

Leaf arrangements are invalid in the taxonomy of orchid species

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The selection and validation of proper distinguishing characters are of crucial importance in taxonomic revisions. The classification of orchids, due to the limited availability of genetic tools, is still based for the most part on features related to column structure and general species morphology. One of the widely accepted 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*. 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* 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 the great range of ontogenetic variability and plasticity in *E. purpurata*. Therefore, the distribution of leaves along the stem has no taxonomic significance in classification and delimitations of taxa within the genus *Epipactis*.

1 Leaf arrangements are invalid in the taxonomy of orchid species

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8 Abstract

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12 species morphology. One of the widely accepted vegetative characters quoted in orchid
13 manuals is leaf arrangement. However, phyllotactic diversity and ontogenetic changeability
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17 course of field research and screening of herbarium materials, we indisputably disproved the
18 presence of distichous phyllotaxis in the species *Epipactis purpurata* and confirmed the spiral
19 Fibonacci pattern as the dominant leaf arrangement. In addition, detailed analyses revealed
20 the presence of atypical decussate phyllotaxis in this species, as well as demonstrated the
21 ontogenetic formation of pseudowhorls. These findings confirm the great range of ontogenetic
22 variability and plasticity in *E. purpurata*. Therefore, the distribution of leaves along the stem
23 has no taxonomic significance in classification and delimitations of taxa within the genus
24 *Epipactis*.

25 **Introduction**

26 Understanding plant variability and the underlying genetic and developmental
27 mechanisms are fundamental to modern plant classifications (Batista & Bianchetti, 2002;
28 Jones & Clements, 2002; Rudall & Bateman, 2002; Bateman, Rudall & Moura, 2013).
29 Genotypic and phenotypic variations reflect the adaptation of a plant to diverse and often
30 demanding environments, and are generally accepted as driving forces behind speciation
31 (Stace, 1991). The family Orchidaceae has recently been extensively studied and
32 taxonomically revised. Despite recent advances in molecular techniques, as yet there are no
33 well-defined genetic markers for orchids which would enable the delimitation of e.g.,
34 aggregate taxa (Chung & Chung, 2012). Therefore, taxonomic surveys focus mostly on
35 flower and especially column (gynostemium) structure, acknowledged as the most reliable
36 and stable characteristics in orchid classifications (Mered'a, 1999; Szlachetko & Rutkowski,
37 2000; Claessens & Kleynen, 2011). However, these surveys also include general
38 morphological descriptions which are often used in manuals for the determination of taxa
39 (Dressler, 1993; Szlachetko & Rutkowski, 2000). One of these widely used characteristics is
40 leaf arrangement; however, detailed data on this aspect in Orchidaceae is lacking.

41 The phenomenon of regular and periodic patterning of leaves (or other lateral organs)
42 is called phyllotaxis and has drawn the attention of researchers for centuries (e.g., Jean 1994;
43 Adler, Barabé & Jean, 1997; Reinhardt, 2005; Kuhlemeier, 2007). In the plant kingdom, two
44 major types of leaf arrangements, whorled and spiral (helical) (Zagórska-Marek, 1985, 1994),
45 are recognised. In whorled phyllotaxis, more than one leaf is simultaneously initiated at the
46 meristem, forming a whorl of leaves in a node. The next whorl is circumferentially displaced
47 so that its elements (leaves) are located in a mid-distance between leaves of the previous
48 whorl. A special whorled leaf arrangement, called decussate phyllotaxis, occurs when two
49 leaves are formed per whorl. This is a common pattern in, for example, the families

50 Lamiaceae and Caryophyllaceae (Rutishauser, 1998; Reinhardt, 2005; Gola & Banasiak,
51 2016). Another modification of whorled phyllotaxis is distichy, whereby only one leaf is
52 initiated per whorl, but the next leaf is displaced the half distance around the stem, i.e. 180° ,
53 with respect to the previous leaf. As a result, leaves occur in two opposite ranks along the
54 stem. This leaf distribution is typical, for example, of the family Poaceae (Gola & Banasiak,
55 2016).

56 In the second major type of leaf arrangement, spiral phyllotaxis, successive leaves are
57 initiated separately at the meristem and can be linked along the stem by a spiral line called the
58 ontogenetic spiral. The spatial configuration (chirality) of the ontogenetic spiral can be either
59 clockwise (S chirality) or counterclockwise (Z chirality) (Zagórska-Marek, 1985). Successive
60 leaves are circumferentially displaced at a stable angular distance (divergence angle) and thus
61 do not overlap (Zagórska-Marek, 1985; Jean, 1994). The most frequent spiral phyllotaxis in
62 the plant kingdom (e.g., Zagórska-Marek, 1985, 1994; Jean, 1994; Adler, Barabé & Jean,
63 1997; Rutishauser, 1998) is related to the series of Fibonacci numbers, i.e. 1, 1, 2, 3, 5, 8, ...
64 etc., in which each element is the sum of the two preceding elements. The first number in this
65 series refers to the ontogenetic spiral; however, this is hardly visible in the majority of shoots
66 due to the shortening of internodes between successive leaves. Then the secondary spirals
67 (parastichies), winding towards the apex in both directions, clockwise (S) and
68 counterclockwise (Z), become visible at the stem surface. The most discernible spirals,
69 crossing at right angles or near-right angles, form a contact parastichy pair, represented by the
70 two succeeding numbers of the phyllotactic series, for example, 1:2 or 2:3 in the Fibonacci
71 pattern (Adler, 1974; Zagórska-Marek, 1985, 1994; Jean, 1994). This formula unequivocally
72 identifies the phyllotaxis of a given shoot (Zagórska-Marek, 1985, 1994).

73 It is generally accepted that in primitive orchid taxa, leaves are spirally arranged,
74 whereas in more advanced species, phyllotaxis is distichous (Withner, Nelson & Wejksnora,

75 1974; Dressler, 1993). Rarely, due to the uneven elongation of internodes, two or more leaves
76 are gathered at the same stem level (Dressler, 1993). In the genus *Epipactis*, the object of our
77 analysis, similarly to other orchids, leaves are usually reported as distichously arranged, but in
78 some species spiral phyllotaxis can also occur (Dressler, 1993; Delforge, 2006). The type of
79 leaf arrangement is even used to distinguish between or to characterise complex taxa, for
80 example, *E. atrorubens*, *E. tremolsii* and *E. helleborine*. Despite this general opinion, accurate
81 data on phyllotaxis in the genus *Epipactis* suitable for use in manuals is lacking. Therefore,
82 the aims of our research were to (i) analyse the variability of leaf arrangements in *E.*
83 *purpurata* in natural conditions; (ii) and quantify phyllotaxis diversity throughout the
84 European range of the species based on herbarium materials, and thus (iii) to validate the
85 usefulness of leaf arrangements in taxa identification within the genus *Epipactis*.

86 **Materials and Methods**

87 Long-term field investigations of natural populations of *E. purpurata* were performed
88 between 2003 and 2016 in permanent research plots in four locations in south-western
89 Poland: in Nieszczyce near Rudna (51°32'14.26"N, 16°23'56.26"E), the "Błyszcz" nature
90 reserve near Pątnów Legnicki (51°15'37.09"N, 16°12'56.95"E), Wałkowa near Milicz
91 (51°30'00.46"N, 17°18'56.04"E) and Straża near Wińsko (51°23'51.40"N, 16°45'52.47"E). In
92 this article, only the results of the analyses carried out in 2015 and 2016 are presented. In
93 addition, relevant specimens from diverse geographical regions deposited in European
94 herbaria (acronyms abbreviations: B, BR, C, FR, G, K, KTU, M, S, WRSL, Z, ZT), were
95 analysed.

96 Leaf arrangements were analysed in both fresh and voucher specimens using the
97 formula of a contact parastichy pair (Adler, 1974; Zagórska-Marek, 1985, 1994). In addition,
98 a series of transverse sections through the mature vegetative shoots were prepared in order to
99 indirectly confirm the leaf arrangements. At the moment when differences in leaf phyllotaxis

100 became macroscopically visible, inflorescences had already been formed and shoot apical
101 meristems were not available for detailed analyses.

102 For anatomical sectioning, shoot fragments which differed in leaf arrangements were
103 collected and fixed in FAA (a formyl-acetic acid–50% ethanol mixture). Following
104 dehydration in an increasing series of tertiary butyl alcohols (50%, 70%, 90%, 96%, and three
105 changes in the pure butanol), the plant material was embedded in Paraplast X-tra (Sigma-
106 Aldrich) and transversely cut, using a rotary microtome (Leica RM2135; Leica Instruments),
107 into 10–20 μm sections. Series of these cross sections were then de-waxed and stained with
108 the Alcian blue-Safranin O mixture (1:1 v/v; O'Brien and McCully, 1981). Sequential digital
109 images were taken using the system: a bright-field microscope Olympus BX 50 – Olympus
110 DP70 camera – Cell^B software (Olympus Optical, Poland). Digital images were processed in
111 Fireworks MX 2004 (Macromedia, USA) and Photoshop CS6 (Adobe Systems, USA). Plant
112 images were also taken in the field using Canon EOS 50D and Nikon D5300 cameras.
113 Experimental studies and material sampling were done with the permissions of the Regional
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115 WPN.6205.122.2016. IL, WPN.6205.122.2016. IL and WPN 6400.29.2016.IL.

116 **Results**

117 In the course of our research, more than 470 ramets of *E. purpurata* were analysed in
118 2015 and 2016 in the field, along with over 800 individual herbarium specimens (Table 1.). In
119 the majority of shoots (ca. 94% of all studied ramets), leaves were separately and spirally
120 arranged along the stem (Figs. 1A and 2, Table 1.). Their arrangement corresponded to 1:2 or
121 2:3 contact parastichy pairs, which are expressions of the main Fibonacci pattern. In the
122 analysed material, the frequencies of both spatial configurations of spiral patterns were
123 similar, with the ontogenetic spiral winding clockwise (S-chirality) in 51.9% and
124 counterclockwise (Z-chirality) in 48.1% of cases.

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

131 In several shoots (ca. 5%), two or three leaves were gathered close to one another,
132 seemingly at the same level of the stem (Figs. 1C, D and 3, Table 1.). However, the leaves in
133 such gatherings did not form opposite pairs and, in extreme cases, were distinctly inserted on
134 one side of the stem (Fig. 3). The analysis of their spatial distribution proved that they were
135 arranged according to the spiral Fibonacci pattern, which was continued along the whole
136 shoot (Fig. 1D and E). Thus they were identified as pseudowhorls.

137 Histological analyses of mature shoots representing different phyllotactic patterns
138 showed the arrangement of vascular tissue at the cross sections in relation to the leaf position.
139 Vascular bundles were scattered throughout the cross section and distributed typically of
140 monocotyledonous plants. In shoots with a spiral leaf arrangement, at one side of the stem,
141 below the node, vascular bundles divided, giving rise to the leaf vasculature (leaf trace).
142 These newly divided vascular bundles, at the level of leaf insertion in the node, diverged to
143 the leaf, forming its supply system (Fig. 4B and C). After leaf departure, in the region of the
144 internode, bundles were again relatively regularly scattered throughout the cross section. In
145 the next node, the successive leaf trace was formed in the stem sector circumferentially
146 distant ca. 137–140° from the previous leaf (Fig. 4B). This pattern repeated along the stem in
147 relation to the successive leaves.

148 In shoots with decussate phyllotaxis, the leaf trace formation for leaves of one pair
149 occurred simultaneously at opposite sides of the stem (Fig. 4C). Vascular bundles in two

150 facing sectors split and departed, forming the vasculature of a given pair. In the subsequent
151 node, the leaf traces for the next pair were again formed by the splitting of existing bundles,
152 but in perpendicular sectors (Fig. 4C).

153 Discussion

154 In taxonomic descriptions of the species belonging to the genus *Epipactis*, the
155 distichous and/or spiral leaf arrangement is usually cited as a typical pattern (Dressler 1993;
156 Delforge 2006). However, during over a decade of research on *Epipactis* morphology, we
157 found no distichous phyllotaxis; instead, in 2016, we discovered an interesting atypical
158 decussate arrangement of leaves in *E. purpurata*. This prompted us to perform a detailed
159 survey of phyllotaxis in this species. In the course of our research, using the formula of a
160 contact parastichy pair, we indisputably disproved the presence of distichy in *E. purpurata*
161 and confirmed the presence of spiral phyllotaxis as the typical leaf arrangement in this
162 species. The phyllotaxis here was identified as that representing the most common Fibonacci
163 pattern in plants (e.g., Jean, 1994; Adler, Barabé & Jean, 1997; Rutishauser, 1998). This
164 pattern occurred in both spatial configurations with comparable frequency, indicating that the
165 direction of the ontogenetic spiral in this species is randomly selected, similarly as in other
166 plant species (Gregory & Romberger, 1972; Gómez-Campo, 1974; Zagórska-Marek, 1985,
167 1994).

168 The only exception to the typical spiral Fibonacci pattern in *E. purpurata* was the
169 occurrence of the whorled decussate phyllotaxis. The decussate pattern in this species was
170 established based on the circumferential arrangement of leaves and further confirmed by
171 analysis of the vasculature. In monocot shoots, *E. purpurata* included (Jakubska-Busse et al.,
172 2012), the vascular tissue forms a complicated network of bundles scattered throughout the
173 cross section, which, in a longitudinal view, are inclined and wind spirally towards the apex
174 (e.g., Pizzolato & Sundberg, 2002; Pizzolato, 2002, 2004). Nevertheless, it is possible to

175 establish the stem sectors in which the subsequent leaf traces are formed. Our results confirm
176 that the arrangement of such stem sectors in *E. purpurata* was in accordance with the position
177 of the leaf insertion at the stem, showing circumferential displacement in shoots with spiral
178 patterns and a regular opposite arrangement in shoots with decussate phyllotaxis. Importantly,
179 we have never observed the decussate pattern along the entire shoot; rather, it emerged during
180 the development of a particular shoot, as leaves at its base, formed earlier in ontogeny, were
181 separately initiated in a spiral sequence. Similarly, leaves above the decussate pattern,
182 especially in the inflorescence, again represented Fibonacci phyllotaxis. These findings
183 illustrate the repeated ontogenetic transitions between different patterns and indicate the
184 developmental plasticity of the *E. purpurata* shoots.

185 Phyllotactic transitions are known to occur spontaneously during plant ontogeny along
186 the same axis, and especially during the change of the developmental phase (Gómez-Campo,
187 1974; Meicenheimer, 1979, 1982; Battey & Lyndon, 1984; Zagórska-Marek, 1985, 1994;
188 Zagórska-Marek & Szpak, 2008; Kwiatkowska, 1995; Banasiak & Zagórska-Marek, 2006), as
189 well as being evoked by chemical factors (e.g., Maksymowych & Erickson, 1977;
190 Meicenheimer, 1981). Among the immediate reasons for phyllotactic pattern transitions are
191 variations in the geometric proportions between the organogenic zone of the meristem, where
192 leaves are initiated, and leaf primordium size (Zagórska-Marek, 1987; Kwiatkowska, 1995;
193 Zagórska-Marek & Szpak, 2008; Wiss & Zagórska-Marek, 2012). In meristems with the
194 relatively wide organogenic zone and small primordia, various arrangements of primordia and
195 thus different phyllotactic patterns are possible, as in magnolia gynoecia (Zagórska-Marek,
196 1994; Zagórska-Marek & Szpak, 2008; Wiss & Zagórska-Marek, 2012), cacti (Gola, 1997;
197 Mauseth, 2004), or capitula of Asteraceae (Hernández & Palmer, 1988; Szymanowska-Pułka,
198 1994). In contrast, if primordia are relatively large compared to the organogenic zone of the
199 meristem, only limited leaf arrangements are possible, as, for example, in grasses. Therefore,

200 ontogenetic changes in apex geometry and the parameters of growth can affect primordia
201 distribution and cause alterations in phyllotaxis. Interestingly, repeated changes in phyllotaxis
202 due to altered meristem proportions have been proven so far only in two mutants, *abphyll* in
203 maize (Jackson & Hake, 1999; Giulini, Wang & Jackson, 2006) and *decussate* in rice (Itoh et
204 al., 2012). The increased diameter of the meristems of these mutants in response to an
205 affected cytokinin signalling pathway causes a phyllotaxis transition upon development from
206 the distichy in seedlings to the decussate pattern (Jackson & Hake, 1999; Giulini, Wang &
207 Jackson, 2006; Itoh et al., 2012). A similar process is observed in *Epipactis*: early in ramet
208 development, the spiral pattern is formed, then transformed during growth progression into a
209 decussate leaf arrangement. However, in *E. purpurata*, this transition is unpredictable and
210 occurs infrequently in populations (>1%). It is impossible to reach an indisputable conclusion
211 about the developmental and/or genetic background of this phyllotactic change due to a lack
212 of molecular tools for this species as well as to the rarity of the phenomenon and of the taxon
213 itself. It can however be speculated that, similarly to *abphyll* and *decussate* mutants,
214 developmental alterations in meristem size cause the observed phyllotaxis transitions. This
215 can partially be confirmed by the fact that *Epipactis* shoots with decussate phyllotaxis always
216 undergo a second transition back to the Fibonacci pattern during the change to the generative
217 phase, during which the meristem size and growth parameters of the shoot are known to be
218 significantly modified (e.g., Kwiatkowska, 2008). Additional evidence for the developmental
219 plasticity of *E. purpurata* shoots is provided by the formation of pseudowhorls, i.e. gatherings
220 of leaves seemingly located at one level of the stem due to the uneven elongation of
221 internodes between them. Pseudowhorls are typical leaf arrangements in some species of
222 *Peperomia* and *Galium* (Kwiatkowska, 1999; Rutishauser, 1999), and may also occur as a
223 result of ontogenetic modifications of shoot growth, as in *Anagallis* (Kwiatkowska, 1995) and
224 *Acacia* (Rutishauser, 1999).

225 **Conclusions**

226 In this article, we document for the first time the presence of decussate phyllotaxis in
227 *E. purpurata*, which is a rare exception to the typical spiral leaf arrangement in this species.
228 We aim to draw the attention of orchid taxonomists to the intraspecific as well as the
229 ontogenetic diversity of phyllotaxes in Orchidaceae. Both phenomena are common in plants;
230 moreover, even when one type of phyllotaxis prevails in a given taxon, it does not exclude the
231 occurrence of other leaf arrangements; thus, the whole spectrum of possible phyllotaxes and
232 their ontogenetic transitions must be considered in the course of taxonomic identification. Our
233 finding of the atypical phyllotaxis is another example of ontogenetic variability in the genus
234 *Epipactis*. In conclusion, the presence of different leaf arrangements in *E. purpurata* has no
235 taxonomic significance in the classification of the genus *Epipactis* and species/taxa
236 delimitations.

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363

364 **Figure legends**

365 **Figure 1.** Diversity of leaf arrangements in *E. purpurata*. (A) Typical spiral phyllotaxis; (B)
366 An atypical decussate arrangement of *E. purpurata* shoots. Leaves are initiated in pairs
367 (indicated by red arrows) which in successive nodes are perpendicular to one another. Note
368 that the lower cauline leaves (indicated by yellow arrows) and bracts are inserted separately at
369 the stem according to the spiral sequence, showing the ontogenetic transitions of the
370 phyllotactic pattern; (C, D) Formation of pseudowhorls. Spirally initiated leaves gather
371 seemingly at one level of the stem due to uneven internode elongation, forming pseudowhorls
372 (indicated by red arrows). However, analysis of the leaf circumferential distribution proves
373 the spiral sequence of leaf initiation (D). (E) Graphic representation of the leaf arrangement
374 along the shoot presented in (C–D); red and blue lines represent parastichies winding toward
375 the apex (black circle), i.e. from older to younger leaves in two opposite directions: clockwise
376 (S chirality, blue lines) and counterclockwise (Z chirality, red lines); successive leaves are
377 numbered, with 1 indicating the youngest leaf/bract and the highest number (13 or 14)
378 indicating the oldest lower cauline leaf. Please note that the two ramets of a single genet
379 presented in (C–D) are characterised by opposite chiralities of the ontogenetic spiral. (F)
380 Developmental aberration in the shoot of *E. purpurata*. Two leaves differing greatly in size
381 are visible at one level of the stem. Scale bars 5 cm (A–D) and 3 cm (F).

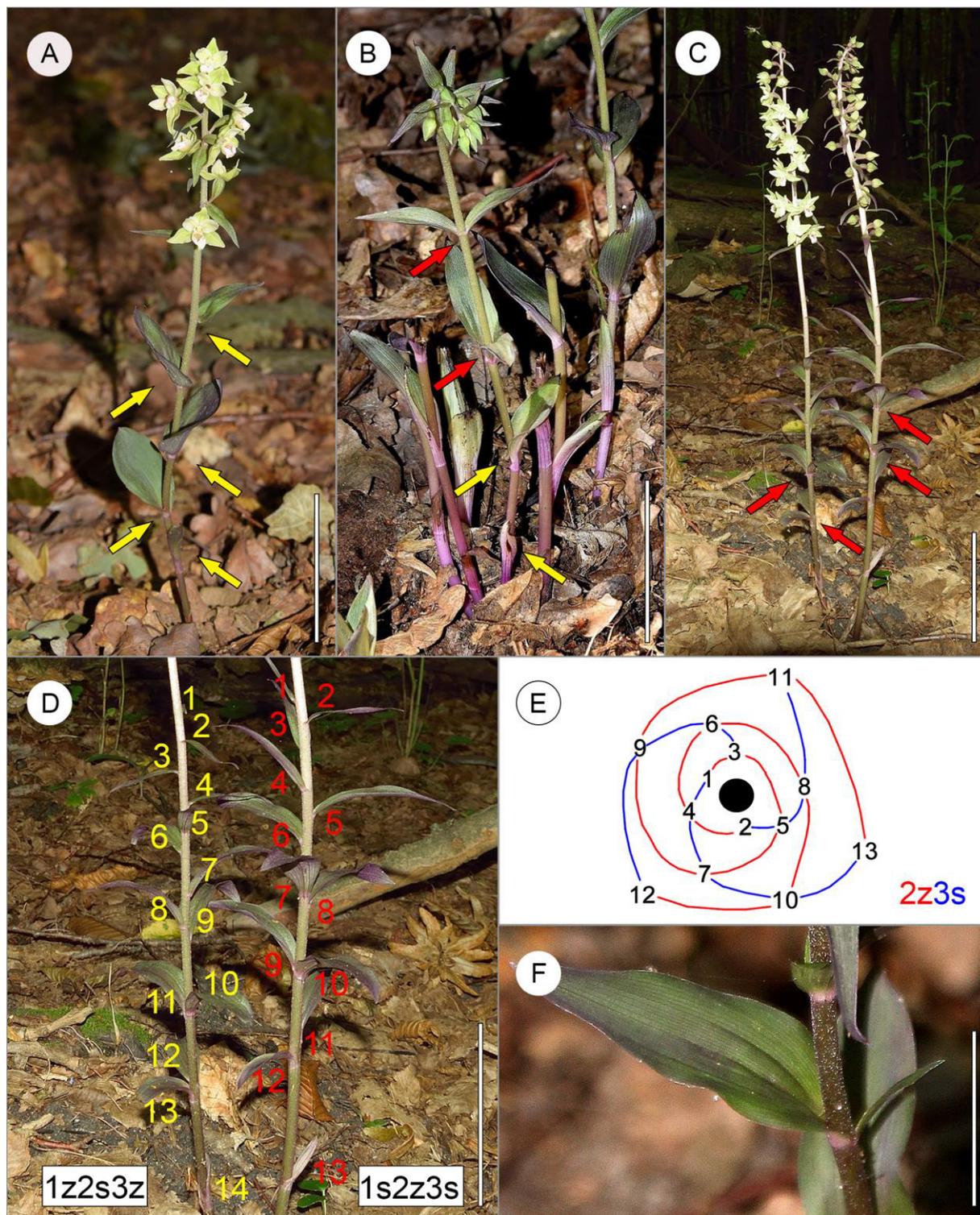


Figure 2. Graphic representation of leaf arrangements observed in *E. purpurata* shoots. (A)

Typical spiral distribution of leaves along the stem; (B) Decussate phyllotaxis; (C) Formation of pseudowhorls as a result of leaves gathering at the same stem level due to the limited growth (elongation) of the internode.

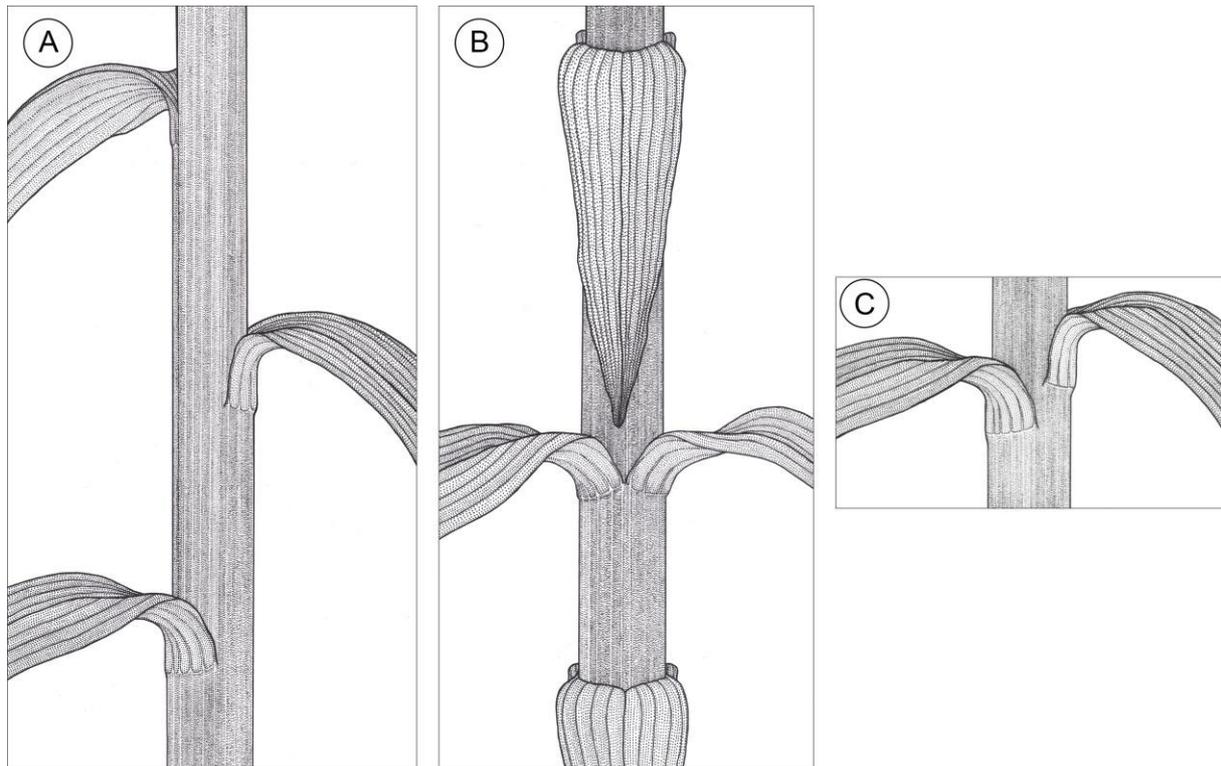


Figure 3. Voucher specimens of *E. purpurata* presenting a number of exemplary abnormalities in leaf arrangement and shoot development. These abnormalities (indicated by filled black arrowheads) include pairs of opposite leaves (A, C, D, F) capable of forming a regular decussate pattern (C), pseudowhorls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

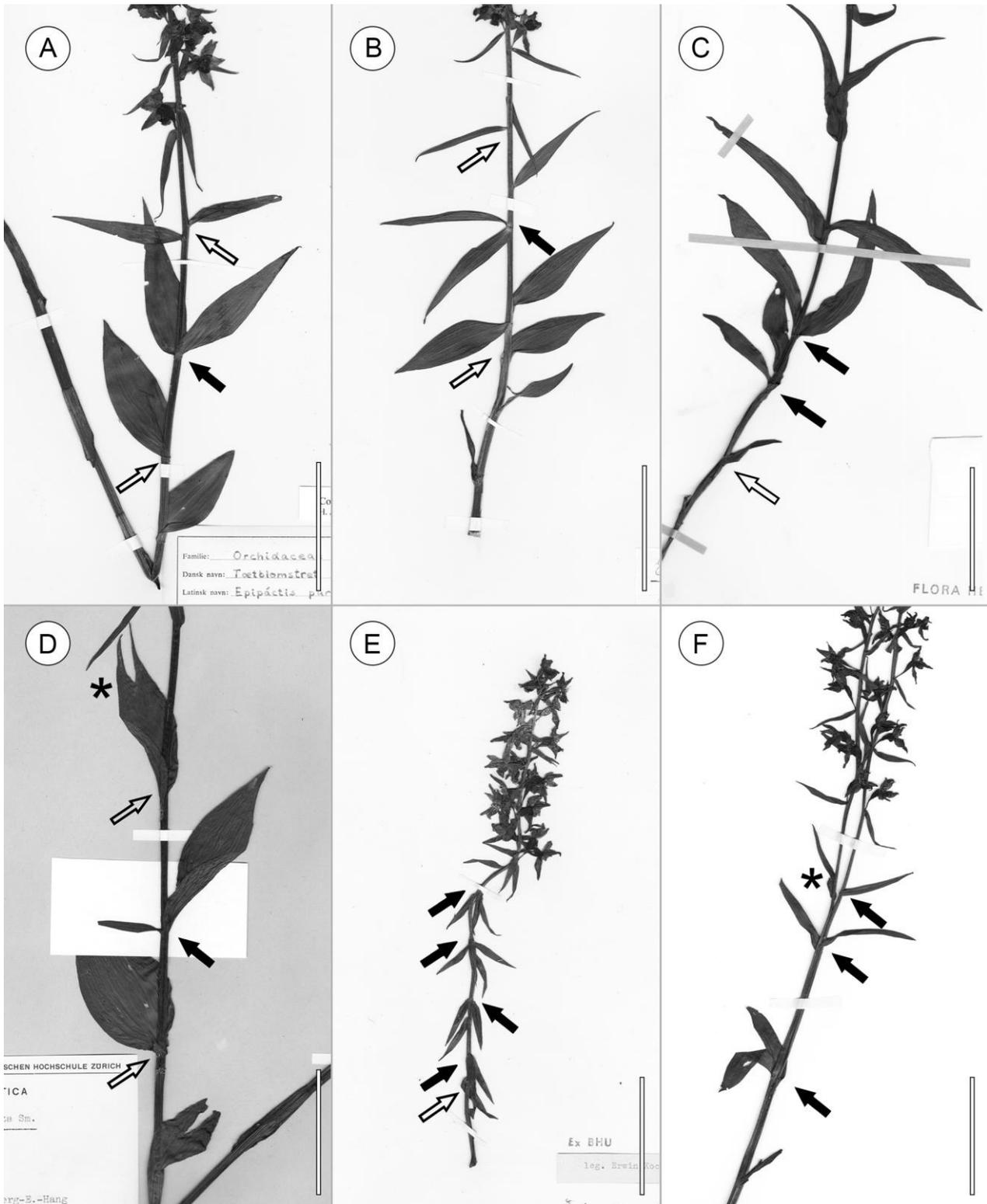


Figure 4. Histological analyses of the leaf vasculature in the shoots of *E. purpurata* differing in phyllotactic patterns. (A) (upper panel) Diagrammatic representation of the internode (1), node (2), and corresponding cross sections; dashed lines refer to the level of the cross section. Vascular bundles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

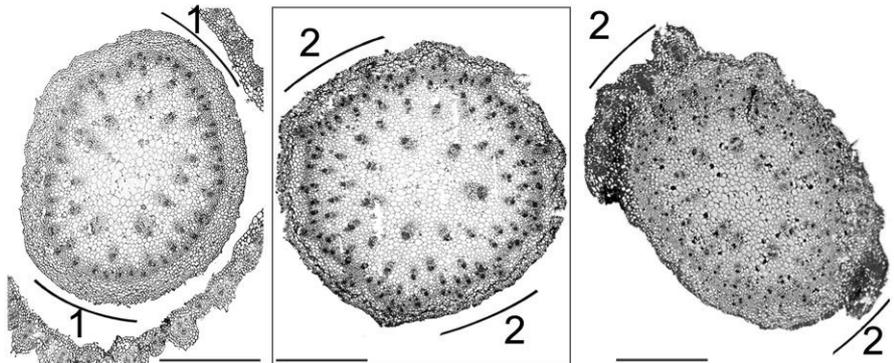
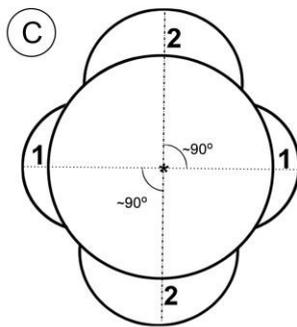
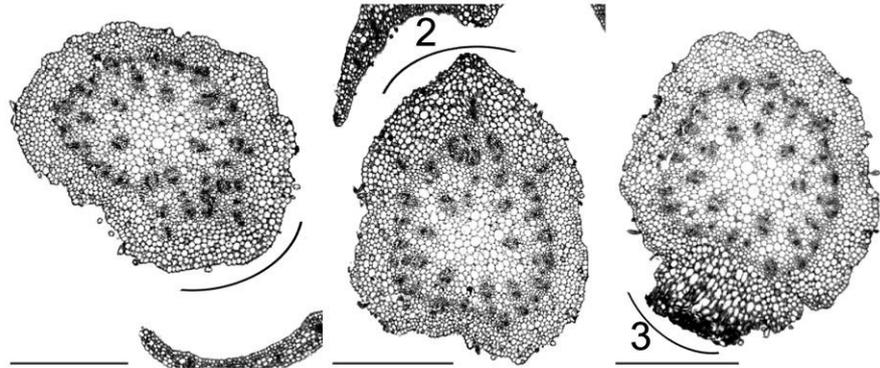
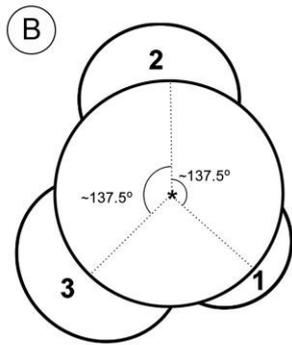
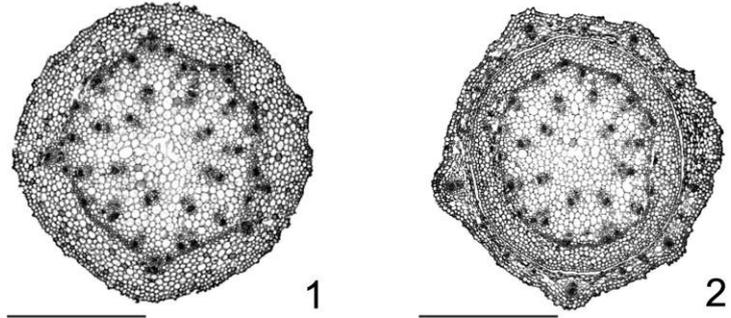
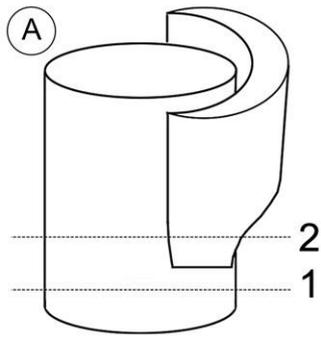


Table 1. Leaf arrangements in the material analysed. In a given shoot, more than one phyllotactic pattern can occur, as, for example, in ramets with a decussate pattern (see the text). In the table, for clarity, shoots with abnormal phyllotaxis (decussate pattern or with pseudowhorls) are counted only once within the total amount of ramets analysed. (A) Ramets from SW Poland: Nieszczyce (two cases) and the Błyszcz nature reserve (single case). (B) Phyllotaxis present in specimens from all herbaria analysed. (C) Herbarium voucher specimen details: Z-000088596; ZT-00071819. (D) Voucher specimens with aberrations in leaf arrangements and the acronyms of 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 ^A	0.6	24	5.0
Herbarium vouchers	806	760 ^B	94.4	2 ^C	0.2	44 ^D	5.4
Total	1283	1210	94.7	5	0.4	68	5.3