Secretory structures in the Plumbaginaceae: origin, evolution and roles in stress tolerance

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Abstract

The Plumbaginaceae (non-core Caryophyllales) is a family well known for species adapted to a wide range of arid and saline habitats. Of its salt-tolerant species, at least 45 are in the genus Limonium; two in each of Aegialitis, Limoniastum and Myriolimon, and one each in Psylliostachys, Armeria, Ceratostigma, Goniolimon and Plumbago. All the halophytic members of the family have salt glands and salt glands are also common in the closely related Tamaricaceae and Frankeniaceae. The halophytic species of the three families can secrete a range of ions (Na+, K+, Ca2+, Mg2+, Cl-, HCO3-, SO42) and other elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn). Salt glands are, however, absent in salt-tolerant members of the sister family Polygonaceae. We describe the structure of the salt glands in the three families and consider whether glands might have arisen as a means to avoid the toxicity of Na+ and/or Cl- or to regulate Ca2+ concentrations with the leaves. We conclude that the establishment of lineages with salt glands took place after the split between the Polygonaceae and its sister group the Plumbaginaceae.

I INTRODUCTION

Exudation is a common phenomenon in plants and specialised tissues have evolved to secrete a variety of substances from nectar to mucilages to salts (Fahn, 1988; Lüttge, 1971). Glands that secrete salt are found across the families of flowering plants with a diversity of structures having evolved to fulfil this role (Dassanayake and Larkin, 2017; Thomson, Faraday & Oross, 1988). Fahn (1988) concluded “(1) During the course of evolution secretory tissues first developed inside plant organs. (2) In the primitive conditions only secretory idioblasts or groups of such cells were scattered among the cells of the ordinary tissues. (3) Later, secretory ducts and cavities developed. (4) The glandular trichomes are the most recently evolved secretory structures.” Here we examine the distribution of salt-secreting structures in the Plumbaginaceae and discuss our observation that all salt-tolerant species within the family utilise glands to secrete salt, whereas salt glands are absent in salt-tolerant members of the related Polygonaceae.

The Caryophyllales is one of the major lineages of flowering plants with about 39 families and approximately 12,500 species (Bremer et al., 2009; Hernandez-Ledesma et al., 2015) and their phylogenetic relationships have been studied over a long time by morphology (Rodman, 1994), targeted gene sequencing (Brockington, Walker, Glover, Soltis & Soltis, 2011; Rettig, Wilson, & Manhart, 1992; Schäferhoff, Müller, & Borsch, 2009), plastome sequencing (Arakaki et al., 2011) and transcriptome sequencing (Leebens-Mack et al., 2019; Walker et al., 2018; Yang et al., 2015). Based on such studies, the Angiosperm Phylogeny Group’s classification, APG III (Bremer et al., 2009) and APG IV (Byng et al., 2016), support the expansion of the traditional

Caryophyllales (i.e., corresponding essentially with the original Centrospermae) to include the noncore-Caryophyllales - the carnivorous families Droseraceae, Drosophyllaceae, Nepenthaceae, Ancistrocladaceae, Dioncophyllaceae, and allies Tamaricaceae, Frankeniaceae, Polygonaceae, and Plumbaginaceae (Brockington et al., 2009; Hernandez-Ledesma et al., 2015).

The Plumbaginaceae Juss. is a cosmopolitan family of perennial herbs, shrubs or small tress, rarely climbers (Kubitzki, 1993) that is well supported as monophyletic (e.g. Cuenoud et al., 2002; Hilu et al., 2003). The family is included in the Polygonids clade of the Caryophyllales (Figure 1 and Supplementary Table 1), which comprises carnivorous (Ancistrocladaceae, Drosophyllaceae, Droseraceae, Nepenthaceae) and other non-carnivorous sister taxa (Frankeniaceae, Tamaricaceae, Plumbaginaceae and Polygonaceae). The Plumbaginaceae is comprised of two subfamilies, the Plumbaginoideae and the Limonoideae. Genera of the Limonoideae are thought to have initially diversified in the Mediterranean and Irano-Turian regions, although a few genera also occur in the Southern Hemisphere (Lledó et al., 2005; Malekmohammadi, Akhani, & Borsch, 2017; Moharrek et al., 2019) (Table 1): *Aegialitis* is the only genus of the Limonioideae with a tropical distribution (two mangrove species in Asia and Oceania). Within the Plumbaginoideae, its members predominantly occur in arid and saline environments and often in coastal habitats (Kubitzki, 1993; Hernandez-Ledesma et al., 2015; Malekmohammadi et al., 2017; Moharrek, Sanmartín, Kazempour,-Osaloo, & Nieto Feliner, 2019). Of the nearly 940 species in the family as a whole, 5% display salt tolerance (Table 1), ranking the family fourth in a list based on the proportion of species within a family that are halophytes (Santos, Al-Azzawi, Aronson & Flowers, 2016).

Halophytes are plants that survive in the presence of significant concentrations of soluble salt in the medium in which they grow. Quite what amounts to a ‘significant concentration of salt’ has been a matter of debate over the years (see, for example, Breckle, 2002; Flowers & Colmer, 2008; Huchzermeyer & Flowers, 2013) with Aronson (1989) selecting a salt concentration of around 80 mM as the dividing line that separates halophytes from more salt-sensitive species, commonly called glycophytes. Flowers & Colmer (2008) used a higher salt concentration, of 200 mM, in order to discriminate higher levels of tolerance. However, even the ability to tolerate 80 mM NaCl is rare with fewer than 1500 species having evolved this ability (https://www.sussex.ac.uk/affiliates/halophytes/index.php?content=plantStats) so that halophytes represent less than 1% of the approximately 351,000 species of plants whose names have been accepted by taxonomists (http://www.theplantlist.org/).

Within the Plumbaginaceae, there are nine genera with salt-tolerant species (recorded in eHALOPH; see Table 1). Both species of *Aegialitis* are salt tolerant trees (mangroves) (Atkinson et al., 1967; Das, 2002) and within the genus*Armeria* , *A. maritima* is found in salt marshes (e.g. Rozema, Gude, & Pollack, 1981), on sandy (Köhl, 1997) and on heavy-metal-rich soils (Farago, Mullen, Cole & Smith, 1980; Heumann, 2002; Neumann et al., 1995). Just one of the seven species of*Ceratostigma* , *C. plumbaginoides* (Borchert, 1989), and one of the 22 species of *Goniolimon* , *G. tataricum* (Faraday & Thomson, 1986c; Pawloski, 1963), is a halophyte. Both species of*Limoniastum* are halophytes (Salama, El-Naggar, & Ramadan, 1999; Zouhaier et al., 2015) as are the two species of *Myriolimon* ,*M. diffusum* (Pount & Revel, 1982) and *M. ferulaceum* , together with one of the 16 species of *Plumbago, P. auriculata*(Faraday & Thomson, 1986a; Faraday & Thomson, 1986c) and one of the four species of *Psylliostachys* (*P. spicatus*). The genus*Limonium* has at least 45 halophytic species that have diversified in coastal and inland-saline and gypsum ecosystems (Hernandez-Ledesma et al., 2015; Kubitzki, 1993).

2 MUCILAGE SECRETING GLANDS

The secretions from plants can be produced from both above and below ground organs for a variety of purposes; secretory structures are found in all angiosperm clades (Brown, George, Neugebauer & White, 2017), with the potential to influence plant microenvironment in various ways (Galloway, Knox & Krause, 2020). Some plant species have trichomes (called colleters by Fahn 1979) that secrete mucilage; examples are seen in young stipules of *Rumex* and *Rheum* (Polygonaceae). The most common colleters have a stalk with at least two rows of cells, side by side (biseriate), supporting several radiate elongated cells (Fahn, 1979). Within the Plumbaginaceae, mucilage glands (see Figure 2) were described, in the nineteenth century, in

Aegialitis ,*Armeria* , *Ceratostigma* , *Limoniastrum* ,*Limonium* and *Plumbago* in comprehensive studies by Wilson (1890) and de Fraine (1916). These mucilage-glands appear in the axils of leaves and on other organs of all the genera, where they are relatively similar in appearance. In some *Limonium* species, these glands occur at the base of the leaf sheath on its upper (adaxial) surface (Batanouny, Hassan, & Fahmy, 1992; de Fraine, 1916; Wilson, 1890; Figure 2A), and secrete large quantities of a transparent colourless, viscous liquid at the base of the petioles. The secreting cells are prismatic, columnar or conical and radiate from basal collecting cells with straight periclinal walls, without pores in the cuticle envelope covering the gland cells (Batanouny et al., 1992; de Fraine, 1916; Wilson, 1890). The mucilages may accumulate either at the cell-wall level or in the space between cell wall and protoplast (Trachtenberg and Fahn, 1981) as well as in vacuoles of epidermal cells (Fahn, 1988). A mucilage histochemical test, tannic acid and iron trichloride (Pizzolato & Lillie, 1973), performed on the lower epidermis of *Limonium multiflorum* leaves, demonstrates the presence of mucilage (non-structural polysaccharides) by the appearance of a black colour at the cell-wall level and inside a few epidermal vacuoles (Figure 2B). In *Armeria, Ceratostigma* and*Limoniastrum* the mucilage glands are of the same type as in*Limonium* , but in *Armeria* the basal cells are comparatively few in number, but larger than in species of *Limoniastrum*(Wilson, 1890). In *Aegialitis* , the mucilage-secreting cells are found in the axils of the leaves, on laminae, bracts, and sepals, and are very numerous, lying in an oval or circular depression, bounded by regularly arranged cells (Wilson, 1890). *Plumbago* species have glandular hairs that secrete a sticky mucilage on the petiole and calyx of flowers (Singh, Naidoo, Bharuth & Baijnath, 2019; Sudhakaran, 2019; Wilson, 1890). It has been hypothesized that the sticky exudates function as an aid to pollination by acting as a barrier for insect predators like ants, so preventing predatory attacks on favoured flying insect pollinators (Panicker & Haridasan, 2016). The calyx glands of non-carnivorous *Plumbago* are anatomically similar to the mucilage glands of carnivorous genera *Drosera* and *Drosophyllum* , suggesting a common ancestral gland structure (Thorogood, Bauer, & Hiscock, 2018).

In carnivorous plants, specific multicellular glands are associated with leaves that have been modified to capture prey. Some of the glands producing secretions are supplied with special vascular strands and the surrounding cells show numerous cell wall plasmodesmata (Guo, Yuan, Liu & Zhu, 2013; Sharifi-Rad et al., 2017), which regulate the transport of substances between adjacent cells. Many internal secretory structures, like glands and ducts of Euphorbiaceae, Papaveraceae, Clusiaceae and Cannabaceae, are associated with vascular bundles, since compound synthesis requires a regular supply of precursors through the phloem. Plastids and photosynthesis itself are known to be involved in the synthetic pathways of many of the compounds secreted (Evans, 2009) and so a localisation near phloem seems to favour the delivery of these different compounds (Sharifi-Rad et al., 2017). However, the presence of vasculature is not an indicator of functional carnivory, since many glands of carnivorous species are not vascularized (e.g. glands of*Nepenthes* ; Renner & Specht, 2013). Apart from glands secreting mucilage, other glands have evolved without vascular connections, glands that secrete salt (Tables 1 and 2).

3 SALT GLANDS

Unlike glands that secrete mucilage, salt-secreting glands are relatively uncommon: they are found in just 12 of the 111 families that contain halophytes. Of the 12 families with salt-secreting halophytes (recretohalophytes), five families contain approximately 90% of the species (Plumbaginaceae, 28%; Poaceae, 21% Amaranthaceae, 20%; Tamaricaceae, 15% and Frankeniaceae, 6%) with seven families containing the remaining 10% (analysis of data in eHALOPH 30/Oct/2019). In all cases, salt glands are epidermal structures, but with anatomical and structural dissimilarities that point to their multiple evolutionary origin (Flowers, Galal, & Bromham, 2010).

Multicellular salt glands have been described in nine genera of the Plumbaginaceae (Table 2); on leaves and stems, as well as other aerial organs, such as rachis, scapes (inflorescences) and spikes (Salama et al., 1999; Wilson, 1890). Among the halophytic species in the family it is likely that all utilise salt glands (see Table 2A), although there are some that thrive in saline habitats/environments, but where the presence of functional glands has yet to be established (e,g.,*Myriolimon diffusum* ; Table 2). There are also species with glands, whose salt tolerance has yet to be established; for example,*Plumbago zeylanica* (Sudhakaran,

2019) and *P. europaea*(Waisel, 1972) as well as non-halophytes like *Armeria caespitosa*(Gimenez-Benavides, Escudero, & Perez-Garcia, 2005) and *A. canescens* (Scassellati, Pasqua, Valletta, & Abbate, 2016), which have structures similar to salt glands (Table 2B). The presence of structures analogous to salt glands in species where salt tolerance has yet to be established is particularly common in the largest genus within the family, *Limonium* (at least 14 species ; see Tables 3A and 3B; there are 34 halophytic species of *Limonium* with salt glands,).

The salt glands of the Plumbaginaceae are complex, consisting of up to 40 cells. Within most species, the glands are composed of 16 cells (Thomson, Faraday, & Oross, 1988); only the glands of*Limoniastrum guyonianum* (with 32 cells; Tables 2 and 3 and Figure 3) and those of *Aegialitis* (24 or 40 cells) have more. Multicellular salt glands have also been described in the noncore Caryophyllales families Frankeniaceae and Tamaricaceae (Dassanayake & Larkin, 2017; Fahn, 1988; Flowers et al., 2010; Grigore and Toma, 2017; Thomson et al, 1988) - in four genera and 58 species (Table 4). Although the number of halophytes within the Frankeniaceae and Tamaricaceae is small, just 2% and 1%, respectively, of all halophytes, 80% of salt-tolerant species within the Frankeniaceae and 67% of salt-tolerant species within the Tamaricaceae have been recorded as having salt glands (eHALOPH, October 2019). Glands within the Frankeniaceae and Tamaricaceae have fewer cells, than genera in the Plumbaginaceae - generally eight (Table 4), rather than 16 in the species within the Plumbaginaceae (Tables 2 and 3). This smaller number of cells per gland (8) is associated with a higher frequency (median of 31 per mm^2; n = 14 within the Frankeniaceae and Tamaricaceae, Table 4) than seen in the Plumbaginaceae (median of 12 per mm^2, n= 22, Tables 2 and 3). Notably, in members of the Polygonaceae, sister group of Plumbaginaceae, no species is recorded as having salt glands.

3.1 Morphological studies of salt glands

Ruhland (1915) using cross and longitudinal sections of leaves of*Statice gmelinii* (syn. *Limonium gmelinii*) illustrated a detailed anatomical description of the complex 16-celled salt glands (with cells arranged in four quadrants; Figure 4) found within the Plumbaginaceae (see Grigore and Toma, 2016 and 2017 for a history of the description of these glandular structures). Ruhland (1915) also reported this cellular arrangement in *Statice incana*(synonym: *Goniolimon incanum*), *S. latifolia* (synonym:*Limonium platyphyllum*) and in *Armeria maritima* (also then known as *A. vulgaris*). Indeed in the Plumbaginaceae this organization is well preserved, with the glands of most species within the family consisting of 16 cells (Table 2; Balsamo & Thomson, 1993; Faraday & Thomson, 1986c; Salama et al., 1999; Thomson et al 1988), characteristically differentiated into secretory, collecting and accessory cells, in a cuticle-lined structure (Figure 4A). There is, however, some disparity in the literature as to whether or not four large basal cells are included in the gland11Note that different authors use the term ‘basal cells’ to refer to collecting cells, and ‘accumulating cells’ to refer to accessory cells. For simplification we synonymize and use the terms secretory cells, collecting cells (=basal cells) and accessory cells (=accumulating or transfer cells)..

In *Armeria* , *Ceratostigma* and*Goniolimon* the 16 cells of the salt glands are arranged in four quadrants. The four central secretory cells are surrounded by accessory cells, both being enclosed by two other layers, each of them consisting of four cells (Fahn, 1988; Faraday & Thomson 1986c; Feng et al., 2014; Salama et al., 1999). In leaves of *Aegialitis annulata* , salt glands appear to be organized into eight rings of five cells (Faraday and Thomson 1986c) whereas in *A. rotundifolia* they are present within a cup-shaped crypt in the epidermal layer (Das 2002) and consist three rings of eight cells (Atkinson et al 1967; Wang, B-S personal communication). In *Limoniastrum guyonianum* leaves, however, each salt gland comprises 32 cells, 16 in cross section (Figure 4B), including two huge collecting cells, six accessory cells, and a central part representing the secretory structure containing eight cells - four collecting cells, each one surmounted by an apical cell (Zouhaier et al., 2015). Although the study by Faraday and Thomson (1986c) reports 16 cells for *Plumbago auriculata* and that of Sudhakaran (2019) of*P. zeylanica* shows eight cells, it is not possible to confirm either number from the published material.

In *Limonium* species a complex 16-celled salt-gland structure is commonly reported (Table 3; Figure 4A; Batanouny et al., 1992; Faraday & Thomson, 1986c; Salama et al., 1999; Vassilyev & Stepanova 1990; Yuan, Leng & Wang, 2016; Ziegler & Luttge, 1966), although other arrangements of cells have also been found. For instance, in *L. aureum* , *L. bocconei* , *L. lojaconi* and in *L. pignattii* the mature gland seems to include 12 cells (four central secretory, adjacent and collecting cells) (Ni, Tan & Shen, 2012). In*L. sinense* and *L. franchetii* the mature salt gland is described as a complex structure with 20 cells - four secreting cells each accompanied by an adjacent cell and bounded by four internal and four accessory cells, and four collecting cells (Xin, Tan & Chu, 2011, 2012). As in previous cases, however, it is possible that the number of cells within a gland is open to different interpretations. Here we show that *L. multiflorum* , *L. narbonense L. nydeggeri* and*L. ovalifolium* all present salt glands with 16 cells, arranged in quadrants (Figure 5), where cells have a spatial and functional differentiation. In each quadrant, there are four cells: a basal collecting cell and two accessory cells, and these surround a secretory cell (Figure 5A).

The glandular complex can vary in its position in the epidermis. In leaves, the glands can be seen on the surface at the level of the other epidermal cells or deeply sunken in the leaves, being then side by side with the mesophyll cells (Tables 2 and 3, Figures 5A & B; Akhani Malekmohammadi, Mahdavi, Gharibiyan & Chase 2013; Salama et al., 1999; Thomson et al, 1988). In stems, scapes and spikelets, the insertion of glands is similar to that in the leaves: glands may protrude from the surface as observed in *A. maritima* (Bernard & Lefebvre, 2001) or even be located on the top of a special elevated cortical structure as reported in *Limonium pruinosum* (Salama et al., 1999). A dense and thick cuticle is present around the whole group of cells forming the gland, which is confirmed by fluorescence microscopy on cross sections of *L. multiflorum* leaves (Figure 5B) and on surface view of *L. narbonense* leaves (Figure 5C). This seems to be fundamental in protecting epidermal and mesophyll cells from salt damage (Thomson et al., 1969). The four secretory cells excrete the mineral solutions through four visible pores (one pore per cell) (Figure 5D), which gives a peculiar appearance to the epidermis, whose cuticle can have different types of wax deposits (Figures 5 E and F). The glands have no direct connection with the vascular bundles (Fahn, 1988).

In summary, reported differences in cell number between 12 and 20 cells appear to us to be an arbitrary difference of interpretation of which cells are included in the gland. We interpret the data as showing most members of the Plumbaginaceae have 16-celled glands; the exceptions are*A. annulata, A. rotundifolia* and *Limoniastrum guyonianum* . Species within the Frankeniaceae and Tamaricaceae have eight-celled glands with each being comprised of six secreting cells and two collecting cells totalling eight cells (Table 4).

3.2 Physiology of salt secretion

In the late nineteenth century, the secretion of $CaCO_3$by glands on the surface of leaves of *Armeria* , *Statice* ,*Goniolimon* , *Limoniastrum* and *Plumbago* was demonstrated (Braconnot, 1836). The organs responsible were drawn by Mettenius (1856) and Licopoli (1866) and called 'Licopoli' (Maury, 1886), 'Mettenius' (Wilson, 1890) or even 'calciferous' glands (Licopoli, 1870). Their illustrations are of structures similar to those we now call salt glands (compare Grigore & Toma 2017 and Wilson, 1890) and indeed Sakai (1974) suggested that "in all probability chalk glands and salt glands are not separate secretory structures" based on the low ion selectivity shown by the way that *Tamarix aphylla* can predominantly secrete either Na or Ca depending on substrate. Advances in analytical techniques, particularly analytical x-ray microscopy, have revealed the range of elements that are secreted by glands (Faraday & Thomson 1986a; Feng et al., 2014; Salama et al., 1999). While the secretions from many species do contain Ca and Mg (Table 2), the glands are capable of secreting a variety of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, $Zn^{2+,}$ Cl^-, HCO_3^-, SO_4^{2-}; see also Thomson et al, 1988) with fluxes of Na^+ and Cl^- reaching values of over 200 pmol/gland/h (Table 3). Crystals on the surface of leaves of *A. maritima* growing on contaminated soil contained Cu and lesser amounts of Zn, Ni, Fe and Mn (Neumann et al., 1995). In plants in solution culture, Heumann (2002) identified Zn within cells of the salt glands. Salts on the surface of leaves of *Tamarix africana* growing on a contaminated salt marsh contained As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn (Santos et al., 2017), while plants of *T. smyrnensis* growing in solution culture have been shown to secrete Pb and Cd (Kadukova, Manousaki, & Kalogerakis 2008). Nevertheless, Na and Cl dominate the secretions of plants growing on maritime saltmarshes.

The presence of salt glands in some, but not all, halophytes raises a question of how some species are able to tolerate salinity without the ability to excrete salt while others utilise glands. Are these glands required for salt tolerance in some species or are they simply a structure that evolved for other reasons and have become adapted to secrete salts when present? Here we examine the case for the more extreme halophytes, the euhalophytes - these are plants that can tolerate at least 200 mM salt and so are amongst the most salt-tolerant species of plants. There are 524 euhalophytes listed in eHALOPH, of which just 74 species are reported to have salt glands - with the Amaranthaceae, Poaceae and Plumbaginaceae accounting for 80% of these species. The Plumbaginaceae is, however, remarkable in that most (86%) of its euhalophytes have salt glands as compared to 31% of the Poaceae and 18% in the Amaranthaceae. In the related Frankeniaceae and Tamaricaceae, the proportion of euhalophytic species with salt glands is also remarkably high (Frankeniaceae, 100% and Tamaricaceae, 60%). So, why the importance of salt glands? The salt glands of the Amaranthaceae and Poaceae are bicellular and so different from the complex glands seen in the other families. Those of the Amaranthaceae are external bladders (Osmond, Luttge, West, Pallaghy & Shacher-Hill, 1969; Shabala, Bose, & Hedrich, 2014) and can be interpreted as a salt-storage system, analogous to the storage cells seen in the succulent leaves in other members of the family. Such bladders are not essential for extreme salt tolerance in the Amaranthaceae; they are absent in genera such as*Salicornia* , *Suaeda* or *Tecticornia* and, where present, do not excrete ions in the way that the glands of the Poaceae do. The microhairs of the Poaceae (present in all but the Pooideae) do secrete salt (see Dassanayake & Larkin, 2017), but again are clearly not essential for tolerance of high salinities in the Poaceae, since there are, euhalophytes in, for example, the genera *Paspalum, Phragmites* and *Puccinellia* that do not have salt glands. So, why are salt glands such a common feature of euhalophytic members of the Plumbaginaceae, Frankeniaceae and Tamaricaceae? Is it that species in these three families are less efficient than the members of the Amaranthaceae in limiting the access of ions to the xylem and so have to excrete salt that reaches the leaves?

To survive salinity, plants have to balance the delivery ions with their growth rate. It is clear that all plants are able to restrict the entry of ions reaching the xylem vessels to some extent (Munns & Tester, 2008) - so called exclusion. Salt-sensitive species, such as rice, appear to be rather poor at this process and rice has been shown to have a 'bypass' flow; a leak of ions through areas of the root system that have a poorly developed endodermis (Faiyue, Al-Azzawi & Flowers, 2010; Yeo, Yeo and Flowers, 1987). Estimates of 'exclusion' can be made by comparing ion concentrations in the xylem sap to those in the medium. Neither parameter is particularly easy to determine: medium concentrations are only easy if plants are growing in a liquid medium while xylem concentrations have to be estimated from change in shoot content over time (hours to weeks) divided by the volume of water transpired in that time. Nevertheless, exclusion' values for halophytes have been calculated and range from 77 to 99 % (Flowers, 1985; Reef & Lovelock, 2015). The apparent 'low exclusion' can be accounted for by the fact that many halophytes utilise Na and Cl in growth so that xylem concentrations of Na^+ can be high (see, for example, Yeo & Flowers, 1986). However, once the delivery of Na^+ and Cl^- exceeds the capacity for the plant to compartmentalise those ions, then growth is reduced. This situation might be avoided or mitigated if excess ions are excreted, but there is too little evidence to allow a clear conclusion. Rozema et al. (1981) showed that excretion by glands can be an important aspect of balancing the salt load in shoots of some species. For plants growing in 200 mM NaCl for three days, they (Rozema et al., 1981) calculated excretion as a proportion of increase in content for *Spartina anglica* to be 1, for *Limonium vulgare* , the ratio was 0.3; for*Glaux maritima* , 0.1 and for *Armeria maritima,* 0.04. It is clear that the two members of the Plumbaginaceae (*Limonium* and*Armeria*) were not particularly reliant on secretion, but even a small proportion could be critical for survival. In a study of mangroves, Reef & Lovelock (2015) concluded that "... salt excretion (the salt gland trait) is not sufficient or necessary to confer high levels of salinity tolerance. The presence of salt glands ... is also not linked to levels of salt exclusion". Unfortunately, there is no systematic data for halophytes that compares secretion as a proportion of uptake or the xylem concentrations of Na^+ and or Cl^- that would allow us to evaluate whether or not glands are required for salt tolerance in some species.

As salt tolerant species in the Plumbaginaceae appear to utilise salt glands and yet there is a significant number of species with glands that are not halophytes (Tables 2B, 3B and below) we have looked for alternative explanations for the presence of glands in members of the family and of species within the Frankeniaceae and Tamaricaceae. One possibility is the regulation of Ca concentrations. Ca is an important determinant of the response of plants to salinity, not only in its role in signalling, but also through its effects on cell walls and membranes (Greenway & Munns, 1980; Hadi & Karimi, 2012). Ca is essential for plant growth (White & Broadley, 2003) and yet the concentration of cytoplasmic free Ca^{2+} is low (sub-micromolar, Broadley et al., 2003; Tang & Luan, 2017), reflecting its role as an important signalling molecule. Since there is a strong inward driving force for Ca^{2+} into cells (Demidchik, Shabala, Isayenkov, Cuin & Pottosin, 2018), plants use a variety of means to regulate their Ca concentrations (Tang & Luan, 2017), amongst which is the ability to precipitate calcium oxalate (Franceschi & Nakata, 2005), seen particularly in the Amaranthaceae and Polygonaceae within the Caryophyllales (White & Broadley, 2003). As far as we are aware, however, little is known of the Ca relations of the Plumbaginaceae, Frankeniaceae, or Tamaricaceae, although shoot Ca concentrations in the Plumbaginaceae and Tamaricaceae, appear to be in the lower range of values seen across plants (Broadley et al., 2003). The range of Ca concentrations seen in plants is 0.11 to 4.41% of shoot dry weight as calculated by Broadley et al. (2003) from data in the literature. The value for the shoots of *Tamarix ramosissima* is in the middle of this range, at 1.97% of the dry weight. In a hydroponic experiment they (Broadley et al., 2003) recorded the shoot Ca of *Armeria maritima*to be 0.59%. Since Ca is secreted from the glands of both *T. ramosissima* and *A. maritima* (Tables 2 and 4), this is a means by which shoot Ca concentrations could be regulated. We hypothesise that multicellular salt glands could have evolved in the Plumbaginaceae, Frankeniaceae and Tamaricaceae to regulate shoot Ca concentrations and perhaps the balance between Ca and Mg (Tang & Luan, 2017). Over the course of time, this allowed species of these families to colonise drier saline soils as well as seawater (*Aegialitis* ; Table 2).

3.4 Evolution of salt glands

The Plumbaginaceae is well-known for salt and drought tolerance, with genera and species adapted to arid environments and a wide range of saline habitats – adaptations involving anatomy (e.g. multicellular glands) and physiology (e.g. osmoprotective compounds; Slama, Abdelly, Bouchereau, Flowers & Savoure, 2015). In the Plumbaginaceae, salt glands occur in species that can grow in tidal areas (including the mangrove *Aegilatis* ; Das, Mishra, & Mohanty, 2006) and in higher drier parts of salt marshes subjected to high salinity (e.g.,*Limonium* and *Limoniastrum* ; Alvarez & Manzanares, 2017; Costa et al., 2014; Dawson & Ingrouille, 1995; de Fraine, 1916; Zhao, Song, Feng, Zhao and Liu, 2011). Glands are also present in genera that grow on rocky coasts in incipient soils exposed to deposition of airborne salt spray (as in *Limonium,* 'rocky species'; Caperta et al., 2014) and in *Armeria* species of coastal sand dunes (Arseni & Diez-Garretas, 2017). At least one *Goniolimon* species that thrives in steppe-like habitats (xerophilous pastures and rocky grounds) in hilly regions has salt glands on its leaves and stems (Buzurovic, Stevanovic, Niketic, Jakovljevic & Tomovic, 2013; Faraday & Thomson, 1986c; Waisel, 1972), although glands are not present in*Acantholimon* that colonized mountainous regions in dry habitats on gravelly and stony soils or on exposed rocks (Moharrek et al., 2019). The gland character is found in species ranging from perennial herbs with slightly fleshy leaves as in *Limonium* , to cushion-forming dwarf shrubs as in *Armeria* , coastal shrubs as in*Limoniastrum* and small trees as in *Aegialitis* (Tables 1 - 3), suggesting a plesiomorphic origin of this halophytic trait. Genetic studies in *A. maritima* provide strong evidence that metallicolous populations have been derived from the ancestral non-metallicolous populations repeatedly and independently in different geographical regions (Baumbach & Hellwig, 2007).

The evolutionary pathways leading to the 'salt glands syndrome' are not well understood. Our phylogenetic reconstruction revealed several main findings. First, salt glands are absent in salt-tolerant members of the Polygonaceae. Second, the most recent common ancestor of the family Plumbaginaceae likely possessed salt glands without vasculature; such glands are also present in the related non-carnivorous families, Frankeniaceae, and Tamaricaceae. Within the Plumbaginaceae, the subfamily *Limonioideae* presents more genera with salt glands and with a greater variety of structures than the subfamily *Plumbaginoideae* : the *Limonioideae*appear to have diversified more and more recently than the*Plumbaginoideae* . In the related carnivorous families (Figure 3), the multicellular glands can be sessile, stalked, or pitted, and may contain xylem and phloem; they have evolved a variety of different functions such as lures, generating trapping glue, providing a drowning mechanism and a digestive (enzyme) medium as well as the absorption of water vapour at night (Juniper, Robins, & Joel, 1989; Renner & Specht, 2013).

Notwithstanding the fact that salt glands are shared by members of Plumbaginaceae and related Tamaricaceae and Frankeniaceae, the divergence date of these families is difficult to estimate since there are no recorded reliable macrofossils of these families. Although there are microfossils from the Plumbaginaceae in the form of pollen grains, those of *Acantholimon* , *Armeria* , *Goniolimon* ,*Limonium* and *Psylliostachys* are difficult to distinguish and are referred to as *Limonium* or *Armeria* type (Baker, 1948, 1953; Skvarla & Nowicke, 1976; Weber-El Ghobary, 1984).Hence, estimating a divergence date using fossil pollen is not feasible. Based on molecular dating, it has been estimated that the split between the Polygonaceae and its sister group the Plumbaginaceae is relatively ancient, occurring about 90.7–125.0 Ma (million years ago, Schuster, Setaro, & Kron, 2013). Bell, Soltis & Soltis (2010) dated minimum and maximum ages for the Tamaricaceae at 60 – 58 Ma compared to the age of Plumbaginaceae at 27 – 57 Ma. Molecular analyses placed the split between *Plumbago* and *Limonium* between 18 – 16 Ma during the Miocene (Lledo et al., 2005), whereas the non-halophyte*Acantholimon sensu lato* diverged from its sister genus about 7.5 Ma, beginning between the Late Miocene and the Pliocene (Moharrek et al., 2019).

In the current revision, a Bayesian inference analysis of the Plumbaginaceae using chloroplast tRNA-Leu (*trnL*) gene and*trnL-trnF* intergenic spacer, shows that genera within the Plumbaginaceae are separated from sister salt-tolerant members of the Polygonaceae (*Polygonum*) (PP=1) and of Tamaricaceae (*Tamarix*), here used as outgroups (Figure 3; Supplementary Table 2). Within the Plumbaginaceae, two major clades represent the two monophyletic subfamilies *Plumbaginoideae* and *Limonioideae*(PP=1). Inside the *Plumbaginoideae* , *Plumbago* , a genus with halophytes with salt glands, is close to *Dyerophytum* , and sister of the *Ceratostigma* clade, which again has halophytes; together they form a clade with the monospecific *Plumbagella* . In the subfamily *Limonioideae* , the genus *Limonium,* with many halophytes and whose members present salt glands, seems to be the oldest genus within the subfamily. *Armeria* , with a halophytic species with glands and non-halophytic species also with glands form a clade together with the halophyte *Psylliostachys* . Another clade with halophytes with salt glands is that grouping *Limoniastrum* , sister to *Ceratolimon* , and close to *Myriolimun* and to*Ikonnikovia* . A clade with non-halophytes with PP=1 is formed by*Acantholimon* , *Dictyolimon* , *Bukiniczia* ,*Chaetolimon* , *Vassilczenkoa* , *Popoviolimon* and*Cephalorhizum* . To sum up, we might presume that the establishment of lineages with salt glands took place upon the split of between the Polygonaceae and its sister group Plumbaginaceae, but how it is that this separation took place is unclear.

Limonium species seemed to diversify at a fast pace during the Messinian (Lledo et al., 2005; this study), when the climatic conditions were drier than in previous ages and plants tolerated low water potentials (Valiente-Banuet, Rumebe, Verdu, & Callaway, 2006). This might have facilitated the diversification of species possessing physiological and anatomical adaptations that allowed colonization of harsh and arid conditions; for example, halophytes from saline steppes, coastal marshes and rocky cliffs, as in *Limonium* and xerophytes of stony soils and exposed rocks, as in *Acantholimon sensu lato* . A study in the *Limonium ovalifolium* (*L. lanceolatum, L. nydeggeri, L. ovalifolium*) and *L. binervosum* (*L. binervosum, L. dodartii, L* . *multiflorum*) complexes and in*L. algarvense* using occurrence records on surface lithology encompassing the entire distribution range of the species from each complex, all over the Atlantic coasts in Europe (Ireland, Britain, France, Iberian Peninsula) and in Morocco, revealed that these species typically thrive on sediments and deposits derived from sedimentary rocks like sandstones, conglomerates and mudstones and carbonate rocks as in limestone (Caperta et al., 2017). Whether salt glands evolved as an ecophysiological innovation to regulate Ca concentrations (discussed above) and adapted to secrete a range of ions (see Tables 2, 3 and 4) including those in saline environments is yet to be clarified. In*Armeria,* secretory glands are present both in halophytes and metalophytes found in non-saline sandy soils (e.g. *A. vulgaris,*synonym *A. maritima* ; Baumbach & Hellwig, 2007; Ruhland, 1915) and in sub-mountain meadows and mountain pastures (e.g. *A. canescens* ; Scassellati et al., 2016). Inland populations of *A. maritima* from sandy and heavy metal soils are salt-tolerant even if less resistant to irrigation with saline water (NaCl) than salt marsh populations (Kohl, 1997). In the related Tamaricaceae, both non-halophytes such as *Myricaria germanica* (Dorken et al., 2017; Table 4) and halophytes such as *Reaumurina* and*Tamarix* species possess salt glands (Abbruzzese et al., 2013; Salama et al., 1999; Weiglin & Winter, 1991; Zhang et al., 2018).

Evolutionary innovations have been correlated with paleopolyploidy (Edger et al., 2015; Soltis & Soltis, 2016; Vanneste, Baela, Maere & Van de Peer, 2014a; Vanneste, Maere & Van de Peer 2014b), such as the paleopolyploidy event at the base of Portulacineae associated with the evolution of succulence (Edwards & Ogburn, 2012; Nyffeler , Eggli, Ogburn & Edwards, 2008; Ogburn & Edwards, 2013), or along the branch leading to the Polygonaceae (Schuster et al., 2013) and the branch leading to carnivorous Droseraceae (Rivadavia, Kondo, Kato & Hasebe, 2003). A phylogenomic sampling based on transcriptomes, show the propensity of polyploidy throughout the evolutionary history of Caryophyllales, and within the non-core Caryophyllales, at least six paleopolyploidy events were inferred (Yang et al., 2018). However, direct connections between genome duplication and biological innovations in the Plumbaginaceae are not yet known.

4 Conclusions

The secretory structures that have evolved on the aerial parts of plants can be differentiated between those that secrete organic compounds and those that secrete inorganic ions. All these glands are multicellular structures in dicotyledonous plants, but their ancestry is uncertain. Although the structure of the glands that secrete organic materials is distinct from that of salt glands, it is unclear whether the former gave rise to the latter or if they arose independently. However, salt glands are less common than mucilage glands across the families of flowering plants. For the Plumbaginaceae, the majority of species that have demonstrable tolerance to salt, have functional salt glands (46 of the 54 species included in eHALOPH). Furthermore, the limited data available suggests that even excretion of a small proportion of uptake is critical for salt tolerance. Nonetheless, there are species within the family that have glands structurally similar to functional salt glands in other species, but with untested tolerance to salt as well as some salt-tolerant species where the presence of salt glands has yet to be investigated. The high proportion of halophytic species with salt glands within the family supports the view that ion-secreting glands are essential for salt tolerance in the Plumbaginaceae.

The question we have not been able to answer is whether ion-secreting glands evolved to secrete NaCl and generated salt tolerance or if ion-secreting glands evolve to regulate Ca^{2+}concentrations and this enabled secretion of Na^{+} and Cl^{-} and salt tolerance. Within the Plumbaginaceae there are species that do not grow on saline soils (e.g. *Armeria caespitosa* , and *A. canescens* , *Plumbago zeylanica, P. europaea, Limonium sogdianum* , *L. nudum* (gypsum soils).listed in Table 3 part B), but with glands that look like salt glands (so-called chalk glands). Their presence on non-saline soils gives some weight to the view that glands could have evolved to regulate Ca concentrations in the plant. However, owing to a lack of data we do not know if these species are tolerant of salt or if they have glands capable of secreting Na^{+} and Cl^{-}.

Most commonly, salt glands in the Plumbaginaceae are formed from 16 cells. Where genera have glands with more than 16 cells, like*Aegialitis* and *Limoniastrum* , they evolved independently as they form non-sister groups (Fig. 3). In the related Frankeniaceae and Tamaricaceae, families where salt tolerance is closely associated with the presence of salt glands, these structures are composed of just eight cells. The absence of a fossil record means that we cannot evaluate the possibility of a common ancestor of the Plumbaginaceae (16-celled glands) and related families of halophytes like non-core Caryophyllales, Frankeniaceae and Tamaricaceae (8-celled glands). The prevalence of salt glands in halophytic members of the Plumbaginaceae, Tamaricaceae and Frankeniaceae and the absence of salt glands in the related Polygonaceae, a family with few halophytes, suggest the evolution of these structure is an aid to salt tolerance. Salt-secreting glands seem to allow the successful colonization of saline habitats albeit maintained in species thriving in non-saline environments, reflecting the evolutionary independence of this halophytic trait. The presence of such glands also appears to have enabled the colonisation of soils high in heavy metals.

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AUTHOR CONTRIBUTIONS

TJF and ADC coordinated the study. GT did the anatomical analyses and ASR performed the molecular analysis. PGC tabulated information. ADC and TJF drafted the manuscript with the input of the co-authors.

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TABLE 1 . Genera assigned to the Plumbaginaceae providing information in terms of number of species, life form/syndrome, plant type and monophyly or phylogenetic relationships. Overall morphologies ("syndromes") are based on Moharrek et al. (2019) and plant type assignments following the database eHALOPH (https://www.sussex.ac.uk/affiliates/halophytes/index.php?content=plantStats). Acronyms: Ac - pulvinate to densely branched caespitose subshrub bearing mostly spike-like or capitate inflorescences, with linear rigid acuminate leaves (*Acantholimon*); Ae - shrubs or small trees (*Aegialitis* ; mangroves); Ar – herbaceous perennial herbs and cushion-forming dwarf shrubs (*Armeria*). Ce – dwarf shrubs (*Ceratolimon*); Dy - shrubs or sub- shrubs (*Dyerophytum*); L - perennial herbs with thick rootstock, rosulate, and slightly fleshy basal leaves, and flowering stems bearing paniculate inflorescences (*Limonium*); Lw - arborescent habit, woody stems up to 3 m (*Limonium dendroides*). s.l. *sensu lato.* *POWO Plants Of the World Online http://www.plantsoftheworldonline.org/. $ includes species listed in eHALOPH and non-halophytic species listed in Table 2B and 3B.

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
A *Limonium* species that are salt tolerant - in eHALOPH	**A *Limonium* species that are salt tolerant - in eHALOPH**	**A *Limonium* species that are salt tolerant - in eHALOPH**	**A *Limonium* species that are salt tolerant - in eHALOPH**		
aureum	12 + 4 collecting cells		12		Ni et al. 2012
axillare	4 rings + 4 basal cells		16		Salama et al. 1999
axillare	Sunken				Akhani et al. 2013
bellidifolium	8 gland cells + 4 subsidiary cells	9	12		de Fraine 1916
bicolor	4 each of outer cup cells, inner cup cells, accessory cells and secretory cells		16	Na, K, Mg. Ca, Cl, SO_4 NO_3 Na: 40-225 pmol/gland/h Cl: 48-226 pmol/gland/h	Feng et al. 2014
bicolor		12-16		Na: 110 pmol/gland/h	Leng, Yuan, Dong, Wang & Wang 2018
binervosum	Not recorded	10			de Fraine 1916; Grigore & Toma 2010;

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
brasiliense	4 secretory cells, 4 collecting cells and remaining accessory cells		16		Bastos, Perazzolo & Gorgen, 1993
brasiliense	12+5 to 8 cells				Gancedo et al. 2018
californicum	4 rings		16		Faraday & Thomson 1986c
caspium	Present but no detail				Akhani et al. 2013
delicatulum	12 cells in 3 rings+ 4 collecting cells	20-29	16		Batanouny et al. 1992; Blazquez 1985
franchetii	4 each of secreting cells, internal and external 'goblet' cells collecting cells	8	20?		Xin et al. 2012
girardianum	Present but no detail				Al Hassan et al. 2017
gmelinii	4 rings	7	16		Faraday & Thomson 1986c; Ruhland, 1915
gmelinii	At surface				Akhani et al. 2013
gmelinii		12			Zhou et al. 2007
gmelinii		10-12		Na$^{:}$ 290 pmol/gland/h	Leng et al. 2018
gmelinii	quadrant		16		Ruhland 1915
gmelinii		6-8			Daraban et al. 2013
gmelinii		11			Zorić, Anačkov, Karanović, & Luković 2013
graecum	Not recorded	10			Waisel 1972
iranicum	Deeply sunken				Akhani et al. 2013
lanceolatum			16		A Caperta Pers. Comm.
lobatum	At surface				Akhani et al. 2013
multiflorum	At surface		16		this study

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
narbonense	At surface		16		Al Hassan et al. 2017 this study
nudum	At surface				Akhani et al. 2013
nydeggeri	At surface		16		this study
oleifolium (syn. = *virgatum*)	Present but no detail				Andres 1989; Colombo 2002
otolepis		9			Zhou et al. 2007
otolepis	At suface	12			Akhani et al. 2013
ovalifolium	Present but no detail				Denaeyer-De Smet 1970
pectinatum			16	Na: 0.2 nmols/gfwt/s	Jung and Luttge 1980; Morales Olmos, Torrecillas, Sanchez-Blanco & Alarcon 2001
perezii	4 quadrants		16		Faraday and Thomson 1986c
perezii				Na, K, Ca, Cl: 20-410 pmol/gland/h	Faraday & Thomson 1986b
platyphyllum	Cross sections**				Vassilyev and Stepanova 1990
pruinosum	16 secretory cells and 4 collecting cells	16	16		Salama et al. 1999; Waisel 1972
reniforme	At surface	12-16			Akhani et al. 2013
santapolense	Present but no detail				Al Hassan et al. 2017
sinense	16 plus 4 collecting cells cells		20		Xin et al. 2011
sinuatum	4 quadrants	10	16		Faraday & Thomson 1986c; Salama et al. 1999; Waisel 1972
stocksii	Sunken				Akhani et al. 2013

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
suffruticosum	Deeply sunken				Akhani et al. 2013
vulgare	16 cells in 4 circles with 4 basal cells		20		Faraday & Thomson 1986c; Salama et al. 1999;
vulgare		30			Rozema et al. 1981
B Limonium **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**		
aegusae			16		Colombo 2002
albidum			16		Colombo 2002; Colombo & Trapani 1992
*bocconei**			12		Colombo 2002
carnosum	Sunken				Akhani et al. 2013
intermedium			16		Colombo & Trapani 1992
dictyophorum (syn. *L. anfractum*)		7			Zorić et al. 2013
lojaconi			12		Colombo 2002
lopadusanum			16		Colombo & Trapani 1992
palmyrense	Deeply sunken				Weiglin & Winter 1991
perfoliatum	At surface				Akhani et al. 2013
*pignanttii **			12		Colombo 2002
ponzoi			16		Colombo 2002
serpentinicum	Present but no detail				Pino Perez, Javier Silva-Pando & Pino Perez 2016
sogdianum	At surface				Akhani et al. 2013

TABLE 2. Genera and species within the Plumbaginaceae (from Plants Of the World Online, POWO) reported to have salt glands (from eHALOPH), their salt tolerance as indicated by inclusion in the database eHALOPH, the position of the glands on the leaf, their frequency and cellular makeup, together with the main elements secreted and rates of efflux. Representatives of the genus *Limonium* are presented separately, in Table 3.

*The presence of glands is recorded with a in column 3; a ? indicates the presence of functional salt glands is uncertain or not known. Entries in bold text relate to genera, rather than individual species.

Genus and species	**Species with salt glands***	**Gland structure**	**No./ mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Reference & notes**
A Species in eHALOPH							
Aegialitis R.Br.	**2**						
annulata		3 rings 8 cells	9	24	Cl 11-62 pmol/cm^2/s	0.79	Atkinson et al. 1967
annulata		8 rings 5 cells		40			Faraday & Thomson 1986c
annulata					Na, Cl, K, Ca	1.1	Faraday & Thomson 1986a
rotundifolia							Seshavatharam & Srivalli, 1989; Yuan, Chen, Leng & Wang 2013
rotundifolia		8 cells per ring	?				Das 2002
rotundifolia				24			Wang Bao Shan, personal communicatio
Armeria (DC.) Willd.	**1**						
maritima					HCO_3, Cl, Na, K, Ca, Mg	0.96	Baumeister & Ziffus 1981
maritima			6		Na, K, Ca Na: 0.7 pmol/gland/h		Rozema et al. 1981
maritima		4 quadrants		16	NaCl		Ruhland 1915 (synonym *A. Vulgaris*)
Ceratostigma Bunge	**1**						

Genus and species	Species with salt glands*	Gland structure	No./ mm^2	Number of cells	Main elements secreted and efflux	Na / Cl	Reference & notes
plumbaginoides					Ca		Borchert 1989
plumbaginoides		4 quadrants		16	Mg, Ca		Balsamo & Thomson 1996; Faraday & Thomson 1986a; Faraday & Thomson 1986c
Goniolimon *Boiss.*	**1**						
tataricum		4 quadrants	31	16			Faraday & Thomson 1986c (mispelled as *tatericum)*; Waisel 1972
Limoniastrum Heist. ex Fabr.	**2**						
guyonianum				32	Mg & Ca or Na & Cl		Zouhaier et al. 2015; Mg & Ca when no NaCl in soil
monopetalum				16	Na, Cl	0.69	Salama et al. 1999
monopetalum					K 0.6-2.1 µmol/g fwt		Ramadan 1997
monopetalum		12+4 cells	22	16			Batanouny & Abo 1977
monopetalum			12		$CaCO_3$, NaCl		Batanouny & Abo 1977
Limonium Mill. See Table 3	**40**						

Genus and species	**Species with salt glands***	**Gland structure**	**No./ mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Reference & notes**
Myriolimon	**2**						
diffusum	?						
ferulaceum	?						
Plumbago Tourn. ex L.	**1**						
auriculata				16?	Mg, Ca		Faraday & Thomson 1986a; Faraday & Thomson 1986c
auriculata			25				Waisel 1972
Psylliostachys	1						
spicatus	?						
B Species NOT in eHALOPH	**B Species NOT in eHALOPH**						
Acantholimon Boiss							
androsaceum		8 cells + 4 subsidiary cells		12			Bokhari 1971
glumaceum		8 cells + 4 subsidiary cells		12			Bokhari 1971
lycopodioides		8 cells + 4 subsidiary cells		12			Bokhari 1971
Armeria (DC.) Willd.							
maritima ssp. *halleri*					K Ca Cu Zn		Neumann et al. 1995
maritima ssp. *halleri*					S, Zn		Heumann 2002
canescens		4 quadrants	2-16	16			Scassellati et al. 2016
Plumbago Tourn. ex L.							

Genus and species	Species with salt glands*	Gland structure	No./ mm^2	Number of cells	Main elements secreted and efflux	Na / Cl	Reference & notes
zeylanica				8			Sudhakaran 2019

TABLE 3. Glandular attributes for species of *Limonium*(A), reported to be salt tolerant (are included in eHALOPH) and (B) without proven salt tolerance.

Species (synonym in parentheses)	Gland structure	Number of glands / mm^2	Number of cells	Main elements secreted and efflux	Reference
A *Limonium* species that are salt tolerant - in eHALOPH	**A *Limonium* species that are salt tolerant - in eHALOPH**	**A *Limonium* species that are salt tolerant - in eHALOPH**	**A *Limonium* species that are salt tolerant - in eHALOPH**		
aureum	12 + 4 collecting cells		12		Ni et al. 2012
axillare	4 rings + 4 basal cells		16		Salama et al. 1999
axillare	Sunken				Akhani et al. 2013
bellidifolium	8 gland cells + 4 subsidiary cells	9	12		de Fraine 1916
bicolor	4 each of outer cup cells, inner cup cells, accessory cells and secretory cells		16	Na, K, Mg. Ca, Cl, SO_4 NO_3 Na: 40-225 pmol/gland/h Cl: 48-226 pmol/gland/h	Feng et al. 2014
bicolor		12-16		Na: 110 pmol/gland/h	Leng, Yuan, Dong, Wang & Wang 2018
binervosum	Not recorded	10			de Fraine 1916; Grigore & Toma 2010;
brasiliense	4 secretory cells, 4 collecting cells and remaining accessory cells		16		Bastos, Perazzolo & Gorgen, 1993
brasiliense	12+5 to 8 cells				Gancedo et al. 2018
californicum	4 rings		16		Faraday & Thomson 1986c

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
caspium	Present but no detail				Akhani et al. 2013
delicatulum	12 cells in 3 rings+ 4 collecting cells	20-29	16		Batanouny et al. 1992; Blazquez 1985
franchetii	4 each of secreting cells, internal and external 'goblet' cells collecting cells	8	20?		Xin et al. 2012
girardianum	Present but no detail				Al Hassan et al. 2017
gmelinii	4 rings	7	16		Faraday & Thomson 1986c; Ruhland, 1915
gmelinii	At surface				Akhani et al. 2013
gmelinii		12			Zhou et al. 2007
gmelinii		10-12		Na⁝ 290 pmol/gland/h	Leng et al. 2018
gmelinii	quadrant		16		Ruhland 1915
gmelinii		6-8			Daraban et al. 2013
gmelinii		11			Zorić, Anačkov, Karanović, & Luković 2013
graecum	Not recorded	10			Waisel 1972
iranicum	Deeply sunken				Akhani et al. 2013
lanceolatum			16		A Caperta Pers. Comm.
lobatum	At surface				Akhani et al. 2013
multiflorum	At surface		16		this study
narbonense	At surface		16		Al Hassan et al. 2017 this study
nudum	At surface				Akhani et al. 2013
nydeggeri	At surface		16		this study
oleifolium (syn. = *virgatum*)	Present but no detail				Andres 1989; Colombo 2002
otolepis		9			Zhou et al. 2007

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
otolepis	At suface	12			Akhani et al. 2013
ovalifolium	Present but no detail				Denaeyer-De Smet 1970
pectinatum			16	Na: 0.2 nmols/gfwt/s	Jung and Luttge 1980; Morales Olmos, Torrecillas, Sanchez-Blanco & Alarcon 2001
perezii	4 quadrants		16		Faraday and Thomson 1986c
perezii				Na, K, Ca, Cl: 20-410 pmol/gland/h	Faraday & Thomson 1986b
platyphyllum	Cross sections**				Vassilyev and Stepanova 1990
pruinosum	16 secretory cells and 4 collecting cells	16	16		Salama et al. 1999; Waisel 1972
reniforme	At surface	12-16			Akhani et al. 2013
santapolense	Present but no detail				Al Hassan et al. 2017
sinense	16 plus 4 collecting cells cells		20		Xin et al. 2011
sinuatum	4 quadrants	10	16		Faraday & Thomson 1986c; Salama et al. 1999; Waisel 1972
stocksii	Sunken				Akhani et al. 2013
suffruticosum	Deeply sunken				Akhani et al. 2013
vulgare	16 cells in 4 circles with 4 basal cells		20		Faraday & Thomson 1986c; Salama et al. 1999;
vulgare		30			Rozema et al. 1981

Species (synonym in parentheses)	**Gland structure**	**Number of glands / mm^2**	**Number of cells**	**Main elements secreted and efflux**	**Reference**
B Limonium **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**	*B Limonium* **species that are not in eHALOPH**		
aegusae			16		Colombo 2002
albidum			16		Colombo 2002; Colombo & Trapani 1992
*bocconei**			12		Colombo 2002
carnosum	Sunken				Akhani et al. 2013
intermedium			16		Colombo & Trapani 1992
dictyophorum (syn. *L. anfractum*)		7			Zorić et al. 2013
lojaconi			12		Colombo 2002
lopadusanum			16		Colombo & Trapani 1992
palmyrense	Deeply sunken				Weiglin & Winter 1991
perfoliatum	At surface				Akhani et al. 2013
*pignanttii **			12		Colombo 2002
ponzoi			16		Colombo 2002
serpentinicum	Present but no detail				Pino Perez, Javier Silva-Pando & Pino Perez 2016
sogdianum	At surface				Akhani et al. 2013

TABLE 4 . Genera and species within the Tamaricaceae and Frankeniaceae (from Plants of the World Online, POWO) reported to have salt glands (from eHALOPH; marked with a), their salt tolerance as indicated by inclusion in the database eHALOPH (also marked with a), the position of the glands on the leaf, their frequency and cellular makeup, together with the main elements secreted and rates of efflux. Data in bold text relate to genera rather than species.

Genus and species (synonym in parentheses)	**Number of species POWO**	**Species In eHALOPH**	**Species with glands**	**Gland structure**	**Number/mm^2**	**No of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Re &**
Frankeniaceae	**78**	**10**	**11**						
Frankenia	**78**	**10**	**11**						
capitata									Gr & To 201
corymbosa (*floribunda*)									Gr & To 201
ericifolia									Gr & To 201
hirsuta (*revoluta*)				Sunken 6+2		8			Gr & To 201 Sal et 199 We & Wi 199
juniperoides				Sunken					Pe Cu & Ca 201
laevis									An 198 Gr ore To 201
palmeri									Gr & To 201
patagonica									Gr & To 201

Genus and species (synonym in parentheses)	Number of species POWO	Species In eHALOPH	Species with glands	Gland structure	Number/mm^2	No of cells	Main elements secreted and efflux	Na / Cl	Re &
pauciflora				6+2		8			Ol 19
pulverulenta									An 198 Cu et 20 Gr ore To 20
salina (*grandifolia*)									Gr & To 20
thymifolia (*reuteri*)							Na, K, Ca, SO_4		De De Sm 19 Gr ore To 20
triandra			?						
Tamaricaceae	**112**	**40**	**29**						
Myricaria Desv.	**14**	**0**	**3**						
bracteata					62				Gu & Mu 198
germanica						8	$CaSO_4$ $CaCO_3$		Dö et 20
germanica					56				Gu & Mu 198
Reaumuria L.	**25**	**5**	**3**						
alternifolia				6+2		8			We & Wi 199

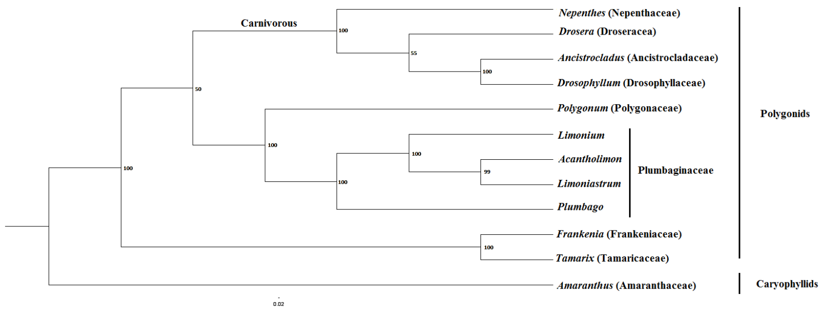
Genus and species (synonym in parentheses)	Number of species POWO	Species In eHALOPH	Species with glands	Gland structure	Number/mm^2	No of cells	Main elements secreted and efflux	Na / Cl	Re &
hirtella							Cl, Na, K, Ca Mg SO_4		Ra 199
negevensis			?						
songarica			?						
trigyna							Cl, Na, K, Ca Mg SO_4 Cl 8 nmol/g/s		Xu Wa 200
Tamarix L.	**72**	**29**	**23**						
africana					26				Ab et 201
africana							Al, Ca, Fe, K, Mg, Mn, Na, Zn		Sa et 201
albiflonum				Raised					
amplexicaulis				6+2		8	Cl, Na, K, Ca Mg SO_4		Sal et 199
androssowii				Raised					Zh et 201
aphylla					43, 23				Gu & Mu 198 Wa 197
aphylla				6+2		8	Cl, Na, K, Ca Mg SO_4		Sal et 199
aphylla							Mg, Ca, Na, K		Th et 196

Genus and species (synonym in parentheses)	**Number of species POWO**	**Species In eHALOPH**	**Species with glands**	**Gland structure**	**Number/mm^2**	**No of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Re &**
aphylla							Na, K, Mg, Ca, Cl, NO3, HCO3, and SO4 B, Mn, Cu, Zn, and Mo		Be 197
aphylla						8			Th & 196
aphylla							Na, Cl, Ca, K and Mg		Ha & Wa 198
arborea			?						
arceuthoides				Raised					Zh et 20
aucheriana			?						
austromongolica				Raised					Zh et 20
boveana			?						
canariensis			?						
chinensis				Sunken					Zh et 20
dioica					48				Gu & Mu 198
elongata				Sunken					Zh et 20

Genus and species (synonym in parentheses)	**Number of species POWO**	**Species In eHALOPH**	**Species with glands**	**Gland structure**	**Number/mm^2**	**No of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Re &**
gallica				Sessile	36	8	K, Ca, Mg and Na		Ab et 20 Ba et 19 De De Sm 19
ganuensis				Sunken					Zh et 20
gracilis					60				Gu & Mu 19
gracilis				Sunken					Zh et 20
hispida				Raised or sunken					Zh et 20
hohenackeri				Raised					Zh et 20
indica			?						
jordanis					22				Wa 19
karelinii				Sunken					Zh et 20
korolkowi				Raised					Zh et 20
laxa				Raised					Zh et 20
leptostachya				Sunken					Zh et 20
mascatensis			?						
meyeri			?						

Genus and species (synonym in parentheses)	Number of species POWO	Species In eHALOPH	Species with glands	Gland structure	Number/mm^2	No of cells	Main elements secreted and efflux	Na / Cl	Re &
nilotica				6+2		8			Sal et 199
nilotica					24				Gu & Mu 198
parviflora									
passerinoides				6+2		8			Sal et 199
polystachya			?						
ramosissima					12				Gu & Mu 198
ramosissima				Raised					Zh et 201
ramosissima							Na and K		Ma al. 201
ramosissima							Ca, K, Mg, Mn and Na		Im Ac & ma 201
ramosissima							K, Ca, Cu, Mn, B, Al, Si, Ti, Mo, Sr and Ba Kleinkopf and Wallace 1974		
smyrnensis					25				Gu & Mu 198

Genus and species (synonym in parentheses)	**Number of species POWO**	**Species In eHALOPH**	**Species with glands**	**Gland structure**	**Number/mm^2**	**No of cells**	**Main elements secreted and efflux**	**Na / Cl**	**Re &**
smyrnensis							Ca, Pb		Ma Ka Pa pac tor & Ka aki 200
taklamakensis				Raised					Zh et 201
tarimensis				Raised					Zh et 201
tetragyna			?		19				Wa 197
troupii					37				Gu & Mu 198
usneoides						6+2	Na, Mg, Al, S, Cl, K Ca		Wi My coo We byo 201



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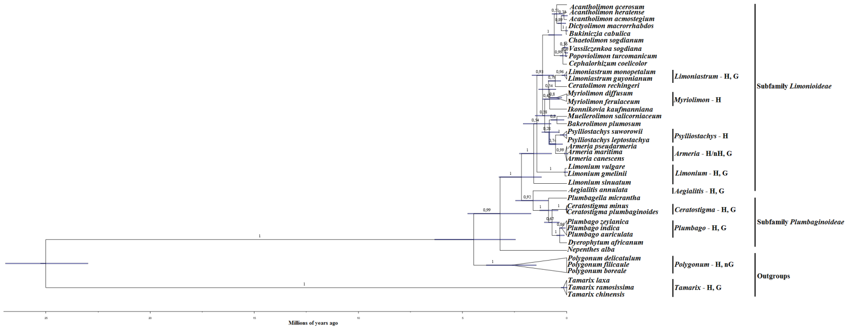

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