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# New video registration of *Autographa pulchrina* (Haworth, 1809) (Lepidoptera, Noctuidae) and *Sphinx pinastri* L., 1758 (Lepidoptera, Sphingidae) pollinating *Platanthera bifolia latiflora* (Orchidaceae) in Norway

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In the Orchidaceae family, many species have a highly specialized floral structure and floral fragrance due to interactions with specific pollinators. Pollinators foraging on orchids have traditionally been monitored by detecting pollen vectors on insects, recording tracks from the moths on the orchids, and by direct observations. In the present study an event triggered video system to monitor *Platanthera bifolia* (L.) L. C. Rich. ssp. *latiflora* (Drejer) Løjtnant. to video register pollinators were used. A total of 16 days of monitoring were conducted, whereas only three nights had visits. Four of the visitors were identified as *Sphinx pinastri* (L., 1758) and two were identified as *Autographa pulchrina* (Haworth, 1809). The visits took place during the night (time-range CET (GMT+2) 21:20–01:16). Observations from the video recordings showed that *S. pinastri* approached and hovered in front of the inflorescence and inserted their proboscises into the spur of the flower, while hovering *S. pinastri* didn't need support by their forelegs to insert their proboscises into the spur. From the recordings both species approached the inflorescence with an uncoiled proboscis, further the proboscises were uncoiled while they hovered from flower to flower.

Key words: *Autographa pulchrina*, digital video monitoring, flower visit, motion detection, pollination, *Platanthera bifolia*, Lepidoptera, Noctuidae, *Sphinx pinastri*.

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## Introduction

The co-evolution between flowering plants and flower visiting insects have fascinated scientists for decades (e.g. Darwin 1877, Wiebes 1979, Schemske & Hortvitz 1984, Thompson & Cunningham 2002, Ramirez *et al*. 2011). In the Orchidaceae family, many species have a highly specialized floral structure and floral fragrance due to interactions with specific pollinators (Darwin 1877, Wasserthal 1997, Whittall & Hodges 2007). Pollinators foraging on orchids have traditionally been monitored by indirect methods, such as detecting pollen vectors on insects and recording tracks from the moths on the orchids (Darwin 1877, Nilsson 1983, Maad & Nilsson 2004), or by direct observations (Robertson & Wyatt 1990, Raguso & Willis 2005, Peter *et al*. 2009) and more recently by continuous video surveillance (Micheneau *et al*. 2008). Direct observations and continuous video monitoring of orchids with low visitation rates in nature is time consuming. Although a more efficient system has been used to monitor flower visiting insects, this system consists of a video motion detection (VMD). The VMD system detects changes in the image captured by the camera, where pollinators trigger recording immediately (Steen & Aase 2011).

In the present study the video system described in (Steen & Aase 2011, Steen 2012) was used to monitor *Platanthera bifolia* (L.) L. C. Rich. ssp. *latiflora* (Drejer) Løjtnant. to register pollinators. *P. bifolia* is a terrestrial orchid with a wide distribution in Eurasia (Hultén & Fries 1986). The flowering season of *P. bifolia* occurs in June and July in northern Europe (Davies *et al*. 1983, Hultén & Fries 1986). This plant is a polycarpic perennial herb, but most individuals do not set florescence every subsequent year (Maad & Nilsson 2004). *P. bifolia* is divided into two subspecies: the one in the present study, *P. bifolia* ssp. *latiflora* which thrives in more nutritious shady habitats as in woodlands, and *P. bifolia* ssp. *bifolia* which is found in light open areas (Løjtnant 1978, Hæggström 1994). The two subspecies differ in their floral scent constitution and are pollinator specific adapted (Tollsten & Bergström 1993, Boberg 2010). The length of the spurs is of higher importance for reproduction success of this species than perianth size, *P. bifolia* ssp. *latiflora* having the longest spurs of these two species, especially on the mid-section of the inflorescence (Boberg & Agren 2009). The two species do not bloom at the same time either. Studies from Sweden (Elin Boberg, 2010) show that *P. bifolia* ssp. *latiflora* develops its flowers about two weeks earlier than *P. bifolia* ssp. *Bifolia* (Boberg 2010). Our purpose was to identify nocturnal insects visiting *P. bifolia ssp. Latiflora*, and provide anecdotal data on hovering behaviour.

## Methods

The study was conducted in Hjartdal municipality in Telemark county in southern part of Norway (16 June–2 July 2011). Two *P. bifolia* ssp. *latiflora* were monitored, the first one was located on a clear-cut with some Scots pine trees (*Pinus sylvestris*), 139m.a.s.l. (N59°37.839' E008°57.082') and second was located in a forest with mainly Norwegian spruce trees (*Picea abies*), close to a stream, 133m.a.s.l. (N59°37.840' E008°56.641).

The first plant had 10 flowers, whereas the average spur length was 31.9mm (±3.0 SD), the second plant had 17 flowers with an average spur length at 19.4mm (±5.9 SD). For the first plant the spur length was evenly distributed along the whole inflorescence, whilst for the latter the lowermost part ranged from 23–26mm (n=8) and the uppermost ranged from 4–20mm (n=9). The total height of the first one was 450mm and the second 500mm.

We placed CCD (charge-coupled device) camera, with a waterproof housing, on a pole, facing towards the capitulum. The camera was connected with a video cable to a mini digital video recorder (mini DVR), the system consists of a video motion detection (VMD) sensor that detects changes in the image captured by the camera, where pollinators trigger recording immediately for details see (Steen 2012).

To identify the nocturnal insects, images taken by Vladimir Kononenko were compared to the screenshots from the video footage. To ensure a precise comparison, the screenshots from the video footage were merged with the images by the use of GIMP (GNU image manipulation program). The image perspective was changed to fit the perspective of the video footage. Further, the transparency of the image was varied to more easily compare the pattern of the wings, the thorax and abdomen from the underlying screenshot of the video footage.

## Results and discussion

A total of 16 days of monitoring was conducted, whereas only three nights had visits. Out of the six visits four were identified as *Spinx pinastri* (Figure 1) and two were identified as *Autographa pulchrina* (Figure 2). From indirect observations, *S. pinastri* is known to visit *P. bifolia* (Nilsson 1978, 1983), and *A. pulchrina* has been reported to being a visitor of *P. chlorantha* (Nilsson 1978), however to our knowledge *A. pulchrina* has not been reported to being a visitor of *P. bifolia*. This is the first study documenting *S. pinastri* and *A. pulchrina* visiting *P.bifolia* by the use of a video

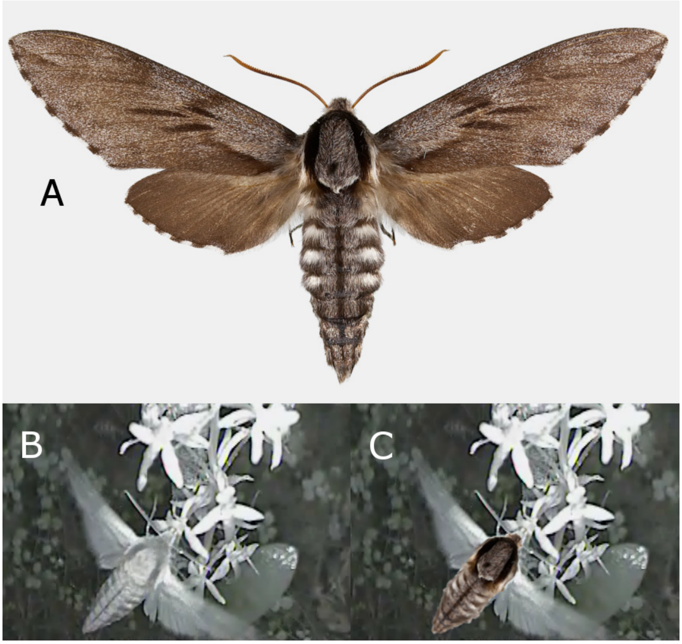

**FIGURE 1**. **A**. *Sphinx pinastri* (L., 1758) (Photo: Vladimir Kononenko). **B**. Screenshot from the video recording, *S. pinastri* (pollinating *Platanthera bifolia* (L.) L. C. Rich. ssp. *latiflora* (Drejer) Løjtnant) the body from the photo merged with the screenshot, manipulated to obtain. **C**.same perspective as the screenshot.

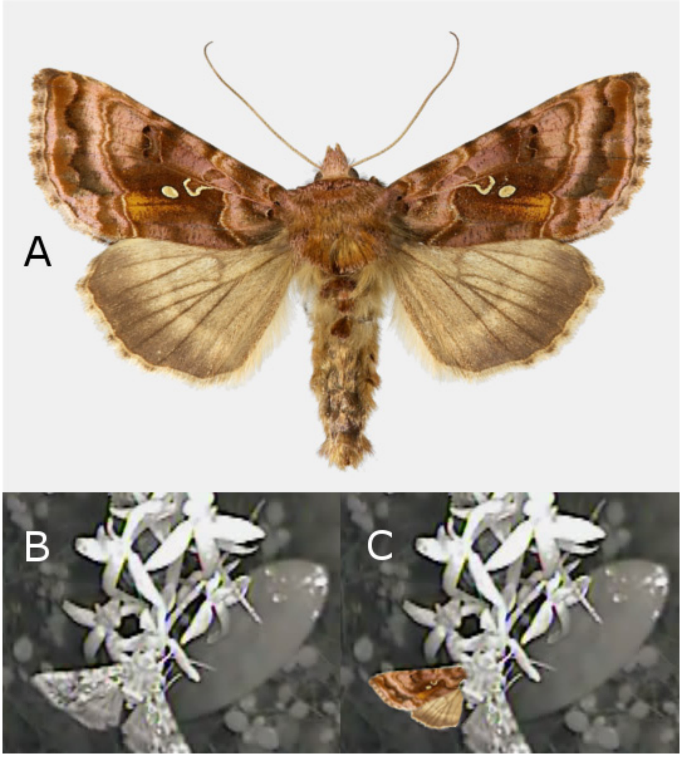

**FIGURE 2**. **A**. *Autographa pulchrina* (Haworth, 1809) (Photo: Vladimir Kononenko). **B**. Screenshot from the video recording, *A. pulchrina* pollinating *P. bifolia* ssp. *latiflora* the left wing from the photo merged with the screenshot, manipulated to obtain. **C**. same perspective as the screenshot.

monitoring system. The proboscis of *S. pinastri* is 28–32mm (Müller 1871), which matches the length of the nectar spur of the *P. bifolia* (Nilsson 1985). The first plant was only visited by *A. pulchrina* once, while the second plant was visited by both species, *A. pulchrina* once and *S. pinastri* four times. At the first location the visit of *A. pulchrina* took place at CET(+2) 01:16:2 –01:16:32 (22 June 2011). At the other location the visit of *A. pulchrina* took place at CET(+2) 00:15:44–00:18:29 (29 June 2011and *S. pinastri* CET(+2) 21:20:44–21:23:56 (29 June 2011) and CET(+2) 22:24:17–22:28:20, 22:35:24–22:37:49, 22:43:02–22:45:12 (2 July 2011). The last three visits might be the same individual.

We observed from the video recordings that *S. pinastri* approached and hovered in front of the inflorescence and inserted their proboscises into the spur of the flower, while hovering *S. pinastri* didn't need support by their forelegs to insert their proboscises into the spur, similar to what has been found for *S. pinastri* pollinating *P. chlorantha* (Steen in 2012). In contrast *A. pulchrina* brought their forelegs and grabbled the flowers to stabilize feeding, this is similar to other hawk-moths pollinating *Habenaria epipactidea* (Peter *et al*. 2009). From the recordings both species approached the inflorescence with an uncoiled proboscis, further the proboscises were uncoiled while they hovered from flower to flower, as reported in other Lepidoptera species visiting plants with inflorescence (Krenn 2008).

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