

ORIGINS OF DOMESTICATION OF *LUPINUS MUTABILIS* IN THE ANDES

Ruth J. Eastwood^{1,2} and Colin E. Hughes¹

¹Department of Plant Sciences, University of Oxford, South Parks Rd, Oxford, OX1 3RB, U.K

²Present address Millennium Seed Bank, Wakehurst Place, Ardingly, Haywards Heath, W. Sussex, RH17 6TN, U.K

Corresponding author's email: colin.hughes@plants.ox.ac.uk

ABSTRACT

Lupinus mutabilis Sweet is apparently unknown in the wild but is cultivated as an important food crop throughout the Andes and is also the focus of crop breeding and development elsewhere in Australia and Europe. The species exhibits many of the typical legume domestication syndrome traits including indehiscent pods, large seeds, water permeable seed coats, reduced seed pigmentation, rapid and uniform germination and growth, and a nearly annual life history, but retains higher seed alkaloid levels than other lupin domesticates. Despite this importance as a crop, the origins of domestication of *L. mutabilis* in terms of where, when, how many times and from what wild progenitors it was domesticated remain unknown. Our phylogenetic studies place *L. mutabilis* in a well-supported Andean clade, ruling out earlier speculations that the crop might have originated from wild North American species. However, within the Andes, research to identify the progenitors of *L. mutabilis* is hampered by chaotic taxonomy, the very low levels of DNA sequence divergence amongst the Andean species, and very limited lupin seed remains retrieved from archaeological sites. Low DNA sequence divergence has been attributed to exceptionally rapid species diversification in the Andes, and makes it difficult to employ conventional DNA sequence data to analyse species relationships among the ca. 81 species in this Andean group and hence to identify the close relatives and putative progenitors of *L. mutabilis*.

Despite these challenges, recent field, herbarium and laboratory investigation of *L. mutabilis* provide new evidence from morphology (leaves, indumentum, habit, flower size, flower colour variation, pod and seed size) and DNA sequence data that suggest *L. piurensis* as the most likely wild progenitor of domesticated *L. mutabilis*. This species has not previously been proposed as a putative progenitor of *L. mutabilis* despite its clear morphological similarities and large seeds. *L. piurensis* is restricted to the western slopes of the Andes between 1650 and 3300 m altitude in northern Peru and southern Ecuador, suggesting that domestication of *L. mutabilis* may have occurred in northern Peru. A limited number of flower and seed colour variants are documented within *L. mutabilis*, but in many respects the species is rather uniform.

KEYWORDS

Andes, crop, domestication, *Lupinus*, *Lupinus mutabilis*, morphology, phylogeny, seed

INTRODUCTION

It has long been known that lupins were domesticated independently as pulse crops in both the Mediterranean and the Andes (Gross, 1982, 1986; Hondelmann, 1984; Plitmann and Heyn, 1984; Cowling, *et al.* 1998; Gladstones, 1998). The origins and likely progenitor of *Lupinus albus*, the species first domesticated in the Mediterranean, are well known. In contrast, the domestication of *Lupinus mutabilis* as an important crop in the Andes (Fig. 1), cultivated especially by mid to high elevation subsistence farmers in Peru and Bolivia, remains poorly understood in common with many other Andean 'Lost Crops of the Incas' (National Research Council, 1989). At the same time, there has been interest in cultivating and improving *L. mutabilis* as a crop in Europe (Hondelmann, 1984), and Australia (Clements *et al.* 2005; Harries, 2005; Sweetingham *et al.* 2005, 2006). Interest in *L. mutabilis* developed in Europe because of the high quality of the seeds. As a protein and oil source (protein 25-50% and oil 21% seed dry matter content rich in polyunsaturated fats), *L. mutabilis* seeds compare well with the quality of soyabean (Blanco, 1982a; Gross, 1988; National Research Council, 1989). However, full crop development and use of *L. mutabilis* has been limited by low and unstable yields, uncertain pod maturation and susceptibility to frost (von Baer and von Baer, 1988; Martins, *et al.* 1992; Hardy, *et al.* 1997). These limitations mean that currently available genotypes cannot compete with other domesticated lupin species.

Despite this actual and potential importance, the origins of domestication of *L. mutabilis*, in terms of where, when, how many times and from what wild progenitors it was domesticated, remain largely unknown. Two main hypotheses have been proposed for the origin of *L. mutabilis*. First, Kazimierski and Nowacki (1961) suggested that *L. mutabilis* arose as a hybrid between two N. American species *L. douglasii* and *L. ornatus*, with seeds subsequently transported to S. America by humans. Current data and evidence provide no support for this. The hypothesis is based on morphological characters that are shared by a large cohort of western New World species. Furthermore, recent phylogenetic studies place *L. mutabilis* in a large

robustly supported group of closely related species all of which are restricted to the Andes (Fig. 2) (Hughes and Eastwood, 2006; Drummond, 2008). Second, Blanco (1982b, 1984, 1986) and Tapia and Vargas (1982) suggested that wild forms of *L. mutabilis* are found around Cuzco in southern Peru. Tapia and Vargas (1982) go further, pointing out the close similarity between *L. mutabilis* and *L. praestabilis* from near Cuzco. These ideas are based on morphological similarities and insights from local names and remain speculative. In fact *L. praestabilis* also shows notable morphological differences from *L. mutabilis* including 40-70 mm-long stipules unique among the Andean lupins.

A clear understanding of the origins and close relatives of *L. mutabilis* is needed to underpin breeding efforts and explore ways to broaden the genetic base, for example through interspecific hybridisation. In addition, understanding the domestication of *L. mutabilis* will contribute important data to the overall picture of crop domestication in the Andes which remains very poorly understood compared to other regions.

The biggest difficulty surrounding studies of *L. mutabilis* is uncertainty about species delimitation and the chaotic taxonomy of the Andean species. There are 480 *Lupinus* species names based on Andean plants, a proliferation brought about in large part by the flawed Species Lupinorum of Smith (1938-1953). Our recent estimate of 85 Andean species (Hughes and Eastwood, 2006) is based on re-examination of species boundaries via field and herbarium work to assemble a new taxonomic account that aims to bring consistency of species delimitation across the genus.

Here we present results of recent field and herbarium surveys and molecular phylogenetic analyses aimed at understanding the origins of *L. mutabilis*. This included: (i) comparative morphological survey to identify putative close relatives of *L. mutabilis*; (ii) taxonomic work to delimit species and resolve synonymy and names; (iii) survey of seed characters and size. Measurements of weight, length, width and thickness and observations of seed shape, colour and seed coat pattern were taken from 25 seeds per accession using standard terms (IBPGR, 1981; Systematics Association, 1962); (iv) DNA sequencing of three nuclear loci (ITS, Cycloidea LEGCYC1A and GPAT) to construct a combined data matrix including 36 of the 85 Andean species. DNA sequencing methods, data sets and analyses are presented elsewhere (Hughes and Eastwood, 2006; Eastwood *et al.* unpubl. data). Results of these investigations are discussed in relation to published data and dates for *L. mutabilis* seed remains from Andean archaeological sites.

RESULTS AND DISCUSSION

Field survey. The present-day distribution of *L. mutabilis* ranges from Venezuela to Bolivia (Fig. 1).

As far as can be ascertained, all these records stem from cultivated plants. This accords with extensive fieldwork in Bolivia, Peru and Ecuador which has so far failed to reveal any occurrences of *L. mutabilis* in anything resembling natural vegetation. Thus, as far as is currently known, *L. mutabilis* is only known in cultivation with occasional individuals persisting immediately thereafter in fallow fields and their margins.

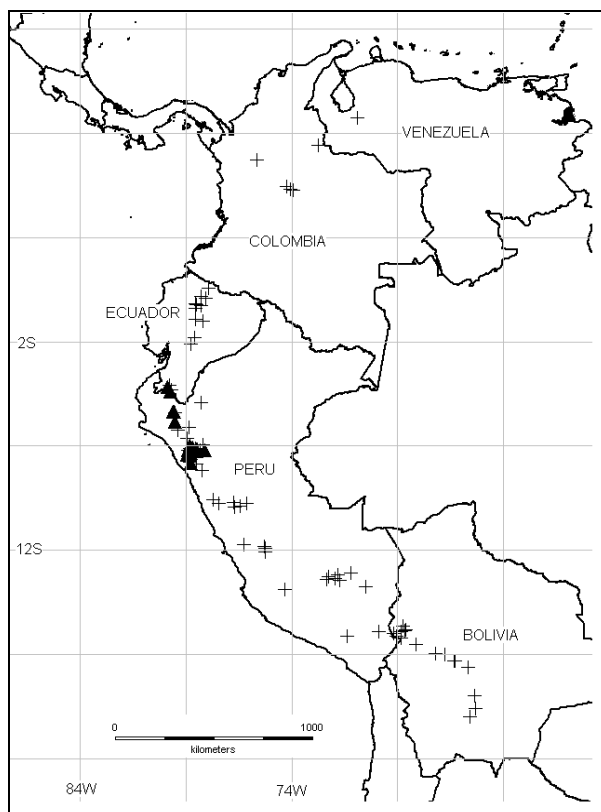


Fig. 1. Map showing the distribution of *L. mutabilis* + and *L. piurensis* ▲.

Morphological Survey. Based on comparative morphological survey of leaves, flowers and fruits, four Andean species: *L. ellsworthianus* C.P.Sm., *L. piurensis* C.P.Sm., *L. praestabilis* C.P.Sm. and *L. semperflorens* Hartweg ex Benth., were identified as similar to *L. mutabilis*. A taxonomic conspectus of these species is presented in Annex 1. These species share **glaucous stems** (especially young shoots), **thin membranous leaflets** with a **glabrous adaxial surface** that is only occasionally sparsely hairy, **mucronate, oblanceolate** rarely narrow-linear or elliptic leaflets, and unusually **large flowers**. Of these species, *L. piurensis* is the most similar. Indeed, it is so similar to *L. mutabilis* that it is usually impossible to confidently separate herbarium specimens of these two species without fruiting material. It is notable that three of these species, *L. semperflorens*, *L. praestabilis* and *L. piurensis* also have **larger and heavier seeds** than the majority of Andean lupin species. In fact all three fall within the top seven Andean species in terms of seed weight (Fig. 2).

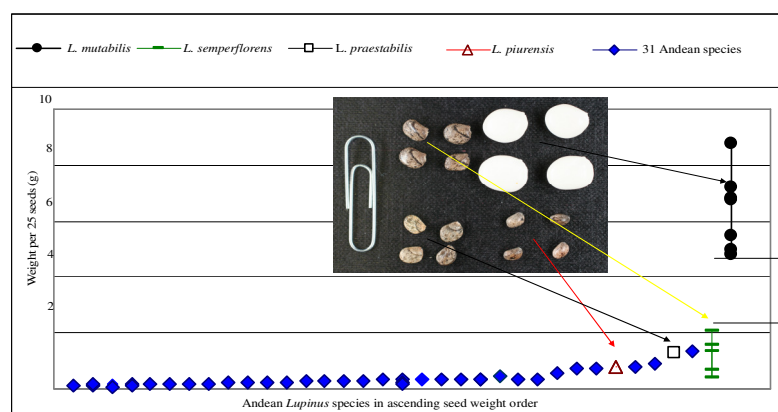


Fig. 2. Seed weights of 32 Andean species showing the weights of putative progenitor species and the dramatic seed weight increase associated with domestication of *L. mutabilis*.

Phylogenetic analyses. Previous analyses of DNA sequence data for lupins have shown a striking lack of sequence divergence in the widely used ITS region of nuclear ribosomal DNA and consequent lack of resolution of relationships amongst the western New World lupins (Ainouche and Bayer, 1999; Ainouche *et al.* 2004). In order to obtain a more resolved phylogeny, we have attempted to select more variable DNA sequence loci. Building on Ree *et al.* (2004), we initially sequenced one copy of the nuclear gene *cycloidea* (*LEGCYC1A*) to produce a new phylogeny for the genus (Hughes and Eastwood, 2006). The *cycloidea* gene sequences are substantially more variable than ITS, providing greater resolution amongst the western New World species and identifying a robustly supported group representing c. 81 Andean species including *L. mutabilis* (Hughes and Eastwood, 2006). However, even by combining the ITS and *cycloidea* data sets, relationships among the Andean species remain almost completely unresolved. This has been attributed to very recent rapid species diversification in the Andes (Hughes and Eastwood, 2006). In an attempt to overcome this, a further DNA sequence locus, *GPAT*, has been sequenced for 90 species, including 35 Andean species. Using all three DNA sequence data sets in combination (2500 bp of sequence for 84 species), some weakly supported resolution within the Andean group is obtained (Fig. 3). The four species identified as morphologically similar to *L. mutabilis* are placed in a weakly supported subgroup of Andean species with *L. mutabilis*. Within this group, lack of resolution is again a serious limitation, but one accession of *L. piurensis* is resolved

with weak support with *L. mutabilis*, suggesting that species as a possible progenitor (Fig. 3).

If this hypothesis is correct, the distribution of *L. piurensis* on the western slopes of the Andes between 1650 and 3300 m in northern Peru (Fig. 1) suggests an origin for *L. mutabilis* in that area. The morphological changes associated with domestication of *L. mutabilis* – increased seed size, non-shattering pods, reduced seed pigmentation, increased seed coat permeability, shift from perennial to annual/biennial habit and rapid and uniform growth – are similar to those observed for Old World lupins. The increase in seed size (six-fold if *L. piurensis* was the progenitor and at least twice the size of any other Andean species) is more dramatic than observed for Old World domesticates where the majority of species have large seeds (Gladstones, 1974; Plitmann, 1981). Lack of resolution and sparse sampling in the Andean clade preclude inferences about whether *L. mutabilis* was domesticated once or multiple times. However, there are no major morphological disjunctions within *L. mutabilis* to suggest more than one domestication event.

The archaeological record of *L. mutabilis* seed remains is summarised in Fig. 4. The earliest presumed wild lupin seeds found in the Andes date to 9,010 - 8,125 BP (Pearsall, 1980) but the first appearance of unequivocally domesticated *L. mutabilis* seeds is much later at 1800 BP from the Mantaro Valley, central Peru (Hastorff, 1993). This leaves a window between 5500-1800 BP for domestication of *L. mutabilis* pending the discovery of additional seed remains that can be assigned to domesticated plants.

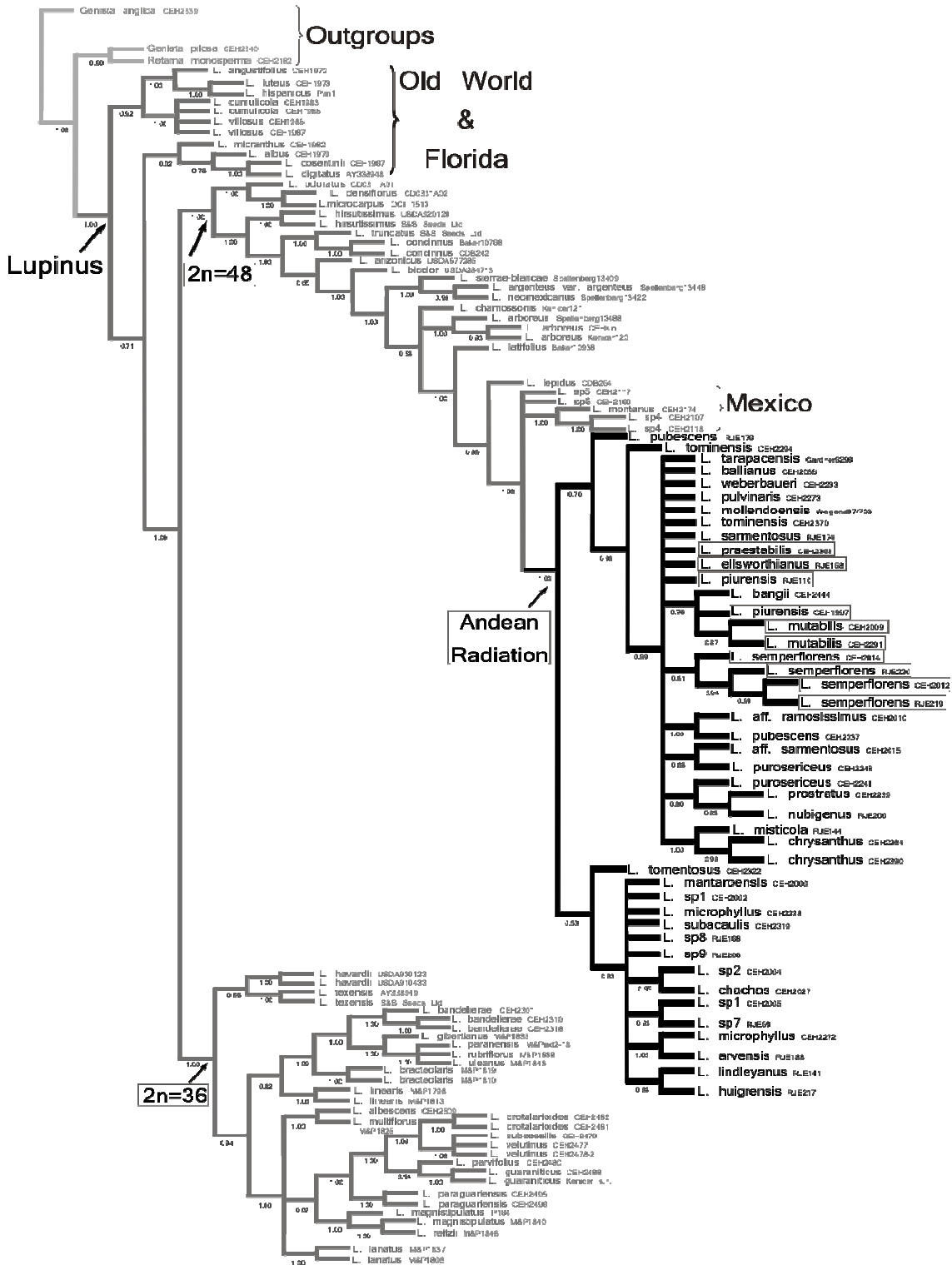


Fig. 3. Phylogeny of *Lupinus* – 50% majority rule Bayesian consensus tree from analysis of three nuclear DNA sequence loci, ITS, LEGCYC1A and GPAT. Values below nodes indicate support based on posterior probability. The two New World clades with chromosome numbers $2n = 36$ and $2n = 48$ are labelled and marked with boxes. The Andean radiation is highlighted in bold.

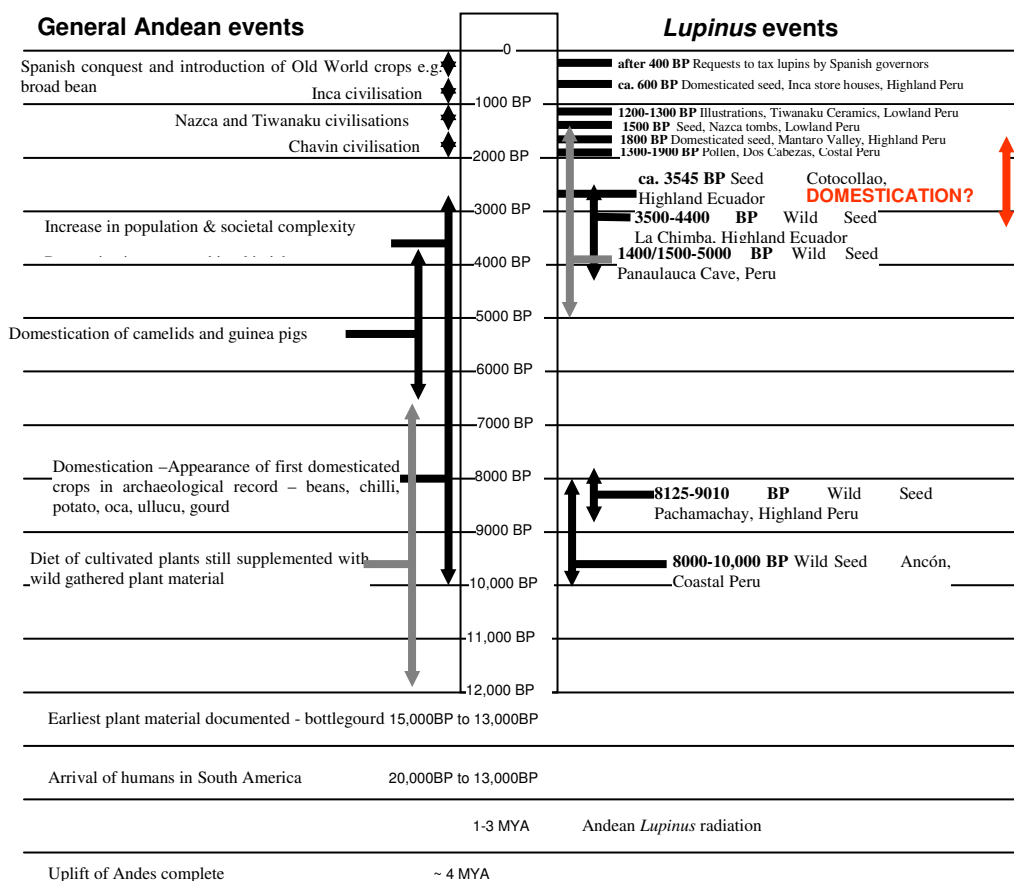


Fig. 4. Andean timeline illustrating the sequence of *Lupinus* seed remains from archaeological sites in relation to the prehistory of Andean crop domestication and agriculture.

CONCLUSIONS

Lack of robust evidence about the origins of domestication of *L. mutabilis* from extensive DNA sequence analysis is disappointing. Building the sort of well-resolved and robustly supported phylogeny that is needed to understand the sister group, and hence origins, of *L. mutabilis* will demand larger genome scale DNA sequence data sets to obtain variation and overcome likely problems of incomplete lineage sorting for the recent and rapid Andean lupin species diversification. Despite these difficulties, evidence from morphology and gene sequences tentatively suggests *L. piurensis* as the most likely progenitor. We propose this as a working hypothesis for further testing.

ACKNOWLEDGEMENTS

We thank the numerous individuals and institutions, and especially Chris Drummond who either provided material or assisted with fieldwork (see Hughes and Eastwood, 2006 for details), Donovan Bailey and Ashley Robertson for assistance in the lab, the authorities in Ecuador, Peru and Bolivia for permission to collect plant material, and the B, CAS, CGE, E, F, FHO, G GH, ISC, K, LPB, M, MO, MOL, MSB, NY,

QCNE, UB, UC, US, USM and USZ herbaria for loan of material. This work was supported by the Royal Society, the Biotechnology and Biological Sciences Research Council, the Genetics Society and the Stanley Smith Horticultural Trust.

LITERATURE CITED

Ainouche, A.-K. and R.J. Bayer. 1999. Phylogenetic relationships in *Lupinus* (Fabaceae: Papilionoideae) based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *American Journal of Botany* 86: 590-607.

Ainouche, A.-K., R. Bayer and M.-T. Misset. 2004. Molecular phylogeny, diversification and character evolution in *Lupinus* (Fabaceae) with special attention to Mediterranean and African lupines. *Plant Systematics and Evolution* 246: 211-222.

Blanco, G.O. 1982a. Genetic variability of tarwi (*Lupinus mutabilis* Sweet). Pp 33-49 *IN* R. Gross and E.S. Bunting (eds.). *Agricultural and Nutritional Aspects of Lupines*. GTZ, Eschborn, Germany.

Blanco, G.O. 1982b. Genetic variability of tarwi (*Lupinus mutabilis* Sweet). Pp. 33-50 *IN* Proceedings of the First International Lupin Conference, Lima, Peru.

- Blanco, G.O. 1984. *Lupinus mutabilis* genetic resources in South America. Pp. 159-164 *IN* Proceedings of the Third International Lupin Conference, La Rochelle, France.
- Blanco, G.O. 1986. Wild stock of *Lupinus mutabilis* Sweet. Pp. 284 *IN* Proceedings of the Fourth International Lupin Conference, Vol 1. Geraldton, Australia.
- Clements, J.C., B.J. Buirchell, H. Yang, P.M.C. Smith, M.W. Sweetingham and C.G. Smith. 2005. Chapter 9: Lupin. *IN* R. Singh and P. Jauhar (eds.), Genetic Resources, Chromosome Engineering, and Crop Improvement, Series-II Grain Legumes, CRC Press.
- Cowling, W.A., B.J. Buirchell and M.E. Tapia. 1998. Lupin. *Lupinus* L. Promoting the conservation and use of underutilised and neglected crops. International Plant Genetic Resources Institute, IPGRI, Rome, Italy. 105 pp.
- Drummond, C.S. 2008. Diversification of *Lupinus* (Leguminosae) in the western New World: derived evolution of perennial life history and colonisation of montane habitats. *Molecular Phylogenetics and Evolution* 48: 408-421.
- Gladstones, J.S. 1974. Lupins of the Mediterranean region and Africa. Technical Bulletin 26. Western Australian Department of Agriculture, Perth, Australia.
- Gladstones, J.S. 1998. Distribution, origin, taxonomy, history and importance. Pp 1-4 *IN* J. S. Gladstones, C. Atkins, and J. Hamblin (eds.). *Lupins As Crop Plants: Biology, Production and Utilisation*. CABI International, Wallingford/New York.
- Gross, R. 1982. El cultivo y la utilización del *Lupinus mutabilis* Sweet. FAO, Rome.
- Gross, R. 1986. First Reinhold von Sengbusch Memorial lecture: lupins in the old and new world - a biological-cultural coevolution. Pp 244-277 *IN* Proceedings of the 4th International Lupin Conference, Geraldton, Western Australia.
- Hardy, A., C. Huyghe and J. Papineau. 1997. Dry matter accumulation and partitioning, and seed yield in indeterminate Andean lupin (*Lupinus mutabilis* Sweet). *Australian Journal of Agricultural Research* 48: 91-101.
- Harries, M. 2005. Lupin and pulse production: agronomy and genetic improvement, *Agribusiness Crop Updates*. Dept. Agriculture and Food, Western Australia.
- Hastorff, C.A. 1993. Agriculture and the onset of political inequality before the Inca. Cambridge University Press, Cambridge.
- Hondelmann, W. 1984. The lupin - ancient and modern crop. *Theoretical and Applied Genetics* 68: 1-9.
- Hughes, C.E. and R.J. Eastwood. 2006. Island radiation on a continental scale: exceptional rates of species diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences, USA* 103: 10334-10339.
- International Board for Plant Genetic Resources. 1981. *Lupin Descriptors*. IBPGR, Rome.
- Martins, J.M.N., P.M.R. Silva and R.F.X.B. Sousa. 1992. Evaluation of *Lupinus mutabilis* accessions for protein and oil in Portugal. Pp 1-10 *IN* *Lupinus mutabilis: its adaptation and production under European pedoclimatic conditions*. Agrimed Research Programme. European Commission, Luxembourg.
- National Research Council, 1989. *Lost Crops of the Incas – Little-Known Plants of the Andes with Promise for Worldwide Cultivation*. National Academy Press, Washington D.C., U.S.A. 407 pp.
- Pearsall, D.M. 1980. Pachamachay ethnobotanical report: plant utilisation at a hunting base camp. Pp. 191-231 *IN* J.W. Rick (ed.) *Prehistoric hunters of the high Andes*. Academic Press, New York.
- Plitmann, U. 1981. Evolutionary history of the old world lupines. *Taxon* 30: 430-437.
- Plitmann, U. and C.C. Heyn. 1984. Old World *Lupinus*: taxonomy, evolutionary relationships and links with New World species. Pp 55-66 *IN* Proceedings of the 3rd International Lupin Conference, La Rochelle, France.
- Ree, R.H., H.L. Citerne, M. Lavin and Q.C.B. Cronk. 2004. Heterogeneous selection on LEGCYC paralogs in relation to flower morphology and the phylogeny of *Lupinus* (Leguminosae). *Molecular Biology and Evolution* 21: 321-331.
- Smith, C.P. 1938-1953. *Species Lupinorum*. Published privately, California, USA.
- Sweetingham M.S., J.C. Clements, B.J. Buirchell, S. Sipsas, G. Thomas, J. Quealy, R. Jones, C. Francis and C.G. Smith. 2005. Preliminary breeding and development of Andean lupin (*Lupinus mutabilis*) for Australian agriculture. *IN* E. van Santen and G.D. Hill (eds.) *Where Old and New World Lupins Meet*. Proc. 11th International Lupin Conference, Guadalajara, Mexico.
- Sweetingham MW, J.C. Clements, G. Thomas, R.A.C. Jones, S. Sipsas, J. Quealy, L. Smith, G. Francis. 2006. Progress in the development of Pearl lupin (*Lupinus mutabilis*) for Australian agriculture *IN* *Crop Updates 2006*, Perth, W. Australia.
- Systematics Association. 1962. Terminology for simple symmetrical plane shapes. *Taxon* 11: 145-156.
- Tapia, M. and C. Vargas 1982. Wild lupines of the Andes of southern Peru. Pp 23-32 *IN* Proceedings of the First International Lupin Conference, Lima/Cuzco, Peru.
- Von Baer, E. and von Baer D. 1988. *Lupinus mutabilis*: cultivation and breeding. Pp. 237-247 *IN* Proceedings 5th International Lupin Conference, Poznan, Poland.

ANEX I

Taxonomic Synopsis of *L. mutabilis* and Putative Allies

Accepted Names in **bold**, synonyms in italics

1. *Lupinus mutabilis* Sweet, Brit. Fl. Gard. Ser. I. ii: t. 130 (1825).

Lupinus cruckshanksii Hook., Bot. Mag. t. 3056 (1832).

Lupinus mutabilis var. *cruckshanksii* (Hook.) Sweet, Brit. Fl. Gard. Ser. 2. 3: t. 203 (1833).

Lupinus mutabilis var. *roseus* C. P. Sm., Sp. Lupinorum 588 (1948).

2. *Lupinus piurensis* C. P. Smith, Sp. Lupinorum 768 (1953).

2.a *L. piurensis* var. *piurensis*

2.b *L. piurensis* var. *cotumazicus* Eastwood & Hughes var. nov, ined.

3. *Lupinus semperflorens* Hartw. ex Benth. Pl. Hartw. 127 (1844).

3.a *Lupinus semperflorens* var. *sempreflorens*

Lupinus macbrideanus C. P. Sm., Sp. Lupinorum 159 (1940).

Lupinus storkianus C. P. Sm., Sp. Lupinorum 606 (1948).

Lupinus semperflorens var. *peruensis* C. P. Sm., Sp. Lupinorum 768 (1953).

3.b *Lupinus semperflorens* var. *amazonus* Eastwood & Hughes subsp. nov. ined.

4. *Lupinus ellsworthianus* C.P. Smith Sp. Lupinorum 159 (1940).

Lupinus albert-smithianus C. P. Smith, Sp. Lupinorum 279 (1941).

Lupinus amboensis C. P. Smith, Sp. Lupinorum 764 (1953).

Lupinus carhuamayus C. P. Smith, Sp. Lupinorum 761 (1953).

Lupinus carpapaticus C. P. Smith, Sp. Lupinorum 181 (1940).

Lupinus cesar-vargasi C. P. Smith, Sp. Lupinorum 538 (1945).

Lupinus diasemus C. P. Smith, Sp. Lupinorum 629 (1948).

Lupinus disjunctus C. P. Smith, Sp. Lupinorum 284 (1941).

Lupinus juninensis C. P. Smith, Sp. Lupinorum 278 (1941).

Lupinus maleopinatus C. P. Smith, Sp. Lupinorum 595 (1948).

Lupinus pachitensis C. P. Smith, Sp. Lupinorum 765 (1953).

Lupinus paucartambensis C. P. Smith, Sp. Lupinorum 542 (1945).

Lupinus quellomayus C. P. Smith, Sp. Lupinorum 592 (1948).

Lupinus semiprostratus C. P. Smith, Sp. Lupinorum 244 (1941).

Lupinus tarmaensis C. P. Smith, Sp. Lupinorum 767 (1953).

5. *Lupinus praestabilis* C.P. Sm. Sp. Lupinorum 587 (1948).

Lupinus proculastrinus C. P. Sm. Sp. Lupinorum 607 (1948).