# DISCUSSIONS

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#### Morphology

The genus Ceropegia, one of the largest genera of Asclepiadaceae with about 200 species (Bruyns 1985) is distributed over most of the Africa, Madagascar, the Arabian peninsula, the Indian subcontinent, the East and into the northern parts of Australia. Huber (1957) in his revision on Ceropegia described about 150 species for whole world. After his revision about 30 species have been described from various parts of the world and India.

Linnaeus proposed genus <u>Ceropegia</u> and distinguished it from allied genera for its tubular corolla which is swollen at the base, funnel shaped and corola lobes more or less connate at the tip. Roxburgh for first time described and illustrated three species of <u>Ceropegia</u> occuring in Bombay in India. Gamble (1921) described about 21 species from precidency of Madras. In recent revision or Indian species of <u>Ceropegia</u>, Ansari (1984) reported 44 species from India out of which 28 are endemic to India.

Cooke (1904) hardly recorded 8 species of <u>Ceropegia</u> for Bombay precidency. Sedgwick (1921) described <u>C. fantastica</u> from N. Kanara. Blatter and McCann (1931) described <u>C. polyantha</u> and <u>C. hispida</u> from Western ghats. Latter it was found that <u>C. polyantha</u> Blatt. and McCann, <u>C. hirsuta</u> wt. & Arn. var. <u>vincaefolia</u>, Hook. <u>C.</u> <u>oculata</u> Hook, var. <u>subhirsuta</u> Huber are the same as <u>C. vincaefolia</u> (Ansari, 1971) and <u>C. hispida</u> Blatt, and McCann as <u>C. hirsuta</u>. Blatter and McCann (1933) described <u>C. panchganiensis</u> from the Panchgani hills. In 1945 McCann described <u>C. evansii</u> from Khandala. In last two decades about 8 species have been described from Maharashtra. It is interesting to note that whatever species described from India after Hooker are either from Maharashtra or Karnataka, however most of them are from Maharashtra. It is probably due to extensive field work and careful search for species of <u>Ceropegia</u> in Maharashtra by Ansari, Kulkarni, Hemadri and Wadhwa. Author feels that such careful and extensive search for species of <u>Ceropegia</u> especially in south India will prove to be fruitful.

Ansari (1968) described an interesting species <u>C. huberi</u> from Amba ghat. It has been also colle cted by author from Gaganbavada of Kolhapur district. <u>C. santapaui</u> has been described by Wadhwa and Ansari (1968) from Mahad Ghat in Satara district. Hemadri (1968) described <u>C. rollae</u> from Dhak Khilla in Poona district. Hemadri and Ansari (1971) described <u>C. mahabalei</u> from Ralegaon hills in Poona district. Ansari and Kulkarni (1971) described <u>Ceropegia sahyadrica</u> from Amboli ghat in Ratnagiri district. Ansari (1972) described <u>C. noorjahaniae</u> from Wai-Panchgani ghat in Satara district. Ansari (1980) added two more species <u>C. jainii</u> and <u>C. maccaunii</u> from Amboli and Sinhagadh hills respectively. Thus from above account it is clear that all the new species of <u>Ceropegia</u> described for India are from Poona, Satara, Ratnagiri and Kolhapur districts of Maharashtra.

In revision on Indian species of <u>Ceropegia</u> Ansari (1984) described 20 species from Maharashtra out of which 17 are endemic to Maharashtra. Although there are number of species of <u>Ceropegia</u> none of them is of common occurrence. Most of species are rare and restricted to small areas. Many of the species of the genus described from Maharashtra are very limited in their distribution so that they may be called not only endemic for Maharashtra but also for a very small area within the state. Most of the species occurring in Maharashtra are endangered. All the new species described from Maharashtra are restricted to small areas and are vulnerable because of the edible tubers destroyed by man and animals, land-slide and destruction of natural habitats. According to Mistry and Almedia (1989) <u>C. huberi, C. oculata</u> and <u>C. sahyadrica</u> are endangered and threatened plant species from Ratnagiri district. It is not the case with only above three species but with all those species endemic to country.

There is threat to survival of <u>Ceropegia</u> species in the wild habitats. Cultivation is the best conservation measure for survival of this natural wealth. Cook (1904) has rightly said that seeds and tubers of the genus would be a valuable contributions to conservatories at home.

Brachystelma is another interesting genus of Genus Asclepiadaceae. The species of this genus are also very mare and endemic to small areas. Hooker (1883) reported 7 species of Brachystelma for British India. Gamble (1921) recorded 6 species south India. Cooke (1904) did not record any species of from Brachystelma from Bombay precidency.

Since publication of Gamble's flora about 3 new species have been described from India and about 13 species have been recorded

from India. Arekal and Ramakrishna (1981) described two new species <u>B</u> kolarenses and <u>B</u>. ciliatum from Karnataka state. Char (1978) described new species <u>B</u>. elenoduensis from Karnataka. Recently Yadav et. al. (1989) have recorded <u>Brachystelma edulis</u> from Kolhapur and Satara district of Maharashtra. During present work author has collected an interesting species of <u>Brachystelma</u> and will be publishing as a new species in short time.

The tubers of <u>Brachystelma</u> are edible. Like <u>Ceropegia</u>, the species of <u>Brachystelma</u> are rare and endemic to small areas. They are threatened and endangered. Vijravelu (1987) has recently collected <u>B. glabrum</u> from Shevaroy hills of Tamil Nadu. Careful search for the species of <u>Brachystelma</u> in south India would yield some new species.

Most of the species of Ceropegia occurring in Maharashtra are found along eastern and western belts of Sahyadri ranges. Most of them are found in Ghat areas in localities away from human and C. varieties of C. bulbosa hirsuta influence. Both are and found distributed through comparatively more common out Maharashtra. C. attenuata seems to be comparatively of common occurrence in Konkan area. It has been collected from Kanheri caves, Deogad, Malwan, Ratnagiri and Vengurla. The remaining species are restricted to small areas.

Out of 13 species studied <u>C. attenuata</u>, <u>C. jainii</u>, <u>C.</u> <u>noorjahaniae</u>, <u>C. maccannii</u> and <u>C. sahyadrica</u> are erect species. The former 3 species have linear to linear lanceolate leaves while later

two species have ovate to ovate lanceolate leaves. C. noorjahaniae is described as erect species (Ansari 1972, 1984) sometimes shows climbing habit. In field as well as under cultivation it showed erect as well as climbing habit. It is found throughout eastern hilly regions of Satara district. Among the erect species under study C. sahyadrica was found to be most robust. It is found growing along steep slopes of Ghat areas. It is reported from Poona and Ratnagiri district. Present author has collected it from Gaganbavada of Kolhapur district. It grows well under cultivaton and profuse flowering was observed in nature as well as in cultivated plants. Stem is thick, flowers are numerous, white and scented but fruiting was found to be rare. Robust erect habit, sweet scented white flowers indicate its primitive nature. C. jainii has been reported from Amboli. Present author has collected it from Kas plataeu of Satara district. Abundant flowering was observed but fruiting was not observed. Ansari (1980) also could not collect the follicels of the species. It raises the question of its propegation and survival. C. maccannii was collected from type locality and flowers are nonattractive.

Out of remaining eight species three showed succulent nature. Both varieties of <u>C. bulbosa</u> and <u>C. woodii</u> show succulent leaves while in <u>C. juncea</u> leaves are reduced or absent and stem is succulent.

<u>C. juncea</u> recorded from Karnataka state is also found in Satara district of Maharashtra (Yadav et. al. 1989). It is cultivated as succulent in home-gardens. It is a singular species of Ceropegia with stem succulent reported from India. Recently Gaikwad et. al. (1989) have described CAM in stem tissue of the species. Such species with succulent stem and reduced leaves are found in Canary Islands. These species include <u>C. dichotoma, C. fusca, C. superba</u> and <u>C. rupicola</u> (Bruyns 1983). There is strong possibility of getting CAM in these species. <u>C. juncea</u> show some climbing shoots and some trailing shoots. When trailing shoots come in contact with soil, root at nodes and form small tubers. Among the species studied <u>C. juncea</u> showed smallest & reduced tubers.

C. woodii is a cultivated species while C. bulbosa is wildly growing species. In C. bulbosa two varieties are recognised such as C. bulbosa and C. lushii. According to Ansari (1984) these two varieties breed true in nature. Present author has also observed that variety lushii grows in similar situations like var. bulbosa but maintains its linear leaves and no intermediate forms between these two varieties were observed. Author supports Ansari's recognition of these two varieties. Govindappa and Ramakrishna (1978) also supports the retension of var. lushii as distinct from var. bulbosa on the basis of their studies on the twin pollinia of these two varieties. It is interesting to note that in single genus Ceropegia, leaf succulent, stem succulent and nonsucculent species have been found. Recently Supate et. al. (1990) has recorded CAM in both varieties of C. bulbosa. Author strongly feels that CAM must be present in leaf succulent species of Ceropegia such as C. woodii.

Remaining five species are climbers. C. hirsuta is widely distributed throughout India while C. huberi, C. media, C. oculata and C. vincaefolia are restricted to Maharashtra and are endemic to state (Ansari 1984). C. hirsuta is found in comparatively drier parts of state. It shows great variation in vegetative as well as floral characters and need biosystematic considerations (Ansari 1984). Other species are found usually at higher altitudes in Sahyadri ranges where there is comparatively high rainfall. C. huberi is unique among all Indian species of the genus because of its reduced corolla tube copying the shape of various African species (Ansari 1968). It is a elegant and fascinating species growing on slopes among grasses and can be easily identified by pure white flower, angular reduced corolla tube and a broad circular flattened, slightely inclined head (Plate II, Fig. 7 & 8). According to Huber (1957) this species might be included into section Buprestis Huber but occupies a scmewhat isolated position.

Huber (1957) in his monograph on the revision of the genus <u>Ceropegia</u> described a new variety of <u>C. evansii</u> McCann known as <u>C. evansii</u> var. <u>media</u> Huber. Ansari (1969) raised this variety to species level - <u>C. media</u> (Huber) Ansari. It is rare and fruiting was not observed by Ansari as well as by author.

<u>C. oculata</u> and <u>C. vincaefolia</u> have beautifully coloured flowers (Plate V, Fig. 16-19). These species also show great variation in flower morphology and colour of flowers. They are of ornamental value. Correct identity of <u>C. vincaefolia</u> is determined by Ansari (1971). Both these species are found on hilly slopes in shrubby vegetation.

All the species of <u>Ceropegia</u> under study bear tubers, however tubers in <u>C. juncea</u> are reduced. The leaves are linear, linear-lanceolate in erect species except <u>C. sahyadrica</u> and <u>C.</u> <u>maccannii</u>. In climbing species the leaves are ovate-lanceolate. The leaves are either absent or reduced to scaly leaves in <u>C. juncea</u>. In <u>C. bulbosa</u> and <u>C. woodii</u> the leaves are fleshy. Leaves are usually petiolate however in erect species like <u>C. jainii</u>, <u>C. attenuata</u> they are almost sessile.

Flowers are produced in lateral umbellate cymes. In <u>C.</u> <u>attenuata</u> and <u>C. jainii</u> the cymes are single flowered while in other species they are 2-3 flowered and some species such as <u>C.</u> <u>sahyadrica</u>, <u>C. vincaefolia</u>, <u>C. huberi</u> show profuse flowering.

Flowers of different species are found to be characteristic of the species, <u>C. huberi C. media</u>, <u>C. maccannii</u> have more or less white flowers. <u>C. attenuata</u> and <u>C. hirsuta</u> (Plate I, Fig. 1-3) show great variation in size as well as colouring patterns of flower. <u>C.</u> <u>bulbosa</u> showed smallest flowers among the species. The flowers in <u>C. huberi</u> are very peculiar in their morphology. <u>C. jainii</u> showed completely red coloured flowers. <u>C. noorjahaniae</u> has green coloured flowers with black throat. Flowers of <u>C. oculata</u> (Plate V, Figs. 16 & 17) and <u>C. vincaefolia</u> (Plate V, Figs. 18-19) have very attractive curious flowers characteristic to the species. Flowers of <u>Ceropegia</u> species are highly specialized and curiously formed. They come under the category of fly trap flowers. (Percival, 1965). Corolla of the species of <u>Ceropegia</u> have slipperysided deep funnel-shaped corolla-tube leading to a chamber wherein the insects are imprisoned and where they come in contact with the stamens and ovaries. Many species bear hairs on corolla-lobes, in corolla tube or in inflated basal portion. They are characteristic for the species. The corolla of <u>C. hirsuta</u>, <u>C. vincaefolia</u> <u>C. oculata</u> and <u>C. bulbosa</u> are mottled and blotched. Colour such as yellow, white and greenish predominates in fly flowers (Percival, 1965), <u>C. hirsuta</u>, <u>C. vincaefolia</u> and <u>C. juncea</u> have flowers with tinge of yellow colour while <u>C. noorjahaniae</u>, <u>C. oculata</u> have tinge of green colours and <u>C. sahyadrica</u>, <u>C. huberi</u>, <u>C. maccannii</u> have white flowers. <u>C. bulbosa</u> and <u>C. jainii</u> have reddish pink to red flowers.

<u>C. hirsuta, C. bulbosa, C. oculata, an C. vincaefolia</u> have "lantern" top to the tube as in <u>C. woodii</u> (Percival, 1965). Similarly almost all the species showed dark lines leading to bottom of corolla probably acting as nectar guides. In all the species studied except <u>C. vincaefolia</u> the inflated corolla base showed 'light window' in the form of vertical translucent strips inbetween dark coloured strips. <u>C. vincaefolia</u> showed characteristic light windows in the form of translucent circular band near the top of inflated portion of corolla and circular translucent areas in dark coloured inflated lower portion of corolla (Text Fig. XVb, Fig. 10). This species showed most elaborate way of illuminating corolla. McCann (1943) described an ingenious method of lighting the interior of fly flowers

by means of "light" windows in the corolla of Ceropegia and Cryptocoryne. According to him flies trapped at the base of long tubes would tend to become inactive in the darkness. This is guarded against by the provision of translucent strips or patches of tissue which enable the light to penetrate & illuminate corona and stamens. Rintz (1979) discussed importance of inflated corolla in С. longkawienss. According to him in C. langkawiensis the flower acts as an insect retainer as a part of its pollination mechanism. Dr. (Botany Dept., Allahabad University) is working Chaturvedi on pollination in Indian species of Ceropegia and various memters of Asclepiadaceae.

In <u>Ceropegia</u> corona is double and staminal. Corona characters of the genus are of diagnostic value. The outer corona is cupular made of 5 lobes, lobes are either entire or bifid. It is either hairy or glabrous. Inner corona is usually of erect linear lobes. They are either hairy or glabrous. The shape of outer corona, lobes of outer corona unfid or entire, glabrous or hairy, the shape of inner corona lobes, glabrous or hairy are the important characters of corona in identification of species. The corona structures (Text Fig. XVa, Figs. 1-15) differ in different species and have diagnotic value. Ansari (1984) has given a comparative account of 18 species diagramatically.

Huber (1957) divided genus <u>Ceropegia</u> into 21 sections. Indian species of <u>Ceropegia</u> represent 10 sections. Section <u>Buprestis</u> is represented by 13 species while section <u>Indopegia</u> is represented by 9 species and section Tiloris by 6 species. Thus out of 44 species from India 30 belong to three section viz. <u>Buprestris</u>, <u>Indopegia</u> and <u>Tiloris</u>. Section <u>Sinopegia</u> is represented by 4 species. Section <u>Ceropegia</u> and <u>Orepegia</u> are represented by 3 species each while section <u>Chinopegia</u> and <u>Janthina</u> are by 2 species each. Section <u>Hylopegia</u> and <u>Phalaena</u> are represent by single species each. <u>C.</u> <u>woodii</u>, (introduced) belongs to Section <u>Ceropegiella</u>. A evolutionary chart of different section given by Huber and Indian species belonging to different sections is given in Table 1.

Genus Brachystelma is represented by about 13 species from India. Like Ceropegia both erect and climbing species are found in the genus. Except Brachystelma brevitubulatum and B. volubile all other Indian species are erect. The Brachystelma species collected from Malwan differs from all Indian species in having broad leaves and long flowering internodes. According to Dr. D.J. Goyder (botanist, Kew Botanical Gardens) it is new species allied to B. edulis. Char (1978) has wrongly described corolline corolla in B. elenaduensis, however in this genus the corolla is double and staminal. The roots are tuberous & similar to Ceropegia. They are edible. Flowers are inconspicuous and most of the species are hysteranthus.

#### Stem Anatomy

Gross histological characters of stem remain more or less same for nonsucculent species of <u>Ceropegia</u> and <u>Brachystelma</u> however some major differences in stem anatomy were observed in succulent and nonsucculent species of <u>Ceropegia</u>. Anatomical characters of the stem of Ceropegia could be summarized as follows :

The epidermis is single layered made up of cubical to rectangular cells. The outer surfaces of epidermal cells are covered with either nonstriated or striated cuticle. In some species some of the epidermis cells produce uniseriate multicellular, nonglandular trichomes. There is no distinct hypodermis. Epidermis is followed by cortex. The cortex could be divided into two zones. The outer cortex is made up of parenchymatous cells. The outermost layers this zone are made up of small oval cells containing few of chloroplasts while the inner layers are made up of large polygonal parenchymatous cells. In inner cortex there are either patches of cellulosic fibres or cellulosic fibres form a continuous zone as in C. hirsuta. The amount and size of fibre patches varies with species. These cellulosic fibres were found to be absent in stem succulent species C. juncea. Cellulosic fibres are followed by 1-3 layers of parenchymatous cells. The parenchymatous cells in inner part of cortex possess starch grains. The starch grains were found to be absent from parenchymatous cortical cells of stem in succulent species as C. bulbosa var. bulbosa, C. bulbosa var. lushii and C. juncea. The thickness of cortex varied with species. Inner cortex is followed by patches of primary phloem and secondary phloem. Laticiferous cells were found scattered inbetween phloem and cellulosic fibre patches. In all the species distinct cambium was observed. Cambium activity varied with species. C. sahyadrica and C. maccannii, both erect species showed prominent secondary growth. In secondary wood, vessels were more numerous in <u>C. sahyadrica</u> than <u>C. maccannii</u>. Secondary xylem of both these species showed radial rows of small

tracheids and fibres. C. attenuata, C. hirsuta C. noorjahaniae, C. C. jainii, C. oculata and C. media showed good amount of secondary growth but it was less than C. sahyadrica and C. maccannii. Secondary wood consisted of vessels, tracheids, fibres and xylem parenchyma. Vascular elements are usually arranged in radial rows. Xylem development was found to be more at two opposite regions in C. attenuata, C. bulbosa, C. noorjahaniae and 4 opposite regions in C. oculata and C. media corresponding to opposite leaves. In C. huberi and C. vincaefolia the secondary growth was found to be negligible and xylem consisted primary xylem and few secondary tracheids. Vessels were found to be absent. Primary xylem of all the species consisted of radially arranged protoxylem & metaxylem, xylem parenchyma and fibres. Patches of intraxylary phloem below protoxylem were observed in all the species. The phloem patches were found to be deeply situated in pith in some of the species. Some laticifers were found associated with intraxylary phloem and among pith cells. Laticifers were found to be absent in pith of stem of C. sahyadrica while they were found abundant in pith region in succulent species such as C. juncea and C. bulbosa. The cells of pith of all the species contained starch grains however in C. sahyadrica and succulent species such as C. juncea and C. bulbosa starch grains were not observed in pith cells.

Among the species studied <u>C. juncea</u> which has succulent stem and reduced leaves showed different anatomical characters. In the stem cellulosic fibres were found to be absent in cortical region which is a constant feature in other species. Vascular tissue was found to be very much reduced and most of the tissue consisted of water storage chlorenchyma. Many laticifers were found to be present in pith but starch grains were found to be absent from all the cells of stem.

Species of <u>Brachystelma</u> showed similar anatomical characters as that of non-succulent species of <u>Ceropegia</u>.

Aerial parts of all the species except C. juncea are annual and secondary growth is limited. Cork found in many members of Asclepiadaceae (Metcalfe and Chalk 1957, Mitra et. al. 1974, Kapoor et. al. 1975, Sabnis 1977, Gupta and Wahl 1978, Gupta 1985) is not developed in any species of Ceropegia or Brachystelma. Collenchyma found in cortex of various members of Asclepiadaceae was not observed in Ceropegia and Brachystelma. Cellulose fibres found in Asclepiadaceae members were found in both the genera except C. juncea. The absence of cellulosic fibres from the cortex of Cynanchum edule Jumelle et. Perr. but present in other members of the genus was reported by Puech (1912). In Ceropegia deeply seated intraxylary phloem was observed in majority of species. Deeply seated phloem in Ceropegia is also described by Metcalfe and Chalk 1957). Similarly some unlignified vascular elements were observed in some species of Ceropegia. Unlignified elements are also reported in the genus by Metcalfe and Chalk (1957). Presence of laticifers, a characteristic of Asclepiadaceae were found in stem tissues such as inner cortex and pith.

#### Petiole anatomy

All the species of Ceropegia and Brachystelma showed similar petiole anatomy with some minor variations. Transection of petiole is concavo-convex structure, however C. bulbosa var. lushii showed V-shaped and C. juncea showed planoconvex shape. The epidermis consisted of thick-barrel-shaped cells. Some of epidermal cells in some species produced uniseriate multicellular nonglandular trichoms. The epidermal cells on ventral side are comparatively larger than on the dorsal side. In ventral groove epidermis is followed by a group of collenchymatous cells. Similarly inside dorsal epidermis there are 2-4 layers of collenchyma. The amount of collenchyma on ventral and dorsal side varies with species. The stele consists of one central large bundle and 2 lateral small bundles. The central bundle is crescentic and bicollateral. The rows of tracheids of central bundle varied with species. Below central bundle 1-3 layers of parenchyma contained starch grains while lateral bundles were encircled by starch sheath. Laticifers were found abundant between ventral collenchyma and ventral phloem. Rest of the tissue of petiole consisted parenchyma. In succulent species all the parenchymatous cells contained chloroplasts and lacked starch sheaths around lateral and below central bundle. Additional lateral vascular bundles were observed in C. bulbosa, C. juncea C. huberi and Brachystelma species.

Petiole in transections exhibited a crescentric, bicollateral vascular strand in all investigated material, notably in species of Asclepias, Calotropis, Ceropegia, Chlorocodon, Cryptostegia

<u>Gorophocarpus</u>, <u>Leptadenia</u>, <u>Marsdenia</u>, <u>Periploca</u>, <u>Stephanotis</u> and <u>Vincetoxicum</u> (Sayeedud-Din and Suxena, 1940) small lateral accessory strands in wings of petiole have been noted by them in <u>Ceropegia</u>, <u>Cryptostegia</u>, <u>Gomphocarpus</u>, <u>Leptadenia</u>, <u>Marsdenia</u>, <u>Pergulavia</u>, <u>Tephanotis</u> and Vincetoxicum.

On the basis of petiole anatomy different species of <u>Ceropegia</u> can not be identified because they exhibit more or less similar structure, however in many other families and genera petiole anatomy was found to be useful in taxonomy (Metcalfe and Chalk, 1957, Howard 1962, Lems 1905, Decker 1967, Schofield 1968, Banerji 1974).

### Leaf anatomy

On the basis of leaf anatomy species of <u>Ceropegia</u> could be grouped into three groups i) species with membranous nonsucculent leaves, ii) species with succulent leaves and iii) species with reduced scale-like leaves. The general anatomical characters remained more or less same for each category.

The membranous nonsucculent leaves of <u>Ceropegia</u> species were found to be dorsiventral and hypostomatic. The cells of both epidermises are covered with smooth or striated cuticle. The cells of upper epidermis in transection were found to be larger than the cells of lower epidermis. In midrib region the cells of both epidermis are cubical and smaller than other epidermal cells. In midrib region a group of collenchymatous cells was found to be present below upper epidermis. The amount of collenchyma varied with different species. In the centre of midrib region there is an

arc of crescent-shaped vascular bundle. The vascular bundle was found to be isobilateral. In midrib region 2-4 layers of collenchyma were found inside lower epidermis. The collenchyma becomes single layered on lateral sides. Remaining tissue of midrib is made up of polygonal parenchymatous cells containing no chloroplasts. Laticifers were found more numerous between upper epidermis and ventral phloem. In lamina region the mesophyll is well distinguished into single layered palisade and 2-4 layered spongy tissue. Inbetween these two tissues are found traces of vascular bundles. Continuity of lower epidermis is broken by stomata.

In above category fall the species such as <u>C. attenuata</u>, <u>C. hirsuta</u>, <u>C. huberi</u>, <u>C. jainii</u>, <u>C. maccannii</u>, <u>C. media</u>, <u>C. oculata</u>, <u>C. sahyadrica</u> and <u>C. vincaefolia</u>. Variation in leaf anatomy was observed in presence or absence of trichoms, amount of <u>Collenchyma</u> size of central vascular bundle, size of epidermal cells and type of cuticle. <u>C. attenuata</u>, <u>C. hirsuta</u>, <u>C. jainii</u> and <u>C. noorjahaniae</u> possessed striated cuticle and trichomes on leaf.

<u>C. bulbosa</u> var. <u>bulbosa</u>, <u>C. bulbosa</u> var. <u>lushii</u> and <u>C.</u> <u>woodii</u> fall under the second category of succulent leaves. Both the species showed isobilateral amphistomatic leaves. Trichomes were found to be absent from both the epidermises. The cells of both epidermis were found to be of similar size and shape. Coller.chyma found below upper and lower epidermis in membranous leaved members was found to be absent in these species. Both upper and lower cuticle was found to be prominently striated. The vascular arc was

found to be reduced and mesophyll consisted of oval parenchymatous cells containing chloroplasts. All the cells including midrib region contained chloroplasts. The major tissue of leaf was water storage chlorenchyma.

<u>C. juncea</u> fall under third category in which leaves are either absent or reduced to scaly leaves. Photosynthetic function is taken by stem. The leaves were found to be isobilateral and amphistomatic. The vascular tissue was found to be very much reduced. Collenchyma was found to be absent both below upper and lower epidermis. The major tissue of leaf consisted of water storage tissue with chloroplasts. Both epidermises showed prominent striations.

Leaf anatomical characters of <u>Brachystelma</u> species differed from that of <u>Ceropegia</u>. The leaves are dorsiventral as that of <u>Ceropegia</u> species with membranous leaves but the palisade layers in <u>Brachystelma</u> varied from 3-4 in number against single layered in membranous leaved <u>Ceropegia</u> species. Secondly the leaves were found to be amphistomatic as seen in succulent species of <u>Ceropegia</u>. Cuticle was found to be prominently striated on both epidermises.

In Asclepiadaceae the leaves are usually dorsiventral, however isobilateral leaves are reported in succulent species of <u>Ceropegia</u> and <u>Hoya</u> and in membranous leaves of <u>Marsdenia</u> (Metcalfe and Chalk, 1957) and according to Sabnis (1919, 1920, 1921) in <u>Leptadenia spartium</u> wt. Colonval-elenkov and Malaisse (1984) studied leaf anatomy of 13 species of Ceropegia from shaba. In all the

species studied by them except <u>C. illlegitima</u> leaves were found to be dorsiventral. Most of the species studied by them possessed single layered palisade, however, <u>C. umbraticola</u>, <u>C. kundelunguensis</u>, and <u>C. nilotica</u> showed 2-3 layered palisade. Leaves were found to be hypostomatic in 5 species and aphistomatic in 8 species studied by them. Collenchyma was found to be absent in 6 species out of 13 species studied by them. Their observations make possible the establishment of a key guide to sterile material. According to them the leaf anatomy is a subject of secondary importance whose usefulness is limited.

Among species of Ceropegia studied by author, C. bulbosa and C. juncea are peculiar in the stem and leaf anatomy. C. juncea possesses peculiar stem and leaf anatomy while C. bulbosa differ in their leaf anatomy from species with membranous leaves. Recently Gaikwad et. al. (1989) have reported CAM in stem of Ceropegia juncea and Supate et. al. (1990) in leaves of C. bulbosa var. bulbosa and C. bulbosa var. lushii. The occurrence of CAM has been reported in several members of the family Asclepiadaceae (Nuernbergk 1961, Milburn et. al. 1968, Szarek & Ting 1977, Szarek 1979). According to Bruyns (1986) the highly succulent reduced stem and the greatly reduced leaves are advanced characters in some Ceropegia species. The development of CAM features in the stem tissue of C. juncea and leaf tissue of both varieties of C. bulbosa represents a major metabolic adaptation. In stem and leaf succulent species such as C. dichotoma, C. fusca, C. illegitima, C. superba, C. woodii there is strong possibility of operating CAM.

C. juncea grows in Badami areas of Karnataka where aridity prevails. It is also interesting to note that Lakshminarayana and Krishnappa (1988) has reported hexaploidy i.e. 2n=66 chromosomes in C. juncea. Polyploidy has been also reported in C. debilis and C. woodii showing tetraploid 2n=44 (Bolkovshish et. al. 1969) It is evident from the literature that polyploid is found in succulent species such as C. juncea, C. woodii, and C. debilis however, C. bulbosa was found to be diploid 2n=22. All other species with membranous leaves showed 2n=22 (Sundara-Rhaghavan & Ansari, 1975) Lakshminarayana and Krishnappa (1990) studied karyomorphology of 11 members of Asclepiadaceae and found that 10 taxa to be diploid with 2n=22 chromosomes whereas Secamone emetica R.Br. showed hexaploidy 2n=66 chromosomes. Arekal and Ramakrishna (1981) have reported 22 as diploid number of chromosomes in Brachystelma B. ciliatum. Thus basic chrosome number kolarenses and for Ceropegia, Brachystelma and majority of Asclepiadaceae seems to be x = 11.

# Cuticle

Cuticular studies of 13 species of <u>Ceropegia</u> and 2 species of <u>Brachystelma</u> revealed that cuticle exhibit many important characters which are of diagnostic value and different species of both the genera could be identified on the basis of cuticular characters such as presence or absence of stomata in upper epidermis, presence or absence of trichomes, cuticular striations; shape of epicermal cells and type of stomata. It is interesting to note that all the nonsucculent species of <u>Ceropegia</u> have dorsiventral and hypostomatic leaves. Dorsiventral hypostomatic leaves were found in <u>C. attenuata</u>, <u>C. hirsuta</u>, <u>C.</u> <u>huberi</u>, <u>C. jainii</u>, <u>C. maccannii</u>, <u>C. media</u>, <u>C. noorjahaniæ</u>, <u>C.</u> <u>oculata</u> and <u>C. vincaefolia</u>. On the other hand all the succulent species showed isobilateral amphistomatic leaves. Trichomes were found to be absent from leaves of all succulent species. On adaxial surface the epidermal cell-walls were found to be straight walled in all the species except in <u>C. media</u> and <u>C. bulbosa</u>. On abaxial surface the epidermal cells walls were found to be more or less wavy in all the species except <u>C. attenuata</u>, <u>C. bulbosa</u> var. <u>lushii</u>, <u>C. juncea</u> and <u>C. woodii</u>. The stomata were usually found to be paracytic, however in <u>C. bulbosa</u>, <u>C. huberi</u>, <u>C. maccannii</u>, <u>C. oculata</u> and <u>C. vincaefolia</u> anomocytic stomata were also observed.

Cuticular striations were found to be present in all the species with membranous leaves except <u>C. media</u>, <u>C. oculata</u> and <u>C. vincaefolia</u>. Cuticular striations were found to be present on both epidermis in <u>C. jainii</u>, <u>C. attenuata</u>, <u>C. noorjahaniae</u> and <u>C. hirsuta</u>, but they were found to be more prominent on upper surface. Striations of <u>C. jainii</u> were found to be very prominent and characteristic to the species (Plate XVII, Fig. 121). It also showed very high stomatal frequency on lower epidermis. <u>C. noorjahaniae</u> also showed prominent striations peculiar to the species (Plate XVII, Fig. 121). All the erect species growing in comparatively xeric conditions showed prominent striations. All of them possessed trichomes on at least upper surface. <u>C. hirsuta</u> which grows in shruby forests ir. low

rainfall areas showed prominent striations and trichomes. Stomatal frequency was found to be higher in these species.

In <u>C. maccannii</u>, <u>C. huberi</u> and <u>C. sahyadrica</u> striations were only found in cells around hair bases. Stomatal frequency was found usually more than  $200/mm^2$  in these species.

Striations were found to be absent completely in <u>C</u>. <u>vincaefolia</u>, <u>C</u>. <u>media</u> and <u>C</u>. <u>oculata</u>. These species usually grow in deep shades in high rainfall areas. Stomatal frequency was found to less than  $200/\text{mm}^2$  in all the three species.

Prominent striations on both epidermises were found to be present in all the succulent species. Stomatal frequency was found to be very low in succulent species as compared to other nonsucculent species of Ceropega.

Brachystelma species showed membranous, amphistomatic leaves. The striations were found to be prominent on both surfaces.

Epidermal characters are considered as having considerable potential in systematics and may also aid in phylogenetic interpretations (Edward 1935, Water 1953, Prat 1960, Borill 1961, Stace 1961, 1965a,b; 1969a,b, 1973, Ramayya and Rajagopal 1968, 1971, Jain and Singh 1974a, b, Singh and Jain 1975).

Cuticular striations have been studied by various workers and found to be of taxonomic value in Apocynaceae (Chandra et. al. 1969, Chandra et. al. 1972, Kapoor et. al. 1969, Sharma et. al. 1970, (Singh in Mitra et. al. 1978) Rosaceae and Jain. 1975) in Asclepiadaceae (Krishnamurthy & Sundaram 1967, 1970) in Combretaceae (Stace, 1961).

Crystals were found associated with wall of upper epidermis in <u>C. hirsuta</u> all other species lacked them. Sphaerocrystals of calcium phosphate have been also recorded in spirit material of Ceropegia and Stapelia by Metacalfe and Chalk (1957).

## Trichomes

Trichomes in Ceropegia and Brachystelma are found to be simple, nonglandular, uniseriate and multicellular. The size of trichomes varied with species. The largest trichomes upto 21 cell in height were found in C. hirsuta and upto 16 cell in height in C. vincaefolia. In both species longest trichomes were found on The presence or absence of trichomes on various parts peduncle. of aerial organs of Ceropegia was found to be of taxonomic value. Similarly the form of trichome-straight or hooked was found to be of taxonomic value in distinguishing Brachystelma from Ceropegia. Brachystelma possessed hooked short trichomes while Ceropegia and straight trichomes. Most of the species of possessed long Ceropegia could be indentified on the basis of number of cells in trichomes & their presence or absence on different parts.

The identification value of trichomes has been recognised by many workers (Small 1913, Zorning and Buch 1926, Singh et. al. 1974, Ramayya 1969, 1972, Singh et. al. 1974 etc.). Colonval-elenkov and Malaisse (1984) has also used presence or absence of trichomes on leaf as taxonomic character to distinguish <u>C. meyeri-johannis</u> and <u>C. muzingana from C. stenantha</u> and <u>C. purpuraseens.</u>

#### Starch grains

The starch grains are excentric and show striations around central nucleus. They are of common occurrence in Ceropegia and Brachystelma species. It seems to be major food reserve in both the genera. Tubers are the major organs which store starch grains, however, starch grains were found in almost all aerial parts of nonsucculant species of Ceropegia and Brachystelma. It is interesting to note that in nonsucculent species of Ceropegia and species of Brachystelma starch was found to be present in cortical and pith cells of stem, below central bundle in leaf and petiole and around lateral bundle in petiole, however, it was found to be absent in all above parts in succulent species of Ceropegia such as C. juncea, C. woodii, C. hulosa. Both the varieties of C. bulbosa, and C. juncea have Crassulean Acid Metabolism (Gaikwad et. al. 1989, Supate et. al. 1990) and author feels that the presence or absence of starch grains in stem, petiole and leaf could be used as one of the anotomical characters to distinguish CAM species from non-CAM species at least in genus Ceropegia.

MIVAJI UNIVERSITY, KOLHAPUR,