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PALYNOLOGICAL STUDY OF THE ENDEMIC WOODY *SONCHUS* FROM THE FLORA OF MADEIRA. A MORPHOLOGICAL AND MOLECULAR APPROACH

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SUMMARY: Palynological analysis of four endemic *Sonchus*, from the subgenus *Dendrosonchus* Webb ex Sch. Bip – *Sonchus ustulatus* Lowe subsp. *ustulatus* and *S. ustulatus* Lowe subsp. *maderensis* Aldr, *S. fruticosus* L .f. and *S. pinnatus* Ait. was performed including morphometric and molecular analysis of their pollen features. Sixteen morphometric parameters were used to determine their pollinic features. The chromatographic separation of pollen wall proteins was done. Results suggest that analysed taxa exhibit closely related features and form a monophyletic group. *S. ust.* subsp. *ustulatus* and *S. ust.* subsp. *maderensis* were previously described as closely related subspecies, with different affinity level to the rest of woody *Sonchus*. Detailed examination of *Sonchus* pollen morphological and molecular features confirms the large similarity between both subspecies. The palynological analysis shows that both groups of woody *Sonchus* – arboreous and semi-shrubby – present the same taxonomical distance to the other *Sonchus*. Based on 16 morphometric characters and 16 features of protein patterns, *Sonchus pinnatus* and *S. fruticosus* appears to have result from a common ancestral with the semi-shrubby woody *Sonchus*.

KEY WORDS: *Sonchus*, endemisms, pollen morphology, wall proteins.

RESÚMEN: Se lleva a cabo un análisis palinológico de 4 *Sonchus* endémico del subgénero *Dendrosonchus* Webb ex. Sch. Bip – *Sonchus ustulatus* Lowe subsp. *ustulatus*, *S. ustulatus* Lowe subsp. *maderensis* Aldr., *S. fruticosus* L. f. *S. pinnatus* Ait. incluyendo análisis morfométrico y molecular de su polen. Para determinar sus características polínicas se utilizaron trece parámetros morfométricos. También se realizó la separación cromatográfica de las proteínas de la pared del polen. Los resultados sugieren que los taxones analizados exhiben características muy próximas y forman un grupo monofilético. *S. ustulatus* subsp. *ustulatus* y *S. ustulatus* subsp. *maderensis* fueron descritos anteriormente como subespecies relacionadas entre si, con diferentes niveles de afinidad con relación a *Sonchus* arbóreos. Un examen detallado de sus características morfológicas y moleculares confirman una gran similitud entre las dos subespecies. El mismo análisis palinológico muestra que los dos grupos de *Sonchus* – el arbóreo y el semi-arbusto – presentan la misma distancia taxonómica con relación a *Sonchus oleraceus* L., *S. pinnatus* y *S. fruticosus* parecen tener un ancestor común con los *Sonchus* semi-arbustívos. Estas conclusiones, tienen sus bases en fenogramas que incluyen 16 patrones morfométricos y 16 de los patrones proteicos.

PALABRAS CLAVE: *Sonchus*, endemísmos, morfología polínica, proteinas de la pared.

INTRODUCTION

The flora of Macaronesia consists of a number of endemic species that reach beyond the vascular vegetation. At the Archipelago of Madeira, almost 30% of native species have an endemic status (VIEIRA, 1992). The greatest number of endemisms belongs to Asteraceae. Among them, *Sonchus* represents a particular case of evolutive radiation in Macaronesian Region, with 30 endemic taxa: 20 species, 6 subspecies, 7 varieties and 3 hybrids (HANSEN & SUNDING, 1993; SENGCHUL *et. al*, 1996). Four of the endemics belonging to *Sonchus* L., subgenus *Dendrosonchus* Webb ex Sch. Bip are found in Madeira Island. These are *Sonchus ustulatus* Lowe subsp. *ustulatus*, *S. ustulatus Lowe* subsp. *maderensis* Aldr., *S. fruticosus* L .fil. and *S. pinnatus* Ait. A detailed description of these taxa can be found in several works (PRESS, 1994; VIEIRA, 1992). *Sonchus fruticosus* and *S. pinnatus* are big shrubby or frequently small trees detected in the native rain forest Laurisilva, mainly in altitude vegetation ecosystems or cliffs under 1000 m. *Sonchus ust.* subsp. *ustulatus* and *S. ust.* subsp. *maderensis* are semi-srubby perennial plants with woody caulex, and are founded in xerophyte areas of the Madeiran coast. They represent woody *Sonchus*, which origin and evolution remains a matter of scientific interest. The palynological study of the endemic *Sonchus* improves our knowledge about their biology and contributes to the understanding of its evolution. Several works concerning the origin and evolution of woody *Sonchus* (SENG-CHUL *et. al.*, 1996) or Lactucoideae pollen descriptions (BOLICK, 1991; BLACKMORE, 1982a; BLACKMORE, 1982b) have been addressed. Pollen morphometry and wall protein patterns are characters of taxonomic importance specially regarding the Lactucoideae organisation (BOLIK, 1991; BLACKMORE, 1982c; SKVARLA *et. al.*, 1977; SAAD, 1961). SAAD (1961) conducted as study concerning *Sonchus* pollen morphology and its evolutive trends. Pollen morphology of several Canarian woody *Sonchus* was also analysed (SAAD, 1961). According our best knowledge, early studies of palynology of the Madeiran plants have not been addressed yet. This study aims to contribute to the characterisation of woody *Sonchus* from the archipelago of Madeira, at the morphological and protein patterns levels.

Taxa	P_o	NL	nLP	sPL	P	E	P/E	Shape	exPT	exER	V_1	AE	SPR
S. fruticosus	3	21	3	10.9 × 4.6	39.0±1.3	38.4±1.3	1.01	Prolate-spherical	8.8±0.6	8.3±0.7	3.3	2.4	5.5
S. pinnatus	3	21	3	9.7×4.1	36.6±1.0	36.2±1.0	1.01	Prolate-spherical	8.0±0.7	7.8±0.7	2.9	2.6	5.6
S ust. maderensis	3	21	3	8.2 × 3.3	42.1±1.1	41.6±1.1	1.01	Prolate-spherical	8.9±0.6	9.1±0.8	3.6	2.6	5.3
S. ust .ustulatus	3	21	3	8.1 × 2.9	40.9±1.1	39.9±0.8	1.02	Prolate-spherical	8.6±0.6	8.4±0.6	3.0	2.3	5.7

TABLE 1. Palynological study of the madeiran endemic woody *Sonchus*. The table shows the mean values and standard deviations obtained from the morphometric analysis of 4 woody Sonchus. The abbreviations means: Po, number of pores; NL, number of lacunae; nLP, number of lacunae per pole; sPL, sizes of polar lacunae; P and E, polar and equatorial axis size; exPT and exER, exine tickness in polar and equatorial outline; V1, distance between spines apices; SPR, number of spines in the paraporal lacuna ridge.

MATERIAL AND METHODS

Pollen wall morphology and protein patterns of the woody *Sonchus* were analysed from field and herbarium material. The herbarium material was provided by MADJ collection (voucher exemplars numbers – R.2506, R.3829, R.3843, R.3846). Pollen samples used in biochemical studies were freshly collected shortly before anthesis.

MORPHOLOGICAL POLLEN ANALYSIS

The pollen was acetolysed, according to Erdtman's modified technique (1960). Acetolysed pollen was mounted in glycerine gelatine (REISTSMA, 1969) and the glass slides deposited in the University Palinotheca. Ten characters were measured – number of pores (P_0), polar (P) equatorial (E) axis, P/E ratio, pollen shape, exine thickness in equatorial outline (exER) and polar thickening (exPT), number of spines in the paraporal lacuna ridge (SPR), distance between spines apices (V_1 and V_2), spines lengths (AE), tectum height (AT), number of lacunae (NL), lacunae per pole (nLP) and size of polar lacunae (sPL). At least 20 pollen grains from each sample were measured. Micrographs have been taken by optical (ME) and scanning electron microscopy (SEM). For scanning electron microscopy (SEM) acetolysed pollen grains were coated with gold. The pollen was examined and photographed in a JEOL, model JSM-5400. Throughout the entire paper terminology proposed by ERDTMAN (1966) and PUNT *et. al.* (1994) has been used.

ANALYSIS OF POLLEN WALL PROTEIN PATTERNS

The sample treatment consisted of removing the anther from the capitulum. Fresh pollen was previously cleaned from anther material and quickly pre-washed through a metallic filter with 125 µm pores. 1.5 g of fresh pollen was suspended in 7.5 ml of extraction buffer I (50 mM Tris, 10 mM NaCl, 0,3 mg/ml EDTA, 0,2 mg/ml phenylmethylsulfonyl fluoride, 1 % toluene; the pH adjusted to 7.5 with HCl). Suspension was slurry shaken for 48 hours in a Heidolph Duomax 2030, at 25ºC. Final suspension was centrifuged, during 10 min at 5000 g in a Hettich EBA 85 centrifuge. The pollen sediment was suspended in buffer I (1:2 w/v) and centrifuged once again for 10 min., at 5000 g. The final supernatant was concentrated in a Seep Vac Plus SC 110A, SAVANT to a final volume of 1.5 ml. Pollen sediment after the first extraction was dried and submitted to a second round of protein

Taxa	Total fraction (mg/g of pollen)	Soluble fraction (mg/g of pollen)	Yield, %	Insoluble fraction (mg/g of pollen)	Yield, %
S. fruticosus	19.4 ± 1.8	13.5 ± 3.5	69.6	5.9 ± 3.1	30.4
S. pinnatus	6.4 ± 1.0	4.6 ± 1.9	71.9	1.8 ± 0.2	28.1
S. ustulatus maderensis	4.5 ± 0.2	2.5 ± 0.2	55.6	2.0 ± 0.5	44.4
S. ust. ustulatus	5.0 ± 0.4	3.1 ± 0.5	62.0	1.9 ± 0.3	38.0

TABLE 2. Palynological study of the madeiran endemic woody *Sonchus*. The table shows the mean values obtained for the extraction and analysis of pollen wall protein fraction. The composition of pollen wall protein fraction - total, soluble and insoluble fractions in mg of protein per gram of fresh pollen is shown.

extraction with a buffer II (50 mM tris, 10 mM NaCl, 0.01% Triton X100, the pH adjusted to 7.5 with HCl). The protein fraction was measured according to LOWRY & ROSEBOURG (1959), using bovine serum albumin (BSA) as a standard.

CHROMATOGRAPHIC ANALYSIS OF PROTEIN EXTRACTS

Five hundred µl of sample was applied into a Superdex HR-75 column K16 (16x40 cm; GradiFrac System, Pharmacia) equilibrated with elution buffer (50 mM Tris, 150 mM NaCl, pH adjusted to 7.5 with HCl). Proteins were separated at a flow rate of 1.5 ml/min and detected by UV-detector (Pharmacia LKB) at 280 nm. Each fraction thus obtained was concentrated to a 1.0 ml final volume and stored at - 20°C. The sizes of native pollen wall proteins were determined through chromatographic separation. The column calibration run was carried out with low molecular weight protein mixture (LMW kit, Pharmacia) as standards. The column void volume was determined with Blue Dextran (2000 kDa) and acetone.

STATISTICS AND DATA ANALYSIS

The data analysis included the statistic analysis of a minimum display, median values calculation and multivariate analysis. Data in the extraction and chromatographic separations represent the mean of three experiences and are reproducible to within ±10% standard error. Morphometric and molecular data were analysed using the grouping NJOIN – WARN method, with the application of the euclidean distances as coefficient. The NTSYSpc 2.0 program was used.

Taxa	Peaks number	Native protein molecular weight, kDa	Nº subunits	Subunits molecular weight, kDa
S. fruticosus	1	95.5 ± 1.5	1	97.0
	2	74.5 ± 3.0	-	58.1 ± 2.1
	3	22.4 ± 1.0	2	34.7 ± 1.7
	4	18.2 ± 1.2	2	12.0 ± 1.0
S. pinnatus	1	107.2 ± 3.5	1	95.0
	2	87.1 ± 2.0	6	14.5 ± 2.0
	3	67.6 ± 1.6	4	
	4	23.4 ± 1.0	2	
	5	18.2 ± 0.5		
S. ust. maderensis	1	89.1 ± 2.0	1	94.0
	2	42.7 ± 1.0	4	14.0 ± 1.7
	3	26.0 ± 0.5	2	
S. ust. ustulatus	1	81.3 ± 2.1	8	14.0 ± 1.9
	2	22.9 ± 1.8	2	

TABLE 3. Palynological study of the madeiran endemic woody *Sonchus*. The table presents the data obtained by the chromatographic analysis of *Sonchus* pollen wall soluble protein fraction, using the exclusion chromatography in a Superdex HR- 75 column K16 (16x40 cm). The molecular weights for the proteins of soluble fractions of woody *Sonchus* were obtained through a calibration curve by comparison of molecular mass of markers from LMW kit (Pharmacia).

RESULTS

POLLEN MORPHOLOGY

Pollen of the four woody *Sonchus* exhibit similar morphological features (Tab. 1). These features are specific when compared with remaining Lactucoideae endemics or other non-endemic *Sonchus* from the Madeiran flora (unpublished data). As a result, a general description of the pollen of woody *Sonchus* from the Archipelago of Madeira can be established. The woody *Sonchus* pollen is echinolophate, median size (P variation between 36.3 – 42.2 µm; E variation 36.8 – 41.3 µm, see table 1), prolate-spheroidal shape, subcircular in equatorial view, tricolporate. Lacunae system is compounded by 21 lacunae: 3 poral, 6 abporal, 6 paraporal and 6 polar. Poral lacunae without complete equatorial ridges, is connected with the abporal ones by small gaps. The ridges and polar thickenings are topped with sharp unperforated conical spines. Polar thickening with triangular shape and broken by 3 half-circular polar lacunae. Shape and sizes of polar lacunae vary among the woody *Sonchus* taxa (between 10.9 x 4.6 µm of *S. fruticosus* and 8.1 x 2.1 µm of *S. ust.* subsp. *ustulatus*). The polar lacunae are clearly delimited in *S. fruticosus* and *S. pinnatus* pollen and of difficult delimitation in pollen

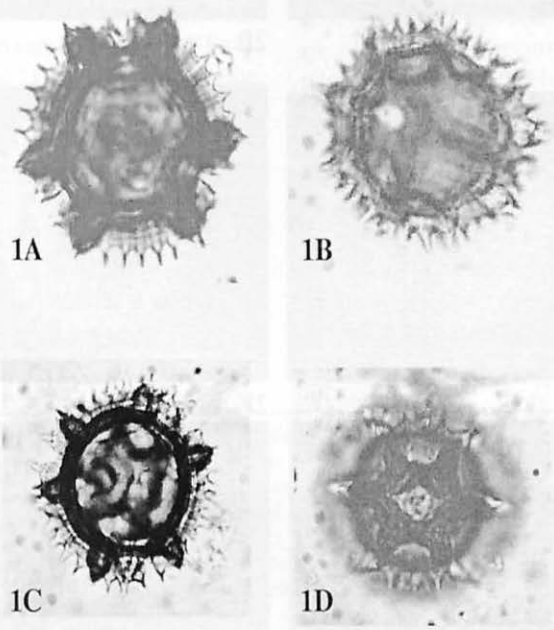
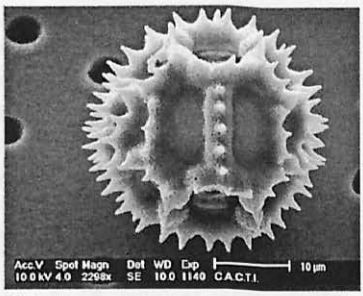
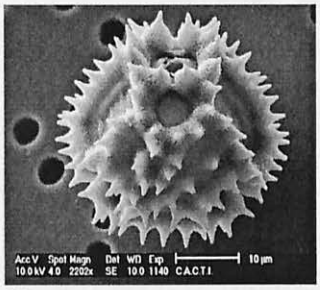


FIGURE 1. Palynological study of the madeiran endemic woody *Sonchus*. Plate shows different aspects of woody *Sonchus* pollen morphology. A. *Sonchus fruticosus* L. fil.; B. *Sonchus pinnatus* Ait; C. *Sonchus ustulatus* Lowe subsp. *maderensis* Aldr.; D. *Sonchus ustulatus* Lowe subsp. *ustulatus*;. MO (x1000). Scale: A-D= 10 µ m.



2A



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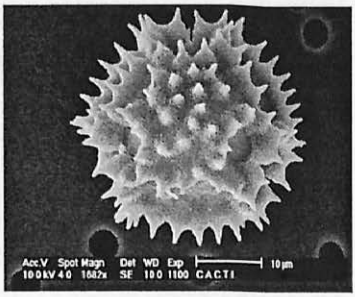

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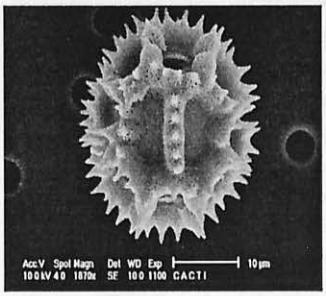

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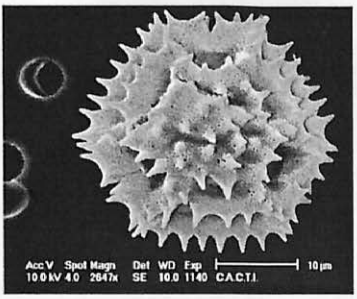

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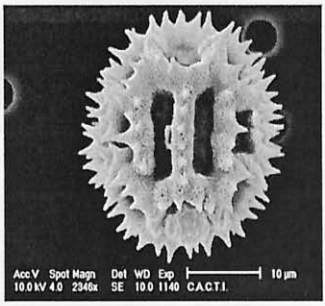

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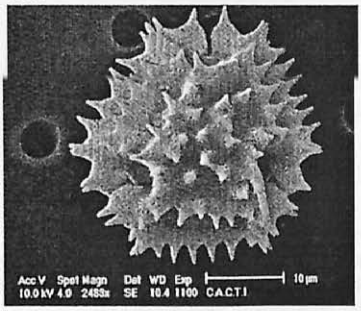
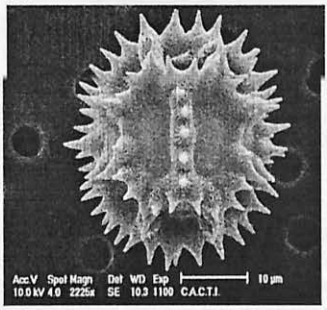


2G



2H

from the remaining taxa. Tectate-perforate exine, with a sexine layer regularly micro-perforated in the ridges and lacuna surfaces. Ectosexine is severe depressed at the lacunae, namely poral and abporal. Different aspects of the pollen morphology of woody *Sonchus* are shown in the plates of figures 1 and 2.

POLLEN WALL PROTEIN PATTERNS

Pollen extractions allowed the determination of the protein fraction from pollen wall of each taxa. The values for soluble and insoluble protein fractions are summarised in Table 2. The data shows that the arboreous woody *Sonchus* – *S. fruticosus* and *S. pinnatus* – contain more protein in the pollen wall when compared with semi-shrubby taxa, 19.4 and 6.4 mg/g of fresh pollen, respectively. Soluble protein fractions accounted for major portion of total protein in the wall. The values of soluble protein vary between 55.6% for *S. ust. ustulatus* and 71.9% of total protein for *S. pinnatus*. The chromatographic separation of pollen extract shows the presence of specific protein patterns for soluble and insoluble fractions of each taxa. The chromatographic profiles obtained for soluble fraction of pollen extracts are shown in Figure 3. A specific number of protein peaks were detected in these chromatograms profiles, 5 peaks for *Sonchus pinnatus*; 4 peaks for *S. fruticosus*; 3 for *S. ust.* subsp. *maderensis*; 2 for *S. ust.* subsp. *ustulatus* (Tab. 3). A major protein peak was found in each pollen extract, with apparent molecular mass of 26.0 ± 0.5 for *S. ust.* subsp. *maderensis*, 81.3 ± 1.8 for *S. ust.* subsp. *ustulatus*, 18.2 ± 1.2 for *S. fruticosus* and 87.1 ± 2.0 kDa for *S. pinnatus*. The number of peaks and protein molecular weights are specific for each woody *Sonchus*. Both arboreous *Sonchus* shows an higher molecular polymorphism of the pollen wall protein fraction, when compared with srubby taxa. The molecular structure of these proteins was studied through separation under dissociating conditions in presence of 10% SDS. A common feature for all *Sonchus* soluble fractions is the presence of proteins which consist of low molecular subunits, with weights between 17.0 and 12.0 kDa. We presume that *Sonchus* wall proteins are oligomers compounded by several subunits or polypeptide chains (Tab. 3). The structure of proteins from *S. fruticosus* pollen wall seems to be more complex. The separation of *S. fruticosus* fraction under dissociating conditions shows the presence of chromatophors, with 58.1 ± 2.1 and 34.7 ± 1.7 kDa. Besides that, a high molecular glycoprotein, with an apparent molecular weight between 97.0 and 94.0 kDa is detected in soluble fractions of

FIGURE 2. Palynological study of the madeiran endemic woody *Sonchus*. Plate shows different aspects of the arboreous *Sonchus* pollen morphology obtained by SEM. (2A-B), *Sonchus fruticosus* L. fil. A, Polar view with an aspect of polar thickening and lacunae. Poral and abporal lacunae are also seen. B, Equatorial views with an aspect of lacunae system (paraporal lacunae) and equatorial ridge with spines. (2C-D), *Sonchus pinnatus* Ait C, Polar views with an aspect of polar thickening and lacunae. Poral and abporal lacunae are also seen. D, Equatorial views with different aspects of lacunae system (paraporal lacunae) and equatorial ridge with spines. (2E-F), *Sonchus ustulatus* Lowe subsp. *ustulatus*. E, Polar views with an aspect of polar thickening and lacunae or poral and abporal lacunae. Spine and tectate-perforate ornamentation of exine is clearly seen. F, Equatorial views with an aspect of lacunae system (paraporal lacunae) and equatorial ridge with spines. (2G-H), *Sonchus ustulatus* Lowe subsp. *maderensis* Aldr. G, Polar views with an aspect of polar thickening and lacunae. H. Equatorial views with an aspect of lacunae system (paraporal lacunae) and equatorial ridge with spines.G, Polar view with an aspect of polar thickening and lacunae. H, Equatorial views with an aspect of lacunae system and equatorial ridge with spines.

all *Sonchus*. The results of chromatographic separation under dissociating conditions were confirmed by in SDS-page electrophoresis (unpublished data).

DISCUSSION

The importance of pollen morphology in the determination of *Sonchus* species relationship and probable genus origin has been discussed early by SAAD (1961). However, attempts to evaluate the importance of molecular markers associated with pollen wall are fairy recent. The data concerning the endemic *Sonchus* palynology may play an important role in the increase of our knowledge concerning its biology. The woody *Sonchus* represent a specific evolutionary event confined to Canaries and Madeira Islands. They are considered to be a linkage between African *Origosonchus* and true *Sonchus*. However, the hypothesis of the *Dendrosonchus* origin from the true *Sonchus* as an unique adaptive radiation event has been recently proposed (SENG-CHUL *et. al*, 1996). Pollen morphology of woody *Sonchus* is related with the Leontodon type (PAUSINGER, 1951) or Sonchus oleraceous type (BLACKMORE, 1982c). Despite that the polar thickening structure and the presence of polar lacunae allows us to classify the *Sonchus* in four subtypes (SAAD, 1961). Whereas primitive *S. afromontanus* subtype appears to be related with *Origosonchus*, *Launaea* or *Crepis*, S. javanicus subtype is related with true *Sonchus* or *Lactuca*. The polar tickening of

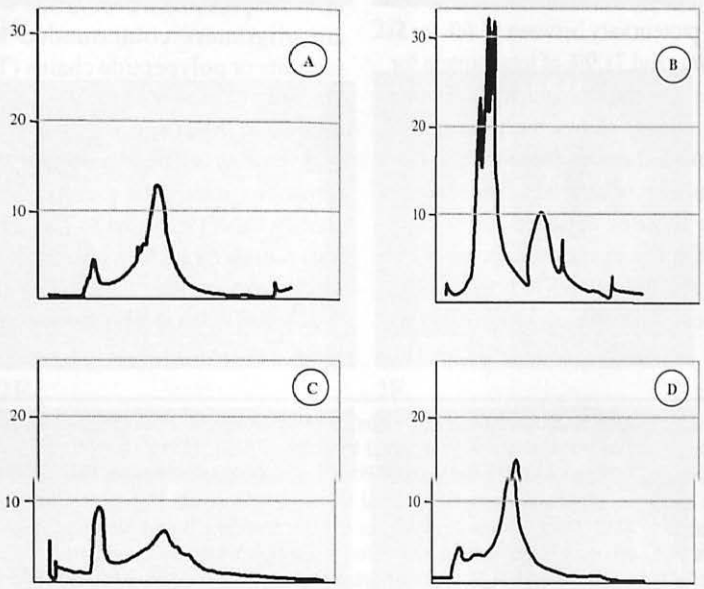


FIGURE 3. Palynological study of the madeiran endemic woody *Sonchus*. The plate shows the chromatograms obtained by the analysis of pollen wall soluble fractions, using the exclusion chromatography in a Superdex HR- 75 column K16 (16x40 cm). A, *Sonchus pinnatus* Ait. B, *Sonchus fruticosus* L.fil. C, *Sonchus ustulatus* Lowe subsp. *ustulatus* D, *Sonchus ustulatus* Lowe subsp. *maderensis* Aldr.

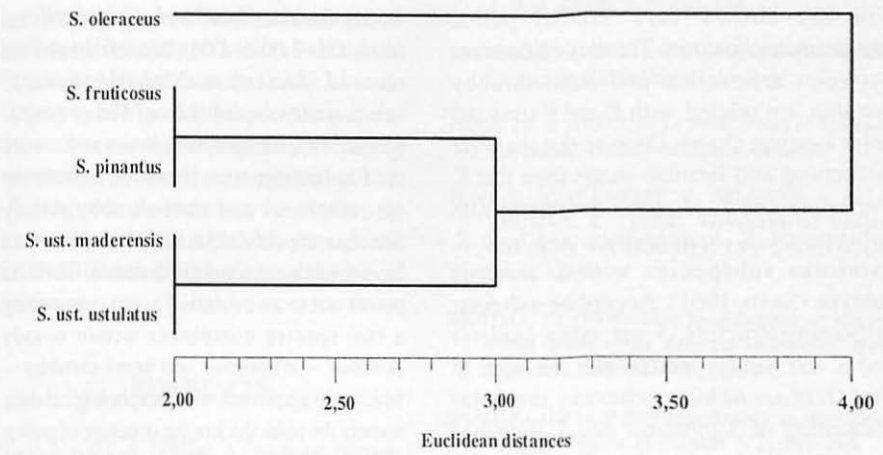


FIGURE 4. Palynological study of the madeiran endemic woody *Sonchus*. Figure shows the phenogram obtained by the analysis 16 morphometric parameters of woody *Sonchus* pollen, analysed through the grouping NJOIN - WARN method, with the application of the euclidean distances as coefficient. The axle scales are given the mean taxonomic distance between the taxa.

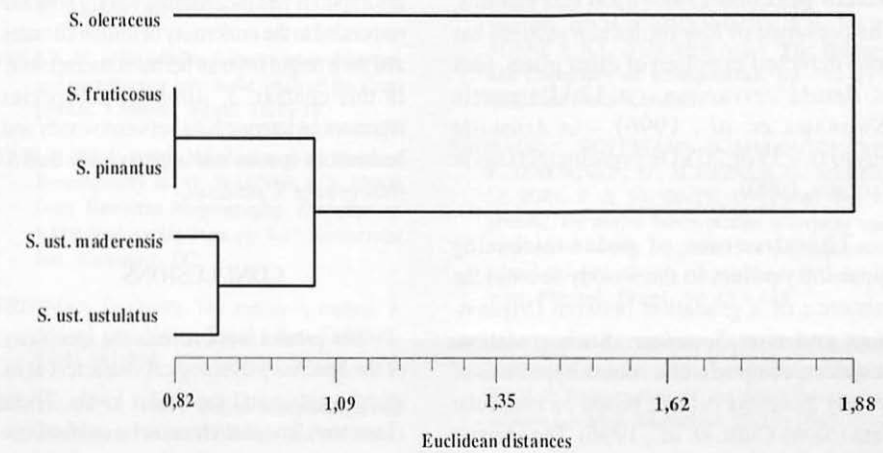


FIGURE 5. Palynological study of the madeiran endemic woody *Sonchus*. Figure shows the phenogram obtained by the analysis 16 morphometric and 16 molecular parameters of woody *Sonchus* pollen, analysed through the grouping NJOIN - WARN method, with the application of the euclidean distances as coefficient. The axle scales are given the mean taxonomic distance between the taxa.

woody *Sonchus* occupies an intermediary position between them. Madeira woody *Sonchus* shows very similar pollen morphometric features. The major differences between arboreous and semi-shrubby *Sonchus* are related with P and E sizes and polar lacunae shape. Despite that the polar thickening and lacunae shape show that *S. fruticosus* and *S. pinnatus* are related with subtype of *S. brachyotus*, and both *S. ustulatus* subspecies with S. arvensis subtype (SAAD, 1961). According with polar thickening structure, *S. ust.* subsp. *ustulatus* and *S. ust.* subsp. *maderensis* are closer to the *Origosonchus*, whereas the polar thickening of *S pinnatus* and *S. fruticosus* are near to the true *Sonchus*. The differences between arboreous and semi-shrubby species are confirmed by pollen wall protein polymorphism. A specific molecular pattern for each of those woody *Sonchus* was detected. Additionally, several common features in protein fractions were also detected, including the presence of similar protein peaks of 17.0 – 12.0 kDa subunits. The presence of low molecular subunits has been detected in pollen of other plants, such us *Betula verrucosa* – a 17 kDa protein (SWOBADA *et. al.*, 1996) – or *Artemisia vulgaris* – 35 or 20 kDa proteins (NIELSEN & PAULSEN, 1990).

The structure of polar thickening apparently points to the woody *Sonchus* the existence of a gradation between *Origosonchus* and true *Sonchus*. Such gradation, however, contradicts a recent hypothesis of woody *Sonchus* origin, based on molecular data (SENG-CHUL *et. al.*, 1996). The analysis of morphometric and molecular data through the NJOIN – WARM method shows that the woody *Sonchus* is a monotypic group, whereas all remain taxa result from an unique event of adaptive evolution (Fig 4). Both phenograms, morphological and molecular (unpublished) show an early divergence of woody *Sonchus* from a common ancestral (distance - 3.00 or 4.03). Similar results are obtained when endemic *Crepis vesicaria* L. subsp. *andryaloides* (Lowe) Babc. (unpublished data) or *Sonchus oleraceus* L. were used as outsider taxa. However, in both cases, arboreous and semi-shrubby woody *Sonchus* appears separated, but their taxa have the same taxonomic distance. Our data points out to an evolutive event originating a two species complexes within woody *Sonchus* – arboreous and semi-shrubby – which is in agreement with morphological data, namely the polar thickening structure of pollen and its evolutive significance (SAAD, 1961). According with analysis of morphometric and molecular data (Fig. 5), the semi-shrubby has an older evolutive age then arboreus woody *Sonchus*, supporting the hypothesis of a radial evolution based on a unique event of island colonisation (SENG-CHUL *et. al.*, 1996). This phenogram confirms CARLQUIST (1995) assumptions that the endemic woody *Sonchus* responded to the uniformity of insular climates and their origin from an herbaceous ancestral. In this context, *S. ustulatus* subspecies represent an intermediate between woody and herbaceous species and appears early then *S. fruticosus* or *S. pinnatus*.

CONCLUSIONS

The present work reveals the specificity of the *Sonchus* palynological characters at its morphometric and molecular levels. These characters allow us to characterise and facilitate the specific distinction of woody *Sonchus*. Some evidences suggest that morphological and molecular pollen characters are a helpfull tools in the study of *Sonchus* phylogeny and island evolutive biology.

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REFERENCES

BLACKMORE, S. (1982a). A Functional Interpretation of *Lactuceae* (*Compositae*) Pollen. **Plant Syst. Evol.** 141(2):153-168.

BLACKMORE, S. (1982b). Palynology of Subtribe *Scorzonerinae* (*Compositae*: *Lactuceae*) and its Taxonomic Significance. **Grana** 21:149-160.

BLACKMORE, S. (1982c). *Compositae*–Lactuceae. In: W. PUNT & G.C.S CLARKE (eds).**The Northwest European Pollen Flora. 4.** pp.45-85. Elsevier, Amsterdam.

BOLICK, M. (1991). Pollen diameter, exine thickness, and ultrastructure type in the tribes of the Compositae. **Compos. Newsl.** 19:17-19.

CARLQUIST, S. (1995). Introduction to the Hawaiian Biogeography In: W. WAGNER & V. FUNK (eds). **Hawaiian Biogeography: Evolution on a Hot Spot Archipelago.** pp. 1-13. Smithsonian Inst. Washington, DC.

ERDTMAN, G. (1960). The acetolysis method. A revised description. **Svenks Bot. Tidskrift.** 54(4):561-564.

ERDTMAN, G. (1966). **Pollen Morphology and Taxonomy.** Hafner Publising Co. New York.

HANSEN, A. & SUNDING, P. (1993). **Flora of Macaronesia. Check-list of Vascular Plants.** Sommerfeltia. Oslo.

LOWRY, O.H. & ROSEBOURG, N.J. (1959). Protein measureament with the folin phenol Reagent. **J. Biol. Chem.** 139:265-275.

PAUSINGER, F. (1951). Die pollengestalitung der Cichorieae.**Carinthia II** 19:3-47.

PRESS. J.R. & SHORT, M.J. (1994). Flora of Madeira. The Natural History Museum. London.

PUNT, W.; BLACKMORE, S.; NILSSON, A. & LE THOMAS, A. (1994). **Glossary of Pollen and Spore Terminology.** LPP Contribution series. Utrecht.

REISTMA, T.J. (1969). Size Modification of Recent Pollen Grains under different treatments. **Rev. Paleobot. Palynol.** 9:175-202.

SAAD, S.I. (1961). Pollen Morphology in the Genus *Sonchus*. **Pollen et Spores** 3(2):247-260.

SENG-CHUL, K.; CRAWFORD, D.J.; FRANCISCO-ORTEGA, J. & SANTO-GUERRA, A. (1996). A common origin for woody *Sonchus* and five related genera in the macaronesian islands: Molecular evidence for extensive radiation. **Proc. Natl. Acad. Sci. USA** 93:7743-7748.

SKVARLA, J.J; TURNER, B.L.; PATEL, V.C. & TOMB, A.S. (1977). Pollen Morphology in the *Compositae* and in Morphologically Related Families. In: H.V. HEYWOOD, S.B. HARBORNE & B.L. TURNER (eds). **The Biology and Chemistry of Compositae.** pp. 141-217. Academic Press. London.

SWOBADA, I.; HOFFMANN-SOMMERGRUBER, K.; O´RÍORDÁIN, G.; SCHEINER, O.; HEBER-LE-BORS, E. & VICENTE, O. (1996). Bet v1 proteins, the major birch pollen allergens and members of family of conserved pathogenesis-related proteins, shows ribonuclease activity in vitro. **Physiol. Plant.** 96:433-438.

NIELSEN, B.M. & PAULSEN, B.S. (1990). Isolation and characterization of a glycoprotein allergen, Art V II, from pollen of mugwort (*Artemisia vulgaris* L.). **Mol. Immunol.** 27(10):1047-1056.

VIEIRA, R. (1992). **Flora da Madeira. O Interesse das Plantas Endémicas.** Serviço Nacional de Parques. Reservas e Conservação da Natureza. Lisboa.