EPIPACTIS × *SCHMALHAUSENII* K. RICHT. *(ORCHIDACEAE)*, A NEWLY IDENTIFIED HYBRID IN SLOVENIAN FLORA

EPIPACTIS × *SCHMALHAUSENII* K. RICHT. *(ORCHIDACEAE)*, NOVO OPISANI KRIŽANEC V SLOVENSKI FLORI

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http://dx.doi.org/10.3986/fbg0112

ABSTRACT

***Epipactis* × *schmalhausenii* K. Richt. *(Orchidaceae)*, a newly identified hybrid in Slovenian flora**

This study analyzes nine specimens of the genus *Epipactis* from the *Orchidaceae* family and classifies them as *Epipactis* × *schmalhausenii* K. Richt (*Epipactis atrorubens* (Hoffm. ex Bernh.) Besser × *Epipactis helleborine* (L.) Crantz). The work provides the first detailed description of this hybrid in Slovenia. The plants were examined using the hybrid index (HI) method. We analyzed six to ten morphological plant characteristics. In addition to the traits summarized from the literature, two new characteristics related to the proportions of the flower labellum were analyzed from digital photographs and included in the study. Both of these showed high discriminatory power between the parental species. We modified the classical definition of the HI to no longer depend on the number of traits used in the analysis. The adopted trait descriptions of typical parental species were compared to six standard works on *Orchidaceae*. We did not observe any apparent contradictions, but the descriptions of some characteristics are somewhat inconsistent, which probably indicates their very high intraspecies variability. A brief assessment of potential geographical, site-related, and temporal barriers to hybridization between the two parental species in Slovenia revealed that none exist. The hybrid is likely quite common in Slovenia and has been overlooked until now, probably due to its substantial phenotypic diversity.

Key words: Orchidaceae hybrid*, Epipactis* hybrid, hybrid index, hybridization barriers, *Epipactis x schmalhausenii*, *Epipactis* × *schmalhausenii* nothosubsp. *schmalhausenii*, flora of Slovenia

IZVLEČEK

***Epipactis* × *schmalhausenii* K. Richt. *(Orchidaceae)*, novo opisani križanec v slovenski flori**

Morfološko opisujemo devet primerkov orhidej iz rodu *Epipactis* Zinn, ki smo jih prepoznali kot križance *Epipactis* × *schmalhausenii* K. Richt. (*Epipactis atrorubens* (Hoffm. ex Bernh.) Besser × *Epipactis helleborine* (L.) Crantz). Križanec je v Sloveniji podrobno opisan prvič. Rastline smo obravnavali z metodo indeksa hibridnosti (HI) na osnovi šestih do desetih morfoloških lastnosti povzetih po literaturi. V analizo smo dodali dve novi lastnosti, vezani na proporce medene ustne cvetov. Obe sta izkazali veliko diskriminatorno moč med obema staršema. Določevali smo jih iz digitalnih fotografij cvetov. Klasično definicijo HI smo preoblikovali tako, da rezultat ni več odvisen od števila opazovanih lastnosti in omogoča primerjavo križancev, ki so bili določeni na osnovi različnega števila opazovanih lastnosti. Privzete opise tipičnih lastnosti staršev smo primerjali z opisi v šestih standardnih literaturnih virih za družino *Orchidaceae*. Očitnih nasprotij nismo opazili, vendar so opisi nekaterih lastnosti nekoliko neenotni, kar verjetno kaže na njihovo veliko variabilnost. Kratka analiza morebitnih geografskih, rastiščnih in časovnih preprek za križanje je pokazala, da jih v Sloveniji ni. Križanec je v Sloveniji zato verjetno relativno pogost in je bil le zaradi velike fenotipske raznolikosti do zdaj bržkone spregledan.

Ključne besede: Kukavičevke križanci, močvirnice križanci, indeks križanja, ovire pri križanju, *Epipactis x schmalhausenii*, *Epipactis* × *schmalhausenii* nothosubsp. *schmalhausenii*, rastlinstvo Slovenije

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INTRODUCTION

Hybridization is expected in the orchid family (*Orchidaceae*) across species and genera, producing more hybrids than in any other seed plant family (Künkele & Baumann 1998). This is mainly due to poorly developed genetic and reproductive barriers between taxa. These hybrids are often fertile; under the right conditions, they cross with each other and back-cross with either parental species. After several generations, the hybrids may show an extensive range of hybridogenous origins or parental-like traits due to introgressive hybridization (Stace 1975). It is, therefore, impossible to verify and validate hybrids by comparing them to a single type specimen (holotype), nor can they be identified using dichotomous keys. Owing to their substantial phenotypic diversity, combined with the high morphological variability of the parental species — which is exceptional in the genus *Epipactis* (Delforge 2006, Łobas et al. 2021) — it is difficult to determine, based solely on morphological traits, which specimens can be attributed to hybridization and which are the result of random deviations from typical forms of parental plants.

The taxon described herein, *Epipactis* × *schmalhausenii* K. Richt., is a hybrid between the dark-red helleborine *Epipactis atrorubens* (Hoffm. ex Bernh.) Besser, and the broad-leaved helleborine *Epipactis helleborine* (L.) Crantz. In this paper, the parental species are referred to as *E. helleborine* and *E. atrorubens*, and hybrid specimens are designated as *E.* ×*schmalhausenii*.

E. ×*schmalhausenii* is one of the first hybrids of the genus *Epipactis* to be reported in botanical literature. Austrian botanist Karl Richter (1855-1891) named it after Ukrainian botanist Johannes Theodor Schmalhausen (1849-1894) – Richter (1890). In his work, the name appears only in its binomial form, without a description. Subsequently, Vollmann (1914) named the taxon (with the then still valid genus name) *Helleborine* ×*schmalhausenii* Richt., i.e., as a hybrid. Similar to other hybrids of the genus *Epipactis*, this hybrid was rarely reported in the literature. It was not mentioned in the standard work by Keller & von Soó (1930-1944), although it discussed hybrids at length. Even Willing (2020), in his comprehensive list of notable publications on the *Orchidaceae* family, spanning more than 600 pages and over 10,000 bibliographic units, did not reference the taxon *E.* ×*schmalhausenii*.

Rare papers on this hybrid from the early and mid-20th century mainly focused on descriptions of individual finds (Künkele & Baumann 1998). The authors discussed only plants that expressed hybridogenous values for most traits, if not all. For example, E. G. Camus & A. Camus (1921-1929) described it as a taxon of hybridogenous origin in all observed traits. As far as we know, Bayer (1986) was the first to discuss hybridization in the genus *Epipactis* based on multiple pre-selected morphological traits. An even more elaborate method for determining the hybrid origin of specimens from this genus was developed by Adamowski (1995). It was also used as a starting point for this article.

Literature sources provide different estimates of the frequency of this hybrid. Some authors consider it rare (Hunt, Roberts & Young 1975, Perazza & Lorenz 2013), while others think it quite common (E.G. Camus & A. Camus 1921-1929). Young (Hunt, Roberts & Young 1975) even questions the possibility of hybridization between *E. atrorubens* and *E. helleborine*. He assumes that the parental species are reproductively and ecologically isolated due to different site requirements and flowering periods and that the evidence suggesting that hybridization has ever occurred is unconvincing. He emphasizes the significant variability in both parental species and the frequent overlapping of morphological traits, which involves the risk of falsely attributing intermediate forms to hybridization. Based on his revision of herbarium specimens, he attributes most previous reports of the hybrid in the UK to *E. atrorubens*. He also notes that the taxon *E.* ×*schmalhausenii* data from Continental Europe are limited, and localities are rarely reported. In Bavaria, for example, only one locality was known at the beginning of the previous century (Vollmann 1914). Subsequent literature brought more reports on the localities of this hybrid. Bayer (1986) already listed 16 localities in Bavaria alone. Peitz (1972) reported localities in eight European countries six years later. Today, the taxon is known from more than 15 European countries. Bayer (1986) and Jakubska-Busse & Gola (2010) believe it to be a common hybrid within the genus *Epipactis*. Adamowski & Conti (1991) listed localities with massive occurrences of the taxon.

The hybrid *E.* ×*schmalhausenii*, on the other hand, was first described in Poland in 1988 (Bernacki 1988) and in Romania only in 2018 (Dulugeac et al. 2019). To our knowledge, the only mention of it in Croatia is by Kranjčev (2005), who reported it for the North and Central Velebit Mts. but without a description or detailed information on the localities. The taxon is not included in the University of Zagreb's Flora Croatica Database (FCD) – Nikolić (2004-2024). As far as we know, there are no detailed published descriptions of this hybrid in Croatia (in lit. Nikolić, 2022; in lit. Rottensteiner, 2022; in lit. Hertel, 2022). Rottensteiner (2014: 624) reported the taxon for Istria, but only tentatively. In his extensive chapter on hybrids, Perko (2004: 261) reported only two finds in the Gailtal Alps in Austrian Carinthia. The hybrid was not included in the chorological atlas of Friuli Venezia Giulia in Italy (Poldini 2002) nor the database of Italian flora Acta Plantarum (Anonymous 2021). The only reference to this hybrid for Slovenia's neighboring region of Friuli Venezia Giulia is by Perazza & Lorenz (2013), also mentioned in Martini et al. (2023: 384): RR: Alt. 700-800 m. Nota: Perrazza & Lorenz (2013): S. Anna di Carnizza in V. Uccea (MTB 9645/4).

According to the available literature, the hybrid *E.* ×*schmalhausenii* occurrence in Slovenia has only been documented once. The photograph of its inflorescence, the find date, and the locality were published in Dolinar (2015: 172). This publication is also the only reference to this hybrid used by Urbanek Krajnc et al. (2020) in their overview of the Slovenian taxa from the group of *E. helleborine* s. lat.

This study aimed to prove the existence of *E.* ×*schmalhausenii* in Slovenia, provide its morphological characteristics, and estimate the frequency of its occurrences based on geographical, site-related, and temporal barriers to hybridization.

METHODS AND MATERIALS

2.1 Assessment of potential barriers to hybridization

The opinion in the literature (Hunt, Roberts & Young 1975) casts doubt on the possibility of hybridization between *E. atrorubens* and *E. helleborine*, at least in certain regions. Given that there are still countries in our vicinity where the hybrid has yet to be described, we wanted to briefly investigate whether there might exist broader geographical, site-related, or temporal barriers to hybridization in Slovenia.

To assess the geographical conditions for hybridization on a broader scale, we studied the occurrence of both parental species based on the relevés obtained from the FloVegSi database (T. Seliškar, Vreš & A. Seliškar 2003). The aim was to determine the extent to which both species occur within the same MTB quadrant. Data from FloVegSi were plotted onto a distribution map, shown in the Results (Fig. 5).

Site-related barriers may also prevent hybridization. Both parental species have significantly different site-related needs (Adamowski 1995, Ravnik 2002, Delforge 2006). The *E. helleborine* occurs in moist, predominantly shady sites with deep, nutrient-rich soil. *E. atrorubens*, on the other hand, usually occurs in dry, sunlit sites with shallow, often skeletal, nutrient-poor soils. This difference may present an ecological barrier to hybridization if the distances between both typical sites are too long for pollinators. In the Results section, we discuss information from the literature and our experiences with both types of sites.

In general, *E. atrorubens* and *E. helleborine* bloom about two weeks apart, with *E. atrorubens* being the first to flower. To estimate how much this delay might prevent hybridization in Slovenia, we analyzed the flowering period of both parental species based on about 2600 observations from 1986 to 2020 in the FloVegSi database. We assumed that most data relates to flowering specimens but not exclusively to them. Therefore, we attempted to eliminate data on non-flowering plants. The flowering season depends on the site's elevation and weather conditions in a specific year. Regrettably, the number of database inputs is still too small to allow a statistically meaningful comparison of the average flowering times in cells defined by a selected elevation and year of observation. To increase the numerus of comparable observations, the data were processed as follows.

First, we excluded the relevés of all evidently non-flowering plants by ignoring the inputs between early September and late April. We then arranged the inputs across seven 200-meter elevation bands ranging from 200 to 1,600 m and, through linear regression, determined the average flowering time in a band as a function of elevation. The linear regression of these averages by elevation showed that the flowering time was delayed by approximately six days for every 200 meters of elevation. The relationship is convincingly linear ($0.85 < R^2 < 0.95$) and almost identical for both species. We were thus able to "project" observations from different elevations to the 600–800-meter elevation band, for which the database also holds the largest number of inputs. In the last step, we again excluded the plants likely to be non-flowering by considering only inputs from May 15 to July 31 for *E. atrorubens* and from June 1 to August 15 for *E. helleborine*. This way, we minimized the number of inputs from non-flowering plants that could compromise the comparison of both parental species.

A comparison of the average flowering times across individual years confirmed that varying weather conditions during those years also significantly impact flowering. The earliest and latest average flowering times for both species from 1986 to 2020 differ by about a month.

This influence, unlike altitude, is stochastic, and its effect on flowering is unpredictable. Therefore, we can only compare data for specific years. We have focused on the results from 2014, 2018, and 2020, as these years have the highest number of recorded observations in the database. The processed data on flowering times is summarized in box plot diagrams that illustrate the relationship between parental plants. The diagrams are presented in the Results (Fig. 6).

2.2 Studied plants

2.2.1. Tentative hybrids

The paper describes nine plants we have found and identified as hybrids. The photographs of the specimen we saw in the Bovec Basin were first published online in 2011 (Anonymous 2024) but under the incorrect name of the 'unusual' *E. atrorubens*. The specimen, whose picture was published in the book of Dolinar (2015), was found in 2014. Subsequent finds were from the vicinity of Kamnik in 2015, the Upper Soča Valley (two), Gorenjska, and the Polhov Gradec Hills (one each) in 2020 and 2021, as well as one from Lake Gradišče near Lukovica in 2020. In 2022, we found two more specimens in the Upper Soča Valley. Five specimens were analyzed in situ; the characteristics of the others were analyzed based on extensive photographic material. The localities of the investigated hybrids are listed below and arranged by the date of relevés.

1. "Javoršček – 2011" (Figure 7A):
   9647/4 (33T UM39) 46,3259 N, 13,5872 E (WGS84). Slovenia, Bovec Basin, the northern slope of Mt. Javoršček; on the side of a forest road, 680 m a.s.l.; Leg. A. Trnkoczy 20. 6. 2011, det. A. Trnkoczy 10. 8. 2020, author's photographs: ID Bot_526/2011_IMG5473, published by CalPhotos, Berkeley Natural History Museums; FloVegSi relevé 286634.

2. "Trnovec – 2014" (Figure 7B):
   9952/1 (33T VM40) 46,09 N, 14,35 E. Slovenia, Polhov Gradec Hills, Trnovec; the edge of a mixed forest, 530 m a.s.l.; Leg. & Det. B. Dolinar 14. 7. 2014; author's photograph; published in Dolinar (2015: 172); FloVegSi relevé 253568.

3. "Kamniška Bistrica – 2015" (Figure 7C):
   9653/4 (33T VM63) 46,3431 N, 14,5725 E (WGS84). Slovenia, Gorenjska, Kamniška Bistrica; clearing above the glacial boulder "Lepi kamen," edge of a clearing in a mixed forest, 910 m a.s.l.; Leg. A. Mihorič 16. 7. 2015, Det. A. Mihorič & A. Trnkoczy 20. 8. 2020; author's photographs; FloVegSi relevé 294626.

4. "Lake Gradišče – 2020" (Figure 8D):
   9854/1 (33T VM71) 46,1595 N, 14,7133 E (WGS84). Slovenia, Gorenjska region, Lake Gradišče; a shallow ditch by a forest road in a mixed forest, 340 m a.s.l.; Leg. A. Mihorič 11. 7. 2020, Det. A. Mihorič & A. Trnkoczy 10. 11. 2020; author's photographs; FloVegSi relevé 294627.

5. "Lower Trenta – 2020" (Figure 8E):
   9648/1 (33T UM39) 46,3601 N, 13,7023 E (WGS84). Slovenia, Soča Valley, Lower Trenta Valley, Na Melu, at the edge of a predominantly beech forest bordering a former pasture, 595 m a.s.l.; Leg. & Det. A. Trnkoczy 3. 8. 2020; author's photographs: ID Bot_1322/2020_DSC05026, published by CalPhotos, Berkeley Natural History Museums; FloVegSi relevé 28663.

6. "Lower Trenta – 2021" (Figure 8F):
   9648/1 (33T UM39) 46,3601 N, 13,7019 E, (WGS84). Slovenia, Soča Valley, Lower Trenta Valley, Na Melu, the edge of a predominantly beech forest bordering a former pasture, 600 m a.s.l.; Leg. & Det. A. Trnkoczy 23. 6. 2021; author's photographs: ID Bot_1386/2021_DSC3722, published by CalPhotos, Berkeley Natural History Museums; FloVegSi relevé 28663.

7. "Osolnik – 2021" (Figure 9G):
   9852/3 (33T VM40) 46,1351 N, 14,3510 E. Slovenia, Gorenjska region, Sora, Osolnik, mixed forest, 665 m a.s.l.; Leg. & Det. B. Dolinar 13. 7. 2021; author's photographs; FloVegSi relevé 286299; herbarium LJS, sheet number 12736.

8. "Lower Trenta – 2022/1" (Figure 9H):
   9648/1 (33T UM39) 46,3558 N, 13,6992 E, (WGS84). Slovenia, Soča Valley, Lower Trenta Valley, left bank of the Soča River downstream from the Matevž farmhouse, Trenta 1, riparian forest, *Salix eleagnos*, *Picea abies* dominant; 525 m a.s.l.; Leg. & Det. A. Trnkoczy 24. 6. 2022; author's photographs: ID Bot_1474/2022_DSC8445; FloVegSi relevé 294628.

9. "Lower Trenta – 2022/2" (Figure 9I):
   9648/1 (33T UM39) 46,3559 N, 13,6995 E (WGS84). Slovenia, Soča Valley, Lower Trenta Valley, left bank of the Soča River downstream from the Matevž farmhouse, Trenta 1, edge of a riparian forest, *Salix*

eleagnos, Picea abies dominant; 525 m a.s.l.; Leg. & Det. A. Trnkoczy 25. 6. 2022; author's photographs: ID Bot_1474/2022_DSC00340; FloVegSi relevé 294629.

The localities of the finds are shown in Figure 1A.

2.2.2 Parental plants

In the hybridity analysis, we introduced two new characteristics of flowers that describe labellum proportions. Since we could not find any literature data on the typical values of these proportions and their variability, we first had to determine these characteristics for both parental species.

To achieve this, we studied 509 flowers from 79 parental species, averaging just over six flowers analyzed per plant. The parental species were categorized into two groups: the "Upper Soča Valley" (SV), which includes plants from the Trenta Valley, Bovec Basin, and surrounding areas, and the "Slovenian" (SI), which consists of those from other regions of Slovenia. We aimed to determine whether the two groups exhibited any differences in these traits.

In the "Slovenian" group of parental plants, we measured 99 flowers on 20 specimens of *E. atrorubens* from 14 different localities and 130 flowers on 24 specimens of *E. helleborine* from 17 localities. In the "Upper Soča Valley" group, we measured 185 flowers on 20 specimens of *E. atrorubens* and 95 flowers on 15 specimens of *E. helleborine*. The plants originate from most phytogeographical regions of Slovenia. Their localities are shown in Figure 1B.

The flowers used in the analysis were selected visually. In each plant, we measured all well-developed flowers that were fully open but showed no signs of withering, irrespective of their position in the inflorescence. Measuring only the flowers with present and fully developed pollinia on the same section of the inflorescence (as traditionally practiced in descriptive morphometry of taxa) would result in a smaller variance of the measured values. However, the number of analyzed flowers would be drastically diminished; hence, the sampling error of the samples would be significantly worsened.

2.3 Hybridity assessment

2.3.1 Hybrid Index Method

The hybridity of the analyzed plants was assessed using the hybrid index method (HI). Developed in the 1930s (Anderson 1936), the method evaluates a preselected set of n morphological traits that are supposed to best discriminate between parental species. Each trait can be assigned to one of three categories: resembling the first parental species, intermediate, or resembling the second parental species. Each category is assigned a weight W. The arithmetic sum of the weights of n observed traits is the HI.

$$HI = \sum_{k=1}^{n} W(k)$$

Based on this definition, the numerical value of HI depends on the number of observed characters n and, therefore, does not allow for direct comparison of HI of plants assessed on a different number of traits. Unfortunately, this is something we are often compelled to do in practice. To avoid this inconvenience, we reformulated the hybrid index HI into the degree of hybridity DH, expressed independently of the number of observed traits.

The HI was transformed into the DH in the following manner. The HI of the plants corresponding to the first parental species in all observed traits was marked HI1, and the HI of those corresponding to the second parental species was marked HI2. The established hybrid index of the studied plant was marked HIX. The DH is defined as:

$$DH(\%) = abs\left(abs\left(\frac{HIX - HI1}{HI2 - HI1} * 100 - 50\right) * 2 - 100\right)$$

This way, the value DH = 0% is attributed to the specimen that completely matches either parental species. In such a case, there is no indication of hybridization. DH = 100%, on the other hand, indicates a specimen that is an ideal intermediate form between both parental species, either in terms of intermediate traits or the retained traits of both parental species. Such a specimen demonstrates maximum hybridity, where both parental species are equally represented. Intuitively, this presentation better illustrates hybridity and allows for a direct comparison of plants, even if the DH is not calculated based on the same number of traits. Numerical values of the DH, however, cannot be directly interpreted as a statistical probability that we are dealing with a hybrid.

Of course, the question immediately arises at what threshold value of DH a plant can be declared a hybrid with reasonable reliability. Generally speaking, the established DH is proportional to an individual's likelihood of being a hybrid. However, the DH "measures" both parental phenotypic deviations and deviations due to (potential) hybridization. We can infer hybridity only when dealing with many substantial deviations that cannot reasonably be attributed solely to chance due to the variability of the parental species. The threshold value, of course, remains a matter of discussion.

Also, even a specimen with a very low DH may still be a hybrid that, through repeated backcrossing to one of the parental species, has become very similar to it. Alternatively, it may also be a parental species that similarly deviates from its typical form. When deviations from the usual form are minor, and DH is low, it is impossible to distinguish between the two possibilities based solely on the morphology of an individual.

This paper assumes the following criterion: a specimen is a hybrid when its DH equals or exceeds 50%. It seems improbable that concurrent deviations from typical traits of the parental species in more than half of them could be attributed solely to their variability and chance.

The chosen criterion was very similar to Bayer's (Bayer 1986). Bayer's first and second conditions for hybrid origin (see next section) numerically correspond to our degree of hybridity DH = 56%. His condition is, therefore, slightly stricter than ours. Adamowski (1995) applied a less stringent condition, identifying the observed plant as a hybrid already at HI = 10, which, considering eight investigated traits, corresponds to our DH = 38%. However, his decision to adopt a less stringent condition is justified because he analyzed a large population of hybrids at a single site where the parental species were hardly present. Based on this, our choice of the threshold value for hybridization seems reasonable.

2.3.2 Selection of the studied morphological traits

The selected traits for calculating the DH are critical to success. They should be as different from each parental species as possible and have as little intraspecies variability as possible. To enhance the reliability of results, they should not be statistically correlated. Quantifiable traits increase objectivity. The more these conditions are satisfied, the greater the discriminatory power of a trait.

According to the available literature, there is no quantitative data on the variability of morphological traits for both parental species (Łobas et al. 2021). The available literature describes only descriptive, qualitative assessments for some of them. It is, therefore, hardly surprising that the selection of traits varies considerably among different authors addressing the subject.

Bayer (1986) discussed hybridization in the genus *Epipactis* in terms of eight morphological traits, focusing primarily on reproductive plant parts. He justified his decision on whether a specimen was a hybrid based on the following logical conditions: a plant is a hybrid if: a) the number of intermediate traits is five or larger, or (if this condition is not satisfied), b) the difference in the number of retained traits attributed to the first parental species and the number of those attributed to the other parental species (i.e., traits other than intermediate ones) does not exceed three. The latter condition reflects the expectation that a hybrid expresses a more or less balanced share of the parental species' typical traits.

Jakubska-Busse & Gola (2010) focused on identifying effective distinguishing traits in plants' vegetative parts, explicitly analyzing leaf morphology and anatomy. Although the researcher did not apply hybrid indices in numerical terms, she clearly stated that analyzing a set of traits was the only way to determine the hybrid nature of this taxon.

Adamovski (1995) analyzed eight traits, focusing on five related to vegetative parts and three concerning reproductive parts. He selected these traits based on standard literature descriptions and his field experience. To quantify the HI, he assigned a weight of one to traits typical of *E. helleborine*, three to traits typical of *Epipactis atrorubens*, and two for traits that fell between the two species. As a result, the HI could range from 8 to 24. An "ideal" hybrid, characterized by a balanced blend of traits, would have an HI of 16. This could represent an individual displaying all eight traits in the intermediate form, one exhibiting four of each parent's typical characteristics, or any other combination of traits where the sum of the weights equals 16.

There are also more complicated ways to assign weights or use more than three categories. However, given the current lack of quantitative knowledge regarding the variability of parental species traits, this does not seem to add to this method's credibility. Baker (1947), for example, criticized the use of such convoluted methods, noting that they are likely to fail.

This paper classifies the investigated traits into three categories using the same weights as Adamovski (1995) and the same eight characteristics. They are shown in Table 1 (rows 1-8). We only defined some of them in more detail or quantitatively. For example, we aligned the description of leaf forms with the standard definitions (Fischer, Adler & Oswald 2005: 79; J. G. Harris & M. W. Harris 1953). We also added two quantitative traits of flower labellum to the list – the hypochile (more precisely, the maximal width of nectarium) to epichile width ratio (H/E) and the ratio of the width of the transition between the hypochile and epichile to the epichile width (P/E) (Table 1, rows 9 - 10).

Due to limited data availability, we used less than ten traits for some of the analyzed specimens. For instance, we could not assess the flower fragrance when only photographic material was available. Similarly, we could not measure certain vegetative traits for two plants with limited photographic records. We usually analyzed nine or ten traits but never assessed fewer than six.

2.4 Description of the morphological traits studied in the analysis

In this chapter, we outline the selected traits of both parental species. Their definitions can be found in Table 1, and most are visually represented in Figure 2. We compared our adopted descriptions with those from five recent and one older standard works on *Orchidaceae*: Baumann, Künkele & Lorenz (2006), Delforge (2006), Künkele & Baumann (1998), Kretzschmar (2008), Lauber, Wagner & Gygax (2021), and Vollmann (1914). The goal was to assess how frequently these sources describe our selected traits, how consistent their descriptions are, and how well they align with ours. We hypothesized that traits described more regularly and consistently possess a higher discriminatory power.

2.4.1 Shape of the Lower Leaves (LVSH)

Differences in the shape of the lower leaves between typical *E. helleborine* and *E. atrorubens* are immediately noticeable (Fig. 2A). However, literature sources and our experience confirm significant variability in this trait, particularly in *E. atrorubens*. Since leaf shape and size vary according to their position on the stem, selecting leaves for analysis influences the assessment. Bayer (1986) studied the "middle leaves," Jakubska-Busse & Gola (2010) examined only the third leaf from the base, and Adamowski (1995) focused on the "lower leaves" without further clarification. For this research, we consider the lower third of leaves (rounding to the nearest whole number), excluding the lowest, smallest, and nearly always orbicular leaf in both parental species. Typically, we analyzed the second, third, and fourth leaves from the ground; in some smaller specimens, we only analyzed the second and third. The average length-to-width ratio of the leaves examined indicated whether the trait was parental-like or intermediate. In borderline cases, we also took into account the position of the widest part of the leaf lamina.

Our descriptions of this trait are only partially consistent with those in the standard sources. Künkele & Baumann (1998) and, to an even greater extent, Vollmann (1914) allow for more intraspecies variability than other sources. Vollmann (1914) describes leaves of both parental species with nearly identical wording, except for allowing the additional nearly orbicular shape attributed to *E. helleborine*. These inconsistencies likely support the presumed substantial variability of this trait. In our experience, however, leaf shape provides a relatively straightforward basis for determining whether the trait resembles one parent species or the other or is of intermediate form.

2.4.2 Leaf arrangement (LVAR)

Leaf arrangement is another immediately recognizable trait that distinguishes the parental species (Fig. 2A). In typical *E. atrorubens*, the leaves are distichous and nearly in the same plane, while they are spiral in *E. helleborine*. Adamowski (1995) assesses this trait by measuring the average angle Ø between leaves. He assigns Ø > 60° to *E. helleborine* and Ø < 30° to *E. atrorubens*. An angle between 30° and 60° suggests a possible hybrid origin. Unfortunately, his article does not clearly define angle Ø. In this paper, we define "angle Ø" as the absolute value of the angle between two consecutive leaves if -90° < Ø < 90°, or the absolute value of the difference between 180° and the angle measured between two successive leaves if -90° > Ø > 90°. The trait LVAR represents the average of angles between all consecutive pairs of stem leaves, quantifying the overall deviation from the strict distichous leaf arrangement.

Our description of this trait aligns with standard descriptions. However, only two sources detail this trait for both parental species, while Vollmann (1914) doesn't mention it.

Based on our experience, the arrangement of stem leaves successfully distinguishes between the two parental species in most cases, with one exception. We often observe *E. atrorubens* individuals showing significant deviations from a distichous leaf arrangement. Other authors have reported similar observations. Whether these cases are linked to very high intraspecies variability of this trait in *E. atrorubens* or arise from past introgressive hybridization (Stace 1975) remains an open question.

2.4.3 The ratio of the length of the uppermost to the last but one internode (INTR)

The ratio r of the lengths of the uppermost (the distance between the attachment of the uppermost stem leaf and the attachment of the lowermost bract) to the last but one internode (the distance between the attachments of the upper two stem leaves) is an easily discernible and quantitatively determinable trait (Fig. 2B). Following Adamowski (1995), we adopted the condition r > 2 for *E. atrorubens* and translated his qualitative description of "more or less equal lengths of both internodes" for *E. helleborine* into r < 1.2.

The sources against which we compared our description of this trait coincide with our definition. However, they describe the trait indirectly as "a large gap between leaves and inflorescence" or "leaves grouped at the base of the stem" for *E. atrorubens* and "stem leaves evenly arranged" for *E. helleborine*. Even though this trait is not quantitatively referenced in standard literature, Bayer (1986) and Adamowski (1995) included it in their hybrid index calculations.

2.4.4 Stem pubescence in the inflorescence (STPB)

The pubescence of the inflorescence axis noticeably differs between the typical parental species (Fig. 2C); however, this trait demonstrates substantial variability. Following Adamowski (1995), we characterize it as "poor" in *E. helleborine* and "strong" in *E. atrorubens*. This is undeniably true for 'typical' individuals. Nevertheless, pubescent *E. helleborine* and *E. atrorubens*, which have no conspicuously strong, dense, and bright hairs (on a significantly darker purple stem), seem quite common. Bayer (1986) made a similar observation and attributed it to potential introgressive hybridization.

All standard sources used to compare our approach describe this trait of both parental species, but they do so in different ways. While the descriptions for *E. atrorubens* are consistent with one another and with our findings, the descriptions for *E. helleborine* differ significantly. For example, Delforge (2006) identifies strong pubescence as a characteristic trait of the entire *E. helleborine* group, including *E. helleborine* (L.) Crantz, whereas Vollmann (1914) hardly distinguishes between the parental species, describing both as downy and adding "softly" for *E. atrorubens*.

2.4.5 Stem color in the inflorescence (STCO)

The stem color in the inflorescence clearly differentiates typical parental species (Fig. 2C). Our descriptions follow Adamowski (1995), who noted it as "green" in *E. helleborine* and "purple" in *E. atrorubens*. Adamowski (1995) did not specify which part of the stem he analyzed. We frequently encounter plants where the stem color at the base differs significantly from that in the inflorescence. Therefore, defining which part of the stem is being considered is important. Following Lauber, Wagner & Gygax (2021), we decided to analyze the color of the stem in the inflorescence.

The sources we compared with our descriptions do not align well with the adopted definition (green and purple). Only Lauber, Wagner & Gygax (2021) and Baumann, Künkele & Lorenz (2006) agree on 'green' for *E. helleborine*, and there is only one instance of agreement on 'purple' for *E. atrorubens,* specifically Künkele & Baumann (1998). Other sources permit a broad spectrum of colors, especially for *E. atrorubens*. Some even suggest that the primary stem color of *E. atrorubens* is green, possibly tinged with red-brown or purple. This likely indicates a very high intraspecies variability of this trait, at least in *E. atrorubens*.

2.4.6 Flower color (FLCO)

The color of the flowers is undoubtedly the most noticeable trait in the field. Although it clearly distinguishes between the parental species (Fig. 2D), it also shows variability, especially in *E. helleborine*, sometimes to an exceptional degree (Kretzschmar 2008: 125, Arbeitskreis Heimische Orchideen Bayern 2014). However, such extreme cases are rare. All cited literature sources report this trait. The descriptions align with ours without significant differences. Generally, the decision regarding the category to which it belongs is relatively straightforward.

2.4.7 Flower Smell (FLSM)

Smell is a highly subjective trait that strongly depends on the anthesis stage, weather conditions, and other abiotic factors. The standard sources we used to compare our descriptions refer to this trait only for *E. atrorubens*, described as having a "vanilla scent." The smell of *E. helleborine* is not mentioned in these sources. However, it has been described, for example, by Dulugeac et al. (2019) and Pantu (1915), who suggested it smells like members of the valerian family (*Valerianaceae*), while *E. atrorubens* is characterized as clove-scented (*Syzygium aromaticum*).

Adamowski (1995) described this trait as "unsmellable" in *E. helleborine* and "distinct, similar to that of lily of the valley (*Convallaria majalis*)" in *E. atrorubens*. However, based on our field experiences, we can assert that *E. helleborine* often has a detectable smell and that the distinct smell of *E. atrorubens* often does not evoke vanilla, lily of the valley, or clove. Due to these ambiguities, we have simplified the description of this trait to "none or faint" for *E. helleborine* and "distinct" for *E.*

atrorubens. Identifying this trait as intermediate remains a very challenging and subjective decision.

2.4.8 Surface structure of labellum protuberances at the base of the epichile and their transition to the epichile (ECON)

The parental species exhibit distinctly different labellum protuberances at the base of the epichile (Fig. 2E). Adamowski (1995) describes the surface of the labellum protuberances as "smooth" in *E. helleborine* and "clearly wrinkled" in *E. atrorubens*. All sources we compared to our descriptions support this trait; however, designations for *E. helleborine* vary. Only descriptions in Lauber, Wagner & Gygax (2021) and Vollmann (1914) align with "smooth," while three others label it as "wrinkled." This contradiction may be explained by the observation that, as also evident in Figure 2E, even in *E. helleborine*, the surface of the labellum protuberances is not entirely smooth. It appears as such only when compared to the much more wrinkled typical appearance of *E. atrorubens*. Baumann, Künkele & Lorenz (2006) and Delforge (2006) do not comment on this trait for *E. atrorubens*. The absence of this comparison between the two species likely accounts for the conflicting descriptions.

In our experience, it is important to consider not only the surface structure but also the transition of the labellum protuberances into the surface of the epichile. In *E. helleborine*, this transition is gradual, while in the typical *E. atrorubens*, it is abrupt, often featuring a pronounced edge. Consequently, our description of this trait encompasses both the surface structure of the labellum protuberances and their transition to the epichile. We describe these traits as "almost smooth/gradual" in *E. helleborine* and "clearly wrinkled/abrupt" in *E. atrorubens*. We find that assessing this trait in practice is not difficult.

2.4.9 Labellum Proportions (H/E and P/E)

The reliability of the hybrid index method is proportional to the number of traits observed, so we aimed to enhance it. According to Adamowski (1995), the eight traits describe more vegetative parts than reproductive parts of the plants, which is why we focused on flowers. We selected five parallel line segments to define the dimensions of flower parts or the distances between them. Figure 3 illustrates these segments.

The lengths of the line segments were measured from high-resolution digital photos using software (Photoshop and the Ruler tool). The measured distances depend on the type of camera used (sensor resolution, focal length of the lens, and types of digital files) and the spatial relationship between the camera and the object (distance from the focal plane, position, and rotation of the flowers relative to the lens's optical axis). Consequently, the numerical values of distances measured in pixels from photos taken under varying conditions and with different cameras are not comparable. Therefore, we use length ratios instead of raw lengths to avoid this incompatibility. The ratios of the lengths of two parallel line segments remain unaffected by the factors above, allowing for the analysis of photos from various sources.

Only certain photographs are permitted for sufficiently accurate measurements. They must be sharp, and the flowers should be positioned close enough to the camera to ensure adequate resolution of their smallest measured details. Fortunately, we gathered enough suitable photographs from many of our images and those generously provided by other wild orchid enthusiasts (see Acknowledgements).

Measured data was processed as follows. In the first step, for each parental plant and hybrid, we calculated the average value, standard deviation, and coefficient of variance of the following ratios: SS/PP, P/E, H/E, PP/E, and SS/E. In the second step, we calculated the same descriptive statistics for all four groups of parental plants.

The standard box-and-whisker plots in Figure 3, which summarize the statistics of the measured ratios of parental species from all four groups, illustrate their variability and the effectiveness of a particular ratio in distinguishing parental species. The discriminatory power of the H/E and P/E ratios is significantly greater than that of the other ratios. The SS/PP and PP/E ratios, which include the distances between petals and/or sepals, show partial overlap. This is likely because the distances between petals and sepals change during anthesis. Despite our best efforts to measure only the flowers at the optimal flowering stage, we could not completely eliminate this source of variability. The SS/E ratio demonstrates slightly higher discriminatory power, but some overlap still exists. Based on these findings, we decided to include only the H/E and P/E ratios in the calculation of the DH.

The differences in average ratios among parental species groups were analyzed using a two-tailed t-test, assuming similar sample variances and sizes. For *E. atrorubens*, we could not reject the null hypothesis that the "Slovenian" and "Upper Soča Valley" groups do not differ in H/E and P/E ($p > 0.05$). However, in *E. helleborine*, the differences were significant ($p < 0.05$). Therefore, we compared hybrids from the Upper Soča Valley with the "Upper Soča Valley" parental group and others with the "Slovenian" sample.

We utilized a one-tailed Welch's t-test due to substantially different sample variances and sizes to assess the statistical significance of the differences between H/E and P/E of potential hybrids and parental species. A trait value that significantly deviated from both parental species ($p < 0.05$) was identified as intermediate. In this way, the traits were objectively assigned to one of the three DH method categories.

Figure 4 illustrates the results of measuring labellum proportions in parental species. The diagram displays both traits, with H/E ratio values on the x-axis and P/E values on the y-axis. The symbols in the chart represent the average ratios of all measured flowers per individual.

RESULTS

3.1 Assessment of geographical, site-related, and temporal barriers to hybridization

Figure 5 illustrates the results of our evaluation of potential large-scale geographical barriers to hybridization. The red circles on the map represent MTB quadrants with *E. atrorubens*, the green circles represent *E. helleborine*, and the black circles indicate the MTB quadrants containing both parental species. Both parental species are found in more than half of the quadrants associated with either species. Based on this information, we can conclude that there are no geographical barriers to hybridization between the two species in many areas of Slovenia.

Regarding site-related barriers, Bayer (1986) described a location where he found hybrids and parental species growing separately under very different site conditions yet within a relatively short distance. We encountered a similar situation in the Lower Trenta Valley. Both sites, typical of their respective parental species, were less than 100 meters apart. Additionally, we observed areas where both species grow together, such as patches of riparian forest (moist alluvium, shady) along the Soča River in the Lower Trenta Valley, where we spotted the hybrids "Lower Trenta–2022/1" and "Lower Trenta–2022/2," along with both parental species. These locations are far from typical for *E. atrorubens*. This species appears to be more tolerant of site conditions than generally thought. Due to its geographic diversity, areas where both parental species grow close enough to facilitate hybridization seem relatively common in Slovenia.

Regarding the temporal barriers to hybridization caused by the differing flowering times of the parental species, it is essential that weather conditions significantly influence these timings in individual years. The flowering times from the FloVegSi data observed in 2014, 2018, and 2020 are displayed in Figure 6. The figure shows at least some overlap in the flowering times of both parental species across all three years. Our field experience also supports this observation since we occasionally encounter flowering individuals of both species simultaneously. Therefore, there are no temporal barriers to hybridization in Slovenia.

We can conclude that the taxon *E. ×schmalhausenii* in Slovenia is likely due to the absence of geographical, site-related, or temporal barriers to hybridization.

3.2 Applied traits from the perspective of their discriminatory power

Although we used the same weights to calculate DH for all observed morphological traits, the variability of these traits—and their capacity to differentiate between the two parents—is certainly not uniform. Unfortunately, aside from a few rare qualitative descriptions, we did not find qualitative data in the literature regarding the discriminatory power of the traits. Therefore, we evaluate the relevance of individual traits based on our experience and the consistency of descriptions found in the standard literature.

We can identify the newly introduced traits of labellum proportions (H/E and P/E) as having some of the highest discriminatory power. The difference between the two parental species is clear, and trait variability is small. As shown in Figure 4, the values for both parental species do not overlap, at least not in the examined sample of plants. Traits such as leaf arrangement (LVAR), the ratio of the length of the uppermost internode to the penultimate one (INTR), and flower color (FLCO) are reliable characteristics that effectively distinguish the parental species. The descriptions of these traits in the literature align well with our findings and with one another. The shape of the lower leaves (LVSH) can also be considered a trait with relatively high discriminatory power, although descriptions in standard literature are not entirely consistent. The same applies to the trait regarding the surface structure of labellum protuberances and their transition to the epichile (ECON).

Weaker discriminatory power could be attributed to the traits of stem pubescence (STPB) and stem color (STCO). The overlap of values in the parental species appears substantial for both traits. The descriptions of these characteristics in the standard literature also differ to some extent. Similarly, flower smell (FLSM) usually does not possess high discriminatory power. This trait is highly subjective and influenced by various biotic and abiotic conditions. Identifying the intermediate state between parental species is difficult. However, a strong flower scent certainly indicates *E. atrorubens*.

3.3 Descriptions of the hybrids

Figures 7, 8, and 9 present the plants we analyzed and identified as *E. ×schmalhausenii*. Plants labeled "Lower Trenta—2020," "Lower Trenta—2021," "Osolnik—2021," "Lower Trenta—2022/1," and "Lower Trenta—2022/2" were examined in situ; the remaining plants were analyzed using photographic material. The figures depict each plant's habitus, inflorescence, and lower stem leaves, except two that include only inflorescence and flower images. The top-left ring diagram illustrates our estimates of the traits, with codes detailed in Table 1. Each plant's degree of hybridity (DH) is noted at the center of the ring. The right section of the ring displays vegetative traits, while the left side presents reproductive characteristics. Brown indicates intermediate expression; green signifies similarity to *E. helleborine*; red denotes similarity to *E. atrorubens*; and gray represents missing data. The bottom-left graph depicts the relationship between the average H/E and P/E values of the analyzed individual (black dot) and the ranges of both parental species (either the "Soča Valley" or the "Slovenian" group). The figures are self-explanatory, so we will only comment on those plants with low DH or exceptional circumstances.

The image of the specimen "Trnovec – 2014" (Figure 7B) was previously published (Dolinar 2015: 172). Because only two photographs of the inflorescence were available, the list of investigated traits is incomplete, making the assessed DH less reliable. However, the calculated DH and the ring graph suggest an almost ideal intermediate expression between the parental species. The proportions of the labellum are also clearly intermediate. The differences between the average values of H/E and P/E in the analyzed individual and the averages of both parental species are highly statistically significant ($p < 0.01$). Therefore, this individual is undoubtedly the hybrid *E. ×schmalhausenii*.

The photographic material for the specimens "Kamniška Bistrica—2015" (Figure 7C) was also limited. It allows us to evaluate only two out of five vegetative traits. To increase the total number of analyzed traits, we added the ratio of the distance between the tips of the lateral sepals to the width of the epichile (SS/E), which, in this case, is statistically significant ($p < 0.01$) compared to both parental species (not shown in Figure 7C). Most of the investigated traits were identified as intermediate, indicating a high degree of hybridity. Despite the fewer traits examined, we consider the assessment that this individual is a hybrid justified.

The DH of the plant "Lake Gradišče – 2020" (Figure 8D) is 56%, making it one of the lowest among the analyzed specimens. At first glance, its habit resembles that of *E. atrorubens*. Most traits align with this parent species, but the shape of the lower leaves differs distinctly from typical *E. atrorubens*. Additionally, three intermediate traits suggest *E. ×schmalhausenii*. The likelihood of this identification being accurate appears greater than that of it being a variant of *E. atrorubens*; however, we cannot completely rule out that possibility.

The specimen "Lower Trenta – 2022/1" (Figure 9H) has a DH of 50%, the lowest among all individuals studied. The only trait attributed to *E. helleborine* is the shape of the lower leaves. The habit of this plant bears a visual resemblance to *E. atrorubens*, but the lower leaves are conspicuously atypical and are almost certainly inherited from *E. helleborine*. Both parental species were present where the specimen was discovered (a riparian forest), increasing the likelihood of hybridization. Interestingly, this plant closely resembles the specimen "Lower Trenta – 2022/2," which grew in the same habitat just a few dozen meters away. Despite the low DH, we still believe that both are hybrids.

DISCUSSION

4.1 Morphological patterns of investigated hybrids

The nine individuals examined in this paper exhibit distinctly different phenotypes. This is anticipated, as the parental plants already show significant intraspecies variability. The hybrids' diverse morphologies may result from various factors, including back-crossing and molecular processes such as accidental mutations, deletions, crossovers, and the impact of numerous abiotic factors.

The specimens in our sample grew in different years on sites that were mainly distant from each other and offered various abiotic and edaphic conditions. The frequency and ratio of the parental species in their vicinity were also likely different. All these factors remain unknown. Moreover, the sample size of the analyzed hybrids is modest. Therefore, the causes for their morphological diversity cannot be depicted. Nevertheless, we were interested in whether we could infer any 'floristic laws' from the studied plants. Therefore, we summarized the behavior of the traits in two graphs, as shown in Figure 10.

Figure 10A illustrates, for each analyzed hybrid (the abscissa), the proportions of intermediate expressions of traits and those from both parental species (the ordinate). The diagram arranges individuals from left to right in ascending order based on the number of intermediate characteristics. The number of traits expressed as intermediate varies significantly from plant to plant. Only 20% of characteristics were intermediate in the "Osolnik – 2021" individual, while in the "Kamniška Bistrica - 2015" individual, 85% exhibited intermediate expressions. Only two out of nine individuals had more than half of all examined traits in the intermediate form. This contradicts expectations and the traditional assumption that hybrids exhibit intermediate morphology in all or at least most cases. Another observation is that hybrids in our sample retained characteristics of *E. atrorubens* more frequently than those of *E. helleborine*. Only two hybrids out of nine exhibited a greater retention of characteristic traits from *E. helleborine*. Consequently, the overall appearance of the hybrids was, on average, more visually similar to *E. atrorubens* than *E. helleborine*.

Figure 10B illustrates the percentage of individuals in our sample (the ordinate) that possess a trait (the abscissa) that is either parent-like or intermediate. The diagram reveals that certain morphological traits are significantly more likely to be inherited unchanged or expressed as intermediate than others. For instance, in our sample, the shape of the lower stem leaves (LVSH) was unchanged from *E. helleborine* in all observed hybrids. Similarly, the inflorescence axis (STCO) color is primarily derived from *E. helleborine*. In contrast, the labellum protuberances and their transition to the surface of the epichile (ECON) most frequently corresponded to typical *E. atrorubens*, with only one hybrid exhibiting this trait aligning with the typical *E. helleborine*. Likewise, the labellum ratios matched typical *E. helleborine* only in rare instances. Conversely, flower color (FLCO) was intermediate across all individuals. This is primarily because the unusual flower color of an *Epipactis* is the most striking feature, quickly drawing a field botanist's attention to the possibility of hybridization, and prompting a closer inspection of the specimen. Therefore, the proportion of specimens displaying intermediate flower colors is much higher than expected from a 'random sampling' of the studied plants. Finding hybrids with flower colors resembling parental species is much less likely. This is why our sample is notably skewed regarding this trait.

CONCLUSION

This research examines nine flowering specimens from the genus *Epipactis* (*Orchidaceae*) that display morphological traits suggesting a hybrid origin between the dark-red helleborine (*Epipactis atrorubens* (Hoffm. ex Bernh.) Besser) and the broad-leaved helleborine (*Epipactis helleborine* (L.) Crantz). Each specimen's degree of hybridity (DH) was determined by analyzing six to ten morphological characteristics. The results support their hybrid origin. A brief review of potential geographic, site-related, and temporal hybridization barriers indicates that the occurrence of this hybrid in Slovenia was anticipated. The parent species rank among the most common allogamous taxa within the *Epipactis helleborine* aggregate and the *Epipactis atrorubens* section in Slovenia; both species occupy extensive areas of Slovenia in the same MTB quadrants, and their flowering periods partially overlap, which increases the likelihood of hybridization.

The hybrids examined exhibit significant phenotypic diversity, which likely explains why they have rarely been identified and discussed in the literature and have been almost overlooked in Slovenia. Nevertheless, they seem to be a relatively common taxon. It is hardly coincidental that the locations of individuals discussed in this paper are close to the authors' homes, where most of their botanical activities are concentrated.

A single trait almost never possesses sufficient discriminatory power to differentiate between a parent species and a hybrid (Jakubovska & Gola 2010, among others). Thus, it is essential to consider a broader array of morphological characteristics to facilitate reasonably reliable assessments. By evaluating multiple traits, the hybrid index method has proven to be an effective complement to assessing overall morphology and decision-making based on expert knowledge.

The method's main weakness lies in the insufficient understanding of the range of intraspecies variability in the traits of parental species. This inevitably leads to a somewhat subjective selection of traits considered in hybridity assessment. Improving quantitative knowledge of intraspecies variability would enable a more informed and efficient selection of characteristics for analysis. This would certainly enhance the credibility of determining the degree of hybridity in individual plants.

POVZETEK

Uvod

V družini orhidej je hibridizacija med vrstami in rodovi pričakovana. V njej poznamo več križancev kot v kateri koli drugi družini semenk (Künkele & Baumann 1998). Vzrok za to so predvsem šibke genetske in reprodukcijske ovire med taksoni. Križanci so pogosto plodni; pri ugodnih pogojih se križajo med seboj in vzvratno s starševskimi vrstami. Po več generacijah lahko zaradi introgresivne hibridizacije kažejo širok spekter lastnosti (Stace 1975). Zato je te križance pogosto nemogoče določevati s primerjanjem z enim samim tipskim primerkom (holotipom) in jih tudi ni mogoče določati z dihotomnimi ključi.

Obravnavani takson *Epipactis* × *schmalhausenii* K. Richt., ki je v tem prispevku prvič podrobno opisan za Slovenijo, je hibrid med temno rdečo močvirnico *Epipactis atrorubens* (Hoffm. ex Bernh.) Besser in širokolistno močvirnico, *Epipactis helleborine* (L.) Crantz. Prvi ga je opisal Karl Richter (Richter 1890). Kasneje je bil, podobno kot drugi iz rodu *Epipactis*, v literaturi redko obravnavan. Avtorji so menili, da gre za redek takson, nekateri so celo dvomili o možnosti križanja (Hunt, Roberts & Young 1975). Kasnejše vedno številčnejše najdbe v večini evropskih držav so pokazale, da temu ni tako.

Članki o tem hibridu iz začetka in sredine 20. stoletja so se osredotočali predvsem na opise posameznih najdb (Künkele & Baumann 1998). Obravnavali so le rastline, ki so kazale hibridogene vrednosti za vse ali vsaj za večino opazovanih lastnosti. Kolikor nam je znano, je bil Bayer (1986) prvi, ki je obravnaval hibridizacijo v rodu *Epipactis* na podlagi opazovanja več vnaprej izbranih morfoloških lastnosti in številskem ocenjevanju opaženega. Še bolj razvito metodo je opisal Adamowski (1995). Metodo in nabor njegovih opazovanih lastnosti smo privzeli kot izhodišče tudi v pričujočem prispevku.

Materiali in metode

Glede na dvome o možnosti hibridizacije med vrstama *E. atrorubens* in *E. helleborine* in dejstva, da so v naši bližini še območja, kjer hibrid še ni bil opisan, smo želeli najprej na kratko preveriti, ali morda obstajajo širše geografske, rastiščne ali časovne ovire za hibridizacijo starševskih vrst v Sloveniji.

Možnosti širših geografskih preprek smo ocenili na podlagi analize vseh vnosov obeh starševskih vrst v bazi FloVegSi. Ugotovili smo, da se v številnih MTB kvadrantih v večjem delu Slovenije nahajata obe starševski vrsti in da zato širših geografskih preprek za križanje ni (Slika 5).

Glede rastiščnih preprek za križanje je sicer res, da se oba starša razlikujeta po nekaterih značilnostih rastišč, predvsem glede vlage, hranljivosti tal in osončenja. Vendar smo iz lastnih izkušenj in navedb v literaturi ugotovili, da so razdalje med njimi pogosto kratke in zato niso ovira za opraševalce. Menimo, da preprek za križanje iz tega razloga v geografsko zelo razgibani Sloveniji ni.

Za osvetlitev časovnih preprek smo analizirali datume vnosov obeh starševskih vrst v bazi FloVegSi. Po izločitvi vnosov, ki se nanašajo na ne cvetoče rastline, smo za leta 2014, 2018 in 2020 statistično primerjali porazdelitev datumov vnosov obeh staršev in ugotovili, da se prekrivajo. Zato menimo, da tudi časovnih preprek za križanje pri nas ni.

Hibridnost analiziranih rastlin smo ocenjevali z metodo hibridnega indeksa (HI) – Anderson (1936). V postopku ocenjujemo vnaprej izbran nabor morfoloških lastnosti. Vsako od opazovanih lastnosti rastline, ki jo analiziramo, razvrstimo v eno od treh kategorij: je podobna prvi starševski vrsti, vmesna, ali podobna drugi starševski vrsti. Vsaki kategoriji dodelimo svojo utež. HI je aritmetična vsota uteži vseh opazovanih lastnosti. Če leži vrednost za opazovano rastlino nekje v sredini med vrednostnima za prvo in drugo starševsko vrsto, rastlino prepoznamo kot križanca. Na ta način določena vrednost HI pa je žal odvisna od števila opazovanih lastnosti, kar onemogoča neposredno primerjavo rastlin, ki smo jih ocenili na osnovi različnega števila opazovanih lastnosti. Temu pa se pri terenskem delu ne moremo vedno izogniti. Zato smo izračun HI aritmetično preoblikovali v stopnjo hibridnosti (SH) na način, da so take primerjave mogoče. SH zavzame vrednot 0%, če je opazovana rastlina v vseh lastnostih podobna prvi ali drugi starševski vrsti in vrednost 100%, kadar leži točno med njima in je zato skrižana v največji možni meri.

Pri kateri pragovni vrednosti SH lahko opazovano rastlino prepoznamo kot križanca je seveda stvar presoje. Odločili smo se za vrednost SH = 50%. Zdi se, da je verjetnost, da bi polovica ali več opazovanih lastnosti zavzele vmesno stanje, oziroma, da bi primerek kazal četrtino tipičnih lastnosti alternativne starševske vrste, zgolj zaradi lastne znotraj vrstne variabilnosti, zanemarljivo majhna. Izbran kriterij smo primerjali z tistimi, ki so jih uporabili drugi avtorji (Bayer 1986, Adamowski 1995) in ugotovili podobno izbrane pragovne vrednosti.

Zanesljivost metode je brez dvoma sorazmerna številu opazovanih lastnosti. Več jih je, bolj zanesljiv je rezultat. Zato smo v privzet nabor opazovanih lastnosti po Adamowski (1995) dodali še dve lastnosti medene ustne cvetov: razmerje največje širine medovnika (aka hipohila) in širine epihila (P/E) in razmerje širine prehoda med hipohilom in epihilom in širino epihila (P/E). Izbrali smo jih iz petih opazovanih lastnosti (Slika 3), ker sta izkazali največjo diskriminatorno moč med obema starševskima vrstama. Za vsako opazovano rastlino smo določili povprečje H/E in P/E vseh cvetov v polnem cvetu. Te vrednosti smo statistično primerjali s tistimi od starševskih rastlin. V primeru, da so se vrednosti H/E in/ali P/E statistično značilno razlikovale od obeh starševskih vrst ($p<0,05$), smo jim pripisali vmesno stanje. Ker kvantitativnih podatkov o teh razmerjih v literaturi nismo našli, smo jih določili sami z analizo 509 cvetov 79 rastlin iz večine fitogeografskih območij Slovenije. Njihova nahajališča so prikazana na Sliki 1B. Razmerja smo določali fotometrično iz fotografij cvetov. Opazili smo, da se vrednosti H/E in P/E pri primerkih *E. helleborine* iz zgornjega Posočja („Upper Soča Valley“ skupina; SV) in tistimi iz drugih delov Slovenije („Slovenian“ skupina; SI) statistično signifikantno razlikujejo ($p<0,05$). Zato smo opazovane križance iz Zgornjega Posočja primerjali s skupino starševskih rastlin od tam, križance iz drugih delov Slovenije pa s skupino „Slovenian“. Ugotovljene povprečne vrednosti H/E in P/E posameznih rastlin so podane na Sliki 4 v obliki diagrama, ki prikazuje odnos obeh starševskih vrst. Vrednosti za vrsti *E. atrorubens* (trikotniki) in *E. helleborine* (kvadratki) so jasno razmejene in se ne prekrivajo.

V analizo zajete morfološke lastnosti in njihov kratek opis tipičnih oblik pri obeh starševskih vrstah so podane v Preglednici 1. Njihov grafična predstavitev je podana na Sliki 2.

Rezultati

Podrobne podatke o nahajališčih devetih primerkov, ki smo jih prepoznali kot križance med 2011 in 2022 navajamo v poglavju 2.2.1. angleškega besedila. Njihova nahajališča prikazuje Slika 1A. Pet primerkov smo analizirali in vivo, štiri po +/- obširnem fotografskem materialu.

Slike 7, 8 in 9 prikazujejo habitus, socvetje in spodnje liste obravnavanih rastlin (v dveh primerih nismo imeli na voljo fotografij vseh aspektov), njihovo stopnjo hibridnosti SH (sreda obroča levo zgoraj) in ocene posameznih opazovanih lastnosti (obod obroča). Leva stran obroča prikazuje reproduktivne lastnosti in desna vegetativne. Zelena barva segmentov pomeni podobnost z vrsto *E. helleborine*, rdeča podobnost z vrsto *E. atrorubens*, rjava označuje odločitev za vmesno stanje in siva pomeni manjkajoč podatek. Diagram levo spodaj prikazuje ugotovljeno vrednost H/E in P/E (črna pika) primerka v odnosu do starševskih vrst. Črni trikotniki označujejo vrsto *E. atrorubens*, rdeči kvadratki vrsto *E. helleborine*.

Opisani križanci izkazujejo veliko fenotipsko raznolikost. Njihove lastnosti so zbirno prikazana na Sliki 10. Delež vmesnih stanj (rjavo), ohranjenih lastnosti vrste *E. helleborine* (zeleno) in ohranjenih lastnosti vrste *E. atrorubens* (rdeče) za vsakega prikazuje diagram A. Vidimo, da se delež vmesnih stanj močno spreminja, od 20% (Osolnik - 2021) do 85% (Kamniška Bistrica - 2015). Tudi razmerje ohranjenih lastnosti obeh starševskih vrst se močno spreminja od 1 : 1 (Osolnik – 2021) do 1 : 6 (Spodnja Trenta – 2022/1).

Diagram B za vsako opazovano lastnost prikazuje delež križancev, pri katerih smo jo pripisali eni od starševskih vrst ali vmesnemu stanju. Opazimo, da nekatere lastnosti pogosteje kažejo hibridogen izgled kot druge. Npr., oblika spodnjih listov (LVSH) pri vseh križancih v našem vzorcu ustreza vrsti *E. helleborine*. Podobno tudi barva stebla v socvetju (STCO) večinoma ustreza temu taksonu in niti enkrat tipični obliki pri vrsti *E. atrorubens*. Na drugi strani pa je struktura grbin na epihilu (ECON) pri vrsti *E. atrorubens* ohranjena skoraj pri vseh opazovanih primerkih. Le v enem primeru ustreza vrsti *E. helleborine* in le en primerek izkazuje vmesno stanje. Obratno pa barvo stebla v socvetju (STCO), razmerje internodijev (INTR) in vonj cvetov (FLSM) pogosteje opažamo v vmesnem stanju. Barvo cvetov (FLCO) smo pri vseh primerkih ocenili za vmesno. Vendar je to le posledica tega, da je neobičajna barva cvetov najočitnejša in najprej opozori terenskega botanika na možnost križanja in vzpodbudi podrobnejši pregled primerka. Zato je delež obravnavanih primerkov z vmesno barvo cvetov daleč večji, kot bi bil pri statistično naključnem vzorčenju opazovanih rastlin.

Zaključek

V prispevku opisujemo devet rastlin, pri katerih njihova morfologija kaže, da gre za križance *Epipactis* × *schmalhausenii*. Najdbe tega taksona v Sloveniji niso presenečenje. Kratka analiza morebitnih geografskih, rastiščnih in časovnih preprek za križanje je pokazala, da jih pri nas ni. Obe starševski vrsti sta med najpogostejšimi alogamnimi v rodu *Epipactis*. Analiza pojavljanja obeh je pokazala, da rasteta v večjem delu Slovenije v istih MTB kvadrantih in da se njun čas cvetenja pogosto prekriva. Priložnosti za križanje, vsaj glede teh pogojev, torej ne manjka. Da je bil v Sloveniji do sedaj ta križanec skoraj popolnoma spregledan, je po vsem sodeč vzrok prav v njegovi izredni fenotipski variabilnosti, ki otežuje njegovo prepoznavanje na terenu. Zelo verjetno je, da ta takson v Sloveniji ni redek.

ACKNOWLEDGMENT

The authors wish to express their sincere gratitude to Mag. Jernej Kavšek, Ivan Papež, Marjan Šenica, and Dr. Igor Dakskobler for providing numerous photographs of the two parental species of the studied hybrid. Their contributions enabled us to incorporate these images into our photometric analysis of labellum proportions and enhance the hybrid index method used. We also appreciate the many data contributors to the FloVegSi database. The extensive entries across MTB quadrants throughout the year and at various elevations allowed us to analyze potential barriers to hybridization between the parental species in Slovenia. Our special thanks go to Dr. Igor Dakskobler for his unwavering support while writing this article, including his review, advice, and assistance with its publication. We also thank the anonymous reviewers for their corrections and valuable suggestions. Additionally, we thank Dr. Branko Vreš, the caretaker of the FloVegSi database, for his support. Furthermore, we appreciate Andreja Šalamon Verbič's help partially translating this paper into English. Thank you to everyone involved.

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CAPTIONS AND TABLE

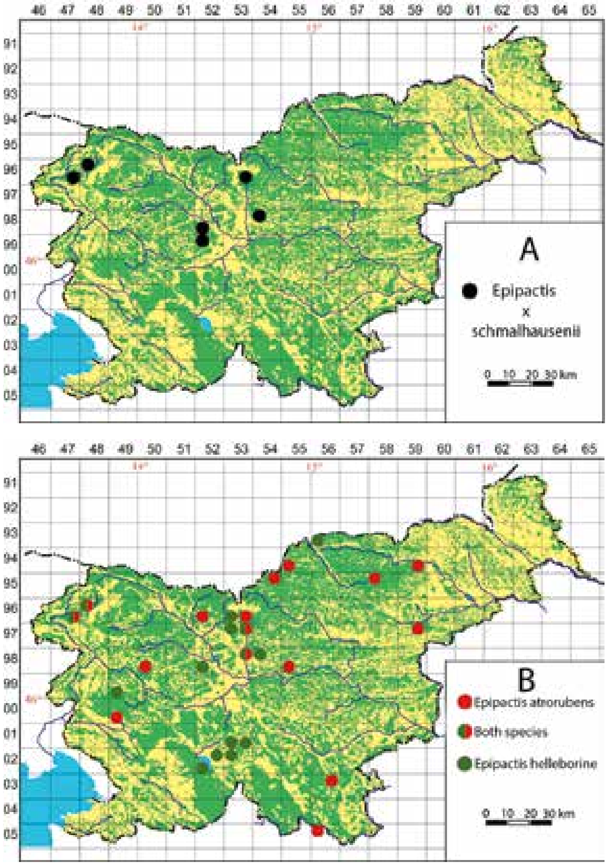

Figure 1: Visual representation of the localities of the studied taxa from the FloVegSi database. A: MTB quadrants with Epipactis × schmalhausenii; B: MTB quadrants with the parental plants.
Slika 1: Vizualni prikaz nahajališč obravnavanih taksonov v podatkovni bazi FloVegSi. A: MTB kvadranti z Epipactis × schmalhausenii; B: MTB kvadranti s starševskimi rastlinami.

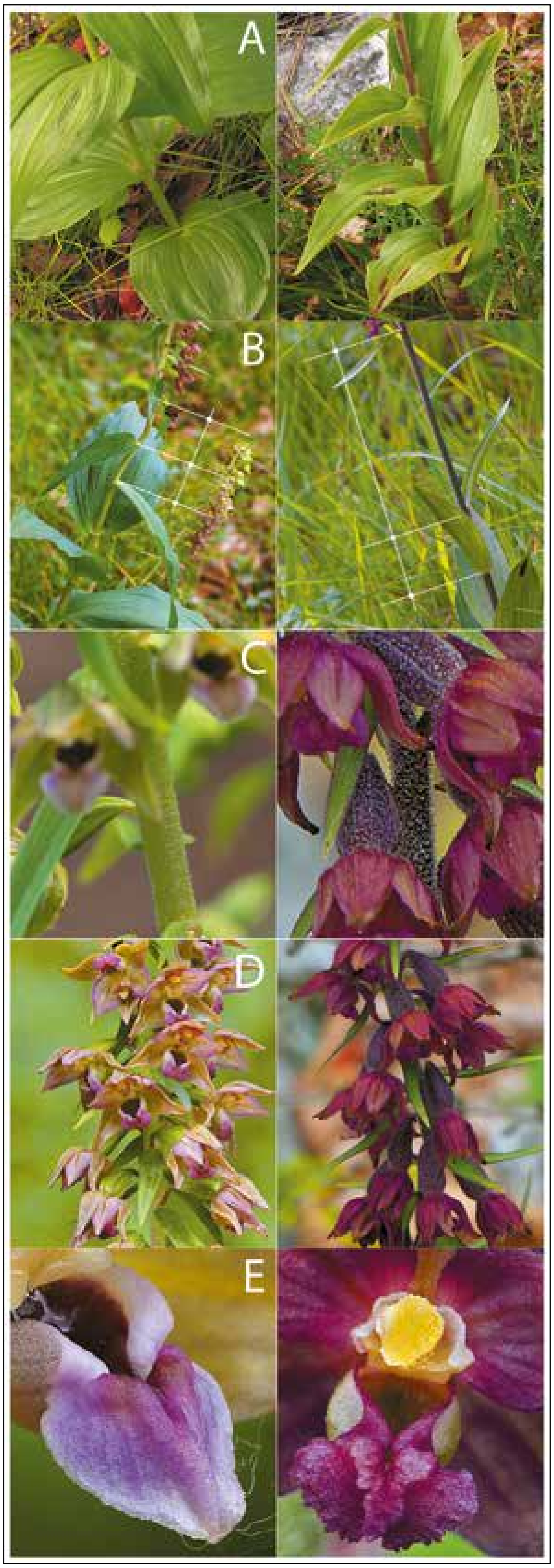

Figure 2: The typical traits of parental plants (left Epipactis helleborine, right Epipactis atrorubens) considered in assessing the degree of hybridity (DH). A: shape and arrangement of lower leaves (LVSH, LVAR); B: the ratio of the length of the last to the length of the last but one internode (INTR); C: pubescence and color of the inflorescence axis (STPB, STCO); D: flower color (FLCO); E: surface structure of protuberances at the base of epichile and their transition to the epichile surface (ECON). Photo: A. Trnkoczy.
Slika 2: Tipične lastnosti starševskih rastlin (levo Epipactis helleborine, desno Epipactis atrorubens) upoštevane pri ocenjevanju stopnje hibridnosti (DH). A: oblika in razporeditev spodnjih listov (LVSH, LVAR); B: razmerje med dolžino zadnjega in predzadnjega internodija (INTR); C: dlakavost in barva osi socvetja (STPB, STCO); D: barva cvetov (FLCO); E: površinska struktura grbin in njihov prehod v površino epihila (ECON). Foto: A. Trnkoczy.

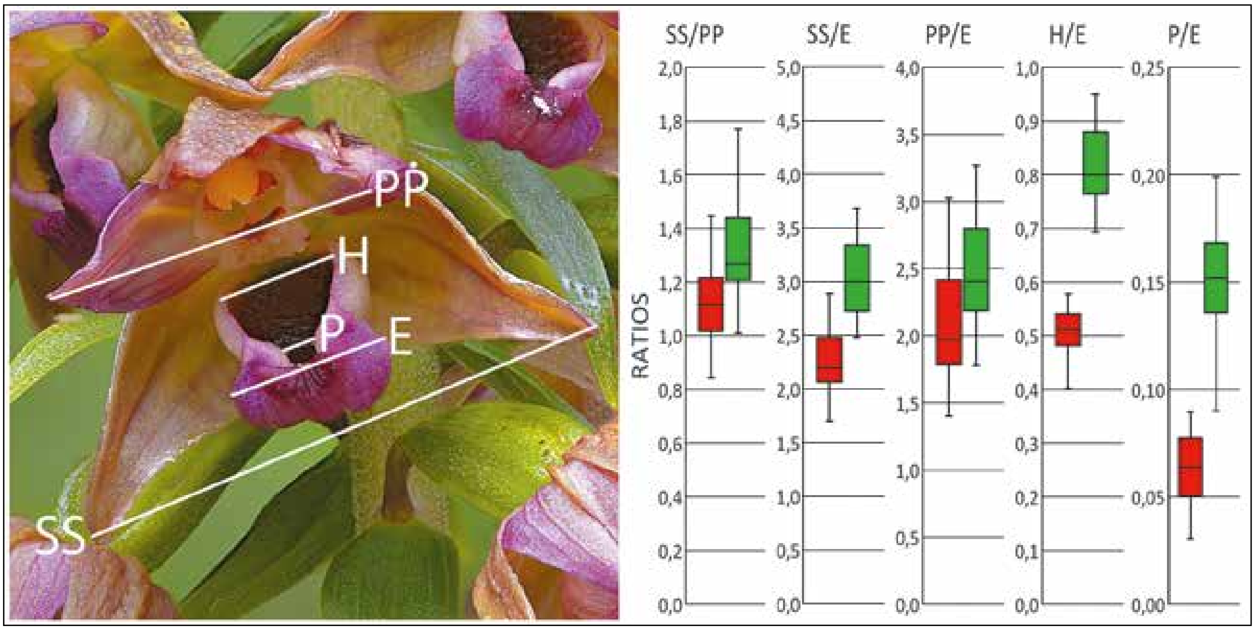

Figure 3: Measured lengths of line segments in the flower and statistics of their ratios (red Epipactis atrorubens, green Epipactis helleborine). SS distance between tips of lateral sepals, PP distance between tips of lateral petals, H maximal distance between the edges of the nectary on the hypochile (aka 'hypochile width'), P width of the gutter-shaped transition between the hypochile and epichile, and E epichile width. Photo: A. Trnkoczy.
Slika 3: Merjene daljice v cvetu in statistika njihovih razmerij (rdeča Epipactis atrorubens, zelena Epipactis helleborine). SS razdalja med konicama stranskih sepalov, PP razdalja med konicama stranskih petalov, H največja razdalja med robovoma nektarija na hipohilu (aka ‚širina hipohila'), P širina žlebastega prehoda med hipohilom in epihilom in E širina epihila. Foto: A. Trnkoczy.

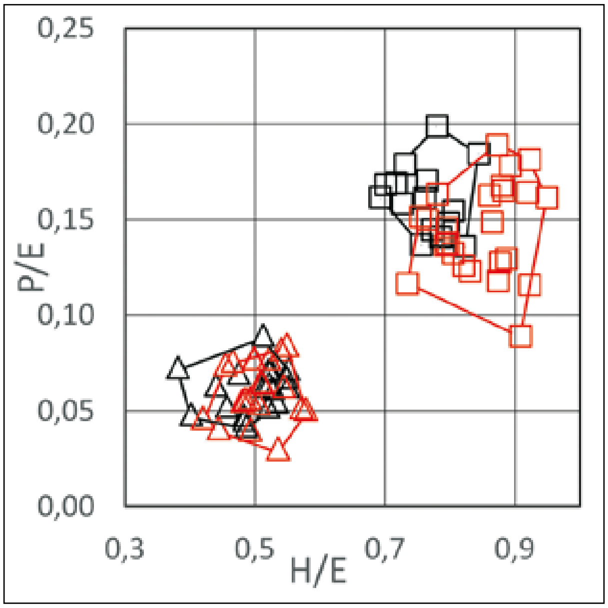

Figure 4: Proportions of the labellum in parental species regarding H/E (abscissa) and P/E (ordinate) ratios. Triangles represent Epipactis atrorubens; squares represent Epipactis helleborine. Both groups of parental species, the "Upper Soča Valley" (depicted in black) and the "Slovenian" (represented in red), are shown separately.
Slika 4: Proporci medene ustne pri starševskih vrstah izraženi z razmerji H/E (abscisa) in P/E (ordinata). Trikotniki predstavljajo Epipactis atrorubens; kvadrati predstavljajo Epipactis helleborine. Obe skupini starševskih vrst, »zgornjesoška« (črna) in »slovenska« (rdeča), sta prikazani ločeno.

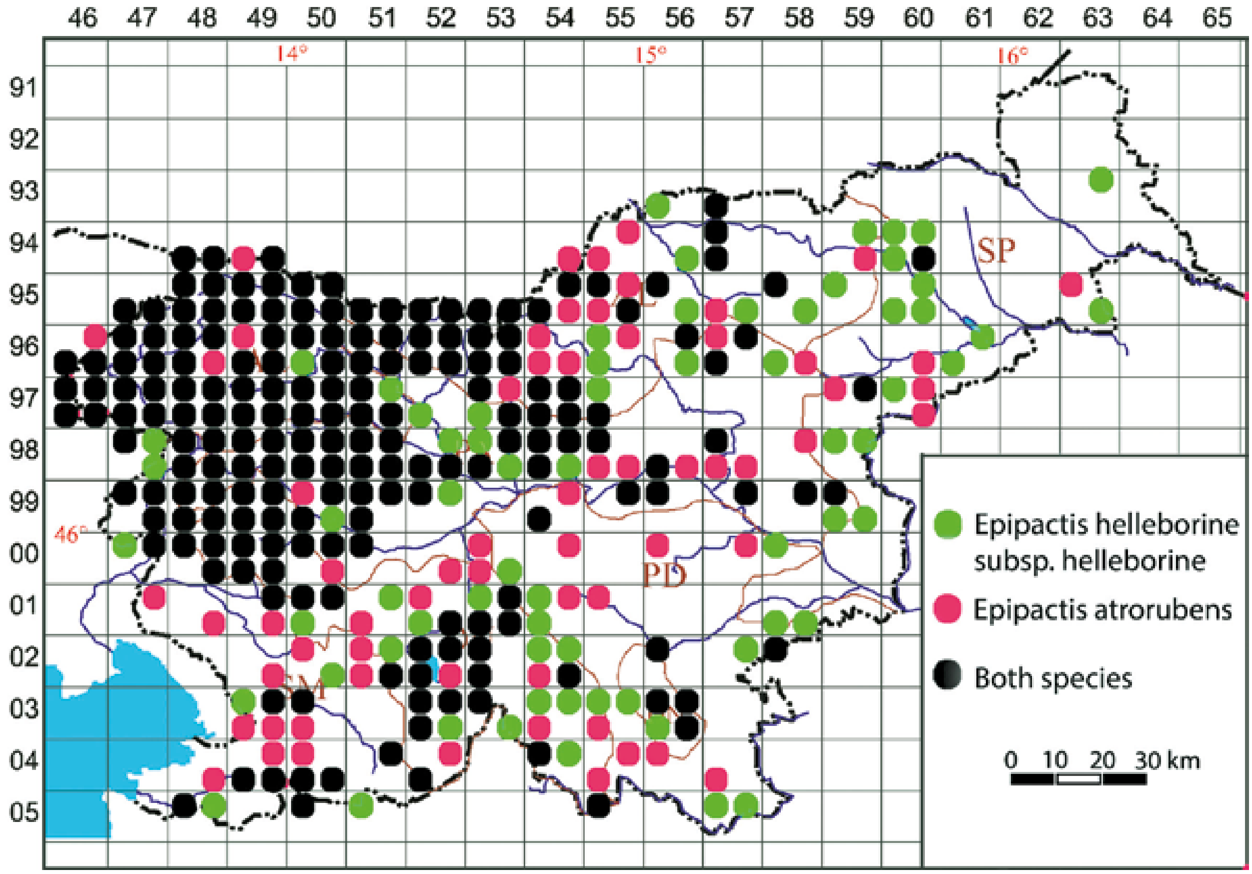

Figure 5: MTB quadrants from the FloVegSi database showing the occurrences of Epipactis atrorubens and Epipactis helleborine in Slovenia.
Slika 5: MTB kvadranti iz baze FloVegSi s prikazom pojavljanja Epipactis atrorubens in Epipactis helleborine v Sloveniji.

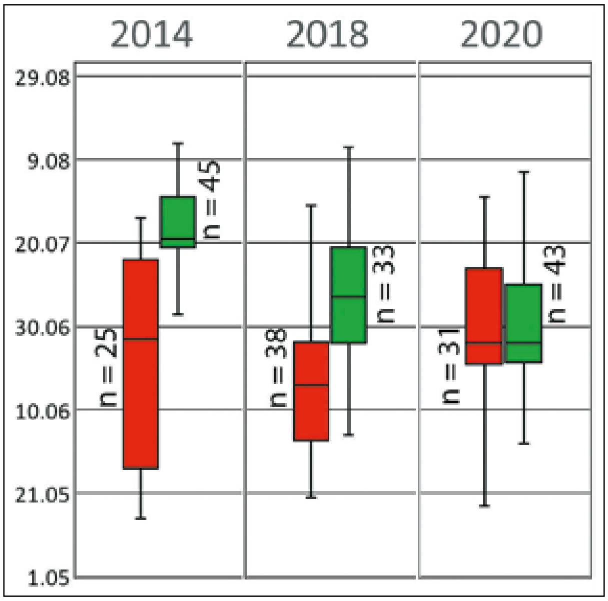

Figure 6: A box plot illustrating the flowering time of Epipactis helleborine (green) and Epipactis atrorubens (red) retrieved from the FloVegSi database for the years 2014, 2018, and 2020 projected to elevation band 600 - 800 m; n = numerus. The vertical axis denotes the observation dates. Notably, the flowering periods of parental species exhibit significant overlap across all three years.
Slika 6: Box-plot diagrami, ki prikazujejo čas cvetenja Epipactis helleborine (zelena) in Epipactis atrorubens (rdeča), pridobljen iz baze podatkov FloVegSi za leta 2014, 2018 in 2020. Datumi opažanj so projicirani na višinski pas 600 – 800 m; n = numerus. Navpična os označuje datume opažanj. Obdobji cvetenja obeh starševskih vrst se v vseh treh letih znatno prekrivata.

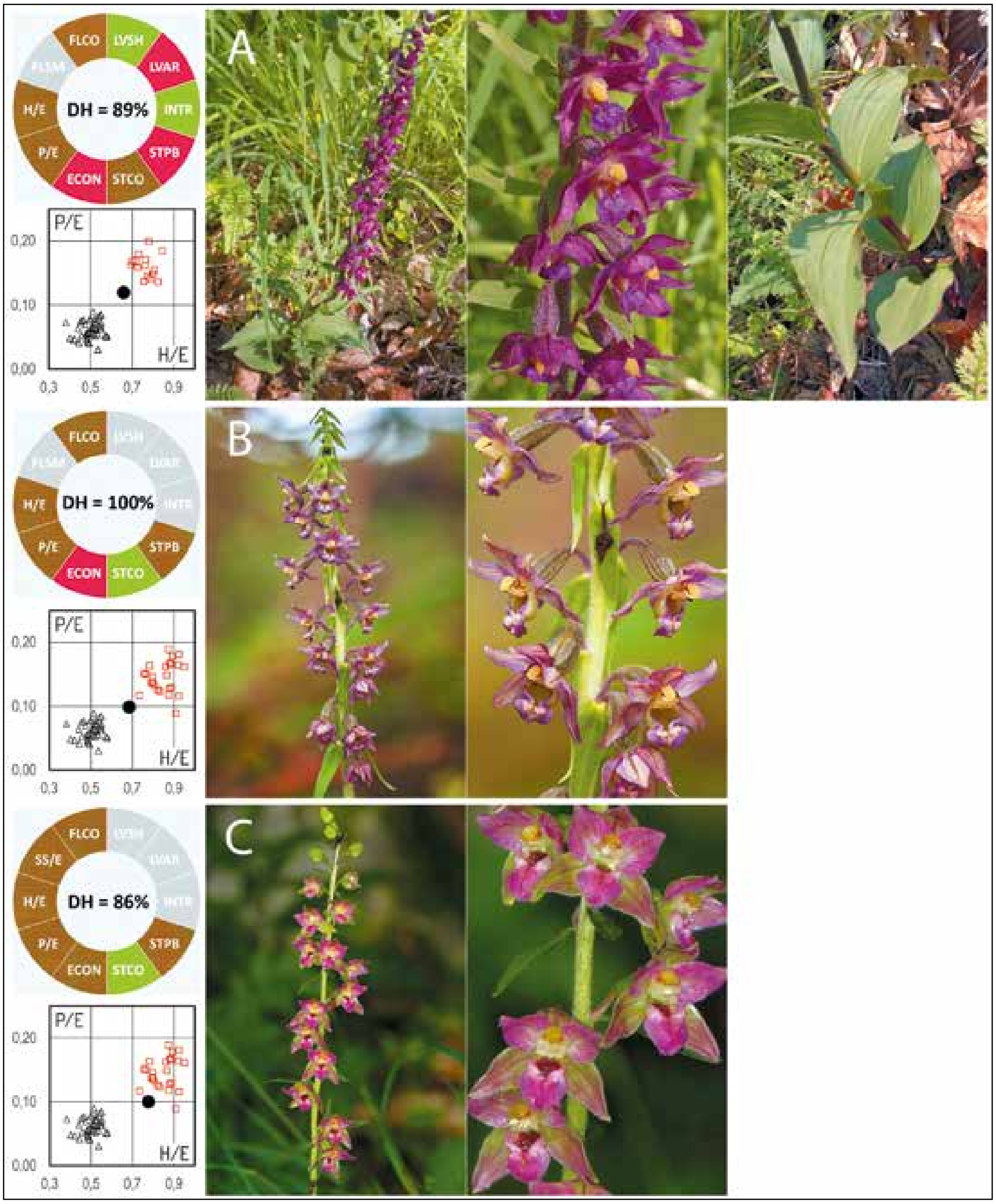

Figure 7: Investigated specimens: A: "Javoršček – 2011", B: "Trnovec – 2014", C: "Kamniška Bistrica – 2015". The top-left ring chart in the pictures of an individual specimen illustrates the degree of its hybridity (DH) and assessments of its traits. Green corresponds to similarity to Epipactis helleborine; red illustrates similarity to Epipactis atrorubens, and brown indicates intermediate expression; grey represents missing data. The codes used for the characteristics are explained in Table 1. The bottom-left diagram depicts the proportions of the labellum relative to the parental species, where the black dot represents the investigated plant, the red squares represent Epipactis helleborine, and the black triangles represent Epipactis atrorubens. Photo: A - A. Trnkoczy, B - B. Dolinar, C - A. Mihorič.

Slika 7: Obravnavani primerki: A: »Javoršček – 2011«, B: »Trnovec – 2014«, C: »Kamniška Bistrica – 2015«. Kolobar v zgornjem levem kotu slik posamezne obravnavane rastline ponazarja njeno stopnjo hibridnosti (DH) in ocene posameznih lastnosti. Zelena barva ponazarja podobnost z Epipactis helleborine; rdeča podobnost z Epipactis atrorubens, rjava označuje vmesno stanje; siva predstavlja manjkajoče podatke. Kode za posamezne lastnosti so razložene v Preglednici 1. Spodnji levi diagram prikazuje proporce medene ustne opazovane rastline v primerjavi s starševskima vrstama. Črna pika predstavlja obravnavano rastlino, rdeči kvadrati predstavljajo Epipactis helleborine, črni trikotniki ponazarjajo Epipactis atrorubens. Foto: A - A. Trnkoczy, B - B. Dolinar, C - A. Mihorič.

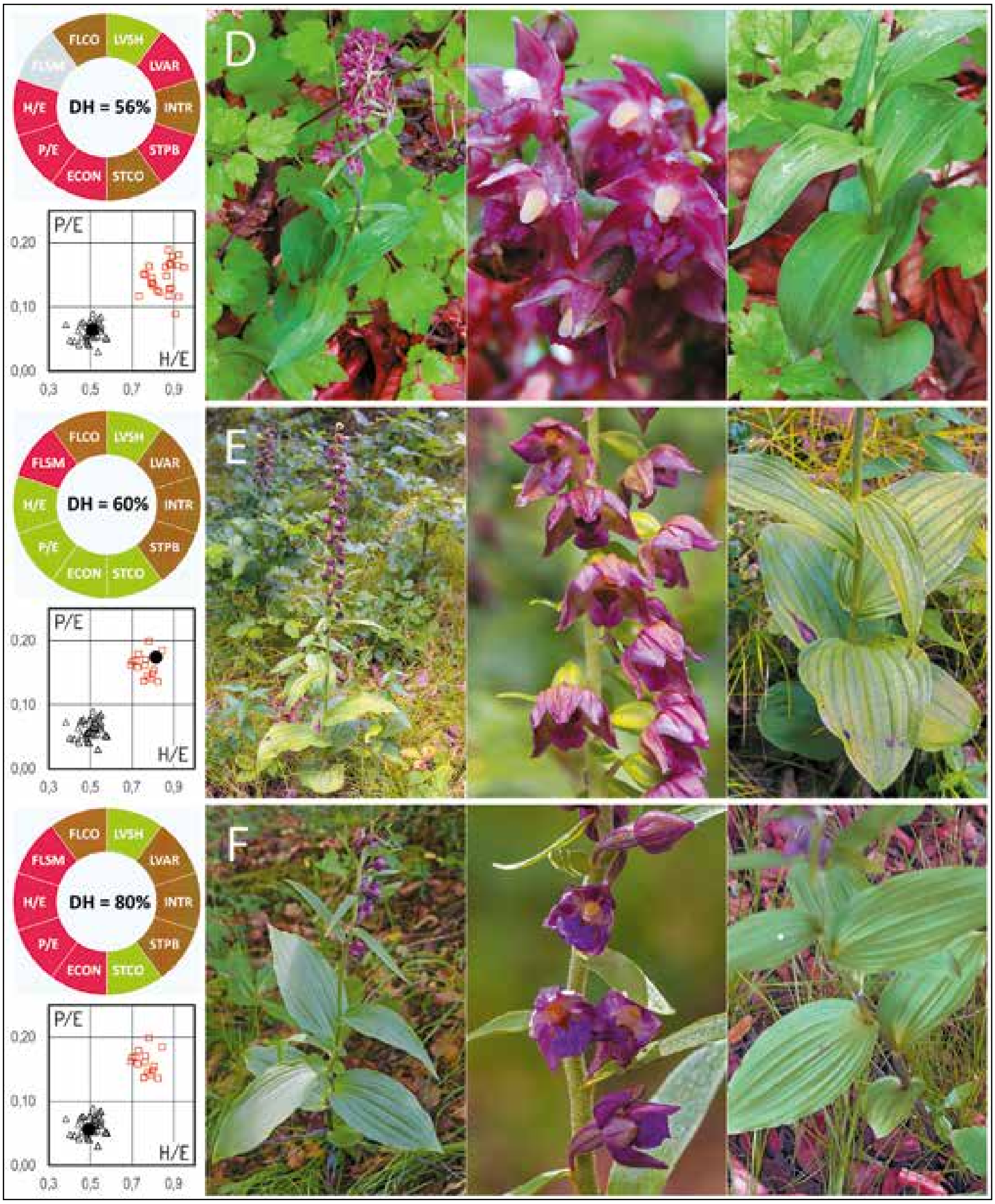

Figure 8: Investigated specimens: D: "Lake Gradišče – 2020", E: "Lower Trenta – 2020", F: "Lower Trenta – 2021". The top-left ring chart in the pictures of an individual specimen illustrates the degree of its hybridity (DH) and assessments of its traits. Green corresponds to similarity to Epipactis helleborine; red illustrates similarity to Epipactis atrorubens, and brown indicates intermediate expression; grey represents missing data. The codes used for the characteristics are explained in Table 1. The bottom-left diagram depicts the proportions of the labellum relative to the parental species, where the black dot represents the investigated plant, the red squares represent Epipactis helleborine, and the black triangles represent Epipactis atrorubens. Photo: D - A. Mihorič, E and F - A. Trnkoczy.

Slika 8: Obravnavani primerki: D: "Lake Gradišče – 2020", E: "Lower Trenta – 2020", F: "Lower Trenta – 2021". Kolobar v zgornjem levem kotu slik posamezne obravnavane rastline prikazuje njeno stopnjo hibridnosti (DH) in ocene posameznih lastnosti. Zelena barva ponazarja podobnost z Epipactis helleborine; rdeča podobnost z Epipactis atrorubens, rjava označuje vmesno stanje; siva predstavlja manjkajoče podatke. Kode za posamezne lastnosti so razložene v Preglednici 1. Spodnji levi diagram prikazuje proporce medene ustne opazovane rastline v primerjavi s starševskima vrstama. Črna pika predstavlja obravnavano rastlino, rdeči kvadrati predstavljajo Epipactis helleborine, črni trikotniki ponazarjajo Epipactis atrorubens. Foto: D - A. Mihorič, E in F - A. Trnkoczy.

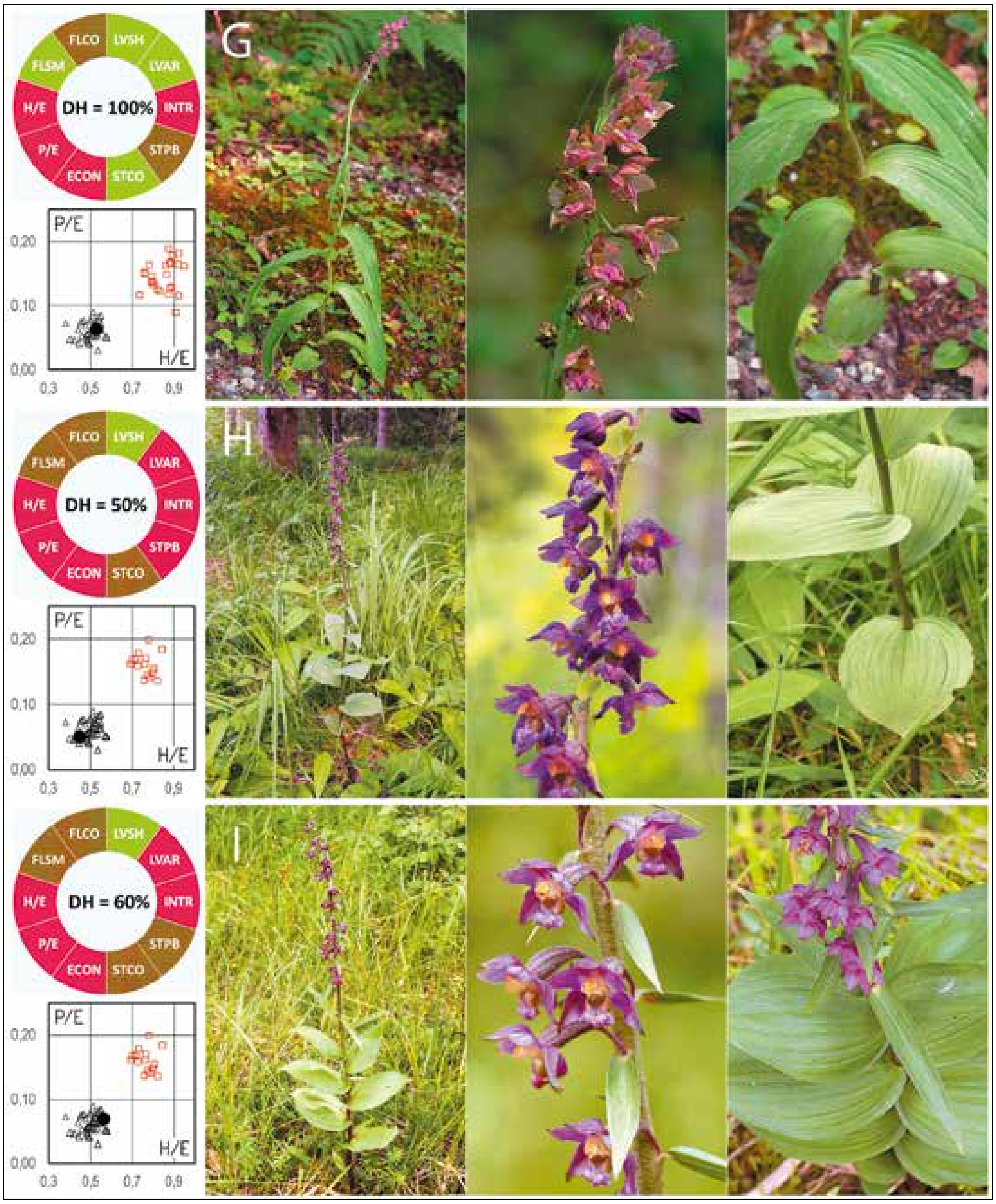

Figure 9: Investigated specimens: G: “Osolnik – 2021,” H: “Lower Trenta – 2022/1,” I: “Lower Trenta – 2022/2.” The top-left ring chart in the pictures of an individual specimen illustrates the degree of its hybridity (DH) and assessments of its traits. Green corresponds to similarity to Epipactis helleborine; red illustrates similarity to Epipactis atrorubens, and brown indicates intermediate expression; grey represents missing data. The codes used for the traits are explained in Table 1. The bottom-left diagram depicts the proportions of the labellum relative to the parental species, where the black dot represents the investigated plant, the red squares represent Epipactis helleborine, and the black triangles represent Epipactis atrorubens. Photo: G - B. Dolinar, H and I - A. Trnkoczy.

Slika 9: Obravnavani primerki: : “Osolnik – 2021,” H: “Lower Trenta – 2022/1,” I: “Lower Trenta – 2022/2.” Kolobar v zgornjem levem kotu slik posamezne obravnavane rastline prikazuje njeno stopnjo hibridnosti (DH) in ocene posameznih lastnosti. Zelena barva ponazarja podobnost z Epipactis helleborine; rdeča podobnost z Epipactis atrorubens, rjava označuje vmesno stanje; siva predstavlja manjkajoče podatke. Kode za posamezne lastnosti so razložene v Preglednici 1. Spodnji levi diagram prikazuje proporce medene ustne opazovane rastline v primerjavi s starševskima vrstama. Črna pika predstavlja obravnavano rastlino, rdeči kvadrati predstavljajo Epipactis helleborine, črni trikotniki ponazarjajo Epipactis atrorubens. Photo: G - B. Dolinar, H in I - A. Trnkoczy.

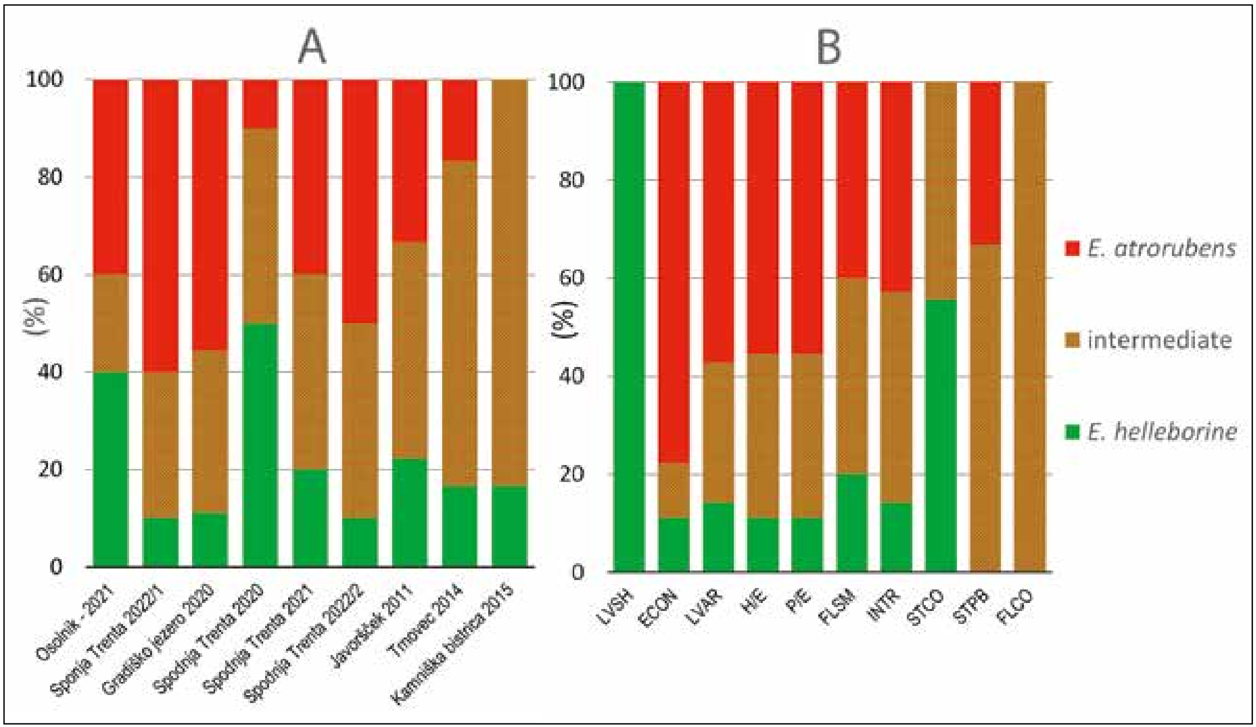

Figure 10: A: Diagram illustrating the shares of intermediate expressions of traits and those retained from Epipactis helleborine or Epipactis atrorubens (ordinate) for each analyzed hybrid (abscissa); B: Diagram showing the proportions of the studied hybrids (ordinate) with either parental or intermediate expression of the investigated trait (abscissa). Codes for investigated traits are explained in Table 1.
Slika 10: A: Diagram prikazuje deleže vmesnih stanj lastnosti in tistih, ki so se ohranile od Epipactis helleborine oziroma Epipactis atrorubens (ordinata) za vsak obravnavani hibrid (abscisa); B: Diagram prikazuje za vsako opazovano lastnost (abscisa) delež obravnavanih rastlin (ordinata), ki so izkazovale ohranjeno starševsko ali vmesno stanje. Kode za preiskovane lastnosti so razložene v Preglednici 1.

	Code	**Trait**	**Typical *Epipactis helleborine***	**Typical *Epipactis atrorubens***
1	**LVSH**	average shape of the bottom third of leaves excluding the first (l = leaf length, w = leaf width)	oval (l/w = 1.5-3), broadly ovate (l/w = 1.5-2), ovate (l/w = 2-2.5), +/- obtuse, the widest in or just under the middle of the leaf length	broadly lanceolate (l/w = 3.2-4), lanceolate (l/w = 4-5.5), narrowly lanceolate (l/w = 6-8), the widest nearer to the base
2	**LVAR**	leaf arrangement (average Ø angle of divergence from distichous leaf arrangement)	Ø > 60^0	Ø < 30^0
3	**INTR**	ratio (r) of lengths of the last, the uppermost, and the last but one internode	r < 1.2	r > 2
4	**STPB**	pubescence of the inflorescence axis	poor	strong
5	**STCO**	color of the inflorescence axis	green	purple
6	**FLCO**	flower color	whitish-green, yellowish, greenish-pink, pink-green	intense purple to brown-red
7	**FLSM**	flower smell	none or faint	distinct
8	**ECON**	surface structure of protuberances and their transition to epichile surface	almost smooth/gradual	clearly wrinkled / abrupt
9	**H/E**	average hypochile to epichile width ratio	SV=0.77, SI=0.85	0.50
10	**P/E**	average ratio of the transition between the hypochile and epichile width to epichile width	SV=0.153, SI=0.148	0.06

Table 1: Description of the morphological traits used in the degree of hybridity calculations and their codes for both typical parental species. The intermediate value of a trait of the studied specimen between those described in the table (for H/E and P/E statistically different from both parents, p<0,05) indicates its possible hybrid origin. Average H/E and P/E values in Epipactis helleborine are stated separately for the "Upper Soča Valley" (SV) and "Slovenian" (SI) groups of plants.

	Koda	Opazovana lastnost	Pri tipični *Epipactis helleborine*	Pri tipični *Epipactis atrorubens*
1	**LVSH**	oblika spodnje tretjine stebelnih listov brez najnižjega (l = dolžina, w = širina listov)	ovalna (l/w = 1.5-3), široko jajčasta (l/w = 1.5-2), jajčasta (l/w = 2-2.5), +/- topa, najširša na sredini lista ali malo nižje	široko suličasta (l/w = 3.2-4), suličasta (l/w = 4-5.5), ozko suličasta (l/w = 6-8), najširša bližje listnega dna
2	**LVAR**	razporeditev listov (Ø povprečen kot odstopanja od razporeditve listov v dveh redeh)	Ø > 60^0	Ø < 30^0
3	**INTR**	razmerje (r) dolžine zadnjega in predzadnjega internodija	r < 1.2	r > 2
4	**STPB**	dlakavost stebla v socvetju	rahla	močna
5	**STCO**	barva stebla v socvetju	zelena	purpurna
6	**FLCO**	barva cvetov	belo-zelena, rumenkasta, zeleno-rožnata, rožnato-zelenkasta	intenzivno purpurna do rjavo rdeča
7	**FLSM**	vonj cvetov	brez ali komaj zaznaven	močan
8	**ECON**	struktura površine grbin in način njihovega prehoda v epihil	skoraj gladka / postopen	razločno nagrbančena / skokovit
9	**H/E**	povprečno razmerje H/E	SV=0.77, SI=0.85	0.50
10	**P/E**	povprečno razmerje P/E	SV=0.153, SI=0.148	0.06

Preglednica 1: Opis upoštevanih morfoloških lastnosti pri izračunih stopnje hibridnosti in njihove kode za obe tipični starševski vrsti. Vmesna vrednost lastnosti proučevanega primerka med tistimi, ki so opisane v preglednici (statistično signifikantno različna od staršev pri H/E in P/E, p<0,05), kaže na njen možni hibridni izvor. Povprečne H/E in P/E vrednosti pri Epipactis helleborine so navedene ločeno za »zgornjesoško« (SV) in »slovensko« (SI) skupino rastlin.