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PLANT TAXONOMY

A new species of *Brassica* sect. *Brassica (Brassicaceae)* from Sicily

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Abstract
Among the suffruticose cabbages of *Brassica* sect. *Brassica*, a new species from Sicily, named *B. raimondoi*, is described and illustrated. It is a chasmophyte restricted to some steep limestone cliffs near Taormina (NE Sicily) and is morphologically related to *B. incana*, with which it shares densely hairy, broad, amplexicaul leaves and winged petioles, but differs principally in its white corolla, larger floral pieces, more developed stigmatic papillae, shorter siliquae with keeled valves and a smaller, seedless beak, smaller seeds differing in testa microsculpture. A key for the identification of the currently known Sicilian taxa of the section is provided.

Keywords: Brassica, Brassica oleracea *group, endemic, karyology, Sicily, taxonomy*

Introduction

Brassica includes many species with economic and agricultural importance. The wild taxa belonging to *B.* sect. *Brassica* are characterized by a robust, suffruticose habit, very large and thick leaves, well-developed racemes, big flowers with white or yellow petals, ellipsoidal to linear siliquae with convex, woody valves ending in a 0- to 2-seeded beak, and globose, ± reticulate seeds (Gómez-Campo 1999), as well as a diploid chromosome complement with $2n = 2x = 18$ chromosomes. According to Harberd (1972, 1976) and Lannér et al. (1997), the section forms a single cytodeme, the wild and cultivated taxa being all inter-fertile to various degrees (Kianian & Quiros 1992; Bothmer et al. 1995).

In addition to gross morphology, seed shape and especially testa micromorphology, as seen by scanning electron microscope (SEM), are important diagnostic characters for *Brassica* taxa (Stork et al. 1980; Snogerup et al. 1990; Koul et al. 2000; Tantawy et al. 2004; Zeng et al. 2004; Kasem et al. 2011). Seed sculpture is known to be conservative and stable, of relevance for taxonomic, phylogenetic and evolutionary studies (Vaughan & Whitehouse 1971; Zeng et al. 2004; El Naggar 2005). Moreover, significant differences with taxonomic implications have been detected in the compounds of seeds of various taxa of *B.* sect. *Brassica* (Scialabba et al. 2010). Some taxa have been studied from the genetic point of view in perspective of genetic resources management (Geraci et al. 2004).

Wild species of *Brassica* sect. *Brassica* are widespread along the Atlantic coast of Europe and in the Mediterranean basin, with Sicily, hosting the highest number of species, as its main centre of diversity. According to current literature (Snogerup et al. 1990; Raimondo et al. 1991; Heywood & Akeroyd 1993; Gustafsson & Lannér-Herrera 1997; Raimondo & Mazzola 1997; Gómez-Campo 1999; Gladis & Hammer 2001), the section comprises up to 22 taxa: *B. oleracea* L., *B. montana* Pourr., *B. bourgeaui* (Webb) Kuntze, *B. cretica* Lam. [subsp. *cretica*, subsp. *aegaea* (Heldr. & Hal.) Snogerup et al., subsp. *laconica* M. A. Gust. & Snogerup], *B. hilarionis* B. D. Post, *B. incana* Ten. (including *B. cazzae* Ginzb. & Tayber, *B. botteri* Vis. and *B. mollis* Vis.), *B. macrocarpa* Guss., *B. rupestris* Raf. (subsp. *rupestris*, subsp. *hispida* Raimondo & Mazzola), *B. villosa* Biv. [subsp. *villosa*,

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subsp. *bivoniana* (Raimondo & Mazzola) Raimondo & Mazzola, subsp. *tinei* (Lojac.) Raimondo & Mazzola, subsp. *brevisiliqua* (Raimondo & Mazzola) Raimondo & Geraci], *B. drepanensis* (Caruel) Damanti, *B. insularis* Moris [including *B. atlantica* (Coss.) O. E. Schulz] and *B. tyrrhena* Giotta et al.

During field work in Sicily, some specimens of a peculiar, densely hairy *Brassica* were collected from the limestone cliffs near Taormina, where they stood out by their white flowers. This feature was surprising, because all Sicilian taxa belonging to the *Brassica oleracea* group show a yellow corolla. The only Mediterranean wild taxa characterized by white petals are *B. insularis* (Sardinia, Corsica, Tunisia, Algeria and Pantelleria), *B. hilarionis* (Cyprus) and *B. cretica* subsp. *cretica* (Crete), but they are glabrous or subglabrous. Due to its semi-amplexicaul, pubescent leaves and petiole with several lobes or ± winged, our plant seems most closely related to *B. incana,* which, however, usually has bright yellow flowers and also differs in many other features. Besides, individuals with white flowers grow together with typical *B. incana* on the same cliffs without giving rise to natural hybrids, suggesting the existence of reproductive barriers preventing cross-fertilization. This reproductive isolation, added to morphological differences, justify the treatment of these populations as distinct species. Thus, this Sicilian population of *Brassica* is here described as a species new to science, named *Brassica raimondoi.*

Materials and methods

Morphological analyses were performed on living plants from the respective *loci classici* and some other Sicilian localities, as well as on herbarium material kept in CAT [for *Brassica incana*: Valle dell'Anapo, 14.4.1988, *Bartolo* et al.; Monti Climiti, Cava Sorciaro, 23.3.1989, *Minissale* et al.;. Cavagrande del Cassibile, Avola, 6.6.1973, *Brullo*; Cava dell'Anapo, Ferla, 14.6.1977, *Brullo*; Rupi tra Gliaca e Gioiosa Marea (ME), 20.4.1988, *Brullo & Minissale*; Taormina, 22.6.1974, *Brullo*; Torrente Rosmarino, Messina, 14.7.1973, *Brullo*; Pressi Mongiuffi (ME), Ampelodesmeto, 30.5.1988, *Minissale*; Capo S. Alessio, 15.5.1987, *Minissale & Spampinato*].

The material was examined under a Zeiss Stemi SV 11 Apo stereomicroscope at 6× to 66× magnification. The seed coat micro-morphology was studied on 20 mature dry seeds randomly selected from a lot of 500 seeds coming from 30 different individuals; analyses were made under a SEM Zeiss EVO LS10, according to the protocol reported by Stork et al. (1980); the terminology of seed coat sculpturing follows Barthlott (1981) and Gontcharova et al. (2009).

Karyological analyses were performed on mitotic plates from root-tip cells of Petri-dish germinated seeds, pre-treated for 3 h with a 0.3% colchicine aqueous solution, then fixed for 12 h in a freshly prepared mixture of absolute ethanol with glacial acetic acid (3:1) and stored in 70% ethanol. Treated root tips were hydrolyzed in 1 N HCl for 7 min at 60°C, and stained according to the Feulgen technique. Micrographs of good quality metaphase plates were taken using a Zeiss Axioskop 2 microscope equipped with a monochrome CCD camera and an Axiocam MRc5 high-resolution digital camera. The somatic chromosome number and complement were studied in 10 metaphase plates from 10 different germinated seeds sampled at the type locality. Vouchers of all material examined are kept in CAT.

Results

Brassica raimondoi Sciandr., C. Brullo, Brullo, Giusso, Miniss. & Salmeri, **sp. nov.** (Figures 1 and 2). – Holotype: Sicilia**,** Rupi di Castelmola (Messina), 21.6.2012, *C. Brullo, S. Brullo, Giusso del Galdo & Sciandrello* (CAT; isotypes: CAT, FI, PAL).

Diagnosis. Planta *Brassicae incanae* similis sed sepalis longioribus, glabris vel subglabris, petalis majoribus, albis, staminibus longioribus, ovario longiore, papillis stigmaticis majoribus, siliqua breviore, valvis dorso carinatis, pedicello fructifero breviore, rostro minore aspermo, seminibus minoribus differt.

Etymology. The new species is dedicated to Francesco Maria Raimondo, active botanist from Palermo and expert of Sicilian *Brassica* species.

Description. Suffrutex 100–150 cm tall, with a robust main stem often branching from the base. Stem woody, up to 20 mm in diameter at the base, green to pale green, hairy below, glabrous or subglabrous in the upper part. Leaves rigid, slightly thickened, green, densely pubescent when young, the adult ones adult laxly hairy to subglabrous, with hairs longest on veins and midrib; basal and lower cauline leaves 10–40 cm long (including the 5–18 cm long petiole); petiole with several pairs of lobes, usually winged and auriculate at base; lamina ovate to ovate-lanceolate, 8–25 cm × 6–12 cm, lobed or basally pinnatifid to pinnatisect, with rounded lobes and irregularly, coarsely dentate margin; midrib strong, lateral veins conspicuous; upper cauline leaves gradually smaller, shortly petiolate to sessile, their lamina often linear, entire, only 1–3 cm long. Inflorescence loosely paniculate, ± branched. Pedicels 7–12 mm long, glabrous, erecto-patent to patent. Sepals green, glabrous, sometimes with scattered hairs at apex, the outer ovate-lanceolate, 18–19 mm × 6.5–7 mm,

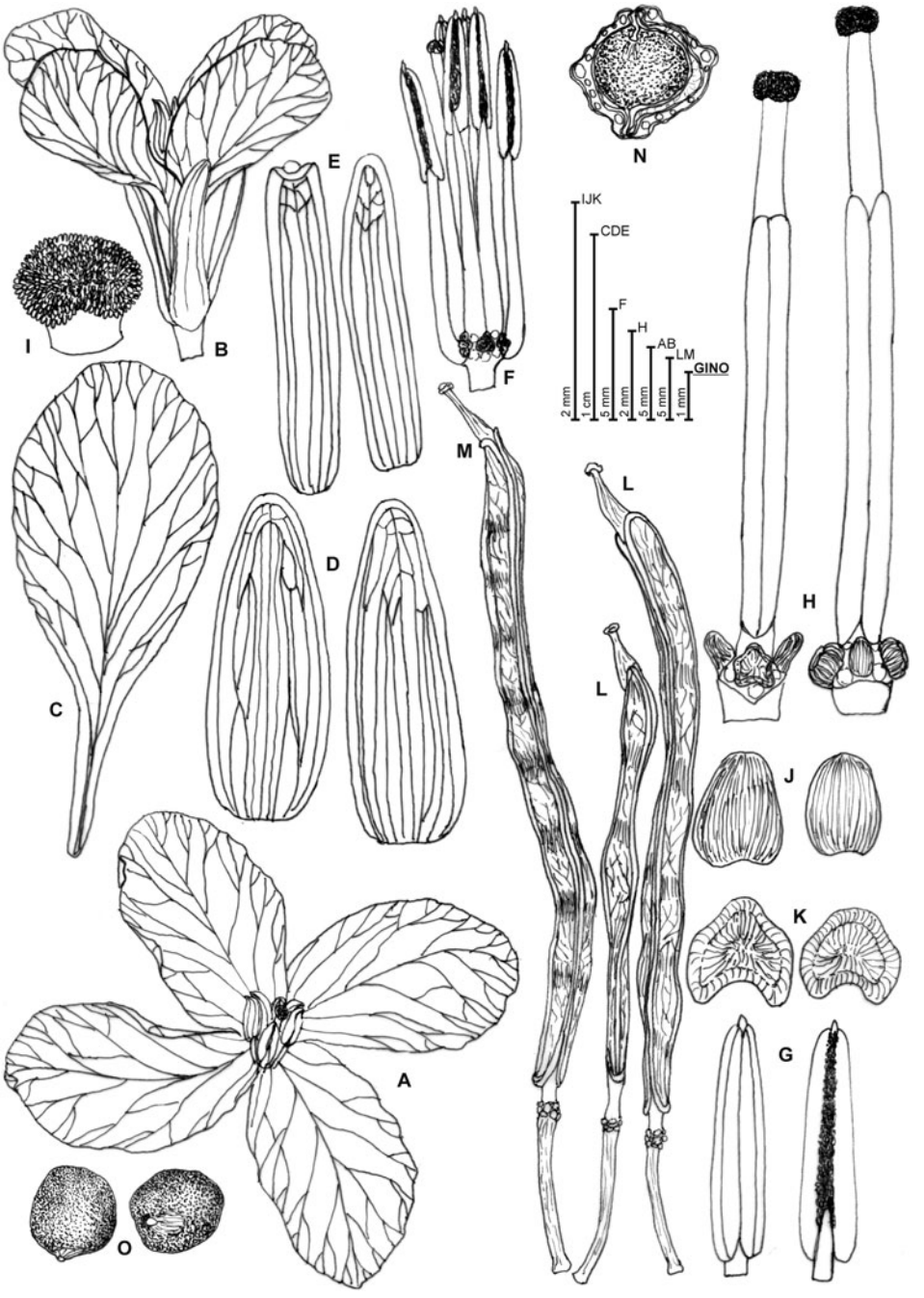

Figure 1. *Brassica raimondoi* Sciandr. et al.: (A) flower, upper view; (B) flower, lateral view; (C) petal; (D) outer sepals; (E) inner sepals; (F) stamen and pistil; (G) anthers; (H) pistils; (I) stigma; (J) smaller nectaries; (K) larger nectaries; (L, M) siliquae; (N) siliqua, cross section; (O) seeds (drawings by S. Brullo).

the inner linear to linear-lanceolate, 17–18 mm × 3–3.5 mm, cucullate, slightly saccate at base. Petals spathulate, white, 25–27 mm long; claw 7–8 mm long; limb 10–11 mm wide, rounded. Stamens exceeding petal claws by 5–6 mm; outer filaments 8–9 mm long; inner filaments 9–10 mm long; anthers yellow, 4.5–5.5 mm long, with a conspicuously tipped connective. Pistil shorter than or ± equalling the stamens; ovary 9–10 mm long, greenish-white, glabrous; style green, 2.5–3.5 mm long, subcylindrical; stigma 0.5–0.6 mm × 0.8–0.9 mm, subcapitate, minutely papillose with 60–100 μm high, greenish papillae. Nectary glands dark green, the larger 0.8–1 mm × 0.8–0.9 mm, the smaller 0.9–1 mm × 0.6–0.8 mm. Fruiting pedicels 14–20 mm, erecto-patent. Siliqua body linear, torulose, striate, (25)30–65(80) × 2.5–3 mm, the valves thin, hardened, usually ribbed dorsally, well filled with 10–32 seeds; beak conic, 4–10(12) mm long, seedless, rarely with 1 seed. Seeds subglobose, 1.7–2 mm in

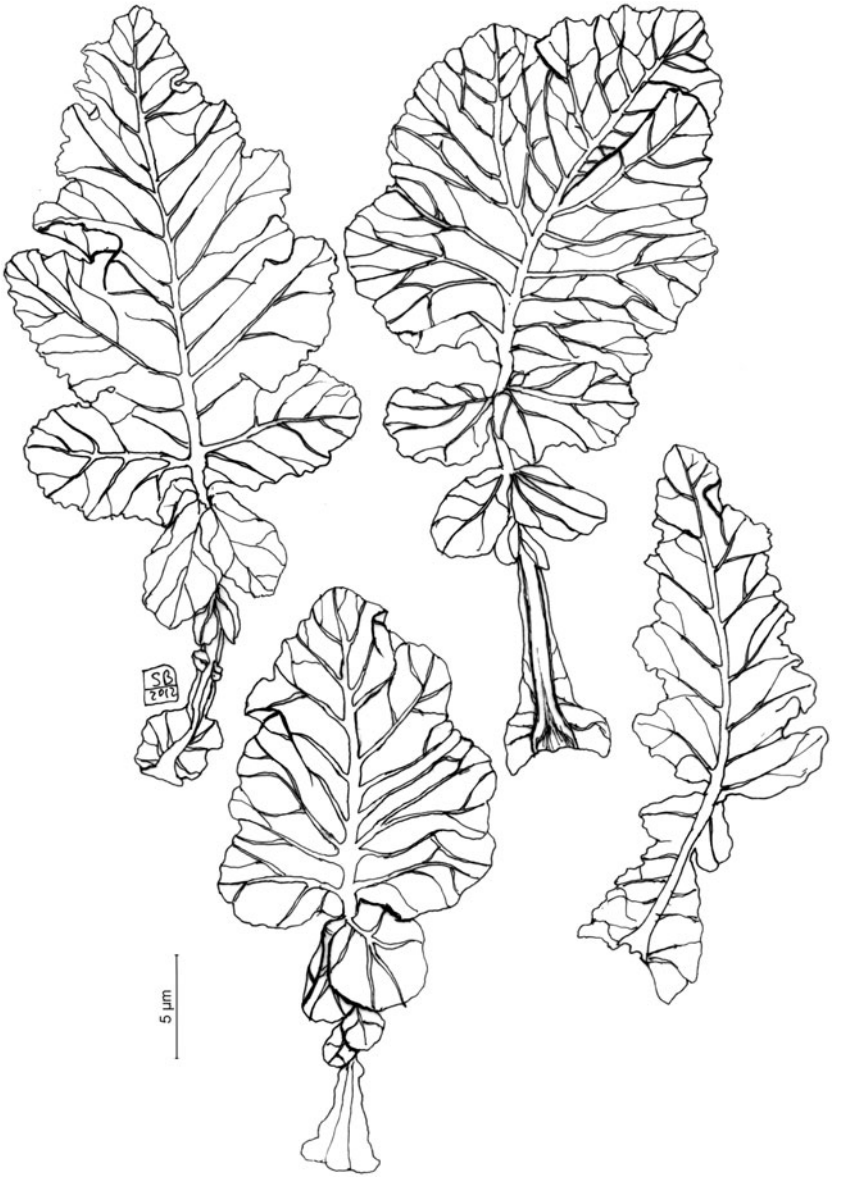

Figure 2. Basal leaves of *Brassica raimondoi*, showing variability (drawings by S. Brullo).

diameter, brownish-black, minutely reticulate, arranged in a single row in each loculus.

Other specimens examined (paratypes). Sicily: Rupi di Castelmola (Messina), 7.4.2012, *Minissale* (CAT); Rupi presso Castelmola (Messina), 8.4.2012, *Sciandrello & D'Agostino* (CAT); Rupi di Castelmola (Messina), 21.4.2012, *Sciandrello & D'Agostino* (CAT); Rupi di Castelmola (Messina), 3.5.2012, *Minissale & Sciandrello* (CAT).

Phenology. Flowering March to May, fruiting June and July.

Testa micromorphology. The seed coat of *Brassica raimondoi* is irregularly reticulate-glebulate, formed of ± polygonal cells 60–80 µm wide; the anticlinal walls are raised, very thick and irregular, with undulate-striate folds over their entire surface; the outer periclinal walls are shallowly depressed, flat and covered by small colliculi (Figure 3(A),(B)). Testa microsculpture of *B. incana*, studied for comparison, differs markedly: the seed coat is irregularly and indistinctly reticulate, with slightly raised, very thin, smooth or weakly striate anticlinal walls and shallowly depressed periclinal walls with a minutely reticulate surface (Figure 3(C),(D)).

Karyology. Brassica raimondoi is diploid, with a somatic chromosome number of $2n = 18$. This number coincides with that commonly reported for other taxa of *B.* sect. *Brassica* (Anderson & Warwick 1999; Warkick et al. 2009). Mitotic metaphase plates (Figure 4) show very small chromosomes, except for two bigger pairs, closely resembling the chromosome

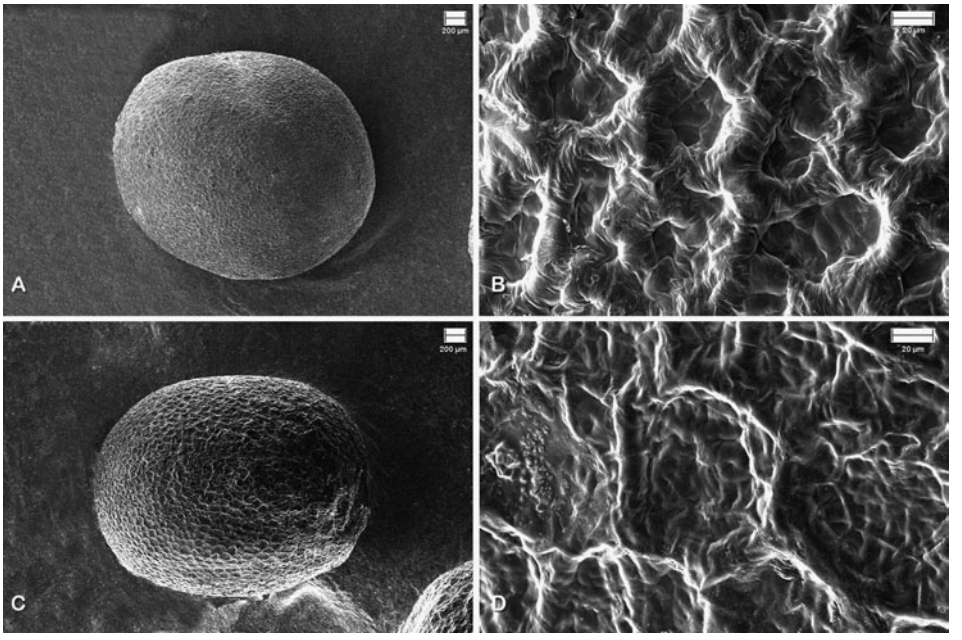

Figure 3. Scanning electron micrographs of seed sculpturing in *Brassica raimondoi* (A, B) and *B. incana* (C, D).

complement given for *B. incana* (Ferrarella et al. 1979).

Distribution and ecology. *Brassica raimondoi* was found on steep limestone cliffs facing north or east, at an altitude of 400–500 m a.s.l., in natural habitats along the road near Castelmola, a little town close to Taormina (NE Sicily). It is a typical chasmophyte, growing in rock crevices together with other endemic or rare species such as *Colymbada tauromenitana* (Guss.) Holub, *Erucastrum virgatum* (C. Presl) C. Presl, *Dianthus rupicola* Biv. subsp. *rupicola*, *Brassica incana* Ten., *Lomelosia cretica* (L.) Greuter & Burdet, *Silene fruticosa* L., *Antirrhinum siculum* Mill. (Lantieri et al. 2012; Raimondo et al. 2012b). From a phytosociological point of view, *B. raimondoi* is a member of the association *Erucastretum virgati* Brullo and Marcenò 1979 (Brullo et al. 2004), belonging to the *Dianthion rupicolae* Brullo and Marcenò (1979).

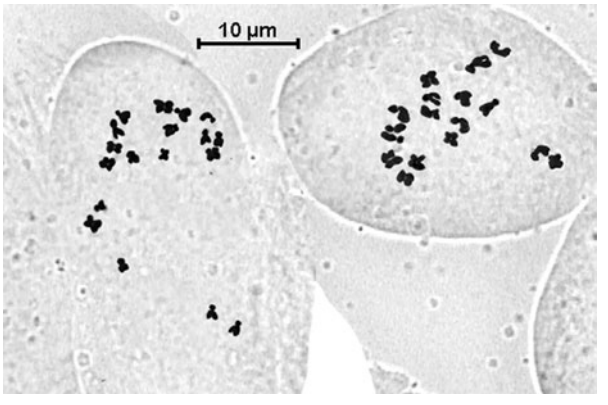

Figure 4. Chromosome complement ($2n = 18$) from mitotic metaphase plates of *Brassica raimondoi*.

Conservation status. Like other Sicilian species of *Brassica* linked to cliff habitats (Raimondo et al. 2012b), *B. raimondoi* is in need of special conservation measures. Due to the low number of individuals (<100) and the very circumscribed distribution (c. 2 km^2), according to the criteria of the IUCN Red list and (IUCN 2010), we suggest that *B. raimondoi* be assigned to the category Critically Endangered [CR = C2 a (i)].

Discussion and conclusion

Within *Brassica* sect. *Brassica*, *B. raimondoi* shows close relationship with *B. incana* (Figure 5), a wild cabbage widely distributed in north-eastern Sicily, central and southern Italy, along the Adriatic coast of southern Croatia and in northern Greece (Snogerup et al. 1990; Raimondo et al. 1991; Gustafsson & Lannér-Herrera 1997; Brullo et al. 2011). They share the same type of leaf, usually densely pubescent and with a winged, basally auriculate petiole bearing several pairs of lobes. Several morphological features allow their easy distinction, both in flower and fruit. The differences are listed in Table I.

Brassica raimondoi is sympatric with *B. incana*, both species growing together at one and the same place. This is surprising because, based on personal observations and literature data, the known populations of the taxa of *B.* sect. *Brassica*, even when they occur in the same geographical area (e.g. Sicily or Greece), never share the same locality. The evident lack of natural hybrids suggests the presence of reproductive barriers, preventing crosses and

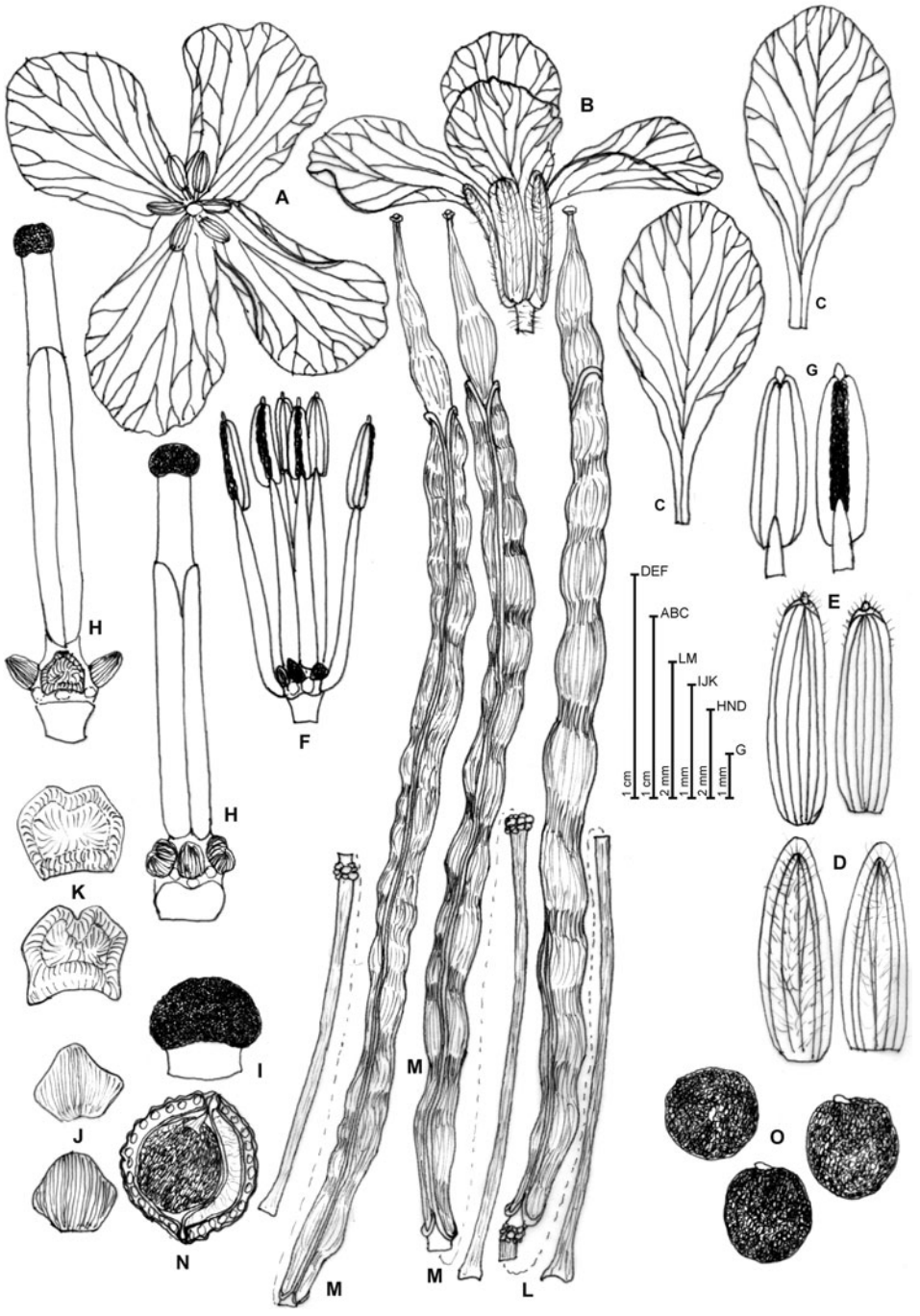

Figure 5. *Brassica incana* Ten.: (A) flower, upper view; (B) flower, lateral view; (C) petals; (D) outer sepals; (E) inner sepals; (F) androecium; (G) anthers; (H) pistils; (I) stigma; (J) smaller nectaries; (K) larger nectaries; (L, M) siliquae, with detached fruiting pedicel (L, dorsal view; M, lateral view); (N) siliqua, cross section; (O) seeds (drawings by S. Brullo).

consequent gene flow between *B. raimondoi* and *B. incana*. Perhaps, some of the observed differences, e. g. in petal colour and size of stigmatic papillae, might affect pollinator attraction, pollen adhesion and pollen tube development (Edlund et al. 2004; Lowry et al. 2008), and limit cross-pollination. Nevertheless, the possibility of post-pollination reproductive isolation mechanisms (Widmer et al. 2009), including chromosomal and genetic factors, should not be discarded and is worthy of further study.

This new species further enriches the Sicilian endemics greatly increased in the last years (Raimondo & Di Gristina 2007; Raimondo & Spadaro 2008; Brullo et al. 2009, 2012a,b, 2013; Troia & Raimondo 2009; Castellano et al. 2012; Cataldo et al. 2012; Marino et al. 2012; Raimondo et al. 2012a).

Table I. Comparison of morphological features of *Brassica raimondoi* and *B. incana*.

Features	*B. raimondoi*	*B. incana*
Stem indumentum	Loosely pubescent	Densely pubescent
Adult leaf indumentum	Loosely hairy to subglabrous	Densely pubescent
Pedicel length (mm)	7–12	14–35
Pedicel indumentum	Glabrous	Hairy
Size of outer sepal (mm)	18–19 × 6.5–7	9–10 × 2.6–3
Size of inner sepal (mm)	17–18 × 3–3.5	8.5–9.5 × 2–2.5
Petal size (mm)	25–27 × 11–12	17–18 × 8–8.5
Petal claw (mm)	7–8	4.5–5
Petal colour	White	Bright yellow
Stamens exceeding petal claws by (mm)	5–6	2.5–3
Inner stamens, filament length (mm)	10–11	9–10
Anther length (mm)	4.5–5.5	4–4.5
Ovary length (mm)	9–10	12–13
Style length (mm)	2.5–3.5	3.5–4
Stigma size (mm)	0.8–0.9	0.9–1
Size of stigmatic papillae (μm)	60–100	20–40
Length of large nectaries (mm)	0.8–1	0.7–0.8
Length of small nectaries (mm)	0.9–1	0.6–0.7
Siliqua length without rostrum (mm)	(25)30–65(80)	60–100
Siliqua width (mm)	2.5–3	3–5
Valve, dorsal rib	Usually present	Usually absent
Seed number	10–32	20–36(–40)
Rostrum length (mm)	4–10(–12)	10–20
Number of rostrum seeds	0(1)	0–2
Fruiting pedicel length (mm)	14–20	25–35
Seed diameter (mm)	1.7–2	2–2.4

Key to the Sicilian taxa of *Brassica* sect. *Brassica*

The following analytical key makes use of those proposed by Raimondo et al. (1991) and Raimondo and Mazzola (1997).

1. Leaves glabrous or hispid with bulbose, ± scattered hairs 2
1. Leaves villous or pubescent 6
2. Petals white; siliqua laterally compressed ..... *B. insularis*
2. Petals yellow; siliqua isodiametric or dorsally compressed 3
3. Leaf teeth obtuse; siliqua navicular, 25–40 mm × 8–12 mm; valves thickened, spongy, dorsally smooth; rostrum widely conic, 10–20 mm, 1–2-seeded ..... *B. macrocarpa*
3. Leaf teeth acute; siliqua linear, 35–65 mm × 3–7 mm; valves thin, dorsally ribbed; rostrum subulate to narrowly conic, 4–11 mm, seedless 4
4. Petals pale yellow, 14–20 mm × 5–8 mm; siliqua 35–45 mm × 6–7.5 mm ..... *B. villosa* subsp. *brevisiliqua*
4. Petals yellow, 18–27 mm × 7–13 mm; siliqua 35–70 mm × 3–4.5 mm 5
5. Leaves green, glabrous; leaf lamina ovate-lanceolate, acute, deeply incised, margin loosely dentate; siliqua valves slightly ribbed ..... *B.rupestris* subsp. *rupestris*
5. Leaves glaucescent, hispid; leaf lamina ovate-elliptical, obtuse, lobed, margin minutely dentate; siliqua valves prominently ribbed ..... *B. rupestris* subsp. *hispida*
6. Petiole auriculate basally, up to 15(18) mm 7
6. Petiole not auriculate, up to 30 mm 8
7. Pedicels hairy, 14–35 mm; petals yellow; siliqua body 60–100 mm × 3–5 mm, not ribbed dorsally; rostrum 10–20 mm; fruiting pedicels 25–35 mm ..... *B. incana*
7. Pedicels glabrous, 7–12 mm; petals white; siliqua body 30–65 mm × 2.5–3 mm, ribbed dorsally; rostrum 4–10 mm; fruiting pedicels 14–20 mm ..... *B. raimondoi*
8. Leaf lamina lyrate, margin crispate-denticulate; petiole winged; siliqua 5–6.5 mm wide ..... *B. drepanensis*
8. Leaf lamina lobed, margin dentate, with petiole unwinged; siliqua 3–4.5 mm wide 9
9. Leaf lamina minutely dentate; body of siliqua laterally compressed, 25–30 mm ..... *B. villosa* subsp. *tinei*
9. Leaf lamina broadly dentate; body of siliqua not laterally compressed, 30–75 mm 10
10. Leaf margin crenate-dentate; sepals 12–15 mm; petals 24–26 mm; siliqua 4–4.5 mm wide, dorsally ribbed ..... *B. villosa* subsp. *villosa*
10. Leaf margin irregularly dentate; sepals 8–11 mm; petal 16–22 mm; siliqua 3.5–3.8 mm wide, dorsally not ribbed ..... *B. villosa* subsp. *bivoniana*

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