

## Selective insectivory in *Nymphaea nouchali* Burm. f.

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**Carnivorous plants comprise roughly 0.24 per cent of the flowering plants, or 640 species represented in 12 families<sup>1-3</sup>. Yet they are regarded as *miracula naturae*<sup>4</sup>. Over fifty per cent of these taxa are represented in a single family, namely Lentibulariaceae<sup>5</sup>. Carnivorous plants are generally insectivorous<sup>6</sup>, and carnivory in flowering plants is generally found in taxa that are adapted to nutrient-deficient habitats<sup>7</sup>. The extra nutrients such plants acquire by special ways serve merely as supplements. The origin and evolution of carnivorous plants is a mystery in the phylogenetic tree of angiosperms, they often appear without a clear linkage<sup>8</sup>. Here, we report that *Nymphaea nouchali* Burm. f. (a cultivar of var. *cernua*), a large aquatic member of the family Nymphaeaceae, indulges in a primitive form of insectivory and represents the missing evolutionary link. To the best of our knowledge, an insectivorous flower was not reported in flowering plants before.**

The early history of the evolution of angiosperms is believed to have been shaped by a series of radiations and quick diversification, which led to the formation of many new groups. Palaeobotanical studies<sup>9-13</sup> support the view that the so-called ANITA clads (Amborellaceae, Nymphaeales, Illiciales, Trimeniaceae, Austrobaileyaceae) were the first line to diverge from the main branch of the angiosperm phylogenetic tree.

Carnivory requires appropriate morphological features to attract, retain, trap, kill, and digest the prey. Darwin postulated several independent origins among carnivorous flowering plants<sup>4</sup>. Genotypic studies also reveal that carnivory is polyphyletic<sup>8</sup>. Nymphaeaceae is a primitive family, fossil record for which goes back to the early Cretaceous period<sup>9</sup>. Phylogenetic trees prepared on the basis of taxonomy suggest a strong evolutionary linkage between some carnivorous families such as Nepenthaceae and Sarraceniaceae to Nymphaeaceae and indicate the line of evolution; however, no hard evidence is available to support the contention. While investigating pollination mechanisms in the genus *Nymphaea*, we repeatedly observed dead insects in the stigmatic cups of *N. nouchali* (Fig. 1). Although Barthlott *et al.*<sup>14</sup> also found dead insects in *N. cernua* – now merged in *N. nouchali* as var. *cernua* – they attributed the phenomenon to pollination—an explanation incompatible with the results of our study.

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Flowers of *N. nouchali* var. *cernua* are bluish to bluish-purple, 15 cm across when completely open, and remain afloat. They are faintly fragrant, open throughout the day (from 8 a.m. to 9 p.m.) and closed at night, and remain functionally open for 3–4 days, after which the peduncle sinks into the water and develops into a fruit. The fruit is covered with a persistent calyx and ripens under water. The stamens, 140–150 of them, are arranged in 9–11 spiral rows. The filaments are petaloid, flattened, dilated at the base, yellow with a bluish distal sterile appendage; in outer rows, they are up to 3.4 cm long. Height of the stamens gradually decreases. Stamens of the two innermost rows are short, 1.5–1.6 cm long, smooth, clavate, and bent inwards to form a loose S-shaped structure. The anthers are inflexed. The ovary is 22-loculed, fused around a central core of receptacular tissue, and sloped inwards to form a cup-shaped depression. The inside of the stigmatic cup is covered with minute hair, each comprising 3–5 cells. The cup's surface is slippery, shiny, and golden yellow with a protuberant receptacular tissue forming a small white knob at the centre. The rim of the cup is surrounded by a row of 3-mm-long clavate-shaped appendages, curved inwards, which are smooth and glabrous and produced at the tips of locules (Supplementary Fig. 1-3).

Sepals control the opening and closing of the flower. The process of blooming begins at about 7 a.m. with the opening of sepals. The flowers are fully open by 8.30 to 10.30 a.m. On the first day of opening, stamens are compact; positioned vertically around the stigma, they form a stigmatic cup 2.5–3.5 cm in diameter. The cup holds 2.2–3 ml of a watery fluid, secreted mostly from a longitudinal groove formed by joining of the carpel walls of receptacle. The fluid is acidic (pH 4–4.5) whereas the water in the pond was neutral (pH 7).

This carpellary groove is covered with oil glands and hydropote-like structures. The oil released from the glands settles above the fluid, forming a thin layer. Subsequently, a mild fragrance is released and anthers in the outer row dehisce. The cup is refilled even when it is deliberately emptied.

As soon as an insect lands on the stamen, the filament bends inwards to make the insect fall into the stigmatic cup filled with the watery fluid. The insect desperately swims around the circular brim to find a path to escape. In doing so, it tries to grasp the clavate-shaped stigmatic appendages - a futile effort, given that the appendages are slippery and curved inwards. The hairs on the inner walls of the cup, the stigmatic appendages, and the inner rows of stamens with their unique shape and structure act as insect-retaining mechanisms. The wet wings and body parts make swimming, flying or climbing up the anthers even more difficult and the insect finally succumbs (Supplementary Movie 1).

We recorded six species of insects visiting the flower to collect pollen. The species were confined to Diptera and Hymenoptera, the most common being two species of honeybees (*Apis florea* and *Apis mellifera*) and two species of solitary bees (Supplementary Fig. 4). Although *Apis dorsata* was observed to visit flowers of other plants growing in the surrounding area, it left those of *N. nouchali* var. *cernua* alone.

Other visitors included some unidentified flies. The maximum number of insects trapped in each flower was 5, the average being 2.25 in summer and 0.90 in the rainy season. (Supplementary table 1) Insects are trapped only on the first day of opening. The watery fluid is normally drawn back the same night by the receptacular tissue, and the stigmatic cup remains dry for the next couple of days; no insects are trapped during these days. The stamens bend inwards over the stigmatic cup on the first night and close the cup, forming a cone-like structure; the next morning, they dehisce steadily in phases, starting from the outer spiral. This process coincides with the visits of pollinators. The number of pollinators, particularly honeybees, is much more on the second day. On the third day, the stigmatic appendages bend inwards at 45° to form a hood. Their golden colour changes to buff. The dead insects in the stigmatic cup shrink slightly. The flower is closed and the peduncle falls into water. The flower floats for a day or two before sinking to the bottom of the pond. The stigmatic cup quickly disintegrates into a brownish mucilaginous mass. One-week-old submerged flowers, when removed and observed under the microscope, revealed insect cadavers embedded in the slimy floral parts. We observed this phenomenon with no other variety of *N. nouchali* and *N. rubra* Roxb. ex Andrews growing in the same pond. Nutrients are absorbed after the flowers are submerged, possibly to support fruit or seedling development. Two features, namely the stigmatic cup functioning as a trap for insects and the insects being digested in the submerged flowers, distinguish this form of insectivory from the rest.

Despite their great diversity, angiosperms are united by a suite of synapomorphies. Recent molecular systematic studies based on lineage from the water lily (Nymphaeales) offer an important key to the understanding of ancestral angiosperm morphology and are of considerable interest to the origins of the angiosperms<sup>15,16</sup>. Fossil evidence also helps in assessing the evolutionary linkages<sup>17,18</sup>. Recent fossil evidence from the Turonian age (90 million years B.P.) includes fossil flowers with characters that, upon rigorous analysis, confirm the affinities with Nymphaeaceae and are closely related to the modern Nymphaealean genus *Victoria*. Detailed comparison of floral morphology of the fossil and that of modern *Victoria* flowers suggests that a mechanism to trap pollinating beetles was present in the earliest part of the late Cretaceous, and studies have further revealed that water lilies were more diverse in the past than they are today<sup>19</sup>.

In brief, the study suggests that the insects are trapped through aquaplaning. The stigmatic cup functions like a pit trap. The flowers selectively trap flies, honeybees, and solitary bees. The form of insectivory seems primitive, given the simple mechanisms for luring, trapping, and killing followed by digestion in submerged flowers. The process is unique, found neither in the other species of the genus *Nymphaea* nor in other groups of plants. Further, we postulate that carnivory and insect entrapment in flowering plants evolved in parallel during early stages of evolution of the flowering plants. The emergence and the subsequent divergence of the two phenomena is seen among ancestral lineages and eminently reflected in ANITA clads. We believe that palaeoclimatic conditions, particularly during the early Cretaceous period, offered a more conducive environment for the evolution and success of carnivorous plants, and that diversity among carnivorous plants was greater during this period. *N. nouchali* is the missing link in the evolutionary history of other highly evolved carnivorous plant families. In fact, the

recent discovery of *Archaeophora longicervia* Li from the early Cretaceous Yixian Formation of China supports this view<sup>20</sup>.

## Methods

Field work was conducted in an artificial pond located in the premises of the Naoroji Godrej Centre for Plant Research, near Pune, India, where two species of water lilies (*N. nouchali* and *N. rubra*) are being cultivated.

Taxonomic identification of the species was provided by Botanical Survey of India (ACC No. 125175). The taxonomy is as described<sup>21</sup>.

The experiment was conducted over a period of 16 months beginning from January 2006. Ecological studies in the flower-visiting insects, their types and number of visits, floral morphology, and the mechanisms to lure, trap, kill, and digest the insects were studied in detail. A total of six species of insects were found visiting the flower for pollen collection: two of honeybees (*Apis florea* and *Apis mellifera*), two of solitary bees, and two unidentified species.

Anatomy of the trapping mechanisms of the stigmatic cup was studied initially with a compound microscope and then by a scanning electron microscope (JOEL, Japan; Model No. JSM 6063A). The standard protocol followed for preparing the sample includes tissue fixation (2.5 % glutaraldehyde) and dehydration.

Preliminary experiments on insecticidal properties of the liquid collected from the stigmatic cup showed 40% mortality in houseflies.

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## Author contributions

Mr. Shrikant Sutar worked on this project as a summer trainee as part of his master's degree programme in botany. The authors declare no competing financial interests. Correspondence should be addressed to [ngcpr@lawkimindia.com](mailto:ngcpr@lawkimindia.com).

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**Figure 1 | Insectroy in *Nymphaea nouchali* Burm. f.** **a.** Insects trapped in stigmatic cup. **b.** Dry stigmatic cup with dead insects **c.** Decaying insect.



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