

What is Pollinating the Critically Threatened Calicolous Plants in the Waitaki Valley?

Supplementary Material

Appendix S1. Supplemental Methods

Glycerine Jelly

To make the glycerine jelly, a glycerine stain solution was created first. To create the stain solution, a 2-dram vial was filled with 10ml of glycerine. Using a small pipette, three drops of Safranin-O, also known as basic red 2, were added to the 2-dram vial and mixed well with a wooden toothpick. The more drops of Safranin-O added, the darker the pollen grains will stain. To make the glycerine jelly, one part gelatine to two parts glycerine Safranin-O mixture was used: 50ml of gelatine to 100ml of glycerine. The gelatine was mixed with enough cold water to hydrate it, left for approximately 10 minutes and then heated to dissolve. Next, the gelatine was placed into a resealable container to protect it from dust while it cooled. It was then mixed well, spread out in a thin layer, and left for 12 hours at room temperature to solidify (Figure 1).

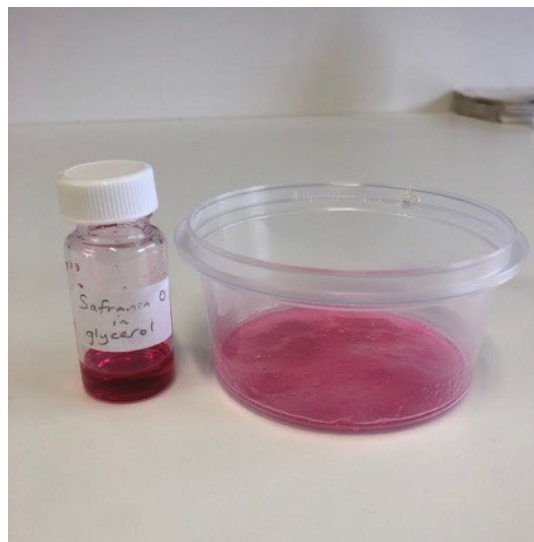


Figure S1: Safranin-O stain mixed with glycerol (left), Safranin-O jelly, set with gelatine (right).
Photo credit: Sharn Milliken.

Appendix S2. Supplemental Data

Observed Arthropod Orders

Table S1: Number of arthropod taxa observed on *Lepidium sisymbrioides* (N = 127, 7 observations days), *Gentianella calcis* subsp. *calcis* (N = 102, 6 observation days), and *Carmichaelia hollowayi* (N = 94, 6 observations days), by taxonomic order. Twelve different taxonomic orders were identified in field observations. Identification was made on-site in the field.

Order	Number	%
<i>Lepidium sisymbrioides</i>		
Diptera	48	37.8
Lepidoptera	3	2.4
Hymenoptera	52	40.9
Thysanoptera	6	4.7
Orthoptera	6	4.7
Trombidiformes	9	7.1
Araneida	2	1.6
Hemiptera	1	0.8
<i>Gentianella calcis</i> subsp. <i>calcis</i>		
Diptera	61	59.8
Hymenoptera	20	19.6
Lepidoptera	15	14.7
Orthoptera	1	1
Trombidiformes	1	1
Hemiptera	3	2.9
Araneida	1	1
<i>Carmichaelia hollowayi</i>		
Hymenoptera	76	80.8
Coleoptera	3	3.2
Diptera	12	12.8
Orthoptera	1	1.1
Araneae	2	2.1

Table S2: Number and origin of captured and identified arthropod taxa observed on *Lepidium sisymbrioides*, sorted by type: endemic, native, exotic, and unknown origin. This list is comprised of

the arthropod taxa that were caught during observational periods and identified in the lab. Taxa were identified down to species level if possible, if not, the next highest classification was used. The unknown category reflects there being both native and introduced species of a taxon in New Zealand.

Type	Order	Family	Insect Taxon	Number	Origin
Endemic native	Diptera	Asilidae	<i>Neoitamus melanopogon</i>	1	New Zealand
	Hymenoptera	Halictidae	<i>Lasioglossum sordidum</i>	7	New Zealand
	Orthoptera	Acrididae	<i>Phaulacridium marginale</i>	1	New Zealand
	Lepidoptera	Geometridae	<i>Asaphodes abrogata</i>	2	New Zealand
	Diptera	Tachinidae	<i>Mallochomacquartia vexata</i>	6	New Zealand
Native	Diptera	Tachinidae	<i>Pales</i> sp.	6	New Zealand
	Orthoptera	Trigonidiidae	<i>Bobilla</i> sp.	1	New Zealand, Australia
	Lepidoptera	Geometridae	<i>Anachloris subochraria</i>	1	New Zealand
	Thysanoptera	Thripidae	<i>Thrips obscuratus</i>	3	New Zealand, Australia
	Orthoptera	Tettigoniidea	<i>Conocephalus</i> sp.	3	New Zealand
Exotic	Hymenoptera	Encyrtidae	<i>Copidosoma</i> sp.	20	South America
	Diptera	Polleniidae	<i>Pollenia pediculata</i>	1	Unknown
	Diptera	Sciarodae	Sciaridae	4	Unknown
	Bibionomorpha	Sciarodea	Superfamily Sciaroidea	4	Unknown
Unknown	Diptera	Tachinidae	Tachinidae	3	Unknown
	Araneida	Arachnida	Arachnida	2	Unknown
	Diptera	Dolichopodidae	Dolichopodidae	1	Unknown
	Hymenoptera	Braconidae	Microgastrinae	4	Unknown
	Trombidiformes	Tetranychidea	Tetranychidea	8	Unknown
Grand Total				78	

Table S3: Number and origin of captured and identified insect taxa observed on *Gentianella calcis* subsp. *calcis*, sorted by type: endemic, native, exotic, and unknown origin. This list is comprised of the insect taxa that were caught during observational periods and identified in the lab. Taxa were identified down to species level if possible, if not, the next highest classification was used. The unknown category reflects there being both native and introduced species of a taxon in New Zealand.

Type	Order	Family	Insect Taxon	Number	Origin
Endemic native	Diptera	Calliphoridae	<i>Calliphora quadrimaculata</i>	1	New Zealand
	Diptera	Syrphinae	<i>Melanostoma fasciatum</i>	29	New Zealand
	Lepidoptera	Geometridae	<i>Samana acutata</i>	1	New Zealand
	Hymenoptera	Halictidae	<i>Lasioglossum sordidum</i>	2	New Zealand
	Araneae	Thomisidae	<i>Diaea ambara</i>	1	New Zealand
Native	Orthoptera	Trigonidiidae	<i>Bobilla</i> sp.	1	Australasia and Pacific islands
	Lepidoptera	Pterophoroidea	Pterophoridae	3	New Zealand
Exotic	Diptera	Polleniidae	<i>Pollenia pediculata</i>	2	Unknown
	Diptera	Sarcophangidae	<i>Oxysarcodexia varia</i>	4	South America,
	Hymenoptera	Apidae	<i>Apis mellifera</i>	1	Unknown
	Hymenoptera	Encyrtidae	<i>Copidosoma</i> sp.	2	South America
Unknown	Diptera	Dolichopodidae	Dolichopodidae	3	Unknown
	Diptera	Tachinidae	Tachinidae	1	Unknown
	Araneida	Arachnida	Arachnida	1	Unknown
	Diptera	Tephritidae	Tephritidae	1	Unknown
	Lepidoptera	Pyraloidea	Pyraloidea	1	Unknown
	Hemiptera	Cicadellidae	Cicadellidae	1	Unknown
	Hymenoptera	Formicidae	Formicidae	2	Unknown
	Diptera	Diptera	Diptera	5	Unknown
	Trombidiformes	Tetranychidea	Tetranychidea	1	Unknown
Grand Total				63	

Table S4: The numbers and origins of captured and identified insects observed on *Carmichaelia hollowayi* sorted by type: endemic, native, exotic, and unknown origin. This list is comprised of the insects that were caught during observational periods and identified in the lab. Insects were identified to down to species level if possible, if not, the next highest classification was used. The unknown category reflects there being both native and introduced species of a taxon in New Zealand.

Type	Order	Family	Insect Taxon	Number	Origin
Endemic native	Coleoptera	Coccinellidae	<i>Coccinella leonina</i>	1	New Zealand
	Hymenoptera	Colletidae	<i>Leioproctus pango</i>	29	New Zealand
	Diptera	Syrphidae	<i>Melangyna novaezelandiae</i>	1	New Zealand
Native	Orthoptera	Tettigoniidea	<i>Conocephalus</i> sp.	1	New Zealand
Exotic	Coleoptera	Coccinellidae	<i>Coccinella undecimpunctata</i>	1	Asia
Unknown	Diptera	Dolichopodidae	Dolichopodidae	1	Unknown
Grand Total				34	

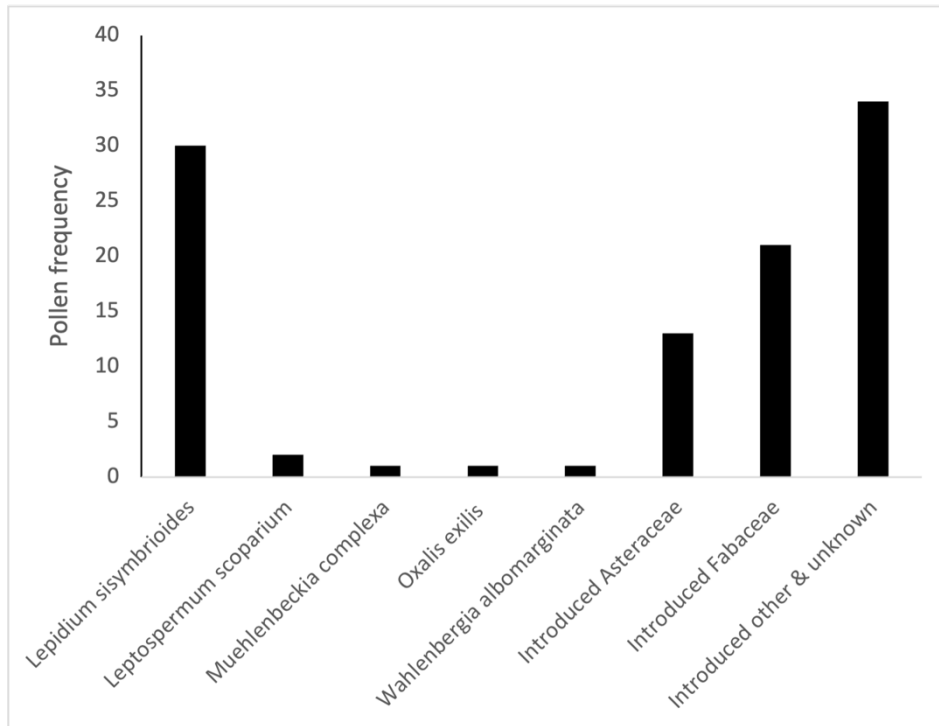


Figure S2: The number of times a pollen species or type was observed on an insect captured at Wai O Toura Scenic Reserve while visiting *Lepidium sisymbrioides*. N= 78.

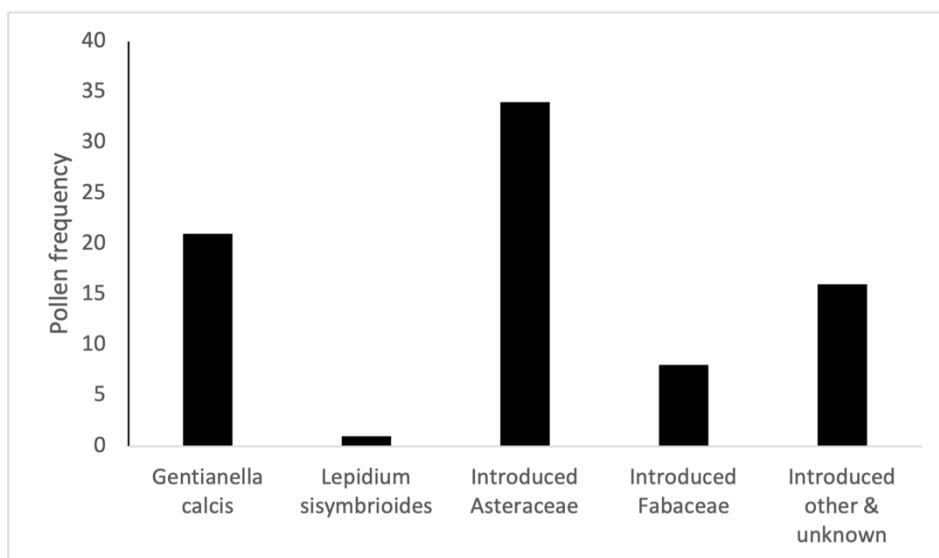


Figure S3: The number of times a pollen species or type was observed on an insect captured at Waipata Scientific Reserve while visiting *Gentianella calcis* subsp. *calcis*. N= 63.

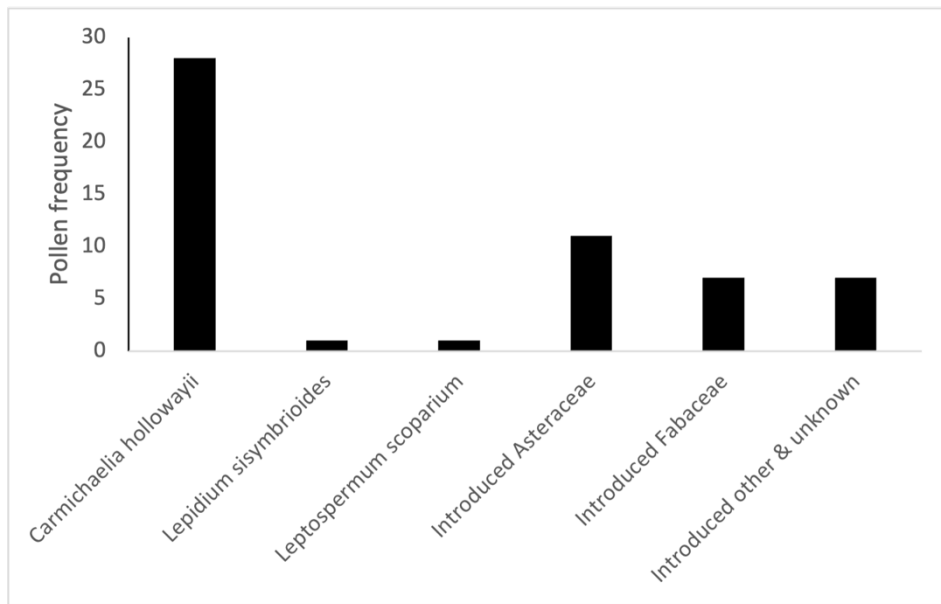


Figure S4: The number of times a pollen species or type was observed on an insect captured at the Wai O Toura Scenic Reserve while visiting *Carmichaelia hollowayi* plant. N= 34.

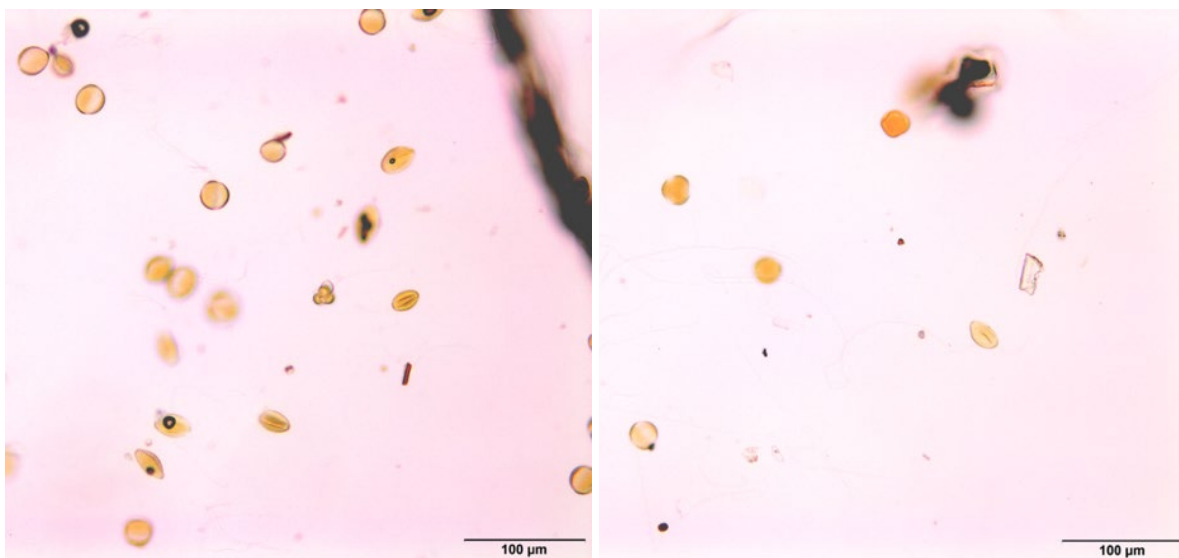


Figure S5: Images of pollen grains removed from *Lepidium sisymbrioides* stigmas after single pollinator visits: left image is from a flower visited by *Pales* sp.; right image is from a flower visited by *Lasioglossum sordidum*. Photo credit: Sharn Milliken.

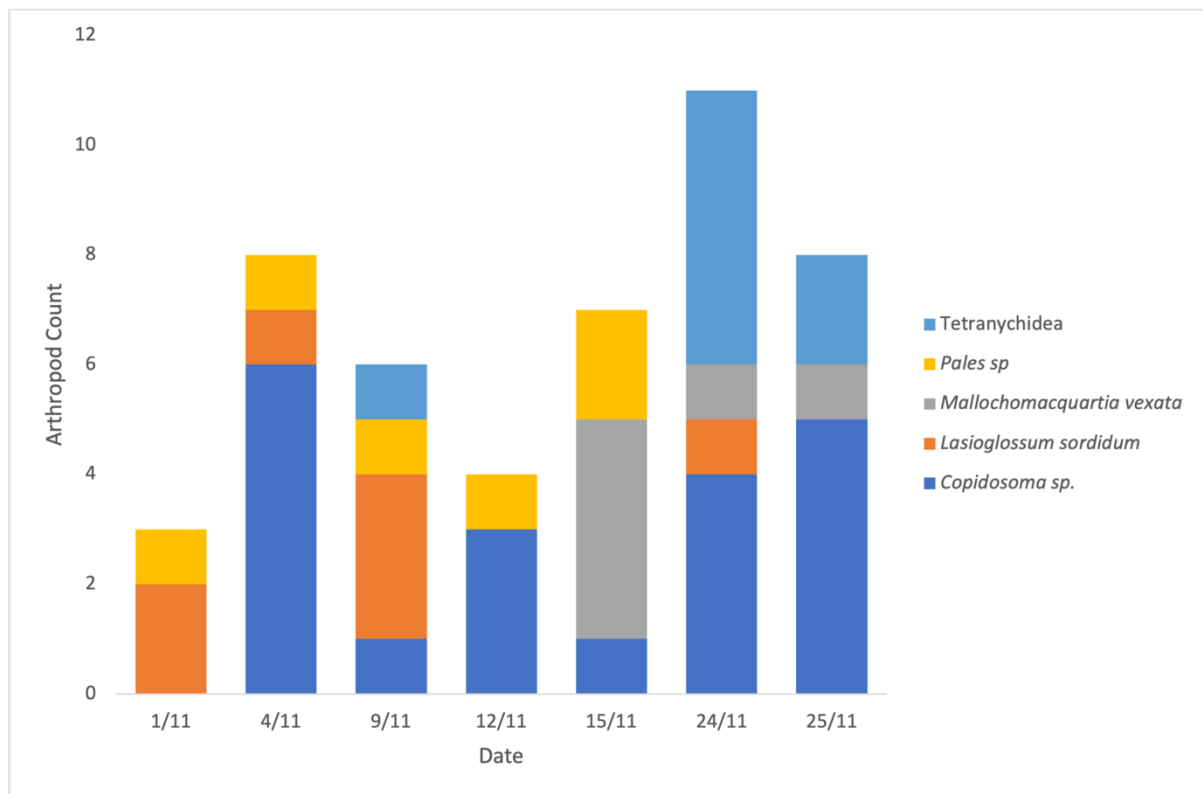


Figure S6: Top five arthropod taxa observed on *Lepidium sisymbrioides* over the 2021 sampling period.

Appendix S3 Supplemental Library

Pollen Library for Wai O Toura Scenic Reserve and Waipata Scientific Reserve, created by extracting pollen from every flowering plant at both sites between November 2021 and March 2022 (see Milliken 2023). Thirty seven plant species are included.

Table of Contents: Pollen Library

<i>Achillea millefolium</i> :	12
<i>Carmichaelia hollowayi</i> :	14
<i>Carmichaelia petriei</i> :	15
<i>Cirsium arvense</i> :	16
<i>Cirsium vulgare</i> :	17
<i>Convolvulus waitaha</i> :	18
<i>Crepis vesicaria</i> :	19
<i>Cytisus scoparius</i> :	19
<i>Echium vulgare</i> :	21
<i>Erodium cicutarium</i> :	22
<i>Fumaria bastardii</i> :	22
<i>Gentianella calcis subsp. calcis</i> :	24
<i>Hypochaeris radicata</i> :	25
<i>Lepidium sisymbrioides</i> :	26
<i>Leptospermum scoparium</i> :	27
<i>Lycium ferocissimum</i> :	28
<i>Malva neglecta</i> :	28
<i>Malva sylvestris</i> :	29
<i>Marrubium vulgare</i> :	30
<i>Medicago lupulina</i> :	32
<i>Medicago sativa</i> :	33
<i>Muehlenbeckia complexa</i> :	34
<i>Pilosella officinarum</i> :	35
<i>Reseda luteola</i> :	36
<i>Rumex acetosella</i> :	37
<i>Salvia verbenaca</i> :	37
<i>Sedum acre</i> :	38

<i>Oxalis exilis:</i>	39
<i>Sisymbrium officinale:</i>	40
<i>Stellaria graminea:</i>	41
<i>Taraxacum officinale:</i>	43
<i>Trifolium arvense:</i>	44
<i>Trifolium pratense:</i>	45
<i>Trifolium repens:</i>	46
<i>Verbascum Thapsus:</i>	47
<i>Vittadinia gracilis:</i>	48
<i>Wahlenbergia albomarginata:</i>	48

Achillea millefolium:

Common name: Yarrow

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Carmichaelia hollowayi:

Common name: Holloway's Broom

Biostatus: native

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Carmichaelia petriei*:**

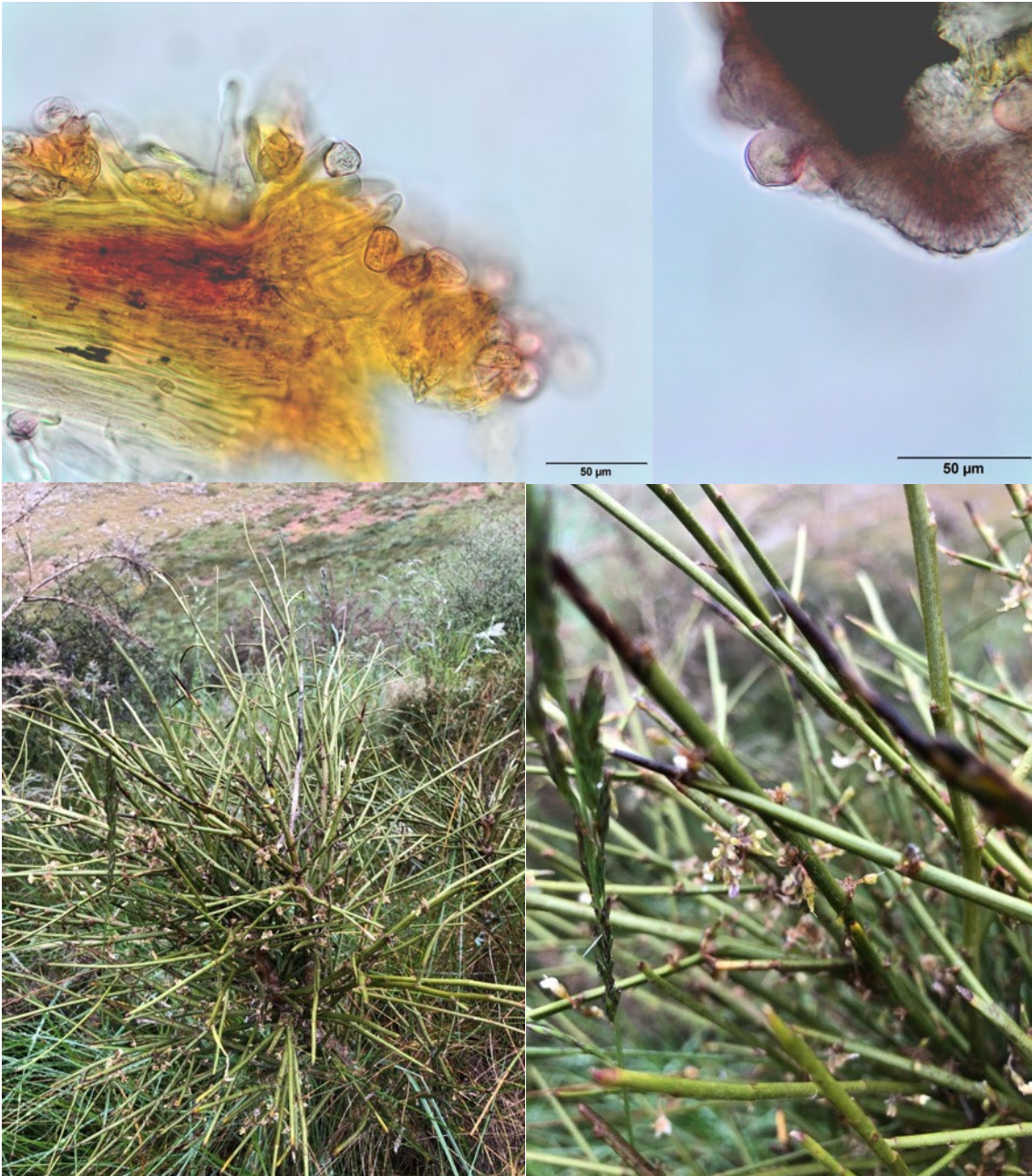
Common name: Desert Broom

Biostatus: native

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Cirsium arvense*:**

Common name: Californian Thistle, Creeping Thistle

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Cirsium vulgare:

Common name: Scottish Thistle

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 14/12/21 Wai O Toura



Convolvulus waitaha:

Common name: Grass Convolvulus

Biostatus: native

Taxonomic Family: Convolvulaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Crepis vesicaria:

Common name: Beaked Hawksbeard

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Cytisus scoparius:

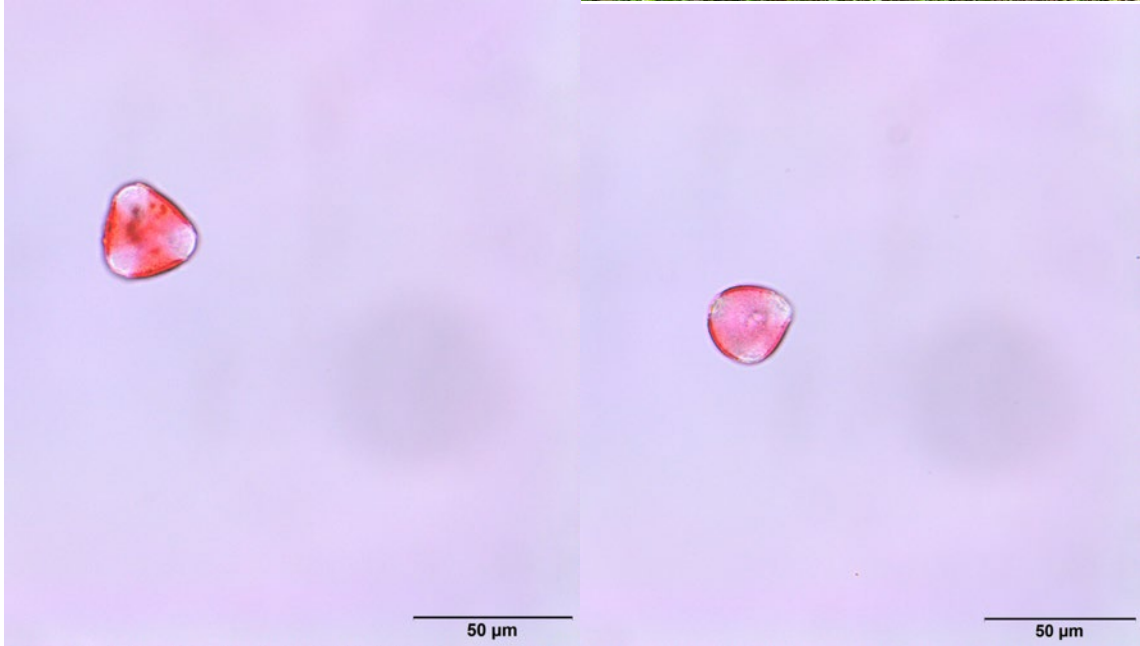
Common name: Broom

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Echium vulgare:

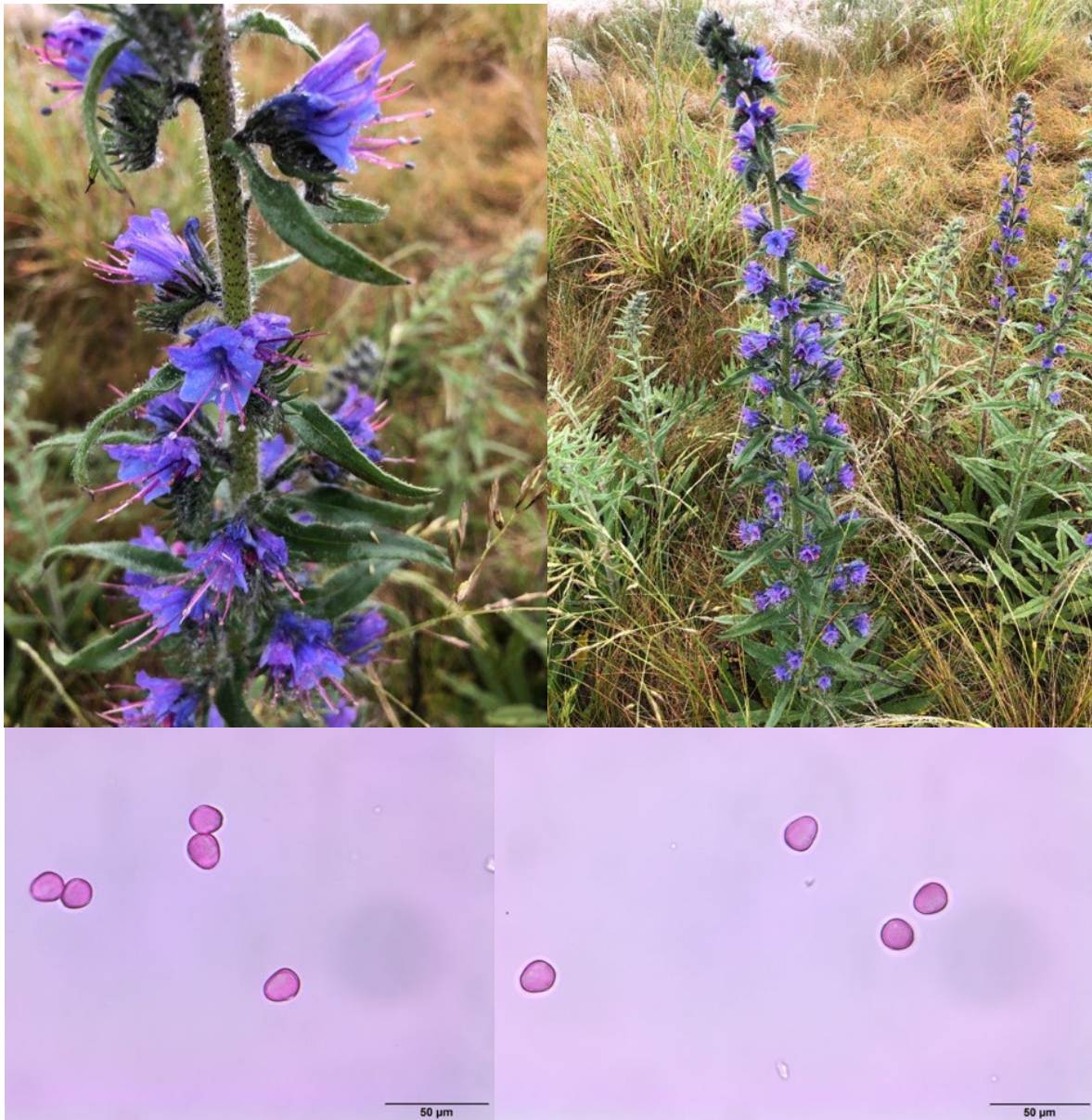
Common name: Viper's-bugloss, Blueweed

Biostatus: introduced

Taxonomic Family: Boraginaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



***Erodium cicutarium*:**

Common name: Stork's Bill

Biostatus: introduced

Taxonomic Family: Geraniaceae

Location: Waipata Scenic Reserve, Waitaki valley

Collected: 1/12/21



Fumaria bastardii

Common name: Tall Ramping Fumitory

Biostatus: introduced

Taxonomic Family: Papaveraceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 14/12/21



Gentianella calcis subsp. calcis:

Common name: Limestone Gentian

Biostatus: native

Taxonomic Family: Gentianaceae

Location: Waipata Scenic Reserve, Waitaki valley

Collected: 25/3/22



Hypochaeris radicata:

Common name: Catsear

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Lepidium sisymbrioides:

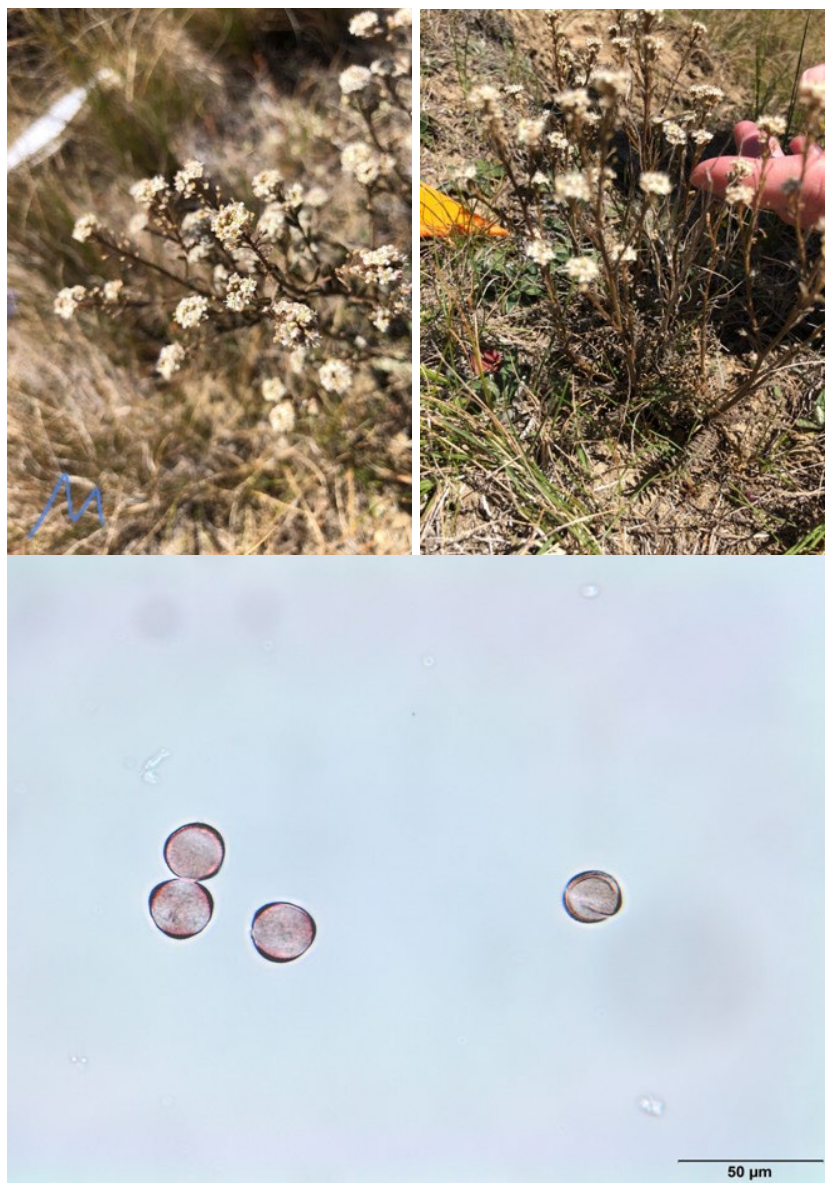
Common name: Peppergrass

Taxonomic family: Brassicaceae

Biostatus: native

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Leptospermum scoparium:

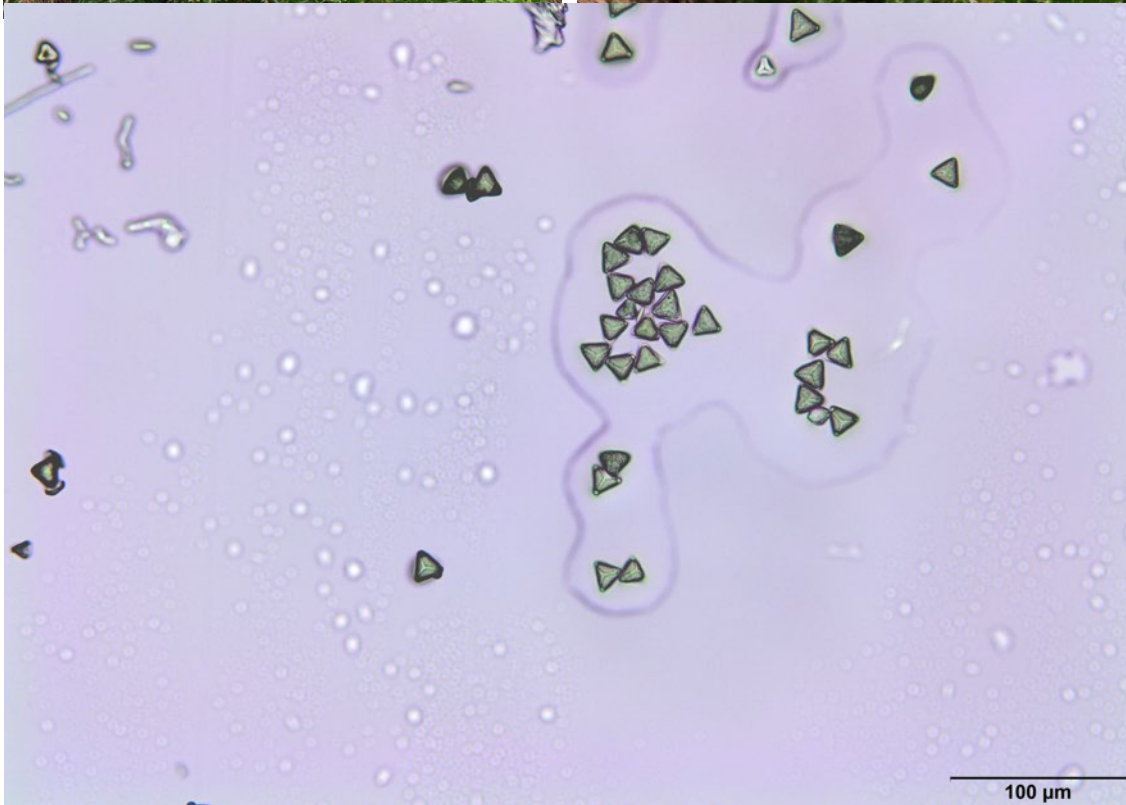
Common name: Manuka, NZ Teatree

Biostatus: native

Taxonomic Family: Myrtaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Lycium ferocissimum*:**

Common name: African Boxthorn

Biostatus: introduced

Taxonomic Family: Solanaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



***Malva neglecta*:**

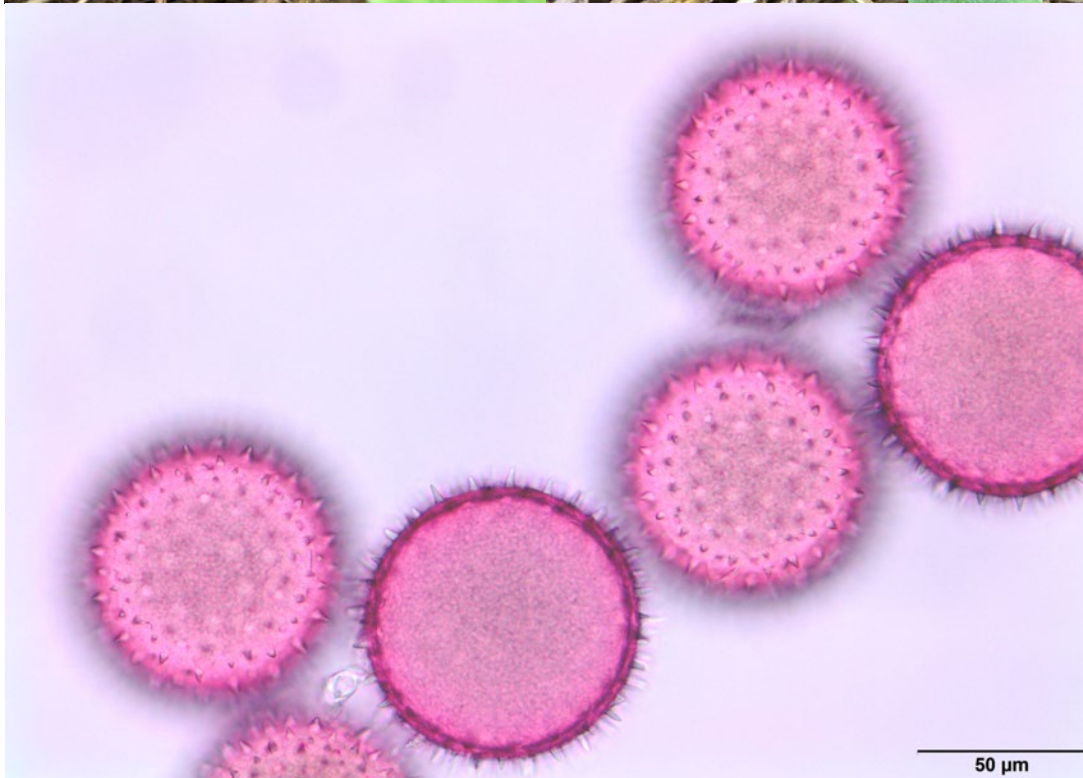
Common name: Dwarf Mallow

Biostatus: introduced

Taxonomic Family: Malvaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 14/12/21



Malva sylvestris:

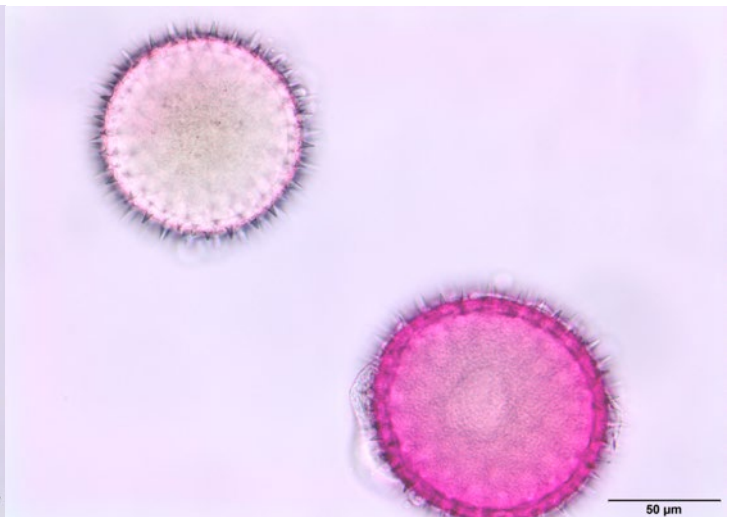
Common name: Common Mallows, Cheeses

Biostatus: introduced

Taxonomic Family: Malvaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Marrubium vulgare:

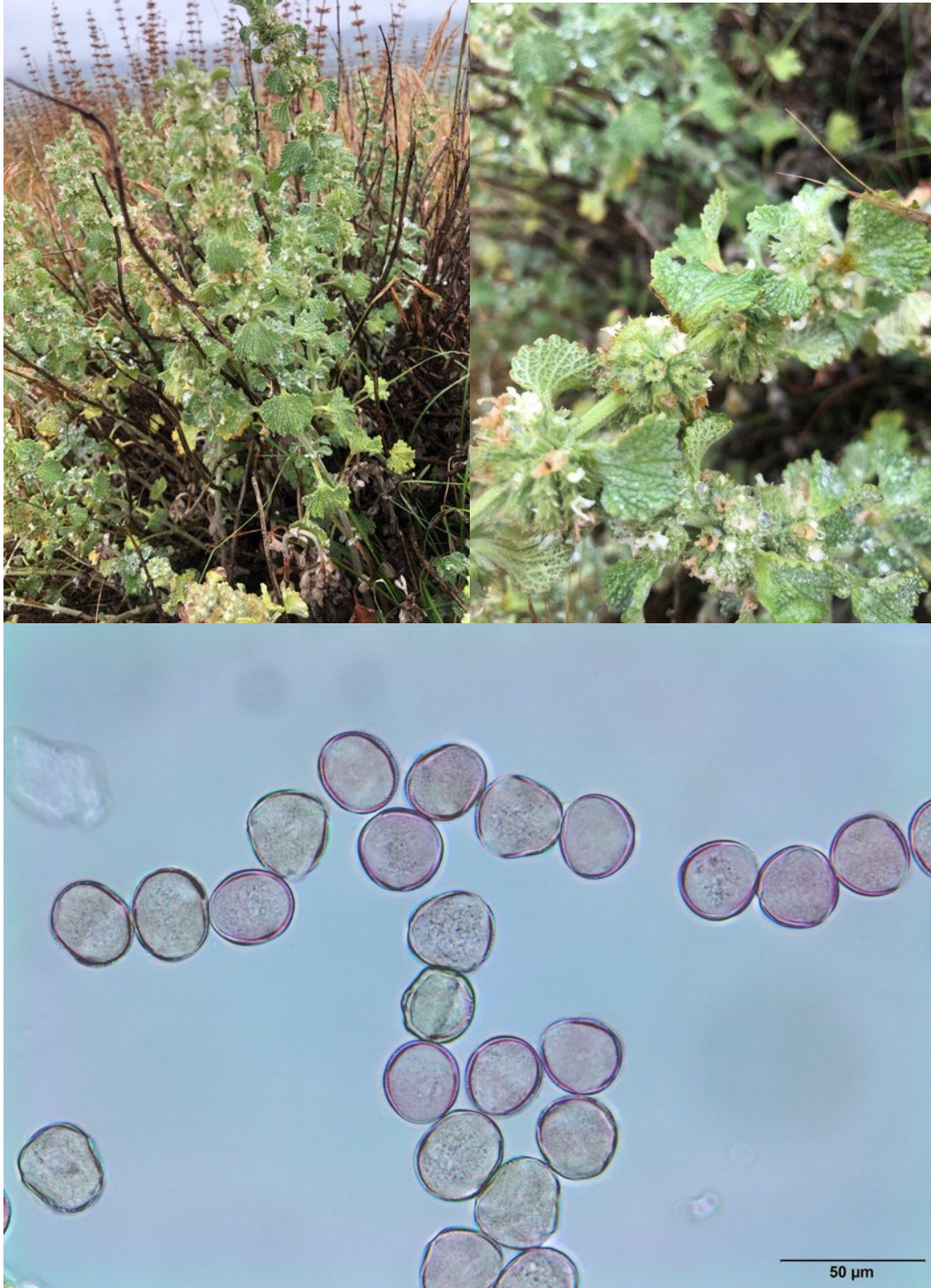
Common name: White Horehound

Biostatus: introduced

Taxonomic Family: Lamiaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Medicago lupulina:

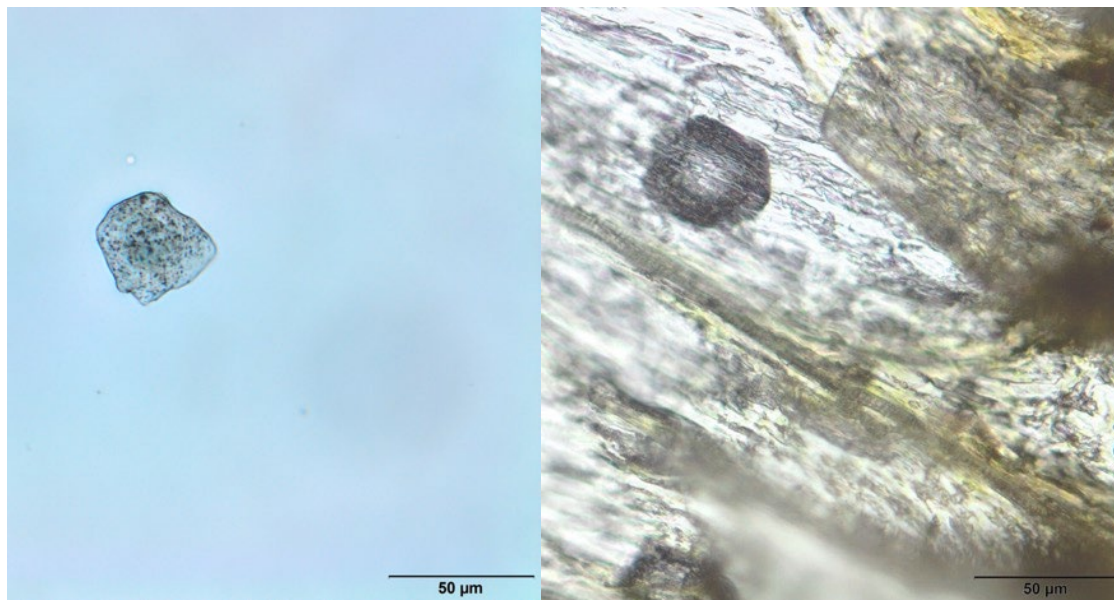
Common name: Black Medick

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Medicago sativa:

Common name: Alfalfa or Lucerne

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



***Muehlenbeckia complexa*:**

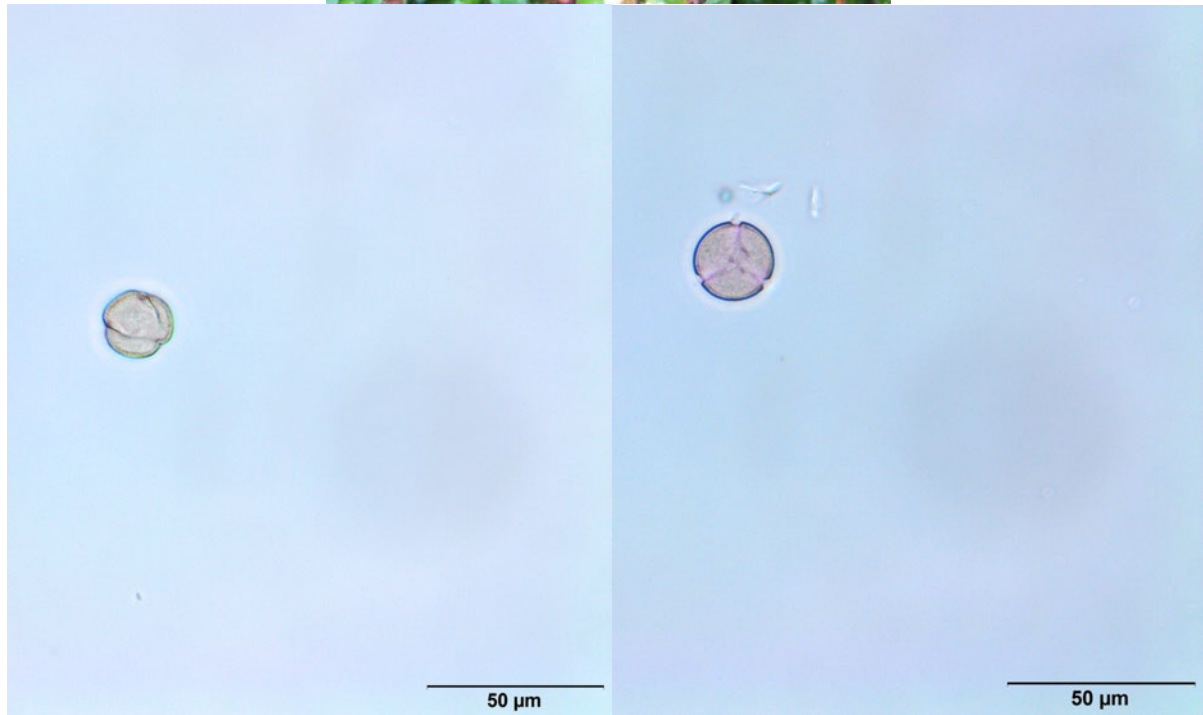
Common name: Scrambling Pohuehue, Maidenhair Vine

Biostatus: native

Taxonomic Family: Polygonaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Pilosella officinarum*:**

Common name: Mouse-eared Hawkweed

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Reseda luteola:

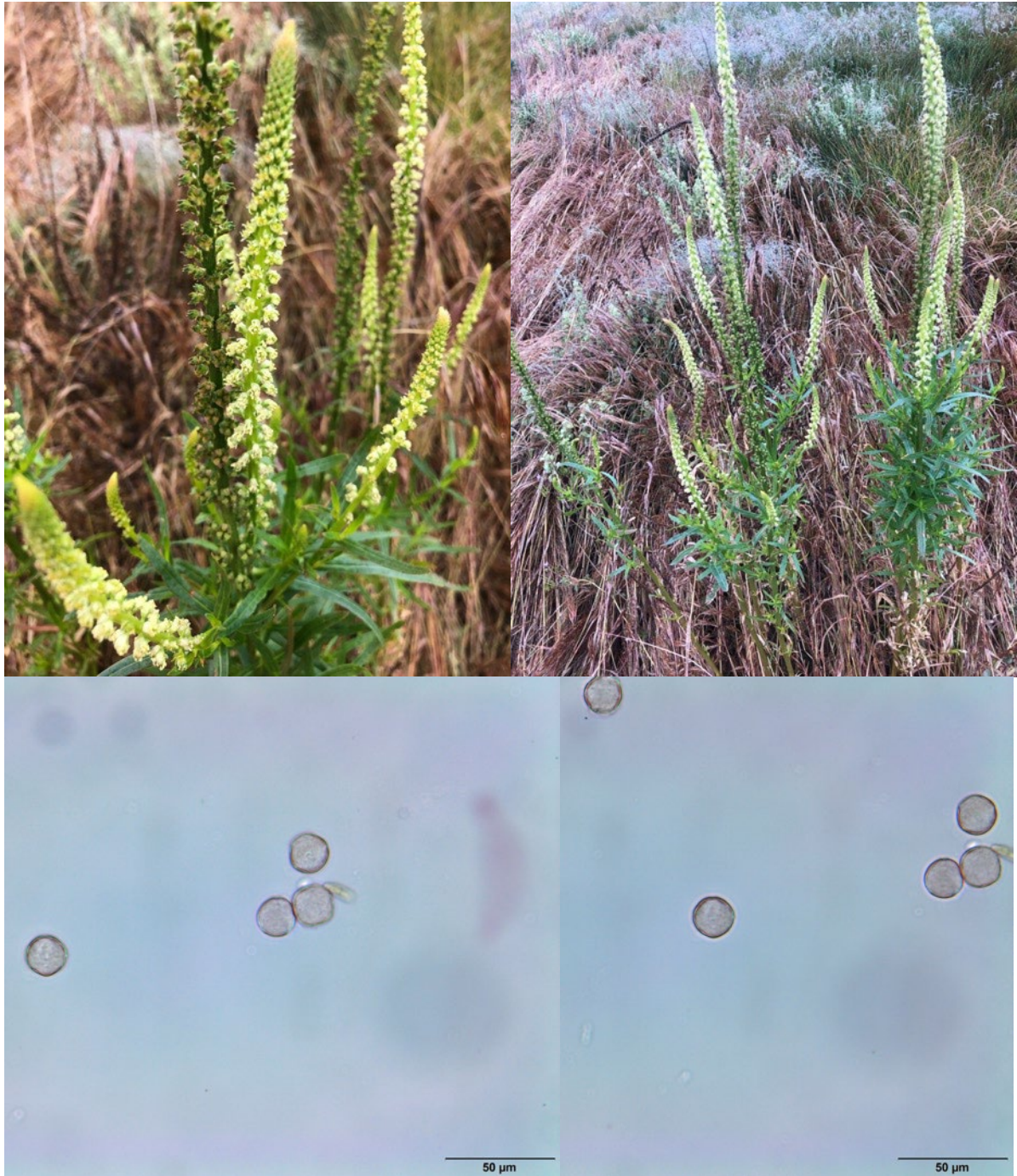
Common name: Wild Mignonette, Dyers Rocket

Biostatus: introduced

Taxonomic Family: Resedaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Rumex acetosella*:**

Common name: Sheep's Sorrel

Biostatus: introduced

Taxonomic Family: Polygonaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Salvia verbenaca:

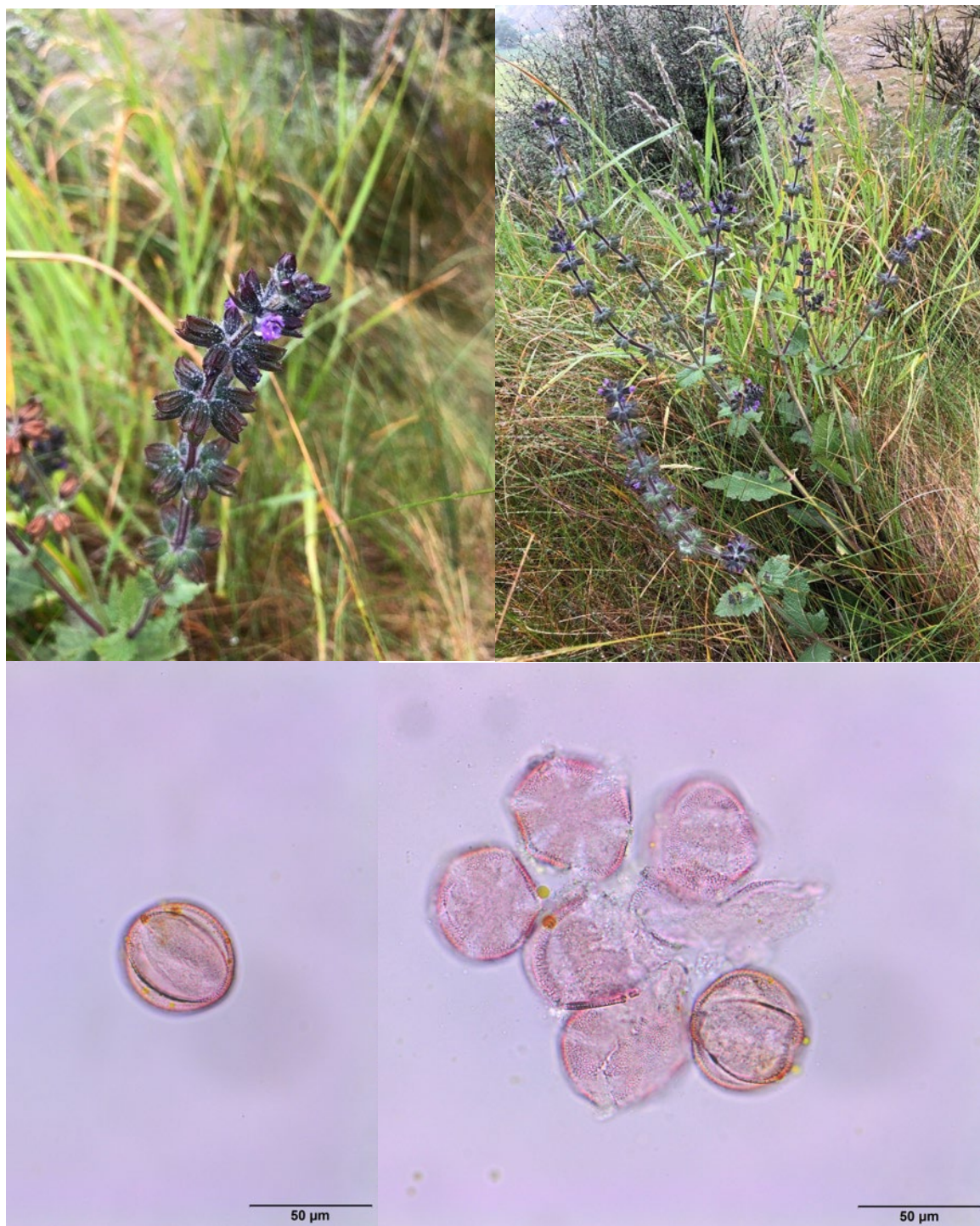
Common name: Wild Clary, Wild Sage

Biostatus: introduced

Taxonomic Family: Lamiaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Sedum acre:

Common name: Biting Stonecrop, Gold Moss

Biostatus: introduced

Taxonomic Family: Crassulaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 14/12/21



***Oxalis exilis*:**

Common name: Least Yellow Sorrel

Biostatus: native

Taxonomic Family: Oxalidaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 14/12/21



Sisymbrium officinale:

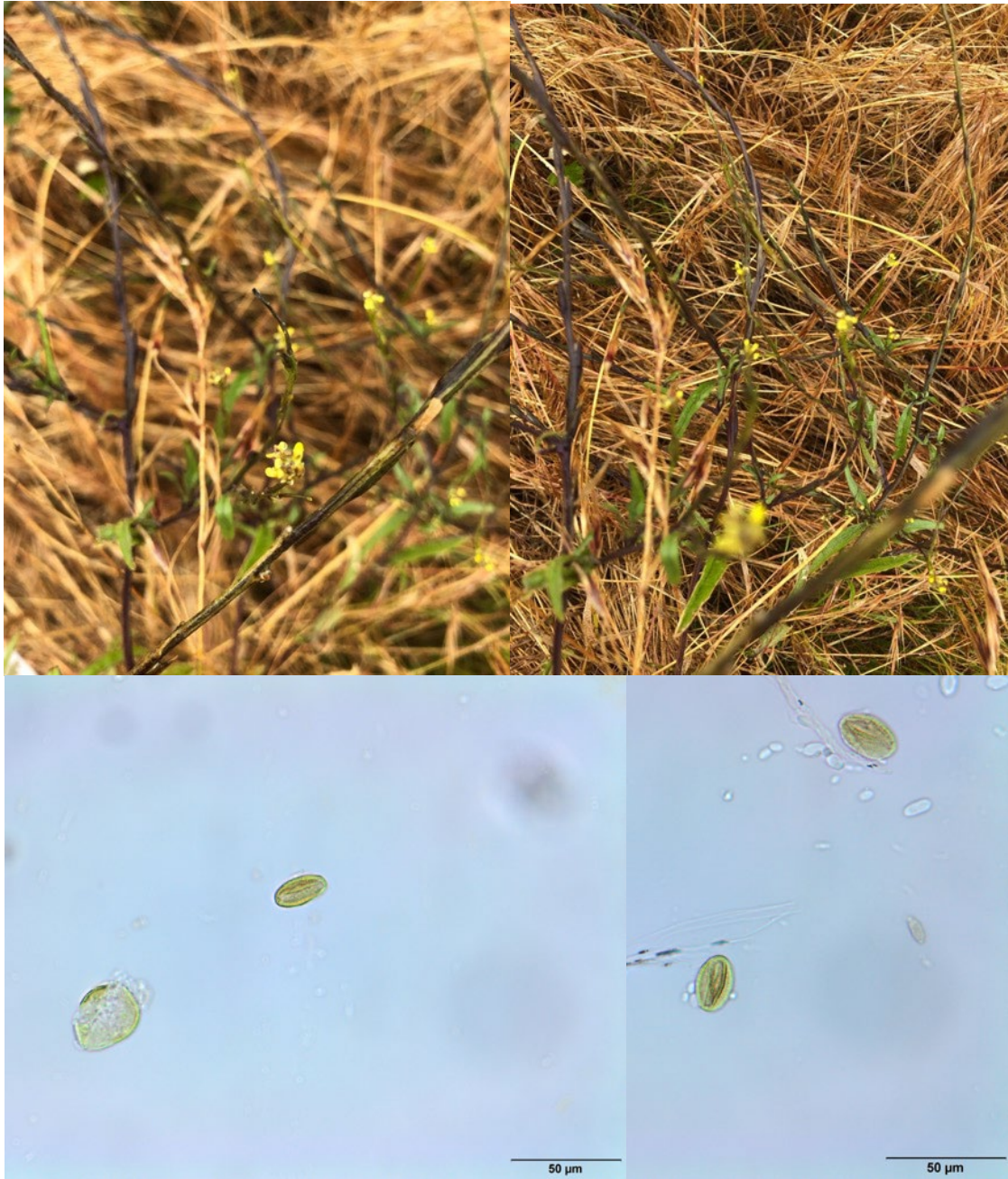
Common name: Hedge Mustard

Biostatus: introduced

Taxonomic family Brassicaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Stellaria graminea:

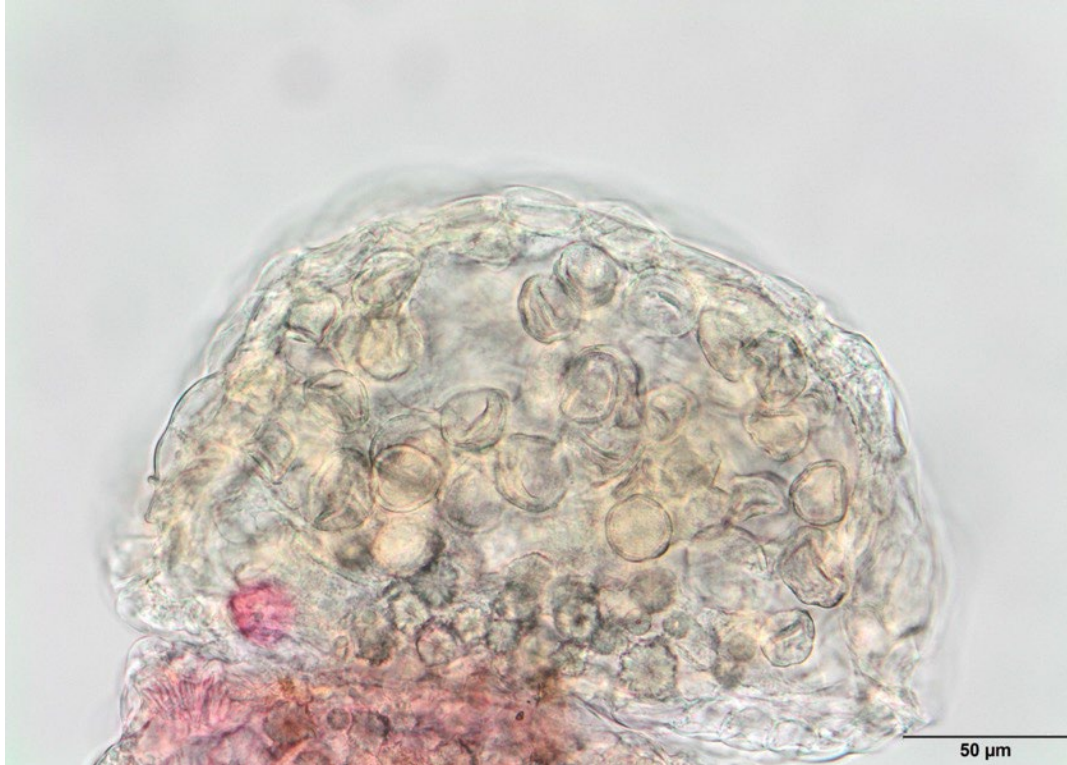
Common name: Lesser Stitchwort

Biostatus: introduced

Taxonomic Family: Caryophyllaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Taraxacum officinale:

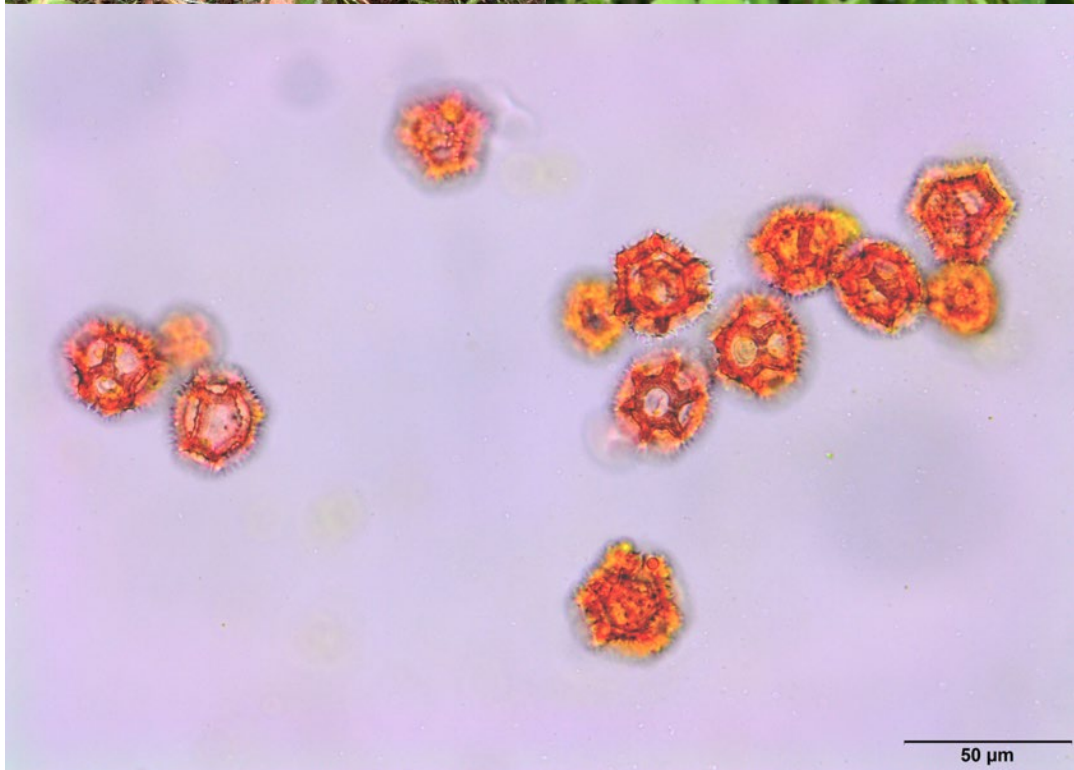
Common name: Common Dandelion

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



Trifolium arvense:

Common name: Hares Foot Trefoil

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



Trifolium pratense:

Common name: Red Clover

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 14/12/21 Wai O Toura



Trifolium repens:

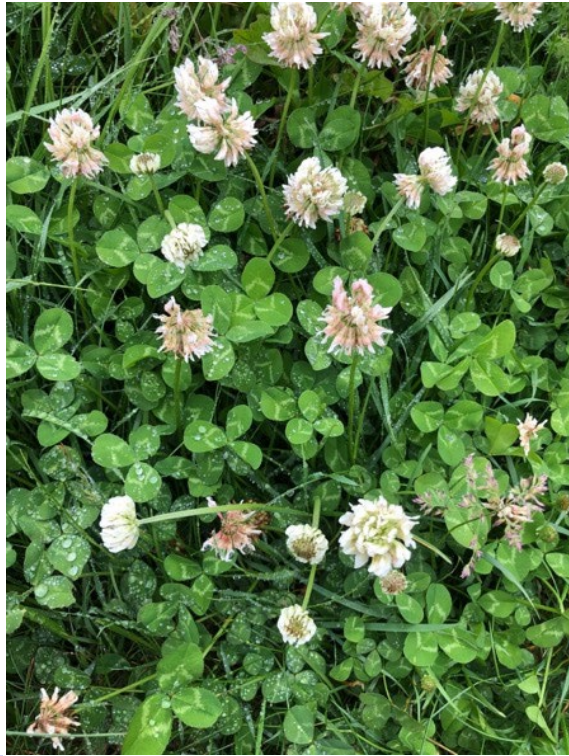
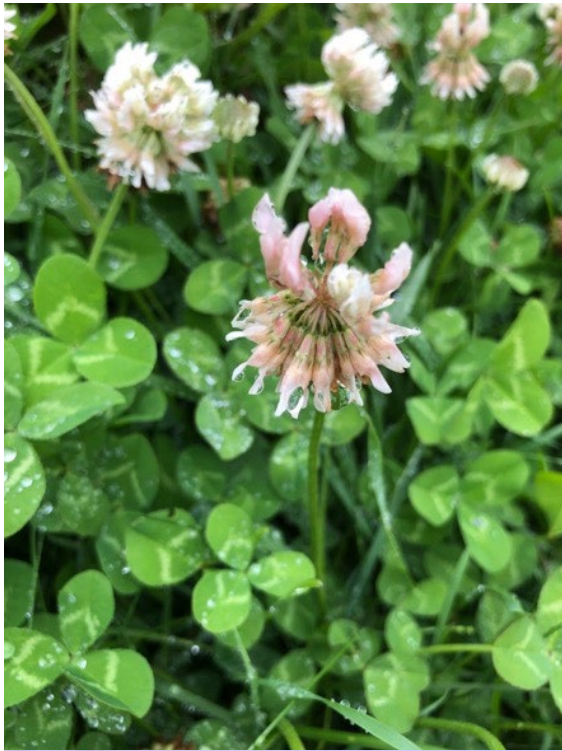
Common name: White clover

Biostatus: introduced

Taxonomic Family: Fabaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



***Verbascum Thapsus*:**

Common name: Woolly Mullein

Biostatus: introduced

Taxonomic Family: Scrophulariaceae

Location: Wai O Toura Scenic Reserve and Waipata Reserve, Waitaki valley

Collected: 1/12/21 Wai O Toura



***Vittadinia gracilis*:**

Common name: Purple Fuzzweed

Biostatus: introduced

Taxonomic Family: Asteraceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21



***Wahlenbergia albomarginata*:**

Common name: New Zealand Harebell

Biostatus: native

Taxonomic Family: Campanulaceae

Location: Wai O Toura Scenic Reserve, Waitaki valley

Collected: 1/12/21

