

Report on the outcomes of a Short-Term Scientific Mission¹

Action number: **CA18201**

Grantee name: **Marta Barberis**

Details of the STSM:

Title: Assessing the pollinator assemblage of a rare and endemic species as potential pre-pollination barrier to hybridization with sympatric and closely related congener.

Start and end date: 11/05/2023 to 19/05/2023.

Description of the work carried out during the STSM:

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

The study on the floral visitor assemblages of the two sympatric species *Salvia pratensis* and *S. saccardiana* was conducted in Brod na Kupi (Croatia), where the two grow in close proximity (45°28'01"N 14°48'38"E; 45°28'29"N 14°47'58"E).

Three aspects regarding floral visitors can impart isolation between the two species: i) the floral visitor assemblages include different insect species, if not, ii) the same insect species behave in different ways on the two species (e.g. one insect taxon may act as nectar robber on one species and as legitimate visitor on the other), and – if no substantial difference can be recognize in the behavior of visit – then iii) pollen may be deposited on different body parts due to the considerable difference in flower size between the two plant species.

To address the first two aspects, we carried out observations on the floral visitor assemblages as well as recording behaviors of visit through visual observations. A total of five days were spent in the field: three were dedicated to performing the protocol for visual behavioural observations, two were dedicated to the observation of the flower visitor assemblages. We recorded all floral visitors interacting with flowers of both *Salvia* species between the following intervals: 8:00am-9:00am, 10:00am-11:00am, 12:00am-1:00pm, 2:00pm-3:00pm, 4:00-5:00pm (see Table 1 for a preliminary report of the taxa observed). When a certain taxon was not recognizable in the field, we caught one or a few specimens in separate vials with ethyl acetate and brought them to the laboratory where they will be inspected under a dissecting microscope for a more accurate taxonomic identification. For what concerns visual behavioural observations, we recorded a total number of 478 insect-flower interactions. From these data we calculated a first preliminary percentage of success of visit per each insect taxon (see Table 2).

¹ This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Insect taxon	Functional group	<i>S. pratensis</i>	<i>S. saccardiana</i>
<i>Apis mellifera</i> Linnaeus, 1758		✓	✓
<i>Anthophora</i> Latreille, 1803 sp. 1		✓	✓
<i>Anthophora</i> Latreille, 1803 sp. 2		✓	✓
<i>Melecta albifrons</i> (Forster, 1771)		✓	
<i>Bombus pascuorum</i> aggr.	Pollinator, potentially able to trigger the lever mechanism.		
<i>Bombus hortorum</i> aggr.		✓	✓
<i>Bombus terrestris</i> aggr.			
<i>Xylocopa violacea</i> Linnaeus 1758		✓	✓
<i>Anthidium</i> Fabricius, 1805 sp.		✓	
<i>Andrena</i> Fabricius, 1775 sp. 1			✓
<i>Halictus</i> Latreille, 1804 sp. 3		✓	✓
<i>Ceratina cucurbitina</i> (Rossi, 1792)		✓	
<i>Ceratina cyanea</i> (Kirby, 1802)		✓	✓
<i>Lasioglossum</i> Curtis, 1833 sp.	Very small bee, style or filament climber, pollen collector.	✓	✓
<i>Halictus</i> Latreille, 1804 sp. 4			✓
<i>Andrena</i> Fabricius, 1775 sp. 2			✓
<i>Hylaeus</i> Fabricius, 1793 sp.		✓	
<i>Osmia</i> Panzer, 1806 sp.		✓	✓
<i>Halictus</i> Latreille, 1804 sp. 1	Fairly small bee/fly/hoverfly, never triggers the lever mechanism, pollen collector	✓	
<i>Halictus</i> Latreille, 1804 sp. 2		✓	✓
<i>Megachile pilidens</i> Alfken, 1924			✓
Syrphidae (Latreille, 1802) sp.		✓	✓
<i>Bombylius</i> (Linnaeus, 1758) sp.		✓	✓
<i>Gonepteryx rhamni</i> (Linnaeus, 1758)	Long proboscis, nectar robber, never triggers the lever mechanism	✓	✓
<i>Pieris napi</i> (Linnaeus, 1758)		✓	✓
<i>Lysandra bellargus</i> (Rottenburg, 1775)		✓	✓
<i>Iphiclides podalirius</i> (Linnaeus, 1758)		✓	
<i>Tropinota</i> (Mulsant, 1842) sp.	Flower burglar, can destroy the flower to reach the nectar, very static behaviour of visit	✓	

Table 1. Presence/absence of the different flower visitor taxa recorded interacting with the two species in 2022 and 2023.

Since the focus of this work was to identify potential reproductive barriers between the two species, in addition to the study on floral visitors, we also carried out a study on the population phenology, to assess how long their blooming period overlaps. To do this, we marked 20 plants per population, and we recorded, every other day, the number of open flowers out of the total number of flowers carried on the stem (ongoing but see Table 3 for initial results).

	<i>Salvia saccardiana</i>	<i>Salvia pratensis</i>
<i>Xylocopa violaceae</i>	0.95	0.87
<i>Apis mellifera</i>	0.17	0.86
<i>Anthophora</i> sp.	0.44	0.45
<i>Bombus</i> sp.	0.70	-
Megachilidae	0.61	0.50
Halictidae	0.00	0.07
Syrphidae	0.00	0.00
Bombiliidae	0.03	0.00
Pieridae	0.00	0.00

Table 2. Percentages of successful flower-insect interactions for the main taxa of floral visitors observed in spring 2023.

	9.05.2023	11.5.2023	13.5.2023	15.5.2023	17.5.2023	19.5.2023
<i>Salvia saccardiana</i>	21	35	62	97	118	148
<i>Salvia pratensis</i>				11	51	69

Table 3. Population flowering phenology recorded in spring 2023 for the two species.

Description of the STSM main achievements and planned follow-up activities.

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

As initially planned, the activity of this STSM represents only a part of a wider study started in 2022. During field work, a considerable amount of time has been also spent recording videos which will then be analyzed with the Software Mangold Interact to investigate whether or not the pollen is deposited in different parts of the body when the insect taxon visiting the two plant species is the same.

This will be done at the Natural History Museum Rijeka (Croatia), as well as the preparation and identification of the insect specimens collected during the surveys. At the moment, additional behavioural observations, video recording and flower phenology monitoring are still being carried out by the people I collaborated with.

Afterwards, when all activities will be done, all data collected within the project will be analyzed and collated in a final paper. For both the analyses and the paper drafting the collaboration will continue.