



Dianthus borbonicus (Caryophyllaceae), a new species from Sicily

SALVATORE BRULLO^{1*}, CRISTIAN BRULLO¹, PAOLO COLOMBO², GIANPIETRO GIUSSO DEL GALDO¹, VINCENZO ILARDI³ & ROSARIA PERRONE²

¹ Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania, via A. Longo 19, I 95125 Catania, Italy; salvo.brullo@gmail.com

² Dipartimento di Scienze Terrestri e Marine, Divisione di Ecologia, Università di Palermo, Viale delle Scienze, Ed. 16, I-90128 Palermo, Italy

³ Dipartimento di Scienze della Terra e del Mare, Università di Palermo, via Archirafi 26, I 90123 Palermo, Italy

* Author for correspondence

Abstract

Dianthus borbonicus a new species occurring in North-Western Sicily is described and illustrated. It is a rare chasmophyte belonging to the *D. sylvestris* group, which is exclusive of a rupestrian stand near Rocca Busambra (Ficuzza). Its macro- and micromorphological features (seed testa sculptures, and leaf anatomy), ecology, conservation status and a comparison with the related species are provided too.

Key Words: Anatomy, *Dianthus*, seed testa, Sicily, taxonomy

Introduction

Dianthus Linnaeus (1753: 409) is one of the largest genera of Caryophyllaceae comprising approximately 600 species, which are widespread distributed in Europe, Asia and North Africa, while some species occur in North America and South Africa (see e.g., İlçim *et al.* 2013). Owing to their ornamental properties, several taxa (species, subspecies, varieties, cultivars or hybrids) are cultivated for gardening. Concerning the native species, they mainly occur in rupestrian habitat, which are usually considered as refuge sites for ancestral plants, as well as grasslands, garigues, steppes, mesic meadows, etc. (Bacchetta *et al.* 2010).

An important diversity centre of the genus *Dianthus*, mainly regarding the *Dianthus sylvestris* group, is the central Mediterranean area (Sardinia, Corsica, Sicily, Italian Peninsula and Croatia) where the number of taxa is high (see e.g., Bacchetta *et al.* 2010).

Recent field investigations carried out in some poorly known areas of Sicily led us to discover of an unusual and quite isolated population of *Dianthus*, which appears to be morphologically well different from the other taxa included in the *D. sylvestris* group. Taxonomical investigations carried out on living plants, and anatomical analyses on the leaves and seed surface, allowed to establish that the population found can be proposed as a distinct species, named *D. borbonicus*.

Materials and methods

Morphological analyses are carried out on both herbarium material (Herbarium CAT, acronym according to Thiers 2015 continuously update) and living plants (from *locus classicus*) (Table 1).

Seed testa morphology was examined on mature and dried seeds by 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 Bartholot (1981) and Gontcharova *et al.* (2009) (see Table 1).

TABLE 1. Herbarium specimens of *Dianthus borbonicus* and allied species.

Species	Locality	Date	Collector(s)	Herbarium
<i>Dianthus borbonicus</i>	Corona del Re (Pizzo Morabito)	10 June 2014	<i>S. Brullo & V. Ilardi s.n.</i>	CAT
<i>Dianthus borbonicus</i>	Corona del Re (Pizzo Morabito)	29 June 2011	<i>S. Brullo et al. s.n.</i>	CAT
<i>Dianthus minae</i>	Passo Scuro (Castelbuono)	29 June 2011	<i>S. Brullo et al. s.n.</i>	CAT
<i>Dianthus busambrae</i>	Rocca Busambra (Ficuzza)	24 June 1999	<i>G. Giardina s.n.</i>	CAT
<i>Dianthus cyathophorus</i>	Gole di Su Gorroppu (Urzulei)	28 May 2002	<i>S. Brullo et al. s.n.</i>	CAT
<i>Dianthus cyathophorus</i>	Foresta Marganai (Iglesias)	04 June 2002	<i>G. Bacchetta et al. s.n.</i>	CAT
<i>Dianthus arrostii</i>	Cozzo del Pellegrino (Madonie)	05 July 2011	<i>C. Brullo et al. s.n.</i>	CAT

Concerning the anatomical study, the most developed leaves were considered and fixed in FAA (90% ethanol, 5% formalin, 5% acetic acid) (Sass 1958), dehydrated in graded ethanol series and embedded in paraffin, following the protocol of Beccari and Mazzi (1966). Cross sections (about 10–20 µm thick) were prepared with Jung-R 2050-Supercut Microtome (Reichert-Jung, Leica) and stained by Safranin; permanent slides were mounted in Canada balsam, then examined under light microscope Leica DMLS and photomicrographed by a NIKON DS Head DS-Fi1 digital camera.

Taxonomic treatment

Dianthus borbonicus Brullo, C. Brullo, Colombo, Giusso, Ilardi & Perrone, *sp. nov.* (Figs. 1, 2A–C).

Type:—SICILY. [Palermo] Ficuzza, Corona del Re presso Pizzo Morabito, su pareti calcaree esposte a Nord, a ca. 1150 m s.l.m., 37° 50' 44" N, 13° 26' 37" E, 10 June 2014, *Brullo & Ilardi s.n.* (holotype CAT!, isotypes CAT!, FI!).

Diagnosis:—*A* *Dianthus cyathophorus* differt caudice compacto, foliis basalibus 2–10(–11) cm longis, 2–4 mm latis, foliis caulinis 2–5(–6) cm longis, caule 6–25 cm longo, 1–4-floro, bracteis calycinis divaricatis vel patentibus, mucrone 0.5–2.0 mm longo, interioribus 10 mm longis, 12–13 mm latis, exterioribus 8–10 mm longis, 5.0–7.5 mm latis, calyce 26–30 mm longo, 6.0–7.5 mm diametro, anthera 3.5 mm longa.

Description (macro-morphology):—Suffrutex, 15–30(–35) cm tall; woody stocks densely branched, with branches 1–4 cm long. Basal leaves inserted along the woody stock branches, flat, 2–10(–11) cm long, 2–4 mm wide, acute at the apex; cauline leaves 2–5(–6) cm long. Stems 1–4-flowered, 6–25 cm long, with 4–5 internodes; epicalyx scales 4, divaricate to patent, with mucro 0.5–2.0 mm long; the inner ones subrounded to obovate, 10 mm × 12–13 mm, the outer ones ovate to subrounded, 8–10 × 5.0–7.5 mm; calyx 26–30 mm long, 6.0–7.5 mm in diameter, with teeth triangular, slightly membranaceous on the margin, acute, not overlapping, 6–7 mm long; petals 40–45 mm long, claw 25–30 mm long, limb purplish-pink, cuneate-rounded, 15.0 × 11–14 mm, with 20–34 teeth, 0.3–2.0 mm long, simple, triangular; anther pink-lilac, 3.5 mm long; ovary 8–10 mm long; style with stigma 20–24 mm long. Capsule cylindrical, 14–16 mm long included in the calyx.

Seed testa micro-morphology:—According to literature (e.g., Yildiz 2002, Vural 2008, Yildiz & Güzel 2008, İlçim et al 2013, Hamzaoğlu *et al.* 2015a, 2015b), the seeds of *Dianthus* are black, flat, peltate, cuspidate at apex, with coat made by elongate cells, which are undulate at the margins, and more or less minutely papillose. As concerns *D. borbonicus*, the seeds show a suborbicular to orbicular-ovate outline, and they are covered by irregularly elongate cells, which are loosely undulate at the margins, and have anticlinal walls represented by a deep and wide grooves, irregularly trabeculate, with U- to S-like undulations, while the periclinal walls are minutely papillose and colliculate, with one or two series of colliculi (Fig. 3A). In addition, the cells on the dorsal face are shorter than those in the centre (75–110 µm) (Fig. 3A2) and longer than the cells on the margins (130–260 µm) (Fig. 3A3), while they are morphologically uniform in ventral portion (60–130 µm) (Fig. 3A5).

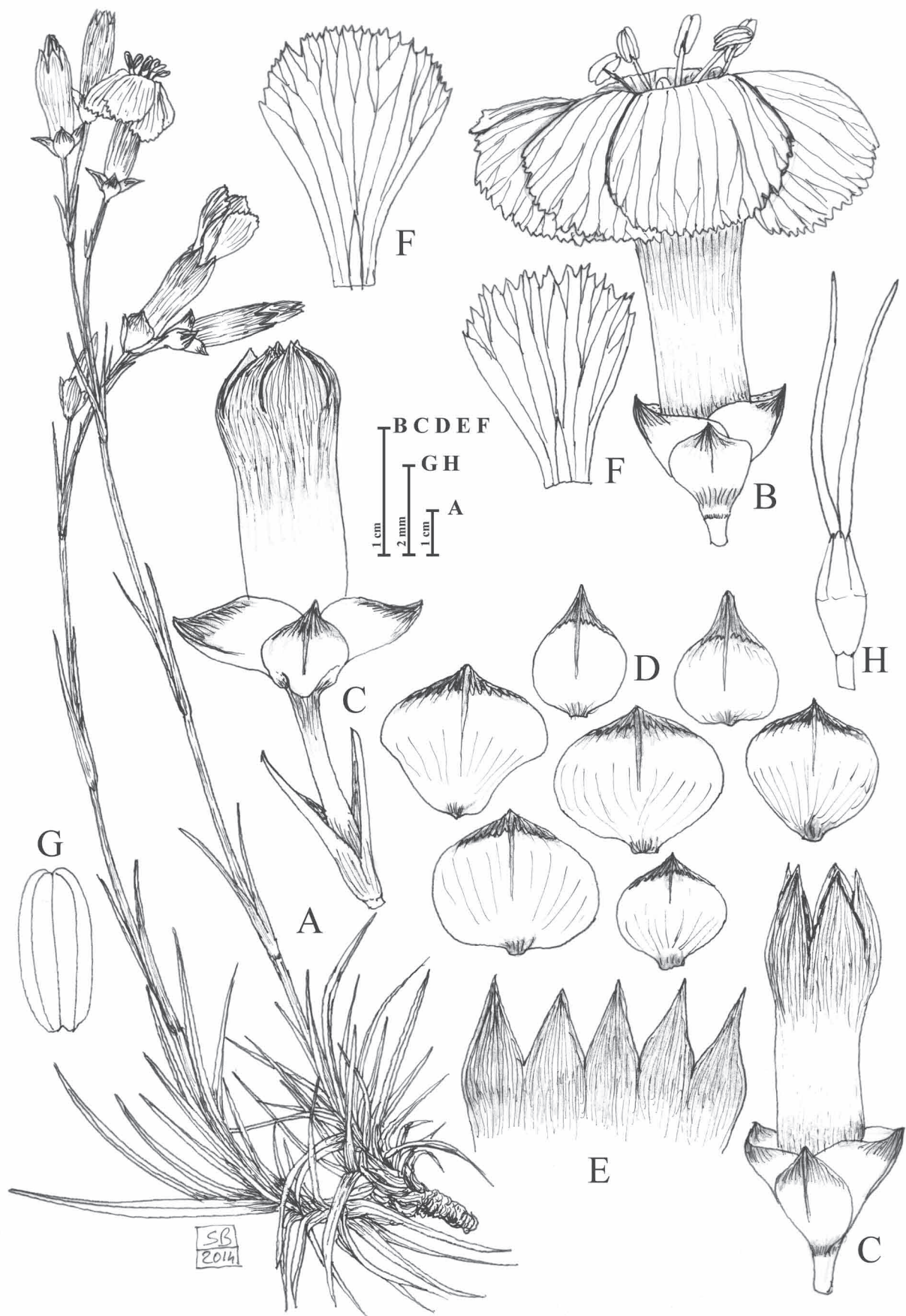


FIGURE 1. Diagnostic features of *Dianthus borbonicus*. **A)** Habit, **B)** Flower, **C)** Calyces and scales, **D)** Epicalyx scales, **E)** Calyx teeth, **F)** Petal limbs, **G)** Anther, **H)** Pistil (drawing by S. Brullo based on living material coming from the type locality).

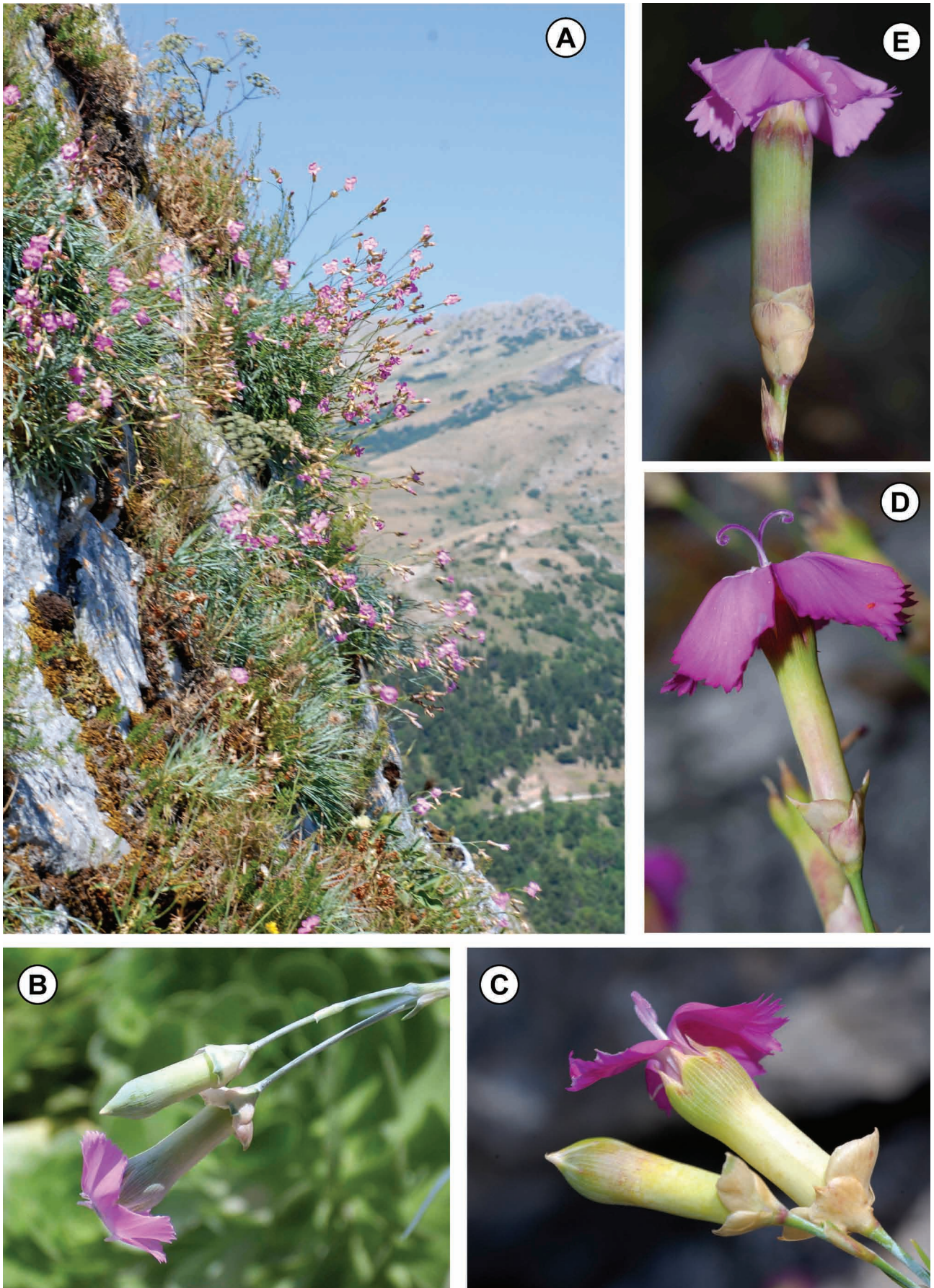


FIGURE 2. Habitat of *D. borbonicus* (A). Flowers of *D. borbonicus* (B–C), *D. minae* (D) and *D. busambrae* (E) (photos by V. Ilardi).

The seeds of the two closely related species [*D. busambrae* Soldano & F. Conti (2005: 18) and *D. minae* Mazzola, Raimondo & Ilardi (2004: 307)] were examined for a comparison. Actually, they are quite similar to those ones of *D. borbonicus* (Fig. 3B), but some important differences occur. *D. busambrae* shows seeds ovate, with testa characterized by very irregular cells in the dorsal face, isodiametric to elongate in the central part (55–100 µm), tightly undulate at the margin, having anticlinal walls represented by a deep and narrow grooves, not trabeculate, with U- or Ω-like undulations (Fig. 3B2), while in *D. minae*, the peripheral cells are elongated (55–130 µm), with anticlinal walls having S- or U-like undulations (Fig. 3B3). As concerns the periclinal walls, they are very similar to those ones of *D. busambrae*, but always with one series of colliculi. Cells of the ventral face are very homogeneous, elongated (80–150 µm), with anticlinal walls usually having U-like undulations (Fig. 3B5). Seeds of *D. minae* are, instead, ovate-lanceolate (Fig. 3C), with irregularly elongated cells in the dorsal face, shorter in the centre (75–120 µm), tightly undulate at the margin (Fig. 3C2). Anticlinal walls are made by deep and very narrow grooves, not trabeculate, with V- or U-like undulations, while the periclinal walls are very similar with only one series of colliculi (Fig. 3C3). Cells of the ventral face are elongated and more or less uniform (130–200 µm), with grooves of the anticlinal walls more spaced showing usually U-like undulations (Fig. 3C5).

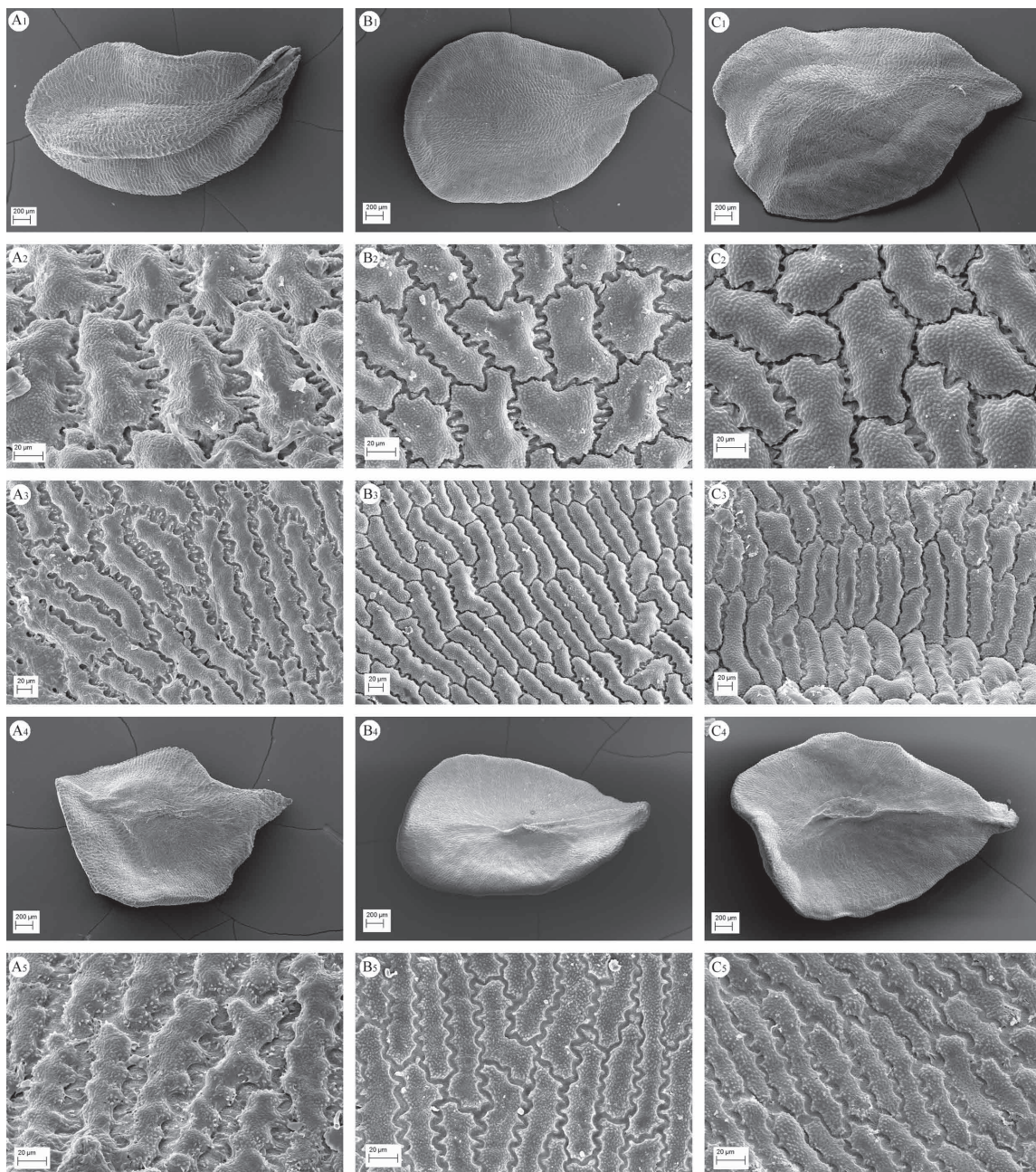


FIGURE 3. SEM micrographs of the seed coat of *Dianthus borbonicus* (A1–5), *D. busambrae* (B1–5) and *D. minae* (C1–5). 1. Seed (dorsal face, ×30). 2. Seed coat (central part of dorsal face, ×500). 3. Seed coat (marginal part of dorsal face, ×250). 4. Seed (ventral face, ×30). 5. Seed (ventral face, ×500), from material coming from the type localities.

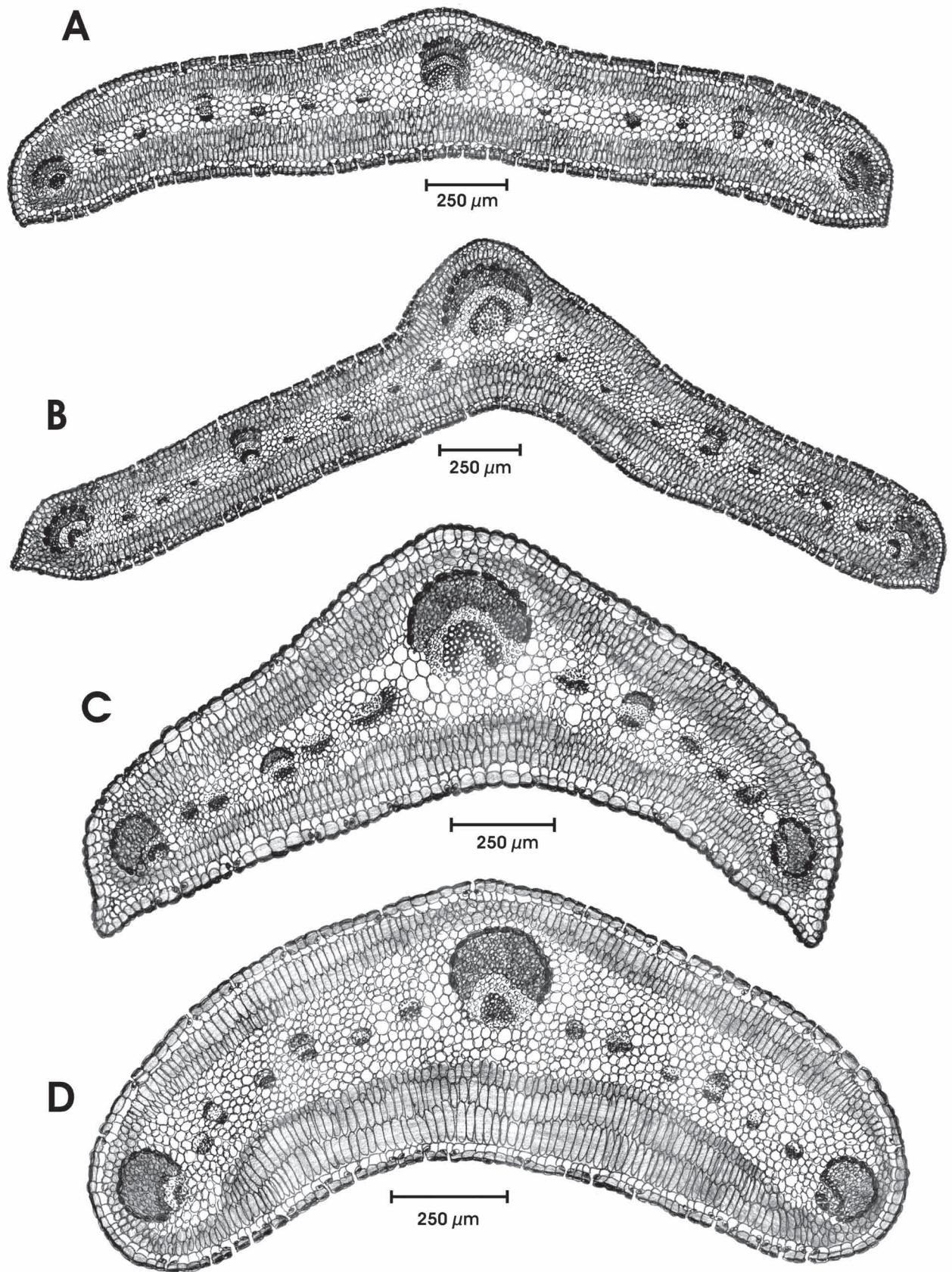


FIGURE 4. Leaf cross sections of *Dianthus borbonicus* (A), *D. busambrae* (B), *D. minae* (C) and *D. arrosti* (D) from living material coming from the type localities (CAT).

Leaf anatomy:—Leaves in *Dianthus borbonicus* appear asymmetrical from the macro-morphological point of view. However, in cross section, they show the typical arrangement of the isobilateral structure (Fig. 4A). It can be considered amphistomatous, with the palisade layer distributed along the whole surface, the abaxial face differing from the adaxial one only concerning a central prominence due to the midrib. The epidermis is 1-layered with a well developed cuticle and numerous sunken stomata. The external parenchyma is represented by a palisade, usually 3-layered (sometimes 2-layered), more developed in the adaxial face, thinning at the margins and on the abaxial face in correspondence of the midrib. The spongy tissue is non-chlorenchymatous and occupies the central part of the leaf wrapping the vascular bundles. There are three main vascular bundles, of which the larger one is placed in correspondence of the midrib and two smaller ones at the margins. Other small vascular bundles (six for each side) are distributed among the main three. In particular, the three largest vascular bundles, which like all the others have the phloem toward the abaxial face, are surrounded externally by a crescent-shaped calotte of sclerenchymatous fibres. Moreover, a sheath of large cells containing drusen of calcium oxalate is set along the outer surface of the calotte. This anatomical structure is very similar to that concerning the leaves of *D. busambrae*. Main differences concerning: the smaller size in *D. borbonicus*, with a shape almost flat with an angle of ca. 170°–180°, midrib little protruding, the vascular bundle corresponding much smaller, a more developed palisade (usually 3-layered) and not more than six secondary vascular bundles for each side. On the contrary, *D. busambrae* is characterized by larger leaves, canaliculate with an angle of ca. 140°, midrib much more developed and protruding, the vascular bundle corresponding much larger with diameter almost double, the palisade less developed (usually 2-layered) and 7–8 secondary vascular bundles per side (Fig. 4B). Much more remarkable differences can be observed in the leaves of *D. borbonicus* if they are compared with those of *D. minae*. This latter species shows leaves less wide and very thick, canaliculate with an angle of 135°–140°, main vascular bundles with a diameter more than twice and the vascular bundles of the second order less numerous (3–5 per side), epidermis with larger cells and thicker cuticle, spongy tissue wider (Fig. 4C). Overall, *D. minae* seems to be more closely related to *D. arrosti* C.Presl (1822: 60) (Fig. 4D). Actually, the two species show remarkable similarities in the leaf shape and tissue arrangement, even if significant structural differences can be observed, such as the leaf thickness and outline, distribution and size of the palisade and spongy tissues, and number of the second order vascular bundles.

Etymology:—The epithet refers to the Bourbon Royal Family (in Latin “Borbonicus”), who had a game reserve and a Royal Palace in Ficuzza, (near Palermo) where the new species grows.

Phenology:—Flowering late May–early June; fruiting late June–July.

Habitat and distribution:—*Dianthus borbonicus* is a very rare chasmophyte localized on Mesozoic north-facing limestones of a rocky walls at 1100–1150 m a.s.l. (Fig. 2A). On the basis of our field investigations, it grows exclusively on a rocky outcrop near Pizzo Morabito at the forested area of Ficuzza (Palermo Province, North-Western Sicily). It is interesting to note that other rare endemics occur, such as *Anthemis cupaniana* Todaro ex Nyman (1879: 360), *Centaurea busambarensis* Gussone (1845: 873), *Helichrysum pendulum* (C.Presl 1822: 97) C.Presl (1826: 29), and *Athamanta sicula* Linnaeus (1753: 244).

Conservation status:—Only one site is currently known for *Dianthus borbonicus* (the Ficuzza-Rocca Busambra Natural Reserve, Fig. 5). Despite the population is small, being represented by about 100 individuals, which occupy an area of about 10.000 m², it does not seem that it are seriously threatened by human activities. Furthermore, considering the remarkable conservativeness of the rupestrian environments and the long life span of the plant, only occasional events (such as fires), may cause a decreasing of the number of mature individuals. According to the criterion “D”. we propose to consider this species as “Endangered” and to include it, for its rarity and punctiform distribution, into the category EN D (IUCN 2014).

Discussion:—*Dianthus* is one of the most critical genera in Caryophyllaceae Juss., being represented by several and poorly investigated groups. From the morphological point of view, important characteristics concern the shape of calyx, calyx, epicalyx scales and corolla (Bacchetta *et al.* 2004, 2010). Based on these features, *D. borbonicus* has to be included into the *D. sylvestris* group, which shows a high diversity in the central Mediterranean area. The new species seems to be closely related to *D. cyathophorus* Moris (1852: 32) from Sardinia, with which it shares the epicalyx scales markedly divaricate, morphological feature rather unusual in *Dianthus*. However, *D. cyathophorus* differs from *D. borbonicus* in having a larger size (40–70 cm tall), flat leaves, 10–25 cm long, epicalyx scales with mucro 3–4 mm long, calyx 22–25 mm, petals 30–35 mm long, with limb 9–10 mm wide and anthers 4 mm long (Fig. 6A3–D3). A further similar species is *D. minae*, which was described from northern Sicily (Mts. Madonie). Due to its remarkable morphological similarity, *D. minae* was previously considered by Bacchetta *et al.* (2010) a synonym of *D. cyathophorus*, but ongoing field investigations revealed that they are two well distinct species (features concerning the shape and size of leaves, epicalyx scales, calyx, petals and anthers, see Table 2). *D. borbonicus* shows significant

differences in comparison with both *D. cyathophorus* and *D. miniae*, since its compact woody stock, shorter stems and leaves, different shape and size of the epicalyx scales, longer petals and shorter anthers. In particular, *D. borbonicus* differs from *D. miniae* also in other features, such as leaves more or less flat not canaliculate, few-flowered stems (1–4), epicalyx scales larger and usually subrounded, calyx with a greater diameter, petals longer and wider, with teeth much more numerous (Fig. 6A2–D2). The phenology, leaf anatomy and seed testa sculptures represent further differences. In fact, *D. miniae* is an early flowering species (from early May to early June), with leaves anatomically very different from those ones of *D. borbonicus*, but very similar to those of *D. arrosti*, and seed coat with anticlinal walls formed by deep and very narrow grooves, not trabeculate. On the contrary, the suffruticose habit, well developed calyx, shortly mucronate epicalyx scales and a very similar leaf anatomy of *D. borbonicus* make the latter species more similar to *D. busambrae*, a chasmophyte localized on the rupestrian stands of the neighbouring Rocca Busambra. Actually, it has to be emphasized that the morphological differences between the two species are quite remarkable. They are mainly concerning the woody stock very loose with more developed stems, leaf canaliculate and longer, epicalyx scales appressed and transversally elliptical, calyx teeth overlapping, petals very shorter, cuneate, with limb narrower and having few teeth, anthers and ovary shorter in *D. busambrae* (Fig. 6A4–D4). Furthermore, the latter is characterized a later flowering period (mid June to July), by relevant differences in the leaf anatomy and seed coat, which are characterized by anticlinal walls with deep and narrow grooves, not trabeculate, with U- or Ω -like undulations.

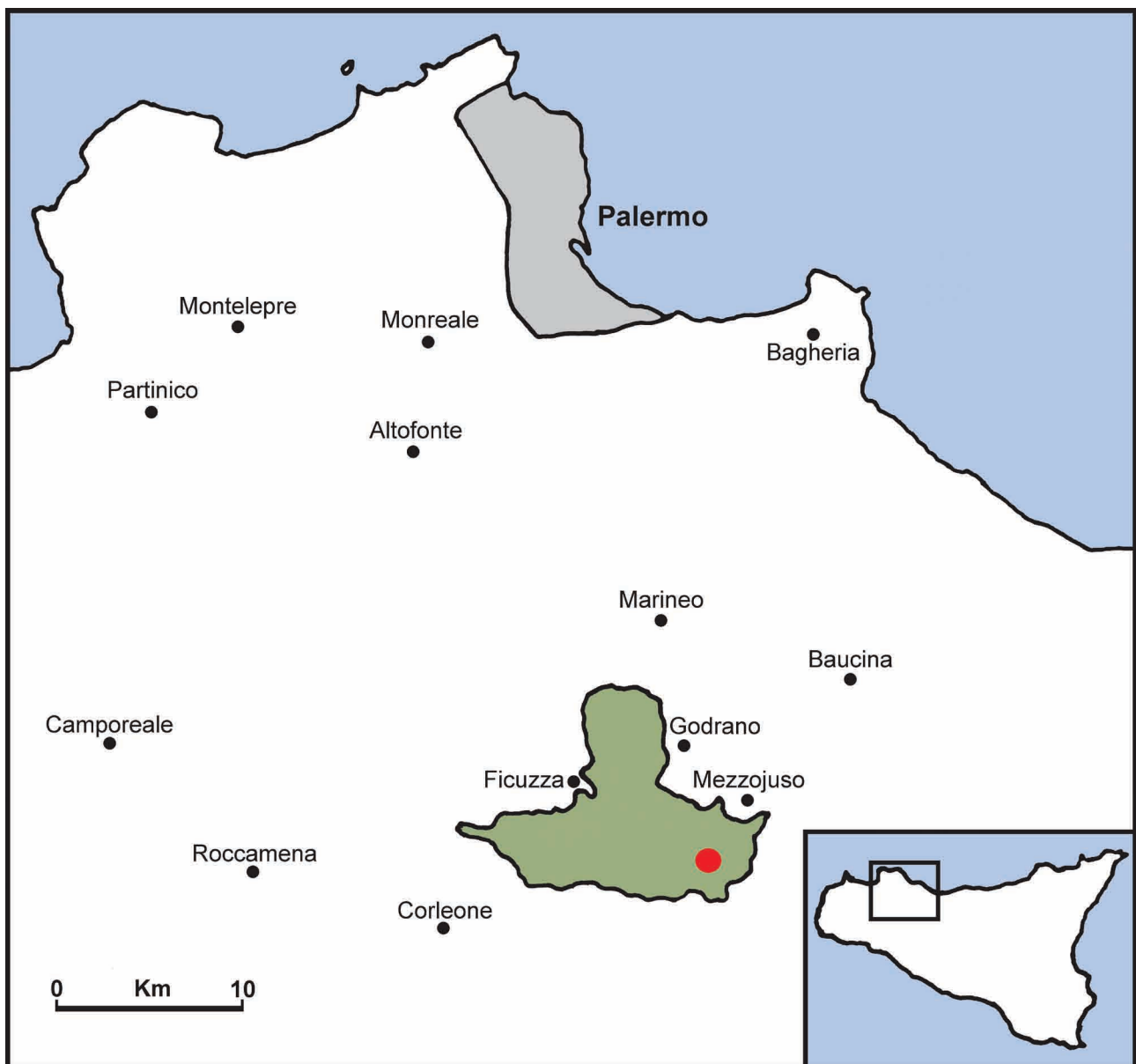


FIGURE 5. Geographical distribution of *Dianthus borbonicus* (red dot). Ficuzza-Rocca Busambra Natural Reserve (green area).

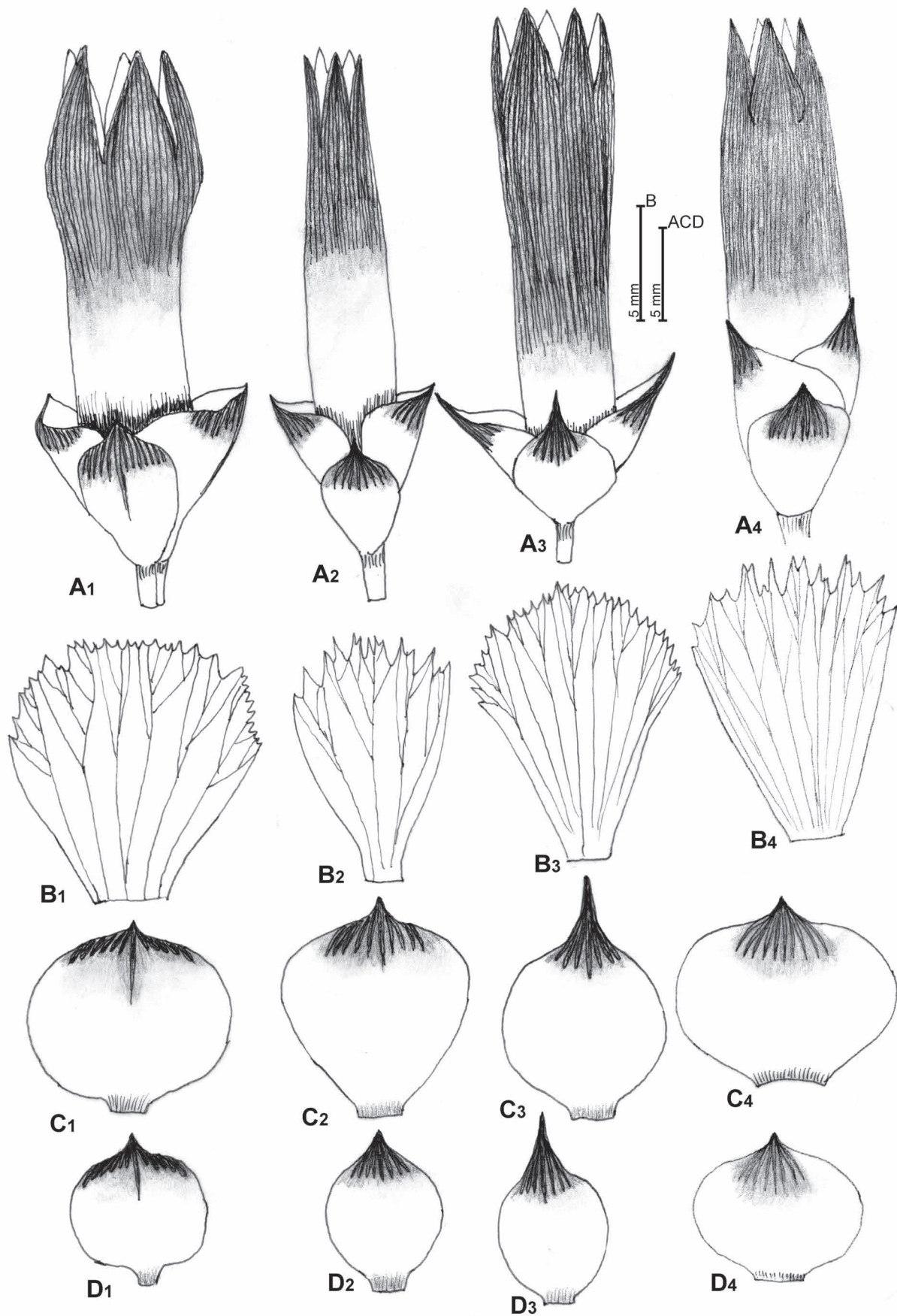


FIGURE 6. Calyces (A), petal limbs (B), inner (C) and outer epicalyx scales 1 (D) of *Dianthus borbonicus* (1), *D. minae* (2), *D. cyathophorus* (3) and *D. busambrae* (4) (drawing by S. Brullo based on living material coming from the type localities).

Additional specimen examined (paratype):—SICILY. Ficuzza, Corona 1 del Re presso Pizzo Morabito, su pareti calcaree esposte a Nord, a ca. 1150 m. di altitudine, 29 June 2011, *Brullo, Colombo, Perrone & Iardi s.n.* (CAT!).

TABLE 2. Main differential characters of *Dianthus borbonicus* and allied species

Characters	<i>D. borbonicus</i>	<i>D. cyatophorus</i>	<i>D. minae</i>	<i>D. busambrae</i>
Plant size (cm)	15–30(35)	(20)40–70	50–70	(15)30–60
Woody stock	compact	loose	loose	loose
Branch length (cm)	1–4	2–7(10)	3–10	3–10
Leaf shape	flat	flat	canaliculate	canaliculate
Leaf length (cm)	2–10(11)	10–25	4–18	3–16
Leaf width (mm)	2–4	1.5–3	2–4	2–3(4)
Number of flowers per stem	1–4	2–5(6)	2–8	(1)2–5
Epicalyx scales arrangement	divaricate	divaricate	divaricate	appressed
Epicalyx scales mucro length (mm)	0.5–2	3–4	0.7–1	1–1.3
Outer scale shape	ovate to subrounded	obovate	elliptical	transversally elliptical
Outer scale length (mm)	8–10	9–10	5.5–8	7–8.5
Outer scale width (mm)	5–7.5	6–6.5	5–6	7–10
Inner scale shape	subrounded to obovate	subrounded	obovate	transversally elliptical
Inner scale length (mm)	10	10–12	8–9	9–10
Inner scale width (mm)	12–13	9–11	8–9	9–13
Calyx length (mm)	26–30	22–25	28–32	26–30
Calyx teeth length (mm)	6–7	6–6.5	5–6	6–7.5
Petal length (mm)	40–45	30–35	34–40	25–30
Petal limb shape	cuneate	cuneate	cuneate–rounded	cuneate
Petal limb width (mm)	11–14	9–10	8–9	8–9
Petal teeth number	20–34	20–30	11–13	9–15
Petal teeth length (mm)	0.3–2	0.3–1	0.8–1.5	0.7–2.5
Anther length (mm)	3.5	4	5–5.5	2.6–2.8
Ovary length (mm)	8–10	8–9	8.5–9	6

Diagnostic key to the species of the *Dianthus sylvestris* group occurring in Sicily:

1. Epicalyx scales always divaricate2
- Epicalyx scales appressed to the calyx3
2. Stems 15–30(–35) cm long, outer scales 8–10 mm long, petal 40–45 mm long, with limb 11–14 mm wide, anthers 3.5 mm long.*D. borbonicus*
- Stems 50–70 cm long, outer scales 5.5–8 mm long, petal 34–40 mm long, with limb 8–9 mm wide, anthers 5.0–5.5 mm long.....*D. minae*
3. Epicalyx scales usually 4 (rarely 6).....4
- Epicalyx scales usually 6–10 (rarely 4).....5
4. Woody stock loose, with branches 3–10 cm long, petal 25–30 mm long, with limb 8–9 mm wide*D. busambrae*
- Woody stock contracted, with branches 0.5–1.0 cm long, petal 38–40 mm long, with limb 12–14 mm long.....*D. arrosti*
5. Woody stock contracted with leaves 1.0–4.5 mm long, stems 2–8-flowered, outer scales 5.0–7.5 mm long, ovary 9–10 mm long.*D. siculus*

- Woody stock loose with leaves 4–20 mm long, stems with 1–2-flowered, outer scales 7–11 mm long, ovary 5–8 mm long6
- 6. Outer scales lanceolate 2–3 mm wide, inner scales ovate-lanceolate 6–7 mm wide, petal 35–40 mm long, with limb 9–10 mm wide, anthers 3 mm long *D. gasparrinii*
- Outer scales obovate 3.0–3.5 mm wide, inner scales obovate 8.0–8.5 mm wide, petal 26–33 mm long, with limb 6–8 mm wide, anthers 3.5 mm long *D. graminifolius*

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