

# Exploring Variations: Leaf Traits, Stomatal Features, and Pigment Composition in Terrestrial Orchid Species

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**Abstract-** This study investigates the variations in leaf traits, stomatal features, and pigment composition in three terrestrial orchid species: *Geodorum densiflorum*, *Nervilia crocifformis*, and *Pecteilis susannae*, collected from the same geographical location in Shimoga, Karnataka, India. Leaf characteristics were analyzed using both visual inspection and microscopic techniques, while stomatal features were examined through detailed microscopic measurements. Stomatal density and index, stomatal dimensions, and pigment levels, including chlorophyll and carotenoids, were quantified. *G. densiflorum* exhibited the largest stomata and moderate pigment levels, *N. crocifformis* had slightly smaller stomata with balanced pigment composition, and *P. susannae* showed the smallest stomata but the highest pigment concentration, suggesting diverse adaptations in gas exchange and photosynthetic efficiency. This comparative analysis provides insights into the ecological strategies of these orchid species and their potential responses to environmental conditions.

**Keywords—** *Geodorum densiflorum*, *Nervilia crocifformis*, and *Pecteilis susannae*, Stomatal density, chlorophyll

## I. INTRODUCTION

*Geodorum densiflorum* (Lam.) Schltr. (Figure 1 A, B) terrestrial species have epigeal globose tubers that appear above ground only during the rainy season. The pseudobulbs are large, conical, exposed, and persistent. Vegetative and floral shoots are separate, with the plant reaching 20-30 cm in height. The leaves are 2-nerved or many-nerved. The inflorescence is a sub-cylindrical, compact raceme with a lateral scape that droops terminally. The flowers are pinkish-white or white, with a pale purple lip marked with darker streaks, 3-lobed, and spurred (Akter et al., 2015). It is distributed in the Eastern and Western Himalayas, Burma, Malaysia, Thailand, Andaman Islands, and Sri Lanka. It is widely found in India at lower elevations of the Western Ghats. In South India, it occurs in Ponmudi, Aryankavu, South Wyanad, Balamur, Nelliampathy, Kodagu, Kudremukh, Gulbarga, Mysore, Hassan, and Uttara Kannada. The flowering and fruiting season is from March to June. The roots of this orchid are reputed to have insecticidal properties, and the pseudobulb is used to regulate the menstrual cycle and has anti-diabetic and antimicrobial properties (Keerthiga et al., 2015).

*Nervilia crocifformis* (Zoll. ex Mor.) Seidenf. (Figure 1 C, D) is a terrestrial species with hystarantous tubers, which means it remains leafless during flowering. The leaf is adpressed to the ground, orbicular, cordate, petiolate, plicate, hairy on the upper surface, and glabrous beneath. The flowers are resupinate, solitary, white, and terminal, with spreading ovate sepals, smaller and narrower lanceolate petals, and a long, 3-

lobed purple lip with a median yellowish callus. The column is curved. This species is widely distributed across Gujarat, Rajasthan, Kerala, Tamil Nadu, and Sikkim (La Chung Valley). It can be found in Mysore, Kodagu, Hassan, Shimoga, and Uttara Kannada in Karnataka. Flowering and fruiting occur from March to April, with flowers appearing with the first showers and lasting only for a day (Arisdason et al., 2014; Lakshminarasimhan et al., 2014).

*Pecteilis susannae* (L.) Rafin. (Figure 1 E, F) is a tall and robust herb that can reach up to 90 cm in height. It has large, coriaceous, glaucous leaves that are 10-15 cm long and 5 cm wide. The flowers are notably beautiful, among the largest of the South Indian orchids, measuring up to 8 cm across, and emit a delightful fragrance only at night. The inflorescence bracts are green, and the slender spur is 10-12 cm long, half-filled with nectar, and concealed inside the large bract. This species is widely distributed across the Garhwal Himalayas, Arunachal Pradesh, Khasia, Nagaland, Manipur hills, Burma, Malaysia, Indonesia, and Thailand. It is found in the Deccan Peninsula and Kerala in South India. Flowering and fruiting occur from August to October (Lasmini et al., (2016).

Leaf traits, stomatal features, and pigment composition are important features of orchid to find diverse Orchid genera. The present study aimed to identify differences in leaf characteristics, stomatal and pigment levels of three orchid species collected from same geographical location.

## II. MATERIAL AND METHODS

### 2.1 Collection of sample

The three terrestrial orchid species, *G. densiflorum*, *N. crocifformis* and *P. susannae* were collected from Shimoga, Karnataka, India. Three samples of each species were collected from locations minimum 1km<sup>2</sup> away from each other. These locations were Sagar, Kogar and Linganmakki Reservoir. They were identified by a taxonomist at the time of collection. Plant leaves were stored in a zip lock plastic cover and transported to the research lab.

## 2.2 Study of leaf:

The surface, orientation, and texture of the leaf were studied by the naked eyes as well as under a simple microscope. The type of Stomata and surface having a high number of stomata was also assessed.

## 2.3 Study of stomata

A thin layer of tissue was carefully peeled from the dorsal side of a leaf. Thin layer of the tissue was kept on the microscope slide and a few drops of Saffranin followed by cover slip was put over the tissue. The preparations were analyzed under an Eclipse E800 microscope (Nikon Corp., Japan) equipped with a Panasonic video camera. Image grabbing, counting the number of stomata and stomatal guard cell length measurements were done with Lucia G software (Laboratory Imaging Ltd.).

## 2.4 Stomatal Density and index

Stomatal density is a simple calculation of a total number of stomata present per millimeter square of tissue. Grids of 100 μm<sup>2</sup> were composed on the microscopic image to count the number of stomata and epidermal cells (E) within that. Stomatal density was calculated using the following formula: Stomatal Density = Number of Stomata present in 1 grid (S) X 100.

The stomatal index (SI) was calculated by using the formula:

Stomatal Index (SI) =  $N/N+E \times 100$

N= Number of Stomata

E= Number of Epidermal Cells

## 2.5: Stomatal length, breath and area

Stomatal length (SL, μm) and width (SW, μm) were measured using the scale in the software from 30 stomata selected randomly.

Stomatal area (SA) was calculated using the following formula.

$$SA = \frac{\pi \times SL}{4}$$

## 2.6 Stomatal aperture and Gaurd cell length

The stomatal aperture and guard cell length were measured with the help of scale in the software. Stomatal aperture length and width are the areas of the stomata that exclude guard cells.

## 2.7 Estimation of chlorophyll and carotenoids

Chlorophyll and carotenoids were estimated using Holm's method (1954). The concentration of chlorophylls was measured using Porra's (2002) equations, which are mentioned below.

Chlorophyll a = 12.25 (A663) - 2.55(A646)

Chlorophyll b = 20.31 (A646) - 4.91 (A663)

Total Chlorophyll = 17.76 (A646) + 7.34 (A663)

Carotenoid concentration was calculated by using Lichtenthaler and Welburn equation (1983): Carotenoid =  $\{[1000 A470 - 3.27 \text{ Chl a}] - [104 \text{ Chl b}]\} / 227$

## III. RESULTS AND DISCUSSION

### 3.1 Study of leaves:

*Geodorum densiflorum* typically had thin and smooth leaves. However, *Nervilia crocifformis* features leaves with a slightly thick and velvety texture. Leaves of *Pecteilis susannae* had both the qualities smooth and somewhat leathery texture. The leaves of *Geodorum densiflorum* were arranged in a rosette form, and they are slightly glossy and smooth. The leaves are typically heart-shaped and lie close to the ground, with a soft and velvety texture.

**3.2 Features of stomata:** The stomata of *Geodorum densiflorum* were relatively large, with an average length of  $48.32 \pm 1.72 \mu\text{m}$  and a width of  $36.31 \pm 1.33 \mu\text{m}$ , resulting in a substantial stomatal area of  $1376.23 \pm 15.75 \mu\text{m}^2$ . The stomatal aperture measures  $20.48 \pm 1.66 \mu\text{m}$ , and the guard cells have a width of  $9.43 \pm 0.81 \mu\text{m}$ .

Study of *Nervilia crocifformis* exhibited slightly smaller stomata with an average length of  $45.81 \pm 2.34 \mu\text{m}$  and a width of  $33.91 \pm 1.55 \mu\text{m}$ , leading to a stomatal area of  $1218.86 \pm 21.82 \mu\text{m}^2$ . The stomatal aperture averages  $18.28 \pm 1.96 \mu\text{m}$ , while the guard cells are  $8.51 \pm 0.74 \mu\text{m}$  in width. These features indicate a balanced approach to gas exchange and water regulation, adapting well to environmental conditions.

Third species included in the study, *Pecteilis susannae* had the smallest stomata among the studied species, with an average length of  $40.54 \pm 3.13 \mu\text{m}$  and a width of  $30.13 \pm 2.81 \mu\text{m}$ , resulting in a stomatal area of  $996.36 \pm 16.74 \mu\text{m}^2$ . The stomatal aperture measures  $15.00 \pm 1.63 \mu\text{m}$ , and the guard cells are  $7.76 \pm 0.92 \mu\text{m}$  wide. This configuration of *Pecteilis susannae* suggests a highly regulated gas exchange process, optimizing water retention while maintaining necessary physiological functions. Similar results were published in *Cymbidium aloifolium* by Mulgaonkar, (2005).

### 3.3 Type of Stomata

On comparing stomata of three species, *G. densiflorum* stomata were paracytic, accompanied by two subsidiary cells parallel to the opening, however *N. crocifformis* stomata were anomocytic, which means they have no distinct subsidiary cells, making them harder to distinguish from other epidermal cells and *P. susannae* stomata were diacytic, with each stoma flanked by two perpendicular subsidiary cells. The diacytic stomata in *P. susannae* represent a more complex adaptation that might provide refined control over the leaf microenvironment. Which was earlier mentioned by Chomiki et al., (2014).

### 3.4 Pigment analysis

The biochemical analysis of *G. densiflorum*, *N. crocifformis*, and *P. susannae* showed statistically insignificant results. *G. densiflorum* had a total chlorophyll content of  $5.10 \mu\text{g/g FW}$ , a chlorophyll-a/chlorophyll-b ratio of 1.24, a chlorophyll/soluble protein ratio of 0.19, a carotenoid content of  $0.74 \mu\text{g/g FW}$ , and an anthocyanin content of  $0.33 \mu\text{g/g FW}$ .

*N. crocifformis* exhibited a total chlorophyll content of  $5.24 \mu\text{g/g FW}$ , a chlorophyll-a/chlorophyll-b ratio of 1.72, a chlorophyll/soluble protein ratio of 0.32, a carotenoid content

of 0.80 µg/g FW, and an anthocyanin content of 0.25 µg/g FW.

*P. susannae* showed the highest values with a total chlorophyll content of 7.77 µg/g FW, a chlorophyll-a/chlorophyll-b ratio of 2.11, a chlorophyll/soluble protein ratio of 0.15, a carotenoid content of 1.65 µg/g FW, and an anthocyanin content of 0.44 µg/g FW. These increases suggest enhanced pigment production and possibly improved photosynthetic efficiency in all three species. Among these species *P. susannae* with medium texture of leaf had maximum pigments. Similar results were published in *Aerides* species by Srivastava et al., (2013a).

**Table.1: Characterization of stomata in the leaf of *G. densiflorum*, *N. crociformis* and *P. susannae***

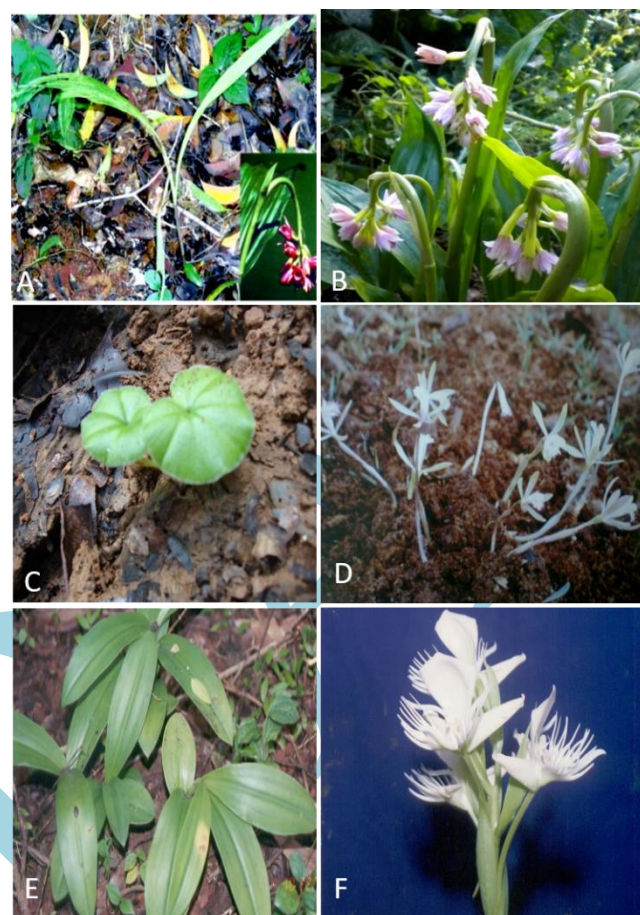
Characters	<i>G. densiflorum</i>	<i>N. crociformis</i>	<i>P. susannae</i>
Stomatal length (µm)	48.32± 1.72	45.81±2.34	40.54±3.13
Stomatal width (µm)	36.31 ±1.33	33.91± 1.55	30.13± 2.81
Stomatal area (µm <sup>2</sup> )	1376.23±15.75	1218.86 ± 21.82	996.36±1 6.74
Stomatal aperture (µm)	20.48±1.66	18.28±1.96	15.00±1.63
Guard cell width (µm)	9.43±0.81	8.51±0.74	7.76±0.92

Note: All the data is mean±SD.

**Table. 2: Pigments in the leaf of *G. densiflorum*, *N. crociformis* and *P. susannae***

Characters	<i>G. densiflorum</i>	<i>N. crociformis</i>	<i>P. susannae</i>
Total Chlorophyll (µg/g FW)	5.10 ± 1.32	5.24 ± 0.78	7.77 ± 1.00
Chlorophyll-a/Chlorophyll-b ratio	1.24 ± 0.03	1.72 ± 0.07	2.11 ± 0.08
Chlorophyll / soluble protein ratio	0.19 ± 0.08	0.32 ± 0.06	0.15 ± 0.08
Carotenoid (µg/g FW)	0.74 ± 0.02	0.80 ± 0.06	1.65 ± 0.10
Anthocyanin (µg/g FW)	0.33 ± 0.04	0.25 ± 0.03	0.44 ± 0.06

Note: All the data is mean±SD.



**Figure 1: Three orchid species in their natural habitat**  
*G. densiflorum* (A, B), *N. crociformis* (C, D), *P. susannae* (E, F).

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