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***Bolboschoenus* (Cyperaceae) in the Maltese Islands – an enigma of taxonomic interest**

Abstract

Mifsud, S.: *Bolboschoenus* (Cyperaceae) in the Maltese Islands – an enigma of taxonomic interest. — Fl. Medit. 32: 53-71. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

This work aimed for a taxonomic investigation conducted on populations of *Bolboschoenus* (Cyperaceae) growing in wetlands throughout the Maltese Islands. A detailed account of the ecology and morphometric analysis of eleven flower-bearing populations is provided and supplemented by images. Emphasis was made on the inflorescence and the achenes, whereas palaeobotanical observations were also included. No significant taxonomic difference has been found between ray-bearing and rayless plants and all examined material did not correspond to *B. maritimus*, the only species reported in previous floristic literature. Findings instead showed two distinct morphotypes that are undocumented. The one prevailing in mainland Malta was completely sterile (a unique finding for the genus) except for one small population, which harboured fragment pockets of fertile plants corresponding best to *B. glaucus* s. l. The other morphotype occurred only on the Island of Gozo and consisted of achene-producing plants that did not match any of the described *Bolboschoenus* species. Morphometrics indicated an intermediate position between *B. glaucus* and *B. maritimus*. Further investigations that are more diagnostic from morphometrics are advocated for reliable taxonomic conclusions of both populations, possibly leading to the first records of the hybrid *B. glaucus* × *maritimus*, as indicated in this morphological study.

Key words: *Bolboschoenus maritimus*, *Bolboschoenus glaucus*, wetland species, halophytes, glycophytes, Malta, Gozo.

Introduction

The genus *Bolboschoenus* (Asch.) Palla was erected after being segregated from its sectional ranking of *Scirpus* L. and accommodated the single species *B. maritimus* (L.) Palla (Palla 1905). In mainland Europe, the genus only gained a widespread taxonomic recognition by the late 20th century after the pioneer works of Van der Veken (1965); Oteng-Yeboah (1974); Browning & al. (1993, 1996, 1997b); Hroudová & al. (1998b, 1999a, 1999b) and Browning & Gordon-Gray (2000). Disagreement on this segregation initially persisted for a while, until phylogenetic sequencing provided better support for the delimitation of *Bolboschoenus* and other genera previously nested in *Scirpus* s. l. (Muasya & al.

2009; Jung & Choi 2011). A distinct morphological differentiation was already well documented for these segregated genera, where *Bolboschoenus* was characterised with spikelets longer than 1 cm, noded culms and long leaf-like bracts (Browning & al. 1988; Pignotti 2003). *Bolboschoenus* has a worldwide distribution with three to five species representing each continent, where the Southeast Asian, Amazonian and Russian regions are the least studied (Browning & Gordon-Gray 2000).

B. maritimus represented the only species in Europe (e.g. Pereira Coutinho 1976; DeFilipps 1980; Pignatti 1982; Rothmaler 1982; Jeanmonod & Gamisans 2009; etc.) and so is the case in the Maltese Islands (Haslam & al. 1979; Lanfranco 1989; Weber & Kendzior 2006, Casha 2017, Brullo & al. 2020). Inevitably, the genus has been later questioned and investigated by more dedicated *Cyperaceae* authorities in central Europe. Several infraspecific rankings have been used in literature and attributed for some well-defined morphotypes of *B. maritimus* (De Filipps 1980; Hroudová & al. 1998a; Pignotti 2003; Marhold & al. 2004), initially based on the inflorescence's architecture such as the length and number of ray-borne spikelets (Casper & Krausch 1978; Defillips 1980;) and then on the morphology and anatomy of the achenes (Browning & al. 1993, 1995, 1997a, 1997b; Hroudová & al. 1998a, 1998b; Browning & Gordon-Gray 2000).

The diversification of the genus shaped up when new characters were being considered, the most important being the morphology of the achenes (Browning & Gordon-Gray 1993; Hroudová & al. 1998b), and within a few years, five taxa were reported from Europe: *B. maritimus*, *B. glaucus* (Lam.) S.G.Sm., *B. laticarpus* Marhold & al., *B. planiculmis* T.V. Egorova and *B. yagara* (Ohwi) Y.C.Yang & M.Zhan (e.g. Hroudová & al. 1999b; Hroudová & al. 2001; Marhold & al. 2004). The morphology, distribution and ecology of these five European taxa were documented in great detail in a monograph by Hroudová & al. (2007b), which led to further taxonomic revisions of *Bolboschoenus* in some European countries, the latest being in the Netherlands (Simons & al. 2016), Serbia (Nikolić & al. 2019) and Italy (Di Natale & al. 2020). Most of these works corrected numerous herbarium accessions labelled as the “*B. maritimus*” to any of the five species mentioned above.

Bolboschoenus has never been investigated in the Maltese Islands, and the aim of this study was primarily to investigate species diversity occurring in Malta, based on morphological analysis. The study commenced in early January 2021 on all the populations known by the author, using the available literature for character selection and final determination.

Records in Malta

A literature review on the Maltese vegetation spanned on the last 150 years conveyed that only *Bolboschoenus maritimus* (= *Scirpus maritimus*) was recorded in the Maltese Islands. Only in a very recent work by Brullo & al. (2020) was the species assigned to *B. maritimus* subsp. *compactus* (Hoffm.) Hejný.

The first record from the Maltese Islands dates back to Grech Delicata (1853) from Marsa and (Duthie 1874) from Wied tal-Gnejna. New records appeared shortly after, namely from Wied Bingemma-San Blas valley system in Gozo (Sommier & Caruana Gatto 1915) and from the Imsellit-Għajn Mula-Rihana valley system (Borg 1927). During this time, the population in Marsa was already considered locally extinct (Sommier & Caruana Gatto 1915). Marsa used to

be a large marshland wetland area featuring brackish vegetation during Delicata's period but it was drained and dried completely due to public health issues by 1869, and gradually the area became developed into shipbuilding and industrial estates (Haslam & Borg 1998).

More than half a century later, the same records were mentioned in Maltese literature (Lanfranco 1969; Haslam & al. 1977; Haslam & Borg 1998) and in the Red Data Book (Lanfranco 1989). However, in the early eighties, Michael Briffa (pers. comm., Apr-2016) has already found some new populations at Wied il-Fiddien, Wied tas-Salini and Wied Gerżuma in mainland Malta and at Wied Sara and Wied Marsalforn in Gozo, and recently reconfirmed by Tabone (2007, 2008), Casha (2017) and the present author (e.g. Mifsud 2011). The populations from Marsa, Wied Bingemma, Wied Gerżuma, and Salini, were so far not substantiated, including surveys conducted in this study. However, new records have been reported during the last 20 years and includes Wied il-Bahrija and Ramla valley (Tabone 2007), whereas Mistra valley (Wied tal-Kalkara) and Ta' Cianti valley are first published in this study. Overall, the species is locally frequent in some valleys, but these wetland habitats are continuously declining through water extraction for agriculture, climate change, and pollution.

Methodology

The fourteen examined *Bolboschoenus* populations known by the author from the Maltese Islands are given in Table 1 and displayed in map Fig. 1. For each population, the pH, conductivity (μ S) and total dissolved solids (ppm) level of the water the plants were found in was recorded during January 2021 using a portable water tester Hanna HI98130. During visits, the population size was estimated, photographs of plants and inflorescences were taken, and the ecological and environmental data were recorded. Seven representative plants were sampled and studied ex-situ. In four populations, plants with both rayless and ray-bearing spikelets were treated as different samples (as indicated in Table 1) in case each form corresponds to a different species. Samples of inflorescences were collected and examined during March and April, whereas the achenes were harvested at the end of May and June. This study also incorporated a trial study on the pollen to increase the knowledge of the genus since palynology in *Cyperaceae* is lacking (pers. comm. Jane Browning, Dec. 2021) and maybe useful to indicate sterility. The pollen of rayless and ray-bearing samples was also compared in three populations.

Most of the morphological characteristics taken into account are given in Table 2, which explains how the measurements or characters were assessed. Most of these characters are considered as diagnostic in recent taxonomic investigations (e.g. Hroudová & al. 2007a, 2007b; Simons & al. 2016; Pisová & al. 2017; Nikolić & al. 2019; Di Natale & al. 2020). Styles of old spikelets were rehydrated in 3% KOH, which provided an easier assessment. Information from the works of Hroudová & al. (1999a, 2007b) and Marhold & al. (2004) was used for general morphology, ecology and distribution; Nikolić & al. (2019) for statistical measurements of the inflorescences and achenes; and Browning & al. (1995), Hroudová & al. (2007b) and Nikolić & al. (2019) for detailed data on the achenes of *B. glaucus*.



Fig. 1. Map of locations of the examined populations of *Bolboschoenus* in the Maltese Islands (BLB01-14) some of which are found along common valley systems, namely, Wied Imsellit-Mula-Rihana in mainland Malta and Wied Sara-Cianti-Marsalforn in Gozo. (map provided online by Worldometer.info, 01-May-2021).

Table 1. Locations of examined populations, including population size and date of sample collections and water analyses. Populations marked by * denote that rayless plants were present and were examined and compared separately with ray-bearing plants (in case they represented a different taxon).

Sample code	Island	Locality	Toponym (valley name)	Valley system	Population size in terms of length along the valley (metres) [f = fragmented]	Sample collection date	Date of water analyses
BLB01*	MALTA	Mellieħa	Wied tal-Mistra	A	50–100 (f)	26/04/2021	25/01/2021
BLB02*	MALTA	San Pawl il-Baħar	Wied tal-Hżejjen	B	50–100	05/05/2021	25/01/2021
BLB03	MALTA	San Pawl il-Baħar	Wied tal-Arkata	B	300–400	01/04/2021	25/01/2021
BLB04	MALTA	San Pawl il-Baħar	Wied Riħana	B	80–100	01/04/2021	25/01/2021
BLB05	MALTA	Mġarr	Wied tal-Ġnejna	C	400 – 500 (f)	01/04/2021	07/02/2021
BLB06	GOZO	Għasri	Wied Sara	D	> 500	29/03/2021	01/02/2021
BLB07	GOZO	Victoria	Wied tal-Kapuċċini	D	20–30	10/04/2021	01/02/2021
BLB08*	GOZO	Victoria	Wied ta' Ċianti	D	300–400	29/03/2021	01/02/2021
BLB09*	GOZO	Żebbuġ	Wied ta' Marsalforn	D	20–30	10/04/2021	01/02/2021
BLB10	GOZO	Żebbuġ	Marsalforn (shore)	D	50–100	10/04/2021	01/02/2021
BLB11	GOZO	Nadur	Wied tar-Ramla	E	50–100	26/04/2021	09/02/2021
BLB12	MALTA	Rabat	Wied tal-Baħrija	F	20–30	No flowering	07/02/2021
BLB13	MALTA	Rabat	Wied tal-Qlejgha	G	10–20	No flowering	25/01/2021
BLB14	GOZO	Xagħra	Wied Ta' San Blas	H	5–10	No flowering	09/02/2021

Table 2. List of characters examined for the taxonomic assessment of *Bolboschoenus* occurring in the Maltese Islands, including brief explanation of how they are evaluated or measured during inflorescence.

Character	Remarks
Number of sessile spikelets	Sessile spikelets are defined as those borne on rays (=peduncles) that are shorter than their own length. A ray is hence defined as those being longer from the longest subtending spikelet in a given inflorescence.
Number of ray-borne spikelets	Total sum of spikelets born on rays.
Length of sessile and ray-borne spikelets	Measurement in mm from the insertion of the peduncle at the base of the spikelet to the summit, excluding bristles and anthers.
Width of sessile and rayed spikelets	Measurement in mm of the widest part of the spikelet, excluding bristles and anthers.
No. of rays	Total number of rays in an inflorescence.
Length of rays	Average length of rays in mm (the total length of all rays divided by the number of rays).
No. of spikelets per ray	The total number of all ray-borne spikelets divided by the number of rays.
Length of achene	Measurement in mm from the base to the tip of the achene, including the beak.
Width of achene	Measurement in mm along the widest part of the achene.
Length of beak	Measurement in mm from the curvature of achene's summit to the tip of beak. Under the stereomicroscope, it has a slightly different colour tone from the achene.
Mesocarp/exocarp ratio	Ratio of the thickness of the exocarp and the underlying mesocarp.
Cross section shape	Cross-sectional shape is assessed along the widest part of the achene.

Pollen examination

Pollen from three plant samples from each population were studied. Fresh inflorescences (or up to 48 hours after collection) were shaken or tapped over a 10 × 10 cm clean aluminium foil on which pollen was released and deposited over. A small amount of pollen was transferred on a drop of water on a microscope slide and examined with the × 40 and × 100 magnification of a Carl-Zeiss AxioLab RE compound microscope. Microphotographs of the pollen using an eyepiece reticule as a scale bar were taken at × 100 magnification. Using the image software Piximètre V5.10 by A. Henriot and J.-L. Cheype, the length and width of at least 31 pollen grains per sample were measured. Statistical data, including a X-Y scatter plot, were used to compare and detect any variation between presumed different taxa, such as the ray-bearing and rayless specimens.

Achenes examination

Achenes from various spikelets of a single sampling plant were examined when fully ripe and hence when they have hardened and darkened to a brown colour. Five sample plants per population were examined, and results were averaged down. When a population comprised of rayless and ray-bearing plants, five samples from both of each morphotype were studied. The morphology and sizes of eleven achenes per sample were examined and photographed with a binocular microscope, using a calibrated eyepiece reticule as a scale

at the $\times 6$ magnification. The length and width of the achene and the length of the beak was carried out on the microphotographs using the software Piximètre V5.10 (*ibid.*). The thickness of the exocarp and mesocarp is highly diagnostic and was measured using agar to hold the transversally-sectioned achenes in place while being examined and photographed under the microscope. The thickness of the exocarp:mesocarp layers and the anatomy of the cells of the exocarp of five achenes per sample were examined under a Carl-Zeiss AxioLab RE light microscope under the $\times 40$ and $\times 100$ magnification, with auxiliary top-light illumination. The imaging software Piximètre (*ibid.*) was again employed to measure the mesocarp and exocarp thickness and calculate their ratio. Usually, this was carried out from three places along the pericarp wall and averaged out.

Results

Ecology and other observations on the examined populations

The field surveys allowed the collection of inflorescences and pollen from 11 out of the 14 populations that were surveyed. The small populations of BLB12 (Wied tal-Qlejgha), BLB13 (Wied tal-Bahrija) and BLB14 (Wied San Blas) did not produce inflorescences despite several staggered visits and hence were not considered in the results. The ecology and vegetative parts were found to be similar to the other populations except that they were heavily shaded by strands of *Arundo donax* L. and which may inhibit flowering as it had already been previously observed (Hroudová & al. 1997b, 2007b).

The populations in the Maltese Islands seemed to share the same habitat of fluvatile or/and stagnant parts of valleys, preferring exposed areas to sunlight. The conductivity and total dissolved solids (see Table 3) were more or less in the same range between 1800 to 5900 μS , with the majority having values of approximately 2500 μS . Slightly polluted waters explain this elevated value, generally originating from leaching of artificial fertiliser seeping from fields lining the valley sides were examined material resided. Population BLB01, had a relatively low conductivity value of 410 μS since it was situated where the valley was not lined by cultivated fields. Moreover, the valley was rather dry, and the small population was in an isolated water pond formed from surrounding, uncontaminated, run-off water. Only the shore-dwelling population at Marsalforn (BLB10) had an elevated conductivity value of 7180 μS , but however, the population did not progress further towards the sea, where water ponds had values of 13,000 μS and above. Hence, all *Bolboschoenus* populations in the Maltese Islands occurred in freshwater wetlands.

Inflorescence and spikelets

Populations were predominantly composed of specimens with three to five distinctly long rays that were on average, between 25 and 52 mm long with 5 or more spikelets each. However, there were smaller individuals within most populations without or at most three short rays (< 18 mm long) with only 1–3 spikelets each. In addition, several populations included intermediate forms, exhibiting a gradual variation from the dominant umbellate inflorescences to the rayless and compact morphotype, most remarkable in BLB01. The morphometric results of each flowering population are given in the upper half of Table S1 in the Electronic Supplementari File 1 (ESF1) and summarised in Table 4.

Table 3. Conductivity (μS), pH and TDS (ppm) readings of water sampled from waterbodies of each examined *Bolboschoenus* population.

Sample code	Locality	Toponym	Conductivity (μS)	pH	TDS (ppm)
BLB01	Mellieħa	Wied tal-Mistra	410	8.01	0.25
BLB02	San Pawl il-Baħar	Wied tal-Hżejjen	4300	7.88	2.16
BLB03	San Pawl il-Baħar	Wied tal-Arkata	3570	8.02	1.78
BLB04	San Pawl il-Baħar	Wied Rihana	5900	7.72	3.01
BLB05	Mġarr	Wied tal-Ġnejna	3320	7.33	1.66
BLB06	Għasri	Wied Sara	2420	7.83	1.21
BLB07	Victoria	Wied tal-Kapuċċini	2450	7.94	1.23
BLB08	Victoria	Wied ta' Ċianti	1780	8.09	0.89
BLB09	Żebbuġ	Wied ta' Marsalforn	3500	7.05	1.72
BLB10	Żebbuġ	Marsalforn (shore)	7180	7.81	6.26
BLB11	Nadur	Wied tar-Ramla	2970	7.51	1.49
BLB12	Rabat	Wied tal-Bahrija	2830	8.1	1.41
BLB13	Rabat	Wied tal-Qlejgħa	1840	8.22	0.92
BLB14	Xagħra	Wied Ta' San Blas	3590	7.73	1.79

Most of the plants exhibited an umbellate inflorescence, consisting of 6–13 sessile spikelets overtopped with rays bearing about 3–6(–8) spikelets each, these slightly shorter than the sessile spikelets (Fig. 2a). However, the length differs upon the maturity of the spikelets, where spikelets are always longer during the infructescence. The variation of spikelet length has also been documented (Browning & al. 1998; Hroudová & al. 2005; Nikolić & al. 2019), where some examples with long catkin-like spikelets have been described under the epithet *macrostachys*. When some spikelets were measured during the fruiting period, they were about 40% longer and up to 35 mm long, but maintained more or less the same width (Fig. 2b). The length of the rays varied from about 10 – 50 mm and on average, they were 25 mm long. Secondary branching has been observed in only one individual at Ta' Ċianti valley (Fig. 2d). The total number of spikelets borne on peduncles was about 2 to 3 times as much as the number of sessile spikelets. However, other plants that were smaller in size and formed compact, capitate, rayless inflorescences or with only a few short rays were consistently encountered within most populations (Fig. 2e). Since these inflorescence characters were considered diagnostic (Hroudová & al. 2007b; Pisová & al. 2017; Nikolić & al. 2019), this 'rayless' morphotype has been preliminary investigated. Intermediates between the two forms have also been observed (Figs. 2c, 2g).



Fig. 2. Variation in the inflorescences from different populations of *Bolboschoenus* in the Maltese Islands (year 2021): [a] typical form with 4–5 long rays each bearing 3–7 spikelets (BLB05, Wied tal-Ġnejna, 28-Mar); [b] robust form with large and multi-clustered spikelets (BLB08, Wied ta' Cianti, 1-Jun); [c] long-rayed (left), compact and rayless (right) and intermediate forms with short rays (middle) (BLB01, Wied tal-Mistra, 2-Apr) ; [d] specimen with secondary branches (BLB08, Wied ta' Cianti, 1-Jun); [e] semicompact form with short rays and many small dark spikelets (BLB04, Wied Rihana, 1-Apr) [f] head-like inflorescence without rays similar to *B. maritimus* (BLB09, Wied ta' Marsalforn, 15-Apr); [g] lax inflorescences with long rays (left) and compact form with short rays (BLB02, Wied tal-Ħżejjen, 4-May)

Pollen morphology

The pollen mass in fresh flowers of all populations was found to be fragrant - described best as a farinose and rose-flower scent. In addition, when examining population BLB11 (30-May-2021), several bees, namely, *Apis mellifera* s. l. and bee species from the Halictidae family (pers. comm. Thomas Cassar, June 2021) were attracted to the spikelets and harvested pollen from them (see ESF1, Fig. S1). The fragrance and insect-relationship were unexpected from a wind-pollinated genus (De Fillips 1980) which seems that it evolved to gain an extra advantage in territories where insect pollination is predominant.

The pollen shape is generally conical-ovate, with one pole rounded to subtruncate and wide, tapering gently to the other pole, which is obtuse to subacute, as shown in Fig. 4. The surface seems to be unevenly verrucose, and sometimes, there is a shallow, plug-shaped, protrusion at the wide pole (Fig. 4). The germinative cell is large and granular, either central and free in many of the pollen examined or less frequently as a stout and T-shaped body, where its central limb adheres at the wide pole, and the other two perpendicular limbs are in contact with the lateral sides just above the other pole. As a result, three empty vacuoles, two laterally at the wide pole and a larger one below the germinative cell are formed (Fig. 4). This pattern was found homogenously within all examined samples.

The average size was found to vary between $51.8 \times 30.4 \mu\text{m}$ (BLB05) to $62.4 \times 32.9 \mu\text{m}$ (BLB09), with further statistical details given in ESF1, Table S1 and illustrated in ESF1, Fig. S2. The pollen sizes between the compact/rayless forms and the ray-bearing morphotypes were compared in three populations. The results, displayed as scatter-plot diagrams and quartile statistical data (see ESF1, Fig. S3), show a major overlap between the two types. Consequently, no diagnostic differentiation could be interpreted from a palynological point of view.

Since very limited literature dealing specifically with the palynology of *Bolboschoenus* was found, interpretations on these ‘vacuoles’ cannot be made. For example, they were not shown or discussed in pollen studies of *B. maritimus* by Sosam & Al-mayyahi (2018). The resulting measurements cannot be critically compared and discussed at present, but this data can be used for future comparisons and supply data in what seems to be a knowledge gap on the palynology not only in *Bolboschoenus*, but within many genera of the *Cyperaceae* (pers. comm., Jane Browning, Dec-2021).

Achene morphology

The size and morphology of the achenes were reported in the lower half of ESF1, Table S1, based on a sample of 55 achenes per population (eleven achenes/five specimens). There was a remarkable difference in fertility between the populations in Gozo and mainland Malta. Most plants within the Gozitan populations (BLB6–BLB11) were fertile, with a few plants (mostly the rayless forms) having poor or no production of achenes. Regarding the Maltese populations, BLB01 was slightly fertile, where a small pocket of plants had a few achenes at the base of some spikelets, no achenes could be found when examining some 50 to 100 plants within populations BLB02–BLB05.

Upon studying the morphology of the achenes, two defined morphotypes are distinguished based on the features described below:

Achenes of the populations in Gozo (Fig. 3a) were distinct for their larger size and thicker exocarp layer. Achenes measured $2.74 - 3.80 \times 1.66 - 2.21$ (mean 3.14×1.94) in size with a distinct nipple to mucro shaped beak about 0.3 mm long (Fig. 3a.1). The meso-

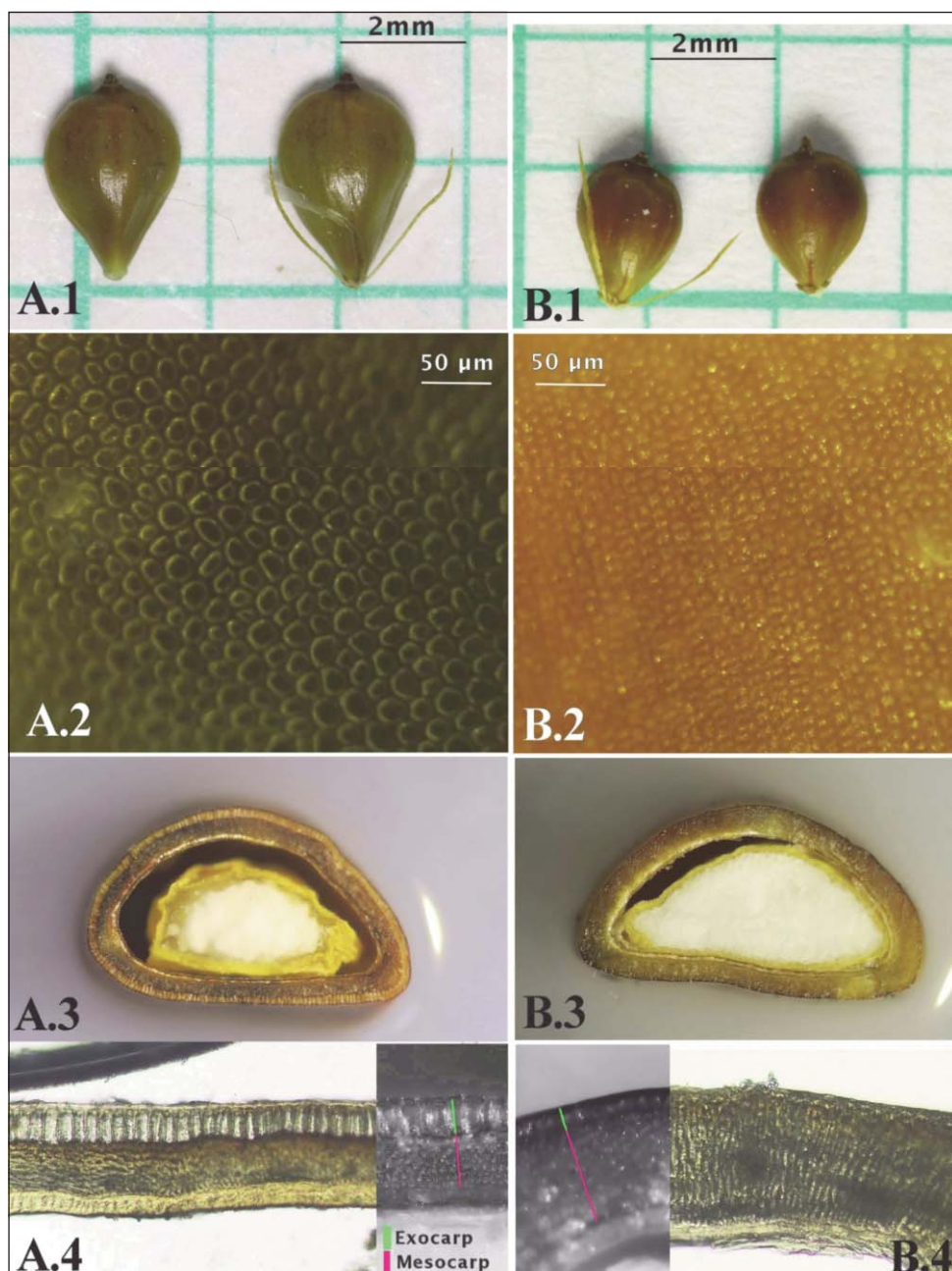


Fig. 3. Comparison of achenes from population BLB01 (Wied tal-Mistra, mainland Malta) [A1—4] and population BLB08 (Wied ta' Cianti, Gozo) [B1—4]: A,B.1: Shape and size of achene; A, B.2: pattern on the coat of the achene; A,B.3: cross-sectional shape and layers of the pericarp; A,B.4: Section of pericarp coat at $\times 100$ magnification showing the three layers of the exocarp (green), mesocarp (purple) and thin layer of endocarp (unmarked).

carp was 1.5 to 2.1 times the thickness of the exocarp layer (Fig. 3a.4), the latter composed of rectangular cells, two to three times longer as broad (Fig. 3a.3). The arrangement of the exocarp cells formed a circular to elliptic pattern measuring 20–30 μm across and grouped in a regular grape-like pattern (Fig. 3a.2). Small air bubbles were formed in the exocarp cells shortly after being immersed in water – a character given importance by Browning & al. (1998) and Browning & Gray (2000).

Population BLB01 in Mistra (Fig. 3b) had smaller achenes, measuring $2.12 - 2.58 \times 1.66 - 2.21$ mm (mean 2.34×1.68 mm), including a beak of about 0.24 mm long (Fig. 3b.1). The exocarp was very thin and hardly visible, approximately one-fifth of the thickness of the mesocarp layer (Fig. 3b.4). The exocarp cells were squarish, with the breadth equal to their length (Fig. 3b.3). The surface of the exocarp consisted of rectangular to irregular polygon-shaped cells, 10–12 μm long, and arranged in short rows resembling ladders (Fig. 3b.2). No air bubbles were seen in the cells when immersed in water.

The outline shape of the nut was similar in both morphotypes, obovate with a gently curved constriction at the lower third and attenuating and tapering to an acute base. The apex is broadly rounded to subtruncate and ends with a distinct, dark-coloured beak at the summit. The cross-section is generally lenticular to plano-convex, sometimes compressed sub-trigonal, usually with the abaxial side flat or gently convex and the adaxial side broadly convex but never distinctly trigonal or triangulate. The perigon bristles were equal in length in both morphotypes (approx. 2.0–3.0 mm). There are 4–6 perigon bristles in both morphotypes, and at least three are persistent when the achene falls out. All specimens examined had a trifid style.

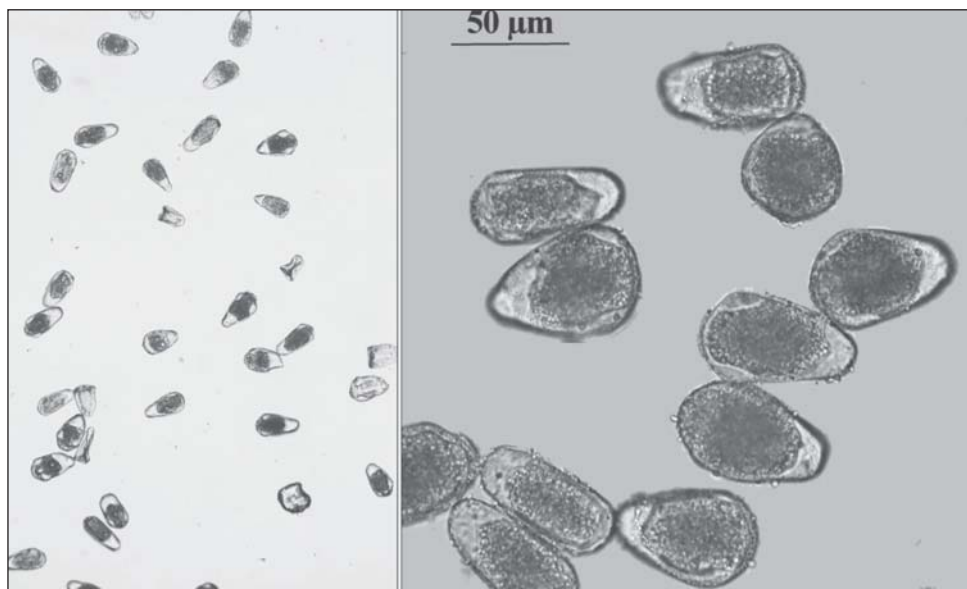


Fig. 4. Pollen grains at $\times 40$ (left) and $\times 100$ magnifications (right) from population BLB09 (Wied ta' Marsalforn). All populations had a similar pollen morphology regarding to the outline shape and size.

Discussion

The morphological analysis of the Maltese populations identified two distinct morphological differences: one in the architecture of the inflorescences and a more important ones in the achenes.

Most plants had inflorescences with long rays in all populations, but a small number of plants had compact, head-like inflorescences, hence either without rays or a few short ones. As repeatedly advocated by recent taxonomical studies, achene morphology is much more diagnostic and reliable for *Bolboschoenus*; primarily postulated by Browning & Gordon-Grey (1993; 2000) and Hroudová & al. (1998b, 1999b), reconfirmed in subsequent works (e.g. Hroudová & al. 2007b) and later supported by genetic (Pisová & al. 2017) and statistical analysis (Pisová & al. 2017; Nikolić & al. 2019). Indeed, when the achene of the rayless plants was examined, as discussed below, these forms did not correspond with *B. maritimus*, which have a different achene morphology (refer to Table 4).

The pooled morphometric data gained from the Maltese material (two morphotypes) was compared with *B. maritimus*, *B. laticarpus* and *B. glaucus* (Fig 4.) in which morphological data was retrieved from Marhold & al. 2004; Hroudová & al. 2007b and Nikolić & al. 2019). The morphotype ‘astericum’ of *B. glaucus* has been selected from the four presented by Nikolić & al. (2019) as it had the best overall resemblance with the Maltese material. The species *B. yagara* and *B. planiculmis* have been excluded from a critical comparison. Firstly, Malta is far away from the distributional range of these two species, with the nearest records reported from northern regions of Italy bordering Austria (Di Natale & al. 2020). Secondly, they are morphologically the most different amongst the *Bolboschoenus* taxa recorded in Europe – *B. planiculmis* achenes have the lateral sides distinctly concave and possess a very thick exocarp layer, whereas *B. yagara* has narrow achenes that are strongly trigonous (Browning & Gordon-Gray 2000; Hroudová & al. 2007b; Wollstonecroft & al. 2011; Pisová & al. 2017).

The morpho-anatomical characteristics of achenes of the rayless forms were identical to those of the umbellate forms within each population. In fact, all examined material (including the rayless forms) the exocarp layer of the achenes was consistently narrower than the mesocarp, and hence, none of the populations (including the rayless forms) corresponds to *B. maritimus*, which instead has a characteristic thicker exocarp. Likewise, pollen morphology showed no differentiation between rayless and pedunculate plants within three tested populations (ESF1 Figs. S2 and S3). Therefore, it can be concluded that *B. maritimus* does not presently occur on the Maltese Islands.

These rayless individuals likely correspond to ecotypes, either formed by younger, first-flowering plants, or plants growing in drier parts or deprived ground where they do not reach the full vigour of the adult plants, a condition already observed by Browning & al. (1998) in some *Bolboschoenus* species in Africa.

Once concluded that the rayless individuals are not *B. maritimus*, the *Bolboschoenus* populations in the Maltese Islands are considered to be one wide-sense taxon split in two distinct morphotypes based on the different characters of the achenes. However, a second and more puzzling question had emerged from the present investigation - why is there a high degree of female-sterility (plants failing to produce achenes), particularly within the populations of mainland Malta? Since *Bolboschoenus*

Table 4. Comparison of the two morphotypes, one in Malta, and the other in Gozo, with the respective characters of the inflorescences and achenes of *Bolboschoenus glaucus*, *B. laticarpus* and *B. maritimus*.

Plant material or taxon	Maltese population (excl. rayless ecotypes) Morphotype 1: mainland Malta	Maltese population (excl. rayless ecotypes) Morphotype 2: Island of Gozo	<i>B. glaucus</i>	<i>B. laticarpus</i>	<i>B. maritimus</i>
Ecological preference	Glycophyte		Glycophyte	Glycophyte	Halophyte
Habitat	Valley beds with freshwater streams that dry completely in summer. Not present in shores with high salinity.		Freshwater wetlands that dry completely in summer, such as river banks, small streams and flood plains.	Wide range of wetland habitats preferring river banks, floodplains and temporarily flooded land	Seashore and marshland areas with high salinity, much less frequently in freshwater wetlands
Distribution	Malta (Central Med. Region)		South Europe and the Med. Region	Central Europe	Throughout Europe
INFLORESCENCES					
$\bar{x}$ =mean; r=range					
No. of sessile spikelets	6–12(–13)	5–13(–16)	(5)–6–10(–15)	(4)–5–8(–11)	(1)–3–7(–10)
Length of sessile spikelets (range, mm)	7–18(–20.5)	9–22(–26)	(7)–9–13(–17)	(9)–11–16(–23)	(8.5)–11–25(–37.5)
Length of sessile spikelets (average, mm)	14.2	15.3	11.74	13.7	18.4
No. of rays	(3)–4–6(–7)	(3)–4–7(–8)	(3)–6–8(–11)	(1)–3–5(–6)	0–2(–5)
No. of rays ($\bar{x}$)	5.3	4.8	7	3	1.32
Length of rays (r, mm)	(10)–18–36(–50)	(9)–17–39(–58)	(12)–16–24(–32)	(10)–17–34(–41)	0–23(–40)
Length of rays ($\bar{x}$, mm)	27.5	28.7	20.18	25.6	12.3
Total no. of ray spikelets	(8)–11–42	(9)–12–45	(16)–23–48(–77)	(3)–6–18(–32)	0–7(–18)
Total no. of ray spikelets ($\bar{x}$)	23.2	23.0	36	12.6	3.4
Number of spikelets per ray	4.4 (2–8)	4.9 (3–9)	(2)–3–7(–10)	(1)–2–4(–6)	0–3(4)
Ray spikelet length (r, mm)	8.7–15.8(–18.4)	10.3–18.8(–20.3)	(7)–9–13(–17)	(8)–10–14(–19)	11–21(–34)
Ray spikelet length ($\bar{x}$, mm)	10.7	12.3	11.2	12.35	11.1
ACHENES					
$\bar{x}$ =mean; r=range					
Achene production	Mostly sterile	Mostly fertile	Fertile	Fertile	Fertile
Achene length ($\bar{x}$, mm)	2.34	3.10	2.59	2.81	2.91
Achene width ($\bar{x}$, mm)	1.68	1.97	1.81	1.91	2.01
Achene length (r, mm)	2.12–2.58	2.84–3.37	2.12–3.05	2.55–3.17	2.22–3.83
Achene width (r, mm)	1.52–1.84	1.74–2.11	1.53–2.24	1.67–2.22	1.41–2.5
Beak length ($\bar{x}$, mm)	0.25	0.28	0.18	0.31	0.33
Mesocarp/Exocarp ratio	× 4.5–6.0	× 1.7–2.4	× (2)–4–5 (–10)	× 3.0	× 0.5
Bristles	4–5 persistent bristles	3–4 persistent bristles	4–6 persistent	2–3 persistent	Caducous, 0 (–1)
Length of bristles	Reaching the top of the achene (1.8–2.9 mm long)	Reaching between half and almost the top of the achene (1.7–3.0 mm long)	Half to two-thirds of achene length	half to two-thirds of achene length	Half of the achene length
Outline shape	Obovate with a broadly rounded to sub-truncate summit and a slight constriction at the basal third.	Obovate with a rounded to a broadly rounded summit and a slight constriction at the basal third.	Elliptic-obovate with rounded and almost truncate summit	Broadly obovate	Elliptic-obovate to broadly obovate
Surface of achenes (x40 magnification)	Glossy, smooth, cellular pattern made up of irregular polygonal units arranged in short rows, 12–18 µm across	Glossy, smooth to minutely pitted, cellular pattern made up of distinct rounded units in clusters, 20–32 µm across	Glossy, cellular	Smooth, forming a fine network structure	Glossy, cellular, highly visible polygonal network
Achene colour	Varies with maturity, initially light olive-brown, finally reaching a cinnamon brown colour when old	Varies with maturity initially medium olive-brown, finally reaching a dark brown to almost black	Golden to dark brown	Medium to dark brown, sometimes blackish	Medium to rust-brown, rarely dark brown
Exocarp cells L/W ratio	Squarish (isodiametric), as long as wide when, slightly air-filled or not.	Rectangular, longer side about twice as much the shorter, distinctly air-filled.	Rectangular to about twice to three times long as wide; rarely isodiametric	Shortly rectangular, about twice long as wide	Rectangular, three times long as wide
Cross-section	Variable – Planoconvex to compressed subtrigonal, abaxial edge flattened, corners rounded or not developed	Variable - lenticular to compressed subtrigonal, abaxial edge flattened, corners rounded to vaguely developed	Lenticular, planoconvex or compressed to faintly trigonal	Obtusely trigonal, well-defined rounded edges, the abaxial sometimes flattened.	Lenticular or less often compressed obtusely trigonal
Number of styles	Always trifid		Trifid	Bifid and trifid	Bifid and trifid

is primarily a wind-pollinated genus, failed fertilisation due to deficiency by pollinators is not among the plausible reasons.

Owing to their shorter rays and less vigorous production of ray-born spikelets, the inflorescences of the Maltese populations are grossly more similar to *B. laticarpus* than that of *B. glaucus*; however, when compared with *B. glaucus* morphotype “astericus” sensu Nikolić & al. (2019), there is a closer resemblance in the inflorescences. At least two morphological characters are judged crucial to exclude *B. laticarpus* from the examined material. Firstly, the cross-sectional shape of the achene of *B. laticarpus* is too much trigonous when compared with that of the Maltese plants (Marhold & al. 2004; Hroudová & al. 2007b; Simons & al. 2016; Rumsey & al. 2019; Nikolić & al. 2019; Di Natale & al. 2020). Secondly, the mesocarp/exocarp ratio of about $\times 3$ reported (e.g. Hroudová & al. 2007b; Nikolić & al. 2019) is considerably different from that of approximately $\times 4$ – 5 found in one morphotype and of $\times 1.5$ – 2 in the other.

Hence, as compared in Table 4, the best match for the examined material in Malta is *Bolboschoenus glaucus*. This is a thermophilic species originating from the Old World (described from Senegal) and widely distributed in Africa (Browning & Gordon-Gray 2000), south Europe (Browning & Gordon-Gray 2000; Hroudová & al. 2007b) and the Near East (Wollstonecroft & al. 2011), hence its presence in Malta is within the current distribution. The plano-convex, non-trigonous cross-sectional shape of the achene, the mesocarp:exocarp ratio of $\times 1.7$ – 2.3 ($\times 4$ – 5 in one population) of the pericarp and the consistent 3-fid style found in Maltese material are strong characteristics of *B. glaucus* (Browning & Gordon-Gray 2000; Hroudová & al. 2007a, 2007b; Nikolić & al. 2019). The few fertile plants (achenes available) in BLB01 match well with *B. glaucus* however, the sterile populations in mainland Malta (approx. 99.5% of the plants) could not be assessed, since they do not produce achenes.

Regarding the Gozitan populations, the morphological and biological findings are closest to *B. glaucus* amongst the five European taxa but are not fully congruent with the characteristics of *B. glaucus* as reported in the literature (*op. cit.*). First of all, the achenes with an average size of 3.2×2.0 mm are significantly larger when compared to *B. glaucus*, which on average are 2.5×1.7 mm. In addition, and more importantly, the mesocarp is only 1.6–2.3 times thicker than the exocarp and remarkably different from the reported ratios of three times as much (Browning & al. 1995; Hroudová & al. 2007b & Nikolić & al. 2019) to five times or more (Hroudová & al. 2007b; Di Natale & al. 2020). Nevertheless, collection from North America (specimen Smith & Taylor 3134) was found to have the mesocarp is 1.5 times thicker than the exocarp (Browning & al. 1995), and hence similar to those from Gozo. The high variability of *B. glaucus* was well reported by Nikolić & al. (2019), who assigned four morphotypes and hinted that *B. glaucus* s. l. might consist of an aggregate of unnamed taxa. The present study similarly confirms the variability of *B. glaucus* in locally-distinct morphotypes that require further investigations employing better diagnostic tools from morphometrics to determine any significant taxonomic units.

Finally, the high degree of female sterility, specifically, pollen-producing florets that fail to develop into a fruit was generally observed in all populations in mainland Malta. In exhaustive field examination, some 200 plants were checked in populations BLB02, BLB03, BLB04 and BLB05 and all spikelets resulted being fruitless. Only a few plants

(0.5 % on a rough estimation) had 2–8 achenes in a few spikelets in population BLB01. Some sterile plants have been found in the Gozitan populations (especially the rayless forms), but these populations were generally fertile. It is noteworthy to mention that extensive sterile populations of *Bolboschoenus* (>99% of plants) have not been reported in the literature reviewed in this study or witnessed by some experts that has been consulted (pers. comm. Richard Lansdown, Mar-2021; Jane Browning, May-2021; Danijela Nikolić, Jun-2021; Zdenka Hroudová, Dec-2022).

Summing up, at least two taxonomic interpretations can be postulated for the *Bolboschoenus* populations in the Maltese Islands. The simplest one would be to attribute all the Maltese populations to *B. glaucus* s. l. with the one in mainland Malta assigned to the nominate form (it resembles the ‘asteriscus’ morphotypes defined by Nikolić & al. 2019) but unexplainably sterile, and the Gozitan population assigned to a new morphotype here provisionally labelled as ‘macrocarpus’ for its large achenes compared to the other morphotypes of *B. glaucus*.

However, a more interesting morphological relationship exists when comparing carefully the achenes of the Gozitan material with that of *B. maritimus* and *B. glaucus*. The mesocarp/exocarp ratio of about $\times 2$ in the Gozitan populations is intermediate between a thin exocarp (ratio ca. $\times 5$) in *B. glaucus* to a thicker one in *B. maritimus* (ca. $\times 0.5$). The achene size (average 3.1 mm) is larger than that reported for *B. glaucus* (2.6 mm), and smaller than *B. maritimus* (3.5 mm). The beak is well developed like *B. maritimus*, but the inflorescence architecture is rayed and similar to that of *B. glaucus*, yet less vigorous in terms of the number of rays and the number of spikelets per rays. Rayless forms (like *B. maritimus*) have been observed within the larger populations.

The second interpretation is hence the formation of complex hybrid patterns between long-existing putative parent species: *B. glaucus* in freshwater valleys and *B. maritimus* in marshlands. This can, at least in part, be an explanation of the elevated sterility in the Maltese populations. From a distributional range point of view, both species co-exist in the southern Mediterranean region (Browning & Gordon-Gray 2000: Fig.8; Hroudová & al. 2007b; Di Natale & al. 2020); with *B. glaucus* extending further south into Africa (Browning & Gordon-Gray 2000) and *B. maritimus* north into mainland Europe (Hroudová & al. 2007b). The high degree of variation in the inflorescences’ architecture (Fig. 2a-g) and the occurrence of pockets *B. glaucus* s. str. detected in few fertile specimens in population BLB01 (thus flagging the presence of one of the putative parents in Malta) are further arguments supporting hybridisation.

Although *B. maritimus* has not been retrieved in this study, its absence does not necessarily imply that it never existed in the Maltese Islands. *B. maritimus* might have been present in brackish and marshland wetlands that once existed in Marsa but were destroyed in the mid-19th century for public health concerns. As a matter of fact, Grech Delicata (1853) recorded ‘*Bolboschoenus maritimus*’ from Marsa, which used to be a brackish marshland at that time. There is currently no evidence to confirm whether Grech Delicata’s record is *B. maritimus* or not. Still, ecological findings on *Bolboschoenus* throughout Europe are consistent: *B. maritimus* prefer (or grow) on brackish water, while the other species are glycophytes and prefer freshwater habitats (Hroudová & al. 1999a, 2007b).

No herbarium material from Marsa has been found to confirm Grech Delicata’s record. The only voucher specimen at Argotti Botanic Gardens [ARG] was from Wied tal-Ġnejna

collected on the 6th of April 1914 and was recorded as *B. maritimus* and was reported as rare. The specimen consisted of leaves and blossomed inflorescences and did not bear any achenes. This locality corresponds to population BLB05 and was covered in this study.

Morphometric analyses alone are not enough for the formal description of a *B. glaucus* × *maritimus* hybrid or to explain the predominant sterility in one of the morphotypes which is a new observation of *Bolboschoenus*. Further molecular and perhaps karyological investigations are hence advocated to understand in greater detail the taxonomy and biology (including sterility) of the Maltese populations of *Bolboschoenus*.

Conclusion

Morphological investigation of eleven populations of *Bolboschoenus* in the Maltese Islands resulted that *B. maritimus* reported in floristic literature does not occur. Two distinct populations are ascribed to the thermophilic *B. glaucus* one exhibiting polymorphism (possibly by introgression of another species forming a hybrid complex) and the other with a high degree of sterility. This study could not determine whether this phenomenon is driven by external ecological influences or intrinsic genetic factors. Deeper investigations using better diagnostic tools from morphometrics are required to establish exactly what these forms genetically represent. The present author is looking forward in contributing and working with other authorities studying this genus, particularly *B. glaucus* s. l., while concurring with Nikolić & al. (2019) that the variability of *B. glaucus* should be investigated more carefully for cryptic taxa. In conclusion, local conservation of *Bolboschoenus* and its wetland habitats are strongly advocated because the *Bolboschoenus* population/s in the Maltese Islands might correspond to a taxon novelty not yet reported anywhere in Europe. Maltese valleys and wetlands are increasingly facing threats and pressures, including habitat loss, water abstraction, nitrogen pollution, introduction of alien species and increased drought by climate change.

Acknowledgements

This work has benefited from conversations and correspondences with botanists who had worked on the genus, particularly (alphabetical order) Jane Browning, Zdenka Hroudová, Richard Lansdown and Danijela Nikolić, who are here greatly thanked for their time and for sharing their knowledge and experience on this genus. Thomas Cassar helped identify some photographed bees while Tony Meli and Christian Borg helped provide voucher specimen from Argotti Botanic Garden whose contribution is greatly appreciated.

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