CONSERVATION BIOLOGY OF *ATUNA INDICA* (BEDD.) KOSTERM. AND *HYDNOCARPUS LONGIPEDUNCULATUS* ROBI ET AL., TWO ENDEMIC TREE SPECIES OF WESTERN GHATS OF KERALA

Thesis submitted to the University of Calicut in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY in BOTANY

By
SUBIN K

Supervising Guide
Dr. P. A. JOSE

DEPARTMENT OF TREE PHYSIOLOGY
SUSTAINABLE FOREST MANAGEMENT DIVISION
KSCSTE-KERALA FOREST RESEARCH INSTITUTE
PEECHI, THRISSUR, KERALA, INDIA

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Under the Supervision and Guidance of

Dr. P. A. Jose

Department of Tree Physiology, Sustainable Forest Management Division

Kerala Forest Research Institute, Peechi, Thrissur district,

Kerala state, India 680 653

May 2023

KSCSTE-Kerala Forest Research Institute
(An Institution under Kerala State Council for Science, Technology and Environment)
Peechi - 680 653, Thrissur, Kerala, India

CERTIFICATE

This is to certify that the thesis entitled **"Conservation biology of *Atuna indica* (Bedd.) Kosterm. and *Hydnocarpus longipedunculatus* Robi *et al.*, two endemic tree species of Western Ghats of Kerala"** is an authentic record of research work carried out by Mr. Subin K under my supervision and guidance in the Tree Physiology Department of KSCSTE- Kerala Forest Research Institute, in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the University of Calicut. This has not been previously for the award of any degree, diploma, associateship or other similar titles to any candidate of any university.

Dr. P.A. Jose
Supervising Guide
Principal Scientist & HoD
Tree Physiology Department
KSCSTE- Kerala Forest Management Division

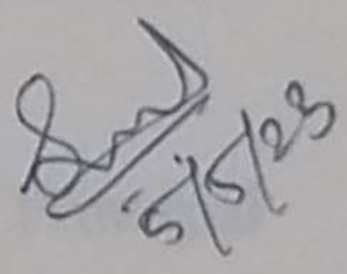
Place: Peechi
Date: 02/05/2023

Dr. P. A. Jose
Principal Scientist & Head
Tree Physiology Department
KSCSTE - Kerala Forest Research Institute
Peechi – 680 653, Thrissur, Kerala

Phone : 0487-2690100; Fax : 0487-2690111, e-mail : kfri@kfri.org, Website: www.kfri.res.in

DECLARATION

I hereby declare that the thesis entitled **"Conservation biology of *Atuna indica* (Bedd.) Kosterm. and *Hydnocarpus longipedunculatus* Robi *et al.*, two endemic tree species of Western Ghats of Kerala"** submitted to the University of Calicut for the award of the degree of Doctor of Philosophy is a record of independent research work carried out by me, under the supervision and guidance of Dr. P. A. Jose, Principal Scientist, KSCSTE-Kerala Forest Research Institute, Peechi, Kerala. This thesis has not been previously submitted for the award of any degree, diploma, associateship, or other similar titles.



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ABSTRACT

The conservation biology of two endemic and threatened tree species such as *Atuna indica* and *Hydnocarpus longipedunculatus*, distributed in the Southern Western Ghats were studied first of its kind. The ecological aspects of both species were analyzed which include the population structure, diversity, and demography along with climate and edaphic factors *in situ.* The vegetative and reproductive phenophases, reproductive biology, fruit/ seed dispersal along with entomological associations of the species were documented as part of the population dynamics. The population genetics including genetic diversity and gene flow analysis were accomplished using the Inter Simple Sequence Repeats primers. Development of vegetative and seed propagation protocols, habitat niche modeling, restoration, etc. was carried out as a comprehensive approach to the conservation of species.

The small population size, extremely poor seedling bank, reproductive constraints such as low fruit set, flower and seed pest, etc. were recorded as factors responsible for the rarity of *A. indica*. The lowest number of flowering individuals, Dam construction-induced population fragmentation are added factors for the decline of the populations. Small population size along with poor natural regeneration, high flower/ seed pest incidence, low fruit set, seed coat-induced dormancy, high seed predation, etc. recorded as factors leading rarity of *H. longipedunculatus*. The population decline in the Kulamavu MPCA is evident and probably due to the formation of Dam reservoir associated with the Idukki hydroelectric project. The results of vegetative propagation, seed characterization, and storage studies could be taken as baseline information for the multiplication and germplasm

storage of both species. The habitat niche modeling, followed by the restoration success recorded could be used for the large-scale restoration of these species in the future. The interdisciplinary study conducted and the data generated could be utilized for the effective conservation and management of these species from untimely extirpation.

Chapter 1

INTRODUCTION

Species and ecosystem conservation is getting better attention in the first quarter of the 21st Century. United Nations declared 2021- 2030 as the decade of ecosystem restoration. Different organizations like World Wide Fund for Nature, The Nature Conservancy, United Nations Environment Programme, Wildlife Conservation Society, etc accelerated their support towards conservation. Apart from these, local governmental and non-governmental organizations bestowed maximum efforts into species and ecosystem conservation. Added efforts of these initiatives showed promising results. According to the Forest Resource Assessment Report (FAO, 2020), the rate of forest decline is two million hectares lesser in 2015-2020 than that of the 2010-2015 period, but the loss is still in million hectares (10 MH in 2015-2020).

In this scenario, the term Conservation biology gains better relevance. Conservation biology is often known as a "discipline with a deadline" or a "crisis discipline". It is defined as the study of the conservation of nature and the earth's biodiversity to protect species, their habitats, and ecosystems from excessive rates of extinction and erosion of biotic interactions (Soule, 1980; Soule, 1985). According to Soule (1985), conservation biology is a multi-disciplinary subject mainly comprised of population biology, genetics, physiology, social sciences, historical biogeography, and eco-philosophy. Conservation biology contributes to maintaining processes that support biosphere function (Given, 1993). Life on earth has a long geological history but there is no guarantee of its continuance if extensive biodiversity losses continue (Given, 1990; Wilson, 1988). Conservation biology

needs the integration of research, management, and a range of relevant skills along with flexible funding (Given, 1993). Gaps between intention and practice are the current barrier to the effective implementation of principles of conservation biology.

Before the enactment of conscious conservation programmes, the ancient cultures followed rules and regulations in natural resource procurement, guided by the clan head. A typical example is the limited collection practice of Sockeye Salmon (Van Dyke & Fred, 2008). Decades later, the first science-based conservation programme (forest) was implemented in British India, by the botanist Alexander Gibson in the year 1982 (Barton, 2002). Thereafter, Governor General Dalhousie implemented large-scale conservation programmes in India. This conservation model spread to other British colonies leading to the establishment of the world's first National Park 'Yellow stone' in 1872, in the United States (Hainis, 1974). The remarkable biological diversity due to the presence of Himalayan hill ranges, and the Eastern and Western Ghats regions are the plausible driving factor behind the commencement of conservation programmes in British India.

The Western Ghats of India are remarkable for their floristic diversity and endemism though it faces an array of threats. It is estimated that the region holds around 7,400 flowering species, of which 15 percent are currently on the brink of endangerment (Nayar et al., 2008; Sasidharan, 2017). Myriad studies are conducted in the Kerala part of the Western Ghats and the area represents the lion's share of species richness and endemism observed in Western Ghats. Out of 4,078 indigenous flowering plants recorded in the State, 1,568 species are endemic to the Western Ghats with 553 species under various IUCN Conservation categories (Sasidharan, 2017). The richness of endemic species in the area indicates biogeographical peculiarities and which in turn draw attention to the possibilities

for speciation or adaptive evolution in the region, especially in the era of climate change. Moreover, the loss of an endemic species leads downturn in existing genetic resources and unpredictable menaces for other community elements like pollinators, pests, etc.

Autecology is the study of single species and it is one of the two branches of ecology. The other branch is synecology and which is further classified into population ecology, community ecology, and ecosystem ecology (Odum, 1959). The major application of autecology lies in the conservation field. The species-specific details, such as population size, structure, dynamics, tolerances and habitat requirements, and species-level interactions are helpful in the conservation of endangered plant species (Pérez-García et al., 1995). The Extinction of species is one of the greatest threats to biodiversity. Unfortunately, many species are threatened due to anthropogenic activities such as habitat fragmentation, over-exploitation, invasive species introduction, and global climate change. The alarmingly high rate of species extinction, as high as 1,000–10,000 times compared to ancient times is far beyond the estimated natural extinction rate. If the process continues at the same pace, the species richness will reduce by around 30-50% by mid-century (Myers, 1980; Chivian & Berstein, 2008). The small population size of endemic plant species is the potential reason behind prognosticated genetic bottleneck and extinction vulnerability (Fischer & Matthies, 1998; Keller & Waller, 2002). Therefore, the endemic and IUCN red-listed species are the high-priority species seeking immediate conservation measures.

Understanding plant rarity has been an important as well as a baseline task in the field of conservation biology. Every threatened plant has its own story to tell about the enlistment in IUCN red list, some are human-induced and others are plants' intrinsic factors. Reveal (1981) states that plant ecology and reproductive biology are the two crucial

factors behind the rarity. The demographic structure of a population including seedling count, spatial and horizontal distribution, diversity of the community, and IVI of the species among associates are the integral elements that come under the plant ecology discipline (Pascal, 1988, Pandurangan, 2003). The reproductive biological studies include the flowering frequencies of the plant, anthesis time, pollen viability and longevity, stigma receptivity, pollen-ovule ratio, rate of fertilization, mode of pollination, count and frequency of pollinating agents, incidents and intensity of pests/predators on flower and fruit, etc. Remadevi (2022) emphasized impairments caused by defoliators and leaf eaters, fruit and seed borers, etc. As per Jose et al. (2014), out of 760 red-listed flowering plants reported from the southern Western Ghats, more than 500 species are yet to be studied for deriving any kind of conservation strategy. Several other reports on Rare, Endangered and Threatened (RET) plants of the Western Ghats, Peninsular India, and southern Western Ghats including Kerala are also available (Ramesh et al., 2003; Sasidharan, 2004, 2017). The literature urges the need for conducting further research on conservation, sustainable management, and effective utilization of threatened plant resources in the Western Ghats.

Conservation genetics is the discipline that applies genetic methods to the conservation of biodiversity. Genetic information plays an important role in developing management strategies for threatened species with small populations. Genetic diversity is one of the three fundamental levels of biodiversity which influences both population dynamics and the long-term survival of species. Low levels of genetic diversity result in reduced fitness by causing high juvenile mortality, diminished population growth (Leberg, 1990), reduced immunity, and ultimately leading to higher extinction risk (Frankham, 2005). Apart from human-induced habitat fragmentation, reproductive events in small

populations are the prime movers behind reduced fitness, as it causes inbreeding and which eventually contributes to reduced genetic diversity further to failed evolvability towards upcoming environmental odds. Thus, the maintenance of genetic variation is one of the fundamental requirements in initiating *in situ* and *ex situ* conservation efforts and effective management of the surviving germplasm (Falk et al., 1991; Hoelzel, 1992). Understanding the genetic variation within and among populations is one of the prerequisites before initiating restoration programmes in any threatened plant species (Hamrick & Godt, 1996). Recent advances in molecular marker techniques enabled the use of DNA sequence to be employed in the field of conservation at affordable costs even in developing countries, in addition, the molecular marker data enables deeper understanding as well as connecting the dots in ecological and biological studies. 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 higher-stringency 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 & 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 & Sun, 1999; Culley & Wolfe, 2001). To complete the picture of conservation biology, active restoration programmes are inevitable. Vegetative propagation, seed propagation, and seed storage

studies are essential in any conservation project (Jose, 2001; Deepu, 2015). Habitat niche modelling is currently employed in many eco-restoration initiatives because of the importance of species niches (Baruah et al., 2019).

In this background, the conservation biology of two endemic and threatened tree species of southern Western Ghats viz. *Atuna indica* (Bedd.) Kosterm. and *Hydnocarpus longipedunculatus* Robi et al. were carried out with the following Objectives :

1. Identification and mapping of the populations of *Atuna indica* and *Hydnocarpus longipedunculautus* in the Kerala part of the Western Ghats
2. Identification of ecological, reproductive biological and environmental factors of target spp. *in situ*
3. Detection of diverse genotypes in the populations of target spp.
4. Development of propagation, niche modelling, and restoration protocols for the target spp.

Chapter 2

REVIEW OF LITERATURE

2.1. Western Ghats

The Western Ghats is a chain of mountains extending from the Tapti River valley in Gujarat to Kanyakumari at the southernmost tip of the Indian peninsula. It is about 1600 km in length in the North-South direction and 5-10 km broad on average. The region lies between 8°20′- 8°40′ North latitudes and 73°-77° East longitude. The highest point is the Anamudi peaks (2695m) and the Nilgiris and Palanies are high-rising Eastern offshoots of the Western Ghats. Several studies were conducted on the biodiversity of the Western Ghats, such as by Myers et al. (2000), Ramesh et al. (2003), Davidar et al. (2005), etc. Many authors like Pascal (1988), Ramachandran and Swarupanandan (2013), Sasidharan (2003, 2011, 2017), Nayar (1996, 1997), Pascal (1988, 1991), and Jose (2001) focused on floral components. The faunal components are studied by Gadagkar et al. (2000), Subramanian (2007), Dahanukumaret al. (2004), Daniels (1992, 1997), Grimmet et al. (1999), Vijayan and Vijayan (2006), Blanford et al. (1901).

The Western Ghats flora is often compared with Island flora due to the high level of species diversity and endemism. The unique features of this mountain system, including the Southern Western Ghats area, are highly discussed and interpreted by several authors (Pascal, 1988; Nayar, 1996, 1997; Myers et al., 2000; Jose, 2001; Ramesh et al., 2003). The Western Ghats has around 7400 species of flowering plants of which nearly 2200 species are endemics (Myers et al., 2000; Sasidharan, 2011, 2017). The state of Kerala harbours more than 5200 species of flowering plants including exotics which are about one-

fourth of the flora of India (Sasidharan, 2004, 2017; Nayar et al., 1996, 2014; Jose et al., 2020). More than 70% of the plants known from the whole of Western Ghats are recorded from the southern Western Ghats itself (Mani, 1974; Sasidharan, 2004). Out of 490 trees found inhabiting the low and medium-elevation evergreen forests, 308 are endemic to the Western Ghats (Ramesh & Pascal, 1991; Ramesh et al., 1997). Looking at the floral elements of Kerala, which indeed accommodate a larger segment of the Western Ghats, has around 553 red-listed species, which includes 151 tree species (Sasidharan, 2017).

2.2. Conservation biology

Michael E. Soule, the founder of conservation biology defined conservation biology as "a new state in the application of science to conservation problems, address the biology of species, communities, and ecosystems, that are perturbed either directly or indirectly by human activities or other agents, its good is to provide principles and tools for preserving biological diversity" (Soule, 1985). Four of the major books in Conservation biology are edited and co-edited by Michael E. Soule, namely *Conservation Biology: An Evolutionary-Ecological Perspective* (1980), *Conservation and Evolution* (1981), *Conservation Biology: the Science of Scarcity and Diversity* (1986) and *Viable Populations for Conservation* (1987). These books are mainly discussing the trends in population genetics, species distribution modeling, and ethical as well as philosophical discussions on topics like the value of nature.

Other well-known literature in the field of conservation biology is *Essentials of conservation biology* (Primack, 1993), which cover key ecological ideas relating to biodiversity, ecological interactions, genetic aspects, etc as well as mentions the threats to

biological diversity (human-caused extinctions), the need of population and species level conservation efforts, issues with small population size and about *ex-situ* conservation strategies. *Fundamentals of Conservation Biology* (Hunter & Gibbs, 2006), *Conservation Biology for All* (Sodhi & Ehrlich, 2010), *Practical Conservation Biology* (Lindenmayer & Burgman, 2005), *Quantitative Conservation Biology* (Morris & Doak, 2002) are some other relevant kinds of literature in this field.

2.3. Rarity

Biodiversity is a future resource pool, the full potential of which has not been folded. The benefits of biodiversity conservation are discussed partially, in terms of the returns to mankind. The species are important not only for their projected value for mankind but also for their role in governing the functioning of ecosystems and the living environment. This emphasizes the need for biodiversity conservation.

The Rare/Endangered/Threatened (RET) plant species are defined as those encountered only in traces at specific sites in their natural habitats for the past 50 years (Lucas & Synge, 1978). RET species constitute the weaker sections of the biota. They become rare either due to reproductive abnormalities, pest infestation, or due to anthropogenic factors such as over-exploitation, land use change, changing management practices, etc. Some of the studies reported poor viable seeds (*Dipterocarpus bourdillonii*- Swarupanandan et al., 2013), an extremely low number of individuals (*Syzygium palghatense, Dialium travancoricum, Hopea sasidharani,* etc.- Jose et al., 2020), fragmented populations in (*Vateria macrocarpa* - Sanil, 2022).

Among the previous studies on threatened trees of Western Ghats, authors focused on distribution and population analysis (Chandran et al., 2008; Ramachandran & Swarupanandan, 2014; Sreekumar et al., 2020; Balan et al., 2019; Rahul et al., 2020; Jose et al., 2018), phenology and reproductive biology (Kasi & Ramasubbu, 2021; Anto et al., 2018; Ramasubbu & Irudhyaraj, 2016; Reghunath & Raju, 2020; Nadarajan & Pujari, 2019; Shivaprasad, 2017), seed biology (Tambat et al., 2005; Sinu & Shivanna, 2016), genetic diversity analysis (Priya et al., 2016; Saini et al., 2018; Dev et al., 2019), propagation and restoration (Jose et al., 2009; Jose et al., 2017; Sivakumar et al., 2015) aspects. Community-level studies are also carried out in *Myristica* Swamps (Chandran et al., 2010) and sacred grooves (Chandran et al., 1998).

Other than biological reasons, several anthropogenic factors were contributing to plant rarity. The Man-made problems ranging from Habitat destruction or conversion into farmlands, over-exploitation of species, the introduction of exotic species, etc., were the widely recognized threats limiting the survival of endangered species (Tilman et al., 1994; Raven, 1988; Rabinovitz et al., 1986; Lucas & Synge, 1978; Pandurangan, 1995).

2.4. Population Ecology

2.4.1. Population structure

Stand structure is generally described as the horizontal and vertical distribution of components of a stand, including the height, diameter, crown layers, and stems of trees, shrubs, and herbaceous understory elements (Helms, 1998). Most of the population structure studies conducted in the Western Ghats focussed on limited forest areas and they investigate the species diversity along with the general population structure of species

(Swamy & Sundarapandian, 2000; Parthasarathy, 1999, 2001; Giriraj et al., 2008; Anitha et al., 2010; Jayakumar & Nair, 2013; Reddy et al., 2008; Elouard et al., 1997; Bharathi & Devi Prasad, 2015; Gunaga et al., 2015). Species-specific structural studies are also carried out in Dipterocarps (Ayyappan & Parthasarathy, 2001), *Dipterocarpus indicus*, *Vateria indica*, *Diospyros montana*, *Diospyros sylvatica*, *Garcinia talbotii* (Somanathan & Borges, 2000), *Artocarpus hirsutus* (Sarkar et al., 2011), *Elaeocarpus venustus* (Irwin et al., 2013), *Kingiodendron pinnatum* (Jose et al., 2018).

Population status, spatial occurrence, and habitat relationships of threatened plants in the tropics have not been well documented (Elias, 1986; Batianoff & Burgess, 1993; Bawa & Seidler, 1998; Scariot, 1999; Shapcott, 1999; Keith, 2000; Vormisto, 2002) even large proportions of tropical species are rare and occur at very low population densities (Bawa & Ashton, 1991; Shapcott, 1999; Vormisto, 2002).

Spatial distribution

Spatial/horizontal distribution is the spatial arrangement of individuals in a forest patch. Tree species can be seen as clumped, random, or in a uniform pattern. Clumped distribution is most common in natural forests, particularly tropical forests (Hai et al., 2014; Condit et al., 2000, Li et al., 2009; Zhang et al., 2013). The spatial distribution has so many things to convey about the species, like habitat specificity. Such information can help us to understand potential ecological processes that control species co-existence and community structure (Hai et al., 2014; Song et al., 2017). The spatial distribution is influenced by spatial and temporal heterogeneity in resource availability (Denslow, 1987; Kohyama, 1993; Bazzaz & Wayne, 1994; Harms et al., 2001). The influential resources are light

(Swaine & Whitemore, 1988; Bazzaz, 1991), soil nutrients (Denslow et al., 1990; Clark et al., 1999), Slope and elevation aspects (Ye et al., 2011; Svenning, 2001), and canopy gaps (Vitousek & Denslow, 1986; Nunez-Farfan & Dirzo, 1988). Other than habitat heterogeneity, niche segregation (Pielou, 1961), dispersal limitation (Hubbel, 1979), within and between species competition (Bruno et al., 2003), and negative density dependence (Wright, 2002) leads to aggregated/ clumped distribution. Species attributes also influence spatial patterns, like trees with larger trunk diameters are less aggregated (Condit et al., 2000; Li et al., 2009) indicating self-thinning.

The vertical distribution

It is the height-based arrangement of trees. Quantifying the vertical distribution of foliage/trees is of major importance to estimate light interception at the tree crown scale (Niinemets, 2007, 2010). Analysis of the vertical distribution of trees helps to understand the amount of light absorbed by emergent, canopy, sub-canopy, and understorey species in an evergreen forest patch (Monteith, 1972; Medlyn, 1998; Jose, 2001; Binkley et al., 2013). The positive correlation between absorbed light and tree growth is well known (Medlyn, 1998; Binkley et al., 2013;), and the knowledge of light requirements of species can be used to develop growth and yield models in forest management programmes (Wang & Javis, 1990; Landsberg & Waring, 1997; Coates et al., 2003). The vertical distribution under the crown is influenced by surrounding environments and by the level of competition. The majority of studies were done in coniferous species. Such studies showed that under-canopy trees usually have a higher concentration of foliage than the dominant trees (Maguire & Bennett, 1996; Gilmore & Seymour, 1997; Garber & Maguire, 2005;

Weiskitted et al., 2009). Shade-tolerant coniferous species concentrate their foliage lower in the crown when compared to light-demanding species (Garber & Maguire, 2005; Horn, 1971; Nelson et al., 2014; Guisasola et al., 2015).

Age class distribution/ Ontogenic stages

Limited studies so far reported the age class distribution in evergreen forests. Age-class distributions classify the species into a set of future (pre-reproductive), set of present (reproductive), and set of past (post-reproductive). The highest number of pre-reproductive and reproductive individuals indicates better health in the population. A reduced number of pre-reproductive individuals indicate poor health of the population and possible extirpation in the future (Jose, 2001).

Importance Value Index (IVI)

IVI is the relative position of a species in its ecosystem, and it is calculated by summing the relative frequency (rf), relative density (rd), and relative dominance (rD) (Philips, 1959; Curtis, 1959) of species and it calculated using software INVENT NTFP (Sivaram et al., 2006). The IVI values provide an identity of dominant species and its ecological importance as well as competitive behavior in the ecosystem, thus helpful in elucidating the ecological characteristics of an ecosystem for prioritizing species conservation (Kacholi, 2013; Zegeye et al., 2006; Pascal, 1988; Jose, 2001; Turkis & Elmas, 2018).

2.4.2. Population dynamics

Population dynamics of a species include phenology and reproductive biology, the latter involves the detailed studies of flowering, pollination, seed dispersal, insect-pest associations, etc.,

Phenology is the study of the periodicity or timing of recurring biological events, related to short-term climatic change. In the case of plants, phenological events involve leaf flushing, flowering, fruiting, and seed germination (Leith, 1974). The knowledge of phenological changes is important in understanding specific functions of plants in natural populations (Aronson et al., 1994), the evolution of species and communities as timing control survival and reproductive success (Newstrom et al., 1994), *in-situ* management and conservation. It also affects faunal elements which consume young leaves, flowers, and mature or immature fruits (Van Schaik et al., 1993).

Many authors studied the phenology of tree species in Western Ghats, some are general phenological studies (Bhat, 1992; Bhat & Murali, 2001; Nadarajan & Pujari, 2019; Patel, 1997; Sundarapandiyan & Chandrasekharan, 2005; Sellamuthu & Lalitha, 2010), others are focused in fruit phenology (Ganesh & Davidar, 1997; Kannan & James, 1999; Aruna & Balasubramanian, 2014; Anbarasu & Balasubramanyan, 2013), seed dispersal and seedling growth (Pannell, 1989; Howe, 1990; Chapman et al., 1992; Wrangham et al., 1994; Swaine & Whitmore, 1988; Schupp, 1990; Brown & Whitmore, 1992), seed predation (Howe, 1989; Schupp, 1990), some other works compile vegetative phenology with reproductive biology (Keshavanarayanan et al., 2015) or with fruit and seed studies (Kasi & Ramasubbu, 2021). The reproductive phenology and reproductive biology are almost the same, with most of the studies incorporating flowering time, pollinators, pollen,

and stigma studies. In addition, studies of plant phenology in tropical forests have been conducted to describe resource availability for consumer animals (Frankie et al., 1974; Croat, 1975; Putz, 1979; Opler et al., 1980; Foster, 1982; Koptur et al., 1988; Murali & Sukumar, 1994; Justiniano & Frederickens, 2000; Morellato et al., 2000).

According to Borchert et al. (2002), the duration and intensity of seasonal drought control the seasonality of tropical phenology. The degree of drought experienced by tree species varies widely, depending on temperature, soil-water availability, and tree rooting depth (Van Schaik et al., 1993). Phenological events such as germination, flowering, and seed dispersal at the wrong time cause high leaf predation rates (Aide, 1992), flower predation (Augspurger, 1981), and low seedling survival rates (Tevis, 1958).

Reproductive biology

According to Maynard (1978), reproduction is the key process that ensures the perpetuation of life and genetic diversity. Genetic diversity is generated through the recombination process in sexual reproduction, which is a process of fundamental importance for population and species biology. Reproductive biology mainly focuses on flowering phenology, floral biology, pollen-pollinator interactions, breeding systems, and gene flow through pollen and seed (Gopalakrishnan & Thomas, 2014). The flower bud initiation, development, maturity, anthesis, pollination/pollinating agents, stigma receptivity, pollen ovule ratios, fruit development, maturity, ripeness, seed predation, dispersal, and seedling regeneration are very crucial in determining the reproductive efficiency, aggressive capacity and ecosystem functioning of the area where the species growing (Richards, 1952; Sathyanarayan & Muthaliyar, 1959; Janzen, 1969; Whitehead,

1969; Harper, 1977; Van der Pijl, 1961; Borchert, 1983; Armstrong & Drummond, 1984; Bawa & Webb, 1984; Bawa & Beach, 1983; Bawa et al., 1990; Richards, 1997; Appanah, 1986, Liberman et al., 1987; Parthasarathy et al., 1988; Ashton et al., 1988; Kuruvila, 1989; Kageyama, 1990; Swarupanandan & Sasidharan, 1992; Murali & Sukumar, 1994; Lokesha & Vasudeva, 1997; Lodhiyal et al., 1998).

The majority of the tropical flowering studies have emphasized community-level questions (Croat, 1969, 1975; Alencar et al., 1979; Putz, 1979; Koptur et al., 1988; Newstrom et al., 1994), and a few species-level studies are reported (Borchert, 1980; Augspurger, 1980, 1981; Bullock & Bawa, 1981; Bullock, 1982; Reich & Borchert, 1982; Bullock et al., 1983; Newstrom et al., 1994). Reproductive biological studies were conducted in several tree species viz. *Ochreinauclea missionis* (Jose & Pandurangan, 2012), *Gluta travancorica* (Jose & Pandurangan, 2013), *Terminalia chebula* (Talwar & Bhatnagar, 2014), *Pterospermum reticulatum* (Keshavanarayan & Rajkumar, 2015), *Canarium strictum* (Kala et al., 2014; Kumar et al., 2015), *Cinnamomum sulphuratum* (Shivaprasad, 2015), *Vateria macrocarpa* (Keshavanarayan et al., 2015), *Lagerstroemia speciosa* (Sivadas & Pandurangan, 2015), *Elaecarpus blascoi* (Ramasubbu & Irudhyaraj, 2016), *Saraca asoca* (Smitha & Thondaiman, 2016), *Dipterocarpus indicus* (Shivaprasad, 2017), *Humboldtia vahliana* (Kumari & Sreekala, 2017), *Garcinia imberti* (Kandhasamy & Puttaramaiah, 2017), *Syzygium occidentale* (Vargheese & Sreekala, 2017), *Garcinia gummi-gutta* (Aswathi et al., 2018), *Monoon tirunelveliense* (Viswanathan et al., 2019), *Chloroxylon swietenia* (Ayyanar et al.. 2021), *Prosopis cineraria* (Sigh & Bangarwa, 2021).

One of the worth mentioning researchers working in tree reproductive biology is K.S. Bawa. He authored/co-authored several articles relating to plant reproduction, reproductive biology, and pollination systems (Bawa et al., 1985), Kin selection and evolution in reproductive traits (Bawa, 2016), relationships between time, frequency, and duration of flowering in tropical rainforests (Bawa et al., 2003), self-incompatibility studies (Seavey & Bawa, 1986),

Research to date shows that many tropical species are hermaphroditic and are obligatory outcrossing as a result of various mechanisms like herkogamy (spatial separation of anther and stigma in a flower), dichogamy (temporal separation of anther and stigma in a flower) and self-incompatibility mechanisms (Jain, 1976; Lloyd, 1982; Richardson et al., 1990; Bawa et al., 1990). Plant density and distribution patterns in tropical communities play a key role in reproductive success. In addition, pollination efficiency, phenology, and the number of flowering plants at a time are also relevant in evaluating reproductive success (Hubbell & Foster, 1986; Bawa & O'Malley, 1987; Martinez-Ramos & Alvarez-Buylla, 1995). Very few studies documented flowering synchronization, and reproductive success of tropical plants with different sexual systems and spatial distribution patterns (Augspurger, 1983; Bawa & Hadley, 1990; Murawski & Hamrick, 1992; Lepsch-Cunha & Mori, 1999).

Knowledge of reproductive biology is a prerequisite for both evolutionary and conservation studies (Moza & Bhatnagar, 2007). Ideas that concern species conservation and recovery will remain ineffective without adequate knowledge of breeding systems and pollination mechanisms. Reproductive biology studies will be helpful in understanding

genetic structure and variations (Costich, 1995), breeding systems, and seed quality (Nagarajan et al., 1996, 1998).

Pollination

Pollination and reproductive phenology are two important aspects of the reproductive biology of plant species (Rathcke & Lacey, 1985). The flower shape, color, flower architecture, and offering of rewards determine visiting fauna and the efficiency of pollen transfer (Faegri & Van der Pijl, 1979; Muchhala, 2003). The timing of flowering can determine the available potential visitors (Augspurger, 1981; Rathcke & Lacey, 1985; Bishop & Schemske, 1998). Entomophily is the predominant mode of pollination which involves several groups of insects such as bees, butterflies, ants, etc. Vertebrate groups also take part in pollination like birds (Ornithophily) and bats (Chiropterophily) etc. (Proctor et al., 1996). It is possible to associate floral traits with particular pollinator groups because of a series of pollination syndromes (Campbell et al., 1996; Galetto et al., 1998). For understorey species, the general impression is that the anemophily is least preferred in the tropics (Whitehead, 1969, 1983) conversely, some good studies in tropical forests reported wind pollination (Bawa & Crisp, 1980). Irrespective of the mode of pollination, the ultimate aim of cross-pollination is better gene flow and increased population fitness of the species in the future (Herrera, 1987; Campbell, 1989; Kenta et al., 2004).

The habitat change and fragmentation of wild plant populations influence the pollination dynamics (Bond, 1994; Somanathan & Borges, 2000; Ghazoul & McLeish, 2001; Pandit & Choudhury, 2001; Lennartsson, 2002; Murren, 2002). The aftermath of the specific exploitation/loss of wild plants on pollination ecology is still a little-known subject.

The plant-animal interactions are important in understanding ecosystem structure and functions (Odum, 1959) including the insect pest attacks in the vegetative and reproductive phases. The herbivory does remove resources or damage the plant's capacity to accumulate resources (Bowels & Whlan, 1994; Ehrlen, 1995). An array of damages are caused by the insects belonging to categories like defoliators, leaf rollers, leaf miners, floral eaters, shoot/stem borers, fruit/seed feeders, sap suckers, etc., The plant-damaging insects found in the Western Ghats belong to the families such as Parasitidae, Epiplemidae, Bostrychidae, Agromyzidae, Chrysomelidae, Curculionidae, Pyralidae, Cicadellidae, etc., (Janzen, 1971; Kevan, 1972; Mani, 1973; Nayar & Selisker, 1976; Richards & Davies, 1977; Barlow, 1982; Holloway, 1987; Dayanandan et al., 1990; Chathurvedi & Haribal, 1992; Mathew & Rahmathulla, 1993; Gadgil, 2000).

The climate and edaphic factors are important in understanding the ecology of an area, as these factors influence the type of vegetation and their zonation. Moreover, these factors influence germination, growth, the timing of flushing and flowering, etc. The main edaphic factors include soil pH, nutrients, and moisture content, climatic factors are rainfall, humidity, light, and temperature. The microclimate has a decisive role in the existence of the populations of many endemic species, which are habitat-specific and narrowly distributed (Ashton, 1958; Borchert, 1980; Odum, 1959; Murali & Sukumar, 1993; Balakrishnan et al., 200; Gupta & Malik, 1996; Parthasarathi & Sethi, 1997).

2.5. Population Genetics

The quantitative measure of variability or the "equilibrium between mutation and lack of variation" is known as genetic variability/diversity (Hughes et al., 2008; Leffler

et al., 2012). Genetic diversity studies give an idea about the past, present, and future stages of a species as well as help in developing in-situ conservation strategies (Falk, 1990).

Most of the previous genetic diversity studies were not considering the ecology and reproductive characteristics of species, and the majority of the authors conclude that the low genetic variation is only due to a small population size/ habitat fragmentation. Indeed, fragmentation is responsible for increasing self-pollination (or between related individuals), followed by inbreeding and reduced gene flow, and low diversity values (Aguilar et al., 2008), but may not always be the reason for reduced genetic diversity, and vice versa.

The germplasm characterization using genetic markers is a prerequisite that is helpful in the conservation as well as sustainable utilization of threatened plant species (Dawson & Powell, 1999; Hegde et al., 2018). Genetic diversity is characterized by differences in the composition or frequency of genes or alleles among individuals in a species or population (Namkoong et al., 1996). Some relevant indices of genetic diversity are the number of alleles, the number of haplotypes (k), haplotype diversity (h), nucleotide diversity (π), observed and expected heterozygosity H_O and H_E, and population structuring, such as F_{ST}, G_{ST} (Wright, 1965; Wright, 1984). A 'good genetic marker for diversity study is defined as a marker that can show available genetic variability and it should have the ability to generate multiple loci data from the genome (Anne, 2006). The earlier used marker is the isoenzymes (Tanksley, 1983), which provided access to genetic diversity as well characterization of accessions and germplasm for genetic improvement (Mohan et al., 1997). Earlier genetic variability works are focused on plants of agricultural importance, and gradually ecologically important plants were taken into consideration (Rajaseger et al.,

1997; Erayman et al., 2014; Miranda et al., 2016; De Paula et al., 2017; Rebolledo et al., 2018).

The ISSR markers came as a solution to various limitations of PCR-based markers like AFLP, RAPD, and SSRs back in 1994 by Zietkiewicz et al. The AFLPs (Vos et al., 1995) are labor intensive, and the operational, as well as developmental costs, are high, even though it shows medium reproducibility (Karp et al., 1997). The RAPDs (Williams et al., 1990) lack reproducibility, but they are quick and easy to develop (Karp et al., 1997; Hansen et al., 1998; Virk et al., 2000). The SSRs require knowledge of genomic sequence to design primers, as they are specific and highly polymorphous (Karp et al., 1997). They are employed in economically important species. The ISSR markers have 92-95% reproducibility and are highly polymorphic (Zietkiewicz et al., 1994) doesn't require prior DNA sequence information, the laboratory procedures are simple and development costs are minimal (Barth et al., 2002).

The ISSR markers are the flanking region found between two microsatellites, with 100-3000 bp size, and consist of repeats of two, three, or four nucleotides The ISSRs are interspersed evenly throughout the genome (Godwin et al., 1997) with regions either unanchored of or anchored at 5′ or 3′ end by two or four arbitrary nucleotides (Wu et al., 1994). The band polymorphism is detected when loss or gain of binding sites is formed due to inserts or deletes in the microsatellites region. Nowadays the plant's chloroplast DNA (cpDNA) markers are predominantly used in ecological studies (Gao et al., 2018; Bai et al., 2017). New generation sequencing (NGS), makes genetic diversity analysis robust as

it can accumulate large quantities of molecular data through the analysis of marker regions (Goodwin et al., 2016).

The ISSR markers are mainly used in genetic diversity, DNA fingerprinting (Shen et al., 2006; Fang et al., 1997), taxonomy and phylogeny studies (Fernandez et al., 2002; Iruela et al., 2002). The markers have been used in the following species in diversity studies, *Commiphora wightii* (Kulhari et al., 2015), *Eucalyptus tereticornis* (Nishad et al., 2014), *Jatropha curcas* (Joshi et al., 2013), *Tectona grandis* (Ansari et al., 2012), *Dalbergia sissoo* (Arif et al., 2009), *Salvadora persica* (Monfared et al., 2018), *Melia dubia* (Rawat, 2018), *Eucalyptus tereticornis* (Adarsh et al., 2014), *Shorea robusta* (Surabhi et al., 2017), *Grevillea robusta* (Priyanka et al., 2019), *Calamus guruba* (Meena et al., 2018).

2.6. Conservation

2.6.1. Seed studies

The majority of the studies conducted on tree species of Western Ghats are slightly skewed toward seed biology. The works of Anil Kumar et al. (2002, 2008), Sinha and Davidar (1992), Gopal et al. (2021), Osuri and Sankaran (2016), Tambat et al. (2006), Radha et al. (2010), Jose and Pandurangan (2013), Sivadas and Pandurangan (2022) are some among them. Despite doing normal storage and viability studies, works conducted after 2000 were mainly focusing on applied aspects like cryopreservation of excised embryonic axes of *Nothapodytes nimmoniana* (Radha et al., 2010), as well as interesting findings such as Non-viable seed set in *Lagerstoemia speciosa* (Sivadas & Pandurangan, 2022).

The seeds represent a unique form of life in the plant kingdom. They serve as the delivery system for the transfer of genetic material from one generation to the next. Seed germination as well as the early growth of seedlings is the most important stage in the plant life cycle (Pathak et al., 1980), and seed studies are helpful in perceiving community processes of plant recruitment, succession, as well as developing strategies for the conservation of tropical forests (Khurana & Singh, 2001).

Seeds that are sensitive to desiccation and low temperatures are classified as recalcitrant and those seeds with low moisture content as orthodox (Roberts, 1973). Intermediate seed type, which is a category reported by Ellis et al. (1991), in which species drastically lose viability below certain seed-specific moisture content. Variations in the distribution and intensity of rainfall, light, temperature, soil nutrients, and ardency of predation and disturbance influence seed as well as seedling traits across tropical forest biomes (Khurana & Singh, 2001). Seeds have evolved mechanisms to act as per the environment for better establishment and survival (Khurana & Singh, 2001). Seed production, dispersal, predation, and germination are important aspects that regulate the population size as well as influence community-level interactions (Alexander et al., 2001; Baskin & Baskin, 1998; Beckman & Rogers, 2013).

Seed germination is defined as the resumption of active growth in an embryo which results in its emergence from the seed, and the development of those structures essential to plant development (Bonner, 1984). Suitable combinations of temperature, moisture, and light conditions are considered major determinants of germination (Funes & Venier, 2006; Bewley & Black, 1994; Baskin & Baskin, 1998). In tropical tree species, the seeds show

delayed or non-uniform seed germination because of the impermeability of seed coats towards water or oxygen and due to chemical inhibitors (Malavasi, 1988; Khan, 2015). The seeds with hard seed coats or seed dormancy are subjected to seed pretreatments for their early germination. Scarification is a well-employed pre-treatment method (Babely, 1985; Sadhu & Kaul, 1989; Kandya & Kandya, 1990; Leonor, 1992; Todd-Bockarie & Duryea, 1993).

The reduced number of mature individuals and low seed production are two of the major problems found in threatened species (Fischer & Matthies, 1998; Luijten et al., 2000). Information on the germination, storage, and ecological requirements of many of the threatened tree species seeds remains unexplored (Iralu et al., 2019). Seed dispersal is an important event in the life cycle of a species as it determines the plants' future distribution type and population structure (Levin, 1981; Wang & Smith, 2002). According to Hutchings (1986), most of the seedlings are deposited near the parent plant, particularly in the case of wind-dispersed seeds. In this scenario, most of the seedlings cannot survive because of the frequency of predators and pathogens operating near the parent (Augspurger, 1983, 1984). In other cases, seed dispersal happens across a wide variety of microhabitats covering a range of biotic and abiotic conditions which influence their germination (Castro et al., 2005). The abiotic and biotic factors prevalent in the distribution area act as a selective force that determines the germination and establishment of dispersed seeds (Bazzaz, 1991). Seed dispersal and germination generally happen either after escape from pests/ pathogens, predators, and herbivores (Connel, 1970; Janzen, 1970) or after satiating seed predators at a high rate of seed production (Nilsson & Wastljung, 1987; Sork, 1993; Sperens, 1997; Shibata et al., 1998, 2002; Curran & Leighton, 2000; Curran & Webb, 2000). The

advantages of seed dispersal to a different area include reduced predation and seedling mortality, (Howe & Smallwood, 1982), colonization in new or better habitats (Hamilton & May, 1977; Redford, 1992; Corlett, 1997; Hoch & Adler, 1997), and better seed-mediated gene flow (Jordan et al., 2007).

Dependence on seed storage methods is inevitable for raising plants per necessity (Pandurangan, 2003). Seed longevity is influenced by several factors like storage temperature, relative humidity, seed moisture content, etc. Better storage conditions are a prerequisite for raising healthy seedlings (Roberts, 1973; Agarwal, 1980).

2.6.2. Macro propagation

To maintain and enrich the genetic diversity of a species, the preferred way of propagation is through seeds. If the seed availability is less/poor germination or any factors hindering sexual reproduction, then the next dependable way of propagation is through stem cuttings and layering. Vegetative or asexual propagation can be defined as the production of a plant so that the offspring will contain the exact characteristics of the mother plant in genotype as well as health status (Macdonald, 1986; Hartmann et al., 2001). Vegetative propagation is an important tool for crop improvement. Its aim is the formation of adventitious roots for successful plant regeneration. According to Hartmann et al. (2001), vegetative propagation is possible because the living cells contain genetic information in their nuclei necessary to reproduce the entire plant.

Propagation by stem cuttings is one of the major methods of clonal propagation in horticultural as well as forest species. Stem propagation has been employed in forestry

species for hundreds of years and is useful in creating large planting stock of forest species. Yet many economically important plants have a low genetic and physiological capacity for adventitious root formation (Hartmann, 2014). Several technologies are developed to successfully manipulate environmental conditions to maximize rooting i.e. intermittent mist and fog systems, temperature, and light manipulation (Hartmann, 2014).

Stem cuttings propagation is an ancient technique and it has been traced as far back as ancient China. Vegetative propagation by stem cuttings can produce a large number of young plants from a single parent plant, thus it is a useful technique in the conservation of endangered plants (Macdonald, 1986) and the rapid propagation of new cultivars. Vegetative propagation is ideal for the rapid multiplication of species under threat while trying to maintain certain desired characteristics (Hartman et al., 2001). Several such studies were standardized for threatened species like *Premna corymbosa* (Raghu et al., 2005), *Cinnamomum heyneanum* (Shareef et al., 2005), *Podophyllum hexandrum* (Kharkwal et al., 2008), *Taxus baccata* (Maden, 2003), *Eugenia singampattiana* (Sarcar et al., 2006), *Hildegardia populifolia* (Saradha & Paulsamy, 2012) and *Salacia oblonga* (Deepak et al., 2016), *Elaeocarpus serratus*, *Hypericum gaitii*, *Bambusa nagalandiana*, *Lasiococca comberi*, *Vanilla borneensis*, *Zanthoxylum armatum* (Panda et al., 2019). The rooting efficiency of tree species propagated by stem cuttings is greatly influenced by factors such as hardness/ age of cutting, the season of collection, growth regulators used, and their concentration, etc (Husan & Pal, 2006).

Stem or branch cutting is the most important and common type of cutting for the vegetative propagation of woody species. According to Hartmann et al. (2001), stem

cuttings can be grouped into hardwood, semi-hardwood, softwood, and herbaceous. The use of hardwood cuttings is one of the least expensive and easiest methods of vegetative propagation, especially in deciduous plants. Semi-hardwood cuttings are usually employed for woody broad-leaved evergreen species, but leafy summer cuttings from partially matured wood of deciduous plants can also be considered semi-hardwood cuttings. Softwood cuttings are prepared from the soft, succulent, new spring growth of deciduous or evergreen species. Leafy semi-hardwood and softwood cuttings require to be rooted in a high-humidity environment (Hartmann et al., 2001). In propagation by stem cuttings, rooting is influenced by many factors including the type of wood, the stage of growth when cuttings are made, the time of year when cuttings are taken, rooting medium, rooting auxin, and physical factors (Macdonald, 1986; Hartmann et al., 2001; Wilson, 1993).

Plant growth regulators and other chemicals are used to improve rooting, survival, and subsequent growth in plant species when propagated vegetatively (Nautiyal et al., 1992; Pijut & Moore, 2002; Henselova, 2002; Henselova et al., 2002; Das, 2006). Various classes of growth regulators such as auxins, cytokinins, gibberellins, and ethylene influence root initiation in cuttings, among these the auxins induce root formation in cutting (Krul, 1968; Thimann & Poutasse, 1941; Tillberg, 1974). The Auxin enters cuttings predominantly via the cut surface even in micro cuttings (Guan & De Klerk, 2000) then it is rapidly taken up in cells by pH trapping (Rubery & Sheldrake, 1973) and by influx carriers (Delbarre et al., 1996). Auxins stimulate cell division in the cambium and which is a prerequisite for the differentiation of cambial initials into form root primordial (Haissig, 1974). IBA is most commonly used for rooting in commercial productions. The other auxins used commercially are IAA and NAA. Many chemical analogs have been

synthesized and examined for auxin-like activity (Jonsson, 1961), but none of them are being used on a large scale for rooting.

Combinations of root-promoting substances are sometimes more effective than either component alone. For example, equal parts of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) when used on a widely diverse species were found to induce a higher percentage of cuttings to root and more roots per cutting than either auxin alone (Ellyard, 1981). However, there are many plant species, cuttings of which do not root even with the application of auxin (Nanda & Anand, 1970). Several investigators (Nanda et al., 1970; Pal, 1980; Nautiyal et al., 1992) have studied the effect of auxins on the rooting of stem cuttings. Pal (1980) found that the highest percentage of rooting occurred in cuttings obtained from the basal part of the seedling even without auxin treatment. Seasonal variations in the effectiveness of auxins have been reported by many workers (Nanda, 1970; Roberts & Fuchigami, 1973). Thus in the same plant species, an auxin may stimulate rooting of cutting in one season, may be ineffective in the other season, and even inhibitory in another season. Again, the maximum rooting of the same plant species may be caused by two different auxins in two different seasons (Nanda, 1970).

2.6.3. Habitat niche modeling

Predictive Species Habitat Distribution Modeling, otherwise called Environmental niche modeling, is defined as the process of modelling the species distributions on the earth with the help of computer-based mathematical algorithms and their realized ecological niches.

The Realized ecological niche is well-described by climatic data (such as precipitation and temperature), but other variables such as soil type, soil moisture, and land use can also be used in its description. With the help of these models, it is possible to simulate the required habitat conditions with even a limited number of species occurrence records (Pearson & Dawson, 2003; Pearson et al., 2007).

The accuracy of the modeled data depends upon numerous factors. They are the complexity, nature, and efficacy of the mathematical algorithms used, the quality and accuracy of the acquired environmental data; the availability of adequate and impeccable species distribution data as model inputs; and the influence of biotic interactions, which raise the inequality between the realized niche and the fundamental niche. Thus, environmental niche modeling is considered a branch of 'Biodiversity Informatics' (Pearson & Dawson, 2003).

The Ecological Niche Models generally used are Generalized Linear Models (GLMs), Generalized Additive Models (GAMs), the Genetic Algorithm for Rule Set Prediction (GARP) model, and the Maxent model. Maxent is a maximum entropy-based machine learning program that analyses the most likely species distributions based on several ecological criteria. It demands only species occurrence records and it doesn't require species absence data. This model requires ecological data layers (continuous or categorical), for the area under study. Thus, the Maxent model illustrates a raster image where each pixel's suitability is a function of the environmental variables at that place (Philips et al., 2004). Distribution model-based restoration was done in species, *Elaeocarpus serratus* (Baruah et al., 2019), *Ilex khasiana* (Adhikari et al., 2012), *Mesua*

assamica (Baruah et al., 2016), *Vanilla borneensis* (Deka et al., 2017), *Brucea mollis* (Borthakur et al., 2018), *Calamus nambariensis* (Deka et al., 2018), etc.

2.6.4. Restoration

As an effective and long-lasting conservation methodology, the process of restoration is getting increased priority in conservation studies. The process involves the reintroduction or restocking of a plant community by means of which the former species diversity of that community is constructed (Bramwell, 1991; Groombridge, 1992; Young, 2000).

As the biodiversity degradation process increases day by day, an alternate gene stock development is essential for their conservation and long-term use for the existing and future generations. In this background, restoration would enhance either to increase the number of individuals in a population to a level at which is no longer in danger or to help in the establishment of new populations (Howe, 1976; Dodson, 1981; Rubluo et al., 1989; Parenti & Guerrant, 1990; Whitten, 1990; Lesouef, 1991; Young, 2000; Owadally et al., 2012).

Efforts to reverse biodiversity decline and mitigate climate change through large-scale reforestation of the tropics have gained global prominence through agreements and missions such as the Bonn Challenge and Paris climate accord (Lewis et al., 2019) and the ongoing UN decade on ecosystem restoration (2021-2030). Tropical forest recovery on degraded lands is constrained by various factors including unfavorable abiotic conditions, competition from grasses and invasive species, and disruption of seed dispersal and other

animal-plant mutualisms (Elgar et al., 2014; Gunaratnae et al., 2014). Restoration might also adopt “active” strategies to assist recovery or reconstruction of degraded ecosystems, using interventions such as invasive species management and tree planting (Chazdon & Guariguata, 2016; Atkinson & Bonser, 2020). Studies have shown that an overstorey of planted trees can contribute to overcoming recovery barriers by shading out competitors, attracting seed dispersers, and creating favorable microhabitats for the regeneration and survival of native species (Ashton et al., 2014).

An interesting study conducted by Sawka et al. (2013) on summer energy conservation has proven that planted trees adjacent to houses decreased electricity consumption, in that study, 25-year-old planted trees near houses reduced electricity consumption between 435 and 483 kWh, that power earlier used for cooling.

A systematic study of identifying the causes for the reduction of the species and developing appropriate conservation measures are plausible means to save these plants from the brink of extinction and for their sustainable utilization.

In this context, a study on population structure, dynamics, genetics, and conservation of two rare and endemic species of the Western Ghats of Kerala, namely *Atuna indica* and *Hydnocarpus longipedunculatus* was carried out. The interdisciplinary approach could help to find out the distribution, ecological characteristics, and biological constraints responsible for the rarity of species in their natural habitats further aiding the management of the populations. The conservation methods developed through the vegetative, seed, and restoration practices could be utilized for their multiplication,

germplasm preservation, and creation of new populations in the predictive population range of the species.

Chapter 3

Population Ecology

Objective 1 : Identification and mapping of the populations of *Atuna indica* and *Hydnocarpus longipedunculautus* in the Kerala part of the Western Ghats

Objective 2 : Identification of ecological, reproductive biological and environmental factors of target spp. *in situ*

3.1. Introduction

The identification and mapping of threatened plant populations are the first steps in any conservation biological studies. The population structural and dynamic aspects come as the sequel stage, which gives information on how the species stands in natural habitats and how they perform reproductively. Thirdly, the environmental factor analysis (climatic and edaphic data) facilitates rational ecology data interpretations as well as helpful in reaching valuable conclusions regarding population health, and possible ups and down in the future (Jose, 2001; Swarupanandan et al., 2013).

The rare and endemic plants of the Western Ghats invite special attention as many of these species exist as isolated and in small populations. In addition, they face various threats in their natural habitats. Habitat loss/ modifications, overexploitation, flowering irregularities, low fruit set, and poor natural regeneration are the major constraints of these species (Drury, 1974; Rabinovitz et al., 1986; Nayar, 1996; Sasidharan, 2017). Population ecological studies include analysis of population structure, diversity, and dynamics of a species. The population ecology and genetics studies (discussed in chapter 4) give a basic knowledge of the life history and population trends of the species (Jose, 2001; Ramachandran et al., 2014). The population ecological studies on threatened tree species such as *Humboldtia bourdillonii* (Ramachandran et al., 2014), *Kingiodendron pinnatum* (Jose et al., 2018), *Syzygium travancoricum* (Sreekumar et al., 2020) found small fragmented populations are the major issue faced by the species. Whereas, poor regeneration and low seedling establishment were reported in *Elaeocarpus venustus* (Irwin et al., 2013), *Hopea glabra,* and *Hopea utilis* (Sanil et al., 2017). These results emphasize

the need for habitat protection and ecological studies on threatened species for developing better conservation and management programs. In the case of newly reported species, intensive population search and population ecological studies facilitate more attention from researchers, conservation biologists, and governmental and non-governmental organizations. *Hydnocapus longipedunculatus*, one of the target species is discovered in the recent past (Robi et al., 2014).

Plant reproductive biology (PRB) is a relevant and less explored domain helpful in understanding the growth and survival of species *in situ.* The PRB studies are essential to interpret genetic structure, evolution, and ecology as well as to frame conservation strategies (Bawa et al., 1985; Jose, 2001). Reproductive characteristics such as the flowering age of the tree, flowering time, number of flowers and seeds formed, features of stigma and pollen grains, pollination, seed dispersal, seed germination, and other biotic interactions can play a key role in determining the reproductive success of a species (Moza & Bhatnagar, 2007; Sreekala, 2017). The PRB studies of threatened species provide insights into their reduced fitness/population size. For example, *Elaeocarpus blascoi*, an endangered species reported with low seedling recruitment, was found with fungal attacks on seeds (*Fusarium* sp., *Lasiodiplodia* sp., and *Pencilium* sp.) (Ramasubbu & Irudhyaraj, 2016). Studies conducted on the endangered *Elaeocarpus gaussenii* and *Elaeocarpus recurvatus* reported fruit damage caused by the Malabar giant squirrel and lion-tailed macaque (Kasi & Ramasubbu, 2021). In *Talbotiella gentii*, a critically endangered tree, the stigmatic surface was found infected by fungi, thus reducing its reproductive potential (Dompreh et al., 2015). In addition, the low number of compatible pollen in *Butea monosperma* (Tandon et al., 2003), seed abortion in *Syzygium cumini* (Krishnamurthy et al.,

1997), difficulties in seed germination and seedling vigor in *Lactoris fernanndeziana* (Bernardello et al., 1999) are some other constraints reported in different PRB studies. These limitations reduce the efficiency of reproduction, the genetic diversity, subsequently leading to the inability for adapting to future environmental odds and finally ending up in reduced genetic fitness against environmental stochasticities (Sreekala, 2017).

The present chapter covers the first two objectives of the study i.e. identification and mapping of the population of *A. indica* and *H. longipedunculatus* along with population ecology and reproductive biology studies. In addition, the study incorporates climate data (Average precipitation and temperature availed in the state of Kerala) to discuss the phenological changes of target species. The state of Kerala experiences different, but almost stable climatic seasons such as summer, monsoon, and winter. The phenological studies including reproductive biology will give an idea of the influence of climate seasons on reproductive performances and the survival of species. The atmospheric temperature, rainfall pattern, diurnal variations, etc., are signaling the episodes of flushing and flowering. These changes may lead to a cascade of positive or negative influences on depending fauna or eventually on plant species' survival.

3.2. Materials and Methods

Species taxonomic details

Atuna indica (Bedd.) Kosterm. Evergreen trees with smooth, brown bark. Leaves are simple, lanceolate, alternate, and stipulate. Stipules are free, lateral, and petioles are 6-12 mm long and glabrous. The lamina measures 17-21 x 5.5-7.5 cm, and it's oblong, with an acuminate apex. The margin is entire, and the lamina is glabrous and chartaceous. The lateral nerves are 12-18 pairs, and the intercostae are reticulate. The flowers are bisexual,

white, and grow in axillary or terminal corymbose racemes. Bracts are brown and hairy, and the calyx tube is funnel-shaped and pubescent. There are 5 lobes, imbricate; 5 white petals that are inserted at the mouth of the calyx tube, and many stamens. The filaments are basally connate, anthers are small, and the ovary is adnate to the side of the calyx tube. It is 2-celled, and the ovules are 2 and erect. The style is filiform, basal, and the stigma is truncated. The fruit is a drupe that measures 3.5-4 x 2.5-3 cm, ovoid, and smooth.

Hydnocarpus longipedunculatus Robi., Sasidh & Jose*:* Medium to large-sized evergreen trees, and their branchlets are angular, covered with dense, rusty-stellate tomentum when young. The mature parts are glabrous and lenticellate, flushing greenish-yellow. The leaves are alternate, measuring 9–25 × 3–8 cm, and are oblong and elliptic-lanceolate with a chartaceous texture. The flowers are greenish-white, monoecious, and hanging in nature. There are 5 sepals, 5 petals, and 5 stamens. The fruit is ovoid-oblong, 5-angular, with an apex that is beaked. The peduncle is 50–65 mm long, and the pedicel is 35–50 mm long.

Atuna indica: Branch showing inflorescence

Hydnocarpuslongipedunculatus: Female inflorescence

3.2.1. Population identification and mapping

Reconnaissance field surveys were conducted based on the literature and previous collection records of the species. GPS data acquired from the field are used for mapping

purposes. The IUCN criteria for assessing the conservation status is followed for estimating the extent of occurrence (the extent of distribution of a species within the shortest continuous imaginary boundary of the species) and area of occupancy (the area occupied by the species within its extent of occurrence wherein the species satisfy its survival) of the species (IUCN, 2022).

3.2.2. Population structure

Demographic structure

The demographic structure covering mature individuals, saplings, and seedlings was enumerated from the populations. The individuals were classified into four categories based on GBH and height, *viz.* mature individuals [girth at breast height (GBH) ≥30cm], saplings (GBH 10-30cm), seedlings (GBH <10cm) of two height classes, ≥100cm and <100cm (Richards, 1952; Halle et al., 1978; Pascal, 1988; Jose, 2001).

Spatial, Vertical distribution, and ontogenic stages

Spatial distribution is the pattern of distribution of individuals of the species in the forest area. Apart from the three distribution types (such as uniform, random, and clumped), microhabitat features like presence slopes, water courses, and trek paths were also noted. The vertical distribution is the vertical positioning of species in a forest community. Based on the height, species are classified as the first layer (reaching 26-35 m ht.), the second layer (16-25 m ht.), the third layer (5-15 m ht.), and below which constitutes shrubs and herbs. The species' height was measured using a clinometer. The vertical distribution of species in the ecosystem gives knowledge of the level of sunlight

dependency/influence on species (Martin-Ducup et al., 2018). The ontogenic stages of *A. indica* and *H. longipedunculatus* are documented based on flowering behavior. Trees shown flowering are classified as 'reproductive' and others as 'pre-reproductive'. In the case of *Atuna indica*, only two trees showed flowering, hence the minimum GBH range of flowering individuals is considered as the line between pre-reproductive and reproductive individuals.

Floristic diversity and Importance Value Index (IVI)

The plant community diversity and relative abundance of target species were perceived by laying sample plots as per basic ecological standards (Odum, 1959). All the species above 30 cm GBH were counted and measured. The density, frequency, and dominance were calculated to find out the IVI, which is the relative position of a species in its ecosystem, and calculated by summing of relative frequency (rf), relative density (rd), and relative dominance (rD) (Philips, 1959; Curtis, 1959) of species using INVENT NTFP software (Sivaram et al., 2006) (sum of IVI values of all the species is equal to three).

3.2.3. Population dynamics

Reproductive phenology

Data on reproductive phenology concerning the number of inflorescences per branch, number of flowers per inflorescence, flower/ inflorescence development, blooming period, fruit initiation, and development was recorded on a day-to-day basis. Five inflorescences per branch were tagged and monitored for flower development from bud to full bloom. The average days taken from bud to bloom were calculated and recorded.

Pollen viability

Pollen grains from fully matured flower buds were dusted into a cavity slide containing a solution of acetocarmine and kept for one hour, later observed under a compound microscope. The pollen grains stained were treated as viable and others as non-viable. The viability test was carried out in two-hour intervals.

Pollen germination

Pollen grains from fully matured flower buds were transferred to a cavity slide containing germination medium (Sucrose 10%). The pollen germination was counted after one hour using a compound microscope. The pollen tubes with longer lengths than pollen diameters were treated as germinated. The experiment was repeated in two-hour intervals from the anthesis.

Stigma receptivity

Both physical (hand lens) and chemical (hydrogen peroxide) tests were conducted. In the hand lens method, the stigma with wetness, turgidity, and oily nature was considered receptive and the rest as non-receptive. Whereas a drop of hydrogen peroxide was added to the stigma of a freshly opened flower, and the effervescence resulting from peroxidase enzyme activity was identified, the test was repeated in different intervals to identify the stigma receptivity period (Dafni & Maués, 1998).

Pollen - Ovule ratio

The number of pollen grains in anthers per flower was counted using Haemocytometer (Shivanna & Rangaswamy, 2012). The number of ovules per ovary was

counted by the cross-section of the ovary (Cruden, 1977). The Pollen- Ovule ratio was calculated by the following formula:

$$\text{Pollen} - \text{ ovule ratio} = \frac{\text{Pollen count per anther} \times \text{No. of anthers per flower}}{\text{No. of ovules per flower}}$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. Physical observations were made throughout the flowering period and recorded insect interactions during the day and night hours. The taxonomic identification of insects was made with the available literature and experts' help.

Fruit phenology

Fruit phenology was monitored and recorded viz. initiation, development, and maturity along with premature abscission, and pest incidence.

3.2.4. Environmental factor analysis

The climatological data of the species covering atmosphere temperature (day and night - ℃) and atmospheric humidity (day and night- %) in three prominent seasons of a year (summer, monsoon, and winter) were recorded and the average values taken for representation (Jose, 2001).

The edaphological data viz., soil texture (from three soil depth levels such as surface, 15cm deep as middle, and 30 cm deep as the bottom), pH, major macronutrients

such as N, P, and K, soil moisture content and temperature in three seasons of a year recorded and average values taken for representation (Gupta & Malik, 1996).

3. 3. Results

3.3.1. Population identification and mapping

Atuna indica

Three populations were identified and located in the Kerala part of the Western Ghats. Two populations are located at Kakkayam forests namely, Kakkayam dam site and Charangad belonging to Malabar Wildlife Sanctuary, Peruvannamoozhi Forest Range, Kozhikode Forest Division. The third population at Nadugani Ghats, Vazhikkadavu Forest Range, Nilambur North Division (Fig. 1). The extent of occurrence and area occupancy of the species are 39.14 km^2 and 16 km^2 respectively (Fig. 3), suggested placement under Endangered conservation status as per 'D' criteria of IUCN Guidelines. The voucher specimens were submitted to the KFRI herbarium (Kakkayam Dam site- 23260, Charangad- 23261, Nadugani- 23262).

Hydnocarpus longipedunculatus

Two populations were identified and located in Idukki District. viz. Kulamavu MPCA belongs to Nagarampara Forest Range, and at Cheri forest area in Thodupuzha Forest Range belonging to High Range Circle, Kottayam (Fig. 2). The extent of occurrence and area occupancy of the species are 0.597 km^2 and 12 km^2respectively (Fig. 4), suggested placement under the Endangered category as per 'D' criterion of IUCN Guidelines. The

voucher specimens were submitted in KFRI herbarium (Kulamavu MPCA- 23263, Cheri-23264).

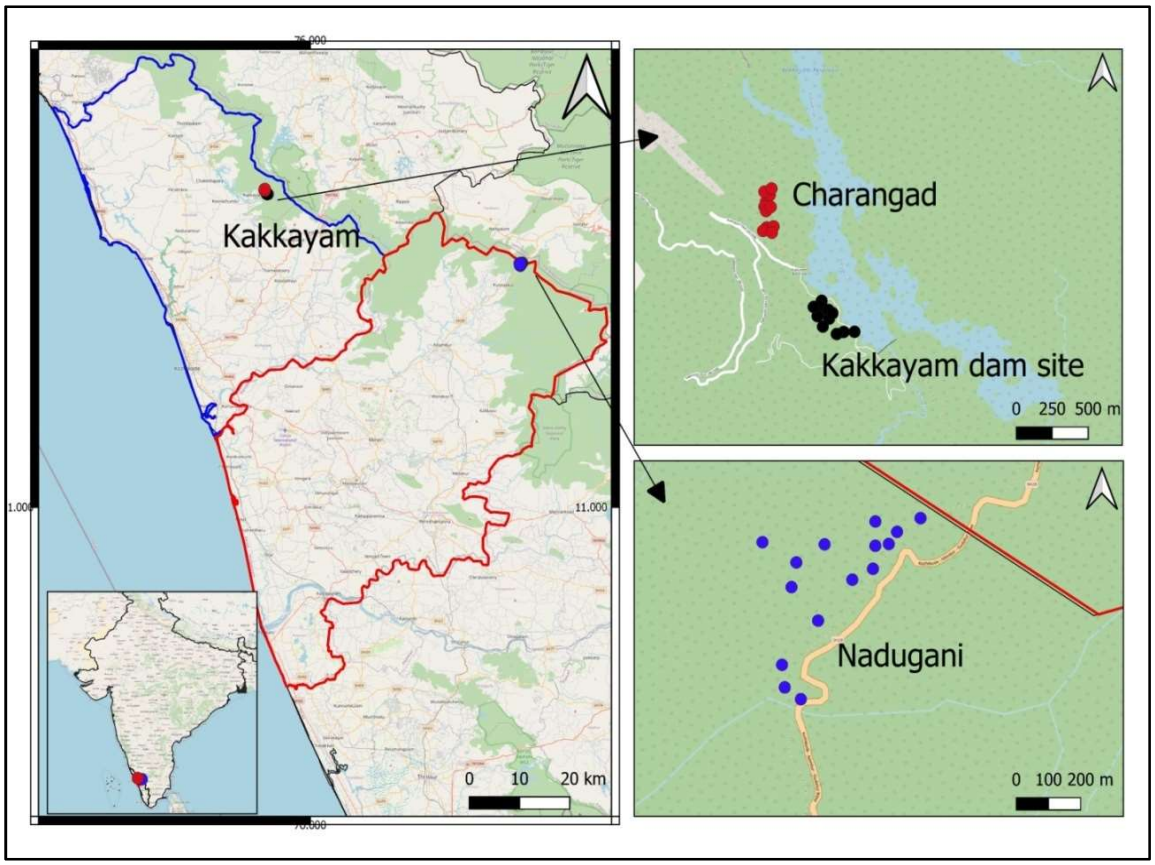

Figure 1. Distribution map of *Atuna indica* in Kerala part of Western Ghats

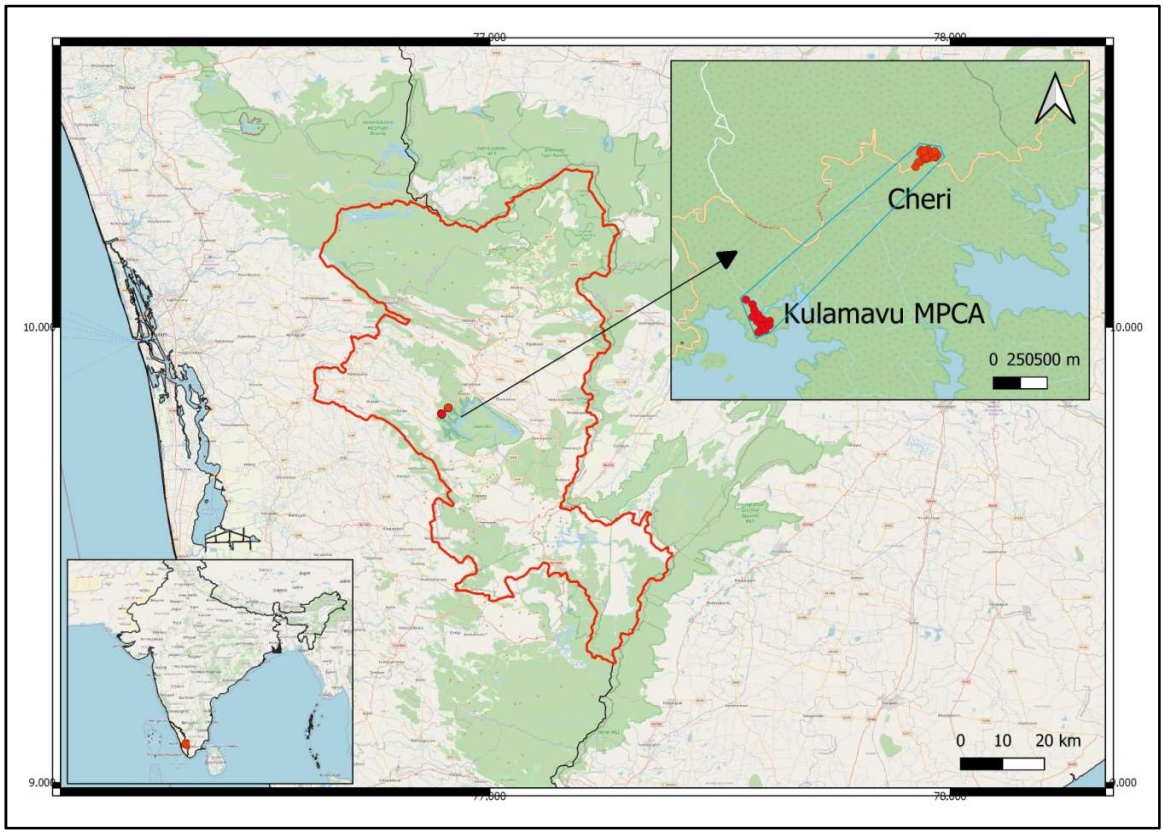

Figure 2. Distribution map of *Hydnocarpus longipedunculatus* in Kerala part of Western Ghats

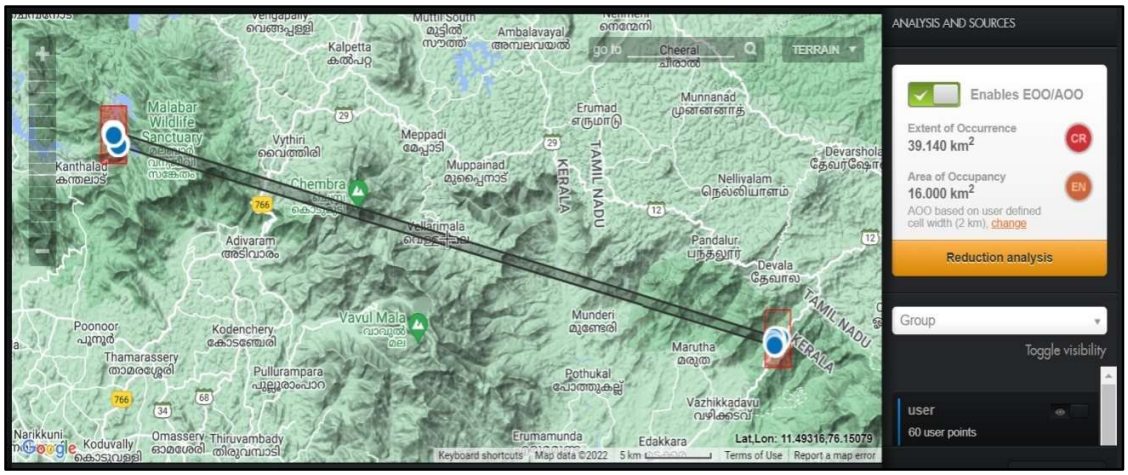

Figure 3. Extent of Occurrence and Area of Occupancy of *Atuna indica*, calculated using GEOCAT software, according to IUCN criteria.

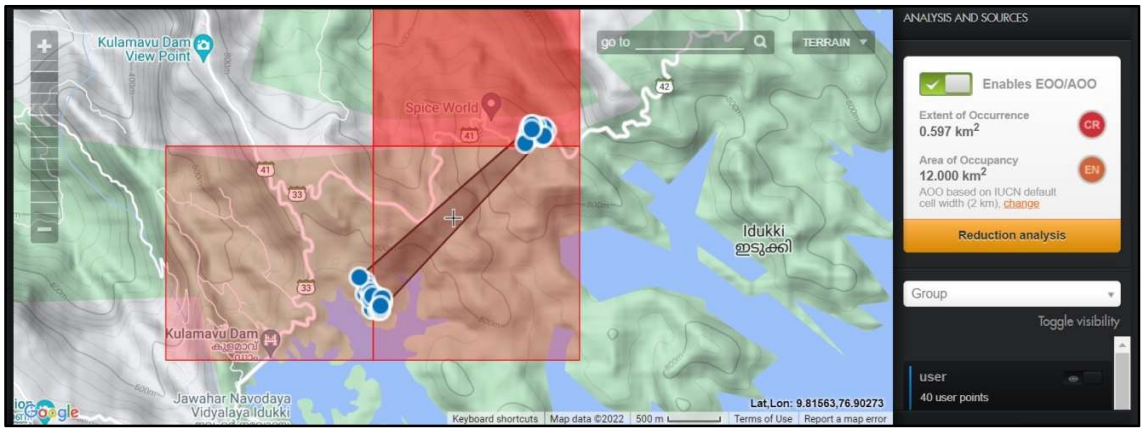

Figure 4. Extent of Occurrence and Area of Occupancy of *Hydnocarpus lonipedunculatus*, calculated using GEOCAT software, according to IUCN criteria.

3.3.2. Population structure

Atuna indica

Seventy-nine matured individuals (>30 cm GBH) of *Atuna indica* and 32 saplings (10-30cm GBH range) were counted from all three populations. Seedlings above 100 cm height are 27 nos. and below 100 cm height are 9 nos. (Fig. 12). Findings on the population structural studies of *Atuna indica* populations ie, Kakkayam Dam site, Charangad, and Nadugani are presented. (Fig. 11- Population view)

Kakkayam dam site

The Kakkayam Dam site population was recorded with two individuals above 30 cm GBH, ten saplings, and seven seedlings below 100 cm in height. No seedlings of above 100 cm height range were observed (Table 1).

(a) Horizontal / Spatial distribution:

Atuna indica showed clumped distribution pattern adjacent to a watercourse (Kakkayam dam reservoir) (Fig. 5).

(b) Stratification / Vertical distribution

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as *Poeciloneuron indicum* Bedd., *Bischofia javanica* Blume, *Dipterocarpus indicus* Bedd., *Holigarna grahamii* (Wt.) Kurz, *Alstonia scholaris* L. R. Br., *Vateria indica* L., *Schleichera oleosa* (Lour.) Merr., etc. as first layer species reaching a height range of 26 to 35m. The second layer is represented by *Syzygium mundagam* (Bourd.) Chithra, *Aglaia barberi* Gamble, *Melicope lunu-ankenda* (Gaertn.) Hartley,

Knema attenuata (Hook. f. & Thoms.) Warb, *Symplocos racemosa* Roxb., *Cinnamomum verum* Presl, *Holigarna arnottiana* Hook.,etc. with a height range of 16 to 25 m. The third layer is occupied by *Haldina cordifolia* (Roxb.) Ridsd., *Olea dioica* Roxb., *Humboldtia brunonis* Wall. var. *raktapushpa* Udayan, *Memecylon umbellatum* Burm., *Syzygium laetum* (Buch.-Ham.) Gandhi, etc with a 6 to 15 m height range along with the target species, *Atuna indica* (Fig. 6) The Shrubby layer consists of *Ixora brachiata* Roxb. ex DC., *Vernonia* sp., etc. The herb layer is mainly dominated by *Cyanotis cristata* (L.) D. Don, *Commelina benghalensis* L., *Dictyospermum montanum* Wight.,etc.

(c) Age distribution/ Ontogenic stages

The ontogenic stages are measured based on the flowering nature of species. Only two individuals showed flowering at the Kakkayam dam site with a mean GBH range of 45 cm, hence 45 cm GBH is treated as the line between pre-reproductive and reproductive individuals. The Kakkayam dam site is the smallest population of *Atuna indica*. Two trees are reproductive and the remaining 10 individuals are saplings.

(d) Crown projections

The crown projections (vertical) showed the placement of individuals such as *Bischofia javanica, Dipterocarpus indicus, Holigarna grahamii, Persea macrantha,* and *Atuna indica,*etc. just below the tallest individual of *Vateria indica.* The crown projections (horizontal) displayed canopy coverage of these species under the tallest canopy of the *Vateria indica.*

(e) Abundance/Importance Value Index (IVI)

The floristic diversity analysis covered individuals of forty-four species with GBH ≥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 *Vateria indica* had the highest IVI of 0.3092 and thus became the dominant species in the particular quadrant, whereas, *Atuna indica* represented 26th position with IVI of 0.0421 (Table 2).

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

(List of individuals with G≥10 cm represented)

Sl. No.	GBH (cm)	Basal area (cm)	Basal cover (m)	Age phase	First branching seen at (m)	Height of stand (m)
1	59	277.45	6.0	Set of present	1.4	8.5
2	10	8.04	2.0	Set of future	1.0	7.0
3	11	9.62	1.6	Set of future	2.5	6.0
4	20	31.75	1.8	Set of future	4.5	7.0
5	14	15.61	1.9	Set of future	0.6	8.0
6	13	13.45	2.0	Set of future	4.5	6.5
7	26	53.82	4.0	Set of future	3.0	8.0
8	25	49.74	4.0	Set of future	6.0	8.0
9	22	38.47	4.0	Set of future	3.0	8.0
10	17	23.06	2.0	Set of future	1.9	6.5
11	19	28.83	3.0	Set of future	2.5	6.0
12	48	183.28	5.5	Set of present	1.7	7.0

Table 2. Floristic diversity / Importance value index of *Atuna indica* : Kakkayam Dam site
(List of individuals with G ≥ 10 cm represented)

Sl. No	Species	Family	rf (%)	rd (%)	rD (%)	IVI
1.	*Vateria indica* 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.	*Garcinia gummi-gutta* (L.) Robs.	Clusiaceae	0.0450	0.0381	0.0561	0.1393
6.	*Xanthophyllum arnottianum*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 ciliate* 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.	*Knema attenuata* (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.	*Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don	Apocynaceae	0.0270	0.0205	0.0217	0.0692
18.	*Holigarna grahamii* (Wight) Kurz	Anacardiaceae	0.0270	0.0205	0.0213	0.0689
19.	*Erythrina variegata*L.	Fabaceae	0.0180	0.0176	0.0261	0.0618
20.	*Memecylon umbellatum* Burm.	Melastomataceae	0.0090	0.0469	0.0045	0.0604
21.	*Macaranga peltata* (Roxb.) Muell.-Arg.	Euphorbiaceae	0.0180	0.0205	0.0153	0.0538
22.	*Nothopegia beddomei* Gamble	Anacardiaceae	0.0270	0.0176	0.0086	0.0532
23.	*Olea dioica* Roxb.	Oleaceae	0.0180	0.0147	0.0121	0.0448
24.	*Haldina cordifolia* (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
30.	*Schleichera oleosa* (Lour.) Oken	Sapindaceae	0.0090	0.0059	0.0179	0.0328

31.	*Syzygium mundagam* (Bourd.) Chithra	Myrtaceae	0.0180	0.0059	0.0064	0.0303
32.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	0.0090	0.0088	0.0091	0.0269
33.	*Cinnamomum malabatrum* (Burm. f.) Blume	Lauraceae	0.0090	0.0088	0.0053	0.0231
34.	*Melicope lunu-ankenda* (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.	*Elaeocarpus serratus* L.	Elaeocarpaceae	0.0090	0.0059	0.0051	0.0200
37.	*Persea macrantha* (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.	*Syzygium laetum* (Buch.-Ham.) Gandhi	Myrtaceae	0.0090	0.0029	0.0035	0.0154
41.	*Pajanelia longifolia* (Willd.) K. Schum.	Bignoniaceae	0.0090	0.0029	0.0028	0.0147
42.	*Dysoxylum malabaricum* 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

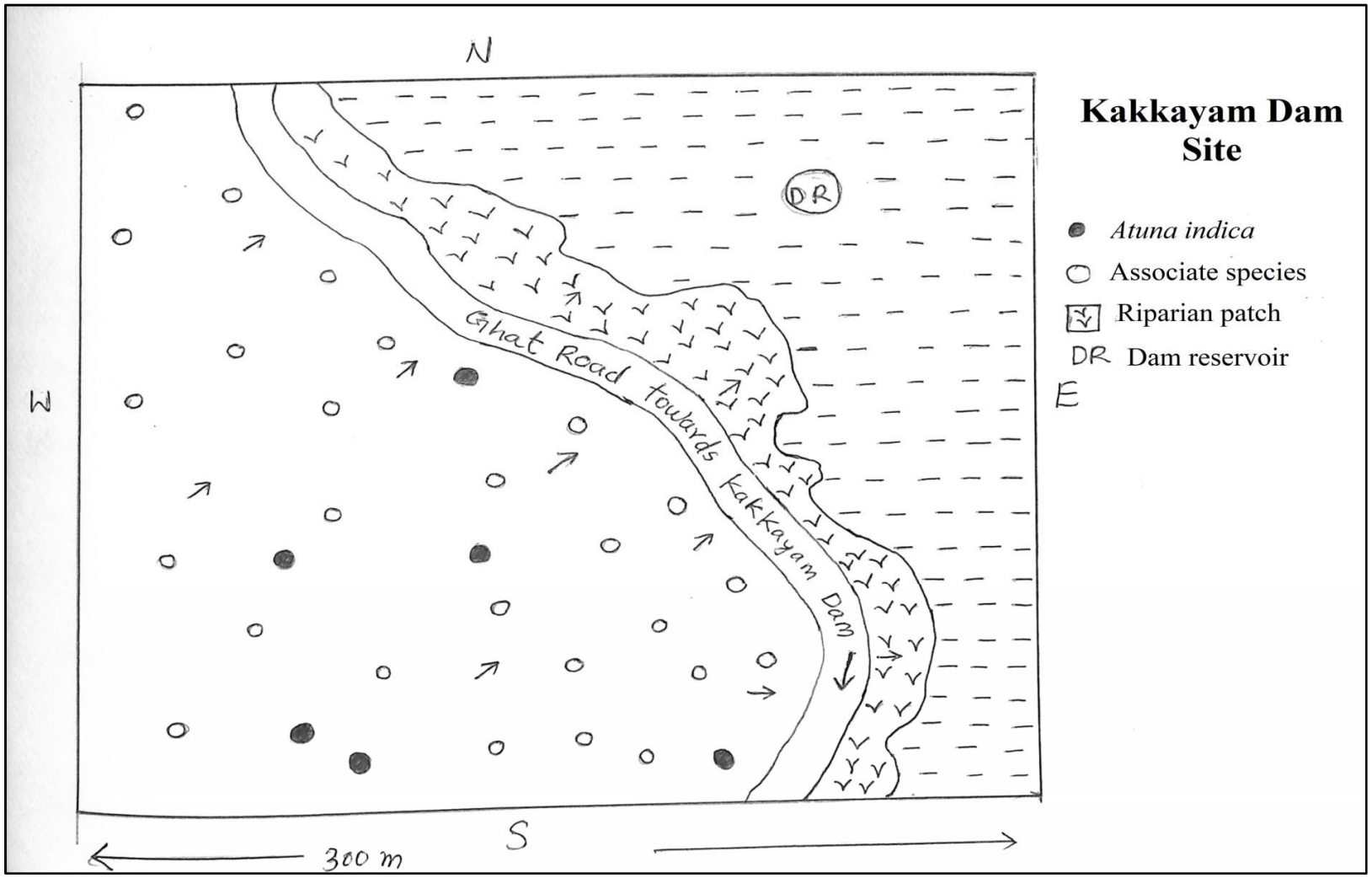

Figure 5. ***Atuna indica*** **: Spatial distribution pattern at Kakkayam Dam site population**

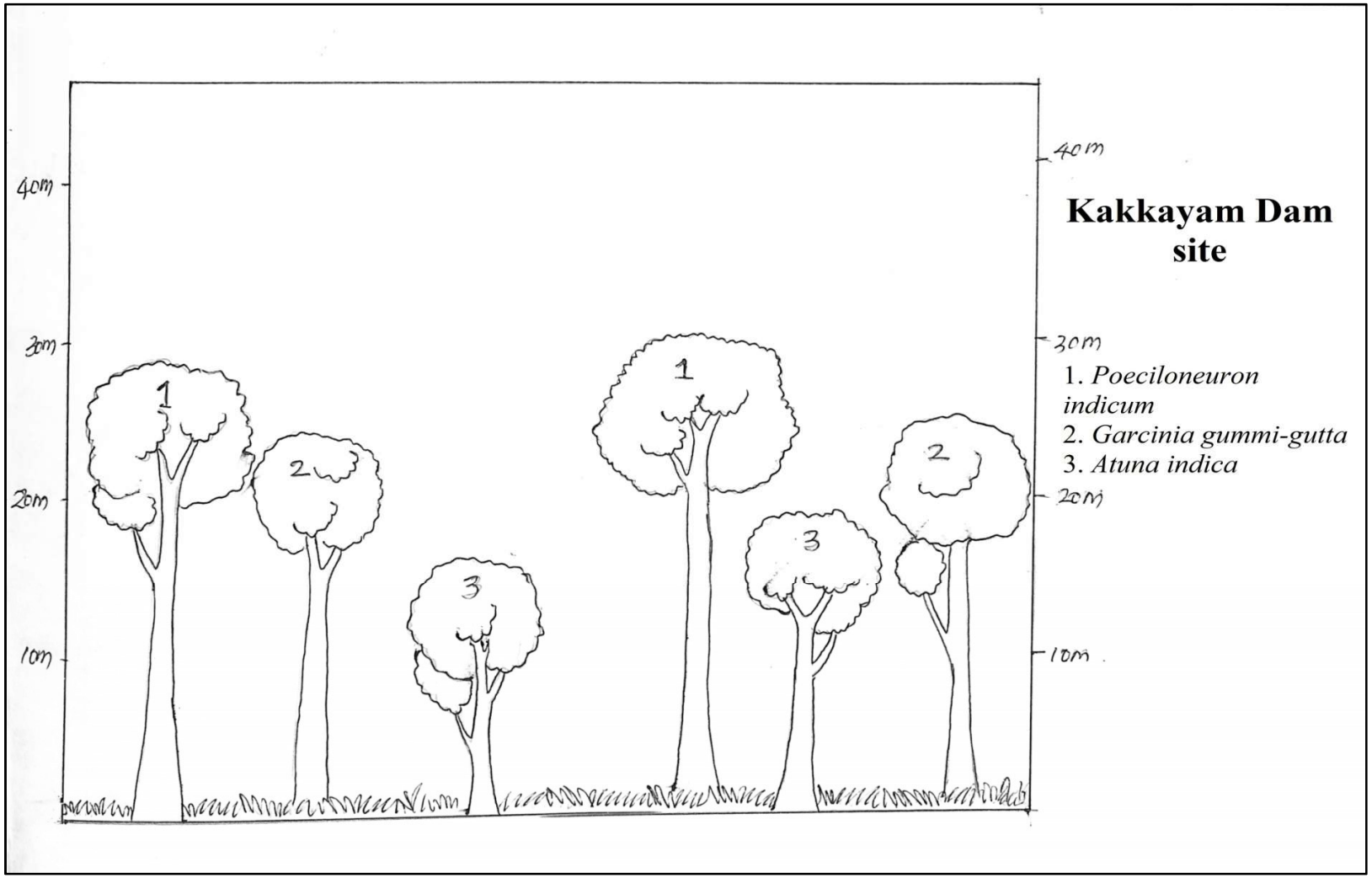

Figure 6. ***Atuna indica:*** **Vertical distribution pattern at Kakkayam dam site population**

Charangad

Thirty-nine matured individuals were enumerated along with 13 saplings (Table 3). Two seedlings below 100 cm in height and 19 seedlings above 100 cm were found.

(a) Horizontal/spatial distribution:

The horizontal profile of the population exhibited scattered arrangements of the individuals of *Atuna indica* along with their associates, adjacent to a watercourse (wetland with bamboo and *Calamus* sp.) (Fig. 7)

(b) Stratification / Vertical distribution

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as *Vateria indica, Hopea parviflora, Bischofia javanica, Elaeocarpus serratus*, etc. as the first layer reaching a height range of 26 to 35 m. The second layer is represented by *Schleichera oleosa, Cinnamomum verum, Holigarna arnottiana*, *Dimocarpus longan, Sterculia guttata,* etc with 15-25 m height range. The third layer is occupied by *Haldina cordifolia*, *Olea dioica*, *Memecylon umbellatum*, *Garcinia morella*, etc with a 6-15 m height range along with *Atuna indica* (Fig. 8). *Humboldtia brunonis* var. *raktapushpa, Ixora brachiata, Vernonia* sp., etc. were the major shrubby associates of the candidate species. The herbaceous layer was dominated by the seedlings of *Sonerila versicolor, Globba* sp*., Pellionia heyneana, Rhynchotechum permolle, Rhynchoglossum notonianum*,etc.

(c) Age distribution/ Ontogenic stages

Out of 39 mature individuals of *A. indica*, six are pre-reproductive and 33 are reproductive. No flowering was recorded during the study period 2017-2022 even though most of the individuals attained ≥ 45 cm GBH.

(d) Crown projection

The vertical crown projection showed the placement of individuals such as *Bischofia javanica, Holigarna grahamii, Persea macrantha,* and *Atuna indica,* etc. just below the tallest individual of *Dipterocarpus indicus.* The horizontal crown projections displayed canopy coverage under the canopy of the tallest individual*, Dipterocarpus indicus.*

(e) Abundance/ Importance Value Index (IVI)

The floristic diversity analysis covered individuals of forty-two species with GBH ≥ 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 community were worked out and found that *Schleichera oleosa* with the highest IVI of 0.2956 and thus became the dominant species in the particular community, whereas, *Atuna indica* represented 29th position with IVI of 0.0315 (Table 4).

Table 3. Population structure of *Atuna indica*: Charangad

(List of individuals with G≥30 cm represented)

Sl. No.	GBH (cm)	Basal area (cm^2)	Basal cover (m)	Age phase	First branching (m)	Height of stand (m)
1.	90	642.1	6.0	Set of present	2.5	15.0
2.	46	167.3	7.0	Set of present	2.0	9.0
3.	45	81.7	4.0	Set of present	0.5	9.0
4.	39	52.8	3	Set of future	0.5	5.0
5.	35	75.4	4	Set of present	1.0	6.0
6.	42	63.6	3	Set of future	1.0	7.0
7.	46	120.7	6	Set of present	1.5	8.0
8.	60	289.4	7	Set of present	3.0	14.0
9.	47	128.6	5	Set of present	2.0	11.0
10.	60	283.4	6	Set of present	3.0	14.0
11.	69	373.1	7	Set of present	3.0	13.0
12.	90	642.1	6	Set of present	3.0	8.0
13.	45	162.8	5	Set of present	1.0	10.0
14.	65	339.6	6	Set of present	8.0	15.0
15.	61	295.4	4	Set of present	1.5	10.0
16.	40	32.2	3	Set of future	1.5	4.0
17.	63	320.3	5	Set of present	3.5	14.0
18.	51	75.4	3	Set of present	3.5	9.0
19.	86	589.3	6	Set of present	0.5	18.0
20.	81	522.5	7	Set of present	6.0	15.0
21.	47	88.2	6	Set of present	3.0	7.0
22.	70	393.8	9	Set of present	3.5	16.0

23.	45	128.6	6	Set of present	3.0	10.0
24.	30	72.3	3	Set of future	1.0	5.0
25.	48	91.6	4	Set of present	1.5	7.0
26.	46	102	3.5	Set of present	1.5	7.0
27.	32	81.7	6	Set of future	2.0	7.5
28.	47	88.2	4.5	Set of present	2.0	7.5
29.	49	98.5	5	Set of present	4.0	10.0
30.	51	132.7	6	Set of present	3.0	10.0
31.	34	75.4	3	Set of future	2.5	6.0
32.	46	140.9	3	Set of present	2.0	7.0
33.	48	181.4	7	Set of present	2.5	5.0
34.	68	366.2	7	Set of present	3.0	13.0
35.	86	589.3	8	Set of present	4.0	16.0
36.	79	498.5	7	Set of present	3.5	15.0
37.	89	633.1	7	Set of present	3.0	15.0
38.	93	687.8	8	Set of present	3.5	16.0
39.	67	359.5	6.5	Set of present	2.5	13.5

Table 4. Floristic diversity / Important value index of *Atuna indica*: Charangad

(List of individuals with G≥30cm represented)

Sl. No.	Species	Family	rf (%)	rd (%)	rD (%)	IVI
1.	*Schleichera oleosa* (Lour.) Oken	Sapindaceae	0.0420	0.0638	0.1897	0.2956
2.	*Persea macrantha* (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.	*Olea dioica* Roxb.	Oleaceae	0.0420	0.0346	0.0550	0.1316
6.	*Knema attenuata* (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0420	0.0638	0.0234	0.1292
7.	*Macaranga peltata* (Roxb.) Muell.	Euphorbiaceae	0.0336	0.0399	0.0552	0.1287
8.	*Vateria indica* 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.	*Sterculia guttata* Roxb. ex DC.	Sterculiaceae	0.0336	0.0266	0.0257	0.0859

13.	*Gmelina arborea* Roxb.	Verbenaceae	0.0336	0.0213	0.0272	0.0821
14.	*Memecylon umbellatum* 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.	*Xanthophyllum arnottianum* Wight.	Polygalaceae	0.0252	0.0186	0.0267	0.0706
17.	*Cinnamomum malabatrum* (Burm. f.) Blume.	Lauraceae	0.0168	0.0213	0.0285	0.0666
18.	*Diospyrous bourdillonii* 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.	*Syzygium laetum* (Buch.-Ham.) Gandhi.	Myrtaceae	0.0252	0.0186	0.0209	0.0647
21.	*Poeciloneuron indicum* Bedd.	Clusiaceae	0.0168	0.0160	0.0256	0.0584
22.	*Myristica beddomei* King.	Myristicaceae	0.0084	0.0426	0.0044	0.0553
23.	*Syzygium mundagam* (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.	*Garcinia gummi-gutta* (L.) Robs.	Clusiaceae	0.0168	0.0106	0.0139	0.0413

26.	*Stereospermum colais* (Buch.-Ham. ex Dillw.) Mabb.	Bignoniaceae	0.0168	0.0133	0.0098	0.0399
27.	*Mangifera indica* L.	Anacardiaceae	0.0084	0.0213	0.0067	0.0364
28.	*Dysoxylum malabaricum* Bedd. ex Hiern.	Meliaceae	0.0084	0.0239	0.0039	0.0363
29.	***Atuna indica* (Bedd.) Kosterm.**	**Chrysobalanaceae**	**0.0168**	**0.0080**	**0.0067**	**0.0315**
30.	*Syzygium cumini* (L.) Skeels.	Myrtaceae	0.0168	0.0133	0.0013	0.0314
31.	*Terminalia bellirica* (Gaertn.) Roxb.	Combrutaceae	0.0084	0.0053	0.0176	0.0313
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.	*Chrysophyllum cainito* L.	Sapotaceae	0.0084	0.0080	0.0047	0.0211
35.	*Elaeocarpus serratus* L.	Elaeocarpaceae	0.0084	0.0053	0.0071	0.0209
36.	*Ceiba pentandra* (L.) Gaertn.	Bombacaceae	0.0084	0.0053	0.0059	0.0196
37.	*Caryota urens* 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.) Muell.	Euphorbiaceae	0.0084	0.0053	0.0040	0.0177
41.	*Pajanelia longifolia*	Bignoniaceae	0.0084	0.0080	0.0009	0.0172

	(Willd.) K. Schum.					
42.	*Lepisanthes tetraphylla* (Vahl) Radlk.	Sapindaceae	0.0084	0.0027	0.0034	0.0145

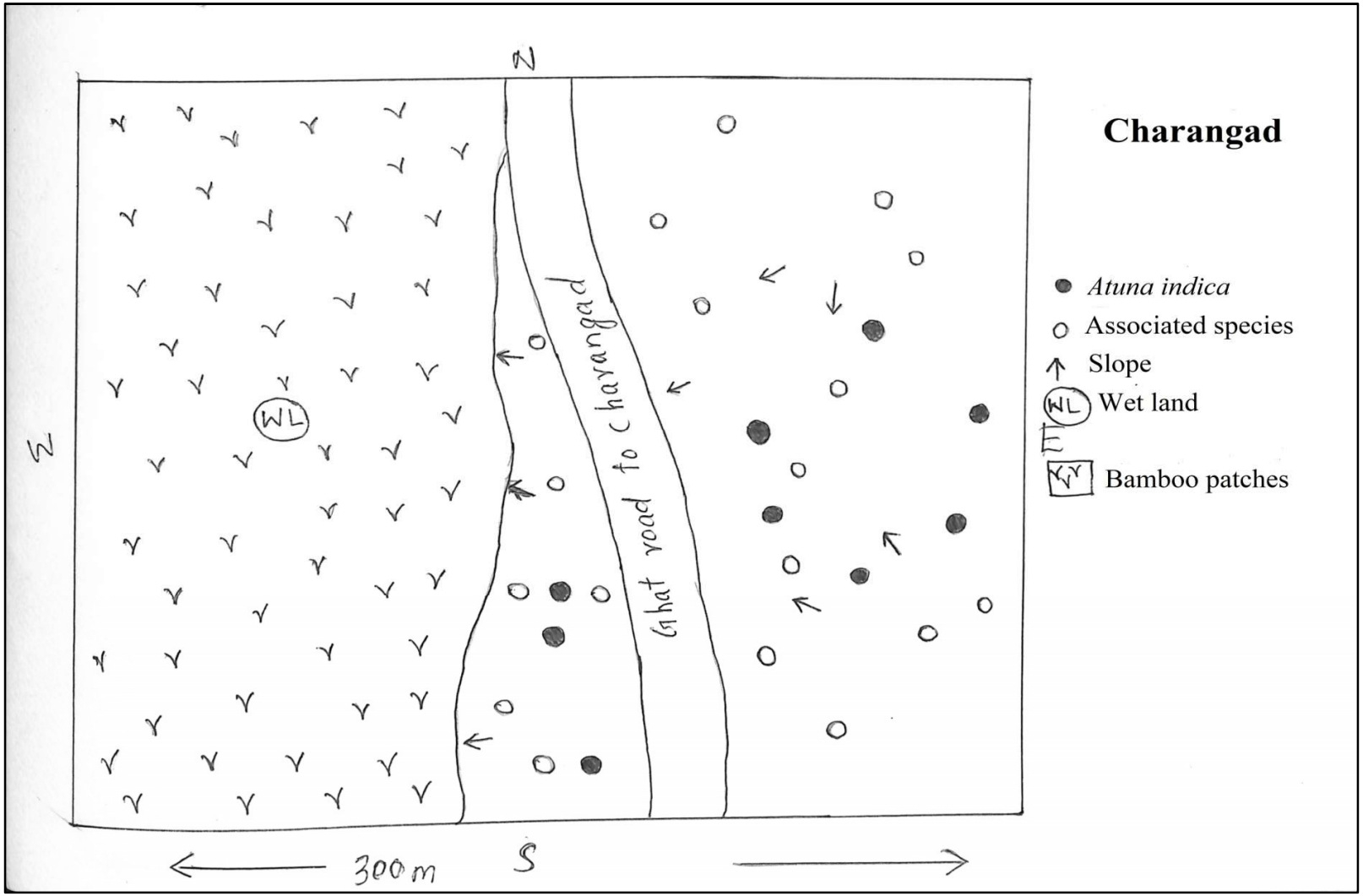

Figure 7. *Atuna indica* : Spatial distribution pattern at Charangad population

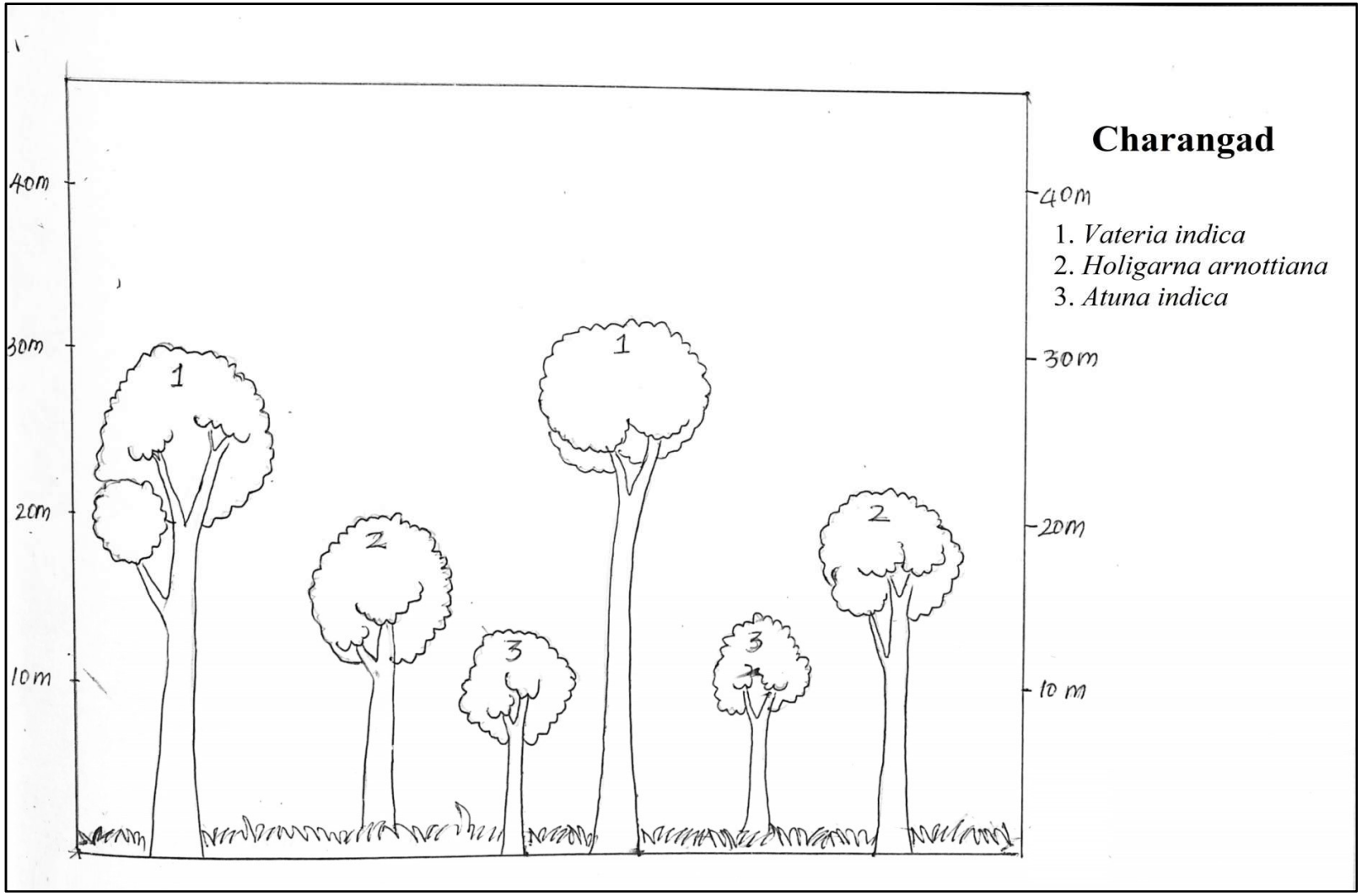

Figure 8: *Atuna indica*: Vertical distribution pattern at Charangad population

Nadugani

Thirty-eight matured individuals along with nine saplings were recorded from the Nadugani population (Table 5). Nine seedlings counted below 100 cm in height and 27nos. with above 100 cm height.

(a) Horizontal/ spatial distribution

The horizontal profile of the population exhibited the arrangement of the individuals of *Atuna indica* in a scattered manner along with its associates, adjacent to a small seasonal water course (Fig. 9).

(b) Stratification / Vertical distribution

The vegetation profile (vertical) of the population showed the occurrence of major tree species such as *Hopea racophloea* Dyer, *Palaquium ellipticum* (Dalz.) Baill*, Calophyllum calaba*L., *Cullenia exarillata* Robyns*, Dysoxylum malabaricum*Bedd. ex Hiernetc. as first layer species reaching a height range of 26 to 35 m. The second layer is represented by *Aglaia barberi*, *Knema attenuate* (Hook. f. & Thoms.) Warb., *Artocarpus hirsutus*Lam.,*Prioria pinnatum*(Roxb. ex DC.) Harms, *Atuna indica* (Bedd.) Kosterm., *Diospyros bourdillonii* Brandis, *Cinnamomum malabatrum*(Burm. f.) Blume Third layer is occupied by *Baccaurea courtallensis* (Wight) Muell*,* small trees of *Dillenia pentagyna*Roxb., *Lagerstroemia microcarpa* Wight, and some individuals of *Atuna indica* (Fig. 10)with a height range of 6 to 15. *Humboldtia brunonis* Wall, *Ixora brachiata* Roxb. ex DC*., Lepisanthes sp., Meiogyne pannosa* (Dalz.) Sinclair*, Goniothalamus* sp., *etc.* are the major shrubby associates of the candidate species. The herb layer is composed of *Argostemma anupama* Sivar, *Begonia integrifolia* Dalz*., Globba* sp*., Rhynchotechum permolle* (Nees) Burtt*, Rhynchoglossum notonianum* (Wall.) Burtt etc **.,**

(c) Age distribution/ Ontogenic stages

The individuals of *A. indica* exhibited two age classes such as pre-reproductive and reproductive. The Population did not show flowering during the study period from 2017 to 2022. Hence the classification is solely based on the GBH. GBH ≥45 cm is treated as reproductive and below as pre-reproductive. Among thirty-eight individuals of the species presented on the site, thirty individuals represented the reproductive class eight individuals represented pre-reproductive.

(d) Crown projections

The vertical crown projections showed the placement of individuals such as *Bischofia javanica, Holigarna grahamii, Cullenia exarillata,*and *Atuna indica* just below the tallest individual of *Dipterocarpus indicus.* The horizontal crown projections displayed an overlapping of canopy coverage under the tallest individual*, Dipterocarpus indicus*. Interestingly, all the species in Nadugani showed better vertical growth. Except for *Cullenia exarillata,* all other trees have shown poor flowering behavior including *Atuna indica.*

(e) Abundance

The floristic diversity analysis covered individuals of 25 species with GBH ≥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 the highest IVI(0.2803) and thus became the dominant species in the particular quadrat, whereas, the *Atuna indica* represented 21st position with IVI of 0.0828 (Table 6).

Table 5. Population structure of *Atuna indica*: Nadugani

(List of individuals with G≥30 cm represented)

Sl. No.	GBH (cm)	Basal area (cm)	Basal cover (m)	Age phase	First branching seen at (m)	Height of stand (m)
1	82	538.9	5	Set of present	7.0	20.0
2	81	522.5	6	Set of present	6.0	24.0
3	73	422.5	4	Set of present	5.5	18.0
4	91	660.2	8	Set of present	2.8	22.0
5	77	475.1	5.5	Set of present	4.5	19.0
6	69	373.1	6.5	Set of present	2.0	21.0
7	39	116.8	1.5	Set of present	7.0	16.0
8	81	522.5	4.5	Set of present	4.5	22.0
9	58	265.8	5	Set of present	3.5	15.0
10	66	346.2	4.5	Set of present	6.5	23.0
11	73	422.5	5	Set of present	6.5	18.0
12	51	206	3	Set of present	2.6	13.0
13	73	422.5	4	Set of present	4.0	16.0
14	72	412.4	2	Set of present	3.5	19.0
15	32	81.7	1.5	Set of future	2.1	5.0
16	72	415.3	5	Set of present	6.2	23.0
17	42	162.8	2	Set of future	3.0	21.0
18	37	109.3	2	Set of future	2.0	18.0
19	61	295.4	3.5	Set of present	3.5	14.0
20	83	547.1	4	Set of present	5.2	23.0
21	61	295.4	4	Set of present	2.5	17.0
22	83	547.1	5	Set of present	17.0	19.0
23	102	834.3	5.5	Set of present	21.0	25.0
24	107	907.5	5.3	Set of present	20.0	24.0

25	41	132.7	2.3	Set of future	3.0	13.0
26	109	950.7	4.8	Set of present	13.0	24.0
27	82	530.7	4	Set of present	2.0	19.0
28	94	697.1	4	Set of present	7.0	19.0
29	86	589.3	3.8	Set of present	8.0	18.0
30	42	140.9	1.9	Set of future	4.0	13.0
31	93	687.8	3.1	Set of present	7.0	19.0
32	94	697.1	3.4	Set of present	9.0	18.5
33	67	352.8	2.3	Set of present	3.0	14.0
34	61	295.4	2.1	Set of present	2.0	15.0
35	41	132.7	1.7	Set of future	7.0	13.0
36	40	181.4	2.4	Set of future	6.0	13.0
37	43	145.2	1.3	Set of future	3.0	12.0
38	99	725.5	3.8	Set of present	2.0	17.0

Table 6. Floristic diversity / Important value index of *Atuna indica*: Nadugani

(List of individuals with G≥30cm represented)

Sl. No	Species	Family	rf	rd	rD	IVI
1.	*Hopea erosa* (Bedd.) van Sloot	Dipterocarpaceae	0.0356	0.1069	0.1377	0.2803
2.	*Cullenia exarillata* Robyns	Bombacaceae	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.	*Palaquium ellipticum* (Dalz.) Baill	Sapotaceae	0.0356	0.0776	0.0211	0.1344
8.	*Diospyros peregrine* (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.	*Cinnamomum malabatrum* (Burm. f.) Blume	Lauraceae	0.0345	0.0335	0.0511	0.1204
12.	*Artocarpus heterophyllus* Lam.	Moraceae	0.0457	0.0293	0.0532	0.1183

13.	*Mesua ferrea* L.	Clusiaceae	0.0356	0.0524	0.0237	0.1118
14.	*Persea macrantha*(Nees) Kosterm.	Lauraceae	0.0257	0.0335	0.0413	0.1105
15.	*Knema attenuate* (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.	*Dysoxylum malabaricum* 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.	*Terminalia bellirica* (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.	*Lagerstroemia microcarpa* Wight	Lythraceae	0.0358	0.0252	0.0197	0.0806
23.	*Syzygium laetum* (Buch.-Ham.) Gandhi	Myrtaceae	0.0294	0.0255	0.0043	0.0651
24.	*Macaranga peltata* (Roxb.) Muell.	Euphorbiaceae	0.0427	0.0084	0.0156	0.0597
25.	*Symplocos racemosa* Roxb.	Symplocaceae	0.0296	0.0084	0.0148	0.0589

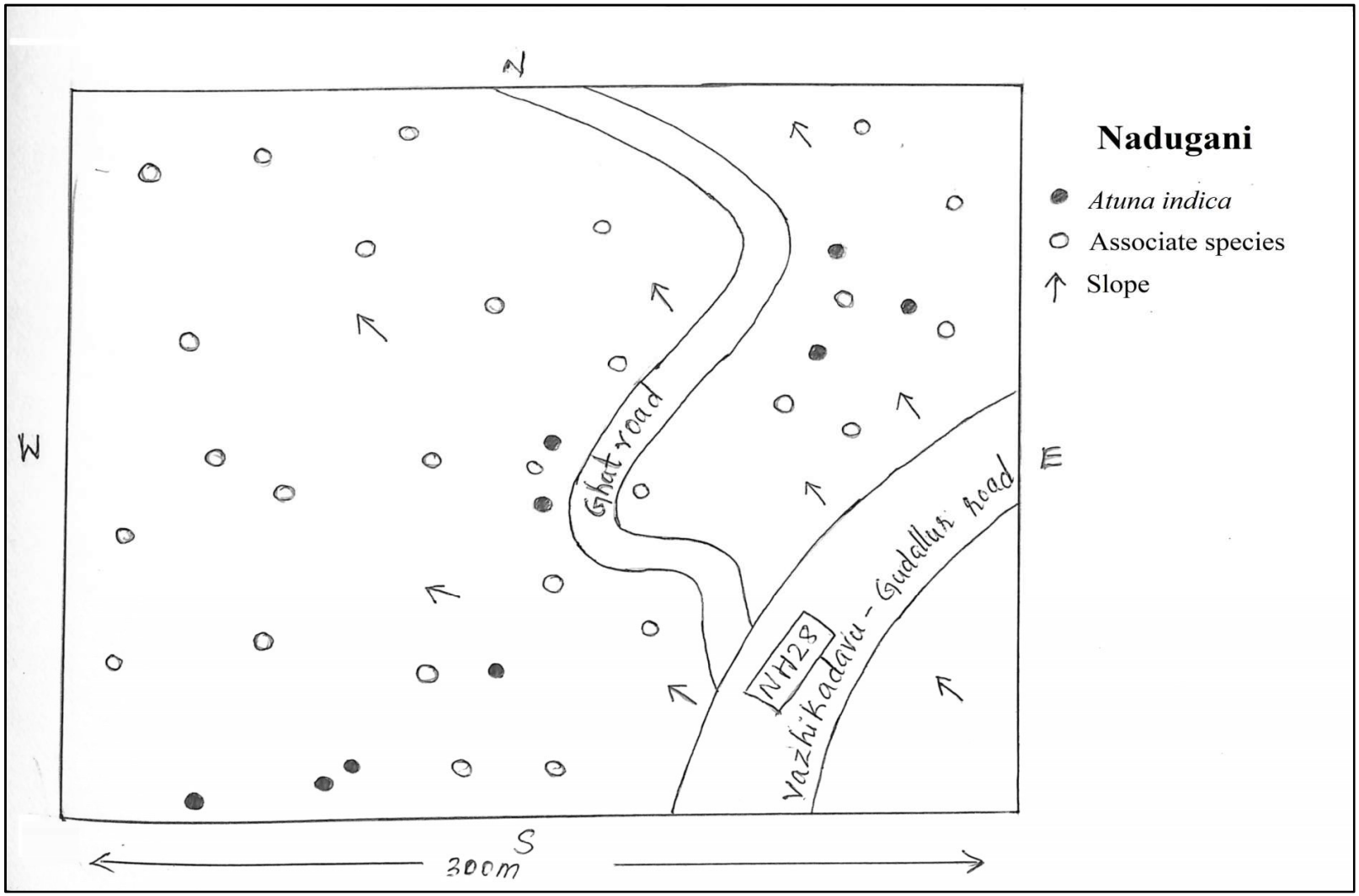

Figure 9: Spatial distribution map of *Atuna indica* at Nadugani population

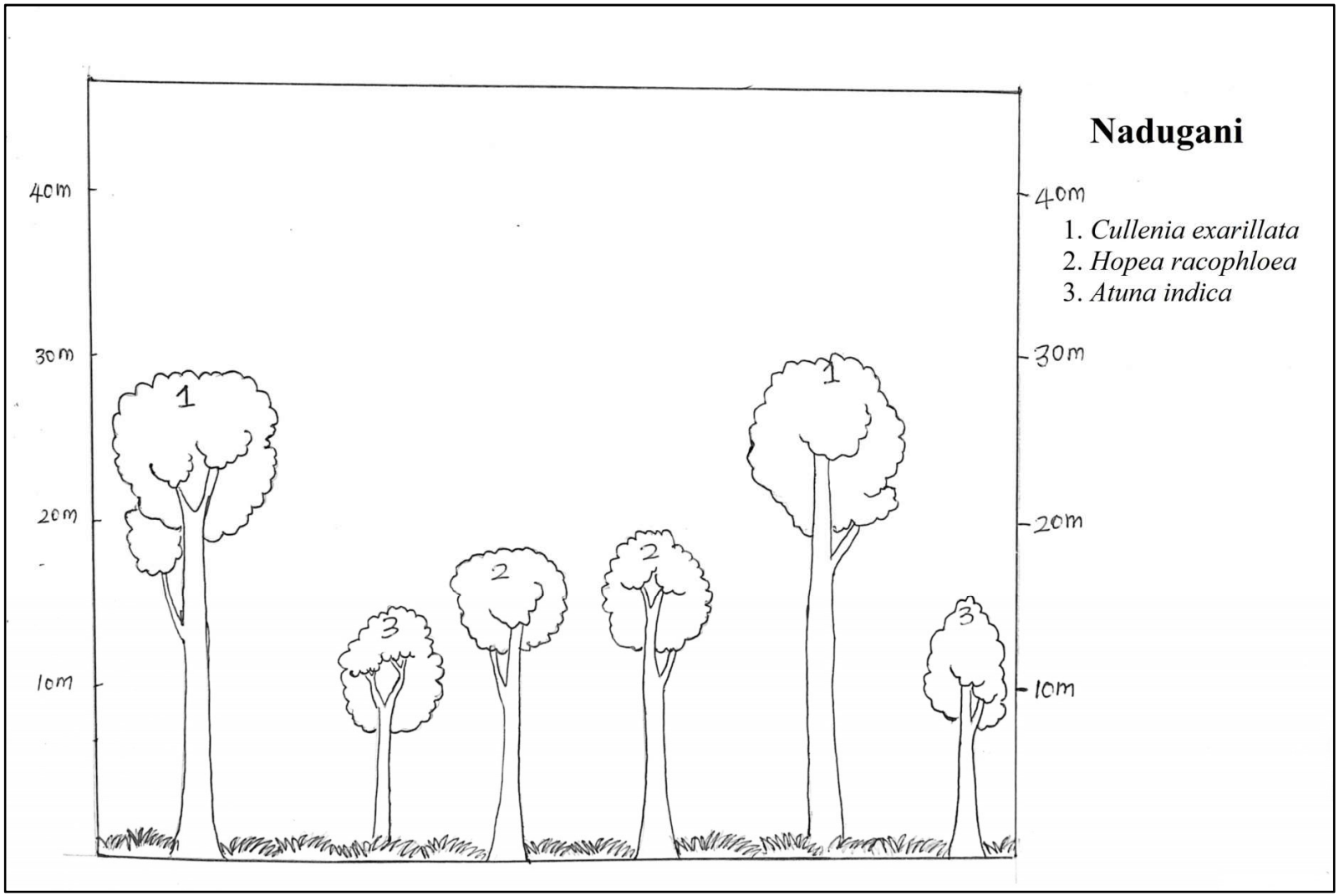

Figure 10: *Atuna indica* : Vertical distribution pattern at Nadugani population

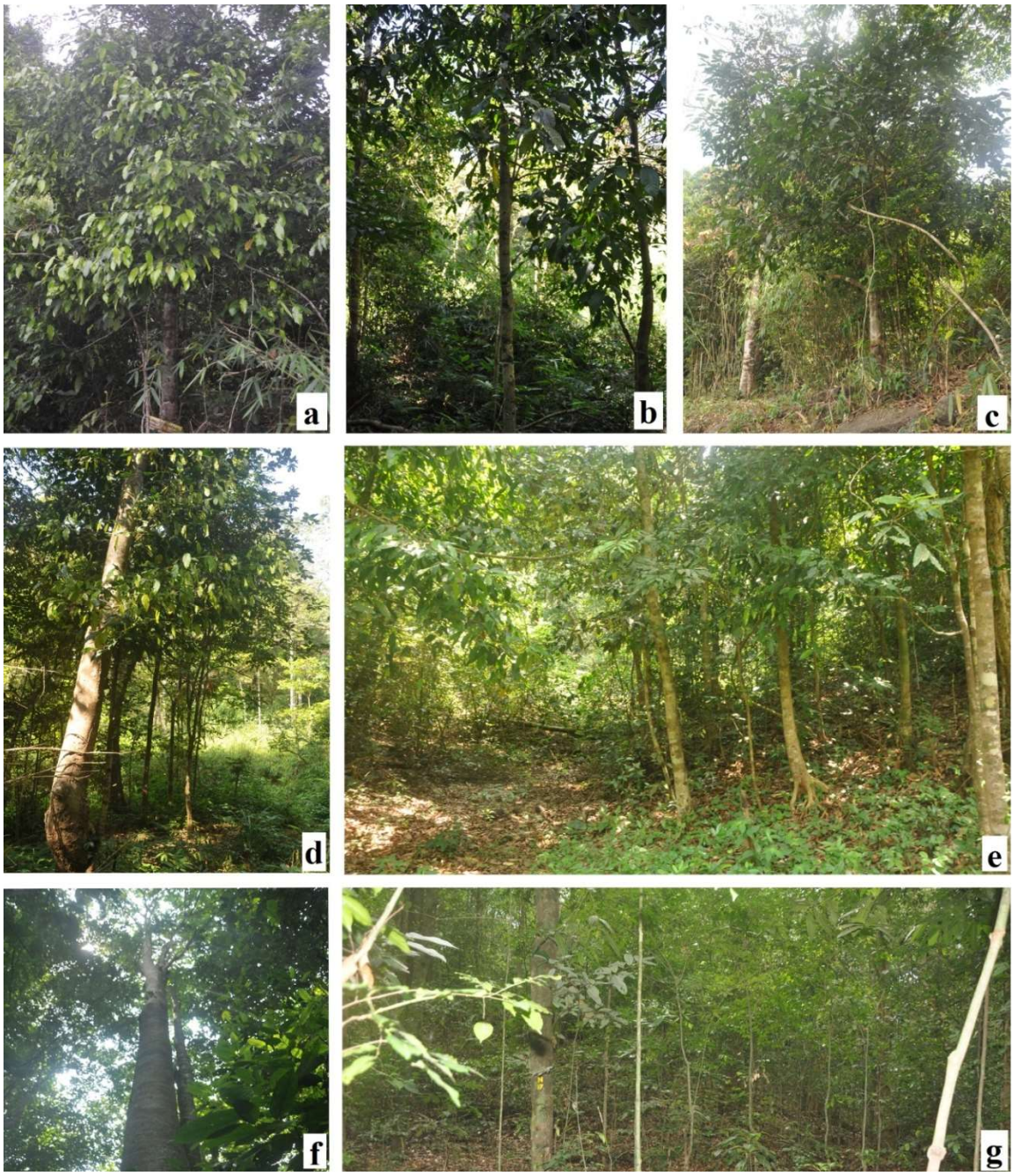

Figure 11. **Population structure of *Atuna indica*. a,b,c**- Views from the Kakkayam Dam site population, **d, e**- Views from the Charangad, **f,g**- Views from the Nadugani

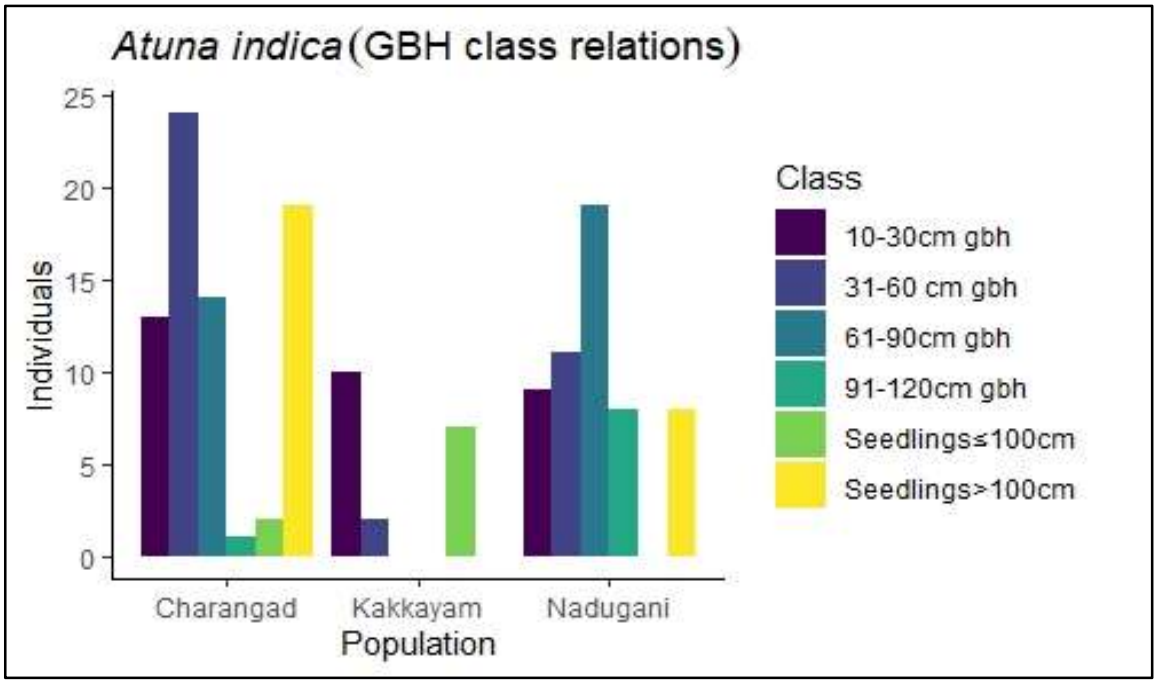

Figure 12. Demographic structure of *Atuna indica* in the three populations.

Hydnocarpus longipedunculatus

Seventy-six matured individuals (>30 cm GBH) and 11 saplings (10-30cm GBH range) of *Hydnocarpus longipedunculatus* were counted from the two populations. Fourteen seedlings above 100 cm in height were recorded and no seedlings were observed below 100 cm in height (Fig. 18).

Findings on the population structural studies of *Hydnocarpus longipedunculatus* in populations atKulamavu MPCA and Cheri are given below (Fig. 17- A view of populations).

Kulamavu MPCA

Thirty-six matured individuals along with five saplings were enumerated from the population (Table 7). In addition, 8 seedlings above 100 cm were also noted.

(a) Horizontal/Spatial distribution

The individuals of *H. longipedunculatus* showed clumped distribution pattern adjacent to the Idukki dam reservoir (Fig. 13).

(b) Stratification/ Vertical distribution

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 first layer species reaching a height range of 26 to 35 m. The second layer was represented by *Actinodaphne malabarica*, *Olea dioica*, *Calophyllum calaba*, *Poeciloneuron indicum, Aglaia barberi,*

Holigarna arnottiana, Madhuca longifolia, etc with a height range of 16-25 m. The third layer was occupied by *Baccaurea courtallensis***,** *Garcinia gummi-gutta*, *Melicope lunu-ankenda*, *Chionanthus courtallensis***,** *Litsea floribunda***,** along with the target species, *Hydnocarpus longipedunculatus* with 5-15 m height range. The herbaceous layer was mainly dominated by the seedlings of *Lepidagathis* sp.*, Gymnostachyum* sp.*,*etc. (Fig. 14).

(c) Age distribution/ Ontogenic stages

The population showed fairly good flowering behavior. The younger individuals which not showed flowering were treated as pre-reproductive. The number of pre-reproductive individuals and reproductive individuals is 10 and 26 respectively.

(d) Crown projection

The vertical crown projections showed the placement of individuals such as *Buchanania axillaris*, *Palaquium ellipticum***,** *Garcinia gummi-gutta,* and *Hydnocarpus longipedunculatus,* just below the tallest individual of *Calophyllum polyanthum.* The horizontal crown projections displayed the overlapping of these species under the canopy of the tallest individual*, Calophyllum polyanthum***.**

(e) Abundance/ Importance Value Index (IVI)

The floristic diversity analysis covered individuals of 49 species of GBH ≥ 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 community were worked out and noted that *Calophyllum polyanthum* had the highest IVI of 0.1909 and thus became the dominant species in the particular quadrat, whereas, *H. longipedunculatus* represented 33rd position with IVI of 0.0382 (Table 8)

Table 7. Population structure of *H. longipedunculatus*: Kulamavu MPCA

(List of individuals with G ≥ 30 cm represented)

Sl. No	GBH (cm)	Basal area (cm^2)	Basal cover (m)	Age phase	First branching (m)	Height of stand (m)
1.	35	98.5	4	Set of future	1.0	5.0
2.	30	45.3	2.5	Set of future	0.75	6.5
3.	55	243.2	3.5	Set of present	1.5	6.5
4.	43	147.3	4.5	Set of future	2.5	7.0
5.	88	615.4	6	Set of present	3.0	13
6.	118	1098	9	Set of present	0.5	17
7.	63	314	4.5	Set of present	2.5	9.0
8.	54	232.2	4	Set of present	2.5	13.0
9.	62	307.8	5	Set of present	2.0	14.0
10.	45	162.8	3.5	Set of present	1.0	6.0
11.	87	606.7	8	Set of present	3.0	13.0
12.	85	606.7	6	Set of present	2.5	12.0
13.	65	339.6	4	Set of present	1.5	10.0
14.	43	123.8	3.5	Set of future	1.5	9.0
15.	109	950.6	10.5	Set of present	0.5	18.0
16.	77	475.1	7.5	Set of present	1.5	12.0
17.	56	248.7	4.5	Set of present	3.5	11.0
18.	74	444.7	7	Set of present	4.5	13.0
19.	49	191	3.5	Set of future	0.5	8.0
20.	52	216.3	4	Set of present	1.5	8.0
21.	54	232.2	3.5	Set of present	2.0	14.0
22.	108	928.9	9	Set of present	1.o	17.0
23.	64	326.7	5	Set of present	3.0	10.0

24.	87	606.7	7	Set of present	1.0	12.0
25.	42	138.4	4.5	Set of future	2.0	7.0
26.	56	248.7	6	Set of present	1.5	13.0
27.	25	47.8	5	Set of future	3.0	6.0
28.	36	102	3	Set of future	4.0	7.0
29.	48	181.4	3.5	Set of future	1.0	7.0
30.	57	257.2	4	Set of present	0.5	8.0
31.	68	366.2	4.5	Set of present	2.0	10.0
32.	73	422.5	5	Set of present	1.0	13.0
33.	87	606.7	5.5	Set of present	1.5	13.0
34.	92	669.3	6.5	Set of present	1.5	14.0
35.	98	764.2	7	Set of present	8.0	16.0
36.	36	102	3.5	Set of future	0.5	6.0

Table 8. Floristic diversity / Importance value index of *H. longipedunculatus*: Kulamavu MPCA

(List of individuals with G ≥ 30cm represented)

Sl. No	Species	Family	rf (%)	rd (%)	rD	IVI
1	*Calophyllum polyanthum* 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	*Poeciloneuron indicum* Bedd.	Clusiaceae	0.0253	0.0378	0.0734	0.1366
4	*Persea macrantha* (Nees) Kosterm.	Lauraceae	0.0253	0.0268	0.0798	0.1319
5	*Syzygium cumini* (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	*Actinodaphne malabarica* Balakr.	Lauraceae	0.0253	0.0488	0.0433	0.1175
8	*Olea dioica* 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	*Knema attenuata* (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0253	0.0315	0.0251	0.0818
13	*Myristica malabarica* Lam.	Myristicaceae	0.0126	0.0189	0.0437	0.0753
14	*Aglaia elaeagnoidea* (A. Juss.) Benth.	Meliaceae	0.0253	0.0236	0.0232	0.0722
15	*Cinnamomum verum* Presl.	Lauraceae	0.0253	0.0205	0.0231	0.0689

16	*Cullenia exarillata* Robyns.	Bombacaceae	0.0126	0.0331	0.0210	0.0668
17	*Hopea parviflora* Bedd.	Dipterocarpaceae	0.0253	0.0315	0.0091	0.0659
18	*Carallia brachiata* (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	*Litsea bourdillonii* Gamble.	Lauraceae	0.0126	0.0094	0.0275	0.0496
23	*Aglaia barberi* Gamble.	Meliaceae	0.0253	0.0173	0.0059	0.0487
24	*Crataeva magna* (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	*Litsea floribunda* (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	*Chionanthus courtallensis* Bedd.	Oleaceae	0.0126	0.0205	0.0078	0.0409
30	*Holigarna arnottiana* Hook.	Anacardiaceae	0.0253	0.0126	0.0018	0.0398
31	*Schefflera capitata* (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	*Litsea glabrata* (Wall. ex Nees) Hook.	Lauraceae	0.0126	0.0189	0.0048	0.0364
36	*Mesua thwaitesii* Planch. & Triana	Clusiaceae	0.0253	0.0063	0.0042	0.0359
37	*Melicope lunu-ankenda* (Gaertn.) Hartley	Rutaceae	0.0126	0.0141	0.0073	0.0342
38	*Cinnamomum malabatrum* (Burm. f.) Blume	Lauraceae	0.0126	0.0126	0.0052	0.0304
39	*Garcinia gummi-gutta* (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	*Trichilia connaroides* (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	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	0.0126	0.0016	0.0014	0.0156

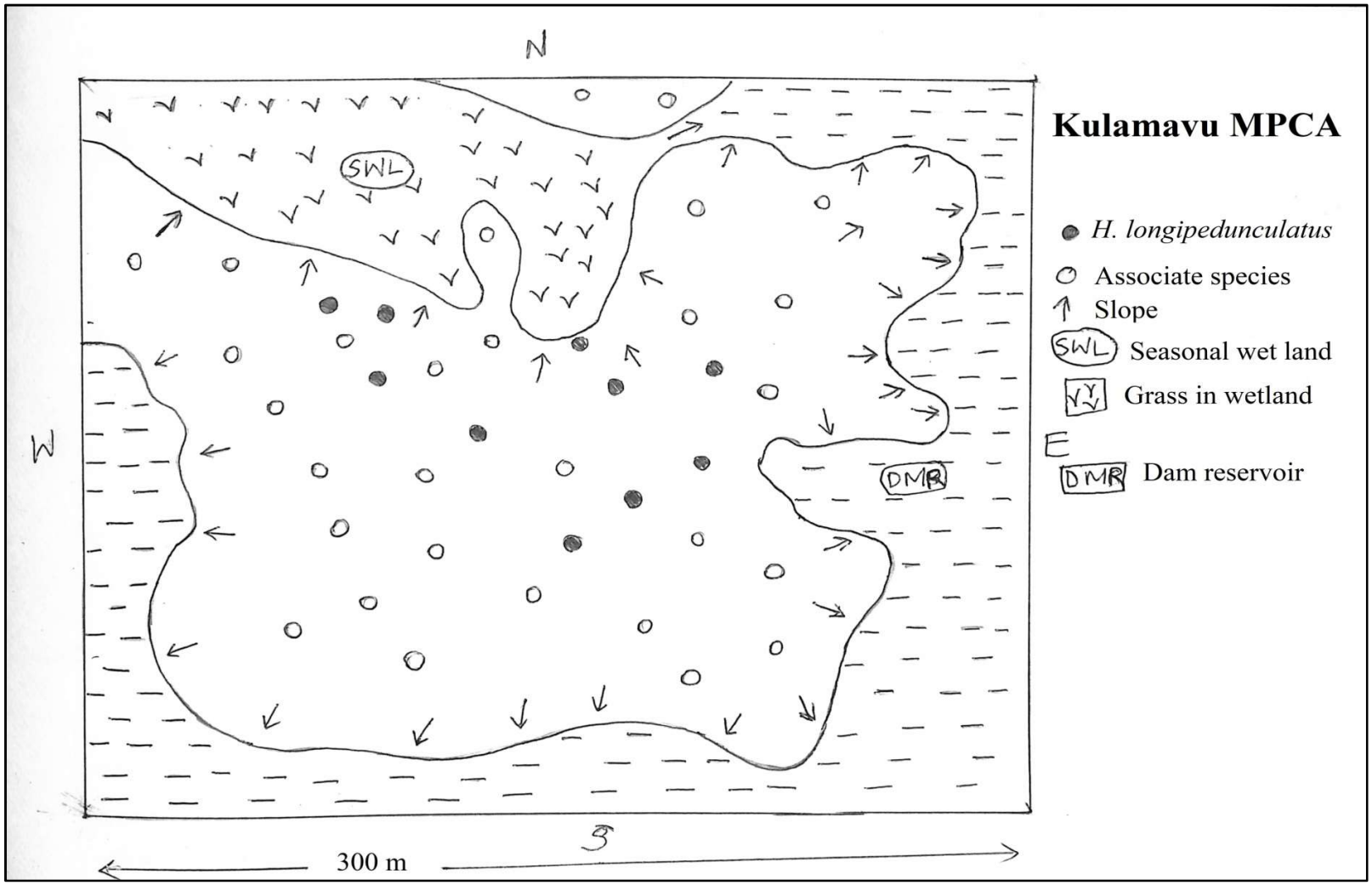

Figure 13. ***Hydnocarpus longipedunculatus*** **: Spatial distribution map pattern at Kulamavu MPCA population**

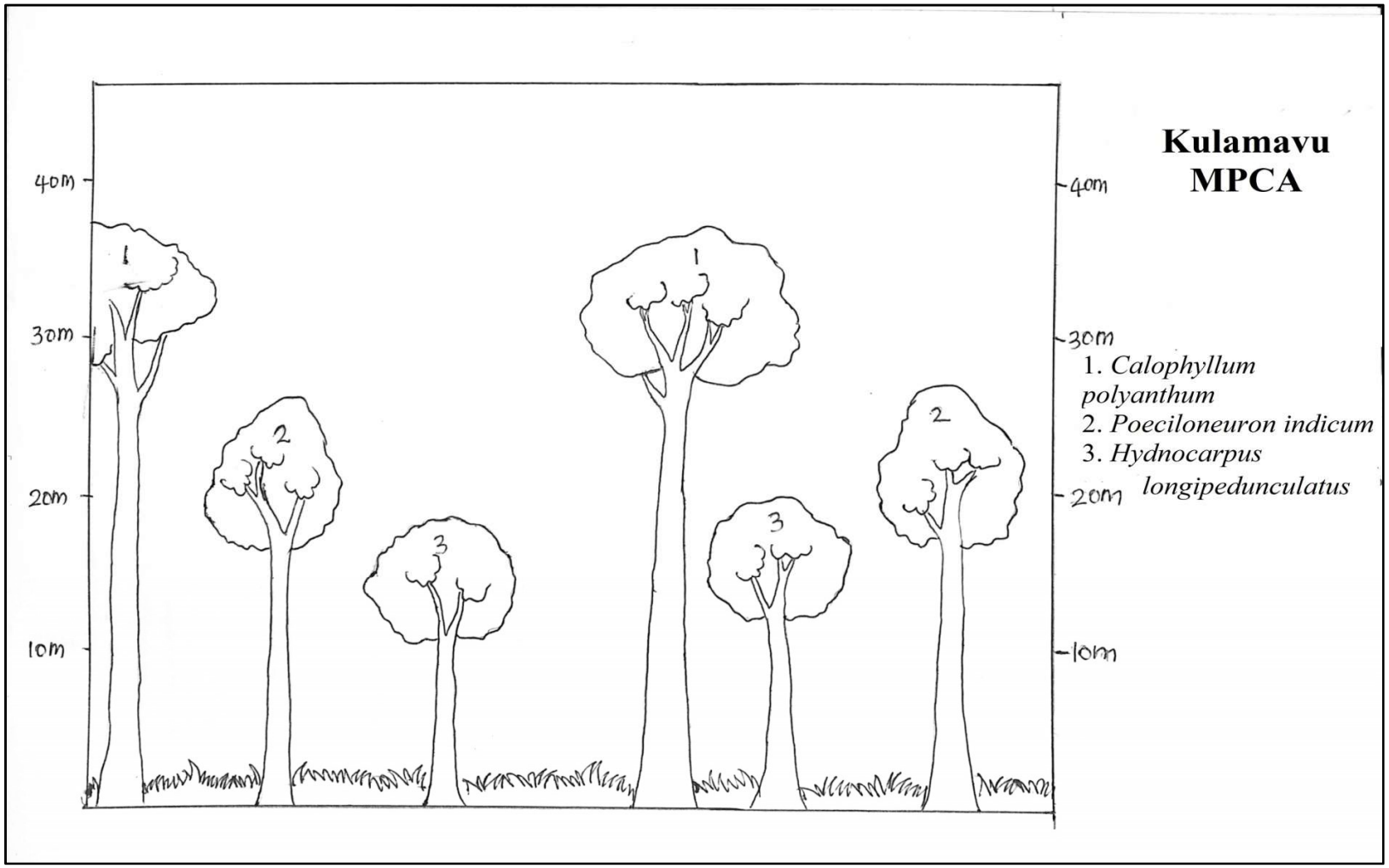

Figure 14: *Hydnocarpus longipedunculatus* : Vertical distribution pattern at Kulamavu MPCA population

Cheri

Forty matured individuals along with six saplings were enumerated from the population (Table 9). Six seedlings above 100 cm in height were also recorded.

(b) Horizontal/Spatial distribution

The individuals of *H. longipedunculatus* were distributed in a clumped manner in deep eastern-facing slopes. A small rivulet is seen on the downside of the slope (Fig. 15).

(a) Stratification/ Vertical distribution

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 first layer species reaching a height range of 26 to 35 m. The second layer specieswere represented by *Drypetes malabarica***,** *Dimocarpus longan***,** *Olea dioica*, *Carallia brachiata***,** *Knema attenuata*, *Myristica malabarica*along with *Hydnocarpus longipedunculatus* with a height range of 16-25 m. Third layer was occupied by *Baccaurea courtallensis***,** *Symplocos racemosa*, *Melicope lunu-ankenda*, *Vitex altissima***,** *Litsea glabrata***,** *Cinnamomum malabatrum***,** *Aporosa cardiosperma***,** and small individuals of *Hydnocarpus longipedunculatus* with 5-15 m height range. The herbaceous layer was mainly dominated by *Strobilanthes aurita*, *Justicia sp., Ophiorriza mungos, Mycetia acuminata,* etc. (Fig. 16).

(c) Age distribution/ Ontogenic stages

The population exhibited good flowering behavior. The younger individuals without flowering were treated as pre-reproductive. The number of pre-reproductive individuals and reproductive individuals is 8 and 32 respectively.

(d) Crown projection

The vertical crown projections showed the placement of individuals such as *Actinodaphnae malabarica*, *Knema attenuata***,** *Palaquium ellipticum***,** *Symplocos racemosa,*and *Hydnocarpus longipedunculatus,*just below the tallest individual of *Calophyllum polyanthum.* The horizontal crown projections displayed overlapping of these species under the tallest canopy of *Calophyllum polyanthum.*

(e) Abundance/ Importance Value Index (IVI)

The floristic diversity analysis covered individuals of 41 species of GBH ≥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 community were worked out and noted that *Calophyllum polyanthum* having the highest IVI of 0.1944 and thus became the dominant species in the particular quadrant, whereas, *H. longipedunculatus* represented 30^{th} position with IVI of 0.0480 (Table 10).

Table 9. Population structure of *H. longipedunculatus*: Cheri

(List of individuals with G ≥ 30cm represented)

Sl. No	GBH (cm)	Basal area (cm^2)	Basal cover (m)	Age phase	First branching (m)	Height of stand (m)
1.	62	307.8	4	Set of present	2.0	14.0
2.	45	162.8	3	Set of future	1.0	6.0
3.	109	950.7	6	Set of present	4.5	18.0
4.	50	195.9	3	Set of future	2.0	8.0
5.	47	176.6	3.5	Set of future	2.5	5.0
6.	140	1561.5	11	Set of present	3.0	20.0
7.	102	824	4	Set of present	6.5	16.0
8.	138	1505.9	10	Set of present	3.5	17.0
9.	124	1218.6	8.5	Set of present	2.5	17.0
10.	118	1109.8	7	Set of present	7.0	16 .0
11.	45	162.8	2.5	Set of future	6.0	8.0
12.	48	171.4	3	Set of future	5.0	8.0
13.	67	359.5	3.5	Set of present	3.0	9.0
14.	79	498.5	4.5	Set of present	2.5	10.0
15.	92	669.3	5	Set of present	2.5	11.5
16.	98	764.2	5	Set of present	3.0	12.0
17.	103	844.5	5.5	Set of present	1.0	12.0
18.	107	907.5	6	Set of present	1.5	13.0
19.	108	928.9	7	Set of present	4.0	13.0
20.	112	994.9	7.5	Set of present	6.0	14.0
21.	55	243.2	4	Set of present	2.0	7.0
22.	59	277.5	4.5	Set of present	3.5	8.0

23.	68	366.2	5	Set of present	1.5	9.0
24.	74	437.2	6	Set of present	2.0	9.0
25.	83	547.1	6	Set of present	3.5	9.5
26.	87	606.7	7	Set of present	4.0	12.0
27.	88	615.4	6	Set of present	3.0	10.0
28.	96	735	7	Set of present	2.0	12.0
29.	103	844.5	8.5	Set of present	6.0	15.0
30.	112	994.8	105	Set of present	6.0	17.0
31.	130	1345.5	12	Set of present	7.5	19.0
32.	42	140.9	3	Set of future	2.0	6.0
33.	44	153.7	3.5	Set of present	3.0	8.0
34.	48	183.4	4	Set of present	4.5	8.0
35.	68	366.2	5	Set of present	2.5	7.0
36.	74	437.2	5.5	Set of present	3.0	7.5
37.	42	140.9	4	Set of future	3.0	7.0
38.	49	191	5	Set of future	2.0	7.5
39.	74	437.2	6	Set of present	3.5	9.0
40.	78	51.3	6.5	Set of present	2.5	11.0

Table 10. Floristic diversity / Importance Value Index of *H. longipedunculatus*: Cheri

(List of individuals with G ≥ 30cm represented)

Sl. No	Species	Family	rf (%)	rd (%)	rD (%)	IVI
1.	*Calophyllum polyanthum* Wall. ex Choisy	Clusiaceae	0.0248	0.0289	0.1417	0.1944
2.	*Calophyllum calaba* L.	Clusiaceae	0.0247	0.0193	0.1123	0.1554
3.	*Poeciloneuron indicum* Bedd.	Clusiaceae	0.0247	0.0289	0.0909	0.1436
4.	*Persea macrantha* (Nees) Kosterm.	Lauraceae	0.0246	0.0385	0.0778	0.1401
5.	*Garcinia gummi-gutta* (L.) Robs.	Clusiaceae	0.0245	0.0963	0.0099	0.1299
6.	*Buchanania axillaris* (Desr.) Ramamoorthy	Anacardiaceae	0.0244	0.0385	0.0639	0.1263
7.	*Actinodaphne malabarica* Balakr.	Lauraceae	0.0243	0.0482	0.0411	0.1131
8.	*Olea dioica* Roxb.	Oleaceae	0.0242	0.0561	0.0289	0.1089
9.	*Vateria indica* L.	Dipterocarpaceae	0.0241	0.0193	0.0610	0.1041
10.	*Xanthophyllum arnottianum* Wight	Polygalaceae	0.0240	0.0642	0.0101	0.0981
11.	*Palaquium ellipticum* (Dalz.) Baill.	Sapotaceae	0.0239	0.0193	0.0451	0.0881
12.	*Knema attenuate* (Hook. f. & Thoms.) Warb.	Myristicaceae	0.0239	0.0337	0.0278	0.0853
13.	*Myristica malabarica* Lam.	Myristicaceae	0.0238	0.0289	0.0309	0.0836
14.	*Cinnamomum verum* Presl.	Lauraceae	0.0238	0.0241	0.0211	0.0690
15.	*Hopea parviflora* Bedd.	Dipterocarpaceae	0.0238	0.0225	0.0218	0.0682

16.	*Cullenia exarillata* Robyns.	Bombacaceae	0.0238	0.0112	0.0326	0.0676
17.	*Carallia brachiata* (Lour.) Merr.	Rhizhophoraceae	0.0238	0.0337	0.0099	0.0674
18.	*Myristica beddomei* King	Mristicaceae	0.0237	0.0193	0.0181	0.0612
19.	*Meliosma pinnata* (Roxb.) Maxim.	Sabiaceae	0.0237	0.0193	0.0172	0.0603
20.	*Dimocarpus longan* Lour.	Sapindaceae	0.0237	0.0257	0.0084	0.0579
21.	*Litsea bourdillonii* Gamble	Lauraceae	0.0237	0.0241	0.0098	0.0576
22.	*Aglaia barberi* Gamble	Meliaceae	0.0237	0.0241	0.0094	0.0573
23.	*Bischofia javanica* Blume	Euphorbiaceae	0.0237	0.0225	0.0088	0.0551
24.	*Litsea floribunda* (Blume) Gamble	Lauraceae	0.0237	0.0225	0.0087	0.0549
25.	*Vitex altissima* L.	Verbenaceae	0.0235	0.0193	0.0114	0.0545
26.	*Schleichera oleosa* (Lour.) Oken	Sapindaceae	0.0235	0.0225	0.0082	0.0544
27.	*Holigarna arnottiana* Hook.	Anacardiaceae	0.0235	0.0128	0.0142	0.0509
28.	*Schefflera capitata* (Wight & Arn.) Harms	Araliaceae	0.0234	0.0225	0.0039	0.0503
29.	*Symplocos racemosa* Roxb.	Symplocaceae	0.0233	0.0209	0.0038	0.0485
30.	*Hydnocarpus longipedunculatus* Robi *et al.*	Flacourtiaceae	0.0232	0.0193	0.0049	0.0480
31.	*Litsea glabrata* (Wall. ex Nees) Hook.	Lauraceae	0.0230	0.0161	0.0057	0.0456
32.	*Mesua thwaitesii* Planch. & Triana.	Clusiaceae	0.0228	0.0161	0.0025	0.0424

33.	*Melicope lunu-ankenda* (Gaertn.) Hartley.	Rutaceae	0.0227	0.0128	0.0053	0.0419
34.	*Cinnamomum malabatrum* (Burm. f.) Blume	Lauraceae	0.0225	0.0112	0.0068	0.0418
35.	*Aporosa cardiosperma* (Gaertn.) Merr.	Euphorbiaceae	0.0224	0.0128	0.0046	0.0412
36.	*Baccaurea courtallensis* (Wight) Muell.	Euphorbiaceae	0.0222	0.0128	0.0018	0.0384
37.	*Artocarpus heterophyllus* Lam.	Moraceae	0.0221	0.0080	0.0059	0.0378
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* (Bedd.) Airy Shaw.	Euphorbiaceae	0.0218	0.0048	0.0015	0.0301
41.	*Lagerstroemia speciosa* (L.) Pers.	Lytheraceae	0.0217	0.0032	0.0010	0.0281

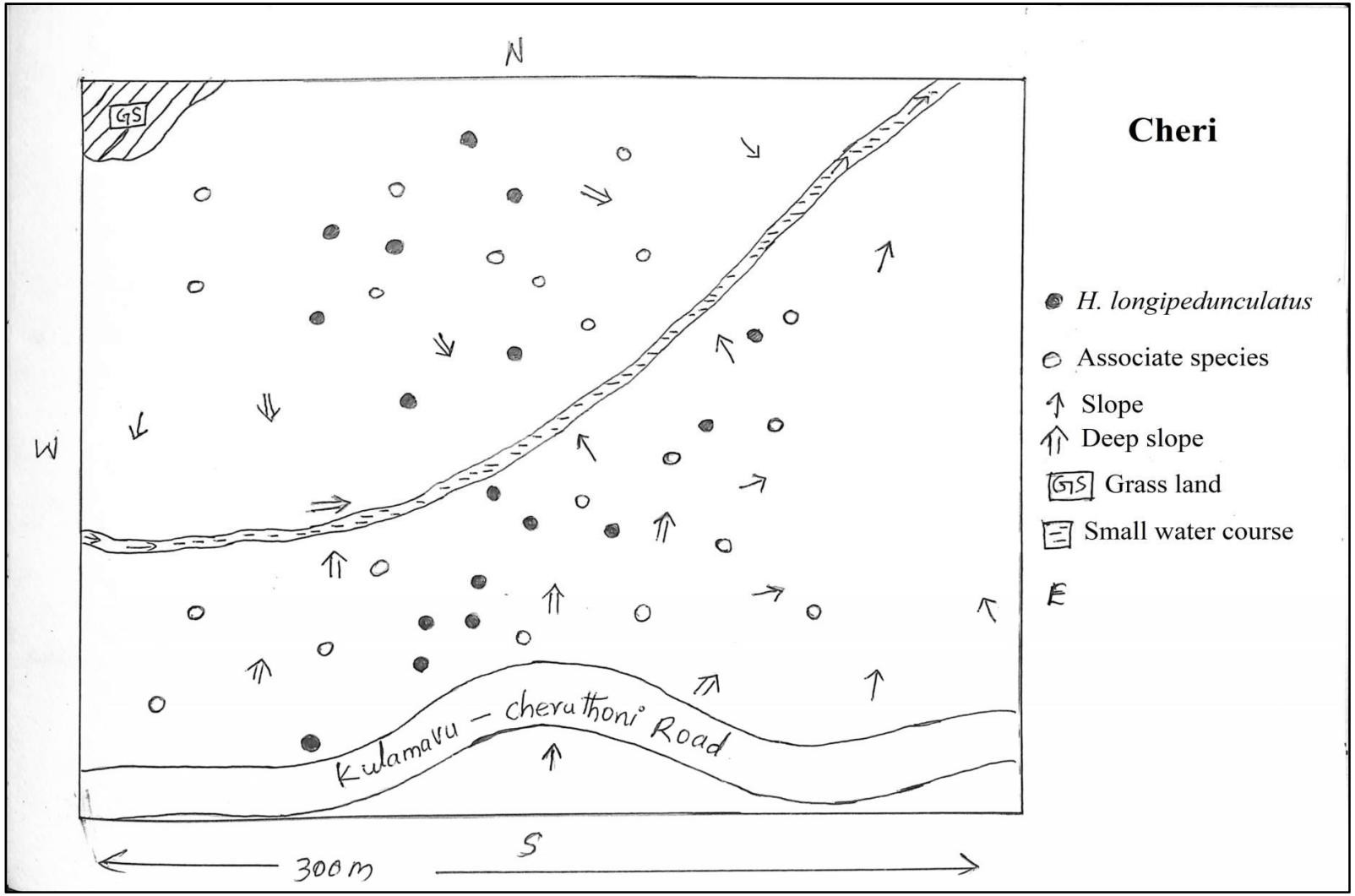

Figure 15: *Hydnocarpus longipedunculatus* : Spatial distribution pattern at Cheri population

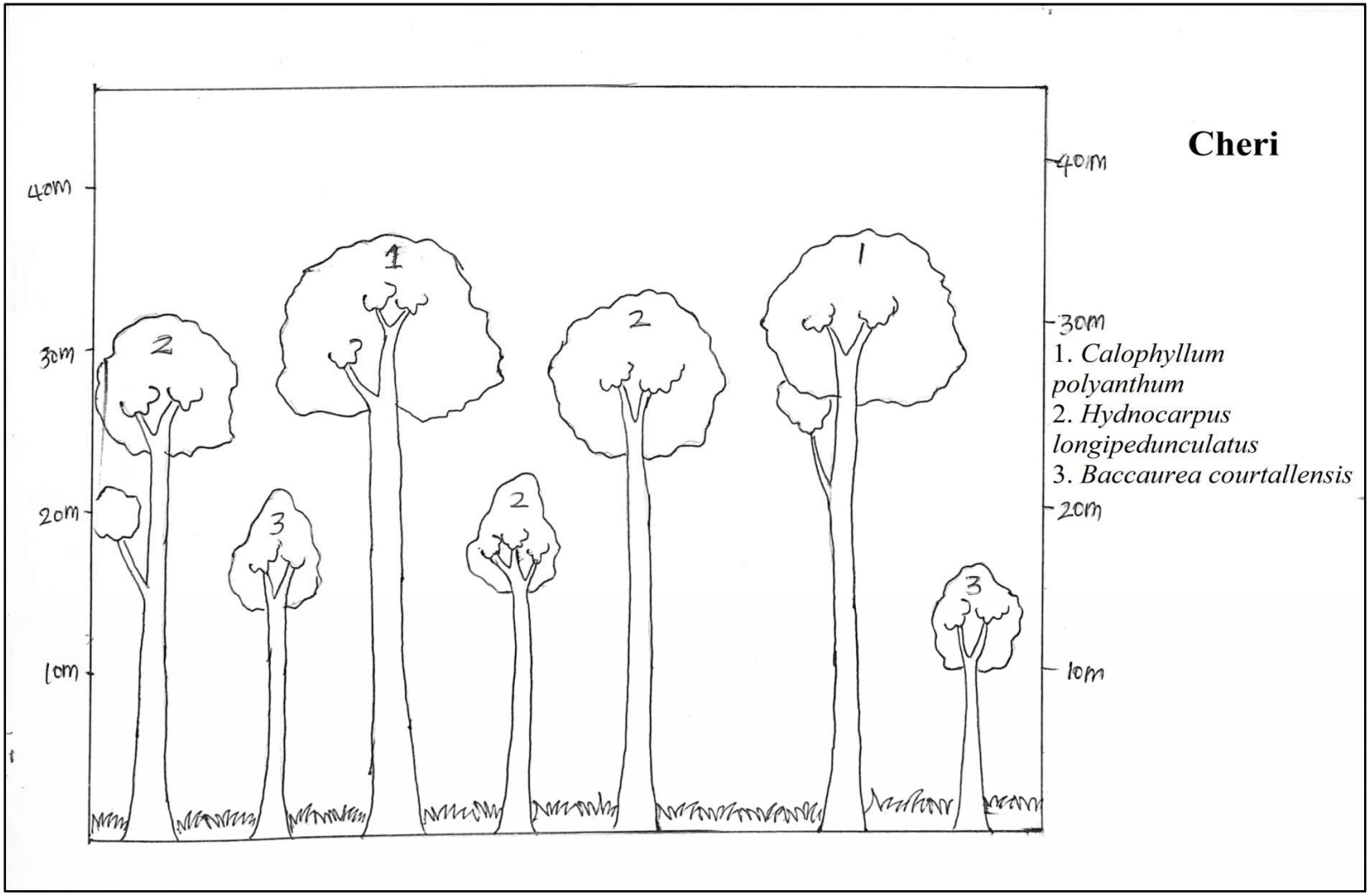

Figure 16: *Hydnocarpus longipedunculatus* : Vertical distribution pattern at Cheri population

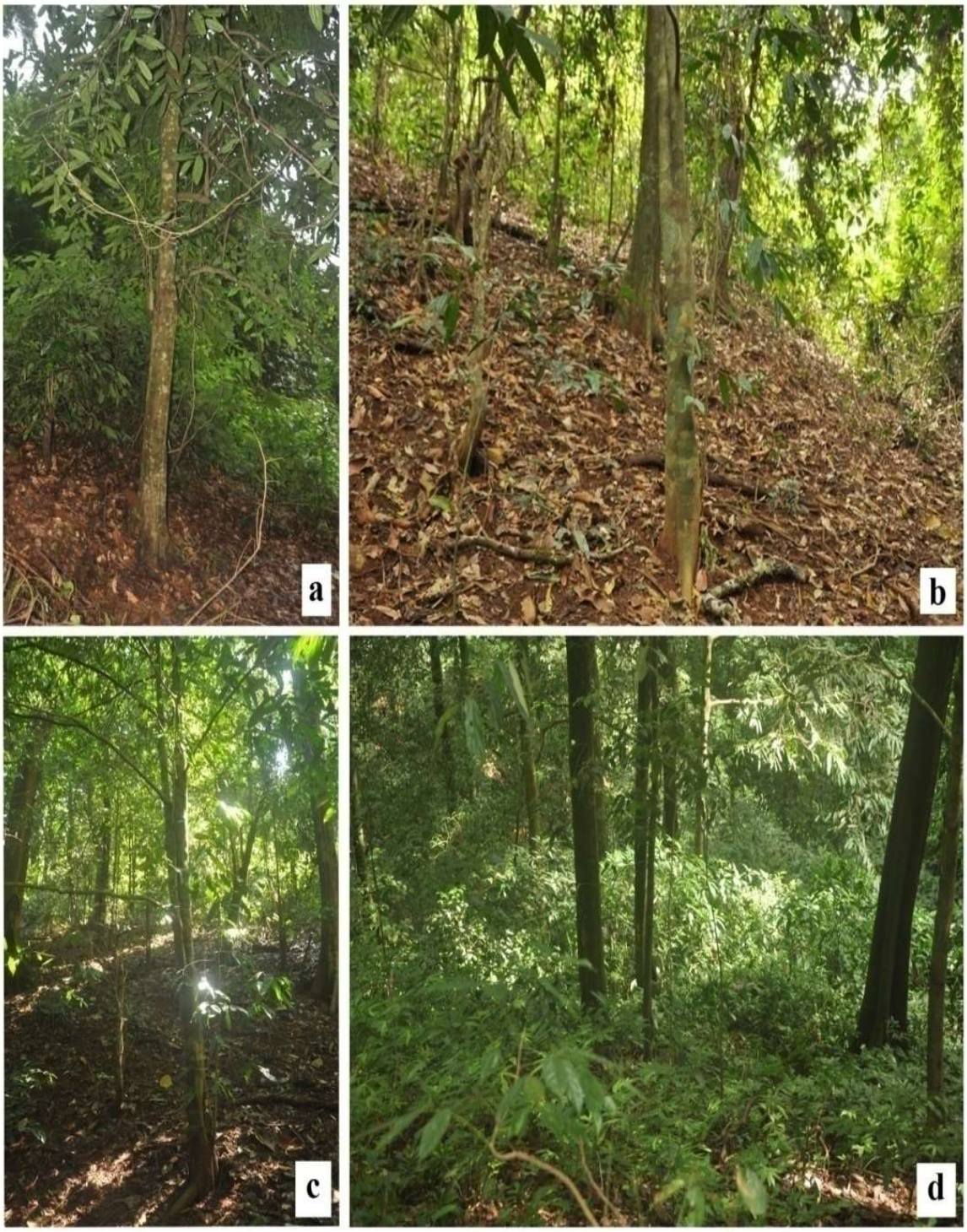

Figure 17: *Hydnocarpus longipedunculatus* View of populations. a & b – Kulamavu MPCA; c & d- Cheri

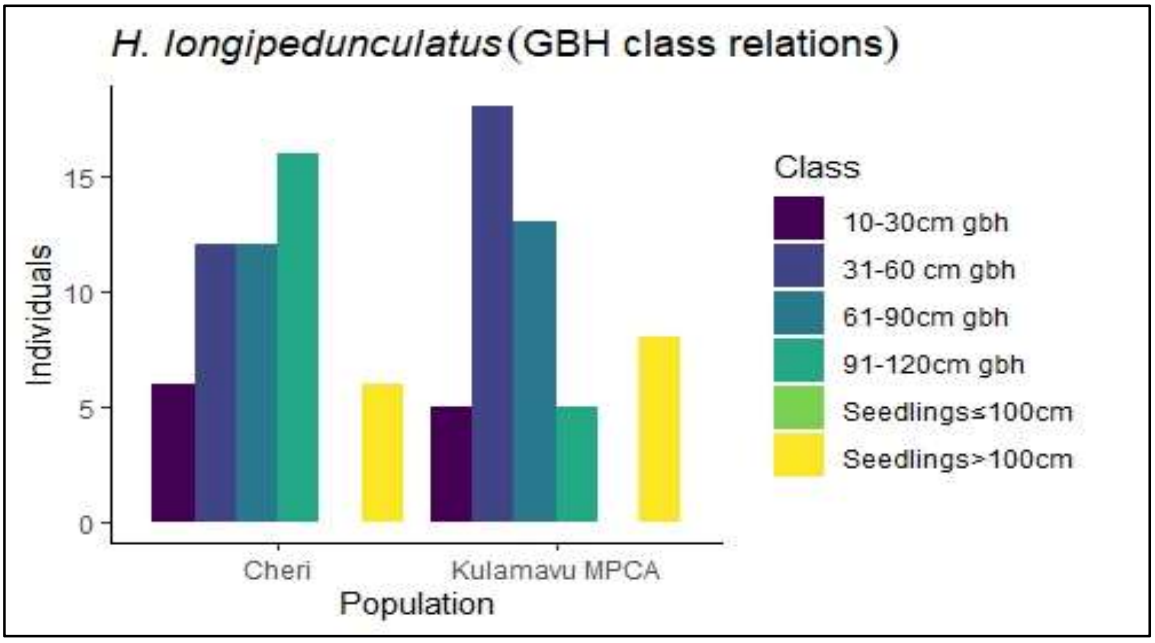

Figure 18: Demographic structure of *Hydnocarpus longipedunculatus* in the Western Ghats, India

3.3.3. Population dynamics

Atuna indica

Flowering was observed along with flushing from October – December months. Fruit development started in January and fruits matured in April. The trees displayed a vegetative phase from May to September (Fig. 19 & 20). Only one tree out of 79 trees among the three populations showed flowering. A total of 13 branches showed flowering which included 113 inflorescences with 521 flowers.

Reproductive phenology

Pale green-colored flower buds were recorded during the first week of October and mass blooming was noted after two weeks in the year, 2017 (The tree showed differential flowering behavior in different branches, in 2018, the North-eastern, sun-facing branch flowered first, and after two to three weeks the opposite branches were flowered). Flower opening started from 0600 to 0615 and fully opened by 0915. Anther dehisced through the vertical slit from 0900-0930. Stigma was found receptive before the anther dehiscence (0800-0830, protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis- 0615) showed 98.32% viability and gradual reduction was noticed to 93.6, 91.3, 88, and 87.5% after 1, 2, 3, and 4 hours respectively. A drastic decline to 20% was noted after 12 hours. The hydrogen peroxide application followed by effervescence formation confirmed the stigma receptivity up to 14 hours, later turned brown-black color, loses turgidity, and becomes non-receptive.

Pollen germination

At the time of anthesis, 58% of pollen grains were found germinated in 10% sucrose solution. A gradual decrease in pollen germination was observed to 47.8 % 23.2 %, and 9.74 % after 1, 2, and 8 hours respectively.

Pollen - Ovule ratio

A flower contains 12-13 anthers and approximately 288± 51.3 pollen grains were present per anther. Hence, the pollen count per flower was calculated as 3744± 667. A flower has two ovules and hence the pollen-ovule ratio was calculated as 1872:1.

Pollination and insect interaction

The bagging experiment was found to be a failure; all the bagged inflorescences had fallen off. Pollinator documentation was done in 2017 and 2019. Observations were made from 0500 to 1800 continuously. During night hours, observations were made in one-hour intervals to record the nocturnal visitors. *Xylocopa* sp. was the first visitor at 0600-0700 (2 times). 0800-0900 is the peak time of pollinators, *Apis mellifera, Idea malabarica, Eurema* sp*., Papilio polymnster, Euploea core, Graphium* sp*.,* are the key visitors, and they recorded in multiple intervals (Fig.21& 22). No flower visitors were recorded between 1100 to 1500. *Xylocopa* sp. was found foraging from 1500 to 1700. In 2019, *Macroglossum stelletarum* was found as a frequent visitor during flowering which wasn't recorded back in 2017. *Xylocopa* sp., *Macroglossum stelletarum,* and *Apis mellifera* were found visiting many flowers one by one, spending two to three seconds per flower. Butterflies found forage flowers and cover two to three flowers per visit. Mouth parts of pollinators collected from the study site were observed through a compound microscope and photos were taken

with Scanning Electron Microscope, the images were compared with SEM images of *A. indica* pollen grains and confirmed the role as a pollinator (Fig.21 D-J). Herds of monkeys were visited during the flowering season and their jumping and movements in flowering branches caused flower fall. Inflorescence fall was recorded and the fallen inflorescence was observed with larvae (unidentified). Cut-opened fruits were found with Pyralidae larvae which caused 30-40% fruit loss (Fig. 21). The adults possibly lay eggs in the late flowering stage.

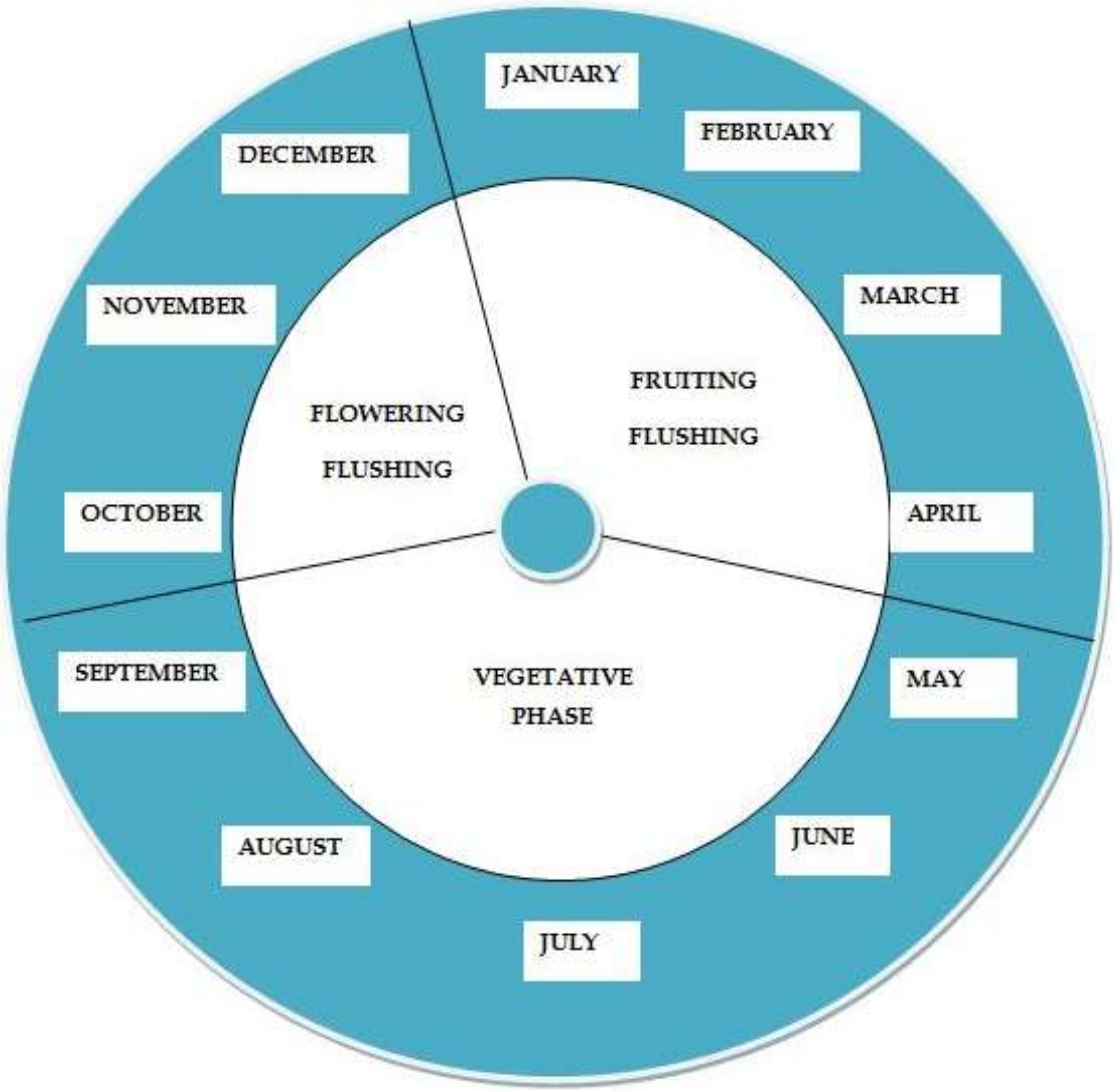

Figure 19: *Atuna indica*: Phenology calendar

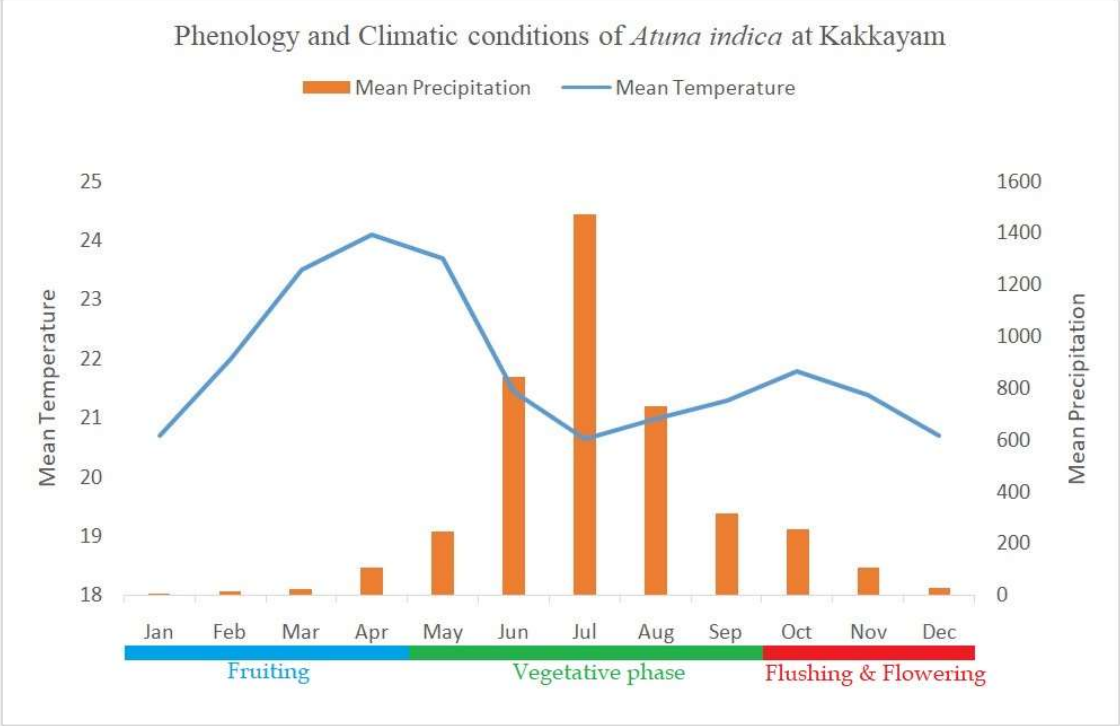

Figure 20: *Atuna indica* : Phenology with respect to climatic conditions at Kakkayam Dam site.

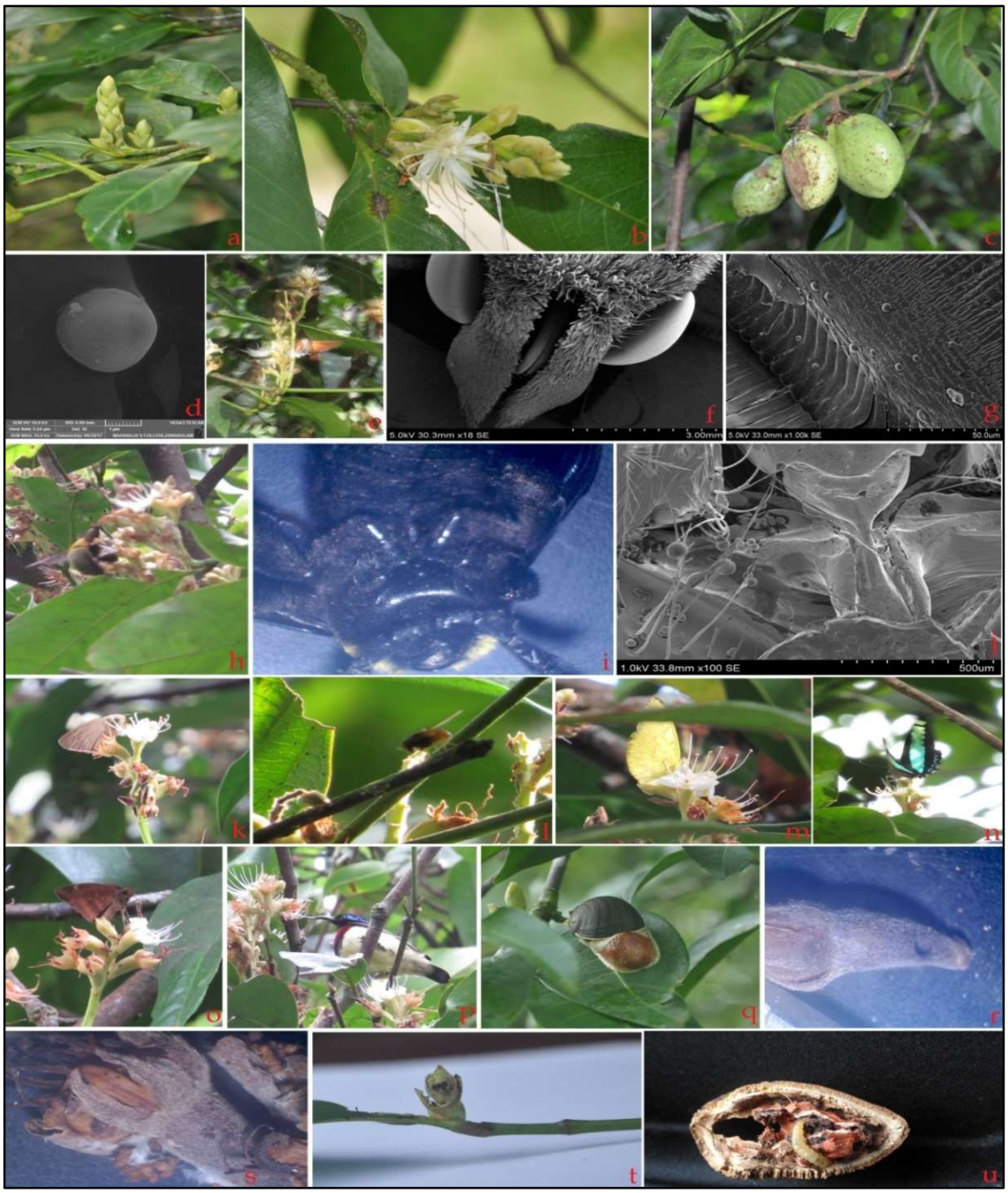

Figure 21: Reproductive biology: *Atuna indica*. a- Flower buds, b- Flower, c- Fruits, d- Pollen grain, e,f,g- Humming bird hawk moth, mouth parts, SEM image of mouth parts showing pollengrains, h, i, j- *Xylocopa* sp., Mouth parts, SEM image of mouth parts, k- *Badamia* sp., l- *Apies mellifera*, m- *Eurema* sp., n- *Graphium* sp., o- Unidentified, p- Sunbird, q- *Indrella ampula,* r-Pierced flower bud, s- Pyralid larvae in flower, t-flower bud eaten by snail, u- Pyralid larvae in fruit

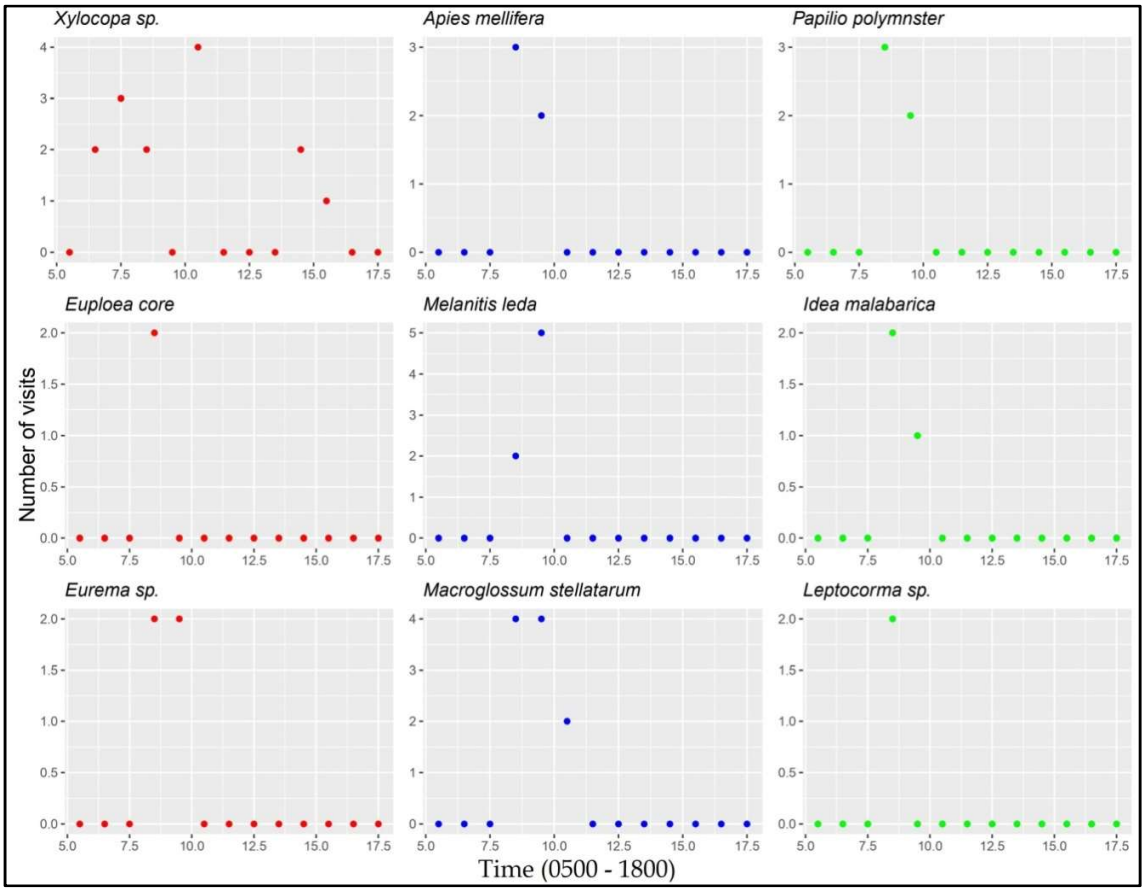

Figure 22: *A. indica*: Insect visitors during flowering from 0500 to 1800

Hydnocarpus longipedunculatus

Phenology

Leaf flushing was observed along with flowering from December to February months during the years from 2016 to 2019. Fruiting primordia appeared in March and ripened fruits were recorded after six to eight months (Fig. 22 & 23), from August-October. Trees were in the vegetative phase from September to November months. Generally, the trees are distributed as under-canopy species in the study site. The majority of the individuals showed synchrony in phenological changes except for one tree grown in a highly insolated area. The individual tree showed irregular leaf flushing, and two floweringand fruiting seasons within a calendar year (in 2017).

Reproductive biology

The species is monoecious, with male flowers without pistils and female flowers with non-functional anthers (staminodes). The pistil is 10 mm in length and appears similar to a fruiting primordial (Fig. 25-b). Both male and female flowers are drooping in nature and the numbers of female flowers are fewer than the male flowers. The flower opens at 0200 and is completely open by 0600 (repeated data recorded on two consecutive days). The pollen grains are in globose shape, with a length of 32.5±2.08 µm, a breadth of 27.5 ± 2.06 µm, and reticulate exine ornamentation (Fig. 25-d). The pollen viability at 0600 was 97% and decreased gradually and was found nonviable after 36 hours. Pollen germination was 83.33% at the time of blooming and decreased gradually, ending after 36 hours. The Anther dehiscence by forming longitudinal slits, each flower has five anthers and four to five seeds per fruit. One anther has 364±21 pollen grains, hence the pollen-

ovule ratio was calculated to364:1. The stigma is receptive up to 36- 48 hours, and the receptivity stigma was shiny and light green. Detailed studies are restricted because the number of female flowers is very low and most of them are infected with Cecidomyiidae larvae.

Pollinator incidence was less at the initial hours of anthesis (0200-0600). *Cirrochroa* sp., *Xylocopa* sp., *Apis mellifera*, *Eurema* sp., and Wasps, were frequently visited the tree from 0600 to 1000 hours. *Xylocopa* sp. and *A. mellifera* were found foraging more often. *Cirrochroathais* visited as a flock of 5-10 individuals and were found browsing each flowering tree during 1000-1100 hours. *Cirrochroathais* showed high activity when sunlight hit the forest floor (1100- 1400). *Leptosia* sp. and *Campanotus* sp. were visiting flowers at 1800-1900 hours. Fruiting primordia appear in March and fruits mature after six-eight months of development.

Developing fruits showed vertical slits on the surface however on the course of maturity slits were found faded (Fig. 25-e). . (Fig. 25-j). The seeds showed dormancy on sowing because the thick and white seed coat delayed the germination process. However, the removal of the seed coat resulted in cent percent germination.

Biotic constraints

The larvae of *Cirrochoroa thais* were recorded during the flowering period of the trees, in December 2016. They were found to consume the flushing leaves (Fig. 26- a). The flower buds were also found eaten (Fig. 26-b) by *C. thais* larvae or land snails.

Cecidomyiidae larvae/Gall midges (adult to be identified; Arthropoda/ Diptera) were found damaging the stigma and petals of female flowers leading to the fusing of petal tips (Fig. 26-c&d), further resulting in nearly70% loss in female flowers. In addition, the trees showed a low number of female flowers compared to male flowers. The male and female flower buds are identical in appearance except for an increase in the length of male flowers. The larvae selectively choose female flower buds indicating the nectar as the allure. The larval incident was noted at the beginning of the summer season, during February 2018, with climatic conditions of low precipitation and moderate temperature (Fig. 24). The Cecidomyiidae larvae were not found in the tree with asynchronous phenological events. An unidentified biotic agent (probably an insect) was found to pierce through the thick fruit rind of matured fruits and found to damage the seeds inside (Fig. 26- e, f & g), the incident was observed in December 2016 in the phenologically asynchronous tree (other trees of the species were in flowering stage).

During development, partially and fully matured fruits were found eaten by *Ratufa indica* and flying squirrels. The hard fruit rind pierces in two to four cm diameter (Fig. 25-h & i), and the seed inside the fruit was found damaged (Fig. 25-k).

The fallen fruits with intact rinds reported larval incidence. The larvae were collected, reared, and identified as Lance flies (Lonchaeidae/ Diptera). The larvae were collected during the first week of January 2020 and adult flies formed during 1st week of February. The Lance flies larvae cause serious loss of viable seeds (Fig. 26- h, i & j). The Unidentified flies (probably Lance flies) were also found *in situ* conditions within the fallen fruit (Fig. 26-k). The fallen fruits were later sheltered by the ants (Fig. 26-l).

On average, 30% of fruit set are only recorded in a flowering period. However, on maturity, the number of fruits/ seeds was reduced further due to the negative influence of the biotic interactions. Seedlings (below 100 cm height)were not recorded from the populations of *H. longipedunculatus* (Subin et al. unpublished) indicating extremely poor seed bank *in situ*.

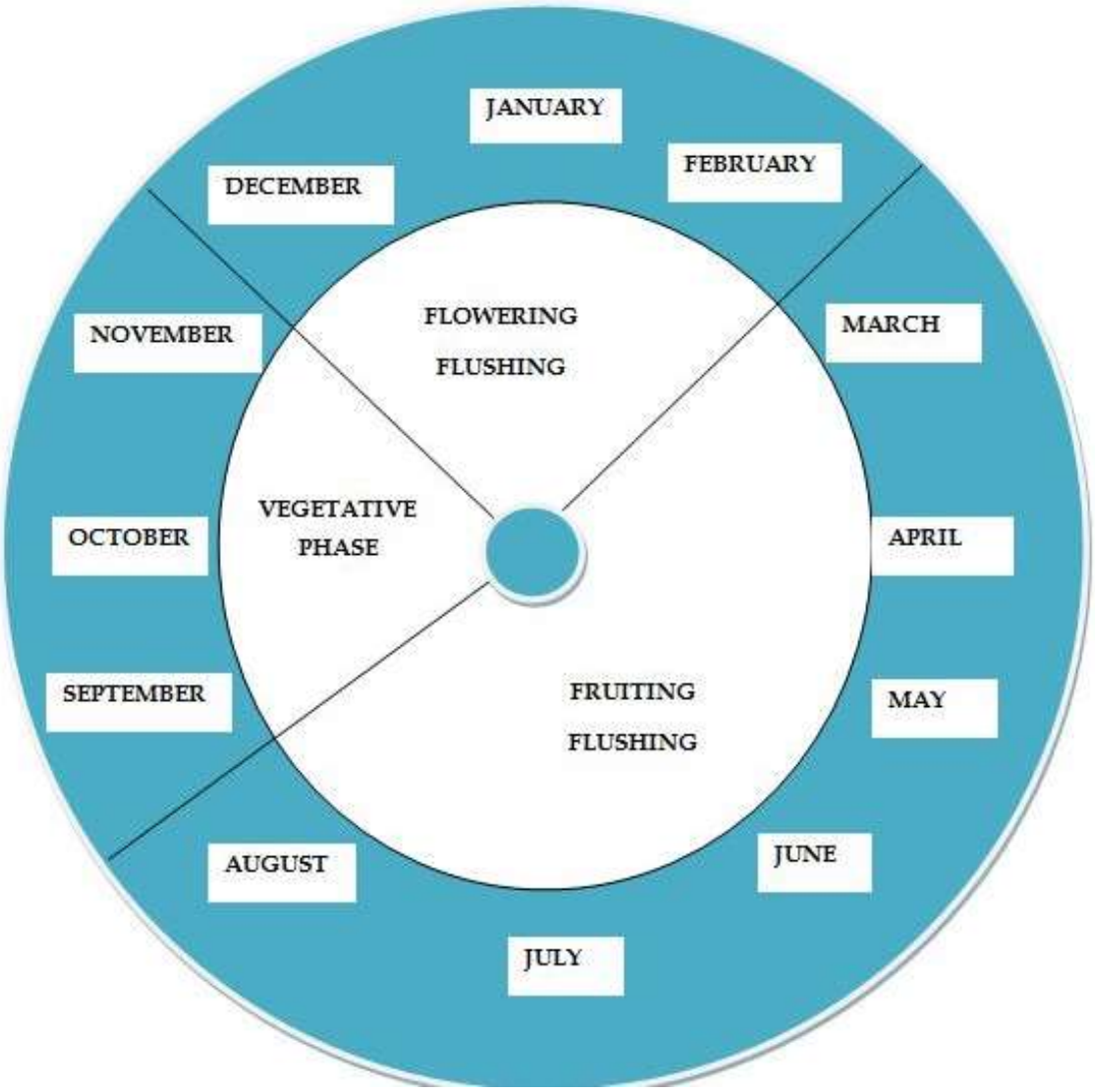

Figure 23: ***Hydnocarpus longipedunculatus*****. Phenology calender**

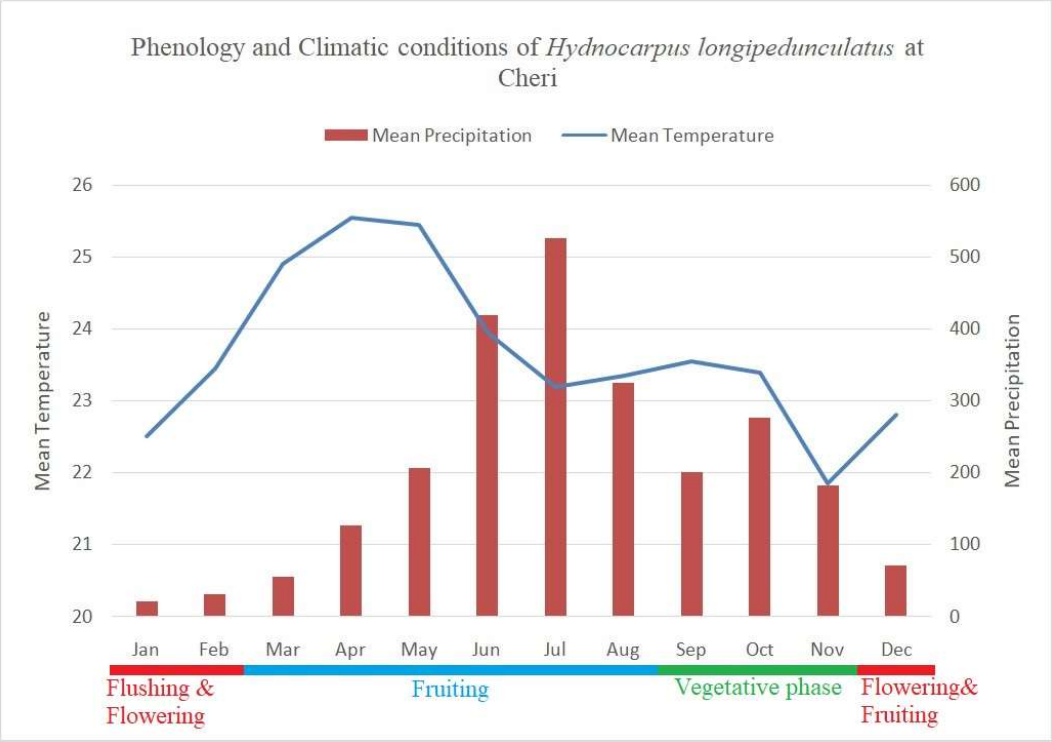

Figure 24: *Hydnocarpus longipedunculatus*:Phenology with respect to climatic conditions at Cheri population site

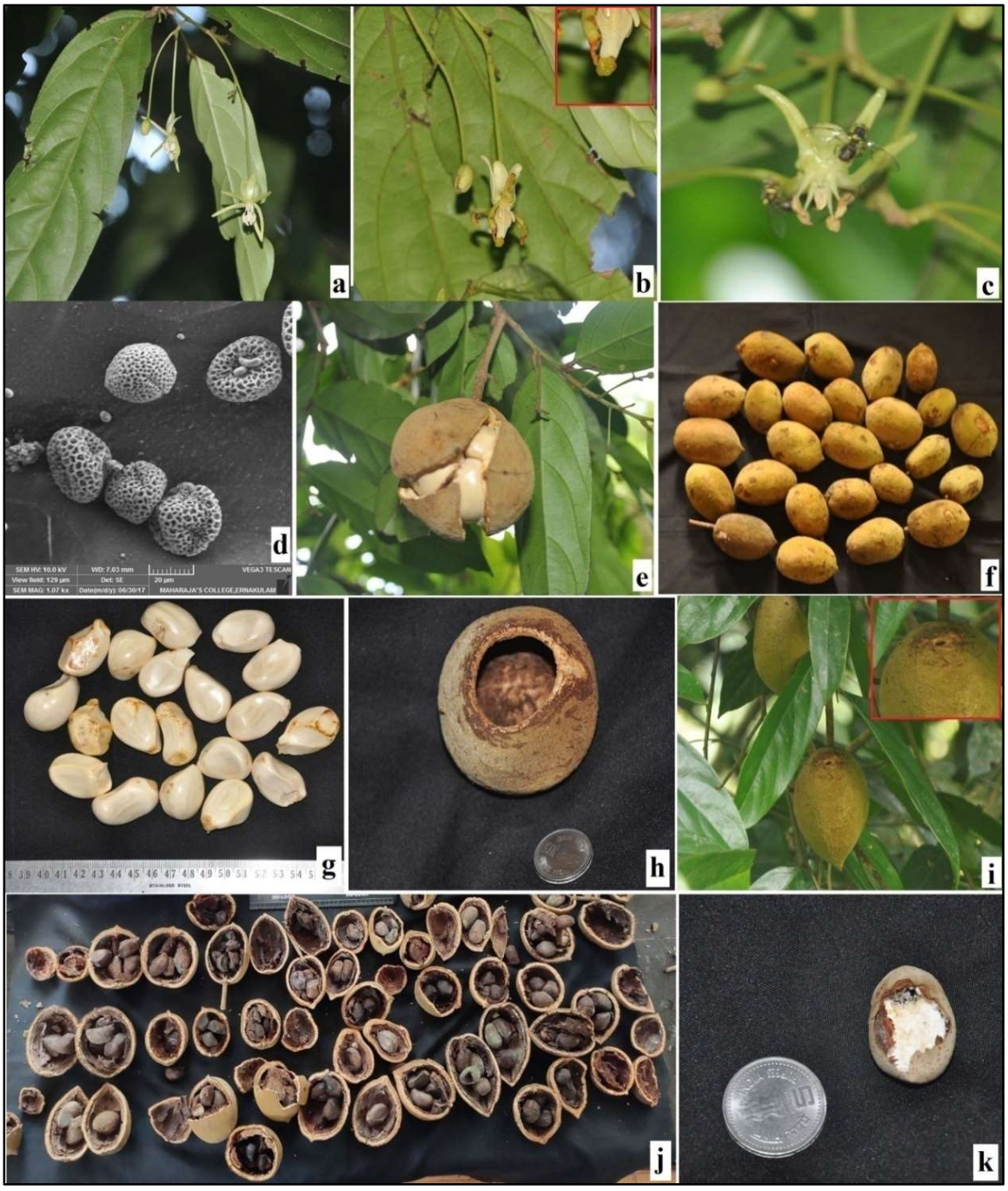

Figure 25: Reproductive dynamics: a- Male flowers and flower bud, b- Female flower with receptive stigma (inset), c- Male flower with wasps, d- Pollen grains, e- Slit formed matured fruit, f- Matured fruits, g- Matured seeds with fleshy seed coat, h & i- vies of fruit damage j- Seeds damaged inside unbroken fruit, k- *Ratufa indica*/flying squirrel damaged seed.

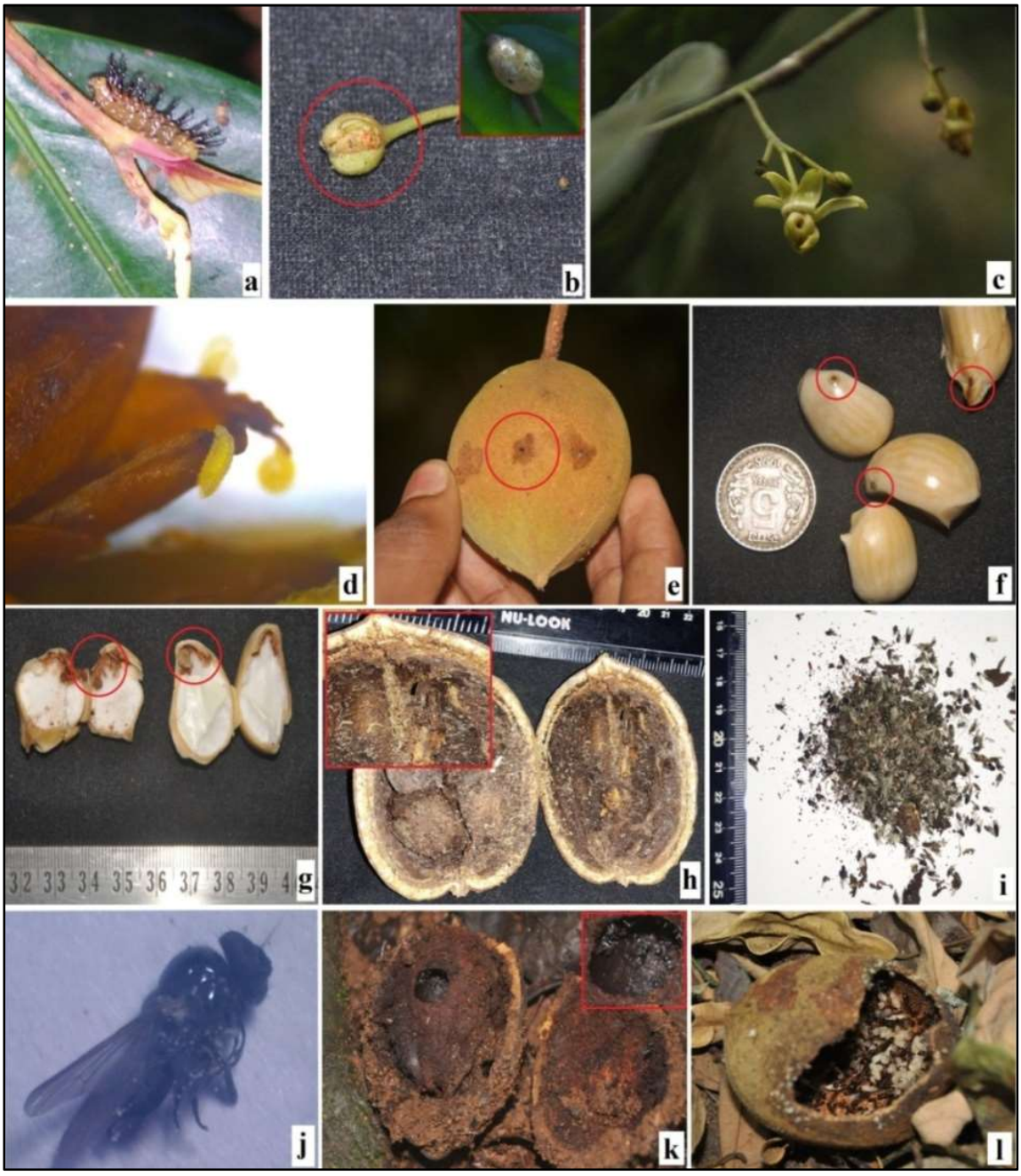

Figure 26: Insect-pest induced reproductive constraints in *H. longipedunculatus:* a- *Cirrochroa thais* larvae feeding flush, b- Flower bud damage, (inset- land snail found during flowering season), c-Webbed corolla of female flower, d- Cecidomyidae larvae in flower, e, f & g- view of fruit puncturing, seed and cut opened seed, h- Lance flies larvae, i & j- reared Lance fly adults and single fly, k- unidentified flies inside fallen fruit, l- Ants sheltering in fallen damaged fruit.

3.3.4. Environmental factor analysis

The climatic and edaphic factors were documented season-wise and used to identify the *in-situ* requirements of the species. The climatological and edaphological data of the Kakkayam dam site (Tables 11 & 12), Charangad (Tables 13 & 14), Nadugani (Tables 15 & 16), Kulamavu MPCA (Tables 17 & 18), Cheri (Tables 19 & 20) are presented below.

Table 11. Climatological data of *Atuna indica*: Kakkayam

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 12. Edaphological data of *Atuna indica*: Kakkayam

Season	Soil Level	Texture	pH	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Temp. (°C)	Moisture (%)
Summer	Surface	Silt loam	5.8	513.2	2.5	336.4	23	21.5
	Middle	Sandy	6.0	500.1	3.5	200.5		
	Bottom	loam	5.4	490.2	3.6	208.4		
		Loam						
Monsoon	Surface	Silt clay loam	4.8	630.2	6.8	476.1	22	30.2
	Middle	Silt clay	5.0	515.8	5.3	520.6		
	Bottom	Silt clay loam	5.5	503.1	3.8	296.4		
Winter	Surface	Silt clay	5.4	450.6	4.6	240.6	20	24.6
	Middle	Loam	5.8	416.5	2.3	200.5		
	Bottom	Silt loam	5.1	396.1	2.1	230.4		

Table 13 . Climatological data of *Atuna indica*: Charangad

Season	Atm. Temperature (°C)	Night Temperature (°C)	Atm. Humidity Day (%)	Atm. Humidity Night (%)
Summer	31	25	61	78
Monsoon	28	22	78	90
Winter	30	20	65	91

Table 14. Edaphological data of *Atuna indica*: Charangad

Season	Soil Level	Texture	pH	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Temp. (°C)	Moisture (%)
Summer	Surface	Silt loam	5.6	534	2.4	340.1		
	Middle	Sandy loam	5.8	536	3.7	210.4	22	22
	Bottom	Loam	5.2	515.5	3.8	221.2		
Monsoon	Surface	Silt clay loam	4.7	648.2	6.7	482.5	20	32
	Middle	Silt clay	5.1	532.3	5.5	531.7		
	Bottom	Silt clay loam	5.2	522.6	3.6	302.4		
Winter	Surface	Silt clay	5.3	465.2	5.1	253.2		
	Middle	Loam	5.6	430.1	2.1	210.6	21	26.5
	Bottom	Silt loam	5.0	410.2	2.3	242.5		

Table 15. Climatic data of *Atuna indica*: Nadugani

Season	Atm. Temp. (ºC)	Night Temp. (ºC)	Atm. Humidity (Day - %)	Atm. Humidity (Night- %)
Summer	34	28	54	62
Monsoon	24	21	78	84
Winter	26	23	68	76

Table 16. Edaphological data of *Atuna indica:* Nadugani

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

Table 17. Climatological data of *H. longipedunculatus*: Kulamavu MPCA

Season	Atm. Temp. (ºC)	Night Temp. (ºC)	Atm. Humidity (Day - %)	Atm. Humidity (Night- %)
Summer	30	25	68	85
Monsoon	26	21	82	88
Winter	27	22	70	82

Table 18. Edaphological data of *H. longipedunculatus*: Kulamavu MPCA

Season	Soil level	Texture	pH	N (Kg/Ha)	P (Kg/Ha)	K (Kg/Ha)	Temp. (°C)	Moisture (%)
summer	Surface	Silty clay loam	4.5	564.5	1.8	121		
	Middle	Silty clay	4.6	504.9	5.0	150.1	23	19.5
	Bottom	Sandy loam	4.5	573.9	6.8	172.5		
monsoon	Surface	Silty clay loam	4.8	637.6	3.3	636.9		
	Middle	Sandy clay Loam	5.0	557.5	4.4	550	21.5	27.67
	Bottom	Silt loam	5.0	301.8	2.0	190.3		
winter	Surface	Silty clay loam	4.9	627.2	2.1	142.2		
	Middle	Silt loam	4.6	439	3.4	181.4	22.8	24.56
	Bottom	Sandy clay Loam	4.9	561.3	3.1	211.7		

Table 19. Climatological data of *H. longipedunculatus*: Cheri

Season	**Atm. Temp. (ºC)**	**Night Temp. (ºC)**	**Atm. Humidity (Day - %)**	**Atm. Humidity (Night- %)**
Summer	32	26	66	84
Monsoon	27	22	80	86
Winter	28	23	69	81

Table 20. Edaphological data of *H. longipedunculatus*: Cheri

Season	Soil level	Texture	pH	N (Kg/Ha)	P (Kg/Ha)	K (Kg/Ha)	Temp. (°C)	Moisture (%)
summer	Surface	Silty clay loam	4.6	523.2	2.8	116		
	Middle	Silty clay	4.8	502.6	6	147.3	25	17
	Bottom	Sandy loam	4.6	568.7	4.6	163.5		
monsoon	Surface	Silty clay loam	4.8	632.8	2.9	586.2		
	Middle	Silty clay Loam	5.1	552.6	5.1	541	22	25
	Bottom	Silt loam	5.2	296	2.3	187		
winter	Surface	Silty clay loam	6.0	616	2.2	153		
	Middle	Silt loam	5.9	428	3.6	176	23.4	24.5
	Bottom	Sandy clay Loam	6.05	554	3.2	197		

3.4. Discussion

Population structure and Diversity

The small population size, reduced number of individuals, and low seedling count causes demographic and population genetic consequences. The genetic consequences in the form of reduced genetic diversity, gene flow, and altogether impaired genetic fitness of the species, further result in reduced evolvability against upcoming environmental odds (Ellstrand & Elam, 1993)

Generally, the semi-evergreen/evergreen forest microclimatic conditions support fair seed germination as the herbaceous and under canopy layer are less developed due to the canopy coverage of old-growth forests (Tripathi & Khan, 1990), better seed germination in semi-evergreen forests is also reported by Mohanta et al. (2021). Hence the extremely low number of seedlings of *A. indica* (9 unestablished seedlings and 27 established seedlings) and *H. longipedunculatus* (14 established seedlings only) indicate either reproductive inefficiency of the species or unfavorable external factors in successful seed production and germination. Subin et al. (2022) reported fruit damage by Pyralid larvae in *A. indica*, which caused a 50-70% loss in available seeds. Female flower damage by Cecidomyidae larvae, seed coat-induced germination hurdles, and fruit predation by arboreal mammals extends greater loss of available seeds *in-situ* in *H. longipedunculatus* (Subin & Jose Unpubli.). Hence the reproductive constraints could be considered a major factor for the reduced seedling count of the species. In addition, cattle herds owned by local farmers were found grazing in Kulamavu MPCA. The cattle foraging/livestock incidence was reported to be a reason for seedling loss by Mohammed

et al. (2022). Cattle browsing could be an additional reason for the low number of seedlings in *H. longipedunculatus*.

Both species prefer wet areas near rivulets and watercourses. According to Nayar (1996) and Swarupanandan et al. (2013), specialized habitats are prerequisites for the growth and reproduction of endemic and threatened plants. Species that are sensitive to extreme water stress preferably inhabit low-altitude wet patches of the ecosystem (neither inundated nor dry) as clumps (Chuyong et al., 2011) as seen in target species except in the Nadugani population. The species did not show obligate specialized habitat preference as reported in *Humboltia bourdillonii*, the Western Ghats' endemic tree seen only in areas adjacent to the watercourse (Rahul et al., 2020). According to Rodriguez et al. (2005), the species diversity and density of an area are dependent on microclimatic conditions. Out of the five forests sampled, four areas namely the Kakkayam dam site, Charangad, Kulamavu MPCA, and Cheri unveiled better species diversity. These areas are composed of diverse geographical features, like dam reservoirs, water sources, deep slopes, and trek paths. The least diverse area, the Nadugani population has comparatively uniform geographical features (excluded a slight slope) and a poor herbaceous layer.

The influence of first-layer species on the growth of second and third-layer species in a forest patch has not been well studied. As a third layer species, the absence of flowering at the Charangad and Nadugani populations of *A. indica* is presumably due to the low availability of sunlight because of the canopy species (Subin et al., 2022). Decreased light availability to the understorey plant community (shrubs) due to the successive layering of the canopy by first-layer species was previously reported in the southern Western Ghats

(Krishnan, 2001). Reduced productivity was also reported in coffee plantations as an impact of tree shade (Estivariz-Coca & Muschler, 1998). *A. indica* has only two flowering individuals at Kakkayam dam site populations which were situated in the north-eastern slope of the forest fringes where they got higher sunlight thorough out the year. (Out of two flowering stands, one tree fell in 2017),

The IVI provides the identity of dominant species as well as their ecological importance and competitive behavior in the ecosystem, thus helpful in elucidating the ecological characteristics of an ecosystem (Pascal, 1988; Jose, 2001; Zegeye et al., 2006; Kacholi, 2013; Turkis & Elmas, 2018). In the diverse forest patches, both the studied species showed low IVI as compared to their associates indicating the inefficiency in utilizing available resources effectively. According to Turkis and Elmas (2018), the high IVI-holding species can effectively utilize the resources or withstand environmental odds in its habitat like in the case of *Abies nordmanniana* with a high IVI could tolerate the long spell of dry period in its ecosystem.

Reproductive biology

Atuna indica

The major climatic variables that alter phenological phenomena are rainfall, temperature, insolation, and water stress (Dutta & Devi, 2015; Ashton et al., 1988; Stevenson, 2004; Borchert, 1994). The flushing along with flowering in October-December months in *A. indica* indicates that the flowering happens onset of the northeast monsoon season (Fig. 19 &20). The fruit ripens and senescence was observed during April-

May. The hard seed coat found delays the seed germination *in situ*. However, seeds found germinated in the South -West monsoon (June –September). The pollen-ovule ratio of the species supports cross-pollination [according to Cruden (1977) P:O ratio=31.9 - 396 for autogamy].

The biotic interactions are influenced by phenology, population density, and inter-population distance (for pollinators) (Lennertsson, 2002). Phenological variations mediate available pollinators, seed dispersal agents, and florivorous insects (Lobo et al., 2003; McCall & Irwin, 2006), this could also influence adaptation in primary and secondary consumers (Van Schaik, 1993). The most frequent pollinators are *Xylocopa* sp. and *Apis mellifera.* Pollinator abundance and behavior is the key influential factor in seed set for entomophily-depending flowers (Larson & Barret, 2000). Apart from this, the abscission of fruiting primordia (monkey induced) was also found. Honey bees are common pollinators found in target species, even though they are reported as less efficient pollinators because they collect pollen from various pollen resources and resulting in the deposition of multiple species-pollen in the stigma surface (Westerkamp, 1991). Colored petals, nectar, scent, discoid-shaped stigma, etc facilitate biotic pollination among flowering plants (Raju & Rao, 2006) as seen in *A. indica.* The common pollinators of the target species are carpenter bees (*Xylocopa* sp.) and *Apis mellifera*. The *Xylocopa* sp. prefers medium-sized, yellow-white colored flowers with odor and nectar (Roubik, 2002), which are found in *A. indica* flowers. Carpenter bees are effective pollinators that support and maintain genetic variability as it travels long distances (Roubik, 2002). The *Apis mellifera* was reported to have high efficiency in pollination (Solomon & Ezradanam, 2002) as reported in *Jatropha curcus* and

Jatropha mutabilis (Kollas et al., 2012). The large number of floral visitors reported in *A. indica* may be due to the exposed nature of reproductive organs and which is preferred by pollinators like *A. mellifera.* Heavy loss in developing inflorescence was also found caused by caterpillars (adults to be identified).

The general reasons for reduced fruit set are stressful environment as well as resource limitation (Friedman & Barret, 2011). Apart from the minimal fruit set, both species have restrictions in forming viable seedlings. The Pyralid larvae incidence in *A. indica* caused a loss of 50-70% in the fruit set. The Pyralid moths (Snout moths) were also reported damaging cereals and dry fruits (Pyralidae- Wikipedia n.d.). The fruits of *A. indica* are hard and devoid of a specific fruit dispersal mechanism. As per Tad Walkar et al., (2012) seeds of 70% of tropical forest species are dispersed by animals, found to aid the natural regeneration of species. The limitations in dispersal agents could be added constraints of *A. indica* for its rarity. The occurrence of an extremely low number of flowering individuals, limited to two stands was found to be the most important finding in *A. indica*. The natural fall of one flowering tree was recorded in 2017. Thus reproductive biological data collected from a single tree was one other limitation of the work. Based on the microhabitat conditions, that lower sunlight availability (as 3rd layer species) is the possible reason behind the low number of flowering individuals. The tree which showed flowering was situated on the forest fringe near the trek path facing the east side, while the non-flowering individuals are in the shade of canopy species. In-depth studies on the relationship between light and flowering are required to untangle the flowering behavior of the species.

Hydnocarpus longipedunculatus

Knowledge of the habitat and phenology of endemic species has a crucial role in understanding their limited distribution (Schwartz & Walker, 1986; Chuine et al., 2000). The major climatic variables that alter phenological events in plants are rainfall, temperature, sunlight, and water stress (Dutta & Devi, 2015; Stevenson, 2004). In *H. longipedunculatus,* the functional dynamics are suitably aligned with the climatic factors. Flowering was observed in the winter season (November- February) as reported in other tree species such as *Terminalia cuneata* (Chauhan et al., 2008), *Pongamia pinnata* (Dillon et al., 2009), *Madhuca neriifolia* (Prasannakumar et al., 2013). Fruit phenology takes six to eight months for maturation and ripeness. Fruit dehisces in August- September (the ending months of the Southwest monsoon- rainy season), could have positively influenced the seed germination performance. The phenology, population density, and inter-population distance (for pollinators) have a role in controlling plant-biotic interactions (Lennertson, 2002). The species is dependent on specific pollinators, seed dispersal agents, and florivorous insects during the natural dynamics (Lobo et al., 2003; McCall & Irwin, 2006), and it also influences the faunal lifecycle adaptation according to resource availability/environmental odds (Van Schaik et al., 1993). The downward-facing flower of *H. longipedunculatus* has the advantage that the inner organs are not directly exposed to precipitation (Corbet, 1990; Aizen & Harder, 2007), this could be an adaptation to protect inner floral organs from mist/occasional rains in the habitat, even though the precipitation is comparatively low during the flowering period.

According to De Jong et al. (2008), hermaphroditism evolves to andromonoecy (male and bisexual flowers on the same plant) and then to monoecy. Generally, the monoecious species have small inexpensive flowers, large costly fruits, and high fertilization rates. The monoecious nature seen in *H. longipedunculatus* helps maintain genetic diversity to an extent as it requires obligatory cross-pollination. Commonly, lower pollen longevity indicates that the species grows in a denser area while higher pollen longevity represents species distributed in vast areas (Dafni & Firmage, 2000). The 36 hours of pollen longevity in *H. longipedunculatus* can be considered as average as in *Mercurdialis annua* and *Festuca arundinaceae* (Pacini et al., 1997), supporting the merely clumped distribution range of the species. No relevant correlations were found between habit and pollen longevity, as the herbaceous *Oryza sativa* and *Triticum aestivum* has four and five minutes of pollen longevity whereas the herbaceous orchid *Calopogon tuberosus* has eight days of pollen longevity (Dafni & Firmage, 2000). The pollen diameter of bee-pollinated flowers in the tropics comes in a range of 10 -100 µm, with an average of 34 µm (Roubik, 1992). *H. longipedunculatus* pollen diameter is about 27-32 µm range. Zhou et al. (2008) also reported that entomophilous plants have rough pollen surfaces for adherence to insect legs. Most of the species either with reticulate pollen ornamentation or large size or both, show entomophilous character as in *Hemerocallis citrine* (Po. size.- 49×76 µm) and *Lilium tigrinum* (Po. size- 47×74 µm) (Lu et al., 2022), the similar reticulate ornamentation and comparatively larger pollen in *H. longipedunculatus* is pointing towards its entomophilous nature.

According to Cruden (1977), P/O ratio of 31.9 - 396 implies that the species prefer autogamy, higher P/O ratio stands for cross-pollination. The low pollen ovule ratio of *H. longipedunculatus* along with its monoecious (obligatory cross-pollination) nature points out that the species is highly dependent on insect pollinators. The pollen germination, pollen viability, and stigma receptivity are normal in *H. longipedunculatus* when compared with other tropical evergreen tree species, *Elaeocarpus blascoi, Canarium strictum,*and *Pittosporum dasycaulon* (Ramasubbu & Irudhyaraj, 2016; Kumar et al., 2015; Gopalakrishnan & Thomas, 2014).

Cirrochroa sp.*, Xylocopa* sp.*, A. mellifera* are the major pollinators of *H. longipedunculatus*. Pollinator abundance and behavior is the key influential factor in the seed set for entomophilous flowers (Larson & Barret, 2000). *A. mellifera* was reported to have high efficiency in pollination (Roubik, 2002) and shown better pollination in monoecious species like *Jatropha mollissima* and *Jatropha mutabilis* (Solomon & Ezradanam, 2002). The *Xylocopa* sp. prefers a medium-sized, yellow-white colored flower with odor and nectar as seen in *H. longipedunculatus*. *Xylocopa* species are effective pollinators that support and maintain genetic variability because they can travel long distances (Raju & Rao, 2006). The vast number of floral visitors reported may be due to the exposed nature of reproductive organs, these kinds of flowers are easily pollinated by bees like *A. mellifera.* Colored petals, nectar, scent and discoid-shaped stigmas as seen in *H. longipedunculatus* are promoting biotic pollination (Dompreh et al., 2015), emphasizing the adaptation of flowers for cross-pollination, and the pollination is effective with the aid of *A. mellifera* and *Xylocopa* sp.

The fleshy seed coat possibly is an evolutionary advantage for the seeds to retain their viability for a long time. But in the current scenario, the seed coat drastically reduces the seed germination. Generally, when fruit/seed falls to the ground, the seeds may land on rocky substrates which in turn cause natural scarification of the seed and aid natural germination (the species is distributed in the sloppy area). A thorough study is required on the evolutionary significance of seed coats in the genus *Hydnocarpus,* as all the species got fleshy seed coats.

Biotic constraints

The reproductive efficiency parameters viz. pollen viability, longevity, germination, pollen-ovule ratio, stigma receptivity, and pollination per se are adequate for the survival of the species. But the biotic interactions, especially harmful ones put the species' survival at risk.

Cirrochroa thais is a common butterfly reported in the genus *Hydnocarpus*. The larvae were found feeding the leaves of *Hydnocarpus venenata* (Jayasinghe et al., 2014) from Sri Lanka and *Hydnocarpus wightiana* (Gupta & Manoj, 2022) from India. The associations between *C. thais* and *Hydnocarpus* genus are yet to be investigated. The high flower loss causing Cecidomyiidae larvae (Gall midge) also act as pests in crops like Rice, Wheat, Sorghum, Coffee, etc. (Cecidomyidae- Wikipedia n.d.). Gall midges were also reported in damaging leaves and branches of the Olive tree (Batta, 2019) and found in trees like Parkinsonia, Jojoba, Alfalfa, and Guar (Gagne & Woods, 1988). Different species of gall midges were also reported from the Western Ghats (Vasanthakumar et al., 2019). No other gall midges were reported in damaging flowers of Western Ghats endemics. A

thorough study of its lifecycle and synchrony with flowering is needed. The alternative host plant of Cecidomyiidae larvae could not be identified from the study area. The results of changes in the flowering season of *H. longipedunculatus* have many repercussions on the life cycle of the flower inhabiting Cecidomyiidae larvae, even though it is destructive.

The general reasons for the reduced fruit set are a stressful environment as well as resource limitations (Kollas et al., 2012; Friedman & Barret, 2011). Apart from the reduced fruit set, *Ratufa indica* predates the available seed source. The hard fruit pericarp is limiting access to the developing fleshy seeds, but *R. indica* managed to pierce through the hard fruit rind and get the seed. The manipulative ability of *R. indica* is well known as it can choose large fruits for predation (Gautier-Hion et al., 1985). They are reported in damaging several tropical tree species (Kasi & Ramasubbu, 2021). Generally, seeds of 70% of tropical forest species are dispersed by animals (Tad Walker et al., 2012) but in the case of *H. longipedunculatus* mammals are mainly involved in fruit predation, while its spherical shape helps in gravity-mediated dispersal as the tree seen in slopes.

The Lance flies larvae are reported in feeding the fresh tissues of syconia in fruits of ficus species like *Ficus erecta*, *F. thunbergii*, *F. variegata* and *F. benguetensis*, The larvae continue feeding even after the fruit fall (Arimoto et al., 2020). A similar kind of larval behavior is seen in *H. longipedunculatus*, the larvae were found feeding on fallen intact fruit. This implies that the larval behavior persists until the fruit

The majority of the arthropod incidence was found in December- February months. The study site has a monsoon climate, hence the December –March months are devoid of

high precipitation (Summer months). The summer months and synchronous flowering behavior is causing such a vast array of arthropod-induced damage in target species, as the study site experiences high precipitation in other months, which impedes the existence of dependent insects. In addition, less arthropod incidence was noted in the tree with asynchronous flowering and fruiting events.

Apart from the mutualistic interactions made by pollinators, the major biotic interactions are found to be harmful to the species. The dependency of *Cirrichroa thais*, Gall midges, Lance flies and other unidentified insects seem to be natural in an undisturbed forest patch. This shows the evolutionary disadvantage of the tree species to withstand hurdles in reproduction, but at the same time, the survival risk of the species puts the dependent insects at some level of risk and the climate change scenario has unexpected implications. Detailed life cycle studies of these insects are required which may unravel complex community-level interactions in tropical semi-evergreen forests like the study site. The seed coat-induced dormancy and its relationship between fruit dispersal time and climate events also need to be studied.

Chapter 4

Population genetics

Objective 2: Detection of diverse genotypes in the populations of target spp.

4.1. Introduction

The Western Ghats of India, one of the biodiversity hotspots, currently faces an alarming rate of species extinction. The flora of these mountain ranges is compared to the island flora because of the high level of species diversity and endemism (Subramanyan & Sasidharan, 1991). The Western Ghats has around 7500 species of flowering plants, of which nearly 2200 species are endemic to the region (Nayar et al., 2014; Sasidharan, 2017). Hunting, livestock grazing, extraction of firewood/fodder, presence of exotics and invasive species, timber felling, and forest fire are the most proximate threats to biodiversity reported in the Western Ghats (Myers et al., 2000; Jose, 2001; CEPF n.d.). Habitat degradation has drastically affected the survival and growth of endemic and threatened flora of the region (Raman, 2006).

Endangered species' generally have small and discrete populations which hinder cross-breeding among populations and lead to genetic erosion resulting in elevated extinction risks. The resultant reduced genetic diversity often compromises the evolutionary responses to impending environmental changes (Frankham, 1995). Extreme reductions in genetic diversity were thus reported in endangered tree species such as *Metrosideros boninensis* (Kaneko et al., 2008), *Metrosideros bartletti* (Drummond et al., 2000), *Ostrya rehderiana* (Li et al., 2012) and *Abies yuanbaoshanensis* (Yan et al., 2003), etc. Conversely, adequate levels of genetic diversity as compared to widespread species have been reported in threatened plants like *Rhododendron protistum* var. *giganteum* (Wu et al., 2015), *Prunus africana* (Farwig et al., 2008), *Eryngium alpinum* (Gaudeul et al.,

2000), *Litsea szemaois* (Ci et al., 2008) and *Tecomella undulata* (Bhau et al., 2007). Though, several ecological studies were conducted in the IUCN Red listed tree species of the Western Ghats, such as *Gluta travancorica*, *Ochreinaucleae missionis*, *Dipterocarpus bourdillonii*, *Humboldtia bourdillonii,* etc., (Jose, 2001; Pandurangan, 2003; Swarupanandan et al., 2013), the genetic structure of such species has rarely been investigated.

Among the anonymous DNA markers (RAPD, ISSR, AFLP) employed for estimating genetic diversity, ISSRshave more reproducibility than RAPD and cost-effective than AFLP markers (Zietkiewicz et al., 1994; Ng & Tan, 2015). ISSR markers have been efficiently utilized to analyze the genetic diversity pattern of plant species like *Begonia fimbristipulata* (Zhao et al., 2016), *Calamus vattayila* (Priya et al., 2016), *Senna reticulata* (De Lima et al., 2015), *Shorea platyclados* (Javed et al., 2014), *Jatropha curcas* (Warrior et al., 2013), *Tamarindus* sp. (Sarmiento et al., 2017), among others. In this context, the present study intends to estimate the genetic structure and diversity of *Atuna indica* and *Hydnocarpus longipedunculatus*, two endemic and threatened tree species with small discrete populations, in the Western Ghats.

4.2. Materials and Methods

Purposive sampling was followed for the selection of mature individuals (GBH ≥30 cm, except *A. indica* at Kakkayam dam site- GBH ≥ 10cm) from three populations of *A. indica* [Kakkayam dam site (10 individuals), Kakkayam-Charangad (30 individuals) and Nadugani (20 individuals)]. For *H. longipedunculatus*, 20 individuals each from Kulamavu MPCA and Cheri populations were collected (Fig. 1).

Fresh leaves were dried and stored in silica gel. Total DNA was extracted using the CTAB method (Doyle & Doyle, 1987), and DNeasy Plant Mini Kit (Qiagen, Germany) was used for difficult samples following the manufacturer's protocol. Quantitative and qualitative analyses of genomic DNA were performed using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, UK) and visualized in 1.0 percent Agarose gel under ultraviolet-light (UVP BioImaging Systems, Upland, CA). Polymerase Chain Reactions (PCR) were performed in a PTC-100 thermal cycler (BIO-RAD, India) with 20 µL reaction mixture containing 50-100 ng DNA, 10X Taq buffer with 1.5 mM $MgCl_2$, 200 mM dNTPs, 20 pm of each primer and 2U Taq DNA polymerase (Invitrogen, Bengaluru). PCR cycling conditions standardized were initial denaturation at 94°C for 5 min., then 35 cycles of denaturation at 94°C for 45 sec., annealing (41.3-59.3°C for *A. indica*; 41.3-53.8°C for *H. longipedunculatus*) for 1 min. depending upon the primers, extension at 72°C for 2 min., and a final extension at 72°C for 20 min. FifteenISSR markers (Zietkiewicz et al., 1994) commercially obtained from the University of British Columbia (Vancouver, Canada) were selected for the study. The amplified products were electrophoresed in 1.5% agarose gel for 60 minutes at 65 V. ISSR fragments were visualized by staining with ethidium bromide and documented using a gel documentation system (Alpha Innotech, USA). A 3000 bp DNA ladder (Sigma-Aldrich) was used as a standard marker to estimate the size of the amplified ISSR fragments.

Since ISSR markers are dominant, each band represents the phenotype at a single biallelic locus. Distinct, reproducible, well-resolved fragments were scored as present (1) or absent (0) for each ISSR reaction and were documented as binary matrices. The following

population genetic parameters were estimated to assess the genetic diversity *via. the* percentage of polymorphic loci (PPL) observed number of alleles (Na), the effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's genetic diversity index (I) (Lewontin, 1972), genetic differentiation (G_{ST}), gene flow (Nm) and Nei's genetic distance (Gd). Gene flow (Nm) was calculated based on G_{ST} [$Nm=(1-G_{ST})/4G_{ST}$] (Wright, 1951). Principal coordinate analysis (PCoA) and Mantel test were conducted using GenALEx 6 software (Peakall & Smouse, 2006). Unbiased genetic distance was used to construct a dendrogram with unweighted pair group arithmetic mean with arithmetic average (UPGMA) as implemented in POPGENE v1.3.1 (Yeh et al., 1999). Allelic profile data of genotypes was analyzed to determine subpopulations of both species, using an admixture model in the Bayesian analysis tool STRUCTURE v2.3. This was used to analyze the separation of total individuals into clusters (K) that represent the number of gene pools (Pritchard et al., 2010). A burn-in period of 50000 and 50000 Markov chain Monte Carlo (MCMC) iterations were set with an admixture model. K values were set from 1 to 7 followed by 15 independent runs for each simulated value of K. Optimal K value was determined using a simulation method (Evanno et al., 2005) in the software Structure Harvester vA.2 (Earl, 2012).

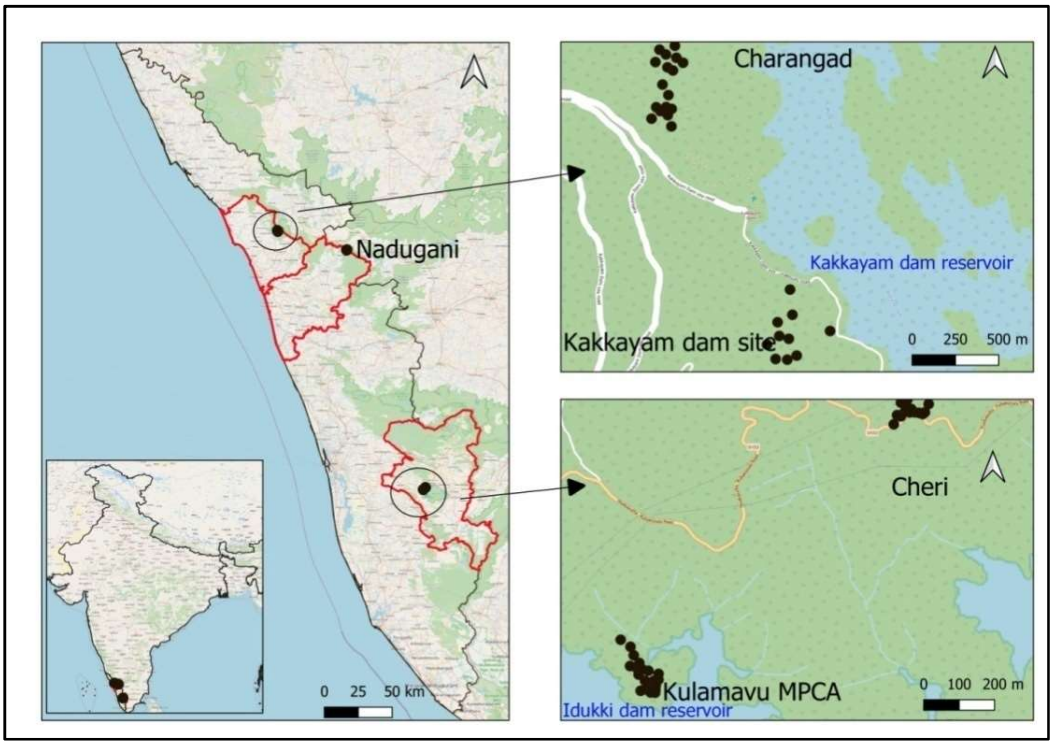

Figure 1. Distribution of *A. Indica* and *H. longipedunculatus* in southern Western Ghats, Kerala. Right upper figure shows *A. indica* individuals in Kakkayam dam site and Charangad as represented by black dots. Right lower figure shows *H. longipedunculatus* individuals in Kulamavu and Cheri sites

4.3. Results

Atuna indica

Out of 15 ISSR primers tested, 11 primers resulted in polymorphic amplified products(Table 1). The observed number of alleles (Na) per locus ranged from 1.53 to 1.72 with a mean of 1.64. The mean effective number of alleles (Ne) was 1.27 and it ranged from 1.19 to 1.31. The percentage of polymorphic loci ranged from 53.19% to 71.63%, with a mean of 64.3%. Shannon diversity index ranged from 0.19 to 0.29 with a mean of 0.26 (Table 2). Kakkayam dam site displayed the highest Nei's genetic diversity index (0.190) followed by Nadugani (0.188) and the least was in Charangad (0.123). The total genetic differentiation residing among populations (G_{ST}) of *A. indica* was 0.2231. The gene flow (Nm) indirectly estimated based on G_{ST} was 0.871. PCoA analysis indicated three well-defined clusters in respective of the three populations. The Charangad population showed two subclusters, coordinate axis 1 explained 20.3% of the variation, and two and three explained 12.6% and 11.4% variation (Fig. 2). The optimal K value was two (K=2) with a good population structure and least admixture pattern. A similar population structure was observed between the Kakkayam dam site and Nadugani populations whereas the Charangad population was genetically very distinct (Fig. 3 & 4). The Mantel test revealed a slight positive correlation between genetic and geographic distances with R^2 value of 0.25 (Fig. 5).

Table 1: ISSR primers used for *A. indica* with annealing temperatures and total number of bands produced by primer

	Primer code	Sequence	Annealing temperature	Total bands produced
***Atunaindica*(11)**	UBC 810	$(GA)_8T$	41.3	12
	UBC 811	$(GA)_8C$	41.3	13
	UBC 812	$(GA)_8A$	46.7	14
	UBC 825	$(AC)_8T$	42.5	14
	UBC 834	$(AG)_8YT$	50.9	14
	UBC 890	$VHV(GT)_7$	53.8	14
	UBC 818	$(CA)_8G$	52.6	15
	UBC 808	$(AG)_8C$	59.3	12
	PRIMER 3	$(GA)_8TC$	45.0	12
	PRIMER 5	$(CA)_8AG$	56.6	11
	ISSR 6	$(GTG)_7C$	49.4	10

Table 2. Genetic diversity indices of three populations of *A. indica.*

Population	Number of Individuals	Observed number of alleles (N_a)	Effective number of alleles (N_e)±S.D	No. of polymorphic loci	Percentage of polymorphic loci	Nei's genetic diversity± S.D	Shannon diversity index(*I*)± S.D
Kakkayam dam site	10	1.681±0.47	1.3066 ±0.322	96	68.09	0.1905 ±0.1778	0.2982 ±0.2527
Nadugani	20	1.716±0.45	1.3052 ±0.3394	101	71.63	0.1881±0.1798	0.2953 ±0.2525
Charangad	30	1.532±0.50	1.1909 ±0.2815	75	53.19	0.1232 ±0.1604	0.1980 ±0.2363

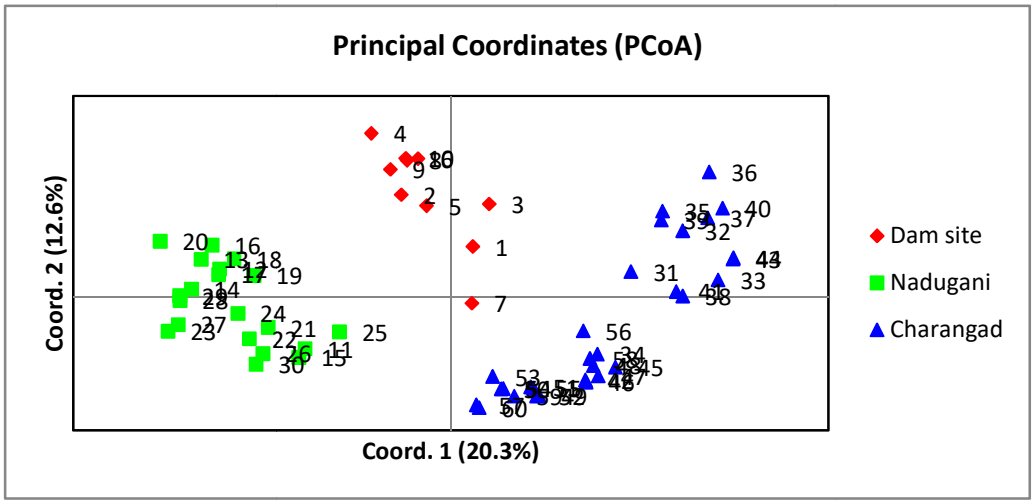

Figure 2. Two-dimensional plot of principal coordinate analysis (PCoA) showing clustering of individual samples of *A. indica.*

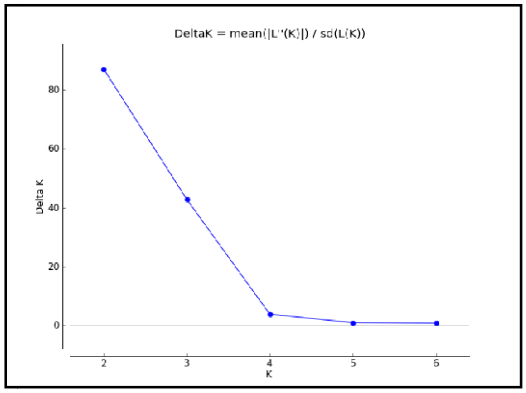

Figure 3. Result of Bayesian analysis of *A. indica* suggesting K = 2 as most likely number of clusters as delta K value maximum at K = 2

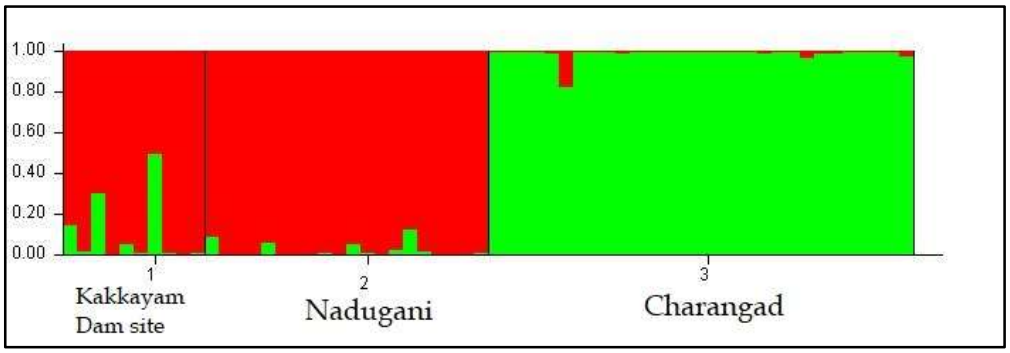

Figure 4. Population structure pattern of three populations of *A. indica* (generated using STRUCTURE program when *K* = 2).

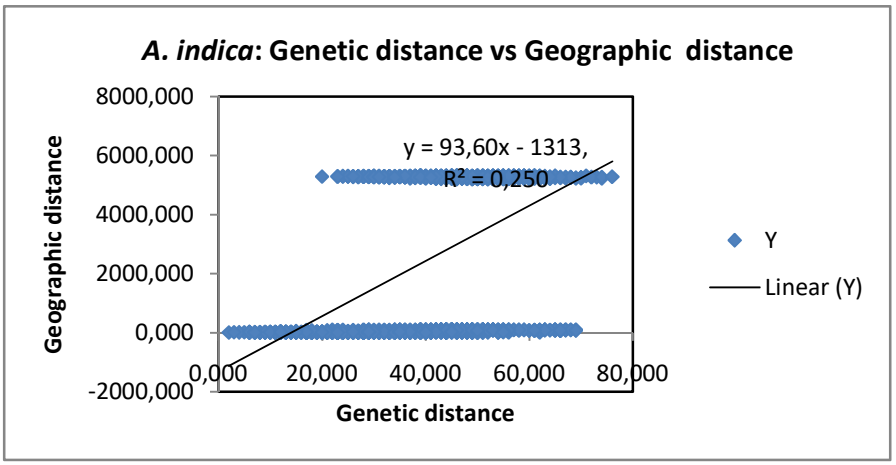

Figure 5. Graph showing correlation between genetic and geographic distances among the two populations of *A. indica.*

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H. longipedunulatus

Out of 15 primers tested ten showed polymorphic bands (Table 3), which were used for further diversity analysis. The observed number of alleles was 1.87 and 1.58, the effective number of alleles was 1.41 and 1.31, the percentage of polymorphic loci was 86.4 % and 58.4%, the Shannon diversity index was 0.38 and 0.28, Nei's genetic diversity was 0.25 and 0.19 in Kulamavu MPCA and Cheri populations, respectively (Table 4). The total genetic differentiation among populations (G_{ST}) of *H. longipedunculatus* was 0.1112. The gene flow (Nm) based on G_{ST} was found to be 1.998. Principal coordinate analysis of the two populations showed three separate clusters along with some scattered individuals. The three clusters represented individuals from Kulamavu MPCA, Cheri, and a mixture of individuals from both populations (coordinate axis 1 explained 33.23% of the variation, and two and three explained 25.7% and 6.4% of variations) (Fig. 6). STRUCTURE analysis showed a K value of 3 indicating three ancestral subpopulations (K=3) (Fig. 7). Both populations showed genotype admixture pattern (Fig.8). Mantel test showed a slight positive correlation between geographic and genetic distance (R^2= 0.147) (Fig. 9).

Table 3: ISSR primers used for *H. longipedunculatus* with annealing temperatures and total number of bands produced by primer.

	Primer code	Sequence	Annealing temperature	Total bands produced
***Hydnocarpus longipedunculatus*(10)**	UBC 810	$(GA)_8T$	41.9	14
	UBC 811	$(GA)_8C$	41.3	13
	UBC 812	$(GA)_8A$	49.0	11
	UBC 825	$(AC)_8T$	42.3	12
	UBC 830	$(TG)_8G$	51.0	11
	UBC 834	$(AG)_8YT$	50.9	13
	UBC 890	$VHV(GT)_7$	53.8	13
	UBC 818	$(CA)_8G$	49.0	9
	UBC 808	$(AG)_8C$	53.8	9
	PRIMER 6	$(CT)_8GC$	53.3	8

Table 4. Genetic diversity indices of two populations of *H. longipedunculatus*

Population	**Number of Individuals**	**Observed number of alleles (N_a) ±S.D**	**Effective number of alleles (N_e) ±S.D**	**No. of polymorphic loci**	**Percentage of polymorphic loci**	**Nei's genetic diversity ±S.D**	**Shannon diversity index(*I*) ±S.D**
Kulamaavu MPCA	20	1.867 ±0.34	1.4082 ±0.3495	98	86.73	0.2465 ±0.1787	0.3804 ±0.2416
Cheri	20	1.584 ±0.50	1.3067 ±0.3432	66	58.41	0.1854 ±0.1911	0.2823 ±0.2752

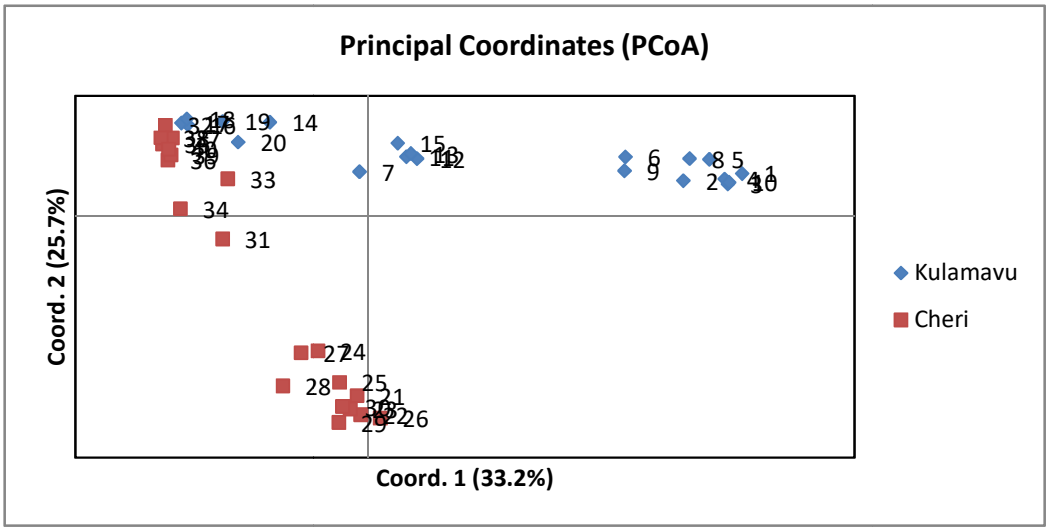


Figure 6. Two-dimensional plot of principal coordinate analysis (PCoA) showing clustering of individual samples of *H. longipedunculatus*

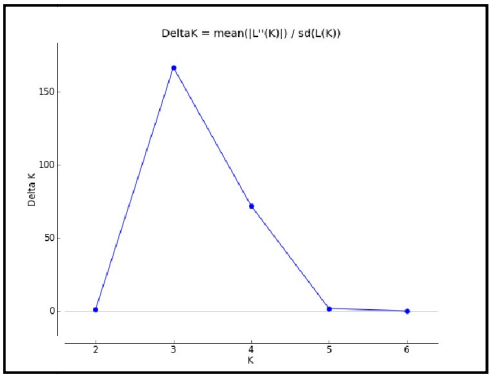


Figure 7. Result of Bayesian analysis of *H. longipedunculatus* suggesting *K* = 3 as most likely number of clusters as delta K value maximum at K = 3

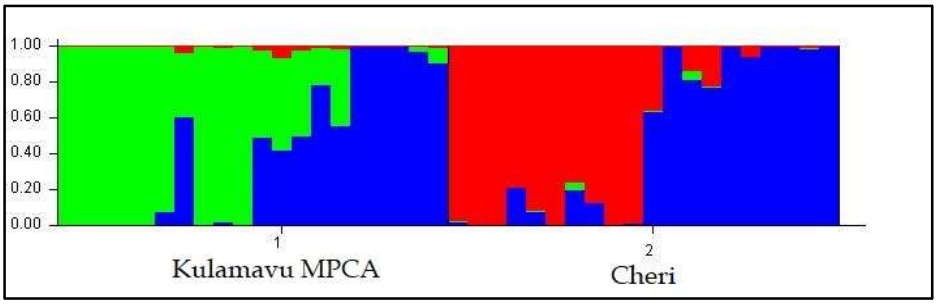


Figure 8. Population structure pattern of two populations of *H. longipedunculatus* (generated using STRUCTURE program with K=3)

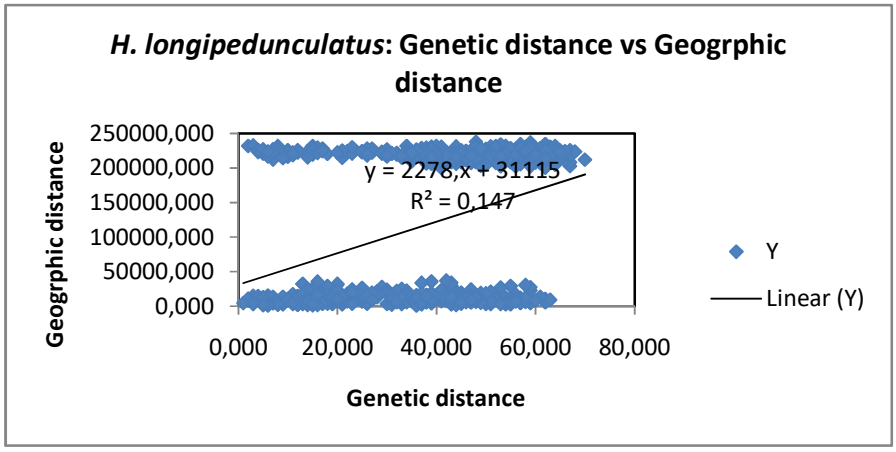


Figure 9. Graph showing correlation between genetic and geographic distances among two populations of *H. longipedunculatus*

4.4. Discussion

The observed values of genetic diversity parameters in *A. indica* and *H. longipedunculatus* are higher than the mean genetic diversity of long-lived plants, 0.077 (Gamba & Muchhala, 2020). Subin et al. (2022) reported entomophily, endemic distribution, and mixed breeding systems in *A. indica.* The species has equal or better G_{ST} values as compared to other species with similar ecological traits, such as species with a mixed breeding system (G_{ST}- 0.243), entomophilous (G_{ST}- 0.227), and endemic nature (G_{ST}- 0.227) (Loveless & Hamrick, 1984). *Hydnocarpus longipedunculatus* has lower G_{ST} values as compared to species with similar ecological traits although the lowest values are reported in monoecious species (Loveless &Hamrick, 1984). The existing small and fragmented populations of both species also showed poor differentiation among populations indicating adequate gene flow among the populations. Both *A. indica* and *H. longipedunculatus* had comparatively high gene flow among the populations *viz.* Nm=0.871, and Nm=1.998, respectively. Woody perennials have a high potential for gene flow due to their large size, massive pollen count, and seed production (Nason & Hamrick, 1997; Hamrick, 2004). Generally, the Gene flow (Nm) >1 implies little differentiation, and the species is expected to maintain genetic stability over time (Lowe et al., 2004; Zong et al., 2015; Matesanz et al., 2017).

The anthropogenic effects can influence the Nmand G_{ST} of species even in reserved forests. The Kulamavu MPCA population has a higher percentage of polymorphic loci, Nei's genetic diversity, and Shannon diversity index, though it has a low number of individuals when compared to the Cheri population. The better diversity indices and lower

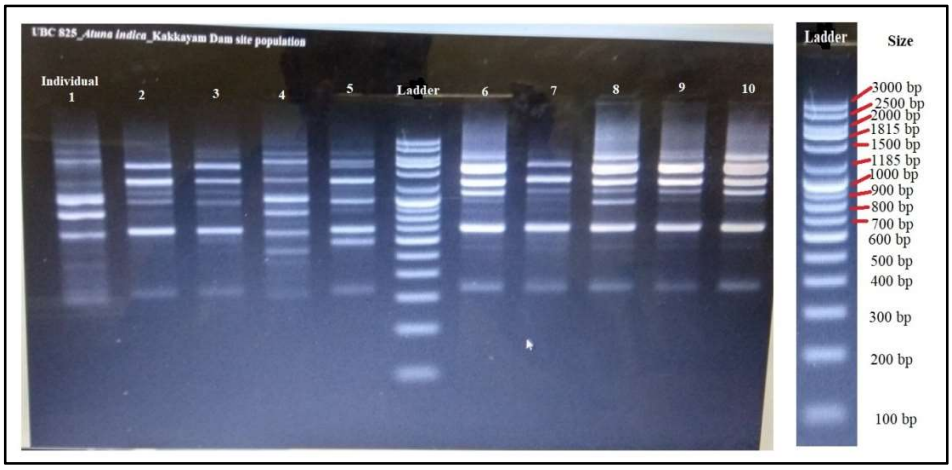
number of individuals indicate a recent population bottleneck event in the Kulamavu MPCA population. This population of *H. longipedunculatus* is situated near the dam reservoir (Idukki dam, commissioned in 1973). The dam construction-driven water level expansion at the upstream area might have affected the survival of individuals of the species. The STRUCTURE results also support this argument, in which among the three ancestral subpopulations, the Kulamavu MPCA population is either part of a larger population that is eventually wiped out due to anthropogenic pressures. A similar scenario is also observed in *A. indica*. The Kakkayam Dam site population is situated near the dam reservoir (Kakkayam dam, established in 1972). Though the number of individuals is the lowest in the population, Nei's genetic diversity and Shannon diversity indices are the highest as compared to the other two populations. It is reported that high genetic diversity in fragmented populations may be possible, when population reduction occurs in the recent past, substantiating the assumptions drawn in the present study (Zhao et al., 2012).

Among the three populations of *A. indica*, the Kakkayam dam site, and Charangad are geographically closer than that of the Nadugani population (Fig. 1), yet the STRUCTURE bar plot showed Nadugani and Kakkayam to share a similar population structure indicating related genetic origins of these populations. Likewise, weak positive correlations between genetic and geographic distances were also reported in *Haloxylon salicornicum* (R^2=0.18) (Al Salameen et al., 2018) and *Magnolica wufengensis* (R^2=0.29) (Chen et al. 2014).

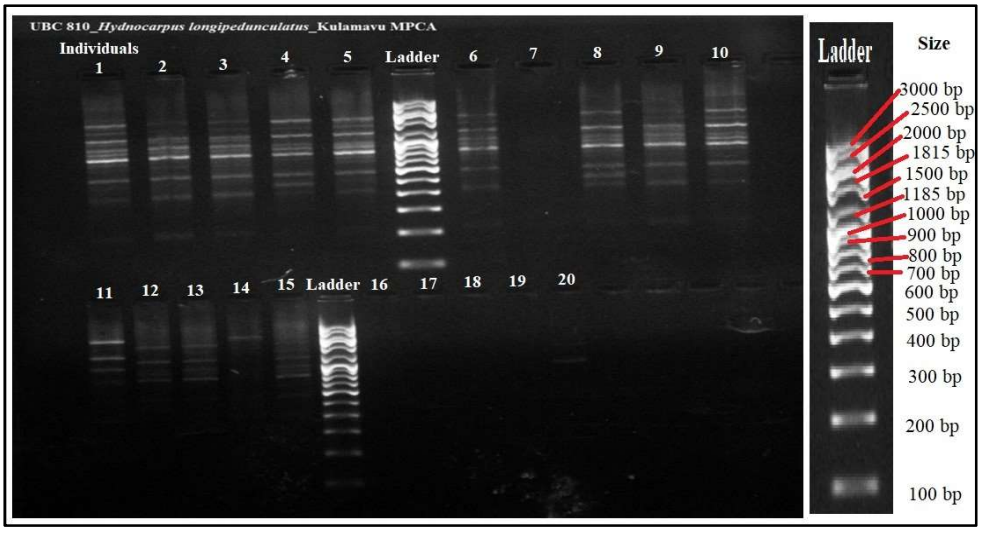
Reproductive biological studies conducted in *A. indica* (Subin et al., 2022) and *H. longipedunculatus* (Subin & Jose, *unpubli.*) reported reproductive constraints in these species. A low number of flowering individuals (sunlight influence) and seed loss due to

pyralid larvae infestation were evident in *A. India* whereas *Cecidomyidae* larvae induced flower damage, fruit and seed losses caused by arboreal mammals, and poor seed germination in *H. longipedunculatus,* impede the seedling recruitment and threatens the survival of these populations (Subin et al., 2022; Subin & Jose, *unpubli*.)

The population bottleneck events in the recent past, probably due to the dam construction and formation and expansion of water reservoirs at the Kakkayam Dam site and Kulamavu MPCA populations, might have drastically affected the viable population size of these species. The existing adequate genetic diversity and gene flow are the remains of a flourished reign of these species. The extremely narrow distributed populations with reproductive constraints affect the long-term survival of these species and are extremely vulnerable to extinction. The study warrants immediate *in situ* and *ex-situ* conservation efforts through habitat protection and appropriate restoration programs.

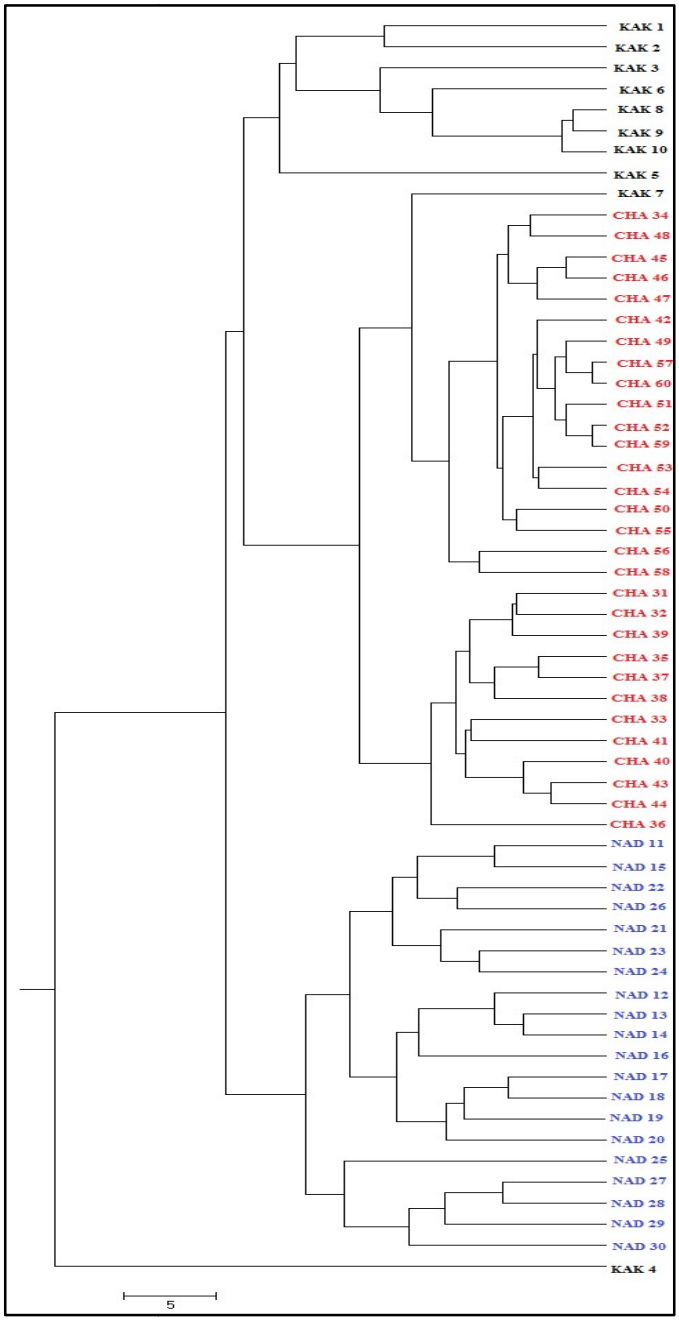


Appendix 1. ISSR fingerprinting of *Atuna indica* individuals at Kakkayam Dam site , ISSR Primer: UBC 825

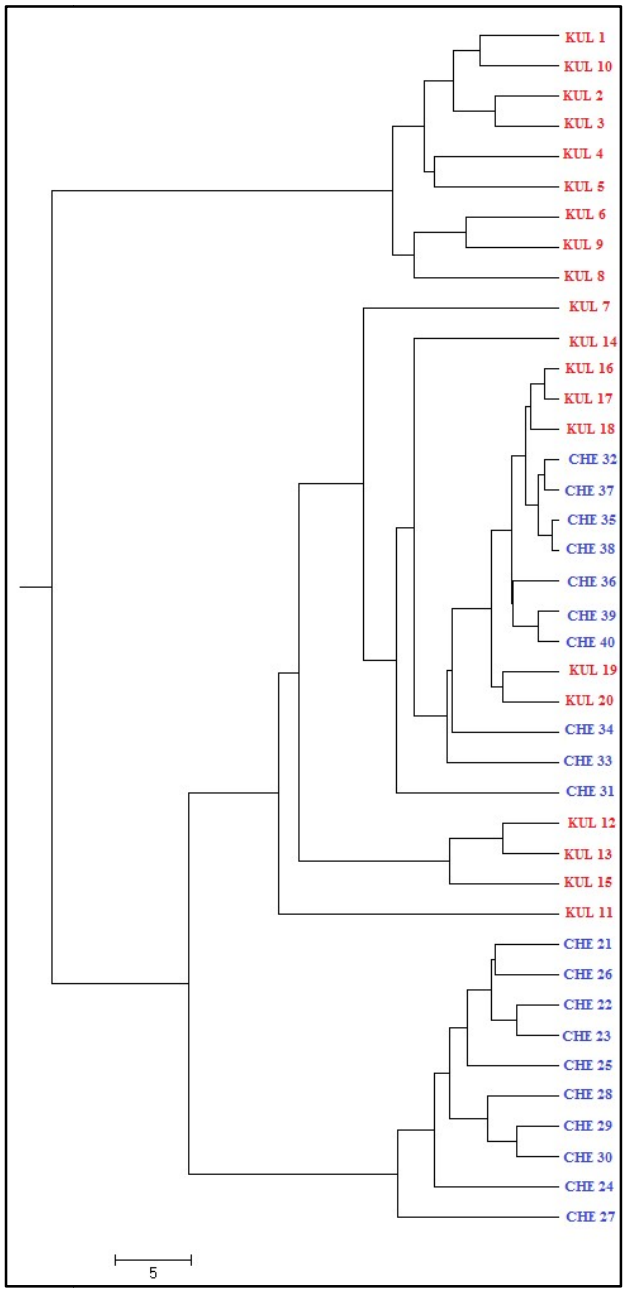


Appendix 2. ISSR profiling of Kulamavu MPCA population of *H. longipedunculatus*, Primer: UBC 810

Appendix 3. UPGMA Dendrogram of *A. indica* showing the genetic clustering of individual genotypes. (KAK- Kakkayam Dam site, CHA- Charangad, NAD-Nadugani).



Appendix 4. UPGMA Dendrogram of *H. longipedunculatus* showing the genetic clustering of individual genotypes. (KUL- Kulamavu MPCA, CHE- Cheri)



Chapter 5

Conservation

Objective 4. Development of propagation, niche modelling and restoration protocols for the target spp.

5.1. Introduction

Atuna indica and *Hydnocarpus longipedunculatus*, are two endemic and threatened tree species distributed in the southern Western Ghats, India. *A. indica* with endangered status (IUCN, 2021), belongs to the family Chrysobalanaceae and reported with anticancerous compound Umbelliferin (Asish et al. 2013). *H. longipedunculatus* is a recently described monoecious tree that belongs to the family Achariaceae (Robi et al., 2014), so far only two populations of the species recorded on repeated field surveys. Both these species were found above 700 m asl.

Restoration of threatened species is one of the easiest and most effective ways to replenish fragmented populations as well as to develop new populations in similar niches. Habitat niche models are the well-known methods used in predicting suitable habitats of the species (Philips et al., 2006). The Maximum entropy model (MAXENT) can predict suitable habitats for threatened species with which having a few numbers of species occurrence records (Pearson, 2007). Habitat niche modeling employed in the restoration of *Elaeocarpus serratus* (Baruah et al., 2019), *Ilex khasiana* (Adhikari et al., 2012), *Mesua assamica* (Baruah et al., 2016), *Vanilla borneensis* (Deka et al., 2017), *Brucea mollis* (Borthakur et al., 2018), *Calamus nambariensis* (Deka et al., 2018), etc.

To accomplish the restoration of threatened tree species, propagule collection and seedling production are two initial activities. Vegetative propagation is considered ideal for the rapid multiplication of a species while trying to maintain certain desired characteristics (Hartmann et al. 2001; Tchoundjeu et al. 2004). Plantlets raised from seeds could improve

the genetic diversity of the new population as the seeds are collected from different populations or locations.

5.2. Materials and Methods

Species distribution modeling

Species distribution data were collected using GPS (Garmin) from identified populations. Nineteen bioclimatic variables were downloaded from the WorldClim website (http://www.worldclim.org) and physiographic factors like slope, topography and hill shade were extracted from Digital Elevation Model raster file (Japan aerospace exploration, ALOS world 3D), solar radiation raster file downloaded from Global solar atlas website (global horizontal radiation), then used for prediction. But only 18 out of 25 variables are taken for model preparation (Table 1).

The physiographic factors like slope and topography were extracted from the Digital Elevation Model raster file. The spatial resolution was 30m, which was then resampled into 100m resolution. The bioclimatic variables were downloaded from the WorldClim website with ~1km spatial resolution, which was resampled to 100m resolution. The solar radiation (global horizontal radiation) is in a 250 m grid size which is then resampled to a 100 m size.

The number of populations is very low in target species. If considering a 1km grid size, *A. indica* may have only 3-4 occurrence points and *H. longipedunculatus* has two. The downscaling from 1 km to 100 m spatial resolution is not a much-recommended way as the average value of a 1 km grid is distributed into ten 100 m grids within. But it is the only

possible way to make species distribution modeling for threatened species like *A. indica* and *H. longipedunculatus*.

The pixel dimensions were 100m×100m in grid cells and the model was developed using Maximum entropy modeling (Maxent version 3.4.1). To validate model robustness, we executed five replicated model runs. In the replicated runs we used a cross-validation technique, samples were divided into replicate folds and each was used for test data. Model quality was evaluated based on Area Under Curve (AUC) was graded following (Thuilleret al., 2006) poor (AUC<0.8), fair (0.8<AUC<0.9), good (0.9<AUC<0.95) and very good (0.95<AUC<1.0).

Propagation

Stem rooting and Air layering experiments were conducted. Different plant growth regulators such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), and Naphthalene Acetic Acid (NAA) in 1000, 3000, 5000, and 7000 ppm concentrations were used for rooting. Air layering experiments were conducted in young branches of small trees. The bark girdled from 20-30cm away from the branch tip. The girdling is done in 2-3 cm length without harming the vascular cambium. Coir pith was used as a layering medium. Ten to fifteen cm-long terminal cuttings were collected from small trees. The leaves were trimmed and the cut ends of the branch cuttings were dipped in liquid media for one minute and planted in sand-filled clay pots and Kept in the mist chamber (Temperature, 28-29°C; Relative Humidity 85%).

Matured fruits of *A. indica* were collected during April- May and *H. longipedunculatus* in August- September. The seeds of *A. indica* were sown in the sand medium and kept in the mist chamber *H. longipedunculatus* seeds have shown dormancy due to a thick fleshy seed coat, which was sown after scarification. Germinated seeds were transplanted into the polybags filled with sand, cow dung, and topsoil in a proportion of 2:1:1.

Restoration and post-restoration monitoring

The established polybagged plants were used for restoration purposes. Planting sites were identified based on habitat niche modeling predictions. Planting was done in the monsoon seasons (June- July months) of 2021. Post-restoration monitoring and seedling survival data were recorded after six months.

Seed storage and Viability

The study includes fruit collection, seed processing, germination, and storage (Anilkumar et al., 2002, 2008; Chacko & Pillai, 1997; Jose & Pandurangan, 2013; Pillai et al., 2016).

5.3. Results

Species distribution modeling

The model calibration (AUC) test for *A. indica* and *H. longipedunculatus* are 0.986 ±0.015 and 0.998 ±0.002 respectively. Out of 19 bioclimatic variables and five physiographic and other variables, 18 selected variables are used for Niche modeling. The variables selected are based on the percentage of contribution. The highly suitable area,

moderately suitable area, marginal and non-suitable area for species restoration are given in Figures 1 and 2.

For *A. indica*, the environmental variable solar radiation has the highest percent contribution (46.7%), and the second is the precipitation of the coldest quarter (16.7%). The permutation importance is higher for the temperature annual range (48.4%) and solar radiation has the second highest (6.2). For *H. longipedunculatus*, the precipitation of the driest quarter (33.9%) and isothermality are second (16.1%). The permutation importance is higher for the temperature annual range (83.3%) and the second is the mean diurnal range (7.6%) (Table 1).

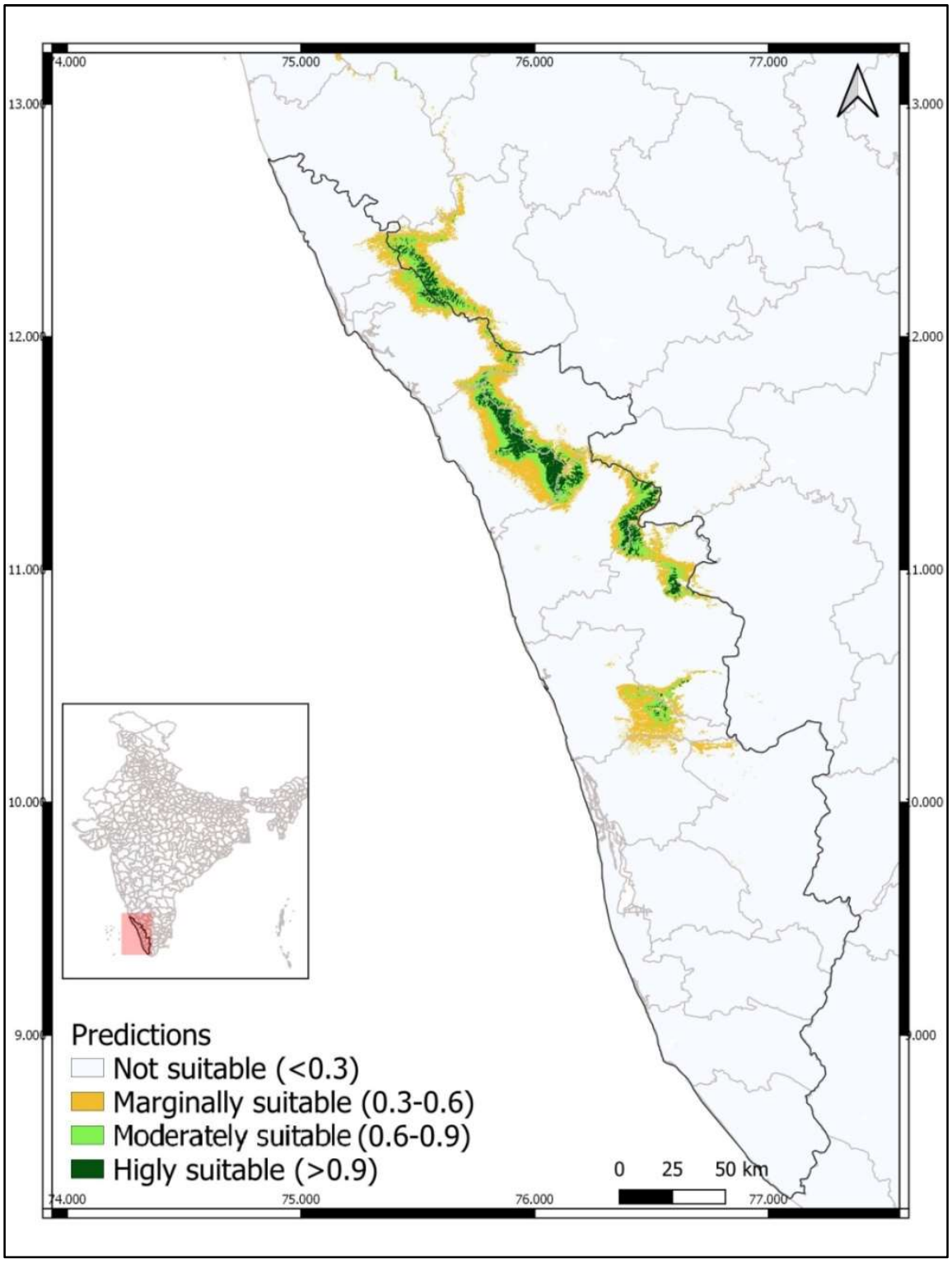


Figure 1. Map and species Distribution model of *A. indica*.

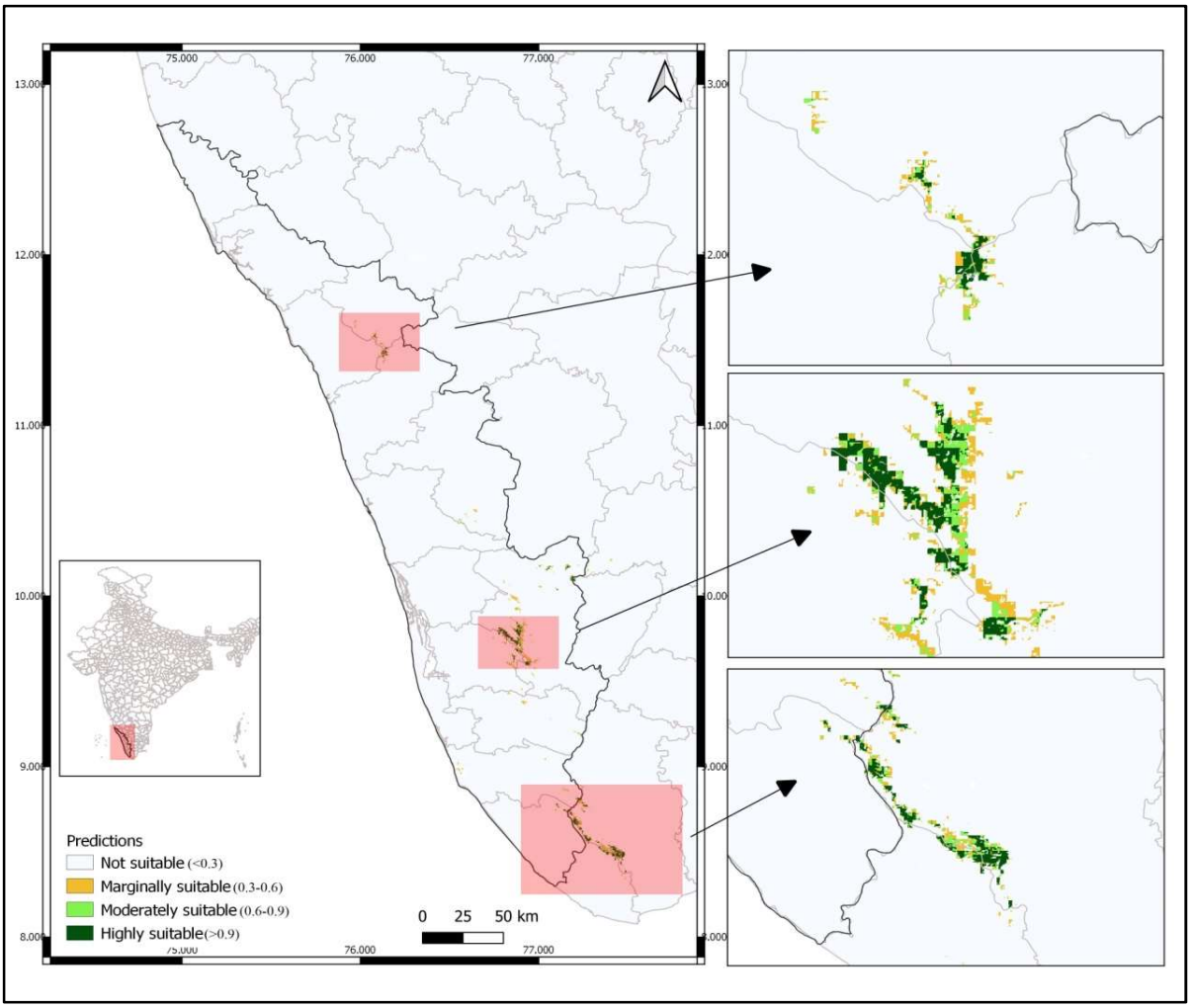


Figure 2. Map and species Distribution model of *H. longipedunculatus*

Table 1. The parameters used for Species Distribution Model, and percent contribution of selected parameters.

Code	Parameters	Used for *A. indica* & Percent contribution (%)	Used for *H. longipedunculatus* & Percent contribution(%)
BIO1	Annual Mean Temperature	-	-
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	0.3	4.1
BIO3	Isothermality (BIO2/BIO7) (×100)	0.7	16.1
BIO4	Temperature Seasonality (standard deviation ×100)	6.8	13.2
BIO5	Max Temperature of Warmest Month	-	-
BIO6	Min Temperature of Coldest Month	-	-
BIO7	Temperature Annual Range (BIO5-BIO6)	15.4	2.4
BIO8	Mean Temperature of Wettest Quarter	0.8	2.7
BIO9	Mean Temperature of Driest Quarter	-	-
BIO10	Mean Temperature of Warmest Quarter	0.5	0
BIO11	Mean Temperature of Coldest Quarter	-	-
BIO12	Annual Precipitation	-	-
BIO13	Precipitation of Wettest Month	0.1	3.6
BIO14	Precipitation of Driest Month	7.5	1.2
BIO15	Precipitation Seasonality (Coefficient of Variation)	0	9.2
BIO16	Precipitation of Wettest Quarter	0.4	0
BIO17	Precipitation of Driest Quarter	0.5	33.9
BIO18	Precipitation of Warmest Quarter	-	-
BIO19	Precipitation of Coldest Quarter	16.7	3.4
DEM	Digital Elevation model	2.3	4.5
Topography	-	0.1	0.8
Aspect	-	0	0
Slope	-	1.2	3.2
Hillshade	-	0.1	0.5
Solar radiation	-	46.7	1.1

Propagation

Atuna india: Stem cuttings taken from juvenile plants below 5 years old resulted in 20% rooting with the application of IBA 3000, NAA 3000, and NAA 5000. While increasing the concentration of auxins no improvement in rooting was noted (Fig. 3 & 4).

The fresh seeds showed an initial Moisture Content (MC) of 45% with 90 % germination in the nursery conditions. The seeds on desiccation were found viable up to 20% germinability at 10-12% MC. At the same time, seeds were susceptible to chilling conditions and found to lose viability and hence categorized under the Intermediate type. During storage, seeds were found viable for up to six months (MC 15% showed 30% germination) while kept in a polycarbonate bottle at room conditions (25- 27℃ / 50-55% RH). However, further seed storage could not possible as seeds started germination in the container itself.

H. longipedunculatus: Vegetative propagation through stem cuttings and air layering methods was conducted with the aid of auxins. Callus formation was only noted in both methods (Fig. 3).

The fresh seeds with MC, 47% along with fleshy seed coat couldn't result in any germination. However, after removing the seed coat, it showed germination up to 100% with 12.91 MC. The seeds were found susceptible to chilling conditions and tolerant to desiccation, therefore categorized under intermediate type. The white fleshy seed coat found in *H. longipedunculatus* hinders seed germination. The extremely low seed availability is a restricting factor in further seed studies of the target species.

Restoration and post-restoration monitoring

Restoration of target species carried out based on Niche modeling viz. Attapadi and Vavul mala areas, (Palakkad and Kozhikode districts respectively, in Kerala). Fifty seedlings of both species were planted. Post-restoration monitoring after six months recorded 50-60% survival of seedlings (Fig. 3).

In addition, seedlings were planted at Poyilkavu and Muchukunnu kavu (Kozhikode district) and KFRI Medicinal garden, Peechi Campus, and KFRIsub-center at Nilambur. The seedling survival was recorded at 60- 70% after 1 year of planting (Fig. 3).

Figure 3. Propagation and restoration: a- *A. indica* air layering, b- *H. longipedunculatus* air layering, c & d- *A. indica*&*H. longipedunculatus* callus formation in air layering, e, f & g- stem rooting in *A. indica* , h & i- Seed germination in *A. indica* and *H. longipedunculatus*, j- planting stock, k & l- planted seedlings of *A. indica*&*H. longipedunculatus* m & n- seedlings of *A. indica* & *H. longipedunculatus* after six month of planting, o- post restoration monitoring

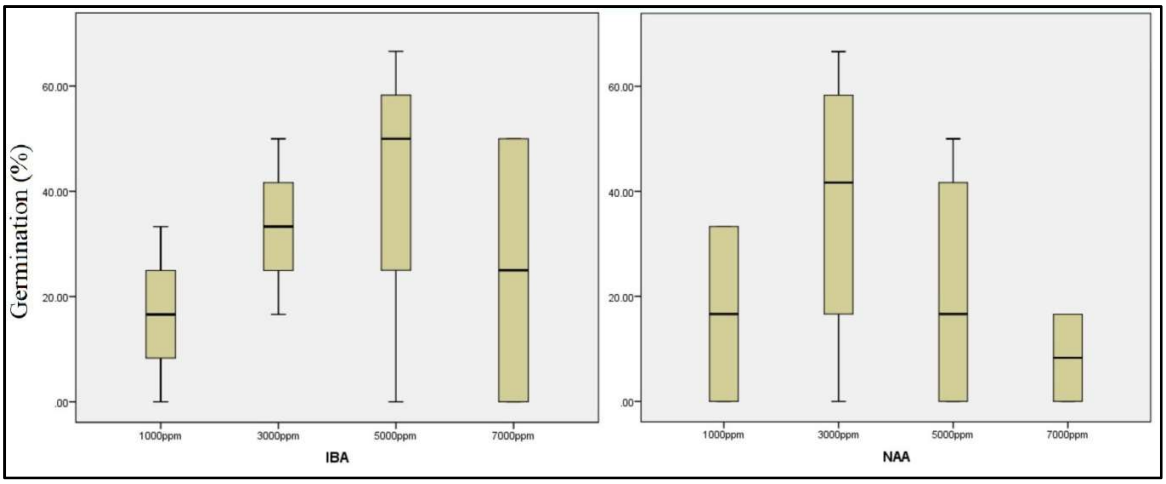


Figure 4. *Atuna indica*: vegetative propagation results

5.4. Discussion

The habitat niche modeling of both species is good as the area under curve value is 0.986 and 0.998 respectively. Solar radiation (46.7%) and precipitation of the coldest quarter (16.7%) have the highest contribution to the model prediction of *A. indica*. The high influence of solar radiation in limiting species distribution is also reported in *Colophospermum mopane* (41.8 % contribution) (Ngarega et al., 2021) and in *Baccaurea angulata* (Gunawan et al., 2021). The high influence of precipitation in the coldest quarter was reported in *Codonopsis pilosula* (Guo et al., 2017) and *Daphnae mucronata* (Abolmaali et al., 2017). The coldest quarter coincides with the flowering period of the species (October – November). The influence of precipitation in the coldest quarter has some possible role in the flowering of the species.

Precipitation of the driest quarter (33.9%) and isothermality are the first and second influential variables in the niche model of *H. longipedunculatus*. The *Zanthophyllum armatum* (Xu et al., 2019) and *Taiwania cryptomerioides* (Zhao et al., 2020) are influenced by precipitation of the driest quarter in limiting distribution prediction. *Dalbergia cultrate* (Liu et al., 2019) and *Paeonia delavayi* (Zhang et al., 2018) species distribution prediction are limited by isothermality. These high percent contributions of temperature and precipitation indicating the species are highly sensitive to temperature and precipitation fluctuations. The *H. longipedunculatus* has dependencies in topographic factors like slope and elevation compared to *A. indica*, as both populations of *H. longipedunculatus* is confined to medium-high altitude location with moderate to high slope.

Propagation studies in the genus *Atuna* are scanty. The specificity of auxins was found critical for the vegetative propagation success of the species. The application of auxins viz. IBA and NAA were found ideal for *A. indica* as shown in *Robinia pseudoacacia* and *Grewia optiva* (Swamy et al., 2002). The insignificant role of IAA compared to IBA and NAA was also reported by Jose et al. (2009) in *Hydnocarpus macrocarpa* and *Syzygium mundagam*. The Allied species *H. macrocarpa* not shown rooting in tender, semi-hard and hard cuttings while juvenile cuttings resulted in rooting with NAA. We tried tender and semi-hard cuttings from shrubby forms of the species due to the unavailability of juvenile plants, which could result in callus formation.

The reduced performance of IAA over IBA was also reported in *Lasiococca comberi* (Kamila & Panda, 2019) due to the photosensitive nature of the IAA. IBA is less prone to non-biological degradation such as photo-oxidation (Epstein & Ludwig- Muller, 1993). IBA and NAA have reported rooting plants within a short period (Bose et al., 1986).

The air layers were found more adaptable to field conditions as they experienced both biotic and abiotic stress by being part of a mature plant (Eganathan et al., 2011). The two species resulted only in callus formation with the application of auxins. The callus formation is a common stage as the stem girdling stimulates the accumulation of carbohydrates and auxins in the basal portion of girdled branches. This added food serve promotes callus formation and subsequently forms the roots (Hartmann et al., 2014). But the studied species not showed any rooting primordial even after six-month duration.

The reintroduction of threatened species will help in their long-term survival by maintaining genetic diversity in the small populations which helps to face future

environmental stochasticity (Martins et al., 2011). The restoration is done at the Vavul mala forest area showed better survival than that of the Attapadi region.

The habitat niche model is a prediction of the fundamental niche of a species, not realized niche. The biotic factors/interactions will be considered while dealing with the realized niche of a species. An ecological study of species is essential before making niche modelled restoration since the knowledge of biotic interactions (need for specialized pollinators etc.) could reveal only during the autecological approach. The ecological and reproductive biological studies of the target species do not show any specialized requirements (Subin et al., 2022; Subin & Jose *unpubli.*).

Appendix 1. Three year old *Atuna indica & H. londipedunculatus* growing in KFRI campus (Planted on June 5, 2019).


Appendix 2. Restoration at Poyilkavu Sacred groove and Vanamoolika (An NGO), Wayanad


Appendix 3. Fungal attack in *H. longipedunculatus* fruits (*Lasiodiplodia theobromae*)

Appendix 4. Micropropagation trials (Additional information- Not included in PhD objectives): 0.1 % Bavistin +2 mg BAP gave single shoot in *Atuna indica*, 0.1 % Bavistin +3 mg BAP gave callus in *H. longipedunculatus in* MS media

Atuna indica

H. longipedunculatus

Summary and Conclusions

Conservation biology is need of the hour discipline for safeguarding endangered flora and fauna from the threat of their extinction. The biological hotspot 'Western Ghats' faces biodiversity depletion at an alarming rate than ever before, mainly because of human-induced causes. The present study focuses on the conservation of two threatened trees (*Atuna indica* and *Hydnocarpus longipedunculatus*), endemic to the southern Western Ghats to prevent their untimely endangerment. The study encompasses a survey and mapping of populations, demographic and population structural analyses, phenology, reproductive biology, and genetic diversity analysis to identify the actual situation of the species in natural stands. Further, the vegetative and seed propagation practices, habitat niche modeling, and restoration were done in order to ensure the species' survival *in situ*. The reduced number of matured individuals, extremely poor natural regeneration, low number of flowering individuals, habitat loss/ degradation, low Importance Value Index, poor fruit/ seed dispersal mechanism, high predation of fruits/ seeds, severe pest incidence during flowering and fruiting, etc. were found to be the ecological and biological constraints of these species. Even though better genetic diversity and gene flow exist among the individuals/ populations of both species, it is the remains of a flourished population before the dam construction. Hence population bottleneck events are foreseeable. It is evident that the Hydroelectric Dam constructions (Kakkayam dam- *A. indica* and Kulamavu dam – *H. longipedunculatus*) in the source population areas

severely affected the population size of both species. The habitat niche model and subsequent restoration could be treated as visible conservation initiatives of the target species aides the large-scale restoration in the future. The seed coat-induced dormancy in *H. longipedunculatus* is an evolutionary advantage to prevent rapid viability and found to restrict natural regeneration however removal of the seed coat enables germination *ex situ.*

Recommendations

The conservation biological studies were carried out in two endemic and threatened tree species viz. *Atuna indica* and *Hydnocarpus longipedunculatus*, distributed in the Kerala part of the Western Ghats resulted in the identification and documentation of the causes of the rarity at species, ecosystem, and gene levels. The study also enabled to develop the conservation and restoration protocols as part of the sustainable management practices of these species. In this context, the following recommendations are presented.

- The three populations identified of *Atuna indica* consist of 79 adult individuals, however, only two individuals at the Kakkayam Dam site population were recorded with the flowering of which one tree was found to fall during the study period. The present study points toward the critical role of the sunlight flowering behavior of species. *Atuna indica* were found growing in the medium-high elevation forests in the southern Western Ghats as sub-canopy species, in-depth studies are recommended to explore the behaviour of flowering.
- The low seed availability of both species became a constraint for seed germplasm storage. The baseline data generated during the present study could be taken for the detailed seed biological studies.
- The poor rooting performance invites multiple trials for vegetative multiplication of these species and plant production *ex-situ.*

- The Habitat protection of target species is to be prioritized as the populations of both species are located in reserve forests and face a high degree of habitat threats. Even though the population of *A. indica* (Kakkayam Dam site) is within the Malabar WLS, the tourist interventions pose a threat to the species. Extreme care is also to be taken during fire protection work carried out in the premises of the population areas of the target species.
- It is also to be considered the sampling of seedlings and saplings to arrive at a better understanding of the population genetics of the species.
- Many biotic incidences were recorded during the flowering and fruiting of both species. Climate change/weather-induced shifts were seen in reproductive cycles and detailed studies are required on the repercussions of flowering cycle shifts on dependent fauna.

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Rarity analysis of an endangered tropical tree species of the Western Ghats, India

K. Subin, P. A. Jose* and A. S. Vivek

Sustainable Forest Management Division, Kerala Forest Research Institute, Peechi 680 653, India

The rarity of *Atuna indica*, an endemic and endangered tropical tree species of the Western Ghats, India has been analysed in this study. The phenology, floral biology, including pollen–ovule ratio, pollinators, fruit development and biotic interactions in natural dynamics were studied. Extreme reduction in the number of flowering individuals, microhabitat conditions, low fruit set, seed infestation and fruit predation were identified as the factors leading to rarity of the species *in situ*.

Keywords: *Atuna indica*, endangered species, phenological changes, rarity analysis, reproductive biology.

THIS study aims to document the reproductive biology as well as phenological changes in *Atuna indica*, an endangered tree species of the Western Ghats, India. Studies on the reproductive biology of threatened tree species provide insights into their reduced fitness/population size. For example, *Elaeocarpus blascoi*, an endangered species reported with low seedling recruitment, was found with fungal attacks on seeds (*Fusarium* sp., *Lasiodiplodia* sp. and *Pencilium* sp.)[1]. Studies conducted on the endangered *Elaeocarpus gaussenii* and *Elaeocarpus recurvatus* reported that fruit damage caused by Malabar giant squirrel and lion-tailed macaque is one of the reproductive constraints[2]. In *Talbotiella gentii*, a critically endangered tree, the stigmatic surface was found infected by fungi, thus reducing its reproductive potential[3]. Even the Evans *et al.*[4] reported reproductive constraints in endangered perennial herbaceous species such as *Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae.*

The present study incorporates climate data (average precipitation and temperature in Kerala, India) to discuss the phenological changes of *A. indica*. Kerala experiences different but almost stable climatic seasons such as the southwest monsoon, northeast monsoon, winter and summer. So phenological studies, including reproductive biology, will highlight the influence of climate on the reproductive performance and survival of species. The variations in atmospheric temperature, rainfall and difference in day length, etc. could signal flushing, flower initiation, etc. These changes may lead to a cascade of positive or negative influences on the depending fauna and eventually on the survival of the plant species, as the plant reproductive cycle depends on pollinators, parasites and pests, which are obligatory and species specific in nature.

Reduced reproductive potential is considered one of the driving forces towards extinction. High reproductive potential may increase seedling recruitment, subsequently resulting in flourishing of the population[5,6]. The reasons for rarity may vary from one species to another. The mode of pollination and type of dispersal impact the future population by influencing the genetic as well as the physical constitution of a population. Abnormalities in these events may result in rarity of the species *in situ*. The factors leading to species decline include reduced pollinator-driven low fruit set, self-pollination driven inbreeding depression[7], and loss of genetic variability as a compound effect. Documenting the reproductive biology of endangered plant species could help unravel their constraints. The rarity analysis of *A. indica* with an emphasis on its reproductive biology will be useful for government and non-governmental organizations in their conservation efforts of endangered tree species.

A. indica is an endemic and endangered tropical tree species of the Western Ghats, India[8]. It is distributed in the evergreen forests in the 500–800 m altitude range and grows up to 15 m height. Flowers are bisexual and cream-coloured. Slopes adjacent to water courses are the microhabitat preference of this species. Umbelliferin (an anticancer drug) has been isolated from *A. indica*[9].

Materials and methods

The population located adjacent to Kakkayam dam site, Kozhikode district, Kerala was chosen for this study (Figure 1). Monitoring and recording of flowering phenology, viz. flower-bud initiation, development, anthesis, stigma receptivity, pollen viability, pollen–ovule ratio, pollination, pollinators, blooming period, pest incidence and fruit set, was done. The data were represented as the average value of each trial[10–14].

Reproductive phenology

Data on reproductive phenology with respect to the number of inflorescences per branch, number of flowers per inflorescence, flower/inflorescence development, blooming

*For correspondence. (e-mail: pajosekfri@gmail.com)

period, fruit initiation and development were recorded daily. Five inflorescences per branch were tagged and monitored for flower development from bud to full bloom. The average number of days taken for each bud to bloom was calculated and recorded. The monthly mean temperature and mean precipitation of the respective area were obtained from WorldClim database using DIVA GIS.

Pollen viability

Pollen grains from fully mature flower buds were dusted into a cavity slide containing acetocarmine solution and kept for 1 h. Later observed under a compound microscope. The pollen grains stained were treated as viable and the others as non-viable. Viability test was carried out in 2 h intervals.

Pollen germination

Pollen grains from fully mature flower buds were transferred to a cavity slide containing a germination medium (sucrose 10%). Pollen germination was counted after 1 h using a compound microscope. The pollen grains with tubes longer than the diameter were considered germinated. The experiment was repeated in 2 h intervals from anthesis.

Stigma receptivity

Both physical (through hand lens) and chemical (using hydrogen peroxide) tests were conducted. In the former method, stigma with wetness, turgidity and oily nature was considered as receptive and the rest as non-receptive. In the latter method, a drop of hydrogen peroxide was added to the stigma of freshly opened flowers and the effervescence resulting from the peroxidase enzyme activity was observed for the duration of stigma receptivity[15].

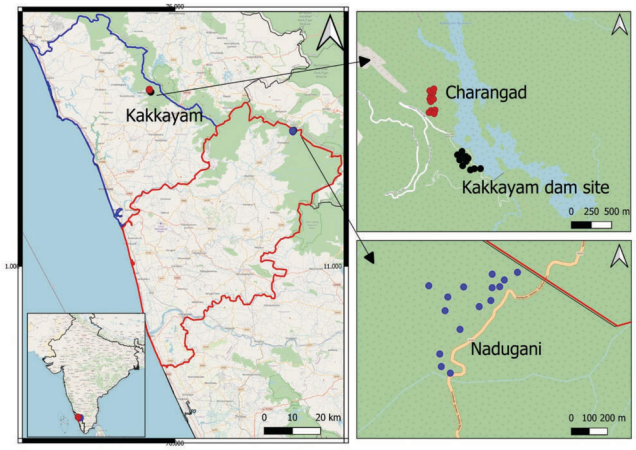

Figure 1. Distribution of *Atuna indica* in the Western Ghats, India. Study conducted on the Kakkayam dam site population.

Pollen–ovule ratio

The number of pollen grains in anthers per flower was counted using a haemocytometer[16]. The number of ovules per ovary was counted by taking sections of the ovary[17]. The pollen–ovule ratio was calculated as follows

$$\text{Pollen–ovule ratio} = \frac{\text{Pollen count per anther} \times \text{No. of anthers per flower}}{\text{No. of ovules per flower}}.$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. Physical observations were made throughout the flowering period and insect interactions were recording during day and night hours. The taxonomic identification of insects was made using the available literature and with the help of experts.

Fruit phenology

Fruit phenology was monitored and recorded, viz. fruiting primordia, period of development, including premature abscission and pest incidence.

Results

Flowering was observed along with flushing during October–December. Fruit development started in January and fruits matured in April. The trees displayed a vegetative phase from May to September. Only one out of 89 trees in the population showed flowering. A total of 13 branches showed flowering, which included 113 inflorescences bearing 521 flowers.

Reproductive phenology

Pale green-coloured flower buds were recorded during the first week of October and mass blooming was noted after two weeks in 2017 (trees showed differential flowering in branches: in 2018, the northeastern, sun-facing branch flowered first, and 2–3 weeks later, the opposite branches flowered). Flower opening started from 0600 to 0615 h and opened fully by 0915 h. Anther dehisced through vertical slit from 0900 to 0930 h. Stigma was receptive prior to anther dehiscence (0800–0830 h, protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis – 0615 h) showed 98.32% viability and gradual reduction was noticed to 93.6%,

Figure 2. ***a***, Flower buds; ***b***, flower; ***c***, fruits; ***d***, pollen grain; ***e–g***, SEM image of mouth parts of humming bird hawk moth showing pollen grains; ***h–j***, SEM image of mouth parts of *Xylocopa* sp.; ***k***, *Badamia* sp.; ***l***, *Apies mellifera*; ***m***, *Eurema* sp.; ***n***, *Graphium* sp.; ***o***, unidentified; ***p***, purple throated sunbird; ***q***, *Indrella ampula*; ***r***, pierced flower bud; ***s***, Pyralid larvae in flower; ***t***, flower bud eaten by snail; ***u***, Pyralid larvae in fruit.

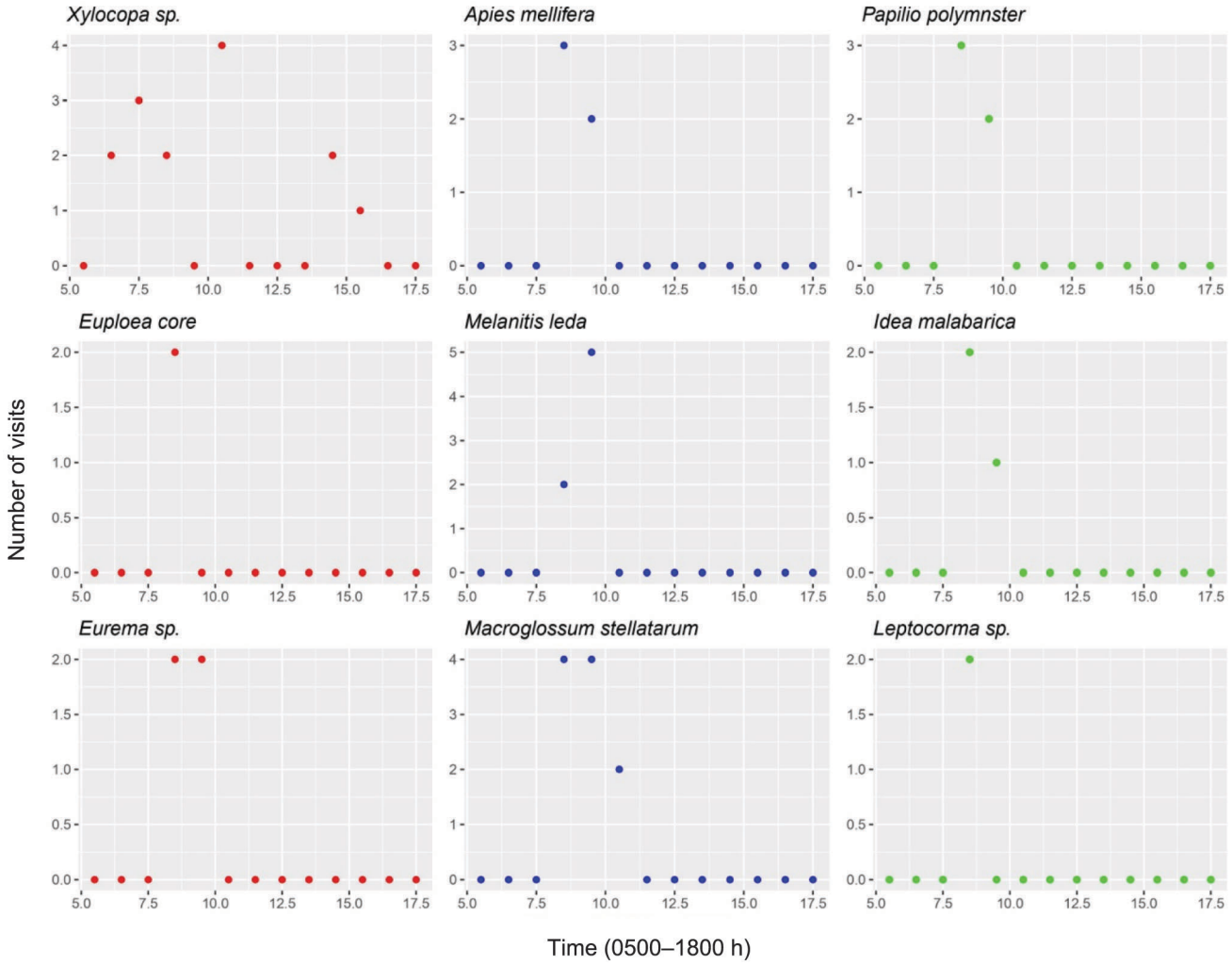

Figure 3. Flower visitors of *A. indica* from 0500 to 1800 h.

91.3%, 88% and 87.5% after 1, 2, 3 and 4 h respectively. A drastic decline to 20% was noted after 12 h. Hydrogen peroxide application followed by effervescence confirmed stigma receptivity up to 14 h, which later turned brown–black in colour, lost its turgidity and became non-receptive.

Pollen germination

At the time of anthesis, 58% of the pollen grains were found germinated in a 10% sucrose solution. A gradual decrease in pollen germination was observed at 47.8%, 23.2% and 9.74% after 1, 2 and 8 h respectively.

Pollen–ovule ratio

A flower contains 12–13 anthers and approximately 288 ± 51.3 pollen grains per anther. Hence pollen count per flower was estimated as 3744 ± 667. A flower has two ovules and hence the pollen–ovule ratio was estimated as 1872 : 1.

Pollination and insect interaction

The bagging experiment had failed; all the bagged inflorescences had fallen off. Pollinator documentation was done in 2017 and 2019. Observations were made from 0500 to 1800 h continuously. The peak time of pollinators incidence was recorded between 0800 and 1000 h and *Xylocopa* sp. was first visited during 0600–0700 h. *Apies mellifera*, *Idea malabarica*, *Eurema* sp., *Papilio polymnster*, *Euploea core*, *Graphium* sp., were the key pollinators seen multiple times (Figures 2 and 3). No flower visitors were recorded between 1100 and 1500 h. *Xylocopa* sp. was found foraging from 1500 to 1700 h. Though the *Macroglosum stelletarum* was not recorded during 2017, it was a frequent floral visitor in 2019.

Xylocopa sp., *M. stelletarum* and *A. mellifera* were found visiting many flowers, spending 2–3 sec per flower. Butterflies visited 2–3 flowers each time. Mouth parts of pollinators collected from the study site were observed through a compound microscope and photographs taken with a scanning electron microscope (SEM). The images

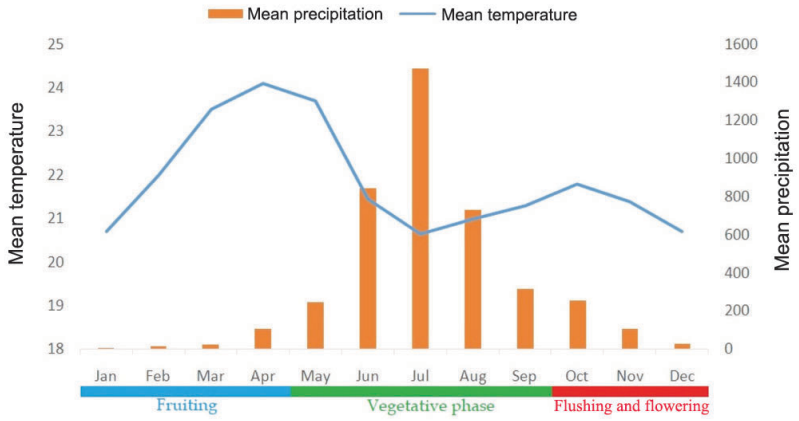

Figure 4. Phenological changes and climatic conditions of *A. indica* at the Kakkayam dam site, Kerala.

were compared with the SEM images of *A. indica* pollen grains, thus confirming their role as a pollinator (Figure 2 *d–j*). A troop of monkeys visited during the flowering season, jumping on the branches and causing flowers to fall. Inflorescence fall was recorded and the fallen inflorescence found with larvae (unidentified).

Cut-open fruits were found with Pyralidae larvae, which caused 30–40% fruit loss (Figure 2). The adult possibly lays eggs in the late flowering stage.

Discussion and conclusion

The major climatic variables that cause phenological changes are rainfall, temperature, insolation and water stress[18–21]. The flushing along with flowering in October–December in *A. indica*, reveals that flowering occurs after the monsoon season (Figure 4), (southwest monsoon, June–September in the southern Western Ghats). Kerala experiences a cool climate during the early weeks of October without any rainfall. Then the northeast monsoon sets in with lightning and thunder in the evening hours. Senescence of ripened fruits is observed during April and May. The hard seed coat delays seed germination *in situ*. However, at the start of the southwest monsoon, the seeds begin to germinate. The pollen–ovule ratio of the species supports cross-pollination (according to Cruden[17], the pollen–ovule ratio = 31.9–396 for autogamy).

The biotic interactions are influenced by phenology, population density and inter-population distance (for pollinators)[22]. Phenological variations mediate available pollinators, seed dispersal agents and florivorous insects[23,24]. This could also influence adaptation in primary and secondary consumers[25]. The most frequent pollinators are *Xylocopa* sp. and *A. mellifera.* Pollinator abundance and behaviour is the key factor in the seed set for entomophily-depending flowers[26]. Abscission of fruiting primordia (monkey-induced) was also observed. Honey bees are common pollinators found in target species, even though they are reported as less efficient because they collect pollen from various resources, resulting in deposition of pollen from multiple species on the stigma surface[27]. *A. indica* possesses coloured petals, nectar, scent and discoid-shaped stigma, which are found favourable for biotic pollination[3], it emphasizes cross-pollination of the flowers. The common pollinators of the target species are carpenter bees (*Xylocopa* sp.) and *A. mellifera*. *Xylocopa* sp. prefers medium-sized, yellow–white-coloured flowers with odour and nectar[28], which is characteristic of *A. indica* flowers. Carpenter bees are effective pollinators which support and maintain genetic variability as they travel long distances[28]. *A. mellifera* showed high efficiency in pollination[29], as reported in *Jatropha mollissima* and *Jatropha mutabilis*[30]. The large number of floral visitors reported in *A. indica* may be due to the exposed nature of the reproductive organs, as preferred by *A. mellifera.* Heavy loss in the developing inflorescence by the caterpillar lead to reduced number of flowers (adult to be identified).

The reasons for the reduced fruit set are stressful environment as well as resource limitation[31,32]. Apart from the minimal fruit set, the species showed restrictions in forming viable seedlings. The Pyralid larvae incidence in *A. indica* caused 50–70% loss in fruit set. Pyralid moths (snout moths) were also reported damaging cereals, dry fruits, etc.[33]. The fruits of *A. indica* are hard and therefore not eaten by birds or mammals. Generally, seeds of 70% of tropical forest species are dispersed by animals[34], but *A. indica* does not have any seed dispersers (the fruits are found only under the flowering tree).

The major limitation of this study is the extremely low number of flowering individuals, i.e. only two. One tree fell in 2017, and thus data collected from a single tree are presented here. The flowering phenology based on a single flowering individual may not be adequate to prove the behaviour of the species. However, it emphasizes the need for immediate conservation measures. Based on the microhabitat conditions, we conclude that less availability of sunlight is the reason for the low number of flowering

individuals. The flowered individuals are in forest edges facing the eastern side, while the non-flowering individuals are under a canopy of other species. In-depth studies on light and flowering relations are recommended.

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Distribution, ecology and status assessment of *Hydnocarpus longipedunculatus* Robi, Sasidh. & Jose (Achariaceae: Flacourtiaceae s.l.) – an endemic tree of the southern Western Ghats, Kerala, India

K. Subin[1,3], P.A. Jose*[1], K.V. Jithin[1] and Suma Arun Dev[2]

[1]Sustainable Forest Management Division, [2]Forest Genetics and Biotechnology Division
Kerala Forest Research Institute, Peechi- 680 653, Kerala, India
[3]University of Calicut, Thenhipalam, Kerala- 673 635
*Corresponding author: pajosekfri@gmail.com

दक्षिणी पश्चिमी घाट, केरल, भारत में एक स्थानिक वृक्ष *हिडनोकार्पस लॉन्गिपिडेंकुलेटस* रॉबी, ससिध. एवं जोस (अकारिएसी: फ्लैकौरसिएसी, एस.एल.) का वितरण, पारिस्थितिकी और स्थिति का आकलन

के. सुबिन, पी.ए. जोस, के.वी. जितिन एवं सुमा अरुणदेव

सारांश

हिडनोकार्पस लॉन्गिपिडेंकुलेटस रॉबी, ससिध. एवं जोस (अकारिएसी: फ्लैकौरसिएसी, एस.एल.) दक्षिणी पश्चिमी घाट, भारत के केरल भाग में वितरित एक स्थानिक वृक्ष है। जनसंख्या संरचना और विविधता विश्लेषण के साथ-साथ जनसांख्यिकीय जानकारी ने वन परिदृश्य में वितरण स्वरूप, आयु वर्ग / ओन्टोजेनी, महत्व मूल्य सूचकांक (आईवीआई), वयस्कों की संख्या, पौधों और प्रजातियों के रोपण की समझ को सक्षम किया है। आईयूसीएन दिशानिर्देशों के अनुसार प्रजातियों की उपस्थिति और अधिभोग के क्षेत्र की सीमा का पता लगाया गया है। छोटी आबादी का आकार, पौधों की बेहद कम संख्या, कम माला में होने और कम आईवीआई मूल्यों के साथ अधिभोग का क्षेत्र प्रजाति की प्रमुख बाधाएं थीं। हम इस प्रजाति को आईयूसीएन लुप्तप्राय श्रेणी में शामिल करने का सुझाव देते हैं क्योंकि यह 'डी' मानदंड को पूरा करती है। आधारभूत अध्ययन प्रजाति के प्राकृतिक आवासों के संरक्षण सहित तत्काल संरक्षण उपायों की सिफारिश करता है।

ABSTRACT

Hydnocarpus longipedunculatus Robi., Sasidh. & Jose (Achariaceae: Flacourtiaceae s.l.) is an endemic tree distributed in the Kerala part of the Southern Western Ghats, India. The population structure and diversity analysis along with demographical information enabled the understanding of the distribution pattern, age classes/ontogeny, Importance Value Index (IVI), number of adult individuals, saplings and seedlings of the species in the forest landscape. The extent of occurrence and area of occupancy of the species were found out as per IUCN guidelines. The small population size, extremely low number of seedlings, lesser extent of occurrence and area of occupancy along with lower IVI values were the major impediments of the species. We suggest the inclusion of the species in the IUCN endangered category as it meets the threshold under criterion 'D'. The baseline study recommends immediate conservation measures including protection of the natural habitats of the species.

Keywords: Conservation, Distribution, Ecology, IUCN status, Kerala, Western Ghats

INTRODUCTION

Western Ghats of India are well known for their biological diversity although it faces various threats. Western Ghats enlisted in UNESCO World Heritage site as well as one of the eight biodiversity hotspots in the world (Myers & al., 2000). Nayar & al. (2006) reported 7402 flowering plants in the Western Ghats, among those, 1273 species are endemic to the region. Hunting, livestock grazing, presence of exotics and invasive species, extraction of firewood/fodder, timber felling and forest fire were the most proximate threats to biodiversity reported in Western Ghats (CEPF n.d.). The flowering plants face severe threats like habitat loss/ modifications,

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overexploitation, irregularities in flowering, low fruit set, and poor natural regeneration (Drury, 1974; Rabinovitz & al., 1986; Nayar, 1996; Sasidharan, 2017). In addition, species also face threats from biotic agents like pest incidence as in fruits of *Atuna indica* (Subin & al., 2022), *Cynometra beddomei* (Jose & al., 2016) and *Gluta travancorica* (Jose, 2001).

Basic population studies of newly reported species are helpful in getting attention from researchers, conservation biologists, and governmental and non-governmental organizations. The autecological studies include analysis of population structure, diversity, dynamics and genetics of a species. This altogether gives a basic knowledge of the life history and population trends (Jose, 2001; Ramachandran & al., 2014). In this work, we carried out the population structure and diversity studies along with assessing IUCN criteria of *H. longipedunculatus,* a newly reported species (Robi & al., 2014) from Western Ghats, India.

The flushing of *Hydnocarpus longipedunculatus* Rabi, Sasidh. & Jose starts in purple colour later turned into green. The leaves are chartaceous and straight. Lateral nerves are 4–6 pairs and arranged distantly. The inflorescence is pseudo-cymes, peduncle slender and 5–6.5 cm in length. Pedicels are sparsely stellate, pubescent and 5 cm long. Flowers are greenish-white and fragrant. Sepals are 12 × 5 mm and sparsely pubescent, petals 15 mm long and margins strongly incurved. The fruit is ovoid-oblong, pentangular and the apex is beaked (Fig.1). The species plausibly has medicinal properties. The genus *Hydnocarpus* is well known for its seed oil, which is used against leprosy and has antimicrobial properties (Sahoo & al., 2014). The local people use seeds as a fungicide in Ginger farming, and collect them as Non-timber forest produce.

METHODS

Field survey

Extensive field surveys were carried out in the Western Ghats of Kerala region from 2015 to 2018. Primarily, the adjacent locations of type locality were thoroughly searched to find out more populations of the species. Suggestions from the taxonomists were also employed for population search.

Demography, Population structure, diversity & abundance

The total number of mature individuals (≥ 30 cm GBH), Saplings (10-30 cm GBH), established seedlings (≥1m height), and unestablished seedlings (<1 m height) were enumerated from the populations. Spatial distribution, vertical distribution (height range of *H. longipedunculatus* among the associated tree species in the community), age class distribution/ontogenic classes (such as pre-reproductive and reproductive) were analyzed in identified populations (Parthasarathy & Sethi, 1997; Pascal, 1988; Jose & al., 2018).

Species diversity and abundance of *H. longipedunculatus* in the community were assessed by laying seven random sample plots of 50×20 m in each population covering 7000 m^2 area per population. The species with GBH of more than 30 cm were counted, and measured for diversity analysis. The Importance Value Index (IVI) was calculated, which is the sum of relative frequency, relative density, and relative dominance. Each parameter is calculated out of one and IVI is out of three. The IVI is calculated by using the INVENT NTFP software (Sivaram & al., 2006).

The extent of occurrence and area of occupancy of the species was calculated using GeoCAT software (VAST, 2019) based on IUCN criteria.

RESULTS

The population survey resulted in the identification of two populations of *H. longipedunculatus* in the Kulamavu forests, Idukki District, Kerala (Fig. 2). One was the type locality, Cheri forest area (N 9^0 49′ 21.71″, E 76^0 54′ 35.1″, 820m asl) and the other was at Kulamavu Medicinal Plant Conservation Area (MPCA) (N 9^0 48′ 32.66″, E 76^0 53′46.43″, 834 m asl). The populations are situated within ~3 km distance.

The species has a low number of mature individuals and seedlings. Trees having GBH ≥30 cm are treated as mature individuals. A total of 76 mature individuals were counted from both populations (36 individuals from the Kulamavu MPCA and 40 from the Cheri). Five saplings and eight established seedlings were noted from Kulamavu MPCA. Six saplings and six established seedlings were counted from Cheri. Seedlings below 1m in height (unestablished) were absent in both populations.

Hydnocarpus longipedunculatus has a poor stand in population structural aspects like spatial distribution, vertical distribution and age class distribution. Kulamavu MPCA population is situated adjacent to the Idukki Dam reservoir in a slight slope landscape and occurred in a clumped manner. In vertical distribution, *Calophyllum polyanthum* is the dominant tree species with a height greater than 30 m. *H. longipedunculatus* occupied as the third layer species with a height of 5–15m along with individuals of *Actinodaphnae malabarica*, *Vateria indica* etc. Twenty-six individuals were recorded in the flowering stage (reproductive class) and ten individuals are in the pre-reproductive stage. Cheri population has situated in

Fig. 1. A–E. *Hydnocarpus longipedunculatus*: A. Habit; B. Flushing along with mature leaves; C. Flowering branchlet; D. Fruit; E. Cut opened fruit.

a deep slope area adjacent watercourse and observed in clumped manner. *Calophyllum polyanthum* is occupied as the first-storey species with a 30-35m height range. *H. longipedunculatus* grows as second-storey species along with *Actinodaphnae malabarica* with a height range of 15-25 m. *Baccaurea courtallensis* and *Xanthophyllum arnottianum* were the major third-storey species with a 5-15 m height range. Thirty-two individuals are in the reproductive phase and eight are in the pre-reproductive stage. No individuals in the post-reproductive stage were noted among the populations.

H. longipeduncalatus has a low Importance Value Index (IVI)/abundance in the community. In Kulamavu MPCA, 49 species were recorded in sample plots of 7000 m^2 area. The Importance Value Index of *H. longipedunculatus* was 0.0382 and relative position of 33rd out of 49 species. The highest IVI-holding species are *Calophyllum polyanthum* and *Calophyllum calaba* with 0.1909 and 0.1719 respectively. *Lagerstroemia speciosa* with the lowest abundance in the community with an IVI of 0.0156 (Table 1). In Cheri, 40 species were recorded from the 7000 m^2 sampled area. The IVI of *H. longipedunculatus* was 0.0480 and relative position of 30th out of 41 species. The highest IVI-holding species are *Calophyllum polyanthum* and *Calophyllum calaba* with 0.1944 and 0.1554 respectively. *Lagerstroemia speciosa* was the least dominant species in the tree community with an IVI of 0.0281 (Table 2). Based on IUCN criteria, we suggest the placement of *H.longipedunculatus* in the Endangered (EN) category under criterion D (Number of individuals less than 250), the extent of occurrence and area of occupancy and the total number of matured individuals are 0.597 km^2, 12 km^2 and 76 respectively.

DISCUSSION

H. longipedunculatus is endemic to the Western Ghats of Kerala having two populations recorded so far with 76 mature individuals cumulatively. The small population size with poor natural regeneration is found inimical for its survival. According to Ellstrand and Elam (1993), small populations are with potential genetic risks such as reduced gene flow, genetic diversity and increased genetic drift and inbreeding. The small population-related genetic risks put the overall fitness of the species in danger.

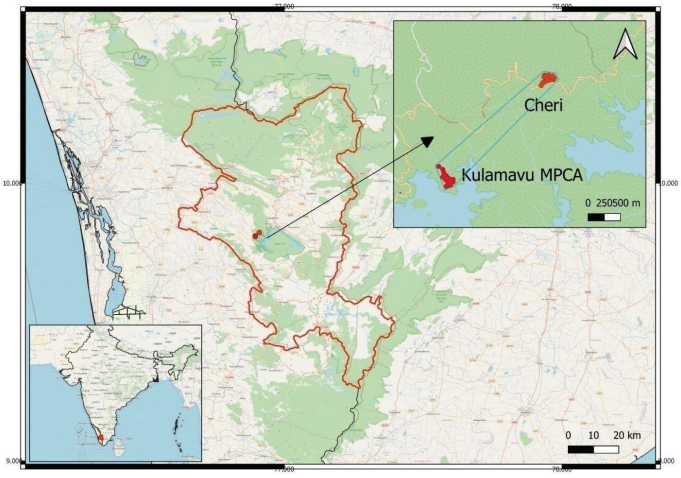

Fig. 2. Distribution map of *Hydnocarpus longipedunculatus* in Western Ghats, India

Table 1. *H. longipedunculatus*: Population diversity analysis at Kulamavu MPCA

Species	Relative density	Relative basal area	IVI
First-storey species (26-35 m)			
Calophyllum polyanthum Wall. Ex Choisy	0.0283	0.1372	0.1909
Poeciloneuron indicum Bedd.	0.0378	0.0734	0.1366
Palaquium ellipticum (Dalz.) Baill.	0.0268	0.0321	0.0843
Cullenia exarillata Robyns	0.0331	0.0210	0.0668
Second-storey species (16-25m)			
Actinodaphne malabarica Balakr.	0.0488	0.0433	0.1175
Calophyllum calaba L.	0.0205	0.1135	0.1719
Vateria indica L.	0.0189	0.0551	0.0993
Aglaia barberi Gamble	0.0173	0.0059	0.0487
Olea dioica Roxb.	0.0157	0.0032	0.1076
Third-storey species (5-15m)			
Melicope lunu-ankenda (Gaertn.) Hartley	0.0141	0.0073	0.0342
Chionanthus courtallensis Bedd.	0.0205	0.0078	0.0409
Hydnocarpus longipedunculatus Robi, Sasidh. & Jose	0.0078	0.0050	0.0382
Garcinia gummi-gutta (L.) Robs. var. gummi-gutta	0.0110	0.0066	0.0303
Other 38 trees	0.6980	0.4875	1.8320
Total	1	1	3

Table 1. H. longipedunculatus: Population diversity analysis at Kulamavu MPCA

Species	Relative density	Relative basal area	IVI
First-storey species (26-35 m)			
Calophyllum polyanthum Wall. Ex Choisy	0.0289	0.1417	0.1944
Calophyllum calaba L.	0.0193	0.1123	0.1554
Vateria indica L	0.0193	0.0610	0.1041
Second-storey species (16-25m)			
Actinodaphne malabarica Balakr.	0.0482	0.0411	0.1131
Persea macrantha (Nees) Kosterm.	0.0385	0.0778	0.1401
Hydnocarpus longipedunculatus Robi, Sasidh. & Jose	0.0193	0.0049	0.0480
Third-storey species (5-15m)			
Melicopelunu-ankenda (Gaertn.) Hartley	0.0128	0.0053	0.0419
Aporosa cardiosperma (Gaertn.) Merr.	0.0128	0.0046	0.0412
Xanthophyllum arnottianum Wight	0.0642	0.0101	0.0981
Other 32 trees			
Total	**1**	**1**	**3**

Reduced seedling count (only 14 seedlings above 1m ht), indicates plausible impediments in the reproductive potential of the species as well as predicts its hampered future. Poor regeneration was reported in other threatened trees like *Humboldtia bourdillonii* (Rahul & al., 2020), *Hopea erosa* and *Hopea glabra* (Sanil & al., 2017) and it indicates reduced population health. The reproductive constraints-driven low seedling count was also reported in other Western Ghats tree species, like *Atuna indica* (Subin & al., 2022), *Dipterocarpus bourdillonii* and *Humboldtia bourdillonii* (Swarupanandan & al 2014). The occurrence of flower-damaging larvae (Cecidomyidae), fruit-eating mammals (Malabar giant squirrel) and seed-coat-induced germination constraints (Subin & Jose *Unpubl.*) was observed in *H. longipedunculatus* proving the role of reproductive performance in the low seedling count. Moreover, the age pyramid of the species is 'Urn' shaped, with a low number of younger pre-reproductive individuals/seedlings, indicating poor population health.

The individuals of the species are spatially distributed in a Clumped manner. The clumped distribution indicates the microhabitat preference of the species mainly resulting from short propagule dispersal distance (Bleher & al., 2002), and widely distributed species have better dispersal modes. Conversely, Odum (1971) states that clumped distribution is normal in natural forest stands. The Kulamavu MPCA population is found adjacent to a Dam reservoir (Idukki Hydro electric project) (Fig. 1). The water rising due to dam construction (1975- Dam established) possibly lead to the loss of forest species in the area including *H. longipedunculatus*.

The vertical distribution of forest communities is determined by the competitive behaviour of tree species towards sunlight. The non-vigorous trees lag behind the race and end up as sub-canopy/under-storey species and this could result in compromising growth as well as reproduction potential (Moutakes & Evans, 2015). In vertical distribution, *H. longipedunculatus* occupies the second storey/under-canopy species in the Kulamavu MPCA population and interestingly, some individuals in the Cheri population (situated in deep slope areas) were found as canopy species growing to a height of 25-30m. It shows the role of landscape slopes in helping sub-canopy species to attain better vertical growth as the species gets enough sunlight in Eastern facing slopes. Moreover, the sloppy habitat of a species generally helps in fruit dispersal wherein the species lacks a well-defined dispersal mechanism as in the case of *H. longipedunculatus*, its seeds are dispersed mainly by gravity-induced methods (Subin & Jose, *Unpubl.*).

The low IVI value of *H. longipedunculatus* indicates the low abundance of the species in the community. The IVI values are used by ecologists to elucidate features of the community (Lamont & al., 1977). Generally, the endemic and endangered plants have low IVI values as reported in *Gluta travancorica* and *Ochreinauclea missionis* (Jose, 2001), *Cynometra beddomei* and *Kingiodendron pinnatum* (Jose & al., 2017) of the Western Ghats, India.

CONCLUSION

The low number of populations (2 Nos.) and mature individuals (76 Nos.), poor natural regeneration, clumped distribution, under-canopy nature, and low Importance Value Index in the community are the possible signs of survival risk in *H. longipedunculatus*. We suggest the inclusion of this species in the Endangered category as it satisfies the 'D' criteria of IUCN. The species warrants immediate conservation measures to prevent untimely endangerment.

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Rarity analysis of an endangered tropical tree species of the Western Ghats, India

K. Subin, P. A. Jose* and A. S. Vivek

Sustainable Forest Management Division, Kerala Forest Research Institute, Peechi 680 653, India

The rarity of *Atuna indica*, an endemic and endangered tropical tree species of the Western Ghats, India has been analysed in this study. The phenology, floral biology, including pollen–ovule ratio, pollinators, fruit development and biotic interactions in natural dynamics were studied. Extreme reduction in the number of flowering individuals, microhabitat conditions, low fruit set, seed infestation and fruit predation were identified as the factors leading to rarity of the species *in situ*.

Keywords: *Atuna indica*, endangered species, phenological changes, rarity analysis, reproductive biology.

THIS study aims to document the reproductive biology as well as phenological changes in *Atuna indica*, an endangered tree species of the Western Ghats, India. Studies on the reproductive biology of threatened tree species provide insights into their reduced fitness/population size. For example, *Elaeocarpus blascoi*, an endangered species reported with low seedling recruitment, was found with fungal attacks on seeds (*Fusarium* sp., *Lasiodiplodia* sp. and *Pencilium* sp.)[1]. Studies conducted on the endangered *Elaeocarpus gaussenii* and *Elaeocarpus recurvatus* reported that fruit damage caused by Malabar giant squirrel and lion-tailed macaque is one of the reproductive constraints[2]. In *Talbotiella gentii*, a critically endangered tree, the stigmatic surface was found infected by fungi, thus reducing its reproductive potential[3]. Even the Evans *et al.*[4] reported reproductive constraints in endangered perennial herbaceous species such as *Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae.*

The present study incorporates climate data (average precipitation and temperature in Kerala, India) to discuss the phenological changes of *A. indica*. Kerala experiences different but almost stable climatic seasons such as the southwest monsoon, northeast monsoon, winter and summer. So phenological studies, including reproductive biology, will highlight the influence of climate on the reproductive performance and survival of species. The variations in atmospheric temperature, rainfall and difference in day length, etc. could signal flushing, flower initiation, etc. These changes may lead to a cascade of positive or negative influences on the depending fauna and eventually on the survival of the plant species, as the plant reproductive cycle depends on pollinators, parasites and pests, which are obligatory and species specific in nature.

Reduced reproductive potential is considered one of the driving forces towards extinction. High reproductive potential may increase seedling recruitment, subsequently resulting in flourishing of the population[5,6]. The reasons for rarity may vary from one species to another. The mode of pollination and type of dispersal impact the future population by influencing the genetic as well as the physical constitution of a population. Abnormalities in these events may result in rarity of the species *in situ*. The factors leading to species decline include reduced pollinator-driven low fruit set, self-pollination driven inbreeding depression[7], and loss of genetic variability as a compound effect. Documenting the reproductive biology of endangered plant species could help unravel their constraints. The rarity analysis of *A. indica* with an emphasis on its reproductive biology will be useful for government and non-governmental organizations in their conservation efforts of endangered tree species.

A. indica is an endemic and endangered tropical tree species of the Western Ghats, India[8]. It is distributed in the evergreen forests in the 500–800 m altitude range and grows up to 15 m height. Flowers are bisexual and cream-coloured. Slopes adjacent to water courses are the microhabitat preference of this species. Umbelliferin (an anticancer drug) has been isolated from *A. indica*[9].

Materials and methods

The population located adjacent to Kakkayam dam site, Kozhikode district, Kerala was chosen for this study (Figure 1). Monitoring and recording of flowering phenology, viz. flower-bud initiation, development, anthesis, stigma receptivity, pollen viability, pollen–ovule ratio, pollination, pollinators, blooming period, pest incidence and fruit set, was done. The data were represented as the average value of each trial[10–14].

Reproductive phenology

Data on reproductive phenology with respect to the number of inflorescences per branch, number of flowers per inflorescence, flower/inflorescence development, blooming

*For correspondence. (e-mail: pajosekfri@gmail.com)

period, fruit initiation and development were recorded daily. Five inflorescences per branch were tagged and monitored for flower development from bud to full bloom. The average number of days taken for each bud to bloom was calculated and recorded. The monthly mean temperature and mean precipitation of the respective area were obtained from WorldClim database using DIVA GIS.

Pollen viability

Pollen grains from fully mature flower buds were dusted into a cavity slide containing acetocarmine solution and kept for 1 h. Later observed under a compound microscope. The pollen grains stained were treated as viable and the others as non-viable. Viability test was carried out in 2 h intervals.

Pollen germination

Pollen grains from fully mature flower buds were transferred to a cavity slide containing a germination medium (sucrose 10%). Pollen germination was counted after 1 h using a compound microscope. The pollen grains with tubes longer than the diameter were considered germinated. The experiment was repeated in 2 h intervals from anthesis.

Stigma receptivity

Both physical (through hand lens) and chemical (using hydrogen peroxide) tests were conducted. In the former method, stigma with wetness, turgidity and oily nature was considered as receptive and the rest as non-receptive. In the latter method, a drop of hydrogen peroxide was added to the stigma of freshly opened flowers and the effervescence resulting from the peroxidase enzyme activity was observed for the duration of stigma receptivity[15].

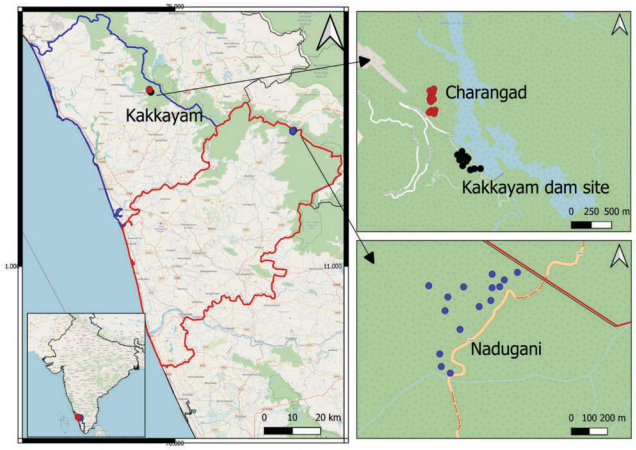

Figure 1. Distribution of *Atuna indica* in the Western Ghats, India. Study conducted on the Kakkayam dam site population.

Pollen–ovule ratio

The number of pollen grains in anthers per flower was counted using a haemocytometer[16]. The number of ovules per ovary was counted by taking sections of the ovary[17]. The pollen–ovule ratio was calculated as follows

$$\text{Pollen–ovule ratio} = \frac{\text{Pollen count per anther} \times \text{No. of anthers per flower}}{\text{No. of ovules per flower}}.$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. Physical observations were made throughout the flowering period and insect interactions were recording during day and night hours. The taxonomic identification of insects was made using the available literature and with the help of experts.

Fruit phenology

Fruit phenology was monitored and recorded, viz. fruiting primordia, period of development, including premature abscission and pest incidence.

Results

Flowering was observed along with flushing during October–December. Fruit development started in January and fruits matured in April. The trees displayed a vegetative phase from May to September. Only one out of 89 trees in the population showed flowering. A total of 13 branches showed flowering, which included 113 inflorescences bearing 521 flowers.

Reproductive phenology

Pale green-coloured flower buds were recorded during the first week of October and mass blooming was noted after two weeks in 2017 (trees showed differential flowering in branches: in 2018, the northeastern, sun-facing branch flowered first, and 2–3 weeks later, the opposite branches flowered). Flower opening started from 0600 to 0615 h and opened fully by 0915 h. Anther dehisced through vertical slit from 0900 to 0930 h. Stigma was receptive prior to anther dehiscence (0800–0830 h, protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis – 0615 h) showed 98.32% viability and gradual reduction was noticed to 93.6%,

Figure 2. ***a***, Flower buds; ***b***, flower; ***c***, fruits; ***d***, pollen grain; ***e–g***, SEM image of mouth parts of humming bird hawk moth showing pollen grains; ***h–j***, SEM image of mouth parts of *Xylocopa* sp.; ***k***, *Badamia* sp.; ***l***, *Apies mellifera*; ***m***, *Eurema* sp.; ***n***, *Graphium* sp.; ***o***, unidentified; ***p***, purple throated sunbird; ***q***, *Indrella ampula*; ***r***, pierced flower bud; ***s***, Pyralid larvae in flower; ***t***, flower bud eaten by snail; ***u***, Pyralid larvae in fruit.

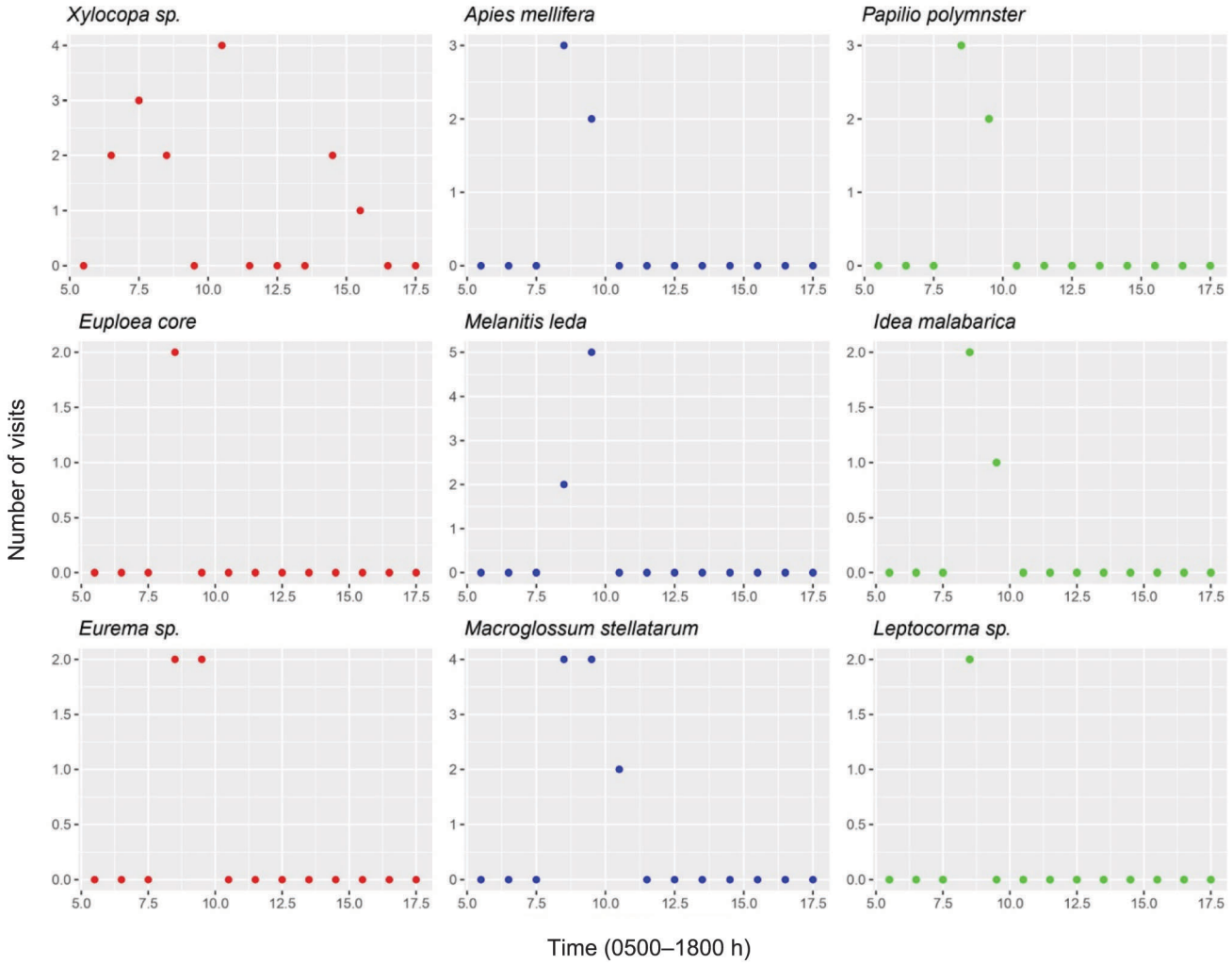

Figure 3. Flower visitors of *A. indica* from 0500 to 1800 h.

91.3%, 88% and 87.5% after 1, 2, 3 and 4 h respectively. A drastic decline to 20% was noted after 12 h. Hydrogen peroxide application followed by effervescence confirmed stigma receptivity up to 14 h, which later turned brown–black in colour, lost its turgidity and became non-receptive.

Pollen germination

At the time of anthesis, 58% of the pollen grains were found germinated in a 10% sucrose solution. A gradual decrease in pollen germination was observed at 47.8%, 23.2% and 9.74% after 1, 2 and 8 h respectively.

Pollen–ovule ratio

A flower contains 12–13 anthers and approximately 288 ± 51.3 pollen grains per anther. Hence pollen count per flower was estimated as 3744 ± 667. A flower has two ovules and hence the pollen–ovule ratio was estimated as 1872 : 1.

Pollination and insect interaction

The bagging experiment had failed; all the bagged inflorescences had fallen off. Pollinator documentation was done in 2017 and 2019. Observations were made from 0500 to 1800 h continuously. The peak time of pollinators incidence was recorded between 0800 and 1000 h and *Xylocopa* sp. was first visited during 0600–0700 h. *Apies mellifera*, *Idea malabarica*, *Eurema* sp., *Papilio polymnster*, *Euploea core*, *Graphium* sp., were the key pollinators seen multiple times (Figures 2 and 3). No flower visitors were recorded between 1100 and 1500 h. *Xylocopa* sp. was found foraging from 1500 to 1700 h. Though the *Macroglosum stelletarum* was not recorded during 2017, it was a frequent floral visitor in 2019.

Xylocopa sp., *M. stelletarum* and *A. mellifera* were found visiting many flowers, spending 2–3 sec per flower. Butterflies visited 2–3 flowers each time. Mouth parts of pollinators collected from the study site were observed through a compound microscope and photographs taken with a scanning electron microscope (SEM). The images

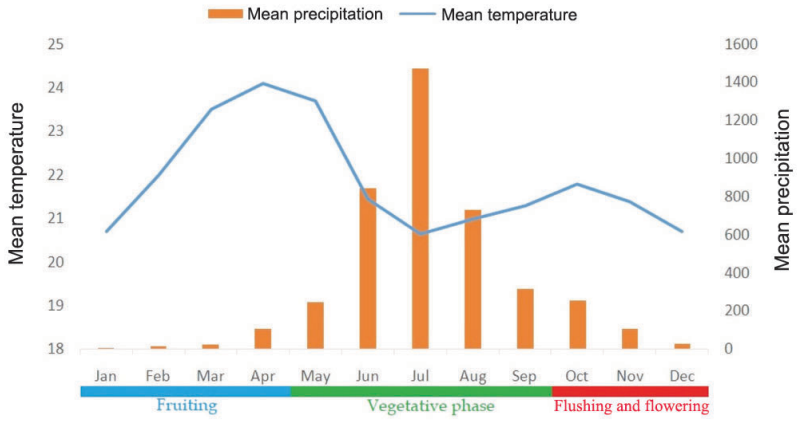

Figure 4. Phenological changes and climatic conditions of *A. indica* at the Kakkayam dam site, Kerala.

were compared with the SEM images of *A. indica* pollen grains, thus confirming their role as a pollinator (Figure 2 *d–j*). A troop of monkeys visited during the flowering season, jumping on the branches and causing flowers to fall. Inflorescence fall was recorded and the fallen inflorescence found with larvae (unidentified).

Cut-open fruits were found with Pyralidae larvae, which caused 30–40% fruit loss (Figure 2). The adult possibly lays eggs in the late flowering stage.

Discussion and conclusion

The major climatic variables that cause phenological changes are rainfall, temperature, insolation and water stress[18–21]. The flushing along with flowering in October–December in *A. indica*, reveals that flowering occurs after the monsoon season (Figure 4), (southwest monsoon, June–September in the southern Western Ghats). Kerala experiences a cool climate during the early weeks of October without any rainfall. Then the northeast monsoon sets in with lightning and thunder in the evening hours. Senescence of ripened fruits is observed during April and May. The hard seed coat delays seed germination *in situ*. However, at the start of the southwest monsoon, the seeds begin to germinate. The pollen–ovule ratio of the species supports cross-pollination (according to Cruden[17], the pollen–ovule ratio = 31.9–396 for autogamy).

The biotic interactions are influenced by phenology, population density and inter-population distance (for pollinators)[22]. Phenological variations mediate available pollinators, seed dispersal agents and florivorous insects[23,24]. This could also influence adaptation in primary and secondary consumers[25]. The most frequent pollinators are *Xylocopa* sp. and *A. mellifera.* Pollinator abundance and behaviour is the key factor in the seed set for entomophily-depending flowers[26]. Abscission of fruiting primordia (monkey-induced) was also observed. Honey bees are common pollinators found in target species, even though they are reported as less efficient because they collect pollen from various resources, resulting in deposition of pollen from multiple species on the stigma surface[27]. *A. indica* possesses coloured petals, nectar, scent and discoid-shaped stigma, which are found favourable for biotic pollination[3], it emphasizes cross-pollination of the flowers. The common pollinators of the target species are carpenter bees (*Xylocopa* sp.) and *A. mellifera*. *Xylocopa* sp. prefers medium-sized, yellow–white-coloured flowers with odour and nectar[28], which is characteristic of *A. indica* flowers. Carpenter bees are effective pollinators which support and maintain genetic variability as they travel long distances[28]. *A. mellifera* showed high efficiency in pollination[29], as reported in *Jatropha mollissima* and *Jatropha mutabilis*[30]. The large number of floral visitors reported in *A. indica* may be due to the exposed nature of the reproductive organs, as preferred by *A. mellifera.* Heavy loss in the developing inflorescence by the caterpillar lead to reduced number of flowers (adult to be identified).

The reasons for the reduced fruit set are stressful environment as well as resource limitation[31,32]. Apart from the minimal fruit set, the species showed restrictions in forming viable seedlings. The Pyralid larvae incidence in *A. indica* caused 50–70% loss in fruit set. Pyralid moths (snout moths) were also reported damaging cereals, dry fruits, etc.[33]. The fruits of *A. indica* are hard and therefore not eaten by birds or mammals. Generally, seeds of 70% of tropical forest species are dispersed by animals[34], but *A. indica* does not have any seed dispersers (the fruits are found only under the flowering tree).

The major limitation of this study is the extremely low number of flowering individuals, i.e. only two. One tree fell in 2017, and thus data collected from a single tree are presented here. The flowering phenology based on a single flowering individual may not be adequate to prove the behaviour of the species. However, it emphasizes the need for immediate conservation measures. Based on the microhabitat conditions, we conclude that less availability of sunlight is the reason for the low number of flowering

individuals. The flowered individuals are in forest edges facing the eastern side, while the non-flowering individuals are under a canopy of other species. In-depth studies on light and flowering relations are recommended.

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Distribution, ecology and status assessment of *Hydnocarpus longipedunculatus* Robi, Sasidh. & Jose (Achariaceae: Flacourtiaceae s.l.) – an endemic tree of the southern Western Ghats, Kerala, India

K. Subin[1,3], P.A. Jose*[1], K.V. Jithin[1] and Suma Arun Dev[2]

[1]Sustainable Forest Management Division, [2]Forest Genetics and Biotechnology Division
Kerala Forest Research Institute, Peechi- 680 653, Kerala, India
[3]University of Calicut, Thenhipalam, Kerala- 673 635
*Corresponding author: pajosekfri@gmail.com

दक्षिणी पश्चिमी घाट, केरल, भारत में एक स्थानिक वृक्ष *हिडनोकार्पस लॉन्गिपिडेंकुलेटस* रॉबी, ससिध. एवं जोस (अकारिएसी: फ्लैकौरसिएसी, एस.एल.) का वितरण, पारिस्थितिकी और स्थिति का आकलन

के. सुबिन, पी.ए. जोस, के.वी. जितिन एवं सुमा अरुणदेव

सारांश

हिडनोकार्पस लॉन्गिपिडेंकुलेटस रॉबी, ससिध. एवं जोस (अकारिएसी: फ्लैकौरसिएसी, एस.एल.) दक्षिणी पश्चिमी घाट, भारत के केरल भाग में वितरित एक स्थानिक वृक्ष है। जनसंख्या संरचना और विविधता विश्लेषण के साथ-साथ जनसांख्यिकीय जानकारी ने वन परिदृश्य में वितरण स्वरूप, आयु वर्ग / ओन्टोजेनी, महत्व मूल्य सूचकांक (आईवीआई), वयस्कों की संख्या, पौधों और प्रजातियों के रोपण की समझ को सक्षम किया है। आईयूसीएन दिशानिर्देशों के अनुसार प्रजातियों की उपस्थिति और अधिभोग के क्षेत्र की सीमा का पता लगाया गया है। छोटी आबादी का आकार, पौधों की बेहद कम संख्या, कम माला में होने और कम आईवीआई मूल्यों के साथ अधिभोग का क्षेत्र प्रजाति की प्रमुख बाधाएं थीं। हम इस प्रजाति को आईयूसीएन लुप्तप्राय श्रेणी में शामिल करने का सुझाव देते हैं क्योंकि यह 'डी' मानदंड को पूरा करती है। आधारभूत अध्ययन प्रजाति के प्राकृतिक आवासों के संरक्षण सहित तत्काल संरक्षण उपायों की सिफारिश करता है।

ABSTRACT

Hydnocarpus longipedunculatus Robi., Sasidh. & Jose (Achariaceae: Flacourtiaceae s.l.) is an endemic tree distributed in the Kerala part of the Southern Western Ghats, India. The population structure and diversity analysis along with demographical information enabled the understanding of the distribution pattern, age classes/ontogeny, Importance Value Index (IVI), number of adult individuals, saplings and seedlings of the species in the forest landscape. The extent of occurrence and area of occupancy of the species were found out as per IUCN guidelines. The small population size, extremely low number of seedlings, lesser extent of occurrence and area of occupancy along with lower IVI values were the major impediments of the species. We suggest the inclusion of the species in the IUCN endangered category as it meets the threshold under criterion 'D'. The baseline study recommends immediate conservation measures including protection of the natural habitats of the species.

Keywords: Conservation, Distribution, Ecology, IUCN status, Kerala, Western Ghats

INTRODUCTION

Western Ghats of India are well known for their biological diversity although it faces various threats. Western Ghats enlisted in UNESCO World Heritage site as well as one of the eight biodiversity hotspots in the world (Myers & al., 2000). Nayar & al. (2006) reported 7402 flowering plants in the Western Ghats, among those, 1273 species are endemic to the region. Hunting, livestock grazing, presence of exotics and invasive species, extraction of firewood/fodder, timber felling and forest fire were the most proximate threats to biodiversity reported in Western Ghats (CEPF n.d.). The flowering plants face severe threats like habitat loss/ modifications,

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overexploitation, irregularities in flowering, low fruit set, and poor natural regeneration (Drury, 1974; Rabinovitz & al., 1986; Nayar, 1996; Sasidharan, 2017). In addition, species also face threats from biotic agents like pest incidence as in fruits of *Atuna indica* (Subin & al., 2022), *Cynometra beddomei* (Jose & al., 2016) and *Gluta travancorica* (Jose, 2001).

Basic population studies of newly reported species are helpful in getting attention from researchers, conservation biologists, and governmental and non-governmental organizations. The autecological studies include analysis of population structure, diversity, dynamics and genetics of a species. This altogether gives a basic knowledge of the life history and population trends (Jose, 2001; Ramachandran & al., 2014). In this work, we carried out the population structure and diversity studies along with assessing IUCN criteria of *H. longipedunculatus,* a newly reported species (Robi & al., 2014) from Western Ghats, India.

The flushing of *Hydnocarpus longipedunculatus* Rabi, Sasidh. & Jose starts in purple colour later turned into green. The leaves are chartaceous and straight. Lateral nerves are 4–6 pairs and arranged distantly. The inflorescence is pseudo-cymes, peduncle slender and 5–6.5 cm in length. Pedicels are sparsely stellate, pubescent and 5 cm long. Flowers are greenish-white and fragrant. Sepals are 12 × 5 mm and sparsely pubescent, petals 15 mm long and margins strongly incurved. The fruit is ovoid-oblong, pentangular and the apex is beaked (Fig.1). The species plausibly has medicinal properties. The genus *Hydnocarpus* is well known for its seed oil, which is used against leprosy and has antimicrobial properties (Sahoo & al., 2014). The local people use seeds as a fungicide in Ginger farming, and collect them as Non-timber forest produce.

METHODS

Field survey

Extensive field surveys were carried out in the Western Ghats of Kerala region from 2015 to 2018. Primarily, the adjacent locations of type locality were thoroughly searched to find out more populations of the species. Suggestions from the taxonomists were also employed for population search.

Demography, Population structure, diversity & abundance

The total number of mature individuals (≥ 30 cm GBH), Saplings (10-30 cm GBH), established seedlings (≥1m height), and unestablished seedlings (<1 m height) were enumerated from the populations. Spatial distribution, vertical distribution (height range of *H. longipedunculatus* among the associated tree species in the community), age class distribution/ontogenic classes (such as pre-reproductive and reproductive) were analyzed in identified populations (Parthasarathy & Sethi, 1997; Pascal, 1988; Jose & al., 2018).

Species diversity and abundance of *H. longipedunculatus* in the community were assessed by laying seven random sample plots of 50×20 m in each population covering 7000 m^2 area per population. The species with GBH of more than 30 cm were counted, and measured for diversity analysis. The Importance Value Index (IVI) was calculated, which is the sum of relative frequency, relative density, and relative dominance. Each parameter is calculated out of one and IVI is out of three. The IVI is calculated by using the INVENT NTFP software (Sivaram & al., 2006).

The extent of occurrence and area of occupancy of the species was calculated using GeoCAT software (VAST, 2019) based on IUCN criteria.

RESULTS

The population survey resulted in the identification of two populations of *H. longipedunculatus* in the Kulamavu forests, Idukki District, Kerala (Fig. 2). One was the type locality, Cheri forest area (N 9^0 49′ 21.71″, E 76^0 54′ 35.1″, 820m asl) and the other was at Kulamavu Medicinal Plant Conservation Area (MPCA) (N 9^0 48′ 32.66″, E 76^0 53′46.43″, 834 m asl). The populations are situated within ~3 km distance.

The species has a low number of mature individuals and seedlings. Trees having GBH ≥30 cm are treated as mature individuals. A total of 76 mature individuals were counted from both populations (36 individuals from the Kulamavu MPCA and 40 from the Cheri). Five saplings and eight established seedlings were noted from Kulamavu MPCA. Six saplings and six established seedlings were counted from Cheri. Seedlings below 1m in height (unestablished) were absent in both populations.

Hydnocarpus longipedunculatus has a poor stand in population structural aspects like spatial distribution, vertical distribution and age class distribution. Kulamavu MPCA population is situated adjacent to the Idukki Dam reservoir in a slight slope landscape and occurred in a clumped manner. In vertical distribution, *Calophyllum polyanthum* is the dominant tree species with a height greater than 30 m. *H. longipedunculatus* occupied as the third layer species with a height of 5–15m along with individuals of *Actinodaphnae malabarica*, *Vateria indica* etc. Twenty-six individuals were recorded in the flowering stage (reproductive class) and ten individuals are in the pre-reproductive stage. Cheri population has situated in

Fig. 1. A–E. *Hydnocarpus longipedunculatus*: A. Habit; B. Flushing along with mature leaves; C. Flowering branchlet; D. Fruit; E. Cut opened fruit.

a deep slope area adjacent watercourse and observed in clumped manner. *Calophyllum polyanthum* is occupied as the first-storey species with a 30-35m height range. *H. longipedunculatus* grows as second-storey species along with *Actinodaphnae malabarica* with a height range of 15-25 m. *Baccaurea courtallensis* and *Xanthophyllum arnottianum* were the major third-storey species with a 5-15 m height range. Thirty-two individuals are in the reproductive phase and eight are in the pre-reproductive stage. No individuals in the post-reproductive stage were noted among the populations.

H. longipeduncalatus has a low Importance Value Index (IVI)/abundance in the community. In Kulamavu MPCA, 49 species were recorded in sample plots of 7000 m^2 area. The Importance Value Index of *H. longipedunculatus* was 0.0382 and relative position of 33rd out of 49 species. The highest IVI-holding species are *Calophyllum polyanthum* and *Calophyllum calaba* with 0.1909 and 0.1719 respectively. *Lagerstroemia speciosa* with the lowest abundance in the community with an IVI of 0.0156 (Table 1). In Cheri, 40 species were recorded from the 7000 m^2 sampled area. The IVI of *H. longipedunculatus* was 0.0480 and relative position of 30th out of 41 species. The highest IVI-holding species are *Calophyllum polyanthum* and *Calophyllum calaba* with 0.1944 and 0.1554 respectively. *Lagerstroemia speciosa* was the least dominant species in the tree community with an IVI of 0.0281 (Table 2). Based on IUCN criteria, we suggest the placement of *H.longipedunculatus* in the Endangered (EN) category under criterion D (Number of individuals less than 250), the extent of occurrence and area of occupancy and the total number of matured individuals are 0.597 km^2, 12 km^2 and 76 respectively.

DISCUSSION

H. longipedunculatus is endemic to the Western Ghats of Kerala having two populations recorded so far with 76 mature individuals cumulatively. The small population size with poor natural regeneration is found inimical for its survival. According to Ellstrand and Elam (1993), small populations are with potential genetic risks such as reduced gene flow, genetic diversity and increased genetic drift and inbreeding. The small population-related genetic risks put the overall fitness of the species in danger.

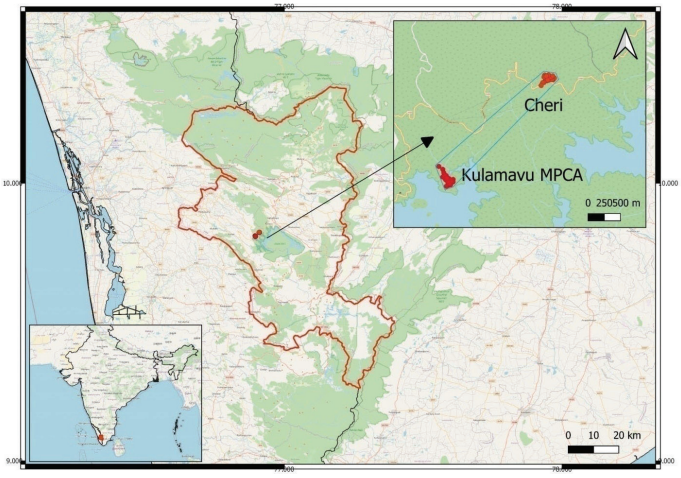

Fig. 2. Distribution map of *Hydnocarpus longipedunculatus* in Western Ghats, India

Table 1. *H. longipedunculatus*: Population diversity analysis at Kulamavu MPCA

Species	Relative density	Relative basal area	IVI
First-storey species (26-35 m)			
Calophyllum polyanthum Wall. Ex Choisy	0.0283	0.1372	0.1909
Poeciloneuron indicum Bedd.	0.0378	0.0734	0.1366
Palaquium ellipticum (Dalz.) Baill.	0.0268	0.0321	0.0843
Cullenia exarillata Robyns	0.0331	0.0210	0.0668
Second-storey species (16-25m)			
Actinodaphne malabarica Balakr.	0.0488	0.0433	0.1175
Calophyllum calaba L.	0.0205	0.1135	0.1719
Vateria indica L.	0.0189	0.0551	0.0993
Aglaia barberi Gamble	0.0173	0.0059	0.0487
Olea dioica Roxb.	0.0157	0.0032	0.1076
Third-storey species (5-15m)			
Melicope lunu-ankenda (Gaertn.) Hartley	0.0141	0.0073	0.0342
Chionanthus courtallensis Bedd.	0.0205	0.0078	0.0409
Hydnocarpus longipedunculatus Robi, Sasidh. & Jose	0.0078	0.0050	0.0382
Garcinia gummi-gutta (L.) Robs. var. gummi-gutta	0.0110	0.0066	0.0303
Other 38 trees	0.6980	0.4875	1.8320
Total	1	1	3

Table 1. H. longipedunculatus: Population diversity analysis at Kulamavu MPCA

Species	Relative density	Relative basal area	IVI
First-storey species (26-35 m)			
Calophyllum polyanthum Wall. Ex Choisy	0.0289	0.1417	0.1944
Calophyllum calaba L.	0.0193	0.1123	0.1554
Vateria indica L	0.0193	0.0610	0.1041
Second-storey species (16-25m)			
Actinodaphne malabarica Balakr.	0.0482	0.0411	0.1131
Persea macrantha (Nees) Kosterm.	0.0385	0.0778	0.1401
Hydnocarpus longipedunculatus Robi, Sasidh. & Jose	0.0193	0.0049	0.0480
Third-storey species (5-15m)			
Melicopelunu-ankenda (Gaertn.) Hartley	0.0128	0.0053	0.0419
Aporosa cardiosperma (Gaertn.) Merr.	0.0128	0.0046	0.0412
Xanthophyllum arnottianum Wight	0.0642	0.0101	0.0981
Other 32 trees			
Total	**1**	**1**	**3**

Reduced seedling count (only 14 seedlings above 1m ht), indicates plausible impediments in the reproductive potential of the species as well as predicts its hampered future. Poor regeneration was reported in other threatened trees like *Humboldtia bourdillonii* (Rahul & al., 2020), *Hopea erosa* and *Hopea glabra* (Sanil & al., 2017) and it indicates reduced population health. The reproductive constraints-driven low seedling count was also reported in other Western Ghats tree species, like *Atuna indica* (Subin & al., 2022), *Dipterocarpus bourdillonii* and *Humboldtia bourdillonii* (Swarupanandan & al 2014). The occurrence of flower-damaging larvae (Cecidomyidae), fruit-eating mammals (Malabar giant squirrel) and seed-coat-induced germination constraints (Subin & Jose *Unpubl.*) was observed in *H. longipedunculatus* proving the role of reproductive performance in the low seedling count. Moreover, the age pyramid of the species is 'Urn' shaped, with a low number of younger pre-reproductive individuals/seedlings, indicating poor population health.

The individuals of the species are spatially distributed in a Clumped manner. The clumped distribution indicates the microhabitat preference of the species mainly resulting from short propagule dispersal distance (Bleher & al., 2002), and widely distributed species have better dispersal modes. Conversely, Odum (1971) states that clumped distribution is normal in natural forest stands. The Kulamavu MPCA population is found adjacent to a Dam reservoir (Idukki Hydro electric project) (Fig. 1). The water rising due to dam construction (1975- Dam established) possibly lead to the loss of forest species in the area including *H. longipedunculatus*.

The vertical distribution of forest communities is determined by the competitive behaviour of tree species towards sunlight. The non-vigorous trees lag behind the race and end up as sub-canopy/under-storey species and this could result in compromising growth as well as reproduction potential (Moutakes & Evans, 2015). In vertical distribution, *H. longipedunculatus* occupies the second storey/under-canopy species in the Kulamavu MPCA population and interestingly, some individuals in the Cheri population (situated in deep slope areas) were found as canopy species growing to a height of 25-30m. It shows the role of landscape slopes in helping sub-canopy species to attain better vertical growth as the species gets enough sunlight in Eastern facing slopes. Moreover, the sloppy habitat of a species generally helps in fruit dispersal wherein the species lacks a well-defined dispersal mechanism as in the case of *H. longipedunculatus*, its seeds are dispersed mainly by gravity-induced methods (Subin & Jose, *Unpubl.*).

The low IVI value of *H. longipedunculatus* indicates the low abundance of the species in the community. The IVI values are used by ecologists to elucidate features of the community (Lamont & al., 1977). Generally, the endemic and endangered plants have low IVI values as reported in *Gluta travancorica* and *Ochreinauclea missionis* (Jose, 2001), *Cynometra beddomei* and *Kingiodendron pinnatum* (Jose & al., 2017) of the Western Ghats, India.

CONCLUSION

The low number of populations (2 Nos.) and mature individuals (76 Nos.), poor natural regeneration, clumped distribution, under-canopy nature, and low Importance Value Index in the community are the possible signs of survival risk in *H. longipedunculatus*. We suggest the inclusion of this species in the Endangered category as it satisfies the 'D' criteria of IUCN. The species warrants immediate conservation measures to prevent untimely endangerment.

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