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**Synopsis of the thesis entitled**

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**SYSTEMATIC STUDIES ON ORCHIDACEAE OF GUJARAT**

**Submitted to**  
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For the Degree of  
**DOCTOR OF PHILOSOPHY**  
in  
**BOTANY**

By  
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## Chapter 1: INTRODUCTION

This chapter deals with the general introduction about Orchidaceae, threats and its systematic treatment by various scientists.

The orchids with approximately 26,567 species (Govaerts *et al.*, 2017), encompasses one of the leading, diverse and greatly evolved families of economically important plants - the Orchidaceae, which has out-manuevered its counterparts in adaptive significance of morphological traits. Major speciation, in these plants, has revolved around floral diversification and specialization (Hapeman *et al.*, 1996) and they still continue to be in an evolutionary flux due to (i) poorly developed barriers of reproductive isolation and (ii) high survival frequency of neotypes. The orchids have contributed immensely to the floriculture, due to their beautiful lustrous foliage, and long-lasting flowers of myriad shapes, sizes and colours. Being rich in alkaloids these have high therapeutic value and widely used in the traditional system of medicine to cure a variety of ailments (Lawler, 1984; Sureshkumar *et al.*, 1994; Toh, 1994; Liu *et al.*, 1992).

The orchids are cosmopolitan in distribution exhibiting variety of life modes, i.e. terrestrial/ epiphytic/ lithophytic/ saprophytic, mainly occurring in the tropical and sub-tropical environs due to the prevalence of thick vegetation and high humidity, both the factors are necessary for their growth and development (Vij, 1995a). However, there is gradual decline in the population of plants due to its slow growing nature, habitat-specificity, habitat destruction and trading which has detrimentally affected their natural populations. The orchids now figure prominently in the Red Data Book by the International Union for Conservation of Nature and Natural Resources (IUCN, 1991). Orchid forms the important part of biodiversity and according to Kull *et al.* (2006) and Swarts and Dixon (2009), orchids are at the front-line of extinction with more species under threat globally than any other plant family. Therefore periodic field surveys are more important for these plants as they cannot tolerate even smaller fluctuation in their habitat.

The state of Gujarat is located on the western coast of the Indian Peninsula and lies between 20°1' N to 24°7' N and 68°4' E to 74°4' E. The state covers an area of 1,96,024 sq. km. with the longest coastline of 1600 km. Administratively it comprises of 33 Districts and 249 Talukas. Gujarat is bounded by Arabian Sea in the West and Southwest, Pakistan in the North,

Rajasthan in the Northeast, Madhya Pradesh in the Southeast and Maharashtra and Union Territories of Daman, Diu and Nagar Haveli in the South.

The biodiversity of any area is markedly influenced by environment and various anthropogenic pressures which act as driver of climate/microclimate change. A periodic evaluation is thus greatly needed to keep check on the current status of biodiversity in any area. As orchids are at the front-line of extinction an attempt was made for surveying and documenting the orchid wealth of Gujarat for conserving the threatened species of orchids and to create awareness among the people about the importance of the orchids aesthetically as well as medicinally that can reduce over exploitation of indicator species of an ecosystem (endemic and threatened orchid taxa from the wild habitat).

The main objectives of the study are:

1. Diversity and Distribution of Orchids in Gujarat.
2. DNA Barcoding for Phylogenetic studies
3. *Ex-situ* conservation of orchids
4. Anatomical studies
5. Preliminary phytochemical analysis of medicinally important species

## **Chapter 2: DIVERSITY AND DISTRIBUTION OF ORCHIDS**

This chapter gives an insight into the historical account of Orchidaceae, its diversity and distribution in India with special reference to the study area.

Orchidaceae is represented by about 36 species under 15 genera in Gujarat (Shah, 1978; Anonymous, 1996; Singh & Parabia, 2003; Tadvi, 2014). From 34 species 10 species are endemic to India (Nayar *et al.*, 2014). In the present study 29 species of Orchids were collected from the state including four new records. The voucher specimens prepared were authenticated from BSI Pune and BSI Jodhpur. The authenticated specimens were deposited in the herbaria at BSI (Botanical Survey of India), Jodhpur and at BARO, Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara. The detailed description and an easy identification keys for the genus and species was provided as this family is very complicated.

### **Chapter 3: DNA BARCODING**

This chapter deals with the identification of species using DNA Barcodes.

DNA barcoding was carried out for all the collected species of Orchids using three barcode loci *i.e.* rbcL (ribulose-bisphosphate carboxylase/oxygenase gene) and matK (maturase K) from the plastid genome and ITS (Internal transcribed spacer) from nuclear genome. The three candidate barcode loci were evaluated and compared individually for their amplification and sequencing success rates. The species resolution for each candidate locus was calculated based on genetic distances, phylogenetic tree method, and through BLAST (Basic Local Alignment Search Tool) analysis. The amplification rate was 99% for all markers but sequencing success rate was 80 – 85%. The species resolution through blast analysis was less in rbcL compared to matK and ITS region. The sequences were submitted to BOLD (Barcode of Life Database) to generate the true barcode.

### **Chapter 4: ANATOMICAL STUDIES**

The chapter gives an insight into the anatomical characters that can be used in identification of species even in vegetative stage.

For anatomical aspects taxonomically identified species were fixed in FAA solution. The fixed samples were further processed for microtomy using Leica rotary microtome and permanent slides were prepared. The vasculature, size and shape of epidermal cells, stomata, size of mesophyll cells and presence of sclereids or raphides forms the diagnostic feature for each species.

### **Chapter 5: PHYTOCHEMICAL STUDIES**

This chapter deals with the preliminary phytochemical analysis of four medicinally important species viz., *Nervilia plicata*, *N. concolor*, *Eulophia ochreatea* and *E. herbacea*.

Preliminary phytochemical screening revealed the presence of secondary metabolites like alkaloids, flavonoids, saponins and phenolic compounds in the tuber of all the four orchids. The fingerprint was also developed for this species and their antioxidant property was also verified by means of HPTLC.

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