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A TAXONOMIC STUDY OF THE *BLEPHARIS EDULIS* COMPLEX (ACANTHACEAE) IN EASTERN AFRICA

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ABSTRACT

The variable species *Blepharis edulis*, as well as the recently described *B. boranensis*, is investigated in Eastern Africa using phenetic analysis of gross morphological data supplemented with field observations and pollen morphology studies. Two species, *B. edulis* and *B. boranensis*, occuring in the drylands of Eastern Africa are confirmed, with the latter occuring in Borana region (Kenya, Ethiopia and Somalia) and Sodere, Ethiopia, while the former remains variable and widespread. The two species are separated by number and distribution of spines on leaf margin: fewer, sparse or restricted towards leaf base in *B. edulis* whereas more and evenly distributed in *B. boranensis*. An identification key and species descriptions are provided.

INTRODUCTION

Blepharis Juss. (Acanthaceae) is composed of 129 species, distributed predominantly in tropical Africa, about half occuring in Southern Africa, and extending through Arabia and India to S. E. Asia (Vollesen, 2000). In Eastern Africa, *Blepharis edulis* (Forssk.) Pers. is commonly found in the arid regions, in habitats that are fragile and undergoing the processes of desertification, which necessitates systematic studies to establish species biodiversity status (Claridge *et al.*, 1997; Bates, 2000).

The genus has been divided into three subgenera, namely *Blepharis, Ebracteata* Vollesen and *Acanthodium* (Del.) Oberm. Subgenera *Blepharis* and *Acanthodium* are further subdivided into three (*Blepharis*, *Inopinata* Vollesen and *Scorpioidea* Oberm. Ex Vollesen) and two sections (*Acanthodium* (Del.) T.Anderson and *Biflora* Vollesen) respectively. *Blepharis edulis* is placed in the Sect. *Acanthodium* (Furness, 1996; Vollesen, 2000).

Taxonomic studies of *Blepharis* in tropical Africa are few, and include that of Clarke (1899–1900), Napper (1970), Agnew & Agnew (1994) and Vollesen (2000). Some specimens of *B. edulis* in various herbaria (*e.g.*, EA and ETH) have been identified as *B. ciliaris* (L.) B.L.Burrt. or *B. linariifolia* Pers. Recently, Vollesen (2000) also separated *B. boranensis from B. edulis,* based on position and number of spines or flower sizes. *B. edulis* has a wide variation in gross morphology, at times blurring the species boundaries with related taxa. For example, in Ethiopia near Bale-Harerge massif where some unique *B. boranensis* forms are found, collections of *B. edulis* are characterized by large flowers.

According to Vollesen (2000), *B. edulis* and allied taxa are more or less geographically separated in Eastern Africa. *B. edulis* grows in northern Tanzania, Kenya, Ethiopia and into Saudi Arabia. *B. boranensis* grows in the Borana region of Kenya/Ethiopia and overlaps with *B. edulis*. *B. linariifolia* is distributed from southern Sudan westwards, whereas *B. ciliaris* is found only in Asia (Oman, Iran and Pakistan).

This study re-examines the infraspecific taxonomy of *B. edulis* complex, based on phenetic analysis of gross morphological data.

MATERIALS AND METHODS

Morphological studies were based on herbarium and field observations. Specimens from EA, ETH, MAL, NU and WAG were investigated for 21 morphological characters (table 1). The vegetative and reproductive parts were measured using a ruler and a WILD M3 dissecting microscope. Between five and ten measurements were taken per specimen for every part observed at standardized positions and an average calculated. A total of 171 specimens (Operational Taxonomic Units, OTUs) were analysed for phenetic relationships using STATISTICA (version 4.1), prior to character standardization. In cluster analysis, the degree of similarity between pairs of OTUs was measured using the Euclidean distance coefficient. Pairs of OTUs were then clustered by Unweighted Paired-Group Method using arithmetic Averages (UPGMA). Principal Component Analysis (PCA) was performed on the data to elucidate patterns of relationship and test separation of groups obtained in cluster analysis.

Pollen grains were obtained from mature flowers of herbarium specimens. The acetolysis method described by Erdtman (1969), using a mixture of glacial acetic anhydride and concentrated sulphuric acid (3:1), was followed in preparation of grains for Light Microscopy (LM) and Scanning Electron Microscopy (SEM) studies. Measurements of the LM pollen were taken using an ocular micrometer at magnification x400 and micrographs made at x1000, respectively. In SEM dry pollen was placed on clean bronze (stub) and coated with gold. The pollen were scanned and investigated using a Jeol JSM 840 Scanning Electron Microscope.

RESULTS AND DISCUSSION

Gross morphology

Intuitive interpretation of gross morphology is the basis of the previous species concepts on the *Blepharis edulis* complex. Phenetic relationships of specimens fitting broadly into *B. edulis* complex below are analysed and presented here, without *a priori* allocation of the specimens into taxonomic groups.

(i) Cluster analysis

The cluster analysis yielded two major clusters above 17.5 Euclidean linkage distance, delineated as B1 and B2 in figure 1. The clusters, B1 and B2, differ in some gross morphological characters and can be separated by vegetative and floral characters. There is discrete variation in number of spines on leaf margins and length of terminal spine on bracts, numbering 2–18 versus 20–36, and length below 4 mm versus 5–9 mm, respectively. In addition, there is geographical separation between the clusters, with B2 having a wide distribution from northern Tanzania through Kenya and Ethiopia into Eritrea whereas B1 is predominantly restricted to Borana region of Kenya/Ethiopia. Cluster B1 has peculiar inclusion of two specimens from Southern Kenya, which have overall gross morphological similarity to B2.

Specimens (OTUs) studied in the cluster analysis above were assigned into the corresponding clusters (B1 and B2) and phenetic relationships further evaluated using Principal Component Analysis (PCA).

(ii) Principal Component Analysis (PCA)

The variance attributed to each of the four principal components (PC) was found to be 42.07%, 9.14%, 7.66% and 6.15% for PC 1, 2, 3 and 4, respectively (table 1). Thus, PC 1 and 2 contributed up to 51.21% of the total variance. A scatter plot of PC1 against PC2 resulted to segregation of the clusters (figure 2). When the data was plotted in three dimensions, based on PC 1 and 2, the two clusters were observed to be unambiguously separated (figure 3). The partial overlap observed in figure 2 is interpreted to be a product of diagrammatic presentation of pattern in two dimensions.

Table 1. Factor loading of the gross morphological characters included in cluster and principal components (PC) analysis

	PC1	PC2	PC3	PC4
Leaf margin spine number	-.708620	.242020	-.214988	.148265
Bract length	-.658309	.170862	.130438	-.479591
Bract length / width	.141566	.378410	-.065338	-.755718
Bract spine length	-.711161	.174444	-.487341	-.088250
Bract length / spine number	-.228695	-.743574	-.007986	-.225794
Bractiole length	-.757717	-.422272	-.232721	-.087505
Bractiole length / width	-.320957	.518671	.182924	-.239506
Anticous calyx length	-.876954	.061251	-.101589	.017006
Anticous calyx length / width	-.617951	-.234957	.315540	-.145810
Posticous calyx length	-.889260	-.088653	-.164651	.073983
Posticous calyx length / width	-.687175	-.217953	.233313	.104824
Ventral calyx length	-.742064	.034804	-.296624	.031452
Corolla length	-.897245	.119383	.154178	.008338
Corolla length / width	.147617	.083368	-.745357	-.144175
Filament length	-.656531	.267216	-.023920	.137086
Process length	-.500841	-.173179	-.149015	.245945
Anticous anther length	-.853582	.135202	-.031912	.020108
Anticous anther length / width	-.249498	.125429	.318528	-.355310
Posticous anther length	-.819098	.088809	.081081	-.008983
Posticous anther length / width	-.156811	.513822	.474052	.238117
Style length	-.854121	.166648	.009147	.145332
% Variance	42.07215	9.13867	7.65748	6.14562

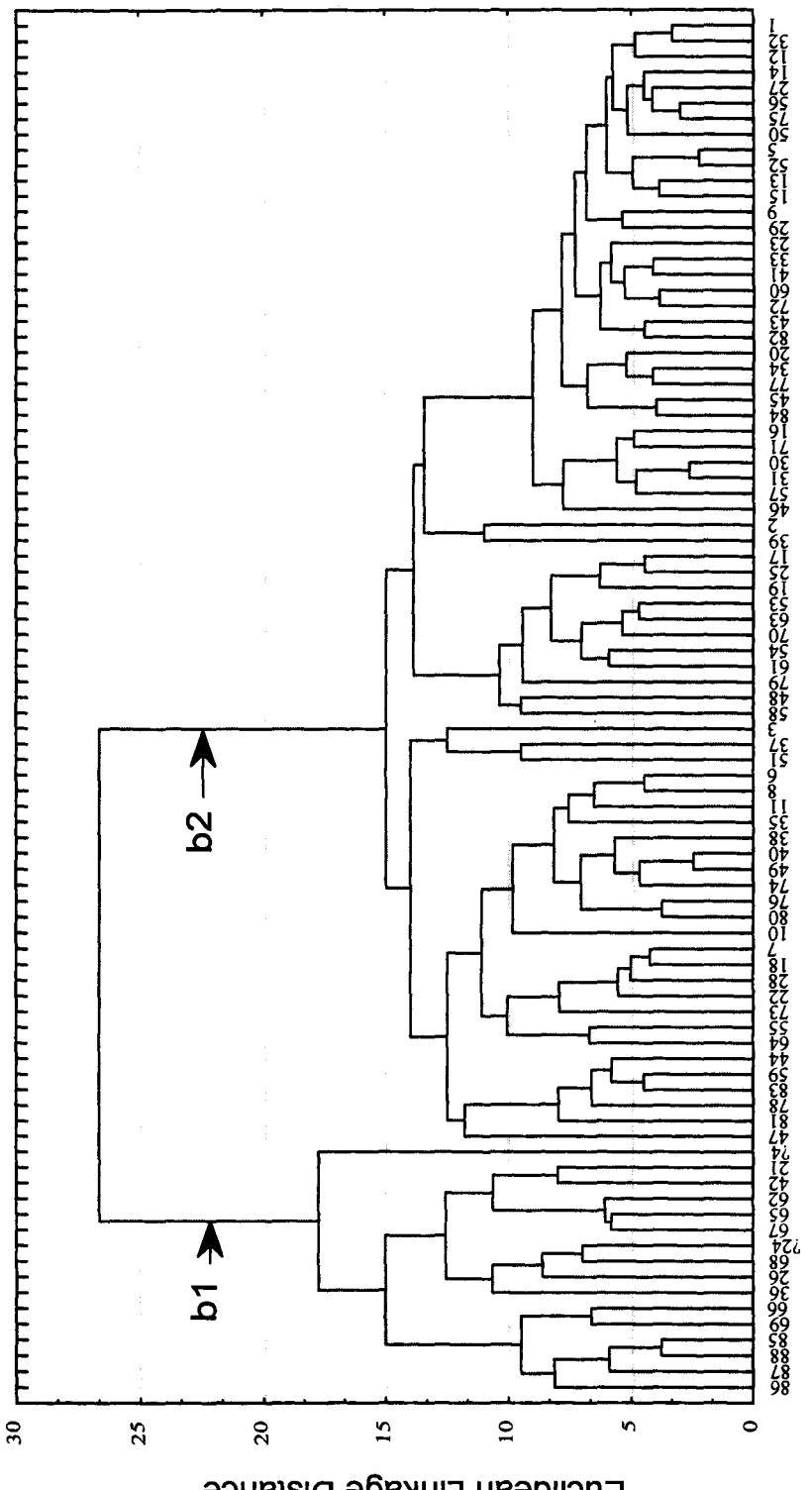

Figure 1. Dendrogram showing cluster pattern of the Blepharis *specimens studied. ? indicates unexpected position of the two collection from southern (K4 in Tsavo area) Kenya.*

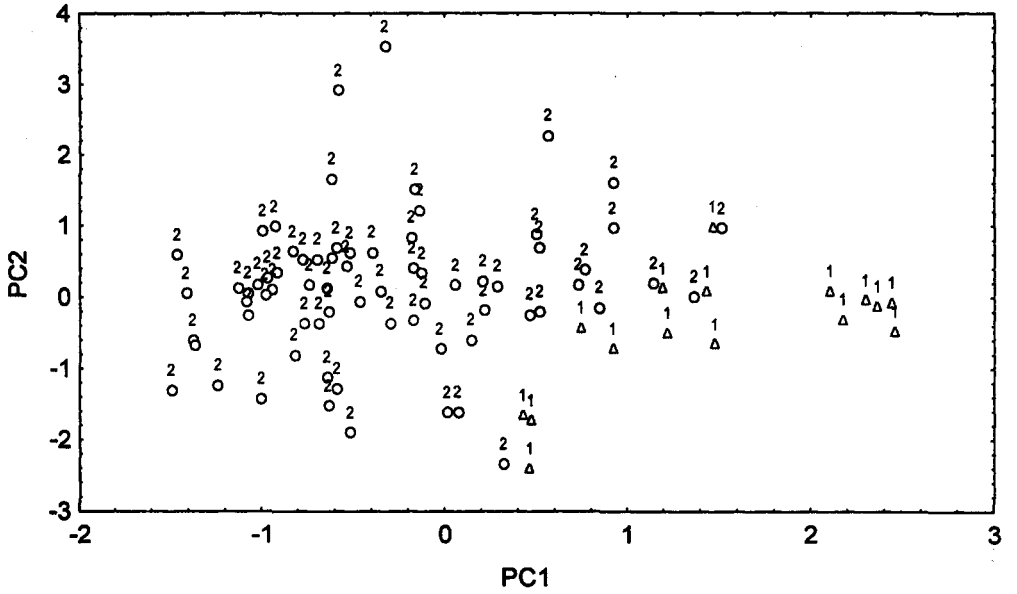

Figure 2: Scatter plot of the two clusters B1 (Δ) and B2 (O) along PC1 and PC2 ordination space

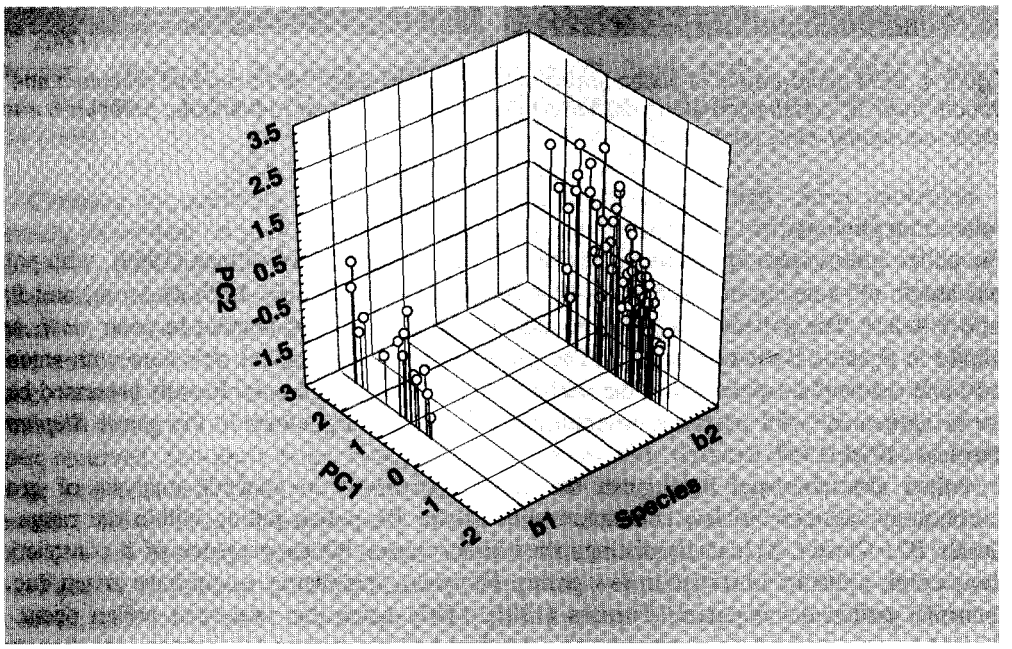

Figure 3: Three dimensional scatterplot showing differences of B1 and B2 along PC1 axis.

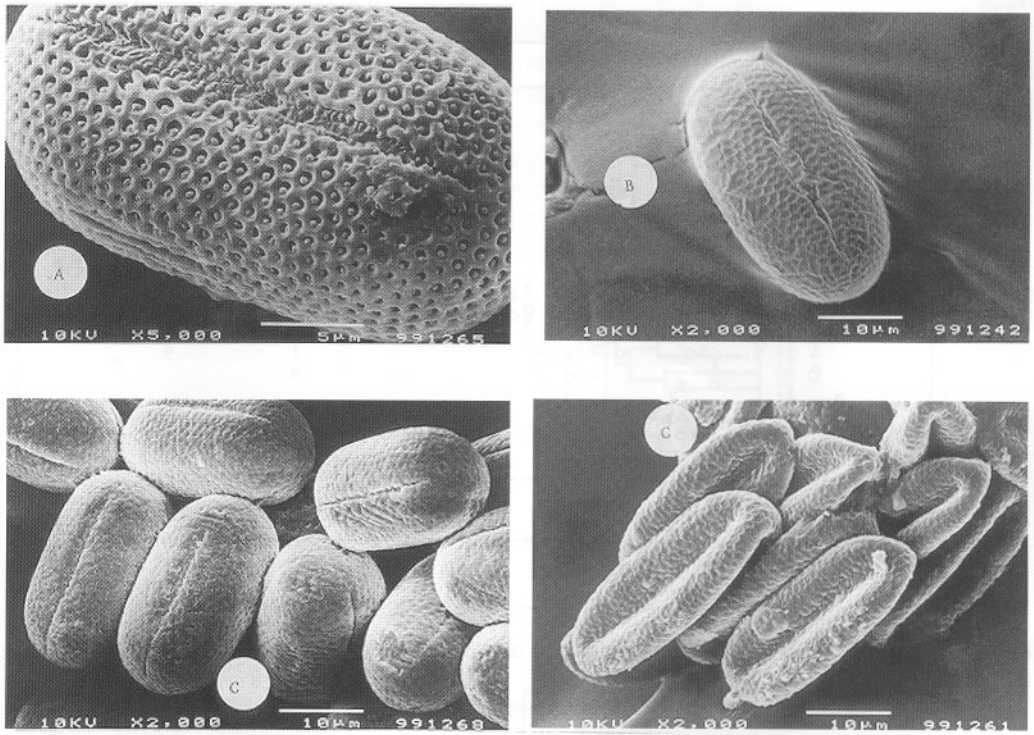

Figure 4. SEM micrographs of pollen grains of B. edulis complex. A: hexagonal reticulum and smooth muri, B: constriction along colpi in b1 pollen and C: circular to triangular polar view and elliptic equatorial view, respectively

Pollen morphology

The pollen grains were of medium size, based on terminology by Erdtman (1969), with polar axis length (P) ranging between 36.4–48.4 µm, equatorial axis (E) 15.9–24.1 µm, and P:E ratio 1.9–2.5. The pollen is isopolar, tri-colpate and circular to triangular in polar view, and elliptic in equatorial view (figure 4). The pollen exine has hexagonal reticulum with smooth muri and columellae appearing in the perforations of lumen (figure 4). Results presented here are in agreement with previous observations on pollen morphology in the genus *Blepharis* (Furness, 1996, 1997).

Pollen size does not distinguish the clusters observed in phenetic analysis of gross morphology, as there is size continuum with cluster B1 pollen falling within the range of cluster B2. Cluster B1 can be distinguished from cluster B2 by presence of a constriction along colpi, a feature observed in few grains. However, this feature might have arisen due to abnormal pollen development. Furness (1996, 1997) notes that abnormal pollen occur in *Blepharis* sect. *Acanthodium* where grains are few and the sculpturing patterns are disrupted near the apertures. Examples of taxa with abnormal pollen include *B. mitrata* C.B. Clarke , *B. capensis* (L.f.) Pers., *B. diversispina* (Nees) C.B.Clarke, *B. grossa* (Nees) T.Anderson, *B. obmitrata* C.B.Clarke and *B. subvolubilis* C.B.Clarke

TAXONOMIC TREATMENT

Two species are recognized based on phenetic analysis of gross morphological data and pollen morphological studies. Cluster B1 is treated as *B. boranensis* Vollesen and B2 circumscribed as *B. edulis* (Forssk.) Pers.

Taxonomic key

Annual herb; bract spines under 4.4 mm long; leaf marginal spines absent or 2–18, rarely more, stem glabrous to puberulous, sericeous .....**1. *B. edulis***

Perennial herb; bract spines 4.4–9.2 mm long; leaf marginal spines 20–36; stem puberulent to densely pubescent, ascending.....**2. *B. boranensis***

1. *Blepharis edulis* (Forssk.) Pers., Syn. Pl. 2: 180 (1806); Spreng., Syst. Veg. (ed. 16) 2: 820 (1825); T. Anderson in J. Linn. Soc., Bot. 7: 36 (1863); Lindau in Engler & Prantl, Nat. Pflanzenfam. IV, 3b: 318 (1895) & in Engler, Planzenw. Ost-Afr. C: 369 (1895) & C. B. Clarke in Thiselton-Dyer, Fl. Trop. Afr. 5: 102 (1899). Type: Yemen, *Forsskal* 905 (C, holotype; K, phototype!).

Acanthus edulis Forssk., Fl. Aegypt-Arab.: 114 (1775); Vahl, Symb. Bot. 1: 48 (1790); Wild., Sp. (ed. 4) 3: 400 (1801).

Blepharis linariifolia sensu Lindau, Ann. R. Ist. Bot. Roma 6: 76 (1896); C. B. Clarke in Thiselton-Dyer, Fl. Trop. Afr. 5: 100 (1899), p.p.; Agnew, Upl. Kenya Wild Fl.: 579 (1974); Kokwaro, Med. Pl. East Afr. (ed. 2): 22 (1993); Furness, Rev. Palaeobot. & Palyn. 92: 256 (1996).

Blepharis ciliaris (L.) B.L.Burtt, sensu Collenette, Fl. Saudi Arabia: 29 (1985); Furness, Rev. Palaeobot. & Palyn. 92: 256 & 263 (1996); Agnew & Agnew, Upl. Kenya Wild Fl.: 274 (1994).

Creeping to ascending annual herbs growing up to 45 cm high. Stem puberulent to densely puberulent on grey young branches and becoming glabrate, sericeous to puberulous, glandular, with age. Leaves green; midrib glaucous; abaxial surface puberulent to densely puberulent; lamina lanceolate to elliptic; 1.9–10.4 × 0.3–1.8 cm; acute to acuminate; apiculate; sessile to subsessile; margin lacerate, spines usually 2–18 or absent, scattered from base up to two-thirds towards apex, occasionally to tip of leaf; terminal spine 0.2–4.5 mm long, straight or on young plants falcate. Spikes erect, strobilate, each comprising of at least 2 mature flowers; 1.8-17.7 × 1.4–7.0 cm. Bracts green to glaucous, 1.4–3.5 × 0.4–1.6 cm; apex recurved, 0.4-2 cm long; marginal spines usually 3-8, terminal one less than 4.0 mm long; bracteole 6.5–20.0 × 0.2–1.5 mm, linear to lanceolate. Sepals densely pubescent; dorsal 1–2.5 × 0.2–1.0 cm, broadly ovate; ventral 0.8-1.9 × 0.2–0.8 cm, oblong; lateral pair 0.4–1.0 cm long, broadly ovate. Corolla blue to purplish; 1.2–3.1 × 0.5–1.8 cm; puberulent with dense strip of indumentum from base towards apex; veins blue, conspicuous; 5 lobed, two lower most lateral lobes vestigial, terminal lobe 0.1–0.6 mm long. Filament purple striped, 5.3–12 mm long with basal tuft of hair, dorsal pair flattened with apical appendages and broader anthers. Style 3.5–11.0 mm long. Capsule 4.3–10.0 × 2.5–5.8 mm, lanceolate to elliptic. Seed 2 per capsule; yellow; discoid; 4.4–6.8 × 3.0–4.5 mm; covered with white stellate hygroscopic hairs 0.3–5.3 mm long.

Distribution and habitat
In eastern Africa *B. edulis* is distributed from central Tanzania through Kenya to Eritrea and Somalia; altitude 20–1,900 m altitude. It grows in sandy or rocky, granite lava on open grounds in semi desert vegetation dominated by *Acacia* spp. or *Commiphora* woodland and annual grasses.

Selected specimens.
Ethiopia. Arsi Region: Sodere, Chilalo Awraja, 3 May 1971, *Thulin* 1309; Harerge Region: Dire Dawa, Isa, 18 Sept. 1985, *Ensermu* 1349; Dire Dawa, Melka Jelda, 29 June 1982, *Mesfin* 2908; Gursum Awr, 20 Jan. 1987, *Ensermu & Petros* 1869; Batie, 9 July 1966, *de Wilde* 9722; Shewa Region: Walenchiti, 26 Sept. 1980, *Ensermu & Tamra* 401; Koka Dam, 14 Mar. 1971, *Ash* 749.

Eritrea. Ocule, 15 Apr. 1902, *Pappi* 1272; Gahatli Ailet, 19 Mar. 1989, *Ryding* 1816; Zula, 6 Mar. 69, *Robertson* 1089; Zula, 6 Mar. 1969, *Bally* B6909.

Kenya. Rift Valley Province: Baringo, Marigat, 23 Oct. 1964, *Leippert* 5187; L. Baringo, Ol Kakwa Is, 2 June 1977, *Gilbert* 4711; Kajiado, Nairobi–Magadi Rd., *Bally* B9767; Turkana, Kainuk, 30 Dec. 1981, *Coppock* 24; *Muasya & Malombe* 1587; L. Turkana, Central Is., *Modha* 11. North Eastern, Marsabit, Milgis, 21 May 1970, *Magogo* 1442; Garissa, 29 May 1977, *Gillett* 21201. Eastern Province: Machakos, Kiboko, 22 Dec. 1960, *Ossent* 533; Mwingi, 4 Mar. 1973, *Sanagi* 934. Coast Province: Manyani, 15 Sep. 1967, *Ivens* 2236; Voi, 22 Feb. 1955, *Assent* 32.

Tanzania. Lushoto, Mkomazi, 1955, *Evens* 572; *Evens* 572; Njooro, 25 Mar. 1977, *Peterson* 433; Lushoto, 1 May 1953, *Drummond & Hemsley* 2324.

Notes
Although this study reconfirms separation of *B. edulis* complex as described, the taxon remains to represent variable forms, which cannot be separated phenetically. As Vollesen (2002) observed, the species can be very small in arid conditions to semi-perennial multi branched ascending semi-woody stems upto 45 cm in a favourable climate. Three major forms were observed in figure 1 but lacked discontinuous characters for separation. One form was characterized by a few widely spaced leaf spines and large bracts (up to 3.5 cm long). Its distribution includes Ethiopia and Eritrean Rift Valley from Zula through Dire Dawa, Kombolcha sites to Northern Kenya in Lake Turkana area such as Koobi Fora and near the Ferguson Gulf. The second form has the smallest flower sizes (up to 1.3 cm wide) and lacks spine or are very few restricted to first half of the leaf. This group is widely distributed in the region mainly in Kenya and Gamo Gofa Region of Ethiopia. The third form is closely related to *B. boranensis* on the basis of leaf spines but differ on the number of spines on the bracts. It is distributed in Kenya (Voi and Taita–Taveta districts), Tanzania and Ethiopia from Jijiga eastwards towards Somali mainly Harerge Region. Therefore, the specimen *Beentje* 1860, from Tsavo in southern Kenya was considered to be more closely related to *B. edulis* even though clustered with *B. boranensis* specimens in figure 1. More material from Tsavo should be examined to further ascertain their affinity.

B. edulis is found in degraded areas and can be a useful indicator species for ecological change. According to *Itani 78/78* (EA) it provides forage for all livestock and flowers are used for staining fat. Leaves are grinded and burned to treat mouth diseases in children

(*Mus 181*, EA). Water from boiled seeds is mixed with milk and taken to cure inflammation of the liver (*Newbould 5936*, EA). Dry inflorescence or root concoction is used to treat mouth wounds in children, kidney ailments, hasten childbirth, and against scorpion stings among the Kalenjin, Maasai, Somali and Turkana communities, respectively.

2. *Blepharis boranensis* Vollesen. Vollesen, Blepharis, 110-112 (2000). Type, Ethiopia, *Burger 3560* (K, holotype!, EA, ETH, isotype!)

Creeping to ascending perennial herb, emanating from a woody rootstock, up to 35 cm high. Stem densely puberulent on the young branches and becoming glabrate, sericeous to puberulous, glandular, with age. Leaves green sometimes with purplish patches, midrib glaucous; abaxial surface glabrous, puberulent to densely puberulent; lamina lanceolate to elliptic; 5.8–11.6 × 0.7–1.6 cm; acute, acuminate; mucronate; sessile to subsessile; dentulate, rarely lacerate; spines 20–36, evenly distributed from base to apex, terminal spine 0.1–3.7 mm long, straight. Spike erect, strobilate, each comprising of at least 2 mature flowers per spike; 3.8–13.2 × 1.7–4.8 cm. Bracts glaucous, 1.3–3.3 × 0.6–1.6 cm; apex slightly recurved, 0.8-1.7 cm long; marginal spine 5–11, terminal spine up to 9.2 mm long; bracteole 9.3–19.0 × 0.2–1.8 mm, linear to lanceolate. Sepals densely pubescent; dorsal 1.5–2.4 × 0.6–7.8 cm, elliptic; ventral 1.1–1.8 × 0.3–0.8 cm, oblong; lateral pair, 0.7–1.0 cm long, ovate. Corolla blue with yellowish base; 2.1–3.3 × 0.8–1.9 cm; puberulent with a brown dense strip of indumentum from base towards apex; vein dark bluish, conspicuous; 5-lobed, two lower side lobes vestigial, terminal lobe 0.3–0.8 cm long. Filament purple striped, 6.1–11.6 mm long with basal tuft of hair, dorsal pair flattened with apical appendages and broader anthers. Style 9–12.2 mm long. Capsule 7.5–10.4 × 3.0–5.8 mm, elliptic. Seeds 2 per capsule; yellow; ovoid, 4.5–6 × 3.0–4.3 mm; covered with white hygroscopic stellate hairs 1.8–8.7 mm long.

Distribution and habitat

Blepharis boranensis is found in Borana region (Kenya, Ethiopia and Somalia) and also around Sodere, Ethiopia between 1,350–1,850 m in limestone soil of *Acacia-Commiphora* woodland.

Selected specimens

Ethiopia. Sidamo Region: 33 km on Neghelle-Filtu road, 20 May 1982, *Friis, Mesfin & Vollesen* 3138; Negelle, Borana Awraja, 8 May 1987, *Puff et al.* 870508; Negelle, Borana Awraja, 20 Dec. 1998, *Ensermu & Aschalew* 4012; Malka Guba, near Dawa Parma River, 30 May 1996, *Ensermu & Dessalegn* 3798; Arsi Region: Sodere, 27 Jan. 1980, *Mesfin* 925.

Kenya. Eastern Province: Marsabit District, Koloba Hill, Jan. 1990, *Powys* 927.

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