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

Stephen Mifsud

Received: 25 August 2020 / Accepted: 11 November 2020 / Published online: 8 March 2021

**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from horticultural advances make the determination of *Carpobrotus* even harder, especially with the application of outdated identification keys which do not take hybrids into account.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.

**How to cite:** Mifsud, S. 2021. Morphology of the invasive *Carpobrotus* (Aizoaceae) in Europe: Malta as a case study. Mediterr. Bot. 42, e71195. https://doi.org/10.5209/mbot.71195

**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, 1993),...
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 Hamra in Gozo (MT00000005), 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 acinaciformis) 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; Žahra, 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 represent 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 Gwann' - and was reported as naturalized in a few places already at the beginning of the 20th century (Sommer & 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 Ghajn Mhelhel     | 01-May      | Abandoned cultivation                            |
| CRB11     | Gozo   | Munxar. Xlendi Bay             | 01-May      | Naturalized on coastal rocky ground             |
| CRB12     | Gozo   | Sannat. Mgarr 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  | Mellieha. Aside chapel at l-Ahrax tal-Mellieha | 07-May | Embellishment                                   |
| CRB15     | Malta  | Mellieha. 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. Ghar Lapsi, traffic island near police station | 08-May | Embellishment                                   |
| CRB19     | Malta  | Qrendi. Haġar Qim restaurant  | 08-May      | Cultivation                                     |
| CRB20     | Malta  | Mellieha. Selmun Palace        | 14-May      | Cultivation                                     |
| CRB21     | Gozo   | Sannat. Ta’ Sabbara area       | 21-May      | Naturalized from abandoned cultivation          |
| CRB22     | Gozo   | Għasri. San Gużepp area        | 21-May      | Naturalized from abandoned cultivation          |
| CRB23     | Malta  | Marsascala. Saint Thomas Bay   | 23-May      | Embellishment                                   |
| CRB24     | Malta  | Xghajra. Coastal promenade     | 23-May      | Naturalized on rocky ground from embellishment |
| CRB25     | Malta  | Xghajra. 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 $1 \times 1$ 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. acinaciformis*; 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 $3 \times 3$ 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.
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         | 21.4| 34    | 159   |       | 12.2 | No   |      |
| CRB02 | Yellow                       | Bright yellow              | Base or lower half  | 22.2| 46.6  | 595   | 9.6  | Yes  |      |      |
| CRB03 | Purple with a white base     | White to cream              | Upper half          | 22.4| 36.8  | 207   |       | 11   | No   |      |
| CRB04 | Purple with a white base     | Light yellow               | Upper half          | 20.6| 31.4  | 117   |       | 12.4 | No   |      |
| CRB05 | Purple with a white base     | White                      | Upper half          | 19  | 28.4  | 88    |      | 11.8 | No   |      |
| CRB06 | Purple with a white base     | Pale yellow                | Upper half          | 19.8| 27    | 52    |      | 11   | Slight|      |
| CRB07 | Purple with a white base     | White                      | Upper half          | 21.4| 31    | 92    |      | 11.4 | No   |      |
| CRB08 | Purple with a white base     | Light yellow               | Upper half          | 17.6| 28.2  | 112   |      | 11.4 | Slight|      |
| CRB09 | Purple with a white base     | Pale yellow                | Upper half          | 20.2| 29.8  | 92    |      | 11.4 | No   |      |
| CRB10 | Purple with a white base     | Light yellow               | Upper half          | 21.6| 31.2  | 92    |      | 12   | No   |      |
| CRB11 | Purple with a white base     | Light yellow               | Upper half          | 18.4| 31.8  | 180   |      | 11.8 | Slight|      |
| CRB12 | Purple with a white base     | Light yellow with a lilac tinge at the apex | Upper half | 14.8| 27    | 149   |      | 12.6 | No   |      |
| CRB13 | Purple with a white base     | Light yellow               | Upper half          | 17  | 25.2  | 67    |      | 12   | Slight|      |
| CRB14 | Purple with a white base     | White                      | Upper half          | 18.8| 29.4  | 112   |      | 12.2 | No   |      |
| CRB15 | Purple with a white base     | Golden yellow              | Upper half          | 20.4| 29    | 74    |      | 11   | No   |      |
| CRB16 | Yellow                       | Bright yellow              | Lower half (base)   | 22  | 47.6  | 655   | 9.6  | Yes  |      |      |
| CRB17 | Yellow                       | Bright yellow              | Lower half          | 16.2| 32.4  | 262   |      | 10   | Yes  |      |
| CRB18 | Purple with a white base     | Light yellow               | Upper half          | 21.4| 34.6  | 174   |      | 11.6 | Slight|      |
| CRB19 | Yellow                       | Bright yellow              | Lower half          | 19.2| 36.4  | 296   | 10.4 | Yes  |      |      |
| CRB20 | Yellow                       | Bright yellow              | Base                | 20.2| 36.8  | 276   | 10.2 | Yes  |      |      |
| CRB21 | Purple with a white base     | Bright yellow              | Upper half          | 22  | 33.8  | 139   |      | 11.4 | Slight|      |
| CRB22 | Purple with a white base     | Light yellow               | Upper half          | 20  | 32.4  | 154   |      | 11   | Slight|      |
| CRB23 | Purple with a white base     | Light yellow               | Upper half          | 23.8| 37.2  | 180   |      | 10.8 | No   |      |
| CRB24 | Purple with a white base     | White                      | Upper half          | 21  | 35    | 196   |      | 12.2 | No   |      |
| CRB25 | Purple with magenta base     | Pink-purple                | Upper half          | 19  | 25.6  | 44    |      | 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 = (CL - 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 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. edulis than in C. acinaciformis (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 justifiable.

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 isosceles cross-sectional 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 been 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” (Grunsvsen 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 Carpobrotus 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 2f), but various tonalities of yellow (including white) in C. aff. acinaciformis (Figure 2H). In one example (CRB02), 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. Carpobrotus 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.)

2. 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. acinaciformis 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.

**References**

Bartomeus, I., Bosch, J. & Vilà, M. 2008. High Invasive Pollen Transfer, Yet Low Deposition on Native Stigmas in a Carpobrotus-invaded Community. *Ann. Bot. London* 102: 417–424. doi:10.1093/aob/mcn109

Borg, J. 1927. Descriptive Flora of the Maltese Islands. Government Printing Office, Malta.

Campoy, J.G., Acosta, A.T.R., Affre, L., Barreiro, R., Brundu, G., Buissin, E., Gonzalez, L., Lema, M., Novoa, A., Retuerto, R., Roiola, S.R. & Fagúndez, J. 2018. Monographs of invasive plants in Europe: Carpobrotus. *Bot. Lett.* 165(3–4): 440–475. doi: 10.1080/23818107.2018.1487884

Cash, A. 2017. Flora of the Maltese Islands, 2nd ed. Self published, Malta.

Chenot, J., Affre, L., Passetti, A., & Buissin, E. 2014. Consequences of iceplant (Carpobrotus) invasion on the vegetation and seed bank structure on a Mediterranean island: response elements for their local eradication. *Acta Bot. Gallica* 161(3): 301–308.

Clegghorn, H. 1869. Notes on the Botany and Agriculture of Malta and Sicily. Trans. & Proc. Bot. Soc. Edinburgh 10(1–4): 106–139.

Gonçalves, M.L. 1990. Carpobrotus N. E. Br. In: Castroviejo, S. et al. (Eds.). *Flora Iberica* vol 2. Pp. 82–85. R. Jard. Bot. C.S.I.C., Madrid.

Gulia, G. 1856. Repertorio Botanico Maltese. Malta.

Grunsven, (van) R.H.A., Bos, F., Ripley, B.S., Suhehs, C.M. & Veendaal, V.M. 2009. Release from Soil Pathogens Plays an Important Role in the Success of Invasive Carpobrotus in the Mediterranean. *S. Afr. J. Bot.* 75(1): 172–175. doi:10.1016/j.sajb.2008.09.003

Haslam, S.M., Sell, P.D. & Wolseley, P.A.W. 1977. *A Flora of the Maltese Islands*. Univ. Press, Malta.

Lanfranco, E. 1974. Wild Succulents in Malta. *Kakti u Sukkulenti Oħra 17*: 12–26.

Lanfranco, G. 1969. Field guide of the Wild flowers of Malta. *Kakti u Sukkulenti Oħra* 17: 12–26.

Lanfranco, E & Bonett, G. 2018. Wild flowers of the Maltese Islands. Nature Guide Series (Reprint from first ed. in 2015). BDL Publ., Malta.

Mifsud, S. 2019. New records, taxonomic updates, and new locations for some alien species occurring in the Maltese Islands. XVI OPTIMA Meeting, October 2–5, 2019, Agric. Univ. Athens, Greece.

Ortiz, D.G., Lumbreras, E.L. & Rosselló, J.A. 2008. *Flora alóctona suculenta valenciana: Aizoaceae y Portulacaceae* [Alochtonous succulent Valencian flora: Aizoaceae and Portulacaceae]. Monogr. Boteloa 7: 1–68.

Pignatti, S. 1982. *Flora d’Italia*. Vol. 1. Edagricole, Bologna.

Pignatti, S. 2019. *Flora d’Italia* Vol. 4, second ed. Edagricole, Bologna.

Preston, C.D. & Sell, P.D. 1988. The Aizoaceae Naturalized in the British Isles. *Watsonia* 17(3): 217–245.

Sarmati, S., Conti, L. & Acosta, A.T.R. 2019. *Carpobrotus acinaciformis* vs *Carpobrotus edulis*: Are there any differences in their impact on coastal dune plant biodiversity? *Flora* 257, 151422. doi: 10.1016/j.flora.2019.151422

Schemperi, P.J. & Lanfranco, E. 1996. Introduced species in the Maltese Islands. In: Baldacchino, A.E. & Pizzuto, A. (Eds.) Introduction of alien species of flora and fauna. [Proceedings of a seminar held at Qawra, Malta, 5 March 1996], Floriana, Malta. Pp. 29–54.

Spectives, T., Moragues, E., Traveset, A. & Rita, J. 2007. Clonal growth dynamics of the invasive *Carpobrotus affine* acinaciformis in Mediterranean coastal systems: A non-linear model. *Ecol. Model.* 206(1-2): 110–118.

Somnier, S. & Caruana Gatto, A. 1915. *Flora Melitensis Nova*. Stab. Pellia, Firenze.

Suhehs, C.M., Affre, L. & Médaillé, F. 2004a. Invasion Dynamics of Two Alien Carpobrotus (Aizoaceae) Taxa on a Mediterranean Island: I. Genetic Diversity and Introgression. *Heredity* 92 (1): 31–40. doi: 10.1038/sj.hdy.6800374

Suhehs, C.M., Affre, L. & Médaillé, F. 2004b. Invasion Dynamics of Two Alien Carpobrotus (Aizoaceae) Taxa on a Mediterranean Island: II. Reproductive Strategies. *Heredity* 92 (6): 550–556. doi: 10.1038/sj.hdy.6800454

Verlaque, R., Affre, L., Diadema, K., Suhehs, S.M. & Médaillé, F. 2011. Unexpected Morphological and Karyological Changes in Invasive Carpobrotus (Aizoaceae) in Provence (S-E France) Compared to Native South African Species. *C. R. Biol.* 334(4): 311–319. doi: 10.1016/j.crvi.2011.01.008

Vilà, M. & D’Antonio, C.M. 1998. Fruit choice and seed dispersal of invasive vs. non-invasive *Carpobrotus* (Aizoaceae) in coastal California. *Ecology* 79: 1053–1060.

Weber, H.C. & Kendzior, B. 2006. *Flora of the Maltese Islands*. A field guide. Margraf Publ., Weikersheim.

Wisura, W. & Glen, H.F. 1993. The South African Species of *Carpobrotus Mesembryanthema-Aizoaceae*. Contri. Bolus Herb. 15: 76–107.

Zahra, R. 2012. *Kaktus u Sukkulenti Oħra. Kullana Kulturali No.79*. PIN Publikazzjonijiet Indipendenza, Malta.

**Websites**

Etsy.com 2019. Listing of *Carpobrotus edulis* by Kauia Garden. https://www.etsy.com/sg-en/listing/666109510/carpobrotus-edulis-highway-ice-plant [Accessed on 15-May-2020].
Native Plant Communities in Terrestrial Settings in the Maltese Islands. https://era.org.mt/guidelines-on-managing-native-plant-invaders-and-restoring-native-plant-communities-in-terrestrial-settings-in-the-maltese-islands/
Mifsud, S. 2002–2020. Aizoaceae in Maltawildplants.com. http://www.maltawildplants.com/wildplants_index_pg05.php#AIZO [Accessed 25-Oct-2020].

Portela, R. 2017. Especies invasoras (V): La uña de gato o Carpobrotus: un problema ornamental. https://cienciaybiologia.com/carpobrotus-edulis/ [Accessed on 15-May-2020].
Rignanese, L. 2005. Carpobrotus edulis N.E.Br. http://serneportal.org/portal/taxa/index.php?taxon=carpobrotus%20edulis [Accessed on 15-May-2020].