The distribution and host range of *Thecaphora melandrii*, with first records for Britain

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**Summary.** *Thecaphora melandrii* (Syd.) Vánky & M.Lutz infects species in the Caryophyllaceae forming sori with spore balls in the floral organs. We report new finds from Britain, supported by phylogenetic analysis, that confirm its occurrence on *Silene uniflora* Roth. We review published and web accessible records and note the relatively few records of this smut, its sparse distribution, confined to Europe but scattered predominantly from central to eastern Europe. Analysis of the rDNA ITS and 28S sequences demonstrates little variability among specimens, even those parasitising different host genera, which suggests that the species has evolved relatively recently. Some *Microbotryum* species infect the same host plants, and we found two species, *M. lagerheimii* Denchev and *M. silenes-inflatae* (DC. ex Liro) G.Deml & Oberw., in the same locations as *T. melandrii*, identified by morphology and molecular phylogenetic analysis. These species may form a stable multi-species community of parasites of *Silene uniflora*.

**Key Words.** Caryophyllaceae, gall, Glomosporiaceae, *Microbotryum*, *Silene uniflora*, smut.

**Introduction**

The Caryophyllaceae is a large family of dicotyledonous plants (Greenberg & Donoghue 2011), and its species are hosts for many plant-parasitic microfungi, among them at least 38 species of smut fungi assigned to the genera *Microbotryum* Lév. and *Thecaphora* Fingerh. (Vánky 2012), with additional species in Denchev et al. (2009); Denchev & Denchev (2011); Piątek et al. (2012, 2013); Denchev et al. (2019); Kemler et al. (2020),) which form their sori in the floral organs. Although there are similarities in the appearance of the infections and the affected hosts, these two genera are not closely related; *Microbotryum* is in the Microbotryaceae within the subphylum Pucciniomycotina, and *Thecaphora* is the only genus in the Glomosporiaceae within the Ustilaginomycotina. The genus *Thecaphora* contains plant-parasitic microfungi infecting hosts belonging to a range of dicotyledonous families. The species and their current nomenclature are summarised by Vánky et al. (2008) and Vánky (2012). Recently, three new species were described in Crous et al. (2018), Kruse et al. (2018) and Piątek et al. (in press). *Thecaphora* species are characterised by having spores in balls (or rarely single), generally without sterile cells, and infections are found in a range of different organs of their host plants. Here we focus on species of *Thecaphora* infecting hosts in the Caryophyllaceae, and specifically on *T. melandrii* (Syd.) Vánky & M.Lutz. Vánky (2012) lists five species of *Thecaphora* with hosts in this family, all destroying the inner floral organs; most remain within the outer floral envelope (the calyx), but *T. alsinearum* (Cif.) Vánky & M.Lutz also spreads to the uppermost leaves. *Thecaphora melandrii* is known to infect *Silene latifolia* Poir., *S. nutans* L., *S. vulgaris* (Moench) Garcke and *Stellaria graminea* L. as confirmed by genetic analyses of smut specimens from the respective hosts (Vánky & Lutz 2007; Vánky 2012), and is tentatively reported from several other host species, based on morphological analyses only, although we have traced the sources of only some of these reports below. Some of these tentatively reported hosts are doubtful, and need confirmation. *Thecaphora melandrii* deforms and partially replaces the inner floral organs, and is therefore gall-forming. The infection remains within the calyx, but causes the buds to deform and remain closed, so that the infection is clearly detectable externally. Here we report recent finds which confirm its occurrence on a new host species (*Silene uniflora* Roth), based on molecular evidence of nuclear rDNA ITS and 28S sequences, and evaluate its geographical distribution based on published records. Vánky (2012) reported *T. melandrii* on *S. vulgaris* subsp. maritima (With.) A.Löve & D.Löve (which is a synonym of *S. uniflora*)
but the source of this report is unknown. This fungus-host combination was however not verified by molecular methods.

*Microbotryum* has been found to consist of many similar species, distinguished by DNA sequence analysis and in some cases by subtle morphological differences. Vánky (2012) lists 28 species in hosts of the Caryophyllaceae, including 20 species in the host’s anthers. More segregates have been identified subsequently, thus there are now 33 species, including 23 species of so-called anther smuts. *Microbotryum* anther smut species have co-evolved with their hosts (Refrégier et al. 2008), and are generally strongly host species specific. Several species have been described in recent years throughout the world, but here we consider only some of the European taxa, which have been worked out by Lutz et al. (2005, 2008); Denchev (2007a, b); Le Gac et al. (2009); Denchev et al. (2009); Denchev & Denchev (2011) and Piątek et al. (2012, 2013).

**Materials and Methods**

**Specimens**

Specimens used for molecular analyses were of *Thecaphora* and *Microbotryum* species infecting *Silene uniflora* (= *S. maritima* With., *S. vulgaris* subsp. *maritima*), collected in 2019, and preserved by pressing. Specimens were photographed in situ. The collection details are given in Table 1 (*Thecaphora*) and Table 2 (*Microbotryum*). The voucher specimens are deposited in the fungarium at the Royal Botanic Gardens, Kew (K(M)) and in the herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM). An example habitat of *Silene uniflora* where infected individuals were found is shown in Fig. 1.

**Morphological analyses**

The morphology of *Thecaphora* and *Microbotryum* species were studied using dried specimens. The microscopic characters were analysed by light microscopy (LM), using a Nikon Eclipse 80i light microscope. For this purpose spore balls and spores were placed in 80% lactic acid, heated to boiling point, cooled and then examined under a light microscope. The spore sizes of *Microbotryum* specimens were measured using NIS-Elements BR 3.0 imaging software. 30 spores were counted for each collection and the extreme measurements were adjusted to the nearest 0.5 μm. The species descriptions include the combined values and standard deviation for all measured specimens of the respective species. LM micrographs were taken with a Nikon DS-Fi1 camera. The morphology of *Thecaphora* and *Microbotryum* species are depicted in Figs 2 and 3.

| Host                  | Genbank accession no. (ITS 28S) | Reference specimen |
|----------------------|---------------------------------|--------------------|
| *Silene uniflora*    | MN929139/MN929241               | England, Stokes Bay, VC1, S255710, 08 Jul 2019, Paul A. Smith, K(M) BRIP: HUV 12681 |
| *Silene uniflora*    | MN929048/MN929232               | England, Hook with Warsash LNR, VC11, SU44932 53297, 1 June 2019, Paul A. Smith, K(M) BRIP: HUV 12682 |
| *Silene uniflora*    | MN929049/MN929235               | England, Gilkicker Point, VC11, SU51126 52262, 1 July 2019, Paul A. Smith, K(M) BRIP: HUV 12683 |
| *Silene uniflora*    | MN929050/MN929239               | England, Hook with Warsash LNR, VC11, SU45800 53877, 1 June 2019, Paul A. Smith, K(M) BRIP: HUV 12684 |
| *Silene uniflora*    | MN929051/MN929240               | England, Hook with Warsash LNR, VC11, SU44932 53297, 1 June 2019, Paul A. Smith, K(M) BRIP: HUV 12685 |
| *Silene uniflora*    | MN929052/MN929241               | England, Gilkicker Point, VC11, SU51126 52262, 1 July 2019, Paul A. Smith, K(M) BRIP: HUV 12686 |
| *Silene uniflora*    | MN929053/MN929232               | England, Hook with Warsash LNR, VC11, SU45800 53877, 1 June 2019, Paul A. Smith, K(M) BRIP: HUV 12687 |
| *Silene uniflora*    | MN929054/MN929239               | England, Hook with Warsash LNR, VC11, SU44932 53297, 1 June 2019, Paul A. Smith, K(M) BRIP: HUV 12688 |
| *Silene uniflora*    | MN929055/MN929240               | England, Gilkicker Point, VC11, SU51126 52262, 1 July 2019, Paul A. Smith, K(M) BRIP: HUV 12689 |

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Table 2. Microbotryum specimens from the localities in England searched for Thecaphora melandrii during this study.

| Species          | Host            | Genbank accession no. (ITS) | Reference specimen                  |
|------------------|-----------------|-----------------------------|-------------------------------------|
| M. lagerheimii   | Silene uniflora | MN922355                    | Gilkicker Point, vc11, SZ60334 97729, 17 July 2019, Paul A. Smith, KRAM F-59668 |
| M. silenes-inflatae | Silene uniflora | MN922356                    | England, Hook with Warsash LNR, vc11, SU48890 04877, 1 June 2019, Paul A. Smith, KRAM F-59669 |
|                  |                 |                             | (this clump was also infected with Thecaphora melandrii) |
| M. silenes-inflatae | Silene uniflora | MN922357                    | Hayling Island, vc11, SZ71649 98757, 20 July 2019, Paul A. Smith, KRAM F-59670 |
|                  |                 | N/A                         | Hayling Island, vc11, SZ71760 98741, 20 July 2019, Paul A. Smith, KRAM F-59671 |

Phylogenetic analyses

Methods of DNA extraction, PCR, and sequencing of the nuclear rDNA ITS1-5.8S-ITS2 region (ITS) and the nuclear rDNA 28S D1–D2 region (28S) followed Lutz et al. (2004) and Vasighzadeh et al. (2014), and molecular phylogenetic analyses followed the techniques used in Ziegler et al. (2018). To elucidate the phylogenetic position of the Thecaphora specimens their concatenated ITS+28S sequences were analysed along with all the available sequences of T. aisinearmum and T. melandrii from GenBank, and three representative specimens of T. saponariae (Syd.) Vánky & M.Lutz. To clarify the relationship of the Microbotryum specimens within the genus Microbotryum, their concatenated ITS+28S sequences were analysed within datasets covering all the Microbotryum species available in GenBank (data not shown). The GenBank accession numbers of the Thecaphora sequences used and generated in this study are given in Table 1 and Fig. 4, the GenBank accession numbers of the Microbotryum sequences generated in this study are given in Table 2.

A Bayesian inference of the phylogenetic relationships within the sampled Thecaphora specimens was undertaken using Markov chain Monte Carlo analysis of an alignment of the concatenated ITS+28S sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and an estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. To illustrate the results, the consensus tree of one run of the Bayesian Approach is presented in Fig. 4. A 50% majority-rule consensus tree is shown computed from 75,000 trees that were sampled after the process had become stationary. The topology was rooted with the Thecaphora saponariae specimens. Numbers on branches before slashes are estimates for a posteriori probabilities, numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site.

Distribution and hosts

Records of Thecaphora melandrii were sought through an extensive review of the online accessible literature and a selection of European smut floras and checklists, also considering the synonyms (taken from Vánky & Lutz (2007)) Sorosporium melandrii, So. silenes-inflatae and So. stellariae, and also So. saponariae or T. saponariae only where the host was in Silene L. or Stellaria L. Other online sources (such as online fungarium information, including records from Herbarium Ustilaginales Vánky (HU; https://collections.daff.qld.gov.au/) and the Solheim Mycological Herbarium) were also investigated. Details of the locations and hosts were abstracted. The distribution of Thecaphora melandrii is shown in Map 1.

Thecaphora melandrii in Britain

There are many records of Microbotryum spp. from Britain and Ireland recorded in the Fungal Records Database of Britain & Ireland (FRDBI; http://www.frdbi.info/). They (almost) all infect only the anthers (among species known in Britain & Ireland (Legon & Henriici 2005), only M. major on Silene ņütis (L.) Wibel deforms other floral parts too). So the first author (PAS) was surprised to find several plants of Silene uniflora on the shingle beach at Stokes Bay, Hampshire (see Table 1 for full record details) with deformed, unopened flowers (Fig. 2A, B, D) inside which most of the floral parts were swollen, and there were copious smut spores. The spores were in balls, although held together very loosely and disintegrating easily when mounted on microscope slides. The individual spores were verrucose (Fig. 2C), without the reticulate sculpturing characteristic of most Microbotryum species. This was clearly Thecaphora melandrii, which is not included in Legon & Henriici (2005) or its updates, and is therefore the first record for Britain & Ireland. Infected plants were present along approx. 1 km of the beach.

Further searching showed Thecaphora melandrii to be frequent infecting Silene uniflora on shingle at Hook with Warsash (SU4804), approx. 11 km from the Stokes Bay location, but it could not be found in searches on Hayling Island to the east of Stokes Bay. Nevertheless, it seems possible that T. melandrii may be in further suitable places along the south coast of England. All the searched locations had S. uniflora.
plants with anthers infected by Microbotryum sp., and we return to these under Microbotryum species below.

The habitat in both locations (for a habitat example see Fig. 1) consists of stable shingle beaches with sparse vegetation consisting of Beta vulgaris L. subsp. maritima (L.) Arcang., Crambe maritima L., Glauccium flavum Crantz, Plantago lanceolata L. and Silene uniflora; this belongs to the Rumex crispus–Glauccium flavum shingle community (National Vegetation Classification SD1 (Rodwell et al. 2000: 128–132)). Silene uniflora is frequent in this habitat, which may help T. melandrii to persist in relative abundance.

Several observations suggest further details of the life cycle of Thecaphora melandrii. In most infected clumps of its host, every flower is infected (Fig. 2A, E, F), which suggests that the infection is systemic. Occasionally there are a few uninfected flowers in a clump, and then it is unclear whether some flowers are able to escape infection and develop normally, or whether the clump contains more than one individual of the host. One host clump at Hook with Warsash was infected with both T. melandrii and Microbotryum silenes-inflatae (Fig. 2E, Microbotryum species below); it appeared that this was a single plant, but the possibility of two individuals growing together cannot be ruled out.

Although all the parts of flowers within the calyx are normally infected and swollen (Fig. 2D), occasional flowers on infected plants still produce exserted corollas (Fig. 2F), and the same phenomenon was reported by Kruse et al. (2020). So there seems to be some variation in infection intensity even within a host plant.

Silene uniflora is regularly robbed for its nectar by insects (presumably short-tongued bees, although this was not observed) which drill holes in the calyx. The same sort of holes were observed in some of the flowers infected by Thecaphora melandrii (Fig. 2F), and this suggests a mechanism for infections to spread to new hosts, since infected flowers do not otherwise open. A reviewer noted similar holes on infected specimens of S. latifolia subsp. alba (Mill.) Greuter & Burdet, possibly made by caterpillars. Spores are also exposed as the flowers senesce, and could also be wind-distributed.

The ITS and 28S sequences extracted from the specimens from southern England were compared with all the available sequences for Thecaphora melandrii, the latter included in the analysis of Thecaphora species on caryophyllaceous hosts by Vánky & Lutz (2007). Details of the collections and accession
numbers are given Table 1. No further sequences for *T. melandrii* were available in GenBank.

A Bayesian inference of the phylogenetic relationships within the sampled *Thecaphora* specimens is shown in Fig. 4. The *ITS* and *28S* sequences are remarkably homogenous, with almost no differences. This is surprising, given that it infects several different host species across two genera; of course there may be differences in other areas of the genome, but on the evidence in *ITS* and *28S* it looks as if this is a recently evolved species, so that there has been insufficient time for divergence of the *ITS* and *28S* sequences; this would also suggest that a single pathogen is involved in infecting these host species. It would be worthwhile undertaking artificial infection experiments to confirm that *T. melandrii* does not vary in its infection ability on different hosts.

The lack of differences confirms that the specimens from England are consistent with the species concept for *T. melandrii*, and this also confirms the tentative assignment of *Silene uniflora* as a host by Vánky & Lutz (2007).

**Microbotryum species**

The localities searched for *Thecaphora melandrii* also supported *Microbotryum* specimens, and the morphology as well as the rDNA *ITS* and *28S* sequences for these collections were also examined. The collection details are given in Table 2. According to our molecular phylogenetic analyses, the clump with infections of both *T. melandrii* and *Microbotryum* sp. at Hook with Warsash was infected by *M. silenes-inflatae,*
Fig. 2. *Silene uniflora* plants infected by *Thecaphora melandrii* showing the deformed, unopened flowers. Note that all flowers in a clump are infected. B close-up of infected head (note that *S. uniflora* has forms with and without anthocyanins (Marsden-Jones & Turrill 1957: 289 – 298), which is why there is a difference in the colours). C spore balls, seen by LM, showing the verrucose sculpturing of the spores in contrast to the reticulate pattern on spores of most *Microbotryum* spp.; D section through infected flower showing the swollen inner floral parts; E single clump of *S. uniflora* infected with *T. melandrii* and *Microbotryum silenes-inflatae*; F infected plant with some infected flowers (e.g., upper right) showing corollas, some infected flowers (circled) have holes in their calyces where insects have attempted to rob them for nectar.
and the other site for T. melandrii at Gilkicker Point produced M. lagerheimii. At Hayling Island where no Thecaphora spp. infection was found, the anther smut was M. silenes-inflatae. The morphology showed differences between the two species detected by molecular analyses. The sori of M. lagerheimii in host anthers were light violaceous, spores were pale violet in transmitted light, globose, subglobose, to broadly ellipsoid, 5.0–7.5 × 5.0–6.5 (± 7.0) μm [av. ± sd, 6.3 ± 0.7 × 5.9 ± 0.6 μm, n = 39/1]; the wall was reticulate, c. 0.5–1.0 μm high, the meshes were more or less polyhedral, usually irregular, and the number of meshes per spore diameter was 5–8. The sori of M. silenes-inflatae in host anthers were relatively dark violaceous, spores were pale violet or violet in transmitted light, globose, subglobose, to broadly ellipsoid, 6.0–8.5 (± 9.5) × (5.5–) 6.0–7.5 (± 8.5) μm [av. ± sd, 7.4 ± 0.8 × 6.8 ± 0.6 μm, n = 90/3]; the wall was reticulate, c. 0.8–1.3 μm high, the meshes were more or less polyhedral, usually irregular, and the number of meshes per spore diameter was 6–9. The second specimen from Hayling Island, not investigated by molecular methods, could be identified as M. silenes-inflatae based on morphology. Microbotryum lagerheimii and M. silenes-inflatae are differentiated based on the colour of the sori (pale violaceous vs dark violaceous) and average of spore sizes (<7 μm vs >7 μm) (Fig. 3). This morphology is consistent with data of Denchev (2007a) for both species and Smith et al. (2017) for M. silenes-inflatae in the Outer Hebrides. Chung et al. (2012) have already shown that there are sympatric populations of these two Microbotryum species in southern England, so the sites are not necessarily differentiated by these records. By contrast, in the northern part of the UK, in the Outer Hebrides, only M. silenes-inflatae has so far been found on Silene uniflora (Smith et al. 2017). Abbate et al. (2018) show that different Microbotryum spp. occur widely in low- and high-altitude populations of S. uniflora and S. vulgaris. Nevertheless, it is clear from our limited records and previous research that populations of any pair of these three species of smut found in this study are able to co-exist in a site, even though they share a host and a similar niche. Further sampling is needed to examine whether all three parasite species can form a stable system.

Distribution and scarcity of Thecaphora melandrii

Table 3 lists all of the records (worldwide) for Thecaphora melandrii which we traced in our literature search (excluding those already listed in Table 1 and doubtful records in Table 4).

Biogeography

In total there are 90 published and herbarium records, and because there are some duplicate collections, even fewer localities. Records based only on the online catalogue of HUV contain at most country-level information. There are undoubtedly collections which have not been published, and some, particularly earlier, collections may be under names which applied to multiple species under current taxonomic concepts, and therefore are not clearly identifiable as Thecaphora melandrii. Nevertheless, it seems that this is a scarce species, with few records anywhere; several authors comment specifically on its scarcity (Sydow 1934; Liro 1938; Lindeberg 1959). There has been a surge in recent records, particularly a clutch of records from Germany (Kruse et al. 2015, 2017, 2020), probably as result of renewed interest in smuts (but possibly also affected by changes in the publication of records), but the number of recent records is still small by contrast with Microbotryum spp. The records of T. melandrii are mapped in Map 1. Thecaphora melandrii is apparently restricted to Europe, although there are eastern European records from Russia and Ukraine, which suggests that the species may yet be found in Asia, and there is a report from Kazakhstan (Schwartzman 1962, Table 4), although on a host species which requires further evidence to assess whether the smut belongs to this species rather than to another host species-specific taxon. Similarly, reports from America (see Table 4) are not clearly referable to this species without further evidence.

The most recent records from England are notable in being coastal; many of the remaining records (where there is sufficient detail to make an assessment) are inland (although there are also coastal records from Sweden), and largely in continental climates. This is despite the occurrence of its hosts in a wide range of habitats. The English locations are therefore in quite a different habitat type, and show that Thecaphora melandrii potentially has a wide biogeographical range and should be sought in further locations.

Since we have well-founded information on the recorded distribution we are able to make a provisional assessment for Thecaphora melandrii against the IUCN red list criteria. There is some evidence of decline in the area occupied as shown by recent records (Map 1), but we strongly suspect that this a result of fluctuation in recording rather than fluctuation in the occurrence of T. melandrii. There are many individuals (systemically infecting plants of the hosts) in each locality, so the total population of mature individuals is likely to be large. Finally, no specific threats have been identified. We therefore provisionally suggest that this species should be classified as LC least concern.

Host range

Thecaphora melandrii has been found infecting Silene vulgaris, S. uniflora, S. latifolia and S. nutans, and Stellaria graminea. The first three Silene species are quite closely related (particularly S. uniflora and S. vulgaris) (Greenberg & Donoghue 2011, Fig. 1), but S. nutans is more distantly related within the
many species in the genus *Silene*. The infection of *Stellaria graminea* is perhaps more surprising, as it is considerably more distantly related to *Silene* (Greenberg & Donoghue 2011, Figs 2, 4, 5). Liro (1938) records *T. melandrii* on *Stellaria graminea* from a range of sites scattered across Finland and nearby parts of Russia, and there is a further cluster of records on this host from Romania in HUV. The minimal variation in the DNA from *T. melandrii* on any of these hosts suggests that it is a single taxon with the ability to infect a range of host species. Infection of further species might therefore also be possible; however, other species of *Silene* are also infected by segregate species (such as *T. italica* M.Lutz & Vánky on *Silene italica* (L.) Pers.).

Table 4 lists infections on a small number of additional hosts which could represent *Thecaphora*...
Table 3. Literature and web-accessible records of *Thecaphora melanidrii* (records from Table 1 are not repeated).

| Country | Location | Host           | Source                  | Date       |
|---------|----------|----------------|-------------------------|------------|
|        |          | *Silene vulgaris* | HUV1764                 | July 1920  |
|        |          | *Silene vulgaris* | HUV5677                 | 6 Aug. 1972|
|        |          | *Stellaria graminea* | HUV8851                 | 6 July 1961|
|        |          | *Stellaria graminea* | HUV1779                 | 10 July 1968|
|        |          | *Stellaria graminea* | HUV1780                 | 12 July 1968|
|        |          | *Stellaria graminea* | HUV10785                | 4 July 1982 |
| Bulgaria |        | *Silene latifolia* | HUV1744                 | June 1885  |
| Croatia |        | *Silene vulgaris* | Ivč et al. (2013)       | 1916       |
| Denmark | Lyngby, Seeland | *Silene vulgaris* | Lind (1913), HUV1761, HUV1772 | 4 July 1912 |
| Finland |        | *Stellaria graminea* | HUV8851, Liro (1938), Vánky & Lutz (2007) | 7 July 1913 |
| Finland |        | *Stellaria graminea* | Liro (1938), HUV1772    | 13 July 1904|
| Finland |        | *Stellaria graminea* | Liro (1938)             | 3 Aug. 1933 |
| Finland |        | *Stellaria graminea* | Liro (1938)             | 3 Aug. 1933 |
| France | Chateau Evrem | *Silene latifolia* | Dauphin (1993)          | 4 July 1993 |
| Germany | Rheinland, Köln-Zollstock | *Silene latifolia* | Vánky & Lutz (2007)     | 29 Sept. 1932|
| Germany | Rheinland, Köln-Zollstock | *Silene latifolia* | Ustilagineen Europas #129, Vánky & Lutz (2007), HUV1746, HUV9788, HUV10815 | 21 June 1934 |
| Germany | Rheinland, Köln-Zollstock | *Silene latifolia* | Solheim Mycological Herbarium | 16 July 1935 |
| Germany | Rheinland, Köln-Zollstock | *Silene latifolia* | HUV10815                | 14 June 1937 |
| Germany | Rheinland, Köln-Zollstock | *Silene latifolia* | HUV10815                | 15 Oct. 2016 |
| Germany | Hessen, Frankfurt am Main, Oberrad, N of Offenbacher Landstraße, fields S of Buchrain, embankment | *Silene latifolia subsp. alba* | Kruse et al. (2017) | 3 Nov. 2015 |
| Germany | Hessen, Main-Taunus-Kreis, Flörsheim/Main, Hochgewann, meadows by railway | *Silene latifolia subsp. alba* | Kruse et al. (2015) | 10 May 2014 |
| Germany | Hessen, Main-Taunus-Kreis, Flörsheim/Main, Hochgewann, meadows by railway | *Silene latifolia subsp. alba* | Kruse et al. (2015) | 17 May 2014 |
| Germany | Hessen, Main-Taunus-Kreis, Flörsheim/Main, Hochgewann, meadows by railway | *Silene latifolia subsp. alba* | Kruse et al. (2015) | 27 July 2014 |
| Germany | Saxony, Eisleben | *Silene vulgaris* | Örtel (1886), Săvulescu (1957), Vánky & Lutz (2007), HUV1760 | 1875 |
| Germany | Bottendorfer Schachtberg, between Bottendorf and Rosslaben | *Silene vulgaris* | Örtel (1886) | c. 1875 |
| Germany | Ahrtal | *Silene vulgaris* | Niesen (1937) | ≤1937 |
| Germany | Bonn | *Silene vulgaris* | Niesen (1937) | ≤1937 |
| Germany | Rodenberg | *Silene vulgaris* | Niesen (1937) | ≤1937 |
| Germany | Bayern, Oberfranken, Lkr. Haßberge, S of Hassfurt | *Silene vulgaris* | Kruse et al. (2017) | 28 July 2016 |
| Germany | Bayern, Oberfranken, Lkr. Lichtenfels, c. 2.7 km WSW of Bad Staffelstein | *Silene vulgaris* | Kruse et al. (2017) | 10 Aug. 2016 |
| Germany | Bayern, Oberfranken, Lkr. Lichtenfels, c. 2 km N of Zapfendorf | *Silene vulgaris* | Kruse et al. (2017) | 18 Aug. 2016 |
melandrii, but also could be a different species. Further specimens and DNA analysis are needed to assess whether T. melandrii is also found in the wild on further species.

**Discussion**

Thecaphora melandrii is seen to be a scarce species, with relatively few records, and is apparently restricted to Europe. It does, however, have quite a wide range,
Table 4. Literature records which have been excluded from maps and analyses in the main part of the paper. Some are known errors, most were recorded on hosts from which *Thecaphora melandrii* has not been confirmed by DNA analysis. Given the frequency of strict host specificity in smuts, it would be unwise to assume that these are the same species without further evidence.

| Country | Location | Host | Source | Date | Notes |
|---------|----------|------|--------|------|-------|
| Germany | Hettstedt, Burgörner | *Silene latifolia* | Scholz & Scholz (2012) | | This record is apparently a misidentification of *Microbotryum hychnidis-dioicae* (Kruse et al. 2015). |
| Kazakhstan | | *Silene alexandrae* B.Keller | Schwartzman (1902) | | For this would be a new host; confirmation from DNA required. |
| Spain | Santiago de Compostela and Padrón | *Silene legionensis* Lag. | Losa España (1952) | | This would be a new host; confirmation from DNA required. |
| Spain | Valle de Ordesa | *Silene saxifraga* L., *Silene nutans* | Losa España (1948) | Aug. 1946 | Reported in the anthers with no mention of other parts of the flower, which suggests that this (despite the name) refers to a species of *Microbotryum*, although *S. nutans* is an accepted host for *Thecaphora melandrii*. Piątek et al. (2013) describe *M. silenes-saxifragae* from the eponymous host. Several similar records from Spain are taken as referring to *Microbotryum* by Almaraz (2002). |
| USA | Mt Majestic, Big Cottonwood Canyon, Utah | *Silene menziesii* Hook. | Garrett (1910); Jackson (1920) | 27 Aug. 1906 | All as 'Sorosporium saponariae', confirmation from DNA required. |
| USA | nr Mt Rose, Nevada | *Silene sargentii* S. Watson (as *S. watsonii*) | Jackson (1920) | 21 July 1918 | |
| USA | La Sal Mts, nr Gold Basin, Utah | *Stellaria curtisi* (Ryd.) | Garrett (1914); Jackson (1920) | 8 July 1911 | |
| USA | Golden, Colorado | *Pseudostellaria jamesiana* (Torr.) W.A.Weber & R.Hartm. (as *Stellaria jamesiana*) | HUV22012 | 6 July 1974 | This would be a new host; confirmation from DNA required. |
particularly within central and eastern Europe, and here we demonstrate an extension of the known range to England. The ITS and 28S regions of the genome show little variation, suggesting that *T. melandrii* is homogenous across this range. However, it has a wide host range including species in two genera within the Caryophyllaceae, and on quite distantly related species within the genus *Silene*. These conditions all suggest that it may be distributed more widely, both geographically and across hosts, and that further searching for this species would be valuable.

The coexistence of *Thecaphora melandrii* with species of *Microbotryum* is interesting; the *T. melandrii* infection is systemic and deforms the anthers (and other floral organs) in the host, presumably excluding plants from infection by *Microbotryum* spp. But it would be interesting to examine whether infection by *Microbotryum* spp. has any relation to subsequent infection by *T. melandrii*, and how these relations play out in a population over time. This would be a valuable supplement to the evidence for coexistence of different *Microbotryum* species on the same hosts in Abbate et al. (2018).

**Fig. 4.** Bayesian inference of phylogenetic relationships within the sampled *Thecaphora* specimens: see Phylogenetic analyses under Materials and Methods for details. Numbers on branches before slashes are estimates for a posteriori probabilities, numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. The specimens from England are highlighted in orange. *T. = Thecaphora*
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