Sec.

STUDIES ON LENTILS AND THEIR VARIATION, I. THE TAXONOMY OF THE SPECIES¹

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Introduction

For the past four years work has been in progress in Birmingham on the cultivated lentil, *Lens culinaris*. Conclusions presented by Zohary (1972) are supported by experimental work that was lacking in his paper.

Lentils have been cultivated since antiquity. Both Renfrew (1973) and Zohary (1972) recount sites in the Near East and C. E. Europe where carbonised remains have been found. Theophrastus (370-285 BC) wrote that they were grown and used by Greeks, Egyptians and Romans, though he stated that they were not grown in India. However, evidence of lentil cultivation in C. India comes from the Chalcolithic occupation at Navdatoli 2,000-1,400 BP (Allchin 1969). Strabo (66 BC-24 AD) reported that lentils were the staple diet of those building the pyramids in Egypt; remains of lentils have been found in Egyptian tombs of the twelfth dynasty 4,400-4,200 BP and lentil soup preparation is depicted on a fresco of 3,200 BP.

The present-day distribution of the cultivated lentil encompasses the Mediterranean, Near and Middle East, Africa (particularly Ethiopia) and extends east through Pakistan, to India and Bangla Desh, where it is an important dietary constituent.

Linguistic evidence suggests that European lentils were introduced originally by the Romans. The Greek name was fakos—food for the poor, but similar words are not found in other languages so the Greeks may not have been instrumental in the spread of the lentil. The Hebrew adaschum, adaschim has its present-day counterparts in Arabic, ads, adas, ades and Berber adis. In Algeria another Berber word bersim is known. The Turkish word is

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merschmek (or mecumeck). In Sanskrit masura, renuka, mangalaya were used, whereas now Hindustani, Bengali and Punjabi share the common word mussour. In Afghanistan the lentil is called nusk.

Lentils pose interesting problems because the relationship between the cultivated and wild species is uncertain, despite it being one of the earliest domesticated plants. Of the wild species L. nigricans, L. orientalis, L. ervoides and L. montbretii, the probable progenitor of the cultivated L. culinaris has been suggested as L. nigricans, because of its occurrence in two archaeological sites (Renfrew 1969). Barulina in 1930 described L. orientalis as the wild species most closely related to the cultivated small-seeded form. Previous investigations on cultivated lentils have been biased by accepting 2 major types; large-seeded and small-seeded. Theophrastus did not mention two seed types, and this could suggest that only one seed type was known at that time (small-seeded). Large-seeded forms could have arisen in cultivation from small-seeded types, or they may have had a separate centre of origin. Barulina suggested that the centre of origin of the small-seeded lentil lay between the Hindu-Kush and the Himalayas; and that Transcaucasia, Asia Minor and the Mediterranean represented a secondary centre of diversity.

Zohary (1972) concluded that available evidence pointed to *L. orientalis* as the progenitor, and that domestication took place in the Near East arc.

1) Taxonomy and synonomy of the species

In the most recent taxonomic treatment of the genus (Davis and Plitmann 1970), 5 species are recognised, showing close morphological similarities, except for *L. montbretti* (which is of doubtful affinity and links with *Vicia*, Sect. *Ervum*, and *Lathyrus*). A survey of the literature gives the synonymy of the species as below;

- 1. Lens culinaris Medik. in Vorles Churpf. Phys. Ges., 2, 361 (1787).
- Syn. L. esculenta Moench, in Meth., 131 (1794); L. camelorum Webb & Berth., L. disperma Webb and Berth., in Phyt. Canar., 2, 97 (1835); L. abyssinica Alef. L. nummularia Alef. in Bonplandia, 9: 130, 131 (1861); L. sativa Hell. in Fl. Wirceb, 2, 169 (1810); L. vulgaris Delanb in Fl. Auv., ed 2, 472 (1800); Ervum lens L. in Sp. Pl., 738 (1753); Vicia lens (L.) Cosson and Germ in Fl. Par. ed. 1, 143 (1845).
- 2. Lens ervoides (Brign.) Grande in Bull. Ort. Bot. Nap., 5: 58 (1918).
- Syn. Lens hohenackeri Webb and Berth. in Phyt. Canar., 2: 97 (1835); L. lenticula Alef. in Bonplandia, 9: 129 (1861); L. uniflora Schur. in Enum. Pl. Transs., 171 (1866); Cicer ervoides; Brign. in Fasc. Rar. Pl. Forojul, 27 (1810); Ervum lenticula Schreb. ex Hoppe in Slurm, Deutschl. Fl. Heft: 32 (1812); Vicia lenticula lanka in Termesz. Fusetek., 9, 138 (1885).
- 3. Lens montbretii (Fisch and Mey.) Davis and Plitm. in Fl. Turkey, 3, 325 (1970).
- Syn. Lens kotschyanus (Boiss.) Nab. in Publ. Fac. Sci. Univ. Masaryk Brno, 35: 100 (1923); Ervum kotschyanum Boiss. in Diagn. ser. 1(6): 48 (1846); Vicia bombycina Stapf. ex Post in Fl. Syria: 290 (1896); V. montbretii Fisch. and Mey. in Ind. Sem. Horti Petrop., 10: 59 (1845).

- 4. Lens nigricans (Bieb.) Godr. in Fl. Lorraine, 1: 173 (1843).
- Syn. Lens biebersteinii Lamotte in Prod., 1: 220 (1877); L. culinaris Medik. subsp. nigricans (Bieb.) Thell. in Mem. Soc. Nat Sci. Math. Cherbourg, 38: 346 (1911-12).
 L. lentoides Webb and Berth. in Phyt. Canar., 2: 97 (1835), L. tenorei Lamotte in Prod., 1: 220 (1877), Ervum nigricans Biebrin in Fl. Taur.-Cave., 2: 164 (1808), Vicia nigricans (Bieb.) Cosson and Germ. in Fl. Par., ed. 1: 143 (1845).
- 5. L. orientalis (Boiss.) Hand.-Mazz. in Ann. Nat. Hofmus, Wein, 27(4): 40 (1913).
- 5a. L. orientalis (Boiss) Hand.-Mazz. subsp. brachycalyp Post in F1. Syria (1896), differentiated by the length of the calyx teeth (½ to 2 as long as the corolla).
- 5b. L. orientalis (Boiss.) Hand.-Mazz. var. cyaneum M. Popov; (the status of this variety needs further study). Ball (1968) and Davis and Plitmann (1970), do not subdivide the species.

2) The morphology of the species

A survey of herbarium specimens was made, using sheets loaned from several herbaria (BM, CGE, E, GAT, HAL, K and SLO). Measurements were taken on the characters listed in Table 1 in which the mean values and range of variations are also shown. Table 1 shows wide variation within each taxon, and taxa overlapped in some characters. However, characters considered to be diagnostic for one species tended to vary in the direction of another, except for *L. montbretii* which is distinct. Comparisons were made using those characters which showed no large overlap (scored on a 0-5 scale) as hybrid indices (Fig. 1).

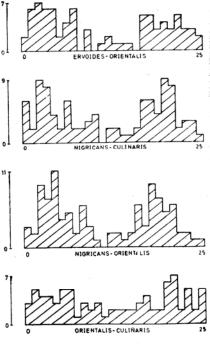


Fig. 1. Hybrid indices.

Table 1. Summary of data (mean \pm standard deviation and range, in mm)

Character	L. culinaris	L, ervoides	L.montbretii	L. nigricans	L. orientalis
Upper leaf: length	29.42±7.01 (21-45)	$ 7 \pm 2.72 \\ (3.75-13) $	45.64 ± 7.42 $(32-53)$	13.46±3.50 (7-21)	11.86±4.27 (5-22)
: petiole length	$^{11.4}_{\ \ (1-4.5)}^{\pm 2.96}$	$^{10.55\pm2.87}_{(0.5-2.5)}$	10.64 ± 2.36 $(2-6)$	$8.51\pm1.72\ (0.5-2)$	$^{6.36\pm1.59}_{(0.5-2)}$
Basal leaflet: length	$11.4 \pm 2.96 \\ (8-18.5)$	10.55±2.87 (5-18)	$10.64\pm2.36\ (8-15)$	$8.51\pm1.72\ (4-12)$	$6.36\pm1.59\ (4-9)$
: breadth	$^{2.47\pm0.87}_{(1.5-4.5)}$	$^{1.27\pm0.45}_{(0.75-2)}$	3.32 ± 0.77 $(2-4)$	$^{1.68\pm0.43}_{(1-2.5)}$	$^{1.48\pm0.45}_{(1-2)}$
: L/b	4.76 ± 0.89 (3.33-6)	8.60±3.11 (3.75-15.33	3.25 ± 0.47 5)(2.75-4)	5.32 ± 1.62 (3.33-9.5)	4.59 ± 1.30 $(3-8)$
Peduncle length	$22.73 \pm 6.65 \ (11.5 - 33)$	$^{22.08\pm6.01}_{(9.5-31)}$	$^{19}_{(12-25)}^{\pm4.72}$	$23.17 \pm 6.39 \ (11 - 35)$	$^{15.43\pm4.44}_{(8-20)}$
Leaf length/peduncle length	$^{1.40\pm0.40}_{(0.77-2.85)}$	0.33 ± 0.14 (0.12-0.61)	$2.51\pm0.76\ (2-4.17)$	$^{0.61\pm0.21}_{(0.3-1.2)}$	$_{(0.5-1.11)}^{0.79\pm0.21}$
Arista length	3.77 ± 2.60 $(2-6)$	absent	$^{1.43\pm1.90}_{(0-5)}$	5.11 ± 1.47 $(2.75-9)$	$2.05\pm1.08\ (0-3.5)$
Corolla length	5.37 ± 1.17 (3.5-7)	$3.91 \pm 0.64 \ (2.5 - 5.2)$	7.5 ± 1.47 (5.75-9)	5.99 ± 1.11 $(3.6-9)$	4.94 ± 0.67 (3.5-6)
Calyx length	6.44±4.19 (5-8)	3.57 ± 0.6 $(2.5-5)$	$6.5 \pm 1.18 \ (5-8)$	$^{6.33\pm1.05}_{(4.8-9)}$	$^{4.25\pm0.61}_{(3-5.5)}$
Corolla length/Calyx length	$ \begin{array}{c} 0.83 \pm 0.11 \\ (0.63 - 1.07) \end{array} $	1.11 ± 0.14 (0.75-1.42)	1.15±0.10 (1.05-1.29)	$_{(0.44-1.3)}^{0.96\pm0.17}$	$^{1.89\pm0.20}_{(0.7-1.56)}$
Calyx teeth length	5.13±2.47 (3-6.25)	2.37 ± 0.59 (1.5-3.6)	3.58 ± 0.49 $(3-4)$	$^{4.82\pm1.03}_{(3.2-7.5)}$	$ \begin{array}{ccc} 3.04 & 0.57 \\ (2-3.5) \end{array} $
Corolla tube length	1.69±0.60 (1-3.5)	$^{1.18\pm0.38}_{(0.5-1.8)}$	$\substack{2.92 \pm 1.02 \\ (2-4)}$	$^{1.5\ \pm0.40}_{(0.8-2)}$	$^{1.18\pm0.38}_{(0.5-2)}$
Calyx teeth L/corolla tube L	3.12±1.13 (2-6)	2.35 ± 1.46 $(1.33-7)$	$^{1.36\pm0.48}_{(0.75-2)}$	$3.51\pm1.40\ (1.57-6)$	$^{2.79\pm1.32}_{(1.33-4)}$
Pod length	12.13±2.36 (7-19)	$\substack{7.81 \pm 1.02 \\ (5.6 - 9.5)}$	19.75±0.99 (19-21.5)	8.82±1.05 (7-11)	$8.40\pm1.38\ (6-11)$
Pod breadth	6.34±1.31 (4-9)	3.63 ± 0.56 (2.5-4)	$\substack{7.83 \pm 0.52 \\ (7-8.5)}$	$4.13\pm0.61\ (3-5)$	$4.13\pm0.74\ (3-5)$
Pod L/b	1.92±0.19 (1.67-2.25)	2.17 ± 0.24 (1.75-2.67)	2.52 ± 0.11 (2.38-2.71)	2.17±0.28 (1.8-3)	$2.06\pm0.24\ (1.7-2.67)$

Comparisons between *L. ervoides* and *L. culinaris*, and *L. ervoides* and *L. nigricans* showed *L. ervoides* to be distinct from the other two and there was no evidence of hybridisation.

When *L. ervoides* and *L. orientalis* were compared, intermediate forms suggested hybridity with indications of backcrossing into *L. ervoides*. When the species pairs, *L. nigricans-L. culinaris*, *L. nigricans-L. orientalis*, *L. orientalis-L. culinaris* were compared, the evidence suggested that multiple hybrids may have been formed and putative hybrids are marked in Fig. 2 where two diagnostic characters are plotted,

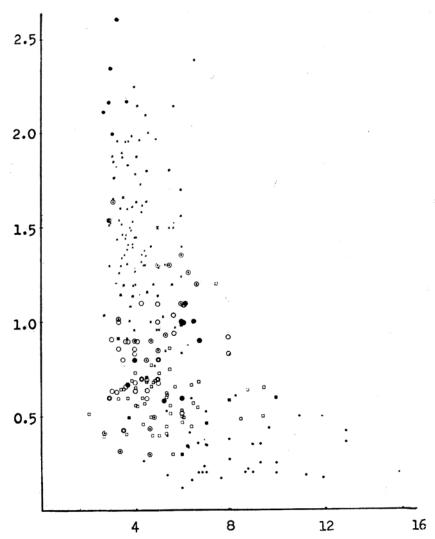


Fig. 2. Variation in the species of Lens. Leaf length/Peduncle length for ordinate, and Length/breadth of leaflet for abscissa.

**—L. culinaris, **—L. ervoides; *—L. montbretii; —L. nigricans;
-L. orientalis. Combinations of symbols indicate putative hybrids.

Introgression can enrich the gene pool of species and many of the morphological differences are quantitive. In *Lens*, introgression has occurred between several species, and the cultivated lentil is very closely associated with *L. nigricans* and *L. orientalis*. Past hybridity and backcrossing has tended to blur species boundaries, e.g. *L. ervoides* subsp. *leiocarpum* could well be hybrid.

3) Experimental investigations

The variation in *L. culinaris*, *L. nigricans* and *L. orientalis* was assessed under uniform growing conditions which were agriculturally meaningful

including the supply of a suitable Rhizobium. The accessions of *L. culinaris* included both primitive and selected forms of both seed sizes. The following characters were scored: date of flowering; presence or absence of anthocyanin; leaf colour (light green, green, or grey green); habit; erect (semi-erect or prostrate); pubescence of upper and lower leaflet surfaces; stipule shape; number of flowers; standard petal; colour; standard petal; vein colour; extent of colouring of laterals and keel; weight of seeds; colour of testa; degree of mottling of testa; colour of cotyledons.

At standardised positions on the plants a large number of measurements were made on leaves, stipules, tendrils, flowers, aristas and seeds. The data were reduced to coded form and subjected to a principal components analysis without weighting any characters. The data were plotted using the scaled vectors 1 and 2, and vectors 1 and 3 of the transformed matrix as co-ordinates, and vectors 1 and 2 are shown in Fig. 3. To see if the wild species and the two seed types of *L. culinaris* were distinguishable simply on vegative and floral characters, the data were subjected to analysis excluding seed characters (Fig. 4).

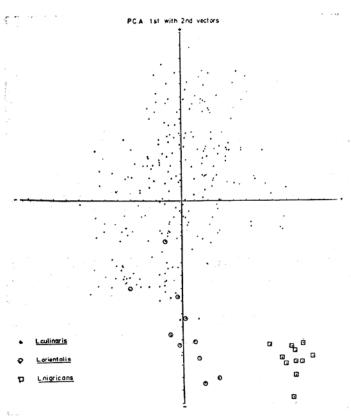
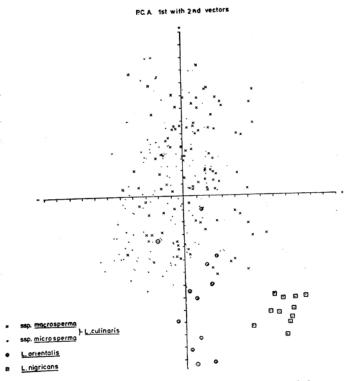


Fig. 3. Principal component analysis.



Eig. 4. Principal component analysis excluding seed data.

In the analyses, the scatter of *L. nigricans* separated from the two species but the scatter of *L. orientalis* overlapped with *L. culinaris*. The scatter of *L. culinaris* takes the form of a wide diagonal belt and the opposite extremes represent small-seeded small-leaved types and large-seeded large-leaved. There was no clear split into two distinct groups and both types occurred in the central area. Some *L. culinaris* forms separated from the others and were closer to *L. orientalis*: these were composed entirely of Indian accessions.

The relative position of the points did not alter between the full analysis and that when seed characters were omitted, so relationships based on vegetative and floral characters are supplemented by similar relationships in seed characters. The gradient that can generally be described as being from large to small seed and leaf size, is valid even without using seed characters. This evidence along with seed size data (Fig. 5) suggests that the division of *L. culinaris* into subspecies is purely arbitrary. The character that most nearly splits the scatter belt is seed shape (Fig. 6). This character, unlike seed size, is dichotomous but its predictive value is only slightly greater than seed size since this does not represent a natural, separated division with respect to other characters.

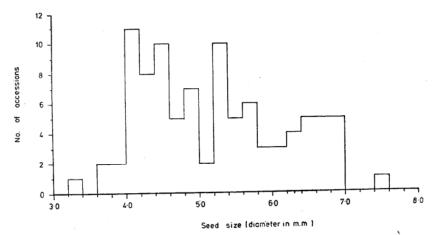


Fig. 5. Variation of seed size in L. culinaris.

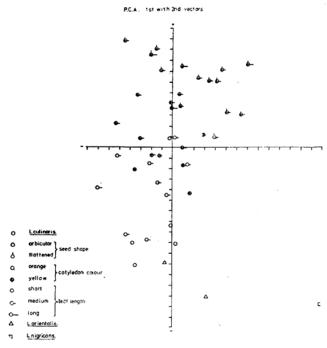


Fig. 6. Principal component analysis of mean accession data for seed characters.

4) Detailed studies on the relationship between the species

(a) Karyotype analyses

Although L. culinaris is diploid 2x=2n=14 (Heitz 1926; Miranda 1931; Bhattacharjee 1951; 1953; Sharma and Muckhopadyay 1963), no counts have been made of L. nigricans and L. orientalis. Cytological preparations were made of the 3 species using PDB pretreatment, staining with Feulgen and mounting, using the technique of Kordan and Jackson (1972).

Karyotype analyses are shown in Fig. 7. In L. culinaris, the karyotype consists of three metacentric and four telocentric pairs, three of which are identical with respect to position of centromere and total length of chromosome. The karyotype of L. orientalis (2n=14) is the same as that of L. culinaris. In L. nigricans (2n=14) the karyotype consists of four metacentric, two telocentric and one acrocentric pairs. L. nigricans has three metacentric and two telocentric pairs in common with the other two species, but whereas L. culinaris and L. orientalis have a triplicate set of telocentrics, L. nigricans has only one equivalent pair. The remaining two are a large metacentric and an acrocentric pair of chromosomes.

The more similarity that exists between two karyotypes, the greater the probability that the two are interfertile. Species of *Lens* normally inbreed but there is evidence of some hybridisation between *L. culinaris* and *L. orientalis*, and *L. orientalis* and *L. nigricans*. Thus the karyotype dissimilarities observed do not entirely prohibit fertile crosses.

Sharma et al. (1963) noted differences in chromatin length within 14 lines of L. culinaris, recognised 5 different types of chromosomes, and also a secondary constriction on one of the chromosomes. The present authors have not observed this latter phenomenon, nor do their karyotype analyses agree with those presented by Sharma et al. This may be due to the use of different material and different treatments. The constancy found in chromosome number is interesting in view of the ideas of Darlington (1956), who stated that pulses (as well as cereals and oil-seed crops) are propagated only by seed and grown mainly for seed. Thus, seed fertility is a major facet of their evolutionary strategems. In this context, it is not surprising that they are functional diploids and chromosome abnormalities are rare.

Further work on meiotic divisions would establish whether the three telocentric pairs in *L. culinaris* and *L. orientalis* are fully equivalent, or just phenotypically similar. Also, further work on karyotype compatability should

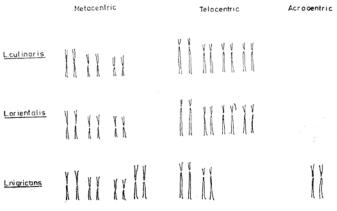


Fig. 7. Karyotypes of three Lens species.

be carried out on hybrids, but as Zohary pointed out, the small flower size and prevalence of cleistogamy make artificial crossing very difficult. The authors have not been able to cross the species successfully due to sensitivity to mutilation.

(b) Pollen grain morphology

There is no description in the literature of the morphology of the pollen grains of the species of *Lens*, even though its significance is argued by David and Heywood (1963). Fresh pollen of *L. culinaris*, *L. nigricans* and *L. orientalis* was collected and examined by scanning electron microscopy.

The three *Lens* species were tricolpate and the pollen shapes were ovate-ellipsoid. The three colpae were also similar, being ca. 2/3 of the pole to pole length and distributed regularly about the polar axis. Differences were observed in the exine sculpture with *L. culinaris* and *L. orientalis* being similar and somewhat different from *L. nigricans* (Fig. 8). Exine types are thus diagnostic.

Discussion

The evidence provided confirms *L. orientalis* as the progenitor of the cultivated lentil. The alternative, *L. nigricans*, was based on seed sizes, but identification on this basis cannot eliminate either of the wild species. No seed characters were found to be exclusive to any of the species. Analysis suggested a very close relationship between *L. orientalis* and *L. culinaris* notably with plants of Indian origin, whilst *L. nigricans* was separate from both. These same relationships were suggested by karyotypes and pollen morphologies.

Within *L. culinaris* there was little evidence of subgroupings, and division into subspecies on seed size is untenable. A more natural division could be into varieties based on seed shape. Other attempts, not reported here, to analyse the variation within *L. culinaris*, e.g. phenolic compounds by chromatography, showed that no relationship existed between them and seed size.

If introgression has occurred between species, some outcrossing must occur even if limited. It has been estimated at 0.01-0.08% by Wilson et al. (1972). Flowering in L. nigricans occurred experimentally when L. culinaris and L. orientalis had set seed, and only overlapped slightly with L. orientalis. Such a situation will cause effective temporal isolation between L. culinaris and L. nigricans.

In *L. culinaris* the amount of pre-harvest dehiscence is one indication of the degree of selection that has taken place. Whilst the Indian lentils are among the most highly selected, they are also the most similar to *L. orientalis*. Since the only characters that distinguish these two groups are amount of

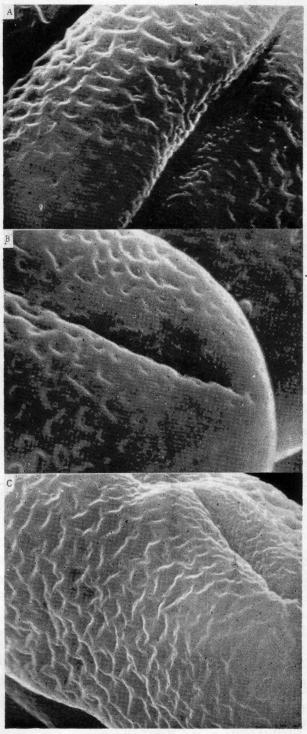


Fig. 8. The surface of polien grains $\times 13 \mathrm{K}$ A -L culinaris,

B L nigricans,

C-L orientalis.

dehiscence, and presence or absence of dormancy, it is suggested that L. culinaris and L. orientalis are best considered as con-specific, each representing a subspecies, viz. L. culinaris Medik. subsp. culinaris comb. nov., and L. culinaris Medik. subsp. orientalis Boiss. comb. nov. including var. brachycalyx (Post) and var. cyaneum (M. Popov).

Results presented in this paper add weight to the origin of the cultivated lentil as a selected form of a wild one, with continued gene-flow from other wild species. *L. culinaris* can therefore be regarded as a compilopecies (Harlan and De Wet, 1963). The descriptions of the species in the summary represents a résumé of the data presented in this paper. A second paper in this series will discuss protein contents in relation to breeding and genetic conservation.

Summary: Key to the genus Lens (Ball 1968, revised)

1.	Stipules	semi-hastate,	entire	or	toothed
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- 2. Legume 7-11×3-5 mm, glabrous; peduncle markedly aristatenigricans
- 2. Legume 5.6-9.5×2.5-4, ciliate; peduncle with no arista, or if present 1 mmervoides
- 2. Legume 19-21.5×7-8.5 mm, villous......montbretii
- 1. Stipules oblong-lanceolate and entire

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