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Generativní rozmnožování a funkční vlastnosti semen parazitických Orobanchaceae

Bakalářská práce

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Abstrakt

Parazitické rostliny tvoří důležitou součást suchozemských ekosystémů. Některé parazitické druhy jsou plevely a představují hrozbu pro zemědělství rozvojových zemí. Tudíž je pochopení těchto rostlin nezbytné pro ochranu přírody a ekonomiky. V této práci se zaměřuji na ekologii a evoluci velikosti semen v čeledi *Orobanchaceae*, jedné z největších monofyletických skupin parazitických rostlin. V práci používám data o hmotnosti semen poskytnutá Seed Information Database (Kew RBG) pro analýzu variability hmotnosti semen napříč jednotlivými rody v čeledi. Pokouším se sledovat evoluci velikosti semen v čeledi použitím rekonstrukce ancestrálních stavů. Dle ní se prachová semena musela v čeledi vyvinout alespoň třikrát. Dalším bodem zájmu jsou tzv. trpasličí semena. Pokouším se kultivovat rostliny druhu *Parentucellia viscosa* a *Bellardia trixago* ve snaze zjistit, zdali samovolně klíčí. Oba druhy ochotně klíčí bez dormance nebo chemického stimulu.

Abstract

Parasitic plants form an integral part of terrestrial ecosystems. Some of the species are weeds threatening agriculture particularly in the developing world. Understanding their biology is paramount for conservation and the economy. In this thesis, I focus on the ecology and evolution of seed size in the family *Orobanchaceae*, one of the largest monophyletic groups of parasitic plants. I use seed mass data provided by the Seed Information Database (Kew RBG) to analyze the seed mass variation of individual genera in the family. I attempt to track the evolution of seed size in the family using ancestral state reconstruction. According to it, dust seeds evolved at least 3 times in the family. Another point of interest are dwarf seeds. I attempt to cultivate *Parentucellia viscosa* and *Bellardia trixago* to find out, whether dwarf seeds germinate autonomously. Both species germinate readily without dormancy or need for a chemical stimulus.

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Generativní rozmnožování a funkční vlastnosti semen parazitických Orobanchaceae

Generative reproduction and functional traits of seeds of parasitic Orobanchaceae

Parazitické rostliny představují významnou funkční složku suchozemských ekosystémů. Pro většinu parazitických rostlin je typický větší význam generativního rozmnožování semen oproti ostatním krytosemenným. Tomu odpovídají i investice do kvetení a produkce semen. Jednotlivé druhy parazitických rostlin se ovšem zásadně liší ve velikosti semen, strategiích jejich rozšiřování, způsobu klíčení a napojení na hostitele.

Cílem bakalářské práce bude zpracování literární rešerše na téma strategie generativního rozmnožování a funkční vlastnosti semen u parazitických rostlin z čeledi Orobanchaceae. Čeleď Orobanchaceae zahrnuje okolo 40% parazitických druhů, přičemž většinu z nich tvoří zelení poloparaziti. Jednoduché druhy v rámci čeledi se výrazně liší právě ve funkčních vlastnostech semen a strategiích klíčení. Mimo jiné jsou zde přítomny i druhy, které mají prachová semena (tzv. „dust seeds“) a pro klíčení vyžadují indukci hostitelem. Evoluční proces vedoucí k prachovým semenům není stále plně pochopen a plánovaná rešerše by mohla pomoci formulovat otázky, které povedou k pokroku v této oblasti.

Jedním ze zajímavých směrů by mohlo být studium druhů s tzv. trpasličími semeny, tj. semeny, která jsou velmi malá, ale stále ještě obsahují dostatek zásobních látek pro samostatné klíčení. Kořenový poloparazit *Parentucellia viscosa* představuje jeden z druhů, jehož semena mají přesně tyto vlastnosti. Experimentální část bakalářské práce se proto zaměří na biologii klíčení tohoto druhu.

Metody a postup práce

1. Rešerše literárních zdrojů (podzim 2018, zima 2019)
2. Analýza evoluce velikosti semen v Orobanchaceae (podzim 2018, zima 2019)
3. Kultivace *Parentucellia viscosa* ve skleníku (zima 2019)

Literatura:

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Proclamation

I hereby declare I have written this thesis on my own with the use of literature sources cited in references.

Brno 31st July 2020

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Maroš Šlachtič

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1. INTRODUCTION

1.1 Ecology of seed size

The traditional ecological understanding of seed size says, there is a tradeoff between seed size and the number of seeds. This is based on the logical assumption, that a plant of limited resources can either produce a smaller number of larger seeds or a larger number of smaller seeds. Since seed size has a positive relationship with nutritional storage, larger seeds have a clear advantage in establishing themselves in suboptimal conditions. This is consistent with the idea of R-selection and K-selection. While this model vastly holds true to this day, recent research suggests, it is incomplete (Moles and Westoby, 2004, 2006). Moles (2004) notably points out, that although larger seeds do have a higher survival rate through the seedling stage, this advantage is not enough to counter-balance the sheer number of seeds small-seeded plants can produce for the same reproductive effort. Furthermore, while small-seeded plants produce a much higher number of seeds per unit of canopy, large-seeded plants, in general, produce more biomass and therefore more square metres of canopy, while also each individual produces seeds for a longer amount of time, than those with smaller seeds. Moles (2004) concludes, there is no detectable relationship between seed size and the number of seeds produced per adult plant over its lifespan. In addition to the seed-size/seed number trade-off, Moles (2005) states, seed size depends on the life history, dispersal syndrome (e.g. an anemochoric species is likely to have smaller seeds than a barochoric species), ability to form a persistent seed bank (smaller seeds tend to last longer in seed bank), and other characteristics. In her 2006 paper, Moles (2006) traces the impact of seed size on the survival rate of plant species through each life stage. She finds a negative relationship between seed size and the presence of the species' seeds in the seed rain. This numeric advantage of small-seeded plants is however evened out in the seedling phase, where both large-seeded seedlings and small-seeded seedlings are represented equally (statistically speaking). In this thesis, I focus on the strategy used by some groups of plants of developing extremely small seeds with a reductionist morphology – dust seeds. It's important to point out that Moles (2006) does not specifically account for dust seeds. While the overall number of seedlings emerged from small-seeded plants is comparable to that of large-seeded species according to her findings, the numerical advantage should

not be discounted. Plants with dust seeds are by definition specialists (resorting to obligatory parasitism or mycoheterotrophy), therefore the sheer number of seeds poses a tremendous advantage in finding ideal conditions to flourish. This might be a contributing factor to her finding, that more individual small-seeded plants make it to adulthood than large-seeded plants.

1.2 Extremely small seeds

Eriksson (2011) describes two categories of very small seeds: dwarf seeds and dust seeds. Dust seeds often possess an undifferentiated embryo consisting of only a few cells and exhibit either a considerable reduction or a complete lack of endosperm. Dust seeds are often produced in millions per plant per year (Arditti and Ghani, 2000). Dwarf seeds are somewhat larger, but still very small seeds, where the embryo and endosperm are reduced to a considerably smaller extent. These categories are polyphyletic, dust seeds having evolved independently in at least 12 families (Eriksson and Kainulainen, 2011). Of these families, Orobanchaceae are the only demonstrated as haustorial parasites, the rest is either mycoheterotrophic, or more research is needed regarding their hosts. There is, therefore, no single morphology of a dust seed and the similarities between different lineages are the result of convergence. It is always an issue to define a polyphyletic trait and many authors have used arbitrary boundaries (usually seed length, or dry weight) to determine, whether a seed is a dust seed, or not. While this method is useful in practice, it makes no sense from the evolutionary standpoint. Therefore in this thesis, I define dust seeds as extremely small seeds, that have lost their ability to reach a photosynthetically active state in nature without the need for an external host (be it plant, or fungus) at the germination phase, while dwarf seeds are substantially larger, but still very small seeds, that however do have enough nutritional storage to germinate and photosynthesize without a host. This is consistent with Eriksson's (2011) idea of dwarf seeds being an evolutionary stepping stone towards dust seeds. One author, in particular, provides a useful hint on how to apply this definition in practice. Raven (1999) determines, that there is a minimum seed mass that a seed needs to possess in order for the plant to be able to acquire its first non-seed energy through photosynthesis. Raven determines this threshold to be around 5 μg of dry weight, with the plant *Sonerila heterostemon* of *Melastomaceae* as an example. This species therefore by my definition represents the smallest example

of dwarf seeds and any seed with a lower dry weight must be parasitic/heterotrophic at least to some extent.

1.3 Parasitism in plants

Parasitic plants acquire at least a part of their resources from another organism while harming the other organism. There are two types of parasitic plants: haustorial parasites and mycoheterotrophs. Haustorial parasites use specialized organs called haustoria to penetrate the tissues of another plant and acquire water, sugars and other resources directly from a host. Mycoheterotrophs abuse mycorrhizal relationships by either not giving resources back to the fungus, or providing significantly fewer resources in return. Parasitic plants, that produce their own organic compounds through photosynthesis are mixotrophic, those that do not are heterotrophic. In haustorial parasites, mixotrophs are traditionally called hemiparasites, while heterotrophs are called holoparasites. It should be noted the traditional classification into holoparasites and hemiparasites should be taken with a grain of salt since the trophism of parasitic plants exists on a scale. For example, the species *Cuscuta reflexa* produces a photosynthetic apparatus, that is largely inactive in the wild since the plant relies on its host for the source of carbon (Choudhury and Sahu, 1999). The plant has however been successfully cultivated without a host and forced to photosynthesize in a laboratory setting (Choudhury and Sahu, 1999). This also demonstrates the flaws of another traditional classification: obligatory and facultative parasitism. While in some cases, it is useful to classify parasites on whether they require a host to function, or not, one should always bear in mind nothing is black and white in biology when using these terms. Facultative parasites rarely grow without a host in the wild, or suffer a significant disadvantage without a host (Těšitel, 2016), while species like *Cuscuta reflexa*, considered obligatory can be grown as autotrophs in isolation (Choudhury and Sahu, 1999).

1.4 Parasitism in Orobanchaceae

Orobanchaceae is one of the two largest monophyletic groups (the other being *Santalales*) containing haustorial parasites with roughly 2000 parasitic species out of a total of 4500 total haustorial parasite species in angiosperms (Těšitel, 2016). While *Orobanchaceae* are widely classified as root parasites, there are instances, where species

of *Orobanchaceae* can penetrate both roots and rhizomes (Heide-Jørgensen, 2013), therefore caution should be used when talking about root and stem parasites in *Orobanchaceae*.

It is still up for debate, whether all species of *Orobanchaceae* are parasitic, or not. 2 basal genera, namely *Mimulus* and *Lindenbergia* have been used as examples of free-living *Orobanchaceae* (Westwood *et al.*, 2010), however, *Mimulus* was recently reclassified in the family *Phrymaceae* and *Lindenbergia* is sometimes classified as *Plantaginaceae* (*Tropicos* / *Lindenbergia indica* (L.) Vatke, 2020). However, whether the family contains free-living species, or not, it is still the only family of haustorial parasites, that contains a full range of parasitic life forms – facultative hemiparasites (*Triphysaria*), obligate hemiparasites (*Striga*) and obligate holoparasites (*Orobanche*) (Westwood *et al.*, 2010). Furthermore, the family spans over 6 orders of magnitude in seed size (Royal Botanic Gardens Kew, 2019) containing regularly-sized seeds (e.g. *Rhinanthus*, *Odontites*, *Melampyrum*), dwarf seeds (e.g. *Bellardia*, *Parentucellia*), and dust seeds (*Alectra*, *Orobanche*, *Striga*). This makes it a unique family for studying the evolution of dust seeds.

1.5 Reproduction in *Orobanchaceae*

The most common mode of reproduction in *Orobanchaceae* is sexual reproduction. Since the vast majority, if not all species of *Orobanchaceae* are parasitic, this is in-line with the Red Queen Hypothesis. The hypothesis states, there is an ongoing evolutionary arms race between the host and the parasite, where the host is constantly trying to reject the parasite, while the parasite is trying to break the host's defences (Ladle, 1992). Since the rate of evolution depends on the rate at which new traits and genetic variation emerge, there is a selective pressure for both sides to reproduce sexually. While sexual reproduction and outcrossing is the most common reproductive strategy in *Orobanchaceae*, some of the species are capable of cross-pollination as well as self-pollination, and even apomixis (Joel, 2013). This has been demonstrated in the genus *Cistanche* as well, as *Orobanche uniflora*, both of which have dust seeds (Pazy, 1998). These species are likely polyploids and the apomixis is a result of meiotic failure and inability to produce sexually. This could, however, be reinforced selectively, as not having to pollinate and fertilize a large number of dust seeds could be an advantage.

Therefore it seems reasonable to assume, some degree of self-pollination and/or apomixis is selective in species with dust seeds, as while keeping the genetic variation through sexual reproduction is integral for the parasite, it is likely the number of ovules per flower is so high, some ovules will never get pollinated in the traditional way.

In Orobanchaceae obligate parasites tend to produce a larger number of smaller seeds (0.2–2 mm), with of higher longevity (up to 15 years), than the seeds of facultative parasites (Joel, 2013). This is likely due to the need of obligatory parasite to find a suitable host, as while the distribution through space is provided by their numbers and ease of dispersal, it is also highly beneficial to distribute the seeds through time via dormancy and the formation of a seed bank. The efficient dispersal through both time and space is what makes obligatory parasites of Orobanchaceae such noxious agricultural pests, according to Eriksson (2011) infection of a field by *Striga* or *Orobanche* in the developing world often leads to the farmer abandoning the land altogether.

It has long been known, that species with dust seeds within Orobanchaceae only germinate when their seeds detect a suitable host around. We now know that the dust seeds won't germinate until they sense a host nearby. The most common germination stimulants for dust seeds in Orobanchaceae are strigolactones. While we have known parasitic plants can sense strigolactones exuded by host's roots since the 1960s, it wasn't until 2005 that their benefit for the host plant was discovered. We now know that plants exude strigolactones from their roots to attract mutualistic fungi forming arbuscular mycorrhiza (Akiyama, Matsuzaki and Hayashi, 2005). Using strigolactones as germination stimulants is a very efficient strategy, as their combinations show high specificity (Westwood *et al.*, 2010), so the parasite can sense, whether there's a suitable host nearby very reliably. Furthermore, this strategy makes a lot of evolutionary sense, because abandoning the use of strigolactones to avoid parasitism would require to overcome the selective pressure for retaining the ability to attract mycorrhizal fungi.

In autonomously germinating species, attachment to the host occurs after an independent seedling stage (Těšitel, 2016). This can take multiple weeks. This mode of germination is typical for root hemiparasites and stem parasites. Dormancy or cold stratification is common in root-hemiparasitic Orobanchaceae, particularly the rhinanthoids (Borg, 2005). Cold stratification is most common in the temperate region

and provides a phenological advantage, as the species germinate in late winter/early spring when competition for light is low.

Since the dwarf seeds are likely an evolutionary stepping stone to dust seeds, it would be interesting to find out, whether they also rely on a chemical stimulus to germinate. This has not been the focus of many studies, that is why I test this on two Orobanchaceae species with dwarf seeds in the practical part of my thesis. Since both species (*Parentucellia viscosa* and *Bellardia trixago*) are from the Mediterranean region, it is unlikely they require cold stratification.

1.6 Observed species

Part of this thesis is attempting to cultivate two species with dust seeds: *Parentucellia viscosa* and *Bellardia trixago* (Fig.1). These annual species are closely related grassland hemiparasites native to the Mediterranean region but also invasive in subtropical regions around the world including the United States (USDA, 2020a, 2020b), Japan (Suetsugu *et al.*, 2012), and even New Zealand (Johnson, 1982).

The seeds of both species are oval-shaped measuring roughly half a millimetre in length. At this size, the seeds are a typical representation of dwarf seeds. As facultative hemiparasites, their host profile is expected to be broad. According to an older study on the differences in fecundity and wellness (Atsatt and Strong, 1970), both species are facultative parasites able to grow without a host, although with a significant reduction in wellness. Furthermore, while *Parentucellia* does well on a broad range of phylogenetically unrelated hosts, *Bellardia* does considerably better on the genus *Trifolium*, than other genera. This has likely influenced the invasive potential, as *Parentucellia* appears to be spreading much more rapidly and readily, than *Bellardia*.



Figure 1: Left: *Bellardia trixago*, Right: *Parentucellia viscosa*

Source:

https://commons.wikimedia.org/wiki/File:Bartsia_trixago_%3D_Bellardia_trixago.JPG

https://commons.wikimedia.org/wiki/File:Parentucellia_viscosa_Planta_2009-4-26_SierraMadrona.jpg

2. AIMS

This thesis consists of a theoretical and a practical part. In the theoretical part, I aim at conducting a literature review on the strategies parasitic plants of Orobanchaceae deploy in generative reproduction and how this relates to functional traits of seeds in these plants with the focus on seed size. Furthermore, I aim at providing a quick look at the evolutionary trends of changing seed size in the family using ancestral state reconstruction.

An evolutionarily interesting seed size category in the family are so-called dwarf seeds. It is currently unknown, whether these seeds germinate autonomously, the practical part of my thesis is therefore focused on attempting to germinate and cultivate two of these species: *Bellardia trixago* and *Parentucellia viscosa*.

3. METHODOLOGY

3.1 Seed size across Orobanchaceae

For the seed size analysis, I have used data publicly available in the Seed Information Database maintained by the Royal Botanical Garden (Royal Botanic Gardens Kew, 2019). I searched the database for taxa within Orobanchaceae that have an entry for seed mass. The Seed Information Database (SID from now on) contains 408 total taxa within Orobanchaceae, of which 351 have a seed mass entry. These taxa¹ are organized into 41 total genera. The seed mass data within the database is given in the form of the average weight of 1000 seeds in grams.

3.2 Ancestral state reconstruction

To visualize evolutionary trends in seed mass, I have conducted an ancestral state reconstruction. An ancestral state reconstruction uses phylogenetic data in conjunction with trait values found in recent species to estimate values found throughout their phylogeny. I have used the maximum likelihood approach, which works by statistically finding the least unlikely scenario out of all possible reconstructions.

I have used phylogeny provided by (Mcneal *et al.*, 2013) pruned to contain only taxa for which seed mass values were available in the Seed Information Database (Royal Botanic Gardens Kew, 2019).

3.3 Cultivation experiment

Seeds of *Parentucellia viscosa* and *Bellardia Trixago* were germinated on wet filter paper placed Petri dishes. The Petri dishes have subsequently been placed inside of a climatic chamber. The light cycle of the climatic chamber was set to 14 hours on, 10 hours off, with the temperature during the day set to 18 °C, and during the night to 15 °C. The chamber was set to keep 55% air relative humidity during the day and 50% during the night. The photon flux density, while the light was on, was set at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. I have chosen five species of plants to serve as potential hosts for the hemiparasitic *P. viscosa*

¹It should be noted, I am using the word “taxa” and not “species”, as there are multiple cases of subspecific taxa within the dataset. Furthermore, I was able to find and correct a number of outdated taxa within the database, but most likely not all of them, therefore I feel that talking about the number of species in the dataset would be unwise.

and *B. trixago*. The chosen species were as follows: *Festuca arundinacea*, *Bromus erectus*, *Poa bulbosa*, *Trifolium pratense* and *Trifolium fragiferum*. These were chosen out of seeds available to me, based on the preference for the families of *Fabaceae* and *Poaceae* in *P. viscosa* (Suetsugu *et al.*, 2012). The hosts were germinated in the same climatic chamber as the parasites and later transplanted into planters filled with a 9:1 mixture of vermiculite and sand (9 parts of vermiculite to 1 part of sand). This medium was chosen, because it is nutritionally inert, light, and holds moisture very well due to the porous nature of vermiculite. An inert substrate is important for a controlled experiment, as it grants the ability to control the amount of nutrients provided to the plants, making the experiment repeatable. I have established 3 planters of potential hosts for each parasite (6 in total), these were grown in a greenhouse under very stable conditions chosen to encourage successful recruitment. The photoperiod during cultivation was 11 hours on and 13 hours off. The temperature was kept at 17 °C during the day and 12 °C during the night. Each planter was watered every 3–4 days with 100 ml of distilled water mixed with a precise dose of fertilizer. The dose of fertilizer was adjusted so that over a 4 week period, each planter would receive 0.02 g of nitrogen. The fertilizer used had an NPK(Mg) rating of 15+10+15(+2).

3.4 Data analysis

Seed size data were visualized by dotcharts and boxplots. Seed size evolution in Orobanchaceae was modelled by maximum likelihood ancestral state reconstruction (Cunningham, Omland and Oakley, 1998).

Data analysis was conducted in R, version 3.6.2 (R Core Team, 2019). Ancestral state reconstruction was computed in R package “phytools” (Revell, 2012) The analysis was done with the help of my supervisor.

4. RESULTS

4.1 Seed size across Orobanchaceae

I have analyzed the average seed mass of 351 taxa within the Orobanchaceae family of plants. The seed mass varied massively spanning over six orders of magnitude with the smallest seeds belonging to *Alectra vogelii* weighing 0.3 μg and the largest seeds belonging to *Melampyrum variegatum* with average seed mass of 14 mg. This is a strikingly wide range, the lowest part of which represents some of the smallest seeds among angiosperms. The following figure (Fig. 2) graphically demonstrates seed mass variability within individual genera of Orobanchaceae. At first glance, it can be seen that most of the genera do not have species with seed mass below a certain well-defined threshold (roughly 0.05 mg). Only three genera contain species with seed mass lower than this threshold: *Alectra*, *Orobanche*, and *Striga*, all of which are known to include species exhibiting dust seeds. Considering the number of species analyzed, it is very likely, there is a physical obstacle that needs to be surpassed by evolving specialized reductionist

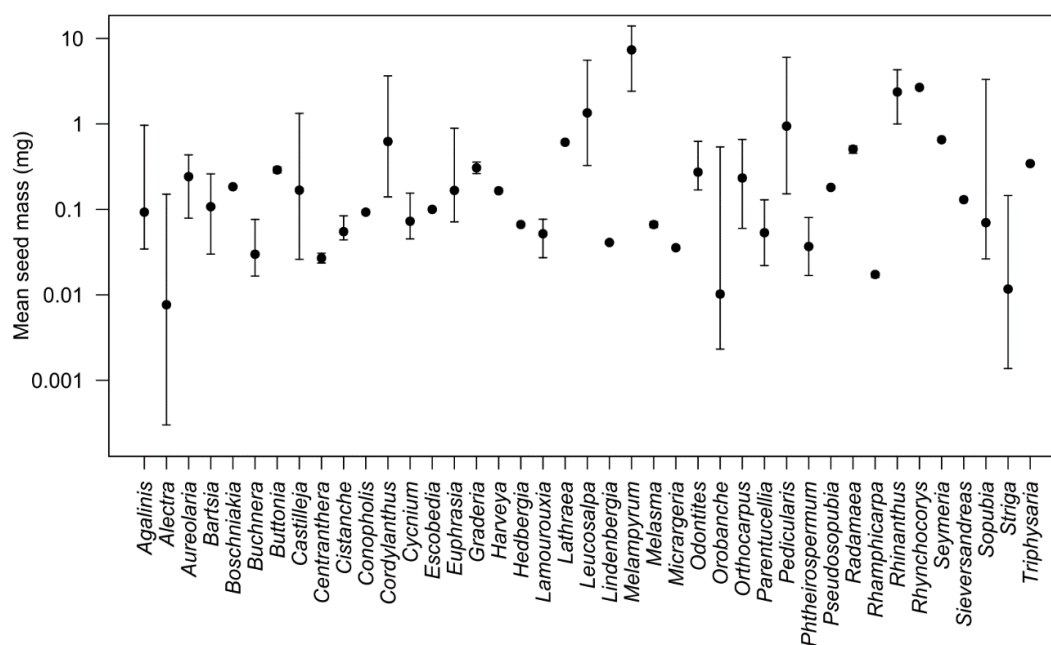


Figure 2: Dotchart demonstrating intrageneric variability of mean single seed mass in individual genera of Orobanchaceae. Seed mass values are displayed on a logarithmic scale. The points denote the geometric mean across the species within the genera and the bars represent minimum and maximum seed mass.

morphology, should a species have smaller seeds than 0.05 mg. Figure 3 shows the distribution of seed mass in the 3 genera with dust seeds.

A surprising finding was the reach of seed mass into high values within the genus *Orobanche*, which is known for its dust seeds. It should be noted, that this is mainly caused by a single species *Orobanche tarapacana* with the seed mass value of 0.54 mg.

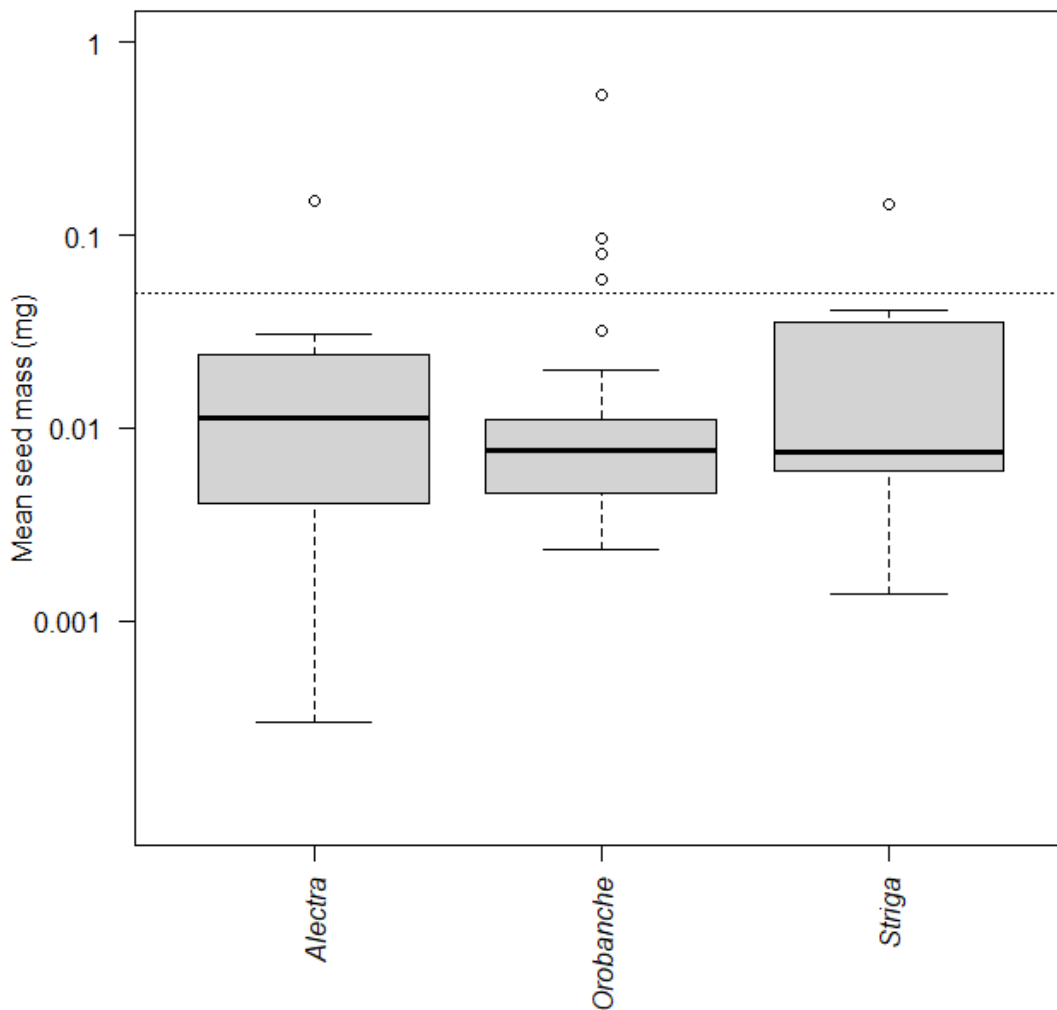


Figure 3: Box plots of the three observed genera exhibiting dust seeds. The dotted line denotes a theoretical physical boundary in seed mass, beyond which a seed must evolve a reductionist morphology (0.05mg). The Y-axis is on a logarithmic scale. Median was used to construct the boxplots, boxes denote upper and lower quartiles, outliers lie outside 1.5 times the interquartile range above the upper quartile and below the lower quartile.

The second-highest value in the genus *Orobanche* is held by *Orobanche hermonis* at 0.1mg, all of the other species are in the order of 0.01 mg, or even less.

4.2 Ancestral state reconstruction

The ancestral state reconstruction (Fig. 4) reveals that dust seeds in the family of Orobanchaceae are a result of convergent evolution, having evolved at least three times - in the species *Alectra*, *Orobanche*, and *Striga*. It would, therefore, be perfectly on point to expect structural differences between the dust seeds of these genera. There also appear to be multiple genera exhibiting dwarf seeds namely *Buchnera*, *Centranthera*, *Sopubia*,

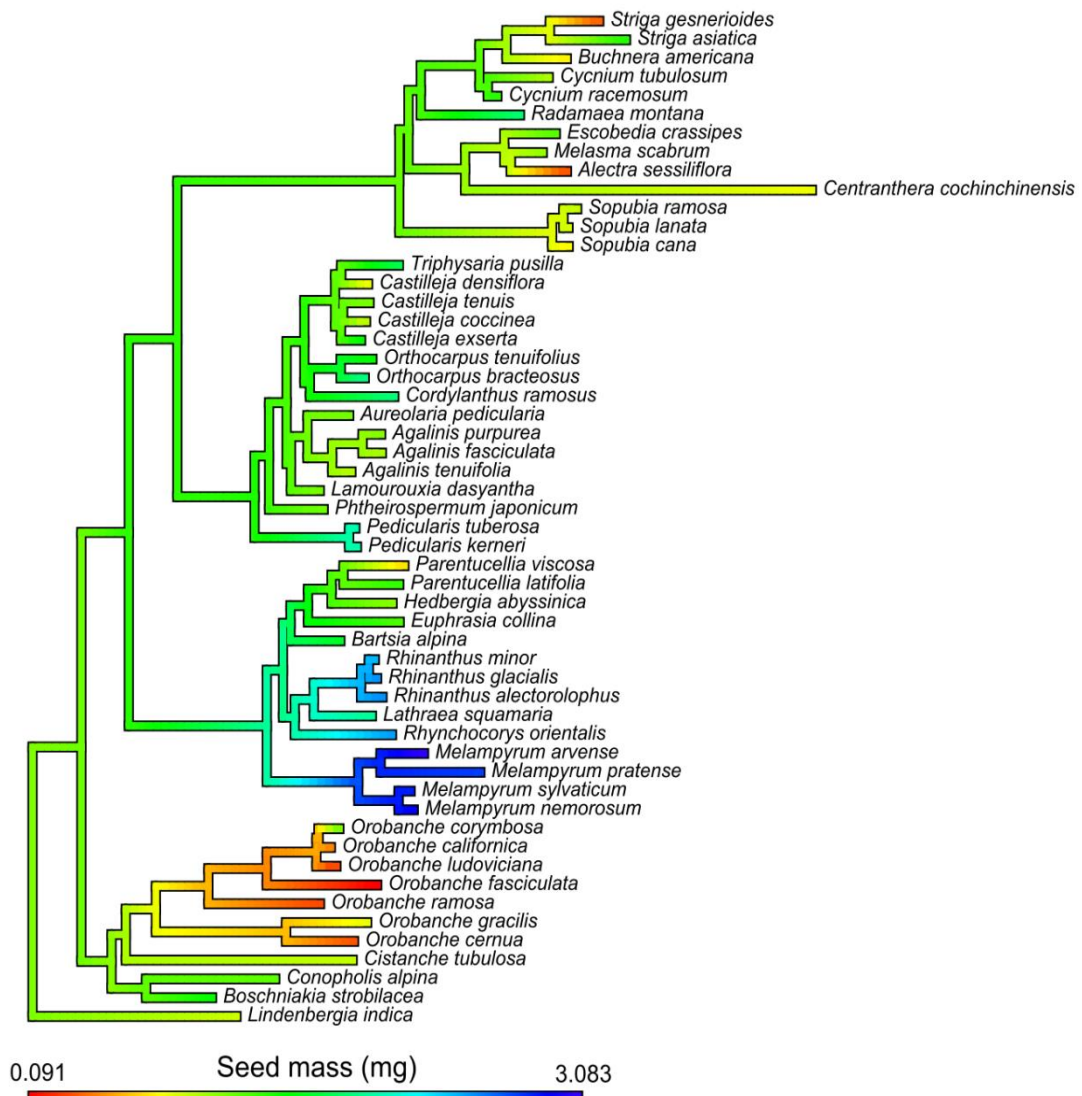


Figure 4: Maximum likelihood reconstruction of seed mass evolution in *Orobanchaceae*. The color scale of seed mass is multiplicative due to analysis based on log-transformed seed mass data.

Castilleja, *Parentucellia* (*Bellardia* is a sister genus), and possibly *Orobanche*, though the last one appears to be due to secondary enlargement. The largest seeds appear within the tribe *Rhinantheae*, though according to data, the genera *Melampyrum*, *Rhynchospora*, and *Rhinanthus* have developed them independently.

4.3 Cultivation experiment

The cultivation experiment has been a moderate success. The experiment showed, that both *Parentucellia viscosa* and *Bellardia trixago* germinate very readily without the need for any kind of chemical stimulus, or stratification (Fig. 5, 6). The germination time of both species was approximately two weeks. When examining the sprouted seeds under a binocular microscope, the vast majority of seeds appeared to have germinated. As an interesting side note, although both plants are covered in glandular trichomes in adulthood, the epithet of *Parentucellia viscosa* meaning sticky, with its common name being yellow glandweed, only *Bellardia trixago* produces said trichomes on the cotyledons.

After the seeds have germinated, I have transferred them into planters containing a mix of potential hosts. Throughout the following weeks, the hemiparasites continued to live but never seemed to thrive. I was never able to find any proof of attachment to the potential hosts and due to the plants mostly staying the same size, it is very unlikely such an attachment occurred. Most of the seedlings eventually succumbed to mould and I was forced to end the experiment before any visible signs of recruitment. This proves both species are autonomously germinating and only attach to a host after living as autotrophic seedlings for a period of time.

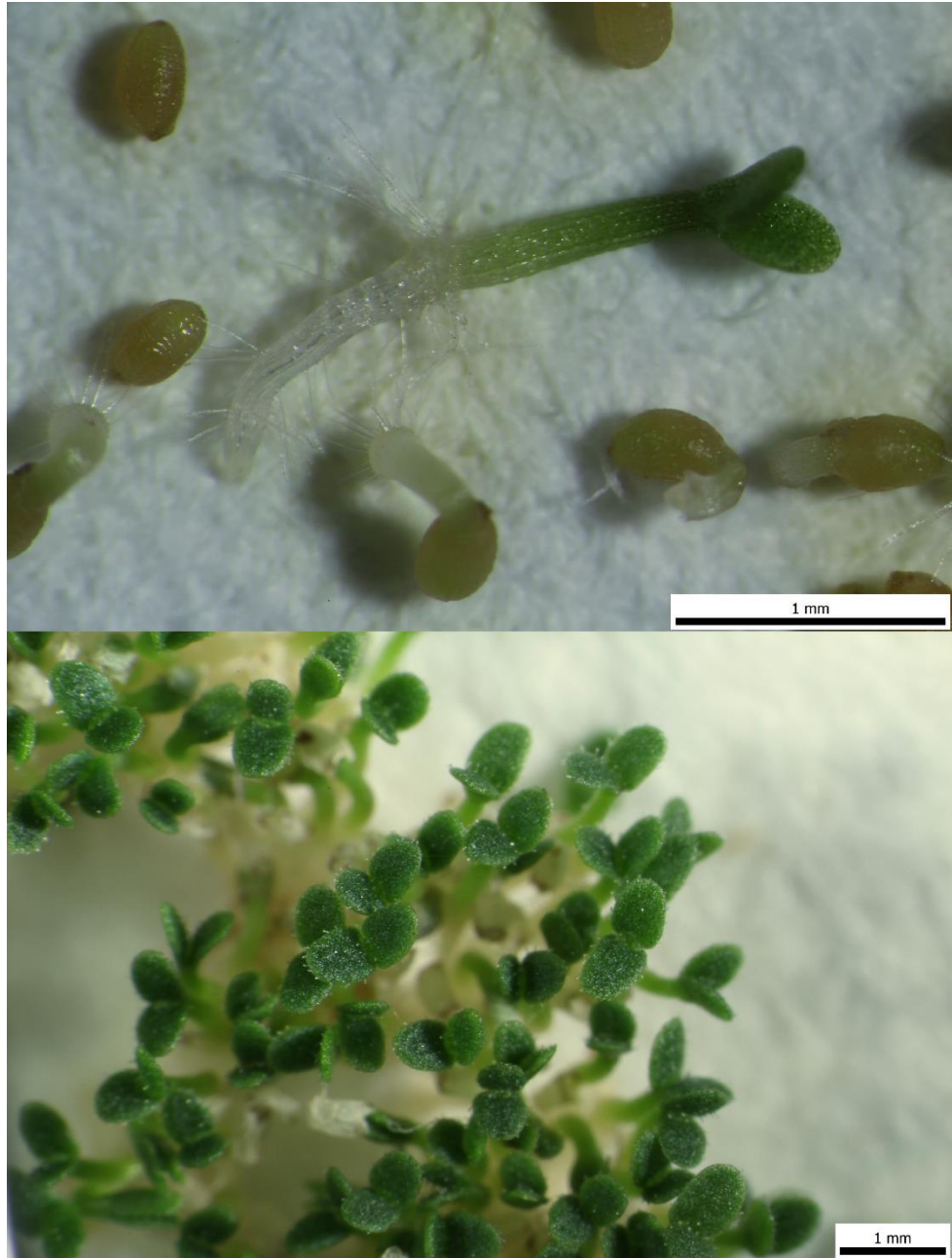


Figure 5: Freshly sprouted seedlings of *Parentucellia viscosa* (top) and *Bellardia trixago* (bottom). Note that *Bellardia* shoots are covered with glandular trichomes.



Figure 6: Seedlings of *Parentucellia viscosa* (top) and *Bellardia trixago* (bottom) 2 weeks after being cultivated with their potential hosts

5. DISCUSSION

5.1 Seed size across Orobanchaceae

My analysis of seed mass showed a large range of seed sizes in Orobanchaceae, spanning from 0.3 μg to 14 mg. This can without a doubt be attributed to the huge variation in life and dispersal strategies in the family. The genus *Melampyrum* represents the largest seeds in the family. Sources from both central Europe and North America demonstrate that *Melampyrum* species are predominantly myrmecochorous, though occasionally capable of endozoochory (Gibson, 1993; Chlumský *et al.*, 2013). The species of *Melampyrum* are hemiparasites with very low host-specificity. This is likely facilitated by the fact, they are xylem parasites (Piehl, 1962) since xylem is a dead tissue with little to no immune response (Irving and Cameron, 2009). Despite notable differences in quality of individual host species (Matthies, 2017), generalist hemiparasites are rarely limited by the availability of suitable hosts. This generates rather low selective pressure to invest in efficient seed dispersal in space and time. By contrast, the smallest seeds belong to species of *Alectra*, *Orobanche*, and *Striga*, all of which are obligatory parasites with a terminal haustorium and have a much narrower host profile, often specializing to one species in particular (Joel, Gressel and Musselman, 2015). Since their hosts are spatially and/or temporally rare, it is in their best interest to produce a large number of small seeds to cover as many potential habitats as possible. Furthermore, their seeds are well-suited to last multiple seasons and form a seed bank (Joel, 2013), which further helps them to multiply their odds of finding a suitable host by spreading through time.

In my data, it appears there is a physical boundary of about 50 μg of dry seed mass, below which only species with dust seeds are able to go. This is at odds with Raven's (1999) theoretical boundary set at 5 μg . As demonstrated by the bottom dotted line (Fig. 7) according to Raven's theoretical boundary, many species with incapable of independent germination would not be considered small enough to be considered as dust seeds. Raven however never intended for his findings to be used as a definition of dust seeds, he merely defined the physiological lower limit of a plant being able to germinate and function independently. It is therefore entirely possible for seeds larger than 5 μg to be reliant on an external source of water and/or carbon during the germination to seedling

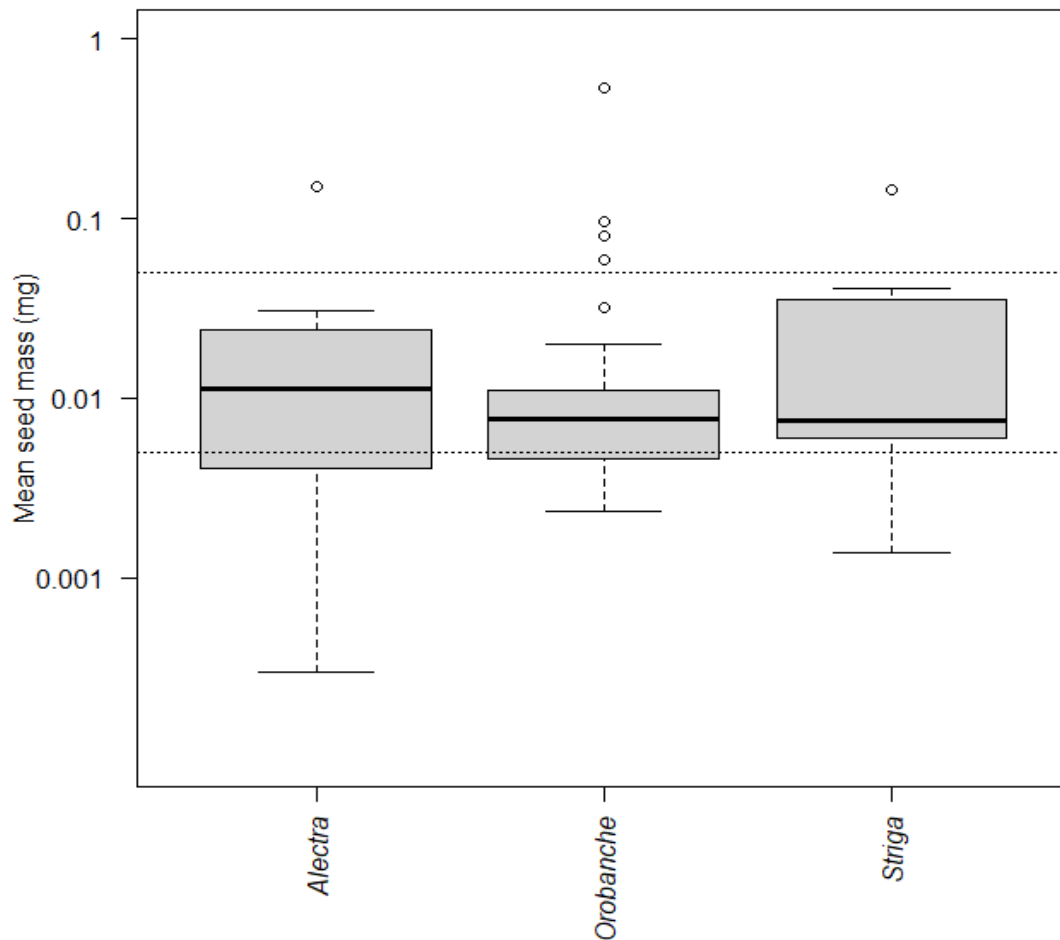


Figure 7: When Raven's (1999) theoretical boundary (bottom dotted line) is laid over Figure 3, a large number of species with dust seeds do not meet the criteria.. Furthermore *Orobanche tarapacana* can be seen as the highest outlier in *Orobanche*.

stage. Therefore while his definition can be reliably used to prove a seed is small enough to be a dust seed, it can't be used to determine, whether a seed is too big to be considered a dust seed.

One of the surprising outliers was *Orobanche tarapacana* with a seed mass of 0.54 mg. This is highly unusual for the genus *Orobanche* and falls out of any weight-based definition of a dust seed. This value raised enough suspicion to investigate it. With the help from my supervisor, I was able to find a paper regarding this species in Spanish literature (Zollner and Greissl, 1980). While this paper states the seeds weigh 2.5 mg each (which is admittedly even less believable, than the SID value), it also provides measurements: 400 μm in length and 270 μm of width. Luckily, an article published by my supervisor has demonstrated, there is a very good correlation between seed dimensions and seed mass in Rhinanthoid Orobanchaceae (Těšitel *et al.*, 2010). While *Orobanche* is not in the Rhinanthoid clade, there is little reason, why the correlation should not apply at least approximately. Using this relation between seed dimensions and mass should provide at least a rough estimate of the seed mass. Based on the correlation the seed mass of *Orobanche tarapacana* can be estimated to 0.02 mg. This is comparable to *Parentucellia viscosa* at 0.022 mg per seed, and would therefore comfortably land the species within the dwarf seed margins. While this is not a direct measurement, it demonstrates that both the SID value and the one found in Spanish literature are unlikely to be correct.

5.2 Ancestral state reconstruction

The results show dust seeds must have evolved at least three times independently in *Orobanchaceae*. This is a typical case of evolutionary convergence and poses the question, whether the dust seeds of *Alectra*, *Striga*, and *Orobanche* are morphologically and functionally equivalent. We know, all three genera use strigolactones as germination stimulants (Yoneyama, Ruyter-Spira and Bouwmeester, 2013). An interesting area of further research would be the evolution of using strigolactones to sense a nearby host. Since seed size is tied to the mode of germination, the most parsimonious theory would be, the induction by strigolactones has also evolved individually for each genus. This shows, that both dust seeds and induction by strigolactones are highly effective strategies. The Rhinanthoid clade has developed significantly larger seeds, than the rest of the family. According to Těšitel (2010), larger seeds (3-4 mg) are the ancestral state for this

group. This would mean the smaller seeds in *Parentucellia* and *Euphrasia* are a result of the secondary reduction, rather than keeping the ancestral state as my analysis shows. This can be attributed to the smaller number of Rhinanthoid species used in the ancestral state reconstruction. The small seeds size in *Parentucellia* and *Euphrasia* is likely the cause of their large geographical range of distribution. Both genera (in case of *Parentucellia* – *Bartsia* s. l.) originate in the Mediterranean area and have found their way to South America (Gussarova *et al.*, 2008; Těšitel *et al.*, 2010).

5.3 Cultivation experiment

I was able to show, that both *Parentucellia viscosa* and *Bellardia trixago* germinate readily without dormancy or the need for a chemical stimulus. This is not particularly surprising for hemiparasitic plants but constitutes an important piece of information for the studies of dwarf seeds. While there are no obligate parasites closely related, we can imagine developing host-induced germination could be the next step on the way to obligatory parasitism. While it's tempting to generalize this finding to other species with dwarf seeds, I would advise caution. Dwarf seeds are a result of evolutionary convergence and while there is some likelihood, that unrelated species with dwarf seed will share similar biology, every phylogenetic group should be studied independently. It should be noted, there are cases of seeds outside of the dwarf, or dust seed range, requiring a chemical stimulus from the host to germinate. These include *Hydnora triceps* and *Dactylanthus taylorii*, both of which are holoparasites lacking in chlorophyll (Ecroyd, 1996; Bolin *et al.*, 2009).

6. CONCLUSION

The seed size in *Orobanchaceae* spans over six orders of magnitude. This is largely thanks to some groups in the family evolving extremely small seeds – dwarf seeds and dust seeds. Producing large numbers of smaller seeds is a very effective strategy for specialized parasitic plants allowing them to maximize their chances of finding a suitable host.

Using maximum likelihood ancestral state reconstruction, I have managed to visualize the evolutionary trends of seed size in the family. The Rhinanthoid clade has evolved the largest seeds in the family. This is likely due to the predominantly hemiparasitic nature of the clade. By contrast, three genera have evolved dust seeds: *Alectra*, *Striga*, and *Orobanche*. The ancestral state reconstruction suggests, these genera have evolved dust seeds independently of each other. It is therefore likely there are morphological and functional differences between the seeds of these genera.

I have attempted to cultivate two species with dwarf seeds (*Parentucellia viscosa* and *Bellardia trixago*) to find out, whether they germinate autonomously. Both species germinate readily without a chemical stimulus or stratification of any kind in approximately two weeks of time. After germination, I attempted to cultivate these plants to maturity with potential hosts available, however, I was not successful. I advise caution when trying to generalize my finding to other species with dwarf seeds due to their polyphyletic nature – more research is required.

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ATTACHMENT

Seed mass data, note that seed mass is for 1000 seeds (Royal Botanic Gardens Kew, 2019)

Species	trophism	thousand seeds weight [g]
<i>Agalinis_aphylla</i>	hemi	0.1136
<i>Agalinis_aspera</i>	hemi	0.2404
<i>Agalinis_densiflora</i>	hemi	0.9660
<i>Agalinis_edwardsiana</i>	hemi	0.0344
<i>Agalinis_fasciculata</i>	hemi	0.0516
<i>Agalinis_genistifolia</i>	hemi	0.0748
<i>Agalinis_heterophylla</i>	hemi	0.1168
<i>Agalinis_kingsii</i>	hemi	0.0596
<i>Agalinis_maritima</i>	hemi	0.0668
<i>Agalinis_paupercula</i>	hemi	0.0741
<i>Agalinis_purpurea</i>	hemi	0.0712
<i>Agalinis_tenuifolia</i>	hemi	0.0480
<i>Alectra_avensis</i>	hemi	0.0008
<i>Alectra_ibityensis</i>	hemi	0.0172
<i>Alectra_orobanchoides</i>	hemi	0.0112
<i>Alectra_pseudobarleriae</i>	hemi	0.1506
<i>Alectra_rigida</i>	hemi	0.0304
<i>Alectra_sessiliflora</i>	hemi	0.0100
<i>Alectra_sessiliflora</i>	hemi	0.0046
<i>Alectra_vogelii</i>	hemi	0.0003
<i>Aureolaria_flava</i>	hemi	0.4300
<i>Aureolaria_grandiflora</i>	hemi	0.4340
<i>Aureolaria_laevigata</i>	hemi	0.2324
<i>Aureolaria_pedicularia</i>	hemi	0.0790
<i>Bartsia_alpina</i>	hemi	0.2600
<i>Bartsia_aspera</i>	hemi	0.1596
<i>Bartsia_trixago</i>	hemi	0.0300
<i>Boschniakia_strobilacea</i>	holo	0.1840
<i>Buchnera_americana</i>	hemi	0.0250
<i>Buchnera_asperata</i>	hemi	0.0380
<i>Buchnera_capitata</i>	hemi	0.0166
<i>Buchnera_ciliata</i>	hemi	0.0398
<i>Buchnera_cryptocephala</i>	hemi	0.0764
<i>Buchnera_dura</i>	hemi	0.0269
<i>Buchnera_foliosa</i>	hemi	0.0236
<i>Buchnera_hispida</i>	hemi	0.0230
<i>Buchnera_lastii</i>	hemi	0.0212
<i>Buchnera_linearis</i>	hemi	0.0218
<i>Buchnera_quadrifaria</i>	hemi	0.0272

<i>Buchnera_ramosissima</i>	hemi	0.0544
<i>Buchnera_randii</i>	hemi	0.0179
<i>Buchnera_speciosa</i>	hemi	0.0531
<i>Buttonia_natalensis</i>	hemi	0.2672
<i>Buttonia_superba</i>	hemi	0.3136
<i>Castilleja_affinis</i>	hemi	0.2220
<i>Castilleja_affinis</i>	hemi	0.1568
<i>Castilleja_ambigua</i>	hemi	0.1290
<i>Castilleja_ambigua</i>	hemi	2.5282
<i>Castilleja_angustifolia</i>	hemi	0.3360
<i>Castilleja_applegatei</i>	hemi	0.2470
<i>Castilleja_applegatei</i>	hemi	0.2622
<i>Castilleja_applegatei</i>	hemi	0.2460
<i>Castilleja_applegatei</i>	hemi	0.1280
<i>Castilleja_attenuata</i>	hemi	0.1130
<i>Castilleja_coccinea</i>	hemi	0.0420
<i>Castilleja_cusickii</i>	hemi	0.0776
<i>Castilleja_densiflora</i>	hemi	0.0310
<i>Castilleja_densiflora</i>	hemi	0.0304
<i>Castilleja_elmeri</i>	hemi	0.2500
<i>Castilleja_exserta</i>	hemi	0.2012
<i>Castilleja_flava</i>	hemi	0.9932
<i>Castilleja_foliolosa</i>	hemi	0.2460
<i>Castilleja_haydenii</i>	hemi	0.2884
<i>Castilleja_hispida</i>	hemi	0.1070
<i>Castilleja_indivisa</i>	hemi	0.0828
<i>Castilleja_integra</i>	hemi	0.2108
<i>Castilleja_lacera</i>	hemi	0.0260
<i>Castilleja_lanata</i>	hemi	0.1300
<i>Castilleja_latifolia</i>	hemi	0.2140
<i>Castilleja_linariifolia</i>	hemi	0.1830
<i>Castilleja_lineariloba</i>	hemi	0.0340
<i>Castilleja_miniata</i>	hemi	0.2110
<i>Castilleja_miniata</i>	hemi	0.2720
<i>Castilleja_minor</i>	hemi	0.1350
<i>Castilleja_nana</i>	hemi	0.1830
<i>Castilleja_occidentalis</i>	hemi	0.2668
<i>Castilleja_oresbia</i>	hemi	0.1948
<i>Castilleja_pilosa</i>	hemi	0.1150
<i>Castilleja_pilosa</i>	hemi	0.1770
<i>Castilleja_pruinosa</i>	hemi	0.2804
<i>Castilleja_purpurea</i>	hemi	0.1268
<i>Castilleja_rhexifolia</i>	hemi	0.4932
<i>Castilleja_sessiliflora</i>	hemi	0.1790

<i>Castilleja_subinclusa</i>	hemi	0.2540
<i>Castilleja_sulphurea</i>	hemi	0.4056
<i>Castilleja_tenuiflora</i>	hemi	0.1230
<i>Castilleja_tenuis</i>	hemi	0.0760
<i>Castilleja_thompsonii</i>	hemi	0.0632
<i>Castilleja_unalascensis</i>	hemi	0.5396
<i>Castilleja_wightii</i>	hemi	0.1210
<i>Centranthera_cochinchinensis</i>	hemi	0.0307
<i>Centranthera_grandiflora</i>	hemi	0.0236
<i>Cistanche_mongolica</i>	holo	0.0448
<i>Cistanche_salsa</i>	holo	0.0840
<i>Cistanche_tubulosa</i>	holo	0.0440
<i>Conopholis_alpina</i>	holo	0.0928
<i>Cordylanthus_filifolius</i>	hemi	0.1400
<i>Cordylanthus_laxiflorus</i>	hemi	0.6834
<i>Cordylanthus_maritimus</i>	hemi	0.4780
<i>Cordylanthus_parviflorus</i>	hemi	3.6512
<i>Cordylanthus_ramosus</i>	hemi	0.4820
<i>Cordylanthus_rigidus</i>	hemi	0.4320
<i>Cordylanthus_rigidus</i>	hemi	2.7628
<i>Cordylanthus_rigidus</i>	hemi	0.2480
<i>Cordylanthus_wrightii</i>	hemi	0.3948
<i>Cycnium_cameronianum</i>	hemi	0.0568
<i>Cycnium_herzfeldianum</i>	hemi	0.0452
<i>Cycnium_racemosum</i>	hemi	0.1548
<i>Cycnium_recurvum</i>	hemi	0.1309
<i>Cycnium_tubulosum</i>	hemi	0.0448
<i>Cycnium_tubulosum</i>	hemi	0.0586
<i>Cycnium_veronicifolium</i>	hemi	0.0548
<i>Escobedia_crassipes</i>	hemi	0.1000
<i>Escobedia_peduncularis</i>	hemi	0.1000
<i>Euphrasia_amphisyssepala</i>	hemi	0.1904
<i>Euphrasia_andicola</i>	hemi	0.2516
<i>Euphrasia_anglica</i>	hemi	0.1129
<i>Euphrasia_antarctica</i>	hemi	0.4292
<i>Euphrasia_arctica</i>	hemi	0.1880
<i>Euphrasia_arctica</i>	hemi	0.1916
<i>Euphrasia_arctica</i>	hemi	0.2016
<i>Euphrasia_campbelliae</i>	hemi	0.1730
<i>Euphrasia_caucasica</i>	hemi	0.1104
<i>Euphrasia_caudata</i>	hemi	0.1245
<i>Euphrasia_collina</i>	hemi	0.0050
<i>Euphrasia_collina</i>	hemi	0.2504
<i>Euphrasia_collina</i>	hemi	0.1468

<i>Euphrasia_collina</i>	hemi	0.1550
<i>Euphrasia_collina</i>	hemi	0.0588
<i>Euphrasia_collina</i>	hemi	0.0384
<i>Euphrasia_confusa</i>	hemi	0.0924
<i>Euphrasia_crassiuscula</i>	hemi	0.1608
<i>Euphrasia_crassiuscula</i>	hemi	0.1530
<i>Euphrasia_drosocalyx</i>	hemi	0.2544
<i>Euphrasia_eichleri</i>	hemi	0.2964
<i>Euphrasia_foulaensis</i>	hemi	0.2964
<i>Euphrasia_frigida</i>	hemi	0.2300
<i>Euphrasia_gibbsiae</i>	hemi	0.2900
<i>Euphrasia_gibbsiae</i>	hemi	0.1818
<i>Euphrasia_gibbsiae</i>	hemi	0.1344
<i>Euphrasia_hookeri</i>	hemi	0.1400
<i>Euphrasia_kemulariae</i>	hemi	0.8892
<i>Euphrasia_lasianthera</i>	hemi	0.1937
<i>Euphrasia_lebardensis</i>	hemi	0.0715
<i>Euphrasia_minima</i>	hemi	0.1775
<i>Euphrasia_nemorosa</i>	hemi	0.1600
<i>Euphrasia_officinalis</i>	hemi	0.1300
<i>Euphrasia_ostenfeldii</i>	hemi	0.1212
<i>Euphrasia_pectinata</i>	hemi	0.0977
<i>Euphrasia_petiolaris</i>	hemi	0.1236
<i>Euphrasia_phragmostoma</i>	hemi	0.1276
<i>Euphrasia_pseudokernerii</i>	hemi	0.1891
<i>Euphrasia_ramulosa</i>	hemi	0.1000
<i>Euphrasia_rostkoviana</i>	hemi	0.2196
<i>Euphrasia_rostkoviana</i>	hemi	0.1956
<i>Euphrasia_salisburgensis</i>	hemi	0.1660
<i>Euphrasia_scabra</i>	hemi	0.0812
<i>Euphrasia_semipicta</i>	hemi	0.0834
<i>Euphrasia_slovaca</i>	hemi	0.3528
<i>Euphrasia_striata</i>	hemi	0.2408
<i>Euphrasia_stricta</i>	hemi	0.1600
<i>Euphrasia_stricta</i>	hemi	0.1690
<i>Euphrasia_stricta</i>	hemi	0.1312
<i>Euphrasia_tetraquetra</i>	hemi	0.2012
<i>Euphrasia_tetraquetra</i>	hemi	0.1459
<i>Euphrasia_vigursii</i>	hemi	0.1356
<i>Graderia_scabra</i>	hemi	0.2620
<i>Graderia_subintegra</i>	hemi	0.3582
<i>Harveya_obtusifolia</i>	hemi	0.1654
<i>Hedbergia_abyssinica</i>	hemi	0.0665
<i>Lamourouxia_dasyantha</i>	hemi	0.0767

<i>Lamourouxia_longiflora</i>	hemi	0.0272
<i>Lamourouxia_nelsonii</i>	hemi	0.0468
<i>Lamourouxia_viscosa</i>	hemi	0.0740
<i>Lathraea_squamaria</i>	holo	0.6100
<i>Leucosalpa_madagascariensis</i>	hemi	5.5679
<i>Leucosalpa_poissonii</i>	hemi	0.3260
<i>Lindenbergia_indica</i>	auto	0.0410
<i>Melampyrum_arvense</i>	hemi	13.3800
<i>Melampyrum_barbatum</i>	hemi	12.3056
<i>Melampyrum_caucasicum</i>	hemi	11.9060
<i>Melampyrum_cristatum</i>	hemi	5.9900
<i>Melampyrum_cristatum</i>	hemi	5.2368
<i>Melampyrum_lineare</i>	hemi	2.4140
<i>Melampyrum_nemorosum</i>	hemi	7.1182
<i>Melampyrum_nemorosum</i>	hemi	11.3816
<i>Melampyrum_pratense</i>	hemi	5.3884
<i>Melampyrum_pratense</i>	hemi	5.9000
<i>Melampyrum_sp.</i>	hemi	5.0200
<i>Melampyrum_subalpinum</i>	hemi	4.8520
<i>Melampyrum_sylvaticum</i>	hemi	7.2500
<i>Melampyrum_variegatum</i>	hemi	14.0064
<i>Melasma_physalodes</i>	hemi	0.0716
<i>Melasma_scabrum</i>	hemi	0.0616
<i>Micrargeria_filiformis</i>	hemi	0.0356
<i>Odontites_asturicus</i>	hemi	0.5164
<i>Odontites_aucheri</i>	hemi	0.6256
<i>Odontites_cyprius</i>	hemi	0.1799
<i>Odontites_glutinosa</i>	hemi	0.1688
<i>Odontites_longifolius</i>	hemi	0.2472
<i>Odontites_luteus</i>	hemi	0.2283
<i>Odontites_vernus</i>	hemi	0.2516
<i>Odontites_vernus</i>	hemi	0.1900
<i>Odontites_vulgaris</i>	hemi	0.2520
<i>Orobanche_amethystea</i>	holo	0.0027
<i>Orobanche_arenaria</i>	holo	0.0052
<i>Orobanche_bulbosa</i>	holo	0.0200
<i>Orobanche_californica</i>	holo	0.0110
<i>Orobanche_camptolepis</i>	holo	0.0077
<i>Orobanche_caryophyllacea</i>	holo	0.0077
<i>Orobanche_cernua</i>	holo	0.0079
<i>Orobanche_cernua</i>	holo	0.0072
<i>Orobanche_chilensis</i>	holo	0.0152
<i>Orobanche_corymbosa</i>	holo	0.0797
<i>Orobanche_crenata</i>	holo	0.0040

Orobanche_elatior	holo	0.0046
Orobanche_fasciculata	holo	0.0040
Orobanche_flava	holo	0.0063
Orobanche_gracilis	holo	0.0320
Orobanche_hederæ	holo	0.0066
Orobanche_hermonis	holo	0.0970
Orobanche_ludoviciana	holo	0.0060
Orobanche_ludoviciana	holo	0.0060
Orobanche_lutea	holo	0.0023
Orobanche_minor	holo	0.0043
Orobanche_picridis	holo	0.0030
Orobanche_pubescens	holo	0.0076
Orobanche_purpurea	holo	0.0110
Orobanche_ramosa	holo	0.0070
Orobanche_rapum-genistæ	holo	0.0043
Orobanche_reticulata	holo	0.0090
Orobanche_sanguinea	holo	0.0592
Orobanche_schultzii	holo	0.0080
Orobanche_tarapacana	holo	0.5380
Orobanche_uniflora	holo	0.0110
Orthocarpus_bracteosus	hemi	0.5280
Orthocarpus_cuspidatus	hemi	0.5720
Orthocarpus_cuspidatus	hemi	0.6144
Orthocarpus_cuspidatus	hemi	0.7898
Orthocarpus_erianthus	hemi	0.2440
Orthocarpus_erianthus	hemi	0.0830
Orthocarpus_floribundus	hemi	0.1750
Orthocarpus_hispidus	hemi	0.0600
Orthocarpus_luteus	hemi	0.2100
Orthocarpus_pachystachyus	hemi	0.5850
Orthocarpus_purpurascens	hemi	0.1570
Orthocarpus_pusillus	hemi	0.1900
Orthocarpus_tenuifolius	hemi	0.1808
Orthocarpus_tolmiei	hemi	0.3996
Orthocarpus_versicolor	hemi	0.1810
Orthocarpus_versicolor	hemi	0.1430
Parentucellia_latifolia	hemi	0.2400
Parentucellia_latifolia	hemi	0.0187
Parentucellia_viscosa	hemi	0.0220
Pedicularis_anthemifolia	hemi	0.8924
Pedicularis_ascendens	hemi	1.0484
Pedicularis_aspleniifolia	hemi	0.4056
Pedicularis_atropurpurea	hemi	1.1040
Pedicularis_attollens	hemi	0.5550

<i>Pedicularis_bracteosa</i>	hemi	2.1190
<i>Pedicularis_canadensis</i>	hemi	0.9390
<i>Pedicularis_caucasica</i>	hemi	0.3788
<i>Pedicularis_centranthera</i>	hemi	4.3169
<i>Pedicularis_condensata</i>	hemi	6.0200
<i>Pedicularis_crassirostris</i>	hemi	0.3924
<i>Pedicularis_crenulata</i>	hemi	0.5204
<i>Pedicularis_densiflora</i>	hemi	3.3850
<i>Pedicularis_dolichorrhiza</i>	hemi	0.6328
<i>Pedicularis_elegans</i>	hemi	1.4192
<i>Pedicularis_elongata</i>	hemi	0.8880
<i>Pedicularis_eriantha</i>	hemi	1.3776
<i>Pedicularis_foliosa</i>	hemi	1.4564
<i>Pedicularis_groenlandica</i>	hemi	0.6000
<i>Pedicularis_gypsicola</i>	hemi	1.7720
<i>Pedicularis_gyroflexa</i>	hemi	1.0000
<i>Pedicularis_hacquetii</i>	hemi	1.6880
<i>Pedicularis_kernerii</i>	hemi	0.7163
<i>Pedicularis_labordei</i>	hemi	0.2182
<i>Pedicularis_longicaulis</i>	hemi	0.1520
<i>Pedicularis_ludwigii</i>	hemi	0.6888
<i>Pedicularis_mixta</i>	hemi	0.7644
<i>Pedicularis_oederii</i>	hemi	1.3792
<i>Pedicularis_olgae</i>	hemi	2.0679
<i>Pedicularis_palustris</i>	hemi	0.8290
<i>Pedicularis_petiolaris</i>	hemi	0.7996
<i>Pedicularis_portenschlagii</i>	hemi	0.6456
<i>Pedicularis_procera</i>	hemi	1.9284
<i>Pedicularis_pyrenaica</i>	hemi	1.0156
<i>Pedicularis_pyrenaica</i>	hemi	1.3400
<i>Pedicularis_racemosa</i>	hemi	1.2124
<i>Pedicularis_recutita</i>	hemi	0.8200
<i>Pedicularis_resupinata</i>	hemi	0.9600
<i>Pedicularis_rex</i>	hemi	2.2580
<i>Pedicularis_rosea</i>	hemi	0.6020
<i>Pedicularis_rostratocapitata</i>	hemi	1.0550
<i>Pedicularis_sceptrum-carolinum</i>	hemi	0.2980
<i>Pedicularis_schizocalyx</i>	hemi	0.9260
<i>Pedicularis_sibthorpii</i>	hemi	1.6700
<i>Pedicularis_sylvatica</i>	hemi	0.8222
<i>Pedicularis_sylvatica</i>	hemi	0.9570
<i>Pedicularis_tuberosa</i>	hemi	0.7520
<i>Pedicularis_verticillata</i>	hemi	0.6299
<i>Pedicularis_wilhelmsiana</i>	hemi	0.7400

<i>Phtheirospermum_japonicum</i>	hemi	0.0804
<i>Phtheirospermum_tenuisectum</i>	hemi	0.0168
<i>Pseudosopubia_hildebrandtii</i>	hemi	0.1808
<i>Radamaea_latifolia</i>	hemi	0.5196
<i>Radamaea_montana</i>	hemi	0.4548
<i>Radamaea_perrieri</i>	hemi	0.5520
<i>Rhamphicarpa_australiensis</i>	hemi	0.0160
<i>Rhamphicarpa_fistulosa</i>	hemi	0.0180
<i>Rhamphicarpa_medwedewii</i>	hemi	0.0180
<i>Rhinanthus_alectorolophus</i>	hemi	3.0700
<i>Rhinanthus_apuanus</i>	hemi	1.5445
<i>Rhinanthus_borbasii</i>	hemi	0.9986
<i>Rhinanthus_colchicus</i>	hemi	2.0219
<i>Rhinanthus_glacialis</i>	hemi	2.9856
<i>Rhinanthus_minor</i>	hemi	1.6018
<i>Rhinanthus_minor</i>	hemi	2.1610
<i>Rhinanthus_minor</i>	hemi	2.5600
<i>Rhinanthus_ovifugus</i>	hemi	2.8988
<i>Rhinanthus_pulcher</i>	hemi	4.3014
<i>Rhinanthus_serotinus</i>	hemi	2.7964
<i>Rhinanthus_serotinus</i>	hemi	2.1401
<i>Rhinanthus_subulatus</i>	hemi	2.9694
<i>Rhynchocorys_orientalis</i>	hemi	2.6736
<i>Seymeria_macrophylla</i>	hemi	0.6530
<i>Sieversandreas_madagascarianus</i>	hemi	0.1300
<i>Sopubia_cana</i>	hemi	0.0264
<i>Sopubia_gracilis</i>	hemi	3.3176
<i>Sopubia_karaguensis</i>	hemi	0.0777
<i>Sopubia_lanata</i>	hemi	0.0380
<i>Sopubia_mannii</i>	hemi	0.0296
<i>Sopubia_mannii</i>	hemi	0.0638
<i>Sopubia_parviflora</i>	hemi	0.0290
<i>Sopubia_ramosa</i>	hemi	0.0442
<i>Sopubia_simplex</i>	hemi	0.0620
<i>Sopubia_trifida</i>	hemi	0.0330
<i>Sopubia_triphylla</i>	hemi	0.0870
<i>Striga_asiatica</i>	hemi	0.1453
<i>Striga_aspera</i>	hemi	0.0055
<i>Striga_brachycalyx</i>	hemi	0.0014
<i>Striga_curviflora</i>	hemi	0.0060
<i>Striga_forbesii</i>	hemi	0.0124
<i>Striga_gesnerioides</i>	hemi	0.0080
<i>Striga_hermonthica</i>	hemi	0.0071
<i>Striga_macrantha</i>	hemi	0.0408

<u>Striga_magnibracteata</u>	hemi	0.0356
<u>Striga_squamigera</u>	hemi	0.0071
<u>Triphysaria_pusilla</u>	hemi	0.3436