

# Leaf arrangements are invalid in the taxonomy of orchid species

Anna Jakubska-Busse <sup>Corresp.</sup> <sup>1</sup>, Elżbieta Żołubak <sup>1</sup>, Zbigniew Łobas <sup>1</sup>, Edyta Magdalena Gola <sup>2</sup>

<sup>1</sup> Department of Botany, Institute of Environmental Biology, University of Wrocław, Wrocław, Polska

<sup>2</sup> Department of Developmental Plant Biology, Institute of Experimental Biology, University of Wrocław, Wrocław, Polska

Corresponding Author: Anna Jakubska-Busse  
Email address: anna.jakubska-busse@uwr.edu.pl

The selection and validation of proper distinguishing characters are of crucial importance in taxonomic revisions. The modern classifications of orchids utilize the molecular tools, but still the selection and identification of the material used in these studies is for the most part related to general species morphology. One of the vegetative characters quoted in orchid manuals is leaf arrangement. However, phyllotactic diversity and ontogenetic changeability have not been analysed in detail in reference to particular taxonomic groups. Therefore, we evaluated the usefulness of leaf arrangements in the taxonomy of the genus *Epipactis* Zinn, 1757. Typical leaf arrangements in shoots of this genus are described as distichous or spiral. However, in the course of field research and screening of herbarium materials, we indisputably disproved the presence of distichous phyllotaxis in the species *Epipactis purpurata* Sm. and confirmed the spiral Fibonacci pattern as the dominant leaf arrangement. In addition, detailed analyses revealed the presence of atypical decussate phyllotaxis in this species, as well as demonstrated the ontogenetic formation of pseudowhorls. These findings confirm ontogenetic variability and plasticity in *E. purpurata*. Our results are discussed in the context of their significance in delimitations of complex taxa within the genus *Epipactis*.

# 1 **Leaf arrangements are invalid in the taxonomy of orchid species**

2 Anna Jakubska-Busse<sup>1,\*</sup>, Elżbieta Żołubak<sup>1</sup>, Zbigniew Łobas<sup>1</sup>, Edyta M. Gola<sup>2</sup>

3 <sup>1</sup>Department of Botany, Institute of Environmental Biology, University of Wrocław, Kanonia 6/8,  
4 50-328 Wrocław, Poland (\*corresponding author's e-mail: [anna.jakubska-busse@uwr.edu.pl](mailto:anna.jakubska-busse@uwr.edu.pl))

5 <sup>2</sup>Department of Plant Developmental Biology, Institute of Experimental Biology, Faculty of  
6 Biological Sciences, University of Wrocław, Kanonia 6/8, 50-328 Wrocław, Poland

## 7 **Abstract**

8 The selection and validation of proper distinguishing characters are of crucial importance in  
9 taxonomic revisions. The modern classifications of orchids utilize the molecular tools, but still  
10 the selection and identification of the material used in these studies is for the most part related to  
11 general species morphology. One of the vegetative characters quoted in orchid manuals is leaf  
12 arrangement. However, phyllotactic diversity and ontogenetic changeability have not been  
13 analysed in detail in reference to particular taxonomic groups. Therefore, we evaluated the  
14 usefulness of leaf arrangements in the taxonomy of the genus *Epipactis* Zinn, 1757. Typical leaf  
15 arrangements in shoots of this genus are described as distichous or spiral. However, in the course  
16 of field research and screening of herbarium materials, we indisputably disproved the presence of  
17 distichous phyllotaxis in the species *Epipactis purpurata* Sm. and confirmed the spiral Fibonacci  
18 pattern as the dominant leaf arrangement. In addition, detailed analyses revealed the presence of  
19 atypical decussate phyllotaxis in this species, as well as demonstrated the ontogenetic formation  
20 of pseudowhorls. These findings confirm ontogenetic variability and plasticity in *E. purpurata*.  
21 Our results are discussed in the context of their significance in delimitations of complex taxa  
22 within the genus *Epipactis*.

## 23 **Introduction**

24 Understanding plant variability and the underlying genetic and developmental mechanisms are  
25 fundamental to modern plant classifications (Batista & Bianchetti, 2002; Jones & Clements,  
26 2002; Rudall & Bateman, 2002; Bateman, Rudall & Moura, 2013). Genotypic and phenotypic  
27 variations reflect the adaptation of a plant to diverse and often demanding environments, and are  
28 generally accepted as driving forces behind speciation (Stace, 1991). The family Orchidaceae has  
29 recently been extensively studied in attempt to find the phylogenetic relationships within this  
30 family (Byng et al., 2016). Although some orchid taxa have been revised based on molecular  
31 markers (e.g. Tranchida-Lombardo et al., 2011; Bateman, Rudall & Moura, 2013; Fajardo, de  
32 Almeida Vieira & Molina, 2014; Zhao, Tang & Bi, 2017), there is still a lack of consensus  
33 regarding the delimitation of other genera (Byng et al., 2016). Specifically, as yet there are no  
34 well-defined genetic markers for orchids which would enable the separation of e.g., aggregate  
35 taxa (Chung & Chung, 2012; Fajardo, de Almeida Vieira & Molina, 2014), especially regarding  
36 their phenotypic variability (Jakubská-Busse et al., 2017). Furthermore, the validation of the  
37 correct identification of plant materials for genetic analyses is mostly based on morphological  
38 traits. Therefore, taxonomic surveys focus mostly on flower and especially column  
39 (gynostemium) structure, acknowledged as the most reliable and stable characteristics in orchid  
40 classifications being related to the pollination systems (Mered'a, 1999; Szlachetko & Rutkowski,  
41 2000; Lovisa, Verola & Antonelli, 2010; Claessens & Kleynen, 2011; Jin et al., 2014). However,  
42 these surveys also include general morphological descriptions which are often used in manuals  
43 for the determination of taxa (Dressler, 1993; Szlachetko & Rutkowski, 2000; Delforge, 2006).  
44 One of these characteristics in taxa circumscription is leaf arrangement (e.g. Delforge, 2006);  
45 however, detailed data on this aspect in the Orchidaceae is lacking.

46         The phenomenon of regular and periodic patterning of leaves (or other lateral organs) is  
47 called phyllotaxis and has drawn the attention of researchers for centuries (e.g. Jean 1994; Adler,  
48 Barabé & Jean, 1997; Reinhardt, 2005; Kuhlemeier, 2007). In the plant kingdom, two major

49 types of leaf arrangements, whorled and spiral (helical) (Zagórska-Marek, 1985, 1994), are  
50 recognised. In whorled phyllotaxis, more than one leaf is simultaneously initiated at the  
51 meristem, forming a whorl of leaves in a node. The next whorl is circumferentially displaced so  
52 that its elements (leaves) are located in a mid-distance between leaves of the previous whorl. A  
53 special whorled leaf arrangement, called decussate phyllotaxis, occurs when two leaves are  
54 formed per whorl. This is a common pattern in, for example, the families Lamiaceae and  
55 Caryophyllaceae (Rutishauser, 1998; Reinhardt, 2005; Gola & Banasiak, 2016). Another  
56 modification of whorled phyllotaxis is distichy, whereby only one leaf is initiated per whorl, but  
57 the next leaf is displaced the half distance around the stem, i.e.  $180^\circ$ , with respect to the previous  
58 leaf. As a result, leaves occur in two opposite ranks along the stem. This leaf distribution is  
59 typical, for example, of the family Poaceae (Gola & Banasiak, 2016).

60         In the second major type of leaf arrangement, spiral phyllotaxis, successive leaves are  
61 initiated separately at the meristem and can be linked along the stem by a spiral line called the  
62 ontogenetic spiral. The spatial configuration (chirality) of the ontogenetic spiral can be either  
63 clockwise (S chirality) or counterclockwise (Z chirality) (Zagórska-Marek, 1985). Successive  
64 leaves are circumferentially displaced at a stable angular distance (divergence angle) and thus do  
65 not overlap (Zagórska-Marek, 1985; Jean, 1994). The most frequent spiral phyllotaxis in the plant  
66 kingdom (e.g. Zagórska-Marek, 1985, 1994; Jean, 1994; Adler, Barabé & Jean, 1997;  
67 Rutishauser, 1998) is related to the series of Fibonacci numbers, i.e. 1, 1, 2, 3, 5, 8, ... etc., in  
68 which each element is the sum of the two preceding elements. The first number in this series  
69 refers to the ontogenetic spiral; however, this is hardly visible in the majority of shoots due to the  
70 shortening of internodes between successive leaves. Then the secondary spirals (parastichies),  
71 winding towards the apex in both directions, clockwise (S) and counterclockwise (Z), become  
72 visible at the stem surface. The most discernible spirals, crossing at right angles or near-right  
73 angles, form a contact parastichy pair, represented by the two succeeding numbers of the

74 phyllotactic series, for example, 1:2 or 2:3 in the Fibonacci pattern (Adler, 1974; Zagórska-  
75 Marek, 1985, 1994; Jean, 1994). This formula unequivocally identifies the phyllotaxis of a given  
76 shoot (Zagórska-Marek, 1985, 1994).

77 It is generally accepted that the spiral leaf arrangement is a plesiomorphic feature in  
78 orchids, whereas distichous phyllotaxis or the presence of only one or two leaves per pseudobulb  
79 is an apomorphic trait (Withner, Nelson & Wejksnora, 1974; Arditti, 1992; Dressler, 1993).  
80 Rarely, due to the uneven elongation of internodes, two or more leaves are gathered at the same  
81 stem level (Dressler, 1993). In the genus *Epipactis*, the object of our analysis, leaves are usually  
82 reported as distichously arranged, but in some species spiral phyllotaxis can also occur (Dressler,  
83 1993; Delforge, 2006; Brullo, D'Emerico & Pulvirenti, 2013; Byng et al., 2016; Lipovšek,  
84 Brinovec & Brinovec, 2017). Despite this general opinion, accurate data on phyllotaxis in the  
85 genus *Epipactis* suitable for use in manuals is lacking. This knowledge is however useful as the  
86 additional indirect confirmation of the species identity, especially during the field work when  
87 sometimes plants are available only in the vegetative phase and/or during revisions of the  
88 collections of plant specimens (vouchers). Therefore, the aims of our research were to (i) analyse  
89 the variability of leaf arrangements in *E. purpurata* in natural conditions; (ii) and quantify  
90 phyllotaxis diversity throughout the European range of the species based on herbarium materials,  
91 and thus (iii) to validate the usefulness of leaf arrangements in taxa identification.

## 92 **Materials and Methods**

93 Long-term field investigations of natural populations of *E. purpurata* were performed  
94 between 2003 and 2016 in permanent research plots in four locations in south-western Poland: in  
95 Nieszczyce near Rudna (51°32'14.26"N, 16°23'56.26"E), the "Błyszcz" nature reserve near  
96 Pątnów Legnicki (51°15'37.09"N, 16°12'56.95"E), Wałkowa near Milicz (51°30'00.46"N,  
97 17°18'56.04"E) and Straża near Wińsko (51°23'51.40"N, 16°45'52.47"E). In this article, only the  
98 results of the analyses carried out in 2015 and 2016 are presented. In addition, relevant specimens

99 from diverse geographical regions deposited in European herbaria (acronyms abbreviations after  
100 Thiers (2017): B, BR, C, FR, G, KTU, M, S, WRSL, Z, ZT), were analysed.

101 Leaf arrangements were analysed in both fresh and voucher specimens using the formula  
102 of a contact parastichy pair (Adler, 1974; Zagórska-Marek, 1985, 1994). In addition, a series of  
103 transverse sections through the mature vegetative shoots were prepared in order to indirectly  
104 confirm the leaf arrangements. At the moment when differences in leaf phyllotaxis became  
105 macroscopically visible, inflorescences had already been formed and shoot apical meristems were  
106 not available for detailed analyses.

107 For anatomical sectioning, shoot fragments which differed in leaf arrangements were  
108 collected and fixed in FAA (a formyl-acetic acid–50% ethanol mixture). Following dehydration  
109 in an increasing series of tertiary butyl alcohols (50%, 70%, 90%, 96%, and three changes in the  
110 pure butanol), the plant material was embedded in Paraplast X-tra (Sigma-Aldrich) and  
111 transversely cut, using a rotary microtome (Leica RM2135; Leica Instruments), into 10–20 µm  
112 sections. Series of these cross sections were then de-waxed and stained with the Alcian blue-  
113 Safranin O mixture (1:1 v/v; O'Brien & McCully, 1981). Sequential digital images were taken  
114 using the system: a bright-field microscope Olympus BX 50 – Olympus DP70 camera – Cell^B  
115 software (Olympus Optical, Poland). Digital images were processed in Fireworks MX 2004  
116 (Macromedia, USA) and Photoshop CS6 (Adobe Systems, USA). Plant images were also taken in  
117 the field using Canon EOS 50D and Nikon D5300 cameras.

118 Experimental studies and material sampling were done with the permissions of the Regional  
119 Director for Environmental Protection, Nos.: WPN.6400.27.2015.IW.1., WPN.6205.122.2016. IL  
120 and WPN 6400.29.2016.IL

## 121 **Results**

122 In the course of our research, more than 470 ramets of *E. purpurata* were analysed in  
123 2015 and 2016 in the field, along with over 800 individual herbarium specimens (Table 1). In the

124 majority of shoots (1210 shoots, i.e. 94.7% of all studied ramets), leaves were separately and  
125 spirally arranged along the stem (Figs. 1A and 2, Table 1). Their arrangement corresponded to 1:2  
126 or 2:3 contact parastichy pairs, which are expressions of the main Fibonacci pattern. In the  
127 analysed material, the frequencies of both spatial configurations of spiral patterns were similar,  
128 with the ontogenetic spiral winding clockwise (S-chirality) in 51.9% and counterclockwise (Z-  
129 chirality) in 48.1% of cases.

130         In five cases (<1%), leaves were initiated in pairs (whorls) and oppositely inserted at the  
131 stem. Successive pairs were perpendicular to one another, forming a regular decussate pattern  
132 (Figs. 1B and 2, Table 1). In such shoots, two or, rarely, three whorls were present along the stem,  
133 while lower cauline leaves (below the decussate pattern) as well as bracts were arranged  
134 according to spiral phyllotaxis (Figs. 1B and 3). Interestingly, shoots with both decussate and  
135 spiral phyllotaxes were found in the ramets of one genet (Fig. 1B).

136         In several shoots (68 shoots, 5.3% of all analysed ramets), two or three leaves were  
137 gathered close to one another, seemingly at the same level of the stem (Figs. 1C, D and 3, Table  
138 1). However, the leaves in such gatherings did not form opposite pairs and, in extreme cases,  
139 were distinctly inserted on one side of the stem (Fig. 3). The analysis of their spatial distribution  
140 proved that they were arranged according to the spiral Fibonacci pattern, which was continued  
141 along the whole shoot (Fig. 1D and E). Thus they were identified as pseudowhorls.

142         Histological analyses of mature shoots representing different phyllotactic patterns showed  
143 the arrangement of vascular tissue at the cross sections in relation to the leaf position. Vascular  
144 bundles were scattered throughout the cross section and distributed typically of  
145 monocotyledonous plants. In shoots with a spiral leaf arrangement, at one side of the stem, below  
146 the node, vascular bundles divided, giving rise to the leaf vasculature (leaf trace). These newly  
147 divided vascular bundles, at the level of leaf insertion in the node, diverged to the leaf, forming  
148 its supply system (Fig. 4B and C). After leaf departure, in the region of the internode, bundles

149 were again relatively regularly scattered throughout the cross section. In the next node, the  
150 successive leaf trace was formed in the stem sector circumferentially distant ca. 137–140° from  
151 the previous leaf (Fig. 4B). This pattern repeated along the stem in relation to the successive  
152 leaves.

153 In shoots with decussate phyllotaxis, the leaf trace formation for leaves of one pair  
154 occurred simultaneously at opposite sides of the stem (Fig. 4C). Vascular bundles in two facing  
155 sectors split and departed, forming the vasculature of a given pair. In the subsequent node, the  
156 leaf traces for the next pair were again formed by the splitting of existing bundles, but in  
157 perpendicular sectors (Fig. 4C).

## 158 **Discussion**

159 In taxonomic descriptions of the species belonging to the genus *Epipactis*, the distichous  
160 and/or spiral leaf arrangement is usually cited as a typical pattern (Dressler, 1993; Delforge,  
161 2006; Byng et al., 2016). However, during over a decade of research on *Epipactis* morphology,  
162 we found no distichous phyllotaxis. Importantly, it is contradictory to the data commonly used in  
163 manuals, where leaf arrangement – spiral or in two opposite rows (distichous), is even sometimes  
164 given as an indirect feature to distinguish between or to characterise separate species as well as  
165 complex taxa (aggregates), for example, *E. atrorubens* (Hoff.) Besser, *E. tremolsii* Pau and *E.*  
166 *helleborine* (L.) Crantz (Delforge, 2006). Interestingly, in 2016, we discovered an atypical  
167 decussate arrangement of leaves in *E. purpurata*. This prompted us to perform a detailed survey  
168 of phyllotaxis in this species. In the course of our research, using the formula of a contact  
169 parastichy pair, we indisputably disproved the presence of distichy in *E. purpurata* and confirmed  
170 the prevalence of spiral phyllotaxis as the typical leaf arrangement in this species. The phyllotaxis  
171 here was identified as that representing the most common Fibonacci pattern in plants (e.g. Jean,  
172 1994; Adler, Barabé & Jean, 1997; Rutishauser, 1998). This pattern occurred in both spatial  
173 configurations with comparable frequency, indicating that the direction of the ontogenetic spiral

174 in this species is randomly selected, similarly as in other plant species (Gregory & Romberger,  
175 1972; Gómez-Campo, 1974; Zagórska-Marek, 1985, 1994).

176         The only exception to the typical spiral Fibonacci pattern in *E. purpurata* was the  
177 occurrence of the whorled decussate phyllotaxis. The decussate pattern in this species was  
178 established based on the circumferential arrangement of leaves and further confirmed by analysis  
179 of the vasculature. In monocot shoots, *E. purpurata* included (Jakubska-Busse et al., 2012), the  
180 vascular tissue forms a complicated network of bundles scattered throughout the cross section,  
181 which, in a longitudinal view, are inclined and wind spirally towards the apex (e.g. Pizzolato &  
182 Sundberg, 2002; Pizzolato, 2002, 2004). Nevertheless, it is possible to establish the stem sectors  
183 in which the subsequent leaf traces are formed. Our results confirm that the arrangement of such  
184 stem sectors in *E. purpurata* was in accordance with the position of the leaf insertion at the stem,  
185 showing circumferential displacement in shoots with spiral patterns and a regular opposite  
186 arrangement in shoots with decussate phyllotaxis. Importantly, we have never observed the  
187 decussate pattern along the entire shoot; rather, it emerged during the development of a particular  
188 shoot, as leaves at its base, formed earlier in ontogeny, were separately initiated in a spiral  
189 sequence. Similarly, leaves above the decussate pattern, especially in the inflorescence, again  
190 represented Fibonacci phyllotaxis. These findings illustrate the repeated ontogenetic transitions  
191 between different patterns and indicate the developmental plasticity of the *E. purpurata* shoots.

192         Phyllotactic transitions are known to occur spontaneously during plant ontogeny along the  
193 same axis, and especially during the change of the developmental phase (Gómez-Campo, 1974;  
194 Meicenheimer, 1979, 1982; Battey & Lyndon, 1984; Zagórska-Marek, 1985, 1994; Kwiatkowska,  
195 1995; Banasiak & Zagórska-Marek, 2006; Zagórska-Marek & Szpak, 2008), as well as being  
196 evoked by chemical factors (e.g. Maksymowych & Erickson, 1977; Meicenheimer, 1981).

197 Among the immediate reasons for phyllotactic pattern transitions are variations in the geometric  
198 proportions between the organogenic zone of the meristem, where leaves are initiated, and leaf

199 primordium size (Zagórska-Marek, 1987; Kwiatkowska, 1995; Zagórska-Marek & Szpak, 2008;  
200 Wiss & Zagórska-Marek, 2012). In meristems with the relatively wide organogenic zone and  
201 small primordia, various arrangements of primordia and thus different phyllotactic patterns are  
202 possible, as in magnolia gynoecia (Zagórska-Marek, 1994; Zagórska-Marek & Szpak, 2008; Wiss  
203 & Zagórska-Marek, 2012), cacti (Gola, 1997; Mauseth, 2004), or capitula of the Asteraceae  
204 (Hernandez & Palmer, 1988; Szymanowska-Pułka, 1994). In contrast, if primordia are relatively  
205 large compared to the organogenic zone of the meristem, only limited leaf arrangements are  
206 possible, as, for example, in grasses. Therefore, ontogenetic changes in apex geometry and the  
207 parameters of growth can affect primordia distribution and cause alterations in phyllotaxis.  
208 Interestingly, repeated changes in phyllotaxis due to altered meristem proportions have been  
209 proven so far only in two mutants, *abphyll* in maize (Jackson & Hake, 1999; Giulini, Wang &  
210 Jackson, 2004) and *decussate* in rice (Itoh et al., 2012). The increased diameter of the meristems  
211 of these mutants in response to an affected cytokinin signalling pathway causes a phyllotaxis  
212 transition upon development from the distichy in seedlings to the decussate pattern (Jackson &  
213 Hake, 1999; Giulini, Wang & Jackson, 2004; Itoh et al., 2012). A similar process is observed in  
214 *Epipactis*: early in ramet development, the spiral pattern is formed, then transformed during  
215 growth progression into a decussate leaf arrangement. However, in *E. purpurata*, this transition is  
216 unpredictable and occurs infrequently in populations (>1%). It is impossible to reach an  
217 indisputable conclusion about the developmental and/or genetic background of this phyllotactic  
218 change due to a lack of molecular tools for this species as well as to the rarity of the phenomenon  
219 and of the taxon itself. It can however be speculated that, similarly to *abphyll* and *decussate*  
220 mutants, developmental alterations in meristem size cause the observed phyllotaxis transitions.  
221 This can partially be confirmed by the fact that *Epipactis* shoots with decussate phyllotaxis  
222 always undergo a second transition back to the Fibonacci pattern during the change to the  
223 generative phase, during which the meristem size and growth parameters of the shoot are known

224 to be significantly modified (e.g. Kwiatkowska, 2008). Additional evidence for the  
225 developmental plasticity of *E. purpurata* shoots is provided by the formation of pseudowhorls,  
226 i.e. gatherings of leaves seemingly located at one level of the stem due to the uneven elongation  
227 of internodes between them. Pseudowhorls are typical leaf arrangements in some species of  
228 *Peperomia* and *Galium* (Kwiatkowska, 1999; Rutishauser, 1999), and may also occur as a result  
229 of ontogenetic modifications of shoot growth, as in *Anagallis* (Kwiatkowska, 1995) and *Acacia*  
230 (Rutishauser, 1999).

### 231 **Conclusions**

232 In this article, we prove that in *E. purpurata* the spiral phyllotaxis is dominant; the  
233 presence of distichous leaf arrangement has not been confirmed. We document for the first time  
234 the presence of decussate phyllotaxis in *E. purpurata*, which is a rare exception to the typical  
235 spiral leaf arrangement in this species. We aim to draw the attention of orchid taxonomists to the  
236 intraspecific as well as the ontogenetic diversity of phyllotaxes in the Orchidaceae. Both  
237 phenomena are common in plants; moreover, even when one type of phyllotaxis prevails in a  
238 given taxon, it does not exclude the occurrence of other leaf arrangements; thus, the whole  
239 spectrum of possible phyllotaxes and their ontogenetic transitions must be considered in the  
240 course of taxonomic identification. Our finding of the atypical phyllotaxis is another example of  
241 ontogenetic variability in the genus *Epipactis*. In conclusion, we confirmed that spiral phyllotaxis  
242 is typical of *E. purpurata* and that the presence of other leaf arrangements has no taxonomic  
243 significance; this is another illustration of the developmental plasticity of the genus *Epipactis*.

### 244 **Acknowledgments**

245 We thank to the Curators and Staff of the herbaria of B, BR, C, FR, G, KTU, M, S, WRSL, Z, ZT  
246 for loans of the specimens; and to the Reviewers for valuable comments on the manuscript.

### 247 **References**

- 248 Adler I. 1974. A model of contact pressure in phyllotaxis. *Journal of Theoretical Biology* 45:1-79.  
249 DOI:10.1016/0022-5193(74)90043-5.
- 250 Adler I, Barabé D, Jean RV. 1997. A history of the study of phyllotaxis. *Annals of Botany* 80:  
251 231-244. DOI: 10.1006/anbo.1997.0422.
- 252 Arditti J. 1992. *Fundamentals of orchid biology*. John Wiley & Sons.
- 253 Banasiak AS, Zagórska-Marek B. 2006. Signals flowing from mature tissues to shoot apical  
254 meristem affect phyllotaxis in coniferous shoot. *Acta Societatis Botanicorum Poloniae* 75:113-  
255 121. DOI: 10.5586/asbp.2006.014.
- 256 Bateman RM, Rudall PJ, Moura M. 2013. Systematic revision of *Platanthera* in the Azorean  
257 archipelago: not one but three species, including arguably Europe's rarest orchid. *PeerJ* 1:e218.  
258 DOI: 10.7717/peerj.218.
- 259 Batista JAN, Bianchetti LB. 2002. A review of *Habenaria* (Orchidaceae) in Pabst and Dungs'  
260 Orchidaceae Brasilienses. *Lindleyana* 17:75-84.
- 261 Battey NH, Lyndon RF. 1984. Changes in apical growth and phyllotaxis on flowering and  
262 reversion in *Impatiens balsamina* L. *Annals of Botany* 54:553-567. DOI:  
263 10.1093/oxfordjournals.aob.a086826.
- 264 Brullo C, D'Emerico S, Pulvirenti S. 2013. Karyological and taxonomical considerations on  
265 *Epipactis cupaniana* sp. nov. (Orchidaceae) from Sicily. *Nordic Journal of Botany* 31:577-589.  
266 DOI: 10.1111/j.1756-1051.2012.01666.x.
- 267 Byng JW, Chase MW, Christenhusz MJM, Fay MF, Judd WS, Mabberley DJ, Soltis DE,  
268 Sennikov AN, Soltis DE, Soltis PS, Stevens PF. 2016. An update of the Angiosperm Phylogeny  
269 Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal*  
270 *of the Linnean Society* 181:1-20. DOI: 10.1111/boj.12385.

- 271 Chung MY, Chung MG. 2012. A review of the use of genetic markers in orchid systematics with  
272 emphasis on allozymes. *Biochemical Systematics and Ecology* 41:62-73. DOI:  
273 10.1016/j.bse.2011.12.012.
- 274 Claessens J, Kleynen J. 2011. *The flower of the European orchid: form and function*. Voerendaal:  
275 Jean Claessens & Jacques Kleynen.
- 276 Delforge P. 2006. *Orchids of Europe, North Africa and the Middle East*. London: A&C Black  
277 Publishers Ltd.
- 278 Dressler RL. 1993. *Phylogeny and classification of the orchid family*. Cambridge: University  
279 Press.
- 280 Fajardo CG, de Almeida Vieira F, Molina WF. 2014. Interspecific genetic analysis of orchids in  
281 Brazil using molecular markers. *Plant Systematics and Evolution* 300:1825-1832.  
282 DOI:10.1007/s00606-014-1009-9.
- 283 Giulini A, Wang J, Jackson D. 2004. Control of phyllotaxy by the cytokinin-inducible response  
284 regulator homologue ABPHYL1. *Nature* 430:1031-1034. DOI: 10.1038/nature02778.
- 285 Gola E. 1997. Phyllotactic spectra in cacti: *Mammillaria* species and some genera from *Rebutia*  
286 group. *Acta Societatis Botanicorum Poloniae* 66:237-257. DOI: 10.5586/asbp.1997.030.
- 287 Gola EM, Banasiak A. 2016. Diversity of phyllotaxis in land plants in reference to the shoot  
288 apical meristem structure. *Acta Societatis Botanicorum Poloniae* 85:3529. DOI:  
289 10.5586/asbp.3529.
- 290 Gómez-Campo C. 1974. Phyllotactic patterns in *Bryophyllum tubiflorum* Harv. *Botanical Gazette*  
291 135:49-58. DOI: 10.1086/336729.

- 292 Gregory RA, Romberger JA. 1972. The shoot apical ontogeny of the *Picea abies* seedling. I.  
293 Anatomy, apical dome diameter, and plastochron duration. *American Journal of Botany* 59:587-  
294 597.
- 295 Hernandez LF, Palmer JH. 1988. Regeneration of the sunflower capitulum after cylindrical  
296 wounding of the receptacle. *American Journal of Botany* 75:1253-1261.
- 297 Itoh JI, Hibara KI, Kojima M, Sakakibara H, Nagato Y. 2012. Rice DECUSSATE controls  
298 phyllotaxy by affecting the cytokinin signaling pathway. *The Plant Journal* 72:869-881. DOI:  
299 10.1111/j.1365-313x.2012.05123.x.
- 300 Jackson D, Hake S. 1999. Control of phyllotaxy in maize by the *abphyll* gene. *Development*  
301 126:315-323.
- 302 Jakubska-Busse A, Proćków J, Górniak M, Gola EM. 2012. Is *Epipactis pseudopurpurata*  
303 distinct from *E. purpurata* (Orchidaceae)? Evidence from morphology, anatomy, DNA and  
304 pollination biology. *Botanical Journal of the Linnean Society* 170: 243-256. DOI: 10.1111/j.1095-  
305 8339.2012.01288.x.
- 306 Jakubska-Busse A, Żołubak E, Jarzembowski P, Proćków J. 2017. Morphological variability in  
307 *Epipactis purpurata s. stricto* (Orchidaceae) – an analysis based on herbarium material and field  
308 observations. *Annales Botanici Fennici* 54: 55-66. DOI: 10.5735/085.054.0310.
- 309 Jean RV. 1994. *Phyllotaxis. A systemic study in plants morphogenesis*. New York: Cambridge  
310 University Press. DOI: 10.1017/cbo9780511666933.
- 311 Jin XH, Ren ZX, Xu SZ, Wang H, Li DZ, Li ZY. 2014. The evolution of floral deception in  
312 *Epipactis veratrifolia* (Orchidaceae): from indirect defense to pollination. *BMC Plant Biology*  
313 14:63. DOI: 10.1186/1471-2229-14-63.

- 314 Jones DL, Clements MA. 2002. A review of *Pterostylis* (Orchidaceae): 2. A new classification of  
315 *Pterostylis* R. Br. (Orchidaceae). *Australian Orchid Research* 4:64-124.
- 316 Kuhlemeier C. 2007. Phyllotaxis. *Trends in Plant Science* 12:143-150. DOI:  
317 10.1016/j.tplants.2007.03.004.
- 318 Kwiatkowska D. 1995. Ontogenetic changes of phyllotaxis in *Anagallis arvensis* L. *Acta*  
319 *Societatis Botanicorum Poloniae* 64:319-327. DOI: 10.5586/asbp.1995.041.
- 320 Kwiatkowska D. 1999. Formation of pseudowhorls in *Peperomia verticillata* (L.) A. Dietr. shoot  
321 exhibiting various phyllotactic patterns. *Annals of Botany* 83:675-685. DOI:  
322 10.1006/anbo.1999.0875.
- 323 Kwiatkowska D. 2008. Flowering and apical meristem growth dynamics. *Journal of*  
324 *Experimental Botany* 59:187-201. DOI: 10.1093/jxb/erm290.
- 325 Lipovšek M, Brinovec T, Brinovec M. 2017. *Epipactis helleborine* (L.) Crantz subsp. *moratoria*  
326 A. Riechelmann & A. Zirnsack., a new subspecies of Broad-leaved Helleborine in Slovenia.  
327 *Hacquetia* 16: 13-18. DOI: 10.1515/hacq-2016-0011.
- 328 Lovisa AGS, Verola CF, Antonelli A. 2010. Reassessing the temporal evolution of orchids with  
329 new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare  
330 South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). *Evolutionary Biology*  
331 10:177. DOI: 10.1186/1471-2148-10-177.
- 332 Maksymowych R, Erickson RO. 1977. Phyllotactic change induced by gibberellic acid in  
333 *Xanthium* shoot apices. *American Journal of Botany* 64:33-44.
- 334 Mauseth JD. 2004. Giant shoot apical meristems in cacti have ordinary leaf primordia but altered  
335 phyllotaxy and shoot diameter. *Annals of Botany* 94:145-153. DOI: 10.1093/aob/mch121.

- 336 Meicenheimer RD. 1979. Relationships between shoot growth and changing phyllotaxy of  
337 *Ranunculus*. *American Journal of Botany* 66:557-569.
- 338 Meicenheimer RD. 1981. Change in *Epilobium* phyllotaxy induced by N-1-naphthylphthalamic  
339 acid and  $\alpha$ -4-chlorophenoxyisobutyric acid. *American Journal of Botany* 68:1139-1154.
- 340 Meicenheimer RD. 1982. Change in *Epilobium* phyllotaxy during reproductive transitions.  
341 *American Journal of Botany* 69:1108-1118. DOI: 10.2307/2443085.
- 342 Mered'a P. 1999. Kl'úč na určovanie druhov rodu *Epipactis* Zinn publikovaných z územia  
343 Slovenska. Identification key to the species of the genus *Epipactis* Zinn published from Slovakia.  
344 *Bulletin Slovenskej Botanickéj Spoločnosti* 21:131-142.
- 345 O'Brien TP, McCully ME. 1981. *The study of plant structure: principles and selected methods*.  
346 Melbourne: Termarcarphi Pty Ltd.
- 347 Pizzolato TD. 2002. Procambial initiation for the vascular system in the shoot of *Raddia*  
348 *brasiliensis* (Poaceae, Bambusoideae). *International Journal of Plant Sciences* 163:877-889.  
349 DOI: 10.1086/342629.
- 350 Pizzolato TD. 2004. Procambial initiation for the vascular system in the shoot of *Alstroemeria*  
351 (Alstroemeriaceae, Liliales). *International Journal of Plant Sciences* 165:11-25. DOI:  
352 10.1086/380981.
- 353 Pizzolato TD, Sundberg MD. 2002. Initiation of the vascular system in the shoot of *Zea mays* L.  
354 (Poaceae). II. The procambial leaf traces. *International Journal of Plant Sciences* 163:353-367.  
355 DOI: 10.1086/339514.
- 356 Reinhardt D. 2005. Regulation of phyllotaxis. *The International Journal of Developmental*  
357 *Biology* 49:539-546. DOI: 10.1387/ijdb.041922dr.

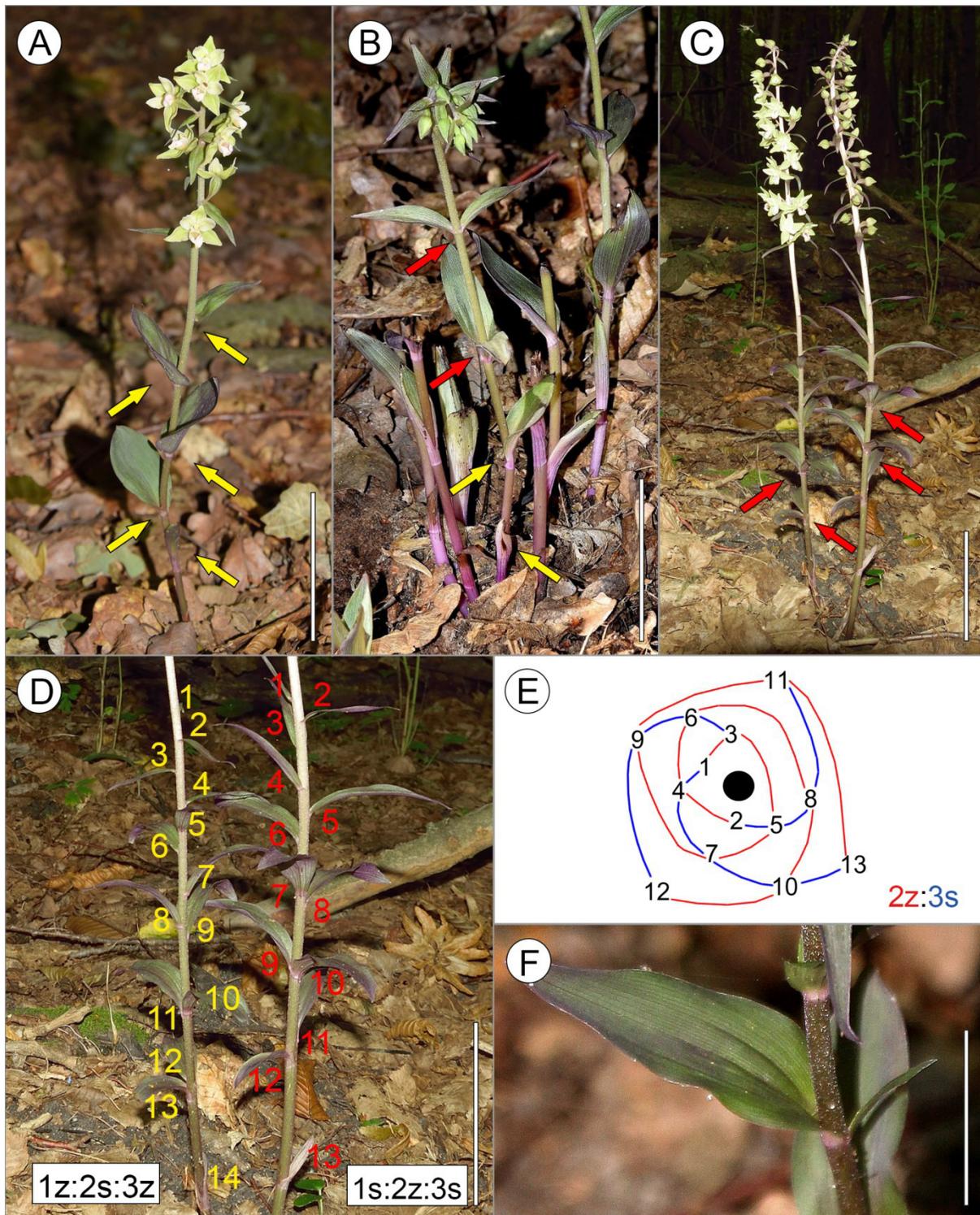
- 358 Rudall PJ, Bateman RM. 2002. Roles of synorganisation, zygomorphy and heterotopy in floral  
359 evolution: the gynostemium and labellum of orchids and other lilioid monocots. *Biological*  
360 *Reviews* 77:403-441. DOI: 10.1017/S1464793102005936.
- 361 Rutishauser R. 1998. Plastochrone ratio and leaf arc as parameters of a quantitative phyllotaxis  
362 analysis in vascular plants. In: Jean RV, Barabé D, eds. *Symmetry in plants*. Singapore: World  
363 Scientific Publ, 171-212.
- 364 Rutishauser R. 1999. Polymerous leaf whorls in vascular plants: developmental morphology and  
365 fuzziness of organ identities. *International Journal of Plant Sciences* 160:81-103. DOI:  
366 10.1086/314221.
- 367 Stace CA. 1991. *Plant taxonomy and biosystematics*. Cambridge: Cambridge University Press.
- 368 Szlachetko DL, Rutkowski P. 2000. *Gynostemia orchidalium* I. Helsinki: Finnish zoological and  
369 botanical publishing board.
- 370 Szymanowska-Pułka M. 1994. Phyllotactic patterns in capitula of *Carlina acaulis* L. *Acta*  
371 *Societatis Botanicorum Poloniae* 65:229-245. DOI: 10.5586/asbp.2011.043.
- 372 Thiers B. 2017. Index Herbariorum: A global directory of public herbaria and associated staff.  
373 New York Botanical Garden's Virtual Herbarium. Available at  
374 <http://sweetgum.nybg.org/science/ih/> (accessed 22 June 2017).
- 375 Tranchida-Lombardo V, Cafasso D, Cristaudo A, Cozzolino S. 2011. Phylogeographic patterns,  
376 genetic affinities and morphological differentiation between *Epipactis helleborine* and related  
377 lineages in a Mediterranean glacial refugium. *Annals of Botany* 107:427-436. DOI:  
378 10.1093/aob/mcq256.
- 379 Wiss D, Zagórska-Marek B. 2012. Geometric parameters of the apical meristem and the quality  
380 of phyllotactic patterns in *Magnolia* flowers. *Acta Societatis Botanicorum Poloniae* 81:203-216.  
381 DOI: 10.5586/asbp.2012.029.

- 382 Withner CL, Nelson PK, Wejksnora PJ. 1974. The anatomy of orchids. In: Withner CL, eds. *The*  
383 *orchids: scientific studies*. New York: Wiley-Interscience, 267-347.
- 384 Zagórska-Marek B. 1985. Phyllotactic patterns and transitions in *Abies balsamea*. *Canadian*  
385 *Journal of Botany* 63:1844-1854. DOI: 10.1139/b85-259.
- 386 Zagórska-Marek B. 1987. Phyllotaxis triangular unit; phyllotactic transitions as the consequences  
387 of the apical wedge disclinations in a crystal-like pattern of the units. *Acta Societatis*  
388 *Botanicorum Poloniae* 56:229-255. DOI: 10.5586/asbp.2011.043.
- 389 Zagórska-Marek B. 1994. Phyllotaxic diversity in *Magnolia* flowers. *Acta Societatis*  
390 *Botanicorum Poloniae* 63:117-137. DOI: 10.5586/asbp.1994.017.
- 391 Zagórska-Marek B, Szpak M. 2008. Virtual phyllotaxis and real plant model cases. *Functional*  
392 *Plant Biology* 35:1025-1033. DOI: 10.1071/FP08076.
- 393 Zhao Y, Tang M, Bi Y. 2017. Nuclear genetic diversity and population structure of a vulnerable  
394 and endemic orchid (*Cymbidium tortisepalum*) in Northwestern Yunnan, China. *Scientia*  
395 *Horticulturae* 219:22-30. DOI: 10.1016/j.scienta.2017.02.033.

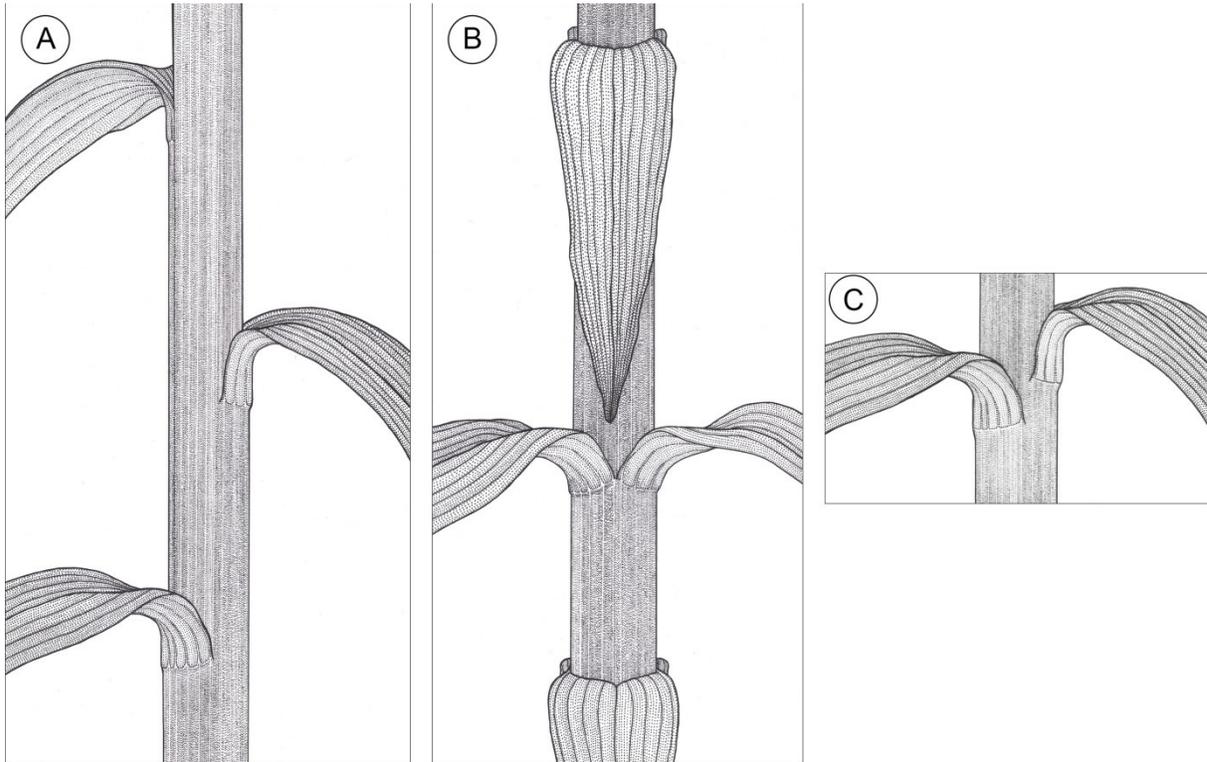
## 396 **Figure legends**

397 **Figure 1.** Diversity of leaf arrangements in *E. purpurata*. (A) Typical spiral phyllotaxis; (B) An  
398 atypical decussate arrangement of *E. purpurata* shoots. Leaves are initiated in pairs (indicated by  
399 red arrows) which in successive nodes are perpendicular to one another. Note that the lower  
400 cauline leaves (indicated by yellow arrows) and bracts are inserted separately at the stem  
401 according to the spiral sequence, showing the ontogenetic transitions of the phyllotactic pattern;  
402 (C, D) Formation of pseudowhorls. Spirally initiated leaves gather seemingly at one level of the  
403 stem due to uneven internode elongation, forming pseudowhorls (indicated by red arrows).  
404 However, analysis of the leaf circumferential distribution proves the spiral sequence of leaf

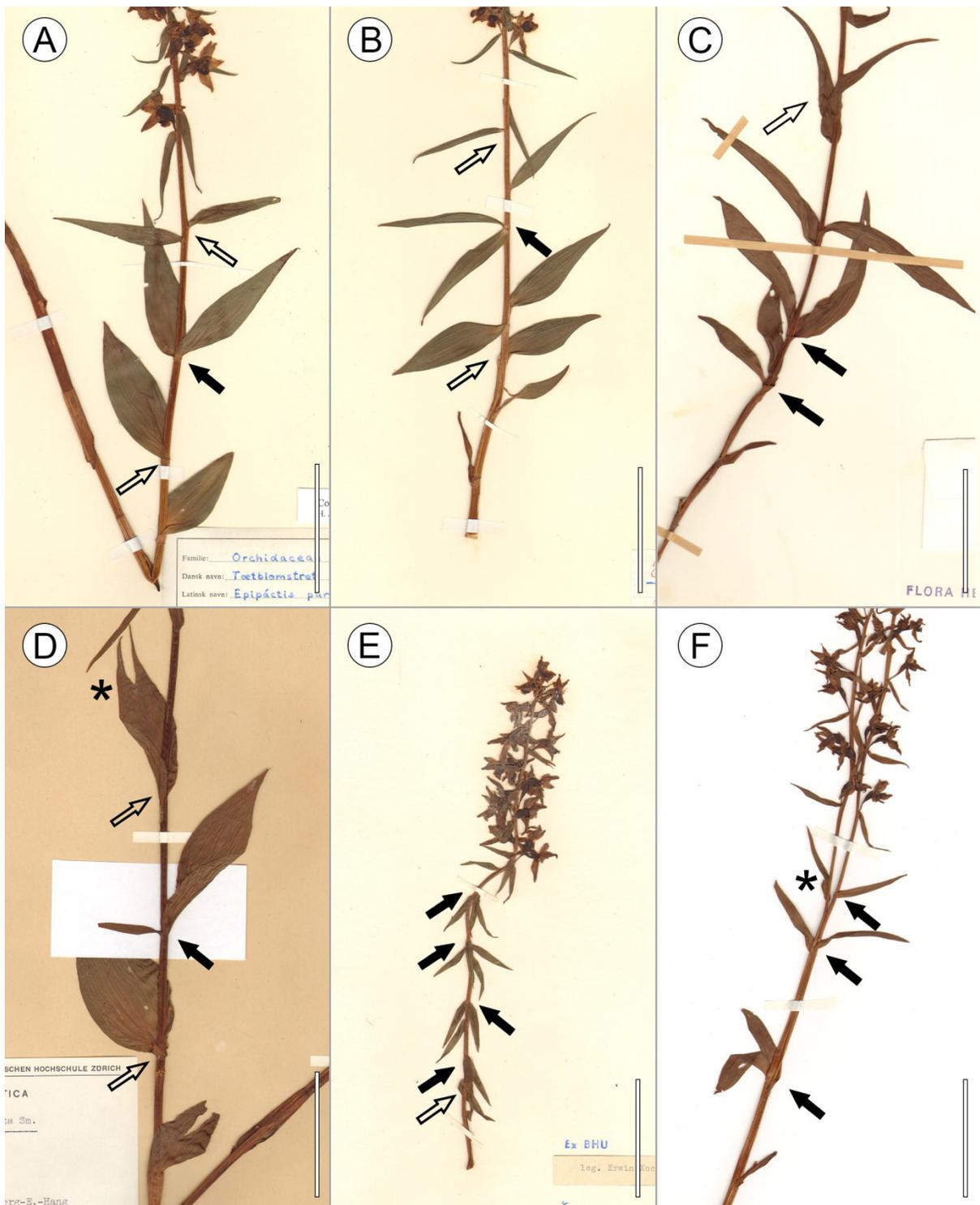
405 initiation (D). (E) Graphic representation of the leaf arrangement along the shoot presented in (C–  
406 D); red and blue lines represent parastichies winding toward the apex (black circle), i.e. from  
407 older to younger leaves in two opposite directions: clockwise (S chirality, blue lines) and  
408 counterclockwise (Z chirality, red lines); successive leaves are numbered, with 1 indicating the  
409 youngest leaf/bract and the highest number (13 or 14) indicating the oldest lower cauline leaf.  
410 Please note that the two ramets of a single genet presented in (C–D) are characterised by opposite  
411 chiralities of the ontogenetic spiral. (F) Developmental aberration in the shoot of *E. purpurata*.  
412 Two leaves differing greatly in size are visible at one level of the stem. Scale bars 5 cm (A–D)  
413 and 3 cm (F).



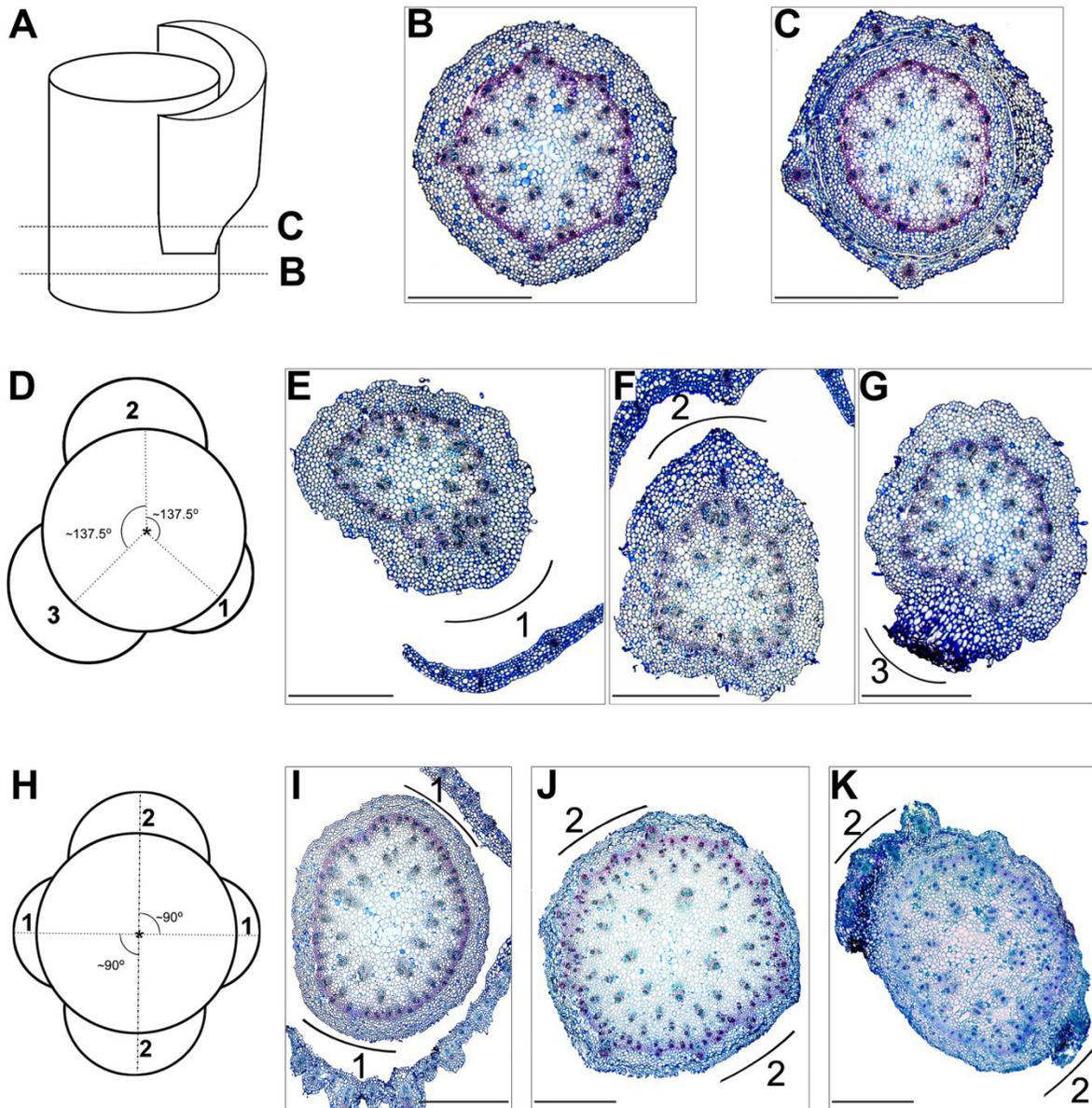
414 **Figure 2.** Graphic representation of leaf arrangements observed in *E. purpurata* shoots (drawn by  
415 Z. Łobas). (A) Typical spiral distribution of leaves along the stem; (B) Decussate phyllotaxis; (C)  
416 Formation of pseudowhorls as a result of leaves gathering at the same stem level due to the  
417 limited growth (elongation) of the internode.



418 **Figure 3.** Voucher specimens of *E. purpurata* presenting a number of exemplary abnormalities in  
419 leaf arrangement and shoot development. These abnormalities (indicated by filled black  
420 arrowheads) include pairs of opposite leaves (A, C, D, F) capable of forming a regular decussate  
421 pattern (C), pseudowhorls (B, E), extremely diversified sizes of leaves and split leaf tips (labelled  
422 with an asterisk, D), and a bifurcating shoot (F). Typically formed and arranged leaves below and  
423 above the nodes with decussate phyllotaxis or pseudowhorls are indicated by clear arrowheads.  
424 The acronyms of the herbaria and the voucher numbers are as follows: (A) DK-0005389, (B)  
425 DK-0005409, (C) Z-000088596, (D) ZT-00071775, (E) B 10 0591214, (F) FR-0001004. Scale  
426 bars 5 cm.



427 **Figure 4.** Histological analyses of the leaf vasculature in the shoots of *E. purpurata* differing in  
428 phyllotactic patterns. (A-C) Diagrammatic representation (A) of the internode (B), node (C), and  
429 corresponding cross sections (B, C); dashed lines refer to the level of the cross section. Vascular  
430 bundles which will be incorporated into the leaf are already split in the internode (B) and visible  
431 in the cortical part of the stem. Later, in the node (C), they depart to form the leaf vasculature.  
432 (D-G) Vascular structure of a shoot with spiral phyllotaxis. (D) a graphic interpretation of the  
433 shoot, viewed from the top, shows the circumferential arrangement of three successively  
434 developed leaves (numbered 1, 2, and 3); the angle (circumferential displacement) between them  
435 is close to  $137.5\text{--}140^\circ$  and corresponds to the divergence angle for Fibonacci phyllotaxis. The  
436 cross sections (E-G) present the same shoot at the levels corresponding to the nodes of three  
437 successively developed leaves (numbered 1–3). The positions of successive leaves are marked  
438 outside the cross sections as arcs. (H-K) Vascular structure of a shoot with decussate phyllotaxis.  
439 (H) a graphic interpretation of the shoot, viewed from the top, shows the circumferential  
440 arrangement of two pairs (numbered 1 and 2) of opposite leaves. Leaves of the second pair are  
441 circumferentially shifted and located halfway between those of the first pair; as a result, both  
442 pairs, the first and the second, are mutually perpendicular. Cross sections (I-K) present the same  
443 shoot at the levels corresponding to the nodes of the first and the second pairs (numbered 1 and 2)  
444 of opposite leaves and the internode between them. The positions of successive leaf pairs are  
445 marked outside the cross sections as arcs. Scale bars (B-C, E-G, I-K)  $1000\ \mu\text{m}$ .



446 **Table 1.** Leaf arrangements in the material analysed. In a given shoot, more than one phyllotactic  
 447 pattern can occur, as, for example, in ramets with a decussate pattern (see the text). In the table,  
 448 for clarity, shoots with abnormal phyllotaxis (decussate pattern or with pseudowhorls) are  
 449 counted only once within the total amount of ramets analysed. (A) Ramets from SW Poland:  
 450 Nieszczyce (two cases) and the Błyszcz nature reserve (single case). (B) Phyllotaxis present in  
 451 specimens from all herbaria analysed. (C) Herbarium voucher specimen details: Z–000088596;  
 452 ZT–00071819. (D) Voucher specimens with aberrations in leaf arrangements and the acronyms of  
 453 the herbaria collections are listed in Appendix A.

	no. of ramets	Leaf arrangement (phyllotaxis)					
		Spiral		Whorled decussate		Pseudowhorls	
		no. of ramets	%	no. of ramets	%	no. of ramets	%
Fresh material	477	450	94.4	3 <sup>A</sup>	0.6	24	5.0
Herbarium vouchers	806	760 <sup>B</sup>	94.4	2 <sup>C</sup>	0.2	44 <sup>D</sup>	5.4
<b>Total</b>	<b>1283</b>	<b>1210</b>	<b>94.7</b>	<b>5</b>	<b>0.4</b>	<b>68</b>	<b>5.3</b>