



Phylogenetic and morphological analysis of a new cliff-dwelling species reveals a remnant ancestral diversity and evolutionary parallelism in *Sonchus* (Asteraceae)

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Abstract

We describe a new cliff-dwelling species within *Sonchus* (Asteraceae): *Sonchus boulosii* and analyze its systematic position and evolutionary significance; in addition, we provide a key to the species of *Sonchus* in Morocco. Both morphological and ecological characteristics suggest a close relationship of *S. boulosii* with taxa of section *Pustulati*. However, ITS nrDNA and cpDNA *matK* markers indicate its uncertain position within the genus, but clear genetic differentiation from the remaining major clades. ITS phylogenetic trees show that likely evolutionary shifts to rocky habitat took place at least five times within genus *Sonchus* and that sect. *Pustulati* and *S. boulosii* clades have a clearly independent evolutionary origin. We postulate that the strong resemblance of *S. boulosii* to other rocky species reflects a phenomenon of homoplasy, probably driven by parallel evolutionary adaptations to the severe ecological constraints of its cliff face habitat. Therefore, a new section is also described, which includes *S. boulosii* as its sole representative: section *Pulvinati*. According to phylogenetic trees, the new clade may share its common ancestor with the clade comprising sections *Maritimi* and *Arvenses*, from which it is widely divergent in morphology and ecology, with the exception of *Sonchus novae-zelandiae*. However, the latter is a derived taxon, with high level of polyploidy unlike *S. boulosii* that shows $2n = 18$, basal chromosome number of the genus. Since sections *Pulvinati* and *Pustulati* seem to be quite old in *Sonchus*, we also hypothesize that some similarities, such as fruit morphology, may reflect the persistence of some primitive characteristics.

Keywords Homoplasy · Karyogram · *matK* cpDNA · nrDNA ITS · Parallel evolution · Western Mediterranean

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Introduction

Predictions of biodiversity loss associated with the looming sixth mass extinction crisis (van Vuuren et al. 2006; Barnosky et al. 2011; Regnier et al. 2015) have placed an increased urgency on taxonomic efforts regarding the discovery and description of new species. Achieving an accelerated rate of taxonomic discovery requires significant funding support (Valdecasas and Camacho 2003; Mora et al. 2011), and also the development of novel and effective taxonomic strategies (Wägele et al. 2011; Wege et al. 2015), especially within poorly studied areas. In this respect, the initiative of the Emirates Center for Wildlife Propagation (ECWP) of promoting botanical field surveys in regions that have been highlighted as being under-explored (Dobignard 2002), such as Eastern and Saharan Morocco, as a part of a research program on natural habitats should be welcomed. To date, this research program has yielded its most significant results

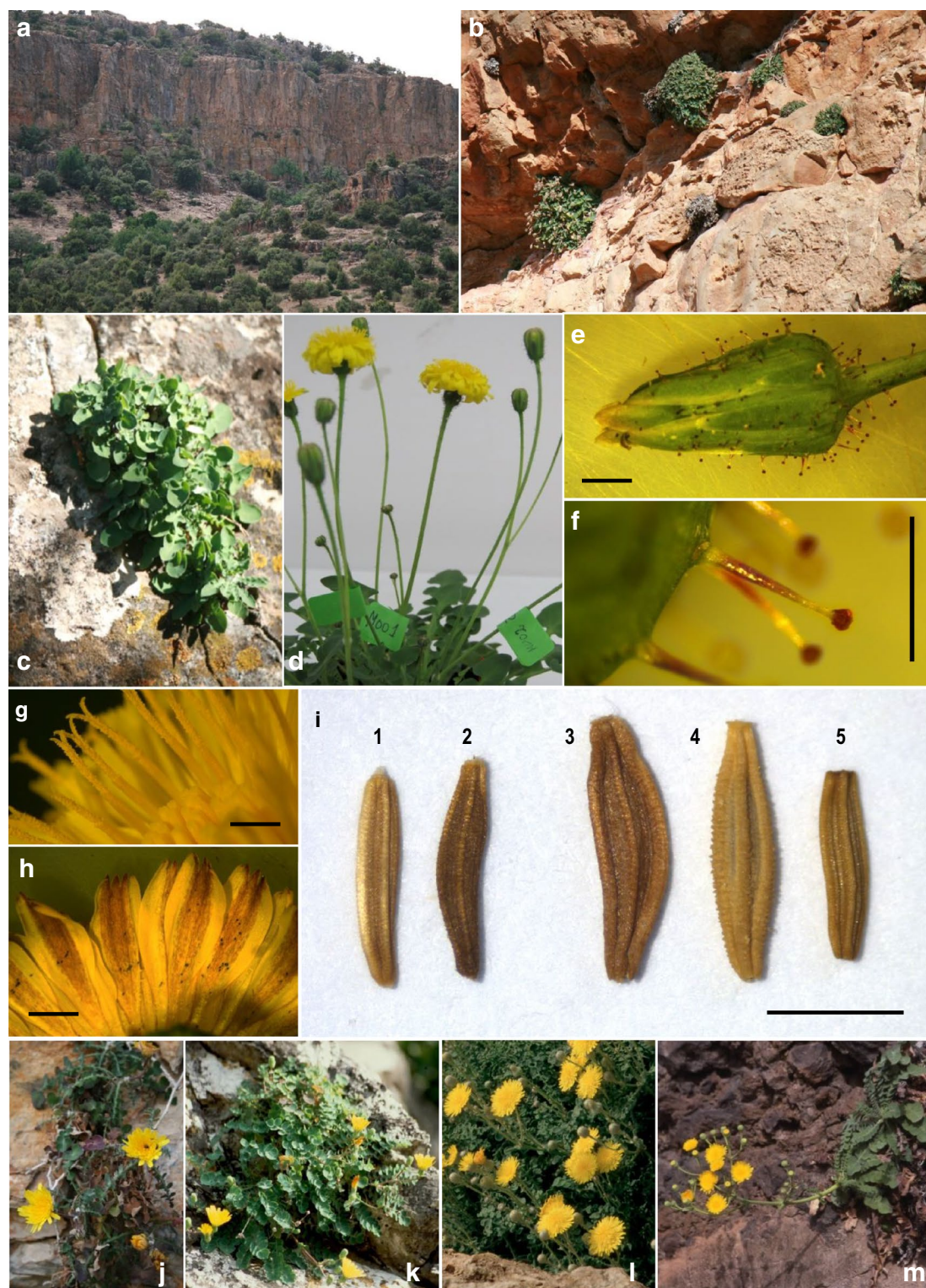


Fig. 1 Ecology and morphology of *Sonchus boulosii* and other cliff-dwelling *Sonchus* species: **a** cliffs in the Debdou Mountains which sustain one of the known populations of this new species; **b** detail of natural habitats of the population in the Debdou Mountains; **c** vegetative plant within its natural habitat; **d** plant from Jbel Tsiouant (Middle Atlas) cultivated in a greenhouse; **e** capitulum of a plant from the Debdou Mountains during the maturation of achenes; **f** detail of

a glandular hair; **g** anthers and style during anthesis; **h** external florets with a purple band on the back; **i** achenes of *S. boulosii* (1, Jbel Tsiouant, SEV 286736; 2, Debdou Mts., SEV 286734), *S. pustulatus* (3, SEV 120991), *S. fragilis* (4, SEV 285880) and *S. masquindalii* (5, SEV 285890); **j–m** appearance of several cliff-dwelling *Sonchus* species: *S. pustulatus* (**j**), *S. fragilis* (**k**), *S. masquindalii* (**l**) and *S. radicans* (**m**). Scale bars: 2 mm (**1e, g–i**), 0.5 mm (**1f**)

from the Lamrija plain and the Debdou mountains (Eastern Morocco) with the discovery of three new plant taxa (Chambouleyron et al. 2015a) here. Two of these have been described previously (Chambouleyron et al. 2014, 2015b), with the third, a species of the genus *Sonchus* L., being presented here.

The new species was initially detected in the Debdou Mountains, with its presence also being confirmed later in the Middle Atlas and the Eastern High Atlas. It bears clear morphological similarities to the components of *Sonchus* sect. *Pustulati* Boulos (Fig. 1), three endemic species from the Baetic–Rifan–Tellian region in the western Mediterranean (Boulos 1973; Silva et al. 2015a) that constitute a well-defined clade within *Sonchus* (Kim et al. 2007). Similarly to these, it possesses a typical rupicolous ecology, colonizing rock crevices in (mainly north facing) calcareous cliffs (Fig. 1a–c). Therefore, it could be plausibly proposed that the new species should be placed within this clade. However, the incorporation of a new component with a distribution extended to the Atlas System and the mountain massifs of the Oran Meseta would force a reinterpretation of the biogeography of sect. *Pustulati*, whose disjunctive distribution is thought to be the result of a tight biogeographical association with the geologic history of the Baetic–Rif–Tell or Baetic–Maghrebides orogen (Rosenbaum et al. 2002; Chalouan et al. 2008; Silva et al. 2015a). This geological terrain includes most Andalusia (Spain) in the northern side and the Rif Mountains (Morocco) and the Tell Coastal Ranges (Algeria) in the southern side, two regions at present separated by the Mediterranean Sea but with a common geologic origin and high floristic affinities (Valdés 1991; Médail and Quézel 1997). They seem to be originated on the European plate from terrains attached to the Iberian Peninsula and southern France in the Oligocene that migrated and rotated across the western Mediterranean Sea during Alpine orogeny (Rosenbaum et al. 2002; Chalouan et al. 2008). The Atlas Mountains, meanwhile, are an intracontinental, autochthonous system which developed over the crust of the African plate during the same period of Alpine orogenesis (Frizon de Lamotte et al. 2008), and the Eastern Morocco mountains system, which is part of the Oran Meseta, comprises Paleozoic massifs of the cited African plate, located to the south of the Rif–Tell system (Michard et al. 2008). It should be considered whether the morphological resemblance of the new species to the representatives of sect. *Pustulati* reflects a pattern of niche conservatism among phylogenetically close related taxa (Wiens and Graham 2005; Crisp and Cook 2012; Pyron et al. 2015), or a case of homoplasy depending on an evolutionary process of parallelism or convergence by ecological coincidence (Pearce 2012; but see Arendt and Reznick 2008).

In view of these considerations, the main goals of this paper are: (1) to formally describe this new species; (2) to

analyze its phylogenetic relations and discuss its systematic position; (3) to address the significance of the species within the evolutionary history of the genus; and (4) assess the conservation status of the new species according to its rarity. Additionally, we also provide a key to the *Sonchus* species of Morocco.

Materials and methods

Morphological study

Our morphological study was based on measurements taken from 54 dried specimens of *Sonchus boulosii* Chamboul., Mejías & J.F.Léger collected in the field during seven fieldwork visits to the four currently known populations, respectively located within the Oran Meseta mountains (Debdou Mountains), Middle Atlas (Jbel Tsiouant) and Eastern High Atlas (Bourdim and Chabkat Bou Ghrara) (Online Resource 1). The specimens are kept in the herbariums ECWP, G, P, RAB, SEV. For measurements of macro-characters an electronic digital Mitutoyo caliper C107127 was used. Measurements of micro-characters were completed using a stereoscopic microscope Leica S6D. Pictures of micro-characters were taken using a technical Leica MCD170 HD Photomicroscope.

Molecular sampling, DNA extraction, PCR amplification, sequencing and alignment

Fresh leaf tissue samples were collected in the wild and dried in silica gel, with four individuals being taken from each of the populations of the Debdou Mountains and Jbel Tsiouant in July 2014, four individuals from the population of Bourdim in July 2017 and two individuals from Chabkat Bou Ghrara population in May 2017. To avoid clonal repetition, plants from which material was collected were distanced at least 5 m from each other. Total DNA was isolated from silica-gel-dried leaf tissue using DNeasy plant mini kits (QIAGEN, Carlsbad, California, USA). PCR amplification of ITS of nrDNA and *matK* gene was the same as described previously (Lee et al. 2005; Kim et al. 2007). PCR products were purified with the QiaQuick PCR Purification kit (QIAGEN). DNA was sequenced at Geno Tech. Corp. (Daejeon, Korea) using the same sequencing primers that we used in the previous study (Lee et al. 2005). Base calling and sequence editing were performed with Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). All the DNA sequences of *Sonchus boulosii* obtained were deposited in GenBank with the following accessions numbers: MH203754 - MH203767 for nrDNA ITS sequences and MH203768 - MH203781 for cpDNA *matK* sequences. Previously published sequences were deposited in GenBank as indicated in Lee et al. (2005)

and Kim et al. (2007). Newly obtained ITS and partial *matK* sequences (5' region 1108 bp) were combined and aligned manually to the previously existing Sonchinae data matrices (Lee et al. 2005; Kim et al. 2007) (Online Resources 2 and 3). Pairwise sequence divergence was calculated using the Kimura 2-parameter method (Kimura 1980) using PAUP* version 4.0a150 (Swofford 2002).

Phylogenetic analysis

For the ITS data set, a total of 126 accessions (including 14 newly sequenced *Sonchus boulosii* accessions) were analyzed, with *Hyoseris* L. being used as an outgroup. In case of the *matK* gene data set, two genera (*Lactuca* L. and *Prenanthes* L.) were selected as outgroups, and a total of 109 accessions (including 14 newly sequenced *S. boulosii* accessions) were analyzed. For each ITS and *matK* data set, phylogenetic analyses using maximum parsimony were performed with PAUP* using the heuristic search option with TBR branch swapping and MULPARS selected. Gaps were treated as missing data. Support for groups was examined by 500 bootstrap replicates (Felsenstein 1985) using the heuristic search option from a simple addition sequence with TBR branch swapping. We also performed Bayesian Inference analysis (BI) using the program MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003).

The best-fit model (GTR + G+I for both ITS and *matK*) was selected based on the Akaike information criterion (AIC) implemented in the program MrModeltest v2.2 (Nylander 2004). Two independent Markov chain Monte Carlo (MCMC) analyses with four Metropolis-coupled chains were from 4 million generations, sampling every 100 generations. The program Tracer v1.5 (Rambaut and Drummond 2009) was used to evaluate the burn-in and to examine log likelihoods, ensuring that the run was in the stationary phase and that adequate effective sample sizes (ESS) were attained. A conservative 25% burn-in was removed from the sampled set of trees, and a 50% majority-rule consensus tree was generated from the remaining trees.

Karyological study

Mitotic studies were conducted on root tips of plants cultivated in a greenhouse, which were grown from seeds or cuttings collected in the field. The root tips were treated with 0.002 M 8-hydroxyquinoline for 3.5 h at room temperature (Tjio and Levan 1950), fixed in Farmer's fluid—ethanol-acetic acid (3:1) (Löve and Löve 1975). They were kept for a week at room temperature and then stored at 4 °C. Material was stained in cold alcoholic-hydrochloric carmine solution for 3–5 days. Chromosome numbers were studied in six plants from the population in the Debdou Mountains and three plants from the population in the Middle Atlas (Jbel

Tsiouant). For chromosome morphology and size analysis, we first took three (Jbel Tsiouant population) and five (Debdou population) clear pictures of mitotic metaphase plates. These were obtained using a Leica DFC490 camera connected to a Zeiss Axiophot microscope. We then identified chromosome pairs in each plate and developed a general haploid idiogram, as well as a corresponding idiogrammatic formula, for each population, following the terminology of Levan et al. (1964) and Küpfer (1974). We also estimated the mean value and range of chromosome size and asymmetry coefficients, according to the procedures of Stebbins (1938, 1971) and Romero Zarco (1986).

Voucher specimens of *Sonchus boulosii* for DNA phylogeny and karyological studies are conserved in the herbariums ECWP and SEV (Online Resource 1).

Results

Morphological observations

A general overview of morphological characters in the new plant species (*Sonchus boulosii*) and the eight clades proposed by Kim et al. (2007) for *Sonchus* are shown in Table 1. This species possesses a suffrutescent structure with pulvinate shape and pinnatisect leaves arranged in dense rosettes. Similar features are found in the components of sect. *Pustulati*, several members of the woody *Sonchus* alliance, a few representatives in sect. *Sonchus*, subg. *Origosonchus* Boulos, and the Pacific endemic *Sonchus novae-zelandiae* (sect. *Maritimi* (Kirp.) Boulos—sect. *Arvenses* (Kirp.) Boulos clade). The species described here possess unbranched or sometimes sparsely branched flowering stems and thus one or a few flowering heads per rosette, similarly to some other clades of the genus (mainly sect. *Pustulati*, *S. bulbosus* (L.) N.Kilian & Greuter, and some representatives of sect. *Maritimi*—sect. *Arvenses* clade). Its fruits are elliptic to oblong achenes with thick marginal ribs and a central main rib on each side (Fig. 1i), and an early deciduous non-dense pappus; a combination of traits also present in sect. *Pustulati*.

Sequence divergence and molecular phylogenetic analysis

Lengths of ITS1 and ITS2 in the new species *Sonchus boulosii* were within the size range reported previously in the subtribe Hyoseridinae (formerly Sonchinae); 254 and 225 bp for ITS1 and ITS2, respectively. No variation among individuals within each population was found, but four populations showed a total of eight bp substitutions/polymorphisms (three bp in ITS1, one polymorphism in 5.8S region, and four bp in ITS2). The average Kimura 2-parameter distance among four populations was 0.53%.

Table 1 Main morphological and ecological characters of phylogenetic clades considered within *Sonchus* L. sensu lato (Kim et al. 2007), and comparison of *S. boulosii* Chamboul., Mejias & J.F.Léger. Data from clades were obtained from Allan et al. (1961), Boulos (1972, 1973, 1974a, b), Carlquist (1966, 1967), Johow (1896), Mejias (2017) and Skottsberg (1922)

Clade	Size. Life cycle, habit	Leaves	Pith	Capitula grouping	Florets per capitulum. Color	Achene morphology	Achene ribs/side	Pappus	Ecology
<i>Sonchus bulbosus</i> (L.) N.Kilian & Greuter	4–65 cm. Perennial, tuberous, stoloniferous and scapose	1.6–25 × 1–17 cm. Entire to lobate. In rosettes	Solid	Solitary or loose panicles (up to 5 heads)	20–110. Bright yellow to pale	3–5.6 × 0.3–0.8 mm. Fusiform to linear. ± Compressed	2(3) furrowed ribs	5.2–10.5 mm. Persistent	Sandy soils, stony grounds
<i>Sonchus</i> sects. <i>Sonchus</i> L. and <i>Asperis</i> Boulos	7–150 cm. Annuals to perennials, rarely suffruticose cent.	2.5–37 × 0.2–10 cm. Entire, lobate to pinnatisect (4–20 ovate to linear lobes)	Hollow	Corymbiform panicles (up to 20 heads)	45–330. Bright yellow to pale	1.9–5.8 × 0.5–1.8 mm. Oblanceolate, obovate or oblong; marginal ribs or wings. Compressed	2 furrowed ribs or 3(–5) simple ribs	5–9.8(11.2) mm. Persistent or slightly deciduous	Weeds and road plants, cliff ecotypes in <i>S. tenerrimus</i>
<i>Sonchus</i> subg. <i>Origosonchus</i> Boulos	3–300 cm. Perennials to suffruticose cent.	2–35 × 0.2–10 cm. Entire, lobate or pinnatisect (4–12 triangular or ovate lobes)	Hollow	Corymbiform panicles	40–200. Pale yellow to yellow-orange	2.5–6.5 × 0.8–1.4 mm. Elliptical to obovate; thick marginal ribs. ± Compressed.	1 thick central rib per side	8–10 mm. Persistent or deciduous	Grasslands, some creepers and rare rocky plants
<i>Sonchus</i> subg. <i>Dendroseris</i> (D Don) S.C.Kim & Mejias	150–600 cm. Shrubs to small trees.	15–65(150) × 1.5–36(57) cm. Entire or pinnate (6–32 lanceolate leaflets). In rosettes	Solid or hollow	Corymbiform panicles, few or hundreds heads	12–100. Creamy white to orange	2–5.5 × 0.7–5 mm. Oblong, obovate or irregular, often winged or triquetrous. ± Compressed	Tuberculate, rugose or striated	Short and early deciduous, sometimes short and persistent	Stony and rocky grounds, neglected fields
<i>Sonchus</i> sect. <i>Martini</i> (Kirp.) Boulos, sect. <i>Arvenses</i> (Kirp.) Boulos and Pacific endemics	10–180 cm. Perennials rhizomatous, sometimes taprooted plants.	3.2–60(150) × 0.4–14 cm. Entire to lobate. Often in rosettes	Hollow, rarely solid	Loose spiciform or corymbiform panicles	30–235. Yellow, often bright yellow	2–4.5(7) × 1–1.5 (3) mm. Elliptical to oblong, with marginal ribs or wings. ± Compressed	Several, often a thick central main rib	5.5–15 mm. Persistent	Weeds in deep or damp soils, rocky places, cliffs and sandbanks
<i>Sonchus</i> sect. <i>Pustulati</i> Boulos	10–45 cm. Suffruticose or suffruticulose perennials.	2–10 × 0.5–3.5 cm. Pinnatisect: 4–12 ovate to reniform lobes. Often in rosettes	Solid	Solitary or loose panicles (up to 4 heads)	25–200. Bright yellow	2.2–5 × 0.6–1.5 mm. Elliptic to oblong, with thick marginal ribs. Compressed.	1 thick central main rib	6–9.2 mm. Early deciduous	Cliff-dwelling species

Table 1 (continued)

Clade	Size. Life cycle, habit	Leaves	Pith	Capitula grouping	Florets per capitulum. Color	Achene morphology	Achene ribs/side	Pappus	Ecology
<i>Sonchus palustris</i> L.	100–250 cm. Rhizomatous perennials.	10–35 × 3–20 cm. Entire, lobate or pinnatisect (± 6 lanceolate lobes)	Hollow	Corymbiform panicle (up to 25 heads)	± 85. Yellow	± 3.8 × 1.2 mm. Elliptical with marginal thick ribs. ± Compressed	1 thick central main rib	± 7.5 mm. Persistent	Deep and damp soils
Macaronesian woody <i>Sonchus</i> alliance	20–250 cm. Perennials to arborescent shrubs.	3–60 × (0.5)1–10 cm. Entire, lobate or pinnatisect (up to 26 ovate to linear lobes)	Hollow or solid	Corymbiform panicles	12–550. Pale yellow to yellow orange	1.7–6 × 0.6–1.5 mm. Elliptical to oblong or oblanceolate, rarely rostrate. ± Compressed	1–5 ribs, sometimes a main central one	4–14 mm. Usually deciduous	Rocky places, cliffs, cultivated fields
<i>Sonchus boulosii</i> Chamboul, Mejías & J.F.Léger	3–17 cm. Suffrutescent plant.	0.6–17 × 0.4–2.6 cm. Pinnatisect (4–12 ovate to reniform lobes). In rosettes	Solid	Solitary, sometimes loose panicles (up to 3 heads)	40–160. Bright yellow	2.2–3.4 × 0.4–0.7 mm. Elliptic to oblong, with thick marginal ribs. Compressed.	1 thick central main rib	3.2–4.9 mm. Early deciduous	Cliff-dwelling species

The average pairwise sequence divergence between major sections of subg. *Sonchus* ranged from 10.39% (between sects. *Maritimi/Arvenses* and *Pustulati*) to 12.89% (between sects. *Sonchus/Asperi* and *Pustulati*). The average pairwise sequence divergence between *S. boulosii* and major clades representing sections of subg. *Sonchus* ranged from 8.51% (with sects. *Maritimi/Arvenses*) to 10.18% (with sects. *Sonchus/Asperi*). We found 9.83% average pairwise sequence divergence between *S. boulosii* and the morphologically closely related sect. *Pustulati*. In general, these values are well above the average pairwise sequence divergence within major sects. of subg. *Sonchus* (e.g., 3.97 and 3.57% in sects. *Maritimi/Arvenses* and *Sonchus/Asperi*, respectively). Therefore, we found substantial ITS sequence divergence of *S. boulosii* from other major sections of subg. *Sonchus*.

A total of 505 manually aligned characters of ITS sequences were used for phylogenetic analyses. We found 192 constant characters (38.0%), 47 variable parsimony-uninformative characters (9.3%), and 266 parsimony-informative characters (52.7%). The heuristic search resulted in more than 50,000 trees, with a tree length (TL) of 846, a consistency index (CI) of 0.5523 (excluding uninformative characters), and a retention index (RI) of 0.8935. The MP strict consensus tree (not shown) of the subtribe Hyoseridinae was somewhat poorly resolved with regard to the phylogenetic position of *Sonchus boulosii*. Nevertheless, the tree showed that two genera of Hyoseridinae, *Launaea* Cass. and *Reichardia* Roth, diverged first within the subtribe and that the monotypic genus *Aetheorhiza* Cass. (= *Sonchus bulbosus*) was sister to the clade containing sects. *Asperi* and *Sonchus* of subg. *Sonchus*. *S. boulosii* appeared as a distinct lineage within the subtribe Hyoseridinae [posterior probability (PP) = 1.00 in BI tree and 100% bootstrap support (BS), Fig. 2], but its relationship to other sections of subg. *Sonchus* and island endemics was uncertain. The MP 50% majority-rule consensus tree (not shown), however, showed that *S. boulosii* was somewhat closely related to the clade containing the Pacific endemics [*Sonchus megalocarpus* (Hook. f.), *S. grandifolius* Kirk, and *S. novae-zelandiae* (Hook. f.) B.D. Jacks] and two sections of subg. *Sonchus* (*Maritimi* and *Arvenses*) (98% value in MP 50% majority-rule consensus tree). This relationship was also supported by the Neighbor-Joining (NJ) tree (not shown). Like the strict consensus tree, the BI tree (Fig. 2) showed an uncertain relationship of *S. boulosii* relative to other lineages within subtribe Hyoseridinae. Within *S. boulosii*, the Chabkat Bou Ghrara population was closely related to the Debdou Mountain population (PP = 1.00). However, phylogenetic relationships among the remaining populations were poorly resolved.

For *matK* gene sequences, we generated the first 1100 bp in 5' region of *matK* gene, including the starting codon base pairs, and compared them to the previously generated *matK* gene sequences for the subtribe Hyoseridinae. We found

a total of five substitutions among four populations of *S. boulosii* (Debdou Mountains, Jbel Tsiouant, Bourdim, and Chabkat Bou Ghrara). No sequence variation was found in two populations: Debdou Mountains and Bourdim, while one substitution was found within each population of Jbel Tsiouant and Chabkat Bou Ghrara. The average pairwise sequence divergence between major sections of subg. *Sonchus* ranged from 0.32% (between sects. *Sonchus/Asperi* and *Pustulati*) to 0.78% (between sects. *Sonchus/Asperi* and *Maritimi/Arvenses*). The average pairwise sequence divergence between *S. boulosii* and major sections of subg. *Sonchus* ranged from 0.29% (with sect. *Pustulati*) and 0.69% (with sects. *Maritimi/Arvenses*). Within two major clades, one containing sects. *Maritimi/Arvenses* and the other *Sonchus/Asperi*, the average pairwise sequence divergence was 0.78 and 0.12%, respectively. As with ITS sequences, we found significant *matK* gene sequence divergence of *S. boulosii* from the rest of major sections in subg. *Sonchus*.

Of a total of 1108 aligned characters of *matK* gene, we found 979 characters to be constant (88.4%), 53 were variable but parsimony-uninformative (4.78%), and 76 were parsimony-informative characters (6.86%). The heuristic search found six equally most parsimonious trees with TL of 146, CI of 0.9022 (excluding uninformative characters), and RI of 0.9778. The MP strict consensus tree (not shown) was poorly resolved, and the phylogenetic position of *S. boulosii* was uncertain relative to other major lineages within Hyoseridinae, such as *Aetheorhiza* (= *Sonchus bulbosus*), three Pacific endemics (*Sonchus megalocarpus*, *S. grandifolius*, and *S. novae-zelandiae*), sect. *Pustulati*, sects. *Sonchus/Asperi* and sects. *Maritimi/Arvenses*. The BI tree (Fig. 3) showed that *S. boulosii* is not monophyletic, showing two major groups within *S. boulosii*; each lineage was strongly supported based on BI PP value of 0.98, but weakly by BS (60 and 65%). One group included the populations sampled from Bourdim and Debdou Mountains, while the other group included the Jbel Tsiouant and Chabkat Bou Ghrara populations.

Karyological observations

All the plants studied showed $2n = 18$ as somatic number (Fig. 4a, c). The karyotype consists mainly of metacentric chromosomes plus some submetacentric ones, being two pairs satellized (Fig. 4b, d). These chromosomes are medium-small in size, with the exception of the shortest pair in the population from the Debdou Mountains, which is small. Therefore, the karyotype of the new species is quite symmetric and fits the type 1A (Stebbins 1971). Significant differentiation in chromosome morphology between the two populations studied was detected, with the plants from the Debdou Mountains possessing more asymmetric and smaller chromosomes than those from Jbel Tsiouant (Table 2).

Discussion

Both ITS and *matK* phylogenetic trees clearly indicate that this newly discovered plant (*Sonchus boulosii* Chamboul., Mejías & J.F.Léger) is a representative of the genus *Sonchus* subg. *Sonchus* L. but is not part of the clade comprising sect. *Pustulati* (Figs. 2 and 3), despite the strong morphological resemblance to the species of this section, particularly to *S. pustulatus* Willk. and *S. fragilis* Ball. It is also not part of any of the eight main clades identified by Kim et al. (2007) in the phylogenetic classification of the genus *Sonchus*. Moreover, the average pairwise divergences between sequences of the new plant samples analyzed and major sections of subg. *Sonchus* ranged 8.51–10.18% for ITS and 0.27–0.66% for *matK* gene, which are quite similar to the values estimated among clades in the subgenus (10.39–12.89 and 0.32–0.78%, respectively). Therefore, it is stated that it clearly represents a differentiated lineage of the genus and a separate new clade within *Sonchus* subg. *Sonchus* L.

Systematic position

Even given the solid phylogenetic differentiation of *Sonchus boulosii*, available trees do not suggest any clear systematic position for it. ITS data are suggestive of close relationship between *S. boulosii* and the clade comprising the sects. *Maritimi* (Kirp.) Boulos and *Arvenses* (Kirp.) Boulos along with some Pacific islands endemic species formerly included in the genera *Kirkianella* Allan, *Embergeria* Boulos and *Actites* N.Lander (Kim et al. 2007). Most representatives of this group are rhizomatous plants found on deep, damp, sometimes brackish or salty, soils. Therefore, to all appearances, they seem unrelated to *S. boulosii*. Exceptionally, the New Zealand endemic *S. novae-zelandiae* (Hook.f.) B.D.Jacks. (\equiv *Kirkianella novae-zelandiae* (Hook.f.) Allan) shows remarkable similarities to *S. boulosii* in morphology and ecology. It is a small scapigerous perennial with a stout, often multicapital, taproot bearing dense rosettes of lobed to pinnatisect leaves that produce solitary flower heads on ± 15 cm scapes. This species often colonizes open, stony habitats such as rock crevices, slopes and rock ledges (Allan et al. 1961). However, karyological data do not support a phylogenetic relationship between the two species or clades, nor help to clarify systematic relations of *Sonchus boulosii*. The new species is diploid with $2n = 18$ as chromosome somatic number, which is considered the base number of *Sonchus* and the most common in the Mediterranean, probably the primary center of diversification of the genus (Mejías and Andrés 2004). The same number has also been indicated for the representatives of sect. *Maritimi* (Mejías and Valdés 1988), a group mainly restricted to the Mediterranean. But a high incidence of polyploidy ($2n = 36, 54, 90, 126$) is present

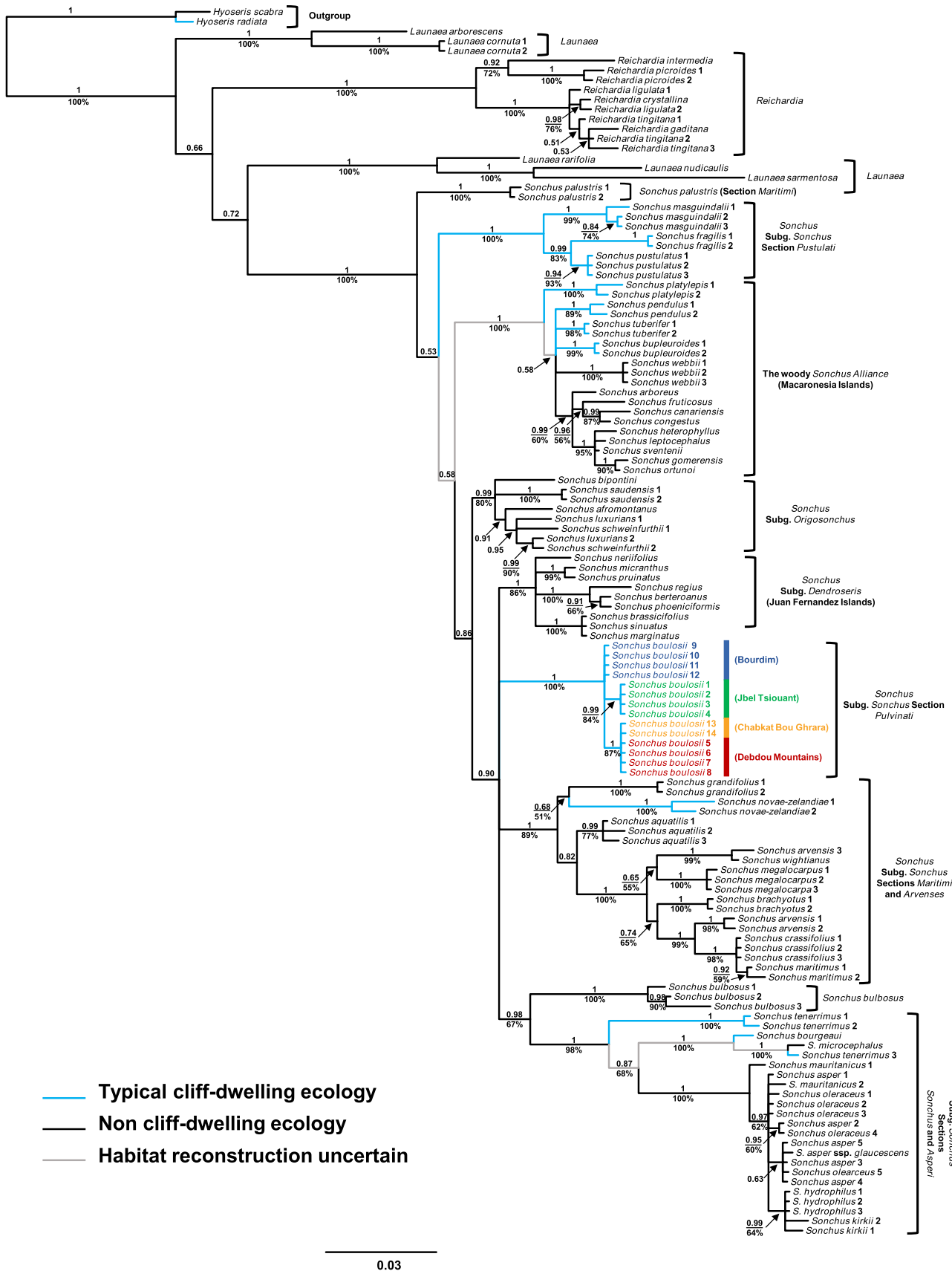


Fig. 2 Phylogenetic tree of genus *Sonchus* based on Bayesian Inference analysis of ITS nrDNA sequences, with indication of the position of *S. boulosii* samples analyzed (colored names). Posterior probabilities (PP) and bootstrap support (BS) values ($\geq 50\%$) are shown above and below branches, respectively. Color-coded branches: typical cliff-dwelling ecology, blue; non cliff-dwelling ecology, black; gray, uncertain phylogenetic reconstruction of habitat diversification

among the non-Mediterranean taxa of the clade comprising sects. *Maritimi* and *Arvenses* and the Pacific endemics (Roux and Boulos 1972; Mejías and Andrés 2004), particularly in *S. novae-zelandiae*, which shows the numbers $2n$ ($10x, 14x$) = 90, 126 (Beuzenberg and Hair 1984). Since the polyploidy has small general incidence in *Sonchus*, a possible polyploid condition of *S. boulosii* would have reinforced the hypothesis of its close relationship with the clade including *S. novae-zelandiae*. However, the present karyological observations, as well as the biogeographical data, do not support any assumption in this respect.

The chloroplast *matK* data also suggest an uncertain position of *S. boulosii* within the Sonchinae; *S. boulosii* is part of highly unresolved clade (PP = 0.98 in Fig. 3) containing *S. bulbosus* and sections *Pustulati*, *Maritimi*, *Arvenses*, *Sonchus*, and *Asperi*. The hybrid origin of *S. pustulatus* in sect. *Pustulati* was postulated based on capturing of chloroplast DNA from sections *Sonchus* and *Asperi* (Kim et al. 2008). It is unknown whether morphologically similar *S. boulosii* may also have hybrid origin, given morphological similarity with *S. pustulatus*. Therefore, it is necessary to further resolve phylogeny of *Sonchus* based on quickly evolving regions of chloroplast DNA and determine the precise position of *S. boulosii*.

Evolutionary significance

It appears that the precise phylogenetic position of *S. boulosii* is uncertain within genus *Sonchus* (Figs. 2 and 3), but we can cautiously estimate the divergence time of this lineage. Bearing in mind the dating of Silva et al. (2015a) for sect. *Pustulati* (5.96–5.33 Mya) and divergence time estimations in Cichorieae (Tremetsberger et al. 2013), we can hypothesize that the clade including *S. boulosii* most probably diverged from its sister lineage some 6.57 My ago (95% HPD of 3.36–10.39 My ago), during the late Tertiary (Kim S.-C., unpublished data). This is consistent with the striking divergence in chlorotypes and ribotypes found among the samples analyzed, which is unusual in many other species of *Sonchus*. With consideration to this genetic variability and its presence in disjunctive, spatially limited areas (as far as we know), it seems logical to propose that *S. boulosii* is an ancient taxon, i.e., a paleoendemic species (Thompson 2005), with a relict distribution similar to the representatives of the sect. *Pustulati* (Silva et al. 2015a). The vast extent of

occurrence calculated ($\geq 15,000 \text{ km}^2$) and the very restricted area of occupancy (probably $\leq 0.5 \text{ km}^2$) support this hypothesis in a species with very early deciduous pappus and, consequently, of limited dispersal ability.

Rocky habitat is common among *Sonchus* species. Besides sect. *Pustulati* and *S. boulosii* clades, whose representatives are exclusively cliff-dwelling species, it is present in at least four more clades (Figs. 2 and 3). Specifically, several species of the Macaronesian *Sonchus* alliance clade are also cliff-dwelling plants and, in addition, some derived cases should be considered within sect. *Maritimi/Arvenses* and sect. *Sonchus/Asperi* clades. Based on the current ITS phylogeny (Fig. 2) and estimation of divergence times, it is conceivable that the origin of cliff-dwelling *Sonchus* species traced back to the Tertiary (i.e., the late Miocene or the Pliocene) during early diversification of the genus (Tremetsberger et al. 2013; Silva et al. 2015a, b). However, given the fact that the earliest diverged clade of *Sonchus* is currently represented exclusively by *S. palustris*, a rhizomatous species colonizing freshwater damp soils, and weak support for the major lineages within *Sonchus*, it may be difficult to determine whether cliff-dwelling ecology is a plesiomorphic condition within the genus. Based on highly resolved and robust phylogenetic framework, the plesiomorphic hypothesis of cliff-dwelling ecology is yet to be determined. Nevertheless, according to ITS phylogenetic trees (Fig. 2), probably evolutionary shifts to rocky habitat took place at least five times along the genealogic history of *Sonchus*, and sect. *Pustulati* and *S. boulosii* clades do not share a common origin. Therefore, neither the morphological similarity between these two clades reflects conservative niche evolutionary processes (Wiens and Graham 2005; Crisp and Cook 2012; Pyron et al. 2015), nor is the general high diversity in rocky species within *Sonchus* the result of this phenomenon.

The noted resemblance between *Sonchus boulosii* and sect. *Pustulati* rather seems to reflect a case of homoplasy; i.e., the strikingly similar traits in both clades have been produced as a result of independent evolutionary processes determined by high niche similarity (Bailey et al. 2015; Lin et al. 2015) and the strong evolutionary constraints of cliff ecosystems (Larson et al. 2005). Such similarities are also evident in some cliff-dwelling representatives of the Macaronesian *Sonchus* alliance clade, in vegetative characters at least (Fig. 1m), and in *S. novae-zelandiae*. Molecular phylogenetic analysis has extensively shown that homoplasy is quite common (e.g., Rothfels et al. 2008; Plata et al. 2011; Guo et al. 2013; Moen et al. 2013; Steffen and Kadereit 2014), hampering efforts to obtain evolutionary consistent plant classifications (e.g., Rothfels et al. 2008; Guo et al. 2013; Steffen and Kadereit 2014). In this case, the difficulty is stressed by the high similarity in fruit features among species. Bristle composition and deciduousness of the pappus is

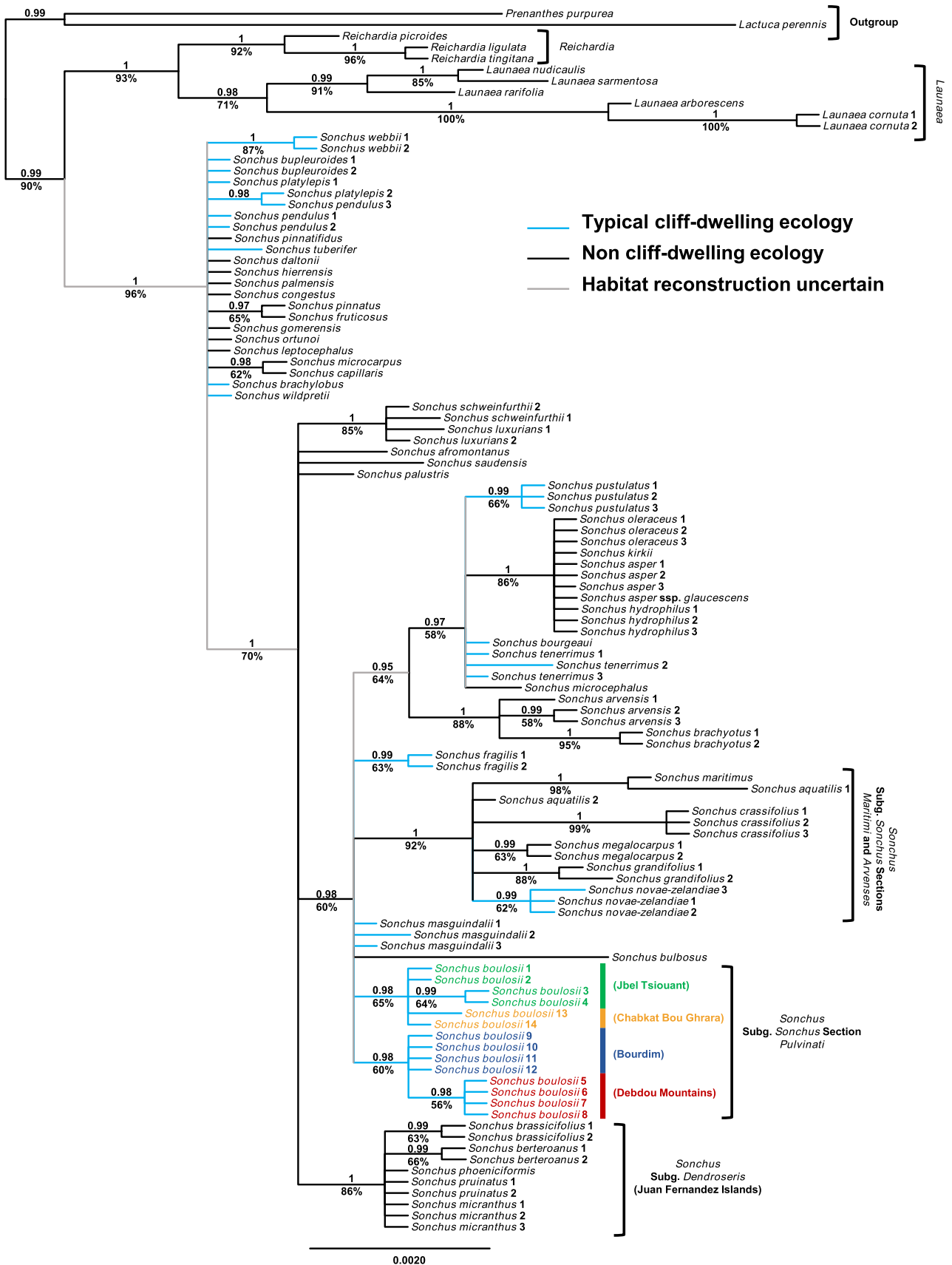


Fig. 3 Phylogenetic tree of genus *Sonchus* based on Bayesian Inference analysis of *matK* cpDNA sequences, with indication of the position of *S. boulosii* samples analyzed (colored names). Posterior probabilities (PP) and bootstrap support (BS) values ($\geq 50\%$) are shown above and below branches, respectively. Color-coded branches: typical cliff-dwelling ecology, blue; non cliff-dwelling ecology, black; gray, uncertain phylogenetic reconstruction of habitat diversification

strongly determined by habitat type. On the contrary, achene morphology, with a thick central rib on each side and clearly defined marginal ribs in the four species (Fig. 1i) as in the putative basal *S. palustris*, probably reflects the retention of ancient characteristics.

Determining whether the presence of these possibly homoplastic traits is the result of parallelism (on the base of the same genetic and developmental mechanisms) or convergence (on the base of different genetic and developmental mechanisms) is difficult and requires a thorough knowledge (Pearce 2012) that we do not have for these species at present. Many biologists consider that homology in closely related taxa, as it is the case here, is usually due to parallel evolution, but this conception is disputed and occasionally violated (Arendt and Reznick 2008; Pearce 2012). At any rate, we believe it is difficult to assume that the high resemblance between *Sonchus boulosii* and sect. *Pustulati* could be the result of a convergence process.

Cliff habitats as source of taxonomic novelties

The discovery of *S. boulosii* and several other taxonomic and floristic novelties by ECWP (Chambouleyron et al. 2015a) illustrate the interest of maintaining well-planned biodiversity field surveys to address the description of hitherto unknown biodiversity (Wägele et al. 2011; Wege et al. 2015). During forthcoming decades, it seems essential to maintain these efforts, especially within global biodiversity ‘hotspots’ such as the Mediterranean region (Myers et al. 2000), and a careful analysis of the results obtained to optimize these efforts. For example, the three new taxa described during the aforementioned surveys are colonizers of open rocky habitats (Chambouleyron et al. 2014, 2015a), which highlights the value of prospecting these habitats further, at least in the Mediterranean region. Indeed, high levels of endemism are sometimes indicated for rocky slopes (Lavergne et al. 2004; Bragazza 2009; Pérez-García et al. 2012) due to endemic taxa association with high habitat specificity, low dispersal ability and their naturally fragmented distribution (García et al. 2002; Lavergne et al. 2004; Thompson 2005). This suggests that habitat type is a factor to be taken into account in the planning of plant biodiversity prospecting, with rocky habitats representing a high priority for future survey efforts.

Conclusion

The phylogenetic study of the new plant material found at several locations within the Atlas system and the Oran Meseta shows that they represent a differentiated lineage within genus *Sonchus*. This genetic divergence is sufficient not only for the description of a new species but also for a new section within *Sonchus* subg. *Sonchus*. We hypothesize that the new material represents a remnant of an ancient diversity, with biogeographical affinities associated with the Northwest of the African plate. The similitude in habitat of the new species to the representatives of *Sonchus* sect. *Pustulati* is determinant for the high morphological resemblance to these. The case is considered to represent a phenomenon of parallel evolution, which involves notable difficulties in taxonomic diagnosis and the achievement of consistent plant classifications. Therefore, a new species and a new section within the genus are described here.

Taxonomic treatment

Sonchus* L. sectio *Pulvinati S.C.Kim, Mejías & Chamboul., **sect. nov.**—TYPE: *Sonchus boulosii* Chamboul., Mejías & J.F.Léger.

Etymology: The Latin word *pulvinatus* refers to the cushion-shaped growth habit of the sole representative of the section.

Description: Perennial herb, cushion-shaped, with a woody rootstock, densely branched, unarmed, with pluricellular, uniseriate, woolly hairs and often unicellular glandular hairs. Leaves in basal rosettes arranged, spiral alternate, simple, with pinnate-reticulate venation and woolly hairs in the axil. Capitula terminal, usually solitary, with scapiform peduncles, sometimes in loose panicles, typically covered by simple glandular hairs. Involucre non-acrescent, of adpressed, plane, lanceolate to linear bracts arranged in three roughly defined series. Receptacle alveolate. Florets ligulate, hermaphrodite, hairy in the upper part of the tube and the base of the ligule, with anthers sagittate at the base and style branches linear. Achenes laterally compressed, not beaked, with a thick central main rib on each side and thick marginal ribs. Pappus of scabrid bristles, dimorphic; outer bristles fine and flexuose, inner ones thicker and straight.

Sonchus boulosii Chamboul., Mejías & J.F.Léger, **sp. nov.**—TYPE: Morocco, Debdou Mountains, Province of Taurirt, Municipality of Sidi Ali Belkasssem, near the village of Sebâat ou Rijal, at a place called “Jorf el Medjam”, on vertical cliffs, elevation 1400–1500 m a. s. l., N 34° 00' 33", W 02° 50' 28.8", 3 June 2013, *M. Chambouleyron* s.n. (holotype: ECWP!; isotypes: G!, P!, RAB!, SEV 286733!) (Fig. 5).

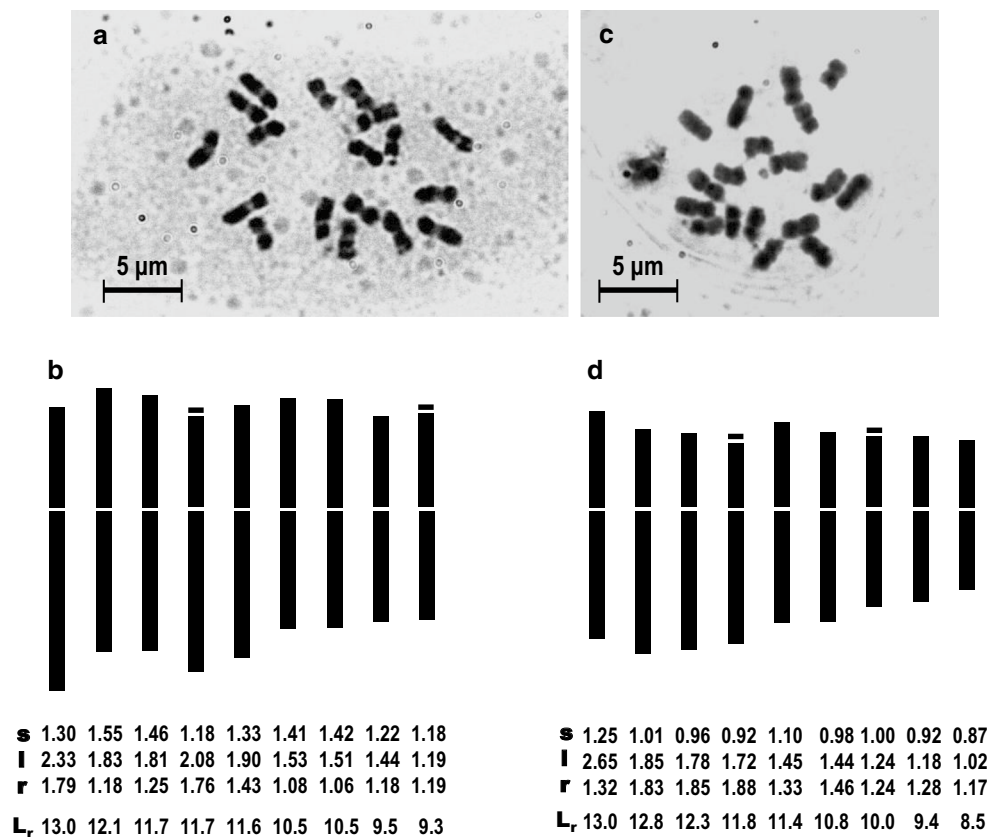


Fig. 4 Somatic metaphases and karyograms of *Sonchus boulosii*: **a, b**: plants from Jbel Tsiouant (SEV 286735); **c, d**: plants from the Debdou Mountains (SEV 286734). *s* mean length of short arm (µm), *l*

mean length of long arm (µm), *r* *l*/*s*, *L_r* relative length of the chromosome (% chromosome length/total length of the haploid karyotype)

Table 2 Idiogrammatic formula, asymmetry (A_1 and A_2 coefficients: mean values and general range) and chromosome apparent length (mean values and general range) of the karyotypes for the two populations studied of *Sonchus boulosii*

Population	Idiogram	A_1 (range)	A_2 (range)	Chromosome length (µm)
Jbel Tsiouant ($N=3$)	4M+8m+2m ^{sat} +4m-sm+2m-sm ^{sat}	0.21 (0.17–0.24)	0.12 (0.11–0.13)	2.59–3.63 (2.29–4.3)
Debdou Mountains ($N=5$)	2M-m+8m+2m ^{sat} +4sm+2sm ^{sat}	0.30 (0.29–0.32)	0.17 (0.15–0.22)	1.89–2.91 (1.65–3.21)

N number of metaphase plates analyzed

Eponymy: The species name honors the Egyptian botanist Loufty Boulos (1932–2015), who published a comprehensive worldwide revision of genus *Sonchus* in the 1970s (Boulos 1972, 1973, 1974a, b).

Description: Perennial herbs, 3–17 cm high, with a woody rootstock, stemless, cushion-shaped, unarmed, with long, pluricellular, uniseriate, woolly hairs and other unicellular glandular hairs. Rootstock densely branched, covered with leaf remains, with numerous erect or ascending branches of 4–30 mm in diameter. Leaves 0.6–5.2(17.0)×0.4–2.6 cm, arranged in basal rosettes, spiral alternate, simple, pinnatifid to pinnatisect, ±lyrate, narrowly obovate in shape, attenuate

to the base with poorly defined petiole, with pinnate-reticulate venation, margin entire with some small, cartilaginous teeth sparsely distributed, with white, woolly hairs in the axil, green colored, sometimes purple-stained; (2)4–7 lateral lobes 0.2–0.9×0.3–0.8 cm, ovate to rhomboidal, often rounded at the apex, sometimes acute and mucronulate; terminal lobe 0.6–2.6×0.4–2.6 cm, ovate to deltoid or, more rarely, reniform. Capitula terminal, often solitary with scapiform peduncles, sometimes in loose panicles up to 3 flower heads, including (41-)60-138(160) florets; peduncles up to 14 cm in length and 0.2–0.4 mm in diameter, erect, cylindrical, angled, covered by simple glandular hairs

0.2–0.7 mm, more abundant in the upper third, sometimes glabrous, commonly with 1 or 2 bracteoles of 1.3–4.2 mm, woolly in the axil. Involucre 7.0–11.0 × 3.8–8.3 mm, non-acrescent, cylindrical during anthesis and conical-campanulate during the maturation of fruits, with 16–26 involucre bracts per capitulum, arranged in three roughly-defined series. Involucre bracts adpressed, plane, acute to erose at the apex, green colored; outer and middle ones—5 to 9—of 1.4–7.4 × 0.5–1.7 mm, lanceolate, often with glandular hairs mainly in the median axis (sometimes glabrous), with narrowly membranous margin; inner ones—8 to 18—of 7.0–11.0 × 0.0–2.6 mm, lanceolate to linear, glabrous, with a well-developed membranous margin. Receptacle ± alveolate (Fig. 5l), with few small scales sparsely distributed. Florets ligulate, hermaphrodite. Corolla 6.1–11.9 mm; tube 2.1–5.3 mm, hairy in the upper part; ligule of the outer florets 4.2–8.1 × 1.5–3.2 mm, shorter inwards the flower head, oblanceolate to ± linear, with 5 teeth at the apex, commonly bright yellow in color but the external ones with a purple band on the back (Fig. 1h). Anthers 2.0–3.6 mm, sagittate at the base and rounded at apex, commonly longer inwards the flower head, yellow. Style branches 0.7–1.9 mm, linear, yellow. Achenes (Figs. 1i, 5m–o) 2.2–3.4 × 0.4–0.7 mm, fusiform, linear or narrowly oblanceolate, not beaked, somewhat laterally compressed, with clearly defined outline by two thick marginal ribs, and a thick central main rib plus up to 4 secondary ribs on each side, in general transversally tuberculate-spinulose. Pappus 3.2–4.9 mm, of scabrid-scabridulous bristles, dimorphic, early deciduous, white; outer bristles 1.8–3.1 mm, fine and flexuose, connate at the base; the inner ones thicker, straight, not connate.

Diagnosis: Morphological identification of *Sonchus boulosii* is difficult because of the high similarity with the taxa of sect. *Pustulati* (Table 1). However, *S. boulosii* shows the most markedly cushion-shaped (pulvinate) habit among all these species, because all leaves are grouped in basal rosettes in the extreme of short stout, lignified stems, while the representatives of sect. *Pustulati* commonly show branches bearing leaves along their lengths. Regarding reproductive characters, florets and achenes of this new species are not readily distinguishable in morphology, but the presence of glandular hairs in the involucre bracts of most individuals of *S. boulosii* allows them to be distinguished from those of *S. pustulatus* and *S. fragilis*. In addition, the pappus is extremely short in *S. boulosii*. Ecology may be another distinctive criterion, as *S. boulosii* grows in mountains from Meso- to Montane-Mediterranean vegetation belts, while the species included in *Sonchus* sect. *Pustulati* are restricted to the Thermo-Mediterranean belt close to the sea. A determination key for the *Sonchus* species occurring in Morocco, including *S. boulosii*, is provided below.

Chromosome number: $2n = 18$ (Fig. 4).

Phenology: Flowering from end-April to late July (depending on elevation); mature achenes are available from end-May to August.

Habitats: *Sonchus boulosii* is a cliff-dwelling species that colonizes sub-vertical, mainly north-facing (NNW–NNE) limestone crags (Fig. 1a–c) at elevations of 1400–2400 m.

It occurs in a wide range of climatic conditions, having been found from upper Meso-Mediterranean to Montane-Mediterranean vegetation belts, in areas receiving between c. 400 mm (semi-arid) to c. 700 mm (sub-humid) annual precipitation (after Mokhtari et al. 2014).

Distribution area: Only four populations have so far been found (Fig. 6), but these occur in three distinct biogeographic zonations of Morocco: the Eastern Morocco mountains (corresponding to “Om-3” according to the classification of Fennane and Ibn Tattou 2005), the Middle Atlas (“MA-2” according to Fennane and Ibn Tattou, *op. cit.*) and the High Atlas (“HA-6” according to Fennane and Ibn Tattou, *op. cit.*). Given that many other locations of appropriate cliff habitat can be found in the areas between these distant populations (80–140 km between the localities), it is likely that further populations occur, especially in the Middle and High Atlas.

Additional specimens examined: Morocco, Middle Atlas, Province of Boulemane, Municipality of Almisse Marmoucha, Jbel Tsiouant, 2300 m a. s. l., N 33° 20' 11.5", W 04° 02' 24.7", 27 Jun 2014, *M. Chambouleyron* s.n. (ECWP s.n.!; SEV 286735!); High Atlas, Province of Midelt, Municipality of En-Nzala, Bourdim, 2280 m a. s. l., N 32° 35' 11.5", W 04° 08' 55.7", 21 May 2008, *J.-F. Léger* s.n. (ECWP s.n.!); High Atlas, Province of Figuig, Municipality of Mâatarka, Chabkat Bou Ghrara, 1690 m a. s. l., 4 May 2017, *M. Chambouleyron* s.n. (ECWP s.n.!).

Conservation status: As with many cliff specialist plants, *Sonchus boulosii* is very rare on a wide geographical scale but locally abundant within appropriate habitat (Larson et al. 2005; Silva et al. 2015b). The four currently known populations delimit a vast extent of occurrence, probably reaching 15,000 km², but the high habitat specificity of the species determines an area of occupancy of ≤ 0.5 km². The largest and best known population is that of the Debdou Mountains, which comprises approximately 1000 mature individuals and an area of occupancy of ± 0.1 km², while the Jbel Tsiouant population (Middle Atlas) is estimated at approximately 200 individuals, and the two Eastern High Atlas populations, from Bourdim and Chabkat Bou Ghrara, both comprise approximately 500 mature individuals. No information on population viability and dynamics is available yet, although

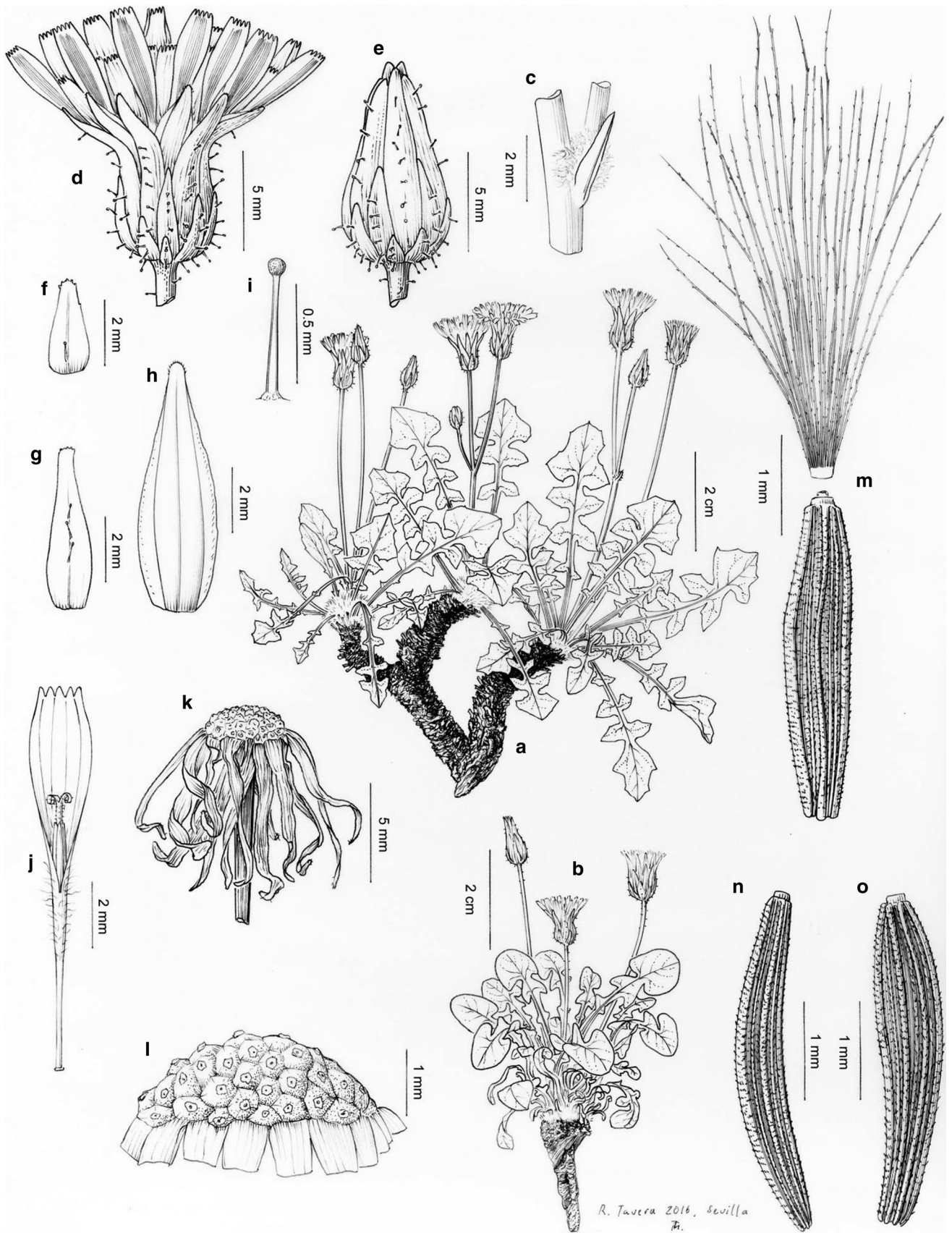


Fig. 5 *Sonchus boulosii*: **a, c–o** Debdou Mountains, Municipality of Sidi Ali Belkassam, village of Sebâat ou Rijal, “Jorf el Medjam” (ECWP s.n., holotype: **a, c–j**; SEV 286733; **k–o**); **b** Middle Atlas, Jbel Tsiouant (ECWP s.n.); **a, b** habit; **c** detail of a ramification in the peduncle; **d** capitulum in anthesis; **e** capitulum during achenes maturation; **f–h** outer, middle and inner involucre bracts, respectively; **i** glandular hair; **j** outer floret without ovary nor pappus; **k** flower head after achenes dispersal; **l** flower head receptacle; **m** inner achene with pappus; **n, o**: outer achene in ventral and dorsal view, respectively

our observations (data not shown) suggest that most mature individuals are reproductive and that many juveniles are present in the populations, indicating effective sexual recruitment. Accordingly, it appears that the most significant factor for assessing the conservation status of this new species is its very small area of occupancy (IUCN 2017). However, given the dispersed distribution of the known populations and that a very significant part of the cliffs from Eastern Morocco and Atlas Range have not been prospected, we think it is premature to determine the conservation status of the new species. We simply suggest that it should be added to the list of rare, threatened and endemic plants of Morocco proposed by Fennane and Ibn Tattou (1998) under the ‘Very Rare’ (RR) category. Monitoring populations would be necessary to evaluate whether *S. boulosii* is in decline or not; achieving this would allow for a more accurate categorization of extinction risk (IUCN 2017).

Uses: No uses are known for this species.

Key to the species of *Sonchus* in Morocco

- 1a. Woody plant at least at the base; shrub, dwarf shrub or perennial with woody rootstock 2
- 1b. Herbaceous plant, annual to perennial 8
- 2a. Achenes with two longitudinal channels; pappus ± persistent *S. tenerrimus*
- 2b. Achenes with one to several ribs, without channels; pappus often early deciduous, sometimes persistent 3
- 3a. Achenes with wide, thick marginal ribs. Plant up to 40 cm high with capitula in groups of 2–4 or solitary 4
- 3b. Achenes without thick marginal ribs. Plant 20–200 cm high, capitula in terminal corymbs with more than four flower heads 7
- 4a. Capitula with more than 100 florets, involucre whitish tomentose. Leaves ± succulent, usually with reniform terminal lobes *S. masguindalii*
- 4b. Capitula up to 100 florets, involucre green, glabrous or bearing some glandular hairs. Leaves not succulent, with ovate to deltoid terminal lobes 5

- 5a. Plant up to 15 cm, clearly pulvinate. Inflorescence unbranched, sometimes with 1(2) branches; often with glandular hairs *S. boulosii*
- 5b. Plant 15–40 cm, pulvinate or not. Inflorescence usually with 2 or more branches; glabrous 6
- 6a. Adult plant with woody branches. Achenes smooth or slightly rugose. Leaf-lobes triangular to ovate *S. pustulatus*
- 6b. Woody parts only at the base. Achenes rugose. Leaf-lobes broadly ovate *S. fragilis*
- 7a. Shrubby plant up to 200 cm, densely branched at base. Leaf-lobes clearly triangular. Peduncles swollen at top *S. pinnatifidus*
- 7b. Subscapose plant up to 80 cm, little branched. Leaf-lobes triangular to rounded, the terminal one being cordate. Peduncles not swollen *S. radicans*
- 8a. Perennial stoloniferous herb with globular underground tubers. Scapose with solitary capitulum *S. bulbosus*
- 8b. Annual or perennial herb without tubers, sometimes rhizomatous. Leafy stems, sometimes subscapose. Capitula several, very rarely solitary 9
- 9a. Achenes strongly compressed, winged, with 3(-5) thin ribs per side 10
- 9b. Achenes weakly compressed, with thin marginal ribs or rounded margins 12
- 10a. Anthers less than 2.0 mm. Annual *S. asper subsp. asper*
- 10b. Anthers at least 2.0 mm. Perennial, sometimes biennial 11
- 11a. Achenes covered with very thin whitish hairs. Plant rhizomatous *S. mauritanicus*
- 11b. Achenes glabrous or slightly hairy at the base. Taprooted plant, sometimes rhizomatous *S. asper subsp. glaucescens*
- 12a. Achenes without longitudinal channels. Rhizomatous perennial with leaves toothed or runcinate 13
- 12b. Achenes with two longitudinal channels. Perennials to annuals, sometimes rhizomatous, leaves entire to pinnatisect 14
- 13a. Achenes elliptic, with wide, thick marginal ribs plus 1–5 ribs per side, the central one clearly thicker. Stylar branches greyish, rarely pure yellow *S. aquatilis*
- 13b. Achenes oblong or narrowly elliptic, with thin marginal ribs plus 3–5 ribs per side, the central one ± thicker. Stylar branches yellow ... *S. maritimus*
- 14a. Anthers up to 2.2 mm long. Ligule of outer florets 5.3–8.4 × 1.0–1.4 mm, equaling the tube in length. Leaves entire to pinnatisect *S. oleraceus*
- 14b. Anthers at least 2.2 mm long. Ligule of outer florets (7.7)8.5–14.0 × 1.5–3.2 mm, longer than the tube. Leaves pinnatisect 15

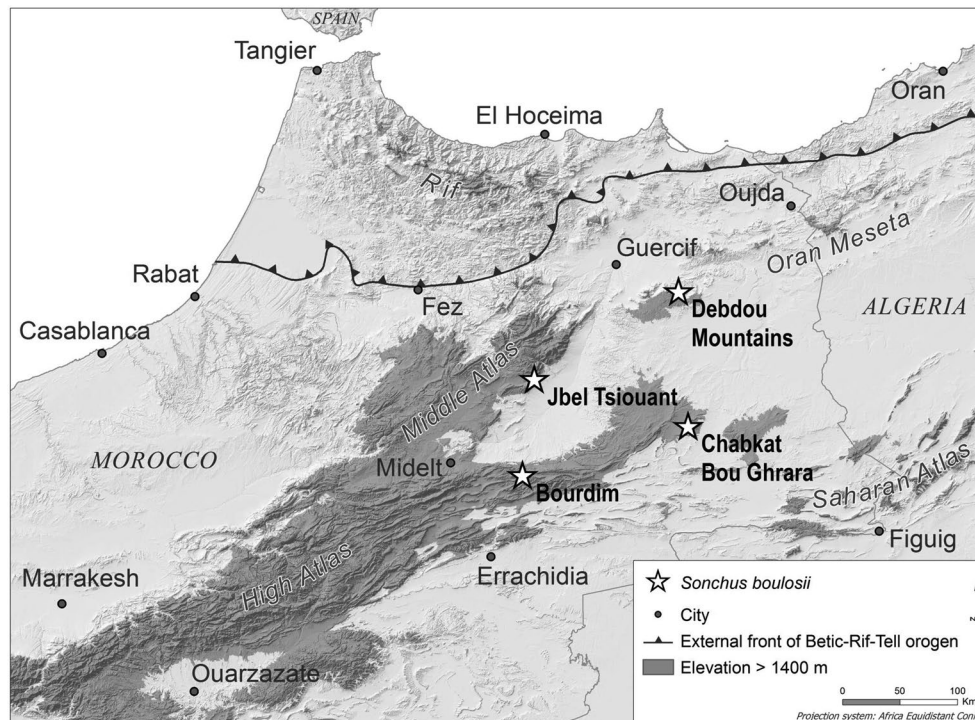


Fig. 6 Known localities of *Sonchus boulosii*, with localization of the Southern external front of Baetic–Rifan–Tell orogen (serrated black line), according to Chalouan et al. (2008)

- 15a. Commonly perennial. Leaf-lobes linear to oval, strongly constricted at the base. Achenes smooth to rugose-tuberculate *S. tenerrimus*
- 15b. Annual. Leaf-lobes triangular. Achenes markedly transversely rugose *S. bourgeaui*

Sonchus briquetianus Gand. has been mentioned for Morocco by Dobignard and Chatelain (2011: 383) and Fennane et al. (2014: 321). However, we are doubt the value of this taxon, which seems to be only a coastal ecotype of the highly variable *S. tenerrimus* (Mejías 1988).

Sonchus maculigerus H.Lindb. has been mentioned as a doubtful Moroccan endemic by Dobignard and Chatelain (2011: 385) and Fennane et al. (2014: 318). We also consider this taxon doubtful because its diagnosis is based on leaf morphology (Lindberg 1932), which is difficult to conclusively separate within this group, as some species display extreme variability in vegetative characters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests.

Information on Electronic Supplementary Material

Online Resource 1. Collections of *Sonchus boulosii* for this study.

Online Resource 2. Sequence Alignment of ITS region for the Sonchiae.

Online Resource 3. Sequence Alignment of *matk* region for the Sonchiae.

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