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Psoralea bituminosa var. *atropurpurea* (Psoraleae, Fabaceae) from Morocco recognised as a distinct species in *Bituminaria*

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Abstract

Within taxonomic studies on the genus *Bituminaria* in Morocco, a population previously attributed to *Psoralea bituminosa* var. *atropurpurea* is examined. Basing on morphological investigations carried out on living and herbarium material coming from the type locality, it is treated as a distinct species and here proposed as *Bituminaria atropurpurea* comb. et stat. nov. This species differs from *B. bituminosa* s.str., as well as from other known taxa of the genus, in several features of the leaves, inflorescence, flowers and pods, as well as the micro-morphology of seeds, pods and pollen grains. Its distribution, ecology and relationships with *B. antiatlantica* and other allied species, such as *B. basaltica* and *B. tunetana*, are also examined. Besides, its detailed iconography and an analytical key of the species currently ascribed to this genus are provided.

Keywords: Endemic, Mediterranean flora, *Psoralea*, taxonomy, tederia

Introduction

Taxonomical investigations of *Bituminaria* Heist. ex Fabricius (1759: 165) in the Mediterranean region have improved the knowledge on this genus, which is represented by some poorly known taxa mainly belonging to the *B. bituminosa* (Linnaeus 1753: 763) C.H. Stirton (1981a: 318) species complex, formerly attributed to the genus *Psoralea* Linnaeus (1753: 762). According to literature (Stirton 1981a, b, Greuter *et al.* 1989, Minissale *et al.* 2013, Giusso *et al.* 2015, Brullo *et al.* 2016, 2017a, b, Bogdanović *et al.* 2016) within this genus, apart from *B. bituminosa*, several species are currently recognised, i.e. *B. morisiana* (Pignatti & Metlesics 1976: 53), Greuter (1986: 108); *B. flaccida* (Nábělek 1923: 74), Greuter (1986: 108); *B. basaltica* Minissale, C. Brullo, Brullo, Giusso & Sciandrello (2013: 2); *B. kyreniae* Giusso, C. Brullo, Brullo, Cambria & Minissale (2015: 288), *B. palaestina* (Bassi 1768: 14) Brullo, C. Brullo, Minissale, Salmeri & Giusso (2016: 65); *B. plumosa* (Reichenbach 1832: 869) Bogdanović, C. Brullo, Brullo, Ljubičić & Giusso (2016: 348); *B. antiatlantica* Brullo, C. Brullo, Cambria, Cristaudo & Giusso (2017a: 111); and *B. tunetana* C. Brullo, Brullo, Cambria, El Mokni & Giusso (2017b: 222). *Psoralea acaulis* Steven ex Bieberstein (1808: 206), referred by Stirton (1981a: 318) to *Bituminaria* subgenus *Christevenia* Barneby ex C.H. Stirton, was transferred into the new monospecific genus *Kartalinia* by Brullo *et al.* (2018: 15).

From field investigations of the genus *Bituminaria* in Morocco, it has been possible to verify that the populations usually attributed to *B. bituminosa* show a remarkable morphological variability. In particular, Maire (1927, 1936, 1938) described three distinct taxa within *Psoralea bituminosa* from Morocco, represented by var. *atropurpurea* Maire (1927: 25), var. *rotundata* Maire (1936: 222) and var. *laxa* Maire & Weiller in Maire (1938: 415). Recently, Brullo *et al.* (2017a) treated *P. bituminosa* var. *rotundata* as a new species and named it *B. antiatlantica*. As concerns the other two varieties, the var. *laxa* seems to fall within the variability of *B. bituminosa*, while the var. *atropurpurea* seems to

be morphologically distinct from *B. bituminosa* s.str. In order to verify the correct taxonomical position of the varieties, we have collected living material at its *locus classicus* at Imintanoute (southwards of Marrakech, High Atlas).

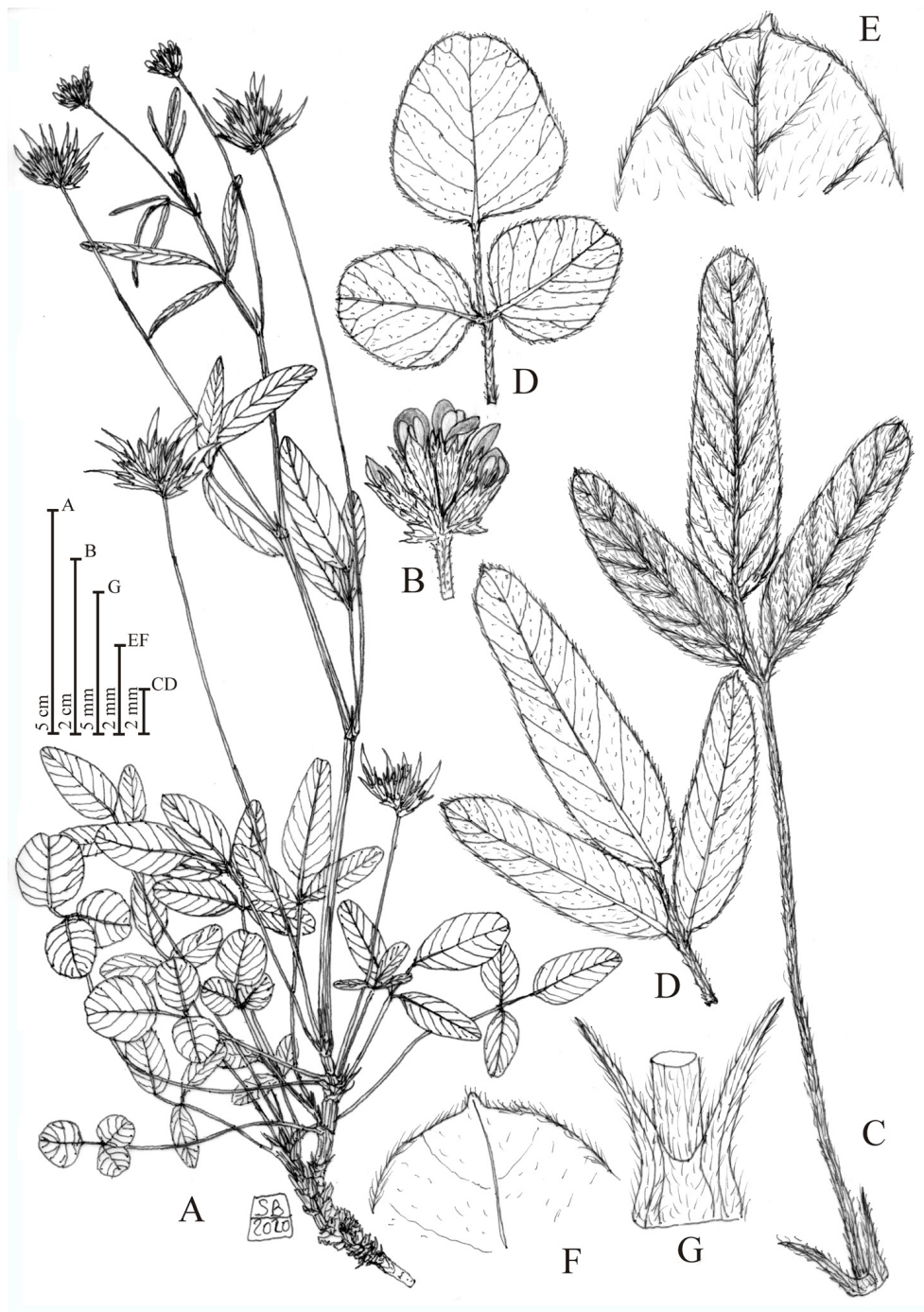


FIGURE 1. *Bituminaria atropurpurea*. A. Habit. B. Inflorescence. C. Leaf abaxial face. D. Leaflets adaxial face. E. Leaf apex abaxial face. F. Leaf apex adaxial face. G. Stipules. Illustration by S. Brullo based on living material coming from type locality (CAT!).

In-depth morphological investigations carried out on herbarium specimens and living plants cultivated in the Botanical Garden of Catania emphasized that the var. *atropurpurea* is distinct from all other *Bituminaria* species. The most relevant distinguishing features observed in the *locus classicus* population chiefly regard the very small flower size and the corolla colour, which is tinged with pink to dark purple, while in the other species the corolla is usually bigger and shows a color variable from white to dark blue-lilac through intermediate shades of lilac. These morphological characteristics are absolutely unique in the genus and therefore differentiate it from the other known species. On this basis it is treated here as a distinct taxon and raised to specific rank, as *B. atropurpurea*.

Materials and methods

Morphological analyses on *Bituminaria atropurpurea* were carried out on herbarium specimens (CAT) and also available online (MPU, RAB, WAG), as well as on living plants cultivated in the Botanical Garden of Catania, coming from pods specimens of plants collected in the *locus classicus*. The characters used for this study are listed in the Table 1. Morphological comparison with *B. antiantlantica*, *B. tunetana*, *B. basaltica* and *B. bituminosa* s.str. was carried out on materials used for the investigations of the above-mentioned species and already reported in literature (see Minissale *et al.* 2013, Brullo *et al.* 2017a, b).

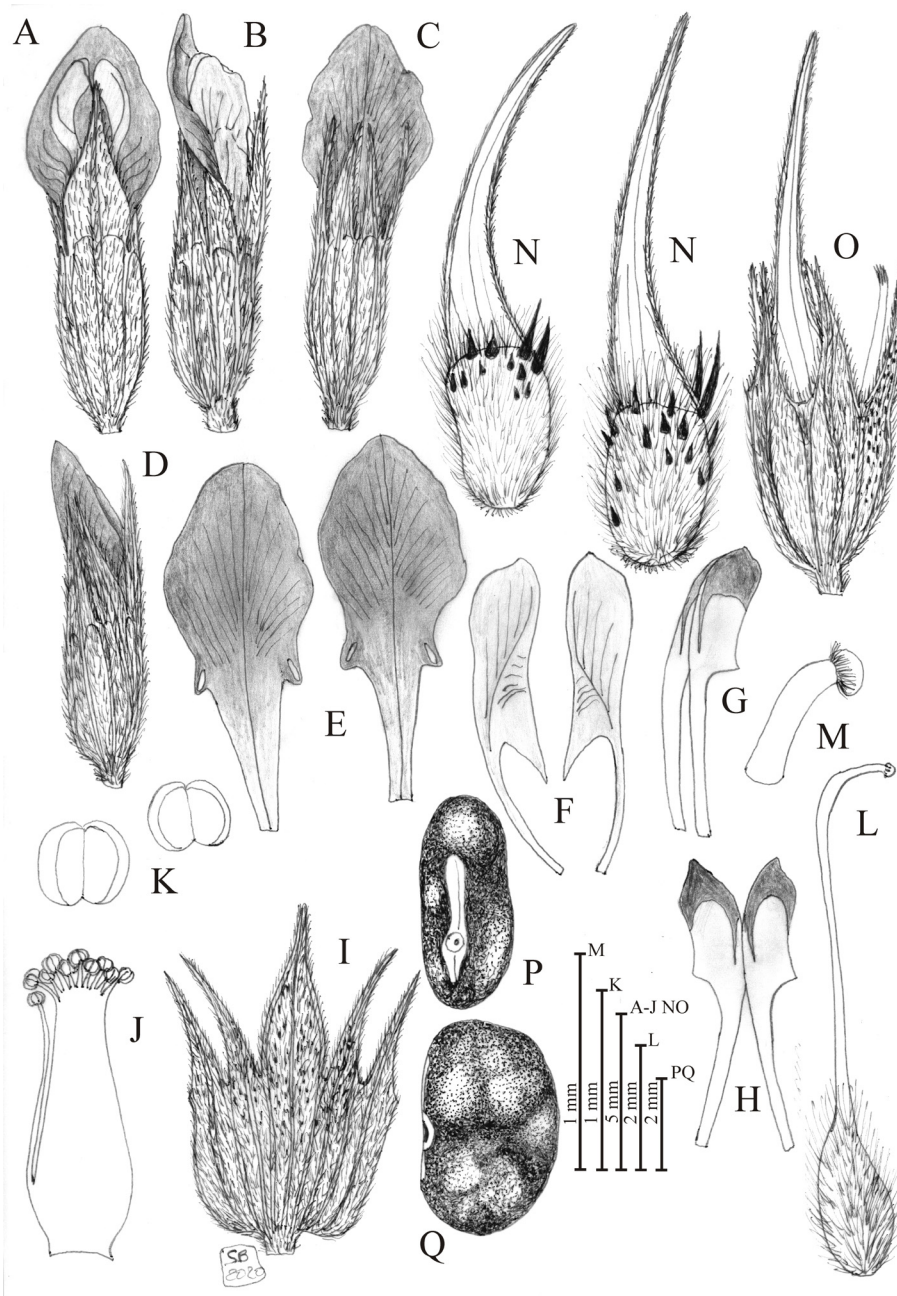


FIGURE 2. *Bituminaria atropurpurea*. **A.** Flower (ventral view). **B.** Flower (lateral view). **C.** Flower (dorsal view). **D.** Bud. **E.** Standards. **F.** Wings. **G.** Keel (lateral view). **H.** Open keel. **I.** Open calyx (external face). **J.** Staminal tube. **K.** Anthers. **L.** Pistil. **M.** Stigma. **N.** Pods. **O.** Pod with calyx. **P.** Seed (hilar view). **Q.** Seed (lateral view). Illustration by S. Brullo based on living material coming from type locality (CAT!).

Pod indumentum and seed testa micro-morphology, regarding the hairs and the sculptures, were studied on mature and dried material using a scanning electron microscope (SEM) Zeiss EVO LS10, according to the protocol reported by Stork *et al.* (1980), while terminology of the seed coat sculpturing follows Barthlott (1981) and Gontcharova *et*

al. (2009). Pollen morphology obtained from herbarium material (CAT) in hydrated state was examined according to Walker & Doyle (1975), Punt *et al.* (1994, 2007) and Hesse *et al.* (2009). Dry pollen was mounted on stubs without any preparation and observed using a scanning electron microscope (SEM) Zeiss EVO LS10.

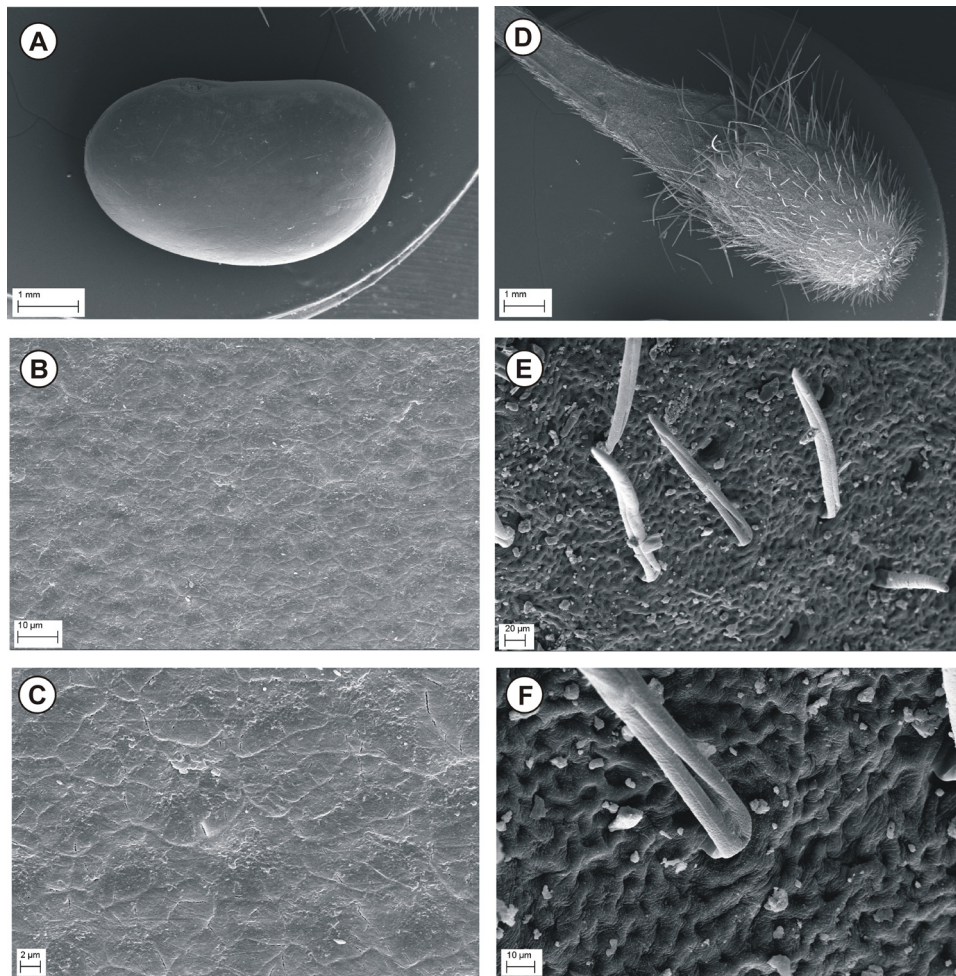


FIGURE 3. SEM micrographs of the seed coats (A–C) and pod indumentum (D–F) of *Bituminaria atropurpurea*. **A.** Seed at low magnification (x 15). **B.** Seed coat at medium magnification (x 1000). **C.** Seed coat at high magnification (x 2500). **D.** Pod at low magnification (x 10). **E.** Pod indumentum at medium magnification (x 250). **F.** Pod indumentum at high magnification (x 700). Material coming from type locality (CAT!).

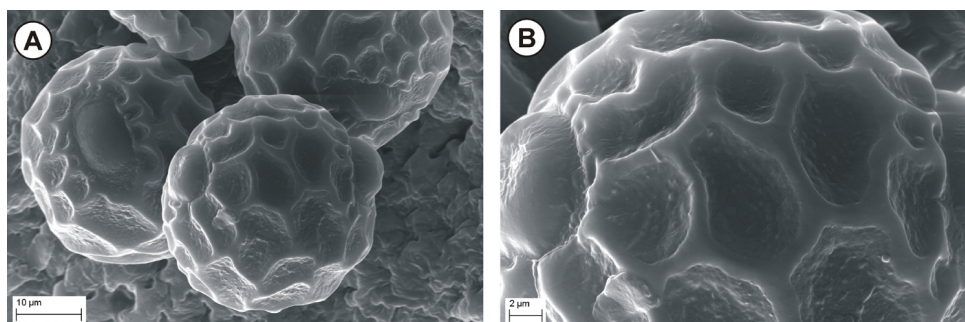


FIGURE 4. SEM micrographs of pollen grains of *Bituminaria atropurpurea* from type locality (CAT!). **A.** pollen grains at medium magnification (x 1600). **B.** Detail of pollen grain at high magnification (x 4000).

Taxonomic treatment

Bituminaria atropurpurea (Maire) Bogdanović, C. Brullo, Brullo, Cambria & Giusso **comb. et stat. nov.** (Figs. 1, 2)

Basionym:—*Psoralea bituminosa* L. var. *atropurpurea* Maire (1927: 25). Type (lectotype, designated here):—Morocco: Imi-n-Tanout M. Grand Atlas, rochers calcaires, 900 m, fl. pourpre foncé, 24 April 1922, R. Maire s.n. (MPU001749!), sub. *Psoralea bituminosa* L. subvar. *atropurpurea* Maire; (isotype: RAB044557!).

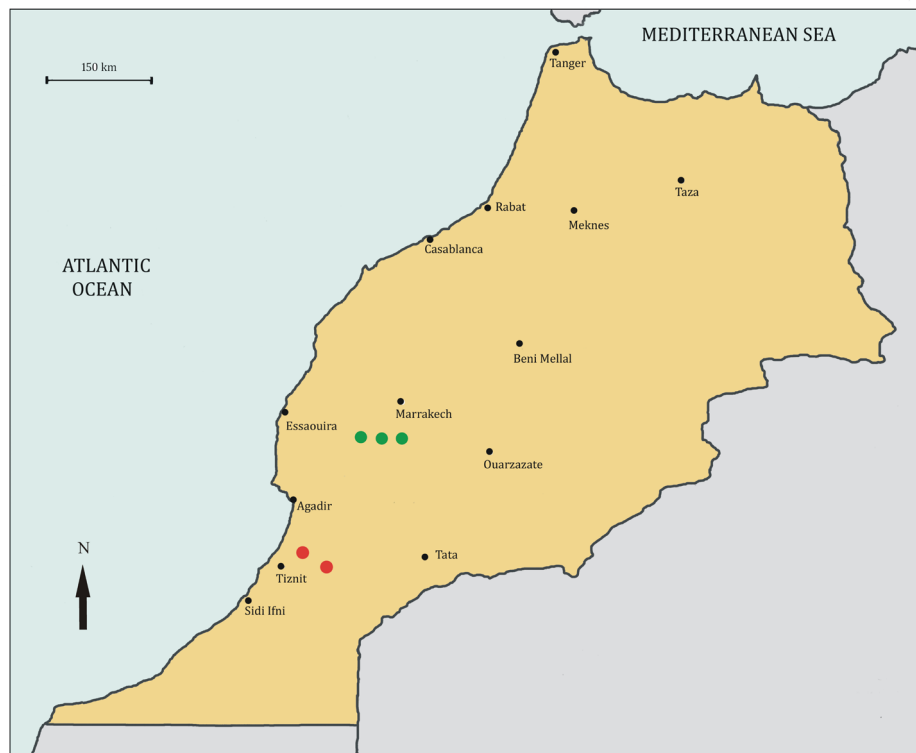


FIGURE 5. Distribution map of *Bituminaria atropurpurea* (green dots) and *B. antiatlantica* (red dots) in Morocco based on herbarium materials.

Description:—Herb perennial, woody and branched at the base, greenish-grey, hirsute, up to 50 cm tall, no or faint smelling of pitch. Stems numerous, prostrate-ascending to erect, rigid, striate. Leaves digitately 3-foliolate, petiolate; stipules linear-triangular, hairy, 5–10 mm long, adnate to the petiole; petiole 3–10 cm long, longer in the basal leaves; leaflets elliptical-oblongate to ovate-rounded, green to greenish-grey, 10–50 × 3–16 mm, subglabrous to sparsely hairy on adaxial face, and more densely hairy on abaxial face mainly on the veins, rounded to retuse and slightly mucronate at the apex, with mucro 0.1–0.3 mm long, the central leaflet longer with petiole 2–10 mm long, the lateral ones shorter, more or less symmetrical, with petiole 0.5–2 mm long. Raceme subcapitate, 10–12 mm long, 5–12-flowered, covered usually by white hairs on the bracts, calyces and upper part of the peduncle, rarely mixed with a few black hairs; peduncles 4–16 cm long, longer than the leaf. Bracts 3–6 mm long, 1–3-toothed. Calyx 10–11 mm long, hairy for short white hairs, 0.1–0.5 mm long, sometimes mixed with minute black hairs, 10-nerved with unequal triangular-subulate teeth; tube 4.5–5 mm long; lower tooth 5–5.5 mm long; lateral teeth 4–4.5 mm long. Corolla pink to dark purple, 11–11.5 mm long, slightly longer than the calyx; standard spatulate, rounded at the apex, 11–11.5 × 4.5–4.7 mm, uniformly dark purplish, sometime pink-purplish above; wings 10–10.5 mm long, with limb pinkish to purplish-pink, 2–2.2 mm wide; keel 9–9.5 mm long, with limb 1.5–1.6 mm wide, having a macula dark purple on inner face towards the apex. Staminal tube 7.5–8.5 mm long; anthers 0.3–0.4 mm long. Pistil 8–8.5 mm long, ovary hairy, style glabrous; stigma subcapitate, ciliate at the base. Pod indehiscent, exserted from the calyx, 16–17.5 mm long (beak included), densely covered in the corpus by setaceous hairs, 0.5–2.5 mm long, mixed with some rigid black prickles; beak flat, falcate, 10–12 mm long, glabrous and ciliate at the margin. Seeds adherent to pericarp, laterally compressed, subreniform, 4.5–5 × 3–3.2 mm, blackish-brown.

Seed and pod micro-morphology:—The micro-morphology of seed coats in Fabaceae can provide an important character in the taxonomic treatment of the critical groups or in species complexes. In particular, several authors have carried out investigations on the seed testa ornamentations of many genera of Fabaceae emphasizing the remarkable importance of this feature in their systematic and phylogenetic approach (Murthy & Sanjappa 2002, Kirkbride *et al.* 2003, Salimpour *et al.* 2007, Bacchetta & Brullo 2010, Fawzel 2011, Gandhi *et al.* 2011, Brullo *et al.* 2011, 2013).

Within the genus *Bituminaria*, SEM investigations of the seed testa of *B. bituminosa*, *B. basaltica*, *B. kyreniae*,

B. palaestina, *B. plumosa*, *B. tunetana* and *B. antiatlantica* have been made by Minissale *et al.* (2013), Giusso *et al.* (2015), Brullo *et al.* (2016, 2017a, b) and Bogdanović *et al.* (2016). Overall, all these species show many relevant micro-morphological differences in ornamentations of their seed testa. In the case of *B. atropurpurea*, the micro-sculptures of the seed coat were very different from the aforesaid species. The seed testa of *B. atropurpurea* is characterized by a fine and irregular reticulum bounding the single cells, which have a very variable outline, both as regards the size and the shape. In particular the cells have a width varying from 2.8 to 7.1 μm . The anticlinal walls are slightly grooved and sometime lacerate, while the periclinal ones are flat with a slightly scabrous epidermis (Fig. 3, A–C). The surface of the pod corpus is deeply rugose-foveolate, while the hairs are slightly scabrous and the longitudinal furrow is widened at the base with a basal diameter of ca. 25 μm (Fig. 3, D–F).

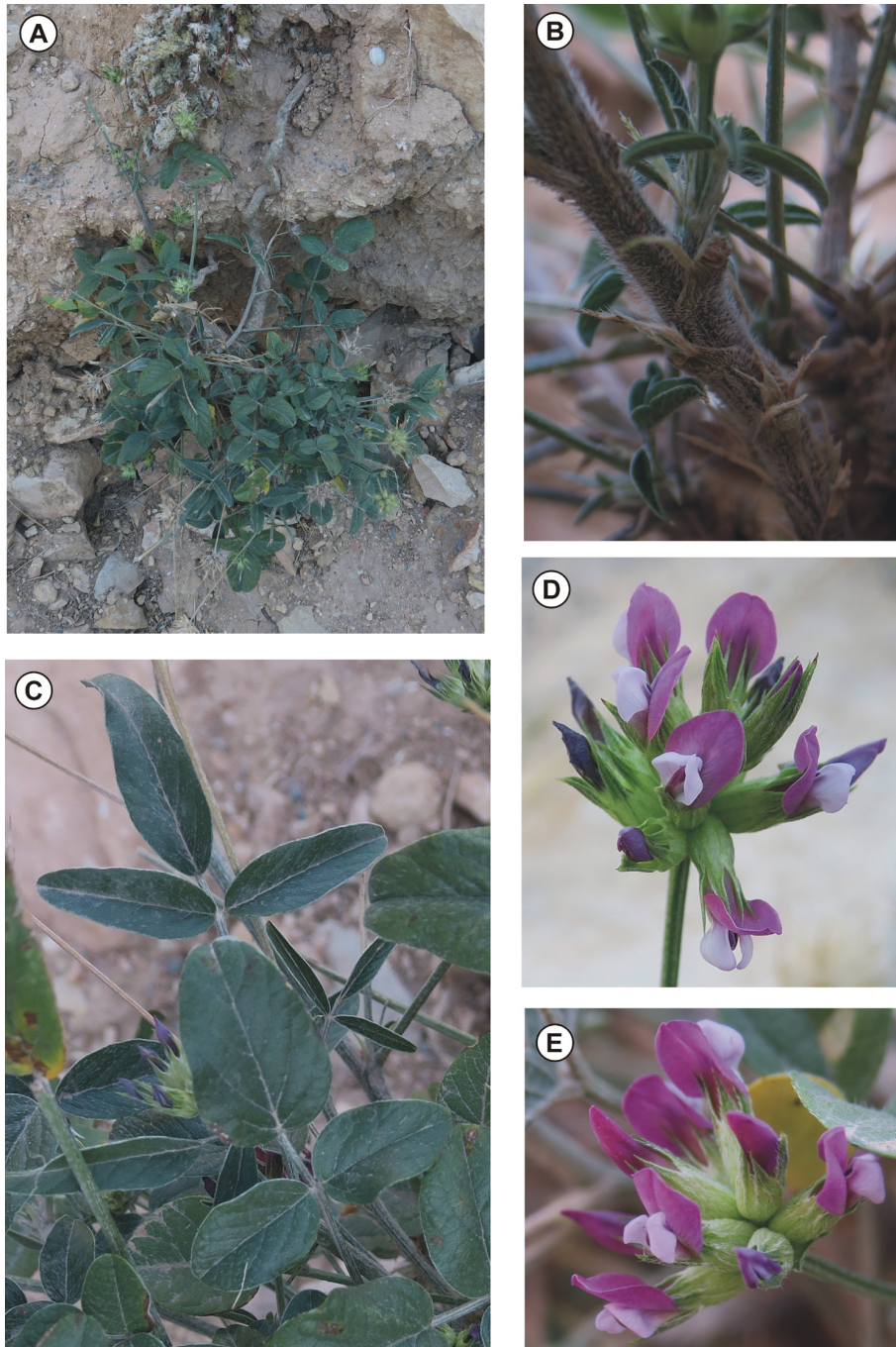


FIGURE 6. Phenological features of *Bituminaria atropurpurea* from the type locality. **A.** Habit. **B.** Detail of the basal part of stem. **C.** Detail of the leaves. **D.** and **E.** Inflorescence detail. (Photos by S. Cambria).

Pollen morphology:—The pollen morphology of *Bituminaria* was examined by La Serna Ramos & Gó Mez Ferreras (2006) and Halbritter & Weis (2015), who provided SEM pictures of *B. bituminosa* s.l., while Brullo *et al.* (2016, 2017a, 2018) examined the pollen grains of *B. bituminosa* s.str., *B. palaestina* and *B. antiatlantica* emphasizing

the morphological differences in the pollen among these species. The pollen grains of *B. atropurpurea* are quite similar to the other investigated species of *Bituminaria*, since they are monad, spheroidal, with a circular outline mainly in equatorial view, angularaperturate, tricolporate, brevicolporate, colpus elliptical with indistinct ora, pore elliptical, protruding, with distinct margin, exine ornamentations coarsely reticulate, slightly heterobrochate, with brochi variable in shape and size, much smaller near the colpus, lumen flat and slightly papillose, muri smooth, almost perpendicular, at the base not separated from the lumen (Fig. 4). However, when *B. atropurpurea* is compared to *B. bituminosa* from Sicily (Brullo *et al.* 2016, Fig. 6A) and *B. antiatlantica* from Morocco (Brullo *et al.* 2017a, Fig. 6), it differs in some small differences regarding the size and ornamentations. In particular, the pollen grains of *B. atropurpurea* shows a medium size 33.5–34.5 µm, pore elliptical 10–11 µm long, brochi 4–13 µm wide, with densely papillose-rugose lumen, muri 0.5–0.8 µm deep, and 1.1–1.7 µm wide (Fig. 4). Conversely, the pollen grains in *B. bituminosa* are smaller with a size of 25–30(34) µm, with brochi up to 17 µm large, and fewer in number, the lumen is laxly papillose, while those of *B. antiatlantica*, are larger (37–38 µm) with brochi reaching a maximum of 11 µm large, and more numerous, with a lumen sparsely papillose and muri deeper (1.4–1.8 µm).

Distribution and ecology:—Based on literature and herbarium investigations, *B. atropurpurea* is currently known only from few localities near Marrakech, in the southern part of the High Atlas (Fig. 5). According to Maire (1927), it occurs at an altitude of 900–2000 m a.s.l. on calcareous rocks, in rupestrian habitats. In particular, it was recently collected at Imintanoute (Fig. 6), where it grows at about 900 m a.s.l. together with other chasmophytes, such as *Bellis caerulescens* Cosson ex Ball (1878: 495), *Crambe kralikii* Cosson (1885: 307), *Deverra scoparia* Cosson & Durieu in Cosson (1855: 248), *Silene boryi* Boissier (1838: 19), *Paronychia chlorothyrsa* Murbeck (1897: 48), *Rhodanthemum atlanticum* (Ball 1873: 366) Wilcox, Bremer & Humphries in Bremer & Humphries (1993: 142), *Salvia taraxacifolia* Cosson & Balansa in Cosson (1874: 253), *Sideritis cossoniana* Ball (1878: 622), *Teucrium rupestre* Cosson & Balansa in Cosson (1874: 259), *Micromeria macrosiphon* Cosson (1880: 72), etc.

Etymology:—The specific epithet *atropurpurea* (Latin) means dark purple (RGBA-135, 31, 120, 1) and refers to the flower colour of the plant.

Phenology:—Flowering April to early July, fruiting May to July.

Discussion:—According to herbarium investigations and morphological observations on living plants cultivated from seeds collected in several Mediterranean localities (see Brullo *et al.* 2017a, Brullo *et al.* 2017b: Table 1, Brullo *et al.* 2018: Appendix 1), the genus *Bituminaria* is currently represented by 10 species of which *B. bituminosa* is the most widespread species across the Mediterranean, whereas the other species have a more circumscribed or punctiform distribution.

Bituminaria atropurpurea is well differentiated from the other species of this genus in several features; mainly the very small inflorescences and flowers, and the standard tinged with dark purple (Table 1). In particular, there are a number of morphological diacritical characters distinguishing *B. atropurpurea* from *B. bituminosa*. The former is a perennial, woody perennial shrublet up to 50 cm tall, much branched at the base, (versus a usually erect stem up to 150 cm tall); with leaflets elliptical-oblongate to ovate-rounded, subglabrous to sparsely hairy on adaxial face, and more densely hairy on abaxial face mainly on the veins (versus leaflets rounded-elliptical to lanceolate, densely hirsute on both faces); inflorescences smaller 10–12 mm long, subcapitate, bearing 5–12 dark purple to pink flowers (versus inflorescences larger 20–28 mm long bearing 15–30 blue-violet flowers); calyx 10–11 mm long, with short white hairs, 0.1–0.5 mm long, sometimes mixed with minute black hairs, (versus calyx 14–18 mm long, densely covered by hairs >1 mm long); standard spatulate, rounded at the apex, 11–11.5 mm long (versus standard ovate-elliptical, emarginate at the apex, 15–20 mm long); pod beak glabrous and ciliate at the margin (versus pod beak wholly hairy); seeds smaller, 4.5–5 × 3–3.2 mm (versus seeds larger, 5–7 × 3–4 mm). Rather *B. atropurpurea* shows more relationships with *B. basaltica* and *B. tunetana*, since they share leaves glabrous or subglabrous on the adaxial surface, small and few flowered-inflorescences, quite small calyx and corolla, quite short staminal tube and pistil, yet distinct morphological differences allow them to be distinguished from each other. *Bituminaria basaltica* is characterized by rounded-elliptical to linear-lanceolate basal leaflets and linear cauline leaflets, with apical mucro 0.5 mm long, calyx covered by long hairs and lower tooth 6–9 mm long, corolla white, with standard 11–13 × 5–6 mm long, pistill shorter 6–7 mm long, pistill shorter 6–7 mm long, pod (beak included) 9–10 mm long, beak subglabrous beak, seeds 3.4–4 mm long. By contrast, *B. tunetana* is differentiated by leaflets lanceolate to linear-lanceolate, sparsely hairy on both surfaces, rounded to acute at the apex, with mucro 0.5–1.2 mm long, inflorescence 1.5–2 cm long, calyces covered by long hairs, with teeth 5.5–8 mm long, corolla pinkish-lilac, longer than the calyx, with standard 13–14 mm long, retuse at the apex, shorter, pod (beak included) 12–15 mm long, with beak subglabrous, seeds smaller, 3.5–4.5 mm long. *Bituminaria antiatlantica*, which also occurs in Morocco in rupestrian habitats though in more xerophilous bioclimatic conditions, has several morphological features differentiating it from *B. atropurpurea*. The more important diacritic

characters of this species are leaves coriaceous, subrounded to ovate, obtuse to acute, inflorescence 1.5–2 cm long, calyx 12–13 mm long, covered by long hairs, with longer teeth, corolla whitish-pink to whitish-lilac, with standard 16–16.5 mm long, obtuse, staminal tube, pistil and pod longer, pod beak hairy, seeds 6–7 mm long. Apart from these differentiating characters the contrasting species also show significant differences in their ecological requirements; *B. bituminosa*, *B. basaltica* and *B. tunetana* are terricolous plants growing in steppic grasslands or synanthropic habitats, while *B. antiatlantica* and *B. atropurpurea* are chasmophytes exclusive of rupestrian stands. Besides, *B. antiatlantica* occurs in territories characterized by very arid bioclimatic conditions on quarzarenitic substrata, while *B. atropurpurea* is linked to limestones of the mountain belt with a more mesic bioclimate.

Additional specimens examined:—MOROCCO. In Atlantis Majoris monte Gourza supra oppidum Amismiz: prope Imi-n-Tala in rupestr. calcareis, corolla atropurpurea, 30 June 1925, *Maire s.n.* (MPU001747!), sub *Psoralea bituminosa* L. var. *atropurpurea* Maire; In Atlantis Majoris monte Erdouz supra oppidum Amismiz: prope Anamrou in glareosis 1800 m, vexillum atropurpureum alae atroviolaceae carinae albido-violaceae rostrum atropurpureum, 1 July 1925, *Maire s.n.* (MPU001748!), sub *Psoralea bituminosa* L. var. *atropurpurea* Maire; In Atlantis Majoris monte Gourza supra oppidum Amismiz: in rupestr. calcareis prope Imi-n-Tala, 1500–1600 m, 30 June 1925, *Maire s.n.* (RAB044556!); 5 km NE of Ouirgane. Route Youkak-Asni. ± 900 m. alt. Haut. Atlas. Fl. dark purple. Badly eroded red soil, 10 May 1961, *J.J.F.E. et P.A.W.J. De Wilde & J. Dorgelo 2249A* (WAG 1005435!); Morocco, Grande Atlante, Imintanoute, affioramenti calcarei lungo la strada, 31°9'59.62"N, 8°50'56.91"W, 24 June 2016, *S. Cambria s.n.* (CAT!).

Key to the known species of *Bituminaria*

1. Corolla 11–14 mm long.....2
- Corolla 15–24 mm long4
2. Basal leaflet ovate-rounded, cauline leaflets elliptical-oblongate, calyx with hairs 0.1–0.5 mm long, standard dark purple, pod (beak included) 16–17.5 mm long, with beak 10–12 mm long, glabrous on lateral faces*B. atropurpurea*
- Basal leaflet rounded-elliptical, lanceolate to linear-lanceolate, cauline leaflets lanceolate to linear, calyx with hairs more than 1 mm long, standard pinkish-lilac or white, pod (beak included) 9–15 mm long, with beak 5.5–10 mm long, sparsely hairy on lateral faces.....3
3. Corolla white, 11–13 mm long, subequalling the calyx, pod (beak included) 9–10 mm long, beak 5.5–6 mm long.....*B. basaltica*
- Corolla pinkish-lilac, 13–14 mm long, longer than the calyx, pod (beak included) 13.5–14.5 mm long, beak 9–10 mm long.....*B. tunetana*
4. Raceme 2–10 flowered.....5
- Raceme 10–30 flowered.....7
5. Corolla blue-violet to violet, oblongate-spathulate, pod beak thin and soft*B. kyreniae*
- Corolla whitish-pink to whitish-lilac, elliptical to ovate-elliptical, pod beak thick and rigid.....6
6. Stems and leaves greyish-glaucous, hirsute, cauline leaflets obovate to linear, calyx 9–12 mm long, pod (including beak) up to 15–16 mm long *B. flaccida*
- Stems and leaves dark green, sparsely hairy to glabrous, cauline leaflets semi-rotund to ovate, calyx 12–13.5 mm long, pod (including beak) 21–23 mm long*B. antiatlantica*
7. Stems with patent hairs, basal leaflets widely ovate-subcordate, pod beak 5–8 (10) mm long.....*B. palaestina*
- Stems with appressed hairs, basal leaflets not ovate-subcordate, pod beak 10–19 mm long8
8. Stems and leaves densely villous, leaflet mucro 1–1.5 mm long, corolla purplish-pink, pod beak 10–12 mm*B. plumosa*
- Stems and leaves hirsute to subglabrous, leaflet mucro 0.3–0.5 mm long, corolla white-violet to blue-violet, pod beak (10) 12–19 mm long.....9
9. Leaflets sparsely hairy to glabrous, raceme 3–4.5 mm long; corolla white- violet, with standard ovate-lanceolate, apex obtuse*B. morisiana*
- Leaflets hirsute, raceme 2–2.8 mm long; corolla blue-violet, with standard ovate-elliptical, apex emarginate.....*B. bituminosa*

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