INTRODUCTION

CHAPTER - I

1.1 Research Background

The word pteridophyta is derived from Greek words "*pteron*" means "feather" and "*phytes*" means "plants" (*i.e*. feather like plants). They are 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 20^{th} century, vascular cryptogams are known by the new name 'ferns and fern-allies or Lycophytes and ferns'. They represent an intermediate position between bryophytes and seed-bearing vascular plants. They are evolved with the presence of independent branched or unbranched sporophyte, lignified vascular tissue, spore bearing structures and have successfully adapted on the land during mid-Palaeozoic era in Late Silurian and Devonian periods *i.e. ca*. 438 million years ago (Chaloner & Sheerin 1979; Kenrick & Crane 1997). Palaeobotanical and Stratigraphic data decorated the initial diversification of the vascular cryptogam of simple fossil from Rhyniophytes such as *Cooksonia* and *Rhynia* (Banks 1975; Chaloner & Sheerin 1979). Furthermore, palaeobotanical data supports hypotheses of two main linage of evolution of clubmosses and other vascular plants which includes horsetails, ferns and seed plants (Banks 1975; Chaloner & Sheerin 1979). The ancestors of living pteridophytes along with their extinct seedless vascular relatives flourished well and grew to greatest heights during the early Carboniferous and Devonian period of Palaeozoic era and forming the first forests which is also known as age of pteridophytes.

In 1737, Linnaeus coined the term Cryptogams (secret marriage or hidden reproduction) which means plants without flower or seeds. The term pteridophyta was first introduced by a German philosopher Ernst Haeckel (1866). Subsequently, Englar (1886) treated the group pteridophyta as a division of subkingdom of Embryophyta. Thereafter, this group of plants was recognized for the presence of vascular system that contains tracheids or tracheary elements and termed as tracheophyta (Sinnott 1935). Soon after, Takhtajan (1953) gave the name *Telomobionta* and Cronquist *et al*. (1966) recognized them as *Embryobionta*. In recent morphological and molecular phylogenetic studies, division tracheophyta is divided into two separate clades *viz*., Lycophytes and Euphyllophytes (Raubeson & Jansen 1992; Kenrick & Crane 1997; Pryer *et al*. 2001, 2004a). Further, Euphyllophytes divided into monilophytes and spermatophytes. The extant monilophytes (or moniliformopses) commonly known as 'ferns' in the broad sense, which comprises four major class *viz*., Equisetopsida (horsetails), Psilotopsida (whisk ferns including Ophioglossoid ferns), Marattiopsida (marattiod ferns) and Polypodiopsida or Filiopsida or Leptosporangiatae (leptosporangiate ferns), and spermatophytes include seed plants (Scotland & Wortley 2003; Pryer *et al*. 2004b).

Pteridophytes grow luxuriously in different habitat during rainy season due to the presence of high humidity, moisture content in the atmosphere and enough shade due to less penetration of sunlight. They grow vigorously in mid-high altitudinal places particularly in the forest area. They are flourishing in shady and moist area as lithophytes and epiphytes particularly on stony walls, clefts and clothed tree trunk associated with leafy mosses. They are wide spread in distribution; plenty in tropical forests and very few in arctic regions. Pteridophyte stands at second position in terms of species richness while, their diversity and distribution ranks next to the angiosperms in 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).

The term gymnosperm is attaining from a Greek "*gymnospermos*" means "naked seeds" (*i.e*. seeds not encircled with cotyledons). Gymnosperm is the group of tracheophyta where, ovules are is not encircled in carpel, unlike angiosperms (Singh & Srivastava 2013). The ovules instead forms a leaf-like constitution (homologous to a leaf), or on a scale or megasporophylls (homologous to the shoots) or on apical part of a (dwarf) shoot. They have slow rate of development and long duration between pollination and seed ripeness. Cycads and gnetophytes are pollinated with the help of insects, whereas wind pollination is occurring in all conifers and *Ginkgo* (Singh & Shrivastava 2013). Gymnosperms form a significant group of plants due to its enormous ecological, socio-economic and evolutionary value. This group of plants are cosmopolitan in distribution the world, extensively diversified and distributed in the tropics and they forms most dominant plant group in the temperate forest area of northern and southern hemisphere (Fragniere *et al*. 2015). In contrast to other group of plant, living gymnosperms are very fewer in number; they comprise *ca*. 1,106 species under four major lineages *viz*., Ginkgo, Gnetophytes, Cycads and Conifers (Calonje *et al*. 2020; WCSP 2020).

Gymnosperm occurs in a different habitat throughout the world with a unique character of particular region referred as biogeographic zones. The term biogeographic regions were formerly coined by the English Ornithologist, Philip L. Sclater (1829-1913) and German botanists H. G. Adolf Engler (1844-1930), are the huge areas with specific flora and fauna, for their segregation through continental drift. Initially, six biogeographic regions were recognized *viz*., Australian (Australia and New Guinea), Ethiopian (Africa), Indian or Oriental (Southeast Asia, Indonesia), Nearctic (North America), Neotropical (Central, South America and Mexico), Palearctic (Asia and Europe) (Kreft & Jetz 2010). Currently, eight biogeographic zones are recognized due to the accumulation of Oceania (Micronesia, Fiji and Polynesia) and Antarctica (Seton *et al*. 2012).

Biogeographically, India is enormously diversified country in terms of flora and fauna. The Indian subcontinent encompasses the most area by Indian plate which is the part of Indo-Australian plate (Crawford 1970). India is the 7^{th} largest country in the world by its area. Asia is second largest country with total land mass area is 3,287,263 km^2. The mainland extends between 8°4'-37°6'N latitudes and 68°7'-97°25'E longitudes (Anonymous 2021a). The tropic of Cancer passes through the Indian Ocean between the Arabian Sea on the west side and Bay of Bengal on east side and the country shares land border with Pakistan to the west; in north-east side with Bhutan, Nepal and China, and to the east side with Bangladesh and Burma. It has a land border of about 15,200 km. The peninsular India is bounded by Arabian Sea in the southwest side and southeast by Bay of Bengal. India's Andaman and Nicobar Islands is a segment of maritime border with Indonesia and Thailand. Furthermore, in the Indian Ocean, India is in the vicinity of Sri Lanka and the Maldives. Coastline of the mainland of country including Andaman and Nicobar and Lakshadweep Islands is 7,517 km. Country has varied climate and terrain characterized by diverse landscape. It is extended from north with snow covered mountains of Himalayan heights to the tropical rain forests in the south including hot dry deserts, hills, plateaus and plains in the west, central and east sides. These huge biogeographically land areas and seas give the country a distinct geographical entity (Anonymous 2021a).

India is one of the 12 mega-biodiversity centers of the world and having 4 hotspots *viz*., the Himalaya, the Indo-Burma (includes entire north-east India, except Assam and Andaman group of island), Sundaland (Nicobar group of island) and the Western Ghats, amongst 35 hotspots identified in the world (Anonymous 2021a). These hotspots possess a unique climatic condition, which makes India as one of the biodiversity rich countries of the world due to a variety of flora and fauna. In its 2.4% of global area, India consists 8% of the world's total biodiversity which include 47,000 species of plants and fungi and 89,000 species of animal (Khoshoo 1995, 1996; Singh & Kushwaha 2008; MoEF 2009). It includes three major ecological zones *i.e.* temperate, tropical, sub-tropical zones comprising various ranges of habitat from tropical rain forest to alpine floristic vegetation and from temperate to coastal wetlands. According to Champion & Seth (2005), India has six major types of forests *viz*., Moist Tropical Forests, Dry Tropical Forests, Alpine Scrub, Montane Temperate Forests, Montane Subtropical Forests and Sub-alpine Forests. In broad term, ecological zones are divided into nine sub zones *viz*., Western Himalaya, Eastern Himalaya, Northeastern Hills, Indo-Ganga Plains, Desert Zone, Central India, Deccan Plateau or South India, West Coast or Malabar Zone and Bay Island (Negi 2002). These ecological zones are house of many rare, threatened and endemic flora and fauna of the country. It covers nearly 981 protected areas in 1,71,921 km^2 areas with 5.03% coverage of land and coastal lines of the country, which includes 104 national parks, 566 wildlife sanctuaries, 97 conservation reserves and 214 community reserves (Anonymous 2021b).

In India, pteridophytes are diversified and flourished in all the biogeological 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). Endemic taxa are mainly concentrated in the diversity rich areas of the peninsular India, the Western Ghats, north-east India, the Islands of Andaman and Nicobar and western Himalayas. In India, extant gymnosperms diversity is estimated about 149 species and 8 varieties (indigenous/introduced) belonging to 46 under belonging to 12 families (Singh & Srivastava 2013; Sharma & Singh 2015; Akhtar *et al*. 2019).

Gujarat is the western most state of the country, lies between 20°07'-24°43' N latitudes and 68°10'-74°29' E longitudes covering area of 1,96,244 km^2, which is 5.97% of the total geographic area of the country. It covers total 21,647 km^2 of forest area, of which 14,373 km^2 is reserved forest, 2,886 km^2 protected forest and 4,388 km^2 areas falls unclassified forests. State is fenced by the Arabian Sea in the west and south-west having longest coastline *ca*. 1,650 km, amongst the Indian states (ISFR 2019). The most conspicuous feature 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 the marine life and coastal wetlands. It shares international border with Pakistan in the north side, which is also known as 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 of the country, the saline deserts of Kachchh (Rann), grassland and wetlands. State has four National Parks, 23 Wildlife Sanctuaries and one Conservation Reserve *i.e*. Rann of Kachchh Biosphere Reserve (RKBR). It comprises the network of Protected Area of the state which is covering 8.83% of the total geographical area of Gujarat (ISFR 2019).

***1.2 Molecular Identification and DNA Barcoding*:**

Earlier taxonomists followed traditional method of identification *i.e*. morphological characters due to which cryptic species created 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 in the field of taxonomic research. This is leading to the development of 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). It is one of the useful tools for species identification, its phylogeny and understanding the systematic relationships. It involves sequencing of short universal gene sequence of a particular standardized region of the genome and comparing them within and between the species to represent a 'barcode' for species identification (Hebert *et al*. 2003). This technique demonstrated a promising tool for species discernment in animals by using the mitochondrial gene *i.e.* cytochrome c oxidase I (COI) (Cowan *et al.* 2006). However, in plants, mitochondrial genes are not used much because of difficulties to analyse nuclear genome due to the presence of the most variable loci, problems with multi-gene families and single-copy genes that create technical difficulties in the process of amplification (Ebihara *et al*. 2005). Therefore, Chase *et al*. (2007) suggested that chloroplast genome as a potential aspirant for barcoding genes with characteristic features *viz*., high copy number, conserved gene structures, diversity of substitution rates across genes, introns, and intergenic spacers and easy amplification by polymerase chain reaction (PCR). Furthermore, multi-locus approach is useful for the species identification. The multi-loci of chloroplast genome has been evident to be used as an effective for barcode of various land plants (Levin *et al*. 2003; Kress & Erickson 2007; Fazekas *et al*. 2008). Hence, standard barcodes for plants have been assessed intended for DNA barcoding (Chase *et al*. 2007; Kress &

Erickson 2007; Lahaye *et al*. 2008; CBOL 2009). A variety of loci of nuclear and plastid genomes have been suggested as DNA barcodes for plants, including coding and non-coding regions. In plants, Consortium for the Barcode of Life (CBOL) also suggested plastid genes such as maturase K (matK), trnH-psbA and ribulose bisphosphate carboxylase (rbcL). Currently, these genes have been widely used to produce DNA barcodes due to the easy recovery of rbcL gene and the high discrimination power of matK. The loci that are most prevalent in plant systematics are rbcL, trnL-F, matK and atpB (CBOL 2009). The ultimate use of DNA barcoding as an identification tool is result in creation of DNA barcode sequence libraries that could be used as a standard and high-quality reference database for species identification (Ratnasingham & Hebert 2007; Hollingsworth *et al*. 2011). Genetic sequences assimilated in the framework of DNA barcoding have also been used for preparation phylogenetic analysis and are also used for the verification of medicinally important plants products and wood identification (Rachmayanti *et al*. 2009; Kress *et al*. 2009, 2010; Asahina *et al*. 2010; Chen *et al*. 2010).

As compared to other groups of plant, angiosperms have received great attention for its 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 groups of plant (particularly angiosperms) studies on this 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*. 2016a). Few species are known from 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* L. 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, to understand the diversity and distribution of pteridophytes and gymnosperms occurring in the Gujarat state is undertaken.

OBJECTIVES

The present study was intended to explore and document the pteridophyte and gymnosperm diversity in Gujarat with 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.