

Warionia (Asteraceae): a relict genus of Cichorieae?

by

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Abstract

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The genus *Warionia*, with its only species *W. saharae*, is endemic to the northwestern edge of the African Sahara desert. This is a somewhat thistle-like aromatic plant, with white latex, and fleshy, pinnately-partite leaves. *Warionia* is in many respects so different from any other genus of Asteraceae, that it has been tentatively placed in the tribes Cardueae, Cichorieae, Gundelieae, and Mutisieae. Until now, a comprehensive study of *Warionia* to have a complete context for discussing its taxonomic position is lacking. The general morphology, anatomy, palynology and chromosome number of *W. saharae* are investigated here, and the species is described and illustrated. Laticifers in leaves and stems indicate a relationship with Cichorieae, and are associated with the phloem, in contact with it or with the surrounding sclerenchyma sheath. The pollen features indicate a strong relation with Cardueae, namely the structure with Anthemoid pattern where the columellae are joined to the foot layer, the ectosexine with thin columellae, the endosexine with stout and ramified columellae, the conspicuous spines with globose bases and conspicuous apical channels, and the tectum surface very perforate. Chromosomal counts resulted in $2n = 34$. The morphological and palynological evidence positions *Warionia* between the tribes Cardueae and Cichorieae suggesting that it could be a remnant of the ancestral stock that gave rise to both tribes.

Keywords: Africa, Cardueae, chromosome number, Cichorieae, laticifers, pollen, taxonomy.

Introduction

Warionia Benth. & Coss. is a monotypic genus of Asteraceae, endemic to the northwestern edge of the African Sahara desert. The species *Warionia saharae* Benth. & Coss., known by the vernacular name of “afessas” or “abessas”, may be found in several localities on dry shale in northwestern Africa, in Morocco

Resumen

Katinas, L., Tellería, M.C., Susanna, A. & Ortiz, S. 2008. *Warionia* (Asteraceae): un género relicto de Cichorieae? *Anales Jard. Bot. Madrid* 65(2): 367-381 (en inglés).

El género *Warionia*, y su única especie, *W. saharae*, es endémico del noroeste del desierto africano del Sahara. Es una planta semejante a un cardo, aromática, con látex blanco y hojas carnosas, pinnatipartidas. *Warionia* es tan diferente de otros géneros de Asteraceae que fue ubicada en las tribus Cardueae, Cichorieae, Gundelieae y Mutisieae. Hasta ahora, no existía un estudio global de *Warionia* como contexto para discutir su posición taxonómica. Se ha investigado aquí su morfología, anatomía, palinología y número cromosómico, y se incluyen una descripción y una ilustración de la especie. Los laticíferos en hojas y tallo están asociados al floema, en contacto directo con él o con sus casquetes esclerenquimáticos y lo relacionan con Cichorieae. El polen indica una gran relación con Cardueae, i.e., la estructura con patrón anthemoides donde las columelas se unen al estrato basal, la ectosexina con delgadas columelas, la endosexina con columelas gruesas y ramificadas, las espinas conspicuas con bases globosas y notorios canales apicales, y la superficie del tectum muy perforada. Los recuentos cromosómicos resultaron en $2n = 34$. La evidencia morfológica y palinológica sitúa a *Warionia* entre las tribus Cardueae y Cichorieae, sugiriendo la posibilidad de que el género sea el remanente de un stock ancestral que dio origen a ambas tribus.

Palabras clave: África, Cardueae, Cichorieae, laticíferos, número cromosómico, polen, taxonomía.

and Algeria (Lewalle, 1986; Audissou, 1999) (Fig. 1). This is a thistle-like aromatic plant, with white latex, and pinnately-partite, somewhat fleshy leaves. The capitula are homogamous with tubular corollas, the anthers are caudate, and the style branches are dorsally covered by acute collecting hairs extending somewhat below the branches bifurcation.

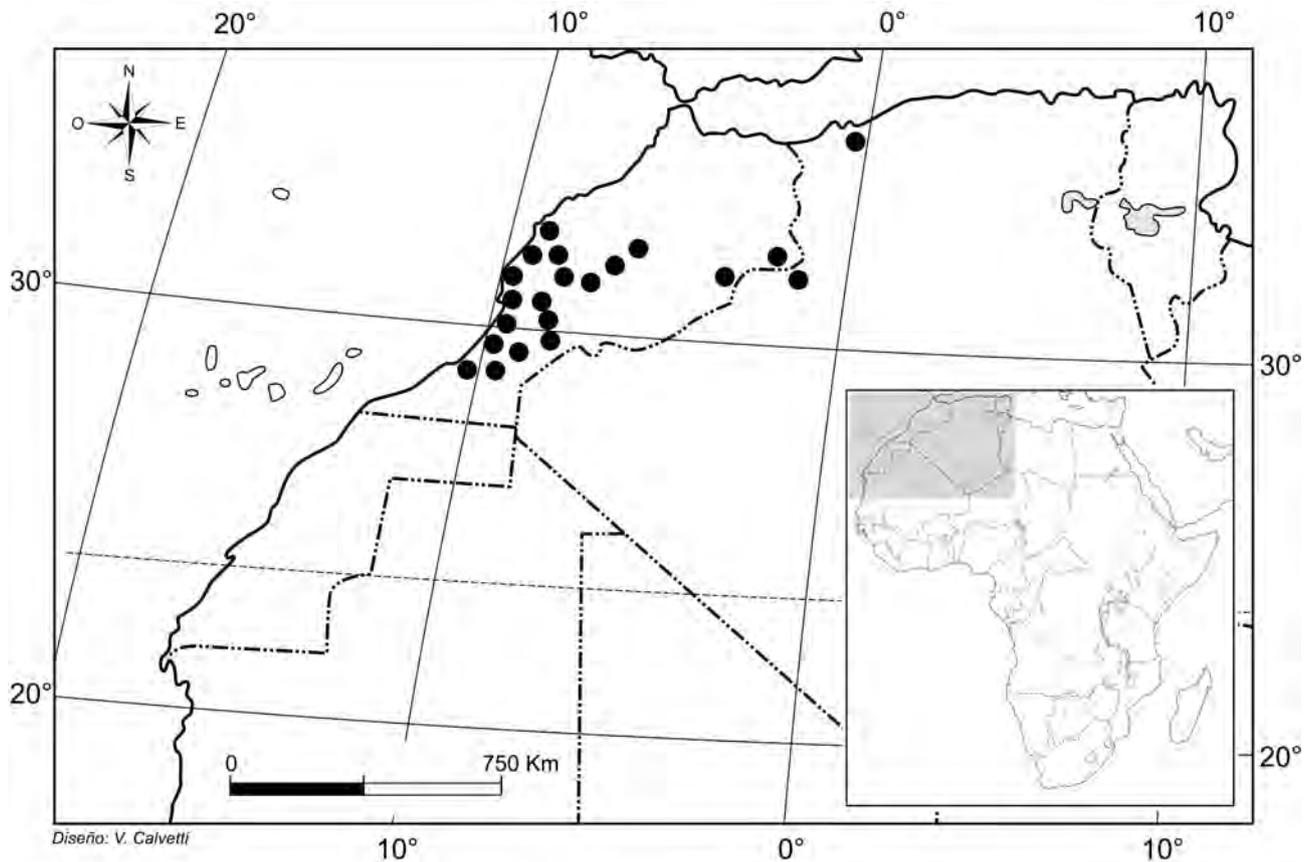


Fig. 1. Distribution map of *Warionia saharae*.

One peculiarity of this plant is the penetrating odor that some people find unpleasant, given by the glandular hairs in the epidermis of the leaves at the slightest contact. The plant is considered to have medicinal properties mainly by its essential oils (Watillon & al., 1987). Decoction of dried leaves is used as antirheumatic and against epileptic crisis (Bellakhdar & al., 1986). Crude extracts of the plants showed antibacterial and cytotoxic activities against a cancer cell line (KB cells) (Hilmi, 2002). In addition, the local women anoint themselves with the perfume of the leaves, and believe that the supernatural powers attributed to the plant make them more seductive (Audisou, 1999).

The occurrence of latex is another peculiarity of the plant. Members of Cichorioideae sensu lato produce characteristic triterpene-rich latex, which is unique in Asteraceae (Mabry & Bohlmann, 1977). Latex is found in all genera of the tribe Cichorieae, and in some members of the tribes Arctoteae (e.g., *Berkbeya* Ehrh., *Gazania* Gaertn., *Gorteria* L.), Cardueae (e.g., *Berardia* Vill., *Carlina* L., *Onopordum* L.), Gundelieae (*Gundelia* L.), and Liabeae (e.g., *Cacosmia* Kunth, *Erato*

DC., *Sinclairia* Hook. & Arn.) (Bremer, 1994; Lewinsohn, 1991). In addition, laticiferous cells were found in some Guayana-Highland centered genera of the tribe Mutisieae sensu lato, such as *Duida* S.F. Blake, *Gongylolepis* R.H. Schomb., *Neblinaea* Maguire & Wurdack, and *Quelchia* N.E.Br. (Carlquist, 1958).

Finally, the pollen of *Warionia* has another particular feature such as the exinal dimorphism detected by Dimon (1971), i.e., the specimens have pollen grains with different exine thickness; although this feature is not mentioned in other works (e.g., Wortley & al., 2007).

Warionia is in many respects so different from any other genus of Asteraceae, that it has been described and tentatively placed in several tribes. In the original description of the genus and its species, Bentham and Cosson (1872) included the new taxa in the Cardueae, but further authors (Dimon, 1971; Cabrera, 1977; Ditrlich, 1977) considered *Warionia* a member of the tribe Mutisieae sensu lato. Hansen (1991a), in his morphology-based cladistic analysis of Mutisieae sensu lato, suggested again a possible relationship of *Warionia* with the Cardueae by the acute style-hairs and the bell-

shaped corolla. The relationship with Cardueae was also supported in a scanning electron microscopy palynological study of Mutisieae (Hansen, 1991b) due to the spiny grains of *W. sabarae*. In the morphology-based cladogram of Karis & al. (1992), *Warionia* appears as an independent branch between the Cardueae and the Vernonioid complex (tribes Arctoteae, Cichorieae, Liabeae, and Vernonieae). Bremer (1994) placed *Warionia* among the genera unassigned to a tribe in the subfamily Cichorioideae. In a monographic study of the Mutisieae sensu lato (Katinas & al., in press), the exclusion of *Warionia* was confirmed. Some molecular phylogenetic studies place *Warionia* as sister to the tribe Cichorieae. Funk & al. (2004) placed *Warionia* in the tribe Gundelieae, together with the monotypic *Gundelia*. This branch had a low support, but the sister relationship of this tribe with Cichorieae resulted in 100 % support. *Gundelia* had been previously included in the Cichorieae by Karis & al. (2001). In the ITS molecular phylogenetic analysis of Goertzen & al. (2003) *Warionia* appears as the only member of a branch sister to the tribe Cichorieae, although in this case not well supported. This result was repeated in the supertree of Funk & al. (2005). Finally, the combined analysis of 10 chloroplast DNA loci of Panero & Funk (2008) yielded a tribe Gundelieae, constituted by *Warionia* and *Gundelia*, sister to Cichorieae with stronger statistical support.

Until now, a comprehensive study of *Warionia* to have a complete context for discussing its taxonomic position is lacking. The main references to *Warionia* are phytochemical studies and floristic treatments (e.g., Ozenda, 1991, Gómez, 2001), although there are references to pollen (e.g., Wortley & al., 2007), laticifers (e.g., Augier & Mérac, 1951) and chromosomes (e.g., Oberprieler & Vogt, 1993). A critical investigation of *Warionia* that gathers all the relevant information on the genus, i.e., morphological, anatomical, palynological, and chromosomal, is needed and have led us to the present study.

Materials and methods

Vegetative and reproductive organs were obtained from herbarium (BC, MA, MO, US; Holmgren & al., 1990) and living specimens. Plants for anatomical and part of the palynological studies were cultivated from seeds of the specimen *Romo & al. 10543* (BC) in the Greenhouse of the Botany Department, University of Wisconsin-Madison. Samples from herbarium specimens were reconstituted in boiling water. Whole foliar segments and phyllaries were cleared according to Strittmatter (1973). The midregion of plant organs

were isolated and free hand cut transversely. Some sections were stained with Safranin while the rest were left unstained. Observations and drawings of morphological and anatomical features were carried out on a Wild M5 stereomicroscope and on a Leitz SM Lux light microscope equipped with a camera lucida; the drawings of the plant were performed by the authors (LK). Photographs were taken with a Nikon Coolpix S10. For scanning electron microscopy (SEM), portions of styles of FAA-fixed samples were taken, critical-point dried in CO₂, and examined in a JEOL/EO JSM-6360.

Pollen grains of *Warionia sabarae* were obtained from herbarium specimens deposited at BC, MO, and US. Pollen of *Gundelia tournefortii* L. was also analyzed (*Barbey 522*, BC) to compare with the pollen of *Warionia sabarae*. For light microscopy (LM), pollen grains were acetolyzed and mounted in glycerol jelly. Measurements of equatorial and polar diameters exclude the spines. For scanning electron microscopy (SEM), acetolyzed pollen grains were suspended in 90 % ethanol, mounted on stubs, sputter-coated with gold palladium and examined with a JEOL JSM T-100 microscope. Pollen terminology follows Punt & al. (1994).

Chromosome counts were made on somatic metaphases using the squash technique. Young receptacles from plants cultivated in pots in the Botanical Institute of Barcelona from germinated cypselae collected in the field were used. Receptacles were coarsely triturated and pre-treated with 0.002 M 8-hydroxyquinoline solution at 4 °C for 8 h. After a distilled water wash, the material was fixed in fresh Carnoy I (3 : 1 v/v absolute ethanol: glacial acetic acid) overnight at -20 °C, and stored in 70% ethanol at -20 °C. This material was hydrolysed with 5 N HCl for 50 min at room temperature, washed with distilled water and stained with 1% acetic orcein and squashed in 45% acetic acid. Preparations were made permanent by freezing with CO₂, ethanol-dehydrating and mounting in Canada balsam. Metaphase plates were photographed using an Olympus 3030 digital camera mounted on an Olympus microscope U-TV1 X. The chromosome preparations are preserved in the Botanical Institute of Barcelona.

Results

Warionia Benth. & Coss., Bull. Soc. Bot. France 19: 165. 1872

TYPE: *Warionia sabarae* Benth. & Coss.

A monotypic genus distributed in northwestern Africa, in Morocco and western Algeria.

Etymology: The generic name is derived from (Jean Pierre) Adrien Warion (1837-1880), a French military physician and botanist who made extensive collections while stationed in North Africa.

Species description

Warionia saharae Benth. & Cosson, Bull. Soc. Bot. France 19: 166. 1872

TYPE. Morocco: Sahara Marocain, Figuig, coteaux rocailleux, 1 Avril 1866, *Warion* (lectotype, designated here, P; digital photograph LP!). **Algeria:** S. O. pr. d'Oran, Tyout, roches, 22-9-1865, *Warion* (syntype, P; digital photograph LP!); sud de la prov. d'Oran, Bou Semghroun, coteaux rocailleux, en allant vers le Dj. Lembah, 9 fevrier 1865, *Warion* (syntype, P; digital photograph LP!). (Figs. 2, 3)

Shrubs 0.15-3 m high, with a taproot, aromatic, with white, milky latex, anastomosed laticifers; stems with corky, grey bark, with 2-seriate glandular hairs when young. Leaves 2-13 cm long, 1-3 cm wide, oblong to oblanceolate, alternate, somewhat fleshy, sinuate to pinnately-partite, lobes mucronate, pinnately-veined, acute at the apex, basally attenuate, sessile, pubescent, glandular hairs especially at the margins. Capitula solitary or in 2-3-headed cymes, 3-4 cm long, 4.5-6 cm wide, campanulate, homogamous, discoid; receptacle flat, epaleate, alveolate, glabrous; involucre 4-5-seriate, phyllaries green, purple at the apex, pubescent especially at the margins; first series 6-7 mm long, 1.5-2 mm wide, lanceolate, longly attenuate at the apex; second series 8-9 mm long, 2-2.5 mm wide, lanceolate, longly attenuate at the apex; third series 11-13.5 mm long, 2-2.5 mm wide, lanceolate, attenuate at the apex, margin scarious, involute; fourth series 17 mm long, 2 mm wide, oblong-linear, attenuate at the apex, margin scarious, involute; fifth series 21-23 mm long, 1.5-2 mm wide, oblong-linear, attenuate at the apex, margin scarious, involute. Florets 25-40, bisexual, corolla yellow, 22-25 mm long, actinomorphic, campanulate, deeply 5-lobed, occasionally with some deeper splits, straight or S-shaped, tube 10-11 mm long, narrow, abruptly dilated into a limb, lobes 7-8 mm long, coiled, pubescent, with a tuft of hairs at the apex, twin hairs and 2-seriate glandular hairs. Anthers 11-12 mm long, basally sagittate, tails 1.5-2 mm long, adjacent tails connate, glabrous, apical appendage 2 mm long, lanceolate, acute, endothelial tissue radial, filament of stamens glabrous, inserted at the corolla throat. Style 24-26 mm long, bifid, yellow, with stylopodium, basal nectary, branches 4-6 mm long, linear, radially arranged, vernonioid type of style with the inner sur-

face completely covered by stigmatic papillae, outer surface covered by acute sweeping hairs extending somewhat below the branches bifurcation point. Cypsela 4.5-5 mm long, 1.5-3 mm wide, truncate at the apex, with apical rim, epicarp cells with elongated crystals, shaggy, with white, long, filiform, non-mucilaginous twin hairs, occasionally 2-seriate glandular hairs. Pappus biseriate, white-yellowish, caducous, bristles rigid, somewhat paleaceous, denticulate, inner and most of the outer series with bristles 15-18 mm long, part of the outer bristles shorter, 2.5 mm long. Pollen spheroidal to suboblate, large size, tricolporate, exine ecaveate, echinate, tectum very perforate. $2n = 34$.

Etymology: The specific epithet refers to the distribution area, in the African Sahara desert.

Distribution and ecology: *Warionia saharae* is endemic to northwestern Africa (Fig. 1), in Morocco, including Ijoukaka (High Atlas), Cape Ghir, Tamanar, Tazerwalt, Tiznit, Agadir, Ifni, Bou Izakarn, Oued Ouarksiz, Figuig, and Erfoud, and in Algeria at Béni-Ounif (South Oran). This species grows on slopes of the High Atlas, Anti-Atlas and Saharian Atlas, in the coast of western Morocco, and in desert areas on basic and siliceous rocks, from 0 to 1300 m.

Biology: Flowering is abundant and lasts several months. The flowering season has been recorded from April to June, while it may extend to July or August if the spring rains are abundant and well spaced (Audissou, 1999; Gómez, 2001). In cultivation (USA) the plants flowered in November. The florets have perfume. In habitat *W. saharae* loses its leaves in the dry season (Audissou, 1999).

Observation: There are two specimens at herbarium K, labelled as types in the Royal Botanic Gardens Kew database (accessed: September 2008), collected by Merdoché in southwestern, in 1875. They have a label with the inscription: "Par les soins de M. Beaumier. Herb. E. Cosson, Recd. 8/77". Despite the fact that they might belong to Cosson's collections, these specimens do not correspond to type specimens because they have a different place of collection and collector than those indicated in the protologue.

Representative specimens

AFRICA. Algeria: Beni-Ounif, V-1922, *s. leg.* (BC 137845). **Morocco:** Grand Atlas, cuestas à Imerghdal, Gontafa, 4-VI-1936, *Gattefosé 1340* (MO); Anti-Atlas, above Ait Baha, 30°5'N, 9°20'W, 4-VI-1974, *Reading Univ./BM Exped. 278* (MO); province d'Agadir, Oued Noun, 25 km W Goulimine, 10°14'W, 28°59'N, 1986, *Förther s.n.* (US 3299743); environs de Mogador, Djebel Amsitten, sur la piste d'Agadir, 6-IV-1921, *Romieux 1367* (MO); inter Marrakech et saltum Tizi n°Test dictum, pr. oppidulum Tagadirt n°Bour, 31°09'N,

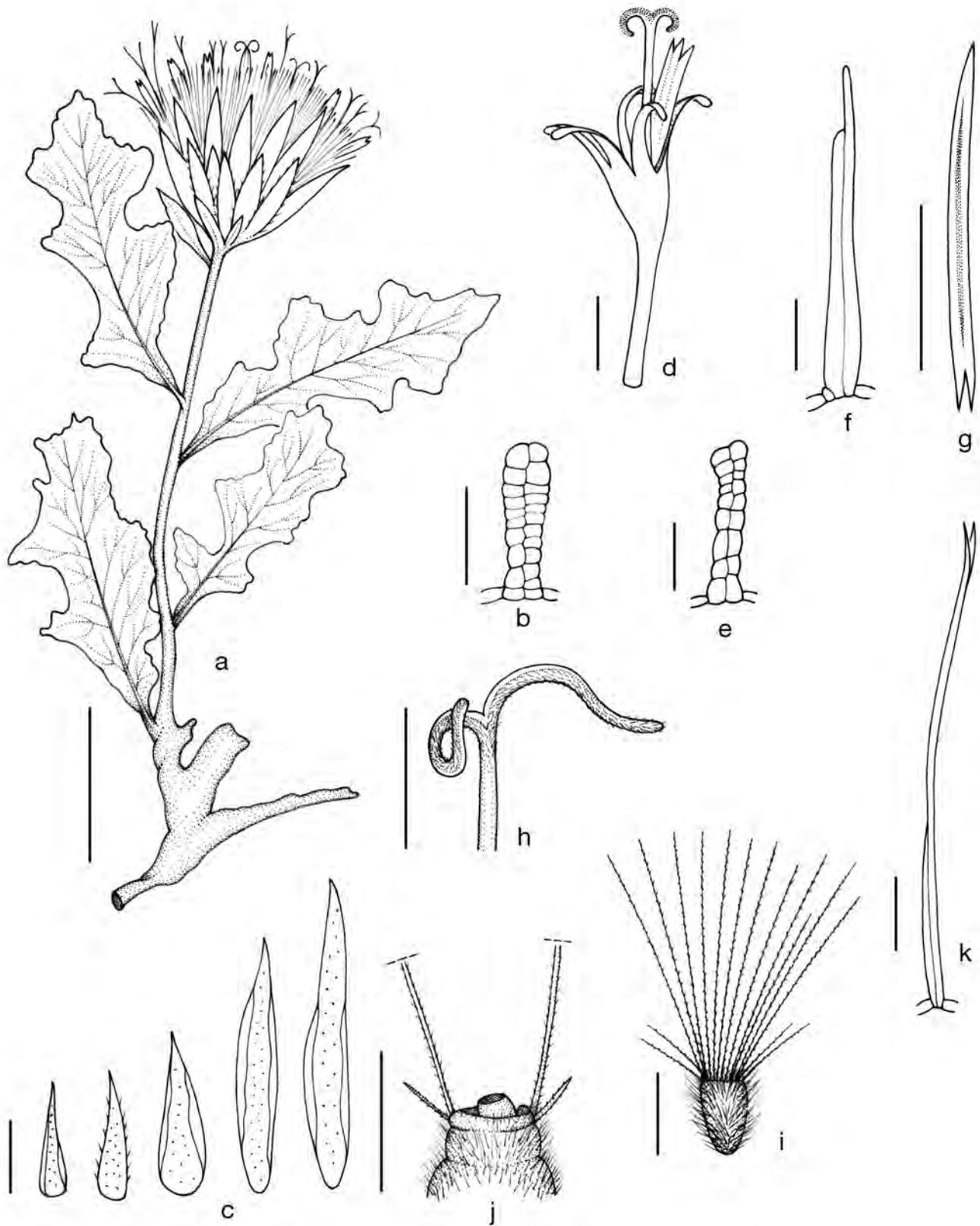


Fig. 2. *Warionia saharae*: **a**, habit; **b**, stem glandular hair; **c**, phyllaries of the involucre (outer phyllaries to the left, inner phyllaries to the right); **d**, floret; **e**, corolla glandular hair; **f**, corolla twin hair; **g**, stamen without filament; **h**, upper part of the style; **i**, cypselas and pappus; **j**, upper part of cypselas; **k**, cypselas twin hair. [A, Bianchi & al. 9457 (BC); B, Blanchet & al. s.n. (BC 813743); C, I, cultivated from seeds of Romo et al. 10543 (BC); D-G, J, K, Förther s.n. (US 3299743); H, Gatefossé 1340 (MO)]. Scale bars: A = 3 cm; B = 100 μm; C, D, G, I = 5 mm; E, F = 50 μm; H, J = 3 mm; K = 200 μm.

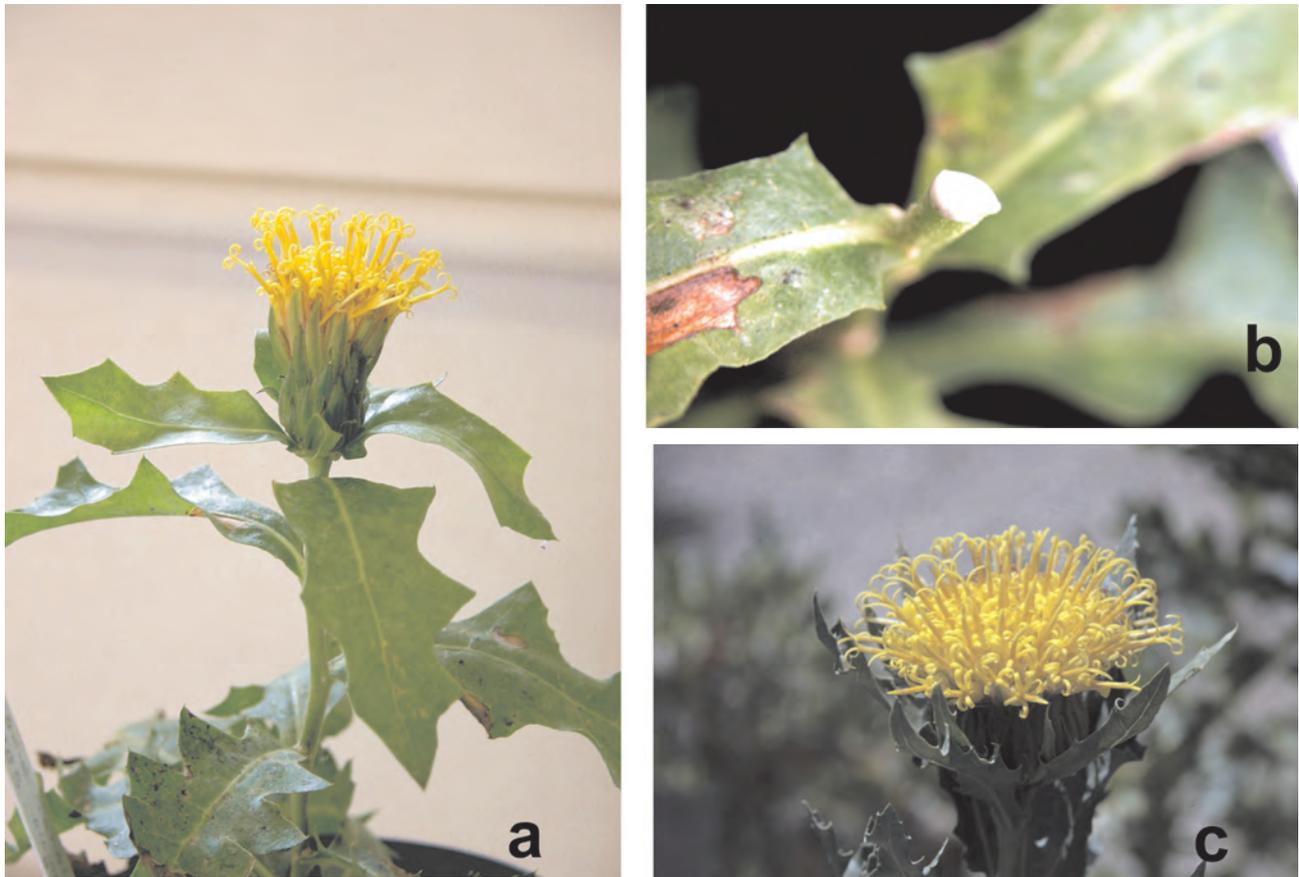


Fig. 3. *Warionia saharae*: **a**, plant in pot; **b**, sectioned stem showing the white, milky latex; **c**, detail of the capitulum [a-c, cultivated from seeds of Romo & al. 10543 (BC)]. Photographs: a, b, L. Katinas; c, A. Susanna.

8°6'W, 27-V-1985, *Blanché & al.* 9457 (BC); S side of Tizi n'Test mountain pass, 24-VII-2002, *Romo & al.* 10543 (BC); Agadir, vallée d'Imouzzev des Ida-ou-Tamame, entre Aït Bifradane et Aït Bauka, 10-VI-1988, *Molero & al. s.n.* (BC 820340); Solana cerca de Tafraoute (Tiznit), 23-IV-1994, *Gómez s.n.* (BC 831919); Taz-zouguert, IV-1923, *Humbert s.n.* (BC 137846).

Anatomy

Leaf transection (Fig. 4 a, b) shows an adaxial and abaxial epidermis with 2-seriate glandular hairs and slightly exerted stomata of the anomocytic type (seen in paradermal section). There is a layer of hypodermis on the adaxial surface. Collenchyma at the midvein level forms extensions from the hypodermis and the abaxial epidermis to the three vascular bundles. The mesophyll consists of a central layer of colorless parenchyma, an upper, and a lower layer of palisade. A sheath of sclerenchyma fibers surrounds the veins. Laticifers are closely associated with the phloem, in contact with it or with the surrounding sclerenchyma sheath. Paradermal sections of leaves show the laticifers associated with the vascular tissue. Parenchyma cells separate tangentially anastomosed strands of laticifers.

The primary stem in transection (Fig. 4 c, d) is characterized by the epidermis covered with scarce 2-seriate glandular hairs, followed by a layer of collenchyma. Vascular bundles are embedded in the parenchyma, and surrounded by sclerenchyma sheath. Lignified parenchyma constitutes the pith. Laticifers with dense content are somewhat larger than the neighboring cells, and are present in the cortex surrounding the sclerenchyma sheath of the phloem.

Pollen

Pollen is radially symmetrical and isopolar; spheroidal to suboblate; more or less circular in equatorial and polar view (Figs. 5 a-d, 6 a, b); large sized (see below); tricolporate; ora lalongate, lolongate or more or less circular; mesoaperturate. Colpi are long with acute ends and psilate membrane.

Exine is tectate, echinate, ca. 12 µm thick at the equator slightly slender at the poles; spines are ca. 7 µm long, with globose bases, acute or rounded tips and conspicuous apical channel (Fig. 6 a-d); tectum surface is very perforate excepting the spine tip. Ectosexine

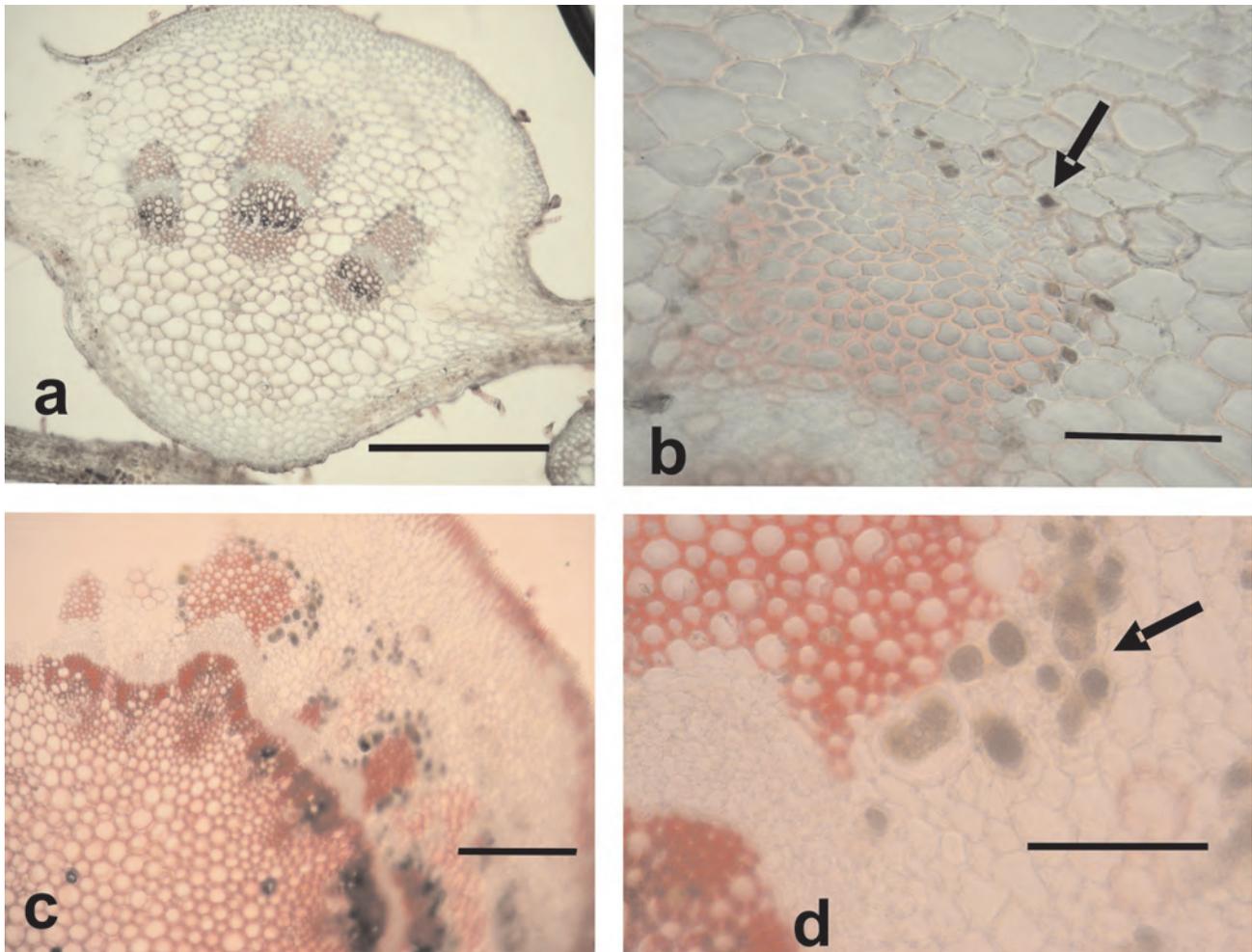


Fig. 4. LM photographs of leaf and stem anatomy of *Warionia saharae*: **a, b**, leaf transection; **a**, midvein level, note the epidermal glandular hairs, and the laticifers surrounding the sclerenchyma tissue; **b**, detail of the laticifers (arrow); **c, d**, stem transection; **c**, some vascular bundles and the laticifers surrounding the sclerenchyma sheath of the phloem; **d**, detail of the laticifers (arrow). [a-d, cultivated from seeds of *Romo & al.* 10543 (BC)]. Scale bars: a = 500 μ m; b = 100 μ m; c = 300 μ m; d = 75 μ m.

presents thin columellae, and ectosexine stout and ramified columellae (Fig. 6 c); nexine is slightly thickened at level of apertures.

Pollen size measurements are: *Förther s.n.* (US 3299743): P \times E = 78-83 \times 78-89 μ m; *Gómez s.n.* (BC 831919): P \times E = 54-73 \times 61-75 μ m; *Romieux 1367* (MO): P \times E = 49-56 \times 54-62 μ m; *Romo & al.* 10543 (BC) (cultivated): P \times E = 70-75 \times 63-74 μ m.

Pollen size is variable among specimens, and strongly variable in the specimens *Gómez s.n.* (BC) and *Gattefosée 1340* (MO). Pollen grains from *Gattefosée 1340* (MO) are unusually spiny (and therefore, it was not possible to take measures of the diameters) having two types of spines: (1) acute tips, and (2) rounded tips or dome-shaped apex (Fig. 6 d). Both spine types have many apical channels. Rounded spines are rare in Asteraceae, generally spines have an acute tip. Similar spine morphology was observed

in apparently aborted pollen of some *Eupatorium* species (Skvarla & al., 2001), and it was attributed to stress conditions during pollen development.

Chromosomes

Our results agree with three previous counts (Reese, 1957; Humphries & al., 1978; Oberprieler & Vogt, 1993; recorded in Watanabe, 2007), all of them with $2n = 34$ (Fig. 7). The chromosome number $n = x = 17$, frequent in Asteraceae (Jeffrey, 2007a), is very common in Cardueae (e.g., *Cirsium*, *Cynara*, *Jurinea*, *Notobasis*, *Onopordum*) but very high for the Cichorieae, in which $x = 9$ (and lower numbers) predominates, with a few genera having $x = 10$ (Lack, 2007). Perhaps the most striking feature is the extreme asymmetry of the karyotype. The combination of a very high chromosome

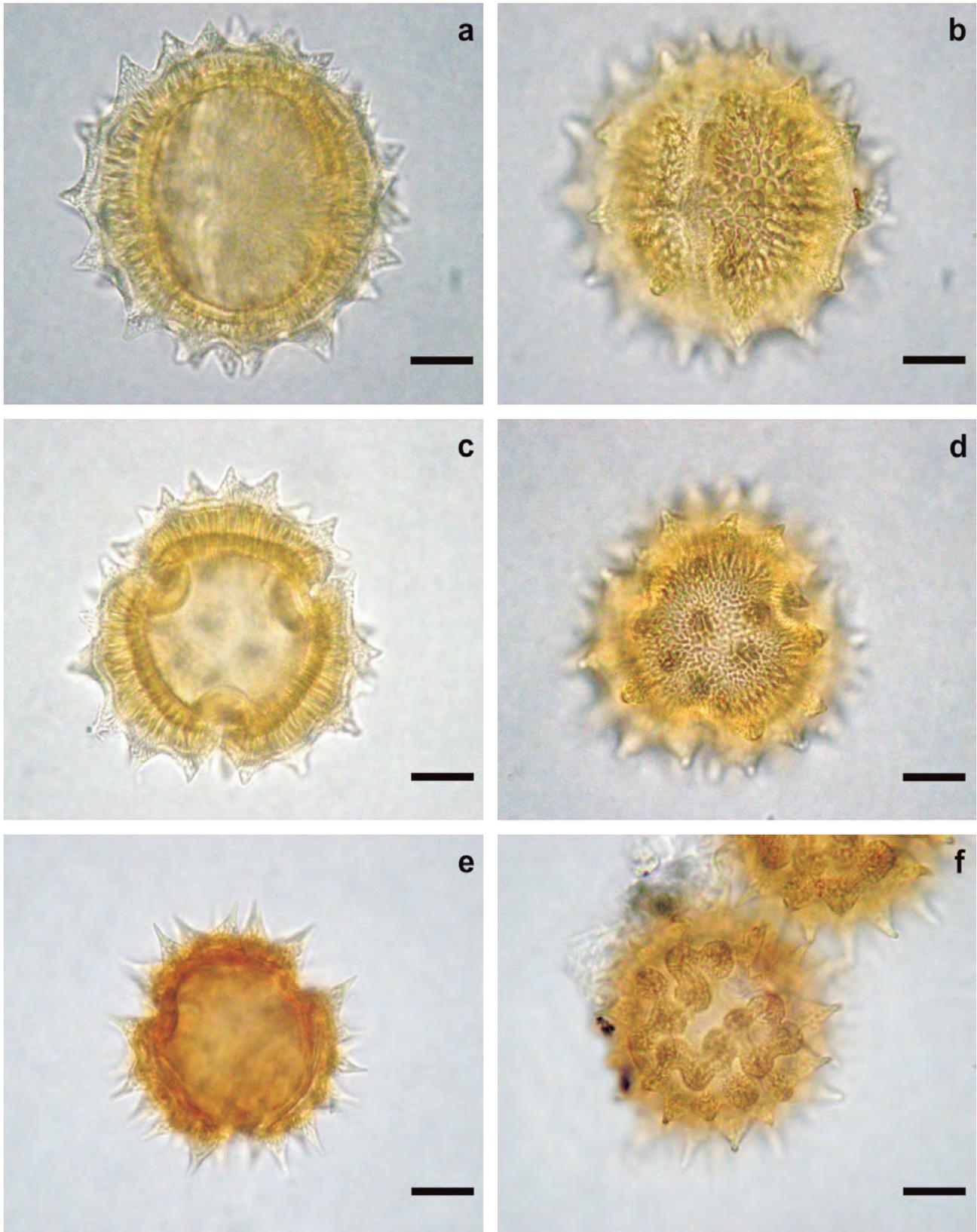


Fig. 5. LM photographs of pollen of *Warionia saharae* and *Gundelia tournefortii*. **a-d**, *Warionia saharae*: **a**, equatorial view, optical section showing the wall structure; **b**, equatorial view, intermediate focus showing thick columellae in section; **c**, polar view, optical section; **d**, polar view, intermediate focus. **e, f**, *Gundelia tournefortii*: **e**, polar view showing the wall structure; **f**, slightly tilted polar view showing the spines fused at the basis. [a-d, Romo & al. 10543 (BC); e, f, Barbey 522 (BC)]. Scale bars: a-f = 15 μ m.

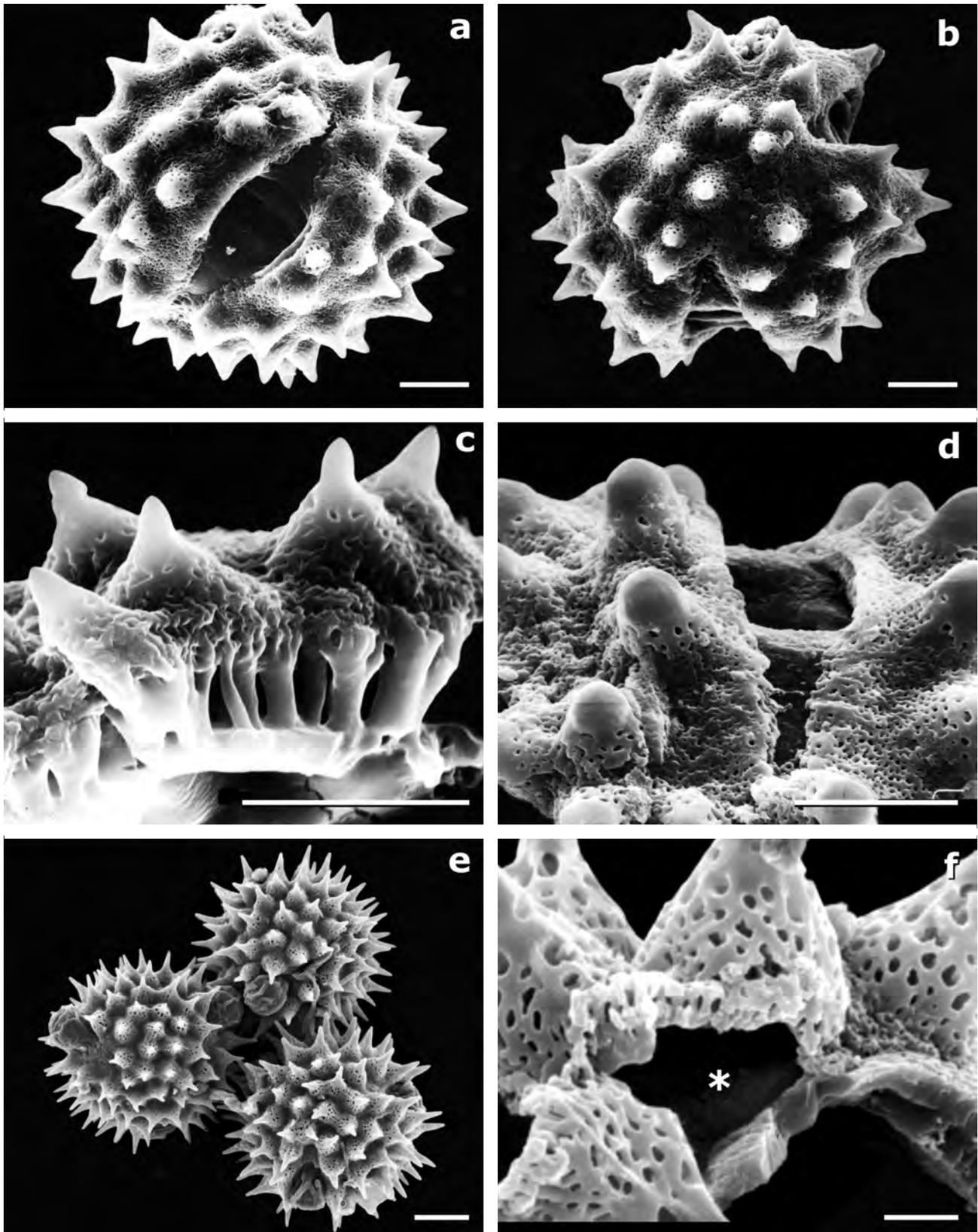


Fig. 6. SEM images of pollen of *Warionia saharae* and *Gundelia tournefortii*. **a-d**, *Warionia saharae*; **a**, equatorial view showing the aperture; **b**, polar view; **c**, detail of pollen wall with Anthemoid pattern in a broken grain; **d**, detail of rounded spines of pollen. **e, f**, *Gundelia tournefortii*; **e**, general aspect of pollen grains; **f**, detail of pollen grain with Heliantoid pattern in a broken grain, *cavus. [a-c, Förther s.n. (US 3299743); d, Gattefosée 1340 (MO); e, f, Barbey 522 (BC)]. Scale bars: a-e = 10 μ m; f = 2 μ m.

number and extreme asymmetry suggests processes of dysploidy in *Warionia*. Descending dysploidy was first detected and classically studied in another Cichorieae genus, *Crepis* L. (Stebbins, 1971). We suggest two events of polyploidy followed by dysploidy to explain both asymmetry and high number. A first duplication of an ancestor with $x = 5$ would have led to $x = 10$, which by descending dysploidy would have resulted in $x = 9$. A second polyploidization event would result in $x = 18$ followed by the loss of a chromosome by dysploidy.

Discussion

This is the first global study of *Warionia*. Although previous chromosome counts in *Warionia* were reported (Reese, 1957; Humphries & al., 1978; Oberprieler & Vogt, 1993) and some pollen studies developed (Dimon, 1971; Wortley & al., 2007), we re-analyzed these features. We were particularly interested in comparing the pollen of *Warionia* with that

of *Gundelia* in order to add another criterion for analyzing the taxonomic relationship between both genera. Considering this information together with the existing molecular data (e.g., Goertzen & al., 2003; Funk & al., 2004) we intended to provide a general overview for discussing the systematics of the genus. In this sense, it is crucial that we discuss some points here: the sister relationship between *Warionia* and *Gundelia*, the tribal position of *Warionia*, and some insights about the probable evolution of the genus.

The Warionia-Gundelia affinity

Gundelia with its unique species *G. tournefortii* was considered by Robinson (1994) the unique genus of the tribe Gundelieae, and he considered it a connecting link between Arctoteae and Cardueae. Later, Funk & al. (2004) added *Warionia* to Gundelieae, and showed the tribe sister to Cichorieae. The thistle-like habit of *Gundelia*, the presence of latex, the spurred

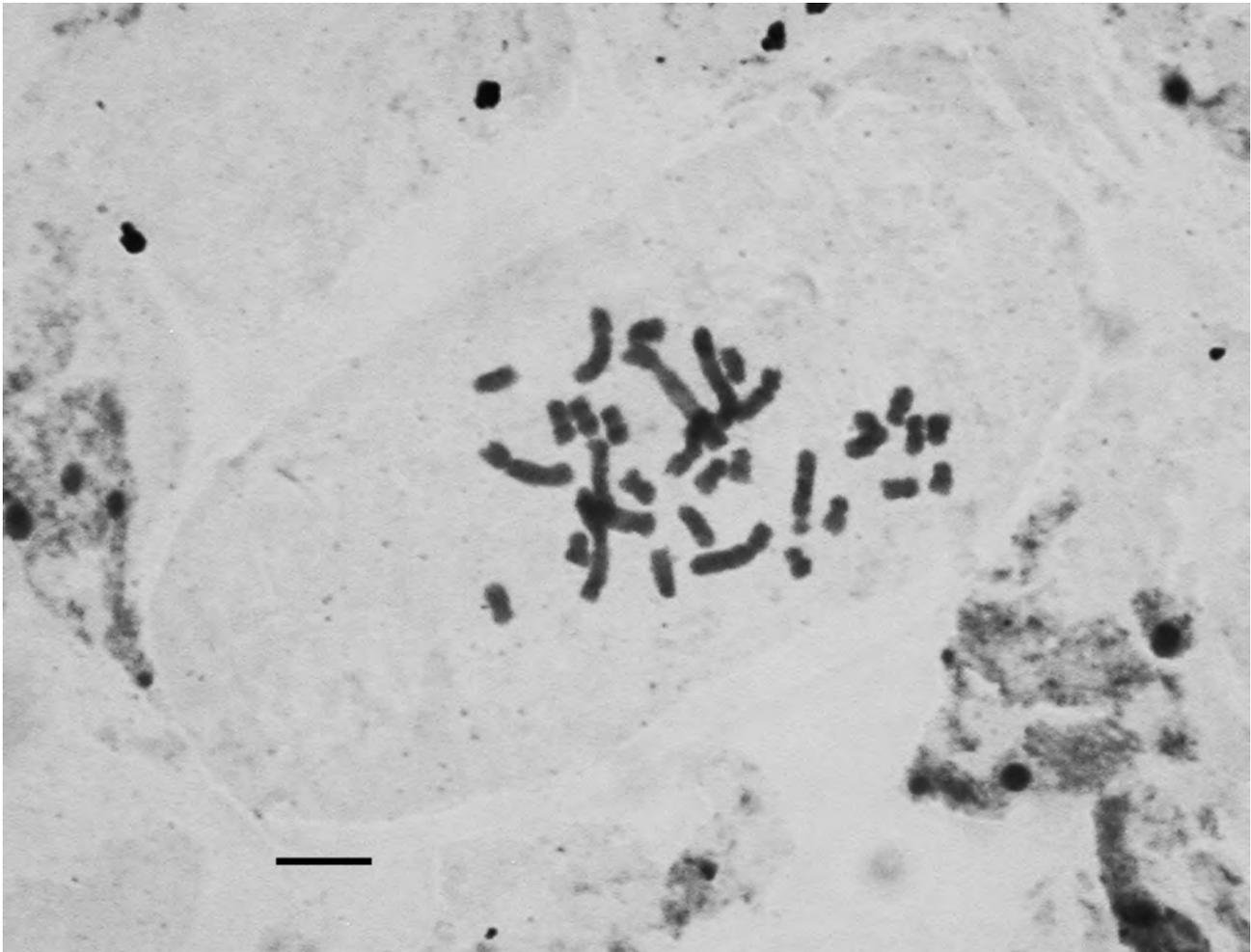


Fig. 7. Somatic metaphase of *Warionia saharae*, $2n = 34$ [pop. Romo & al. 10543 (BC)]. Scale bar: 10 μ m.

anther thecae, and the vernonioid style (Karis & al., 2001) would suggest some resemblances with *Warionia*. However, *Gundelia* also shows important morphological differences regarding *Warionia* such as the secondary heads (vs. solitary or 2-3-headed in *Warionia*), the functionally male peripheral florets (vs. all bisexual florets), the annuliform pappus (vs. capillary pappus) and, most important, the pollen features. Pollen of *G. tournefortii* has Helianthoid pattern, distinguished mainly by the presence of cavea (Fig. 6 f) (Skvarla & al., 1977; Robinson, 1994; Wortley & al., 2007), where the layer of columellae is separated from the foot layer from aperture to aperture. *Warionia sabarae*, on the other hand, has an Anthemoid or ecaveate pattern (Fig. 6 c). Although the pollen sculpture is echinate in both genera, they differ in the shape of the spines. In *Gundelia*, the spines are conical with subglobose-shaped bases (Fig. 6 e), whereas in *Warionia* the spines are mameliform with globose-shaped bases (Fig. 6 a, b). Another remarkable feature of the spines of *Gundelia* is their tendency to unite at their bases (Fig. 5 f) as happens in species with subechinolophate pollen of Cichorieae, Vernonieae, and Arctoteae (Blackmore, 1984; Skvarla & al., 1977). We agree with Wortley & al. (2007) in that it is strongly incongruent to link *Warionia* and *Gundelia* into the tribe Gundelieae. Our observations suggest that the pollen morphology of *Warionia* shows a close relationship with the *Serratula* type of Cardueae (Wagenitz, 1955), whereas pollen of *Gundelia* shows that it is related to some Cichorieae, Vernonieae and Arctoteae.

Molecular phylogenetic analyses are not congruent regarding the degree of support of the branch *Warionia-Gundelia*. Combined molecular data (*trnL-F*, *ndhF*, ITS) display weak support (less than 50%) for the sister relationship between both genera (Funk & al., 2004), whereas the strict consensus tree resulting from maximum parsimony of combined data of 10 chloroplast loci (Panero & Funk, 2008) shows higher support (86%). Based on the overall evidence, we consider *Warionia* to be a separate lineage of *Gundelia* and thus *Warionia* should be excluded from Gundelieae.

Tribal position of *Warionia*

Warionia, as commented before, was included by some authors (Dimon, 1971; Cabrera, 1977; Dittrich, 1977) within the Mutisieae sensu lato. Some characters coincide with those of the African Mutisieae, principally with the members of the tribe Dicomeae (Ortiz, 2000, 2001), whereas many characters of *Warionia* show a departure from that group. A comparison be-

tween *Warionia* and the African Dicomeae shows that: (1) Leaves of *Warionia* are sinuate to pinnately-partite whereas all the African Dicomeae have entire leaves; (2) *Warionia*'s involucre is rather similar to that of the core Dicomeae (*Dicoma* Cass., *Macleodium* Cass., *Cloiselia* S. Moore, *Pasaccardoa* Kuntze) but its phyllaries are not coriaceous and have not an acuminate-pungent apex as in those genera; (3) *Warionia*'s corolla tube dilates abruptly into the limb (being very similar to that of the genera *Pleiotaxis* Steetz and *Erythrocephalum* Benth. and *Pasaccardoa*) whereas in *Dicoma* and *Macleodium* that separation is gradual; (4) the corolla epidermal cell cuticle ornamentation is conspicuously longitudinally striate and transversely undulate ("intestine-like", see Karis & al., 1992) as in almost all the genera of the Dicomeae. The corolla lobes of *Warionia*, however, do not have thick-bundled apical veins, whereas these are present in the African Dicomeae (with the exception of some species of *Dicoma* and *Pleiotaxis*); (5) the anther apical appendage of *Warionia* is acute and thin, whereas in Dicomeae it is apiculate to acuminate and enlarged; (6) style of *Warionia* is covered by acute sweeping hairs as in almost all the African Dicomeae, but extending somewhat below the branches bifurcation point, whereas in the African Dicomeae the sweeping hairs are subapical, apical or lacking. Katinas & al. (in press), finally excluded *Warionia* from the Mutisieae s. l. mainly on the basis of style and pollen features.

The morphological and palynological evidence would place *Warionia* in a middle position between the tribes Cardueae and Cichorieae, since the genus has features of both tribes (Table 1).

The characters that link *Warionia* with Cardueae are mainly the type of corolla, the morphology of the cypsela apex, and the pollen features. The thistle-like habit of these plants, with its coarsely lobed leaves and large capitula of campanulate corollas probably led Bentham and Cosson (1872) to include *Warionia* in the Cardueae and relate it with the European genus *Berardia*. The thistle habit, however, may also be found in genera of Cichorieae. The discoid, homogamous capitula, with all campanulate florets are another feature typical of the Cardueae. Also, S-shaped corollas, as described by Dittrich (1977) for some genera of Cardueae, are found in *Warionia*. The apical plate at the end of the cypsela pericarp in *Warionia* is also characteristic of the Cardueae, as described by Dittrich (1977). Apically, the cypselae of *Warionia* have a crown-like structure or apical plate upon which are inserted the pappus and the nectary disc. More importantly, the pollen features indicate a strong relation with Cardueae, namely the structure with Anthemoid

Table 1. Comparison of the main morphological and palynological characters between *Warionia saharae*, and the members of the tribes Cardueae and Cichorieae.

Character	<i>Warionia saharae</i>	Cardueae	Cichorieae
Laticifers	present	absent, occasionally with latex sacs	present
Corolla morphology	tubular	tubular	ligulate
Style: type	vernonioid	carduoid	vernonioid
branches orientation	radial	radial	tangential
Pollen: structural pattern	anthemoid	anthemoid, helianthoid	helianthoid
sculpture	echinate	echinate, microechinate	echinate, echinolophate, subechinolophate
spines shape	with globose base	with globose base	conical with sharp end

pattern where the columellae are joined to the foot layer, the ectosexine with thin columellae, the endosexine with stout and ramified columellae, the conspicuous spines with globose bases and conspicuous apical channels, and the tectum surface very perforate. Its highly perforate exine surface led Zao & al. (2006) to relate the pollen of *Warionia* with some *Gochnatiinae* (tribe Mutisieae s. l.). Pollen of *Warionia* can be placed among the echinate types of Cardueae (Tormo & Ubera, 1995) and, to some degree, it is related to the genus *Macroclinidium* Maxim. (tribe Mutisieae s. l.), which was regarded near the Cardueae by Katinas & al. (in press). The pollen of *Warionia* is very different from that of Cichorieae whose members have cavate exine (helianthoid pattern) with either echinate, echinolophate (with large window-like spaces lacking a tectum, and echinate ridges), or subechinolophate sculpture. When echinate, the spines do not have globose bases, as occurs in *Warionia*. Another feature that would link *Warionia* with Cardueae is the radially arranged style branches (Fig. 3 a, c), different for example from the tangentially arranged branches in other tribes such as Cichorieae (Robinson, 1984). The radial condition of the style occurs when the branches diverge along a radius of the head, and the tangential condition when the branches spread laterally and transversely to a radius of the head.

On the other hand, the characters that link *Warionia* with Cichorieae are the presence of laticifers, the type of style, and the molecular evidence. Although latex has been reported in Cardueae, it is stored in latex sacs rather than in elaborate laticifers as in the Cichorieae (Metcalf & Chalk, 1965; Bremer, 1994). Leaves and stems of *Warionia* have conspicuous laticifers. As hap-

pens with the palynological evidence, the style features are another important criterion when reevaluating the systematic position of taxa. *Warionia* has the typical vernonioid style of Cichorieae, Liabeae and Vernoniaeae, very different from the carduoid style characterized by a ring of hairs on the shaft below the branches. The style of *Warionia* has slender, long, filiform style branches and acute collecting hairs, distributed all along the branches extending down on the shaft of the style (Fig. 8). Finally, the molecular data represents another line of evidence regarding the position of *Warionia* close to Cichorieae. In the ITS analysis of Goertzen & al. (2003), *Warionia* appears at the base of Cichorieae, sister to the genera of the tribe although not well supported. In the phylogenetic analyses of Funk & al. (2004) and Panero & Funk (2008), the position of *Warionia* is also basal to Cichorieae, with higher support; in the later study, the Gundeliaeae branch, which includes *Warionia* and *Gundelia*, has the lowest support value when compared with the other tribes of the tree.

In summary, the morphological and palynological evidence positions *Warionia* between the tribes Cardueae and Cichorieae, whereas the phylogenetic molecular analyses place the genus at the base of the tribe Cichorieae. These two views are not contradicting but complementary, suggesting the following possibilities: (1) to regard *Warionia* as a primitive, basal genus of Cichorieae, or (2) to regard *Warionia* as the only member of a new, distinct tribe, sister to Cichorieae. The second option would need fewer assumptions regarding evolutionary changes of key features within Cichorieae, such as type of corollas and pollen grains. However, we cannot disregard the molecular data and

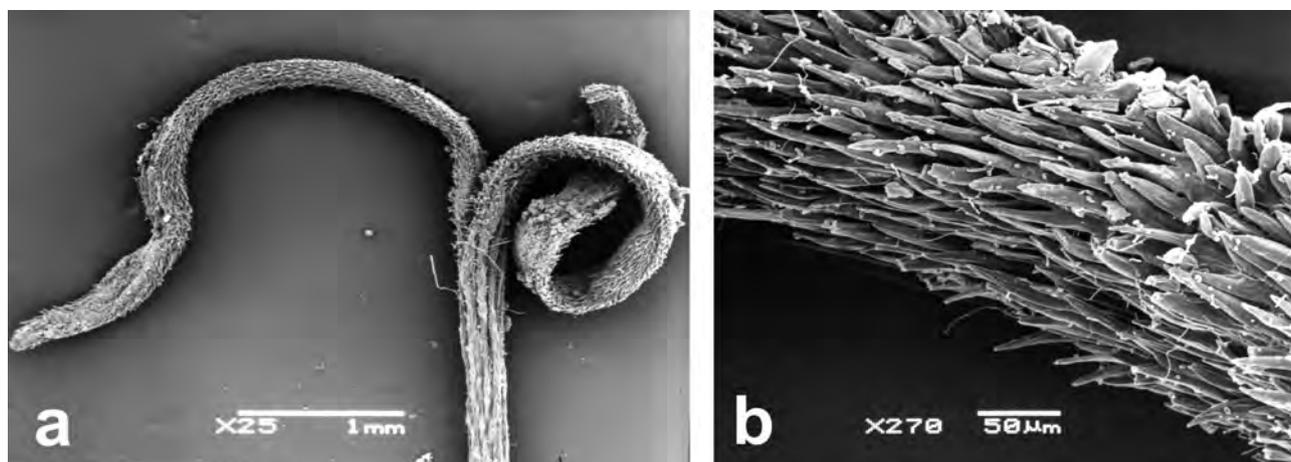


Fig. 8. SEM photographs of the style of *Warionia saharae*: **a**, upper part of the style showing the vernonioid type morphology; **b**, detail of one branch showing the acute collecting hairs. [a, b, Gattefossé 1340 (MO)].

prefer to maintain *Warionia* as a member of Cichorieae until new molecular studies determine other position.

Evolution of *Warionia*

Direct dispersal easterly from South America to Africa was postulated by Panero & Funk (2008) as a plausible sweepstakes route across the Atlantic where early lineages of Asteraceae reached Africa. This may have given origin to the earliest African branching clades outside South America. African clades are represented by Tarchonantheae, Dicomeae, and *Warionia*; mostly African/Eurasian clades are Cichorieae, Gymnarrheneae; and the Cardueae are a Mediterranean/Central Asian clade. According to Panero & Funk (2008) the African dispersal gave rise to explosive radiation of Asteraceae across the world. The basal lineages of Cardioideae and Cichorioideae appear to be African or Mediterranean. Understanding the means by which *Warionia saharae* evolutionary arose is a challenge. Some chromosomal rearrangements may trigger speciation (Levin, 2002), particularly when geographical barriers to gene flow are absent. In these situations, the rearrangements may decrease gene flow sufficiently among diverging populations to allow selected differences or hybrid incompatibilities to accumulate (Riesberg & al., 1999). Hybrid speciation by means of allopolyploidy in which reproductive isolation arises as an instantaneous by-product of hybrid genome duplication could be one of such mechanisms. It is not possible to formulate any founded hypothesis about hybrid speciation in *Warionia* between parental species of Cardueae and Cichorieae since it would require, for example, crossing experiments. It is also possible that

from an ancestral stock some genera remained in isolated pockets as relicts, conserving primitive set of features. The Sahara endemic *Warionia* could be a remnant of the ancestral stock that gave rise to Cardueae and Cichorieae, among other groups, and conserved morphological features of both tribes. Other genera of Asteraceae appear to have undergone similar processes. In North America, for example, Panero & Funk (2008) postulated that the monotypic genus *Hecastocleis* A. Gray endemic to western United States, linked to Mutisieae, could be the only relict of an Asteraceae lineage that reached North America from the Old World probably via the Bering land bridge. Jeffrey (2007b) placed the Asian *Dipterocome* under the carduoid genera of uncertain placement. He postulated that the carduoid pollen and cypsela features are probably plesiomorphic in *Dipterocome*, and that this genus, like the north African and Middle East *Gymnarrhena*, would lie at or below the base of Cichorioideae as a relict of a distinct line. Thus, monotypic genera such as *Dipterocome*, *Gymnarrhena*, *Hecastocleis*, and *Warionia* may illustrate that not all sweepstakes dispersing organisms may be equally successful in terms of speciation after colonization, depending on where and when they arrive and on their capacity to compete with other organisms.

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