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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Mauve Stinger *Pelagia noctiluca* by Swaathi Na. Medium used is soft pastels and gelly roll.

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ARTICLE

Floral biology of *Baccaurea courtallensis* – an endemic tree species from peninsular India

Karuppiah Nandhini [1], Vincent Joshuva David [2], Venugopal Manimekalai [3] & Perumal Ravichandran [4]

[1,2,4] Department of Plant Science, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu 627012, India.
[3] Department of Botany, Sri Parasakthi College for Women, Courtallam, Tamil Nadu 627802, India.
[1] nandhuwin19@gmail.com, [2] joshuvadavi@gmail.com, [3] sehimaravi@gmail.com, [4] grassravi@msuniv.ac.in (corresponding author)

Abstract: *Baccaurea courtallensis,* a member of the Phyllanthaceae family is a tree species endemic to peninsular India. Despite the fact that this plant is naturally propagated through seeds, there is no information on its reproductive biology. To understand the reproductive biology of this species, its floral biology is very important. Hence, this study was conducted to comprehend the detailed aspects of its flowering and fruiting characters. Blooms occur during February–May; fruits develop and mature from June to September. Flowers are unisexual, and dioecious. The present study reports on the rare occurrence of monoecious flowers in many inflorescences of a few trees. Crimson red fruits are arranged in a racemose type of inflorescence and hang in symmetric clusters. Inflorescence clusters are observed all along the trunk from base upwards. Wind and insect pollinations were observed in this species during field visits: honey bees and black ants were observed as the major floral visitors. Pollen grains showed 96.24% fertility in the acetocarmine glycerin test and 80% viability in the fluro-chromatic reaction test. Pollen germination was 63.1% in Brewbaker and Kwack's medium containing 10% sucrose. The detailed aspects of flower and fruit morphology and anatomy respectively are reported for the first time.

Keywords: Anthesis, blooms, frugivores, phenology, pollen germination, monoecious, pollen viability, pollination, seed biology.

Tamil: *Baccaurea courtallensis* (மூட்டுப்பழம்), Phyllanthaceae குடும்பத்தைச் சேர்ந்த ஒரு மர சிற்றினம். தீபகற்ப இந்தியாவில் மட்டுமே வாழும் இட வாரிய தாவரம். விதைகள் மூலமாக மட்டுமே இனப்பெருக்கம் இம்மரத்தில் நடைபெறுகிறது. எனினும் இம்மரத்தை பற்றிய இனப்பெருக்க உயிரியல் ஆய்வுகள் போதுமானதாக இல்லை. ஒரு தாவரத்தின் இனப்பெருக்க உயிரியல் பற்றி அறிந்து கொள்ள அத்தாவரத்தின் மலர் பண்புகள், மகரந்தச் சேர்க்கை நிகழ்வுகள், கருவுறுதல் மற்றும் கனி நிலை வளர்ச்சி போன்றவை மிகவும் அவசியமானதாக கருதப்படுகின்றன. எனவே, அவற்றை விரிவாக புரிந்துகொள்ள இந்த ஆய்வு நடத்தப்பட்டது. ஆண் மற்றும் பெண் மரங்கள் தனித்தனியாக காணப்படுகின்றன. ஒரு பால் மலர்கள், ஈரில்லத் தாவரங்களில் காணப்படுகிறது. மலர்களின் பூக்கும் காலம் பிப்ரவரி முதல் மே வரை ஆகும். பழங்கள் ஜூன் முதல் செப்டம்பர் வரை உருவாகி முதிர்ச்சியடையும். பொதுவாக மலர்கள் ஒருபாலினம், அரிதாக இருபாலின மலர்கள் சில மரங்களில் காணப்படுகின்றன. சிவப்புநிற பழங்கள் ஒரு வகை நுனி வளர் மஞ்சரிகளில் அமைக்கப்பட்டு சீரான கொத்துகளில் அடிப்பகுதியில் இருந்து மேல்நோக்கி தண்டு முழுவதும் காணப்படுகிறது. அயல் மகரந்தச்சேர்க்கை காற்று மற்றும் பூச்சிகளின் மூலமாக நடைபெறுகிறது. குறிப்பாக தேனீக்கள் மற்றும் கருப்பு எறும்புகள் முக்கிய மலர் வருகையாளர்களாகும். மகரந்த துகள்கள் அசிட்டோகார்மைன் கிளிசரின் சோதனையில் 96.24% மற்றும் ∴ப்ளூரோ-குரோமடிக் சோதனையில் 80% வளமானதாக காணப்படுகிறது. 10% சுக்ரோஸைக் கொண்ட ப்ரூபேக்கர் மற்றும் குவாக்கின் ஊடகத்தில் மகரந்த முளைப்புத்திறன் 63.1% ஆக இருந்தது. மலர் மற்றும் பழங்களின் உருவவியல் மற்றும் உள்ளமைப்பியல் பற்றிய விரிவான ஆய்வுக்கட்டுரை இதுவே முதன்முறையாகும்.

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INTRODUCTION

Baccaurea courtallensis (Wight). Müll. Arg., an endemic species to peninsular India (Kumar 2012; Narasimhan & Irwin 2021) requires special conservation measures to increase its population. The species survives the wet tropical biome and occasionally grows along river or stream banks in the moist deciduous forests of southern India. It is locally called Muttakaipu or Muttathuri in Malayalam and Mootupazham, Pulichampazham in the Tamil language. The habit is a medium-sized tree, growing to a height of 15–25 m, and produces crimson-red edible fruits, sour to sweet in taste when fully ripe. The fruit is reported to be a good source of vitamin 'C' and antioxidants (Nazarudhin 2010). This tree species has ornamental value as well, at full bloom it is a treat to watch the color contrast of inflorescences (Yogeesha et al. 2016). Tropical evergreen forests of the southern Western Ghats, Eastern Ghats, and Odisha (Balakrishnan & Chakrabarty 2007; Narasimhan & Irwin 2021) are the home of this evergreen tree. It is distributed in evergreen and semi-evergreen forest areas in the Western Ghats especially the southern Sahyadri and central Sahyadri (up to the Coorg region). Understory trees in low and medium-elevation evergreen forests up to 1,000 m (Abhishek et al. 2011). *B. courtallensis* is an underutilized fruit tree. In the broadest sense, the term "underutilized fruit tree" refers to a group of fruit trees that are currently growing in a dispersed and unattended manner on roadsides, homestead land, and wasteland despite having the potential for intensive utilization (Jisha et al. 2015). Fruits are crimson red in colour and acidic in taste. Only the local tribal population of the Western Ghats region consumes these fruits and it's not widely known to others. Fruits of *B. courtallensis* are eaten for their medicinal properties too. Fruits are used to induce fertility in men and women (Daniel et al. 2005). Fruits are also used to prepare jam, squashes, and wine. The fruits are also consumed in greater quantities by tortoises and Sloth Bears, which possibly reduces the likelihood of natural regeneration (Mohan 2009). The flowering period of *B. courtallensis* starts in February and extends up to April; the peak flowering month is March and the fruits develop and mature from June to September, during the rainy season. Literature on the reproductive biology or floral biology of this tree is sporadic. The present investigation was carried out to study plant morphology, flower, fruit morphology, anatomy, pollen biology, and fruit set.

MATERIALS AND METHODS

Study area

The present research work was carried out from 2018 to 2022, in the southern Western Ghats of Tamil Nadu and Kerala. The Western Ghats is a magnificent mountain range, next only to the Himalaya, and has rich biological wealth with a high degree of endemism. Southern Western Ghats is one of the richest areas of India in the context of floristic, diversity, composition, holding a large number of endemic taxa. *B. courtallensis* is one among the endemic species of south Western Ghats and is distributed in Tenkasi, Tirunelveli, and Kanyakumari districts of Tamil Nadu and in Kerala, it is located in all districts but more abundantly in Kollam, Thiruvananthapuram, Pathanamthitta, and Idukki districts. To locate the candidate species, field expeditions were made to the forests in the KMTR zones, especially the Mundanthurai range of Ullaru, Kannikatti, and Kodamaadi beats, Kadayam range of Kadana beat, Therkumalai, and Mylodai estate, as well as at Kollam forest division of Achenkovil forest range. A total of about 2,500 individuals were found in the Ullaru (1700), Kodamadi (600), and Achenkovil (200) forest areas. Detailed field investigations on the reproductive biology and phenology of the species were conducted in these forest areas (Figure 1).

Selection of trees

Baccaurea courtallensis is an evergreen medium-sized tree that grows up to 15–25 m in height. It is a dioecious tree; the morphological characters are similar in both male and female trees, except for the stem region. The distinguishing factor for the identification of male and female trees during the non-flowering season is based on the scars on the trunk. The inflorescence-arising zones of the male tree are numerous, while those of the female tree are fewer. The trunk size of the female tree is larger than the male tree and scars of several floral primordia regions were observed in the entire male tree. For the purpose of observing plant morphological traits, 25 female, and 25 male trees from fourteen population sites were chosen. The tree's height and width, stem, bark colour, branching pattern, leaf arrangement, and the places where inflorescences emerge in both sexes were all documented in the field.

Floral phenology

Flowering phenology was observed in a sequential manner from the bud to the seed maturity stage. Twenty-five healthy trees were selected randomly from the study

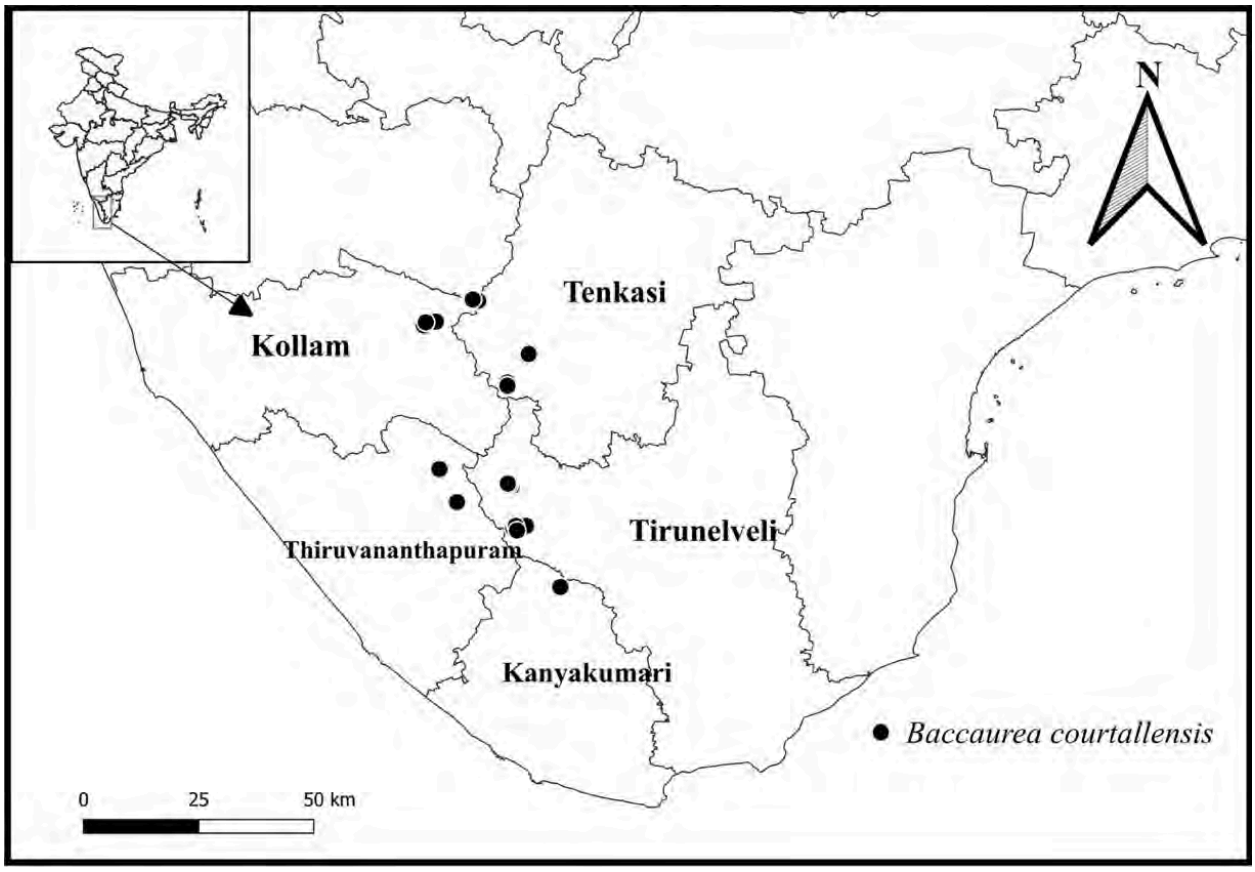

Figure 1. Distribution of *Baccaurea courtallensis* populations from southern Western Ghats.

sites, during the period 2018–2022. Observations were made on the reproductive phases of plants, with respect to the ontogeny of the floral primordium, development of flowers, maturation, anthesis, pollination, and natural withering of flowers. Floral visitors were also recorded among these plants (Image 1 F, G & Image 2 I, J).

Inflorescence and floral morphology

Male and female inflorescences and floral morphologies were noted. The inflorescence type, position, average number of inflorescences per cluster, floral characteristics, and flowering time were all closely monitored. A Nikon SMZ 800 stereomicroscope fit with a digital dark box and facilitated with Nikon NIS-Elements basic research imaging software was used for standard research applications such as analysis and photo documentation of fluorescent imaging, four-dimensional acquisition, and advanced device control capabilities.

Male and female flowers

Flowers are one of the key characters to distinguish male and female trees of *B. courtallensis*, however, it is hard to distinguish male and female inflorescences at the very early stages of their development and it's difficult to count male and female inflorescences from cluster racemes of primordial regions due to several clusters. Twenty-five flowering trees, as mentioned earlier, of both sexes were selected in a population for counting inflorescences and flower buds. The mean number of clustered inflorescences and flower buds of males and females was calculated and tabulated (Table 3).

Fruit morphology and anatomy

The fresh and mature fruits were harvested and brought to the laboratory immediately. After harvest, the collection was completely randomized without seeing the size. The collected fruits were stored in plastic bags and kept in a refrigerator. Fruits' fresh and dry weights were measured using a digital balance. The maximum length (linear distance between peduncular and stylar ends) and maximum diameter (linear distances across the width of the fruits) were measured using a digital Vernier caliper.

Free hand cross and longitudinal sections of fruit and seed were taken and observed under Nikon 80i fluorescent microscope fitted with a digital photography

workstation and SNZ stereo microscope. Specific stains and reagents such as Toluidine Blue 'O', Safranin, and oil red were used to detect specialized structures. After staining, sections were mounted on clean micro-slides using glycerin, as a mounting solution. The anatomical features were observed and microphotographs were taken.

Pollen Biology

Pollen-Ovule ratio

Healthy, undehisced mature flower buds were taken to determine the pollen-ovule ratio. Mature and fresh anthers from 20 flowers were randomly collected during the early morning and squashed separately in a mixture of ethanol (0.5%), methylene blue, and detergent (0.9ml+0.3ml+0.4ml) (Dafni et al. 2005). Thus, collected pollen grains were mixed thoroughly by the repeated drawing of the liquid into a disposable syringe and expelling it with force. Pollen suspensions of 100 µl were taken on a clean hemocytometer and the total number of pollen grains were counted in a sample under the light microscope. The procedure was repeated with 10 samples of a suspension and the average number of pollen grains was calculated. Twenty young pistils were collected from the flowers used for ovule count and the average number of ovules per ovary was determined by dissecting the ovaries under the microscope. The pollen-ovule ratio was calculated by the following formula (Cruden 1977). The percent of fruit set was calculated by dividing the mean number of matured fruits by the mean number of female mature flowers in an inflorescence.
The pollen-ovule ratio is the mean number of pollen grains per flower/mean number of ovules per flower.

Pollen fertility

The dye acetocarmine and glycerine reagent method was used to determine the fertile pollen grains by the colour change of cytoplasm alone into deep red and the pollen wall remained uncoloured (Shivanna & Rangaswamy 1992). Fresh pollen grains were collected from ten flowers and transferred to a clean slide and two drops of the acetocarmine glycerine mixture were added in a ratio of 3:1 and mixed thoroughly. After 15 minutes the slides were examined under a light microscope. The number of stained and unstained pollen grains was counted. The stained pollen grains were considered as fertile and the unstained as sterile.

Pollen viability

The viability of pollen grains was examined by three different reagents such as flurochromatic reaction test (FCR), 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) and Iodine-Potassium-iodide test (I_2KI). The pollen grain was considered viable if it appeared green in FDA (Fluorescein diacetate) under a fluorescent microscope (Heslop-Harrison, 1970), dark red in TTC test (Shivanna & Rangaswamy 1992), and brown in the I_2KI test (Sulusoglu & Cavusoglu 2014) under a light microscope. Viable and non-viable pollen grains were counted in each field of view for calculating the percentage of viable and non-viable pollen grains.

In vitro pollen germination

Fresh pollen grains were collected on the day of anthesis for pollen germination studies. *In vitro* pollen germination was conducted in Brewbaker and Kwack (BK) medium (Brewbaker & Kwack 1963) in different levels of sucrose (5, 10, 15, and 20% solutions), in order to determine the effect of different nutrients like boron, and calcium nitrate at various concentrations of sucrose. The fresh pollen samples were placed into the BK medium and kept in petri dishes lined with moist filter paper and incubated for 12 hours. After incubation for twelve hours, pollen samples were observed under a light microscope. The observed data was recorded and the percentage of pollen germination was calculated using the following formula.

Percent of pollen germination = Number of pollen grains germinated / Total number of pollen grains observed × 100

Floral visitors

During the flowering period field visits were carried out to record floral visitors and pollinators. The flowers are small and red in colour and male flowers emit mild musky fragrance. Floral visitors and their behaviour were recorded from 0700 h to 1330 h at each study site using Canon D-SLR Camera. Insect visitation starts around 0730 h. Observations were made on insect floral visitors, visiting time, the purpose of visiting, foraging activity and time spent on each flower. Floral visitors were photographed using a Canon D-SLR camera. Some of these floral visitors were collected and preserved for identification.

RESULTS

Distribution and morphology of the plant

The selected trees were found at altitudes between 180 to 1,000 m in forest areas of Kadana, Kannikatti, Ulllaru, Ingikuzhi, Kodamadi, Ueipattrai and Valaiyaru,

of Tirunelveli District and Courtallam of Tenkasi District (as per state government orders) of Tamil Nadu. In Kerala, it is located from Achenkovil and Thenmala of Kollam District and Kallaru, and Bonacaud Estate on the way to Ponmudi, at an elevation of 400 to 700 m in the Thiruvananthapuram District (Figure 1). It is a shade-loving understory tree that can reach a height of 15 to 25 m, like other evergreen trees found in the Western Ghats. It is a slow-growing, dioecious tree and rarely monecious. Few monoecious trees were identified in Ullaru and Kodamadi forest regions. *B. courtallensis* trees are closely associated with *Antidesma menasu*, *Elaeocarpus venustus*, *Eugenia singampattiana*, *Polyalthia korintii*, *Celosia polygonoides*, *Calamus* sp., *Hydnocarpus pentandra,* and *H. alpina*.

Fourteen population sites were selected for observing the stand height, GBH, and density of the selected candidate species. Among the 14 populations, only a few forest areas had a large number of trees specifically Ullaru, Kodamadi, and Achenkovil. The distribution of the population was observed continuously to a stretch of 10 km in Ullaru, while in Kodamadi the distribution was discontinuous and broken into smaller groups. The population size in Ullaru and Kodamadi forest areas was about 250 individuals per 5 km radius whereas in Kadana, Achenkovil, and Kallaru to Ponmudi the population size was 50–100 (Table 1). The number of adult individuals, stand height and GBH was quantified using population structural data. In every population, there were more male trees (77.56%) than female (22.43%) trees. The height of male trees ranges from 10–25 m, whereas the female trees were 10–20 m. Male trees' mean GBH was 54 cm, whereas female trees' mean GBH was 58 cm.

The main threats to candidate species are natural landslides, habitat disturbance, formation of roads and dams, and other non-forestry activities, since mud roads have been extended and anthropogenic activities have made the species more susceptible. The majority of populations were found along roadsides and in neighboring regions. If no specific precautions are taken for this plant across the sites, the species will be more vulnerable to catastrophic events resulting in a reduction of populations.

Baccaurea courtallensis grows up to 15–25 m tall (Image 1A & Image 2A). The colour of the bark is grey usually smooth or scaly, blaze light orange covered by lichens. Branchlets terete, glabrous. Leaves simple, alternate, clustered at twig ends, stipules ovate, acute, hairy, and caducous. 1.2–3.8 cm long, swollen at both ends, terete, puberulous when young. Leaf lamina 7.5–17.8 × 3–7.6 cm, oblanceolate, apex bluntly caudate acuminate, base cuneate, glabrous, midrib slightly raised above, secondary nerves 4–8, ascending, tertiary nerves slender, distantly per count. As per earlier reports, the trees are unisexual and dioecious. The present study reports for the first time that the trees are rarely monoecious (Image 4). At two different forest locations namely Kodamadi and Ullaru a few trees were observed

Table 1. *Baccaurea courtallensis* populations in selected forest areas of Western Ghats.

	District	Forest location	GPS coordinates			No. of Individuals	
			Latitude	Longitude	Altitude (in m)	Male	Female
1	Tirunelveli	Kannikatti	8.63083	77.27417	795	26	12
		Ullaru	8.62875	77.29455	615	160	50
		Ingikuzhi	8.61955	77.27647	644	24	7
		Kodamadi - Ueipattrai	8.70730	77.26643	496	60	12
		Vethalakan odai	8.71018	77.26237	457	40	19
		Kalkatodai	8.71197	77.25870	482	44	23
		Kadana	8.966466	77.299725	181	13	4
2	Tenkasi	Courtallam-Therkumalai estate	8.90412	77.25780	569	9	2
		Courtallam- Mayilodai estate	8.91028	77.25760	734	36	9
3	Kollam	Achenkovil	9.07085	77.20057	382	65	7
		Thenmala	9.02872	77.09707	672	27	5
4	Thiruvananthapuram	Kallaru	8.74087	77.12062	394	14	2
		Ponmudi - on the way	8.74045	77.12377	482	11	2
		Bonacaud estate	8.67570	77.15845	514	7	1
	Percentage distribution of male and female trees					77.56%	22.43%

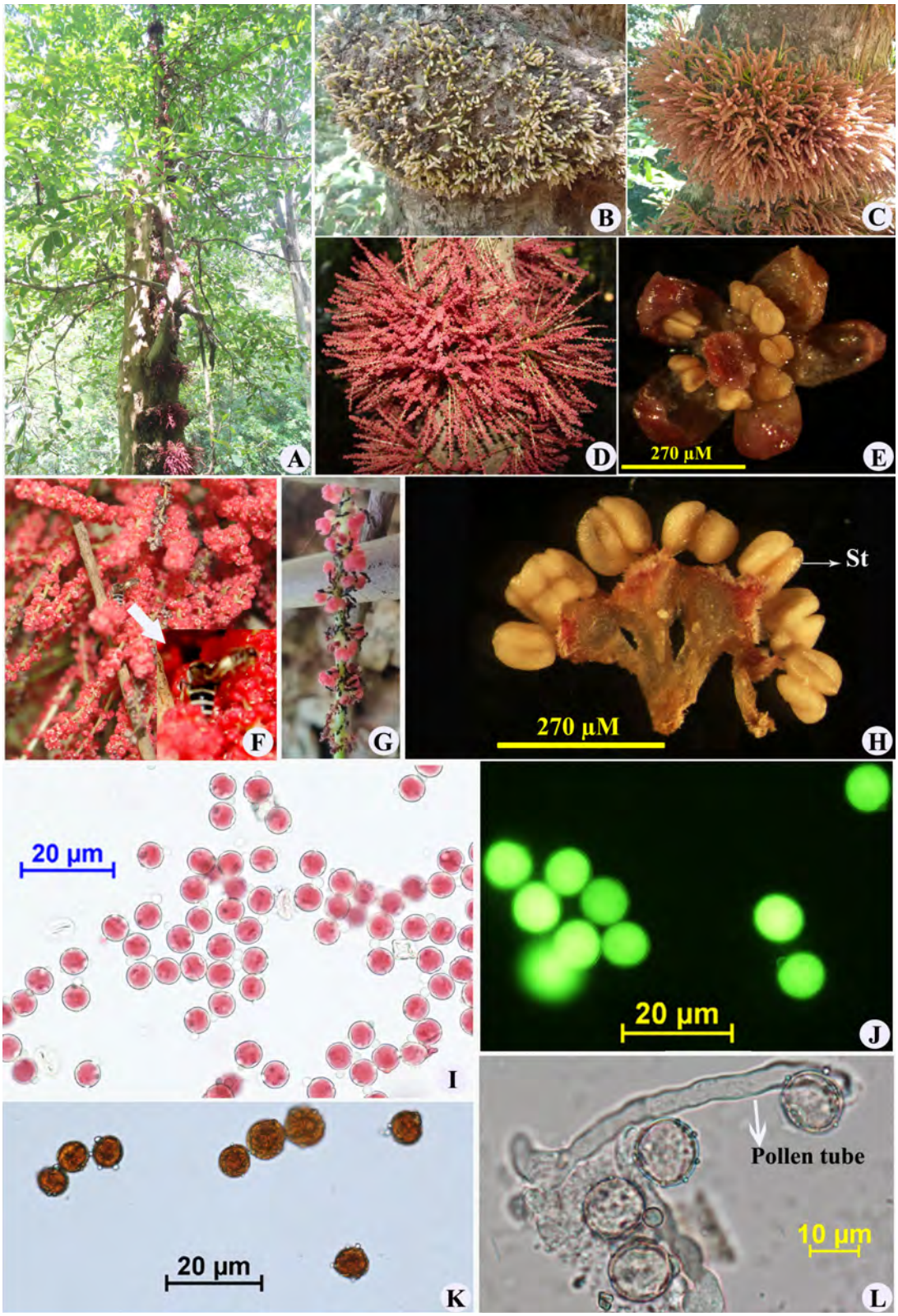

Image 1. A—Habit of 15 m tall male tree in flowering | B—Five day's old male inflorescence arose from the male tree trunk | C—Fourteen days old male inflorescence | D—Twenty-four days old male inflorescence with a drastic change in colour | E—Close up view of a male flower with seven stamens | F—Honey bee taking nectar from male flowers | G—Activity of Black ants on male flowers | H—Dissected male flower bud showing prominent stamens | I—Light microscopic image of viable pollens stained with Acetocarmine | J—Fluorescent microscopic image of Pollen grains - FCR test for viability | K—Light microscopic view of viable pollen grains - I2KI test for viability | L—In vitro pollen germination in 10% sucrose in BK medium | St—Stamen. © All authors at MSU- Tirunelveli and SPC- Courtallam.

with monoecious flowering conditions.

Floral phenology

The term 'phenology' describes how living things react to climatic and seasonal changes in a cyclic manner. Seasonal patterns are observed in a variety of reproductive processes known as pheno-events in flowering plants, including the commencement of flowering, fruiting, and seed distribution (Shivanna & Tandon 2014). The phenological data of *B. courtallensis* was observed and recorded based on repeated field observations. When the tree attains maturity (nearly 10–12 years), floral primordia appear from the base to the middle portion of the main trunk, from which crimson-red flowers emerge (Yogeesha et al. 2016). *Baccaurea courtallensis* was confirmed as unisexual, dioecious, and infrequently monoecious trees. The seasonal and climatic changes influence the flowering phenology of evergreen trees as witnessed over a period of four years. *Baccaurea courtallensis* begins flowering in the month of February and extends up to May, however, reaches mass flowering during March. Both male and female floral buds primordial are eventually developed into brown buds and then mature into dark red coloured buds (Image 1B,D). The flower buds take 16–24 days from initiation to full bloom. Flowers open in the morning from 6.30–10.00 h and anther dehiscence was noticed around 1330 h after anthesis. However, seasonal and climatic changes have made a strong impact on the flowering of *B. courtallensis* (Image 4). Continuous observations helped us to determine the flowering period, occasionally flowering occurred in early February and in some other times cauliflorous initiates from March and extended up to May (Image 4). These changes occur due to rainfall and other environmental factors and such factors significantly influence the flowering phenomenon of these trees.

Floral morphology

The inflorescence is cauliflory, a large population of trees that produce dark crimson colour flowers, in densely clustered slender racemes on the old stem. Rarely trees were observed in the monoecious status where male and female flowers are produced in the same inflorescence stalk but such cases have yielded fewer fruits (Image 5). The flower size of candidate species was observed in female flowers with 3–4 mm (Image 2C,D) and in male flowers 1 mm to 1.5 mm (Image 1E). Female flowers are relatively larger than male flowers and the tepals of the female are also larger than the male flower (Image 5). Both male and female flowers emit a mild musky odour. Floral traits of *B. courtallensis* are listed in (Table 2).

Table 2. Floral characteristics of *Baccaurea courtallensis*.

	Criteria	Result
1.	Flowering period	February to March/ May
2.	Flower type	Unisexual, dioecious, and rarely monoecious
3.	Flower colour	Crimson red
4.	Odour	Musky fragrance
5.	Presence of nectar	Present, at the base of stamens
6.	Anthesis time	0630–1000 h
7.	Anther dehiscence time	0900–1145 h
8.	No. of anthers per flower	7 to 8
9.	Total no. of pollen grains in a male flower	Approximately 1,500
10.	No. of ovule per flower	6 (3 locules, 2 ovules in each locule)
11.	Pollen size	10 to 12 µM
12.	Stigma type	3-flabellate
13.	Pollen fertility	Fertile (tested by I_2KI, TTC, Acetocarmine and FDA)
14.	Fruit type	Capsule/Fleshy berry
15.	Pollination	Wind assisted insect pollination
16.	Floral visitors	Honey bees, flies, black ants, spiders

Male flowers

The male inflorescence appears in clustered racemes on short tubercles all over the trunk, red in colour. Bracts, linear lance-shaped or triangular, free, conduplicate, encircling the base of lateral branches; tepals 4–5, 1.5–2 x 1 mm, linear, oblong, elliptic, nearly round or inverted lance-shaped, glabrous or sparsely puberulous; stamens 6–7, free and fertile; anthers basifixed; pistillode club-shaped (Image 1H).

Several clustered racemes are found all over the trunk. The stem shows several dark brown coloured scars, where the floral primordium abscised. The male flower buds take 5 to 24 days from initiation to maturation (Image 1B,C,D). At the time of blooming each cluster produces numerous inflorescences. Each cluster has a maximum of 96 male inflorescences, a minimum of 13, and an average was 58.32. The single inflorescence consists of many of floral buds, maximum numbers of floral buds were 85, the minimum was 39 and the average number of buds was 64.32. The length of male inflorescence was also measured; the maximum length was 19 cm; the minimum was 7.5 cm and the average was 12.10 cm (Table 3).

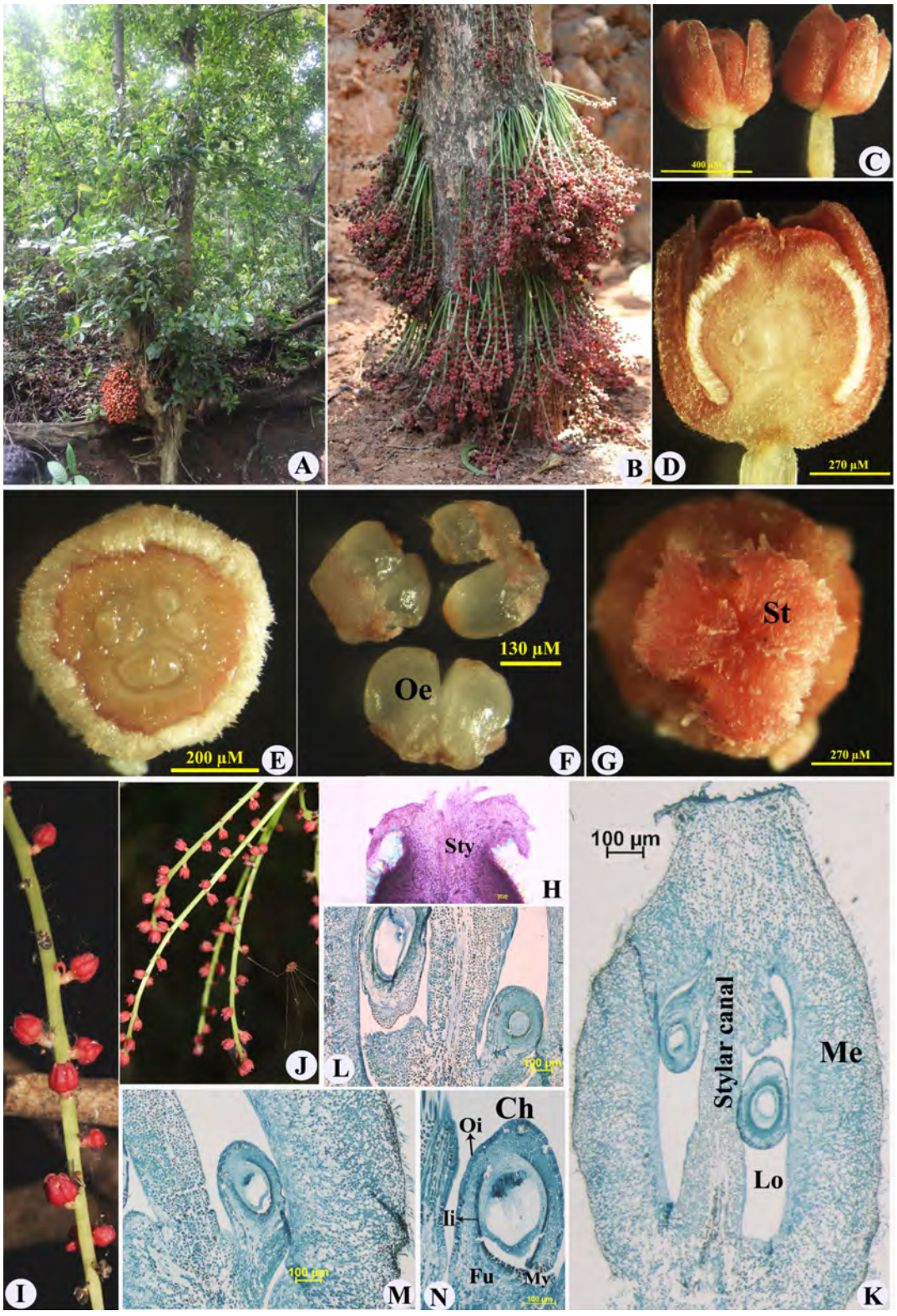

Image 2. A—Female tree showing bunches of ripened fruits at the base of trunk | B—Clustered female inflorescences emerged from the base of tree trunk | C—Close up view of female flower buds | D—Longitudinal section of a female flower bud | E—Cross section of female flower bud showing trilocular ovary and immature ovules | F—Dissected immature ovules from a female flower | G—Close up top view of stigma | H—Longitudinal section of portion of ovary, style and stigma | I–J—Foraging activity of honey bee and a spider in female inflorescence | K—Longitudinal section of fertilized female flower with ovules in 0.8 cm size fruit | L–M—Close up view of female flower showing 2 distinct ovules | N—Enlarged view of fertilized ovules | Oe—Ovule | St—Stigma | Ch—Chalazal region | My—Micropylar region | Oi—Outer integument | Ii—Inner integument | Fu—Funicle | Lo—Locule | Me—Mesocarp. © All authors at MSU- Tirunelveli and SPC- Courtallam.

Table 3. Number of inflorescences, buds, and length of female and male flowers.

Traits	Sex of the trees	Sample size (n)	Mean±SD	Std. Error	Minimum	Maximum
No. of Inflorescences in a cluster	Female	25	10.56±3.428	.68576	6.00	19.00
	Male	25	58.32±22.24	4.4496	13.00	96.00
	One-way ANOVA		*F* = 112.535, *P* <0.01%			
Length of Inflorescences in cm	Female	25	18.22±2.665	.53314	13.00	25.00
	Male	25	12.10±3.034	.60690	7.50	19.00
	One-way ANOVA		*F* = 57.396, *P* <0.01%			
No. of flower buds	Female	25	34.00±5.751	1.1503	23.00	45.00
	Male	25	64.32±13.06	2.6132	39.00	85.00
	One-way ANOVA		*F* = 112.765, *P* <0.01%			

Female flowers

Female inflorescences are born on clustered racemes mostly confined to the base of the trunk (Image 2B). Small projections were observed on such trunks where the female inflorescence arises. Each cluster had a maximum of 19 female inflorescences, a minimum of 6, and an average of 10.56. The single inflorescence contained many flower buds with a maximum number of 45 buds, a minimum of 23, and an average of 34. The length of female inflorescence was also measured by using a scale; the maximum length was 25 cm, the minimum was 13 cm, and the average was 18.22 cm (Table 3).

Bracts 1–1.5 mm long, lance-shaped; tepals 4–5, 2.5–3 x 0.6–1.5 mm, linear, oblong or oblong-elliptic, sparsely puberulous to hairless, fringed with hairs; the ovary is superior, woolly, 3-angled, 2.5 x 2–3 mm, ovoid or sub-spherical, trilocular; ovules 2 in each locule (Image 2F); as the flower is trimerous stigma is with three flabellate sticky surfaces to be successful by wind pollination (Image 2G,H). Style and stigma are reduced close to the gynoecium. The ovule structure of *B. courtallensis* is anatropous with amphistomal making up the micropyle, crassinucellate, origin from axile placentation, bitegmic integuments, and obturator forming a nucellar beak. The position of the ovule is ventral epitropous. The ovary normally has two ovules in a locule. The embryo is chlorophyllous and the endosperm is cellular in nature. Seeds are arillate with white mucus fibers on the surface. The cross-section of the flower showed three distinct locules (Image 2E), in each fruit two–three seeds are produced at the time of maturation and the remaining ovules are either aborted or unfertilised.

Fruit morphology and anatomy

The fruit is a capsule and Bacca (fleshy fruit) thus the name *Baccaurea* (Image 3A). Fruits are crimson to brown after maturity, globose, beaked, 1.5–2.5 cm across; ribbed, pubescent when young, seeds broad composed with fleshy aril and have exocarp, mesocarp, and stony endocarp (Image 3B). The fruit shows tricarpellary ovary with well-developed three locules (Image 3D). The average fruit size in length is 25.36 mm and its width is 25.30 mm. The thickness of the fruit varies from place to place with a range of 23.91 to 35.38 mm. Similarly, the fresh weight of the fruit varies from 19.5 to 26.52 g (average 22.77 g), and the dry weight has an average of 5.22 g. The fruit has about 77.06 % of moisture content at the time of harvest.

Exocarp is made of a single layer of epidermis with thin wall cells (Image 3F), rectangular in shape, it contains more amount of anthocyanin pigment, and chloroplast is deposited within the epidermis cells. The size of the epidermis has a maximum length of 229.79 µm and a minimum of 103.08 µm (average 155.93 µm) and width maximum of 134.28 µm and a minimum of 60.04 µm (average 106.45 µm). Fruit cover has stomata of cyclocytic type. Stomata are surrounded by one or two narrow rings of subsidiary cells and two guard cells. The pubescent nature of fruits is due to the presence of trichomes and hooked hair-like structures with thick-walled cells (Image 3E). Mesocarp is 2 to 3 mm thick and has approximately 40–50 layers of parenchymatous cells. Mesocarp consists of two types of cells, thick-walled cells, and thin-walled cells. The size of thin-walled cells has a maximum of 267.62 x 239.82 µm and a minimum of 118.94 x 124.42 µm. Consequently, the thick-walled cells have a maximum of 195.13 x 179.48 and a minimum of 63.63 x 76.49 µm.

Seeds are covered with an extra, fleshy layer outside the seed coat. This fleshy layer more or less envelops the seed coat and it is known as aril (Image 3C). In transverse sections, the mucilage layer is present below the testa, and mucilage cells are thin-walled in nature, with fiber-

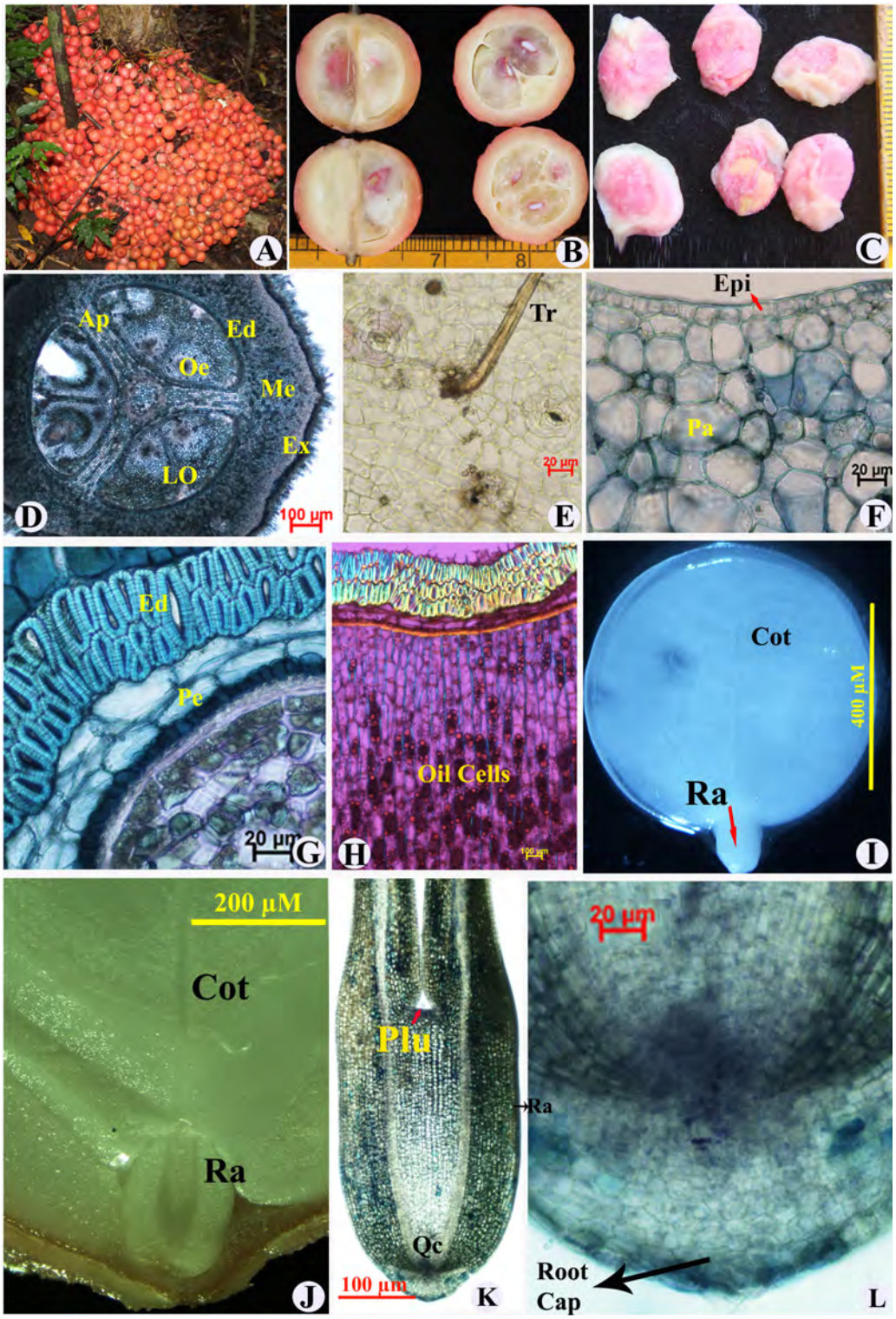

Image 3. A—Clustered crimson red mature fruits | B—Longitudinal and cross sections of mature fruits | C—Fleshy arillate seeds | D—Cross section of immature fruit stained with TBO observed under a light microscope showing tricarpellary ovary with ovules | E—Epidermal peeling of immature fruit rind shows cyclocytic type of stomata and hooked trichomes | F—Cross section of mesocarp region stained with TBO showing parenchymatous cells | G—Cross section of endocarp stained with TBO showing mucilage layer and testa | H—Longitudinal section of seed observed under polarized light microscope showing testa (stone cells) and oil cells in the endosperm | I—Close-up view of an immature embryo showing well-developed radicle and etiolated cotyledon | J—Longitudinal section of a mature embryo showing developed radicle region and faint plumule region | K—Longitudinal section of radicle showing root cap, cortex, and central cylinder | L—Enlarged view of the root cap. Ex—exocarp | Pe—perisperm layer | Me—mesocarp | Ap—axile placentation | Lo—locule | Pa—parenchyma cells | Mu—mucilage layer | Tr—trichome | Cot—cotyledon | Pu—plumule | Ra—radicle. © All authors at MSU- Tirunelveli and SPC- Courtallam.

like structures containing polyphenol compounds (Image 3G,H). Endocarp or testa is made of stone cells, it covers the endosperm, and two to five layers of cells are tightly packed; the length of a stone cell varies 73.04–37.85 µm (average 52.89 µm), and its thickness is between 95.61–216 µm (average 140.82 µm). The testa is followed by a palisade layer of thin-walled cells, 2 to 6 layers occur below the endocarp; a group of large-size druse crystals is located in the palisade layer. The length of the palisade cell varies from 86.54–343.21 µm (average 205.29 µm) and its width is 49.35–133.58 µm. Almost the entire region of the endosperm is occupied by the developing embryo with large etiolated cotyledons which are fleshy in texture and circular in shape (Image 3I). The length of endosperm cells varies from 94.04–167.95 µm (average 128.39 µm) and its width is between 48.75–198.9 µm (average 108.2 µm). The longitudinal section of the embryo axis shows developing cotyledons, hypocotyl region, and radicle with root primordium (root meristematic region) and well-developed root cap region (Image 3J,K,L).

Pollen Biology

Pollen morphology and production

The male flowers have a reduced pistillode and well-developed anthers (Image 1H). Pollen grains are spherical, trilobed, or four-lobed, circular in polar view, tricolporate to tetracolporate, colpus slender, and pore elongate with an unclear outline. The estimation of pollen production is important to understand many aspects of pollen biology. The amount of pollen produced in each anther/flower varies greatly. In *B. courtallensis* each anther produces a minimum of 1,648 to a maximum of 2,144 pollen grains; the mean number of pollen grains in a single anther was 1,446. The sizes of pollen grains vary from 10–12 µm in diameter.

Pollen ovule ratio

The study on pollen ovule ratios was used to predict the efficiency of pollination in a particular species (Oskay 2017). The cross-section of a pistil shows three locules and each locule has two ovules. The total number of ovules in an ovary is six. A single anther consists of around 1,446 pollen grains and thus a flower has around 10,122 pollen grains. The pollen ovule ratio was counted as 1687:1. This study indicates that external agents are needed for pollination and successful fruit set. The percent of fruit set for the candidate species is 74.

Pollen fertility and viability

Pollen fertility and viability is a critical factor, which is considered important parameter of pollen quality (Dafni & Firmage 2000). Pollen grains treated with reagents such as acetocarmine, TTC, and I_2KI essentially impart colouration to the cytoplasmic contents of the pollen to indicate whether the pollen grains are fertile

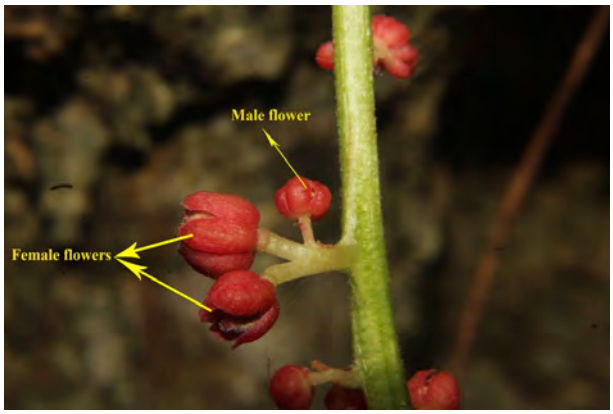

Image 4. *Baccaurea courtellensis* male and female flowers. © All authors at MSU- Tirunelveli and SPC- Courtallam.

Image 5. *Baccaurea courtallensis*: Evidence for variation in in flowering and fruiting period. © All authors at MSU- Tirunelveli and SPC- Courtallam.

or not. The stained pollen grains were considered fertile and the unstained as sterile (Image 1I). This technique revealed that 96.24% of pollen grains are fertile on the day of anthesis and this is used to know the pollen longevity. Pollen viability was determined by using the Flurochromatic reaction test (FCR), 2, 3, 5-Triphenyl Tetrazolium chloride (TTC) test, and the Iodine Potassium iodide (I_2KI) test. The percentage of pollen viability on the day of anthesis in the FCR test showed 80.00% (Image 1J) TTC test was 86.72% and the I_2KI test was 91.03% (Image 1K). Among the three staining tests I_2KI expressed the highest pollen viability (91.03%).

In vitro pollen germination

Pollen germination was carried out in Brewbaker and Kwack medium at different concentrations of sucrose such as 5%, 10%, 15%, and 20%. The maximum percentage of pollen germination was observed in 10% sucrose after 12 hours of incubation. Pollen germination was low in 5, 15, and 20% levels of sucrose-supplemented BK medium. BK medium augmented with 10% of sucrose, showed 63.15% of pollen germination. (Image 1L). Increasing the concentration of sucrose beyond 10% inhibits pollen germination in *B. courtallensis*. A similar kind of response was reported in *Tectona grandis* tree (Hine et al. 2019) and this study corroborates such a finding.

Floral visitors

The candidate species is a unisexual-dioecious tree; male and female trees produce flowers separately, so obviously, cross-pollination is predetermined. Dioecious plants have a great advantage over other plants wherein their cross-fertilisation is guaranteed (Renner 2014). Further, the cluster of inflorescences and the miniature size of flowers also designate that they may be cross-fertilised by wind and biotic means. During the flowering period, many floral visitors were observed. In female flowers; black ants and spiders were found however in male flowers, honeybees were also observed. During many such field visits, black ants and honeybees were detected in male flowers abundantly (Image 1F,G Image 2 I,J). Though, floral visitors were observed in large numbers, the presence of nectar glands or nectar organs in either male or female flowers could not be located. Floral visitation happens from 0730 h to 1000 h. The flowers are small, dark crimson colour with a mild musky fragrance. The floral visitors are attracted by the mass flowering with contrasting colour combinations.

DISCUSSION

The knowledge of phenology, and flower biology is essential to understand the persistence, dispersion, pollination, and breeding systems of plants (Munguia-Rosa et al. 2011). Phenological studies are essential to increase knowledge of the specific functions of plants in natural populations and they must be taken into account in conservation and rational management schemes (Oskay 2020). The development of successful conservation and sustainable use strategies for endangered, indigenous plants depends on comprehensive knowledge of their reproductive biology. The current findings revealed the species floral phenology, male and female flower morphology, and pollen biology. The selected species is an understory semi-evergreen to evergreen tree, endemic to Southern Western Ghats and Eastern Ghats. Fruits of this tree are edible and used by Kani, Kadar, Muthuvan tribes for medicinal purposes specifically to treat infertility problems (direct conversation with them).

An altitudinal gradient, which supports the significance of elevation in the distribution of a tree species, is the foundation upon which a forest community depends for its survival (Liu et al. 2007; Barni et al. 2012). Based on our random and repeated field observations this tree occurs from 200 m to 1000 m altitude in Tamil Nadu and Kerala forest areas. This tree prefers a canopy region to grow and reach up to 15 m in height. The current investigation revealed that the vegetative phase of both sexual plants displayed similar phenological characteristics throughout the season. Both male and female trees share a similar kind of morphological pattern except for the stem portion. Only the flowering phenomenon helps to identify both sexes.

Baccaurea courtallensis is found to be unisexual and dioecious tree in abundance however; monoecious trees were also recorded sporadically based on our findings. Though, Monoeceae status was reported by Yogeesha et al. (2016) where the male and female flowers were observed in different inflorescence stalks, however, the present study reports that both male and female flowers are present in the same inflorescence stalks. A similar kind of morpho-variation was observed in *Jatropha curcas* flowers (Singh et al. 2010). This tree begins to flower in February and continues till April, with a peak flowering period being recorded in March. In comparison to female flowers, male trees begin flowering earlier. These changes might be influenced by local climate and edaphic conditions. In dioecious species, the timing of flowering varies between the sexes, with the male

typically starting to flower before the female tree (Lloyd & Webb 1977; Beach 1981). In dioecious species, male plants often produce a greater number of flowers than female plants (Vaughtton & Ramsey 1998) to provide sufficient pollen grains for successful pollination. Fruit growth and development were observed from April and need 4–5 months to attain full maturation. Fruits that have matured fall off from June to September. Fruits are eaten by frugivores especially elephants, sloth bears, monkeys, squirrels, bats, and tortoises. The tortoises possibly consume a greater number of fruits than any other due to the nature of cauliflory and close abundance to the floor. As a consequence of this behavior, seeds are also dispersed by these frugivores. The plant and animal interactions signify a mutualistic network of seed dispersal systems in *B. courtallensis*.

Studying anthesis and anther dehiscence is essential for understanding how pollen grains disperse into the atmosphere (Bhattacharya & Datta 1992). Flowers of *B. courtallensis* open in the morning 6.30–10.00 hours, male flowers are short-lived compared to females. Dehiscence of anthers was observed after 30–45 minutes of a flower opening. The mean number of pollen grains in a single anther was 1,446. In the acetocarmine glycerin and the flurochromatic tests, pollen grains displayed a 96.24% fertility rate and an 80% of viability rate, respectively. However, I_2KI test showed 91.03% of pollen viability, it may be due to more amount of starch content present in the pollen grains. This method is commonly used to stain the starch content in pollen grains (Bolat et al. 1999). Iodine broke up in a watery arrangement of potassium iodide the tri-iodide-anion edifices with starch, and it distinguishes the viable and non-viable pollen grains. The same kind of result was observed in hybrid banana pollen grains (Ssali et al. 2012). It has been known that pollen viability is an important factor in assessing the good quality of pollen (Sulusoglu et al. 2014). Brewbaker and Kwack's medium supplemented with 10% sucrose had a pollen germination rate of 63.1%. Pollen germination rate was higher in 10% sucrose-containing medium in many Euphorbiaceae species. 71% of pollen germination rate was observed in *Jatropha curcas* in a medium containing sucrose (Abdelgadir et al. 2012). Sucrose serves as a nutritive resource for pollen germination and assists in the maintenance of the osmotic balance between pollen cytoplasm and germinated medium (Johri & Vasil 1961). However, a higher concentration of sucrose inhibits the growth of pollen tubes (Hine et al. 2019). Pollination efficiency and pollinator availability are influenced by the timing of flowering (Bawa 1983). Female flowers have a lifespan of 5–7 days, while male flowers have a lifespan of 2–3 days after anthesis. Female flowers have pistils and reduced staminodes, and male flowers have well-developed anthers and diminished pistillode. Cross-pollination is facilitated in *B. courtallensis* by the flower architecture.. In the present study honey bees, black ants, and spiders were observed in male and female flowers. Black ants and spiders were considered floral visitors because they were found on both male and female flowers. A male flower offers nectar and pollen grains as food resources to floral visitors. There is a possibility for pollination by these floral visitors. A comparable type of insect visitation and pollination that occurred in *Phyllanthus emblica* was reported by Halder et al. (2019). Amla tree flowers are mostly cross-pollinated by wind and honey bees. The pollen ovule ratio is an important factor that determines the fruit set rate (Cruden 1977). The mean number of pollen/ovule ratio was 1687:1 and the percentage of fruit set was 74. Successful pollination and fruit set are greatly influenced by pollinators, as well as, by the abundance of male trees (Dafni et al. 2005).

The fruit is a bacca (berry) which has a thick and juicy pericarp with a coloured exocarp, fleshy mesocarp, and a membranous endocarp. The contrasting inference is offered by a few authors that fruits of phyllanthaeceae are dehiscent schizocarps (Fahn & Zohary 1955; Roth 1977; Gagliardi et al. 2013). However, the fruit of *Baccaurea courtallensis* is a bacca which is dehiscent naturally. The mesocarp encloses a fragile aril that develops from one of the structures like funiculus, raphe, or integuments (Fahn 1967). Arils are very common in tropical and subtropical plants. These arillate seeds are well adapted to dispersal by animals (Corner 1976). The fruit is a dehiscent type and develops from three carpels. Species of Phyllanthaceae have tricarpellary ovary with two ovules in each locule (Gagliardi et al. 2013). Though, fertilization takes place uniformly in a flower the number of seeds set at maturity is either one or two per fruit. Thus, out of six ovules, only one or two become fertile seeds and the rest either abort or are underdeveloped. As the flower and fruit set ratio is unbalanced a large number of flowers are produced on the tree trunks in order to increase the availability of fertile seeds. The fruits are fleshy and edible and hence, they attract animals which subsequently help in seed dispersal. Fleshy fruits are opened by dispersing animals or naturally by the rotting of the outer tissues (Mauseth 1988).

The seed is covered by a protective layer of cells called testa which is made of an outer thick layer

with macrosclerides and thin inner layers (Gagliardi et al. 2013). The scleride nature of these cells is easily distinguished while observing under polarized light facilitated with first order red plate. The thickness of the testa varies from region to region within a seed. Below the testa, two degrading layers of cells are found during the developmental stage which may be the perisperm and endosperm (Image 3G). Just beneath the digesting endosperm layer, the storage cells of cotyledon are found abundantly. These storage cells house starch and oil bodies enormously. Oil bodies in cotyledons are distinguished by rhodamine stain and Sudan III (Image 3H).

There is inadequate information in the literature on most of the aspects of the reproductive biology of *B. courtallensis* like phenology, floral description, pollen biology, pollination, and embryo development. The present study has carried out floral biology, phenology, pollen biology, and some aspects of fruit development for the first time in *B. courtallensis*, which may be useful for efforts on the conservation of this endemic, underutilized wild edible fruiting tree.

CONCLUSION

Baccaurea courtallensis is one of the few interesting and important members of the family Phyllanthaceae, which is endemic and occurs from 180 m to 1,000 m elevations in the Western Ghats of peninsular India. This taxon attracts attention due to its cauliflory and the bright red flowers and fleshy fruits that are attributed with curative properties. Apart from the ornamental purpose, the fruits of this tree are rich in nutritional value and possess medicinal properties. Due to anthropogenic activities and habitat destruction, this species has reduced in population. Being devoured by animals and exploited by local trade, the species is represented sparsely by fewer individuals. Successful management of forest resources requires knowledge of blooming phenology, the time, duration, and frequency of distinct phenoevents, particularly for species of forest trees. The conservation of this species depends heavily on the understanding of its reproductive biology, floral biology, pollen biology, and pollination. Thus, the flowering phenology, floral morphology, fruit anatomy, and pollen biology are reported in the current study in detail so as to better understand them for future applications. Future efforts to protect this unique tree might benefit a lot from this report.

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Author details: Karuppiah Nandhini is a Ph.D. research scholar & worked as Assistant Professor (Temporary) in the Department of Plant Science at Manonmaniam Sundaranar University. She is very passionate about the field of Plant Tissue culture and is also strongly interested in the fields of plant reproductive biology and molecular biology. She is keenly interested in the conservation of IUCN red-listed plants of Western Ghats. Vincent Joshuva David, a DBT Junior Research Fellow, is currently working on his Ph.D. in the Department of Plant Science, Manonmaniam Sundaranar University, Tirunelveli. He has a keen interest in practical exposure to Biodiversity, Plant Reproductive Biology, Plant Molecular and Biotechnology, Plant anatomy, and Conservation of Biodiversity. Venugopal Manimekalai is currently working as an Assistant Professor in the Department of Botany, Sri Parasakthi College for Women, Courtallam. Her fields of research include Plant anatomy and histochemistry, Natural dyeing of miscellaneous plant fibres, Plant tissue culture, and Plant reproductive biology. She was a recipient of the DST young women scientist (WOSB) award. She has bagged three times best student's research project awards from the Tamil Nadu State Council for Science and Technology. Perumal Ravichandran is working at present as a Professor in Manonmaniam Sundaranar University. His fields of research are the Conservation of Biodiversity, Agrostology, Grassland Management, Natural Dyeing, and Plant Biotechnology. He has over 25 years of teaching and research experience. He has extensively surveyed the Western Ghats and other forest areas in India for Botanical and Ecological investigations. He was a member of IUCN – SSC- Indian Sub-Continent Plant Specialist Group (ISPSG).

Author contributions: KN – Field & experimental study, data collection, compilation, writing, VJD- Field study, Field Photography, and Photo editing, Map preparation, VM-writing, proofreading and editing, PR- Field study, Hypothesis, conceptualization, Microphotography, writing, editing, and supervision.

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