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Xanthoria calcicola (Teloschistaceae, Ascomycota) still present on bark in Sweden

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For almost twenty years *Xanthoria calcicola* was considered extinct on bark in Sweden. Here, we report *X. calcicola* growing on bark at 14 localities in Skåne, southernmost Sweden. In total, ca 300 thalli were observed on bark, and the populations vary from 1 to 200 thalli. In all localities except one *X. calcicola* was also present and more abundant on neighboring substrates made of stone, such as churchyard walls, church walls or tombstones. Preliminary results from fungal ITS data reveal that haplotypes found on bark are always present in the surrounding wall populations. We conclude that trees are suboptimal habitats for *X. calcicola* and only colonized when in close vicinity of an established wall population. The most obvious threat to epiphytic *X. calcicola* is the cutting down of host trees.

Almost twenty years ago, a comprehensive survey of the status of the redlisted ('protection-worthy') lichens of southwestern Sweden was published (Arup et al. 1997). This publication quickly became an important baseline for subsequent Swedish red lists (Gärdenfors 2000, 2005, 2010) as well as further studies of the lichen flora of the region. The characteristic lichen *Xanthoria calcicola* Oxner, at that time redlisted as Vulnerable (IUCN category 2), is one of the species included in the study (Arup et al. 1997). It was reported to grow on slightly alkaline substrates, mainly on churchyard walls, but also on church walls. It was refound at seven out of 13 earlier known localities (ca 50%), and 12 new localities were discovered (Arup et al. 1997). It was pointed out that there was no modern observation of *X. calcicola* growing on bark, in spite of several earlier reports between 1939 and 1948 of the species occurring on various deciduous trees (*Aesculus hippocastanum*, *Acer* spp., *Fraxinus excelsior*, *Tilia* spp. and *Ulmus* spp.). Arup et al. (1997) postulated that the disappearance of *X. calcicola* from the suboptimal bark substrate further corroborated its status as being in decline, probably referring to the hypothesis that ecological retardations start with marginal populations (Kawecki 2008).

Xanthoria calcicola belongs to a European southern-temperate element (Almborn 1948, as *X. aureola*), extending from southern Scandinavia to the Mediterranean, and from eastern Great Britain to Ukraine and the middle East (Wirth 1995, Hitch et al. 2009). In Sweden, the species has a southern distribution in the lowlands of the southernmost regions; Skåne, Gotland, eastern Småland and Halland (Arup et al. 1997, Nordin et al. 2016). The Swedish localities constitute the northern rim of the total geographical distribution of *X. calcicola*.

We made an attempted complete survey of the supposedly rare (included in all Swedish red lists before 2015; ArtDatabanken 2015) churchyard lichen *X. calcicola* within the south and west parts of Skåne, a total area of ca 5500 km^2 (Fig. 1). Primarily all churchyards and churches, abandoned churchyards, ruin churches and castle ruins were investigated. *Xanthoria calcicola* was searched for at all habitat types, including trees, at each locality, the number of thalli estimated, and occurrence of apothecia noted (Lindblom and Blom 2009).

Of totally 331 investigated localities, *X. calcicola* was found at 125 localities (38%). Fourteen of the localities harbour thalli growing on bark (11%) (Table 1, Fig. 1).

Table 1. Visited localities where *X. calcicola* was found on bark, arranged by year of first observation. All localities are churchyards, except Månstorps gavlar (Månstorp gables), which is a castle ruin since 1678. The two rediscoveries of *X. calcicola* on bark in our study (2010–2014) are growing on the same tree species as the original observation (1938–1939). *) total ITS haplotype. **) in our study (2010–2014).

Locality	Year of first obs.	Year revisited	Substrate tree spp.	Note (2010-2014)	No. thalli bark**	No. thalli stone**	Haplotypes* bark**	Haplotypes* stone**	Apotheciate bark**	Apotheciate stone**
Mölleberga	1938	2010	*Tilia* sp.	one tree only.	17	0	C1, C3	-	N	n/a
Barkåkra	1939	2012	*Aesculus hippocastanum*	also on churchyard wall.	3	180	C3	C1, C3, C5	Y	Y
Mellan-Grevie	1942	2012	*Fraxinus excelsior*	not found.	-	-	-	-	n/a	n/a
Hemmesdynge	1943	2011	*Aesculus hippocastanum*	only on churchyard wall.	-	5	-	C1, C2, C3, C6	n/a	Y
Lilla Isie	1943	2011	*Ulmus* sp.	only on tombstones/ churchyard wall.	-	47/962	-	C1, C3	n/a	N
Skabersjö	1946	2012	*Ulmus* sp.	only on churchyard wall.	-	80	-	C1, C3, C5	n/a	Y
Hyllie old	2000	2014	*Ulmus* sp.	only on churchyard wall. Trees removed 2000-2014.	-	11	-	C3, C5	n/a	Y
Kyrkoköpinge	2011	-	*Ulmus laevis*		2	5130	C1	C1, C3	Y	Y
Virke	2011	-	*Tilia* sp.		20	875	C2	C2	N	N
Hofterup	2011	-	*Tilia* sp.		24	1833	C3	C1, C2, C3	N	Y
Maglarp old	2011	-	*Aesculus hippocastanum*		4	8120	C1	C1, C2, C3, C5	N	Y
Fleninge	2012	-	*Aesculus hippocastanum*		6	347	C3	C1, C3	N	Y
Skegrie	2012	-	*Tilia* sp.		200	2092	C3	C1, C3, C5	N	Y
Månstorps gavlar	2012	-	*Fraxinus excelsior*		2	4998	C3	C1, C2, C3	N	Y
Oxie	2012	-	*Tilia* sp.		2	15	C2	C1, C2, C3	N	N
Bunkeflo	2012	-	*Tilia* sp.		8	184	PCR failed	C1, C3	N	Y
Båstad	2012	-	*Tilia* sp.		5	93	C3	C3	N	Y
Öja	2013	-	*Tilia* sp.	small thallus, no genetic sample	1	10	-	C1, C3	N	Y
Östra Nöbbelöv	2013	-	*Tilia* sp.		1	25	C3	C3	N	N

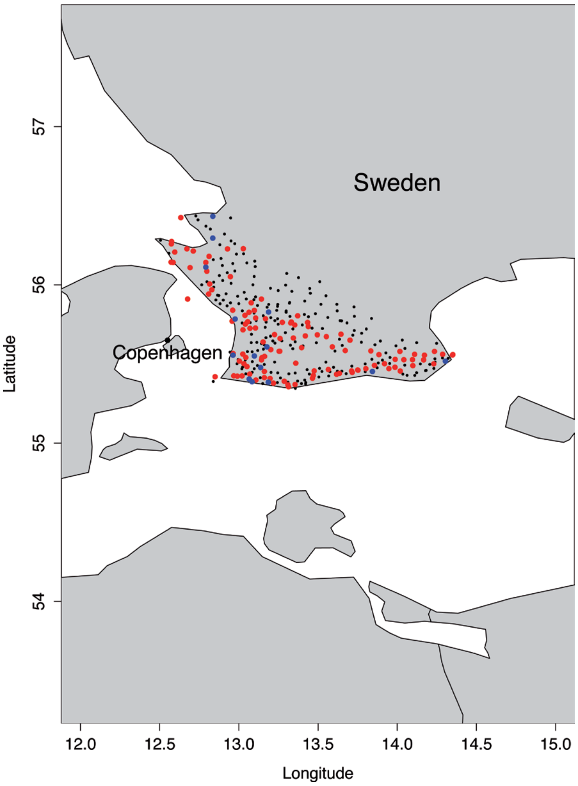

Figure 1. Map of the study area within the south and west parts of Skåne, southernmost Sweden. Localities where *X. calcicola* was searched for are marked with dots. Black – not found. Blue – found on tree bark. Red – found on wall substrates.

The populations found on bark vary in size from 1 to ca 200 thalli (Fig. 2–5). All bark populations are small (1–24 thalli), except one (Skegrie churchyard, Fig. 5). Two are rediscoveries at localities where it has previously been collected on bark and 12 are new discoveries. In total, we have observed ca 300 thalli of *X. calcicola* growing on bark. At six of the previously known localities, including one where it was reported relatively recently (Malmqvist 2000), the species was not found (Table 1). At all localities except one *X. calcicola* in addition to bark was present on the surrounding churchyard walls. The only exception was Mölleberga churchyard (Fig. 4B), where there were virtually no lichens on the wall, indicating that it had been recently whitewashed (Lindblom and Blom 2010). The wall populations at localities where *X. calcicola* grows on bark were, on average, larger than other wall populations (1706 ± 2559 versus 489 ±1360 thalli), and a larger proportion of them possessed thalli with apothecia (71.4% versus 50.5%). Thus, localities with tree populations seem to have important source populations on walls in relation to localities with populations on walls only. Seemingly suitable churchyard trees were present at most of the investigated localities, indicating that colonization of *X. calcicola* on trees is a rare event. The trees harbouring *X. calcicola* were always growing close to the wall habitat, up to ≤ 5 m (Fig. 3). These results add to the hypothesis that trees are actually suboptimal habitats for *X. calcicola*. Hence, bark populations are sinks (sensu Dias 1996) sustained by continuous supply of diaspores from the connected source population on walls (sensu Dias 1996).

Figure 2. Epiphytic population consisting of one single thallus of *X. calcicola* on a *Tilia* sp. (Östra Nöbbelöv church).

Figure 3. Two trees (*Tilia* sp.) with *X. calcicola* growing close to the churchyard wall with a large *X. calcicola* population (Hofterup church).

Heterothallic species require two compatible partners to produce sexual diaspores and, hence, outbreeding is promoted. Interestingly, we have, although extremely rarely, observed thalli of this heterothallic species with apothecia on bark (Table 1, Fig. 4A). Thus, *X. calcicola* can potentially disperse by ascospores even when growing on a suboptimal substrate as tree bark. However, whether these ascospores are viable, able to resynthesis with a photobiont, and establish the lichen thallus remains to examine.

It is notable that we never observed *X. calcicola* growing on trees in tree avenues and parks with an environ-

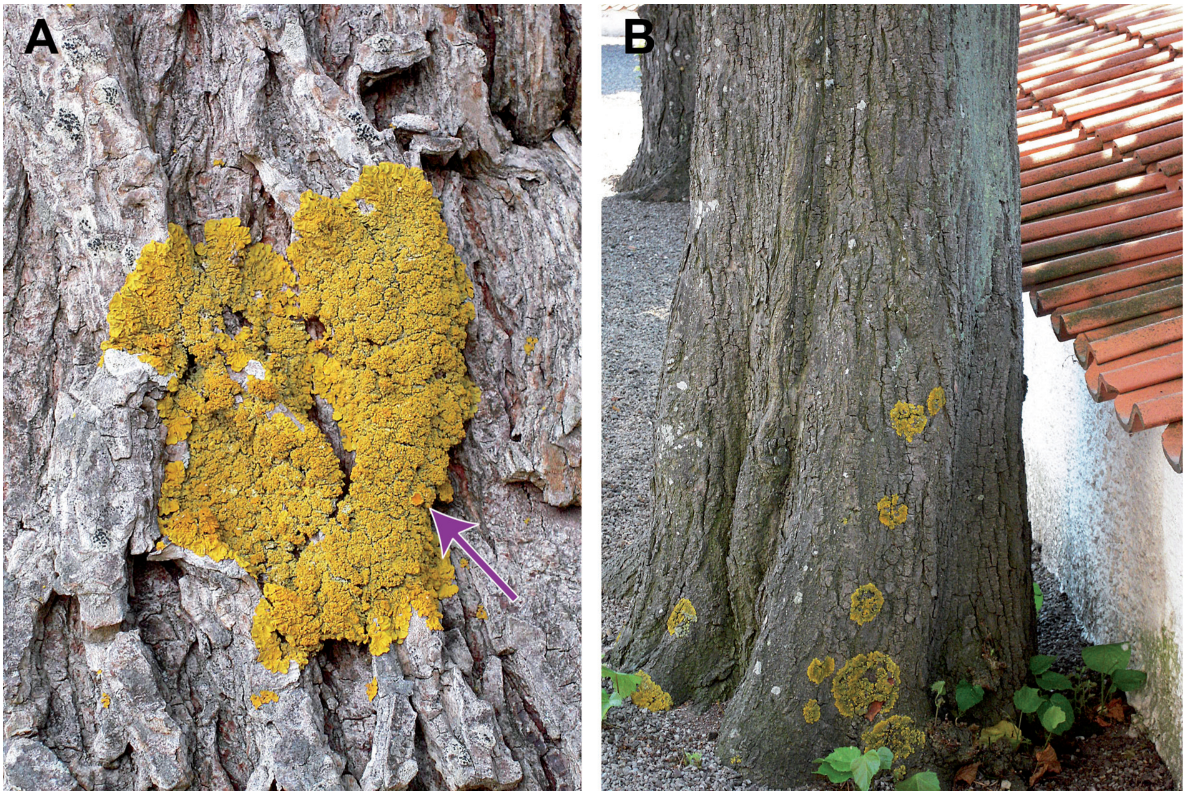

Figure 4. *Xanthoria calcicola* on bark. (A) Apotheciate thallus on *Ulmus laevis* growing close to the churchyard wall, which harbours a large population (Kyrkoköpinge church), (B) Small population growing on one *Tilia* sp. close to the churchyard wall, which seems recently whitewashed and almost free of lichens (Mölleberga church, Lindblom and Blom 2010).

ment closely resembling churchyards. Such habitats are very common in the open and predominantly cultural landscape of Skåne. During our many travels across Skåne before and during our field study we have searched countless random deciduous trees (of the same species as those colonized by *X. calcicola*) outside churchyards without ever finding *X. calcicola*. Neither in the extensive field work of Arup et al. (1997) were any tree populations discovered. Observations of young colonizing populations (small thalli) on walls, cemented posts, etc., at distances up to more than 1 km from known source populations, as well as preliminary statistical analyses from our study, indicate that *X. calcicola* is not strongly delimited by dispersal distances at the scale of our investigation area. We argue that the reason why *X. calcicola* has not yet been found on trees away from wall populations is a combination of diaspore density dilution by distance from sources and a clearly suboptimal habitat; there are simply so few diaspore hits on such trees that establishment becomes extremely rare.

Preliminary results from fungal ITS data of samples from the localities with populations of *X. calcicola* (methods following Lindblom and Ekman 2005) reveal that haplotypes found in bark samples are always present in the surrounding wall populations (Table 1). The molecular data suggest that each population of *X. calcicola* occurring on bark is a subsample of the connected wall population.

Our results support the hypothesis that establishment on trees are dependent of wall populations in close vicinity, continuously bombarding the suboptimal tree habitats with diaspores. The Mölleberga churchyard locality, where the churchyard wall has recently been whitewashed and the wall population are now without *X. calcicola*

Figure 5. The largest bark population found, with approximately 200 thalli growing on bark of 22 *Tilia* sp. trees. The surrounding wall population is also very large (Table 1) (Skegrie church).

thalli, would be an interesting case study for investigating whether a population on bark can sustain over time without continuous supply of diaspores.

Xanthoria calcicola is no longer redlisted in Sweden, based on the results of our field investigation submitted to the Swedish expert committee for lichens (ArtDatabanken 2015). Clearly, conservation of this beautiful lichen should focus on the churchyard wall populations, addressing the main threats of whitewashing and pressure cleaning.

Conclusions

Xanthoria calcicola in Skåne is more frequently occurring on tree bark than previously assessed.

Trees are suboptimal habitats for *X. calcicola* and only colonized when in close vicinity of an established wall population.

Even when growing on a suboptimal susbtrate, in this case tree bark, the heterothallic species *X. calcicola* is able to produce apothecia.

The most obvious threat to epiphytic *X. calcicola* is the cutting down of the host tree. In addition, thorough cleaning of walls near the trees is a threat, because it could eradicate the potential source population.

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