AN INTRIGUING MORPHOLOGICAL VARIABILITY OF *PLATANthera* s.l.

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**ABSTRACT**

The morphology of 38 species of the genus *Platanthera* s.l. was investigated by means of light and electron microscopy. Particular attention was paid to the structure of gynostemium and pollinia. The species investigated represent 10 major lineages of the genus, all being recognized as sections or segregate genera in various taxonomical treatments. Considerable morphological variability was confirmed for *Platanthera* s.l. The most notably variable structures are tuberoids (root-like, stoloniferous, spherical, etc.), lip (entire, lobed, fimbriate etc.), rostellar lateral lobes (flat, concave, bursicle-like), lateral stigma lobes (flat, protruding, etc.) and viscidia (orbicular, lanceolate, sub-linear, deeply concave, reduced). The morphology of several taxa confirms that they do not belong to *Platanthera* s.l., of them *Platanthera camtschatica* displays relation to *Galearis* s.l. and *P. latilabris, P. clavigera* and *P. biermanniana* – to subtribe Habenariae. In many cases the morphology confirms the monophyly of the lineages within *Platanthera* s.l., as *Tulotis, Limnorchis* etc., although more thorough sampling is needed. Some of those groups correspond to the clades as defined by previously published molecular phylogenies (Hapeman and Inoue 1997, Bateman et al. 2009). The morphological polymorphism in *Platanthera* s.l. (especially gynostemium structure) seems to be higher than in many other large genera of the subtribe *Orchidinae*, as *Dactylorhiza, Ophrys or Orchis*, and led to recognition of several segregate genera within *Platanthera* s.l. However, lesser genetic polymorphism revealed by molecular markers gives evidence for morphological plasticity (in particular, in gynostemium structure) and rapid morphological evolution of this group.

**Keywords:** Platanthera, gynostemium, morphological plasticity, viscidium, bursicle

**Introduction**

The genus *Platanthera* Rich. belongs to subtribe *Orchidinae* (subfamily *Orchidoideae*), and taken *sensu lato*, it encompasses about 120 species. They are terrestrial herbs, except for several humus epiphytes that grow on tree trunks near to ground level. The distribution of the genus covers mostly temperate zone of Northern Hemisphere. Several species are tropical, but they mostly inhabit mountainous forests at higher altitudes and do not represent tropical species *de facto*. Taxonomically *Platanthera* is a rather poorly known genus, which is mainly due to the paucity of taxonomic knowledge of numerous Asiatic and some American representatives of the genus. To certain extent, inconspicuous habit of the plant along with usually small flowers might be a reason that this genus has not been investigated thoroughly before.

Detailed taxonomical studies of the genus based on thorough morphological analysis were undertaken for Japan and Taiwan by Ken Inoue (Inoue and Lin 1980; Inoue 1982, 1983). 15 species reaching Russia were also reviewed in detail (Efimov 2006, 2007a, 2007b), the same as for Korean peninsula (Lee and Choi 2005). Other taxonomical studies of the genus include mainly treatments in different 'floras', of which 'Flora Reipublicae Popularis Sinicae', 'Flora of China' and 'Flora of North America north of Mexico' cover the most species-rich territories for *Platanthera* in the world.

Generally, *Platanthera* s.l. is the subject of significant taxonomical controversy. This is mainly due to the problems with generic affiliation of its species, because many segregate genera were described within *Platanthera* s.l. on the basis of various, more or less significant morphological distinguishing features. Those genera are *Limnorchis, Tulotis, Lysiella, Mecosa, Fimbriella, Blephariglottis, Gymnadeniopsis and Pseudodiphyllum*. Monotypic *Neolindleya* was also merged into *Platanthera*-complex by some authors. Furthermore, several genera were assigned to this complex on the basis of new molecular phylogenetic data, such as *Piperia* and *Diphyllax* (Bateman et al. 2009). Probably their list will be enlarged in the future with further sampling of the taxa. Three molecular phylogenies of *Platanthera* s.l. have been published (Hapeman and Inoue 1997; Bateman et al. 2003; Bateman et al. 2009).

Main aim of this study was to describe the morphology of species belonging to *Platanthera* s.l. in relation to the taxonomy of this group. Especially gynostemium structure of many representatives of this group remains not sufficiently investigated. But it is very important for taxonomy, because gynostemium is traditionally viewed as taxonomically informative in this genus. However, it should be mentioned that in this study we covered only part of morphological variability of this group, both concerning organs that were analyzed in detail, and concerning the sampling of the taxa. Especially impor-
tant is further sampling of North American taxa, as our sampling of the species from that continent proved to be rather superficial.

For the purpose of simplicity, I divided the genus *Platanthera* into the formal groups. Some of them are named after the names of smaller genera of *Platanthera* s.l. to which they were once assigned. Others are named after the names of corresponding sections. Few groups have been formed here for the first time and named by listing all taxa which they include.

**Materials and Methods**

The study is based on the analysis of herbarium specimens and flowers preserved in 70% alcohol. For the study of the flower morphology from dried specimens, 1–2 young flowers (or flower buds ready to open) were immersed into the hot water (80–100 °C) for 5–15 minutes and examined under the light microscope. Herbarium specimens from Asia were verified by the author in relation to his revision of the genus for Pan-Himalayan flora (in prep.), or corresponding to the "flora of North America North of Mexico" (Sheviak 2003), for the purpose of simplicity, I divided the genus *Platanthera* into the formal groups. Some of them are named after the names of smaller genera of *Platanthera* s.l. to which they were once assigned. Others are named after the names of corresponding sections. Few groups have been formed here for the first time and named by listing all taxa which they include.

**Results**

**Morphological descriptions of the subgroups in *Platanthera* s.l.**

1. *Platanthera* s.s.

*Platanthera* sensu stricto remains largely polymorphic in its morphology. I divided it further into smaller groups and discuss them separately. Those groups were either treated as taxonomic sections (sect. *Mecosa*, sect. *Stigmatotusae*) or just represent the associations of species erected here on the basis of the species morphology.

1a. *Platanthera* sensu strictissimo, including *Lysiella* (*P. bifolia, P. chlorantha, P. densa, P. dyeriana, P. mandarinorum, P. metabifolia, P. oligantha, P. oreophila, P. tipuloides*). Fig. 1a; Fig. 2a–b.

Orthotropic shoot 1. Tuberoids ovate to narrowly fusiform (but in *Lysiella* not thickened and root-like), with solitary root hairs (in *P. dyeriana* tuberoids are hairy).

Table 1 List of voucher specimens for the investigations of gynostemium structure.

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Data from the label</th>
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<tbody>
<tr>
<td>1</td>
<td><em>P. bifolia</em></td>
<td>Russia, VII 2004, Efimov (flowers in alcohol)</td>
</tr>
<tr>
<td>2</td>
<td><em>P. chlorantha</em></td>
<td>Russia, VI 2005, Efimov (flowers in alcohol)</td>
</tr>
<tr>
<td>3</td>
<td><em>P. densa</em></td>
<td>Russia, VI 2006, Efimov (flowers in alcohol)</td>
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<tr>
<td>4</td>
<td><em>P. dyeriana</em></td>
<td>China, VIII 2006, Jin 8368 (PE)</td>
</tr>
<tr>
<td>5</td>
<td><em>P. mandarinorum</em></td>
<td>Russia, VII 2007, Efimov (flowers in alcohol)</td>
</tr>
<tr>
<td>6</td>
<td><em>P. metabifolia</em></td>
<td>Russia, VI-VII 2007, Efimov (flowers in alcohol)</td>
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<tr>
<td>7</td>
<td><em>P. oligantha</em></td>
<td>Russia, VII 1928, Sukaczew and Bryzhchev s.n. (LE)</td>
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<tr>
<td>8</td>
<td><em>P. oreophila</em></td>
<td>China, VII 1937, Yu 7178 (PE)</td>
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<tr>
<td>9</td>
<td><em>P. tipuloides</em></td>
<td>Russia, VII 2007, Efimov (flowers in alcohol)</td>
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<td>10</td>
<td><em>P. epiphytica</em></td>
<td>Vietnam, X 2005, Averyanov et al. (LE)</td>
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<tr>
<td>11</td>
<td><em>P. elliptica</em></td>
<td>New Guinea, s.d., Hongfan s.n. (PE)</td>
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<td>12</td>
<td><em>P. sингgalan-gensis</em></td>
<td>China, X 1935, Indonesia, Zollinger 1706 (LE)</td>
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<td><em>P. bakeriana</em></td>
<td>China, VIII 1981, collector unknown 3016 (PE)</td>
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<td>14</td>
<td><em>P. exelliana</em></td>
<td>China, VIII 1938, Yu 22219 (PE)</td>
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<td>15</td>
<td><em>P. handel-mazettii</em></td>
<td>China, VIII 2006, Jin 8369 (PE)</td>
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<td>16</td>
<td><em>P. junceae</em></td>
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<td><em>P. leptocaaulon</em></td>
<td>China, VII 1981, collector unknown 01381 (PE)</td>
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<td><em>P. roseotincta</em></td>
<td>China, VII 2010, Jin et al. 1291 (PE)</td>
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<td>19</td>
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<td>China, VII 2010, Jin et al. 2833 (PE)</td>
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<td>26</td>
<td><em>P. finetiana</em></td>
<td>China, VII 1958, Yang 58846 (PE)</td>
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<td>27</td>
<td><em>P. fuscescens</em></td>
<td>Russia, VI 1909, Diukina 172 (LE)</td>
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<td>28</td>
<td><em>P. holoaglotis</em></td>
<td>Russia, VII 2005, Efimov (flowers in alcohol)</td>
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<td>29</td>
<td><em>P. japonica</em></td>
<td>China (PE)</td>
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<td>30</td>
<td><em>P. sinica</em></td>
<td>China, VIII 1981, collector unknown 02492 (PE)</td>
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<td>31</td>
<td><em>P. ussuriensis</em></td>
<td>Russia, VII 2005, Efimov (flowers in alcohol)</td>
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<td>32</td>
<td><em>P. whangsha-nensis</em></td>
<td>China, VII 1985, Chen and Ma 1143 (PE)</td>
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<td>33</td>
<td><em>P. elongata</em></td>
<td>USA, VII 1896, Hansen 1864 (LE)</td>
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<td>34</td>
<td><em>P. chirissiana</em></td>
<td>Russia, VII 2007, Efimov (flowers in alcohol)</td>
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<td>35</td>
<td><em>P. camtschatica</em></td>
<td>Russia, VII 2007, Efimov and Andronova (flowers in alcohol)</td>
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<td>36</td>
<td><em>P. integra</em></td>
<td>USA, VIII 1939, Godfrey and Tryon 936 (LE)</td>
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<td>37</td>
<td><em>P. psycodes</em></td>
<td>USA, s.d., Martz s.n. (LE)</td>
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<td>38</td>
<td><em>P. plebhangiottis</em></td>
<td>USA, s.d., Wiedemann s.n. (LE)</td>
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<tr>
<td>39</td>
<td><em>Habenaria lineatifolia</em></td>
<td>Russia, VII 2005, Efimov (flowers in alcohol)</td>
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European Journal of Environmental Sciences, Vol. 1, No. 2
Flowers green, white or yellowish. Hood usually present, in some species (*P. mandarinorum, P. minor*) absent. Margin of tepals entire, except for *P. oreophila*, where it is densely papillose. Lip simple, in some species (*P. oligantha, P. minutiflora, P. mandarinorum*) slightly dilated at the base. Rostellum median lobe long or short depending on the position of the viscidia, flat. Rostellar arms situated face-to-face. Lateral stigma lobes small, hardly distinguishable, somewhat inside spur entrance. Viscidia orbicular to suborbicular. Pollen massulae smooth in *P. bifolia, P. oligantha, and P. tipuloides*.

**1b. Platanthera sect. Mecosa** (*P. epiphytica, P. elliptica, P. singgalangensis*). Fig. 1b.
Fig. 2 Morphology of gynostemium (SEM). a – *P. mandarinorum*; b – *P. metabifolia*; c – *P. sachalinensis*; d – *P. convallariifolia*; e – *P. hologlottis*; f – *Habenaria linearifolia*; g – *P. chorisiana*; h – teratologic gynostemium of *P. sachalinensis* with a tendency to actinomorphy. Scale bars: 500 μm.
Herbs with 2 orthotropic shoots, one bearing inflorescence and another with leaf rosette, forming inflorescence in next year. Tuberoids usually fusiform, densely covered with root hairs. Flower structure is the same as in the group 1a.

1c. Platanthera sect. Stigmatosae (P. bakeriana, P. exelliana, P. handel-mazzetti, P. juncea, P. leptocaulon, P. roseotincta). Fig. 1c.


1d. Platanthera stenantha. Fig. 1d.


1e. Platanthera sachalinensis. Fig. 1e; Fig. 2c; Fig. 3a–c.

Orthotropic shoot 1. Tuberoids fusiform with root-end, more or less covered with root hairs. Flowers greenish. Hood present. Petals glabrous. Lip simple, at the base with small lateral teeth and a small knob above median line. Rostellar arms almost completely turning into concave viscidia. Stigma laterales lobes prominent, slightly convex.

1f. Platanthera latilabris, P. clavigera, P. biermanniana. Fig. 1f.

Orthotropic shoot 1. Tuberoids ovate, densely covered with thin and long root hairs. Flowers greenish or yellowish. Hood present. Petals glabrous. Lip divided into slightly concave hypochile and flat epichile, with a strong bend between. Rostellar arms flat, in P. latilabris and P. clavigera somewhat elongated parallel to lateral stigma lobes, which are in all 3 species substantially protruding forward, attached to thin lateral appendages of gynostemium and also to a lip. Viscidia orbicular, with central stipe placement.

2. Limnorchis (P. convallariifolia, P. dilatata). Fig. 1g; Fig. 2d.

Orthotropic shoot 1. Tuberoids fusiform, gradually narrowed to a root-end, without root hairs. Flowers green, white or yellowish. Hood present. Petals glabrous. Lip simple, strongly concave and sometimes dilated at the base. Rostellar arms flat or slightly concave. Stigma lateral lobes generally hardly distinguishable, forming a slightly concave or flat common stigma plate. Lateral walls of column forming wide space near spur entrance. Viscidia ovate to lanceolate.

3. Tulottis (P. finetiana, P. fuscescens, P. holoqottis, P. japonica, P. sinica, P. ussurensis, P. whangshanensis). Fig. 1h; Fig. 2e; Fig. 3d.

Orthotropic shoot 1. Tuberoids stoloniferous, up to at least 15 cm lg, almost lacking root hairs. Flowers green, white or yellowish. Hood present. Tepals glabrous. Lip simple, with prominent basal tooth or tubercle at each side, rarely without teeth (P. holoqottis), sometimes with a knob above medial vein. Rostellar arms deeply concave, situated below stigmatic surface. Stigma lateral lobes indistinguishable, united into a common convex stigma. Viscidia strongly twisted.

4. Piperia (P. elongata). Fig. 1i.


5. Pseudodiphyllum (P. chorisiana). Fig. 1j; Fig. 2g.


6. Neolindleya (P. cantschatica). Description is based on Efimov et al. (2009). Fig. 1k.

Orthotropic shoot 1. Tuberoids narrowly fusiform, hairy. Leaves margin crenulate. Flowers purple, rarely white. Hood present. Tepals glabrous. Lip apicantly three-lobed. Rostellum forming two bursicles which are in the same time reduced as a consequence of obligate autogamic nature of the plant. Stigma lateral lobes spreading to the sides. Viscidia reduced.

7. Gymnadeniopsis (P. integra). Fig. 1l.

Orthotropic shoot 1. Tuberoids fusiform or stoloniferous, glabrous. Flowers white or orange (P. integra). Hood present. Tepals glabrous, margin sometimes slightly crenulate. Lip entire or three-lobed at the apex. Rostellar arms flat or slightly concave. Stigma lateral lobes protruding to the sides. Viscidia orbicular to narrowly-lanceolate.

8. Fimbriella (P. psycodes). Fig. 1m.

Orthotropic shoot 1. Tuberoids fusiform, with long root-end, glabrous. Flowers purple or white. Hood pres-
ent. Tepals glabrous, margin sometimes crenulate. Lip deeply three-lobed, each lobe fimbriate (dentate-crenulate in *P. peramoena*). Rostellar arms long, elongated along with anther and lateral walls of the column. Stigma lateral lobes entire, distinct, more or less flat, not protruding. Viscidia suborbicular, rarely lanceolate.

9. **Blephariglottis (P. blephariglottis)**. Fig. 1n.

Similar to previous group. Differs by orange flowers (white in *P. blephariglottis*) and fimbriate, but entire, not three-lobed lip and always suborbicular viscidia.

**Discussion**

**An overview of the variability of main morphological features which are useful for phylogenetic study within Platanthera s.str.**

**Shoot structure**

All species of *Platanthera* s.l. represent herbs with sympodial growth and more or less thickened tuberoids. Distinctive shoot structure is specific for *Platanthera* sect. *Mecosa*, a small group of species which occur in tropical East Asia (*P. angustata, P. singgalangensis, P. elliptica, P. kinabaluensis, P. stapfii*, etc.). They have 2 orthotropic shoots at different ontogenetic stages: one bearing inflorescence and another with leaf rosette forming inflorescence next year. Speculatively, such shoot structure might have evolved in tropical regions as an adaptation to diminish the tranpiration from leaves during the warmest period of a year.

**Tuberoids**

Most common shapes of tuberoids are ovate and fusiform, gradually narrowed into a root-end, similar to the tuberoids of *Dactylorhiza* and *Gymnadenia*, but not palmate. They are clearly divided into a short (up to 1.5 cm long) rhizomatic part of shoot origin, bearing a bud at its distal end, and thickened root part of tuberoid (Fig. 4a–d). This typical structure of tuberoids is widely represented in *Platanthera* s.str. (*P. bifolia*, *P. mandarinorum, P. convallariifolia*, etc.). Some species have thin, root-like tuberoids, which do not differ substantially from roots (*‘Lysiella’* clade of *Platanthera* s.str., *Gymnadeniopsis*, etc.).

In some groups tuberoids are lacking root-end, becoming spherical or ovate, as in *Herminium*. Such tuberoids are characteristic to *Piperia*, to small group of Asiatic species (*P. latilabris, P. clavigera, and P. biermanniana*) and also to *Bhutanthera* (Pearce et al. 2001). Tuberoids
without root-end are characteristic for morphologically similar but phylogenetically distant genus Habenaria, and therefore, along with gynostemium structure, might help to reclassify species between Platanthera and Habenaria in the absence of molecular phylogenetic data.

The most specific type of tuberoid is a stoloniferous tuberoid. Its structure was studied in detail by Kumazawa (1958). Usually it is a plagiotropic fusiformy thickened structure that bears a bud near its central part, which later forms a shoot of the next generation (Fig. 4e). As it was shown by Kumazawa, part of stoloniferous tuberoid between shoots of two generations possesses a unique stela with features intermediate between stem and a root. This part might be rather long (up to 10 cm) and contributes to vegetative spread of a plant. Such species often form clones up to 1 m in diameter, with up to 70 individuals within one clone, as in P. fuscens (Tatarenko 1996). Distal part of the tuberoid has root origin, and might be not plagiotropic.

In many species of Platanthera s.str. tuberoids are densely covered with root hairs.

**Leaves**

Leaves vary from roundish to almost linear. Piperia has leaves withering by the flowering time which might be due to xeromorphosis (Ackerman 1977). Anatomical investigation has not been done.

**The flower color**

Flower colour in Platanthera s.l. is highly diverse showing a great variation in green, white and yellowish hues in Platanthera s.str., Tulotis, Limnorchis, Lysieilla, Pseudodiphyllum, and Piperia. Purple flowers are characteristic for majority of Fimbriella species, and orange for majority of Blephariglottis.

**Hood**

The upper tepals of the majority of species of Platanthera s.l. are connivent into a hood. They are spreading in Piperia and in some species of Platanthera s.str. (P. mandarinorum, P. ophryoides, P. amabilis, P. takedae, P. tipuloides, and some others).

**Margin of the petals**

Some Asiatic species of Platanthera s.str. have tepals with papillose margin (majority of sect. Stigmatosae, P. oreophila). Species of Fimbriella and Blephariglottis with fimbriate lip sometimes also have fimbriate to papillose petals.

**Lip shape**

Typical lip in Platanthera s.l. is entire. Deeply three-lobed lip is typical for Fimbriella. Moreover, in this group the margin of the lip is fimbriate (or dentate-crenulate in P. peramoena). In Blephariglottis lip is entire, but with fimbriate margins, too.

The base of the lip in Tulotis usually bears small but distinct dent at each side (such dents are absent in P. holoiglottis, and P. brevicatarata). In some species of Platanthera s.str. such dents are also present, but they are very small and inconspicuous, or the lip is only dilated at the base (P. oligantha, P. blumei, etc). Lip is dilated also in some species of Limnorchis clade.

In some species (P. sachalinensis, P. oligantha) there is a small knob above central vein of the lip near spur entrance which is thought to be adaptive, allowing pollinator to take only one pollinium at a time (Hapeman and Inoue 1997).

Specific lip shape was found in Platanthera latilabris, P. clavigera and P. biermanniana. Here it is divided into concave hypochile and flat epichile, separated by a sharp bend. Hypochile is conjoined with the basal appendages of gynostemium.

Spur length in Platanthera s.l. vary greatly, from 6 cm lg. (e.g. in P. japonica) to very short (1 mm in P. chorisiana, P. inumae, P. oligantha, etc.). seems to be rather evolutionary plastic and here is not discussed in detail. Sometimes it is curved or clavate at the distal end.

**Anther thecae**

Anther thecae might be either situated side-by-side or distant from each other, depending on the width of the connective. This trait correlates with viscidia position, divergence of anther locules, length of the caudicle and shape of lateral walls of column. It was clearly shown that this combination of features is connected with a pollination mechanism, and corresponds to the two variants of the pollinaria placement on the head of pollinator: to the eyes or to the base of proboscis (Nilsson 1983; Hapeman and Inoue 1997 etc.). It was shown that this feature evolves rapidly, resulting in pairs of species which differ by this particular character only, for instance, P. bifolia and P. chlorantha (Nilsson 1983, 1985), P. metabifolia and
P. densa (Efimov 2006), P. leucophaea and P. praeclara (Sheviak and Bowles 1986), P. psycodes and P. grandiflora (Stoutamire 1974). Therefore, this feature hardly can serve as a source of phylogenetic signal for Platanthera s.l.

**Lateral walls of column**

In some species lateral walls of the column are curved inwards which is drawing viscidia closer to each other. That allows an adaptation to various types of pollinia placement on pollinator’s body which was described above. Some species (P. stenantha and Limnorchis) possess wide spreading lateral walls of column near spur entrance forming a wide space above the lip blade. This feature might be also somehow connected to a pollination mechanism.

**Auricles**

Auricles are most probably present in all members of Platanthera s.l. As it was shown by Kurzweil (1987) in P. chlorantha, the auricles are formed from the anther tissue, and do not represent staminodia. I found that auricles slightly differ in diameter and shape among the species. Very specific auricles are characteristic for Diaphylax (Szlachetko and Rutkovski 2000). They are very long, finger-like, only slightly shorter than the anther thecae. Alternatively, in Piperia (Ackerman 1977) they were thought to be absent, although I found that they exist, although very tiny.

**Rostellum median lobe**

I here accept term ‘rostellum’ following Dressler (1986), i.e. define it as a modified part of stigma median lobe. Rostellum median lobe forms a thin or relatively thick rim, surrounding the stigma from above. It is either long or short, depending from the position of viscidia. In species with thick and protruding rostellum median lobe (Limnorchis, Platanthera chorisiana) it may function as a barrier between the pollen and stigma of the same flower preventing autogamic pollination.

**Rostellum lateral lobes (‘rostellar arms’)**

Rostellum lateral lobes are marginal parts of rostellum, bearing viscidia. Depending from the viscidia position, origin and shape, rostellum lateral lobes vary in shape and occupy different position within gynostemium. Usually rostellar arms are flat and situated parallel to the flat surface of the stigma or ‘face to face’ to each other if lateral walls of column are curved inwards (P. bifolia, P. metabifolia, P. tipuloides, sect. Stigmatosae). But in species of Tulotis rostellar arms occupy the bottom of the gynostemium below stigmatic surface and they prove to be deeply concave after the removal of viscidia. Such specific rostellar arms were misdetermined for bursicles (Nevski 1935; Lang 1999; Su 2000). The classical bursicles, as those that are present e.g. in Dactylorhiza, were found in Platanthera cantschatica (Efimov et al. 2009), which proved to be the crucial point for establishing a taxonomic position for this species (see ‘discussion’).

**Stigma lateral lobes**

In the most of Platanthera s.str., in P. sachalinensis, Lysiella, Mecosa, Fimbriella, and Blephariglottis, it is possible to distinguish stigma lateral lobes in the lower part of the common stigma. They look as small hardly visible structures, sometimes sunk into the basal part of the spur. In this case, most of the fertile stigma surface is formed, most probably, by fertile part of median lobe of the stigma. In Limnorchis, Piperia, Tulotis and Pseudodiphyllum it was hardly possible to distinguish a boundary between the lobes of the stigma. It means that in those groups, stigma lateral lobes are either reduced, or completely fused with fertile part of median lobe. Lastly, there are species where lateral stigma lobes are well-distinguishable, protruding laterally (Gymnadeniopsis, Platanthera sect. Stigmatosae), protruding upwards (Platanthera cantschatica) or protruding down and conjointed to basal appendages of gynostemium and probably also to a lip (P. latilabris, P. clavigera, P. biermanniana).

**Viscidia**

Viscidia of Platanthera s.l. vary greatly in shape. In the majority of Platanthera-clade, viscidia are more or less roundish and include most of the rostellar arm tissue. In P. sachalinensis (Fig. 3a–c) viscidia are twisted, but also include the most (if not all) of the rostellar arm tissue. Viscidia of Platanthera sect. Stigmatosae vary from ovate to narrowly-lanceolate. Very specific structure of the viscidia is typical for some representatives of Tulotis group, where viscidia include only minor part of the rostellar arm tissue (Fig. 3d). They are more (P. fuscescens, P. usuriensis) or less (P. japonica, P. sinica) twisted. In some autogamic taxa viscidia might be reduced at all (as in Platanthera cantschatica and possibly also in some species of Blephariglottis). Stipe is usually not attached to the viscidium disc centrally, with an exception at least in P. latilabris, P. clavigera, and P. biermanniana.

**Caudicle**

Caudicle may be long or short. This structure is probably subjected to rapid evolution, the same as the position of anther thecae, corresponding to viscidia placement on pollinator’s body. The shortest, almost invisible caudicle was found in Piperia (Ackerman 1977). In Platanthera cantschatica caudicle is highly reduced, and solid pollinia are not formed at all, in connection with autogamy (Efimov et al. 2009).

**Pollen massulae**

This character was examined in 8 species of Platanthera s.l. only (Fig. 5). Four species of Platanthera s.str. had more or less laevigate surface of exine (P. bifolia, P. oligantha, P. tipuloides, P. sachalinensis). The exine of P. holoiglottis, and P. fuscescens (“Tulotis”) is hamulate, and
P. convallariifolia (‘Limnorchis’) – ornate (all in terminology by Schill and Pfeiffer 1977). More species sampling along with statistical investigation of the variability of exine surface within one species is needed for the correct analysis of this feature.

**Seed surface**

Seed shape, texture of periclinal walls and the presence/absence of lamella allow distinguishing between Limnorchis and Platanthera s.str. (incl. Piperia) within Platanthera-complex (Gamarra et al. 2008). That study was based on 5 species of Platanthera s.str., one species of Piperia and two representatives of Limnorchis. I sampled and investigated three more species of Platanthera s.str. (P. oligantha, P. tipuloides, and P. sachalinensis), one species of Limnorchis (P. convallariifolia), Pseudodiphryllum (P. choristiana), and two species of Tulotis (P. hologlottis and P. fuscescens). My results (Fig. 6) contradict those from Gamarra et al. (2008). Firstly, I found smooth periclinal walls in P. bifolia, P. oligantha, and P. tipuloides (vs. reticulate found by Gamarra et al.). Secondly, two studied species of Tulotis were different from each other in seed surface which was smooth in P. hologlottis and reticulate in P. fuscescens). That diminishes an importance of the seed coat features in delimitating groups within Platanthera s.str. Further sampling and investigation of the influence of seed maturity on seed surface are important for final decisions on the subject.

**Implications for taxonomy**

Substantial morphological polymorphism is characteristic for Platanthera s.l. This involves many traits which are traditionally taken as phylogenetically informative in Orchidaceae at genus level, such as structure of tuberoids, shape of the lip, structure of rostellar arms, structure of lateral lobes of the stigma, shape of viscidia.

Of species studied, a detailed analysis of Platanthera camtschatica flower was presented previously by (Efimov et al. 2009). It was shown that this species did
not belong to Platanthera s.l., and should be placed as a separate genus Neolindleya which is more related to Galea-ris-Amerorchis clade.

Futhermore, some other species traditionally regarded within Platanthera, might be also excluded from this genus. On the molecular phylogeny published by Bateman et al. (2003), first candidate for this is Platanthera latilabris, which falls in subtribe Habenarinae. Our morphological study of this species has shown peculiarities in its morphology, as rounded tuberoids, rostellar lateral lobes that are projected forward, and elongated lateral stigma lobes that are partly conjoined to a lip. Moreover, the structure of its lip, which might be divided into a hypochile and epichile with a strong fold between, is unique in Platanthera s.l. Such morphological specificity along with molecular phylogenetic data, corroborate its exclusion from subtribe Orchidinae. Perhaps wisely, Szlachtuko (2006) transferred this species to a genus Habenella, along with P. clavigera, P. cumminsiana, P. edgeworthii, and some other species of Habenaria). Morphology of the gynostemium of P. clavigera and P. biermanniana is almost the same as in P. clavigera, which suggests that all those species are related to each other and should be better placed in subtribe Habenarinae.

This study also enables us to recircumscribe the section Stigmatosae that was established by K.-Y. Lang (1998) in the rank of a subgenus. Firstly, as it was shown before, P. latilabris, P. clavigera, and P. edgeworthii didn’t belong to Platanthera and should be treated within subtribe Habenarinae. Also P. stenantha should be excluded from this group, because it has different structure of the gynostemium. I assume also that two closely related species, P. juncea and P. handel-mazzetti, fall into this group on the basis of the similarity of their morphology with other members of this section.

Comparison with other genera

Platanthera s.l. shows significantly higher morphological polymorphism comparing to other large genera of subtribe Orchidinae (as outlined, e.g., by Pridgeon et al. 2001 and by Bateman et al. 2003) – Dactylorhiza, Orchis, Anacamptis, and Ophrys.

In genus Dactylorhiza, the gynostemium structure is not polymorphic, however pollen exine and seed surface vary significantly (Averyanov 1987). There is some polymorphism in lip structure, but species with an entire lip (D. incarnata s.l.) are rather closely related to 3-lobed species, and even within D. incarnata a variety with 3-lobed lip (var. trifurca) was described.

In Orchis and Anacamptis only slight polymorphism in gynostemium structure was detected (Kretzschmar et al. 2007) along with significant variability in exine surface in Orchis s.l. (Schill and Pfeiffer 1977; Barone Lumaga et al. 2006). Lip structure in Orchis and Anacamptis displays some polymorphism in the number of lip lobes. However, lip shape is an evolutionary plastic character within genera, which is for example the case with Anacamptis, where molecular phylogeny indicated close relationship of three-lobed species A. pyramidalis with 5-lobed species, represented by the majority of this genus (Bateman et al. 1997).

The similar level of variability was observed in Ophrys. As far as I know, no major differences were reported for the gynostemia of this genus. The variability of testa surface is also weak, representing different variations of reticulate pattern (Aybeke 2007). Pollen morphology is more polymorphic, with several morphological types found within the genus (Schill and Pfeiffer 1977; Barone Lumaga et al. 2006; Aybeke 2007). Lip represents high polymorphism concerning its colour and shape, but this is thought to be subjected to rapid evolution in connection with peculiar pseudocopulational pollination mechanism of Ophrys.

In general, it is possible to conclude that variation of gynostemium structure is significantly higher in Platanthera s.l. than in other genera listed here. Variability of lip structure is also more prominent in Platanthera s.l. than in other genera, although to a lesser extent than the gynostemium structure. Concerning seed surface, the variability in Platanthera s.l. is similar to that of Dactylorhiza and Orchis, and higher than in Ophrys.

The geographical distribution of Platanthera s.l. is significantly wider than that of any other genus of the subtribe Orchidinae. This might be either explained by an ancient origin of this group, or by its rapid morphological evolution and diversification. It should be also mentioned that Dactylorhiza, Orchis, Anacamptis, and Ophrys are connected with Europe since their origin and during diversification, whereas diversification centers of Platanthera s.l. are located in subtropical East Asia and in North America.

Molecular phylogenies of the subtribe (Bateman et al. 2003) argue for recent diversification in Orchis and probably Dactylorhiza, whereas in Anacamptis, Orchis, and especially in Platanthera higher genetic polymorphism in ITS region was observed, which indicates more ancient diversification of those groups.

Morphological evolution in Ophrys is driven by pollination mechanisms, resulting in rapid evolutionary changes of lip structure, which enabled description of numerous species with weak differences in the lip structure, which might in reality represent interspecific taxa or hybrid swarms (Bateman et al. 2011). In Dactylorhiza, variability of pollination mechanisms is low, and evolution is mostly driven by ecological and geographical isolation and allopolyploid speciation. In Orchis and Anacamptis, it is possible to suggest a high influence of pollination strategies for morphological evolution. However, in Platanthera very deep specializations to various pollinators, from small beetles to large moths, were discovered (Hapeman and Inoue 1997). Specializations to various methods of pollination by the same type of pollinators are also known in this genus, probably along with numerous, independent cases of transition to au-
togy. All those specializations might have challenged deep shifts in the structure of a flower, including lip and gynostemium. At the same time, exact mechanisms of pollination in many species are still under-described, which makes it difficult to explain the intriguing variability of gynostemium occurring within Platanthera s.l. Given large number of mostly Asiatic species with peculiar morphology of the flower, more studies explaining connections between morphology and pollination strategy are needed in this group. Judging from high fruit set of some species (as *P. fuscescens, P. choristiana*, etc.) many independent cases of shifts to autogamy might be supposed here to exist.

**Conclusions**

Comparing to other large genera of subtribe Orchidinae, Platanthera s.l. is characterized by highest morphological polymorphism. At least partly it can be explained by more ancient diversification in this group, by wider geographic distribution and by wider diversification to pollination mechanisms. High morphological polymorphism of Platanthera s.l., along with the possibility to divide this genus into distinct clades (Hapeman and Inoue 1997), supports the possibility of recognition of the smaller genera within this group. However, in many cases those genera are weakly delimited both by molecular and morphological methods, and for certain species we still do not have enough neither molecular nor morphological data to properly classify them. Moreover, we have evidence for rapid morphological evolution in connection with pollination shifts. Those facts, along with inconveniences in the use of new names argue for treating all species within one genus Platanthera taken sensu lato. Further morphological studies along with the studies of taxonomy of Platanthera are needed.

**Acknowledgements**

The study is supported by the Ministry of Education of the Russian Federation (contract 16.518.11.7071). My sincere thanks to Liudmila Kartsava for the technical support with scanning electron microscopy, to Irina Tatrenko for discussions and to Michael Dodd for checking English.

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