Synopsis of the thesis entitled

Systematics and Molecular Studies on Diversity of Pteridophyte and Gymnosperm of Gujarat

Submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

By

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2021

INTRODUCTION

The word Pteridophyta is derived from the Greek words "*pteron*" means "feather" and "*phytes*" means "plants" (*i.e.* feather-like plants). They are the spore-bearing, seedless vascular cryptogams characterized by the self-regulating heteromorphic alternation of generation. The word pteridophyte does not include all the vascular cryptogams; therefore, in the 20th century, vascular cryptogams are known by the new name '*ferns and fern-allies or Lycophytes and ferns*'. Pteridophytes stand at second position in terms of species richness while their diversity and distribution rank next to the angiosperms in the plant kingdom. It is estimated that, about 11,916 species representing 337 genera in 51 families showing cosmopolitan distribution in different biogeographic zones of the world (PPG-I 2016). They grow luxuriously in different habitat during the rainy season due to the presence of high humidity, the moisture content in the atmosphere and enough shade due to less penetration of sunlight.

Gymnosperms form a significant group of plants due to their enormous ecological, socio-economic and evolutionary value. This group of plants are cosmopolitan in distribution throughout the world, extensively diversified and distributed in the tropics and they form the most dominant plants group in the temperate forest area of the northern and southern hemisphere (Fragniere *et al*. 2015). In contrast to other plant groups, extant gymnosperms are very less in number; they comprise *ca*. 1,106 species under four major lineages *viz*., *Ginkgo*, Gnetophytes, Cycads and Conifers (Calonje *et al*. 2020; WCSP 2020).

In India, pteridophytes are diversified and flourish in all the bio-geographical and ecological zones of the country. A cosmopolitan distribution of pteridophytes in India comprises about 1,138 species and 114 subspecies belonging to the 125 genera, of 34 families (Fraser-Jenkins et al. 2017, 2018a, 2020; Fraser-Jenkins 2020). From which, 47 species of 20 genera belonging to 15 families of pteridophytes are endemic to the country (Fraser-Jenkins 2008a; Fraser-Jenkins et al. 2017, 2018a, 2020; Fraser-Jenkins 2020). These are mainly concentrated in the diversity rich areas of peninsular India, the Western Ghats, North-East India, Western Himalayas and Islands of Andaman and Nicobar. In India, extant gymnosperms diversity is estimated at about 149 species and 8 varieties (indigenous/introduced) belonging to 46 genera belonging to 12 families (Singh & Srivastava 2013; Sharma & Singh 2015; Akhtar *et al*. 2019).

Gujarat is the westernmost state of India, lies between 20°07'-24°43' N latitudes and 68°10'-74°29' E longitudes covering an area of 1,96,244 km^2, which is 5.97% of the total geographic area of the country. The state is fenced by the Arabian Sea in the west and south-

west having the longest coastline *ca*. 1,650 km, amongst the Indian states (ISFR 2019). The most conspicuous features of the coastline are two Gulfs (2/3 Gulfs of the country) *i.e*. Gulf of Khambhat and Gulf of Kachchh which are crowded with marine life and coastal wetlands. It shares an international border with Pakistan on the north side, which is also known as the Sir Creek line. North and north-east side of the state is shared with Rajasthan, Madhya Pradesh in the east side and Maharashtra and Dadra Nagar Haveli in the south side. The unique features of the state are climatic and geomorphologic conditions such as the longest coastline in the country, the saline deserts of Kachchh (Rann), grassland and wetlands. The state has four National Parks, 23 Wildlife Sanctuaries and one Conservation Reserve *i.e.* Rann of Kachchh Biosphere Reserve (RKBR). It comprises the Protected Area network of the state which is covering 8.83% of the total geographical area of Gujarat (ISFR 2019).

Earlier taxonomists followed the traditional method of identification *i.e.* morphological characters due to which cryptic species created the problem and appeared as new species or merging of distinct species. To overcome these problems, various other allied fields such as anatomy, biochemistry, cytology and molecular methods are exploited extensively recently, which is giving new insights into the field of taxonomic research. This is leading to the development of a new field of systematic research known as molecular systematics and DNA barcoding. DNA barcoding is a potential tool for species identification using molecular methods (Hebert *et al*. 2003).

As compared to other groups of plants, studies on angiosperms have received great attention for their diversity and distribution in the state. Similar studies on pteridophytes are not being reflected in botanical literature due to the lack of enumeration/excursion studies. Unlike other group of plants (particularly angiosperms) studies on these group of plants have been neglected by earlier field taxonomists as under the notion that numbers of pteridophytes are lower than other states due to lack of higher elevation of mountains and poor rainfall, lack of suitable climate, temperatures etc. (Rajput *et al.* 2016). Few species are known from the state usually has information only from a few localities and no detailed studies are carried out on this group of plants in Gujarat state due to aforesaid reasons. In similar case for gymnosperm diversity and only *Ephedra foliata* is documented from few places of Saurashtra and Kachchh. However, we feel that there is an existence of more than one species of *Ephedra* from the Gujarat state. Therefore, understanding the diversity and distribution of pteridophytes and gymnosperms occurring in the Gujarat state is undertaken with the following ***objectives***.

- To explore and document the diversity of pteridophytes in different forest regions of the Gujarat state during different seasons.
- To study the distribution pattern of pteridophyte in different parts of Gujarat.
- To study the habitat diversity of pteridophytes in relation to different climatic zones of Gujarat state.
- To explore the additional locality of pteridophytes and *Ephedra* sp. already documented from Gujarat state.
- Molecular identification and generation of barcode for collected pteridophyte and gymnosperm.
- To analyze the regional threats to their diversity.

REVIEW OF LITERATURE

Pteridophytes and gymnosperms flora of Gujarat state has been studied by various researchers *viz*., Saxton, Sedgwik, Mahabale, Gaekwad, Deshmukh, Chavan, Mehta, Padate, Sabnis, Inamdar, Shah, Vaidya, Thaker etc., studied its occurrence, distribution, micromorphology, anatomy and structure of stomata etc. In the recent past, there are sporadic reports on the common pteridophytes like *Marsilea* and *Azolla*. However, these studies were restricted to adjoining areas of the cities and town and neglected the forest areas of the whole state for the total diversity and its distribution of pteridophytes and gymnosperms flora. A review of literature reflects the sporadic reports on some wild and exotic species. Few of them were misidentified and recorded from the state *i.e. Actiniopteris australis*, *Athyrium solenopteris*, *Tectaria cicutaria*, *Ophioglossum fibrosum* and *Azolla africana*. Studies on pteridophyte and gymnosperm diversity, their distribution status in the state, morphology and molecular studies are indicative of lacuna. Some exotic species of gymnosperms were reported from the various places of the state like *Thuja occidentalis*, *Cycas revoluta*, *Cycas circinalis* etc.

STUDY AREA

Gujarat is located between 20°01’-24°07’N latitudes and 68°04’-74°04’E longitude and covers an area of 195,984 km^2. On the basis of geology, climatic variation, forest types, soil, and drainage patterns of the state, it is further sub-divided into five regions *viz*., Saurashtra, Kachchh, North Gujarat, Central Gujarat and South Gujarat. Topographically, the state shows a wide range of geographical features ranging from forests covers, drainage, desert, soil to the coastal and marine environment. The climatic conditions are pretty diverse in the state. Mostly, the state has tropical climate *viz*., sub-humid, semi-arid to arid that

spread over different regions of the state. North and north-west part of Gujarat receives arid climate (North Gujarat and Kachchh Region) Saurashtra and northern part of mainland region has a semi-arid climate, while the central and southern part of mainland falls under sub-humid climate region. Rainfall in Gujarat is greatly variable over time and space leading to flooding situation while sometimes faces drought too. The average annual rainfall for the year 2013-19 of Gujarat state was recorded 1006.5, 605.6, 584.3, 604.9, 815.0, 485.3 and 1067.8 mm respectively (Kaur & Purohit 2014, 2016a, b; Purohit & Kaur 2017; Yadav et al. 2018, 2020, 2021). Gujarat covers 14,857.33 km^2 area of forest, which is 7.57% of the state geographical area of the state. As per Champion and Seth (1968), the forests in the state belongs to five major sub-groups *viz.*, 3B (South Indian Moist Deciduous Forests), 4A (Littoral Forests), 4B (Swamp or Tidal Forests of (Southern Tropical Dry Deciduous Forests), 6B (Northern Tropical Thorn Forests, which are Mangroves) and 5A further divided into 31+1 different forest types (Champion and Seth 2005; FSI 2019). The major soil types of Gujarat state are Alluvial soil, Black soil, Lateritic, Hill soils and Desert soils (Kulkarni 1985).

MATERIALS AND METHODS

An extensive and critical survey of concerned literature on pteridophyte and gymnosperm flora of Gujarat state has been made. Comprehensive information on pteridophytes and gymnosperms of the state and their occurrence, distribution, status and diagnostic features etc., were collected through referring to various research publications, state and national floras, monographs, books and electronic sources. Critical notes on each species were prepared by consulting recent literatures and various specimen records deposited in different herbaria.

On the basis of available literature and herbarium information, several explorative field survey trips of short and long duration were undertaken at various localities in Gujarat state for seven years (2013-2020). For the period of field exploration, observations were made on life form, ecology, distribution and occurrence and variations in species and threat encountered by the species in their natural habitat. Specimens were collected for the micromorphology and molecular studies. For morphological identification, collected specimens were observed by referring to standard literature, state and national floras, monographs, books and efloras. For molecular identification, a modified CTAB method of DNA isolation, amplification, purification and sequence analysis was followed. Further, specimens were identified, confirmed and authenticated by several field experts. Later,

generated sequences were uploaded to the BOLD System v3 and NCBI database. Species distribution mapping was carried out by using QGIS software and rare and threatened species were analyzed by using standard guidelines of IUCN (IUCN 2016).

RESULTS AND DISCUSSION

Exclusive and extensive fieldwork was carried out for the collection of pteridophytes and gymnosperms to document the diversity and species richness in different areas of the Gujarat state. Theses group of plants constitutes an obvious component in the flora of the entire state and flourishes well during the rainy season and is at its best from June to October. The present study resulted in the collection and identification of 47 taxa (6 lycophytes and 39 ferns) belonging to the broader category in the pteridophytes, 21 genera under 13 families in the wild from the state. There are 15 species under 10 genera belonging to 9 families were under cultivation in botanical garden and arboretum. Hence, a total of 60 taxa belonging to 29 genera under 18 families which includes wild and ornamental species of pteridophytes in the state were documented. On the other side, two species of gymnosperms belonging to one genus (*i.e*. *Ephedra*) and one family were documented in the wild from the state. Eleven species under 8 genera belonging to 6 families were documented as ornamental or exotic species of gymnosperms cultivated in the botanical gardens and arboretum of the state due to their aesthetic value. Therefore, a total of 13 taxa belonging to 9 genera under 7 families which include wild and cultivated species of gymnosperms in the state are documented. All the biotypes, morphotypes, synonymy and ecologically variable species were merged to the universally accepted taxon.

Voucher specimens are deposited in BARO herbarium of the Botany Department, The Maharaja Sayajirao University of Baroda-Vadodara (BARO); Arid and Semi-arid regional centre, Jodhpur, Rajasthan (BSJO); Western Regional Centre, Pune, Maharashtra (BSI); The Blatter Herbarium, Mumbai, Maharashtra (BLAT) and Central National Herbarium, Kolkata, West Bengal (CAL). The detailed description and easy identification keys for the genus and species are provided along with its phenology, distribution, ecology and IUCN category for both pteridophytes and gymnosperms of the state. Habitat diversity of pteridophytes was studied in all the agro-climatic zones of the state. Species were found in all habitats ranging from terrestrial, lithophytes and epiphytes to hydrophytes.

Pteridophytes flora of the state displays, Polypodiales is the most species-rich order in the state covering 6 families under 13 genera and 24 species, followed by Ophioglossales with single-family and single genus with 12 species. Equisetales and Schizaeales possess

only one family belonging from one genus and one species. Pteridaceae is the most specious family, comprises 13 species under 7 genera, followed by Ophioglossaceae shows 12 taxa under a single genus. However, Equisetaceae, Lygodiaceae, Marsileaceae, Hypodematiaceae and Tectariaceae show single family with single taxa in each. In diversity point of view, the richest genus in the term of a number of species is *Ophioglossum* (12 taxa) in the state, followed by *Athyrium* (5 taxa), *Selaginella* (4 taxa), *Aleuritopteris* (4 taxa), *Adiantum* (3 taxa), *Isoetes* (2 taxa), *Ceratopteris* (2 taxa) and *Thelypteris* (2 taxa). Only single species were encompassing genera *viz*., *Equisetum*, *Lygodium*, *Azolla*, *Salvinia*, *Marsilea*, *Actiniopteris*, *Anogramma*, *Pteris*, *Cheilanthes*, *Hypodematium*, *Tectaria*, *Lepisorus* and *Microsorum*. In the state, two species of gymnosperms were reported from the Kachchh, Saurashtra and North Gujarat regions.

Gujarat is divided into six bio-geographical zones *viz*., 1) Desert; 2) Semi-Arid; 3) the Western Ghats (Malabar Plains); 4) the Western Ghats (Western Mountains); 5) Deccan Peninsula and 6) Coasts. The most pteridophyte species-rich bio-geographical zone of the state is Semi-Arid region represents total 41 species under 20 genera from 13 families. Thereafter, the Western Ghats (Malabar Plains) shows 30 species with 15 genera under 11 families and the Western Ghats (Western Mountains) encompassing 26 species under 13 genera with 9 families. Subsequently, 10 species represented by 7 genera under 5 families occurs in Desert. While, the least species are occurring in the Deccan Peninsula and Coastal areas of the state which shows, two taxa representing two genera and families. Gujarat is divided into five regions *viz*., North, Central, South, Saurashtra and Kachchh. The most species-rich region of the state is central Gujarat, which encompassing 35 species belonging to 18 genera under 12 families. The least species-rich region is Kachchh, which shows distribution of 9 species representing 6 genera under 4 families.

Pteridophytes are a habitat-specific group of plants with unique niche preferences within their range of distribution. In the state, the distribution of pteridophytes is characterized under four major categories. The highest species diversity was reported in the terrestrial habitat with 40 taxa. Followed by, 22 taxa as lithophytic, 6 taxa as hydrophytes and 2 of the taxa were epiphytes.

For molecular identification, morphologically distinct and complex taxon of pteridophytes and gymnosperms were selected and processed by using five barcode loci *i.e.* rbcL (ribulose-bisphosphate carboxylase/oxygenase gene), trnE (trnF-trnR), trnLF (trnLF-F-R), psbA-trnH (psbA3_f- trnHf_05) from the plastid genome and ITS2 (Internal transcribed spacer) from nuclear genome. The four candidate barcode loci were evaluated and compared

individually for their amplification and sequencing. Total 28 DNA barcodes of 18 taxa were generated. The generated sequences were analyzed and compared by using BLAST (Basic Local Alignment Search Tool) analysis. Successful DNA barcodes of pteridophytes and gymnosperms were submitted to BOLD SystemV3 (Barcode of Life Database) and NCBI, to generate the true barcode.

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Signature of the Candidate

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