

Anther smuts on Butterworts (*Pinguicula* spp.)

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Abstract

Microbotryum pinguiculae has recently been reported from Wales as new to Britain, and we present details of locations and infection rates, and also report a first record for Scotland. We expand previous molecular phylogenetic analyses to include a specimen from Wales, and also examine specimens from Austria infecting *Pinguicula leptoceras*. All these new collections are referable to *M. pinguiculae* s.str., demonstrating that this smut species can infect these two closely-related host species.

Introduction

Smut species in the genus *Microbotryum* infect a range of different plant organs (Kemler *et al.* 2020). There is a particularly wide range of cryptic species found in the anthers of different host plants, many of which are in the Caryophyllaceae. But a recent paper (Ziegler *et al.* 2018) focussed on the anther smuts in butterworts (*Pinguicula* spp.), which have previously been found to be quite distantly related to anther smuts infecting Caryophyllaceae (Lutz *et al.* 2005; Kemler *et al.* 2006, 2009). Ziegler *et al.* (2018) considered specimens from three host species, *Pinguicula alpina* L., *P. villosa* L. and *P. vulgaris* L. and discovered that each supported a different segregate of *Microbotryum*. *P. vulgaris* is the type host of *M. pinguiculae* (Rostr.) Vánky, a name which had hitherto been used as a catch-all for anther smuts on *Pinguicula* spp. The smut on *P. alpina* was described as *M. alpinum* R. Ziegler, M. Lutz & Piątek, and that on *P. villosa* as *M. liroi* R. Ziegler, M. Lutz & Piątek, separating them into three species.

***M. pinguiculae* in Britain and Ireland**

In Britain and Ireland there are three native *Pinguicula* species: *P. lusitanica* L. and *P. vulgaris* are relatively widespread, and *P. grandiflora* Lam. is restricted to southern Ireland. There were no records for *M. pinguiculae* s.l. in the Fungus Records Database of Britain and Ireland (FRDBI), and the species is not included in Legon & Henrici (2005) or its updates until update 9 (Ainsworth & Henrici 2020), so seemed to be previously unknown in Britain. However, the Biological flora of the British Isles account of *Pinguicula* (Heslop Harrison 2004) says that “The anthers of *P. vulgaris* are attacked by *Ustilago pinguiculae* Rostrup in Europe [...]; a brownish discoloration of the anthers has sometimes been observed in British plants, perhaps also owing to this infection”, so it seems that it has previously been suspected to be present in Britain, if not formally recorded.

Setting this as a challenge for the Welsh Rust Group led very quickly to its discovery by RGW at Pentrosfa Mire, vc43 (see table 1 for full details) on *P. vulgaris*, apparently the first formal record for Britain (Woods *et al.* 2018). The spore deposit of *M. pinguiculae* is pale violet (or brownish violet) as described by Ziegler *et al.* (2018), but in some specimens can appear more brown because of the purple colour of the corolla (Fig. 1). Detailed spore pictures are available in Ziegler *et al.* (2018).



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Fig. 1. Brownish tinged spores of *M. pinguiculae*; Pentrosfa Mire (see Table 1 for full details).

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Fig. 2(a). Longitudinal section through infected flower showing the way the infected anthers are hidden. Flush on moorland slope 3.5 km E of Ffair-rhos, Ceredigion, SN773680, 400 m, 1 June 2018.



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Fig. 2(b). Two corollas (left) pulled apart to expose the infected anthers; an uninfected flower is shown on the right. Flush on moorland slope, Bryn Llestydd, 6 km E of Tal-y-bont, Ceredigion, SN718896, 350 m, 2 June 2018.

The main challenge in detecting the infection is that the throat of the corolla is very closed in *Pinguicula vulgaris*, in contrast to *P. alpina*, so that it is necessary to pull the corolla apart to see the discoloured anthers inside (Fig. 2).

Some infected flowers have the upper lip somewhat contorted, which narrows the entrance to the flower even further (Fig. 3; Woods *et al.* 2018, p41).

M. pingiculae has now been found from several sites in Wales (Fig. 4). Woods *et al.* (2018) reported high rates of population infection, and high rates of infection within infected populations, and Fig. 3 shows the proportions of each population examined that were infected. Some of these populations of *Pinguicula vulgaris* were tiny,

so the percentages are only very crude approximations, but they do demonstrate that the infected plants were in the majority in many populations. Many populations of *Pinguicula vulgaris* are relatively isolated from each other, so the question of how the smut may be transmitted between



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Fig. 3. Infected flowers of *P. vulgaris* showing the somewhat flattened corollas.

them is an open one. Heslop-Harrison (2004) says that they are pollinated by bees, but it is still unlikely for a single bee to transfer spores between separated populations.

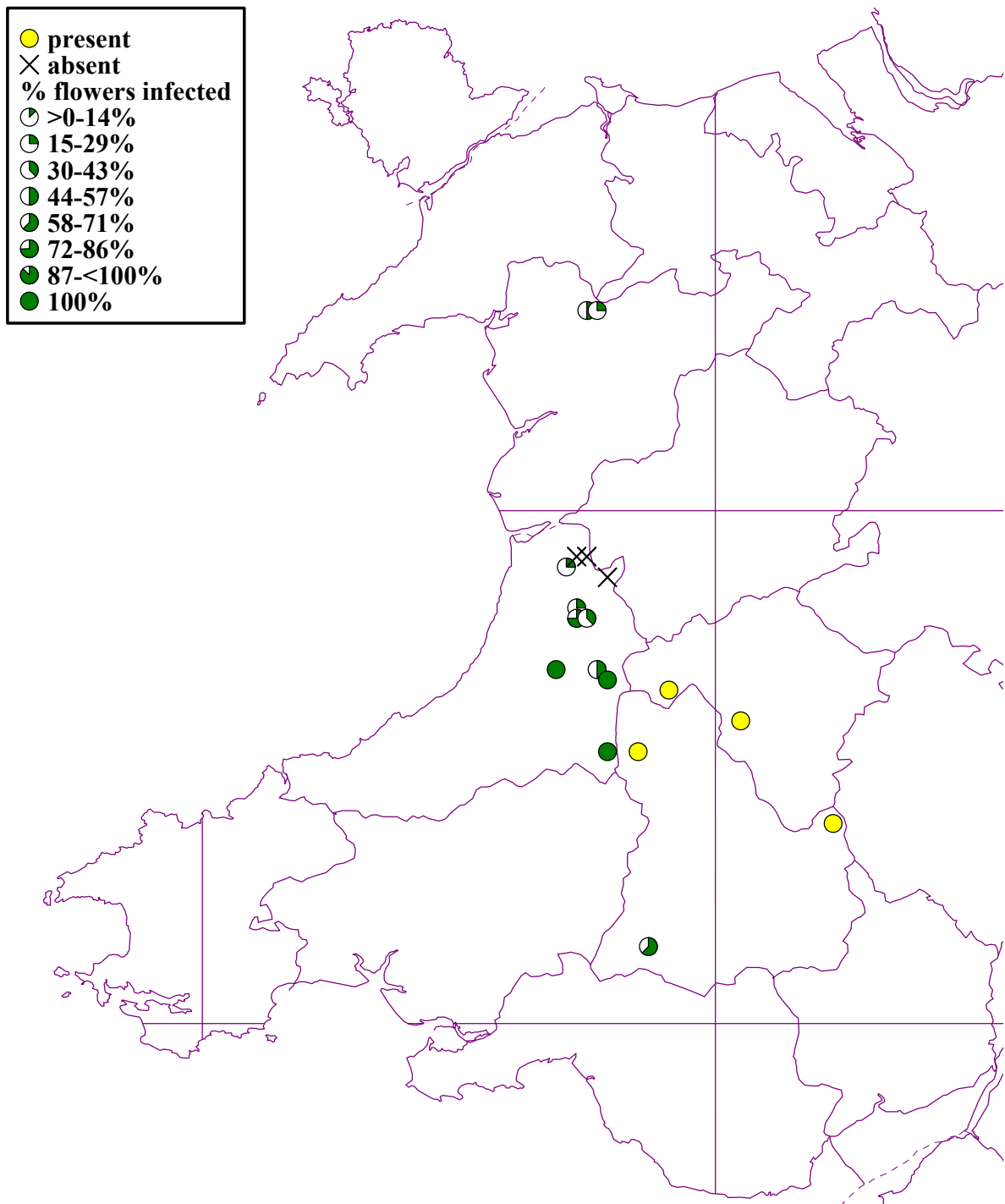


Fig. 4. Distribution of *Microbotryum pinguiculae* in Wales, and (where recorded) infection rates of examined populations of *Pinguicula vulgaris*.

Even within a population it is interesting to consider how *M. pinguiculae* persists. Schellenberg (1911) observe that all the flowers on a single plant are infected, and that the same stock produces infected flowers in all years, and therefore concludes that the mycelium is systemic in the stock; Lind (1913) also says that the infection is systemic. AOC found several flies well within the flowers of an uninfected population of *P. vulgaris* on Pumlumon, above Llyn Llygad Rheidol (600 m altitude), vc46. These were determined by Mike Howe as *Empis verralli* Collin, a relatively widespread species. These

are potentially vectors for within population transfer of infections, but not likely to effect exchange between populations.

Pinguicula vulgaris is an early-flowering species, but some finds have been made slightly later in the season from corollas still attached to the stem. Krieglsteiner (2002) discovered *Microbotryum pinguiculae* at Sippenauer Moor in Germany in late June and commented that the only flowering plants were infected ones. So the smut may extend or delay the flowering period. But this does not seem a useful adaptation to dispersal by insect pollination.

The effect of the smut on seed production in its host is also interesting. From visits to Pentrosfa Mire it seems that infected flowers (which are rendered male sterile) do not set seed. Some populations in Cardiganshire appeared to be in decline, though this could be for reasons unconnected with infection by *M. pinguiculae*. Nordin (2015) found similarly high rates of infection and low rates of seed set for *M. alpinum* on *Pinguicula alpina* in Gotland.

Karlsson *et al.* (1990) study resource allocation in reproduction in *Pinguicula alpina*, *P. villosa* and *P. vulgaris*. They discover that only small proportions of plants flower in any year and suggest that “it is possible that the low flowering frequency in *Pinguicula* is an adaptation to avoid diseases or pathogens coupled to the reproductive cycle,” and mentioning *M. pinguiculae* (as *Ustilago*). Some examination of the population dynamics of *Microbotryum* smuts and their hosts would be valuable.

Host	Country	GenBank accession nos. (ITS/28S)	Collection details
<i>Pinguicula leptoceras</i>	Austria	MT663526/MT663522	Tirol, Ötztal, Vent, Quellflur north of the village, 46°51'58.32"N, 10°54'55.41"E, ca. 1970 m asl., 23 June 2018, leg. H. Fischer & M. Lutz, KR-M-0005958
<i>Pinguicula leptoceras</i>	Austria	MT663527/MT663523	Tirol, Ötztal, Vent, Wildspitzliftweg, wayside, 46°51'45.88"N, 10°54'52.26"E, ca. 1950 m asl., 23 June 2018, leg. H. Fischer & M. Lutz, KR-M-0019504
<i>Pinguicula leptoceras</i>	Austria	MT663529/MT663525	Tirol, Ötztal, Vent, street to Rofen, roadside, 46°51'27.01"N, 10°54'25.02"E, ca. 1985 m, 23 June 2018, leg. H. Fischer & M. Lutz, KR-M-0019505
<i>Pinguicula vulgaris</i>	UK	MT663528/MT663524	Pentrosfa Mire, Llandrindod Wells, vc43, SO059597, 30 May 2018, leg. R.G. Woods, KR-M-0019510

Table 1. Specimens used in this study, additional to those in Ziegler *et al.* (2018). Accession numbers are for mycological herbarium of the Staatliches Museum für Naturkunde Karlsruhe (KR-M).



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Fig. 5(a). Infected flower of *P. leptoceras* *in situ*.



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Fig. 5(b). Infected flower of *P. leptoceras* showing the swollen anthers and spores.

PAS found *M. pinguiculae* on *P. vulgaris* in Scotland in 2019 (at Loch Ashie, vc96, NH6234 and NH631357, 25 June 2019), and a first formal record for England has recently appeared on the web at <https://www.inaturalist.org/observations/47569365>. So far, we know of no reports of infections on the other species of *Pinguicula* which are found in Britain and Ireland. *P. lusitanica* is quite distantly related to all the other species considered in this paper, but *P. grandiflora* is in the same clade as the other hosts considered here (Degtjareva *et al.* 2006).

Molecular phylogenetic analysis of anther smuts on *Pinguicula leptoceras* and *P. vulgaris*

Meanwhile, ML managed to collect some further infected *Pinguicula* plants, this time of *P. leptoceras* Rchb., from a cluster of three sites near to each other in Austria (Fig. 5a & b). The ITS and 28S D1/D2 sequences were extracted from these collections and from a specimen from the first-discovered Welsh site at Pentrosfa Mire (collection details and GenBank sequence accession numbers are given in Table 1). The concatenated sequences were used in a phylogenetic analysis alongside the specimens previously analysed by Ziegler *et al.* (2018), and using the same methods (which are therefore not redescribed here). The tree was rooted with *Microbotryum betonicae* (Beck) R. Bauer & Oberw.

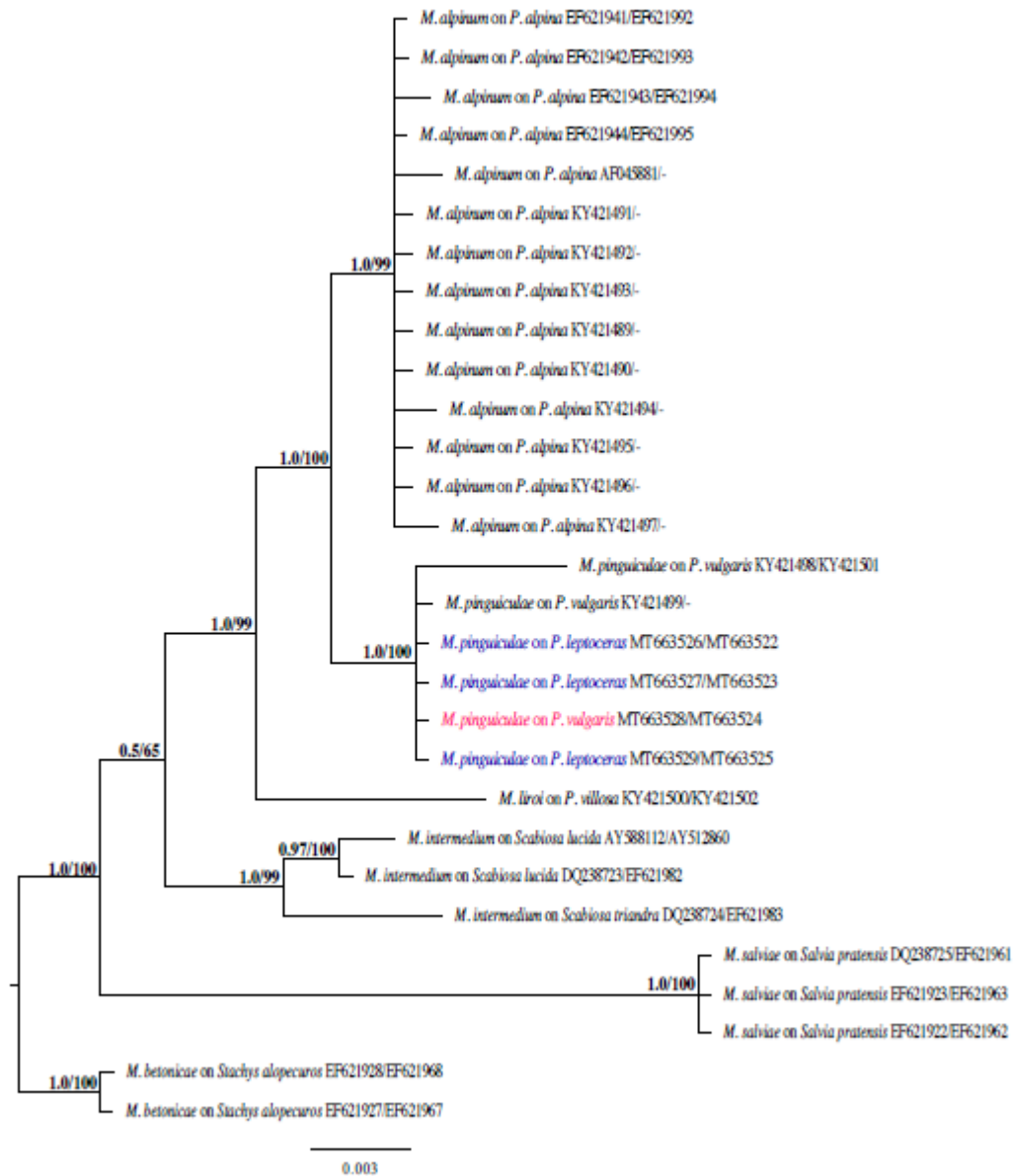


Fig. 6. Bayesian inference of phylogenetic relationships within the sampled *Microbotryum* species. Markov chain Monte Carlo analysis of an alignment of concatenated ITS+28S sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and an estimated proportion of invariant sites, random starting trees, and default starting parameters of the DNA substitution model. 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 *Microbotryum betonicae*. 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. *M.* = *Microbotryum*; *P.* = *Pinguicula*. The new specimens in this study are shown in blue (on *P. leptoceras*) and pink (on *P. vulgaris*).

A phylogenetic hypothesis is shown in Fig. 6. All the new specimens show both identical ITS and 28S sequences and cluster closely with the previously sequenced collections of *M. pinguiculae* s.str. The differences between the new specimens from *P. leptoceras* and *P. vulgaris* and the two specimens available in Genbank (both from *P. vulgaris*: STU 10004567401, KY421498/KY421501 (ITS/28S), Germany; GLM 046919, KY421499 (ITS), Austria) are small (ITS: 1 bp, 28S: 5 bp), and the presented consensus tree as well as the ITS and 28S data suggest that the anther smut on the two different

host plant species are not differentiated on the basis of the genes considered. We infer that *M. pinguiculae* is a species capable of infecting multiple closely-related species in the genus *Pinguicula*. Therefore *P. leptoceras* can be added to the account of Ziegler *et al.* (2018) as a further host species, and the UK can be added to the known distribution of *M. pinguiculae*.

These relationships amongst infections on *Pinguicula* spp. closely mirror relationships within the host family itself. Degtjareva *et al.* (2006) show that *P. leptoceras* and *P. vulgaris* are closely related within the same clade within *Pinguicula*, and that *P. alpina* and *P. villosa* are both distantly related, falling in separate clades. This is one of many examples of parasitic fungi acting as plant taxonomists (e.g., Savile 1954, Kukkonen & Timonen 1979).

European distribution

M. pinguiculae was separated from *Ustilago violacea* s.l. by Rostrup in 1890, and there was a period at the end of the 19th century when it was frequently recorded. This was followed by a period when it was either forgotten or became unfashionable, and there were relatively few records. An upsurge in modern records is associated with the description of new species. The European distributions of species on hosts dealt with here and by Ziegler *et al.* (2018) are shown in Fig. 7. *M. alpina* seems to infect *P. alpina* throughout its range (as summarised by Casper 1972), and *M. liroi* similarly seems to have been recorded across the range of *P. villosa*. *P. vulgaris*, however, the most widely distributed species in the genus in Europe (Casper 1972) has not been recorded as infected across its range. Indeed several authors have commented that they did not find it while specifically searching (e.g., Liro 1924, Kemler *et al.* 2005). In contrast, the recent (formal) discovery in Britain, and the relatively high rate of finds among the searched populations, suggests that it awaits discovery in other places in the range of *P. vulgaris*. Further targeted searching is needed to expand our knowledge of the distribution of *M. pinguiculae*.

Conclusions

It seems that *M. pinguiculae* will prove to be widespread in Britain, and is potentially present elsewhere in Europe, and we encourage micromycologists to actively search for it – it is very unlikely to be discovered by casual observation. There are several aspects of the ecology and life history of *M. pinguiculae* which seem to be unknown, and which would benefit from some observational research. And there is plenty of scope for examination of further relationships within *Microbotryum* taxa on *Pinguicula* spp. – Ziegler *et al.* (2018) list several other species from which *M. pinguiculae* has been reported. We would be interested to hear of infections on any other species of *Pinguicula*.

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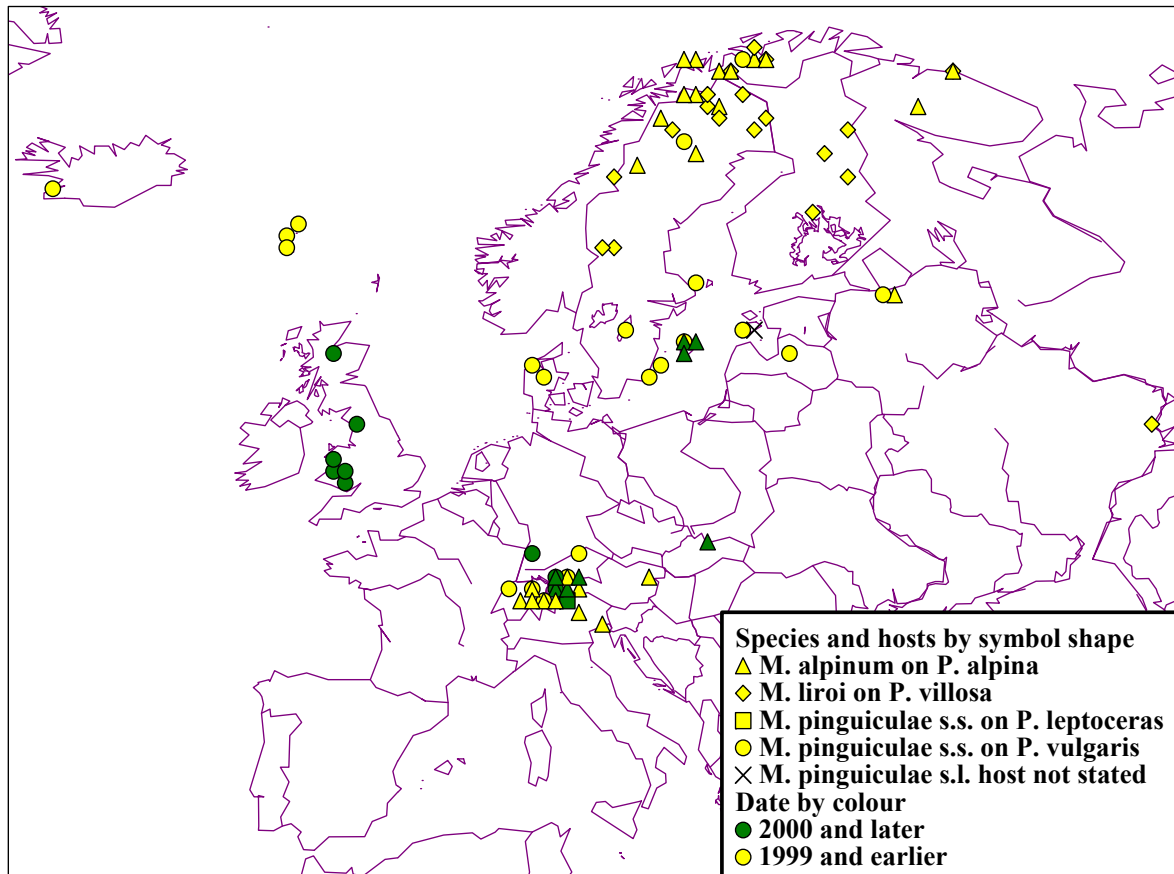


Fig. 7. European distributions of *Microbotryum* spp. on *Pinguicula* spp. covered in this paper and in Ziegler *et al.* (2018), based on published and web-accessible records. Symbols indicate one or more records in a 30' × 45' square. Note that some symbols are superimposed. Map produced with DMAP.

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