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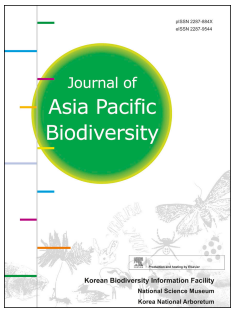

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Original Article

Reproductive biology of East Indian Satin Wood (*Chloroxylon swietenia* DC., Rutaceae: Sapindales), a threatened timber yielding tree

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Abstract

Chloroxylon swietenia DC. (Rutaceae) is widely used in folk medicine and provides a commercial timber. In the present study, the phenological events like flowering and fruiting phenology, pollinator visitation, anthesis, pollen viability, and pollen tube germination tests were analysed in 11 populations, in Tamil Nadu, India. Number of flowers was recorded as 82.6±1.4 to 162.2±4.2 during flower bud formation and reduced to 25.4±0.5 to 61.8±1.0 at maturity. Number of fruits per twig was 21.0±0.8 to 47.2±0.7 in initial stages and slightly reduced to 16.2±1.8 to 34.6±1.4 at maturation. Sixty-two species of insects were visiting the flowers with bees/ants as effective pollinators. The MTT assay revealed more viable pollen grains in five populations and fluorescence dye assay displays maximum viable pollen grains in six populations. For pollen tube germination test, 60% sucrose concentration provides significant results with a high number of germinated pollen grains. Principal component analysis of flowering and fruiting phenology, floral visitors, and pollen viability showed 79.5, 79.3, 56.7 and 41.7% for first factor respectively. Our results indicated that *C.swietenia* depend heavily on pollinators for its reproduction. Hence, there is a need to expand this study to related species which are having agronomic, pharmacological, and economic potential.

Keywords: *Chloroxylon swietenia*, Floral visitors, Phenology, Pollination, Pollen viability, Rutaceae.

Introduction

Anthropogenic growth in Earth's climate is affecting many aspects of ecological systems (Traill et al 2010) including seasonal timing of biological events which includes leaf, flowering and fruiting phenology. Association between phenology and reproductive events in plants with diverse climatic conditions have been well understood, mainly flowering phenology has a major impact on plant reproductive biology and its success was concerned with evolutionary and ecological consequences (Tadey 2020). Flowering phenology is driven by climatic conditions and this enables the plants to bloom when the surrounding climatic features are most suitable, ensuring effective sexual reproduction (Theobald et al 2017). The importance of phenology to the science of global change has been progressively renowned during the last few decades (Schwartz 1998; Fitter and Fitter 2002; Tandon et al 2003; Neil and Wu 2006; Selwyn and Parthasarathy 2006; Singhal et al 2011; Tadey 2020).

Pollination biology is a prime process in plant reproductive achievement, frequency and identity of floral visitors, the ability for autonomous self-pollination and the magnitude of pollen limitation in which pollinators transmit vital ecosystem facilities to diverse ecosystems like natural and agro-ecosystems (Tandon et al 2003; Aizen and Harder 2007; Tadey 2015; Richardson et al 2017; Figueiredo et al 2020; Impe et al 2020). Diversity of wildflowers is maintained by pollination and it plays an important role to resolve floral resource-related problems like herbivores and seed predators (Potts et al 2006). Most of the plants depend on animal pollination for their sexual reproduction via insects, birds, bats, etc. in which insects playing a vital role in pollination. It is well accepted that diversity of pollinators or number of interactions established by a specific plant is defined by different features. For example resource availability (quantity of nectar or pollen), morphological traits like colour, size, and

shape of flowers and flowering time (Bosch et al 1997), especially significant association was found between flower colour and flowering phenology (Warren and Billington 2005).

Chloroxylon swietenia DC. (East Indian Satin Wood) is a moderate-sized tree belongs to the family Rutaceae, distributed in deciduous forests throughout the Peninsular India and Ceylon with scattered population (Senthilkumar and Venkatesalu 2013). Wood of the plant has valued for its timber and due to the presence of essential oil, the plant has vast application as a phytopharmaceutical formulation in various therapeutic uses (Ravi Kiran et al 2008). The plant provides a decorative timber which is commonly used for furniture, pattern making, interior trim, cabinet work, flooring, boxes, interior joinery, carvings, toys, musical instruments and luxury goods. Ethnic groups in India use various parts of this plant to prepare herbal formulations as a topical application to treat infectious wounds, bacterial, and fungal infections (Ravi Kiran et al 2008). Kani tribal people in Agasthiyamalai hills of Tamil Nadu use the leaves of *C. swietenia* to treat constipation, snakebite and rheumatism (Ayyanar et al 2014). The plant is reported to have major phytochemicals like alkaloids, furoquinolines, lignans, xanthoxyletin, xanthyletin, coumarins, 7-demethylsuberosin, and skimmianine (Palani et al 2010). Various parts of the plant have been investigated for antimicrobial, antioxidant, anti-inflammatory hepatoprotective, antihelmintic, larvicidal, and antifertility activities (Patchimatla et al 2015).

During the last few decades, the population of this plant has declined rapidly and appeared in the IUCN red list category as ‘vulnerable’ species due to its slow-growing nature and over-exploitation for timber which has become very scarce in wild habitats (The IUCN Red List of Threatened Species 1998). Though, *C. swietenia* has diverse use in herbal market and has a considerable decline in its natural populations, there is virtually no evidence about the pollination biology of this species. Information on phenology and reproductive biology of

a plant is extremely essential for determining barriers to seed and fruit set ratio, conservation, and understanding pollination and breeding systems which regulate the genetic structure of particular population (Tandon et al 2003). Also, detailed studies on reproductive biology of Indian plants are limited, although the country is reported to have much varied ecosystems and vegetations with thousands of medicinally important as well as endemic and threatened plants. Hence, the present research was aimed to study the reproductive biology of *C. swietenia*. The study has been designed to understand the phases of flowering and fruiting phenology, pollinators who frequently visits the flower for the success of pollination and viability of pollen grains in different populations of *C. swietenia*.

Material and methods

Study site

The present study was carried out in 11 populations of *C. swietenia* from Eastern Ghats, India (altitude ranges from 300-1200 MSL) during July 2018 to August 2019 (Figure 1E). In which five populations were selected above 600 MSL and six populations were selected from lower altitudes of scrub jungle region. Each population has been selected on the basis of minimum distance of about 10-60 km (Table 1). In each population, five individuals were selected and marked with a tag to study the phenological events and reproductive biology.

Studied plant species

The plant selected in the study is *Chloroxylon swietenia* DC., widely distributed in deciduous forests and dry regions with hard yellowish wood (Gamble 1881). Young parts and inflorescence are clothed with short grey pubescence. Leaves are paripinnate, leaflets 10-20 pair, gland-dotted, unequal-sided, obtuse, and ~1 inch long; flowers are bisexual, cream coloured, in small terminal and axillary pubescent panicles; calyx is short, 5 lobed, corolla of 5 petals, clawed, spreading and imbricate in bud; disk is fleshy and 10 lobed; stamens are 10, inserted outside the disk at its base, anthers cordate, apiculate, and versatile; ovary is immersed in the disk, 3-lobed, 3-celled, ovules 8 in each cell; capsule is oblong, coriaceous, 3-celled, loculicidally 3-valved and the dissepiments remains attached to the valves; seeds imbricate, oblong, and winged (Figure 1A-D). It forms dense population in the dry deciduous forests and found in varied vegetation types including evergreen forest, scrub jungle and disturbed environments. Flowering occurs in March-April and leaves are renewed in May. During blooming, petal colour was white in colour in juvenile stage and after one to three hours it turned into light yellow in colour.

Phenological studies

The study sites were visited every week to monitor the number of flowers blooming in the marked twigs. The newly formed young flowers and flowers falling from the marked twigs were noted. Stages of flower growth were recorded with number of flowers per twig, the extent of flower buds, flowers in anthesis and fruits were recorded per individual and averaged for each population. The total number of open flowers and fruits per twig during the whole season was averaged for each population and compared with other studied populations. For fruiting phenology, the origin of young fruits from mature flower and up to fruit ripening

was recorded. Fruit development in marked twigs was recorded with the number of fruits per twig.

Floral events. Time of flower blooming, anthesis and abscission were observed in the tagged twigs from early in the morning to late evening. During the flowering period, most of the flowers in the tree (including marked twigs) were fell down and the flowers remains in the branches were assumed as fertile/viable. Mostly all the viable flowers produce fruits and in a few cases some biotic and abiotic factors troubling the fruiting phenology during its juvenile stages which reduces the viability of flowers.

Floral visitors. The frequency of floral visitors was studied throughout the study period and observations were made on selected tagged plants for the study of pollination biology. The daily observation period was from 06.00 to 18.30 h. At the end of each observation day 20 flowers were re-examined in the next day to detect the viable nature of the flower.

Pollen production. To determine the number of pollen grains per anther, five flowers (before anthesis) from each tagged branch were selected and anthers from each flower were gently removed and vortexed in a tube containing 200µl of 0.5M sucrose solution. The pollen suspension was injected into haemocytometer and the total number of pollen grains per anther was measured.

Pollen viability. The viability pollens were assessed through staining tests and *in vitro* germination of pollen grains. Freshly collected dehisced anthers from fresh flowers were treated with different stains as given below. Fertile pollen grains were identified as those take up the deep and uniform stain. The pollen grains which are incompletely filled or empty and do not taken up deep and uniform stain are considered as sterile. The number of fertile and

151 sterile pollen grains was scored using a microscope and percentage of fertile pollen grains
152 was calculated.

153 *Carmine acetic acid (CAA) test.* Pollen samples were stained with one drop of 4%
154 CAA on a microscopic slide. After 1 or 2 min, viable pollen grains which are stained and
155 observed as red in colour were recorded (Stanley and Linskens 1974).

156 *MTT (2,5-diphenyltetrazolium bromide) test.* Pollen grains were fixed with the test
157 solution made up of a 1% of substrate 2,5-diphenyltetrazolium bromide (MTT or thiazolyl
158 blue) with 5% sucrose. The pollen grains were considered viable if they turned into deep pink
159 or colourless and showed irregular black lines over its surface (Khatum and Flowers 1995).

160 *Fluoresceine diacetate (FDA-test).* Pollen grains were added with a 0.5M sucrose
161 and FDA (2 mg dissolved with 1 ml of acetone) solution (Kison and Franke 1980) and
162 incubated for 30 min at room temperature. After incubation, a few drops of water were added
163 to the sample to dilute the solution mixture. This process was repeated until a clear and
164 colourless solution is obtained. Then, the solution was decanted and excess water was
165 removed with the help of filter paper. Stained and unstained pollen grains were observed
166 under the microscope.

167 *In vitro germination of pollen grains.* A water solution with various concentrations
168 of sucrose (20, 40, 60, 80 and 100 %) and 50 ppm H_3BO_4 was served as the test medium. The
169 pollen grains were incubated for 16 h under dark at room temperature. After incubation, it
170 was smeared on a microscopic slide and excessive moisture was removed using a filter paper.
171 The percentage of germinated pollen grains was observed under the microscope (Shivanna
172 and Rangaswamy 1992).

173

Data analysis

All statistical (univariate (ANOVA) and multivariate (Cluster and Principal Component Analysis)) and graphical analyses were performed with base R ver.3.5.3 (R Development Core Team 2016). For the data on pollen viability, the descriptive analysis was performed using the Boxplot statistical algorithm. The principal component analysis and cluster analyses were performed using the "ggplot2″ package in the R program. All the experimental studies at various populations were carried out by replicate.

Results

Flowering phenology and floral biology

The *C. swietenia* plants produce fragrance during the flowering stage with a small amount of sugary content to attract pollinators. Flower blooming was started in early March and ends in April. At lower elevations, flowering was started during February and ends in March. The months March to April were recorded as an ideal blooming season in studied forest populations. It was noted that some trees are blooming even towards the end of the fruiting season and it was observed in very few individuals. Flowering and fruit set was not synchronized during the first season. New inflorescences were formed to instigate shoot tip and subsequent inflorescence formation in the twigs was started at the nodes. Inflorescences produced in delayed season always grew on the abaxial side of early produced ones.

The flowers are actinomorphic and the anthesis of flowers was monitored up to 40 hours. The changes in fully matured flower buds were monitored on selected inflorescence twigs from 06:00 h. The floral buds of *C. swietenia* are started to open by 06:00 h and the overall floral opening process lasted about two hours so that by 08:00 h all the flowers in

198 tagged inflorescences were fully open. Among the 10 stamens, five are situated towards the
199 pistil of flower and the remaining five are back to the first whorl of stamens. The floral
200 anthesis was lasted up to four hours and it was observed mostly between 10.30 to 14.30 h.
201 The deposition of pollen grains on the pistil was monitored from 10.30 h. During this period,
202 flower colour was changed from white to yellowish-white (Figure 2A-C).

203 The fleshy filaments with dehisced anthers were observed with the nature of pollen
204 grains. Mostly the pollen grains are eaten or collected by various floral visitors like bees,
205 ants, beetles, flies, and wasps. So, the pollen accumulation on stigma was slightly reduced in
206 the second day of floral anthesis. In the second day of flowering, during 09.30 to 14.00 h, the
207 petal colour was changed into dark yellowish-brown which indicated the maturation of petals
208 and the flowers produce attractive aromatic smell in the environment similar to lemongrass
209 oil aroma. The ratio of viable flowers was monitored based on the presence of mature flowers
210 in the tagged inflorescence twig. Every day it was monitored to detect the stability of flowers
211 and also for the success rate of fruit set.

212 During the initial stage of flowering in *C. swietenia*, the number of flowers was
213 recorded as 82.6±1.43 to 162.2±4.16 in an inflorescence, in which populations 1, 2, 4, and 5
214 were noted with an average of 102 flowers (Table 2). The number of flowers in populations
215 3, 7, 8, 9, 10, and 11 were recorded with about 140 to 165. After nine days of flower bud
216 initiation, number of flowers per inflorescence was different with 118.2±2.05 to 170.2±2.08.
217 The reduction in number of flowers was started from 16^{th} day of flowering i.e. 106.6±2.24 to
218 143.3±2.18. After 23 days, it was reduced to 78.8±0.86 to 119.6±1.55 and after 32^{nd} day, it
219 was 39.8±1.15 to 82.3±1.89. After 40 days i.e. during the maturation of flowers, the number
220 of flowers per inflorescence was highly reduced to 25.4±0.51 to 61.8±1.0. There was a
221 significant difference recorded at different stages of flowering with the *p* value of <2.22-16.

222

223 *Fruiting phenology*

224 The fruiting in *C. swietenia* was commenced at the end of March and number of fruits
225 in this stage was 21.0±0.83 to 47.2±0.73. The fruit set ratio was determined based on the
226 number of fruits in the marked twig and it was more or less similar in 44^{th} and 55^{th} days. A
227 significant difference was observed on all the stages (p<2.2e-16) and in 8^{th} and 18^{th} day, it
228 was not significantly different. Moderate level of difference was recorded on 30^{th} day of
229 fruiting. Natural fruit set ratio was very less in populations 1 and 2 with 16.2±1.77, 16.4±1.03
230 respectively and high in quadrat 3 with 34.6±1.36 (Table 3). In some populations, fruit set
231 ratio was disturbed by the environmental conditions like temperature, moisture, rainfall, etc.
232 For example, untimed blooming of flowers in the month of December does not produce fruits
233 and the fruit set was very rare in these cases since in that time temperature was very low.
234 Fruiting was started from April and ends during the last week of August (Figure 2 D-I).

235

236 *Floral visitors*

237 The flowers of *C. swietenia* were visited by 62 species of insects and most of them are
238 recognized as pollinators (Figure 3A-I) which includes 8 species of bees, 8 species of ants, 14
239 species of beetles, 11 species of wasps, 9 species of butterflies, and 12 species of other flies
240 (for details please refer supplementary data). In which bees and ants visits during the whole
241 flowering period and considered as effective pollinators and specialists in pollination. Most
242 of the ants and bees contact with anthers and stigmas with its ventral region of body while
243 collecting nectar. Though, bees are the chief pollinators of *C. Swietenia*. The frequent visitor
244 of flowers was different species of ants on all the populations and most them are generalists.
245 The remaining pollinators like wasps and beetles were approached the flowers for food; some

bugs use the inflorescence as shelter and food resource. About 17 species of pollinators visit more than 20 times a day during observation period and pollinator visitation rate was scarce during the first week of flowering.

The bees visit the flowers several times than other pollinators in populations 1, 2, and 9 and moderate visitation of bees was recorded in the populations 4, 10 and 11 (Table 4). Mostly, ants visit the flowers during the day time (~08.30 to 16.00 h). The beetles visit the flower to eat honey as their food and some beetles eat the fleshy petals (in populations 1 and 2) and filaments of androecium (in populations 3, 7, and 9). In some cases, butterflies and wasps also act as major pollinators in the studied populations especially in 1, 5, and 7. In the populations 3, 4 and 8, flies are helpful in pollination success. Significance values exhibited a variation between pollinators of different populations. The visitation of ants in the studied populations was too high in all the tagged branches. The number of pollinators, types of pollinators and number of visits per pollinator was moderately high in the populations of higher altitude than lower altitude and scrub jungle region. Number of visits by beetles, wasps, butterflies and flies was very minimal than bees and ants and there was a significant difference (p<2.2e-16) of pollinator visitation on all the studied populations.

Pollen viability

The results of pollen viability and pollen tube germination tests were summarized in table 5. The number of pollen grains was measured in five *C. swietenia* populations from the study area which ranged from 397.6±15.4 to 722.7±12.4. The number of pollen grains was high in lower elevations and scrub jungle region. Among the staining methods, MTT assay exhibited more number of viable pollen grains in populations 1, 3, 4, 5, and 6 (Figure 4 B-D); fluorescence dye assay (FDA) method displays more number of viable pollen grains in the

populations 2, 7, 8, 9, 10, and 11 (Figure 4E); and CAA test showed a moderate number of pollen grains in the populations 1, 2, 4, 7, and 8 (Figure 4A). In pollen tube germination test (Figure 4F), 60% sucrose concentration provides significant results especially in the populations 1, 3, 4, 5, 7, and 8 with number of germinated pollen grains as 79.8±4.4, 71.6±2.8, 94.4±2.3, 125.4±3.6, 73.8±1.7, and 74.8±4.1 respectively. At 100% of sucrose concentration almost all the pollen grains do not grow and 20% concentration showed marginal tube germination ratio.

Principal component analysis (PCA) and cluster analysis

The PCA for the flowering and fruiting phenology, floral visitors, pollen viability and pollen tube germination test were elaborated in figure 5. The PCA of flowering phenology showed 79.5% of the variance for the first principal component followed by 12.1% and 3.85% for second and third principal components (Figure 5B). Factor 1 showed a strong correlation with all the flowering stages. The factor 2 was negatively correlated with day 16 and positively correlated with day 40 and 32. In fruiting phenology of PCA, the first factor displayed 79.3% of the variation and the second factor showed 15.3% of variation with a cumulative of 94.6% variance. The first factor showed a positive correlation with all the stages of the investigation except the day 1 and the second factor strongly correlates with day 1 (Figure 5A). The PCA of pollinator data showed the variance of 56.7% and 21.3% in factor 1 and 2 respectively and the third factor showed 9.1% of the variance. Factor 1 showed a strong correlation with all the pollinators observed during the study and bee pollinator was strongly correlates with the second factor (Figure 5C). The PCA of pollen viability data showed 41.74% of the variance in factor 1 followed by second and third factors with the variance of 23.14% and 10.4% respectively. Factor 1 strongly correlates with FDA, MTT,

and pollen tube germination at different concentrations. In which FDA, pollen tube germination (at 80 and 100% sucrose concentrations) were negatively correlated with dimension 1 (Figure 5D). The MTT, pollen tube germination (at 20, 40 and 60% sucrose concentrations) were positively correlated for factor 1 and the second factor strongly correlates with MTT and CAA methods.

In the present study, positive correlation was observed between the number of flowers and studied days. There was a significant positive correlation found between the number of flowers with the day 7 (0.93), 16 (0.86) and 23 (0.88) with the significance as $p \leq 0.001$ (Figure 6A). Also there was strong relationship observed between the number of days and number of fruits. Day 55 showed maximum correlation (0.99) followed by day 44 and day 30 (0.97 each) with the significance of $p \leq 0.00$ (Figure 6B). For pollination visitation, wasps and ants showed higher correlation in the study area with the value of 0.74 followed by beetles and ants with the 0.72 (Figure 6C). In the pollen viability tests, 60% of sucrose concentration showed positive correlation with the number of viable pollen grains (0.87) followed by MTT assay with the value of 0.75 (Figure 6D).

The UPGMA clustering method for flowering phenology on 11 populations of *C. swietenia* revealed two principal clusters (Figure 7A) based on the number of flowers. Cluster 1 consists of populations 1, 2, 3 and second cluster contain three sub-clusters. Cluster analysis of fruiting phenology has been divided into two main clusters. The first cluster contains ten populations with three sub-clusters (2 to11) and the second cluster contains a single population (Figure 7B). Cluster analysis of pollinator data was categorized into two clusters with two sub-clusters in each cluster (Figure 7C). The UPGMA clustering method for pollen viability tests revealed two principal clusters three sub-clusters. In which, cluster 1 comprised six populations (6-11) and the second cluster was divided into two sub-clusters

318 (Figure 7D). Each cluster was further separated into sub-clusters based on the similarities and
319 differences among the populations.

320

321 **Discussion**

322 *Chloroxylon swietenia* is having the ability to survive at different environmental
323 conditions and blooms at two seasons, first usual season starts from March to April and
324 second irregular season during the month of December. This extended duration of blooming
325 exhibits lack of seasonal differences in resource availability, as in the case for understorey
326 plants in the tropic regions (Rathcke and Lacey 1985). The number of flowers was higher
327 during the initial stages and it was reduced gradually after 15 days of flower bud initiation in
328 *C. swietenia* among the studied populations. At higher altitude, the plants retain 29-32% of
329 produced flowers, whereas it was about 37-42% in the pants of lower altitude populations.
330 This number was also reflected in fruit set ratio and it may be due to various environmental
331 conditions. It was reported that plant size and altitude has a major factor for the production of
332 flowers and fruits in *Pterocereus gaumeri*, a columnar cactus of Yucatan peninsula in Mexico
333 (Mendez et al 2005), in which the plants of lower altitude forests produce a large number of
334 flowers than the plants of higher altitude forests.

335 In our study, 62 species of insects are identified as floral visitors in *C. swietenia*
336 populations, in which 17 species of insects often visits the flower throughout its flowering
337 period. Most of the flowering plants depend on different kinds of animals for sufficient pollen
338 dispersal as their main mode of reproduction (Buchmann and Nabhan 1996; Ashman et al
339 2004). When the number of pollinator visitation was decreased in an environment, the pollen
340 dispersal also disturbed which leads to a decrease in fruit set ratio (Willcock and Neiland

2002). Meyer et al. (2007) reported that fruit and seed set ratio was very low due to minimal number of visitation of pollinators in *Hipppocrepis comosa*.

Among the recorded floral visitors in the present study, bees, ants, and butterflies were identified as major pollinators due to their frequent visit in the flower. Mostly, insects had a key role in pollination for thousands of flowering plants and some plant species requires a specific group of insects for successful pollination. For example, the sweet cherry plant always depends on insects for its pollination and when the deficiency of insects in its environ during the flowering period, there was a decline of 40-90% of fruit set ratio (Klein et al 2007; Holzschuh et al 2012). Six species of ants were visited the *C. swietenia* flowers in the entire flowering season in all the studied populations and ants has a significant role in the pollination of the plant. The ants use volatiles which was produced by plants as signal for orientation towards food sources (Youngsteadt et al 2008) and due to the presence of a large amounts of ants in most of the environments and its potential impact on ecosystems particularly in pollination biology, the role of ants as pollinators offers more attention in the recent years (McMullen 2011).

The bees and ants visit the plant during the whole flowering period and considered as effective pollinators of *C. swietenia*. Selwyn and Parthasarathy (2006) also revealed bees have a major role in pollination of several flowering plants especially in the flowering plants of tropical dry evergreen forest regions. In some plants like oilseed rape (Jauker and Wolters 2008) and *Linum lewisii* (Kaerns and Inouye 1994), flowers are predominantly pollinated by several species of flies, since some flies have the potential to perform pollination services in particular environments which was highly efficient in fruit set ratio too. Likewise, 21 species of flies including 9 species of butterflies are considered as effective pollinators in our study.

It was recorded that beetles were visiting the flowers of *C. swietenia* to eat the petals and this behaviour leads to damaging the flowers and make them not as much attractive to primary pollinators like bees and ants. We also noticed that the visitation by bees and ants was not as much in the populations where the visitation of beetles is higher. The similar kind of observation was made by McMullen (2009) in *Ipomoea habeliana,* a night-flowering plant endemic to Galapagos Islands. In contrast, *Annona squamosa* a medicinally important fruit yielding plant mostly pollinated by beetles (Kishore et al 2012).

Pollen viability is considered as an important factor for the analysis of pollen quality and fruit set ratio. In the present study, the number of pollen grains was too high in populations of lower altitude plants in *C. swietenia* across all seasons. Likewise, a higher number of pollen grains with the highest amount of viable pollen grains was reported in the plants like *Ziziphus mauritiana* (Tel-Zur and Schneider 2009) and *Physalis peruviana* (Chauta-Mellizo et al 2012). It was well known that fruit production and seed set ratio in flowering plants were highly caused by the reduced pollinator visitation, inactive pollinators, low pollen quantity and low viability or stigma receptivity. Prasad et al. (1999) reported that, reduced pollen quantity and viability being the major factor in lower fruit production in *Arachis hypogaea*. In coffee plants also, the presence of a higher number of pollinators promotes the fruit set ratio as reported in Mexican coffee plantations (Vergara et al 2008).

Conclusions

To the best of our knowledge this is the first report containing comprehensive data on the reproductive biology of *C. swietenia*, though the plant has been valued as much economic and medicinal importance. The wild population of the plant is under great pressure due to over-exploitation for their hardwood to make agricultural products and herbal preparations.

The results of our study revealed that there is a need for detailed explorations with a network approach which seems promising to explore distinct ecological process i.e., the interaction between plants and pollinators in relation to the phenological structure of plant communities and flower visitors in a similar kind of ecosystems. Our multivariate analysis results were used to find best or viable populations based on the phenological character, pollen viability, and pollinator diversity as well. In the studied 11 populations of *C. swietenia*, 5 samples were considered as potential populations since the fruit set ratio were increased when there was an increase in number of viable pollen grains, pollinator diversity, and their visitation rate. *C. swietenia* is highly dependent on pollinators and reproductive success is more sensitive to environmental changes especially temperature (based on altitude) and rainfall. The results of the reproductive biology of *C. swietenia* carried out in the study will be helpful for pollination biology studies of other related species of medicinal and economic importance.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal

411 relationships that could have appeared to influence the work reported in this paper.

412

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530

531 **Figure legends**

532 **Figure 1.** Morphology and distribution map of *C. swietenia* in Western & Eastern Ghats of
533 Tamil Nadu, India: A, habit; B, a vegetative branch; C, flowering twig; D, fruiting twig;
534 E, locality of *C. swietenia* populations in the study area

535 **Figure 2.** Different stages of flowering & fruiting in *C. swietenia* in the study area: A-B,
536 Flower bud formation and young flowers in the end of 1st week; C-D, Maturation of
537 flowers after 6th week; E, Initiation of fruit set and young fruits; F-I, Maturation of
538 fruits and dehiscence of mature fruits

539 **Figure 3.** Floral visitors/pollinators of *C. swietenia*: A-B and D, Different types of beetles
540 visiting the flowers and feed the petals of flowers; C and E-G, Different types of flies
541 visits the flower; H-I, Ants visits the flower

542 **Figure 4.** Different stain tests for pollen viability in *C. swietenia*: A, CAA test for pollen
543 viability; B-D, MTT test for pollen viability; E, FDA test for pollen viability; F, Pollen
544 tube germination test.

545 **Figure 5.** Principal component analysis (PCA) of *C. swietenia* in the studied eleven
546 populations in the study area: A, Fruiting phenology; B, flowering phenology; C,
547 pollinator visitation; D, pollen viability.

548 **Figure 6.** Correlation analysis of *C. swietenia* in the studied populations: A, Fruiting
549 phenology; B, flowering phenology; C, pollinator visitation; D, pollen viability.

550 **Figure 7.** Cluster analysis of *C. swietenia* showing variations and differences among the
551 studied populations in eleven quadrats: A, Flowering phenology; B, fruiting phenology;
552 C, pollinator visitation; D, pollen viability.

553 **Table 1**

554 List of quadrats/populations selected for the study of reproductive biology in *C. swietenia*

Studied populations	Place	Forest range	Latitute	Longitute	Elevation
Population 1	Mettupalayam (Coimbatore Dist.)	Western Ghats	11^0 18’ 50’	76^0 54’00’	360 m
Population 2	Sathyamangalam (Erode Dist.)	Western Ghats	11^0 35’ 09’	78^0 31’08’	290 m
Population 3	Palamalai (Salem Dist.)	Eastern Ghats	11^0 42’ 22’	$77^{0\,4}$44’05’	1050 m
Population 4	Servalar (Tirunelveli Dist.)	Western Ghats	08^0 41’ 07’	77^0 18’ 12’	331 m
Population 5	Kolli malai (Namakkal Dist.)	Eastern Ghats	11^0 18’ 21’	78^0 20’40’	1170 m
Population 6	Palamalai (Salem Dist.)	Eastern Ghats	11^0 41’ 09’	77^0 41’49’	307 m
Population 7	Pachchamalai (Tiruchirappalli Dist.)	Eastern Ghats	11^0 20’ 21’	78^0 32’04’	315 m
Population 8	Kalappanaikkan patti (Namakkal Dist.)	Eastern Ghats	11^0 19’ 58’	78^0 18’18’	265 m
Population 9	Pethamm palayam (Erode)	Plains	11^0 20’ 32’	77^0 34’10’	270 m
Population 10	Arasanamalai (Tirupur Dist.)	Scrub Jungle	11^0 12’ 08’	77^0 30’31’	401 m
Population 11	Kollangarai (Thanjavur Dist.)	Sacred Grove	11^0 21’ 04’	78^0 43’00’	65 m

555

556

Table 2

Flowering phenology of *C. swietenia* in selected quadrats in the study area

	Day 1	9th day	19th day	23rd day	32nd day	40th day
Population 1	102.3±1.7	128.0±1.2	117.2±1.2	81.6±2.5	55.6±0.9	30.8±0.9
Population 2	100.2±1.3	121.6±2.5	114.4±3.0	78.8±0.9	51.6±0.5	25.4±0.5
Population 3	156.2±3.8	170.2±1.6	140.0±1.9	115.6±1.6	75.6±1.2	61.8±1.0
Population 4	102.2±1.3	122.4±1.4	110.0±2.4	95.8±2.1	60.8±1.8	41.8±1.1
Population 5	103.8±0.8	121.0±0.8	113.2±1.6	78.4±0.9	39.8±1.2	27.8±1.1
Population 6	82.6±1.4	118.2±2.1	106.6±2.3	80.4±1.1	57.4±2.5	41.4±0.7
Population 7	147.4±1.9	170.2±2.1	140.0±.0.2	117.6±1.7	80.8±2.6	49.2±1.7
Population 8	162.2±4.2	164.0±2.4	140.8±1.5	106.6±1.8	60.6±2.1	43.8±1.3
Population 9	154±3.13	168.1±2.1	143.3±2.1	119.6±1.5	82.3±1.9	58.4±1.1
Population 10	153.3±4.2	172.3±1.1	142.4±2.1	111.3±1.7	78.5±1.9	55.7±1.1
Population 11	145.4±2.2	161.3±2.1	139.3±1.9	118.5±1.9	76.2±1.5	56.3±1.2

560 **Table 3**

561 Fruiting phenology of *C. swietenia* in selected quadrats in the study area

	Day 1	8th day	18th day	30th day	44th day	55th day
Population 1	25.6±1.2	22.0±1.1	19.6±1.7	18.0±1.9	16.8±1.9	16.2±1.8
Population 2	23.4±0.5	21.0±0.8	19.6±1.1	18.4±1.2	17.2±0.9	16.4±1.0
Population 3	52.8±1.3	47.2±0.7	43.0±0.7	39.0±1.7	36.0±0.9	34.6±1.4
Population 4	38.0±0.5	34.4±0.9	31.4±1.8	29.8±1.8	28.2±1.9	26.2±1.3
Population 5	24.4±1.2	22.2±1.4	21.2±1.4	20.8±1.5	20.0±1.5	19.6±1.6
Population 6	32.6±1.6	29.4±2.4	26.0±2.5	23.6±2.6	21.4±2.4	20.6±2.4
Population 7	42.4±1.1	38.2±1.1	35.4±1.1	30.2±1.6	26.8±1.1	25.6±1.0
Population 8	36.4±1.7	32.6±1.9	29.8±1.4	27.4±0.8	26.2±1.1	25.6±1.1
Population 9	33.3±1.2	29.2±1.4	25.6±1.3	24.5±1.5	25.6±1.7	25.6±1.7
Population 10	29.3±1.1	26.4±1.1	22.4±1.9	21.3±1.9	22.4±1.9	21.1±1.1
Population 11	31.4±1.3	27.6±1.0	25.8±1.5	23.2±1.1	22.3±1.1	21.9±1.2

562

563

564 **Table 4**

565 Pollinator visitation in the studied populations of *C. swietenia*

	Ants	Bees	Beetles	Butterflies	Other flies	Wasps
Population 1	114.0±9.9	76.7±6.9	21.7±1.5	24.3±5.2	11.3±1.2	33.0±3.2
Population 2	103.0±10.4	76.7±6.4	18.7±1.9	14.0±1.5	24.3±5.2	18.7±1.9
Population 3	76.66±5.8	40.0±2.1	13.0±0.5	7.7±1.7	33.3±5.5	24.0±0.6
Population 4	88.0±5.8	61.0±9.1	12.7±1.2	13.7±0.9	47.3±9.6	19.0±1.7
Population 5	74.7±6.7	44.0±5.5	8.7±0.3	26.3±3.8	24.7±3.2	26.0±4.0
Population 6	148.0±4.0	19.0±1.2	3.6±0.9	11.7±0.3	17.3±2.9	38.7±4.8
Population 7	87.7±4.9	27.7±7.3	16.3±0.9	26.0±2.5	24.0±2.1	17.0±2.6
Population 8	129.7±6.7	26.3±1.7	05.0±0.6	03.3±0.7	40.0±3.6	33.0±0.6
Population 9	119.4±6.1	78.4±5.3	14.5±4.5	12.2±1.3	22.3±1.9	22.5±0.9
Population 10	103.5±6.1	58.2±4.9	12.3±3.1	14.3±1.8	21.3±1.1	22.4±1.6
Population 11	123.7±5.1	56.8±3.7	09.7±0.9	08.6±1.2	1.09±1.8	17.5±1.9

566

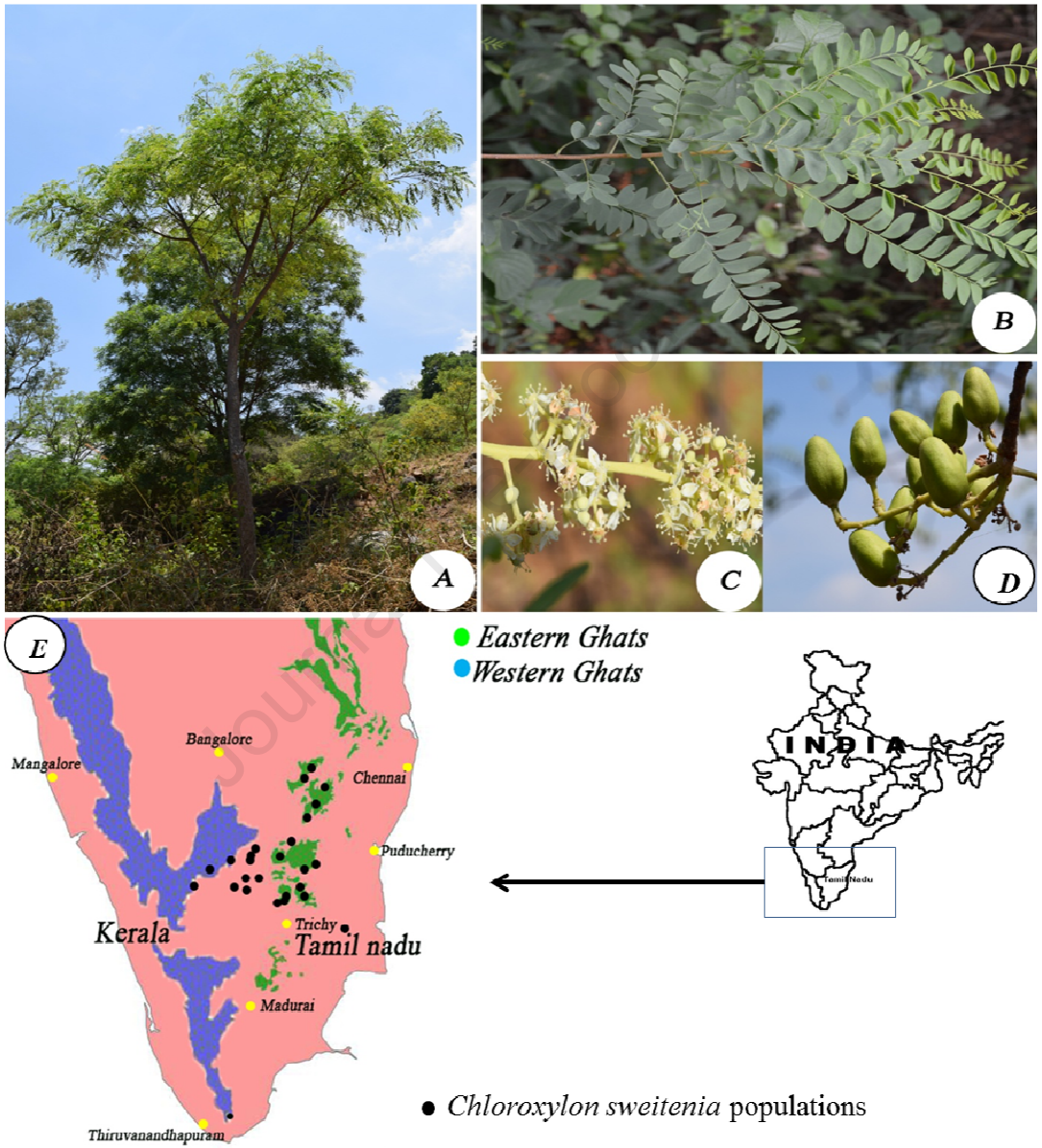
567 **Table 5**

568 Pollen viability of *C. swietenia* using various staining techniques

	Number of pollen grains	Pollen viability tests			Pollen tube germination test				
		CAA	FDA	MTT	20% SC	40% SC	60% SC	80% SC	100% SC
Population 1	557.4±9.3	56.0±0.8	53.0±6.5	62.8±3.9	23.2±2.3	40.0±2.0	79.8±4.4	42.0±1.3	7.2±0.9
Population 2	466±11.3	44.4±0.5	45.0±4.3	43.2±2.2	15.2±0.8	30.0±1.3	57.4±1.3	37.2±1.6	9.8±0.7
Population 3	692.8±38.0	35.6±6.8	66.4±7.6	66.6±2.5	28.2±2.2	38.0±1.6	71.6±2.8	54.2±0.3	3.0±1.4
Population 4	477.8±9.4	43.6±0.7	39.8±1.8	56.8±1.2	26.6±2.0	56.8±0.7	94.4±2.3	34.4±4.1	0
Population 5	397.6±15.4	23.0±0.7	38.8±5.9	73.6±2.2	33.2±1.5	90.0±4.1	125.4±3.6	17.0±2.3	4.0±1.9
Population 6	528.0±9.9	40.2±4.0	59.2±2.8	69.6±3.9	13.2±0.8	38.6±3.3	68.8±1.1	28.2±2.7	11.4±0.8
Population 7	702.0±13.8	48.2±9.6	60.8±5.0	39.0±2.1	20.2±1.2	52.4±3.4	73.8±1.7	15.2±2.1	10.4±1.6
Population 8	616.2±10.2	38.6±8.0	44.4±2.5	7.4±1.3	33.4±3.2	58.6±1.6	74.8±4.1	24.0±0.7	3.2±0.6
Population 9	722.7±12.4	28.4±4.6	64.2±2.4	13.0±0.7	12.2±0.6	24.2±0.9	38.8±1.6	61.4±1.7	6.6±1.8
Population 10	688.3±11.6	28.5±4.2	59.3±2.1	10.2±0.7	16.6±0.9	36.0±1.5	59.8±2.1	81.8±2.8	12.8±1.1
Population 11	678.7±12.31	22.4±2.7	61.2±2.9	12.0±0.8	17.4±2.1	46.8±1.9	66.0±1.1	13.4±2.2	0

569

Figure 1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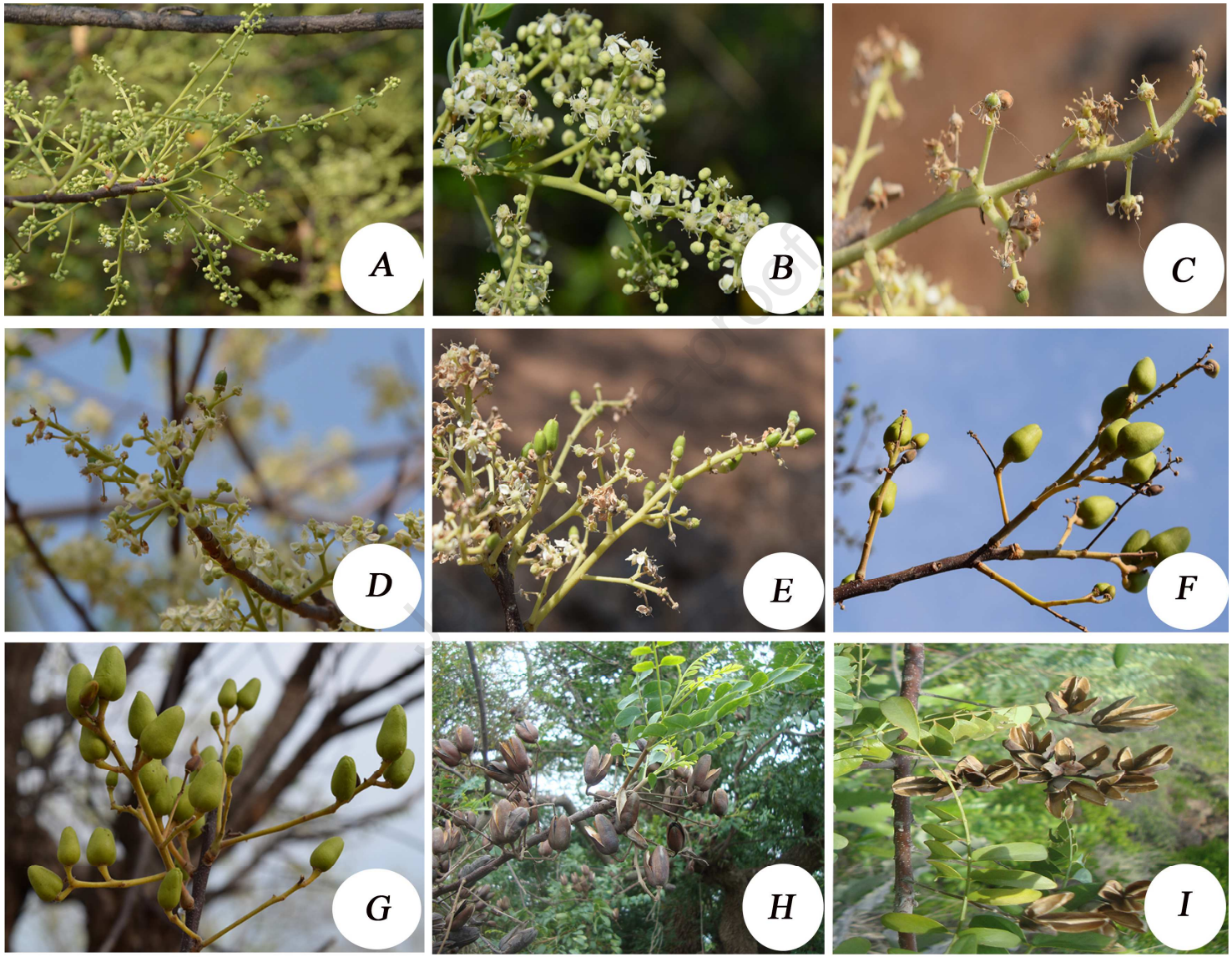
● Chloroxylon sweitenia populations

Figure 2



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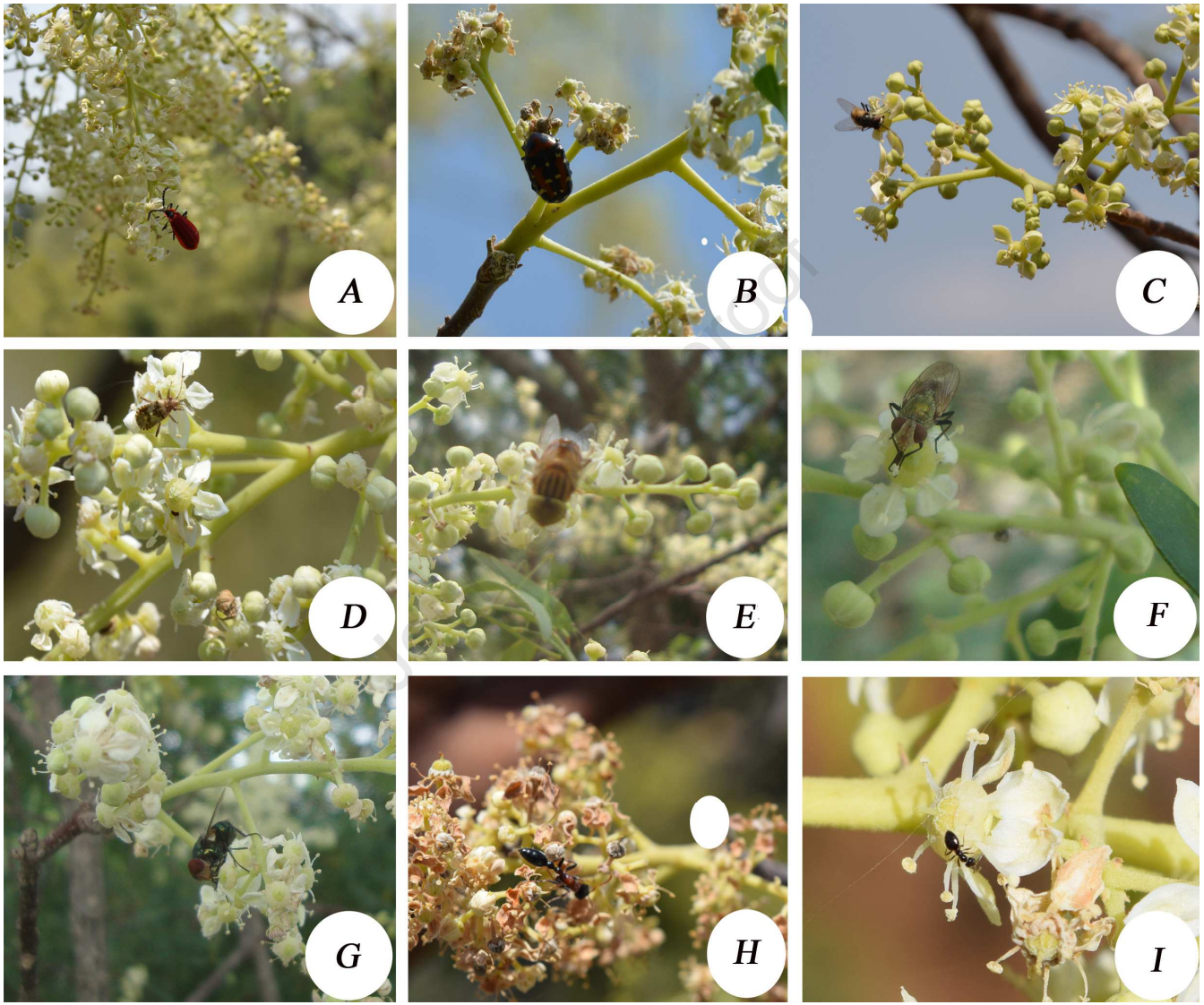

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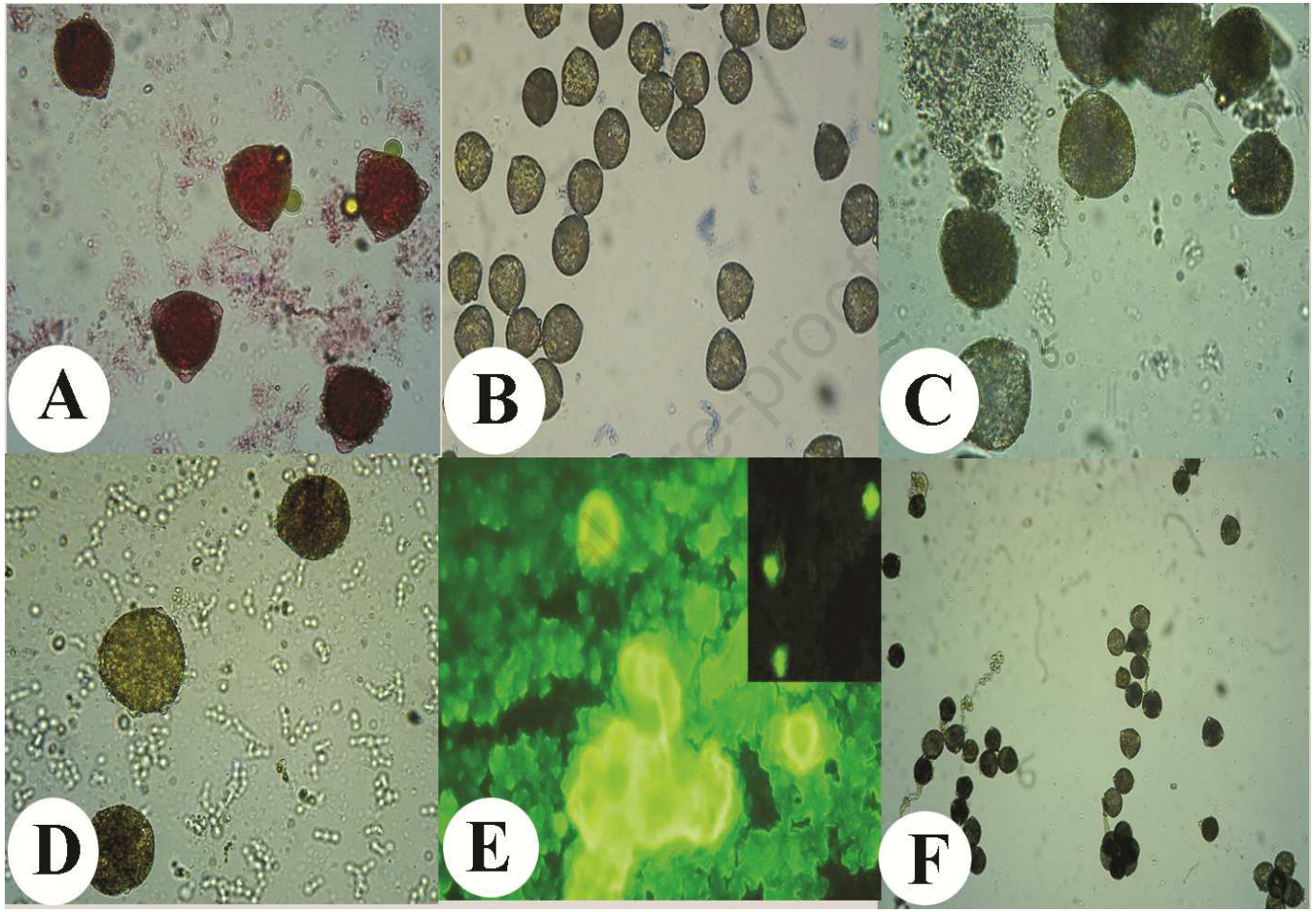

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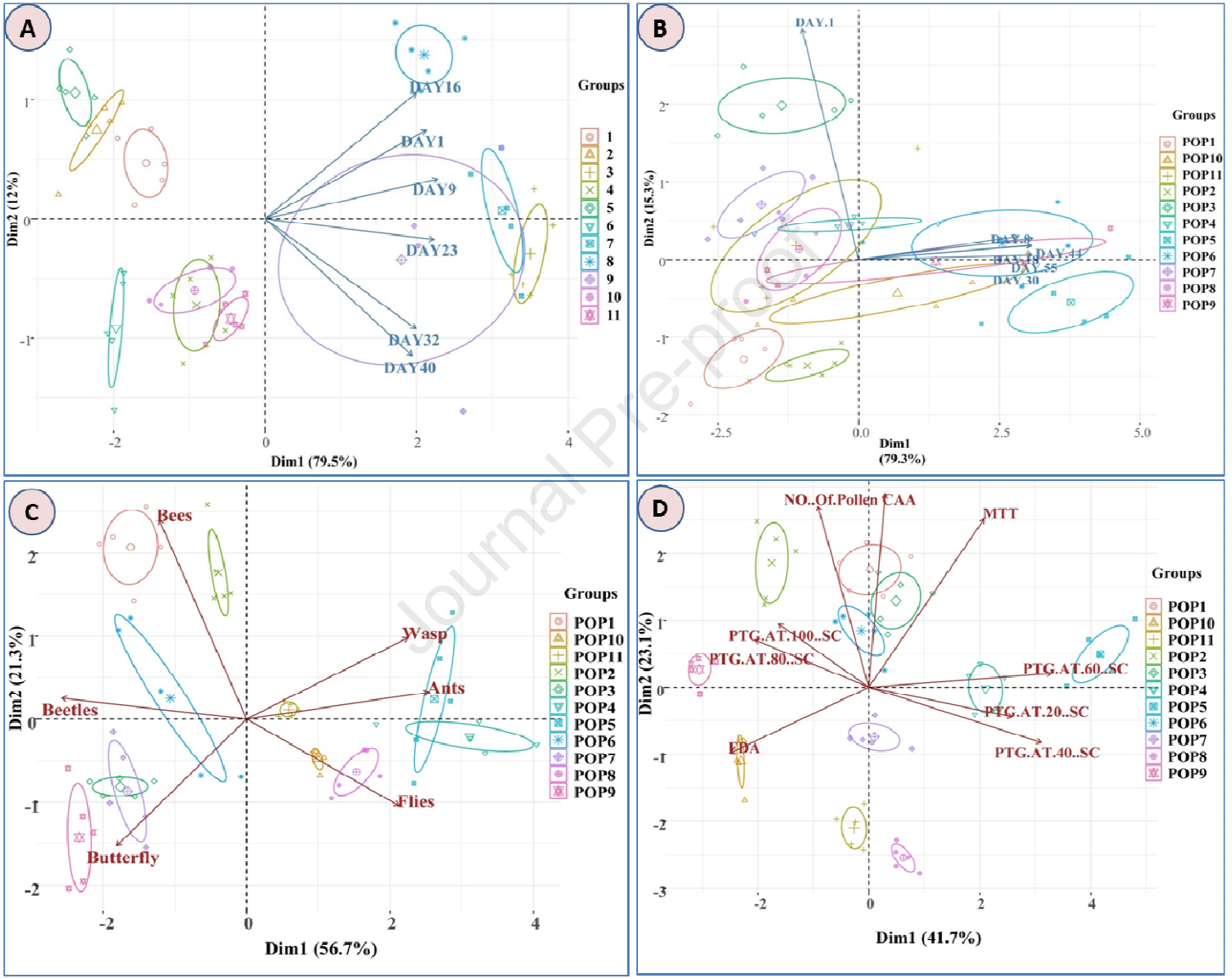

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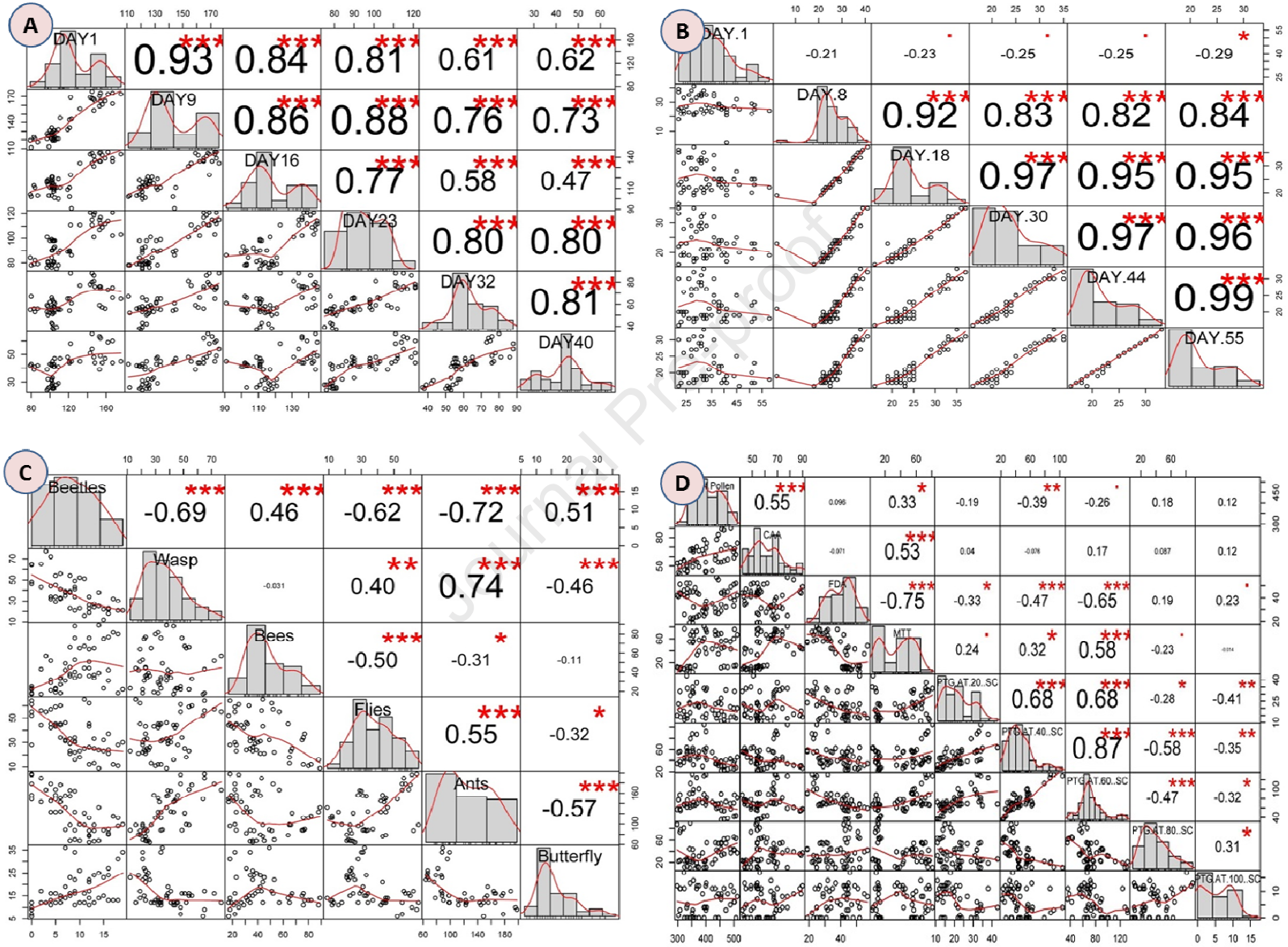
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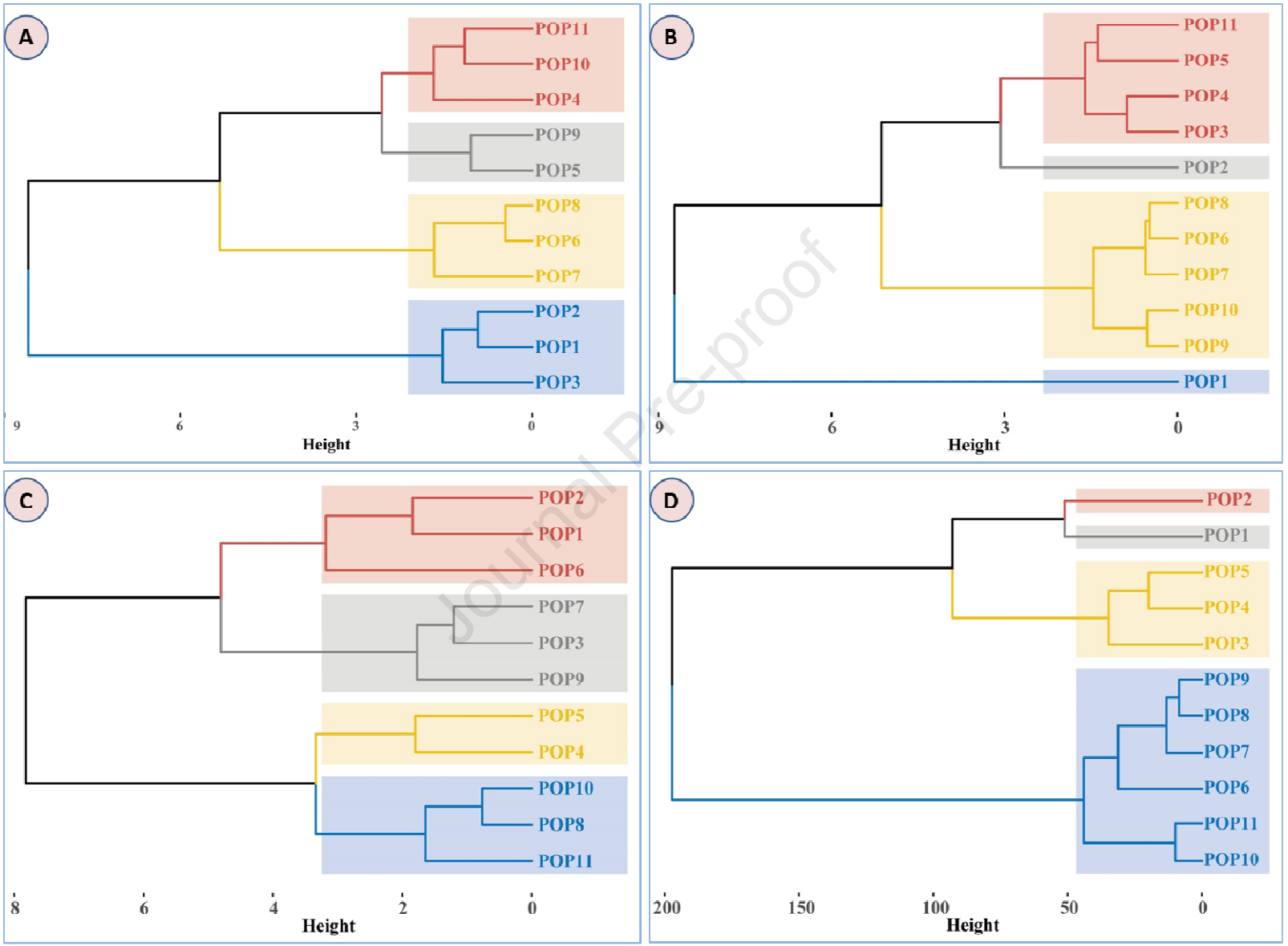
Figure 7



Highlights

- *Chloroxylon swietenia* is a deciduous tree with limited population.
- Reproductive biology of *C. swietenia* was reported for first time.
- Much variation in flowering and fruiting phenology in varied altitudes.
- Flowers are actinomorphic and are visited by 62 species of insects (pollinators).
- Fruit set ratio was disturbed by the environmental conditions.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: