

Novel techniques in non-invasive pollen collection and the use of Scanning Electron Microscopy for the analysis of native pollinator preferences.

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Introduction : Entomophily plays a crucial role in the pollination of thousands of species of angiosperms globally (87.5%) and is of critical importance to food security with around 75% of global domestic crops being pollinated by insects [1;2]. Traditional studies in pollinator preference use direct observational strategies [3] and may not typically represent the full range of flowering plant species visited by pollinating insects. Therefore in this study we use minimally invasive techniques to collect and analyse the pollen from key pollinating insects, including Hymenoptera (Bees), Diptera (Flies), Coleoptera (Beetles) and Lepidoptera (Butterflies and Moths). Using Scanning Electron Microscopy (SEM) we are able to catalogue and identify both qualitative and quantitative details about the pollen collected, giving insights into floral associations and species preferences demonstrated by key native pollinators in South Wales.



Figure 1- Pollen collection from *Bombus pascuorum*.

Methods: Native pollinators were opportunistically collected using sweep netting from wildflower meadows at the University of South Wales Glyntaff. Bees, beetles and flies were placed in sterile 30ml glass collection jars and the opening was covered with a glass slide, insects were allowed to move freely in the container for 3 minutes and then released, the pollen was collected from inside the jar and glass slide using adhesive pads mounted on 1cm SEM stubs. Stub samples were coated in gold and visualised under 0.4-7.0kx magnification using Scanning Electron Microscopy. Pollen species were identified using morphometric analysis of micro-characteristics, in comparison with a pollen reference collection.

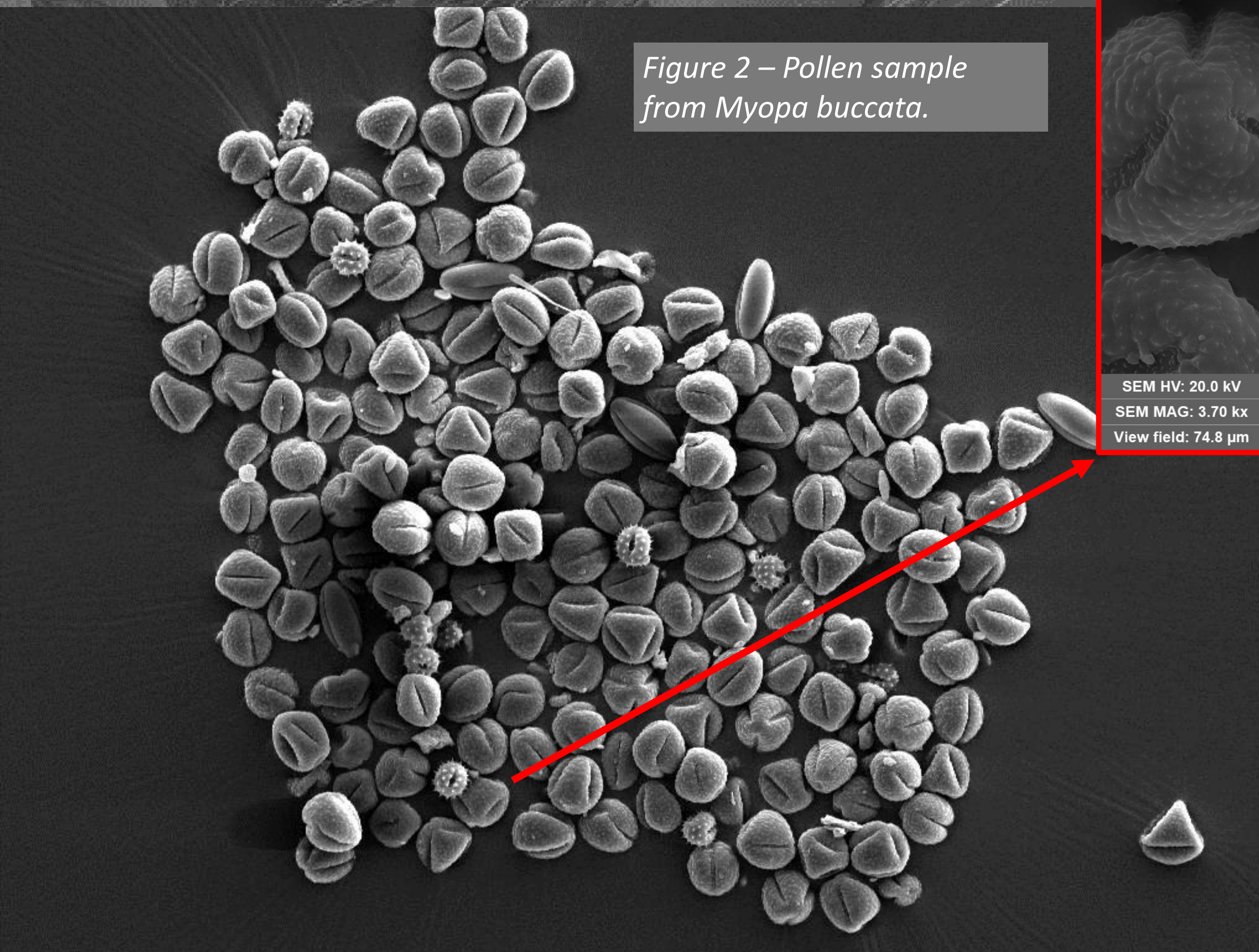
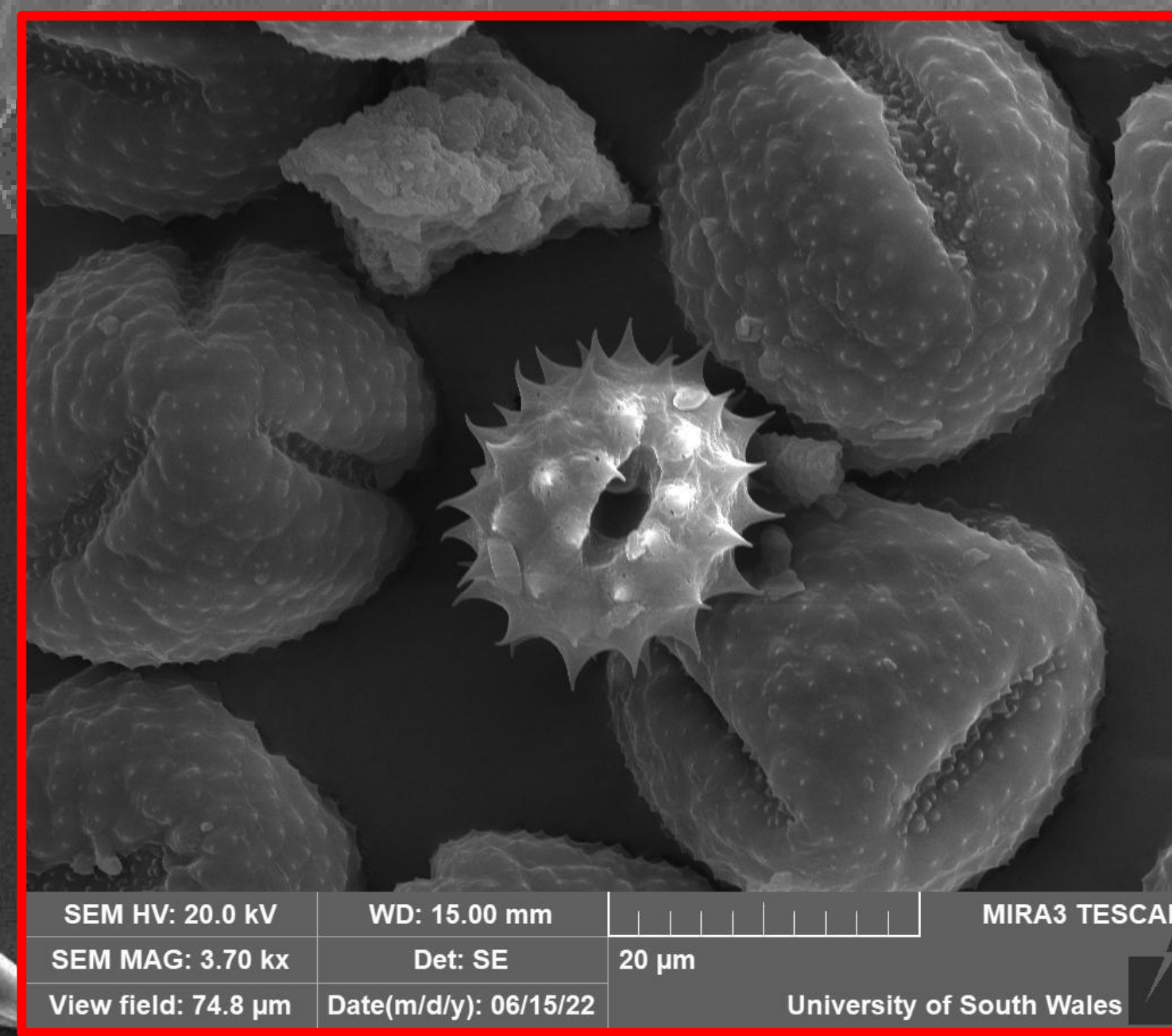


Figure 2 – Pollen sample from *Myopa buccata*.

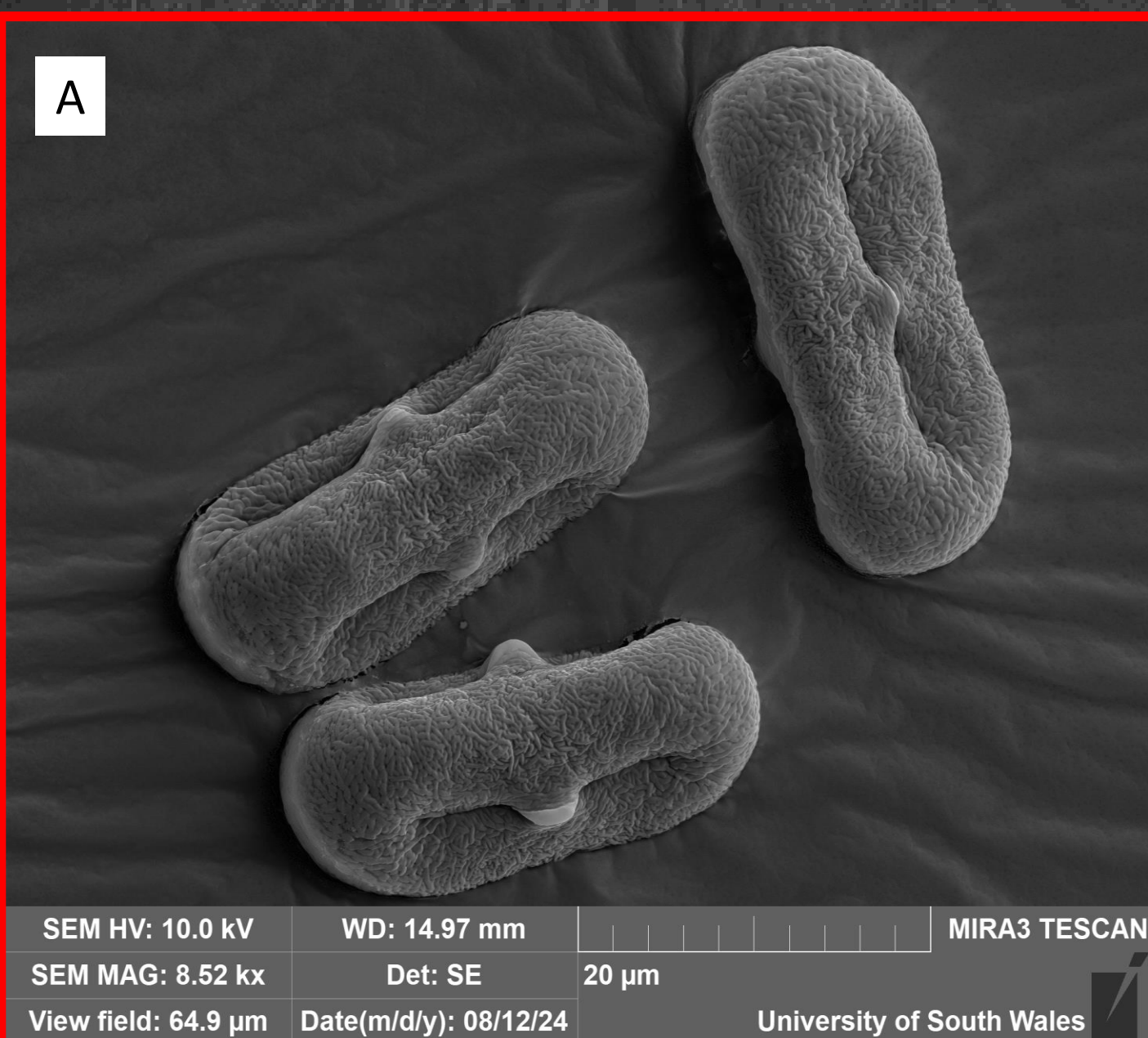


SEM HV: 20.0 kV WD: 15.00 mm MIRA3 TESCAN
SEM MAG: 3.70 kx Det: SE 20 µm
View field: 74.8 µm Date(m/d/y): 06/15/22 University of South Wales

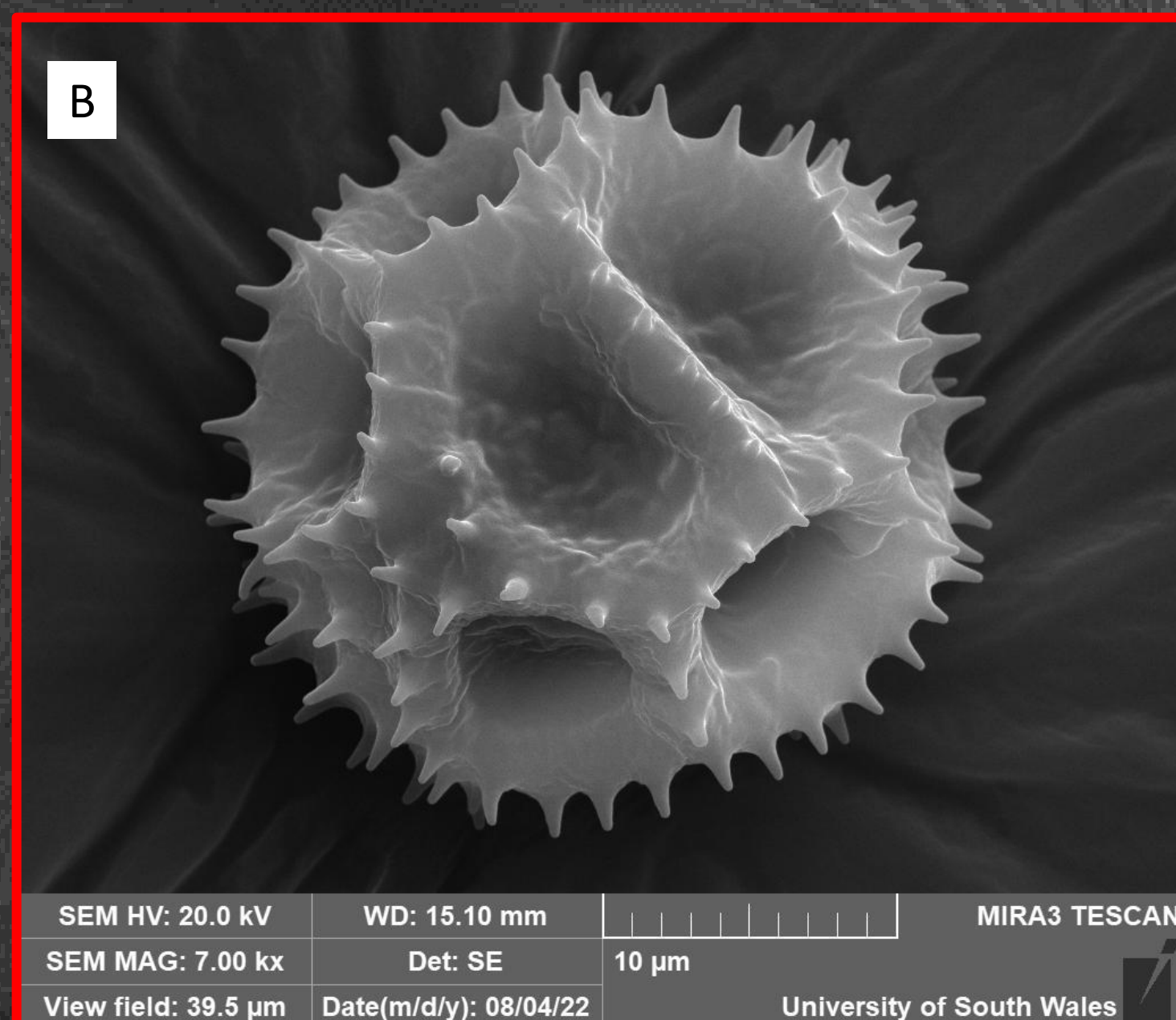
Results & Discussion:

- Scanning Electron Microscopy is a technique at the forefront of pollen analysis in a wide variety of scientific disciplines.
- Pollen can be removed from pollinators in a minimally invasive manner, which allows the pollinator to be released unharmed into its environment, without detrimental removal of foraged resources.
- Weather conditions (rain) can be a limiting factor in successful pollen recovery.
- Most pollinator species were found to be polylectic foragers with pollen from an average of 2.14 (± 1.15) plant families per sample.

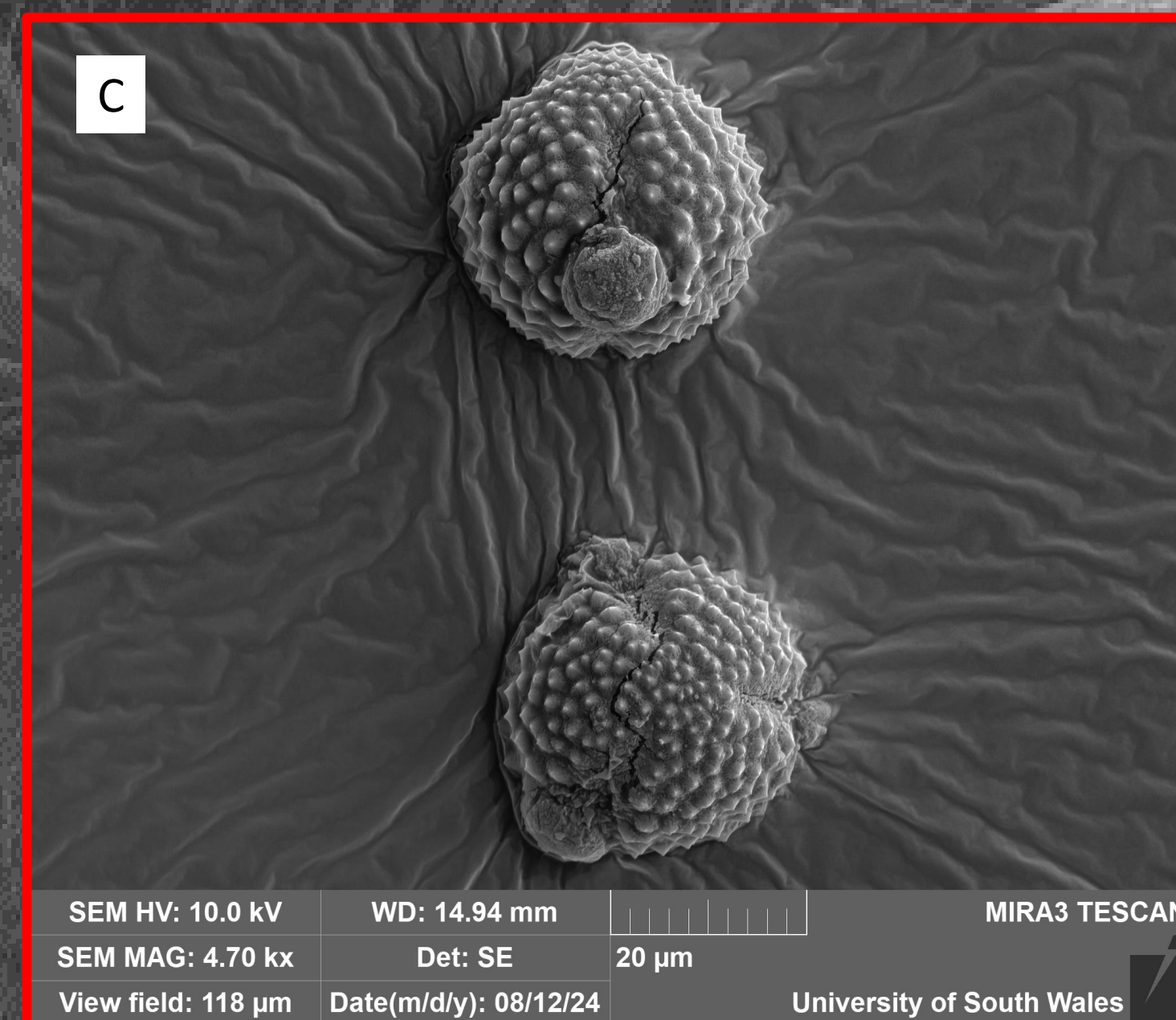
SEM HV: 20.0 kV WD: 15.00 mm MIRA3 TESCAN
SEM MAG: 400 x Det: SE 200 µm
View field: 692 µm Date(m/d/y): 06/15/22 University of South Wales



SEM HV: 10.0 kV WD: 14.97 mm MIRA3 TESCAN
SEM MAG: 8.52 kx Det: SE 20 µm
View field: 64.9 µm Date(m/d/y): 08/12/24 University of South Wales



SEM HV: 20.0 kV WD: 15.10 mm MIRA3 TESCAN
SEM MAG: 7.00 kx Det: SE 10 µm
View field: 39.5 µm Date(m/d/y): 08/04/22 University of South Wales



SEM HV: 10.0 kV WD: 14.94 mm MIRA3 TESCAN
SEM MAG: 4.70 kx Det: SE 20 µm
View field: 118 µm Date(m/d/y): 08/12/24 University of South Wales



SEM HV: 10.0 kV WD: 15.08 mm MIRA3 TESCAN
SEM MAG: 7.59 kx Det: SE 20 µm
View field: 72.9 µm Date(m/d/y): 08/12/24 University of South Wales

Figure 3: Examples of Pollen collected from pollinator species. A – *Daucus carota*; B – *Pilosella aurantiaca*; C - *Centaurea nigra*; D – *Prunella vulgaris*

Conclusions & Future Research: Sampling techniques for rare and endangered species of invertebrates should take into account the impact on populations. Minimally invasive techniques, such as the one identified here, demonstrate a significant advantage in sampling from pollinators, in that populations are not detrimentally impacted by collection activities. Future studies will examine the use of this technique in different habitat settings, examine specific species in more detail including rare or data deficient species, and quantitatively evaluate this method against other traditional pollen collection methods.