Beatriz da Cunha Ferreira

Interactive Seed and Mucilage Identification Key for Lamiaceae in Portugal

Dissertação de Mestrado em Biodiversidade e Biotecnologia Vegetal, orientada pela Professora Doutora Maria de Fátima Matias Sales Machado e coorientada pelo Doutor Daniel Montesinos Torres, apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

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Abstract

The Herbarium of the University of Coimbra holds a vast Seed Collection which was initiated in the 60's and is now greatly expanded. We described the macromorphological characters, including mucilage production of nutlets from the Lamiaceae portuguese native flora, in the framework of the long-term Herbarium project for the online identification of the seeds of the entire Portuguese native flora.

The macrocharacters chosen for this investigation were nutlet size, shape, colour, presence and position of abscission scar, nutlet surface pattern and gloss, and mucilage presence and size after hydration. The macromorphological investigation was supported by a high-performance stereomicroscope (Leica MZ9.5). Nutlet size was measured using *Digimizer 4.7.0*. For the assessment of mucilage presence and size, five nutlets of each species were dry-weighed and subsequently immersed in water for 24 hrs and then weighed again. A second test in a smaller subset of samples was made to assess the speed of hydration and saturation point of a representative species from each subfamily.

The macromorphological investigation provided with data that allowed to construct a nutlet interactive key and proved its efficiency in the identification of nutlets to the genus and species level, demonstrating the large importance of external macromorphological characters for nutlet identification within this family. Mucilage assessment confirmed the presence of mucilage in 47% of the studied Nepetoideae and included assessments for several species which had never been evaluated before. Our results highlighted the large variability of the presence and importance of mucilage in this subfamily, even within genus, with important taxonomical and ecological implications.

Key-words: Nutlet identification, Macromorphology, Nepetoideae, COI Seed Collection, Portuguese native flora

Resumo

O Herbário da Universidade de Coimbra possui uma vasta coleção de sementes, iniciada nos anos 60 e que agora encontra-se largamente expandida. Foram descritos os caracteres macromorfológicos, incluindo produção de mucilagem das núculas de Lamiaceae da flora nativa portuguesa, no contexto do projeto a longo termo de identificação de sementes online de toda a flora nativa de Portugal.

Os macrocaracteres selecionados para esta investigação foram tamanho das núculas, forma, cor, presença e posição da cicatriz de abscissão, padrão e brilho da superfície da núcula, e presença e tamanho da mucilagem depois de hidratação. A investigação macromorfológica teve o auxílio de um estéreo-microscópio de alta performance (Leica MZ9.5). O tamanho das núculas foi medido usando o *Digimizer 4.7.0*. Para a avaliação da presença e tamanho da mucilagem, cinco núculas de cada espécie foram pesadas a seco e posteriormente imersas em água durante 24 horas e então pesadas novamente. Um segundo teste numa amostra menor foi realizado para avaliar a rapidez de hidratação e pontos de saturação em espécies representativas de cada espécie.

A investigação macromorfológica forneceu dados que permitiram a construção de uma chave de identificação interativa e mostrou eficiência na identificação das núculas até ao nível de género e espécie, demonstrando a grande importância dos macrocaracteres externos para a identificação de núculas desta família.

A avaliação da mucilagem confirmou a presença de mucilagem em 47% das Nepetoideae estudadas e incluiu avaliações para muitas espécies nunca avaliadas anteriormente. Os nossos resultados realçaram a elevada variabilidade da presença e importância da mucilagem nesta subfamília, até dentro dos géneros, com importantes implicações taxonómicas e ecológicas.

Palavras Chave: Identificação de núculas, Macromorfologia, Nepetoideae, Coleção de Sementes de COI, Flora nativa portuguesa

Introduction

Seeds are the result from sexual reproduction by two major classes of plants – Gymnosperms and Angiosperms (Pinopsida and Magnoliopsida) - the Spermatophyta (literally “plants that bear seeds”) - and for these species, seeds are the main way of dispersal. The evolution of seeds is considered one of the most important adaptations of plants to life on land (Ingrouille & Eddie, 2006).

Seeds are very dry – typical average water content is c. 10% (Angelovici *et al.*, 2010), their embryo is dormant, they have a thick protective seed coat and are most suitable for perennation through unfavourable periods. They have adaptive strategies to get dispersed to new habitats and colonise them (Willson *et al.*, 1990). Because they are the result of sexual reproduction, seeds carry a combination of the genetic richness of their parents, which is essential for their adaptability to diverse environmental conditions. Seeds have reserve nutrients for nourishing the young seedlings till they become nutritionally independent. These nutrients constitute a food resource for many animal species and were the motor for the domestication of plants and the development of agriculture, when humans learnt to eat, store and sow seeds of many species (Navazio, 2012). Seeds can be stored and used as food supply throughout the year and to overcome drought and famines. Agriculture was the turning point for evolution of human civilisation via the production of surplus food that could be stored, and thus used to feed specialized workers, and marked the start of complex societies (Martínez-Ainsworth & Tenaillon, 2016).

Seeds result from the development of ovules after fertilisation (Esau, 1977). The ovule derives from a megasporangium containing a single megaspore, which is never released (Fig. 1A) (Ingrouille & Eddie, 2006). The megasporangium is surrounded by 1-2(-3) protective layers of tissue, the integuments. The endosperm, and in some cases the perisperm, are nutritive tissues that support germination and the initial growth of the seedling (Esau, 1977). The perisperm and the seed coat result both from maternal tissue, developing, respectively, from the nucellus (megasporangium wall) and the integuments (Werker, 1997). Mature seeds comprise the young sporophyte, the embryo, its nutritive tissues, endosperm (occasionally also perisperm) often absorbed by the embryo’s cotyledons, and the protective layers, the testa (Esau, 1977).

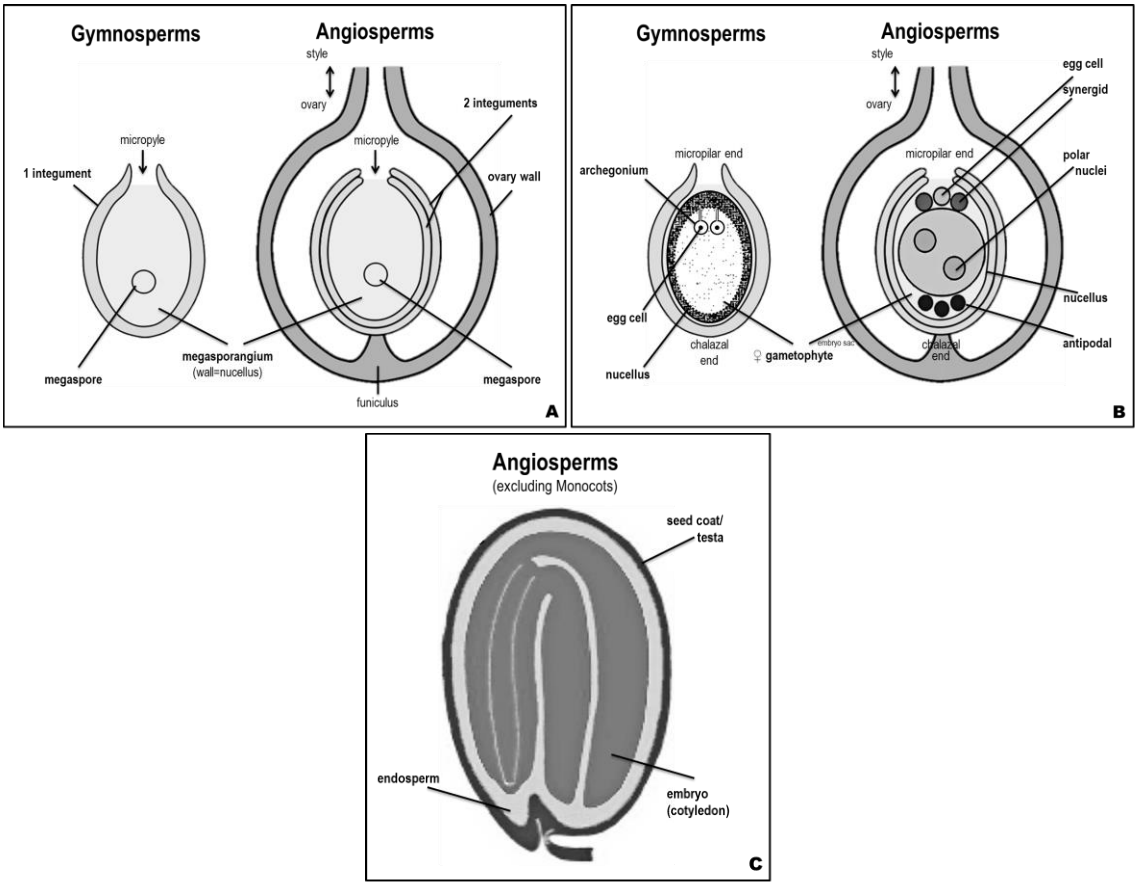

Figure 1 - *Basic aspects of the reproduction in the Spermatophyta and the female gametophyte. A. Developing ovules. B. Mature ovules. C. Mature seed; embryo with the cotyledons derives from the fertilised egg cell; endosperm derives from the fertilised polar nuclei; testa derives from the integuments (illustration adapted from Wikimedia Commons contributors, retrieved 29 May 2018).*

In Angiosperms, there is double fertilization (Fig. 1B). This leads to the beginning of embryogenesis and the development of the endosperm, which is triploid (Western, 2012), and derives from the fertilisation of two cells from the female gametophyte (polar nuclei) and one of the sperm nucleus (Judd *et al*., 1999). The endosperm surrounds the embryo at the time of seed dispersal or disappears, absorbed by the first leaves of the embryo, the cotyledons. Double fertilization also leads to the differentiation of the integuments into a mature seed coat, the testa, responsible for seed protection (Fig. 1C) (Ingrouille, 1992).

SEED COLLECTION AT COI

The Herbarium of the University of Coimbra (COI) has an extended seed collection, mainly of taxa belonging to the Portuguese native flora. It is held at room temperature in glass tubes with cork tops (Fig. 2) and is regularly fumigated and frozen for pest control. The seed collection began with 2,552 accessions/tubes assembled and identified by Luís

Cabral in the 1960s to aid plant identification. The collection was recently expanded to include (1) the seeds collected by Arménio Matos which has approximately 1,460 accessions collected in 2013 for the last *Index Seminum*, published by the Coimbra Botanic Garden and (2) 2,500-2,800 accessions assembled over the years also for identification purposes. Currently, the collection is being prepared for databasing and it will be organised following APG IV (Angiosperm Phylogeny Group, 2016).

Figure 2 - *Seed Collection at the Herbarium of the University of Coimbra at Department of Life Sciences, FCTUC.* ***A.*** *The classical glass tubes with labels* ***B.*** *Seed sorting in the Herbarium (courtesy of COI).*

LAMIACEAE

The Lamiaceae is a cosmopolitan family with 239 genera and approximately 6,500 species (Cappers & Bekker, 2013). This family is especially common in the Mediterranean (47 genera and 857 species in the European Mediterranean Region only, Greuter *et al.* 1989), Southwest Asia, China, Australia and South America (Mabberley, 2008). The genera with larger number of species are *Salvia* L. (900), *Scutellaria* L. (360), *Stachys* L. and *Plectranthus* L'Hér. (300), *Hyptis* Jacq. (280), *Teucrium* L. (250), *Thymus* L. (220), *Nepeta* L. (200), *Sideritis* L. (140), *Isodon* (Schrad. ex Benth.) Spach, *Leucas* R.Br., *Prostanthera* Labill. and *Phlomis* L. (100) (Castroviejo, 2010).

Many of its species are well-known culinary herbs on the account of the essential oils they produce in often abundant and morphologically variable glandular trichomes (Fig. 3). The secondary metabolites in these essential oils have many other uses (Nieto, 2017).

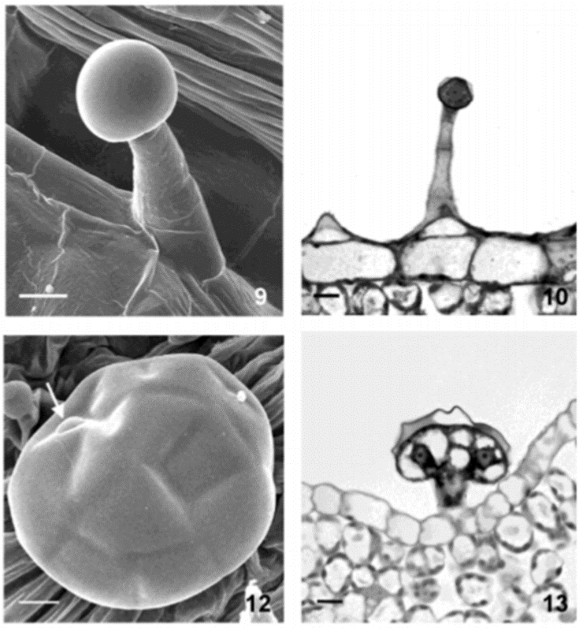

Figure 3 - *SEM and LM micrographs of capitate glandular trichome (9-10) and peltate glandular trichome (12-13) on leaf of Salvia aegyptiaca* L.*, some of the most common glandular trichomes in the Lamiaceae.* ***9 –*** *SEM micrographs of mature trichomes with a large spherical secretory head;* ***10 –*** *semi-thin section of capitate trichomes showing the secretory head cell with dense cytoplasm and small dark vesicles;* ***12 –*** *SEM micrograph of peltate trichome with a wrinkled surface; ↑: decrease in secreted material;* ***13 –*** *semi-thin longitudinal section of peltate trichome with wrinkled cuticle and small subcuticular space (adapted from Janošević et al. 2016).*

The family name Lamiaceae, or Labiatae *nom. conser*., is based on the genus *Lamium* (Lanjouw *et al*., 1961). The names have their origin in the bilabiate, usually pentamerous, flower (Fig. 4c) (Harley *et al*., 2004). The Lamiaceae usually have 4-sided stems (Fig. 4b), the leaves are opposite (occasionally whorled). The inflorescences are complex with cymes organised in terminal thyrsi, or more or less spaced out verticillasters or solitary flowers in the axils (Fig. 4a) (Mabberley, 2008). The flowers are hermaphrodite, usually bracteolate and the ovary is mostly superior, deeply 4-lobed (Fig. 4d) caused by the formation of “false septa” that almost totally separate the four carpels of the gynoecium. At fruiting stage each lobe detaches at maturity and is often referred to as nutlet.

The nutlets are dry, indehiscent and monospermous and, in fact, they could be called achenes. Their dorsal surface is rounded, the ventral surface has two slanted surfaces that develop against the other three nutlets (Fig. 4e), and where the abscission scar, where the nutlet was attached to the receptacle, is located. This kind of fruit is a schizocarp and is the most common fruit type in the family (Cappers & Bekker, 2013), but there are also berries and drupes (Harley *et al*., 2004; Simpson, 2010), all remaining in the persistent calyx.

Nutlets in the Lamiaceae provide one of the best series of macrocharacters for the identification at genera and/or species level (Hedge, 1992). Hedge referred to the nutlets as having many differences in character state, both in terms of size, shape, colour, surface ornamentation, abscission scar shape and mucilage production, when in hydration.

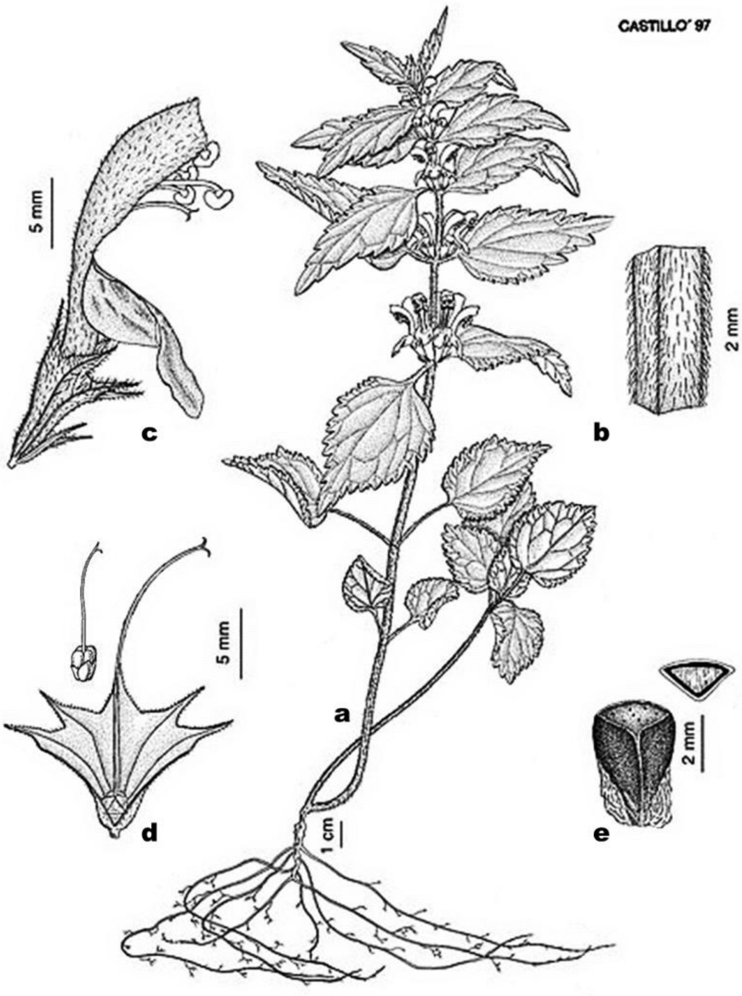

Figure 4 - *Lamium galeobdolon* (L.) L. *subsp. montanum* (Pers.) Hayek. ***a.*** *Habit with opposite leaves and bracteolate verticillasters.* ***b.*** *4-sided stem.* ***c.*** *bilabiate, usually pentamerous, flower.* ***d.*** *superior ovary deeply 4-lobed.* ***e.*** *nutlet ventral surface and transversal section. a, b, MA 145945; c, d, MA 338981; e, MA 540459. (adapted from Castroviejo, 2010, Lam. 53).*

Martin's (1946) classical investigation on the inner structure of seeds described some Lamiaceae, on the form, size and position of the embryo and the endosperm. The genera studied point out that in the family there is little or none endosperm, and the nutrients are transferred to and stored in the cotyledons which are, therefore, expanded. The embryo, although differing in shape, lies in the axis of the seed (axile) and it is large (foliate), occupying more or less the entire seed (Fig. 5A). All the Lamiaceae illustrated in Martin (1946) come into the category of spatulate embryo (Fig. 5B).

Diagram	Embryo classification; inner seed structure
	Axile, foliate, spatulate Embryo spatulate, erect; cotyledons thin to thick and slightly expanded to broad.
	Axile, foliate, bent Embryo bent, spatulate but bent in jack-knife shape; cotyledons generally thick.
	Axile, foliate, investing Embryo investing, erect; cotyledons thick, overlapping, encasing the somewhat dwarfed hypocotyl for at least half its length; endosperm absent or much reduced.

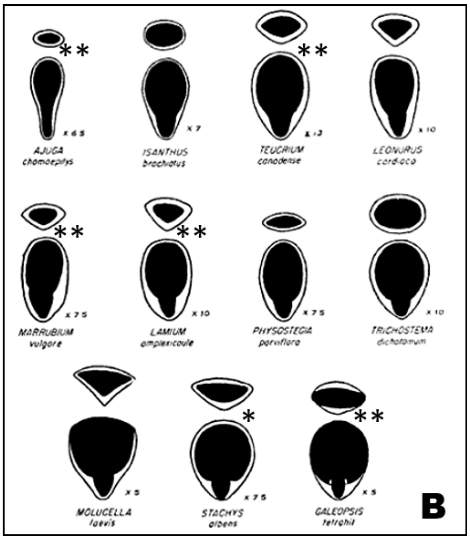

Figure 5 - *Seed inner structure in the Lamiaceae.* ***A.*** *The three embryo types present in the family.* ***B.*** *Diagrams of the transversal and longitudinal sections of the nutlets of 11 species in the family, all with axile, foliate, spatulate embryos;* ******* *genus studied in this thesis,* ******** *species studied in this thesis. In black is the embryo, in white the endosperm. (adapted from Martin, 1946).*

MUCILAGE

Different environmental conditions can lead to the development of different adaptations of the outermost surface of fruits and seeds (Western, 2012). One of those adaptations is myxodiaspory, which is the capability of fruits and seeds to produce a hydrophilic, pectinaceous mucilage when hydrated (Yang *et al*., 2012). The presence of seed mucilages is known as myxospermy and occurs in a wide range of families such as Acanthaceae, Brassicaceae, Linaceae and Plantaginaceae. The presence of fruit mucilages is known as myxocarpy, and can be found in families such as Asteraceae, Lamiaceae and Poaceae (Ryding, 2001; Kreitschitz *et al.*, 2009; Western, 2012). These traits are present in many plant families and species around the world, being predominantly found in Angiosperms (Grubert, 1981).

Mucilage has been described to provide with various ecological advantages (Western, 2012; Yang *et al*., 2012), including facilitating seed hydration (Western, 2012), establishment of the seedlings in environments with low precipitation and disturbed habitats (Yang *et al*., 2012), anchoring the seed to the ground and consequently aiding water diffusion from the substrate to the seed (Harper & Benton, 1966 cited in Werker, 1997) and supporting seed and fruit dispersal by adhesion to soil or animal vectors (Ryding, 2001; Kreitschitz *et al.*, 2009; Western, 2012).

In the Lamiaceae, fruit mucilage can be found in approximately 70-75% of the genera and species in the subfamily Nepetoideae (Ryding, 1992). It is, therefore, a taxonomic characteristic that contributes to separate this subfamily from all the other subfamilies of Lamiaceae.

TOOLS FOR IDENTIFICATION

The refinement of agriculture, nature conservancy and citizen science has emphasised the importance of giving access to botanical information to a wider audience and not only to specialists. The classic tools for identification are essentially dichotomising or multi-access keys and identification guides, mainly designed for botanists and taxonomists. They are not very practical identification and diagnostic tools since they use technical terminology. The development of informatics provided the knowledge to create new, user-friendly tools such as online publications of electronic identification keys, created with specialised software.

Interactive, multimedia keys are becoming increasingly popular. According to Norton *et al.* (2012) this type of keys offer a mechanism for transferring taxonomic knowledge into an easier format for non-taxonomists. Currently there are many programs available to produce this kind of keys, either as freeware or comercially such as LucID (http://www.lucidcentral.org/); Meka (http://ucjeps.berkeley.edu/-Meacham/meka/index03.html); Navikey (http://www.huh.harvard.edu/databases/-legacy/navikey/); PollyClave (http://prod.library.utoronto.ca:8090/polyclave/) and XID (http://www.xidservices.com/).

Seed identification is important in plant taxonomy (*e.g.* Gabr, 2018; Hedge, 1992; Vaughan, 1968), in ecology and conservation (Vandvik *et al*., 2016), and in agriculture. Alternative means of weed control are very important for an integrated, desirable greener and organic agriculture. Soil and crop management practices can influence the environment of the seeds present in the seedbank, either by managing those seeds longevity or their germination behavior (Hussain & Begum, 2015). Various technological methods have been recently explored (e.g. Hemming & Rath, 2001) but identification of the weed species, even before the plants develop, provides the best information for weed control. This means identifying the seedbank in the soil before plantation and/or sowing.

The combination of various aspects mentioned above determined the scope of this project:

- the major importance of seed identification;
- the need for user-friendly tools for their identification, sufficiently clear, to be used by different professionals (mainly agronomists and ecologists);
- the unique resource that is Seed Collection of the Herbarium of the University of Coimbra (COI) and the availability of Portuguese nutlet material of the Lamiaceae.
- the ecological relevance of fruit and seed mucilage.

The objectives of this research are, then:

1.1. to investigate the macromorphological characters of nutlets to create an interactive nutlet and mucilage identification key for Lamiaceae in Portugal,

1.2. to initiate the long-term Herbarium project for the online identification of the seeds of the entire native flora, the *Portuguese Seed Identification Key – Online* (PSIKO);

2.1. to assess the presence and importance of nutlet mucilage in the different species of the Lamiaceae, and

2.2. to experimentally assess the importance of mucilage for water retention.

Materials & Methods

ORGANISING AND DATABASING THE COLLECTION

This study is based on mature nutlets from the Seed Collection provided by the Herbarium of the University of Coimbra (COI). The Collection has just been expanded and is being organised. It includes 237 accessions of Lamiaceae, involving 39 genera and 136 species. The material of Lamiaceae was at three different stages of organisation (Fig. 6): (1) the material from the 1960's assembled and identified by Luís Cabral, all stored in glass tubes with old, outdated names on labels, (2) the remains from the last *Index Seminum* (2013) already transferred to glass tubes with updated names on labels, and (3) the collection assembled by Arménio Matos for identification purposes, with outdated names on labels and still in paper packets.

Figure 6 – *Organising the Seed Collection (Lamiaceae).* ***a, b*** *- Packets with material;* ***c*** *– Copies of the old glass tubes with cork tops;* ***d*** *– Transferring nutlets to the glass tubes;* ***e*** *– Old collection with outdated labels;* ***f*** *- Placing the barcode into the glass tube;* ***g, h*** *– Seed Collection (Lamiaceae) organised, all with barcodes and databased.*

The whole material of Lamiaceae was retrieved. That still in paper packets was transferred to glass tubes. All material received a barcode and was databased using the software *Specify 6.6.05*. This software is a database platform utilized at the Herbarium of Coimbra. The purposes of this software are: (1) to research data, since it manages species and specimen information for computerizing biological collection, (2) to track herbarium specimen transactions, c) to link images to specimen records and (3) to publish catalogue data to the internet.

The accessions had their taxonomic nomenclature updated following (1) the most recent classification, by the Angiosperm Phylogeny Group IV (2016), for the delimitation of the family, and (2) the World Checklist of Selected Plant Families (WCSP, 2018), for the most recent synonymy. The *Flora Iberica* (Castroviejo, 2010) is, in some respects, outdated.

As the main objective of this thesis was to study the nutlets of the Portuguese native flora, the accessions were selected according to this specific criterion. In the end we had a total of 66 species from 25 genera belonging to 4 subfamilies to work with, as shown in Table 1.

Table 1 – Species of Lamiaceae belonging to the Portuguese native flora and the corresponding subfamily in the Seed collection of the Herbarium.

Subfamily	Genus	Species
Ajugoideae	*Ajuga*	*A. chamaepitys* (L.) Schreb.
		A. iva (L.) Schreb.
		A. pyramidalis L.
		A. reptans L.
	Teucrium	*T. salviastrum* Schreb.
		T. scorodonia L.
		T. spinosum L.
Lamioideae	*Ballota*	*B. nigra* L.
		B. nigra subsp. *foetida* (Vis.) Hayek
	Betonica	*B. officinalis* L.
	Galeopsis	*G. tetrahit* L.

	Lamium	*L. amplexicaule* L. *L. maculatum* L. *L. purpureum* L. *L. purpureum* var. *hybridum* (Vill.) Vill.
	Marrubium	*M. vulgare* L.
	Melittis	*M. melissophyllum* L.
	Sideritis	*S. hirsuta* L.
	Stachys	*S. arvensis* (L.) L. *S. germanica* L. *S. ocymastrum* (L.) Briq. *S. palustris* L.
Nepetoideae	*Cleonia*	*C. lusitanica* (L.) L.
	Clinopodium	*C. alpinum* (L.) Kuntze *C. nepeta* subsp*. spruneri* (Boiss.) Bartolucci & F. Conti *C. vulgare* L. *C. vulgare* subsp*. arundanum* (Boiss.) Nyman
	Lavandula	*L. latifolia* Medik. *L. multifida* L. *L. pedunculata* (Mill.) Cav. *L. stoechas* L. *L. stoechas* subsp*. luisieri* (Rozeira) Rozeira *L. pedunculata* subsp*. sampaiana* (Rozeira) Franco *L. viridis* L'Hér.
	Lycopus	*L. europaeus* L.
	Melissa	*M. officinalis* L.

	Mentha	*M. longifolia* (L.) L. *M. pulegium* L. *M. suaveolens* Ehrh.
	Micromeria	*M. graeca* (L.) Benth. ex Rchb. *M. juliana* (L.) Benth. ex Rchb.
	Nepeta	*N. caerulea* Aiton *N. cataria* L. *N. nuda* L. *N. tuberosa* L.
	Origanum	*O. vulgare* subsp. *virens* (Hoffmanns. & Link) Ietsw. *O. vulgare* L.
	Prunella	*P. grandiflora* L. *P.* × *intermedia* Link
	Salvia	*S. officinalis* L. *S. sclarea* L. *S. sclareoides* Brot. *S. rosmarinus* Spenn. *S. verbenaca* L. *S. viridis* L.
	Satureja	*S. hortensis* L. *S. montana* L.
	Thymbra	*T. capitata* (L.) Cav.
	Thymus	*T. caespititius* Brot. *T. capitellatus* Hoffmanns. & Link *T. mastichina* (L.) L. *T. pulegioides* L.

		T. villosus L.
		T. zygis subsp*. sylvestris* (Hoffmanns. & Link) Cout.
Scutellarioideae	*Scutellaria*	*S. galericulata* L.
		S. minor Huds.

MORPHOLOGICAL STUDY

The morphological data was collected from 10-12 mature nutlets from each species, except from *Thymus villosus* for which only 5 nutlets were available. The nutlets were disposed in paperboard with double-faced adhesive tape to facilitate their handling. The nutlets were photographed and examined with a high-performance stereomicroscope (Leica MZ9.5). The macrocharacters selected for this study were those referred to by Hedge (1992): size, shape, colour, surface ornamentation, abscission scar shape and mucilage production. All nutlets were positioned with their basal end being the abscission scar location.

SIZE

Both nutlet length and width were taken from ten apparently mature nutlets. After being photographed with the stereomicroscope which had a scale incorporated, the nutlets were measured with the help of an image analysis software - *Digimizer 4.7.0.* This software was developed by *MedCalc Software* and can be found free online. This software is an easy-to-use and flexible image analysis software. It allows precise manual measurements and automatic object detection, with measurements of the object characteristics.

The length was measured from the base to the apex (Fig. 7a) and the width was the largest axis perpendicular to the latter, as shown in Figure 7b. Both mean length and mean width were calculated, and these means were used to create three size ranges for the interactive key: (1) less than 1,5 mm, (2) between 1,5 and 3 mm and (3) more than 3 mm.

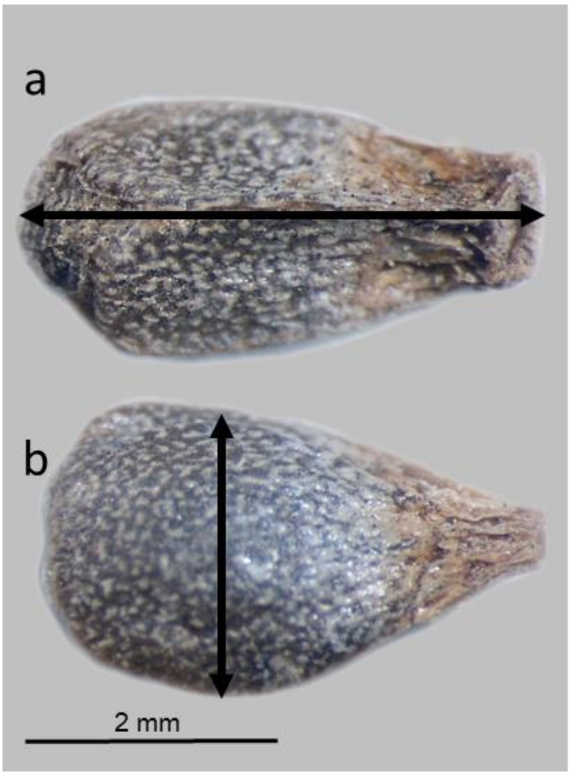

Figure 7 –*Lamium maculatum. Measurements taken for nutlet description. Length (**a**) was measured from the base to the apex, and width (**b**) was the largest axis perpendicular to the length.*

SHAPE

The shape was described following the terminology used in Anderberg (1969). Alongside with the nutlet shape, its base and apex shape were described following Werker (1997) and Bojňanský and Fargašová (2007).

COLOUR

Nutlet colour was described in a simplified way because, due to variation, the character lacks taxonomic importance. It is frequent that a species can show variation of colour in different nutlets (Martin Mosquero, 2002; Guerin, 2005; Hassan, 2015). Mature nutlets have, in their majority, colours varying from different shades of brown to black.

ABSCISSION SCAR

The abscission scar was described based on its shape and size. The shape follows the terminology of Anderberg (1969).

NUTLET SURFACE

For the surface description, pattern and brightness of the nutlet were considered. The surface pattern was described following Murley (1951) in Bojňanský and Fargašová (2007). Regarding brightness, the nutlets were classified as shinny or dull.

MUCILAGE

The objective of this investigation was (1) to assess the presence and importance of nutlet mucilage in the different species of the Lamiaceae and (2) the nutlet change in weight in the presence of water.

For this purpose, we used nutlets of numerous species of the Nepetoideae subfamily (the only subfamily known to include species which have mucilage) plus nutlets of three other subfamilies present in our seed collection, which were helpful as controls, to evaluate the increment in weight of nutlets with no mucilage.

In a first experiment, five dry-nutlets of each species were weighed to the nearest 0.0001 g using an analytical scale - KERN ABS-N/ABJ-NM - *ABS 120-4N*. The nutlets, were subsequently submerged in distilled water for 24 hrs, and then weighed in the same analytical scale.

In a second experiment, and to assess in detail the mucilage expansion over time of a selection of representative species from each subfamily (Table 2), we developed an additional test weighing the submerged nutlets at intervals of 15, 45, 60, 120, 180, and 240 minutes. These data were used to draw saturation curves of percentage increases in mucilage size over time.

Table 2 – Total species investigated, and sub-set selected to investigate mucilage expansion over time.

Subfamily	Species no.	Species selected for additional mucilage expansion test
Ajugoideae	7	*Teucrium scorodonia* L.
Lamioideae	15	*Betonica officinalis* L.
Nepetoideae	42	*Nepeta cataria* L. *Salvia rosmarinus* Spenn.
Scutellarioideae	2	*Scutellaria galericulata* L.

We combined data from seed dry-weight and weight after hydration to calculate percentage of increase in nutlet weight, and to characterize the importance of this trait in the different species, genus, and subfamilies. We statistically analysed the data in IBM SPSS Statistics, version 25.0 by means of General Linear Models in which the study variable was either initial seed weight, or percentage increase in weight after 24h of hydration, and the fixed factors were species, genus, and subfamily. Tamhane post-hoc tests were used to assess for statistically significant differences among species, genus, and subfamilies.

INTERACTIVE KEY

The online interactive identification key was created for the identification of 25 genera and 66 species from the Portuguese native Lamiaceae. The key was built using the software *LucID v3.3*.

LucID was developed at the University of Queensland and is a multimedia identification tool. According to Norton *et al.*, (2012), this software is easy to use, both on creating identification guides and as a source of information. It was originally designed for taxonomic identification but currently is being used for much wider diagnostic purposes as well. This software is also a research, educational and decision support tool (Norton, *et al.*, 2012). *LucID* includes two components, *LucID* Builder and *LucID* Player. This program comprises four basic elements for creating and using an interactive random-access key: (1) a list of entities, (2) a list of features and states that may be used to identify those entities, (3) a matrix of score data for the features associated with each of the entities, and (4) various attachments (images, further information, etc.) to provide extra information to users.

LUCID BUILDER

This component provides all the necessary tools to create the entity, features lists and respective feature states, to encode the score data and to attach information files to items.

In this research, the entities consisted of the 66 species already mentioned. The features chosen, and the respective character states are shown in table 3.

Table 3 - Features and respective feature states used for the construction of the interactive key.

Features		Feature state
Nutlet dimension	**Length (mm)**	Less than 1,5 mm
		Between 1,5-3 mm
		More than 3 mm
	Width (mm)	Less than 1,5 mm
		Between 1,5-3 mm
		More than 3 mm
Shape	**Nutlet shape**	Narrowly obovate
		Obovate
		Broadly obovate
		Narrowly elliptic
		Elliptic
		Broadly elliptic
		Circular
	Base and apex Shape	Acute
		Apiculate
		Rounded
		Truncate

	Transversal cross-section shape	Concave 2-convex Angular
Nutlet Colour		Brown Light brown Dark brown Brown with greyish areas Brown with darker spots Dark brown with darker spots Black Dark brown with whitish spots Brown with whitish spots Light brown with whitish spots Yellowish brown
Abscission scar	**Visible**	Yes No
	Shape	Biconvex Elliptic Circular Triangular Reniform
	Size	Small Medium Large
Nutlet surface	**Pattern**	Areolate Colliculate

	Foveolate
	Granulate
	Punctate
	Reticulate
	Smooth
	Smooth with one longitudinal rib
	Smooth with three or more longitudinal ribs
	Tuberculate
	Verrucate
Shine	Shinny
	Dull

LucID Player

This component comprises a main window displaying four lists: (1) *Features Available*, all features and states available to describe the specimen to be identified, (2) *Features Chosen*, features that have already been chosen, (3) *Entities Remaining*, the entities in the key that remain into play, given the features that have been chosen, and (4) *Entities Discarded*, the entities that have been excluded as result of the chosen features.

When the user first start using the key, all *Features Chosen* and *Entities Discarded* are empty. The user chooses states of features by ticking their checkbox. In response, LucID player progressively removes from *Entities Remaining* those that do not match the choices made. In the end, only one entity should remain – the identification has been accomplished.

Results

ORGANISING AND DATABASING THE SEED COLLECTION

The organisation of the Lamiaceae Seed Collection resulted in the placement of all accessions in their final place, with their corresponding identification and barcode. This organisation was made to follow a uniformity within the entire seed collection (Fig. 8). This organisation allowed us to simplify the databasing of this family, which is now available on the Herbarium website (appendix 1).

Figure 8 - *Seed Collection (Lamiaceae) organised, all with barcodes and databased.*

NUTLET DESCRIPTION

The seed description was one of the most important stages of this project and provided with the data to construct the interactive key (appendix 2). The nutlets descriptions are listed by: (1) subfamily, (2) genus and (3) species. For each species it is given the habit, *i.e.*, whether the species is annual, biennial or perennial. Nutlet description follows the sequence: (1) dimensions, (2) general shape, (3) apex shape, (4) base shape, (5) transverse cross section shape, (6) colour, (7) surface shine, (8) surface pattern, and (9) abscission scar features.

AJUGOIDEAE

Ajuga

***A. chamaepitys* (L.) Schreb.** (Fig. 9a)
Habit: annual
Description
Nutlets c. 3 x 1,3 mm. Shape: narrowly obovate. Apex: slightly rounded or sub truncate. Base: rounded. Transverse cross section shape: concave. Colour: from brown to dark brown. Surface: shinny. Surface pattern: Reticulate. Abscission scar: large and elliptic.

***A. iva* (L.) Schreb.** (Fig. 9b)
Habit: perennial
Nutlet description
Nutlets c. 3 x 1,7 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light to dark brown. Surface: shinny. Surface pattern: reticulate. Abscission scar: large and elliptic.

***A. pyramidalis* L.** (Fig. 9c)
Habit: perennial
Nutlet description
Nutlets c. 3 x 1,9 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: reticulate. Abscission scar: large and elliptic.

***A. reptans* L.** (Fig. 9d)
Habit: perennial
Nutlet description
Nutlets c. 3 x 1,9 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: reticulate. Abscission scar: large and elliptic.

Teucrium

***T. salviastrum* Schreb.** (Fig. 9e)
Habit: perennial
Description
Nutlets c. 1,9 x 1,5 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: smooth. Abscission scar: large and circular.

***T. scorodonia* L.** (Fig. 9f)
Habit: perennial
Nutlet description
Nutlets c. 1,9 x 1,6 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown to black. Surface: shinny. Surface pattern: smooth. Abscission scar: medium and slightly circular.

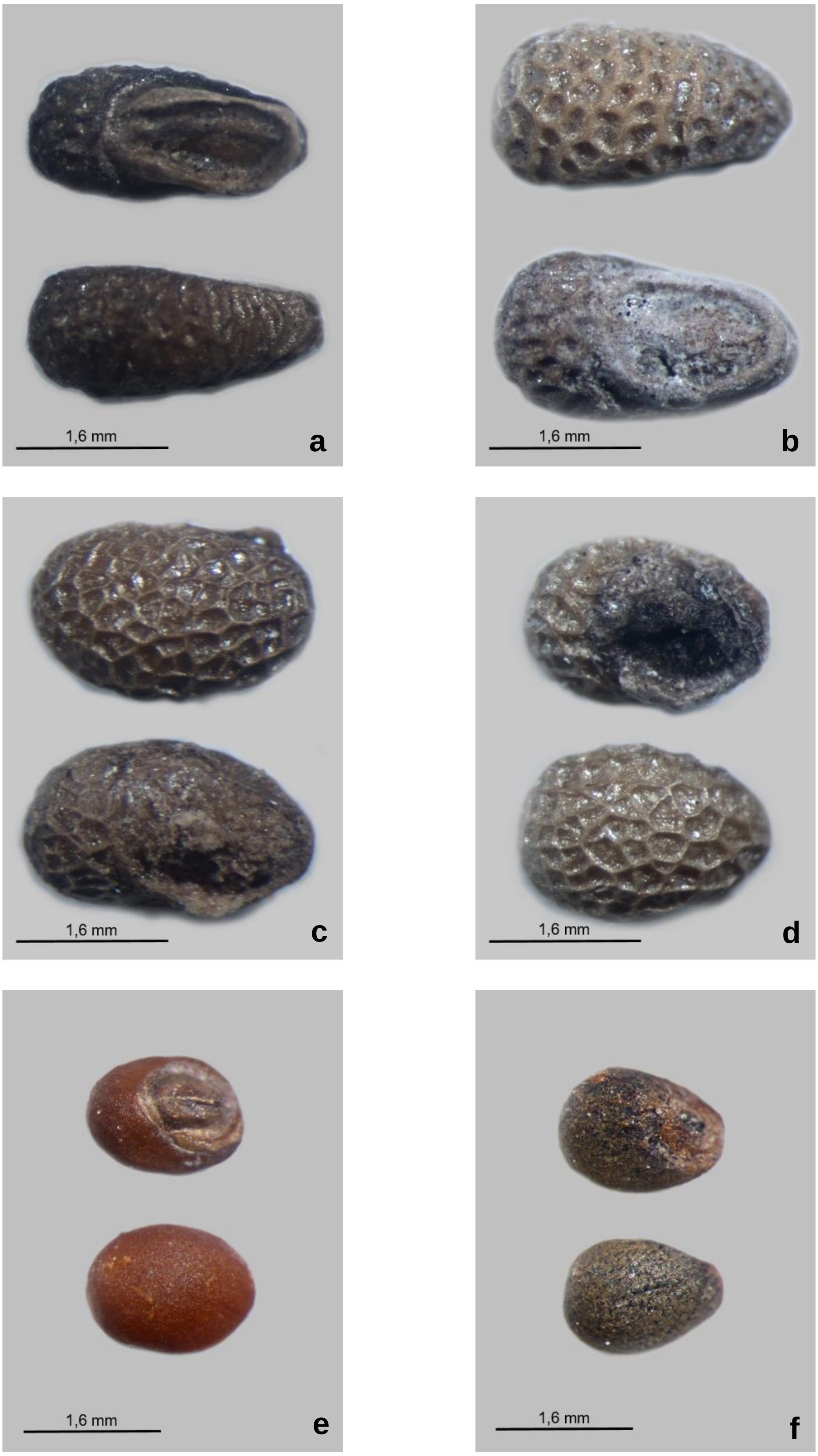

Figure 9 - *a) Ajuga chamaepitys; b) A. iva; c) A. pyramidalis; d) A. Reptans; e) Teucrium salviastrum; f) T. scorodonia.*

***T. spinosum* L.** (Fig. 10a)

Habit: annual

Nutlet description

Nutlets c. 1,5 x 1,2 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown to black. Surface: shinny. Surface pattern: smooth. Abscission scar: medium and slightly triangular.

LAMIOIDEAE

Ballota

***B. nigra* L.** (Fig. 10b)

Habit: perennial

Nutlet description

Nutlets c. 2,6 x 1,4 mm. Shape: obovate. Apex: rounded or slightly sub truncate. Base: obtuse and slightly apiculate. Transverse cross section shape: 2-convex. Colour: dark brown. Surface: shinny. Surface pattern: smooth to slightly foveolate. Abscission scar: small and slightly circular.

***B. nigra subsp. foetida* (Vis.) Hayek** (Fig. 10c)

Habit: annual

Description

Nutlets c. 2,7 x 1,4 mm. Shape: obovate. Apex: slightly rounded or sub truncate. Base: rounded. Transverse cross section shape: 2-convex. Colour: from dark brown to black. Surface: shinny. Surface pattern: smooth to slightly foveolate. Abscission scar: small and slightly circular.

Betonica

***B. officinalis* L.** (Fig. 10d)

Habit: perennial

Nutlet description

Nutlets c. 3,5 x 2,2 mm. Shape: obovate. Apex: irregular. Base: rounded. Transverse cross section shape: 2-convex. Colour: brown. Surface: dull. Surface pattern: smooth. Abscission scar: small and slightly triangular.

Galeopsis

***G. tetrahit* L.** (Fig. 10e)

Habit: annual

Nutlet description

Nutlets c. 3,8 x 2,9 mm. Shape: broadly obovate. Apex: rounded. Base: acute. Transverse cross section shape: concave. Colour: brown with grey areas. Surface: shinny. Surface pattern: colliculate. Abscission scar: small and circular.

Lamium

***L. amplexicaule* L.** (Fig. 10f)

Habit: annual

Nutlet description

Nutlets c. 2,5 x 1,3 mm. Shape: obovate. Apex: rounded to slightly truncate. Base: acute. Transverse cross section shape: 2-convex. Colour: dark brown with whitish spots. Surface: shinny. Surface pattern: colliculate. Abscission scar: small and biconvex.

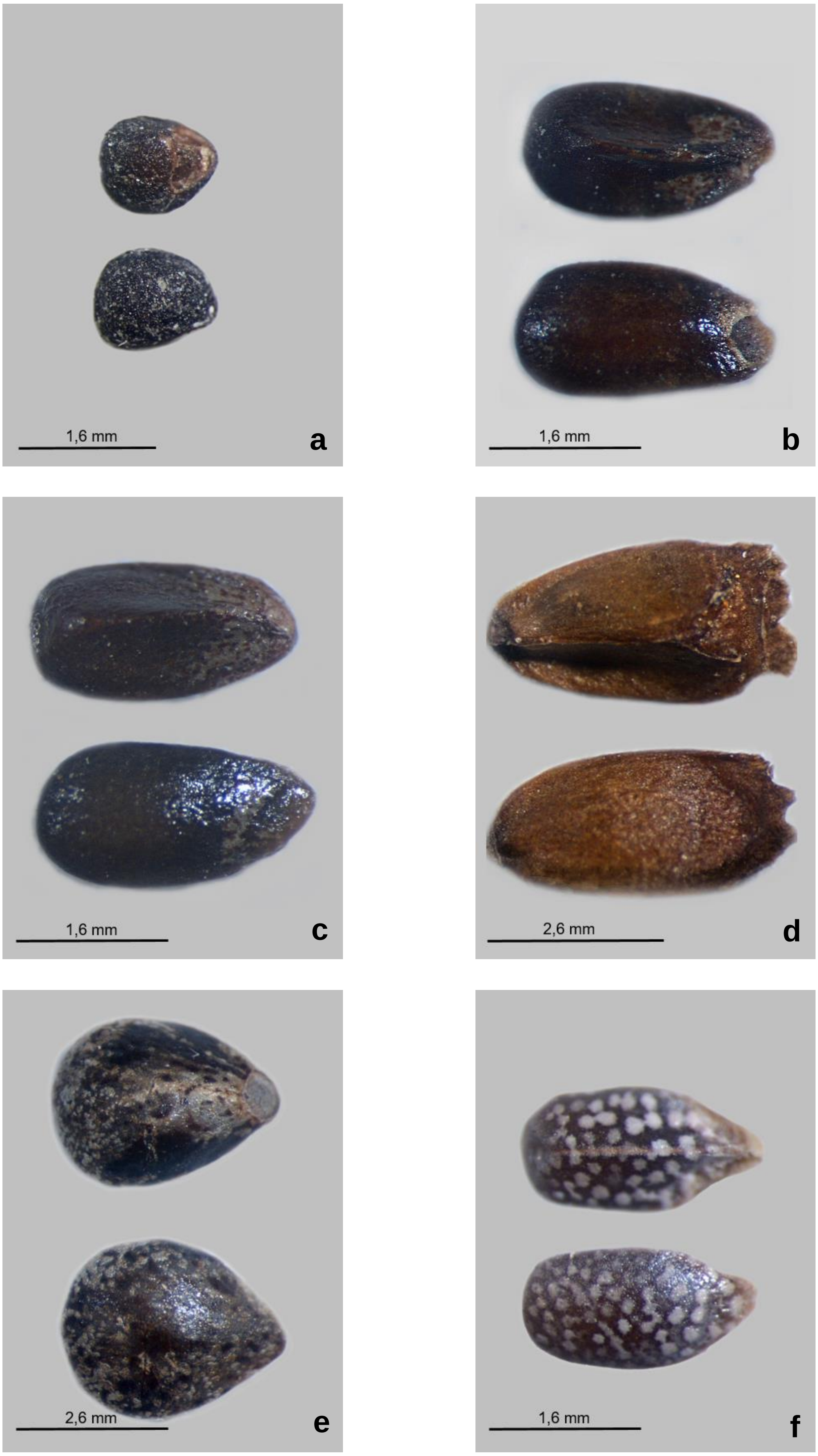

Figure 10 - *a) Teucrium spinosum; b) Ballota nigra; c) B. nigra subsp. foetida; d) Betonica officinalis; e) Galeopsis tetrahit; f) Lamium amplexicaule.*

***L. maculatum* L.** (Fig. 11a)

Habit: perennial

Description

Nutlets c. 3,8 x 2,1 mm. Shape: obovate. Apex: rounded. Base: truncate. Transverse cross section shape: 2-convex. Colour: brown with whitish spots. Surface: shinny. Surface pattern: colliculate. Abscission scar: small and elliptic.

***L. purpureum* L.** (Fig. 11b)

Habit: annual

Description

Nutlets c. 2,6 x 1,6 mm. Shape: obovate. Apex: rounded. Base: truncate. Transverse cross section shape: 2-convex. Colour: brown with whitish spots. Surface: shinny. Surface pattern: colliculate. Abscission scar: small and elliptic.

***L. purpureum var. hybridum* (Vill.) Vill.** (Fig. 11c)

Habit: annual

Nutlet description

Nutlets c. 2,9 x 1,6 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: 2-convex. Colour: brown with whitish spots. Surface: shinny. Surface pattern: colliculate. Abscission scar: small and elliptic.

Marrubium

***M. vulgare* L.** (Fig. 11d)

Habit: perennial

Nutlet description

Nutlets c. 2,6 x 1,5 mm. Shape: obovate to elliptic. Apex: rounded to slightly truncate. Base: rounded to acute. Transverse cross section shape: angular. Colour: dark brown with greyish areas. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and slightly triangular.

Melittis

***M. melissophyllum* L.** (Fig. 11e)

Habit: perennial

Nutlet description

Nutlets c. 4,6 x 4,5 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded to acute. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

Sideritis

***S. hirsuta* L.** (Fig. 11f)

Habit: perennial, sometimes annual

Nutlet description

Nutlets c. 2,8 x 1,9 mm. Shape: obovate. Apex: rounded. Base: slightly acute. Transverse cross section shape: angular. Colour: light brown to dark brown. Surface: shinny. Surface pattern: punctate. Abscission scar: small and slightly circular.

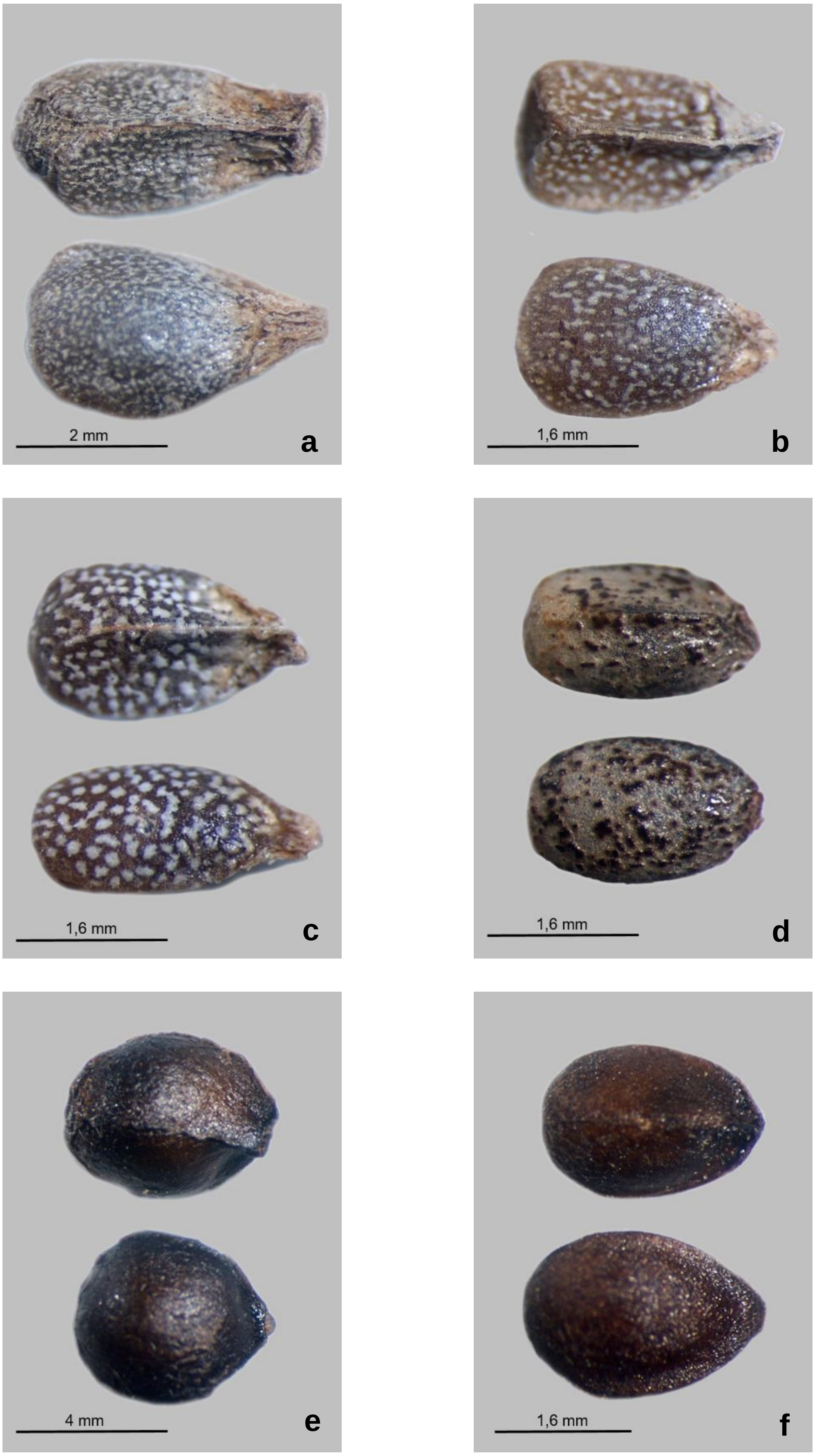

Figure 11 - *a) Lamium maculatum; b) L. purpureum; c) L. purpureum* var. *hybridum; d) Marrubium vulgare; e) Melittis melyssophylum; f) Sideritis hirsuta.*

Stachys

***S. arvensis* (L.) L.** (Fig. 12a)

Habit: annual

Nutlet description

Nutlets c. 2,2 x 1,7 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown with darker spots. Surface: dull. Surface pattern: slightly tuberculate. Abscission scar: small and slightly circular.

***S. germanica* L.** (Fig. 12b)

Habit: perennial

Description

Nutlets c. 3,6 x 2,8 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: angular. Colour: brown with darker spots. Surface: shinny. Surface pattern: slightly tuberculate. Abscission scar: small and slightly circular.

***S. ocymastrum* (L.) Briq.** (Fig. 12c)

Habit: annual, rarely biennal

Nutlet description

Nutlets c. 2,4 x 1,9 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: angular. Colour: dark brown. Surface: shinny. Surface pattern: slightly tuberculate. Abscission scar: small and slightly circular.

***S. palustris* L.** (Fig. 12d)

Habit: perennial

Nutlet description

Nutlets c. 2,8 x 2,1 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: angular. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

NEPETOIDEAE

Cleonia

***C. lusitanica* (L.) L.** (Fig. 12e)

Habit: annual

Nutlet description

Nutlets c. 2,3 x 1,8 mm. Shape: broadly obovate. Apex: apiculate. Base: rounded to slightly truncate. Transverse cross section shape: concave. Colour: light brown to brown. Surface: dull. Surface pattern: smooth. Abscission scar: small and triangular.

Clinopodium

***C. alpinum* (L.) Kuntze** (Fig. 12f)

Habit: perennial

Nutlet description

Nutlets c. 2,2 x 1,1 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: angular. Colour: light brown. Surface: shinny. Surface pattern: areolate. Abscission scar: medium and biconvex.

Figure 12 - *a) Stachys arvensis; b) S. germanica; c) S. ocymastrum; d) S. palustris; e) Cleonia lusitanica; f) Clinopodium alpinum.*

***C. nepeta subsp. spruneri* (Boiss.) Bartolucci & F. Conti** (Fig. 13a)
Habit: perennial
Nutlet description
Nutlets c. 1,2 x 0,9 mm. Shape: obovate to elliptic. Apex: rounded. Base: rounded to acute. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and reniform.

***C. vulgare* L.** (Fig. 13b)
Habit: annual
Description
Nutlets c. 1,5 x 1,3 mm. Shape: broadly obovate to circular. Apex: rounded Base: rounded. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and reniform.

***C. vulgare subsp. arundanum* (Boiss.) Nyman** (Fig. 13c)
Habit: perennial
Nutlet description
Nutlets c. 1,5 x 1,3 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and reniform.

Lavandula

***L. latifolia* Medik.** (Fig. 13d)
Habit: perennial
Nutlet description
Nutlets c. 2,6 x 1,4 mm. Shape: elliptic. Apex: truncate. Base: rounded. Transverse cross section shape: 2-convex. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and elliptic.

***L. multifida* L.** (Fig. 13e)
Habit: perennial
Nutlet description
Nutlets c.2,2 x 1,5 mm. Shape: broadly elliptic. Apex: rounded. Base: slightly truncate. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: granulate. Abscission scar: medium and slightly triangular.

***L. pedunculata* (Mill.) Cav.** (Fig. 13f)
Habit: perennial
Nutlet description
Nutlets c. 2,0 x 1,4 mm. Shape: elliptic. Apex: rounded. Base: slightly truncate. Transverse cross section shape: 2-convex. Colour: brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and elliptic.

Figure 13 - *a) Clinopodium nepeta subsp. spruneri; b) C. vulgare; c) C. vulgare subsp. arundanum; d) Lavandula latifolia; e) L. multifida; f) L. pedunculata.*

***L. stoechas* L.** (Fig. 14a)

Habit: annual

Nutlet description

Nutlets c. 1,9 x 1,4 mm. Shape: broadly elliptic. Apex: slightly truncate. Base: rounded. Transverse cross section shape: 2-convex. Colour: brown to dark brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and elliptic.

***L. stoechas subsp. luisieri* (Rozeira) Rozeira** (Fig. 14b)

Habit: perennial

Description

Nutlets c. 1,9 x 1,3 mm. Shape: elliptic. Apex: slightly truncate. Base: rounded. Transverse cross section shape: angular. Colour: brown to dark brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and elliptic.

***L. pedunculata subsp. sampaiana* (Rozeira) Franco** (Fig. 14c)

Habit: perennial

Nutlet description

Nutlets c. 2,2 x 1,5 mm. Shape: elliptic. Apex: rounded. Base: slightly truncate. Transverse cross section shape: angular. Colour: brown to dark brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and elliptic.

***L. viridis* L'Hér.** (Fig. 14d)

Habit: perennial

Nutlet description

Nutlets c. 2,6 x 1,0 mm. Shape: elliptic to broadly elliptic. Apex: rounded to slightly truncate. Base: truncate. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and elliptic.

Lycopus

***L. europaeus* L.** (Fig. 14e)

Habit: perennial

Nutlet description

Nutlets c. 1,7 x 1,2 mm. Shape: obovate. Apex: truncate. Base: acute. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

Melissa

***M. officinalis* L.** (Fig. 14f)

Habit: perennial

Description

Nutlets c. 2,3 x 1,1 mm. Shape: narrowly obovate. Apex: rounded. Base: acute. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: slightly foveolate. Abscission scar: small and slightly circular.

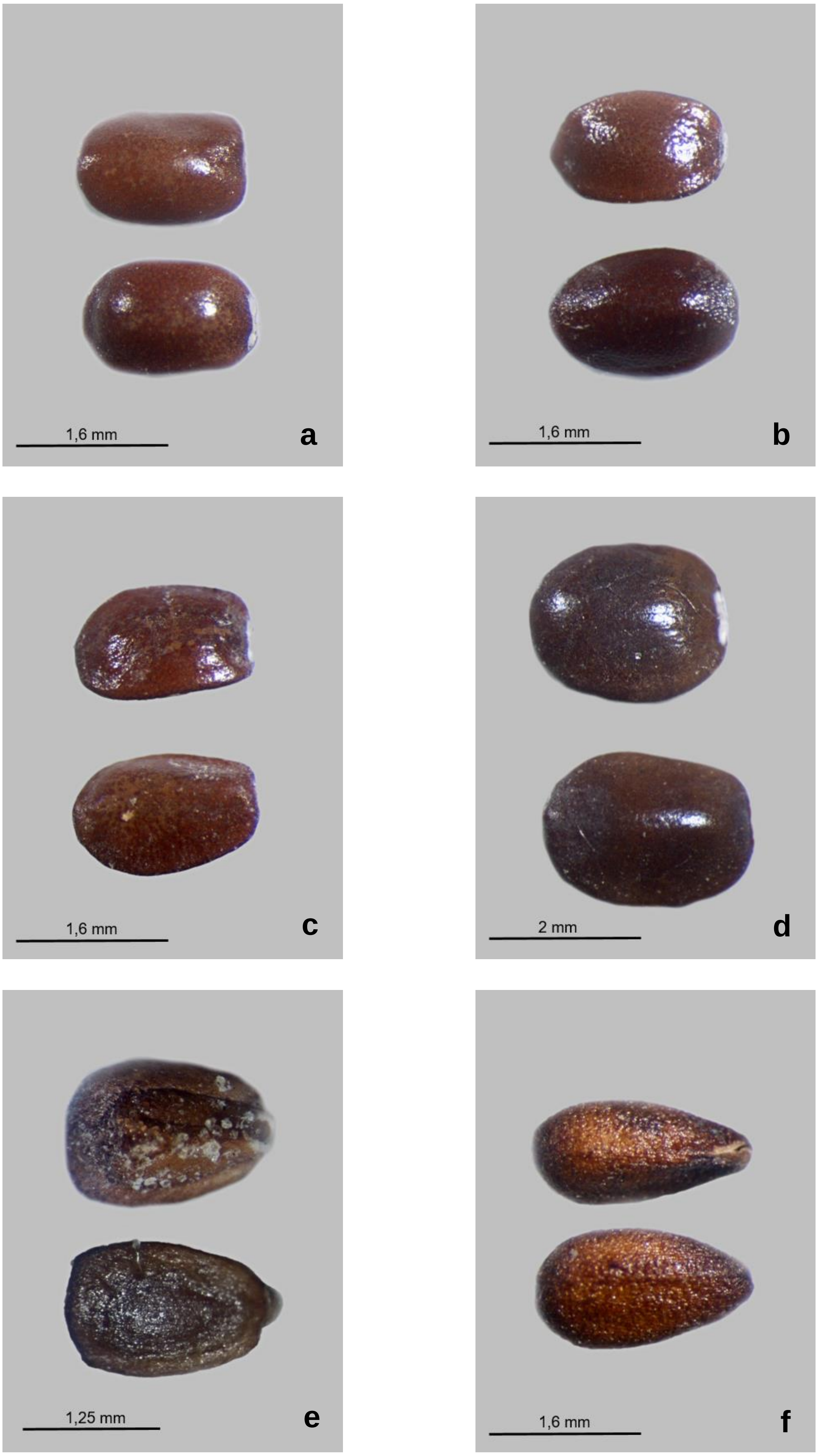

Figure 14 - *a) Lavandula stoechas; b) L. stoechas subsp. luisieri; c) L. pedunculata subsp. sampaiana; d) L. viridis; e) Lycopus europaeus; f) Melissa officinalis.*

Mentha

***M. longifolia* (L.) L.** (Fig. 15a)

Habit: perennial

Nutlet description

Nutlets c. 1,0 x 0,7 mm. Shape: obovate. Apex: acute. Base: acute. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and reniform.

***M. pulegium* L.** (Fig. 15b)

Habit: perennial

Nutlet description

Nutlets c. 0,8 x 0,6 mm. Shape: obovate. Apex: rounded. Base: rounded to acute. Transverse cross section shape: concave. Colour: light brown to dark brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and reniform.

***M. suaveolens* Ehrh.** (Fig. 15c)

Habit: perennial

Nutlet description

Nutlets c. 0,9 x 0,7 mm. Shape: obovate. Apex: rounded. Base: slightly apiculate. Transverse cross section shape: angular. Colour: brown to dark brown. Surface: shinny. Surface pattern: areolate. Abscission scar: small and reniform.

Micromeria

***M. graeca* (L.) Benth. ex Rchb.** (Fig. 15d)

Habit: perennial

Description

Nutlets c. 1,2 x 0,6 mm. Shape: narrowly elliptic. Apex: rounded to acute. Base: acute to slightly apiculate. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and slightly triangular.

***M. juliana* (L.) Benth. ex Rchb.** (Fig. 15e)

Habit: perennial

Nutlet description

Nutlets c. 1,1 x 0,4 mm. Shape: narrowly elliptic. Apex: apiculate. Base: apiculate. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and slightly triangular.

Nepeta

***N. caerulea* Aiton** (Fig. 15f)

Habit: perennial

Nutlet description

Nutlets c. 1,2 x 0,9 mm. Shape: obovate to elliptic. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: black. Surface: shinny. Surface pattern: tuberculate. Abscission scar: medium and reniform.

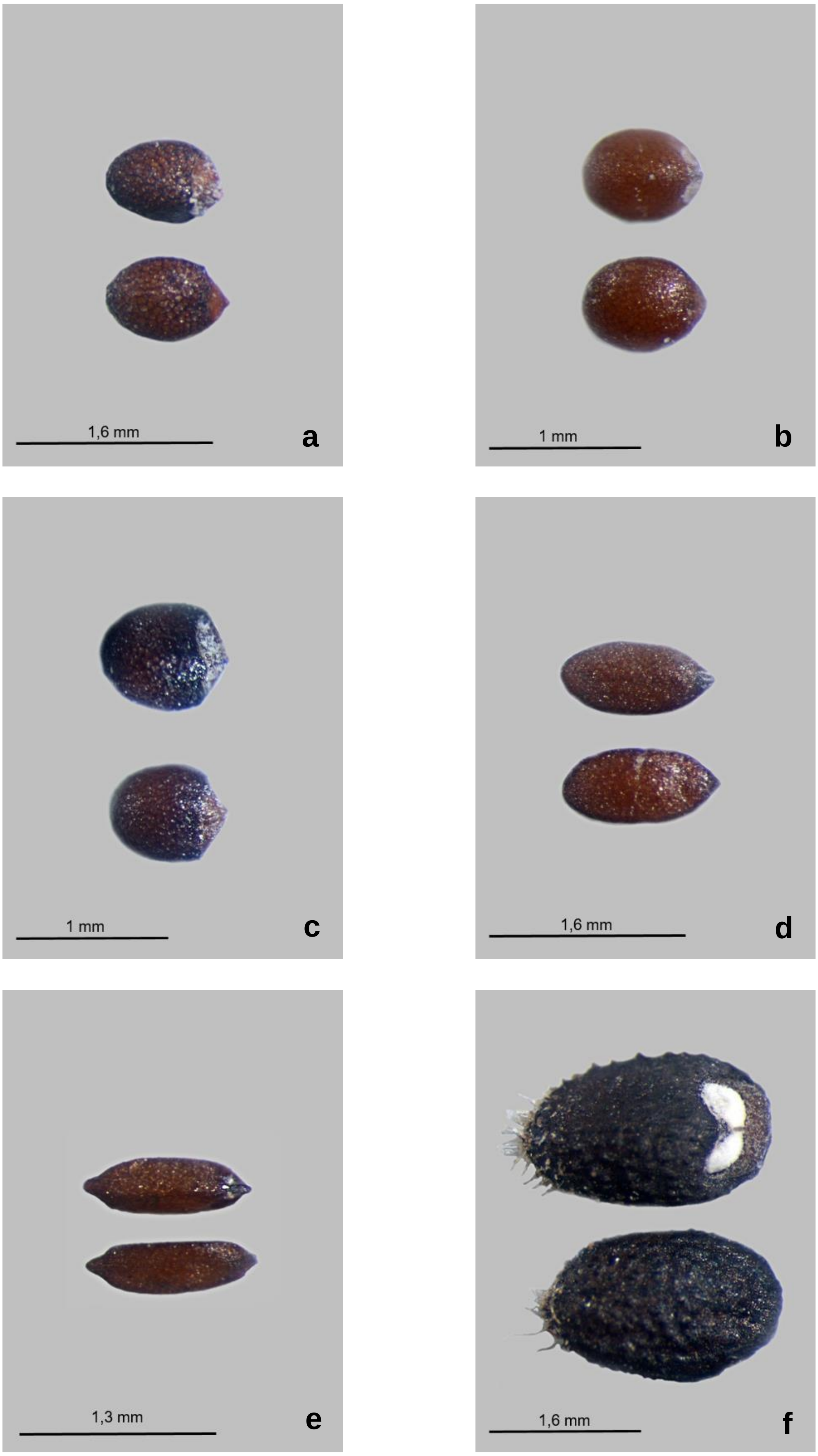

Figure 15 - *a) Mentha longifolia; b) M. pulegium; c) M. suaveleons; d) Micromeria graeca; e) M. juliana; f) Nepeta caerulea.*

***N. cataria* L.** (Fig. 16a)

Habit: perennial

Nutlet description

Nutlets c. 2,1 x 1,6 mm. Shape: elliptic. Apex: rounded to slightly truncate. Base: rounded to slightly truncate. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: medium and reniform.

***N. nuda* L.** (Fig. 16b)

Habit: perennial

Nutlet description

Nutlets c. 2,2 x 1,5 mm. Shape: obovate to elliptic. Apex: rounded to slightly truncate. Base: rounded. Transverse cross section shape: angular. Colour: brown. Surface: shinny. Surface pattern: slightly tuberculate. Abscission scar: medium and reniform.

***N. tuberosa* L.** (Fig. 16c)

Habit: perennial

Description

Nutlets c. 1,9 x 0,8 mm. Shape: elliptic. Apex: rounded. Base: rounded to slightly acute. Transverse cross section shape: concave. Colour: black. Surface: shinny. Surface pattern: tuberculate. Abscission scar: medium and reniform.

Origanum

***O. vulgare* L.** (Fig. 16d)

Habit: perennial

Nutlet description

Nutlets c. 1,1 x 0,8 mm. Shape: obovate. Apex: rounded. Base: acute to slightly apiculate. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and slightly triangular.

***O. vulgare subsp. virens* (Hoffmanns. & Link) Ietsw.** (Fig. 16e)

Habit: perennial

Nutlet description

Nutlets c. 1,2 x 0,8 mm. Shape: obovate. Apex: rounded. Base: slightly acute. Transverse cross section shape: concave. Colour: light brown to dark brown. Surface: shinny. Surface pattern: foveolate. Abscission scar: small and slightly triangular.

Prunella

***P. grandiflora* L.** (Fig. 16f)

Habit: perennial

Nutlet description

Nutlets c. 3,5 x 2,1 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: smooth with longitudinal ribs. Abscission scar: small and slightly circular.

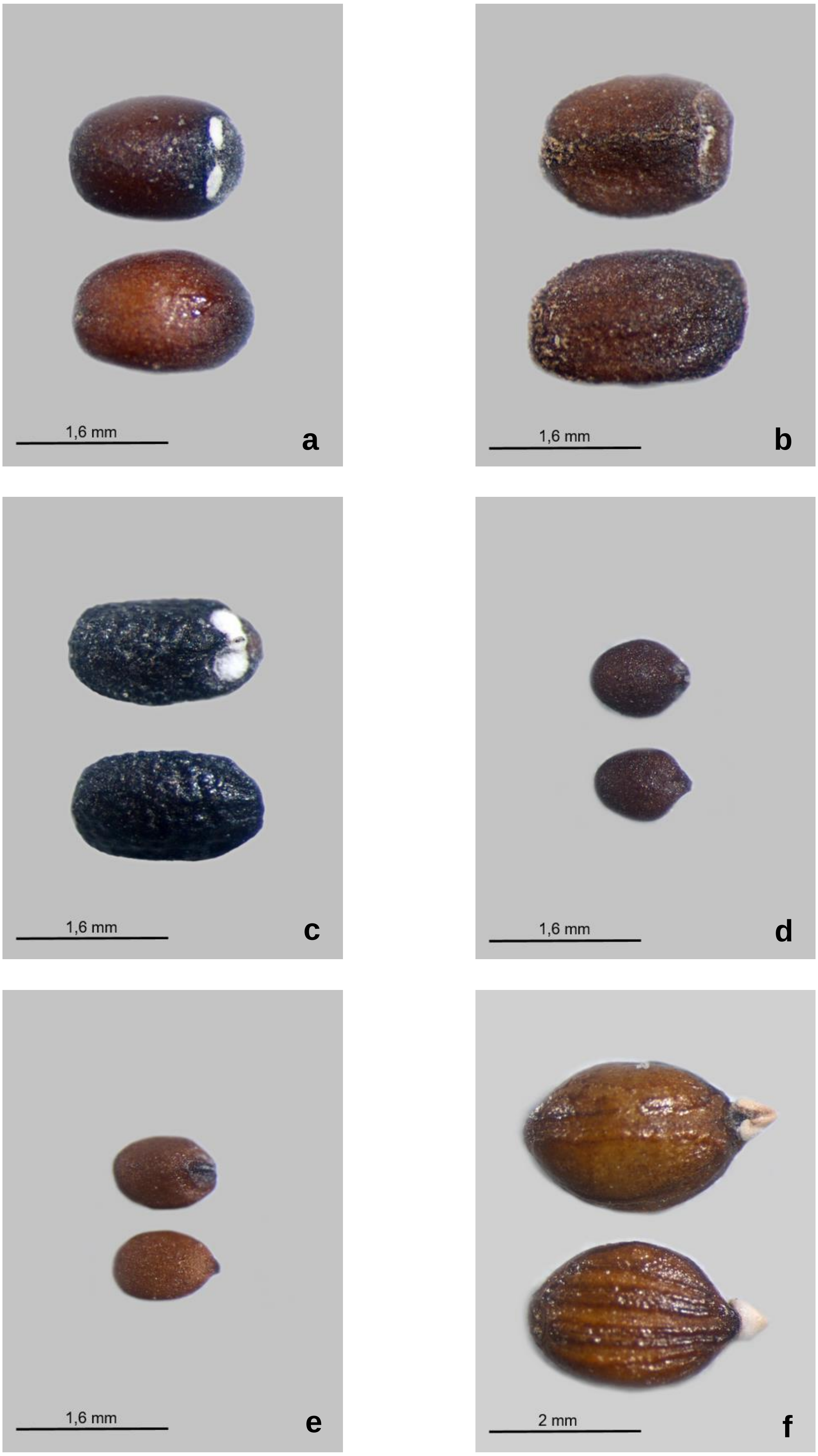

Figure 16 - *a) Nepeta cataria; b) N. nuda; c) N. tuberosa; d) Origanum vulgare; e) O. vulgare subsp. virens; f) Prunella grandiflora.*

***P. × intermedia* Link** (Fig. 17a)
Habit: perennial
Nutlet description
Nutlets c. 3,1 x 1,9 mm. Shape: obovate. Apex: rounded. Base: acute. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: smooth with 1 longitudinal rib. Abscission scar: small and slightly circular.

Salvia

***S. officinalis* L.** (Fig. 17b)
Habit: annual, biennial or perennial
Nutlet description
Nutlets c. 4,3 x 3,3 mm. Shape: broadly obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and narrowly elliptic.

***S. sclarea* L.** (Fig. 17c)
Habit: perennial
Nutlet description
Nutlets c. 3,5 x 2,6 mm. Shape: broadly obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

***S. sclareoides* Brot.** (Fig. 17d)
Habit: biennial or perennial
Nutlet description
Nutlets c. 3,5 x 2,1 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: slightly verrucate. Abscission scar: small and slightly circular.

***S. rosmarinus* Spenn.** (Fig. 17e)
Habit: perennial
Description
Nutlets c. 3,4 x 1,7 mm. Shape: obovate to elliptic. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: slightly verrucate. Abscission scar: large and circular to slightly triangular.

***S. verbenaca* L.** (Fig. 17f)
Habit: perennial
Nutlet description
Nutlets c. 2,9 x 2,3 mm. Shape: obovate. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: slightly verrucate. Abscission scar: small and slightly circular.

Figure 17 - *a) Prunella x intermedia; b) Salvia officinalis; c) S. sclarea; d) S. sclareoides; e) S. rosmarinus; f) S. verbenaca.*

***S. viridis* L.** (Fig. 18a)

Habit: annual, rarely biennial or perennial

Description

Nutlets c. 3,8 x 2,1 mm. Shape: narrowly obovate to elliptic. Apex: rounded. Base: acute. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

Satureja

***S. hortensis* L.** (Fig. 18b)

Habit: annual

Nutlet description

Nutlets c. 1,8 x 1,3 mm. Shape: obovate to elliptic. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: brown with grey areas. Surface: shinny. Surface pattern: granulate with darker longitudinal lines. Abscission scar: small and reniform.

***S. montana* L.** (Fig. 18c)

Habit: perennial

Nutlet description

Nutlets c. 1,9 x 1,5 mm. Shape: obovate to broadly elliptic. Apex: rounded. Base: rounded to slightly truncate. Transverse cross section shape: angular. Colour: brown. Surface: shinny. Surface pattern: granulate with darker longitudinal lines. Abscission scar: small and reniform.

Thymbra

***T. capitata* (L.) Cav.** (Fig. 18d)

Habit: perennial

Nutlet description

Nutlets c. 1,1 x 1,0 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown. Surface: shinny. Surface pattern: granulate. Abscission scar: small and slightly circular.

Thymus

***T. caespititius* Brot.** (Fig. 18e)

Habit: perennial

Nutlet description

Nutlets c. 1,0 x 0,8 mm. Shape: obovate to broadly elliptic. Apex: rounded. Base: truncate. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: slightly reticulate. Abscission scar: small and slightly circular.

***T. capitellatus* Hoffmanns. & Link** (Fig. 18f)

Habit: perennial

Nutlet description

Nutlets c. 1,1 x 0,9 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded to slightly apiculate. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: medium and elliptic.

Figure 18 - *a) Salvia viridis; b) Satureja hortensis; c) S. montana; d) Thymbra capitata; e) Thymus caepititius; f) T. capitellatus.*

***T. mastichina* (L.) L.** (Fig. 19a)
Habit: perennial
Description
Nutlets c. 1,3 x 1,2 mm. Shape: broadly obovate. Apex: rounded to slightly truncate. Base: rounded. Transverse cross section shape: concave. Colour: brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

***T. pulegioides* L.** (Fig. 19b)
Habit: perennial
Nutlet description
Nutlets c. 0,9 x 0,8 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown. Surface: shinny. Surface pattern: smooth. Abscission scar: small and slightly circular.

***T. villosus* L.** (Fig. 19c)
Habit: annual
Nutlet description
Nutlets c. 1,2 x 1,1 mm. Shape: broadly obovate to circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: dark brown to black. Surface: shinny. Surface pattern: smooth. Abscission scar: small and circular.

***T. zygis subsp. sylvestris* (Hoffmanns. & Link) Cout.** (Fig. 19d)
Habit: perennial
Nutlet description
Nutlets c. 0,9 x 0,8 mm. Shape: broadly obovate. Apex: rounded to slightly truncate. Base: rounded. Transverse cross section shape: concave. Colour: brown to dark brown. Surface: shinny. Surface pattern: slightly reticulate. Abscission scar: small and slightly circular.

SCUTELLARIOIDEAE

Scutellaria

***S. galericulata* L.** (Fig. 19e)
Habit: perennial
Nutlet description
Nutlets c. 1,9 x 1,6 mm. Shape: circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown to yellowish brown. Surface: shinny. Surface pattern: tuberculate. Abscission scar: small and slightly circular.

***S. minor* Huds.** (Fig. 19f)
Habit: perennial
Nutlet description
Nutlets c. 1,4 x 1,1 mm. Shape: circular. Apex: rounded. Base: rounded. Transverse cross section shape: concave. Colour: light brown to yellowish brown. Surface: shinny. Surface pattern: tuberculate. Abscission scar: small and slightly circular.

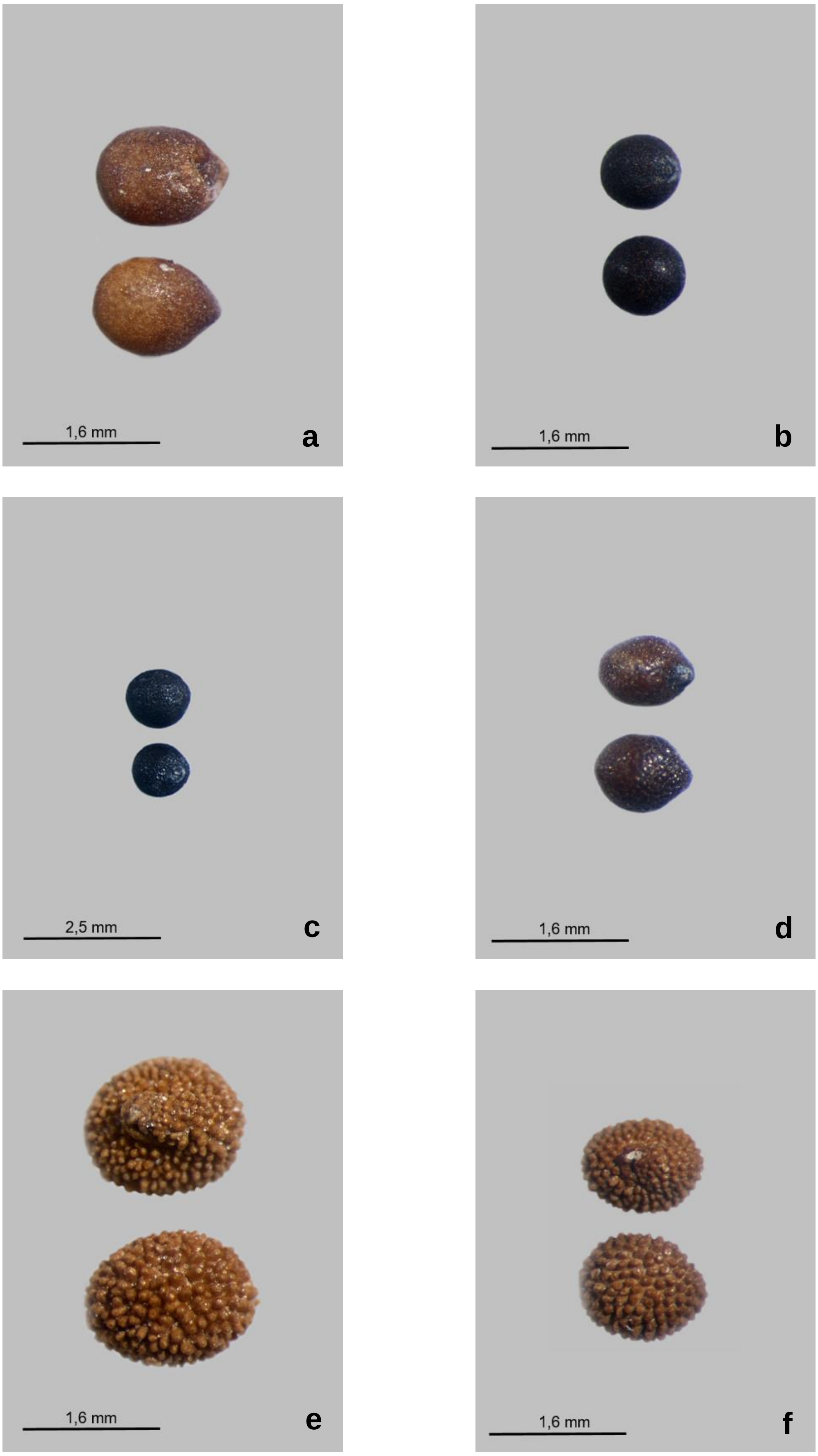

Figure 19 - *a) Thymus mastichina; b) T. pulegioides; c) T. villosus; d) T. zygis subsp. sylvestris; e) Scutellaria galericulata; f) S. minor.*

INTERACTIVE KEY

To create the Interactive Key in LucID Builder, we had (1) to create a tree in the *Tree View*, and (2) to fill in the *Spreadsheet Scoring.* In the *Tree View* (Fig. 20) we added the characters studied and the respective character states (Fig. 20, left), and the entities (Fig. 20, right), *i.e.,* the taxa studied. For the different character states, we added illustrations, as well as, descriptions to help to understand and select the character states. Images of the nutlets were also added to illustrate each taxa. The *Spreadsheet Scoring* (Fig. 21) was filled in with the results obtained from the correspondent nutlet descriptions.

LucID Builder provides a Score Analyser where nutlet differences are analysed after completing the aforementioned stages. Figure 22 shows the Score Analyser for taxa with zero differences between them.

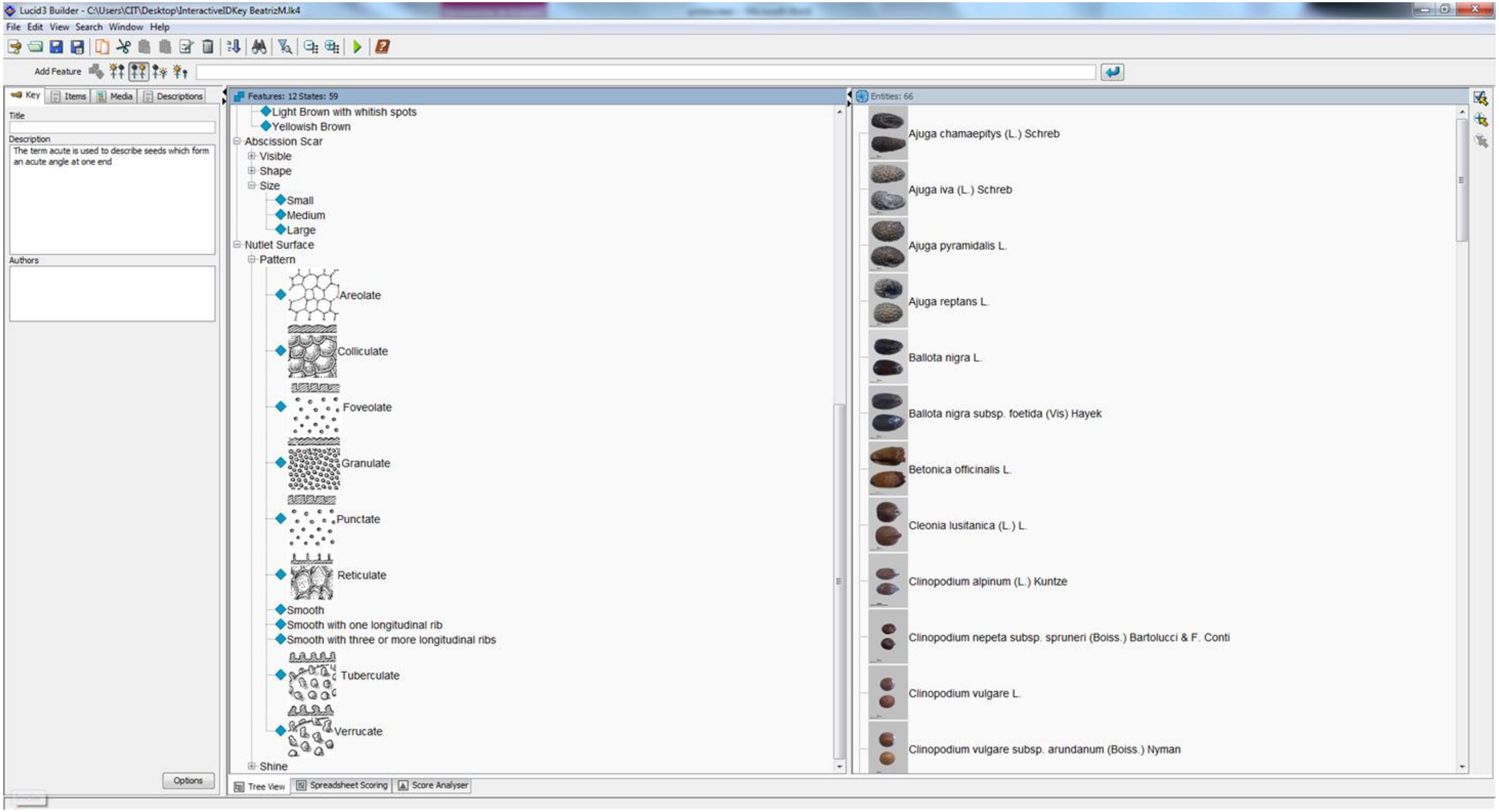

Figure 20 - *LucID Builder; Tree view with some of the characters studied and correspondent character states (left) and entities (right), i.e. taxa studied.*

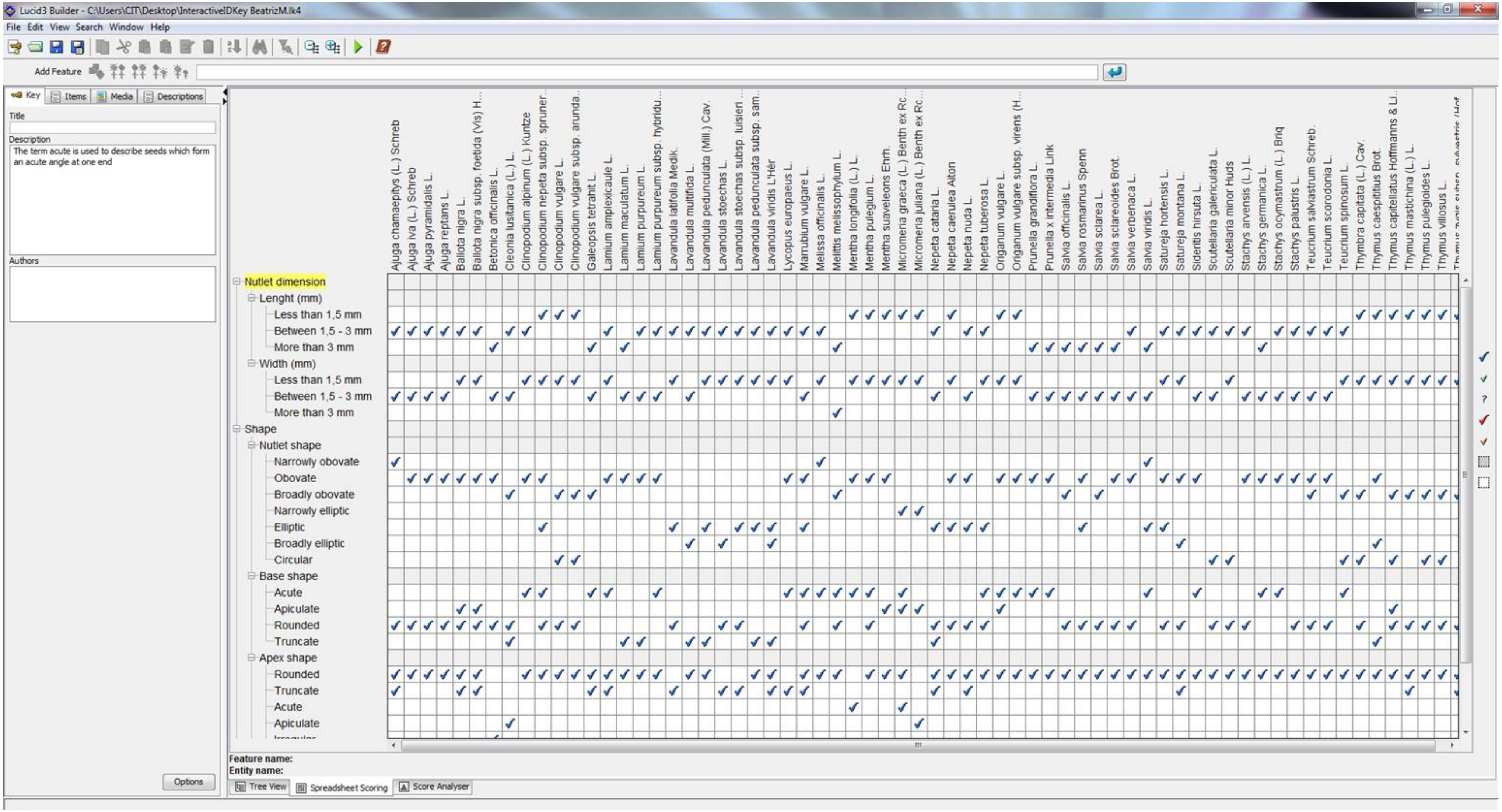

Figure 21 - *LucID Builder; Spreadsheet Scoring filled in with the results obtained from the correspondent nutlet descriptions.*

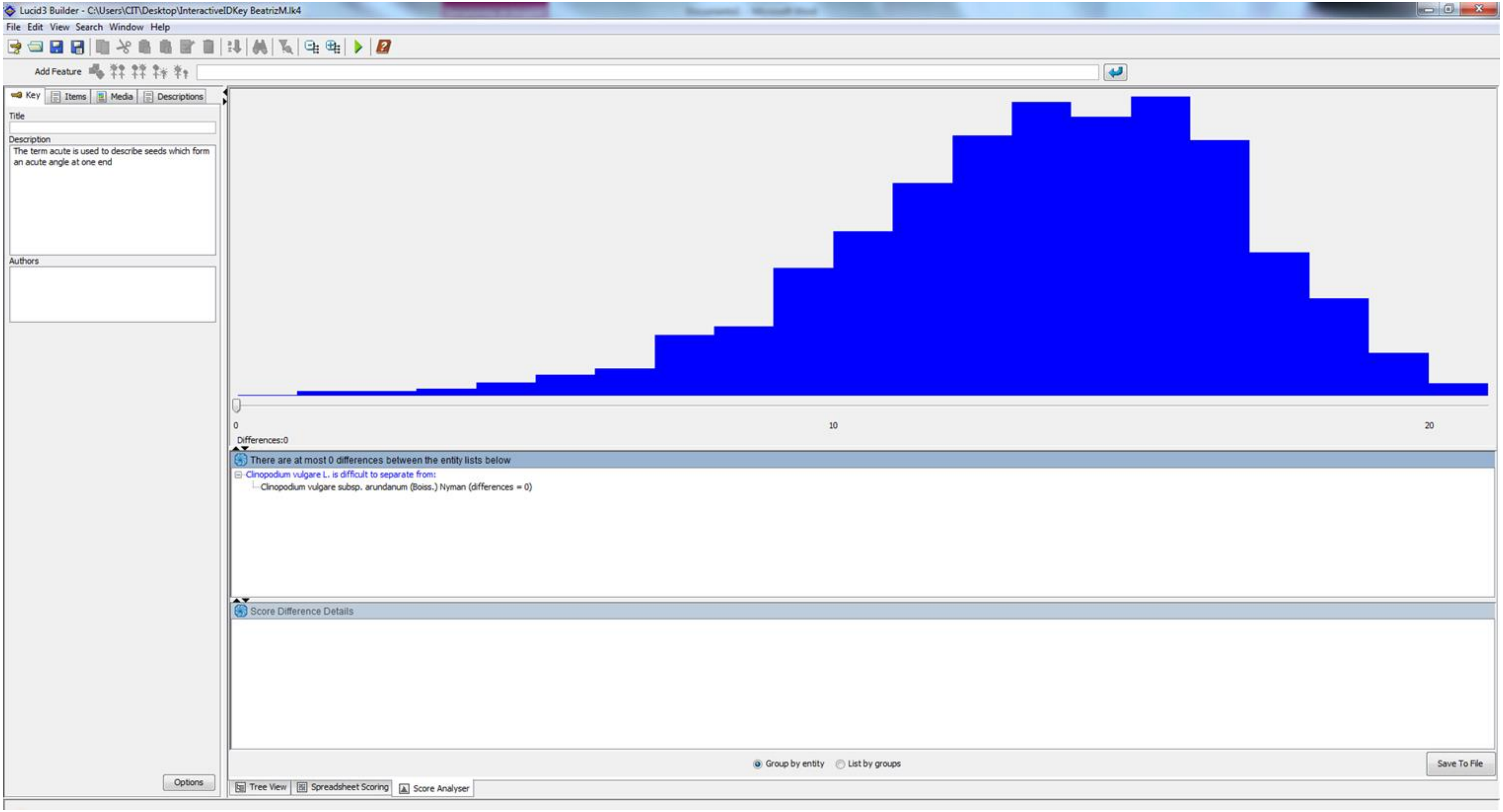

Figure 22 - *LucID Builder; Score Analyser for zero differences between taxa.*

MUCILAGE TESTS

Statistical analyses of initial dry nutlet weight showed significant differences in the dry weight of the nutlets belonging to different subfamilies ($F_{1,3}$=7.610; $p \leq 0.0001$).

The post-hoc tests (Fig. 23) showed that the subfamily Lamioideae is the group with significantly ($p \leq 0.05$) heavier nutlets than the remaining subfamilies, and the Scutellarioideae the group with lighter nutlets.

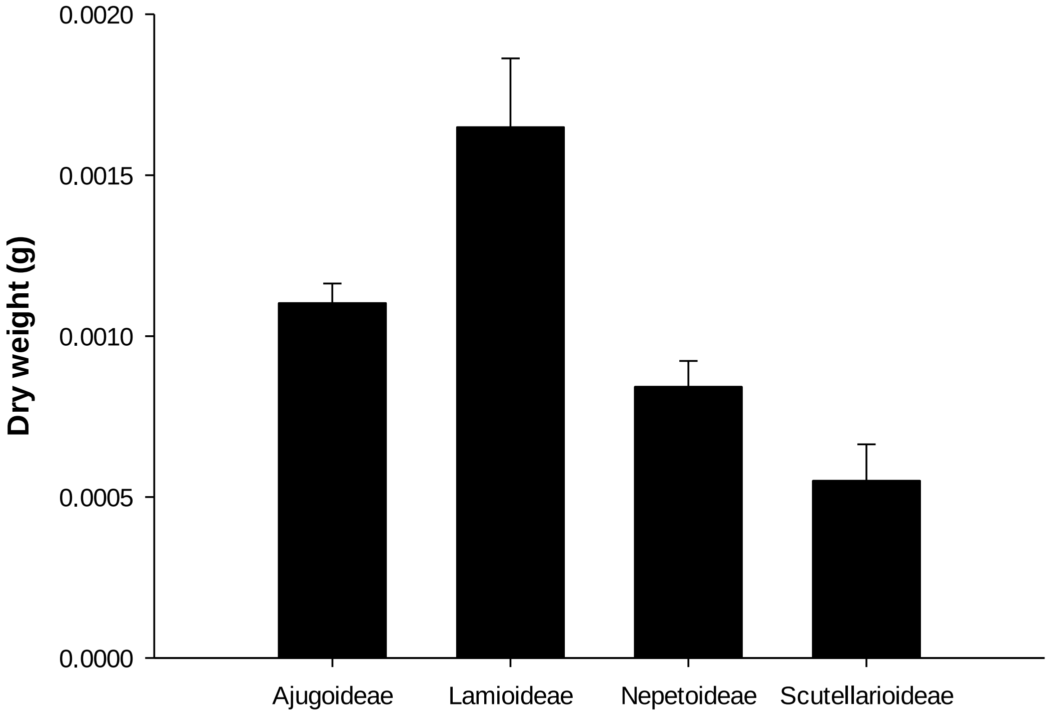

Figure 23 – *Initial dry weight (grams, mean±se) for each studied subfamily.*

The statistical analyses concerning the proportional increase in weight after 24 hrs of hydration showed significant differences among subfamilies ($F_{1,3}$=10.808; $p<0.0001$).

Post-hoc tests (Fig. 24) categorized the subfamilies in three significantly different groups ($p \leq 0.05$), with Nepetoideae nutlets being significantly larger than the other subfamilies after hydration for 24 hrs, up to four times more than the other subfamilies – in fact, up to twelve times its initial weight.

Since the Nepetoideae was the only subfamily producing mucilage, we can infer that this difference in weight increase of the hydrated nutlets was mostly due to the mucilage activity.

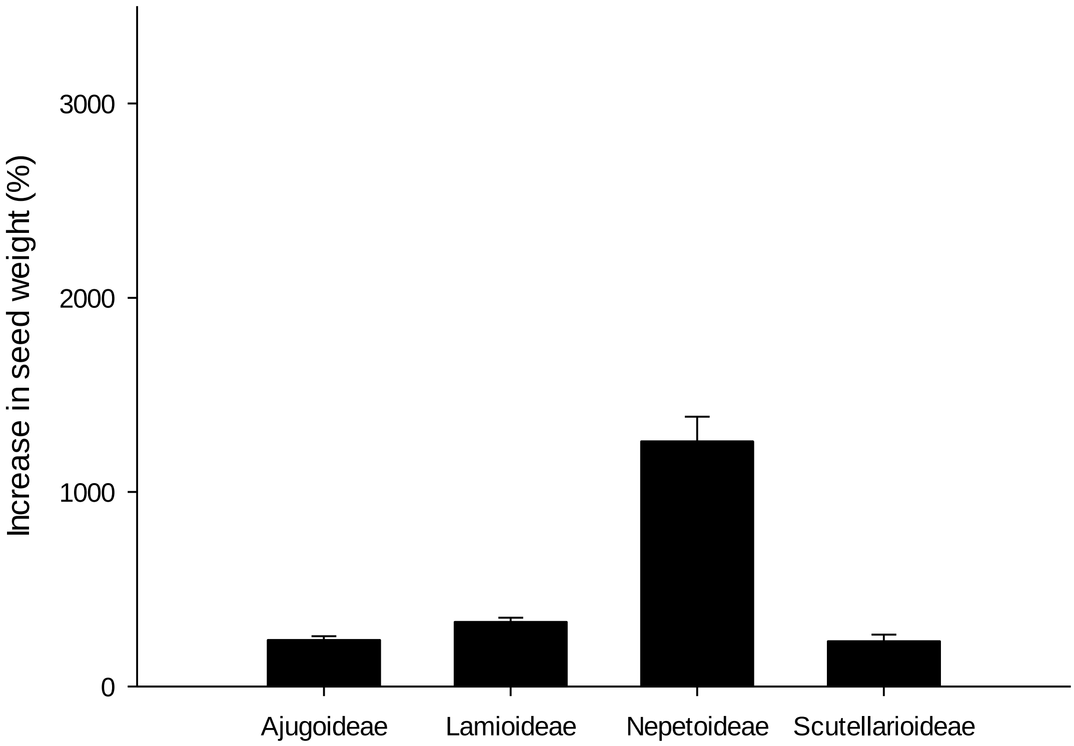

Figure 24 - *Proportional increase in weight (%, mean±se) after 24 hrs of hydration for each studied subfamily.*

The statistical analyses showed significant differences in the initial weight (g) among the different genera ($F_{1,24}$=24.980; p<0.0001).

Post-hoc analyses (Fig. 25) established six significantly different groups (p≤0.05). The genus with the largest initial weight was *Melittis*, followed by another group composed by *Galeopsis* only. *Salvia* constitutes the third group. The following group shows no differences among the genera and is constituted by *Clinopodium, Satureja*, *Scutellaria*, *Sideritis*, *Nepeta*, *Ballota*, *Marrubium*, *Lavandula*, *Lamium*, *Teucrium*, *Cleonia*, *Ajuga*, *Betonica*, *Prunella*, *Stachys.* These genera show overlapping results with the seven remaining genera (Fig. 25).

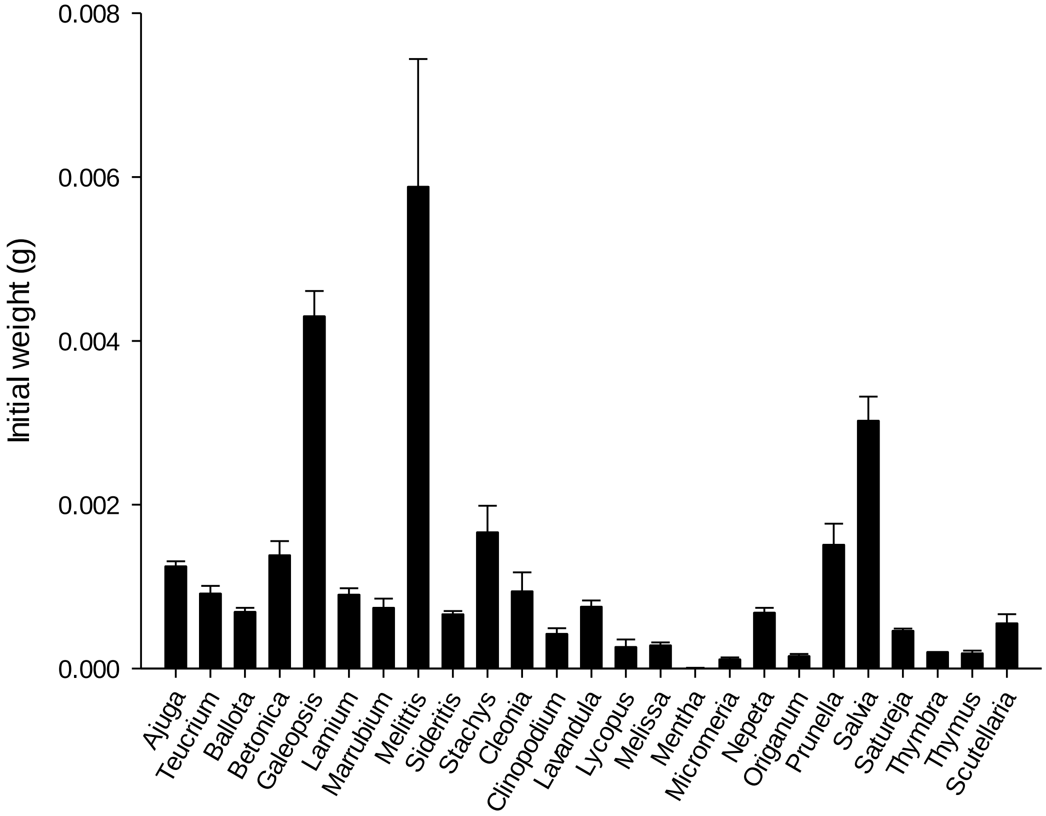

Figure 25 *-Initial dry weight (g, mean±SE) of each studied genus.*

When considering our data at generic level, the statistical analyses showed significant differences in weight increases among genera ($F_{1,24}$=8.729; p<0.0001).

Post-hoc analyses (Fig. 26) confirmed four significantly different (p≤0.05) but partially overlapping groups. The genera with the most weight increase after hydration for 24 hrs were *Cleonia* and *Thymus*. *Thymus* was closer to *Salvia*, which in turn was intermediate between *Thymus* and a group formed by *Marrubium*, *Clinopodium*, *Lavandula*, *Lycopus*, *Melissa*, *Mentha*, *Origanum* and *Prunella*. The fourth group is formed by the remaining genera, with very modest weight increase after hydration.

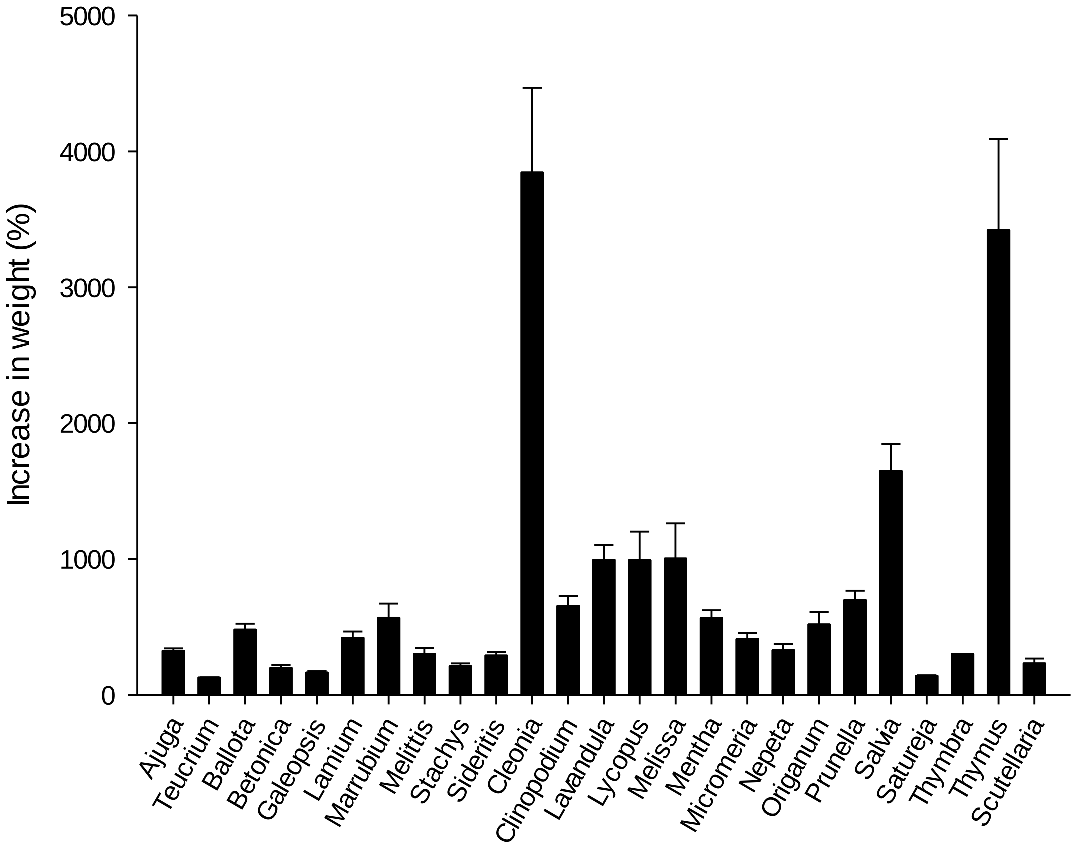

Figure 26 - *proportional increase in weight (%, mean±se) after 24 hrs of hydration for each studied genus.*

The statistical analyses showed differences in initial weight (g) at species level ($F_{1,65}$=21.542; p<0.0001).

Post-hoc tests (Fig. 27) categorized the results in nine significantly different (p≤0.05) but overlapping groups. The species with the largest initial weight was *Melittis melyssophylum*, followed by another group comprising *Stachys germanica*, *Galeopsis tetrahit*, *Salvia officinalis* and *Salvia sclarea*. This group overlapped with the next, in which *Salvia viridis* was present. The fourth group comprises *Prunella grandiflora*, *Salvia verbenaca*, *Salvia sclareoides* and *Salvia viridis*. For the remaining species, some small differences emerged, but the magnitude of those differences was negligible.

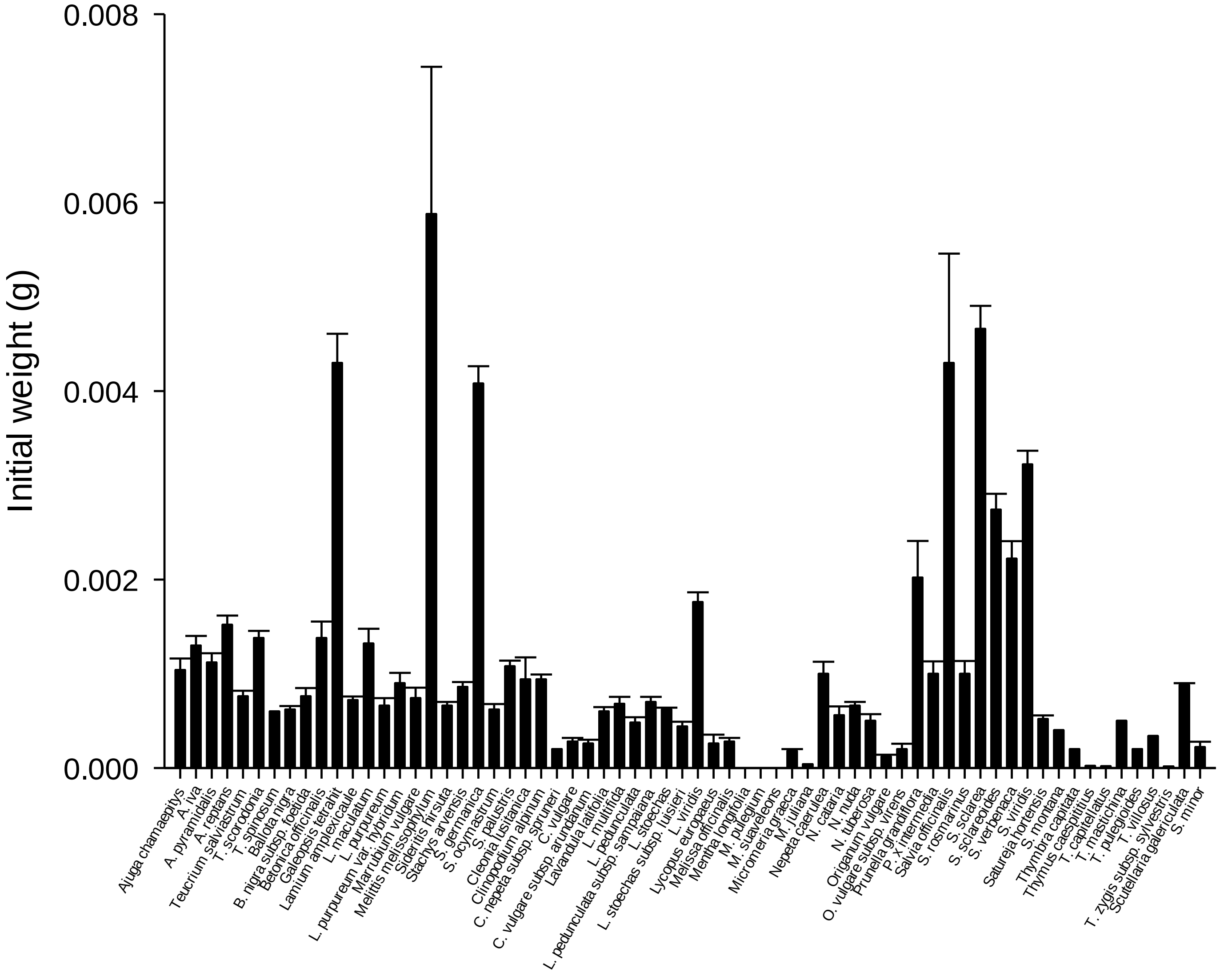

Figure 27 – *Initial dry weight (g, mean±se) for each studied species.*

Statistical analyses showed significant differences in weight increase (%) at species level ($F_{1,65}$=55.976; p<0.0001).

Post-hoc analyses (Fig. 28) showed up to five significantly different (p≤0.05) but partially overlapping groups. Nutlets of *Thymus zygis* subsp. *sylvestris* and *Thymus caespititius* had the largest weight increase after hydration. Their size more than doubled that of *Cleonia lusitanica*, which was intermediate between the first group and that formed by *Thymus capitellatus*, *Salvia viridis*, *Salvia verbenaca* and *Salvia sclareoides*.

Partially overlapping with the previous group, were *Salvia rosmarinus*, *Lavandula multifida*, *Lavandula pedunculata*, *Lavandula pedunculata* subsp. *sampaiana*, *Lavandula stoechas* subsp. *luisieri*, *Lycopus europaeus*, *Melissa officinalis*, and *Thymus villosus*. The remaining species had very little weight increase after hydration.

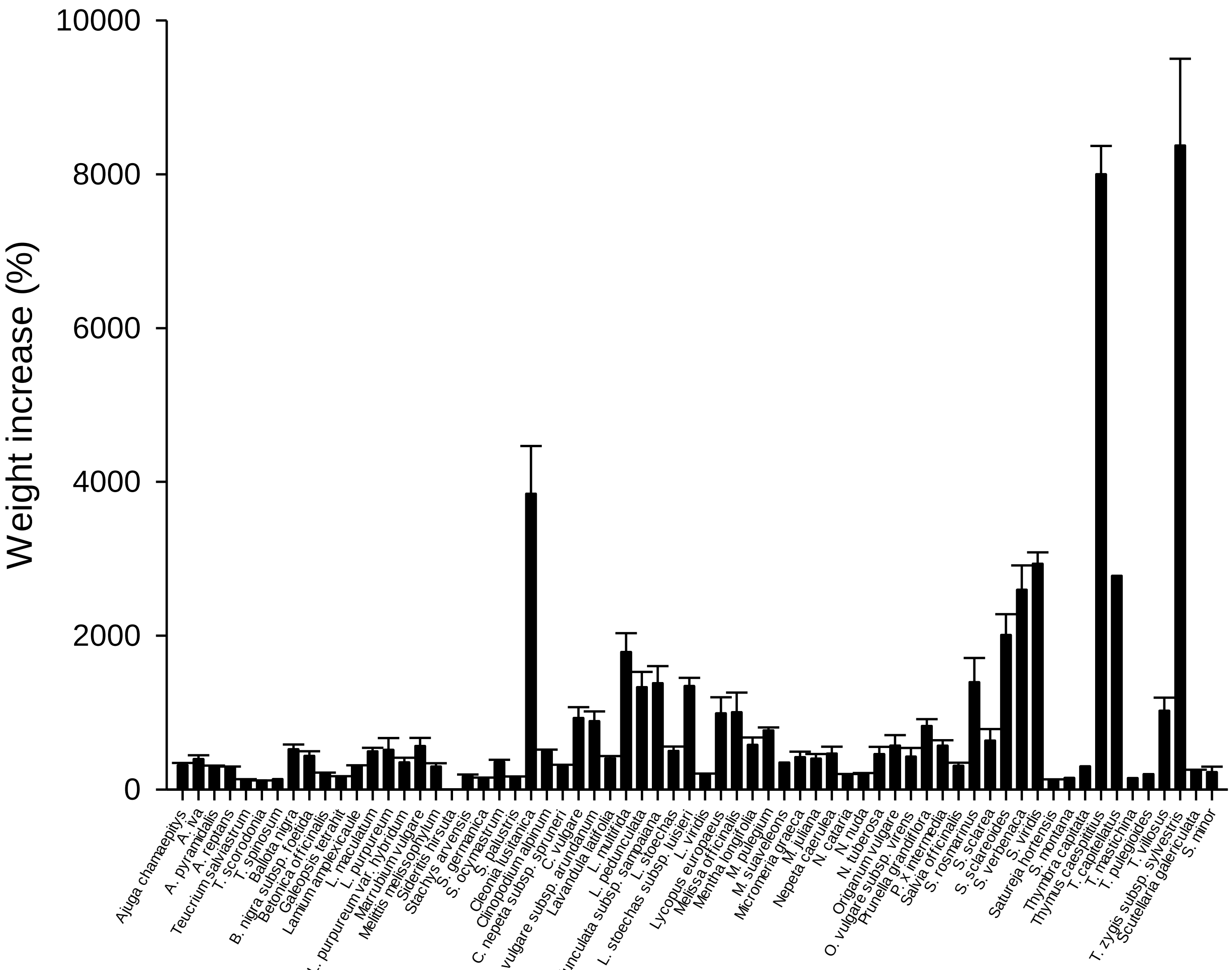

Figure 28 - *Proportional weight increase (%, mean±se) after 24 hrs of hydration for each studied species.*

Our research confirmed information collected from other studies on the nutlet morphology and properties of the studied species. A summary of the literature describing mucilage presence and characteristics for each of our species can is in Table 4.

Table 4 - Mucilage presence or absence according to available literature, and according to our results. Species in bold correspond to first records based on our study, or to conflicting reports.

Species	Mucilage presence/absence according to available literature	Current study
Cleonia lusitanica	Present (Martín Mosquero, 2002; Martín Mosquero *et al.,* 2004)	Present
Clinopodium alpinum	Present (Ryding, 1992)	Present
C. nepeta subsp*. spruneri*	**-**	**Absent**
C. vulgare	Absent (Lauševiæ & Marin, 1999)	Absent
C. vulgare subsp*. arundanum*	**-**	**Absent**
Lavandula latifolia	**-**	**Absent**
L. multifida	Present (Martín Mosquero, 2002)	Present
L. pedunculata	Present (Martín Mosquero, 2002)	Present
L. stoechas	Present (Martín Mosquero, 2002)	Present
L. stoechas subsp*. luisieri*	**Present (Martín Mosquero, 2002)**	**Absent**
L. pedunculata* subsp. *sampaiana	Present (Martín Mosquero, 2002)	Present
L. viridis	**Present (Martín Mosquero, 2002)**	**Absent**
L. latifolia	**-**	**Absent**
Lycopus europaeus	**-**	**Absent**
Melissa officinalis	Present (Lauševiæ & Marin, 1999)	Present
Mentha longifolia	**Present (Ryding, 1992; Lauševiæ & Marin, 1999)**	**Absent**
M. pulegium	Present (Ryding, 1992; Lauševiæ & Marin, 1999; Martín Mosquero, 2002)	Present
M. suaveolens	Present (Lauševiæ & Marin, 1999; (Martín Mosquero, 2002)	Present
Micromeria graeca	Present (Doroszenko, 1986; Martín Mosquero, 2002)	Present

M. juliana	**Present (Doroszenko, 1986; Lauševiæ & Marin, 1999)**	**Absent**
Nepeta caerulea	**-**	**Absent**
N. cataria	**Present (Lauševiæ & Marin, 1999); Absent (Martín Mosquero, 2002)**	**Absent**
N. nuda	**Present (Lauševiæ & Marin, 1999)**	**Absent**
N. tuberosa	**-**	**Absent**
Origanum vulgare subsp. *virens*	**Present (Martín Mosquero, 2002)**	**Absent**
O. vulgare	Absent (Lauševiæ & Marin, 1999)	Absent
Prunella grandiflora	Present (Lauševiæ & Marin, 1999)	Present
P. × intermedia	**-**	**Present**
Salvia officinalis	Absent (Ryding, 1992; Martín Mosquero, 2002)	Absent
S. sclarea	Present (Martín Mosquero, 2002)	Present
S. sclareoides	Present (Martín Mosquero, 2002)	Present
S. rosmarinus	Present (Ryding, 1992; Martín Mosquero, 2002)	Present
S. verbenaca	Present (Martín Mosquero, 2002)	Present
S. viridis	Present (Martín Mosquero, 2002)	Present
Satureja hortensis	Present (Doroszenko, 1986; Lauševiæ & Marin, 1999)	Absent
S. montana	**Absent (Doroszenko, 1986) Present (Lauševiæ & Marin, 1999)**	**Absent**
Thymbra capitata	**Present (Martín Mosquero, 2002)**	**Absent**
Thymus caespititius	**-**	**Present**
T. capitellatus	**-**	**Absent**
T. mastichina	Absent (Martín Mosquero, 2002)	Absent
T. pulegioides	**Present (Lauševiæ & Marin, 1999)**	**Absent**
T. villosus	**-**	**Present**
T. zygis* subsp. *sylvestris	Present (Martín Mosquero, 2002)	Present

We visually verified the presence and expansion of mucilages in each species and confirmed the presence of this trait in 20 of the 43 species assessed in the Nepetoideae (47%).

Statistical analyses of the species that produced mucilage showed significant differences in weight increase (%) ($F_{1,19}$=44.603; p≤0.0001).

The post-hoc tests (Fig. 29) separated the species into five different groups (p<0.05) with some species overlapping different groups. *Thymus caespititius* and *Thymus zygis* subsp. *sylvestris* formed the group of species with the larger increase in weight, followed by the group of *Cleonia lusitanica*, *Salvia viridis* and *Salvia verbenaca*. These last two species showed similarities with *Lavandula pedunculata*, *Salvia rosmarinus*, *Lavandula multífida* and *Salvia sclareoides*. The remaining species presented small-sized mucilage and were grouped together.

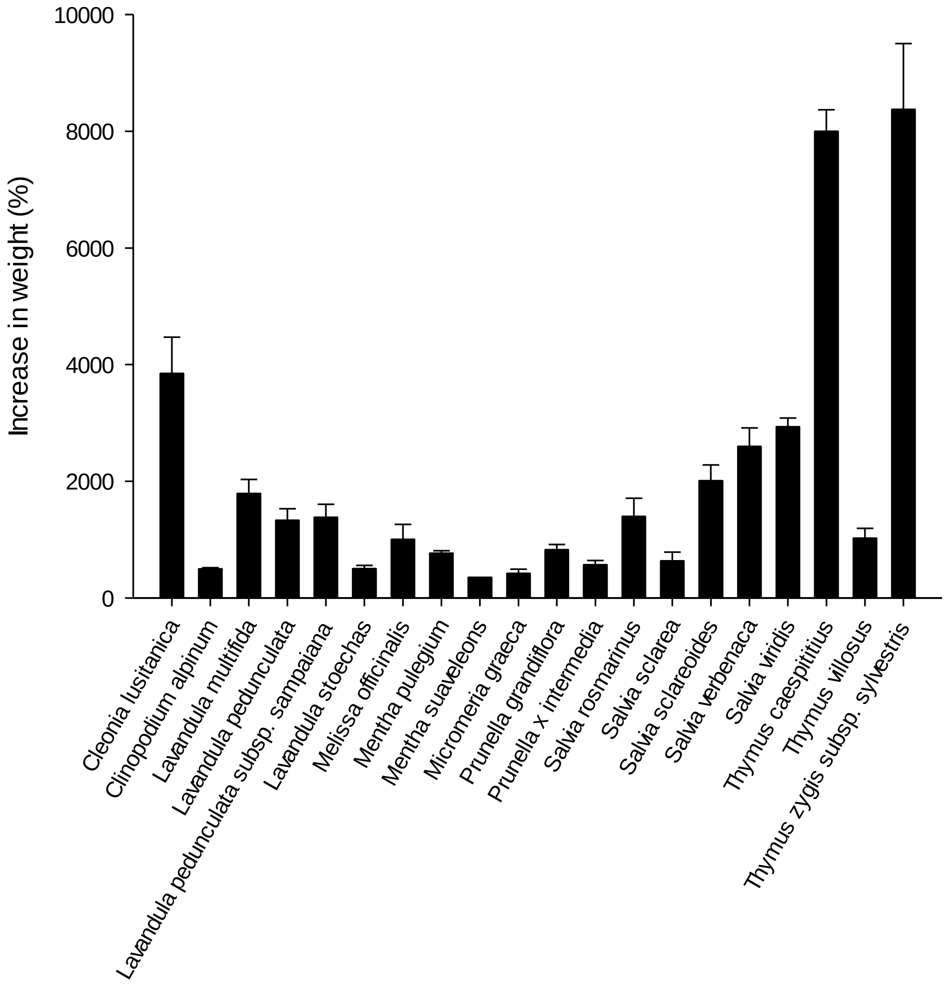

Figure 29 - *Proportional weight increase (%, mean±se) in species with mucilage production.*

For the **second test**, in which we measured weight at intervals for 4 hrs, we produced saturation curves of percentage of weight increase (g) versus time (min) in a sub-set of five species representing the four subfamilies here studied (Fig. 30). For the Nepetoideae two species were selected. Although mucilage is an exclusive trait to this subfamily, not all its species produce mucilage. Therefore, one species reportedly with mucilage (*Salvia rosmarinus*) and one reportedly without mucilage (*Nepeta cataria*) were selected.

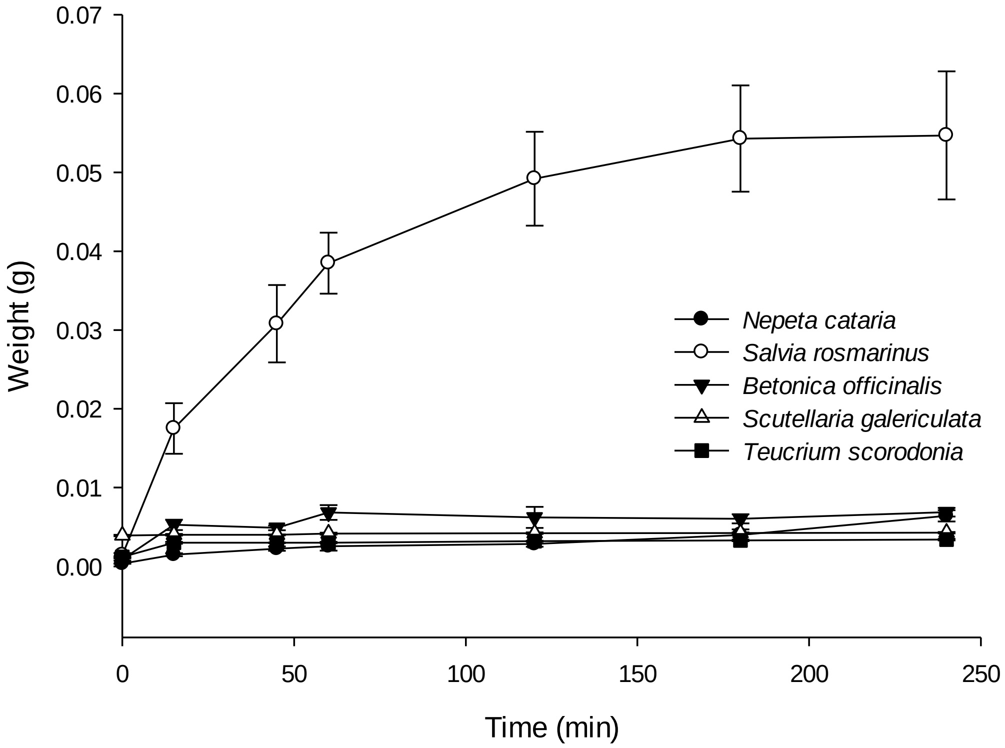

Figure 30 - *Saturation curves of weight increase (%, mean±se) in intervals of 15, 45, 60, 120, 180, 240 minutes.*

Discussion

In the studied Lamiaceae collection, all nutlets were schizocarps. Their size showed an appreciable variation within this family (0.84-4.6 mm). Variation was large not only at the genus level but also within genera and in some cases even at the species level. This pattern was also reported in the literature (*e.g.* Martín Mosquero, 2002). Nutlet size is in part determined genetically, but it is also influenced by maternal factors and by the environment, during the period of development (Werker, 1997). For those reasons, nutlet size has not usually been regarded so important in taxonomic studies (Mourad, 1988 in Hassan, 2015).

Lamiaceae nutlet shape was elliptic or obovate and varied within their subtypes. Shape is, for the majority of the species, consistent within species and within genera. However, it is possible some variation in shape at the species level. Werker (1997) stated that both seed shape and nutlet shape are determined genetically, but at the final stages of their development, shape is moulded according to the space available for its growth. This can well explain the slanted ventral surfaces, caused by growth against other nutlets.

Nutlet colour varied from different shades of brown to black and is considered of little taxonomic value in taxa delimitation. This lack of taxonomic value can be explained by: (1) the little variation among the different genera and species and (2) in some cases it can be inconsistent within the same species (Guerin, 2005, Hassan, 2015). Nevertheless, in some of the species studied here, it is possible to distinguish some genera because of their colour or colour pattern, as in *Lamium* which nutlets have white spots on the surface, a particularity not seen in any other genus studied.

For the abscission scar, size and shape can be used to distinguish some genera. In fact, despite the majority of nutlet had a small abscission scar, a large abscission scar is present in a small number of genera, specifically in all species of *Ajuga*, in some species of *Teucrium* and in *Salvia rosmarinus*.

Surface pattern is a very useful character in the differentiation of the various taxa. However, while in some genera, *i.e.*, *Ajuga* and *Scutellaria*, the pattern is prominent and easily observed, in others it is difficult to confirm the minute pattern. Due to our objective to develop a user-friendly interactive key, the nutlets were studied at low magnification. Anatomy or SEM would bring in a further perception of features and differences but would not be used for this identification tool.

Some characters individually lacked taxonomic importance but the combination of them and of their states showed to be efficient in the identification of the different genera and species. Still, the Score Analyser inbuilt in LucID revealed that two taxa in *Clinopodium*

(*C. vulgare* and *C. vulgare* subsp. *arundanum*) have zero differences for the characters investigated. This seems to us a really minor issue to the usefulness of the nutlet characters for identification purposes, given the fact that these taxa are at the subspecific level.

MUCILAGE

Mucilage production has been associated with the establishment of the seedlings in environments with low precipitation and disturbed habitats (Yang *et al*., 2012), anchoring the seed to the ground and consequently aiding water diffusion from the substrate to the seed (Harper & Benton, 1966 cited in Werker, 1997) and supporting seed and fruit dispersal by adhesion to soil or animal vectors (Ryding, 2001; Kreitschitz *et. al*, 2009; Western, 2012).

We confirmed Nepetoideae as the only subfamily in the Lamiaceae containing species with mucilaginous nutlets, as previously reported by Ryding (1992). Mucilage is present in approximately 70-75% of the genera/species of the Nepetoideae (Ryding, 1992). However, our tests and visual assessment abled us to confirm the presence of this trait in only 47% of our sample (20 out of the 43 species). The Nepetoideae include 3100-3700 species across the world. Consequently, we can consider that our study species set might be a good representation of Portuguese Lamiaceae, but still just a small fraction of the entire family. In fact, we provide for the first time with mucilage assessments for 11 species not previously described, or 26% of the total species studied here.

Nutlets of non-mucilaginous subfamilies increased their dry-weight about two or three times after exposure to water for 24 hrs. However, hydration increased nutlet weight approximately five times more in the Nepetoideae than in the other subfamilies, a pattern that was confirmed at the genus level. Consequently, we can infer that approximately 20% of the weight increase observed in the Nepetoideae could be attributed to hydration of non-mucilaginous elements of the nutlets, suggesting that water retention is an important ecological property of mucilages. However, even mucilaginous nutlets presented differentiated responses to the presence of water, pointing out to divergence in the relative importance of water retention versus other biological functions of mucilages, like anchoring to the ground or dispersal, across the different study species (Harper & Benton, 1966 cited in Werker, 1997; Ryding, 2001; Kreitschitz *et. al*, 2009; Western, 2012).

For two of our studied species, the literature provided with conflicting reports about the presence of mucilage, and for nine species our study found contrary evidence to what has been previously reported (Table 4). Specifically, no mucilage production had been described for *Satureja montana* by Doroszenko (1986) but as having mucilage production by Lauševiæ & Marin (1999). Our assessment agreed with Doroszenko (1986). In the study of Lauševiæ & Marin (1999), the mucilage activity in both species was described as "very weak or only swolling reaction" with less than 0,05 mm of expansion which can suggest to some misperception of the viewer. Additionally, there are clear conflicting reports about mucilage presence in *Nepeta cataria*. *Nepeta cataria* nutlets were reported by Lauševiæ & Marin (1999) to produce mucilage and by Martín Mosquero (2002) as not having mucilage production. Our results agree with the latter and we emphasise the fact that their investigation involved a larger number of samples. These apparent contradictions between our assessment and literature records could be due to various causes. For example, in some species, nutlets can produce large volumes of mucilage*, e.g*. *C. lusitanica* and *Thymus zygis* subsp. *sylvestris*, but in other cases, the amount produced is very small. This makes mucilages hard to observe and increases the chances of contradictory visual identification of the presence or absence of mucilage. Differences in mucilage density could be another issue, since in some cases mucilages can be very dense, *e.g*. *Cleonia lusitanica*, *Salvia viridis*, whereas in others the mucilage is very weak, *e.g. Mentha*. This fragility could potentially cause inadvertent mucilage destruction during nutlet management. Finally, mucilage production is a trait which, like any other biological trait, experiences population variability, not only in size, but also on expression. Our assessment found important variability in mucilage size within species, and in some cases nutlets from the same species and sample presented mucilage whereas others did not (e.g. *C. lusitanica*). Additionally, we have to consider that our samples come from a historical Herbarium, and thus that differences in conservation could be responsible, too, for some of these disparities.

Our work systematically and quantitatively compared the nutlet morphology and their mucilage production in the Lamiaceae using a unified methodology, which allowed to describe interesting patterns across the main subfamilies. Nepetoideae was the only subfamily where mucilages were present, even though its prominence and presence were highly variable even within this subfamily. This suggests that different selective pressures might have selected for mucilage presence or absence, or for the large differences observed in mucilage size and properties, even among closely related species in the Nepetoideae. Our results provide with critical information for future studies, with important implications for the fields of taxonomy, ecology, dispersal, and restoration, among others.

Conclusions

In conclusion, our rather systematic investigation provided enough data to construct the Interactive Key and was efficient to identify the Lamiaceae nutlets to the genus and to the species level.
Our results confirm Hedge (1992) comments pointing out to the importance of the nutlet characters for species identification in the Lamiaceae.

It is our ambition to further improve this interactive key to which we intend to add some other features to provide the nutlet identification with a stronger efficiency. Also, the remaining taxa of the Lamiaceae in the Portuguese flora will eventually be added.

As aforementioned, this investigation constitutes the first step of the long-term Herbarium project for the development of the Portuguese Seed Identification Key – Online (PSIKO) based on the native flora material only of the Seed Collection. As other families will be added to the Key, some characters will have to be considered to separate the nutlets of this family and sheets with added information about each species will be made available in LucID online.

We confirmed Nepetoideae as the only subfamily presenting mucilages in the Lamiaceae, Comparative assessments of nutlet weight after hydration allowed us to observe up to an 80-fold increase in size for some mucilage bearing nutlets, but we also found a large variability on the presence and prominence of mucilages within this subfamily, with important taxonomical, ecological, and evolutionary implications.

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Appendix

Appendix I

Cataloged Date	COI no	Full Name	Species Author	State	Locality Name	Locality and Habitat Notes (Original)	Specimen Description (Original)	Collectors/Last Name	Collectors/First Name	Determiner Name	Comment
2017/10/04	COI00079399	Thymus mastichina	(L.) L.	Beira Alta	Estrada Guarda a Manteigas, perto do Parque Eólico	Na berma da estrada. Solo inculto		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 929.
2017/10/04	COI00079397	Thymus caespititius	Brot.	Beira Alta	Rampa de estrada que vai de S. João do Monte para o Caramulo	Solo inculto		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 927.
2018/05/24	COI00079396	Thymus capitellatus	Hoffmanns. & Link	Ribatejo	Estrada de Benavente a S. Estevão, a 5km. de S. Estevão	Solo inculto com pinhal		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: s.n.
2017/10/04	COI00079395	Salvia verbenaca	L.	Ribatejo	Alcanena, Serra de Santo António, na descida para a Aldeia	Solo inculto e pedregoso		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 903.
2017/10/04	COI00079394	Thymus capitatus	Hoffmanns. & Link	Beira Litoral	Coimbra, Rio de Galinhas	Solo inculto e com mato		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 928.
2018/05/24	COI00079393	Prunella laciniata	(L.) L.	Beira Litoral	Figueira da Foz, Serra da Boa Viagem, perto da fábrica de cimento	Na berma da estrada. Solo inculto		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: s.n.
2017/10/04	COI00079392	Salvia verticillata	L.	Beira Litoral	Coimbra, Jardim Botânico, Escola N.3	Colhida no canteiro N.112 L.23	Cultivado	Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 904.
2017/10/04	COI00079391	Stachys germanica	L.	Beira Litoral	Estrada de Eiras para Vilarinho de Baixo	Solo inculto e com muita vegetação		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 917.
2017/10/04	COI00079390	Teucrium arduini	L.	Beira Litoral	Coimbra, Jardim Botânico, Escola N.3	Colhido no canteiro N.110 L.12	Cultivado	Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 921.
2018/05/24	COI00079389	Teucrium scordium ssp. scordioides	L.	Beira Litoral	Coimbra, Vil de Matos	Solo húmido e inculto		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 925.
2017/10/04	COI00079388	Teucrium spinosum	L.	Beira Litoral	Coimbra, Vilarinho de Baixo	Solo inculto com oliveiras		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: s.n.
2017/10/04	COI00079387	Salvia sclareoides	Brot.	Beira Litoral	Monte de Fonte Coberta	Solo inculto com pouca vegetação		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 900.
2017/10/04	COI00079386	Rosmarinus officinalis	L.	Beira Litoral	À saída de Fátima para Tomar, no monte	Solo inculto e com vegetação		Matos	Arménio	Matos, Arménio; Fernandes, Abílio; Paiva, Jorge; Ormonde, José & Coutinho, António	There is a label of "Hortus Botanicus Coimbra - Portugal"; Index seminum number: 893.

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