# Ecology and Conservation genetics of *Atuna indica* and *Hydnocarpus longipedunculatus* - two rare and endemic trees in the Kerala part of the Western Ghats



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# ECOLOGY AND CONSERVATION GENETICS OF ATUNA INDICA AND HYDNOCARPUS LONGIPEDUNCULATUS - TWO RARE AND ENDEMIC TREES IN THE KERALA PART OF THE WESTERN GHATS

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(An Institution under Kerala State Council for Science, Technology& Environment)



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- : Ecology and conservation genetics of *Atuna indica* and *Hydnocarpus longipedunculatus* two rare and endemic trees in the Kerala part of Western Ghats
- : Kerala Forest Research Institute, Peechi.
- : i. Population survey and Mapping
  - ii. Population structure
  - iii. Population dynamics (Vegetative and Reproductive dynamics)
  - iv. Climatic and edaphic factors analysis in situ
  - v. Population genetics (Through DNA markers)
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#### ABSTRACT

A study on the ecology and conservation genetics of two rare and endemic tree species viz. Atuna indica and Hydnocarpus longipedunculatus of the Kerala part of the Western Ghats were carried out. The population survey enabled to locate three populations of A. indica in the evergreen forests at an altitude above 400. asl viz. Kakkayam, Kozhikode District and Nadugani Ghat of Malappuram District. Sample plots were laid and population structure and diversity of the species were analyzed. A total of 89 adult individuals of girth  $\geq$ 30 cm and 68 seedlings/ saplings were recorded from the three population sites of which 9 seedlings are below 1m and 59 are above 1m height. The age-wise distribution revealed a decrease in pre reproductive individuals (23%). The population diversity analysis showed relatively low IVI values for the species in three forest areas. During three year study period, only two trees were found flowering while monitoring 89 mature individuals of the species. The nature of extreme low number of flowering individuals among populations required in depth studies. Pest incidence were noted during flower/ fruit stages, resulted in extreme low seed output. Based on data generated (IUCN guidelines), an up gradation of conservation status is suggested from Endangered to the Critically Endangered (CR). The presence of moderate to high Nitrogen, low to moderate Phosphrous and moderate to high Potassium in the soil were identified as the edaphic requirement of the species in situ.

The population survey of *H. longipedunculatus* located two populations at Kulamavu forests in Idukki district. The two populations consist of 76 adult individuals with girth  $\geq$ 30 cm and 25 seedlings/ saplings in 1m height were recorded. The age-wise distribution revealed a moderate decrease in pre reproductive individuals (36%). The population diversity analysis showed

relatively low IVI values for the species in the two population sites. The assessment of as per IUCN guidelines, the species suggested for placing under Critically Endangered (CR). A moderate to high N, low P and a moderate to high K in the soil were identified as the edaphic requirement of the species *in situ*. The flower infestation, fruit damage by giant squirrels, poor seedling bank *in situ*, etc. the reproductive barriers of the species.

The percentage of polymorphic loci, total genetic diversity and genetic diversity within population were comparatively low for both species. However, the percentage of effective alleles, genetic diversity among population was higher than that of other woody species. In general, the diversity analysis pointing towards unfitness of the populations and subsequent low adaptiveness to the changing environments of the species. The gene flow among the populations of *Hydnocarpus longipedunculatus* has shown a peak value compared to other species of same kind indicating the thriving/ competing nature of the populations.

#### 1. Introduction

The Western Ghats of India is remarkable for its floristic diversity and endemism. At the same time it is considered as one of the threatened landscapes. It is estimated that the region holds around 7,400 flowering species, of which 15 per cent are currently facing multiple threats (Nayar *et al.*, 2008; Sasidharan, 2017). The forests of Kerala along southern Western Ghats are considered to be the most species rich area along with highest rate of endemism in the Western Ghats. Out of 4078 indigenous flowering plants recorded in the State, 1568 species are endemic to the Western Ghats with 553 species under various threat categories (Sasidharan, 2017). The endemic species in the flora of a geographical region reveal the biogeography of the area and serve as a centre of speciation with a key role in adaptive evolution. Moreover, loss of an endemic species, leads to simultaneous decline in the existing genetic resources.

Extinction of species is considered as one of the greatest threats to biodiversity. Unfortunately, many species are threatened due to anthropogenic activities such as habitat fragmentation, resource exploitation and global climate change. The alarming high rate of species extinction as high as 1,000–10,000 times is far beyond the estimated natural extinction rate. If the process continues in the same pace, we will be losing as many as 30–50 per cent of the species richness by mid-century (Myers, 1980; Chivian and Berstein, 2008). The populations of endemic tree species having few individuals are likely to experience genetic drift and are more vulnerable to extinction (Fischer and Matthies, 1998; Keller and Waller, 2002). Therefore, thrust has to be given for the conservation and management of endemic and threatened plants on a priority basis for their sustainable use.

Understanding plant rarity has been an important task among plant ecologists. There are many ways by which a species can become rare, and the process has diverse ecological consequences. According to Reveal (1981), plant rarity is a two-fold phenomenon, associated with ecology and biology of the species. The associative species in a community, relative dominance of each species, spatial and stratic distribution and size of population in general are the integral elements affecting rarity of species (Pascal, 1998, Pandurangan, 2003). The studies on floral biology help in understanding the breeding behaviour and reproductive biology of the species. This includes flowering features, pollination, anthesis, pollen viability and fertility, stigmatic receptivity, pollen-ovule ratio, rate of fertilization, among others. The dispersal and regeneration mechanisms along with habitat conditions are significant episodes regarding the dynamics of species. Insects and pests are other determining factors on the population behaviour of species. Defoliators and leaf eaters, fruit and seed borers, etc. are the major threats to the species distribution and survival (Pushpangadhan, 1992). As per the studies of Jose et al., (2014), out of 760 red listed flowering plants reported from southern Western Ghats, more than 500 species are yet to be studied for deriving any kind of conservation strategies. Several other reports on Rare, Endangered and Threatened (RET) plants of the Western Ghats, Peninsular India, southern Western Ghats including Kerala are also available (Blasco, 1979; Henry et al., 1979; Ramesh et al. 2003; Sasidharan, 2004, 2011, 2017). The available literature urges the need for conducting research towards conservation, sustainable management and effective utilization of the threatened plant resources in the Western Ghats.

Genetic information plays an important role towards developing management strategies and conservation efforts of threatened species with small effective populations. Genetic diversity is one of the three fundamental levels of biodiversity which influences both dynamism and long-term survival of populations. Low levels of genetic diversity generally associate with reduced fitness, such as high juvenile mortality, diminished population growth (Leberg, 1990), reduced immunity (Ferguson *et al.*, 1990) and ultimately, higher extinction risk (Frankham and Richard, 2005). One of the necessary consequences of evolution in small population is the loss of genetic variation via 'genetic drift'. The future evolutionary adaptation depends on the existence of available genetic variation; loss of variation reduces the adaptive potential. A second consequence of loss of genetic variation is that the number of homozygous individuals increases within a population which may lead to heterozygote deficiencies with a consequent reduction in reproductive fitness. Thus, maintenance of genetic variation is one of the fundamental requirements in initiating *in situ/ ex situ* conservation efforts and effective management of the surviving germplasm (Falk and Holsinger 1991; Hoelzel, 1992).

Understanding of the genetic variation within and among populations is one of the pre-requisites before initiating restoration programmes in any threatened plant populations (Hamrick and Godt, 1996). Recent advances in molecular marker techniques enable the utility of DNA sequences in addition to general profile fingerprinting tools and can provide deeper insights into the population genetic structure. Among the universal markers, Inter-Simple Sequence Repeats (ISSRs), have several advantages as a candidate tool for assessing genetic diversity (Gupta et al., 1994; Zietkiewicz et al., 1994). ISSR analyses are more specific than RAPD analyses, due to the longer SSR based primers, which enable higherstringency amplifications (Wolfe et al., 1998). The high stringency reduces reproducibility issues, a common criticism against Randomly Amplified Polymorphic DNA (RAPD) marker technique with decamer primers (Yang and Meerow, 1996). The shortcoming of ISSR markers, as with RAPDs, is that most bands are scored as dominant markers, giving no possibility to distinguish between homozygotes and heterozygotes directly at the loci level. However, ISSR markers have also demonstrated their potentiality as a hyper-variable marker with great potential in population genetic analysis (Ge and Sun, 1999; Culley and Wolfe, 2001).

In this context, the present study was carried out in two endemic and threatened tree species of southern Western Ghats viz. *Atuna indica* (Bedd.) Kosterm. and *Hydnocarpus longipedunculatus* Robi *et al.* 

### 2. Objectives

- 1. Survey and identification of populations of *Atuna indica* and *Hydnocarpus longipedunculatus* in the Kerala part of the Western Ghats.
- 2. Ecology of the species viz. population structure, diversity along with environmental factors *in situ*
- 3. Population dynamics covering vegetative and reproductive phenology, insect association including reproductive constraints faced by the species.
- 4. Population genetic analysis in the existing population of the species through DNA markers
- 5. To assess the causes of rarity and further to recommend appropriate management strategies towards conservation and sustainable utilization of the species.

## 3. Study area

The study areas were selected after referring species literature from district floras, herbaria and other leading publications. In addition, field experience of the investigators also immensely helped to locate the sites (Fig.1).

#### 4. Materials and Methods

#### 4.1. Species selected

Atuna indica (Bedd.) Kosterm., Reinwardtia 7: 423. 1969; Ratheesh Narayanan, Fl. Stud. Wayanad Dist. 344. 2009; Sasidh. & Sujanpal, Rheedea 21: 81.2011. Parinarium indicum Bedd., Ic. t. 109. 1868-1874; Hook. f., Fl. Brit. India 2:311.1878; Gamble, Fl. Pres. Madras. 437 (310). 1919.

Evergreen trees, bark brown, smooth, thin; blaze reddish. Leaves simple, alternate, stipulate; stipules free, lateral, lanceolate; petiole 6-12 mm long, stout, glabrous; lamina 17-21 x 5.5-7.5 cm, oblong, elliptic-lanceolate, elliptic-oblong or elliptic-ovate, base acute, apex acuminate or obtusely acuminate, margin entire, glabrous, chartaceous; lateral nerves 12-18 pairs, pinnate, prominent, intercostae reticulate, prominent. Flowers bisexual, white, in axillary or terminal corymbose racemes; bracts brown, hairy; calyx tube funnel-shaped, pubescent; lobes 5, imbricate; petals 5, white, inserted to the mouth of calyx tube; stamens many; filaments basally connate, anthers small; ovary adnate to the side of calyx tube, 2-celled, ovules 2, erect; style filiform, basal; stigma truncate. Fruit a drupe, 3.5-4 x 2.5-3 cm, ovoid, smooth.

The species has been Gazette notified as per biological diversity act, 2002 by the Ministry of Environment and Forests, New Delhi in the plant list on the verge of extinction.

#### Hydnocarpus longipedunculatus Robi, Sasidh. & Jose, Webbia, 69: 243-247, 2014.

Evergreen tree of 12 m height; Leaves alternate,  $9-25 \times 3-8$  cm.Oblong, margins entire; inflorescence pendulous, in axillary pseudocymes. Male flowers greenish white, fragrant; female flowers creamy white, fragrant; pedicel long. Ovules many, Fruit ovoid – oblong, apex beaked, pentangular, densely covered by stellate hairs. Pedicel to 4 cm long; seeds 5-9, angular ovoid to rounded, pale brown, smooth, glossy.

#### 4.2. Methods

#### 4.2.1. Population survey and mapping

Intense population survey was conducted throughout the Western Ghats of Kerala based on the literature and expert opinion, subsequently detailed distribution map of the species was prepared.

#### **4.2.2.** Population structure

The sampling quadrat size was worked out as per species area curve method to determine the vertical, horizontal, age wise distribution and crown projections of candidate species along with associate species in a forest community. Populations of the two species were studied in relives (sample plots) of 0.1 ha (50 m x 20 m) size. All trees having diameter at breast height (gbh)  $\geq$ 30 cm in the study plots were identified, enumerated and recorded. The gbh  $\geq 10$  cm was taken for individuals of A. *indica* at Kakkayam-Dam site population, as individuals displayed low diameter classes. Individuals within the gbh range 10 - 30cm were categorized as saplings, and gbh below 10 cm as seedlings. Seedlings were further divided into two classes; height > 100 cm as established seedlings and height < 100 cm as un-established seedlings (Swarupanandan and Sasidharan, 1992). Each candidate species was enumerated in quadrates covering a total enumerated area of 7000 m<sup>2</sup> according to their availability within the forest areas in order to arrive at a realistic conclusion on the relative abundance of the species in a community. The floristic diversity in terms of relative frequency, relative density, relative dominance and IVI of each individual of species with  $gbh \ge 30$  cm was calculated (Misra, 1968 and Sivaram *et al.*, 2006). The extent of occurrence and area of occupancy were estimated as per IUCN guidelines. The mature individuals were physically counted in the sampled and nonsampled areas.

*Density:* Number of individuals of a species per unit area gives its density (d). This is usually computed as trees per hectare (tr ha<sup>-1</sup>).

*Frequency:* The chance of finding a species at a particular area in a particular trial sample is called its frequency (f) and is expressed as the number of quadrats in which a species is found per total number of quadrats studied.

**Dominance** (Basal area): Cover is usually the area covered by crown or shoot area, or the stem. For trees and shrubs, the area occupied by the stem is taken as the cover and is known as the basal area. Basal area =  $\pi r^2$ ,  $r = gbh/2\pi$ .

The *Importance Value Index* (IVI): It is defined as the sum of relative density (rd), relative frequency (rf) and relative Dominance (rD) (Muller-Dombois and Ellenberg, 1974). This expresses the relative importance of species in a community. Thus, IVI = rd + rf + rD, where,

rd = (Density of the species) / (Density of the stand)

rf = (Frequency of the species)  $/ \sum$  (frequency of all the species)

rD = (Basal area of the species) / (Basal area of all species)

Strata was classified as per the height stands. Girth size was used to determine the age wise distribution. Populations were categorized into set of future, set of present and set of past depending upon the reproductive nature of the species. Crown projections were measured by four perpendicular radii of the tallest individuals of the species in the quadrat (Pascal, 1988; Parthasarathi and Sethi, 1997).

Extent of occurrence is the extent of distribution of a species within the shortest continuous imaginary boundary of the species. Area of occupancy is the area occupied by the species within its extent of occurrence wherein the species' satisfy its survival.

#### 4.2.3. Population dynamics

It covers both vegetative and reproductive stages of the species in their natural life cycle (Davy and Jefferies, 1981). Observations on vegetative dynamics were made for leaf initiation, growth, maturity, senescence and insect-pest associations. In reproductive dynamics, different episodes such as flowering, fruiting, seedlings including insect-pest, dispersal and regeneration phases were monitored and recorded (Murali and Sukumar, 1994; Daniel and Jayanthi, 1996; Vivek Menon, 2003; Jose *et al.*, 2000; Jose and Pandurangan, 2002; Jose *et al.*, 2003, 2004).

#### 4.2.4. Climatic and edaphic factors

The climatological data of the species covering atmosphere temperature (day and night -  $^{\circ}$ C) and atmospheric humidity (night -  $^{\circ}$ ) in three prominent seasons of a year (summer, monsoon and winter) were recorded and average values taken for representation. The edaphological data on soil texture (from three soil depth levels such as surface, 15 cm deep as middle and 30 cm deep as bottom), p<sup>H</sup>, major macro nutrients such as N, P and K; soil moisture content and temperature in three seasons of a year were recorded and average values represented (Bawa, 1983; Gupta and Malik, 1996).

#### 4.2.5. Population genetics

#### 4.2.5.1. DNA extraction and PCR amplification

The leaf samples (20% of the population) were collected from mature trees and stored in silica gel for DNA extraction. Total genomic DNA was extracted using modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1990) as well as using DNeasy Plant Mini Kit (Qiagen, Germany) for difficult samples. DNA quality and quantity were determined through NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, UK). The purity and integrity of DNA were later checked by performing 1.0 per cent agarose gel electrophoresis and documented (Syngene, UK). DNA samples

were diluted to 100 ng/µL and stored at - 20°C for further use. Amplification of genomic DNA was performed in a PTC-100 thermocycler (Biorad, India) in final volume of 20 µl reaction buffer containing 50-100 ng template DNA, 10X PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, 1 µM primer and 0.6 U Taq DNA polymerase (Invitrogen, Banglore). The ISSR primers selected for amplification were UBC-810, UBC-811, UBC-812, UBC-825, UBC-830, UBC-834, UBC- 890, Primer 1, Primer 2, Primer 3, Primer 5, Primer 6 and ISSR 6, obtained commercially from the University of British Columbia (Vancouver, Canada).The amplified products were resolved in 2 per cent agarose gel and documented using a gel documentation system (Syngene, UK).

#### 4.2.5.2. Data Analysis

Distinct, reproducible, well resolved fragments for each ISSR reaction were scored as present (1) or absent (0) and were recorded as part of a binary matrix. The data matrices obtained were analyzed using POPGENE version 1.31 (Yeh *et a.l.*, 1999). Genetic parameters such as percentage of polymorphic band(s) (PPB), observed number of alleles (*N*a), the effective number of alleles (*N*e), Shannon's genetic diversity index (*I*), gene diversity (*H*t), gene diversity within population (*H*s), Nei's genetic differentiation index among populations (*Fst*), and gene flow estimates between populations (*N*m) were determined. Genetic divergence between populations of the target species was also investigated using Nei's unbiased genetic distances and genetic identities (Nei, 1978). An analysis of molecular variance (AMOVA) was performed to estimate the variance components and their significant levels of genetic variation within and among populations using GenALEx version 6.5 (Peakall and Smouse, 2012). The unbiased genetic distance was utilized for the construction of a dendrogram using UPGMA (Unweighted Pair Group Arithmetic Mean) method in POPGENE program version 1.31. Principal coordinate analysis (PCoA) was performed using GenALEx version 6.5.

#### 5. Results

#### **5.1**. Atuna indica

#### 5.1.1. Population survey and mapping

The populations of Atuna indica were identified and located in the following sites;

1. Kakkayam Dam site ; N 11.5 54157°, E 75.919519°

(Malabar WLS, Peruvannamuzhi Range, Kozhikode Division), 14 km away from Kakkayam junction; Alt.,  $740 \pm 5$  m asl; the population is located in a semievergreen forest patch and habitats are found often disturbed by tourists.

2. Kakkayam-Charangad ; N 11.560327°, E 75.915842°

(Peruvannamuzhi Range, Kozhikode Division), 19 km away from Kakkayam junction; Alt.,  $740 \pm 5$  m asl; The population is located in an evergreen forest and habitats are negligibly intervened from human activities.

3. Nadugani Ghats; N 11.429483°, E 76.384216°

(Vazhikadavu Range, Nilambur North Division), 42 km away from Nilambur Town enroute to Goodallur town. Alt. 450 m asl; The population is located in an evergreen forest, in the fringes of State highway.

The extent of occurrence and area of occupancy of the species were estimated as 42.517 and 0.088 km<sup>2</sup> respectively.

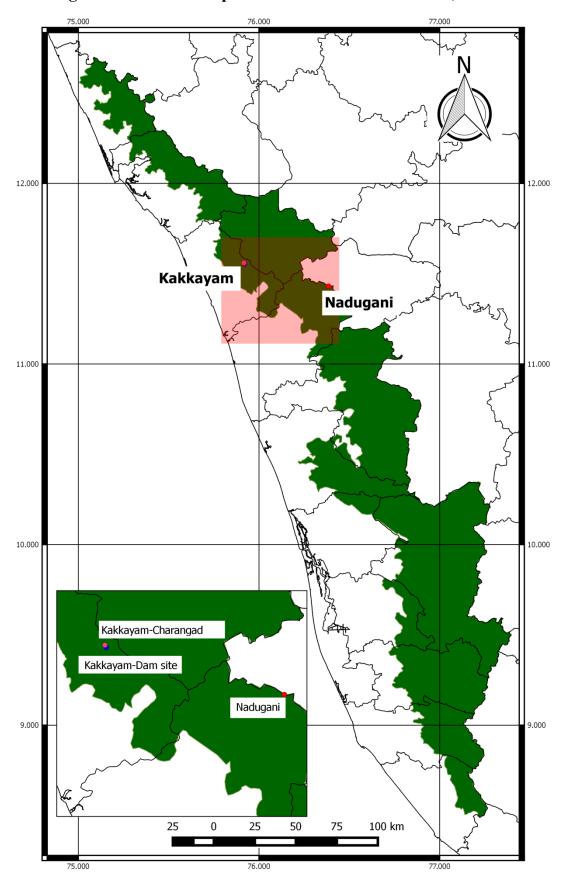


Fig.1. Atuna indica: Populations in the Western Ghats, Kerala

#### 5.1.2. Population structure

The population structure and floristic diversity analyses in the identified sites viz., Kakkayam -Dam, Kakkayam-Charangad and Nadugani are detailed below.

#### 1. Kakkayam -Dam site

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as Poeciloneuron indicum, Dipterocarpus indicus, Holigarna grahamii, Alstonia scholaris, Vateria indica, Artocarpus heterophyllus, Elaeocarpus serratus, among others as top layer/ first storey reaching a height range of 26 to 35 m. The second storey represented by species such as Syzygium mundagam, Aglaia barberi, Melicope lunuankenda, Knema attenuata, Symplocos racemosa, Bischofia javanica, Cinnamomum verum, Holigarna arnottiana, Persea macrantha, Schleichera oleosa, etc. with 16-25 m height range. The third storey occupied by Haldina cordifolia, Olea dioica, Memecylon umbellatum, Syzygium laetum, among others with 6-15 m height range along with Atuna indica. The shrubby layer consisted of major species' such as Humboldtia brunonis var. raktapushpa, Ixora brachiata, small plants of Vateria indica, Knema attenuata, among others. The herbaceous layer was mainly dominated by Cyanotis cristata, Commelina benghalensis, Dictyospermum montanum, etc. The horizontal profile of the population exhibited the arrangement of Atuna indica individuals in a scattered manner along with their associates, adjacent to the water course. The individuals of A. indica exhibited two age classes such as set of future (pre-reproductive individuals) and set of present (reproductive individuals) with a height range from 6 to 8.5 m and a diameter from 10 to 59 cm. Two individuals represented set of present with height of 7 and 8.5 m, gbh ranged from 48 to 59 cm respectively. While set of future was represented by ten individuals covering a height range of 6 to 8 m and gbh of 10 to 26 cm. The crown projections (vertical) showed the placement of individuals such as Bischofia javanica, Dipterocarpus indicus, Holigarna *grahamii, Persea macrantha,* etc. just below the tallest individual of *Vateria indica*. The crown projections (horizontal) had displayed an overlapping canopy coverage for these species under the canopy of the tallest individual, *Vateria indica*. Natural regeneration was very low and seven seedlings of height < 100 cm were counted in the particular forest area.

The population structure of *Atuna indica* in the forest area was analysed by recording gbh, basal area, basal cover, age phases and height of each individuals (Table 1). The floristic diversity analysis covered individuals of forty four species with gbh  $\geq$ 30 cm size in 7000 sq.m. (Table 2). The aggregated values of relative frequency (rf), relative density (rd) and relative dominance (rd) of each species in the quadrat were worked out and noted that *Vateria indica* had the highest index value of 0.3092 and thus became the dominant species in the particular quadrant, whereas, *Atuna indica* represented 26<sup>th</sup> position with IVI of 0.0421.

#### ii . Kakkayam-Charangad

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as *Vateria indica, Hopea parviflora, Bishofia javonica, Elaeocarpus serratus,* etc. as top layer/ first storey reaching a height range of 26 to 35 m. The second storey represented by *Schlecherea oleosa, Cinnamomum verum, Holigarna arnottiana, Dimocarpus longan, Sterculia guttata* etc with 15-26 m height range. Third storey occupied by *Haldina cordifolia, Olea dioica, Memecylon umbellatum, Garcinia morella,* etc with 6-15 m height range along with *Atuna indica. Humboldtia brunonis* var. *raktapushpa, Ixora brachiata, Vernonia* sp. *etc.* were the major shrubby associates of candidate species. The herbaceous layer was mainly dominated by the seedlings of *Sonerilla versicoor, Globba* sp., *Pellionia heyneana, Rhynchotechum permolle, Rhynchoglossum notonianum,* etc. The horizontal profile of the population exhibited scattered arrangement of the individuals of *Atuna indica* along with their associates, adjacent to a water course. The individuals of *Atuna indica* exhibited two age classes such as set of future and set of present. Among thirty nine

individuals of the species enumerated in the site; thrity three individuals represented the set of present with height range of 6- 18 m and gbh range 31- 93 cm. Set of future was represented by six individuals with height range from 4 - 7.5 m and gbh range 30-32 cm. The vertical crown projection showed the placement of individuals such as *Bischofia javanica, Holigarna grahamii, Persea macrantha,* etc. just below the tallest individual of *Dipterocarpus indicus.* The horizontal crown projections displayed overlapping canopy coverage under the canopy of the tallest individual, *Dipterocarpus indicus.* Two seedlings were noted with a height <100 cm and nineteen seedlings of >100 cm in the forest patch.

The population structure of *Atuna indica* was analyzed by recording gbh, basal area, basel cover, age phases and height of each individuals. The floristic diversity analysis covered individuals of forty two species with gbh  $\geq$  30 cm in 7000 sq.m. The aggregated values of relative frequency (rf), relative density (rd) and relative dominance (rD) of each species in the quadrat were worked out and noted that *Schleichera oleosa* had the highest index value of 0.2956 and thus became the dominant species in the particular quadrat, whereas, *Atuna indica* represented 29<sup>th</sup> position with IVI of 0.0315.

#### iii. Nadugani

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as *Hopea racophloea*, *Palaquium ellipticum*, *Calophyllum calaba*, *Cullenia exarillata*, *Dysoxylum malabaricum* etc. as top layer/ first storey reaching a height range of 26 to 35 m. The second storey was represented by *Aglaia barberi*, *Knema attenuata*, *Artocarpus hirsutus*, *Hydnocarpus longipedunculatus*, *Diospyros bourdillonii*, *Cinnamomum malabatrum* along with *Atuna indica*, with a height range 16-25 m. The third storey was occupied by *Baccaurea courtallensis*, *Dillenia pentagyna*, *Lagerstroemia microcarpa*, etc. with 6-15 m height range along with *Atuna indica*. *Humboldtia brunonis*, *Ixora brachiata*, *Lepisanthes* sp., *Meiogyne pannosa*, *Goniothalamus* sp. etc. were the major shrubby associates of *Atuna indica*. The herbaceous layer was mainly dominated by

*Argostemma anupama, Begonia integrifolia, Globba* sp., *Rhynchotechum permolle, Rhynchoglossum notonianum*, etc. The horizontal profile of the population exhibited the scattered arrangement of individuals of *Atuna indica* along with their associates in sloppy areas. The individuals of *A. indica* exhibited two age classes such as set of future and set of present with a height range from 5 to 25 m and diameter of 32 to 109 cm. Among thirty eight individuals of the species presented in the site; thirty individuals represented the set of present covering a height range of 16 to 25 m and gbh range of 39 to 109 cm. Set of future was represented by eight individuals covering a height range of 5 to 14 m and gbh of 32 to 48 cm. The vertical crown projections showed the placement of individuals such as *Bischofia javanica, Holigarna grahamii, Persea macrantha,* etc. just below the tallest individual of *Dipterocarpus indicus.* The horizontal crown projections displayed an overlapping of canopy coverage under the canopy of the tallest individual, *Dipterocarpus indicus.* Eight seedlings > 100 cm were noted in the forest patch.

The population structure of *Atuna indica* was analyzed by recording gbh, basal area, basal cover, age phases and the height of each individual. The floristic diversity analysis covered of individuals of 25 species with gbh  $\geq$ 30 cm in 7000 sq.m. The aggregated values of relative frequency (rf), relative density (rd) and relative dominance (rD) of each species in the quadrat were worked out and noted that *Hopea erosa* had highest index value of 0.2803 and thus became the dominant species in the particular quadrat, whereas, the *Atuna indica* represented 21<sup>st</sup> position with IVI of 0.0828

#### **5.1.3.** Population Dynamics

#### 5.1.3.1. Vegetative phenology

The flushing noted from October onwards and the process continued upto April along with matured leaves. The young foliage was observed in grayish purple colour later changed to creamy white, finally turned into green. The tree exhibited vegetative phase from May to September (Fig.2).

#### 5.1.3.2. Reproductive phenology

Flower bud initiation noted from October and the process of flowering phase continued upto December (Fig. 2). The flower buds in a raceme took about one month for maturity and flowering. The flowers are bisexual in nature. The blooming time was noted from 5 am to 9 am. At 5 am the petals started to open and fully opened at 9 am. The flowers are protandrous (anther dehisce prior to stigma receptive). Anther dehisced by around 8.30 am and stigma was receptive after a while. The pollen viability was higher (98 %) at the time of anthesis and gradually declined up to 50 % as upto 10hrs and lost its viability after 30 hrs. The pollens in sucrose solution exhibited 58- 60 per cent germination. However, the percentage of germination was getting reduced in consecutive intervals of 2 hours. The stigma receptivity lasted till 6 -7 pm in the same day (9-10 hours), after that the stigma turned to black colour and became non-receptive. Each flower had 12 anthers and two ovules. The number of pollen grains per anther was 1,224 and hence the pollen-ovule ratio was estimated into 7344:1. Insect viz. honey bees, butterflies, ants were noted (they could be the potential pollinators) during flowering. The peak time of insect incidence was 8 am -11.30 am and 4 -6 pm. The insects identified included Apies mellifera, Papilio polymnstor, Melanitis leda, Idea malabarica, Euploea sp., Eurema sp., Xylocopa sp., Camponotus sp., etc. Abscission of few developing inflorescence was also noted during the flowering period. The percentage of fruit set was around 25 per cent. Apart from the above, rare incidence of the terrestrial snail, Indrella ampula, (Mollusca: Gastropoda) was recorded during flowering which was found to feed either flower buds or tender leaves of the species. The fruit initiation was noted from January and attained maturity by April. During the course of development, fruits were found infested by caterpillars of the Pyralid moths (adult to be collected) which fed seeds and

caused a seed loss to 40-50 per cent of the species. Fruits were oval shaped, elongated fleshy drupes with a pleasant smell.

#### 5.1.3.3. Climatic and edaphic factors

Climatological and edaphological data of the species were recorded in three seasons of the year. Average value of climatic data such as atmospheric day temperature, atmospheric humidity, night temperature, night humidity of each seasons of respective population sites were recorded and are presented in Tables 7 & 9. Similarly, soil factors such as texture, pH, macro nutrients, soil moisture content, soil temperature of each season from the respective population sites are presented in Tables 8 & 10.

#### 5.1.3.4. Population genetics

Eleven ISSR markers were selected for final analysis out of the 15 ISSR primers initially screened for genetic diversity analysis. The number of scorable bands amplified by each primer varied from 10 (ISSR 6) to 14 (UBC 890) (Table 11). The percentage of polymorphic loci was 68.09 per cent in Kakkayam-Dam site population; 53.19 per cent in Kakkayam-Charangad and 71.63 per cent in Nadugani. The ISSR band size generated ranged from 200 bp to 3000 bp. On an average, effective number of alleles were 1.31 out of 1.68 number of alleles observed in Kakkayam-Dam site; 1.19 out of 1.53 in Kakkayam- Charangad and 1.31 out of 1.72 in Nadugani population. The gene diversity was 0.191 in Kakkayam- Dam site, 0.123 in Kakkayam- Charangad and 0.188 in Nadugani (Table 12). Partitioning of genetic variation within and among populations using analysis of molecular variance (AMOVA) showed 65 per cent genetic variation within the populations and 35 per cent variation among populations (Fig. 3). The UPGMA dendrogram based Nei's genetic identity showed that Kakkayam- Charangad population is more closely related to Nadugani (0.9329) than Kakkayam-Dam site (0.9134) even though Dam site and Charangad populations are located

in a same forest landscape (Fig.1). The results highlighted, a total gene diversity (Ht) of 0.2153 over the species as a whole; genetic diversity (Hs) of 0.16673 within populations; relative magnitude of genetic differentiation among populations (Fst) = 0.2231 and estimated gene flow among populations (Nm) = 1.7407.

#### **Casual factors of population reduction**

- Extremely low number of flowering individuals in the populations.
- Long intervals in flowering behaviour (above three years).
- Low per cent of pollen germination.
- Low per cent of pre-reproductive individuals.
- Extremely low natural regeneration.
- Incidence of seed pest.
- Predation and abscission of developing inflorescence
- Low genetic diversity within and among populations.

# Table 1. Population structure of Atuna indica: Kakkayam Dam site

Sl. No.	gbh (cm)	Radius (cm)	Basal area (cm)	Basal cover (m)	Age phase	First branching seen at (m)	Height of stand (m)
1	59	9.40	277.45	6.0	Set of present	1.44	8.5
2	10	1.60	8.04	2.0	Set of future	1.02	7.0
3	11	1.75	9.62	1.6	Set of future	2.50	6.0
4	20	3.18	31.75	1.8	Set of future	4.50	7.0
5	14	2.23	15.61	1.9	Set of future	0.60	8.0
6	13	2.07	13.45	2.0	Set of future	4.50	6.5
7	26	4.14	53.82	4.0	Set of future	3.00	8.0
8	25	3.98	49.74	4.0	Set of future	6.00	8.0
9	22	3.50	38.47	4	Set of future	3.00	8
10	17	2.71	23.06	2	Set of future	1.9	6.5
11	19	3.03	28.83	3	Set of future	2.5	6
12	48	7.64	183.28	5.5	Set of present	1.7	7

(List of individuals with G $\geq$ 10 cm represented)

SI. No	Species	Family	rf (%)	rd (%)	rD (%)	IVI
1.	<i>Vateria indica</i> L.	Dipterocarpaceae	0.0450	0.0704	0.1937	0.3092
2.	Myristica beddomei King.	Myristicaceae	0.0450	0.0762	0.0663	0.1876
3.	Hopea parviflora Bedd.	Dipterocarpaceae	0.0450	0.0439	0.0679	0.1569
4.	Poeciloneuron indicum Bedd.	Clusiaceae	0.0450	0.0323	0.0721	0.1494
5.	<i>Garcinia gummi-gutta</i> (L.) Robs.	Clusiaceae	0.0450	0.0381	0.0561	0.1393
6.	<i>Xanthophyllum arnottianum</i> Wight.	Polygalaceae	0.0450	0.0703	0.0239	0.1392
7.	Cinnamomum verum Presl.	Lauraceae	0.0360	0.0439	0.0564	0.1364
8.	Holigarna arnottiana Hook.	Anacardiaceae	0.0541	0.0440	0.0374	0.1355
9.	Toona ciliata Roem.	Meliaceae	0.0450	0.0352	0.0313	0.1116
10	Bischofia javanica Blume	Euphorbiaceae	0.0450	0.0587	0.0057	0.1094
11.	Sterculia guttata Roxb. ex DC.	Sterculiaceae	0.0360	0.0293	0.0262	0.0916
12	<i>Knema attenuata</i> (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0360	0.0293	0.0230	0.0884
13	Dipterocarpus indicus Bedd.	Dipterocarpaceae	0.0270	0.0234	0.0290	0.0795
14	Arenga wightii Griff.	Arecaceae	0.0270	0.0205	0.0273	0.0748
15	Mangifera indica L.	Anacardiaceae	0.0270	0.0205	0.0244	0.0720
16	Dimocarpus longan Lour.	Sapindaceae	0.0180	0.0234	0.0291	0.0706
17.	<i>Holarrhena pubescens</i> (Buch Ham.) Wall. ex G. Don	Apocynaceae	0.0270	0.0205	0.0217	0.0692
18	<i>Holigarna grahamii</i> (Wight) Kurz	Anacardiaceae	0.0270	0.0205	0.0213	0.0689
19	<i>Erythrina variegata</i> L.	Fabaceae	0.0180	0.0176	0.0261	0.0618
20	Memecylon umbellatum Burm.	Melastomataceae	0.0090	0.0469	0.0045	0.0604
21	<i>Macaranga peltata</i> (Roxb.) MuellArg.	Euphorbiaceae	0.0180	0.0205	0.0153	0.0538
22	Nothopegia beddomei Gamble	Anacardiaceae	0.0270	0.0176	0.0086	0.0532
23	<i>Olea dioica</i> Roxb.	Oleaceae	0.0180	0.0147	0.0121	0.0448
24	<i>Haldina cordifolia</i> (Roxb.) Ridsd.	Rubiaceae	0.0180	0.0117	0.0142	0.0439
25	Alstonia scholaris (L.) R. Br.	Apocynaceae	0.0180	0.0147	0.0010	0.0427
26	Atuna indica (Bedd.) Kosterm.	Chrysobalanaceae	0.0090	0.0323	0.0008	0.0421
27	Syzygium cumini (L.) Skeels	Myrtaceae	0.0090	0.0235	0.0068	0.0393
28	Aglaia barberi Gamble	Meliaceae	0.0180	0.0147	0.0013	0.0340
29	Dalbergia latifolia Roxb.	Fabaceae	0.0180	0.0088	0.0068	0.0337

Table 2. Floristic diversity / Important value index of Atuna indica : Kakkayam-Dam site<br/>(List of individuals with  $G \ge 30$  cm represented)

30	<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	0.0090	0.0059	0.0179	0.0328
31.	<i>Syzygium mundagam</i> (Bourd.) Chithra	Myrtaceae	0.0180	0.0059	0.0064	0.0303
32.	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	0.0090	0.0088	0.0091	0.0269
33.	<i>Cinnamomum malabatrum</i> (Burm. f.) Blume	Lauraceae	0.0090	0.0088	0.0053	0.0231
34	<i>Melicope lunu-ankenda</i> (Gaertn.) Hartley.	Rutaceae	0.0090	0.0059	0.0073	0.0222
35	Artocarpus heterophyllus Lam.	Moraceae	0.0090	0.0059	0.0060	0.0209
36	<i>Elaeocarpus serratus</i> L.	Elaeocarpaceae	0.0090	0.0059	0.0051	0.0200
37	<i>Persea macrantha</i> (Nees) Kosterm.	Lauraceae	0.0090	0.0029	0.0072	0.0192
38	Symplocos racemosa Roxb.	Symplocaceae	0.0090	0.0059	0.0041	0.0189
39	Ixora brachiata Roxb. ex DC.	Rubiaceae	0.0090	0.0059	0.0041	0.0189
40	<i>Syzygium laetum</i> (BuchHam.) Gandhi	Myrtaceae	0.0090	0.0029	0.0035	0.0154
41	<i>Pajanelia longifolia</i> (Willd.) K. Schum.	Bignoniaceae	0.0090	0.0029	0.0028	0.0147
42.	<i>Dysoxylum malabaricum</i> Bedd. ex Hiern	Meliaceae	0.0090	0.0029	0.0010	0.0130
43	Humboldtia brunonis Wall.	Fabaceae	0.0090	0.0029	0.0004	0.0123

# Table 3. Population structure of Atuna indica: Kakkayam-Charangad(List of individuals with G≥30 cm represented)

Sl. No.	gbh (cm)	Radius (cm)	Basal area (cm <sup>2</sup> )	Basal cover (m)	Age phase	First branching (m)	Height of stand (m)
1.	90	14.3	642.1	6.0	Set of present	2.5	15.0
2.	46	7.3	167.3	7.0	Set of present	2.0	9.0
3.	34	5.1	81.7	4.0	Set of present	0.5	9
4.	30	4.1	52.8	3	Set of future	0.5	5
5.	35	4.9	75.4	4	Set of present	1.0	6
6.	32	4.5	63.6	3	Set of future	1.0	7
7.	39	6.2	120.7	6	Set of present	1.5	8
8.	60	9.6	289.4	7	Set of present	3.0	14
9.	40	6.4	128.6	5	Set of present	2.0	11
10.	60	9.5	283.4	6	Set of present	3.0	14
11.	69	10.9	373.1	7	Set of present	3.0	13
12.	90	14.3	642.1	6	Set of present	3.0	8
13.	45	7.2	162.8	5	Set of present	1.0	10
14.	65	10.4	339.6	6	Set of present	8.0	15
15.	61	9.7	295.4	4	Set of present	1.5	10
16.	30	3.2	32.2	3	Set of future	1.5	4
17.	63	10.1	320.3	5	Set of present	3.5	14
18.	31	4.9	75.4	3	Set of present	3.5	9
19.	86	13.7	589.3	6	Set of present	0.5	18
20.	81	12.9	522.5	7	Set of present	6.0	15
21.	33	5.3	88.2	6	Set of present	3.0	7
22.	70	11.2	393.8	9	Set of present	3.5	16
23.	40	6.4	128.6	6	Set of present	3.0	10
24.	30	4.8	72.3	3	Set of future	1.0	5
25.	34	5.4	91.6	4	Set of present	1.5	7
26.	36	5.7	102	3.5	Set of present	1.5	7
27.	32	5.1	81.7	6	Set of future	2.0	7.5
28.	33	5.3	88.2	4.5	Set of present	2.0	7.5
29.	35	5.6	98.5	5	Set of present	4.0	10
30.	41	6.5	132.7	6	Set of present	3.0	10
31.	31	4.9	75.4	3	Set of future	2.5	6
32.	42	6.7	140.9	3	Set of present	2.0	7
33.	48	7.6	181.4	7	Set of present	2.5	5
34.	68	10.8	366.2	7	Set of present	3.0	13
35.	86	13.7	589.3	8	Set of present	4.0	16
36.	79	12.6	498.5	7	Set of present	3.5	15
37.	89	14.2	633.1	7	Set of present	3.0	15
38.	93	14.8	687.8	8	Set of present	3.5	16
39.	67	10.7	359.5	6.5	Set of present	2.5	13.5

# Table 4. Floristic diversity / Important value index of Atuna indica :Kakkayam-Charangad(List of individuals with G≥30cm represented)

Sl. No.	Species	Family	rf (%)	rd (%)	rD (%)	IVI
1.	<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	0.0420	0.0638	0.1897	0.2956
2.	<i>Persea macrantha</i> (Nees) Kosterm.	Lauraceae	0.0420	0.0691	0.0649	0.1761
3.	Bischofia javanica Blume.	Euphorbiaceae	0.0420	0.0399	0.0665	0.1483
4.	Dimocarpus longan Lour.	Sapindaceae	0.0420	0.0293	0.0706	0.1419
5.	<i>Olea dioica</i> Roxb.	Oleaceae	0.0420	0.0346	0.0550	0.1316
6.	<i>Knema attenuata</i> (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0420	0.0638	0.0234	0.1292
7.	<i>Macaranga peltata</i> (Roxb.) Muell.	Euphorbiaceae	0.0336	0.0399	0.0552	0.1287
8.	<i>Vateria indica</i> L.	Dipterocarpaceae	0.0504	0.0399	0.0367	0.1270
9.	Holigarna arnottiana Hook.	Anacardiaceae	0.0420	0.0319	0.0307	0.1046
10	Hopea parviflora Bedd.	Dipterocarpaceae	0.0420	0.0532	0.0056	0.1008
11	Cinnamomum verum Presl.	Lauraceae	0.0420	0.0346	0.0237	0.1003
12	DC.	Sterculiaceae	0.0336	0.0266	0.0257	0.0859
13	<i>Gmelina arborea</i> Roxb.	Verbenaceae	0.0336	0.0213	0.0272	0.0821
14	<i>Memecylon umbellatum</i> Burm.	Melastomaceae	0.0252	0.0212	0.0284	0.0749
15	Alstonia scholaris (L.) R. Br.	Apocynaceae	0.0252	0.0266	0.0224	0.0742
16	<i>Xanthophyllum arnottianum</i> Wight.	Polygalaceae	0.0252	0.0186	0.0267	0.0706
17	<i>Cinnamomum malabatrum</i> (Burm. f.) Blume.	Lauraceae	0.0168	0.0213	0.0285	0.0666
18	<i>Diospyrous bourdillonii</i> Brandis	Ebenaceae	0.0084	0.0558	0.0018	0.0660
19	Holoptelea integrifolia (Roxb.) Planch.	Ulmaceae	0.0252	0.0186	0.0212	0.0650
20	<i>Syzygium laetum</i> (Buch Ham.) Gandhi.	Myrtaceae	0.0252	0.0186	0.0209	0.0647
21	<i>Poeciloneuron indicum</i> Bedd.	Clusiaceae	0.0168	0.0160	0.0256	0.0584
22	Myristica beddomei King.	Myristicaceae	0.0084	0.0426	0.0044	0.0553
23	<i>Syzygium mundagam</i> (Bourd.) Chithra	Myrtaceae	0.0252	0.0160	0.0084	0.0496
24	Holigarna nigra Bourd.	Anacardiaceae	0.0168	0.0133	0.0118	0.0419
25	<i>Garcinia gummi-gutta</i> (L.) Robs.	Clusiaceae	0.0168	0.0106	0.0139	0.0413

26	Stereospermum colais	Bignoniaceae	0.0168	0.0133	0.0098	0.0399
	(BuchHam. ex Dillw.)					
	Mabb.					
27	Mangifera indica L.	Anacardiaceae	0.0084	0.0213	0.0067	0.0364
28	Dysoxylum malabaricum	Meliaceae	0.0084	0.0239	0.0039	0.0363
	Bedd. ex Hiern.					
29	Atuna indica (Bedd.)	Chrysobalanaceae	0.0168	0.0080	0.0067	0.0315
	Kosterm.					
30	Syzygium cumini (L.)	Myrtaceae	0.0168	0.0133	0.0013	0.0314
	Skeels.					
31	Terminalia bellirica	Combrutaceae	0.0084	0.0053	0.0176	0.0313
	(Gaertn.) Roxb.					
32	Ficus racemosa L.	Moraceae	0.0084	0.0080	0.0090	0.0253
33	Humboldtia brunonis Wall.	Caesalpiniaceae	0.0084	0.0080	0.0052	0.0216
34	<i>Chrysophyllum cainito</i> L.	Sapotaceae	0.0084	0.0080	0.0047	0.0211
35	<i>Elaeocarpus serratus</i> L.	Elaeocarpaceae	0.0084	0.0053	0.0071	0.0209
36	<i>Ceiba pentandra</i> (L.)	Bombacaceae	0.0084	0.0053	0.0059	0.0196
	Gaertn.					
37	<i>Caryota urens</i> L.	Arecaceae	0.0084	0.0053	0.0050	0.0187
38	Arenga wightii Griff.	Arecaceae	0.0084	0.0026	0.0071	0.0182
39	Dalbergia latifolia Roxb.	Fabaceae	0.0084	0.0053	0.0040	0.0177
40	Cleistanthus patulus (Roxb.)	Euphorbiaceae	0.0084	0.0053	0.0040	0.0177
	Muell.					
41	Pajanelia longifolia (Willd.)	Bignoniaceae	0.0084	0.0080	0.0009	0.0172
	K. Schum.					
42	Lepisanthes tetraphylla	Sapindaceae	0.0084	0.0027	0.0034	0.0145
	(Vahl) Radlk.					

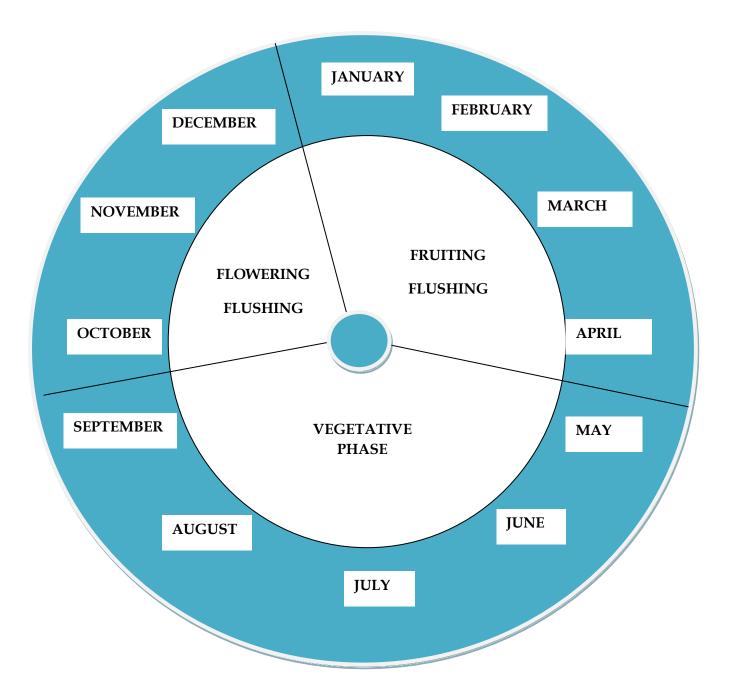
Sl. No.	gbh (cm)	Radius (cm)	Basal area (cm)	Basal cover (m)	Age phase	First branching seen at (m)	Height of stand (m)
1	82	13.1	538.9	5	Set of present	7	20
2	81	12.9	522.5	6	Set of present	6	24
3	73	11.6	422.5	4	Set of present	5.5	18
4	91	14.5	660.2	8	Set of present	2.8	22
5	77	12.3	475.1	5.5	Set of present	4.5	19
6	69	10.9	373.1	6.5	Set of present	2	21
7	39	6.1	116.8	1.5	Set of present	7	16
8	81	12.9	522.5	4.5	Set of present	4.5	22
9	58	9.2	265.8	5	Set of present	3.5	15
10	66	10.5	346.2	4.5	Set of present	6.5	23
11	73	11.6	422.5	5	Set of present	6.5	18
12	51	8.1	206	3	Set of present	2.6	13
13	73	11.6	422.5	4	Set of present	4	16
14	72	11.5	412.4	2	Set of present	3.5	19
15	32	5.1	81.7	1.5	Set of future	2.1	5
16	72	11.5	415.3	5	Set of present	6.2	23
17	45	7.2	162.8	2	Set of future	3	21
18	37	5.9	109.3	2	Set of future	2	18
19	61	9.7	295.4	3.5	Set of present	3.5	14
20	83	13.2	547.1	4	Set of present	5.2	23
21	61	9.71	295.4	4	Set of present	2.5	17
22	83	13.2	547.1	5	Set of present	17	19
23	102	16.3	834.3	5.5	Set of present	21	25
24	107	17	907.5	5.3	Set of present	20	24
25	41	6.5	132.7	2.3	Set of future	3	13
26	109	17.4	950.7	4.8	Set of present	13	24
27	82	13	530.7	4	Set of present	2	19
28	94	14.9	697.1	4	Set of present	7	19
29	86	13.7	589.3	3.8	Set of present	8	18
30	42	6.7	140.9	1.9	Set of future	4	13
31	93	14.8	687.8	3.1	Set of present	7	19
32	94	14.9	697.1	3.4	Set of present	9	18.5
33	67	10.6	352.8	2.3	Set of present	3	14
34	61	9.7	295.4	2.1	Set of present	2	15
35	41	6.5	132.7	1.7	Set of future	7	13
36	48	7.6	181.4	2.4	Set of future	6	13
37	43	6.8	145.2	1.3	Set of future	3	12
38	99	15.8	725.5	3.8	Set of present	2	17

**Table 5. Population structure of** *Atuna indica*: **Nadugani** (List of individuals with G≥30 cm represented)

# Table 6. Floristic diversity / Important value index of Atuna indica: Nadugani (List of individuals with G≥30cm represented)

S1.	Species	Family	rf	rd	rD	IVI
No	-	-				
1.	Hopea erosa (Bedd.) van Sloot	Dipterocarpaceae	0.0356	0.1069	0.1377	0.2803
2.	Artocarpus heterophyllus Lam.	Moraceae	0.0714	0.0566	0.0675	0.1955
3.	Aglaia barberi Gamble	Meliaceae	0.0714	0.0461	0.0688	0.1863
4.	Baccaurea courtallensis (Wight) Muell	Euphorbiaceae	0.0357	0.0734	0.0735	0.1826
5.	Calophyllum calaba L.	Clusiaceae	0.0372	0.0629	0.0545	0.1531
6.	Dillenia pentagyna Roxb.	Dilleniaceae	0.0357	0.0314	0.0776	0.1447
7.	<i>Palaquium ellipticum</i> (Dalz.) Baill	Sapotaceae	0.0356	0.0776	0.0211	0.1344
8.	<i>Diospyros peregrina</i> (Gaertn.) Gurke	Ebenaceae	0.0714	0.0209	0.0331	0.1255
9.	Bischofia javanica Blume	Euphorbiaceae	0.0357	0.0314	0.0565	0.1236
10.	Artocarpus hirsutus Lam.	Moraceae	0.0637	0.0419	0.0429	0.1205
11.	<i>Cinnamomum malabatrum</i> (Burm. f.) Blume	Lauraceae	0.0345	0.0335	0.0511	0.1204
12.	Cullenia exarillata Robyns	Bombacaceae	0.0457	0.0293	0.0532	0.1183
13.	<i>Mesua ferrea</i> L.	Clusiaceae	0.0356	0.0524	0.0237	0.1118
14.	<i>Persea macrantha</i> (Nees) Kosterm.	Lauraceae	0.0257	0.0335	0.0413	0.1105
15.	<i>Knema attenuata</i> (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0354	0.0440	0.0187	0.0984
16.	Hopea racophloea Dyer	Dipterocarpaceae	0.0357	0.0209	0.0366	0.0933
17.	<i>Dysoxylum malabaricum</i> Bedd. ex Hiern	Meliaceae	0.0357	0.0398	0.0161	0.0916
18.	Schleichera oleosa (Lour.) Oken.	Sapindaceae	0.0357	0.0335	0.0222	0.0914
19.	Diospyros bourdillonii Brandis	Ebenaceae	0.0617	0.0335	0.0161	0.0854
20.	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	0.0337	0.0252	0.0242	0.0851
21.	Atuna indica (Bedd.) Kosterm.	Chrysobalanaceae	0.0357	0.0377	0.0094	0.0828
22.	<i>Lagerstroemia microcarpa</i> Wight	Lythraceae	0.0358	0.0252	0.0197	0.0806
23.	<i>Syzygium laetum</i> (BuchHam.) Gandhi	Myrtaceae	0.0294	0.0255	0.0043	0.0651
24.	<i>Macaranga peltata</i> (Roxb.) Muell.	Euphorbiaceae	0.0427	0.0084	0.0156	0.0597
25.	Symplocos racemosa Roxb.	Symplocaceae	0.0296	0.0084	0.0148	0.0589

## Fig. 2. Phenology calendar of Atuna indica



Season	Atm. Temperature (°C)	Night Temperature (°C)	Atm. Humidity Day (%)	Atm. Humidity Night (%)	
Summer	32	25	60	78	
Monsoon	28	23	79	91	
Winter	31	20	65	92	

Table 7. Climatic data of Atuna indica: Kakkayam

Table 8. Edaphological data of Atuna indica: Kakkayam

Season	Soil Level	Texture	рН	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Temp. (°C)	Moisture (%)
Summer	Surface Middle Bottom	Silt loam Sandy loam Loam	5.8 6 5.4	513.2 500.1 490.2	2.5 3.5 3.6	336.4 200.5 208.4	23	21.5
u	Surface	Silt clay loam	4.8	630.2	6.8	476.1	22	30.2
Monsoon	Middle	Silt clay	5	515.8	5.3	520.6		
M	Bottom	Silt clay loam	5.5	503.1	3.8	296.4		
Winter	Surface Middle Bottom	Silt clay Loam Silt loam	5.4 5.8 5.1	450.6 416.5 396.1	4.6 2.3 2.1	240.6 200.5 230.4	20	24.6

Table 9.	Climatic	data o	of <i>Atuna</i>	indica:	Nadugani
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Season	Atm. Temp. ( <sup>0</sup> C)	Night Temp. ( <sup>o</sup> C)	Atm. Humidity (Day - %)	Atm. Humidity (Night- %)
Summer	34	28	54	62
Monsoon	24	21	78	84
Winter	26	23	68	76

Season	Soil Level	Texture	pН	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Temp. (°C)	Moisture (%)
Summer	Surface Middle Bottom	Silt clay loam Sandy loam Loam	5.4 5.3 6	510 480.1 520.6	2.6 2.4 2.2	340.6 220.5 202.4	26	23.6
Winter	Surface Middle	Silt clay loam Sandy clay Loam	5.3 5.8	440.5 436.6	3.3 2.8	260.6 180.5	21	21.4
	Bottom	Silt clay loam	5.4	398.5	2.2	280.4		
Monsoon	Surface Middle Bottom	Silt clay loam Silt clay Silt clay loam	5.3 5.6 5.9	584.5 480.2 443.1	3.2 2.9 2.1	323.2 224.1 204.6	21	29.8

Table 10. Edaphological data of Atuna indica: Nadugani

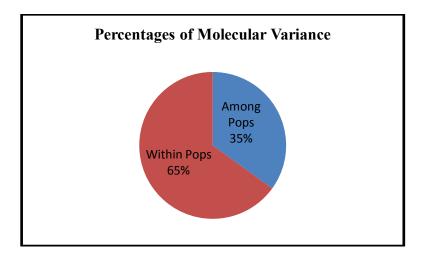
Table 11. Atuna indica: ISSR primers vs. polymorphism

Sl. No	Primer code	Annealing temperature ( <sup>0</sup> C )	Total bands produced	Polymorphic bands	Polymorphism (%)
1.	UBC 810	41.3	12	12	100
2.	UBC 811	41.3	13	13	100
3.	UBC 812	46.7	14	14	100
4.	UBC 825	42.5	14	14	100
5.	UBC 834	50.9	14	14	100
6.	UBC 890	53.8	14	14	100
7.	PRIMER 1	52.6	15	15	100
8.	PRIMER 2	59.3	12	12	100
9.	PRIMER 3	45.0	12	12	100
10.	PRIMER 5	56.6	11	11	100
11.	ISSR 6	49.4	10	10	100

Sl. No	Population	Number of Individuals	Observed number of alleles ±S.D.	Effective number of alleles ±S.D.	No. of poly- morphic loci	Poly- morphic loci (%)	Nei's gene diversity ±S.D	Shannon information index ±S.D
1	Kakkayam- Dam site	10	1.681 ±0.47	1.3066 ±0.322	96	68.09	0.1905 ±0.1778	0.2982 ±0.2527
2	Nadugani	20	1.716 ±0.45	1.3052 ±0.3394	101	71.63	0.1881 ±0.1798	0.2953 ±0.2525
3	Kakkayam- Charangad	30	1.532 ±0.50	1.1909 ±0.2815	75	53.19	0.1232 ±0.1604	0.1980 ±0.2363
4	All populations together	60	-	1.2837 ±0.2369	-	-	0.1969 ±0.1337	0.3286 ±0.1825

 Table 12. Atuna indica: Population genetic diversity parameters

Fig.3. Atuna indica : Genetic diversity within and among populations



## Plate 1. Atuna indica : Population structure



Habit and Habitats: Different views from the Kakkayam- Dam site



Different views from the Kakkayam-Charangad site



Different views from the Nadugani forest

## Plate 2. Atuna indica : Population dynamics







Leaf phenology



Flower phenology



Single flower



Fruiting primordia



**Developing fruit** 

## Plate 3. Atuna indica : Population dynamics







Views of flower damage by *Indrella ampulla* 

Closer view of Indrella ampulla



Fruit phenology



**Abscised fruits** 



**Matured fruits** 



Views of infested fruits/ seeds by Pyralidae larvae

## 5.1. Hydnocarpus longipedunculatus

## 5.1.1. Population survey and mapping

- Kulamavu Medicinal Plant Conservation Area (MPCA); N 9.808793°, E 76.8958° (Nagarampara Range, High Range Circle, Kottayam); 17 km away from Painavu enroute to Kulamavu town. Alt., 840±5 m asl; The habitat of the species is located in a semi-evergreen secondary type forest patch.
- 2. Cheri ; N 9.823329°, E 76.91304°

(Thodupuzha Range, High Range Circle, Kottayam); 10 km away from Painavu town enroute to Kulamavu town. Alt; 830±5 m asl; The habitat of the species is located in an evergreen secondary type forest patch.

The extent of occurrence and area of occupancy of the species were estimated as 0.5937 and 0.056 km<sup>2</sup> respectively.

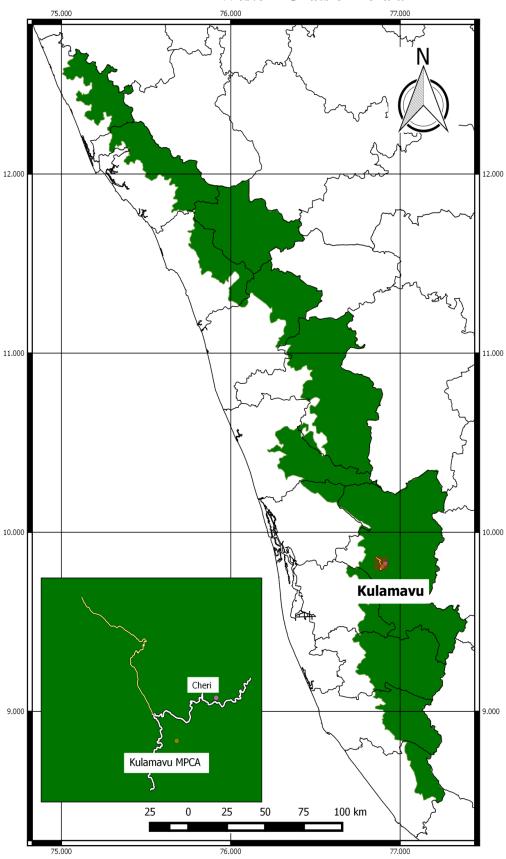


Fig. 3. *Hydnocarpus longipedunculatus*: Populations in the Western Ghats of Kerala

## **5.2.2.** Population structure

Population structure and floristic diversity analysis in two population sites viz. Kulamavu MPCA and at Cheri are detailed below.

### i. Kulamavu MPCA

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as Calophyllum polyanthum, Dipterocarpus indicus, Vateria indica, Persea macrantha, Buchanania axillaris, Cullenia exarillata, etc. as top layer/ first storey reaching a height range of 31 to 40 m. The second storey was represented by Actinodaphne malabarica, Olea dioica, Calophyllum calaba, Poeciloneuron indicum, Aglaia barberi, Holigarna arnottiana Madhuca longifolia, etc. Third storey was occupied by Baccaurea courtallensis, Garcinia gummi-gutta, Melicope lunu-ankenda, Chionanthus courtallensis, Litsea floribunda, etc with 11-20 m height range. The herbaceous layer was mainly dominated by the seedlings of *Lepidagathis* sp., *Gymnostachyum* sp. etc. The horizontal profile of the population exhibited the arrangement of the individuals of H. longipedunculatus in a clumped manner and is adjacent to a water course. The individuals of H. longipedunculatus exhibited two age classes such as set of future and set of present with a height range from 5 to 17 m and a girth of 30 -118 cm. Among thrity six individuals of the species enumerated, twenty six individuals represented the set of present covering a height range of 6-17 m and gbh range of 48-118 cm. Set of future was represented by ten individuals covering a height range of 5-7 and gbh of 30-48 cm. Eight seedlings > 1 m were noted. The vertical crown projections showed the placement of individuals such as Buchanania axillaris, Palaquium ellipticum, Garcinia gummi-gutta, etc. just below the tallest individual of Cullenia exarillata. The horizontal crown projections displayed the canopy overlapping of these species under the canopy of the tallest individual, Cullenia exarillata.

The population structure of *H. longipedunculatus* was analysed by recording gbh, basal area, basal cover, age phases and height of each individuals. The floristic diversity analysis covered individuals of forty nine species of gbh  $\geq$  30 cm size in 7000 sq.m. The aggregated values of relative frequency (rf), relative density (rd) and relative dominance (rd) of each species in the quadrat were worked out and noted that *Calophyllum polyanthum* had the highest index value of 0.1909 and thus became the dominant species in the particular quadrat, whereas, *H. longipedunculatus* represented 33<sup>rd</sup> position with IVI of 0.0382.

## ii. Cheri

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as Palaquium ellipticum, Calophyllum polyanthum, Persea macrantha, Buchanania axillaris, etc. as top layer/ first storey reaching a height range of 31 to 40 m. The second storey was represented by Drypetes malabarica, Dimocarpus longan, Olea dioica, Carallia brachiata, Knema attenuata, Myristica malabarica etc. Third storey was occupied by Baccaurea courtallensis, Symplocos racemosa, Melicope lunu-ankenda, Vitex altissima, Litsea glabrata, Cinnamomum malabatrum, Aporosa cardiosperma, etc. with 11-20 m height range. The herbaceous layer was mainly dominated by Justicia sp., Ophiorriza mungos, Mycetia acuminata, etc. The horizontal profile of the population exhibited the arrangement of the individuals of H. longipedunculatus in a clumped manner in the deep sloppy areas adjacent to a water course. The individuals of H. longipedunculatus exhibited two age classes such as set of future and set of present. Among forty individuals of the species enumerated in the site, thirty two individuals represented the set of present covering a height range of 7-20 m and gbh ranged from 44-140 cm. Set of future was represented by eight individuals covering a height range of 2.5 -6 m and gbh from 42-56 cm. Six seedlings >1 m height were noted. The vertical crown projections showed the placement of individuals such as Persea macrantha, Palaquium ellipticum, Symplocos racemosa, etc just below the tallest individual of Calophyllum polyanthum. The horizontal crown projections displayed an overlapping of canopy coverage of these species under the tallest canopy of *Calophyllum polyanthum*.

The population structure of *H. longipedunculatus* was analysed by recording gbh, basal area, basal cover, age phases and the height of each individuals. The floristic diversity analysis covered individuals of forty one species of gbh  $\geq$ 30 cm size in 7000 sq.m. The aggregated values of relative frequency (rf), relative density (rd) and relative dominance (rD) of each species in the quadrat were worked out and noted that *Calophyllum polyanthum* had the highest index value of 0.1944 and thus became the dominant species in the particular quadrant, whereas, *H. longipedunculatus* represented 30<sup>th</sup> position with IVI of 0.0480

## **5.2.3.** Population Dynamics

## 5.2.3.1. Vegetative phenology

Flushing along with matured leaves was observed from December to August. Young foliage appeared in yellowish green changed into reddish brown then to pale green and finally to dark green (Fig.4).

### 5.2.3.2. Reproductive phenology

Generally, flowering was noted from the month of December to February on individuals above 60 cm gbh (Fig. 4). The trees are polygamo- diocious in nature (trees having either male flower only or with male and bisexual flowers). However, both the populations generally showed extremely low number of male trees. In male flowers, the pedicel, sepal, petal and stamens are longer than that of bisexual flowers. Bisexual flowers are somewhat globular in shape. Both male and bisexual flowers are hanging in nature. The ratio between bisexual and male flowers was 1:3.

Single flower bud in a raceme took around three weeks for its development and bloom. The blooming started at night hours, from 2 am onwards, and gradually opened up by 6 am, the

sepals and petals were bent backwards and the stamens were seen as drooping as the flower is hanging type. In the case of bisexual flowers, the pistil is similar to fruiting primodia.

The anthesis was noted by 6 to 6.30 am. The pollen viability was higher (97 %) at the time of anthesis and viability lost within 36 hrs. The pollen germination was noted as 83.33 per cent during anthesis and gradually declined by next day. A flower had 5 anthers and ovules each and one anther contained 364 pollen grains. The pollen-ovule ratio was estimated as 364:1. It was also noted that pollen grains were absent in a few flowers. The stigma receptivity period couldn't assess as the number of bisexual floweres was either low or mostly infected. The larvae of Cecidomyidae sp. (Arthropoda : Diptera) in bisexual feed the floral parts severely and subsequently affected the fruit set. The insect flowers such as Cirrochroa sp., Apies mellifera, Euresma sp., Xylocopa sp., etc. were repeatedly observed during morning and evening hours of flowering period. The Leptosia sp., *Campanotus* sp., along with some other unidentified moths were also visited the flowers during night hours. The fruit phenology extended from March to August. The fruits were often predated by giant and flying squirrels. They either damaged the fruits or consumed the developing seed. It was noted that the fallen and empty fruits were sheltered by secondary invaders such as ants and termites. Both biological and physical interventions met in reproductive phase resulted only in 30 per cent fruit set in the species.

## 5.2.3.3. Climatic and Edaphic factors

Climatological and edaphological data of the species were collected in three seasons of the year. Average values of climatic data such as atmospheric day temperature, atmospheric humidity, night temperature, night humidity for each seasons of the respective population sites were recorded and are presented in table 15. Similarly, soil factors such as texture, pH, macro nutrients, soil moisture content, soil temperature of each season recorded from respective population sites are presented in table 16.

## 5.2.3.4. Population genetics

Out of the 15 ISSR primers initially screened, 10 ISSR markers were selected for genetic diversity analysis. The number of scorable bands amplified by each primer varied from 8 (Primer 6) to 14 (UBC 810) (Table 19). The percentage of polymorphic loci was observed as 86.73 per cent in Kulamavu MPCA and 58.41 per cent in Cheri. The band size generated from primers ranged from 200 bp to 2500 bp. On an average, effective number of alleles were 1.41 out of 1.87 observed number of alleles in Kulamavu MPCA and 1.31 effective alleles otut of 1.58 observed alleles of Cheri. The gene diversity was 0.247 and 0.185 in Kulamavu MPCA and Cheri respectively (Table 20). Partitioning of variation within and among populations using analysis of molecular variance (AMOVA) showed 70 per cent of genetic variation within the populations and 30 per cent of variation among the populations (Fig. 6). The UPGMA dendrogram based Nei's genetic identity showed that Kulamavu MPCA and Cheri had the highest genetic identity (0.9329) values. Total genediversity (Ht) overall the species was 0.2430; within population (Hs) was0.2160. Relative magnitude of genetic differentiation among the populations (Gst) was 0.1112 and the gene flow estimated among the populations (Nm) was 3.9965.

## **Casual factors of population reduction**

- Low per cent of pre- reproductive individuals
- Low rate in natural regeneration
- Incidence of insect- pest during flowering
- Fruit/seed predation
- Low genetic diversity within and among populations

## Table 13. Population structure of *H. longipedunculatus*: Kulamavu MPCA<br/>(List of individuals with $G \ge 30$ cm represented)

Sl.No	gbh	Radius	Basal	Basal	Age phase	First	Height of
	(cm)	(cm)	area	cover		branching	stand
			(cm <sup>2</sup> )	(m)		(m)	( <b>m</b> )
1.	35	5.6	98.5	4	Set of future	1	5
2.	30	3.8	45.3	2.5	Set of future	0.75	6.5
3.	55	8.8	243.2	3.5	Set of present	1.5	6.5
4.	43	6.9	147.3	4.5	Set of future	2.5	7
5.	88	14	615.4	6	Set of present	3	13
6.	118	18.7	1098	9	Set of present	0.5	17
7.	63	10	314	4.5	Set of present	2.5	9
8.	54	8.6	232.2	4	Set of present	2.5	13
9.	62	9.9	307.8	5	Set of present	2	14
10.	45	7.2	162.8	3.5	Set of present	1	6
11.	87	13.9	606.7	8	Set of present	3	13
12.	85	13.9	606.7	6	Set of present	2.5	12
13.	65	10.4	339.6	4	Set of present	1.5	10
14.	43	6.8	123.8	3.5	Set of future	1.5	9
15.	109	17.4	950.6	10.5	Set of present	0.5	18
16.	77	12.3	475.1	7.5	Set of present	1.5	12
17.	56	8.9	248.7	4.5	Set of present	3.5	11
18.	74	11.9	444.7	7	Set of present	4.5	13
19.	49	7.8	191	3.5	Set of future	0.5	8
20.	52	8.3	216.3	4	Set of present	1.5	8
21.	54	8.6	232.2	3.5	Set of present	2	14
22.	108	17.2	928.9	9	Set of present	1	17
23.	64	10.2	326.7	5	Set of present	3	10
24.	87	13.9	606.7	7	Set of present	1	12
25.	42	6.7	138.4	4.5	Set of future	2	7
26.	56	8.9	248.7	6	Set of present	1.5	13
27.	25	3.9	47.8	5	Set of future	3	6
28.	36	5.7	102	3	Set of future	4	7
29.	48	7.6	181.4	3.5	Set of future	1	7
30.	57	9.1	257.2	4	Set of present	0.5	8
31.	68	10.8	366.2	4.5	Set of present	2	10
32.	73	11.6	422.5	5	Set of present	1	13
33.	87	13.9	606.7	5.5	Set of present	1.5	13
34.	92	14.6	669.3	6.5	Set of present	1.5	14
35.	98	15.6	764.2	7	Set of present	8	16
36.	36	5.7	102	3.5	Set of future	0.5	6

# Table 14.Floristic diversity / Important value index of *H. longipedunculatus*:Kulamavu MPCA

(List of individuals with  $G \ge 30$ cm represented)

Sl.	Species	Family	rf	rd	rD	IVI
No			(%)	(%)		
1	<i>Calophyllum polyanthum</i> Wall. ex Choisy	Clusiaceae	0.0253	0.0283	0.1372	0.1909
2	Calophyllum calaba L.	Clusiaceae	0.0379	0.0205	0.1135	0.1719
3	<i>Poeciloneuron indicum</i> Bedd.	Clusiaceae	0.0253	0.0378	0.0734	0.1366
4	<i>Persea macrantha</i> (Nees) Kosterm.	Lauraceae	0.0253	0.0268	0.0798	0.1319
5	<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	0.0253	0.0378	0.0583	0.1215
6	Buchanania axillaris (Desr.) Ramamoorthy	Anacardiaceae	0.0379	0.0536	0.0274	0.1189
7	<i>Actinodaphne malabarica</i> Balakr.	Lauraceae	0.0253	0.0488	0.0433	0.1175
8	<i>Olea dioica</i> Roxb. Oleaceae		0.0886	0.0157	0.0032	0.1076
9	Vateria indica L.	Dipterocarpaceae	0.0253	0.0189	0.0551	0.0993
10	Xanthophyllum arnottianum Wight	Polygalaceae	0.0253	0.0615	0.0105	0.0973
11	Palaquium ellipticum (Dalz.) Baill.	Sapotaceae	0.0253	0.0268	0.0321	0.0843
12	<i>Knema attenuata</i> (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0253	0.0315	0.0251	0.0818
13	<i>Myristica malabarica</i> Lam.	Myristicaceae	0.0126	0.0189	0.0437	0.0753
14	<i>Aglaia elaeagnoidea</i> (A. Juss.) Benth.	Meliaceae	0.0253	0.0236	0.0232	0.0722
15	<i>Cinnamomum verum</i> Presl.	Lauraceae	0.0253	0.0205	0.0231	0.0689
16	<i>Cullenia exarillata</i> Robyns.	Bombacaceae	0.0126	0.0331	0.0210	0.0668
17	Hopea parviflora Bedd.	Dipterocarpaceae	0.0253	0.0315	0.0091	0.0659
18	<i>Carallia brachiata</i> (Lour.) Merr.	Rhizophoraceae	0.0253	0.0220	0.0108	0.0582
19	Myristica beddomei King.	Myristicaceae	0.0253	0.0173	0.0155	0.0582
20	Meliosma pinnata (Roxb.) Maxim.	Sabiaceae	0.0253	0.0205	0.0119	0.0577
21	Dimocarpus longan Lour.	Sapindaceae	0.0253	0.0189	0.0084	0.0527
22	<i>Litsea bourdillonii</i> Gamble.	Lauraceae	0.0126	0.0094	0.0275	0.0496
23	Aglaia barberi Gamble.	Meliaceae	0.0253	0.0173	0.0059	0.0487
24	<i>Crataeva magna</i> (Lour.) DC.	Capparaceae	0.0126	0.0252	0.0081	0.0460

25	Bischofia javanica Blume	Euphorbiaceae	0.0126	0.0236	0.0094	0.0457
26	<i>Litsea floribunda</i> (Blume) Gamble	Lauraceae	0.0126	0.0221	0.0085	0.0433
27	Vitex altissima L.	Verbanaceae	0.0126	0.0221	0.0085	0.0433
28	Schleichera oleosa (Lour.) Oken.	Sapindaceae	0.0126	0.0221	0.0079	0.0427
29	<i>Chionanthus courtallensis</i> Bedd.	Oleaceae	0.0126	0.0205	0.0078	0.0409
30	<i>Holigarna arnottiana</i> Hook.	Anacardiaceae	0.0253	0.0126	0.0018	0.0398
31	<i>Schefflera capitata</i> (Wight & Arn.) Harms	Araliaceae	0.0126	0.0126	0.0138	0.0391
32	Symplocos racemosa Roxb.	Symplocaceae	0.0126	0.0221	0.0038	0.0386
33	Hydnocarpus longipedunculatus Robi et al.,	Flocourtiaceae	0.0253	0.0078	0.0050	0.0382
34	Aporosa cardiosperma (Gaertn.) Merr.	Euphorbiaceae	0.0126	0.0205	0.0036	0.0368
35	<i>Litsea glabrata</i> (Wall. ex Nees) Hook.	Lauraceae	0.0126	0.0189	0.0048	0.0364
36	<i>Mesua thwaitesii</i> Planch. & Triana	Clusiaceae	0.0253	0.0063	0.0042	0.0359
37	<i>Melicope lunu-ankenda</i> (Gaertn.) Hartley	Rutaceae	0.0126	0.0141	0.0073	0.0342
38	<i>Cinnamomum</i> <i>malabatrum</i> (Burm. f.) Blume	Lauraceae	0.0126	0.0126	0.0052	0.0304
39	<i>Garcinia gummi-gutta</i> (L.) Robs.	Clusiaceae	0.0126	0.01101	0.0066	0.0303
40	Diospyros buxifolia (Blume) Hiern.	Ebenaceae	0.0126	0.0126	0.0044	0.0297
41	Artocarpus hirsutus Lam.	Moraceae	0.0126	0.0126	0.0043	0.0296
42	Baccaurea courtallensis (Wight) Muell.	Euphorbiaceae	0.0126	0.0142	0.0022	0.0291
43	Artocarpus heterophyllus Lam.	Moraceae	0.0126	0.0094	0.0061	0.0282
44	Mangifera indica L.	Anacardiaceae	0.0126	0.0079	0.0058	0.0264
45	Madhuca longifolia (Koenig) Macbr.	Sapotaceae	0.0126	0.0079	0.0045	0.0251
46	Caryota urens L.	Arecaceae	0.0126	0.0094	0.0025	0.0246
47	<i>Trichilia connaroides</i> (Wight & Arn.) Bentv.	Meliaceae	0.0126	0.0047	0.0014	0.0188
48	Drypetes malabarica (Bedd.) Airy Shaw.	Euphorbiaceae	0.0126	0.0032	0.0010	0.0168
49	<i>Lagerstroemia speciosa</i> (L.) Pers.	Lythraceae	0.0126	0.0016	0.0014	0.0156

## Table 15. Population structure of *H. longipedunculatus*: Cheri(List of individuals with $G \ge 30$ cm represented)

Sl.	gbh	Radius	Basal	Basal	Age phase	First	Height of
No	(cm)	(cm)	area	cover		branching	stand
			$(cm^2)$	(m)		(m)	(m)
1.	62	9.9	307.8	4	Set of present	2	14
2.	45	7.2	162.8	3	Set of future	1	6
3.	109	17.4	950.7	6	Set of present	4.5	18
4.	50	7.9	195.9	3	Set of future	2	8
5.	47	7.5	176.6	3.5	Set of future	2.5	5
6.	140	22.3	1561.5	11	Set of present	3	20
7.	102	16.2	824	4	Set of present	6.5	16
8.	138	21.9	1505.9	10	Set of present	3.5	17
9.	124	19.7	1218.6	8.5	Set of present	2.5	17
10.	118	18.8	1109.8	7	Set of present	7	16
11.	45	7.2	162.8	2.5	Set of future	6	8
12.	48	7.6	171.4	3	Set of future	5	8
13.	67	10.7	359.5	3.5	Set of present	3	9
14.	79	12.6	498.5	4.5	Set of present	2.5	10
15.	92	14.6	669.3	5	Set of present	2.5	11.5
16.	98	15.6	764.2	5	Set of present	3	12
17.	103	16.4	844.5	5.5	Set of present	1	12
18.	107	17	907.5	6	Set of present	1.5	13
19.	108	17.2	928.9	7	Set of present	4	13
20.	112	17.8	994.9	7.5	Set of present	6	14
21.	55	8.8	243.2	4	Set of present	2	7
22.	59	9.4	277.5	4.5	Set of present	3.5	8
23.	68	10.8	366.2	5	Set of present	1.5	9
24.	74	11.8	437.2	6	Set of present	2	9
25.	83	13.2	547.1	6	Set of present	3.5	9.5
26.	87	13.9	606.7	7	Set of present	4	12
27.	88	14	615.4	6	Set of present	3	10
28.	96	15.3	735	7	Set of present	2	12
29.	103	16.4	844.5	8.5	Set of present	6	15
30.	112	17.8	994.8	105	Set of present	6	17
31.	130	20.7	1345.5	12	Set of present	7.5	19
32.	42	6.7	140.9	3	Set of future	2	6
33.	44	7	153.7	3.5	Set of present	3	8
34.	48	7.6	183.4	4	Set of present	4.5	8
35.	68	10.8	366.2	5	Set of present	2.5	7
36.	74	11.8	437.2	5.5	Set of present	3	7.5
37.	42	6.7	140.9	4	Set of future	3	7
38.	49	7.8	191	5	Set of future	2	7.5
39.	74	11.8	437.2	6	Set of present	3.5	9
40.	78	12.4	51.3	6.5	Set of present	2.5	11

#### IVI Sl.No Species Family rf rd rD (%) (%) (%) 0.1417 0.1944 Calophyllum polyanthum Clusiaceae 0.0248 0.0289 1. Wall. ex Choisy Calophyllum calaba L. Clusiaceae 0.0247 0.0193 0.1123 0.1554 2. 3. Poeciloneuron indicum Clusiaceae 0.0247 0.0289 0.0909 0.1436 Bedd. 4. Persea macrantha (Nees) Lauraceae 0.0246 0.0385 0.0778 0.1401 Kosterm. 5. Garcinia gummi-gutta (L.) Clusiaceae 0.0245 0.0963 0.0099 0.1299 Robs. Buchanania axillaris 0.0244 0.1263 Anacardiaceae 0.0385 0.0639 6. (Desr.) Ramamoorthy 7. Actinodaphne malabarica Lauraceae 0.0243 0.0482 0.0411 0.1131 Balakr. Olea dioica Roxb. 0.0242 0.0561 0.0289 0.1089 8. Oleaceae *Vateria indica* L. 0.0193 0.0610 0.1041 9. Dipterocarpaceae 0.0241 10. Xanthophyllum arnottianum Polygalaceae 0.0240 0.0642 0.0101 0.0981 Wight 11. *Palaquium ellipticum* Sapotaceae 0.0239 0.0193 0.0451 0.0881 (Dalz.) Baill. 12. Knema attenuata (Hook. f. Myristicaceae 0.0239 0.0337 0.0278 0.0853 & Thoms.) Warb. 13. Myristica malabarica Lam. Myristicaceae 0.0238 0.0289 0.0309 0.0836 14. Cinnamomum verum Presl. 0.0241 0.0690 Lauraceae 0.0238 0.0211 15. Hopea parviflora Bedd. 0.0238 0.0225 0.0218 0.0682 Dipterocarpaceae 16. Cullenia exarillata Robyns. Bombacaceae 0.0238 0.0112 0.0326 0.0676 Carallia brachiata (Lour.) Rhizhophoraceae 17. 0.0238 0.0337 0.0099 0.0674 Merr. 18. Myristica beddomei King Mristicaceae 0.0237 0.0193 0.0181 0.0612 19. *Meliosma pinnata* (Roxb.) Sabiaceae 0.0237 0.0193 0.0172 0.0603 Maxim. 20. Dimocarpus longan Lour. 0.0237 0.0257 0.0084 0.0579 Sapindaceae 21. *Litsea bourdillonii* Gamble Lauraceae 0.0237 0.0241 0.0098 0.0576 0.0094 22. Aglaia barberi Gamble Meliaceae 0.0237 0.0241 0.0573 23. *Bischofia javanica* Blume Euphorbiaceae 0.0237 0.0225 0.0088 0.0551 24. *Litsea floribunda* (Blume) 0.0237 0.0225 0.0087 0.0549 Lauraceae Gamble 0.0235 0.0193 0.0545 25. Vitex altissima L. Verbenaceae 0.0114 26. Schleichera oleosa (Lour.) 0.0235 0.0225 0.0082 0.0544 Sapindaceae Oken 27. Holigarna arnottiana Anacardiaceae 0.0235 0.0128 0.0142 0.0509 Hook. 28. Schefflera capitata (Wight Araliaceae 0.0234 0.0225 0.0039 0.0503 & Arn.) Harms

## Table 16. Floristic diversity / Importance value index of *H. longipedunculatus*: Cheri(List of individuals with $G \ge 30$ cm represented)

29.	Symplocos racemosa Roxb.	Symplocaceae	0.0233	0.0209	0.0038	0.0485
30.	Hydnocarpus	Flacourtiaceae	0.0232	0.0193	0.0049	0.0480
	longipedunculatus Robi et					
	al.					
31.	<i>Litsea glabrata</i> (Wall. ex	Lauraceae	0.0230	0.0161	0.0057	0.0456
	Nees) Hook.					
32.	Mesua thwaitesii Planch. &	Clusiaceae	0.0228	0.0161	0.0025	0.0424
	Triana.					
33.	1	Rutaceae	0.0227	0.0128	0.0053	0.0419
	(Gaertn.) Hartley.					
34.		Lauraceae	0.0225	0.0112	0.0068	0.0418
	(Burm. f.) Blume					
35.	Aporosa cardiosperma	Euphorbiaceae	0.0224	0.0128	0.0046	0.0412
	(Gaertn.) Merr.					
36.		Euphorbiaceae	0.0222	0.0128	0.0018	0.0384
	(Wight) Muell.					
37.	Artocarpus heterophyllus	Moraceae	0.0221	0.0080	0.0059	0.0378
	Lam.					
38.	Mangifera indica L.	Anacardiaceae	0.0220	0.0080	0.0047	0.0365
39.	Caryota urens L.	Arecaceae	0.0219	0.0064	0.0049	0.0351
40.	Drypetes malabarica	Euphorbiaceae	0.0218	0.0048	0.0015	0.0301
	(Bedd.) Airy Shaw.					
41.	Lagerstroemia speciosa	Lytheraceae	0.0217	0.0032	0.0010	0.0281
	(L.) Pers.					

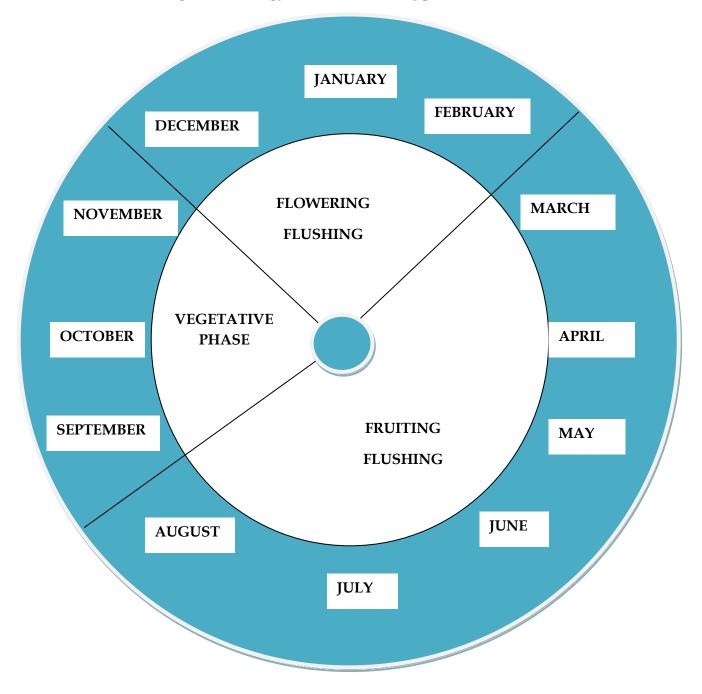


Fig. 5. Phenology calendar of *H. longipedunculatus* 

Season	Atm. Temp. ( <sup>0</sup> C)	Night Temp. ( <sup>0</sup> C)	Atm. Humidity (Day - %)	Atm. Humidity (Night- %)
Summer	30	25	68	85
Monsoon	26	21	82	88
Winter	27	22	70	82

 Table 17. Climatological data of H. longipedunculatus: Kulamavu

 Table 18. Edaphological data of H. longipedunculatus: Kulamavu

u	Soil level	Texture	p <sup>H</sup>	N	Р	K	Temp.	Moisture
Season				(Kg/Ha)	(Kg/Ha)	(Kg/Ha)	(°C)	(%)
	<b>A C</b>	0.1	1.5	<b>.</b>	1.0	101		
<u>ب</u>	Surface	Silty clay loam	4.5	564.5	1.8	121		
mei	Middle	Silty clay	4.6	504.9	5.0	150.1	23	19.5
summer	Bottom	Sandy loam	4.5	573.9	6.8	172.5		
	Surface	Silty clay	4.8	637.6	3.3	636.9		
		loam						
on	Middle	Sandy clay	5.0	557.5	4.4	550	21.5	27.67
OSL	D	Loam		201.0	•	100.0		
monsoon	Bottom	Silt loam	5.0	301.8	2.0	190.3		
	Surface	Silty clay	4.9	627.2	2.1	142.2		
er	~~~~~	loam		02712				
winter	Middle	Silt loam	4.6	439	3.4	181.4	22.8	24.56
	Bottom	Sandy clay	4.9	561.3	3.1	211.7		
		Loam						

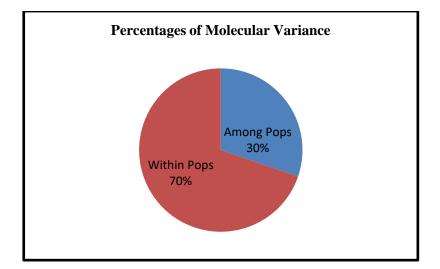
Sl. No	Primer code	Annealing temperature (°C)	Total bands produced	Polymorphic bands	Percentage of polymorphism
1.	UBC 810	41.9	14	14	100
2.	UBC 811	41.3	13	13	100
3.	UBC 812	49.0	11	11	100
4.	UBC 825	42.3	12	12	100
5.	UBC 830	51.0	11	11	100
6.	UBC 834	50.9	13	13	100
7.	UBC 890	53.8	13	13	100
8.	PRIMER 1	49.0	9	9	100
9.	PRIMER 2	53.8	9	9	100
10.	PRIMER 6	53.3	8	8	100

Table 19. H. longipedunculatus: ISSR primers vs. polymorphism

 Table 20. H. longipedunculatus: Population genetic diversity parameters

Sl. No.	Population	Sample size	Observed number of alleles	Effective number of alleles ±S.D	No. of poly- morphic loci	Percentage of poly- morphic loci	Nei's gene diversity ±S.D	Shannon informat ion index ±S.D
1	Kulamavu MPCA	20	1.867 ±0.34	1.4082 ±0.3495	98	86.73	0.2465 ±0.1787	0.3804 ±0.2416
2	Cheri	20	1.584 ±0.50	1.3067 ±0.3432	66	58.41	0.1854 ±0.1911	0.2823 ±0.2752
3	All populations together	40	-	1.3679 ±0.2646	-	-	0.2430 ±0.1377	0.3905 ±0.1807

## Fig. 6. *H. longipedunculatus* : Genetic diversity within and among populations



## Plate 4. *Hydnocarpus longipedunculatus* : population structure



Habit and Habitats: Different views from the Kulamavu MPCA



Habit and Habitats: Different views from the Cheri -Kulamavu



Views of field study

## Plate 5. *Hydnocarpus longipedunculatus* : population dynamics



Views of vegetative phenology along with insect incidence



Male flowers along with insect incidence



Views of female flowers and Cecidomycidae larvae in flower

## Plate 6. *Hydnocarpus longipedunculatus* : Population dynamics



Fruit primordia



Half matured fruits



**Dehisced fruits** 



**Matured Fruit** 



 37
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**Processed seeds** 

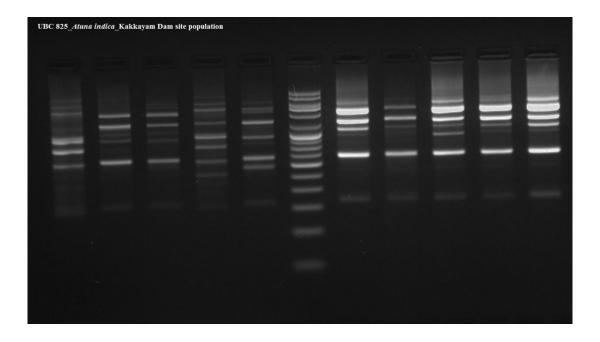


Infestated fruit

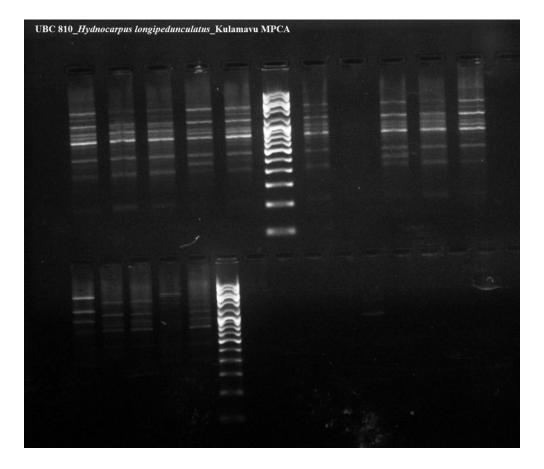


## Fallen degraded fruit

## Plate 7: Genetic diversity analysis of target species



PCR profile of *A. indica* at Dam site population using ISSR primer (UBC 825)



PCR profile of *H. longipedunculatus* at Kulamavu MPCA population using ISSR primer (UBC 810)

## 6. DISCUSSION AND CONCLUSION

The populations of Atuna indica and Hydnocarpus longipedunculatus are distributed isolated forest areas in Kerala part of the Western Ghats which generally face high degree of habitat modifications and loss. The populations of A. indica are confined to the northern part of Kerala whereas; *H. longipedunculatus* is restricted to the high ranges of Idukki District. The high degree of isolation/fragmentation of populations/habitats often result in diversity loss which can lead to the endangerment of species (Zhu et al. 2004; Bhatt et al. 2015). The limited and localized distribution of the species may also be possibile due to lesser level of ecological amplitude (Krishnamoorthi et al., 2015). . In any plant species, maintenance of genetic variation is a pre-requisite to adapt to the changing environments and to develop resistance towards pest as well as disease outbreaks. The loss of genetic variation will gradually lead the species to untimely endangerment and local extinction in due course (Barrett and Kohn, 1991). The limited distribution may also possibile due to lesser level of ecological amplitude and thereby resulted in localized distribution of the species (Krishnamoorthi et al., 2015). Further, small and isolated populations are prone to heterozygote deficit and chances of genetic drift are high for such species. Therefore, conservation strategies need to be formulated in such species to better equip the germplasm from possible extinction.

In an ecological analysis of a threatened species, age class distribution plays a significant role in estimating the population size. The number of adults covering pre-reproductive and reproductive individuals determines the effective population size of a species. The reduction in set of pre-reproductive individuals and poor rate of natural regeneration accelerates the processes leading to the decline in population size of a species (Swarupanandan *et al.*, 2013; Jose and Pillai, 2014). The reduced number of pre-reproductive individuals and poor natural regeneration observed in the endemic *A. indica* 

and *H. longipedunculatus* may lead to local endangerment of these species in the absence of any conservation efforts. In a population diversity analysis, the lower IVI values among the associated species in a given forest landscape are associated with poor dominance and competitive behavior (Pascal, 1988; Jose, 2001). The extreme low abundance values of *A. indica* and *H. longipedunculatus* point towards the low occurrence of these species in the identified forest ecosystems. Specialized habitats are prerequisites for the growth and reproduction of endemic and threatened plants (Nayar, 1996; Jose, 2001; Pandurangan, 2003; Swarupanandan *et al.*, 2013). The altitude specificity, integrity in species association, spatial and temporal distribution pattern, soil factors, among others are essential to meet niche specialties of rare and threatened species'. The two target species displayed their integral association in the evergreen ecosystems, habitat specificity towards altitude gradients and spatial preference towards watercourses and thereby indicate that the species are passing through rarity process.

The reproductive constraints faced by species also lead to untimely endangerment and local extinction (Bawa, 1983; Primack, 1994; Daniel and Jayanthi, 1996; Davy and Jefferies, 1981). The two target species exhibit reproductive complexities such as abnormal flowering periodicities, flowering of isolated individuals among populations (*A. indica*), stray flowering nature, abscission of fruiting primordia, low fruit set, seed pest infestation, etc. The seed pest infestation severely affected the germination and subsequently poor natural regeneration is reported in tropical trees such as *Dipterocarpus retuses* (Senthilkumar *et al.*, 2009), *Cinnamomum sulphuratum* (Manivannan *et al.*, 2010), *Gluta travancorica* (Jose *et al.*, 2004) and *Humboldtia vahliana* (Jose *et al.*, 2008). The density values of seedlings and saplings are considered as an indicator of regeneration potential of the species. The presence of good regeneration potential shows suitability of a species to the environment (Choudhury *et al.*, 2007). The poor regenerative ability coupled with seed pest infestation in the studied species drastically reduced the seedling bank *in situ* and warrants urgent conservation measures.

The climatic factors have a crucial role in the flowering of trees (Doorn and Meeteren, 2008). The flower which blooms in early morning is driven by the increased temperature and sunlight and decreased humidity (or humidity is irrelevant). In A. indica, only two flowering individuals were recorded across the three populations during the period of study ie. Kakkayam Dam site population. Interestingly, it is noted that these individuals are located in the fringes of evergreen forests where light and temperature are sufficient (high irradiance) for flowering as compared to Charangad and Nadugani Ghat populations (Low irradiance). In H. longipedunculatus, the flowering started at mid night, influenced by a hike in the relative humidity. The habitats of the species possess dense canopy with higher humidity and are therefore favour flowering of the species. Habitat modification/ degradation along with unprecedented climate change can induce drastic changes in flowering phenology with subsequent reproductive complexities. Studies on regeneration patterns of forest tree species revealed the significance of canopy gaps in seedling establishment as well as in increasing the sapling size (Clark and Clark, 1999). The old growth forests in Kakkayam-Charangad and Nadugani forest area are therefore believed to be suppressing the regenerative performance of sub canopy species like A. indica.

The relationship of Pyralid moth with *Atuna indica* can be believed to a mutualism/Parasitism. The *Pyralidae* larvae were observed within the matured fruits of *Atuna indica*. The flowers were pollinated while adult moths were laying eggs in the soft tissues of flower. The eggs developed into larvae which later completed its lifecycle by feeding on the growing endosperm of seeds. Similarly, *Upig virescence (Pyralidae)* had a mutualistic interaction with its host plant, *Lophocereus schotti* (Holland and Fleming, 1999). The interaction of pyralid moth with *A. indica* could be either of mutualistic or parasitic. Likewise Cecidomyiidae sp. which was earlier reported as flower pest in lentils

and alfalfa (Hill, 1987) were observed to damage the bisexual flowers of *Hydnoarpus longipedunculatus*.

In the ecological context, both climate and soil factors plays a key role in the establishment, growth and reproduction of a species. In plants, the nutrient elements are essential for performing various functions such as chlorophyll synthesis, protein synthesis, lignifications, etc. (Ram *et al.* 2004). Microsite conditions such as atmospheric temperature, humidity, rainfall, etc. can often control germination and subsequent establishment of a plant species (Dhaulkhandi, 1996). The variation in these conditions alter the phenological phases such as initiation and development of leafing, flowering, fruit development, dispersal and regeneration, etc. (Kallarackal and Chandrasekhara, 2008). It is estimated that the macronutrients level was low to medium in the populations of target species. Since natural regeneration is affected by the microsite conditions particularly by the avaibility of the soil nutrients, the low soil macronutrients may result in poor regeneration of the species (Khumbongmayum *et al.*, 2005, Sarkar and Devi, 2014). The soil among the populations exhibited medium level of N, low content in P and moderate to high K content for both the species (Kerala State Planning Board, 2013).

The population genetic structure of narrowly distributed endemic species in a specific microhabitat is directly influenced by the historical origin of the population, fragmentation, effective population size, mating system, gene flow, among others (Schaal *et al.*, 1998). Narrowly distributed species generally tend to have a low genetic diversity compared to widely distributed species (Lovelss and Hamrick, 1994). Results revealed that in general, the genetic diversity of the two studied endemic species was almost similar or more than the average in long lived woody species with large populations. Hamrick *et. al.* (1992) compared the genetic diversity data (allozyme) in respective of their life forms, ecological and life history traits (geographical range, breeding system, seed dispersal and mode of reproduction). In long lived woody species with large number of populations, the

average/standard Percentage of Polymorphic Loci (PPL) is reported with 65.0. In *A. indica,* similar values were recorded in PPL (64.3), whereas, *H. longipedunculatus* has got comparatively better PPL values (72.57).

The population genetic diversity analysis is generally performed assuming random breeding under Hardy-Weinberg Equilibrium (HWE). In *A. indica* and *H. longipedunculatus*, the effective number of alleles was less than the observed number of alleles indicating a slight deviation from the general assumptions of random breeding under HWE. In both the species, the estimated values of allelic richness (79.5 % and 79.2% respectively, for *A. indica and H. hydnocarpus*) was significantly higher than average values reported for long woody perennials (55.9%) and is even higher than that of wide spread species (65.9%). This reveals that the populations are free from any population bottlenecks owing to fragmentation, human disturbance or founder effects even though the effective population size is small.

The genetic structure of plant populations is mainly influenced by population history, genetic drift, breeding system, gene flow, among others (Barrett and Kohn, 1991). The genetic differentiation among populations in *H. longipedunculatus* (Gst = 0.1112) was significantly lower than the average value reported for outcrossing plant species (Gst = 0.22) while that of *A. indica* had a similar value (0.2231) (Michael and David, 1999). The species with low inter population genetic differentiation is reported to have more overall diversity (Hamrick et al., 1979; Hamrick and Godt, 1996). In *H. pedunculatus*, the high gene flow in (3.9965), low genetic differentiation and consequently high genetic diversity with more number of flowering individuals is sufficient enough to conserve the species *in situ*. The comparatively low rate of gene flow in *A. indica* is due to the reduced sexual and asexual recruitment with extreme low number of flowering individuals (2), low fruit set and absence of effective seed dispersal which eventually resulted in reduced amount of horizontal gene transfer (1.7407).

Both the species had pollinators with great range of foraging activities. *Apies mellifera* is the key pollinator in both the target species with a foraging range of 40-5983 m (Hagler *et al.*, 2011). In *A. indica*, two populations at Kakkayam are located within 3-4 Km. Even though the foraging of *Apies mellifera* noticed during flowering, gene dispersal was not effective due to the less number of flowering trees. In *H. longipedunculatus*, the two populations are located in proximity (4-5 km) and many flowering trees were found which in turn increased the rate of gene flow (3.9965) and consequently reduced the genetic differentiation among the populations. Since the target species lack efficient seed dispersal mechanisms, the pollen transport becomes the major factor facilitating the gene flow.

There is no immediate risk of population bottle neck effects as the both these endemic species' are self incompatible, outcrossing and perennial in nature. Population with highest diversity indices can be targeted for *in situ* conservation efforts to enrich the species natural resources in both the narrowly distributed species. Additionally, the existing natural resources especially populations with low number of mature individuals can be enriched with more number of migrants so that it can effectively support long term evolution and survival of both the species. *Ex situ* conservation efforts in a suitable microclimate adopting vegetative/ *in vitro* propagation protocols and targeting genetically rich population can be a viable option for expanding the natural resources for future survival and evolution of both these species.

### Salient findings:

## 1. Atuna indica

- A distribution map of *Atuna indica* covering 3 major populations in two forest areas (Kakkayam and Nadugani) in the Kerala part of Western Ghats was prepared based on GPS coordinates.
- The population structure covering number of adult individuals, gbh classes, basal area, age phase and height of individual trees was analyzed within the three sampled populations viz. Kakkayam-Dam site, Kakkayam-Charangad, Nadugani. A total of 89 adult individuals of the species were enumerated from the respective forest areas.
- Population diversity analysis was carried out in an enumerated area of 21,000 m<sup>2</sup> covering three populations and relative dominance among associates of the species was estimated.
- Only 36 seedlings altogether were recorded from the three populations.
- Extreme low number of flowering individuals, abscission of fruiting primordia, low fruit set (25%), incidence of seed pest, low percentage of pre-reproductive individuals (23%), etc. are indicative of the reproductive constraints in the species.
- Based on the distribution and ecological data generated (IUCN guidelines), a conservation status is assessed as Critically endangered (CR) from the endangered category.
- Altogether 9 soil samples *in situ* representing three season of a year were analyzed with respect to texture, pH, N,P,K, temperature and moisture from the three populations as part of identification of *in situ* requirements of the species.
- Genetic diversity value (0.2153) and gene flow (1.7407) were comparable with that of open pollinating outcrossing perennial species.

## 2. Hydnocarpus longipedunculatus

- A distribution map of *Hydnocarpus longipedunculatus* covering two populations in Kulamavu forests of the Kerala part of Western Ghats was prepared based on GPS coordinates.
- Population structure covering vertical/ horizontal distribution, number of adult individuals, gbh classes, basal area, age phase and height of individual trees was analyzed in the two sampled forest areas viz., Kulamavu MPCA ad Cheri. A total of 76 adult individuals of the species were enumerated within the study area.
- Population diversity analysis covering an enumerated area of 14,000 m<sup>2</sup> comprising two population sites viz. Kulamavu MPCA and Cheri revealed relative dominance among associates of the species.
- Seedling recruitment was very low, only 14 seedlings could be enumerated from the existing two populations.
- Polygamo-diocieous nature, pest incidence at flowering stage, fruit predation, low fruit set (35%), low percentage of pre reproductive individuals (23%) etc. were the major reproductive hurdles faced.
- Based on the distribution and ecological data generated (IUCN guidelines), Critically Endangered (CR) conservation status is suggested.
- The soil samples in *situ* representing three season of a year were analyzed with respect to texture, pH, N,P,K, temperature and moisture as part of identification of *in situ* requirements of the species.
  - Genetic diversity in the species (0.2465) was higher than that of average in long lived woody trees and the high gene flow (3.9965) can prevent genetic differentiation of the existing populations.

## Recommendations

- ✓ Since the populations of two target species were found intervened by various physical factors, the habitats of the species to be prioritized for protection and management.
- ✓ As flowering stands of *Atuna indica* are extremely few among the populations, long term monitoring/ experiments of populations is essential to identify flowering individuals and factors associated including light irradiance affecting flowering phenomenon.
- ✓ Genetically diverse populations can be targeted for selection of genotypes/ propagules for *ex situ* conservation adopting vegetative/ *in vitro* propagation protocols
- ✓ Since the populations are small and isolated in their distribution range, creating new populations through restocking/ reintroduction is significant to minimize local endangerment of the species. As the populations are dwindling in nature development of alternate populations *ex situ* through appropriate genebanks for evaluation programmes.
- ✓ Long term monitoring/ experiments are essential to identify the reproductive constraints and to enhance the seedling recruitment for retaining a viable germplasm.

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## 8. References

- Barrett, S. C. H. and Kohn, J. R. 1991. Genetic and Evolutionary consequences of small population size in plants: Implication for conservation. In; Dunald A. Falk and Kent E. Holsinger (Eds.), *Genetics and Conservation of rare plants*. Oxford University, New York.
- Bauert, M.R., Kalin, M., Baltisberger, M. and Edwards, P.J. 1998. No genetic variation detected within isolated relic populations of *Saxifraga cernua* in the Alps using RAPD markers. *Mol. Ecol.*, 7:, 1519–1527.
- Bawa, K.S. 1983. Patterns of flowering in tropical plants. In: C.E. Jones and R.J. Little (eds.), *Handbook of Experimental Pollination Biology*. Van Nostrand Reinhold, New York. pp. 394-410.
- Bhatt, G.D., S.P.S. Kushwaha, S. Nandy, K. Bargali, P.S. Nagar and D.M. Tadvi .2015. Analysis of fragmentation and disturbance regimes in south Gujarat forests, India. *Tropical Ecology*, 56: 275-288.
- Blasco, F. 1979. *Montagnes du sud de l'Inde Forests, Savanes, Ecologies*. Travaux de la section scientifique et technique 11. Institut Francais de Pondichery.
- Clark, D.A. and Clark, D.B., 1999. Assessing the growth of tropical rain forest trees: issues for forest modeling and management. *Ecol. Appl.* 9: 17.
- Chivian, E. and Bernstein, A. (eds.), 2008. Sustaining Life : How human health depends on biodiversity, Oxford University Press, New York.
- Clegg, M.T. 1990. Molecular diversity in plant populations. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. Plant population genetics, breeding and genetic resources. Massachusetts: Sinauer Associates, Inc., 98±115.

- Culley, T.M. and Wolfe, A.D. 2001. Population genetic structure of the cleistogamous plant species, *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity*, 86: 545-556.
  - Daniel, R.J. and Jayanthi, M. 1996. Biology and Conservation of endangered plants. The need to study breeding systems. *Trop. Ecol.*, 37(1): 39-42.
  - Davy, A.J. and Jefferies, R.L. 1981. Approaches to the monitoring of rare plant populations. In: H. Synge (eds.), *The Biological Aspects of Rare Plant Conservation*. John Wiley & Sons Ltd., New York. pp. 219-232.
- Dennis, S. Hill. 1987 Agricultural Insect Pests of tropics and their control Cambridge. University Press, New York ISBN 9780521294416.
- Dhaulakhandi, M. 1996. A study on structure, phytosociology and regeneration of an oak forest of Bhagirathi Valley, Garhwal Himalaya. Ph.D. Thesis, Garhwal University, Srinagar (Garhwal).
- Dong, Y.H., Chen, J.M., Gituru, R.W. and Wang, Q.F. 2007. Gene flow in populations of the endangered aquatic fern *Ceratopteris pteridoides* in China as revealed by ISSR markers. *Aquat. Bot.*, 87, 69–74.
- Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytoch. Bull.* 19:11-15.
- Doorn VWG and Meeteren, U. 2003. Flower opening and closure: a review . *Journal of Experimental Botany* 54, 1801–1812.
- Falk D. A. and Holsinger K. E. (Eds.) 1991. Genetics and Conservation of Rare Plants, Oxford University Press, New York.
- Ferguson, Moira M., Drahushchak, Lenore R.1990. Heredity- Abstract of article: Disease resistance and enzyme heterozygosity in rainbow trout. *Heredity*, 64 (3): 413–417. <u>doi:10.1038/hdy.1990.52</u>. <u>ISSN 0018-067X</u>.

- Fischer, M. and Matthies, D. 1998. Effects of population size on performance in the rare plant, *Gentianella germania*. *Journal of Ecology*, 86: 195-204.
- Frankel, O.H. and Soulé, M.E. *Conservation and Evolution*; Cambridge University Press: Cambridge, UK, 1981.
- Frankham
   and
   Richard
   2005.
   Biological
   Conservation, 126 (2):

   131–140. doi :10.1016/j.biocon.2005.05.002.
   131–140.
   131–140.
   101–140.
   101–140.
   101–140.
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   101–140.
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   101–140.
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   101–140.</td
- Ge, X.J. and Sun, M. 1999. Reproductive biology and genetic diversity of a crypto viviparous mangrove Aegiceras corniculatum (Myrsinaceae) using allozyme and Inter Simple Sequence Repeat (ISSR) analysis. Molecular Ecology, 8: 2061-2069.
- Godt, M.J.W., Caplow, F. and Hamrick, J.L. 2005. Allozyme diversity in the federally threatened olden paintbrush, *Castilleya levisecta* (Scrophulariaceae). *Conserv. Genet.*, 6, 87–99.
- Gupta, M., Chyi Y.S., Romero-Severson J. and Owen J.L. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, 89: 998-1006.
- Gupta, S.R. and Malik, V. 1996. Soil ecology and sustainability. *Trop. Ecol.* 37(1): 43-55.
- Hagler, J.R., Mueller,S., Teuber L.R., Machtley S.A. and Deynze, A.V. 2011. Foraging range of honey bees, *Apis mellifera*, in alfalfa seed production fields. J. Insect Sci., 11 (2011), p. 144
- Hansson B. and Westerberg L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Mol.Ecol.* 11: 2467–2474. 10.1046/j.1365-294X.2002.01644.x
- Hamrick, J.L., Godt, M.J.W. and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests*, 6: 95–124.

- Hamrick, J.L. and Godt M.J.W. 1996. Conservation genetics of endemic plant species. In: Avise JC, Hamrick JL, eds. Conservation genetics: case histories from nature. New York: Chapman and Hall, 281±304.
- Henry, A.N., Vivekanandan, K. and Nair, N.C. 1979. Rare and threatened flowering plants of South India. *J. Bombay Nat. Hist.Soc.* 75: 684-697.
- Hill, R. R., Jr. 1987. Alfalfa. W. R. Fehr ed. Principles of cultivar development. Vol. 2. Macmillan Publishing Co., New York, NY .pp 11–39.
- Hoelzel, A.R., 1992. Molecular Genetic Analysis of Populations A Practical Approach. Oxford University Press, New York, pp: 50-58.
- Holland, J. N., and Fleming, T. H. 1999. Mutualistic interactions between Upiga virescens (Pyralidae), a pollinating seed-consumer, and Lophocereus schottii (Cactaceae). *Ecology*, 80: 2074–2084.
- IUCN, 2001. IUCN redlist categories and criteria: version 3.1. IUCN Species Survival Commission, IUCN. Gland, Switzerland and Cambridge. 30p.
- IUCN, 2012. IUCN Red List of Threatened Species (ver. 2012.2). Available at: http://www.iucnredlist.org. (Accessed: 17 October 2012).
- Javed, A.M., Cannon, C.H. and Wickneswari, R., 2014. Microsatellite DNA markers in Shorea platyclados (Dipterocarpaceae): genetic diversity, size homoplasy and mother trees. J. For. Sci., 60: 18–27. <u>https://doi.org/10.17221/71/2013-JFS</u>.
- Jose, P.A. 2001. A Study on the Population structure, dynamics and conservation of two Rare and endemic trees of Western Ghats of Kerala. Ph.D. Thesis, Kerala University, Thiruvananthapuram. pp. 184.
- Jose, P.A. and P.K.C. Pillai 2014. Conservation through restoration of two endemic endangered trees of Western Ghats of Kerala. Final Project Report, No. 473. Kerala Forest Research Institute, Peechi, Thrissur.

- Jose, P.A. and Pandurangan, A.G. and Thomas, J. 2000. Ecology and Conservation of Ochreinauclea missionis : A Case Study for the Rare and Endemic Trees of Western Ghats. In: M.R. Das (ed.), Proceedings of the Twelfth Kerala Science Congress. State Committee on Science, Technology and Environment. Thiruvananthapuram, Kerala. pp. 576-580.
- Jose, P.A., and Pandurangan, A.G. 2002. Conservation biology of *Gluta travancorica*: A system approach for management and utilization of rare and endemic trees of Western Ghats. In: M.K. Janarthanan and D. Narasimhan (eds.), *Plant Taxonomy, Human Welfare and Conservation*, Goa University. pp. 321-328.
- Jose, P.A., Hussain, K.H. and Sreekumar, V.B. 2014. Developing an information system for the Rare, Endangered and Threatened (RET) plants of Southern Western Ghats. KFRI Research Report No.492. Kerala Forest Research Institute, Peechi, Thrissur. p.33.
- Jose, P.A., Mohanan, N., and Hussain, A. 2008. New record of seed pest, *Cryptorhynchus indicus* Motschulsky (Coleoptera: Curculionidae) in *Humboldtia vahliana* Wight. *Ind. For.*, 134 (6): 849-850.
- Jose, P.A., Pandurangan, A.G. and George Mathew. 2004. Insects associated with population dynamics of *Ochreinauclea missionis* (Wall. ex G.Don) Ridsd.-A rare and endemic tree species of the Western Ghats,India- *Journal of Non-Timb. For. Prod.* 11(3) : 166-169.
- Kallarackal. J. and Chandrasekhara, U.M. 2008. Water and light use characteristics of the vegetation in the different strata of a moist deciduous forest. KFRI Research Report No.310, KFRI, Peechi, Kerala. India.
  - Keller, L. and Waller, D.M. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution, 17: 230-241.

- Kerala State Planning Board. 2013. Soil fertility assessment and information management for enhancing crop productivity in Kerala. Kerala State Planning Board. Thiruvananthapuram. pp.514.
- Khumbongmayum A.D., Khan M.L. and Tripathi R.S. 2005. Survival and growth of seedlings of a few tree species in the four sacred groves of Manipur, Northeast India. *Curr. Sci.*, 88:1781–1788.
- Kingston, N., Waldren, S. and Smyth, N. 2004. Conservation genetics and ecology of Angiopteris chauliodonta Copel. (Marattiaceae), a critically endangered fern from Pitcairn Island, South Central Pacific Ocean. *Biol. Conserv.*, 117: 309– 319.
- Krishnamoorthi H., Ramakrishna H., Ahir, K.C. and Shrikanth G., 2015. Population structure of *Knema attenuata* (J. Hk. & Th.) – A red listed medicinal tree species in Northern Western Ghats of Karnataka, India. *Medicinal Plants*, 7(1): 41-47.
- Krukebery, A. K. and Rabinowitz, D. 1985. Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics*, 16: 447-479.
- Lande, R. and Barrowclough, G.F. 1987. Effective Population Size, Genetic Variation, and Their Use in Population Management. In *Viable Populations for Conservation*; Soulé, M.E., Ed.; Cambridge University Press: Cambridge, UK; pp. 87–123.
- Leberg, P. L. 1990. Influence of genetic variability on population growth: implications for conservation. *Journal of fish biology*. **37**:193–195. doi:10.1111/j.1095-8649.tb05036.x. ISSN 1095-8649.
- Leimu R., Mutikainen P., Koricheva J. and Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol., 94: 942–952. 10.1111/j.1365-2745.2006.01150.x

- Lima, R.A. de, Lopes, M.T.G., Bentes, J.L. da S., Valente, M.S.F., Pereira, J.O. and Muniz, G.I.B. de, 2015. Genetic diversity and structure of *Senna reticulate*. FLORESTA 45, 507. <u>https://doi.org/10.5380/rf.v45i3.38079</u>
- Loveless, M.D. and Hamrick, J.L. 1984. Ecological determinants of genetic structure of plant populations. *Annu. Rev. Ecol. Syst.* 15: 65–95.
- Luan, S.S., Chiang, T.Y. and Gong, X. 2006. High genetic diversity vs. low genetic differentiation in *Nouelia insignis* (Asteraceae), a narrowly distributed and endemic species in China, revealed by ISSR fingerprinting. *Ann. Bot.*, 98:583–589.
- Manivannan, S., Nagaveni, H.C. and Sundararaj, R. 2010. Record of weevil, *Alcidodes* sp. damaging the seeds of *Cinnamomum sulphuratum*. *Pest Management in Horticultural Ecosystems*, 16 (1): 25-28.
  - Michael, C.W. and David, E.M. 1999. Indirect measures of gene flow and migration:  $FST \neq 1/(4Nm+1)$ ," Heredity, 82: 117-125.
- Milligan, B.G., Leebens-Mack, J. and Strand, A.E. 1994. Conservation genetics: beyond the maintenance of marker diversity. *Molecular Ecology*, 12: 844-855.
- Misra, R. 1968. Ecological Work Book. Oxford and IBH Publishing Co., Calcutta.
- Mouhaddab, J., Ait Aabd, N., Achtak, H., Msanda, F., Zahidi, A., Filali-Maltouf, A., Ferradouss, A., El Modafar, C., Nejmeddine, M. and El Mousadik, A. 2015.
  Patterns of genetic diversity and structure at fine scale of an endangered Moroccan endemic tree (*Argania spinosa* L. Skeels) based on ISSR Polymorphism. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 43. https://doi.org/10.15835/nbha.43.2.9842
- Murali, K.S. and Sukumar, R. 1994. Reproductive phenology of a tropical forest in Mudumalai, Southern Ind. J. Ecol., 82: 759-767.

- Myers, N. 1980. Address to the world future society Third General Assembly. *Futurist*. 14(5) :13.
- Nayar, M.P. 1996. *Hot Spots of Endemic Plants of India, Nepal and Bhutan*. Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram.
- Nayar, T.S., Sibi, M., Rasiya Beegam, A. Mohanan, N. and Rajkumar, G. 2008. Flowering plants of Kerala: Status and Statistics. *Rheedea*, 18(2): 95-106.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89 (3): 583–590.
- Pandurangan, A.G. 2003. Rescue and restoration of endemic and RET medicinal plants of Agasthyamalai, Kulamavu and Wayanad MPCAs, Kerala, India. Final Project Report. Tropical Botanic Garden and Research Institute, Thiruvananthapuram.
- Parthasarathy, N. and Sethi, P. 1997. Trees and liana species diversity and population structure in a tropical dry evergreen forest in South India. *Trop. Ecol.*, 38(1): 19-30.
- Pascal, J. P. 1988. Wet Evergreen Forests of the Western Ghats of India: Ecology, Structure, Floristic Composition and Succession. Institut Francais de Pondichery, India.
- Pawar, U., Baskaran, J., Ajithkumar, I. and Panneerselvam, R. 2013. Genetic variation between Xylocarpus spp. (Meliaceae) as revealed byRandom Amplified Polymorphic DNA (RAPD) markers. *Emir. J. Food Agric.*, 25: 597 https://doi.org/10.9755/ejfa.v25i8.15403.
- Peakall, R. and Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research— an update. *Bioinformatics*, 28 (19): 2537–2539.

- Primack, R.B. 1994. *Essentials of Conservation Biology*. Sinauer Associates, Sunderland, Massachusetts.
- Priya, K., Indira, E.P., Sreekumar, V.B. and Renuka, C.2016. Assessment of genetic diversity in *Calamus vattayila* Renuka (Arecaceae) using ISSR markers, *Journal of Bamboos and Rattan*, Vol.15, Nos 1-4, pp.61-69.
- Pushpangadan, P. 1992. On Conservation Biology, Domestication and Commercial Cultivation of Wild Medicinal and Aromatic Plants. In: S.P. Raychoudari (ed.), *Recent Advanced in Medicinal, Aromatic and Spice Crops*. Today and Tomorrow's Printers & Publishers, New Delhi. pp. 431-436.
- Ram, J., Kumar A. and Bhatt J. 2004. Plant diversity in six forest types of Uttaranchal, Central Himalaya, India. *Curr. Sci.*, 86: 975-978.
- Ramesh, B. R., Karunakaran, P. V. and Balasubramanian, M. 2003. Conservation strategy for RET and endemic plant species of Kerala. *In:* J. Kallarackal, K. Swarupanandan & J. K. Sharma (eds.), *Conservation and Research Needs of the Rare, Endangered and Threatened (RET) Tree Species in Kerala Part of the Western Ghats*. Kerala Forest Research Institute, Peechi. pp. 13-17.
- Ramesh, B.R. and Pascal, J.P. 1991. Distribution of the endemic arborescent evergreen species of Western Ghats. In : Karunakaran C.K. (ed.), Pro. Symposium on Rare endangered and endemic plants of the Western Ghats. Kerala Forest Department Wildlife Wing, Thiruvananthapuram. pp-20-29.
- Reveal, J.L. 1981. The concept of rarity and population threats in plants communities. In:E. Larry Morse and Mary Sue Henifin (eds.), *Rare Plant Conservation: Geographical Data Organization*. New York. pp. 41-47.
- Rossetto, M., Weaver, P.K. and Dixon, K.W. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera*. *Mol. Ecol.*, 4: 321–329.

- Rossetto, M., Weaver, P.K. and Dixon, K.W. 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera*. *Mol. Ecol.*, 4: 321–329.
- Saccheri, Ilik Kuussaari, Mikko, Kankare, Maaria, Vikman, Pia, Fortelius, Wilhelm, Hanski, Ilkka. 1998. Inbreeding and extinction in a butterfly metapopulation.*Nature*. 392 (6675):491– 494. doi:10.1038/33136. ISSN 0028-0836.
- Sarkar, M. and Devi, A. 2014. Assessment of diversity, population structure and regeneration status of tree species in Hollongapar Gibbon Wildlife Sanctuary, Assam, Northeast India. *Tropical Plant Research*, 1(2): 26–36.
- Sarmiento, L., Pérez-Almeida, I., Díaz, B., Álvarez, H. and Viera, W., 2017. Molecular marker-based characterization of ecuadorian dry forest tamarind plus trees 10.
- Sasidharan, N. 2004. *Biodiversity documentation for Kerala. Part 6: Flowering plants.* Kerala Forest Research Institute, Peechi.
- Sasidharan, N. 2011. Flowering Plants of Kerala. Digital Version 2.0. Kerala Forest Research Institute, Peechi.
- Sasidharan, N. 2017. A Handbook on the Red listed species and their conservation status in Kerala. Final technical report. Kerala Forest Research Institute, Peechi. pp.527.
- Schaal, B.A., Hayworth, D.A., Olsen, K.M., Rausher, J.T. and Smith, W.A. 1998.
   Phylogeographical studies in plant: problems and prospects. *Molecular Ecology*, 7(4): 465-474.
- Schaal, B.A., Leverich, W.J. and Rogstad, S.H. Comparison of Methods for Assessing Genetic Variation in Plant Conservation Biology. In *Genetics and*

*Conservation of Rare Plants*; Falk, D.A., Holsinger, K.E., Eds.; Oxford University Press: New York, NY, USA, 1991; pp. 123–134.

- Senthilkumar, N., Barthakur, N.D. and Singh, A.N. 2009. Record of seed insect pests of *Dipterocarpus retusus* in Hollongapar reserve forests. J. Trop. For. Sci., 21(1): 8 - 12.
- Sivaram, M., Sasidharan, N., Shine, S., Sreedevi E.P., and Soumya Ravi. 2006. Invent NTFP Ver 1.0. Computer aided inventory analysis for sustainable management of Non-timber forest product resources. Kerala Forest Research Institute, Peechi.
- Smith, R. L. 1976. Ecological genesis of endangered species: The philosophy of preservation. Annual Review of Ecology and Systematics 7: 33-55.
- Swarupanandan, K., Indira, E.P., Muralidharan, E.M., Pandalai, R.C., Jose, P.A. and Sanjappa, M. 2013. Species recovery of *Dipterocarpus bourdillonii* and *Humboldtia bourdillonii*, two critically endangered, endemic trees of Western Ghats. Final Project Report No. 463. Kerala Forest Research Institute, Peechi.
- Vivek Menon. 2003. A Field Guide to Indian Mammals. Dorling Kindersley (India) Pvt. Ltd. pp. 201.
- Warrier, R., Devika, N.B., Savitha, C., Anandalakshmi, R., Nicodemus, A. and Singh, G., 2013. Assessment Of Macrogeographical Genetic Variations In Jatropha Curcas L In India Using Allozyme And Rapd Markers. *Trop. Agric. Res. Ext.*, 15, 24. https://doi.org/10.4038/tare.v15i1.5239
- Wolfe, A,D,, Xiang, Q.Y. and Kephart, S.R. 1998. Diploid hybrid speciation in Penstemon (Scrophulariaceae). Proceedings of the National Academy of Sciences of the USA 95: 5112±5115.

- Yang, S.L. and Meerow, A.W. 1996. The *Cycas pectinata* (Cycadaceae) complex structure and gene flow. *International Journal of Plant Sciences*, 157: 468-483.
- Yao, X.H., Ye, Q.G., Kang, M. and Huang, H.W. 2007. Microsatellite analysis reveals interpopulation differention and gene flow in endangered tree *Changiostyrax dolichocarpa* (Styracaceae) with fragmented distribution in central China. *New Phytol.*, 176: 472–480.
- Yeh, C. F., Yang, R. and T. Boyle. 1999. POPGENE VERSION 1.31. Microsoft Windows-Based Freeware for Population Genetics Analysis, University of Alberta and Centre for International Forestry Research, Alberta, Canada.
- Zhu, H., Xu, Z..F., Wang, H. and Li, B.G. 2004. Tropical rain forest fragmentation and its ecological and species diversity changes in southern Yunnan. *Biodiversity & Conservation* 13: 1355-1372.
- Zietkiewicz, E., Rafalski, A. and Labuda, D., 1994. Genome fingerprinting by Simple Sequence Repeat (SSR) - Anchored Polymerase Chain Reaction Amplification. *Genomics* 20: 176– 183.https://doi.org/10.1006/geno.1994.1151.

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