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AN OVERVIEW OF RECENTLY DISCOVERED INTRA AND INTER-GENERIC ORCHID HYBRIDS AS NEW ADDITIONS TO ROMANIAN FLORA

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Abstract

We describe, for the first time, fourteen of the most spectacular intra- and inter-generic orchid hybrids, new to Romanian flora. As crosses between species of the same or different genera, their occurrence is exceptionally rare in the wild, both in Romania and throughout temperate Europe. Discovering and having the chance to study them in detail in the wild, represents a remarkable privilege. During our three-year study (2020-2023), we encountered several hybrids belonging to the following genera, Anacamptis (A.× menosii; Anacamptis × olida nothosubsp. paparistoi), Ophrys (O. × minuticauda), Orchis (O. × loreziana nothosubsp. kisslingii), Neotinea (Neotinea × dietrichiana), Dactylorhiza (D. × ruppertii) and nothogenera × Dactylodenia (× D. lebrunii; × D. heinzeliana; × D. illyrica nothosubsp. siculorum; × D. sinaiensis) and × Pseudadenia (× P. schweinfurthii; × P. vitosana). Out of these, × Dactylodenia illyrica nothosubsp. siculorum (Dactylorhiza cordigera subsp. siculorum × Gymnadenia frivaldii) and × Dactylodenia sinaiensis (Dactylorhiza saccifera × Gymnadenia conopsea), are regarded as new-to-science taxons, hereby described for the first time. To give a complete overview, we included the inter-generic hybrid belonging to × Pseudorhiza nothogenus (× P. nieschalkii nothosubsp. siculorum). In addition, information regarding their distribution, habitat, ecology, phenology and IUCN conservation assessments are provided.

Key words*: orchids, intergeneric, hybrids, × Dactylodenia, × Pseudadenia, conservation, intrageneric.*

INTRODUCTION

In this article, we document the first reported natural occurrence of **six *intra-generic hybrids*** belonging to genera, ***Anacamptis*** - ***Anacamptis*** × ***menosii*** (Chr. Bernard & G. Fabre) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*A. coriophora* × *A. papilionacea*), ***Anacamptis*** × ***olida*** **nothosubsp.** ***paparistoi*** (Gölz & H.R. Reinhard) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*A. coriophora* × *A. morio* subsp. *caucasica*), ***Ophrys*** - ***Ophrys*** × ***minuticauda*** Duffort, 1902 (*O. apifera* × *O. scolopax* subsp. *cornuta*), ***Orchis*** - ***Orchis*** × ***loreziana*** **nothosubsp.** ***kisslingii*** (Beck) Potucek, 1976 (*O. mascula* subsp. *speciosa* × *O. pallens*), ***Neotinea*** - ***Neotinea*** × ***dietrichiana*** (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr., 2007 (*N. tridentata* × *N. ustulata*) and ***Dactylorhiza*** - ***Dactylorhiza*** × ***ruppertii*** (M. Schulze) Borsos & Soó, 1960 (*D. majalis* × *D. sambucina*). Additionally, we included **eight**, exquisitely rare ***inter-generic hybrids***, members of the nothogenera × ***Dactylodenia*** (*Dactylorhiza* × *Gymnadenia* genera) and × ***Pseudadenia*** (*Pseudorchis* × *Gymnadenia* genera). The chances of these specific hybridisation events occurring simultaneously are exceedingly rare: × ***Dactylodenia lebrunii*** (E.G. Camus) Peitz, 1972 (*Dactylorhiza majalis* × *Gymnadenia conopsea*); × ***Dactylodenia heinzeliana*** (Reichardt) Garay & H.R. Sweet, 1969

(*Dactylorhiza fuchsii* × *Gymnadenia conopsea*); × ***Dactylodenia illyrica* nothosubsp. *siculorum*** L. Balogh & Mih. Balogh, N. Anghelescu, N. Kigyossy, 2023 nothosubsp. *nov*. (*Dactylorhiza cordigera* subsp. *siculorum* × *Gymnadenia frivaldii*); × ***Dactylodenia sinaiensis*** N. Kigyossy, N. Anghelescu, L. Balogh & Mih. Balogh, 2023 nothosp. *nov.* (*Dactylorhiza saccifera* × *Gymnadenia conopsea*), × ***Dactylodenia* cf. *vitosensis*** Jagiello, 1988 (*Dactylorhiza cordigera* × *Gymnadenia conopsea*) and × ***Pseudadenia schweinfurthii*** (Hegelm. ex A. Kern.) P.F. Hunt, 1971 (*Gymnadenia conopsea* × *Pseudorchis albida* subsp. *tricuspis*); × ***Pseudadenia vitosana*** (H. Baumann) O. Gerbaud & W. Schmid, 1999 (*Gymnadenia frivaldii* × *Pseudorchis albida* subsp. *tricuspis*). Out of these, × *Dactylodenia illyrica* nothosubsp. *siculorum* (*Dactylorhiza cordigera* subsp. *siculorum* × *Gymnadenia frivaldii*) and × *Dactylodenia sinaiensis* (*Dactylorhiza saccifera* × *Gymnadenia conopsea*), are regarded as new-to-science taxons, hereby described for the first time. Furthermore, another unique notho-population of nine individuals of × *Pseudadenia vitosana* (*Gymnadenia frivaldii* × *Pseudorchis albida* subsp. *tricuspis*) is described for the first time, demonstrating the successful propagation and proliferation of inter-generic combinations. Additionally, an incipient nothopopulation of two × *Dactylodenia illyrica* nothosubsp. *siculorum* hybrids was recorded between 2022-2023. These two nothopopulations may be regarded as unique in Europe and represent a novel addition to the orchid flora of Romania. Regrettably, during the summer of 2023, between the 15th and 30th of June, ***two out of the total of nine × Pseudadenia vitosana inter-generic hybrids were discovered missing at the specified locations,*** within the highly protected Harghita Mădăraș area. It is crucial to emphasize that collection of rare plant taxa, such as our rare orchid hybrids from the highly protected Harghita Mădăraș ROSCI00090 area, is illegal, even when granted a study or work permit. These taxa are strictly protected not only by international law but also by regional (Romanian) regulations. Moreover, for laboratory research or deposition of herbarium vouchers, only 1-3 individual flowers and, exceptionally, one leaf may be collected from a single individual, the holotype. In recent years, photographs of the holotype(s) are also accepted as herbarium vouchers, to ensure full protection and preservation of these extremely rare and vulnerable plants.

To give a complete overview of all natural orchid hybrids reported in Romania, we included one newly described inter-generic orchid hybrid belonging to nothogenus × ***Pseudorhiza*** (*Dactylorhiza* × *Pseudorchis* genera), recently named × ***P. nieschalkii* nothosubsp. *siculorum*** nothosubsp. *siculorum* H.Kertész & N.Anghelescu, 2020 (*Dactylorhiza fuchsii* subsp. *sooana* × *Pseudorchis albida* subsp. *tricuspis*) (Anghelescu *et al*., 2021a).

The hybrids were found/studied in two major protected natural areas of Romania, **Bucegi Natural Park** ROSCI0013, Prahova County (Hedrén et al., 2022; Anghelescu et al., 2023b) and **Harghita Mădăraș**, ROSCI00090, Harghita County, as well as in various other regions, known for their rich orchid diversity, such as **Hunedoara**, **Argeș** and **Mehedinți Counties**. Over the three-year study, from **2021 to 2023**, the authors conducted extensive fieldwork in the above regions to document and map the orchid flora present within the newly discovered, remote hybridogenic zones (Anghelescu et al., 2020; 2023a; 2023b). Our observations show that the main floral features of the nothotaxa, are generally, intermediate between the parental species.

The aims of the present study are: (1) to describe in detail the main morphological characteristics of the two nothospecies (especially those of the flowers), by comparing them to the respective parental species; (2) to give information on various aspects of their biology, relevant to understanding their ecological requirements, such as distribution, habitat, communities, phenology, reproductive requirements and conservation; (3) to present information regarding the IUCN (2021) conservation status of all the taxa considered, accompanied by photographs of the holotypes.

Taking into consideration the importance and rarity of these newly discovered nothotaxa, we strongly propose the two nothopopulations as new additions to the Romanian flora.

MATERIALS AND METHODS

Sites Studied

The studies were conducted in various natural parks and protected areas:

(1) Bucegi Natural Park, Southern Carpathians, Central Romania, is a protected area included within Natura 2000 ROSCI0013, IUCN category V. It covers Prahova, Dâmbovița and Brașov Counties, with an area of ca. 32,663 ha/326.63 km^2 and the highest elevation at Omu Peak of 2,505-2,514 m. a.s.l (Ielencz, 2005).

(2) Natura 2000 protected area ROSCI00090 Harghita-Mădăraș, located in the central-eastern part of Harghita County (EU Environment, 2014) and covers an area of 13,373 hectares. It is located at an altitude between 1,500-1,800 meters a.s.l (above sea level) (Mikfalvi & Vifkori, 1979; Marcu, 1986; Cavruc, 2000). The site is a natural area covered by deciduous, coniferous and mixed forests, natural meadows, heathlands, bogs (peat bogs) and steppes together with a rich hydrographic network that consists of several lakes and watercourses (Ciocârlan 2000; 2009).

(3) Hunedoara County - the southern range of Apuseni Mountains, with the highest peak being Poienița Peak, with an elevation of 1,437 meters a.s.l. The field study included full sun to partial shade, subalpine meadows, short grasslands, forest fringes and open woodland, on calcareous substrate, up to 1,000 meters a.s.l.

(**4) Argeș County** - studies included similar habitats, dry to moist grassland (swampy areas), forest margins, meadows and pastures on calcareous substrates, up to 700-900 meters a.s.l.

(5) Mehedinți County - the species studied prefer full sun, grassy, alkaline marsh, prone to flooding (wet meadows), forest clearings, on calcareous substrates, up to 100 meters a.s.l.

Morphometric methods/comparisons

Special attention was given to the characters that proved to be taxonomically informative and those that involve the differentiating details in the morphology of the leaves, labellum and tepals. Measurements of the vegetative and floral parts were made from living plants and fresh flowers. (Anghelescu et al., 2021b).

Digital Photographic Equipment

Digital images of individual plants and floral parts were taken using Canon 5D Mark III, Nikon D3 and Nikon D850 camera bodies equipped with Nikon Micro NIKKOR 60 mm and NIKKOR 24.0-70.0 mm lenses, Venus Optics Laowa 100 mm 2X Ultra Macro. Additional equipment included a Manfrotto Tripod and Litra Torches 2.0s. Images were analysed using Adobe Photoshop® CC 2023, Zerene Stacker Software, Vers.2021-11-16 (Anghelescu et al., 2021c).

RESULTS AND DISCUSSIONS

Overcoming the premating (prezygotic) and postmating (postzygotic) isolation barriers, species boundaries might easily be disrupted, allowing cross-pollination (Marques et al., 2014). In the cases where these isolation mechanisms are not fully effective, cross-pollination leads to hybridisation (Goulet et al., 2017). Consequently, in natural conditions, hybridization represents an evolutionary engine, which breaks down the reproductive barriers that separate different species. By definition, hybridization refers to the mating or cross-breeding of two parental lineages, P1 and P2, who possess different/distinct genetic backgrounds. When P1 and P2 reproduce (P1 × P2), their genes combine in the first offspring generation (primary hybrids), known as F1 (Scopece et al., 2007). In the resulting hybrids, each genetically inherited trait/character can be exhibited as dominant (prevailing), recessive (masked) or intermediate (Ramsey et al., 2003). Consequently, this phenomenon can have profound effects on the genetic variation within populations and the evolution of species, leading to the creation of hybrids with unique combinations of traits (Marques et al., 2014). In certain instances, these hybridization events may even lead to the emergence of entirely new species (Mayr, 1942).

Description of the fourteen nothotaxa studied

The totality of all hybrid individuals resulting from the cross-breeding of the same parental taxa (natural species, not hybrids themselves) is termed a hybrid lineage or a ***nothotaxon*** (Lowry, 2008; Gill, 1989; Scopece et al., 2008).

Following is the full description of the ***fourteen intra and inter-generic orchid hybrids*** included in this study, named according to POWO (2022), Eccarius (2016; 2022). They are all terrestrial, perennial, rhizomatous autotrophic, sympodial herbaceous geophytes (Figures 1-14).

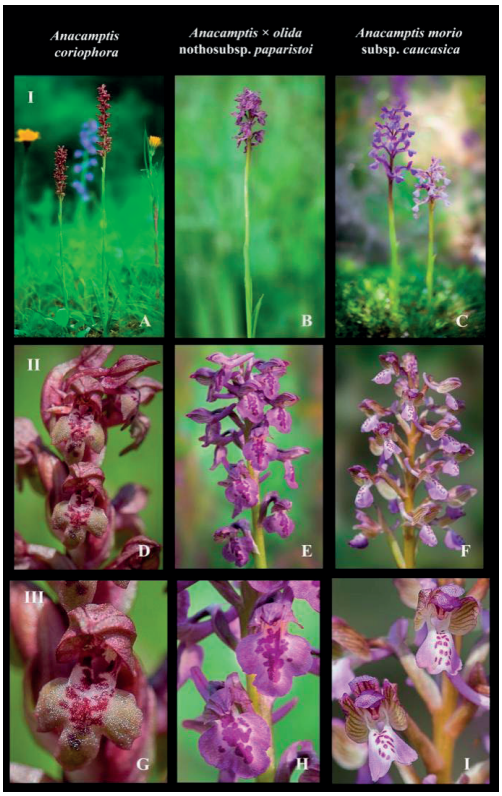


Figure 1. ***Anacamptis* × *olida*** **nothosubsp.** ***paparistoi*** **(Gölz & H.R. Reinhard) H. Kretzschmar, Eccarius & H. Dietr., 2007**

I. Full plants in their natural habitats; **II**. Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Anacamptis coriophora*; **B., E., H.** - *Anacamptis* × *olida* nothosubsp. *paparistoi*; **C., F., I.** - *Anacamptis morio* subsp. *caucasica*. Photos © Nora E. Anghelescu, 5 May 2018, Mehedinți County

Hybrid formula: *A. coriophora* × *A. morio* subsp. *caucasica.* **Discovered:** 5 May 2018, Nora E. Anghelescu. **Locus classicus:** Mehedinți County. **Flowering time:** April-May. **Native to:** Romania, Albania. **Description:** Stem (epigeal), 21 cm, erect, purple-tinged in the upper part (inflorescence). One basal sheath, 1-3 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem. Inflorescence lax to dense, elongated raceme, floriferous. Flowers intermediate between parental species: labellum shape, median hyperpigmented markings and the strong purple shades of its margins resemble *A. coriophora*. The thicker, robust and cylindrical spurs partially resemble *A. coriophora*, but also *A. morio* subsp. *caucasica* in the upward curving of the acuminated tip. The hood is tighter, similar to that of *A. coriophora*. **Population counts:** This particular individual was discovered in 2017, in Mehedinți County, and it is the only hybrid of this type known to occur in Romania (De Angelli & Anghelescu, 2020). **Proposed conservation status:** Endangered (EN).

Figure 2. ***Neotinea* × *dietrichiana*** **(Bogenh.) H. Kretzschmar, Eccarius & H. Dietr., 2007**

I. Full plants in their natural habitats; **II**. Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Neotinea tridentata*; **B., E., H.** - *Neotinea* × *dietrichiana*; **C., F., I.** - *Neotinea ustulata*. Photos B., E., H. © Lori Balogh; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 5 June 2023, Prahova County

Hybrid formula: *N. tridentata* × *N. ustulata.* **Discovered:** 2 June 2023, Lori & Mihaela Balogh. **Locus classicus:** Prahova County. **Flowering time:** May-June. **Native to:** Europe to Caucasus, Romania. **Description:** Stem (epigeal), 20-40 cm, erect, vivid green. 2-4 basal leaves, forming a rosette, 2-3 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem purple-tinged at the tip (resembling *N. tridentata*). Inflorescence lax to dense, 4-8(10) cm elongated raceme, very floriferous, purple-tinged (resembling a hyperchromatic *N. tridentata*), with a burnt tip (resembling *N. ustulata*). This individual presents a significantly elongated raceme, resembling *N. ustulata* parent (*N. tridentata* has a much shorter, roundish raceme). Labellum shape, as well as the labellar purple

markings, which are larger, roundish and purple, resemble *N. ustulata*. Middle labellar lobe is divided into two smaller lobules resembling both *N. ustulata* and *N. tridentata* (Kretzschmar et al., 2007). The helmets are tighter, purple-coloured, resembling *N. tridentata* (in *N. ustulata*, they are dark-red to almost black-coloured). **Population counts:** single individual, discovered in June 2023, in Prahova County. **Proposed conservation status:** Endangered (EN).

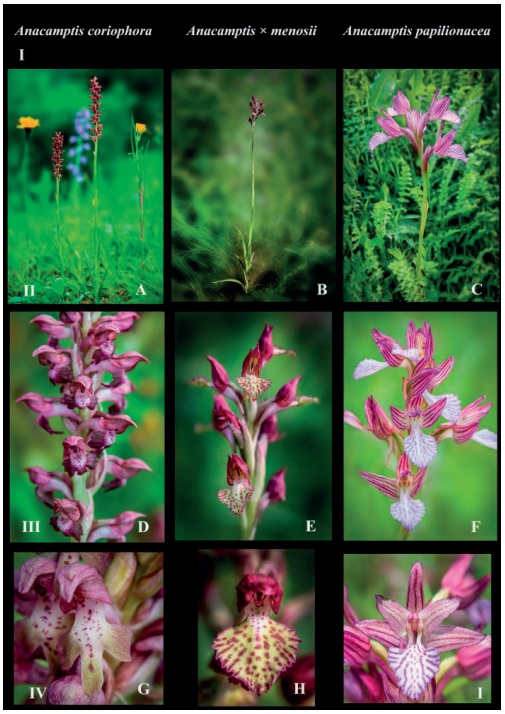


Figure 3. *Anacamptis* × *menosii* (Chr. Bernard & G. Fabre) H. Kretzschmar, Eccarius & H. Dietr., 2007
I. Full plants in their natural habitats; **II**. Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Anacamptis coriophora*; **B., E., H.** - *Anacamptis* × *menosii*; **C., F., I.** - *Anacamptis papilionacea*. Photos © Nora E. Anghelescu, 31 May 2023, Mehedinți County

Hybrid formula: *A. coriophora* × *A. papilionacea*. **Discovered:** 31 May 2023, Nora E. Anghelescu. **Locus classicus:** Mehedinți County. **Flowering time:** May-June. **Native to:** France, Portugal, Sardegna, Romania. **Description:** Stem (epigeal), 10-14 cm, erect, vivid green, no purple pigmentation. 1-2 basal leaves, sheathing the stem, 3-5 cauline leaves, unspotted, sessile, vivid-green, acuminate, sheathing the stem (resembling *A. coriophora*; in *A. papilionacea*, the leaves are veined, purple-tinged at the tips). Inflorescence lax raceme (resembling *A. papilionacea*), less floriferous, 8-10 medium-sized flowers, resembling *A. coriophora*. Helmet deep-purple pigmented, tight, with elongated tip, resembling *A. coriophora*. Labellum entire, heart-shaped (in upper flowers, resembling *A. papilionacea*) to mildly three-lobed (in basal flowers, resembling *A. coriophora*), smaller in size than *A. papilionacea*. Labellar stripes and dots are deep-purple to red (heterosis effect) resembling *A. papilionacea*. The spur is conical, thick resembling *A. coriophora*. **Population counts:** single individual, discovered in May 2023. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410363*. Proposed conservation status:** Endangered (EN).

Figure 4 *Ophrys* × *minuticauda* Duffort, 1902
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail - lateral view; **A., D., G.** - *Ophrys apifera*; **B., E., H.** - *Ophrys* × *minuticauda*; **C., F., I.** - *Ophrys scolopax* subsp. *cornuta*. Photos © Nora E. Anghelescu, 16 June 2023, Bucegi Natural Park ROSCI0013, Prahova County

Hybrid formula: *O. apifera* × *O. scolopax* susp. *cornuta*
Discovered: 5 June 2021, Ramy Maalouf. **Locus classicus:** Bucegi Natural Park ROSCI0013. **Flowering time:** May-June. **Native to:** France, Greece, Crimea, North Caucasus, Sardegna, Spain, Transcaucasus, Turkey, Romania. **Description:** Stem (epigeal), 25-30 cm, erect, vivid green. 1 basal leaf, sheathing the stem, 3-5 cauline leaves, unspotted, sessile, green, acuminate (resembling *O. apifera*). Inflorescence lax raceme, less floriferous, 4-5 medium-sized flowers, resembling *O. apifera*, smaller in size than *O. scolopax* ssp. *cornuta*. Sepals light-pink, centrally green veined, resembling *O. apifera*. Lateral petals, greenish, villous (resembling *O. apifera*, triangular and equal to those of *O. scolopax* subsp. *cornuta*. Labellum three-lobed, median lobe slightly elongated, speculum bluish, resembling *O. scolopax* subsp. *cornuta*. Lateral lobes pointed,

conical-elongated, shorter, resembling *O. scolopax* subsp. *cornuta*. Basal field orange-brown, wider, resembling *O. apifera*. Appendix oblique to the labellum, intermediate between parents. Pollinia with shorter caudicle, overhanging resembling *O. apifera*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited Herbarium of the Botanical Garden Bucharest: ***BUC 410370*. Proposed conservation status:** Endangered (EN).

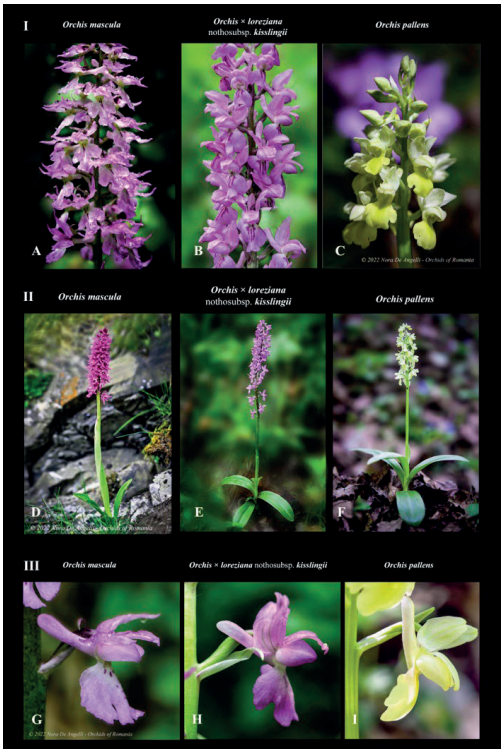


Figure 5. *Orchis* × *loreziana* nothosubsp. *kisslingii* (Beck) Potucek, 1976

I. Inflorescences details; **II.** Full plants in their natural habitats; **III.** Individual flower detail - lateral view; **A., D., G.** - *Orchis mascula* subsp. *speciosa*; **B., E., H.** - *Orchis* × *loreziana* nothosubsp. *kisslingii*; **C., F., I.** - *Orchis pallens*. Photos © Nora E. Anghelescu, 5 May 2023, Hunedoara County

Hybrid formula: *O. mascula* susp. *speciosa* × *O. pallens.*
Discovered: 5 May 2021, Nicoleta Kigyossy, Camelia & Cornel Alexandru. **Locus classicus:** Hunedoara County **Flowering time:** May-June. **Native to:** Romania, Austria, Czechoslovakia, Crimea. **Description:** Stem (epigeal), 20-55 cm, erect, purple-tinged in the upper part (inflorescence). 3-4 basal leaves, forming a rosette, 1-3 cauline leaves, unspotted (3 hybrids - resembling *O. pallens*) and basally purple spotted (2 hybrids - resembling *O. mascula* subsp. *speciosa*), sessile, vivid-green, acuminate, sheathing the stem. Inflorescence lax to dense, 10-20 cm raceme, floriferous (resembling *O. mascula* subsp. *speciosa*). Labellum shape, purple colour and hyperpigmented parallel stripes resemble *O. mascula* subsp. *speciosa* parent. Median and lateral lobes scalloped, intermediate Spur near-perpendicular to labellum, yellowish-purple, resembling *O. pallens*. The green parallel elongated stripes on lateral and median sepals resemble *O. pallens*. **Population counts:** ***Nothopopulation of five hybrids***. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410372*. Proposed conservation status:** Endangered (EN).

Figure 6. *Dactylorhiza* × *ruppertii* (M. Schulze) Borsos & Soó, 1960

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza majalis*; **B., E., H.** - *Dactylorhiza* × *ruppertii*; **C., F., I.** - *Dactylorhiza sambucina*. Photos © Nora E. Anghelescu, 10 June 2023, Harghita County

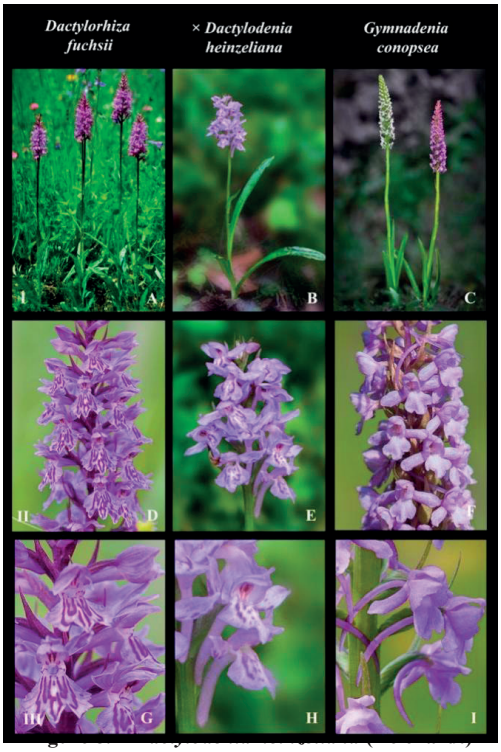
Hybrid formula: *D. majalis* × *D. sambucina.*
Discovered: 10 June 2021, Hajnalka Kertész. **Locus classicus:** Harghita County. **Flowering time:** May-June. **Native to:** Austria, Czechoslovakia, France, Germany, Switzerland, Romania. **Description:** Stem (epigeal), 12-25 cm, erect, vivid green, mildly pigmented at the tip (resembling *D. majalis*). 1 basal leaf, sheathing the stem, 2-3 cauline leaves, sessile, vivid-green, acuminate, narrow-elongated, sheathing the stem (resembling *D. sambucina*), with minute purple maculae at the tips, resembling *D. majalis*. Inflorescence dense raceme, less floriferous, resembling *D. majalis*. Flowers large resembling *D. sambucina*. Labellum widely spread (resembling *D. sambucina*) and purple-pink coloured

with mildly circular purple loops resemble *D. majalis*. Labellar central purple-pink dots and yellowish base resemble *D. sambucina*. Spur thicker, sacciform resembling more *D. sambucina*. Basal flower bracts longer than the flowers, purple pigmented, resembling *D. majalis*. **Population counts:** ***Nothopopulation of five hybrids***, including f**our dark-red hybrids between *D. majalis* × *D. sambucina* f. *rubra***. **Herbarium voucher specimen:** deposited Herbarium of Botanical Garden Bucharest: ***BUC 410367.*** **Proposed conservation status:** Endangered (EN).

Figure 7. × *Dactylodenia sinaiensis* N. Kigyossy, N. Anghelescu, L. Balogh & Mih. Balogh, 2023 nothosp. *nov.*

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza saccifera*; **B., E., H.** - × *Dactylodenia sinaiensis*; **C., F., I.** - *Gymnadenia conopsea*. Photos © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park ROSCI0013, Prahova County

Hybrid formula: *D. saccifera* × *G. conopsea*. **Discovered:** 2 July 2023, Nicoleta Kigyossy. **Locus classicus:** Bucegi Natural Park. **Flowering time:** June-July. **Native to: Romania, *endemic*, *nothosp. nov.*** **Description:** Stem (epigeal), 22-24 cm, erect, vivid green, pigmented at the tip, resembling *D. saccifera*. 1 basal, 3 cauline leaves, purple-pigmented, arched, oval-acuminate resembling *D. saccifera*. Upper cauline leaf, vivid green, narrow-lanceolate. Inflorescence dense raceme, 6-7 cm, floriferous, cylindrical, resembling *D. saccifera*. Flowers pinkish, medium-sized, 7-9 mm diameter. Lateral sepals wide-spread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. saccifera*. Labellum three-lobed, equal in size, resembling *G. conopsea*, marked with purple stripes resembling *D. saccifera*. Spur elongated, twice as ovary, pointed similar to *G. conopsea*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited Herbarium of the Botanical Garden Bucharest: ***BUC 410366.*** **Proposed conservation status:** Endangered (EN).



Garay & H.R. Sweet, 1969

I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza fuchsii*; **B., E., H.** - × *Dactylodenia heinzeliana*; **C., F., I.** - *Gymnadenia conopsea*. Photos B., E., H. © Remus Dulugeac, 01 July 2023, Argeș County; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park.

Hybrid formula: *D. fuchsii* × *G. conopsea.* **Discovered:** 1 July 2023, Remus Dulugeac. **Locus classicus:** Argeș County. **Flowering time:** June-July. **Native to:** Austria, Belgium, Czechoslovakia, France, Germany, Great Britain, Ireland, Netherlands, Poland, Switzerland, Yugoslavia, Romania. **Description:** Stem (epigeal), 10(12) cm, erect, vivid green, purple-tinged at the tip, resembling *D. fuchsii*. 2 basal leaves, 2-3 cauline leaves, purple-pigmented, arched, oval-acuminate resembling *D. fuchsii*. Upper cauline leaf, vivid green, narrow-

lanceolate, non-pigmented. Inflorescence dense raceme, less floriferous, flowers pinkish, medium-sized, 7-9 mm diameter, resembling *D. fuchsii*. Lateral sepals wide-spread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. fuchsii*. Labellum three-lobed, lateral lobes scalloped (resembling *G. conopsea*), median lobe pointed, marked with purple loops and stripes resembling *D. fuchsii*. Spur longer than the ovary, pointed downwards, resembling *G. conopsea*. **Population counts:** single individual. **Proposed conservation status:** Endangered (EN).

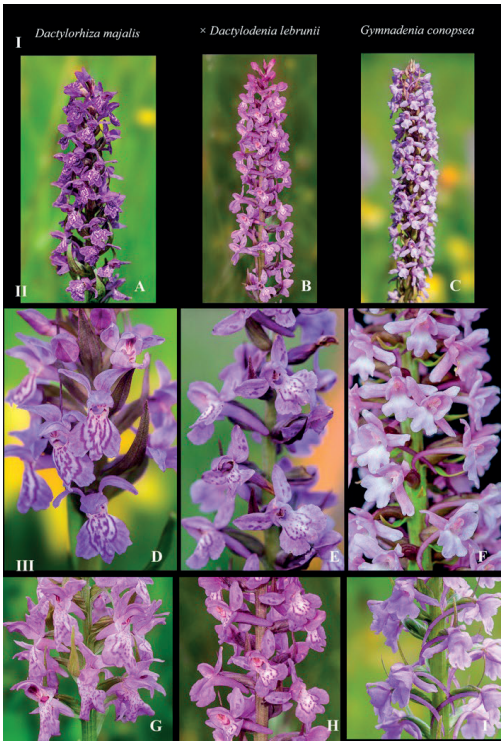


Figure 9. × *Dactylodenia lebrunii* (E.G. Camus) Peitz, 1972
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza majalis*; **B., E., H.** - × *Dactylodenia lebrunii*; **C., F., I.** - *Gymnadenia conopsea*. Photos B., H. © Lori Balogh; Photos A., C.-G., I. © Nora E. Anghelescu, 2 June 2023, Vlăhița, Harghita County

Hybrid formula: *D. majalis* × *G. conopsea*. **Discovered:** 2 June 2019, Lori & Mihaela Balogh. **Locus classicus:** Vlăhița, Harghita County. **Flowering time:** May-June. **Native to:** Austria, Czechoslovakia, France, Germany, Poland, Sweden, Switzerland Romania. **Description:** Stem (epigeal), 23(27) cm, erect, vivid green, mildly purple-tinged at tip, resembling *D. majalis*. 1 basal leaf, 2-3 cauline leaves, purple-pigmented, arched, elongate-acuminate resembling *D. majalis*. Inflorescence dense raceme, very floriferous, cylindrical-elongated, resembling *G. conopsea.* Flowers pinkish-purple, medium-sized, 5-8 mm diameter, resembling *D. majalis*. Lateral sepals roundish-ovoidal, widespread (resembling *G. conopsea*), dorsal sepal and lateral petals forming a loose hood, resembling *D. majalis*. Labellum flat, wide, three-lobed, lateral lobes scalloped (resembling *G. conopsea*), median lobe narrower, pointed, marked with dark purple loops and stripes resembling *D. majalis*. Spur equal/longer than the ovary, pointed downwards, resembling *G. conopsea*. **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410365.*** **Proposed conservation status:** Endangered (EN).

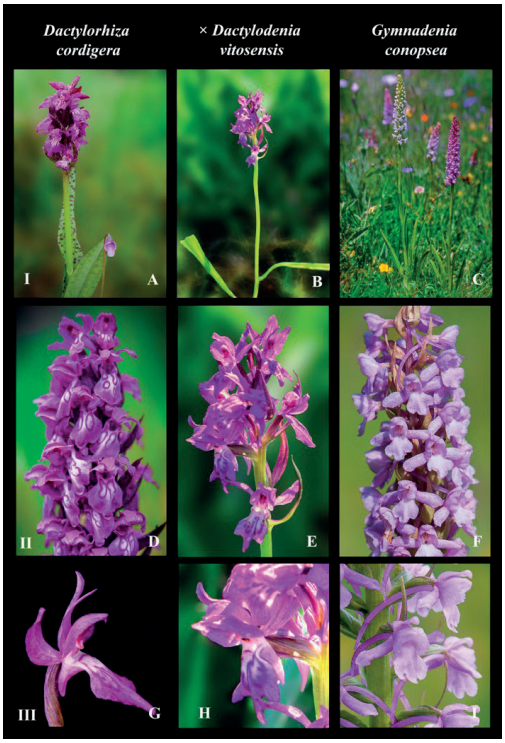


Figure 10. × *Dactylodenia* cf. *vitosensis* Jagiello, 1988
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza cordigera;* **B., E., H.** - × *Dactylodenia* cf. *vitosensis;* **C., F., I.** - *Gymnadenia conopsea*. Photos B., E., H. © Remus Dulugeac, 26 May 2018, Argeș County; Photos A., C., D., F., G., I. © Nora E. Anghelescu, 10 July 2023, Bucegi Natural Park

Hybrid formula: *D. cordigera* × *G. conopsea.* **Discovered:** 26 May 2018, Remus Dulugeac. **Locus classicus:** Argeș County. **Flowering time:** May-June. **Native to:** Bulgaria, Romania. **Description:** Stem (epigeal), 14(16) cm, erect, vivid green, spindly, resembling *G. conopsea*. 1-2 basal and 1 cauline leaves, vivid-green, non-pigmented, narrow-lanceolate, acuminate, widest in the middle, resembling *G. conopsea*. Inflorescence short, dense raceme, less floriferous, flowers purple, small sized, resembling *D. cordigera.* Lateral sepals elongated, reflexed to

spreading, resembling *D. cordigera*. Dorsal sepal and lateral petals forming a loose hood, resembling *D. cordigera*. Labellum three-lobed, very similar in shape and labellar markings to *D. cordigera.* Spur rather more elongated than that of a typical *D. cordigera*, with a mild resemblance to *G. conopsea*. This unique individual is still under study. Its full identification is still to be confirmed, since, except for the leaves and possibly the lengths of the spur, this hybrid resembles more a *D. cordigera* individual with abnormally non-pigmented, elongated leaves. **Population counts:** single individual. **Proposed conservation status:** Endangered (EN).

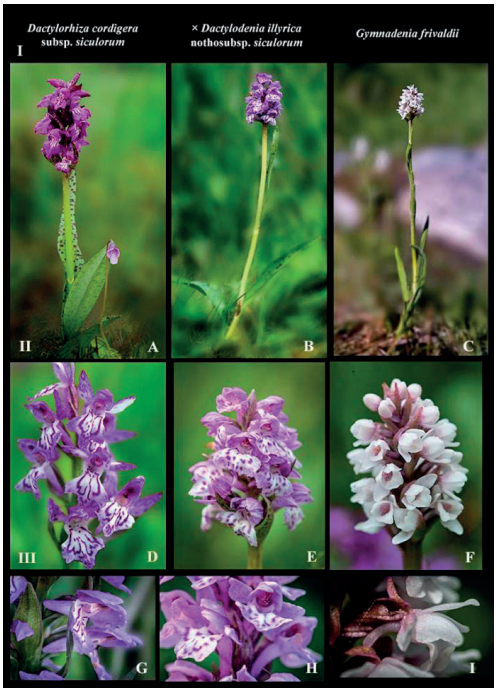


Figure 11. × *Dactylodenia illyrica* nothosubsp. *siculorum* L.Balogh & Mih.Balogh, N.Anghelescu, N. Kigyossy, 2023 nothosubsp. *nov*.
I. Full plants in their natural habitats; **II.** Inflorescences details**; III.** Individual flower detail; **A., D., G.** - *Dactylorhiza cordigera* subsp. *siculorum***; B., E., H.** - × *Dactylodenia illyrica* nothosubsp. *siculorum*; **C., F., I.** - *Gymnadenia frivaldii*. Photos © Nora E. Anghelescu, 9 June 2023, Harghita Mădăraș, Harghita

Hybrid formula: *D. cordigera* subsp. *siculorum* × *G. frivaldii***. Discovered:** 20 june 2022, Lori & Mihaela Balogh. **Locus classicus:** Harghita Mădăraș, ROSCI00090. **Flowering time:** June-July. **Native to:** Romania, endemic, ***nothosubsp. nov***. **Description:** Stem (epigeal), 10(12) cm, erect, vivid green, spindly, resembling *G. frivaldii*. 1 basal and 1-2 cauline leaves, vivid green, purple-pigmented with minute roundish-elongated, acuminate, purple-brownish maculae, wider toward the middle, resembling *D. cordigera* subsp. *siculorum*. Inflorescence short, dense raceme, resembling *G. frivaldii* in shape and size. Flowers small, pinkish, with flat labellum, wider than long, whitish in the middle, pink-purple towards the margins, marked with dark-purple dots, resembling *D. cordigera* subsp. *siculorum*. Lateral sepals spreading, resembling *G. frivaldii*. Hood flat and tight resembling *G. frivaldii*. Spur elongated-cylindrical, purplish, resembling *D. cordigera* subsp. *siculorum*. Flower bracts, wide, triangular, keeled, marked with purple-black maculae resembling *D. cordigera* subsp. *siculorum*. **Population counts: *Nothopopulation of two hybrids***. **Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410364***. **Proposed conservation status:** Endangered (EN).

Figure 12. × *Pseudadenia vitosana* (H. Baumann) O. Gerbaud & W. Schmid, 1999
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Gymnadenia frivaldii*; **B., E., H.** - × *Pseudadenia vitosana*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis*. Photos © Nora E. Anghelescu, 1 July 2023, Harghita Mădăraș, Harghita County

Hybrid formula: *G. frivaldii* × *P. albida* subsp. *tricuspis*
Discovered: 20 Iunie 2022, Lori & Mihaela Balogh. **Locus classicus:** Harghita Mădăraș, ROSCI00090. **Flowering time:** June-July. **Native to:** Albania, Bulgaria, Yugoslavia, Romania. **Description:** Stem

(epigeal), 15-30 cm, erect, vivid green, spindly, resembling *P. albida* subsp. *tricuspis*. 1 basal and 4-6 cauline leaves, vivid green, keeled, wider towards the upper half, resembling *P. albida* subsp. *tricuspis*. Upper cauline leaves sheathing the stem, resembling *G. frivaldii*. Inflorescence elongated-cylindrical, floriferous raceme, resembling *P. albida* subsp. *tricuspis*. Flowers small, whitish-yellowish, with flat, three lobed-labella, with lobes near equal, resembling *P. albida* subsp. *tricuspis*. Lateral sepals spreading horizontally, the hood flat and tight resembling *G. frivaldii*. Spur intermediate, whitish, nectariferous. **Population counts:** ***Nothopopulation of nine hybrids***. **Herbarium voucher specimen:** deposited at the Herbarium Botanical Garden Bucharest: ***BUC 410374***. **Proposed conservation status:** Endangered (EN).

Figure 13. × *Pseudadenia schweinfurthii* (Hegelm. ex A. Kern.) P.F. Hunt, 1971
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Gymnadenia conopsea*; **B., E., H.** - × *Pseudadenia schweinfurthii*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis.* Photos © Nora E. Anghelescu, 1 July 2023, Harghita Mădăraș, Harghita County

Hybrid formula: *G. conopsea* × *P. albida* susp. *tricuspis*

Discovered: 20 June 2023, Nora E. Anghelescu. **Locus classicus:** Harghita Mădăraș, ROSCI00090. **Flowering time:** June-July. **Native to:** Austria, Czechoslovakia, Germany, Norway, Sweden, Switzerland, Romania. **Description:** Stem (epigeal), 25-35 cm, erect, vivid green, spindly, resembling *G. conopsea*. 1 basal and 3-4 cauline leaves, vivid-green, keeled, narrower, acuminate, resembling *G. conopsea*. Inflorescence cylindrical, floriferous raceme, resembling *P. albida* subsp. *tricuspis*. Flowers small, yellowish-pink (resembling *G. conopsea*), with flat, three lobed-labella, with lobes nearly equal, tooth-like, resembling *P. albida* subsp. *tricuspis*. Lateral sepals spreading horizontally, the hood flat and tight resembling *G. conopsea*. Spur intermediate, whitish, nectariferous. **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium of Botanical Garden Bucharest: ***BUC 410373***. **Proposed conservation status:** Endangered (EN).

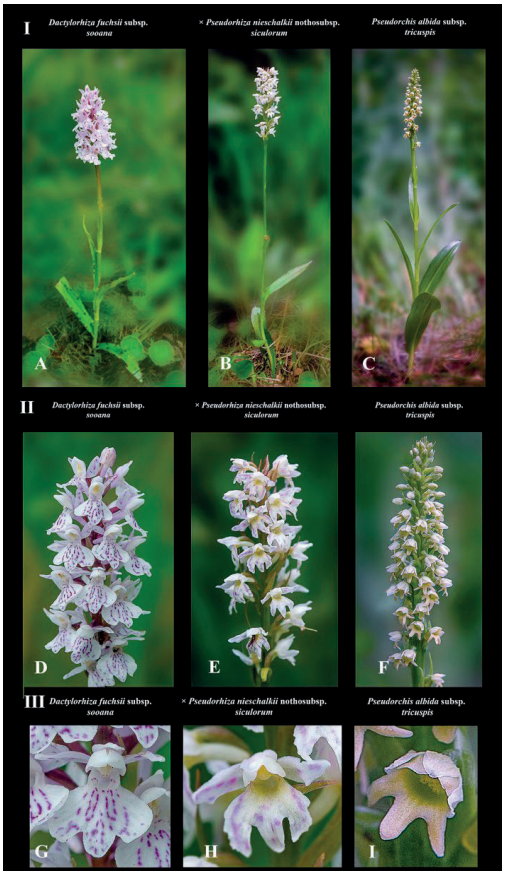


Figure 14. × *Pseudorhiza nieschalkii* nothosubsp. *siculorum* H. Kertész & N. Anghelescu, 2020
I. Full plants in their natural habitats; **II.** Inflorescences details; **III.** Individual flower detail; **A., D., G.** - *Dactylorhiza fuchsii subsp. sooana*; **B., E., H.** - × *Pseudorhiza nieschalkii* nothosubsp. *siculorum*; **C., F., I.** - *Pseudorchis albida* subsp. *tricuspis*. Photos © Nora E. Anghelescu, 3 July 2020, Harghita Mădăraș.

Hybrid formula: *D. fuchsii* subsp. *sooana* × *P. albida* subsp. *tricuspis*. **Discovered:** 30 June 2020, Hajnalka Kertész. **Locus classicus:** Harghita Mădăraș, ROSCI00090. **Flowering time:** June-July. **Native to: Romania, *endemic***. **Description:** Stem (epigeal), 28(29) cm, erect, vivid green, spindly, resembling *P. albida* subsp. *tricuspis*. 1 basal leaf, 2-3 cauline, unspotted leaves, resembling *P. albida* subsp. *tricuspis*. Upper cauline leaf bract-like, resembling *D. fuchsii* subsp. *sooana.* Inflorescence short, dense raceme, resembling *D. fuchsii* subsp. *sooana* (De Angelli & Anghelescu, 2020). Flowers small, yellowish-white, with flat, three-lobed labellum with three equal lobes (length and width), resembling *P. albida* subsp. *tricuspis*. Lateral sepals spread laterally, white with pinkish dots and stripes, resembling *D. fuchsii* subsp. *sooana.* Labellum and lateral sepals are marked with purple-pinkish dots and lines, resembling *D. fuchsii* subsp. *sooana.* The white background of the tepals and labellum resembles *D. fuchsii* subsp. *sooana.* The hood is tight, formed of dorsal sepal and lateral petals resembling *D. fuchsii subsp. sooana* (Anghelescu *et al.,* 2021a). **Population counts:** single individual. **Herbarium voucher specimen:** deposited at the Herbarium of the Botanical Garden Bucharest: ***BUC 410375***. **Proposed conservation status:** Endangered (EN).

CONCLUSIONS

The genetic diversity within a species or population is the outcome of long-term evolutionary processes and forms the foundation for its survival and adaptability (Soltis & Soltis, 2009). Various species exhibit varying degrees of genetic diversity and variation, allowing them to adapt to various environmental conditions (Wu et al., 2023). The formation of new species (a phenomenon known as speciation), is primarily driven by the development of the reproductive isolating barriers that gradually diminish and ultimately prevent significant gene flow between distinct lineages (Christie et al., 2022). There is a general consensus that the speed and manner of speciation are significantly influenced by the type of isolating barriers (Coyne & Orr, 2004). In plants, these barriers are most conveniently categorized as either premating (prezygotic) or postmating (involving both prezygotic and postzygotic) isolation mechanisms (Grant, 1981; Snow, 1994).

Hybridization was demonstrated to represent a dynamic process in evolution that can lead to genetic diversity, adaptation, and the formation of new nothospecies. Novel genetic lineages occur in many, specific hybrid zones implying that hybrids are fertile and can coexist with the parental species.

These results, together with the prediction of suitable conditions for the future occurrence of these hybrid zones, highlight the importance of conserving these geographic areas as sources of novel taxonomic entities. The high frequency of hybrids suggests low efficiency of reproductive barriers in genera such as *Anacamptis* and *Ophrys* or between genera such as *Dactylorhiza, Gymnadenia* and *Pseudorchis*. Given that a high number of viable and fertile hybrid seeds were produced, hybridization generated several genetic novelties in all hybrid zones.

Our studies highlight the complex relationships between different species and their genetic material over geological time scales, contributing to the rich tapestry of life on Earth.

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