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The systematic value of the leaf indumentum in *Lobostemon* (Boraginaceae)

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ABSTRACT

The character states pertinent to the indumentum of *Lobostemon* Lehm. leaves are tabulated and discussed. *Lobostemon* possesses similar trichome and indumentum types as described for *Echium* L., with the exception of the 'Alpine' indumentum type. Due to the environmentally induced variation, the leaf indumentum characters of *Lobostemon* are viewed to be of limited phylogenetic value, although they do diagnose a number of taxa.

INTRODUCTION

In terms of *Lobostemon* Lehm. systematics, the most recent revision by Levyns is noteworthy because she delimits five sections based on floral characters and presents a branching diagram (Levyns 1934: 412) to elucidate relationships within the genus. The aforementioned constitute falsifiable hypotheses.

One of the most striking features of the herbaceous forms of the Boraginaceae is the covering of thick-walled, harsh, unicellular trichomes (Metcalfe & Chalk 1950: 945), so much so that Klotz (1959) was of the opinion that one cannot ignore these characters when studying the Boraginaceae. Numerous studies of the leaf indumentum in the Boraginaceae have been undertaken e.g. Revedin (1902), Jonová (1926), Bider (1935), Klotz (1959), Lems & Holzapfel (1968), Bramwell (1972) and Selvi & Bigazzi (2001). Yet, opinions differ with regard to the taxonomic value of these characters. Because the largely European *Echium* L. is considered to be a sister taxon of the South African endemic *Lobostemon* (Böhle *et al.* 2001), the focus henceforth will for the moment fall on *Echium*. Klotz (1959), during his revision of the genus, used indumentum and trichome characters to key out species, series and sections. Lems & Holzapfel's (1968) in depth study of the genus on the Canary Islands, leads to an optimistic view about their use, claiming that there are at least 14 criteria which can be applied in comparing the Canary Island *Echium* species with one another, including not only the types of trichomes present, but their relative abundance, their total coverage of the leaf surface, the size of the pustules, and the orientation of the trichomes. Gibbs (1971: 31, 32), confining himself to the Spanish echiums, formed three groups based on indumentum characters and used a number of characters to key out species. Bramwell (1972), working in Macaronesia, chose to differ and suggested that the indumentum: 'is more or less useless and certainly of secondary importance ... in the consideration of the evolutionary and ... the phenetic relationships'.

Levyns (1934), for the first time in *Lobostemon*, mentioned that indumentum and trichome characters are particularly influenced by environmental and temporal factors. Failure to realise this earlier led especially De Candolle (1846) and Wright (1904) to describe a myriad of names, most of which were placed in synonymy by Levyns (1934: 403). Levyns (1934), in certain instances, still made use of vegetative characters in her key, e.g. in the section *Trichotomi* Levyns. Difficulty in identifying species using this key invariably occurs when using vegetative characters like indumentum or trichome type.

While it is true that like all taxonomic criteria, epidermal characters must be interpreted with great circumspection, Barthlott (1981) voiced the opinion that the major problem in their systematic application is that we do not yet have enough data [compare also Cole & Behnke (1975)]. Despite the vast amounts of SEM micrographs published, many of the data are not comparable because of a lack of standardized terminology and often no structural interpretation of the characters illustrated.

In the light of the aforementioned, this paper aims to contribute to epidermal related data in the Boraginaceae. Existing terminology is followed as far as possible, and for this reason is expanded in Material and methods below. Two issues are investigated, namely: 1, can SEM analyses of indumentum characters reveal states that can diagnose *Lobostemon* species, and 2, does the grouping of taxa based on these characters correlate with existing hypotheses of infrageneric relationships within the genus?

MATERIAL AND METHODS

Voucher specimens were collected for all taxa examined and are listed in Table 1.

Material collected from plants in their natural habitat in the field was fixed in FAA. The indumenta of the fixed leaves were studied with a JOEL scanning electron microscope (SEM), using secondary-electron detection and an acceleration voltage of 4–5 kV. All fresh material used in the SEM study was collected during the peak flowering season between August and October. Additional observations were made with a light microscope on herbarium

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specimens as well as on potted plants. In the latter case, both young to mature leaves were analysed.

Terminology

Lems & Holzapfel (1968) and Bramwell (1972) identified three basic trichome types in Macaronesian species of *Echium*:

Glandular trichomes (Gl) occur mainly on the abaxial surface of the midrib or more rarely over wide areas of the abaxial surface of the leaf in most of the Macaronesian species of *Echium*. Selvi & Bigazzi (2001) distinguished two types of glandular trichomes in the genera of the tribe Boragineae: Type 6 trichomes consisting of a single stalk cell, and Type 7 trichomes consisting of three or more stalk cells.

Simple trichomes (Si) correspond to the Type 3 trichomes of Selvi & Bigazzi (2001). They are mostly short, stiff, hollow trichomes without a large pustular base, and are present in the cotyledons of all the *Echium* species. However, they do not persist into the adult stage in all species. Simple trichomes occurring in mature leaves are usually short, often curved and closely appressed to the leaf surface [the appressed trichomes correspond to the Type 2 trichomes of Selvi & Bigazzi (2001)]. Though the base of the trichome is usually swollen, there is generally little or no cell differentiation of the surrounding epidermal cells. Lems & Holzapfel (1968) record simple trichomes longer than 400 mm always possessing one or more rows of differentiated cells around the base. It is well established that the number of rows of epidermal cells involved in the pustule formation in the Boraginaceae is a product of environmental and temporal variables.

Pustular trichomes (Pu), a term coined by Lems & Holzapfel (1968), correspond to the *Hügelborsten* of Bider (1935), nodular bristles of Metcalfe & Chalk (1950), *Höckerborsten* of Klotz (1959) and Type 1 trichomes of Selvi & Bigazzi (2001). One or more concentric rings of strongly differentiated epidermal cells which contain cystolith-like structures and whose walls are strongly impregnated with calcareous material surround the bases of the hollow, pustular trichomes. In very large trichomes, some of the cells of the upper palisade layer of the mesophyll are also involved in the pustule structure and may also be calcified. Uphof (1962) referred to these subepidermal areas as pedestals.

Lems & Holzapfel (1968) furthermore discerned four main indumentum types in the Boraginaceae:

Spinous indumentum (Sp) consists of stiff spines and is found on the leaf surface or is often confined to the margins and the midrib of the leaf;

Appressed to ascending silky indumentum (Ap). Here the leaf surface is covered with a dense layer of appressed trichomes that are either simple or with small basal cells;

Umbonate indumentum (Um), spinous, consisting of relatively sparsely distributed pustular trichomes with a large, round, basal region on the otherwise glabrous leaf surface. I designate the term umbonate to describe this type of indumentum. Klotz (1959) referred to these as

TABLE 1.—Distribution of indumentum types and trichome characteristics in *Lobostemon* (with voucher specimens housed in NBG)

Taxa	Voucher specimens	Indumentum type			Trichome type			Trichome length		Trichome distribution		Micropapillae presence and type		
	MHB	Sp	Ap	Um	Gl	Si	Pu	1	2	Ad	Ab	No	Sm	Un
L. argenteus	436	•	•		•	•	•		•	•	•		•	
L. belliformis	432	•			•		•	•		•	•			•
L. capitatus	503	•			•		•	•		•	•		•	
L. collinus	505	?			?		•	•		•	•		•	
L. curvifolius	392	•	•		•		•	•		•	•		•	
L. daltonii	501	•			•	•	•	•		•	•		•	
L. decorus	422	•			•		•	•		•	•	•	•	
L. echioides	403	•	•		•	•	•	•		•	•		•	
L. fruticosus	385	•	•		•	•	•		•	•	•		•	
L. glaber	417	•		•	?		•	•		•	•		•	
L. glaucophyllus	384	•		•	•	•	•	•		•	•		•	
L. gracilis	443		•		•	•	•	•		•	•		•	
L. hottentoticus	379	•		•	?	•	•	•		•	•		•	
L. laevigatus	519	•		•	?	•	•	•		•	•		•	
L. lucidus	446	•			•		•	•		•	•		•	
L. marlothii	419	•			•		•	•		•	•	•	•	
L. montanus	382	•	•		•		•	•		•	•		•	
L. muirii	413	•			•		•	•		•	•	•		
L. oederiaefolius	396	•			•		•	•		•	•	•		
L. paniculatus	421	•		•	•	•	•		•	•	•		•	
L. paniculiformis	508	•		•	•	•	•	•		•	•		•	
L. pearsonii	515	•		•	?	•	•	•		•	•		•	
L. regulareflorus	439			•	•		•	•		•		•		
L. sanguineus	447			•	?		•	•		•	•	•		
L. stachydeus	420	•			•	•	•		•	•	•		•	
L. strigosus	387	•			•		•	•		•	•		•	
L. trichotomus	377		•		?	•	•	•		•	•		•	
L. trigonus	425	•	•		•		•	•		•	•		•	

MHB, M.H. Buys; Sp, spinous; Ap, appressed; Um, umbonate; Gl, glandular; Si, simple; Pu, pustular; 1, homomorphic or with a continuous variation; 2, dimorphic; Ad, adaxial; Ab, abaxial; No, without papillae; Sm, smooth papillae; Un, undulate papillae.

FIGURE 1.—*Lobostemon regulareflorus*: t/s through leaf showing involvement of underlying parenchyma in pustule formation. Scale bar: 50 µm.

diskusartig bases. Selvi & Bigazzi (2001) referred to the trichomes forming this indumentum type as Type 4 trichomes;

Alpine indumentum (Al), dense, ascending to erect, with long trichomes with small bases, found only in subalpine zone species.

Trichome and indumentum types were identified according to those recognized by Lems & Holzapfel (1968). All observations of indumentum presence, abundance and type were confined to the surface of the lamina (excluding margins). Leaves were considered to be glabrous when no sign of any trichomes could be seen (including on the midrib) with a light microscope or SEM.

Transverse sections of paraffin wax-embedded laminae were cut with a rotary microtome and stained with a mixture of Safranine O and Alcian green (Joel 1983). Sections were taken through the middle of the laminae.

The cluster analysis was done using the Statistica 6.1 package with the following settings: tree clustering; Ward's (1963) method of minimum-variance clustering under the amalgamation rule and percentage disagreement as a measure of distance. Characters were coded as qualitative presence/absence data. Data for the spinous indumentum type and glandular trichome type were excluded from the analysis due to the presence of unknown/uncertain states in one or more taxa.

RESULTS

Table 1 presents a summary of the various leaf indumentum and trichome characters codified for *Lobostemon*.

Trichomes

In *Lobostemon*, the tendency for epidermal cells at the base of the trichomes to become organized into pustules extends sometimes to several concentric rows, the number of which seem to vary with the climatic conditions under which the plant grew and the stage of ontogenetic development. Trichomes typically longer than 400 mm tend to develop pustular bases. In trichomes with a large base, the underlying parenchyma also becomes involved in pustule formation (Figure 1). The pustular trichome type occurs in all *Lobostemon* species (Figure 2). Most of the species in *Lobostemon* possess glandular tri-

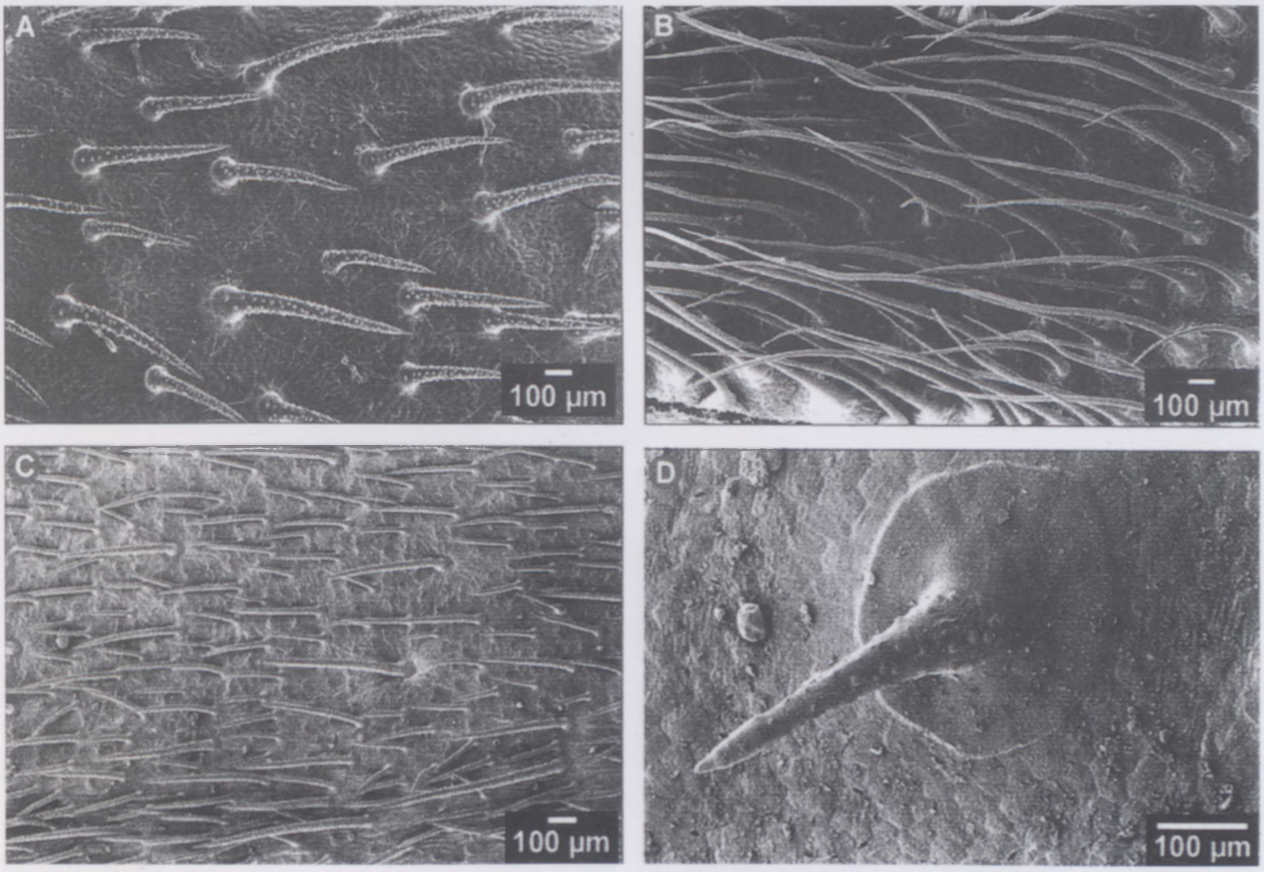

FIGURE 2.—Indumentum types in *Lobostemon* leaves. A, spinous in *L. belliformis*; B, appressed in *L. montanus*; C, appressed in *L. trichotomus*; D, umbonate in *L. paniculatus*. Scale bars: 100 µm.

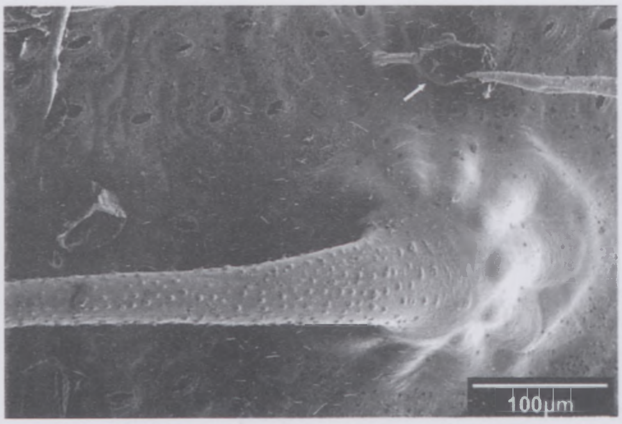

FIGURE 3.—Collapsed glandular trichomes in *Lobostemon fruticosus* indicated by arrow. Scale bar: 100 µm.

chomes on their leaf surfaces. These trichomes have a tendency to fall flat from an early age (Figure 3).

Length

With regards to the leaves, only four species of *Lobostemon*, namely *L. argenteus* (P.J.Bergius) H.Buek, *L. fruticosus* (L.) H.Buek, *L. paniculatus* (Thunb.) H.Buek and *L. stachydeus* A.DC. clearly possess a dimorphic indumentum in the adult stage (Figure 4A). The shorter trichome is usually of the simple trichome type. Some forms of *L. echioides* Lehm., *L. gracilis* Levyns, *L. trichotomus* (Thunb.) A.DC. and *L. paniculiformis* A.DC. possess a heteromorphous indumentum. Here the variation in trichome length is discerned to be continuous (Figure 4B).

Distribution

Species with trichomes on both the abaxial and adaxial leaf surfaces are the most common. *L. regulareflorus* (Ker Gawl.) M.H.Buys is unique in only its adaxial leaf surface being hairy. *L. collinus* C.H.Wright is a good example of how climate or age can influence trichome distribution. Plants collected in spring generally have both sides of the leaf hairy. Those collected in late summer (January–April) have glabrous adaxial surfaces. In *L. capitatus* (L.) H.Buek, however, the opposite holds true. Young leaves collected in spring appear to be hairy only on the abaxial surface. Older leaves from the previous year's growth are hairy on both the adaxial and abaxial surfaces.

Micropapillae

Only a limited number of species seem to have trichomes without micropapillae (Figure 5A). In the majority of taxa, trichomes examined in young leaves as well as flower buds display micropapillae. Micropapillae may either be smooth (Figure 5B) or undulated (Figure 5C). In terms of shape, both round and elongate micropapillae have been observed on single trichomes (Figure 5D). The round micropapillae in the aforementioned figure are confined to the base of the trichome, whereas the elongate micropapillae occupy the distal parts. *L. decorus* Levyns and *L. marlothii* Levyns have been observed to possess trichomes with or without micropapillae.

Indumentum

Three main indumentum types can be recognized in the genus. The spinous indumentum type (Figure 2A) is the most prevalent. The appressed indumentum type (Figure 2B) is commonly found on those taxa exhibiting silvery leaves. Forms of *L. argenteus, L. curvifolius* H.Buek, *L. echioides, L. fruticosus* and *L. trigonus* (Thunb.) H.Buek that grow in less arid and more sheltered conditions tend to have appressed trichomes. Generally appressed indumenta also become more spinous as the season progresses, i.e. as it becomes drier and warmer. *L. trichotomus* and *L. gracilis* appear to be the only species with an appressed indumentum without an accompanying complement of spinous indumentum (Figure 2C). The umbonate indumentum type is the most difficult to identify due to the general absence of calcified cells around the trichome bases (Figure 2D). This indumentum type appears to be absent from sections *Argentei* Levyns and *Fruticosi* Levyns, but most prevalent in section *Trichotomi* Levyns. Only *L. regulareflorus* and *L. sanguineus* Schltr. appear to possess an umbonate indumentum type to the exclusion of a spinous indumentum on the leaf surfaces. The remainder of species generally have a spinous indumentum on the margins in addition to the umbonate indumentum of both leaf surfaces. Although the leaves of *L. glaucophyllus* (Jacq.) H.Buek appear to be glabrous to the naked eye, this study has shown the prevalence of minute umbonate indumentum on especially the abaxial surface (Figure 6).

A cluster analysis of the data in Table 1 (excluding characters with unknown/uncertain states) created a number of clusters with taxa possessing identical character states viz.:

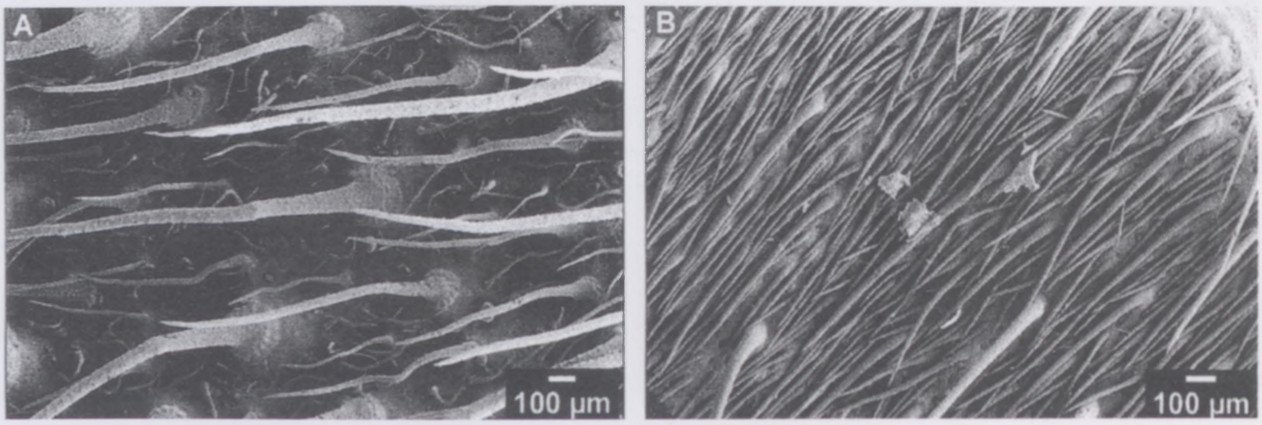

FIGURE 4.—Trichome lengths in *Lobostemon*. A, dimorphous in *L. argenteus*; B, continuous in *L. echioides*. Scale bars: 100 µm.

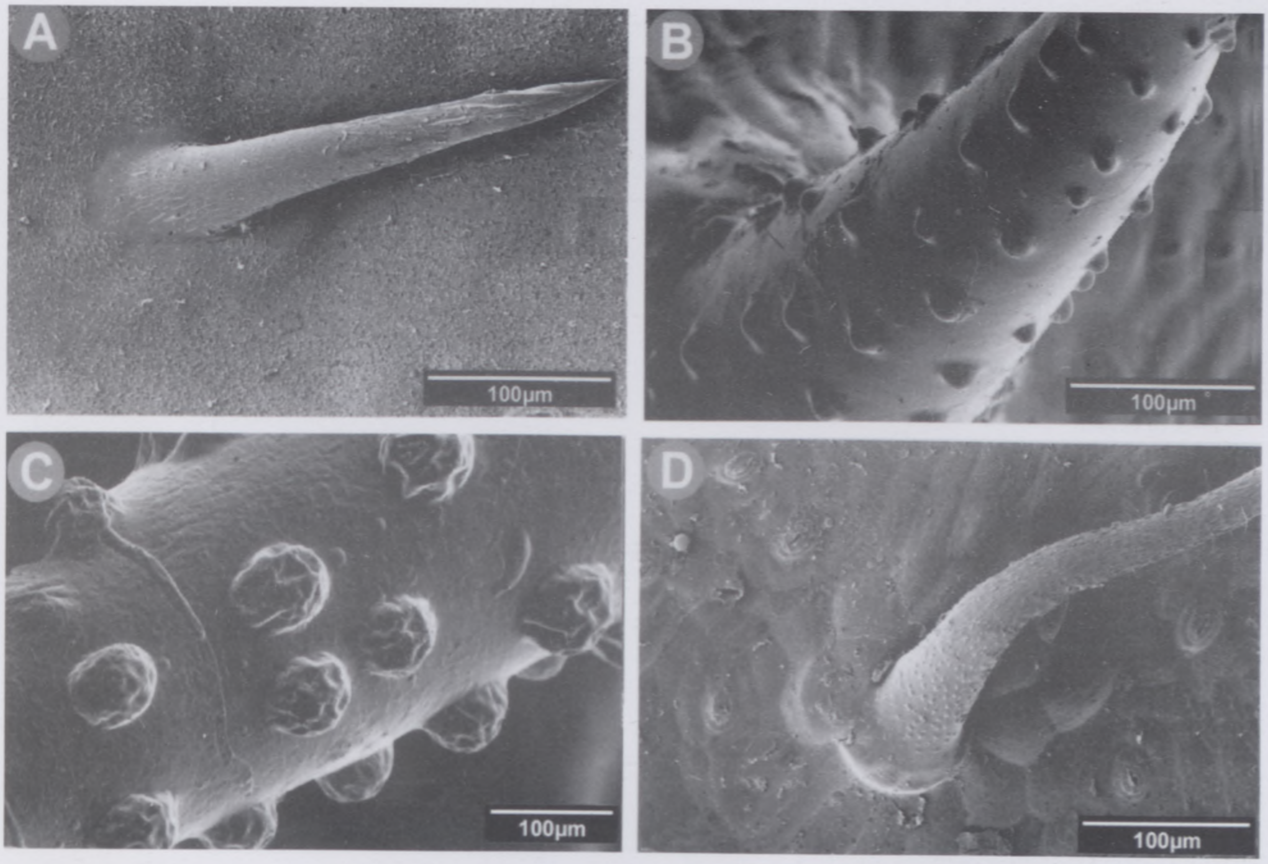

FIGURE 5.—Trichome micropapillae in *Lobostemon*. A, no micropapillae in *L. muirii*; B, round and smooth in *L. paniculatus*; C, round and undulate in *L. belliformis*; D, round and elongate smooth in *L curvifolius*. Scale bars: 100 µm.

L. argenteus and *L. fruticosus* cluster; *L. capitatus*, *L. collinus*, *L. lucidus* (Lehm.) H.Buek and *L. strigosus* (Lehm.) H.Buek cluster; *L. curvifolius*, *L. montanus* H.Buek and *L. trigonus* cluster; *L. echioides*, *L. gracilis* and *L. trichotomus* cluster; *L. glaucophyllus*, *L. hottentoticus* Levyns, *L. laevigatus* (L.) H.Buek, *L. paniculiformis* A.DC. and *L. pearsonii* Levyns cluster; *L. muirii* Levyns and *L. oederiaefolius* A.DC. cluster (Figure 7).

DISCUSSION

I alluded above to Barthlott's (1981) reasons as to why indumentum and trichome characters have not been applied to systematics with great success. Following this study, and those by Levyns (1934) and Bramwell (1972), it seems clear that in the absence of data to the contrary, a lack of knowledge concerning the influence of habitat and ontogenetic development probably more than anything else places a damper on the use of indumentum and trichome data for systematic studies in the Boraginaceae. However, even though there is uncertainty about their systematic use, and grouping of plants based on epidermal features does not agree with those based on reproductive features, the need remains to provisionally describe and organize into a system, all data whereby relationships between the various patterns can be meaningfully approached in the future (Klucking 1995).

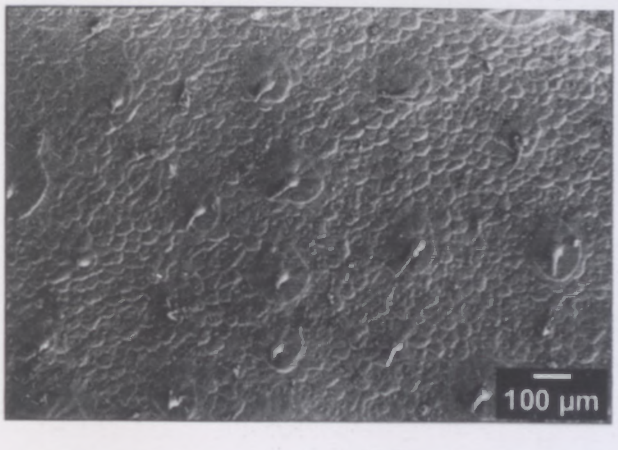

FIGURE 6.—Minute umbonate indumentum on abaxial leaf surface of *Lobostemon glaucophyllus*. Scale bar: 100 µm.

This study has revealed that trichomes in *Lobostemon* should not be defined as purely epidermal but rather as emergences. In *Lobostemon*, young plants possess straight, simple, unicellular trichomes (Table 1: Si) whose swollen bases are part of the epidermal layer. From this simple type, present on juvenile leaves, different developments may occur both in the ontogeny and in the transition from juvenile to adult foliage.

Most of the species in *Lobostemon* possess glandular trichomes on their leaf surfaces. By contrast, Klotz (1959) in his revision of the genus *Echium*, found the chief occurrence of glandular trichomes to be on the stems of *E. humile* Desf. and *E. trygorrhizum* Pomel, although *E. gaditanum* Boiss. was observed with glandular trichomes on their leaf surfaces.

Levyns (1934) correctly indicated that *Lobostemon* tends to undergo a second growing period later in the

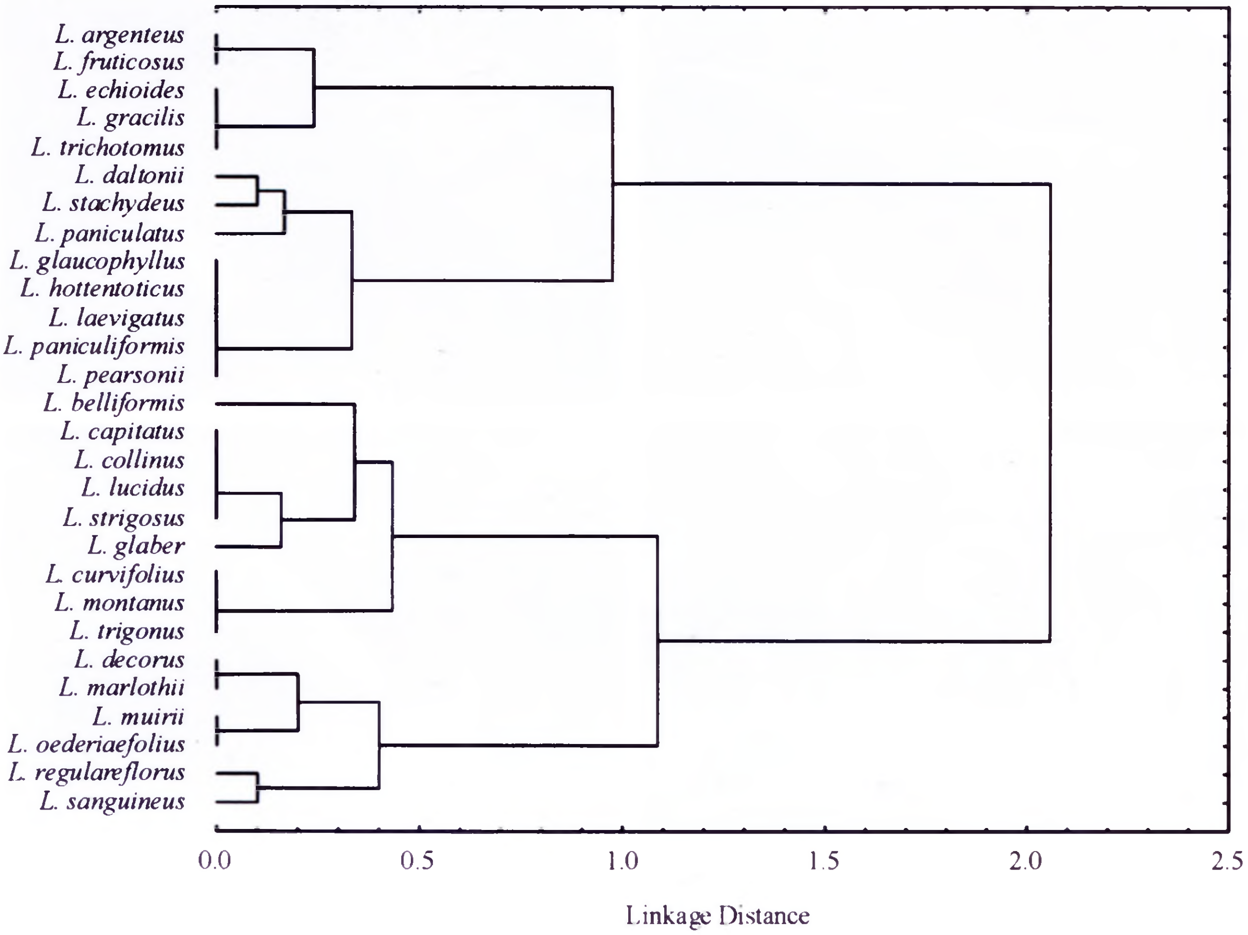

FIGURE 7.—A phenogram of *Lobostemon* based on indumentum characters employing Ward's method of minimum-variance clustering and percentage disagreement as a measure of distance.

season that results in the indumentum becoming sparser as the leaf size increases. Moreover, this study has shown that trichomes tend to fall or break off in some species of *Lobostemon*, leaving behind their hardened bases and manifesting a sparser coverage.

Some taxa can be diagnosed on a single or a combination of indumentum characters. The possession of a dimorphic indumentum in *Lobostemon* is confined to *L. argenteus*, *L. stachydeus*, *L. paniculatus* and *L. fruticosus*. *L. curvifolius* and *L. fruticosus* have often been mistaken for each other: the two taxa are distinguishable—the dimorphic indumentum being absent in *L. curvifolius*. *L. regulareflorus* and *L. belliformis* M.H.Buys, although morphologically similar, differ in that *L. belliformis* possesses trichomes on both leaf surfaces whereas *L. regulareflorus* possesses trichomes confined to the adaxial surface.

Keeping the shortcomings of phenetics in mind, the aforementioned cluster analysis created groups that could not be correlated to current sectional divisions *sensu* Levyns (1934: 412). Some clusters, however, can be found as subgroups within Levyns' sections e.g. the *L. decorus*, *L. marlothii*, *L. muirii* and *L. oederiaefolius* cluster and to an extent the *L. glaucophyllus*, *L. hottentoticus* Levyns, *L. laevigatus* and *L. paniculiformis* cluster. The *L. echioides*, *L. gracilis* and *L. trichotomus* cluster, although not all members of the same section, represent the basal taxa in Levyns' branching diagram.

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