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DISCUSSION

**5.1.1 Taxonomy and distribution:**

A number of workers described *B. mollis* from time to time. But none of the descriptions provided was comprehensive as these were based only on the male plant except by Hua and Thomas (2008). As the material collected by Wallich (1873) was a twig of male plant only, therefore, the first ever description given by Kurz (1875) was incomplete. He described leaf, petiole, inflorescence and flower of a male plant with the description of fruit. Bennett (1875) provided a comparative description of leaves and fruits of *B. mollis* and *B. javanica*. He also appended the description of the inflorescence and fruit by defining the raceme as axillary panicle having many flowered secondary cymes and the fruit by adding size, colour and texture. The subsequent description of Kurz (1877) was also incomplete. He described that the inflorescence length is much shorter than the leaf length, petals ciliate towards the base which is not reported in any of the subsequent works and the present authors also have not recorded ciliated petals either. Kurz also (1877) noted the larger green disc than the glabrescent ovary and broadly linear, deflexed style which infers that he observed the female plants also but had not included the description of the male and female reproductive parts comprehensively.

There is a series of publications by Merrill (1906, 1908, 1915 and 1917) and Li (1943) describing *B. luzoniensis* Merr., *B. membranacea* Merr., *B. macrobotrys* Merr., *B. stenophylla* Merr. and *B. acuminata* as new species which were subsequently considered conspecific to that of *B. mollis*.

Observations of Vidal (*cf.* Merrill, 1906) and Merrill (1906) himself appeared to be inconsistent. *B. membranacea* Merr. was reported to be 5-6 m tall while *B. mollis* is only 2-3 m tall. Merrill (1906) mentioned as leaves alternate, margin sub entire or toothed and are strikingly different from *B. mollis* where leaves has 1/3 phyllotaxy and leaf margin is entire or sometimes wavy. It was also reported by Merrill (1906) that the shorter inflorescence length *i.e.* only 10 cm in *B. membranacea* is strikingly different from *B. mollis* where male inflorescence of 15-163 cm long and female inflorescence of 16-40 cm long have been observed by the present authors. This indicates that he might have described the features of inflorescence from a female plant or from both male and female plants without considering the fact that *B. mollis* is dioecious species. Further, he also described that in *B. membranacea* both the staminate and pistillate flowers are similar but provided only a brief description of stamen. In the description of Merrill (1906) neither the differences of male and female flower nor the differences of inflorescences of male and female plants were provided rather general accounts of flowers and inflorescence were given. He also provided a note stating that in both *B. membranacea* and *B. luzoniensis* have short inflorescence along with the differences in both the species.

Merrill and Rolfe (1908) examined the materials collected from Luzon and compared with the Indian materials of *B. mollis* at Kew and concluded that *B. luzoniensis* cannot be maintained as a separate species from *B. mollis* and considered as conspecific. This implies that *B. mollis* exhibits morphological variability with a series of transitional forms.

Lecomte (1911) briefly described leaf, inflorescence, petals and fruit of *B. mollis* collected from Vietnam. But he neither provided any measurements nor any

distinctive features of the reproductive parts. On the basis of only much developed cyme than that of true variety he also described the new variety of *B. mollis* viz., *B. mollis* var. *tonkinensis* H. Lec. This implied that he established the variety based on male inflorescence only.

In 1915, Merrill described another species *B. macrobotrys* which differ from *B. mollis* in having leaflet number nine and larger leaflet size of 12-17 cm X 5-6cm. But in the present study it is found that these variations in leaflet number and size are common features not only between the male and female plants but also in a plant of either sex in *B. mollis*. However, he pointed out for the first time about the presence of marginal glands and may represent incipient teeth. He noted that the length of inflorescence is 80 cm without considering dioecious nature of the species and the observations made by the present authors confirmed that only inflorescences of male plant can be so long. Interestingly, Merrill (1915) described the male flower as having 3 sepals, a feature neither recorded by the present workers nor by any of the earlier workers.

Merrill (1917) again described *B. mollis* as *B. stenophylla* with a brief description about branch, branchlet, leaf, leaflet and inflorescence. Though the description has many similarities with *B. mollis* but some noticeably different and unusual characters were given. Later, he again mentioned the fruit size as 12 mm in the same description. This work was prepared casually and the author was confused about the proper taxonomic identification and relatedness of the species with *B. mollis*, *B. luzonensis* and *B. membranacea*.

Li in 1943 described *B. mollis* as *B. acuminata* on the basis of difference in size and shape of leaflets and length of inflorescence. In his description he mentioned the leaflet number 15 and inflorescence 15cm long. However, he added a

note that though the new species resembles with *B. mollis* reported from India and its variety *B. mollis* var. *tonkinensis* Lecomte being recorded from Indo-China and south China are significantly different from the both in having smaller and narrower leaflets and slender inflorescence (Li, 1943).

Nootebom (1962) described taxonomy, habit, distribution and ecology of *B. mollis* which mostly coincides with our findings. However, he mentioned some doubtful characters such as 1-8m high shrub which is not noticed by the present authors. His description about flower mentioning as similar to *B. javanica* is highly contradictory as flower of *B. javanica* is monoecious bisexual and that of *B. mollis* is dioecious unisexual. As he mentioned further about the flower colour as white, creamy, green or red is also doubtful as red coloured flower is never found in *B. mollis* and about white and green colour of flower is the indication of his confusion between male and female plants. Brandis (1906) briefly described the leaf and fruit of the species only. Hua and Thomas (2008) briefly described about branch, leaf, leaflet, petiole, inflorescence and both male and female flowers. He also mentioned that disk is flat and globose in male whereas shallowly disk shaped in female which is also observed by the present authors.

From the analysis of facts and the detail studies carried out by the authors on *B. mollis* it is evident that the species has so far been described partially because most of the earlier workers have eluded the dioecious nature of the species.

The distributional pattern of the species clearly shows the effects of available ecological conditions and several biological factors such as Xenogamy, inefficient pollen and seed dispersal and crucial phenology of flowering. The species also exhibits combination of both the primitive and advanced characters inferring a transitional state in the evolution. Moreover, *B. mollis* has been facing threats due to

habitate distruction and other anthropogenic factors, which is resulting in shrinkage of the germlasm of the species. Thus, the endemics is intermediate between paleo and neo as plants which are not of recent origin but have retained a narrow distribution and can be regarded as Holoenedemic (Richardson, 1973) and are extinction-pronged, due to conversed radial adoption (Ahmedullah and Nayar, 1986).

### **5.1.2 Leaf Morphology:**

Leaflet number, size and shape in the same plant or between and among the male and female plants within the species *B. mollis* are significant and can be used as taxonomic criteria either for identification of the species or for further intraspecific categorization of the species. There are four distinct variations are there in leaves bearing 7, 9, 11 and 13 leaflets. The same plant may bear leaves with different numbers of leaflets. Similarly, leaflet shape ranges from lanceolate to obovate or even bilobed or round. Blade classes of the leaves are either microphyll or nanophyll. The variation in size and number (5-12) of secondary ( $2^0$ ) veins of leaflets might have bearing to leaflet number and vice versa.

Leaflet margins of *B. mollis* are characteristically either entire or undulating which appears to be in contrast with the description of the leaflets of the species described as toothed, bluntly serrate or crenate by Steenis (1950) (Kakati and Barthakur, 2016). This characteristic feature of the leaflets distinguishes the species from *B. javanica* where it is distinctly toothed, serrate or crenate. This feature of leaflet margin is an important character for the quick differentiation of both the species.

### 5.1.3 Leaf Architecture:

The plasticity of venation varies in different species and sometimes in a species with the variation in leaf architectural features as pointed out by Arber (1950) in *Campanula rotundiflora* L. and *Gaya lyallii* E.G. Baker, which show a range of palmate to pinnate venation even within a single plant. Similarly, Carr *et al.* (1986) observed that two fundamentally different venation patterns co-exist within the genus *Eucalyptus*, and sometimes even within a single species

Angiosperms have greatest diversity in vein structure but hierarchy of vein orders forming a reticulate architecture are fundamental features without any variations (Hickey, 1973; Gourlay *et al.*, 2009). Leaf venation in angiosperm varies both in pattern (Hickey 1973) and regularity (Hickey & Doyle, 1972). According to Pray (1954), the veins of first, second and third order form major venation pattern and those of subsequent orders constitute minor venation patterns. Three lower order veins constitutes 'major veins' viz. first order veins run from the petiole to the leaf apex, second order veins branching at intervals, and third order veins branching between. All are often ribbed with sclerenchyma (Esau, 1977). The patterns formed by the venation have secondary importance in Hickey's scheme, but Pole (1991) suggests it is the behaviour of the veins which expresses their identity, not their thickness. The underlying principle of his approach is to look at leaf venation as a coherent configuration or set of patterns formed by groups of elements rather than the behaviour of individual elements. He also considered the growth and developmental view of leaf by Thompson (1952) and Arber (1950) as a conceptual tool for ordering the patterns of leaf venation. Veins of equal width along their length which terminated against a lower order vein at each end and presence of "percurrent" tertiaries indicates that leaves of *B. mollis* is simultaneous as per the

terminology suggested by Pole (1991). Besides providing taxonomic criteria it also determines the type and function of plant resistance against environmental elements (Zarinkamar and Sharifsadat, 2016).

Vein systems have been classified with 'types' for each order. In *B. mollis*, the major venation pattern is pinnate type, first order vein type includes brochidodromous, second order vein type include percurrent or cascade and third order vein type is lattice. The classification proceeds with progressively higher order veins until reach areolation where the vein system terminates into fine 5° branching within the areola.

According to the terminology proposed by Hickey (1973) the 3° venation (second order venation) pattern is 'Percurrent sinuous venation' as it repeatedly changing the direction of curvature. But following terminology modified by Pole (1991) the 3° venation can be termed as 'Percurrent joining veins' as they join two adjacent lateral veins. It cannot be termed as interangular veins as 3° veins do not join lateral veins with the midrib directly. Here, a special case was recognized by Corner (1958) where interangular veins are not directly percurrent, but are staggered so that the end of a vein originating at the midrib terminates along a vein originating on a lateral, and vice versa. However, according to Pole (1991) such type of pattern occurs due to the typical growth initiated in the angle between midrib and laterals. But, in *B. mollis* formation of ununiform cascades with higher order venations is noticed. From the present study it can be inferred that a more comprehensive terminology to describe such type of venation pattern in angiosperm in general and *B. mollis* is yet to be coined to utilize taxonomic criterion.

According to Pole (1991) third order venation refers to repeating patterns of venation consistently found between two percurrent veins. But, in *B. mollis* the third

order venation is lattice type. It is formed by a zigzag vein connecting two rows of offset veins, but there is no marked trend in the size of its constituent veins in any particular direction, and it is not oriented towards the axil between two major veins.

Loops present in the lamina of *B. mollis* have been studied following terminology suggested by Pole (1991). In lateral loops midrib forms the base, and the arch include first order lateral, first order external and second order external. First order external loops have first order externals as their origin and are based on first order laterals. Arch comprises of first and second order externals. Second order external loops are based on first order external forming arch with first and second order externals. Third order external loops are based on a second order external comprising the arch with third order externals. Internal basisopic loops are also there. They may arise from midrib, first order lateral, first order external or second order external forming arches with extralaterals, first order laterals, first order externals and second order externals. Lateral and external loops do not show any particular distortion and are elongated widthwise *i.e.* perpendicular to the midrib. Internal basisopic loops may be elongated both widthwise and lengthwise *i.e.* parallel to the midrib or randomly occur within lateral loop.

In *B. mollis* the areoles are variable in size and shape *viz.* triangular, tetragonal or pentagonal in outline and with dichotomously branched vein endings. No areole has been seen without any veinlets or loop like structure. Therefore, dichotomously branched vein endings are a constant feature of taxonomic significance in *B. mollis*.

Nicely (1965) reported significant variations within the same leaf as regards the size and shape of areoles and number of vein endings in each veinislet. According to Inamdar and Shenoy (1981) the number of vein endings is not



correlated with the size of the areole, as the nearby areoles even though more or less equal in size vary in their number of vein endings. They used the term 'enlarged terminal tracheary idioblasts' for dilated enlarged terminal tracheids at the vein endings and in intimate continuation with the underlying normal or conventional tracheary elements. Thompson (1912) and Scheit (1883) reported occurrence of transfusion tracheids in *Ephedra altissima*. Scheit (1883) termed them 'tracheid border'. Subsequently, Foster (1972) also reported the presence of such cells in *Ephedra foliate* Boiss. and *E. altissima* Desf.. The occurrence of transfusion tracheids perhaps attributed to adaptive features. However, their presence in specific leaves alone indicates the localised need to compensate and assist the water distributing system (Inamdar and Shenoy, 1981). Sack and Scoffoni (2013) reviewed the structural and functional role of tracheids as accessory transport and supporting tissue. In *B. mollis* there is no evidence of isolated veins and isolated tracheids within the areole. But terminal tracheids and transfusion tracheids are prominent. They increase the mechanical strength of the leaf and prevent shrinkage during dehydration. Their presence may infer the development of adaptive strategy for better distribution system of water in the plant. They may act as water storage to regulate the water potential to cope with high transpiration.

Development of the lamina is disproportional in basal, suprabasal or interlateral segments in *B. mollis*. Therefore, it can be recognized as acrodromous development which is a common pattern of development in camptodromous venation (Pole, 1991).

There are two main functions of leaf venation- transport of substances and mechanical stabilization. The thick and mechanically stiff midribs and the

architectural structure of leaf venation in *B. mollis* perform these two major tasks and other functional properties. The variations in number, size and shape of leaflets in the plant may be due to the environmental pressure as the plants are growing along hill slopes where soil water is usually scanty. Therefore, variation in venation pattern is inversely proportional to the number, size and shape of leaflets in *B. mollis*.

#### **5.1.4 Micromorphology of trichome:**

Taxonomic significance of structural diversity of trichomes in plants has been emphasized by a number of workers (Bureau and Schumann, 1897; Seibert, 1948; Gentry, 1980; Vilhena –Potiguara *et al.*, 2012; Nogueira *et al.*, 2013; Gama *et al.*, 2013). Trichome characters in medicinal plants have also been reported to act as biomarkers for identification (Ragusa *et al.*, 2001, Gohil *et al.*, 2007, Jayeola, 2009). The presence of glandular trichomes in medicinal plants is correlated with the concentration of secondary metabolites with pesticidal, pharmacological, and fragrant properties (Duke, 1994).

The present study examined the structural features of the trichomes of leaflet, petiole and rachis of *B. mollis*. Trichomes in *B. mollis* are unbranched *i.e.* simple type consisting of both glandular and non glandular trichomes. They may be unicellular or multicellular. There are twenty different types of trichomes are observed in *B. mollis* including both glandular and non glandular trichomes. Non glandular trichomes are of four types – short unicellular, short multicellular, long unicellular and long multicellular. Glandular trichomes are of eight types- capitate, stipitate, peltate, fusiform, cupular, patelliform and digitate. The glandular trichomes are the primary sites of biosynthesis, secretion and storage of secondary metabolites (Weiss, 1997). In the present study capitate trichomes are found to be sessile or

stalked. Following the terminology of Abu-Asab and Cantino (1987) the simple glandular trichomes of *B. mollis* can be termed as capitate with short stalk and stipitate with stalk longer than the half the head length. Head of stalked capitate trichomes may be either smooth or folded or wrinkled or calcified. Stipitate glandular trichomes may or may not have swollen basal cell. Peltate trichomes are of four types depending on its head cell assembly. It may have calcified, smooth, micropapillate or wrinkled head. Wrinkled headed peltate trichomes may be sunken or sub sessile.

Distribution of unicellular trichome is restricted to lower epidermis of leaflet and petiole while that of multicellular trichome is abundant in foliar epidermis, petiole and rachis. Glandular trichomes are more abundant in leaf than in petiole and rachis. Petiole has two types of glandular trichomes *viz.* capitate stalked glandular trichome with smooth or folded or wrinkle head and stipitate glandular trichome without swollen basal cell. There is no glandular trichome observed under LM in rachis. This indicates that the active principle responsible for the therapeutic property of the plant might be synthesized in the leaflet, petiole and rachis of *B. mollis*.

Presence of patelliform and cupular trichomes in leaf blades infers the presence of nectaries (Fróes *et al.*, 2015). Presences of these glands are abundant in adaxial and abaxial leaf surfaces. This infers that presence of large number of ants on leaflets is possibly due to the presence of patelliform and cupular trichomes.

Extrafloral nectaries play an important role in plant insect relationship, but the trichomes may also provide defensive strategies (Fróes *et al.*, 2015). Stipitate trichomes may secrete a sticky substance which was observed by Nogueira *et al.*,

(2013). Therefore, presence of stipitate trichome in *B. mollis* may also have some defensive role.

Franceschinelli *et al.*, 2017 has preclude the criteria of taxonomic separation of papillate and non-papillate species groups in the genus *Simarouba Aublet* (Simaroubaceae) depending on the presence of papillate trichomes on the abaxial leaf surface, as in *Simarouba amara* both types of trichomes can be observed in scattered or reduced form. In the present study no papillate trichome has been recorded in *B. mollis*. Rather some simple non glandular trichomes exhibit micro-ornamentation of micro papillae.

The present study reflects that the diversity in the type and distribution of trichomes in *B. mollis* can be used in solving the taxonomic problem in generic level in *Brucea* as well as in species level in *B. mollis*. It may also provide taxonomic insight into intraspecific level of *B. mollis* at population level. The results emphasizes the correlation of distribution and types of glandular and non glandular trichomes of *B. mollis* with the site of production and storage of active principle of medicinal property, plant insect relationship and probable defensive strategy adopted by the plant. These inferences could have important significance in understanding the taxonomic relationship between and within the species of *Brucea* in future studies.

#### **5.1.5 Micromorphology of RMGN:**

Despite of the potent antimalarial activity of the plant the crucial microstructures that act as a site for the secondary metabolites are not known. This is the first comprehensive study of the micro morphological and anatomical features of the raised marginal nodule present in the leaf margin of *B. mollis*. In Flora Malesiana (1950) various aspects of the family Simaroubaceae related to the taxonomic relatedness of this family to others were briefly described.

Simaroubaceae is closest related to the family Rutaceae. The bitter principle of Simaroubaceae (except Irvingioideae and Surianoideae) is also present in some members of Rutaceae and Meliaceae (*Trichilia*). There are many striking resemblance in phytochemical constituents of Simaroubaceae with Rutaceae and Umbelliferae. Steenis (1950) mentioned the presence of another common character *i.e.* concave or flattish but sunken spot glands on the adaxial surface of the leaves in the members of Simaroubaceae which are also found in meliaceous genus *Trichilia*. The presence of large marginal gland just beneath the leaf margin in a row at the tip of the 2° and some 3° veins of *B. mollis* corroborated the observations made by Merrill (1915).

The schizogenous marginal gland of *B. mollis* has striking similarity with the characteristic schizogenous secretory cavities of *Haplophyllum telephioides* in the process of its origin and development. Both originate with the development of a schizogenous sac gap lined by secretory cells. The gap continues to widen forming a hollow schizogenous sunken gland in *H. telephioides*. But in *B. mollis* the schizogenous sac get filled up with translucent secretory cells and continues to grow beyond the adaxial epidermis as a raised nodule. As a result the glandular nodule of the *B. mollis* becomes submerged or half sunken in leaf mesophyll tissue and visible as a raised gland in the adaxial surface.

Liu and Hu (1998) extensively studied the secretory cavities present in the members of Rutaceae and suggested three types – notch type, mesophyll type and mixed type. Considering the study of Liu and Hu (1998) raised glandular nodules of *B. mollis* can be classified as mesophyll type.

Dark glands or black nodule present beneath the leaf margin of *Hypericum perforatum* and *H. perforatum* (Clusiaceae) (Curtis and Lersten, 1990; Perrone *et*

*al.*, 2013) share few similarities with raised glandular nodule of *B. mollis*. Though both are different in their morphoanatomy, origin, and development but both have at least one major or minor vascular bundle running to them and ending at their base. According to Green (1884) 'black glands' in *H. perforatum* had a different and special relationship with vascular bundles, especially with vein endings. This relationship raises a confusion of transpiratory function of this gland. However, the findings of Curtis and Lersten (1990) regarding black dots in the leaf margins of *H. perforatum* did not supported Green's statement as they found that the black dots typically get surrounded by an irregular vascular reticulum which includes one or more vein endings. Other similarities are that both are filled with secretory cells and are separated from the adaxial epidermis by a flattened parenchyma cells. Like *Hypericum perforatum* and *H. perforatum* the RMGN of *B. mollis* also exhibits certain periodicity of 3:1.

Clark (1990) reported that undersurface of the leaf of *Walsura trichostemon* shows one glandular body (Ohn Shwe 5037; Burma). Small (100-310 $\mu$  diameter) prominent circular black bodies were observed by Clark in species of *Walsura*, *Heynea* and *Ekebergia*. They were often present near midrib or sporadically distributed over the lamina. These structures were recognized by them as extrafloral nectaries. The internal secretory cells reported by Solereder (1908) in members of *Trichilieae* and by Pennington (1981) in species of *Trichilia* were quite different from EFN of *Walsura*, *Heynea* and *Ekebergia*. Their function is unknown and considered to be quite different. Though Bentvelzen (1962) reported occurrence of black glands on the undersurface of leaf in *Hynea* and one unidentified species of *Trichilia*, but Clark (1990) found that the gland is common in *Walsura* and *Hynea*

but rare in *Trichilia*. However the glandular structure in *B. mollis* has similarities with that of the *Walsura* in having a no porous opening to the outside.

Another interpretation of RMGN of *B. mollis* shows similarities with the extrafloral nectaries (EFN) present in the rachis of *Erythrophleum* (Pascal *et al.* 2000). Leaves of *B. mollis* are often visited by black ants which indicate the presence of extrafloral nectaries in the plant. EFN of *Erythrophleum* is also dome shaped but with a central pore (Pascal *et al.* 2000). The base of the gland is surrounded by large number of eglandular trichomes like in the RMGN of *B. mollis*. Vascular tissue is separated from the EFN of *Erythrophleum spp.* by a thin layer of sclerenchyma except *E. suaveolens*, allowing the passage of materials from vascular tissue to secretory cells (Pascal *et al.* 2000). Secretory tissue in RMGN of *B. mollis* and *E. suaveolens* is separated from the vascular tissue by a thick layer of parenchyma indicating less expensive vascular supply to the gland. Though the EFN function of the gland in *B. mollis* is not clear but less exposed secretory tissue and poor connection to the vascular tissue suggested less probability of the structure to be EFN.

From anatomical investigation it can be suggested that the RMGN is definitely a site of synthesis of bioactive metabolites. Minute granules get accumulated and formed clumps and later druses. It is also evident that the secretions are transported from the site of production to the site of accumulation through the elongated cells. In LM and SEM the special girdle like opening of the nodule to the exterior is a peculiar distinguishing character.

In this study some new morphoanatomical characters of the leaflet of *B. mollis* are recognized and by using these characters the taxonomical description of the plant could possibly be extended. And these results can be used to evaluate the

diagnostic features of *B. mollis* as well as to further study the exact functionality and role of this structure in the production of biologically active compounds.

#### **5.1.6 Floral morphology:**

Floral morphology of *B. mollis* reported here agrees with the previous workers (Narayan and Sayeeduddin, 1958; Nair and Jain, 1956; Alves *et al.*, 2016). However, there arise some differences. Present structural study provides new data and further insights.

In *B. mollis* there is occasional occurrence of pentamerous and even hexamerous flowers. However, flowers of *B. mollis* have vascular traces 12-16 indicating tetramery. The tetramerous condition has been reported for Simaba (Engler, 1874; Cronquist, 1944) and hexamery has also been reported for several groups of Sapindales, other rosid groups, early diverging asterids and other angiosperms (Pirani *et al.*, 2010; Ronse De Craene and Smets, 2016). Tetramery and pentamery often co-occur in some eudicots even in the same inflorescence, as found here (Ronse De Craene & Smets, 2016). However, the variation in merism in *B. mollis* is present only in androecium. Such variation has not been observed in other whorls. Similar variations in merism are also reported in some members of Sapindales (Pirani *et al.*, 2010).

Puberulous to densely pubescent perianth is also another significant character of *B. mollis* resembling other members of Simaroubaceae. *B. mollis* exhibits variation in organ sterility in male and female flower, a condition reported in few other members of Simaroubaceae and Sapindales (Alves *et al.*, 2016). The presence of the four lobed intrastaminal nectariferous disc surrounding stamens resembles with the presence of similar disc surrounding the ovary base in several families of Sapindales.



The ovary in Simaroubaceae is described as apocarpous or syncarpous (Ramp, 1988) and apocarpy has been considered as secondary from an evolutionary perspective (Endress *et al.*, 1983). From such perspective apocarpous gynoecium of *B. mollis* can be considered as an adaptive feature for insect pollination. As the mechanism of pollination in *B. mollis* is ambophilly representing a transitional state between insect and wind pollination, apocarpous gynoecium may add advantage to crawling insects by increasing visiting area. The absence of any indumenta or thick hairs over the carpel also facilitates the access of insects to stigmatic crest of *B. mollis* flower.

Stomata are observed in both sepal and petal of male flower of *B. mollis* indicating the classical interpretation of the flower.

Morphological nature of the intrastaminal consolidated disc of *B. mollis* can be described as nectary similar to that of *Pilocarpus pennatifolius* Lem. (Rutaceae) in having stomata and secretory tissue of minute cells (Souza *et al.*, 2005). Cronquist (1981) described the disc in some Sapindales appears to be modified gynophore. However the consolidated disc present in *B. mollis* has vascularization near the base while vascular tissue absent towards the apex. This suggests the receptacular nature of the disc.

The consolidated intrastaminal disc of male flower of *B. mollis* receives vascular supply from two sources- branches from the antisealous staminal traces and branches from the petal traces. The disk in *B. mollis* may be considered to have been formed by fusion of the bases of the antisealous stamens and petals. Considering the fact that prominent disc of bisexual flower of *B. javanica* receives vascular supply from three sources *i.e.* antisealous staminal traces, whorl of traces from petal radii and whorl of traces from secondary margins of carpel, the unisexual

flowers of *B. mollis* can be considered to have been evolve from bisexual flowers of *B. javanica* or some intermediate ancestorhaving bisexual flower, due to complete abortion of either of the essential whorls.

Ovaries of homochlamydeous female flower of *B. mollis* get mature at different times. Thus, ovaries remain open in various degrees. This corroborated with the findings of Nair and Joshi (1958).

As per our study and available literature *B. mollis* is dioecious species having unisexual flower assuring the cross pollination. But, the occurrence of false fruiting in male plants in some cases reveals the need for more accurate investigation of floral structure and biology regarding possible occurrence of monoecy, polygamy or bisexuality in this species.

The floral axis of *B. mollis* continues to grow above the levels of the essential whorls. It is also supplied by traced of well developed vascular branches which fade up near the tip. These features resembles with the similar features reported in *B. sumatrana* and members of *Samadera* and *Quassia* (Nair and Joshi, 1958; Nair and Joseph, 1957; Nair and Jain, 1956). Such findings are in favor of the classical interpretation of the flower.

Completely free carpels in *B. mollis* are primitive in nature. Apocarpous condition in Simaroubaceae was earlier reported to be secondary resulting from the early splitting of the syncarpous gynoecium (Saunders, 1939). However, later the primitive nature of the apocarpous gynoecium of *Brucea* followed by a graded series in the connate nature of the styles present in *Alianthus*, *Samadera* and *Quassia* was explained (Nair and Joshi, 1958; Nair and Joseph, 1957; Nair and Jain, 1956). Thus,

*B. mollis* lies near to the completely apocarpous primitive ancestral flower of the Simaroubaceae.

## **5.2 Floral biology:**

Endemic species with restricted geographic distributions and small population has always been a central concern of biologists faced with the problem of conserving rare species endangered by habitat destruction and fragmentation (Navarro and Guitian, 2002). Knowledge of floral biology as well as reproduction in such species is important to study the spread of the species. There is a widespread consent that studies on the reproductive biology of endangered, rare or threatened species may be useful for understanding the reason behind such conditions (Schemske *et al.*, 1994). Such studies could provide better insight which may be useful for evaluating and developing alternative in situ and *ex situ* conservation and management strategies (Menges, 1986). Also all part of the plant at all stages of their development may produce valuable evidence which could be used in studying their taxonomy and phylogeny (Thorne, 1958).

Study of floral biology reveals *B. mollis* exhibits mostly advanced floral characters along with few primitive ones. Dioecy, aphanisis in female flowers, oligocarpy, reduced style, tricolpate pollen and production of drupe are advanced floral characters whereas apocarpy is considered as primitive (Smith, 1967; Hutchinson, 1973). After pollination two or three carpels fall off leaving only one or two carpel per flower. This tendency to reduce the number of fertilized carpel could indicate the evolution towards monocarpy. This also could be a reason of low seed production in the plant.

Floral morphology of *B. mollis* exhibiting the unisexual flowers rich in nectaries and glandular epidermal and sub epidermal appendage are important

features of the floral biology favoring extensive cross pollination and hence expected to result genetic diversity. Xenogamous plants are considered to have less reproductive assurance than the autogamous (Huang *et al.*, 2012). Thus, xenogamous plants are expected to have low propagation rate. Such low reproductive success is also observed in *B. mollis*. However, xenogamous plants also exhibit better adaptability and thus widespread distribution (Huang *et al.*, 2012). In spite of having combination traits such as xenogamy and apomixes, *B. mollis* exhibits restricted distribution.

From the study of the developmental stages of both pistilate and staminate flower it is evident that the stamens arises from the lateral sides of the floral disc, whereas the carpels arises from the centre of the apical meristem pushing the floral disc outside. Vascular traces present in the floral disc also support this.

Entomophily is considered primitive than anemophily. But, surprisingly *B. mollis* exhibits both type of pollination *i.e.* ambophily. In *B. mollis* such observation indicates transitional stage of development towards true anemophily.

Absence of good mechanism of seed dispersal results in poor genetic variability, particularly when population size is small (Navarro, 1996). Thus, even with effective cross-pollination in *B. mollis* majority of crosses may be inbreeding.

Less fruit production, short dispersal distances and low habitat availability are the limiting factors for the survival of the population. However, observing the considerable rates of seed germination and ability of the plants to produce fruit by combining insect and wind pollination, *B. mollis* may be more resilient than others to habitat fragmentation.

### **5.3 Anatomy of root, stem and leaf:**

Stem and root of *B. mollis* exhibits prominent secondary growth. Distinct wide circular layers of cambium are observed in both root and stem. Secretory canals are scattered in the pith of stem and cortex of root in *B. mollis*. In petiole large secretory ducts are present. Presence of microsphenoidal calcium oxalate crystals and druses in all the vegetative parts of the plant exhibits potent medicinal value of the species. Numerous glandular trichomes spreading over the vegetative parts of the plant also coincides with the medicinal property of the species.

### **5.4 Seed germination:**

Fruits of *B. mollis* borne in small bunches consisting of 1-5 fruits in each bunch. Even though the plant produces abundant fruits and seeds during the peak fruiting season in April, yet very less number of seedlings is found growing in the forest floor. The germination behaviour of the seeds has not been studied yet. Present work deals with the preliminary investigation of the seed germination and viability of *B. mollis*.

The results of TZ seed viability test exhibits similarity with that of germination test for examining the seed viability of *B. mollis*.

From the results it is evident that maturity of seeds plays an interesting role in germination. Mature ripe seeds grown in soil: sand mixture germinated better than mature unripe seeds. Fully ripe brick red seeds exhibits better germination percentage and CVG than partially ripe, green or light orange seed. Seeds of same plant attain maturity simultaneously. This results the lack of variation in maturity of the fruits of a plant which in turn maximizes the competition for substrate for successful seed germination at the forest floor. Sometimes seeds may involve in controlling germination by restricting it to the most favorable periods and conditions

for seedling growth Viluer (1972). Delay in germination or low percentage of germination of immature seeds is due to lower available gibberellin content in embryo-cotyledon at young stage resulting in slower rate of water imbibitions (Rudrap *et al.*, 1992). Lower gibberellin concentration in younger seeds may be the reason for lower seed germination percentage of mature unripe *B. mollis* seeds. However, there is no significant difference in the incubation period before germination between the ripe and unripe seeds.

Seeds grown in field germinated better than the seeds incubated in laboratory (Fig: 4.4.3). The highest germination percentage among the seeds incubated in laboratory is only  $26.6 \pm 0.57$ . However, seed grown in field exhibits highest germination percentage  $85.6 \pm 1.7$ . Thus germination in laboratory is proved not suitable for *B. mollis*.

The fleshy coating of some seeds has chemicals that prevent germination (Crocker and Barton, 1953; Evenari, 1949). The experimental results indicate that presence of fruit wall strongly inhibit the seed germination in *B. mollis* (Table: 11). Most of the inhibition compounds that inhibit seed germination usually present in the fruit wall or seed coat (Edwards, 1968). Hence, this explains failure of *B. mollis* seeds with fruit wall.

However, all the seeds of *B. mollis* exhibit a considerable amount of imbibition period ranging from 10 to 90 day. It may be due to high degree of impermeability of the endocarp to water or oxygen or to both. Similar phenomenon is also reported in various seeds (Rudrapal *et al.*, 1992; Rolston, 1978).

Seeds require adequate moisture and optimum favorable temperature for germination (Scharpf and Parmeter, 1962; Beckman and Roth, 1968). Seed

germination of *B. mollis* is poor in case of sowing in December than in May. The low temperature may be a major factor for such delaying in germination.

Different chemicals are used to stimulate the germination of seeds in many plants (Crocker and Barton, 1953; Srinivasan and Subramanian, 1960). Plant growth regulator gibberellic acid (GA) helps in stimulating seed germination in two species of the parasitic genus *Orobanche* (Nash and Wilhelm, 1960). Sometimes application of chemical stimulants may not stimulate germination of seeds or growth of seedlings (Bonga, 1965). Soaking the seeds of *B. mollis* in GA also did not exhibit any appreciable effect on seed germination.

From above discussion it is evident that the low germination rate and restricted distribution of *B. mollis* seed may depend on various factors. Low germination rate may be due to the simultaneous maturity attainment among the seeds of the same plant. The resulting competition for the forest floor reduces the germination percentage in natural population. Another potent factor for seed germination is its hard endocarp which may take long imbibition period to germinate the seed. Favorable temperature for germination is also a potent factor for seed germination in *B. mollis*. Since the seeds of *B. mollis* germinate much better in summer while growing in field and there is no significant difference in the growth pattern of the seedlings, the *ex situ* plantation could be an efficient method for increasing population and distribution of the endemic plant.

### **5.5 Traditional uses:**

Study of traditional knowledge about the use of plants by ethnic communities has always been the centre of attraction for the botanist. It provides better insight about the plant as well as helps in designing suitable conservation strategies. Although use of *B. mollis* in the treatment of fever is no longer popular

against antibiotics or over the desk medicines, yet it is still used in some localities of Karbi Anglong. Present investigation finds that the indigenous knowledge of *B. mollis* is only restricted to the old generation. Young ones are not much aware of the plant and its uses. Moreover, the population of the plant is not so abundant and anthropogenic pressure also threatens the population. Therefore, it is necessary to arrange researches and workshops regularly to generate awareness about traditional knowledge and species identification among youth. Tissue culture, construction of gene sanctuary, protected area management, harvest management plans, restoration, recovery and rehabilitation of the habitat are few strategies that can be undertaken by researcher and scientific community in future for conservation of this endemic plant.

#### **5.6 Significant findings:**

1. Taxonomy: Lectotype and Epitype have been selected for the species.
2. Gross morphology:
  - Variations in the shape, size and number of leaflets present in leaf and variations in shape and size of leaflets have been recorded, which are grouped into six and eight types respectively.
  - Stem sucker has been recorded
  - Gross morphology of Raised marginal glandular nodule present in the leaflet is described.
3. Micromorphology:
  - Detail Leaflet architecture including pattern, order and type of venation, analysis of tertiary vein origin, vein architecture, leaflet rank and features of terminal trachieds have been recorded.



- Nineteen various types of glandular and non glandular trichomes have been recorded and studied under LM and SEM.
- Raised marginal glandular nodule present in the leaflets are described with special reference to the micromorphology, anatomy and SEM.

4. Anatomy:

- Presence of secretory canals in stem, midrib, petiole and epicarp of the fruit has been recorded.
- Presence of druses and microsphenoidal crystals has been recorded throughout the stem, root and fruits.

5. Seed germination:

- Seeds collected and sown in May exhibits greater germination percentage than the seeds collected and sown in December.
- Seeds without fruit wall exhibits better germination percentage than seeds with fruit wall intact.
- GA treatment has no significant effect on seed germination.

6. Floral Biology:

- Parthenocarpic fruit development in male plants has been recorded.
- Distinct phenological pattern and blooming peak of male and female flowers have been recorded.
- Anthesis is recorded as both diurnal and nocturnal.
- Anther is recorded to exhibit nine and carpel is recorded to exhibit six distinct developmental stages.
- Pollination syndrome of *Brucea mollis* is recorded to be ambophilic
- Fertilization is recorded to be porogamic.

- Ovaries have been recorded to get mature at different times and remain open in various degrees.

7. Traditional knowledge:

- Method of preparation and doses by the ethnic groups of Karbi Anglong have been documented.