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ECOLOGICAL CORRELATES OF SEED SIZE AND DISPERSAL IN *HABENARIA* (ORCHIDACEAE)

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ABSTRACT:

The Orchidaceae family is renowned for its minute, dust-like seeds, characterized by a round to oval-shaped embryo enclosed within a thin, transparent, spindle-shaped testa. These morphological traits reflect the evolutionary adaptations of the family. Given the taxonomic value of seed morphometric characteristics, the present study employs microscopic analysis to investigate the seeds of Habenaria *species, belonging to the Orchidaceae family. Both qualitative and quantitative aspects of seed morphology are explored, focusing on surface features such as size, shape, embryo visibility, and the variation in seed length-to-width ratios.*

The data suggest that an increase in seed volume is directly associated with greater seed length and breadth. However, the embryo occupies a significantly smaller volume compared to the testa, resulting in substantial internal air spaces. These air pockets give the seed a balloon-like structure, enhancing its ability to disperse via wind and water currents. Seeds with larger dimensions tend to spread across wider geographic regions, whereas smaller seeds remain localized. An increased air-to-embryo ratio improves seed buoyancy, promoting wider dispersal.

This study highlights a positive correlation between seed buoyancy and dispersal range. Specifically, species like Habenaria digitata *and* H. gibsonii, *which possess elongated, lightweight seeds, exhibit widespread distribution. In contrast,* H. grandifloriformis *remains geographically restricted due to its relatively smaller seeds.*

Keywords: *Habenaria*, seed morphology, scanning electron microscopy, seed dispersal, Western Ghats.

INTRODUCTION:

The Orchidaceae family is distinguished by its exceptionally small, dust-like seeds, which typically possess a round, oval, or ellipsoidal embryo enclosed in a thin, transparent, spindle-shaped (fusiform) testa. These structural traits reflect the evolutionary refinement and developmental trends of the family. The considerable variation in seed length and width, particularly at the genus and species levels, has been highlighted for its taxonomic relevance (Arditti & Ghani, 2000).

Several studies (Clifford & Smith, 1969; Vij et al., 1992; Rasmussen, 1995; Swamy et al., 2004; Verma et al., 2013) have explored the correlation between seed size and plant growth habit, noting that epiphytic orchids generally produce smaller seeds than terrestrial species. However, Arditti & Ghani (2000) emphasized that this trend is not universal. Molvray & Kores (1995), after assessing seed dimensions across various orchid species, observed that seed size typically ranges between 0.5 and 0.8 mm, depending on the species.

Seed morphology within the subfamily Epidendroideae displays notable variation, especially among advanced orchids, while primitive Cypripedioideae species tend to maintain a more uniform fusiform shape (Arditti et al., 1979; Vij et al., 1992). It has been proposed that fusiform seeds represent an ancestral form present across all subfamilies, from which other seed types likely evolved (Arditti et al., 1979; 1980; Healey et al., 1980; Rasmussen, 1995; Verma et al., 2013). These authors argued that seed volume, rather than length or width alone, serves as a more accurate measure of seed size. Additionally, Arditti et al. (1980) and Augustine et al. (2001) suggested that the length-to-width (L/W) ratio could indicate seed truncation, with values below 6.0 denoting truncated forms and those above 6.0 indicating elongated seeds.

Advanced imaging techniques, including light and scanning electron microscopy, have enabled researchers (e.g., Arditti et al., 1980; Barthlott & Ziegler, 1981) to provide comprehensive accounts of seed structure and surface characteristics. Most species examined to date exhibit quadrilateral testa cells. Studies by Clifford & Smith (1969), Vij et al. (2006), and Verma et al. (2013) confirmed the prevalence of such fusiform quadrilateral testa cells in both epiphytic and terrestrial orchid species. Kurzweil (1993) further described the testa cells as generally elongated and concave, with straight or slightly wavy anticlinal walls and varied ornamentation.

Arditti et al. (1979) also postulated a direct relationship between seed size and volume, where a higher seed volume-to-embryo volume ratio indicates greater buoyancy. Seeds with more extensive air spaces are generally lighter and capable of dispersing over broader geographic areas. Conversely, seeds with limited internal air space tend to have restricted distribution (Augustine et al., 2001). Arditti & Ghani (2000) attributed the increase in air space to elongation of the testa cells.

Despite the taxonomic and ecological significance of seed morphometry, limited attention has been given to the genus *Habenaria*. Its seed traits remain understudied or overlooked as diagnostic tools. This study aims to address this gap by examining the detailed seed morphology of *Habenaria* species and investigating how these features influence their distribution patterns.

MATERIALS AND METHODS

Between 2014 and 2021, mature seeds from eighteen *Habenaria* species were collected from both the germplasm bank of the Departmental Botanical Garden and naturally dehiscing capsules found in the wild. Light microscopy and imaging were conducted using an Olympus DM2000 compound microscope. Detailed qualitative and quantitative analyses of seed morphology were performed, focusing on traits such as seed size, shape, color, embryo visibility, testa structure, curvature, surface ridges, and the ornamentation of anticlinal and periclinal walls. Additionally, variations in seed length-to-width (L/W) ratio were recorded.

To account for intraspecific variability, a large number of seeds per species were observed, and mean values were derived. Seed volume was calculated using the formula for an ellipsoid: 43πab2\frac{4}{3} \pi ab^234πab2, where *a* = ½ length and *b* = ½ width, based on the assumption of an elliptical embryo cross-section. Seed and surface morphological terminology followed the conventions set by Arditti et al. (1980).

RESULTS AND DISCUSSION

SEED SHAPE

Microscopic analysis identified three distinct seed shapes:

1. **Spathulate** – observed in *H. brachyphylla*, *H. grandifloriformis*, *H. roxburghii*, *H. suaveolens*, and *H. rariflora*.
2. **Fusiform** – found in *H. commelinifolia*, *H. crinifera*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, and *H. plantaginea*.
3. **Filiform** – present in *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii*, and *H. ovalifolia*.

All species exhibited centrally located, oval to elliptical embryos that were visible under the microscope.

SEED SIZE

Seed lengths ranged from 0.29 ± 0.14 mm in *H. grandifloriformis* to 1.69 ± 0.35 mm in *H. digitata*. Widths varied from 0.08 ± 0.02 mm (*H. commelinifolia*) to 0.33 ± 0.02 mm (*H. diphylla*). Based on length, seeds were categorized into:

- **Small** (< 0.7 mm): *H. brachyphylla*, *H. diphylla*, *H. grandifloriformis*, *H. roxburghii*
- **Intermediate** (0.7–0.9 mm): *H. commelinifolia*, *H. crinifera*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, *H. rariflora*, *H. suaveolens*
- **Large** (0.9–2.0 mm): *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii*, *H. ovalifolia*

Most species belonged to the intermediate group, aligning with prior observations by Molvray and Kores (1995). These morphological distinctions have potential taxonomic utility.

SEED LENGTH-TO-WIDTH RATIO (L/W)

The study revealed that *H. commelinifolia*, *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii*, *H. ovalifolia*, and *H. rariflora* had elongated seeds (L/W > 6), consistent with Arditti et al. (1980) and Augustine et al. (2001). In contrast, species like *H. brachyphylla*, *H. crinifera*, *H. diphylla*, *H. grandifloriformis*, and others had truncated seeds (L/W < 6). The highest L/W ratio was found in *H. gibsonii* (17.67 ± 3.84), while *H. diphylla* had the lowest (1.75 ± 0.69). This trait serves as an effective taxonomic marker.

SEED VOLUME

Consistent with Arditti et al. (1979), seed volume was found to be directly proportional to size. Although *H. diphylla* had the largest volume (16.97 ± 2.14 $mm^3 \times 10^{-3}$) due to its greater width, *H. crinifera* had the smallest volume (0.08 ± 0.03 $mm^3 \times 10^{-3}$). Findings confirm that increased seed width, rather than testa length, contributes to greater seed volume, echoing results from Augustine et al. (2001) in *Bulbophyllum*.

EMBRYO MORPHOMETRY

Embryo dimensions varied across species, with lengths from 0.17 ± 0.09 mm (*H. grandifloriformis*) to 0.34 ± 0.17 mm (*H. roxburghii*), and widths from 0.06 ± 0.01 mm (*H. crinifera*) to 0.19 ± 0.10 mm (*H. roxburghii*). L/W ratios ranged from 1.09 ± 0.09 (*H. diphylla*) to 5 ± 2.98 (*H. crinifera*). Embryo sizes remained consistent within species but varied significantly in volume, aligning with previous findings (Healey et al., 1980; Augustine et al., 2001). In most species, the embryo occupies a minor portion of seed volume, although species like *H. grandifloriformis*, *H. rariflora*, and *H. suaveolens* had embryos occupying a relatively larger fraction, increasing seed weight.

SEED-TO-EMBRYO VOLUME RATIO

Embryo volume ranged from 0.4 ± 0.09 mm^3 in *H. gibsonii* to 16.29 ± 4.36 mm^3 in *H. diphylla*. *H. gibsonii* and *H. furcifera* exhibited the highest seed-to-embryo volume ratios (6.63 and 6.57, respectively), facilitating greater air space and wider dispersal. Conversely, species like *H. brachyphylla* and *H. roxburghii* had low S/E ratios (1.2 ± 1.02 and 1.24 ± 0.65), indicating limited distribution.

AIR SPACE PROPORTION

The role of internal air space in enhancing seed buoyancy is well established (Arditti et al., 1980; Arditti & Ghani, 2000; Augustine et al., 2001). According to Arditti & Ghani (2000), increased testa cell length contributes to higher air volume. In the current study, *H. gibsonii* (84.91 ± 11.34%) and *H. furcifera* (84.77 ± 9.45%) had the highest air space percentages, while *H. grandifloriformis* had the lowest (0.86 ± 0.46%).

CONCLUSION:

The present morphometric analysis of *Habenaria* seeds confirms that the examined seed traits hold significant diagnostic value, contributing both to phytogeographical research and to the taxonomic differentiation of species. Based on the length-to-width (L/W) ratio, seven species exhibit truncated seeds, while the others possess elongated ones. Species with greater seed length, higher seed-to-embryo volume ratios, and increased internal air space are better adapted for long-distance dispersal. Seed volume, influenced by both length and breadth, further enhances this capability.

Notably, the embryo occupies a much smaller volume compared to the testa, resulting in abundant internal air cavities that lend the seeds a balloon-like form. This structural feature promotes dispersal through air and water currents. While species with smaller seeds tend to remain localized, those with larger seeds are capable of colonizing broader geographic ranges. An increased proportion of air space relative to embryo volume enhances seed buoyancy, thereby improving their dispersal potential.The findings of this study underscore a positive correlation between seed morphology—particularly buoyancy-related traits—and dispersal patterns. For instance, *Habenaria digitata* and *H. gibsonii*, with their elongated, lightweight seeds, are widely distributed, whereas *H. grandifloriformis*, possessing comparatively smaller seeds, remains geographically restricted.

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Legends

Plate – I: Seed Diversity in *Habenaria* species.

a- *Habenaria brachyphylla*, b- *H. commelinifolia*, c- *H. crinifera*, d- *H. digitata*, e- *H. diphylla*, f- *H. foetida*, g-

H. furcifera, h- *H. gibsonii*, i- *H. grandifloriformis*, j- *H. heyneana*, k- *H. longicorniculata*, l- *H. longicornu*, m-

H. marginata, n- *H. ovalifolia*, o- *H. plantaginea*, p- *H. rariflora*, q- *H. roxburghii*, r- *H. suaveolens*

(Scale bar = 100 μm)

Plate - I

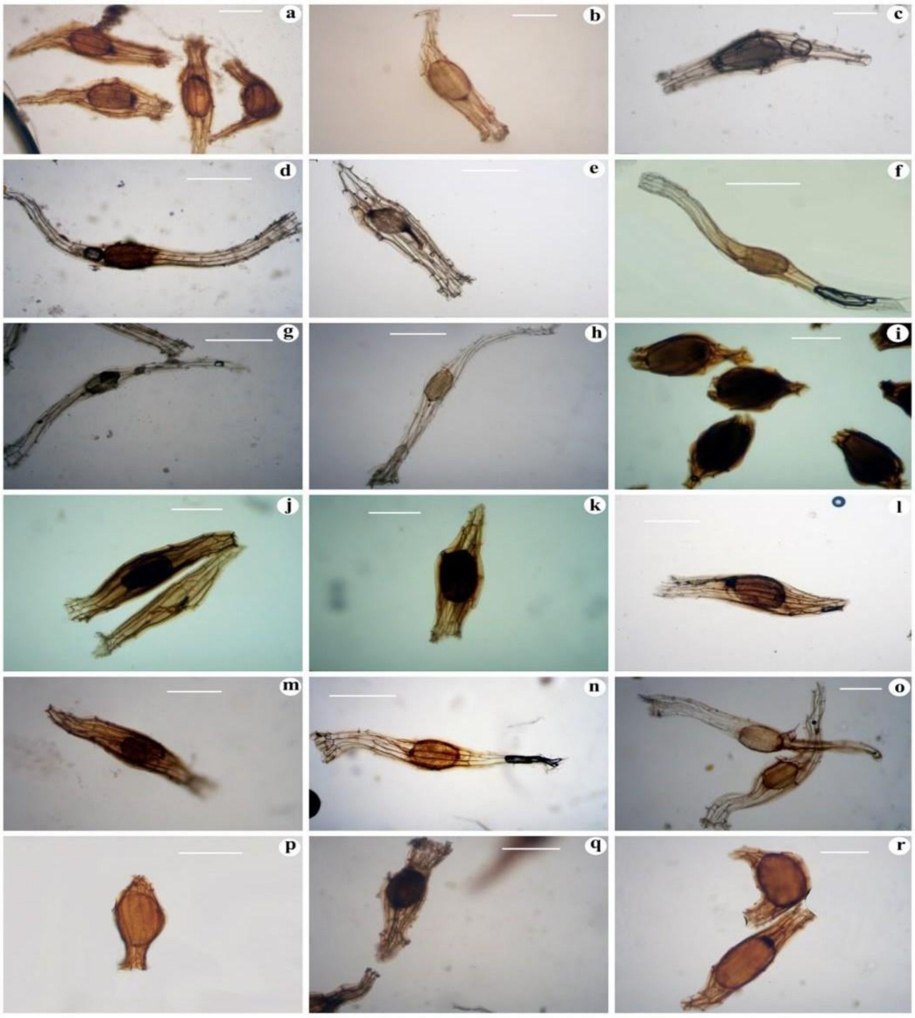

Table -1. Comparative features of the seeds in *Habenaria* species

Sr. No.	Name of the species	Length (L) (mm)	Width (W) (mm)	Length/ Width ratio of seed (L/W)	Volume of Seed (S) (mm^3 X 10^{-3})
1	H. brachyphylla	0.39 ± 0.16	0.11 ± 0.08	3.50 ± 1.06	1.26 ± 0.50
2	H. commelinifolia	0.75 ± 0.22	0.08 ± 0.02	9.00 ± 1.23	1.36 ± 0.62
3	H. crinifera	0.47 ± 0.16	0.08 ± 0.03	5.67 ± 2.56	0.86 ± 0.12
4	H. digitata	1.69 ± 0.35	0.11 ± 0.05	15.25 ± 3.54	5.48 ± 0.95
5	H. diphylla	0.58 ± 0.24	0.33 ± 0.02	1.75 ± 0.69	16.97 ± 2.14
6	H. foetida	1.44 ± 0.34	0.11 ± 0.04	13.00 ± 2.56	4.67 ± 0.74
7	H. furcifera	1.44 ± 0.54	0.14 ± 0.05	10.40 ± 2.69	7.29 ± 1.49
8	H. gibsonii	1.47 ± 0.28	0.09 ± 0.03	17.67 ± 3.84	2.68 ± 0.86
9	H. grandifloriformis	0.29 ± 0.14	0.16 ± 0.04	1.82 ± 0.54	1.85 ± 0.74
10	H. heyneana	0.54 ± 0.28	0.14 ± 0.06	3.86 ± 1.25	2.77 ± 0.95
11	H. longicorniculata	0.53 ± 0.32	0.14 ± 0.05	3.80 ± 1.65	2.66 ± 0.80
12	H. longicornu	0.67 ± 0.31	0.14 ± 0.04	4.80 ± 1.36	3.37 ± 0.94
13	H. marginata	0.47 ± 0.21	0.11 ± 0.02	4.25 ± 2.11	1.53 ± 0.85
14	H. ovalifolia	0.97 ± 0.46	0.11 ± 0.06	8.75 ± 2.25	3.14 ± 0.86
15	H. plantaginea	0.72 ± 0.40	0.17 ± 0.05	4.33 ± 1.23	5.25 ± 1.06
16	*H. rariflora*	0.78 ± 0.35	0.11 ± 0.04	7.00 ± 1.68	2.51 ± 0.65
17	*H. roxburghii*	0.61 ± 0.24	0.22 ± 0.08	2.75 ± 0.098	7.90 ± 1.24
18	*H. suaveolens*	0.47 ± 0.21	0.14 ± 0.06	3.40 ± 1.56	2.38 ± 0.56

Table -2. Comparative features of the Embryo in Habenaria species seeds

Sr. No.	Name of the species	Length L (mm)	Width W (mm)	Length/ Width ratio (L/W)	Volume of Seed (S) (mm^3 X 10^{-3})	Embryo Volume (E) (mm^3 X 10^{-3})	Seed Volume/ Embryo Volume ratio (S/E)	Air Space (%)
1	H. brachyphylla	0.28 ± 0.16	0.08 ± 0.02	3.33 ± 2.13	1.26 ± 0.50	1.01 ± 0.26	1.24 ± 0.65	19.67 ± 4.62
2	H. commelinifolia	0.28 ± 0.18	0.08 ± 0.02	3.33 ± 2.16	1.36 ± 0.62	1.01 ± 0.28	1.35 ± 0.86	25.95 ± 6.65
3	H. crinifera	0.28 ± 0.14	0.06 ± 0.01	5.00 ± 2.98	0.86 ± 0.12	0.45 ± 0.08	1.91 ± 0.98	47.73 ± 8.52
4	H. digitata	0.28 ±	0.08 ±	3.33 ±	5.48 ±	1.01 ±	5.42 ±	81.56
		0.13	0.03	2.42	0.95	0.30	2.85	±11.23
5	H. diphylla	0.33 ± 0.19	0.31 ± 0.12	1.09 ± 0.09	16.97 ± 2.14	16.29 ± 4. 36	1.04 ± 0.24	4.00 ± 2.56
6	H. foetida	0.31 ± 0.17	0.08 ± 0.04	3.67 ± 2.54	4.67 ± 0.74	1.11 ± 0.22	4.20 ± 2.13	76.21 ± 9.59
7	H. furcifera	0.31 ± 0.16	0.08 ± 0.03	3.67 ± 2.64	7.29 ± 1.49	1.11 ± 0.26	6.57 ± 2.45	84.77 ± 9.45
8	H. gibsonii	0.25 ± 0.14	0.06 ± 0.02	4.50 ± 2.78	2.68 ± 0.86	0.40 ± 0.09	6.63 ± 3.45	84.91 ±11.34
9	H. grandifloriformis	0.17 ± 0.09	0.14 ± 0.09	1.20 ± 1.06	1.85 ± 0.74	1.83 ± 0.45	1.01 ± 0.19	0.86 ± 0.46
10	H. heyneana	0.26 ± 0.12	0.12 ± 0.08	2.17 ± 1.64	2.77 ± 0.95	1.96 ± 0.69	1.41 ± 0.90	29.27 ± 8.36
11	H. longicorniculata	0.19 ± 0.14	0.11 ± 0.07	1.75 ± 1.26	2.66 ± 0.80	1.26 ± 0.31	2.12 ± 1.20	52.86 ± 6.53
12	H. longicornu	0.28 ± 0.16	0.11 ± 0.08	2.50 ± 1.84	3.37 ± 0.94	1.79 ± 0.40	1.88 ± 1.56	46.68 ± 6.24
13	H. marginata	0.22 ± 0.15	0.08 ± 0.04	2.67 ± 1.95	1.53 ± 0.85	0.81 ± 0.16	1.89 ± 1.65	47.08 ± 8.95
14	H. ovalifolia	0.22 ± 0.15	0.08 ± 0.03	2.67 ± 1.90	3.14 ± 0.86	0.81 ± 0.20	3.89 ± 1.59	74.29 ±11.23
15	H. plantaginea	0.25 ± 0.16	0.14 ± 0.09	1.80 ± 1.32	5.25 ± 1.06	2.52 ± 0.89	2.08 ± 1.24	51.94 ± 8.26
16	H. rariflora	0.22 ± 0.14	0.08 ± 0.03	2.67 ± 1.87	2.51 ± 0.65	0.81 ± 0.22	3.11 ± 1.69	67.87 ± 6.35
17	H. roxburghii	0.34 ± 0.17	0.19 ± 0.10	1.71 ± 1.20	7.90 ± 1.24	6.60 ± 2.12	1.20 ± 1.02	16.50 ± 4.62
18	H. suaveolens	0.22 ± 0.15	0.11 ± 0.09	2.00 ± 1.56	2.38 ± 0.56	1.44 ± 0.67	1.66 ± 1.13	39.78 ± 6.52

(± SD, n= 10)