MATERIALS AND METHODS

Various species of Ceropegia and Brachystelma were collected from different localities in Maharashtra. The species of Ceropegia were collected in vegetative as well as in flowering and fruiting stages through frequent visits to the places of collection during months of June to October for last 2 years. Species of Brachystelma were collected during rainy - season (June - September) in vegetative stages and in flowering and fruiting stage during summer (May - June) The details of the species collected along with places and dates of collections are given in Table - 1. The distribution of Ceropegia and Brachystelma species, collected from Maharashtra is shown in Map - I & II.

Tubers of different species were planted in earthern pots and botanical gardens of the department Most of the species grow well under cultivation. Majority of the species under study are growing in departmental garden. Careful observations on the vegetative growth, morphology, habit, habitat and floral pecularities were made in field as well as on plants growing in the departmental garden. Sketches of the vegetative and reproductive parts were made in the laboratory using fresh materials.

For anatomical and cuticular studies, few plants of each species along with tubers were preserved in FAA (70% Alcohol 90 c.c. + Acetic acid 5 c.c. + Formaline 5 c.c.). Preparation of herbarium of Ceropegia species during rainy season is difficult due to delicate nature of leaves and flowers. Therefore the plants were first killed and partially dehydrated in 70% alcohol and then dried by usual way using old news papers. Special methods for preservation of flower colours were tried. Drying flowers in between two slides on Calcium

chloride was found to be suitable for preserving colours of flowers. Similarly Chitlay's technique for preservation of Ceropegia flowers was found to be most suitable. The fresh flowers were kept in equal parts of castor oil and kerosin for 4-6 hours. Then excess oil was blotted with blotting papers. The flowers were kept inbetween two sheets of polythene paper and pressed with iron at suitable temperature. With this technique, the flower colours and external morphology is preserved well. Light-windows were studied by dissecting the corolla under dissecting microscope. The sketches of small flower structures such as corona, pollinia were made with the help of prism type of Camera lucida.

For anatomical studies of various plant parts conventional techniques of fixation, dehydration, infilteration, embedding sectioning and staining were followed. (Johansen, 1940). Similarly hand-cut sectioning was also tried to study various anatomical characters of stem, petiole and leaf. Study of vascular elements such as fibres. tracheids, vessels were made by maceration technique. (Jaffrey, 1928) . Leaf, stem and petiole anatomy was studied by microtome sectioning as well as hand cut sectioning. Aqueous safranin and fast green (Johnson, 1940) was found to be most suitable for stem petiole and leaf anatomy.

For cuticular study, the peels of fresh and preserved leaves were taken. The peels were taken from various parts of the leaves for keeping constancy. Fresh peels were taken under water and dehydrated, stained with safranin or fast green and made permanent by passing through usual alcohol xylol grades. For preserved leaves the KOH treatment was given. The leaves were kept in the 10% KOH for 10-15 minutes. The removal of peel becomes easy due to the action

of KOH. These peels were dehydrated, stained and made permanent by passing through usual alcohol xylol grades, stained with fast green in clove oil and mounted in DPX. These permanent slides of peels were used for the determination of stomatal size, length and breadth of epidermal cells. For stomatal density, at least 50 readings were taken from various parts of the peels, from lower and upper epidermis. Stomatal index was determined by the following formula.

$$\text{Stomatal Index (SI)} = \frac{S}{S + E} \times 100$$

Where SI = Stomatal Index

S = Number of stomata mm^{-2}

E = Number of epidermal cells per unit area (mm^{-2})

For starch grain analysis, material from tuber was taken on slide. It was crushed and stained with I_2KI solution. The various types of starch grains were studied under microscope.

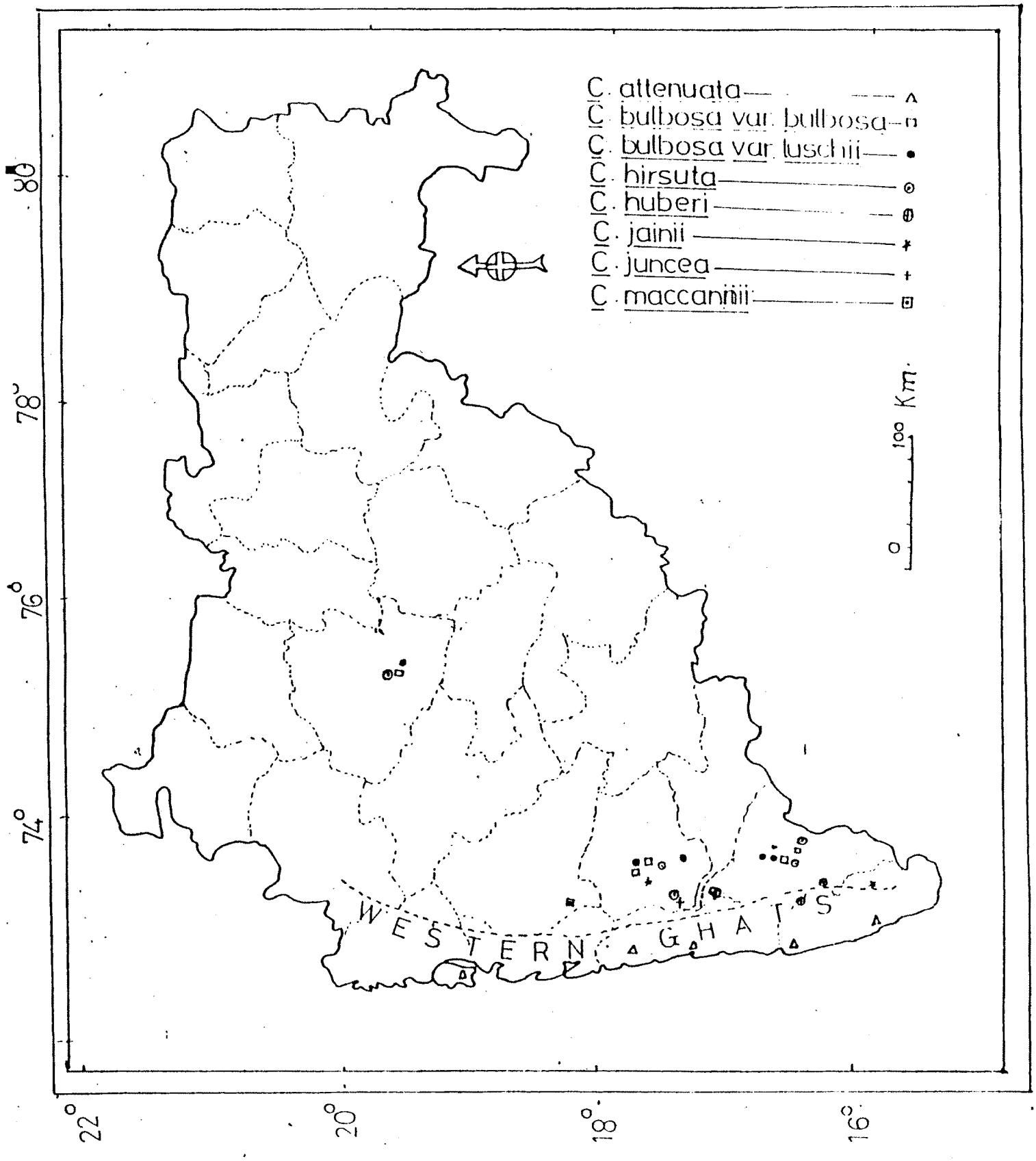
Drawings of anatomical structures such as stem, petiole, leaf, cuticle, trichomes and starch were made by using Hamburg microscope and Erama's camera lucida at suitable magnification. Photomicrographs were taken by using MFAK's system of Jenaval Carlzeiss microscope. Photographs of plants, plant parts were taken by Asai Pentax camera using NP-55, black-white film and coloured film of Kodak or Konica.

Table : 1 - Distribution of Ceropegia Linn. and Brachystelma R.Br. Species in India.

Sr. No.	Name of the Species	Places of collection	Remarks
1	2	3	4
1.	C. attenuata Hook.f	Kanheri caves, Malwan Deogad, Dapoli, Ratnagiri.	Endemic to Maharashtra
2.	C. bulbosa Roxb.var. bulbosa	Kolhapur, Appachiwadi, Kartiki-swami(Pusegaon) Khatav, Saikade (Patan) Panhala.	Throughout India.
3.	C. bulbosa(Roxb.) var. lushii (Grah.) Hook.f.	Kolhapur, Kagal, Aurangabad Pusegaon, Karad, Panhala	Throughout India
4.	C. hirsuta Wt.et Arn.	Kolhapur, Appachiwadi, Panhala, Saikade, Surli-Ghat Pusegaon, Aurangabad.	Throughout India.
5.	C. huberi Ansari.	Gaganbavada, Amba-Ghat.	Endemic to Maharashtra.
6.	C jainii Ansari.	Kas plateau, Chaukul.	Endemic to Maharashtra.
7.	C. juncea Roxb.	Saikade(Patan), Manewadi (Patan).	Throughout India.
8.	C. maccannii Ans.,	Singhgarh hills (Pune)	Endemic to Maharashtra.
9.	C. media (Huber)Ans.	Amba-ghat, Bhimashankar hills (Pune).	Endemic to Maharashtra.
10.	C. noorjahaniae Ans.	Rethare, Kartikishwami (Khatav).	Endemic to Maharashtra.

1	2	3	4
11.	C. oculata Hook.	Malwan, Ratnagiri, Panhala Petlond, Radhanagari.	Endemiic to Maharashtra,
12.	C. sahyadrica Ans. et. Kul.	Gaganbavada.	Endemic to Maharashtra .
13.	C. vincaefolia Hook.	Kas, Radhanagari, Singhgad Kanheri- caves.	Endemic to Maharashtra .
14.	Brachystelma edulis. Coll. & Helmsl.	Appachiwadi (Kolhapur).	New Record in Maharashtra .
15.	Brachystelma species.	Malwan.	It is a new species.

MAP NO.-1: Distribution of Ceropegia species in MAHARASHTRA



MAP NO-2: Distribution of Ceropegia & Brachystelma species in MAHARASHTRA