

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 pollinaria. 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 *Habenariinae*. 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 taxonomic 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 *Pseudodiphryllum*. 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 *Diphylax* (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 collections of LE (Saint-Petersburg, Russia), PE (Beijing, China) and K (Kew, Great Britain) were studied.

Fresh flowers of some species were collected by the author in Russia (Leningrad Region, Primorye Territory, Sakhalin Region) and analyzed under the light microscope or scanning electron microscope JSM 6390-LA. For SEM, the material was initially subjected to a critical point drying following a standard procedure of ethanol, acetone and isoamylacetate treatments and sputter-coated with platinum/palladium alloy. The details of seed and pollen surface were investigated under the electron microscope of older version (JEOL JSM 35C) without critical point drying procedure.

In total, the gynostemium structure of 38 species of *Platanthera* was examined (Table 1). All identifications of 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 American taxa.

Results

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

1. *Platanthera* s.str.

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. *Stigmatosae*) 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*,

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

| No | Species | Data from the label |
|----|-------------------------------|---|
| 1 | <i>P. bifolia</i> | Russia, VII 2004, Efimov (flowers in alcohol) |
| 2 | <i>P. chlorantha</i> | Russia, VI 2005, Efimov (flowers in alcohol) |
| 3 | <i>P. densa</i> | Russia, VI 2006, Efimov (flowers in alcohol) |
| 4 | <i>P. dyeriana</i> | China, VIII 2006, Jin 8368 (PE) |
| 5 | <i>P. mandarinorum</i> | Russia, VII 2007, Efimov (flowers in alcohol) |
| 6 | <i>P. metabifolia</i> | Russia, VI-VII 2007, Efimov (flowers in alcohol) |
| 7 | <i>P. oligantha</i> | Russia, VII 1928, Sukaczew and Bryzhzhev s.n. (LE) |
| 8 | <i>P. oreophila</i> | China, VII 1937, Yü 7178 (PE) |
| 9 | <i>P. tipuloides</i> | Russia, VI 2007, Efimov (flowers in alcohol) |
| 10 | <i>P. epiphytica</i> | Vietnam, X 2005, Averyanov et al. (LE) |
| 11 | <i>P. elliptica</i> | New Guinea, s.d., Hongfan s.n. (PE) |
| 12 | <i>P. singgalangensis</i> | China, X 1935, Indonesia, Zollinger 1706 (LE) |
| 13 | <i>P. bakeriana</i> | China, VIII 1981, collector unknown 3016 (PE) |
| 14 | <i>P. exelliana</i> | China, VIII 1938, Yü 22219 (PE) |
| 15 | <i>P. handel-mazettii</i> | China, VIII 2006, Jin 8369 (PE) |
| 16 | <i>P. juncea</i> | China, VIII 2007, Jin 9154 (PE) |
| 17 | <i>P. leptocaulon</i> | China, VII 1981, collector unknown 01381 (PE) |
| 18 | <i>P. roseotincta</i> | China, VII 2010, Jin et al. 1291 (PE) |
| 19 | <i>P. stenantha</i> | China, VIII 2010, Jin et al. 2833 (PE) |
| 20 | <i>P. sachalinensis</i> | Russia, VI 2007, Efimov (flowers in alcohol) |
| 21 | <i>P. latilabris</i> | China, VIII 2010, Jin et al. 2591 (PE) |
| 22 | <i>P. clavigera</i> | China (PE) |
| 23 | <i>P. biermanniana</i> | China, VIII 2007, Jin 9132 (PE) |
| 24 | <i>P. convallariifolia</i> | Russia, VII 2007, Efimov (flowers in alcohol) |
| 25 | <i>P. dilatata</i> | Russia, VIII 2000, Tatarenko (LE) |
| 26 | <i>P. finetiana</i> | China, VII 1958, Yang 58846 (PE) |
| 27 | <i>P. fuscescens</i> | Russia, VI 1909, Diukina 172 (LE) |
| 28 | <i>P. hologlottis</i> | Russia, VII 2005, Efimov (flowers in alcohol) |
| 29 | <i>P. japonica</i> | China (PE) |
| 30 | <i>P. sinica</i> | China, VIII 1981, collector unknown 02492 (PE) |
| 31 | <i>P. ussuriensis</i> | Russia, VII 2005, Efimov (flowers in alcohol) |
| 32 | <i>P. whangshanensis</i> | China, VII 1985, Chen and Ma 1143 (PE) |
| 33 | <i>P. elongata</i> | USA, VII 1896, Hansen 1864 (LE) |
| 34 | <i>P. chorisiana</i> | Russia, VII 2007, Efimov (flowers in alcohol) |
| 35 | <i>P. camtschatica</i> | Russia, VII 2007, Efimov and Andronova (flowers in alcohol) |
| 36 | <i>P. integra</i> | USA, VIII 1939, Godfrey and Tryon 936 (LE) |
| 37 | <i>P. psycodes</i> | USA, s.d., Martz s.n. (LE) |
| 38 | <i>P. blephariglottis</i> | USA, s.d., Wiedemann s.n. (LE) |
| 39 | <i>Habenaria linearifolia</i> | Russia, VII 2005, Efimov (flowers in alcohol) |

P. mandarinorum, *P. metabifolia*, *P. oligantha*, *P. oreophila*, *P. tipuloides*). Fig. 1a; Fig. 2a–b.

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

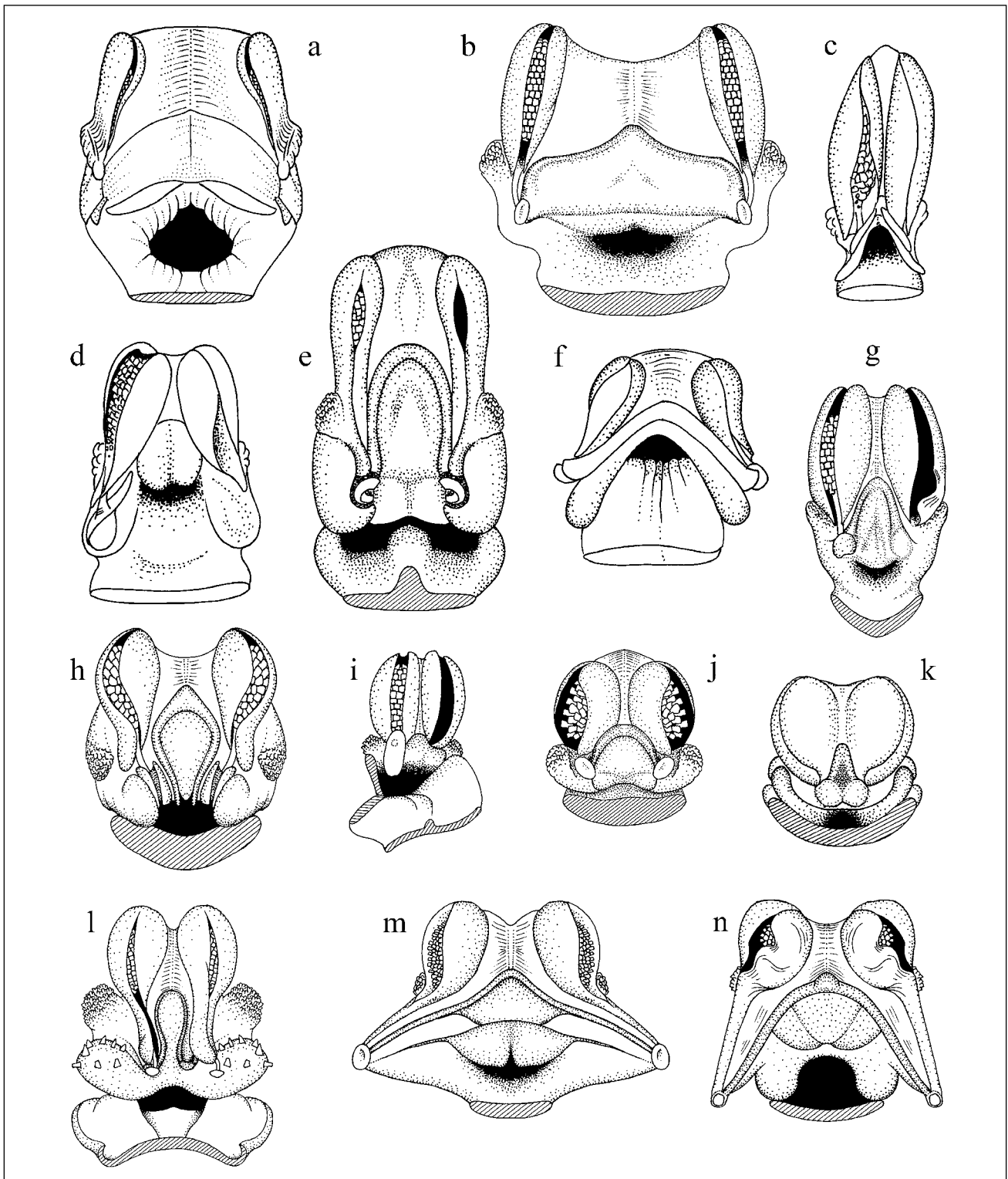


Fig. 1 Morphology of gynostemium. a – *P. mandarinorum*; b – *P. singgalangensis*; c – *P. leptocaulon*; d – *P. stenantha*; e – *P. sachalinensis*; f – *P. latilabris*; g – *P. dilatata*; h – *P. hologlottis*; i – *P. elongata*; j – *P. chorisiana*; k – *P. camtschatica*; l – *P. integra*; m – *P. psycodes*; n – *P. blephariglottis*.

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.

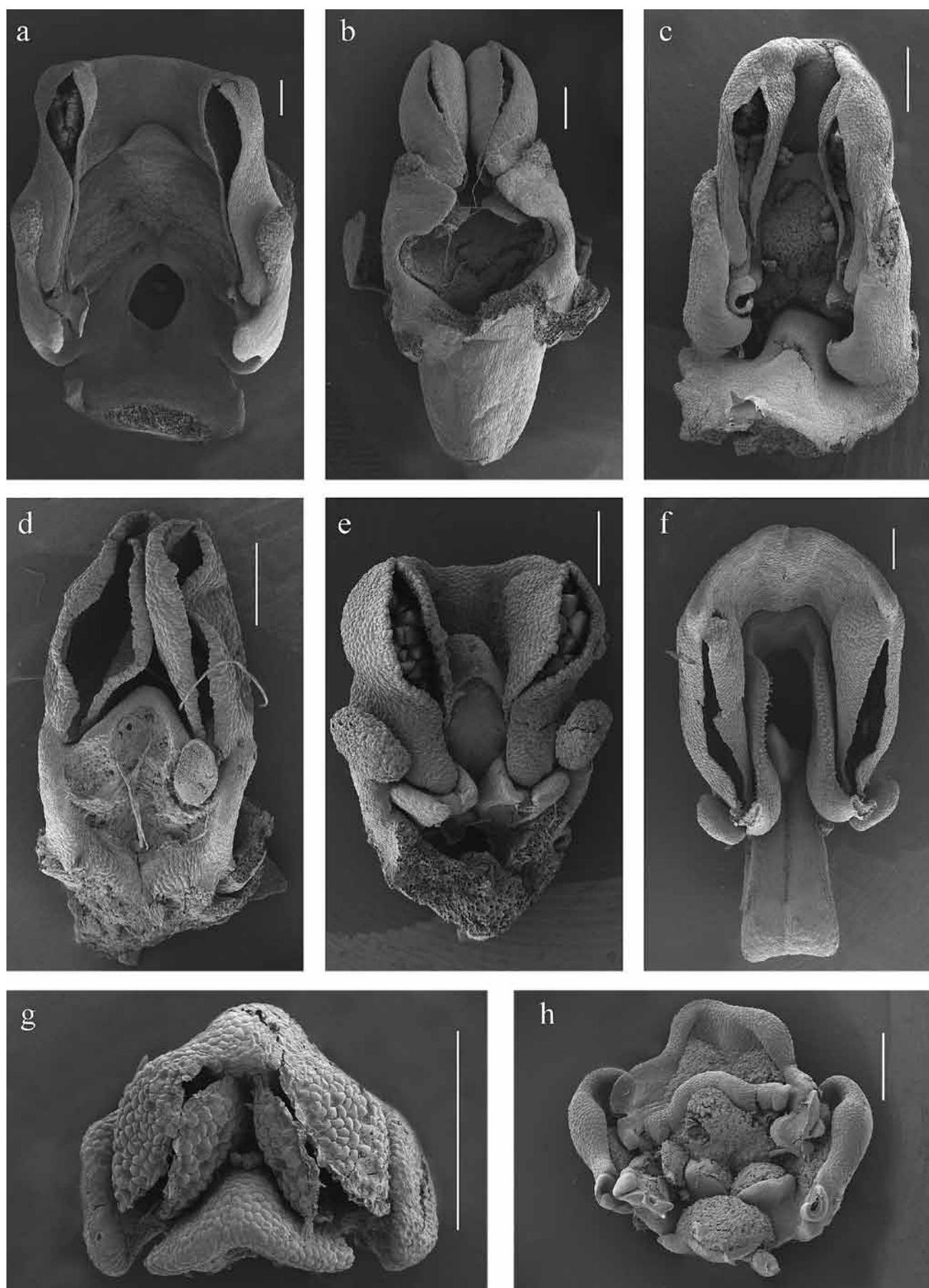


Fig. 2 Morphology of gynostemium (SEM). a – *P. mandarinorum*; b – *P. metabifolia*; c – *P. sachalinensis*; d – *P. convallariifolia*; e – *P. hologlottis*; f – *Habenaria linearifolia*; g – *P. chorisiana*; h – teratologic gynostemium of *P. sachalinensis* with a tendency to actinomorphy. Scale bars: 500 μm.

Herbs with 2 orthotropic shoots, one bearing inflorescence and another with leaf rosette, forming inflorescence in next year. Tuberooids 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-mazettii*, *P. juncea*, *P. leptocaulon*, *P. roseotincta*). Fig. 1c.

Orthotropic shoot 1. Tuberooids fusiform with root-end, in *P. bakeriana* somewhat stoloniferous. Tuberooids are sparsely covered with root hairs. Flowers green, yellowish or white, in *P. roseotincta* sometimes purplish. Hood present. Margin of tepals usually papillose, in *P. juncea* and *P. handel-mazettii* smooth. Lip simple, thick, in *P. roseotincta* somewhat dilated near its base. Rostellum short, almost completely turning into viscidia. Stigma lateral lobes protruding forward. Viscidia usually big, lanceolate to almost linear, situated in front of each other with small median part of rostellum between.

1d. *Platanthera stenantha*. Fig. 1d.

Orthotropic shoot 1. Tuberooids fusiform with root-end, densely covered with root hairs. Flowers green or yellowish. Hood present. Petals glabrous. Lip simple. Rostellar arms wide, slightly twisted. Stigma lobes hardly distinguishable, confluent to the common convex stigma. Lateral walls of the column forming wide space near spur entrance. Viscidia narrowly ovate, slightly twisted.

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

Orthotropic shoot 1. Tuberooids 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 lateral lobes prominent, slightly convex.

1f. *Platanthera latilabris*, *P. clavigera*, *P. biermaniana*. Fig. 1f.

Orthotropic shoot 1. Tuberooids 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. Tuberooids 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. *Tulotis* (*P. finetiana*, *P. fuscescens*, *P. hologlottis*, *P. japonica*, *P. sinica*, *P. ussuriensis*, *P. whangshanensis*). Fig. 1h; Fig. 2e; Fig. 3d.

Orthotropic shoot 1. Tuberooids 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. hologlottis*), 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.

Orthotropic shoot 1. Tuberooids spherical, densely covered with root hairs. Leaves almost wither by the flowering time. Flowers greenish to yellowish. Upper tepals spreading or connivent into a hood. Tepals glabrous. Lip simple. Auricles almost absent. Rostellar arms flat. Stigma lateral lobes almost indistinguishable, united into a common stigma. Viscidia suborbicular to elliptical.

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

Orthotropic shoot 1. Tuberooids narrowly-lanceolate, almost root-like, glabrous. Flowers green. Hood present. Tepals glabrous. Lip simple, dilated at the base. Rostellar arms flat. Stigma lobes indistinguishable, united into a common convex stigmatic surface, which is as long as $\frac{1}{2}$ of the gynostemium and strongly protrudes forward. Viscidia suborbicular.

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

Orthotropic shoot 1. Tuberooids narrowly fusiform, hairy. Leaves margin crenulate. Flowers purple, rarely white. Hood present. Tepals glabrous. Lip apically 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. Tuberooids 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. psychodes*). Fig. 1m.

Orthotropic shoot 1. Tuberooids fusiform, with long root-end, glabrous. Flowers purple or white. Hood pres-

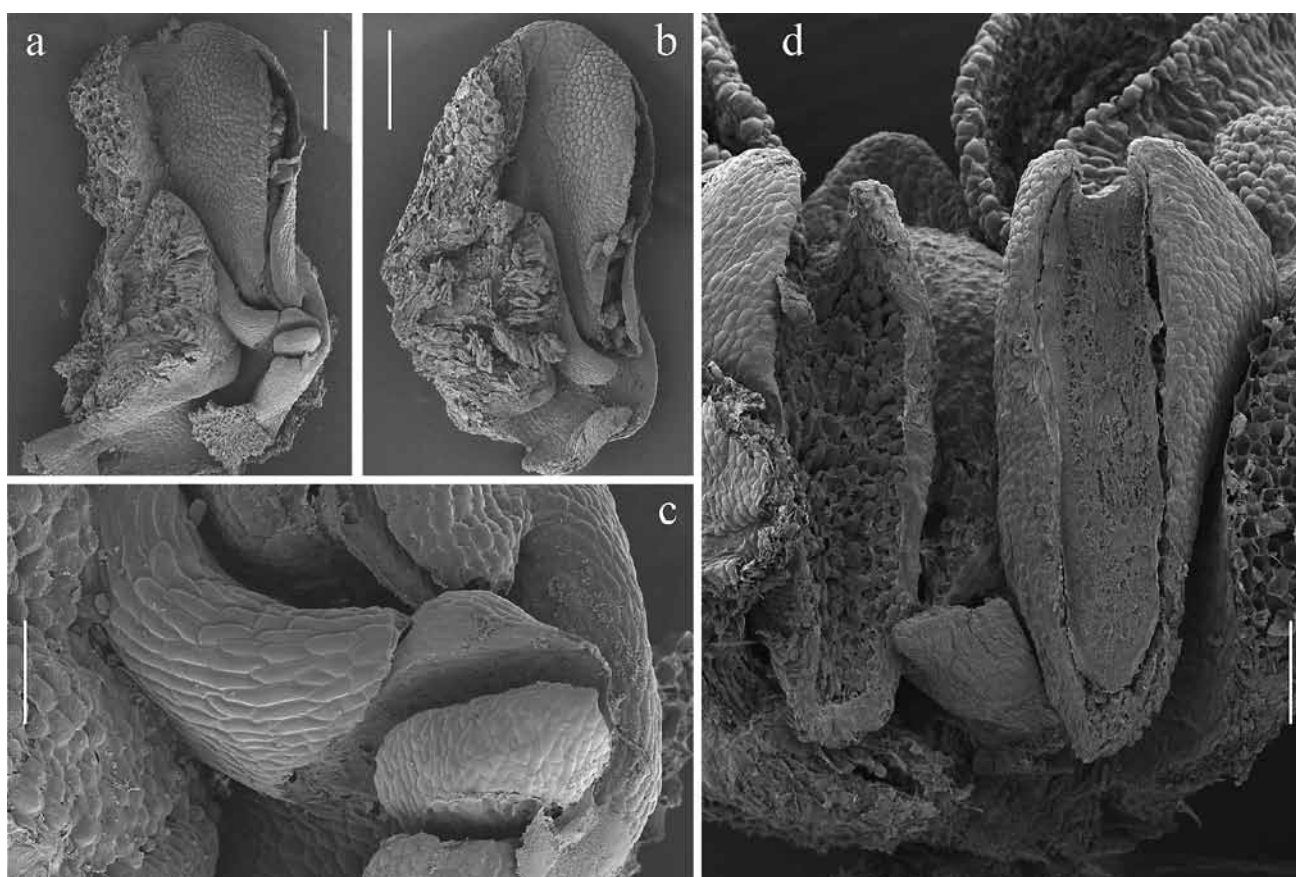


Fig. 3 Viscidia of *Platanthera sachalinensis* and *P. fuscescens* (SEM). a – *P. sachalinensis*, cross-section of gynostemium with intact viscidium; b – *P. sachalinensis*, cross-section of gynostemium with removed viscidium; c – *P. sachalinensis*, one viscidium enlarged; d – *P. fuscescens*, bottom part of the gynostemium with one viscidium removed and one viscidium remained intact. Scale bars: a and b – 500 μm , c – 100 μm , d – 200 μm .

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 transpiration 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

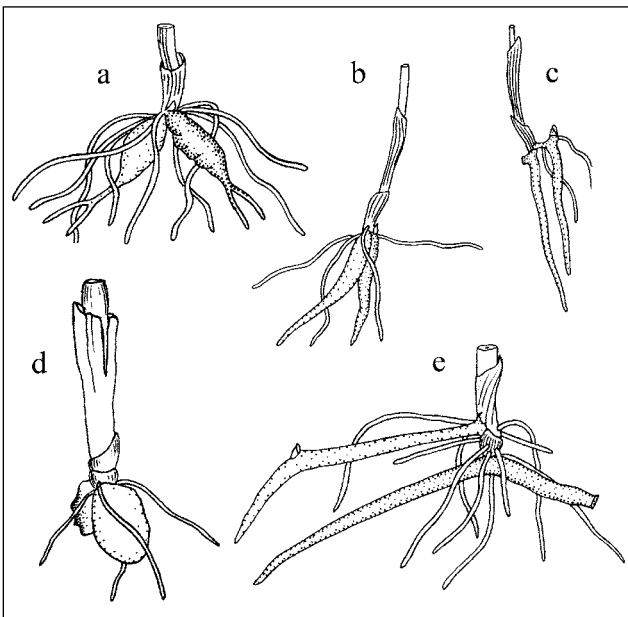


Fig. 4 Tuberoids. a – *P. chlorantha*; b – *P. mandarinorum*; c – *P. oligantha*; d – *P. elongata*; e – *P. hologlottis*.

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 fusiformly 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. fuscescens* (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*, *Lysiella*, *Pseudodiphryllum*, 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. ophrydioides*, *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. hologlottis*, and *P. brevicealcarata*). 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. iinumae*, *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 *Diphylax* (Szlachetko and Rutkowski 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 camtschatica* (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 *Pseudodiphryllum* 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 camtschatica*) or protruding down and conjoined 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. ussuriensis*) or less (*P. japonica*, *P. sinica*) twisted. In some autogamic taxa viscidia might be reduced at all (as in *Platanthera camtschatica* 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 camtschatica* 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. hologlottis*, and *P. fuscescens* ('*Tulotis*') is hamulate, and

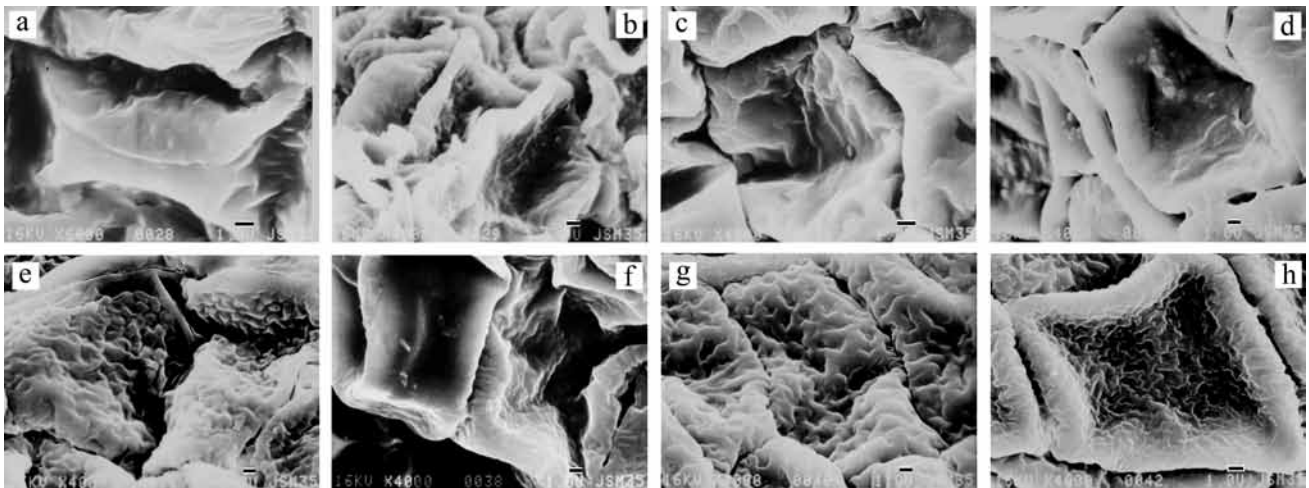


Fig. 5 Pollen morphology. a – *P. bifolia*; b – *P. oligantha*; c – *P. tipuloides*; d – *P. sachalinensis*; e – *P. convallariifolia*; f – *P. chorisiana*; g – *P. holo-glottis*; h – *P. fuscescens*. Scale bars: 1 μ m.

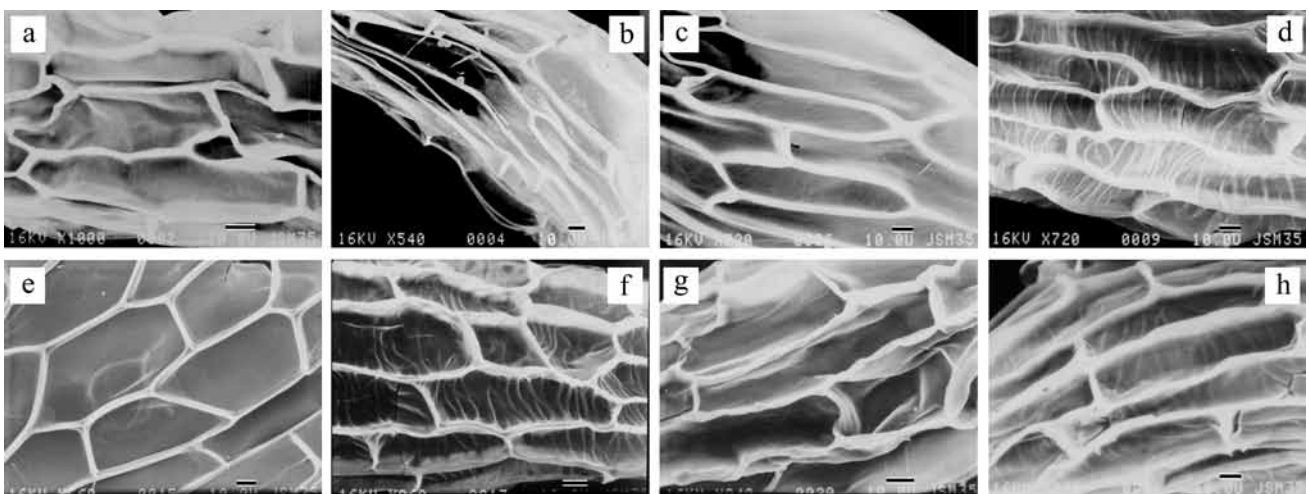


Fig. 6 Morphology of seed testa. a – *P. bifolia*; b – *P. oligantha*; c – *P. tipuloides*; d – *P. sachalinensis*; e – *P. convallariifolia*; f – *P. chorisiana*; g – *P. holo-glottis*; h – *P. fuscescens*. Scale bars: 10 μ m.

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. chorisiana*), and two species of *Tulotis* (*P. holo-glottis* 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. holo-glottis* 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 *Galearis*+*Amerorchis* clade.

Furthermore, 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 *Habenariinae*. 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, Szlachetko (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 *Habenariinae*.

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 *Habenariinae*. 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-mazzettii*, 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 gynostemium 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 pseudocopulatory 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-

togamy. 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. chorisiana*, 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.

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