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Comparative Morphological Assessment of Seven Species of Zingiberaceae from Garbhanga Reserve Forest of Assam, India

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ABSTRACT

Zingiberaceae, the largest family in the order Zingiberales, is a taxonomically diverse group of aromatic, rhizomatous herbs represented by 4022 species under 114 genera globally. The present study deals with a comparative morphological assessment of seven Zingiberaceae species—*Alpinia nigra* (Gaertn.) B.L.Burtt, *Boesenbergia hamiltonii* Mood, S.Dey & L.M. Prince, *Curcuma aromatica* Salisb., *Globba racemosa* Sm., *Hedychium coccineum* Buch.-Ham. ex Sm., *Hedychium stenopetalum* G. Lodd., and *Zingiber zerumbet* (L.) Sm. collected from Garbhanga Reserve Forest of Assam, India. Detailed observations of vegetative and reproductive traits, including leaves, calyx, corolla, labellum, stamens, staminodes, and gynoecium were recorded, and diagnostic features were tabulated. A dichotomous key based on the species morphology was prepared to facilitate the identification of the studied genera. The study revealed both interspecific variations and shared characteristics, remarkably in floral structures crucial to taxonomic demarcation. Moreover, the incorporation of high-resolution colored comparative photoplates of the floral parts adds significant value to species identification and taxonomic verification. The findings provide baseline data valuable not only for taxonomic verification but also for ecological monitoring and conservation of Zingiberaceae within this biodiversity-rich yet underexplored forest ecosystem.

Keywords Comparative morphology, Floral characters, Morphological diversity, Species delimitation, Zingiberaceae.

INTRODUCTION

Zingiberaceae is a monocotyledonous family belonging to the order Zingiberales, distributed worldwide throughout tropical and subtropical regions across Africa, Asia, and America, with Southeast Asia recognized as its center of diversity (Windarsih *et al.* 2021). Zingiberaceae members are perennial aromatic rhizomatous herbs that typically grow in shaded habitats, although some species also occur under direct sunlight (Mans *et al.* 2019). It is the largest family within Zingiberales, comprising

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about 4022 species under 114 genera globally (Yadav and Gowda 2024).

In India, around 178 species belonging to 22 genera have been recorded (Jain and Prakash 1995). Barooah and Ahmed (2014) reported 15 genera and 45 species of Zingiberaceae from Assam.

The family holds substantial economic and ecological importance. Rhizomes of many species are widely used as a source of food, spice, cosmetics, dye, and perfumes. A number of species are cultivated for ornamental purposes due to their showy flowers. The family is known to exhibit various pharmacological activities due to the bioactive compounds it possesses (Windarsih *et al.* 2021). Traditionally, members of Zingiberaceae such as *Alpinia calcarata, Alpinia malaccensis, Alpinia nigra, Amomum aromaticum, Amomum koenigii, Amomum maximum, Curcuma amada, Curcuma caesia, Curcuma longa, Curcuma picta, Hedychium coccineum, Hedychium coronarium,* and *Hedychium thyrsiforme* have been utilized as the natural sources of herbal medicine (Saha *et al.* 2020). Four tribes are recognized within Zingiberaceae: Alpinieae, Hedychieae, Globbeae, and Zingibereae. Based on the molecular data, Zingiberaceae is classified into four subfamilies: Alpinioideae, Siphonochiloideae, Tamijioideae, and Zingiberoideae (Benedict *et al.* 2015).

Even though floristic accounts and taxonomic studies have been carried out on Zingiberaceae from Assam, those studies primarily focus on descriptive morphology, phenological observations, and other aspects, with less visual representation. Despite the significance of studying detailed floral structures supported by colored photoplates in the delimitation of the species, such resources remain scarce.

Therefore, the present work aims to document the key morphological variation and similarity among seven Zingiberaceae species from Garbhanga Reserve Forest of Assam, along with high-resolution colored comparative photoplates of the floral parts. These species were selected as they were widely distributed in the present reserve forest and collectively represent different genera of the family, thereby reflecting its morphological diversity in the region. To date, no comparative analysis of this kind has been documented from this reserve forest. The present study is expected to aid future identification and taxonomic verification of Zingiberaceae, and thereby add critical value to the morphological understanding of the family in this region.

MATERIALS AND METHODS

Study Area

Garbhanga Reserve Forest (GRF) covers an area of 110 sq km and lies between 26º1'40" N and 91º43'31" E (Barua *et al.* 2004, Dey *et al*. 2016) (Fig. 1). The forest is situated adjacent to greater Guwahati and Southern bank of the river Brahmaputra (Saikia and Saikia 2015) and holds a distinct topographical position bounded by the Meghalaya hill ranges to the East and South, Rani reserve forest to the West, Guwahati city and Deepor Beel wildlife sanctuary to the North. The area lies at an altitude of 100-200 m (Barua *et al.* 2004) and exhibits a distinct climatic phase characterized by pre-monsoon, monsoon, post-monsoon, and winter (Lahkar *et al.* 2010).

Field survey and specimen collection

An extensive field survey to different parts of GRF was carried out during the flowering and fruiting season between 2023 and 2024 to record the occurrences of Zingiberaceae. Samples were collected for laboratory analysis. Standard herbarium techniques (Jain and Rao 1977) were followed for the collection, pressing, drying, and preservation of specimens.

Morphological analysis

Detailed morphological observations were made on freshly collected specimens as well as preserved herbarium materials. Both vegetative (habit, leaf type, arrangement, surface, shape, and apex) and reproductive (inflorescence position, flower, calyx, corolla, labellum, staminodes, stamens, gynoecium, and stylodes) characteristics were examined (Figs. 2, 3, 4, 5, 6, 7, 8, 9, and 10).

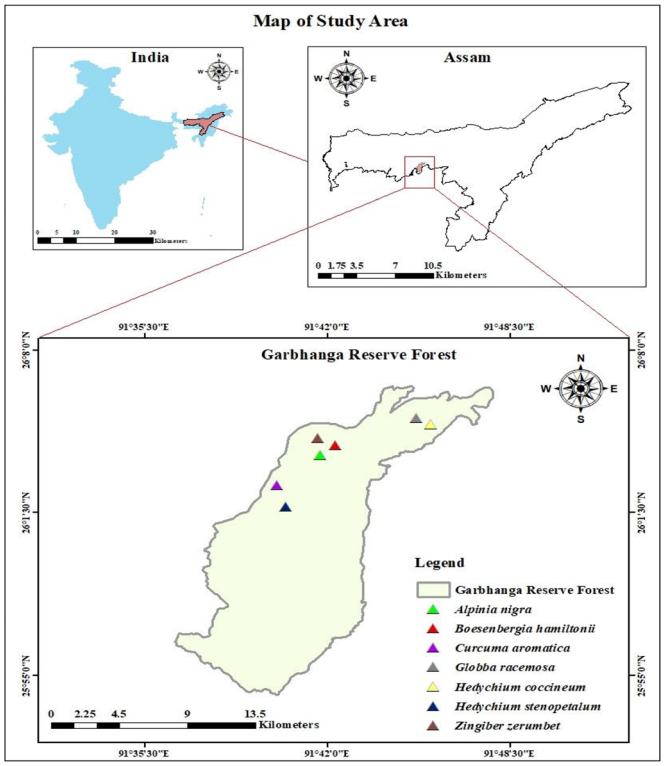

Fig. 1. Map of the study area.

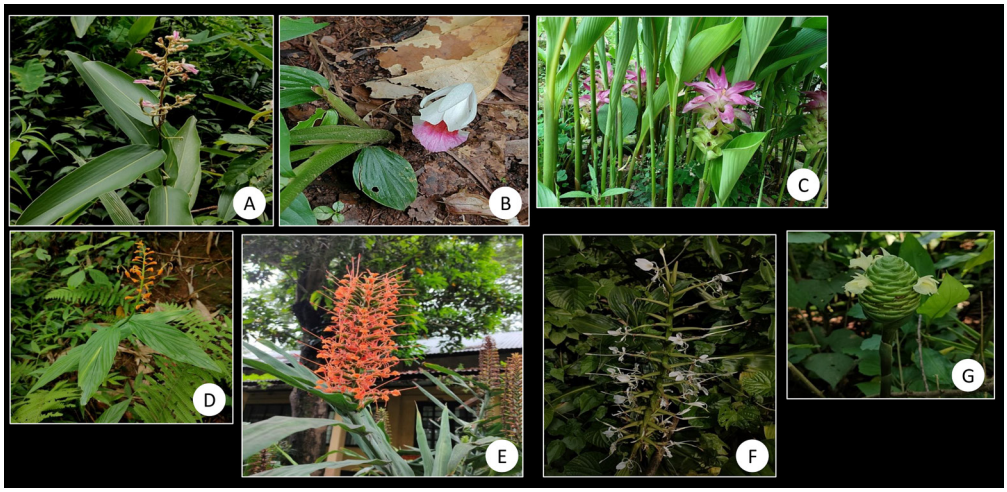

Fig. 2. Habit. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L .M. Prince. **C)** *Curcuma aromatica* Salisb. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

Fig. 3. Comparative flower morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L .M. Prince. **C)** *Curcuma aromatica* Salisb. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

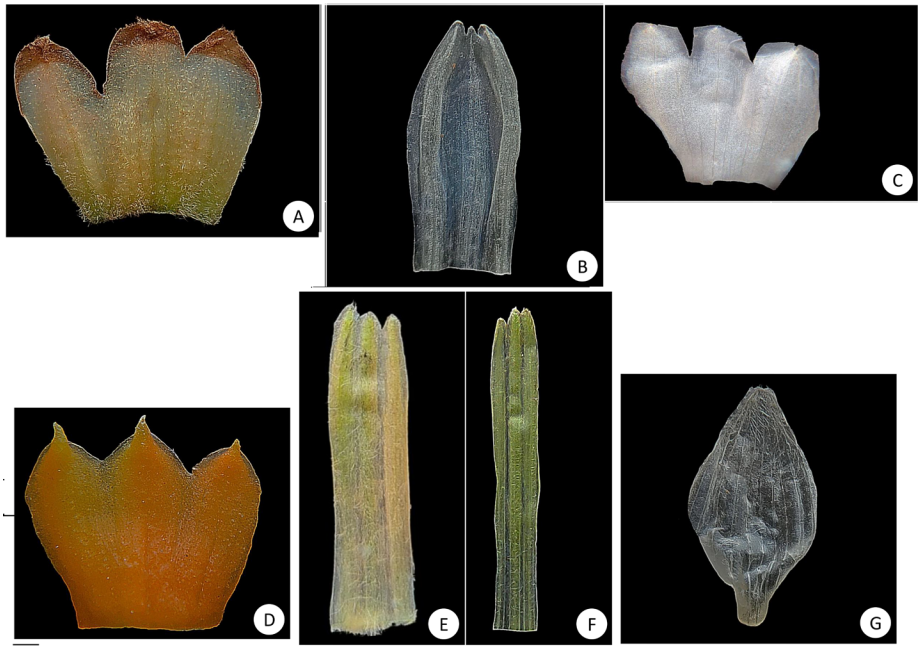

Fig. 4. Comparative calyx morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince. **C)** *Curcuma aromatica* Salisb. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

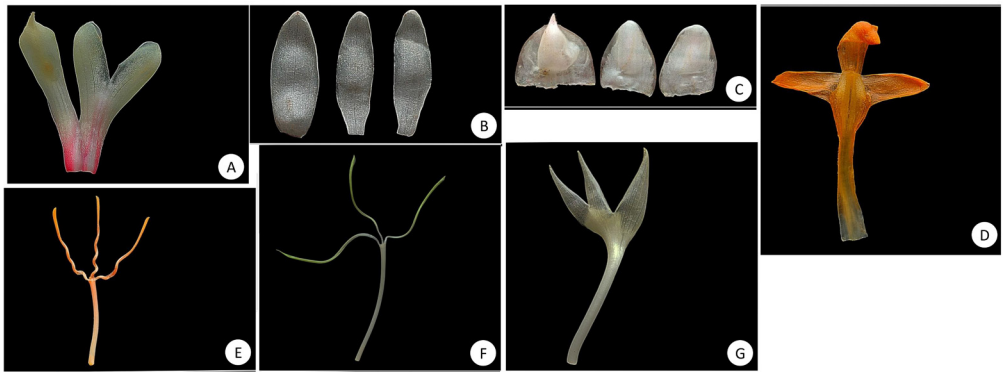

Fig. 5. Comparative corolla morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince. **C)** *Curcuma aromatica* L. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

Fig. 6. Comparative labellum morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince. **C)** *Curcuma aromatica* L. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

Photographic documentation

High-resolution photographs were taken to prepare comparative photoplates of floral parts. All images were processed uniformly for clarity and scale. Smaller floral structures were captured using a simple dissecting microscope (RDM-4) at a magnification of 20X, while larger parts were photographed directly without magnification, and the images were edited using Adobe Photoshop CS5.

Data organization and comparative analysis

Morphological characteristics were tabulated to facilitate comparison among the species. Taxonomic determination of the species was done based on relevant taxonomic literature and consultation with authentic specimens housed in different Herbaria (ASSAM, BM, CAL, KEW, MO, NYBG). A dichotomous taxonomic key to the studied genera was constructed based on diagnostic characters. Obser-

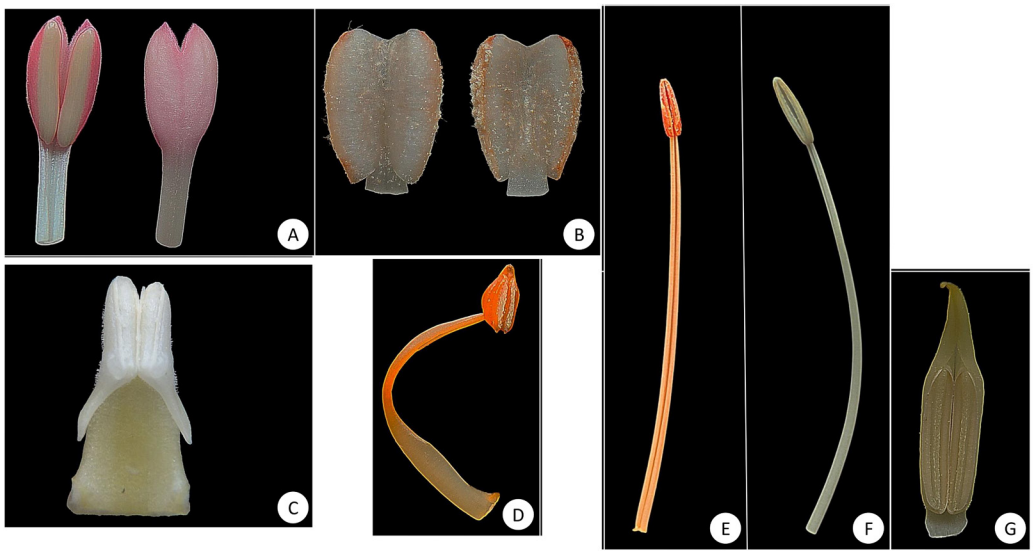

Fig. 7. Comparative stamen morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B.L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince. **C)** *Curcuma aromatica* L. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbe*t (L.) Sm.

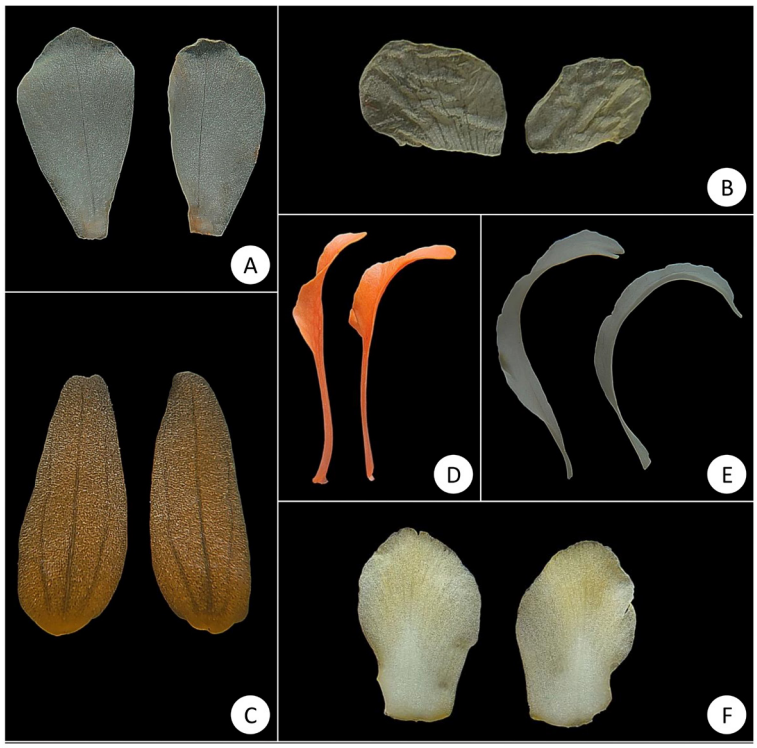

Fig. 8. Comparative staminode morphology in the studied species. **A)** *Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince. **B)** *Curcuma aromatica* L. **C)** *Globba racemosa* Sm. **D)** *Hedychium coccineum* Buch.-Ham. ex Sm. **E)** *Hedychium stenopetalum* G. Lodd. **F)** *Zingiber zerumbet* (L.) Sm.

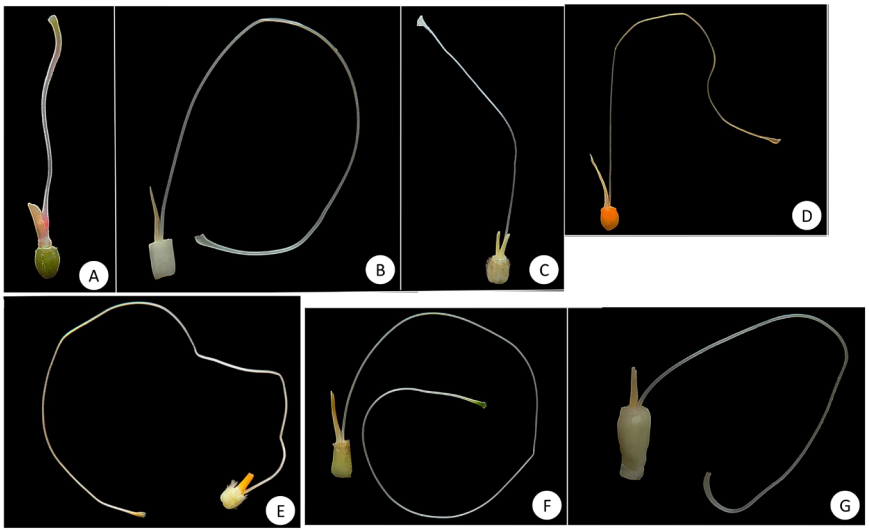

Fig. 9. Comparative gynoecium morphology in the studied species. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S.Dey & L.M. Prince. **C)** *Curcuma aromatica* L. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

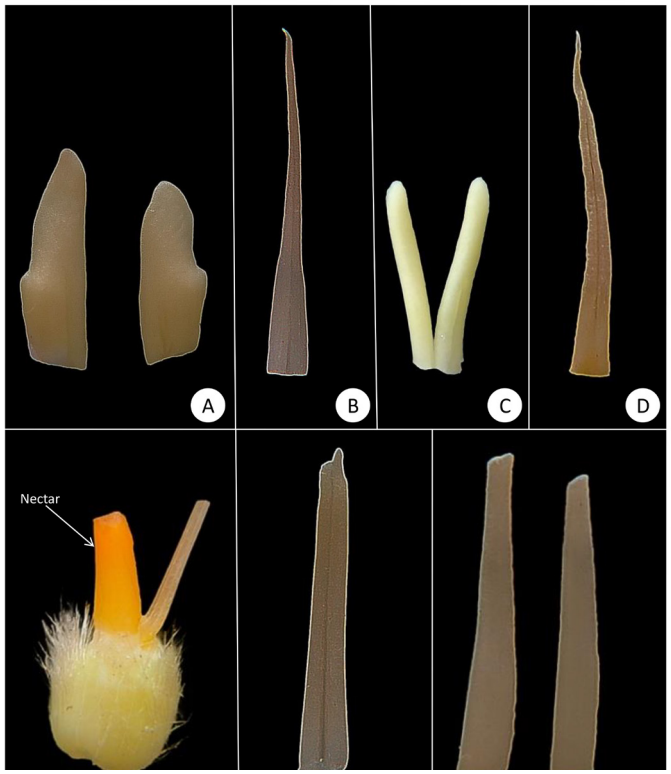

Fig. 10. Comparative stylodes structure. **A)** *Alpinia nigra* (Gaertn.) B. L.Burtt. **B)** *Boesenbergia hamiltonii* Mood, S.Dey & L.M. Prince. **C)** *Curcuma aromatica* L. **D)** *Globba racemosa* Sm. **E)** *Hedychium coccineum* Buch.-Ham. ex Sm. **F)** *Hedychium stenopetalum* G. Lodd. **G)** *Zingiber zerumbet* (L.) Sm.

vations were interpreted to assess interspecific variation and identify key traits for delimitation of the species. For updated nomenclature of the species, online databases like Plants of the World Online and International Plant Names Index were consulted. Voucher specimens have been deposited at the Botanical Survey of India (BSI).

RESULTS

Key to the genera studied

1a. Labellum conspicuously bilobed or clawed; corolla lobes linear.....*Hedychium*

1b. Labellum not clawed, entire or bilobed; corolla lobes not linear.....2

2a. Anther spurred.....*Curcuma*

2b. Anther not spurred.....3

3a. Anther connective terminating into a beak like appendage.....*Zingiber*

3b. Anther connective not terminating into an appendage.....4

4a. Calyx membranous.....*Boesenbergia*

4b. Calyx not membranous.....5

5a. Labellum reflexed.....*Globba*

5b. Labellum not reflexed.....*Alpinia*

Phenological and voucher data

The flowering and fruiting periods of the seven Zingiberaceae species collected from Garbhanga Reserve Forest were recorded through regular field observations. The details of phenological data and voucher specimens are presented in Table 1.

Comparative morphological assessment

Habit

All the species observed (*Alpinia nigra, Boesenbergia hamiltonii, Curcuma aromatica, Globba racemosa, Hedychium coccineum, Hedychium stenopetalum,* and *Zingiber zerumbet)* are rhizomatous herbs.

Leaf

Observations on the leaf morphology showed that all the species bear simple, alternate, and sheathing leaves. Leaf surface is hairy in *Hedychium stenopetalum* and *Zingiber zerumbet,* glabrous in *Alpinia nigra, Boesenbergia hamiltonii, Curcuma aromatica,* and *Hedychium coccineum,* and either hairy or glabrous in *Globba racemosa.* Leaf apex is acuminate across all the species, and the blade shape varies as follows: lanceolate in *Alpinia nigra, Hedychium stenopetalum,* and *Zingiber zerumbet;* ovate-lanceolate in *Boesenbergia hamiltonii* and *Globba racemosa,* and oblong-lanceolate in *Curcuma aromatica* and *Hedychium coccineum*.

Calyx

Calyx consists of three united, tubular sepals in all the species. Calyx is hairy in *Alpinia nigra, Curcuma aromatica, Hedychium coccineum, Hedychium stenopetalum,* and *Zingiber zerumbet;* a membranous calyx is observed in *Zingiber zerumbet* and *Boesenbergia hamiltonii*. Calyx color varies among the species: greenish yellow in *Alpinia nigra;* white in *Boesenbergia hamiltonii, Curcuma aromatica,* and *Zingiber zerumbet;* orange in *Globba racemosa,* and greenish in *Hedychium coccineum* and *Hedychium stenopetalum.*

Table 1. Phenology and voucher examined of the species.

Species	Phenology	Voucher examined
Alpinia nigra (Gaertn.) B.L.Burtt	June–August	GRF, B. Das 241, date 23.06.2024
Boesenbergia hamiltonii Mood, S. Dey & L. M. Prince	July– August	GRF, B. Das 242, date 24.07.2023
Curcuma aromatica Salisb.	June–July	GRF, B. Das 154, date 06.04.2024
Globba racemosa Sm.	April–June	GRF, B. Das 229, date 23.05.2024
Hedychium coccineum Buch.-Ham. ex Sm.	August –October	GRF, B. Das 163, date 13.08.2023
Hedychium stenopetalum G. Lodd.	August –September	GRF, B. Das 263, date 16.09.2023
Zingiber zerumbet (L.) Sm.	August – October	GRF, B. Das 253, date 15.08.2023

Corolla

All the species bear united and tubular corollas with three free lobes. Color is white in *Alpinia nigra, Boesenbergi hamiltonii,* and *Curcuma aromatica*; orange in *Globba racemosa* and *Hedychium coccineum;* pale green in *Hedychium stenopetalum,* and pale yellow in *Zingiber zerumbet*. Corolla lobes vary in shape: oblong in *Alpinia nigra*; ovate-oblong, reflexed and hooded in *Globba racemosa;* linear and reflexed in the species of *Hedychium*; lanceolate in *Zingiber zerumbet* and oblong in *Curcuma aromatica.* The corolla tube is slender in the species of *Hedychium* and *Boesenbergia hamiltonii.*

Labellum

In *Alpinia nigra,* the labellum color is pink; white with dark red throat and purplish apex in *Boesenbergia hamiltonii*; orange in *Globba racemosa* and *Hedychium coccineum*; white in *Hedychium stenopetalum,* yellow in *Curcuma aromatica,* and pale yellow in *Zingiber zerumbet.* Labellum is obovate and bilobed in *Alpinia nigra*; obovate and entire in *Boesenbergia hamiltonii;* obovate with three shallow lobes having the middle lobe emarginated in *Curcuma aromatica*; obcuneate in *Globba racemosa*; suborbicular, two-cleft and clawed in the species of *Hedychium*, and emarginate in *Zingiber zerumbet.*

Stamen

Stamen morphology of the species reveals variable features: *Alpinia nigra* has linear filaments with curved anthers; filament is longer in the species of *Hedychium; Globba racemosa* has curved filaments, while *Zingiber zerumbet* shows short filaments with the anther connective terminating into a beak-like appendage; in *Curcuma aromatica*, filament is short and broad with anthers spurred at the base. The species show differences in anther color: anther color is pink in *Alpinia nigra;* pale yellow in *Boesenbergia hamiltonii*; orange in *Globba racemosa* and *Hedychium coccineum*; off-white in *Hedychium stenopetalum;* yellow in *Zingiber zerumbet,* and white in *Curcuma aromatica.*

Staminode

Staminode is obovate in *Boesenbergia hamiltonii*; obovate-oblong in *Curcuma aromatica;* lanceolate in *Globba racemosa* and *Hedychium coccineum*; oblanceolate and linear in *Hedychium stenopetalum.* Staminode color is white in *Boesenbergia hamiltonii* and *Hedychium stenopetalum*; orange in *Globba racemosa* and *Hedychium coccineum;* pale yellow in *Zingiber zerumbet,* and yellowish in *Curcuma aromatica.*

Gynoecium

All the species bear a thin, long style located in a furrow in the filament and between anther locules. Two stylodes varying in length occur at the apex of the ovary. Ovary hairy in *Alpinia nigra, Curcuma*

Table 2. Growth habit and leaf morphology of the species.

Species	Growth habit	Leaf				
		Type	Arrangement	Surface	Shape	Apex
Alpinia nigra (Gaertn.) B. L Burtt	Rhizomatous herb	Simple	Alternate	Glabrous	lanceolate	Acuminate
Boesenbergia hamiltonii Mood, S.Dey & L. M. Prince	Rhizomatous herb	Simple	Alternate	Glabrous	ovate-lanceolate	Acuminate
Curcuma aromatica Salisb.	Rhizomatous herb	Simple	Alternate	Glabrous above and hairy beneath	oblong-lanceolate	Acuminate
Globba racemosa Sm.	Rhizomatous herb	Simple	Alternate	Glabrous or hairy	ovate-lanceolate	Acuminate
Hedychium coccineum Buch.-Ham. ex Sm.	Rhizomatous herb	Simple	Alternate	Glabrous	oblong- lanceolate	Acuminate
Hedychium stenopetalum G. Lodd.	Rhizomatous herb	Simple	Alternate	Hairy	lanceolate	Acuminate
Zingiber zerumbet (L.) Sm.	Rhizomatous herb	Simple	Alternate	Hairy	lanceolate	Acuminate

Table 3. Comparision of key characters in floral morphology among the species.

Characters	*Alpinia nigra* (Gaertn.) B. L. Burtt	*Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince	*Curcuma aromatica* Salisb.	*Globba racemosa* Sm.	*Hedychium coccineum* Buch.-Ham. ex Sm.	*Hedychium stenopetalum* G. Lodd.	*Zingiber zerumbet* (L.) Sm.
Inflorescence position	Terminal	Lateral	Terminal	Terminal	Terminal	Terminal	Lateral
Calyx hair	Present	Absent	Present	Absent	Present	Present	Present
Corolla lobes color	White	White	White	Orange	Orange	Pale green	Pale yellow
Labellum color	Pink	White with dark red throat and purplish apex	Yellow	Orange	Orange	White	Pale yellow
Staminodes color	__	White	Yellowish	Orange	Orange	White	Pale yellow
Anther color	Pink	Pale yellow	White	Orange	Orange	Off-white	Yellow
Anther spur	Absent	Absent	Present	Absent	Absent	Absent	Absent

aromatica, and *Hedychium coccineum;* glabrous in *Zingiber zerumbet* and *Globba racemosa.*

The comparative morphological assessment of the species is presented in Table 2 (Growth habit and leaf morphology) and Table 3 (Comparison of key characters in floral morphology).

Representative floral measurements of the studied species

In Table 4, representative floral measurements for each species are provided, based on a single, well-developed flower. Remarkable variation was observed among the species. *Boesenbergia hamiltonii* possessed the longest flower (11.9 cm), while *Globba racemosa* exhibited the shortest (3.2 cm). Maximum length in calyx (4.2 cm), stamen (6.7 cm), staminode (2.5 cm), and gynoecium (10.6 cm) was recorded in *Hedychium stenopetalum.* In contrast, minimum values for these respective floral parts were recorded in *Globba racemosa* (0.7 cm), *Curcuma aromatica* (0.8 cm), *Zingiber zerumbet* (0.6 cm), and *Alpinia nigra* (3.3 cm). Stylode length ranged from 0.3 cm (*Zingiber Zerumbet*) to 0.8 cm (*Boesenbergia Hamiltonii*).

DISCUSSION

The use of morphological characteristics remains crucial in the taxonomic identification and species delimitation within Zingiberaceae. The current research reveals that the seven species examined exhibit both preserved and deviating features.

A number of common characters, such as rhizomatous herbaceous habit; simple, alternate, and sheathing leaves with acuminate apices; floral structures including a united, tubular, three-lobed calyx and corolla; similarly structured gynoecium with a thin, elongated style situated in a filamentary and anther groove, and an ovary with two erect stylodes,

Table 4. Representative floral measurements of the studied Zingiberaceae species.

Floral parts	*Alpinia nigra* (Gaertn.) B. L.Burtt	*Boesenbergia hamiltonii* Mood, S. Dey & L. M. Prince	*Curcuma aromatica* Salisb.	*Globba racemosa* Sm.	*Hedychium coccineum* Buch.-Ham. ex Sm.	*Hedychium stenopetalum* G. Lodd.	*Zingiber zerumbet* (L.) Sm.
Flower	4.2 cm	11.9 cm	4.9 cm	3.2 cm	5.7 cm	9.7 cm	5.6 cm
Calyx	1.1 cm	1.7 cm	1 cm	0.7 cm	2.4 cm	4.2 cm	1.8 cm
Corolla	1.7 cm	2.8 cm	1.5 cm	0.5 cm	3 cm	3.5 cm	2.5 cm
Stamen	2 cm	1.1 cm	0.8 cm	1.3 cm	5.7 cm	6.7 cm	1.7 cm
Staminodes	---	1.4 cm	1.7 cm	0.6 cm	2.4 cm	2.5 cm	0.6 cm
Labellum	2.4 cm	2.6 cm	1.7 cm	0.7 cm	2 cm	2.6 cm	1.6 cm
Gynoecium	3.3 cm	9.9 cm	3.8 cm	4.7 cm	8.2 cm	10.6 cm	4.7 cm
Stylodes	0.5 cm	0.8 cm	0.4 cm	0.6 cm	0.3 cm	0.7 cm	0.3 cm

indicate the shared family-level trait.

Despite these shared characters, substantial interspecific variation was observed in multiple vegetative and floral features critical for taxonomic delimitation. The shape of the leaf blade varied from lanceolate to oblong-lanceolate and ovate-lanceolate, with differences in surface pubescence. Differences were observed in calyx morphology in both surface texture and coloration, while corolla lobes exhibited variation in color and shape. Labellum morphology shows species-specific differences in both shape and color providing strong distinguishing characteristics among the genus and species. Interestingly, the presence of an anther spur in *Curcuma aromatica* and anther connective terminating into a beak-like appendage in *Zingiber zerumbet* are distinguishing characters for the genus *Curcuma* and *Zingiber*, respectively. Marked variation was also observed in filament structure, anther color, and staminode morphology. Although floral measurements were based on single representative specimens, noticeable differences in size among the species highlight the potential taxonomic value of floral dimensions.

The species-specific morphological features combined with shared characters highlight the value of integrated morphological assessment in the taxonomy of Zingiberaceae. The addition of detailed, comparative colored photoplates of the floral parts of each species is one of the major contributions of this study. Despite their significance in supporting morphological comparisons and serving in accurate species identification, these visual aids are often missing in many floristic literature. Documentation of the characters, such as the morphology of calyx and corolla, labellum shape, anther morphology, and staminode structure, with clear visual data supports the significance of this study in the taxonomy of Zingiberaceae from Garbhanga Reserve Forest. Furthermore, these visual data improve the consistency of morphological assessments as well as provide a baseline for future revisions, floristic surveys, and digital herbarium databases.

CONCLUSION

The present study provides a comprehensive comparative morphological assessment of seven species of Zingiberaceae from Garbhanga Reserve Forest of Assam, India, integrating both qualitative characteristics and representative floral measurements, together with the phenological data and voucher documentation. By documenting both shared and species-specific traits along with the incorporation of the comparative tables and high-resolution colored photoplates of the floral parts, this work establishes a morphological and ecological baseline that will aid in future floristic study, taxonomic revisions, biodiversity monitoring, ecological research, and conservation planning. In light of increasing anthropogenic pressures on tropical forests, such integrative taxonomic documentation is necessary for preserving the diversity and ecological integrity of Zingiberaceae in Assam.

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AUTHOR CONTRIBUTIONS

B.D. designed the study and conducted field surveys, specimen collection, and herbarium preparation. Morphological characterization, comparative taxonomic analyses, diagnostic tables, taxonomic keys, and photographic documentation were performed by B.D. The initial manuscript draft was written by B.D. HR reviewed and revised the manuscript. All authors read and approved the final manuscript.

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