1. INTRODUCTION

1.1. Polygonaceae A. L. Jussieu

Polygonaceae is cosmopolite to temperate region (Tackholm 1974) *i.e*., geographically dispersed from the tropical region to the arctic, from montane to lowland and from arid to aquatic condition although majority of the taxa are confined to the northern temperate region. The name of the family "Polygonaceae" is based on its type genus *Polygonum*. It is a large family comprising around 48 genera and 1200 species (Freeman and Reveal 2005; Sanchez and Kron 2008).

Polygonaceae is morphologically diverse and habit ranges from trees, shrubs, vines, lianas to herb. The presence of membranous or hyaline sheath uniting the stipules is the characteristic feature of the family. The sheath more precisely the ochreae are present in majority of the genera except in the largest genus, *Eriogonum* Michaux., *Antigonon* Endl. and *Fallopia* Adans. In various genera the stem is typically swollen at the nodes. Assimilating stems with rudimentary leaves are observed in *Calligonum* and *Muehlenbeckia* but in *Muehlenbeckia platyclada* striate flattened cladodes are present. Phyllotaxy is typically alternate but in some taxa they can be opposite (e.g. *Pterostegia* Fischer & C. A. Meyer., some species of *Polygonum* L. and *Eriogonum* Michaux.). The flowers are habitually actinomorphic with two to six uniform tepals, often in two whorls of three, persistent in fruit, ovule is predominantly orthotropus, fruit is trigonous nut and seeds have excentric copious endosperm (Cronquist 1981).

1.2. Distribution of Polygonaceae

The utmost diversity of Polygonaceae is seen in the northern temperate region which is the most favoured habitat of the family. But some taxa are also dominating the vegetation at sub alpine region. Nevertheless, it is universally found in temperate region of America and Asia. Polygonaceae are broadly distributed from China to Japan in Northern Asia while in south, it occurs in Afganistan, Bhutan, Nepal, Pakistan and India. The family distributed in most part of the Kazakhstan, Korea, Kyrgyzstan, Mongolia, Myanmar, New guinea, Pakistan, Philippines, Tajikistan, Thailand, Turkmenistan, Uzbekistan, Vietnam, North Africa, Australia, Europe, North America. In India Polygonaceae has extensive range of distribution stretching from the Eastern Himalayan region including Kashmir, the subtropical and temperate to coastal region. Majority of the species are confined to various altitudes of Himalaya and North East India (Assam, Meghalaya, Nagaland and Manipur). The data about the number of the species and their distribution in the Eastern and Western Himalaya are gradually increased since the time of Hooker (1886). Srivastava (2014) reported 121 species and 29 varieties belonging to 12 genera of Polygonaceae from India. The checklist has been made based on the available herbarium specimen of different herbaria of India. Darjeeling-Kalimpong Himalaya and Terai-Duars region of West Bengal forms a fundamental part of the Eastern Himalayan region. Polygonaceae widely distributed from the Darjeeling-Kalimpong Himalaya to the Terai- Duars and also in the Gangetic Delta of West Bengal. Unfortunately through literature survey, no complete floristic database of Polygonaceae was obtained from this region. As the study on Polygonaceae has not yet done from this tremendously rich and diverse vegetation, so there are still some confusions about the phylogeny and affinity of some taxa which are widely distributed in this region and their uncertain positioning and inter relationship are still remains unsolved. Hence, the present study will aiming to clarify the taxonomic repositioning and will enrich the floristic and systematic database of family for this entire region.

1.3. Taxonomic delimitation of Polygonaceae

The family Polygonaceae is a very interesting family, especially regarding their most diverse morphological variations (Ronse Decraene and Akeroyd 1988; Brandbyge 1993). To describe the species and their systematic positions, various authors were frequently segregating this family into subfamilies or tribes in different times based on some selected morphological characters. Jussieu (1789) accepted the distinctiveness of the family as a natural group. Arnott (1832) was published subfamily Eriogonoideae and then subfamily Polygonoideae (Eaton 1836) while third subfamily Coccoloboideae was suggested by Luerssen (1882).

Bentham and Hooker (1880) were directly classifying the family into three tribes: Coccolobeae, Eupolygoneae and Rumiceae based on their morphological characteristic features. Dammer (1893) on the basis of morphological evidences divided it into three subfamilies like Polygonoideae, Rumicoideae and Coccoloboideae, where he included Eriogonoideae as a tribe under the subfamily Polygonoideae. Gross (1913) proposed Eriogonoideae, Coccoloboideae and Polygonoideae as subfamilies and considered Rumicoideae as a tribe under Polygonoideae.

Jaretzky (1925) recognized two subfamilies in the family Polygonaceae based on their foliar and floral morphological evidences *i.e.*, Eriogonoideae and Polygonoideae and proposed Coccolobeae as a tribe in Polygonoideae. Buchinger (1957) suggested three subfamilies: Polygonoideae, Rumicoideae and Eriogonoideae of Polygonaceae. Polygoneae and Coccolobeae were the tribes under Polygonoideae while Rumiceae placed under Rumicoideae.

On the basis of the morphological evidences Roberty and Vautier (1964) postulated that Polygonaceae can be divided into three subfamilies such as Calligonoideae, Eriogonoideae and Polygonoideae and the sub-family Polygonoideae was also segregated into four tribes *i.e.*, Coccolobeae, Polygoneae, Rumiceae and Rheae.

Haraldson (1978) based on anatomy of vegetative parts first time attempted to divide the family Polygonaceae into two subfamilies Eriogonoideae and Polygonoideae. She further divided Eriogonoideae into two tribes: Eriogoneae and Pterostegieae while Polygonoideae into five tribes Polygoneae, Persicarieae, Rumiceae, Coccolobeae and Triplareae.

The most recent and generally accepted consensus is to recognize two subfamilies, Eriogonoideae Arnott and Polygonoideae Eaton (Brandbyge 1993; Sanchez and Kron 2008) with different circumscription.

1.4. Taxonomic convolution of the Genus *Persicaria* and *Polygonum*

Polygonum, had been presented a great taxonomic challenge over the years. Various classification systems had been proposed to resolve the problem and sometimes same names had been suggested to moderately different groups and at different taxonomic hierarchial ranks (Haraldson 1978). Therefore nearly every botanist was contented to evade the underlying issues by suggesting *Polygonum sensu lato*.

In the first edition of 'Species Plantarum' (1753) Linnaeus described the genus *Polygonum* where he included seven different genera as its species viz., *Aconogonon* (Meisn.) Reichb., *Bistorta* (L.) Scop., *Fagopyrum* Mill., *Fallopia* Adans., *Persicaria* Mill., *Polygonum s.s*., and *Reynoutria* Haraldson. The first attempt to divide the genus *Polygonum* was done by Meisner (1826) who segregated the genus into eight sections such as *Amblyogonon*, *Aconogonon*, *Avicularia*, *Cephalophilon*, *Fagopyrum*, *Persicaria*, *Bistorta*, and *Tiniaria*. Later in 1857 he added the section *Echinocaulon* and *Tephis* in his studies and distinguished *Echinocaulon* and *Cephalophilon* from *Persicaria* due to capitate inflorescence. Meisner's delimitation into section became the preliminary point for many various workers who wish to subdivide the complex genus *Polygonum*. Moreover, Bentham and Hooker (1880) predominantly followed Meisner's sectional division with addition of some new sections (*Pleuropterus*, *Pseudopolygonella* and *Pseudomollia*).

Gross (1913) also tried to resolve the confusion among *Polygonum* and *Persicaria* based on the gross morphology and palynology. He retained few of the Meisner's sections and also removed 7 genera from *Polygonum*. He distinguished the genus *Persicaria* Mill. from *Polygonum* L. and suggested *Aconogonon, Amblygonon, Cephalophilon, Echinocaulon* and *Tovara* as sections of *Persicaria* and separated the genus *Fagopyrum* Mill. into two sections (*Tiniaria* and *Eufagopyrum*).

Subsequently, Jaretzky (1925) on the basis of presence or absence of anthraquinones proposed five genera viz., *Avicularia*, *Polygonum*, *Polygonella*, *Persicaria* and *Fagopyrum* which had previously been considered as sections of *Polygonum*. He also supported Bentham and Hooker's (1880) classification *i.e.*, *Pleuropterus* and *Tiniaria* as the sections of *Polygonum* and *Typhis* as the sections of *Avicularia*. On the basis of pollen morphology Hedgeberg (1946) suggested that *Persicaria* is a separate genus. He established eight genera from *Polygonum s.l.* (*Koenigia*, *Fagopyrum*, *Bistorta*, *Persicaria*, *Polygonum s.s.*, *Pleuropteropyrum* and *Tiniaria*).

Clapham et al. (1962) accepted in the genus *viz*., *Polygonum*. They proposed this based on a combination of plant habit, leaves shape, perianth segments and stamen number. Roberty and Vautier (1964) also suggested *Persicaria* as a section and divided the genus based on their structural variation in stigmas and propagules into nine sections *viz*., *Typhis*, *Avicularia*, *Persicaria*, *Polygonum*, *Bistorta*, *Aconogonon*, *Cephalophilon*, *Echinocaulon* and *Eleutherosperma* and three genera: *Bilderdykia*, *Reynoutria* and *Antenoron*. Webb and Chater (1964) further abridged the number of sections and treated the genus containing four sections (*Aconogonon*, *Bistorta*, *Polygonum*, and *Persicaria*). Graham and Wood (1965) also recommended *Persicaria* as the sections of *Polygonum* along with *Tiniaria*, *Cephalophilon* and *Echinocaulon* whereas *Fagopyrum* and *Polygonella* were considered to be separate genera.

Hara (1966) based on the morphological characteristics proposed that *Persicaria* along with *Aconogonon*, *Antenoron*, *Bistorta*, *Bilderdykia*, *Fagopyrum*, *Koenigia* were not the section of *Polygonum s.l.* but also treated as the separate genera. Haraldson (1978) after thoroughly reviewed the existing data and with the additional support from anatomy of the vegetative character suggested that *Polygonum s.l.* should be separated into two tribes (Polygoneae and Persicarieae) and she placed *Polygonum*, *Fagopyrum*, *Polygonella*, *Fallopia*, *Reynoutria* in the tribe Polygoneae while *Bistorta*, *Aconogonon* and *Persicaria* in Tribe Persicarieae. She also retained sectional division of *Polygonum* (*Duravia*, *Polygonum* and *Tephis*). Ronse Decraene and Akeroyd (1988) also supported the division of *Polygonum* L. *s.l.* into two tribes as anticipated by Haraldson (1978). On the basis of the morphology of floral characters of *Polygonum* and related genera of Polygonaceae they proposed that the tribe Polygoneae should contain *Polygonum* L. *s.s.*, *Fallopia* Adans., *Oxygonum* Burch., *Pteropyrum* Jaub. and Spach., *Atraphaxis* L. *and Calligonum* L. while *Fagopyrum* Mill., *Harpagocarpus* Hutch. and Dandy, *Persicaria* Mill. and *Koenigia* L. were included in the tribe Persicarieae. Li *et al*. (2003) investigated the genus *Polygonum* from China and documented two tribes for the taxa of *Polygonum viz*., Polygoneae and Persicarieae. While studying Polygonaceae for the flora of North America, Freeman and Reveal (2005) suggested *Persicaria* Mill as separate genera and *Polygonum* L. segregated into two sections (*Polygonum* and *Duravia*).

1.5. Taxonomic tool for resolving the systematic position of Polygonaceae

Flora is the most priceless gift of nature which provides all kinds of fundamental requirements for our continued existence, including food, medicine, fuel, fodder, timber, resins, oils etc. Natural assets investigation like floristic study plays imperative role in the economic up gradation of developing country (Ganorkar and

Kshirsagar 2013). Beside this, floristic survey of a particular province is also generating the whole information on different family, genus and species, dominant taxa and major life-forms occupying a particular habitat (Sasidharan 2002). Therefore, floristic study is one of the most important provisions from the taxonomic point of view. In recent years there has been a reinforcement of interest in floristic studies from different regions in India, as it is one of the seventeen mega diversity countries of the world. Till now, large number new taxa have been reported from various parts of the country (Eshuo and Chaturvedi 2011; Chowdhury *et al*. 2013; Paul and Chowdhury 2016; Mondal and Chowdhury 2019). This is due to insufficient previous documentation, progress in processes of evolution and changes of floristic elements of vegetation with time. Exotic species of a flora have some impact that in most cases it becomes offensive for the local taxa. However, several exotics are progressively naturalizing (Chowdhury *et al*. 2013). Conversely many inhabitant species are slowly but surely becoming endangered and even getting extinct from the different floristically rich regions. As the result number and the identity of taxa included in earlier floras are now changing hurriedly so, it is necessary to explore the flora in regular interval. Other than that numerous floristically important regions are not yet explored rather under explored. Therefore, floristic study is one of the most important provisions from the taxonomic point of view. West Bengal is the major part of IUCN recognized 'Himalaya Biodiversity Hotspot' (Conservation International 2005). However, Darjeeling - Kalimpong Himalaya, Terai and Duars are forming a proximate and comprehensive part of the prosperous vegetation of the Eastern Himalaya. Extensive survey through available literature covering the flora of East Himalayan region shows that Polygonaceae is one of the dominant families in this region (Hooker 1886; Prain 1906; Maiti and Sikdar 1985; Chowdhury 2013). Regrettably, there is no comprehensive floristic work accessible on these important plants occurring West Bengal. Thus, in the present study an endeavour has been made to investigate the distribution of Polygonaceae in this region.

From the primitive time recognition and reconstruction of relationships between plants have been based mainly on characteristic features of their reproductive organs. The floral characters have recognized very helpful for identification (Wilkinson 1979), but sometimes these organs are not easily accessible for study.

Therefore, fossils record viz., leaf impression and compression are the most recurrent macroscopic residues of extinct plants, but they are generally not attached to other plant organs when those are excavated. Because of their profusion and impenetrable strata graphic occurrence, fossil leaves can provide massive information about the composition and diversity of primordial floras. Leaves are very important as those are extremely polymorphic organs and provide sets of diverse features. The term “leaf architecture” was coined by Hickey (1973) to denote the placement and outline of those elements constituting the outward expression and lamina structure, as well as leaf shape, size, blade class, venation pattern, marginal configuration and gland position etc. Venation is the vasculature formed by veins and veinlets. Hence, venation patterns are significant characteristics attribute used to resolve many controversies in plant systematics (Larano and Buot 2010). So, leaf architecture like venation pattern were studied in different families viz., Asteraceae (Banerjee and Deshpande 1973), Berberidaceae (Singh *et al.* 1978), Betulaceae (Frank 1979), Euphorbiaceae (Sehgal and Paliwal 1974), Bignoniaceae (Jain 1978), Labiatae (Tyagi and Kumar 1978), Rosaceae (Merrill 1978), Solanaceae (Inamdar and Murthy 1978) and Scrophulariaceae (Varghese 1969), Hydrocharitaceae, Taccaceae, Dioscoreaceae, Smilacaceae, Araceae, Alismataceae, Aponogetonaceae (Inamdar *et al.* 1983), Lauraceae (Chowdhury 2015).

Alongside venation, stomata and epidermal cells also provide taxonomically imperative diagnostic features, such as the occurrence of stomata on the adaxial or abaxial surface of lamina and arrangement of epidermal cells adjoining to the guard cells (Tripathi and Mondal 2012). Usually, the small openings in the epidermal layers which involve with gaseous exchange among intercellular spaces and the atmosphere are known as stomata. These are surrounded by guard cells, which organize the pore size. The morphology of the stoma was first studied by Stresburger (1866) followed by various workers such as Vesque (1989) who reported four basic types of stomata based on the presence and arrangement of subsidiary cells along with their mode of development namely Rannanculaceous, Cruciferous, Rubiaceous and Caryophyllaceous. Metcalfe and Chalk in 1950 described main four types of stomata, i.e. Anisocytic, Anomocytic, Diacytic and Paracytic whereas Stace (1980) observed 31 different types of stomata among dicotyledonous plants.

One more fundamental character of foliar micro morphology is indumentums and it plays a noteworthy role in plant systematics, especially of particular groups at generic and specific levels (Hardin 1979). The presence or absence of peltate hairs and their shape, size could be used as distinguishing features of different genera and species (Cooper 1931; Spring 2000). Undoubtedly in recent studies advancement of approaches like bioinformatics, biotechnology, biochemistry, biomolecular etc. are necessary, but the conventional morphological disciplines should not be neglected. Now a day, leaf architecture is also a neglected feature. Conversely, importance of leaf architectural data like other countless parameters remains unquestionable. Therefore in the present study comprehensive account of leaf architectural data is undertaken as one of the parameter for resolving the confusion about the systematic position of Polygonaceae.

The floral character plays an important role in angiosperm taxonomy. The vegetative parts of the plant may differ in different climate conditions, geographical location and various biotic and abiotic stresses. In these cases, the floral parts or the floral formula can play an incredibly significant role in the authentic identification at the species level. The great Swedish botanist Linnaeus was first introduced the term pollen (Knox 1984). Pollen refers to the highly reduced male gametophyte enclosed within the microspore wall of seed plants or flowering plants. It is the most vital unit of the angiosperm flower, both with regard to form and function, and represents the sole medium through which the entire male genetic attributes are transmitted to the next generation; and thereby, it enters the perpetuation of species (Erdtman 1952; Nair 1970). The term Palynology was coined by Hyde and Williams (1944) who refer to the study of external morphological features of mature pollen grains. The term palynology has been derived from the word palymein, meaning ‘to scatter’ as pollens are often spread by wind, and logos meaning study. Comparative studies of pollen morphology have provided constructive attributes for resolving the inter-relationships and delimiting genera and species. Palynology is diverse and widely accepted to reflect phylogenetic relationships (Lee *et al*. 2011). Unique morphological features of pollen grains were moderately conserved and recurrently used in solving taxonomic inconvenience at family, genus, species and varietal rank since long and have become part of the multidisciplinary and mutual approach in plant systematic and evolution (Carlo *et al*. 2004; Giveyrel *et al*. 2000; Persson

1996). Constant exine ornamentation and aperture features of pollen grains are appreciably recognizable for parent genera or species (Harris 1955; Moore *et al.* 1978). Different researchers from different parts of the world have worked on the pollen morphology of Polygonaceae but very little work was done on this family from this part of the world. Present study reports detailed morphology of pollen grains of the Polygonaceae from West Bengal using LM (Light microscopy) and SEM (Scanning Electron Microscopy).

In recent years, the understanding of phylogenetic relationship among angiosperms has significantly increased with the coalescence of molecular data accumulated through different conventional techniques. Large-scale barcoding projects through molecular data coupled with the rapid expansion of bioinformatics tools, recommended a unique circumstance for species delimitations (Monaghan *et al*. 2009; Fujita *et al*. 2012). The chloroplast genome of terrestrial plants typically harbours a conserved set of approximately 120 genes in a 120–160 kb pair genome (Kaneko *et al*. 1996). Although chloroplast genomes contain highly conserved indispensable genes for the growth and development of plant, they also contain variable regions, *i.e*., intergenic regions and structural variations. In addition, they restrain one of the few sets of characters that can exceed the life history of green plants and hence produce important evolutionary information. Therefore, chloroplast genome sequences can be used for comparative evolutionary studies within and between different groups of plants (Timmis *et al*. 2004; Greiner and Bock 2013). Among molecular markers, the *rbcL* gene has been extensively used for systematics at higher levels (Olmstead *et al.* 1992; Chase *et al*. 1993; Qiu *et al*. 1993; Nickrent *et al.* 2002).

Taking this into consideration the present study deals with the sequencing of *rbcL*, *matK* and *trnL-F* region of chloroplast genome of some members of Polygonaceae that were not sequenced earlier.

1.6. Research Objectives

The objectives of the present research work:

- Floristic survey to understand the present status of different taxa of Polygonaceae from West Bengal along with their habitat types and recognition of economically important taxa.
- Construction of distribution map of those taxa of Polygonaceae
- To Record the exact flowering and fruiting periods for different recorded taxa of Polygonaceae in study area.
- To study and analyze the macro and foliar micro-morphological characters of collected taxa.
- To study and analyze the ultra-morphology of pollen grains of recorded taxa.
- To assess inter and intraspecific relationship in selected taxa of Polygonaceae through barcode primers (*matK, trnL-F, rbcL*) of chloroplast genome.
- Analysis and draw a significant phylogeny among the taxa using both Phenetic and molecular taxonomic attributes and easy identification key were made and it helps to reduce the confusion at least at species level of Polygonaceae.