CHAPTER III

MATERIALS AND METHODS

Equipment Used

Skin-dive equipment: diving mask, fins and snorkel.

Specimen containers: plastic bags and 6 oz. specimen bottles.

Binocular microscope.

Compound microscope.

Micrometer.

Camera and accessories for microphotography.

Equimpent for free-hand sectioning, e.g. petri-dishes, razor blades, dissecting needles, forceps, scissors, slides, cover-glasses, medicine dropper, etc.

Preserved solution: 4% formaldehyde solution.

Dye: 2% Safranin O dye solution (2.0 gm. of Safranin O in 10 ml. 95% ethyl alcohol and add distilled water to 100 ml.).

Collected Specimens

Preserved and dried specimens used in this study were provided by the Faculty of Fisheries, Kasetsart University, and the Department of Botany, Chulalongkorn

University, those fresh materials were personally collected. All the localities of the collections are the followings:

Locality	Geog. position	
	North lat.	East long.
Chon Buri Prov.:		
Patthaya	12° 53′	100° 49′
Patthaya Tai Beach		
Sattahip	12° 41′	100° 49′
Ao Dong Tan		
Sri Racha	13° 20′	100° 50′
Laem Tao Thewa		
Rayong Prov.:		
Ban Phe	12° 38′	101° 26′
Hard Hin Khao		
Marine Fisheries Station		
Suan Son		
Trat Prov.:		
Koh Chang	12° 5′	102° 23′
Koh Mak	11° 48′	102° 30′
Laem Ngob	12° 11′	102° 24′
Ao Tan		

Locality	Geog. position	
	North lat.	East long.
Surat Thani Prov.:		
Koh Samui	9° 30′	99° 59′
Sila Ngu		
Phuket Prov.:		
Ao Makham	7° 50′	98° 23′
Ban Patong	7° 53′	98° 18′
Laem Sing		
Laem Panwa	7° 48′	98° 24′
Songkhla Prov.:		
Koh Nu	7° 14′	100° 36′
Koh Yaw	7° 9′	100° 33′

Method

For identification, the whole fertile plant must be examined first with the un-aided eyes or the binocular microscope. For the dried or herbarium specimens, a piece of frond will be cut off and soaked in water for a few minutes before sectioning.

The characteristics used for examining are:-

(i) the height and width of the whole plant.

(ii) the calcification on surfaces of the blade.

(iii) the length and stupa of the stipe.

(iv) the branching or dividing of the blade.

(v) the development of piliferous zones on both surfaces.

(vi) the width of sterile and fertile glabrous zones.

(vii) the bearing of tetrasporangial, oögonial and antheridial sori.

(viii) the width and position of sori in the fertile glabrous zones and the distribution of sori among them.

(ix) the present of indusia.

If the piliferous zones and indusia are not well seen, a few drops of 2 percent Safranin O dye solution should be added. Minute pieces from various parts of the frond about 2 by 4 mm. were used for sectioning in order to study the details by the usual freehand process. These pieces were used both for radial and transverse sections; the following features were recorded:

(i) number of cell layers forming the blade.

(ii) length and width of cells of the superficial layers.

(iii) present and persistance of indusia.

(iv) shape and size of reproductive organs.

(v) occurring of hairs and rhizoids.