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Studies on Floral Biology, Pollination Ecology, Pollen Viability, Pollen Rewards and HPTLC Fingerprinting of *Couroupita guianensis* Aubl.

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Abstract

Couroupita guianensis Aubl. (Lecythidaceae), commonly known as the cannonball tree, is a distinctive tropical tree characterized by cauliflorous inflorescences, large strongly scented flowers and an unusual reproductive architecture. The floral organization of the species is particularly remarkable because the androecium is differentiated into a staminal ring bearing fertile pollen and a specialized androecial hood bearing morphologically and functionally distinct pollen. Such structural specialization provides an important basis for investigating the relationship between floral morphology, pollen presentation, floral rewards and pollinator behaviour. The present study examines the floral biology and pollination ecology of *C. guianensis*, with particular emphasis on flower morphology, anthesis, pollen production, pollen–ovule ratio, pollen viability, pollen rewards and the possible participation of nocturnal visitors, including bats. In addition, HPTLC fingerprinting of pollen was undertaken to provide a chromatographic profile of the investigated plant material. Detailed observations were conducted on flowering individuals at Amravati during November to December. Floral development, timing of anthesis, anther dehiscence, pollen presentation, stigma condition and floral visitor activity were documented throughout the flowering period. Pollen production and viability were evaluated using quantitative and microscopic methods, while the pollen–ovule ratio was determined as an indicator of reproductive investment. HPTLC analysis generated a characteristic chromatographic fingerprint of the investigated extract, providing a complementary chemical dimension to the floral biological observations. The integrated approach adopted in the present study provides a comprehensive assessment of the reproductive biology of *C. guianensis* and emphasizes the functional significance of its unusual floral architecture. The findings are relevant to the understanding of plant–pollinator interactions, pollen–reward specialization and reproductive ecology in Lecythidaceae.

Keywords: *Couroupita guianensis*; Lecythidaceae; floral biology; pollination ecology; anthesis; pollen viability; pollen production; pollen–ovule ratio; pollen reward; bats; HPTLC.

Introduction

The reproductive success of flowering plants depends upon a complex interaction between floral architecture, pollen production, pollen viability, stigma receptivity, floral rewards and the behaviour of pollinating organisms. Flowers are not merely reproductive organs in a structural sense; they represent highly integrated biological systems in which morphology, timing, chemistry and ecological interactions operate together to facilitate the transfer of male gametes between compatible individuals. Consequently, the study of floral biology provides an important foundation for understanding the reproductive strategy and evolutionary ecology of angiosperms.

Floral characteristics such as flower size, symmetry, colour, scent, anthesis, anther dehiscence, pollen presentation, stigma morphology and the nature of floral rewards can strongly influence the identity and behaviour of floral visitors. At the same time, the production of pollen involves considerable reproductive investment, and the amount and quality of pollen available to visitors may influence both pollinator attraction and pollen transfer. The distinction between pollen that contributes directly to fertilization and pollen that functions primarily as a food reward is particularly significant in several specialized plant–pollinator systems. Within this broader context, the family Lecythidaceae represents an important group for investigating the relationship between floral structure and pollination ecology. Several members of the subfamily Lecythidoideae possess highly specialized androecia and distinctive mechanisms of pollen presentation. In some genera, the pollen itself constitutes the primary floral reward, whereas in others sterile or nutritionally attractive pollen, often termed fodder pollen, is presented to floral visitors. Research on Lecythidoideae has demonstrated that floral symmetry and reward type are closely associated with the diversification of pollination systems.

Among the members of this group, *Couroupita guianensis* Aubl. is particularly noteworthy. The species is currently accepted taxonomically within Lecythidaceae and has a native distribution extending from Panama through tropical South America. It is also widely cultivated in tropical regions beyond its native range. The species is commonly recognized by its massive woody fruits and flowers produced directly from the trunk and larger branches. The cauliflorous habit gives the flowering phase a conspicuous appearance and brings the flowers into a distinctive spatial relationship with visiting organisms. The flowers of *C. guianensis* are structurally unusual. They are relatively large, zygomorphic and perigynous, with a perianth consisting of several sepals and petals. The androecium is not uniform throughout the flower. Instead, numerous stamens form a staminal ring surrounding the gynoecium, while a laterally developed androecial structure extends over the reproductive centre of the flower. This structure, generally referred to as the androecial hood, contains numerous staminodes. Recent research describes approximately 600–700 stamens in the staminal ring and approximately 180–220 staminodes in the hood. The two regions differ not only morphologically but also in the pollen they produce. Pollen from the staminal ring is fertile and occurs as monads, whereas pollen from the androecial hood occurs in tetrads and is sterile. This differentiation is fundamental to an understanding of the pollination biology of the species. Earlier studies recognized the existence of two morphologically and functionally different pollen types in *C. guianensis*. Pollen from the staminal ring has been reported to possess a relatively smooth exine and lipid coating and to exhibit germination capacity, whereas hood pollen is characterized by a different morphology and lacks normal germination ability. Observations summarized by the New York Botanical Garden indicate that the distinction between ring pollen and hood pollen has been repeatedly confirmed by earlier investigations.

A recent experimental study published in 2025 has added an important new dimension to the reproductive biology of *C. guianensis*. Ribeiro et al. revisited the breeding system of the species using controlled pollination experiments and microscopic observations of pollen germination and pollen-tube growth. Their results demonstrated that naturally pollinated and cross-pollinated flowers were capable of producing mature fruits, whereas self-pollinated flowers-initiated fruit development but subsequently aborted. The authors interpreted the reproductive behaviour as evidence of postzygotic, late-acting self-incompatibility and self-sterility. The same study recorded a very large number of ovules per ovary, averaging approximately 1,128 ovules, while naturally pollinated mature fruits contained approximately 274 developed seeds on average. The resulting difference between ovule number and mature seed number demonstrates that reproductive success in the species involves substantial attrition between ovule formation and mature seed production. These findings are significant for the interpretation of pollen–ovule ratios. The P/O ratio is a useful comparative parameter in reproductive ecology because it relates male reproductive investment to potential female reproductive units. Cruden's classical work demonstrated that P/O ratios can provide an indication of breeding strategy, although such ratios should not be treated as independent proof of a breeding system. They are most informative when interpreted together with direct observations of pollination, pollen viability, pollen transfer and fruit or seed production.

Pollen viability is equally important because pollen production alone does not guarantee successful fertilization. The physiological competence of pollen changes with developmental stage, environmental conditions and duration after anthesis. Earlier investigations of *C. guianensis* have reported temporal changes in pollen viability and pollen histochemical characteristics during floral opening. In one study, high pollen viability coincided with the early period of floral opening and with the availability of other floral resources. The relationship between pollen and nocturnal visitors is another aspect requiring careful consideration. Floral characteristics such as strong fragrance, temporal opening, abundant pollen and substantial energetic rewards can provide clues concerning pollination syndrome, but these characteristics alone cannot establish a pollinator. Earlier observations discussed the possibility of bat involvement while also reporting relatively infrequent bat observations. Contemporary pollination research therefore emphasizes the need to distinguish between floral visitation and effective pollination. A visitor should be regarded as an effective pollinator only when there is evidence of pollen acquisition followed by deposition on a receptive stigma and, ideally, subsequent reproductive success.

The chemical component of the present investigation provides an additional dimension to the study. Floral rewards are chemically mediated, and the volatile and non-volatile constituents associated with flowers can influence interactions with visiting organisms. High-performance thin-layer chromatography provides a convenient fingerprinting technique for complex botanical extracts. HPTLC has previously been applied to *C. guianensis*, including phytochemical fingerprinting of fruit material. However, an HPTLC investigation specifically integrated with the floral biology and pollen-reward system may provide useful complementary information, particularly if the analysis is directed towards flower or pollen material. The present investigation therefore approaches *C. guianensis* as an integrated reproductive system rather than merely as a taxonomic or morphological subject. The study combines observations of floral structure and anthesis with quantitative assessment of pollen production, pollen–ovule ratio and pollen viability, examination of floral rewards and pollinator behaviour, assessment of possible nocturnal or bat visitation, and HPTLC fingerprinting of the selected floral material. Such an integrated approach can contribute to a more comprehensive understanding of how the unusual architecture of *C. guianensis* functions within its reproductive ecology.

Materials and Methods

1. Study site and plant material

The investigation was carried out on mature individuals of *Couroupita guianensis* Aubl. growing at Amravati, Maharashtra, India. The study was conducted from November 2025 to December 2025, covering the principal flowering period of the species at the study site.

A total of 10 mature trees were selected for observation. The selected individuals were healthy, mature and actively flowering. Each tree was assigned a field identification number and repeatedly monitored throughout the study period. Observations were made on flowers representing different developmental stages in order to document temporal changes in floral morphology and reproductive function.

The botanical identity of the species was confirmed using standard taxonomic literature and comparison with authenticated herbarium material. The accepted name *Couroupita guianensis* Aubl. is currently recognized within Lecythythaceae.



2. Floral Morphology and Structural Organization

Fresh flowers were examined using direct observation, a hand lens and a stereomicroscope. Floral dimensions were measured using a digital vernier calliper. The morphology of the perianth, androecium and gynoecium were documented in detail. Particular attention was given to the structural relationship between the staminal ring,

androecial hood and gynoecium. The number and arrangement of stamens were recorded, together with the dimensions and structural characteristics of the hood and its associated staminodes.

3. Anthesis and Floral Phenology

Flower buds approaching anthesis were tagged and monitored at regular intervals. Observations were made from the period immediately preceding flower opening until the completion of floral senescence. The timing of the following events was recorded: bud opening-petal expansion-anther dehiscence-pollen presentation-stigma exposure-floral visitor activity-androecium senescence. The duration of anthesis was calculated from the beginning of visible floral opening to the point at which the flower lost its functional reproductive structures. Recent controlled-pollination work recorded anthesis beginning at approximately 07:00 h and complete shedding of the corolla and androecium at approximately 17:00 h at the study site. The present investigation therefore treated anthesis as a field-dependent character and determined the timing independently at the study site.

4. Pollen Production and Pollen-Ovule Ratio

Pollen production was determined from mature anthers collected at the appropriate developmental stage. Individual anthers were placed in a known volume of pollen-dispersing medium and thoroughly agitated to release the pollen grains. Pollen grains were counted using haemocytometer. Multiple flowers from different individuals were examined to obtain representative estimates. The pollen production per flower was calculated from the mean pollen number per anther and the number of fertile anthers per flower. The pollen-ovule ratio was calculated according to:

$$P/O = \frac{\text{Total pollen grains per flower}}{\text{Total ovules per ovary}}$$

The P/O ratio was subsequently interpreted in relation to the observed pollination system rather than being used independently as evidence of the breeding system. This approach is particularly appropriate for *C. guianensis* because recent controlled-pollination research has demonstrated self-sterility and late-acting incompatibility in the species.

5. Pollen Viability

Pollen viability was examined at different floral stages, including pre-anthesis, anthesis and post-anthesis. Fresh pollen was collected separately from the fertile staminal ring and the androecial hood. Viability was determined using Alexander's stain method, while pollen germination, where performed, was assessed using in-vitro germination method.

The percentage of viable pollen was calculated as:

$$\text{Pollen viability (\%)} = \frac{\text{Number of viable pollen grains}}{\text{Total pollen grains observed}} \times 100$$

Where germination tests were conducted, germination percentage was calculated separately.

The two pollen types were not treated as biologically equivalent. This distinction is essential because pollen produced by the staminal ring is associated with fertilization, whereas the pollen produced in the androecial hood has been reported to be sterile.

6. Pollination Ecology and Floral Visitors

Floral visitors were observed during different periods of the day and night. Direct observations were supplemented by photographic and video documentation wherever possible. For each visitor, the following behavioural characteristics were recorded: arrival time, visitation frequency, duration of visitation, feeding behaviour, contact with pollen-bearing structures, contact with the stigma and movement between flowers. Particular attention was given to the sequence of events during visitation.

7. Assessment of Possible Bat-Mediated Pollination

Nocturnal observations were conducted during the flowering period to investigate possible bat visitation. Observations were carried out between 8 pm and 10 pm using direct observation and, where available, night-vision or infrared video recording. The presence of bats was recorded together with their visitation frequency, duration of flower handling, contact with the androecial structures and contact with the stigma. Where feasible, evidence of pollen transfer was investigated. The interpretation of bat visitation was deliberately conservative. The presence of bats at flowers was not considered sufficient evidence of bat pollination. Evidence of effective bat-mediated pollination requires demonstration of contact with fertile pollen, subsequent contact with receptive stigmatic tissue and preferably reproductive consequences such as fruit set. This approach is consistent with the broader literature, which has discussed bats as potential visitors to *C. guianensis* while also emphasizing the importance of direct observations.

8. Pollen Rewards

One of the most significant aspects of the floral biology of *C. guianensis* is the differentiation of pollen according to its location within the flower. Pollen from the staminal ring and pollen associated with the androecial hood were therefore examined separately. The comparison included pollen abundance, morphology, viability and, where feasible, histochemical characteristics. The distinction is biologically important because the hood pollen has been interpreted as a food reward rather than a direct reproductive resource. In Lecythidoideae, such sterile pollen rewards are commonly referred to as fodder pollen and represent a specialized mechanism through which flowers attract and retain pollinators. Thus, the floral architecture of *C. guianensis* can be interpreted as a functional system in which a reward-bearing structure and a reproductive pollen-bearing structure occur within the same flower.

9. HPTLC Fingerprinting

For chemical characterization, pollen material was collected separately, shade-dried and powdered. The powdered material was extracted using ethanol by Soxhlet extraction. The extract was filtered, concentrated and reconstituted

at a known concentration before HPTLC analysis. Chromatographic separation was carried out on silica gel 60 F254 HPTLC plates using an optimized mobile phase consisting of ethanol solvent system. The developed plates were examined under appropriate wavelengths, including 254 nm and/or 366 nm, where applicable. Densitometric scanning was subsequently performed to obtain the chromatographic fingerprint.

The HPTLC profile was evaluated on the basis of number of detectable bands, R_f values, band colour, peak area, peak height, and relative abundance of individual chromatographic zones.

The HPTLC procedure should be reported with sufficient detail to permit reproducibility. The HPTLC Association emphasizes standardized analytical procedures and appropriate system suitability/validation considerations for HPTLC methodology. Importantly, chromatographic bands were treated as fingerprint features rather than definitive compound identifications unless authentic standards or additional spectroscopic evidence were available.

Results and Discussion

1. Floral architecture

The flowers of *C. guianensis* exhibited a highly specialized organization that clearly differentiated them from flowers possessing a simple, radially symmetrical androecium. The cauliflorous arrangement of the inflorescences, together with the large and conspicuous flowers, produced a distinctive visual and spatial flowering display. The most significant structural feature was the differentiation of the androecium into the staminal ring and the androecial hood. The staminal ring surrounded the gynoecium and represented the principal source of fertile pollen, whereas the hood extended over the reproductive centre and contained specialized staminodes.

This organization is consistent with previous descriptions of the species, in which approximately 600-700 fertile stamens have been reported in the staminal ring and approximately 180-220 staminodes in the androecial hood. The structural differentiation of these two regions is unlikely to be merely morphological. It has a direct functional significance for pollen presentation and visitor behaviour. The presence of two distinct pollen types suggests that the flower allocates reproductive resources in two different ways: fertile pollen contributes to male reproduction, whereas sterile pollen may contribute to pollinator nutrition.

2. Anthesis and temporal organization

The observed anthesis pattern demonstrated a clearly defined temporal sequence of floral events. The transition from bud to fully functional flower involved coordinated changes in petal position, anther dehiscence, pollen availability and stigma exposure. The temporal organization of these events is important because pollination can occur successfully only when viable pollen and receptive stigmatic tissue overlap with effective pollinator activity.

Earlier investigations have documented both diurnal and nocturnal components of the floral biology of *C. guianensis*. A recent controlled study observed anthesis beginning around 07:00 h and floral structures being shed by approximately 17:00 h. In contrast, earlier research highlighted floral reward characteristics during nocturnal activity and reported high pollen viability and nectar-related measurements around the beginning of floral opening. These observations illustrate that anthesis should not be regarded as a single instantaneous event. Rather, it represents a period during which the functional properties of the flower change continuously.

3. Pollen production and reproductive investment

The quantitative pollen analysis revealed 1,11,000 pollen grains per flower. The number of fertile stamens was 650 while the mean pollen production per anther was 1800. The relatively high pollen production observed in *C. guianensis* should be considered in relation to its specialized pollination mechanism. A large quantity of pollen does not necessarily imply inefficient pollination. Where pollen is collected as a reward, a substantial fraction may be removed by visitors without contributing directly to fertilization. The existence of two pollen types provides an especially interesting explanation for this apparent reproductive investment. The flower can simultaneously present pollen as a nutritional reward while protecting or positioning fertile pollen in a manner conducive to transfer between compatible flowers.

4. Pollen-ovule relationship

The calculated P/O ratio was 1034:1. The significance of this value becomes clearer when considered alongside direct reproductive observations. P/O ratios provide a comparative estimate of reproductive investment, but they cannot independently establish whether a species is self-compatible, self-incompatible or predominantly outcrossing. The recent demonstration of late-acting self-incompatibility in *C. guianensis* is particularly relevant here. Ribeiro et al. showed that self-pollinated flowers could initiate fruit development but subsequently aborted, whereas naturally pollinated and cross-pollinated flowers were capable of producing mature fruits.

Thus, the P/O ratio in the present study should be interpreted as one component of a broader reproductive strategy involving pollen production, pollen viability, pollinator-mediated transfer and post-pollination reproductive selection.

Conclusion

The present investigation demonstrates the importance of treating *Couroupita guianensis* as an integrated floral and reproductive system. Its unusual floral architecture is closely associated with the organization and functional differentiation of pollen resources. The staminal ring represents the principal source of fertile pollen, whereas the androecial hood provides a specialized pollen resource that has been interpreted as sterile or fodder pollen.

The temporal organization of anthesis further contributes to the reproductive strategy by coordinating pollen availability, stigma function and floral visitor activity. Quantitative assessment of pollen production and pollen-ovule ratio provides information concerning reproductive investment, while pollen viability establishes the functional period during which male gametes remain capable of participating in fertilization. The pollination system is particularly significant because floral visitors interact with structurally differentiated pollen resources. The

possible participation of nocturnal visitors, including bats, requires direct behavioural and pollen-transfer evidence and should not be inferred solely from floral characteristics. The reproductive significance of these observations is strengthened by recent experimental evidence demonstrating self-sterility and late-acting self-incompatibility in *C. guianensis*. Consequently, effective pollination and appropriate pollen transfer between compatible individuals appear to be central components of reproductive success. The HPTLC fingerprinting component adds a complementary chemical perspective to the investigation and provides a basis for characterizing the selected floral material chromatographically. When combined with floral morphology, pollen biology and pollination observations, the chromatographic profile contributes to a broader characterization of the species.

Overall, *C. guianensis* represents a valuable system for studying the interaction between floral specialization, pollen differentiation, pollinator behaviour and reproductive success. The integration of morphological, phenological, palynological, ecological and chromatographic approaches provide a more comprehensive understanding of its floral biology than any single approach alone.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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