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GENUS *OPHRYS* L., 1753 IN ROMANIA – TAXONOMY, MORPHOLOGY AND POLLINATION BY SEXUAL DECEPTION (MIMICRY)

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Abstract

Pollination by sexual deception is thought to be the most remarkable mechanism of pollination, mainly characteristic to Orchidaceae, which has independently evolved throughout Europe, Australia, Asia, South Africa and Central- and South-America. Sexual deceptive mimicry is a complex deceptive mechanism in which the orchid flowers went through remarkable evolutionary morphological changes in their structure and function, especially that of the labellum, in order to achieve visual (shape and colour), tactile (texture and pilosity) and olfactory (sex-hormones or *pheromones) mimicry of the signals used by various insect female species in breeding condition. Because insect mating signals are usually very specific, pollinator attraction by sexual deception is also very specific, each orchid only attracting one or two insect species. In Romania, Ophrys genus is represented by four species only: Ophrys insectifera, Ophrys sphegodes and Ophrys oestrifera (all cross-pollinated by insects) and Ophrys apifera (self-pollinated). This article describes and illustrates for the first time in Romania, the phenomenon of pollination by pseudocopulation of Ophrys insectifera and its unique pollinator, the solitary male wasp Argogorytes mystaceus, and Ophrys oestrifera and its male bee pollinator Eucera longicornis. In the same time, it is described in detail the self-pollination mechanism employed by Ophrys apifera. Using advanced techniques of ultra-macro photography, details of floral organs are illustrated in order to show the spectacular morphological modifications of the bee-like flower labellum.*

Key words: *Orchidaceae; pollination; pollinaria; reward; sexual deceptive mimicry; pseudo-copulation; functional morphology.*

INTRODUCTION

"Whenever, […] *people are deceived* […] *it is clear that the error slid into their minds through the medium of certain resemblances to the truth"* - **Socrates (470-399 BCE)**

Distribution: *Ophrys* is a monophyletic genus of orchids. Following the lead of Kew Botanical Garden's, the World Checklist of Selected Plant Families, the genus is represented by ***148 species*** and ***nothospecies*** (species of hybrid origin). They are widespread across much of Europe, from the Canary Islands to the Caspian Sea, from southern Scandinavia to North Africa, east to Western Asia (North West Iran), with the greatest diversity in the Mediterranean Basin (Pridgeon *et al*., 2001; Delforge, 2016). The genus is close to the *Serapias-Orchis-Himantoglossum* assemblage, from which it derives. However, this genus is genetically very isolated, therefore, it does not form intergeneric (bigeneric hybrids) with any other orchid genus. (Anghelescu *et al*., 2021a; Delforge, 2006).

Generic name: The official, accepted name of the *Ophrys* genus was established and published in **1753**, by the Swedish botanist **Carl Linnaeus (1707-1778)**, author abbreviated as **L.** The original publication details are: *Sp. Pl.*: 948. **1753**. **Etymology:** The ***generic name***, ***Ophrys***, originates in the ancient Greek word ***ophrys*** (eyebrow, eyelid) and was first used by Roman naturalist and philosopher, **Gaius Plinius Secundus (23-79 BCE)**, known as **Pliny the Elder**, for a plant of uncertain origin. ***Ophrys*** (eyebrow) is a reference to the ***hairy rim*** or the ***hairy margin***, a reference to the ***hairy margins of the labellum*** that ***mimic the pilosity of the female bees***, hence the generic vernacular name of this genus, the **Bee Orchids**. *Ophis* was also a name given by the Swiss botanist **Gaspard Bauhin (1560-1623)** to the genus *Neottia* (previously *Listera*), which flowers have a green hood and forked labellum, resembling the *head* and *tongue of a snake*.

Evolution & diversification

Hymenopterans (bees and wasps) diversified in the Cretaceous **110-140 million years ago** (mya), in parallel with the diversification of **Angiosperms**, about **150 mya** (Danforth *et al*., 2013). **Genus *Ophrys*** only appeared about **4.9-5 million years ago** (Breitkopf *et al.,* 2015) but soon after, it experienced episodes of massive diversification through pollinator transitions, allowing both the exploitation of new ecological niches and the establishment of reproductive isolation between lineages. The explosive speciation rate of the genus *Ophrys* (bee orchids) is among the highest reported in Angiosperms, with diversification rates peaking at between 4 and 8 lineages per million years in some clades (Breitkopf *et al*., 2015). This spectacular radiation has given rise to several hundred species in the Mediterranean region of the western Palaearctic (Delforge, 2016). According to Baguette *et al*. (2020), this spectacular adaptive radiation is due to the particular coevolutionary dynamics between these plants and their pollinators. Many plant species are generalists, they attract and can be pollinated by many different pollinator species. But some families have evolved highly specialised plant-pollinator interactions. One of those families is the Orchidaceae, a very diverse flowering plant family with approximately 27,500-29,000 species (Chase *et al*., 2015). About ***one-third*** of these has evolved a ***food-*** or a ***sexually deceptive pollination strategy***.

Sexual Deceptive Mimicry

Ophrys are ***non-rewarding*** species with ***nectarless flowers*** that ***do not offer any recompense*** to the pollinating insects. As a consequence, their pollination is based exclusively on ***deceit*** and ***mimicry***, which in this case becomes one of the most refined, sophisticated and fascinating strategies in the Plant Kingdom, known as the ***sexual deceptive mimicry***. ***Sexual deception*** in orchids occurs when the plant sends a sexual signal to the pollinator, tricking it into thinking the flower is a female of its own kind available for sex. ***Sexual deceptive mimicry*** is a complex deceptive mechanism in which the orchid flowers undergo remarkable evolutionary, morphological changes in their structure and function, especially in that of the labellum, in order to achieve visual and olfactory mimicry of the signals used by insect females in breeding condition. It involves a wide array of pollinator attractants and stimulants including ***olfactory***, ***visual*** and ***tactile*** floral signals. ***Sexually deceptive orchids*** mimic the ***pheromones, shape, size, colour, texture and pilosity of a large variety of female insects***, mainly of the orders **Hymenoptera** Linnaeus, 1758 (bees, wasps) and **Diptera** Linnaeus, 1758 (flies). The resulting convergent resemblance among two unrelated kingdoms, ***plants*** and ***animals*** - ***orchids*** and ***insects***, is staggering even to human perception (De Angelli & Anghelescu, 2020).

First mentions of *Sexual Deceptive Mimicry*

Charles Robert Darwin

"*I never was more interested in my life in any subject than this of orchids*", wrote Darwin, **1861** (Darwin, 1861) in a letter addressed to his friend **Sir Joseph Dalton Hooker (1817-1911)**, director of The Royal Botanic Gardens, Kew from 1865 until 1885.

Although earlier botanists including French naturalist, **Bernard de Jussieu (1699-1777)**, the younger brother of **Antoine Laurent de Jussieu (1748-1836)** [who fully established the **Orchidaceae** family, in **1789**, (Anghelescu *et al*., 2020a) and Scottish botanist **Robert Brown (1773–1858)**, had described the structure of orchid flowers and observed flowers explored by insects, the nature and variations of pollination mechanisms in orchids were firstly fully studied by the English naturalist, explorer and geologist **Charles Robert Darwin (1809-1882)**. In **1862**, Darwin published the first edition of his book named ***Fertilisation of Orchids***, with the subtitle ***On the Various Contrivances by which British and Foreign Orchids Are Fertilised by Insects and on the Good Effects of Intercrossing***. This was his first essential contribution to the understanding of the pollination strategies employed by orchids to attract/deceive pollinators and accomplish successful cross-fertilization, explaining in the same time, how such complex ecological relationships could result in the ***co-evolution of orchids and insects*** (Darwin, 1862). Among the various deceptive British orchid species investigated by Darwin were the intriguing *Ophrys* orchids, to which he devoted a chapter description in his book, ***Fertilisation of Orchids***. On page 56, he included a short footnote mentioning a description by Church of England cleric and botanist **Mr. Gerard Edwards Smith (1804-1881)**, of some interesting observations by Mr. Price, perhaps the **Reverend Ralph Price**, Rector of Lyminge and Paddlesworth, Kent, to whom Smith dedicated his book, ***A Catalogue Of Rare Or Remarkable Phaenogamous Plants, Collected In South Kent*** **(1829)** (Vereecken & Francisco, 2014), admitting that he was not able to fully understand the insect's behaviour: "*Mr. Price has frequently witnessed attacks made upon the Bee Orchis by a bee, similar to those of the troublesome Apis* [*Bombus*] *muscorum*." (Smith, 1829; p. 25), "*What this sentence means I cannot conjecture*" (Darwin, 1862; p. 56). Darwin was obviously bewildered by these results, but failed to provide a rational, scientific explanation in accordance with his ***theory of natural selection*** for the insect-like flowers, a theory which did not tolerate mutual errors or waisted resources (deceiving pollinators or the lack of nectar). He incorrectly rejected the idea that plants attract their pollinators through an '*organized system of deception*' (Darwin, 1862; p. 45) on the grounds that insects, particularly bees, would be too intelligent to fall for '*so gigantic an imposture'* (Yam & Arditti, 2009). In his understating, the (co)adaptations between plants and insects had to be based on ***mutual benefits***, at least to some extent, and regarded the ***deceiving orchids*** as impossible to further proliferate/reproduce, being already engaged in an evolutionary dead end. However, Mr. Price's intriguing '*attacks*' of a bee on an *Ophrys* flower (Vereecken & Francisco, 2014), were probably the very first references to the *pseudocopulation* attempts by pollinating male insects, which remained largely unknown until the beginning of the 20th century.

Henry Correvon & Alexandre Pouyanne

By the early 20th century, Darwin's orchid book, ***Fertilisation of Orchids*** had already been edited several times and translated in several languages, inspiring various amateur and professional botanists to further investigate in much greater detail, their native orchid flora and orchid pollination mechanisms. The eminent Swiss botanist **Henry Correvon (1854-1939)**, creator of the first alpine botanical garden of Switzerland and author of the ***Album des orchidées d'Europe centrale et septentrionale*** **(1899)**, was particularly interested in the fertilization of orchids, especially in the pollination of *Ophrys apifera* and *Ophrys insectifera* (Vereecken & Francisco, 2014). During the preparation of a new edition of his book, Correvon contacted one of his collaborators, Maurice-Alexandre Pouyanne, to gather more facts on the biology of the *Ophrys* orchids, which were not native to Switzerland. **Maurice-Alexandre Pouyanne (1867-mid 20th century)** was an amateur botanist and President of the Court of Appeal in Sidi-Bel-Abbès, Algeria, who performed detailed observations on the fertilization of several *Ophrys* orchids in his home region of North Africa. In **1916**, Correvon introduced Pouyanne's findings to the famous **National Botanical Society of France**, (today known as the **F.F.O. - Fédération France Orchidées**), in a threefold, co-authored paper (Correvon & Pouyanne, 1916). In this pioneering, ground-breaking, first report, Pouyanne describes with astonishing, accurate detail how the flowers of ***Ophrys speculum***, the **Mirror Orchid (Fig. 1)** are pollinated by the orchid's only known pollinator, the male scoliid wasp, ***Dasyscolia ciliata*** Fabricius, 1787 (Family Scoliidae Latreille, 1802; Order Hymenoptera Linnaeus, 1758). He noticed that the solitary ***male*** insects often attempt ***copulation*** with the ***pseudo-female flowers***, a phenomenon later termed as ***precopulatory courtship behavior***, ***pseudocopulation*** or ***Pouyannian mimicry*** **(Fig. 2)**. Stimulated by the striking resemblance to a wasp female, whose ***shape, size, color, texture*** and ***pilosity*** are perfectly mimicked by the orchid, the male reaches high levels of sexual stimulation on the orchid labella (Correvon & Pouyanne, 1923).

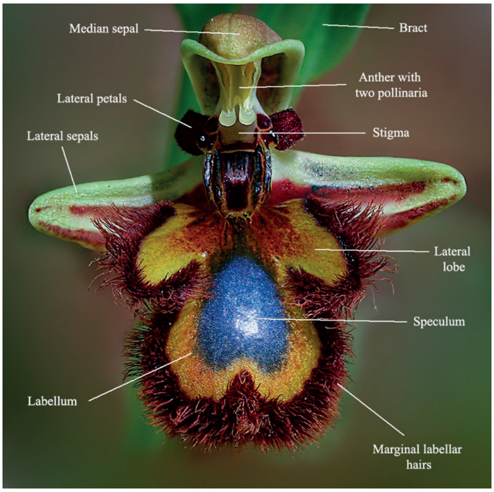


Fig. 1. *Ophrys speculum* - detail of the flower and the central area of the labellum, the ***speculum***. The ***intense brilliant blue colour of the speculum***, which has fascinated scientists and naturalists for many years, and the ***glossiness of the labellum***, have been hypothesized to improve the *sexual mimicry* of the flower by resembling the *sheen* on the *folded wings of an insect at rest* (Viniolini *et al*., 2012). Photo courtesy of Helmut Presser (Greece)

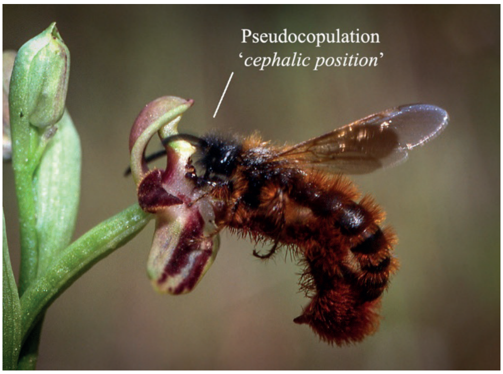


Fig. 2. A male scoliid wasp, *Dasyscolia ciliata*, pseudocopulating the flower of the Mirror Orchid, in ***cephalic position*** on the labellum. Guided by '*the reddish-brown colour of the hairs of the labellum [that] matches almost miraculously the colour of the body pubescence of the female wasp'* (Paulus, 2006), the male is positioned with the head toward the centre of the flower, thus collecting the pollinaria on its head. Photo courtesy of Helmut Presser (Greece)

After several unsuccessful attempts to mate, the ignored, naïve male will take flight, often with the ***pollinia*** firmly attached to its body, usually the head or abdomen. During a successive visit, the pollinator will transfer the pollinia to the stigma of another flower, during pseudocopulation. Since mating signals are usually specific to a certain species of insects, pollinator attracttion by sexual deception is also ***very specific***, with each orchid species only attracting one or two insect species (Stökl *et al.*, 2009). In this report, Pouyanne also provides the first adaptive explanation for the *Ophrys* insect-like flowers, which he suggests were devoid of nectar but instead they were able to attract the insects by a ***complex sexual mimicry strategy***, involving ***visual***, ***tactile*** and ***chemical (olfactive - pseudo-pheromones) signals***.

John Godfery & Bertil Kullenberg

The pioneering findings of Correvon and Pouyanne were further confirmed and extended on other representatives of the genus *Ophrys* from southern France, by British naturalist **Masters John Godfery (1856-1945)**. Godfery's classification of the genus *Ophrys* introduced the section *Pseudophrys* (Godfery, 1928), which encompasses species that deliver the pollinaria on their ***pollinators' abdomen*** tip during pseudocopulation, hence the term ***abdominal position*** **(Fig. 3)**. This pollination strategy is used by sympatric orchid species which have the same pollinator, in order to achieve reproductive isolation (and prevent hybridization), by a mechanical barrier (*abdominal* vs. *cephalic* pseudocopulation), provided by a different micromorphological pattern of the labellum (Schlüter & Schiestl, 2008; Cortis *et al.,* 2009). The rest of the species, characterized by ***cephalic position***, were included in section *Euophrys* (syn. *Ophrys*, Godfery, 1928). However, both sections, *Pseudophrys* (Godfery, 1928) and *Euophrys* (syn. *Ophrys*, Godfery, 1928) are no longer in use today.

Edith Coleman & Bertil Kullenberg

It was not long before the extraordinary findings on sexual deception mimicry, which were first met with disbelief by their contemporaries since they stroke as ***highly anthropomorphic***, were confirmed on other continents as well. During the late 1940s, the Swedish entomologist **Bertil Kullenberg (1913-2007)** performed a series of studies on *Ophrys* pollination, both in Sweden but also, in Morocco. He reconfirmed Pouyanne's and Godfery's findings/results on the deceiving nature of the nectarless/rewardless

Fig. 3. Copulation attempts of a male chaffer of the genus *Blitopertha* Reitter, 1903, (Superfamily Scarabaeoidea Latreille, 1802; Order Coleoptera Linnaeus, 1758) in the ***abdominal position*** on the flower labella of *Ophrys urteae* Paulus, with ***pollinaria*** on its abdomen. Photo courtesy of Helmut Presser (Greece)

Ophrys species and on the emission of their almost imperceptible scent. Kullenberg also published the first photograph of a male scoliid wasp *Dasyscolia ciliata* pseudocopulating on a flower of *Ophrys speculum* (Kullenberg, 1949; Vereecken & Francisco, 2014). Kullenberg was among the first to start using modern techniques and technologies in his multidisciplinary approaches in the study orchid pollination, such as a spectrophotometer to measure the relative reflectance curves of the colours and gas chromatography (GC) to analyse the of volatiles emitted by the flowers and insects. He was particularly interested in insect chemical ecology and behaviour. Based on Pouyannes' observations/studies, Kullenberg was also the first to establish the hierarchical importance of the different floral stimuli (olfactory, visual, and tactile), demonstrating that the specific floral scent emitted by each orchid species is crucial in long distance attraction of specific pollinators.

Once landed, the pollinators are further stimulated by the micromorphological pattern of the labellum, which tactilely stimulates the male insects (***thigmotaxis***) and guides them into the right positioning for the most efficient uptake of the orchid's pollinaria. Following Kullenberg observations/studies, Borg-Karlson (1990), used electrophysiological tests [gas chromatography coupled with electro-antennographic detection (GC-EAD) and mass spectrometry (GC-MS)] to identify organic compounds emitted by *Ophrys* flowers or by females of their pollinators, paved the way for a new generation of studies (Vereecken & Francisco, 2014).

Aims of the present study are: (1) to briefly summarize the history of orchid pollination by sexual deceit from Darwin to the present day; (2) to give a detailed taxonomical overview of *Ophrys* genus in Romania; (3) to describe in detail the main morphological characteristics of Romanian *Ophrys* taxons, by using advanced techniques of ultra-macro photography; (4) to illustrate this exceptional ***deceptive pollination strategy*** on the particular Romanian representative species included in this study.

MATERIALS AND METHODS

1. Study species: All four representatives of the Romanian orchid flora are the subject of this study **(Table 1).**

Table 1. Detailed list and taxonomical classification of the Romanian taxons, which belong to four separate clades or metaspecies (Bateman, Sramkó, & Paun, 2018)

Ophrys Clades				
Clade *Insectifera*	Clade *Apifera*		Clade *Scolopax*	Clade *Sphegodes*
Ophrys insectifera L., 1753	*Ophrys apifera* Huds., 1762	*Ophrys apifera* Huds., var. *aurita* (Moggr.) Gremli, 1887	*Ophrys oestrifera* (Steven) K.Richt., 1890	*Ophrys sphegodes* Mill., 1768

2. Study sites, populations counts and time frames: Studies of several populations of the four mentioned taxons were conducted between 2017-2021, in various locations across Romania, where they occurred in more or less stable populations, each year **(Table 2).**

Table 2. Describes in detail the locations (names), altitudes (a.s.l.- above the sea level), substrates, types of habitats, number of individuals, year(s) and the number of hours of pollination monitoring

Location(s)	Altitude (a.s.l.)	Substrate	Type of habitat	No. of individuals	Year	No. of hours
Ophrys insectifera						
Bucegi Natural Park	1020 m	Alkaline/ calcareous	Mixed forest Partial shade	20-25	2017 2018 2019 2020 2021	5 4 3 5 4
Nistorești, Prahova County	520 m	Alkaline/ calcareous	Deciduous forest Partial shade	2-3	2017 2018 2019 2020 2021	3 4 4 3 5
Breaza, Prahova County	530 m	Alkaline/ calcareous	Mixed forest Partial shade	1-2	2017 2018 2019 2020 2021	3 5 3 4 5
Ophrys apifera						
Iron Gates Natural Park	80-85 m	Alkaline/ calcareous	Grassy meadow Full-sun	12	2017 2018 2019 2021	3 4 3 5
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	2	2021	6
Ophrys apifera* var. *aurita						
Bozioru, Buzău County	410-415 m	Alkaline/ calcareous	Grassy meadow Full-sun	2	2018	1
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	2-3	2021	6
Ophrys oestrifera						
Bucegi Natural Park	990-1010 m	Alkaline/ calcareous	Grassy meadow Full-sun	2-3	2017 2018 2019 2020 2021	5 4 4 3 6
Nistorești, Prahova County	528 m	Alkaline/ calcareous	Deciduous forest Partial shade	4-6	2017 2018 2019 2020 2021	4 4 3 4 5
Breaza, Prahova County	545 m	Alkaline/ calcareous	Grassy meadow Full-sun	3-4	2017 2018 2019 2020 2021	5 4 3 5 4
Tărlung River Brașov County	518-522 m	Alkaline/ calcareous	Grassy, swampy meadow Full-sun	14-18	2018 2019 2020 2021	5 4 5 5
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	3-4	2021	6
Ophrys sphegodes						
Tinăud, Cluj County	240-250 m	Alkaline/ calcareous	Grassy meadow Full-sun	100-120	2017 2018 2020 2021	4 8 6 8

3. Pollination monitoring: The inflorescences were studied from the beginning to just after the peak of anthesis, when the flowers are freshly opened and most attractive to the pollinators. The observer (NA) was initially located approximately 6-8 meters from the subjects. Once the insects were observed to have landed on the labellum, the observer (NA) recorded in writing the frequency of the visiting insects, the time spent on the flower/ inflorescence and the pollinia removal. On several occasions, the observer (NA) approached the flowers and the behaviour of visitors was recorded on digital photographs, from the moment they landed on the labella, until they left flowers/inflorescence.

4. Digital photographic equipment: Individual plants were photographed with body cameras: Canon 5D Mark III, Nikon D3 and Nikon D850. lenses: Nikon Micro NIKKOR 60mm, Venus Optics Laowa 100mm 2X Ultra Macro and Canon MP-E 65mm 1-5x Macro Lens. Additional equipment: Manfrotto Tripod, Litra Torches 2.0s. Adapted Helion FB tube was used for Automated Focus Bracketing. Images were analysed using Adobe Photoshop CC 2021, Helicon Focus and Zerene Stacker Software (previously used by Anghelescu *et al*., 2021b).

RESULTS

1. General description: *Ophrys* are long-lived herbaceous, terrestrial, perennial, geophytes.

2. Habitats: Most *Ophrys* species occur in mesic to dry grasslands, shrublands, verges, open pine woods and wooded meadows, where the cover and height of the herbaceous layer are limited, hence the competition with other herbaceous species is rather low (Eber, 2011; Jacquemyn & Hutchings, 2015). In Romania, *Ophrys* grow on nutrient-poor, warm and dry to fairly moist, alkaline/calcareous substrates (chalk and limestone), sometimes (slightly) acidic bogs (*Ophrys insectifera*, *Ophrys oestrifera*), in full sunlight, on grassy meadows, short grasslands or open woodland (pine, oak, beech). They can also be found growing adjacent to deciduous/mixed forest margins, in partial to heavy shade (*Ophrys insectifera*). The altitude preferred by Romanian *Ophrys* species is from 150-1200 meters a.s.l.

3. Morphology: Non-reproductive organs: They may present two to three ovoid-ellipsoid, entire ***root-tubers***. ***Stems*** are flexuous, spindly (*Ophrys insectifera* **- Fig. 4a**) or thicker (*Ophrys apifera*), fleshy, glabrous (hairless), yellowish-green (*Ophrys insectifera*, *Ophrys sphegodes*) to vivid green. ***Leaves*** are green, entire, sometimes yellowish-green (*Ophrys insectifera*) or glaucous, silvery or greyish-blue (*Ophrys oestrifera* - **Fig. 4b**), near erect, round to linear-oblong, unspotted and usually form a basal rosette (except *Ophrys insectifera*). ***Bracts*** are concave, leaf-like, smaller. ***Inflorescences*** are lax, few flowered (*Ophrys sphegodes* - bearing up to 3-4 flowers) to floriferous (*Ophrys insectifera*, *Ophrys oestrifera* - bearing up to 16-18 flowers). ***Flowers*** are usually showy, vividly coloured, insect-shaped (insectiform). ***Sepals*** are large, greenish, rose-pinkish to whitish, with the large median sepal bending over the ***gynostemium***.

Fig. 4a. *Ophrys insectifera* & **4b.** *Ophrys oestrifera* – full plants in their natural habitats © NA

Petals are smaller, triangular (*Ophrys apifera*, *Ophrys oestrifera*) or filiform/antenna-shaped (*Ophrys insectifera*), glabrous or hirsute, rose-pinkish to whitish. Their margins are fimbriate (having a fringe or border of hairlike or finger-like projections), sometimes with wavy edges (*Ophrys sphegodes*). ***Labellum*** is insect-shaped, flattened or arcuate to convex, sometimes with two conical protuberances at the base. The surface is brownish coloured, velutinous and short haired (downy). The labellum is three lobed, with two smaller

lateral lobes (with longer haired margins) and a large ***median lobe***, which serves as a landing platform for pollinators. ***Median lobe*** may be bilobed, the lobes being separated by a ***notch*** (incision, groove). The central area of the median lobe is usually adorned with lines and markings or with a glabrous, metallic blue-silvery area, known as the ***speculum*** (*mirror*). The shiny speculum mimics the iridescent folded wings on the back of a female insect. Above the speculum, there is a shield-shaped, vividly coloured area, marked by a line, known as the ***basal field***. On other side of the basal field, there are two small, shiny, dark bulges, known as ***pseudo-eyes***. They were interpreted as mimics of the eyes of insects or the tegulae of their wings (Paulus 2006). Darwin was among the first to notice these shining bulges with '*an almost metallic luster, appearing like two drops of fluid*' (Darwin, 1862, p. 57), near the base of the *Ophrys* labellum and regarded them as a likely example of '*sham nectaries'* (Sprengel, 1793). However, their location close to the stigmatic surface of the flower led him to suppose that these shining protuberances, were the attractive feature that would draw the insects' attention (Vereecken & Francisco, 2014). The labellum has ***no spur*** and ***no nectar*** is secreted. It ends into a distinct, deflexed, horizontal or upturned (apical) green, fleshy ***appendage***/***appendix***, which presumably provides a tactile stimulation to the male insects during pseudocopulation. The ***appendage***, which shows great species-specific variability in size and shape, is considered a putative ***osmophore*** believed to be responsible for much of the highly volatile long-range attractants (***pseudopheromones***) production in the flower.

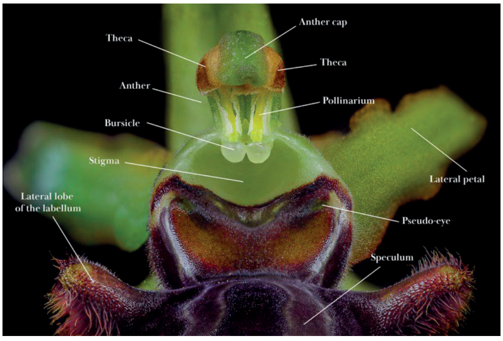


Fig. 5. *Ophrys sphegodes* - flower close up. © NA

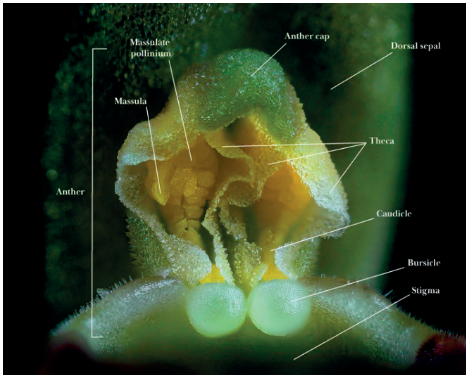


Fig. 6. *Ophrys sphegodes* - dehiscent anther frontal view (pollinarium & anther cap); inside the globular ***bursicle***, the ***viscidium*** is protected from drying © NA

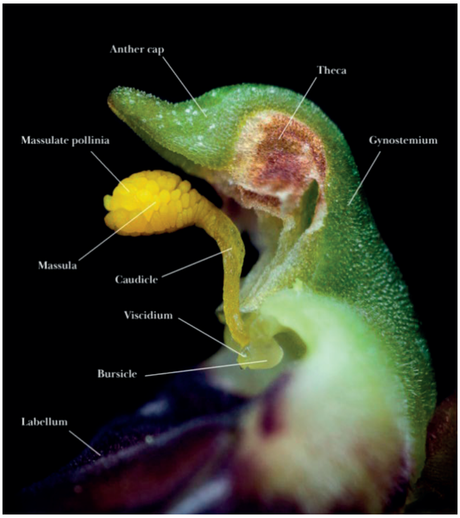


Fig. 7. *Ophrys sphegodes* - ***dehiscent anther*** side view; the ***thecae*** are dehiscent, each containing a ***pollinarium*** that terminates in a globular ***bursicle*** which contains the adhesive ***viscidium***. © NA

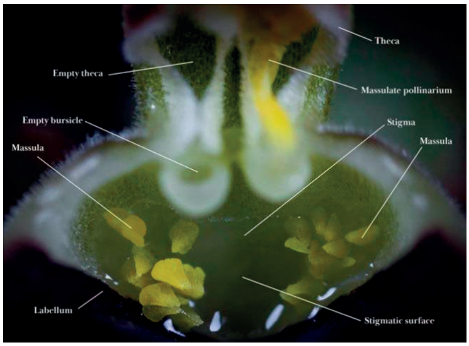


Fig. 8. *Ophrys sphegodes* - ***stigmatic cavity*** containing several ***massulae*** that started to germinate on its wet, sticky surface. © NA

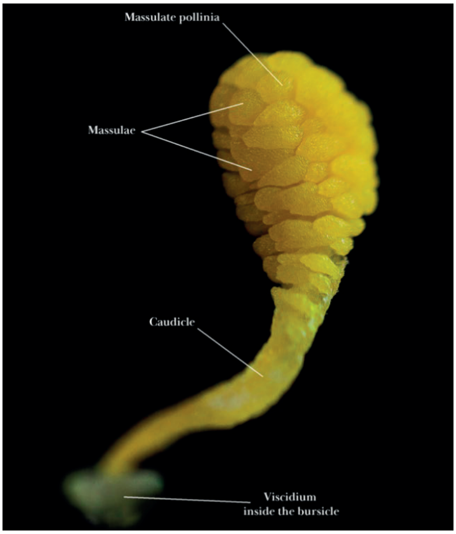


Fig. 9. *Ophrys sphegodes* - entire ***massulate pollinarium*** composed of several ***compacted blocks of pollen grains***, the ***massulae*** that form the ***pollinium***, followed by a ***caudicle*** and the adherent disc, the ***viscidium*** © NA

Reproductive organs. *Gynoecium* (represented by the ***stigma***, placed at the base base) and ***androecium*** (represented by the ***unique anther***, placed on the top of the stigmatic cavity) are fused into an elongated, central organ, known as the ***gynostemium***. ***Anther*** is bithecal/dithecal (bi-chambered). Each ***theca*** contains a yellow, club-shaped ***pollinarium*** (two parallel pollinaria are thus present per *Ophrys* flower). Each ***pollinarium*** is composed of a flat, translucent, adhesive disc - the ***viscidium***, followed by an elongated stalk - the ***caudicle*** and ending with a swollen, apical part - the ***pollinium***. In *Ophrys* genus, the pollinium is termed as ***massulate pollinium***, being composed of numerous, compacted blocks of pollen grains, which cohere well and form the wedge-shaped ***massulae***. The only other plant group which also has developed pollinia also is the milk weeds,

Asclepiadiaceae. Presumably for the same reasons, they have also developed a large number of deceptive flowers (Ollerton & Liede, 1997). There are ***no auricles*** and ***no staminodia*** present (Claessens & Kleynen, 2011; 2016). ***Stigma*** is roundish, elliptic, concave, three-lobed, with a large median lobe, the ***rostellum***. ***Rostellum*** is three lobed and roof-like, placed above the stigma, preventing the contact of the pollinia with the stigma (preventing self-pollination). ***Median rostellar lobe*** is small and the ***lateral rostellar lobes*** carry a ***globular bursicle***, each. Each ***bursicle*** contains an individual, round, flat, adhesive and translucent ***viscidium***. ***Ovary*** is sessile, not or slightly twisted, glabrous, bent over, bringing the lip into a downwards position. (Woods, in Pridgeon *et al*., 2001; Delforge 2005; 2006).

4. Chromosome numbers: 2n = 2x = 36. However, tetraploidy was shown in the case of *Ophrys fusca*: **2n = 4x = 72** (D'Emerico, in Pridgeon *et al*., 2001).

5. Flowering time: *Ophrys* species flower once a year, from April to June, depending on the altitude (De Angelli & Anghelescu, 2020). It is common that individual plants do not necessarily flower every year (Hutchings, 2010) and the proportion of plants that flower within a population greatly varies from year to year. For example, during long-term studies in southern England, it has been observed that the average frequency of plants flowering from one year to another was 27.4% in *Ophrys apifera* and 83.7% in *Ophrys sphegodes* (Wells & Cox, 1989; Hutchings, 2010). Successful flowering usually depends on tuber size, leaf number and the amount of rains during autumn, when the leaves start to appear above ground (Wells & Cox, 1989). ***Drought*** represents a very impotant limiting factor, as it prevents inflorescence formation and also causes premature sensecence of the flowers, thus reducing the chances of pollination and seed production (Neiland, 1994).

6. Pollinators: In Romania, the most currently known pollinators of *Ophrys* species are male bees and wasps of the **Order Hymenoptera** Linnaeus, 1758. They belong to the widely distributed families: **Apidae -** *Eucera longicornis* (Linnaeus, 1758), unique pollinator of *Ophrys oestrifera*, **Andrenidae** - *Andrena nigroaenaea* (Kirby, 1802), unique pollinator of *Ophrys sphegodes* and **Crabronidae** Latreille, 1802 - *Argogorytes mystaceus* (Linnaeus, 1761), unique pollinator of *Ophrys insectifera*. In the Mediterranean Basin, *Ophrys apifera* is accidentally visited *by Eucera longicornis.*

7. Pollination strategies: All *Ophrys* species are self-compatible (able to self-pollinate). However, except *Ophrys apifera* (self-pollinated), all other species employ pollination by ***sexual deception*** to attract their highly specific male pollinators. A single pollination event implies the removal of the pollinium and the deposition of the pollinia on a second flower.

Ophrys insectifera L., 1753 - Fly Orchid

The ***specific epithet***, ***insectifera***, originates in the Latin ***insectum*** (insect) and ***phór(os)*** (to carry) meaning ***carrying an insect***, a reference to ***insect-shaped labellum*** that mimics the shape, size, color, pilosity, texture and smell (***pseudo-pheromones***) of a female insect.

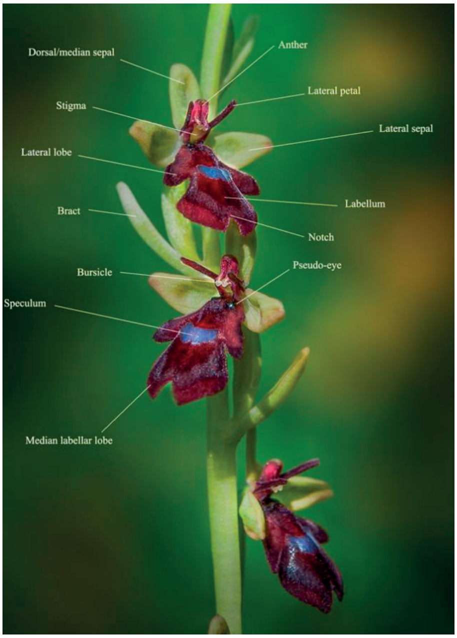


Fig. 10. *Ophrys insectifera* - inflorescence detail. The brown-reddish flowers resemble bees perching on a blade of grass. The ***lateral petals*** are long and filiform, ***mimicking insect antennae***. © NA

Fig. 11. *Ophrys insectifera* - ***speculum*** detail. It is metallic-blue colored (sometimes grayish) and shiny, mimicking the ***folded wings of a female insect.*** © NA

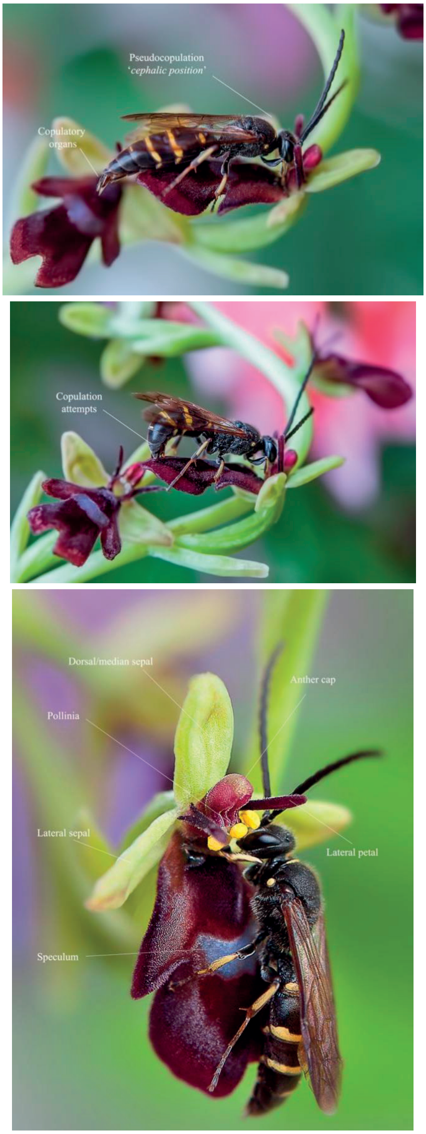


Fig. 12-14. *Ophrys insectifera* - pollinated by pseudocopulation by the solitary wasp, *Argogorytes mystaceus*. Attracted by the ***orchid's pheromones***, it is seduced by the visual stimuli offered by the labellum that resemble a female wasp. Aroused by the ***sexually-stimulating hairs on the labellum***, the male aligns with the labellum and ***attempts to copulate*** with the orchid flower. After several unsuccessful attempts to mate and frustrated by the lack of interest from the insensible (***pseudo***) ***female,*** the male flies off with the ***pollinia*** (yellow pollen sacs) firmly attached to its body, in frantic search of more authentic female companionship. The process of **pseudocopulation** took about **7-8 minutes**, during which the males wasp pseudocopulated with all freshly opened flowers, spending up to **72 seconds on each flower**. © NA

Ophrys oestrifera M.Bieb., 1808 - Botfly Orchid

The ***specific epithet***, ***oestrifera***, comes from the family of botflies, **Oestridae** Leach, 1815, and the Latin ***phór(os)*** (to carry) meaning ***carrying a (furry) fly***, a reference to ***insect-like furry labellum*** that mimics the botflies/maggot flies.

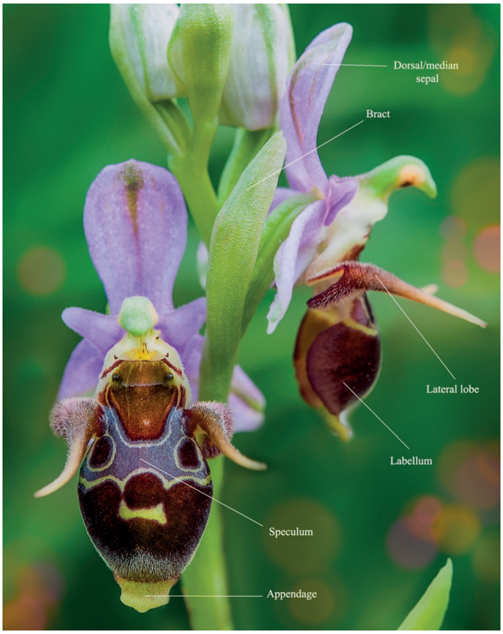


Fig. 15. *Ophrys oestrifera* – ***inflorescence*** detail. The flowers are large, with pinkish-violet lateral petals and sepals. The ***labellum*** is brounish, furry and has a shiny, metallic-blue, edged yellowish, ***speculum***, in the form of ***H or X***; the ***green appendage*** is prominent. © NA

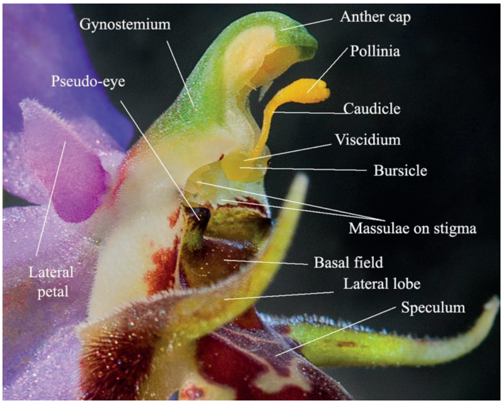


Fig. 16. *Ophrys oestrifera* – ***stigmatic cavity*** entirely filled with ***massulae***, as a result of successful pollination. © NA

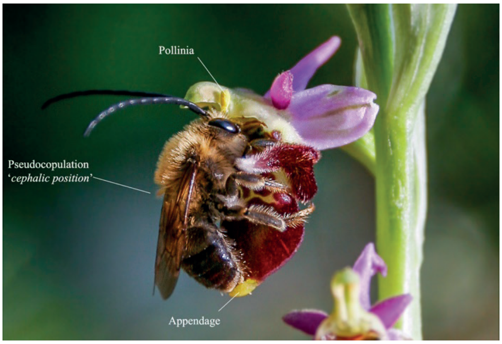


Fig. 17. *Ophrys oestrifera* pollinated by pseudocopulation by a ***male Eucera longicornis***. A **Long-horned** male bee embraces the labellum in a ***pseudocopulation attempt***, touching the ***green appendage*** (which mimics ***female reproductory organs***) with its copulatory organs. Trying to find the female's head, the male sticks its head in the center of the flower, touching the stigma and thus removing the ***pollinia***. The ***pseudocopulation*** process took **6 minutes**, with approximately **68 seconds** per flower.

Fig. 18. *Ophrys oestrifera* – new individual plant in process of getting pollinated by a pollinia-carrier, male *Eucera longicornis.* The **long-horned male bee**, with the ***pair of pollinia*** stuck on its head (from a previous ***pseudocopulation attempt***) is approaching a new, unpollinated inflorescence. **Photos 17-18** courtesy of **Dan Anghelescu & George Avanu**.

Ophrys sphegodes Mill., 1768 - Spider Orchid

The ***specific epithet***, ***sphegodes***, originates in the ancient Greek word ***sphêx, sphec*** (wasp), *ad litteram* meaning ***carrying a wasp***, a reference to the ***wasp-like labellum***. However, *Ophrys sphegodes* is not pollinated by wasps, but by ***bees only***. Due to a distinctive, highly variable **H** markings on the labellum (even among the flowers of the same inflorescence), which resemble a ***spider***, and its early-spring flowering time, *Ophrys sphegodes* is also known as the **Early Spider Orchid**.

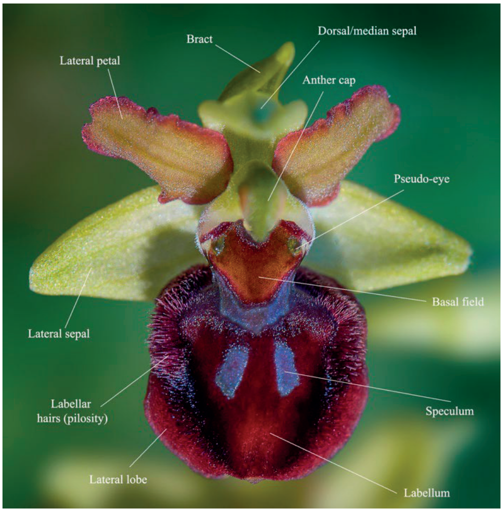
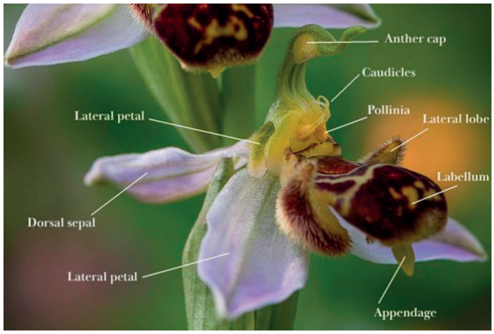


Fig. 19. *Ophrys sphegodes* – flower detail. The ***flowers*** are medium-sized. The ***sepals*** are greenish yellow. The ***lateral petals*** are auriculate, greenish, washed red. The ***labellum*** is brownish red to dark brown, with furry margins and a velvety surface, trilobed. The ***median lobe*** has a central H-shaped bluish-grey to reddish-brown speculum. © NA

Fig. 20. *Ophrys sphegodes* – insect visitors. Its usual pollinator is the mining bee, *Andrena nigroaenaea*; the flowers are often visited by numerous insects, e.g., **wood ants**, *Formica (Serviformica) cinerea* Mayr 1853, which rob the orchid's pollen by chewing the pollinium. © NA

Ophrys apifera **Huds., 1762 - Bee Orchid**

The ***specific epithet***, ***apifera***, originates in the Latin ***apis*** (bee) and the Greek ***phór(os)*** (to carry), meaning ***carrying a bee***, a reference to the ***bee-like labellum***. Despite its name, *Ophrys apifera* is never pollinated by bees, this orchid being an ***exclusively autogamous orchid*** that ***self-pollinates***.



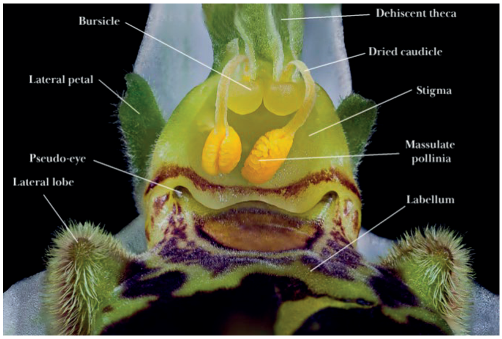


Fig. 21-22. *Ophrys apifera* – ***self-pollination***. Shortly after anther dehiscence (within mimutes), the long ***caudicles*** wither and bend forwards and downwards, until the ***massulate pollinia*** reach their own ***stigma***, under below. A gentle breeze is often enough to blow them onto the sticky stigmatic surface. © NA

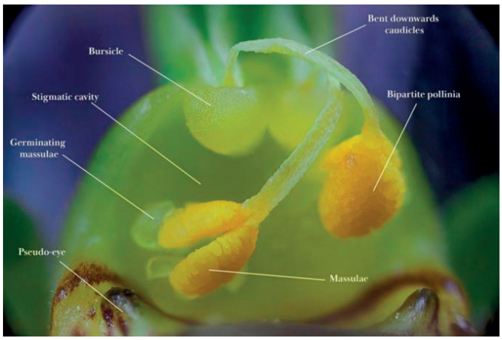


Fig. 23. *Ophrys apifera* – ***stigmatic cavity*** filled with ***massulae***, which adhered to the ***stigmatic surface*** and started to germinate (self-pollination). © NA

Fig. 24. ***Ophrys apifera*** **var.** ***aurita*** **(Moggr.) Gremli, 1887**. The ***infraspecific epithet*** (***variety epithet***), ***aurita***, originates in the Latin ***aurīta*** (long ears), meaning ***ear-shaped***, a reference to the ***filiform, elongated lateral petals***, which resemble ***two pricked up ears***. © NA

8. Fruit: Many *Ophrys* species can flower and fruit for several years in succession (Hutchings, 1989; Wells & Cox, 1991). Therefore, although the species are capable of producing fruit more than once, all orchid plants are effectively ***monocarpic*** because they may flower only once before dying. Each fertilised *Ophrys* flower produces a single, elongated, ovoid, erect, ***greenish fruit pod***. ***Fruit set*** varies from year to year, greatly depending on the presence of the pollinators and on the exposure to sunlight. Our observations/counts show relatively high fruit set, e.g., *Ophrys insectifera* (20-35%), *Ophrys oestrifera* (30-32%), *Ophrys sphegodes* (17-26%) and *Ophrys apifera* (35-45%).

9. Seeds: The ***seeds*** usually mature by July-August/September, depending on the species. The greenish ***fruit pod*** matures and transforms into a dehiscent, brownish ***seed capsule***, closed at both ends. *Ophrys* seed capsules are fairly large and may contain in average 5000-20.000 tiny seeds per capsule (Arditti & Ghani, 2000), which is more than any other European orchid genera (Neiland & Wilcock, 1995).

DISCUSSIONS

The use of DNA analyses for *Ophrys* systematics led to major controversies regarding the number of species, ranging from **251 species** (Delforge, 2006) to **9-11 macrospecies** or ***clades*** (***molecularly cohesive monophyletic groups***) (Bateman *et al*., 2018): *Insectifera*, *Apifera*, *Umbilicata*, *Fuciflora* (incl *Scolopax*), *Sphegodes*, *Speculum*, *Bombyflora*, *Tenthredinifera* and *Fusca*. The species richness is mostly due to a high degree of hybridisation, with parents and hybrids (partially) sharing the pollinator communities, a phenomenon common to other genera (Anghelescu *et al*., 2020b). Although most researchers accept that some taxonomic exaggeration exists, ***intrageneric hybridization*** is considered a source of evolutionary novelties that could ultimately lead to ***pollinator shifts*** and ***reproductive isolation*** (***speciation***) (Baguette *et al*., 2020).

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