Hacquetia 22/2 • 2023, 247–262 DOI: 10.2478/hacq-2022-0018 sciendo

Diversity of *Epipactis palustris* (L.) Crantz (*Orchidaceae*) pollinators and visitors in conditions of Kyiv city (Ukraine)

Alla M. Gnatiuk[1], Mykola B. Gaponenko[1], Hanna Y. Honchar[2] & Andrii M. Gaponenko[3]

Key words: orchids, insects, arthropods, protection, plant ecology.

Ključne besede: orhideje, žuželke, členonožci, zavarovanje, rastlinska ekologija.

Abstract

Epipactis palustris (L.) Crantz is a protected species in Ukraine. In order to organize optimal protection measures comprehensive studies of various aspects of its biology are necessary. The aim of this study was to determine the pollinators and visitors of *E. palustris.* The study was conducted in Kyiv city (Ukraine) between 2020 and 2022. The research focused on arthropod visitors in two human-made populations. Specimens of 31 families and 48 genera from 9 arthropod orders were identified and established. The greatest number of species represented *Hymenoptera, Coleoptera* and *Diptera*. Evaluation of visitor activity show that *Hymenoptera* were the most frequent visitors. *E. palustris* in the conditions of Kyiv is mainly characterized by entomophily. *Halictidae*, *Apidae* and *Coccinellidae* played an important role in *E. palustris* pollination. It was found that populations of *E. palustris* in the conditions of the Kyiv city form a unique self-regulating biocenosis that provides shelter and food for 53 identified species of arthropods.

Izvleček

Epipactis palustris (L.) Crantz je v Ukrajini zavarovana vrsta in da bi zagotovili njeno optimalno varovanje, so potrebne raziskave različnih vidikov njene biologije. S to raziskavo smo hoteli ugotoviti, katere vrste jo oprašujejo in obiskujejo. Raziskavo smo izvedli v mestu Kijev (Ukrajina) med letoma 2020 in 2022. Osredotočili smo se na obiskovalce iz skupine členonožcev v dveh umetnih populacijah. Osebki so pripadali 31 družinam in 48 rodovom iz 9 redov členonožcev. Največ vrst je bilo iz skupin *Hymenoptera, Coleoptera* in *Diptera*. Spremljanje obiskovalcev je pokazalo, da so bili najbolj pogosti obiskovalci iz skupine *Hymenoptera*. Vrsta *E. palustris* je v razmerah v Kijevu večinoma entomofilna. *Halictidae*, *Apidae* in *Coccinellidae* so pomembne za opraševanje vrste *E. palustris.* Ugotovili smo, da je v populacijah *E. palustris* v mestu Kijev nastala samo regulirajoča biocenoza, ki nudi zavetje in hrano 53 vrstam členonožcev.

Corresponding author:
A. M. Gnatiuk
E-mail: gnatiukalla@gmail.com

Received: 30. 10. 2022
Accepted: 23. 2. 2023

1 M. M. Gryshko National Botanical Garden of National Academy of Science of Ukraine, Kyiv, Ukraine.
2 Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine, Kyiv, Ukraine.
3 National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine.

Introduction

Epipactis palustris (L.) Crantz (*Orchidaceae)* is a Palearctic orchid species that is included in Appendix II of CITES and the Red Book of Ukraine (Didukh, 2009). It occasionally occurs in Ukrainian Polissia, Roztochcha, Opillia, Forest-steppe, Steppe, the Carpathians and the Mountainous Crimea. It rarely occurs in northern part of Polissia, and in the south of the Forest-steppe. In the Steppe, it is known in the basin of the Dnipro and Siverskyi Donets, in the Kherson region on the Tendrivska (Tendra) Spit and on Dzharylgach. It occurs frequently in the Carpathians, found in the Chornogora, in the Gorgans, in the Eastern Beskids. In Transcarpathia, it grows from the lowlands to the foothills. Plants very rarely occur on the Southern coast of Crimea. *E. palustris* often settle in peat bogs, marshy meadows and are phytocoenotically associated with sedge-sphagnum plant communities, as well as with grass-herbaceous marsh meadows (Gaponenko, 2013). Natural populations of the species were found in the vicinity of Kyiv (the village of Bobrytsia, Kyiv-Svyatoshinsky district and Svyatoshin Lakes) (Sobko, 1989; Parnikosa & Shevchenko, 2006).

The study of antecological features of *E. palustris* in the conditions of Kyiv is extremely important, as they demonstrate the prospects of preserving this species outside its natural habitats and its role in the formation of urban ecosystems. Anthropogenic habitat transformation regarded as a principal cause of pollinator decline on a global scale, as well as an important factor directly and indirectly changing the species structure of pollinating insects (Rewicz et al., 2017). The presence of a forage base in urban conditions is decisive in preserving the biodiversity of useful insects (Honchar & Gnatiuk, 2020).

Epipactis palustris is a nectar-bearing orchid that is visited by a large number of insects. Marsh Helleborine's nectar is rich in aromatic compounds. The main attractants found in nectar are: nonanal (pelargonaldehyde), decanal, eicosanol and its derivatives (Jakubska-Busse & Kadej, 2011). Nectar of *Epipactis* is a rich source of amino acids and carbohydrates, which are essential for the development of animals (Jakubska et al., 2005). Studies of pollinators and visitors of this orchid in different regions have shown that the species that dominate in pollination differ depending on the geography of the region of the research conducted (Jacquemyn et al., 2014).

In a semi-wild population in Haren, the Netherlands, *E. palustris* was visited by honeybees, sawflies, parasitic hymenoptera, ants, hoverflies and other flies. Bumblebees rejected the flowers. Of the hoverflies, only *Lejogaster metallina* (Fabricius, 1777) and males of *Syritta pipiens* (Linnaeus, 1758) were pollinators. *S. pipiens* was common enough (20% of the visits) to substantially effect pollination. Small flies of several families were effective pollinators but in low abundance (1%). Ants were the most frequent visitors (50%) and effected self- and cross-pollination. Honeybees (25%) pollinated and collected pollinia for larval food. Other hymenopterans were very rare on the flowers (1%). *Vespula vulgaris* (Linnaeus, 1758) neglected the flowers. *E. palustris* was not autogamous here. The epichile bent down under heavy insects, and worked as a 'springboard' for light hymenopterans. On the epichile a taste nectar guide was found, effective for flies, ants, experienced honeybees and other hymenopterans (Brantjes, 1981).

Epipactis palustris is well visited by insects, primarily by honey bees, solitary bees and hoverflies (Claessens & Kleynen, 2016). Van der Pijl and Dodson (1966) state that Marsh Helleborine is pollinated mostly by nectar feeding solitary wasps (*Hymenoptera: Eumenidae*) as well as by ants, bumblebees and hoverflies.

Brantjes (1981) notes that *Syrphidae* (Diptera), *Myrmica* sp. (*Hymenoptera*), *Sarcophaga carnaria* (Diptera) and *Coelopa frigida* (Fabricius, 1805) (Diptera) also belong to *E. palustris* pollinators.

Observations on the pollination of *E. palustris* from the island of Oland (southern Sweden) reported a broad spectrum of insects feeding on the nectar too. Ants, bumblebees and hover-flies were noted as the most frequent visitors, but generally played a minor role as pollinators. Solitary wasps (*Hymenoptera, Eumenidae*) were the most important pollen vectors, although present only at low frequencies. Ants were important pollinators in one locality. Entomogamy prevails and the frequency of visits is much lower if the plant is growing in association with entomophilous competitors. On Oland in Sweden, *Hymenoptera* (49.7%) and *Diptera* (40.8%) were the most frequent visitors, whereas *Coleoptera* (5.8%), *Lepidoptera* (2.8%) and *Araneida* (0.9%) were only occasionally observed visiting flowers of *E. palustris* (Nilsson, 1978).

Kryvosheev (2012) notes that in the southern Urals, *E. palustris* is highly specialized to *Vespidae*. Research carried out in natural populations of Crimea also showed pollination by *Vespidae*: *Polistes dominulus* (Christ, 1791), *Vespula vulgaris* (Ivanov et al., 2009).

Vast research on representatives of genus *Epipactis* and their pollination peculiarities were provided by A. Jakubska-Busse et al. in Poland, Czech Republic and Lithuania (Jakubska et al., 2005a, Jakubska-Busse & Kadej, 2011; Jakubska-Busse, 2011). It has been proven that Hymenoptera (*Apis*, *Bombus*) and representatives of Diptera (*Empis, Episyrphus*) are the main pollinators of *E. palustris* (Jakubska et al., 2005b; Jakubska-Busse & Kadej, 2011). Jakubska-Busse (2011) notes that

Hymenoptera: *Apis mellifera* (Linnaeus, 1758), *Bombus lapidarius* (Linnaeus, 1758), *Bombus lucorum* (Linnaeus, 1758), Diptera: *Empis livida* (Linnaeus, 1758), *Episyrphus balteatus* (De Geer 1776), *Cetema cereris* (Fallen 1820), *Chloromyia formosa* (Scopoli, 1763) belong to the true pollinators in Central Europe. On the other hand Diptera: *Calyptrata* sp., Coleoptera: *Anomala dubia* (Scopoli, 1763), *Cantharis pellucida* (Fabricius, 1792), *Rhagonycha fulva* (Scopoli, 1763), *Gaurotes virginea* (Linnaeus 1758), *Oedemera femorata* (Scopoli, 1763) and Lepidoptera: *Ochlodes sylvanus* (Esper, 1777), *Aphantopus hyperantus* (Linnaeus, 1759) belong to the flower visitors, that can carry out the pollination.

In order to organize optimal protection measures for *E. palustris* in Ukraine, comprehensive studies of various aspects of its biology and ecology are necessary. The interaction of plants and animals is one of the links to adaptation to environmental conditions and coexistence with other living organisms.

The aim of this study was to determine the visitors (pollinators and concomitant arthropods) of *E. palustris* plants in Kyiv (Ukraine).

Material and methods

Kyiv is located on the border of the Forest and Forest-steppe physiographic zones. The climate is moderately continental, with mild winters and warm summers. Average monthly temperatures in January are –3.5 °C, in July +20.5 °C. The average annual amount of precipitation is 649 mm, with the maximum precipitation falling in July (88 mm), the minimum in October (35 mm). In winter, Kyiv has a snow cover with an average height of 20 cm of snow in February and a maximum of 44 cm (Osadchiy et al., 2010). The study of *E. palustris* was carried out on two artificial populations: 1) in M.M. Gryshko National Botanical Garden of NAS of Ukraine (hereinafter¸– NBG) and 2) in the private garden that is inside the natural range of *E. palustris*. The area belongs to the Right-Bank Forest-steppe of Ukraine, a temperate climate zone.

NBG is located in the Pechersk District of Kyiv in South-Eastern part of city. The population is located at the collection plot "Rare species of Ukrainian Flora" of the Natural Flora Department where *E. palustris* was planted since 1978 (plants originate from Vorokhta, which is an urban-type settlement located in the Carpathian Mountains on the Prut River) (Gaponenko, 2013). The population is local with vegetative reproduction. Plants grow along the pathway near the artificial pond, which provides soil moisture in a semi-shaded condition. The soil in the study area is dark gray, sandy, with a humus content of about 2.6%, pH 6.5. The number of the population is not stable and depends on the lighting and moisture conditions during the growing season. The population had 55–75 generative shoots on a 0.9 m^2 in 2020–2022.

Private garden is located in Solomianskyi District of Kyiv in the western part of the city, in the basin of Lybid river near the Sovki Lakes. The soil in the study area is gray, podzolic, sandy, with a low humus content, pH 6.5–7. The artificial population is local with vegetative reproduction. Plants originate from NBG, planted in 1995. Artificial irrigation is provided during the growing season for soil moisture. There are 60–83 generative stems in population on a 1.4 m^2 area in a semi-shaded condition.

The research was carried out in the growing seasons of 2020–2022. Observations of entomofauna related to Marsh Helleborine were carried out in field conditions during the flowering period. They were made over a span of 2–6 h, covering daylight hours (08.00–18.00 h). The sites were visited during sunny days, when pollinators have been shown to be most active. The arthropods were observed and photographed in natural conditions (Appendix 1–4). For the documentation purposes and identification some of observed insects were caught using entomological net. In the case when the species affiliation could be determined visually, the capture was not carried out, and the fact of the visit was only recorded in the field diary. Uncaught insects, the species of which cannot be determined from the photo, are assigned to the genus.

The species composition of insects was studied based on collection materials and photos. Species were identified using special literature (Medvedev, 1978; Gaetan du Chatenet, 1990; Oosterbroek, 2006; Pauly, 2015) as well as through specialized communities (iNaturalist, 2022) and a database (GBIF.org, 2022). Species are listed in an alphabetical order.

Arthropod representatives observed during the study on *E. helleborine* have been divided into 5 groups (status) using a modified classification system introduced by Jakubska et al. (2005). Arthropods: 1) N – potential pollinators: nectar feeding insects visiting the flowers, but not demonstrated to carry pollen; 2) P – effective true pollinators or pollen vectors: flowers visitors touching the androecium and demonstrated to carry *Epipactis* pollen or pollinia on the body; 3) H – aphid hunters; 4) P – pests of *Epipactis* plants; 5) V– other visitors on *Epipactis* plant. The activity of visitors was evaluated on a 5-point scale: 1) very rare visitors – occur once or twice per observation season; 2) rare visitors – three or more occurrences; 3) uncommon visitors – do not occur often or there are few of them; 4) common visitors – present on the plants almost every day, but their number is not large; 5) active visitors – occur every day in fairly large quantities.

Figure 1: Locations of the *Epipactis palustris* populations in Kyiv: 1) M. M. Gryshko National Botanical Garden of NAS of Ukraine (hereinafter – NBG); 2) private garden.
Slika 1: Lokacije populacij vrste *Epipactis palustris* v Kijevu: 1) Nacionalni botanični vrt M. M. NAS Ukrajine (v nadaljevanju – NBG); 2) zasebni vrt.

The surface of the flower's lip and the structure of pollinia were studied by the Stemmi 2000 light microscope using the "Axio Vision" program (Figure 2).

Results and Discussion.

Epipactis palustris (L.) Crantz is the most showy and conspicuous orchid of the genus. Together with *E. veratrifolia* Boiss. & Hohen., it belongs to the section *Arthrochilium* Irmisch, characterised by a non cup-shaped hypochile with lateral lobes and a hinged lip (Claessens & Kleynen, 2011). *E. palustris* is a perennial herbaceous plant. The plant consists of separate stems growing from a common rhizome. In studied populations the stems of plants reach a height of 20–60 cm and produce 15–30 flowers. The inflorescence is panicle-like, 8–19 cm long, drooping before flowering, then straight, sparse. The flowers are hanging, without a noticeable smell; the main color is white (due to the color of the lip). The flower perianth is bell-shaped and has a bipartite lip with the epichilium (part that serves as a landing place for insects) and a basal, cup shaped part where nectar is secreted (hypochile) (Figure 2). Perianth leaves are brownish-green or greenish-gray, greenish-white or reddish from the inside, 9–12 mm long. The lip is almost as long as the outer petals of the perianth; hypochile is 4.5–5.5 mm long, grooved, with two blunt lobes in front, pinkish-white below, white above, with light purple veins, and yellow relief dots at the base. The epichile is 5.5–6.5 (8) mm wider than the hypochile, with which it is connected by a narrow bridge. The epichile is almost flat, broadly ovoid or round, blunt, wavy-toothed, white, with 2 longitudinal, front yellow combs at the base, its surface is smooth. Pollinaria about 1.8 mm posterior, yellow 2-lobed, without a stalk, connected by a small sticky viscidium. The flowers produce small amounts of nectar that is present on the labellu. The viscidium glues pollinia to the pollinator and prevents pollinia from sliding out of the anther and contacting the stigmatic surface. The conspicuous flowers produce nectar in the hypochile mostly. In order to enable autogamy the pollinia must be able to contact the stigma.

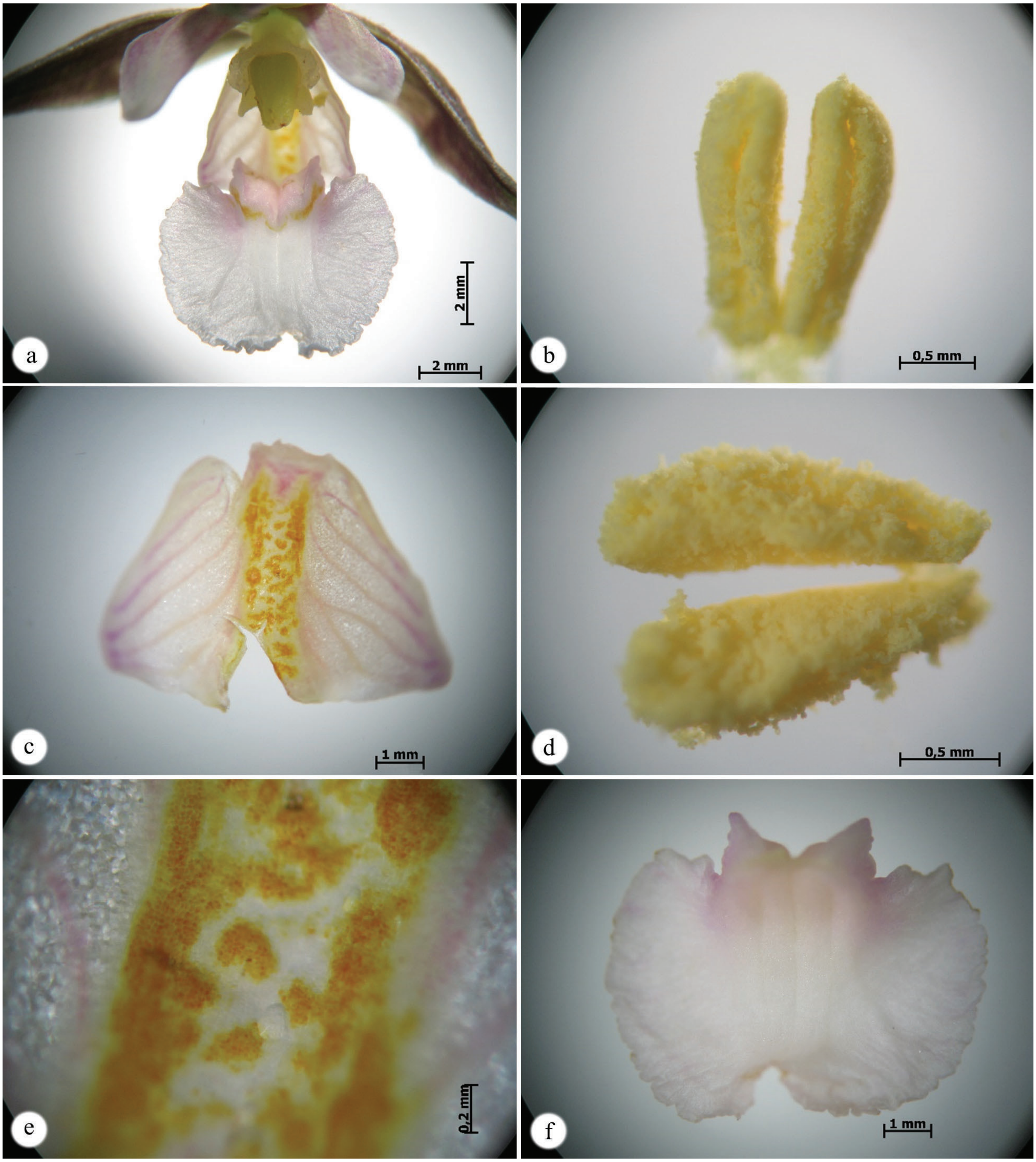


Figure 2: Parts of *Epipactis palustris* flower: a – labellum; b, d – pollinia; c, e – hypochile; f – epichile.
Slika 2: Deli cveta *Epipactis palustris*: a – medena ustna; b, d – poliniji; c, e – hipohil; f – epihil.

Epipactis palustris has a flowering period from the mid-June until the end of 1st decade of July. In the years 2020–2022, flowering in populations in Kyiv starts between June 13 and 20, peak flowering took place between June 25 and 30. The earliest record for the end of flowering was 6 July 2020 and the latest was 10 July 2021. Plants of *E. palustris* are very attractive for insects in Kyiv. We noted visitors, pollinators, and other arthropods of *E. palustris* (Table 1).

Table 1: List of visitors of *Epipactis palustris* and their activity on population in Kyiv (Ukraine) during 2020–2022.
Tabela 1: Seznam obiskovalcev vrste *Epipactis palustris* in njihova aktivnost v populaciji v Kijevu (Ukrajina) med 2020–2022.

	Order, Family, Species	*Status/ Activity*		
		2020	*2021*	*2022*
Order Araneida				
Phalangiidae:	*Phalangium opilio* (Linnaeus, 1758)	*V/3*	*V/3*	*V/3*
Thomisidae:	*Misumena vatia* (Clerck, 1757)	*V/4*	*V/4*	*V4*
	Thomisus sp.	*V/3*	*V/3*	*V/3*
Order Coleoptera				
Cantharidae*:*	*Cantharis livida* (Linnaeus, 1758)	*V/3*	*-*	*N/1*
Cerambycidae:	*Pseudovadonia livida* (Fabricius, 1776)	*N/1*	*-*	*N, V/1*
Coccinellidae:	*Coccinella septempunctata* (Linnaeus, 1758)	*-*	*H/4*	*H,P/5*
	Harmonia axyridis (Pallas, 1773)	*H, P/5*	*H/5*	*P, H/5*
	Propylea quatuordecimpunctata (Linnaeus, 1758)	*H/4*	*-*	*H,P/4*
	Scymnus frontalis (Fabricius, 1787)	*-*	*H,P/1*	*-*
	Tytthaspis sedecimpunctata (Linnaeus, 1758)	*-*	*H,P/3*	*H/2*
Curculionidae:	*Gymnetron rostellum* (Herbst, 1795)	*-*	*-*	*V/1*
	Otiorhynchus ligustici (Linnaeus, 1758)	*-*	*V/1*	*-*
Oedemeridae:	*Oedemera virescens* (Linnaeus, 1767)	*N/1*	*-*	*N/1*
Order Diptera				
Anthomiidae:	*Leucophora* sp.	*N/3*	*N/3*	*N/3*
	Hydrophoria lancifer (Harris, 1780)	*V/1*	*-*	*-*
Bombyliidae:	*Hemipenthes morio* (Linnaeus, 1758)	*N/3*	*N/3*	*N/3*
Polleniidae:	*Pollenia* sp.	*N/3*	*N/2*	*N/3*
Sarcophagidae:	*Sarcophaga carnaria* (Linnaeus, 1758)	*N/3*	*N/3*	*N/3*
Stratiomyidae:	*Chloromyia formosa* (Scopoli, 1763)	*V/2*	*V/2*	*V/1*
Syrphidae:	*Eupeodes corollae* (Fabricius, 1794)	*N,H/1*	*N,H/2*	*N,H/2*
	Sphaerophoria scripta (Linnaeus, 1758)	*H,N/3*	*H,N/4*	*N/4*
	Syritta pipiens (Linnaeus, 1758)	*-*	*N/4*	*N/4*
Order Hemiptera				
Aphidae:	*Aphis epipactis (Theobald, 1927)*	*A/5*	*A/5*	*A/5*
Flatidae:	*Metcalfa pruinosa (Say, 1830)*	*A/3*	*A/4*	*A/3*
Miridae:	*Deraeocoris ruber (Linnaeus, 1758)*	*H/3*	*H/5*	*H/5*
	Lygus rugulipennis (Poppius, 1911)	*-*	*-*	*N/1*
	Stenotus binotatus (Fabricius, 1794)	*-*	*-*	*N/1*
Pentatomidae:	*Palomena prasina (Linnaeus, 1761)*	*V/1*	*V/1*	*V/1*
Rhopalidae:	*Corizus hyoscyami (Linnaeus, 1758)*	*-*	*-*	*V/1*
Order Hymenoptera				
Apidae:	*Apis mellifera* (Linnaeus, 1758)	*P/5*	*P/5*	*P/5*
	Bombus hortorum (Linnaeus, 1761)	*V/1*	*N/2*	*N/2*
	Bombus terrestris (Linnaeus, 1758)	*N/1*	*-*	*N/1*
	Bombus pascuorum (Scopoli, 1763)	*V/1*	*-*	*V/1*
Halictidae:	*Lasioglossum malachurum* (Kirby, 1802)	*P/4*	*P/4*	*P/4*
	Lasioglossum morio (Fabricius, 1793)	*P/4*	*P/4*	*P/5*
	Lasioglossum politum (Schenck, 1853)	*P/5*	*P/5*	*P/5*
	Lasioglossum zonulum (Smith, 1848)	*P/4*	*P/4*	*P/4*
Colletidae:	*Hylaeus confusus* (Nylander, 1852)	*-*	*-*	*N/1*

	Order, Family, Species	Status/ Activity		
		2020	2021	2022
Chrysididae:	*Elampus panzeri* (Fabricius, 1804)	-	N/1	N/1
Crabronidae:	*Cerceris arenaria* (Linnaeus, 1758)	-	-	N/2
	Pemphredon sp.	H/3	H,P/4	P,H/3
Eumenidae:	*Eumenes pedunculatus* (Panzer, 1799)	-	-	N/1
	Euodynerus quadrifasciatus (Fabricius, 1793)	-	N\1	-
	Stenodynerus chevrieranus (Saussure, 1855)	-	-	N/2
Formicidae:	*Formica cunicularia* (Latreille, 1798)	N,P/5	N/5	N/5
	Tetramorium caespitum (Linnaeus, 1758)	-	N/1	-
	Lasius niger (Linnaeus, 1758)	N/5	N/5	N/5
Order Lepidoptera				
Pieridae:	*Pieris napi* (Linnaeus, 1758)	N/2	N/2	N/2
Order Neuroptera				
Chrysopidae:	*Chrysoperla carnea* (Stephens, 1836)	H/1	-	H/1
Order Odonata				
Coenagrionidae:	*Enallagma cyathigerum* (Charpentier, 1840)	V/3	V/3	V/-
Platycnemididae:	*Platycnemis pennipes* (Pallas, 1771)	V/5	V/5	V/5
Order Orthoptera				
Acrididae:	*Chorthippus* sp.	-	-	V/1
Tettigoniidae:	*Phaneroptera* sp.	N/1	V/1	N/1

Notes:
Status: 1) N – potential pollinators; 2) P – true pollinators; 3) H – aphid hunters; 4) P – pests; 5) V – other visitors.
Activity: 1 – very rare visitors; 2 – rare visitors ; 3 – uncommon visitors; 4 – common visitors; 5 – active visitors.

Epipactis palustris was visited by honeybees, bumblebees, parasitic hymenopterans, ants, wasps, hover flies, soldier flies, flesh flies, dung flies and other flies, bugs, grass bugs, aphids, butterflies, dragonflies, spiders and grasshoopers (Apendix 1–4). 53 species from 28 families and 46 genera were identified and established: *Phalangiidae* (*Phalangium* Linnaeus, 1758), Thomisidae (*Misumena,* Latreille, 1804*, Thomisus* Walckenaer, 1805), *Cantharididae* Imhoff, 1856 (*Cantharis* Linnaeus, 1758), *Cerambycidae* Latreille, 1802 (*Pseudovadonia* Lobanov, Danilevsky & Murzin, 1981), *Coccinellidae* Latreille, 1807 (*Harmonia* Mulsant, 1850; *Coccinella* Linnaeus, 1758*; Propylea* Mulsant, 1846*; Scymnus* Kugelann, 1794*; Tytthaspis* Crotch, 1874), *Curculionidae* Latreille, 1802 (*Otiorhynchus* Germar, 1822*, Gymnetron* Schönherr, 1825*)*, *Oedemeridae* Latreille, 1810 (*Oedemera* Olivier, 1763), *Stratiomyidae* Latreille, 1802 (*Chloromyia* Duncan, 1837); *Sarcophagidae* Macquart, 1834 (*Sarcophaga* Meigen, 1826); *Syrphidae* Latreille, 1802 (*Sphaerophoria* Le Peletier & Serville, 1828; *Syritta* Le Peletier & Serville, 1828; *Eupeodes* Osten Sacken, 1877*)*, *Bombyliidae* Latreille, 1802 (*Hemipenthes* Loew., 1861), *Anthomiidae* Robineau-Desvoidy, 1830 (*Leucophora* Robineau-Desvoidy, 1830; Hydrophoria Robineau-Desvoidy, 1830*)*, *Polleniidae* Brauer & Bergenstamm, 1889 *(Pollenia* Robineau-Desvoidy, 1830)*, Aphididae* Latreille, 1802 (*Aphis* Linnaeus, 1758), *Miridae* Hahn, 1831 (*Lygus* Hahn, 1833; *Deraeocoris* Kirschbaum, 1856), *Rhopalidae* Amyot & Serville, 1843 (*Corizus* Fallén, 1814), *Pentatomidae* Leach, 1815 (*Palomena* Mulsant & Rey, 1866); *Flatidae* Spinola, 1839 (*Metcalfa* Caldwell & Martorell, 1951), *Apidae* (*Apis* Linnaeus, 1758; *Bombus* Latreille, 1802), *Halictidae* Thomson, 1869 (*Lasioglossum* Curtis, 1833), *Colletidae* Lepetier,1841 (*Hylaeus* Fabricius, 1793), *Crabronidae* (*Pemphredon* Latreille, 1796, *Cerceris* Latreille, 1802*)*, *Eumenidae* Leach, 1815 (*Stenodynerus* Saussure, 1863*, Euodynerus* Dalla Torre, 1904, *Eumenes* Latreille, 1802), *Chrysididae* Latreille, 1802 (*Elampus* Spinola, 1806), *Formicidae* Latreille, 1802 (*Lasius* Fabricius, 1804; *Formica* Linnaeus, 1758; *Tetramorium* (Mayr, 1855), Pieridae Duponchel, 1835 (*Pieris* Schrank, 1801), *Chrysopidae* Schneider, 1851 (*Chrysoperla* Steinmann, 1964*)*, *Platycnemididae* Yakobson & Bianchi, 1905 (*Platycnemis* Burmeister, 1839; *Enallagma* Charpentier, 1840), *Acrididae* MacLeay, 1819 (*Chorthippus* Fieber, 1852, *Tettigoniidae* Krauss, 1902, *Phaneroptera* Serville, 1831).

Taxonomic diversity of visitors in Kyiv on *E. palustris* show that *Hymenoptera* (34%), *Coleoptera* (19%) and *Diptera* (17%) are represented by the greatest diversity, whereas, *Neuroptera* (2%) – by the smallest number of species (Figure 2.). Evaluation of visitor activity show that *Hymenoptera* (36%) were the most frequent visitors, whereas, *Lepidoptera* (2%) and *Neuroptera* (0%) were only occasionally visiting of *E. palustris* (Figure 3, 4). It makes our data somewhat different from those reported in the literature.

The species diversity of wild bees in the parks of Kyiv is about 115 species (Radchenko & Gonchar, 2019).

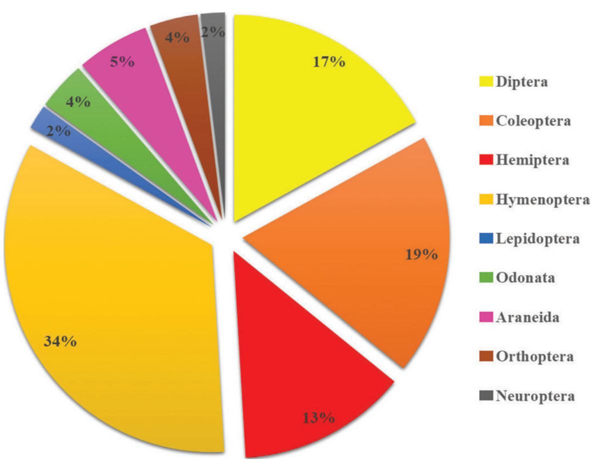


Figure 3: Taxonomic diversity of visitors on *E. palustris* in 2020–2021.
Slika 3: Taksonomska raznolikost obiskovalcev vrste *E. palustris* med 2020–2021.

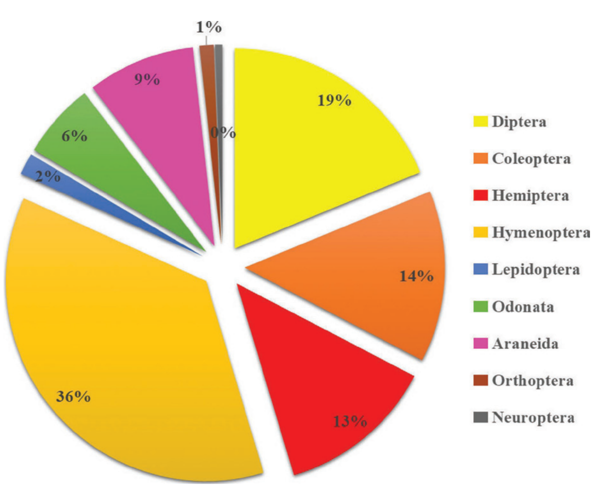


Figure 4: Activity of visitors on *E. palustris* in 2020–2021.
Slika 4: Aktivnost obiskovalcev vrste *E. palustris* med 2020–2021.

147 species from 6 families and 35 genera were found in NBG (Gonchar & Gnatiuk, 2018). However, bees from only three genuses (*Apis, Halictus* and *Lasioglossum*) were observed on *E. palustris.* Only *Apis* and *Lasioglossum* were active pollinators. Surveys of pollination of *Orchidaceae* in the NBG in previous years also indicate *Halictidae*: *Lasioglossum*: *L. zonulum*, *L. politum*, *L. morio*, and *A. mellifera* as main pollinators of *E. palustris* (Gnatiuk et al., 2017; Gaponenko et al., 2017). The *Apis mellifera* and species of *Lassioglossum* are in the lists of *E. palustris* visitors in the south-western Poland (Jakubska-Busse, 2005; Jakubska-Busse & Kadej, 2011), British Isles (Jacquemyn et. al., 2014), and Crimea (Fateryga & Ivanov, 2012).

Hymenoptera *Apis* and *Lasioglossum* were active pollinators that were purposefully interested in the flowers of *E. palustris* and often carried pollinia. Bees from the genus *Lasioglossum* (body length ≈ 0.5 cm) carried pollinia on their backs, crawling from flower to flower, as they often could not fly away under their weight. The presence and frequent visitation of flowers by these bees is not surprising, since these are the most common polylectic species, which occur in different biotopes. Despite their non-food specialization, these bees play an important role in the pollination of many plants, and this species of orchid is probably no exception. Bumblebees visited flowers mostly by accident and did not linger on them for a long time. Wasps (*Crabronidae, Eumeninae*) and flies visited flowers, fed on nectar and could transfer pollen, but we did not manage to see these insects with pollinia (except for *Pemphredon*). Flowers with nectar attracted beetles (*Oedemeridae* and *Coccinellidae*) and butterflies. From 10 to 25 insects were observed per hour on the studied population of *E. palustris* flowers, and by 1:00 p.m., all open flowers had no pollinia.

Nilsson (1978) noted, that «deposition and frequency of pollinaria, morphology and action of the hinged labellum, and the time of flowering all suggest that *E. palustris* is adapted to pollination by solitary wasps, perhaps primarily to males of the genus *Eumenes* Latr.». However, our observations have not confirmed the main role of *Vespidae* in pollination. Solitary wasps were the rare visitors on *E. palustris* flowers.

We would like to note the symbiosis of ants and the aphids, which plays a significant role in the life of these orchids. Among the recorded species of arthropods 13 species (25%) prey on aphids or feed on their secretions. Every year there are aphids on the *E. palustris* shoots. They populate the inflorescence zone even at the stage of budding. In the population, 15–40% of the shoots have aphids every year. Ants were regular visitors to *E. palustris* in all years of observation: they cared for aphids and drank nectar from flowers. We observed an ant with a pollinia fragment only once. The presence of aphids in the inflorescences attracted insects that prey on them even before the flowers open. During the flowering of plants, *Syrphidae* and *Coccinellidae*, both adults and their larvae, were active consumers of the *Aphis*. *Coccinellidae* adults often had pollinia or pollen attached to their backs and visited flowers. The *Deraeocoris ruber*, which simultaneously numbered in 3–7 adults at the peak of flowering of

Epipactis population, had a significant effect on the number of aphids. Mirid Bugs visited flowers rarely. Wasps of genus *Pemphredon* also prey on aphids quite actively, they sometimes sat on the flowers. In all the years of observation, even before the end of flowering, the aphids were completely eradicated by predator insects. The presence of aphids had almost no effect on the formation of fruits and seeds.

Other researchers also confirm the presence of aphids, particularly *Aphis ilicis* (Kaltenbach, 1843), on *E. palustris* (Jakubska et al. 2005, Jakubska-Busse & Kadej, 2008) and connect it with the presence of *Chrysopa carnea* (Stephens, 1836) and *Syrphidae*. Some authors separate the aphids that settle and feed on *E. palustris* into a species *Aphis epipactis* (Jacquemyn et al., 2014), which is close to other species of "long-haired black aphids" of *Aphis fabae* group: *A. ilicis, A. viburni* (Scopoli, 1763), and *A. mordwilkoi* (Börner, 1926) in terms of morphological and biological characteristics (Jorg et al., 1995).

A vital role of ladybirds in pollination biology of *Epipactis* species were noted by A. Jakubska and others in Poland. They consider that: «the presence of *Coccinellidae* should not be connected only with the plant louses which occur often on the vegetative sprouts of the orchids. Ladybirds were present numerously on the plants without plant louses» (Jakubska et al., 2005). However, we observed the presence of ladybirds mostly in correlation with aphids. The largest number of *Coccinellidae* and their larvae (like other aphid-hunters) was observed mostly during the period of maxim growth of aphid colonies on *E. palustris* stems. At the same time several species of aphidophagous hoverflies (the females of which sometime lay eggs on the flowers) and wasps actively visit flowers. The fact that hoverfly's females lay eggs on the flowers of the orchid may point to specific flower attraction. During the flowering time we observed imago and larvae of *Coccinellidae* actively visit open flowers. Based on this fact we suggested that the *E. palustris* might attract aphidophagous by generalized mimicry of aphid pheromone in floral odour profile as it is described for *Epipactis veratrifolia* Boiss. & Hohen (Stökl J. et al., 2011).

Colonies and young individuals of *Metcalfa pruinosa* (Say, 1830) were found on the shoots of *E. palustris* in Kyiv. This polyphage is an invasive pest that has recently spread in Ukraine. We have not established the role of other *Hemiptera* as pests of *E. palustris*.

E. palustris plants proved to be a convenient shelter for dragonflies and spiders, which actively prey on flies and bees. Some *Phaneropterinae* nymphs feed on flower's nectar and *Phaneropterinae* imago sit on the plants.

Among *Lepidoptera*, flowers with nectar attracted only *Pieris napi* butterflies in all years of our observations. Anna Jakubska-Busse noted Ochlodes sylvanus (Esper, 1777) butterflies as flower visitors (Jakubska-Busse, 2011). In "Biological Flora of the British Isles" *Autographa gamma* (Linnaeus, 1758) noted as a pollinator (Jacquemyn et al., 2014). In the conditions of Kyiv, these butterflies are quite numerous, but they ignored the flowers of *E. palustris* in the studied populations.

We were interested in the research of Jennifer Brodmann with colleagues (2008). They investigated that *E. helleborine* (L.) Crantz flowers emit four common "green leaf volatiles" (GLVs) that can also be found in cabbage infested by Pieris caterpillars. GLVs are emitted by plants while herbivorous insects, for example caterpillars, feed on them. *E. helleborine* flowers produce GLVs in order to attract preyhunting social wasps for pollination (Brodmann et al., 2008). The fact that only *Pieris napi* butterflies were observed on *E. palustris* flowers in Kyiv led us to believe that its flowers might produce similar GLVs that could attract these butterflies.

Epipactis helleborine is distant from *E. palustris*, both chemically and genetically (Jakubska-Busse & Kadej, 2011). However, plants of the same genus may have similar adaptations, in particular chemical mimicry for the attraction of pollinating insects and predatory insects for biological protection against pests. But, the presence of such mimicry in *E. palustris* should be checked by detailed chemical analyses.

All insects that visited *E. palustris* flowers accidentally or purposefully can be pollinators or pollen vectors. *E. palustris* has a rather sticky viscidium and after 1–2 visits by insects the pollinia are removed from the flower. On larger insects (especially *A. mellifera*), it sticks to the head, in smaller insects to the back (*Lassioglossum* spp., *Pemphredon sp.*) or to the elytra (*Coccinellidae*). Often pollinia simply lie removed from the flower on the leaves and stems of *E. palustris* or nearby plants. In our study, only 23.4% of insect species were recorded with pollinia or their fragments on the body. We observed geitonogamy induced by bees, bugs and ants on *E. palustris*. *Lassioglossum* and *Coccinellidae* mainly ensured self-compatibility within the same inflorescence by crawling from flower to flower.

Conclusions

It was found that populations of *E. palustris* in the conditions of the Kyiv city form a unique self-regulating biocenosis that provides shelter and food for 53 identified species of arthropods. Taxonomic diversity of visitors on *E. palustris* show that *Hymenoptera* (34%), *Coleoptera* (19%) and *Diptera* (17%) are represented by the greatest diversity, whereas, *Neuroptera* and *Lepidoptera* by the smallest number of species. Evaluation of visitor activity shows that *Hymenoptera* (36%) were the most frequent visitors, whereas, *Lepidoptera*, *Orthoptera* and *Neuroptera* were only occasionally observed on *E. palustris.*

E. palustris in the conditions of Kyiv is mainly characterized by entomophilia (melitophilia and cantharophilia). Potential pollen vectors or pollinators belong to *Coleoptera, Diptera*, part of *Hemiptera*, most of *Hymenoptera*. They have an interest in nectar and visit flowers.

Among the recorded species of arthropods, more than 75% of the species are potential pollinators or pollen vectors, of which about 35% prey on aphids or feed on their secretions. Further studies of the trophic relationships of *E. palustris* in artificial and natural populations of Ukraine will surely reveal a larger spectrum of its visitors and additional ways of attracting beneficial insects.

Acknowledgments

We are sincerely grateful to Dr. Stanislav Stukalyuk for his help in identifying some insects.

A. M. Gnatiuk https://orcid.org/0000-0001-5001-971X
M. B. Gaponenko https://orcid.org/0000-0003-1513-1065
H. Y. Honchar https://orcid.org/0000-0002-3429-5500
A. M. Gaponenko https://orcid.org/0000-0003-4215-2216

Reference

Brantjes, N.B. (1981). Ant, bee and fly pollination in *Epipactis palustris* (L.) Crantz (Orchidaceae). *Acta Botanica Neerlandica, 30*, 59–68.

Brodmann J., Twele R., Francke W., Hölzler G., Zhang Q. H., & Ayasse M. (2008). Orchids mimic green-leaf volatiles to attract prey-hunting wasps for pollination. *Current biology, CB*, *18*(10), 740–744. https://doi.org/10.1016/j.cub.2008.04.040

Claessens, J., & Kleynen, J. (2011). *The flower of the European orchid.* Form and function, 440.

Claessens, J. & Kleynen, J. (2016). Many ways to get happy: pollination modes of European Epipactis species. *Orchid Digest, 80*, 142–150.

Didukh, J.P. (Ed.). (2009). *Red Data Book of Ukraine. Plant Kingdom.* Globalconsalting, Kyiv (in Ukrainian).

Doust, J.L., Dous,t L.L. (Eds.). (1988). *Plant reproductive ecology: patterns and strategies.* New York: Oxford University Press.

Fateryga, A.V., Ivanov, S.P. (2012). Pollination ecology of the species from the genus Epipactis (Orchidaceae) in the Crimea. *Optimization and Protection of Ecosystems, 6*, 136–150.

Gaetan du Chatenet (1990). *Guide des Coléopterés d'Europe*. Délacrois et Niestlé, Paris 480 p.

Gaponenko, M. (2013). Features of introduction of species of genus Epipactis Zinn (*Orchidaceae* Juss.) in M.M. Gryshko National Botanical Gardens of the NAS of Ukraine. *Plant Introduction, 57*, 27–32. https://doi.org/10.5281/zenodo.1490417

Gaponenko, M.B., Gnatiuk, A.M. & Loja, V.V. (2017). Biomorphological, antecological features and prospects for preservation of orchids of the natural flora of Ukraine ex situ. In Cherevchenko T.M. et al. (eds.), *Adaptation of introduced plants in Ukraine* (pp. 27–45). – Kyiv. Phytosociocenter. (in Ukrainian)

GBIF.org (2022). The Global Biodiversity Information Facility. Available at: https://www.gbif.org

Gnatiuk, A.M., Gaponenko, M.B., & Honchar, H.Yu. (2017). The Role of Hymenoptera in pollination of introduced Orchidaceae in M.M. Gryshko National Botanic Garden of NAS of Ukraine. In Koval I.M. et al. (eds.), *The gene pool of collections of botanical gardens and arboretums is the key to sustainable phytocenoses in conditions of climate change. The book of articles from the international scientific conference dedicated to the 150th anniversary of the Academician V.I. Lipsky Botanical Garden of I.I. Mechnikov Odesa National University* (pp. 147–150). Odessa. ONU. (in Ukrainian)

iNaturalist (2022). Available at: https://www.inaturalist.org

Honchar, H.Yu., & Gnatiuk, A.M. (2018). The diversity of wild bees (Hymenoptera: Apoidea) in the M. M. Gryshko National Botanic Gardens of the NAS of Ukraine. *The Kharkov Entomological Society Gazette*, *26*(2), 33–42. https://doi.org/10.36016/KhESG-2018-26-2-5

Honchar, H. Yu, & Gnatiuk, A. (2020). Urban ornamental plants for sustenance of wild bees (Hymenoptera, Apoidea). *Plant Introduction, 85/86*, 93–108. https://doi.org/10.46341/PI2020014

Ivanov, S.P., Kholodov, V.V., & Fateryga, A.V. (2009). Crimean Orchids: pollinators composition, pollination systems diversity and its effectiveness. *Uchenye zapiski Tavricheskogo Natsionalnogo Universiteta im. V. I. Vernadskogo. Series «Biology, chemistry»*, *22*(61)1, 24–34.

Jacquemyn, H., Brys R., & Hutchings, M.J. (2014). Biological Flora of the British Isles: Epipactis palustris. *Journal of Ecology, 102*(5), 1341–1355. https://doi.org/10.1111/1365-2745.12287

Jakubska, A., Kadej, M., Prządo, D., & Steininger, M. (2005a). Ekologia zapylania Epipactis helleborine (L.) Crantz (Orchidaceae, Neottieae) w poudniowo-zachodniej Polsce. *Acta Botanica Silesiaca, 2*, 131–144. (in Polish)

Jakubska A., Smoczyk M., & Kadej M. (2005b). Kruszczyk błotny *Epipactis palustris* (L.) Crantz na Ziemi Kłodzkiej. *Przyroda Sudetow 8*, 3–12. [in Polish].

Jakubska, A. & Kadej, M. (2006). Preliminaries to the study of pollination ecology of *Epipactis palustris* (L.) Crantz (Orchidaceae, Neottieae) in Poland, pp. 34–38. [In:] Kočarek P., Plašek V. & Malachova K. (eds.), 2006: *Environmental changes and biological assessment* III. Scripta Facultatis Rerum Naturalium Universitatis Ostraviensis Nr. 163, Ostrava, 355 pp.

Jakubska-Busse, A. & Kadej, M. (2008). Pollination ecology of Marsh helleborine *Epipactis palustris* (L.) Crantz on the Polish side of the Orlickie Mts.(Central Sudety Mts.). Environmental changes and biological assessment IV. *Scripta Fac Rerum Natur Univ Ostraviensis*, *186*, 247–252.

Jakubska-Busse, A., & Kadej, M. (2011). The pollination of *Epipactis* Zinn, 1757 (Orchidaceae) species in Central Europe – the significance of chemical attractants, floral morphology and concomitant insects. *Acta Societatis Botanicorum Poloniae, 80*(1), 49–57.

Jakubska-Busse, A. (2011). Biologia zapylania wybranych gatunków z rodzaju Epipactis Zinn, 1757 (Orchidaceae, Neottieae) w Europie Środkowej – „kwiatowi goście" a faktyczni zapylacze. In: Brzosko E, Wróblewska A, Jermakowicz E (eds). *Storczykowate w Polsce: biologia i ochrona*, pp. 5–11. (in Polish)

Jorg, E. & Lampel, G. *et al.* (1995). *Studies on the Aphis fabae group (Homoptera, Aphididae). Inaugural Dissertation.* Zoologisches Institut der Universitat Freiburg, Sektion Entomologie, Freiburg, Switzerland. 55.

Kryvosheev, M.M. (2012). *Reproduction ecology of some species of orchids (Orchidaceae Juss.) of the Southern Urals*: Autoref. Thesis ... candidate biological of science. Ufa. 16. (In Russian).

Medvedev, G. S. (Ed.). (1978). *Identification key to Insects of the European part of USSR. Vol. 3: Hymenoptera.* Leningrad: Nauka Press. (in Russian).

Nilsson, L.A. (1978). Pollination ecology of Epipactis palustris Orchidaceae. *Botaniska Notiser, 131*(3), 355–368.

Oosterbroek, P. (2006). *The European Families of the Diptera: Identification – Diagnosis – Biology.* KNNV Publishing; Illustrated Edition. DOI: 10.1163/9789004278066

Osadchyy, V. I., Kosovets, O. O., & Babichenko, V. M. (Eds.). (2010). *Climate of Kyiv*. Ukrainian Research Hydrometeorological Institute, Central Geophysical Observatory. Nika-Center. (In Ukrainian)

Parnikoza, Y.U., & Shevchenko, Ì.S. (2006). Cenopopulations of rare plant species of the Svjatoshin Lakes (Kyiv). *Ukrainian phytocenological collection*, C, *4*, 110 –114.

Pauly, A. (2015). *Clés illustrées pour l'identification des abeilles de Belgique et des régions limitrophes (Hymenoptera: Apoidea)*. Document de Travail du Projet BELBEES, Bruxelles.

Pijl, L. van der, & Dodson, C.H. (1966). *Orchid flowers: their pollination and evolution.* Published jointly by the Fairchild Tropical Garden and the University of Miami Press. 214.

Radchenko, V., & Honchar, H. (2019). Species diversity of wild bees (Hymenoptera: Apoidea) in parks of Kyiv. *Bulletin of Taras Shevchenko National University of Kyiv Series Biology*, *2*, 40–49. https://doi.org/10.17721/1728_2748.2019.78.40-49

Rewicz, A., Jaskuła, R., Rewicz, T., & Tończyk, G. (2017). Pollinator diversity and reproductive success of Epipactis helleborine (L.) Crantz (Orchidaceae) in anthropogenic and natural habitats. *PeerJ*, *5,* e3159. https://doi.org/10.7717/peerj.3159

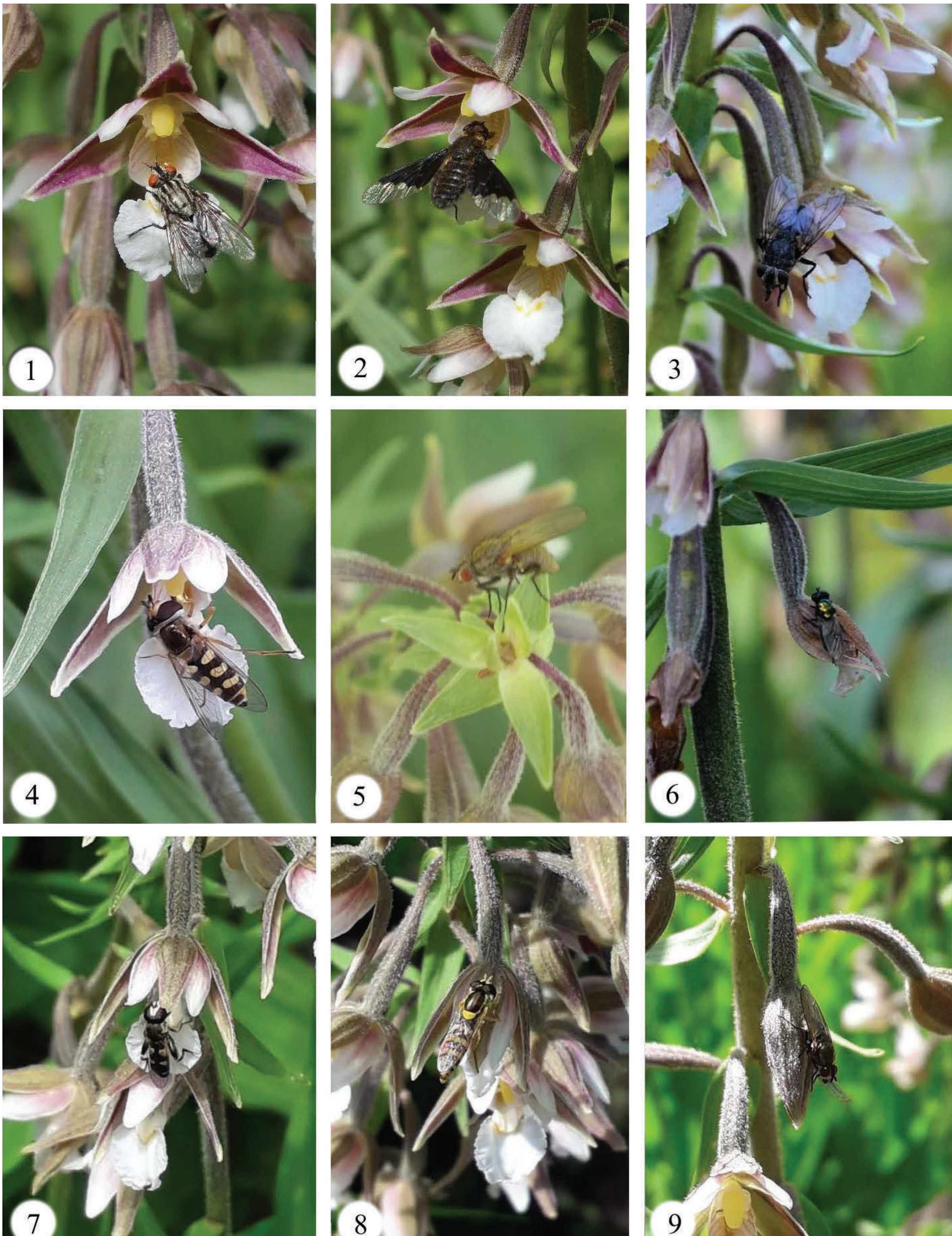
Sobko, V.G. (1989) *Orchids of Ukraine.* Kyiv. Naukova Dumka. (in Ukrainian).

Stökl, J., Brodmann, J., Dafni, A., Ayasse, M., & Hansson, B.S. (2011). Smells like aphids: orchid flowers mimic aphid alarm pheromones to attract hoverflies for pollination. Proc. R. Soc. B.278, 1216–1222. http://doi.org/10.1098/rspb.2010.1770

Tałałaj, I., & Brzosko, E. (2008). Selfing potential *in Epipactis palustris, E. helleborine and E. atrorubens (Orchidaceae)*. *Plant Syst Evol,* 276, 21–29 https://doi.org/10.1007/s00606-008-0082-3

Appendix I

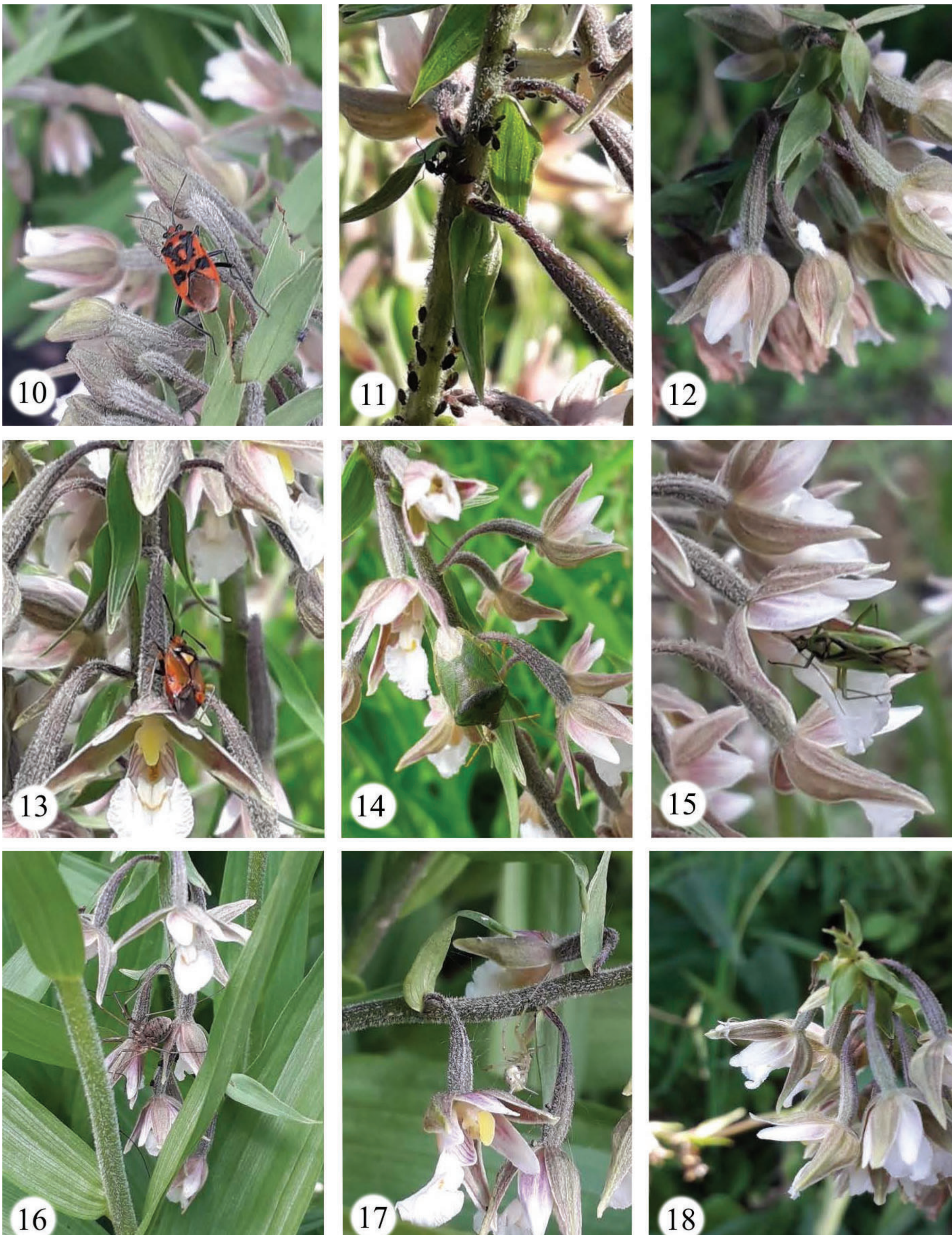
1 – *Sarcophaga carnaria*; 2 – *Hemipenthes morio*; 3 – Pollenia sp.; 4 – *Eupeodes corollae*; 5 –_Hydrophoria lancifer; 6 – *Chloromyia Formosa; 7 – Syritta pipiens*; 8 – *Sphaerophoria scripta* ♀; 9 – *Leucophora* sp.



Hacquetia 22/2 • 2023, 247–262

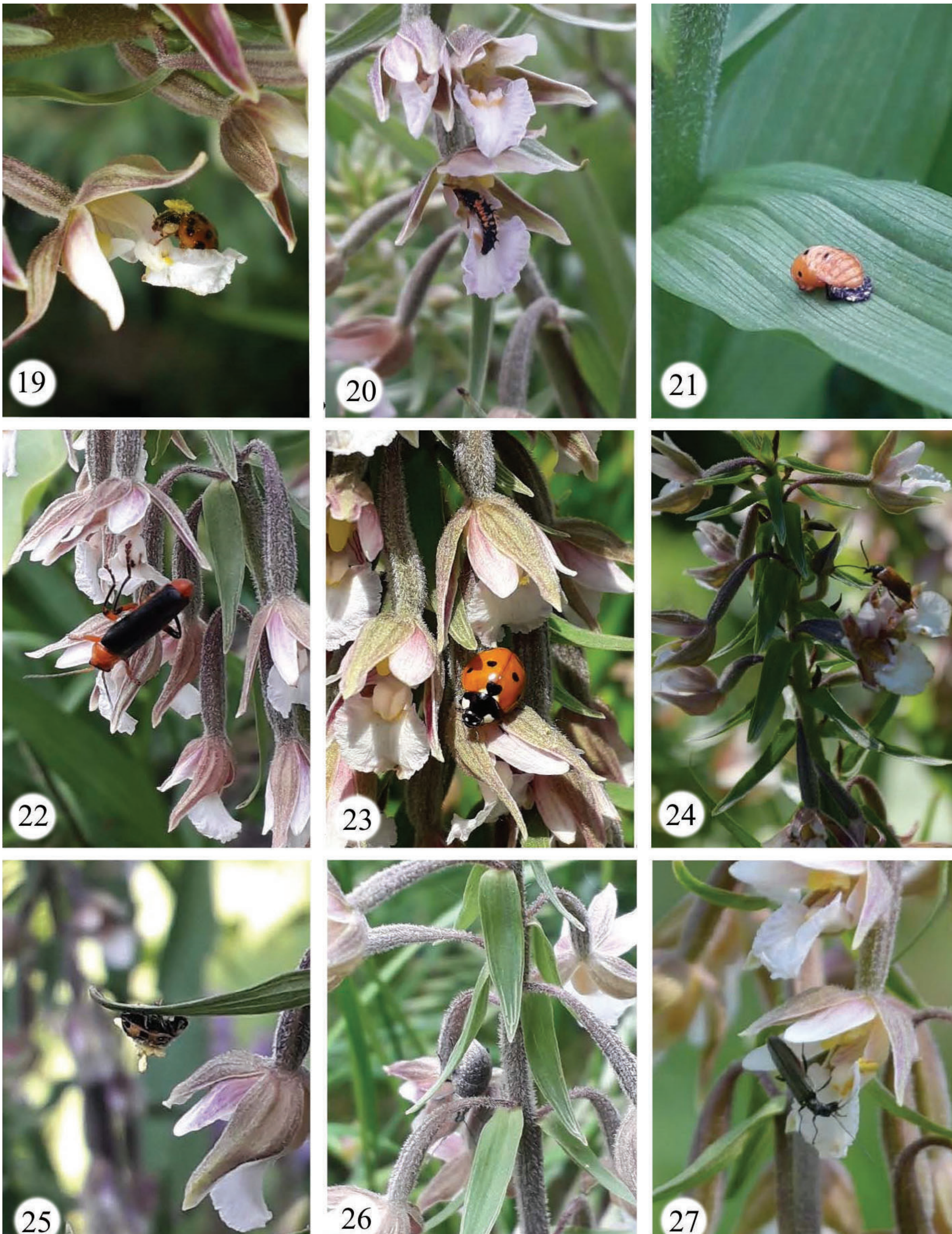
Appendix II

10 – *Corizus hyoscyami*; 11 – *Aphis epipactis*; 12 – *Metcalfa pruinosa*; 13 – Deraeocoris ruber; 14 – *Palomena prasina*; 15 – Stenotus binotatus; 16 – *Phalangium opilio*; 17 – *Misumena vatia*; 18 – *Thomisus* sp.



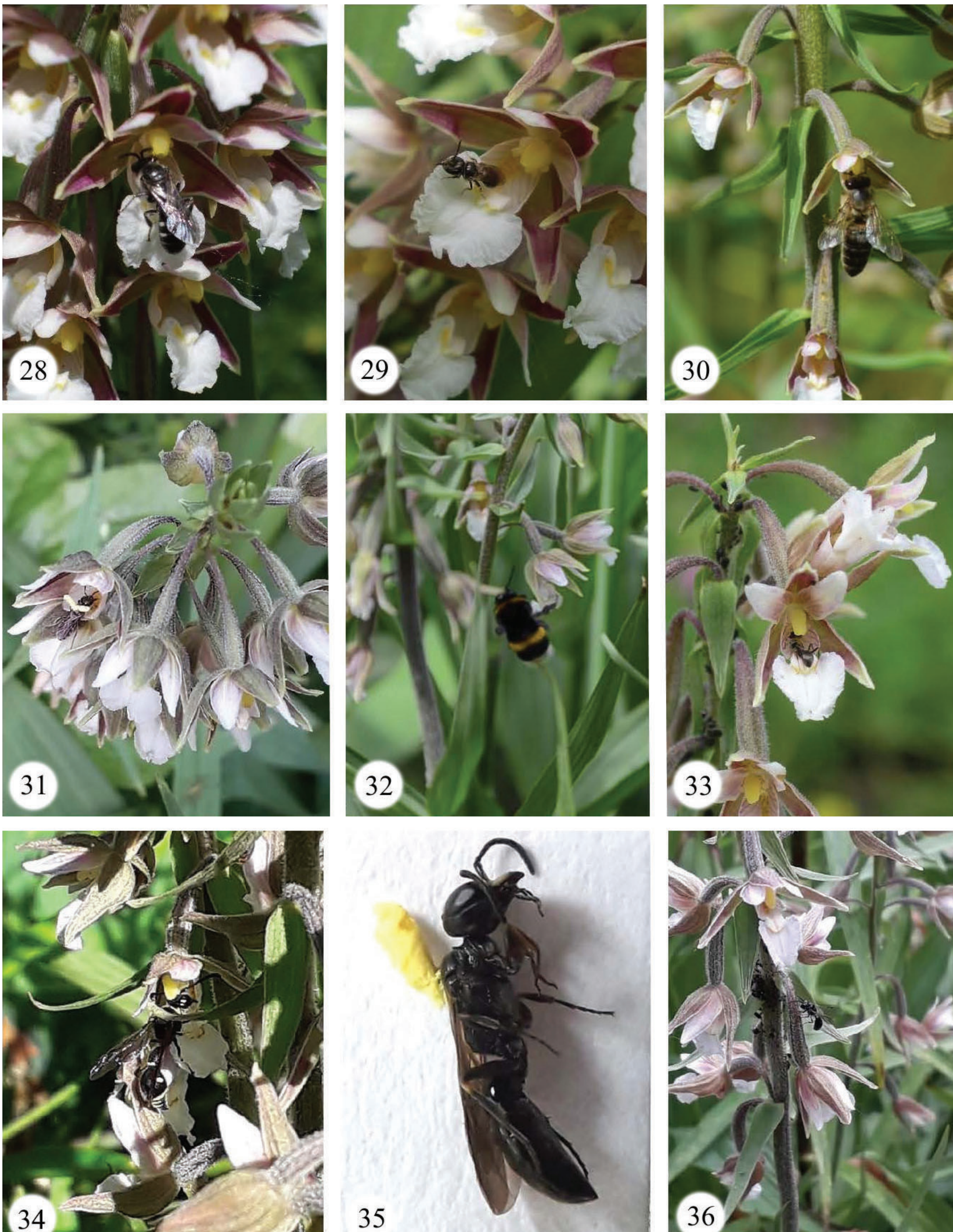
Appendix III

19 – Harmonia axyridis; 20 – *Harmonia axyridis larvae; 21 – Coccinellidae pupae;* 22 – *Cantharis livida; 23 – Coccinella septempunctata*; 24 – *Pseudovadonia livida*; 25 – *Propylea quatuordecimpunctata*; 26 – *Otiorhynchus ligustici*, 27 – *Oedemera virescens*.



Appendix IV

28 – *Lasioglossum zonulum*; 29 – *Lassioglossum politum*; 30 – *Apis mellifera*; 31 – *Lasioglossum morio*; 32 – *Bombus terestris*; 33 – *Formica fusca*; 34 – *Eumenes pedunculatus*; 35, 36 – *Pemphedron* sp.



Appendix V

37 – *Lasioglossum malachurum*; 38 – *Hylaeus confusus*; 39 – *Tetramorium caespitum*; 40 – *Pieris napi*; 41 – *Platycnemis pennipes*; 42 – *Phaneroptera* sp. nymph; 43, 44 – *Chorthippus* sp.; 45 – *Chrysoperla carnea*.