.1
Precipitation of wettest quarter (Bio16)	2.8	17.6
Precipitation seasonality (Bio15)	2.6	0.3
Precipitation of wettest month (Bio13)	2.2	68.1
Mean temperature of coldest quarter (Bio11)	1.7	0.9
Mean monthly temperature (Bio2)	1.3	0.3
Annual mean temperature (Bio1)	1.3	1.6
Temperature annual range (Bio7)	0.8	0
Precipitation of driest quarter (Bio17)	0.6	0.1
Maximum temperature of warmest month (Bio5)	0.5	1
Mean temperature of wettest quarter (Bio8)	0.4	0
Maximum temperature of coldest month (Bio6)	0.3	0.1
Precipitation of warmest quarter (Bio18)	0.1	0.2
Mean temperature of warmest quarter (Bio10)	0.1	0.1
Mean temperature of driest quarter (Bio9)	0	0.1

The results of the Jackknife test for the importance of variables indicate that the environmental variable (BIO4) shows the highest gain in the regularized training among the nineteen Bioclimatic variables when it was used in isolation. However BIO4, the temperature seasonality showed consistently high gain in jackknife analysis of train, test as well as in AUC (Fig. 4).

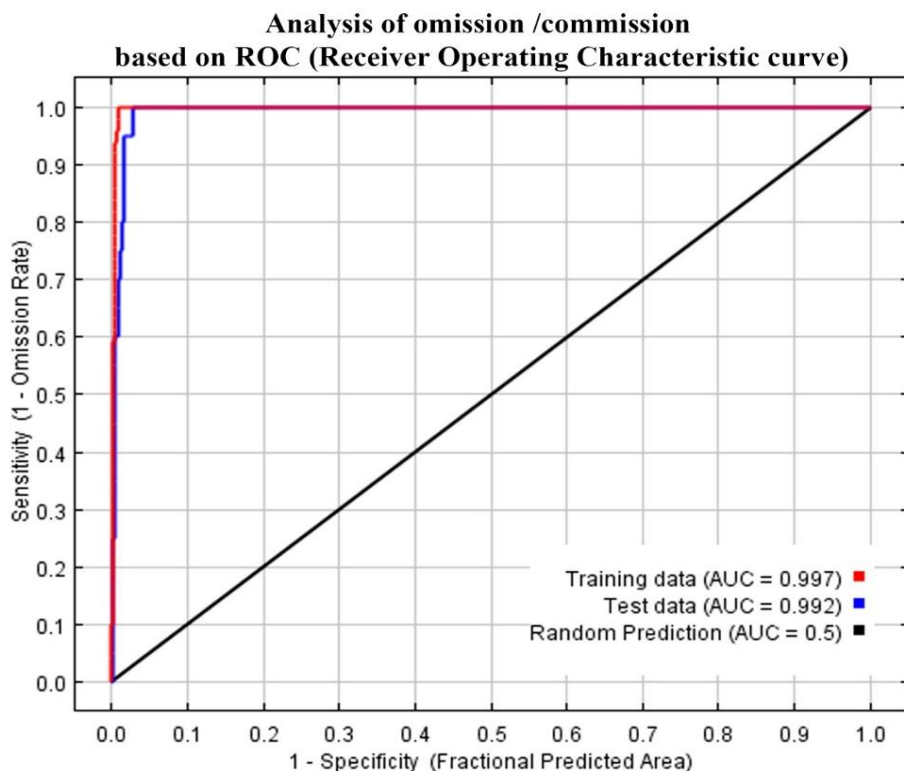


Figure 3. Analysis of omission/commission based on ROC (Receiver Operating Characteristic) curve that shows significance of AUC (Area Under Curve) between test and train data for *N. nimmoniana*

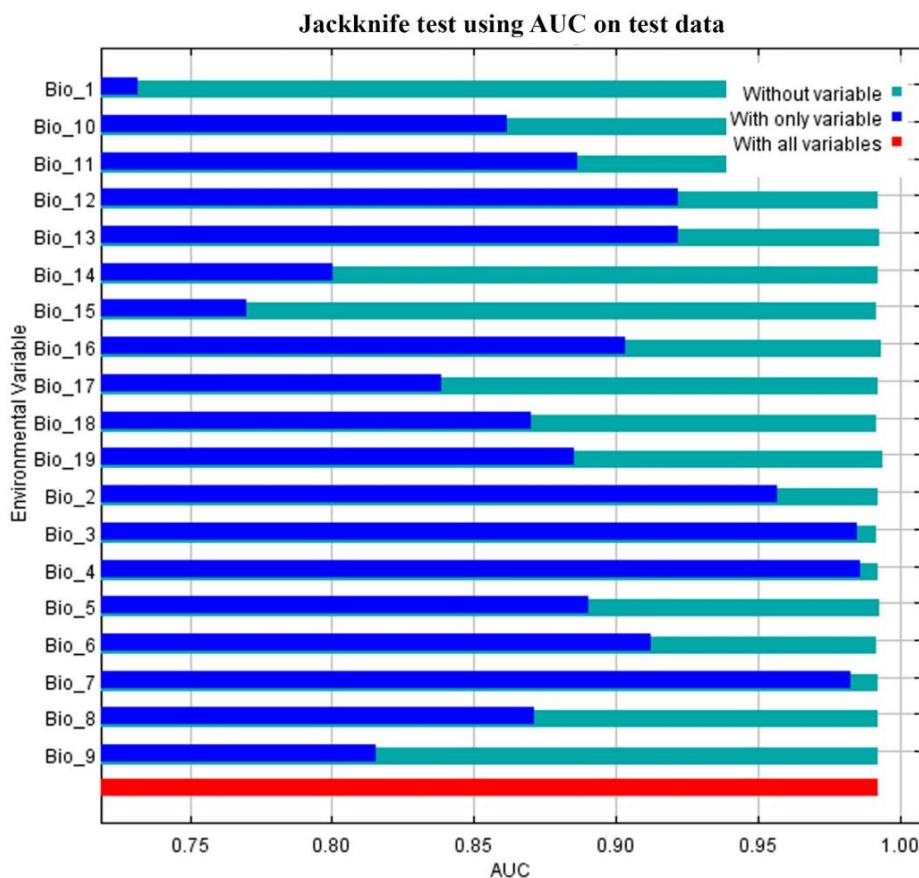


Figure 4. Bar diagram indicates the significance of AUC using test data against the Bioclimatic variables based on Jack-Knife simulations.

3.3. Correlation of ecological attributes against niche modeling predictions

3.3.1. Fluctuating leaf asymmetry (FLA)

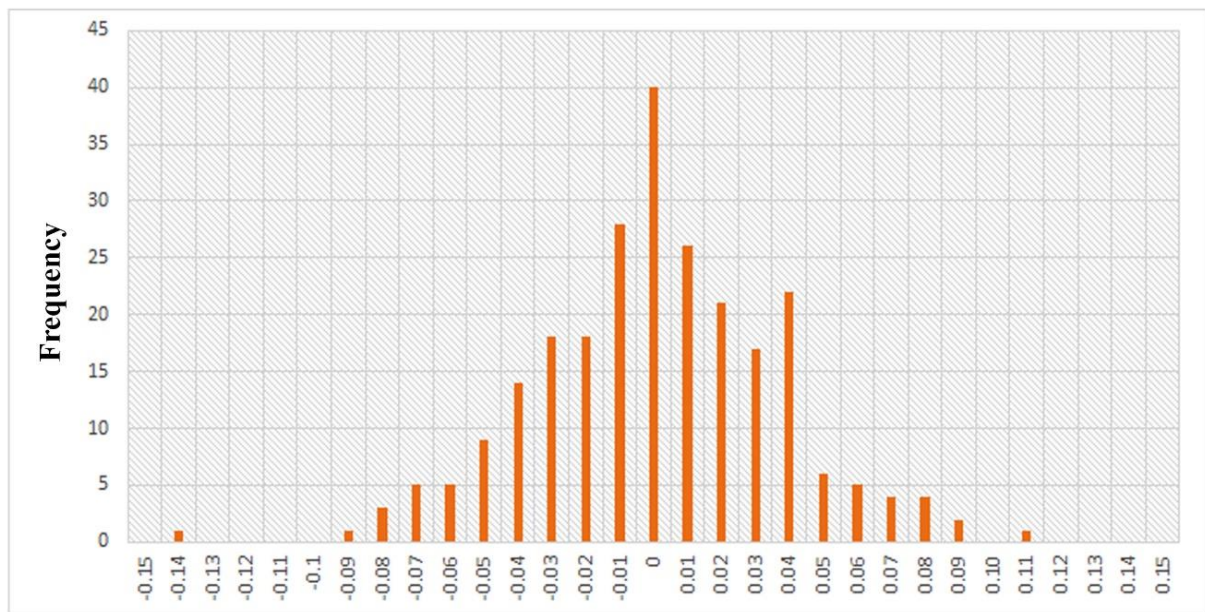
Fluctuating leaf asymmetry for all 25 individuals was grouped together and plotted to see the nature of asymmetry in *N. nimmoniana*. The frequency distribution of FLA across 250 leaves of 5 ecotypes is shown in Fig. 5a. The deviation of laminal length from mid-rib at both left and right side of the leaf ranged from -0.14 to 0 cm (at right side of the leaf) and from 0 to 0.11 cm (at left side of the leaf) as shown in Fig. 5a. Mean deviation of the symmetry in laminar growth was 0.0178 ± 0.004 cm in *M. foetida* and 0.01 ± 0.009 cm in *M. ovata* that falls into low suitable niche categories (Fig. 5b), whereas the mean fluctuating leaf asymmetry significantly decreased (0.006 ± 0.005 in *M. wightiana*) in highly suitable habitat (Fig. 5b).

The distribution of individuals with perfect leaf symmetry was segregated and grouped across three niche categories (Fig. 5b). Among the individuals (n=25) 35% of individuals were from excellent, 30% from medium and 35% were from low suitable niche categories. The significance of FLA among the ecotypes of *N. nimmoniana* against the habitat suitability index (HSI) yielded a result of P-value - 0.00001 based on Pearson correlation (Fig. 7a), which is significant at $p < 0.01$ ($R = 0.9683$, $n = 25$).

3.3.2. Specific Leaf Weight (SLW)

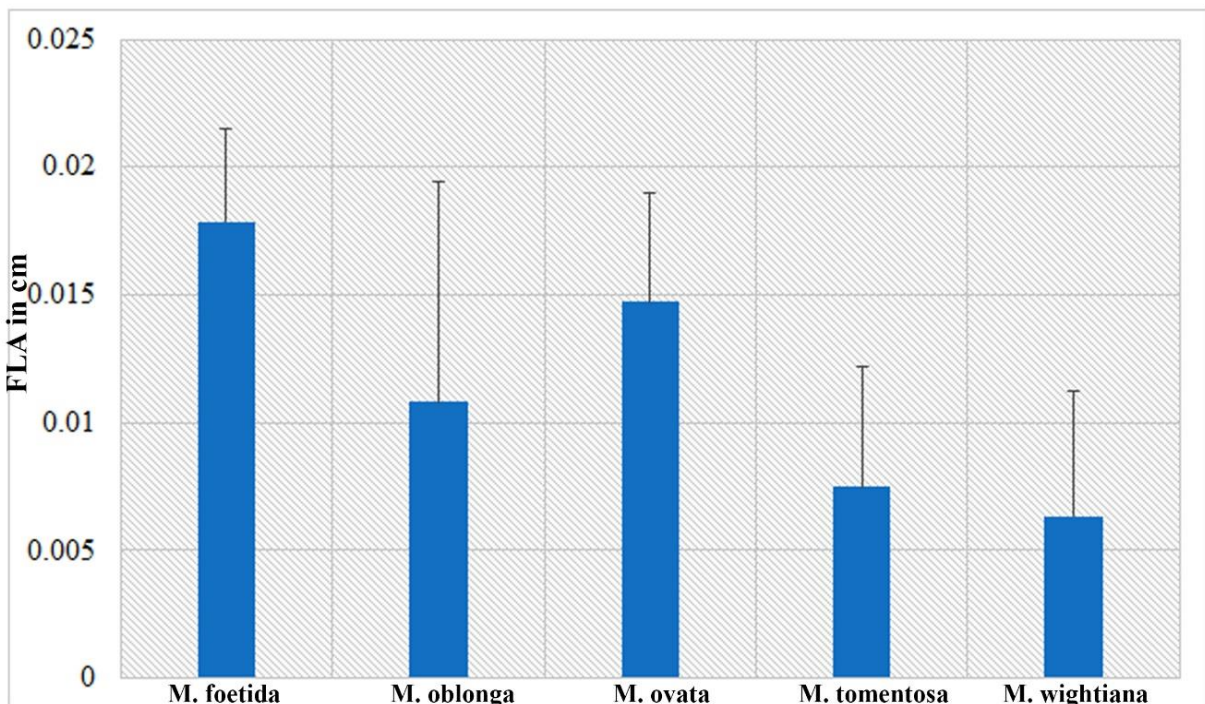
The frequency of SLW using 375 leaf discs from 15 individuals showed that the 76% of the leaf discs across ecotypes ranged from 5-9 mg/mm² (Fig. 6a). Mean specific leaf weight (leaf mass/ leaf area) was 9.68 ± 4.4 mg/mm² in excellent, 6.02 ± 1.68 mg/mm² in medium and 4.78 ± 0.4 mg/mm² in low suitable niche categories (Fig. 6b). The results clearly show that mean specific leaf weight was significantly higher in highly suitable habitat (*M. wightiana*) compared to medium (*M. oblonga*) and low suitable (*M. foetida*) niche categories.

Among the individuals of the five different ecotypes (n=15), about 30% of individuals were from excellent while about 40% from medium and about 30% of individuals were from low suitable niche categories respectively. The significance of SLW among the ecotypes of *N. nimmoniana* against the habitat suitability index (HSI) showed the P-value of 0.00001 (Fig. 7b), which is significant at $p < 0.01$ ($R = 0.968$, $n = 15$).



a

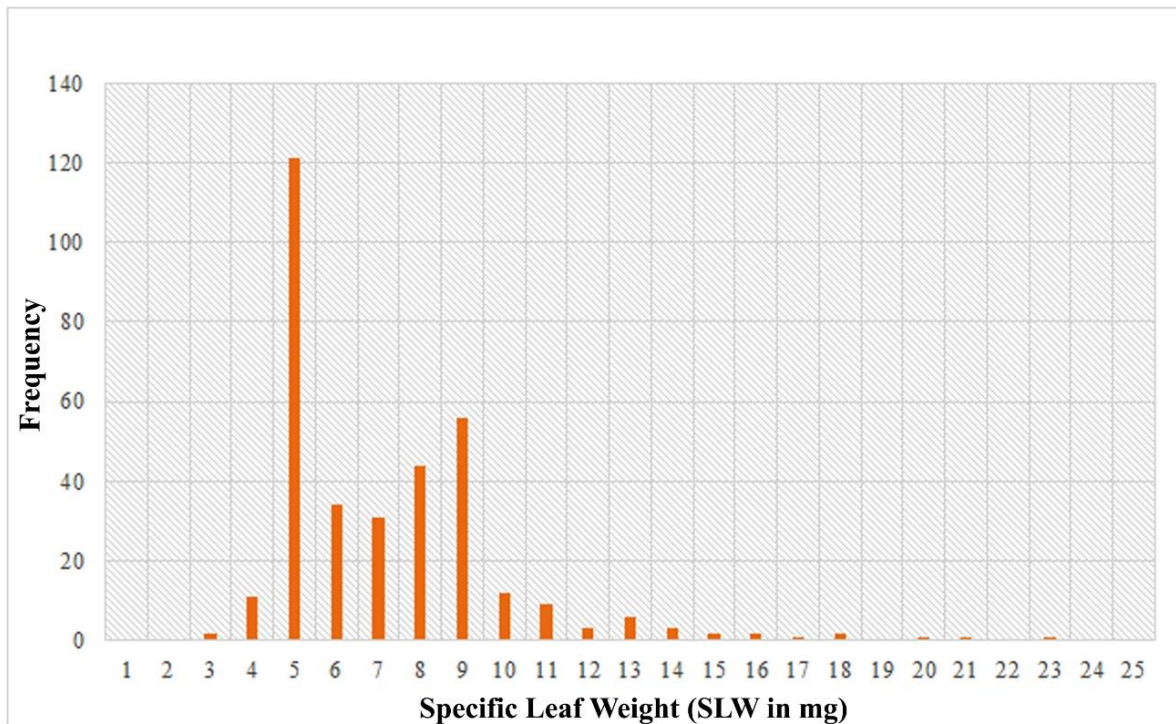
Fluctuating Asymmetry (in cm)



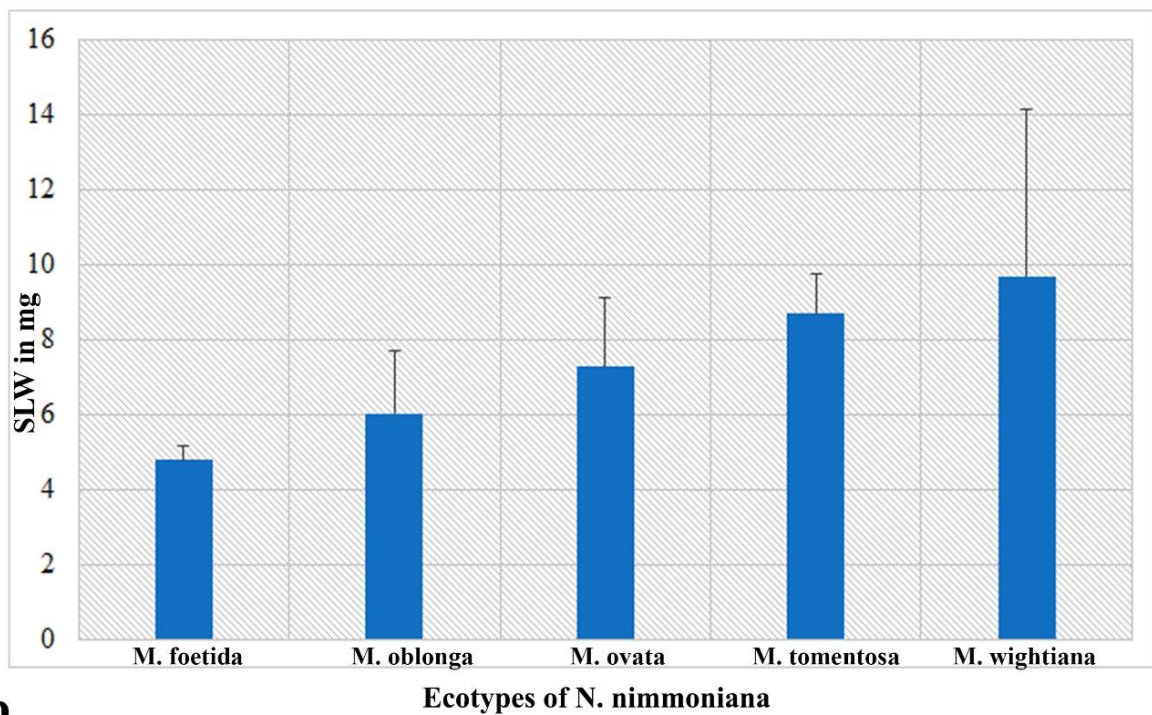
b

Ecotypes of *N. nimmoniana*

Figure 5. Analysis of the functional trait- Fluctuating Leaf Asymmetry (FLA) in *N. nimmoniana*:- a. Frequency of FLA across different ecotypes of *N. nimmoniana*, b. Correlation between Fluctuating leaf asymmetry and ecotypes of *N. nimmoniana*.



a



b

Figure 6. Analysis of the functional trait- Specific Leaf Weight (SLW) in *N. nimmoniana*:- a. Frequency of SLW across different ecotypes of *N. nimmoniana*, b. Correlation between Specific Leaf Weight and the ecotypes of *N. nimmoniana*.

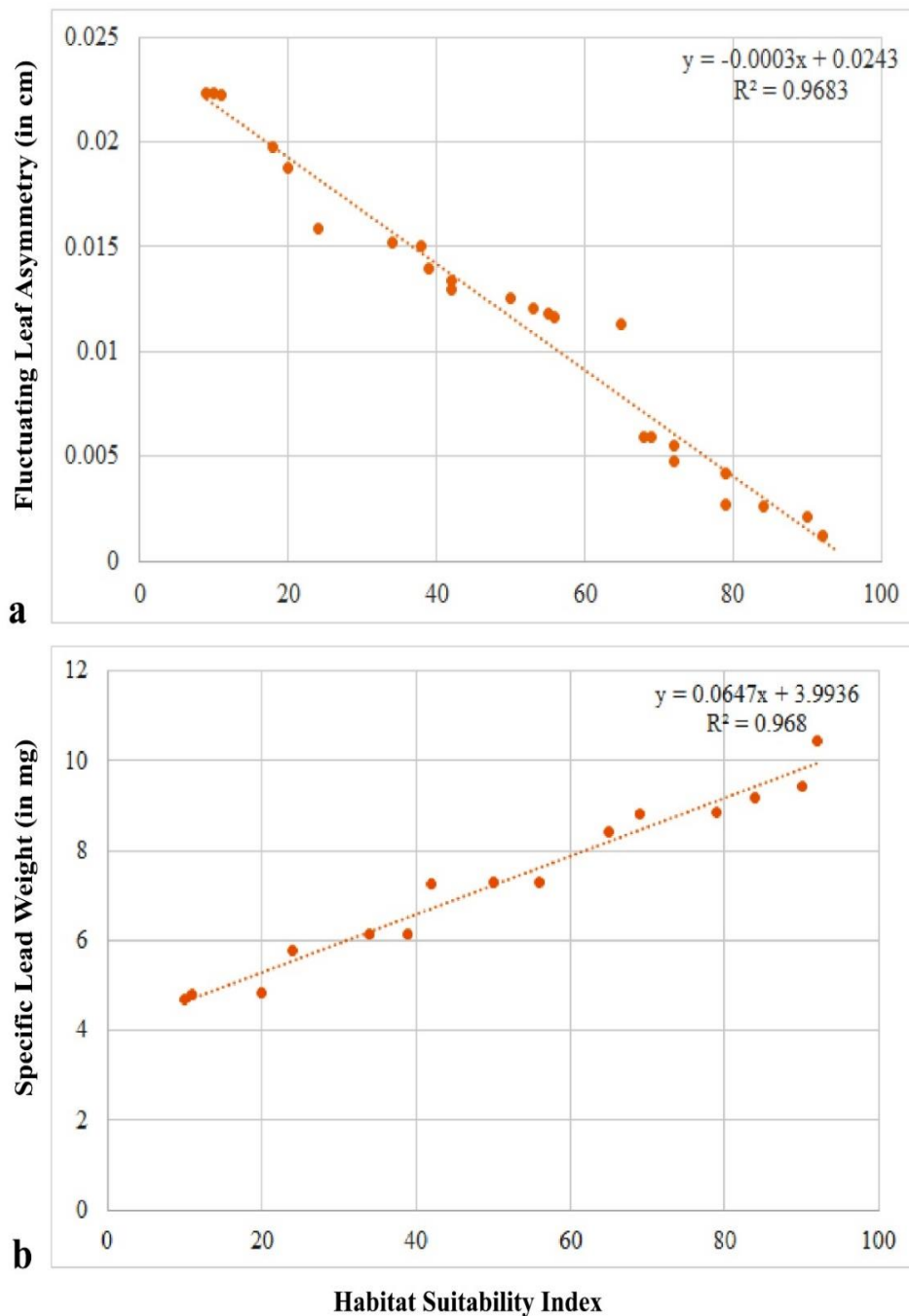


Figure 7: Correlation of the functional traits against the different ecotypes of *N. nimmoniana*. a. Correlation of fluctuating leaf symmetry and habitat suitability index show the significant linear regression at $p < 0.01$ ($p < 0.00001$); b. Correlation of specific leaf weight against habitat suitability index show the significant P-value (< 0.00001) at $p < 0.01$.

IV. DISCUSSION

4.1. Habitat structure

The five ecotypes of *N. nimmoniana* identified in the Western Ghats occurred in the areas of floristically rich wet evergreen/disturbed wet evergreen/semi-evergreen and deciduous forest types (Fig. 1). All the ecotypes were found in the high elevation habitats except for *M. foetida* which was only 100m above

the cut-off limit (i.e.) low elevation forest types of Courtallam, and Sirsi. Floristically these forests showed disturbed formations with common associated species such as *Agailea* spp., *Phyllanthus polyphyllus* and *Lea indica*. Many differences were found in the habitat structure where these ecotypes exist along the Western Ghats. However, these ecotypes were distant to each other and restricted to smaller pockets of forests such as *M. wightiana* and *M. tomentosa* belonged to Nilgiri biosphere reserve whereas *M. ovata* from Amboli Ghat. But, *M. foetida* is widespread and have been found throughout Western Ghats and Sri Lanka. In general, *N. nimmoniana* survives under a varied range of light levels and regenerates naturally well in those areas, but show more population density in wet evergreen forests than deciduous forests as reported by Ramesh et al. (2008).

4.2. Ecological niche predictions

The predictive distribution map developed for Western Ghats adopting Maxent agreed with the observed distributional pattern of the species. Only hilly slopes of the Western Ghats with high rainfall (Nilgiri biosphere reserve) were predicted to be suitable for the species (Fig. 2). This indicates high habitat-specificity and restricted distribution of certain ecotypes (*M. wightiana* and *M. tomentosa*). The excellent niche for the occurrence of *M. wightiana* and *M. tomentosa* (high percentile with red colour pixels in the map) was indeed corroborated in the field study. Apart from these, hill slopes of Silent Valley, Neliampathy, Wynad were also predicted to be of high-suitability (Orange). One of the basic assumptions of the ecological niche modeling is that the areas identified as 'highly suitable niche' support better growth and regeneration of the species. This result of the present study along with several other collateral studies on *Myristica malabarica*, *Dysoxylum malabaricum* and *Coscinium fenestratum* (Shivprakash et al., 2013; Thriveni et al., 2017; Priti et al., 2016), validates the fact that plants growing in highly suitable habitats were fit. Very high niche suitability was also predicted in many other adjacent geographical areas but the populations in these areas were not assessed in the field study, due to the sparse distribution and high orogenic nature of these sites. However, the predictions offer platform to undertake finer scale exploration of these areas and to identify remnant populations of the respective ecotypes, if any, and use the same as source for conservation before they become locally extinct.

4.3. Functional traits vs ENM predictions: Is there a correlation?

The present study evaluated the functional traits such as FLA & SLW with the augury on the habitat suitability for the ecotypes of *N. nimmoniana* by ENM. Fluctuating asymmetry is a widely used to measure developmental instability (Palmer and Strobeck, 1986). According to Shivaprakash et al., 2013, these instability could be either genetical (developmental lethals) or environmentally caused (stressful environment). Our study showed that individuals occurring in highly suitable habitats were significantly less asymmetric in their leaf morphology (*M. wightiana*) compared to those in poorly suitable habitat (*M. foetida*). In other words, it appears that individuals and hence ecotypes in highly suitable habitats might be more "fit"

than those from the poorly suitable habitat. The difference in FLA between the habitats might reflect the underlying genetic adaptations of respective ecotypes. Several studies have reported a positive association between FLA and environmental stress (Wilsey et al., 1998; Valkama and Kozlov, 2001; Nagamitsu et al., 2004; Shivaprakash et al., 2013).

Specific leaf weight (SLW), is the extent of mesophyll development that determines the photosynthetic capacity of a leaf, although other intracellular factors may also influence photosynthetic capacity (Jurik et al., 1977). Clearly a higher SLW can be expected to contribute to the net fitness of a plant through enhanced resource supply for seed production. A significantly higher SLW of plants in the highly suitable habitat compared to plants in the poorly suitable habitats once again indicates the relative adaptive landscape traits of the plants from wet-evergreen (*M. wightiana*) to deciduous forests (*M. foetida*). Similarly, the percentile contribution of Bio4 and Bio13-16 from the ENM predictions shows that the precipitation influences the fitness in highly suitable sites (wet-evergreen) compared to low suitable habitats (deciduous). Besides, contributing to the net photosynthates, SLW can also contribute to deterring herbivore damage thereby conferring fitness gains (Steinbauer et al., 1998; Howlett et al., 2001).

It is therefore evident from the present study that not all regions in the Western Ghats are uniformly suitable for the ecotypes of *N. nimmoniana*. In fact within the Western Ghats, certain areas are highly suitable and others are unsuitable. Areas that have highest “habitat suitability index” would be ideal locations for either domestication/cultivation of the species or for the selection of elite germplasms for the discovery of phytochemicals. The outputs of this study, specifically the prediction of the ENM provide a robust platform for identifying sites for establishing such enrichment plantations or any other silvicultural practices under species recovery to avoid direct extraction of the species from the wild.

V. ACKNOWLEDGEMENT

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