CHAPTER II

Literature Review

Topography

Thailand is a tropical country in Southeast Asia situated between latitudes $5^{\circ}40'$N to $20^{\circ}30'$N and longitude $97^{\circ}30'$E to $105^{\circ}45'$E. The major geographic regions can be divided into four natural regions by the pattern of rivers and mountains. They are northern, northeastern (Korat Plateau), central (Chao Phraya basin), and southern region (Peninsula). The climate is a monsoonal alternation of wet and dry seasons. The basin pattern is determined by the moisture-laden southwest wind which blows in from the sea from May to October and the dry northwest wind which comes down out of the continental land mass during the rest of the year. The narrow southern peninsula receives rain almost throughout the year, with the bulk of it falling between October and January. So rain-fall varies greatly from place to place. The average annual rain fall is about 59 inches in Phetchaburi (central region) and 177 inches in Narathiwat (southern region). This condition is suitable for the stoneworts to grow all year round (Harris G.L. 1963).

Previous works of Characeae of Thailand

The first record of Characeae of Thailand appeared in the " Flora of Koh Chang " by Schmidt (1901). Two species of *Chara* were mentioned, they were *Chara gymnopithys* Al.Br. and *C. fluccida* Al.Br. He noted that the specimens collected from muddy rice field near Lam Dan, Trad, were too young for a certain determination.

Another work was done by Suwatabandhu (1950) in the "Weeds in Paddy Field in Thailand". He mentioned Chara sp. family Characeae, as fresh water algae commonly found in rice field where water level is low and stagnant. The heat of the sun during the hot day made the surrounding water of this algae warmer and is quite injurous to the rice crop.

Yongboonkerd (1975) included two species of Characeae growing in rice field in "Some Weeds in Paddy Field", they were Chara zeylanica Kl. ex Willd and Nitella sp. She noted that these weeds grew abundantly and interfered the growth of rice. They also increased water temperatures in the rice field.

Chokenukool (1974) did a Master's thesis on "A study on the distribution of fresh-water algae in the district of Talingchan, Bangkok". He found two members of Characeae grew in canals and rice fields. They are Nitella sp. and Tolypella sp.

In "The Charophyta of Malaysia" by Zaneveld (1940) gave a taxonomic survey of the Charophyta of the Netherlands Indies, including some note on their history, distribution, classification, ecology and economy. This dealed not only with the Charophyta occurring in Malaysia, but at the same time with those found in British India (Ceylon incl.), Siam (Thailand) and French Indo-China. Among 61 species reported in this study only 3 species were found in Siam (Thailand), they were, Chara corallina, C. hydropitys f. major, and C. zeylanica f. typica.

General information on Characeae

The plant body of Characeae composes of an erect vertical axis which differentiates into a regular succession of nodes and internodes. Each node produces a whorl of branchlets of limited growth and lateral branch of unlimited growth may arise axillary to the leaves. Sexual reproduction of Characeae is oogamous. The sex organs normally occur at nodes of branchlets and rarely at the base of branchlets. The oogonia are one-celled, solitary, surrounded by a sheath of spirally twisted cells. The antheridia are one-celled, united in uniseriate filaments, of which several are surrounded by a common spherical envelop composed of eight cells (Smith, 1955 and Prescott, 1972).

The members of the family Characeae, are widely distributed in water which is not subject to much movement and which affords the sandy or muddy substratum in which the anchoring rhizoids can find a suitable foothold. Most of them are confined to fresh, some to brackish water (Chara baltica), while the others occur in both habitats (Chara fibrosa). They are able to exist with a small supply of oxygen and can thrive where the substratum contains much decomposing matter (frequently including H_2S). They usually form extensive subaquatic meadows and in larger bodies of water constitute characteristic zones extending to a considerable depth in clear water, Nitella in general going deeper than Chara. The abundant incrustation with carbonate of lime that characterises most species of the latter genus often leads to the accumulation of considerable calcareous deposits. The presence of calcium is essential, although the demand of the different species is varied (Fritsch, 1965).

Vegetative structure

The thallus is an erect branched axis attached to the substratum by rhizoids. The rhizoids are uniseriately branched filaments, with or without a differentiation into nodes and internodes. The erect axis has an Equisetum-like differentiation into nodes and internodes. Each node bears a whorl of several branches that cease to grow after they have attained a certain length. The leaves may be simple or divided, and with or without a differentiation into nodes and internodes. In some genera, as Nitella, an internode always consists of a single cell, many times longer than broad. In other genera, as Chara a majority of the species have this internodal cell ensheathed (corticated) by a layer of vertically elongated cells of much smaller diameter. The ensheathing layer (cortex) of an internode is always one cell in thickness.

Terminal growth of an axis is initiated by a single domeshaped apical cell which cuts off derivatives at its posterior face. Each derivative cut off by an apical cell develops into a node and its underlying internode. This begins with a transverse division of a derivative soon after it is cut off from the apical cell. The inferior daughter cell remains undivided, elongates to many times its original length, and maturates into an internodal cell. The superior derivative, the nodal initial, divides and redivides to form the nodal tissue and also the corticating tissue of species in which the internode is corticated. The first division of a nodal initial is vertical, and the two daughter cells also divide vertically and in a plane intersecting the first plane of division. Successive divisions are also vertical and in planes intersecting the preceding plane of division. The nodal tissue produced by these divisions consists of two central cells and an encircling ring of 6 to 20 peripheral cells. The central cells may remain undivided or may divide two or three times. All the peripheral cells divide periclinally. The inner daughter cells produced by these periclinal divisions may remain undivided or may divided periclinally. The outer daughter cells function as initials that give rise to leaves. Among species with corticated internodes, half of the corticating tissue of an internode is derived from the node above and the other half is derived from the node below. The basal cell of each leaf of a node produces a single ascending corticating initial and a single descending one. Each ascending corticating initial is an apical cell, that lies closely applied to the internodal cell, and each apical cell produces a corticating filament differentiated into three-celled nodes and one-celled internodes. All nodal and internodal cells of a young corticating filament are at first approximately the same length but eventually the two lateral nodal cells of a node and the internodal cell elongate to many times of their original length. The median cell of a node does not elongate, and it may or may not form one-celled spines. Leaves develop from the peripheral cells of a node. Some genera have leaves that consist of a simple or branched uniseriate row of cells. Other genera, as _Chara_, have leaves with the same structure as the axis. Here the apical cell of a leaf cuts off derivatives in the same manner as an apical cell of the main axis. The apical cell of a leaf becomes conical and ceases division after it has cut off 5 to 15 derivatives. In _Chara_, the first derivative from a leaf's apical cell becomes the basal node in all leaves; all other derivatives divide to form a nodal initial and an internodal cell. Internodal cells of a leaf maturate in the same manner as those of an axis, except that they do not become so long. Development of nodal tissue from a nodal initial is much the same as in an axis. Nodes of leaves have but one central cell, and the peripheral cells never function as apical cells. Instead, all or certain of embryonic peripheral cells maturate into one-celled, spine-like structures. Cortication of a leaf may be similar to that of an axis, or the corticating initials at a leaf node may elongate without dividing (Smith, 1955).

Cell Structure

Cells near the branch apex are without conspicuous central vacuoles and are always uninucleate. Greatly enlarged cells of mature regions, as those of an internode, have a large central vacuole and may have a few large irregularly shaped nuclei because of nuclear division by constriction. The cytoplasm external to the central vacuole contains many small ellipsoidal chloroplasts that lie in longitudinal spirally twisted parallel series. The portion of the cytoplasm next to the central vacuole streams continuously in a longitudinal direction. There are ascending and descending longitudinal streams of cytoplasm laterally separated from each other by a motionless streak of cytoplasm without chloroplasts (Smith, 1955).

Asexual Reproduction

None of the Characeae produces zoospores, but many of them produce asexual reproductive bodies of a vegetative nature. Vegetative propagation may be effected by

1. star-shaped aggregates of cells developed from the lower nodes, and frequently called amylum stars because they are densely filled with starch;
2. bulbils developed upon rhizoids;
3. protonema-like out growth from a node (Smith, 1955).
4. by means of fragmentation (Zaneveld, 1940).

Sexual Reproduction

All genera reproduce sexually. The male and female fructifications are usually called, respectively, antheridia and oogonia, but these names are inappropriate because the structures so designated include both the sex organ and an enveloping multicellular sheath derived from cells below the sex organ. According to the old terminology, the male fructification is a globule and the female is a nucule. These names are more appropriate since they do not imply that the entire fructification is a sex organ.

In almost every case, the fructifications are borne on the leaves. The vast majority of species are homothallic, and only relatively few are heterothallic. Homothallic species usually have two kinds of fructification borne adjacent to each other. There may be one of each and the globule above, Nitella, or below, Chara, the nucule. There are several nucules flanking a single globule, as in Tolypella. Chara may be taken the manner in which globules and nucules develop. Here the fructifications are borne at nodes of leaves and on the adaxial side.

A mature oogonium contains a single egg with a nucleus at the base and with the cytoplasm densely packed with starch. Even before elongation of the oogonial parent cell, there is an upgrowth of the five lateral initials of the nodal tier to form a protective sheath enclosing the oogonial parent cell. The sheath soon becomes transversely divided into two tiers of five cells each. Cells of the upper tier elongate but little and maturate into the five cells, elongate to many times their original length and become spirally twisted about the oogonium. In certain genera, including Nitella, the corona consists of two tires and has five cells in each tier. The spirally twisted tube cells of a mature sheath separate from one another just below the corona to make five small angular slits. Antherozoids swim through these openings in the sheath of a nucule and down to the oogonium. One of them penetrates the gelatinized wall and unites with the egg. Male gamete nuclei have been observed within gamete nuclei at the base of an egg (Smith, 1955).

The Zygote and Its Germination

The zygote secretes a thick wall, and the inner periclinal walls of tube cells also become thickened. Other portions of walls of a sheath decay, leaving the thickened inner tube cell walls projecting from the zygote like the threads on a screw. The zygote, with the surrounding remains of the sheath, falls to the bottom of the pool and then germinates after a period of a few weeks. The zygote nucleus migrates to the apical pole of a zygote and there divides into four daughter nuclei. This division into four nuclei suggests that division is meiotic. According to such an interpretation, the thallus is gametophytic and the zygote is the only diploid cell in the life cycle. The rhizoidal initial develops into a colorless rhizoid differentiated into nodes and internodes, and one with a whorl of secondary rhizoids growing out at each node. The protonematal initial develops into a green filament also differentiated into nodes and internodes. Appendages produced by the lower most node of a primary protonema become either rhizoids or secondary protonema. The second node of a primary protonema bears a whorl of appendages. All but one of them are simple green filaments; the remaining appendage develops into a typical axis in which growth is as in an adult plant (Smith, 1955).

The Use and Damage

The utilization of Charophyta was reported by Zaneveld (1940) in " The Charophyta of Malaysia and adjacent countries ". His report is quoted as follow:

1. F i s h - c u l t u r e .

In the tropics it might often be observed that already in the forenoon the stagnant waters, in which Charophyta and other submerged plants grew were in a state of supersaturation with O_2, shown by the rise of oxygen bubbles to the surface; the converse activity, i.e. the oxygen consumption for respiration, being less intensive. As the amount of oxygen dissolved in water was raised by the photosynthesis of green plants, these might for that reason be recommended for use in fish-cultures.

2. P u r i f i c a t i o n o f w a t e r .

The water in which the Charophyta grew was always extremely clear. This might at least partly be ascribed to the fact that Charophyta were able to purify the water by retaining mud particles between the whorls of their branchlets.

3. F o o d .

A great number of insects, crustaceans, snails and other organisms, took shelter in the dense masses of Charophyta and/or fed on them, thus providing a rich supply of food for fishes at the same time. In addition, it must be stated that some fishes made nests among Charophyta.

Zaneveld (1940) referred to Backer that Najas tenuifolia (limcet siarang) was used at lake Toba as a food for hogs. This lake had a rich Charophyta flora and it was very well possible that Charophyta were also used for this purpose. He also referred to

Macatee that all parts of various Charophyta were eaten by 14 species of ducks occurring in North Carolina (U.S.A.). More than 1100 root-bulbils were observed in the stomach of one single golden-eye and more than 1,500 in that of a pintail. Therefore he recommended Charophyta as food for wild duck. In Malaysia too, it would furnish a cheap and readily accessible food and might therefore be introduced in native ducks.

4. M a n u r e .

Zaneveld (1940) mentioned a photograph made by Prof. A. Thienemann, representing a heap of Charophyta at the bank of Lake Batoer, Kedisan, Bali. These were dredged by the natives, who look in it for snails which were used as a duck's food. Prof. Thienemann informed his opinion that the Charophyta were afterwards spread upon the land as manure.

The importance of using the decayed Charophyta for manure or especially for correcting the acidity of soils, may appear from the subjoined analyses of Charophyta. From table I and II showed that the content of calcium oxide and carbonate was very high and it required no further comment that Charophyta enoumous banks were formed at the bottom of the waters from which these plants were not collected for agricultural purposes. At the same time, a minor part was played by the decaying Charophyta which were accumulated at the bottom of waters, and by which action the bottom was raised. Therefore the plants might be useful in land reclamation.

5. P o l i s h i n g - p a s t e .

Zaneveld (1940) cited the work of Dalechamps, in " Historia Generalis Plantarum ", that the inhabitants of Lyon, France, made use of a plant with the popular name " Chara " to polish plates and other domestic utensils.

6. M u d - b a t h i n g .

Zaneveld (1940) quoted the work of Prosper that in Spain in a pool people bathed, " attributing the cure to their maladies to the action of the deposit of saltpetre on the banks, the saltpetre being the white masses of dry Chara which surround the pool ".

7. T h e r a p y .

In connection with the foregoing, it might be added that Charophyta were sold in Germany as chemicals, which, when taken, should prevent a number of diseases. It seemed not impossible that Charophyta in some forms were sold in Malaysia druggist's shops.

8. I n s e c t s .

In some parts of Java a plant, named ganggeng or gnaggang, was used to lure noxious insects, especially the nauseous-smelling bug, Leptocorisa varicornis (walang sangit, Jav.). Bushes of this plant including Charophyta, were attached to a stick which was placed in the rice-fields. The walang sangit then alighted upon it and may thus be caught (Zaneveld, 1940).

Although the agreement of habitats was very striking, a number of authors have stated that larvae of mosquitoes have never been found in localities where these plants occurred. The first who observed this phenomenon was Caballero. The theory was that Chara vulgaris, produced toxic substances which spread through the water and which were lethal to the larvae of mosquitoes.

Zaneveld (1940) believed that in tropical countries the problem was first studiedby Blow in Madagascar. The Charophyta themselves possess larvicidal properties, but some other substances occurring in the localities of these plants. Moreover, dilute solutions of glucoside from dried Chara zeylanica took no effect. Nitella furcata var. Roxburghii, Chara fibrosa ssp. gymnopitys and C. zeylanica appeared to keep the water quite free of the larvae of Theobaldia annulata, Culex pipiens, and Anopheles maculipennis. He also quoted the experiment of Buhot with Nitella phauloteles and various species of mosquitoes, viz. Stegomyia fasciata, Culex fatigans and Anopheles nyssorhynchus. " All gave the same and pleasing results, not laying their eggs upon the surface of the water in the aquarium where the Nitella grew ". In addition, this author showed that water in which this Nitella grew, had no toxical activity on rats, fish and men.

In Burma, Pal (1932) experimented with Nitella acuminata, N. oligospira and Chara fibrosa ssp. gymnopitys and the mosquito Culex fatigans. Nether species proved to have any lethal effect on the insects. In nature, however, ponds containing mosquito larvae never contained Charophyta, and conversely. Pal arrived at the conclusion that this was due to the occurrence of larvae of Libellulidae (dragon flies), which had a greenish tinge or some other protective camouflage. They watched for prey, e.g. mosquito larvae, which often occurred between the branchlets of the Charophyta. " This would account satisfactorily for the absence of mosquito larvae from waters containing Charophyta ".

Pal (1932) referred to Senior-White, who investigated the chemical factors of Ceylon breeding places, but also studied in this connection the algal distribution. They found that mosquito larvae in general only occur at a hydrogen number varying between 5.8 and 8.6, and in waters with a conductivity, varying between 62 and 922 mhos/cm measured at 25°C. They found that mosquito larvae do not appear to be liable to supersaturation of the water, as fish do; Anopheles maculatus was still found in water with 14.84 mg oxygen per litre (the minimum lies at 0.87 mg/l). This stated of supersaturation frequently occurred in the stagnant waters, where dense masses of Charophyta were found growing, and the water might sometimes become highly alkaline caused by the CO_2 consumption by photosynthesis. This was the reason why $CaCO_3$ may be precipitated. In connection with this, Senior-White mentions an important cause of larval mortality. Larvae of Anopheles listoni died when the pH under the above mentioned conditions rose to 8.6 and they were found covered by spherical bodies, with a dark centre and a broad translucent edge.

For the damage of Charophyta was reported by Dr. Mukherji (1970) that about one million acres of lowland paddy in the West Bengal alone was affected by stoneworts. It grew and acted as a serious weed to cause a terrible loss of the paddy crop by using the mineral food of rice plants. Otherwise, it interfered with tiller formation, intercultural operations and harvesting. It was found that the yield of this area was reduced in 10 - 25 % and about 2-4 tons per acre of these algae could be easily collected from the field.

For the control of these algae, in pool, pond and irrigation canal, machanical control can be applied because of its fragile stem, but in paddy field, mechanical control is almost impossible; as it is very difficult to remove all traces of algae clinging closely to paddy stalks which again multiply rapidly. Their growth cannot be prevented by any means, as their resistant spores over-winter in soil and pools and spread and grow with the onset of the rains. The only practical means of controlling these algal weeds is by chemical.

Mukherji and Sengupta (1964) have described the usual chemical control with 4-6kg of copper sulphate per acre and found that half the dose of any 50% wettable copper fungicides will also do the work. Normally 2 ppm of copper sulphate is enough, but in the muddy water of paddy fields some may become inactivated and there is no residual action.

Mukherji and Roy (1966) have shown subsequently that, among other chemicals, a formulation containing 60% of triphenyl tin acetate (Brestan-60, Hoechst) was highly effective in killing these algal growths within a short time at the dose of 700g/acre.

Mukherji (1972) used pentachlorophenol and sodium pentachlorophenate mixed into the soil with a wooden plough. The two chemicals were applied at 6.25kg/ha. It is clear that both pentachlorophenol and sodium pentachlorophenate were good algicides, whether applied to the soil 3 weeks before transplanting, or as post-transplanting treatments after the algae had developed. The only apparent effects on the crop were small spots which appeared on the young leaves; there was no adverse effect on growth. The soluble sodium salt appeared to act more rapidly than pentachlorophenol, but was inactivated sooner. The minimum effective doses appear to be 2.5-3.75 kg/ha for sodium pentachlorophenate and 3.75-4.4 kg/ha for pentachlorophenol. Even with doses of 3.75 kg/ha, small snails and fish were found to be killed, and it is clear that these compounds should not be used in water in which fish are likely to be reared unless at least a month can elapse after application.