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Morphology of the invasive *Carpobrotus* (Aizoaceae) in Europe: Malta as a case study

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Abstract. Since the late 19th century, different taxonomic views have been reported for *Carpobrotus* species occurring in the Maltese Islands, where the latest treatments imply two varieties of *C. edulis* and the doubtful occurrence of *C. acinaciformis*. Taxonomic inconsistencies are possibly derived from the poor understanding or misinterpretation of morphological characters. Moreover, the hybridogenous morphotypes resulting from horticultural advances make the determination of *Carpobrotus* even harder, especially with the application of outdated identification keys which do not take hybrids into account. The difficulty in determining *Carpobrotus* spp. has been expressed in several recent accounts within the Mediterranean region. A taxonomic investigation was carried out by applying eleven morphological characters adopted from recent monographs on 25 populations present in Malta. Three taxonomic units have been retrieved from the analyzed sample, where *C. acinaciformis* s.l. has been confirmed to represent all the purple-flowering populations and *C. edulis* s.s. for the yellow-flowering ones. In this account, emphasis was given on the interpretation and understanding of distinctive morphological characters by employing a standardized method of assessment aided by images. This comparative morphological study resulted in a new characteristic in the leaves of *C. edulis*, by which it could be easily distinguished from *C. acinaciformis* in the vegetative state. A detailed discussion about the two different morphotypes of *C. acinaciformis* s.l. and dichotomous keys to distinguish *Carpobrotus* taxa are also provided.

Keywords: *Carpobrotus*; invasive alien species; Mediterranean region.

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Introduction

Carpobrotus are native to coastal areas of Cape Town, South Africa and were introduced in the Mediterranean region in the 17th century as ornamental garden plants (Preston & Sell, 1988), which gradually spread further north and became invasive in many coastal areas, not only in the Mediterranean but throughout the five continents as well explained in great detail by Campoy *et al.* (2018). The taxonomic repertoire of *Carpobrotus* spp. is quite unstable for Malta and has been subjective to different taxonomists. The difficulty in determining *Carpobrotus* plants is not only exhibited in Malta (see history chapter below) but also in many parts of the Mediterranean region. From the literature review, the morphological distinction between the two main European species - *Carpobrotus acinaciformis* (L.) L. Bolus and *C. edulis* (L.) N.E. Brown is still either not well documented or not easy to understand when applied in the field; such difficulties have been expressed even in recent work (Campoy *et al*., 2018; Sarmati *et al*., 2019). Hybridization between the two species had also been expressed (Suehs *et al.*, 2004a,b), making their determination even harder (Campoy *et al*., 2018) due to broader overlapping of characters.

This study is an extension and application of the excellent paper by Campoy *et al*. (2018), who monographed in great detail several biological aspects of the invasive *Carpobrotus* species occurring in Europe, including hybrids referred to as *C.* aff. *acinaciformis*. They provided a section about the morphological characters of both species, including some distinctive characteristics reviewed from previous literature (Gonçalves, 1990; Wisura & Glen, 1993). This data was tested in the field on trial *Carpobrotus* populations present in the Maltese Islands and served as a taxonomic exercise to investigate what *Carpobrotus* morphotypes occur. This study emerges from the general assumption that the populations in the Maltese Islands consist of yellow and purple flower forms of *C. edulis*, but a few simple trials carried out in 2018–2019 have revealed the strong presence of *C. acinaciformis* in Malta (Mifsud, 2019).

One aim of this study was, therefore, to examine and test the reported distinctive characters listed by Campoy *et al*. (2018) on specimens in the field and give annotated observations of how diagnostic and useful each character is to distinguish *Carpobrotus* taxa *in situ*. It is understood that while Campoy *et al*. (2018) excelled in highlighting the differences extracted from various literature (e.g. Gonçalves, 1990; Wisura & Glen,

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1993), they have not tested them in the field. Hence, this work expands on the work done by Campoy *et al*. (2018) on various populations of *Carpobrotus* occurring in the Maltese Islands as a pilot study.

The occurrence of *Carpobrotus* in Malta follows the same trend for other Mediterranean countries in Europe. Plants were introduced as an ornamental succulent to embellish public gardens, parks and later private gardens. Eventually, they escaped and naturalized in the wild through the dumping of unwanted plants or deliberate cultivation in fields or natural ecosystems. For example, the population of *Carpobrotus* found invading the sand dune of a Natura 2000 site in Ramla Ħamra in Gozo (MT0000005), was deliberately planted by an individual in the mid-eighties (anonymous informant, May, 2018).

History and Taxonomy of *Carpobrotus* in Malta

Carpobrotus was initially recorded as *Mesembryanthemum* (=*Carpobrotus*) *acinaciforme* L. in the early 20th century (Sommier & Caruana Gatto, 1915; Borg, 1927) and updated to *C. acinaciformis* by Lanfranco (1969). A few years later, Lanfranco (1974) postulated that in Malta, *Carpobrotus* was represented by two forms of *C. edulis:* the yellow-flowering form (var. *edulis*) and the purple-flowering form (var. *rubescens* Druce). Lanfranco's treatment was adhered to till recent literature (Schembri & Lanfranco, 1996; Żahra, 2012; MEPA, 2013; Casha, 2017; Lanfranco & Bonett, 2018). Weak and unsuccessful attempts indicating that both *C. edulis* s.l. and *C. acinaciformis* occur in Malta were reported by Haslam *et al.* (1977), who probably only cited historic records without any investigation, and by Weber & Kendzior (2006) who stated that *C. edulis* var. *rubescens* is widely distributed in Malta. Casha (2017) refers to a personal communication by Lanfranco that *Carpobrotus* specimens that were examined resulted in being *C. edulis* s.l., and illustrates photos of *Carpobrotus* with purple and yellow flowers captioned to represents different flowering forms of *C. edulis*. The same stance was taken by Mifsud (2002–2020) until a preliminary investigation, in which purple-flowering specimens were assigned to be *C. acinaciformis* (Mifsud, 2019) upon using the latest diagnostic characters (Campoy *et al*., 2018).

This inconsistency clearly shows that there is a misconception or poor understanding of the aforementioned taxa, perhaps better referred to as morphotypes (e.g. Vilà & D'Antonio, 1998). There seems to be a misconception that pinkish-purple stamens and glaucous leaves are characters attributed to *C. acinaciformis* (Lanfranco, 1974; Haslam *et al*., 1977; Pignatti, 1982; Casha, 2017), and since the Maltese *Carpobrotus* plants do not exhibit these characters, it was then deduced that they must be referable to *C. edulis*. This argument can be well demonstrated by the key of Pignatti (1982), which differentiates *C. edulis* from *C. acinaciformis* by the colour of the stamens, but this distinction was discarded in the second edition of his work (Pignatti, 2019) and concurrently not included amongst the critical characters for distinguishing these two species by Campoy *et al*. (2018). Therefore, such inaccurate keys have probably contributed and influenced early local botanists.

The introduction of *Carpobrotus* to the Maltese Islands is estimated to have taken place in the middle of the 19th century. Earlier records of closely related Aizoaceae species, namely *Mesembryanthemum tenuifolium* Lin (Gulia, 1856) and *Mesembryanthemum deltoideum* L. (Cleghorn, 1869), might have been misidentified records of *C. acinaciformis*. Old floristic literature show that *Carpobrotus* already had a Maltese name - 'Xuxet San Ġwann' - and was reported as naturalized in a few places already at the beginning of the 20th century (Sommier & Caruana Gatto, 1915; Borg, 1927).

Most likely, the European species of *Carpobrotus* were introduced by the British who were stationed and had military bases in Cape Town, South Africa, where these species are native to. Indeed, historical records by Borg, as well as Sommier and Caruana Gatto (*op. cit*.), include Valletta, Balluta, and the British military hospital at Bighi, all of which were managed or influenced (populated) by British rule. Nowadays, *Carpobrotus* spp. are declared as invasive alien species in Malta (MEPA, 2013) and are found naturalized in several coastal areas, including Natura 2000 sites such as in Comino, Xlendi, Ta' Ċenċ, Dwejra and Żurrieq.

Materials and methods

An investigation on 25 randomly selected populations of *Carpobrotus* spp. - 1 in Comino, 10 in Malta and 14 in Gozo - was carried out (see Table 1 for location and habitat, and Figure 1 for map). Recent literature was consulted to obtain the morphological characters reported to distinguish *C. edulis* from *C. acinaciformis* and related taxa (Gonçalves, 1990; Wisura & Glen, 1993; Preston & Sell, 1988; Campoy *et al*., 2018; Pignatti, 2019). The morphological characteristics selected in this study were: the shape of the leaf; the colour of leaves; the cross-section of leaves; the length of the calyx lobes; the shape and size of the receptacle (longitudinal section); the shape of the lateral side of fruit; the colour of petals; the colour of filaments; and the number of locules per ovary (transversal section). These characters were also tested to check if they are reliable and how valuable they are to discriminate between the different taxa of *Carpobrotus*. This assessment is recorded in four states: none, low, medium and high, based on reliability, discrimination strength, consistency throughout the analyzed samples and ease to measure or judge the character states. Moreover, observations were made to standardize the recording of the state of some characters or provide recommendations to obtain optimal measurements or observations. During the morphological study of various specimens, other unreported morphological characters which were found helpful and practical to differentiate the *Carpobrotus* taxa are also documented.

Table 1. Data from 25 populations of *Carpobrotus* spp. occurring in the Maltese Islands.

Ref. Code	Island	Locality and area where found	Date (2020)	Habitat
CRB01	Comino	Għajnsielem. Close to disused tennis court	25-Apr	Naturalized on coastal rocky ground
CRB02	Gozo	Għasri. Opposite Ta' Pinu church	28-Apr	Neglected cultivation
CRB03	Gozo	Żebbuġ. Coast near Qbajjar	28-Apr	Naturalized on coastal rocky ground
CRB04	Gozo	Xagħra. Marsalforn Road	28-Apr	Abandoned cultivation
CRB05	Gozo	Żebbuġ. Qbajjar playing fields and promenade	28-Apr	Embellishment
CRB06	Gozo	Għasri. Opposite Ta' Pinu church	28-Apr	Abandoned cultivation
CRB07	Gozo	San Lawrenz. Inland sea at Dwejra	28-Apr	Naturalized on coastal rocky ground
CRB08	Gozo	San Lawrenz. Close to Kempinski Hotel	28-Apr	Cultivation
CRB09	Gozo	Sannat. Near ta Ċenċ Hotel	28-Apr	Abandoned cultivation
CRB10	Gozo	Żebbuġ. Triq Għajn Mhelhel	01-May	Abandoned cultivation
CRB11	Gozo	Munxar. Xlendi Bay	01-May	Naturalized on coastal rocky ground
CRB12	Gozo	Sannat. Mġarr ix-Xini Bay	01-May	Naturalized on coastal rocky ground
CRB13	Gozo	Sannat. Ta' Ċenċ cliffs	01-May	Naturalized on coastal rocky ground
CRB14	Malta	Mellieħa. Aside chapel at l-Aħrax tal-Mellieħa	07-May	Embellishment
CRB15	Malta	Mellieħa. Selmun Palace	07-May	Embellishment
CRB16	Malta	Wardija. Promontorio Gardens	07-May	Cultivation
CRB17	Malta	Burmarrad. Outside Sherries Garden Centre	07-May	Embellishment
CRB18	Malta	Siġġiewi. Għar Lapsi, traffic island near police station	08-May	Embellishment
CRB19	Malta	Qrendi. Ħaġar Qim restaurant	08-May	Cultivation
CRB20	Malta	Mellieħa. Selmun Palace	14-May	Cultivation
CRB21	Gozo	Sannat. Ta' Sabbara area	21-May	Naturalized from abandoned cultivation
CRB22	Gozo	Għasri. San Ġużepp area	21-May	Naturalized from abandoned cultivation
CRB23	Malta	Marsascala. Saint Thomas Bay	23-May	Embellishment
CRB24	Malta	Xgħajra. Coastal promenade	23-May	Naturalized on rocky ground from embellishment
CRB25	Malta	Xgħajra. Smart City	23-May	Embellishment (abandoned?)

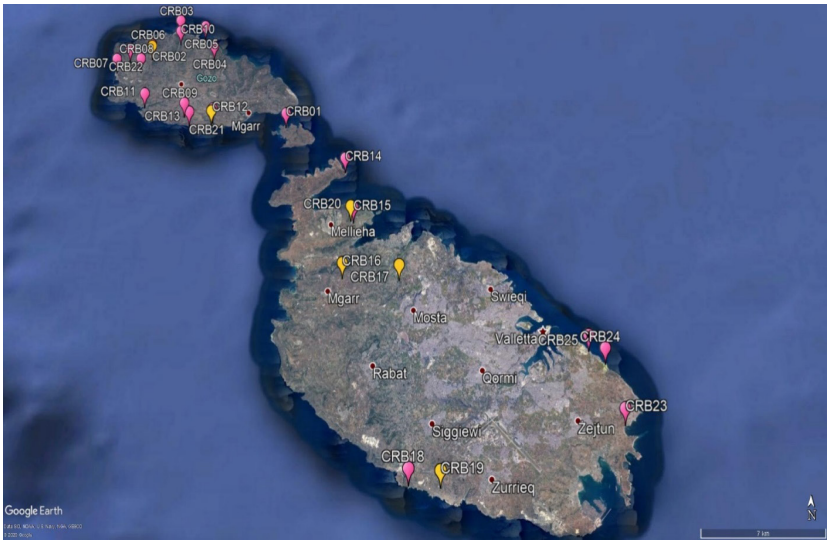

Figure 1. Map of the Maltese Islands showing the location of the studied populations of *C. edulis* (yellow mark), and *C. acinaciformis* s.l. (purple mark).

Herbarium specimens are bulky and unsuitable for the study of *Carpobrotus* (destructive analysis, colour not restored, the requirement of analyzing more than one organ or sample, etc. (Preston & Sell, 1988). Therefore, a representative photo was instead taken for each specimen over a 1 × 1 cm grid mat (Figure 2A-B) comprising of the following structures: five developed leaves located at least three nodes below the flower or apical leaves; five cross-sections of leaves; abaxial side of the flower to show calyx lobes; transversal

section of flower to show the petals (including their bases), stamens and ovary; and cross-section of five to seven ovaries of mature flowers or young fruit to expose the number of locules. Other photos of the entire plants, flowers, fruit, and other organs were also taken *in situ*. From preliminary studies (Mifsud, 2019), the methodology employed here was that of checking at least five samples and taking an averaged morphological assessment due to the variability and inconsistency of some characters.

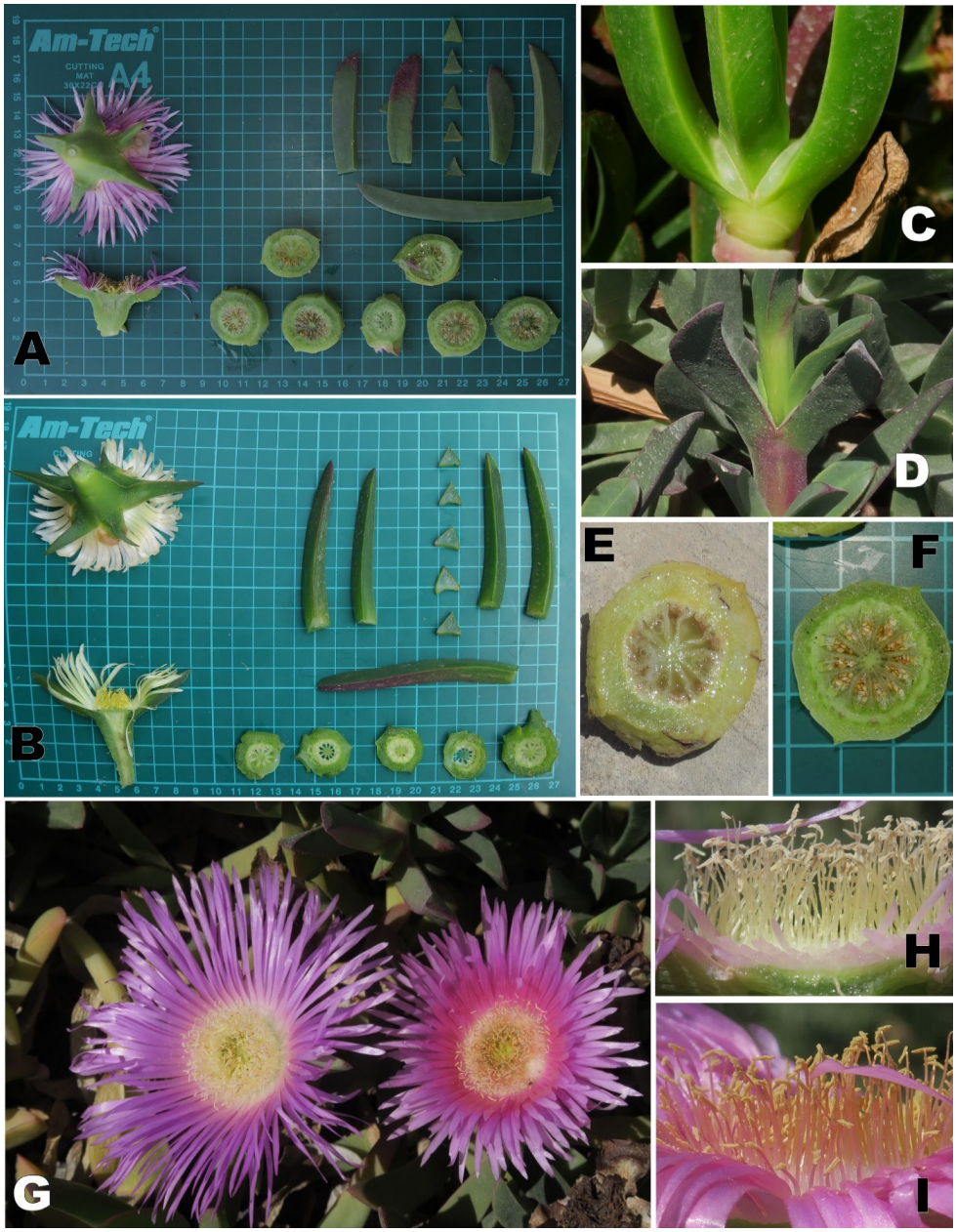

Figure 2. *Carpobrotus* spp. from the Maltese Islands: A-B, representative study images taken for each population comprising the most significant morphological characters, A, *C.* aff. *acininaciformis*; B, *C. edulis*; C, swollen subhyaline lips at base of leaves of *C. edulis*; D, unspecialized leaf bases (sometimes slightly swollen) of *C.* aff. *acinaciformis*; E, seedless and sterile ovary of *C.* aff. *acinaciformis*; F, developed seeds in ovaries of *C. acinaciformis* s.s.; G, comparison of flowers of *C.* aff. *acinaciformis* (left) and *C. acinaciformis* s.s. (right); H, yellow stamens of *C.* aff. *acinaciformis*; I, purple-pink filaments of *C. acinaciformis* s.s.

Samples of leaves, flowers or fruit were taken randomly from scattered locations within the studied population, usually spaced equidistantly from each other except for small populations (less than 3 × 3 m^2 surface area) that are often found as embellishment or as garden plants (e.g. CRB02, CRB08 and CRB18). Small populations were assumed to be the same clone or plant, while for large populations which formed dense mats (e.g. Figure 3 H-I), it was difficult to ascertain if samples were taken from the same or different plants. However, it was assumed that any single population was represented by one morphotype. The selection of populations was rather random; either known previously by the author or encountered during surveys. For each population, at least five samples were measured, and the results were averaged out, although a larger sample size was permitted in larger populations. The location, habitat and date of collection are given in Table 1.

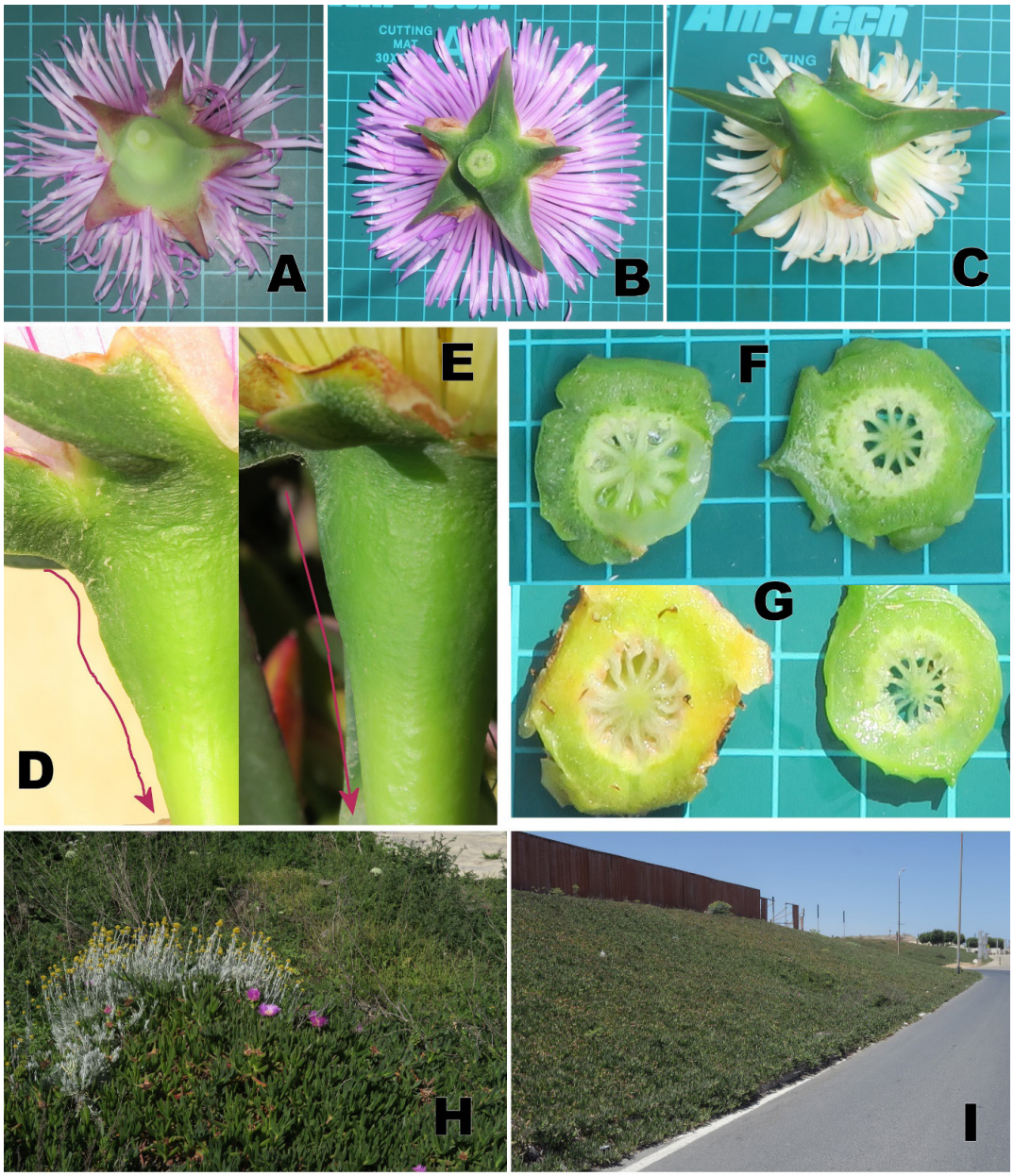

Figure 3. *Carpobrotus* spp. from the Maltese Islands: A-C, Abaxial side of flower showing calyx lobes, A, subequal lobes of *C. acinaciformis* s.s.; B, unequal lobes of *C.* aff. *acinaciformis*; C, unequal lobes of *C. edulis*; D, profile of receptacle (ovary) of *C. acinaciformis* s.l. having a slightly swollen side; E, profile of receptacle of *C. edulis* having a rather straight and tapering side; F, locules of two ovaries of *C. edulis* (normally 9–10 locules); G, locules of two ovaries of *C. acinaciformis* s.l. (normally 11–13 locules); H, endemic shrub of *Helichrysum melitense* (Pignatti) Brullo et al. smothered by an invasive population of *C.* aff. *acinaciformis* at the Natura 2000 site of Dwerja, Gozo (28/Apr/2020); I, huge population of *C. acinaciformis* s.s. at Smart City, Kalkara (23/May/2020).

Results

Three principal outcomes resulted from this investigation: i) a sound understanding of the three morphotypes of *Carpobrotus* occurring in the Maltese Islands; ii) a better interpretation of the most taxonomically significant characters used in the field, and iii) an unreported morphological characteristic that easily distinguishes *Carpobrotus* taxa in the vegetative state without the need for any dissection. The set of character states found for each population is presented in Table 2. Both *C. edulis* and *C. acinaciformis* s.l. have been confirmed as had already been indicated (Mifsud, 2019), however, the latter has been found to be represented by two distinct morphotypes as discussed below.

Table 2. Morphological characters for 25 populations of *Carpobrotus* spp. studied. Abbreviations are: CL, Calyx Lobes; CSL, Cross section of leaves; ALSCL, Average length of shortest CL (mm, n=5); ALLCL, Average length of longest CL (mm, n=5); CLDI, CL Differential Index (ALLCL-ALSCL)^2; LSR, Lateral shape of receptacle; ANL, Average number of locules per ovary (n=5); SLBL, Swollen lips at base of leaves.

Code	Flower colour	Filaments colour	Widest part of leaf	CSL	ALSCL	ALLCL	CLDI	LSR	ANL	SLBL
CRB01	Purple with a white base	Golden Yellow	Upper third	Isosceles	21.4	34	159	Amphora	12.2	No
CRB02	Yellow	Bright yellow	Base or lower half	Equilateral	22.2	46.6	595	Deltoid	9.6	Yes
CRB03	Purple with a white base	White to cream	Upper half	Isosceles	22.4	36.8	207	Amphora	11	No
CRB04	Purple with a white base	Light yellow	Upper half	Isosceles	20.6	31.4	117	Amphora	12.4	No
CRB05	Purple with a white base	White	Upper half	Isosceles (few sub-equilateral)	19	28.4	88	Amphora	11.8	No
CRB06	Purple with a white base	Pale yellow	Upper half	Isosceles	19.8	27	52	Amphora	11	Slight
CRB07	Purple with a white base	White	Upper half	Isosceles	21.4	31	92	Amphora	11.4	No
CRB08	Purple with a white base	Light yellow	Upper half	Isosceles	17.6	28.2	112	Amphora	11.4	Slight
CRB09	Purple with a white base	Pale yellow	Upper half	Isosceles	20.2	29.8	92	Amphora	11.4	No
CRB10	Purple with a white base	Light yellow	Upper half	Isosceles	21.6	31.2	92	Amphora	12	No
CRB11	Purple with a white base	Light yellow	Upper half	Isosceles	18.4	31.8	180	Amphora	11.8	Slight
CRB12	Purple with a white base	Light yellow with a lilac tinge at the apex	Upper half	Isosceles	14.8	27	149	Amphora	12.6	No
CRB13	Purple with a white base	Light yellow	Upper half	Isosceles	17	25.2	67	Amphora	12	Slight
CRB14	Purple with a white base	White to cream	Upper half	Isosceles	18.8	29.4	112	Amphora	12.2	No
CRB15	Purple with a white base	Golden yellow	Upper half	Isosceles	20.4	29	74	Amphora	11	No
CRB16	Yellow	Bright yellow	Lower half (base)	Equilateral	22	47.6	655	Deltoid	9.6	Yes
CRB17	Yellow	Bright yellow	Lower half	Equilateral	16.2	32.4	262	Deltoid	10	Yes
CRB18	Purple with a white base	Light yellow	Upper half	Isosceles	21.4	34.6	174	Amphora	11.6	Slight
CRB19	Yellow	Bright yellow	Lower half	Equilateral	19.2	36.4	296	Deltoid	10.4	Yes
CRB20	Yellow	Bright yellow	Base	Equilateral	20.2	36.8	276	Deltoid	10.2	Yes
CRB21	Purple with a white base	Bright yellow	Upper half	Isosceles	22	33.8	139	Amphora	11.4	Slight
CRB22	Purple with a white base	Light yellow	Upper half	Isosceles	20	32.4	154	Amphora	11	Slight
CRB23	Purple with a white base	Light yellow	Upper half	Isosceles	23.8	37.2	180	Amphora	10.8	No
CRB24	Purple with a white base	White	Upper half	Isosceles	21	35	196	Amphora	12.2	No
CRB25	Purple with magenta base	Pink-purple	Upper half	Isosceles	19	25.6	44	Amphora	12.4	No

The documented distinctive morphological characters that were critically explored in this field study are reported below. Each includes relevant observations or adaptations for optimal assessment and the resulting states for the different taxa. For economizing typing space, *C. edulis* is abbreviated to 'C.ed' and *C. acinaciformis* (senso lato) to 'C.ac'. The character states of the morphologies recorded for each of the 25 populations are reported in Table 2, and a summary of these states for each of the three taxa is summarised in Table 3.

Table 3. Significant morphological characteristics to distinguish *Carpobrotus* spp. (adopted from Campoy *et al.*, 2018) indicating the strength of taxonomic significance (STS: none, low, medium, high) in terms of consistency, distinctness and ease of assessment (* data from one population only - CRB25; measurements produced from 5 readings within the same large population).

Character	*C. acinaciformis* s.s.	*C. acinaciformis* complex	*C. edulis* s.s.	STS and other notes
Widest part of leaf	Upper (distal) third	Upper (distal) third	Lower (proximal) half, usually close to the base	Medium
Cross-section of leaf	Isosceles	Isosceles	Equilateral	High
Colour of leaf	Variable, usually dark	Variable, usually dark	Variable, usually bright green	None
Length of shortest calyx lobes (mm)	(16–)19(–21) *	(14.8–)20.1(–23.4)	(16.2–)20.0(–22.5)	None
Length of longest calyx lobes (mm)	(22–)25.6(–29) *	(25.8–)31.4(–37.2)	(32.4–)40.0(–46.6)	Medium (difficult to assess)
Calyx Lobes Differential Index (CLDI)	44 *	(52–)130(–207)	(262–)412(–655)	High (if a mean is taken)
Flower colour	Purple only	Purple only	Yellow or purple	Low (yellow excludes C.ac s.l.)
Colour of base of petals	Purple, slightly darker	White	Cream to pale yellow	Strong
Colour of filaments	Pink to mauve	White, pale to golden yellow or cream with lilac apex	Cream to yellow	Low
Profile shape of the receptacle	Slightly swollen laterally like the shape of an amphora.	Slightly swollen laterally, like the shape of an amphora.	Tapering linearly towards the pedicel, deltoid shape.	Low (difficult to assess but once understood it is helpful)
Number of locules per ovary	12–14 mean = 12.4	(10–)11–14 mean = 11.6	9–10(–11) mean = 9.9	High (if a mean is taken)
Swollen lips on the adaxial surface at base of leaves	Flat or very slightly raised above surface of leaf, never distinct	Flat or very slightly raised above surface of leaf, never distinct	Distinctly swollen-like lips embracing the stem, at least 1 mm thick, subhyaline.	High
Seed development in young fruit	Well developed	Poor to none	Moderately to well developed	Medium

Shape of leaf

The difference between the two taxa is not always distinct and sometimes not consistent in all leaves. It was found that the difference is most pronounced in mature (but not the oldest) leaves, namely 3 to 4 nodes below the flower bud or the apical leaves. The margins of the leaves of C.ed are straight to very slightly arcuate (Figure 2B), the widest part being close to the base to about the proximal third from the base, while the margins of C.ac are moderately and visibly curved at the distal half and their shape is often referred to as scimitar-shaped (Figure 2A). It is sometimes hard to judge whether the leaf curvature is uniformly straight or scimitar-shaped, so instead, the location of the widest part of the leaf is more reliable and easier to assess. At least five random leaves have to be examined for a sound judgement since C.ac can occasionally have a few leaves that look like those of C.ed.

Colour of leaves

The leaves are described in some outdated literature as green in C.ed and strongly glaucous in C.ac. Field surveys showed very marginal and uninterpretable colour differences between the two species, although they are brighter green in C.ed. Most populations of both species had dark green leaves sometimes tinged in purple at the margin. This character was therefore found unsuitable and with minimal strength to discriminate between the two species.

Cross-section of leaves

This is among the most reliable and essential characters to distinguish the taxa apart. In various taxonomic accounts, it is reported as isosceles in C.ac and equilateral in C. ed, which was consistently confirmed in this study with only rare occasions of leaves of C.ac having a quasi-equilateral cross-section in a few plants. For consistent and standardized assessment, the cross-section should be carried out along the widest part of any leaf of the plant except the youngest ones at the tip of branches. Cross-section of three to five different leaves is suggested for a better evaluation. Unfortunately, this character cannot be assessed on photographed plants.

Difference in the length of the calyx lobes

The calyx lobes are defined to be unequal in C.ed and subequal in C.ac, but when both species were assessed,

their calyx lobes were generally unequal without providing an evident distinctive result (Figure 3 B-C). According to Wisura & Glen (1993) and Campoy *et al.* (2018), the shortest lobes of C.ed are 10–35 mm long and the longest 30–70 (80) mm, whereas in C.ac, they both vary between 10–35mm (≈subequal). Field observations showed a wide overlapping in the respective measurements, which vary according to the age of the flowers because the calyx lobes keep growing during the maturity of the flowers and early fruit formation. Only in one population (CRB25) were the calyx lobes subequal (see discussion), as shown in Figure 3A. However, the difference between the shortest and longest calyx segment was found to be consistently larger in C.ed. The length of the herbaceous part of the calyx lobes (hyaline flap excluded) was used to formulate an index, here referred to as the "Calyx Lobes Difference Index" (CLDI). This provided a quantitative difference following a more standardized method. CLDI is the square of the difference between the longest and shortest calyx lobes and is calculated by measuring the average length of the longest calyx lobe of five flowers (CL) and that of the shorter calyx lobes of the same flowers (CS) and used in the following equation: $CLDI = (\overline{CL} - \overline{CS})^2$. An index below 210 resulted in all C.ac specimens, and one above 260 in C.ed.

Shape and size of receptacle

A 20–40 mm long turbiniform receptacle was reported for C.ed and an oblong or sub-globose one about 12–20 mm long for C.ac (Wisura & Glen, 1993; Campoy *et al.*, 2018). Field studies showed negligible differences to separate both taxa. Besides that, the longitudinal dissection of the flowers is rather difficult, time-consuming and at times a hazardous operation. This character was found to have no significant value.

Shape of hypanthium or fruit

A minor but rather consistent difference was found in the shape of the hypanthium or immature fruit of the two species. The difference might be undetectable to the untrained eye but eventually the shape in C.ac - resembling and here termed as a Phoenician amphora (Figure 3D) - has the sides slightly swollen and arching out, whereas in C.ed the sides are straight and tapering gradually towards the pedicel (Figure 3E).

Colour of petals

Flower colour provides the easiest distinction, where, simply put, C.ed has yellow flowers whereas they are purple in C.ac. Unfortunately, since C.ed have varieties with purple flowers (Preston & Sell, 1988), this character alone has limited use. At least, all yellow-flowered specimens can be ascertained as C.ed, but not the other way round - purple-flowering *Carpobrotus* could be from either of the two species. All purple-flowered populations in this study had a white base, generally observed as a white ring around the receptacle, except for population CRB25 which was dark and forming a magenta ring or halo around the receptacle when seen from above.

Colour of filaments

Literature postulated that C.ac has lilac-pink filaments whereas they are yellow in C.ed (Wisura & Glen, 1993; Campoy *et al.*, 2018). Results in this investigation show that all populations have golden, bright, light, or pale yellow filaments, sometimes white (Figure 2H). Nevertheless, population CRB25 was the only one that which had flowers with perceivable pink to mauve filaments (Figure 2I), while population CRB12 had pale yellow filaments which turn to lilac at the apex. *Prima facie*, this character seems to be unreliable or inconsistent to differentiate the two taxa, but as discussed below, it is a key character for C.ac s. str.

Number of locules per ovary

This character was best assessed on mature flowers or unripe fruit. Mature fruit are often tough to dissect, while ovaries are small in the youngest flowers or buds. There was an obvious and strong trend that C.ed had a smaller number of locules; 9–10(–11) per ovary (Figure 3F), while C.ac had (10–)11–13(–14) locules (Figure 3G). A marginal overlap is exhibited and hence a better assessment of this character was conducted by taking an average value of at least five ovaries. The threshold index of 10.7 was employed to discriminate between the two taxa, where an average count of less than 10.7 is used to confirm C.ed and 10.7 or larger (usually >11) for C.ac. It was noted that the last flowers of a population produced smaller flowers with a lower number of ovaries, hence this character is most reliable during periods of full bloom.

Swollen lips at the base of leaves

In this study, a strong and constant morphological character which distinguishes both species is reported for the first time. The base of most leaves of C.ed is furnished with a subhyaline (wax-white), swollen lip embracing the stem, whereas it is flat or very slightly raised above the adaxial surface of the leaf in C.ac. This character, combined with the cross-section of the leaves, can differentiate between both species in their vegetative state. A few leaves of some populations of C.ac had rudimentary or slightly developed lips, but never reaching the dimensions of C.ed which are found in every single leaf. Therefore, checking five leaves is suggested and the presence of distinct lips in at least four leaves would confirm the identification of C.ed. This character can be observed without dissecting the leaves and readily visible from the images taken at high to medium resolution (e.g. Rignanese, (2005); Portela, (2017); etsy.com, (2019)), portraying its valuable taxonomic importance.

Seed development

When ovaries were dissected to count the locules, important observations were obtained on the fertility and development state of the seeds. Seeds were better developed in C.ed than in C.ac (Figure 2E), except for CRB25, of which the seeds were fully developed and hard in all examined fruit (Figure 2F). Therefore, this character can be regarded to have some taxonomic value, especially to distinguish hybridogenous morphotypes from fertile species.

Determination of the Maltese populations

In the Maltese Islands, two species of Carpobrotus occur and are represented by three taxa: *C. edulis* s.s. (CRB02, CRB16-17, CRB19-20), with yellow flowers fading to a cream colour at the base; *C. acinaciformis* s. str. (CRB25), with entire purple flowers but exhibits a vivid magenta zone or halo at the base of the petals; and an altered form of *C. acinaciformis*, characterized by light purple petals fading abruptly to a white base, forming flowers with a white ring around the receptacle. Most of the discussion below deals with the latter taxon.

Discussion

This investigation conveyed a full picture of the status of *Carpobrotus* in Malta, and most likely it is applicable for other stations in Europe and the Mediterranean region. The full understanding of the morphological characters reported in the reviewed literature to discriminate *Carpobrotus* taxa can now explain the past confusion in the Maltese flora, which is somewhat justified.

The purple-flowering variety of *Carpobrotus edulis* (= var. *rubescens*) has been suggested to represent the purple-flowering *Carpobrotus* in Malta. However, this variety has petals that are purple throughout (Preston & Sell, 1988) and leaves with an equilateral cross-section characteristic for *C. edulis* s.l. (Wisura & Glen, 1993; Campoy *et al.,* 2018), and has not been detected in the studied material. Hence this taxon has to be excluded from the alien flora of Malta until its presence is confirmed.

When applying the key by Preston and Sell (1988), the purple-flowering *Carpobrotus* might refer to *C. glaucescens* (Haw.) Schwantes, a species native to Australia that is characterized by its purple petals with a white base and white to pale yellow filaments. Despite the overall similarity, *C. glaucescens* do not match with the Maltese populations, as *C. glaucescens* has pedicels only up to 1 cm long, smaller flowers up to 45 mm (rarely up to 60 mm) across; longest calyx lobes up to 20 mm long; stamens' filaments mostly white (occasionally pale yellow); and the fruit is well developed and fertile (Preston & Sell, 1988). The Maltese populations, on the other hand, have longer pedicels of 12–25 mm in length; flowers 50–90 mm in diameter; longest calyx lobes around 40 mm long; filaments mostly yellow; and the fruit is sterile and dries out after anthesis.

Based on the sterility of these populations (except for population CRB25), the option of a hybrid between *Carpobrotus acinaciformis* and *C. edulis* was explored, and it seems to be the most plausible explanation. This hybrid has already been reported in the Mediterranean region and it is believed to have be introduced in Europe in its hybrid state as an ornamental, maybe with the first introductions as a garden plant in the late 17th century (Preston & Sell, 1988). This *Carpobrotus* is believed to be a complex hybridogenous taxon with multiple backcrosses with the putative parents and offspring lineages forming a hybrid aggregate (Suehs *et al*., 2004a,b; Ortiz *et al*., 2008; Campoy *et al*., 2018). Upon naturalizing it further hybridized with *C. edulis*, forming more invasive offspring such as reported on the island of Bagaud in France (Suehs *et al*. 2004a,b; Verlaque *et al*., 2011). Interestingly, the hybrid has not been found to have a specific binomial (undescribed?), and in the reviewed literature, it is referred to as "*C.* aff. *acinaciformis*." (Suehs *et al.*, 2004a,b; Sintes *et al.*, 2007; Bartomeus *et al*. 2008; Verlaque *et al*., 2011; Campoy *et al*., 2018) or "*C.* × cf. *acinaciformis*" (Grunsven *et al*., 2009).

The aforementioned accounts, amongst others, mention the presence of hybrid swarms invading the Mediterranean shores, but they do not give a clear morphological picture or distinct features to tell them apart from the putative parents, although they commonly suggest a closer relationship to *C. acinaciformis*. A thorough comparative morphological analysis of the purple-flowering populations in Malta suggests that they are represented by at least two taxa.

First of all, population CRB25 from Kalkara (Smart City) matched perfectly with *Carpobrotus acinaciformis* in its strict sense, with typical purple-pink stamens, subequal calyx lobes (very low CLDI of 44), and isosceles cross-sectional leaves. This population was probably introduced and cultivated with the development of the Smart City business complex in 2007, and was of a different origin from the previously introduced populations in Malta, some of which were reported at the beginning of the 20th century.

The hypothesis that the other purple-flowering populations in Malta (CRB01, CRB03-15, CRB18, CRB21-24) represent the hybrid form (here referred to as *Carpobrotus* aff. *acinaciformis*) is backed up by aspects of its morphology and biology. The most significant evaluation is the variability and intermediate states of some characters, namely the number of locules per ovary, the difference between the shortest and longest calyx lobes, ranging between those of the putative parents. For instance, the 10 to 14 locules per ovary in the hybrid form is relatively variable compared to a more stable count in *C. edulis* (9–10) and *C. acinaciformis* (12–14). Moreover, the CLDI of 52–207 happens to be intermediate between 44 of *C. acinaciformis* and 262–655 in *C. edulis*. The inconsistent shape of the leaves is also an indication of variability between the two parents.

From a biological aspect, the pure species produced hard fertile seeds (Figure 2F) and most of the fruit was well developed and swollen when mature. Conversely, the fruit of the presumed hybrid morphotypes never reached maturity and shrivelled within two weeks after

anthesis. When the ovaries were dissected to count the locules, only a few hardened and viable-looking seeds could be traced in the locules of the presumed hybrid plants (Figure 2E). This compares well with a previous study carried out in the island of Bagaud (southeastern France), where the seed fertility of *Carpobrotus* aff. *acinaciformis* was found to be considerably lower than that of *C. edulis* (Chenot *et al*., 2014)

One of the most important characters to differentiate *Caprobrotus* aff. *acinaciformis* from *C. acinaciformis* s.s. is the colour of the filaments. The latter forms purple-pink filaments (Lanfranco, 1974; Wisura & Glen, 1993; Pignatti, 1982; Preston & Sell, 1988) (Figure 2I), but various tonalities of yellow (including white) in *C.* aff. *acinaciformis* (Figure 2H). In one example (CRB12), the filaments had a faint lilac tinge at the apex of the otherwise yellow filaments, possibly indicating another example of an intermediate state between that of the two parents. Additionally, another important difference which is easier to judge even from photographs is the white colour at the base of the petals in *C.* aff. *acinaciformis* forming a white ring around the receptacle, whereas in *C. acinaciformis,* the petals are completely purple with a somewhat darker or more vivid hue at the base forming an intense magenta halo around the receptacle (see Figure 2G - in this photograph, one of the flowers was removed from its mother plant and placed next to the other for a direct comparison). The diameter of the flowers of the parent seems to be smaller, but no quantitative measurements have been taken in this study. *Caprobrotus edulis* can be distinguished from *C. acinaciformis* s.l. by its yellow flowers; an equilateral cross-section of the leaves (Figure 2B); unequal calyx lobes (Figure 3C); lower number of locules per ovary, usually between 9 and 11 (Figure 2F); and the possession of swollen subhyaline lips at the base of each leaf (Figure 2C). But the enigma of *Carpobrotus* spp. is however not completely solved as this investigation brings forth a new question: the fruit of *C. edulis* (CRB02) also showed some sterile fruit - is this also due to some complex hybridogenous horticulturally-produced plants, or can it be explained by a decreased success of fertilization (e.g. inability of self-pollination)?

Identification keys

Key to determine *Carpobrotus* in the vegetative state (leaves only)

1. Cross-section of all leaves shaped equilateral triangle; base of all leaves possesses swollen subhyaline lips ..... *C. edulis* s.l.
1'. Cross-section of most leaves shaped isosceles triangle; base of most leaves without distinctly swollen lips ..... *C. acinaciformis* s.l.

Key to determine *Carpobrotus* in the reproductive state (flowers and fruit)

1. Flowers yellow; CLDI > 250; mean number of locules per ovary ≤ 10.7..... 2. (*C. edulis* s.l.)
1'. Flowers purple; CLDI < 220; mean number of locules per ovary >10.7..... 3. (*C. acinaciformis* s.l.)
2. Flowers yellow, base of petals concolorous or cream..... *C. edulis* var. *edulis*
2'. Flowers purple, base of petals concolorous..... ..... *C. edulis* var. *rubescens**
2''. Flowers purple, base of petals yellow..... ..... *C. edulis* var. *chrysophthalmus**
3. Stamen filaments pink-purple; CLDI < 50; base of petals purple with a darker magenta hue; seeds and fruit developed ..... *C. acinaciformis* s.str.
3'. Stamen filaments yellow or white; CLDI >50; base of petals white, forming a flower with a white ring around receptacle; seeds undeveloped, most fruit small and shrivelled . *C. acinaciformis* × C. *edulis* complex (*C.* aff. *acinaciformis*)

* Taxa not detected in this study and possibly do not occur in Malta. CLDI is the square of the difference between the average longest and shortest calyx lobes of at least five flowers.

Conclusion

The taxonomic status of *Carpobrotus* in the Maltese Islands is expected to be parallel with the rest of the Mediterranean basin, Portugal, the Azores, the Canary Islands, Macaronesia and Madeira. Taxonomy plays an important role in understanding the specific biology and behaviour of *Carpobrotus* morphotypes and taxa in this region, enabling an effective control and management plan for these invasive plants.

Morphological analyses suggest that morphotypes identified from this investigation on 25 populations from Malta belong to three different taxa: yellow-flowering *Carpobrotus edulis* var. *edulis; C. acinaciformis* s.s. with completely purple flowers, and their sterile hybrid complex referred to in scientific literature as *C.* aff. *acinaciformis* (or *C.* × cf. *acinaciformis*) also with purple flowers but with white bases. While before this study, most, if not all of the naturalized populations of *Carpobrotus* in Malta were referred to as *C. edulis*, it is now clear that there is a strong presence of naturalized *C. acinaciformi*s s.l. (including hybrids). These taxa are also likely the main component of *Carpobrotus* spp. in north Africa and Europe, but other taxa which have been introduced later and not yet established as invasive include *C. glaucescens* introduced from Australia and other varieties of *C. edulis* namely var. *chrysophthalmus* C.D. Preston & P.D. Sell and var. *rubescens* (Preston & Sell, 1988).

This investigation also provided a more comprehensive understanding of the morphological characters, resulting in a new characteristic useful to differentiate between *C. edulis* which have swollen subhyaline lip-like border at the base of leaves, and *C. acinaciformis* s.l., where it is absent or vestigial.

These conclusions are limited to a morphological approach. DNA analysis can be helpful to resolve the

hybridization complex and their parentage. The reduced fertility of at least one population of *Carpobrotus edulis* s.s. also left some doubt about its purity as a species and it may be interesting to investigate further to understand its genotype. On the other hand, the relevance of such resolution may be unnecessary for the scientific community, where priority is given to the management and control of these invasive plants.

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