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First contribution to the comparison of flower morphology of six *Arbutus* species (*Ericaceae*), one hybrid and one undefined origin cultivar of the Mediterranean Basin and North America

Abstract

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Three Mediterranean Arbutus species and one natural hybrid, as well as three North American species and one Californian hybrid of unknown origin were studied microscopically; a comparison of the internal anatomy of the flowers of these species is reported for the first time. Anatomical features were found to be similar in general but unique to the Arbutus. However, the length of the anther appendages, the spurs, seems to provide a heritage trait linking the Californian hybrid Arbutus 'Marina' to the A. canariensis and support other morphological findings between the East Mediterranean hybrid, $A. \times andrachnoides$, and its parents A. andrachne and A. unedo.

Key words: strawberry tree, anther appendages, blossom, spur.

Introduction

Fossil findings of plants from the upper Cretaceous period in a clay pit in New Jersey have shown the presence of spurs in the anthers of flowers having some characteristics similar to modern *Ericales* and even *Ericaceae* (Crepet 2009). Present day *Ericaceae* have been studied including the development of the stamens and their appendages (Hermann & Palser 2000). Many of these were found to have "awns" and "spurs". The prevalence of appendages in *Ericaceae* have suggested a different mechanism involving the pollination process.

Arbutus (Ericaceae) includes many popular shrubs or tree species in both hemispheres. A. unedo L. is prominent in the Mediterranean region ("Strawberry Tree"). It is being utilized in the northwest coast of the western hemisphere and in the Mediterranean region in landscaping as an attractive and simply maintained bush. Its bell-shaped blossoms in the fall are generally white with a rare variety originated in Ireland with a pink to ruby color called Arbutus unedo var. rubra W.T. Aiton. A. andrachne L. of the central and eastern Mediterranean commonly (Greek or Eastern Strawberry tree), has also potential in land-

scape use, reforestation, honey production and pharmaceutical industry (Bertsouklis & Papafotiou 2013; Bertsouklis & Papafotiou 2016; Yilmaz 2016). *A.* ×*andrachnoides* Link is believed to be a natural hybrid between *A. unedo* and *A. andrachne* and it is native to Greece and the eastern Mediterranean region (Arabatzis 2001; Torres & al. 2002). It is challenging however, to differentiate from *A. andrachne* (Bačić & al. 1992; Cullen & al. 1997). A significant difference has been found in the length/width ratio of the leaves (Bertsouklis & Papafotiou 2016) and in a different pattern of the stomata and the surrounding tissue when studied with electron scanned microscopy (Bačić & al. 1992). *A. canariensis* Duhamel is an indigenous tree of the Canary Islands. It has most recently been assessed for The IUCN Red List of Threatened Species in 2017 and is listed as Near Threatened under criteria B1a+2a (Beech 2017) portraying many of the same traits of the Mediterranean *Arbutus* but offering pink to ruby blossoms instead of white. In the natural habitat, *A. canariensis*, like *A. unedo*, is a fall bloomer whereas *A. andrachne* is a spring bloomer.

In the western hemisphere, *A. peninsularis* Rose & Goldman is indigenous to the Sierra de la Laguna mountains in the southern region of Baja California in Mexico. *Arbutus menziesii* Pursh (Pacific madrone) is prevalent for much of the Pacific Northwest extending from central Vancouver Island south to mid California. *A. menziesii* extends from British Columbia to Baja California along the West Coast of North America (Hileman & al. 2001). It also can grow to immense size; a circumference of 10 m has been reported (Coe 1983); the species is found along the seashore, often in rocky regions, bending and twisting out to open space to obtain the sunshine *A. xalapensis* Kunth (Texas madrone) is found in Texas and northeastern Mexico. The bark of *A. xalapensis* is red but the color turns to a white appearance, depending on the brightness and harshness of its sun exposure during the year.

A mysterious and popular cultivar Arbutus 'Marina' has been propagated in California particularly in the regions with a Mediterranean climate (Fig. 2). The tree is known to have originated in California, at the San Francisco 1915 Panama American Exposition. It is thought to have been brought to the exposition from somewhere in the Mediterranean region. Details of its history after the exposition are described (Baldwin 2020). However, of pertinence here is that in the early 1990' Briggs Nursery Inc. near Olympia WA, USA, contracted to produce plantlets by micro propagation techniques. This action fueled the starting of wide distribution of it in California and apparently elsewhere. Demoly (2004) named and described two hybrids: Arbutus *thuretiana Demoly and Arbutus *reyorum Demoly. The first is a cross between A. canariensis and the A. andrachne and the latter a second cross between the A. ×andrachnoides and the A. canariensis. Further, he suggested that the latter hybrid was displayed in the 1915 Panama American Exposition but doesn't indicate how it could have arrived there (Demoly 2004). World War I was erupting at that time, which added to confusion of normal record keeping. However, Demoly (2004) also writes that a plant called Arbutus 'Marina' was introduced in France in 1993 from the Madrona Nursery located in England. Liam Mackenzie the 1986 founder of that nursery revealed he used to travel in the early 1990's to the Courson Flower Show, held south of Paris, to sell plants and probably sold A. 'Marina' there. However, he had himself acquired A. 'Marina' plants from Peter Catt of Liss Forest Nursery in Hampshire, UK (Mackenzie 2018 pers. comm.). Mr. Catt, formerly of the Liss Forest Nursery, though unsure of dates, said he had bought many plants from Briggs Nursery in that early time frame (Catt 2018 pers. comm.). Demoly compared the morphology of the A. 'Marina' to the A. 'revorum

and feels justifying maintaining the name of the cultivar as $Arbutus \times reyorum$ 'Marina' (Demoly 2018 pers. comm.). Nevertheless, there is no other direct evidence that they are the same. This investigation was prompted finding the link between the spurs of the A. $\times reyorum$ 'Marina' and the A. canariensis compared to other Arbutus trees.

Materials and Methods

Flowers of the *Arbutus* species were obtained from various regions for examination. *A. unedo*, *A. andrachne* and *A. × andrachnoides* were obtained in the region of Tatoi Former Royal Palace (Varympompi, Athens, Greece, N 38°08'14.0", E 23° 47'59.0". *A. menziesii* and *A. unedo* were acquired near Anacortes, WA, USA (N 48° 30'54.011 W 122° 37'10.169); *A. peninsularis* blossoms were obtained from the Sierra de la Laguna mountains (Baja California, Mexico, N 23° 32'44.489" W 110°00'37.68"); *A. xalapensis* flowers were picked and sent from Dripping Springs TX, USA (N 30°11'31" W 98° 05'07") near Austin; *A. canariensis* and *A. ×reyorum* 'Marina' were obtained, with permission, from the Golden Gate Botanical Garden in San Francisco.

The significance of the results was tested by one-way analysis of variance (ANOVA). The spur length means were compared by Student's t test at $P \le 0.05$ (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The blossoms of all studied species of Arbutus had a similar form (Fig. 1). The corolla of the studied species was urceolate similar to most genera of the Arbutoidae subfamily (Matthews & Knox 1927) (Fig. 1A). Stamens furnished with awn (bristle-like appendages) were enclosed in the corolla. It required cutting and removing sections of it with dissecting scissors to expose the inner parts of the blossom (Fig 1B). Ten anthers with each having two pollen sacs and two spurs were found in all species. The filaments of the stamens were well developed and broadened at increasing distance from the anther until it suddenly reduced in diameter just before it attached to the corolla. White dense hairs, almost luminous in color, encompassed the lower one third of each stamen. Its purpose is not known. The spurs tapered from their attachment point to the anther to their tip. The internal parts of the blossoms of A. ×reyorum 'Marina' and A. canariensis have similar appearances (Fig 3, 4). The spurs seem to be shorter and thicker where they attach to the anther, and with less complicated curvature compared to A. unedo (Fig. 1) and the other Arbutus species (Fig. 5, 6). A. unedo had the longest spur length (Table 1). A. × andrachnoides had a value closer to A. andrachne, along with other traits found in a previous study (Bertsouklis & Papafotiou 2016). The length of all three of these species were found to be different and longer that both A. canariensis and A. ×reyorum 'Marina'. According to Pascual & al. (1993) A. canariensis in the Canary Islands had thick and short spurs like what we found in the blossoms from San Francisco.

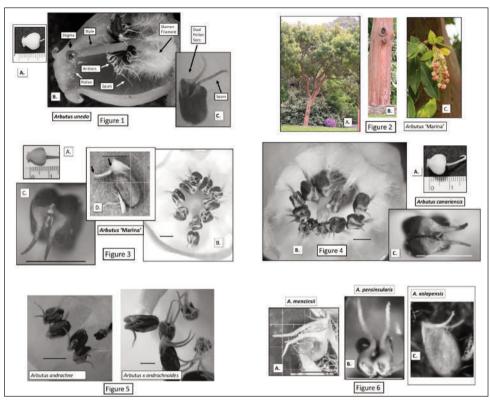


Fig. 1. Arbutus unedo A) Blossom; B) Dissected blossom; C) Single anther with dual pollen sacs and the dual spurs. Fig. 2. Arbutus ×reyorum 'Marina' tree: A) Tree; B) expanded insert of the trunk; C) cluster of blossoms, picture taken April 25, 2019 at Gold0en Gate Botanical Garden San Francisco. Fig. 3. A. ×reyorum 'Marina': A) Blossom; B) View into the blossom with the opening end of the corolla removed exposing the anthers and their spurs; C) A magnified view similar orientation as B. Single anther, D) Single anther. Fig. 4. A. canariensis A) blossom; B) Stamens; C) Single anther with its dual spurs like the view of Figure 3C for the A. 'Marina'. Fig. 5. Blossoms of A. andrachne and A. × andrachnoides. Bars represent 1mm. Fig. 6. Anthers and spurs of three western hemisphere Arbutus trees. A) A. menziesii, an oblique view of two spurs; B) A. pensinsularis, apical view of single anther; C) A. xalapensis, a lateral view of an anther showing the curved spurs.

Table 1. Comparing the spur length measure (mm) of the spurs of various species.

Species	Mean Length (mm)
A. unedo	1.27 ± 0.18 a
$A \times andrachnoides$	$1.19 \pm 0.18 b$
A. andrachne	$1.10 \pm 0.13 \text{ b}$
A. ×reyorum 'Marina'	0.69 ± 0.10 c
A. canariensis	$0.69 \pm 0.14 c$

Mean separation in columns by Student's *t*-test, $P \le 0.001$. Means followed by the same letter are not significantly different, n=15-66.

Conclusions

The dominate morphology of the dual spurs in the *Arbutus* species studied and their definitive structure found implies that the hybrid and cultivar *A.* × *reyorum* 'Marina' have the *A. canariensis* in their heritage line. The color of the blossoms and the peeling bark further supports this indication. Additionally, this finding confirms the premise of Demoly (2004) that it is the hybrid *Arbutus* × *reyorum* Dem. However, by itself it does not rule out that it could be *Arbutus* × *thuretiana* Demoly that he also has identified as a hybrid of the *A. canariensis*. The spur length of the Greek hybrid, *A.* × *andrachnoides*, agrees with other reported morphology findings that place *A.* × *andrachnoides* closer to *A. andrachne*.

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