SUMMARY

The present work embodied in this thesis deals with the morphology-anatomy, *in-vitro* seed germination, some aspects of reproductive biology, ecology and Ecological Niche Modeling of three aquatic plants – *Polypleurum stylosum* var *laciniata*, *Zeylanidium lichenoides* and *Willisia selaginoides* of the family Podostemaceae.

The plants are collected from different locations of Kerala, India. *P. stylosum* var *laciniata* was collected from the river Kunthipuzha, Silent Valley; Pooyamkutty, *Z. lichenoides* was collected from Silent Valley, Pooyamkutty and Cheeyappara and *W. selaginoides* was collected from Companywada, Pooyamkutty. *P. stylosum* var *laciniata* and *Z. lichenoides* grow in a few sites however *W. selaginoides* is represented by only one population.

Water quality of rivers is determined by a diverse range of catchment variables and by local environmental conditions near sampling point. A number of human activities like domestic and industrial effluents, use of water for washing and bathing, drainage and other management activities alter the biological and chemical status of the habitat of the aquatic vegetation. Chemical attributes of water can affect the quality such as how water looks smells and tastes.

Washing of clothes, bathing and washing of vehicles are observed at the collection sites of Pooyamkutty and Cheeyappara fall in the present work. Many tourists are also encountered in the collection sites like Silent Valley and Cheeyappara fall which may disturb the habitat of the podostemads. Sewage and drainage pollution is a threat to the water spots due to increase in number of tourists.

The present work shows no significant variations in the dissolved oxygen between the Pooyamkutty river and Cheeyappara fall. The high value of oxygen may be due to the abundance of vegetation post monsoon. The effect of waste discharge in a water body is largely determined by the oxygen balance of the system. *Willisia selaginoides* is restricted to Pooyamkutty dam which has non-polluted habitat. The free carbondioxide value of the present study in Pooyamkutty river was 0.97mg/l and the Cheeyappara waterfall was 1.8mg/l which is low or moderate that indicates lower

pollution of the water. *Zeylanidium lichenoides* was found in Cheeyappara fall which is suitable for the plant to flourish. It was found that the value of alkalinity was found to be below 15mg/l which indicate a nutrient poor habitat. This shows that podostemads require low nutrient for their growth. Chloride is generally undertaken as major factor to aquatic cation-anion balance of the ecosystem. The value of chloride in Pooyamkutty was found to be 10mg/l and 9.8 mg/l in Cheeyappara that were well within the limit. Both the stations have hard water which indicates that the studied plants can flourish well in it. These values remain normal during rainy season and after the rains but increase substantially during February to May. Cheeyappara falls recorded a lower temperature than Pooyamkutty which may be due to its high altitude. These findings show that the podostemads grow in well oxygenated, non-pollutant and nutrient poor habitats. Some algae belonging to families of Cyanophyceae, Chlorophyceae and Bacillariophyceae were found growing with the podostemads studied here. Some water animals/insects were also found with these plants which may help in their pollination.

Podostemaceae grow attached to the surface of rocks. They also fix themselves to wooden pieces and fallen branches which are entrapped and wedged in the river. The plants prefer sunshine and are conspicuously absent from the perennially shaded areas of the river bank.

P. stylosum var *laciniata*, *Z. lichenoides* and *W. selaginoides* are thalloid angiosperms with no true leaf, stem or root. The thallus is long and broad in *Polypleurum* and attached to the rock with the rhizoids on its ventral surface while the tip remain swaging in the water current. In *Zeylanidium,* the thallus is tightly attached to the rock and the thallus has many lobes. A small crust like thallus is found in *Willisia* that bears a tuft of flowering shoots on the dorsal surface. The thallus is made up of parenchymatous ground tissue surrounded by an epidermal covering. The clear differentiation of xylem and phloem, cambium and aerenchyma (commonly found in hydrophytes) are altogether absent. Although, poorly developed vasculature can be seen. The ground tissue stores abundant starch. Intercellular spaces are lacking. In the plants occurring in the natural habitat, one or two groups of small parenchyma cells which show some annular thickenings on their walls have been observed. These

parenchyma strands may function as conductive tissue. The gummy substance exudes from the lower surface of the thallus that helps in attachment to the substratum.

Leaf like structures originate in groups of 3-4 along the margins of the thallus in *Polypleurum* and *Zeylanidium* which are called secondary shoots. However, in *Willisia* the secondary shoots are nearly 5-7cm long and leaves are arranged in four rows in an opposite-decussate manner and closely appressed to the stem/shoot. They lack petiole, mesophyll tissue, aerenchyma, venation and stomata.

The seeds are tiny and embryo lacks a distinct plumule and radicle. During the process of *in-vitro* seed germination, the seed coat swells on soaking and secretes a copious amount of a gummy substance which helps the seed in securing a firm adherence to the hard and stable substratum such as rocks and boulders. Seeds are germinated using the technique developed by Vidyashankari and Mohan Ram (1987). Various dilutions of M.S. medium supplemented with 2% sucrose are tested. The medium with 1/20 of the original strength was found to be most suitable for the seed germination and growth of seedlings in *Polypleurum* and *Zeylanidium*. The medium with 1/5 of the original strength was found to be most suitable for *Willisia*. The pH of the medium was adjusted between 7-7.5 before autoclaving. Seed cultures were maintained at 26±2˚C with illumination of 1500 lux for 8-10hrs a day length. In nature, seeds start germinating with the arrival of the first monsoon showers in May.

In the in-vitro culture, highest percentage of germination was found in *Polypleurum* followed by *Zeylanidium* and *Willisia* in the suitable medium. During germination the radicular pole emerges from the seed coat, followed by the elongation of the cotyledons. Numerous rhizoids arise from the peripheral cells of the radicular pole. Numerous rhizoids are found developing from the cotyledons in *Polypleurum*. Growth at the apical end of the seedling can be seen when a leaf primodium is visible between the two cotyledons in the 14 days old seedling in *Polypleurum* and 15 days in *Willisia*. In case of *Zeylanidium*, a leaf primodium can be seen in 17 days old seedling. Unlike in the majority of angiosperms and gymnosperms, the apical meristems of the studied plants do not contribute to the formation of the plant body.

In *Polypleurum*, the cells of the primary axis below the cotyledons i.e the hypocotyl region become meristematic and initiate the development of thallus in the 22days old

seedling. In *Zeylanidium,* the mid of the hypocotyl swells up because of the meristematic activity towards horizontal direction and form a lateral bulge in the 24 days old plant. In *Willisia,* the radicular base of the hypocotyl expands horizontally and gives rise to a thalloid plant body after about 28 days of seed culture. The thermocoles supporting the young plants are transferred to jars containing 0.8% agar over which MS medium are pour. It has been possible to maintain these plants in culture over three months.

The adhensive hairs or rhizoids are produced from the ventral surface of the thallus. The tips of the rhizoids are swollen and secrete a gummy substance which helps in the attachment to the substratum.

Flowers of all the three studied species are zygomorphic, lack sepals, petals, bear only the essential organs and are covered by a spathella. Spathella is made up of 3-4 layers of cells. In the young flowers the anthers and staminoides are placed close to the ovary. The anthers lie below the bifid stigma before pollination. However, the filament of the anthers increase double fold at the time of pollination and overgrown the stigma. There are two stamens in a flower whose filaments are united at the base forming a Y-shaped structure called andropodium. The plants collected from natural habitat show silica deposition in many of their parenchyma cells.

Study of SEM shows dyad pollen grains with an echinate ornamentation and bifid stigma in the studied plants. A single flower produces about ca. 7000 pollens and ca.356 ovules in *Polypleurum*, ca. 4938 pollens and ca. 78 ovules in *Zeylanidium* and ca. 8135 pollen and ca. 252 ovules in *Willisia*.

The pollen:ovule ratio in *Polypleurum* is found to be 20:1 with an ovule:seed ratio of 8:5, pollen:ovule ratio in *Zeylanidium* is 63:1 with an ovule:seed ratio of 6:5 and pollen:ovule ratio in *Willisia* is found to be 32:1 with an ovule:seed ratio of 8:5. This relatively low Pollen: Ovule ratio in the studied plants and high seed set suggests the possibility of self pollination. The seeds are non-endospermous and minute. A single fruit, which is called capsule, contains ca. 205 seeds in *Polypleurum*, ca.50 seeds in *Zeylanidium* and ca.184 seeds in *Willisia*.

Unlike in the majority of the angiosperms, the embryo sac is of 4-nucleate cells. There is no double fertilization and no endosperm. Antipodal cells are absent. The

development and organization of the embryo sac follows different patterns in different species. They are classified into 3 types as the *Apinagia* type, the *Polypleurum* type and the *Podostemum* type. The mature ovary bears numerous anatropous, tenuinucellate and bitegmic ovules in a swollen axile placenta.

In *Polypleurum*, the megaspore mother cell divides meiotically and produces two uninucleate dyad cells. The micropylar dyed cells degenerate while the chalazal dyad cells divide meiotically to produce two megaspore nuclei. These two nuclei divide and produce four free nuclei which organize into two synergids at the chalazal end, an egg in the median cell and a polar cell at the micropylar end. The development of embryo sac follows the *Polypleurum* type.

In *Zeylanidium*, the megaspore mother cell divides meiotically to produce two uninucleate dyads cells. The micropylar dyad cell degenerates soon and the chalazal dyad cell divides meiotically to produce two nuclei. Out of these two nuclei, the nucleus at the chalazal end degenerates. The micropylar megaspore divides twice and four nuclei are produced. These four nuclei participate in the formation of the embryo sac with two synergids at the micropylar end, a central egg cell and a polar cell at the chalazal end. The sequence of development of embryo sac corresponds to the Apinagia type B.

In *Willisia*, the megaspore mother cell divides meiotically to produce two dyads cells. The micropylar dyad cell degenerates and the chalazal dyad cell divides meiotically to produce two nuclei. These two nuclei divide resulting in the formation of four nuclei that participate in the organization of the embryo sac. The embryo sac consists of two synergids at the micropylar end, a central egg cell and a polar cell in the chalazal end. The sequence of development of embryo sac corresponds to the *Podostemum* type.

From these findings it is known that the embryo sac of these three podostemads also consists of only four-nucleate cells and there are no antipodal cells, which is the characteristic feature of this family. The presence of pseudo-embryo sac/ nucellar plasmodium is the characteristic feature of all podostemaceae so far investigated. It is believed that the nucellar plasmodium nourishes the developing embryo as endosperm is absent and protects it from desiccation.

The study of ENM has provided a predicted habitat distribution map of *Polypleurum stylosum* var. *laciniata*, *Zeylanidium lichenoides* and *Willisia selaginoides* in India. Most suitable habitat for *Polypleurum stylosum* var. *laciniata* is in Western Ghats whereas for *Willisia selaginoides,* it is in Kerala, Tamil Nadu and Karnataka. It is predicted that Kerala and southern part of Karnataka show the most suitable habitat for *Zeylanidium lichenoides*. The habitat distribution map of these species can help in discover new populations, identify top priority survey sites and set priorities to restore the natural habitat for effective conservation of *Willisia* and *Polypleurum.*

The study of this family Podostemaceae has been fascinating. There is much need for the early developmental, pollination and phylogenetic studies and conservation of this plant in view of its vulnerable habitats. Shortage of time and the unavailability of the material nearer to my work place were some im portant limitations that encountered during my present work. There are many other aspects that mentioned above which were worthy to study. There are hundreds of other little-known plants in the fragile ecosystem throughout the world waiting for conservation and scientific study.