Summary of thesis entitled

SYSTEMATIC STUDIES ON ORCHIDACEAE OF GUJARAT

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By

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1.1. INTRODUCTION

The word 'Orchid' has been originated from the Greek word '*Orchis*' meaning testicles (Bedford, 1969; George, 1999; Rittershausen et al., 2002). Orchidaceae is one of the largest and most advanced family in the plant kingdom. The family shows pantropic distribution and comprises approximately 28,484 species. (Christenhusz and Byng, 2016; Govaerts et al., 2017). Orchids are considered to be the highly evolved among all the flowering plants (Waller, 2016). They are inhabitant of tropical countries, which includes tropical forest of South and Central America, Mexico, India, Ceylon, Burma, South China, Thailand, Malaysia, Philippines, New Guinea and Australia (Irawati, 2013). Orchids are annual or perennial herbs, lacking permanent woody structure (Randhawa and Mukhopadhyay, 1986). Depending on the mode of habits, they can be terrestrial (growing on the ground) epiphytic (growing on trees), lithophytic (growing on rocks) or mycoheterotrophic (growing on the dead and decaying matter).

The vegetative features in orchids are very diverse, but in general, their common components are root, stem/pseudobulb, leaf, flower and fruit. Few distinguishing features of the members of Orchidaceae are

- The presence of an odd petal called labellum with spur or without spur.
- The presence of a column called as Gynostemium.
- Pollens are packed together into the pollinia or pollinium, a mass of waxy pollen.
- The seeds are minute, dust like and numerous.
- The seeds can only germinate in symbiosis with specialized fungi called as mycorrhizae under natural circumstances.

The classification of Orchidaceae was given by number of scientist but the most accepted one was given by Chase et al. (2015). According to their classification, the family Orchidaceae was divided into five subfamilies – Apostasioideae, Vanilloideae, Cypripedioideae, Epidendroideae and Orchidoideae based on both morphological and molecular data (Figure 1).

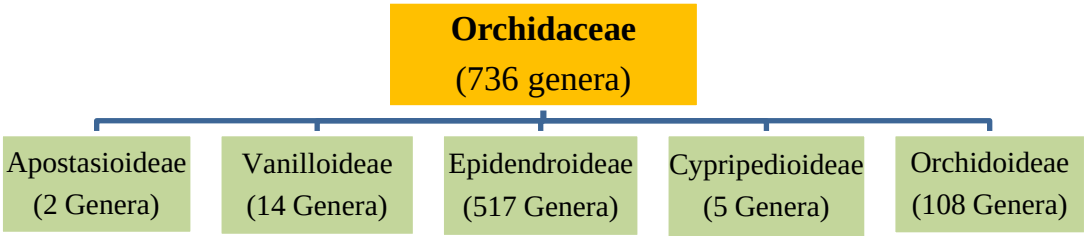


Figure 1: Classification of Orchidaceae

Orchids are very sensitive towards the change in habitat which leads to a restricted distribution of certain species. Various anthropogenic activities and change in global climatic conditions are responsible for the habitat loss (Kumar et al., 2007, 2006). Furthermore, the monoculture plantations, collection of orchid bulbs for livelihood and medicinal purposes, mass over-collection due to fascination, leads to exploitation of orchids.

The aim of the present study was to explore the orchid diversity in Gujarat and to establish an authentic data for the purpose of taxonomical evaluation as well as their molecular identification using DNA barcoding techniques, anatomical studies and phytochemical profiling of ethnobotanically important orchids. Additionally, emphasis on their preservation was done by *ex-situ* conservation (Figure 2).

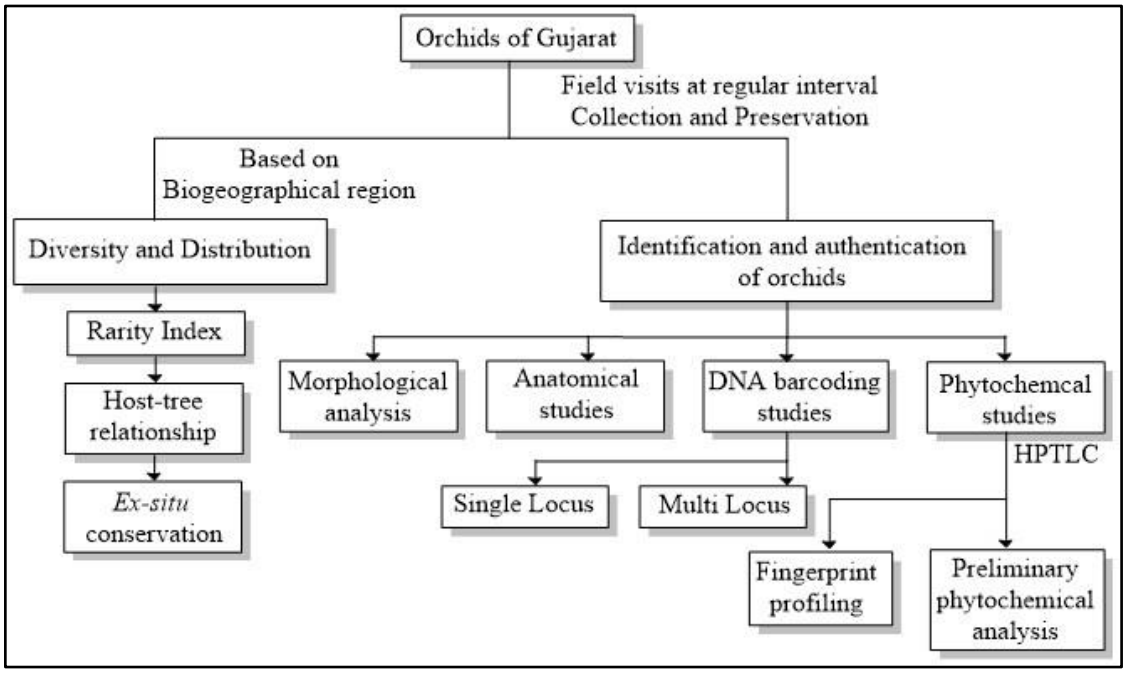


Figure 2: Summarized work flowchart

In order to achieve these goals, the objectives were elucidated in 5 chapters

Chapter 1 comprised of introduction about the family Orchidaceae, their general morphology, systematics, threats and objectives of the present study.

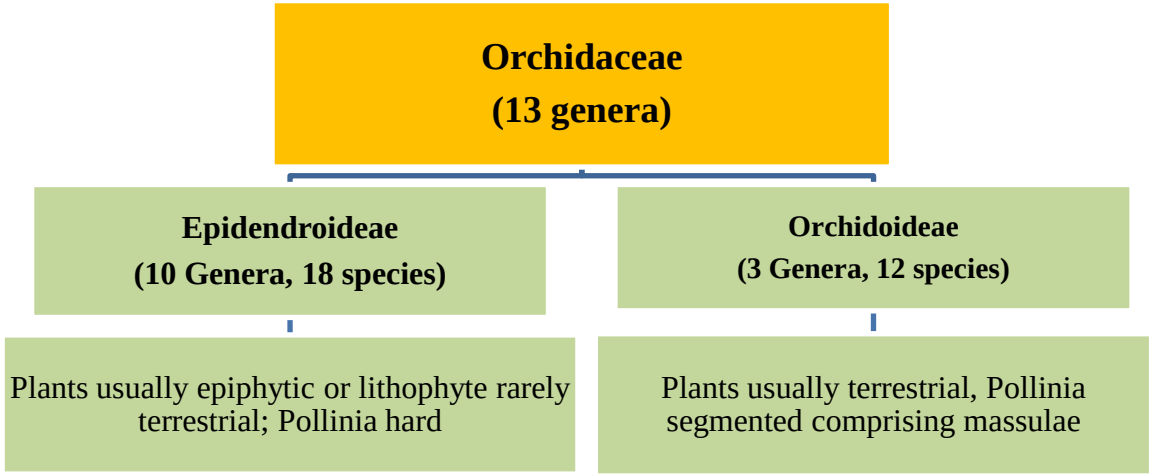
An attempt was made to understand the diversity and distribution of orchids in **Chapter 2**. The chapter includes review on Orchidaceae of India and Gujarat, description of study area on the basis of biogeographical region with its forest type, methods for identification and results. The current study resulted into 30 species of orchids representing 13 genera from Gujarat of which 12 are epiphyte, 17 terrestrial and one is lithophyte (Table 1). Among the 13 reported genera, five (*Acampe*, *Crepidium*, *Geodorum*, *Rhynchostylis* and *Zeuxine*) are represented by single species, four (*Aerides*, *Eulophia*, *Nervilia* and *Oberonia*) by two species and two (*Dendrobium* and *Peristylus)* by four species. The key for genera and species were provided for easy identification. The elaborated description for each species were also provided along with the information about its flowering and fruiting period, habit, habitat, distribution pattern in Gujarat with mapping and global distribution. All the herbarium specimens were submitted at BARO, The Maharaja Sayajirao University of Baroda and Arid Zone Herbarium, Jodhpur (BSI).

This chapter also deals with the distribution pattern of orchids in Gujarat, their rarity status and their conservation at Arboretum of Maharaja Sayajirao University of Baroda, Vadodara and Waghai Botanical Garden, Dangs.

Table 1: List of Orchids of Gujarat

SN	Orchid Name	Habit
1.	*Acampe praemorsa*	Epiphyte
2.	*Aerides maculosa* (Endemic)	Epiphyte
3.	*A. ringens*	Epiphyte
4.	*Crepidium mackinnonii*	Terrestrial
5.	*Dendrobium barbatulum* (Endemic)	Epiphyte
6.	*D. microbulbon* (Endemic)	Epiphyte
7.	*D. ovatum* (Endemic)	Epiphyte
8.	*D. peguanum*	Epiphyte

SN	Orchid Name	Habit
9.	*Eulophia herbacea*	Terrestrial
10.	*E. ochreata* (Endemic)	Terrestrial
11.	*Geodorum laxiflorum* (Endemic)	Terrestrial
12.	*Habenaria furcifera*	Terrestrial
13.	*H. gibsonii* (Endemic)	Terrestrial
14.	*H. grandifloriformis* (Endemic)	Terrestrial
15.	*H. longicorniculata*	Terrestrial
16.	*H. marginata*	Terrestrial
17.	*H. plantaginea*	Terrestrial
18.	*H. rariflora* (Endemic)	Lithophyte
19.	*Nervilia concolor*	Terrestrial
20.	*N. plicata*	Terrestrial
21.	*Oberonia falconeri*	Epiphyte
22.	*O. mucronata*	Epiphyte
23.	*Peristylus constrictus*	Terrestrial
24.	*P. lawii*	Terrestrial
25.	*P. plantagineus*	Terrestrial
26.	*P. stocksii* (Endemic)	Terrestrial
27.	*Rhynchostylis retusa*	Epiphyte
28.	*Vanda tessellata*	Epiphyte
29.	*V. testacea*	Epiphyte
30.	*Zeuxine strateumatica*	Terrestrial



Chapter 3 comprise of general introduction on DNA barcoding and its importance. In the present study three barcode loci *viz.* rbcL, matK and ITS were used to identify 31 orchidaceous taxa. The sequences obtained were analyzed and subjected to NCBI blast followed by their submission to BOLD. The sequences were processed in MEGA 7 (v. 7.0.26) for further analysis. The species resolution for each locus was calculated using three different methods *viz*. genetic distances, phylogenetic tree and BLAST analysis. As none of the locus (rbcL, matK and ITS) yielded 100% species discrimination, different multilocus combinations were also assessed. The inter-specific variations and species discrimination rates among the congeneric species were calculated using multilocus combinations *viz.* rbcL+matK, rbcL+ITS, matK+ITS and matK+rbcL+ITS. The ITS (single locus) and rbcL+matK+ITS (multilocus combination) were recommended as best loci for differentiating orchids.

The **Chapter 4** gives an insight of the anatomical variations in leaves of epiphytic and terrestrial species of orchids. The chapter embraces general information about plant anatomy, review on vegetative anatomy of orchids, and detailed leaf anatomical study of 29 species of orchids. The comparative study of the structure and arrangement of different cell layers like cuticle, epidermis, hypodermis, mesophyll, vascular tissues were the main characteristics utilized to differentiate the orchids. All species from different genus provide several traits which would help in their correct identification, authentication as well as to prevent adulteration of plant samples.

Chapter 5 was an approach to evaluate the nature of different phytochemical groups present in selective abundant orchids using modern technique *i.e.* HPTLC. Tubers of four different species of orchids namely *Nervilia concolor, N. plicata, Eulophia ochreata* and *E. herbacea* were processed for preliminary phytochemical analysis using HPTLC technique. HPTLC fingerprint profiling of samples concluded that **toluene: ethyl acetate: glacial acetic acid (16: 4: 0.5 v/v/v)** was the best solvent system for better resolution of all the extracts. The primary screening of phytochemicals using HPTLC revealed that all the four species of orchids are rich in essential oils, glycosides, phenolics, saponins, steroids and triterpenes. Alkaloids and flavonoids were only present in *N. plicata* and *E. ochreata*. All the four species are having efficient potential to scavenge the free radical, which could be related to its good antioxidant activity.

1.2. CONCLUSION

The systematic execution of Orchidaceae in Gujarat revealed 30 species (belonging to 13 genera) of orchids inclusive of five new records. However, according to earlier review, 35 species were reported from the Gujarat, out of which 10 species were not be located during the present study. This could be owing to either mis-interpretation or local extinction due to habitat alternation and anthropogenic threats. The orchid diversity of Gujarat state is higher than that of neighboring state i.e. Rajasthan and Madhya Pradesh. However, it is less than adjoining state, Maharashtra.

The issue pertaining to identification of orchids in vegetative condition was resolved by DNA barcoding and anatomical study. The ITS (single locus) and multilocus combination of rbcL+matK+ITS were recommended as best loci for the identification of orchids. The anatomical study concluded that the selective traits like cuticle, epidermis, hypodermis, sclerenchyma bands, fibre bundles and fibre caps in vascular bundles provides useful information for the correct identification and authentication of different species of orchids. The HPTLC fingerprint and Preliminary phytochemical analysis of four species of orchids provides useful information regarding quality control, standardization of herbal formulations, identification of adulterants and biomarkers. Moreover, these data provide an additional information to support their ethnobotanical applications.

The present study also emphasizes that all the documented species of orchids are regionally threatened. Thus, an initiation step was taken for e*x-situ* conservation of orchids at the M. S. University (Vadodara) and Waghai Botanical Garden (Dangs). A total of 24 species were successfully conserved at both the sites.

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