Studies on the foliage of *Myricaria germanica* (Tamaricaceae) and their evolutionary and ecological implications

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Abstract

Key message Ancestral halophytic traits such as salt glands and leaf deciduousness have facilitated the adaptation of *Myricaria germanica* to non-saline calcium-rich soils.

Abstract *Myricaria germanica* is a scale-leaved, deciduous shrub from the Tamaricaceae, a salt gland family of halophytes, xerophytes and rheophytes usually from xeric, saline areas. Atypically, the genus *Myricaria* is usually from mesic, non-saline areas. In this study, we describe the shoot morphology and anatomy of seedlings and adult plants of *Myricaria germanica* in order to explore its adaptation to the environment. It is a species of montane to sub-alpine-flooded riverine areas on non-saline limestone and dolomite soils. The adult leaves show strong leaf reduction but no other significant xeromorphic or scleromorphic features. While the salt glands of most Tamaricaceae secrete NaCl, our SEM EDS investigations show that *Myricaria germanica* secretes large amounts of Ca and Mg, probably as CaSO₄ and as Mg-containing CaCO₃, rather than NaCl. This suggests that the evolution of salt glands in a halophytic ancestor may have been an enabling trait that facilitated the adaptation of *Myricaria germanica* to non-saline

Ca-rich soils. Because leaf deciduousness can also be an adaptation for reduction of plant NaCl content, the same may apply to this *Myricaria germanica* trait. Similarly, leaf reduction can evolve as a response to osmotic stress in saline areas. Its persistence in *Myricaria germanica* may no longer have any adaptational significance. Our work highlights the dichotomy of the stress-tolerant family Tamaricaceae into two types of stressful habitats, one lowland (e.g. *Tamarix*) and one montane to alpine (*Myricaria*). Similar range fragmentation is known in Mediterranean taxa like *Armeria* and *Astragalus*.

Keywords Calcium secretion · Deciduousness · Dimorphic leaves · Leaf glands · *Myricaria*

Introduction

Reduction in leaf size is widely distributed among extant seed plants; the stage prior to leaflessness results in small, scale-like leaves closely appressed to the stem. This stage is especially common in gymnosperms and is frequently called “cupressoid foliage” (Krüssmann 1983; Salmon 1986; Farjon 2005, 2010a, b; Eckenwalder 2009; Dörken 2013, 2014). It can also be found in several angiospermous groups (e.g. Krüssmann 1976, 1977, 1978; Kubitzki et al. 1993; Kubitzki 2004). Such leaf reduction is widely recognized as an adaptation to reduce water loss via the lamina in dry conditions (e.g. Blum and Arkin 1984; Blum 1996; Bosabalidis and Kofidis 2002; Seidling et al. 2012), but can also be correlated with stress from both low temperatures and water deficit in subalpine and alpine areas (e.g. Korner 2003; Parsons 2010). As well, it can be correlated with low soil phosphorus levels along with other scleromorphic features (e.g. Loveless 1961, 1962; Beadle 1966;

Communicated by K. Masaka.

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Seddon 1974; Hill and Merrifield 1993; Hill 1998; Salleo and Nardini 2000; Dörken and Parsons 2016) as is typical for many Ericaceae (e.g. Düll and Kutzelnigg 2011) and for some gymnosperms (Dörken and Jagel 2014). For the gymnosperm species studied showing strongly reduced scale leaves when mature, the earliest seedling leaves are quite different and needle-like (e.g. Feustel 1921; de; Laubenfels 1953; Langner 1963; Napp-Zinn 1966; Dörken 2013; Dörken and Parsons 2016). This change in the leaf type involves species-specific ontogenetic changes which are not well understood, especially in angiosperms.

This study is one of a series where we explore these subjects by describing the shoot morphology and anatomy of seedlings and adult plants. So far, we have published work on species of the gymnosperms *Thuja* and *Thujaopsis* (Dörken 2013), *Dacrycarpus* and *Dacrydium* (Dörken and Parsons 2016) and on species of the angiosperm *Casuarina* (Dörken and Parsons 2017, in press); all these are evergreens. This time, in contrast, we are dealing with *Myricaria germanica* (L.) Desv., a deciduous species from the Tamaricaceae with strongly reduced imbricate leaves, despite a habitat almost never suffering from water deficit. Most members of this family are representing characteristic species of Mediterranean areas and the central Asian and African steppes and deserts (Brunswik 1920; Hegi 1975; Heywood 1982). The species of this family are usually described as halophytes, xerophytes or with a strong connection to riverine flooding ('rheophytes') (Gaskin 2003; Mabblerley 2008). However, the known adult root systems are deep (up to 30 m depth), and often tap saline or brackish water at depth (Gaskin 2003; Carlquist 2010), so that Carlquist (2010) describes Tamaricaceae as a family of halohydrophytes, which can secrete an excess of NaCl via external 'leaf salt glands'. Such glands are also developed in leaves of *Myricaria germanica*, despite the fact that the soils the plants inhabit are always non-saline.

Among the taxa of the salt-secreting Tamaricaceae family, the leaf deciduousness of some species may function primarily as an important adaptation to reduce tissue NaCl levels, as is known for some species of *Tamarix* (Gaskin 2003) and in various mangrove families (Saenger 2002). In this study, we also explore this subject and consider whether in the case of *Myricaria germanica*, there is evidence of a switch from Na secretion to a possible Ca secretion, given its clear preference for non-saline, but calcareous soils, given that most references record *Myricaria germanica* exclusively from highly calcareous soils (Hegi 1975; Petutschnig 1994) or from limestone and dolomite (Kiermeier 1993; Müller 1988, 1991, 1993; Müller and Bürger 1990). Only Bachmann (1997) described exceptionally a record on silicate gravels at a single small site. Among species growing on highly calcareous soils, it is not uncommon that the excess of Ca in the plant tissue

is secreted via the leaves, as is carried out by the laminar hydathodes of several *Saxifraga* species (Saxifragales, Angiospermae) (e.g. Schmidt 1930; Köhlein 1980; Webb and Gornall 1989; Conti et al. 1999).

To explain the ecological and evolutionary forces leading to leaf reduction and deciduousness in *Myricaria germanica*, leaves in different ontogenetic stages will be investigated to document the morpho-anatomical changes that occur between cotyledons, primary leaves, juvenile leaves and adult leaves. Also, those findings will be used to explore any important differences between the deciduous species *Myricaria germanica* and the evergreen species studied previously. Finally, the *Myricaria germanica* leaf gland secretions will be analysed to determine the presence and the relative amounts of the different elements that are secreted via the leaf glands, and the significance of those data will be discussed in the context of leaf reduction and deciduousness.

Materials and methods

Material

Seeds of *Myricaria germanica* (L.) Desv. (Tamaricaceae) were obtained from the Eberhard Karls-Universität Tübingen, Germany (IPEN No. xx-0-TUEB-2694). They were germinated and grown in a temperate glass-house with long day conditions in the Botanic Garden Konstanz (Germany). Seeds were sown in a mixture of compost and vermiculite (5:1). The glasshouse temperature ranged from 25 °C (day) to 10 °C (night). Material from mature individuals was collected with special permission from the "Amt für Natur, Jagd und Fischerei" Kanton St. Gallen, Switzerland on a gravel bank in the alpine Rhine near Vilters-Wangs (Kanton St. Gallen, Switzerland).

Methods

Freshly collected material was photographed and then fixed in FAA (100 ml FAA=90 ml 70% ethanol+5 ml acetic acid 96%+5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The leaf anatomy was studied from serial sections using the classical paraffin technique and subsequent astrablue/safranin staining (Gerlach 1984). Macrophotography was accomplished using a digital camera (Canon PowerShot IS2) and microphotography with a digital microscope (Keyence VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright-field illumination (Keyence VH-S5).

SEM was done with a Zeiss Auriga. For SEM investigations, freshly collected material was fixed in FAA, later dehydrated in FDA (formaldehyde-dimethyl-acetal),

critical-point dried (CPD 030, Balzer) and sputtered with gold–palladium, thickness 5 nm (SCD 030, Baltec Sputter-Coater).

For SEM EDS elemental analysis, leaf samples were air-dried before small areas containing glands were cut out and attached with conductive tape to SEM sample mounts. The sample was then inserted into the preparation chamber (Oxford Instruments 1500CT, Oxford Instruments, High Wycombe, Buckinghamshire, UK) attached to a SEM (JEOL JSM 840A, JEOL, Tokyo, Japan) and evaporatively coated with Al (10 nm). The sample was transferred into the SEM specimen chamber and analysed at 15 kV and a beam current of 2×10^{-10} A. X-ray microanalysis was carried out with an Aztec analyser and a 150 mm² X-MAX detector (Oxford Instruments, High Wycombe, Buckinghamshire, UK) with a take-off angle of 40°. Qualitative elemental images (maps) were obtained from selected regions of the sample. Elemental images are shown in terms of counts per second (cps) corrected for background, peak overlaps and pulse pile-up events. X-ray spectra were obtained from small areas on elemental images by summing the spectral data for each pixel. Samples of analytical grade, calcium sulphate (CaSO₄·2H₂O) crystals were attached to sample mounts and treated in the same manner as the leaf samples. Quantitative data were obtained from horizontal faces of leaf crystals and CaSO₄ crystals using the Aztec XPP software.

Soil was sampled from a depth of 0–120 cm from a stand of *Myricaria germanica* on a gravel bank in the alpine Rhine near Vilters-Wangs (Kanton St. Gallen, Switzerland). It was analysed by the GeoNatura-Laboratory (Filderstadt, Germany) after being air-dried and sieved to 2 mm. The investigations were performed according to the accepted methods of the “Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VD-LUFA)”.

To test for the presence of carbonates, leaf gland tissue was immersed in 1 M HCl to see if the secretions effervesced as CO₂ was released.

Results

Soil properties

The geological map shows that the dominant rock types in the mountains surrounding the sampling location are limestone and dolomite (Schälchli et al. 2001). Those are the dominating types of rocks forming the alluvium, which our investigated *Myricaria germanica* specimen inhabits. Apart from limestone and dolomite, argillite, breccia, gneiss, granite, marl, radiolarite, sandstone and verrucano also occur quite frequently. The samples were from

a typical alpine sandy alluvium where large gravel stones occur in the topsoil. The groundwater level was at a depth of 1.2 m. The analyses show that the soil is alkaline and very low in organic matter and nearly all of the nutrients analysed (Table 1). Loss of weight of soil samples on thorough digestion with HCl showed that approximate carbonate contents ranged from 40% at the soil surface to 60% at a depth of 1.2 m, which was ground water level.

Leaf morphology and leaf anatomy

Cotyledons

Seedlings show a well-developed hypocotyl. The two linear cotyledons are flattened structures (Fig. 1a) 2.1–2.4 mm long and 0.5–0.6 mm wide. Glands and secretions are lacking in the cotyledons. Stomata show an amphistomatic distribution, with the majority developed on the adaxial side. They are slightly sunken in the epidermis. The epidermal cells of both leaf sides are equal in size and shape. The epidermis is covered with a weakly developed cuticle. The mesophyll, consisting of more or less isodiametric cells, shows large intercellular spaces. There is no differentiation between palisade and spongy parenchyma. The leaf is supplied with a single vascular bundle that branches several times. Xylem is located towards the sunny adaxial and phloem towards the shaded abaxial surface. There is no distinct bundle sheath (Fig. 1b).

Table 1 Analyses of the soil from 0 to 120 cm depth/from a stand of *Myricaria germanica* at a gravel bank in the alpine Rhine near Vilters-Wangs (Kanton St. Gallen, Switzerland)

General features		Fertility/notes	Method
Texture	Sandy	–	D 2.1
pH	7.8	Alkaline	A 5.1.1
Organic matter (%)	0.104	Low	Calculated
Organic C (mg kg ⁻¹)	603	–	A 2.2.5
C:N ratio	2.9	–	Calculated
Macronutrients			
Total N (mg kg ⁻¹)	208	Very low	A 2.2.5
P ₂ O ₅ (mg kg ⁻¹)	0.0216	Very low	A 13.1.1
K ₂ O (mg kg ⁻¹)	7.14	Very low	A 13.1.1
MgO (mg kg ⁻¹)	30.2	Very low	A 13.1.1
Trace elements			
B (mg kg ⁻¹)	0.0212	Very low	A 13.1.1
Cu (mg kg ⁻¹)	1.52	Adequate	A 13.1.1
Mn (mg kg ⁻¹)	15.3	Very low	A 13.1.1
Fe (mg kg ⁻¹)	29.0	Low	A 13.1.1
Zn (mg kg ⁻¹)	0.65	Very low	A 13.1.1

The methods are those of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

◀**Fig. 1** *Myricaria germanica*, morphology and anatomy of leaves in different ontogenetic stages; **a–d** juvenile leaves; **a** seedling; **b** cotyledon (cross-section) **c** primary leaf (cross-section); **d** young subsequent leaf (cross-section); **e–f** mature leaves; **e** leaves at a mature branch are imbricate, abaxial leaf surface with several secretions (arrows); **f** mature leaf (cross-section) (*C* cuticle, *E* epidermis, *G* gland, *M* mesophyll, *SP* spongy parenchyma, *P* phloem, *PP* palisade parenchyma, *S* stoma, *X* xylem)

Primary leaves

The epicotyl is about 1.1–1.9 mm long. The primary leaves are flattened, linear, 1.7–2.1 mm long and 0.3–0.4 mm wide. The adaxial surface is orientated towards the shoot axis (Fig. 1a). The leaves lack glands and secretions. They are amphistomatic with the highest density of stomata on the adaxial side. Stomata are only weakly sunken in the epidermis. There is no difference between the cells of the upper and lower epidermis. A weakly developed cuticle covers the epidermis. The mesophyll is monomorphic and shows large intercellular spaces. Primary leaves are supplied with a single vascular bundle strand which branches only weakly a few times. There is no distinct bundle sheath. Xylem is placed towards the shaded adaxial and phloem towards the light-exposed abaxial surface (Fig. 1c).

Subsequent juvenile leaves

The alternating subsequent juvenile leaves are imbricate and slightly adpressed to the shoot axis. They are linear to slightly oblong linear, 1.7–2.4 mm long and 0.3–0.5 mm wide. There are a few glands and some whitish secretions, developed exclusively on the abaxial side of the leaves (Fig. 1a, d). The leaves are hypostomatic, with stomata slightly sunken in the epidermis. The epidermal cells of the sun-exposed abaxial side are slightly larger than those of the shaded adaxial side. The epidermis is covered with a weakly developed cuticle. The mesophyll is monomorphic and shows large intercellular spaces. A distinct vascular bundle sheath is absent. Xylem is placed towards the shaded adaxial side and phloem towards the sunny abaxial side (Fig. 1d).

Adult leaves

The alternating leaves developed at branches of mature individuals are imbricate and strongly adpressed to the shoot axis. The abaxial side of the leaves is the light-exposed one, the adaxial side the shaded one. The leaves are linear to oblong-linear, 2.4–4.8 mm long and 1.1–1.8 mm wide. Only on the abaxial side of the leaves several multicellular glands, slightly sunken in the epidermis, are developed; they are absent on the adaxial surface (Figs. 1f, 2a). There is no connection between the glands

and a vascular bundle. The distal leaves at the shoot axis show the highest density of glands. Their secretions are visible as white dots (Fig. 1e). The leaves are epistomatic. Stomata are exclusively developed on the shaded adaxial side of the leaf. They are sunken in the epidermis. The epidermal cells are papillae-shaped (Fig. 2b). Those of the sun-exposed abaxial surface are about double the size of those of the shaded adaxial surface. A well-developed cuticle covers the epidermis. The cuticle waxes are curled spirals (Fig. 2c). The mesophyll is dimorphic, with palisade parenchyma located towards the abaxial and spongy parenchyma towards the adaxial side. A single vascular bundle strand, which branches several times, supplies the leaf. There is no distinct bundle sheath. Xylem is located towards the adaxial and phloem towards the abaxial side.

Glands and secretions on the surface of adult leaves

Glands and secretions are always absent in the cotyledons (Fig. 1a, b) and primary leaves (Fig. 1a, c). In subsequent juvenile leaves, they are weakly developed (Fig. 1a, d), and in mature leaves, they show a high density (Figs. 1e, 2a) on the abaxial side of the leaves. Especially leaves developed in distal parts of the shoot axis show a huge density of glands and secretions (Fig. 1e). The secreting surfaces of the sunken glands are about 20–30 µm in diameter and are surrounded by crown-like epidermal cells (Fig. 2d). Including these cells, the glands reach about 60–90 µm in diameter (Fig. 2d). They are always multicellular and consist of 2 basal-collecting cells which are in contact with the mesophyll and (2–) 4–6 upper secretory cells (Fig. 3a, b). Glands consisting of 8 cells (2 collecting and 6 secretory cells) represent the most common type, glands consisting of only 2 collecting cells and 2 secretory cells were rarely found. The upper secretory cells are enclosed by a cuticle showing several narrow pores through which the secretions emerge. The secretions on the leaf surface are about 50–70 µm in diameter (Fig. 2c, d). The crystal morphology within a secretion shows orthorhombic and fusiform crystals (Fig. 2e, f).

Immersion in 1 M HCL produced vigorous effervescence in the gland secretions, indicating the presence of carbonates.

The crystal deposit shown in Fig. 4a was composed of thin orthorhombic and granular clusters of fusiform crystals. Calcium was distributed throughout the deposit (Fig. 4b), whereas S was restricted to the orthorhombic crystals (Fig. 4c,d) and Mg was confined to the granular clusters (Fig. 4d). Spectra were extracted from the image shown in Fig. 4e. The spectrum shown in Fig. 4f is from a S-rich orthorhombic crystal and has prominent peaks indicating O, S and Ca. The small Al peak is from the Al coat applied to the sample, and there are small C and Mg

◀**Fig. 2** *Myricaria germanica*, details of mature leaves; **a** leaves at the distal part of a shoot axis; abaxial surface with several secretions; adaxial surface without secretions; **b** papillae-shaped epidermal cells; **c** cuticular waxes strongly curled; **d** gland without secretion; the secreting surface is surrounded by crown-like cells; **e** Detail of a whole secretion in situ; **f** Crystal morphology within a secretion showing orthorhombic and fusiform crystals

peaks. In Fig. 4g, the spectrum is from a Mg-rich granular cluster. The spectrum contains prominent O, Ca, C and Mg peaks. A small S peak is also present. The elemental images suggest the presence of orthorhombic crystals containing CaSO_4 and granular clusters of fusiform crystals of Mg-containing CaCO_3 (Fig. 6d). The presence of small S and Mg peaks indicates mutual contamination of the two types of secretions.

Further evidence of the distribution of CaSO_4 and Mg-containing CaCO_3 is shown in Fig. 5. The deposit shown was composed of large orthorhombic crystals and granular clusters of fusiform crystals (Figs. 5a, 6a, b). Elemental images are shown that represent element intensity distribution, in terms of X-ray counts. These in turn are a reflection of concentration distributions. Calcium intensity (Fig. 5b) was highest in the area of granular clusters of fusiform crystals and corresponded to the highest Mg intensity (Fig. 5c), whereas the highest S intensity corresponded to the lowest Ca intensity (Fig. 5d).

Confirmation of the likely presence of CaSO_4 in orthorhombic crystals (Fig. 6a, b) is shown in Fig. 6c. This selected area spectrum was similar to that obtained from a crystal of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Fig. 6e, f). The small difference in the O peaks was probably attributable to topographic effects arising from crystal orientation. The spectrum from the granular cluster of fusiform crystals was suggestive of Mg-containing CaCO_3 .

A comparison of quantitative analyses of analytical grade $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ crystals and analyses of orthorhombic crystals (Table 2) indicate a close similarity.

Crystal deposits were removed from leaves and mounted so that the bases of the crystals were presented uppermost, i.e. the now upper surfaces (Fig. 7a) prior to removal had been adjacent to the surfaces of the secretory gland cells. The elemental intensity images of Ca, S and Mg (Fig. 7b–d) suggest that CaCO_3 is secreted centrally and CaSO_4 is secreted peripherally.

Discussion

The morpho-anatomical changes from juvenile to adult leaves

In contrast to the linear, flattened cotyledons, all subsequent leaves are greatly reduced in size. The cotyledons and both

types of young leaves have undifferentiated mesophyll but in the cotyledons, the adaxial surface is light exposed and the abaxial surface shaded, while in both young leaf types the adaxial surface is orientated towards the shoot axis. Mature leaves are, however, strongly addressed to the shoot axis (Fig. 1e). Their mesophyll is dimorphic with palisade parenchyma developed towards the abaxial and spongy parenchyma towards the adaxial surface (Fig. 1f). Such a strong shift from juvenile to mature scale or imbricate leaves is also reported for several gymnosperms where this shift can take place in different ontogenetic stages; for example, when the first lateral shoots are developed, e.g. in thujoid Cupressaceae, or just after years as is the case in cupressoid Cupressaceae or some Podocarpaceae (e.g. Foster and Gifford 1974; Salmon 1986; Dörken 2013; Dörken and Parsons 2016).

In *Myricaria germanica*, all leaves, except the cotyledons, are orientated with their adaxial side towards the shoot axis. Thus, the morphologically abaxial side of these leaves becomes the new light-exposed surface, and the morphologically adaxial side the new shaded one. This means that the morphologically adaxial leaf surface becomes the new functionally lower leaf side and the morphologically abaxial leaf surface the new functionally upper leaf side. This change in the exposition of the leaf surface is accompanied by a functional transformation in the mesophyll, the epidermis and in the distribution of the stomata. In these leaves (Fig. 1f), the palisade parenchyma is located towards the new light-exposed surface and the spongy parenchyma towards the shaded surface. To reduce the water loss via the lamina in these leaves, the epidermal cells of the light-exposed side become thicker and are about double the size of the epidermal cells of the shaded side. The stomata are only developed on the shaded surface. These important functional transformations lead to the situation as is typical for bifacial leaves with palisade parenchyma located towards the light exposed and spongy parenchyma located towards the shaded surface. Our results clearly indicate that this functional transformation is exclusively caused by the solar radiation depending on which part of the leaf is shaded and which one is light exposed. Stomata are developed exclusively on the shaded parts of the leaf whether or not they are the morphologically ad- or abaxial surface. These results concur with earlier studies in gymnosperms where such a functional transformation in the leaf morphology and leaf anatomy depending exclusively on the solar radiation could be proven for single leaves, cladode formations and also for complete orthotropic shoot systems (e.g. Strasburger 1872; Schneider 1913; Imamura 1937; Fitting 1950; Napp-Zinn 1966; Tetzlaff 2005; Dörken and Stützel 2011; Dörken 2013). The latter case can be found in several scale- and imbricate-leaved Cupressaceae. In this study, the bifaciality is no longer correlated with the morphological

Fig. 3 *Myricaria germanica*, leaf gland structure; **a** longitudinal section of a leaf gland with 4 secretory cells; **b** schematic drawing of a leaf gland with 6 secretory cells (*C* cuticle, *CC* collecting cell, *E* epidermis, *M* mesophyll, *SC* secretory cell, *VB* vascular bundle)

ad- or abaxial side of a single leaf. It is correlated to the complete plagiotropic shoot, which is showing a light exposed and shaded side. In this case, it is clearly shown that for such a “superimposed bifaciality” of leaves, the orientation of a plagiotropic shoot system to the orthotropic main axis is of greater relevance than the orientation of a single leaf to its axis (e.g. Dörken 2013). Such a “superimposed bifaciality” of leaves is absent in *Myricaria germanica*, irrespective of whether the leaves are developed at a plagiotropic or orthotropic shoot axis. Thus, contrasting to scale- and imbricate-leaved Cupressaceae, the orientation of a plagiotropic shoot system to the orthotropic main axis is not of relevance for the functional transformation of leaves in *Myricaria germanica*. In this study, only the orientation of a single leaf to its axis is of most relevance, and the exposure of each single leaf to the light.

Climate, soils and leaf reduction

So far, this series of studies has focussed on leaf reduction mainly in terms of the water factor (xeromorphy) and the nutrient factor (scleromorphy) and has studied only

evergreen plants. Studying a deciduous species in this study presents some contrasts as we are dealing with plants with leaf lifespans of 4–4.5 month from May to October and which are leafless for the rest of the year, whereas the lifespan of most evergreen sclerophyllous leaves exceeds 2 years and can be 5–6 years (Blondel et al. 2010).

Firstly, with the water factor, the genus *Myricaria* is the most mesic in the Tamaricaceae, occurring in the Northern Temperate zone of Eurasia, mainly along the Asian mountains (Zhang et al. 2014). *M. germanica* is native to montane to subalpine riverine floodplains in Europe and west Asia (Moor 1958; Hegi 1975; Endress 1975; Kudrnovsky 2002; Kammerer 2003; Egger et al. 2014). The *M. germanica* floodplains present a case where water table levels are always above, at or close to the soil surface so that the deep adult *M. germanica* root systems (Bill et al. 1997) must (nearly) always have ready access as was the case for our investigated plants. The only water deficiency problem concerns summer drought deaths when the upper topsoil can dry out before *M. germanica* seedling roots have reached the water table (Müller 1995a, b; Lener 2011). It is hard to assess the importance of such a short-lived drought

problem to long-term selection for reduced leaf size. In terms of other possibly xeromorphic features, *M. germanica* has stomata only in the shaded parts of the leaves and an epidermis with strongly thickened cell walls in light-exposed parts, but a thick cuticular layer, trichomes, deeply sunken stomata, a distinct vascular bundle sheath, etc. could not be found. Thus, there is no clear evidence for a xeromorphic response.

Secondly, with the nutrient factor, low soil nutrient status may follow from the gravelly and sandy soil textures and the low availabilities of P, Fe, etc. in such alkaline soils (Marschner and Marschner 2012) as it is also the case for our investigated, highly alkaline soil (Table 1) showing a sandy texture correlated with very low levels of soil nutrients. However, it is not clear if such levels could be related to leaf reduction which can be a response to other factors as well. In any case, in assessing scleromorphic features, many criteria for scleromorphy may well only become manifest in the presence of the long leaf lifespans of evergreen plants rather than of the very short lifespans of deciduous leaves dealt with here. Typical scleromorphic foliar features like collenchyma and sclerenchyma are absent from *M. germanica*. In summary, there is no clear evidence for scleromorphy. Also, regarding the soil nutrient status, it is not clear if periodic nutrient enrichment from flooding occurs. In the present case, evidence discussed later suggests a halophytic ancestor for *Myricaria*. Because leaf reduction is a well-known adaptation to osmotic stress (Hanson et al. 1994), this is a likely factor here.

Leaf glands and their secretions

Until the present work, the leaf gland structure of the Tamaricaceae was known largely from very detailed work on *Tamarix aphylla* (Fahn and Cutler 1992; Evert 2006). The work here shows clearly that the gland structure of *Myricaria germanica* is extremely similar. For example, regarding the number of cells in the gland, *T. aphylla* has six secretory cells and two collecting cells (Evert 2006), while *M. germanica* is identical except that occasionally the number of secretory cells is reduced from six to four or two. In other families, the leaf glands of *Frankenia revoluta* Forsk., in the closely related Frankeniaceae, are virtually also identical (Salama et al. 1999), while the leaf glands of the mangrove *Avicennia* (Acanthaceae) show some differences to the genera above but are basically similar (Evert 2006). The calcium-secreting hydathodes of *Saxifraga ligulata* Wall. with their terminal tracheids of vein endings have a completely different structure (Evert 2006).

Turning to the nature of the gland secretions, the crystal deposits secreted on the surface of *M. germanica* leaves are composed of orthorhombic crystals that vary in size and shape, and granular clusters that appear to have developed

by epitaxial growth of fusiform crystals. The orthorhombic crystals are most likely composed of calcium sulphate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and the granular clusters are probably Mg-containing calcium carbonate similar to some forms of calcite. Sakai (1974) noted the presence in CaCO_3 rich secretions on leaves of *Plumbago auriculata* that had a “rice grain appearance” of similar dimensions to the fusiform crystals described here. X-ray diffraction analysis indicated the presence of $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ in these secretions, but spectra from X-ray microanalysis show that considerably more Mg was present relative to Ca than in the analyses from *M. germanica* crystals.

Fusiform crystals of CaCO_3 have been reported to occur on the CaCO_3 skeletons of scleractinian corals (Gladfelter 1983; Hidaka 1991; Clode and Marshall 2003), but to date no satisfactory analysis of these has been reported.

The deposition of crystals together with evidence of epitaxial growth indicates that the processes of crystallization must occur in an aqueous medium. It is not apparent how this occurs on the surface of leaves or how the components of the crystals are secreted. The analysis of the adaxial surface of isolated crystal deposits suggests that CaSO_4 is deposited peripherally and CaCO_3 is deposited centrally. The cells that might be responsible for this segregation have not been identified.

Current work on the ecology and evolution of the Tamaricaceae places it in the “salt gland clade” (Carlquist 2010) and strongly emphasizes the secretion of toxic levels of NaCl as being the role of the leaf glands (e.g. Fahn and Cutler 1992; Gaskin 2003). In this study, for the first time, we focus on a species of Tamaricaceae found only on non-saline, but highly calcareous soils. The results of the X-ray microanalyses make clear that the “salt glands” said to be a characteristic feature of this family can function principally as an adaptation to secrete Ca in a highly calcareous soil in a habitat where NaCl is ecologically unimportant.

This finding is not surprising, given that as long ago as 1856, Mettenius (in Sakai 1974) showed that in some species in the Plumbaginaceae (also in the “salt gland clade”), the secretions can be dominated by calcium compounds; he referred to the glands as “chalk glands”. Further early work followed until Brunswik (1920) recorded $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (gypsum) crystals in taxa of *Myricaria*, *Reaumuria* and *Tamarix* (all Tamaricaceae). Then the subject was neglected (Sakai 1974) until it became clear that *Tamarix aphylla* (L.) Karsten can secrete mostly calcium compounds or mostly NaCl depending on the contents of calcium and sodium in the substrate (Berry 1970; Storey and Thomson 1994).

In the detailed work on the Tamaricaceae, the predominant compound usually recorded in the secretions is NaCl, e.g. in *Reaumuria* (Ramadan 1998) and *Tamarix* including *T. aphylla* (Salama et al. 1999) (both on

Fig. 4 Element distribution in *Myricaria germanica* crystal deposit; **a** secondary electron image of crystal deposit on abaxial surface of leaf showing orthorhombic and clusters of fusiform crystal forms; **b** Ca distributed throughout the crystal; **c** S distribution confined to orthorhombic crystals; **d** Mg is present in fusiform crystals contrasting with S in orthorhombic crystals; **e** showing analysis sites in Mg-containing fusiform region and S-containing orthorhombic region; **f** spectrum from orthorhombic crystal region showing high concentrations of Ca, S and O suggestive of CaSO_4 ; **g** spectrum from fusiform region showing high concentrations of Ca, O, C and Mg suggestive of Mg-containing CaCO_3 , possibly calcite; the Al peaks are generated by coating on the samples

saline soils). One exception is the work on *T. aphylla* on calcium-rich, non-saline soils where Ca is the predominant ion, most likely as $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$; NaCl is virtually

absent (Storey and Thomson 1994). The other exception is the present work, where we record crystals likely to be both Mg-containing CaCO_3 and CaSO_4 (and the absence of NaCl) in *Myricaria germanica* from non-saline soils rich in CaCO_3 . In non-Tamaricaceae, the only leaf gland secretion analytical work we know is that of Sakai (1974) who recorded CaCO_3 and $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ as the main secretions of *Plumbago auriculata* Lam. (in Sakai 1974) as *Plumbago capensis*.

The low ion selectivity shown by the way that *Tamarix aphylla* can predominantly secrete either Na or Ca depending on substrate has led Sakai (1974) to suggest that “in all probability chalk glands and salt glands are not separate secretory structures”. Also, it may help to

Fig. 5 Element distribution in *Myricaria germanica* crystal deposit; **a** secondary electron image of crystal deposit on abaxial surface of leaf showing orthorhombic and fusiform crystal forms; element X-ray intensity is shown in terms of a colour scale where *black* represents minimum X-ray counts and *white* represents maximum X-ray counts;

X-ray counts are an indication of element concentrations modified by topographic effects; **b** Ca concentration is highest in the fusiform crystal region where Mg is also concentrated (**c**) and S concentration is very low (**d**)

◀**Fig. 6** **a** and **b** showing the same crystal deposit as in Fig. 5; **a** is a FESEM image obtained at 2 kV, whereas **b** is an image obtained under analytical conditions in a conventional SEM at 15 kV; a region of a large orthorhombic crystal and a region of fusiform crystals were analysed; **c** spectrum from orthorhombic crystal showing high concentrations of Ca, S and O suggestive of CaSO_4 ; **d** spectrum from fusiform region showing high concentrations of Ca, O, C and Mg suggestive of Mg-containing CaCO_3 , possibly calcite; **e** secondary electron image of analytical grade CaSO_4 crystals showing an analysed region; **f** spectrum from analysed region of CaSO_4 crystal; note the similarity to the spectrum in **c**

explain why *Tamarix aphylla* can grow on a wide range of soil types (Storey and Thompson 1994).

A related subject is that NaCl tolerance and heavy metal hyperaccumulation and secretion are often associated in angiosperm families and can be found in the same species (Moray et al. 2016), including *Tamarix smyrnensis* Bunge (Kadukova et al. 2008). Exposure to excess NaCl and heavy metals both induce osmotic stress, so that evolution of an adaptation, in this case-specialized salt glands, can be seen as an “enabling trait” which allows *Tamarix smyrnensis* to hyperaccumulate and secrete Cd and Pb (see Moray et al. 2016). Similarly, evolution of salt glands in a halophytic ancestor may have been the enabling trait that facilitated the adaptation of *Myricaria germanica* to non-saline calcareous soils. Neither NaCl nor heavy metals occurred in the *M. germanica* secretions we sampled.

Some deciduous *Tamarix* species exclude significant amounts of excess NaCl in their annual leaf fall which forms a thick saline duff on the soil surface; this can inhibit germination of other species (Gaskin 2003). Similarly, from general observations, it is clear that *M. germanica* excludes a large amount of Ca compounds from its canopy during annual leaf fall. Its short leaf lifespan is 4–4.5 months from May to October (authors’ observations).

More generally, the adaptational relationship between NaCl secretion and deciduousness can involve the shedding of parts of leaves, as in the bladder cells of the leaf trichomes of *Atriplex* (Evert 2006) or in the whole leaves of many Tamaricaceae (see above) and at least three deciduous genera of mangroves (Saenger 2002). Similarly, in some taxa-lacking salt glands, leaf deciduousness appears to be the main strategy of NaCl regulation; this applies to herbaceous basal rosette salt marsh species of *Aster*, *Plantago*, *Scorzonera* and *Triglochin*, where leaf lifespans can be only a few weeks (Albert 1975).

Considering the overall evolution and ecology of the Tamaricaceae, it seems likely that the leaf glands evolved originally in response to high soil NaCl levels and later became an enabling trait allowing colonization of calcium-rich soils. It is possible that the same scenario also applies to the evolution of leaf deciduousness in some taxa from the family.

Relating these ideas to the literature on the evolutionary history of *Myricaria*, the stock from which *Myricaria* arose is thought to have occurred along the eastern shore of the Tethys Sea (“the palaeo-Mediterranean sea”) in the early Tertiary (Liu et al. 2009). Such an area could provide saline habitats as a basis for leaf gland evolution. Subsequent uplift caused the retreat of the Tethys westwards and produced the Qinghai-Tibet region of the Himalayas and the diversification and dispersal of *Myricaria* as a montane to alpine genus (Liu et al. 2009). Westwards migration from there led to the present range of *M. germanica* (Zhang et al. 2014). This background provides us with the halophytic ancestor that we suggested above was responsible for the leaf glands and deciduousness found in present day *Myricaria*.

The emphasis on westward migration above is supported by recent work on another stress-tolerant group of Mediterranean species, the group of xerophytes comprising section *Tragacantha* of *Astragalus* (Leguminosae). The species are all from stressful, xeric, open habitats, either from exposed coasts or from rocky sites at high altitudes. This same dichotomy into those two types of stressful habitats also applies to other groups, including *Armeria* (Plumbaginaceae) (Hardion et al. 2016). This is interesting because, like the Tamaricaceae, the Plumbaginaceae is a highly stress-tolerant family, with the species adapted to a range of harsh environments and with salt glands being found in all species of the family, regardless of their habitat (Hanson et al. 1994). The division of Mediterranean *Armeria* species into coastal and mountain habitats (Hardion et al. 2016) is strongly reminiscent of the division of Mediterranean Tamaricaceae into coastal species (*Tamarix* spp.) and the mountain species (*M. germanica*). Thus, the Mediterranean species of Tamaricaceae may fit into the same (coastal/mountain) range fragmentation scenario described by Hardion et al. (2016). To partly relate this to leaf gland behaviour, firstly in the Tamaricaceae, for *Tamarix* on lowland sites, the cation secretion can be dominated by Na on saline soils (Salama et al. 1999) or by Ca on non-saline, lime-rich soils (Storey and Thomson 1994), whereas the only data from mountain sites are the Ca-dominant *Myricaria germanica* secretions reported here from plants from limestone soils.

In contrast, in the Plumbaginaceae, the data are more incomplete and complex. In lowland areas, *Limonium* from highly saline sites can secrete large amounts of Na, while *Armeria maritima* (Mill.) Willd. from less saline sites unaccountably has a higher secretion preference for K and Ca than for Na (Rozema et al. 1981). For the Italian montane to subalpine species *Armeria canescens* (Host) Boiss., on both high and low Ca soils, by far the predominant cation secreted is K, followed by Ca. In this case, “the meaning of the presence of salt glands is still

Table 2 The mean analysed composition of analytical grade $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ crystals and orthorhombic crystals from *Myricaria germanica* compared to the calculated composition of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$

Crystal composition in weight percent							
	C	O	Mg	P	S	K	Ca
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ crystals $n = 5$	6	51	0	0	19	0	23
<i>Myricaria germanica</i> crystals $n = 11$	11	45	0.2	0.1	15	0.1	21
Calculated $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0	56	0	0	19	0	23

Fig. 7 Secondary electron image of isolated *Myricaria germanica* crystal deposits; element X-ray intensity is shown in terms of a colour scale where *black* represents minimum X-ray counts and *white* represents maximum X-ray counts; X-ray counts are an indication of element concentrations modified by topographic effects; **a** crystal

deposits showing the surface normally attached to the leaf surface; **b** Ca intensity; **c** S intensity; **d** Mg intensity; the distribution of S and Ca suggests that CaSO_4 is deposited peripherally, whilst Ca and Mg distributions suggest that CaCO_3 is deposited centrally

unclear” (Scassellati et al. 2016). These authors suggest that the glands are derived from a halophytic ancestor as we have suggested here for *Myricaria*. For *A. canescens*, at least on the low Ca soils, it is likely that they no longer have a function. As an aside, it has been suggested that the high secretion preference for K in the Plumbaginaceae

is because K may play a role in the secretion process (Faraday and Thomson 1986).

To now examine the occurrence of leaf glands in the other 12 or so *Myricaria* species, of the 10 species listed in the Flora of China, all are recorded as occurring on riverbanks and river valleys but none are recorded with

leaf glands (eFloras 2008). However, the Flora of Pakistan records one of them as being “impregnated with salt glands” (eFloras 2008). Clearly, leaf glands and how they relate to salinity and calcareous soils need to be studied throughout the genus.

Another subject requiring further study in the present context is that of the ecological effects of calcareous soils. To explain why they are unfavourable for many plant species, most research focuses on their high alkalinity causing low nutrient availabilities, e.g. of Fe and P (see Zohlen and Tyler 2004), whereas in the present case, secretion of Ca by leaf glands is more suggestive of Ca-toxicity problems. That subject is much underworked and poorly understood (Marschner and Marschner 2012).

Concluding discussion

So far in this series of studies on species showing extreme leaf reduction, the interpretation has centred on the expected environmental factors of water and nutrients. The present case is completely different; in this study, we deal with a species from mesic non-saline areas but which, being from the salt gland family Tamaricaceae, originated from ancestors adapted to xeric, saline conditions. This ancestry has left its mark; the original halophytic traits such as salt glands and leaf deciduousness may now serve as adaptations to avoid Ca-toxicity on Ca-rich soils, while it seems very likely that extreme leaf reduction in this case developed originally from osmotic stress on saline sites; it may no longer have a functional role in *Myricaria germanica*. To confirm and extend these findings, work is badly needed to document the habitat and the adaptational response to it of the other 12 or so *Myricaria* species.

Author contribution statement The experiments were constructed and designed by all three authors, who analysed the data and wrote the paper together. V. M. Dörken performed the microtome sections and the SEM analysis, A. T. Marshall the SEM EDS elemental analysis, and R. F. Parsons searched and organized important literature.

Acknowledgements We are grateful to Mr. Otmar Ficht and Mrs. Anne Kern (Botanic Garden, University of Konstanz, Germany) for producing the seedlings. Furthermore, we thank the Botanic Garden of the Eberhard Karls Universität Tübingen (Germany) for providing research material; the “Amt für Natur, Jagd und Fischerei” (Kanton St. Gallen, Switzerland) for the special permission to collect material in the natural habitat; Dr. Michael Laumann and Mrs. Lauretta Nejedli (Electron Microscopy Center, Department of Biology, University of Konstanz, Germany) for technical support (paraffin technique and SEM). Finally, we thank Dr. Volker Hellmann for his helpful discussions and our field-trips to *M. germanica* in the northern Alps and Dr. N.C. Uren (Department of Animal, Plant and Soil Sciences, LaTrobe

University, Australia) for helpful discussions and advice concerning soil properties.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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